INVESTIGATION OF

ELECTROSTATIC ION CHROMATOGRAPHY

FOR THE SEPARATION OF INORGANIC IONS

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degree of Master of Science

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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 Eadaoin Twoh U ID No 95008853

Date 18/04/02

For Mum

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To begin with, I would like to thank Brett for all his help, guidance and ideas over the last couple of years Thank you also to Enterprise Ireland and the School of Chemical Sciences for their financial support I must also thank all the technical staff, who have been absolutely brilliant, especially Maurice, without whom I would never have got this far Thanks also go to the girls next door (Ka, Emily, Niamh, Aoife and Maire) who always helped out when I needed to 'borrow' something! Thanks also go to the boys in the 'BETTER centre' (not) Liam, Brendan and especially Aogan who never said no when asked for help, even if he didn't know what he was doing! To the rest of the lab (especially Damien!), sorry about the temper tantrums! Hope you won't miss me too much! To Eimear, Michaela and Marion, thanks for all the lunches and coffee breaks and for the sympathetic ears when I needed them To Michael and Garret, thanks for your encouragement and for giving me a link to the outside world! To my family, especially Dad, thanks for your support and patience Last but not least, I want to give a big thank you to Marion Without your friendship and support, I never would have survived the last two years You've been great!

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LIST OF ABBREVIATIONS

EDL	Electrical double layer
EIC	Electrostatic ion chromatography
GC-MS	Gas chromatography - mass spectroscopy
GLC	Gas liquid chromatography
HETP	Height equivalent to a theoretical plate
HPLC	High performance liquid chromatography
IC	Ion chromatography
IIR	Ion-interaction chromatography
MTA	Myristyltrimethylammonium
ODS	Octdadecylsilica
% RSD	Percentage relative standard deviation
Rt	Retention time
UV	Ultraviolet
WMP-IC	Water mobile phase ion chromatography
ZEDL	Zwitterionic electrical double layer

ABSTRACT

The new technique of 'electrostatic ion chromatography' (ion chromatography using a zwitterionic stationary phase) has been applied to the separation of ions using pure water as an eluent, without the addition of any inorganic buffers or organic modifiers The nature of the separation, i e cationic or anionic, is dependent upon the nature of the zwitterionic stationary phase In the work presented here, the zwitteriomc surfactant Zwittergent 3-14 was used to functionalise an octadecylsilica stationary phase with its subsequent application to the separation of common inorganic anions, using direct conductivity detection and/or UV absorption detection

Ion redistribution, a major drawback of electrostatic ion chromatography, has been eliminated by development of an off-line sample pre-treatment procedure, employing a cation exchange resin in the sodium form This resulted in the quantitative exchange of analyte cations to sodium ions When the method was applied to water samples, such as tap water, mineral water or river water, the various mono- and divalent cations present in the water samples were quantitatively exchanged, resulting in single ion - pairs for all analyte anions and hence a simpler separation was achieved The beneficial effect this had upon peak retention, resolution and efficiency is described and explained

Another advantage of electrostatic ion chromatography is that as water can be used as the eluent, the entire system can be set up in such a way that the eluent can be constantly recycled This negates the need for re-coating of the column due to 'column bleeding', and hence improves the problem of poor reproducibility, inherent in any method based on dynamic coating Work here employed the use of post-detector cation

and anion exchange columns in the acid and hydroxide forms to remove sample anions and cations from the eluent stream, allowing complete recycling of the water eluent In addition, since Zwittergent 3-14 passed undetected and unretained through the complete chromatographic system, small amounts could be added to the water eluent When this eluent was recycled, the resulting system provided superior separations and much improved reproducibility for all anions tested With the addition of a UV absorption detector, the method was successfully applied to the determination of chloride, sulphate and nitrate in drinking and river waters

The developed method proved extremely sensitive Detection limits were found to be in the ppb range for the common inorganic anions such as sulphate, chloride, nitrite, nitrate and iodide When the method was compared to standard ion exchange chromatography, the agreement was excellent The concentration of nitrate in a sample of mineral water was found to be 0 18 mM by electrostatic ion chromatography and also by ion exchange chromatography, while the level of chloride in the same sample was found to be 1 17 mM by ion exchange chromatography and 1 18 mM by electrostatic ion chromatography The method was also suited to the analysis of complex matrix samples such as seawater where the high concentration of chloride salts would normally cause problems for ion chromatographic techniques Using electrostatic ion chromatography, iodide was successfully separated from chloride in samples of iodised sodium chloride table salt The trace iodide in the sample was easily quantified using direct UV detection Application of the developed method was shown with the determination of iodide at a concentration of 77 µg/L in a sample solution containing 20 g/L of sodium chloride

CHAPTER ONE

CHROMATOGRAPHY OF INORGANIC IONS

Chromatography is the single most popular analytical technique in use today From reversed-phase liquid chromatography to gas chromatography coupled with mass spectrometry (GC-MS), it is invaluable in many industries, including the pharmaceutical, biochemical, environmental, food, healthcare and nuclear industries Despite the earlier development of gas chromatography, liquid chromatography has become extremely popular, due to its simplicity and application to a wider range of analytes

First developed and named in 1903 by Russian botanist Mikhail Tswett [1], it took many years to reach the level that we know today Starting with Tswett's separation of plant pigments, adsorption chromatography was born, and continued into the 1930's where it was used to separate natural products Classical column or open bed chromatography, where a column was usually used only once, could take up to several hours to achieve separation The process was long, tedious and expensive[2] as a result of the production and packing of the column, gravity feeding of the solvent into the column and then the manual detection and quantification once the samples had been collected

