Pharmaceutical Process Analysis by Mass Spectrometry

by

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Dedicated

to

Duncan Bryant

Acknowledgements

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

Rebecca Clinton, April 2005.

Abstract

The optimisation of chemical and pharmaceutical production is achieved by having full control of the manufacturing process. Novel and developing processes must be thoroughly characterised to determine the reaction mechanisms, reaction rates and critical operating parameters. Mass spectrometry has the potential to be a powerful technique for process analysis, but reaction mixtures frequently pose a problem for online monitoring by mass spectrometry, because the concentrations of the reactants and products exceed the working limits of the spectrometer. This thesis describes the development and evaluation of membrane interfaces for mass spectrometry with potential for the direct monitoring of organic process reactions.

A single-stage microporous membrane-based interface was developed for real-time mass spectrometric monitoring of the starting materials and products of a highly concentrated process reaction mixture of pharmaceutical importance. The liquid-liquid interface was directly connected to the atmospheric pressure chemical ionisation source of a quadrupole mass spectrometer (APCI-MS). A significant level of dilution of the concentrated reaction mixture was achieved in a single step using the interface. The combination of the membrane inlet with APCI-MS was demonstrated for the Michael Addition reaction of phenylethylamine and acrylonitrile in ethanol using a hydrophobic polyvinylidene fluoride microporous membrane. The reaction was monitored throughout its course, allowing the endpoint to be determined based on the relative concentrations of the reaction precursors and products. The device required

minimal analyst intervention, reducing sample preparation and handling prior to real-time MS analysis.

The preparation of supported semi-permeable silicone membranes has been investigated. Supported semi-permeable silicone membranes were cast onto both nylon and polypropylene net support materials and incorporated into a membrane probe device. The supported silicone membranes were evaluated for their potential in process analysis and for screening, however the membrane probes took too long to detect a variation in the analyte concentration. This time scale was not appropriate for process monitoring where the analyte concentration must be monitored in as close to real-time as possible.

A membrane inlet device incorporating a semi-permeable silicone membrane was developed as a diluting interface between a concentrated reaction mixture and the electron ionization source of a quadrupole mass spectrometer. The potential of the interface for the headspace analysis of the volatile components of the mixture was demonstrated. The reactants and products of an early stage, pharmaceutical intermediate process were analysed by headspace membrane inlet mass spectrometry, with dilution of the concentrated reaction mixture achieved in a single step. Headspace sampling combined with membrane inlet mass spectrometry allowed the reaction components of the Mannich reaction of dimethylamine with formaldehyde and parahydroxyacetophenone to be monitored on-line in real time.

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Chapter 1

Introduction

1.1 Process Monitoring

The optimisation of chemical and pharmaceutical production is achieved by having full control of the manufacturing process. Novel and developing processes must be thoroughly characterised to determine the reaction mechanisms, reaction rates and critical operating parameters. Once in production, these process should be maintained at optimal capacity by monitoring in real-time to obtain quantitative and qualitative information on reagents, products, impurities and intermediates. This real-time data would allow informed decision making when adjusting parameters that affect product quality, such as reaction time, modification of reaction conditions and detection of process deviations, as soon as possible to minimise contamination and/or side reactions. Process monitoring should therefore be an integral part of process design and day-to-day plant operation. Automated feedback control loops may then be installed to control and optimise the yield of the product and minimise impurity formation. The optimised process will have reduced production costs, minimised environmental impact and maximised product quality.

1.1.1 Process Characterisation

The type of process analysis used can be classified according to where and how the analysis is performed.[1] The four common approaches are outlined below.

1.1.1.1 Off-Line Analysis

This term is applied when a sample is withdrawn from the reaction vessel and transported to a remote laboratory for analysis.[1] This may be the process chemistry laboratory in the next room, next building or even a different site. Here the samples may be analysed using a range of specialised instrumentation by highly trained analysts. However, this sampling regime provides only discrete data and there is a time delay associated with the result generation. The more remote the testing laboratory, the longer the time between taking the sample and the results being generated, the greater scope there is for sample degradation, loss or contamination in transit. Long delays in results reporting are far from ideal as by the time the result has been transmitted back to the process chemist, a significant time period has elapsed and the process has moved on.

1.1.1.2 At-Line Analysis

The analytical instrument may be placed next to the reaction vessel, possibly in a protective plant room shelter.[1] This at-line analyser would be a dedicated plant room instrument operated by the process chemists. There still remains the delay between sampling and analysis and opportunities for sample contamination or degradation, but there may be a significant reduction in the sample transport time and complexity.

1.1.1.3 On-Line Analysis

An on-line analyser automatically removes a sample from the process vessel or from a loop containing the process stream.[1] The sample is then analysed by an automated instrument such as HPLC, GC, a spectroscopic technique (FTIR, UV) or by mass spectrometry. Here the time between sampling and analysis is purely a function of the length of the transfer line, the speed of analyte transport to the instrument and the analysis time. Contamination and degradation are minimised, continuous data on the reaction status can be produced rapidly and can be available for viewing almost immediately.

1.1.1.4 In Line Analysis

An ideal analyser would be in-line with the process. That is, located inside the reaction vessel, completely eliminating the need for sample transportation prior to analysis.[1] In-line analysers usually have an electrical or spectroscopic sensor probe inside the vessel with the analytical information relayed back to the processor, for example via a fibre optic cable. The in-line analyser reduces the opportunity for contamination to a minimum and the instantaneous status of the process may be observed. However the situation of the sensor inside the reaction vessel limits the number of applications with the result that in-line analysis is usually only used for spectroscopic techniques (NIR, FTIR, UV), pH sensors or physical sensors such as for temperature and pressure.

1.1.2 Process Monitoring Criteria

All analytical procedures used to monitor reactions must comply with a number of fundamental criteria. The analysis must be safe and require minimal plant room modification. This keeps the cost to a minimum and reduces interference either by restriction of the process (thermally, pressure-wise or kinetically) or by possible contamination. Low environmental impact is desirable, i.e. low solvent consumption, and additionally low sample consumption. This is important where process costs are high and additionally the product must not be significantly consumed during testing. The data generated must be process-specific, both qualitative and quantitative and available within a timeframe that allows appropriate reaction control measures to be taken. Finally, the system must be robust as it would be in use on a day-to-day basis in a harsh environment.

A monitoring method is appropriate if it monitors only those factors that either affect the system equilibrium or demonstrate the status of the process. These include environmental conditions, such as temperature and pressure, and chemical conditions such as concentrations of the various reactants and products. This thesis will concentrate on the chemical testing side of reaction and process monitoring.

In order that the data is available to process controllers at a rate consistent with the reaction rate, the monitoring device is usually located as close to the process as practicable. The acceptable timescale for this is determined by the rate of the

reaction and the response times required for any corrective measures. In-line monitoring is ideal as continuous data can be generated in real time and is available immediately for the process controller. Unfortunately, not all analytical equipment is designed to operate in the harsh reaction conditions, nor is it necessarily practical, so on-line monitoring is a reasonable substitute. On-line analysis is performed by instruments as close to the reaction vessel as is practicable, using a suitable sampling device to transport analyte to the analytical apparatus.

The technique used to monitor the process obviously depends on the information required and the physical and chemical properties of the system. Recently published research material demonstrates that process/reaction monitoring is an active, expanding science covering a wide range of industrial disciplines as evidenced by the large number of papers published. The journal, Analytical Chemistry, publishes a review of process monitoring techniques approximately every two years[1-6] and the field has been discussed in several recent reviews that describe novel sampling devices or techniques.[7-17]

As with all analytical techniques the sampling method is critical to the generation of meaningful, representative data and much of the literature is concerned with the interface between the reaction vessel and the analytical instrument.

1.1.3 Process Monitoring Techniques

1.1.3.1 Flow Injection Analysis

Ruzicka and Hansen[18] first described flow injection analysis (FIA) in 1975. FIA uses a sequence of pumps and valves to extract samples continuously from a process mixture, quench the reaction and extract the analyte prior to detection/quantification by a suitable detection method. Since its inception this sample handling approach has been applied to many analytical techniques such as HPLC, mass spectrometry, spectrometry, pH measurement etc. [2-6,15]

There will always be a time delay between the reaction vessel sampling and the production of an analytical result, but FIA has typically reduced this time to between 1-2 minutes.[8] Examples include the use of FIA coupled to enzyme electrodes and amperometric detection[19] to monitor lactic acid fermentation. Dantan, Frenzel and Kuppers[20] combined FIA with HPLC and CE to perform on-line derivatisations in order to achieve 'near real-time monitoring' of a reaction processes.

One of the problems with FIA is that the reaction is continually sampled resulting in a high sample and reagent usage. This issue was tackled by Ruzicka[21] *et al* who developed the double-injection, single-line FIA which reduced the reagent usage and increased the versatility of the technique. Additionally, sequential injection analysers (SIA) were proposed[22] to reduce the complexity of

the flow injection analysis. FIA and SIA are now commercially available[23] and new applications to process monitoring are still being published.

1.1.3.2 Spectroscopic Methods

UV-visible spectrometry is not a very selective technique and as such has been used mainly as a detection method for samples that have been pre-treated by rigorous sample extraction or after chromatographic separation (e.g. HPLC). However, Buhlman[24] used UV-Visible spectrometry coupled with intricate data analysis algorithms to monitor the dissolution testing of multi-component pharmaceutical formulations. Dissolution testing is usually the domain of HPLC where components are separated before detection and quantification resulting in a significant delay in obtaining results. Buhlman's UV method allows the dissolution to be monitored in real time.

Near-infrared detection (NIR) gives more structural information than UV and increased in popularity in the early 1980s with the development of high quality fibre optic probes, increased computing capacity and improved chemometric methods. This resulted in commercially available Fourier-Transform NIR instruments being targeted at pharmaceutical companies specifically for in-line reaction monitoring. As a non-destructive, non-invasive technique giving near-instantaneous analysis of a sample composition, NIR is a useful process monitoring tool for some applications. To this end Harris and Walker[25] quantified solvents in drier effluent using a fibre optic acousto-optic tunable filter

(AOTF) NIR, and using the same technology, Mid-IR was used to determine end points and impurity formation during a hydrogenation reaction[26] and an organic synthesis.[27]

NIR and Raman spectroscopy were compared for the on-line monitoring of emulsion polymerisation reactions.[28] Both techniques were suitable for monitoring the changing monomer concentrations if subjected to appropriate calibration regimes.

Duan *et al*[29] developed a portable plasma source spectrometer to analyse waste streams on-site and in real time. Fluorescence detection coupled with pressurised hot water extraction has been used to monitor a PAH leaching process and determine the reaction kinetics and the end of the leaching process.[30] Fourier Transform Raman spectroscopy was also used to monitor a pharmaceutical blending process.[31] This in-line non-destructive probe was used to successfully monitor the process and replaced a time consuming HPLC analysis that required samples to be removed from the vessel to be taken away for off-line analysis.

Other spectroscopic techniques that have been employed for process analysis are spectroscopic ellipsometry, (a non-destructive optical technique that measures the polarisation of a monochromatic light beam reflected from the sample surface) and light scattering detectors used mainly in the monitoring of surface interactions.[32,33]

Nuclear magnetic resonance spectroscopy (NMR) has been used to characterise bio-catalysed reactions[34] by placing the reactants in NMR tubes and monitoring the reactions *in-situ* in the NMR magnet. In this way Pokorny *et al*[35] used NMR to monitor the cleavage of acetopyruvate in real-time. Littlejohn used NMR spectroscopy to monitor both a benzene production process[36] and the reaction of 2-butanol with crotonic acid,[37] replacing the off-line gas chromatography method.

1.1.3.3 Chromatography Methods in Process Analysis

Chromatography is one of the most commonly used analytical techniques for process monitoring and HPLC in particular is widely used in pharmaceutical monitoring. HPLC is not very well suited to on-line applications due to the significant time delay caused by sampling, sample preparation and separation, the operator time required and the volume of potentially toxic solvents used. However, the advantage of HPLC as a monitoring technique is its capability as a multi component analyser.

Rehorek *et al* [38] used HPLC with diode array detection to monitor a bioreactor degradation process by a series of electrical switching valves and pumping systems. Liu, Wang and Lee[39] monitored glucose consumption and ethanol formation in a fermentation broth using a by-pass circuit and a membrane ultrafiltration cell to sample the reactor. Two HPLC systems analysed samples simultaneously for different components using diode array and refractive index

detection. This enabled them to create a control feedback loop to regulate the addition of glucose to the reaction vessel. The repeat analysis time was at least 35 minutes due to the HPLC run time and the small amount of sample, but this was an acceptable delay time for this bioreaction process operating for up to 16 hours. With the advent of smaller columns reducing analysis time to 1-2 minutes and increasingly sophisticated interfacing devices (e.g. FIA) the technique is open to development for faster reactions.

Van der Mebel[40] used an in-line ultrafiltration device to sample a fermentation broth. The extracted samples were automatically sampled into an LC system and multiple components of the reaction mixture were monitored in near real-time. Kokkonen *et al* [41] used capillary electrophoresis as an analysis technique for on-line monitoring of multiple components in waste-water streams from pulp and paper machines. The monitoring system was used on eight different systems and continuously monitored the waste streams for up to a month at a time. Non-porous silica chromatography with electrospray mass spectrometry (ESI-MS) detection was used to study mesylation reactions.[42] The technique was rapid, selective and provided suitable feedback for a reaction that was complete in under an hour.

Gas chromatography offers a very simple interface for the analysis of gaseous samples as these are relatively easy to remove from reaction vessels, often with little more than a heated transfer line. Again the technique suffers from the time delay inherent in the time required for chromatographic separation. New fast GC

columns and systems are being introduced bringing the analysis time down to a timeframe more acceptable for multiple sample points per hour. Synovec[43] proposed a two dimensional GC technique as a process control analysis system, while Chang and Her[44] used fast gas chromatography coupled to membrane inlet mass spectrometry (MIMS) for the on-line monitoring of trihalomethanes in water. They achieved a sampling rate of approximately twenty samples per hour which corresponds to a run time of less than three minutes.

1.1.3.4 Mass Spectrometry Methods in Process Analysis

Mass spectrometry has been used widely for monitoring reaction processes as it provides both qualitative and quantitative data on sample composition. The potential of using mass spectrometers as process control instruments has been extensively reviewed.[45-53] Whereas many techniques for on-line analysis, such as HPLC, may take many minutes to separate and detect the components in the sample, mass spectrometry is an almost instantaneous technique with scan times of as little as one second and the resulting data being available on screen immediately. Gaseous components of reaction processes are the simplest to monitor, even though they often involve pre-concentration steps such as purge and trap and headspace analysis. Liquid phases, however, have posed more of a challenge, especially in concentrated reaction mixtures (with concentrations as high as 30% by weight) where multiple analyte levels far exceed the working limits of the instrument. Complex sampling, separation, preparation and dilution systems must be developed to analyse reaction mixtures without compromising

the mass spectrometer operation.

Lee *et al* [54] developed a continuous introduction reaction sampling system for mass spectrometry in 1989. An electrospray needle was directly coupled to the sample vessel and the gravity fed reaction components were successfully monitored in real time. This reaction was a miniature version of a true process scale reaction, but heralded the start of many new reaction systems for reaction monitoring using different interfaces.

Brum and Dell'Orco[55-57] have carried out extensive research on techniques for the on-line analysis of liquid reactions by mass spectrometry. Their reaction monitoring systems involved extracting the sample from a loop outside the reaction vessel. The liquid sample was then diluted, to quench the reaction, and split before introduction to an electrospray mass spectrometer. Reaction starting materials, catalysts, products and intermediates were monitored online. In some instances the whole reaction process was over in less than 30 minutes and this method generated enough data points to demonstrate reaction kinetics and be a rapid, working monitoring system.

Arakawa *et al* [58] coupled a photoreaction cell to a mass spectrometer and passed the reaction mixture through the cell immediately prior to analysis. This coupling allowed labile and active species to be analysed and thus allowed a thorough investigation of the reaction pathway not afforded by more time consuming techniques. Similarly, Xu, Lu and Cole[59] invented a novel probe accessory to

allow the fast analysis of polycyclic aromatic hydrocarbons during oxidation by fast electrochemistry/mass spectrometry. Again reactions were monitored that would otherwise be missed by more distant coupling techniques.

An inverse sampling valve was used by Brodbelt *et al* [60] to monitor both gaseous and liquid process streams. This simple device removed 0.25-1.0 µl sample directly from the sample chamber into the mass analyser. A similar approach was taken to compare total vaporisation with dynamic gas purging headspace analysis, in order to monitor liquid process streams. [61]

Lancaster *et al*[62] developed a programmable temperature vaporising injector to analyse liquid process streams by mass spectrometry. While Hoffmann[63] characterised the gaseous and particulate components of an ozonolysis experiment, on-line using a combination of atmospheric pressure chemical ionisation mass spectrometry (APCI) and atmospheric pressure photoionisation (APPI).

The continuous real-time monitoring of the leaching profiles of dredged sediments was performed by Beauchemin *at al* [64] using flow injection inductively coupled plasma mass spectrometry. Karst[65] compared two techniques to monitor an enzymatic cleavage reaction. A luminescence technique detected the product in real time but ESI-MS was found to be superior in that it was possible to monitor the substrate and products in real time.

On-line mass spectrometry was used to monitor the reaction kinetics for the oxidation of isoprene and the formation of the products.[66] The data produced were compared with the projected model for the reaction and enabled reaction pathways to be determined.

Time of flight (TOF) instruments were used on-line by both Zimmermann[67] and Reinhoudt.[68] In the first instance photonionisation with vacuum UV light was coupled with TOF mass spectrometry to monitor the changes in the organic compounds in the exhaust gases of a motorcycle. Reinhoudt *et al* employed miniaturisation using an on-chip microfluidic device coupled to a matrix-assisted laser desorption ionisation (MALDI) TOF spectrometer. They monitored a Schiff's base process involving the reaction of amines with aldehydes, detecting the imines as they were formed.

1.1.3.5 Membrane Interfaces

Membranes play a valuable role in sample preparation for on-line analytical applications. Membrane inlet mass spectrometry and its uses in process monitoring are discussed in the Membranes section of this chapter (Section 1.3).

1.2 Principles of Mass Spectrometry

1.2.1 Introduction

Mass spectrometry is an analytical technique for the identification and quantification of ions according to their mass to charge ratio (m/z).[69-73] The separation and detection of ions began over ninety years ago when Thomson[74] experimented using a 'positive ray' parabola spectrograph and collected spectra for a number of gases. Aston[75] then developed a "mass spectrograph" and incorporated slits to focus the beam. At around the same time Dempster[76] developed the first electron impact (EI) source and used this with a magnetic sector instrument to determine isotope abundances of magnesium and other elements.

Mass spectrometers were steadily improved and developed for the analysis of organic molecules. The quadrupole analyser was developed in 1953 by Paul[77] and Finnigan produced the first commercially available quadrupole mass spectrometer in 1968. The coupling of a gas chromatograph with a mass spectrometer[78] in 1957 was a significant advance for the analysis of volatile samples.

Fenn's[79] new ionisation techniques expanded the applicability of mass spectrometry through the introduction of thermospray ionisation[80] and then electrospray ionisation[81-82] (ESI) in the 1980's, which revolutionised the analysis

of volatile liquid samples, allowing even involatile, thermally labile peptides and proteins to be analysed by mass spectrometry.

There are three basic stages involved analysis by mass spectrometry: ionisation of the sample to produce gas-phase ions, separation of the ions according to their mass-to-charge ratio and finally detection and recording of the separated ions.

1.2.2 Sample Introduction and Ionisation

Analytes must be ionised before they can be separated and detected. There are several ionisation techniques and their use depends on the physical state of the sample, its volatility, thermal stability and ionisation characteristics produced or required. For example, solid samples may be introduced into the ionisation source using a direct probe with a pressure lock system to protect the high vacuum of the spectrometer. The sample can then be ionised by fast atom bombardment, field desorption or matrix assisted laser desorption.

Liquid samples present a problem because of the great volume of solvent vapour produced if the sample is introduced directly into the spectrometer in a liquid stream such as a liquid chromatography eluent. To avoid compromising the system vacuum, or overworking the pumping system, it is common to ionise the sample at atmospheric pressure, removing the majority of the solvent using a rough pump connected to the source, and allowing only the ionised species to enter the mass analyser. Thermospray was the favoured ionisation technique for

liquid sample streams until electrospray ionisation, atmospheric pressure chemical ionisation (APCI) and atmospheric pressure photon ionisation (APPI) became commercially available. Other ionisation sources available to the mass spectrometrist are chemical ionisation (CI), electron ionisation (EI), field ionisation (FI) and laser desorption (LD). EI, APCI and ESI have been used exclusively in this work and are described in more detail below.

1.2.2.1 Electron Ionisation

Electron ionisation (EI) is an ideal ionisation technique for use when the mass spectrometer is coupled to a gas sample stream or a gas chromatographic (GC) eluent as analytes compatible with GC analysis are generally ionisable by EI. The first EI source was produced by Dempster[76] and was then modified and improved by Bleakney[83] and Nier.[84]

A schematic diagram of an EI source is presented in Figure 1.1. A tungsten or rhenium filament is heated in a vacuum, emitting electrons which are accelerated towards a collecting plate by the application of a potential difference. The electron beam passes through a narrow aperture and crosses the ionisation region.

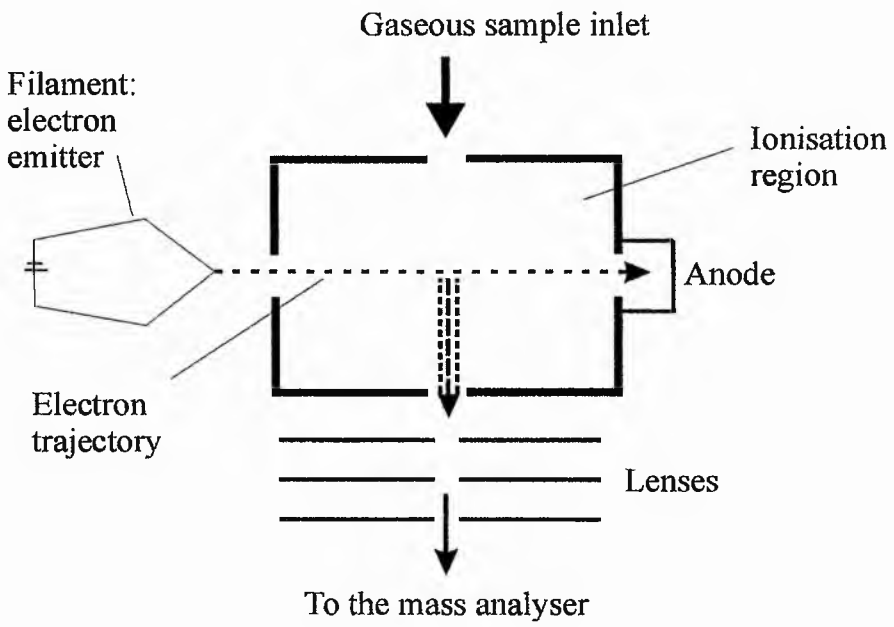

Figure 1.1. A schematic diagram of an electron ionisation source.

Gaseous or vapour phase sample molecules are directed into the ionisation chamber. The electron beam interacts with these molecules imparting energy causing some molecules to lose an electron to form a radical cation ($M^{+\bullet}$) or molecular ion (Equation 1.1).

$$M + e^- \rightarrow M^{+\bullet} + 2e^- \qquad \text{(E.1.1)}$$

Molecular ions with sufficient internal energy following ionisation will undergo fragmentation to yield fragment ions: F_1^+, F_2^+, etc. (Equation 1.2)

$$M^{+\bullet} \rightarrow F_1^+ + F_2^+ + \ldots. \qquad \text{(E.1.2)}$$

The resulting ions are extracted from the ionisation chamber through a series of focusing lenses and into the mass analyser.

The number of ions produced in the EI source depends upon the acceleration potential used for the electron beam. As the electron energy increases the number of ions produced increases to a maximum at approximately 70 eV. Figure 1.2 demonstrates the ion current maximum for different compounds.

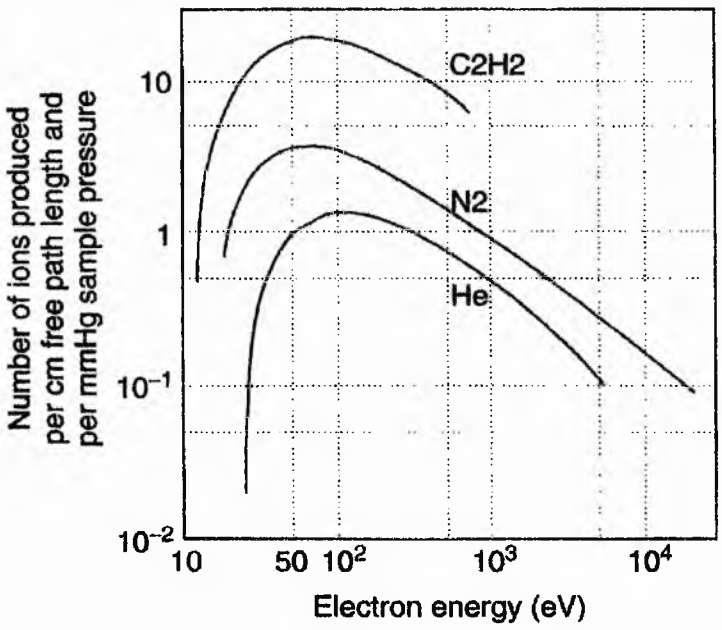

Figure 1.2. Effect of ion energy on the number of ions produced by EI.[73]

The ionisation efficiency for EI is relatively low with less than 1 in 100 molecules in the source being ionised. To maximise the number of ions produced and thus the sensitivity of the instrument, the electron energy or acceleration potential is usually set at around 70 eV. However, 8-12 eV is usually sufficient to ionise most organic molecules and the excess energy often causes the newly

formed ions to fragment further (Equation E1.2), and in some cases leaving no molecular ion. This produces complex spectra for even simple molecules, but is useful for structural studies. Over the many years of EI use, large reference libraries of spectral data have been generated to assist chemists with identification and interpretation of EI spectra, further increasing the use of EI as an ionisation technique.

1.2.2.2 Electrospray Ionisation

Electrospray ionisation is an atmospheric pressure, liquid phase ionisation technique first described by Dole[81] in 1968 but only successfully coupled with mass spectrometry by Fenn[82] in 1984. An analyte solution is passed through a capillary held at a high electrical potential (1-5 kV) and concentrically surrounded by a nebulising gas supply (see Figure 1.3).

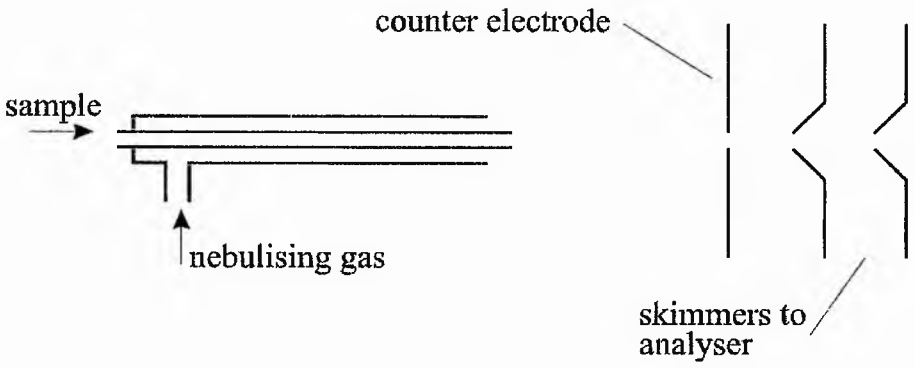

Figure 1.3. A schematic diagram of an ESI source.

As the liquid leaves the end of the capillary it forms an aerosol consisting of charged droplets. Aided by a drying gas flow the solvent evaporates causing the droplets to decrease in size, in effect increasing the charge concentration on the droplet surface. When the droplet reaches a critical size the charge density on the droplet surface equals the surface tension (the Rayleigh limit) causing the droplet to explode into smaller droplets. Finally, as the solvent is driven off, singly or multiply charged sample ions and solvent clusters remain. The production of multiply charged ions is very beneficial in the analysis of high molecular weight compounds that would otherwise be beyond the range of the mass spectrometer (e.g. peptides, proteins and polysaccharides).

1.2.2.3 Atmospheric Pressure Chemical Ionisation

Shahin[85] first described the mass spectrometric analysis of air samples ionised at atmospheric pressure using a corona discharge tube in 1966. Horning[86-87] developed this idea to produce an atmospheric pressure chemical ionisation source for use with liquid chromatographic eluents.

Atmospheric pressure chemical ionisation is a gas phase ionisation technique. The analyte solution is introduced into a nebuliser consisting of a capillary which is concentrically surrounded by a sheath gas supply which converts the liquid stream into a fine spray (Figure 1.4).

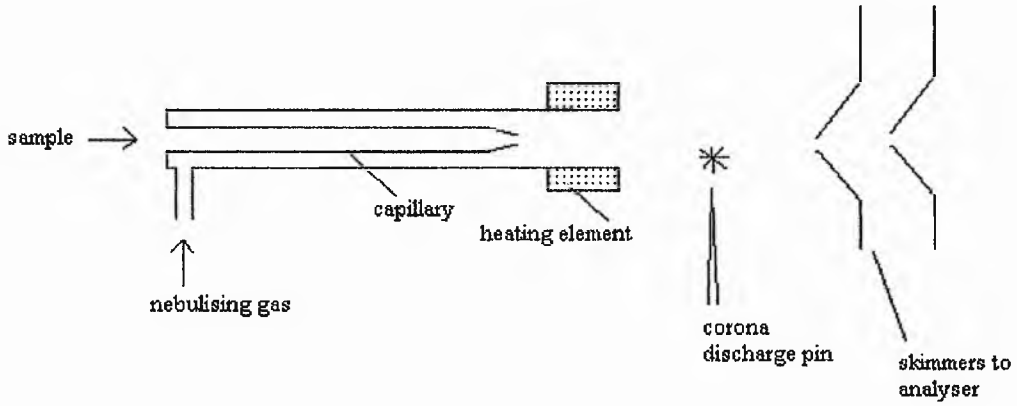

Figure 1.4. A schematic diagram of an APCI source.

