DEVELOPMENTS IN DETECTION METHODS AND USE OF SUPERHEATED WATER AS AN ELUENT FOR LIQUID CHROMATOGRAPHY

By

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Dedication

I would like to dedicate this thesis to my husband Trevor, son Sebastian and my family. Their love and continuous support made hard times bearable.

Acknowledgements

Firstly, I would like to acknowledge Dr. Barber of Peterborough Technical College, whom has remained my main inspiration throughout my "apprenticeship".

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Abstract

A system has been developed to link superheated water liquid chromatography with flame ionisation detection. The system has been demonstrated to be robust, sensitive and have a linear response, therefore having advantages over existing commercial universal detectors, such as refractive index and evaporative light scattering detectors. A wide variety of compounds, such as non-volatiles and volatiles, with and without chromophores have not only been detected by flow injection analysis, but also separations have been demonstrated. The columns used have been polystyrene divinylbenzene, various ion exchange columns, Zirconia-PBD and porous graphitic carbon, all of which have been proven to be stable at the conditions required to produce superheated water. The separation of various mixtures have required pH control and the use of buffers, all of which have been found to be compatible with the system. The results obtained from the system using sugars, amino acids and polysaccharides, which cannot traditionally detected directly by FID or UV without derivatisation, have proven that detection is linear and detection limits are better than existing universal detectors. Also it has been demonstrated that compounds which are not detected by flame ionisation detection, such as ammonia, formaldehyde, dichloromethane and ionic compounds such as sodium nitrite, can be detected by the new system.

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Chapter 1

Introduction

The aim of the project was to develop a universal detector for liquid chromatography which is capable of detecting volatile, non-volatile, chromophore and non-chromophore containing compounds. The intention of this system is that it would overcome many of the problems encountered with existing detection methods, such as the need for derivatisation, and selective responses.

The most commonly used detectors in liquid chromatography are the UV-Vis [1] and fluorescence detectors. The UV-Vis detector relies on the absorption of UV-Vis radiation at a particular wavelength (between 190 – 800 nm) by a covalent unsaturated functional group called a chromophore [2], whose absorption of radiation causes a change in the molecular transition of the molecule.

The fluorescence detector relies on the emission of the absorbed energy as light at a longer wavelength, by a suitable fluorophore. Although the fluorescence detector is more sensitive and selective, as relatively fewer molecules fluoresce than absorb UV-Vis radiation [3], it is also more limited in application. The principal problem both fluorescence and UV-Vis detectors face are that not all compounds contain chromophores or fluorophores which either absorb or emit within the working wavelength range of the detectors (190-800 nm).

For the many compounds, which lack a useful chromophore, one answer to the detection problem has often been derivatisation, in which the analyst chemically attaches a suitable group to the analytes to facilitate detection. The drawbacks of derivatisation are first of all, the increase of the cost of analysis, in terms of chemicals and time required to perform the analysis. Also it is always uncertain as to whether the reaction has gone to completion. Derivatisation can cause problems in the chromatographic separation

because of excess reagent and the derivatised analyte (which may contain one or more derivatized end groups) may be seen with the same response factor [4].

The other options available to the analyst would be to change the method of detection to one of the commercially available universal detectors, which all have their own problems. For example, the refractive index (RI), evaporative light scattering detector (ELSD), mass spectrometry (MS) or a liquid chromatographic interface to a universal detector, such as the flame ionisation detector (LC-FID).

1.1 Universal detectors for liquid chromatography

The universal detectors are so-called because they do not depend on the presence in the analyte of a particular functional groups, or unusual elements and are thus structurally insensitive.

The three principal detection methods that have been used in the past are:

1.1.1 Refractive index detectors
1.1.2 Evaporative light scattering detector (or mass evaporative detector)
1.1.3 LC transport detectors, such as LC-FID and LC-MS

1.1.1 Refractive index (RI) detectors

The refractive index or differential refractive index detector monitors the difference in the refractive index of pure reference mobile phase and column effluent [5]. The detector provides near universal detection for organic compounds, providing that the refractive index of the solute and the mobile phase is different.

The principle of detection [6] is based on the additivity law of refractive index for dilute solutions. Whereby the total refractive index N_c is expressed as the refractive index of

the mobile phase (N_1) and solute (N_2), and the volume of each present (V_1, V_2), in a flow cell.

$$N_c = (N_1V_1 + N_2V_2)/(V_1 + V_2)$$

When mobile phase alone is present in the flow cell, the refractive index is equal to N_1:

$$N_1 = N_1(V_1 + V_2)/(V_1 + V_2)$$

When solute is present in the flow cell the resulting difference signal (S) can be calculated as:

$$S = N_c - N_1$$

Substituting N_c and N_1

$$S = [V_2/(V_1 + V_2)](N_2 - N_1)$$

Therefore the signal of the detector is proportional to the concentration of the solute:

$$S \approx c(N_2 - N_1)$$

Both negative and positive changes in refractive indexes can be obtained, which can lead to confusion when analysing complex mixtures.

The main problems associated with refractive index detectors are that they suffer from low sensitivity because it is a bulk property detector, and are incompatibile with gradient elution, since a change in mobile phase composition results in a change in eluent refractive index, therefore affecting the baseline signal. The refractive index of an eluent is also sensitive to changes in pressure, and hence flow irregularities (for mechanical

rather than chemical reasons), and temperature and these factors must be closely controlled.

There are four designs [7] of refractive index detector namely the a. Fresnel, b. deflection, c. interferometric and the d. Christiansen Effect.

1.1.1.1 The Fresnel refractometer

The Fresnel refractometer operates by monitoring the loss of intensity of a beam of light when reflected near its critical angle.

The disadvantages of this detector are that it requires two prisms to cover the RI range and low sensitivity (1000 times less than UV). However, smaller cell volumes can be used with this detector, which can potentially make it more sensitive [8].

1.1.1.2 The Deflection refractometer

A light source is collimated by a lens to fall onto a detector cell, which is a two compartment cell separating the reference mobile phase and column effluent on either side of a window. The light is refracted, reflected and refracted again as it passes through the cell window, before passing through another lens and then on to the photodetector.
The disadvantages of this detector are that the cell volumes are large therefore making it incompatible with microbore chromatography.

1.1.1.3 The Interferometric refractometer

A light source beam (e.g. 546 nm) is divided into two parts, by a beam splitter, focused by a lens and passed through the reference and sample flow cells (typically 5 μl). The light beams are recombined by a second lens and a beam splitter. This has the effect of correlating the wave fronts, so that they interfere (cannot be in phase as no effect).

Constructive interference occurs when mobile phase is in both the sample and reference flow cells. Partial destructive interference occurs when a solute is in the sample cell, since the difference in refractive index between the solute and mobile phase causes a change in the optical path of the sample. Thus the two beams are out of phase and the intensity and hence sensitivity is reduced.

1.1.1.4 The Christiansen Effect refractometer

This detector is based on the principle that light is transmitted through fluids and solid particles if the refractive index of the fluid is identical to that of the solid. The detector uses a cell packed with a solid which has the same refractive index as the mobile phase, so that light is transmitted through the cell.

A tungsten lamp is used to provide a broad spectral band emission, which is concentrated onto an aperture by an optical condenser. An achromatic lens is used to make the beam parallel, then the light beam is split into two by a double prism. The two parallel beams of light then pass through reference and samples cells and are monitored by a pair of wavelength sensitive photocells. Only a narrow band of wavelengths, closely matching the refractive index of the mobile phase and the solid particles is transmitted through the cell. Therefore, when the refractive index of the solution flowing through the cell changes, the amount of transmitted light is reduced which gives rise to the signal.

1.1.2 Evaporative Light Scattering Detector (ELSD)

The ELSD was first reported by Charlesworth in 1978 [9]. The eluent from a chromatographic column is nebulised by a concentric nebuliser, mixing with a suitable, warm nebulising gas to form an aerosol spray. The spray passes down a heated drift tube, where the solvent within the aerosol droplets evaporates to leave particles of non-volatile solute. The solute particles are carried by the nebulising gas across an intense

light beam, originally from a lamp but now commonly from a laser, where the amount of scattered light is collected by a photocell or photomultiplier or photodiode. The signal is a measure of the amount of non-volatile solute [9-12] in the sample.

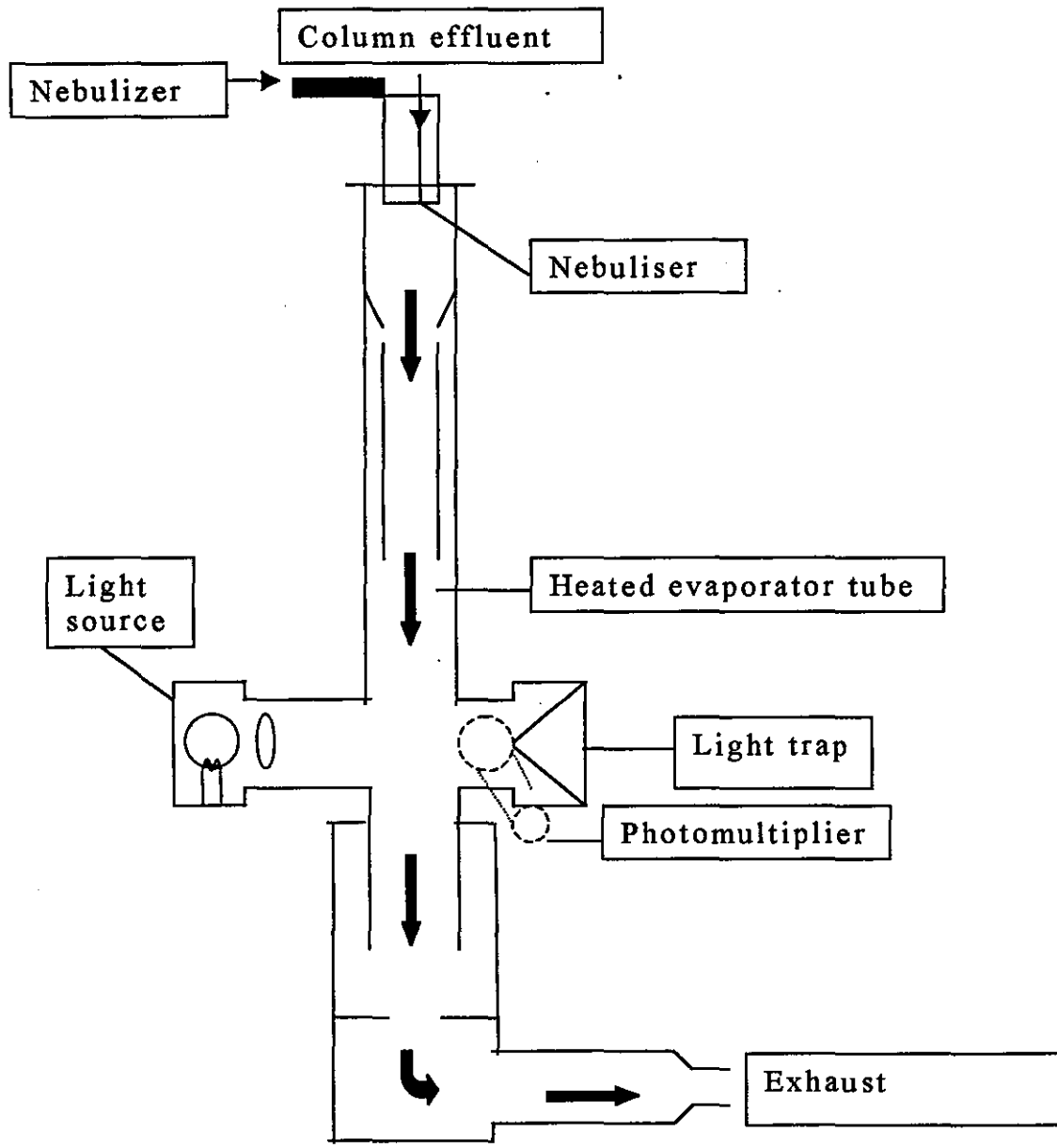


Figure 1.1 Schematic diagram of an evaporative light scattering detector

The mechanism of light scattering is dependent on the number, shape, surface properties and particle size of the solute particles [5, 6, 9, 13 - 15]. When solute particles pass through a beam of light, the effect of the solute in the light beam may be absorption, reflection, refraction, Rayleigh Scattering or Mie Scattering. The process of scattering is dependent upon the radius of the suspended particle in relation to the wavelength of incident light [5].

Radiation is, by definition, the emission or transfer of radiant energy as photons and is produced when light falls on a solid particle, by oscillation of the electrons. All of the radiation is emitted at an angle different from that of the emission direction and interfere destructively, if the particle is homogeneous.

Reflection and refraction occur when the particle size and the wavelength of light are similar [5]. Hence reflection and refraction take over at higher particle sizes, which is more efficient and increases sensitivity.

a. Rayleigh Scattering

Rayleigh scattering, by definition [16], is scattering of light by particles which are very small (the ratio of the particles diameter to the wavelength of incident light is less than 0.1) compared to the wavelength of the radiation being considered. It occurs when radiation is scattered in various directions due to the particle being inhomogeneous.
A feature of Rayleigh scattering is that it is inversely proportional to the fourth power of the wavelength. It is dominant in ELSD when low concentrations are used, as smaller particles are formed as the solvent evaporates from the droplet.
These small droplets $r/\lambda < 0.05$ absorb any light and re-emit energy radiation in all directions.

b. Mie Scattering

Mie scattering [13], is exhibited by particles which are about the same size as the wavelength of radiation under consideration (ratio of particle radius to incident wavelength is greater than 0.1 and 2), with a refractive index significantly different from that of the surrounding media. It predominates when the solute of interest is in high concentrations, when large particles are formed post evaporation. The light re-emitted by the larger particles has different amplitudes and phases than the incident light, and the intensity of the emitted radiation is dependant on whether the separate waveforms combine constructively or destructively [5]. The maximum sensitivity of the ELSD is observed when the ratio of average droplet diameter to wavelength is about 3.5, where diffraction and reflection on the particles surface is dominant. Above this value the sensitivity decreases due to a reduction in the average droplet diameter to wavelength ratio, below this value Mie scattering occurs and the intensity is reduced by interference [6].

The importance of the mean droplet size on the mechanism of light scattering is clear and hence its effect on the intensity of the radiation and detector response.

The main factors which affect the size of the aerosol droplets are:

1.1.2.1 Carrier gas flow rate,
1.1.2.2 Physical properties of the mobile phase,
1.1.2.3 Mobile phase flow rate,
1.1.2.4 Solute concentration / Sample size,
1.1.2.5 Drift tube temperature.

1.1.2.1 Effect of Carrier gas flow rate

If the carrier gas flow rate is too high it results in the production of small aerosol particles, which pass down the drift tube too rapidly to allow complete mobile phase evaporation, leading to a decrease in detector response [8, 9, 17]. The result of using a nebulising gas flow rate that is too low, is the formation of very large droplets, which

vaporise very slowly as they pass down the drift tube, resulting in an increase in background noise and the occurrence of spikes on the baseline. Willoughby and Browner [18] reported, that the application of a carrier gas with a higher thermal conductivity than the mobile phase, resulted in an improvement in the evaporation of the mobile phase from the solute.

1.1.2.2 Physical properties of the mobile phase

The only requirement for light scattering detection is that the mobile phase to be used is more volatile than the analyte of interest and contains no non-volatile components such as inorganic buffers or ion-pair reagents. It has been reported [5, 8, 9] that different physical properties, such as surface tension, viscosity and density of different solvents, result in changes in the surface volume mean diameter, hence droplet size, which causes a change in response. But these properties are not as significant as the changes in carrier gas flow rate [9].

Nukiyama and Tanasawa [19] developed a equation linking the average droplet diameter D_o to the surface tension (σ), viscosity (μ) and density of the solution (ρ):

$$Do = \frac{585\sigma^{1/2}}{(\upsilon_g - \upsilon_l)\,\rho^{1/2}} + 597\left(\frac{\mu}{(\sigma\rho^{1/2})}\right)^{0.45} \left(\frac{1000Q_l}{Q_g}\right)^{1.5}$$

Where υ_l and υ_g are the velocities of the liquid and gas and Q_l and Q_g are the flow rates of the liquid and gas.

1.1.2.3 Mobile phase flow rate

The average droplet diameter decreases with increasing carrier gas flow rate and decreasing mobile phase flow rate [11]. Hence decreasing the mobile phase flow rate results in a decrease in droplet size.

1.1.2.4 Solute concentration / sample size

For solutes at low concentrations, the particle size, after evaporation of the mobile phase, is small, as the ratio of surface area to volume increases [11] and Rayleigh scattering dominates whereas at high solute concentrations, large particles are formed and Mie scattering dominates. The effect of sample concentration in the droplet (C) on the size of the particles entering the optical cell (D) can be explained further by the equation

$$D = D_0 (C)^{1/3}/(\rho_a)$$

Where D_o is the average droplet diameter obtained at the outlet of the nebuliser, ρ_a is the density of the analyte. To tune an ELSD the objective is to form aerosol droplets of a narrow size distribution and of the largest diameter possible without generating a noisy baseline due to incomplete solvent evaporation [11].

Hopia and Ollilanen [20] noted that response factors for the ELSD increased with increasing injection volume, thus indicating an increase in light scattering for an increase in concentration. They further explained their findings by claiming that as particle size changed, the mechanism of scattering changed from non-efficient Rayleigh and Mie scattering to more efficient reflection and refraction scattering. This causes a strong increase in the response factors of the ELSD and can help explain why the calibration curves of the ELSD are often sigmoidal [5, 9, 11, 17].

1.1.2.5 Drift tube temperature and length

The temperature of the drift tube should to be sufficiently high to remove all of the mobile phase during the migration of the droplets down the drift tube [5, 8] to leave a solid particle of analyte. The optimum temperature depends upon the composition of the mobile phase. The temperature rises with increasing solvent heat of vaporisation [9]. It is preferential to use the lowest minimum temperature, not only to prevent vaporisation of analytes, but to produce larger droplet sizes, hence increasing the intensity of light scattering. If the temperature is too high the result could be total vaporisation of the analytes (complete loss of signal) or systemic error (partial vaporisation of analytes). Mourey and Oppenheimer [9] found that the drift tube temperature only had a minor effect between 50 - 100°C and that variations in response caused by temperature were due to differences in the physical properties of the mobile phases. They also found that more reproducible results were obtained when using the lowest temperature.

The length of the drift tube has been found to have an effect on the signal to noise ratio [21] but not on the limit of detection. An increase in signal to noise ratio was observed for shorter drift tube lengths, but this problem could be overcome by increasing both the carrier gas flow and the temperature in the carrier gas preheating element.

1.1.2.6 Applications of the ELSD

The ELSD has become a widely accepted method of detection for relatively non-volatile compounds, such as surfactants [22 – 26], amino acids [27], fatty acid methyl esters [28], polymers [29 – 30], chitosans [31-32], pharmaceuticals [33], triacylglycerols [15], carbohydrates [34 – 36] and peptide mapping of proteins [37].

The advantages of using a ELSD is that it can detect virtually any solute without the requirement of a chromophore or fluorophore and unlike RI, it is compatible with gradient elution. It has also been successfully linked to supercritical fluid

chromatography [12, 38], capillary electrochromatography [39], high temperature micro liquid chromatography [18, 40] and continuous flow systems for screening purposes [41].

However, the ELSD is limited by the requirement of a volatile mobile phase. Difficulties have been cited when using water as a mobile phase due to its high heat of vaporisation, as higher drift tube temperatures are required to evaporate the water, which results in a decrease in sensitivity [5, 8]. Also, due to the mechanism of light scattering, calibration curves are non linear.

1.1.3 Transport detectors for Liquid Chromatography

The flame ionisation detector (FID) responds to most organic compounds, it has a large dynamic linear range, suited to the detection of compounds in a gas stream and is generally viewed as a universal detector for gas chromatography, as almost all organic compounds give similar responses [42]. However, one drawback of gas chromatography is the reliance on the volatility of the analyte for both detection and separation. If a non-volatile sample is injected onto a GC column, it remains at the top of the column where it chars, deteriorating the column and increasing the background signal.

As GC separations rely on the volatility of the analytes, the only method for separation of non-volatile analytes is by LC. Therefore the detection of non-volatile analytes requires an interface to transport the sample to the flame of the FID, post separation. The main problem facing the direct linkage of LC to FID, is that organic solvents, used for LC, give a high response, which swamps the flame of the detector, thereby making detection of solutes impossible. Therefore it is imperative that all organic solvents/ and mobile phases are removed prior to detection by FID.

In order to facilitate the complete removal of mobile phase from the analyte, a number of transport detectors have been developed. The idea behind these is that the column effluent is applied to a transport carrier medium, to produce a thin film of liquid

containing both the mobile phase and the analyte. The carrier medium is then passed into a heated zone, where the mobile phase is removed, leaving the less volatile solute on the carrier. The solute is then further heated or pyrolysed and the resulting vapours are swept by the aid of a gas to the FID.

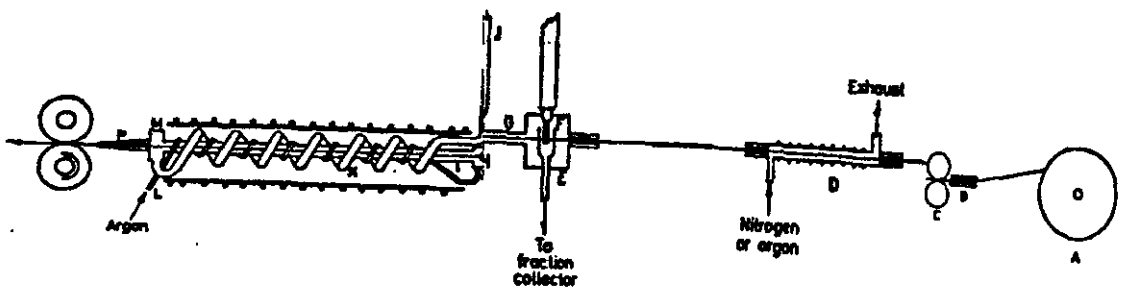
The advantage of transport detectors is that gradient elution can be used as the composition of the mobile phase is not important, as it is evaporated prior to detection. The only drawback of this method of detection is that the mobile phase must be more volatile than the solutes, otherwise systematic losses occur or the background noise increases.

A number of transport detector have been developed to link LC to FID. These are:

1.1.3.1 Moving wire detector [43 – 47]
1.1.3.2 The chain detector [48-49]
1.1.3.3 The rotating disc [50 - 51]
1.1.3.4 The rotating belt [52 – 53]

1.1.3.1 The moving wire detector

Figure 1.2 Schematic diagram of the moving wire detector



A. coiled iron wire B. narrow capillary C. ball races D. quartz tube E. brass block fitted F. hypodermic needle G. stainless steel tube H. brass T piece I. Stainless steel tube J. argon ionization detector K. stainless steel pipe wound round the central tube L. argon inlet through stainless steel tube M. T-piece P. glass capillary through which the wire is led [43, 44].

The first moving wire detector [43, 44] worked by first cleaning a thin iron thermocouple wire by passing it through a quartz tube heated to 750°C through which a suitable gas, such as nitrogen or argon, was used to remove residual lubricant from the wire.

The clean wire was then passed through the effluent of a chromatographic column, so that it was coated with a thin layer of mobile phase and solute. The mobile phase was evaporated by passing the wire into a chamber heated to an appropriate temperature. A heated argon stream flowed through the oven to carry the volatilised eluent away. The solute residue on the wire is then transported by the wire into a oven heated to 600°C, where it was pyrolysed.

Argon was continuously blown through the oven, to carry the low molecular weight pyrolised solute to an argon ionisation detector, which was operated at room temperature. An argon ionisation detector was chosen for the detection of involatiles, as it is capable of detecting non-volatile material in column eluents [43, 44].

The sensitivity of the detector was found to be proportional to the mobile phase flow rate and the speed of the moving wire. The higher the mobile phase flow rate, the faster the speed of the moving wire required to maintain sensitivity. Typical mobile phase flow rates were 0.5 – 5 ml/min and moving wire speeds ranged from 110 – 330 cm/min. Although the detector was demonstrated to work, unfortunately the sensitivity was little better than the refractive index detector (around 5×10^{-6} g/ml [2]), as it suffered from excessive noise and irregularities in the pyrolysis process. The dynamic linear range was found to be less than two orders of magnitude [44]. This configuration was developed further by Pye Unicam, who replaced the argon ionisation detector by a hydrogen flame ionisation detector, and employed nitrogen as the carrier and exhaust gas.

Further modifications (Figure 1.3) to the moving wire detector were made by Scott and Lawrence [45], who instead of relying on pyrolysing the non-volatiles in a heated oven, combusted them with oxygen.The reason behind this change in configuration was because many compounds only produce a small fraction of volatile components after pyrolysis, therefore the FID would only be detecting a very small percentage of the non-volatile analyte. Whereas the combustion of non-volatiles produced carbon dioxide and water, which was then mixed with hydrogen and passed over a nickel catalyst, to convert the carbon dioxide to methane, which was easily detected by the FID.

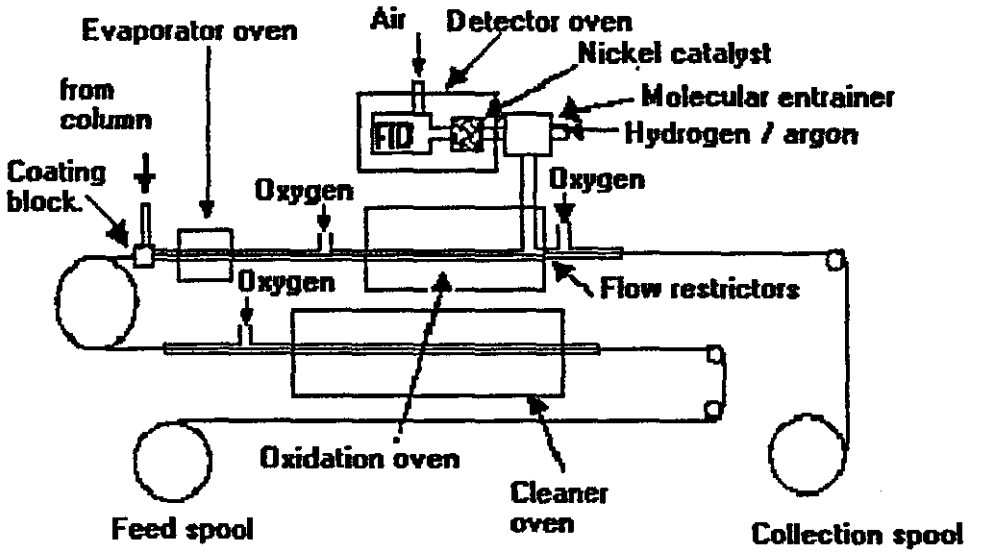


Figure 1.3 Schematic diagram of the modified wire detector [45]

Other modifications made by Scott and Lawrence [45] were to increase the internal diameter of the jet of the FID and the hydrogen lines. All of the modifications resulted in an increase in sensitivity and this was maintained even for compounds with high oxygen content. The response of the detector expressed as a Log [detector ionisation current] to Log [carbon dioxide] plot was found to be linear, therefore enabling quantitative analysis to be performed.

The most crucial part of the system was found to be the temperature of the mobile phase within the oxidation oven. If both volatile and in-volatile compounds were to be analysed together and the temperature was too high, the volatile compounds would volatilise and hence their response would be poor.

A major consideration to be taken into account for this type of detector was that 50% of the noise was attributed to the FID and the majority of the rest of the noise was due to fluctuations in the speed of the wire motor drive.

Other modifications made to detectors include using helical wires [54] to improve the uniformity of the coating of the mobile phase, changing the coating of the wire [55] and changing the method of application of the eluent to the wire [56].

The transport interfaces basically work as described previously, but subtle changes in the design, have brought about large differences in terms of their sensitivity.

Other modifications to the moving wire detector were to replace the tip of the FID jet with a tip made of compressed sodium sulphate [46], which produced an alkali flame sensing element. This modification of the flame enabled the FID to analyse thermally labile halogen derivatives of tetrahydrofurans [46]. During this work, it was found that both the temperature of the FID flame and emission of the alkali increased with an increase in hydrogen gas flow rate. This leads to an increase in the background ionisation current, which in turn increased the response and the background noise. The response of the alkali FID was found to be proportional to the content of halogen. A lower than expected response was found for an increase in halogens per molecule, which can be explained by an increase in volatility of the solute.

Important, work undertaken by Slais and Krejci [46] gave an indication why the sensitivity of the moving wire detectors' was not as high as expected. They showed that only 0.5% of the column effluent was transported by the wire to the detector and only 50% of the air, which was used to sweep the combustion products, reached the FID.

However, the ionisation efficiency of this transport detector using an alkali FID was comparable to that of an AFID used in GC.
Various efforts were therefore made to try to increase the percentage of solute transfer, thus increase the sensitivity of the detector. These were mainly aimed at increasing the surface area available to the effluent / solute and were of two main forms. The first was the helix detector [54], where a second wire was spiralled around the central wire.
The second involved the use of wires coated with sodium silicate, kaolin or copper-kaolin mixture [55], to produce a porous coating on the wire, to trap the solute within.

All of these systems had employed mobile phase flow rates between 0.5 – 5 ml / min and therefore high wire speeds were required to obtain a film of usable thickness.
This problem was overcome by the linkage of microbore HPLC columns to the moving wire detector [47]. At mobile phase flow rates between 60 – 100 µl/min and increased wire speeds of 360 – 480 cm/min, it was reported [47] that all of the column effluent was transported to the detector by the wire. However, the coating of mobile phase and solutes onto the wire still resulted in a non uniform film, which resulted in baseline fluctuation. Nevertheless, this methodology was used to analyse fatty acids, glycerol esters and carbohydrates, for which detection limits of 160 ng for xylose and 400 ng for lactose were reported.

1.1.3.2 The chain detector

The first chain detector [48] was developed around the same time as the moving wire detector. The difference from the moving wire was that the chain detector used a hydrogen flame for the detection of higher boiling point material and a gold chain continuous loop was used, in place of the disposable spool of wire. Unlike the moving wire detector, the detection of volatile and non-volatile analytes relied on the processes of volatilisation and pyrolysis.

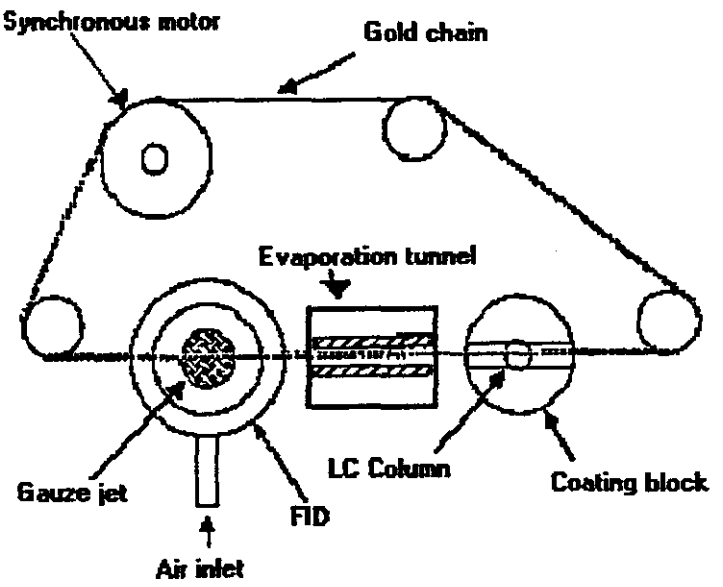


Figure 1.4 Schematic diagram of the chain detector [48]

The circulating gold chain was driven by at a speed of 1 mm/sec, through a coating block, where the effluent from the column was deposited onto it as a thin film. The chain then passed into an evaporation tunnel through which air was blown, to help evaporate any solvent from the chain. The chain then passed into the flame of the FID, where higher boiling point materials were combusted. The products of the combustion process gave rise to an ionisation current, which was monitored by the detector. As with the moving wire detector, the response given by volatile compounds was smaller than that of less volatile compounds, due to partial evaporation immediately before entering the evaporation tunnel. Nevertheless, despite the output from the detector being noisy, due to the absorption of high concentrations of solute in the links of the chain, the analysis of lipids, triglycerides and esters was reported.

Further work undertaken by Karmen [49] noted that as non-volatile lipid residues, approached the flame they were seen to melt and move in the away from the flame, where they formed droplets and subsequently fell off the chain. Therefore, it is clear that

the limits of detection were poorer than would have been expected. Some non-volatile compounds also crystallised as they approached the flame, and spattered, again giving a higher than expected limit of detection due to loss of analyte.

1.1.3.3 The rotating disc

Due to the problems of sample deposition observed for the chain and moving wire detector, alternatives were examined. Dubsky [50] claimed to overcome these problems by continuously depositing the column effluent onto a rotating circular net (disc), which presented a higher surface area for the coating of the effluent in comparison to the moving wire or chain detectors. The disc then entered a flame ionisation detector after evaporation of the mobile phase (figure 1.5). The advantages of using a rotating disc were, firstly, a ten-fold increase in sensitivity when compared to previous configurations. The net could also be impregnated with chemicals to improve volatility and act as a combustion catalyst or an ionisation medium for thermionic detection.

The construction of the detector was substantially different from the moving wire and chain detectors, as the mobile phase was evaporated by heating the disc with an infrared lamp, which was placed ahead of the point of column effluent application. The residual solute was rotated through 180° into the flame of the FID, where electrodes were placed above the disc, to facilitate the detection of ions, which lead to a signal. As with the moving wire and chain detectors, an increase in sensitivity was observed with an increase in the speed of the transport interface.

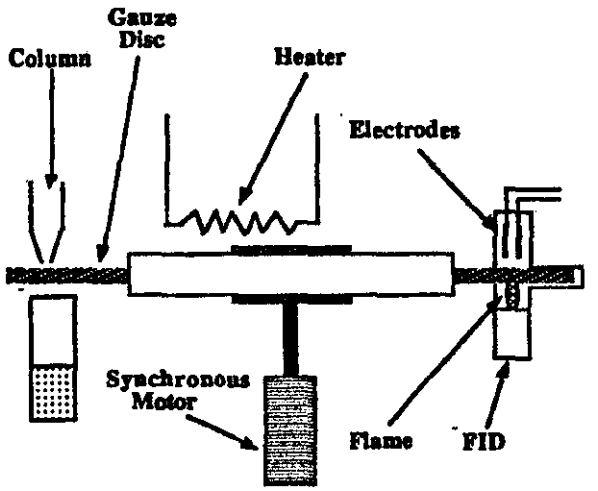


Figure 1.5 Schematic diagram of a rotating disc detector [50]

Szakasits and Robinson [51] claimed that excessive noise was observed due to the diffusion of the sample onto a hot metal conveyor, as previously seen with the chain detector. To overcome this problem, the rotating net was exchanged for a porous alumina disc, which reduced the amount of noise observed. However, small spikes were observed on the side of peaks when n-pentane was used as a solvent. These were thought to be due to occasional gas bubbles ejected from the tip of the applicator and/or the flashing of the solvent.

Other problems associated with the rotating disc detector were the reusability of the alumina disc, due to pore blockage, and the subsequent increase in the background noise due to incomplete removal of analytes.

1.1.3.4 Moving belt detector

Following on from the rotating disc detector, the moving belt detector was developed from the system originally described by McFadden [57 – 60] as an LC interface for mass spectrometry.

For LC-FID there are two main designs, firstly by Simmonite [53] whose configuration was manufactured by Analink Developments (Figure 1.6). The second design was described by Brown [52], which was improved by combining the use of a quartz belt with a rotating disc, and was subsequently manufactured by Tracor [61].

