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PHARMACEUTICAL AND ENVIRONMENTAL ANALYSIS USING MEMBRANE INLET MASS SPECTROMETRY

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This thesis is, to the best of my knowledge, original except where due reference is made.

David Gómez Lamarca, October 2002

Abstract

The development of membrane inlet mass spectrometry strategies for semi-volatile and volatile organic compounds has been investigated. Linear quadrupole and ion trap mass spectrometers have been used for detection of target analytes related to the pharmaceutical industry.

In-membrane pre-concentration membrane inlet mass spectrometry has been employed for the analysis of semi-volatile organic compounds in aqueous samples. A drying stage between in-membrane pre-concentration and thermal desorption has been incorporated into the technique, increasing sensitivity. Matrix and pH studies have also been carried out to optimise the effects caused to MIMS analysis.

The in-membrane pre-concentration procedure has been applied to biodegradation monitoring. The technique compared well with HPLC and ion chromatography methods for the at-line monitoring of 4-FBA and 4-FCA biodegradation process. An on-line monitoring system was also developed and validated. The technique has been demonstrated for the biodegradation of a mixture of nitrogen compounds relevant to the pharmaceutical industry.

Reversed-phase membrane inlet mass spectrometry incorporating a hollow fibre Nafion® membrane has been developed for the determination of methanol and ethanol in chloroform. The hydrophilic Nafion® membrane preferentially transports methanol and ethanol, whilst discriminating against a chloroform. The system has been evaluated for the monitoring of a chloroform recovery process using a residual gas analyser and the data compared to an established GC/MS analyser.

A temperature controlled membrane inlet has been constructed as a universal interface for hollow-fibre silicone membranes. The membrane temperature is controlled by an electrical heater and sub-ambient temperatures were obtained using a flow of cooled nitrogen gas. The device has been linked to mass spectrometry and GC/MS for the determination of volatile and semi-volatile organic compounds in aqueous and air samples at temperatures in the range -70–250°C.

Acronyms

3BHP	3-bromo-hydroxypyridine
3BP	3-bromopyridine
4-FAP	4-fluoroacetophenone
4-FBA	4-fluorobenzoic acid
4-FCA	4-fluorocinnamic acid
APCI	Atmospheric pressure chemical ionisation
CFA	2-chloro-5-trifluormethylaniline
CI	Chemical ionisation
CID	Collision-induced dissociation
COD	Chemical oxygen demand
CSTB	Continuous stirred tank bioreactor
DIMP	Direct insertion membrane probe
DMF	Dimethylformamide
DMSO	Dimethylsulfoxide
EI	Electron ionisation
FBA	Flurobenzoic acid
FMA	3-trifluoromethylaniline
FHA	3-trifluoromethyl-hydroxyaniline
GC	Gas chromatography
HPLC	High performance liquid chromatography
IC	Ion chromatography
IMP	In-membrane preconcentration

IMP-MIMS	In-membrane preconcentration membrane inlet mass spectrometry
IMP-MIMS/MS	In-membrane preconcentration membrane inlet tandem mass spectrometry
LC	Liquid chromatography
MI	Membrane inlet
MS	Mass spectrometry
MSM	Mineral salt medium
MS/MS	Tandem mass spectrometry
MS^n	Multi-stage tandem mass spectrometry
NMP	N-methylpyrrolidinone
RGA	Residual gas analyser
RP-MIMS	Reversed-phase membrane inlet mass spectrometry
S:N	Signal to noise ratio
SVOC	Semi-volatile organic compound
TCMI	Temperature controlled membrane inlet
TCMI-MS	Temperature controlled membrane inlet mass spectrometry
TCMI-GC/MS	Temperature controlled membrane inlet gas chromatography mass spectrometry
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TMEDA	tetramethylethylenediamine
VOC	Volatile organic compound
WWTP	Wastewater treatment plant

CONTENTS

CHAPTER 1

Introduction

1.1. Mass spectrometry

1.1.1. Introduction

Mass spectrometers have been in use since the beginning of this century [1], but mass spectrometry did not become a more commonly used technique until its application to the characterisation of organic molecules in the petroleum industry 40 years later [2]. The coupling of mass spectrometry with electrophoretic [3, 4] and chromatographic [5, 6] separation systems has led to the widespread use of mass spectrometry today.

Mass spectrometers work at low pressures, normally between 10^{-3} and 10^{-7} torr, achieved by multi-stage vacuum pumps. At such low pressures, ion-molecule scattering and the oxidation of hot metallic surfaces such as lenses and filaments is eliminated, therefore lengthening the lifetime of the instrument. As inlet systems are normally held at atmospheric pressure, the system must take this into consideration and be able to provide conditions needed for sample volatilisation. The system used for introduction of the sample into the ion source depends on the physical state of the sample: gas, liquid or solid.

Three different processes take place in any mass spectral analysis: formation of gas-phase sample ions, separation of ions according to their mass-to-charge ratio (m/z) and detection and electronic recording of their relative intensities. In order to obtain a mass spectrum, four different instrumental components are required: a sample inlet system, an ionisation source, a mass analyser and a detector. The

ionisation step requires the introduction of sample molecules into close proximity with the ion source, where destructive ionisation takes place (normally consuming picograms of sample during this step).

1.1.2. Inlet systems

Liquid samples may be introduced into the system via a liquid reservoir, consisting of a glass vial connected to the spectrometer vacuum manifold via a fine-control needle valve and shut-off toggle valve. This method is also suitable for the introduction of other liquids that are volatile at room temperature, even if they are not used as calibration gases. Less volatile liquids can be injected into the mass spectrometer by gently heating them in a vial prior to injection. Gases at atmospheric pressure can be injected by a similar device. The inlet system is flushed with the sample until a valve is opened. Then, part of the sample diffuses into the ion source.

For solids or very involatile liquids, samples are introduced with the help of a probe. The probe is normally made of stainless steel and the sample is loaded inside a quartz or silica tube. This whole assembly is introduced into the entrance of the ionisation chamber, through a vacuum lock. This process requires the four following steps. Firstly, the sample is placed into the clean sample holder. The probe is then introduced into the vacuum lock, which is pumped to the required pressure. The valve between the vacuum lock and the main vacuum chamber containing the ion source is then opened, so that the probe can pass through it in order to bring the sample holder into the source, where the sample is finally

desorbed. Once a spectrum is obtained, the sample holder is withdrawn from the ion source and then from the vacuum lock, making sure vacuum inside the mass spectrometer is maintained during the whole process. Such probes can be independently heated by a probe-controller assembly at various rates, to temperatures in excess of 400°C. The desorption of thermally unstable compounds requires rapid heating, so that the substance can pass into the gaseous state before thermal decomposition takes place.

Another method of introducing samples into a mass spectrometer is by coupling to either gas chromatography (GC/MS) or liquid chromatography (LC/MS). The difficulty in coupling a chromatograph to a mass spectrometer is the increase in pressure in the ion source caused by the carrier gas or liquid eluate from the chromatograph. Two different types of coupling can be used, depending on the type of chromatographic column: direct introduction of the capillary into the ion source is possible when using capillary columns, while packed columns require separators. In direct coupling interfaces, the end of the chromatographic capillary passes into the ion source through a glass-lined stainless-steel capillary that is heated to the required temperature by a resistance heater.

A jet separator can be used when coupling with a gas chromatograph, if the mobile phase flow is high. This kind of separator is based on the fact that the velocity of a gas molecule is a function of the molecular weight (Graham's law of diffusion). As low molecular weight species tend to be carrier gases or eluant solvents, they can be removed by pumping down during their passage towards the ion source. Therefore the gas flow reaching the source is enriched with the

higher molecular weight species. Membrane separators have also been used to interface gas chromatography with mass spectrometry, based on the relative permeability of a membrane for different molecules.

When coupling with a liquid chromatograph, the mobile phase can be easily evaporated, but the mass spectrometer would not be able to handle the large amount of gas produced. A more efficient way of overcoming this problem is by atmospheric pressure ionisation (API), in which the analyte is desolvated and ionised at atmospheric pressure before being introduced into the mass spectrometer.

1.1.3. Ionisation methods

Neutral molecules must be ionised before mass analysis and there are many ionisation methods available, including some for solids and less volatile liquid samples, such as laser desorption, fast atom bombardment, electrospray, inductively coupled plasma and atmospheric pressure chemical ionisation. However, only electron and chemical ionisation have been used in this work and are discussed below.

1.1.3.1. Electron ionisation

Electron ionisation (EI) has been in use for many years, and is still in widespread use. This source was devised by Dempster [7] and improved by Bleakley [8] and Nier (Figure 1.1) [9]. When an electric current (normally between 80 and 100 μA) is passed through a filament (tungsten or rhenium), electrons are emitted

from the surface. A 70 V potential accelerates the electrons across the source housing to a collector plate, creating a 70 eV electron beam. This value is known as the standard energy for ionisation under EI conditions, as it gives good ion yield (on average, one ion is produced for every 1000 molecules entering the source) and reproducibility (Figure 1.2).

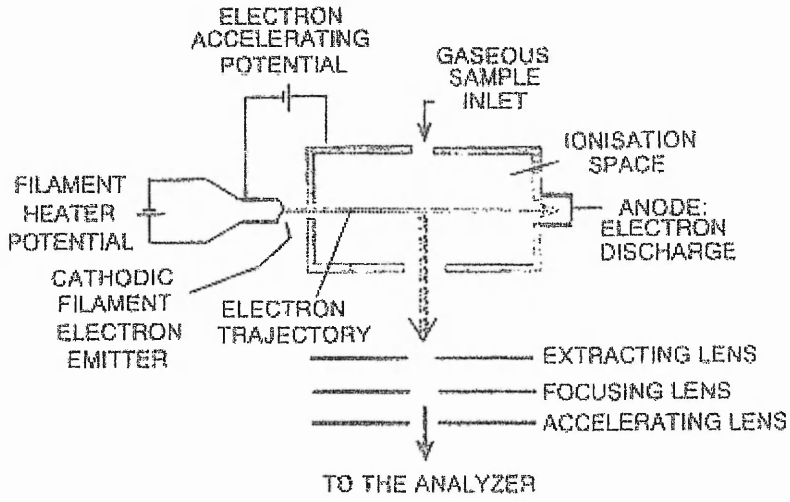

Figure 1.1: An electron ionisation source

In electron ionisation, a gaseous sample molecule (M) will undergo the following process if an electron interacts strongly enough with the sample:

$$M + e^-_{(70\ eV)} \longrightarrow M^{+\bullet} + 2e^- \qquad \text{(eq. 1.1)}$$

This process leads to the formation of a characteristic molecular radical cation ($M^{+\bullet}$) for each compound. EI is quite inefficient, as less than 1% of the molecules are ionised, but the high sensitivity of modern detectors compensates for this inefficiency. However, as the average ionisation energy of most

compounds lies below 15eV, the EI process imparts a large amount of excess internal energy to a significant proportion of the radical cations formed. This leads to a rapid cleavage of chemical bonds in the sample molecule-ion, resulting in the presence of fragment ions in the mass spectrum. Fragmentation is in general a useful tool for structural determination, but in some cases, electron ionisation is too hard and the molecular ion ($M^{+\bullet}$) is not present in the spectrum. A way to determine the molecular weight of such molecules is then by applying a smaller energy to the sample during the ionisation step. This is possible by using chemical ionisation.

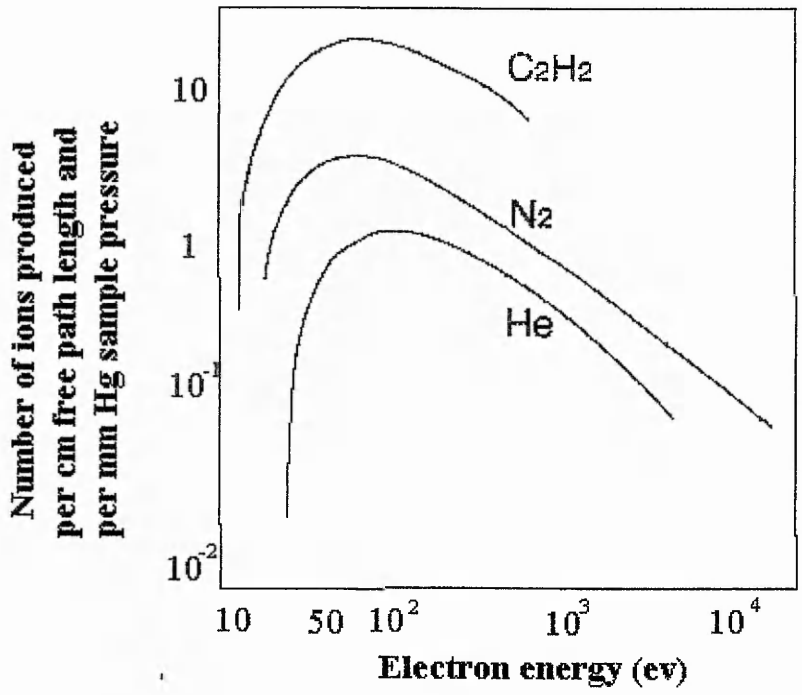

Figure 1.2: Ion current vs. electron energy for electro ionisation

1.1.3.2. Chemical ionisation

Chemical ionisation (CI) has become a widely accepted ionisation technique, used to complement EI and overcome the fragmentation problems associated with EI analysis of some molecules. Chemical ionisation was invented in 1966 by Munson and Field [10] and was quickly commercialised and applied to many different analytes. Instrumentally, the CI source is similar to the EI source, except that the ionisation region must contain a high pressure (0.1 to 2 torr) of reagent gas, which is typically ammonia, methane, iso-butane or water. The introduction of these gases normally takes place using a regulated supply or with the sample effluent. Under these conditions, primary ions rapidly undergo ion-molecule reactions to form secondary reagent ions. For example, CI with ammonia would involve the following process:

Primary ion formation:

$$NH_3 + e^- \longrightarrow NH_3^{+\bullet} + 2e^- \qquad \text{(eq. 1.2)}$$

and then

Secondary ion formation:

$$NH_3^{+\bullet} + NH_3 \longrightarrow NH_4^+ + NH_2^\bullet \qquad \text{(eq. 1.3)}$$

The other common CI gases react in a similar way, producing CH_5^+ for methane, $C_4H_9^+$ for iso-butane and H_3O^+ for water as reagent ions.

On introduction of sample molecules (M) into a CI source, any of the following reactions may occur (using ammonia as an example, but similar reactions occur with other reagents):

$$NH_4^+ + M \longrightarrow NH_3 + [M+H]^+ \text{ (proton transfer)} \quad \text{(eq. 1.4)}$$

$$NH_4^+ + M \longrightarrow [M+NH_4]^+ \text{ (electrophilic addition)} \quad \text{(eq. 1.5)}$$

$$NH_4^+ + M \longrightarrow NH_4A + N^+ \text{ (anion abstraction)} \quad \text{(eq. 1.6)}$$

$$NH_3^{+\bullet} + M \longrightarrow NH_3 + M^{+\bullet} \text{ (charge exchange)} \quad \text{(eq. 1.7)}$$

The most important reaction is proton transfer, leading to a pseudo-molecular ion, $[MH]^+$. The thermodynamics of such reaction are as follows:

$$\Delta H^\circ = -RT \ln K \cong [\text{proton affininty (M)} - \text{proton affinity (CI gas)}] \quad \text{(eq. 1.8)}$$

where proton affinity is defined as:

$$\text{P.A. (M)} = -\Delta H^\circ \ (M + H^+ \rightarrow [M+H]^+) \quad \text{(eq. 1.9)}$$

This has very important consequences. Firstly, the difference in proton affinities for any combination of common reagent and sample molecules is much less than the excess energy associated with electron impact, and the $[M+H]^+$ ions formed will have little excess internal energy. Therefore, chemical ionisation spectra have prominent molecule-ions, with little or no fragmentation, making CI a technique of choice for molecular weight determination. Secondly, by careful choice of reagent gas, the exothermicity can become the key to the selectivity of the process. For example methane has a proton affinity of 536 kJ mol^{-1}, enabling it to protonate most organic molecules, while ammonia, with a proton affinity of 847 kJ mol^{-1}, will only ionise polar molecules, in particular amines. This opens

the possibility of selectively ionising certain components in a mixture at the expense of others. Much work has been done on the selective CI, using many different reagent ions, for the analysis of a wide range of compounds [11-14].

Negative chemical ionisation is possible through an acid-base reaction using reagent ions such as OH^- or NH_2^-. The major mechanisms of ionisation are proton abstraction and nucleophilic addition, both of which are analogous to the processes observed in positive CI. In some cases, the detection sensitivity is better with negative CI than with positive CI. The need to detect tetrachlorodioxins with high sensitivity has contributed to the developments of negative ion chemical ionisation.

1.1.4. Mass analysers

This part of the mass spectrometer performs the separation of the ions according to their mass-to-charge ratio (*m/z*). As ions produced by EI or CI usually have a charge state of one, the *m/z* ratio tends to dictate the mass alone. The three main characteristics of an analyser are the upper mass limit, the transmission and the resolution. The mass limit determines the highest value of the *m/z* ratio that can be measured. The transmission is the ratio between ions reaching the detector and ions produced in the source. The resolving power is the ability of the analyser to yield distinct signals for two ions with a small mass difference. There are a number of methods of mass analysis, all of which are commercially available: linear quadrupole, quadrupole ion trap (QIT), time-of-flight (TOF), magnetic sector and Fourier transform ion cyclotron resonance (FTICR). Only

linear quadrupole and quadrupole ion trap mass analysers were used in this work and will be discussed in detail.

A calibration standard, which the ionisation of gives a characterised response allowing accurate placement of detected mass-to-charge ratios, is needed to calibrate the mass spectrometer. This calibration standard is normally a volatile liquid, and the most widely used is perfluorotributylamine (PFTBA or FC-43).

1.1.4.1. Linear quadrupoles

The design of a linear quadrupole was first reported in 1953 by Paul and Steinwegen [15] and was patented in 1956 [16]. These devices are the most common kinds of mass analysers in environmental laboratories, as they provide powerful detection, mainly for GC separations, and are economically affordable.

Linear quadrupole analysers consist of four rods with circular or, ideally, hyperbolic sections. A schematic diagram of a quadrupole analyser is shown in Figure 1.4 and a picture is shown in Figure 1.5. Diagonally opposite pairs of rods are electrically paired and coupled to direct current voltages (U) and a radio frequency potential (Vcos ωt). A positive ion entering the space between the rods will be drawn toward a negative rod. If the potential changes sign before it discharges itself on the rod, the ion will change direction. Ions travelling along the z axis are subjected to the influence of a total electric field (Φ_0) resulting from the application of the potentials upon the roads:

$$\Phi_0 = U + V\cos \omega t \qquad \text{(eq. 1.10)}$$

while the potential (Φ) at any point (x,y) in the quadrupole field is:

$$\Phi = \Phi_0 (x^2 - y^2)/r_o^2 \qquad \text{(eq. 1.11)}$$

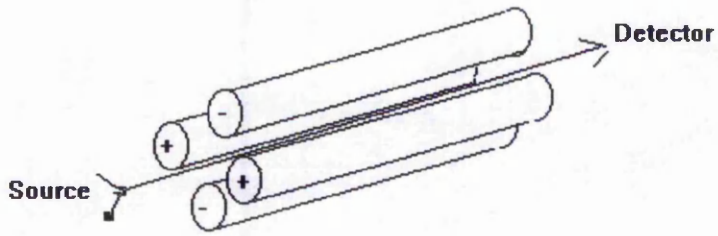

Figure 1.4: Linear quadrupole analyser schematic.

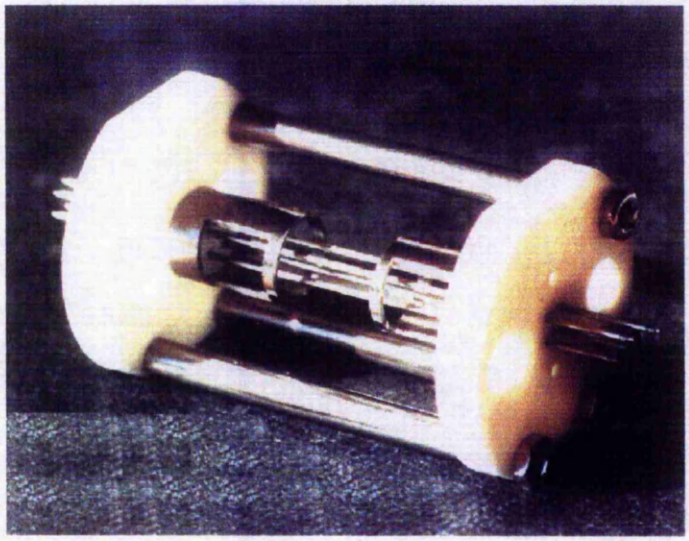

Figure 1.5: Photograph of a linear quadrupole analyser.

Ions travelling down the quadrupole have complex periodic motion, the equations for which can be given under Mathieu's equation (derived from the second law of Newton) [17].

$$\frac{d^2u}{d\xi^2} + (a_u - 2q_u \cos 2\xi)u = 0 \qquad \text{(eq. 1.12)}$$

where u represents either x or y and ξ is πft, t is time; f is frequency; a and q are dimensionless constants, which have the following equations in a quadrupolar field:

$$a_x = -a_y = -\frac{8eU}{mr_o^2\Omega_o^{\ 2}} \qquad \text{(eq. 1.13)}$$

and $$q_x = -q_y = \frac{4eV}{mr_o^2\Omega_o^{\ 2}} \qquad \text{(eq. 1.14)}$$

where an ion of mass m and single charge e travels through a quadrupole of radius r_o, operating with RF and DC potentials $V_{0\text{-}p}$ and U respectively and an RF drive frequency $\Omega_O/2\pi$. For a given quadrupole r_0 is constant. $\omega = 2\pi f$ is maintained constant. U and V are the variables. Therefore, for an ion of any mass, x and y displacements can be determined as a function of U and V. For ions to pass unhindered along a linear quadrupole, they must be stable in both x and y directions and the Mathieu stability diagram, has one such significant region, shown enlarged (Figure 1.6), where this occurs. Ions in this region possess frequencies, Ω:

$$\Omega = \frac{\beta\Omega_o}{2} \qquad \text{(eq. 1.15)}$$

where β is a parameter directly related to the ion frequency, which can take values between 0 and 1. Ions with the same a and q coordinates possess the same fundamental, or "secular", frequency.

From the dimensionless parameters a and q given in equations 1.13 and 1.14, for given values of U, V, ω and r_0, the a and q values will depend on m/e, and since a/q is equal to 2U/V and independent of m/e, ω and r_0, the a, q co-ordinates corresponding to different m/e values will lie on a line of slope a/q drawn in the stability diagram. This line, called working line, cuts the stability boundary at two points, corresponding to two m/e values, m_1/e and m_2/e. All ions with m/e values between these will be transmitted by the filter and detected. Ions having the same m/e value will have the same a,q co-ordinates. The resolution will depend on the slope of the working line. The closer the line comes to the apex of the stability boundary, the greater the resolution, as a narrower "window" of *m/z* values are stable. The apex is at a = 0.237 and q = 0.706. Mass scanning is done by varying U and V simultaneously, while keeping their ratio and ω constant.

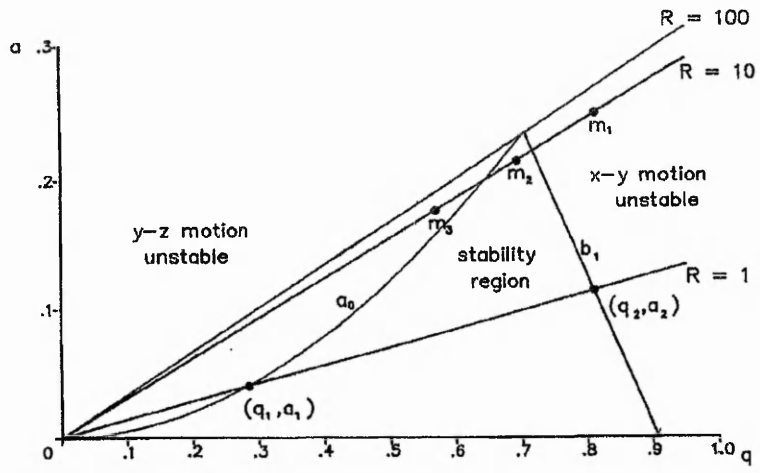

Figure 1.6: Stability diagram for a quadrupole, where a and q are dimensionless solutions derived from the Mathieu equation. The mass scan lines correspond to resolutions 1, 10 and 100: m_1, m_2 and m_3 represent three singly charged ions of increasing mass.

When working under high resolution conditions, transmission will suffer, making the quadrupole an inherently low-to-medium resolution device. The advantages of the quadrupole are the low cost, easy to use and compact size.

1.1.4.2. Quadrupole ion traps

The original public disclosure of the quadrupole ion trap, filed in 1953 is to be found in the same patent as that describing the operating principle of the quadrupole mass spectrometer [16]. The quadrupole ion trap is directly related to the linear quadrupole in that it can be visualised as being a solid of revolution generated by rotating the hyperbolic rod electrodes about an axis perpendicular to the z axis and passing through the centres of two opposing rods. This results in one pair of rods joining up to form a doughnut-shaped ring electrode and the other two forming end-cap electrodes, which are moved closer together. A design of it is shown in Figure 1.7 and two pictures of a quadrupole ion trap are shown in Figure 1.8.

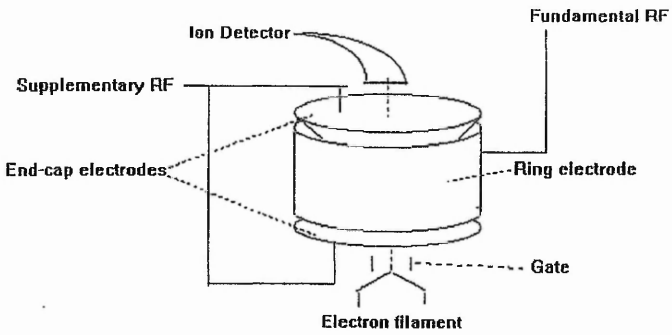

Figure 1.6: Quadrupole ion trap schematic.

As shown in Figures 1.7 and 1.8, the system is axially symmetric, and for ideal field geometry within the trap, the surfaces should also be hyperbolic. The field can be generated by applying the same RF and DC voltages between the ring electrode and the pair of end-cap electrodes as those applied to the linear quadrupole. However, it is more common to keep the end-cap electrodes at ground potential and apply an RF-only supply to the ring electrode.

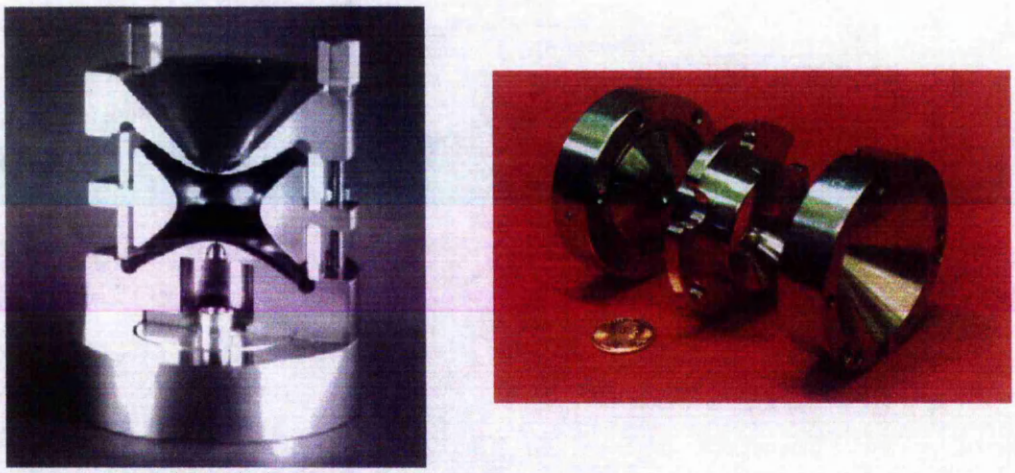

Figure 1.8: Quadrupole ion trap photographs.

Ions inside the trap exhibit complex periodic motion, in three dimensions. Mathieu equations (eq. 1.13 and 1.14) used for linear quadrupoles, can also be applied to ion traps by linking equations 1.13 and 1.14 to equation 1.16 [18].

$$r_0^2 = 2z_0^2 \qquad \text{(eq. 1.16)}$$

where z_0 is the closest distance between opposing end-cap electrodes and r_0 is the internal radius of the ring electrode.

Mathieu equations linked to eq. 1.16 can be solved to give a stability diagram, which is shown in Figure 1.9. Ions that exhibit stable trajectories can be trapped within the trapping volume of the three electrodes [19]. The investigation of ion motions in the ion trap has provided the opportunity to compare the predicted trajectories with those actually observed by photographing charged particles in dynamic equilibrium inside an ion-cage built from copper mesh. This phenomenon is called the Lissajous trajectory (Figure 1.10) [20].

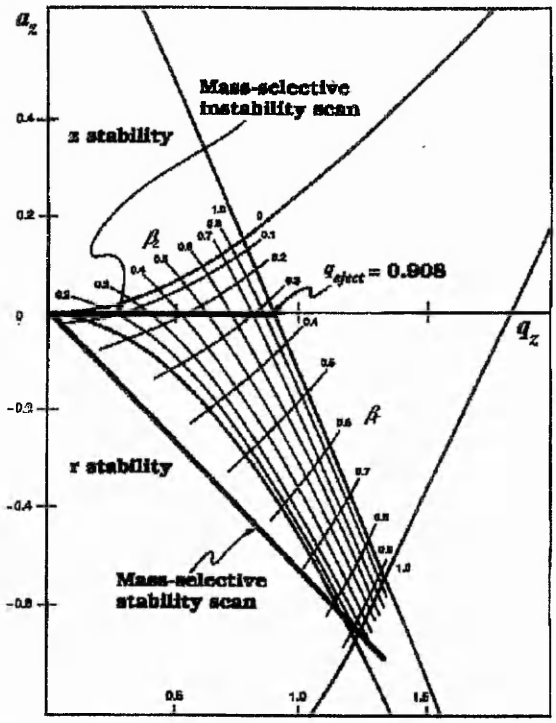

Figure 1.9: Stability diagram for quadrupole ion trap.

Whilst linear quadrupoles were widespread in the laboratory environment, ion trap mass spectrometers found little applications due to the lack of effective scan routines for accurate mass-to-charge ratio of the trapped ions. Some results were obtained [21, 22], but these were limited by long scan times, making the

technique incompatible with chromatographic coupling, that requires a higher scan rate. Ion traps were therefore used for mass-selective detection and for mass-selective storage.

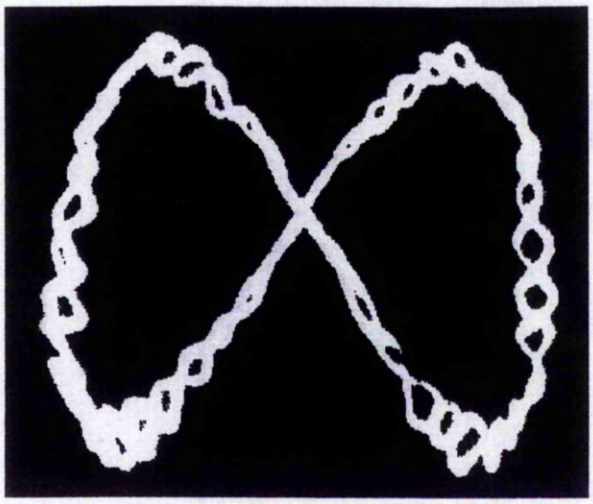

Figure 1.10: Ion motion in a three dimensional trap of metallic particles following a Lissajous trajectory.

However in 1983, Stafford and co-workers announced and patented a new scan method [23, 24]: the mass selective-instability scan. In this simple arrangement, an RF voltage was applied to the ring electrode instead of to all electrodes. After ion formation by electron bombardment of neutral molecules inside the trapping volume from gated electrodes, the RF potential (U) is linearly increased, to a maximum value of 15 kV. The q value of all trapped ions increases proportionally as a function of U until $q_z = 0.908$, at the boundary of the stability diagram (β=1). Resonant coupling of the drive and secular frequency ($\Omega = \frac{\Omega_0}{2}$) occurs, ion oscillations in the z direction exponentially increase until ejection

takes place. Around 50% the population is ejected towards each end cap, so nearly half of all trapped ions are detected, giving ion trap mass spectrometers their inherent sensitivity. Performance of the ion trap was also aided by the discovery that the presence that a light buffer gas such as helium or hydrogen, at a pressure of approximately 0.1 Pa significantly improves sensitivity, resolution and detection limit. This is due to ion-buffer gas collisions, as they tend to cool down the ion volume inside the trap to a dense cloud at the trap centre, minimising the scattering effect. [25] These improvements on the ion trap led to commercialisation of the instruments by Finningan MAT (San Jose, CA, USA), as a GC detector, the ITD and as a research instrument, the ITMS^{TM}. A schematic of a typical scan routine in the ITMS^{TM} is shown in Figure 1.11.

The early ion traps suffered a loss of mass resolution at high analyte concentrations. This was due to a phenomenon known as space charging, which occurs when the total number of trapped ions exceeds a certain limit (generally between 10^5 and 10^6). Above this upper limit, a significant proportion of the potential felt by each ion is due to coulombic repulsion. Therefore ions with same *m/z* values occupy different points in the stability diagram and are ejected at different times during the mass scan, leading to peak broadening. High analyte concentrations also resulted in ion-molecule reactions, mainly leading to enhanced $[M+H]^+$ peak intensities. These problems were overcome by a software modification, called automatic gain control (AGC). [26] This new scan function, developed by Finnigan MAT for ion trap instruments under computer control, allows a brief ionisation pulse of about 200 μs after which the intensity of fragment ions up to those of *m/z* 45 is measured. The total ion intensity is

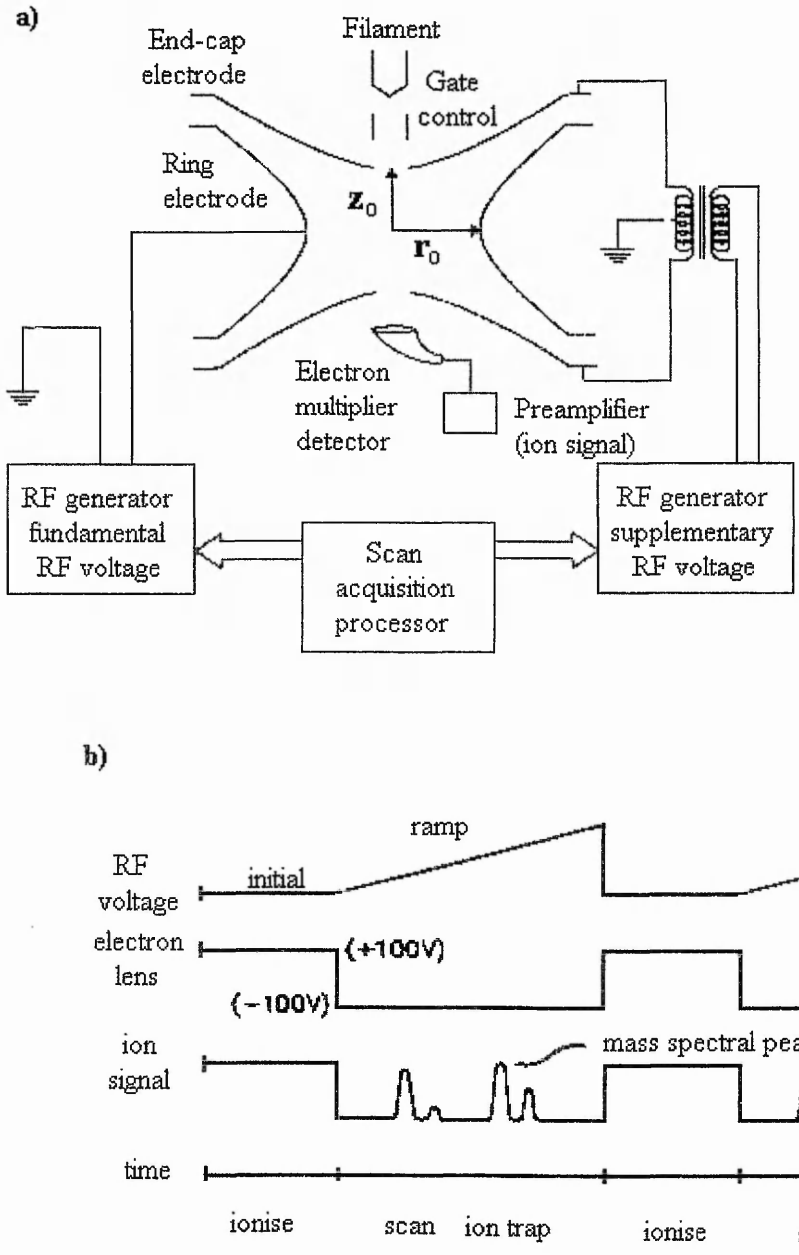

Figure 1.11: a) Schematic diagram of an ion trap mass separator and b) a typical scan routine for elution ionisation.

assessed by the computer, which then determines an appropriate duration for the second ionisation pulse so as to maximise sensitivity at a minimum acceptable level of space-charge perturbation.

