Development of low-pressure ion chromatography for the separation of anions

By

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A thesis submitted to Dublin City University in part fulfilment for the degree of

MASTER OF SCIENCE

Dublin City University.

School of Chemical Sciences.

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December 2005

Authors Declaration

I hereby certify that this material, which I now submit for assessment on the programme of study leading to the award of Master of Science is entirely my own work and has not been taken from the work of others' save and to the extent that such work has been cited and acknowledged within the text of my work

Signed Danielle Victory

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Date 26/06/06

Acknowledgements

I would like to acknowledge a few people who helped me through my MSc project

To my supervisor, Dr Brett Paull for his advice, support, encouragement and guidance throughout my project

I would also like to thank all the technical staff from the School of Chemistry in DCU, in particular Maurice for his help and assistance

To all the chemistry postgraduates who contributed in making my MSc an enjoyable experience

All in S205D thanks for your friendship and tolerance in the last two years of my project

To Cian, whom I tormented with proof reading, thanks for putting up with me!

And finally to Mam, Dad, Luke and Granny (for the prayers) who always believed in me and who provide me with encouragement and support in everything I do Thanks

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Abstract

Ion chromatography on short permanently coated monolithic columns has been investigated with the aim of developing a low-pressure chromatographic method for the separation of inorganic anions Both ion-chromatography and anion-exchange chromatography were studied as were three modes of detection namely, direct UV-Vis using a post-column reaction method, indirect UV-Vis and suppressed conductivity detection

Initially, the investigation of different monolithic columns with the later application of these to low-pressure anion-exchange separations was considered Traditional analytical columns used in chromatographic separations have high backpressures associated with them The introduction of commercial polymeric and silica based has effectively reduced the backpressure restriction associated with the traditional chromatographic stationary phase Hence, the use of short monolithic silica based anion exchange columns (25 mm and 10 mm column lengths) for the low-pressure separation of inorganic anions (fluoride, chloride, nitrite, bromide, nitrate and sulphate) was investigated The instrumental set-up consisted of a basic FIA manifold using simple peristaltic pumps, but incorporated a short low pressure anion exchanger The use of direct conductivity and indirect UV detection was examined for the determination of these inorganic anions using various concentrations of phthalate and *p*-hydroxybenzoate eluents Suppressed conductivity was also possible within the FIA/IC system by incorporation of a second low-pressure cation exchange column in the acid form after the short monolithic analytical column This approach significantly reduced the background conductance of the *p*-hydroxybenzoate eluent, resulting in improved detection limits for the above inorganic anions A two peristaltic pump system was then developed which allowed gradient separations to be carried out with suppressed conductivity detection The potential significance and advantages of this work in relation to low cost portable ion chromatography was evident

Finally, the development of another low-pressure ion chromatographic system (LPIC) using monolithic columns coated with DDMAU surfactant (N-dodecyl-N, N-(dimethylammomo) undecanoate) was also considered The column coating showed

excellent retention time reproducibility, even at elevated temperatures, over an approximate 17,200 column volumes Using a combination of low-pressure pumps and short 1 cm monolithic columns, it was possible to separate and detect both silicate and phosphate in surface water samples using post-column reaction (PCR) and UV/Vis detection at 840 nm Linear range was established between 1 and 10mg/L phosphate with this PCR method Total backpressures associated with this system were < 100 psi The possibility of using a near infrared LED device as the detection mode was also investigated for further miniaturisation of system components

Papers Published

"Low-pressure gradient micro-ion chromatography with ultra-short monolithic anion exchange column " Danielle Victory, Pavel Nesterenko and Brett Paull, **The Analyst**, **129** (2004) 700-701

"Rapid, low pressure and simultaneous ion chromatography of common inorganic anions and cations on short permanently coated monolithic columns " Damian Connolly, Danielle Victory and Brett Paull, **Journal of Separation Sciences**, **Vol. 27** (2004), **10&11**, 912-920

Poster Presentations

Posters were presented at the following conferences.

Combining FIA with Ion Chromatography, separation of inorganic anions on short monolithic ion-exchange columns, 56th Irish Universities Chemistry Research Colloquium, University of Limerick, Ireland, June 2004

Combining FIA with Ion Chromatography, separation of inorganic anions on short monolithic ion-exchange columns, Royal Society of Chemistry, Analytical Research Forum, University of Central Lancashire, Preston, UK, July 2004

Combining FIA with Ion Chromatography, separation of inorganic anions on short monolithic ion-exchange columns, 3rd Biennial Conference on Analytical Science, Ireland, University College Cork, Cork, Ireland, September 2004

Combining FIA with Ion Chromatography, separation of inorganic anions on short monolithic ion-exchange columns, 17th International Ion Chromatography Symposium, Trier, Germany, September 2004

Low-Pressure Ion Chromatography of Inorganic Anions-Determination of Silicate and Phosphate in Surface Water with Post-Column Reaction, Royal Society of Chemisty, Analytical Research Forum, University of Plymouth, UK, July 2005

List of Abbreviations

BCG	Bromocresol Green
CE	Capillary Electrophoresis
CTAB	Cetyltrimethylammonium Bromide
CPC	Cetylpyridinium Chloride
DDAB	Didodecyldimethylammomum Bromide
DDMAU	N-dodecyl-N, N- (dimethylammonio) undecanoate
DVB	Divinylbenzene
DNA	Deoxyribonucleic Acid
EDTA	Ethylenediaminetetraacetic Acid
FIA	Flow Injection Analysis
FWIC	
HPLC	High Performance Liquid Chromatography
IC	Ion Chromatography
IIR	Ion-Interaction Reagent
LC	Liquid Chromatography
LED	Light Emitting Diode
Li-DS	Lithium Dodecylsulphate
LPIC	Low-pressure Ion Chromatography
MeOH	Methanol
KCl	Potassium Chloride
ODS	Octadecyl Silica
PAR	4-(2-pyridylazo) resorcinol
PCR	Post-column Reaction
PEI	Polyethyleneimine
POE	Polyoxyethylene
PS-DVB	Polystyrene Divinylbenzene
RP-HPLC	Reversed-phase high performance liquid chromatography
S/N	Signal-to-noise Ratio
TBA-Cl	Tetrabuytlammonium Chloride
US-EPA	United States Environmental Protection Agency

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CHAPTER 1

Introduction

1.0 High Performance Liquid Chromatography

High Performance Liquid Chromatography (HPLC) is a fundamental analytical technique used today in many industries including environmental, pharmaceutical, food and beverage and power-generation to name but a few It has proven itself as a highly accurate and precise method for both qualitative and quantitative analysis of a large variety of samples A wide range of selective and non-selective detection modes are compatible with HPLC These include UV-Vis detection, conductivity detection (suppressed or non-suppressed), fluorescence detection and mass-selective detection

In HPLC, the liquid eluent is driven under high pressure through the analytical column containing a densely packed stationary phase In reversed-phase HPLC (RP-HPLC) the stationary phase contains a non-polar group chemically bonded onto a particulate support This is the most common mode of HPLC and the preferred method in industries for analytical separations of non-polar analytes

Modern HPLC instrumentation has become highly automated and is now capable of analysing multiple samples unattended, as most modern HPLC systems incorporate an autosampler Both isocratic and gradient separations, in some cases at increased temperatures, are also possible on typical HPLC systems developed by various manufacturers, e g Hewlett-Packard, Waters, Agilent Technologies However, despite these developments, sample backlog is still inevitable if a poor chromatographic method is chosen, hence the productivity of the laboratory suffers Some separations can prove to be particularly time consuming resulting in the need for shorter and more efficient columns to increase sample throughput Figure 1 1 illustrates a typical HPLC system

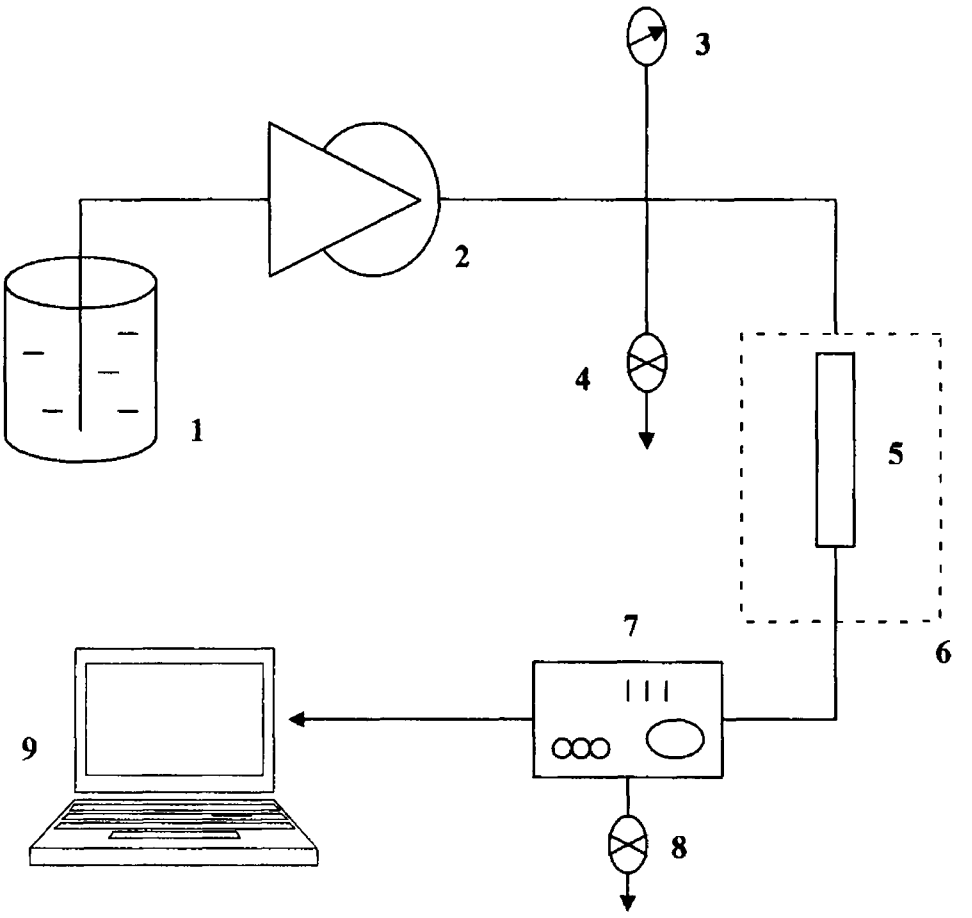

Figure 1.1 Schematic of a high performance liquid chromatography system
1=solvent reservoir, 2=high pressure pump, 3=injection valve, 4=injection waste, 5=separation column, 6=column oven, 7=detector, 8=waste collector, 9=data acquisition (recorder or integrator)

In the early days, Martin and Synge laid down the theoretical foundations for HPLC and developed 'plate theory', which was subsequently used as a formal measurement of the efficiency of the chromatographic process They realised that major increases in column efficiency would arise from decreases in the particle size of packings used as stationary phases [1] Efficiency is a measure of the narrowness of a solute band as it moves through the column Ideally, peaks will be very narrow which suggests that little or no band broadening is occurring during the chromatographic process Two parameters are considered when measuring the efficiency of a system number of theoretical plates (N) and plate height (H) The efficiency of a chromatographic column increases as the number of plates becomes greater and as the plate height becomes smaller [2] Analytes are carried through the stationary phase of the column

by a liquid mobile phase and these separations are based on the differences in migration rates through the column among the sample components The time a component takes to travel the length of the column L, is known as the retention time, t_R Each solute will partition between the stationary phase and the mobile phase based on its affinity for the particular phase, hence the retention time for each solute is different Movement down the column can only occur while the molecule is in the mobile phase Certain molecules travel rapidly by virtue of their inclusion in the mobile phase for a majority of the time, whereas others are retained in the stationary phase for a longer period, therefore resulting in different retention times for each solute The solute will not elute as an infinitely narrow band but rather as a broader band due to combined effect of a number of processes, which will now be examined

The retention mechanism is based upon the equilibrium between the stationary and mobile phases, shown in equation one below

$$\mathbf{A}_{\text{mobile}} \Longleftrightarrow \mathbf{A}_{\text{stationary}}$$

Equation 1

The migration rate is dependent on the partition ratio and is given by

$$\mathbf{K} = \frac{\mathbf{A}_{\text{stationary}}}{\mathbf{A}_{\text{mobile}}}$$

Equation 2

Where $\mathbf{A}_{\text{mobile}}$ = the equilibrium conc of solute A in the mobile phase

$\mathbf{A}_{\text{stationary}}$ = the equilibrium conc of solute A in the stationary phase

K= partition ratio

A theoretical plate represents a single equilibrium step, the greater the number of theoretical plates, the greater the efficiency of the column and the resolving power of the column The plate theory postulates that there are thousands of these equilibria established as a solute traverses through the column A typical high performance separation should render tens of thousands of plates per metre

For column length L, the height equivalent to a theoretical plate (H) is given by

$$H = \frac{L}{N}$$

Equation 3

For high efficiency in a chromatographic column, H should be as small as possible whereas N should be large The number of theoretical plates, N, can be determined from a chromatogram by measuring retention time (t_R) and peak width (W)

$$N = \frac{(4t_R)^2}{W}$$

Equation 4

Factors affecting the number of theoretical plates in a column are,

- Characteristics of column (packing and stationary phase)
- Characteristics of the solute
- Temperature
- Flow rate
- Method of sample introduction [3]

Examination of peaks in a typical chromatogram or the concentration profile of bands on a column reveals that their shapes are similar to Gaussian distribution curves Typical Gaussian shape of a chromatographic band can be attributed to a combination of random motions of the solute molecules as the chromatographic band moves down the column [2]

The diameter of the packing particles and the thickness of the liquid coating on the stationary phase influence plate height and subsequently affect column efficiency Efficiency of a column is inversely proportional to particle size If shorter columns packed with smaller particles are used the linear velocity of the eluent, and the diffusion coefficients of the solute between the mobile and stationary phases will be reduced, thus resulting in a more efficient separation

After an injection is made, a narrow band is broadened during its movement through the column If column band broadening is high, consequently the number of components that can be separated on a particular column will be low The ideal chromatographic process is one in which the components of a mixture form narrow bands which are completely resolved from one another The efficiency of a column is described by the following van Deemter equation[2]

$$H=A+B/u+Cu$$

Equation 5

Constants A, B, C and u represent coefficients of eddy diffusion, longitudinal diffusion along the column, mass transfer and the linear velocity of the eluent, respectively The ultimate width of a peak is determined by the total amount of diffusion occurring during movement of the solute through the system and on the rate of mass transfer between two phases The van Deemter equation summarises on-column effects that contribute to plate height and hence band broadening

Eddy diffusion arises from inconsistencies in flow velocities and path lengths around packed particles Small tightly packed columns give a small A term as the solute molecules must find the shortest pathway through the bed while they travel along the column within the mobile phase Some solute molecules travel through the column in a direct path whereas others will not find a direct pathway through the bed and undergo several diversions along the way, i e eddy diffusion This multiple path term is diminished by reducing particle size and column length

Longitudinal diffusion results in the molecular diffusion of the analyte longitudinally at low mobile phase velocities High diffusion rates of the solute in the mobile phase can also cause the solute molecules to diffuse axially (at right angles to the mobile phase flow), while slowly migrating through the column and subsequently increasing band broadening Longitudinal diffusion results in the migration of a solute from the concentrated centre of a band to more dilute regions, i e diffusion away from the peak centre towards or against the direction of flow of the mobile phase It is independent of the particle size of the stationary phase This type of diffusion is inversely proportional to the velocity of the mobile phase, it can be minimised with

increasing flow rates At low velocity, the elution time is longer and as a result there is more time for diffusion to cause the band width to increase [3]

Mass transfer is the dominant cause of band broadening and is directly related to the velocity of the mobile phase At high velocities, there is less time available for equilibration of the system, resulting in an increase in the mass transfer term Most stationary phases contain pores, which analytes can diffuse into and out of and therefore interact with the stationary phase It is because of the presence of these pores that silica phases have such a high bonded surface area When equilibrium within the column is established the pores are filled with mobile phase, which does not move When a sample molecule enters a pore, it is trapped and will not be carried by the mobile phase outside of the pores, while the sample molecules outside the pores in the intra-particle channels will be carried forward by the mobile phase The trapped molecules inside the pores can only return to the bulk mobile phase by diffusion, and this complete process results in band broadening The smaller stationary phase pores are, the smaller the diffusion distance and the smaller the band broadening effects Diffusion rates of solutes in and out of the pores are higher when the viscosity of the mobile phase is reduced Figure 1 2, the van Deemter curve, demonstrates the band broadening effects in relation to column efficiency

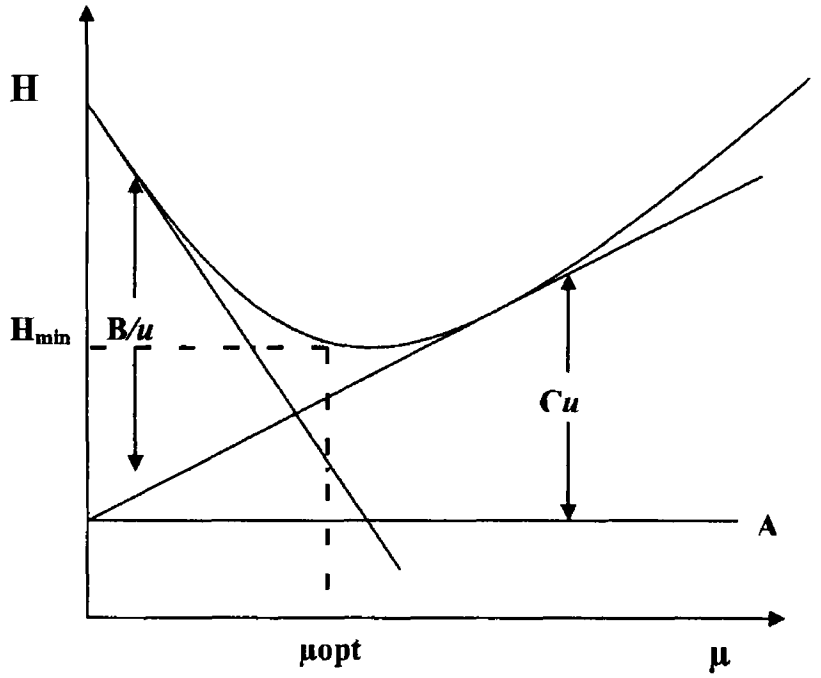

Figure 1.2. Theoretical van Deemter plot [3]

Where A=eddy diffusion, B/*u*=longitudinal diffusion component, C*u*=mass transfer, μopt=optimum eluent flow velocity

The optimum flow rate and hence efficiency is reached at the point on the curve where H is minimised and hence N is optimised Smaller particle sizes reach their μopt at higher flow rates than larger sizes, allowing an equivalent efficiency to be achieved at a faster rate The use of smaller particle sizes results in flatter curves and showing that increased flow rates have less adverse affects on the efficiency of smaller packings

Resolution R, provides a quantitative measure of the ability of a column to separate two analytes For quantitative analysis, a resolution of 1 5 is desirable and corresponds to baseline resolution of 2 bands of similar size

$$R = \frac{2(t_{R1} - t_{R2})}{(W_1 + W_2)}$$

Equation 6

Where t_{R1} and t_{R2} are the retention time of peaks 1 and 2 respectively and W_1 and W_2 are the peak widths at the base height of peak 1 and 2 respectively

1.1 Ion Chromatography

The foundations of modern Ion Chromatography (IC) were laid down by Small, Stevens and Bauman in 1975, when they developed an ion-exchange chromatographic method for the separation and suppressed conductimetric detection of anionic and cationic species[4] Since 1975, there have been continuous advances in this technique, which has resulted in IC being routinely used by a number of industries for inorganic and organic ion analysis [5] This technique was then developed by the Dionex Corporation commercially and IC grew rapidly as an analytical technique as it allowed for the separation and determination of complex mixtures of anions and cations in aqueous samples IC can be used for the determination of morgamc anions and cations, low molecular weight carboxylic acids, organic bases and ionic organometallic compounds, e g tributyltin[6] A typical standard IC method is the determination of the seven common anions in drinking water, detailed within USEPA Method 300 1, which uses a carbonate eluent with suppressed conductivity detection [7] IC offers a wide range of advantages including speed, sensitivity, selectivity, simultaneous detection of multiple sample components and highly stable and efficient separator columns [8]

Modern IC as a mode of liquid chromatography can be based on any of three different separation mechanisms ion-exchange chromatography (high performance ion chromatography), ion-exclusion chromatography (high performance ion exclusion chromatography) and ion-pair chromatography (eluent ion chromatography) [8]

It is possible to separate a range of ionic analytes, which possess hydrophobic character by reversed-phase chromatography when a suitable eluent pH or the use of a pH gradient is used The ionisation of these analytes can be suppressed and they move through the column as neutral molecules and interact with the stationary phase by reversed-phase hydrophobic interactions However, for hydrophilic morgamc anions (e g chloride, nitrate, nitrite, sulphate, phosphate etc) and cations, which have been derived from strong acids and bases and dissociate over a wide pH range, ionisation suppression is not possible within the normal working pH range of these

reversed-phase silica columns (pH 3-7) Hence, little retention, if any, is possible on C_{18} stationary phases when using conventional reversed-phase eluents

There is a growing trend towards moving IC measurements away from the laboratory and to use the technique for real-time in-situ monitoring However, separation times in IC are too long for the continuous monitoring of samples Improvements in separation speed can be achieved by decreasing column length (simultaneously decreasing particle size), so that the analysis time is reduced while maintaining efficiency This method was used by Connolly and Paull to achieve sub-minute separations for nitrate and nitrite and a 2 5 minute separation of nine common anions using a 30 mm x 4 6 mm i d reversed-phase column and ion-pair chromatography [5]

1.2 Ion-exchange

Ion-exchange is based on ion-exchange processes occurring between the eluent and chemically bonded or coated charged functional groups on the stationary phase When an ionic solution is passed over the stationary phase, the ions on it exchange with those in the solution The two main classifications are simply cation and anion exchangers In an ion-exchange separation, the role of the eluent ions are to compete with solute ions for the fixed ions on the stationary phase and to separate the mixture of solute ions into well-defined bands [6] The ion-exchanger is classified as a cation exchanger when the fixed ion is negative, the opposite applies for the anion exchanger Ion-exchange chromatography can be used for the separation of both anions and cations Separation of anions is generally achieved with quaternary ammonium groups attached to the stationary phase, whereas sulphonate, carboxyl or phosphonate groups are used as ion-exchange sites for the separation of cations [8]

In ion-exchange the following factors must be considered if optimum conditions are to be implemented

1 The more ionic groups present on an ion exchanger, the greater its exchange capacity i e its separating ability The exchange capacity of a weak ion exchanger will be altered by varying the pH of the eluent Weak cation exchangers are not dissociated at pH's below 4, as its ionic groups are weak acids At this pH 'the hydrogen form' comes into effect and the protons are

too strongly bound to the stationary phase to exchange places with the sample ions Subsequently resulting in very little stationary phase exchange capacity Strong ion exchangers are preferred as they remain dissociated over a wider range of pH values [9] Table 1 1 below illustrates the exchange capacity of different ion exchangers as a function of pH

Table 1.1: Exchange capacity of different types of ion exchanger as a function of pH[9]

Type of ion exchanger	Full exchange capacity
Strong cation exchanger	Above pH 3
Weak cation exchanger	Above pH 6
Strong anion exchanger	Below pH 9
Weak anion exchanger	Below pH 6

2 As the concentration of the competing ion in the eluent increases, the retention time of the solute ion decreases [6]

3 Eluent pH influences the charges on both the eluent and solute ions Weak acids are commonly used as eluents for IC of anions and weak bases are used as eluents for cation IC The charge on the acid anion increases with pH, and hence the eluting power of weak acid eluents increases with pH until the weak acid completely dissociates For weak acid and base eluents an increased solute charge results in increased retention because the electroselectivity effect for the solute ion is enhanced with higher charge [6]

4 Ion exchange is also influenced by the counter-ion in the eluent The ion exchanger typically prefers an ion with higher charge, smaller diameter and greater polarisability (greater potential for moving electric charge i e better dipole induction capacity) [9]

1.3 Ion-interaction Chromatography

Ion-interaction chromatography is a useful analytical technique that allows for the separation of both inorganic and organic ions, with similar efficiency and resolution values to those obtained by reversed-phase LC Retention and separation of hydrophilic ionic solutes (inorganic cations and anions) on hydrophobic stationary

phases can be achieved by the addition to the eluent of a hydrophobic reagent ion having the opposite charge to that of the solute ion Retention is therefore based upon the formation of a neutrally charged ion-interaction between these two species The dominating separation mechanism in ion-interaction chromatography is adsorption The stationary phase consists of a neutral porous divinylbenzene resin of low polarity and high specific surface area, or a more commonly chemically bonded octadecyl silica phase The eluent contains an organic modifier and an ion-interaction reagent which is selected depending on the chemical nature of the analytes Ion interaction chromatography is suitable for the separation of surface-active anions and cations, sulphur compounds, amines and transition metal complexes [8] The advantages of using ion-pair chromatography as a separation technique include the ability to use reversed-phase systems and to separate acids, bases and neutral molecules A number of parameters influence the adsorption of the ion-interaction reagent (IIR) on the stationary phase and also the retention of the solutes These include the concentration and nature of the IIR The nature of the stationary phase, the concentration of the organic modifier in the eluent and the concentration and nature of the counter-ion present in the eluent

1 The concentration and nature of the IIR

An ion-interaction reagent is typically a long chain alkyl hydrophobic molecule with a charged group at one end (for anion determinations an ammonium group is at the end and in the case of cation determinations a sulphonate group) When the length of molecule increases, the hydrophobicity of the IIR also increases, which results in increased interaction with the stationary phase and consequently an increase in the 'dynamic exchange capacity' of the column This increases the retention of oppositely charged solute ions An increase in the IIR concentration in the eluent will also have this effect Retention increases to a certain point, and then decreases with further additions of IIR, due to the increasing concentration of the IIR counter ion in the eluent, which acts as a competing ion This also results in solubility problems due to the increased concentration of the IIR

2 The nature of the stationary phase

Retention increases with increased hydrophobicity of the stationary phase, e g C_{18} compared to C_8

3. The concentration of the organic modifier

The analyte/IIR ion pair has a neutral charge and retention is based on its ability to interact with the hydrophobic stationary phase An organic solvent is added to the eluent to decrease the retention of oppositely charged analytes, resulting in shorter run times

4. The concentration and nature of the counter ion

An increase in the IIR counter ion concentration will result in a decrease in retention The dynamic ion-exchange retention model suggests that the stationary phase has ion-exchange character when a charged IIR is adsorbed For this reason, the IIR counter-ion (which has the same charge as the analyte) will act as a competing ion and thus contribute to decreased retention A decrease in retention also occurs if a counter ion is chosen which has increased elution strength, i e a divalent ion instead of a monovalent ion

Ion interaction chromatography occurs on the basis of three models the ion-pair model, the dynamic ion exchange and the ion-interaction model

1.4 Ion pair model

This model proposes that a charged IIR forms an ion pair with an oppositely charged analyte and the resultant neutral ion-pair can partition and interact with the stationary phase by reversed-phase hydrophobic interactions

1.5 Dynamic ion-exchange model

This model postulates that the charged IIR is in dynamic equilibrium with the mobile and stationary phases and will absorb onto the stationary phase via hydrophobic interactions This exposes a charge on the stationary phase and results in it acting as an ion-exchanger As already mentioned, the IIR is in dynamic equilibrium with the mobile and stationary phases, hence the charged active sites are not fixed as in conventional ion-exchange Therefore, the column is considered as a dynamic ion-exchanger Changing the nature of the IIR, i e its concentration in the eluent and the organic modifier, can change the dynamic exchange capacity of the column Oppositely charged solute ions will compete with the IIR counter-ions for the active charge sites on the stationary phase [6]

1.6 Ion-interaction model

This is a combination of the two models previously discussed. It incorporates both electrostatic effects, which are the basis of the ion-pair model, and adsorptive effects, which are the basis of the dynamic ion-exchange model. [6] Figure 1.3 illustrates the effects of the ion-interaction model.

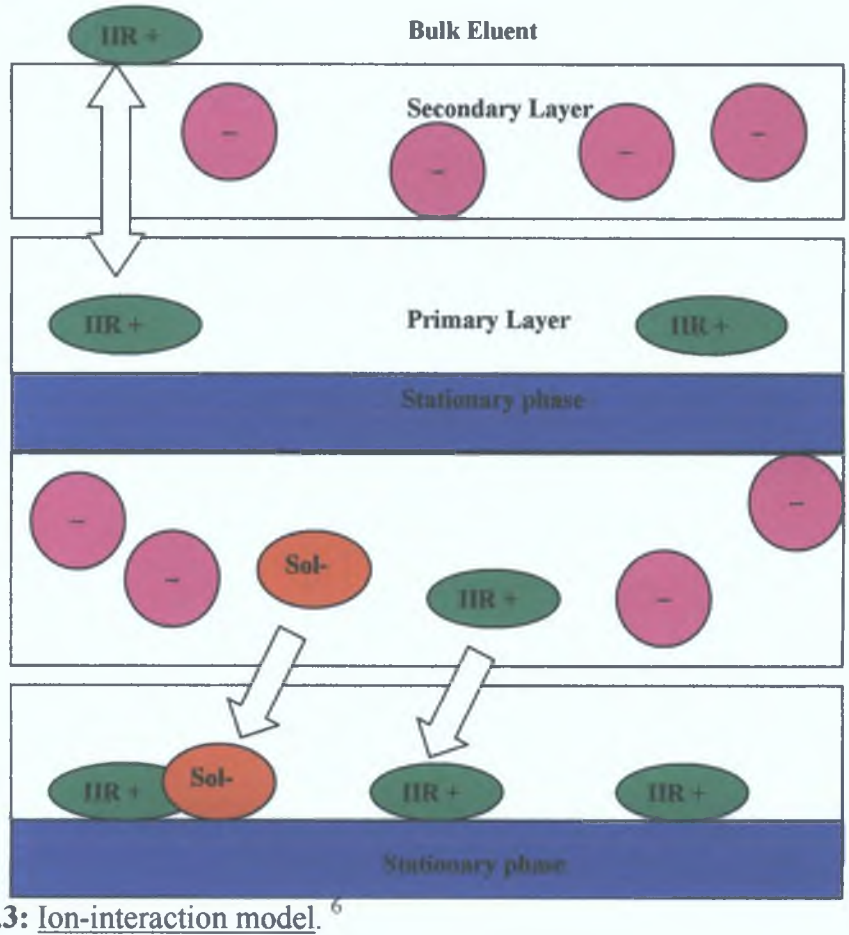

Figure 1.3: Ion-interaction model. [6]

This represents the determination of anions, as the IIR is a cation. The pink circle is the IIR counter-ion.