The solvent is vaporised, by a combination of heat and drying gas, to produce vaporised solute molecules. A pin operating at approximately 3 kV creates a corona discharge in the solvent vapour generating an ionised solvent cloud. The vaporised solute ions undergo a sequence of collisions with water/solute clusters generating proton transfer reactions which results in positive $(M+H)^+$ and negative $(M-H)^-$ ions. Thus desolvation and ionisation occur separately rather than in the same process as happens in ESI. The sample ions are protonated or deprotonated, undergo little fragmentation and are mostly singly charged, so the molecular weight range is limited by the mass range of the analyser (up to 4000 Daltons for a single quadrupole MS). APCI can operate with solvent flows from 0.2-2.0 ml/min and can accommodate a wide range of volatile buffers and solvents making it a good technique to use with HPLC analysis without solvent splitting being required and is thus widely used in the pharmaceutical industry for the analysis of non-polar and semi-volatile compounds.

1.2.3 Mass Analysers

The analyser region of the mass spectrometer is maintained under high vacuum (10^{1}-10^{-8} Pa) in order to reduce the number of gaseous molecules in the path of the ions. Ion-molecule collisions cause the ions to deviate from their trajectory, lose energy and discharge on the sides of the analyser resulting in poor sensitivity, complex spectra and broad peaks. The absence of oxidising gases also protects the filaments and other sensitive components in the instrument, thus prolonging the instrument life and maintaining sensitivity. The MS vacuum is generated and maintained using a system of efficient turbomolecular and rotary-vane pumps. As the samples are initially at atmospheric pressure and the analyser under vacuum there must be an interface between the two regions to protect the vacuum and ensure the pumping capacity is not exceeded. This depends upon the type of ionisation source used.

A series of lenses direct the ions from the ionisation source into the analyser region where they are separated according to their mass-to-charge ratio. There are many types of mass analysers now commercially available including: magnetic sector, linear quadrupole, quadrupole ion trap, time-of-flight (TOF) and Fourier Transform ion cyclotron resonance (FTICR) and combinations of these analysers (for example triple quadrupole TOF-MS). Linear quadrupole mass analysers are the only analysers discussed in detail here due to their exclusive use in this work.

1.2.3.1 Linear Quadrupole Mass Analysers

Paul and Steinwedel[88] described the linear quadrupole mass analyser in 1953 and from this work the commercially available quadrupole mass analysers have developed. Quadrupole mass analysers consist of four concentrically arranged, parallel rods which are either circular or hyperbolic in cross section (see Figure 1.5). The rods are subjected to superimposed radio frequency (RF) and direct current (DC) voltages. The rods are connected such that diagonally opposite rods have a DC current applied whilst the inverse current is applied to the other pair of rods. Likewise, an RF field is applied to one pair of rods and the RF field, shifted by 180° (half a cycle), is applied to the other pair.

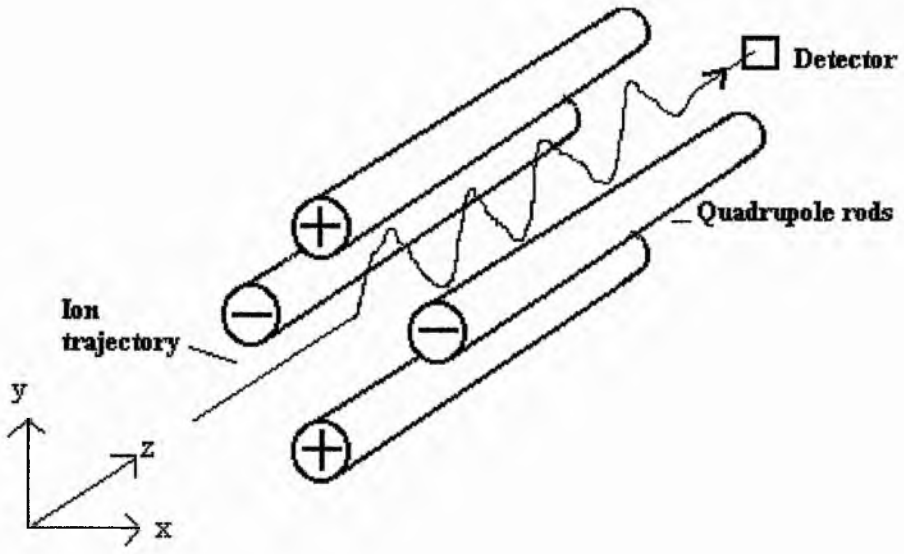

Figure 1.5. Quadrupole mass analyser.

Ions are propelled into the mass analyser from the ion source by means of an accelerating voltage. As the phase of the current applied to the rods changes the ions are alternately attracted and repelled by the rods. The ions follow a complex trajectory down the axis of the quadrupole depending on their mass to charge ratio and the voltages applied to the quadrupole rods. An ion with an unstable trajectory will either discharge on the rods or be repelled out of the quadrupole region. Only when a DC and RF voltage of a specific amplitude is applied will an ion of a particular *m/z* ratio have a stable trajectory and pass along the quadrupole and into the detector.

The path of an ion through the quadrupole field has been described using the generalised form of the Mathieu equation:

$$\frac{d^2u}{d\xi^2} + (a_z + 2q_z \cos 2\xi)\, u = 0 \qquad \text{(E.1.3)}$$

Where: $u = x$, y or z axis

$\xi = \omega t/2$

$\omega = 2\pi f$ (f = RF frequency, MHz)

'a_z' and 'q_z' are dimensionless constants

Two solutions for the Mathieu equation involving a_z and q_z are:

$$a_z = \frac{-8\, e\, z\, U}{m\, r_o^2\, \omega^2} \qquad \text{(E.1.4)}$$

$$q_z = \frac{4\,e\,z\,V}{m\,r_o^2\,\omega^2} \qquad \text{(E.1.5)}$$

Where: V = RF voltage amplitude

U = DC voltage amplitude

r_o = radius of quadrupole arrangement

m = mass of the ion

e z = charge on the ion

Figure 1.6 shows a graph of a_z plotted against q_z (the graph is symmetrical about the q_z axis but only the top half is portrayed here) where there is a region where an ion has a stable trajectory through the quadrupole. That is, the ion travels from the ionisation source to the detector without discharging on the rods.

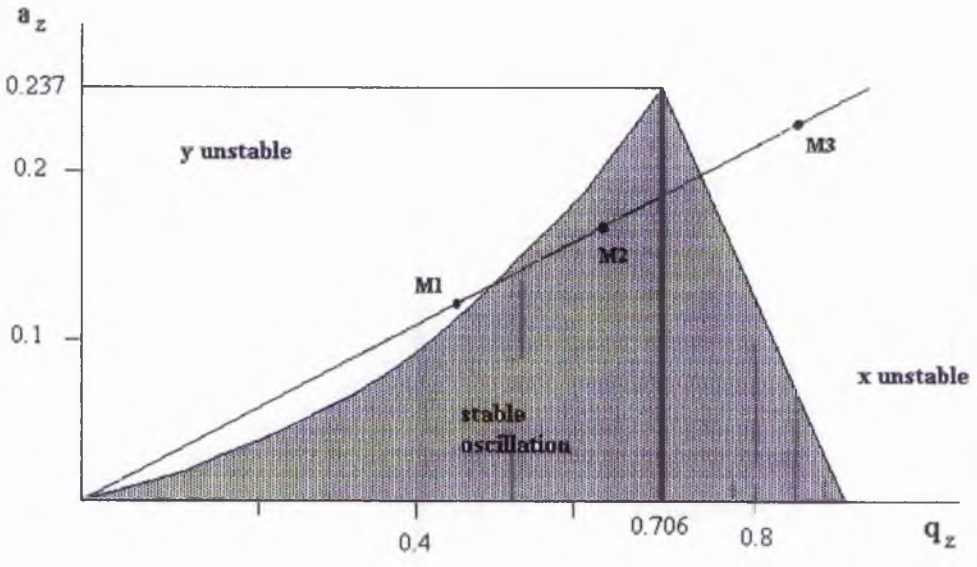

Figure 1.6. Stability diagram for the trajectory of an ion in a linear quadrupole analyser

For an ion of a particular mass and charge in a quadrupole analyser the radius of the quadrupoles (r_o) is constant, as are the mass and charge of the ion (m and ez). Thus:

$$q_z \propto \frac{V}{\omega^2} \qquad \text{(E.1.6)}$$

$$a_z \propto \frac{-2U}{\omega^2} \qquad \text{(E.1.7)}$$

To maintain the stable trajectory through the analyser and into the detector the working point of an ion must be brought into the stable region of the chart. This is usually achieved by changing the DC and RF voltage amplitudes whilst maintaining a constant DC/RF ratio. By changing the voltages it is possible to selectively scan the ions, detecting them in increasing mass order.

Quadrupole mass analysers have a working range of up to 4000 amu for the more expensive instruments. They can scan up to 5000 amu per second and have an accuracy of 0.1-2 amu. This makes them a good work-horse instrument that are easy to use, relatively cheap and usually reliable for a range of applications.

1.2.4 Detectors

Ions leaving the analysers enter the detector region where the ions must be registered and converted into a usable signal. Original mass spectrometers recorded the ions as spots on a photographic plate. These were replaced by more sophisticated digital measuring systems.[70, 73]

1.2.4.1 Faraday Cup

A Faraday cup or cylinder consists of a conversion dynode, a cup shaped plate typically composed of a beryllium-copper alloy, and an amplification device. When an electron collides with the plate it emits a shower of electrons which cause a current to be detected. This current is magnified and recorded via a data collection system. Due to the nature of the signal amplification this detector type is susceptible to ion sensitivity and high noise levels.

1.2.4.2 Electron Multiplier

An electron multiplier works as an extended version of the Faraday cup and consists of a conversion dynode and a horn shaped continuous conversion dynode. A typical electron multiplier is represented in Figure 1.7

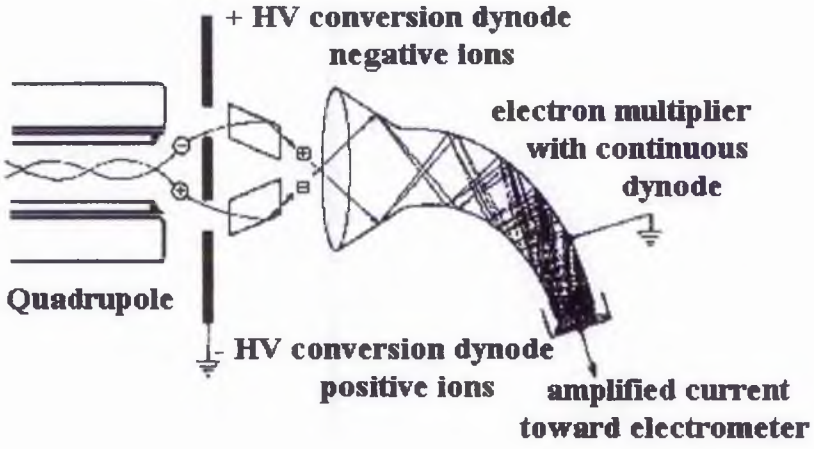

Figure 1.7 An electron multiplier.

As an ion strikes the dynode it emits a burst of electrons which are accelerated towards a continuous conversion dynode. Here the electrons cascade through a series of funnelled plates, with each impact generating several secondary electrons. The electrons move through a series of collisions with further plates until they reach a cathode where the current is measured and reported to a data collection device. The ion signal may be magnified up to a factor of 10^7. Due to the contamination effect of the cations on the dynodes these electron multipliers have a life span of approximately two years when they must be replaced. Electron multipliers are not as precise as a Faraday cup, but they have a lower noise level and a rapid response time allowing for faster scanning.

1.2.4.3 Photon Multiplier

A photon multiplier consists of two dynodes, one for positive ions and one for negative ions. Ions striking the dynodes emit electrons which are accelerated towards a phosphorescent screen which emits photons when an electron strikes the screen. The photons enter a photon multiplier tube where the signal is amplified in a similar way to the operation of the electron multiplier. However the photon multiplier is sealed and maintained under a clean vacuum so resists contamination. Compared with an electron multiplier, the photon multiplier has a greatly extended life span.

1.3 Membranes

1.3.1 Introduction

A membrane can be defined as a structure forming a barrier between two phases, which selectively restricts the passage of chemical components between the two phases. Natural membranes were studied by Nollet[89] in 1748 when he used a pig bladder as a semipermeable membrane in his studies of osmosis. The first synthetic membrane was manufactured from cellulose nitrate by Fick[90] in 1855 in order to study diffusion. Since then membrane production has been transformed into a highly technical science with membranes being manufactured from substances ranging from glass fibres to complex polymeric materials, and their uses becoming the subject of many scientific papers.[89-155]

Synthetic membranes can be classified according to their physical structure (microporous, homogeneous, electrically charged gels and supported liquid membranes), topography (flat sheet or tubular) and their chemical composition (hydrophobic and hydrophilic).

1.3.1.1 Microporous Membranes

Microporous membranes have a uniform structure consisting of a matrix of pores ranging from 0.1 to 20 μm in diameter. They are produced from a range of polymeric materials including polycarbonate, polyethylene, polypropylene,

polytetrafluoroethylene, polyvinylidene fluoride and nitrocellulose. These polymers are generally hydrophobic in nature, but surface treatment of the membrane has been used to convert the membranes into hydrophilic materials.[91] Here a polar substituted acrylate or acrylamide is coated onto the membrane surface along with a cross-linking agent. The surface is then exposed to a high energy ionising electron beam which causes the polymers to react and cross-link generating a polar, hydrophilic membrane surface but retaining the physical characteristics of the original membrane.

Industrial production of microporous membranes uses a variety of techniques.[92-93] The sinter process involves pressing a powder into a thin film then heating it to just below its melting point, at which point the particles fuse forming a membrane structure with a range of pore sizes. Track-etched membranes are formed when a thin film of polymer is irradiated by charged particles which form tracks through the membrane material. These tracks are enlarged by exposure to corrosive chemicals resulting in very precisely defined pore sizes in the membrane sheet. Figure 1.8 presents an example of track etched polycarbonate membrane.[94]

Figure 1.8 A track etched polycarbonate membrane (adapted from Reference 94)

Another method of production is to stretch extruded polymeric material perpendicular to the direction of extrusion. The polymer stretches rather like melted cheese to form a non-uniform pore structure. See Figure 1.9 for an example of a extruded PTFE membrane.[94]

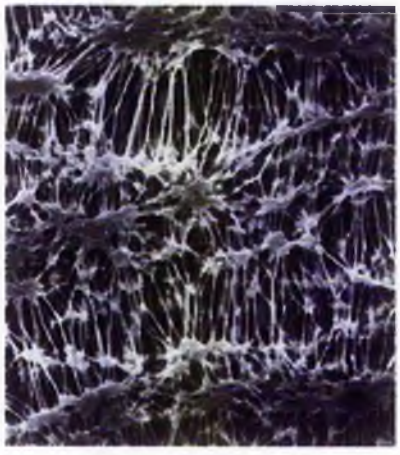

Figure 1.9 An extruded PTFE membrane (adapted from Reference 94)

The most common method of microporous membrane manufacture is phase inversion or film casting. A polymer solution is induced to precipitate by changes in temperature or solvent composition. The polymer separates out into two phases: a polymer rich solid phase that becomes the membrane structure, and a solvent rich, liquid phase filling the pores. Figure 1.10 presents a phase inversion polyvinylidene fluoride membrane.[94]

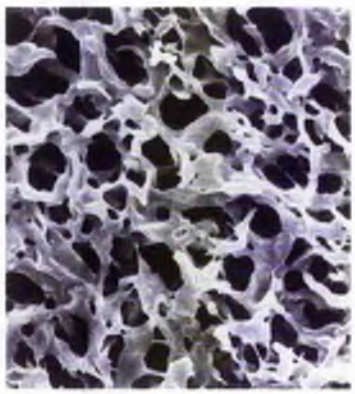

Figure 1.10 A typical polyvinylidene fluoride membrane.
(adapted from Reference 94)

The driving force behind microporous membrane separation is either hydrostatic pressure or a concentration gradient.[93] Hydrostatic pressure results in a sieving action and is dependent on the molecule sizes and membrane pore sizes. It is used to remove particulates in suspensions and is utilised in sterile solution manufacture to remove microbiological material via a filtering mechanism. A concentration gradient results in the diffusion of molecules through the solution

filling the membrane pores or adsorption/desorption of analyte, dependent upon their chemical affinity for the membrane material. Applications include the use of microporous membranes for the separation of specific chemicals from solution, solution degassing, as protein binding agents and, to a lesser extent as sample preconcentration devices (as will be discussed later in this chapter). Membranes are selected according to their pore size, polymeric material and hydrostatic properties and are generally produced as flat sheets.

1.3.1.2 Semi-Permeable Membranes

Semi-permeable or non-porous membranes (homogeneous membranes) have a uniform, solid film structure. The membranes are manufactured from polymer solutions by blow and press moulding or casting into flat sheets or by the extrusion of melted polymer into flat sheets and hollow fibres. The membranes are produced using polymers such as polydimethylsiloxane (silicone rubber), latex and nafion.[95]

Separation of analytes is based upon their solubility in the membrane material, influenced by temperature, pressure, electrical potential and concentration gradient. Mass transfer is by diffusion through the membrane material so the rate of separation is based on analyte affinity for the membrane, membrane thickness and surface area.

Semi-permeable membranes are used for the separation of gas mixtures (e.g. hydrogen separation and natural gas processing and recovery), azeotropic mixtures and the separation of volatile components of liquid mixtures by MIMS analysis.

1.3.1.3 Electrically Charged Membranes

Electrically charged, ion exchange membranes are formed by applying a fixed charge to a polymer gel. The polarity of the charge determines the selectivity of the membrane. Cation exchange membranes have functional groups such as $-SO_3^-$, $-COO^-$, etc., resulting in the selective exchange of cations across the membrane. Anion exchange membranes may have functional groups such as $-NH_3^+$, $-NH_2^+$, etc. and only allow the exchange of anions across the membrane.

1.3.1.4 Supported Liquid Membranes

A supported liquid membrane consists of an organic solvent (such as octane, n-undecane, di-n-hexylether and tri-n-octyl phosphate)[96] held in the pores of a hydrophilic supporting structure (usually a porous membrane) by capillary action. The liquid acts as a diffusion pathway for analyte molecules which can be preconcentrated from a dilute analytical sample into a small liquid volume, prior to analysis.

1.3.2 Membrane Inlet Mass Spectrometry

Membrane inlet mass spectrometry (MIMS) has been used widely as an interface for the analysis of dilute volatile organic compounds in aqueous and gaseous samples by transport across a semi-permeable membrane. The three stage process of analyte transfer across a membrane in MIMS analysis is known as 'pervaporation'.[97] Typically the analyte solution is sampled into a 'donor' stream and is passed across one surface of the membrane. A proportion of the solvent and analyte adsorb onto the membrane surface, diffuse through the membrane and desorb into the 'acceptor' stream flowing across the other surface of the membrane. This is represented schematically in Figure 1.11.

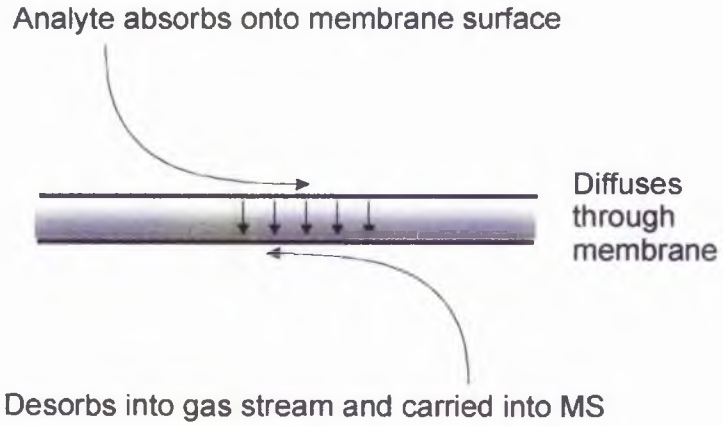

Figure 1.11. The analyte transport process in a MIMS membrane

Siloxane, semi-permeable, hollow fibre membranes are most commonly used in MIMS analysis. They are very hydrophobic and are highly selective in favour of

volatile nonpolar compounds and discriminate against polar matrices, making them highly suitable for the analysis of dilute aqueous solutions of organics.

The popularity of MIMS as an analytical technique lies in the fact that the concentration of very dilute samples can be brought to a measurable level in a relatively short time period with minimal sample handling and solvent use. The enriched analytes can then be quantified using mass spectrometry with the selectivity and speed the technique affords.

Hoch and Kok[98] developed the first MIMS interface in 1963 to study the kinetics of photosynthesis by measuring oxygen and carbon dioxide in liquid samples. This established the principle of interfacing liquid samples with the vacuum of a mass spectrometer via the selective barrier of a membrane. MIMS has been developed into a commonly used technique for sample preconcentration and online analysis through the increasing improvements to the interface design.[99-103]

1.3.2.1 Liquid-Gas Membrane Inlet Mass Spectrometry Development

Westover *et al* [104] designed the first hollow fibre probe device. They sealed a silicone membrane to one end of a transfer line and placed this directly into the sample solution. Analyte molecules selectively diffused through the membrane into the evacuated transfer tube interfaced directly into the vacuum source of a mass spectrometer. This gave a sample enrichment factor of 1 x 10^4 and detection limits of ~10 ppb for chloroform in aqueous solution. This probe is

known as a direct insertion MIMS probe or 'flow by' probe type as the analyte flows past the membrane in the sample vessel.

Instead of allowing the analyte to flow past the membrane surface in a passive manner Cooks pumped the analyte through a loop of membrane tubing located inside the MS source.[105] They went on to support this loop of membrane so that instead of the probe being inserted into the analyte solution the solution was pumped through the hollow fibre held around the end of the probe tip.[106] This "flow through" probe was attached to the leak valve inlet of a CI-MS. The new probe design reduced the detection levels of organic solvents (benzene etc) to ppb levels.

The flow through probe was further developed when the hollow fibre was sealed inside an evacuated tube connected to the MS source via a sampling valve and analyte was pumped through the hollow fibre. Lapack *et al*[107] used this MIMS device to analyse, on-line, both the liquid and gaseous waste streams from a wastewater treatment plant.

The long transfer line connecting the membrane to the MS in these MIMS probes could be problematic, causing long response times due to absorbance of analyte to the transfer line walls. Lauritsen[108] designed a new inlet with a flat sheet membrane mounted close to the MS source. Aqueous sample solution was pumped across the membrane surface and analytes were transferred directly into the vacuum region. The direct insertion membrane probe resulted in a more

efficient sampling method with quicker responses to changing analyte concentrations. This probe type was then used to monitor the liquid stream of a penicillin fermentor in real time.[109]

Most MIMS devices use non-porous (semi-permeable) hydrophobic membranes in order to discriminate against water in the samples, in order to keep the vapour levels in the evacuated MS region to a minimum. Lauritsen *et al*[110] used porous polypropylene membranes with this direct insertion membrane probe. The porous membrane allowed not only the analyte (organic solvents) but also water to pass into the MS source where the water vapour was used as a reagent gas for the chemical ionisation source. Unfortunately the water vapour significantly reduced the life of the filament. This problem was overcome by replacing the filament with a glow discharge allowing organic compounds in water to be monitored over many hours.[111] A polyetherimide-silicone composite membrane was tested in this probe type[112] as the mechanical strength of the combined polymer membrane was more able to withstand the physical demands of the MS vacuum. The membrane gave higher analyte response times and sensitivity for volatile organic compounds compared with the usual silicone membrane.

The MIMS techniques used up until this point were interfaced directly with the ionisation source of the mass spectrometers and were maintained under vacuum. This has the disadvantages of requiring the instruments to be vented in order to maintain the membranes and there were often issues with condensation of the

analyte on the walls of the membrane probes leading to carryover effects and increased response times.

In 1987 Nguyen and Nobe[113] used the novel idea of passing the analyte over the outer surface of the membrane and purging the middle of the hollow fibre with helium. They connected this helium flow to the inlet of a GC column. Slivon's group[114] developed this idea and connected the helium purge to the ion source of a mass spectrometer. The helium flushed the analyte though the transfer lines thus minimising condensation and the associated carry-over effects whilst increasing detection limits and decreasing analysis time.

A jet separator was added to the sampling configuration between the membrane and the mass spectrometer.[115] Creaser and Stygall[116] used a silicone hollow fibre membrane located in a glass-lined stainless steel tube as a flow over MIMS interface to analyse the volatile organic components of aqueous effluents at ppb levels.

The flow-over, helium purged MIMS interface has become a popular interface design and has been used in numerous analysis applications. An example of this interface type is presented in Figure 1.12.

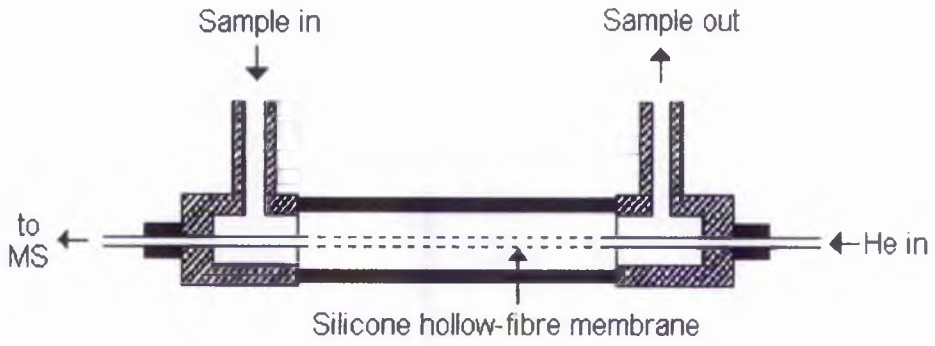

Figure 1.12 A Flow-over, helium purged MIMS interface (from Reference 116)

1.3.2.2 Liquid-Gas MIMS and Process Monitoring

MIMS has been used as a technique for monitoring dynamic processes in real time sampling both gaseous and vapour phase streams. Of particular interest in this project is the analysis of liquid phase process streams.

A remarkable early study into microbial physiology[117] used a membrane as an interface between a bacterial reaction vessel and a mass spectrometer. Dissolved oxygen and carbon dioxide were monitored in real time as the bacteria reacted to changing light levels.

Tou's group[118] used a silicone membrane loop on the end of a probe, suspended in a reaction vessel, to measure the formation of potentially explosive nitrogen trichloride. They investigated a range of membrane materials in order to find one

that was suitable for the harsh conditions the membrane was exposed to. They demonstrated the feasibility of MIMS as an on-line analysis technique in a situation where conventional methods were unsuitable.

A microbial oxidation reaction was monitored by Lauritsen and Gylling.[119] During their research they noted that the siloxane tubing in the peristaltic pump absorbed analyte from the reaction mixture, causing a loss from the system over a period of hours. Alternative pumps were investigated and a piston pump was identified as a superior alternative. However, if a peristaltic pump must be used in a MIMS experiment then it must be positioned on the waste tubing, downstream of the membrane.

Cooks[120] used a MIMS interface to monitor the ethanol content of, initially a 2 litre[121] and, later a 9,000 litre fermentation reactor using a MIMS interface. The reaction was sampled on-line, in real time, every three minutes for an interval of four days with only minimal maintenance of flushing the membrane with ethanol twice daily. Cooks proposed that semi-volatile reaction components may be analysed using the same system.

A feedback system was set up by Hansen and Deng[122] to regulate the phenoxyacetic acid levels (determined by MIMS) in a penicilin fermentor, whilst Kotiaho's group[123] monitored possible contaminants in a waste-water stream using a fully automated MIMS system. Creaser's group[124] have designed a novel, in-membrane thermal desorption MIMS (IMP-MIMS) technique to monitor the

biodegradation of semi-volatile acids as an alternative to HPLC analysis. MIMS and IMP-MIMS were then applied to the online analysis of multiple components of a bioreactor.[125] The techniques were used to identify metabolites and predict degradation pathways.

Online MIMS was used by Nogueira *et al*[126] to monitor phenol and trichloroethylene degradation and carbon dioxide production in water. The extent of degradation and the half lives for three reactions were determined allowing the processes to be realistically compared.

A very complex reaction system; the iodate catalysed decomposition of hydrogen peroxide, was characterised using MIMS with a PTFE membrane.[127] The precise timing of the appearance of different species in the reaction process generated valuable new data to contribute to a new reaction model.

Pedersen *et al*[128] compared MIMS analysis with NIR and fluorescence spectroscopy for the on-line monitoring of a fermentation process. They determined that the combination of the three techniques resulted in a comprehensive system of monitoring for the process with the ability to detect numerous deviations from normal operation.

1.3.2.3 Liquid - Liquid Membrane Interface Development and Applications

The preceding two sections detailed the analysis of volatile and semi-volatile analytes by membrane inlet mass spectrometry. The analysis of non-volatile samples in liquid process streams is a more recent development and on-line membrane analysis for process monitoring has concentrated mainly on sample enrichment.[129-132]

A supported liquid membrane (SLM) is a three phase system comprising of an organic solvent held in the pores of a supporting structure (usually a microporous membrane such as PTFE) by capillary action. The 'donor' sample solution is pumped across one surface of the membrane. The liquid in the membrane pores serves as a diffusion pathway for analyte molecules which are pre-concentrated by their transfer from a dilute analytical sample into the small liquid 'acceptor' volume on the other side of the membrane. This concentrated sample can then be removed for analysis.

Audunsson[133] developed a supported liquid membrane extraction system as a sample enrichment technique. A 'U' shaped groove was cut into two Teflon® blocks and a liquid impregnated membrane was clamped between the grooves. This membrane interface was used to extract analytes from a process stream into an organic solvent in order to increase their concentration by 100-250 times to a quantifiable level. He then went on to use this SLM system to enrich amines

from urine prior to chromatographic analysis.[134] The efficiency of the analyte extraction was improved by extending the contact surface area of the membrane using spiral grooves on the interface plates.[135-136]

Microporous membrane liquid extraction (MMLE) is similar to SLM as the pores are filled with the acceptor solvent, rather than a third phase. MMLE consists of an aqueous 'donor' sample phase separated from an organic 'acceptor' phase by a microporous membrane. Unlike MIMS analysis the two phases are usually stagnant during the extraction period, but are then pumped away to either a sample holding device prior to analysis or an on-line analyser.