In 1941, Martin and Synge [3] published results describing the discovery of liquid – liquid partition chromatography They also introduced the idea of gas - liquid chromatography (GLC) and high performance liquid chromatography (HPLC), along with the concept of height equivalent to a theoretical plate (HETP) During the 50's and 60's, GLC took off as an analytical technique, with liquid chromatography being relatively neglected

In the late 1960's, Kirkland [4], Huber [5], Horvath, Preiss and Lipsky [6] introduced the first HPLC columns Operating at high pressures, which resulted in shorter runtimes, they were comparable with GLC and meant the beginning of the liquid chromatography revolution Over the next decade, HPLC advanced as more and more detectors were developed The original single or dual wavelength UV detectors were replaced with variable wavelength UV spectrophotometers, refractive index detectors, spectrofluorimeters and electrochemical detectors

Development also continued with the analytical column [7] Efficiency increased due to reductions in particle sizes (currently down to ~ 1 5 μm) Slurry packing techniques meant that the packing of columns became more reproducible Stationary phases developed with the introduction of bonded columns Halasz *et al* [8] reacted silica with alcohols and amines, while organosilanes were bonded to the surface of silica, introducing reversed-phase liquid chromatography Columns became commercially available at this stage, which hastened the increase in their use and also in the modes of liquid chromatography developed

1.1 Introduction to Ion Chromatography

Ion exchange was discovered in 1850 by two English agricultural chemists, J T Way and H S Thompson, while monitoring the power of soils to absorb manure [9] Tswett's discovery of chromatography might have been over fifty years later, but it took even longer still to combine the two concepts Originally, synthetic zeolites (dried aluminosilicate gels that were used to soften water) were used as a separation medium But since they were attacked by dilute acids and alkali, their use as a chromatographic

medium was limited In 1935, Adams and Holmes [10] developed the first organic ion exchangers (ion exchange resins) which began the development of ion exchange chromatography

The developments in ion exchange chromatography have closely followed developments in the atomic energy industry World War II gave birth to the Manhattan Project in the United States As most of the elements of the periodic table were formed upon the fission of uranium, separation of all these elements had to be achieved Ion exchange chromatography was the main technique employed From then, and on through the post war years, ion exchangers developed more and more Resins were introduced that could be used over wider pH ranges along with cross-linked polystyrene and chelating resins Many companies across Europe and North America became involved in the synthesis and manufacturing of ion exchangers Work was undertaken to understand and describe the separation process and the kinetics involved in ion exchange chromatography [11]

A major breakthrough occurred when the separation and automated detection of amino acids was successfully accomplished by Moore and Stein [12], which led to the Nobel Prize in chemistry in 1972 This was the beginning of separation with a continuous flow through detector, as is used today With the development of high-pressure columns and automated systems, samples could now be successfully separated and detected However, unless the analytes in question were UV absorbing, or could be pre-treated in some way so as to make them capable of detection by fluorescence or electrochemical

detectors, analysis by ion exchange chromatography was still proving difficult This was especially the case for the analysis of inorganic ions

In 1975, Small, Stevens and Bauman [13] released their results detailing a novel ion exchange chromatographic method They had in fact invented the chemical suppressor, or "stripper" as it was called then This finally allowed the use of conductimetric detection Trace level analysis could now be carried out in environmental, biological and agricultural samples to name but a few The DOW Chemical Company, along with the Dionex Corporation (who manufactured the suppressors) christened this technique "ion chromatography" The first ion chromatograph instrument was displayed in Chicago in 1975 at the American Chemical Society Meeting [11]

This was the beginning of ion chromatography, as it is known today Over the years, numerous column types have been devised for different sample types Suppressed and non – suppressed detection are employed as well as various different separation techniques All are widely embraced under the banner of ion chromatography

1.2 SEPARATION METHODS

Many types of separation techniques exist for the separation and analysis of inorganic ions The choice of technique is made depending on the matrix of the sample to be analysed, the form of the analytes of interest, the equipment available, etc Several of the main techniques are discussed below

1 2 1 Ion Exchange Chromatography

Ion exchange chromatography is the most commonly used separation technique for the analysis of ions Numerous types of exchange columns are available commercially, varying in resin type, pore size, exchange capacity, length, pH tolerance, etc Therefore there is a column type suitable for most sample types

1 2 1 1 Basic Principle

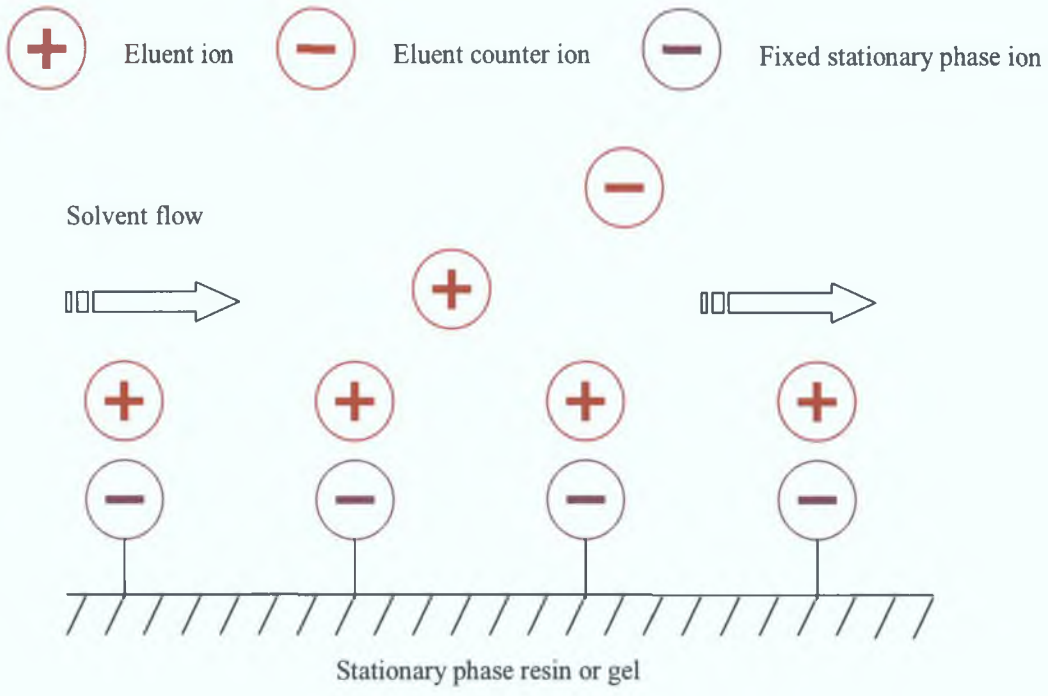
The basic principle of ion exchange is not very different from that of adsorption chromatography [14] With adsorption chromatography, a weak interaction is formed between the active site on the stationary phase e g silanol groups, and any molecule in its surrounding vicinity, once a dipole – dipole interaction is present Since the stationary phase sites are surrounded at all times by mobile phase molecules, and a dipole – dipole interaction occurs between the two, the mobile phase (or solvent) molecules occupy the sites on the stationary phase