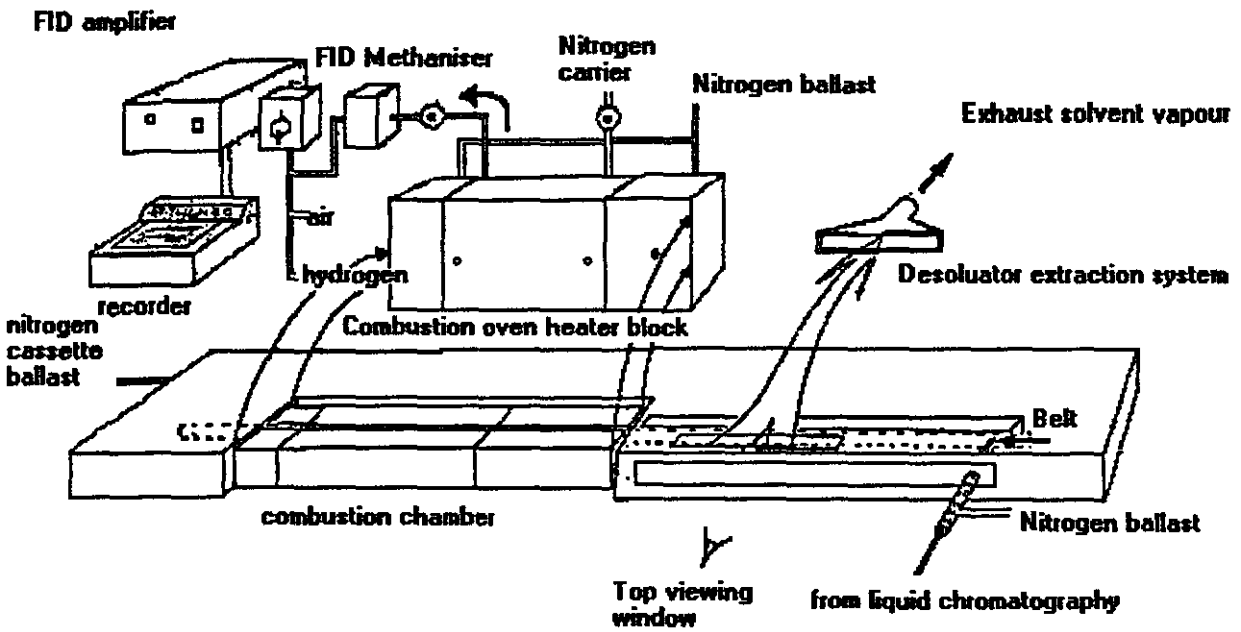


Figure 1.6 Schematic diagram of moving belt detector as manufactured by Analink [53].

For the LC-FID as described by Simmonite [53], the column effluent was deposited in a thin film onto the surface of a rotating quartz belt. The belt passed into a heated chamber and air [53] or nitrogen [52] passed over it to aid the evaporation of the mobile phase, leaving the residual non-volatiles on the belt as a solid or liquid. The solutes were either passed directly into the flame, where they were combusted [52] or the belt passed into a combustion oven where the non-volatiles were pyrolysed or combusted, and passed through a methaniser to form methane which was subsequently detected by the FID.

Both instruments have been demonstrated to detect polymers [52, 53, 62], triglycerides [52, 53], prostaglandins [53], steroids [53], hydrcarbon group types [63, 64], trimer in Z-11-hexadecenal pheromone [65], petroleum waxes [66] and mineral oils [53]. As with previous detectors, the disadvantages of this type of detector are losses of volatiles, due to premature or late vaporisation from the belt and mechanical failure of the belt.

1. 2 Water as a chromatographic eluent

Water is a cheap, common solvent available in large quantities. Its use plays a large part in everyday life for cooking, drinking and cleaning and its availability is generally taken for granted in the western hemisphere. Yet it is the backbone to life, it constitutes a large proportion of the body, where it plays a major role in the structure of DNA[67] and the very functioning of the body. It also represents 71% of the earth's surface and plays a major role in climates, where an extreme lack of or excessive amounts decide the fate of many living organisms. So it can clearly be seen that although it is common, cheap and available in copious amounts, it should never be taken for granted as it is vital to our very own existence.

Water is a simple molecule, combining two atoms of hydrogen and one atom of oxygen to form a single molecule. To form one single droplet requires billions of water molecules [67]. Whilst being one of the simplest of molecules, it is also one of the most

complex and least understood, whose properties show many anomalies. For instance, water has a relative molecular mass of 18, so one could reasonably expect it to exist primarily as a gas at room temperature, but it is a liquid. When solidified, it floats, when one would expect it to sink. As a molecule, water is described as one of the most polar solvents, with its polarity arising from the uneven distribution of electrons. Due to the polar nature of the molecule, it is capable of dissolving most ionic species. However, at room temperature, water cannot solvate non-polar, hydrophobic compounds.

1.3 Background to Superheated water

To superheat a liquid, by definition [68], means to "to heat a liquid above its boiling point without boiling occurring", in other words heating a liquid to a temperature where a change of state should occur, without the change of state occurring. During this thesis superheated water is defined as water heated above 100°C and less than its critical temperature, under sufficient pressure to prevent it from boiling (above 15 bar). The temperature range used for most of the work was 100 - 250°C.
Supercritical water is defined as liquid water heated above its critical temperature and pressure of 374.3°C, and 221 atm [69]. It is also of interest to organic chemists, as it is capable of solubilising organic compounds and oxygen and can be used for many reactions as a unique solvent.

If heated below the critical temperature under sufficient pressure to remain as a liquid, the polarity of water is substantially reduced, thus enabling the dissolution of hydrophobic compounds. This can be partially observed by the measurement of the dielectric constant of water at elevated temperatures, as an approximate measure of the polarity of a solvent.

Initial studies on the effect of temperature on the dielectric constant (ε) of water were undertaken by Wyman [70], who found that on going from 0 to 100 °C the dielectric constant reduced from 88 to 55. Following on from this work, Akerlof [71] undertook studies on the effect of temperature on the dielectric constants of various organic solvent

/ water ratios, across the temperature range of 0 - 100°C (figure 1.7). Again, a gradual decrease in the dielectric constant was observed with an increase in temperature.

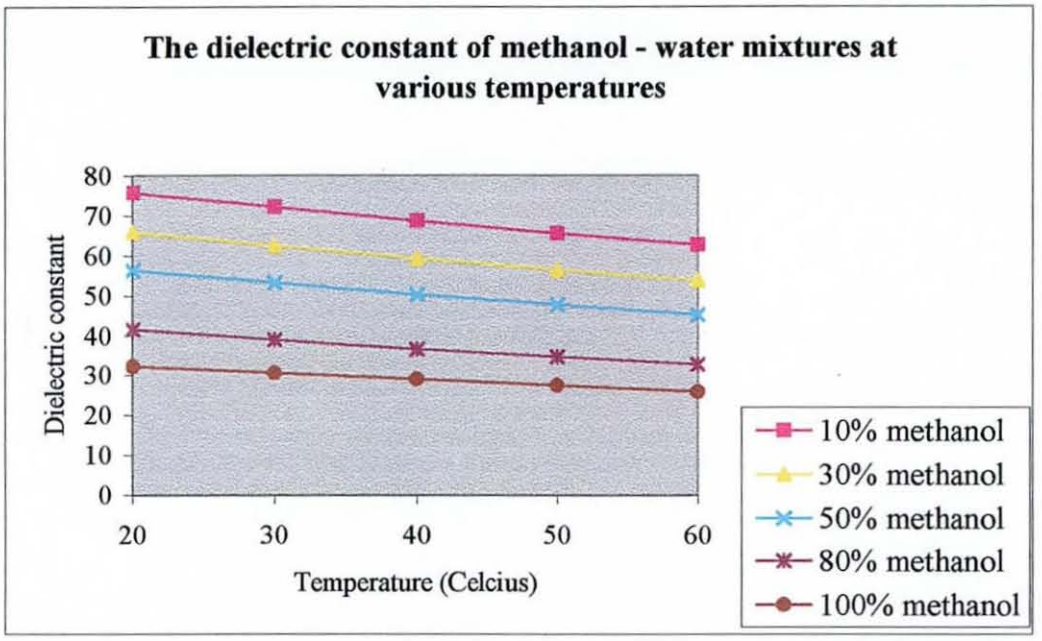


Figure 1.7 the effect on temperature on the dielectric constant of various methanol – water mixtures [71]

From this study, the dielectric constants of various organic solvents were determined, such as methanol whose dielectric constant at ambient conditions was found to be ε = 32.35, which is significantly lower than that of water at 100 °C.

It was work undertaken by Akerlof and Oshry [72], that overcame the problems associated with the measurement of the dielectric constant at temperatures between 100 - 370 °C, and finally determined that when water was heated above its boiling point, under sufficient pressure for it to remain as a liquid, its dielectric constant decreased (figure 1.8). The lowering of its dielectric point was to such an extent, under certain conditions, that its value was similar to that of organic solvents, such as methanol and methanol / water mixtures as traditionally used in reversed phase liquid chromatography.

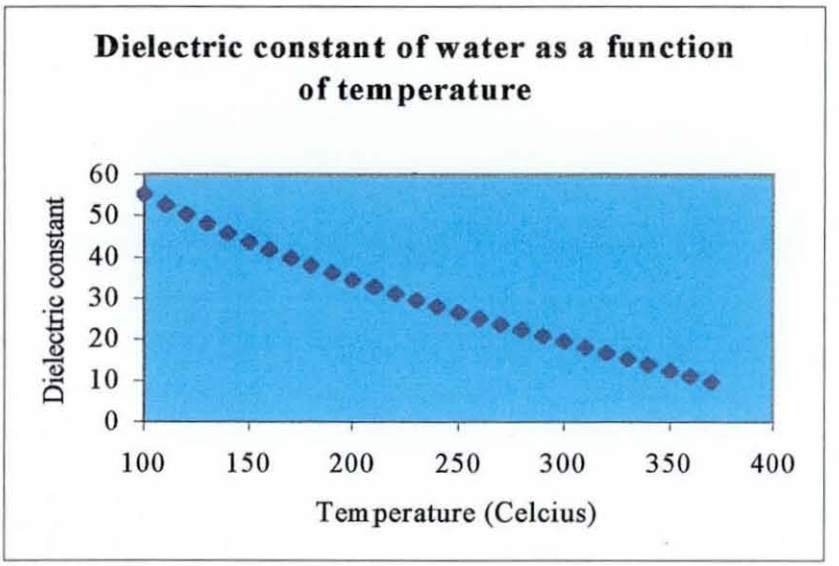


Figure 1.8 The effect of temperature on the dielectric constant of water [72]

It can be clearly seen that when water is heated under pressure to temperatures above its boiling point, it can truly obtain a dielectric constant similar to that of methanol and methanol – water mixtures from ambient up to 60 °C. This can more clearly be seen by comparing the data from figures 1.7 and 1.8 and figure 1.9.)

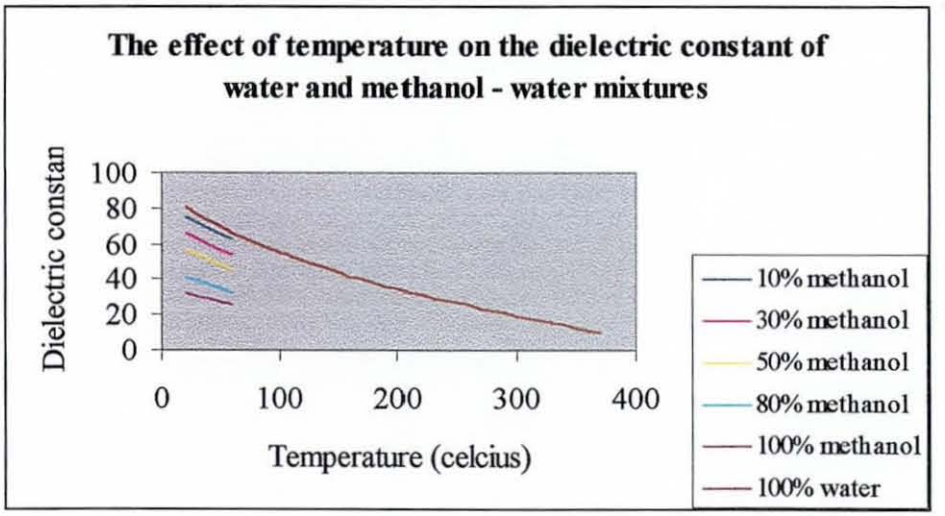


Figure 1.9 culminated data from figures 1.7 and 1.8 [71, 72].

From figure 1.9 it can seen that if water is heated, under sufficient pressure for it to remain as a liquid, to a temperature of 210 °C, its dielectric constant is lowered to ε = 32.93. This is comparable to the dielectric constant of methanol at 20 °C, ε = 32.7.

The influence of temperature and pressure on the dielectric constant of water has far reaching implications, when considering the dielectric constants of other commonly used solvents [73] at ambient temperatures (table 1.1).

Table 1.1. Dielectric constants of common solvents at ambient [73]

Solvent	Dielectric constant (ε)/ F m^{-1}
Acetonitrile	37.5
Ethanol	24.6
Acetone	20.7
Tetrahydrofuran	7.6
Chloroform	4.8
Hexane	1.9

From this data, it can be seen that when water is heated above 100° C at temperatures below its critical temperature, it can mimic the dielectric constants of widely used chromatographic eluents such as acetonitrile, ethanol and acetone.

At 300 °C, water has a similar dielectric constant (ε_{H2O} = 20), polarity and density as acetone. Alongside the reduction of the dielectric constant, with increasing temperature is a reduction in the solubility parameter [74] from 23.4 to 14.5 cal / cm^3 at 300 °C. The effect of the reduction in the solubility parameter means that as the temperature of the water is increased, the dielectric constant decreases and the solubility of non-polar organic compounds in water increases. Therefore this can be used as a key to controlling analyte solubility.

The solubility of a range of polar and non-polar organic compounds were measured in water, up to its critical temperature [75, 76] it was found that non polar compounds exhibited enhanced solubility in water as it approaches its critical temperature. For example, alkyl aromatic compounds are reported to be completely soluble at temperatures of 300 °C and above [75], n-heptane displays a five fold increase in solubility at 350 °C compared to that at ambient and toluene is completely soluble at temperatures above 308 °C.

More recently, this effect has been successfully demonstrated by Miller and Hawthorne [77]. They found that for a non-polar molecule, such as naphthalene, a 6 fold increase in solubility in water was observed at 65 °C. The solubility of chlorothalonil showed a 130 000 fold increase at 200 °C in comparison to its solubility at ambient.

Other changes in the properties of water when heated above its boiling point under pressure are in the negative logarithmic ionic product of water (pK_w) [78, 79]. At 150 °C, the pK_w of water is 11.6, therefore giving a neutral pH of 5.8, whereas at 250 °C, the pK_w is 11, giving a neutral pH of 5.5. Therefore as the temperature of water increases, water becomes both a stronger acid and base, thus enhancing acid and base catalysis due to the natural kinetic increase with temperature [74] and the increased availability of H^+ and OH^- which can act as catalysts for chemical reactions [80]. All of these properties allow water at temperatures above 200° C to act as an acid, base or bi-catalyst therefore reducing the need for neutralisation and catalyst regeneration.

It is clear to see that the properties of subcritical and supercritical water enable it to be used to replace a range of commonly used organic solvents for organic reactions and synthesis. This has been successfully demonstrated for a wide range of reactions, such as hydrolysis [74, 81], catalysed hydrolysis [82 – 84], oxidation [85, 86], cleavage [74, 87], deuteration reactions in superheated deuterium oxide [88], condensation [74] and non-catalytic rearrangements [89].

After the reports of increased solubility of non-polar and polar compounds in subcritical and supercritical water, the natural progression of research was to apply this technology to extraction methodology. Subcritical or superheated water have been successfully demonstrated to extract essential oils from plants [90 – 96], elements from spiked sand or sludge [97], and coal [98, 99], pesticides from foods [100], herbicides from soils [101 – 106], organic pollutants from spiked sand, soils and sediments [107 – 115] and organic carbon types from humic soil [116].

The use of subcritical water as an extractant of oxygenated compounds from plants, for the production of essential oils, is proving to be a powerful tool, when compared to more traditional methods of extraction, such as hydrodistillation, Soxhlet and carbon dioxide supercritical fluid extraction. Although the first of these can be viewed as a very low dielectric constant version of water [95]. The advantages of using subcritical water as an extractant are that the extraction times are lowered [92, 93,96], the efficiency of extraction is better [91 - 93, 95], and cheaper [90 - 92, 95]. An improved quality of the essential oil [90, 95] has been reported as superheated water is more selective than other extraction methods, as unlike steam distillation, it does not extract non-polar alkane terpenes, lipids and large hydrocarbons. It is also more suitable for volatile and thermolabile compounds [91] and is environmentally friendly [90, 92].

It has been demonstrated that the key variable for the extraction of oxygenated compounds from plants, is an increase in temperature (up to an optimum), which results in an increase in the rate of extraction [90-93, 95]. Other observations were that an increase in pressure at temperatures below 200°C gave no significant difference in terms of yield [92, 95], although data produced by Rovio et al. [93] and Ammann et al. [96] claimed the that pressure can be manipulated to optimise the yield. Flow rates have also been demonstrated to affect the yield [92, 95].

These findings were reflected when extracting metal ions using acidified subcritical water [97-99], herbicides using buffered subcritical water [103], pesticides from food using hot pressurised water [100], a range of acidic, non-acidic polar and non-polar

herbicides [101, 102, 104, 105], organic pollutants from sand, soil and sediments [107-115] and organic types from humic soil using subcritical water.

It can be concluded that an increase in pressure has a minimal effect when water is in the condensed liquid phase. Even though it has been demonstrated that an increase in pressure, results in a slight increase in the dielectric constant [107, 113, 115] which should therefore inhibit the extraction of low polarity organic compounds, the effect is small and is not reflected by changes in observed extractions.

1.3.1 Extraction of neutral and non-polar compounds

The methods employed for the extraction of non-polar organic pollutants (such as n-alkanes [107, 109, 115], polychlorinated biphenyls (PCBs), [108] polycyclic aromatic hydrocarbons (PAHs) [109], polychlorinated naphthalenes (PCNs) [113], polychlorinated dibenzofurans (PCDFs) [113], chlorinated phenols [107] and substituted aromatic compounds [109]) employed a liquid-liquid extraction (typically n-heptane was used as the extractant from water) whereby the column effluent was passed into a vessel containing a solvent suitable for GC analysis. Liquid-liquid extraction of the analyte of interest was performed prior to analysis by GC.

The findings from the initial work was that PAHs could be quantitatively extracted using superheated water at 250 – 300°C, above 50 atm and that the results were comparable to sonication and Soxhlet extraction. Better extraction efficiencies of more non-polar compounds, such as higher molecular weight alkanes, PCDFs, PCNs, were achieved using steam, which has a dielectric constant of 1 and this value is closer to the dielectric constant of these compounds. Hence steam will extract these types of compounds more efficiently than superheated liquid water. The effect of temperature on steam extraction has been assessed [108, 109, 113] and it has concluded that temperature has little effect on the dielectric constant of steam. Instead the increased extraction efficiency of steam at elevated temperatures is attributed to a combination of enhanced thermal desorption,

increased analyte vapour pressures and faster extraction kinetics of hydrophobic compounds.

To overcome the poor repeatability associated with liquid – liquid extraction of the extractant from the aqueous extraction into a non-polar solvent for GC analysis two alternatives were examined. Hartonen [110] replaced the liquid-liquid extraction with a solid phase trap, which was packed with Florisil, XAD-4, XAD-7, Tenax GC for PCBs and Tenax-GR and Tenax-TA for PAHs and n-alkanes. Unfortunately Florisil, XAD-4 and XAD-7 produced poor recoveries for PCBs when eluted with n-heptane. Improved repeatability and recovery was observed using the Tenax GC trap. Graphitised Tenax proved to be too retentive for n-alkanes but Tenax-TA gave good recoveries and reproducibilities when steam was used as the mobile phase. Due to the aromatic nature of Tenax, strong interactions were formed between the sorbent and PAHs, and steam proved to be more efficient at extracting PAHs from Tenax-TA, than superheated water. Yang et al. [105, 114] took a slightly different approach and directly linked a solid phase trap to an HPLC system. The sample was extracted with superheated water, the analyte of interest was trapped onto the solid phase trap, then eluted by aqueous – organic mobile phase and injected onto an HPLC column prior to quantitation by a UV-Vis detector.
A wide range of analytes were extracted from sand, soil [114] or paper and sand [105], onto a silica bonded C18 packed solid phase trap. The extraction compared favourably to sonication extraction followed by GC analysis [114] and provided higher sensitivity than the previous off-line methods. It also demonstrated that some peak broadening had occurred, when comparing the peak shapes of non-extracted standards to extracted samples, but the peak shapes were similar and the on-line extraction technique offered a convenient mode of operation.

Hawthorne et al. [112] extracted PCBs from soil using subcritical water (250°C, 15 – 60 minutes). After extraction, the extraction cell was cooled and an aliquot was transferred to an autosampler vial containing a stirrer bar, and an solid-phase microextraction fibre (polydimethylsiloxane coated). The fibre was exposed to the water for 15 minutes, when

it was withdrawn, and inserted into a heated (300°C) split/splitless injection port to volatilise the analyte for detection by GC. The findings from this work were that the extractions were in agreement with Soxhlet extraction. Kipp et al. [111] also used superheated water to extract PAHs from sediments and used enzyme immunoassay for their determination. They also found that the extraction recoveries were comparable to those of Soxhlet extraction.

The intermolecular interactions of a range of polar, non-polar organic solutes with five different sorbents (glass beads, alumina, Florisil, silica bonded C18 and XAD-4) and their elution with subcritical water was undertaken by Yang et al.[117]. For these experiments, the analyte of interest was spiked onto the top of an packed stainless steel column and eluted using water at different temperatures (50 - 250°C) and pressures (5 and 50 bar).

Polar solutes were found to be effectively eluted from normal phase sorbents (Florisil and alumina), using water at low temperatures (50°C), where it is more polar and has a high dielectric constant. This demonstrates that water effectively disrupts and breaks dipole interactions between the sorbent and solutes. However, elution from reversed-phase packing materials (C18 and XAD-4) generally required water which was between 100 - 150°C higher than that required for normal phase packing materials. This can be explained by the fact that more energy is required to break the Van der Waals interactions (for C18) and π-electron interactions (for XAD-4) between the sorbent and solute. In addition, less polar solutes, such as aromatic solutes, were found to require an elution temperature 50°C above that of C18, from XAD-4 since it is capable of π and Van der Waals interactions with the analyte. However, n-decane, which can only form Van der Waals attractions with XAD-4, displayed no difference in elution between C18 and XAD-4, thus demonstrating that water is more effective at disrupting Van der Waals attractions than π-electron interactions.

1.4 Superheated Water Chromatography

For reversed-phase chromatography, a non-polar stationary phase and a polar, largely aqueous, mobile phase are used. Retention of the analyte is dependant on the degree of its partition into the stationary phase and its hydrophobic interactions with the mobile phase [118]. Properties of the analyte, such as its polarity and molecular size play an important role in reversed-phase separation, hence large non-polar analytes are highly retained.

Most reversed-phase sorbents are silica based alkyl bonded phases, but polystyrene divinylbenzene (PSDVB), graphitised carbon and sorbents based on inorganic substrates other than silica also have their place [119]. Water is the weakest mobile phase for reversed-phase separations and gives the greatest retention times, thus mobile phases are typically made with a mixture of water and organic solvents such as methanol, acetonitrile, or tetrahydrofuran.

As superheated water has been demonstrated to extract a wide range of polar and non polar analytes from various substrates and sorbents, it is reasonable to assume that reversed-phase chromatography could be performed using superheated water as a mobile phase, as its dielectric constant can be easily manipulated so as to mimic those of organic-aqueous mixtures. Smith and Burgess [120 – 122] studied the separation of a range of polar and relatively non-polar analytes on a range of reversed-phase sorbents (including C18 bonded silica, PS-DVB, porous graphitic carbon (PGC) and polybutadiene (PBD) coated zirconia columns), using superheated water as the mobile phase. They found that an increase in temperature resulted in an increase in efficiency and a decrease in retention.

As predicted, PS-DVB required an increase of approximately 50°C in the temperature of the superheated water, for the elution of non-polar analytes compared to the temperature

required for C18 bonded silica. They demonstrated that superheated water at a temperature of 180°C gave a separation of phenols, whose separation was equivalent to that achieved when using acetonitrile-water 20:80 mixture. Even when higher temperatures were used (210°C) for the elution of more hydrophobic analytes (propyl and butyl hydroxy benzoate esters) there was no evidence of analyte degradation or sorbent dissolution when using a column packed with PS-DVB. This was in contrast to C18 bonded silica , which after prolonged exposure to superheated water, suffered hydrophobic collapse and sorbent dissolution [122]. However, Burgess [122] also found that when nitrobenzene was eluted from PS-DVB using superheated water at 225°C, at flow rates of 0.3 and 1.0 ml/min, the lower flow rate resulted in an 80% reduction in response, due to analyte degradation.

To try to overcome the problems associated with exposing C18 bonded silica stationary phases to water, Young [123] synthesized a brush and branch phase for specific use with water as the sole eluent.
Subcritical water extractions were performed on sand spiked with a mixture containing benzaldehyde, benzene, toluene and ethyl benzene, followed by the immediate separation by LC, using water as the sole eluent. The branched phase produced 12 000 plates for the unretained analyte, whereas the brush phase produced 16 000 plates. But the branch phase produced over 2.5 times as many plates as the brush phase when the retention factor was one.

Pawlowski and Poole [124] studied the influence of temperature (75 - 180°C) on the solvation properties of pressurised water on PLRP-S 100. They added 1 % acetonitrile to the eluent to improve peak shapes for all solutes at low temperatures. It had little effect on the solvation properties of water, but did reduce interfacial tension and promoted favourable mass transfer with the stationary phase. The general findings from this work were that even at 180°C, water still remained as relatively weak eluent when compared to acetonitrile and methanol. At this temperature superheated water demonstrates the same elution strength as 15 – 25% acetonitrile / water, 50 – 60 % methanol- water or 25 – 35% propanol water mixtures. Therefore, if gradient elution was

compared to temperature gradient elution of water, superheated water still remained the weaker eluent. It was also observed that changes in selectivity with temperature were different to those of various organic/ aqueous mixtures, using PLRP-S 100. This was thought to be attributed to the changes in dipole – dipole interactions for hot water compared to the changes in hydrogen bonding interactions for organic solvents. It was also concluded that superheated water, due to its modest elution strength is most useful for the separation of polar analytes.

The retention factors of alkyl substituted benzenes, phenol, aniline and their derivatives were determined using subcritical water, methanol/water and acetonitrile/water mixtures as eluents on silica bonded C18, alumina and PS-DVB packed columns were studied by Yang et al [125]. The retention factors and times for all analytes were found to decrease with increasing temperature. It was reported that to obtain a similar retention on PS-DVB as C18, a temperature of approximately 50°C higher was required. It was also found that the retention factors for phenol and anilines at 150°C, are similar to those separations using 43% methanol in water or 40% acetonitrile in water and when separations are performed at 200°C, the retention factors of chlorinated phenols and anilines are similar to those obtained by 68-69% organic solvent in water.

More interestingly, in comparison to the work undertaken by Pawlowski [124], who employed a 1% acetonitrile solution, Yang reported that water at 180°C had an elution strength corresponding to 15-25% acetonitrile in water or 50 – 60% methanol in water, discrepancies seem to arise, when comparing PS-DVB phases.
These could have arisen due to either the differences in the manufacturing process of the sorbent (as the sorbents used were from two different suppliers), the configuration of the columns (Pawlowski used 100 x 4.6mm i.d., whereas Yang used 150 x 2.1mm i.d.) leading to enhanced temperature gradients for the wider bore columns or insufficient column equilibration.

A high temperature liquid chromatography system, capable of using flow rates of 15 ml/min was developed by Yan and co-workers [126]. Making use of the reduced

viscosity at higher temperatures, columns of dimensions 50 x 4.6 mm i.d. were packed. The separation of long chain alkylphenones on 2.5μm polystyrene coated zirconia was reported at a flow rate of 15 ml/min, acetonitrile –water mobile phase at 150°C. Also phenol and four alkyl substituted phenols were separated using water at 120°C at a flow rate of 12 ml/min in less than 30 seconds.

Chienthavorn [127] reported the use of superheated water buffered at pH 3, 7 and 11 to separate a range of sulfonamides, using a PS-DVB column and comparing the separations to those obtained using C18 bonded silica. A temperature gradient from 70 - 190°C was used to facilitate the separation of the analytes of interest. From the study, it was found that for temperatures above 100°C, a change in the degree of ionisation of the analytes and the dissociation constant of the buffered superheated water was observed which was lower than that observed at room temperature.

Smith et al.[128, 129] replaced water with deuterium oxide for the separation of salicylamide and barbiturates on a PS-DVB packed column. For the mixture of barbiturates a temperature gradient from 180 - 200°C was employed to speed up elution. Detection of all analytes was confirmed by stop flow mode using proton NMR and the use of a gradient caused no spectral problems.

1.5 Coupling of LC to FID, using water as an eluent

Water is potentially an attractive eluent for flame ionisation detection as it should generate a minimal response. In previous work, steam has been successfully used as a carrier gas for packed column GC (GSC) [130], and mixtures of hydrocarbons, alcohols, organic acids and amines were detected by a hydrogen flame ionisation detector. A range of packing materials were employed (activated alumina, Chromosorb P, sintered magnesia, diatomaceous firebrick) for the separations. This configuration overcame the problems observed when analysing aqueous samples, since water in the sample interferes with the stationary phase, causing peak tailing.

Nonaka [130], Rudenko et al [131] demonstrated the use of the vapours of water, formic acid and methanol as a mobile phase with flame ionisation and electron capture detection. Steam was shown to separate very polar compounds, such as free fatty acids and amines, and to yield symmetrical peaks. The use of steam as a mobile phase was found to decrease analysis times and lower the column temperature required for the elution of polar compounds. Formic acid is also an attractive eluent for flame ionisation detection, since it also produces a low response. Formic acid vapours were used as a mobile phase for the separation of amines and alcohols, with a reported reduction in analysis time. Also, more interestingly, methanol vapours were used as the mobile phase for the separation of haloalkanes using an electron capture detector.

Berezkina et al.[132] reported that the problems associated with steam chromatography were the possibility of chemical reactions of water vapour with the analytes and stationary phases. The advantages were reported to be improvements in the symmetry of polar analytes and the ability to analyse water samples without peak broadening. Polar stationary liquid phases (group 1 salt – water) were investigated with steam chromatography.

It was found that the elution order of a mixture of alcohols (C1 – C8) was governed by their polarites rather than their molecular masses. Alterations in the elution order were observed using different salts and the nature of the salt was found to influence the column retention factors. Column temperature was found to be an important factor on resolution. This can be explained as increasing the temperature decreases the water content in the water-salt in the liquid-stationary phase, which leads to a change in selectivity. Another important factor for elution was the pressure. Increases in pressure resulted in an increase in retention and selectivity due to an increase in the amount of water present in the stationary phase. It was also found that the efficiency of a column packed with sodium nitrite-water phase was similar to that of a column packed with polydimethylsiloxane (SE-30).

Viktorova and co-workers [133] studied the retention of alcohols in steam chromatography using calcium chloride – water as a stationary phase. The stationary phase was found to be stable upto 150°C and have high selectivity for the separation of high boiling point alcohols and diols.

1.6 Coupling of LC to FID using superheated water as an eluent

The first reported attempt to link superheated water LC to FID was by Miller and Hawthorne [134]. The system comprised of a PS-DVB packed column linked to a 10 cm length of glass lined stainless steel tubing. The HPLC column was placed inside a GC oven and the injector was placed outside the oven. The outlet of the HPLC column was connected to a stainless steel supercritical fluid extraction restrictor, which provided sufficient pressure in the column for the water to remain as a liquid at temperatures above 100°C. The end of the restrictor was placed up into heated block of the FID, 3 cm from the tip of the jet. To achieve good peak shapes, a flow rate of 200 µl / min of water was to be used. For this flow rate, a stable flame was obtained using a hydrogen flow rate of 300 ml / min, 430 ml / min for air and a FID detector block temperature of 400°C. Using this system, they demonstrated a detection limit of 5 ng for tert-butyl alcohol and the separation of seven aliphatic alcohols, both isothermally and using a temperature gradient programmed from 120 - 150°C. They also demonstrated the separation of a range of hydroxy-phenols and the detection of amino acids. Therefore demonstrating LC-FID as a potential universal detection system.

Using a similar system set up, Burgess [122] also linked superheated water LC to FID for the separation and detection of a range of parabens, aromatic amides, phenols and D-glucose using both isothermal and temperature gradient elution. Unfortunately, it was reported that the system was not robust and suffered from flame instability, sputtering which led to spiking in the background signal, and frequent capillary blockages.

A slightly different approach was adopted by Bruckner [135], whereby the detection of volatiles and non-volatiles was made possible by dual detection linking UV and FID.

The detection of volatiles by FID, post UV detection, was made possible by the development of a drop headspace interface. The effluent from the conventional HPLC column passes through a capillary, to its tip, to form a droplet. Helium flows around the droplet and becomes enriched with the volatile analytes of successive droplets. The volatile enriched helium stream is then swept to the FID for detection. This system has many advantages, since it allows the detection of coeluting non-volatile and volatile species in complex mixtures. It also could be used at flow rates up to 1 ml /min and due to the stationary phase as developed by Young [123], for use specifically with water, hydrophobic compounds such as naphthalene could be separated at room temperature. Unfortunately, the disadvantages of this system are that the system is not truly universal, since it could not detect non-chromophore containing non-volatile compounds.

Ingelse [136] partially overcame the problems that were found by Burgess [123] by thermostatting the capillary restrictor from the HPLC column. The restrictor was placed in a second GC oven, linked to the HPLC column by a transfer line. Unfortunately, when temperature gradient elution was employed, sputtering occurred. Different designs of restrictors were tried to overcome this problem, but were found to be prone to blockage, therefore the linear restrictor was used. To overcome the problems associated with sputtering, it was found that a relatively low restrictor temperature (75°C) was favourable, since elevated temperatures increased the incidence of the restrictor plugging. Using this configuration, isothermal and gradient elution were demonstrated for the separation of alcohols and aldehydes, which whilst demonstrating detection of these compounds, it did not prove the system to be capable of detecting an extended range of compounds outside that of the normal FID capabilities.

It was reported, using this type of interface, that mobile phase modifiers such as formic acid, trifluoroacetic acid and ammonia strongly reduce the FID signal and increase the chance of restrictor plugging. It was also noted that strongly acidic mobile phases led to the corrosion of the syringe pump used in the analyses. A μLC-FID was proposed by Hooijschuur [137], whereby an inverted FID was linked to a micro LC system by means of an eluent-jet interface. This type of interface is based on the thermospray technique,

as used in LC/MS, whereby a jet of fine liquid particles is produced by heating a liquid stream as it passes through a capillary [138, 139]. The eluent-jet interface produces a stream of droplets by a sharp temperature gradient at the tip of the capillary, caused by RF inductive heating. Unlike thermospray, the tip was cooled by helium passing around it and a second inlet for helium was used to transport the aerosol into the FID. A restriction of 4 - 10μm, 0.5 – 1 cm from the tip of the capillary was found to produce smaller droplets, which had the affect of eliminating spiking, and produce a stable response. Typical flow rates used in this system were 5 - 50μl/min, but at the higher flow rates the RF power had to be increased from between 40-70 W, to maintain a stable baseline. It was concluded that the system could be linked to μLC where flow rates are >10 - 30μl/min successfully.

Separations of a mixtures of alcohols and bis(2-hydroxyethylthio)alkanes, using 1% formic acid were demonstrated showing that volatile polar and non-polar compounds can be efficiently eluted and detected by the system. Improvements in the sensitivity and peak shapes of non-volatile compounds, such as organic acids and amino acids, was found by increasing the RF power, which unfortunately led to rapid blockage of the restrictor.

A comparison was made between the eluent jet interface and a nebuliser, as described by Ganan-Calvo [140, 141] which produces monodispersed size aerosols. It was reported that the eluent jet interface was up to 10 times more efficient at transporting non-volatile compounds to the FID. Although the reports of the linkage of the eluent jet interface to the FID appear to be very favourable, unfortunately it was reported to be non-robust and unstable as the restrictor lasted only up to 4 weeks. Also it could not cope with the mobile phase flow rates more traditionally required for LC.

1.8 Aims of the project

Previous attempts at the development of a universal detection system for LC have taken two separate directions. The original concept was to transport the eluent from the chromatographic column, evaporate the volatile mobile phase and transport the residual

analyte to the flame for detection. The second more recent was to use water as the eluent and produce a thermospray from a capillary, which was positioned up into the jet of the FID, to spray the column effluent up into the flame for detection.

Unfortunately, both directions have yielded limited success. In view of these findings, the current project set out to develop a LC-FID for universal detection of volatile, non-volatile, chromophore and non-chromophore containing compounds. The main objective of the project was to produce a system that was both robust and sensitive, which could be coupled to a superheated water LC system. The effect of detector temperature, gas and mobile phase flow rates were investigated initially, in order to establish flame stability. Once established, various designs on interfaces were adopted and modifications made to the FID, to provide a stable system.