The wide acceptance of ion trap mass analysers after all these improvements to the original design now promotes the sensitive and selective analysis of many complex samples. The principles and applications of ion trap mass spectrometry have been detailed in a number of reviews [27, 28].

1.1.5. Ion trap tandem mass spectrometry

Tandem mass spectrometry (MS/MS) is a method in which a precursor of a selected *m/z* ratio ion is isolated in the ion trap and then fragmented to yield product ions and neutral fragments. The product ions are then mass analysed in the usual way to give the product ion spectrum. This technique was developed to provide structural information from a peak in a mass spectrum and to enhance selectivity in the analysis of complex mixtures.

Fragmentation of selected precursor ions may be carried out using a variety of techniques including collision-induced dissociation (CID). CID consists of two steps. The first step corresponds to the collision between the ion and a target gas (ion activation), while the second step is the unimolecular decomposition of the activated ion (ion dissociation). CID in tandem mass spectrometry can therefore be described by the following processes:

Ion activation: $M_1^+ + N \rightarrow M_1^* + N$ (eq. 1.17)

Ion dissociation: $M_1^* \rightarrow M_2^+ + M_3$ (eq. 1.18)

where

M_1^+ is the accelerated precursor ion with high translational energy

N is the neutral target gas (e.g. helium or nitrogen)

M_1^* is the activated ion ($E>E_0$)

M_2^+ is the resultant fragment ion

M_3 is the neutral fragment formed

The energy and momentum conservations imply that only a fraction of the translational energy is converted into internal energy under inelastic conditions. This energy fraction is given by the following equation:

$$E_{con} = E_{lab} \frac{M_t}{M_t + M_a} \qquad \text{(eq. 1.19)}$$

where M_a is the ion mass, M_t is the target mass, E_{lab} is the ion kinetic energy in the laboratory frame of reference and E_{con} is the maximum energy fraction converted into internal energy. Consequently, an increase in the ion kinetic energy or in the target gas mass increases the energy available for the conversion.

The selectivity of tandem mass spectrometry can be improved by carrying out multi-stage tandem mass spectrometry (MS/MS/MS or MS^n). As the number of ions transmitted in every stage decreases due to the ionic species trajectory

lengths and the presence of the collision gas, the technique is generally limited in the number of stages of tandem mass spectrometry to three or four, although MS^{12} has been reported before [29]. However, at each stage the chemical noise decreases more than the analyte ionic signal, so by using tandem mass spectrometry, the signal to noise ratio improves, enhancing selectivity.

MS^n is most commonly performed in the quadrupole ion trap and FTICR, as ion isolation and product ion analysis in different stages are temporally, but not spatially, separate. This leads the ion trap to be referred as a "tandem-in-time" instrument [30, 31].

1.1.6. Detectors

Once the beam analyser has passed through the mass analyser, it is detected and transformed into a usable signal by a detector. There are different kinds of detectors, divided in two categories: Faraday cage and photographic plate, which allow a direct measurement of the charges that reach the detector, while electron or photomultiplier detectors and array detectors increase the intensity of the signal. During this work only electron multipliers have been employed, so they will be discussed in more detail.

Electron multipliers (see Figure 1.12) are used as detectors, where a positive or negative ion reaching the plate causes the emission of several secondary particles. These second particles can be cations, anions, electrons and neutrals. These secondary particles are accelerated into the continuous-dynode electron multiplier. They strike the cathode with enough energy to dislodge electrons as

they collide with the inner walls. These electrons pass into the electron multiplier, still striking the walls, causing the emission of more and more electrons as they travel toward the ground potential. Thus a cascade of electrons is created that finally results in a measurable current at the end of the multiplier. Although these detectors are not as precise as Faraday plates, due to their great sensitivity (being able to reach up to 10^7 counts), they allow rapid scanning.

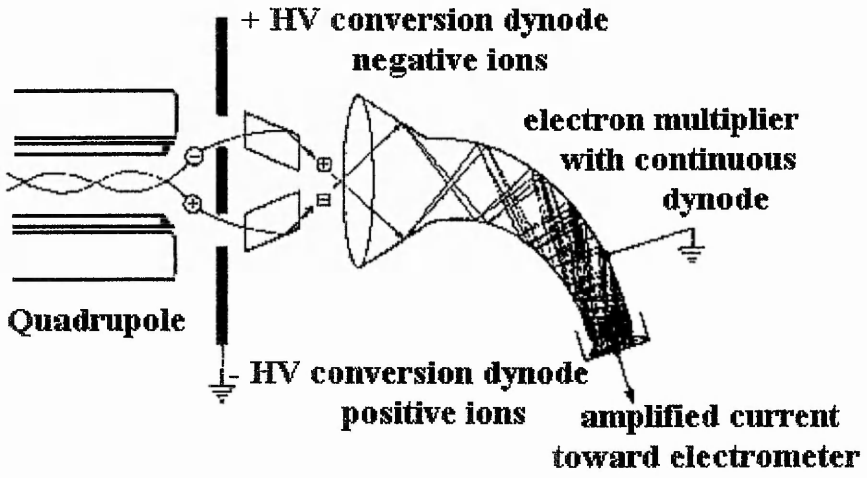

Figure 1.12: Schematic of an electron multiplier.

1.2. Chromatography

1.2.1. Introduction

The term chromatography has been applied to cover the "science of separations". Chromatography embraces techniques that enable samples of chemical mixtures to be separated by exploiting differences in their chemical and physical properties. There are different kinds of chromatographic separations, liquid and gas, but the principle is similar for both types.

1.2.2. Principles of chromatography

Column chromatography is based on the separation of components of a mixture by establishing conditions under which the individual components flow at different rates through the column, under the influence of a mobile and a stationary phase. The stationary phase is the column packing material (the material of the column will interact reversibly with the different analytes), and the mobile phase may be a liquid (liquid chromatography or ion chromatography) or a gas (gas chromatography). The stronger the interactions between the analyte and the stationary phase, the longer it will be retained on the column, therefore the separation process is due to chemical properties of the different analytes. This phenomenon can be quantified and is determined by the partition coefficient K of the analyte, which is defined by the following expression (equation 1.20):

$$K = \frac{C_S}{C_M} \qquad \text{(eq. 1.20)}$$

where C_s and C_m are the concentrations of analyte in the stationary and mobile phase respectively.

The capacity factor (k') is a measure of the amount of solute in each phase and therefore, the amount spent in each, and can be defined as (eq. 1.21):

$$k' = \frac{C_S V_S}{C_M V_M} = K \frac{V_S}{V_M} \qquad \text{(eq. 1.21)}$$

where V_m and V_s are the mobile and stationary phase volumes.

As the capacity factor is specific to a single solute in a given chromatographic system, it can also be calculated by measuring the retention time of two solutes one retained (t_R) and the other unretained (t_m).

$$k' = \frac{t_R - t_m}{t_m} \qquad \text{(eq. 1.22)}$$

In order to describe the solute separation in a chromatographic analysis, two different parameters can be used: resolution and selectivity. Resolution (R_S) is calculated by dividing the distance between adjacent peak maxima (t_{R1} and t_{R2}) by their mean base width (W_{b1} and W_{b2}) (equation 1.23). Selectivity (α) is expressed as the ratio of the capacity factors of the two components of interest (equation 1.24).

$$R_S = \frac{t_{R2} - t_{R1}}{0.5(W_{b2} + W_{b1})} \qquad \text{(eq. 1.23)}$$

$$\alpha = \frac{t'_{R2}}{t'_1} = \frac{k'_2}{k'_1} = \frac{K_2}{K_1} \qquad \text{(eq. 1.24)}$$

where $t'_R = t_R - t_0$

In chromatography, the narrower the width of the eluted peaks, the greater chance of separating a multi-component mixture in a column. The ability of a column to produce sharper peaks is determined by the efficiency of a column. The efficiency in all chromatographic techniques is expressed quantitatively as the number of theoretical plates (N) for the column. This value is calculated from the following expression (eq. 1.25):

$$N = 16\left(\frac{t_R}{W_b}\right)^2 \qquad \text{(eq. 1.25)}$$

where W_b is the base width of the peak.

The concept of a plate has its origin in the theoretical treatment of fractional distillation columns in the petroleum industry. In fractional distillation the greater the number of metal plates and the narrower gap between them, the narrower the boiling point that can be isolated. The concept, in chromatography, is then analogous to fractional distillation.

1.2.3. Gas chromatography

Gas chromatography separates a mixture into its individual components using a gas mobile phase (carrier gas). The invention of the first gas chromatograph took place in 1954 by Cremer [32] for the analysis of volatile organic mixtures, but since then the technique has progressed rapidly. Modern capillary GC is able to achieve a higher resolution than any other chromatographic technique. However analytes must be in the vapour phase, for separation to occur, the column must be at a high temperature, making the technique suitable for only volatile and thermally stable compounds.

A gas chromatograph has the following components: an injector, a column, an oven and a detector. The sample can be introduced using splitless, on-column or direct split injection.

There are two different kinds of GC columns: capillary and packed. Packed columns were used during the early stages of the development of the technique, but they did not offer the resolution that capillary columns give. Capillary GC columns used to link the mass spectrometer to the MIMS device will be discussed in section 1.3.3. Capillary columns are constructed from fused silica with an outer protective coating of polyimide. Dimensions are in the range of 15 to 100 m in length and 0.1 to 0.53 mm internal diameter. The inner surface of the column is coated with a stationary phase. This inner coating is normally based on a polysiloxane backbone with various functional groups, such as methyl, vinyl or phenyl.

The selection of mobile phases in GC is not very important for the separation of the components. Helium, nitrogen or hydrogen can be used, depending upon relative costs, desired speed of analysis and type of detector used. However, it is possible to modify the chromatographic conditions in a GC separation, by altering the column temperature. Therefore, a GC column is normally placed in a GC oven. In general heating enables more volatile compounds to elute first, then as the temperature rises, less volatile compounds begin to elute.

There are several detectors for GC, such as the flame ionisation detector (FID) and mass spectrometer (MS). GC/MS was used in this work, and a schematic of a gas chromatograph using a mass spectrometer as a detector is shown in Figure 1.13. Mass spectrometers have been discussed in section 1.1.4.

Original GC columns consisted of packed glass or steel tubes, with flow rates of up to 40 ml/min. Mass spectrometers could not manage large amounts of gas, so many interfaces were constructed to remove the carrier gas selectively from the eluting analytes, allowing the mass spectrometer to retain a suitable vacuum. However, with the invention of capillary GC columns, interfaces were no longer needed, due to the direct coupling capacity of the capillary columns. GC capillary columns have flow rates of around 1 ml/min of helium, which modern mass spectrometry pumps can cope with, allowing direct coupling of the GC column into the source, giving total column eluant transmission and no analyte loss.

In flame ionisation detectors, analytes leaving the column enter a small metal jet, at the tip of which burns a hydrogen/air flame below a tubular collector electrode. The potential of the collector relative to the flame is generally between −100V and −400V. Analytes entering the flame undergo a series of reactions, producing cationic species, which are attracted to the collector. The current produced is finally converted into a signal.

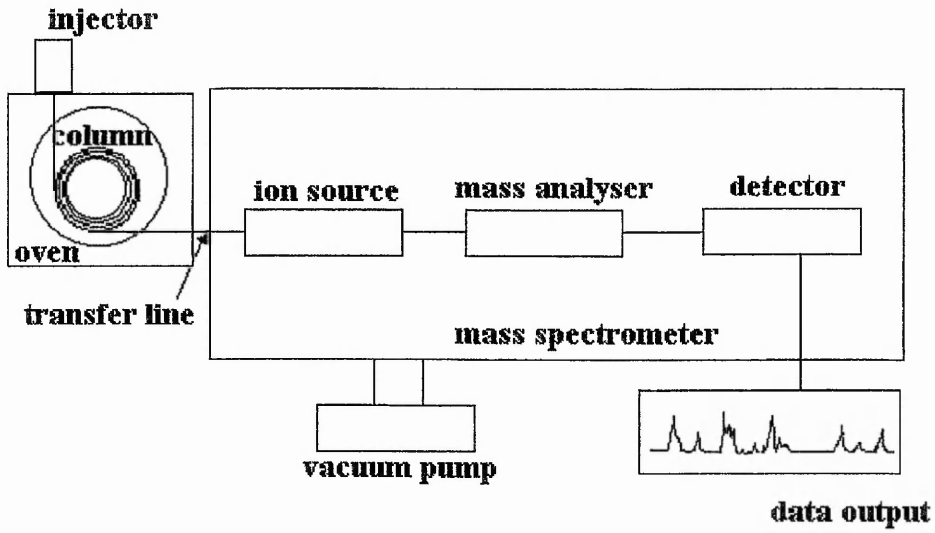

Figure 1.13: Schematic of a gas chromatograph combined with a mass spectrometer detector.

1.2.4. High performance liquid chromatography

High performance liquid chromatography (HPLC) is a method of separation of the components of a mixture by partioning between an immobilised stationary phase and a liquid mobile phase. Separation by HPLC is based on the different affinities of the compounds between the stationary and mobile phases. Some of

the components will travel more slowly than the others, as a result of their affinity for the stationary phase.

Two techniques of elution are used for the separation of a mixture of small molecules supplied to a HPLC column. Analytes may be separated by using isocratic elution where the mobile phase make-up remains constant during the analysis or by a gradient programme, which generally begins with a high aqueous composition and runs to high organic content.

The HPLC instruments consist of four parts: a pump, an injector, a separation column and a detector (Figure 1.14).

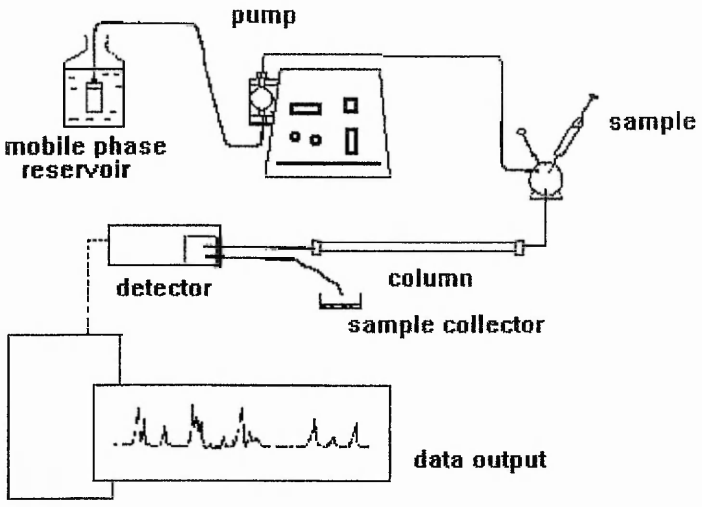

Figure 1.14: Schematic representation of HPLC.

The pump provides a constant flow of liquid through the separation column (the range of flow-rate of eluant solution is between 100 nL min^{-1} to 2 mL min^{-1}

depending on the analytical column). The injector regulates the amount of compound introduced onto the column and also permits automatic injection of samples. The separation takes place in the column, where equilibria of the different compounds between stationary and mobile phase are established. A column is normally between 30 and 150 mm long and has a diameter of around 1 to 5 mm. A typical analytical column uses C_{18} packing (a silica backbone attached to an 18-carbon hydrocarbon chain) (Figure 1.15). The detector can be UV, photometric, fluorescence, refractive index or mass spectrometric. For the work described in this thesis, a UV detector was used. UV detectors respond to substances that absorb ultraviolet light, and the relationship between absorbance of light and solute concentration is given by the Beer Lambert's law:

$$A=\varepsilon lc \qquad \text{(eq. 1.26)}$$

where A is the absorbance, ε is the molar absorptivity of the solute, l is the path length of the flow cell and c is the concentration of the solute.

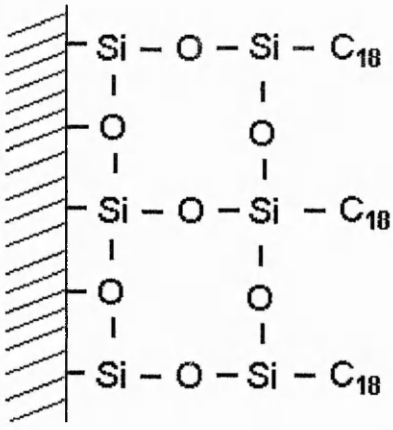

Figure 1.15: C_{18} polymeric HPLC column packing

1.2.5. Ion chromatography

Ion chromatography (IC) is a liquid chromatography technique using ion exchange mechanisms and suppressed conductivity detection for the separation and determination of anions and cations. It is a suitable technique for the determination of trace ionic contaminants. It is also useful for monitoring acidic anion content in the industrial environment. In this work IC was used for the determination of halide ion concentration in samples containing biodegraded halogenated compounds.

The ion chromatograph consists in the following components: a pump, an injector, a separation column, a suppressor column and a detector (Figure 1.16).

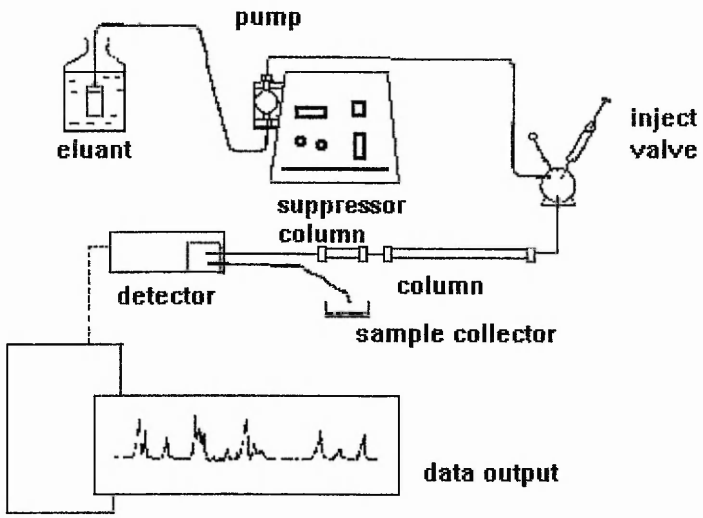

Figure 1.16: Schematic standard ion chromatogram.

The pump provides a constant flow of liquid through the column. The injector regulates the amount of compound introduced onto the column. The main differences between IC and HPLC are the column and the detector. There are two different kinds of ion chromatography: anionic and cationic. Anion exchangers are typically composed of a gel with functional groups such as diethylaminoethyl or quarternary ammonium, whereas cation exchange resins usually have functional groups such as carboxymethyl or methylsulfonate. A suppressor column reduces background conductivity and therefore enhances sensitivity. Suppression consists of the neutralisation of the salt by a weak acid or base. With suppression, the background conductivity of eluant is lowered and the response of the analyte is increased. There are also several types of detectors: conductimetric, amperometric, spectrophotometric, or fluoresence. In this work the detector used was a suppressed conductimetric detector. This type of detector consists of a cell with a small void volume and electrodes with a very small surface area which are placed very close together (the smaller their area and the closer together they are, then the higher the sensitivity).

1.3. Membrane inlet mass spectrometry

1.3.1. Introduction

MIMS was first reported by Hoch and Kok [33] in 1963, with the purpose of sampling gases dissolved in liquid samples. However it was not until the 1980s that MIMS saw wide use for environmental, biological or chemical applications due to the modernisation of mass spectrometric techniques and interfacing devices.

MIMS is a technique based on the transport of analytes across a membrane. In its simplest form, MIMS generally consists on a hydrophobic membrane (usually silicone derivative) linked to a mass spectrometer. One side of the membrane is in contact with the sample (gas or aqueous) at atmospheric pressure, while the other side is interfaced to the vacuum system of a mass spectrometer on a flow of inert gas (He) that is directed towards the mass spectrometer.

The main advantage of MIMS is the sampling simplicity of the technique, as no sample pre-treatment is required. This makes MIMS amenable for on-line monitoring processes. The main disadvantage of the technique is the fact that analytes may not be fully separated, so in very complex mixtures, identification, or monitoring, of certain compounds can become a difficult task. This may be overcome by the selectivity of the membrane for a range of compounds, whilst discriminating against other analytes.

1.3.2. Principles of MIMS

The process of analyte transport across a semi-permeable membrane in MIMS is called pervaporation, which is a three-stage process, shown in Figure 1.17. The analyte is transferred from the sample to the membrane surface by diffusion; it crosses the membrane and then is evaporated from the other side to be directly transported to the mass spectrometer with the help of a stream of inert gas, generally helium.

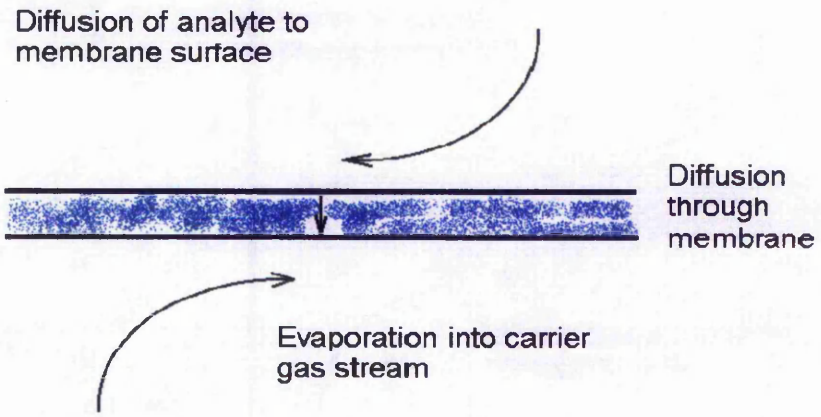

Figure 1.17: Pervaporation process at the membrane surface.

The main criteria for polymeric membranes for MIMS are the discrimination against the matrix, the permeability of the analyte and the maximum flow across the membrane and the time required to complete the analysis of one sample. These parameters determine the response sensitivity and the sampling frequency [34].

Volatile organic compounds (VOCs) diffuse across the membrane readily unless cryotrapping (discussed in section 1.3.6.5.) takes place, but semi-volatile organic

compounds (SVOCs) diffuse slowly through the membrane and tend to accumulate in the membrane.

Fick's law of diffusion describes the behaviour of the analyte in a membrane sampling system. The flux (F) of an analyte across a membrane is directly proportional to the area of the membrane (A), the diffusion coefficient of the analyte (D) and the concentration of the analyte (C), while it is inversely proportional to the membrane thickness (x) (equation 1.27).

$$F(x,t) = -AD\left[\frac{dC_m(x,t)}{dx}\right] \qquad \text{(eq. 1.27)}$$

For a hollow fibre membrane (used for all the work carried out in this work), Fick's first law can be expressed as:

$$F(x,t) = -2\pi L\left[\frac{dC_m(x,t)}{dx}\right] \qquad \text{(eq. 1.28)}$$

where L is the length of the membrane.

The negative sign shows the decrease of analyte in the diffusion direction along the concentration gradient.

In MIMS, it is assumed that the fluid around the membrane is well mixed and that analyte transport across the membrane is one-dimensional. If it is also assumed that the diffusion constant (D) on the membrane surface is constant over time, we can express the following equation, which corresponds to Fick's second law:

$$\frac{\partial C_m}{\partial t} = D\frac{\partial^2 C_m}{\partial x^2} \qquad \text{(eq. 1.29)}$$

When combining the two Fick laws together, the mathematical solution obtained for diffusion through a membrane of thickness l is shown in equation 1.30:

$$F_t = F_{SS}\left\{1 + [2\Sigma(-1)^n \exp(-(\frac{n\pi}{l})^2 Dt\right\} \qquad \text{(eq. 1.30)}$$

where F_{ss} is the flux at steady state.

The permeation process exhibits an asymptotic approach to steady state [35], therefore the time required to achieve steady state may be difficult to determine. If the time taken for the response to rise from 10 to 90% ($t_{10\text{-}90\%}$) is calculated, the following solution applies:

$$t_{10-90\%} = 0.237\left(\frac{l^2}{D}\right) \qquad \text{(eq. 1.31)}$$

Whilst for 50% ($t_{50\%}$) the result is:

$$t_{50\%} = 0.14\left(\frac{l^2}{D}\right) \qquad \text{(eq. 1.32)}$$

It can be seen that both equations are directly proportional to the square of the membrane thickness and inversely proportional to the diffusion coefficient.

In addition, if the concentrations are expressed in partial pressures following Henry's law (equation 1.33), equation 1.34 can be used to calculate the steady state molecular flow of an analyte through the membrane.

$$C_m = SP_s \qquad \text{(eq. 1.33)}$$

$$F_{SS} = 2\pi LDS\left(\frac{P_S}{l}\right) \qquad \text{(eq. 1.34)}$$

where S is the solubility constant and P_s the vapour pressure of the analyte on the sample side, L is the membrane length, D is the diffusion constant and l the membrane thickness.

Despite these theoretical simplifying assumptions, the most important limiting factor for the analyte transport across selective non-porous membranes is that their transport properties are related to the chemical structure of the membrane material. Therefore, the analyte must be able to dissolve in the membrane before the diffusion phenomena can take place.

1.3.3. MIMS interfaces

There are three different kinds of membrane interfaces: the flow-over, flow-through and direct insertion membrane probe (DIMP). The interface geometry is very important, as this determines which material and membrane configuration (flat sheet or hollow fibre) can be used.

1.3.3.1. Flow-over interfaces

These devices were used at early stages of MIMS [36] and consist of a membrane probe situated at the end of a long transfer line. One side of the membrane is in

contact with the bulk sample, while the other side is connected to the evacuated transfer line that goes to the mass spectrometer. Hoch *et al.* [33] first recorded MIMS application for kinetic studies measuring gases, where a simple silicone probe was dipped in the sample.

Despite the fact that this device was used for several applications, including industrial process monitoring [35, 37, 38], the long distance between the membrane probe and the mass spectrometer resulted in a very slow response to changes in analyte concentration at the membrane surface. High detection limits were also observed due to membrane memory effects. [39] Analyte adsorption and condensation in the transfer line were also significant disadvantages, mainly when the system remained unheated.

Flow-over devices were also coupled to gas chromatography packed columns. [40] This type of device had limited success for a variety of reasons, including the selectivity of the membrane, the lack of reproducibility and memory effects (Figure 1.18a).

1.3.3.2. Flow-through interfaces

A significant advance in MIMS was reported by Weaver *et al.* [41], who used a flow-through interface for the analysis of low molecular weight volatile compounds such as methanol, formaldehyde or ethanol. This device allowed the solution to flow through a hollow fibre membrane, with the other side of the

membrane connected to the mass spectrometer vacuum system by a transfer line [42] or a small helium purge [43].

Degn [44] carried out an experimental comparison between the flow-over and flow-through hollow-fibre membrane interfaces, concluding that a higher sensitivity could be achieved with the later device. The main reason was the long connecting transfer line of the flow-over device, as it causes retardation and loss of analyte. There are also minimal memory effects with this type of interface.

This kind of interface is still in use, employing a hollow fibre or a flat membrane. Figure 1.18b shows an example of this interface using a hollow fibre membrane (this interface has been used in this work).

1.3.3.3. Direct insertion membrane probe (DIMP)

A new type of membrane interface that allowed the aqueous sample to flow through the hollow-fibre membrane instead of over the external surface of a flat membrane was introduced by Cooks research group in collaboration with Dow Chemical [45-48].

The probe was constructed by modifying an existing Finnigan TSQ insertion probe allowing the membrane to be introduced through the probe lock into the vacuum system. The device was placed in close proximity to the mass spectrometer ion source. The work on volatile organic compounds in water such

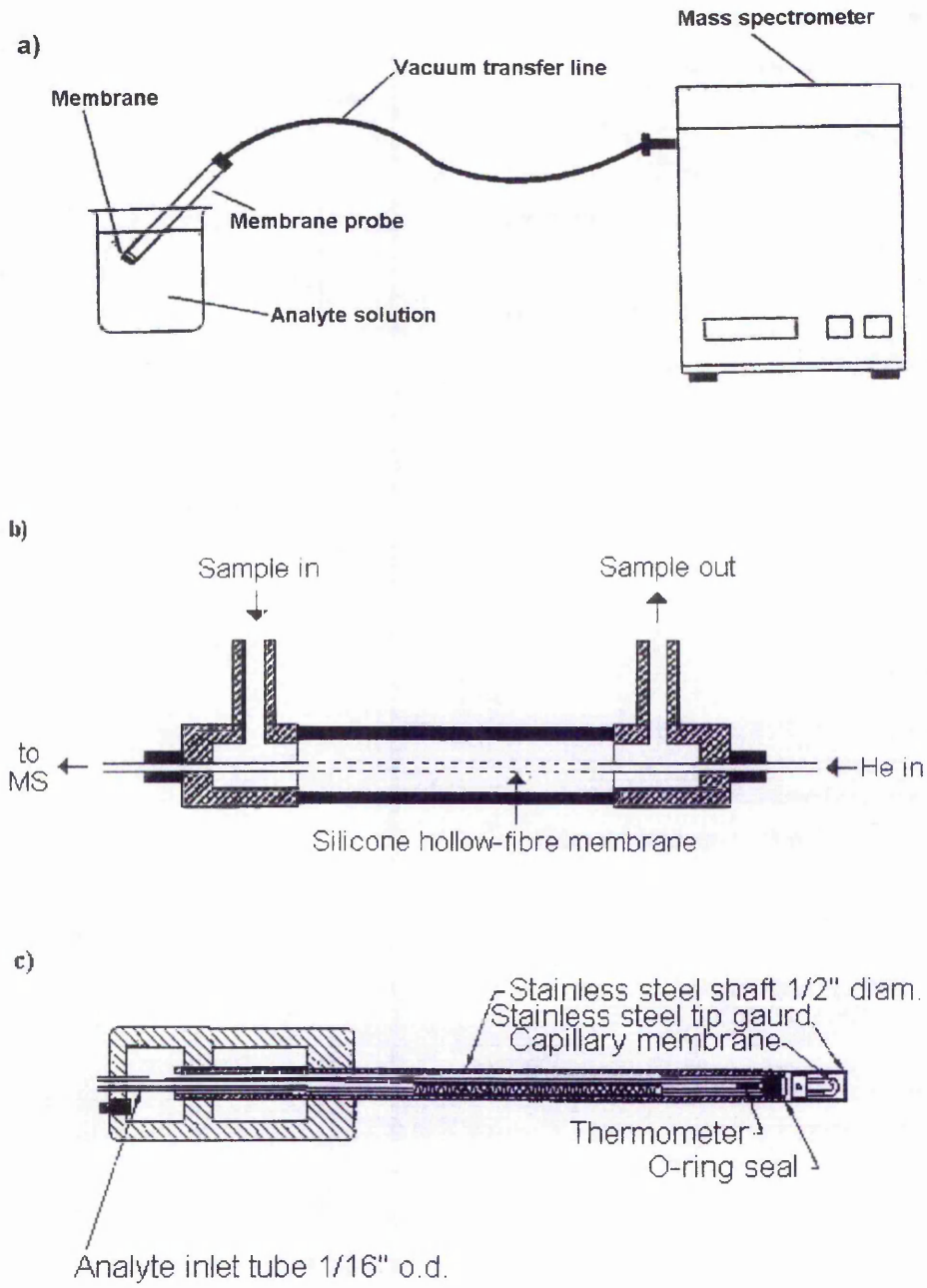

Figure 1.18: MIMS interfaces. a) Flow-over interface, b) flow-through interface. c) direct insertion membrane probe interface.

as tetrahydrofuran or benzene with a direct insertion membrane probe was reported [49]. Figure 1.18c shows a diagram of a DIMP interface.

1.3.4. Membrane types

Polymers used as membrane materials for MIMS are divided in two different categories: microporous and non-porous or semi-permeable membranes.

1.3.4.1. Microporous membranes

Microporous membranes lack the selectivity of non-porous membranes because the transport is determined by laminar flow and size exclusion through the pores rather than diffusion across the membrane. The high sample flow through micro-porous membranes also requires that analyses are carried out using solvent chemical ionisation mass spectrometry (CI-MS) [50].

Examples of the use of a microporous polypropylene membrane include the analysis of organometallics (such as ferrocene molybdenum) in air [51], organic compounds such as methanol, ethanol, dimethylsulfoxide and benzene in hexane [50]. Kasthurikrishnan *et al.* [52] used zeolite-filled polydimethylsiloxane micro-porous membranes for the analysis of benzene, carbon tetrachloride, chlorobenzene, toluene and trans-1,2-dichloroethane in methanol and hexane. The analysis of several gases including oxygen, carbon dioxide, helium and argon was achieved by a polymeric microporous membrane carried out with Krytox (a high-vacuum fluorinated grease) [53].

1.3.4.2. Semi-permeable membranes

Semi-permeable, or non-porous, membranes are chemically selective to different kinds of analytes. They are divided in hydrophilic and hydrophobic membranes.

1.3.4.2.1. Hydrophobic membranes

These are the most common types of membrane used for MIMS applications. Hydrophobic polymers discriminate in favour of polar compounds and against non-polar compounds. This application is called normal phase MIMS. The most used material is a poly(dimethyl)siloxane or silicone rubber membrane. This is due to several reasons: silicone is widely available in a large number of geometries, it is a robust material that can be used for a large number of non-polar compounds showing high selectivity over complex air or water matrices.

Several studies have been carried out evaluating other membrane materials such as latex, poly(vinyl chloride) (PVC), Teflon, polyurethane, polyimide and polyethylene.

Maden *et al.* [54] performed comparative studies on polymer sheet membranes, including silicone, latex, PVC, Teflon, polyurethane, polyethylene and nitrile, for MIMS. Polymide and Teflon showed very poor organic permeability and higher water permeability, and are therefore not suitable for normal phase MIMS. These experiments showed that all materials had a very similar performance to silicone, and as silicone is a more readily available material than the others, this makes it

the most suitable membrane material for MIMS. Similar studies carried out by Stone *et al.* [55] using polyphosphazene materials for the analysis of benzene, toluene, trichloroethylene, isopropanol, tetrahydrofuran and methylethylketone in water. Results from this worked to similar conclusions as those obtained by the Maden group.

Liquid membranes have also been used as hydrophobic membranes. Low vapour pressure liquids sandwiched between microporous membranes offer a flexibility of surface area obtaining any desired thickness or shape [56]. Polyphenyl ether, alkylated cyclopentane, perflurinated ether and silicone oil were the chosen liquids. They were coated on a micro-porous polypropylene substrate for support and mounted on a direct insertion probe. Johnson *et al.* [57] applied these membranes to the study of an on-line reaction monitoring of epochloridine in water.

1.3.4.2.2. Hydrophilic membranes

Hydrophilic membranes discriminate in favour of non-polar compounds against polar compounds. This application is called reverse-phase MIMS (RP-MIMS) and has not been widely exploited.

A poly(ethylene terephthalate) membrane was used by Bohatka *et al.* [58] to determine selectivity traces of water in butanol, hexanol and octanol, and Bauer *et al.* [59] employed a poly(vinyl alcohol) (PVA) membrane in the RP-MIMS analysis of VOCs, including acetone, methylethylketone, methanol and

tetrahydrofuran in hexane. Although the PVA membrane discriminated against the solvent, hexane was used as the reagent gas for CI analysis. Maden *et al.* [54] investigated the use of polyimide membrane for the RP-MIMS analysis of water, ethanol, chloroform, acetone, acetic acid and ethyl acetate in hexane. The polyimide material was permeable to polar compounds, including water or ethanol, but showed limited discrimination against the permeation of non-polar compounds, such as chloroform and ethyl acetate. The flux of hexane permeating the membrane was sufficiently high that EI spectra were observed to have CI characteristics.

1.3.5. Membrane geometry

Two different kinds of membrane geometry have been used in MIMS: flat sheet and hollow-fibre.

Hollow-fibre membranes have been shown to be more efficient for the extraction of analytes from a matrix due to higher exchange area [35, 38, 60], although flat sheet membranes are more useful when they need to be replaced very often.

In comparative studies of different materials [54], sheet membranes were found to be more appropriate, due to the fact that fewer materials are commercially available on the hollow-fibre form.

1.3.6. MIMS assisted desorption

Strategies to widen the range of compounds of amenable analysis to MIMS have been investigated recently, linking MIMS to other techniques and using more sofisticated temperature control systems.

1.3.6.1. Laser-assisted release

Laser desorption MIMS or LD-MIMS is an important technique for extending the range of compounds of analysis by MIMS. In this technique a low-powered carbon dioxide laser (10.4 μm wavelength, < 5 Watts) was used to irradiate the low-pressure side of a silicone membrane during a typical MIMS analysis of an aqueous solution [61]. This resulted in direct membrane heating, enabling rapid desorption of permeate molecules with a sensitivity and response time improvement for compounds with high molecular weight and low volatility. In this study, Soni *et al.* were able to monitor ppb concentrations of naphthalene, anthracene, pyrene, chrysene, indenopyrene and benzofluoranthracene.

An improvement of this technique was achieved by using a KrF eximer laser (λ = 248 nm), and analytes were ionised by resonance-enhanced multiphoton ionisation (REMPI) [62]. This approach was used to monitor polyaromatic hydrocarbons at ppt concentrations. The increase in sensitivity was due to the replacement of electron ionisation by REMPI, since the REMPI technique is selective to this kind of molecular structure, therefore avoiding background

interference from contaminants such as sample matrix or membrane degradation products.