Once a sample molecule is introduced, it can only be adsorbed, that is replace a solvent molecule, if its interaction with the stationary phase molecule is stronger than that of the solvent molecule When this is the case, the solvent molecule takes up a site on the

stationary phase, and remains there until it is removed or exchanged with another solvent molecule This competition between sample and solvent molecules for occupation of sites is the power behind elution The 'stronger' a solvent is, the more it competes for active sites, resulting in less adsorption time (generally referred to as retention time) for sample molecules and therefore quicker elution

With ion exchange, the active sites of the stationary phase consist of ionic sites on the surface of the stationary phase resin Commonly used ionic groups would be sulphonic acid groups or carboxylic acid groups for cation exchange and quaternary amines (NR_4^+) for anion exchange As these groups are 'fixed' onto the stationary phase resin or gel, they are generally referred as 'fixed ions' The mobile phase or solvent ions are referred to as 'eluent ions' and they neutralise the charges of the fixed ions, rendering the system electrically neutral

Upon introduction of a sample, the eluent ions and the sample ions compete for a place on the surface of the stationary phase (Figure 1 1) The sample ions *exchange* with the eluent ions, which are also referred to as competing ions As in adsorption chromatography, the eluting power of a solvent depends on how strongly the eluent ions compete with the sample ions Changing the ionic strength of the solvent will change its eluting power and therefore will also alter the retention times of the sample ions



A sample ion is introduced into the mobile phase stream, upon which it exchanges with an eluent ion.

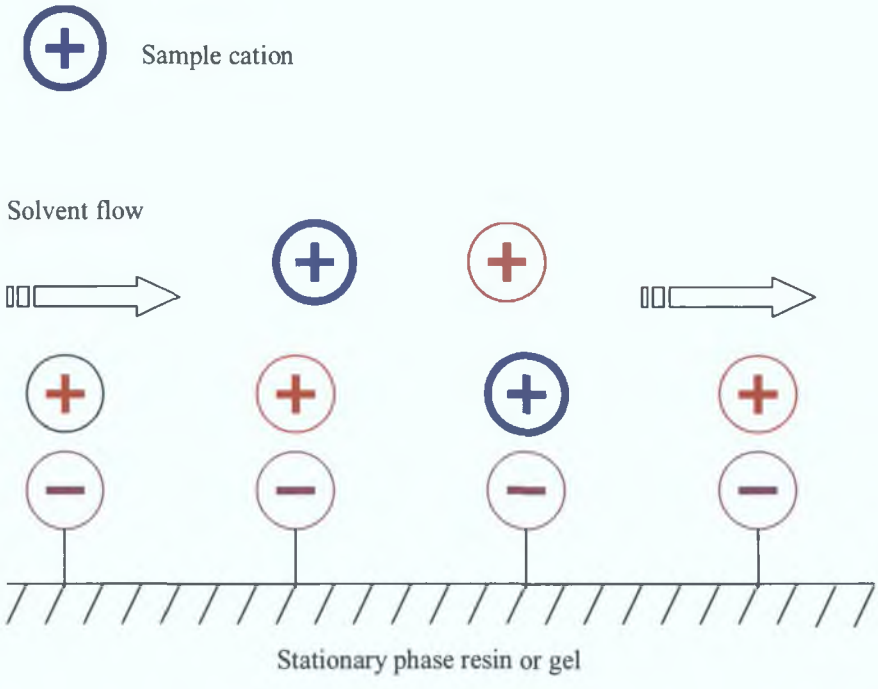


Figure 1.1 Principles of a cation exchanger.

1 2 1 2 Optimising Conditions

As already stated, changing the eluting power of a solvent will alter the retention times of sample analytes In ion exchange chromatography, this is achieved by changing the ionic strength of the eluent As the concentration of the eluent ions is increased, more eluent ions are present to compete with the sample ions, and therefore the retention times of the sample ions are reduced There are other variables to take into account when optimising chromatographic conditions

(a) Ion exchangers

The more fixed groups available on a stationary phase, the more sample ions it can exchange with This is measured by what is called 'exchange capacity' This capacity of an exchanger often varies depending on the pH of the eluent For example, a weak cation exchanger, such as –COOH, depends strongly on the pH of the solvent which is flowing around it [14] At low pH levels, (less than 4), the protons of the acidic groups are too strongly bonded to exchange with sample ions At pH levels higher than 8, the acidic group dissociates fully, leaving the remaining ionic groups available to the sample ions Between pH 4 and 8, partial exchange capacity exists Therefore, the exchange capacity of the anion is at its maximum at a pH of 8 Similar trends are followed for anion exchangers

Alternatively, strong exchangers (both cationic and anionic) are not affected by changes in pH levels, as they are fully dissociated over a wide range But this can lead to longer retention of the sample molecules as an increase in the amount of available exchange sites leads to an increase in retention times Therefore, in optimising the

analysis time for a separation, the choice of ion exchange resin to be used will vary depending on the sample to be separated, its required pH level and its responding retention times.