To determine whether the system was capable of universal detection, flow injection analysis (FIA) of a range of analytes was undertaken followed by investigations into suitable column materials. The possibility of the separation of analyte mixtures using water at ambient was initially investigated, followed by chromatographic separation using superheated water. Finally it was proposed that solid phase extraction (SPE) could be performed using superheated water, following on from the successful extraction studies as performed previously. The aim was to eventually develop an integrated extraction – chromatography system using only water as the solvent thus avoiding the use of organic solvents.

Chapter 2

Experimental

2.1 Chemicals

2.1.1 General Chemicals

Throughout this project, the mobile phase used was *fresh*, triply deionised water at an output of 18.2 MΩ cm^{-1} obtained from an Elga Maxima HPLC purification unit (Elga Ltd. Wycombe, Bucks. UK). Prior to use as a mobile phase the water was ultrasonicated for 10 min and then continuously purged with nitrogen, so to deoxygenate the mobile phase, thereby helping to prevent corrosion in the system.

Ammonium dihydrogen phosphate, ammonia, ammonium nitrate, potassium dihydrogen orthophosphate and formic acid 0.88 specific gravity were of HPLC grade (Fisher Scientific, Loughborough, Leics. UK). Trifluoroacetic acid (TFA) HiperSolv grade was obtained from Merck (Poole, UK).

Nitrogen (oxygen free grade), hydrogen (research grade) and air (zero grade) were from BOC (Manchester, UK).

2.1.2 Standard Chemicals

D-glucose, D-sucrose, maltose, fructose, fucose, mannose, xylose, arabinose, rhamnose, D-ribose, mannitol, sorbitol, maltotetraose (dp4), maltopentaose, (dp5), maltohexaose (dp6), pectin, L-valine, L-methionine, L-proline, L-arginine, L-isoleucine, L-phenylalanine, L-tryptophan, caprolactam, chitosan, benzene sulphonamide, m-toluamide, benzamide, 4-hydroxybenzamide, benzylamine, N-methylamine, cyclohexylamine, hexylamine, methylamine, propylamine, butylamine, benzaldehyde, formaldehyde, acetaldehyde, propionaldehyde, cyclohexanone, cyclohexanol, m-cresol, , benzyl alcohol, dichloromethane, citric acid, oxalic acid

dihydrate, tartaric acid, lactic acid and allantoin were of a purity of at least 98%, obtained from Sigma-Aldrich (Poole, Dorset. UK).

Silver nitrate (SLR grade), acetophenone (SLR grade) and methanol, ethanol, acetic acid, heptane, hexane, cyclohexane, nitrobenzene, isooctane, toluene (HPLC grade) were obtained from Fisher Scientific (Loughborough, Leics. UK).

2.1.2.1 Preparation of standard solutions

The test solutions were prepared by weighing an appropriate amount of analyte and then dissolving in water, giving a typical stock solution concentration of 1000 µg/ml. Sequential dilutions were diluted to volume with water. Amino acid standards were dissolved and sequentially diluted using a 0.02% v/v solution of trifluoroacetic acid.

Pectin stock standards were prepared by weighing an appropriate amount of test compound into a volumetric flask, diluting to volume with mobile phase (0.001 M $NH_4H_2PO_4$+0.02 M NH_4NO_3 adjusted to pH 7 using 0.2 M NH_4OH). The resulting stock solution was then stirred for a period of 1 hour to ensure complete dissolution.

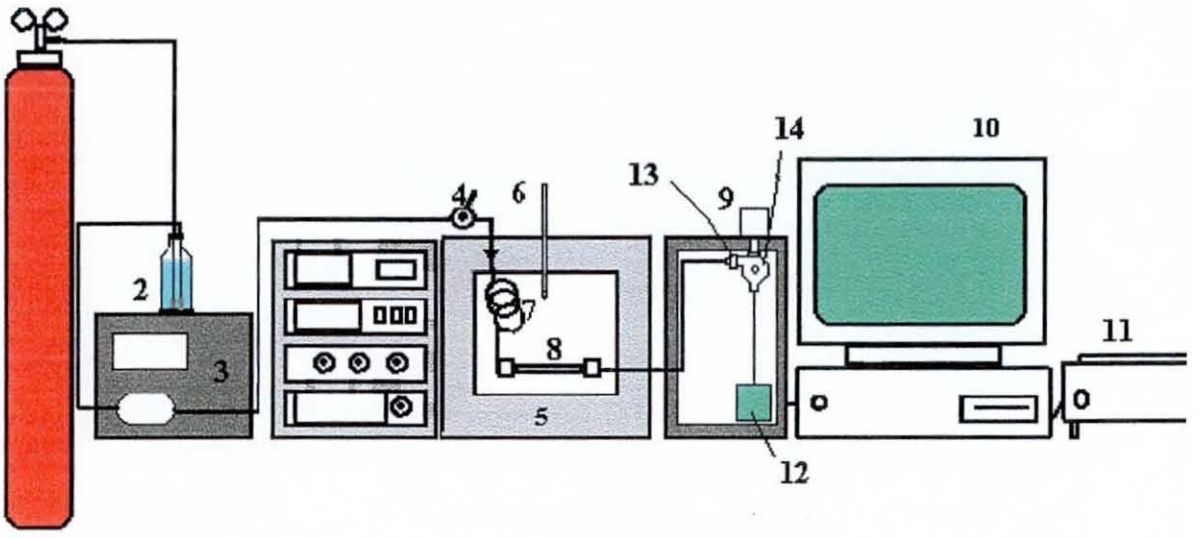
Chitosan stock standards were prepared by weighing an appropriate amount into a volumetric flask and diluting to volume using a solution of 0.1M formic acid, again the resulting stock standard was stirred for a period of 1 hour. A 1 in ten dilution of this standard was performed using water as a diluent, giving a total acid concentration of 0.01 M formic acid. Further dilutions were undertaken using 0.01 M formic acid as a diluent.

2.2 LC-FID instrumentation and conditions

2.2.1 General description

In order to obtain a robust and stable detection system, many configurations and alterations were examined. The final arrangements are shown as a schematic diagram (Figure 2.1) and can be considered as a hybrid of LC, GC and ICP.

Figure 2.1 Schematic diagram of new LC-FID interface



1.	**Gas cylinders**	8.	**Analytical column**
2.	**Solvent reservoir**	9.	**FID**
3.	**Analytical pump**	10.	**Data management system**
4.	**Injector port**	11.	**Printer**
5.	**GC oven**	12.	**Peristaltic pump**
6.	**Thermometer**	13.	**Microconcentric nebuliser**
7.	**Preheating coil**	14.	**Spray chamber**

The main components of both systems were a Jasco PU 980 Intelligent pump (Hachioji, Tokyo, Japan), to which was attached a manual injection port, Rheodyne model 7125 (Cotati, California, USA) fitted with either a 10 or 20 µl loop. From the injector port a length of stainless steel tubing was attached (0.3 mm i.d., 30 cm length), to which the HPLC column was connected.

For superheated water applications, the length of tubing was increased (1 m x 0.3 mm i.d.), coiled and placed inside a GC oven (Phase Separations, UK) to ensure thermal equilibration of the mobile phase prior to entering the analytical column. The column was attached to a length of stainless steel tubing (1 meter x 0.6 mm), along which lengths of copper foil were crimped to the tubing to aid heat dissipation from the system. The columns were connected to the LC-FID interface by a length of

narrow bore PEEK tubing (1 meter, 0.13 mm i.d.), to provide a back-pressure between 35-52 Kg cm^{-2}.

The LC-FID interface was placed in the oven of a Varian GC model 3700 (USA) which was held at ambient temperature (30 °C) . The interface consisted of a MCN microconcentric nebuliser M2S (CETAC Technologies, Cheshire, UK), a 1/16 inch PTFE T-piece (Omnifit, Cambridge, UK) attached to a small cyclonic spray chamber (Loughborough University, Loughborough, UK). The drain of the cyclonic spray chamber was attached, via a stainless steel needle to a peristaltic pump (Ismatec, Zurich, Switzerland) by 0.4 mm i.d. peristaltic pump tubing.

The cyclonic spray chamber was also connected via a short length of glass tubing (2 mm i.d.), to the base on an FID. The temperature of the FID base was maintained between 230 - 420°C (figure 2.1).

The jet of the FID was replaced by alumina tube (2 mm i.d.) Typical gas flow rates for the system were hydrogen at 110 ml/min, air at 475 ml/min and a carrier/ nebuliser gas of nitrogen at 375 ml/min. Typical FID temperatures were between 230 - 420 °C.

The aqueous mobile phase flow rates into the interface were typically between 0.2 – 0.9 ml/min.

2.2.2 HPLC columns used

Columns of differing sources and dimensions were used in the study.

Column Trade name	Stationary Phase	Particle size (µm)	Pore size (Å)	Dimensions (mm)	Manufacturer*
CHO-611	Cationic in Na^+form			300 x 6.5	Interaction
Nucleosil	NH_2 bonded silica	10	100	250 x 4.6	Hypersil
Hypercarb	Porous graphitic carbon	7	250	2.1 x 150	Hypersil

ZirChrom-PBD	Polybutadiene coated zirconia	3	300	150 x 2.1 150 x 4.6	ZirChrom Separations
PL HiPlex H	Cationic PSDVB in H^+ form	8		250 x 4.0	Polymer Laboratories
PL HiPlex Ca	Cationic PSDVB in Ca^{2+} form	8		250 x 4.0	Polymer Laboratories
PL-RPS	PSDVB	5	100	2.1 x 250 4.6 x 250	Polymer Laboratories
PL Aquagel 50	Mixed mode	8	1000	300 x 7.5	Polymer Laboratories
NH2P-50	Polyamine bonded polymer gel	5	200	4.6 x 250	Asahipak
Aqua ™	C18	5	125	250 x 2.0	Phenomenex

* Source

Interaction (Omaha, Nevada, USA)

Hypersil (Runcorn Cheshire, UK)

ZirChrom (Anoka, USA)

Polymer Laboratories (Church Stretton, Shropshire, UK)

Ashai Chemical Industry Co. (Yakoo Kawasaki-Ku, Kawasaki-shi, Japan)

Phenomenex (Macclesfield Cheshire, UK)

2.3 Superheated water extraction instrumentation and conditions

The extraction system (refer to appendix 1) consisted of a Waters 590 pump (Taunton, Massachusetts, USA), attached to a preheating coil of stainless steel (1 m x 0.50 mm i.d.) and a SPE trap. Both of which were placed inside a Pye Unicam series 104 GC oven (Cambridge, UK) controlled isothermally by a Pye Unicam oven programmer. A cooling coil of the same dimensions was connected directly after the

trap, outside of the oven. A back-pressure restrictor was attached to the end of the cooling coil to provide a back pressure between 83 - 152 bar.
The SPE trap was prepared from a refillable guard column (Upchurch Scientific, UK) and was packed with 20 mg of sorbent. Prior to trapping, the traps were conditioned by passing 10 ml of water at a flow rate of 1 ml/min. The flow was then stopped and the temperature of the oven was raised to 150 °C and a back pressure of 97 bar was applied. Once the temperature of the oven had reached 150°C, the flow was re-started for a five minute period, after which, the trap oven was cooled and the deionised water was allowed to flow until the whole system reached room temperature.

2.3.1 HPLC system

Quantification of the extract was carried out using an LC system. This consisted of a Waters 590 pump (Taunton, Massachusetts, USA), a Rheodyne 7125 manual injector (Cotati, CA, USA) fitted with a 20 µl loop, a Jones 7960 block heater (Hengoed, UK) at 27 °C.
The analytes were separated on a Spherisorb ODS 2 column (5µm, 150 x 4.6 mm i.d., Phase Separations, Clwyd, UK) and were detected using a Pye Unicam 4025 variable wavelength UV-Vis detector (Cambridge, UK). All analytes were quantified using a five-point external calibration curve. Data acquisition was obtained by a Spectra Physics SP4270 integrator (San Jose, USA).

2.3.2 Extraction Scheme

The experiment comprised of two stages. The first stage was to load the dilute aqueous sample, at ambient, onto the cold SPE trap at a flow rate of 1 ml/min, without a back-pressure restrictor. The trap was washed with water to remove any residual sample from the solvent lines on to the trap. The flow was then stopped. In the second stage, the back-pressure restrictor was fitted and the temperature of the oven was raised to the desired elution temperature. The flow was restarted and the sample was eluted off the trap and collected after the cooling coil. When steam was used as the extraction solvent the back-pressure restrictor was omitted and the

condensed liquid was collected and injected on to an HPLC system. The mobile phase prior to use was ultrasonicated for a 10 minute period and then continuously purged with nitrogen, to deoxygenate the mobile phase, thereby preventing corrosion to the system.

2.3.3 Sorbents used

Two polymeric sorbents were chosen for the experiments, these were:

Trade name	Stationary phase material	Particle size (μm)	Pore size (Å)	Manufacturer*
PLRP-S	PS-DVB	5	100	Polymer Laboratories
Oasis HLB™	Poly(divinylbenzene-co-N-vinylpyrrolidone	30	30	Waters Ltd

* Source

Polymer Laboratories (Church Stretton, Shropshire, UK)

Waters Ltd (Taunton, Massachusetts, USA)

Chapter 3

Development of initial thermospray LC-FID interface

3. 1 Introduction

The flame ionisation detector has been in use for nearly 40 years and is employed more than any other detector in GC but has rarely been used previously with LC. It is generally viewed as a universal detector for carbon containing compounds, having a linear response over a wide range, high sensitivity, and the optimised response does not vary significantly with detector temperature and gas flow rates [142 –144].
Due to the lack of a universal detector for LC, the linkage to FID is an attractive proposition. This combination would open up the opportunity to analyse compounds containing no or weak chromophores, such as many amino acids, sugars and amines, without the need for derivatisation. The aim of the present investigation, once a stable flame system has been produced, was to develop a method of analysis for the separation of non-volatile, non-chromophore containing analytes.

Previous LC-FID linkages have relied on the complete removal of organic modifiers prior to detection (see Chapter 1). Failure to remove the organic modifiers prior to detection resulted in the swamping of the FID signal, thus making detection of the analyte of interest impossible. Other problems caused by the necessity of the removal of organic modifiers were losses of volatile analytes.
Superheated water has been demonstrated as a suitable replacement for methanol-water and acetonitrile eluents for LC, and can be used as an extraction solvent for many polar and non-polar analytes. As water produces a minimal FID response, its use as a mobile phase for LC-FID is attractive, as it eliminates the need for the removal of organic modifiers from the mobile phase, therefore overcoming the problems associated with the conventional use of the FID with LC.

To understand the requirements for the establishment of the system, we must first look at the operation of the FID. It is based on a hydrogen diffusion flame. Hydrogen gas is fed through a flame jet [145, 146] and is surrounded by an air stream at a much higher flow rate to ensure complete combustion. To improve the transport of the analytes to the detector, a make up or carrier gas is added to the centralised hydrogen flow, mainly to introduce the analyte.

When organic compounds are introduced into the flame through the carrier gas, their combustion produces ions, which are collected at a polarised electrode (collector) positioned around the top of the flame. The ions are collected at the base of the collector, where the electric field is at its greatest, this produces an increase in current across the flame, to produce a response that is proportional to the amount of carbon in the flame.

3.1.1 Proposed FID Response Mechanism

The response from a FID, is generally accepted as being generated by a number of pyrolytic reactions commencing at the tip of the jet [143,144]. The analyte eluting from the column undergoes a series of degradation reactions in the hydrogen rich flame atmosphere, to give a single carbon species [143,144].

$$CH_3^{\bullet} + H^{\bullet} \rightarrow CH_2^{\bullet} + H_2$$

$$CH_2^{\bullet} + H^{\bullet} \rightarrow CH^{\circ} + H_2$$

$$CH^{\circ} + H \rightarrow C + H_2$$

When moved into the oxygen containing zone of the flame, a chemi-ionisation reaction occurs, producing CHO^+,

$$CH + O \rightarrow CHO^+ + e^-$$

CHO^+ is unstable and reacts rapidly with water to generate positively charged hydroxonium ions, which were initially thought to generate the current.

$$CHO^+ + H_2O \rightarrow H_3O^+ + CO$$

More recently, with the aid of mass spectrometry, it has been found that the current is carried by hydrated hydroxonium ions [142]:

$$H_3O^+ + nH_2O \rightarrow H_3O^+,nH_2O$$

3.2 Initial attempt at LC-FID using water as the sole eluent

The main problems facing the linkage of an LC to an FID system using aqueous mobile phases are firstly, the stability of the flame and secondly, a robust interface to transport the analyte from the column to the flame. The instability of the flame is reported to be mainly due to the cooling of the flame by the water vapour. Previous workers have shown that the problem can be overcome by increasing the hydrogen and air gas flow rates as reported by Miller and Hawthorne[134] and Burgess [122], as well as increasing the temperature of the detector body.
The initial interface that was studied was based on a concept similar to the thermospray interface as used for LC-MS, which is capable of handling aqueous mobile phases and non-volatile and thermolabile molecules [138, 139] in which a jet of fine particles is produced by heating a flowing solution. The rapid heating vaporises part of the solution, which then acts as a nebulising gas for the rest of the eluent.

The original instrumentation designs for direct LC-FID were described by Hawthorne. A fused silica capillary (typically 30 μm i.d. 375μm o.d) acted as the transport medium to carry all of the column eluent up into the FID where it was heated to 200-300 °C.
As those initial studies were concerned with using superheated water as the LC eluent, the capillary had a dual function, as it acted not only as a transport medium for the

analyte, but also provided the necessary back-pressure to the system to prevent water boiling within the LC system.

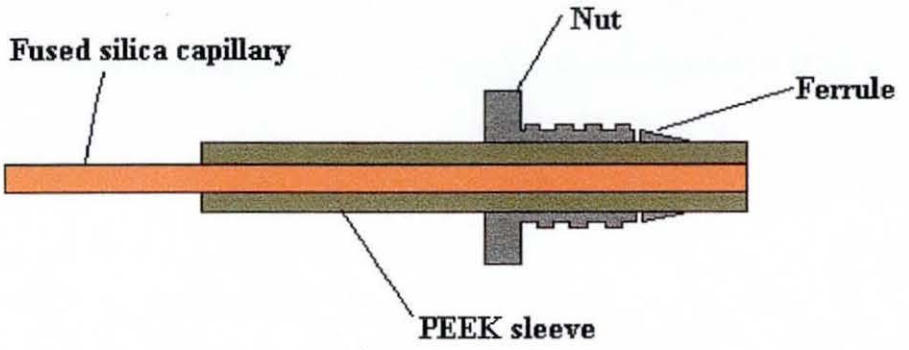


Figure 3.1 Schematic diagram of capillary interface

The present study started with a capillary interface (Figure 3.1) similar to the configuration as used by Burgess [122]. The effluent from the column was linked to the detector by a length of capillary tubing (30 μm i.d., 17.5 cm) and a method had to be determined to hold the capillary in place. To provide a leak free connection to the FID, the capillary was feed through a length of PEEK (2 cm long, i.d. greater than 375μm), which formed a sleeve over the capillary, and was held in place by a 1/16 inch nut and ferrule. Using a zero-volume connector, the nut was tightened sufficiently for the ferrule to crimp onto the PEEK tubing and was then placed into a GC oven at 150 °C for a period of 10 minutes.

At this temperature, the PEEK tubing softened, and after 10 mins heating period, the interface was taken out of the oven and the nut was further tightened, using the zero-volume connector, until no movement was observed when the capillary was pulled. The ferrule was therefore gripping the capillary firmly in place through the PEEK tubing. The interface was then left to cool for 10 minutes, and the zero-volume connector was removed.

The capillary was threaded through a 2 cm length of stainless steel tubing, which was connected to the base of the glass connector via a nut, through the glass connector to the tip of the FID jet. Its position was marked against the bottom of the stainless steel tubing with a marker pen, then pulled down 3 cm, to achieve the optimum positioning of the capillary within the FID, for use with superheated water as reported by Miller and Hawthorne [134].

The interface could now be directly linked to the HPLC column (Figure 3.2), which was held within the GC oven. Alternatively, when used without a column, the capillary was connected to a zero-volume connector, which was itself attached to a preheating coil within the GC oven, and the system could be used as a flow injection analysis (FIA) system.

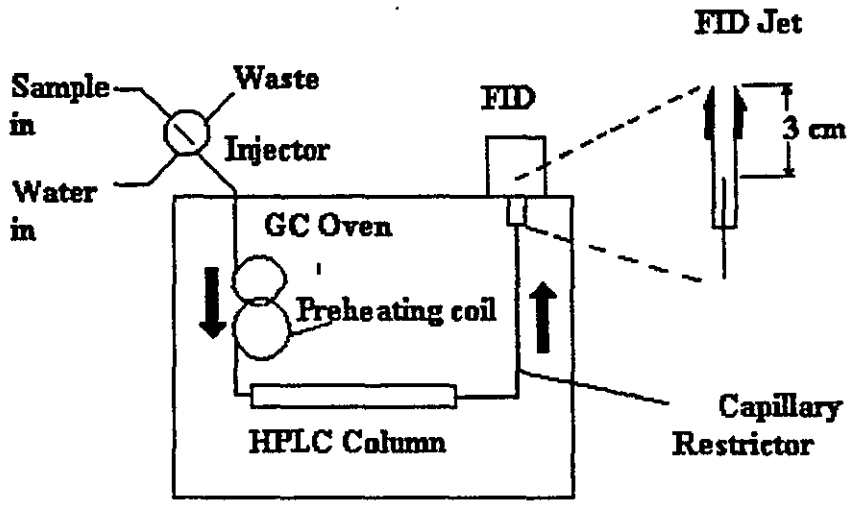


Figure 3.2 Schematic diagram of initial instrumentation, based on GLC and adapted for use with superheated water

3.2.1 Establishment of flame stability

The operating variables of the system, gas flow rates, flow rate of the mobile phase and temperature of the FID detector, were then optimised to obtain a stable flame. The gas

flow rates initially were based on those described by Miller and Hawthorne [134], whereby the air flow rate was kept at a constant 240 ml/min and the hydrogen flow rate increased from 20 ml/min when using a water flow rate of 20 µl/min to a hydrogen flow rate of 300 ml/min for a water flow rate of 200 µl/min.

To aid aerosol formation and analyte transport into the flame, the GC oven, containing the transport capillary, was heated to a temperature of 150 °C, to heat the mobile phase. The system was tested with different hydrogen flow rates and eluent flows by injecting 10 x 10 µl injections of 10% v/v ethanol. The mean of the peak areas of each experiment were calculated (Figure 3.3).

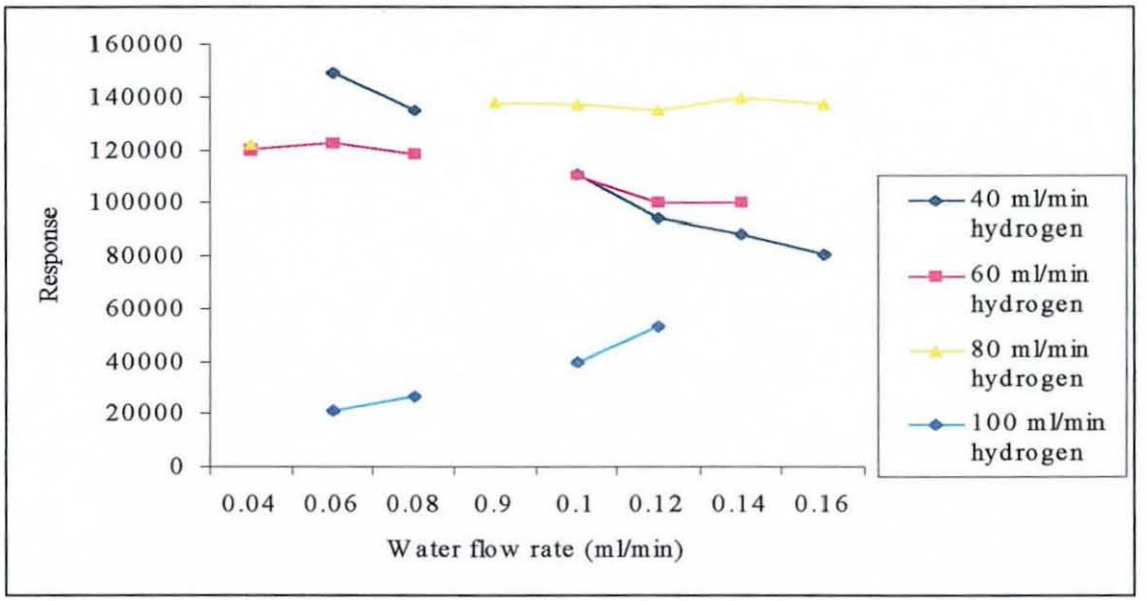


Figure 3.3 Effect on signal intensity for the LC-FID system of systematically altering the flow rate of hydrogen gas and aqueous mobile phase flow rate from the mean peak area of 10 x 10 µl injections of ethanol.

For each different hydrogen gas flow rates, the inlet water flow rate, which provided the optimum response, was selected, and using these conditions the temperature of the detector was varied, to try to optimise the conditions. The results of the experiments

revealed that for water flow rates above 0.1 ml/min, the optimum sensitivity was obtained using hydrogen flow rates in excess of 80 ml/min. Also, in most cases the optimum sensitivity and a stable flame was achieved with detector temperature of 400°C.

However, a number of problems were encountered with this system, especially frequent blockages of the capillary, spiking on the signal and non-reproducible responses obtained from each capillary. Upon inspection of the blocked capillaries, there was a black carbon-like deposit towards the tip of the capillary, which could be due to the burning of the analyte within the capillary. It was also noted that the external polyimide coating of the capillary was frequently stripped or burnt off. This could be due to the temperature of the capillary tip (400 °C) being much higher than the rest of the capillary. This temperature exceeds the normal GLC open tubular column limits and was due to the tip being placed in the heating block of the FID.

Gidding et al. [147] observed a spiking signal (or "sputtering") during their work linking FID to supercritical fluid chromatography, and postulated that it was due to cold spots throughout the FID as a whole. To overcome these problems they suggested running the detector at a temperature higher than that of the GC, to overcome some of the thermal gradient problems within the system. As the LC-FID detector was already being operated at a temperature significantly above the GC, this was not the obvious solution.

However, it was suspected that a cold spot could be present elsewhere within the system. Because glass is poor conductor of heat, the glass rod connecting the capillary to the FID, through which the capillary passed up into the FID, was therefore replaced by a number of different designs of stainless steel connectors, which also controlled the point at which hydrogen was introduced into the sample stream. refer to figure 3.4.

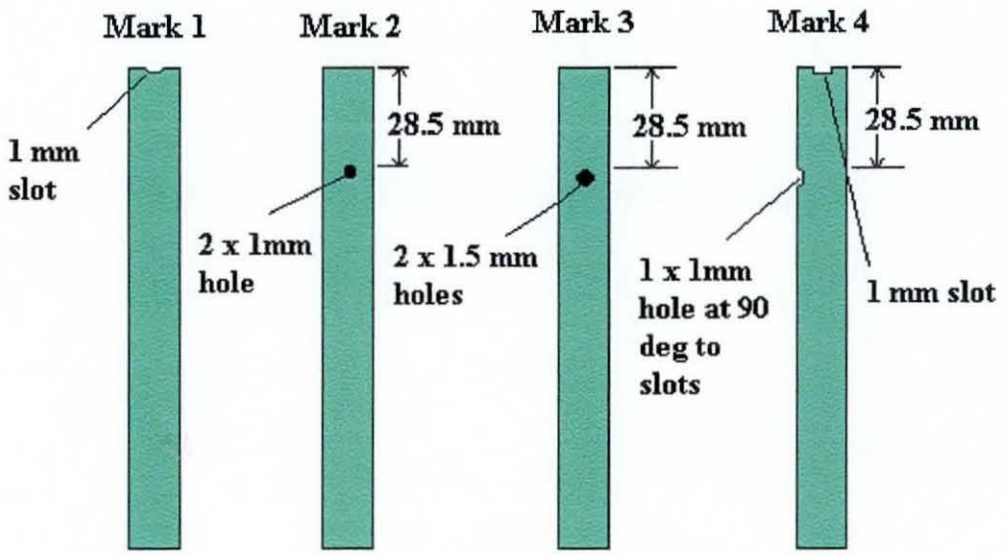


Figure 3.4 Schematic diagrams of the various stainless steel connectors for the FID

The sensitivities obtained with the glass connector and versions Mark 1 and 2 of the stainless steel connectors were compared. The Mark 2 version, which contained 2 x 1 mm holes coinciding with the hydrogen inlet, increased the sensitivity of the system by up to 10% over a range of water flow rates. An added advantage of this connector was that liquid flow rates of up to 0.2 ml/min were now possible and there was a reduction in the frequency of blockage. However, the Mark 1 connector did not offer any advantages, in terms of sensitivity, over the existing glass connector.

The Mark 2 connector allowed an increase in hydrogen flow rates compared to the Mark1 and glass connectors, due to the extra 1mm hole in its trunk. The increase in the flow of hydrogen around the capillary, was thought to be cooling the capillary (as the hydrogen inlet line was not heated), therefore reducing the combustion of ethanol within the capillary.

A further Mark 3 connector was therefore examined. This was similar to the Mark 2 with the exception that the two holes which were drilled through the stainless steel rod

near the hydrogen inlet position had a larger internal diameter of 1.5 mm i.d.. This configuration allowed a even greater flow of hydrogen through the connector, compared to the two previous designs. Unfortunately, Mark 3 displayed no advantage over Mark 2. Mark 3 was expected to increase the response, due to the increase in hydrogen passing through the holes and along the capillary giving a curtain flow around the tip of the capillary.

To investigate the effect of restricting the flow of hydrogen around the capillary, the Mark 4 configuration was constructed. This had a smaller 1 x 1 mm internal diameter hole and a slot across the top of the rod (at 90° to the hole), so that the hydrogen was fed both through the hole and around the tip of the connector. This configuration offered a further 10% advantage over the Mark 2 connector and the Mark 4 connector was therefore used for the further studies.

3.2.2 Positioning of the capillary tip

During the optimisation of the FID for capillary gas chromatography Hyver [148], noted that the position of the end of the column within the detector is important. If the end of the capillary column was placed too far below the flame jet , the peak shapes were impaired due to the column eluates contacting the hot metal surfaces of the heater block of the detector. Whereas if the capillary column was positioned higher and was inside the flame, excessive noise and spiking resulted due to the decomposition of the polyimide coating of the capillary.

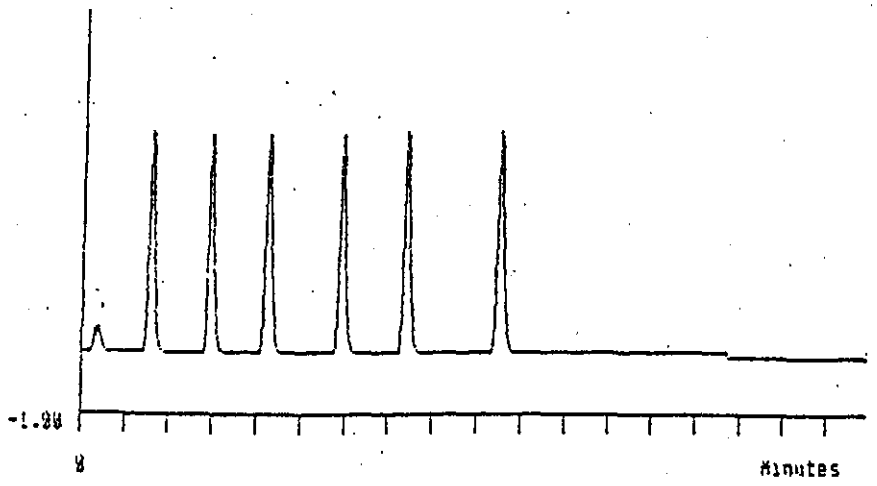
In the present study, the capillary was positioned approximately 3 cm from the tip of the FID jet, approximately as it passes through the heater block, which is set at 400°C. If the capillary was mis-aligned and touched the wall of the heater block, it was thought to be possible that the polyimide coating would to be heated to a sufficiently high temperature for the coating to decompose. It was also thought that the ethanol within the capillary

could reach a high enough temperature for it to combust within the capillary, therefore increasing the background noise and causing "spiking" in the baseline.

The positioning of the capillary tip was reinvestigated, at 3 cm, 3.6 cm and 2.7 cm from the tip of the jet. The results confirmed those of Hawthorne and Miller [134], who suggested that for optimum sensitivity, the capillary should be placed 3 cm from the tip of the jet.

The system was then tested using compounds which were less volatile, to see if they had an effect on the stability of the system.

A 2% w/v solution of glycerol and a 1 % w/v of D-glucose were injected separately in a flow injection mode, as a model of volatile and non-volatile analytes.(refer to Figures 3.5 and 3.6).



Figures 3.5 FIA trace of 10 µl injection of a 2% solution of glycerol. Conditions 100 ml/min hydrogen, 240 ml/min air, GC oven temperature of 150°C, detector temperature of 400°C and water flow rate of 0.18 ml/min. Each division represents 1 minute

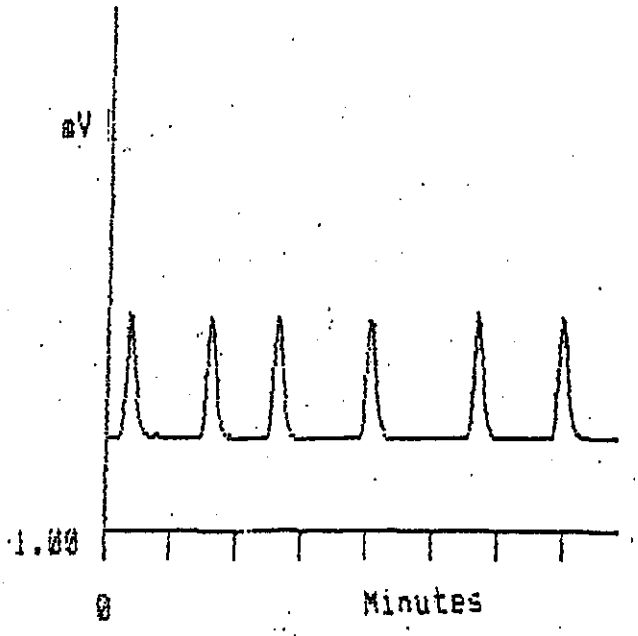


Figure 3.6 FIA trace of 10 µl injection of a 1% solution of D-glucose. Conditions 100 ml/min hydrogen, 240 ml/min air, GC oven temperature of 150°C, detector temperature of 400°C and water flow rate of 0.18 ml/min. Each division represents 1 minute.

As the % RSD of the peak areas for each individual analytes was less than one, it was concluded that the reproducibility of the system was good. However, partial blockage of the capillary was still evident, as indicated by the changes in the back-pressure of the HPLC pump. It was concluded that it would be possible to detect both non-volatile and volatile analytes using this LC-FID configuration.

3.2.3 The effect of changing the internal diameter of the capillary

As the system had been set up using 30 μm fused capillaries, in order to try to optimise the sensitivity of the system, alternative capillaries of internal diameters 20 and 50 μm were tested. Whilst the 20 μm capillary did increase the sensitivity, it suffered from frequent blockages. Although the 50 μm capillary did not suffer as frequently from blockages, the sensitivity was decreased. Therefore further experiments were carried out using the 30 μm capillaries.

For all subsequent experiments, the interface used comprised of a 30 μm capillary 17.5 cm in length was positioned 3 cm down from the tip of the FID jet. The connectors used were stainless steel and of version Mark 4.

3.3 Initial attempts using FIA and Chromatography

Since the detection of glucose and glycerol by FID using superheated water in the FIA mode was possible, the natural progression was to introduce a suitable HPLC column between the injector and FID and examine the effects on a separated peaks.

A suitable column for the separation of glucose was identified as the CHO-611 corn syrup column. The sorbent is a polymeric based resin containing cation exchanger groups in the Na^+ form. It is specifically designed for the separation of mono and polysaccharides using water as the mobile phase at elevated temperatures (typically operated at 80-90°C and 0.5 ml/min flow rate). The separation is achieved in the order of increasing affinity but decreasing with molecular weight. Resolution is increased with increasing temperature [149]. Injection of 1% w/v D-glucose resulted in a peak at 25 minutes.