In MMLE, the selectivity of the system depends upon the nature and size of the membrane pores, the chemical affinity for the acceptor solvent plus any physical or chemical interactions between the analyte and the membrane material. SLE depends more upon the chemical affinity for liquid membrane and is comparable to liquid-liquid solvent extraction.

Jönsson and Mathiasson's group in Lund, Sweden, performed extensive research into the use of microporous membranes as separation devices.[137] They later automated the extraction process to selectively remove anaesthetics from blood plasma and analyse the resultant solutions by GC.[138]

Jönsson *et al* combined SLM with MIMS analysis when they investigated alternatives to solid polymer membranes as the interface for MIMS analysis.

Polypropylene membranes were soaked in a range of organic solvents and their analyte selectivity was investigated.[139-142]

Trace levels of thiophanate-methyl and its metabolites were enriched using a PTFE based, MMLE technique. The system was then automated and once the extraction time was over the analyte enriched acceptor solvent was pumped into a sample loop and switched into a flow of mobile phase for direct HPLC analysis.

Guo and Mitra[143] used a hollow fibre microporous polypropylene membrane to extract semi-volatile organic compounds from water, before continuous, on-line analysis by HPLC. Bjergaard[144] developed a microextraction device for drug analysis in urine. An aliquot of sample was placed in a vial and a piece of hollow fibre polypropylene tube was filled with acceptor solvent and placed in the vial. After 45 minutes the sample was removed from the hollow fibre tubing and analysed by HPLC or CE.

Mullins[145] produced a non-porous silicone membrane probe and used it as an infinite sink to extract sulfonylureas from human plasma samples, prior to analysis by HPLC-MS/MS. Cukrowska's team[146] used a similar device to extract manganese II from biological fluids prior to LC analysis.

MMLE has been used extensively to preconcentrate analytes before analysis by a chromatographic technique.[147-151] Recent applications include Jiangs's extraction of chlorophenols,[152] sulfoylureas[153] and aromatic amines[154] from water samples.

Kuosmanen *et al*[155] coupled pressurised hot water extraction system to an on-line MMLE-GC system producing an effective system for the automated analysis of PAHs in soil samples.

1.4 Project Aims

The aims of the research reported in this thesis were to:

- Explore the use of microporous and semi-permeable membranes in process analysis.

- Develop membrane interfaces using microporous and semi-permeable membranes, and couple these with mass spectrometry.

- Evaluate interfaces for process monitoring of volatile and semi-volatile analytes in liquid and gaseous streams.

- Apply the membrane interfaces to process monitoring of model pharmaceutical reaction systems.

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Chapter 2

Reaction Monitoring Using a Liquid-Liquid Membrane Interface

2.1 Introduction

Membranes interfaces have been used widely in sample preparation,[1-9] and are generally employed as sample enrichment devices. In membrane inlet mass spectrometry (MIMS), volatile or semi-volatile compounds are extracted selectively from a liquid stream, transported across the membrane, desorbed into a helium flow and detected by mass spectrometry.[6-9] Liquid-liquid interfacing, in which two liquid phases are separated by a microporous membrane, has been reported in combination with mass spectrometry.[10,11] The pores of the membrane are filled with a solvent which acts as an interface between a flowing donor solvent, containing the dilute analyte, and the static acceptor solvent into which the analyte diffuses. These systems offer high enrichment factors and selective analyte extraction for dilute sample systems. Microporous membranes have also been investigated extensively for use as pre-concentration devices for dilute samples using supported liquid membrane (SLM) and microporous membrane liquid-liquid extraction (MMLLE).[3,12,13]

A membrane interface may alternatively be used to achieve high dilution factors by controlling analyte transport across the membrane. This allows quantifiable mass spectrometric data to be obtained by bringing the analyte concentrations within the dynamic range of the mass spectrometer (pmol/μl) and thus preventing contamination of the mass spectrometer. In addition, membrane interfaces may be applicable to sample suspensions and heterogenous mixtures, which would

otherwise be impossible to analyse using flow injection analysis or direct sampling.

In this work, a microporous membrane has been used as a novel in-line interface for APCI-MS in order to dilute concentrated solutions of organic reaction mixtures to a level suitable for direct mass spectrometric analysis. The potential of the interface for pharmaceutical process analysis is demonstrated for the Michael Addition reaction of phenylethylamine and acrylonitrile in ethanol, and was investigated for the Mannich reaction of dimethylamine with formaldehyde and parahydroxyacetophenone. The combination of the use of a microporous membrane for dilution of the reaction mixture with mass spectrometric detection, allowed the on-line, real-time analysis of starting materials, reaction products and impurities.

2.2 Materials and methods

2.2.1 Chemicals

Acetonitrile (HPLC grade) and formaldehyde solution (AR grade, 37-40% aqueous solution, stabilised with 9.4% methanol) were obtained from Fisher Chemicals, (Loughborough, UK), ethanol (99.86-100%) from Hayman Ltd, (Witham, UK), and purified water from a Barnstead Nanopure Diamond purification unit (Barnstead, UK). Dimethylamine (60% aqueous solution) and parahydroxyacetophenone (>98%) were obtained from Fluka (Buchs, Switzerland), phenylethylamine (99%) from Lancaster Synthesis UK (Morecambe, UK) and acrylonitrile (99%) from Sigma-Aldrich Company Ltd. (Gillingham, UK).

2.2.2 Membrane Interface Set-up and Sampling

A microporous membrane was cut into a disk of approximately 1.2 cm diameter and clamped firmly between two stainless steel supporting plates carved with matching 'S' shaped grooves. Each groove had a path length of 45 mm and a volume of approximately 140 µl. The end of each groove was connected to inlet and outlet ports fitted with PEEK tubing (0.25 mm i.d., Alltech Associates Applied Science Ltd, Carnforth, UK). Figure 2.1 presents a photograph of the membrane interface.

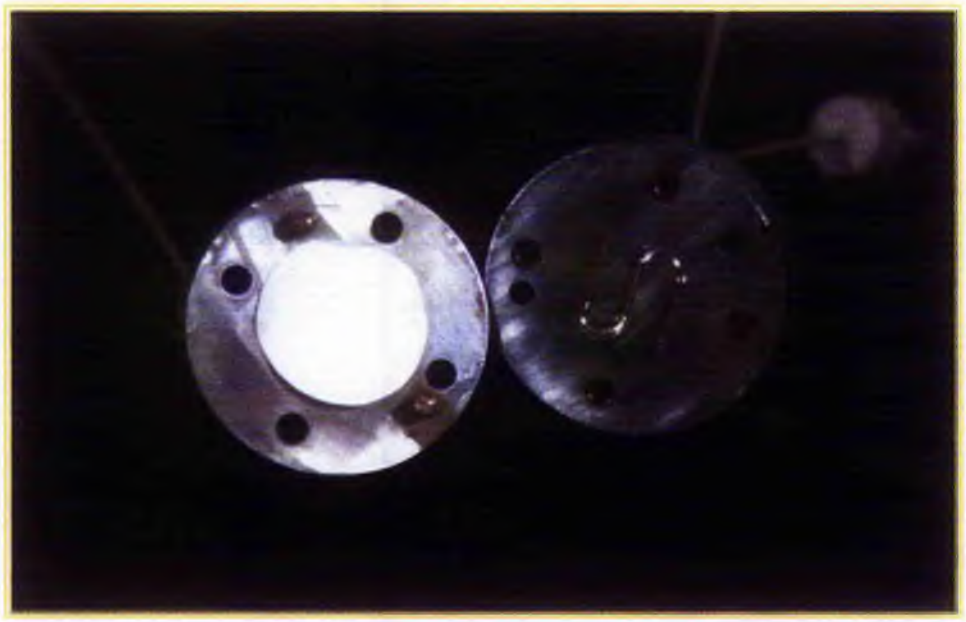


Figure 2.1 A photograph of the liquid-liquid membrane interface showing the membrane on the left plate and the 'S' shaped groove.

An aliquot of the reaction mixture was sampled and introduced into a donor solvent stream by means of a six port injection valve (Rheodyne six port manual injector, Rheodyne Europe GmbH, Bensheim, Germany) fitted with a 20 µl sample loop. The donor solvent was pumped through the 'S' shaped groove on the donor side of the membrane interface at 0.5 ml min^{-1} using an HPLC pump (Waters 501 HPLC pump, Waters, Milford, USA), to the outlet of the interface, which was fitted with a flow restrictor (approximately 110 mm x 100 µm i.d. fused silica capillary tubing held inside 0.25 mm i.d. PEEK tubing; Alltech Associates Applied Science Ltd, Carnforth, UK) and to waste. The acceptor solvent was pumped at 0.5 ml min^{-1} (Waters 2790 HPLC system, Waters, Milford, USA) across the opposite surface of the membrane and directly into the

atmospheric pressure ionisation source (APCI) of the quadrupole mass spectrometer (Platform LC, Micromass, Manchester, UK). The membrane interface was maintained at room temperature in all experiments. A schematic representation of the sampling system is presented in Figure 2.2.

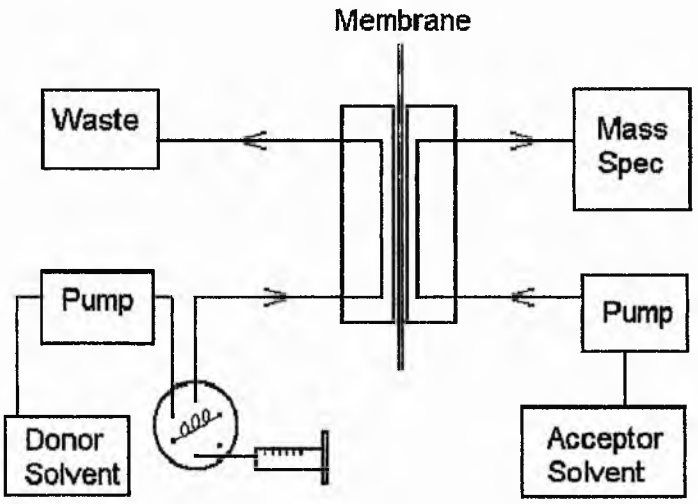


Figure 2.2. Schematic diagram of the membrane interface for APCI/MS

2.2.3 Michael Addition Reaction Monitoring Investigation

2.2.3.1 The Michael Addition Reaction

A bench scale Michael Addition reaction was studied (Scheme 2.1). To a stirred solution of phenylethylamine (20.8 ml, 0.16 moles) in ethanol (54.2 ml), acrylonitrile (25 ml, 0.16 moles) was added and the reaction mixture was stirred continuously and monitored at room temperature (~24°C).

2.2.3.2 Mass Spectrometric Analysis

In a typical experiment an aliquot of the Michael Addition reaction mixture was sampled and injected into a stream of ethanol donor solvent (0.5 ml min^{-1}). The donor solvent was passed across the surface of a hydrophobic polyvinylidene fluoride microporous membrane (Durapore®, Millipore Watford, UK, pore size of 0.1 μm) and a proportion of the solvent and analyte passed through the pores of the membrane and into the acceptor solvent stream (acetonitrile/water 90/10 % v/v) flowing across the other surface of the membrane (Figure 2.3). This acceptor solvent was pumped into the APCI source of the MS at 0.5 ml min^{-1}. The mass spectrometer (Platform LC, Micromass, Manchester, UK) was operated in positive ion APCI mode under the following conditions: mass range: 80-300 amu, APCI pin voltage: ± 3.5 kV, cone voltage: ±10 V, APCI probe heater: 400°C, source heater: 150°C.

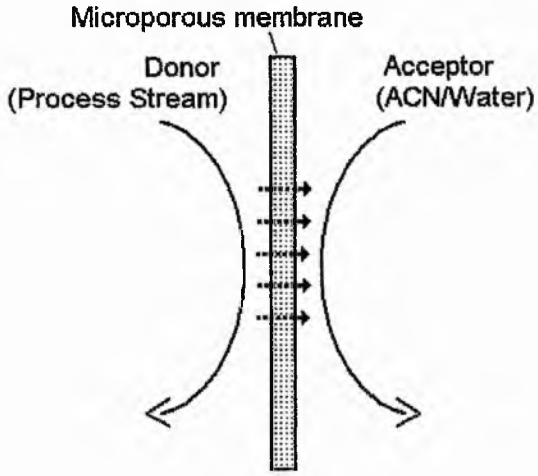


Figure 2.3 A schematic diagram of the donor/acceptor flow across the microporous membrane.

2.2.3.3 Membrane Selection

Various membrane materials including polyvinylidene fluoride (Durapore®, PVDF), polypropylene (PP), polystyrene (PS), polytetrafluoroethylene (PTFE), polyvinyl chloride (PVC), polycarbonate, cellulose ester and nylon were evaluated by examining their compatibility with the proposed solvent systems, their hydophobicity and available pore sizes. Suitable membrane materials were tested in the membrane interface using aliquots of the Michael Addition reaction mixture to determine their ability to transport analyte molecules between the donor and acceptor phases.

2.2.3.4 Acceptor Solvent Selection

Acceptor solvents were prepared by mixing acetonitrile and purified water in a range of compositions from 100% acetonitrile to 50/50% acetonitrile/water. A solution of the reaction mixture was prepared, allowing the reaction to go to completion (Section 2.2.3.1). 20 µl aliquots of this neat reaction mixture were introduced into the membrane interface and analysed according to the procedure in Section 2.2.3.2 using different acceptor solvent compositions.

2.2.3.5 Liquid-Liquid Membrane Dilution Effect

A solution of the reaction mixture was prepared, allowing the reaction to go to completion (Section 2.2.3.1). A 20 µl aliquot of this neat reaction mixture was introduced into the membrane interface and analysed according to the procedure in Section 2.2.3.2. A 20 µl aliquot of the same reaction mixture was serially diluted and introduced directly into the acceptor solvent stream for APCI-MS analysis.

2.2.3.6 Performance of the Liquid-Liquid Membrane Interface

Replicate aliquots (20 µl) of a solution of the Michael Addition reaction mixture, after the reaction had been allowed to go to completion (Section 2.2.3.1) were introduced into the membrane interface and the mass spectrometric responses recorded.

2.2.3.7 Real-Time Michael Addition Reaction Monitoring using the Liquid-Liquid Membrane Interface

A Michael Addition reaction was monitored in real-time using the reaction system detailed in Section 2.2.3.1, the membrane interface sampling system detailed in Section 2.2.2 and mass spectrometric conditions detailed in Section 2.2.3.2. This

system could be easily modified to become an on-line interface by the addition of a feed loop from the reaction vessel to the Rheodyne sampling valve.

2.2.4 Mannich Reaction Monitoring Investigation

2.2.4.1 The Mannich Reaction

A bench scale Mannich Reaction was studied (Scheme 2.2). Purified water (30 ml) was added to dimethylamine (DMA, 17 ml, 60% aqueous, 0.227 moles) the mixture was continuously stirred and cooled to 15°C in a stirred ice/water bath. Parahydroxyacetophenone (pHAP, 12.5 g, 0.092 moles) was added to the reaction vessel. Formaldehyde solution (15 mL, ~40%, 0.185 moles) was added over 30-50 minutes using a burette, with the temperature maintained at 15°C $\pm$ 2°C.

2.2.4.2 Mass Spectrometric Analysis

In a typical experiment an aliquot of the process mixture was sampled and injected into a stream of donor solvent (methanol/water 90/10 % v/v, 0.5 ml min^{-1}). The donor solvent was passed across the surface of the membrane and a proportion of the solvent and analyte passed through the pores of the membrane and into the acceptor solvent (water/methanol, 95/5 % v/v) flowing across the other surface of the membrane. This acceptor solvent was pumped into the APCI source of the MS at 0.5 ml min^{-1}. The mass spectrometer (Platform LC,

Micromass, Manchester, UK) was operated in positive ion APCI mode under the following conditions: mass range: 100-300 amu, APCI pin voltage: ± 3.3 kV, cone voltage: ±15 V, APCI probe heater: 400°C, source heater: 150°C.

2.2.4.3 Membrane Selection for Mannich Reaction Monitoring

Various membrane materials including nylon, polypropylene, hydrophobic Durapore® and hydrophilic Durapore® were evaluated by examining their compatibility with the proposed solvent systems, their hydophobicity and available pore sizes. Suitable membrane materials were tested in the membrane interface using aliquots of the Mannich Reaction mixture to determine their ability to transport analyte molecules between the donor and acceptor phases.

2.2.4.4 Performance of the Liquid-Liquid Membrane Interface

Replicate aliquots (20 µl) of a solution of the Mannich Reaction mixture, after the reaction had been allowed to go to completion (Section 2.2.4.1) were introduced into the membrane interface and the mass spectrometric responses recorded.

2.2.4.5 Investigation of Ion Suppression

The reaction mixture was diluted 10,000 fold with methanol/water (10/90 % v/v). This dilute solution of the reaction mixture was introduced to the mass

spectrometer at 0.2 ml min^{-1} to obtain a system response at a level approximately equivalent to that for a typical analyte. The individual components of the Mannich Reaction mixture (formaldehyde, dimethylamine, parahydroxyacetophenone and the reaction mixture) were diluted 200 fold (5 µl to 1 ml) with methanol and injected separately as 50 µl aliquots into the stream of dilute reaction mixture. The change in response due to the addition of the different components was noted.

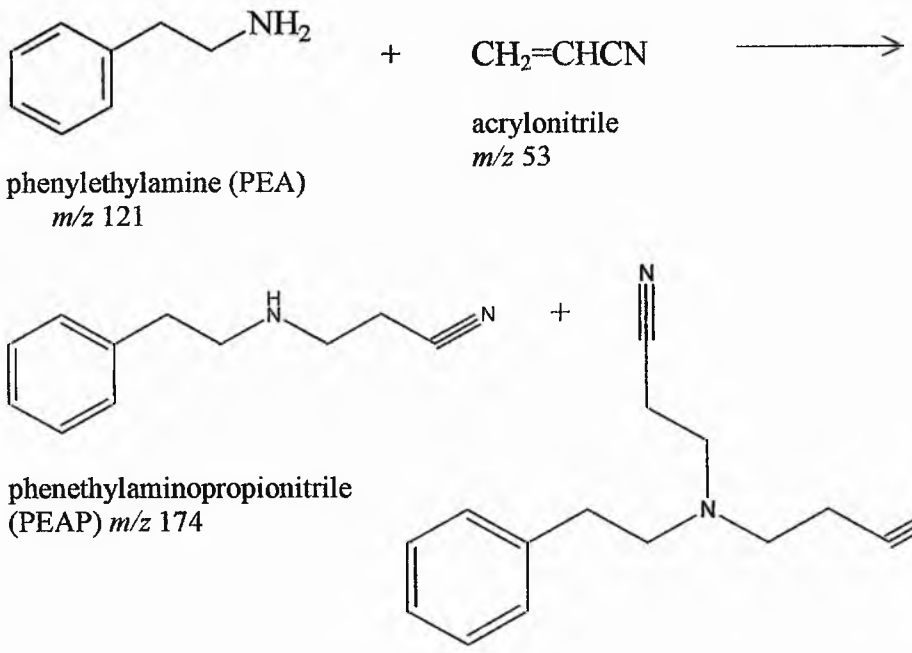
2.3 Results and Discussion

2.3.1 Michael Addition Reaction Monitoring Investigation

2.3.1.1 Selection of a Model Reaction System

The Michael Addition reaction of phenylethylamine with acrylonitrile in ethanol was selected as a model reaction system for preliminary studies. The reaction was chosen for several reasons. It is a simple two component reaction that progresses at room temperature with no special requirements for temperature and pressure control. The reaction gives a yield of approximately 80% after only 5 hours which is a reasonable time for experimental observation. The reaction rate is reagent concentration and temperature dependent and the reagents, products and impurities are compatible with proposed atmospheric pressure ionisation techniques (see Scheme 2.1).

Scheme 2.1 The Michael Addition reaction mechanism for the reaction of phenylethylamine with acrylonitrile in ethanol.



2.3.1.2 Mass Spectrometric Analysis

APCI was selected as the ionisation method for this analysis as it is a relatively soft ionisation technique that does not require the addition of ionisation agents or modifiers. The mass spectrometric conditions (Section 2.2.4.2) were selected to minimise fragmentation of the analyte molecules and optimise the detection of intact $[M+H]^+$ ions.

2.3.1.3 Membrane Selection

A number of microporous membrane materials were considered for the liquid-liquid membrane interface. The membranes were first evaluated for by examining their compatibility with the proposed solvent systems (see Table 2.1).

Table 2.1 Evaluation of Microporous Membranes for Use in the Liquid-Liquid Membrane Interface

Membrane Material	Compatible With Solvents	Hydrophobic/ Hydrophilic	Pore Size Available (μm)
Cellulose ester	No	-	-
Nylon	No	-	-
Polycarbonate	No	-	-
Polypropylene	No	-	-
Polystyrene	No	-	-
Polytetrafluoroethylene	Yes	hydrophilic in alcohol	-
Polyvinyl chloride	No	-	-
Polyvinylidene fluoride (Durapore®)	Yes	both	0.1 - 0.45

The only membrane materials compatible with acetonitrile and ethanol solvent systems were polytetrafluoroethylene and polyvinylidene fluoride. These

materials were further investigated for hydrophobicity (to reduce the transport or the water from the donor phase) and available pore sizes. Polyvinylidene fluoride was available in both hydrophobic and hydrophilic forms and was tested in the membrane interface using aliquots of the Michael Addition reaction mixture to determine its ability to transport analyte molecules between the donor and acceptor phases.

A hydrophobic polyvinylidene fluoride microporous membrane with a pore size 0.1 μm was selected for use in the reaction monitoring studies because of its compatibility with the donor and acceptor solvents and resistance to back flow of the aqueous/acetonitrile acceptor stream across the membrane. The 0.1 μm pore size was selected to keep the membrane flux to a minimum. A suitable analyte flux was demonstrated when the 0.1 μm hydrophobic polyvinylidene fluoride microporous membrane was installed in the membrane interface and an aliquot of the reaction mixture was sampled into the donor stream with the membrane interface acceptor stream connected to the APCI source of the mass spectrometer.

Note: The donor and acceptor streams were configured to flow in the same direction across the membrane, contrary to the design of most extraction systems. Extraction systems are usually configured with the donor flow and acceptor flow running in opposing directions, presenting a concentration gradient most suitable for maximised transfer of the desired components, be it analyte or heat. In these experiments the configuration was selected to minimise the transfer of analyte across the system and to produce narrow analyte bands. Additionally, the solvent

pressures on the membrane were better balanced when the solvents entered the membrane unit in the same position. This configuration reduced the possibility of the donor solvent stream emerging from the transfer line, continuing straight through the membrane and out of the acceptor stream exit port without passing over the membrane surface.

The APCI single ion response (*m/z* 175) for a 20 µl aliquot of a neat reaction mixture, containing the main product PEAP (Scheme 2.1), introduced into the membrane interface is shown in Figure 2.4 The single ion response rises to a peak as the analyte passes across the membrane and a small amount is transferred from the donor to the acceptor stream.

↑ Relative Ion Intensity (%)

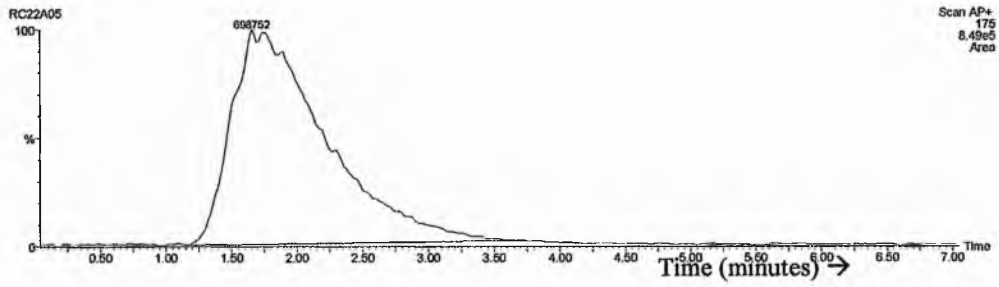


Figure 2.4 Single ion responses for $[M+H]^+$ for PEAP (*m/z* 175) for a 20 µl aliquot of the neat reaction mixture analysed using the membrane interface

Note: The data system used in this work (MassLynx 3.5, Micromass, Manchester, UK) did not label the axes in a manner consistent with good labelling practice. The above mass chromatogram has been manually annotated but the following labelling convention applies to all mass chromatograms in this thesis:

X-axis = Time (minutes)

Y-axis = Relative Intensity (%) - Signal response normalised to most intense signal in the chromatogram.

Features in top right-hand corner of chromatogram:

AP+ = ion mode used for data collection (APCI +)

175 = *m/z* of ion plotted in this trace (TIC = Total ion count)

8.49e5 = intensity of signal maxima in ion counts

Area = integrated peak reported as area units

2.3.1.4 Acceptor Solvent Selection

A range of acceptor solvent compositions (acetonitrile/water) were evaluated for the Michael Addition monitoring system. Figures 2.5 to 2.9 present the single ion plots for the $[M+H]^+$ ion, PEAP (*m/z* 175) with changing acetonitrile content of the acceptor solvent.

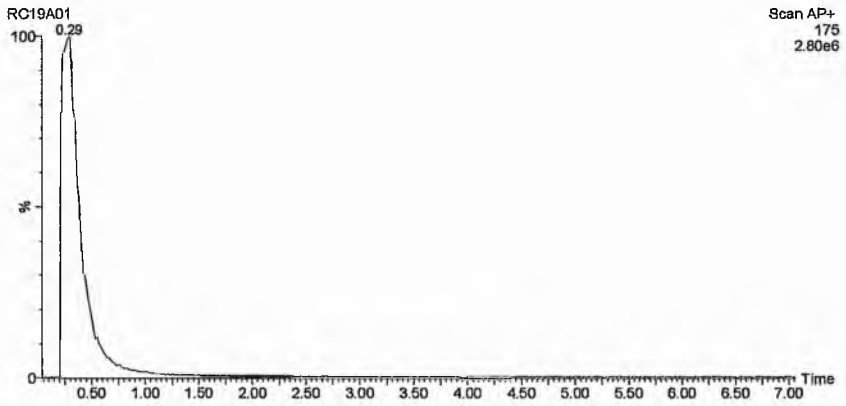


Figure 2.5 Single ion response for PEAP (*m/z* 175) with an Acceptor Solvent Composition of Acetonitrile (100%)

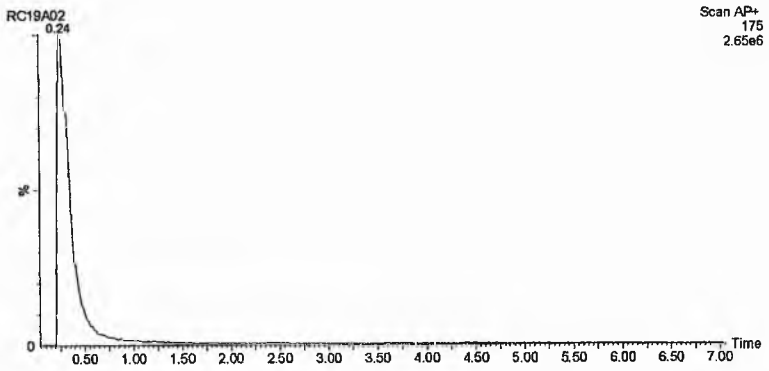


Figure 2.6 Single ion response for PEAP (*m/z* 175) with an Acceptor Solvent Composition of Acetonitrile/Water (90/10 %)

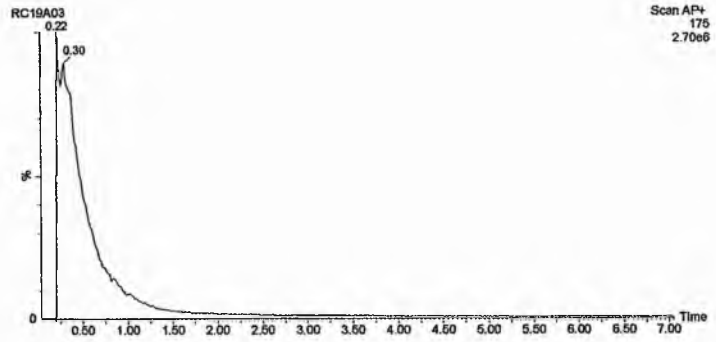


Figure 2.7 Single ion response for PEAP (*m/z* 175) with an Acceptor Solvent Composition of Acetonitrile/Water (80/20 %)

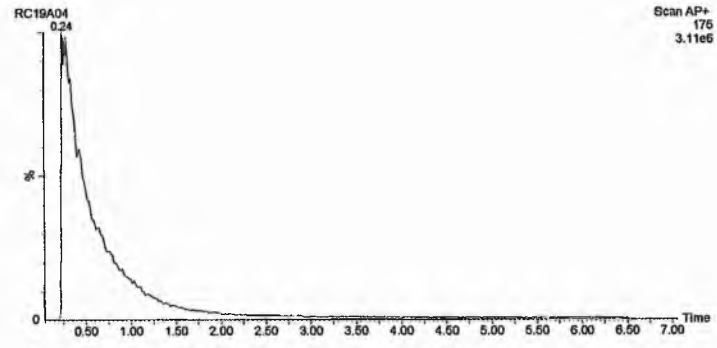


Figure 2.8 Single ion response for PEAP (*m/z* 175) with an Acceptor Solvent Composition of Acetonitrile/Water (70/30 %)

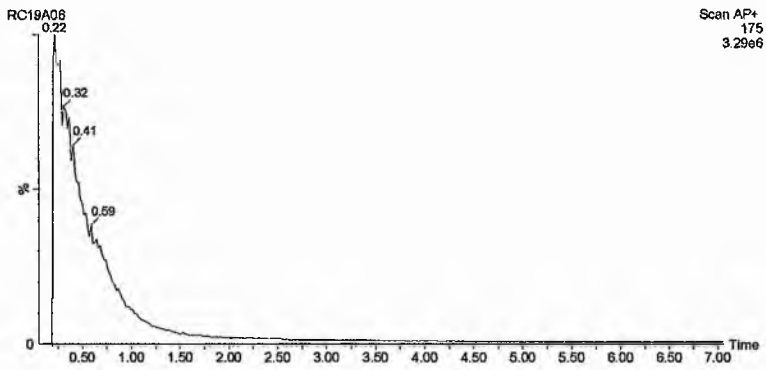


Figure 2.9 Single ion response for PEAP (*m/z* 175) with an Acceptor Solvent Composition of Acetonitrile/Water (50/50 %)

High acetonitrile concentrations gave sharp peaks for the single ion response for PEAP. Sharp peaks enable more reliable integration and reduce the time taken for the system to return to a baseline level response after each aliquot of analyte, thus increasing the possible sampling rate. Water in the acceptor solvent acted as the proton transfer reagent in the atmospheric pressure ionisation source. An optimal acceptor solvent composition of 90% acetonitrile, 10 % water (v/v) was selected and used for subsequent experiments.