(b) Eluent

Several aspects of the eluent may be changed in order to optimise chromatographic conditions, the three main aspects being:

(i) The type of eluent ion used.

The retention time of the analyte ion increases when the eluent ion is replaced by one with greater potential for dipole – dipole induction, i.e. greater polarizability, for example:

In cation exchange, calcium > magnesium > potassium > ammonium > sodium > hydronium

In anion exchange, sulphate > nitrate > phosphate > chloride > hydroxide

(ii) The ionic strength of the eluent.

The retention time of the analyte ion increases when the concentration of the eluent ions (the ionic strength of the eluent) is decreased. As there are less competing ions to exchange with the analyte ions, they remain 'fixed' to the stationary phase for longer.

(iii) The pH of the eluent.

As explained previously, the exchange capacity of the exchange resin depends on the pH level at which it is used, which in turn affects the retention time of the analyte ions. The

pH level can also affect the form in which the analyte ions and the eluent ions exist, with dissociation of the molecules depending on the pH Different forms of an analyte will result in different relationships with the eluent ions and also the stationary phase ions Therefore, retention times of the ions also depend on the pH at which the eluent is prepared and vary with different types of eluent

1 2 2 Ion-Pair/Interaction Chromatography

Although, inorganic analysis is typically carried out using ion exchange columns, it is also possible to achieve separations of inorganic ions using conventional reversed – phase columns Reversed – phase columns are generally cheaper and more robust, making them more popular to work with Also, if a sample contains ions and neutral molecules, a complete analysis cannot be carried out with ion exchange chromatography For these reasons, ion – pair chromatography was developed With ion – pair chromatography, the analysis is carried out on reversed – phase columns, and mixtures of acids, bases and neutral products may be separated simultaneously [14]

1 2 2 1 Basic Principles

The name given to this technique varies It has been called ion - pair, ion-interaction and even dynamic ion exchange chromatography Despite differences in the proposed models, the chromatographic trends do not change and each one utilises the addition of an organic ionic reagent to the eluent Even though the model names vary, for the purpose of simplicity, this reagent shall be known as the ion-interaction reagent or IIR

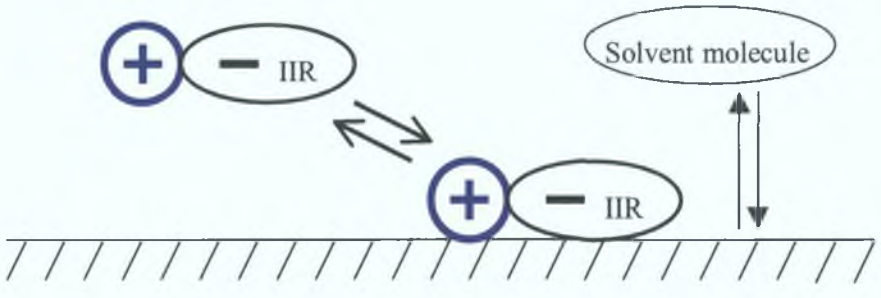
(a) Ion - pair model

As is normal in reversed – phase chromatography, the mobile phase used is generally an aqueous – organic solvent mixture. An organic reagent (IIR) is added to this eluent, which supplies a counter ion to the analyte of interest, i.e. in the analysis of cations, the IIR will have a negative charge. Upon introduction of the sample ion, an ion – pair is formed between the two.

For example, in cation analysis :

Figure 1.2 Ion - pair formation in ion - pair chromatography.

The resulting 'ion - pair' is then free to adsorb onto the stationary phase in the manner that is common in reversed – phase chromatography, competing for position with the solvent molecules (Figure 1.3). All interactions between the reagent ion and the sample ions take place in the eluent.



Reversed - phase stationary phase

Figure 1.3 Sample retention in ion - pair chromatography.

(b) Dynamic ion - exchange model

This model proposes that a portion of the reagent added adsorbs onto the stationary phase and that a dynamic equilibrium is set up between the IIR adsorbed and the IIR remaining in the mobile phase. Upon introduction of the sample ions, a basic ion exchange process occurs, between the sample ions and the counter ions of the IIR. Retention times are dependent on the ion exchange capacity, which in turn is dependant on the amount of IIR adsorbed onto the stationary phase. Since the IIR is constantly interchanging between the eluent and the stationary phase, the stationary phase is considered to be a dynamic exchanger [15] (Figure 1.4).