The initial conditions selected for the linkage of LC to FID were an eluent flow rate of 0.18 ml/min, detector temperature 400°C, column temperature 90°C, hydrogen and air flow rates 100 and 240 ml/min

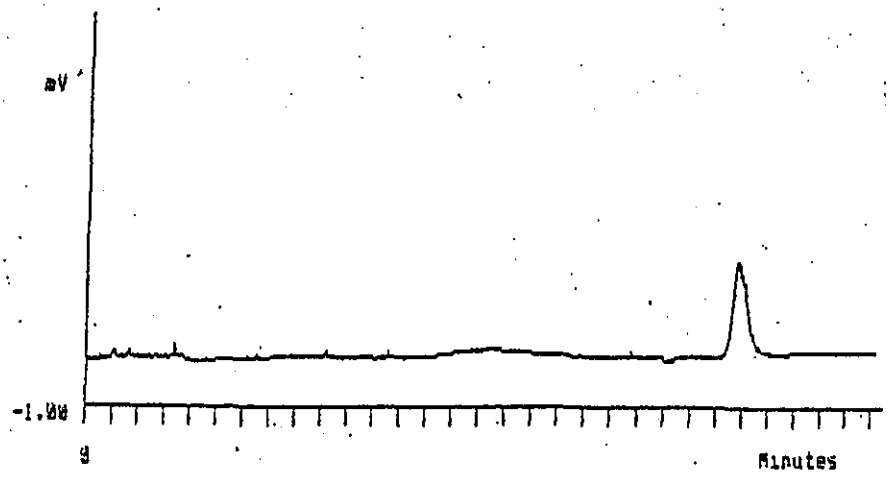


Figure 3.7 Chromatogram demonstrating a 10µl injection of 1%w/v D-Glucose on CHO-611 corn syrup column, water flow rate 0.18 ml/min, detector temperature 400°C, column 90°C.

Unfortunately, a stable flame was not achievable under these conditions. There were sudden drops of the baseline off scale and the signal could only be brought back on scale by re-ignition of the flame. This was probably due to the cooling of the flame by the amount of water entering it at 0.18 ml/min

In the initial studies, this problem had been overcome by increasing the temperature of the detector, but even when the detector temperature was raised to 430°C, flame stability was not achievable and a decrease in peak height and area was evident.

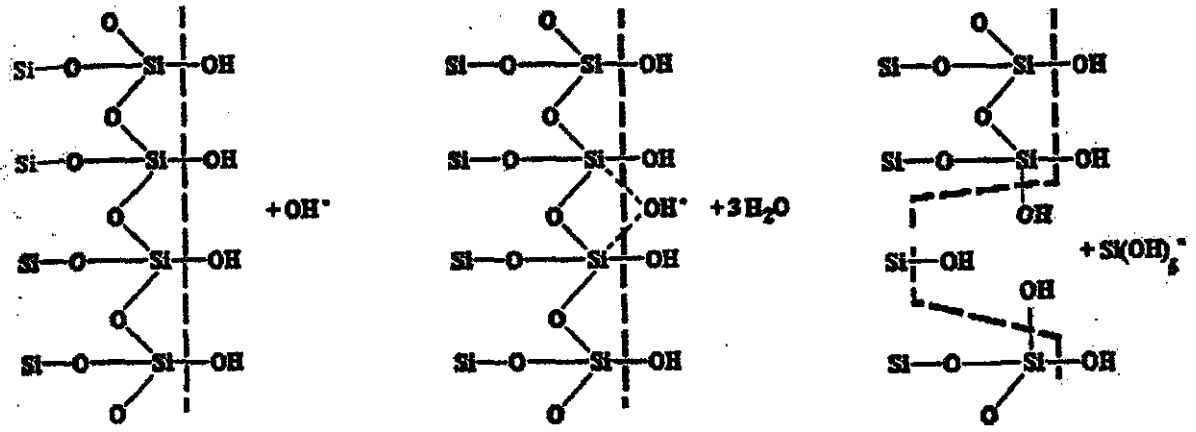
The water flow rate was reduced to 0.08 ml/min, to try to increase flame stability, the column temperature was increased to 100 °C and detector temperature set at 420 °C. To ensure that the water entering the analytical column was the same temperature as that of the oven, a 1 metre length of fused silica lined stainless steel tubing was placed inside the GC oven between the injector and the column inlet. The aim of the preheating coil

was to prevent band broadening due to thermal gradients within the column. An increase in background noise was noted alongside the decrease in the water flow rate using this configuration.

To try to determine if the problem was associated with the stationary phase, separations on a Nucleosil NH_2 column were examined and the experiment was repeated using the same conditions as previously.

This resulted in the immediate blockage of the capillary. Under inspection it was found to be blocked with a white powder, which was presumably silica which had been dissolved from the inlet tubing or the column.
A similar problem was also noted by Burgess [122] who observed that the increase in the ionic product (Kw) of water at elevated temperatures, caused an increase in both acidic and basic nature of the water. Superheated water therefore rapidly dissolved silica based materials due to the increase in the concentration of hydroxyl ions and the higher temperatures employed.

The mechanism of the dissolution of silica, as proposed by Iler [150].



This dissolution occurs along the column, when using superheated water as an eluent. The rapid blockage of the capillary occurred because as the eluent containing the

dissolved silica passes up the capillary, the water flash-evaporates leaving the silica deposited near the tip. The deposition of silica would rapidly build up along the capillary, until total blockage occurred. Silica can be deposited in the jet of the FID.

The frequent blockage of the capillary was thought to be caused through the flash evaporation of water within the capillary. To try to reduce hot spots due to the capillary touching the side of the detector heating block, which might promote deposition, the capillary was passed through a 6cm length of stainless steel tubing, to try to centralise the capillary, as indicated by figure 3.8.

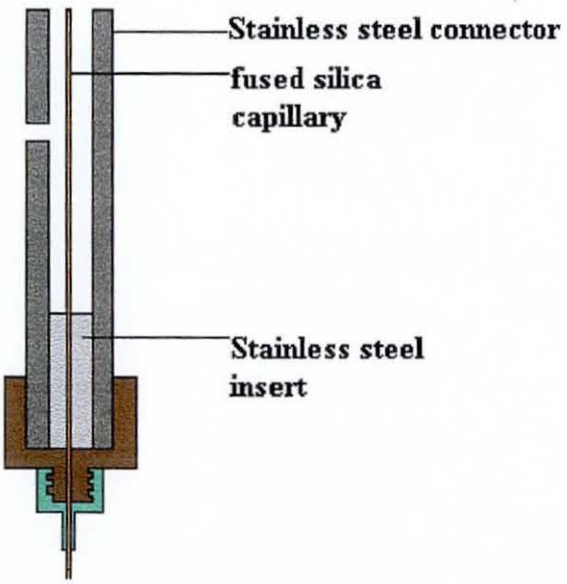


Figure 3.8 Schematic diagram of centralised capillary for LC-FID

This configuration resulted in an increase in capillary lifetime and had the add benefit that higher mobile phase flow rates could be used (up to 0.16 ml/min) compared to previous maximum flow rates of 0.08 ml/min. Therefore, it can be concluded that centralising the capillary helps to prevent the capillary from resting on the side of the heater block, thus reducing flash evaporation of water and silica deposition inside the capillary.

Burgess [122] also observed that as PS-DVB sorbents are polymeric and not silica based, they are not expected to dissolve under the conditions required for superheated water. Sorbent checks were carried out using a PL-RPS-1 column and again the incidence of capillary blockage was reduced, although there was an increase in the amount of background noise.

It was found to be possible to detect oxalic acid, glycerol and D-glucose using the PL-RPS-1 column and a water flow rate of 0.16 ml/min, but unfortunately, all three compounds were effectively unretained, having retention times of 1 min therefore making separation impossible.

To try to increase the retention of these analytes, the PL-RPS column was replaced by a Asahipak Gel NH_2P-50, column, which is an amino bonded vinyl alcohol polymer. Although the both glucose and sucrose could be easily detected, again both had the same retention time and could not be separated. Also the background noise increased.

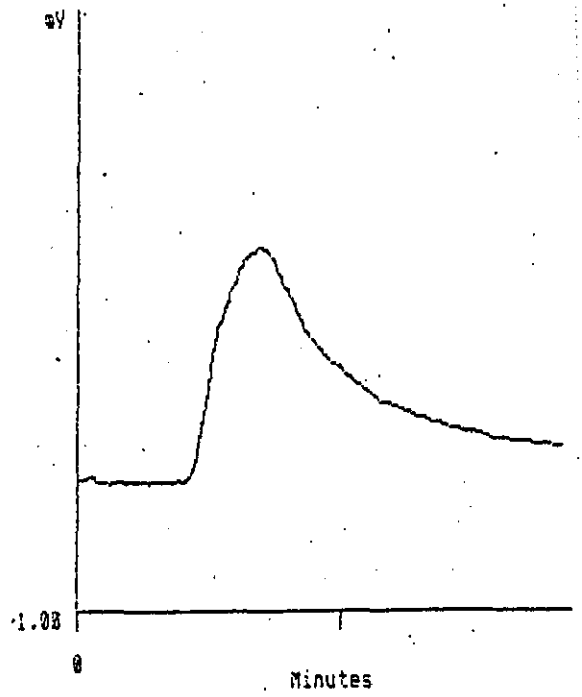


Figure 3.9 Chromatogram of a 1µl injection of 1% D-glucose using Asahipak Gel NH_2 column, hydrogen 100 ml/min, air 240 ml/min, GC oven temperature 90°C, mobile phase 100% water at a flow rate of 0.16 ml/min.

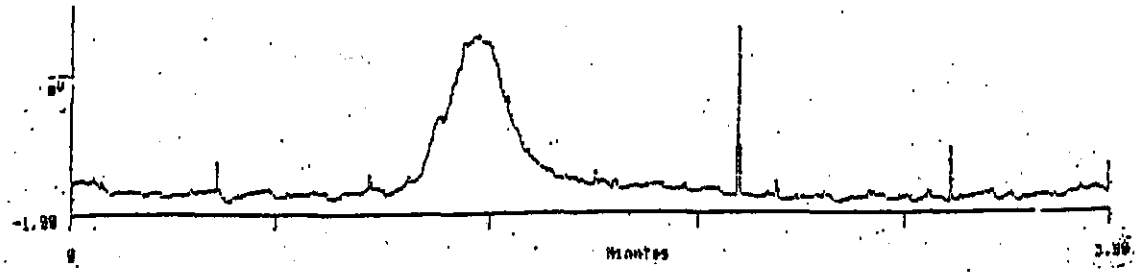


Figure 3.10 Chromatogram of a 1µl injection of a 1% solution of sucrose. Conditions as Figure 3.9

Using the interface configuration as figure 3.8 a high frequency of capillary blockage occurred, and when the capillary was inspected under a microscope, blockages were occurring at regular intervals along the capillary. At approximately 2-3cm from the tip, a white powder was evident, similar to that seen when using the Nucleosil NH_2 column.

However, in this case the column was polymeric and could not be the source of a silica deposition. Therefore, as the only source of silica within the system was that of the silica lined preheating coil, the preheating coil was removed. Upon reinjection of D-glucose, without the preheating coil, a much lower background noise was observed. The fused silica lined preheating coil was therefore replaced by an unlined stainless steel preheating coil. Repeated injections of D-glucose were carried out, but blockages were still occurring. However, instead of appearing periodically along the capillary, the blockages occurred reproducibly 2.5 cm down from the tip of the capillary.

As the blockages were occurring in the same place, the possibility of a hot spot causing excessive heating of one part of the capillary could not be ruled out. As the capillary was positioned level with the top of the stainless steel rod, the capillary was now pulled down 3 cm from the top of the stainless steel rod, to try to avoid the hot spot. It was also

noted that the outside of the capillary appeared to be burnt above the top of the centralising capillary stainless steel insert. Therefore the length of the insert was increased from 6.5 cm to 7.2 cm, to try to prevent this from happening.

The result of increasing the distance of the capillary tip from the jet was that the lifetime of the capillary, before blockage, increased threefold, but sensitivity was reduced. Increased flame instability was evident when using flow rates of 0.14 ml/min. To try to overcome the limited sensitivity problem, the stainless steel connector was changed from Mark 2 to Mark 4, which allowed higher flow rates of gases and water. This permitted aqueous eluent flow rates of up to 0.2 ml/min into the flame, which was more typical of the mobile phase flow rates used when coupled to narrow-bore columns.

From the results already obtained, the maximum flame stability and sensitivity, for D-glucose, was achieved using a detector temperature of 400 °C, GC oven temperature 200 °C, 0.18 ml/min water flow rate and hydrogen and air flow rates of 100 ml/min and 240 ml/min respectively.

3.4 Linkage of initial LC interface to FID

Alternative columns were then examined. A Hypercarb PGC 2.1 x 150 mm column was linked into the system to try to separate D-glucose, ribose and sucrose. The reason for the choice in column material was due to the sorbent being non-silica based and its high temperature stability, as already indicated by Burgess [122]. Again, as with PLRPS-1, all three analytes eluted at the same time, therefore making separation impossible. This result was to be expected, since the findings from Koizumi [151] for the separation of monosaccharides, using PGC column with water as the mobile phase, resulted in similar retention times.

To try to obtain a separation on the analytical column, ammonium hydroxide was added to increase the pH water to 10, to try to obtain retention of the monosaccharides, as indicated by Lu et el. [152]. The reason for the change in pH is due to saccharides

having pKa values ca. 12 or more. Therefore a high pH is required to dissociate the the solutes in the mobile phase. It was therefore hoped that retention of the saccharides would be achieved, unfortunately, within 20 minutes the capillary had blocked. As porous graphitised carbon is stable from pH 1 to 14 [119], it was assumed that the blockage could not be due to the sorbent. However, when the pH of the mobile phase is above 8, it has a tendency to dissolve base silica. As the only part of the system containing silica was the back pressure capillary, this was exchanged for a 0.012 inch i.d. stainless steel tube of the same length. The result of which was that although blockage did not occur whilst using the pH 10 buffer, the background noise did increase.

The poor peak shapes and the increase in the background noise was attributed to the "Christmas Tree effect" [153] which has been observed in GC. During isothermal conditions, the heating element of the GC oven switches on and off in quick bursts to ensure that the temperature within the oven is maintained. Due to the low thermal mass of fused silica, the capillary will respond instantly to the change in temperature. As the bursts are not uniform, where portions of the capillary are shielded and others are not, the front of the chromatographic band is exposed to bursts of radiant heat while the rear of the band is shielded, causing the acceleration of the front of the peak and deceleration at the rear, thus causing malformed peaks.

Once the whole of the interface was lagged with aluminium foil, peak shapes and background noise improved, therefore confirming that the poor peak shapes and background noise was attributed to the Christmas Tree effect, which is in effect the non uniform heating of the interface.

Although the stainless steel capillary could be used with buffers, the sensitivity was poor and the capillary was still prone to blockages. This was thought to be due to sample decomposition and pyrolysis because of the high temperature of the capillary.

3.5 Introduction of a make-up gas to the initial LC-FID interface

As the sensitivity of the system was comparatively low, it was thought that perhaps the current configuration did not supply enough energy to the eluent/analyte droplets to facilitate complete transport to the flame. Therefore, to try to increase the amount of analyte reaching the flame, nitrogen was introduced to the system as a make-up gas as figure 3.11.

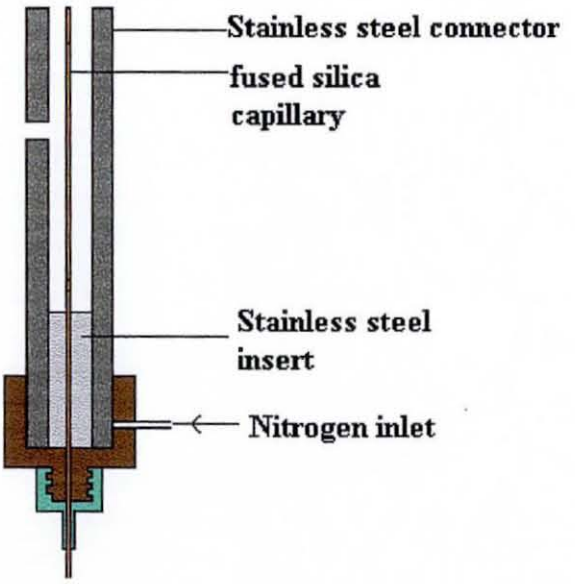


Figure 3.11 LC-FID system using nitrogen as a make-up gas

Initial conditions used were gas flow rates of hydrogen 100 ml/min, air 240 ml/min, nitrogen 120 ml/min, detector temperature 400°C, GC oven temperature 150°C, analytical column Hypercarb PGC and water flow rate of 0.11 ml/min.

Under these conditions, an injection of 1 µl D-Glucose (7 mg/ml) gave a peak at 2 minutes, with the peak off-scale. Upon reinjection the retention time of D-glucose changed to 3 minutes, this time the peak was on scale (Figure 3.12).

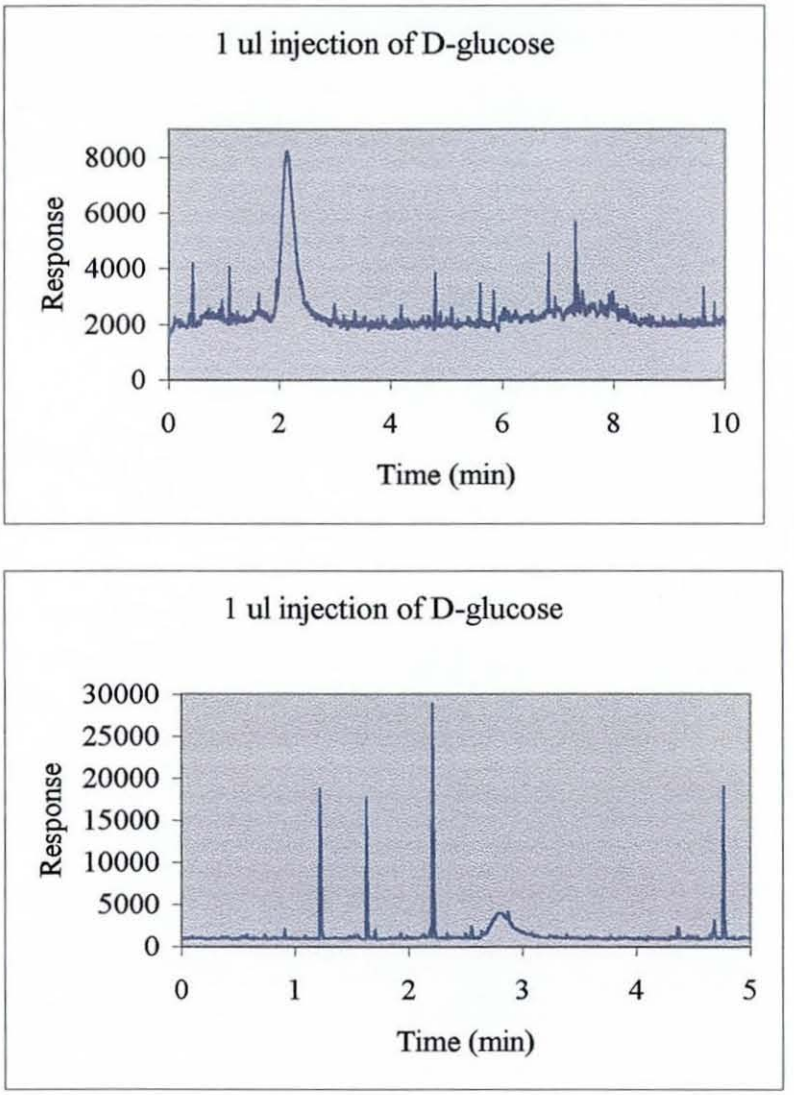


Figure 3.12 Chromatograms of 1 µl injections of D-Glucose taken in succession, detector temperature 420°C, water flow rate 0.16 ml/min, Hypercarb PGC and GC oven temperature 90°C.

To check as to whether the peak was D-glucose or carry over, a 1 µl injection of water was made (Figure 3.13)

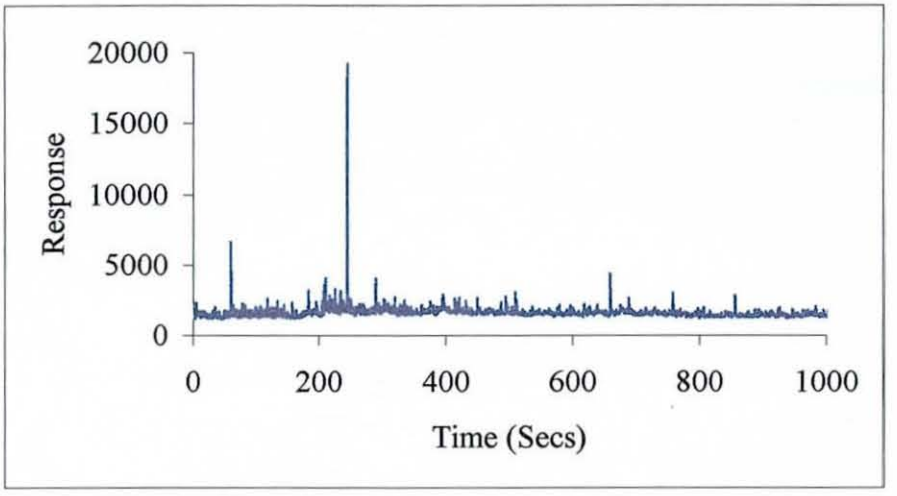


Figure 3.13 1 µl injection of water detector temperature 420°C, water flow rate 0.16 ml/min, Hypercarb PGC 2.1 x 150 and GC oven temperature 90°C.

As it can be seen from the chromatogram, the baseline noise has increased significantly, with regular noise and "spiking" along the baseline was now a problem. The regular baseline noise was found to be due to the presence of an air bubble in the pump head. Once removed, the baseline noise was significantly reduced.

As the sorbent used is carbon based, to determine as to whether the spiking was due to carbon bleed or the configuration of the system, injections were made both with and without the analytical column.

Upon injection of 1 µl injection of 1% D-Glucose in flow injection mode, no peak was found to elute. Upon investigation it was found that the nitrogen flow rate had doubled to 225 ml/min. Therefore an increase in baseline noise was attributed to an increase in nitrogen flow rate which could dilute the hydrogen and cause flame instability, by

altering the air:fuel ratio. When the nitrogen flow rate was reset, a repeat injection of D-Glucose was made, again no response was observed for D-glucose. To determine as to whether the lack of response was due to the analytical column or the system, the column was removed and the system linked as for FIA. As the system was very responsive to methanol, 1µl was injected in to the system, the result of which was a much lower than expected response (Figure 3.14)

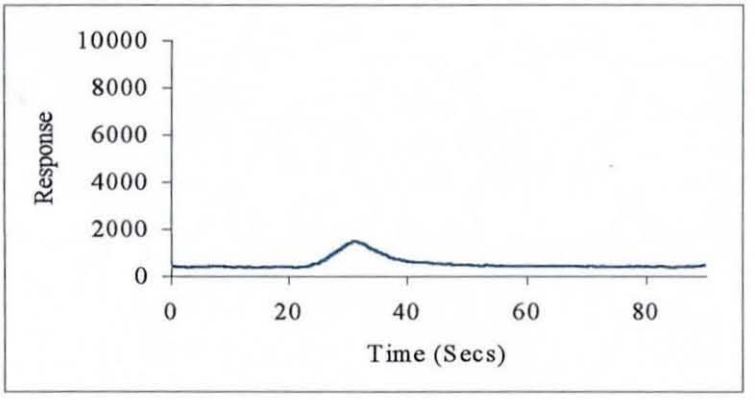


Figure 3.14 FIA trace of a 1µl injection methanol (conditions as Fig 3.12)

From this information, it appeared that the analyte was still not being completely transported to the flame. To try to overcome this problem, the flow rate of nitrogen was gradually increased, but unfortunately, no increase in sensitivity was evident with an increase in carrier gas flow rate.

A further injection of D-glucose was made, in flow injection mode, without any make-up gas, this time a peak for D-glucose was apparent and a reduction in background noise and spiking was noted. As steam is a more aggressive solvent than superheated water and to ensure that the analytes were being eluted by superheated water and not steam, a different configuration of interface was tried (Figure 3.15).

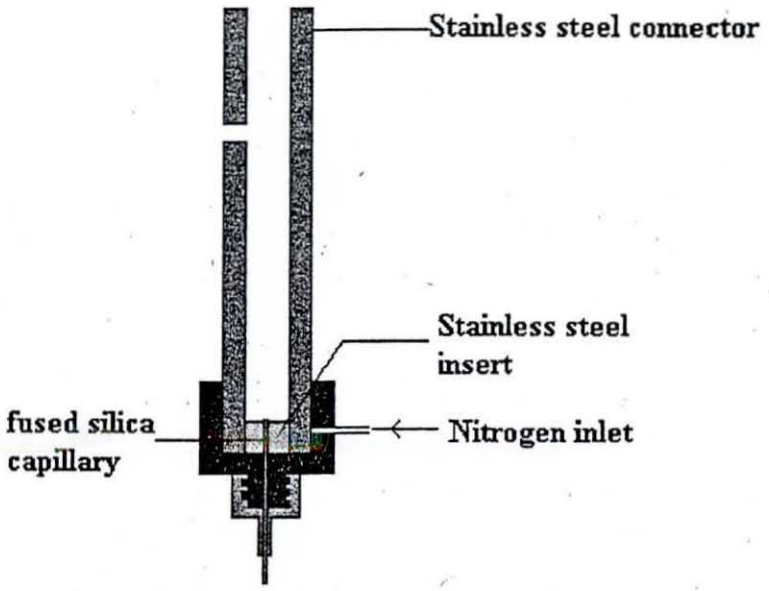


Figure 3.15 Schematic diagram of LC-FID interface using water at ambient

Using this configuration injections of non-volatile D-glucose and volatile benzaldehyde were undertaken (refer to figures 3.16 & 3.17), to assess the response of the detector.

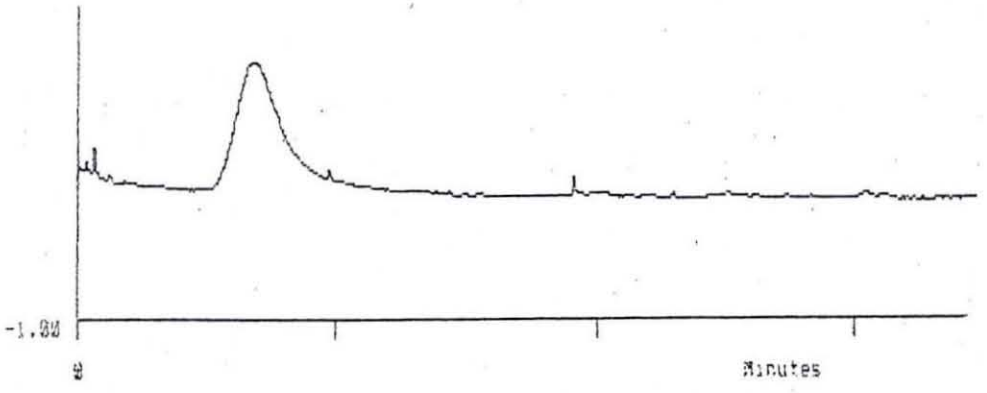


Figure 3.16 1µl injection of D-glucose, hydrogen 100 ml/min, air 240 ml/min, nitrogen 50 ml/min, water flow rate 0.1 ml/min, detector temperature 420°C and GC oven temperature 190°C

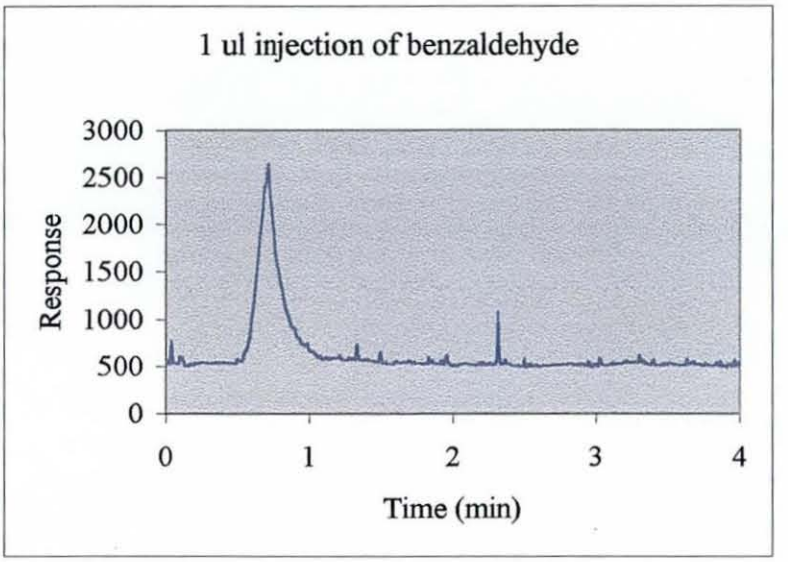


Figure 3.17 1μl injection of 0.1% w/v benzaldehyde, hydrogen 100 ml/min. air 240 ml/min, nitrogen 50 ml/min, water flow rate 0.1 ml/min, detector temperature 420°C and GC oven temperature 190°C.

A threefold increase in sensitivity, in terms of peak area of D-glucose, was achieved using this configuration, also a dramatic reduction in baseline noise, spiking had all but been eliminated as was the frequent blocking of the capillary. However, whilst this configuration lowered the frequency of capillary blockage and a reduction in the baseline noise, it did not wholly overcome these problems. Therefore to totally overcome these problems, an alternative to the thermospray interface needed to be developed, to provide a robust LC-FID.

3.6 Summary

The linkage between superheated water LC and FID using the interface as initially described Miller and Hawthorne has proven to be problematic, due to frequent capillary blockages. The interface has been found to be irreproducible and non-robust. The use of mobile phase modifiers, such as ammonium hydroxide have proven to be incompatible with the system. This had also been observed by Ingelse et al [136], who reported that

subcritical water with FID detection offered an alternative and more sensitive universal detector compared to the refractive index detector. They examined the use of non-FID responding additives, such as formic acid, trifluoroacetic acid and ammonia, and found that the stability of the FID signal was strongly reduced by concentrations of less than 10 mM. They also observed a dramatic increase in the incidence of plugging of the restrictor, especially where buffered solutions, such as ammonia and formic acid were used.

The problems, such as spiking, capillary blockage and increase in background noise have been attributed to the heating of the fused silica capillary and stripping of the polyimide coating. When the analyte was introduced into the capillary at ambient temperatures higher sensitivity and lower background noise was achievable, with a dramatic lowering of the frequency of blockage.

However, whilst this configuration lowered the frequency of capillary blockage and a reduction in the baseline noise, it did not wholly overcome these problems. Therefore to totally overcome these problems, an alternative to the thermospray interface needed to be developed, to provide a robust LC-FID.

Chapter 4

Development and testing of new LC-FID system

4.1 Introduction to microconcentric nebuliser interface

In order to overcome the problems found when using the thermospray type interface linkage between LC and FID, in particular the frequent transport capillary blockage, non-reproducible signals and poor flame stability, it became apparent that an alternative approach to sample introduction to the flame was necessary. This new technique was then optimised by adjusting in turn the construction and operational variables.

Pneumatic nebulisers are routinely used in inductively coupled plasma atomic emission spectrometry (ICP-AES) to introduce the sample solution to the plasma.[154]. The sample introduction system typically consists of a pneumatic nebuliser (such as a microconcentric nebuliser), spray chamber and an injector tube.

Nebulisation is based on the break up of a liquid stream by two processes. The first being the formation of a droplet from the jet of the nebuliser and secondly, the interaction with a high velocity gas jet [18]. The pressure drop observed due to the acceleration of the gas jet is sufficient to make it self feeding, and it is this feature of the nebuliser, along with its ease of use and simple construction, that has established its use in flame spectroscopy [155].

The nebuliser interface is also potentially attractive as an interface between the LC and FID, since it has demonstrated the ability to overcome problems such as irreproducible sample introduction, when using an aqueous media as a mobile phase. As there are several similarities that could be drawn from the problems associated with the linkage between the ICP to AES, a similar interface configuration could be constructed and linked between the LC and FID system. The system consists of a microconcentric

nebuliser which slots into a cyclonic spray chamber. The spray chamber is attached to the stainless steel connector, which allows the passage of the aerosol to the flame.

4.2 Microconcentric nebuliser

The microconcentric nebuliser used in this study has been demonstrated to be a highly efficient pneumatic nebuliser [156] designed for use with samples containing high levels of dissolved inorganic materials, without blocking, and high concentrations of acids [157].The body of the nebuliser is built of a completely inert material, therefore is ideal for use with acids, and can operate at low flow rates from 10 µl/min up to 1 ml/min [158]. This makes the nebuliser attractive for the flow rates required for LC. The nebuliser (Figure 4.1) consists of a nebuliser capillary (125-150 µm i.d.) made of either Teflon or polyimide and a sapphire orifice. It has been designed to be used with a carrier gas flow rate of 1.5 l/min and a back-pressure of 80 psi [159].
At liquid flow rates of 30 – 50 µl/min the nebuliser self aspirates and reported to produce[159] an aerosol with a mean droplet size of less than 5 µm (typically 3 – 4 µm) with a transport efficiency of 90% at 0.1 ml/min and 55% at 1.0 ml/min[159]. At higher flow rates, the transport efficiency is lowered due to the cooling of the plasma caused by the increase in aerosol load to the plasma. Droplets whose mean droplet size was greater than 5 µm were not transported from the nebuliser, instead they are thrown to the sides of the spray chamber and are eliminated as waste.

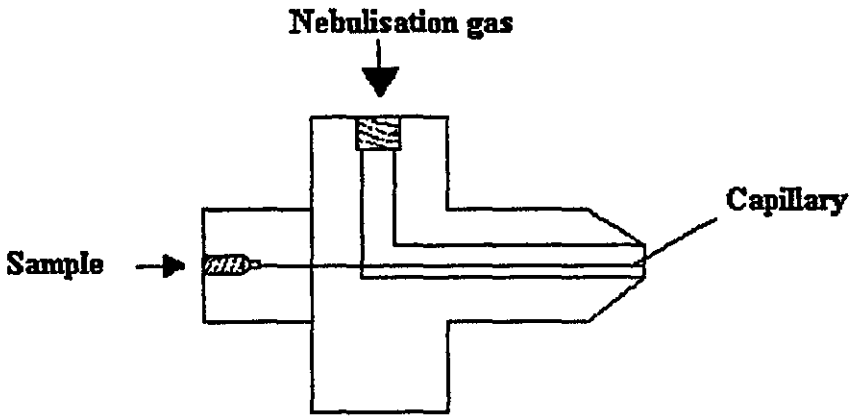


Figure 4.1 schematic diagram of MCN-100 nebuliser body

4.3 Cyclonic spray chamber

The cyclonic spray chamber, as used in ICP-AES or ICP-MS is used in conjunction with the pneumatic nebuliser to remove some of the larger droplets that are produced [155]. This has the effect of reducing the concentration of the aerosol and modifying the particle size distribution.

4.3.1 Mechanisms involved in the modification of droplet size distribution within the cyclonic spray chamber

As the aerosol droplets enter the spray chamber, those which are transported by the carrier gas near to the chamber wall meet a thin boundary layer of viscous stagnant gas,

of which there are three mechanisms by which the droplet can cross and deposit on the surface of the chamber [160].
The first mechanism occurs if the droplets radius is larger than the thickness of the boundary layer, so that contact occurs without further transport being necessary. The second mechanism occurs by the Brownian motion of particles whose internal diameters are <1μm, therefore diffusing through the boundary layer to the wall. The final mechanism occurs when the internal diameter of the droplet is greater than 1μm. Under these conditions, the droplet moves towards the boundary layer with sufficient inertia for it to undergo free flight through the layer to deposit onto the wall of the chamber. These mechanisms acting together allow a select cut of the mean particle size distribution of the droplets to enter into the flame.

A cyclonic chamber (Figure 4.2), as described by Taylor [161] was used in the experiment. The chamber was made of glass with a flattened wheel shaped with a drain at its base and an orifice for the horizontal insertion of the MCN. The spray from the aerosol was directed horizontally where it experienced centrifugal force, which removed the larger droplets from the aerosol. On one side of the chamber a dimple was pressed into the centre of the chamber, which generated turbulence and caused the larger droplets to collide with the dimple and outer wall of the chamber, by disrupting the circulating flow of the aerosol
A further dimple was placed underneath the orifice for the MCN, which acted as a flow spoiler. This feature was designed to prevent the recirculation of the aerosol, which would lead to band broadening by increasing the effective dead volume of the spray chamber.