1.3.6.2. Liquid chromatography MIMS

Liquid chromatography membrane inlet mass spectrometry (LC/MIMS) has been reported by Ouyang *et al.* [63] for the determination of compounds with identical quantitation ions, taking advantage of the good MIMS performance for VOCs. In this work, a C_{18} column and a mobile phase (methanol/water) were used for the chromatographic separation in conjunction with a DIMP interface. The method showed a lower analysis time than purge-and-trap GC/MS and a simpler sample pre-treatment.

1.3.6.3. Fast Gas chromatography MIMS

Combining chromatography with MIMS offers an opportunity for taking advantage of chromatography as a separation method, while MIMS is ideal as a real time interface for analyte identification and quantification [64, 65]. However, coupling MIMS with GC, the on-line monitoring characteristic is lost as it takes over 10 minutes for a conventional capillary GC separation.

With the recent appearance of short columns for fast separation (Fast GC), a significant reduction in separation time can be achieved by the on-line coupling of GC with MIMS, without losing the on-line monitoring characteristic of

MIMS. A study of trihalomethanes in chlorinated water was monitored by a fast GC-MIMS system, achieving low ppt limits of detection [65].

1.3.6.4. Purge and trap or trap and release MIMS

This technique involves membrane cooling by a continuous flow of sample solution passing through the inlet for sample pre-concentration. The membrane is then heated in order to allow analytes to desorb from the membrane. However, in a typical experiment, optimum sample response requires 20 minutes pre-concentration, followed by 20 seconds of heating initiated by injecting plugs of air into the membrane. This technique was reported for the first time by Leth *et al.* in 1995 [66] for the analysis of SVOCs in aqueous solution. Samples were introduced at 0°C (they were placed on ice and pumped using a peristaltic pump). Then the membrane was heated by electron bombardment and analytes dissolved in the membrane were thermally desorbed. Compounds analysed by this technique included phenol and some chlorophenols. Other workers have since reported the use of this technique for VOCs [67-70].

A variation of this technique is the purge-and-membrane MS (PAM-MS) approach. In PAM-MS of VOCs, analytes are purged from the sample with an inert gas, directing the stream towards the membrane interface [71-73].

Another variation of this technique is referred to single-sided MIMS, reported by Riter *et al.* [74]. This system consists on a combination of the features of the trap and release method with surface partioning. The interface allows sample

injection (only useful for air samples) and analytes are retained in the membrane. By fast electrical heating (12V and 3A), compounds that had been preconcentrated in the membrane, are then desorbed and detected by the mass spectrometer. This system is only useful for SVOCs, as VOCs are released immediately from the membrane without pre-concentration. Air samples were prepared using a gas dilution apparatus. Compounds detected by this technique were lindane (a pesticide), hexahydro-1,3,5-trinitro-1,3,5-triazine (an explosive), butylated hydroxitoluene , 1,2-dichlorobenzene, dimethyl ethylphosphonate and naphthalene.

1.3.6.5. Cryotrapping MIMS (CT-MIMS)

This technique consists on placing a liquid nitrogen cooled tube between the membrane interface and the mass spectrometer [75]. The tube is then very rapidly heated releasing the condensed VOCs. The main advantage of this technique compared to purge and trap is the fact that cryotrapping does not use trapping material but it is performed using external liquid nitrogen, followed by fast heating.

Mendes *et al.* [75] reported the application of this technique for the first time. They used it for the analysis of VOCs such as chloroform, benzene, toluene and chlorobenzene in the ppt region. Moraes *et al.* [76] also used this device for the selective quantitation of cyanogenic glycosides by previously hydrolysing the sugars and then monitoring the released ketones (acetone and butan-2-one).

Creaser *et al.* [77] used a cryotrapping system linked to a membrane inlet in one side and a short chromatographic column in the other. The outlet of the capillary GC column was connected directly to the mass spectrometer via a heated transfer line. This system was evaluated for the on-line determination of VOCs and also SVOCs: 4-fluorobenzoic acid, 3,5-fluorobenzoic acid, 2-chlorophenol, p-tert-butylphenol, DMSO and toluene.

1.3.7. Applications of MIMS and recent developments

Membrane inlet mass spectrometry has been applied widely in the chemical and biological fields. There have been several MIMS reviews [78, 79] including applications such as environmental monitoring [80, 81], biological reaction monitoring [82], and process control [83].

There is a general need to widen the range of compounds amenable to be monitored by MIMS (to include less volatile, more polar and a higher molecular weight). Sensitivity is also an important issue.

1.3.7.1. Monitoring of industrial processes

In 1985 Dow Chemical was interested in MIMS for industrial applications, as the technique had potential to be used for reaction monitoring. The use of liquid membranes for on-line monitoring of reactions of epochloridine [57] was an alternative approach to off-line monitoring using solid-phase extraction or solid-phase microextraction followed by gas chromatography. Despite the fact that

MIMS did not offer such low limits of detection, the procedure was much faster and could be used on-line due to the simplicity for sample introduction.

Nogueira *et al.* [84] carried out an on-line photocatalytic degradation study of phenol and trichloroethylene in water, by monitoring the amount of carbon dioxide gas liberated using MIMS. Another on-line monitoring application of MIMS involved a chlorination reaction of organics in water [85]. MIMS has also been applied to the analysis of beverages. Ketola *et al.* [86] carried out a classification of cola beverages by MIMS combined with statistical computing methods. An investigation of the aroma fraction of some Italian wines was also performed using MIMS [87], while Bocchini *et al.* [88] monitored the presence of volatile organohalogens (such as chloroform, bromoform or bromodichloromethane) in drinking water at the ppb levels.

1.3.7.2. Biological applications

The first MIMS report [33] had a biological application for the measurement of gases in a photosynthetic study. Since then, other more complex analytes have been detected by MIMS. The most significant biological publications are described below.

Heindricher *et al.* [89] studied protein oxidative products by MIMS. Products detected were tyrosine, 4-vinylanisole and p-methoxyphenylethylamine. Mono- and sesquiterpenes such as α-pinene, limonene, terpinene or geraniol in aqueous samples were analysed by MIMS and compared by static headspace gas

chromatography (HSGC) [90]. These studies concluded that MIMS is more suitable for on-line analysis and is less time consuming than HSGC.

Lauristen *et al.* [70] detected large fat-soluble biomolecules such as testosterone, cholesterol or vitamin E by MIMS and desorption chemical ionisation. As large biomolecules have too high a polarity for transport across a hydrophobic membrane at ambient temperatures, samples were accumulated in the membrane and then released when the membrane was heated in the ion source of the mass spectrometer at 300°C. Analysis times were close to 30 minutes and limits of detection were around 1 μM, which is too high for blood analysis. Less than a year later the same group were able to increase the limits of detection to low ppb levels in order to determine steroid hormones in birth control pills. The reported improvement included the CI gas by isobutene and by sealing more tightly the ion source with ceramic fittings [91].

Haddad *et al.* [92] were able to determine the total homocysteine concentration directly from human plasma by trap and release MIMS. The limit of detection was 2 μM by monitoring *m/z* 234 (as lower *m/z* ions created higher interferences with other molecules), enough for the 5 μM threshold of homocysteine in human plasma.

Moraes *et al.* [76] were able to monitor cyanogenic glycosides by cryotrap MIMS. They hydrolysed the cyanogenic glycosides and analysed the released ketones (acetone and butan-2-one).

1.3.7.3. Environmental applications

1.3.7.3.1. Soil

In-situ soil analysis techniques consisting of membrane inlets linked to a mass spectrometer via an evacuated tube have been reported [93-96]. Dissolved gas concentrations such as oxygen, methane and carbon dioxide were monitored to obtain ecological data related to gas exchange and bacterial growth. Sensitivity for in-situ processes is limited, so the technique has been mainly focused on the analysis of ambient gases rather than VOCs or SVOCs, where more complex analytical techniques are required.

Ex-situ techniques offer greater sensitivity for dissolved organics using efficient heating and purging of samples prior to analysis. Kostiainen *et al.* [71] analysed VOCs in water and soil samples by purge-and-membrane mass spectrometry (PAM MS). In this method, VOCs are purged from the sample with an inert gas, directing the stream through a sheet membrane module. Toluene, benzene, xylenes, dichlorobenzene and ethyl acetate were detected to ppb levels with this technique.

A different approach consisted of headspace MIMS (HS-MIMS) for trace level analysis of VOCs such as toluene and chloroform in soil [97] or for SVOCs such as phenyl acetic acid by acidifying samples prior to analysis [98]. For this kind of analysis an air-tight oven heated to 90°C is used, releasing analytes from soil and pre-concentrating them in the gas phase prior to analysis.

1.3.7.3.2. Microbiological studies

MIMS has been used for microbiological studies of fermentation or biodegradation products [99-104] and dynamic respiratory measurements [105]. A good example is the on-line monitoring of phenoxyacetic acid (b.p. 285°C), a precursor of penicillin V, during *Penicillum chrysogenum* fermentation. [101] Johnson *et al.* [106] monitored on-line fermentation broths in a pilot plant by MIMS. A 9000-litre fermentation reactor was used and samples were continuously taken by splitting flow from the fermentation broth and analysed for ethanol content by flow injection. Minor components such as acetic acid and lactic acid were also identified.

The identification of VOCs from the growth of the fungus *Bjerkandera adjusta* has been reported [99], allowing chloromethoxybenzaldehyde, a major fermentation product, not previously described, be identified using MS/MS techniques.

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CHAPTER 2

In-membrane preconcentration membrane inlet mass spectrometry (IMP-MIMS) fundamentals

2.1. Introduction

The analysis of volatile organic compounds (VOCs) in both air [1,2] and aqueous [3] samples by MIMS has been widely studied. The technique has been proved to be rapid and sensitive for these compounds, giving limits of detection in the low ppb region for compounds such as benzene and toluene. [1, 3] VOCs diffuse through the membrane by continuous infusion. MIMS has been combined with tandem mass spectrometry (MS/MS) [4] and on-line cryotrapping and rapid GC separation [5] to reduce limits of detection to the ppt region and increase selectivity.

The analysis of semi-volatile organic compounds (SVOCs) has been less studied, as SVOCs diffuse slowly through the membrane at temperatures lower than 100°C (boiling point of aqueous samples). The various strategies developed to overcome this limitation for the analysis of SVOCs were described in section 1.3.6.

The approach used to analyse SVOCs by MIMS in the work presented in this chapter is based on the in-membrane pre-concentration MIMS (IMP-MIMS) technique, and was first reported by Creaser *et al.* for the analysis of compounds such as 2-chlorophenol, 4-fluorobenzoic acid, 3,5-difluorobenzoic acid or dimethylsulfoxide (DMSO) in aqueous samples. [6] In IMP-MIMS the membrane is exposed to the analytes in an aqueous or gaseous matrix for a defined amount of time, by pumping the sample through the membrane at a low temperature (40°C). This is the pre-concentration step, where the SVOCs enter,

but do not diffuse through the membrane. The membrane is then heated in a rapid cycle using the GC oven (40-200-40°C) in order to thermally desorbe the analytes that had been previously preconcentrated in the membrane. The IMP-MIMS technique extends MIMS to a wider range of analytes, but sensitivity is compromised by the water released with the analytes during the desorption step. This chapter describes studies directed at overcoming the limitation and optimising the MIMS technique for VOCs and SVOCs.

2.2. Preliminary work

Preliminary MIMS studies involved the analysis of both air and aqueous samples containing VOCs. The limits of detection for toluene and benzene in air were determined to be approximately 1.5 ppb_v and 2.5 ppb_v respectively in a Varian Saturn 4D ion trap (Varian Associates, Walnut Creek, CA, USA) demonstrating the pervaporation efficiency of these volatile analytes. The responses for less volatile analytes in the same instrument were significantly lower, with limits of detection of 0.5 mg L^{-1} for dimethylformamide (DMF) and 0.3 mg L^{-1} for dimethylsulfoxide (DMSO). Further work was therefore concentrated on the determination of SVOCs, including DMSO, DMF and fluorobenzoic acids in aqueous samples by an IMP-MIMS technique using both the ion trap and the quadrupole mass spectrometers.

Samples of water containing DMSO and DMF were analysed by IMP-MIMS using the method reported previously, but problems often occurred when an aqueous sample was introduced into the membrane followed by thermal

desorption. The system often failed during the desorption stage due to excessive water pressure in the mass spectrometer source. The effect of water in MIMS has been suppressed by the use of a Nafion® membrane (discussed in Chapter 4) after the silicone membrane [7-9]. However, for the analysis of SVOCs, IMP-MIMS requires the membrane to be heated to temperatures higher than 100°C, which are beyond the operating range of Nafion® (it becomes black, rigid and breaks). Therefore a different strategy had to be used to reduce the excessive pressure arising from the thermal desorption step.

2.3. Drying stage

The addition of a drying stage between pre-concentration and thermal desorption was investigated. The following parameters were varied in order to optimise the mass spectrometric sensitivity:

a) Drying time

b) Drying temperature

c) Drying gas

2.3.1. Experimental

Several membrane drying parameters were investigated in order to determine their effect on the mass spectrometric response for DMF using the IMP-MIMS technique. The temperature vs. time profile used is shown in Figure 2.1. The conditions were as follows:

a) Desorption temperature: 200°C selected for all experiments.

b) Preconcentration temperature: 40°C selected for all experiments.

c) Drying time: the membrane drying time was varied from 0 to 54 minutes.

d) Drying temperature: varied between 40 and 80°C.

e) Nitrogen drying gas flow: varied between 15 and 100 ml/min.

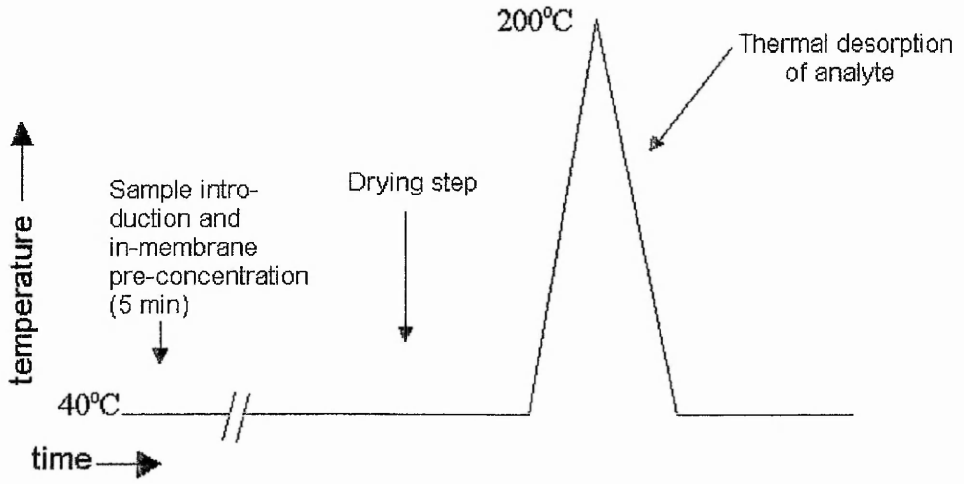

Figure 2.1: Temperature vs. time profile for IMP-MIMS with a sample drying step.

These experiments were carried out using a HP 5970 quadrupole MS coupled to a HP 5890 GC oven (Hewlett Packard, Palo Alto, California, USA). The silicone membrane interface (0.635 mm o.d. x 0.305 mm id, Dow Corning, Silastic, Sanitech, USA) shown in Figure 2.2 was constructed in-house and located inside the GC oven in place of the capillary GC column. [10] The scan range was between *m/z* 50 and 150 at a scan rate of 2.6 scan/s. The addition of a drying stage for IMP-MIMS was tested with water samples containing DMF or DMSO.

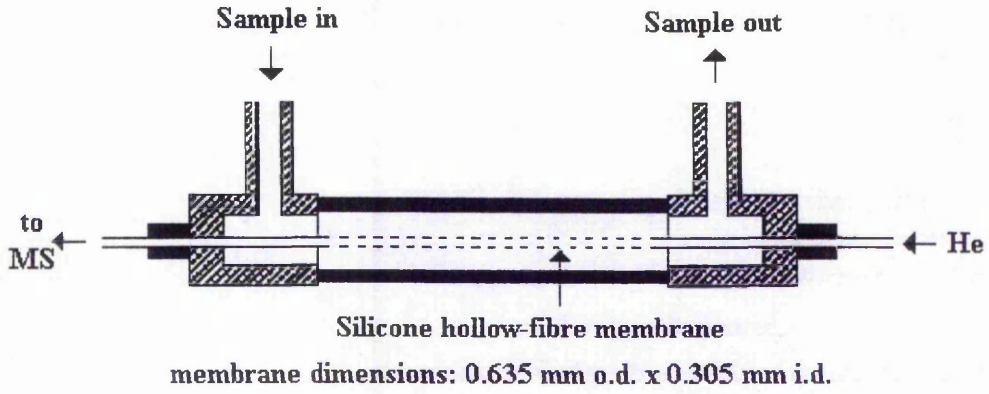

Figure 2.2: Silicone hollow fibre membrane interface schematic.

2.3.2. Results and discussion

2.3.2.1. Effects of the membrane drying time

The effect of the membrane drying time, following analyte preconcentration, is shown in Table 2.1 and Figure 2.3 for aqueous samples of DMF (40 mg L^{-1}) using 50 mL min^{-1} of nitrogen as the drying agent. For drying times greater than 24 minutes no difference was observed in the DMF responses, so it appears that the optimum drying time for DMF under these conditions is 24 minutes. This is a significant length of time for the analysis, so other parameters were investigated in order to reduce the time of the analysis. The MIMS response for DMS using a 24 minute drying time and a 40-200-40°C thermal desorption programme is shown in Figure 2.4.

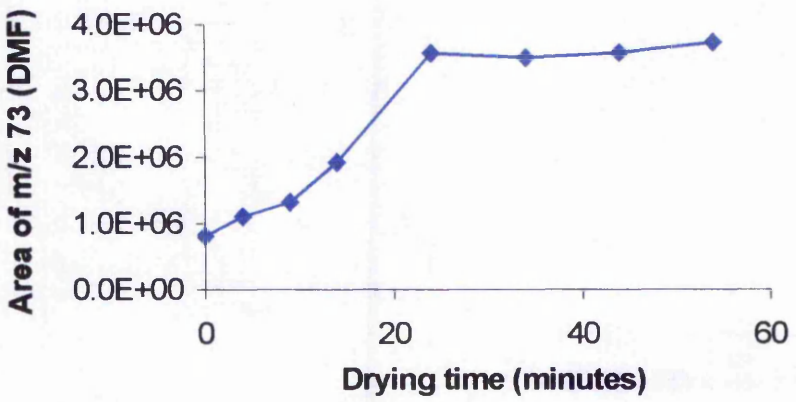

Figure 2.3: Peak area vs. drying time for 40 mg L^{-1} DMF aqueous samples.

Table 2.1: Results for IMP-MIMS analysis of 40 mg L^{-1} aqueous samples of DMF using different drying times. The drying gas was nitrogen at 50 mL min^{-1}.

Drying time (min)	**Retention time** (min)	**Peak width ($W_{1/2}$)**	***m/z* 73 peak area**
0	9.201	0.903	814453
4	13.672	1.017	1105349
9	18.255	1.159	1326697
14	23.467	0.976	1915892
24	33.266	1.068	3566876
34	43.322	1.142	3493864
44	53.393	1.071	3568635
54	63.279	1.047	3725236

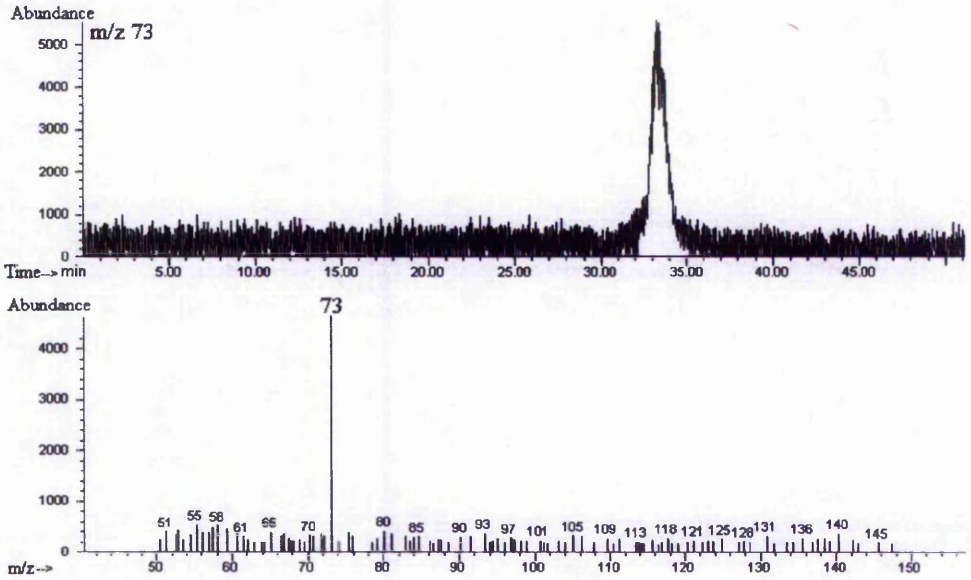

Figure 2.4: DMF single ion response and mass spectrum for a 40 mg L^{-1} aqueous solution obtained on a quadrupole MS using a drying time of 24 min and a nitrogen flow of 50 mL min^{-1}.

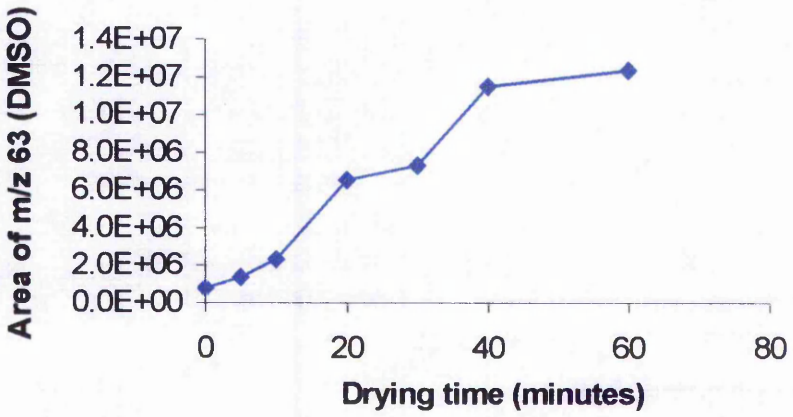

Figure 2.5: Drying time vs. peak area for DMSO IMP-MIMS analysis.

Table 2.2: IMP-MIMS results for the analysis of DMSO (220 mg L^{-1}) in water using different drying times. Drying agent was nitrogen at 50 ml min^{-1}.

Drying time (min)	Retention time (min)	Peak width ($W_{1/2}$)	*m/z* 73 peak area
0	10.090	0.712	766264
5	15.167	0.802	1354828
10	19.981	0.772	2312802
20	29.814	0.935	6539540
30	39.725	0.945	7323978
40	49.705	1.017	11506747
60	69.593	1.087	12354950

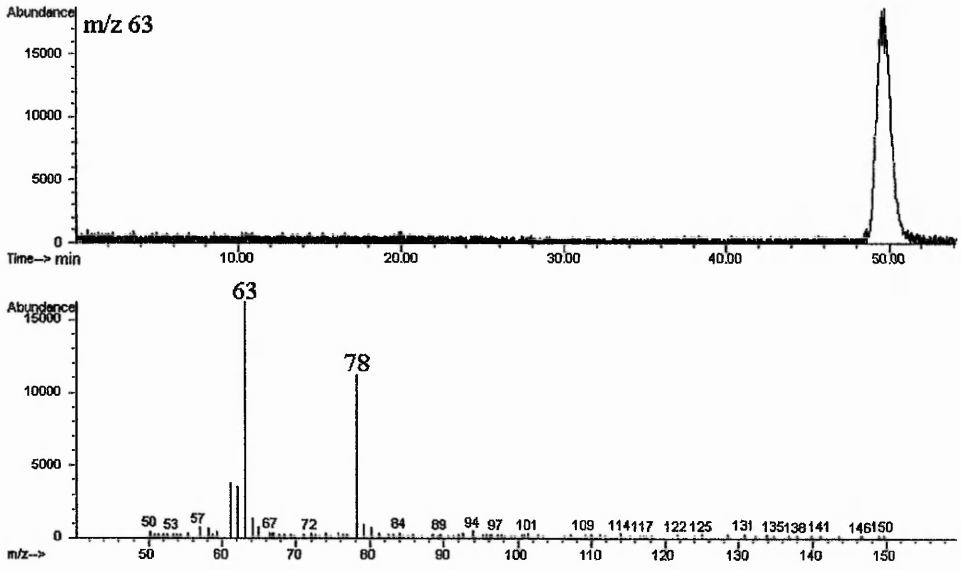

Figure 2.6: DMSO single ion response and mass spectrum for a 220 mg L^{-1} aqueous solution in the quadrupole MS using a drying time of 40 min and a nitrogen flow of 50 mL min^{-1}.

DMSO was analysed using a similar procedure to that for DMF. For samples containing 200 mg L^{-1} of DMSO, the optimum drying time was found to be 40 minutes at 40°C (See Table 2.2 and Figures 2.5 and 2.6).

It can be seen from the results for DMF and DMSO, that although both compounds show a similar trend (the longer the drying time, the greater the analyte response until a steady response is obtained), the drying time is different for each compound. These results show that the membrane drying step reduces suppression of the analyte response resulting from the release of water during thermal desorption step. However, relatively large drying times were required for DMSO and DMF to achieve optimum responses.

2.3.2.2. Effects of drying temperature

The effect of the drying temperature was investigated in order to reduce the drying time. The drying time chosen for these experiments was 24 minutes (optimum drying time), with a nitrogen flow of 50 mL min^{-1}. A range of drying temperatures were investigated in the range 40 to 80°C using the IMP-MIMS profile shown in Figure 2.7. The effect of membrane drying temperature was tested on the DMF response for a 40 mg L^{-1} aqueous sample and the results are shown in Table 2.3.

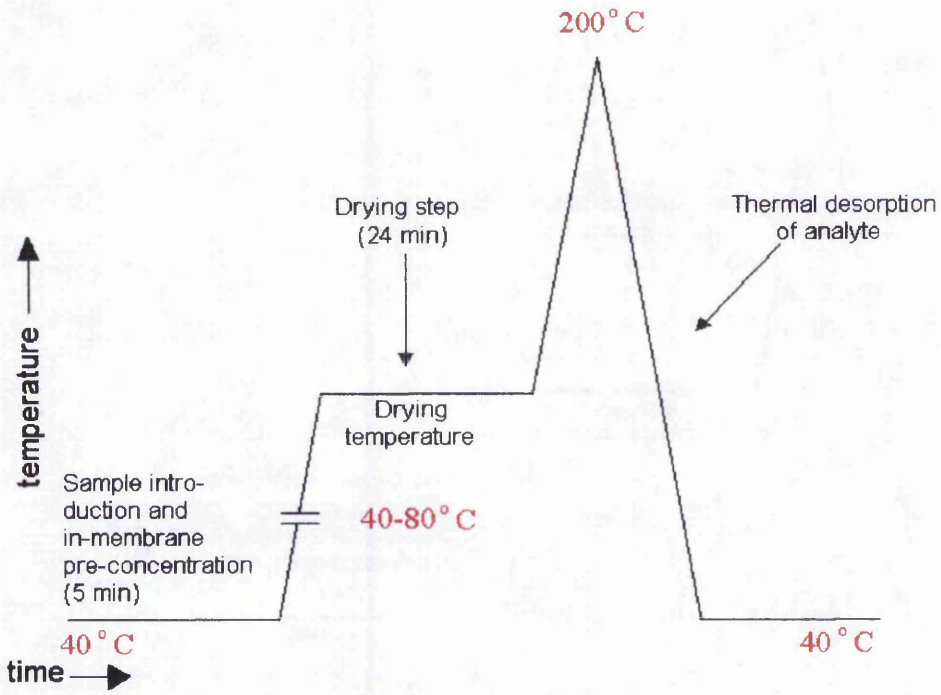

Figure 2.7: IMP-MIMS temperature vs. time profile with changing drying temperature.

Table 2.3: Results for the IMP-MIMS analysis of 40 mg L^{-1} aqueous samples of DMF using different drying temperatures.

Drying temperature (°C)	**Retention time** (min)	**Peak width ($W_{1/2}$)**	***m/z* 73 peak area**
40	33.384	1.096	2706673
60	34.216	1.049	2289599
80	34.414	1.022	856231

The results in Table 2.3 indicate that, as expected, the higher the drying temperature, the lower the signal observed for DMF. This is presumably due to losses of DMF due to diffusion through the membrane and evaporation during the membrane drying step. The use of high drying temperatures (between 60 and 80°C) with shorter drying times also gave lower signals than using a drying temperature of 40°C.

2.3.2.3. Effects of the drying gas

a) Drying agent

The use of an inert gas such as nitrogen has been shown to dry the membrane efficiently and protect it from oxidation when heated, leading to an increased life-time. The results of an experiment carried out to compare nitrogen and air as a drying gas for DMF aqueous samples (40 mg L^{-1}) showed that nitrogen (50 mL min^{-1}) gave a higher MIMS response than atmospheric air pumped through the membrane (3 mL min^{-1}) of almost 20% (see Table 2.4).

Table 2.4: Response obtained for 40 mg L^{-1} DMF in the quadrupole MS using nitrogen or pumped air as the drying agent.

Drying agent	Retention time (min)	Peak width ($W_{1/2}$)	Area
Nitrogen (50 mL min^{-1})	33.378	1.082	2649917
Pumped air (3 ml min^{-1})	33.282	1.210	2228331

b) Effect of the flow of nitrogen drying gas

The nitrogen flow used to dry the membrane was expected to be an important parameter determining the MIMS response for SVOCs. Studies were carried out by changing the nitrogen flow between 10 and 100 mL min^{-1}. Samples containing 40 mg L^{-1} of DMF were analysed using a drying time of 24 minutes. The optimum flow rate was found to be 50 mL min^{-1}, but flow rates between 40 and 70 mL min^{-1} did not show significant differences in analyte responses (see Table 2.5 and Figure 2.8). At a nitrogen flow rate of 90 mL min^{-1}, the DMF response was reduced, presumably as a result of evaporative losses, whilst at flow rates below 40 mL min^{-1} incomplete drying of the membrane resulted in ion suppression in the EI source.

Table 2.5: IMP-MIMS analysis of a 40 mg L^{-1} DMF aqueous sample with N_2 drying gas flow between 15 and 100 mL min^{-1}.

Nitrogen flow (mL min^{-1})	**Retention time** (min)	**Peak width ($W_{1/2}$)**	***m/z* 73 peak area**
15	33.608	0.903	994539
30	33.538	1.017	1379995
40	33.526	1.159	1790277
50	33.440	0.976	1940952
60	33.363	1.068	1753042
70	33.442	1.142	1771632
100	33.459	1.071	1414398

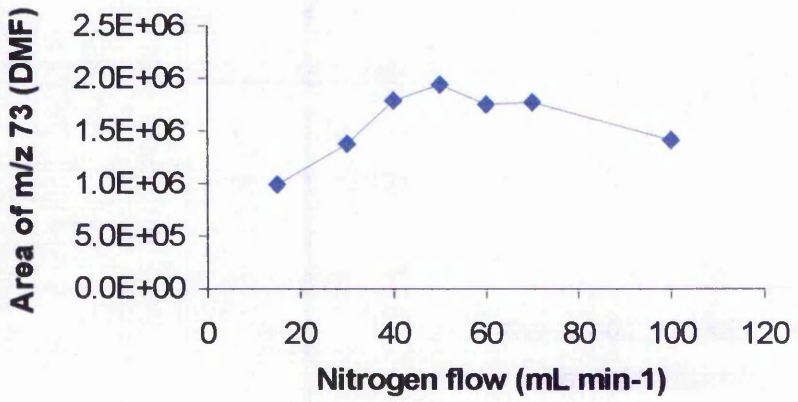

Figure 2.8: Peak area vs. nitrogen flow rate for the IMP-MIMS analysis of DMF.

2.3.2.4. Linearity

A calibration curve was measured for DMSO with quadrupole MS detection, using the optimised IMP-MIMS conditions. Results showed a good linearity (r^2 = 0.9943) in the concentration range between 50 and 220 mg L^{-1} (See Figure 2.9).

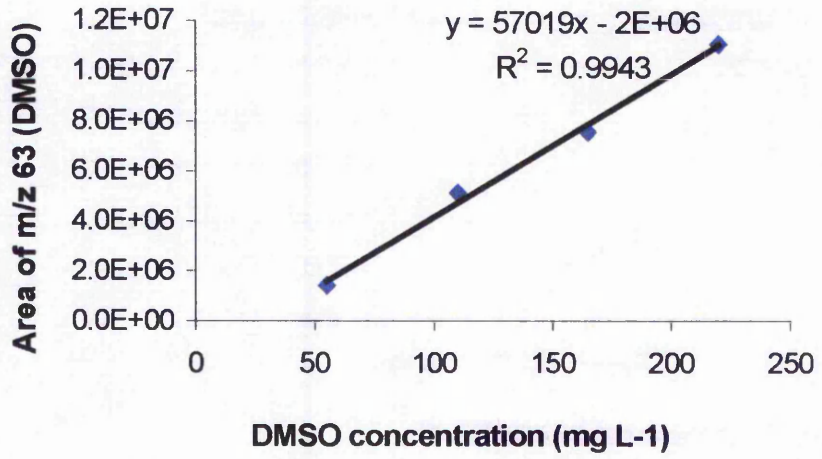

Figure 2.9: Calibration curve for DMSO between 50 and 220 mg L^{-1}.

2.4. Ion trap studies

2.4.1. Introduction

Once the system had been optimised using the quadrupole MS, characterisation of model compounds was carried out using an ion trap MS. The main advantage of using the Varian Saturn 4D ion trap instead of the HP5970 quadrupole spectrometer is the possibility of using tandem mass spectrometry (MS/MS) and chemical ionisation.

2.4.2. Experimental

Experiments were carried out using a Varian Saturn 4D ion trap (Varian Associates, Walnut Creek, CA, USA). Samples were analysed in full scan mode with a *m/z* range between 50 and 150.

In the ion trap experiments were carried out using water reagent chemical ionisation (CI) or electron ionisation (EI). CI using water as the ionisation agent was carried out because ion trap spectral quality is very susceptible to the presence of water, which affected the quality of the EI spectra. In chemical ionisation, water permeating the membrane was used to generate H_3O^+ reagent ions, which yielded protonated molecular ions.

Benzene and toluene were analysed by continuous infusion MIMS with the GC oven at 40°C, while THF was measured by continuous infusion MIMS with the

GC oven at 80°C. DMF, DMSO and fluorobenzoic acids were measured by IMP-MIMS following the temperature profile shown in Figure 2.1. All samples were prepared in water.

2.4.3. Results and discussion

The optimum conditions observed for benzene and toluene in aqueous samples by continuous infusion were obtained using EI. For DMF, DMSO and THF in water the best conditions were obtained using CI, with a sample infusion time of 2.5 minutes. THF infused through the membrane significantly at 80°C by MIMS, but for DMF and DMSO IMP-MIMS had to be used with a thermal desorption cycle of 40-200-40°C. Figure 2.10 shows a single ion response (*m/z* 78+79) and a mass spectrum obtained for the analysis of DMSO.

Better limits of detection were obtained using the ion trap than the quadrupole mass spectrometer. The limits of detection determined for some VOCs and SVOCs using the ion trap mass spectrometer in full scan are shown in Tables 2.6 and 2.7. The limits of detection could be improved for SVOCs by use of a longer sample infusion time. Limits of detection were measured by obtaining a 3:1 signal to noise ratio.