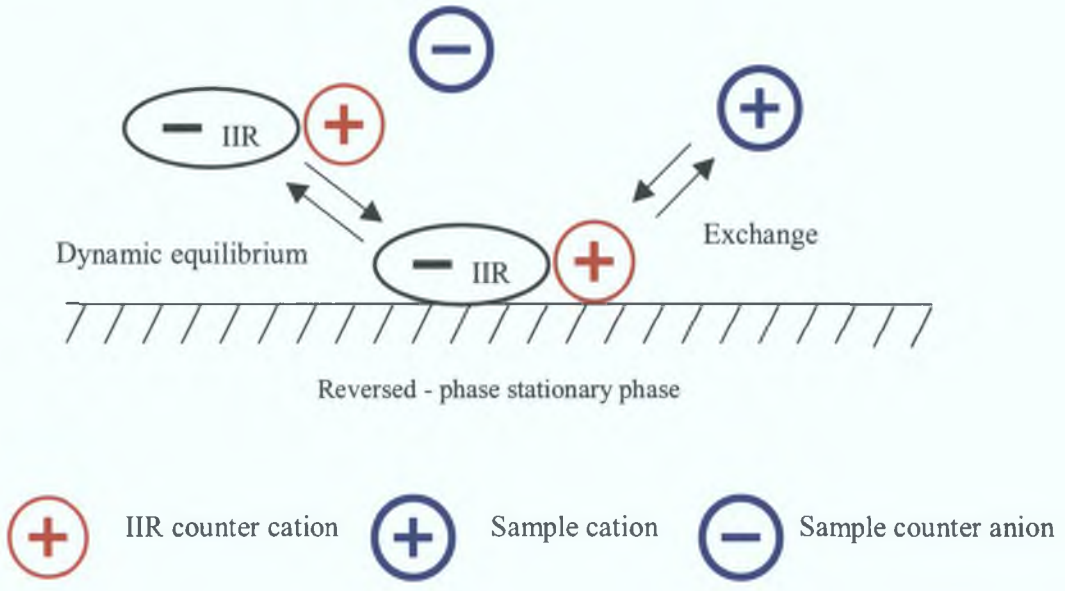


Figure 1.4. Dynamic ion exchange retention mechanism

(c) Ion-interaction model

This model is considered to be a combination of the previous two. As in the dynamic ion exchange model, the IIR forms an equilibrium between the mobile and stationary phase. This time, the counter ions of the IIR are also thought to form a secondary layer next to the stationary phase forming an *electrical double layer* (EDL) (Figure 1.5).

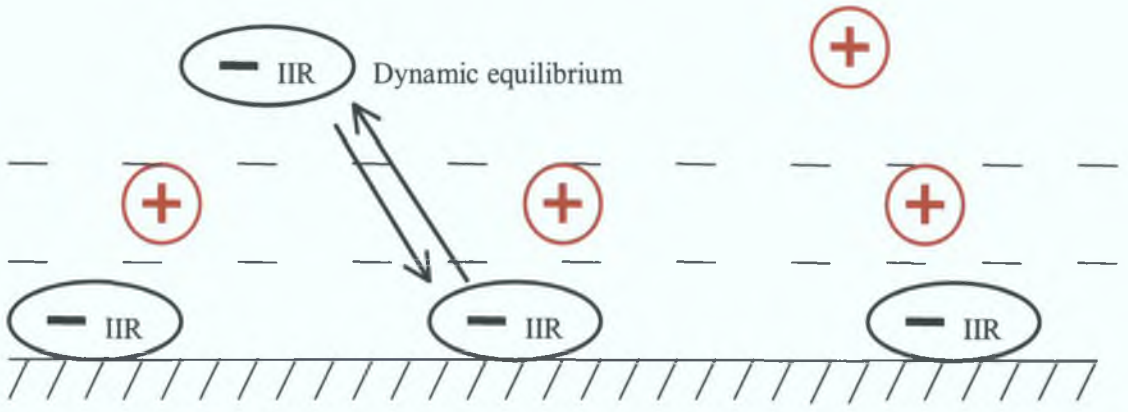


Figure 1.5. Electrical double layer in ion-interaction chromatography.

Movement of the sample ion through this double layer to the stationary phase for retention purposes is due to electrostatic effects (and to the usual reversed – phase chromatographic effects if applicable). That is, a sample cation competes with the counter ion in the secondary layer and moves into the primary layer.

Upon reaching the stationary phase, this cation effectively neutralises an IIR anion, leaving the stationary phase with a decreased charge. To maintain charge balance, another IIR ion must enter the primary layer. This means that overall, for every sample ion that is retained, an IIR ion is also adsorbed onto the surface of the stationary phase and again, when the sample ion is released back into the mobile phase, the IIR ion is also released. Therefore, the two ions are effectively travelling as an 'ion - pair', without actually being bonded together (Figure 1.6).

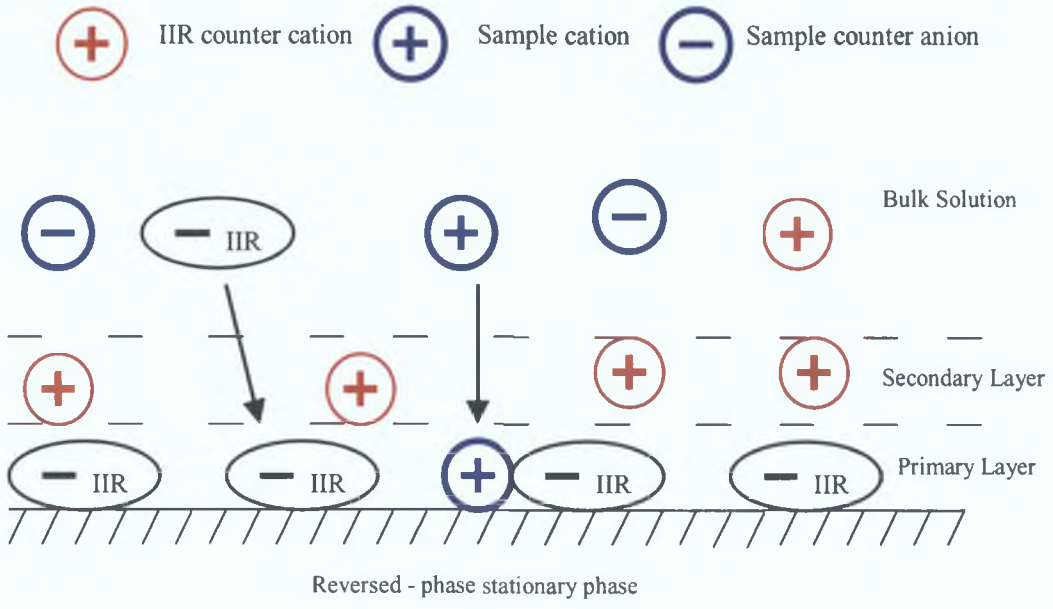


Figure 1.6. Ion-interaction retention mechanism.