Connolly and Paull successfully separated UV absorbing anions by fast ion-interaction chromatography. Tetrabuytlammonium chloride (TBA-Cl) was used as the IIR in the eluent. The eight inorganic and organic anions were determined by direct UV detection at 225 nm. The authors considered the following two parameters very carefully: concentration of the IIR and the concentration of the organic modifier present in the eluent.[10] Connolly and Paull also studied the use of ion-interaction liquid chromatography (using similar IIR and detection method) for the rapid determination of nitrate and nitrite in drinking water. The addition of a peristaltic

pump and in-line filter, proved successful for the analyses of 60 real tap water samples per hour [11]

1.7 Coatings used in Ion Chromatography

Two types of coating mechanisms are implemented in IC, namely 'dynamic modification' and 'permanent coating' of the solid reversed-phase stationary phase

The liquid ion exchangers used for dynamically coated IC vary from moderately hydrophobic to strongly hydrophobic (i e tetrabutylammonium to hexadecyltrimethylammomum ions) This method involves the use of a eluent containing a liquid ion exchanger, which is in dynamic equilibrium with the stationary phase [6] The stationary phase can be considered to possess a 'dynamic exchange capacity' when the column is dynamically coated with a liquid ion exchanger The dynamic exchange capacity is dependant upon the eluent composition i e the concentration of organic modifier and type of liquid ion exchanger

O'Riordain *et al* used this approach in zwitterionic IC by using carboxybetaine surfactants to dynamically coat particle packed and monolithic columns The eluent used in these separations contained 0 2 mM of the carboxybetaine surfactant to stabilise the column coatings and to maintain a constant amount of the carboxybetaine surfactant on the surface of the stationary phase These coated monolithic columns were capable of separating anions rapidly using elevated flow rates up to 4 5 mL/min and flow rate gradients [12] This method of dynamically coating a monolithic column was also considered by Xu *et al* [13] The authors dynamically coated the monolith with lithium dodecylsulphate (Li-DS), which was also present in the eluent, to stabilise the stationary phase This method was suitable for the rapid and efficient separation of hydronium, magnesium and calcium within 4 minutes at a flow rate of 4mL/min in rainwater samples

Dynamic coatings were also used in zwitterionic ion chromatography by Twohill and Paull These authors used a mobile phase recycling method to ensure the stability of

the adsorbed coating (Zwittergent 3 14) The retention times of the sulphate, chloride and nitrate peaks over a period of three weeks varied by less than 2 5% [14]

The second method is to permanently coat the stationary phase with a strongly retained liquid ion exchanger, changing the stationary phase to an ion-exchange phase This liquid ion exchanger is used initially to equilibrate the stationary phase and hence establish a very strongly bound coating of the liquid ion exchanger on the stationary phase This coating is stable for long periods of continual usage and for this reason was termed 'permanent coating ion-exchange chromatography' by Haddad and Jackson[6] Since the stationary phase has now been converted into an ion-exchanger by virtue of the absorbed liquid ion exchanger, the eluents used are identical to those utilised in conventional ion-exchange This method of chromatography offers a range of advantages when compared with conventional ion-exchange which include

- Ion-exchange capacity of the column can be varied by altering the composition of the equilibrating solution, i e hydrophobicity and concentration of the liquid ion exchanger and concentration of organic solvent present in the equilibrating solution
- Adsorbed liquid ion exchanger on the stationary phase can be stripped or renewed as desired by washing of the column with an organic solvent
- High efficiency of reversed-phase packing particles is often retained when these packings are converted into permanently coated ion-exchangers [6]

The capacity of a coated column was estimated by Hatsis and Lucy through the breakthrough curve by the following equation

$$Q=CF\ (t_b-t_o)$$

Equation 7

Where Q is the capacity of the coated column (µmol/column), C is the concentration of liquid ion exchanger in the coating solution, F is the flow rate in mL/min, t_b is the time for complete breakthrough (min) and t_o is the void time of the column (min) These authors used the above expression (equation 7) to determine the stability of a didodecyldimethylammonium bromide (DDAB) coated column by continually flowing 6 mM o-cyanophenol (pH 7 0) through the column at 1 mL/min A

chromatogram was acquired every hour until the separation of the test anions was compromised, i.e., a 10% change in retention time was observed. [5]

Hatsis and Lucy also used this approach of permanently coating a reversed-phase monolithic column with DDAB to perform ultra-fast separations of iodate, chloride, nitrite, bromide, nitrate, phosphate and sulphate in 30 seconds. Separations were performed using a 6 mM o-cyanophenol (pH 7.0) eluent and suppressed conductivity detection. Detection limits within the ppb range were observed for all the anions mentioned. [5]

The possibility of separating seven common anions using a permanently coated ODS analytical column with the surfactant DDAB by fast ion-exchange chromatography and indirect UV detection was considered by Connolly and Paull, which proved to be successful for the determination of ions in real water samples (river, sea).[15]

A reversed-phase column permanently coated with n-cetylpyridinium chloride was used by Jun *et al.* for the determination of inorganic anions and carboxylic acids (lactic, succinic, malonic, malic, maleic and tartaric) in wines using phthalate eluents and indirect UV detection. Satisfactory and reproducible results were obtained even after four months of continual use of this column. [16]

Sato *et al.*, considered the possibility of separating and detecting four oxoanions-vanadate, tungstate, molybdate and chromate. Ion-exchange chromatography and ion-pair chromatography with tetrabutylammonium hydroxide (TBA-OH) and with cetyltrimethylammonium bromide (CTAB) as the ion interaction reagent were compared. The use of a carbon column, a Na_3PO_4 eluent, UV detection and a permanent coating technique resulted in these four oxoanions being separated without any interference from common inorganic anions. [17]

An ODS column permanently coated with cetyltrimethylammonium chloride was capable of separating seven common inorganic anions (fluoride, chloride, phosphate, bromide, nitrite, nitrate and sulphate) in less than 10 min using a 1.5 mM sodium phthalate eluent (pH 6.8) and UV detection at 277 nm. The column was converted to phthalate and salicylate anion forms by the phthalate and salicylate eluents. The

anion-exchange capacities of the cetyltrimethylammonium (CTA^+)-coated columns were determined from the breakthrough volume of phthalate or salicylate eluents. The separation improved with increasing anion-exchange capacity for the phthalate eluent but not for the salicylate.[18]

Double-coated monolithic columns have been employed for the determination of hydroxide from other anions based on a conventional ion-exchange mechanism using sodium sulphate eluents at high pHs. An ODS monolithic column was first coated with a nonionic surfactant (polyoxyethylene (POE)) and then with a cationic surfactant CTAB. The double-coated stationary phase was thought to be superior to a single coating of CTAB. A range of samples comprising of both strong and weak bases were analysed by this method. The double-coated stationary phase was less affected by increased flow-rate than was the single-coated stationary phase. The hydroxide ion could be separated from the eluent dip and from chloride, nitrate and sulphate using this double-coated monolith. [19]

Fritz and co-workers have also reported that double coating of monolithic columns with non-ionic surfactants (Tween 20 or Triton-X-100) and then cationic surfactants (DDAB) dramatically improves the efficiency of the column for anion separations and also reduces the retention times of the ions. It was also found that dynamic coating by incorporating a small amount of an alcohol, diol or zwitterion in the aqueous eluent permits good separations for alkanecarboxylic acids. Anions have been separated previously on HPLC columns coated with cetylpyridinium salts and polyethyleneimine (PEI). These authors found that the most efficient column for anion chromatography was prepared by coating a Phenomenex Synergi column with 5 mM Triton X-100 from 30% acetonitrile and then aqueous 5 mM cetylpyridinium chloride (CPC). The eluent was 2 mM sodium perchlorate and direct UV detection at 210 nm was used. [20,21]

1.8 Detection methods used in Ion Chromatography

1.8.1 Conductivity detection

This mode of detection for IC has been used since 1975. It has two major advantages for inorganic ion detection in that all ions are electrically conducting, resulting in a universal response, and conductivity detectors are reasonably simple to construct and operate.

The theory behind this detection mode is that any electrolytic solution conducts a current when an anode and a cathode are inserted into the solution and a voltage is applied across them. In this case, the detector contains a cell containing the electrodes that allow the electrolytic eluent to pass through it continuously. A voltage is applied and the resulting conductance is measured, i.e. the signal.[4] If the limiting equivalent ionic conductance of the eluent is low, the analyte ion enters the conductivity cell and causes an increase in conductivity thus resulting in detection by direct conductivity, where the analyte ion has a higher conductance than the eluent ion. Indirect conductivity detection is the opposite of this, i.e. a decrease in voltage signal will result from the analyte entering the conductivity cell.

IC with suppressed conductivity detection is most widely used and generally offers the best performance. In this particular mode of detection an eluent containing a suitable electrolyte (NaCl) is passed over the suppressor membrane and then onto the conductivity detector.[22] The suppressor reduces background conductivity by acid-base neutralisation and removal of ions from the eluent. The suppressor modifies both the eluent and the separated analytes coming out of the separator column, so that the eluent conductance is reduced and that of the analytes is enhanced, hence detectability of the analytes is improved giving rise to a large increase in the signal to noise ratio of the detection signal. [22]

If an eluent contains a salt of a weak acid, (sodium bicarbonate) replacement of sodium ions with hydronium ions forms weakly conducting carbonic acid. Bicarbonate eluents have been used in IC for many years. However, the carbonic acid formed after suppression is vulnerable to partial dissociation leading to some

background conductance. Eluents containing hydroxide ions are ideal as they form water after suppression, which has practically zero conductance. However, this eluent also creates two problems; firstly, it readily absorbs carbon dioxide to form carbonate which changes the concentration of hydroxide present, leading to changes in retention times of analytes. Secondly, the presence of this carbonate in the eluent leads to unstable baselines in the chromatograms. Electrolytic eluent generators can be a means of solving this problem as the eluents are generated inside a closed system and hence reduce contamination effects.

Conductivity detectors are sensitive to even minor pump fluctuations during separations. To combat this problem the addition of pulse dampeners was investigated by Jackson *et al.*[23] This proved crucial in obtaining a pulseless baseline for both suppressed and non-suppressed conductivity detection and decreased the height of baseline noise by three. Column temperature control also proved essential in achieving sub-ppb detection limits in non-suppressed IC.

A wide range of applications have been carried out using suppressed and non-suppressed IC. A typical application includes the determination of inorganic anions and divalent cations (Ca^{2+} and Mg^{2+}) using suppressed ion-exchange chromatography and a sodium carbonate-EDTA eluent. [24]

Pyromellitate acid as an eluent was considered for the separation of inorganic anions and divalent cations by Ohta and Tanaka.[25] In their paper, upon introduction of pyromellitate eluent and inorganic anions into a cation-exchange membrane suppressor in the acid form after anion-exchange separation, pyromellitate and the anions were converted into pyromellitic acid and the anions were converted into their corresponding acids. The acids of the anions were detected conductimetrically as an increase in the concentration of the H^+ in the eluent. In contrast, divalent cation-pyromellitate complexes were converted into pyromellitic acid by the suppressor and these divalent cations were detected as an increase in the concentration of pyromellitic acid in the eluent.

Wang *et al.*, were responsible for the simultaneous determination of mono (MFA), di (DFA) and tri (TFA) fluoroacetate using a Dionex anion-exchange column,

potassium hydroxide gradient and suppressed conductivity detection. The total analysis time for this separation was 35 min. The fluoroacetates were successfully separated from the inorganic and organic species usually associated with environmental samples. [26]

Lu *et al.*, investigated the possibility in the determination of nine inorganic and organic acid anions (fluoride, acetate, formate, chloride, nitrite, sulphate, bromide, nitrate and phosphate) in power plant water samples at μg/l levels. The samples were injected using a large-volume direct injection technique and separated on a hydroxide-selective anion-exchange column using high-purity hydroxide eluents and suppressed conductivity detection. Method performance was evaluated by the authors by analysing synthetic water samples containing additives present in the power plant water samples and four water samples from a fossil fuel power plant.[27]

In the pharmaceutical industry, there is an increasing trend to conduct small, rapid, clinical studies to aid in the selection of active pharmaceutical ingredient (API) candidates for further development. Cassidy *et al.* developed a method for the quantification of anionic constituents in APIs by suppressed conductivity anion exchange chromatography. [28]

1.8.2 UV-vis detection

This is another common mode of detection used for the detection of ions. Temperature variance is not an issue as it is in conductivity detection and it can also be used for gradient elution. These are simple and inexpensive instruments, which are easy to use.

Several inorganic anions absorb UV radiation above 200 nm, while fluoride, chloride, phosphate and sulphate absorb below this. Detection selectivity exists and gives rise to common interferences. Detectors are usually operated with fixed or variable lamps. Fixed wavelength lamps can be up to 20 times more sensitive than variable wavelength lamps. However, variable wavelength lamps are more suited to a wider range of analytes. The use of lower wavelengths permits the use of direct UV as the

mode of detection in which all eluent components must be transparent or weakly absorbing at the detection wavelength used.

Direct UV detection occurs when the analyte ion causes an increase in the signal being recorded. The separation of common inorganic cations cannot usually be carried out by direct UV detection, as their molar absorptivities are too small. However, the introduction of a ligand to complex with the cation can make it readily detectable in the UV range. Common eluents for the direct detection of anions include phosphate and bicarbonate/carbonate buffers and sodium chloride. Direct UV detection is also employed for the determination of nitrates and nitrites as these ions have relatively high molar absorptivities.

Ito *et al.*, used this mode of detection for the determination of nitrate and nitrite by IC using monolithic ODS columns connected in series coated with 5 mM cetyltrimethylammonium chloride (CTAC) using 0.5 M NaCl as the eluent. The column efficiency did not decrease even with increased flow rates of eluent. Direct UV detection was carried out at 225 nm for these two analytes. These two anions could be separated without matrix interferences from artificial seawater samples in less than 3 min.[29]

Bromate was successfully determined by Kuldvee *et al.*, using an IC method and direct UV detection at 204 nm. An 11 mM borate buffer pH 8.76 and a Dionex AS9-S6 column were used in this separation. Low µg/l levels of bromate could be easily attained using an experimental arrangement of direct pseudo random injections of large quantities of the sample and UV detection at 204 nm and subsequent decorrelation of the detector signal. This chromatography is correlation chromatography and places a high demand on reproducibility. This chromatography is less sensitive to spikes and baseline drift.[30]

Indirect UV detection occurs when the eluent ion has a higher molar absorptivity than the solute ion (when both are univalent). Common eluents for the detection of anions include phthalate and nitrate. Upon elution of the analyte ions, a decrease in the recorded signal is observed as the ions absorb less strongly. This detection mode

is used for analytes that do not absorb strongly. Eluent pH is an important variable in indirect UV detection. The pH affects the charge on the eluent anion and the molar absorptivity of the eluent; therefore, the use of incorrect eluent pH may lead to a decrease in detection sensitivity.

Ohta and Tanaka investigated the possibility of simultaneous determination of common inorganic anions, magnesium and calcium ions in environmental samples using this detection method.[25] This separation was achieved by using an anion-exchange column and an eluent containing weak acids, trimellitic acid and EDTA. Later, this mode of indirect detection was employed for the photometric detection of mono/divalent cations (Li^+, Na^+, NH_4^+, K^+, Mg^{2+} and Ca^{2+}) by IC, using an unmodified silica gel column by Ohta.[31]

The above workers also investigated the possibility of indirect UV detection and non-suppressed IC for the separation of inorganic anions and divalent cations (Mg^{2+} and Ca^{2+}) using a pyromellitate eluent on an anion-exchange column. Indirect UV detection proved to be the more sensitive of the two detection methods. These ions formed complexes with the pyromellitate eluent that were detected indirectly as anions and directly as cations by UV photometric detection.[32] Litvina *et al.* [33] considered the determination of aluminium in natural waters by ion-exchange chromatography with indirect UV detection. A solution of non-complexing trivalent Ce (III) was used as the eluent. The detection limit for aluminium under optimum conditions was 50 ng/mL; this limit was decreased even further with the inclusion of a preconcentration procedure.

In recent times, ion-exchange has been combined with capillary electrophoresis for anion separations. This was possible by the addition of a cationic polymer to the electrolyte. Indirect detection was then carried out. A system peak formed due to the counter anion of the polymer. The position of the analytes could be changed in relation to the system peak. Conversion of the polymer to replace the counter-ion with the indirect UV detection probe ion was investigated. The optimised electroyte composition (0.28% (poly (diallyldimethylammonium)) PDDA, 16.25 mM chromate buffered with 10 mM histidine (pH 7.70)) containing a chromate probe achieved the separation of 24 inorganic and organic anions in less than 7min.[34]

1.8.3 Post-Column Reaction Detection (PCR)

PCR is mostly used in combination with UV-vis detectors but other techniques like fluorescence or luminescence are possible. Most common routine applications of PCR are still in the area of metal ion analysis.[35] PCR has a wide range of advantages including high selectivity, wide linear range, and is often able to achieve low detection limits. PCR involves a chemical reaction of the analytes with the post-colum reagent, as they are eluted from the column, before they pass through the detector. Low concentrations of analytes within high concentrations of matrix interferences can be detected in this way as this approach enhances the specificity and sensitivity of the detection method. PCR is performed by the introduction of suitable reagents into the column effluent. These reagents are added as a solution, which is mixed directly with the column effluent. PCR systems can increase the baseline noise of a UV-vis detector, the extent to which depends on the post-column delivery method used and the reaction involved. [35]

In 1999, Miura and Hamada considered the possibility of using PCR for the determination of nitrite in aqueous solutions at ppb levels with photometric measurement of iodine formed. A photometric measurement of iodine formed by a reaction of nitrite with iodide has been found to make it possible to determine trace amounts of nitrite without any interference from chloride. The proposed method was based on the separation of nitrite from matrix anions on a silica-based anion-exchange column with a phthalate eluent (pH 5.0), followed by photometric measurement of the iodine (as tri-iodide) formed via a post-column reaction of the separated nitrite with iodide.[36]

Sugrue *et al.*, investigated the possibility of using this method of detection for the separation of alkaline earth and transition metal ions using solvent based ammonium and sodium acetate buffered eluents on a silica monolith. Detection of the transition metal ions was achieved using a post column reaction with 4-(2-pyridylazo) resorcinol at 510 nm.[37]

In 2000, Wasim and Paull examined the possibility of using PCR detection for the determination of Be (II) in water samples at low µg/L, on an iminodiacetic acid (IDA)

functionalised silica gel column Be (II) was detected at 590 nm following a PCR reaction with chrome azurol S (CAS) The eluent used was 0 4 M KNO_3 (pH 2 5) [38]

Bromate is a by-product of the ozomsation of water, is it therefore regulated for human consumption in low µg/L ranges Valsecchi et al , developed an IC method for the determination of bromate on a high capacity anion-exchange column using tetraborate eluents with serially connected conductivity and spectrophotometric detection The bromate present in the water samples was detected by PCR with fucshin at 520 nm Above injection volumes of 1 5 mL, a sample pre-treatment with a cartridge in Ag and H form, followed by a 10 min degassing in an ultrasonic bath was needed due to the overlapping of the chloride present in the samples with the bromate peak response [39]

Jones *et al* [40] investigated the determination of arsenate, silicate, germinate and phosphate by post-column reaction with molybdenum blue Detection was based on the molybdenum blue reaction, which reacts with all four oxoamons sensitively This work will be discussed in detail later in the Chapter 5

1.9 Low-pressure Ion Chromatography (LPIC)

Recently, within the field of analytical chemistry smaller, portable and more inexpensive systems for on-line or in-situ analysis have received increased attention Within this context, the analytical technique of low-pressure IC (LPIC) has been considered High back-pressures associated with traditional HPLC and IC analytical columns have meant the growth of these LC techniques into the fields of portable and micro-scale systems has been hindered by the constant need for expensive high-pressure pumping systems In the past, only a few authors have attempted to develop low-pressure systems for high-performance separations of ions [41] [42], the most successful of which has been based upon the use of ultra-short columns (3-6 cm) packed with relatively large ion exchange particles for the analysis of alkali metals alkaline earth metals, transition-metals, rare-earth elements, inorganic anions, organic acids and common amino acids[42] However, the commercial introduction of polymeric and silica based monolithic stationary phases has effectively reduced the backpressure restriction associated with the traditional chromatographic stationary

phase Now simple peristaltic pumps can be used to obtain significant flow rates through short monolithic stationary phases capable of exhibiting reasonably high separation efficiencies This means that the obstacles once separating the fields of low cost FIA and HPLC have been removed LPIC offers the possibility of maintaining the overall backpressure of a chromatographic system below 100 psi The ability to separate solutes using short monolithic phases with relatively high efficiency whilst generating minimal backpressure across the column presents a number of obvious advantages in terms of simplifying and potentially miniaturising LC equipment [43] The length of the coated monoliths was thought to further reduce the size of the micro-LPIC system

Jiang *et al* [42] considered the use of a low-pressure ion chromatograph for the analysis of alkali metals, alkaline earth metals, transition-metals, and inorganic anions, which operate at low pressures of $1.95 \times 10^5 \sim 2.94 \times 10^5$ Pa Ultra-short columns were used in these separations These columns reduced the system pressure The optimised method achieved high sensitivity and precision for the determination of these analytes An LPIC column whose exchange capacity was 0 01 mmol/g achieved the best separation for the analytes The authors considered the column exchange capacity, column dimensions and particle size of the columns when choosing the optimum column used for the LPIC separations of the analytes mentioned above

1.10 Monolithic columns

Recently, the interest in using porous monolithic stationary phase media for the possibility of high performance separations or inorganic and organic ions has increased All separations in chromatography would benefit from high resolution, high column capacity, fast separations and low backpressures One way of reducing backpressure is to employ monoliths Separation efficiency on a monolithic rod column does not suffer the same detrimental separation performance that is observed in packed columns as the flow rate increases, and the comparative increase in backpressure is only a fraction of that on a packed column of the same length This results in the monolithic column being suitable for use in chromatography and LPIC

Silica and polymer-based monoliths have been produced with either bonded or coated ionic sites for anion and cation separations The use of these monolithic materials facilitated the development of a number of new approaches including low/medium pressure IC, ultra-fast IC and the use of flow/double flow gradient IC to name but a few [43] The monolithic separation medium is made of a continuous, rigid, silica rod with a porous structure The high porosity leads to high permeability or low-pressure drop Conventional size monolithic silica columns typically have 60% external porosity and 85% total porosity higher than particle-packed columns The surface area and capacity factors (k values) are smaller than those of particle-packed columns as there is less silica present in the column (higher porosity) [44] It has been shown that short monolithic phases can exhibit peak efficiencies equivalent to traditional sized particle-packed columns The bimodal porous structure of monolithic stationary phases, consisting of small pores (mesopores-13 nm in diameter) within the monolith skeleton surrounded by larger pores (transport channels-macropores, 2 μm in diameter), in combination with high mechanical stability of the matrix, provides high permeability and low backpressure from the through flow of the liquid phase[43] and thus renders it more suitable for LPIC These columns are able to achieve high efficiencies at higher eluent flow rates than in conventional reversed-phase LC The higher performance at high flow rate is due to the small size of silica skeletons This is mainly due to the relatively small C term (mass transfer) in the van Deemter equation The most important factor that determines the column efficiency is homogeneity of silica skeletons and through-pores [44]

Monolithic columns suffer two important limitations The first being it is difficult to manufacture large volume solid rod monoliths and the second is monoliths are subject to sample overload as a function of injection volume

Figure 1 4 illustrates the pores present inside the silica skeletons and flow through channels of monolithic columns The mesopores are 13 nm in diameter and constitute the silica skeleton whereas the macropores are 2 μm and constitute the flow through channels of these columns [45]

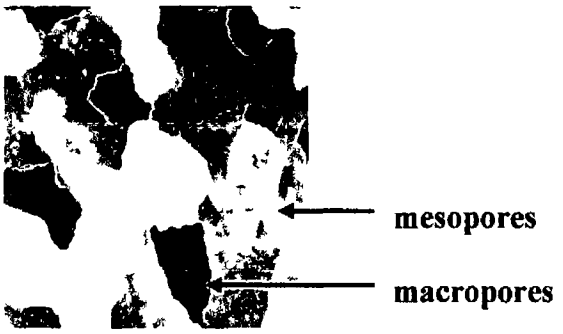

Figure 1.4· Illustration of the mesopores and macropores in monolithic columns[45]

Hatsis and Lucy[46] obtained the ultra-fast separation of eight common inorganic anions on a monolithic column in 15 sec using ion-interaction chromatography (tetrabutylammonium-phthalate as the IIR) with direct conductivity and indirect UV detection The use of short monoliths as very weak anion-exchange chromatographic columns has also been considered and applied for the fast purification of plasmid DNA Plasmid DNA can be purified from bacterial cells using alkaline lysis followed by chromatography on these columns [47] Table 1 2 illustrates some of the applications that monolithic columns can be used for

Table 1.2: Application of monolithic columns for the separation of ions and cations

Ref	Analytes	Stationary phase/column	Eluent	Method	Detection mode	Application
5	IO_3 Cl NO_2 Br NO_3 PO_4^{3-} SO_4^{2}	Chromolith Speed ROD RP 18e column (50mmx4 6mm) permanently coated with the surfactant DDAB	6 mM o-cyanophenol pH 7	Anion exchange chromatography	Suppresed conductivity	High speed ion Chromatography of inorganic anions using a coated monolithic column
12	NO_2 Br NO_3 I	Chromolith performance RP 18e monolithic reversed-phase C18 column (100mmx4 6mm) dynamically coated with 0 2mM carboxybetaine	10 mm KCl 0 2 mM carboxybetaine pH 6	Reversed phase ion chromatography	UV at 214 nm	Zwitterionic ion chromatography for anions using carboxybetaine coated monolithic columns
13	H^+ Mg^{2+} Ca^{2+}	Chromolith 50x4 6mm column coated with 40mM lithium dodecylsulphate (Li-DS)	2mM ethylenediamine and 0 1mM Li-DS pH 6 0	Conventional ion exchange	Conductivity	High-speed separation of H^+ Mg^{2+} and Ca^{2+} in rainwater using a coated monolithic column
19	OH^- NO_3 Cl SO_4^{2}	Monolithic ODS-silica gel column (10cmx4 6mm) coated with anionic surfactant polyoxyethylene and cationic surfactant cetyltrimethylammomumbromide	10mmol/l Na_2SO_4 aqueous solution adjusted to pH 10 3 with NaOH	Ion-exchange	Conductivity	IC determination of hydroxide ion on double-coated monolithic reverse-phase silica gel columns
40	Methylparaben, propylparaben, sodium diclofenac internal std butylparaben	Chromolith Flash RP-18e 25mmx4 6mm monolith	Acetonitrile/water 40 70 (v/v) 0 05% triethylamine pH 2 5 pumped using flow gradient with 8-20µl/s	Reversed-phase sequential injection chromatography	UV at 275nm	Separations using monolithic columns and sequential injection chromatography

Ref.	Analytes	Stationary phase/column	Eluent	Method	Detection mode	Application
41	NO_2^- and NO_3^-	100x4.6mm Chromolith Speed ROD RP-18e monolith and 50x4.6mm Chromolith Speed ROD RP-18e coated with 5mm CTAC aqueous solution	0.5M NaCl and 5mM sodium phosphate buffer (pH 4.7)	Anion–exchange chromatography on a two coated monolithic columns in series	UV at 225nm	IC for determination of NO_3^- and NO_2^- in seawater using monolithic ODS columns
42	Inorganic anion and cations	100x4.6mm silica monolith column modified through the in-situ covalent attachment of lysine (2, 6-diaminohexanoic acid) groups	3mM HNO_3 acid for analysis of alkali and alkaline earth metals and 3mM KCl for transition metal cation analysis 2mM phosphate buffer for anion analysis	Fast ion chromatography	Conductivity for alkali and alkaline earth metals, UV-vis/PCR for transition metal analysis at 495nm. UV for anion analysis at 214nm	Fast ion chromatography of inorganic anions and cations on a lysine bonded porous silica monolith
46	PO_4^{3-}, Cl^-, NO_3^- Br^-, NO_2^- ClO_3^-, I^-, SO_4^{2-}	100x4.6mm Chromolith Speed ROD RP-18e monolith	1.5mM tetrabutylammonium and 1.1mM phthalate at pH 5.5 with 5% (v/v) acetonitrile	Ion-interaction chromatography	Conductivity and direct UV at 255nm	Ultra-fast separation of inorganic anions by ion-interaction chromatography using short monolithic columns in leass than 15secs
47	Plasmid DNA	Convective interaction media disk monolithic column bearing weak diethylaminoethyl (DEAE) anion group	Elution buffer: 25mM 3-[N-morpholino]propansulfonic aicd (MOPS), 1.5M NaCl, pH 7.0, 15% isopropanol (v/v)	Ion-exchange chromatography	UV at 260nm	Application of short monolithic columns for fast purification of plasmid DNA

1.11 Detection Methods in LPIC

The detection modes used in LPIC are similar to those of IC as discussed in section 1.8. However, the possibility of using a light emitting diodes (LEDs) detector can also be investigated if low-pressure pumps are incorporated into miniaturised systems. The presence of an LED detection system will result in further miniaturisation of the LPIC system. They are a subsequent decrease in size and expense. LEDs are attractive sources of light; stable, exhibit long lifetimes, consume little power (can be battery operated) and have high efficiency (little heat generated). They are also robust and inexpensive. However, due to their relatively low intensity they are of limited use in spectroscopy. Recently, these have only been available from the IR region down to the middle of the visible region of the spectrum. Recent breakthroughs have led to the development of the blue (GaN) and UV-LEDs. They are currently being used for the development of optical devices. LED-photodiodes are considerably less sensitive than commercial photodiodes (10-100 times smaller). O'Toole *et al.* suggest the possibility of using a sensing system that employs two LEDs whereby one is used as the light source and the other as a light detector. This sensing concept has been applied in flow analysis by constructing an optical flow cell with a pair of LEDs. Calibration of the integrated optical flow cell using a dye resulted in a linear response. This system was used for the successful determination of pH using bromocresol green (BCG) in the range of 2.5-6.8.[48] Simultaneous determination of phosphate and silicate levels by a stopped flow injection technique with an LED-based photometer as the detection mode was proposed by Grudpan *et al.*, using a laboratory-made semi-automatic stopped-FI analyser.[49]

The work detailed in the following results sections illustrates the use of both ion interaction chromatography (on short monolithic permanently coated columns) and of ion-exchange chromatography (on anion exchange columns) using low pressure systems and various modes of detection for the determination of a wide range of analytes in standards and real samples.