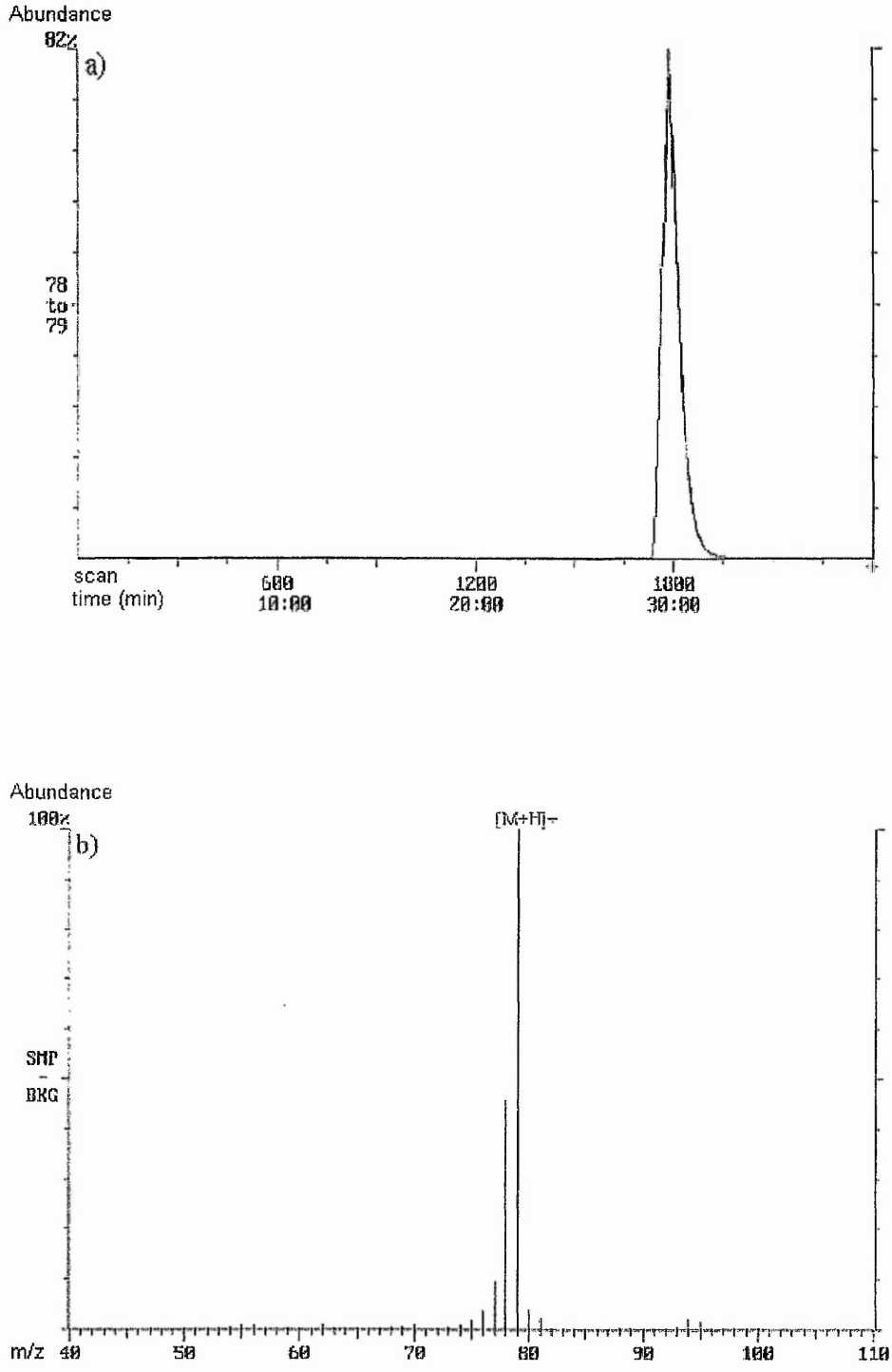

Figure 2.10: 220 mg L^{-1} DMSO using an ion trap in CI mode (a) extracted single ion response for *m/z* 78+79 and (b) mass spectrum.

Table 2.6: Limits of detection obtained in the ion trap mass spectrometer for different VOCs and SVOCs in aqueous samples.

Compound	Limit of detection
Benzene	0.9 μg L^{-1}
Toluene	2.1 μg L^{-1}
DMSO	0.3 mg L^{-1}
DMF	0.5 mg L^{-1}
THF	<0.1 mg L^{-1}

For the analysis of fluorobenzoic acids (FBA), the temperature profile (Figure 2.1) was the same as that for DMF and DMSO: sample injection and membrane drying step were carried out at 40°C, and then the system was heated to 200°C to thermally desorb the FBA, before being cooled back down to 40°C again. The limits of detection are presented in Table 2.7 and Figure 2.11 shows a single ion response (*m/z* 123) and a mass spectrum obtained for the analysis of 4-fluorobenzoic acid (4-FBA). Optimum drying time was 10 minutes for 2-fluorobenzoic acid (2-FBA) and 3-fluorobenzoic acid (3-FBA), while 20 minutes for 4-FBA.

Table 2.7: Limits of detection obtained for FBA using IMP-MIMS

FBA Isomer	Limits of detection (mg L^{-1})	Ionisation mode	Optimum drying time (min)
2-FBA	0.5	EI	10
3-FBA	0.3	EI	10
4-FBA	0.2	EI	20

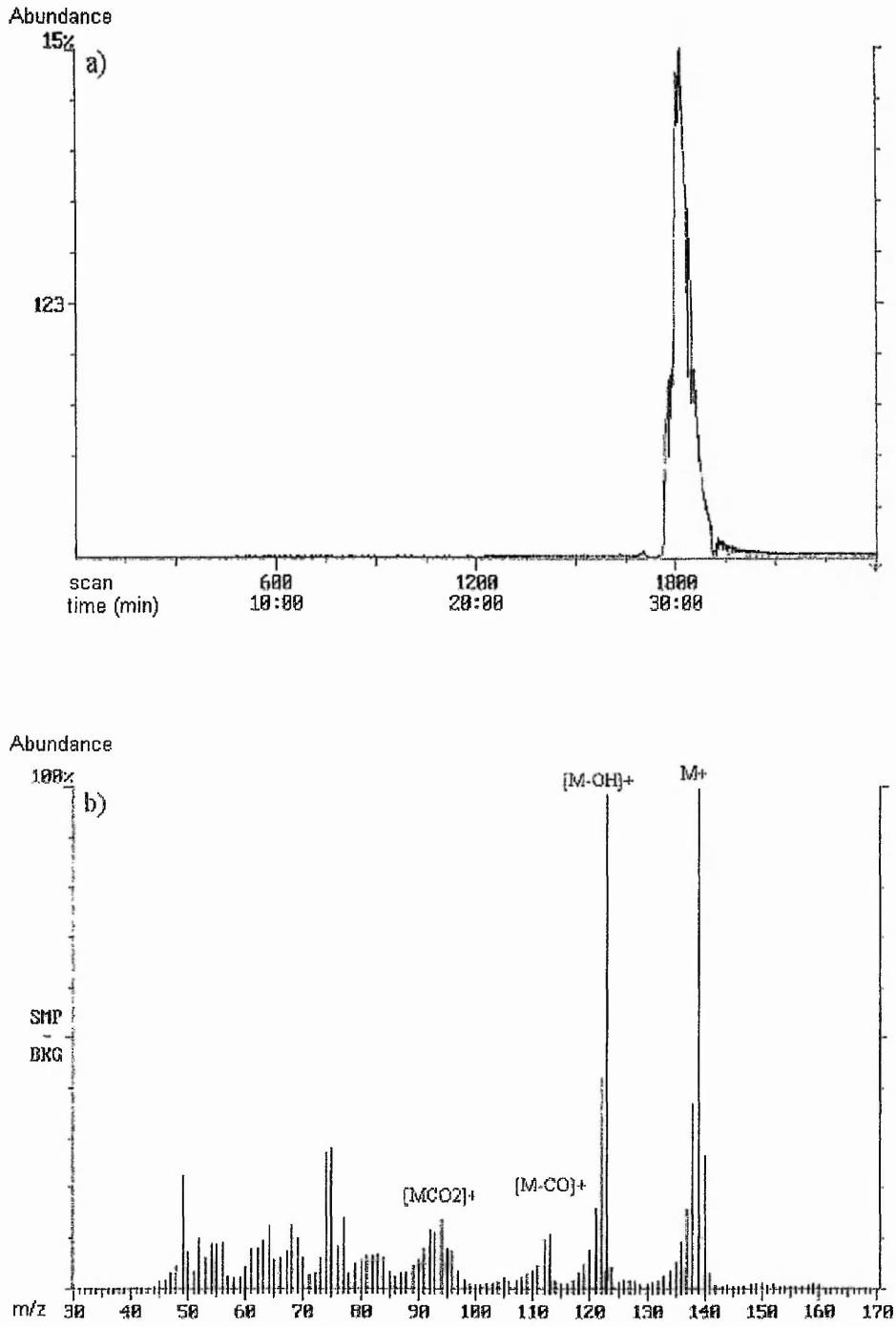

Figure 2.11: 10 mg L^{-1} 4-FBA using an ion trap in EI mode (a) extracted single ion response for *m/z* 123 and (b) mass spectrum.

Limits of detection (S:N 3:1) below 1 mg L^{-1} were observed for all three fluorobenzoic acids. However, it should be noted that whilst DMF and DMSO sensitivities were improved by using CI, better sensitivity for the three fluorobenzoic acid isomers was achieved using EI. It can be seen in Figure 2.11 that the mass spectrum is of lower quality than expected, because of overloading the trap causing space charge effects due to the presence of water in the trap.

2.5. Tandem mass spectrometry

Tandem mass spectrometry (MS/MS) can be used to improve the limits of detection or to gather structural information on selected ions. MS/MS was used for 4-FBA, 4-fluorocinnamic acid (4-FCA) and 4-fluoroacetophenone (4-FAP). Biodegradation studies carried out for 4-FBA and 4-FCA (Chapter 3) showed that 4-FCA biodegrades into 4-FAP before converting into 4-FBA. Therefore a mixture of the three compounds is present when 4-FCA biodegrades. 4-FBA and 4-FCA can be quantified as they have characteristic ions that do overlap with any of the other compounds (*m/z* 140 for 4-FBA and *m/z* 166 for 4-FCA). However, quantification of 4-FAP is problematic, as this compound does not have characteristic ions of its own (it shares *m/z* 123 with 4-FBA and *m/z* 138 with 4-FCA). Therefore MS/MS studies of these compounds were carried out.

2.5.1. Experimental

Experiments were carried out using a Varian Saturn 4D ion trap (Varian Associates, Walnut Creek, CA, USA). Collision induced activation voltage

(CID) applied was 0.5 V for 4-fluoroacetophenone (4-FAP) and 1.00 V for 4-FBA. A CID frequency of 48.00 and a 3 mass unit window were chosen for both compounds. Standards solutions of 4-FBA (3.5 mg L^{-1}) and 4-FAP (100 mg L^{-1}) in water were prepared and run by IMP-MIMS/MS.

Experiments were carried out using the silicone membrane interface shown in Figure 2.2. Analytes were introduced to the membrane at a flow rate of 3 mL min^{-1} for 5 min, followed by a 15 min drying step using pumped air at 3 mL min^{-1} through the outer wall of the membrane at 40°C. For thermal desorption of the analyte the membrane was heated to 200°C at a rate of 45°C min^{-1} and then cooled back down to 40°C.

2.5.2. Results and discussion

MS/MS can give structural information that may be used for the identification of unknown metabolites in processes such as the ones described in Chapter 3. By applying MS/MS, sensitivity can also be improved.

4-FAP cannot be quantified by MIMS when present with 4-FBA and 4-fluorocinnamic acid (4-FCA), as both peak ions for 4-FAP are common to 4-FBA and 4-FCA (see Chapter 3). Therefore 4-FAP and 4-FBA were analysed by MIMS/MS.

The main peaks of 4-FAP by MIMS are *m/z* 138 ($M^{+\cdot}$) (present in 4-FCA) and 123 ($[M-CH_3]^+$) (present in 4-FBA). When IMP-MIMS/MS was applied to *m/z*

123 or *m/z* 138 of 4-FAP a fragment with *m/z* 112 ($[M-C_2H_2]^+$) was observed. When IMP-MIMS/MS was applied to *m/z* 123 ($[M-OH]^+$) or *m/z* 140 ($M^{+\cdot}$) for 4-FBA, a fragment of *m/z* 112 ($[M-CO]^+$) was also formed. Therefore IMP-MIMS/MS could be successfully applied but no individual peaks corresponding to the MS/MS fragmentation of 4-FAP were observed.

IMP-MI$(MS)^3$ was applied successfully for 4-FBA (Figure 2.12) on peaks *m/z* 123 and *m/z* 140, obtaining mainly a fragment on *m/z* 112.

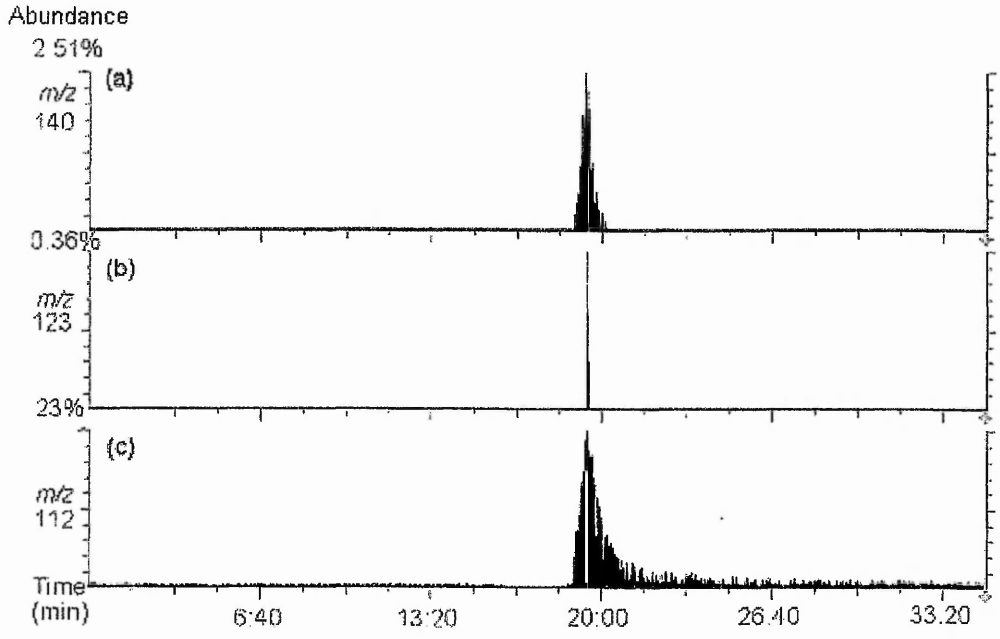

Figure 2.12: IMP-MI$(MS)^3$ single ion response for a 4-FBA (3.5 mg L^{-1}) aqueous sample (a) *m/z* 140, (b) *m/z* 123, (c) *m/z* 112.

2.6. pH and mineral salt effects on 4-FBA and 4-FCA by MIMS

2.6.1. Introduction

As 4-FCA and 4-FBA are both acids, it was considered that the pH of the solution could significantly affect the permeation of the product across the membrane, facilitating the process when 4-FBA and 4-FCA were under acidic conditions. 4-FBA and 4-FCA were chosen to evaluate the importance of the pH and matrix effect by MIMS, as both compounds would be studied during biodegradation monitoring (Chapter 3). When the samples were prepared at GSK for biodegradation, the two analytes were dissolved in a mineral salt medium (MSM) necessary for bacteria to grow efficiently. As the effect of these ions in MIMS was unknown, a test was also carried out in order to find out if the presence of ions in solution affected the response.

2.6.2. Experimental

Standard solutions (100 mg L^{-1}) of the analytes (4-FBA and 4-FCA) were dissolved in water. When necessary these solutions were diluted with an MSM solution (see Table 2.8 for composition) to test the salt effect. pH was regulated using HCl (50-50% v/v concentrated HCl-H_2O) and NaOH (0.5M).

MIMS analysis was carried out using a Hewlett Packard HP5970 quadrupole MS coupled to a HP 5890 GC (Hewlett Packard, Palo Alto, California, USA). The interface containing a silicone hollow-fibre membrane (0.635 mm o.d. x 0.305

mm i.d., Dow Corning Silastic, Sanitech, USA) was constructed in-house and located inside the GC oven in place of the capillary GC column (Figure 2.2) [10]. Aqueous samples were pumped through the interface using a peristaltic pump (Watson-Marlow Bredel Pumps Limited, United Kingdom) for 5 minutes at a rate of 2.5 mL min^{-1} with the oven temperature set to 40°C. The membrane was dried for 15 minutes by drawing air through the interface using the pump (4-FBA) or a nitrogen flow at 50 mL min^{-1} (4-FCA). The oven temperature was then heated to 200°C at 45°C min^{-1} to desorb the preconcentrated analytes before being cooled down to 40°C again. The MS was set to acquire full scan EI mass spectrum in the range *m/z* 70 and 170 at a scan rate of 2.6 scan/second.

Table 2.8: Composition of MSM solution.

	Chemical	**Concentration** ($g\ L^{-1}$)
Stock solution A	KH_2PO_4	8.5
	K_2HPO_4	21.75
	$Na_2HPO_4.2H_2O$	33.4
	NH_4Cl	0.5
Stock solution B	$CaCl_2$	27.5
	$CaCl_2.2H_2O$	36.4
Stock solution C	$MgSO_4.7H_2O$	22.5
Stock solution D	$FeCl_3.6H_2O$	0.25

In order to prepare the MSM solution, 10 mL of stock solution A and 1 mL of stock solutions B, C and D were dissolved in water and made up to 1 litre volume. The MSM solution was prepared as shown in Table 2.8. [11]

2.6.3. Results and discussion

2.6.3.1. Reproducibility

The reproducibility of the MIMS analytes was tested for both 4-FBA and 4-FCA. In each case 6 subsamples of 100 mg L^{-1} solution of each acid were analysed using the method described in Section 2.6.2. The %RSD for 4-FBA and 4-FCA were 3.3% and 3.5% respectively.

2.6.3.2. pH effect for 4-FBA and 4-FCA.

Standard solutions containing 100 mg L^{-1} of 4-FBA or 4-FCA were prepared. pH was modified by adding NaOH or HCl. The pH range used for the different solutions was between 2 and 8, as the membrane degrades rapidly under alkaline or highly acidic conditions. Results are shown in Figures 2.13 and 2.14.

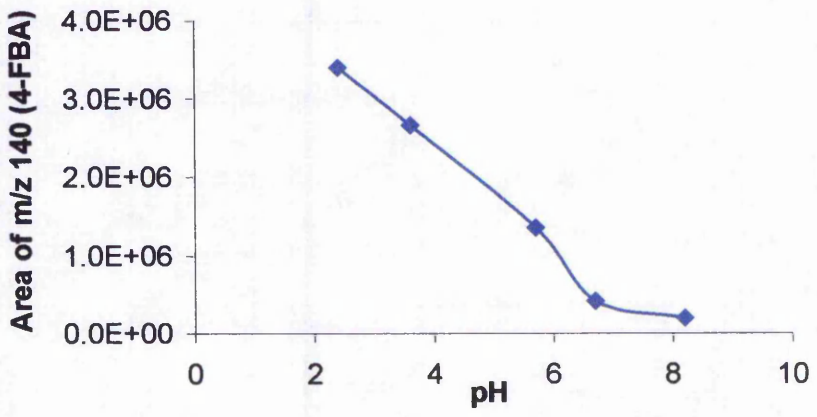

Figure 2.13: pH effect for 100 mg L^{-1} 4-FBA solutions.

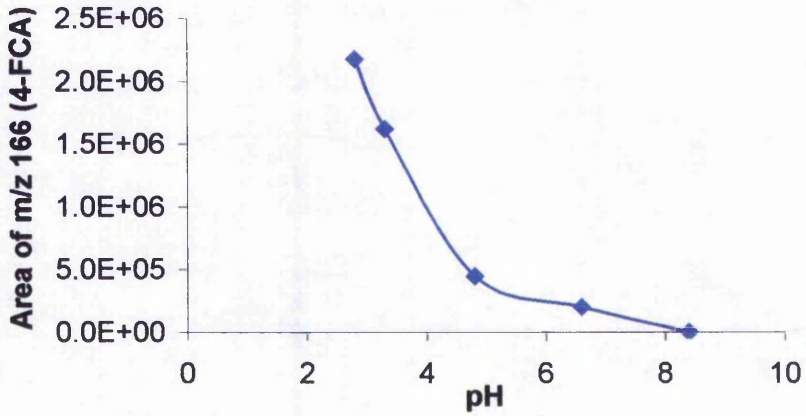

Figure 2.14: pH effect for 100 mg L^{-1} 4-FCA solutions.

As expected, the effect of pH in the analysis of 4-FBA and 4-FCA by MIMS is a significant parameter (see Figures 2.13 and 2.14). For both compounds, acidification of the sample enhances the response by facilitating the transport of the undissociated acids across the silicone membrane. Looking at the profile of both graphs (Figures 2.13 and 2.14), there is a clear indication of a pH signal drop, above the pKa value for both compounds. The pK_a values for 4-FCA and

4-FBA were searched in the literature, but only the value for 4-FBA was reported by Strong et al. to be 4.1 (Table 2.9) [12]. Therefore, it was necessary to calculate the value of the pK_a for 4-FCA and compare it to the one of 4-FBA.

2.6.3.3. Calculation of the pK_a value for 4-FCA

In order to calculate the value of the pK_a of 4-FCA, the Henderson-Hasselbalch equation expression (eq. 2.1) was used:

$$pH = pK_a + \log \frac{[A^-]}{[HA]} \qquad \text{(eq. 2.1)}$$

If in this expression, the concentration of acidic species is equal to the concentration of the acid radical, then we obtain the following equality (eq. 2.2):

$$pH = pK_a \qquad (2.2)$$

There was no chemical having the fluorocinnamate ion available commercially, so a known amount of 4-FCA (20 mg L^{-1} or 0.12 mmolar) and half the number of moles of NaOH were mixed and the pH was measured to determine the pK_a. In order to compare the efficiency of the method, this experiment was repeated also with 4-FBA, to allow the pK_a value obtained to be compared with the literature value.

There is a 0.3 unit difference between the pK_a value from the literature and the one calculated experimentally. The pK_a value for 4-FCA was calculated to be 3.9,

which is very similar to that calculated for 4-FBA (around 3.8). These results are in agreement with the pH graphs shown in Figures 2.13 and 2.14.

Table 2.9: Experimental pK_a values for 4-FBA and 4-FCA

Compound	pK_a
4-FBA	3.8[a]
	4.1[b]
4-FCA	3.9

[a] this work, [b] taken from reference 12.

2.6.3.4. Effect of MSM solution on the MIMS technique for 4-FBA and 4-FCA

Two sets of samples were prepared for each compound in the range between 20 and 50 mg L^{-1}. One set of samples contained water as the solvent, while the other set of samples had a 50% v/v water/MSM solution. The IMP-MIMS responses for 4-FBA and 4-FCA in the water and aqueous MSM solutions are shown in Figures 2.15 and 2.16.

Figures 2.15 and 2.16 show that the responses for the acids are significantly lower in MSM solution than in water. However, the observed responses may be attributed to the effect of pH, as MSM solutions have a pH value close to 6.5, while the samples in water have a pH value close to 3.0. In order to investigate the mineral salt effect, samples in water were prepared by adjusting the pH to the value for the MSM solutions prior to MIMS analysis.

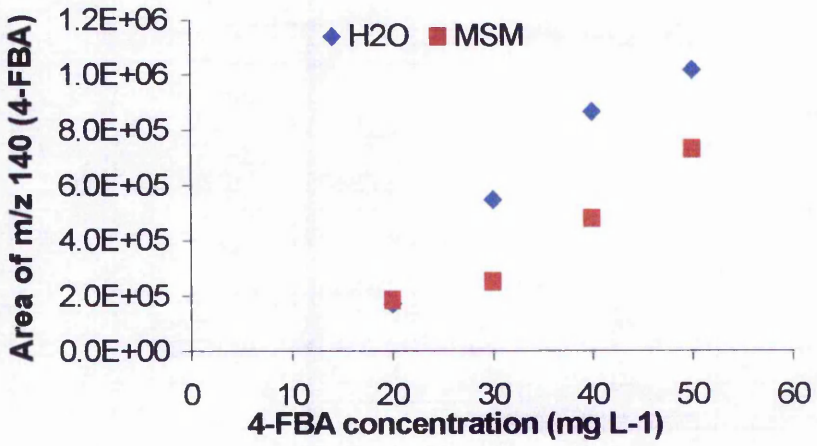

Figure 2.15: Calibration curve results for 4-FBA using water and MSM as solvents.

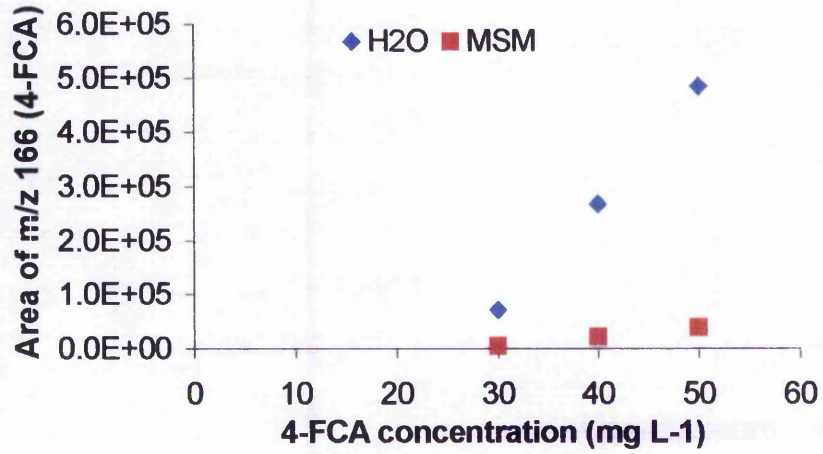

Figure 2.16: Calibration curve results for 4-FCA using water and MSM as solvents.

2.6.3.5. Investigation of the mineral salt effect independent of pH

The aim of this investigation was to evaluate the effect of the salts on the MIMS analysis independently of the pH value. Two different points were chosen, one pH close to that for 4-FBA and 4-FCA in water, and the other with a pH similar to that of the analytes in MSM solution. The results are shown in Tables 2.10 and 2.11.

Table 2.10: IMP-MIMS responses for 4-FBA at pH 2.9 and 6.4 in water and aqueous MSM solutions.

pH of the 50 mg L^{-1} solution	Solvent	Area peak 141
2.9	H_2O	1546325
2.9	MSM	2386624
6.4	H_2O	1169945
6.4	MSM	1550488

Table 2.11: IMP-MIMS responses for 4-FCA at pH 3.3 and 6.6 in water and aqueous MSM solutions.

pH of the 50 mg L^{-1} solution	Solvent	Area peak 166-167
3.3	H_2O	485574
3.3	MSM	509351
6.6	H_2O	4406
6.6	MSM	38598

From the response data in Tables 2.10 and 2.11, it can be seen that for both compounds the addition of salts enhances the MIMS response considerably. A possible explanation for this effect may be that in solutions of high ionic strength these organic acids preferentially partition into the non-polar silicone membrane.

Although the addition of MSM solution tends to show an increase in the response for both 4-FBA and 4-FCA, the main parameter to affect the results is the pH. So in any kind of biodegradation studies for acidic compounds, pH should be closely monitored, or controlled by introducing an on-line addition of acid before analysing the sample, as this would give an improvement in the response for these compounds.

2.7. Conclusions

An IMP-MIMS technique incorporating a novel membrane drying stage has been developed for the determination of SVOCs. The effect of drying time, drying temperature and drying agent has been evaluated, proving that the presence of a drying stage was critical for enhancing mass spectrometric performance, thus the absence of water in the MIMS device avoids signal suppression of the analyte and excessive source pressure in the MS ion source. The presence of an inert gas (drying agent) in the membrane surface during the thermal desorption stage also reduced thermal degradation of the membrane and extended the lifetime of the membrane. Although optimum drying times and hence analytical cycle times were long for some applications, a compromise between length of analysis and sensitivity can be made. The IMP-MIMS technique showed good linearity and

reproducibility for SVOCs, making it a suitable technique for quantitation analysis.

The effect of pH and matrix on the MIMS responses for 4-FBA and 4-FCA in aqueous samples has been evaluated. The main parameter to effect the results was the pH, which is shown to be a critical parameter in MIMS, because the use of semi-permeable membranes discriminates ionic species, showing preferential selectivity for non-polar analytes. As 4-FBA and 4-FCA are acid species, non-ionic species will be predominant under acidic conditions, while in alkali conditions both compound will be mainly under the ionised form. Conversely, alkali conditions would enhance the response of basic species (such as amines) by MIMS. Therefore when quantification must take place by MIMS, the pH should be closely monitored and kept at a constant value during the whole study.

2.8. References

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CHAPTER 3

Biodegradation monitoring by membrane inlet mass spectrometry

3.1. Introduction

Biodegradation can be defined as the biologically catalysed reduction in complexity of chemicals. In the case of organic compounds, biodegradation frequently, although not necessarily, leads to the conversion of much of the C, N, P, S and other elements in the original compound to inorganic products. The complete conversion of an organic substrate to inorganic products is known as mineralisation. The biodegradation of synthetic chemicals by biological processes apply mainly to the result of microbial activity.

Microorganisms carry out biodegradation in many different types of environments such as soil, watercourses or sediments. The ability to carry out biodegradation of toxic and hazardous chemical compounds is exploited by designing systems such as wastewater treatment plants (WWTP), reedbeds or bioaugmenting soils. A varied range of micro-organisms is essential for the effective treatment of wastewaters, since they are responsible for the destruction of a large number of organic pollutants and hazardous chemicals. Natural communities of microorganisms in these treatment processes have a rich physiological versatility. They are able to metabolise and often mineralise a wide range of organic molecules.

Several conditions are required for biodegradation to take place in a particular environment:

1. The microorganisms require the necessary enzyme to bring about the biodegradation of a certain compound.
2. The chemical must be accessible to the microorganism having the required enzyme. Inaccessibility may occur if the substrate is in a different microenvironment from that of the microorganism, e.g. in a solvent not miscible with water or sorbed in solid surfaces or particles. A parameter that gives an idea of the behaviour of chemicals under these circumstances is given by the octanol/water partition coefficient ($\log_{oct}$), which indicates the possibility of a certain chemical to be absorbed by solid particles or in another solvent different than water.
3. Conditions in the environment must be adequate for the proliferation of the most active microorganisms, i.e. enough trace elements, adequate temperature and pH.
4. Intermediate metabolites formed due to the biodegradation must not be toxic to the microbial community.

Prior to the degradation of many complex organic compounds, an acclimation period is usually required. This is defined as the length of time between the addition or entry of the chemical into an environment and evidence of its detectable loss. The length of the acclimation period can vary significantly. It may be between hours and several months. The duration depends on the chemical complexity, their concentration and a number of environmental factors. Biodegradation is only detectable until enough microorganisms develop the ability to degrade a particular compound. A critical amount of biomass is required to cause appreciable chemical loss. In some environmental systems

complex mixtures of organic compounds are present, and one or more may be inhibitory to the microbial growth, increasing the length of the acclimation period or making biodegradation not possible.

In WWTP a mixture of diverse organisms is used for the biodegradation. This mixture is known as activated sludge. Activated sludge is a heterogeneous microbial culture composed mostly of bacteria, protozoa, rotifers and fungi. However, it is the bacteria which are responsible for biodegrading most of the organic compounds, while protozoa and rotifers are mainly responsible for the removal of excess and/or inactive bacteria. Due to the variety of enzymes present in the activated sludge and the fact that WWTP are operated in a continuous manner, the acclimation time is minimised, although still required for new chemical compounds introduced in the feed.

To produce a high-quality final effluent from a WWTP, the activated sludge (after removing the organic materials from the wastewater) must be separated from the liquid stream. A typical WWTP flow diagram is shown in Figure 3.1 [1].

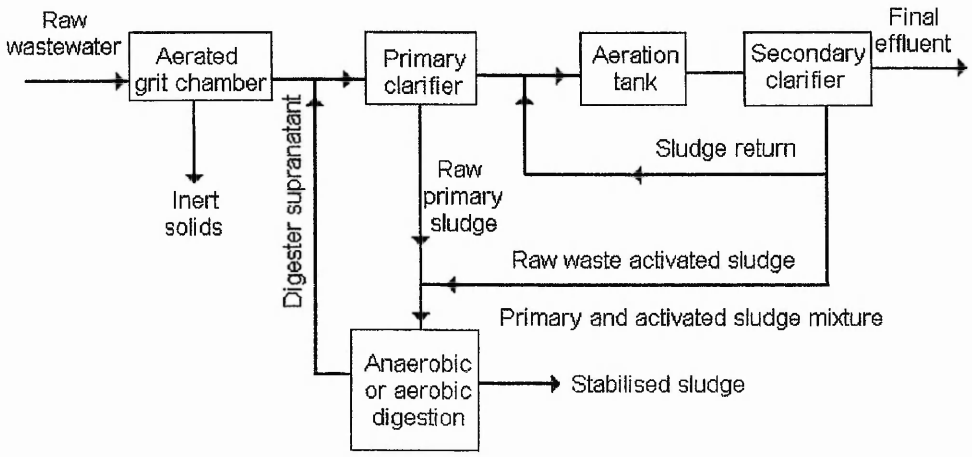


Figure 3.1: Typical flow diagram of a WWTP.

One of the fundamental requirements in the design of a WWTP is to know what type of tank of reactor (aeration tank) is best for a given wastewater. The geometry of the reactor is important as well as having a good aeration system which is necessary to achieve aerobic conditions throughout the reactor medium and to improve mixing.

In the biodegradation studies carried out as part of this thesis, mineral salt medium (MSM) and synthetic sewage were added to mimic a typical feedstock. The activated sludge used in the laboratory studies was collected from a municipal WWTP. MSM was added to the system to provide buffering and trace elements, while synthetic sewage was added to provide a metabolisable source of carbon to the activated sludge while acclimatising to the new organic compounds introduced into the biological medium.

MIMS processes have been reported by several groups for monitoring the biodegradation or fermentation of volatile products and by-products [2,8]. The

on-line monitoring of the major products (acetic acid, acetoin, 2,3-butanediol and ethanol) of fermentation by the *Bacillus polymyxa* and *Klebsiella oxytoca* by MIMS has been reported by Hayden *et al.* [2]. Hansen *et al.* carried out an on-line monitoring of phenoxyacetic acid, a precursor of penicillin V, during *Penicillum Chrysogenum* fermentation [3,4] and Johnson *et al.* monitored on-line fermentation broths in a pilot plant by MIMS, using a 9000-litre fermentation reactor in which samples were continuously taken by splitting flow from the fermentation broth and analysed for ethanol content by flow injection. Minor components such as acetic acid and lactic acid were also identified [5]. The fungus *Bjerkandera adusta* has been used for monitoring volatile halogenated compounds [6,7]. Arcangeli *et al.* monitored the kinetics of *cis*-1,2-dichloro-ethylene biodegradation at μg dm^{-3} levels in a biofilm reactor under the presence of methane-oxidising bacteria [8]. In contrast to the determination of VOCs, the monitoring of SVOCs has received little attention.

3.2. Biodegradation studies of 4-FBA and 4-FCA: an evaluation of MIMS as an alternative to HPLC and IC

3.2.1. Introduction

Aerobic biodegradation of halogenated aromatic compounds has been studied extensively, particularly for the fluorobenzoates [9,10] and chlorobenzoates [11-13]. As a general rule, the strength of resistance to enzymatic cleavage of carbon-halogen bonds is observed to increase with the electronegativity of the substituents, in the order F-C>Cl-C>Br-C>I-C [14]. The exceptional biological

activity of fluorinated aromatic compounds can therefore be explained by the dichotomic chemical behaviour of the fluorine with its mesomeric effects and also with its hydrogen-resembling size [15].

Fluorobenzoic acids have been reported to degrade under both aerobic [10,15,16] and anaerobic [17] conditions. Several pathways have been identified under aerobic conditions, as a function of the different enzymes, but two are most widely reported. One pathway involves the degradation of fluorobenzoic acids into the corresponding fluorocatechol [10,15], whilst in the other pathway fluorobenzoic acid is transformed into hydroxybenzoic acid [10]. It has also been shown that 4-FCA is biotransformed to 4-FBA under aerobic conditions [18,19].

Biodegradation processes have been monitored by several techniques, including gas chromatography/mass spectrometry (GC/MS) and high performance liquid chromatography (HPLC). The measurement of semi-volatile halogenated organic compounds in aqueous solutions using MIMS has been less widely studied than VOCs because of their poor permeation through the membrane, although Leth *et al.* reported the analysis of chlorophenols and Hansen *et. al.* employed an on-line MIMS system to monitor phenoxyacetic acids in a penicillin fermentation [3,20]. No previous studies describing the use of MIMS for the analysis of the biodegradation of semi-volatile organic compounds have been found in the literature.

This section (section 3.2) reports the development of a MIMS method for monitoring SVOCs in a biodegradation medium, using novel IMP-MIMS

procedures described in chapter 2. The method is applied to the determination of fluorobenzoic and fluorocinnamic acids. The MIMS approach is compared with the use of HPLC, ion chromatography (IC) and liquid chromatography/mass spectrometry (LC/MS), which have been previously reported as suitable techniques for monitoring the biodegradation of these acids [19]. MIMS has been shown to be a robust method for batch and on-line analysis and the objective of this work was to evaluate the potential of the IMP-MIMS technique [21] as an alternative to a suite of chromatographic techniques for biodegradation monitoring.

3.2.2. Experimental

3.2.2.1. Biodegradation

All chemicals were obtained from Sigma-Aldrich Chemicals (Gillingham, United Kingdom) and used without further purification. Standard solutions (200 mg L^{-1}) of 4-fluorobenzoic acid (4-FBA) and 4-fluorocinnamic acid (4-FCA) were prepared in an MSM [19] (see Table 2.8). Activated sludge was obtained from a WWTP at a GlaxoSmithKline site in the UK and washed to remove residual carbonaceous material present in the WWTP liquor. The total suspended solids in the activated sludge was determined to be 3 g L^{-1} by drying 10 ml of activated sludge at 104-106°C for one hour. A volume of bacteria inoculum (100 mL) and of a standard solution of 4-FBA or 4-FCA (100 mL; 200 mg L^{-1}) were mixed in a 250 mL flask, giving an initial concentration of 100 mg L^{-1}. Foam stoppers were used to allow passage of air into and out of the flasks. Separate control flasks

were set up containing 4-FBA and 4-FCA (100 ml; 200 mg L^{-1}) and MSM (100 mL), but no activated sludge. All tests were carried out in triplicate.

The flasks were placed in an orbital shaker (New Brunswick Scientific Company, New Brunswick, USA) at a temperature of 30°C and an agitation speed of 150 rpm. The pH was monitored using a bench top pH meter (Jenway 3310, UK). Aliquots (20 mL) were removed from the flasks and filtered with a 0.2 μm filter before being analysed by MIMS, HPLC and IC. A number of samples were also analysed by LC/MS in order to identify metabolites. The data acquired by MIMS and HPLC were quantified relative to the control flask response.