1.2.2.2 Trends in Sample Retention Times

Regardless of which model is employed, the trends that apply upon increase or decrease of IIR reagents or organic modifiers do not change.

(a) Neutral analytes

Neutral analytes are retained in the conventional reversed-phase chromatographic manner. Addition of the reagent to the mobile phase does not affect the retention of these analytes. Decreasing the percentage of organic modifier in the eluent increases their retention times.

(b) Analytes having opposite charge to the IIR

The analyte ions of interest are retained upon addition of the reagent to the mobile phase. The retention times of these ions are increased when the concentration of the reagent is increased and also when its hydrophobicity is increased. Increasing the percentage of organic modifier decreases the retention times.

(c) Analytes having the same charge as the IIR

Generally, these ions are not retained, but any retention that they may have had prior to addition of the IIR is decreased once this addition occurs Increasing the concentration or the hydrophobicity of the IIR also decreases their retention times

1 2 2 3 Permanent Coating Ion – Interaction Chromatography

A different alternative to the dynamic coating of the stationary phase with adsorbed IIR is to use an extremely hydrophobic IIR such as tridodecylmethylammonium bromide, which is strongly bound to the stationary phase and remains coated to it for long periods of use In this way, the column has effectively been changed into an ion exchange column and eluents subsequently reflect this Organic modifiers are not used, as they would strip the IIR from the stationary phase Instead, electrolytes providing a competing ion are used Ion exchange capacities may be varied depending on the composition of the original coating solution As the efficiency of reversed – phase columns remains once they are coated with the IIR, efficiency levels remains high

1 2 3 Miscellaneous Separation Methods

1 2 3 1 Ion Exclusion Chromatography

Developed and employed since the early 1950's, this form of chromatography was introduced mainly for the separation of weakly ionised samples, that do not suit the conventional separation techniques, e g carboxylic acids, weak inorganic acids and bases, etc The main difference in the physical aspects of this chromatographic technique is that the ion exchange resin used has the *same charge* as the analyte of interest Therefore fully

ionised sample ions tend to be unretamed and so are eluted together at the void volume of the column

The principle or mechanism behind this separation method is quite complex and not fully understood [15] The numerous factors, which affect retention when using this technique, are listed below in approximate order of importance for the analysis of carboxylic acids The order may be subject to change in the analysis of other analytes

- the degree of ionisation of the analyte
- hydrophobic interactions between the analyte and the stationary phase
- the size of the analyte
- the degree of crosslinking of the stationary phase
- the separation temperature
- the ion exchange capacity of the resin
- hydrogen bonding interactions between the analyte and the stationary phase
- the ionic form of the ion exchange resin

This method of separation is very suited to the separation of carboxylic acids, and tends to be the chosen method for their determination Most of the earlier work carried out with ion exclusion chromatography was done using water as the eluent However, since the resulting peak shapes were often poor, water is only used nowadays for the separation of very weakly ionised analytes The commonly used eluents are sulphuric acid, hydrochloric acid and aliphatic sulphomc acids The detection method used is often determined by the choice of eluent Sulphuric acid is generally used with absorption detection, while hydrochloric acid is often used with suppressed conductivity detection

Non-suppressed conductivity detection can be used with sulphomc acids, as the background conductance of these eluents is relatively low

1 2 3 2 Electrostatic Ion Chromatography

Electrostatic ion chromatography is a novel separation technique that has only recently been introduced and is still in its development stages It is the main focus of this work and will be explained in depth in Chapter Two

1.3 Detection Methods for Inorganic Ions

1 3 1 Conductivity Detection

As mentioned before, conductivity detection became popular in ion chromatography after Small, Stevens and Bauman [13] developed the 'suppressor' in 1975 Since all ionic groups are electrically conducting, the conductivity detector is universal in response, as well as being simple and inexpensive to construct

The principle behind its mode of operation is that any electrolyte 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 allows the electrolytic eluent to pass through it continuously A voltage is applied and the resulting conductance is measured and recorded Upon introduction of a sample, conductance levels increase or decrease (indirect detection) due to the conducting nature of the ions This change in conductance is recorded in the form of a chromatogram

Using Ohm's Law ($V=IR$) and introducing factors relevant to the chemistry involved, the conductance of a solution can be seen to be equal to,

$$G = \frac{1000\Lambda_o C}{K}$$

Equation 1 1

G = Conductance of a solution (μS)

Λ_0 = Equivalent conductance of electrolyte, (S cm ^{2} equiv^{-1})

C = Concentration of electrolyte (equiv / 1000 cm ^{3})

K = Cell constant (geometrical characteristic) (cm $^{-1}$)

As the electrolyte conductance is contributed to by both the anion and the cation, Λ_0 can be written as ($\lambda_+ + \lambda$ -) representing the limiting equivalent ionic conductances of the cationic and anionic compounds respectively (Table 1 1) Further calculations based on Equation 1 1 have deduced that the signal produced upon introduction of a sample is proportional to the concentration of sample ions and also to the difference in limiting equivalent ionic conductances between the electrolyte and the sample ions For this reason, care must be taken when choosing suitable electrolytes for the analysis of different ions