1.12 Combining Flow injection analysis (FIA) and Chromatography

1.12.1 Flow injection Analysis

In 1975, flow injection analysis (FIA) was first demonstrated as a powerful and useful analytical tool in Denmark by Ruzicka and Hansen.[50] FIA is used as a sampling system that has great potential for miniaturisation. Continuous flow injection systems lead to large amounts of reagent consumption and waste generation because solutions are pumped through continuously. Sequential flow on the other hand, is based on the discontinuous flow of the carrier stream and consumes reagents only when the sample is being treated by exploiting a combination of stopped, reversed, as well as forward flow in microlitre scale.[53]

FIA systems incorporate a range of operations including dilution or mixing, sample enrichment, matrix modification and solvent exchange. FIA systems generate rapid, highly reproducible results. FIA systems have several important advantages: higher analysis rates, enhanced response times, lower solvent costs and much more rapid startup and shutdown times.[2] A typical FIA system consists of a pump (usually a peristaltic pump), an injection valve, a mixing coil and a detector. However, the addition of a separation column to this FIA manifold is the basis of LPIC separations and will be discussed later in chapters 3 & 4.

Highly precise sample volumes are introduced into the carrier flow stream. Immediately after injection, the sample zone in a flow-injection apparatus has a rectangular concentration profile. As the sample moves through the tubing, band broadening or dispersion takes place and the zone profile shape changes to a peak. The width of the zone depends upon the distance between the injector and the detector, as well as on the pumping rate.[2]

A flow injection method was proposed for the determination of silicate in natural waters based on the formation of an ion associate between molybdosilicate and malachite green. The sample was injected into a water carrier stream, which reacted with a molybdate solution, and finally the ion associate was formed from merging

molybdosilicate and malachite green solutions, which was then measured experimentally at 645 nm.[51]

A few authors have utilised the interactions of basic dyes with molybdophosphate/molydosilicate to yield more sensitive methods. Burns *et al.* proposed the use of the basic dye - crystal violet in this instance - for phosphate determination by FIA on reaction with 12-molybdophosphate, which can be spectroscopically determined at 560 nm. [52]

1.12.2 FIA and Chromatography

A chromatographic system incorporates similar equipment, hence the similarity of these two analytical techniques (chromatography and FIA). There is an unexplored area existing between the two flow-based techniques - chromatography and FIA - in so far as both have rarely been used together. FIA is broadening its scope into environmental research and as a tool in both the biotechnology and chemistry fields.[53]

Leach *et al.* demonstrated the use of a combined FIA monolithic system, which incorporated peristaltic pumps, an injection valve, mixing column and a microfluidic injection loop. The authors demonstrated the ability of this FIA system to perform rapid and sensitive assays for the enzymatic hydrolysis of fluorescein diphosphate by alkaline phosphatase.[54]

Karmarkar successfully combined both FIA and IC for the analysis of the anionic and cationic nutrients in wastewater.[55] Ion chromatography is typically used for the determination of nitrate and phosphate levels in wastewater samples whereas the determination of ammonium levels in these samples is usually carried out by wet chemistry, e.g. flow injection analysis. This work describes IC determination of chloride and the two anionic species, nitrate and phosphate, with determination of the cationic nutrient ammonium, by sequential FIA. This method enables quantification in a single injection of the two anionic nutrients using a bulk-property conductometric detector and that of a cationic nutrient using a solute-specific colorimetric detector with a 520 nm interference filter.[55]

Narusawa considered the possibility of simultaneously determining silicate, phosphate and arsenate by FIA with an on-line anion exchange column and spectroscopic detection at 810 nm. This method is based on the measurement of the absorbance of the heteropoly blue formed with ascorbic acid as reducing reagent. $KCl/NH_3/EDTA$ was used as the eluting solution and the limit of detection of the three ions was found to be 10^{-4} mol/L.[56]

A novel reversed-phase sequential injection chromatographic technique with UV spectrophotometric detection was considered by Satinsky *et al.* [57] to separate four different compounds (methylparaben, propylparaben, sodium diclofenac, and internal standard butylparaben) using a monolithic column. An eight-port selection valve and 10 mL syringe were used for these separations.

Combining FIA and chromatography was again the approach taken by Pobozy *et al.* [58] for the separation of trace transition metals in natural waters. A flow-injection preconcentration system was used for on-line preconcentration of the transition metals on a micro-column loaded with functionalised cellulose sorbent Cellex-P prior to introduction of the sample into the ion-pair chromatographic system. This Cellex-P exhibits fast kinetics of sorption processes that allows rapid preconcentration of trace metal ions under flow conditions. A reversed-phase C_{18} column was modified using a sodium dodecylsulphate solution. The transition metals were detected spectrophotometrically at 510 nm after a post-column derivatisation with PAR (4-(2-pyridylazo) resorcinol).

These are typical examples of how chromatography can be used in conjunction with FIA for the separation of selected analytes.

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CHAPTER 2

Equipment and Reagents

2.1 Column coating procedures

The ionic surfactants chosen to permanently coat the columns used throughout this work were 98% didodecyldimethylammoniumbromide (DDAB) and 98% N-dodecyl-N, N-(dimethylammonio) undecanoate (DDMAU). The columns used are mentioned later. A solution of 10 mM concentration of the ionic surfactant was prepared in Milli-Q water and placed in a sonicator to aid dissolution. Due to the nature of these surfactant solutions, excessive shaking and/or agitation were avoided. These ionic surfactant solutions were pumped through the columns at 1 mL/min for approximately 30 min until column saturation occurred (which was previously determined by Connolly and Paull [1]). The ionic surfactant was then permanently coated onto the stationary phase inside the column representing a stable high capacity ion-exchange surface on the column. The column was subsequently washed with Milli-Q water to remove weakly absorbed surfactant from the column. The column was washed with Milli-Q water at the end of each day, and stored as such, to reduce the likelihood of column blockage. It was very important to refrain from the use of organic solvents as these would strip the column of its coating.

2.2 Equipment (Chapter 3)

For the majority of this work a Varian Prostar HPLC system (JVA Analytical, Longmile road, Dublin 12, Ireland) comprising of a Varian Prostar autosampler and a Varian Prostar PDA detector were used. For permanently coating the Chromolith SpeedROD 25 mm x 4.6 mm i.d monolithic column (Merck KGaA, Damstadt, Germany) with DDAB solution, a Waters 501 HPLC pump was used (Waters, Milford, USA). The injection loop was 100 μL. Detection was by indirect UV at 279 nm. Data acquisition was performed using Star (Version 5.52) chromatography software, which allowed for the integration of peaks.

In later work, a low-pressure separation was attempted by connecting the above coated monolithic column to a peristaltic pump (Gilson Minipuls 312, Villiers, France fitted with 0.76 mm i.d peristaltic tubing supplied by Anachem, Bedfordshire, England) and used to deliver the eluent to a Rheodyne injection valve (with a 100 μL loop supplied by Carl Stuart, Tallaght, Dublin 24), and a Hewlett Packard 1050

series UV-Vis detector (HP, Houston, USA) was used for indirect UV at 279 nm Chromatograms were captured using Pico Technology (Cambridgeshire, UK), a high resolution data logger ADC-16 connected to a portable PC

2.3 Reagent and chromatographic conditions (Chapter 3)

All chemicals used were of analytical reagent grade Before coating of the column, the new column was conditioned with 50% MeOH, supplied by Labscan (Dublin, Ireland) at 1 mL/min for 30 min, followed by Milli-Q water for 30 min The 98% DDAB used to permanently coat the column was supplied by Aldrich (Aldrich, USA) The coating solution was filtered using a 0 45 μm filter

For preparation of the eluent, water was obtained from a Millipore Milli-Q water purification system (Millipore, Bedford, MA, USA) Potassium hydrogen phthalate (99 95-100 05%) was supplied by Aldrich The optimised eluent for the separation of the anions consisted of 5 mM potassium hydrogen phthalate adjusted to pH 7 5 with NaOH (ACS 97%) All eluents were degassed and filtered using 0 45 μm filters before use Filters used for eluent filtration and coating solution filtration were 0 45μm nylon membrane filters from Millipore laboratories The flow rate used was 1 mL/min Column temperature was ambient

Stock standard solutions of concentration 1000 ppm were prepared monthly and working standards were prepared twice weekly from each respective stock solution Fluoride, chloride, nitrite, nitrate, sulphate and iodide standards were prepared from their respective 95% sodium salts (Sigma-Aldrich, Gillingham, Dorset, UK) Bromide and Bromate standards were prepared from their respective 98% potassium salts (Sigma-Aldrich, Gillingham, Dorset, UK)

2.4 Equipment (Chapter 4)

For the early section of this work a Varian Prostar HPLC system comprising of a Varian Prostar autosampler and a Varian Prostar PDA detector were used For permanently coating the Chromolith SpeedROD 25 mm x 4 6 mm i d and 10 mm x

4.6 mm i.d guard monolithic column (Merck KGaA, Damstadt, Germany) with the DDAB solutions, a Waters 501 HPLC pump (Waters, Milford, USA) was used. The injection loop was 100 µL. Detection was by indirect UV at 279 nm. Data acquisition was performed using Star (Version 5.52) chromatography software, which allowed for the integration of peaks.

Later in this work, low-pressure separations were attempted by connecting the above coated monolithic column to a peristaltic pump (Gilson Minipuls 312, Villiers, France and/or Perkin Elmer peristaltic pump, fitted with 0.76 mm i.d peristaltic tubing supplied by Anachem, Bedfordshire, England) and used to deliver the mobile phase to a Rheodyne injection valve (with a optimised 75 µL loop), with a Hewlett Packard 1050 series UV-Vis detector used for indirect UV at 279 nm. A 10 cm packed bed suppressor cartridge (Alltech Associates, Lancashire, England) and a conductivity cell (adapted from a Dionex DX100 ion chromatograph-Sunnyvale, CA, USA) were used for suppressed conductivity detection. The processing of chromatograms was performed by using Pico Technology (Cambridgeshire, UK), a high resolution data logger ADC-16 connected to a portable PC.

Concluding this work, low-pressure separations were attempted by using two independently controllable micro-peristaltic pumps (1 cm x 1 cm x 6 cm total dimensions), fitted with 1.016 mm i.d. peristaltic tubing (manufactured by BVT technologies, Euro-link Associates, Tyne & Wear, England). These were used to deliver the eluents. A T-piece was used to mix eluents. A 10 mm coated guard column, coated with 98% DDAB, and suppressed conductivity detection (as mentioned above) was also used. The hot plate used to heat the eluents was a Yellowline MST basic hotplate (Boutersem, Belgium). The processing of chromatograms was again performed by using Pico Technology (Cambridgeshire, UK), a high resolution data logger ADC-16 connected to a portable PC.

2.5 Reagent and chromatographic conditions (Chapter 4)

All chemicals used were of analytical reagent grade. Before coating of the monolithic columns, the new column was conditioned with 50% MeOH, supplied by

Labscan (Dublin, Ireland) at 1 mL/min for 30 min, followed by Milli-Q water for 30 min The 98% DDAB used to permanently coat the monolithic columns was supplied by Aldrich (Aldrich, USA) The coating solution was filtered using a 0 45 μm filter (Millipore, Bedford,USA) For preparation of the eluent, water was obtained from a Millipore Milli-Q water purification system (Millipore, Bedford, MA, USA) *p*-Hydroxybenzoate (4-hydroxybenzoic acid-99%) that was used in the eluent was supplied by Sigma Aldrich, Tallaght, Ireland The optimised eluent for the separation of the anions consisted of 3 mM *p*-hydroxybenzoate pH 6 9 All eluents were degassed and filtered using 0 45 μm filters before use Filters used for eluent filtration and coating solution filtration were 0 45 μm nylon membrane filters from Millipore laboratories (Cork, Ireland) The flow rate used was ~0 33 to 0 65 mL/min depending on the separations Eluent was heated to 25°C and 45°C Column temperature was ambient

The standard mix of anions used for eluent optimisation was as follows fluoride, chloride, nitrite, bromide, nitrate and sulphate Stock standard solutions of concentration 1000 ppm were prepared monthly and working standards were prepared twice weekly from each respective stock solution Fluoride, chloride, nitrite, nitrate and sulphate standards were prepared from their respective sodium salts (Sigma-Aldrich, Gillingham, Dorset, UK)

2.6 Equipment (Chapter 5)

For early work, two persistaltic pumps (Gilson Minipuls 312, Villiers, France and Perkin Elmer peristaltic pump fitted with 0 76 mm i d peristaltic tubing supplied by Anachem, Bedfordshire, England) were used to deliver post column reagents and a Waters 501 HPLC pump (Waters, Milford, USA) was used to deliver the eluent through an anion-exchange column (AG18 IonPac 2 x 50 mm guard column supplied by Dionex, Sunnyvale, CA, USA) The injection volume was increased from 25 μL to 100 μL to increase sensitivity A 1 46 M reaction coil (0 5 mm i d) was connected to a T-piece used for mixing during the post-column reaction This coil was heated in a water bath to 60°C using a Yellowline MST basic hotplate (Boutersem, Belgium) Detection was by direct UV-vis detection at 600 nm and also

by LED detection at 610 nm The LED detector was controlled by a 1-DSP-SCU-000 sensor control unit (www innovada com) The LEDs were supplied by Maplin Electronics in Dublin, Ireland This LED was a miniature detector comprising of 2 near-infrared LEDs (emission and detection wavelengths of 850 nm) and a 20 mL internal volume [2]

The processing of chromatograms obtained by using direct UV-vis detection was again performed by using Pico Technology (Cambridgeshire, UK) a high resolution data logger ADC-16 connected to a portable PC However, the Hyperterminal communicator of windows enabled the data from the LED detector to be converted into a chromatogram

For later work, a larger reaction coil was used (5 M x 0 25 mm i d) (Agilent Technologies) also heated in a water bath to 60°C using a Yellowline MST basic hotplate (Boutersem, Belgium) Injection loop used was 100 µL Detection by UV-vis spectrophotometric detector (Shimadzu SPD-6AV-Tungsten lamp) was at 840 nm The all-in-one reagent[3] was used for post column reaction so two 501 HPLC pumps (Waters, Milford, USA) were used to deliver the post column reagent and the eluent These were again mixed by use of a T-piece The processing of chromatograms obtained by using direct UV-vis detection was again performed by using Pico Technology (Cambridgeshire, UK) a high resolution data logger ADC-16 connected to a portable PC

For final work, a 10 mM Chromolith SpeedROD guard column (10 mm x 4 6 mm i d) (Merck KGaA, Damstadt, Germany) coated with 98% DDMAU surfactant was used The reaction coil mentioned previously was employed again and heated Injection loop used was 100 µL Two MilliGat pumps supplied by Global FIA (Fox Island, WA, USA) MilliGat (USA) were used to pump the all-in-one reagent and the eluent Pressure of the system was monitored by a USG pressure gauge (Bensenville, IL, USA) Detection by UV-vis spectrophotometric detector (Shimadzu SPD-6AV-Tungsten lamp) was at 840 nm in which the Pico Technology (Cambridgeshire, UK) high resolution data logger ADC-16 connected to a portable PC was used to process data Detection using a wavelength specific near infrared LED at 850 nm was also used This used two 850 nm LEDs (emitter and detector) with lights paths of 8 mm x

1 mm i d , to increase the sensitivity of the signals The hyperterminal communicator of MS Windows enabled the data from the LED detector to be converted into the resultant chromatogram

2.7 Reagent and chromatographic conditions (Chapter 5)

All chemicals used were of analytical reagent grade For preparation of the eluent, water was obtained from a Millipore Milli-Q water purification system (Millipore, Bedford, MA, USA) All eluents were degassed and filtered using 0 45μm filters before use Filters used for eluent filtration and coating solution filtration were 0 45 μm nylon membrane filters from Millipore laboratories (Cork, Ireland) Column temperature was ambient

For early work, the optimised eluent was 20 mM KCl (99% ACS reagent) supplied by Aldrich, (USA) 0 6% ammonium molybdate tetrahydrate (Fluka Chemie GmBH, Buchs, Switzerland) and 0 6% L-ascorbic acid (supplied by Biochemical) comprised the post-column reagent The reaction coil was heated to 60°C The flow rate used was 0 3 mL/min for eluent and post-column reagents

For later work, an all-in-one reagent[3] (comprising of AR grade sulphuric acid (Aldrich, Tallaght, Ireland), ammonium molybdate tetrahydrate (Fluka Chemie GmBH, Buchs, Switzerland), potassium antimony tartrate hydrate 99% (Aldrich, USA), L-ascorbic acid (Biochemical) and Milli-Q water was used for the post-column reaction 10 mM KCl (99% A C S reagent) was used as the optimised eluent and pumped through the microbore anion-exchange column at 0 3 mL/min The post-column reagent was pumped through to the detector at a rate of 0 4 mL/min The reaction coil was kept at room temperature and then also heated to 60°C to investigate what effect this would have

For final work, a 10 mm Chromolith SpeedROD column (Merck KGaA, Damstadt, Germany) was coated with N-dodecyl-N, N-(dimethylammonio) undecanoate (DDMAU) surfactant which was supplied by Calbiochem (Lennox Supplies, Naas Rd , Dublin, Ireland)

Before coating of the monolithic columns, the new column was conditioned with 50% MeCN - supplied by Labscan (Dublin, Ireland) - at 1 mL/min for 30 min, followed by Milli-Q water for 30 min The DDMAU was then allowed flow through the column for 30 mm and excess coating was removed by washing the column again with Milli-Q water for a further 30 min The flow rate for the eluent and all-in-one reagent was 0 3 mL/min respectively All other conditions previously mentioned were the same

Stock standard solutions of concentration 1000 ppm were prepared monthly and working standards were prepared twice weekly from each respective stock solution Phosphate standards were prepared from its respective sodium salts (Sigma-Aldrich, Tallaght, Dublin) Arsenate standards were prepared from its potassium monobasic salt (supplied by Sigma, Dublin, Ireland) and silicate standards were prepared from its sodium silicate solution (supplied by Riedel de Haen, Hannover, Germany)

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CHAPTER 3

Phthalate eluents for use with coated monolithic columns

3.1 Aims

The possibility of using short monolithic columns for the LPIC determination of anions in water samples was investigated Phthalate eluents were considered for such anion separations using indirect UV detection Method development and mobile phase optimisation were initially undertaken The properties of the coated monolithic columns were investigated, with regard to improving peak efficiencies As LPIC was the ultimate aim of this work, validation and flow rate optimisation of the peristaltic pumps was also necessary

The interest in using porous monolithic stationary phases for high-performance separations of cations and anions has increased in recent years [1] These porous monoliths have been produced with either bonded or coated ionic sites for ion-exchange separations Short monolithic columns can exhibit peak efficiencies equivalent to, and in some cases better than those previously obtained using the more traditional sized particle-packed columns [1] Monolithic columns can be prepared by the polymerisation of monomers with integrated structures which leads to a much higher external porosity than the normal particulate column and which results in a high permeability and low column pressure drop [2] Conventional-size silica monolithic columns (15-25 cm) typically possess ca 60% external porosity and 85% total porosity, higher than a particle-packed column by approximately 20% Commercial silica-rod columns (e g Dionex Acclaim 300, C18, 3 μm Analytical (4 6 x 50mm)) produce efficiency similar to those packed with 3 5 μm particles and typically gives a pressure drop half that caused by a column packed with 5 μm particles [2] These new monolithic columns have facilitated the development of a number of new approaches to the chromatographic separation of ions including LPIC The ability to separate solutes using short monolithic phases with relatively high efficiency whilst generating minimal backpressure across the column presents a number of obvious advantages in terms of simplifying and potentially miniaturising LC equipment [1]

Coating a monolithic column with ionic surfactants results in ions being retained This also results in sharper peaks for these ions, thus also resulting in improved efficiency for the chromatographic system [3] The DDAB molecule was used for

coating monolithic columns in this work, as shown below in Figure 3 1 This ionic surfactant has two C_{12} chains resulting in strong coating on a reversed-phase monolith and a very high anion exchange capacity, which makes this molecule suitable as a stationary phase for such anion separations

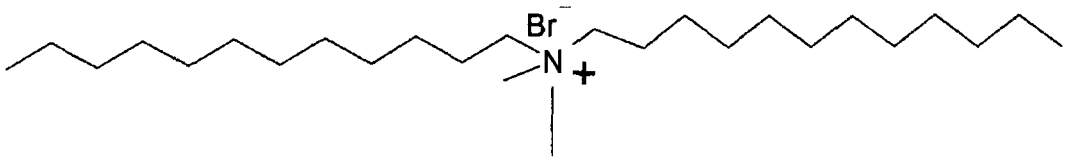

Figure 3.1: DDAB surfactant used to coat monolithic column

3.2 Investigation of backpressures on monolithic columns

The change in the backpressures of using different monolithic columns permanently coated with ionic surfactants was investigated The advantages of monolithic columns were previously discussed in Chapter 1 10

Monolithic columns can be used at flow rates up to 8 mL/min The contribution of the mobile phase mass transfer term when using a monolithic column was greater than when using a particulate column, while contribution of the stationary phase mass transfer term was much less compared with a column that gives a similar pressure drop [2] The combined size of through-pore and skeleton (domain size) was considered as a characteristic length scale in a monolithic column For a silica monolithic column, which has 2 μm through-pore, and 1-1 5 μm silica skeleton size, equivalent particle dimensions of 4 μm were suggested [2] The back pressures generated on a number of monolithic columns were investigated at different flow rates as shown in Table 3 1

Table 3.1: Investigation of backpressures at different flow rates on non-coated monolithic columns and permanently coated monolithic columns

Flow rate in mL/min	**Backpressures on 50 mm x 4.6 mm monolithic column in psi**	**Backpressures on 100 mm x 4.6 mm monolithic column in psi**	**Backpressures on 25 mm x 4.6 mm monolithic column in psi**	**Backpressures on 25 mm x 4 6 mm 10 mM DDAB coated monolith in psi**
0.2	29	44	15	15
0.6	88	118	59	74
0 8	118	147	74	103
1.0	147	191	88	132
2.0	323	411	206	279
3.0	470	617	323	426
4 0	646	837	426	573
5.0	793	1058	558	705

The backpressures on the non-coated Chromolith SpeedROD RP-18e were measured using a Varian star HPLC system and by passing a solution of 60/40 MeCN/H_2O through the column The effect of coating the 25 mm x 4 6 mm i d with 10 mM DDAB was then examined The column was coated as outlined in Chapter 2 1 (column coating procedures) The backpressures of this modified column were measured using distilled water

Monolithic columns exhibit a linear dependence of backpressure versus flow rate Flow rate and backpressure are directly proportional to each other The data in Table 3 1 was plotted and the R^2 values for the 100 mm, 50 mm and 25 mm non-coated monoliths were 0 9995, 0 9995, 0 9986 respectively and 0 9993 for the 25 mm permanently coated monolith as can be seen in Figure 3 2

At flow rates <1 mL/min, the pressures in psi through the 25 mm monolithic column were low enough to use with the low-pressure peristaltic pumps, hence this approach was later investigated for the separation of anions

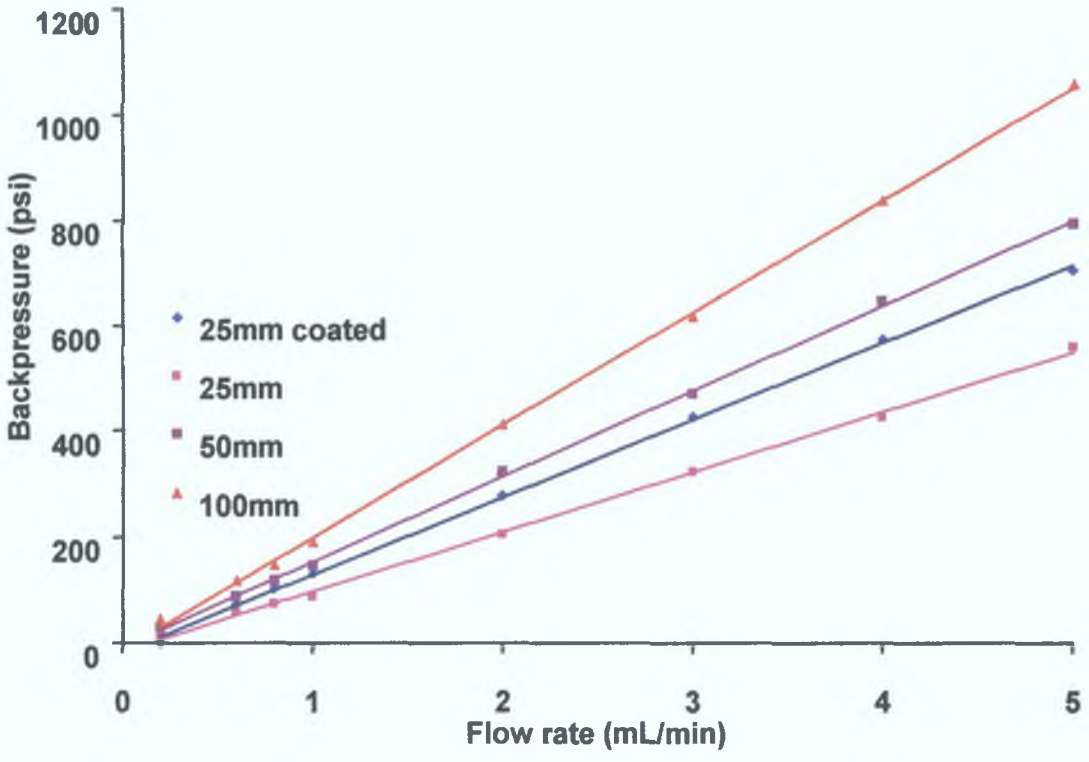

Figure 3.2: Investigation of column backpressures on different monolithic columns (i) non-coated and (ii) coated with an IIR.

Column: Chromolith SpeedROD RP-18e 25mm x 4.6mm i.d., Chromolith SpeedROD RP-18e 50 mm x 4.6 mm i.d., Chromolith SpeedROD RP-18e 100 mm x 4.6 mm i.d., Coating solution: 10 mM DDAB.

3.3 Coated monolithic columns and indirect UV detection

The possibility of using the 25 mm x 4.6 mm monolithic column coated with a 10mM DDAB surfactant for the separation of anions by indirect UV detection was considered. This coated column represented a high capacity anion exchange stationary phase, which was thought to be suitable for the separation and determination of anions. A suitable UV absorbing eluent e.g. potassium hydrogen phthalate (pK_a=2.95) was suggested to facilitate indirect UV detection. Non-UV absorbing anions (chloride, sulphate) can be determined in samples by using a UV absorbing probe in the eluent followed by indirect UV detection. The phthalate eluent anion is shown as Figure 3.3.