3.2.2.2. Analytical methods

a) Membrane Inlet Mass Spectrometry

MIMS analysis was carried out using a Hewlett Packard HP5970/HP5890 or a HP6890/HP5973 GC/MS configuration (Agilent, Palo Alto, California, USA). The interface (Figure 2.2) containing a silicone hollow-fibre membrane (0.635 mm o.d. x 0.305 mm i.d., Dow Corning Silastic, Sanitech, USA) was constructed in-house and located inside the GC oven in place of the capillary GC column [22]. Aqueous samples were pumped through the interface using a peristaltic pump (Watson-Marlow Bredel Pumps Limited, United Kingdom) for 5 minutes at a rate of 3 mL min^{-1} with the oven temperature set to 40°C. The membrane was dried for 15 minutes by drawing air through the interface and the oven temperature was then heated to 200°C at 45°C min^{-1}, to desorb the

preconcentrated analytes, before being cooled to 40°C. The heating cycle was carried out twice. The first cycle desorbed the analyte from the membrane and the second cleaned the system in order to eliminate the possibility of carryover. The mass spectrometer was set to acquire electron ionisation (EI) full scan data between *m/z* 40 and 180.

b) High Performance Liquid Chromatography

HPLC was carried out using a Hewlett Packard HP 1100 chromatograph (Agilent Technologies, Palo Alto Ca, USA) equipped with a Waters Symmetry RP8 column (3.9 x 150 mm; 5 μm particle size; Waters, Watford, UK). The injection volume was set to 10 μL. The flow rate was set to 1 mL min^{-1}, the UV detector wavelength to 230 nm and the column oven temperature to 40°C. The eluent consisted of 70/30 (v/v) 0.1% formic acid in water/acetonitrile. Each analysis was performed isocratically over a 15 minute period.

c) Ion Chromatography

Ion chromatography analysis was carried out using a Dionex DX500 ion chromatograph (Dionex, Sunnyvale, Ca, USA). A Dionex Ionpac Guard AG11 column (50 x 4 mm) was coupled to a Dionex AS11 analytical column (250 x 4 mm). The injection volume was 10 μL, the column temperature was set to 30°C and the flow rate was 1mL min^{-1}. Detection was by suppressed conductivity. A hydroxide gradient was produced using a Dionex EG40 eluent generator equipped with a cartridge containing potassium hydroxide. The gradient was set

as follows: T= 0 min 5 mM hydroxide, T= 4.00 min 5 mM hydroxide, T= 4.01 min 50 mM hydroxide, T= 7.00 min 50 mM hydroxide, T= 7.01 min 5 mM hydroxide and T= 10.00 min 5 mM hydroxide.

d) Liquid Chromatography/Mass Spectrometry

LC/MS was carried out using a Agilent 1100 chromatograph (Agilent Technologies, Palo Alto, Ca, USA) interfaced to a Micromass quadrupole orthogonal acceleration time-of-flight mass spectrometer (Q-TOF, Micromass, Manchester, UK) mass spectrometer fitted with a Z-Spray electrospray ion source. HPLC conditions were as described in a previous paragraph. The mass spectrometer was operated in negative electrospray ionisation, using a spray voltage of 3 kV. The source and desolvation temperatures were set to 100°C and 300°C respectively. The nitrogen desolvation and nebuliser gas flow rates were set to 400 $L \cdot h^{-1}$ and 90 $L \cdot h^{-1}$ respectively. The cone voltage was set to 25 V. Experimental data were acquired over a 40-1000 Da mass range at an acquisition rate of 1 spectrum per second. For MS/MS structural elucidation work the collision energy was at 25 eV.

3.2.3. Results and discussion

MIMS analysis was carried out using a silicone hollow-fibre membrane interface similar to that reported in Chapter 2 (Figure 2.2) [22]. An IMP-MIMS procedure [21] was employed to monitor the biodegradation 4-fluorobenzoic acid (4-FBA) and 4-fluorocinnamic acid (4-FCA), using the GC oven temperature profile

shown in Fig. 3.2. IMP-MIMS was necessary because of the poor MIMS response for these semi-volatile compounds at temperatures <100°C. The aqueous sample was removed from the interface and the membrane was dried between the preconcentration and desorption stages by drawing air over the membrane using a peristaltic pump. A membrane drying step was introduced to reduce the amount of water released with the analytes during the desorption step, since significant suppression of the analyte response was observed if the membrane was heated immediately after removal of the water from the interface. A similar approach has been reported for dry purging solid sorbents used to sample volatile organic compounds in ambient air [23].

The single ion response for *m/z* 140 ($M^{+\cdot}$) extracted from the full scan electron ionisation data for the IMP-MIMS analysis of a bacterial broth is shown in Figures 3.3a and 3.3c. Sample giving single ion response shown in Figure 3.3a was taken at time 0 hours, while sample from single ion response shown in Figure 3.3c was taken 72 hours after the medium was spiked with 4-FBA. The 4-FBA is released as a peak, with a width at half height of approximately 1 minute, as a result of the rapid heating of the membrane during the desorption step. Mass spectra shown in Figures 3.3b and 3.3d match the spectrum of the pure 4-FBA closely showing the discrimination of the membrane inlet against activated sludge, mineral salts and other matrix components. As it can be seen, the response of 4-FBA at time 0 hours is greater than at time 72 hours, due to the biodegradation of 4-FBA by the action of microorganisms.

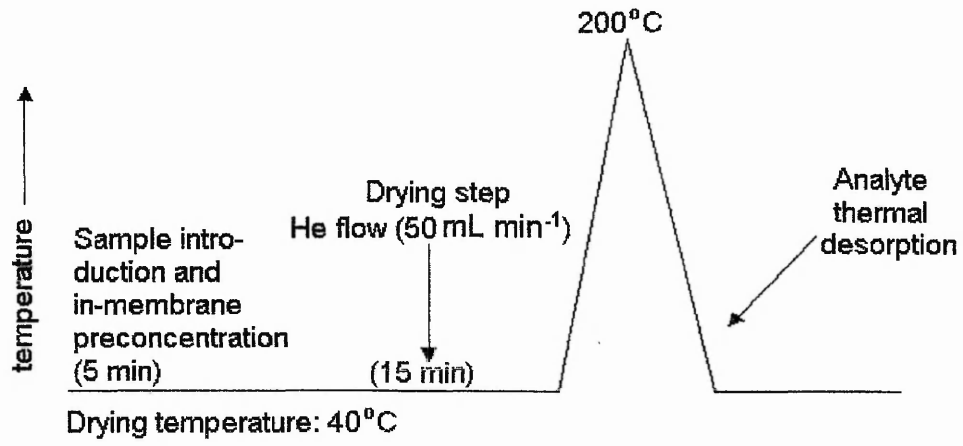


Figure 3.2: The oven temperature programme for IMP-MIMS analysis.

The IMP-MIMS response was calibrated using solutions of 4-FBA and 4-FCA in the range 5 to 100 mg L^{-1} in MSM solution. The limit of detection for 4-FBA was 2 mg L^{-1} (S:N 3:1) and the response for the *m/z* 140 ion ($M^{+\cdot}$) was linear with an r^2 of 0.983. The molecular ion at *m/z* 166 was used to quantify 4-FCA, which gave a limit of detection of 5 mg L^{-1} and a linear response (r^2 = 0.978). The membrane gradually aged during the analysis due to the repeated thermal desorption cycles leading to some variation in analyte response, so for the quantification of 4-FBA and 4-FCA the response obtained during the biodegradation studies was referenced to the control standard. The precision of the IMP-MIMS procedure was 3.3% and 3.5% (n = 6) respectively for 4-FBA and 4-FCA at 100 mg L^{-1}. Samples taken during the biodegradation studies were also subjected to HPLC analysis. The response to standard solutions in the range 5 and 100 mg L^{-1} in MSM solution showed good linearity for 4-FBA and 4-FCA (r^2 of 0.997 and 0.999 respectively), with limits of detection < 1 mg L^{-1} for

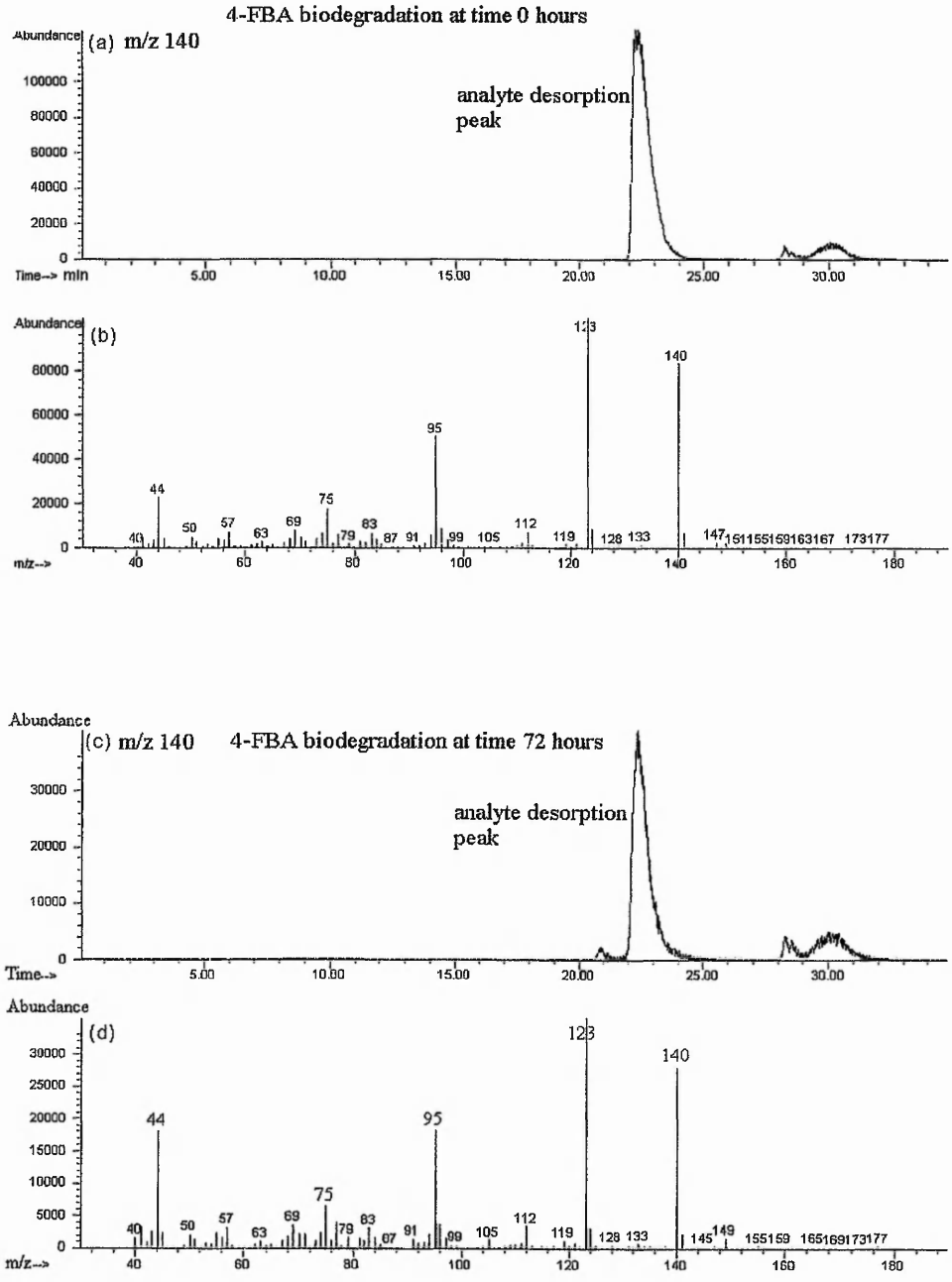


Figure 3.3: Biodegradation of 4-FBA monitored by MIMS at 0 hours (a) extracted single ion response for *m/z* 140 and (b) mass spectrum and at 72 hours (c) extracted single ion response for *m/z* 140 and (d) mass spectrum.

4-FBA and 4-FCA. Sample turnover for HPLC was 25 min, compared to 34 for MIMS analysis.

The pH effect of acids by the MIMS technique has been discussed in section 2.6. The pH of the activated sludge/mineral salt medium remained in the range 6.9 ± 0.2 during the biodegradation studies, which is high compared to the pK_a of the two acids (4.1 for 4-FBA and 3.9 for 4-FCA [24]). This resulted in a significant reduction of the analyte response in the MIMS experiments, because acid dissociation does not favour transport across the non-polar polydimethylsiloxane membrane. However, this was partly offset by an enhancement in sensitivity observed in the higher ionic strength mineral salt medium, presumably because of preferential partitioning of the undissociated acids into the membrane.

A comparison of the degradation profiles for 4-FBA using MIMS and HPLC analysis is shown in Fig. 3.4. These data show good agreement between the two methods with approximately 65% of the initial amount of 4-FBA degraded after 72 hours. The MIMS analysis was not continued beyond this point, while HPLC analysis showed that after 96 hours, 90% of the 4-FBA was degraded and after 144 hours no 4-FBA was detected, consistent with previously reported data for this compound. The concentration of 4-FBA in the control flask remained constant throughout the experiment. No intermediates were observed by MIMS during the biodegradation study. A possible explanation for the absence of intermediates is that these were more polar than the precursor acids and were therefore not transported across the silicone membrane. This membrane

selectivity is supported by the HPLC data, which shows the presence of a number of unidentified polar compounds eluting after the solvent front.

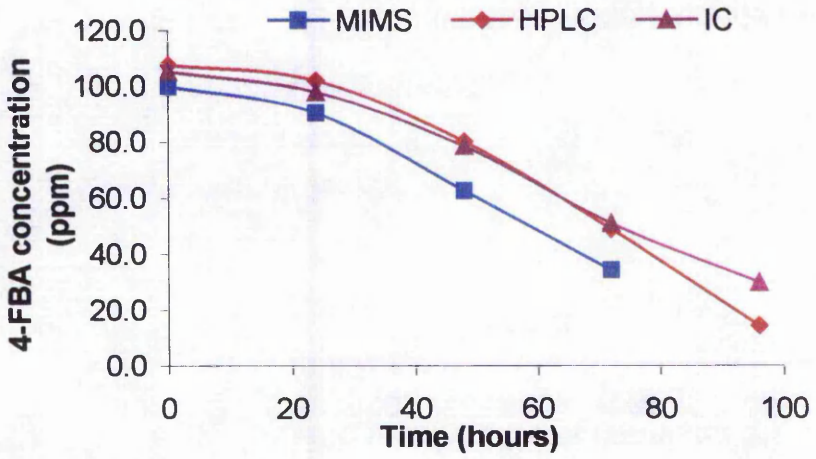


Figure 3.4: MIMS, HPLC and IC data for 4-FBA biodegradation. MIMS and HPLC results are the concentration values for 4-FBA, IC data show fluoride ion concentration expressed as 4-FBA concentration remaining.

The fluoride ion concentration was determined using ion chromatography (IC) during the biodegradation as a way of completing the mass balance and providing evidence of biodegradation rather than chemical degradation or adsorption. Fluoride measurements were carried out in triplicate for the biodegradation of samples containing 4-FBA. Two ion chromatography chromatograms of samples extracted from a shakeflask for the 4-FBA biodegradation at times 0 and 96 hours are shown in Figure 3.5. As it can be seen from the differences between Figures 3.5a and 3.5b, there was an increase in the fluoride concentration (peak at time 1.865 minutes) in the active flask samples as a function of the time, but no increase was observed in the controls. Figure 3.4

shows the temporal variation of fluoride ion expressed in terms of the concentration 4-FBA remaining in the flask. These data agree well with that obtained for the 4-FBA concentrations measured by MIMS and HPLC. This suggests that the initial biodegradation of 4-FBA is the rate-limiting step in the process leading to complete mineralisation resulting in the liberation of fluoride ion.

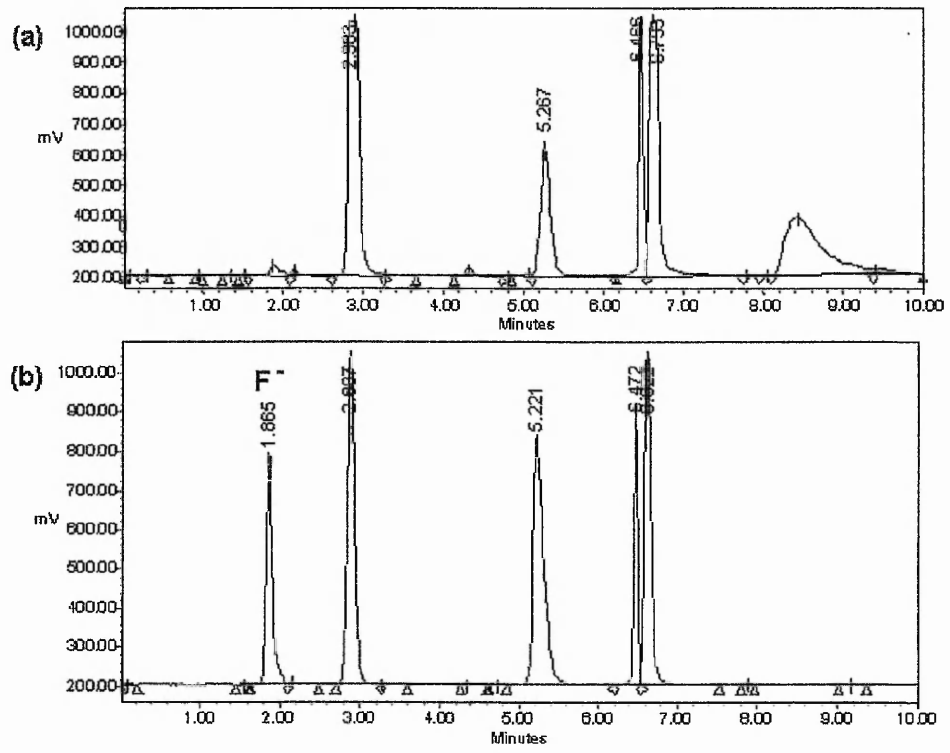


Figure 3.5: Ion chromatograms for samples coming from a shakeflask from 4-FBA biodegradation monitoring at (a) time 0 hours and (b) time 96 hours.

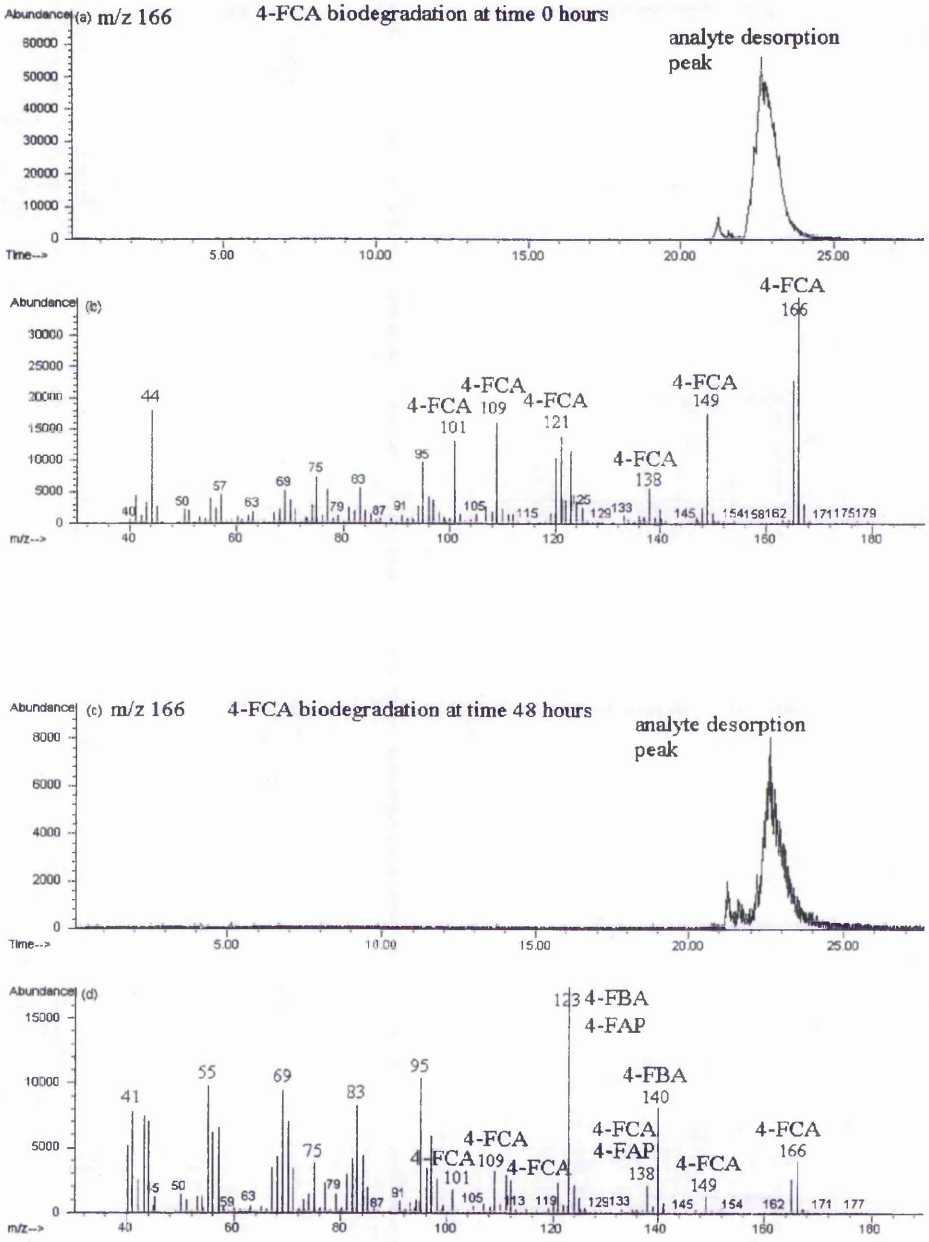


Figure 3.6: Biodegradation of 4-FCA monitored by MIMS (a) extracted single ion response for *m/z* 166 at time 0 hours, (b) mass spectrum at time 0 hours, (c) extracted single ion response for *m/z* 166 after 48 hours, (d) mass spectrum after 48 hours.

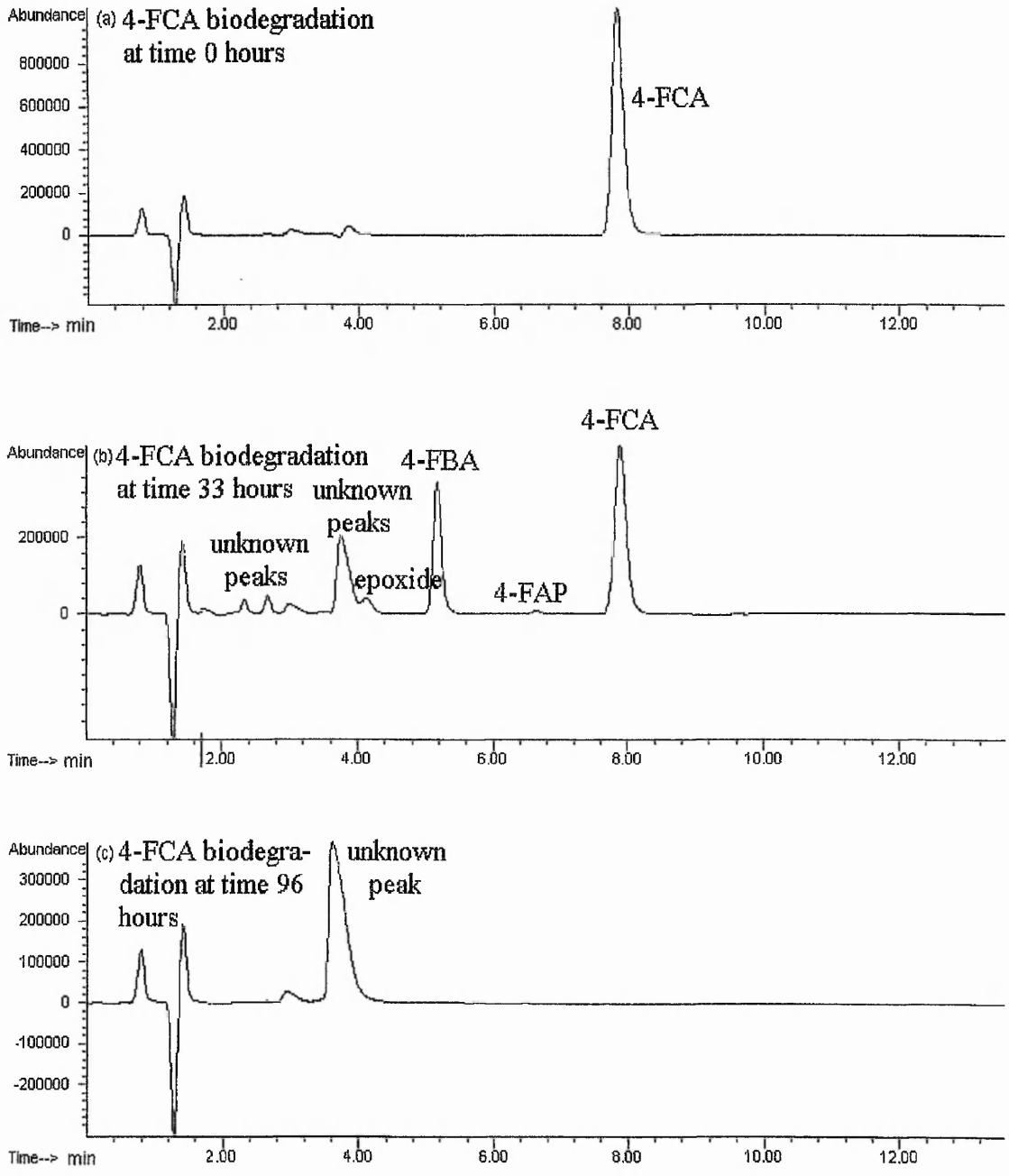
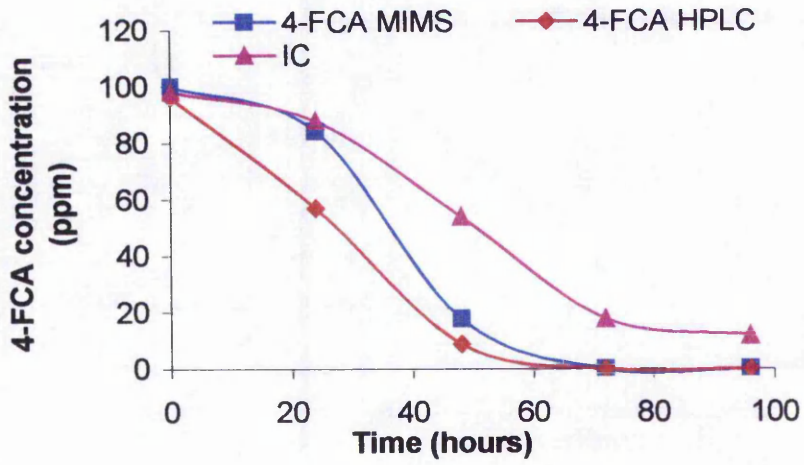


Figure 3.7: Biodegradation of 4-FCA monitored by HPLC (a) chromatogram at time 0 hours, (b) chromatogram after 33 hours, (c) chromatogram after 96 hours.

In order to follow the 4-FCA biodegradation by MIMS, aliquots were removed from the sample and control flasks at times between 0 and 96 hours and analysed by MIMS, HPLC and IC. The MIMS extracted single ion responses (*m/z* 166) and mass spectra observed immediately following addition of the activated sludge and at 48 hours after mixing are shown in Figure 3.6. HPLC chromatograms obtained for the same sample are shown in Figure 3.7. The results of the MIMS, HPLC and IC monitoring experiments are summarised in Figure 3.8.

The mass spectrum from the MIMS analysis at time zero shows ions characteristic of 4-FCA at *m/z* 166 ($M^{+\cdot}$), 149 $(M\text{-}OH]^{+}$), 138 ($[M\text{-}CO]^{+\cdot}$), 121 ($[M\text{-}COOH]^{+}$), 109 ($[C_7H_6F]^{+}$), 101 ($[C_8H_5]^{+}$), 95 ($[C_6H_4F]^{+}$) and 75 ($[C_6H_3]^{+}$), as well as some lower mass ions originating from the activated sludge. After 48 hours, the intensity of these ions has decreased to approximately 10% of its original intensity as a result of biodegradation of 4-FCA and after 72 hours no 4-FCA could be detected. The concentration of 4-FCA in the control flask remained constant throughout the experiment. The spectrum obtained after 48 hours shows the presence of new ions at *m/z* 140 and 123, tentatively assigned to 4-FBA, that has been identified previously as an intermediate [19]. These ions were no longer detected after 96 hours indicating that 4-FBA and 4-FCA had been fully degraded by that stage. 4-fluoroacetophenone (4-FAP), has also been reported to be a metabolite of 4-FCA [18] and has a molecular ion at *m/z* 138. This ion was present in the mass spectrum obtained after 48 hours, but is also an EI fragment of 4-FCA (Figure 3.6). In order to determine whether 4-FAP was being formed, the intensity of *m/z* 138 ion was ratioed to the intensity of *m/z* 166 ion that is present in 4-FCA, but not in 4-FAP. The *m/z* 138:166 ratio increased with time, as the

(a)



(b)

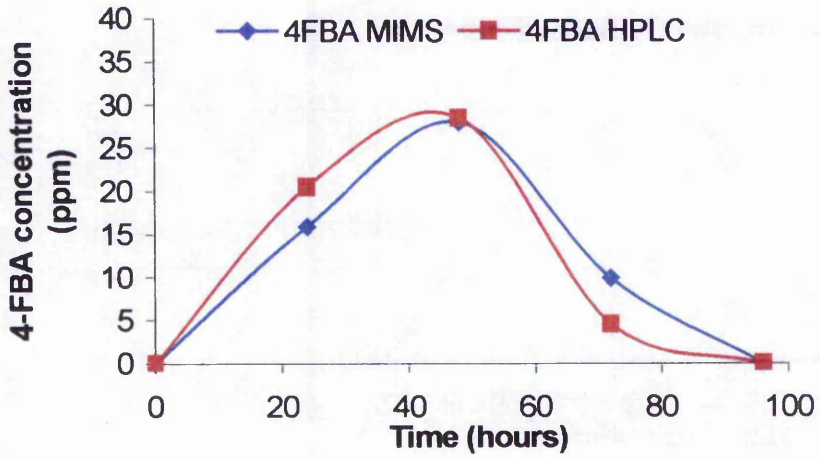


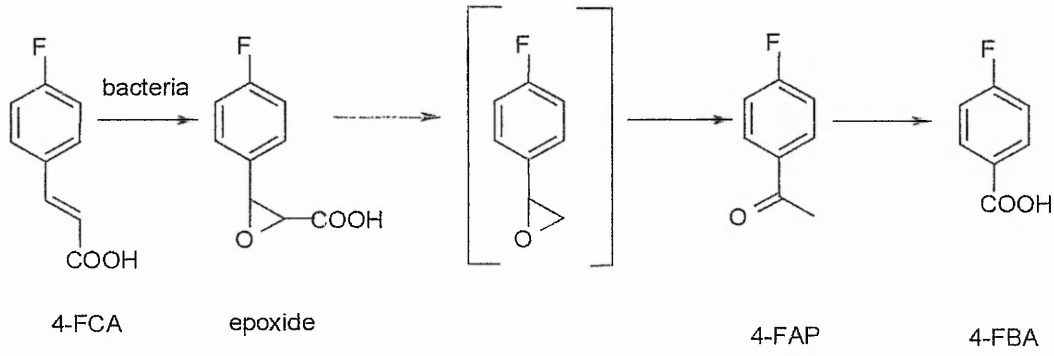
Figure 3.8: (a) MIMS, HPLC and IC data for 4-FCA biodegradation. MIMS and HPLC results are the concentration of 4-FCA, IC data show fluoride ion concentration expressed as remaining 4-FCA concentration, (b) MIMS and HPLC data for 4-FBA formed during the biodegradation of 4-FCA.

4-FCA concentration decreased, supporting the assignment that 4-FAP was present in the biodegradation medium and was detected by MIMS. This observation was confirmed using HPLC (Figure 3.7) by comparing the retention time of the peak at 6.6 min with a standard.

The concentration of 4-FBA formed as a result of the biodegradation of 4-FCA was quantified by both MIMS and HPLC (Figure 3.8 b). The 4-FBA concentration increased to a maximum of 28.5 mg L^{-1} after 48 hours, after which the concentration decreased and by 96 hours no 4-FBA was detected. There is good agreement between the MIMS and HPLC profiles for 4-FBA. No intermediates could be identified from the breakdown of 4-FBA, but a peak due to an unidentified product with a higher polarity than 4-FBA (retention time 3.9 minutes) was observed by HPLC (Figures 3.7 b and 3.7 c), which increased in intensity during the biodegradation process. This peak also appeared in the HPLC chromatograms for the 4-FBA biodegradation studies.

Liquid chromatography/mass spectrometry was used to identify the peaks observed in the HPLC chromatograms, including those that were not clearly characterised from the MIMS analysis. The presence of 4-FBA and 4-FAP was confirmed by LC/MS as intermediates in the biodegradation of 4-FCA. Other intermediates were also observed by LC/MS including an epoxide derived from the 4-FCA with a retention time of 4.19 min. This kind of intermediate can be produced directly by bacteria containing a monoxidase enzyme. A mass isomer of 4-FBA with retention time of 5.23 min was also observed, but this product had a different retention time to those for authentic samples of the 2-FBA and 3-FBA

isomers analysed by HPLC. The proposed pathway for the biodegradation from 4-FCA to 4-FBA based on the MIMS, HPLC and LC/MS data is shown in Scheme 3.1.



Scheme 3.1: Proposed pathway for 4-FCA biodegradation.

Ion chromatographic analysis was also carried out during the biodegradation experiments on 4-FCA, which showed that fluoride ion concentration in the active flask increased as a function of the time, but no increase was observed in the controls. Plotting the concentrations of fluoride ion (as 4-FCA) on the same graph as the MIMS and HPLC data for 4-FCA (Fig. 3.8 a), shows that between 40 and 80 hours after addition of the activated sludge there is less 4-FCA than would be expected from the fluoride ion concentration. This is due to the initial transformation of 4-FCA into 4-FBA, to produce fluoride ion and other metabolites (Figure 3.4).

3.2.4. Conclusions

A IMP-MIMS technique incorporating a novel membrane drying stage that enhances mass spectrometric performance has been used to monitor the biodegradation of 4-FBA and 4-FCA. Intermediates have been identified in the biodegradation of 4-FCA and confirmed by HPLC and LC/MS.

MIMS shows good sensitivity as well as discrimination against the sample medium and the concentrations determined by MIMS compare well with data obtained using HPLC and IC. The selectivity of the membrane discriminates against the more polar degradation products identified by HPLC. These preliminary data suggest that MIMS is a good alternative to chromatographic methods for monitoring semi-volatile compounds during biodegradation studies.

For the HPLC and IC experiments, it was necessary to remove aliquots of solution from the batch fermentation flask prior to analysis. However, in the case of MIMS, it is possible to introduce the sample directly into the interface without pre-treatment, other than in-line filtration to exclude the bacterial sludge from the membrane interface. Returning the sample to the fermentation vessel after passage through the interface would reduce sample consumption, as only a small percentage of the analyte is absorbed in the membrane. This is an initial step towards being able to apply this type of IMP-MIMS experiment to the monitoring of biodegradation reactions on-line.

3.3. On-line biodegradation monitoring of nitrogen containing compounds in a simulated wastewater treatment plant by MIMS

3.3.1. Introduction

The at-line monitoring of 4-FBA and 4-FCA by IMP-MIMS, demonstrated the application of MIMS to biodegradation studies. However, an on-line monitoring system had to be developed. In this section the development of an on-line IMP-MIMS method for monitoring volatile and semi-volatile compounds simultaneously in a continuous stirred tank bioreactor (CSTB) containing activated sludge collected from a municipal wastewater treatment plant (WWTP) is described. A mixture of four industrially relevant nitrogen containing compounds was selected for on-line monitoring: 2-chloro-5-trifluoromethylaniline (CFA), an aniline derivative was chosen, as anilines are commonly used in the manufacture of pharmaceuticals, pesticides and dyes [25,26]. N-methylpyrrolidinone (NMP) is an increasingly common solvent often used to replace chlorinated solvents [27]. Pyridine derivatives, such as 3-bromopyridine (3BP) are also used as solvents or starting materials for pharmaceutical and fine chemicals synthesis [28]. The mixture was completed with the selection of tetramethylethylenediamine (TMEDA), a semi-volatile linear amine. The individual compounds fed to the CSTB were measured on-line by MIMS while the metabolites were identified with a combination of MIMS, gas chromatography/mass spectrometry (GC/MS) and liquid chromatography/mass spectrometry (LC/MS).