Anion λ_- (S.cm^2 eq^{-1})		Cation λ_+ (S.cm^2 eq^{-1})	
OH^-	198	H_3O^+	350
SO_4^{2}	80	K^+	74
Br	78	Pb^{2+}	71
Cl	76	Ce^{3+}	70
NO_2	72	Ca^{2+}	60
NO_3	71	Fe^{3+}	68
PO_4^{3}	69	Zn^{2+}	53
Fl	54	Mg^{2+}	53
HCO_3	45	Na^+	50

Table 1 1 Limiting Equivalent Ionic Conductances for the common inorganic ions and cations in aqueous solutions at 25°C [15, 16]

1 3 1 1 Factors Affecting Conductance

(a) Temperature

Variations in temperature have been shown to affect both the sensitivity and the reproducibility of conductivity detection in ion chromatography Generally, there is a 2 % conductance change for each degree varied in the temperature, when the temperature is between 10 and 25 °C Outside this range, the change in conductance may be as much as 25 % [15] Most modern day systems are equipped with thermal stabilisers for the detectors to keep the conductance levels stable

(b) Concentration

As Equation 1 1 demonstrates, conductance increases with the increasing concentration of the eluent or sample ions At very high concentrations, ion – pairs may form as a result of ion – ion interactions This results in a decrease in the measured conductance Therefore, the relationship between conductance and concentration becomes non - linear at high concentrations However, this only applies at concentration levels higher than those normally encountered in ion chromatography, so does not always affect calibration studies An additional effect of concentration variance is that the sensitivity of the detector increases as the ionic strength of the eluent decreases

(c) Detector cell

As can be seen from Equation 1 1, the cell constant is inversely proportional to conductance The cell constant is a mathematical representation of the dimensions of the cell and the electrodes within Since the cell constant is equal to the length of the cell divided by the surface area of the electrodes, the conductance is increased when the surface area of the electrodes are large and close together As the dimensions of the detector cell do not change from analysis to analysis, this factor can be considered a constant

1 3 1 2 Suppressors

As mentioned earlier, the signal produced upon introduction of a sample is proportional to the difference in limiting equivalent ionic conductances between the eluent and the sample ions In a chromatogram, the baseline is a result of the signal produced by the eluent and the peak is the signal produced by the sample ions, therefore a large

difference between the two conductances provides a larger sample peak Since the limiting ionic conductances of most common electrolytes are high, the resulting detection proves to be quite insensitive, effectively masking the conductance that would be supplied by sample ions A way around this problem is to reduce the conductance of the electrolyte ions, so as to increase the difference between their conductance and that produced by the sample

To reduce the conductance of the electrolyte, a suppressor is placed in–line after the separation column and before the detector The suppressor exchanges the counter-ions present in the eluent with a lesser conducting ion For example, in anion analysis with a sodium hydroxide eluent, the suppressor would provide H^+ ions to exchange with the Na^+ ions The resulting eluent comprises solely of H_2O, which has a very low conductance, and therefore provides a much lower baseline The signal difference between the mobile phase and the sample is now greatly increased, and concentration detection limits decreased

The original suppressors designed by Small, Stevens and Bauman were ion-exchange columns in the H^+ or OH forms Although they successfully reduced the background conductance, they also encountered some drawbacks such that they needed constant regeneration, reduced chromatographic efficiency and in the cases of some analytes, interfered in the retention Common day suppressors are flow-through membrane devices, which allow continuous regeneration They also have a high suppression capacity in relation to the original suppressors, are suitable for gradient elution and are applicable to a wide variety of eluents

Suppression of the background conductance is not required when low conducting electrolytes are used, or when carrying out indirect conductivity detection For example, in anion analysis, aromatic acids can be used as low conducting eluents for direct conductivity detection, with potassium hydroxide being used as an eluent for indirect conductivity In cationic analysis, organic bases, inorganic acids and inorganic eluents are suitable as eluents for these two modes of non-suppressed detection

1 3 2 Spectroscopy and Other Miscellaneous Detection Methods

Many types of spectroscopic detection are used in ion chromatography, the most common being

- UV – vis spectrophotometry
- refractive index detection
- fluorescence detection

As with conductivity detection, the eluent flows through a cell, and the change in eluent composition (i e the presence of an analyte) is recorded as an electronic signal

1 3 2 1 UV – Vis Spectrophotometry

UV – Vis spectrophotometry is the most commonly used detection system after conductivity detection It doesn't show much variation with temperature and can be used for gradient elution Its one major limitation is that it can only be used to detect analyte ions that absorb in the ultraviolet or visible range, i e above 200 nm Many common inorganic ions do not absorb in the given range and are therefore undetectable by direct UV – Vis spectrophotometry For the ions that are suitable to this detection method, (such

as ions containing carbonyl groups, nitrile groups, aromatic groups, etc) UV – Vis spectrophotometry proves to be quite sensitive and operates over a wide linear range

Detectors are normally operated with fixed or variable wavelength lamps While fixed wavelength lamps can be up to 20 times more sensitive than variable wavelength lamps [14], variable wavelength lamps are suitable for a much larger range of analytes In quantitative analysis, the wavelength that results in the maximum absorption of the analyte ions should be chosen