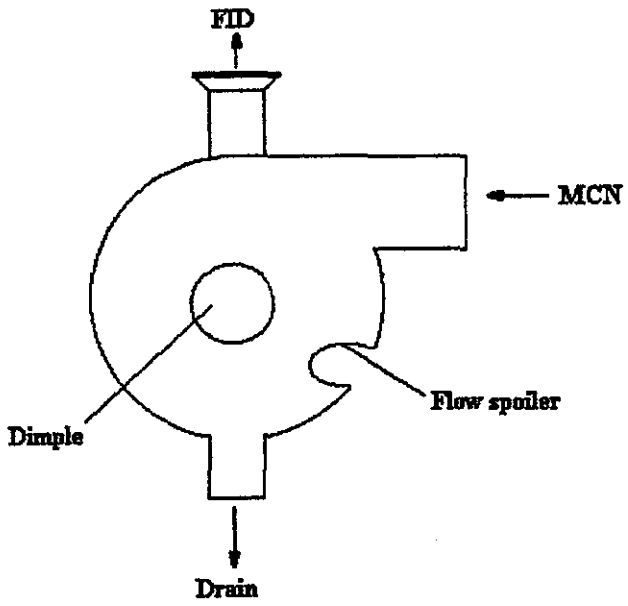


Figure 4.2 Schematic diagram of the cyclonic spray chamber

4.4 Linkage of LC to the FID through a nebuliser

As part of the redesign of the system, the FID construction was examined to handle higher gas and water flow rates. The jet and the collector of a FID form a pair of electrodes above and below the flame, of which the collector is generally selected as the anode and the jet as the cathode. The jet is supplied with a polarising voltage (typically 50-170V) and the resulting current across the flame is amplified and measured [162]. For this reason the jet is normally constructed of stainless, platinum, platinum-iridium, quartz [163] or any suitable inert, heat resistant material. However, the sensitivity of the FID with a quartz jet is lower in comparison to the sensitivity achievable when fitted with a stainless steel jet, due to the poor electrical and heat conduction, low heat capacity and hence low ionisation efficiency [163]. The internal diameter of the jet is also important, since it can affect the sensitivity of the FID. For example, a flame jet of internal diameter 0.025 cm is reported to give double the sensitivity to a tip of internal diameter 0.05 cm [163].

4.4.1 Changes made to the FID prior to linkage to LC

In the present study, a major change made to the FID, was to replace the existing standard jet with a 33 mm length of 2 mm i.d. alumina tubing, to allow the free flow of the gas through the system. The reason for this was to prevent the gas flow from convoluting and causing a turbulent flame [164]. Although this jet was not expected to achieve the sensitivity as a stainless steel jet, it is reasonable to expect that its replacement by a stainless steel jet will increase the sensitivity of the system. Other increases in sensitivity could be achieved by the optimisation of the internal diameter of the jet. But due to time constraints of the project and lack of suitable resources, it was not possible to study alternatives materials or to vary the internal diameter.

To remove the solution that built up in the spray chamber, a stainless steel hypodermic needle was glued at the base of the chamber to form a drain. Because the aqueous solution was held by surface tension a peristaltic pump was attached to the drain. The flow rate of the peristaltic pump was found to be crucial, since if it was too slow, the result was the eventual flooding of the spray chamber, or the appearance of spikes in the baseline of the chromatogram due to a "pressure drop" within the chamber as a droplet was slowly removed by the peristaltic pump.

The vertical side arm of the spray chamber was attached to the base of the Mark IV connector which was in turn connected to the FID through a standard glass ball and socket fitting, so that the connection was readily demountable.

4.4.2 Optimisation of LC-FID linkage using Flow Injection Analysis (FIA)

The system was first tested in a direct inlet FIA mode by connecting an injector to the nebuliser using a 30 cm length of 0.13 mm PEEK tubing.

The system then was optimised, by using different carrier/nebulising gases and flow rates, altering the distance travelled by the aerosol up the glass side arm of the spray chamber to the FID and altering the respective hydrogen, air, nitrogen and water flow rates. As there was no requirement to evaporate the mobile phase from the analyte prior to analysis, the interface was housed in a GC oven at 30°C to stabilise the temperature conditions of the interface.

The system was initially optimised using flow injection analysis (FIA) to quickly be able to assess the optimum sensitivity obtained from the system and its response reproducibility

4.4.3 Use of air as a nebuliser gas

The initial conditions for the system were a hydrogen flow rate 130 ml/min, air 210 ml/min, detector temperature 150°C, a water flow rate of 0.1 ml/min and GC oven temperature of 30°C. with the sample being introduced directly into the nebuliser through an FIA type connection. The air was introduced into the system as the carrier gas for the nebuliser, therefore inverting (premixing) the traditional hydrogen/air flame. With the new interface, an immediate improvement with the signal to noise ratio was observed, compared to the thermospray type interface but although detection of D-glucose was achievable, even when gas flow rates and water flow rates were adjusted to try to optimise the sensitivity, it was still very low (refer to Figure 4.3).

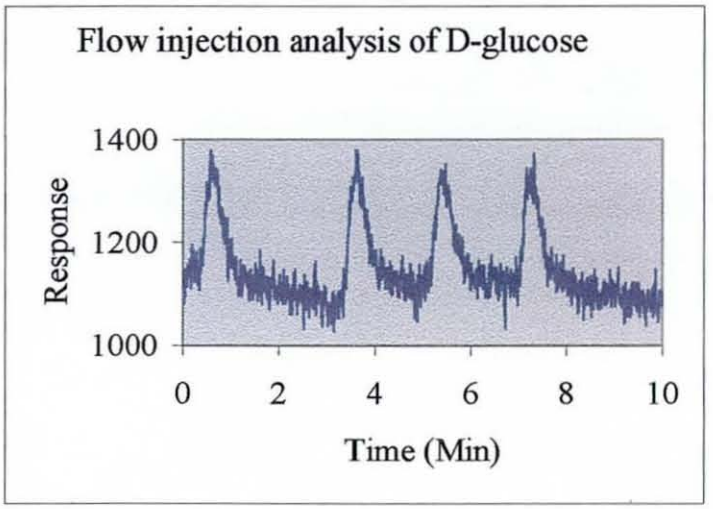


Figure 4.3 FIA trace of four 10µl injections of 0.75% w/v D-glucose

4.4.4 Nitrogen as a nebuliser gas

Because of the effect of using air as a nebulising gas seemed to result in a poor response, nitrogen replaced air and the air was re-connected to its conventional inlet of the FID, externally to the hydrogen flame. The result was an increase in the response of the detector to D-glucose(refer to Figure 4.4)

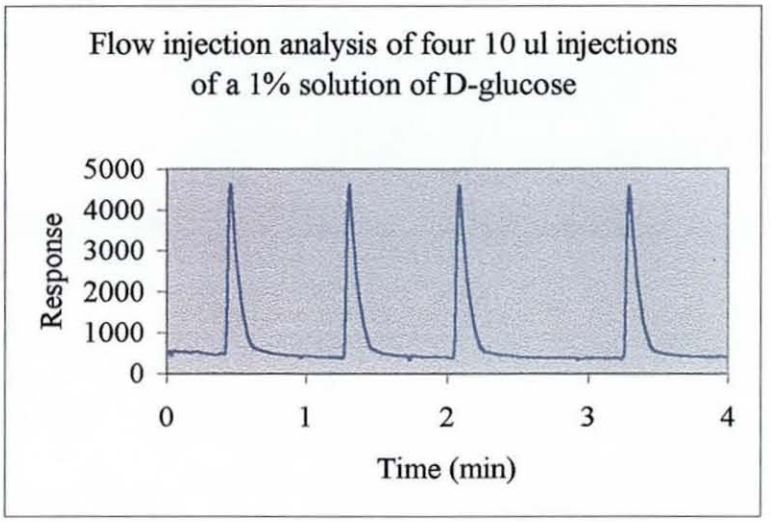


Figure 4.4 FIA trace of four 10μl injection of a 1% solution of D-glucose

4.4.5 Effect of preheating the mobile phase prior to injection

In an attempt to try to sharpen the shape of the glucose peaks, a 1 m length of 0.02 inch stainless steel tubing was connected between the analytical (FIA) pump and injector. The tubing was then placed in a GC oven and heated to 60°C, to preheat the mobile phase prior to injection. However, the result was a 45% reduction in response. Therefore the preheating coil was removed.

4.4.6 Effect of distance travelled by the aerosol droplets to the FID

It was found that the response of the detector appeared to be affected by the distance the aerosol droplets had to travel to the detector from the spray chamber. Every time a small change was made to the connection distance, the gas flow rates, detector temperatures and water flow rates had to be re-optimised to achieve the best response from the detector.

Initial experiments were carried out using a glass socket joint of length 38mm to connect the spray chamber to the Mark IV stainless steel connector. Preliminary conditions for the detector were hydrogen, air and nitrogen flow rates of 120, 300 and 520 ml/min, GC oven temperature 28°C, FID detector temperature 230°C and flow rates of 0.7 and 0.9 were investigated.

A series of 10, 5, 2 and 1µl injections of a 10 ug/ml solution of D-glucose were made to assess the linearity of the detector

The results were compared to those of a glass socket joint 20 mm in length (figure 4.5).

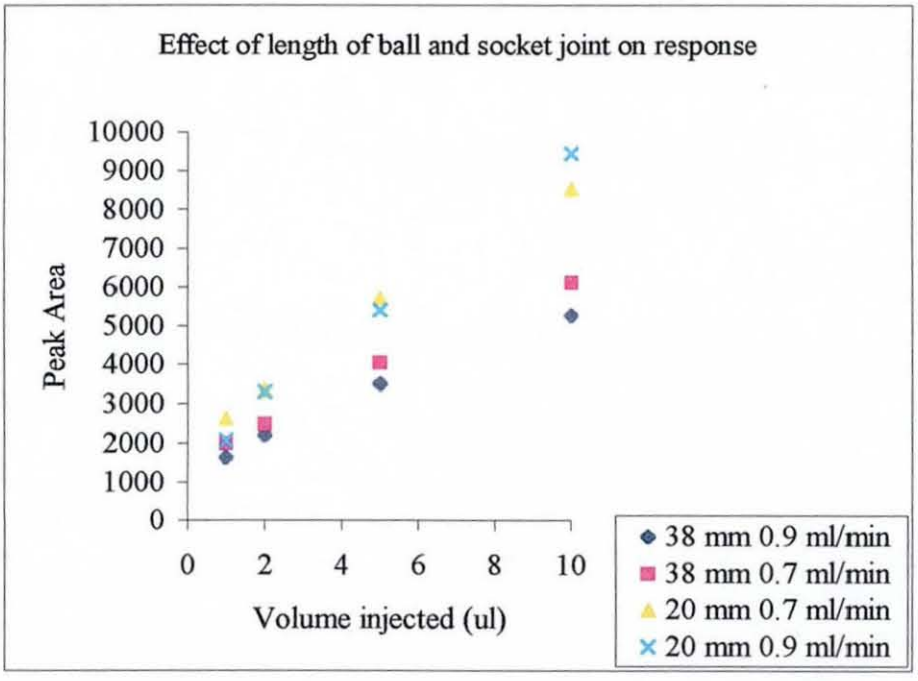


Figure 4.5 Effect of the length of the ball and socket joint on response of varying injection volumes of 10 ug/ml D-glucose. Conditions for the detector were hydrogen, air and nitrogen flow rates of 120, 300 and 520 ml/min, GC oven temperature 28°C, FID detector temperature 230°C

As it can be seen from Figure 4.5 the response from the detector in flow injection mode, is linear r>0.995 and a significant increase in response (up to 80 %) was achieved by using shorter the 20 mm long socket connector.

Unfortunately, the connection between the spray chamber and the mark 4 stainless steel connector was found to be leaking nitrogen. To remedy this the whole socket joint was encased in a polypropylene tube and each end was tied tightly with copper wire. The joins were then tested for leakage before use on a daily basis, using a solution of "Snoop". To compensate for the loss of nitrogen from the joint, all gas flow rates were adjusted, especially the nitrogen gas flow rate, and optimised. The ball and socket joint was eventually removed and replaced with a length of glass tubing, of the same internal diameter as the stainless steel connector, to keep the distance of the spray chamber and stainless steel connector constant. Again, the whole joint was encased in a polypropylene tube that was tied at both ends, using copper wire. Each join was tested for nitrogen leakage, using "Snoop", prior to analysis.
As the previous nitrogen gas flow rates did not take into account the leakage from the ball and socket joint, the gas flow rates and the temperature of the detector were re-optimised to obtained the best response from the system.

4.4.7 Optimisation of the nitrogen flow rate

A series of injections of varying concentrations of D-glucose were performed using different nitrogen flow rates with a hydrogen flow rate of 120 ml/min, air 300 ml/min and detector temperature of 230°C (Figure 4.6). The results show that a nitrogen flow rate of 430 ml/min gave the best response from the system. Under these conditions a limit of quantification of 59 ng on-column was achievable in flow injection mode.

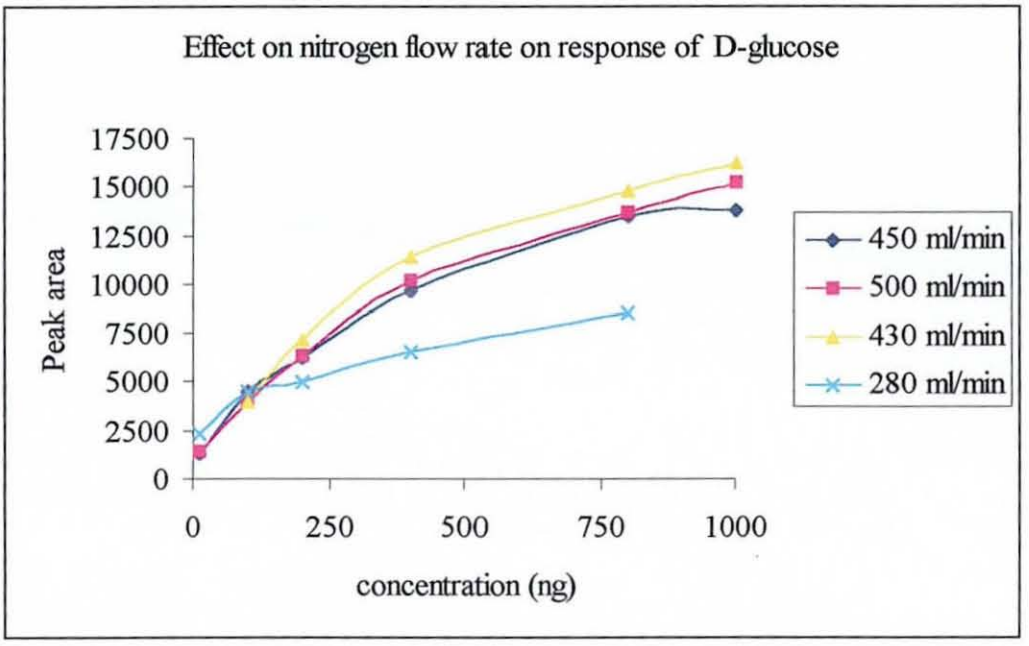


Figure 4.6 Effect of the flow of nitrogen on the response of the FID using varying concentrations of D-glucose. Hydrogen and air gas flow rates 120 and 300 respectively, detector temperature 230°C

It is apparent from Figure 4.6 that the response from the FID was non-linear. This could be attributed to a change in the flame characteristics, from a pure hydrogen/air flame to a hydrocarbon like flame [164], due to the increase in concentration of the analyte of interest. The change in flame characteristics and flame size cause the production of ions to take place higher up in the collector, where the flame is cooler and the electric field is weaker therefore the formation and collection of ions is less efficient, thus causing the response to be non-linear [164].

4.4.8 Re-optimisation of the detector temperature

It was found that at temperatures below 200°C, the flame of the FID became unstable when water flow rates between 0.7 – 0.9 ml/min were used. These water flow rates were chosen, as they were close to the flow rates typically required for reversed-phase liquid chromatography with conventional 4 - 4.6 mm i.d.columns.

Using these analytical parameters, optimum detector temperature lay between 230 - 260°C for D-glucose. The result of increasing the detector temperature above 260°C was a decrease in response.

4.5 Linkage of LC to FID, using various analytical columns

The next stage in the development of the LC-FID was to try to perform separations of mixtures using analytical columns of various dimensions, with water or aqueous buffer solutions as the mobile phase. As with the optimisation of the system when using FIA, various parameters such as column, detector and nebuliser temperature, effect of preheating the mobile phase and the optimisation of gas flow rates were investigated. The initial work used separation at temperature below 100 °C so that no back pressure was needed.

4.5.1 Separation of carbohydrates using PL HiPlex H column

The initial column used for the separation of glucose, maltose and glycerol was the PL HiPlex cation exchange column in the hydrogen form. The sorbent is an 8 % crosslinked polystyrene divinyl benzene, which is typically used with water as the mobile phase and the column is usually heated to 65°C to achieve good resolution of a range of sugars and sugar alcohols.

4.5.1.1 Effect of column temperature

Because the body of the MCN-100 was fabricated from polypropylene, the temperature of the GC oven used for the detector, had to be kept below to 65°C. Therefore the oven could not be heated to the temperatures required to produced superheated water or even hot water.

To obtain hot water, at temperatures suitable for the usage with cation exchange columns, the column was placed inside a separate column heater and a length of 45 cm of 0.13 mm i.d. PEEK tubing was used to connect the outlet of the column to the nebuliser.

A 10 µl injection of a mixture of 100 ug/ml solution of D-glucose, maltose and glycerol was injected onto the column, which was thermostated at 65°C. All three analytes were detected (Figure 4.7) but the baseline was noisy, with negative peaks before the peak of interest.

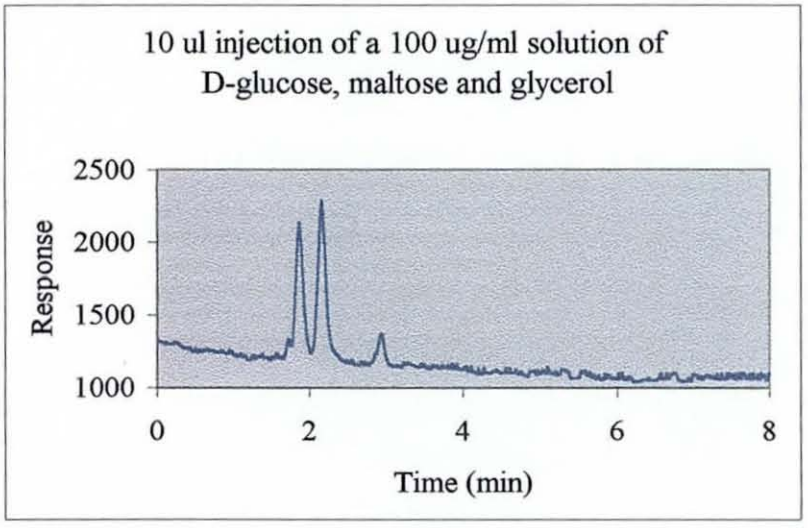


Figure 4.7 Chromatogram demonstrating the separation of glucose, maltose and glycerol using a water flow rate of 0.6 ml/min, detector temperature 230°C, HiPlex H column, GC oven temperature 30°C and hydrogen, air and nitrogen flow rates of 110, 315 and 420 ml/min respectively.

The noisy baseline and negative peaks were eliminated by reducing the temperature of the column oven to 45 °C and reducing the water flow rate to 0.6 ml/min. The identification of the peaks was made by injecting solutions of the three separate analytes.

4.5.1.2 Determination of the effect of cooling of the mobile phase, post separation

As the response of the analytes appeared to be favoured by nebulisation at lower temperatures, the column effluent was cooled prior to nebulisation. This was achieved by replacing the 45 cm of 0.13 mm i.d. PEEK tubing by 126 cm of 0.13 mm i.d. PEEK tubing. The tubing was coiled and placed in an "ice trap", which consisted of a polypropylene box packed with a mixture of ice, salt and water.

The result was a 50% reduction in the response of maltose and glucose, and a 90 % increase in the response of glycerol.

As the cooling of the column effluent had a dramatic but varied effect on the response of each analyte, to try to obtain a general improvement, the column temperature was further lowered to 35°C, the effect of which was an increase in response of all three analytes (Figure 4.8).

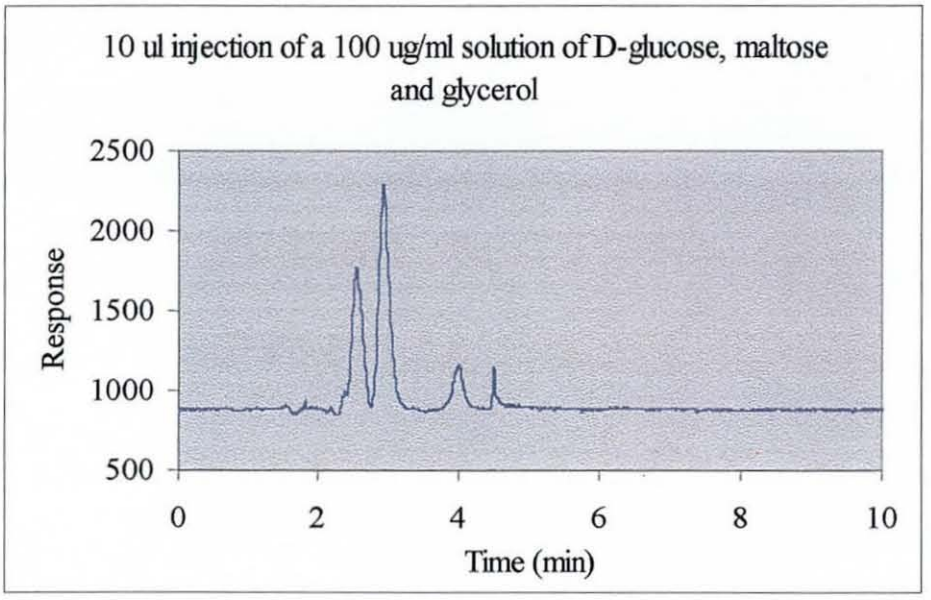


Figure 4.8 Chromatogram of a 10 µl injection of a 100 ug/ml solution of D-glucose, maltose and glycerol. Detector temperature 230°C, column temperature 35°C, water flow rate 0.5 ml/min.

Using the above conditions a range of sugars were injected on to the column and their respective retention times were recorded (Table 4.1) to enable a suitable mixture for further test separations to be selected.

Table 4.1 Retention times of various sugars using PL HiPlex H column

Sugar name	Retention Time (min)
Arabinose	3.4
Fructose	3.2
Fucose	3.5
Glucose	3.0
Glycerol	3.2
Maltose	2.6
Mannose	3.1
Rhamnose	3.2
Xylose	3.1

From Table 4.1, the only other separation for which baseline resolution would be achievable was that of maltose, glucose and arabinose (or fucose). A set of calibration standards were prepared and the response of each standard was measured (Figure 4.09).

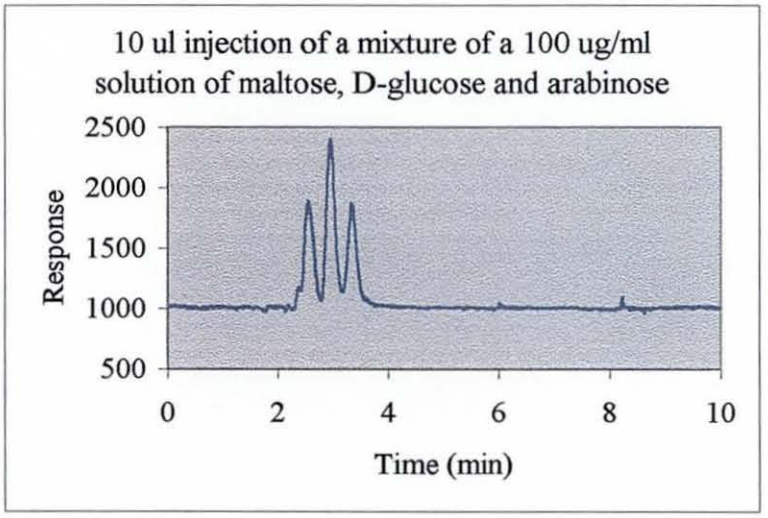


Figure 4.09 Separation of maltose, glucose and arabinose. Conditions as Figure 4.08

4.5.1.3 Effect of temperature of the nebuliser

The temperature of the GC oven, containing the interface was investigated between the temperature range of 35 -65°C. The effect of which was that temperatures above 40°C resulted in a decrease in detector response, was observed for all three analytes. Therefore, subsequent experiments were carried out with the GC oven set to 35°C.

4.5.1.4 Effect of increasing analyte concentration

Nitrogen flow rate was re-set to 420 ml/min to obtain optimum response from all three analytes.

A stock standard containing 100 ug/ml of maltose, glucose and arabinose was prepared in water and serial dilutions were made to prepare a concentration range from 0.1 – 40 ug/ml of all three analytes.

To assess the lowest concentration which could be detected (Figure 4.10), 10 µl injections of each standard was injected, of each concentration.

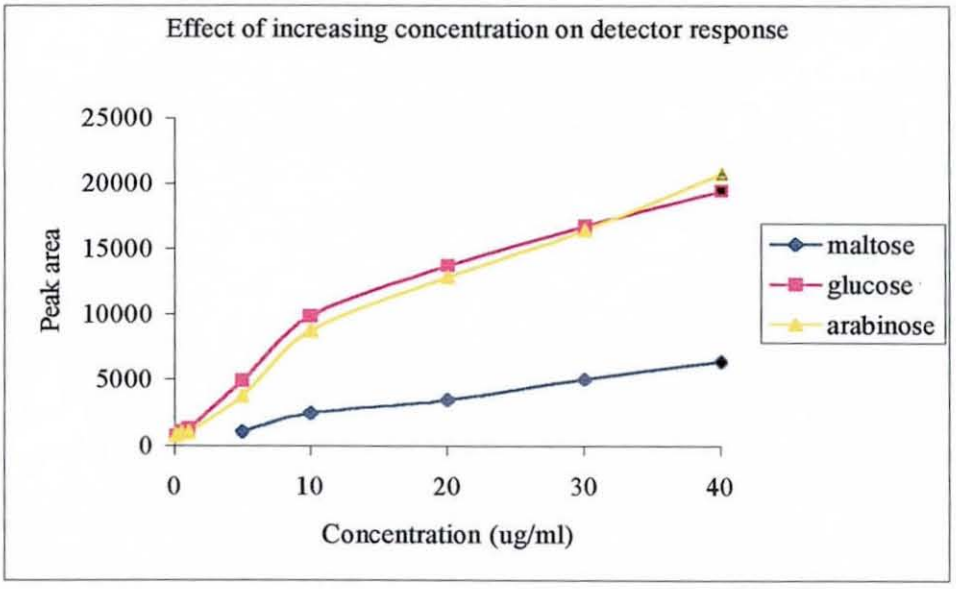


Figure 4.10 Effect of increasing concentration on detector response

As seen previously, the trend of increasing concentration is not wholly linear over the concentration range. Concentrations below 10 ug/ml cause a bias in the response, but the response is linear. Above 10 ug/ml the response appears to be linear. This could reiterate the problems with the FID in terms of the changes in the flame when constant volumes at varying concentrations are analysed [164].

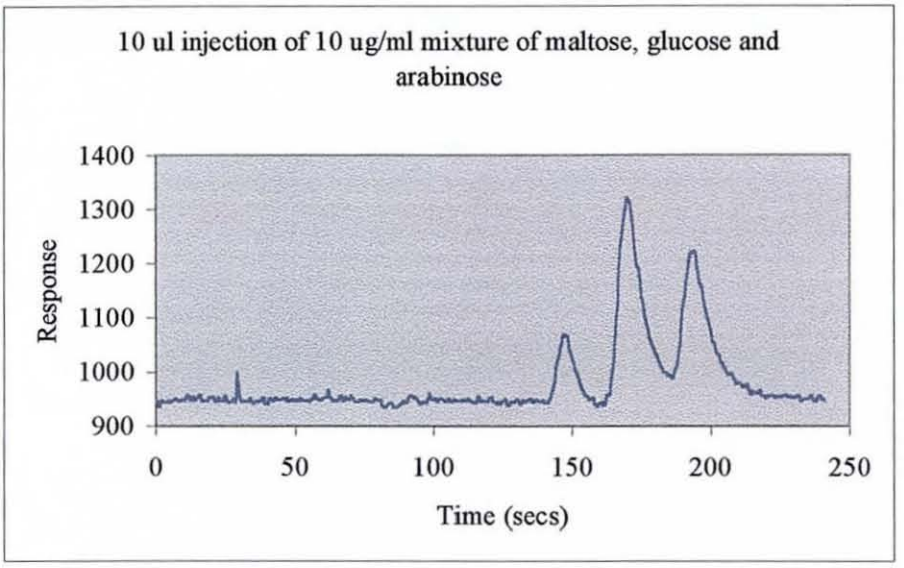


Figure 4.12 Chromatogram demonstrating separation of maltose, glucose and arabinose using HiPlex H column water flow rate 0.5 ml/min, detector temperature 230°C, GC oven 35°C, hydrogen, air and nitrogen gas flow rates 110, 315 and 420 ml/min

4.5.2 Separation of various carbohydrates using PL HiPlex Ca Column

To try to increase the scope of analytes which could be separated and detected, the HiPlex H column was exchanged for a HiPlex Ca column, which is an 8 % crosslinked polystyrene divinyl benzene cation exchange column in the calcium form. This sorbent is recommended for the separation of sugar alcohols (alditols), monosaccharides and oligosaccharides [149]. A test mixture was therefore prepared containing, therefore 10 µl of a 100 ug/ml each of sucrose, glucose, fructose, mannitol and sorbitol

This was injected under the conditions as described by Polymer Laboratories Ltd [165] which required the analytical column to be heated to a temperature of 75°C. The column effluent was cooled prior to nebulisation, due to the findings from the previous set of experiments by using a 1 metre length of 0.13 mm PEEK tubing to connect the column to the nebuliser.

The separation (Figure 4.12) was similar to that reported by Polymer Laboratories [165].

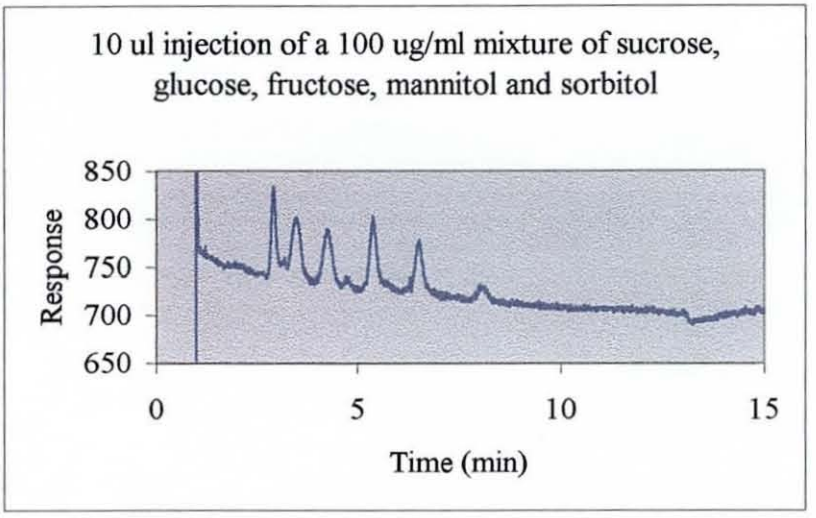


Figure 4.12 Typical chromatogram of a 10 µl injection of a 100 ug/ml mixture of sucrose, glucose, fructose, mannitol and sorbitol using PL HiPlex Ca column, water flow rate 0.45 ml/min, column temperature 75°C, detector temperature 230°C, GC oven temperature 35°C, hydrogen, air and nitrogen flow rates 110, 320 and 420 ml/min.

4.5.2.1 Effect of column temperature

If the temperature of the column was reduced by 15°C to 60°C, there was a 3-4 fold increase in the response of the analytes but the reason is not clear. A further reduction in temperature by 10°C to 50°C, and a reduction in the a flow rate of 0.3 ml/min to prevent a high back pressure on the column because of the higher eluent viscosity. This resulted in a further increase in the response of the analytes. However, the baseline resolution between sucrose and glucose was reduced due to the poor peak shape of glucose (Figure 4.13)

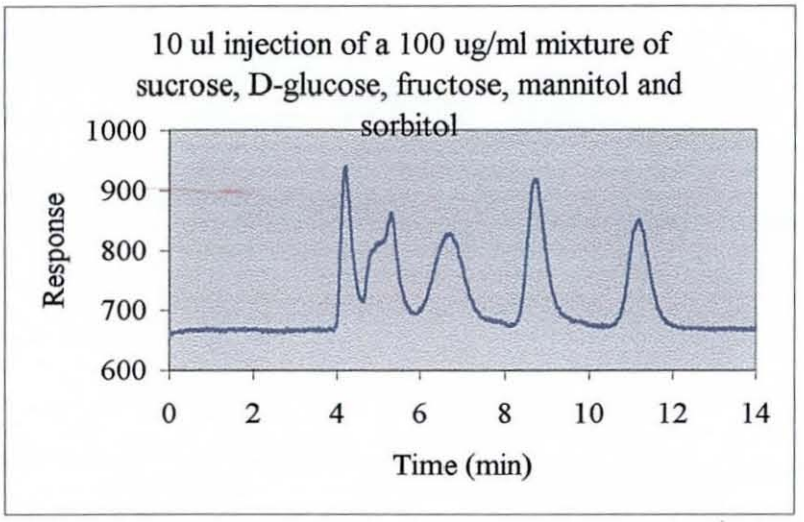


Figure 4.13 Chromatogram demonstrating the separation of sucrose, glucose, fructose, mannitol, and sorbitol, conditions as Figure 4.12 except PL HiPlex Ca column thermostatted to 50°C with a water flow rate of 0.3 ml/min.

To try to increase the response of the analytes further, the column temperature was decreased further to 40°C and the water flow rate was reduced 0.24 ml/min, to prevent any damage to the column through high back pressure within the column. As the glucose peak was most affected by a reduction in temperature, a new 100 ug/ml solution was prepared without glucose. The result of the further reduction in temperature was an increased response (Figure 4.14)

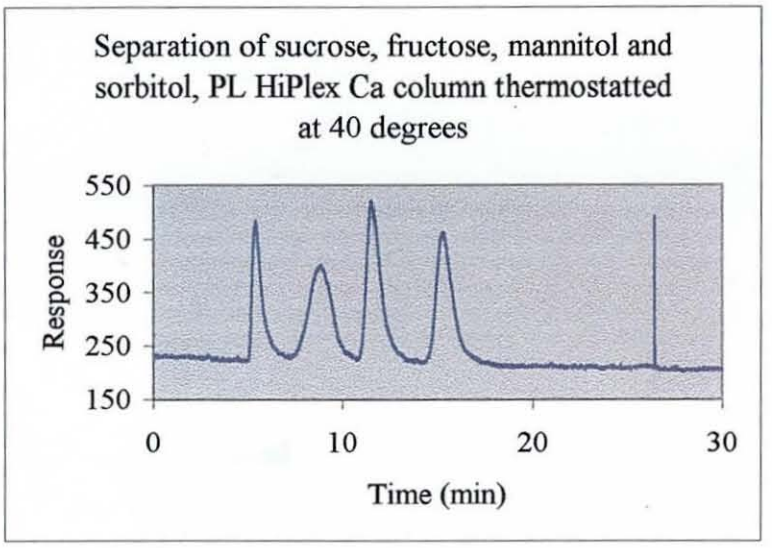


Figure 4.14 Chromatogram demonstrating the separation of sucrose, fructose, sorbitol and mannitol using PL HiPlex Ca column thermostatted at 40°C, water flow rate 0.24 ml/min.

4.5.3 Investigations into the peak shape of D-glucose at different column temperatures

Since it has been identified that an increase in the temperature of the water being nebulised, increased the baseline noise, a cooling water condenser (Figure 4.15) as described by Burgess [122] was placed between the column oven and the nebuliser. This consisted of 20 cm of 0.3 mm stainless steel tubing coiled tightly to increase the surface area available for contact with the circulating water that was thermostated at 35°C.