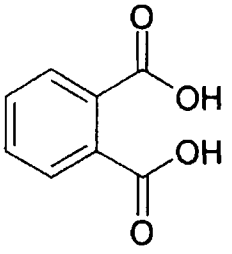

Figure 3.3: Phthalate ion

Indirect UV detection occurs when the eluent ion has a higher molar absorptivity than the solute ion, of the same charge Common eluents for the detection of anions include phthalate and nitrate solutions Upon elution of the analyte ions, a decrease in the recorded background signal is observed as the ions absorb less strongly than the eluent probe anion Hence, this detection mode is used for analytes that do not absorb strongly within the UV Eluent pH is an important variable in indirect UV detection The pH affects the charge on the eluent anion and can also affect the molar absorptivity of the eluent As a result, the use of incorrect eluent pH may lead to a decrease in detection sensitivity

3.3.1 Phthalate as an eluent

The separation and determination of a number of anions using a phthalate eluent was considered (namely bromate, bromide, carbonate, chloride, fluoride, nitrate, nitrite, phosphate and sulphate) A range of phthalate concentrations as the eluent were considered ranging from 2 5 mM to 0 25 mM The higher the concentration of eluent used, the more effectively the eluent displaces solute ions from the stationary phase and thus the solute ions were eluted more rapidly from the column Table 3 2 illustrates the capacity factors (k') for these anions using different concentrations of phthalate in the eluent

Table 3.2: Capacity factors (k') for anions using 0 25 mM-2 5 mM phthalate eluents at a flow rate of 1mL/min using a Chromolith SpeedROD RP-18e 25 mm x 4 6 mm i d coated monolith and using indirect UV detection at 279 nm

Anion	k' 0.25 mM phthalate eluent	k' 0 5 mM phthalate eluent	k' 1 mM phthalate eluent	k' 2 5 mM phthalate eluent
25 ppm Carbonate	1 50	1 18	0 56	0 33
25 pm Chloride	3 54	2 86	1 52	1 00
25 ppm Fluoride	0 69	0 29	0 37	0 17
25 ppm Nitrate	15 51	14 79	5 56	4 63
25 ppm Nitrite	6 42	5 19	2 69	1 75
25 ppm Phosphate	4 13	4 29	1 36	3 39
25 ppm Sulphate	>16	15 02	5 78	4 87

The rate at which retention can be varied by changing eluent concentration depends on the charges carried by both the solute and the eluent ions Figure 3 4 below illustrates the variation of the capacity factors for the anions against eluent concentration and the R^2 values for the anions were 0 8612, 0 8648, 0 9641, 0 9661, 0 9707 and 0 7983 for nitrate, sulphate, nitrite, chloride, carbonate and fluoride respectively

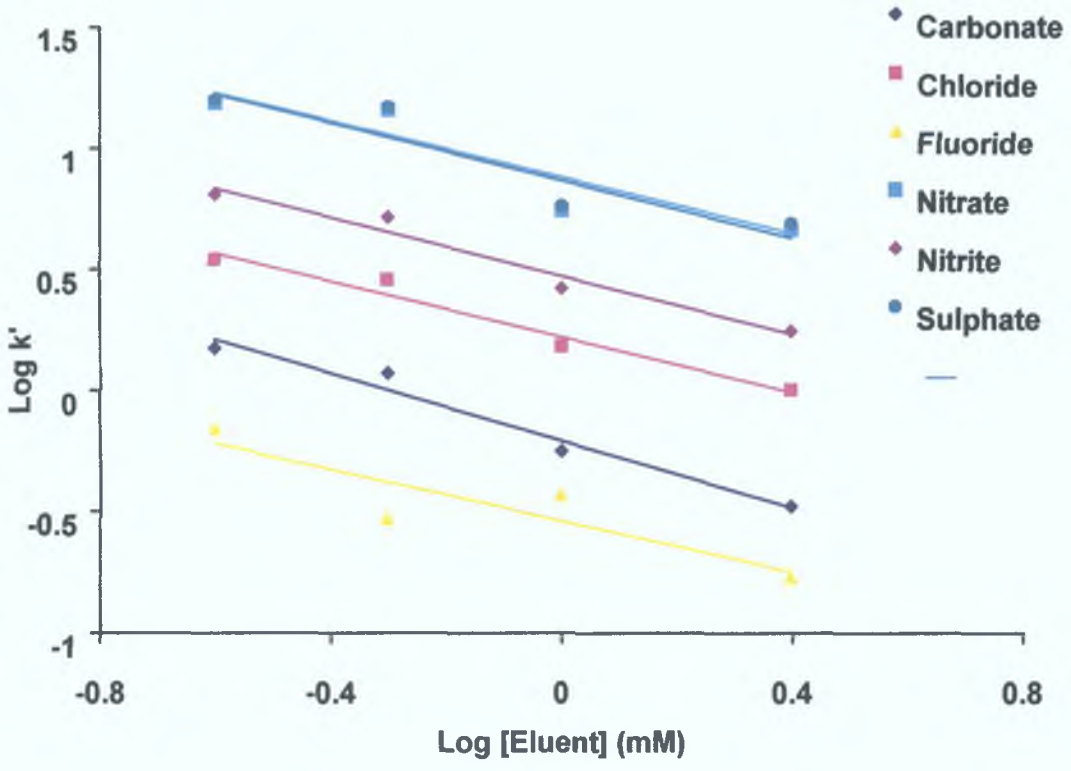

Figure 3.4: Investigation the variation of capacity factors for the anions with eluent concentration using a Chromolith SpeedROD RP-18e 25 mm x 4.6 mm i.d. coated monolithic anion-exchanger for the separation of seven common anions.
fluoride (25 ppm), carbonate (25 ppm), chloride (25 ppm), phosphate (25 ppm), nitrite (25 ppm), nitrate (25 ppm), sulphate (25 ppm). Eluent: 0.25-2.5 mM potassium hydrogen phthalate pH 7.5, Column temperature: Ambient. Flow rate: 1 mL/min. Injection volume: 100 µL Indirect UV detection at 279 nm.

An eluent concentration of 0.5 mM phthalate proved to be particularly successful initially for anion separations in a separation window of less than 8 min. A Varian HPLC system, a 25 mm coated monolith, a 0.5 mM potassium hydrogen phthalate eluent pH 7.5 and indirect UV detection at 279 nm were used for the separation of these anions. These anions were injected singly and as a mixture. However, some of the retention times of the anions were quite similar on this short monolithic column. Co-elution and swamping of the anion signals is evident from Figure 3.5. Of the nine anions injected, seven peaks can be seen and of these seven, three are co-eluting with other anions. The bromate peak is being swamped by the chloride peak. Also, there is a system peak at ~1.2 min. The formation of system peaks, which was evident in Figure 3.5, can be attributed to an eluent pH of 7.5 in this case. This system peak is a

peak which does not represent the elution of a solute ion

However, such system peaks represent a disturbance in the complex equilibria, which exists between the eluent and stationary phase

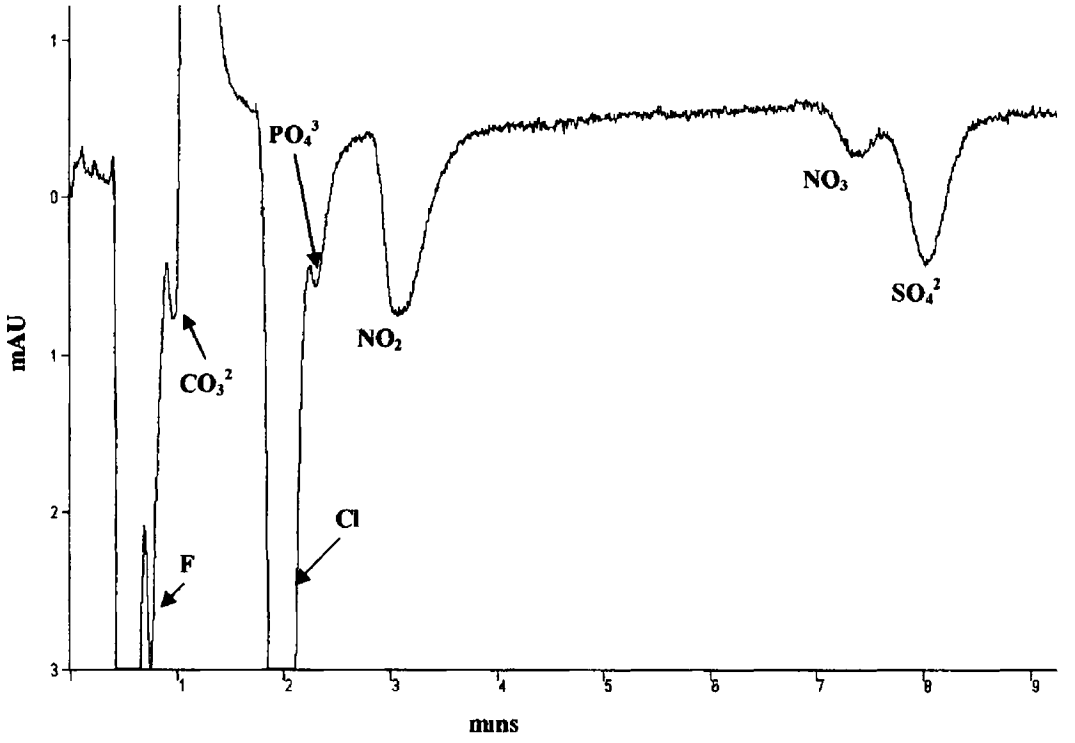

Figure 3.5. Ion chromatogram obtained using a Chromolith SpeedROD RP-18e 25 mm x 4 6 mm i d coated monolithic anion-exchanger for the separation of seven common anions,

fluoride (25 ppm), carbonate (25 ppm), chloride (25 ppm), phosphate (25 ppm), nitrite (25 ppm), nitrate (25 ppm), sulphate (25 ppm) Eluent 0 5 mM potassium hydrogen phthalate pH 7 5, Column temperature Ambient Flow rate 1 mL/min Injection volume 100 µL Indirect UV detection at 279 nm

This elution order observed was presumably due to the highly hydrophobic nature of the DDAB coated stationary phase The nature of the coating results in the weak retention of hydrophilic anions such as fluoride and phosphate with less hydrophilic anions such as bromide and nitrate retained longer As can be seen from Figure 3 5, the elution of phosphate after chloride may interfere with phosphate determinations in real freshwater samples, which contain high levels of chloride The number of theoretical plates for chloride, nitrite, nitrate and sulphate were 21, 26, 322 and 178 N/cm respectively which were calculated using the formula $N=16(t_r/W)^2$ However,

these are very inaccurate as the poor peak shape and baseline resolution was not present in Figure 3 5 Table 3 3 illustrates the capacity factors for these anions using a 0 5 mM phthalate eluent

Table 3.3· Capacity factors (k') for anions using 0 5 mM phthalate eluent pH 7 5 at a flow rate of 1 mL/min on a Chromolith SpeedROD RP-18e 25 mm x 4 6 mm i d coated monolith and using indirect UV detection at 279 nm (t_0=0 49min)

Anion	Capacity factors (k')
25 ppm Bromate	3 23
25 ppm Bromide	12 32
25 ppm Carbonate	1 18
25 ppm Chloride	2 86
25 ppm Fluoride	0 29
25 ppm Nitrate	14 79
25 ppm Nitrite	5 19
25 ppm Phosphate	4 29
25 ppm Sulphate	15 02

3.3.2 Effect of phthalate concentration in eluent and flow rate on retention

The effect of phthalate concentration in the eluent on the retention of the anions (chloride, nitrate and sulphate) was exammed The reduction in retention of all of the anions was expected with an increase in the concentration of the eluent-ion (phthalate ion)

It was thought that retention times could be further reduced by increasing the eluent concentration to 5mM without significantly compromising resolution The possibility of reduced retention time whilst maintaining efficiency would be advantageous in the determination of anions in real sample analysis

When high concentration anion mixtures were injected (100ppm chloride, 100ppm nitrate and 100ppm sulphate) a positive injection/system peak appeared in the chromatogram The possibility of resolving the chloride peak from this positive peak posed another potential problem The use of a 5mM phthalate eluent was found to improve efficiency The number of theoretical plates for chloride, nitrate and sulphate were 144, 470 and 566 N/cm respectively

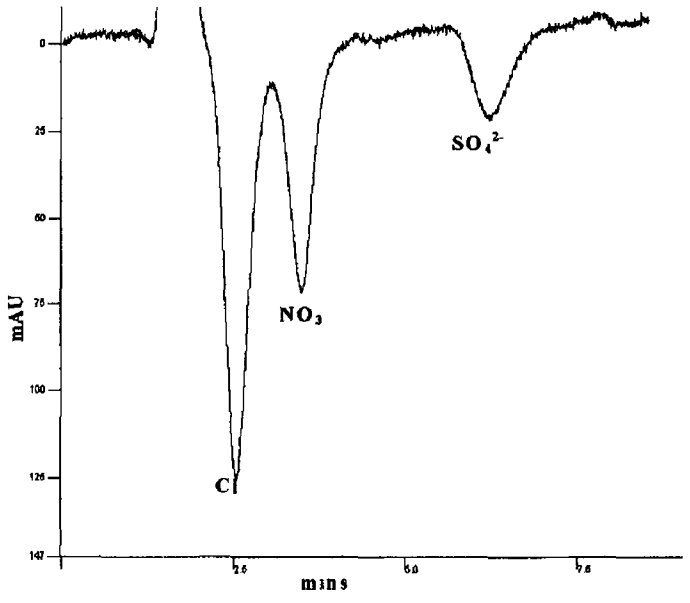

Figure 3.6: Ion chromatogram obtained using a Chromolith SpeedROD RP-18e 25 mm x 4 6 mm i d coated monolithic anion-exchanger for the separation of three common anions,
chloride (100 ppm), nitrate (100 ppm), sulphate (100 ppm) Eluent 5 mM potassium hydrogen phthalate pH 6 5, Column temperature Ambient Flow rate 0 5 mL/min Injection volume 100 µL Indirect UV detection at 279 nm

As can be seen from the Figure 3 6 above, chloride was not baseline resolved from the positive injection peak A lower strength eluent was considered as a solution to this As the phthalate concentration in the eluent and flow rate were decreased the effect upon the overall peak resolution was monitored A flow rate of 0 35 mL/min was successful in baseline resolving the positive injection/system peak and the negative chloride peak This is shown in Figure 3 7 The efficiency of the separation

decreased marginally due to the lower concentration strength of eluent being used The number of theoretical plates (N) for chloride, nitrate and sulphate were 144, 373 and 525 N/cm respectively Table 3 4 shows the effect of flow rate and phthalate eluent concentration on the capacity factors (k') for anions

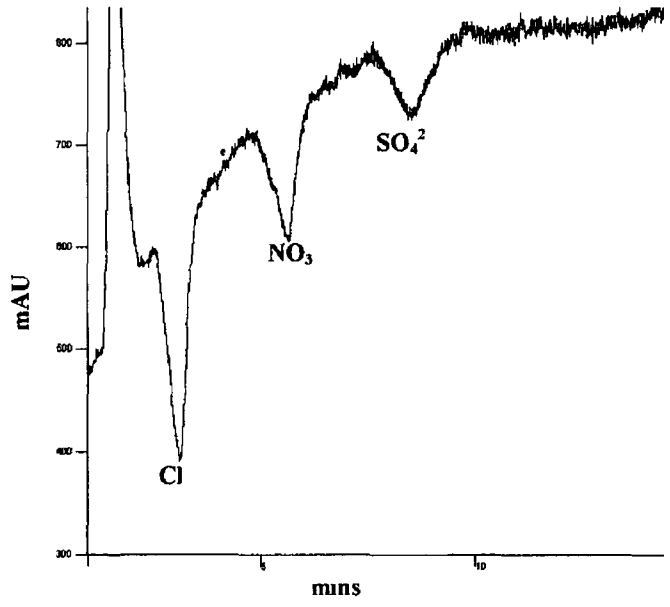

Figure 3.7: Ion chromatogram obtained using a Chromolith SpeedROD RP-18e 25 mm x 4 6 mm i d coated monolithic anion-exchanger for the separation of three common anions,

chloride (100 ppm), nitrate (100ppm), sulphate (100 ppm) Eluent 2 5mM potassium hydrogen phthalate pH6 5, Column temperature Ambient Flow rate 0 35 mL/min Injection volume 100 µL Indirect UV detection at 279 nm

Table 3.4: Effect of flow rate and phthalate eluent concentration on the capacity factors (k') for anions using a Chromolith SpeedROD RP-18e 25 mm x 4 6 mm i d coated monolith (t_0=0 49min)

Anion	**k' using 5 mM phthalate eluent at 0.5mL/min**	**k' using 5 mM phthalate eluent at 0.35 mL/min**	**k' using 2 5 mM phthalate eluent at 0 35 mL/min**
100 ppm Chloride	0 61	0 64	1 00
100 ppm Nitrate	1 22	1 25	2 60
100 ppm Sulphate	2 96	3 03	4 40

A decreased flow rate led to increased band broadening and an increased separation window for the anions, whereas retention times decreased with an increase in eluent concentration A 0 5 mM phthalate eluent was (at a flow rate of 1 mL/min) chosen as the optimum, based on a compromise between the separation of these three anions achieved and the run time

3 3.3 Phthalate as an eluent using low-pressure IC

As the method development and mobile phase optimisation were carried out successfully on a high-performance LC system, the application of using phthalate eluents for LPIC for the in-direct UV determination of anions was then considered This resulted in a further miniaturised, less expensive and portable system, as the high-pressure pump in the previous separation was replaced with a low-pressure peristaltic pump Co-elution with the high-pressure pump for the separation of these anions proved to be a drawback A test mixture was prepared containing fluoride, chloride, phosphate, bromide, nitrate and sulphate and injected into this low-pressure system, which comprised of a low-pressure peristaltic pump that delivered the eluent to a Rheodyne injection valve From there the eluent passed through the 25 mm x 4 6 mm i d 10 mM DDAB coated monolithic column and finally to the UV-vis detector where monitoring of the anions took place at 279 nm

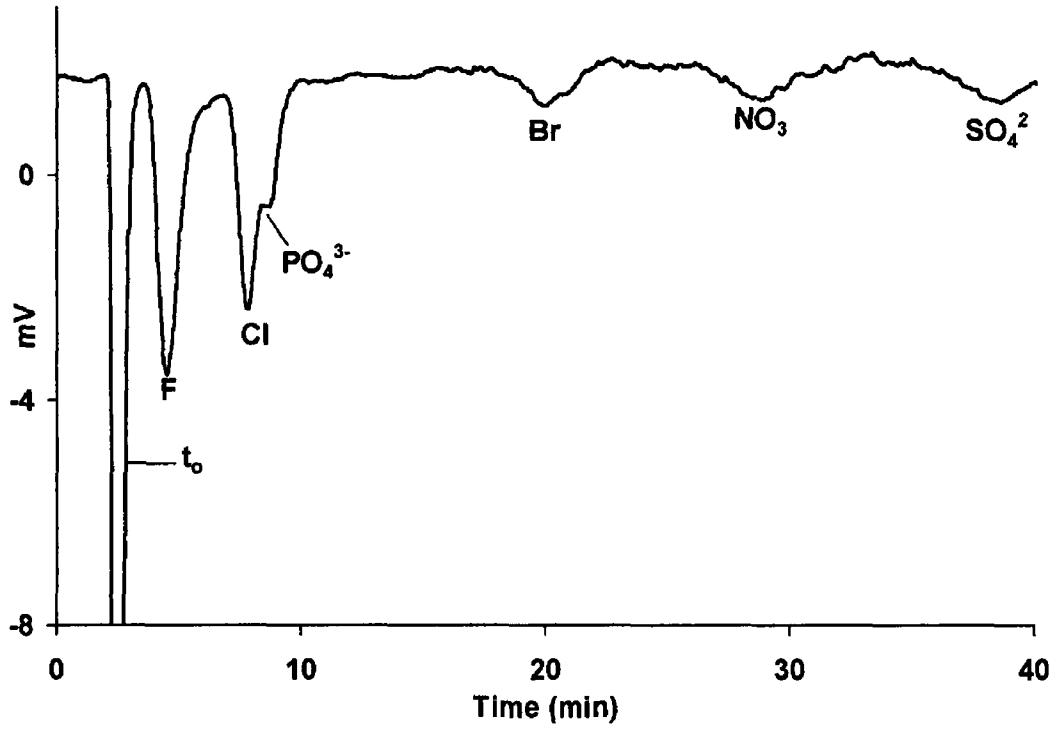

Figure 3 8: Low-pressure ion chromatogram obtained using a Chromolith SpeedROD RP-18e 25 mm x 4 6 mm i d coated monolithic anion-exchanger for the separation of six common anions.

fluoride (25 ppm), chloride (25 ppm), phosphate (25 ppm), bromide (25 ppm), nitrate (25ppm), sulphate (25ppm) Eluent 0 5 mM potassium hydrogen phthalate pH 7 5, Column temperature Ambient Flow rate 0 6 mL/min Injection volume 100 µL Indirect UV detection at 279 nm

From Figure 3 8 it was observed that 5 anions were completely baseline resolved from each other in a separation window of approximately 40 min Retention times using this low-pressure system were significantly longer as the flow rate was decreased due to increased column backpressure, leading to larger elution volumes However, each of the anions was clearly separated apart from the phosphate peak As the column backpressure was a clear limitation when using such peristaltic pumps, column blockage was a constant threat The monolithic column had to be washed with Milli-Q water at the end of each day, and stored as such, to reduce to likelihood of such column blockage The peak efficiency of some of these peaks was acceptable given the length of the column used The number of theoretical plates (N/cm) was calculated using the formula $N=16(t_r/W)^2$ The number of theoretical plates (N/cm) for fluoride, chloride, bromide, nitrate and sulphate were 29, 118, 344, 411 and 1537

respectively The capacity factors for these anions are shown below in Table 3 5

Table 3.5. Capacity factors for anions using 0 5 mM phthalate eluent at a flow rate of 0 6mL/min on a Chromolith SpeedROD RP-18e 25 mm x 4 6 mm i d coated monolith using a low-pressure pump and indirect UV detection at 279 nm (t_0=2 46min)

Anion	Capacity factors (k')
25 ppm Fluoride	0 85
25 ppm Chloride	2 22
25 ppm Phosphate	2 58
25 ppm Bromide	7 06
25 ppm Nitrate	10 70
25 ppm Sulphate	14 67

The possibility of using a higher phthalate eluent concentration of 2mM for the separation of the anions was then considered to reduce the capacity factors and elution volumes

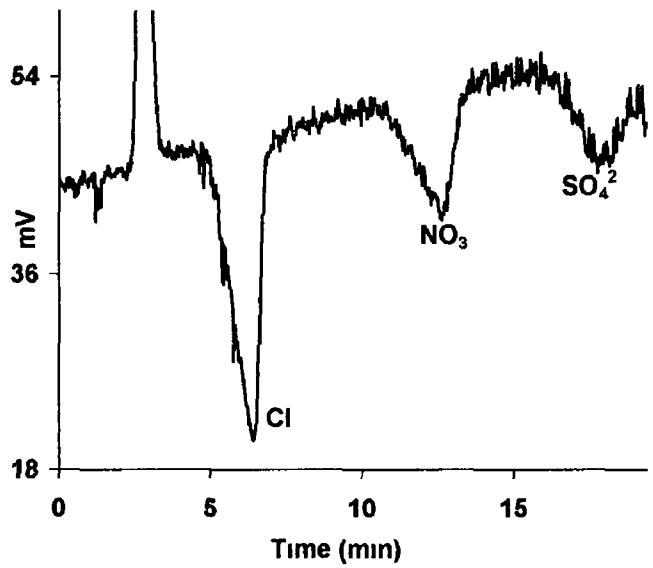

Figure 3.9: Low-pressure ion chromatogram obtained using a Chromolith SpeedROD RP-18e 25 mm x 4 6 mm i d coated monolithic anion-exchanger for the separation of chloride (300 ppm), nitrate (300 ppm), and sulphate (300 ppm) Eluent 2 mM potassium hydrogen phthalate pH 6 5, Column temperature Ambient Flow rate 0 7 mL/min Injection volume 100 µL Indirect UV detection at 279 nm

The retention times of these anions in Figure 3 9 were reduced significantly with the use of higher phthalate concentration The retention times were 6 48 min, 12 66 min and 18 03 min respectively for chloride, nitrate and sulphate The separation window for these anions was halved with no compromise in separation efficiency The number of theoretical plates for chloride, nitrate and sulphate were 210, 389 and 679 N/cm respectively The use of higher eluent concentration also resulted in less band broadening for the early eluting chloride anion The ultimate goal in chromatography is to achieve the highest possible resolution in the shortest possible elapsed time

3.4 Conclusions

It was evident from this chapter that short monolithic anion exchange columns can be used to achieve low-pressure anion separations LPIC proved reasonably successful for the separation of these anions on a 25 mm coated monolithic anion exchange column in a satisfactory timescale The possibility of using LPIC for further separations will be discussed later LPIC provides small, portable and inexpensive systems for chromatographic separations

3.5 References

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CHAPTER 4

Anion separations using *p*-hydroxybenzoate

4.1 Aims

The use of *p*-hydroxybenzoate eluents for the separation of common inorganic anions with the newly developed micro LPIC system was investigated The possibility of detecting anions in tap water using this system was also examined These common inorganic anions could be easily detected by indirect UV-vis and direct conductivity detection The analytical equipment used was relatively simple and straightforward at this stage of the work The advantages of developing such a system included portability and miniaturisation The capacity of a 10mM DDAB coated column was also investigated using *p*-hydroxybenzoate eluents Linearity and van Deemter curves for chloride, nitrate and sulphate were also examined using this eluent

Two of the anions, which were chosen to investigate i e nitrate and nitrite were at this time receiving considerable interest in the environmental field for their adverse effects upon human health IC nowadays has become a highly selective, highly sensitive method for anion determination in real water samples Nitrate and nitrite levels in our natural waters are important indicators of water quality Both of these anions are involved in the nitrogen cycle of soil and higher plants resulting in the leaching of nitrate from fertilisers added to soils giving rise to increased levels of nitrate in ground and surface waters Nitrite can be formed during the biodegradation of nitrate, ammonia and other nitrogenous organic matter It is an important indicator of faecal pollution of natural water systems In addition, nitrite is readily oxidised to nitrate by dissolved oxygen, thus, decreasing oxygen levels in water [1]

Chloride is another major anion found in water Since almost all chloride salts are highly soluble in water, the chloride content ranges from 10 to 100 mg/L Seawater contains over 30,000 mg/L as sodium chloride Chloride is associated with the corrosion of piping because it forms compounds like magnesium chloride, which can generate hydrochloric acid when heated Corrosion rates and the iron dissolved into the water from piping increases as the sodium chloride content of the water is increased The chloride ion (Cl^-) is responsible for breaking down films that protect ferrous metals and alloys from corrosion, and is one of the main causes for the pitting corrosion of stainless steel

Sulphate occurs in natural waters Most sulphate compounds originate from the oxidation of sulphite ores, the presence of shales, and the existence of industrial wastes Sulphate is one of the major dissolved constituents in ram High concentrations of sulphate in drinking water causes a laxative effect when combined with calcium and magnesium, the two most common components of hardness The recommended level from the USEPA for sulphate in secondary drinking water is 250 mg/L

Bromide is found in seawater at a level of approximately 65 mg/L as magnesium bromide Bromides are formed by the reaction of bromine or a bromate with another substance, they are widely distributed in nature Most metal bromides are water soluble, except bromides of copper, lead, mercury, and silver that are only slightly soluble in water Ethylene bromide is used, as an anti-knock additive in gasoline and methyl bromide is a soil fumigant Bromide is extensively used in the pharmaceutical industry, and occurs naturally in blood in the range of 1 5 to 50 mg/L

Fluoride is present in many products e g toothpaste, drinking water, prescribed treatments and other commercially available oral hygiene products as it is thought to strengthen tooth enamel Many local water municipalities fluoridate their water supplies by adding fluoride in concentrations of less than 4 ppm Hexafluorosilicic acid (H_2SiF_6) and its salt (Na_2SiF_6) are more commonly used to fluoridate water Hydrofluoric acid is used in the etching of glass and other industrial applications, including integrated circuit manufacturing High concentrations of fluoride are toxic to humans Fatalities have been reported in humans at doses as low as 71 mg/kg

4.2 Eluent optimisation using indirect UV detection

When choosing an eluent for a separation, it was important to consider compatibility with the DDAB coating on the stationary phase of the monolithic columns used Eluent optimisation was first carried out on a typical LC system It was also important to note that the use of an organic modifier in the eluent would result in the column coating being stripped as the stationary phase of the monolith The eluent pH was kept reasonably constant throughout these experiments at a pH of approx 6 9

The eluent pH influences the charges on both the eluent ions and the analyte ions The effect of pH is particularly important in the separations of anions, where it may effect their ionisation The charge on the acid anion increases with pH, so the eluting power of weak acid eluents increases with pH until the acid is completely dissociated The opposite trend occurs for weak bases in the eluent Similarly, the degree of ionisation of analyte ions that have derived from weak acids or bases will be pH dependent An increase in analyte charge will increase its affinity to the functional groups on the stationary phase and hence increase their retention (e g F , CO_3^{2-}, and PO_4^{2}) The standard mixture of anions consists of fluoride (20 ppm), chloride (20 ppm), nitrite (20 ppm), bromide (20 ppm), nitrate (50 ppm) and sulphate (200 ppm) For this standard mixture of anions, the pH was unlikely to have a major effect upon selectivity in the region of 3-7 (anion selective region) Monolithic columns used for these separations have pH stability in this region It was thought that the use of *p*-hydroxybenzoate (4-hydroxybenzoic acid shown as Figure 4 1) would possess sufficient separating power as the eluent for the standard mixture of anions A UV spectrum was carried out on this molecule and it was found to have a strong absorption peak at 279 nm Hence 279 nm was used as the detection wavelength for indirect UV

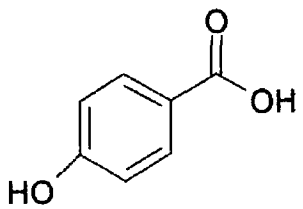

Figure 4.1 *p*-hydroxybenzoate used as the eluent

Monolithic columns are used as they offer a number of advantages for the separation of analytes The use of these monolithic columns facilitates the use LPIC which is the basis for this work

A Chromolith SpeedROD RP-18e (25 mm x 4 6 mm i d) was used for the initial separations A Chromolith SpeedROD RP-18e guard cartridge kit (10 mm x 4 6 mm i d) was used for later separations These monolithic columns were both permanently coated with 10 mM didodecyldimethylammoniumbromide (DDAB) as outlined in Chapter 2 1 (column coating procedures) This results in the stationary

phase of the monolith changing into an anion exchanger Since the stationary phase has now been converted into an anion exchanger by virtue of the absorbed DDAB, the eluents used are identical to those utilised in conventional ion-exchange

4.2.1 Effect of *p*-hydroxybenzoate concentration in eluent

Firstly, the effect that eluent *p*-hydroxybenzoate concentration had upon the retention of this standard mixture of anions was examined The reduction in retention of all of the anions was expected with an increase in the concentration of the eluent ion in the eluent, i e an increase in the *p*-hydroxybenzoate concentration present in the eluent The eluent contains an ion (of opposite charge to the stationary phase), which passes through the column These eluent ions will neutralise the oppositely charged stationary phase, as the eluent will compete for electrostatic interaction with the stationary phase IC separates ions on the basis of electrostatic interactions occurring between the charged functional groups present on the stationary phase (the fixed ionic site and its counter-ion) and the ions present in the eluent (the eluent ion and the analyte ion) The counter-ion of the fixed ionic site must be exchangeable by both the analyte ion and the eluent ion in order to achieve an effective distribution of the analyte ions between the stationary phase and the eluent, thereby leading to separation [2] Increased eluent strength affects the retention of ions in direct proportion to their charge