The biodegradability of pyridine derivatives has been studied extensively and been found to follow the order: pyridinecarboxylic acids > pyridine = monohydroxypyridines > methylpyridines > aminopyridines =chloropyridines [29-35]. The biodegradation of 3BP has not been reported to date, but in general the strength of resistance to enzymatic cleavage of carbon-halogen bond is observed to increase with the electronegativity of the substituents [14], therefore it should be easier to biodegrade a brominated pyridine than a chlorinated pyridine. Biodegradation studies on selected anilines not including CFA have been reported previously [36,37]. Bachofer *et al.* demonstrated that aniline is converted to catechol by a dioxygenase reaction in *Nocardia* sp. [36], while *Pseudomonas* AK20 biodegrade aniline and methylaninlines [37]. Halogenated aromatic compounds have been previously reported as biodegradable, although the presence of a trifluoromethyl and a chloride group can increase the biodegradation resistance of the molecule [14,38]. NMP has been reported to biodegrade to 4-(methylamino)butanoic acid [27]. Linear amines with similar chemical properties as TMEDA (trimethylamine, dimethylamine and methylamine) have been reported to show high resistance to biodegradation [39].

3.3.2. Experimental

3.3.2.1. Preparation of feedstock

All chemicals were obtained from Sigma Aldrich (Gillingham, UK) and used without further purification. Standard solutions (100 mg L^{-1} of each analyte) were prepared in a MSM (Table 2.8) [19] and synthetic sewage (1024 mg L^{-1}

nutrient broth, 105 mg L^{-1} urea, 75 mg L^{-1} yeast extract, 24 mg L^{-1} NaCl, 13.5 mg L^{-1} $CaCl_2.2H_2O$, 7.5 mg L^{-1} $MgSO_4.7H_2O$, 206 mg L^{-1} $NaH_2PO_4.2H_2O$ and 65 mg L^{-1} KH_2PO_4) was added to the solution. Activated sludge was obtained from a municipal WWTP (Thames Park, Roydon, UK) and washed to remove residual carbonaceous material present in the liquor. The amount of total suspended solids (TSS) in the activated sludge was determined to be 4 g L^{-1} by drying 10 mL of washed activated sludge at 104-106°C for 1 hour.

3.3.2.2. Continuous stirred tank bioreactor (CSTB)

The CSTB (Brighton Systems Ltd, Newhaven, UK) shown in Figures 3.9 and 3.10 was filled with 3 litres of activated sludge medium. A peristaltic pump (Watson-Marlow Bredel Pumps Ltd., UK) was used to supply the wastewater feedstock continuously to the CSTB at a flow of 125 mL h^{-1} corresponding to a hydraulic retention time (HTR) of 24 hours. The volume of wastewater in the CSTB was kept constant throughout the experiment.

The CSTB was held at 30°C for optimal activity of the activated sludge and was stirred constantly at 130 rpm. Air was supplied to the CSTB, and dissolved oxygen was monitored continuously in order to ensure aerobic conditions in the CTSB. The average value of oxygen was 6.1 mg L^{-1}. Activated sludge leaving the system was filtered, washed and fed back into the bioreactor. The pH in the CSTB was kept constant at 7.00 for the duration of the experiment. The pH was regulated automatically by adding NaOH or H_3PO_4.

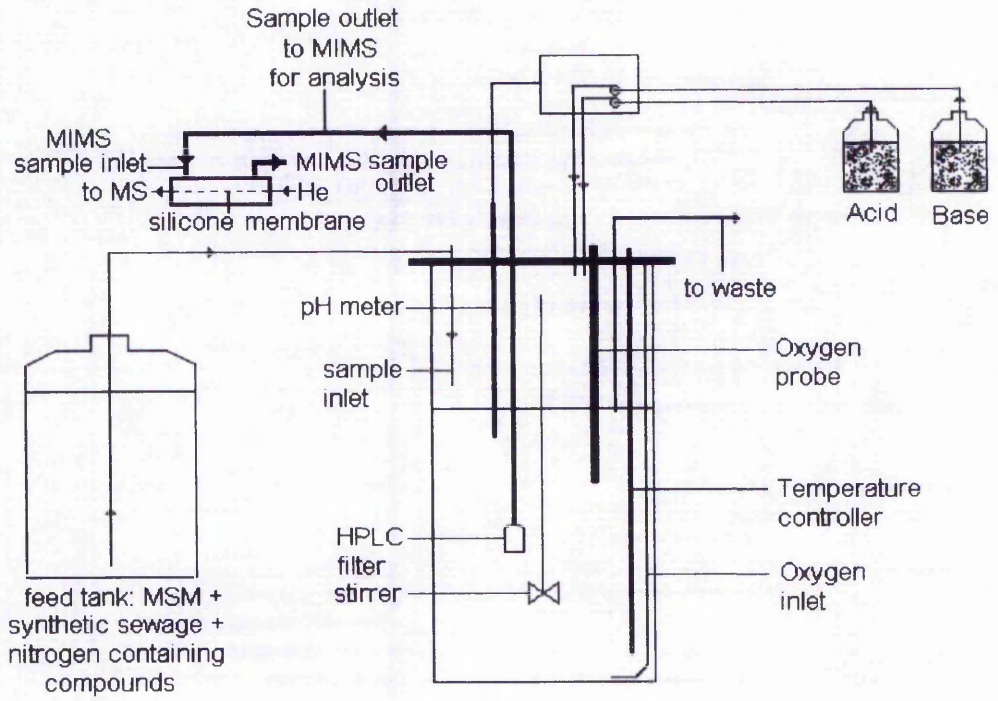


Figure 3.9: Schematic diagram of the CSTB set-up.

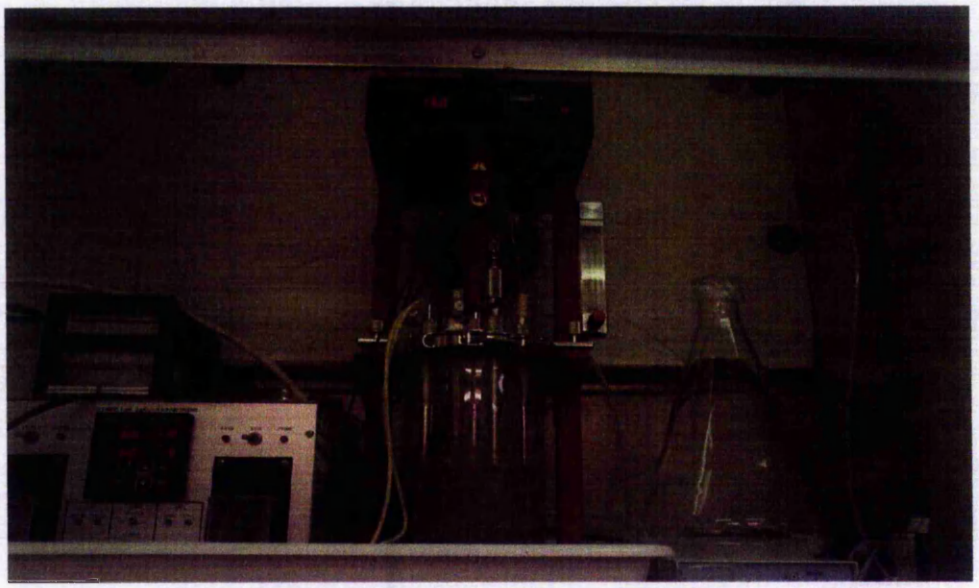


Figure 3.10: Picture of the CSTB set-up

Samples of the aqueous medium were withdrawn from the CSTB and transferred to the MIMS interface via a 1/16" stainless steel line. An in-line HPLC solvent reservoir filter was used for on-line sample filtration. This was required in order to remove solid particles, which could cause blockage of the MIMS interface.

3.3.2.3. Analytical methods

a) MIMS

MIMS analysis was carried out using a Hewlett Packard HP6890/HP5973 GC/MS configuration (Agilent Technologies, Palo Alto, CA, USA). The interface containing a silicone hollow-fibre membrane (0.635 mm o.d. x 0.305 mm i.d., Dow Corning Silastic, Sanitech, USA) was constructed in-house and based on the interface described in Chapter 2. The interface was located inside the GC oven in place of the GC capillary column (see Figure 2.2) [22]. Aqueous samples were pumped through the interface using a peristaltic pump (Cole-Parmer Instrument Co. Vernon Hills, IL, USA) for 5 min at a rate of 3 mL min^{-1}, with the oven temperature set to 40°C. The membrane was dried for 5 minutes by flushing nitrogen through the interface at a flow of 50 mL min^{-1}. The oven temperature was then raised to 200°C at 45°C min^{-1} and immediately cooled to 40°C again (Figure 3.11). A second heating cycle was performed between samples to eliminate possible memory effects in the membrane. The mass spectrometer was set to acquire electron ionisation (EI) full scan data between *m/z* 40 and 200. The MIMS device was connected on-line with the CSTB, so that sampling for analysis could be carried out as and when required by pumping

sample into the MIMS interface. The nitrogen containing analytes were quantified using the peak area for the SVOCs (NMP and TMEDA) and the area of the infusion response for the VOCs (3BP and CFA) compared to the responses for an external standard containing 100 mg L^{-1} of each analyte in MSM solution with synthetic sewage.

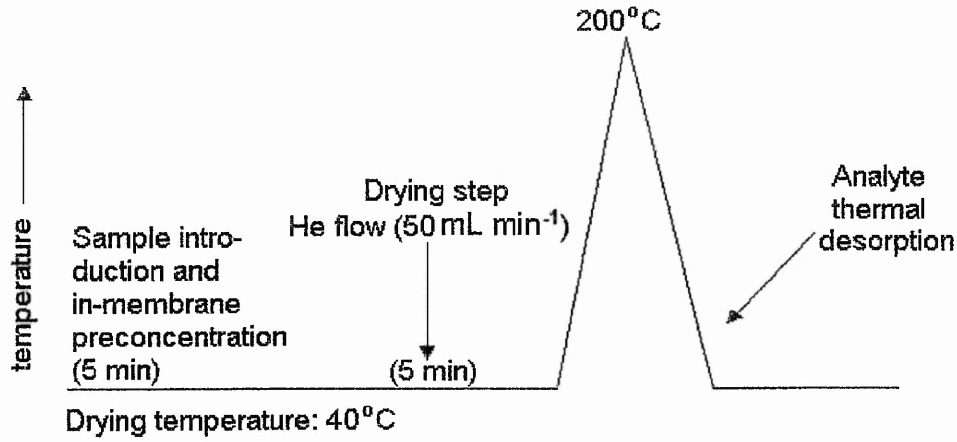


Figure 3.11: Oven temperature program for IMP-MIMS analysis.

b) LC/MS

LC/MS was carried out using a Waters Alliance 2790 chromatograph (Waters, Watford, UK), fitted with a Hypersil BDS C_8 packing column (4.6 mm x 150 mm; 5 μm particle size; Astmoor Runcorn, UK), linked to a Micromass Platform LC single quadrupole (Micromass, Manchester, UK). The injection volume was set to 25 μL, the eluent flow rate to 1 mL min^{-1} and the column oven temperature to 40°C. Analysis was carried out using positive or negative atmospheric pressure chemical ionisation (APCI) under the conditions described below. The mass

spectrometer was set to acquire full scan data between *m/z* 50 and 250 at 1 scan/second.

Positive ionisation:

The eluent was 0.05% trifluoroacetic acid (TFA) in water (mobile phase A) and 0.05% TFA in acetonitrile (mobile phase B). The gradient was set as follows: at time t = 0.00 min, A = 100%; t = 8.00 min, A = 5%; t = 10.00 min, A = 5%; t = 10.10 min, A = 100%; t = 12.00 min; A = 100%. The mass spectrometer was operated using a corona discharge voltage of 3.20 kV. The cone voltage was set to 10 V. The source and probe temperatures were set to 150 and 400°C respectively. The nitrogen gas flow was 259.8 L h^{-1}.

Negative ionisation:

The eluent was water (mobile phase A) and acetonitrile (mobile phase B). The gradient was set as follows: at time t = 0.00 min, A = 100%; t = 8.00 min, A= 5%; t = 10.00 min, A= 5%; t = 10.10 min, A = 100%; t = 12.00 min; A = 100%. The mass spectrometer was operated using a corona discharge voltage of 3.00 kV. The cone voltage was set to 10 V. The source and probe temperatures were set to 150 and 400°C respectively. The nitrogen gas flow was 256.2 L h^{-1}.

c) Chemical oxygen demand (COD)

COD analysis was carried out using a Merck Vega 400 spectrophotometer (Merck, UK), and commercial COD vials (containing potassium dichromate, mercure sulphate and sulphuric acid) (Merck, UK). COD was measured in samples taken from the CSTB and the feed tank.

3.3.3. Results and discussion

3.3.3.1. On-line monitoring by MIMS

MIMS analysis was carried out using a silicone hollow-fibre membrane interface similar to that reported in Chapter 2 (Figure 2.2) [22]. Samples were analysed on-line from the CSTB by pumping the reactor medium through the MIMS interface. The only pre-treatment required for the sample was on-line filtration. MSM was added to the system to provide buffering and trace elements, while synthetic sewage was added in order to provide a source of carbon to the activated sludge during the acclimatising period to the nitrogen containing compounds. Previous studies have shown that the pH is a critical parameter when monitoring acid or basic compounds by MIMS, as ion dissociation does not favour transport across the non-polar silicone membrane (see section 2.6).

Volatile nitrogen containing compounds (CFA and 3BP) were quantified by continuous infusion, as they pervaporate across the membrane at 40°C. Semi-volatile nitrogen containing compounds (NMP and TMEDA) were quantified

using an IMP-MIMS procedure employing the temperature profile shown in Figure 3.11. This approach is required because of the poor MIMS response for these semi-volatile compounds at temperatures lower than 100°C (the boiling point of water). In IMP-MIMS, analytes were accumulated in the membrane at 40°C as the aqueous sample was pumped through the interface. The membrane was dried following the pre-concentration stage by passing nitrogen over the membrane, as suppression of the analyte response can be observed when the membrane is heated immediately after removal of the water from the interface. The use of an inert gas also helped to avoid membrane oxidation at high temperatures, extending the lifetime of the membrane. The analytes were released from the membrane by rapid heating to 200°C (45°C min^{-1}) and transferred to the MS ion source.

Single ion responses extracted from the full scan EI data (*m/z* 58 for TMEDA, *m/z* 98 for NMP, *m/z* 157 for 3BP and *m/z* 195 for CFA) are shown in Figure 3.12. CFA and 3BP infuse through the membrane throughout the time that the sample is introduced into the interface (Figure 3.12b). The peak appearing after 10 minutes is due to the heating of the membrane during the thermal desorption step (Figure 3.11), which results in the release of residual CFA and 3BP from the membrane. In contrast, the responses for NMP and TMEDA were only significant during the thermal desorption step. At this time the peak observed for each component showed a width at half height of approximately one minute.

The MIMS responses for all four compounds were calibrated using standard solutions in the range 5-100 mg L^{-1} in the presence of MSM solution and

synthetic sewage. The response for CFA (*m/z* 195) and 3BP (*m/z* 157) was observed throughout the sample infusion and drying stages and both compounds were quantified using the integrated area of the infusion response (window time: 0-12 minutes) (Figure 3.12b). NMP (*m/z* 98) and TMEDA (*m/z* 58) were quantified using the integrated area of the IMP-MIMS desorption peak (Figure 3.12a). The analytes responses showed good linearity between 5 and 100 mg L^{-1}, with r^2 values of 0.9998 (3BP), 0.9954 (CFA), 0.9871 (TMEDA) and 0.9973 (NMP). The response reproducibility was determined for solutions containing 100 mg dm^{-3} of each analyte. The %RSDs (n = 6) were 6.0% (3BP), 6.8% (CFA), 7.6% (TMEDA) and 5.2% (NMP). Limits of detection were 1.9 mg L^{-1} for NMP, 1.3 mg L^{-1} TMEDA, 120 μg L^{-1} for CFA and 150 μg L^{-1} 3BP. It has been reported previously [27] that longer drying times increase the limits of detection of SVOCs, although in these studies the limits of detection obtained for TMEDA and NMP were within the acceptable range and the analysis time was shortened to 5 minutes, giving a total time of 16 minutes for the analysis.

The concentrations of the four analytes (CFA, 3BP, NMP and TMEDA) were monitored in the feed tank by MIMS during the experiment and the variation in concentration with time is shown in Figure 3.13. The concentration of CFA, TMEDA and 3BP in the feed tank remained constant throughout the study. However, cross-contamination in the feed tank was observed, causing a gradual decrease in NMP (from an initial concentration of 100 mg dm^{-3}) until no NMP could be detected in the feed. After 73 hours, the feed tank was replaced, but cross-contamination occurred again, as the feed tank is directly linked to the

CSTB and no sterilisation measures could be put in place in the laboratory set-up.

The concentrations of CFA, 3BP, NMP and TMEDA in the CSTB obtained by MIMS at different times during the on-line monitoring experiment are shown in Figure 3.14. At time 0 hours the CSTB did not contain any of the nitrogen containing compounds. The wastewater was fed from the feed tank (containing 100 mg dm^{-3} of each analyte) into the CSTB at a flow of 125 mL h^{-1} (HRT = 24 hours), so that steady state was achieved after 96 hours (i.e. 4 reactor volumes). The concentration of all analytes initially increased as expected. NMP concentration rose to 22 mg L^{-1} and then started to decrease until it was below limit of detection (1.9 mg L^{-1}) indicating total removal of NMP at steady state although NMP was also being degraded in the feed tank due to cross-contamination. The concentration of CFA increased to 12 mg L^{-1} and then remained steady at around this concentration for the rest of the experiment corresponding to a CFA removal close to 90%. A similar trend was observed for 3BP, which showed a steady state removal of approximately 70%. TMEDA showed no significant removal after reaching steady state. The loss of compounds due to volatilisation was not quantified, although it may contribute to some of the loss of the nitrogen containing compounds from the CSTB tank. However the concentration of CFA, 3BP and TMEDA remained constant throughout the experiments in the feed tank (see Figure 3.14), indicating that volatilisation of these compounds was not a significant cause of removal for these compounds.

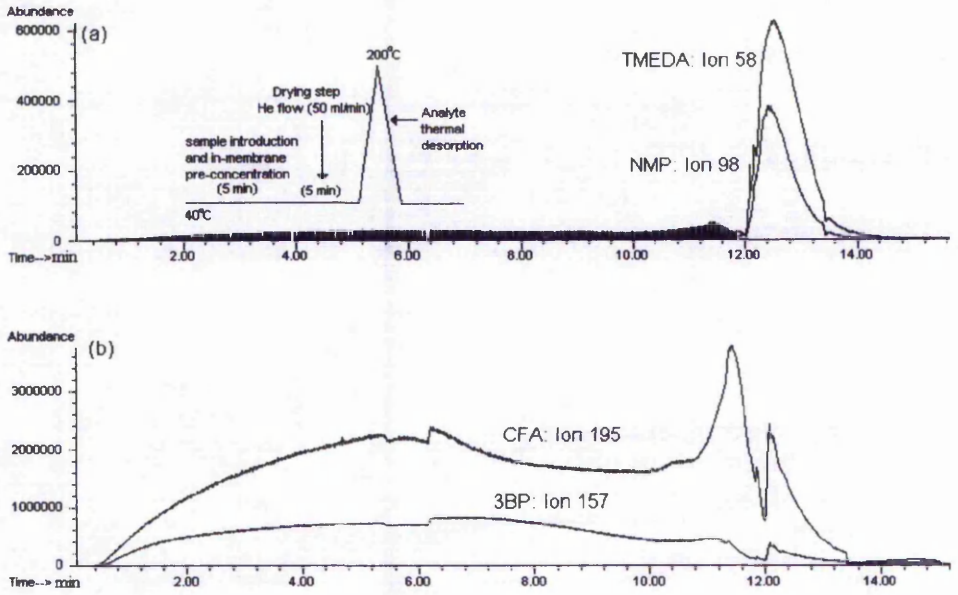


Figure 3.12: Single ion responses for 100 mg l^{-1} of (a) NMP (*m/z* 98) and TMEDA (*m/z* 58) by IMP-MIMS; (b) 3BP (*m/z* 157) and CFA (*m/z* 195) by continuous infusion MIMS.

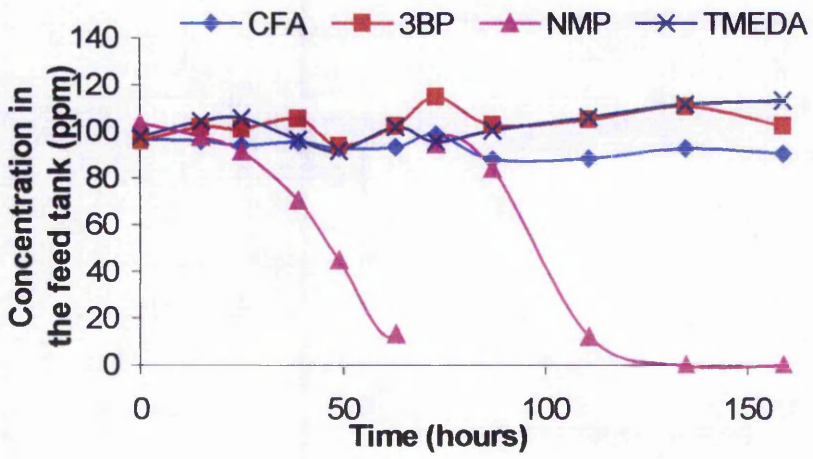


Figure 3.13: Variation of the concentration of NMP, TMEDA, 3BP and CFA with time in the feed tank, monitored on-line by MIMS.

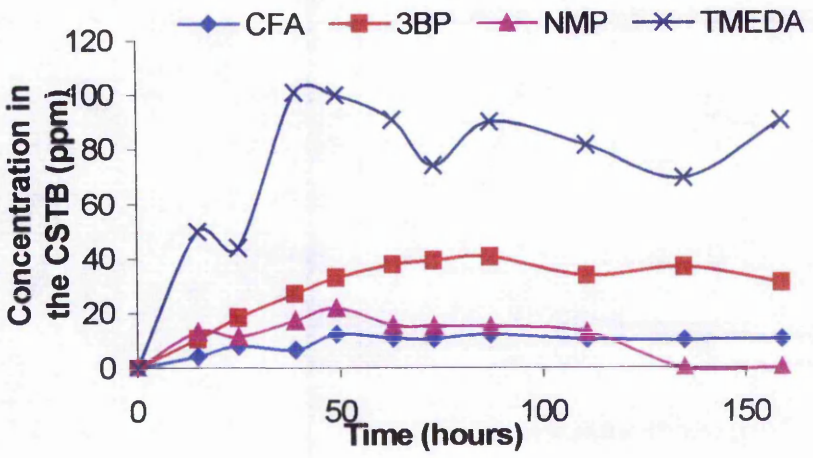


Figure 3.14: On-line monitoring of NMP, TMEDA, 3BP and CFA in the CSTB by MIMS.

3.3.3.2. COD

COD data support the results obtained by MIMS (Figure 3.15). COD shows the amount of chemical oxygen needed to biodegrade the compounds found in solution. If the feed tank composition remains constant, the COD value should not change throughout the experiment. However, there was a gradual decrease in the COD value in the feed tank, corresponding to the removal of NMP. At time 73 hours, when the feed was replaced by a fresh solution, the value of COD increased to its original value before decreasing as cross-contamination started to reduce the NMP concentration once again. In the CSTB, the COD value increased in the first few hours, before reaching steady state. At time 0 hours in the CSTB there was only MSM and synthetic sewage, responsible for the initial COD value of 106 mg dm^{-3}. As the concentration of analytes increased the value

of COD increases too, until it reached a steady state. MIMS and COD results agree well, although the COD data do not provide information on the removal rate of the individual nitrogen compounds that is obtained using the on-line MIMS approach.

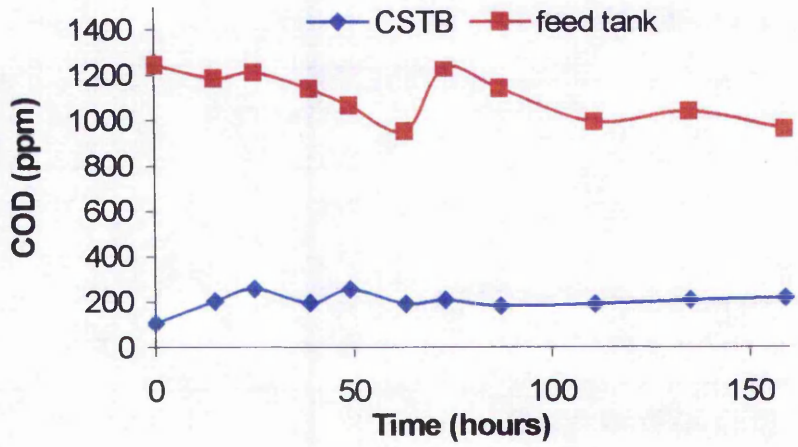


Figure 3.15: Chemical oxygen demand (COD) analysis of the feed tank and the CSTB.

3.3.3.3. Metabolite identification

MIMS, GC/MS, and LC/MS were used for the identification of biodegradation metabolites. No compounds relating to NMP or TMEDA were observed. However, the presence of 3-bromo-hydroxy-pyridine (3BHP), thought to be due to the biodegradation of 3BP, was identified by MIMS and confirmed by GC/MS and LC/MS (positive ionisation). Figure 3.16a shows the EI mass spectrum of the

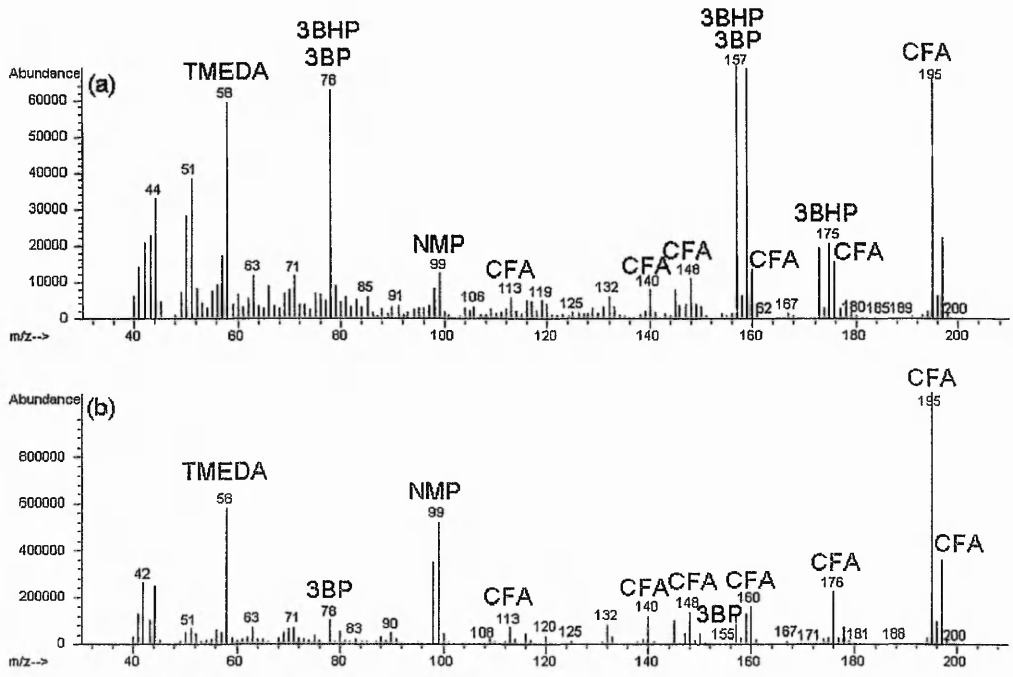
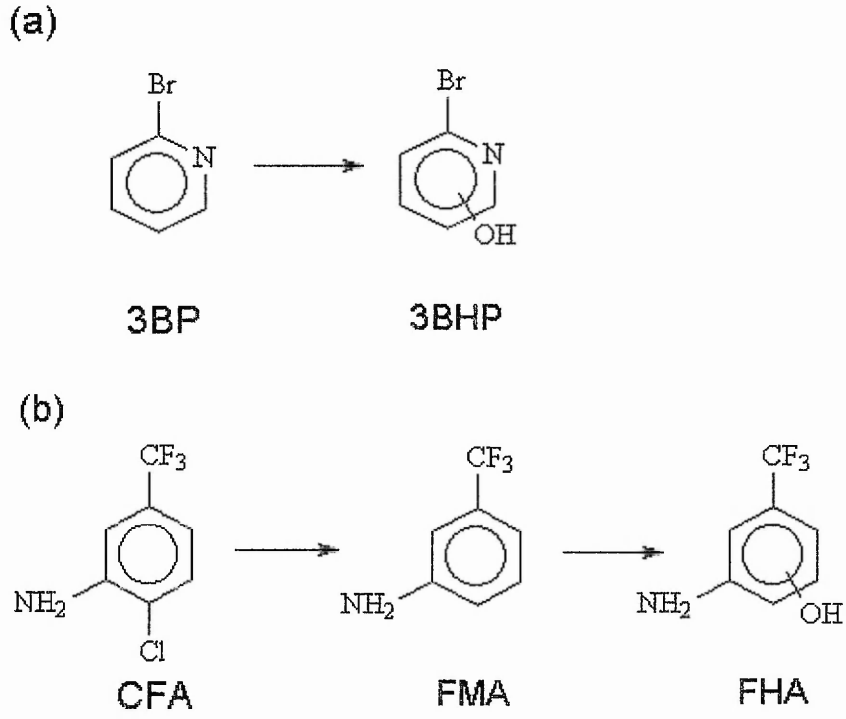


Figure 3.16: MIMS mass spectra at time 25 hours of (a) a bioreactor sample, containing the nitrogen containing feedstock and 3BHP and (b) the feed tank, containing only the nitrogen containing feedstock.

CSTB medium obtained by MIMS at time 25 hours obtained during thermal desorption of the metabolite pre-concentrated in the membrane during sampling. Due to the increasing polarity of 3BHP compared to 3BP, it has a typical SVOCs MIMS behaviour. Figure 3.16b shows a mass spectrum of the feed tank solution at time 25 hours during thermal desorption. Figure 3.16a contains two peaks at *m/z* 173 and *m/z* 175 that do not appear in Figure 3.16b and are assigned to the bromine isotopes of the molecular ion of 3BHP. The metabolite identity was confirmed by mass spectral data obtained by GC/MS (retention time 6.9 min) and by LC/MS (retention time 3.9 min). The EI spectrum of this metabolite showed

ions at *m/z* 173/175 ($M^{+\cdot}$), 157/159 ($[M\text{-}OH]^+$) and 78 ($[M\text{-}OH\text{-}Br]^+$), all of which are observed in Figure 3.16a, as well as some lower mass ions common to bromopyridine.



Scheme 3.2: Proposed pathways for (a) CFA and (b) 3BP initial steps of biodegradation.

Two metabolites were observed from CFA biodegradation, tentatively identified as 3-trifluoromethylaniline and 3-trifluoromethyl-hydroxy-aniline. Both metabolites were identified by LC/MS using negative ionisation and were detected by MIMS. 2-trifluormethylaniline (FMA) showed a retention time of 1.8 min by negative ion APCI and its mass spectra showed its main ion at *m/z* 160 ($[M\text{-}H]^-$). 3-trifluoromethyl-hydroxy-aniline (FHA) showed an HPLC retention

time of 2.3 min and the negative ion APCI mass spectra showed ions at *m/z* 176 ($[M-H]^-$), and 160 ($[M-OH]^-$). Scheme 3.2 shows suggested initial steps for the biodegradation pathways for 3BP and CFA.

3.3.4. Conclusions

An on-line MIMS system has been developed and used to monitor the biodegradation of a mixture of nitrogen containing compounds in a CSTB used to simulate a biological WWTP. On-line filtration was the only sample pre-treatment required. A mixture of volatile (3BP and CFA) and semi-volatile (NMP and TMEDA) nitrogen containing compounds was successfully monitored and quantified simultaneously on-line. Volatile compounds were determined by continuous infusion, while IMP-MIMS was employed for the semi-volatile compounds.

MIMS showed a high degree of discrimination against the sample medium, making it an ideal technique for on-line monitoring of aqueous bioreactor medium. The technique was validated for biodegradation monitoring, with results comparable to COD data. The response of the four analytes by MIMS was linear and showed good sensitivity and reproducibility.

Total removal of NMP was observed once steady state conditions were reached, whereas removal for 3BP was ~ 70% and for CFA was ~ 90%. There was no evidence of significant TMEDA removal. A metabolite of 3BP was detected by MIMS, GC/MS and LC/MS, while two metabolites of CFA were detected by

LC/MS. The identification of metabolites from CFA and 3BP demonstrates biodegradation of both compounds is taking place, although loss due to volatilisation may also contribute to the removal of the nitrogen containing compounds.

This work reports the use of a novel IMP-MIMS system for the biodegradation monitoring of 4-FBA and 4-FCA. The system was monitored at-line, and was validated comparing the results with HPLC and IC. The MIMS analysis showed good linearity, good reproducibility and compared well with HPLC and IC, an on-line system was also developed for the biodegradation monitoring of nitrogen containing compounds. A mixture of 2 SVOCs (NMP and TMEDA) and 2 VOCs (3BP and CFA) were analysed simultaneously. The mixture was monitored in a bioreactor that was linked on-line to the MIMS interface. The only pre-treatment step required was to introduce an on-line filtration system.

3.5. References

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CHAPTER 4

Membrane inlet mass spectrometry applied to the real-time monitoring of low molecular weight alcohols in chloroform.

4.1. Introduction

The use of non-polar membranes in hollow fibre or sheet form to exclude polar solvents and allow the pervaporation of organic analytes is termed normal phase MIMS [1]. Previous work has employed polydimethylsilicone membranes to extract volatile organic compounds (VOCs) from aqueous systems [1-4] and has been discussed in chapters 2 and 3. This separation relies on the higher pervaporation of the VOCs and the hydrophobicity of the silicone membrane to exclude polar analytes. Conversely, the use of a membrane introduction technique that allows the passage of more polar materials, termed reversed-phase MIMS (RP-MIMS), has been less well studied [5-8].

Microporous polypropylene [5] was used by Lauritsen *et al.* for the analysis of organic compounds such as methanol, ethanol, dimethylsulfoxide and benzene in hexane. Kasthurikrishnan *et al.* [6] used zeolite filled polydimethylsiloxane microporous membranes for the analysis of benzene, carbon tetrachloride, chlorobenzene, toluene and *trans*-1,2-dichloroethane in methanol and hexane. However, microporous membranes lack the selectivity of semi-permeable membranes, because transport is determined by laminar flow through the pores rather than diffusion across the membrane. The high sample flow through microporous membranes also usually requires that analyses be carried out using solvent chemical ionisation-mass spectrometry (CI-MS).

A polyethylene terephthalate membrane was used by Bohatka *et al.* [7] to determine selectively traces of water in butanol, hexanol and octanol, and Bauer *et al.* [8] employed a polyvinyl alcohol (PVA) membrane in the RP-MIMS analysis of VOCs including acetone, methylethylketone, methanol and

tetrahydrofuran in hexane. Although the PVA membrane discriminated against the solvent, hexane was used as the reagent gas for CI analysis. Maden *et al.* [9] investigated the use of a polyimide membrane for the RP-MIMS analysis of water, ethanol, chloroform, acetone, acetic acid and ethyl acetate in hexane. The polyimide was permeable to polar compounds, including water or ethanol, but showed limited discrimination against the permeation of non-polar compounds such as chloroform and ethyl acetate. The flux of hexane permeating the membrane was sufficiently high that EI spectra were observed to have CI characteristics.

Nafion® is an ionic polymer with a tetrafluoroethylene backbone and perfluorinated ether side chains terminating in sulfonic acid sites (see Figure 4.1) [10]. The hydrophilic character of the sulfonic acid groups gives a very high affinity for water and other polar compounds such as low molecular weight alcohols, [11-13] which can absorb into a Nafion® membrane, whilst non-polar compounds have a much lower affinity. Properties and selectivity found in Nafion® are based on its microphase separated morphology. Figure 4.2 shows a phase separated morphology of discrete hydrophobic and hydrophilic regions, the hydrophobic region being composed of the polymer fluorocarbon and the hydrophilic region of the sulfonic groups and their counter ions. Once absorbed into the wall of the Nafion® membrane, polar analytes diffuse across the membrane via association with neighbouring sulphonic acid groups. Nafion® has been widely used for drying vapor streams as a consequence of this hydrophilicity [14-17].