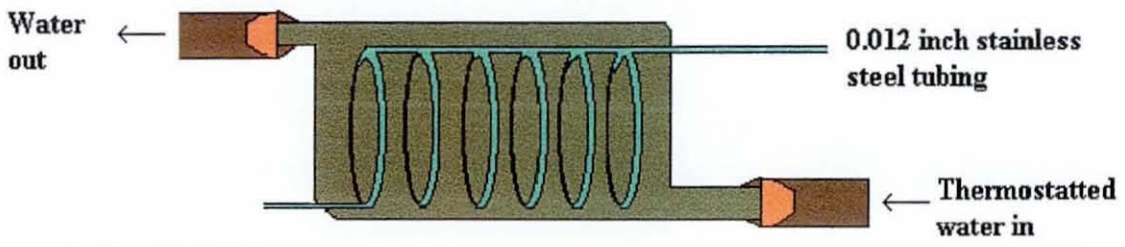


Figure 4.15 Schematic diagram of cooling water condenser

To try to assess the effect of the column temperature on the response and hence the linearity of glucose at various temperatures, a series of calibration standards of concentration range 50 – 250 ug/ml were prepared in water. Each standard was injected on to a PL HiPlex H column at temperatures in the range of 30 - 70°C (Figure 4.16)

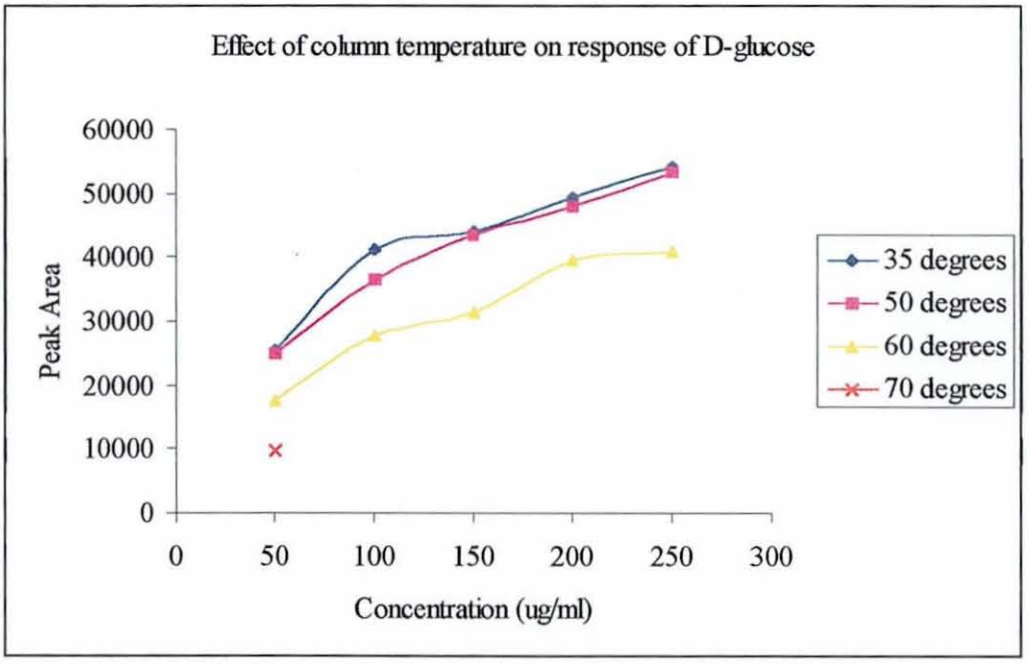


Figure 4.16 The effect of temperature on the response of D-glucose, using HiPlex H column (10 µl injections) using water as an eluent at 0.24 ml/min flow rate.

It is evident that an increase in the column temperature, decreases the response from the detector. .

4.5.4 Further investigations into the effect of temperature using PL HiPlex Ca column

Again, as in the previous experiment, on the effect of the column temperature between 40 and 80°C was investigated using D-glucose as the test analyte on PL HiPlex Ca column. This time, low temperatures of 40°C produced a split peak at all concentration levels but when the temperature of the column was raised a single peak was produced (Figure 4.17) .

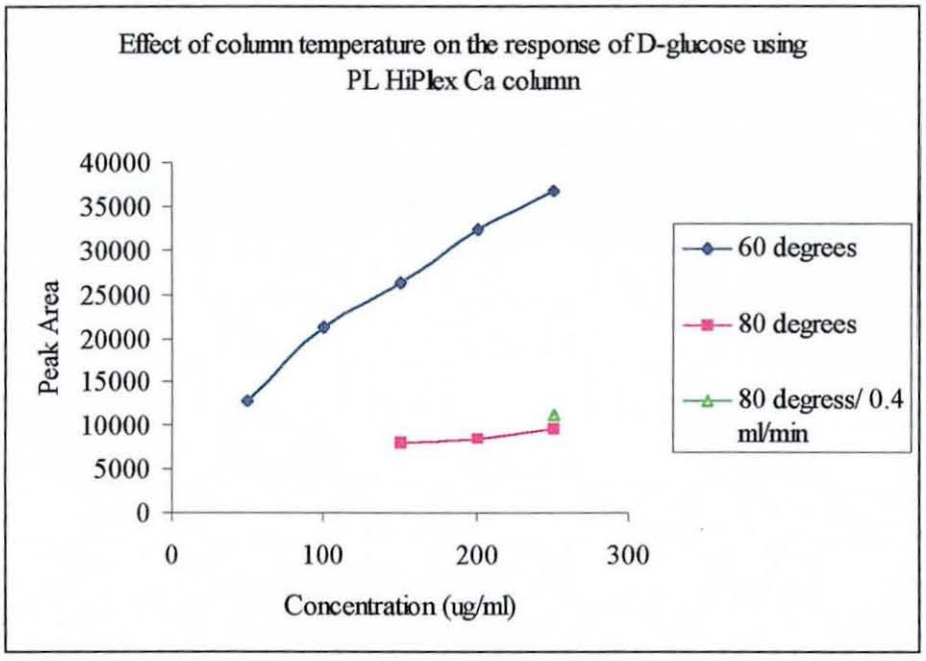


Figure 4.17 Effect of column temperature on the response of D-glucose using PL HiPlex Ca column The temperature range investigated was using a water flow rate of 0.24 ml/min

These experiments clearly demonstrated that whilst the two cation exchange columns required to be run at a high temperature to firstly to lower the eluent viscosity and thus reduce the backpressure to prevent column damage and secondly to obtain good resolution, this is clearly a compromise to the response of this detection system.

Further investigations were carried out into the peak shape of D-glucose using the HiPlex Ca column at 40°C, to try to determine as to whether the peak shape was due to the temperature of the cooling coil or the column. The temperatures of both the column and the cooling coil were reversed from the initial experiment, the column temperature was lowered to 40°C and the temperature of the water condenser was raised to 60°C.

The peak obtained was still of poor shape so the split resulted from the column rather than the temperature of the eluent entering the nebuliser (Figure 4.18) .

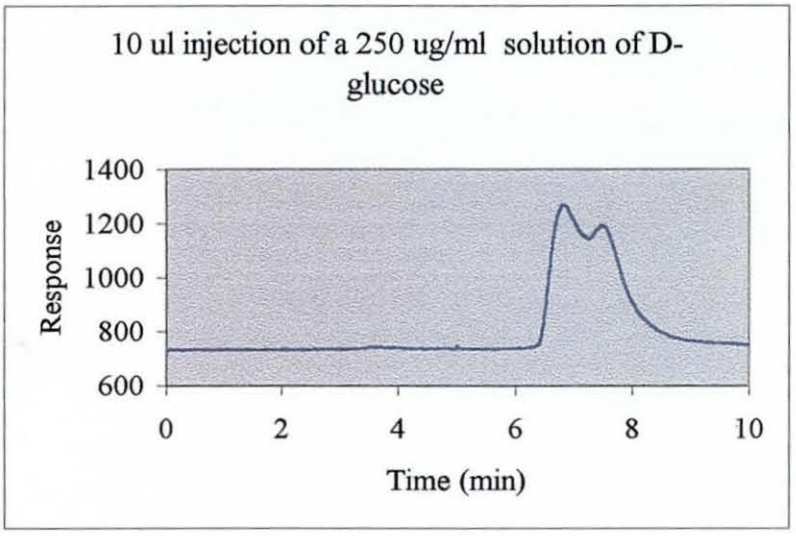


Figure 4.18 Typical chromatogram of a 10 µl injection of a 250 ug/ml solution of D-glucose. Column temperature 40°C and water condenser temperature 60°C.

To try to determine if the sample had deteriorated on standing, two further 250 ug/ml D-glucose standards were prepared at the same time and one was stored in ice prior to injection but the peak shapes were unaltered .A final experiment was to inject 10 µl of a 250 ug/ml solution of D-glucose immediately after dissolution in water.
This resulted in quite a different peak shape, and standing over time the peak shape that was obtained changed (Figures 4.19 – 4.21)

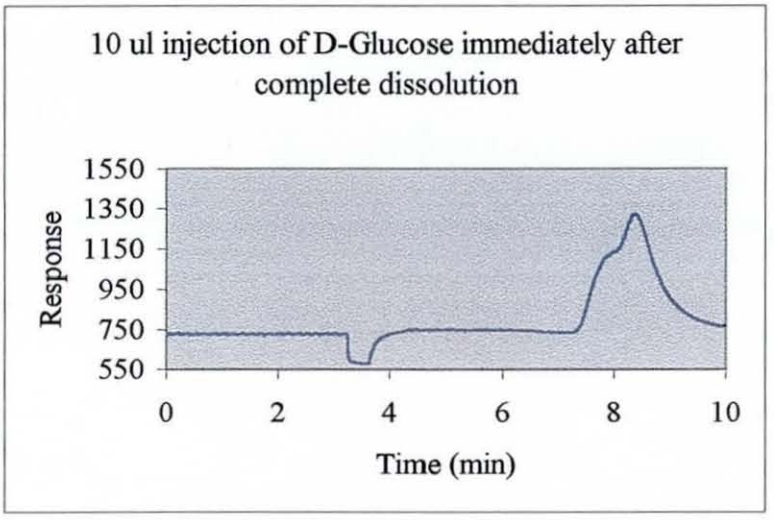


Figure 4.19 A 10 µl injection of D-glucose immediately after complete dissolution

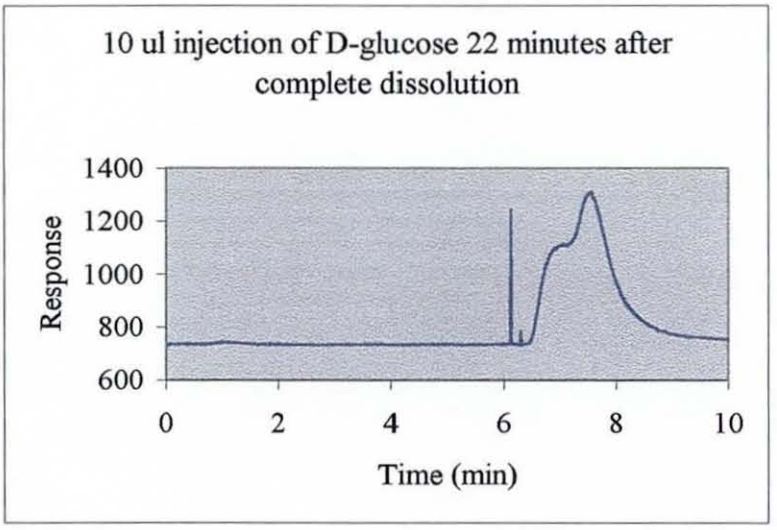


Figure 4.20 A 10 µl injection of D-glucose after 22 minutes elapsed

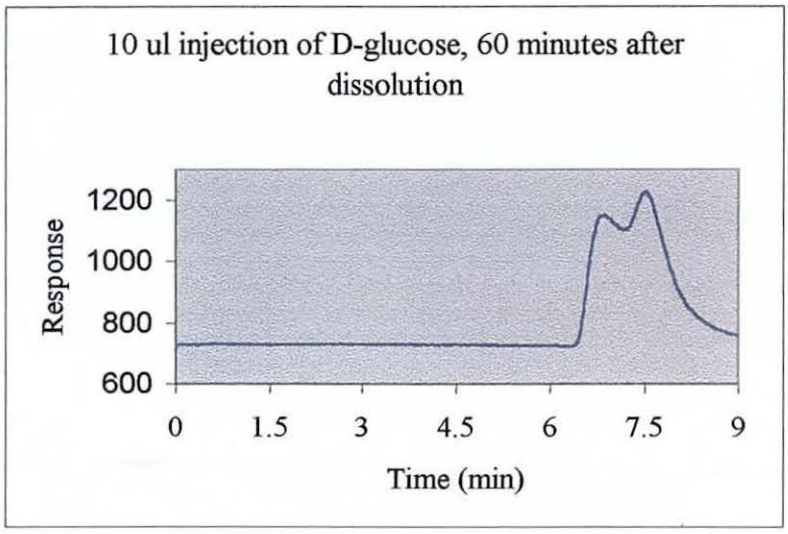
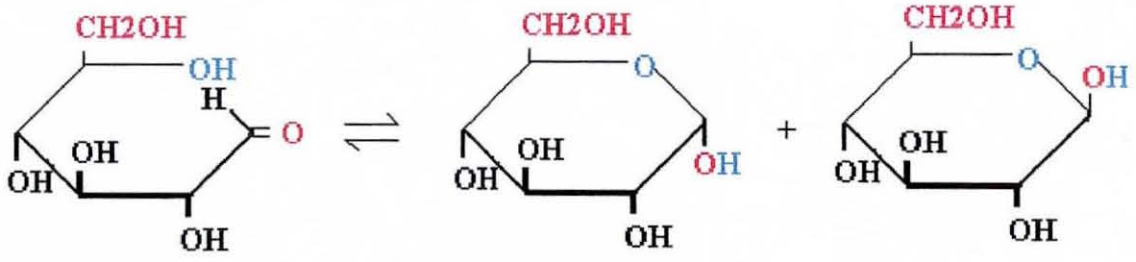


Figure 4.21 10 µl injection of D-glucose after 60 minutes elapsed

These peak shape changes can be explained by the ability of glucose to undergo mutarotation when in solution (Figure 4.22).



D-Glucose **α-D-Glucopyranose (36%)** **β-D-Glucopyranose (64%)**

Figure 4.22 Mutarotation of D-glucose in water

The D-glucose is converted into a mixture of the two anomers in solution. The anomeric carbon centres are formed by the reaction between C5 and the aldehyde group of the open chain structure, forming two hemiacetals whose configuration differs about C1 [166]. The two hemiacetals are diastereoisomers and are readily hydrolyzed by water and via the open chain configuration, into an equilibrium mixture usually a 36:64 mixture of the cyclic anomers [167].

Therefore it can be concluded that if the HiPlex Ca column was employed at temperatures below 40°C, separation of the α-D-glucopyranose and β-D-Glucopyranose would be possible. The first peak is that of the β-D-Glucopyranose and the second peak is that of the α-D-glucopyranose. At higher temperatures, interconversion of the anomers on the column is rapid and only one peak is observed

4.6 Separation of oligosaccharides

Oligosaccharides by definition are polysaccharides of low molecular weight (oligo meaning few units) [166]. Like monosaccharides, they can be separated by cation exchange columns, typically in the calcium form.
The oligosaccharides analysed were a mixture of maltotetraose (dp4), maltopentaose (dp5) and maltohexaose (dp6), whose molecular weights are 666.6, 990.9 and 1153.0 respectively.
Using the conditions set for D-glucose with the PL HiPlex Ca column, the retention times for dp4, dp5 and dp6 under these conditions were 4.76, 4.55 and 4.32, therefore making baseline resolution impossible.
The response given by all three analytes, at a concentration of 100 ug/ml, was half that of the response of a 100 ug/ml solution of D-glucose. To try to increase the response of the detector, the flow rate of air was varied, whilst keeping the other flow rates unchanged. As the response for all three analytes, at the same concentration was similar, all experiments were carried out using dp5.

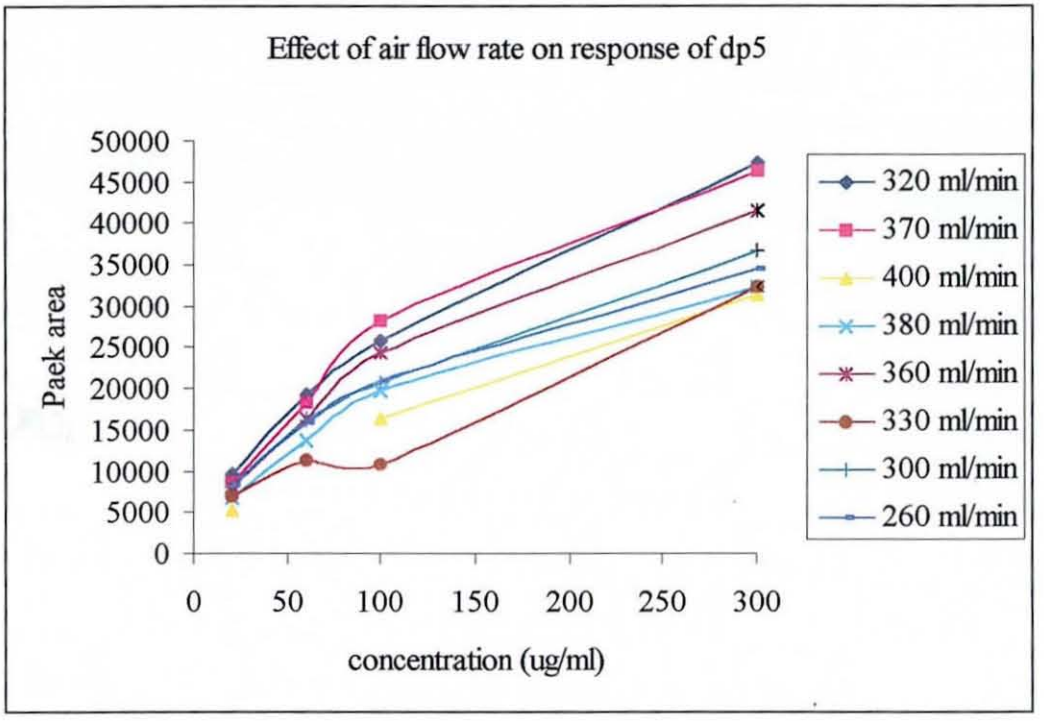


Figure 4.23 Effect of air flow rate on the response of dp5

From Figure 4.23 it is clear to see that the optimum air flow rate for higher molecular weight carbohydrates is 370 ml/min across the concentration range.

The flow rate of hydrogen was changed to 80 ml/min for which the effect was a 4% reduction in response at 60 ug/ml and a 20% reduction in response for 100 ug/ml. Therefore the flow rate of hydrogen was changed back to 110 ml/min. The nitrogen flow rate was also varied, but as previously observed, 420 ml/min proved to provide to optimum response from the detector.

4.6.1 Effect of increasing column oven temperature

To obtain the optimum baseline resolution from the cation exchange column, the column should be thermostatically controlled at 80°C. As previously, when the temperature of

the column is increased, the response of the detector decreases, and at 80°C the reduction in peak area was 80% compared to that at 40°C. The higher temperature gave no advantage in terms of baseline resolution, as demonstrated in figure 4.24 300 ug/ml mixture of dp4, dp5 and dp6 at 80°C

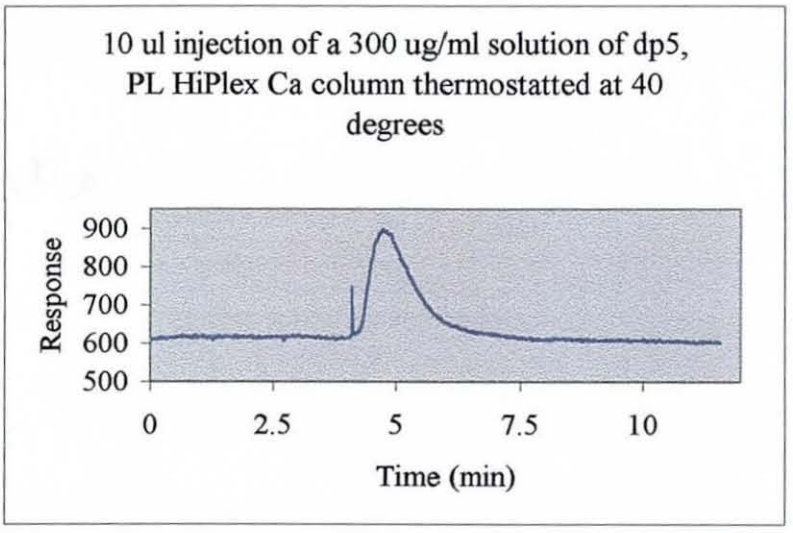


Figure 4.24 Chromatogram demonstrating 10 µl of a 300 ug/ml solution of dp5, column temperature 40°C. of PL HiPlex Ca column temperature 40°C, detector temperature 230°C, water flow rate 0.24 ml/min, nitrogen 420 ml/min, hydrogen 110 ml/min and air 240 ml/min.

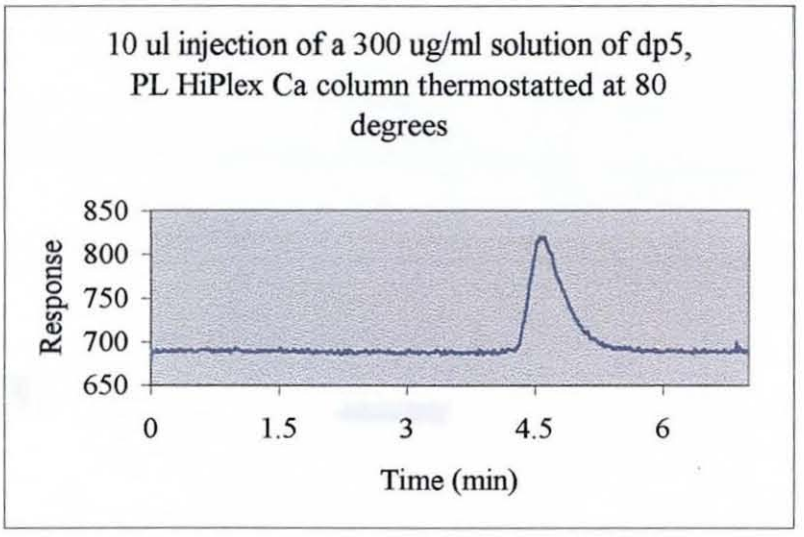


Figure 4.25 Chromatogram demonstrating 10 µl of a 300 ug/ml solution of dp5, column temperature 80°C conditions as previous

4.6.2 Effect of preheating the mobile phase

To try to minimise band broadening caused by any possible temperature gradient along the column, the mobile phase was preheated to 40°C, prior to entering the analytical column (which was thermostatically controlled at 40°C). A 1 metre length of coiled stainless steel tubing (0.020 inch) was attached between the pump and the injector, and placed into the GC oven, which was set to 40°C. The result of the experiment can seen in Figure 4.26.

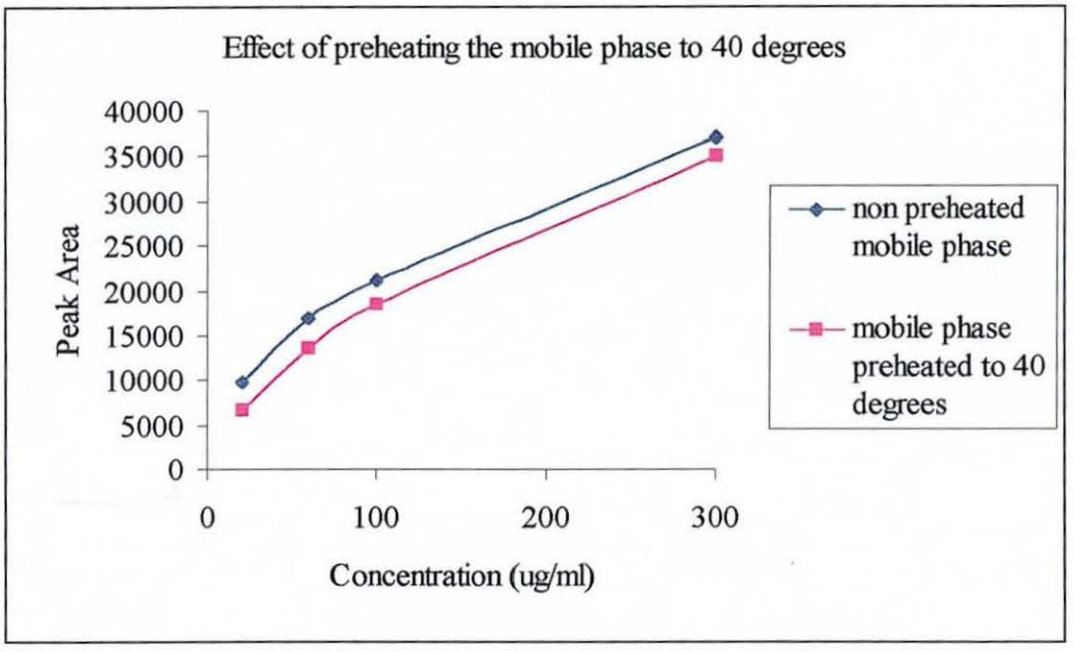


Figure 4.26 Effect of preheating mobile phase on peak area of dp5

As it can be seen from Figure 4.27, the greatest loss is seen at the lower concentration levels, whereas at levels of 300 ug/ml and higher, the net loss due to preheating would be negligible.

4.7 Summary

The new interface between the LC and FID has managed to overcome the problems associated with the "thermospray" type interface, such as robustness, spiking and reproducibility.

The linkage of the two techniques has been demonstrated to separate and detect a range of non-volatile mono-, di- and oligosaccharides using various polymeric cation exchange resins. The possibility of using air as a carrier gas was demonstrated to be not very effective, although the response achieved was lower than that using nitrogen as a carrier gas.

The optimum conditions for the analysis of carbohydrates was gas flow rates of 100-110 ml/min hydrogen, 320 ml/min air and 420 ml/min nitrogen, detector temperature 230°C, water flow rates of up to 0.7 ml/min (dependant on back pressure of the analytical column used), column temperatures as near to ambient as the back pressure would allow.

No advantage was gained by the preheating of the water prior to injection, as losses are observed at low levels.

Chapter 5

Results and discussion

5.1 Determination of the scope of the detector

Although the direct the linkage of LC to FID using a thermospray type interface has previously been successful for volatile analytes, it proved to be problematic when analysing involatile compounds due to frequent blockages of the transfer capillary. The new nebuliser interface has overcome these problems and had worked for model analytes in FIA and LC modes. The aim of the next stage of this study was to demonstrate the capability of the detection system to handle analytes of different molecular weights, volatilities, functional groups and heteroatoms and any affect they had upon the response, linearity and selectivity of the system.

The principal competition for the nebuliser-FID interface is the evaporative light scattering detector. Although it is classed as a universal detector, it cannot detect small volatile compounds, because they are lost during the evaporation of the mobile phase Other problem facing the ELSD is that the response is non-linear and to obtain a linear response a log-log calculation is required (see Chapter 1).

Separations of mixtures of analytes were performed using buffer solutions and water at ambient temperatures and water up to superheated temperatures. The effect of water entering the FID flame on the response was also determined.

The detector characteristics and parameters that will be examined include [168 - 173].

a. Response

The response of a detector is defined as the magnitude of the electrical signal from the detector per unit mass of analyte.

b. Noise

This is referred to the signal that is obtained from the detector which is unrelated to the analyte. For a typical HPLC system, noise could be attributed electronics within the system, fluctuations in the pump, dirt in the flow cell, UV lamp being used after 1000 hours has elapsed etc. Noise effects the detection limits achievable by a detector, whereby the lower the noise experienced by a detector, the lower the detection limit achievable and vice versa.

Detector noise is defined as the standard deviation of the detector when no sample is present, often referred to as the root-mean-square (N_{rms}). For the proposed system, noise will be mostly attributed to the pumping system and the spray chamber.

c. Sensitivity

This is the change in signal with sample size usually measured as the slope of the plot of the sample input against the signal output. Hence a highly sensitive detector will have a high value for the slope.
The sensitivity is often expressed as the limit of detection, where it is defined as the lowest concentration of analyte that can be detected, but not necessarily quantitated. The limit of detection is expressed as a concentration at a specified signal to noise ratio, which in this case was 3:1.

There are two methods for the calculations for the limit of detection.

The first is based on the standard deviation (SD) of the response and the slope of the calibration curve (S), whereby LOD = 3.3 (SD/S).

The second calculation is based on the standard deviation (SD) of the background signal:

$x - xb = 3SD$

Where x represents the signal obtained from the blank sample and x_b is the standard deviation of the blank readings.

For this study, the first method, as quoted for analytical method development and validation [172], the LOD calculated on the basis using 3.3(SD/S), will be used for the purpose of the project.

d. Limit of Quantitation (LOQ)

The LOQ is defined as the lowest concentration of analyte that can be determined both accurately and precisely under the stated operational conditions. A signal to noise ratio of 10:1 is typically used and the calculation of the LOQ is as follows

$LOQ = 10\ (SD/S)$

e. Linear dynamic range

The linear dynamic range is the range over which the sensitivity of the detector is constant and the entire range over which the response varies with concentration or quantity is called the dynamic range of the detector.The upper limit of the detector is determined when the sensitivity falls to zero. At this point the detector is saturated (usually a 10% deviation from the linear response curve). The lower limit of the dynamic range is the detection limit.

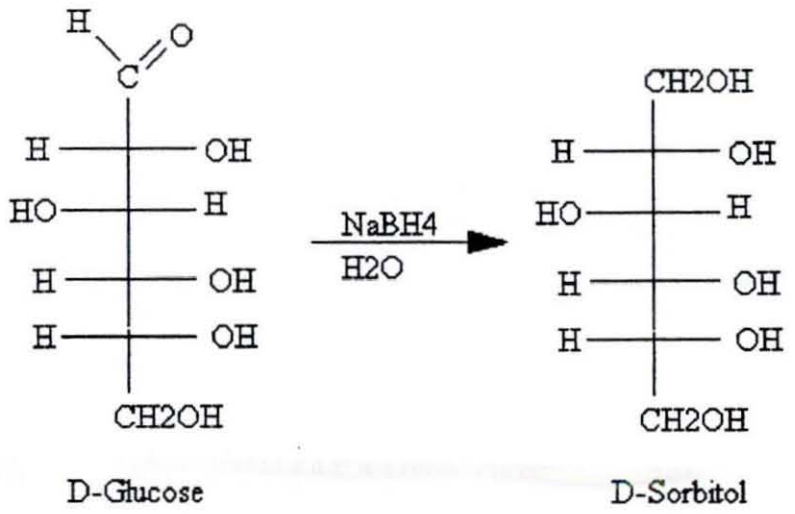
5.2 Detection of Non-volatile, Macromolecule Analytes

5.2.1 Applications using water at temperatures between ambient and 100°C

5.2.1.1 D-Glucose and D-sorbitol

D-glucose is a naturally occurring carbohydrate, as with all other naturally occurring monosaccharides, it has the same stereochemical configuration as D-glyceraldehyde at the chiral carbon farthest from the carbonyl group [167], naturally occurring sugars have the hydroxyl group at the lowest chiral carbon pointing to the right and these are referred to as D sugars. Whereas L-glucose, whose hydroxyl group at the lowest chiral carbon points to the left, does exist, but it is not naturally occurring.

D-glucose was used as a test compound for the initial work on detector development (Chapter 4), due to its in-volatility, which caused many problems for the thermospray type interface.
D-sorbitol or D-glucitol, is the product formed on the reduction of the terminal aldehyde of D-Glucose to an alcohol group .It is used in many foods as an artificial sweetener and a substitute for sugar. D-glucose and D-sorbitol were chosen as test analytes since they are typically found in fruit juices, such as orange, apple and tomato. Each sugar is generally required to be separated to ascertain the varying proportions within a fruit and hence the qualities of the fruit juice.



D-Glucose D-Sorbitol

Previous methods of detection for carbohydrates have involved derivatisation for both GC [174] and HPLC [34]. For example, the determination of sugars by GC requires the conversion into volatile derivatives such as methyl-, acetyl-, silyl-, oxime or oximesilyl-derivatives. The sugar can also be initially converted into their oximes by reaction with hydroxylamine in pyridine and then converted to their trimethylsilyl derivatives, by the reacting with hexamethyldisilazane [174].

In LC, detection is usually the problem and there is a need for the derivatisation of such carbohydrates is because they have no UV or fluorescence chromophore within the molecule. Therefore to be analysed spectroscopically, it is necessary to introduce a suitable absorbing chromophore into the molecule. However, the process usually proves to be tedious and introduces variables into the methodology [34].

Previous direct methods for the determination of carbohydrates involve evaporative light scattering detection [34 – 36] or high performance anion exchange chromatography with pulsed amperometric detection [175, 176].
The initial analytical conditions used for the determination of both sugars by LC-FID, were those as previously determined in chapter 4.

Analytical conditions

Gas flow rates: hydrogen 110 ml/min; air 475 ml/min; nitrogen 380 ml/min

FID detector temperature 230°C

Analytical column PL HiPlex H, 8μm, 250 x 4.0 mm i.d.

Column temperature 50°C

Mobile phase 100% water

Mobile phase flow rate 0.4 ml/min

Injection volume 1μl

a. Determination of the limit of detection

Under the above conditions, 1μl injections of 1000 ug/ml solution of D-glucose and D-sorbitol were performed, (figures 5.1 and 5.2)

Both compounds produced the same mass response values, but similar retention times. Glucose was therefore used to calculate the typical the limit of detection.

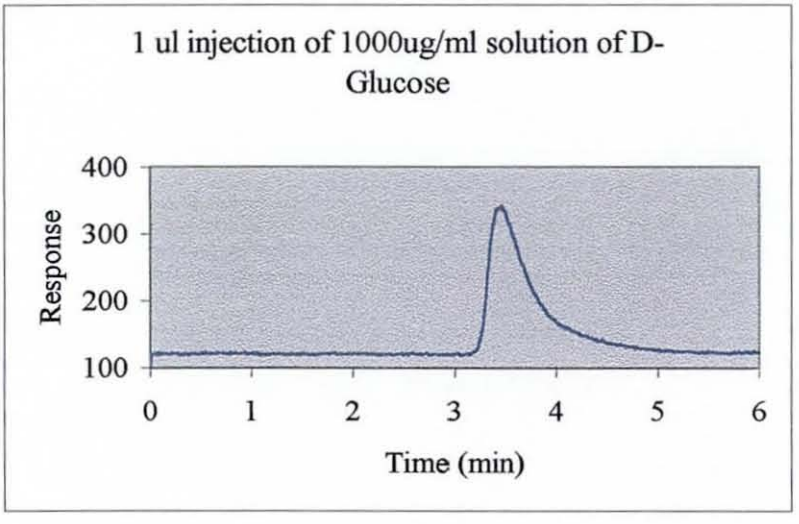


Figure 5.1 Chromatogram of a 1μl injection of 1000 μg/ml solution of D-glucose

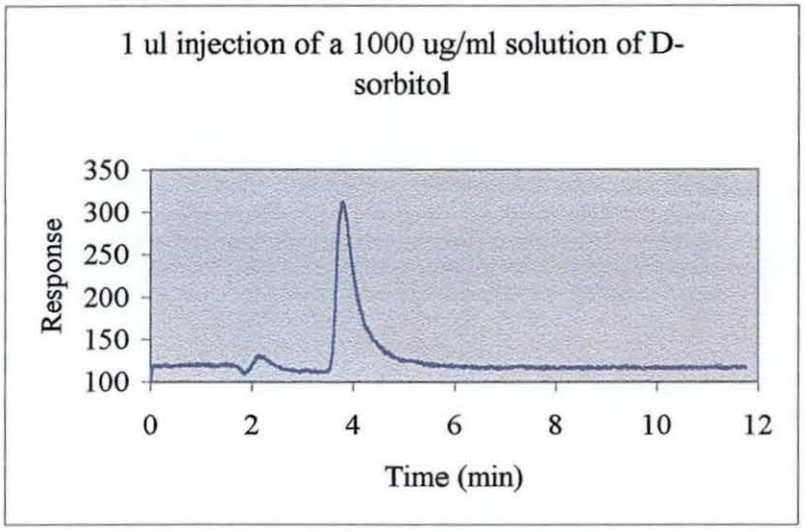


Figure 5.2 Chromatogram of a 1µl injection of 1000 µg/ml solution of D-sorbitol

To determine the limit of detection for D-glucose, serial dilutions of a 1000 µg/ml solution were prepared and injected, until the peak observed began to disappear into the baseline noise. The concentration at which this occurred was 10.4 µg/ml, which for a 1 µl injection, represents 10 ng injected on column. At this level, five 1 µl injections were performed and their peak areas recorded (Table 5.1).

Table 5.1 Calculation of limit of detection on the peak areas of 5 injections of 10 ng D-glucose on column.

Peak Area
407
532
403
579
490
Mean 482
SD 77.2
3.3SD 254.8
LOD 5.5 ng
LOQ 16.6 ng

Using the information from Table 5.1 a set of calibration standards of concentrations 20, 35, 50, 75 and 100 ug/ml were prepared and sequentially injected on to the system.