Indirect UV detection was used in the initial stages of these anion determinations This is shown in Figure 4 2 where the analytes are represented by negative peaks

The concentration of the *p*-hydroxybenzoate was increased in the eluent from 2 mM-4 mM respectively and the effect was studied The optimum concentration chosen for the eluent was 3 mM *p*-hydroxybenzoate as it resulted in the best separation and resolution of the test anions in a suitable time frame

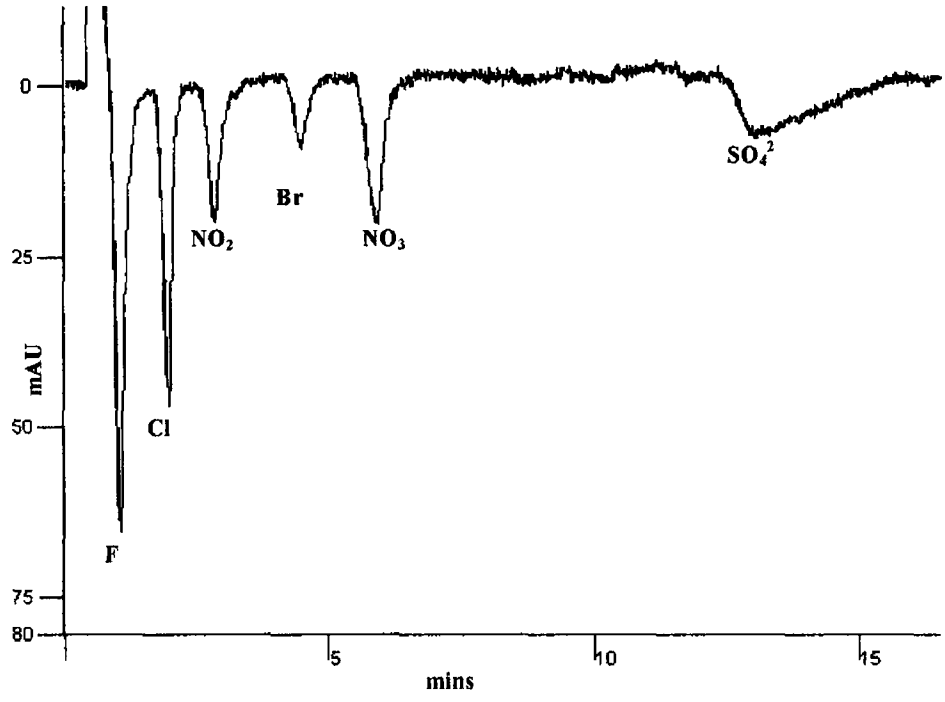

Figure 4.2: Optimised eluent for determination of six UV absorbing anions using a Chromolith SpeedROD RP-18e 25 mm x 4 6 mm i d coated monolith, fluoride (20 ppm), chloride (20 ppm), nitrite (20 ppm), bromide (20 ppm), nitrate (50 ppm) and sulphate (200 ppm) Eluent 3 mM *p*-hydroxybenzoate pH 6 9, Column temperature ambient Flow rate 1 0 mL/min Injection volume 50 µL UV-vis detection 279 nm

The peak efficiencies for the anions were calculated using the formula $N=16\ (t_r/W)^2$ The number of theoretical plates for fluoride, chloride, nitrite, bromide, nitrate and sulphate were 41, 81, 178, 400, 523 and 310 N/cm respectively using these conditions The capacity factors (k') were also calculated for the anions using different concentrations of *p*-hydroxybenzoate eluents and can be seen in Table 4 1

Table 4.1: Capacity factors (k') of the anions using 2 mM, 3 mM and 4 mM *p*-hydroxybenzoate pH 6 8 on a chromolith speedROD RP-18e 25 mm x 4 6 mm i d coated monolith (t_0=0 4828mm)

Test anions	**k' 4 mM *p*-hydroxybenzoate**	**k' 3 mM *p*-hydroxybenzoate**	**k' 2 mM *p*-hydroxybenzoate**
20 ppm fluoride	0 83	1 18	1 61
20 ppm chloride	2 30	3 00	4 61
20 ppm nitrite	3 70	4 71	7 20
20 ppm bromide	6 33	8 26	12 40
50 ppm nitrate	8 38	11 18	17 00
200 ppm sulphate	15 50	-	-

The rate at which retention can be varied by changing eluent concentration depends on the charges carried by both the solute and the eluent ions Figure 4 3 illustrates the variation of the capacity factors for the anions against eluent concentration The R^2 values for the anions were 0 9999, 0 9995, 0 9964, 0 9986 and 0 9823 for nitrate, bromide, nitrite, chloride and fluoride respectively

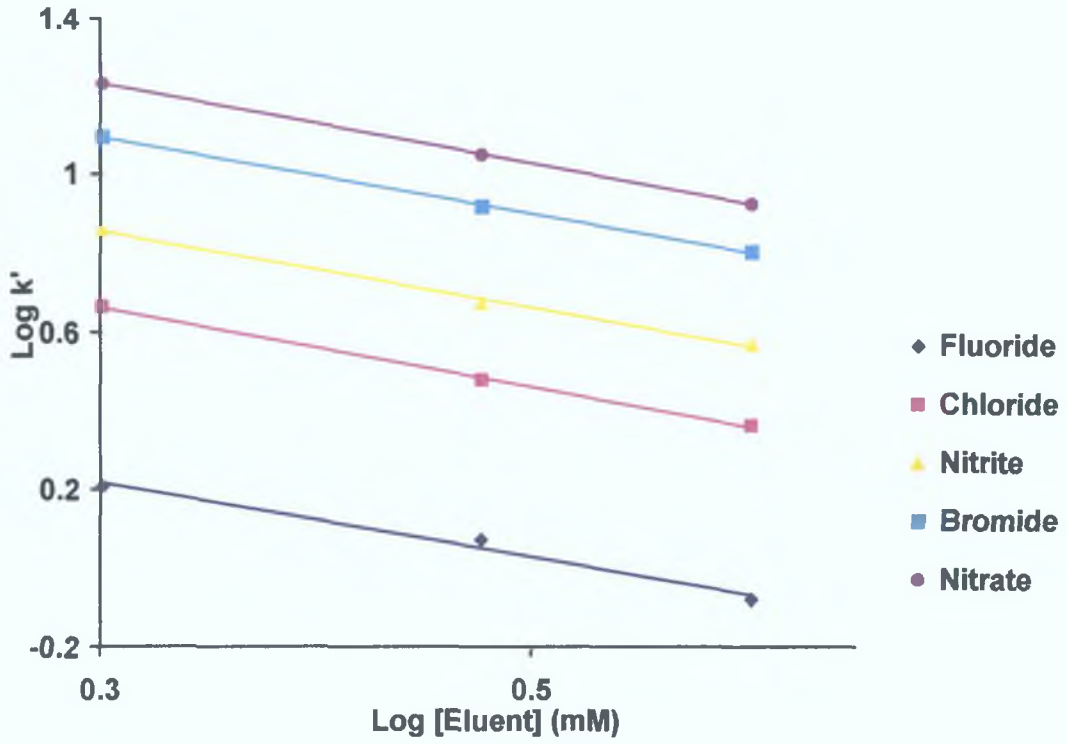

Figure 4.3: Investigation of the variation of the capacity factors for the anions and eluent concentration using a Chromolith SpeedROD RP-18e 25 mm x 4.6 mm i.d. coated monolithic anion-exchanger for the separation of five common anions, fluoride (25 ppm), carbonate (25 ppm), chloride (25 ppm), phosphate (25 ppm), nitrite (25 ppm), nitrate (25 ppm), sulphate (25 ppm). Eluent: 2-4 mM *p*-hydroxybenzoate pH 6.9, Column temperature: ambient. Flow rate: 1.0 mL/min. Injection volume: 50 µL. UV-vis detection 279 nm.

4.3 LPIC using previously optimised eluent

The system mentioned in Chapter 4.2 was modified; the high-pressure pump was replaced with a low-pressure peristaltic pump. The 50 µL injection loop was replaced with 75µL as the peak heights increased with this loop size and column overload was minimal. The flow rate of the system was then reduced to 0.65 mL/min with the introduction of this low-pressure pump and a separation of the common inorganic anions was attempted. Surprisingly, despite using the peristaltic pump to deliver the eluent, baseline pulsations were not as significant as might be expected, probably due to the damping effect of the monolithic column.

Figure 4 4, illustrates the separation of the common inorganic anions in less than 12 mins using this LPIC system with indirect UV-vis detection and the previously optimised *p*-hydroxybenzoate eluent The peak efficiencies for fluoride, chloride, nitrite, bromide and nitrate were 59, 313, 508, 841 and 756 N/cm respectively which were calculated using $N=16\ (t_r/W)^2$

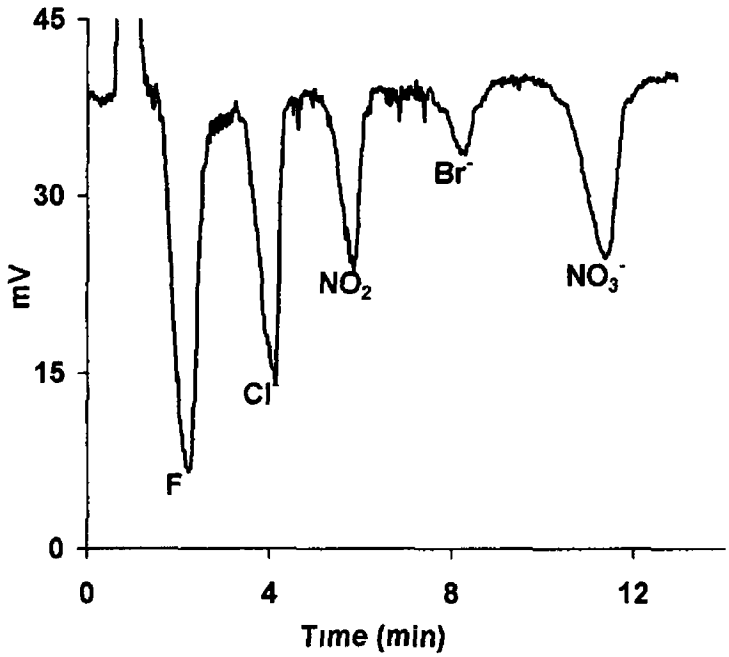

Figure 4.4: Low-pressure ion chromatogram using optimised eluent for determination of five UV absorbing anions and Chromolith SpeedROD RP-18e 25 mm x 4 6 mm i d coated monolithic column,
fluoride (20 ppm), chloride (20 ppm), nitrite (20 ppm), bromide (20 ppm), nitrate (50 ppm) Eluent 3 mM *p*-hydroxybenzoate pH 6 9 delivered by peristaltic pump, Column temperature 50^{0}C Flow rate 0 65 mL/min Injection volume 75 µL UV-vis detection 279 nm

A decreased flow rate from 1 mL/min to 0 65 mL/min led to an increased separation window of 12 mm for the separation of these 5 anions in Figure 4 4 in comparison to Figure 4 3 where 6 anions were separated in a similar time frame This was due to the use of a low-pressure peristaltic pump Backpressure and flow rate are directly related The maximum flow rate for any column is ultimately limited by operating

backpressure The greater the viscosity of the eluent, the higher will be the backpressure at a given flow rate As a coated stationary phase was used for these separations the use of aqueous based eluents was required The use of less viscous organic solvents as eluents was not advised, as the coating would strip from the column High column backpressures can ultimately result in column blockage of the coated DDAB monolith, as this low-pressure pump is not powerful enough to (i) withstand the backpressure of the column and (ii) pump the eluent through the column

4.3.1 Effect of using shorter monolithic column

The possibility of using an even shorter coated (as outlined in Chapter 2 1-column coating procedures) monolithic column was then considered, i e a chromolith guard cartridge kit (10 mm x 4 6 mm i d) As this column was shorter, it resulted in decreased column backpressure in comparison to the longer 25 mm coated monolith previously used for these separations This led to an overall system with lower pressure, which resulted in a further reduction of retention times for the common inorganic anions Column length is directly proportional to retention time and backpressure of the column

Figure 4 5 illustrates a typical low-pressure anion separation using the shorter 10 mm coated monolithic column with *p*-hydroxybenzoate eluent and UV-vis detection

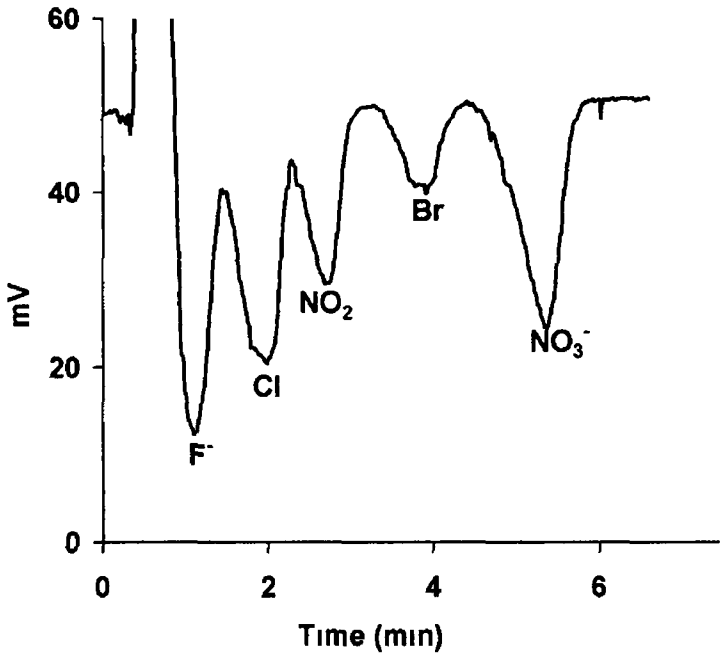

Figure 4.5: Low-pressure ion chromatogram using optimised eluent for determination of five UV absorbing anions and Chromolith SpeedROD RP-18e 10 mm x 4 6 mm i d coated monolithic column,

fluoride (20 ppm), chloride (20 ppm), nitrite (20 ppm), bromide (20 ppm), nitrate (50 ppm) Eluent 3 mM *p*-hydroxybenzoate pH 6 9 delivered by low-pressure peristaltic pump, Column temperature 50^{0}C Flow rate 0 61 mL/min Injection volume 75 µL UV-vis detection 279 nm

Table 4.2: Comparison of the capacity factors for the anions using (i) Chromolith SpeedROD 25 mm x 4 6 mm i d monolithic column and (ii) Chromolith SpeedROD 10 mm x 4 6 mm i d monolithic column and 3 mM *p*-hydroxybenzoate delivered by a low pressure peristaltic pump

Test anions	**k' using 25 mm x 4.6 mm chromolith column and low-pressure peristaltic pump (t_0=0.83min)**	**k' using 10 mm x 4 6 mm chromolith column and low-pressure peristaltic pump (t_0=0 61min)**
20 ppm fluoride	1 71	0 85
20 ppm chloride	3 95	2 27
20 ppm nitrite	6 02	3 50
20 ppm bromide	8 96	5 55
50 ppm nitrate	12 69	7 85
200 ppm sulphate	22 60	14 34

From the results in Table 4 2, using an even shorter monolithic column and a low pressure pump resulted in comparable retention times of the test anions, to that when using a high pressure pump and a longer monolithic column This reinforces the need for smaller, portable and more inexpensive systems for on-line or in-situ sampling for analytical techniques However, the use of a shorter column led to decreased peak efficiencies for the anions in Figure 4 6 The number of theoretical plates for fluoride, chloride, nitrite, bromide and nitrate were 65, 89, 135, 234 and 256 N/cm respectively This was due to minimal baseline resolution among the anions when using the 10 mm coated monolithic column

4.4 LPIC using suppressed conductivity detection

The possibility of using a different mode of detection was then considered Ion chromatography with suppressed conductivity is the most widely used mode of detection Suppressed conductivity detection is needed when conductivity detectors are used and the eluent is strongly conducting, saturating the detector's response A device, called the suppressor, is inserted between the ion-exchange separator column and the detector The suppressor releases hydronium ions or hydroxyl ions

dependent on the characteristics of the eluent, to convert it to the corresponding non-ionised species hence reducing their conductance The suppressor reduces background conductivity by acid-base neutralization and removal of ions from the eluent The suppressor modifies both the eluent and the separated analytes coming out of the separator column, so that the eluent conductance is reduced and that of the analytes is enhanced, hence detectability of the analytes is improved After leaving the suppressor, the eluent and the separated analytes then pass through the detector where they are monitored and detected This mode of detection was considered as it was thought that it would increase the signal of the standard anions In this work, a 10 cm packed bed suppressor cartridge was used which was previously modified for anion exchange separations

4.4.1 Effect of *p*-hydroxybenzoate concentration in eluent

The standard anion mixture was injected using the previously optimised eluent, 3 mM *p*-hydroxybenzoate However, fluoride and chloride were co-eluting and not resolved from each other Therefore, an eluent of lower concentration was considered for the separation of these anions The concentration of the *p*-hydroxybenzoate in the eluent had to be reduced from 3 mM to 1 mM in order for the fluoride and chloride peaks to be baseline resolved The resultant injection can be seen in Figure 4 6 The fluoride peak eluted with the void volume It was observed that five of the anions were separated in less than 15 min, however the peak efficiencies for the anions were low The N/cm for fluoride, chloride, bromide, nitrite and nitrate were 56, 92, 207, 123 and 172 respectively using these conditions

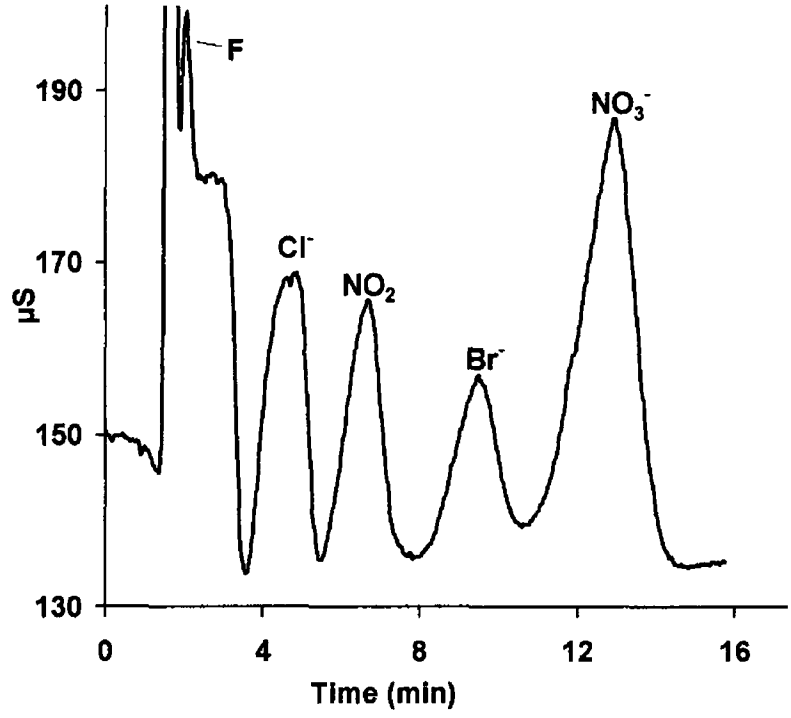

Figure 4.6 Low-pressure ion chromatogram using optimised eluent for determination of five anions and Chromolith SpeedROD RP-18e 10mm x 4 6mm i d coated monolithic column,

fluoride (20 ppm), chloride (20 ppm), nitrite (20 ppm), bromide (20 ppm), nitrate (50 ppm) Eluent 1 mM *p*-hydroxybenzoate pH 6 9 delivered by peristaltic pump, Column temperature Ambient Flow rate 0 65 mL/min Injection volume 75 µL Suppressed conductivity detection

4.4.2 Effect of flow rate

The concentration of the *p*-hydroxybenzoate in the eluent was then reduced to 0 5 mM to examine the effect on retention The flow rate was also decreased from 0 65 mL/min to 0 37 mL/min to investigate whether the fluoride peak could be separated from void volume (t_0) of the column

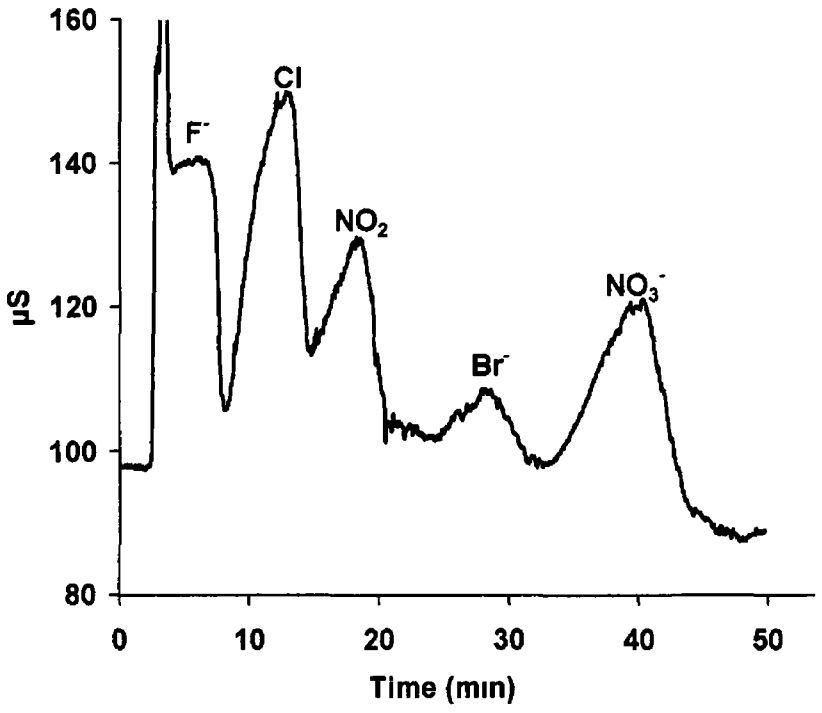

Figure 4.7: Low-pressure ion chromatogram using optimised eluent for determination of five anions and 10 mm coated monolithic column, fluoride (20 ppm), chloride (20 ppm), nitrite (20 ppm), bromide (20 ppm), nitrate (50 ppm) Eluent 0 5 mM *p*-hydroxybenzoate pH 6 9 delivered by peristaltic pump, Column temperature Ambient Flow rate 0 37 mL/min Injection volume 75 μL Suppressed conductivity detection

As can be seen from Figure 4 7, the peak shape and baseline have completely deteriorated in comparison to Figure 4 6 Band broadening is clearly evident and the fluoride peak is still co-eluting with the void volume Due to a decreased flow rate and decreased eluent concentration the total separation time was approximately 45min in comparison to 15 min previously Decreased peak efficiencies resulted The number of theoretical plates for chloride, nitrite, bromide and nitrate were 64, 111, 180 and 170 N/cm respectively

4.4.3 Concentration gradient LPIC

A concentration gradient was then attempted to decrease the separation time and to investigate whether the elution of sulphate in a suitable time frame would be possible An increase in eluent elution strength was necessary so that peaks, which would otherwise be eluted late or not at all, are accelerated [3] The system was then changed to a dual peristaltic pump in order to deliver two eluents, (i) 0 5 mM *p*-hydroxybenzoate and (ii) 3 mM *p*-hydroxybenzoate This low-pressure gradient manifold consisted of two eluent reservoirs, which were pre-mixed by use of a T-piece prior to a Rheodyne injection valve At the beginning of the separation, pump (i) started at 100% and pump (ii) started at 0%, as the early eluting peaks were better resolved from void volume The flow rates of these pumps were decreased and increased respectively, so that at the end of the separation, pump (i) was delivering 0% of 0 5 mM *p*-hydroxybenzoate and pump (ii) was delivering 100% 3 mM *p*-hydroxybenzoate Figure 4 8 shows the low-pressure gradient separation of six common inorganic anions

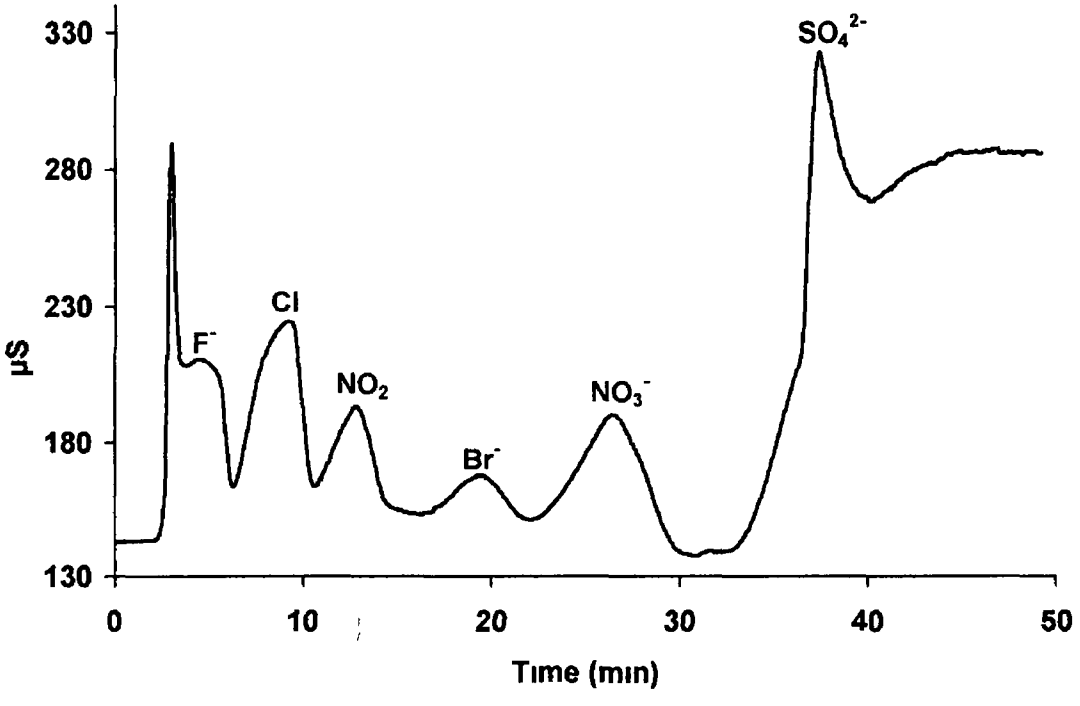

Figure 4.8. Low-pressure conc-gradient ion chromatogram for determination of six anions and 10 mm coated monolithic column,

fluoride (20 ppm), chloride (20 ppm), nitrite (20 ppm), bromide (20 ppm), nitrate (50 ppm), sulphate (50 ppm) Eluent pump (i) 0 5 mM *p*-hydroxybenzoate pH 6 9 and

pump (ii) 3 mM *p*-hydroxybenzoate pH 6 9 delivered by two peristaltic pumps, Column temperature Ambient Flow rate 0 55 mL/min Injection volume 75 µL Suppressed conductivity detection

From Figure 4 8, the fluoride peak is still not satisfactorily separated from the system peak The advantage of using this concentration gradient method is clear, resulting in the six anions being separated in just over 40 min, using a flow rate of 0 55 mL/min However, the constant increase in baseline due to the switching of eluent from 0 5 mM to 3 mM maybe considered to be a downside of this particular method, as it is difficult to calculate the number of theoretical plates and hence the efficiency of the separation

4.4.4 Analytical performance characteristics in LPIC

4.4 4.1 Limits of detection

There is a linear working range where detector response is proportional to solute concentration Above it there is a non-linear but may be useful range where the relationship between solute and response is continuously changing Above this there is saturation and no relationship Linearity of this system was measured for chloride, nitrite, bromide and nitrate in the range of 3-150 ppm A range of standards in this range were injected Each of the standards was made up in the eluent so as to increase sensitivity and decrease baseline noise However, this method was only linear in the range of 5-40 ppm for each of the anions tested

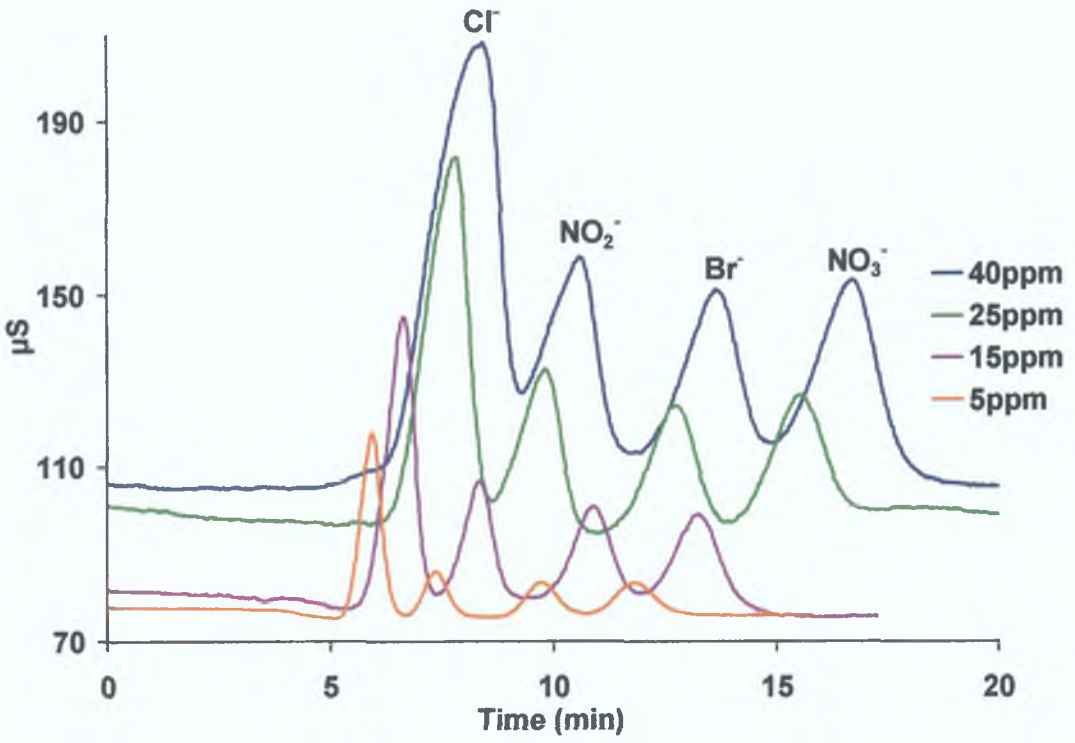

Figure 4.9: Linearity of four anions in the dynamic range of 5-40 ppm using 10 mM DDAB coated anion exchange (10 mm x 4.6 mm i.d.) monolithic column, chloride, nitrite, bromide, nitrate. Eluent: 3 mM *p*-hydroxybenzoate pH 6.9, delivered by a peristaltic pump. Column temperature: Ambient. Flow rate: 0.55 mL/min. Injection volume: 75 µL. Suppressed conductivity detection.