2.3.1.5 Liquid-Liquid Membrane Dilution Effect

To determine the level of dilution achieved by the membrane interface an aliquot of neat reaction mixture containing the main product phenylethylaminopropio-nitrile, was introduced into the membrane interface. The single ion response for the $[M+H]^+$ ion, PEAP (*m/z* 175) is presented in Figure 2.4 with a nominal peak area of 698752. A second 20 µl aliquot of the same reaction mixture was serially

diluted (~3 x 10^5 dilution) and introduced directly into the acceptor solvent stream. Figure 2.10 presents the single ion response for the diluted sample with a nominal peak area of 703587. The similar areas for the two peaks in Figures 2.4 and 2.10 show that the use of the membrane interface resulted in a single stage dilution of approximately five orders of magnitude for the reaction mixture in the acceptor stream compared with the donor stream. This reduced the concentration of the reactants and products to a level compatible with APCI-MS analysis.

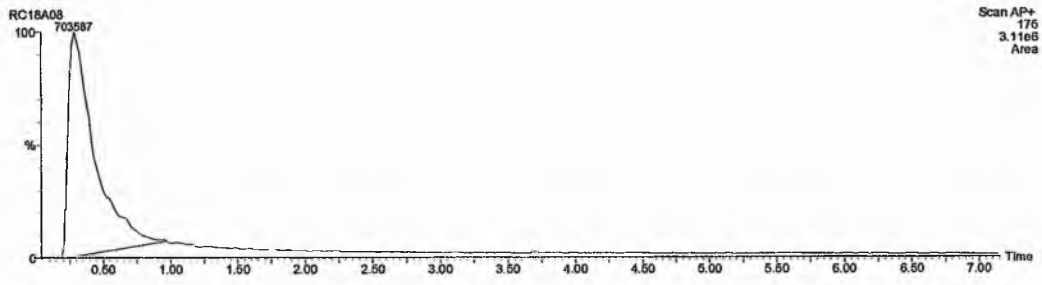


Figure 2.10 Single ion response for PEAP (*m/z* 175) for a 20 µl aliquot of serially diluted reaction mixture (~3 x 10^5 dilution)

2.3.1.6 Performance of the Liquid-Liquid Membrane Interface

Replicate aliquots of the Michael Addition reaction products were introduced into the membrane interface. The single ion responses for the $[M+H]^+$ ions, PEAP (*m/z* 175) and CPEAP (*m/z* 228), are presented in Figure 2.11 and the peak areas obtained are presented in Table 2.2.

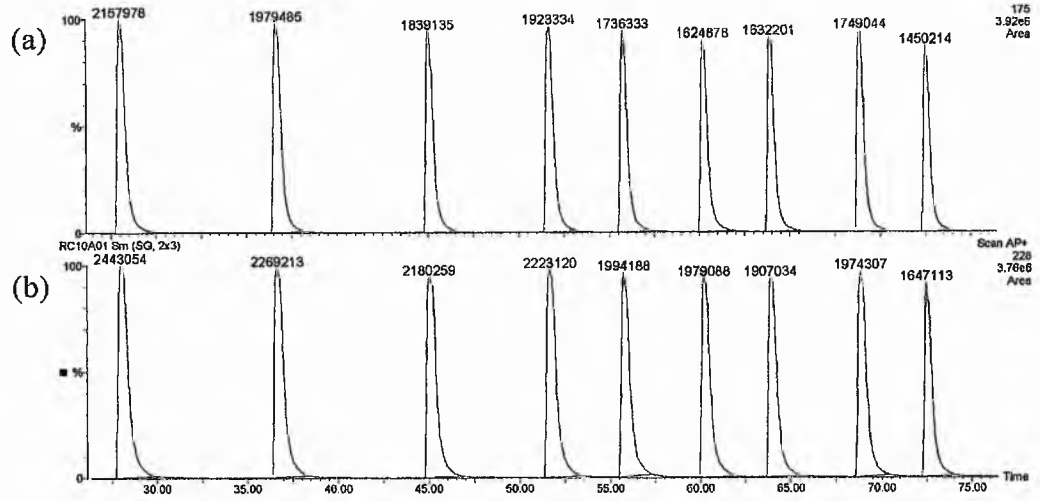


Figure 2.11 Single ion responses for replicate analyses of Michael Addition Reaction Mixture for (a) the main reaction product PEAP (*m/z* 175) and (b) the secondary reaction product CPEAP (*m/z* 228)

The observed analytical responses were very susceptible to small changes in operational pressures, flow rates for the donor and acceptor streams and sample pulsing on both sides of the membrane from the reciprocating HPLC pumps. This lack of robustness led, in some cases, to poor run to run reproducibility and consequently wide variations in absolute ion responses. However, using the ratio of the PEAP to CPEAP peak areas (Table 2.2), rather than the absolute area, gave a calculated RSD of 2.5% (n=9). This is a satisfactory level of reproducibility for the purposes of process monitoring.

In a real-life process monitoring situation it may not be appropriate to take the ratio of these two components due to their changing compositions and the unknown effects of unexpected side reactions. Further development would be

required to minimise the system pressure fluctuations in order to allow the direct monitoring of the individual reaction components.

Table 2.2 Reproducibility of System Response for PEAP and CPEAP

Injection	System Response (Area) PEAP	System Response (Area) CPEAP	Ratio PEAP/CPEAP
1	215978	2443054	0.883
2	1997756	2269213	0.880
3	1839135	2180259	0.884
4	1923334	2223120	0.865
5	1736333	2032152	0.854
6	1624878	1979088	0.812
7	1669100	1907034	0.875
8	1749044	1974307	0.886
9	1450214	1647113	0.880
RSD %	11.8%	11.3%	2.5%

2.3.1.7 Real-Time Michael Addition Reaction Monitoring using the Liquid-Liquid Membrane Interface

Aliquots of the reaction mixture were withdrawn from the reaction vessel and injected into a stream of ethanol donor solvent, which carried the components of

the mixture to the membrane interface. Reactants and products diffusing through the membrane were extracted into the acetonitrile/water acceptor solvent flowing across the other surface of the membrane and transferred to the APCI ion source. Mass spectra of the extracted components of the reaction mixture taken at time 0, 60 minutes and 350 minutes after the addition of acrylonitrile are presented in Figure 2.12.

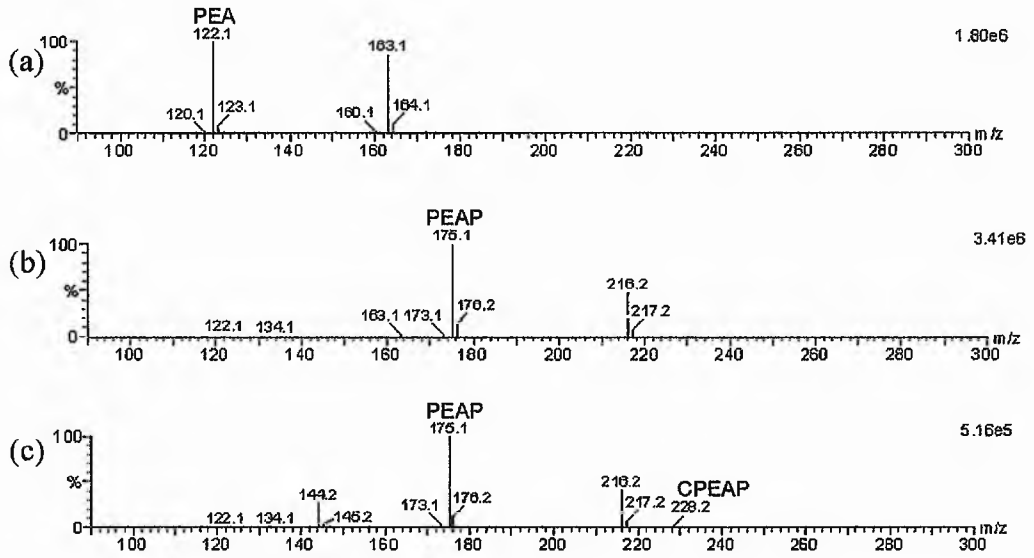


Figure 2.12 Mass spectra for the Michael Addition reaction mixture sampled at (a) 0 min, (b) 60 min and (c) 350 min after the addition of acrylonitrile.

At the start of the reaction the only component detectable in the spectrum is the protonated starting material, PEA (*m/z* 122) and the acetonitrile adduct of PEA (*m/z* 163). After 60 minutes, the protonated main product PEAP (*m/z* 175) has become the dominant species, along with its acetonitrile adduct at *m/z* 216. After 350 minutes the secondary product CPEAP, formed by a second Michael addition

to the phenylethylamine (Scheme 2.1), can be seen at *m/z* 228. Acrylonitrile (MW=53 Da) was not monitored because many other compounds gave signals in this low mass region (high chemical noise levels) resulting in a very poor limit of detection and analytical reproducibility. Also, acrylonitrile is likely to have very a low proton affinity, making it a poor subject for APCI analysis.

The single ion responses for PEA, PEAP and CPEAP, monitored using the membrane interface during the course of the Michael addition reaction are presented in Figure 2.13. It can be seen that the response for the starting material PEA was observed to decrease with time, whilst the responses for the products, PEAP and CPEAP, increased over the same time period.

The ratio of the PEAP product peak area (*m/z* 175) to the PEA starting material peak area (*m/z* 122) plotted against time during the course of the Michael Addition is shown in Figure 2.14 (left hand scale). The primary product to reactant ratio (PEAP:PEA) increased to a maximum at approximately 160 minutes and then began to decline. The secondary product to reactant ratio (CPEAP:PEA, right hand scale), present at a much lower concentration, also increased with time over the first 280 minutes and continued to rise slowly over the course of many hours,

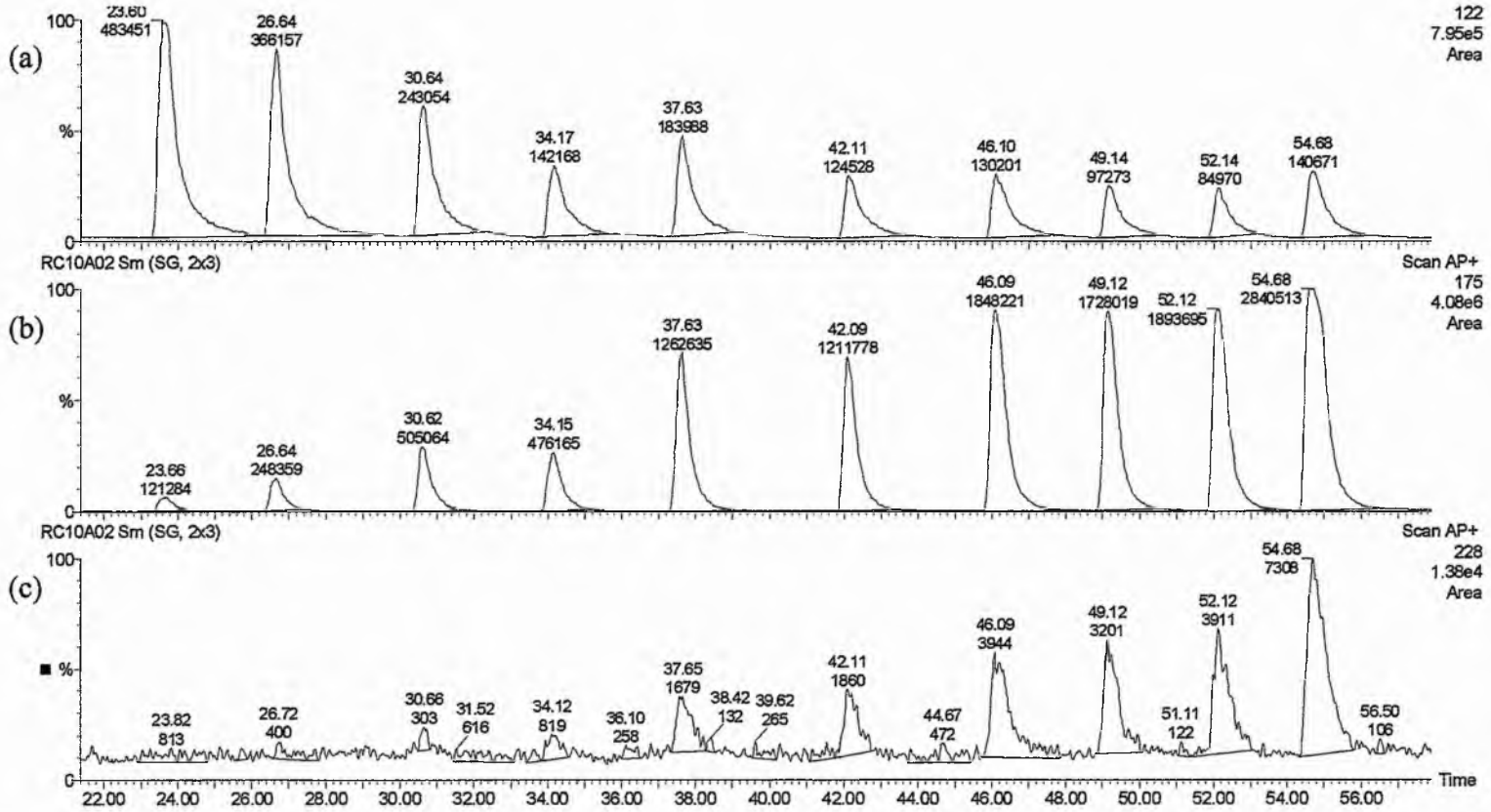


Figure 2.13. Single ion responses for (a) the starting material PEA (m/z 122), (b) the main reaction product PEAP (m/z 175) and (c) the secondary product CPEAP (m/z 228) monitored during the Michael addition reaction.

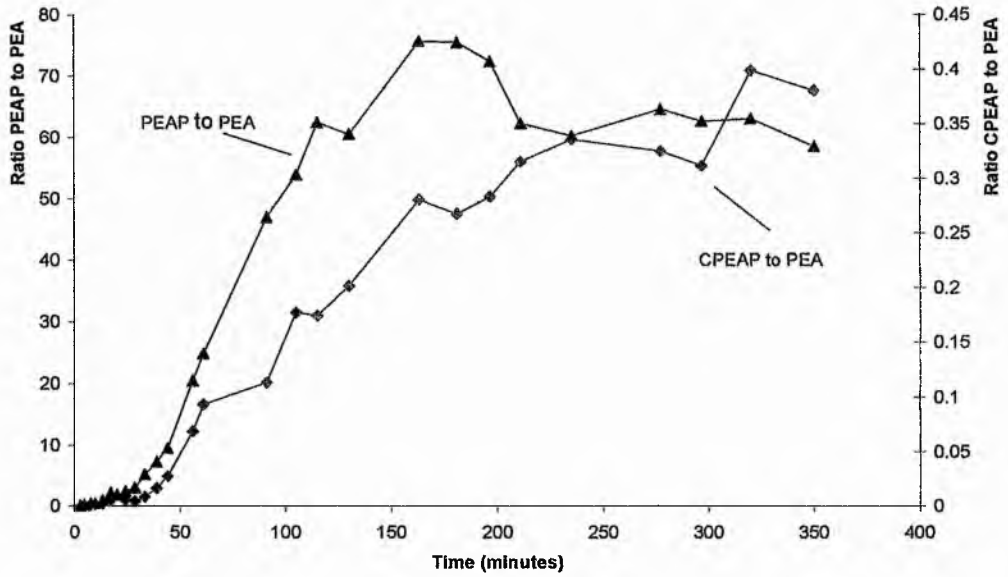


Figure 2.14 Variation in the APCI/MS peak area ratios for a) PEAP to PEA ratio (left axis) and (b) CPEAP to PEA ratio (right axis).

eventually reaching a similar concentration to that of the primary product after circa 36 hours (data not shown). These data suggest that stopping the reaction after 160 minutes would yield an optimal concentration of primary product and minimal level of unwanted secondary product.

The time between switching the Rheodyne valve and the observation of a mass spectrometric response was *circa* 30 seconds. The membrane interface, coupled with APCI/MS, therefore exhibits a minimal time delay between process sampling and the generation of quantitative analytical data, allowing real-time monitoring of the reaction on a timescale compatible with end point determination or operator intervention in the process.

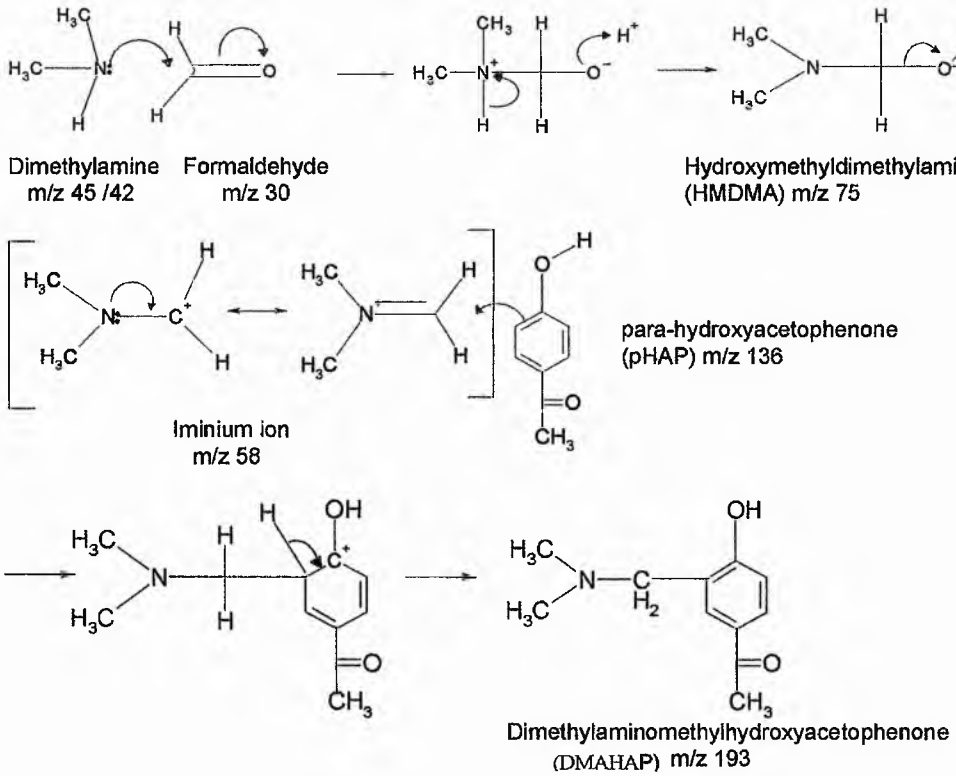
2.3.2 Mannich Reaction Monitoring Investigation

2.3.2.1 Selection of a Model Reaction System

The Mannich reaction of dimethylamine (DMA) with parahydroxyacetophenone (pHAP) and formaldehyde solution in water was selected as a model reaction system. The reaction was chosen for several reasons. This Mannich reaction is an early stage of the manufacturing process for salbutamol sulphate, a drug for the treatment of asthma. Salbutamol sulphate was initially produced by GlaxoSmithKline and marketed as Ventolin™ but is now manufactured by other generic pharmaceutical manufacturing companies.

This reaction is a slightly more complex system than that studied in Section 2.2.3, being a three component reaction that progresses at near-room temperature with a relatively simple temperature monitoring system and no special requirements for pressure control. The reaction gives a good yield of dimethylamino-methylhydroxyacetonphenone (DMAHAP) after only a few hours, which is a reasonable time for experimental observation, but also forms interesting intermediates during the reaction (see Scheme 2.2).

Scheme 2.2 The Mannich reaction mechanism for the reaction of dimethylamine with parahydroxyacetophenone and formaldehyde in water.



2.3.3 Sampling using Liquid-Liquid Membrane Interface with APCI - Single Quadruple Mass Spectrometry

The successful application of the liquid-liquid membrane interface to reaction monitoring of the Michael reaction in an organic solvent (ethanol) suggested that other systems might be amenable to the technique. In particular, it was of interest to investigate a reaction in an aqueous solvent. The Mannich reaction of dimethylamine with formaldehyde and parahydroxyacetophenone was chosen as a model system to study.

2.3.3.1 Membrane Selection

A number of microporous membrane materials and pore sizes were considered for the liquid-liquid membrane interface. A hydrophilic polyvinylidene fluoride (Durapore®) microporous membrane (Millipore Watford, UK) was selected because of its chemical compatibility with the donor and acceptor solvents. A hydrophobic polyvinylidene fluoride membrane was evaluated but did not allow any analyte to pass into the acceptor stream. A 0.1 μm pore size was selected to keep the membrane flux to a minimum.

A suitable analyte flux was demonstrated when the 0.1 μm hydrophilic polyvinylidene fluoride microporous membrane was installed in the membrane interface and an aliquot of the reaction mixture was sampled into the donor stream with the membrane interface acceptor stream connected to the APCI source of the mass spectrometer.

2.3.3.2 Mass Spectrometric Analysis of the Mannich Reaction

APCI is a relatively soft ionisation technique that does not require the addition of ionisation agents or modifiers and the mass spectrometer conditions were selected to minimise fragmentation of the analyte molecules and optimise the detection of intact $[M+H]^+$ ions. The starting material: parahydroxyacetophenone (pHAP, *m/z* 137), used for the Mannich Reaction and the main product: dimethylaminomethylhydroxyacetophenone (DMAMHAP, *m/z* 194) were readily

protonated and detected in positive ion APCI mode as shown in Figure 2.15. Dimethylamine (DMA, Mw 45) and formaldehyde (Mw 30), the other major starting materials, were not detectable using this system because the low mass ions formed were obscured by the solvent background.

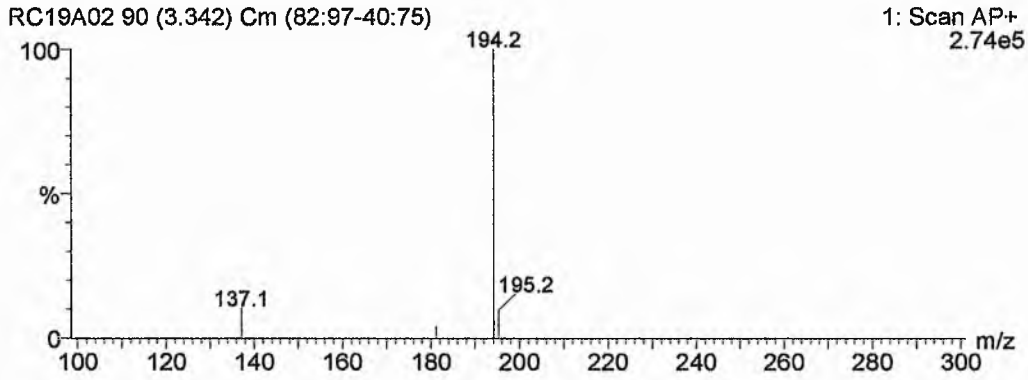


Figure 2.15 Mass Spectrum Obtained for the Mannich Reaction Mixture.

An aliquot of a neat reaction mixture (20 µl) was extracted from the vessel during the course of the reaction and introduced into the membrane interface. The resulting APCI single ion responses for the $[M+H]^+$ ions of pHAP (*m/z* 137) and DMAMHAP (*m/z* 194) are shown in Figure 2.16. The responses rose to a peak as the analyte passed across the membrane and a small amount was transferred from the donor to the acceptor stream. This reduced the concentration of the reactants and products to a level compatible with APCI-MS analysis allowing on-line monitoring of the reaction.

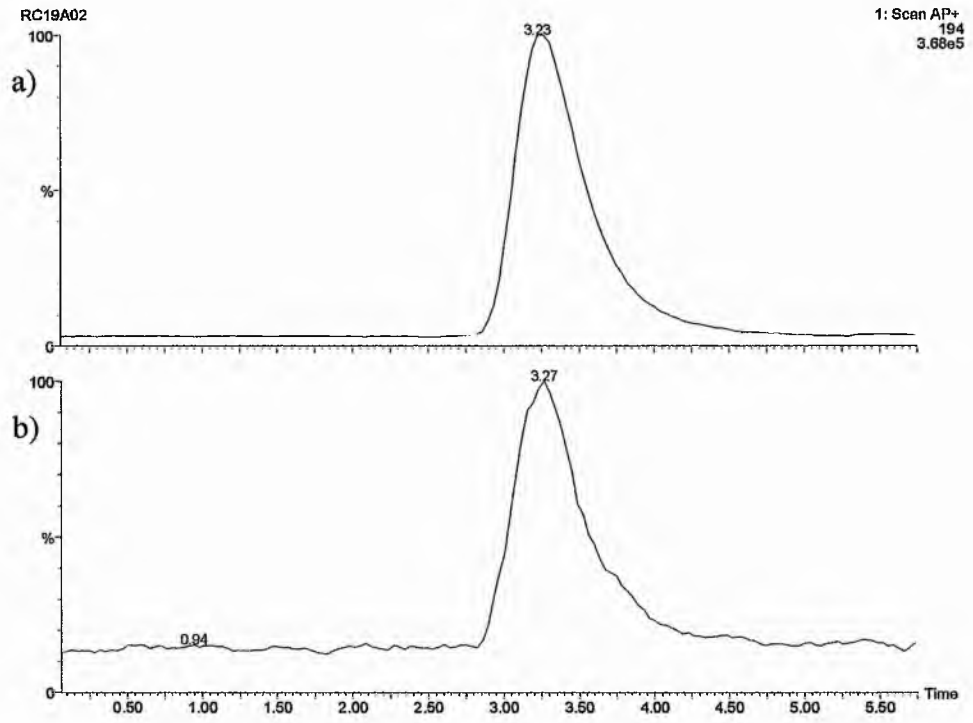


Figure 2.16 Single ion responses for the Mannich Reaction mixture sampled directly into the membrane interface for $[M+H]^+$ for a) pHAP (*m/z* 137) and b) DMAMHAP (*m/z* 194)

2.3.3.3 Performance of the Liquid-Liquid Membrane Interface

The reproducibility of the interface was determined by extracting replicate aliquots of the reaction mixture (after the reaction had been allowed to go to completion) and introducing them into the membrane interface. An example of the single ion responses for the $[M+H]^+$ ions of pHAP (*m/z* 137) and DMAHAP (*m/z* 194), is presented in Figure 2.17.

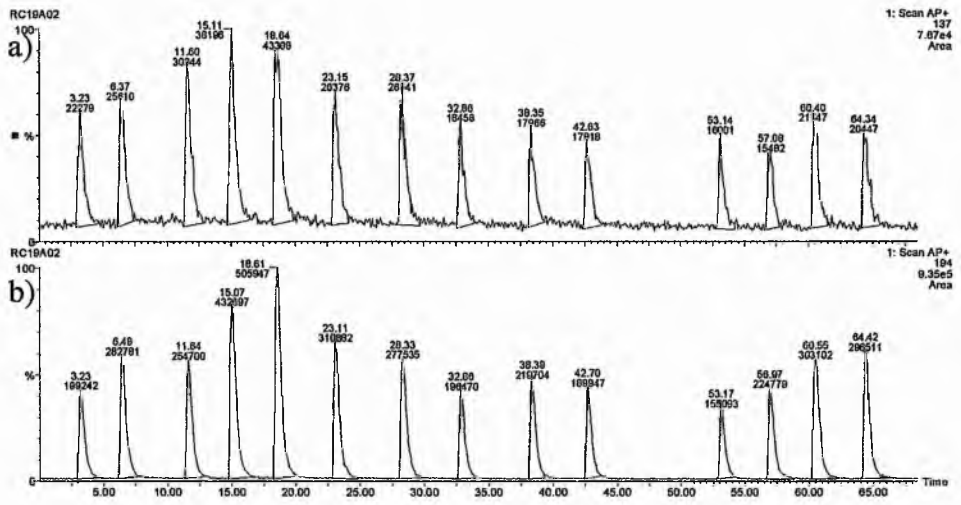


Figure 2.17 Single ion responses for $[M+H]^+$ for a) pHAP (*m/z* 137) and b) DMAHAP (*m/z* 194)

There was considerable variation in the mass spectrometric responses observed for the aliquots of reaction mixture. The data for fourteen replicates are presented in Table 2.3.

Table 2.3 Reproducibility of Response for the Mannich Reaction Components When Monitored Using the Liquid-Liquid Membrane Interface.

Injection	Peak Area *m/z* 137 (pHAP)	Peak Area *m/z* 194 (DMAMHAP)	Peak Area Ratio 194/137
1	22279	199242	8.94
2	25610	282781	11.04
3	30244	254700	8.42
4	38196	432697	11.33
5	43308	505947	11.68
6	26376	310882	11.79
7	26741	277535	10.38
8	18458	196470	10.64
9	17966	219704	12.23
10	17818	189947	10.66
11	16001	155093	9.69
12	15482	224779	14.52
13	21747	303102	13.94
14	20447	296511	14.50
mean	24334	274957	11.4
SD	8274.3	96205.4	1.897
RSD %	34.0	35.0	16.6

A %RSD of approximately 35% (n=14) was achieved for the individual peaks. This level of precision is not acceptable for routine process analysis, so the ratio of the pHAP and DMAHAP responses were calculated, since this was shown to improve precision during monitoring of the Michael Addition reaction (Section 2.3.1.6). The precision was improved (RSD=16.6%) but not to a sufficient extent that reaction monitoring could be carried out with confidence. The most likely cause is variation in the pressure differential for the donor and acceptor streams either side of the membrane, possibly due to pulsing of the HPLC pumps. Precision would be improved by better pressure regulation and pulse-free pumps, but these were not available to the project. In a real-life process monitoring situation it may not be appropriate to take the ratio of these two components due to their changing compositions and the unknown effects of unexpected side reactions. Further development would be required to minimise the system pressure fluctuations in order to allow the direct monitoring of the individual reaction components.