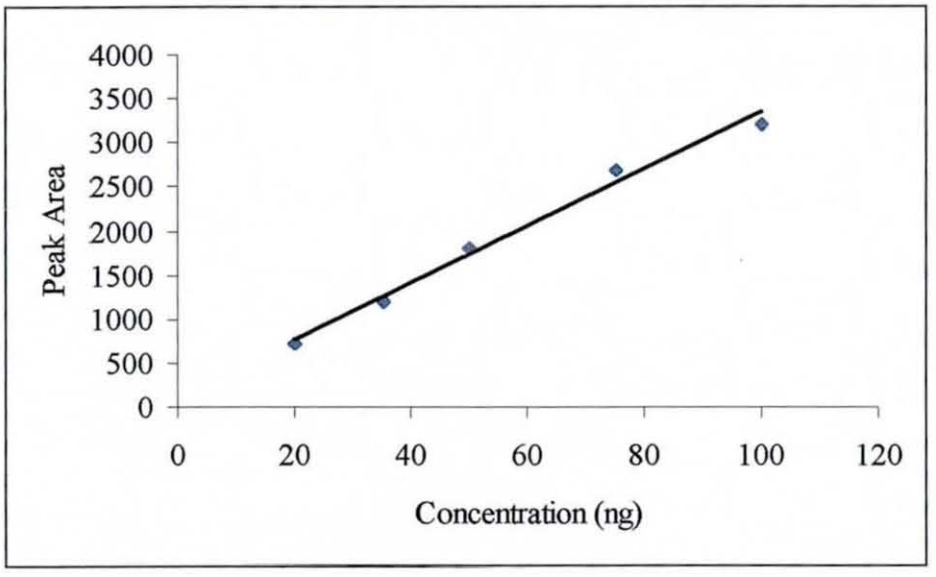


Figure 5.3 Calibration graph of D-glucose 20 – 100 ng on column plotted against peak area. Conditions as page 114.

The response showed a good linearity r = 0.9943, slope = 135.13 and intercept = 31.99 n = 5.

The measured sensitivity of the new system compares favourably against reports of sugars derivatised with ammonical cupric sulphate analysed by HPLC-UV using post column derivatisation, whose sensitivity was quoted as 3 nmol, which is equivalent to 52 ng [149] on column. The detection limit of the ELSD for glucose is reported to be in the region of 80 ng [177], with a non-linear response. Whereas using pulsed amperometric detection the sensitivity is reported to be 0.01 nmol, which is equivalent to 0.17 ng on column, but the problems facing this type of detection are frequent electrode fouling

5.2.2 Applications using mobile phase additives at temperatures between ambient and 100°C

The main point of interest with the LC-FID methodology was to determine if it could be used in conjunction with mobile phase additives and pH control. Volatile buffers are already employed for LC-MS and ELSD and it was important to determine if they could also be used in this case without causing any baseline disturbances.

5.2.2.1 Amino Acids

Amino acid units linked together by amide (or peptide) bonds form proteins, which are large biomolecules that occur in every living organism, of which there are many different types, each having many different functions [178]. Difficulties arise for the analysis of amino acids by liquid chromatography due to their existence as zwitterions in solution. The pH of the mobile phase therefore has to be controlled. Also with the exception of histidine, tryptophan, phenylalanine and tyrosine, they generally lack a suitable chromophore within the molecule. Amino acids contain both an amino and carboxyl group within their structure. Of the 20 commonly found in proteins, all are α-amino acids, whereby the amino group bonds to the carbon next to (α) the carbonyl group. Of the 20 amino acids, 19 are primary amines, the exception being proline, which is secondary, where the nitrogen and α-carbon are part of the pyrrolidine ring.

Due to amino acids having both amino and carboxyl groups, anomalies appear in their physical and chemical properties because of their zwitterionic properties, which are not consistent with their structure. Amino acids typically have high melting points, and are insoluble in non-polar solvent but soluble in water

Whereby the degree of ionisation is depended on the pH :

$$^{+}H_3NCHRCOO^{-} + H_2O \leftrightarrow H_3O^{+} + H_2NCHRCOO^{-}$$

Acid dissociation

$$K_a = \frac{[H_3O^+][H_2NCHRCOO^-]}{[^+H_3NCHRCOO^-]}$$

$$^+H_3NCHRCOO^- + H_2O \leftrightarrow {}^+H_3NCHRCOOH + OH^-$$

Base dissociation

$$K_b = \frac{[^+H_3NCHRCOOH][OH^-]}{[^+H_3NCHRCOO^-]}$$

The analysis of amino acids can be carried out by HPLC or GC. For analysis by GC, due to the non-volatile nature of free amino acids, derivatisation to their volatile acyl esters or N-(O,S)-pentafluoropropionyl isopropyl esters coupled with N-acyl derivatisation is a common method of analysis for GC [168].

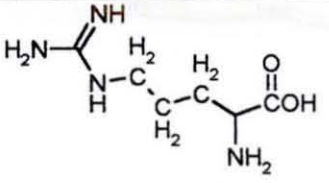
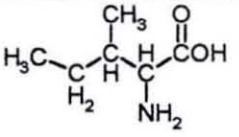
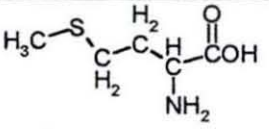
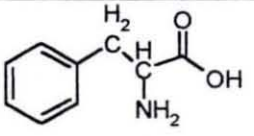
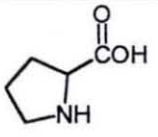
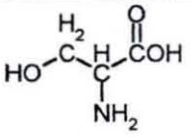
The amino acid derivative must be volatile, stable, separable and detectable [179] to enable the successful detection by GC.

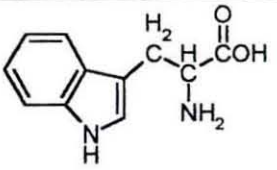
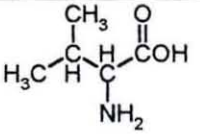
For analysis by HPLC, derivatisation is required due to the lack of a suitable UV absorbing chromophore. For HPLC, there are two main precolumn derivatisation methods for amino acids. The first is by conversion of the amino acid to the stable 3-phenyl-2-thiohydantoin (PTH) derivative, prior to detection. The other main precolumn derivatisation method for HPLC involves reacting the sample with phenylisothiocyanate before reaching the analytical column, to form a phenylthiocarbamyl derivative, which strongly absorbs in the UV [180]. Post column derivatisation for amino acids can be achieved by firstly the separation of the amino acids on a cation exchange column using various buffers. Detection is accomplished by performing a post column colorimetric

reaction using ninhydrin [181] on the separated amino acids. Evaporative light scattering detection has also been used for the analysis of underivatised amino acids [27, 182] where detection limits of 200 pmol for most amino acids were found [27].

A selection of amino acids was chosen for the present study including aliphatic, aromatic and secondary amino acids.

Table 5.2 Selection of amino acids chosen for the present study

Name	Structure	Mol Wt
Arginine		174.20
Isoleucine		132.17
Methionine		150.21
Phenylalanine		165.19
Proline		115.13
Serine		105.09

Tryptophan		204.23
Valine		117.15

The separation of this group of amino acids on a PS-DVB column has been reported using the evaporative light scattering detector [183]. The initial eluent was 0.1% trifluoroacetic acid for 5 minutes followed by a gradient from 0 – 100% acetonitrile : 0.1% TFA (40 / 60 v/v) from 5 – 25 minutes.

These compounds were examined using the LC-FID detector, however, the system was set up for only isothermal elution (equivalent to isocratic elution), using a 0.02% aqueous solution of trifluoroacetic acid as the eluent.
As a result it was not possible to elute tryptophan, within a reasonable time period and it was dropped from the test mixture. It was also found that phenylalanine co-eluted with serine and arginine, it too was removed from the test mixture. The change in relative retention of phenylalanine compared to the gradient study could be due to the change in mobile phase composition.

Analytical Conditions

Gas flow rates: hydrogen 110 ml/min; air 475 ml/min; nitrogen 380 ml/min
Detector temperature 390°C
Mobile phase 0.02% TFA
Mobile phase flow rate 0.5 ml/min
Analytical column PLRP-S, 5μm, 250 x 4.6 mm i.d.

a. Determination of limit of detection

To determine the limits of detection for serine, valine, methionine and isoleucine, serial dilutions of a 1000 ug/ml solution were prepared, using 0.02% TFA as the diluent, and injected, until the peaks observed were beginning to disappear into the baseline noise. The concentration at which this began to occur was around 10 ug/ml for each analyte, which upon 2 µl injection, represents 20 ng injected on column. Five 2 µl injections of each amino acid were performed and their peak areas recorded, (Table 5.3).

Table 5.3 Peak area of multiple injections of approximately 20 ng of each amino acid on column

Peak Areas			
Serine	Valine	Methionine	Isoleucine
1055	263	902	741
1122	522	660	578
858	525	575	480
689	581	824	612
828	619	696	543
Mean = 910.4	Mean = 502	Mean = 731.4	Mean = 590.8
SD = 176.2	SD = 139.6	SD = 130.9	SD = 97.1
3.3SD = 581.6	3.3SD = 460.7	3.3SD = 431.8	3.3SD = 320.5
LOD = 12.7 ng	LOD = 14.7 ng	LOD = 13.5 ng	LOD = 11.7 ng
LOQ = 38.4 ng	LOQ = 44 ng	LOQ = 40.9 ng	LOQ = 35.4 ng

Using the information from table 5.3, a set of calibration standards of concentrations 25, 50, 75, 100 and 150 ug/ml was prepared for each amino acid, which represents 50, 100, 150, 200 and 300 ng on column. 2 µl samples were sequentially injected on to the system and a calibration curve prepared (Figure 5.4).

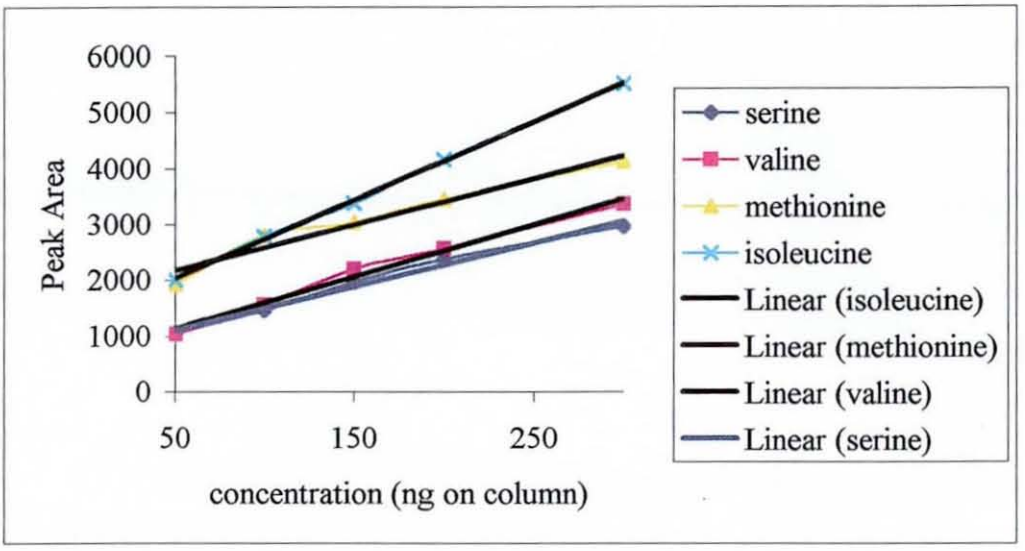


Figure 5.4 Calibration curve Serine, valine, methionine and isoleucine 50 – 300 ng on plotted against peak area. Conditions as page 120.

All regressions for serine, valine, methionine and isoleucine r> 0.99. Again response of the new detection system compares favourably against the evaporative light scattering detector, in terms of increased sensitivity and linear response.

A typical separation of serine, arginine, proline, valine, methionine and isoleucine, using the conditions as page 120 can be seen as Figure 5.5.

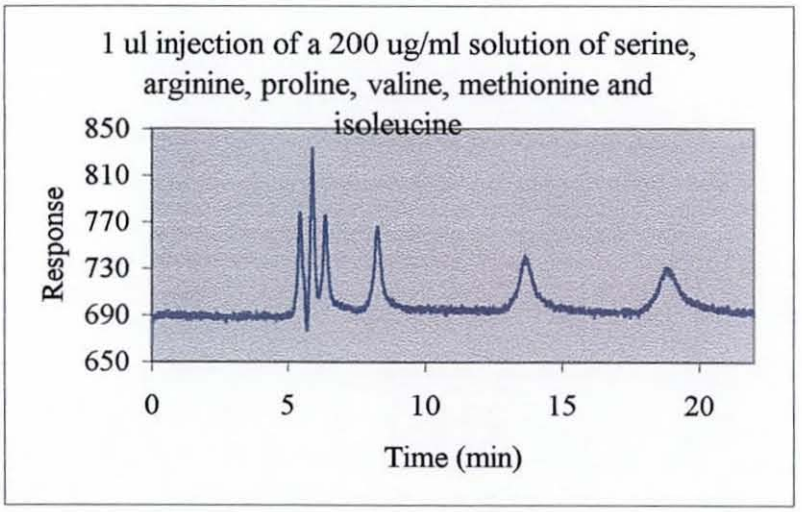


Figure 5.5 Typical chromatogram of the separation of serine, arginine, proline, valine, methionine and isoleucine

5.3 Analysis of non-volatile high molecular weight analytes

Typically non-volatile macromolecules are separated using size exclusion chromatography.

5.3.1 Pectin

Pectin is a polysaccharide composed of many monosaccharide units per molecule, which are held together by glycoside linkages hence its high molecular weight of 20,000 – 40,000. It occurs naturally and is derived from fruits and berries. It was chosen as a test analyte for the detection system due to its in-volatility and high molecular weight. The method developed was based on that described by Polymer Laboratories for use with an evaporative light scattering detector [183].

Analytical conditions

Gas flow rates: hydrogen 110 ml/min; air 475 ml/min; nitrogen 380 ml/min
Detector temperature 390°C
Mobile phase 0.001M $NH_4H_2PO_4$/0.02M NH_4NO_3 adjusted to pH 7 with 0.2 M NH_4OH
Mobile phase flow rate 0.5 ml/min
Analytical column PL Aquagel mixed mode, 8 μm, 300 x 7.5 mm i.d.

a. Determination of the limit of detection

To determine the limit of detection for pectin, serial dilutions of a 1000 ug/ml solution were prepared, using mobile phase as the diluent, and injected, until the peak observed was beginning to disappear into the baseline noise. The concentration at which this began to occur was around 5 ug/ml for each analyte, which upon 10 μl injection, represents 10 ng injected on column.
Five 10 μl injections of the 5 ug/ml solution of pectin were performed and their peak areas recorded (Table 5.4).

Table 5.4 Peak areas of multiple injections of approximately 10 ng of pectin on column. Conditions as page123.

Peak Height Pectin (mm)
8
7.2
6
8
8.1
Mean = 7.5
SD = 0.89
3.3SD = 2.95
LOD = 20.9 ng
LOQ = 63.4 ng

Using the information from Table 5.4 a set of calibration standards of concentrations 10, 20, 30, 75 and 100 ug/ml was prepared, which represented 100, 200, 300, 750 and 1000 ng on column and 10 µl of each standard was sequentially injected on to the system.

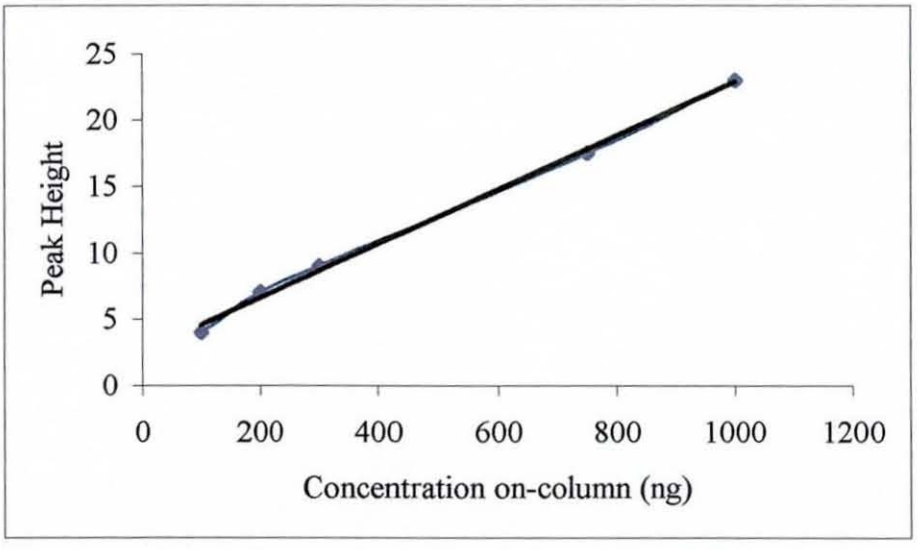


Figure 5.6 Pectin 100 – 1000 ng on-column plotted against peak height

Linear regression r = 0.9986, slope = 2.5192, intercept = 0.2043, n = 5.

A typical chromatogram of a 10µl injection of pectin using the analytical conditions as page 123 can be seen as Figure 5.7.

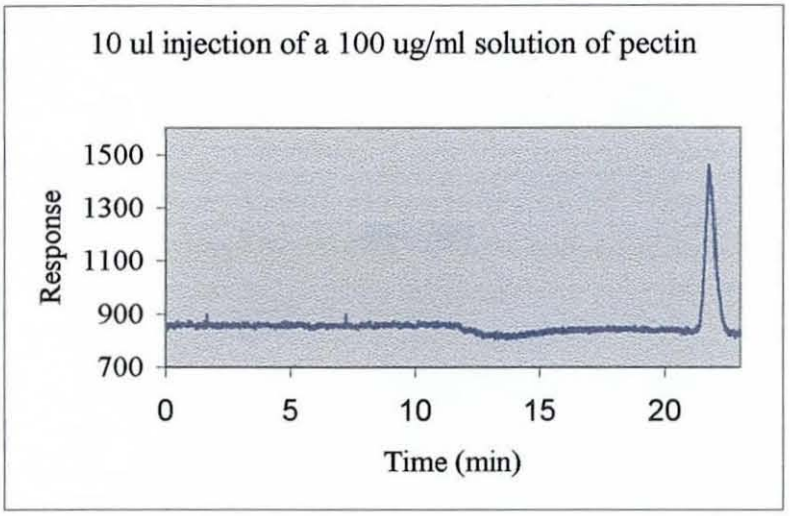


Figure 5.7 Typical chromatogram of 10 µl injection of pectin using mobile phase 0.001 M $NH_4H_2PO_4$/0.02M NH_4NO_3 pH 7.0, conditions as page 123.

5.3.2 Chitosan

Chitosan is a linear polysaccharide consisting of 2-amino-2-deoxy-β-D-glucopyranose and 2-acetamido-2-deoxy-β-D-glucopyranose units. It is prepared commercially by the partial N-deacetylation of chitin (which is insoluble), which forms the exoskeleton of many arthropods, to produce a water soluble and positively charged polysaccharide at low pH. It was chosen as a test analyte due to its high molecular weight and non-volatility.

Chitosan is typically detected using low angle laser light scattering and/or differential refractive index detection [31, 32].

The method developed was based on that described by Polymer laboratories for use with an evaporative light scattering detector.

Analytical conditions

Gas flow rates: hydrogen 110 ml/min; air 475 ml/min; nitrogen 380 ml/min
Detector temperature 390°C
Mobile phase 0.01M Formic acid
Mobile phase flow rate 0.5 ml/min
Analytical column PL Aquagel mixed mode, 8 μm, 300 x 7.5 mm i.d.

a. Determination of the limit of detection

To determine the limit of detection for chitosan, serial dilutions of a 1000 ug/ml solution were prepared and injected, until the peak observed was beginning to disappear into the baseline noise. The concentration at which this began to occur was around 50 ug/ml for each analyte, which upon 10 μl injection, represents 500 ng injected on column.

Five 10 μl injections of 50 ug/ml solution of chitosan were performed and their peak areas recorded (Table 5.5).

Table 5.5 Peak heights of multiple injections of approximately 500 ng of chitosan on column.

Peak Height of Chitosan (mm)
5
4.5
5
5
5
Mean = 4.9
SD = 0.22
3.3SD = 0.74
LOD = 81.5 ng
LOQ = 250 ng

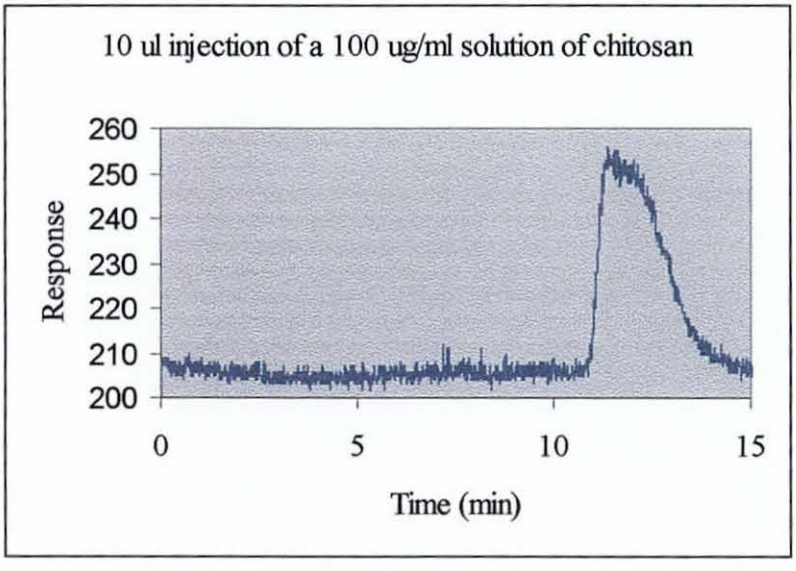


Figure 5.8 Typical chromatogram of a 10 µl injection of a 100 µg/ml solution of Chitosan using mobile phase 0.01M formic acid at flow rate 0.5 ml/min. Conditions as page 126.

5.4 Testing of functional group selectivity of the LC-FID detection system

The detection of the FID is based on its proportional response to the number of -CH_2- groups introduced into the flame, which is equimolar. There is normally little or no response from fully oxidised carbons such as carbonyl or carboxyl groups and the response from carbons attached to amine or hydroxyl groups is lower than that of hydrocarbons. The conventional GLC-FID is also reported to be insensitive to permanent gases such as CO, CO_2, CS_2, SO_2, NH_3, N_2O, NO, NO_2, SiF_4 and $SiCl_4$ [184].

Studies were undertaken to demonstrate the capability of the detection system to handle analytes of different molecular weights, volatilities, functional groups and heteroatoms and any affect they had upon the response and hence selectivity of the detection system. Also the response of the system was further tested by analysing both aromatic and aliphatic compounds, without derivatisation, to determine whether the system was universal or selective in its response.

For the studies, a variety of aqueous solutions of compounds were initially investigated by flow injection analysis. Following detection by flow injection analysis, mixtures of some of the compounds were separated by various analytical columns, using superheated water as the mobile phase.

For each set of analytes, the analytical conditions were set at gas flow rates of hydrogen 110 ml/min, air 475 ml/min; nitrogen 380 ml/min, detector temperature 390°C, mobile phase 100% water, unless otherwise stated

5.4.1 Toluene

Toluene can be analysed using either GC-FID or HPLC-UV. The analysis of toluene for this study was examined previously on a polystyrene divinylbenzene packed LC-column but was too highly retained on the aromatic based phase. Therefore, the less retentive Zirconia -PBD column was chosen. To elute toluene from the analytical column, the typical organic/aqueous mobile phase mixtures were replaced by superheated water at a temperature of 160°C.

Analytical conditions

Mobile phase flow rate 0.7 ml/min
Analytical column Hypersil Zirconia-PBD, 3μm, 300Å, 150 x 4.6 mm i.d.
Analytical column temperature 160°C

To determine the limit of detection for toluene, serial dilutions of a 57 ug/ml solution were prepared, using water as the diluent, and injected, until the peak observed was beginning to disappear into the baseline noise. The concentration at which this began to occur was around 5.7 ug/ml for each analyte, which upon 5 μl injection, represents 29 ng injected on column.Five 10 μl injections of 5.7 ug/ml solution of toluene were performed and their peak areas recorded (Table 5.6)

Table 5.6 Peak area of multiple injections of approximately 29 ng of toluene on column.

Peak Area toluene
9413
8985
9507
9951
9389
Mean = 9449
SD = 344.8
3.3SD = 1137.9
LOD = 3.5 ng
LOQ = 11 ng

Using the information from Table 5.6, a set of calibration standards of concentrations 5, 10, 20, 50 and 100 ug/ml (Figure 5.10) was prepared, which represented, 50, 100, 200, 500 and 1000 ng on column and 10 µl of each standard was sequentially injected on to the system.

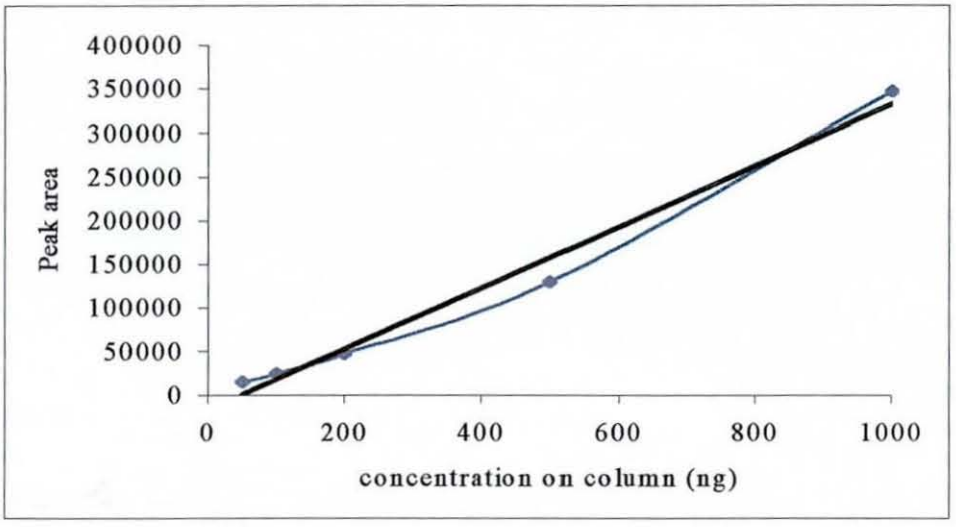


Figure 5.9 Calibration graph of toluene 50 – 1000 ng plotted against peak area

Linear regression r = 0.9916, slope = -16586.51, intercept = 3492.27, n = 5

Headspace gas chromatographic analysis of volatile pollutants in water, using flame ionisation detection, has reported to be sensitive at 0.3-0.5 ppb level [185]. From this information, it appears that the introduction of water into the flame of the FID reduces the temperature and hence lowers the sensitivity of the detection system. This problem could possibly be overcome by optimising the operating conditions of the system.

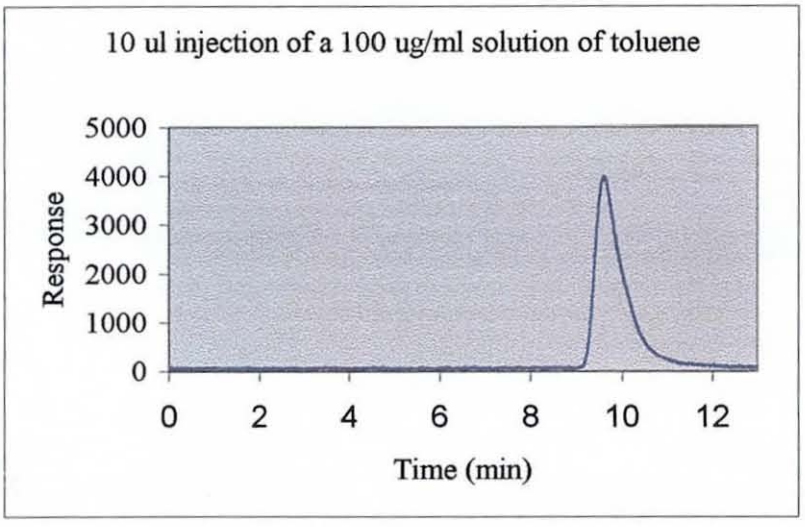


Figure 5.10 Typical chromatogram of a 10µl injection of a 100 ug/ml solution of toluene mobile phase flow rate 0.7 ml/min, conditions as page 129.

Further studies examined functional groups and assumed a linear response and comparable limits of detection and concentrated on differences in response and selectivity towards particular groups

5.4.2 Hydrocarbons

Hydrocarbons were chosen as test analytes. Although aromatic hydrocarbons can easily be detected by UV-Vis, aliphatic hydrocarbons do not contain a chromophore and they are not easily detected. Both aromatic and aliphatic hydrocarbons are easily detected by the flame ionisation detector in GLC.

A selection of aromatic, branched aromatic, aliphatic and branched aliphatic hydrocarbons as well as nitrobenzene, which has a polarity similar to the test compounds, were chosen. The reason for this selection was to ascertain as to whether the response of the system was selective or not.

The test analytes chosen were hexane, heptane, isooctane, nitrobenzene, cyclohexane and toluene. The relative response of hexane, heptane and cyclohexane was determined by

injecting the solute using FIA five times and the mean of the peak area was divided by the concentration injected on to the system.

Table 5.7 Relative response factors of hexane, heptane and cyclohexane in FIA.

Analyte	Relative response factor
Hexane	95470
Heptane	136305
Cyclohexane	77850

Following on from the flow injection analysis determinations, a simple superheated water separation was developed for a mixture of hydrocarbons. Each was injected separately to establish it retention time prior to injection of a mixture of all six analytes.

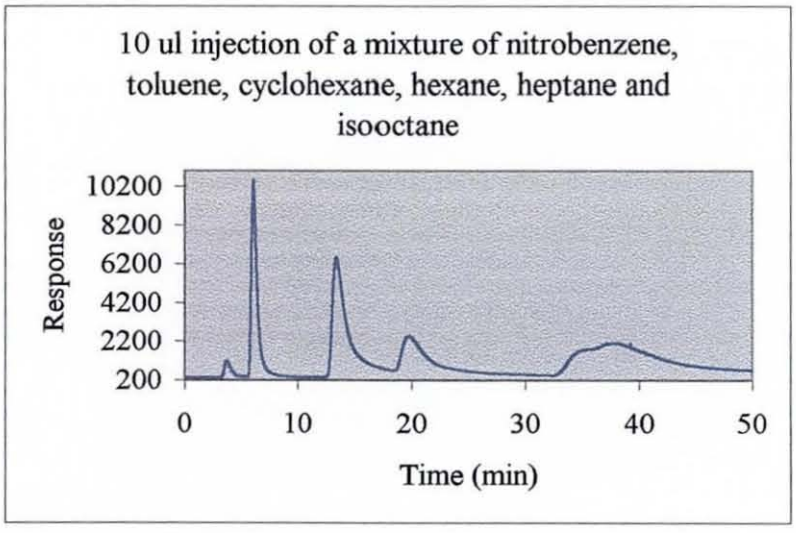


Figure 5.11 Separation of nitrobenzene, toluene, cyclohexane, hexane, heptane and isooctane using Zirconia-PBD, 3µm, 150 x 4.6 mm i.d. column thermostatted at 200°C, mobile phase 100% water at a flow rate of 0.7 ml/min. Detector temperature 390°C

5.4.3 Aromatic and aliphatic alcohols

Aromatic and aliphatic alcohols were chosen as test analytes to determine the effect of the hydroxyl group on detection. As with hydrocarbons, alcohols are volatile and so often cannot be detected by ELSD, but aromatic alcohols can be analysed by UV-Vis due to the presence of a suitable chromophore, which is lacking in aliphatic alcohols.

The test analytes chosen were benzyl alcohol, cyclohexanol and m-cresol. This range of test analytes represent an aromatic, aliphatic and a phenolic hydroxyl. The reason for this selection of alcohols was to determine as to whether the response from the detection system was selective or not.

The relative responses were calculated from the individual injections of each test compound, on-column.

Table 5.8 Response factors of benzyl alcohol, cyclohexanol and m-cresol using PL-RPS 5 μm 2.1 x 250 mm column, mobile phase 100% water at 180°C at a flow rate of 0.2 ml/min

Test analyte	Response factors
Benzyl alcohol	15000
Cyclohexanol	71605
m-Cresol	26720

Upon injection of benzyl alcohol, two peaks were obtained, the first at 12 minutes and the second at 20 minutes. The smaller of the two peaks (at 20 minutes) was thought to be the oxidation product benzaldehyde, which often causes problems with UV detection because of its strong chromophore. But on injection of benzaldehyde under the same

conditions, a single peak at 17.5 minutes was observed. Therefore, the second peak was thought to be due to contamination of the benzyl alcohol sample.

Following on from the injections of each separate analyte, a simple superheated water separation was developed for the mixture of alcohols.

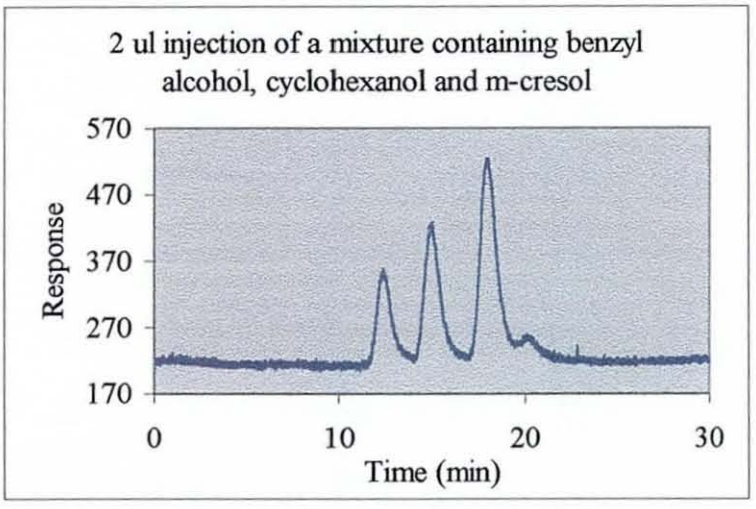


Figure 5.12 Separation of benzyl alcohol, cyclohexanol and m-cresol using Polymer laboratories PL-RP-S, 5μm, 250 x 2.1 mm i.d. column thermostatted at 200°C, mobile phase 100% water at a flow rate of 0.2 ml/min. Detector temperature 390°C

5.4.4 Aromatic and aliphatic amines

Aliphatic and aromatic amines were chosen as test analytes to determine the effect of a molecule containing an amino group on detection. Also, amino groups are of particular interest as they are often present in pharmaceuticals. As previous, the relative responses were calculated from the individual injections of each test compound on-column.
To try to obtain good separations, various analytical columns and mobile phases were tried at different temperatures and the relative responses of the analytes were calculated in Table 5.9.

Table 5.9 Response factors of benzylamine, N-methylaniline, cyclohexylamine, hexylamine using PLRP-S 5μm, 2.1 x 250 mm column and superheated water at various temperatures and a mobile phase flow rate of 0.2 ml/min.

	Superheated water Temperature	
Compound name	**180 °C**	**210 °C**
Benzylamine	281364	
Hexylamine	25528	
N-Methyl aniline		129771

The analytical column was changed from PLRP-S 5μm, 2.1 x 250 mm column to Alltech Zirconia-PBD, 150 x 4.6 mm i.d. column, due to excessively long run times for the aliphatic amines. The effect of pH was also studied, as ionised amines cannot be normally detected by GC-FID

Table 5.10 Response factors of benzylamine, hexylamine, N-methyl aniline, methylamine and cyclohexylamine using Alltech Zirconia-PBD, 150 x 4.6 mm i.d. column and various pH solutions of trifluoroacetic acid as a mobile phase, at various temperatures and flow rates.

	Temperature (°C)					
	140[a]	**120[b]**	**140[c]**	**160[c]**	**180[c]**	**180[d]**
Compound Name		**pH 9.5**	**pH 3.0**	**pH 3.0**	**pH 3.0**	**pH 2.0**
Benzylamine	11562				21665	
Hexylamine	4830				5399	2998
N-Methyl aniline	39500				1122	1671
Methylamine		10118				

Cyclohexyl-amine		18155	24592	245592	28539	2714

[a] mobile phase 100% water at a flow rate of 0.5 ml/min

[b] mobile phase water adjusted to pH 9.5 using ammonium hydroxide, flow rate 0.7 ml/min

[c] mobile phase 1.7 x 10^{-6} M trifluoroacetic acid to produce a mobile phase of pH3, flow rate 0.7 ml/min

[d] mobile phase 1.7 x 10^{-4} M trifluoroacetic acid to produce a mobile phase of pH2, flow rate 0.7 ml/min

From Table 5.10, it is clear to see that the mass response increases with a decrease in mobile phase flow rate and an increase in analytical column temperature. This could be attributed to the cooling of the flame by the increased amount of water entering into it at higher flow rates.