The linearity of these anions was calculated based on peak areas and can be seen in Table 4.3. Peak area is used for this measurement instead of peak height as height is susceptible to peak asymmetry, while area is not. Also, the linear dynamic range for area measurement is greater than for height.[4] It can be calculated by using the mathematical formula for the area of a triangle (0.5 x base x height of peak) given in mm^2.

Table 4.3: Investigation of linearity of anions using peak areas in mm^2

Conc. of anion	Peak area in mm^2 for Chloride	Peak area in mm^2 for Nitrite	Peak area in mm^2 for Bromide	Peak area in mm^2 for Nitrate
5ppm	612	112	120	121
10ppm	672	272	238	275
15ppm	833	351	300	420
20ppm	893	290	265	299
25ppm	924	314	322	363
30ppm	902	347	345	462
40ppm	1225	528	384	558

The peak areas were plotted against concentration and the R^2 value for each anion was determined and hence showed the linearity of each anion If the anions peak area increases directly proportionally to its concentration, i e is 100% linear, an R^2 value of 1 is expected The R^2 values were 0 9131, 0 7756, 0 8188 and 0 7915 for chloride, nitrite, bromide and nitrate respectively Therefore, chloride is the most linear of the anions tested However, it is evident from Table 4 3 that some of the peak areas do not correspond to an increase in concentration of the analyte This may have resulted from errors in the calculation of the peak areas due to baseline disturbances

4.4.4.2 Coated column stability (effect on retention time)

As a low-pressure peristaltic pump was used throughout this work, over time column blockage became a major problem resulting in less repeatable results and thus, changing flow rates, which subsequently corresponded to a change in the retention time of the anions Column performance degrades over time Removal of the old coating with methanol or acetonitrile and recoating the monolithic column regenerated the column Column degradation is clearly evident in Figure 4 10

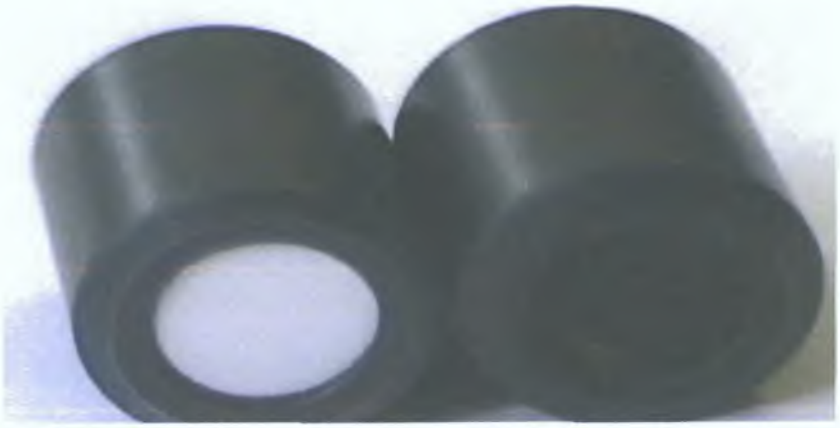

Figure 4.10: Photograph of new monolithic guard cartridge and blocked coated monolith used for separations.

As mentioned, due to column blockage and increased backpressure the flow rates decreased over time through the column, thus resulting in increased retention times of the anions. This was investigated by carrying out five repeatable injections for four anions (chloride, nitrite, bromide and nitrate). The effects can be seen in Figure 4.11.

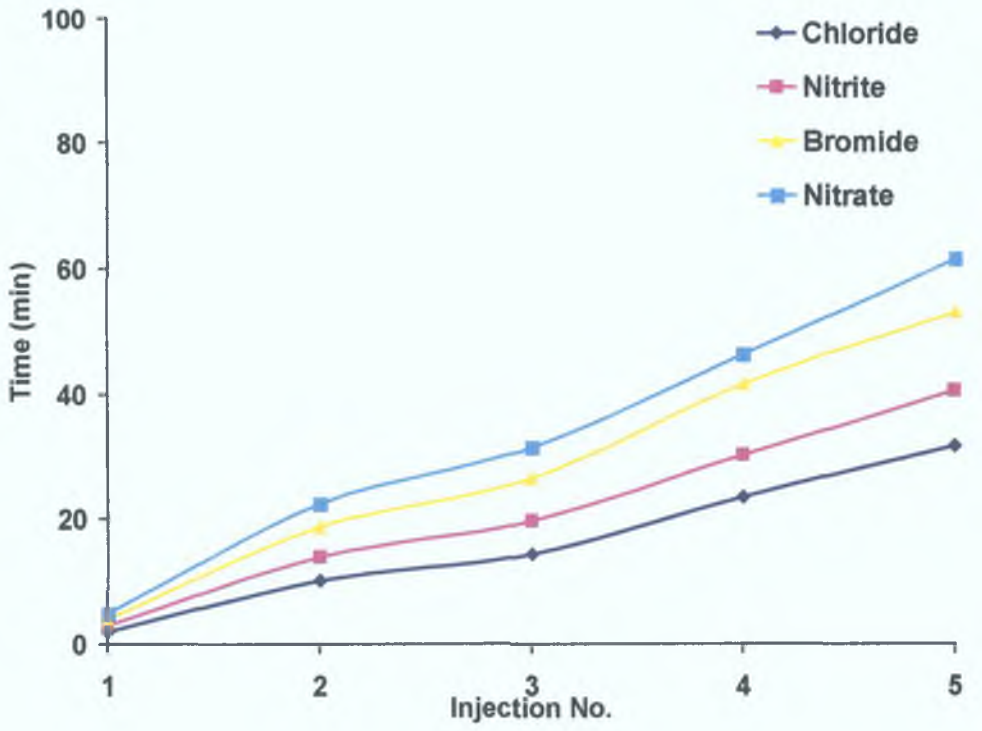

Figure 4.11: Effect on retention of four anions after 5 repeated injections using 10 mM DDAB coated anion exchange (10 mm x 4.6 mm i.d.) monolithic column, chloride (20 ppm), nitrite (20 ppm), bromide (20 ppm), nitrate (50 ppm). Eluent: 0.5 mM *p*-hydroxybenzoate pH 6.9, delivered by a peristaltic pump. Column temperature: Ambient. Flow rate: 0.55 mL/min. Injection volume: 75 µL. Suppressed conductivity detection.

4.5 Micro-LPIC using suppressed conductivity detection

In this work two independently controllable micro-peristaltic pumps were used to deliver the eluent. In isocratic mode, the two pumps were used simultaneously for the same eluent, with the eluent flow joined by a T-piece prior to the Rheodyne injection valve, which was fitted with a 25 µL injection loop. In gradient mode each micro-pump delivered the eluents from individual eluent reservoirs. The maximum flow rates at ambient temperature ranged from 0.22 mL/min (single pump operation) to 0.33 mL/min (dual pump operation). The outlet of the injection valve was connected directly to a coated anion exchange 10 mm monolithic column, coated with 2 mM DDAB (which all of the separations in 4.5 were carried out on). The eluate then passed through a 10 cm packed bed suppressor and into a conductivity

cell. All excessive connecting tubing was reduced to an absolute minimum to reduce the backpressure on the system and extra-column band broadening, [5] thus resulting in a compact, portable micro LPIC system. The micro-LPIC system described is illustrated in Figure 4.12.

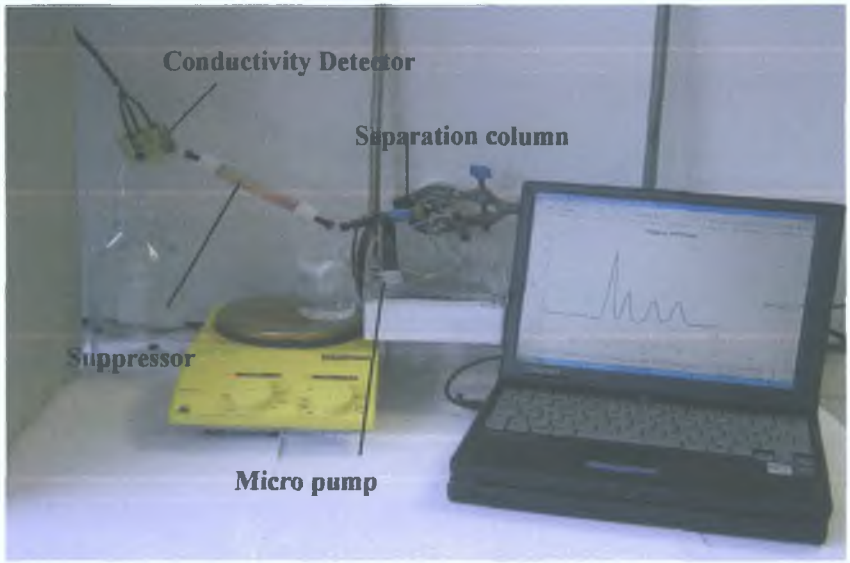

Figure 4.12: Micro LPIC system used for separations

4.5.1 Effect of temperature on peak efficiency and capacity factors

The effect of temperature was determined by varying the eluent temperature from 25°C to 45°C. Temperature has little effect on the weakly retained anions. However, on the later eluting anions like sulphate, a higher temperature results in a shorter retention time. This was to be expected as the retention mechanism for these late eluting anions is partly by electrostatic interaction and hydrophobic interaction with ODS stationary phase on the monolithic column. In these interactions, an increase in temperature increases the diffusion rates due to lower eluent viscosity and therefore improves the mass transfer rate between the eluent and the stationary phase. Equilibrium is shifted to the eluent resulting in reduced band broadening and so improving efficiency. Another reason for using elevated temperatures is to reduce the overall column backpressure by decreasing the eluent viscosity due to heating. Eluent viscosity is reduced by approx. 1% for every 1°C increase in temperature between 10°C and 70°C. Using 2 mM and 5 mM *p*-hydroxybenzoate eluents, the retention, separation and peak efficiencies of the common anions were investigated using this micro LPIC system and can be seen in Table 4.4 and 4.5.

Table 4.4: Capacity factors (k') for anions on low-pressure 1 0 x 0 4 cm monolithic anion exchanger obtained under isocratic conditions with 2 mM and 5 mM eluent (pH 6 5) [a]Temperature of the eluent reservoir

Anion	k' (2 mM) 25°C[a]	k' (2 mM) 45°C[a]	k' (5 mM) 25°C[a]	k' (5 mM) 45°C[a]
Fluoride	0 69	0 68	0 29	0 30
Bromate	1 38	1 64	0 62	0 69
Chloride	1 85	1 85	0 66	0 66
Nitrite	3 06	3 04	1 08	1 12
Phosphate	5 00	3 99	1 21	1 42
Bromide	5 35	5 34	1 84	1 86
Nitrate	7 32	7 34	2 50	2 51
Chlorate	10 91	12 16	4 23	4 46
Sulphate	>15	>15	3 51	4 60

Table 4.5: Peak efficiencies (calculated using $N=16t_r^2/W^2$) for anions on low-pressure 1 0 x 0 4 cm monolithic anion exchanger obtained under isocratic conditions with 2 mM and 5 mM eluent (pH 6 5) [a]Temperature of the eluent reservoir

Anion	N (2 mM) 25°C[a]	N (2 mM) 45°C[a]	N (5 mM) 25°C[a]	N (5 mM) 45°C[a]
Fluoride	216	303	365	523
Bromate	690	847	1302	1277
Chloride	499	459	697	854
Nitrite	608	778	485	433
Phosphate	345	238	588	1116
Bromide	566	442	400	994
Nitrate	682	641	691	961
Chlorate	459	648	507	1242
Sulphate	-	-	818	814
Average N	508	545	650	913

As can be seen from Tables 4 4 and 4 5, improvements in peak efficiency from increased eluent temperature were only marginal when using low strength eluent, but were considerably improved when using the 5 mM eluent When expressed as N/m, an average value of 91,300 was obtained when using the 5 mM eluent at the elevated temperature of 45°C The stability of the capacity factors for most of the anion peaks over the differing temperatures conveys the stability of the DDAB coating even at increased temperatures

A typical chromatogram obtained on a low-pressure micro IC system is shown in Figure 4 13 The isocratic chromatogram labelled (a) shown was obtained at room temperature and with both pumps simultaneously delivering the eluent at a flow rate of 0 33 mL/min By warming only the eluent reservoir to 45°C, the decrease in eluent viscosity of ~15% and increased flow rate, reduces the overall separation window of the anions by approximately 2 mm This is represented by the (b) chromatogram in Figure 4 13

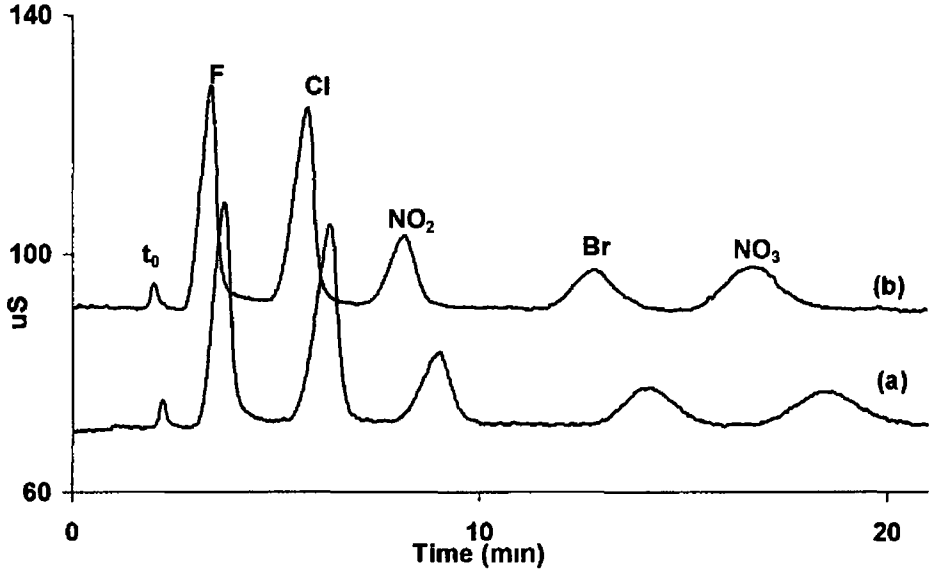

Figure 4 13· Low-pressure ion chromatograms obtained using a 10 mm coated monolithic anion exchanger for the separation of five common anions, fluoride (50 ppm), chloride (50 ppm), nitrite (50 ppm), bromide (50 ppm), nitrate (50 ppm) Eluent 2 mM *p*-hydroxybenzoate eluent delivered by two micro peristaltic pumps, (a) Eluent at 25°C (b) Eluent at 45°C Flow rate 0 33 mL/min Injection volume 25µL Suppressed conductivity detection

As can be seen from Figure 4 13, excellent peak efficiencies were possible on the micro-column, comparable to those commonly achieved with many commercial 15-25 cm anion exchange columns The level of performance for this 10 mm anion exchanger has not been seen previously and is highly significant for the future development and miniaturisation of micro-scale LPIC systems The separation of fluoride from the void volume was particularly impressive as traditionally this would have been a problem with low capacity anion exchange columns

4.5.2 Micro-LPIC concentration gradients

With the 2 mM eluent it is clear that sulphate is retained for an excessively long time Higher strength eluents (3 mM to 5 mM) could be used to easily elute sulphate However, the use of these eluents subsequently reduced the resolution of the fluoride peak from both the void volume and chloride peaks The dual micro-pump design of the system allowed for simple gradient separations to be carried out, with one pump delivering 2 mM eluent and the other delivering 5 mM eluent These eluents were mixed as previously mentioned in 4 4 3 by using a T-piece Individual control of the two micro-pumps allowed a gradual gradient from one to the other without changing the overall flow rate of the system The resultant low-pressure gradient separation can be seen below in Figure 4 14

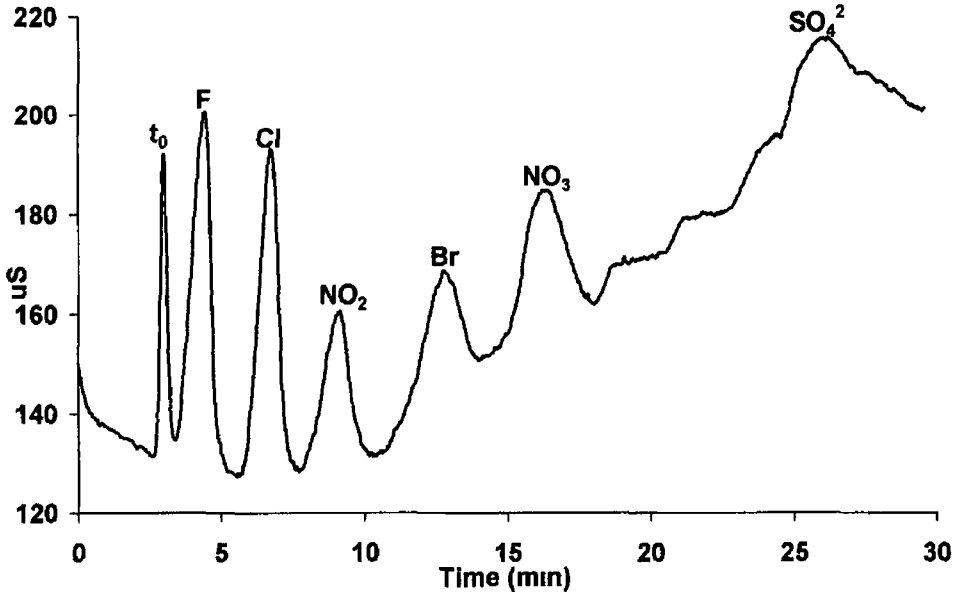

Figure 4.14: Low-pressure gradient ion chromatogram obtained using a 10 mm coated monolithic anion exchanger for the separation of six common anions, fluoride (50 ppm), chloride (50 ppm), nitrite (50 ppm), bromide (50 ppm), nitrate (50 ppm), Sulphate (50 ppm) Eluent 2 mM *p*-hydroxybenzoate eluent, increasing to 5 mM over a 15 min interval from 5-20 min, Eluent at 45°C Flow rate 0 33 mL/min Injection volume 25 µL Suppressed conductivity detection

As can be seen above in Figure 4 14, the gradient separation offers the best method of separating these anions as it allows for both the early eluting anions and the later eluting anions to be separated successfully This chromatogram demonstrates the potential for this micro LPIC in gradient separations and possibly for use in the determination of commonly monitored anions (fluoride, chloride, nitrate and sulphate) in real water samples The peak efficiencies at an increased temperature of 45^{0}C for these anions were reasonable The number of theoretical plates for fluoride, chloride, nitrite, bromide, nitrate and sulphate were 69, 205, 248, 198, 309 and 599 N/cm respectively

4.5.3 Real sample analysis using micro-LPIC

A number of tap water samples were collected from Dublin City University, Dublin 9, Ireland and analysed using this micro LPIC system A number of inorganic anions were detected from the injection of neat tap water as can be seen in Figure 4 15

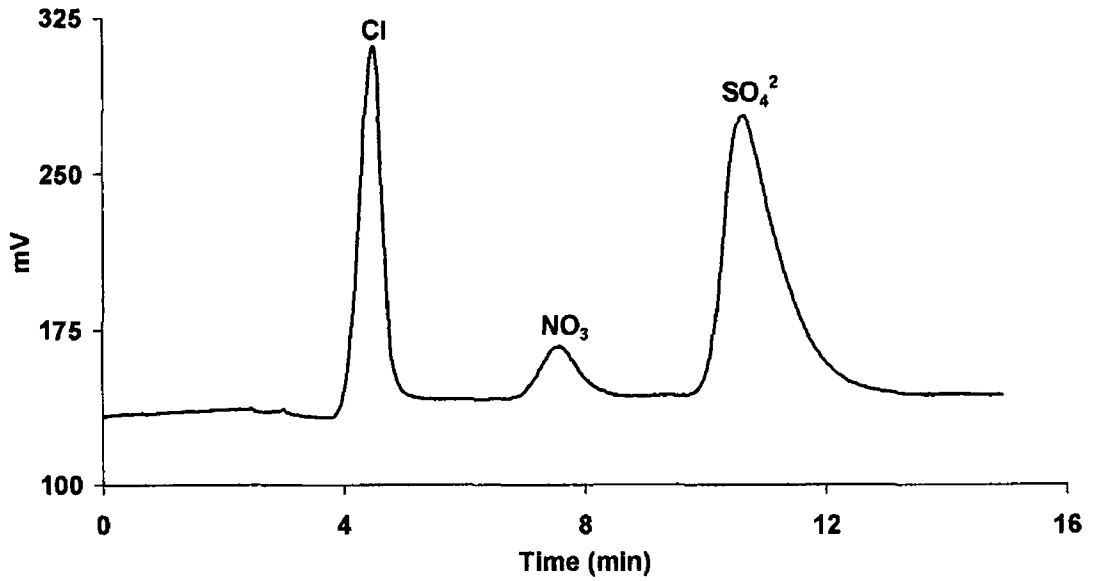

Figure 4.15: Low-pressure ion chromatogram obtained from the injection of a Dublin tap water sample. Eluent 5 mM *p*-hydroxybenzoate eluent delivered using micro-peristaltic pumps. Flow rate 0.33 mL/min. Injection volume 25 µL. Suppressed conductivity detection.

This low-pressure micro-IC system can be used for the determination of commonly monitored anions (fluoride, chloride, nitrate and sulphate) in real samples. LPIC allows for short efficient separations on a micro scale level using inexpensive portable peristaltic pumps. As there were only three anions in this separation, baseline resolution and reasonable peak efficiencies were evident. The number of theoretical plates for chloride, nitrate and sulphate were 196, 320 and 268 N/cm respectively.

4.6 Investigating van Deemter curves for anions and column capacity of DDAB coated columns using *p*-hydroxybenzoate

4.6.1 van Deemter curves for anions

As mentioned previously in Chapter 1, the van Deemter equation shows the relationship between eluent velocity and peak efficiency. The van Deemter equation ($H=A+B/u+Cu$) summarises on-column effects that contribute to plate height and hence band broadening. All the factors contributing to band broademng and the inefficiency of a system have been previously mentioned in Chapter 1. Figure 1.2 illustrates a typical van Deemter curve where band broadening in a system is

minimised. The longer a chromatographic bed, the more theoretical plates it contains and the better the degree of separation of a mixture. This effect is partly counteracted by band broadening. Peaks become increasingly broader, the greater the distance along the column and the longer the retention time. [3]

The van Deemter curves for chloride, nitrate and sulphate were examined and are illustrated as Figure 4.16.

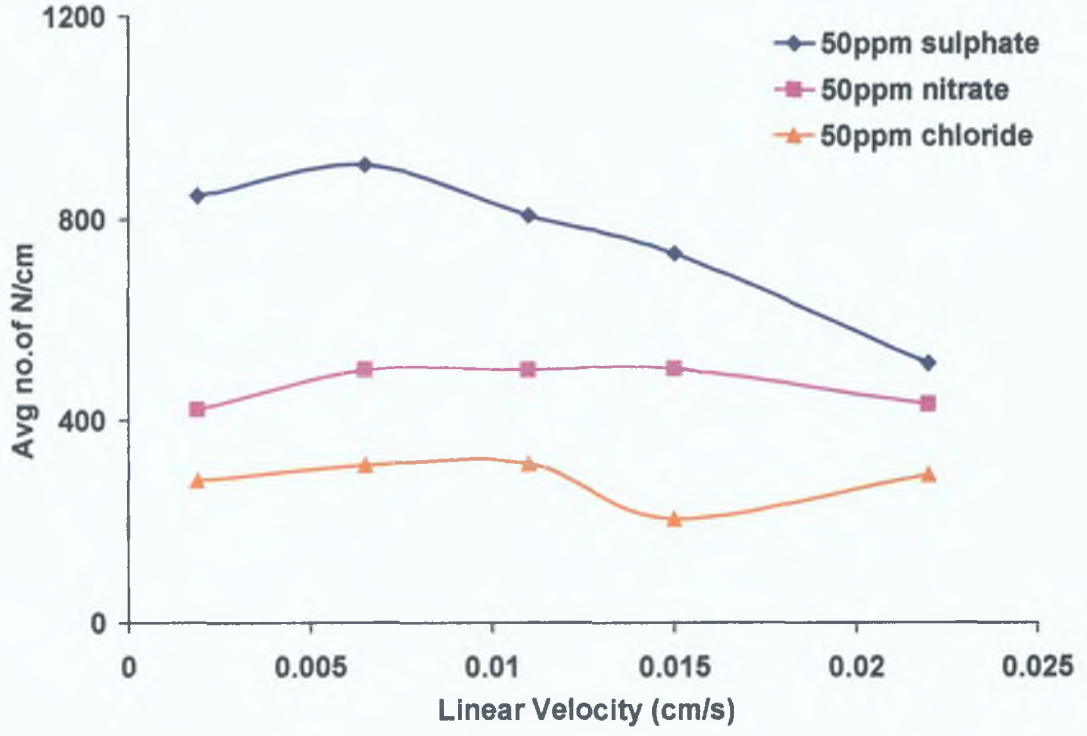

Figure 4.16: van Deemter curves for three anions using high-pressure pump and 10 mM DDAB coated anion exchange (10 mm x 4.6 mm i.d.) monolithic column. chloride (50 ppm), nitrate (50 ppm) and sulphate (50 ppm). Eluent: 5 mM *p*-hydroxybenzoate pH 6.9 heated to 45°C. Velocity: various (cm/s). Injection volume: 25 µL. Suppressed conductivity detection.

These anions were injected singly using the conditions mentioned in the caption of Figure 4.16, before doing so as part of a mixture at various flow rates ranging from 0.1 mL/min to 1 mL/min. The average number of theoretical plates in cm was calculated for each anion ($N=16t_r^2/W^2$). From Figure 4.16 none of the curves shown exhibit typical van Deemter behaviour.