In this chapter, the use of a RP-MIMS configuration incorporating a semi-permeable Nafion® membrane for the selective permeation of the low molecular

weight alcohols methanol and ethanol in a chloroform matrix is reported, and results are compared to the ones obtained by normal-phase MIMS using a silicone membrane.

$$\left[-\left(CF_2-CF_2\right)_n-CF_2-CF\right]_x$$
$$\Big|$$
$$\left(O-CF_2-CF\right)_m-O-CF_2-CF_2-SO_3^- H^+$$
$$\Big|$$
$$CF_3$$

Figure 4.1: Nafion® structure

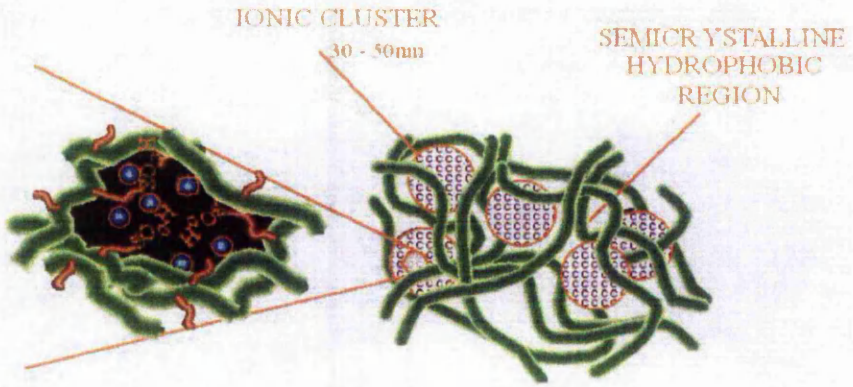

Figure 4.2: View of polar/nonpolar microphase separation in Nafion®

Membrane performance was characterised with respect to response time and the selectivity of the membrane. The RP-MIMS approach, using a portable residual gas analyser (RGA), has been evaluated for a process monitoring application involving distillation of an alcohol/chloroform mixture and referenced against GC based quantification. This particular solvent system was investigated due to an interest in evaluating an on-line analytical system capable of real time

determination of alcohols in chloroform. The concentrations of the alcohols represent quality criteria in a solvent recovery process (via distillation) of the halogenated solvent, making their temporal behaviour an important parameter for process monitoring.

4.2. Experimental

4.2.1. Normal-phase MIMS for analysis of chloroform/alcohol mixtures.

The normal phase MIMS interface used for the analysis of methanol and ethanol in chloroform has been described in section 2.3 and is shown in Figure 2.2 (see chapter 2). The interface was mounted inside the oven of a Hewlett Packard HP 5890 GC, which was coupled to a HP 5970 quadrupole analyser. The interface containing a silicone hollow-fibre membrane (0.635 mm o.d. x 0.305 mm i.d., Dow Corning Silastic, Sanitech, USA) was constructed in-house and located inside the GC oven in place of the capillary GC column [1].

For this analysis, sample pre-treatment was required. A standard solution containing ethanol (1% v/v) and methanol (5% v/v) was prepared in chloroform. 10 mL of the organic sample were mixed with 10 mL of water and the alcohols were extracted into the aqueous phase by liquid-liquid partitioning (manually shaking the mixture for 2 minutes), followed by partial degasification prior to analysis of the aqueous phase by MIMS (flowing nitrogen at 50 ml min^{-1} for 2 minutes). Aqueous samples were pumped through the interface using a peristaltic

pump (5 mL min^{-1}) with the oven temperature set to 40°C. An aliquot (40 μL) of the aqueous solution was introduced into the interface using a syringe.

4.2.2. Characterisation of Nafion® membrane performance for RP-MIMS

The RP-MIMS interface used for the characterisation of the Nafion® membrane (Figure 4.3 a) was based on the design of a normal-phase MIMS configuration previously reported [1]. The interface was mounted inside the oven of a Hewlett Packard HP 5890 GC, which was coupled to a HP 5970 quadrupole analyser. The interface consisted of a Nafion® membrane (0.014" i.d., Omnifit Ltd, UK) located inside a short section (50 mm) of 1/8" stainless steel tubing. The ends of the Nafion® membrane were fitted tightly onto two lengths of 0.25 mm i.d. fused silica tubing, one of which was connected to the GC injector and the other to the MS ion source. The whole assembly was made vacuum and water tight using stainless steel compression fittings (1/16" x 1/16" x 1/8"). The sample was introduced into the interface using a peristaltic pump such that it flowed over the outer surface of the Nafion® membrane. Pervaporating analytes were transferred to the mass spectrometer in a stream of helium and analysed using full scan mode in the mass range *m/z* 10-120 at a scan rate of 2.4 scan s^{-1}. A standard solution containing ethanol (1% v/v) and methanol (5% v/v) was prepared in chloroform. An aliquot (50 μL) of the standard solution was introduced into the interface using a syringe with the membrane maintained at temperature in the range 40 - 100°C.

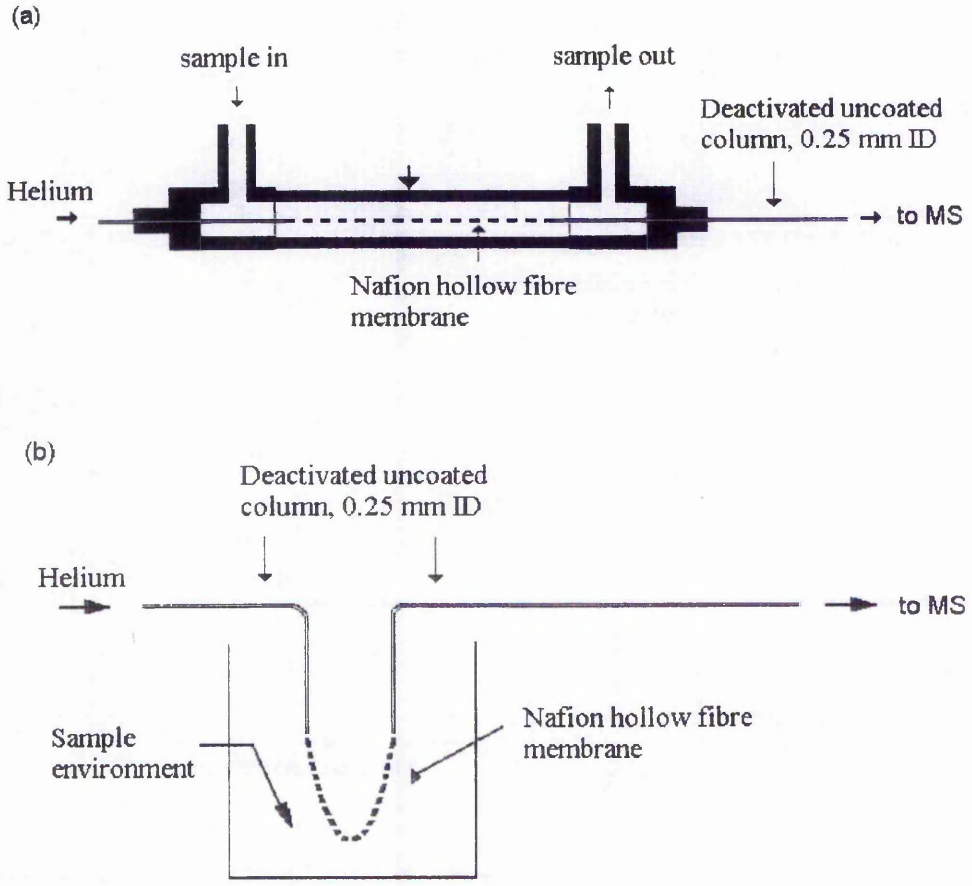

Figure 4.3: Schematic diagram of the RP-MIMS interfaces incorporating a Nafion® hollow fibre membrane employed for (a) system characterisation and (b) real-time process monitoring.

Once the system had been shown to be efficient towards the selective membrane transfer of methanol and ethanol compared to chloroform, a simplified membrane interface was used for on-line applications. (Figure 4.3 b). This interface consisted of the same Nafion® membrane (0.014'' i.d.) fitted tightly onto two lengths of 0.25 mm i.d. fused silica tubing, using wire to secure the membrane to the tubing, which were connected to the GC injector and MS ion source. The membrane was immersed in the sample for analysis. Mass spectra

were recorded in full scan mode over the mass range *m/z* 10-120 at a scan rate of 2.4 scan s^{-1}. Response time and linearity were investigated via a standard additions approach as follows: the membrane was introduced in 100 mL of a stirred solution of 4% methanol in chloroform and stirred. Every five minutes 0.5 mL of ethanol was added to the mixture and the *m/z* 29 and 45 responses were monitored with time.

4.2.3. Distillation of chloroform/alcohol mixtures.

The simplified MIMS interface configuration (Figure 4.3 b) was used for the on-line investigation of chloroform/alcohol mixtures during a distillation process using a QMS 300 quadrupole based RGA system (Stanford Research Systems (SRS), Stanford CA, USA). The RGA was operated under the following conditions: data were typically acquired in SIM mode using *m/z* settings appropriate for the analytes of interest (*m/z* = 45 for ethanol, *m/z* = 29 for methanol, *m/z* = 35 for chloroform). The differentially pumped sampling/transfer line was actively heated to 60-70°C to insure no condensation of solvent vapour. Distillations were run on a 0.5 L scale using a LabMax automated lab reactor system (Mettler Toledo Inc. Hightstown, NJ USA) under typical process conditions. Condensed distillate collected in a receiving flask was monitored in real time by MIMS. Samples for comparative GC analysis were collected from the same flask periodically.

4.2.4. Gas chromatographic analysis

Complimentary quantitative data for reference and comparison was obtained via analysis by GC/MS. A Hewlett Packard HP 6890 GC and HP 5973 quadrupole analyser were employed. The method used a Restek (Bellefonte, PA, USA) DB Stabilwax column (30 m x 0.25 mm i.d.). The oven temperature was programmed as follows: 40°C for 5 minutes followed by a ramp at 20°C min^{-1} to 200°C.

4.3. Results and discussion

The studies described here were pursued with four objectives. The first two were to perform a detailed laboratory based characterisation of the behaviour of the silicone and Nafion® membrane for the analysis of mixtures of low molecular weight alcohols in chloroform. The third objective was to compare the performance of Nafion® and silicone membranes for the analysis of methanol and ethanol in chloroform, while the fourth objective was to design and perform experiments to test the feasibility of a process application of the Nafion® membrane in a RP-MIMS application, involving a recovery process for chloroform by distillation in which methanol and ethanol concentrations represent important parameters.

4.3.1. Characterisation of a silicone membrane for the analysis of methanol and ethanol in chloroform using normal phase MIMS

Normal-phase MIMS was carried out using the interface described in Chapter 2.3 (Figure 2.2). Normal-phase MIMS allowed methanol and ethanol concentrations to be determined after liquid-liquid extraction, although the trace levels of chloroform present in the aqueous phase were preferentially transported across the silicone membrane. However, this normal phase procedure required a two-stage sample pre-treatment procedure prior to MIMS. This had to be carried out off-line and was therefore not readily amenable to on-line real time process monitoring applications.

An aqueous sample containing methanol and ethanol extracted from the organic sample was introduced to the mass spectrometer. The amount of chloroform dissolved in water was enough to create a too great source pressure. Therefore a purge extract using nitrogen was needed in order to analyse the aqueous samples by normal phase MIMS. By flushing the sample with nitrogen for two minutes, enough chloroform was removed from the sample so that there would not be excessive pressure in the source for the mass spectrometer to stop acquiring data.

Mass chromatograms using normal phase MIMS for methanol and ethanol in chloroform are shown in Figure 4.4. As it can be seen in Figure 4.4, at time 3.2 minutes, the maximum response for *m/z* 83 (chloroform) is obtained. There is some signal suppression for *m/z* 29 (methanol) and *m/z* 45 (ethanol) during this period of time, because the silicone membrane gets saturated with chloroform,

which diffuses across the membrane preferably to methanol and ethanol. It can also be seen from the single ion responses shown in Figure 4.4 that chloroform signal rises sharply, while it takes longer for methanol and ethanol to permeate the membrane, due to the higher affinity of silicone for non-polar compounds.

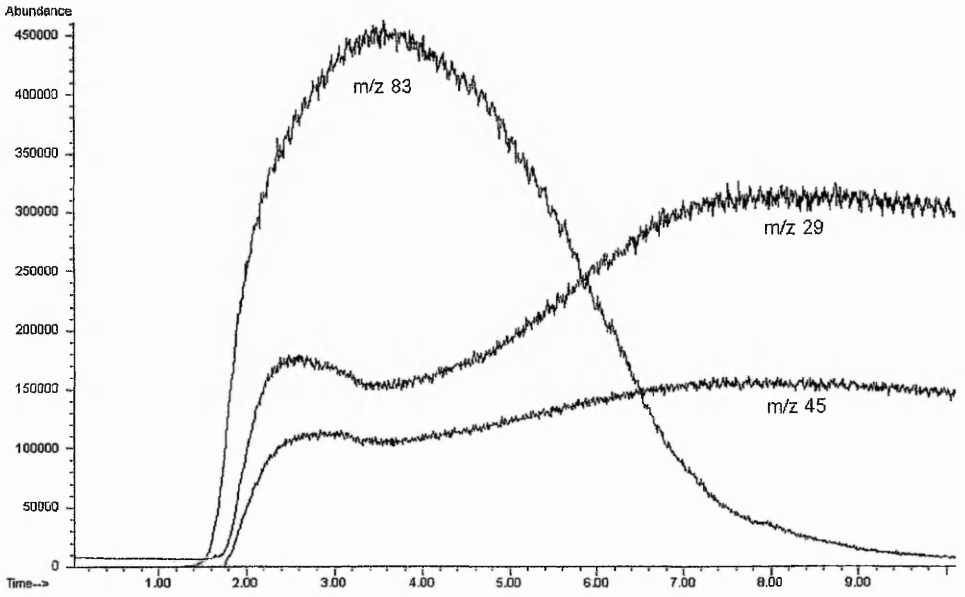

Figure 4.4: Single ion responses for the analysis of 5% v/v methanol (*m/z* 29) and 1% v/v ethanol (*m/z* 45) extracted with water from an organic sample using chloroform (*m/z* 83) as the solvent by normal-phase MIMS using a silicone membrane.

Limits of detection (S:N 3:1) were estimated to be 0.28 % (v/v) for methanol and 0.12% (v/v) for ethanol under the experimental conditions described in section 4.2.1. No quantification measurements were carried out by this technique, as the

method was not useful for on-line monitoring due to the liquid-liquid extraction and chloroform purge extract steps.

4.3.2. Characterisation of Nafion® membrane under RP-MIMS conditions.

The performance of the Nafion® hollow fibre membrane for RP-MIMS was investigated initially using the interface configuration shown in Figure 4.3 a. The interface is based on the design for normal phase MIMS described in chapter 2.3. [1] The mass chromatogram obtained following exposure of the Nafion® membrane to a pulsed sample injection of a mixture of chloroform, methanol (5 %) and ethanol (1 %) is shown in Figure 4.5. The responses for methanol and ethanol (*m/z* 29 and 45) rise rapidly following the introduction of the sample and begin to level off before a significant chloroform response (*m/z* 83) is detected, reflecting the selective permeation of the polar alcohols through the hydrophilic membrane. The higher diffusion coefficient for methanol leads to a faster initial rate of rise in the response curve [18-19]. The ability of the Nafion® membrane to discriminate against the non-polar chloroform solvent is apparent from the relative intensities of the chloroform and alcohol traces, which allows the membrane to be exposed directly to the mixture without the much higher concentration of the chloroform saturating the mass spectrometer.

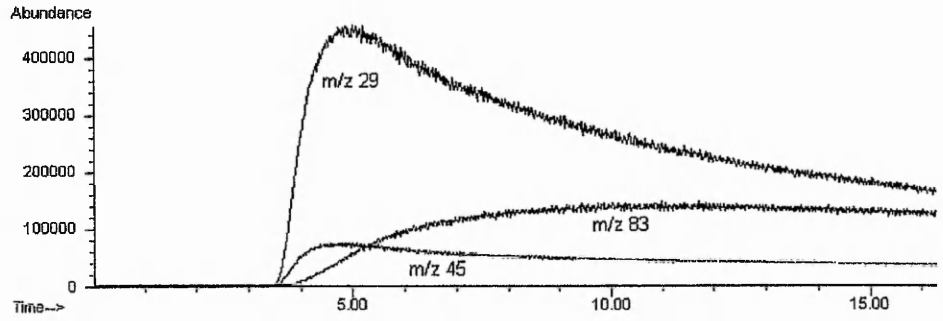

Figure 4.5: Mass chromatograms for the analysis of 5% v/v methanol (*m/z* 29) and 1% v/v ethanol (*m/z* 45) in chloroform (*m/z* 83) by RP-MIMS using a Nafion® membrane.

Ion intensities for *m/z* 29, 45 and 83 indicate that the Nafion® membrane discriminates in favour of methanol and ethanol by a factor of 67 and 55 respectively relative to chloroform, assuming equal analyte responses. This discrimination allowed EI spectra to be recorded, although weak ions corresponding to protonated molecules characteristic of RP-MIMS applications were observed in some cases. The performance of the Nafion® membrane was investigated over the temperature range 40–100°C and the optimum performance for the membrane was observed at 40°C. At higher temperatures, the alcohol and chloroform responses were both reduced and the highest temperature (100°C) led to membrane failure.

The linearity of the response for ethanol using the RP-MIMS method was determined over a working range of concentrations from 0.5-2.5% v/v. Figure 4.6 displays the data obtained during an RP-MIMS analysis where additions of 0.5 mL of ethanol were made to 100 mL of ethanol stabilised chloroform. The

starting concentration of ethanol in chloroform was determined to be 5.7 g L^{-1}. The ethanol response (*m/z* 45, $C_2H_5O^+$) increases with each addition, whilst the *m/z* 29 response associated with methanol falls as a result of the dilution of the sample. A good linear response ($r^2 = 0.9959$) was observed for ethanol over the range by normalising the mass spectrometry response for ethanol to the total ion current and correcting for volume changes.

The limits of detection by RP-MIMS were 0.34% (v/v) for methanol and 0.15% (v/v) for ethanol under the experimental conditions described in section 4.2.2.

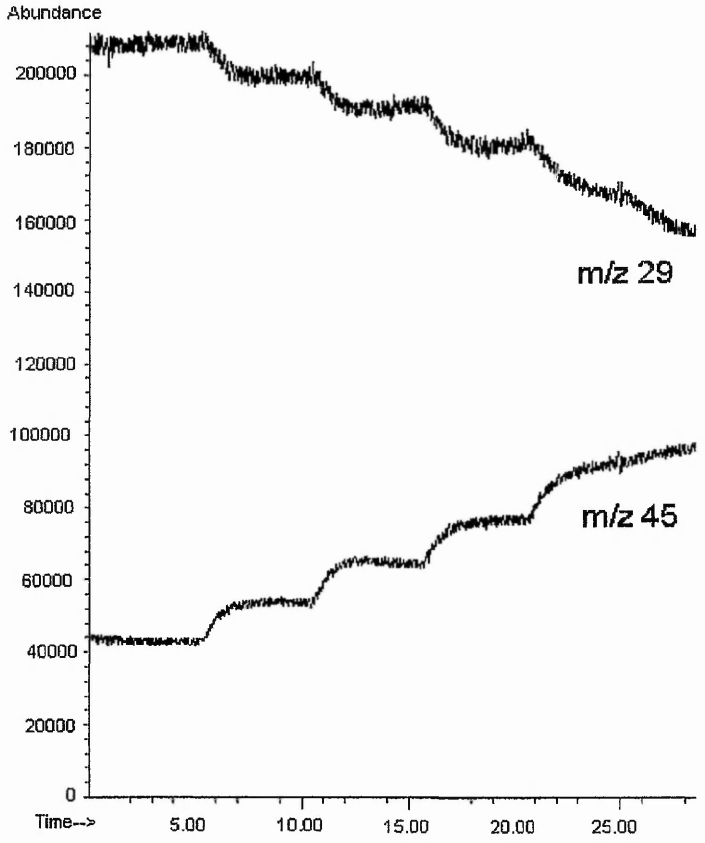

Figure 4.6: Mass chromatograms for the analysis of 5% v/v methanol (*m/z* 29) in chloroform on addition of 0.5 mL aliquots of ethanol (*m/z* 45).

4.3.3. Comparison between normal-phase and RP-MIMS

In normal phase MIMS (Figure 4.4), polar compounds are discriminated against and the chloroform signal rises sharply, while it takes longer for methanol and ethanol to permeate the membrane. This is in contrast to RP-MIMS (Figure 4.5), where non-polar compounds are discriminated against and the alcohols diffuse more rapidly through the membrane. Therefore for this application, RP-MIMS is a more suitable technique, as chloroform (the solvent) is discriminated favouring the transport across the membrane for methanol and ethanol (the polar compounds), which are the analytes that need to be monitored.

Normal-phase MIMS allowed methanol and ethanol concentrations to be determined after liquid-liquid extraction. However, this normal phase procedure required a two-stage sample pre-treatment procedure prior to MIMS. This had to be carried out off-line and was therefore not readily amenable to on-line real time process monitoring applications. When using a Nafion® membrane for RP-MIMS, the sample could be injected for analysis without any pre-treatment. Therefore the simplicity of the analysis makes the RP-MIMS suitable for on-line monitoring, as it can be seen in Figure 4.7.

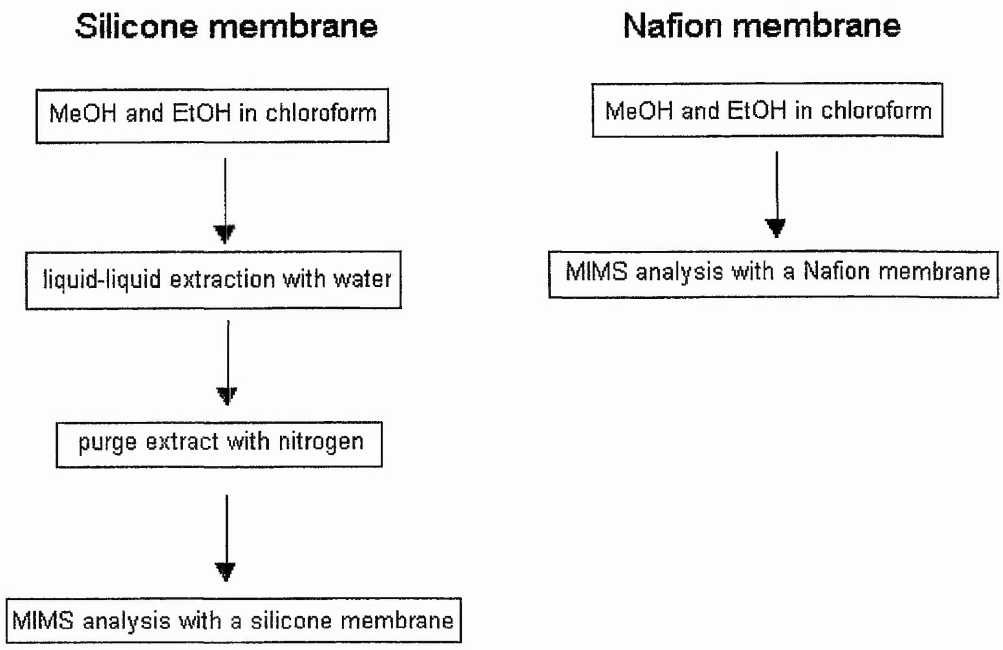

Figure 4.7: Method comparison between normal (silicone membrane) and reverse-phase (Nafion® membrane) MIMS.

4.3.4. Real-time monitoring of chloroform/alcohols distillation

The application of the RP-MIMS interface has been investigated for the real-time monitoring of methanol and ethanol concentrations in chloroform during a process distillation. This ternary system contains azeotropes that hamper the recovery of the halogenated solvent. As such, the alcohol concentrations (in particular ethanol) represent a critical quality parameter in this recovery process. [20] The RGA system was chosen as the instrument since it has realistic process use possibilities.

A distillation of a solution of 4% (v/v) methanol as a co-solvent in chloroform containing 0.75% (v/v) ethanol as a stabiliser was carried out and the condensed distillate was monitored by RP-MIMS using the RGA. Normalised ion ratios

29/35 (COH^+/Cl^+) and 45/35 ($C_2H_5O^+/Cl^+$), relative to chloroform, were monitored for methanol and ethanol respectively. These experiments were carried out in the laboratory at the 0.5 L scale. Sampling via RP-MIMS was carried out as described above. Data for monitoring the change in methanol and ethanol concentration are summarised in Figures 4.8 and 4.9.

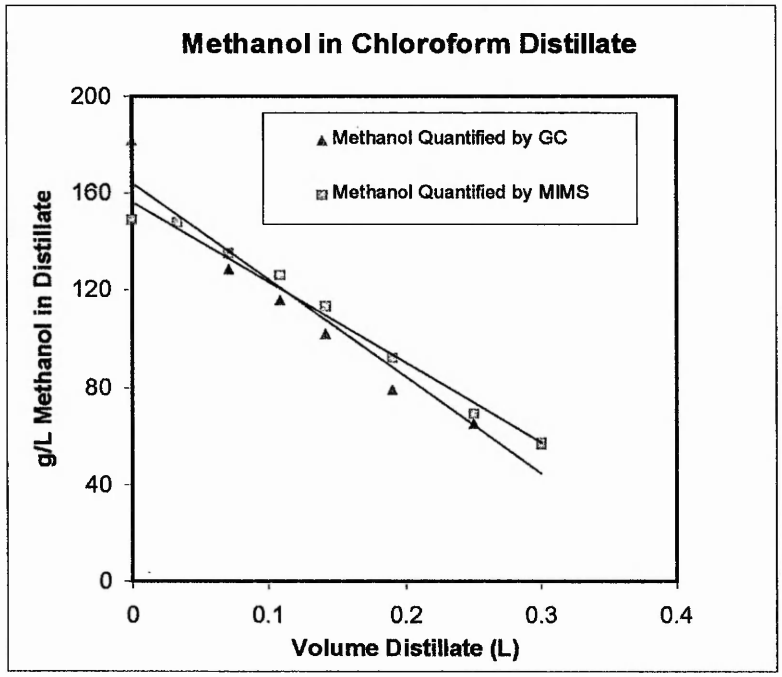

Figure 4.8: On-line monitoring of methanol in a distillation condensate by RP-MIMS and GC.

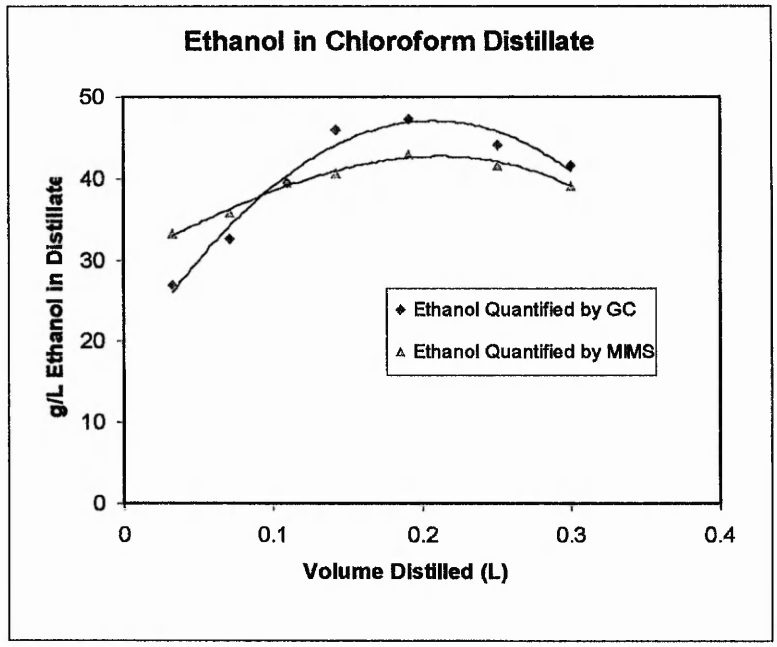

Figure 4.9: On-line monitoring of ethanol in a distillation condensate by RP-MIMS and GC.

The results obtained via mass spectrometry were referenced to data quantified by GC/MS. For referencing purposes the MIMS results were scaled by a single GC/MS quantification. In the data shown in Figure 4.8 the reference point for quantification is the point at 0.03 L of distillate where the data points for the two different techniques overlap. The results for the RP-MIMS and GC analysis show that the methanol concentration decreases during the evolution of the distillation process (Figure 4.8). Early fractions of the distillation are enriched with the more volatile alcohols that subsequently decrease in concentration in the collected distillate as less alcohol enriched fractions are collected. In practice this concentration could be monitored to a predetermined point after which collection for solvent recovery of chloroform would begin. The portion of distillate that was being monitored would be reserved for an alternative recovery treatment being

overly enriched in alcohols. The graph shows that the time evolution of the methanol concentration in chloroform distillate data obtained on-line by RP-MIMS using a Nafion® membrane compares favourably with GC data for the condensate. The main difference is that the slope of the function obtained by using RP-MIMS is slightly less negative. The source of this difference is not yet clear; however, it may be related to a delay in the transfer of the alcohol across the membrane. The variation in the concentration of ethanol during the distillation, monitored by RP-MIMS and GC is shown in Figure 4.9, where the quantitative reference was performed at 0.1 L distillate collected. The data sets for both methods show an initial increase in ethanol concentration, which reaches a maximum when the volume of distillate is ca. 0.2 L, before falling slightly. These results indicate that RP-MIMS method also has potential for monitoring of ethanol. The advantages of the MIMS approach, especially in comparison to an optically based method, is that real time monitoring of methanol and ethanol during the distillation process is possible because of the ability of the MS to distinguish between the alcohols.

4.4. Conclusions

The use of a hydrophilic Nafion® membrane in the RP-MIMS analysis of mixtures of methanol, ethanol and chloroform has been demonstrated. The membrane discriminates against the non-polar chloroform allowing the alcohols to be determined at concentrations below 0.5% v/v. The direct on-line introduction of sample mixtures into the reverse phase membrane interface is a convenient alternative to the lengthy off-line procedure required for normal

phase MIMS. The potential of RP-MIMS with a RGA for process monitoring has been investigated for evolving methanol and ethanol concentrations during a chloroform distillation process. The analyser has been shown to be able to track vapour phase compositions in real time, with reasonable accuracy and sensitivity. A semi-quantitative capability with a minimal approach towards calibration has been demonstrated. A primary focus for further work will be to determine more precise methods towards calibration. Whereas these will not be required in all applications a thorough understanding of this area is important.

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CHAPTER 5

Development of a universal temperature controlled membrane interface for the analysis of volatile and semi-volatile organic compounds

5.1. Introduction

A membrane inlet (MI) has been shown to be a rapid and sensitive technique for the determination of volatile organic compounds (VOCs) in aqueous streams, air samples and process monitoring applications by transport of analytes across a permeable or semi-permeable membrane. The principles and recent developments of membrane inlet mass spectrometry (MIMS) have been discussed in a number of reviews [1-3] and have been shown to be superior in many respects to other techniques, including purge and trap-GC/MS, for the determination of VOCs [4-6]. The sensitivity of MIMS for VOCs is generally high and detection limits in the parts per billion (ppb) range are possible for many compounds, with less polar, low molecular weight analytes showing the lowest detection limits. MIMS has been combined with tandem mass spectrometry (MS/MS), on-line cryotrapping and rapid GC separation to improve selectivity and reduce detection limits to the low parts per trillion (ppt) range for selected VOCs [6,7].

The observation that MI interfaces perform better for non-polar, low molecular weight VOCs than for more polar, less volatile, compounds, particularly with the use of silicone membranes, demonstrates the limit of applicability of the MI technique. A number of procedures have been published on techniques that extend the range of compounds compatible with MI. For example, Lauritsen *et al.* described an in-source membrane inlet system for the detection of semi-volatile organic compounds (SVOCs) in aqueous solution, in which the sample was passed through a hollow-fibre membrane positioned in a modified EI mass

spectrometer source. Interruption of the sample flow led to rapid heating of the membrane by the EI filament situated close to the membrane surface [8]. A second-generation system employing the same principles was also devised, but instead of the sample flow being interrupted, an air plug was passed through the membrane while heating took place [9]. This resulted in a more rapid heating rate, giving a narrower desorption profile. In the configuration adopted by Matz *et al.* in the analysis of fermentation suspensions, analytes were pre-concentrated on a pneumatically driven membrane probe before desorption at 180°C [10].

The use of laser desorption in the determination of SVOCs by MIMS was reported by Soni *et al.* [11,12], who used a low-power carbon dioxide laser to irradiate the vacuum side of a sheet membrane held in a direct-insertion (in-source) membrane probe, resulting in desorption of the permeate molecules with little fragmentation. All these methods are effective in the determination of SVOCs in aqueous samples, but in most cases require elaborate and often lengthy modifications to either the membrane interface or the spectrometer ion source, or both.

The MI technique has been applied successfully to SVOCs in an interface remote from the mass spectrometer ion source using in-membrane pre-concentration (IMP-MI) [13]. IMP-MI analytes are retained in the membrane while the sample is pumped across the surface of the membrane. The membrane is then dried by a flow of air or an inert gas across the surface, to remove water from the membrane and then heated, to release the analytes from the membrane and into a stream of helium, which is directed into the mass spectrometry source or other detector.

A disadvantage of IMP-MI is that existing technology uses a GC oven or the ion source of a mass spectrometer to heat the membrane to the desired temperature and that sub-ambient operation of the MI interface is not possible. If a GC oven is used, the time required to heat (and cool) the mass of the oven significantly increases the analysis time. The release of materials from the membrane heated in this way is slow, resulting in a broad analyte desorption peak which arises a problem when the MI is linked, for example, to a GC.

When VOCs are analysed by MI they may not be retained in the membrane significantly, and continuously infuse through the membrane. However, MI combined with cryotrapping [13-15], would allow VOCs to be concentrated in the membrane. Rapid heating of the membrane releases the condensated VOCs which are transferred to the detector.

This paper describes the development of a novel temperature controlled membrane inlet (TCMI) interface, which may be operated at temperatures in the range of –70°C to 250°C. This interface is heated with the use of a heater element at rates of up to 10°C s^{-1}. The TCMI device may be connected directly to a mass spectrometer or to a GC column linked to a MS. This work also reports the first operation of a MI interface at sub-ambient temperatures for the pre-concentrating of SVOCs and VOCs.

5.2. Experimental

5.2.1. TCMI interface

The TCMI device shown in Figures 5.1 and 5.2 is constructed from three concentric stainless steel tubes. The inner tube, or cartridge (0.1090" o.d. by 0.091" i.d.), contains stainless steel sample inlet and outlet tubes (0.03125" o.d. by 0.020" i.d) at one end. The sample inlet is connected to a liquid or gaseous sample or an inert gas stream (such as helium or nitrogen when a drying stage is required). A polydimethyl(siloxane) membrane (0.635 mm o.d. x 0.305 mm i.d., Dow Corning Silastic, Sanitech, USA) or often silicone hollow fibre membrane, is placed inside the cartridge, and connected to a length deactivated fused silica tubing (0.25 mm i.d., SGE, Milton Keynes, UK) at each end. The fused silica is inserted approximately 5 mm into the membrane. One length of fused silica is connected to a helium supply (for example the injector of a GC can be used for this purpose). The other length of fused silica is connected to a mass spectrometer or GC column linked to a mass spectrometer. PEEK caps are glued to each end to seal the cartridge.

The cartridge is placed inside a T-shaped tube (0.375" o.d. and 0.319" i.d.) containing heating wire and a thermocouple (K type, RS Components Ltd., UK) brazed to the inner wall of the tube. The heater wire (Thermocoax, Suresnes, France) is a thin nichrome alloy heater wire (0.5 mm diameter and around 300 mm long) encased in and isolated from a stainless steel outer sheath that is brazed to the inside of the T-shaped tube. Heating rates up to 20°C s^{-1} may be

achieved, but rates in the range between 5 and 10°C s^{-1} were used in the experiments carried out in this work. The heater wires were terminated to 7 strand 0.3 mm diameter nickel wire insulated with vidaflex sleveing via a stainless steel crimp connection potted with a high temperature ceramic fibre adhesive (Cambridge Scientific Instruments, Ely, UK). The heating wires and the thermocouple were connected to a control box, which was used to regulate the temperature or temperature gradient of the interface to the desired value by controlling the power applied to the heater. Cooled gas (helium or nitrogen) can be introduced into the space between the cartridge and the outer tube to cool the cartridge. The cooling gas is passed through an 1/8'' tube immersed in a Dewar containing liquid nitrogen or other coolant prior to introduction into the MI interface. The outer stainless steel tube (0.700" o.d., 0.621" i.d.) is used to insulate the cartridge and the carrier gas tube, and the space between is filled with insulating material (Figure 5.2b).

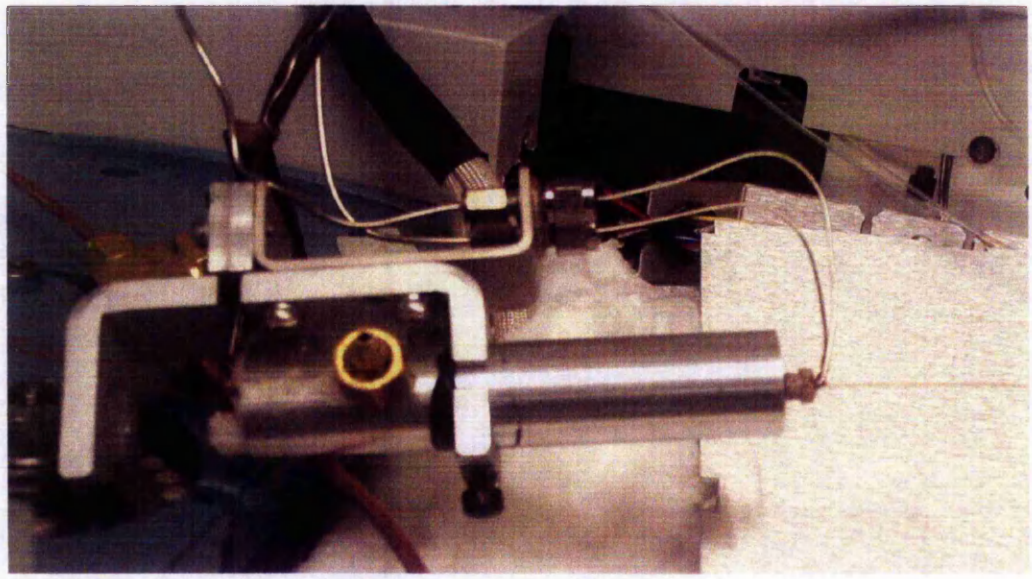

Figure 5.1: Picture of the TCMI device.