5.4.5 Aromatic and aliphatic aldehydes and cyclohexanone

Aliphatic and aromatic carbonyl compounds were chosen as test analytes to determine the effect of the presence of a carbonyl group on detection.
The test analytes chosen were formaldehyde, acetaldehyde, propionaldehyde, benzaldehyde and cyclohexanone. Formaldehyde, in particular, was chosen as a test analyte due to its poor response in GLC.

As previous by the mass responses were calculated from individual 10µl injections of each test compound, on-column.

To try to obtain good separations of the aliphatic aldehydes, the analytical column was thermostatted at different temperatures and mass responses are calculated in Table 5.11.

Table 5.11 Response factors of various aliphatic and aromatic carbonyl compounds, using analytical column Polymer Laboratories PLRP-S 5μm, 2.1 x 250 mm using a mobile phase of 100% water at various temperatures.

Compound	Column Temp (°C)				
	120[a]	160[a]	180[a]	200[a]	200[b]
Formaldehyde	6224	4887	5226	6450	11286
Acetaldehyde	79461	49292	45909		127412
Propionaldehyde	148332				194525
Benzylaldehyde					370523
Cyclohexanone			159366		

[a] 2μl injection volume

[b] 1μl injection volume

From the studies for aldehydes, the most interesting result is that a response was obtained for formaldehyde, for which the FID is usually insensitive. To detect formaldehyde, usually the photoionisation detector with a high-energy lamp is required [186].

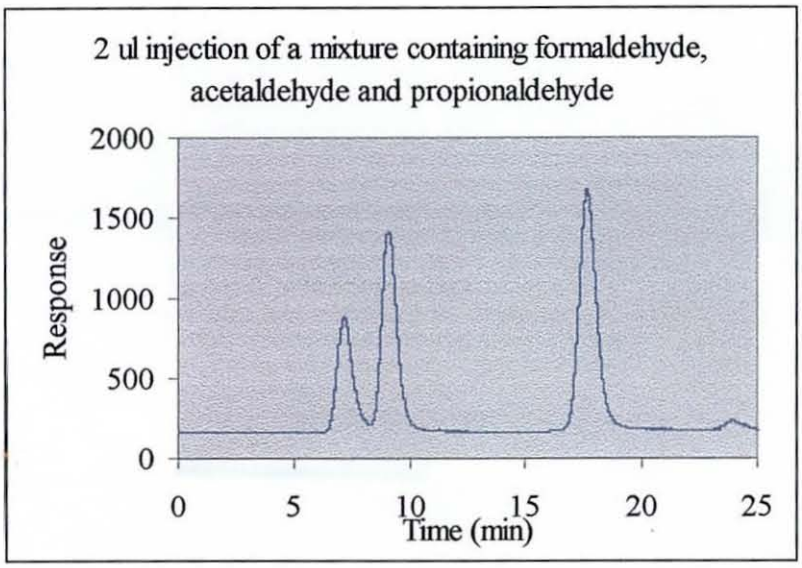


Figure 5.13 Chromatogram demonstrating the separation of formaldehyde, acetaldehyde and propionaldehyde using analytical column Polymer Laboratories PLRP-S 5μm, 2.1 x 250 mm using a mobile phase of 100% water at 120°C

5.4.6 Organic acids

Organic acids were chosen as test analytes to determine the effect of the presence of a carboxyl group upon detection. Detection of organic acids is difficult by GC due to their relative involatility and problems of ionisation, also detection by UV-Vis is troublesome as many of the biochemically interesting acids are aliphatic and have only a weak response. Alternative methods of detection used in the past have included electrochemical detection in ion chromatography and derivatisation to introduce a chromophore.

The test analytes chosen were acetic, tartaric, formic, lactic, citric and oxalic acid. The relative response of each analyte was determined by flow injection analysis, by injecting 10 μl of the solute five times and the mean of the peak area was divided by the concentration injected on to the system.

Table 5.12 Response factors of organic acids using flow injection analysis

Compound name	Response factor
Acetic acid	3031
Tartaric acid	797
Formic acid	166
Lactic acid	797
Citric acid	939
Oxalic acid	350

The response for organic acids was found to be much weaker than any of the other groups, but this could be expected as the formic acid is undetectable in GC-FID.

Although all analytes could be detected, separation proved to be difficult, as the baseline obtained using a Phenomenex Aquagel 4.6 x 250 mm i.d. column, which was recommended for the separation, was extremely noisy. Therefore the analytical column was changed to a PL-RP-S 5μm 2.1 x 250 mm i.d.

Table 5.13 Retention times of various organic acids using Polymer Laboratories PLRP-S 5μm 2.1 x 250 mm column, mobile phase 100% water at a flow rate 0.2 ml/min

Compound name	Retention time
Formic acid	3.21
Acetic acid	5.6
Oxalic acid	2.7
Tartaric acid	2.7
Citric acid	3.2

From the studies on organic acids, it is evident that detection is possible, although the responses are somewhat lower than expected, but this could be overcome by the optimisation of conditions for the organic acids.

5.5 Real Applications – detection of problem compounds from other areas

5.5.1 Allantoin

Allantoin was chosen as a test analyte as it does not contain a chromophore, is non-volatile and a biological sample that has been difficult to detect by conventional LC methods. Methods for its detection include colorimetry methods [187], ion exchange followed by post-column derivatisation [188] and capillary electrophoresis coupled with low UV wavelength detection [189].
The mass response of allantoin was determined by flow injection analysis, by injecting 1µl of a 500 ug/ml solution five times and the mean of the peak area was divided by the concentration injected on to the system.

The response factor for allantoin was found to be 5576 (Table 5.14).

Table 5.14 Determination of limit of detection and quantification, by flow injection analysis, of allantoin by five 1µl injections of a 500 µg/ml solution.

Peak Area
2770
2282
2745
2750
2928
Mean = 2695
SD = 242.9
3.3SD = 801.6
LOD = 154 ng
LOQ = 466.9 ng

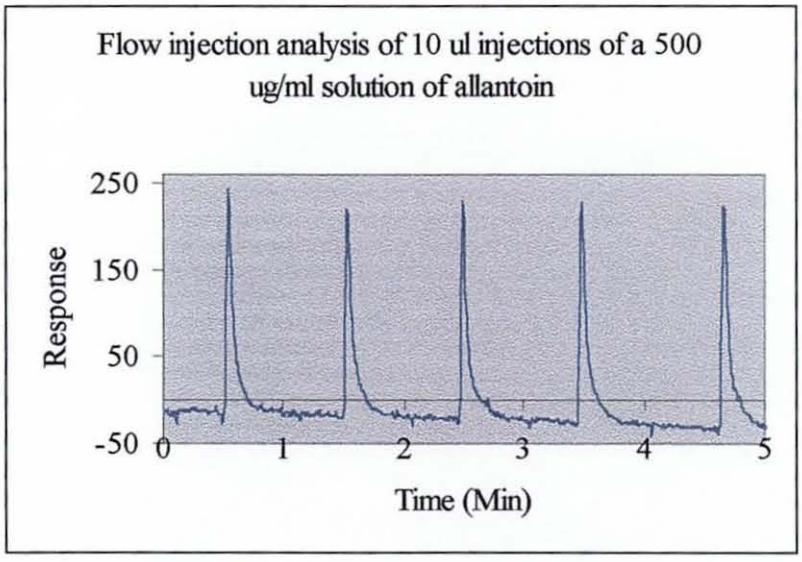
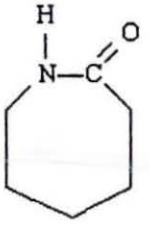


Figure 5.14 Typical trace of 0.5 ng allantoin on column, by flow injection analysis, mobile phase 100% water at a flow rate of 0.4 ml/min.

5.5.2 Caprolactam

Structure



ε-Caprolactam is an important starting material for the production of polyamides. As caprolactam can be detected at 200 nm, it can be analysed by HPLC [190, 191] or planar chromatography [192].

10 µl injection of a 1000 ug/ml solution of caprolactam was injected on to the system, using a Polymer Laboratories PL-RP- analytical column thermostatted at various temperatures, using a mobile phase of 100% water.
Response factors were calculated using the peak areas from the experiments, refer to Table 5.15.

Table 5.15 Response factors for 10µl injection of a 1000 uµg/ml solution of capralactam using a Polymer Laboratories PLRP-S 5µm 2.1 x 250 mm i.d. analytical column thermostatted at various temperatures, and a mobile phase of 100% water at a flow rate of 0.2 ml/min.

Mobile phase temperature (°C)	Response factor
200	2158
160	1771

From Table 5.15, it can be seen that an increase in the temperature of the mobile phase results in an increase in the response factor.

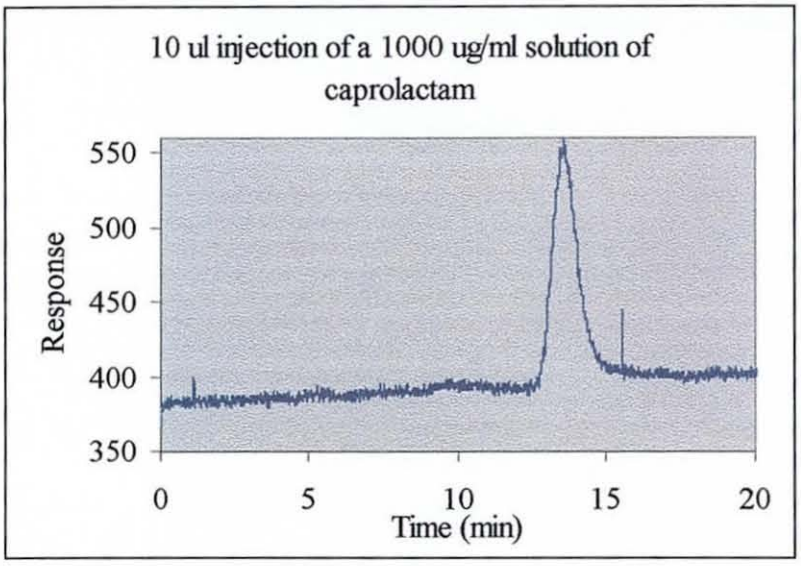


Figure 5.15 Typical chromatogram of a 10 µl injection of a 1000 ug/ml solution of caprolactam using a Polymer Laboratories PLRP-S 5µm 2.1 x 250 mm i.d. analytical column thermostatted at 160°C, using a mobile phase of 100% water at a flow rate of 0.2 ml/min.

5.5.3 Ammonia

Ammonia was chosen as a test analyte as it cannot be analysed directly by FID. In fact it has recently been used as a carrier gas for the GC-FID detection of trichlorophenols [193].

However, in some of the earlier studies when an aqueous solution of ammonia was used to alter the pH of the mobile phase there was a positive shift in the baseline, when compared to the baseline produced by water. It was thought that it might be possible to

detect ammonia using LC-FID.This might be explained due to the different nature of the flame used in the detection system.

The response factor was calculated by injecting 1 µl of a 9.0 M solution of ammonium hydroxide, five times in flow injection mode.

The relative response factor was found to be 348. Although the value is low, it indicates that there is a potential which could be exploited

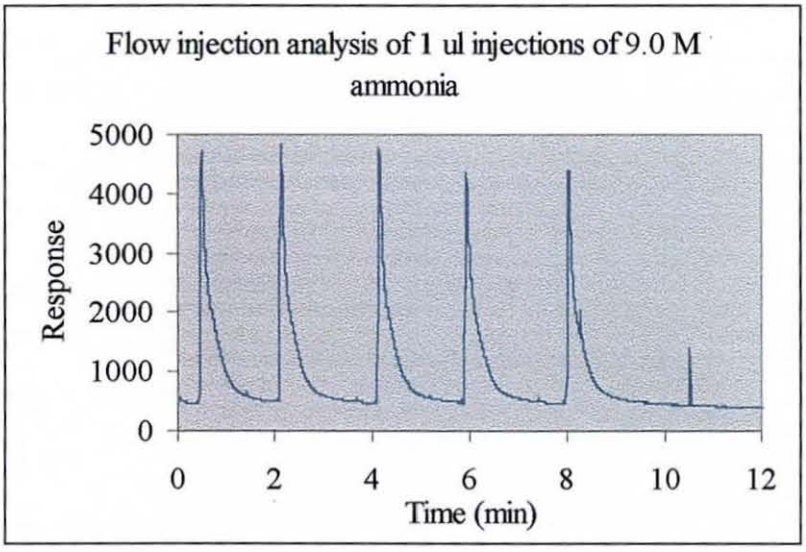


Figure 5.16 Typical FIA trace of five 1 µl of a 9.0 M solution of ammonium hydroxide in flow injection mode

5.5.4 Dichloromethane

Dichloromethane was chosen as a test analyte due to its volatility and it does not contain a UV chromophore and in most conventional FID, is only weakly responsive. Recently, dichloromethane and chloroform have been extracted from blood and urine, using SPME and analysed by GC-FID [194]. Using this methodology, linear calibration curves in the

range of 0.5 - 8μg/ml and detection limits of 0.3 μg/ml in blood and 0.2μg/ml in urine were achieved.

The response to a 1 μl injection of dichloromethane at an attenuation of 10^{-11} was completely off scale, once the attenuation was readjusted to 10^{-10} the response was found to be on scale (Figure 5.17).

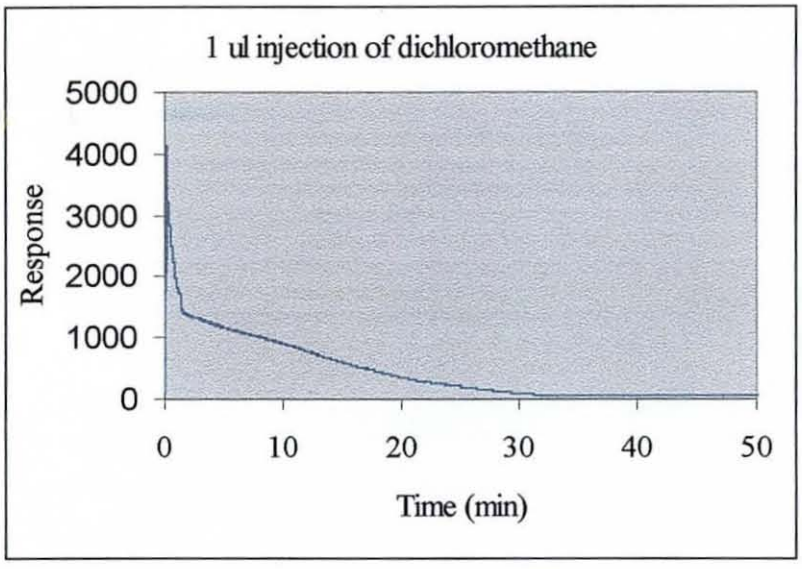


Figure 5.17 Flow injection analysis trace of a 1μl injection of dichloromethane, detector temperature 390°C, mobile phase 100% water at a flow rate of 0.2 ml/min.

5.5.5 Sodium nitrite

Sodium nitrite is used in HPLC as a void volume marker for C18 based analytical columns.

It was chosen as a test analyte, to see if the system was capable of detecting ionised analytes and non-carbon containing compounds, which normally do not give an GC-FID response, due to poor volatility.

Upon a 5µl injection of sodium nitrite, giving a concentration of 52 ug on column, a peak was evident at 5.7 minutes, refer to Figure 5.18.

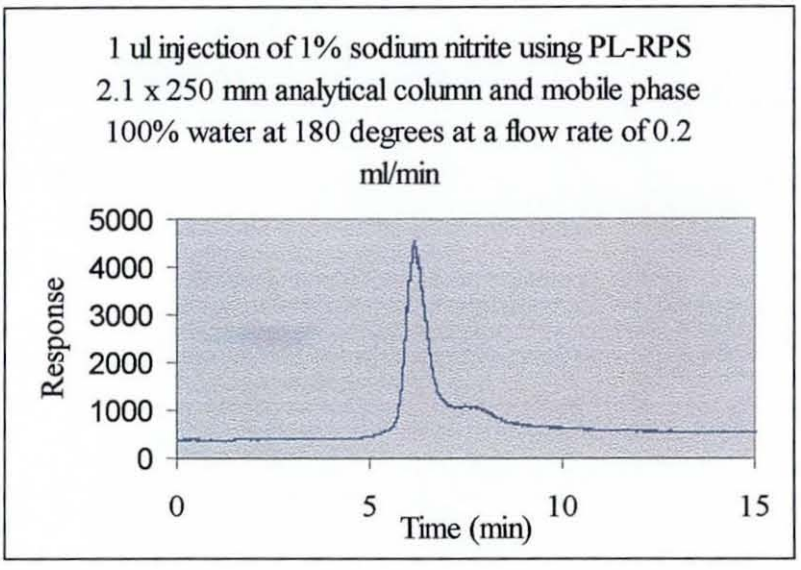


Figure 5.18 Typical chromatogram of a 52 ug injection on-column of sodium nitrite, using a Polymer Laboratories PLRP-S 5µm, 2.1 x 250 mm i.d. using 100% water at a temperature of 180°C at a flow rate of 0.2 ml/min

It has now been demonstrated that using the new LC-FID the detection of inorganic, non-volatile ions is now possible. Previously, detection by GC-FID would have been impossible, due to the non-volatility of the analyte.

5.6 Summary

It has been demonstrated that the developed system is capable of detecting a wide range of compounds, which cannot be traditionally analysed directly by GC-FID or HPLC-UV-Vis, without the need for derivatisation.

Most notable is the response for cyclohexane and cyclohexanol, which are very similar, therefore the effect of the presence of an hydroxyl group has no effect. Whereas the

response of cyclohexylamine demonstrates that, as with traditional FID, the presence of an amino group lowers the relative response factor, although the system was not optimised for its detection. Also, the relative response factors were calculated on-column, from which losses could occur. To further determine the effect of the amino group on response, flow injection analysis under the same conditions as for hydrocarbons and alcohols would have to be undertaken. More interestingly, the response of cyclohexanone was double that of cyclohexane or cyclohexanol. Although low responses were obtained for organic acids, the analytical conditions were not optimised and it expected that much improved detection is achievable up on optimisation of the system.

The detection limits for free amino acids using the new system are better than those achieved by light scattering detection [27] and the response from the LC-FID is linear, which is a distinct advantage over the ELSD.

The new system also demonstrates the possibility of the detection of analytes not normally detected by GC-FID, such as ionised analytes, formaldehyde and ammonia. This further demonstrates the universality of the system, as direct detection of these types of analytes would have been impossible without the need for derivatisation. It also demonstrates that the new detection system is capable of detecting small, volatile analytes, a further advantage over the ELSD.
It is also apparent that the introduction of water into the flame of the FID does lower the sensitivity of the detection system, when comparing the analysis of toluene by headspace analysis and the new detection system. Although the analytical conditions used for the new detection system were not fully optimised.

Chapter 6

Conclusions and further work

The development and use of a universal detector for LC has been demonstrated using water and aqueous buffers as the mobile phase. As well as water and aqueous buffers, superheated water has also been demonstrated as a suitable eluent to be used with this system. The detection system has been shown to be capable of detecting a wide range of molecular weight volatile organic and inorganic compounds, such as ammonia, dichloromethane and formaldehyde, which cannot traditionally be detected by the flame ionisation detector. The detection of small volatile compounds has proven to be a great advantage against universal detectors such as the evaporative light scattering detector, which cannot detect these type of compounds. As well as volatile compounds, the detection system has also demonstrated detection of a wide range of high molecular weight non volatile compounds, such as polysaccharides, amino acids, chitosan, allantoin and caprolactam. It has also been found that unlike traditional GC-FID, the detection of non volatile compounds does not require derivatisation of any form to produce a response.

The developed system has therefore overcome the problems associated with transport detectors and previous superheated water LC-FID linkages. It has proven to be robust and unlike other commercial universal detectors, it has a linear response and is sensitive.

In order to try to establish the full capability of the system, further development will be necessary and comparisons made against other "universal detection systems". Also a wider variety of molecular weight compounds should be analysed and compounds which are not traditionally detected by FID should also be tried.

Stationary phases

To increase the applications for the system, further polymeric stationary phases should be tried as well as different bonded versions of Zirconia-PBD, PGC and PS-DVB, to try to enhance selectivity. Although silica based columns have been found not to be compatible with systems using water as the sole mobile phase, new bonding processes resulting in new "phases" are becoming more occurrent, therefore it would be prudent to try these phases for their increased stability.
Also, there is a need for an ion exchange column, for the separation of sugars, which could be used at room temperature. This would result in an increase the sensitivity of both RI and LC-FID detectors.
Further investigations should be carried out on both injection volume and column internal diameter, to optimised the system.

Improvements to the system

As peak shapes were generally broad, this could be due to inadequate thermal equilibration along the analytical column. Therefore there is a need for a more efficient column/mobile phase heating system. As already discussed, losses were observed on preheating the mobile phase prior to injection, therefore it appears that the system requires a temperature gradient between the injector and column. It is this gradient which should be investigated and optimised. The effect of band broadening should also be investigated and dead volume within the system minimised, to help the peak shapes.

Superheated to supercritical water

The solvating power of water has only been investigated at temperatures up to 250°C. Temperatures above 250°C could offer improved solvating power, due to the lower dielectric constant, for higher molecular weight compounds.

New directions to be studied

Further investigations into pH control and aqueous buffers that could be used with the new LC-FID system should be investigated to determine the most suitable aqueous additives for the system.

The development of a superheated water system capable of supplying a temperature gradient would be a great advantage for the system. As would the linkage of the system to a quaternary pump, whereby enabling the system to run a true gradient.

A comparison of pre and post column derivatisation using superheated water as an eluent and comparing sensitivities against traditional LC and the new system would provide valuable information.

The linkage of solid phase extraction to direct injection or superheated water chromatography FID and/or UV-Vis would be attractive.

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Papers, Presentations and Awards

Papers

R. M. Smith, R. J. Burgess, O. Chienthavorn and J. R. Stuttard, "Superheated water: a new look at chromatographic eluents for reversed-phase chromatography", LC*GC International, 1999, 12, 30 - 36

R. M. Smith, R. J. Burgess, O. Chienthavorn, J. R. Bone superheated water: a new look at chromatographic eluents for reversed phase liquid chromatography, LC*GC North America, 1999, 17, 938 - 945

J. R. Bone and Roger M. Smith, Solid phase extraction and thermal desorption of acetophenone and m-cresol using superheated water or steam as the sole eluent Anal. Commun., 1999, 36, 375 – 376

Presentations

J. R. Stuttard and R. M. Smith, "Determination of the feasibility of solid phase extraction using super-heated water as the eluent", 1998 National congress, and 35th Research and development Topics in Analytical Chemistry, University of Durham, April 6-7th 1998.

J. R. Stuttard and R. M. Smith, "Determination of the feasibility of solid phase extraction using super-heated water as an eluent", presented at SIA'98 organised by the Scientific Unstrument Association, Earls Court, 29th April 1998.

J. R. Stuttard and R. M. Smith, "Liquid phase thermal desorption in solid phase extraction using superheated water as an eluent" presented at 20th International Symposium on Capillary Chromatography, May 26 – 29th, 1998 in Riva del Garda.

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J. R. Stuttard and R. M. Smith, "Solid Phase extraction and thermal desorption using superheated water and steam" 36th Research and Development Topics in Analytical Chemistry, University of Greenwich, April 12-14th 1999.

J. R. Bone and R. M. Smith, "A green universal detector for the future", 23rd International Symposium on Chromatography, London, 1st – 5th October 2000.

R. M. Smith and J. R. Bone, "Novel detection methods in aqueous liquid chromatography", 25th International Symposium on High Performance Liquid Phase Separations and Related Techniques, HPLC 2001, Maastricht, June 17 – 22nd 2001.

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Awards

May 1999 Runner up SOCSA prize for Innovation -

Sept 2000 Winner of Innovative Metrology – Metrology award for World Class Manufacturing

Sept 2000 British Patent application no. 0021567.3 – Analyte detection system

Oct 2000 SMART award – feasibility study for the development of Universal Detection system for liquid chromatography

Solid phase extraction and thermal desorption of acetophenone and m-cresol using superheated water or steam as the sole eluent

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Steam or superheated water can be used to efficiently elute moderately polar and non-polar compounds from polymeric sorbents at temperatures up to 220 °C without degradation of the analyte. Acetophenone and m-cresol, as model compounds, have been concentrated from dilute aqueous solutions, using solid phase extraction (SPE) traps packed with polystyrene–divinylbenzene (PS–DVB) or poly(divinylbenzene-*co*-*N*-vinylpyrrolidone). The technique offers low cost, ease of use and an environmentally friendly eluent.

Introduction

Solid phase extraction (SPE) is widely employed for sample preparation and enables trace enrichment, purification or removal of interferences to be performed on dilute solutions of analytes, prior to chromatographic or spectroscopic analysis.[1] Non-polar sorbents, such as silica based C_{18} phases, are typically used to trap organic compounds from aqueous solutions. The mechanism of sorption is through Van der Waals forces and occasionally secondary interactions, such as dipole–dipole or hydrogen-bonding between the solute and the sorbent.[1] Aromatic polymeric supports, such as polystyrene–divinylbenzene (PS–DVB), generate additional interactions with the π electrons of the analyte. It is for this reason that polymeric sorbents are more retentive than C_{18}. This can provide additional selectivity between analytes. The release of the analytes from the SPE trap requires a much smaller volume of organic solvent that would be needed for liquid–liquid extraction. However, with repetitive assays even this amount can be significant and the solvent is often incompatible with a subsequent separation using reversed-phase LC.

At ambient temperatures, water is a relatively weak chromatographic solvent due to its high polarity (dielectric constant ε_{H_2O} = 79 at 20 °C).[2] Under elevated temperatures and pressures, its polarity and dielectric constant decrease so that ε_{H_2O} = 55 at 100 °C, ε_{H_2O} = 44 at 150 °C, ε_{H_2O} = 31 at 220 °C and ε_{steam} = 1.[2] These dielectric constants are often similar to those of methanol–water mixtures, for example $\varepsilon_{methanol-water}$ = 55 for a 50:50 mixture, $\varepsilon_{methanol-water}$ = 45 for a 70:30 mixture and $\varepsilon_{methanol}$ = 32.[3]

It has been demonstrated that water at supercritical (400 °C, 350 bar) and superheated (250 °C, 50 bar) temperatures can be used as an alternative to organic solvents, for the extraction of polycyclic aromatic hydrocarbons (PAHs), n-alkanes, alkylbenzenes and polychlorinated biphenyls (PCBs) from river sediment and soil samples.[4–7] The cold aqueous extracts were usually concentrated by absorption onto a sorbent trap and the extract was released using organic solvents. In one of these studies, it was found that steam successfully extracted n-alkanes, whereas superheated water was less successful.[7]

It has also been shown that water at temperatures above 100 °C, under sufficient pressure to remain as a liquid, is capable of replacing organic–aqueous mixtures used in reversed-phase HPLC[8–12] and steam has been used as a mobile phase for gas chromatography.[13,14]

The present work examines the trapping of analytes from dilute aqueous solutions onto different polymeric supports and their subsequent release using superheated water or steam, replacing the usual organic modifiers. The effect of different concentrations, load times and thermal desorption conditions have been examined.

Experimental

Reagents

Deionised water, 18.2 MΩ was obtained from an Elga Maxima HPLC purification unit (Elga, Wycombe, UK). Acetonitrile, far-UV grade was from Fisher Scientific (Loughborough, UK). Test compounds were of analytical grade from Sigma–Aldrich (Gillingham, Dorset, UK).

Extraction conditions

The extraction system consisted of a Waters 590 pump (Taunton, Massachusetts, USA), attached to a preheating coil of stainless steel tubing (1 m × 0.50 mm id) and a SPE trap, both of which were placed inside a Pye Unicam series 104 GC oven (Cambridge, UK) controlled isothermally by a Pye Unicam oven programmer (Fig. 1). A cooling coil of the same dimensions was connected directly after the trap outside of the oven. A back-pressure restrictor was attached to the end of the cooling coil to provide a back pressure of 83 bar for acetophenone and 152 bar for m-cresol at 1 ml min^{-1} flow rate. The water was deoxygenated using nitrogen to prevent any oxidation to the sample or corrosion within the system.

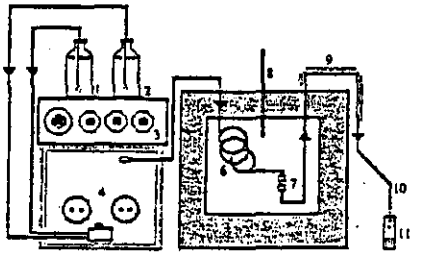

Fig. 1 Equipment for SPE of analytes using superheated water or steam. Components: 1, solvent reservoir containing water; 2, reservoir containing aqueous analyte; 3, oven temperature programmer; 4, pump; 5, oven; 6, preheating coil; 7, SPE trap; 8, thermometer; 9, cooling/condensation coil; 10, back-pressure restrictor; and 11, collection vial.

Extraction scheme

The experiment comprised of two stages. The first stage was to load the dilute aqueous sample, at ambient temperature, on to the cold SPE trap at a flow rate of 1 ml min^{-1}, without a back-pressure restrictor. The trap was then washed with water to remove any residual sample solution. The flow was then stopped. In the second stage, the back-pressure restrictor was fitted and the temperature of the oven was raised to the desired elution temperature. The flow was restarted and the sample was eluted off the trap and collected after the cooling coil. When steam was used as the extraction solvent the back-pressure restrictor was omitted and the condensed liquid was collected.

For these studies, the SPE trap was prepared from a guard column (Upchurch Scientific, UK) and was packed with 20 mg of either PLRP-S (5 μm, 100 Å, PS–DVB, Polymer Labs., Shropshire, UK) or poly(divinylbenzene-*co*-*N*-vinylpyrrolidone) (30 μm, 30 Å, Oasis HLB™, Waters Ltd, Massachusetts, USA). Prior to trapping, the traps were conditioned by passing 10 ml deionised water through at a flow rate of 1 ml min^{-1}. The flow was then stopped and the temperature of the oven was raised to 150 °C for a period of 5 min and a back-pressure of 97 bar was applied. After this period, the trap oven was cooled and the deionised water flow was started until the whole system reached room temperature.

Chromatographic conditions

Quantification of the extract was carried out using a LC system consisting of a Waters 590 pump, a Rheodyne 7125 manual injector (Cotati, CA, USA) fitted with a 20 μl loop, a Jones 7960 block heater (Hengoed, UK) at 27 °C. The analytes were separated on a Spherisorb ODS 2, (5 μm, 150 mm × 4.6 mm id, Phase Separations, Clwyd, UK) column and were detected using a Pye Unicam 4025 variable UV-Vis detector (at 245 nm for acetophenone and 215 nm for m-cresol). All analytes were quantified using a five-point external calibration curve. Data acquisition was obtained by a Spectra Physics SP4270 integrator.

Results and discussion

The aim of the study was to demonstrate the trapping of a dilute aqueous solution of analyte onto a polymeric sorbent and its subsequent release as a concentrated solution using superheated water or steam as the elution solvent.

Elution using superheated water

Concentration of acetophenone using a PS–DVB packed SPE trap. A dilute aqueous solution of acetophenone (0.0024 mg ml^{-1}) was applied to a conditioned trap at a flow rate of 1 ml min^{-1} for a period of 40 min (giving a total sample mass of 0.096 mg). The trap was then washed with 5 ml cold water to remove any residual sample solution. The flow was then stopped and the temperature of the oven was raised to 220 °C, then a back-pressure restrictor (which had previously been calibrated to supply 83 bar at a flow rate of 1 ml min^{-1}) was attached to the cooling coil and the flow was restarted. The first 1 ml of eluent was collected and quantified by HPLC. The sample contained 0.0864 mg, which represents a recovery of 90%. Further 1 ml fractions were collected and analysed by HPLC, but were found not to contain any analyte. A repeatability study was undertaken on the use of the same packed trap and gave a mean recovery of 88.2%, s (%) = 3.87, n = 5.

In order to determine the maximum degree of concentration that could be performed on the sorbent, a 1 l solution of acetophenone (0.096 mg l^{-1}) was pumped through the trap to give the same total loading. The trap was washed with 5 ml of cold water and eluted at 220 °C to give a 76.3% recovery. This suggested that despite the large volume extracted, both the trapping and thermal desorption were efficient and a significant degree of concentration could be obtained.

Concentration of m-cresol using a PS–DVB packed SPE trap. Initial studies found that the optimum elution temperature for m-cresol was 210 °C and the maximum volume of deionised water that could be applied to the trap, as an intermediate wash, without sample loss, was 4 ml. A dilute aqueous solution of m-cresol (3×10^{-4} mg ml^{-1}) was applied to a conditioned trap at a flow rate of 1 ml min^{-1} for a period of 40 min (giving a mass of 0.012 mg). After washing and elution the first 1 ml sample contained 0.011 mg, which represents a recovery of 91%. A repeatability study was undertaken on the same packed trap and gave a mean recovery of 93.5%, s (%) = 3.53, n = 5.

As with acetophenone, the *maximum* degree of concentration was examined. A litre of a dilute aqueous solution of m-cresol (1.2×10^{-5} mg ml^{-1}) was loaded onto a conditioned trap (giving a mass of 0.012 mg) and eluted as described. However, the recovery was only 10%. Further investigations found that even a 200 ml load of a solution of m-cresol (0.012 mg) gave a recovery of only 20%. It was concluded that the analyte was being eluted off the trap at ambient temperature by the large volumes of dilute aqueous sample. This was confirmed when 40 ml of m-cresol solution (0.012 mg) was loaded onto a conditioned trap, followed by a 60 ml wash of deionised water. On subsequent elution, the recovery obtained was only 6%. These results are in agreement with Slobodnik and co-workers,[15] who found that a decrease in concentration resulted in an increase in the breakthrough volume from an SPE trap packed with PS–DVB, resulting in lower recoveries.

Elution using steam

Concentration of acetophenone using a SPE trap packed with Oasis HLB™. This polymeric material is more polar than PS–DVB and it might be expected to have a higher retention of polar analytes. As the sorbent has increased water wettability[16] compared to PS–DVB, it should provide greater trapping capacity for large volumes of aqueous solutions. However, a repeat of the concentration scheme as described above for acetophenone but using a trap packed with Oasis HLB™ resulted in negligible recovery upon elution. An investigation into the cause of the low recovery found that the sorbent appeared to collapse in the liquid flow and so is not suitable for use at the back-pressures and temperatures necessary to generate superheated water.

However, if the back-pressure restrictor was omitted from the system and elution of the analyte was by steam at 210 °C, a recovery of 95% was obtained in the first ml of the condensed water. Although this sorbent was not designed for repeated use,[16] a repeatability study was undertaken on the use of the same packed trap. It was found that by the third consecutive preconcentration, the recovery had decreased from 96.5 to 62.5%. A further study was undertaken using traps, which were freshly packed with Oasis before each preconcentration. The result was a mean recovery of 90%, s (%) = 3.24, n = 5, suggesting that column degradation had occurred in the previous experiment.

A solution of 1 l of dilute acetophenone (0.012 mg l^{-1}) was loaded onto the trap but released using steam as the eluent instead of superheated water. The result of the study was an 83.4% recovery of acetophenone in the first 1 ml fraction, demonstrating efficient trapping and thermal desorption.

Concentration of m-cresol using a SPE trap packed with Oasis HLB™. Again, it was found that a negligible recovery was achieved in the release of m-cresol when using superheated water but extraction with steam resulted in a recovery of 95%. A repeatability study, using the same trap found that after the fourth consecutive extraction the recovery decreased from 95.9 to 25%, again suggesting the collapse of the phase. Good recoveries in the range >90% were only achievable when a freshly packed trap was used for each extraction.

Appendix 1

The maximum degree of concentration was determined by loading a 1 l solution of dilute aqueous m-cresol (0.012 mg l^{-1}) on to a preconditioned trap. The trap was then washed with 4 ml deionised water. m-Cresol was eluted in the first 1 ml using steam at 210 °C. The result of the experiment was a recovery of 84%, suggesting that both trapping and thermal desorption were efficient and a significant degree of concentration had been achieved.

Conclusions

The use of water or steam as an extraction eluent for solid phase extraction has been demonstrated. Reproducible recoveries can be obtained from an SPE trap packed with PS–DVB, using superheated water as the extraction eluent.

Higher recoveries of non-polar and polar analytes, at trace levels, were obtained using an SPE trap packed with Oasis HLB™, using steam as an extraction eluent, whereas with this polymer recoveries using superheated water were low. Further work is being undertaken to examine the concentration of non-polar analytes such as naphthalene.

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