4.6.2 Column capacity of DDAB coated columns

Column capacity essentially refers to the exchange functional groups with which the sample analytes interact as they travel through the column The number of groups can be modified by the column body format and/or by the density of the exchange functional groups in the resin [11]

The absolute capacity of the column is the maximum amount of a solute that can be introduced into a column before significant peak distortion occurs Retention time and peak width are independent of the amount of sample injected up to a point called the sample loading capacity Above this point, retention times (k') decrease and peak widths increase When retention time decreases by 10% of its normal value, the column capacity has been exceeded Overloaded peaks are asymmetric with a leading egde Column capacity is related to exchange functional groups on the stationary phase, column diameter and solute retention Sample loading capacity decreases as solute retention increases, thus two similar polarity solutes may overload the column by different amounts resulting in lower capacity for the later eluting solute [6]

The ability to increase the capacity of the column (peak capacity and sample load capacity) has been suggested by Gray *et al*, by coupling the monoliths serially and/or in parallel The authors found that a serial couple would result in increased number of theoretical plates, while a parallel couple effectively allows splitting of the flow stream and hence a decrease in the apparent sample load in accordance to the number of parallel flow channels [12]

A column capacity study was carried out on a coated 10 mm monolithic guard column coated with 10 mM DDAB and a 25 mm monolithic column coated with 10 mM DDAB The degradation of column capacity was also investigated as the volume of eluent (*p*-hydroxybenzoate) passing through the newly coated 10 mm guard column increases All the sites on the stationary phase were converted into the chloride form by using 50 mM NaCl The system was then washed with Milli-Q water to remove the excess chloride on the stationary phase 1 mM *p*-hydroxybenzoate eluent pH 6 9 was then pumped through the column The volume

required for the absorbance at 279 nm to increase was monitored First derivative plots were drawn using MS Excel and the exact point of inflection, i e the column capacity, could be calculated See Table 4 6

Table 4.6: Investigation of column capacity on 10 mM DDAB coated monolithic columns

Expt No.	**Column Capacity (min) on coated old 10 mm column**	**Column Capacity (min) on coated new 10 mm column**	**Column Capacity (mm) on coated old 25 mm column**
1	11 66	14 75	29 78
2	11 81	14 06	29 46
3	11 01	14 06	28 23

The flow rate of the overall system was 1 mL/min From this, the volume of 1 mM *p*-hydroxybenzoate pH 6 9 in mLs taken for the 10 mM DDAB coated column to reach its capacity could be calculated The column capacity for the old 10 mm column, new 10 mm column and old 25 mm column were 11 5 μmoles, 14 3 μmoles and 29 2 μmoles respectively

The volume of eluent passing through the column affected column capacity and was investigated at a flow rate of 1 mL/min The capacity of the newly coated 10 mm monolithic column was investigated after 500 mLs, 1000 mLs and 2000 mLs of eluent The results are shown in Table 4 7 below It is clearly evident that the column capacity drops significantly as the volume of eluent passing through the column increases After 500 mLs of 1 mM *p*-hydroxybenzoate pH 6 9 passing through the column the column capacity of the 10 mM DDAB coated column was 13 86 μmoles However, the capacity of the column drops considerably to 6 15 μmoles after 2 L of eluent passed through it

Table 4 7: Investigation of column capacity on 10 mM DDAB coated monolithic column after a known volume of eluent passes through it

Volume of eluent passing through the column	**Column Capacity (min) on newly coated 10 mm column**
500 mLs	13 86
1000 mLs	7 68
2000 mLs	6 15

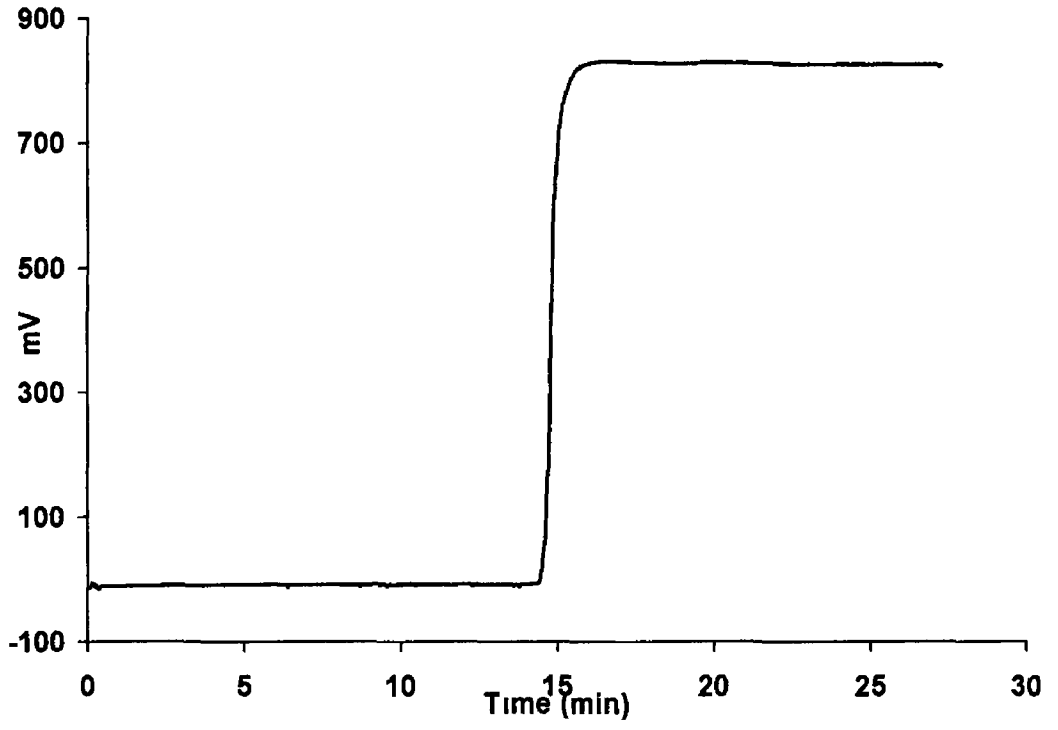

Figure 4 17: Column capacity illustration using a newly coated (10 mM DDAB) Chromolith SpeedROD 10 mm x 4 6 mm i d monolithic column
Eluent 1 mM *p*-hydroxybenzoate pH 6 9 Flow rate 1 mL/min Direct UV detection at 279 nm

The point at which column capacity occurs can be seen clearly from Figure 4 17 i e the point at which the absorbance increases A first derivative plot of Figure 4 17 was used to determine the exact point at which column capacity occurs This is shown as Figure 4 18

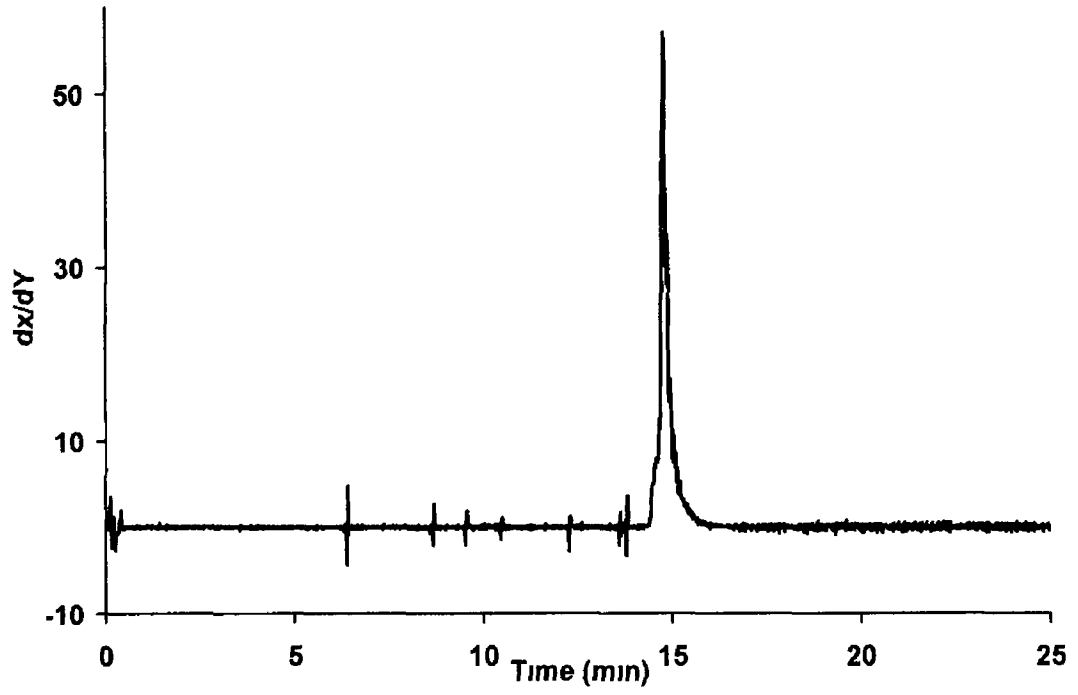

Figure 4.18: First derivative plot of column capacity chromatogram using a newly coated (10 mM DDAB) Chromolith SpeedROD 10 mm x 4 6 mm i d monolithic column

Eluent 1 mM *p*-hydroxybenzoate Flow rate 1 mL/mm Direct UV detection at 279 nm

From Figure 4 18, it is evident that column capacity occurs at approximately 14 76 min A first derivative plot is helpful in determining the exact point at which column capacity is reached on this 10 mM DDAB coated monolithic column The capacity of this column deteriorates rapidly as the volume of eluent passing through the column increases Hence, suggesting that the DDAB coating of the column is not stable for continual use after long periods of time

4.7 Conclusions

The use of *p*-hydroxybenzoate eluents for anion separations on a newly developed LPIC method was successful A low-pressure micro-IC system was also developed and can be used for the determination of commonly monitored anions (fluoride, chloride, nitrate and sulphate) in water samples LPIC allows for short efficient

separations on a micro scale level using inexpensive portable peristaltic pumps Peak efficiencies using this low-pressure system were comparable to those achieved on a 15-25 cm commercial anion-exchange column The level of performance for the short 10 mm monolithic anion-exchanger is highly significant for the future development of LPIC systems The separations obtained with the micro-IC system and 1 0 cm column to-date were extremely promising and of great significance, particularly in a time of maximum interest in miniaturised analytical systems The micro LPIC system was then applied to real water samples Separation of chloride, nitrate and sulphate in this water sample was possible van Deemter curves and column capacity were also studied using these *p*-hydroxybenzoate eluents

4.8 References

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CHAPTER 5

Phosphate, Arsenate and Silicate determination by LPIC and PCR detection

5.1 Aims

The aim of this chapter was to compare and contrast the sensitivity of a PCR detection method with an LED detector and UV-vis detector for the separation of phosphate, arsenate and silicate in water samples and to develop a LPIC-PCR system Optimisation of a method for the separation and determination of phosphate was first developed The length of the reaction coil, velocity of eluent and temperature were carefully considered An anion-exchange column was used for early separations and then the possibility of using a coated 10 mm N-dodecyl-N, N-(dimethylammonio) undecanoate (DDMAU) monolithic column was investigated

Phosphates are introduced into natural waters from inadequately treated sewage containing detergents and water from human and animals If present in excess quantity, they lower the quality of the water and cause the death of living organisms by stimulating the growth of algae [1] Phosphates have traditionally been used as fertilisers Excessive use of phosphate by overfertilisation can result in overabundance of aquatic media in industrial and domestic wastewater Consequently, monitoring of phosphates in waters is essential to control and avoid eutrophication of the aquatic environment This will result in increased dissolved oxygen levels due to the rapid growth of aquatic vegetation and hence cause the death and decay of vegetation and aquatic life Enrichment of aquatic areas with plant nutrients results in oxygen depletion, fish kills, loss of shellfish, decline in seagrasses and corals and toxic blooms Chromatographic analysis of phosphate, as well as common inorganic anions, has traditionally been performed using ion-exchange columns with alkaline eluents (e g carbonate solutions) and conductivity detection

Silicate is considered to be the core parameter of oceanography The dissolved silica is a valuable tracer of water masses, motions or mixing processes Silicate concentrations promote the growth of phytoplankton in the open ocean or coastal environments [2] Silicate serves as a micronutrient for diatomaceous algae This type of algae converts soluble silicate to solid silicate, and thus increases the insoluble material percentage of water, which is finally used for drinking [1]

The toxicity and possible carcinogenicity of arsenate has made it necessary to carefully monitor its level in the environment It has been suggested that arsenate adversely affects human health and therefore poses a health risk It is for this reason that it is continuously monitored in the environment

The simultaneous determination of phosphate, arsenate and silicate in samples by a simple and inexpensive method is difficult, due to mutual interference between the three anions The determination of silicate has proved particularly less sensitive than phosphate and arsenate The main purpose of this work was to develop a method for the determination of phosphate and arsenate by a simple, sensitive and selective method utilising relatively inexpensive LPIC systems

5.2 Method development for the PCR

The possibility of determining of phosphate by anion exchange low-pressure flow-injection chromatography and post-column (molybdenum blue) reaction was investigated

Post-column reaction detection for the determination of phosphate has been previously attempted by Grudpan *et al* [3] using a phosphate-molybdate-ascorbic acid reaction PCR is mostly used in combination with UV-vis detectors PCR has a wide range of advantages including high selectivity, wide linear range, and its ability to achieve low detection limits PCR involves a chemical reaction of solutes, as they are eluted from the column, before they pass through the detector Low concentrations of analytes or high concentrations of interferences can be detected in this way as this approach enhances the specificity and sensitivity of the detection method PCR systems can increase the baseline noise of a UV-vis detector, the extent of which depends on the post-column delivery method used and the reaction involved [4]

An FIA manifold was constructed for this reaction This involved using three pumps (one for the eluent, and two for the post-column reagents (the ascorbic acid and the molybdate solution) A high-pressure pump was initially used to deliver the 20 mM

KCl eluent (flow rate 0 3 mL/mın), whereas two perıstaltıc pumps were used to delıver the post-column reagents These reagents were mıxed wıth each other by usıng a T-pıece before mıxıng wıth the eluate from the mıcro-bore anıon exchange column (AG18) Detectıon was by UV-vıs-PCR at 600 nm The overall flow rate of the system was 1 mL/mın The reactıons for molybdenum blue may ınvolve

$$\textbf{heptomolybdate + phosphate} \overset{\textbf{fast}}{\Longleftrightarrow} \textbf{yellow heteropoly complex}$$

$$\textbf{yellow heteropoly complex + ascorbıc acıd} \overset{\textbf{slow}}{\Longleftrightarrow} \textbf{molybdenum blue}$$

$$\textbf{Mo (VI)} \qquad\qquad \textbf{Mo (V)} \longleftrightarrow \textbf{Mo (III)}$$

The premıxed stream of molybdate and ascorbıc acıd form the reactıve ıntermedıate (yellow heteropoly complex, the reduced form of molybdate), whıch ıs then readıly avaılable and reactıve to the ınjected phosphate to yıeld molybdenum blue very fast, ın the order of seconds [3]

The reactıon was tımed and the molybdenum blue colour started to form after 36 sec A reactıon coıl was needed to ensure complete mıxıng of post-column reagents for the post-column reactıon to occur successfully The resultant phosphate response can be seen ın Fıgure 5 1

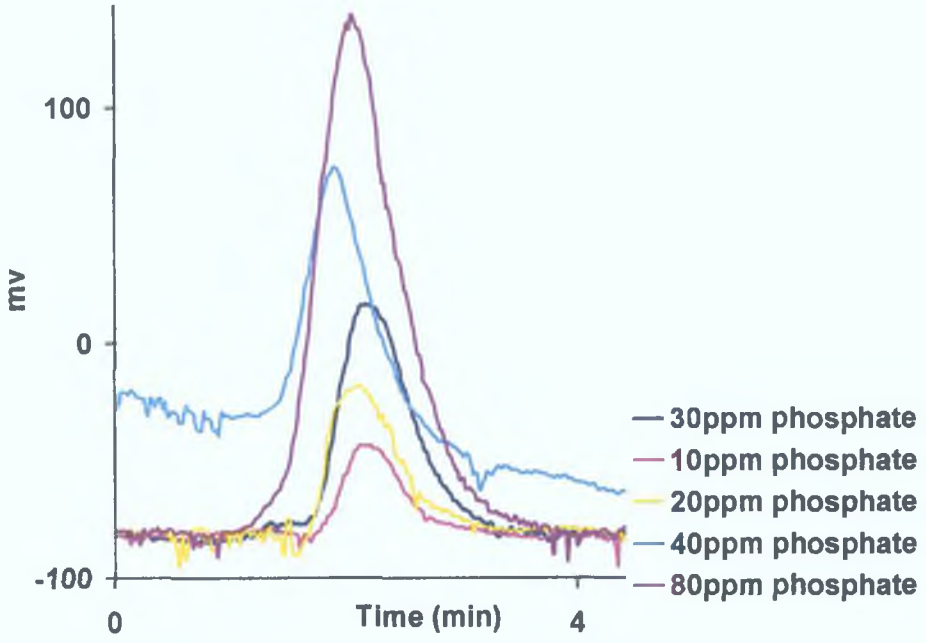

Figure 5.1: Determination of phosphate by anion exchange chromatography and PCR detection, series of phosphate standards. Eluent: 20 mM KCl. Flow rate: 1 mL/min. UV-vis detection by PCR at 600 nm. 146 mm x 0.5 mm i.d. reaction coil to mix 0.6% ammonium molybdate and 0.6% ascorbic acid (PCR reagents), Dionex Anion exchange (AG18) column at ambient temperature, injection volume: 25 µL.

5.2.1 Optimising flow rate and temperature for this PCR

When optimising these parameters, modification of the previous system used in 5.2 was necessary. The two peristaltic pumps previously used to pump the PCR reagents were replaced with a high-pressure pump and a T-piece to ensure mixing of these two reagents. The optimum flow rate of the PCR reagents for this reaction was then investigated in the range of 0.6 mL/min to 1.6 mL/min and found to be 0.9 mL/min. This is illustrated in Figure 5.2. The flow rate of the eluent was kept constant at 0.3 mL/min as a micro-bore column was used for this separation. The temperature at which the optimal PCR reaction occurs was then investigated over a range of 20°C to 60°C; 30°C was deemed the optimum temperature, the resultant phosphate response is shown in Figure 5.3.

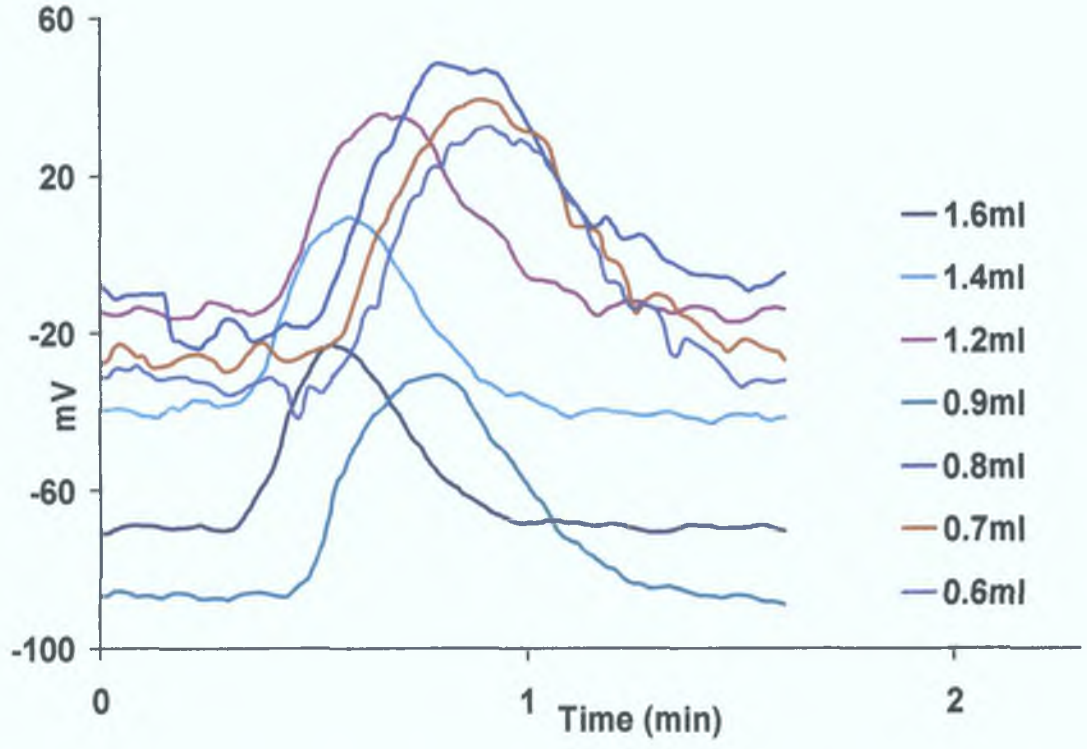

Figure 5.2: Effect of flow rate of the PCR reagents on the phosphate peak using anion-exchange chromatography and UV-vis PCR detection. 20 ppm phosphate. Eluent: 20 mM KCl. Flow rate: Eluent-0.3 mL/min, PCR reagents-various. UV-vis detection by PCR at 600 nm. 146 mm x 0.5 mm i.d. reaction coil to mix 0.6% ammonium molybdate and 0.6% ascorbic acid (PCR reagents) at ambient temperature. Dionex Anion exchange (AG18) column at ambient temperature. Injection volume: 25 µL.

The effect of flow rate upon the signal to noise ratio (S/N) for the phosphate peak was determined. The optimum flow rate was 0.9 mL/min. The signal to noise ratio for the phosphate peak at different temperatures was then calculated and can be seen in Table 5.1.

The smallest measurable peak is the smallest peak, which can be unambiguously measured from baseline noise. It is described in terms of the signal to noise ratio, S/N, which compares the height of a peak to the height of the surrounding noise. If a solute is repeatedly analysed, its peak can still be seen on a chromatogram when the S/N ratio is as low as one, i.e. the peak is as high as the noise amplitude. When the S/N ratio is three, it is unambiguous from the baseline noise and it defines the smallest peak that can be confidently judged as a peak, i.e. the limit of detection

(S/N=3).[5] The signal to noise ratio was measured by taking three points of baseline either side of the peak and measuring them in millimetres, getting an average of these six points. This value represented the noise contribution (N). The height of the signal (S) was then measured in millimetres.

This optimum flow rate was then used to investigate effect of temperature on the phosphate peak. Temperature optimisation was determined by measuring the effect on the phosphate peak of heating the reaction coil at various temperatures. Figure 5.3 shows the effect of heating the reaction coil on the phosphate peak at optimum flow rate.

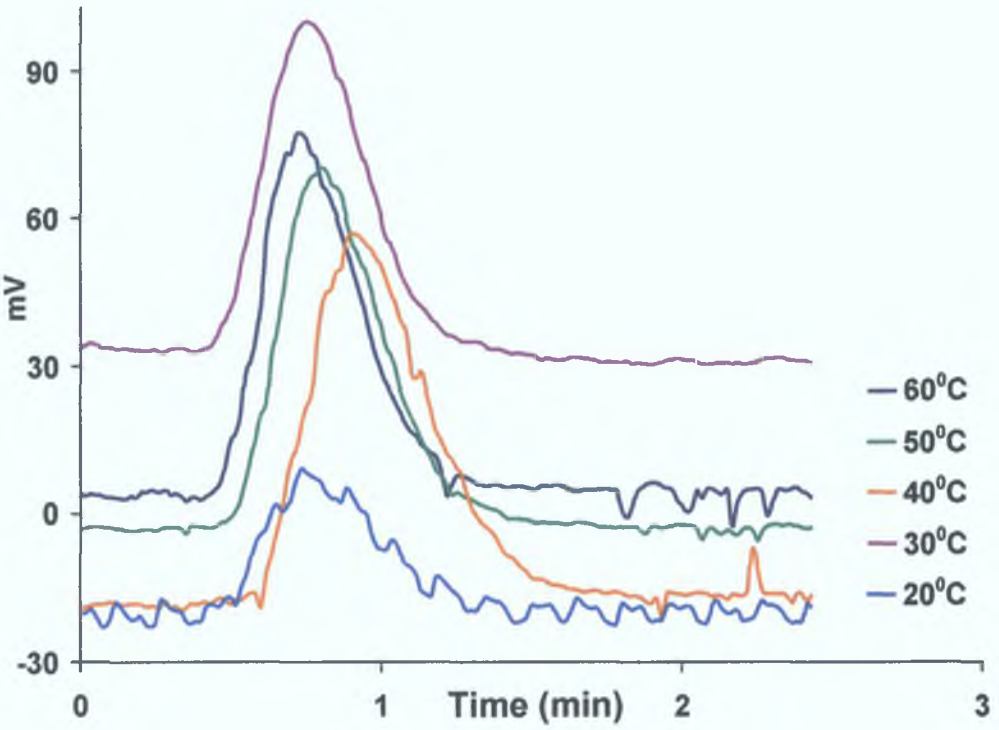

Figure 5.3: Effect of temperature of the reaction coil on the phosphate peak using anion exchange chromatography and UV-vis PCR detection. 20 ppm phosphate. Eluent: 20 mM KCl. Flow rate: Eluent-0.3 mL/min, PCR reagents-0.9 mL/min. UV-vis detection by PCR at 600 nm. 146 mm x 0.5 mm i.d. reaction coil to mix 0.6% ammonium molybdate and 0.6% ascorbic acid (PCR reagents) heated in water bath at a range of temperatures from 20°C to 60°C. Dionex Anion exchange (AG18) column at ambient temperature. Injection volume: 25 µL.

Table 5.1: S/N ratios for 20 ppm phosphate at various flow rates and temperatures using the system described in 5 1

Flow rate in mL/min	**S/N ratio of 20 ppm phosphate**
1.6	17
1 5	13
1.4	17
1.3	8
1.2	15
1.1	7
1.0	17
0.9	**24**
0.8	9
0.7	13
0.6	10

Temperature in °C	**S/N ratio of 20 ppm phosphate**
60	19
55	20
50	44
45	47
40	26
35	44
30	**50**
25	13
20	16

These optimum conditions were then used and a range of phosphate standards from 10 ppm to 100 ppm were analysed using (i) UV-Vis/post-column reaction detection at 600 nm and (ii) LED (light emitting diode)-post-column reaction detection at 630 nm The possibility of this LED device as the detection mode was investigated for further miniaturisation of system components The injection loop was increased from 25 µL to 100 µL to increase the sensitivity of the peak Figure 5 4 illustrates the injection of the phosphate standards using the optimum conditions described

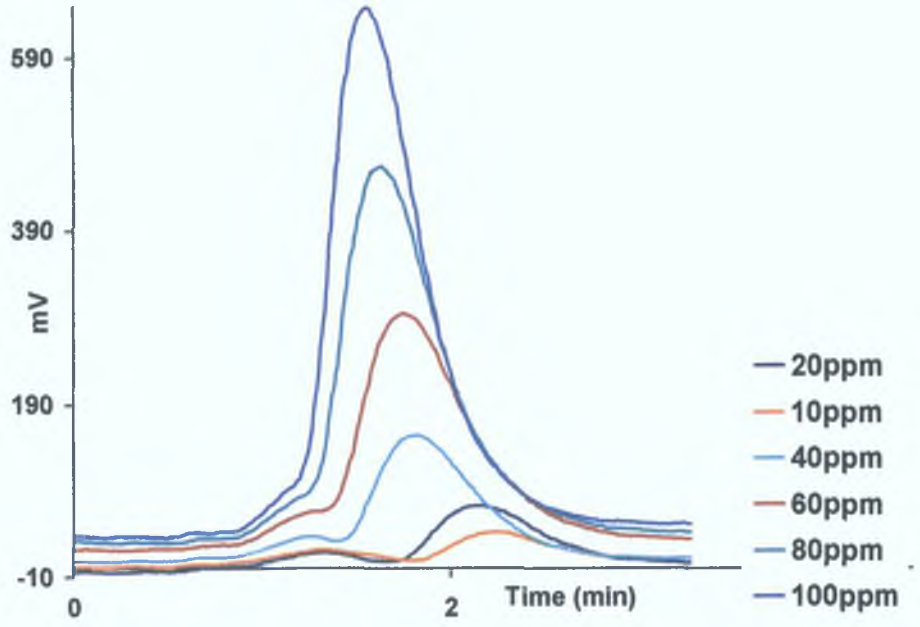

Figure 5.4: Phosphate standards using optimum conditions, anion-exchange chromatography and UV-vis PCR detection. Range of phosphate standards from 10 ppm to 100 ppm. Eluent: 20 mM KCl. Flow rate: Eluent-0.3 mL/min, PCR reagents-0.9 mL/min. UV-vis detection by PCR at 600 nm, 146 mm x 0.5 mm i.d. reaction coil to mix 0.6% ammonium molybdate and 0.6% ascorbic acid (PCR reagents) heated in water bath at 30°C. Dionex Anion exchange (AG18) column at ambient temperature. Injection volume: 100 µL .

It is evident from Figure 5.4, that considerable improvement of peak shape and baseline noise has occurred when using the optimum conditions. This determination of phosphate was then attempted using a red LED detector at 630 nm with post-column reaction detection to further miniaturise the system. However, it was observed from Figure 5.5 that this mode of detection is significantly inefficient for phosphate determination due to increased baseline noise, increased band broadening and lower sensitivity.

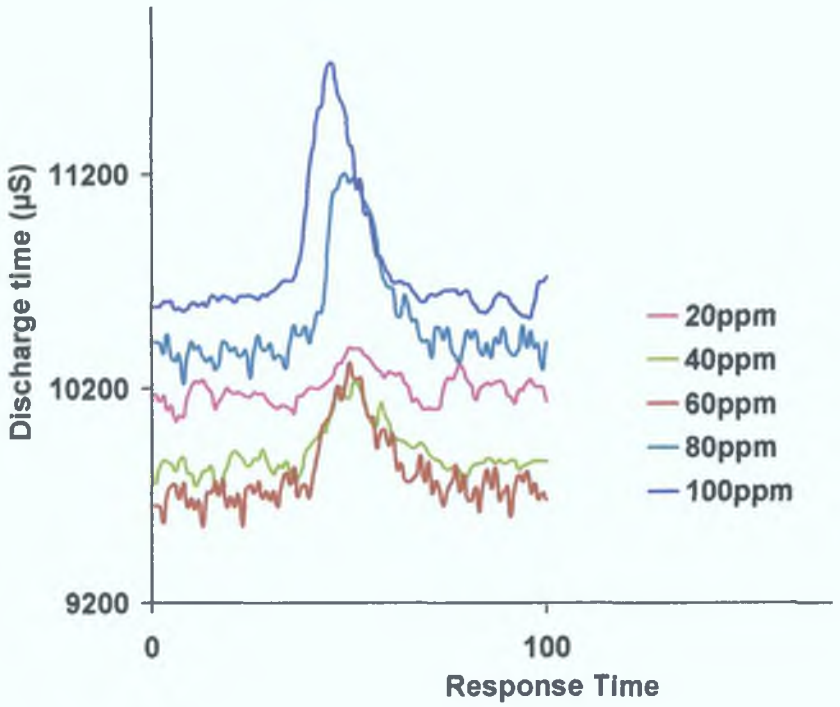

Figure 5.5: Phosphate standards using optimum conditions, anion-exchange chromatography and LED-PCR detection. phosphate standards from 10 ppm to 100 ppm. Eluent: 20 mM KCl Flow rate: Eluent-0.3 mL/min, PCR reagents-0.9 mL/min. LED detection by PCR at 620 nm. 146 mm x 0.5 mm i.d. reaction coil to mix 0.6% ammonium molybdate and 0.6% ascorbic acid (PCR reagents) heated in water bath at 30°C. Dionex Anion exchange (AG18) column at ambient temperature. Injection volume: 100 µL.

The possibility of the determination of silicate was also investigated using this system. However, as previously found by Grudpan *et al.*[3] the phosphate-molybdate-ascorbic acid reaction was faster than that of the silicate-molydate-ascorbic acid reaction and so silicate cannot be determined using this system.

However, Saurina and Hernandez-Cassou proposed a method for the determination of silicate by FIA. This method was based on the formation of an ion associate between molybdosilicate and Malachite Green, which was determined spectrophotometrically at 645 nm. [6]

5.2.2 Optimising flow rate and temperature for this PCR using FIA

The optimum conditions were again examined by FIA (flow-injection analysis) only; removal of anion-exchange column from the system was necessary for this. The effect of heating the reaction coil for the post-column reaction in a water bath at different temperatures (25°C to 60°C) on the phosphate peak was investigated and can be seen in Figure 5.6.

Various flow rates of the PCR reagents (0.3 mL/min to 1.3 mL/min) were also examined using FIA only and their effect on the phosphate peak was studied. This is illustrated in Figure 5.7. Optimum conditions were again determined by S/N ratios. Optimum temperature for the post-column reaction reaction coil was 50°C and optimum flow rate was 0.4 mL/min for the post-column reaction reagents.

The temperature optimum study was carried out first, at the optimum flow rate determined in section 5.2.1 (eluent flow rate was 0.3 mL/min and the post-column reaction flow rate was 0.9 mL/min).