A further problem was that after a few days of membrane analysis the responses for aliquots of the reaction mixture became broad and tailing. This was attributed to the membrane degrading and losing separation efficiency.

2.3.3.4 Investigation of Ion Suppression

Ion suppression of the pHAP and DMAMHAP ion response was suspected when unusual single ion chromatograms were obtained for duplicate injections of the

Mannich reaction mixture (Figure 2.18). The single ion chromatograms showed a rise in analyte response for *m/z* 137 and 194, this response then dropped to little or no signal response when they were expected to continue rising. After a short time period the responses rose again in a manner to the level they were expected to have reached. Ion suppression of the pHAP and DMAMHAP ion response was suspected because of the presence of the DMA, which has a higher proton affinity, and is thus ionised in preference to the other reaction components. Figure 2.18 has been annotated to show the expected signal for the pHAP peak.

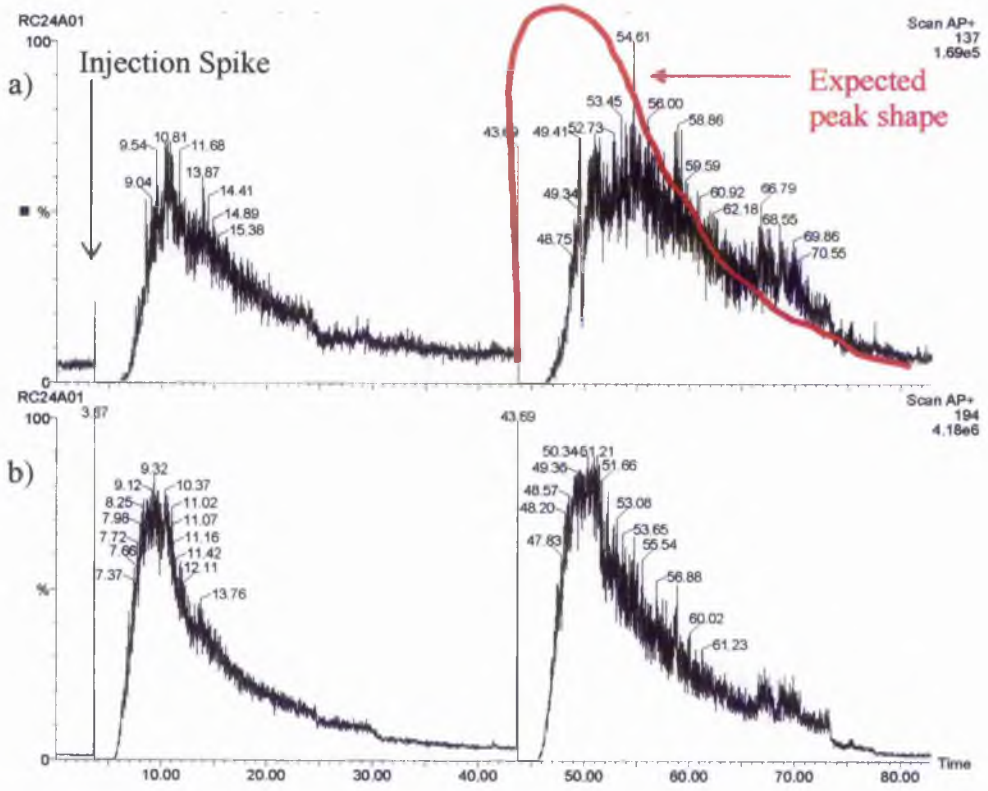


Figure 2.18 The suppressed responses for $[M+H]^+$ a) pHAP (*m/z* 137) and b) DMAMHAP (*m/z* 194) with the expected signal annotated in red for the second injection

Ion suppression was investigated by introducing a dilute solution of the reaction mixture into the mass spectrometer at a constant flow rate and recording the baseline response for this solution. Dilute solutions of the reaction components were injected into the flowing analyte stream.

A small drop in the baseline was observed when methanol and formaldehyde were introduced into the carrier stream, as expected, due to the reduction in the amount of carrier phase present when the analyte was injected. However a large drop in all single ion signal levels was observed when dimethylamine was introduced into the reaction mixture stream. The single ion chromatograms for $[M+H]^+$ for pHAP (*m/z* 137) and DMAMHAP (*m/z* 194) are presented in Figure 2.19.

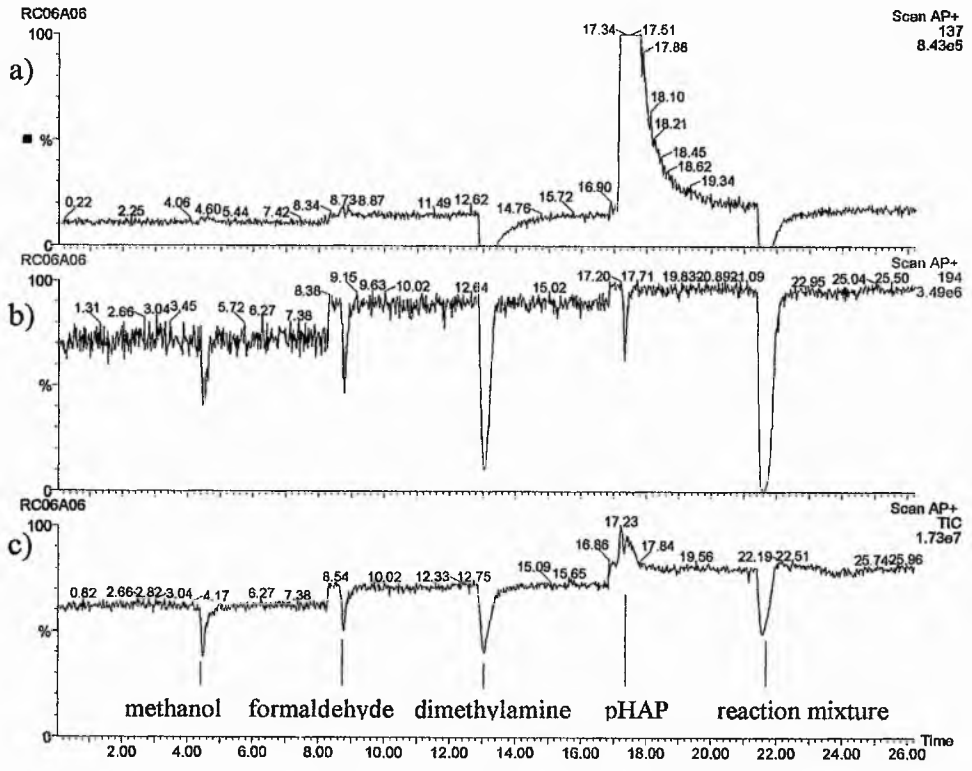


Figure 2.19 Single ion chromatogram for $[M+H]^+$ for a) pHAP (*m/z* 137) (pHAP peak shown offscale) and b) DMAMHAP (*m/z* 194), and c) the total ion chromatogram, showing ion suppression effects in the presence of DMA.

Dilution of the reaction mixture reduced the suppression effect but also reduced the ion responses of the lower level components so that they were not quantifiable above the system noise. An alternative approach to monitoring this reaction by membrane inlet mass spectrometry using a semi-permeable membrane is described in Chapter 4.

2.4 Conclusions

A novel liquid-liquid membrane interface device has been constructed and used for process reaction monitoring in real-time. The microporous membrane provided a simple interface between the concentrated reaction mixture and the aqueous acetonitrile flow directed towards the APCI source of the quadrupole mass spectrometer. The reduced concentration on the acceptor side of the membrane allowed the simultaneous determination of multiple components of a highly concentrated Michael Addition reaction mixture on a real-time basis with minimal delay time between sampling and obtaining the analytical result.

The analytical response was very susceptible to changes in pressure and flow on the donor and acceptor sides of the membrane and careful control of the interface operating conditions and automation by the addition of a feed loop from the reaction vessel at predetermined time intervals would be required for reliable process monitoring. The reproducibility of measurement was found to be improved by the use of reactant to product ion ratios during real-time reaction monitoring. However, in a real-life process monitoring situation it may not be appropriate to take the ratio of these two components due to their changing compositions and the unknown effects of unexpected side reactions. Further development would be required to minimise the system pressure fluctuations in order to allow the direct monitoring of the individual reaction components.

The Mannich Reaction was studied using this microporous membrane, liquid-liquid interface. The monitoring experiments were unsuccessful due to high fluctuations in system pressure preventing reproducible analysis. Also, the starting material, dimethylamine, suppressed the ionisation of the starting material, parahydroxyacetophenone and the reaction product, dimethylaminomethyl-hydroxyacetophenone, preventing their reliable detection using this ionisation technique.

2.5 References

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Chapter 3

Supported Membrane Development for Liquid-Liquid Sample Extraction

3.1 Introduction

Microporous membranes were used in Chapter 2 to extract analytes from a concentrated aqueous analyte stream into an organic phase acceptor stream. The organic solvent filled the pores of the hydrophobic membrane and acted as a transport agent for the analyte through the membrane. The non-polar, hydrophobic membrane repelled the polar analytes and ensured that the transfer of analyte into the donor stream was minimal and thus served as a diluting rather than a concentrating interface.

The affinity of the analyte for the membrane material or the solvent filled pores affects the transfer rate across the membrane and into the donor solvent. In liquid-gas membrane inlet mass spectrometry the membrane is often a hydrophobic non-porous hollow fibre silicone membrane.[1-12] Non-polar analytes preferentially cross the membrane driven by the diffusion gradient and their solubility in the non-polar membrane material.[13-18] Changing the chemistry of the membrane material alters the discriminatory properties of the membrane and this can be used to optimise the selectivity of the membrane device.

This chapter describes an investigation into the preparation of supported silicone membranes for use in membrane reaction monitoring. The aim was to incorporate these new membrane materials into the liquid-liquid membrane interface used in Chapter 2 and investigate the effect of changing the physical properties of the membranes.

The production of non-porous membrane extraction probes, fabricated in-house from polydimethylsiloxane has been reported.[19-21] Mullins[20] used these probes to extract analytes from a complex matrix, held in contact with one surface of the membrane, into an acceptor solvent held inside the probe. The membrane material used in these probes was extremely thin and would not withstand the operating pressures of a liquid-liquid membrane interface with pumped donor and acceptor phases.

In this set of experiments semi-permeable membranes were constructed using a net support material to provide a structural rigidity to the silicone membranes, and to allow assessment of the membranes as interfaces for analyte extraction.

The aim of this study was to evaluate the manufactured supported membranes as simple extraction devices by determining the transport efficiency of PEA across the membrane barriers. The membranes would then be incorporated into the liquid-liquid membrane interface discussed in Chapter 2. The effects of modifying the membrane chemistry and structural properties, membrane extraction solvent systems and different analyte systems was to be studied.

3.2 Materials and methods

3.2.1 Chemicals and Materials

Ethanol (99.86-100%) was obtained from Hayman Ltd, (Witham, UK), acetonitrile (HPLC grade), acetone, methanol and toluene (AR grade) from Fisher Chemicals, (Loughborough, UK). Purified water was taken from a Barnstead Nanopure Diamond purification unit (Barnstead, UK). Phenylethylamine (99%) was purchased from Lancaster Synthesis UK (Morecambe, UK). Silicone adhesive/sealant 3145 (one-part moisture-cure, room temperature vulcanising trimethoxy(methyl)silane, RTV MIL-A-46146) was purchased from Dow Corning, (MI, USA).

Polypropylene net, sheet membranes (80 μm pore size, 100 mm diameter disks) and nylon net, sheet membranes (80 μm pore size, 100 mm diameter disks) were obtained from Millipore (Watford, UK). The membrane holders were 10 ml centrifuge tubes from Fisher Chemicals (Loughborough, UK) with the end of the tube cut off and the centre of the tube cap removed to leave a collar with screw thread.

3.2.2 Supported Membrane Preparation Process

The author would like to thank Frank Mullins and Sally Hannam at Medeval Group Ltd., Manchester, for demonstrating their membrane manufacturing

technique and for the donation of membrane material for analytical trials.

Toluene (20 ml, AR grade) and acetone (10 ml, AR grade) were combined in a glass, stoppered flask and silicone sealant (Dow Corning 3145; approximately 10 g) was added and the mixture was stirred until a homogeneous solution was obtained. The silicone mixture was then cast onto a sheet of PTFE coated onto a glass sheet (for rigidity). The silicone was spread to an even thickness using a straight edged blade, then the sheet was left to cure for 24 hours. The silicone membrane was then peeled carefully from the PTFE backing and stored under nitrogen.

3.2.3 Membrane Material Evaluation

Sections of prepared silicone membrane (section 3.2.2) and the membrane support materials (nylon and polypropylene net) were placed in flasks containing acetonitrile, acetone, methanol, toluene or acetone/ toluene mix (1:2 v/v). The samples were left overnight then dried and examined.

3.2.4 Silicone Solution Preparation

A range of dilute solutions of the silicone sealant were prepared. The silicone sealant was weighed into a stoppered flask, the required volume of acetone/toluene solution (1:2 v/v) was pipetted into the flask, which was shaken until a homogeneous solution was obtained. Silicone casting solutions were

prepared at concentrations ranging from approximately 0.003 g ml^{-1} to 0.10 g ml^{-1}.

3.2.5 Supported Membrane Casting

A variety of membrane casting techniques were investigated. Prior to casting the net membrane support materials were rinsed with toluene/acetone solution and allowed to dry. Dilute silicone solutions were cast onto the net support. In all cases the membranes were left to cure for 24 hours then examined under a microscope (Micro Instruments Ltd., Oxford, UK)

The supported membrane was assembled into a membrane probe. The end of a 10 ml centrifuge tube was removed and the centre of the cap was removed. The membrane was positioned over the screw thread end of the tube and secured in place with PTFE tape and the threaded collar of the screw cap. Figure 3.1 presents a schematic diagram of the membrane probe.

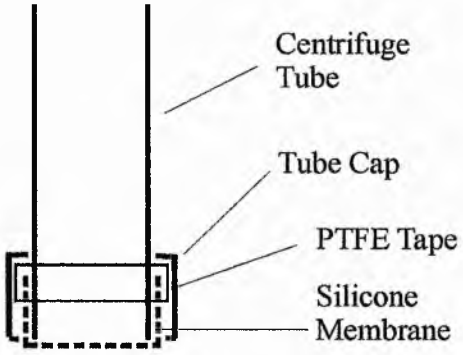

Figure 3.1 The in-house constructed membrane probe.

The membrane probe was filled with purified water then methanol. The membrane was examined for signs of leakage.

3.2.5.1 Membrane Casting Methods

- A section of support net was placed on the PTFE casting sheet. Silicone solution was dispensed onto the surface of the membrane using a glass dropping pipette.

- A section of support net was placed on the PTFE casting sheet then silicone solution was dispensed onto the membrane surface. The solvent was allowed to evaporate then two further aliquots of silicone solution were dispensed before the membranes were left to cure.

- A section of support net was dipped into the silicone solution. The membrane was suspended in a fume cupboard to dry before being dipped in the silicone solutions a further two times. The membrane was hung in the fume cupboard to cure.

- A section of support net was assembled into the membrane probe detailed in Section 3.2.5. The probe was inverted and silicone solution was dispensed onto the outer net membrane surface. The solvent was allowed to evaporate then two further aliquots of silicone solution were dispensed before the membranes were left to cure.

- A section of support net was assembled into the membrane probe. The probe was placed membrane-side down onto the PTFE casting sheet. The probe was clamped firmly into position and silicone solution was dispensed into the membrane probe. The solvent was allowed to evaporate then two further aliquots of silicone solution were dispensed before the membranes were left to cure.

3.2.6 Supported Membrane Evaluation Using the Membrane Probe

Ethanol (donor solvent, 20 ml) was pipetted into a beaker and stirred continuously. The supported silicone membrane probe was positioned so the membrane was immersed just below the surface of the ethanol as shown in Figure 3.2.

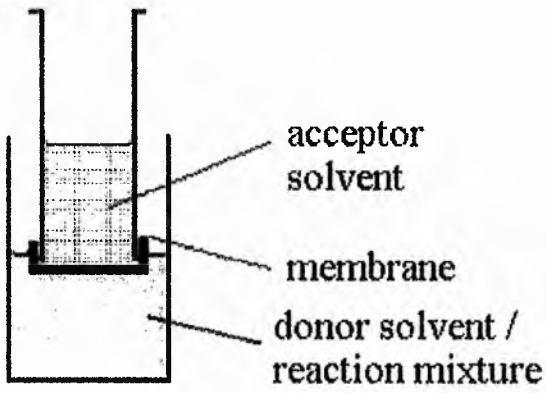

Figure 3.2. Silicone Membrane Evaluation Using the Supported Silicone Membrane Probe

A methanol/water acceptor solution (20/80 % v/v) was pipetted into the membrane probe. An aliquot of this acceptor solvent (300 µl) was removed from the membrane probe and placed in a labelled HPLC vial. Phenylethylamine (PEA, 5.0 ml) was pipetted into the stirred ethanol and the membrane probe height was adjusted. At regular time intervals aliquots of the acceptor solvent were removed from the membrane probe and placed in HPLC vials for off-line analysis.

The extracted solutions were analysed by atmospheric pressure chemical ionisation mass spectrometry (Platform LC, Micromass, Manchester, UK). The analyte solution (50 µl) was injected, in triplicate, into a stream of methanol/water (50/50 % v/v) pumped into the APCI source of the MS at 0.5 ml min^{-1}. The mass spectrometer was operated in positive ion APCI mode under the following conditions: mass range: 100-300 amu, APCI pin voltage: ± 3.5 kV, cone voltage: ±10 V, APCI probe heater: 400°C, source heater: 150°C.

3.3 Results and Discussion

3.3.1 Membrane Material Evaluation

The silicone membrane, cast using the technique described in Section 3.2.2, is extremely thin and would not withstand the pressures applied when incorporated into the liquid-liquid membrane interface used in Chapter 2. Casting the membrane onto a porous support material would give it some structural rigidity and enable it to withstand the pressures associated with the membrane interface and donor and acceptor flow pumping systems.

A search of the membrane suppliers determined that nylon and polypropylene were available as net filters with a pore sizes of 80 μm (Millipore Watford, UK). This structure would give enough support to the membrane to prevent it from rupturing but have large enough pores so that the silicone membrane had contact with the analyte.

The solubility of the silicone membrane and support material were investigated using the solvents expected to be encountered in the membrane preparation and use: acetonitrile, acetone, methanol, toluene and acetone/ toluene mix. Sections of prepared silicone membrane and the membrane support materials; nylon mesh and polypropylene mesh, were left overnight in flasks containing solvents. The membrane sections were then dried and examined for swelling, signs of dissolution or other physical changes. All the materials tested showed no changes

in structure due to contact with the solvents and were suitable for use in the supported membrane experiments.

3.3.2 Supported Membrane Casting

A considerable amount of time and effort was taken investigating various methods of casting the silicon membrane. Initially the membranes placed on the PTFE casting plate and silicone solution dispensed onto the net support membrane surface. After curing the membranes were examined under the microscope for integrity. Initially the membrane did not coat the net sufficiently and was either "clumped" in discrete particles, or later, formed a uniform layer, but with holes in the membrane material, which compromised the integrity of the supported membrane.

Additional coatings with more concentrated solutions were performed until a uniform membrane appeared to be formed, when observed using the microscope. The silicone supported membrane was then mounted into a probe unit to test for integrity. Water was placed inside the probe and leakages were observed. Pinholes in the membrane that had not been seen under the microscope allowed water to pass through the membrane.

A new approach to casting the membrane was therefore tested. The membranes were cast in-situ in the probe unit. Initially the silicone solution was cast onto the

surface of the upturned membrane probe. However, the membranes were found to be flawed when tested for integrity with water and then methanol. Methanol was used because it has a lower surface tension and would flow through smaller holes in the membrane material.

Membranes were then produced by placing the probes membrane side down onto the PTFE casting sheet and placing the silicone solution inside the probe directly onto the membrane surface. These supported membranes did not leak when tested with water and methanol.

3.3.3 Supported Membrane Evaluation Using the Membrane Probe

The supported membranes were evaluated as simple extraction devices prior to use in the liquid-liquid membrane interface discussed in Chapter 2. The membrane probe was filled with an acceptor solvent (methanol/water 20/80 % v/v) and positioned so the supported membrane was just below the surface of the stirred ethanol donor solvent. Phenylethylamine (5 ml), an analyte used in Chapter 2 for the evaluation of the membrane interface, was pipetted into the ethanol donor solution to form a donor solution (PEA, 1.65 mol/l). Aliquots of the acceptor solution were removed over time and placed in HPLC vials for off-line analysis. The extracted solutions were analysed by APCI-MS and the analyte response from phenylethylamine (PEA, *m/z* 122) was determined.

3.3.3.1 Nylon Supported Silicone Membrane Evaluation

The aliquots of acceptor solution from the nylon supported membrane probe were analysed by mass spectrometry to determine the rate of transfer of analyte from the ethanol donor solution to the methanol/water acceptor solution held within the membrane probe system. The mass spectrometric single ion responses, monitored at *m/z* 122, obtained for the extracted phenylethylamine solutions are presented in Table 3.1 and Figure 3.3.

Table 3.1 Observed response for acceptor solution after addition of PEA.

Time from addition of PEA (minutes)	Peak Area (*m/z* 122)
0.0	4142
1.0	5628
1.6	6921
2.0	6501
2.6	68871
3.7	61175
4.8	86094
6.3	70306
15.1	72387
19.1	64031

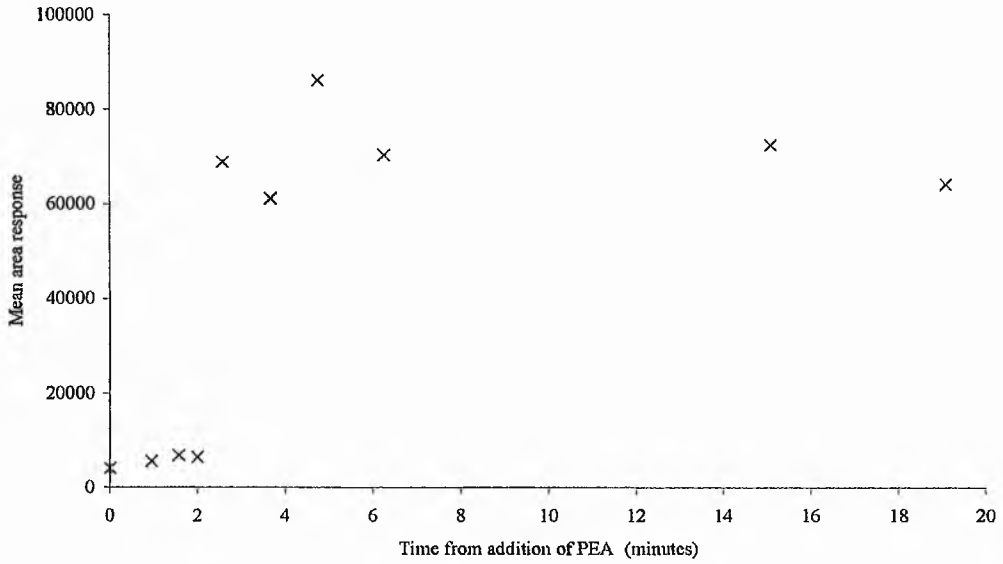

Figure 3.3 Observed responses for acceptor solution after addition of PEA

Two minutes after the addition of phenylethylamine there is a step change in the response seen for the acceptor solution indicating a rapid infusion of PEA into the acceptor solution. As the response did not change gradually over time it is possible that there was a membrane failure after 2 minutes operation. This may have been caused by degradation of the membrane material, resulting in enlarged pores which allowed phenylethylamine to pass directly into the acceptor solution held inside the membrane probe. Alternatively a lack of control in the membrane production may have resulted in a dense, thick membrane through which the PEA took longer than expected to diffuse.

3.3.3.2 Polypropylene Supported Membrane Evaluation

Aliquots of acceptor solution from the polypropylene supported membrane probe

were analysed by mass spectrometry to determine the rate of transfer of analyte from the ethanol donor solution to the methanol/water acceptor solution held within the membrane probe system. The mass spectrometric single ion responses, monitored at *m/z* 122, obtained for the extracted phenylethylamine solutions are presented in Table 3.2 and Figure 3.4.

Table 3.2 Observed response for acceptor solution after addition of PEA.

Time from addition of PEA (minutes)	Peak Area (*m/z* 122)	Time from addition of PEA (minutes)	Peak Area (*m/z* 122)
0.0	5476	5.0	7067
0.7	5925	7.0	9205
1.0	6394	8.2	7424
1.5	5393	9.0	5437
2.0	3946	11.0	6095
2.5	4537	13.0	7049
3.0	4991	15.0	8779
3.5	7929	26.0	14330
4.0	8795	48.0	113562
4.5	7398	65.0	170231

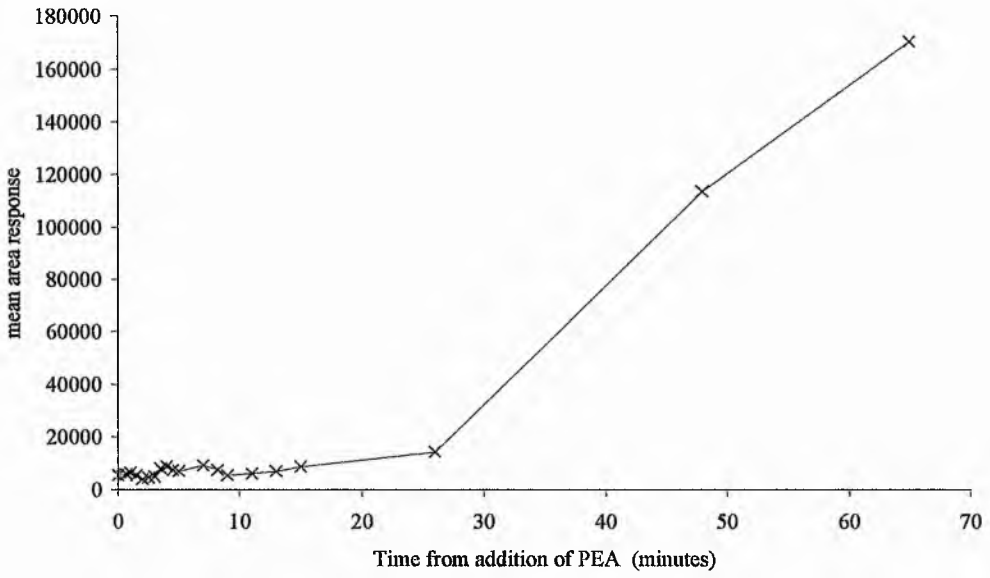

Figure 3.4 Observed responses for acceptor solution after addition of PEA

The addition of phenylethylamine resulted in a gradual change in the response seen for the acceptor solution indicating that the PEA was diffusing into the acceptor solution. The response continued increasing gradually over 25 minutes, indicating a very slow membrane diffusion process. However the PEA concentration increased much more rapidly after 25 minutes, suggesting partial failure of the membrane. For process monitoring applications the variation in analyte concentration must be measured in a time scale appropriate to the changing reaction conditions. A membrane interface with a response time of over hour is of no use in process monitoring applications.

To improve the rate of diffusion through the membrane interface the membrane would need to be thinner, but would need to retain its rigidity and integrity.

Although the process of casting the silicone membrane onto the net membrane support was optimised for the membrane probe, attempts to cast thin silicone membranes with improved integrity and robustness were unsuccessful. Increasing the temperature of the membrane interface would increase the rate of analyte diffusion through the membrane. However this was not possible for the membrane probe nor the liquid-liquid membrane interface.

The investigation proceeded using a commercially available semi-permeable membrane in the form of a hollow fibre silicone membrane. The use of a semi-permeable silicone membrane coupled with mass spectrometric analysis, to monitor a reaction in real time, is discussed in Chapter 4.

3.4 Conclusions

The production of supported semi-permeable membranes was investigated and a variety of casting techniques were evaluated. Semi-permeable silicone membranes were cast onto both nylon and polypropylene net support materials and incorporated into a membrane probe device. The supported silicone membrane probes were evaluated for their application as a liquid-liquid membrane interface for monitoring process changes. Despite considerable effort over several months, with continuous re-evaluation of the supported membrane casting process, membranes could not be produced to a sufficient quality to perform the work intended.

The transport of PEA through supported membrane probes took over 2 minutes for the nylon membrane and up to 60 minutes for the polypropylene membrane. The lengthy response time was attributed to the thickness of the cast silicone membrane. This time scale was not appropriate for process monitoring where the analyte concentration must be monitored in as close to real-time as possible. Increasing the temperature of the extraction system would increase the rate of analyte diffusion through the membrane, but this facility was not available for the membrane probe or liquid-liquid membrane interface.

Response times could be improved if the silicone membrane could be cast as a thinner layer whilst retaining the integrity of the membrane. Unfortunately, facilities were not available to produce thinner membranes.

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Chapter 4

Reaction Monitoring Using a Semi-Permeable Membrane Interface

4.1 Introduction

This chapter describes an investigation into the use of membrane inlet mass spectrometry for the real time monitoring of an early stage, pharmaceutical intermediate process, containing components of typically 20% w/v. For some reactions it is convenient to monitor the volatile reactants or products as a means of process analysis. In these cases membrane inlet membrane spectroscopy (MIMS) using a semi-permeable membrane may be better than the microporous membrane interface used in Chapter 2.