The whole assembly is located in a stainless steel holder, which may be attached to the detector or GC wall (Figure 5.2b).

Samples were pumped through the interface using a peristaltic pump (Watson-Marlow Bredel Pumps Limited, United Kingdom) at a rate of 3 mL min^{-1}. When no sample was being introduced into the interface, the outer walls of the membrane were flushed with inert gas (nitrogen at a flow of 50 mL min^{-1}), in order to dry the membrane.

5.2.2. TCMI-MS

5.2.2.1. Analysis of aqueous samples

For the analysis of aqueous samples, the TCMI device was linked to a Hewlett Packard HP6890/HP5973 GC/MS configuration (Agilent Technologies, Palo Alto, California, USA). The device was located inside the GC oven in place of the capillary GC column. The oven temperature was mantained at 40°C and the membrane was heated directly using the heating wire located inside the device. The TCMI-MS interface was then heated to 220°C at a gradient of 10°C s^{-1}. The membrane was held at 220°C for 30 seconds and then the electrical supply was switched off and the device was rapidly cooled to 40°C. Analytes were introduced to the membrane at a flow rate of 3 mL min^{-1} for 5 min, followed by a 14 min drying step using helium at 50 mL min^{-1} through the outer wall of the membrane. During this time the membrane was held at 40°C.

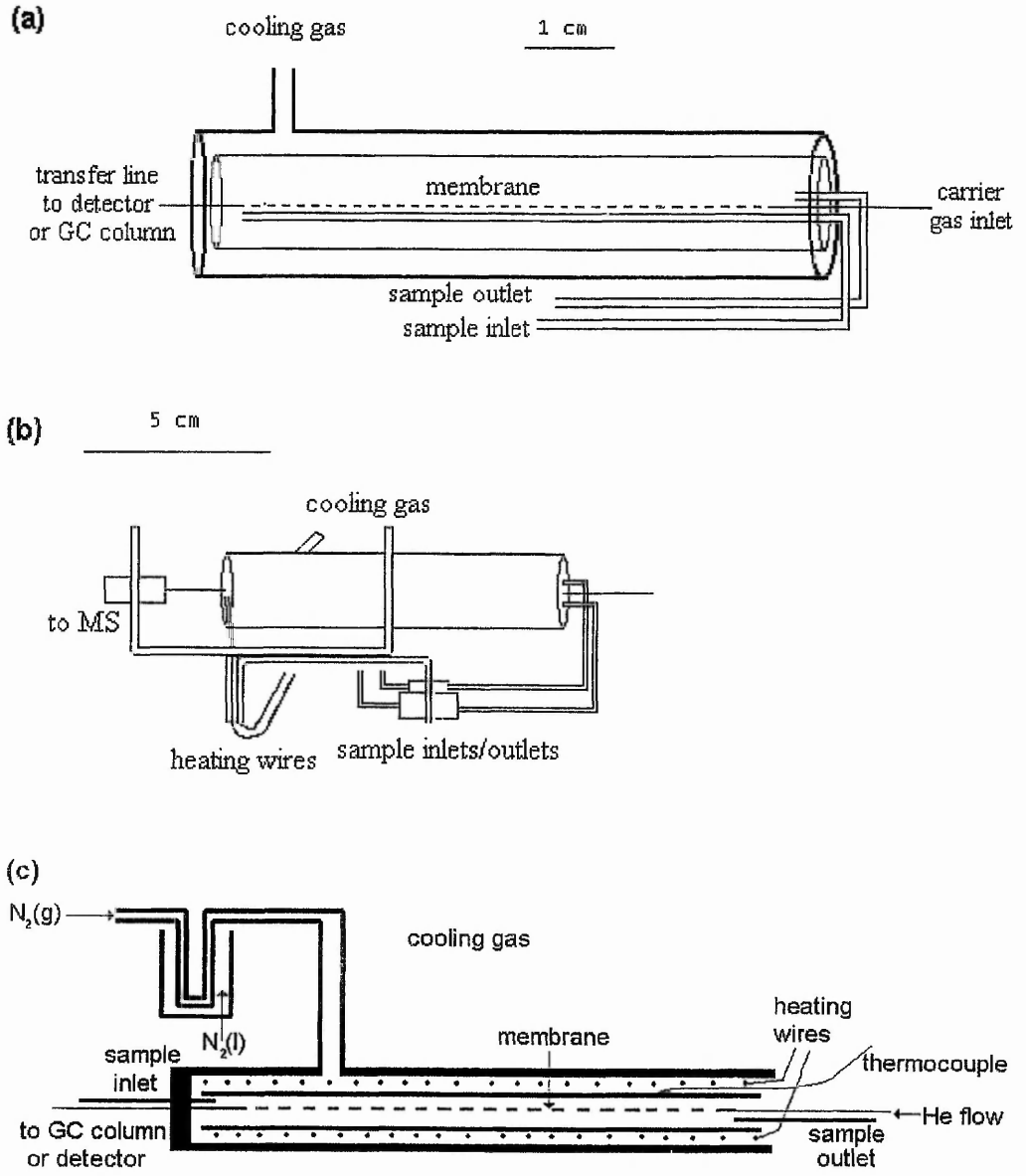

Figure 5.2: Schematic of the TCMI device.

IMP-MIMS experiments were carried out heating the membrane with the GC oven. Analytes were introduced to the membrane at a flow rate of 3 mL min^{-1} for 5 min, followed by a 14 min drying step using helium at 50 mL min^{-1} through the outer wall of the membrane at 40°C. For thermal desorption of the analyte the membrane was heated to 200°C at a rate of 45°C min^{-1} and then cooled back down to 40°C.

5.2.2.2. Analysis of VOCs in air

The TCMI device was operated at sub-ambient temperatures in combination to a Varian Saturn 4D (Varian Associates, Walnut Creek, CA, USA) quadrupole ion trap mass spectrometer. A schematic diagram of the TCMI device for sub-ambient analysis is shown in Figure 5.2c. Air samples were introduced into the interface at 3 mL min^{-1} for 3 min, whilst the membrane was held at –20°C with the aid of the cooling gas (nitrogen at 200 mL min^{-1} passed through a tube immersed in a Dewar containing liquid nitrogen). The membrane was flushed with nitrogen (50 mL min^{-1}) for two minutes and the interface was then electrically heated to 100°C and then cooled to –20°C. The heating cycle was repeated twice to clean the membrane between samples.

5.2.3. TCMI-GC/MS

The TCMI device was located outside the GC oven and connected to a helium supply on the outlet side. The inlet side of the membrane was linked to a GC column. A schematic diagram of the set-up is shown in Figure 5.3.

5.2.3.1. Analysis of aqueous samples at room temperature

The TCMI device was linked to a GC column located inside a GC oven, which was interfaced to a Hewlett Packard HP6890/HP5973 GC/MS (Agilent Technologies, Palo Alto, California, USA). The GC column was a 25 m x 0.22 mm i.d. BP1 column with film thickness of 0.25 μm (SGE, Milton Keynes, UK).

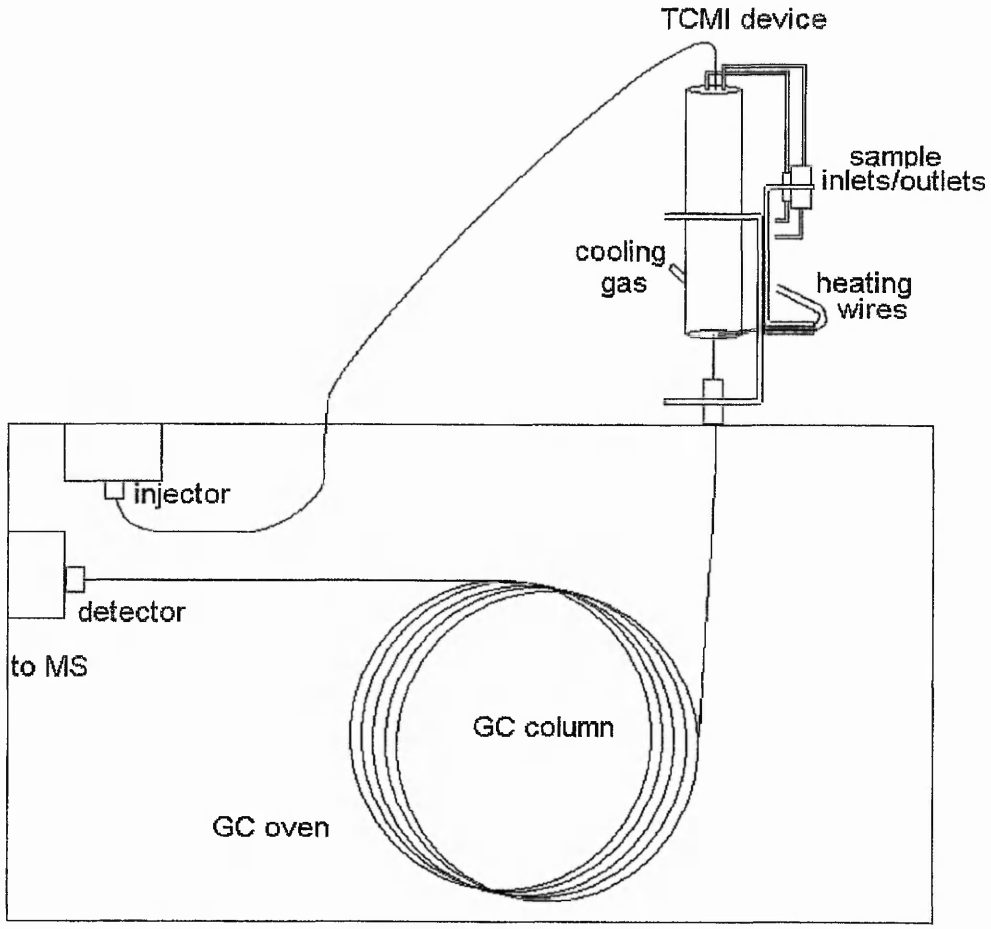

Figure 5.3: Schematic diagram of the TCMI-GC/MS configuration.

Aqueous samples of 5-chloro-2-trifluoromethylaniline (CFA), 3-bromopyridine (3BP), n-methylpyrrolidinone (NMP) and tetramethylethylenediamine (TMEDA) (100 mg L^{-1}) were introduced into the TCMI at room temperature for 5 min, and the membrane was flushed with nitrogen (50 mL min^{-1}) for 5 min. The membrane was then heated to 200°C at a rate of 4°C s^{-1}. The GC program was as follows: 3 minutes at 35°C, heat to 200°C at 25°C min^{-1}, and hold at 200°C for 10 minutes. The GC program was started once the membrane had reached 200°C.

On-line monitoring of a bioreactor was carried out in a 5 L bioreactor containing activated sludge (Chapter 3 Section 3.3). The pH was held at 7.00, the temperature was set to 35°C, and the reactor was stirred to 150 rpm. The bioreactor was spiked with CFA (25 mg L^{-1}), TMEDA (100 mg L^{-1}) and 3BP (100 mg L^{-1}), and a sample of the bioreactor medium was extracted and measured on-line.

5.2.3.2. Analysis of aqueous samples containing VOCs by MI pre-concentration at sub-ambient temperatures

The TCMI device was linked to a GC column located inside a GC oven, which was interfaced to a Hewlett Packard HP5890/HP5971 GC/MS (Agilent Technologies, Palo Alto, CA, USA). The GC column was a 12 m x 0.22 mm i.d. HP1 column with a film thickness of 0.33 μm (Agilent, Palo Alto, CA, USA). The GC program was as follows: 3 minutes at 35°C, heat to 200°C at 25°C min^{-1}, and hold at 200°C for 3 minutes. The GC program was started once the membrane had reached 160°C.

The sample was introduced into the interface for 5 minutes at 3 mL min^{-1} whilst the interface was held at –15°C with the aid of the cooling gas (nitrogen at 200 mL min^{-1} passed through a tube immersed in a Dewar containing liquid nitrogen). The membrane was then dried with nitrogen (50 mL min^{-1}) for 2.5 minutes and the interface was then electrically heated to 160°C and then cooled to –15°C again.

5.2.3.3. Analysis of VOCs from a car exhaust with MI pre-concentration at sub-ambient temperatures

The TCMI device was operated at sub-ambient temperatures in combination with a Varian Saturn 4D (Varian Associates, Walnut Creek, CA, USA) quadrupole ion trap mass spectrometer. The GC column was a 12 m x 0.22 mm i.d. HP1 column with a film thickness of 0.33 µm (Agilent, Palo Alto, CA, USA). Air samples were collected from car exhausts in evacuated glass containers and were introduced into the interface at 3 mL min^{-1} for 10 min, whilst the membrane was held at –20°C with the aid of the cooling gas (nitrogen at 200 mL min^{-1} passed through a tube immersed in a Dewar containing liquid nitrogen). The membrane was flushed with nitrogen (50 mL min^{-1}) for 2 minutes and the interface was heated to 160°C and then cooled to –20°C. The GC program was as follows: 3 minutes at 35°C, heat to 200°C at 25°C min^{-1}, and hold at 200°C for 3 minutes. The GC program was started once the membrane had reached 160°C.

5.3. Results and discussions

5.3.1. TCMI-MS

5.3.1.1. Aqueous samples at room temperature

Experimental work demonstrating the performance of the TCMI device and comparing the response given by IMP-MIMS [13] (Chapter 2) and by TCMI-MS was carried out using DMSO (100 mg L^{-1}). Figure 5.4 shows two overlapped ion chromatograms of mass *m/z* 63 (characteristic ion for DMSO) (a) using MIMS and (b) using TCMI-MS. The peak width at half height obtained by TCMI-MS is over 5 times narrower than the peak obtained by MIMS, resulting in a much more intense peak. This is a result of the faster heating rate for the TCMI interface compared to the GC oven used to heat the membrane in the IMP-MIMS experiment. These data show that TCMI-MS is capable of producing sharper peaks and higher responses. The cycle time for the analysis is also reduced using TCMI because of a reduction in the time required for the desorption step.

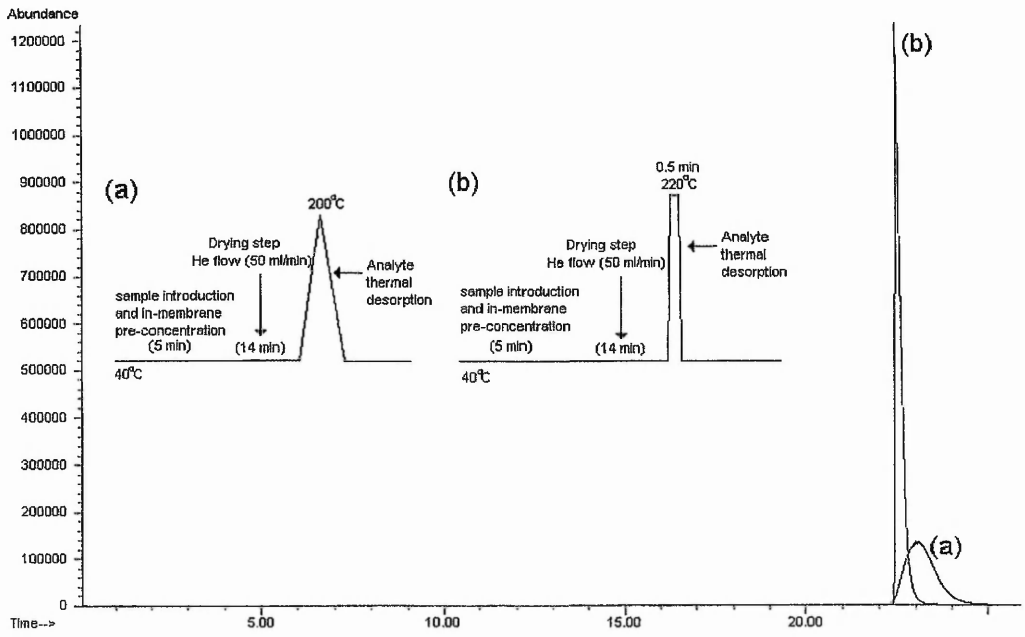

Figure 5.4: Single ion chromatograms for DMSO (100 mg L^{-1}) (*m/z* 63) with a drying time of 5 minutes, a drying time of 14 minutes and N_2 at 50 mL min^{-1} as drying agent by (a) IMP-MIMS (maximum height 130 000 and width at half height 0.990) and (b) IMP-TCMI-MS (maximum height 1 200 000 and width at half height 0.182).

5.3.1.2. Air samples at sub-ambient temperatures

In MIMS analysis VOCs such as benzene and toluene diffuse through the membrane readily at room temperature, giving continuous response at the detector for the analytes, unless cryotrapping is used. However, in the TCMI configuration the membrane may be cooled to sub-ambient temperatures, allowing VOCs to be retained and pre-concentrated in the membrane and then released as a sharp peak by rapid heating of the membrane. Air samples containing benzene and toluene

were analysed by TCMI-MS at sub-ambient temperatures to retain benzene and toluene in the membrane during separation. Both compounds were analysed in the low ppb_v region (33 ppb_v of benzene and 28 ppb_v of toluene) for evaluation of the technique. The single ion responses for toluene (*m/z* 91) and benzene (*m/z* 78) are shown in Figure 5.5. The estimated l.o.d by TCMI-MS at sub-ambient temperatures were 0.15 ppb_v for benzene and 0.10 ppb_v for toluene, while by MIMS they were 3.0 ppb_v for benzene and 2.0 ppb_v for toluene. This shows an increase in sensitivity of ca. 20 times for benzene and toluene by using sub-ambient analysis by TCMI-MS. The main reason for the increase in sensitivity is because at sub-ambient temperatures the VOCs are pre-concentrated in the membrane, whereas the MIMS response at ambient temperature is determined by the steady state flow of the analytes across the membrane by direct infusion.

The analysis time was 6 minutes including a 3 minute sample pre-concentration step. It can be seen in Figure 5.5, that a second heating cycle shows only a small carryover response for the analyte (3.5 % for toluene and 1.5% for benzene), while when using the direct infusion MIMS technique, it can take over 10 minutes to clear the membrane from analytes.

Similar data has been reported using a cryotrap between the MS and the MI interface, but a step is saved by using the TCMI configuration by combining the trapping and pervaporation stages in the interface.

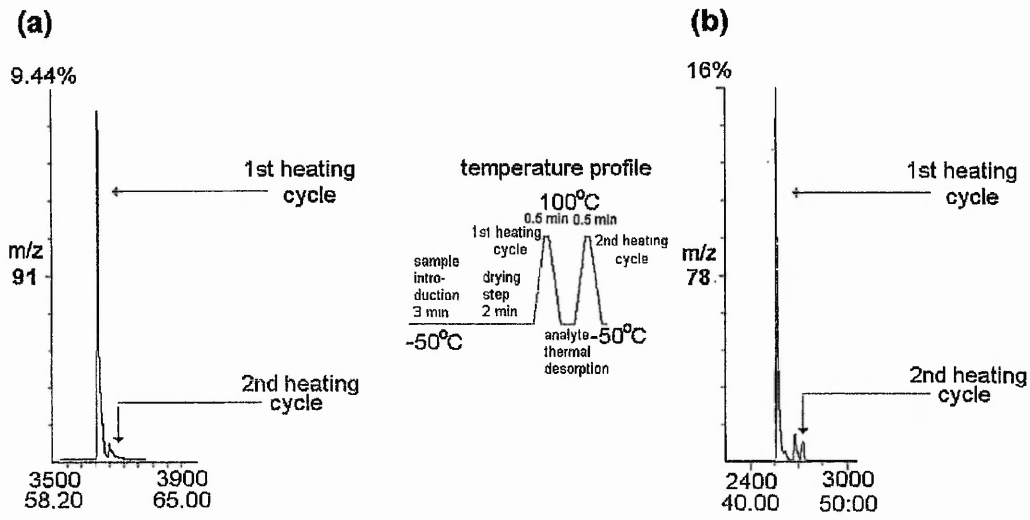

Figure 5.5: Single ion responses obtained by TCMI-MS using sub-ambient analysis for (a) toluene (28 ppb_v) (*m/z* 91) and (b). benzene (33 ppb_v) (*m/z* 78).

5.3.2. TCMI-GC/MS

5.3.2.1. Aqueous samples at room temperature

The sharp analyte desorption peak associated with the TCMI allows the interface to be combined directly with a GC column without a cryotrapping stage between the MI and the detector [6]. The TCMI-GC/MS configuration is demonstrated for the analysis of four nitrogen containing compounds (3BP, CFA, NMP and TMEDA) Although 3BP and CFA diffused through the membrane continuously during the sample injection and the drying time, both analytes were also retained in the GC column, and released as a peak with the less volatile analytes NMP and TMEDA when the membrane was heated. The single ion responses following GC separation (*m/z* 58 for TMEDA, *m/z* 98 for NMP, *m/z* 158 for 3BP and *m/z* 195 for CFA) are shown in Figure 5.6.

The analytes responses showed linearity between 5 and 100 mg L^{-1}, with r^2 values of 0.9985 (3BP), 0.9949 (CFA), 0.9763 (TMEDA) and 0.9599 (NMP). The response reproducibility was determined for solutions containing 100 mg dm^{-3} of each analyte. The %RSDs (n = 6) were 5.5% (3BP), 4.9% (CFA), 10.4% (TMEDA) and 8.9% (NMP).

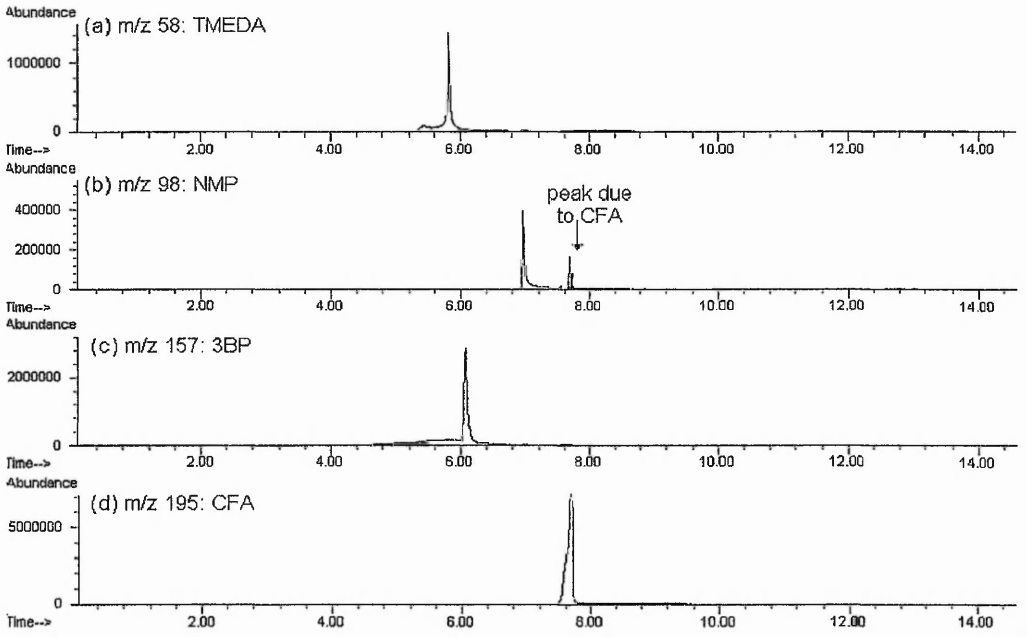

Figure 5.6: Single ion chromatograms for aqueous bioreactor samples (100 mg L^{-1}) of (a) TMEDA (*m/z* 58), (b) NMP (*m/z* 98), (c) 3BP (*m/z* 158) and (d) CFA (*m/z* 198) obtained by TCMI-GC/MS analysis simultaneously.

The TCMI-GC/MS device was linked on-line to a bioreactor containing activated sludge and a minimal salt medium in order to evaluate the response to the nitrogen containing analytes in a complex medium. The bioreactor was spiked

with 25 mg L^{-1} of CFA, 100 mg L^{-1} of3BP and 100 mg L^{-1} of TMEDA. The concentrations obtained by on-line TCMI-GC/MS analysis were 28 mg L^{-1} for CFA (88% accuracy), 96 mg L^{-1} for 3BP (96% accuracy) and 91 mg L^{-1} for TMEDA (91% accuracy).

The TCMI-GC/MS interface was also used to identify metabolites obtained during the biodegradation studies on TMEDA, NMP, 3BP and CFA. The metabolites identified by GC/MS were also identified by TCMI-GC/MS. However it is notable that one of CFA metabolites tentatively identified as 2-trifluoromethylaniline by TCMI-GC/MS was not detected by GC/MS using direct injection of the aqueous sample (Chapter 3 Section 3.3.3.3). The TCMI-GC/MS analysis shows the metabolite with a retention time of 6.8 min, and its mass spectrum contains peaks at *m/z* 161 ($M^{+\cdot}$), *m/z* 142 $(M\text{-}F]^{+}$) and *m/z* 111 ($[M\text{-}CF_2]^{+\cdot}$). A possible explanation for the detection of this metabolite by TCMI-GC/MS (and not by GC/MS) is that the sample is introduced via an injector held at higher temperature in the GC/MS than that used in the thermal desorption step in TCMI-GC/MS analysis, which can cause product decomposition, especially for the more polar, thermally labile compounds. Larger amounts of water will also be introduced into the system by GC/MS than by TCMI-GC/MS analysis. In contrast, by TCMI-GC/MS, the compound is trapped in the membrane, rather than passing through the injector, avoiding the high temperatures and water vapour that could produce thermal decomposition of some compounds.

5.3.2.2. Aqueous samples at sub-ambient temperatures

A mixture of benzene, toluene and xylenes in water was analysed by TCMI-GC/MS. These BTEX compounds diffuse through the membrane at room temperature and do not retain in the GC column at 35°C, so a separate cryotrapping step is normally required in MI-GC/MS methods [6]. The membrane was therefore cooled to sub-ambient temperatures to pre-concentrate the analytes in the membrane prior to thermal desorption. In order to prevent aqueous sample from freezing in the membrane inlet, the interface was held at –15°C. Cooler temperatures froze the aqueous sample inside the interface, interrupting the sample introduction.

A good GC separation was obtained for the desorbed BTEX compounds (see Figure 5.7) and the analyte responses showed good linearity between 1 and 25 mg L^{-1}, with r^2 values of 0.9923 (benzene), 0.9941 (toluene), 0.9996 (m-xylene and p-xylene) and 0.9992 (o-xylene). The response reproducibility was determined for solutions containing 25 mg L^{-1} of each analyte. The %RSDs (n = 3) were 8.4% (benzene), 4.9% (toluene), 6.2% (m-xylene and p-xylene) and 8.2% (o-xylene). Limits of detection (S:N 3:1) were estimated for the compounds, resulting in 20 μg L^{-1} for benzene, 23 μg L^{-1} for toluene, 29 μg L^{-1} for m-xylene and p-xylene and 21 μg L^{-1} for o-xylene for a 5 minutes membrane pre-concentration time.

Table 5.1: Concentrations of benzene, toluene, (m+p)-xylene and o-xylene in aqueous samples collected from the site of a former petrol station site obtained by TCMI-GC/MS.

sample	benzene ($mg\ L^{-1}$)	toluene ($mg\ L^{-1}$)	(m+p)-xylene ($mg\ L^{-1}$)	o-xylene ($mg\ L^{-1}$)
1	20.7	17.3	5.4	6.4
2	12.4	11.8	3.4	4.3
3	17.9	13.8	3.3	3.9
4	3.8	5.4	1.9	2.7

Aqueous samples obtained from a former petrol station site were analysed by TCMI-GC/MS. The concentrations of the BTEX analytes in four different samples collected at the site of a former petrol station are given in Table 1. Peaks A to D (Figure 5.7) have been confirmed by retention time and mass spectral data, therefore corresponding to benzene (*m/z* 78), toluene (*m/z* 92), (m+p)-xylenes (*m/z* 106) and o-xylene (*m/z* 106) respectively. Peaks E to I arise from the presence of alkylbenzene with a molecular weight of m.r. 120 (C_9H_{12}). Peaks J and K show a molecular ion at *m/z* 116 and *m/z* 130 respectively, corresponding to C_9H_8 and $C_{10}H_{10}$. Peak L shows a molecular ion at *m/z* 128 ($C_{10}H_8$) and assigned to naphthalene. Peaks M and N have molecular ions at *m/z* 142 ($C_{11}H_{10}$), assigned to 1-methylnaphthalene and 2-methylnaphthalene.

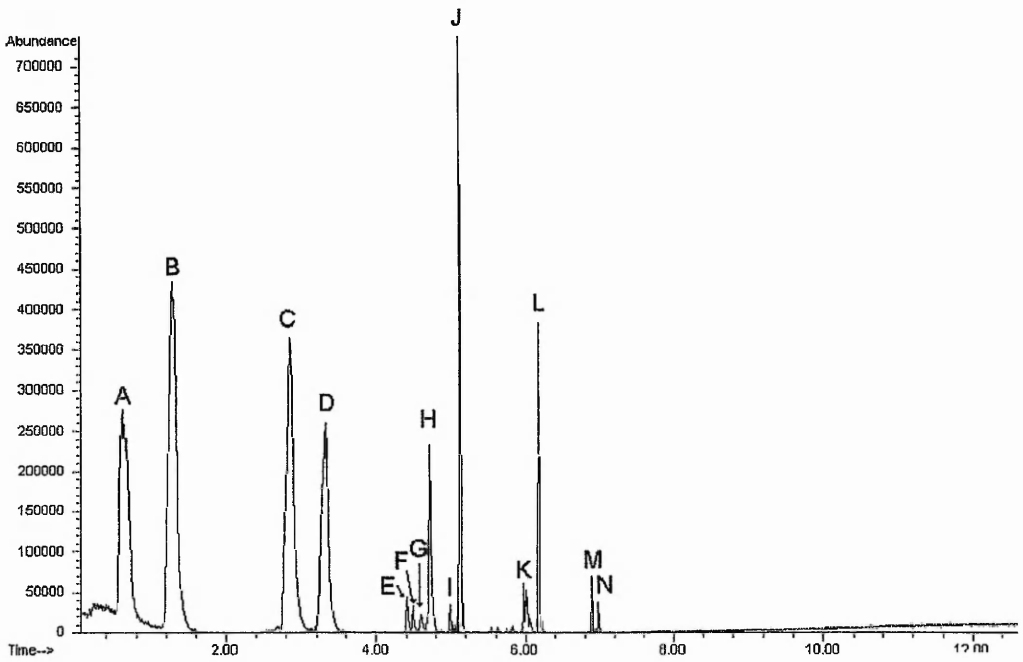

Figure 5.7: TCMI-GC/MS chromatogram of an aqueous sample from a former petrol station site containing benzene (A), toluene (B), (m-p)-xylenes (C) and o-xylene (D).

5.3.2.3. Car exhaust analysis at sub-ambient temperatures

Air samples collected from car exhausts were analysed by TCMI-GC/MS at sub-ambient temperatures in order to retain and pre-concentrate volatile organic compounds in the membrane. During the thermal desorption step the analytes were released from the membrane and chromatographically separated before entering the mass spectrometer.

A car exhaust sample obtained from a Metro car (1989 registration) was analysed by sub-ambient TCMI-GC/MS and the single ion chromatograms are shown in Figure 5.8a. Peaks A to D (Figure 5.8a) have been confirmed by retention time

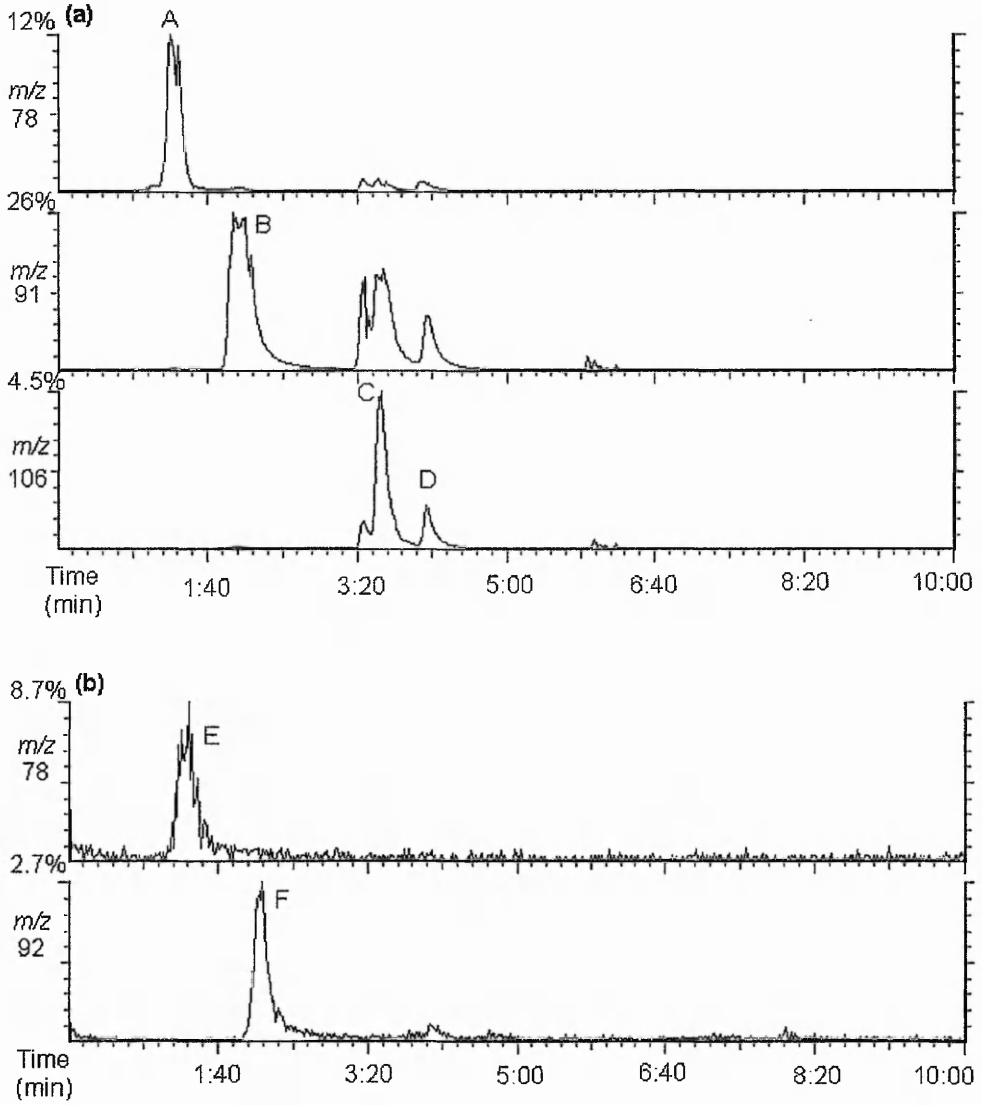

Figure 5.8: TCMI-GC/MS single ion responses for (a) benzene (*m/z* 78 peak A), toluene (*m/z* 92 peak B), (m-p)-xylenes (*m/z* 106 peak C) and o-xylene (*m/z* 106 peak D) from a 1989 car exhaust and (b) benzene (*m/z* 78 peak E) and toluene (*m/z* 92 peak F) from a 2001 car exhaust.

and mass spectral data, and correspond to benzene (*m/z* 78), toluene (*m/z* 92), (m+p)-xylenes (*m/z* 106) and o-xylene (*m/z* 106) respectively.

A car exhaust sample collected from a Ford Focus Van (2001 registration) was also analysed by TCMI-GC/MS (Figure 5.8b). Benzene (*m/z* 78) and toluene (*m/z* 92) could be detected (peaks E and F) but the xylenes were not detected in this sample. The probable reason for xylenes being detected in the old car and not in the new car may be due to the use of a catalytic converter in the newer vehicle.

These data show that real sample monitoring from air samples can be carried out using TCMI-GC/MS, and this has been proved with samples collected from car exhausts.

5.4. Conclusions

A novel temperature controlled membrane inlet (TCMI) has been constructed that allows fast heating and cooling of the membrane. As the rate of heating and cooling is greater by TCMI than IMP-MIMS, peaks obtained are significantly narrower, resulting in more intense peaks.

The interface does not require a GC oven to heat the membrane for thermal desorption of the analytes, allowing the interface to be combined directly with GC/MS without a cryotrap being required. The TCMI-GC/MS configuration has been demonstrated for the on-line monitoring of a bioreactor. As the sample is

not introduced through the GC injector, thermal decomposition of some compounds may be reduced. Pre-concentration of volatile compounds in the membrane prior to their release has been demonstrated for aqueous and air samples.

A universal interface, designed for use with hollow-fibre membranes, has been constructed and evaluated for MIMS. The interface is capable of handling gaseous and liquid samples and is applicable to the determination of VOCs and SVOCs, with or without subsequent GC separation. The interface is also compatible with other gas-phase detection systems.

5.5. References

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Publications

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- Device, Patent application number: GB0219712.7, Glaxo Group Limited. Patent based on the TCMI device described in chapter 5.

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