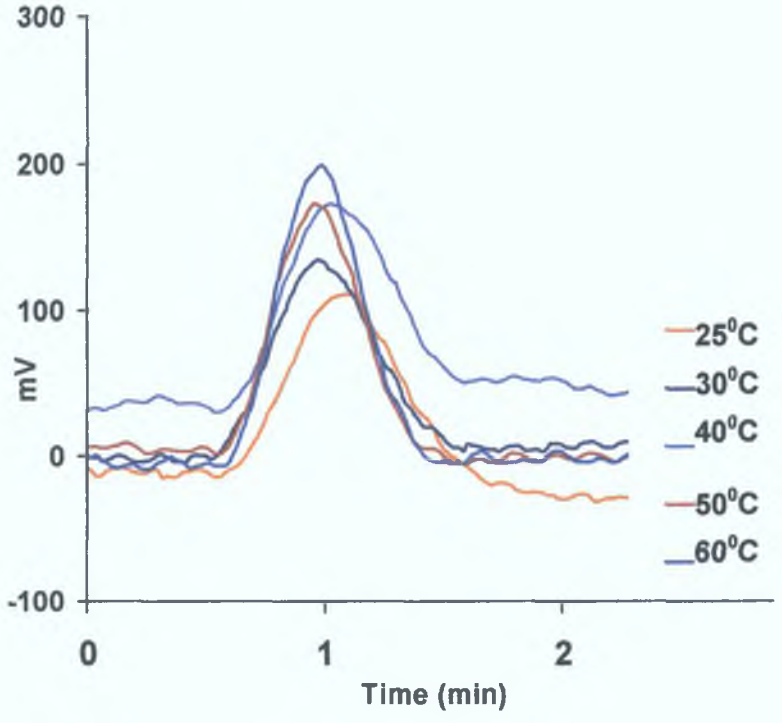

Figure 5.6: Effect of temperature of the reaction coil on the phosphate peak using UV-vis PCR detection and FIA, 20 ppm phosphate. Eluent: 20 mM KCl. Flow rate: Eluent-0.3 mL/min, PCR reagents-0.9 mL/min. UV-vis detection by PCR at 600 nm.

146 mm x 0.5 mm i.d. reaction coil to mix 0.6% ammonium molybdate and 0.6% ascorbic acid (PCR reagents) heated in water bath at a range of temperatures from 20°C to 60°C. Injection volume: 100 µL.

In FIA, there was no retention of the phosphate peak as no column was present. The sample was injected and it moves through the tubing from the injector to the detector. The width of the peak depends upon the distance between the injector and detector and on the flow rate.

The reaction coil was heated to 50°C and the optimum flow rate of the post-column reagents was then investigated while delivering the 20 mM KCl eluent at 0.3 mL/min.

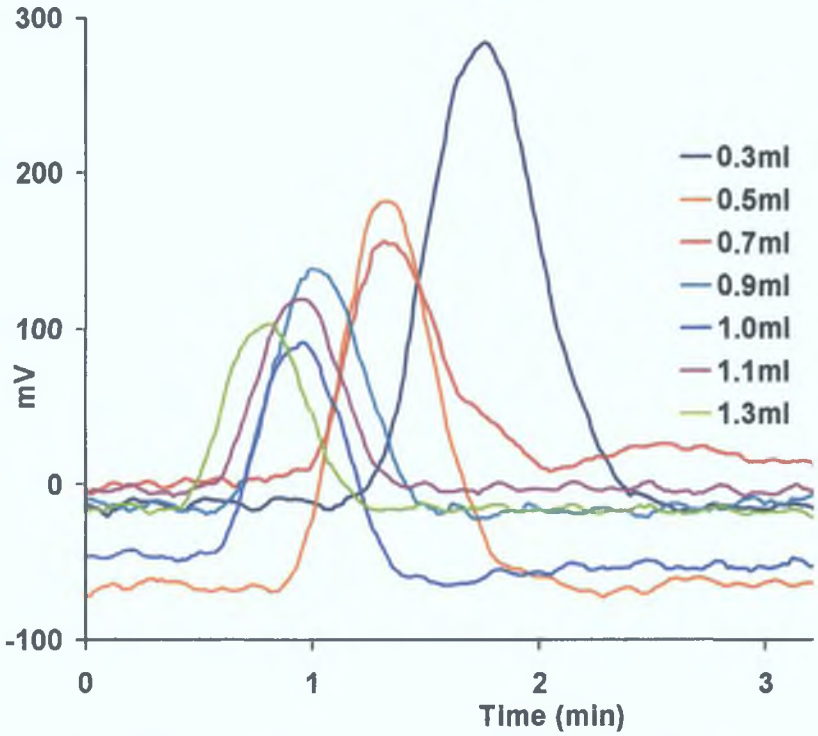

Figure 5.7: Effect of flow rate of the post-column reagents on the phosphate peak using UV-vis PCR detection and FIA. 20 ppm phosphate. Eluent: 20 mM KCl Flow rate: Eluent-0.3 mL/min, PCR reagents-various. UV-vis detection by PCR at 600 nm. 146 mm x 0.5 mm i.d. reaction coil to mix 0.6% ammonium molybdate and 0.6% ascorbic acid (PCR reagents) heated in water bath at 50°C. Injection volume: 100 µL.

Table 5.2· S/N ratios for 20 ppm phosphate at various flow rates and temperatures using this FIA system

Flow rate in mL/min	S/N ratio of 20 ppm phosphate
1 3	31
1.2	33
1 1	33
1.0	29
0.9	23
0.8	21
0.7	21
0 6	24
0 5	45
0.4	**60**
0.3	39

Temperature in °C	S/N ratio of 20 ppm phosphate
60	29
55	27
50	**41**
45	26
40	19
35	22
30	21
25	20

It can be seen from Table 5 2 above that the optimum temperature for heating the reaction coil water bath is 50°C and the optimum flow rate is 0 4 mL/min for the post-column reagents to ensure complete mixing of these two reagents and hence, optimal post-column reaction with phosphate will occur

5.3 PCR with all-in-one reagent and longer reaction coil

Jones and Stanley investigated the determination of arsenate, silicate and phosphate by IC using a post-column reaction (molybdenum blue) detector [7] In this work, the possibility of using a longer reaction coil to ensure that the post-column reaction goes to completion was considered especially in the case of silicate as its kinetics were deemed too slow for use in chromatographic detection as the reaction had not reached equilibrium after 60 mm The possibility of using an all-in-one reagent also allowed for increased sensitivity for this analysis

This application for future analysis of phosphate, silicate and arsenate was considered The all-in-one reagent comprised of 125 mL of sulphuric acid (0 75 M), 37 5 mL of ammonium molybdate (2 5% w/v), 75 mL of ascorbic acid (17 6 g/L), 12 5 mL of antimony potassium tartrate (2 743 g/L-catalyst) and 530 mL of distilled water The reaction coil used was 500 mm x 0 25 mm i d The optimum wavelength for this reaction was investigated by taking a UV scan over the range of 200 nm to 900 nm and was found to be 840 nm The reaction coil was heated in a water bath to 60°C and an injection volume of 100 µL was used for increased sensitivity

5.3.1 Effect of length of reaction coil and temperature on phosphate determination

A range of phosphate standards from 3 ppm to 100 ppm were injected The S/N ratios of the peaks were calculated using the old reaction coil (146 mm x 0 5 mm i d) heated to 60°C and the new reaction coil (500 mm x 0 25 mm i d) at room temperature and heated to 60°C These can be seen in Table 5 3

Table 5.3: S/N ratios using different length reaction coils and temperatures for phosphate standards using the chromatographic conditions from Figure 5 8

PO_4^{3-} conc (ppm)	S/N ratio using 146 mm x 0.5 mm i.d coil at 60°C	S/N ratio using 500 mm x 0.25 mm i.d. coil at RT	S/N ratio using 500 mm x 0.25 mm i.d. coil at 60°C
3	22	2	10
5	37	7	14
10	24	12	27
20	40	20	32
30	25	21	30
40	26	15	31
60	34	12	33
80	43	20	35
100	59	20	39

The mixing of the PCR reagents and consequently the PCR reaction at room temperature led to poor peak shape. Figure 5.8 illustrates the phosphate standards injected using the longer 500 mm x 0.25 mm i.d. reaction coil for the PCR reaction. This longer coil was used for subsequent work as it was thought that the PCR reaction would have increased time to go to completion. The loop size was decreased again to 25 μL when using the longer reaction coil and this micro-bore column to decrease the backpressure of the system.

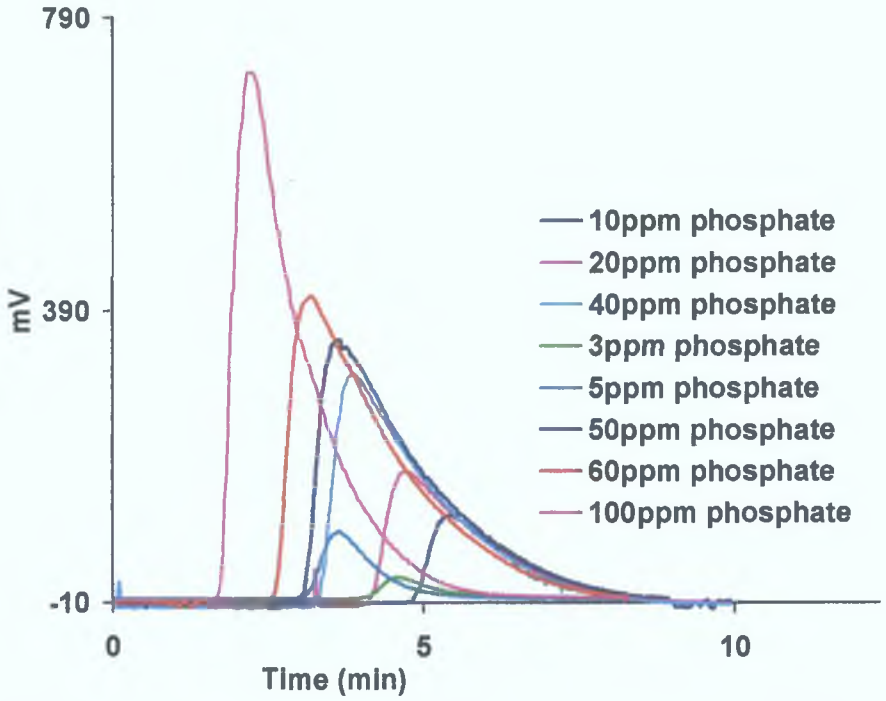

Figure 5.8: Injection of phosphate standards from 3 ppm-100 ppm using this system. Eluent: 10 mM KCl. Flow rate: Eluent-0.3 mL/min, all-in-one PCR reagent 0.4 mL/min. UV-vis detection by PCR at 840 nm. 500 mm x 0.25 mm i.d. reaction coil heated in water bath at 60°C, anion-exchange column. Injection volume: 25 μL.

5.4 PCR with all-in-one reagent, longer reaction coil and DDMAU column

For the final analysis of phosphate, silicate and arsenate, a 10 mM Chromolith SpeedROD guard column (10 mm x 4.6 mm i.d.) coated with 98% DDMAU surfactant was used. The reaction coil mentioned previously was employed again and heated. The pressure of the system was monitored by a USG pressure gauge. Two

low pressure MilliGat pumps were used to pump the all-in-one reagent and the eluent instead of the previous high pressure pumps. The total system backpressure with a flow rate of 0.3 mL/min was <100 psi. This configuration is shown in Figure 5.9.

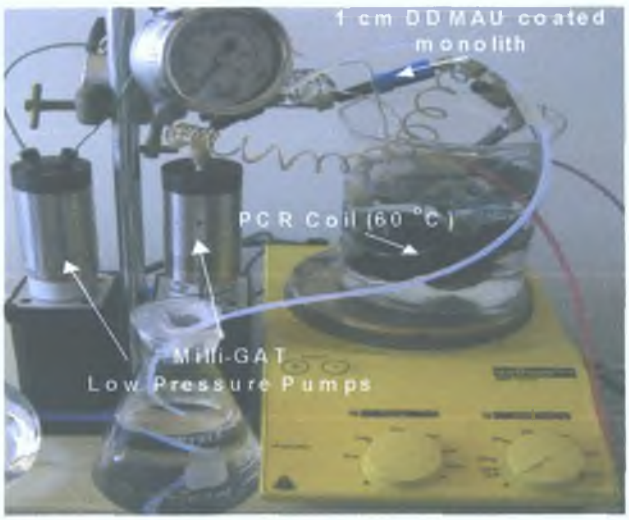

Figure 5.9: Low-pressure system with DDMAU coated monolithic column as described in 5.4 used for phosphate and silicate determinations.

5.4.1 LPIC-PCR method using DDMAU column for phosphate

A range of phosphate standards from 1 ppm to 50 ppm were injected using this system. Figure 5.10 shows an overlay of these injections from 1 ppm to 30 ppm. The eluent and PCR reagents were both at a flow rate of 0.3 mL/min. The reaction coil (500 mm x 0.25 mm i.d.) was heated to 60°C and the eluent used was 10 mM KCl instead of the 20 mM KCl used previously for separations.

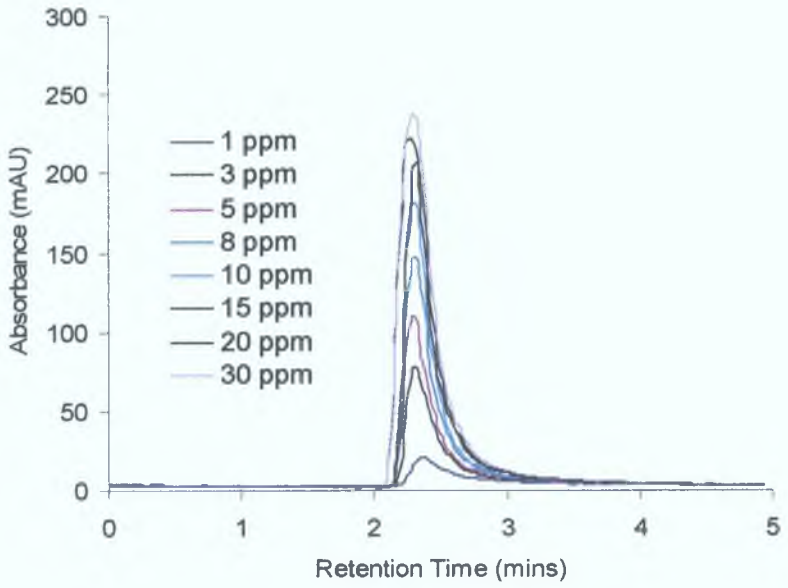

Figure 5.10: Injection of phosphate standards using LPIC-PCR method.
Eluent: 10 mM KCl. Flow rate: Eluent-0.3 mL/min, all-in-one PCR reagent 0.3 mL/min. UV-vis detection by PCR at 840 nm. 500 mm x 0.25 mm i.d. reaction coil heated in water bath at 60°C, 10mM DDMAU coated monolithic column (10 mm x 4.6 mm i.d). Injection volume: 100 µL.

The method was found to be linear from 1-10 ppm. Above this concentration, peak broadening due to column overload affected peak height linearity. Figure 5.11 shows the linearity of the LPIC-PCR method for the analysis of phosphate. The R^2 value for this graph was 0.97.

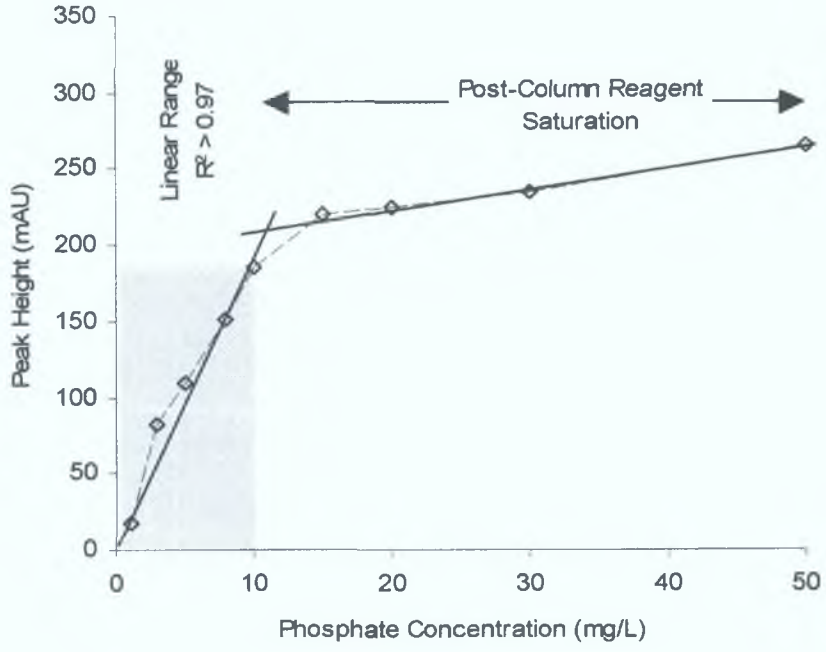

Figure 5.11: Linearity of the LPIC-PCR method for phosphate with the linear region highlighted in grey.

Eluent: 10 mM KCl. Flow rate: Eluent-0.3 mL/min, all-in-one PCR reagent 0.3 mL/min. UV-vis detection by PCR at 840 nm. 500 mm x 0.25 mm i.d. reaction coil heated in water bath at 60°C. 10mM DDMAU coated monolithic column (10 mm x 4.6 mm i.d). Injection volume: 100 µL.

5.4.2 Application of LPIC-PCR method to real samples

A sample of surface water was taken from an abandoned slate quarry in Callan, Co. Kilkenny and diluted 1/10 with Milli-Q water. Using a 100 µL injection loop this diluted sample was injected onto the LPIC-PCR system above (Figure 5.9) and both phosphate and silicate peaks were observed. The first peak in Figure 5.12 was in fact silicate present in the quarry sample.

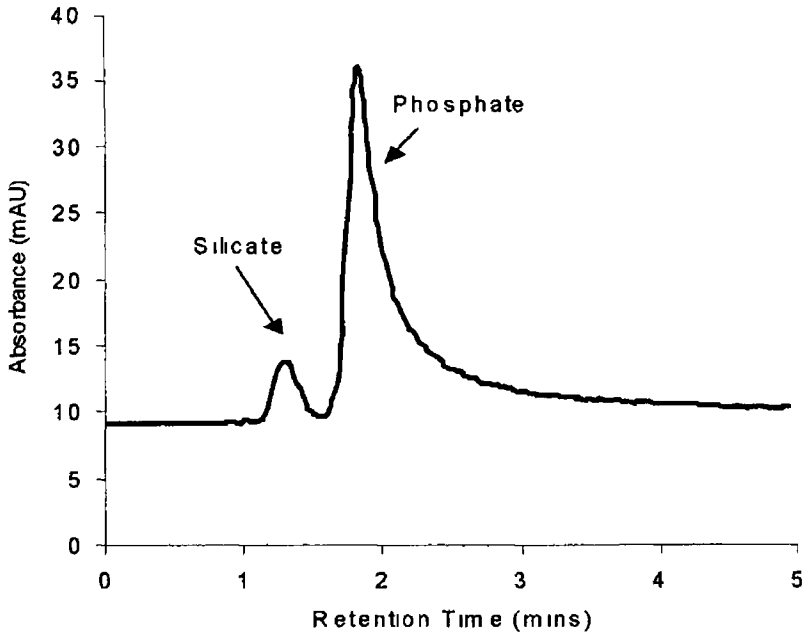

Figure 5.12 Quarry water sample (diluted 1/10) containing silicate and phosphate determined by the LPIC-PCR method

Eluent 10 mM KCl Flow rate Eluent-0 3 mL/min, all-in-one PCR reagent 0 3 mL/min UV-vis detection by PCR at 840 nm 500 mm x 0 25 mm i d reaction coil heated in water bath at 60°C, 10 mM DDMAU coated monolithic column (10 mm x 4 6 mm i d) Injection volume 100 µL

5.4.3 LPIC-PCR with LED detection

In order to reduce system size even further, a miniature detector comprising of two near-infrared LEDs (both the emission and detection wavelength was 850 nm, 20 µL internal volume [8]) was incorporated into the above LPIC-PCR system and a 15 ppm phosphate standard was injected This can be seen from Figure 5 13 The inset in Figure 5 13 illustrates the LED detector used in this separation The previous UV-Vis detection method was more sensitive for the determination of phosphate

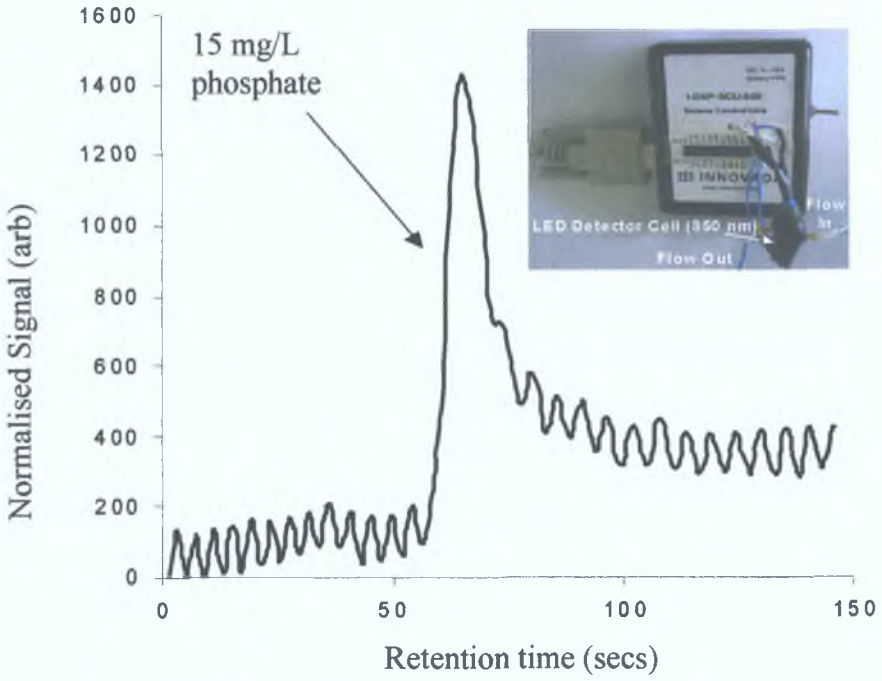

Figure 5.13: Injection of a 15 ppm phosphate standard using LPIC-PCR method and LED detection.

Eluent: 10 mM KCl. Flow rate: Eluent-0.3 mL/min, all-in-one PCR reagent 0.3 mL/min. LED detection by PCR at 850 nm. 500 mm x 0.25 mm i.d. reaction coil heated in water bath at 60°C, 10 mM DDMAU coated monolithic column (10 mm x 4.6 mm i.d). Injection volume: 100 µL.

5.5 Arsenate determination using the LPIC-PCR method

The LPIC-PCR system described in 5.4.1 was then used for the determination of arsenate. This method was linear for arsenate up to 15 mg/L. Increased concentrations of arsenate led to column overload and affected the peak height linearity. The arsenate response was of similar height to that of phosphate. A range of arsenate standards were injected from 3-100 ppm and the results can be seen in Figure 5.14 where peak tailing is a prominent consequence with both phosphate and arsenate separations.

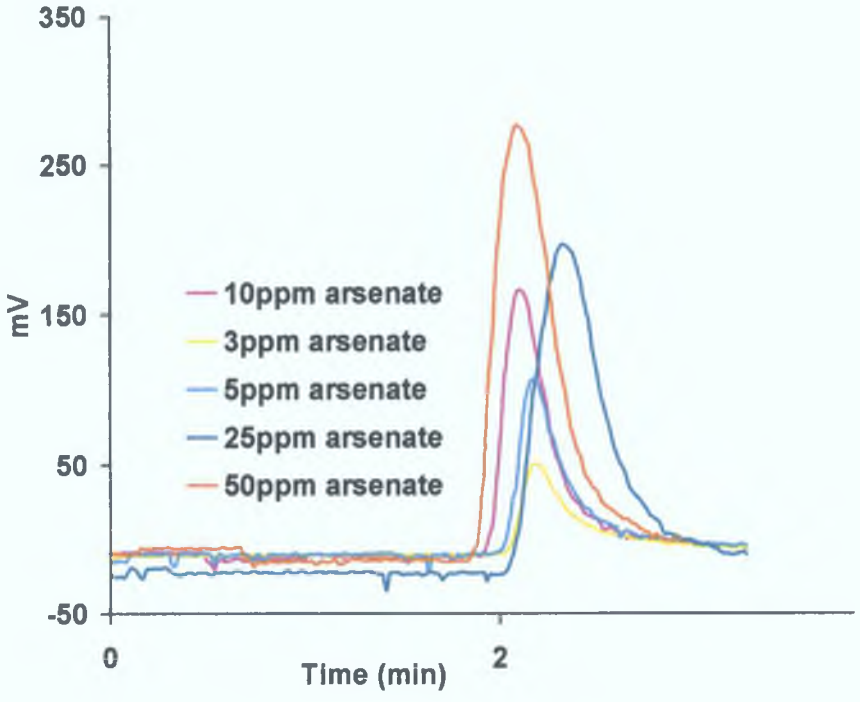

Figure 5.14: A range of arsenate standards determined by the LPIC-PCR method. Eluent: 10 mM KCl. Flow rate: Eluent-0.3 mL/min, all-in-one PCR reagent 0.3 mL/min. UV-vis detection by PCR at 840 nm. 500 mm x 0.25 mm i.d. reaction coil heated in water bath at 60°C, 10 mM DDMAU coated monolithic column (10 mm x 4.6 mm i.d). Injection volume: 100 µL.

5.6 Conclusions

The separation and determination of phosphate and arsenate on the newly developed LPIC-PCR system was successful. The LPIC-PCR method was also applied to real water samples for determination of phosphate and silicate. Method development of this system was first investigated. Careful consideration of the column used, length of the reaction coil, flow rate of the eluent and PCR reagents and temperature at which optimised post-column reaction occurred needed to be investigated. This LPIC-PCR method using UV-Vis detection was compared and contrasted with LED detection. The UV-Vis detection was more sensitive for phosphate determination.

5.7 References

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CHAPTER 6

Future Work and Conclusions

6.1 Conclusions

From this project, it can be seen that LPIC is versatile in a number of applications The development of LPIC was needed before any separations could be undertaken High backpressures associated with traditional HPLC and IC analytical columns have meant the growth of these liquid chromatographic techniques into micro-scale systems has been hindered by the constant need for expensive high-pressure pumping systems However, the commercial introduction of polymeric and silica based monolithic stationary phases has effectively reduced the backpressure restriction associated with the traditional chromatographic stationary phase Peristaltic pumps can now be used to obtain significant flow rates through short monolithic phases capable of exhibiting reasonably high separation efficiencies This means the obstacle once holding apart the fields of low cost FIA and HPLC has been removed

In the first instance, it was demonstrated that LPIC could be used for the separation of common inorganic anions by anion-exchange using phthalate eluents and indirect UV detection at 279 nm on a 25 mm coated monolithic column The investigation as to how monolithic columns operated and the backpressures associated with them was first examined The coating of such a column with ionic surfactants was then explored The use of the coated anion-exchange monolithic columns resulted in the ability to separate and determine common inorganic anions by LPIC as monolithic columns reduced the overall backpressure of the system

In subsequent work, another eluent was used for the separation of common inorganic anions by LPIC, this was *p*-hydroxybenzoate This eluent was first used in conjunction with indirect UV detection at 279 nm However, suppressed conductivity using this eluent resulted in increased separation and sensitivity for the anions A 10 mm coated (10 mM DDAB) monolithic column was employed for this work This LPIC system was further miniaturised with the introduction of in-expensive micro-peristaltic pumps Peak efficiencies (N/m) using this LPIC system were comparable to those achieved on a 15-25 cm commercial anion-

exchange column The level of performance for the short 10 mm monolithic anion exchanger is highly significant for the future development of LPIC systems The micro-LPIC system was successful for the separation of anions in real water samples Column capacity and van Deemter curves using this eluent were also studied

In the final work, post-column reaction for the determination of phosphate, silicate and arsenate was investigated A LPIC method with post-column reaction with UV-vis detection was developed and optimised Optimisation of the post-column reaction was investigated The flow rate of the post-column reagents, eluent, length of the reaction coil and optimised temperature were explored An anion-exchange column was used for initial stages of this work However, a 10 mm coated DDMAU monolithic column was used later The use of the UV-vis detection method was compared with an LED detector The UV-vis detection method was more sensitive for the analysis of phosphate This LPIC-PCR system with UV-Vis detection was applied to a real water quarry sample The silicate and phosphate ions were found to be present in this sample

In conclusion, separations obtained with this LPIC system are extremely promising and of great significance, particularly in a time of increased interest in miniaturised analytical systems and lab-on-a-chip technology Advantages of LPIC in comparison to other methods include portability, miniaturisation, less expense, less reagent consumption and less power supply

6.2 Future Work

As can be seen from previous chapters, separation of common inorganic anions was achieved using this LPIC system

The possibility of using this low-pressure IC system for future work could be considered However, modification of the separation column, eluent and mode of detection would be necessary for the relevant analytes

This robust LPIC system could be adapted and developed for the chemistry market as a mode of chromatographic separations as it would allow for miniaturised, portable, real-time sampling maybe offering the consumer/analyst a cheaper method to HPLC

The possibility of separating cations and/or using different coating solutions could be investigated and considered using this LPIC system There are a number of surfactants that could be tried for coating columns The possibility of double coating monolithic columns has also been employed previously and could be investigated using this system, as double coating a column (firstly with a non-ionic surfactant and then secondly with a cationic surfactant) is thought to dramatically increase the efficiency of the column for anion separations [1]

Separation and determination of inorganic anions and cations in tandem in environmental water samples could also be explored Cations could be detected by UV indirectly as negative peaks if a UV absorbing additive was added to the eluent Such anion-exchange and cation-exchange columns could be coupled in tandem for the simultaneous separation of such ions An eluent consisting of an inorganic acid and weak basic amino acids could be employed for such a separation [2]

6.3 References

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