Membrane inlet mass spectrometry has been used widely as an interface for the analysis of dilute, volatile or semi-volatile organic compounds in aqueous samples by transport across a semi-permeable membrane.[1-4] MIMS has been used in a number of process monitoring studies, where reactants or products have sufficient volatility to permeate a semi-permeable membrane and desorb into the helium flow. Applications include catalytic hydrohalogenation of halogenated aromatics,[5] photocatalytic degradation of phenol and trichloroethylene,[6] the photolysis of benzyl acetate and dimethoxybenzyl acetate[7] and bioreactor monitoring.[1,8,9] The process mixture is sampled into a 'donor' stream (either solvent or vapour). The donor stream is passed across the surface of the membrane and a proportion of the solvent and analyte pass through the membrane and desorb into a helium 'acceptor' stream flowing across the other surface of the membrane. The helium stream, together with any desorbed analytes, is directed towards the source of the mass spectrometer.

A membrane interface may alternatively be used to achieve high dilution factors by controlling analyte transport across the membrane. This allows quantifiable mass spectrometric data to be obtained by bringing the analyte concentrations within the dynamic range of the mass spectrometer and preventing contamination of the mass spectrometer. In addition, membrane interfaces may be applicable to sample suspensions and heterogenous mixtures, which would otherwise be impossible to analyse using flow injection analysis or direct sampling. In this set of experiments a MIMS device, incorporating a semi-permeable membrane, was used to monitor the volatile components of a process mixture from a Mannich reaction and acted as a diluting interface between the concentrated reaction mixture and a quadrupole mass spectrometer.

In preliminary experiments (Chapter 2 Section 2.2.4) the liquid reaction mixture was sampled into an aqueous donor stream, which was interfaced to a methanol/water acceptor stream, via a liquid-liquid microporous membrane interface coupled to an APCI MS source. A major component of the reaction mixture caused suppression of the MS signals for the other reaction components, so a semi-permeable membrane interface and electron ionisation mass spectrometer was evaluated. The membrane interface was operated in two modes. Initially the reaction solution was sampled directly into an aqueous donor stream connected to a MIMS interface. Analyte levels were found to be too high for EI/MS analysis using this configuration, so a post-membrane splitter was added. Volatile components of the reaction mixture were detected and quantified with

this technique, but the membrane had a limited lifetime and the method lacked robustness.

In an alternative approach, designed to preserve the membrane and improve the analyte peak shape, the headspace above the reaction mixture was sampled thought the MIMS device in the vapour phase using air as the donor stream and helium as the acceptor stream.

When a solution of analyte is held in a sealed container the analyte establishes an equilibrium with the layer of vapour above it, known as the headspace.[10] At a constant temperature the concentration of an analyte in the headspace (C_H) is proportional to the concentration in the liquid phase (C_L) for a fixed volume of liquid and headspace (see Equation 4.1).

$$K_D = \frac{C_L}{C_H} \qquad \text{(E.4.1)}$$

Where K_D is the partition coefficient for the analyte which is dependent on the liquid and headspace volume and temperature. The headspace concentration therefore reflects the analyte concentrations in the reaction mixture.

4.2 Direct Liquid Sampling Membrane Inlet Mass Spectrometry

4.2.1 Materials and Methods

4.2.1.1 Chemicals

Parahydroxyacetophenone (>98%) and dimethylamine (60% aqueous solution) were obtained from Fluka (Buchs, Switzerland), formaldehyde solution (AR grade, 37-40% aqueous solution, stabilised with 9.4% methanol) and methanol (AR grade) from Fisher Chemicals, (Loughborough, UK) and purified water from a Barnstead Nanopure Diamond purification unit (Barnstead, UK).

4.2.1.2 Mannich Reaction - early stage Salbutamol Synthesis

A bench scale Mannich Reaction was studied (Scheme 4.1). Purified water (30 ml) was added to dimethylamine (DMA, 17 ml, 60% aqueous, 0.227 moles), the mixture was continuously stirred and cooled to 15°C in a stirred ice/water bath. Parahydroxyacetophenone (pHAP, 12.5 g, 0.092 moles) was added to the reaction vessel. Formaldehyde solution (15 ml, ~40%, 0.185 moles) was added over 30-50 minutes using a burette, with the temperature maintained at 15°C±2°C.

4.2.2 Membrane Interface Set-up and Mass Spectrometric Analysis for Direct Liquid Sampling

A MIMS interface constructed in-house (Figure 4.1) was fitted with a silicone hollow-fibre membrane (0.66 mm OD x 0.305 mm ID x 65 mm, SFMedical, Huston).

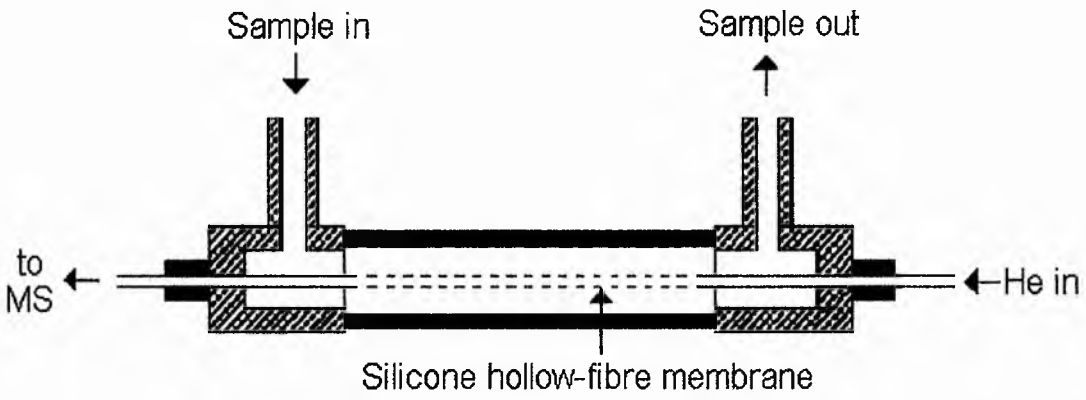

Figure 4.1 The MIMS interface. (From reference 11)

The MIMS interface was installed in a HP5890 GC with HP5971 quadrupole MS (Agilent, Palo Alto, USA). Fused silica tubing (approximately 2 m x 0.25mm ID, SGE Europe Ltd., Milton Keynes, UK) was used to connect the MIMS interface to the GC injector and the MS inlet. Coils of this fused silica were used as flow restrictors to maintain the backpressure for the helium supply before and after the MIMS unit. A schematic diagram of the instrument set-up is presented in Figure 4.2.

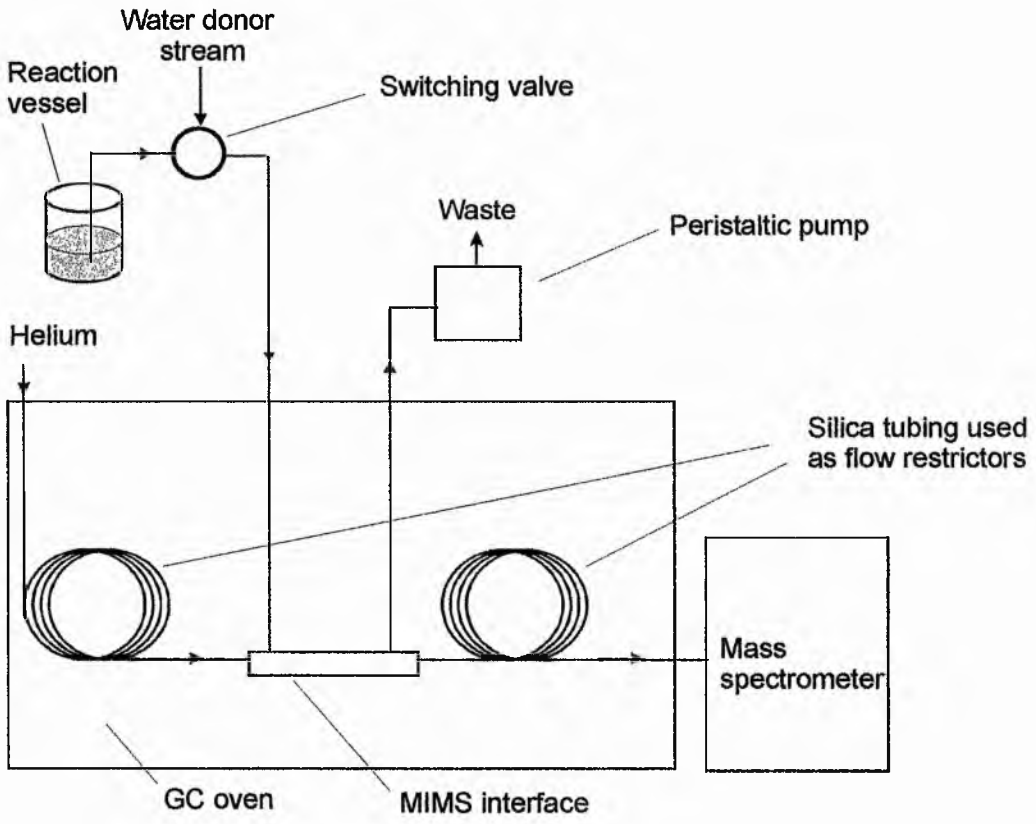

Figure 4.2 A schematic diagram of the instrument set-up for direct liquid sampling membrane inlet mass spectrometry.

Note: The membrane interface required a high degree of dexterity to construct. The membrane tubing was cut to size then one end was pushed onto and over the end of the fused silica tubing. This was then threaded through the interface device and the other end of the membrane threaded onto a second piece of silica tubing. The membrane was then secured in place in the interface unit using stainless steel nuts and soft graphite ferrules. Care was required to ensure a good seal was formed, holding the membrane in place without crushing the silica tubing. The MIMS interface was then suspended inside the GC oven. The full weight of the

interface unit needed to be supported to prevent the fragile silica from snapping (and thus requiring the entire unit to be rebuilt). Securing tapes had to be designed and positioned to avoid scratching or abraiding the silica tubing as the device was moved by the GC oven fan. Once the interface device was constructed and secured in place it was safe and robust but the construction was tricky and often took several hours.

A continuous donor flow (purified water at 4 ml min^{-1}) was pumped through the MIMS device using a peristaltic pump (CL Variable Speed peristaltic pump, Fisher, Loughborough, UK). A helium flow of 2 ml min^{-1} was maintained as an acceptor stream, passing through the hollow fibre membrane and into the EI ion source of the mass spectrometer. At regular intervals the manual switching valve was activated (for a timed interval) to switch from pumping water as the donor solvent to drawing a sample of the reaction mixture from the reaction vessel containing the Mannich reaction. The valve was then switched back to the aqueous flow. Samples withdrawn from the reaction vessel were introduced directly into the MIMS interface. Components of the reaction mixture that diffused through the MIMS membrane and into the helium acceptor stream were carried into the MS for analysis. The GC oven temperature maintained the membrane interface at 80°C. The single quadrupole mass spectrometer (HP5971, Agilent, Palo Alto, USA) was operated in full scan EI mode over the range *m/z* 35-150 with a threshold value of 100.

4.2.3 Effect of Membrane Temperature

The reaction mixture was sampled, using the procedure described in Section 4.2.2, for 6 seconds (equivalent to 400 µl of reaction mixture) at the column oven temperatures of 40, 60, 70, 80 and 90°C.

4.2.4 Results and Discussion

4.2.4.1 Selection of a Model Reaction System

The Mannich reaction of dimethylamine (DMA) with parahydroxyacetophenone (pHAP) and formaldehyde solution in water was selected as a model reaction system for evaluating membrane interface configurations as discussed in Section 2.3.2.1.

4.2.4.2 Mass Spectrometric Analysis

The MIMS interface shown in Figure 4.1 was fitted with a semi-permeable silicone membrane. The interface was installed in the GC oven of a GC-MS instrument to control the membrane temperature. At regular intervals throughout the reaction process replicate aliquots of the concentrated Mannich reaction mixture were introduced directly into the gaseous donor stream and pumped through the MIMS interface. Components of the reaction mixture that diffused through the MIMS membrane into the helium acceptor stream were transported into the MS. Sharp peaks were obtained when the single ion response (*m/z* 42) for DMA was plotted (See Figure 4.3) showing that the volatile amine is readily transported across the membrane and desorbed into the helium stream directed towards the EI source of the mass spectrometer.

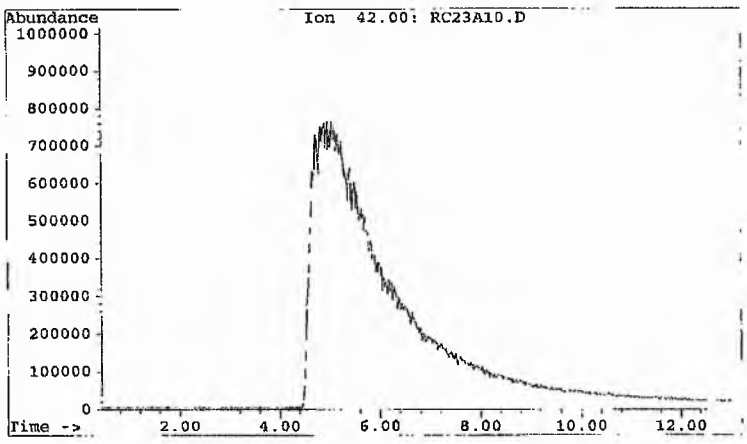

Figure 4.3 Single ion response for the dimethylamine fragment at *m/z* 42

To avoid interference the single ion response for DMA was monitored using a fragment ion at 42 amu ($[C_2H_4N]^+$) because the $[M]^{+\cdot}$ ion at 45 amu for DMA overlaps with the ^{13}C peak for carbon dioxide in air. Even when the solvents used were degassed before use a response attributable to the CO_2 in the system was still observed. The larger, less volatile components of the Mannich reaction, pHAP and HMADMA, were not detected by this system. This was either due to these larger molecules having a lower solubility in the membrane material and thus a lower transport rate across the membrane, or because they were not vaporised efficiently at the relatively low reaction vessel temperatures used in these experiments. A typical spectrum obtained for the Mannich reaction mixture is presented in Figure 4.4

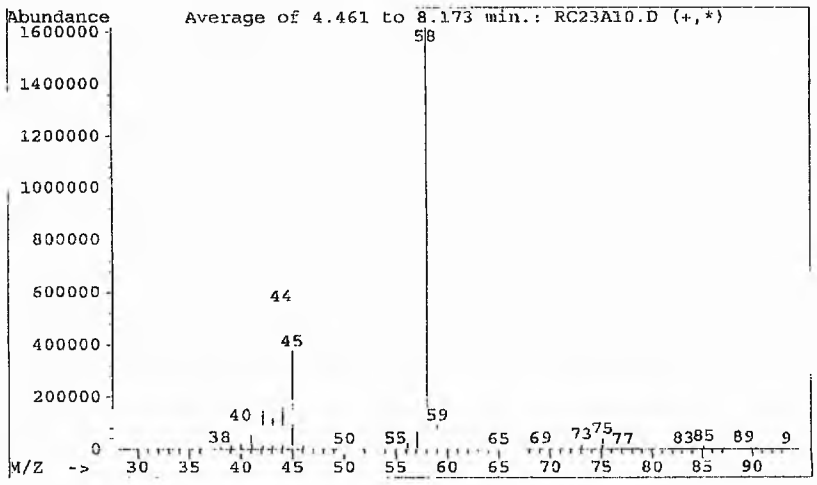

Figure 4.4 A typical spectrum for the Mannich Reaction Mixture obtained using direct liquid sampling membrane inlet mass spectrometry

4.2.4.3 Effect of Membrane Temperature

The effect of membrane temperature on the DMA response was investigated by extracting replicate aliquots (6 seconds, 400 μl) of the concentrated reaction mixture (after the reaction had been allowed to go to completion) and introducing them into the membrane interface at membrane temperatures of 40, 60, 70, 80 and 90°C. Single ion responses for the DMA fragment (*m/z* 42) at 60, 70 and 80°C are presented in Figures 4.5, 4.6 and 4.7. The peak at 40°C was broader than that at 60°C, and the peak at 90°C was similar to that at 80°C.

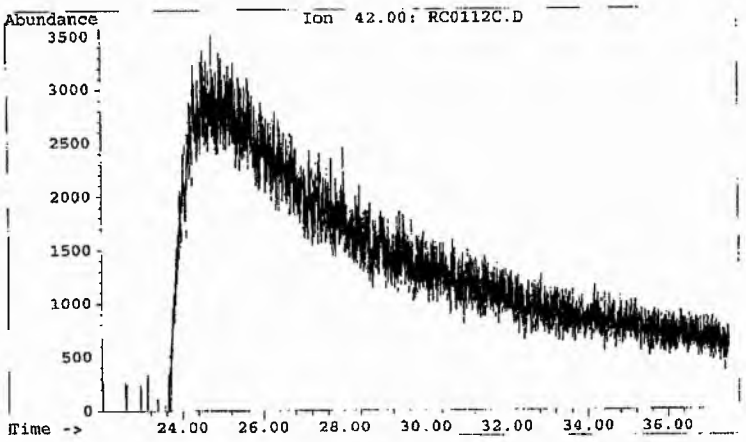

Figure 4.5 Single Ion Response for DMA (*m/z* 42) at 60°C

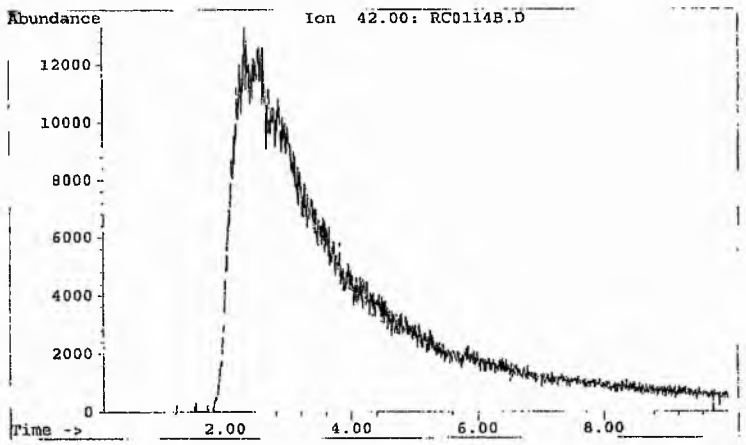

Figure 4.6 Single Ion Response for DMA (*m/z* 42)at 70°C

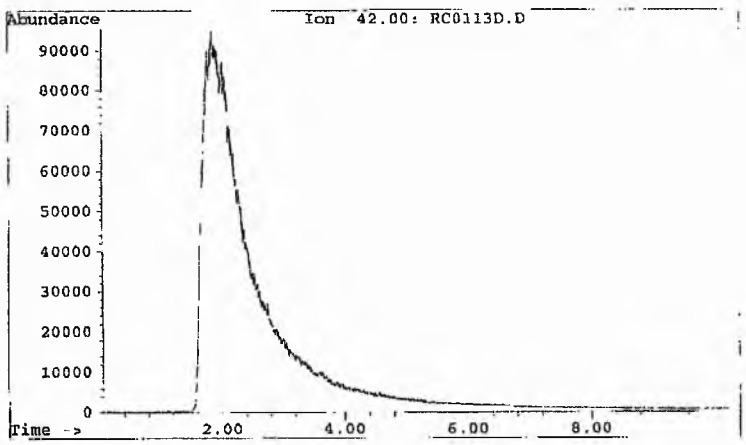

Figure 4.7 Single Ion Response for DMA (*m/z* 42)at 80°C

As the temperature of the membrane interface was increased the DMA peak became sharper and narrower. This is because the rate of diffusion of analyte molecules through the MIMS membrane increases with temperature, as does the rate of desorption of analyte into the acceptor stream. The optimal operational temperature for the MIMS membrane with water donor solvent was 80°C as this gave the sharpest peaks, but was below the boiling temperature of the aqueous donor solvent. Sharp peaks enable more reliable integration and reduce the time taken for the system to return to a baseline level response after each aliquot of analyte, thus reducing the cycle time for reaction monitoring to 2-3 minutes.

These experiments indicated the potential of MIMS for the direct analysis of concentrated reaction mixtures introduced as liquid samples in direct contact with the membrane surface, with certain conditions. The analyte molecules must be small enough to be transported across the membrane, have a suitably high volatility and be chemically compatible with the membrane material so as not to cause membrane deterioration.

However, in these experiments the membrane did not appear to be stable for long-term use under these conditions and deteriorated after a few days, or in some cases a few hours of operation. As the membrane degraded the membrane flux increased causing the mass spectrometer to shut down because the backing pressure rose above the pre-set cut-off. The membrane degradation was attributed to the high load of corrosive analyte passing through the interface as the liquid reaction mixture was sampled. Various attempts were made to reduce the

membrane degradation and MS base pressure by diluting the sample prior to introduction into the interface and by splitting the helium flow between the interface and mass spectrometer. However, none of these modifications to the system resulted in a robust configuration. An alternative sampling regime using headspace analysis combined with MIMS was therefore investigated.

4.3 Headspace Sampling Membrane Inlet Mass Spectrometry

The Mannich reaction studied in Section 4.2 was monitored using headspace sampling membrane inlet mass spectrometry. The chemicals and model reaction system are as described in the Liquid Sampling Membrane Inlet Mass Spectrometry section.

4.3.1 Materials and Methods

4.3.1.1 Membrane Interface Set-up And Mass Spectrometric Analysis for Headspace Sampling

A MIMS interface constructed in-house (Figure 4.1) was fitted with a silicone hollow-fibre membrane (0.66 mm OD x 0.305 mm ID x 65 mm, SFMedical, Huston).

The MIMS interface was installed in a HP5890 GC with HP5971 quadrupole MS (Agilent, Palo Alto, USA). Fused silica tubing (approximately 2 m x 0.25mm ID, SGE Europe Ltd., Milton Keynes, UK) was used to connect the MIMS interface to the GC injector and the MS inlet. Coils of this fused silica were used as flow restrictors to maintain the backpressure for the helium supply before and after the MIMS unit. A schematic diagram of the instrument set-up is presented in Figure 4.8

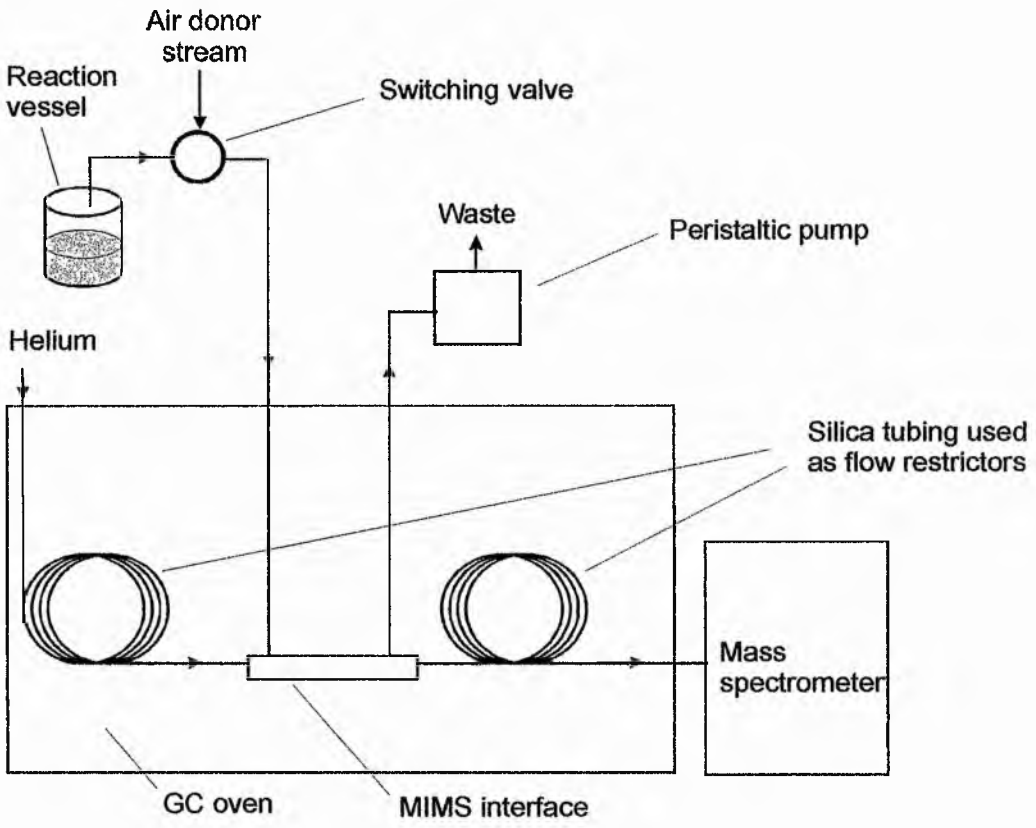

Figure 4.8 Schematic diagram of the instrument set-up for headspace sampling membrane inlet mass spectrometry.

A continuous donor flow of laboratory air (2.2–2.4 ml min^{-1}) was pumped through the MIMS device using a peristaltic pump (CL Variable Speed peristaltic pump, Fisher, Loughborough, UK). A helium flow of 2 ml min^{-1} was maintained as an acceptor stream, passing through the hollow fibre membrane and into the EI ion source of the mass spectrometer. At regular intervals the switching valve was activated for a timed interval to switch from pumping air as the donor, to drawing a sample of the headspace from the reaction vessel containing the Mannich reaction mixture. The valve was then switched back to the air donor flow.

Samples withdrawn from the reaction vessel were introduced directly into the MIMS interface. Components of the reaction mixture that diffused through the MIMS membrane and into the helium acceptor stream were carried into the MS for analysis. The GC oven temperature maintained the membrane interface at 180°C. The single quadrupole mass spectrometer (HP5971, Agilent, Palo Alto, USA) was operated in full scan EI mode over the range *m/z* 35-80 with a threshold value of 100.

4.3.1.2 Effect of Sampling Time and Membrane Temperature

A solution of the concentrated reaction product mixture after the reaction had been allowed to go to completion (Section 4.2.1.2, ~10-20 % w/v) was continuously stirred and aliquots of the headspace were sampled by switching the sampling valve for a timed interval. The membrane temperature, maintained by the GC oven, was set to 120, 150, 180 and 200°C and the headspace mixture was sampled for periods of 5, 10 and 30 seconds.

4.3.1.3 Effect of Donor Gas Flow Rate on Dimethylamine Response

A stock dimethylamine solution (18% v/v) was prepared by adding dimethylamine solution (8.5 ml, 60% aqueous solution) to methanol (0.5 ml) and purified water (18.8 ml). An aliquot of this stock (15 ml) was pipetted into the reaction flask and stirred continuously. An aliquot of the flask headspace was

sampled via the MIMS membrane interface for 10 seconds with a donor gas flow of 5.6 ml min^{-1}. The headspace vapour was then sampled for 5 and 10 seconds at donor gas flow rates of 2.2 to 6.2 ml min^{-1}.

4.3.1.4 Linearity of the System Response for Dimethylamine

The reaction vessel used for the Mannich reaction was charged with purified water (18.8 ml) and methanol (0.7 ml) and was stirred continuously. Aliquots of dimethylamine (0.5 ml, 60% aqueous solution) were added to the flask, using a long needled syringe, to introduce the DMA below the surface of the liquid. Up to a total of 8.5 ml dimethylamine was added to the flask. After each addition the contents of the flask were allowed to equilibrate with the headspace for 2 minutes before sampling via the MIMS interface.

4.3.1.5 Reproducibility of Dimethylamine Response at Different Reaction Temperatures

An aliquot (15 ml) of stock dimethylamine solution (prepared as described in Section 4.3.1.3, ~10-20 % w/v) was pipetted into a reaction flask, placed in an ice/water bath and stirred continuously. The reaction vessel temperature was adjusted to 15°C $\pm$ 0.5°C and the system allowed to equilibrate. Aliquots of the headspace were sampled via the MIMS interface (membrane temperature 180°C). This procedure was repeated, using a fresh flask and aliquot of DMA stock solution, at 10, 13, 15 and 17°C.

4.3.1.6 Effect of Reaction Vessel Temperature on Dimethylamine Response

An aliquot (15 ml) of stock dimethylamine solution (prepared as in Section 4.3.1.3) was pipetted into a reaction flask, placed in an ice/water bath and stirred continuously. The temperature was adjusted to 15°C and the system allowed to equilibrate. Aliquots of the headspace were sampled via the MIMS interface at reaction vessel temperatures of 14 to 17°C.

4.3.1.7 Reproducibility of Hydroxymethyldimethylamine Response

A stock hydroxymethyldimethylamine solution (HMDMA) was prepared by adding the formaldehyde solution (45 ml, ~40% formaldehyde, ~0.60 moles), to the dimethylamine solution (51 ml, 60% DMA, 0.68 moles) and purified water (90 ml) in a stirred flask and leaving them to react overnight. An aliquot (15 ml) of this reaction mixture was pipetted into a reaction vessel in an ice/water bath and stirred continuously. The temperature was adjusted to 15°C and the system allowed to equilibrate. Aliquots of the headspace were sampled via the MIMS interface.

4.3.1.8 Effect of Reaction Vessel Temperature on Hydroxymethyl-dimethylamine Response

An aliquot (15 ml) of the HMDMA solution prepared as described in Section

4.3.1.7 was pipetted into a reaction flask, placed in an ice/water bath and stirred continuously. The temperature was adjusted to 15°C and the system allowed to equilibrate. Aliquots of the headspace were sampled via the MIMS interface at reaction vessel temperatures of 13 to 17°C.

4.3.1.9 Monitoring a Mannich Reaction in Real Time

Mannich reactions were monitored on-line in real-time using the reaction system detailed in Section 4.2.1.2 using the membrane interface sampling system detailed in Section 4.3.1.1. For the first system the reaction vessel temperature was maintained at 15°C $\pm$ 2°C whilst the second was maintained at 15°C $\pm$ 1°C.

4.3.2 Results and Discussion

The headspace/MIMS configuration shown in Figure 4.8 allows the volatile DMA in the reaction mixture headspace to be withdrawn from the reaction vessel and transported to the membrane interface. The DMA is solubilised in the membrane, diffuses across the membrane to the inner surface and is desorbed into the helium stream directed towards the EI source of the mass spectrometer. A silicone membrane was selected because these have been shown to be robust and permeable to a wide variety of analytes.

The single ion response for DMA (*m/z* 42) extracted from the headspace of the reaction vessel is shown in Figure 4.9. As the DMA passes through the interface the intensity rises to a maximum, before returning to the background level.

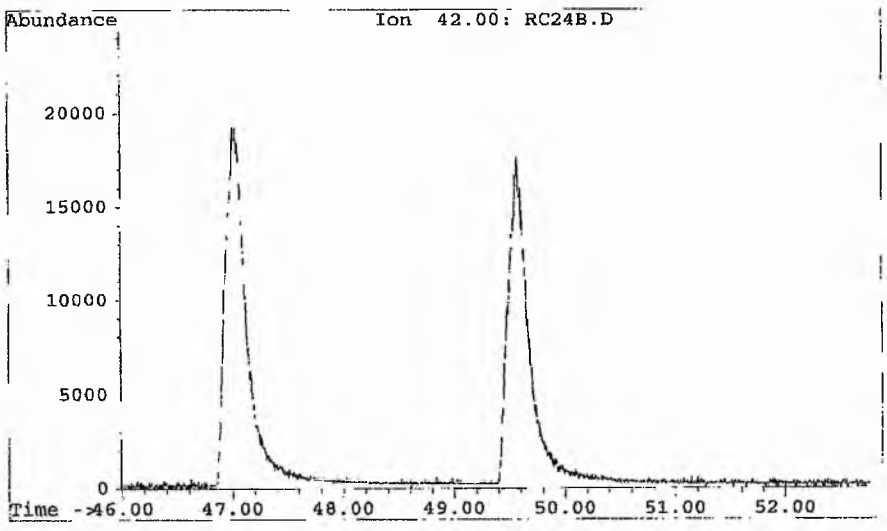

Figure 4.9 Single Ion Responses For Mannich Reaction Headspace Analysed by Membrane Inlet Mass Spectrometry

4.3.2.1 Components of the Mannich Reaction Detected During Analysis with the MIMS Interface

An example of the mass spectrum obtained for an aliquot of reaction mixture analysed at a membrane temperature of 180°C is presented in Figure 4.10. The major components detected were dimethylamine (fragment at *m/z* 42), and the iminium ion (*m/z* 58) and hydroxymethyldimethylamine (*m/z* 75) reaction intermediates. The other reaction components, parahydroxyacetophenone and dimethylamino-methylhydroxyacetophenone were not detected by this system due to their lower rate of diffusion through the MIMS interface.

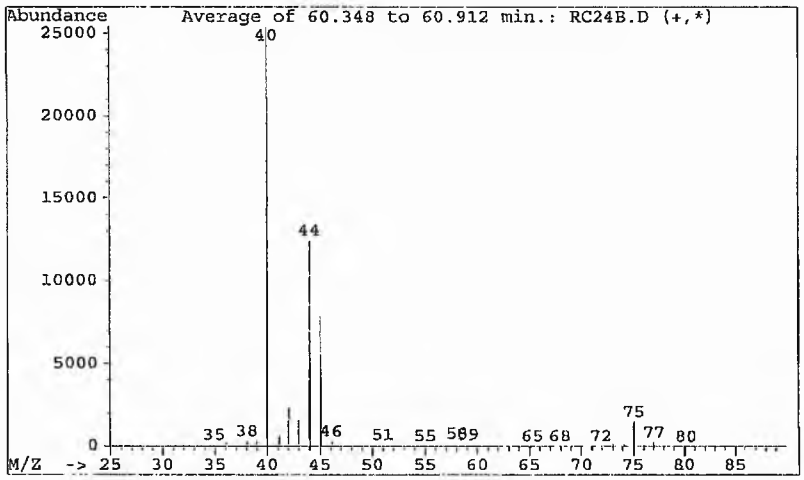

Figure 4.10 Mass Spectrum Showing the Single Ion Response for a 10 Second Aliquot of the Mannich Reaction Mixture.

4.3.2.2 Effect of Sampling Time and Membrane Temperature on the DMA Response

The MIMS response is known to be sensitive to temperature and concentration[12] so the reaction mixture was sampled over a range of membrane temperatures (120, 150, 180 and 200°C) and for different sampling times (30, 10 and 5 seconds). The different peak shapes observed for the experiments are presented in Figures 4.11, 4.12, 4.13 and 4.14. The single ion response for DMA was monitored using the fragment ion at 42 amu ($[C_2H_4N]^+$).

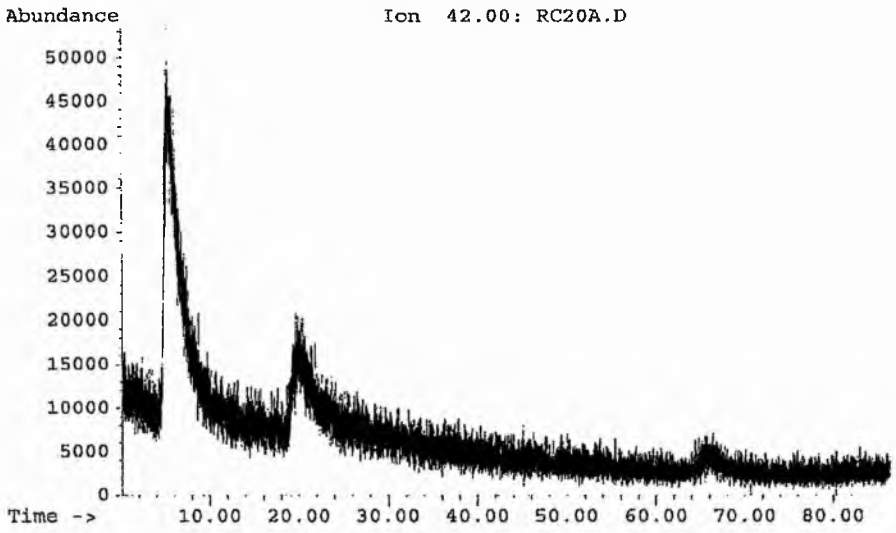

Figure 4.11 Single Ion Response for DMA (*m/z* 42) at 120°C Sampled for 30, 10 and 5 Seconds Respectively.

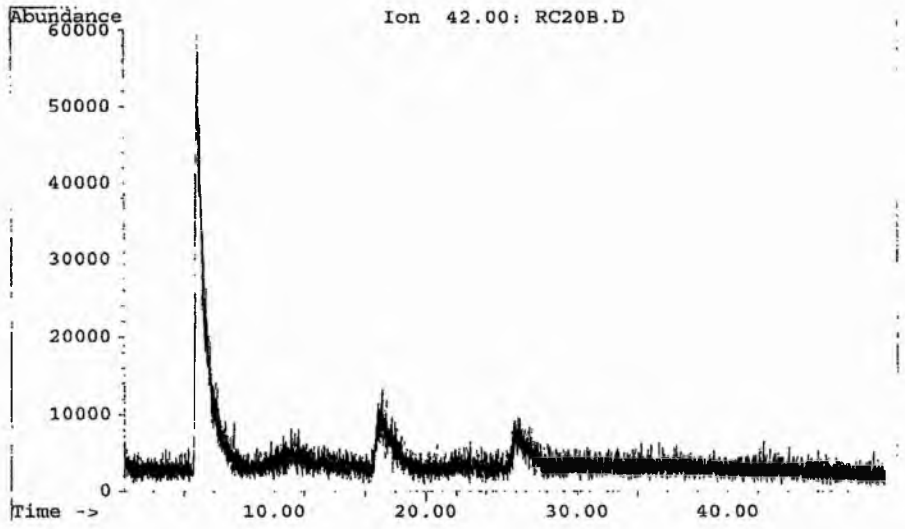

Figure 4.12 Single Ion Response for DMA (*m/z* 42) at 150°C Sampled for 30, 10 and 5 Seconds Respectively.

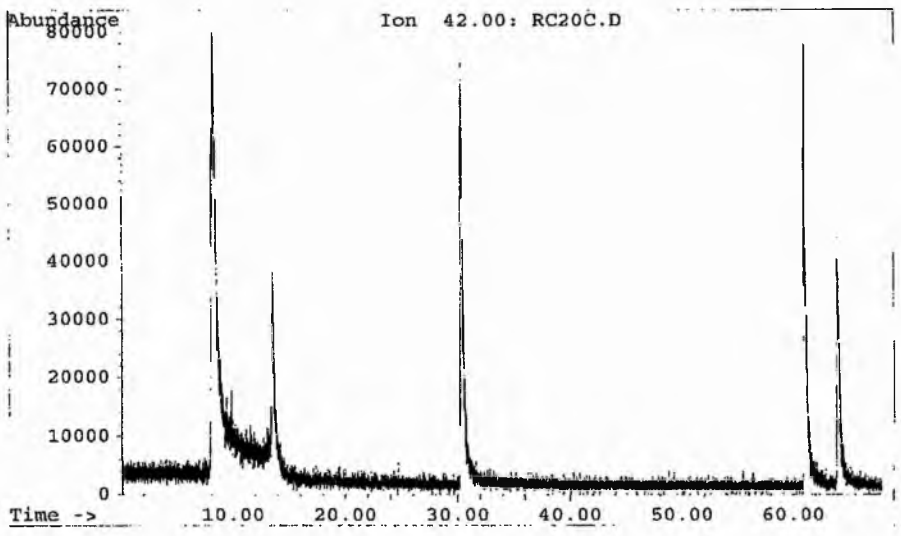

Figure 4.13 Single Ion Response for DMA (*m/z* 42) at 180°C Sampled for 30, 10, 10 and 5 Seconds Respectively.

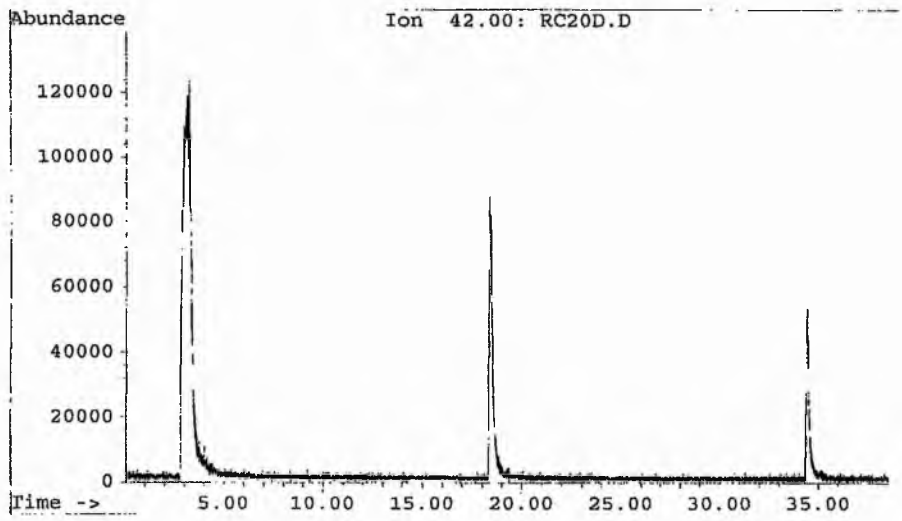

Figure 4.14 Single Ion Response for DMA (*m/z* 42) at 200°C Sampled for 30, 10 and 5 Seconds Respectively.

The rate of diffusion of analyte molecules through the MIMS membrane increases with increasing temperature, as does the rate of analyte desorption into the acceptor stream. In this experiment it was noted that as the temperature of the membrane interface rose the DMA single ion responses became sharper and narrower. Sharp peaks enabled more reliable integration and reduced the time taken for the system to return to a baseline level response after each aliquot of analyte headspace vapour was introduced into the interface, thus increasing the possible sampling rate. Although 200°C produced the sharpest DMA peaks there was concern about the membrane degrading at such high temperatures. The optimal temperature for the membrane system interface was therefore selected as 180°C, which gave sharp analyte responses (with a peak width of 2-3 minutes) at a low enough temperature to avoid membrane degradation.

The peak areas varied as expected with headspace sampling time. All of the peaks obtained for ten second sampling interval were sharp and of a suitable level for quantification. A 5 second sampling period at 120°C resulted in a DMA response level that was unquantifiable, due to the baseline noise level, whilst longer sampling times took more time for the signal to re-equilibrate back to baseline, resulting in a lower possible number of samples per time interval. The optimal sampling conditions were deemed to be a 10 second sampling period at a membrane interface temperature of 180°C, as shown in Figure 4.13 and these conditions were used in all subsequent experiments.

4.3.2.3 Effect of Donor Gas Flow Rate on Dimethylamine Response

The flow rate of the donor gas and the length of the sampling time both affect the amount of analyte sampled and thus the system response. To investigate the combined effect of donor flow rate and sampling time, aliquots of the reaction flask headspace were sampled via the MIMS membrane interface for 5 and 10 seconds with donor gas flow rates of 2.2 to 6.2 ml min^{-1} at a constant membrane temperature of 180°C. The single ion responses for DMA (*m/z* 42) were determined and the results are presented in Table 4.1 and Figure 4.15.

Table 4.1 Effect of Changing Donor Gas Flow Rate and Sampling Time on System Response for Dimethylamine.

Donor Gas Flow Rate (ml min^{-1})	Sampling Time (s)	System Response (Area)
5.6	10	13621162
3.6	10	12660155
2.2	10	12316215
5.6	5	8760956
3.6	5	7978238
3.6	5	7510495
2.2	5	7445440
6.2	5	7412443

With a constant sampling time there was a relatively small rise in the system response for dimethylamine with increasing donor gas flow rates. The rate of analyte transfer through the membrane to the acceptor stream was at optimal rate for the constant analyte concentration. Increasing the donor stream flow rate slightly increased both the volume of headspace sampled and the donor side operational pressure, driving more analyte through the membrane. However, this was countered by the faster removal of the analyte from the membrane interface once the valve was switched back to sampling air. Increasing the analyte sampling time from 5 to 10 seconds approximately doubled the analyte response, as the analyte was sampled from the vessel for twice the time. The response was not exactly doubled as part of the sampling time is dedicated to switching the sampling valve at the start and end of the sampling period.

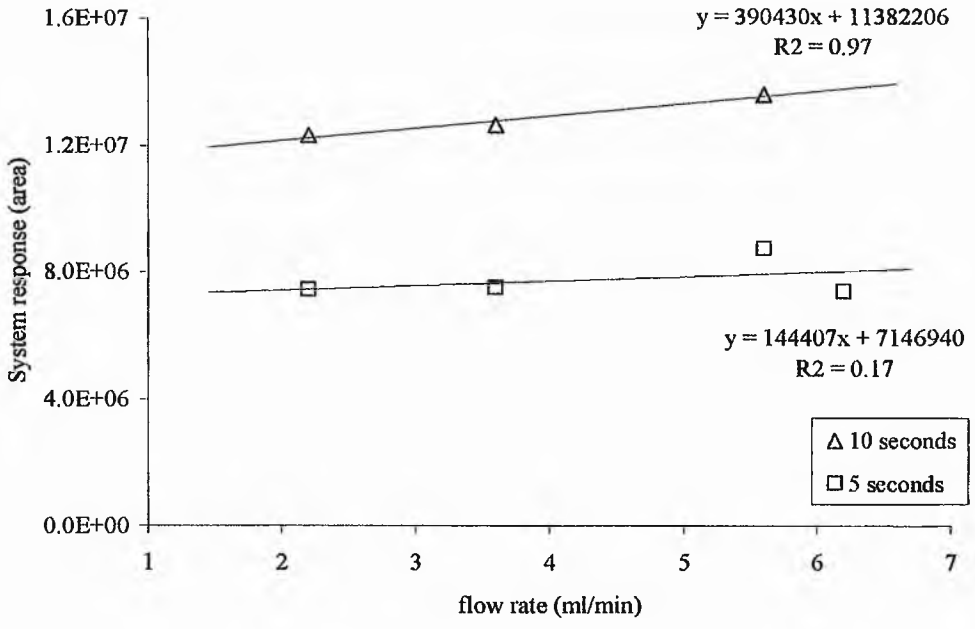

Figure 4.15 Effect of Changing Donor Gas Flow Rate and Sampling Time on System Response for Dimethylamine.

A 10 second sampling interval could be measured with greater confidence and precision than a 5 second interval, whilst sampling at a low donor flow rate reduced the sample consumption to a minimum. The optimal sampling conditions were deemed to be a donor flow rate of approximately 2.2 ml min^{-1}, with the headspace sampled for 10 seconds (equating to a sample volume of approximately 370 µl) and these conditions were used for all subsequent experiments.

4.3.2.4 Linearity of the System Response for Dimethylamine

The linearity of the system response was determined for dimethylamine. Aliquots of DMA were added to a water/methanol solution (at a composition equivalent to that expected in the reaction vessel) to simulate a changing reaction system concentration. After each addition the headspace was sampled (for 10 seconds with a membrane temperature of 180°C) and the system response (*m/z* 42) determined. The results of the linearity experiment are presented in Table 4.2 and Figure 4.16. The line of best fit through the data points and a visual inspection of the data shows a generally linear response to concentration with an R^2 value of 0.96. The variation about the best fit line for the data points is attributable to temperature changes in the system during monitoring. The quantitative effect of the reaction vessel temperature on DMA response was therefore investigated in more detail.

Table 4.2 Linearity of System Response for Dimethylamine using the Modified MIMS Interface.

DMA Added (ml)	DMA Conc. (mol/l)	System Response (Area)	DMA Added (ml)	DMA Conc. (mol/l)	System Response (Area)
0.5	0.33	483679	5.5	2.93	4092865
1	0.65	850793	6	3.14	4395561
1.5	0.95	1313955	6.5	3.33	5729674
2	1.24	1560879	7	3.52	6316124
2.5	1.52	1837417	7.5	3.7	5848584
3	1.78	2208577	8	3.88	6226021
3.5	2.03	2882365	8.5	4.05	6795252
4	2.27	2980091	8.5	4.05	7703914
4.5	2.5	4401811	-		-
5	2.72	4279216	R^2		0.96

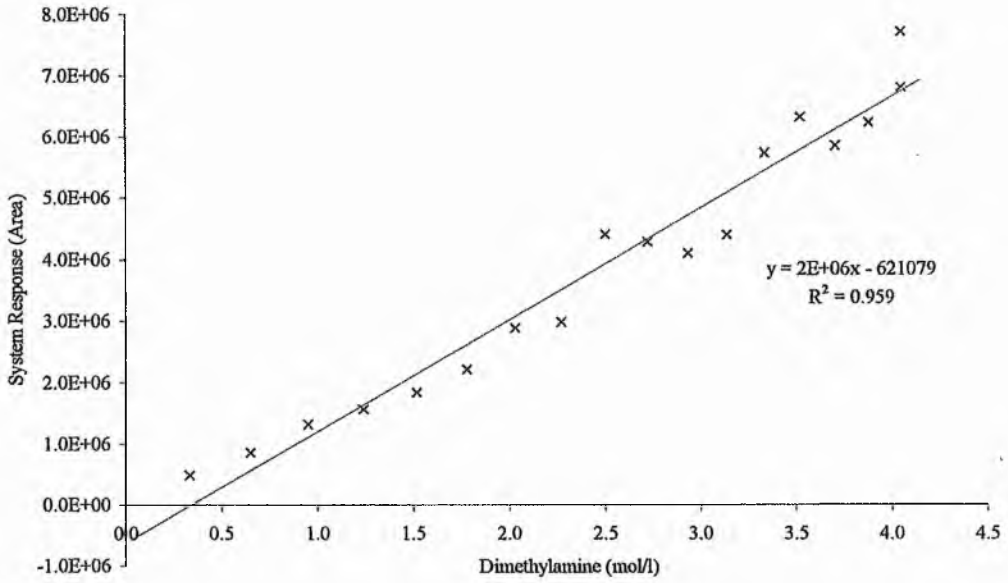

Figure 4.16 Linearity of system response for dimethylamine using the modified MIMS interface.

4.3.2.5 Reproducibility of Dimethylamine Response at Different Reaction Temperatures

Flasks containing dimethylamine solutions (equivalent to the initial Mannich reaction concentration) were sampled at a range of reaction vessel temperatures from 13 to 17°C via the MIMS interface. Table 4.3 presents the system responses for different reaction vessel temperatures. At each reaction vessel temperature the replicate analyses of the DMA solution demonstrated good reproducibility with range errors (E_R) in the range of 2.2 to 7.6%. It should be noted that the reaction flasks used for this experiment were of slightly different sizes for the four temperatures and thus, with a constant volume of sample there would have been a

different headspace volume in each flask. The data have not been corrected for headspace volume.

Table 4.3 Reproducibility of the Single Ion Response for Dimethylamine with Changing Reaction Vessel Temperature.

Temperature (°C)	System Response (Area)	Temperature (°C)	System Response (Area)
10	3118087	15	3528630
10	2888608	15	3414329
10	2968247	15	3617545
mean	2991647	mean	3520168
E_R%	7.6	E_R%	5.8
13	3101316	17	4473858
13	3168328	17	4534440
13	3176656	17	4573702
mean	3148767	mean	4527333
E_R%	2.4	E_R%	2.2
R^2 (mean values)		0.74	

4.3.2.6 Effect of Reaction Vessel Temperature on Dimethylamine Response

The effect of temperature on the reaction system was further investigated for the

extraction of replicate samples from a single reaction vessel. A flask containing dimethylamine solution (equivalent to the initial Mannich reaction concentration) was prepared. Aliquots of the headspace were sampled via the MIMS interface at 14, 15, 16 and 17°C in a randomised order to prevent bias due to sampling. Table 4.4 and Figure 4.17 present the system responses for different reaction vessel temperatures. The variation in the single ion response for dimethylamine (*m/z* 42) with temperature shows a linear relationship with reaction vessel temperature with a correlation coefficient of 0.996, in agreement with headspace theory. It should therefore be possible to correct the DMA response for temperature variations by monitoring the sample vessel temperature and the DMA concentration during the course of the reaction.

Table 4.4 Single Ion Response for Dimethylamine (4.0 mol/l) with Changing Reaction Vessel Temperature.

Temperature (°C)	System Response (Area)
14	2845919
15	3281449
16	3611962
17	3968242
R^2	0.996

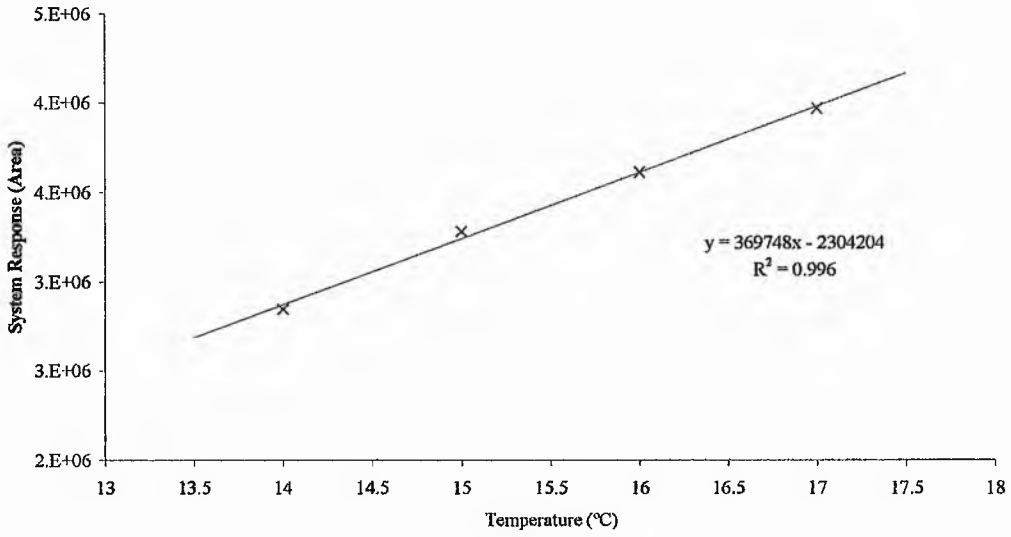

Figure 4.17 Single Ion Responses for Dimethylamine with Changing Reaction Vessel Temperature.

During the GlaxoSmithKline manufacturing plant process the Mannich Reaction is performed in a temperature controlled system, regulated at 15°C $\pm$ 2°C. The variation in DMA response with temperature and the reproducibility data reported in Tables 4.3 and 4.4 suggest that in order to monitor the reaction with a high degree of accuracy, the temperature would have to be regulated more closely or a temperature correction factor applied when determining the concentration of the reaction components.

4.3.2.7 Reproducibility of the Single Ion Response for Hydroxymethyl-dimethylamine

In order to determine the reproducibility of the single ion response for the

Mannich reaction intermediate; hydroxymethyldimethylamine (HMDMA), a solution of HMDMA was prepared by reacting a solution of DMA with formaldehyde (at Mannich Reaction concentrations). Replicate aliquots of the synthesised mixture were analysed and the single ion responses for HMDMA (*m/z* 75) are presented in Table 4.5, demonstrating reproducibility with an E_R of 14.2%, which is slightly poorer precision than that observed for DMA.

Table 4.5 Reproducibility of the Single Ion Response for HMDMA.

Injection	System Response (Area)
1	1206788
2	1132307
3	1135563
4	1045932
mean	1130148
E_R%	14.2

4.3.2.8 Effect of Reaction Vessel Temperature on HMDMA Response

The synthesised HMDMA solution was analysed at reaction vessel temperatures of 13, 15, and 17°C. The single ion responses for HMDMA (*m/z* 75) at different reaction vessel temperatures are presented in Table 4.6 and Figure 4.18.

Table 4.6 Single Ion Responses for HMDMA with Changing Reaction Vessel Temperature.

Temperature (°C)	System Response HMDMA (Area)
13	955065
15	1104601
17	1192603
R^2	0.978
Slope (response per °C)	59385

The single ion response for hydroxymethyl-dimethylamine (*m/z* 75), at the different reaction vessel temperatures, was proportional to the reaction vessel temperature with a correlation coefficient of 0.98. However, the sensitivity of the response, indicated by the slope of the line was significantly lower than that obtained for DMA (Section 4.3.2.6) indicating a lower susceptibility to reaction vessel temperature changes over the ranges monitored due to its lower vapour pressure. These data suggest that controlling the reaction temperature within the range 15°C $\pm$ 1°C would have a significantly smaller effect on the determination of HMDMA than on DMA.

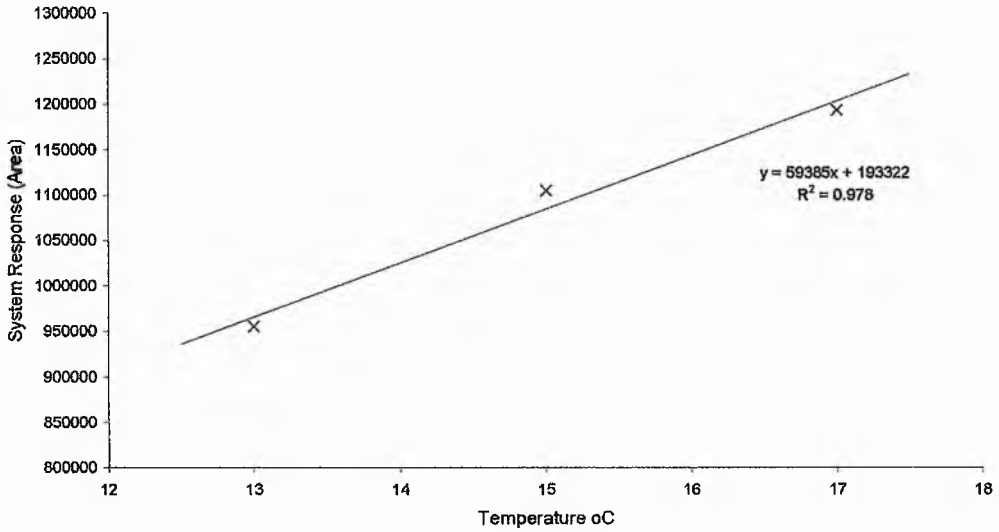

Figure 4.18 Single Ion Responses for HMDMA with Changing Reaction Vessel Temperature.

4.3.2.9 Real-Time Monitoring a Mannich Reaction

The Mannich reaction of DMA with formaldehyde and pHAP was monitored on-line and in real-time using the headspace/MIMS system developed and characterised in the previous sections. The responses due to dimethylamine (*m/z* 42), the iminium ion (*m/z* 58) and the reaction intermediate hydroxymethyl-dimethylamine (*m/z* 75) were monitored along with the reaction vessel temperature. Figure 4.19 presents an overview of the variation in the single ion responses for the three ions monitored during the course of the reaction. The temperature and volume of formaldehyde solution added (actual titre volume) are indicated on the right hand axis.

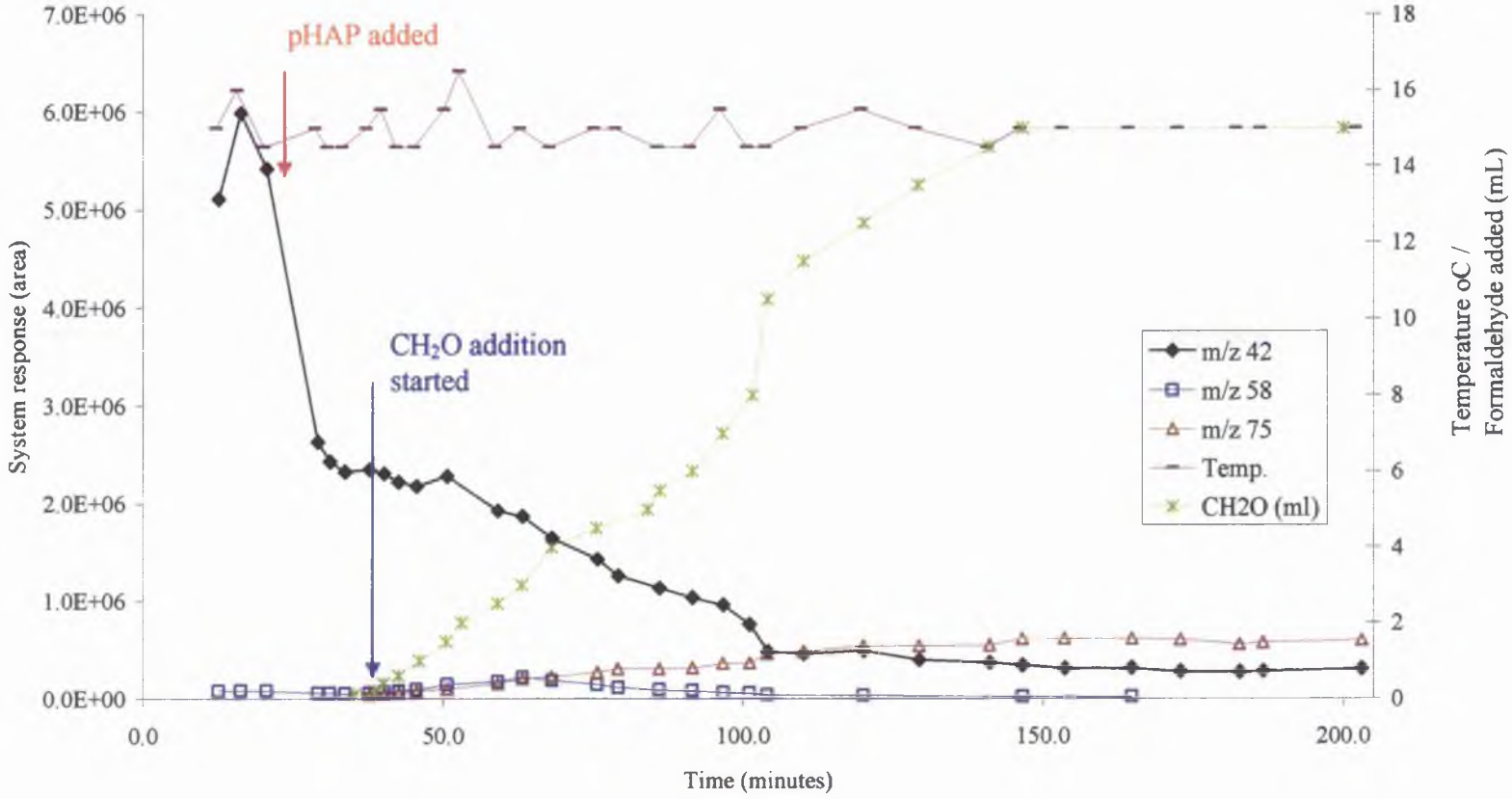

Figure 4.19 Real-Time, On-Line Monitoring of the Mannich Reaction (Maintained at 15°C±2°C).

The single ion response due to DMA (*m/z* 42) initially falls rapidly following the addition of pHAP and then declines more gradually as the formaldehyde solution is added to the reaction vessel. The initial drop in DMA presumably arises because of a fall in the activity of the DMA due to ion pairing with the pHAP which reduces mobility across the membrane. Significant levels of the reaction intermediates; hydroxymethyldimethylamine (HMDMA, *m/z* 75) and the iminium ion (*m/z* 58) were also detected following the addition of formaldehyde as shown in Figure 4.20. As formaldehyde solution was added to the system it reacted with the dimethylamine (Reaction Scheme 4.1) to form the reaction intermediate, HMDMA which rapidly loses water to form an iminium ion. This intermediate is consumed as it reacts with pHAP to form the product, dimethylamino-methylhydroxyacetonphenone (not detected by this system) and so remains at a low concentration throughout the synthesis.

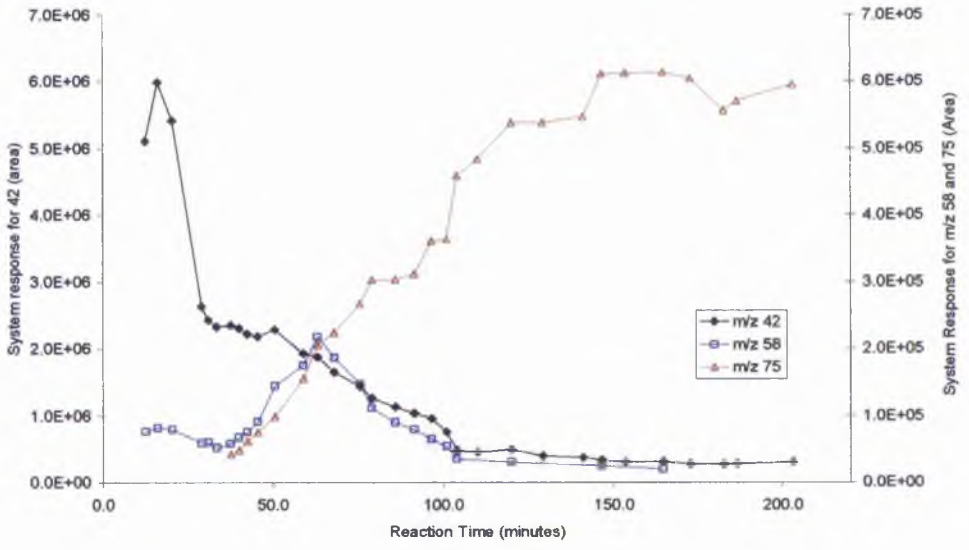

Figure 4.20 Single Ion Responses for the Monitored Reaction Components

Figure 4.21 shows an expanded scale for *m/z* 75, which demonstrates the close relationship between the addition of formaldehyde and the appearance of hydroxymethyldimethylamine (*m/z* 75), the intermediate formed by the reaction of DMA with the formaldehyde. DMA and formaldehyde are present in molar excess over pHAP and consequently the concentration of the HMDMA intermediate (*m/z* 75) reaches a steady state as the reaction progresses and the pHAP is consumed.

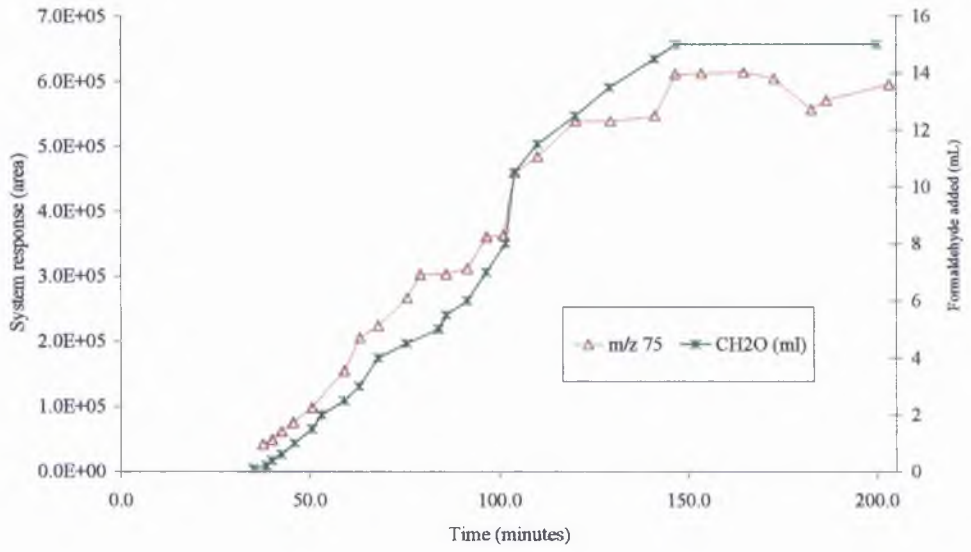

Figure 4.21 The Relationship Between the Addition of Formaldehyde Solution and the Formation of Hydroxymethyldimethylamine

It was observed that the single ion response for DMA during the reaction was affected by the system temperature which was allowed to vary in the range 13.5 to 16.5°C. Figure 4.22 demonstrates that whenever the temperature increased the DMA response was higher than the adjacent responses. This correlation was

expected from the temperature effects observed during the method development stage (Section 4.3.2.6) and may be corrected using a built-in a temperature correction factor for the DMA response.

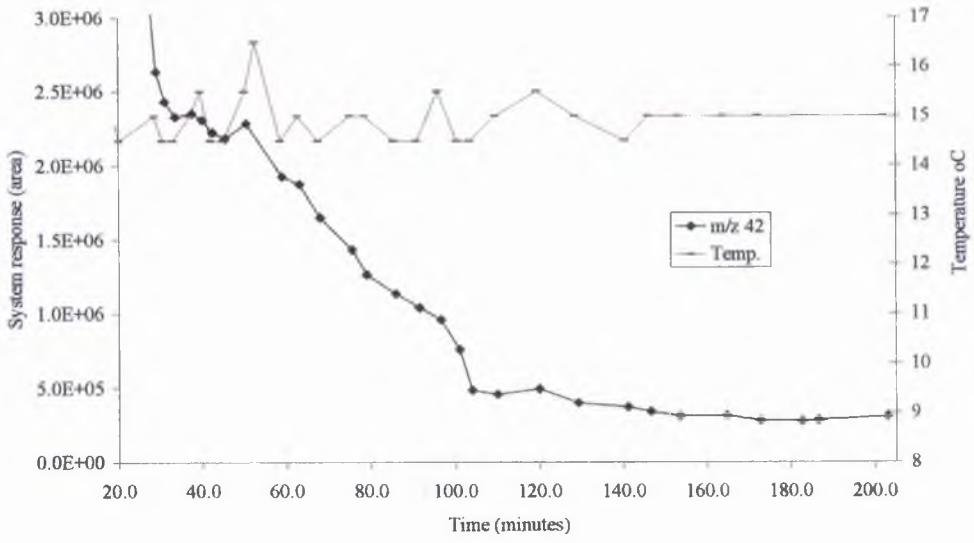

Figure 4.22 Single Ion Response for Dimethylamine with Temperature

A second Mannich reaction was monitored with the temperature controlled to 15°C ± 1°C. Figure 4.23 presents an overview of the variations in the single ion responses for the three ions monitored during the course of the reaction with the temperature maintained within this narrower range. The temperature and volume of formaldehyde solution added are indicated on the right hand axis.

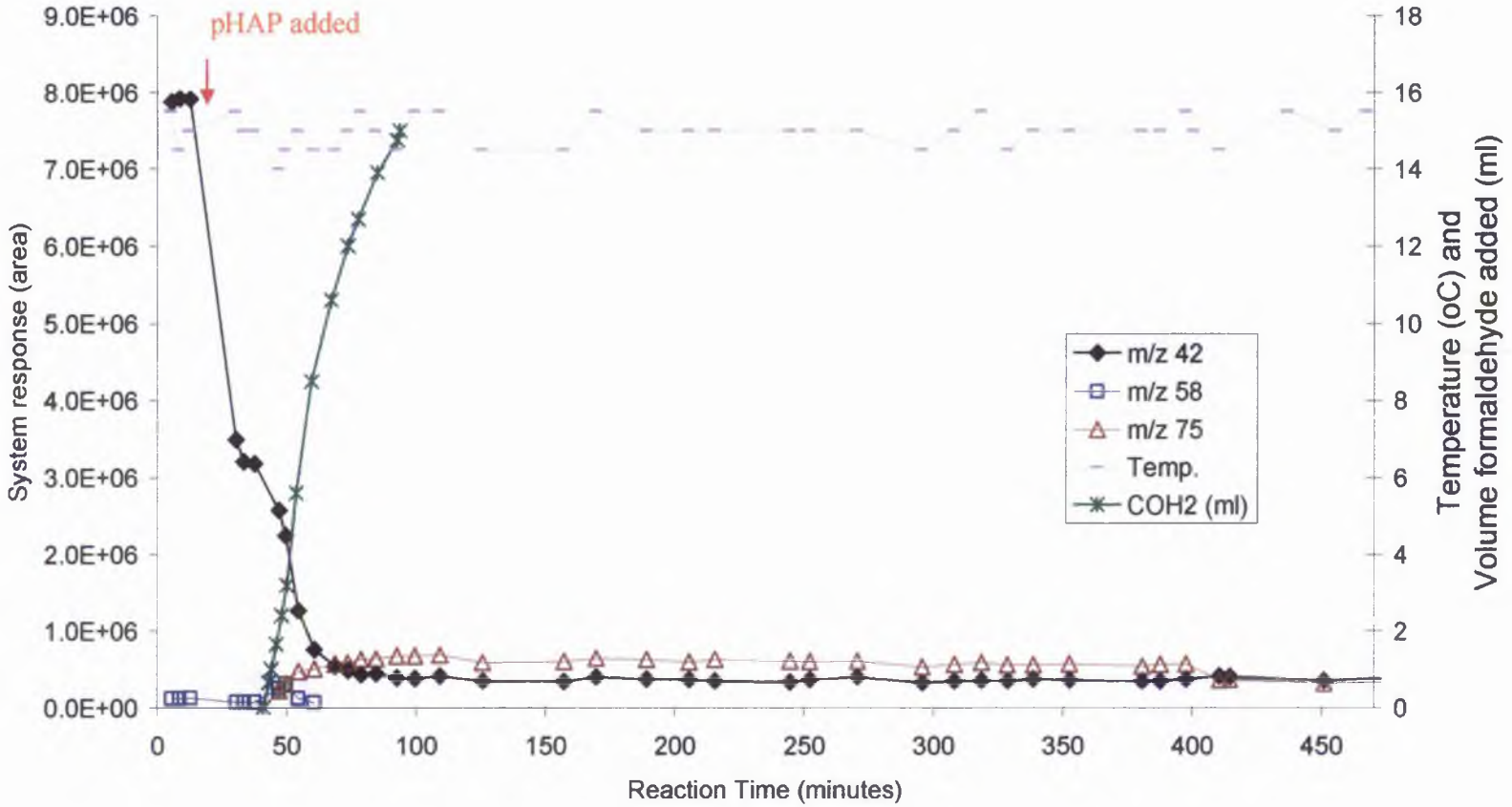

Figure 4.23 Real-Time, On-Line Monitoring of the Mannich Reaction (Maintained at 15°C±1°C)

The data again show the initial fall in DMA headspace concentration following the initial addition of pHAP and the subsequent addition of formaldehyde. Single ion responses for DMA (*m/z* 42) for the first hour of reaction monitoring are presented in Figure 4.24.

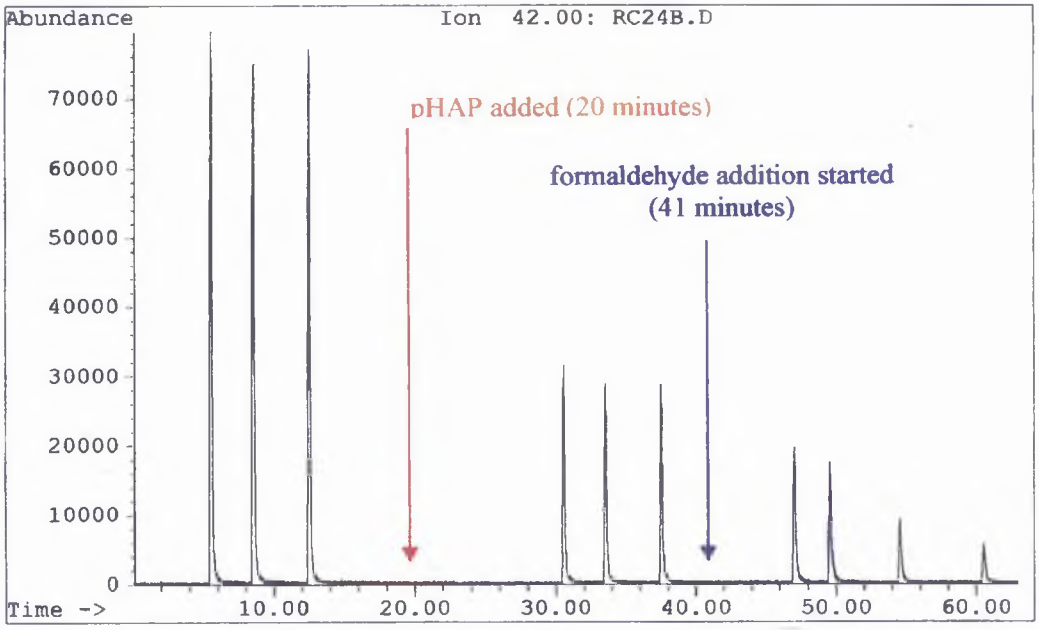

Figure 4.24 Single Ion Responses for DMA During Real-Time On-Line Mannich Reaction Monitoring

Significant levels of the reaction intermediates; hydroxymethyldimethylamine (*m/z* 75) and the iminium ion (*m/z* 58) were also detected in the reaction mixture following the addition of formaldehyde. Figure 4.25 presents the single ion responses for HMDMA (*m/z* 75) during the first hour of reaction monitoring and Figure 4.26 is a graphical representation of the monitored levels of the iminium ion (*m/z* 58) and HMDMA during the course of the reaction.

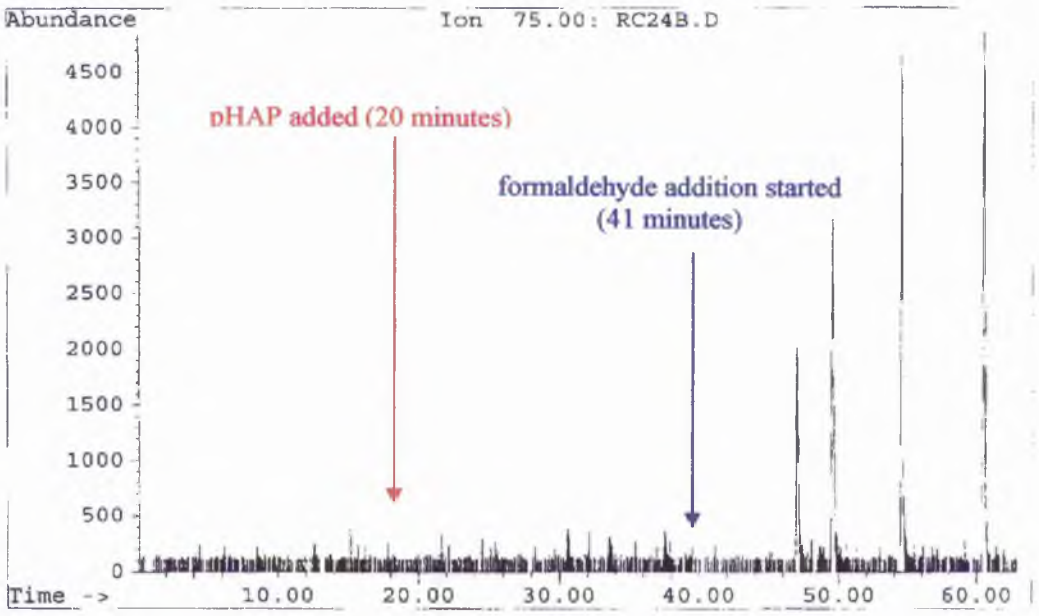

Figure 4.25 Single Ion Responses for HMDMA During Real-Time On-Line Mannich Reaction Monitoring

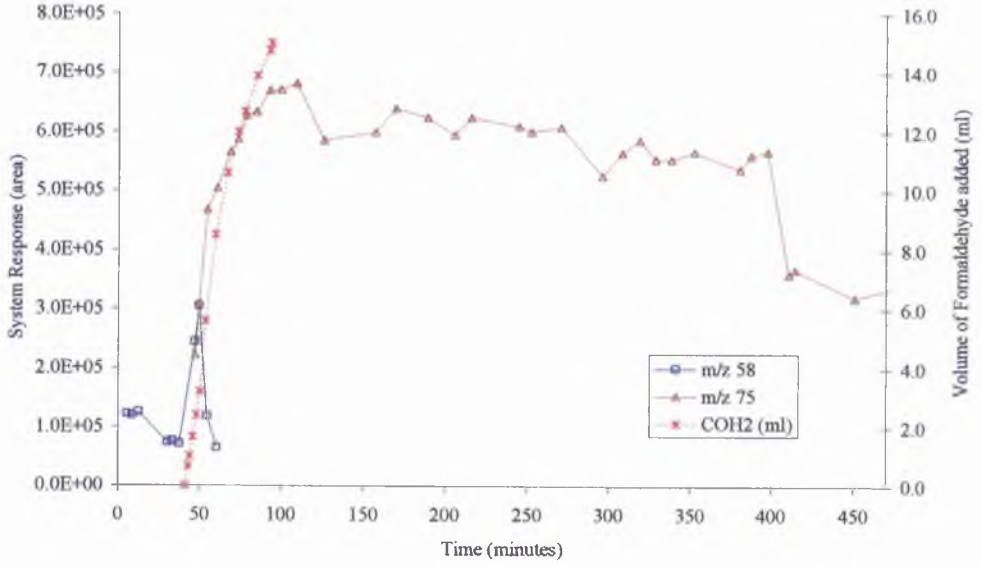

Figure 4.26 Single Ion Responses for the Reaction Intermediates

In theory this headspace sampling membrane interface system would support sampling of the reaction vessel every 2-3 minutes with an analytical result available in the same time frame. Additional data points could have be recorded for the reactant and intermediates through automation of the laboratory reaction system, although this was not implemented in these studies. The requirements for automation are: measure and record the reaction vessel temperature, switch the sampling valve, time the sample addition, switch the sampling valve back to air, record the time sampled on the analysis sheet, record the cumulative volume of formaldehyde added, measure the reaction vessel temperature and adjust to 15°C $\pm$ 1°C if required.

4.4 Conclusions

The reaction components of an early stage, pharmaceutical intermediate process (Mannich Reaction) were studied using membrane inlet mass spectrometry.

Direct liquid sampling membrane inlet mass spectrometry was used to monitor a highly concentrated reaction mixture. In these experimental conditions the membrane did not appear to be stable for long term use and deteriorated after a few days of operation. However, this sampling system showed the potential to be used for the analysis of concentrated reaction mixtures within a set of limitations. The analyte molecules must be small enough to be transported across the membrane, have a suitably high volatility and be chemically compatible with the membrane material so as not to cause membrane deterioration. Within these limitations the selected extraction of a number of components from a liquid reaction mixture would allow their analysis in the absence of interferents.

A headspace sampling membrane inlet mass spectrometry technique was developed to analyse the reaction headspace of a Mannich reaction vessel. Reproducibility, linearity and temperature dependence were demonstrated for the starting material; dimethylamine, and the reaction intermediate; hydroxy-methyldimethylamine. The Mannich reaction of dimethylamine with formaldehyde and parahydroxyacetophenone was monitored on-line in real-time via the headspace MIMS interface. The consumption of the starting material and

reaction intermediates were tracked throughout the reaction in real-time with minimal analyst intervention and sample handling.

The components of the headspace MIMS interface are delicate and the assembly of the device required a high degree of dexterity and care. At this stage in its development the membrane interface is not sufficiently robust for routine use in a process plant environment. However, the interface has been demonstrated to be suitable for use during the early, investigative stages of process development where reaction mechanisms are being characterised and optimised.

4.5 References

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Chapter 5

Conclusions and Further Work

5.1 Reaction Monitoring Using a Liquid-Liquid Membrane Interface

A novel, single-stage microporous membrane-based interface has been developed for real-time mass spectrometric monitoring of the starting materials and products of a highly concentrated pharmaceutical process reaction mixture. The interface was directly connected to the atmospheric pressure chemical ionisation source of a quadrupole mass spectrometer (APCI-MS). Dilution of the concentrated reaction mixture was achieved in a single step using the interface, with a precision of 2.5% for replicate samples. The combination of the membrane inlet with APCI-MS was demonstrated for the Michael Addition reaction of phenylethylamine and acrylonitrile in ethanol using a hydrophobic polyvinylidene fluoride microporous membrane. The reaction was monitored throughout its course, allowing the endpoint to be determined based on the relative concentrations of the reaction precursors and products. There was minimal delay time between sampling the reaction mixture and obtaining the analytical result and the interface required minimal sample handling or operator intervention.

The success of the investigations reported in this work provides a basis for the future development of automated systems for process monitoring by mass spectrometry using a microporous membrane interface. Components of an automated monitoring process would include the addition of a feed loop from the reaction vessel, fitted with an automated switching valve, to sample the reaction

mixture at predetermined time intervals. Several sample streams could be analysed simultaneously using a multi-port ionisation source. Up to eight process streams may be sampled in turn with the aid of a rotating baffle. This would allow the synchronized monitoring from a range of sampling positions in a large reaction vessel, lowering the risk of reporting localised reaction conditions. Alternatively, the multi-port source would allow the simultaneous monitoring of multiple process streams, thereby off-setting the cost of a mass spectrometer installation against several projects.

Additionally, the membrane interface could provide a means of testing non-homogeneous mixtures, acting as a filtering barrier, and preventing solid matter from interfering with the sampling process. Fully automated sampling systems would permit the analysis of toxic, potentially explosive or corrosive process mixtures with minimal operator risk.

5.2 Supported Membrane Development for Liquid-Liquid Sample Extraction

The production of supported semi-permeable membranes was investigated and a variety of casting techniques were evaluated. Semi-permeable silicone membranes were cast onto both nylon and polypropylene net support materials and incorporated into a membrane probe device. The supported silicone membrane probes were evaluated for their application as a liquid-liquid membrane interface

for monitoring process changes. The response times obtained for analyte transport through the membrane were in excess of practical analysis times.

The membranes could not be cast in a layer thin enough to allow transport of analytes through the membrane material without failing due to possible membrane imperfections. Increasing the temperature of the extraction system would increase the rate of analyte diffusion through the membrane, permitting the use of a thicker supported silicone membrane, with the implied structural robustness.

Future investigations into the supported silicone membranes might include casting the silicone onto support structures with smaller pore sizes. This may permit the silicone membrane to form a more robust surface layer, with a larger surface area in contact with supporting structures, but maintaining a large number of pores per unit area, for sample transport.

The membrane probe system, developed for performance testing the supported silicone membrane, may be used for other membrane evaluation experiments. Multiple probe systems could be used simultaneously for rapid off-line testing of membrane materials for solvent compatibility, permeability and speed of analyte transport. Membrane systems could be evaluated with a range of solvents without requiring the membrane interface to be configured directly with a mass spectrometer.

5.3 Reaction Monitoring Using a Semi-Permeable Membrane Interface

Direct liquid sampling membrane inlet mass spectrometry was used to monitor a highly concentrated Mannich reaction mixture. Direct liquid sampling MIMS was, in this case, unsuccessful for the analysis of concentrated reaction mixtures. However, the sampling system showed the potential to be used for the analysis of concentrated reaction mixtures within a set of limitations. The analyte molecules must be small enough to be transported across the membrane, have a suitably high volatility and be chemically compatible with the membrane material so as not to cause membrane deterioration. Within these limitations the selected extraction of a number of components from a liquid reaction mixture would allow their analysis in the absence of interferents.

In these experiments the membrane was not sufficiently robust for long term use under the reaction conditions and deteriorated rapidly on exposure to the concentrated Mannich reaction mixture. Future experiments would need to be directed towards an investigation of the availability of more chemically robust membrane materials. Reducing the length or internal diameter of the hollow fibre membrane, or increasing the rate of donor flow, would decrease the contact time for the corrosive reaction mixture and possibly allow extended analysis times with this system. Splitting the analyte flow into a neutralising solution prior to sampling may reduce the corrosive properties of the analyte solution and further prolong the membrane's useable lifespan.

A headspace membrane inlet mass spectrometry technique has been successfully developed for reaction monitoring using electron ionization mass spectrometry. The progress of the reaction was monitored by the analysis of headspace extracted from the reaction vessel during the course of a Mannich reaction. Reproducibility, linearity and temperature dependence were investigated for the determination of the starting material, dimethylamine, and the reaction intermediate hydroxymethyldimethylamine, during the Mannich reaction of dimethylamine with formaldehyde and para-hydroxyacetophenone. The volatile starting materials and reaction intermediates were monitored on-line via the headspace MIMS interface. The consumption of the starting material, dimethylamine, and reaction intermediates were tracked throughout the reaction in real-time with minimal analyst intervention and sample handling.

The components of the headspace MIMS interface are delicate and the assembly of the device required a high degree of dexterity and care. At this stage in its development the membrane interface is not sufficiently robust for routine use in a process plant environment. However, the interface has been demonstrated to be suitable for use during the early, investigative stages of process development where reaction mechanisms are being characterised and optimised.

With improvements in the interface robustness the membrane device could be automated for use in a routine process environment. This would require further investigation into the system control, including the use of a thermo-regulated

cooling jacket for the reaction vessel, a digital recording thermocouple in the reaction vessel and an automated switching valve for the reaction vessel sampling. This would allow near-complete automation of the process monitoring system, permitting the unattended monitoring of a process. Toxic or potentially explosive reaction systems could be monitored remotely maintaining the safety of the process analysts.

Appendix

Publications and Presentations

Journal paper

Real time monitoring of a pharmaceutical process reaction using a membrane interface combined with atmospheric pressure chemical ionisation mass spectrometry, Rebecca Clinton, Colin S. Creaser and Duncan Bryant, *Anal. Chim. Acta*, **539**, 133 (2005)

Oral presentations

A membrane based approach to on-line reaction monitoring by mass spectrometry, Rebecca Clinton, Colin Creaser and Duncan Bryant, Advances in Process Analytics and Control Technology Conference (APACT 03), York, UK, April 2003.

On-line reaction monitoring using atmospheric pressure chemical ionisation mass spectrometry, Rebecca Clinton, Colin Creaser and Duncan Bryant, 25th Annual Meeting of the British Mass Spectrometry Society, Southampton, September 2002.

Real time liquid-phase reaction monitoring by mass spectrometry, Rebecca Clinton, C.S. Creaser and D. Bryant, Royal Society of Chemistry, Analytical Research Forum, Sunderland July 2003.

Poster presentations

Real time, on-line monitoring of a highly concentrated liquid reaction by membrane inlet mass spectrometry, Rebecca Clinton, Colin S. Creaser and Duncan Bryant, 52nd ASMS Conference on Mass Spectrometry and Allied Topics, Nashville, TN, May 2004.

A membrane based approach to on-line liquid-phase reaction monitoring with APCI mass spectrometry, Rebecca Clinton, Colin S. Creaser and Duncan Bryant, 16th International Mass Spectrometry Conference, Edinburgh, Aug 2003.

On-line reaction monitoring using atmospheric pressure chemical ionisation mass spectrometry, Rebecca Clinton, C.S. Creaser and D. Bryant, Royal Society of Chemistry, Analytical Research Forum, Kingston, 2002.