Two modes of detection are common, direct and indirect Direct detection occurs when the analyte ions cause an increase in the signal being recorded, as previously mentioned Indirect detection results when the eluent ion has a higher absorptivity than the analyte ion (when both are univalent) For example, a given eluent ion absorbs at a high level Upon elution of the analyte ions, a decrease in the recorded signal is observed as the ions absorb less strongly and therefore reduce the overall signal being recorded In this way, it is possible to detect some of those analytes that do not absorb and hence are undetectable using direct UV - Vis spectrophotometry Careful selection must be made while choosing the appropriate eluent for indirect detection

1 3 2 2 Refractive Index Detection

As suggested by the name, this method of detection is based on measuring the difference in refractive indices of the eluent and the analyte This is done by measuring the refractive index of the eluent alone, in what is known as a reference cell A flow-through cell contains the eluent along with the analytes, as they leave the separation

column The change in refractive index of the eluent as the different bands of analytes move through is recorded and generates the peaks

As with conductivity, refractive indices vary with temperature, and the cell must be thermally stabilised This method of detection was more widely used before the development of conductivity detection It is about 1000 times less sensitive than UV - Vis spectrophotometry and is not suited to gradient elution

1 3 2 3 Fluorescence Detection

This detection method measures the change in fluorescence caused by the presence of analyte ions and is well known to be extremely sensitive Its major limitation is that most common inorganic ions do not fluoresce However, this can be overcome if the analyte can be suitably derivatised Derivatisation may be carried out during sample preparation, post-column or by the addition of a suitable reagent to the eluent For example, 8-hydroxyquinoline-5-sulfonic acid has been used in this way for the determination of certain cations [17]

BIBLIOGRAPHY

1 M Tswett, Proc Warsaw Soc Nat Sci (Biol) (**14**), No 6, 1903

2 Introduction to Modern Liquid Chromatography, by L R Snyder and J J Kirkland, 1979 A Wiley Interscience publication

3 A J P Martin and R L M Synge, Journal of Biochemistry, (**35**), 1358 - 1368, 1941

4 J J Kirkland, Journal of Chromatographic Science, (**7**), 7 - 12, 1969

5 J F K Huber, Journal of Chromatographic Science, (**7**), 85 - 90, 1969

6 C G Horvath, B A Preiss and S R Lipsky, Analytical Chemistry, (**39**), 1422 - 1428, 1967

7 Applications of High Performance Liquid Chromatography, by A Pryde and M T Gilbert, 1979 Published by Chapman and Hall, Ltd London

8 O E Brust, I Sebestian, and I Halasz, Journal of Chromatography, (**83**), 15 - 24, 1973

9 Ion Exchange Chromatography, edited by H F Walton, 1976 Benchmark Papers in Analytical Chemistry, Vol 1

10 B A Adams and E L Holmes, J Soc Chem Ind (London), (**54**), 1-6T, 1935

11 Ion Chromatography by Hamish Small, 1989 Series Modern Analytical Chemistry, Plenum Press, New York

12 S Moore and W H Stein, Journal of Biological Chemistry, (**192**), 663 – 681, 1951

13 H Small, T S Stevens and W C Bauman, Analytical Chemistry, (**47**), 1801 – 1809, 1975

14 Practical High Performance Liquid Chromatography, by Veronika R Meyer, 2nd edition, published by Wiley

15 Ion Chromatography, by Paul R Haddad and Peter E Jackson Journal of Chromatography Library – volume 46 Published by Elsevier

16 CRC Handbook of Chemistry and Physics, 76th Edition 1995 – 1996

17 W Bashir, B Paull and E Twohill, Journal of Chromatography A, (**877**), 123 – 132, 2000

CHAPTER TWO

ELECTROSTATIC ION CHROMATOGRAPHY

2.1 INTRODUCTION

Electrostatic ion chromatography (EIC) was first named by Wenzhi Hu, Toyohide Takeuchi and Hiroki Haraguchi [1] in 1993 when the first paper on the subject was published Since then several other research groups have taken up the challenge of developing this novel analytical technique As with any new analytical methodology, there is some uncertainty as to the mechanisms involved in EIC, but those proposed by Hu and Haddad [2] are taken, for the purposes of this review, to be correct

Since the development of ion chromatography, many different branches of the technique have arisen There is always a search for the perfect technique for each individual sample, from seawater samples with an abundance of matrix ions to biological sample with their interfering proteins Research has always been aimed at trying to improve on existing techniques by making them faster, more efficient and simpler By using just pure water as an ion chromatographic eluent, it was thought this could be achieved When using pure water, there is no need for complex mobile phase preparation or pH adjusting Laboratory time is saved and the overall cost of the analysis is also reduced The simplicity of using water as a mobile phase means that detector sensitivity is improved and systems can be easily automated

Water mobile phase ion chromatography (WMP – IC) has been tried by several research groups with limited success [3 4] Separation efficiency tends to be low due to the inability to manipulate retention without adding any foreign agents In ion exchange chromatography, the eluent must provide competing ions in order to cause the elution of any analytes ions that have exchanged with the functional sites on the exchange resin

Pure water does not provide these competing ions and therefore analytes will not elute However, if the need for competing ions can be overcome, and a way can be found in which to remove the analyte ions from the stationary phase then pure water may be used to 'carry' the analytes out of the separating column This is the basis of EIC

2.1 1 Basic Principle

In conventional ion exchange chromatography, the stationary phase consists of a layer of charged particles on the stationary phase and a layer of competing ions, supplied by an electrolytic eluent, that together provide an electrical double layer (EDL), (Figure 2 1) In EIC, a zwitterionic surfactant is dynamically coated or bonded onto stationary phase support, generally an ODS reversed-phase material This zwitterionic surfactant generally contains both a sulphonic group (negative charge) and an ammonium group (positive charge) in close proximity to each other (Figure 2 2) The eluent can be simply pure water or a dilute electrolyte solution

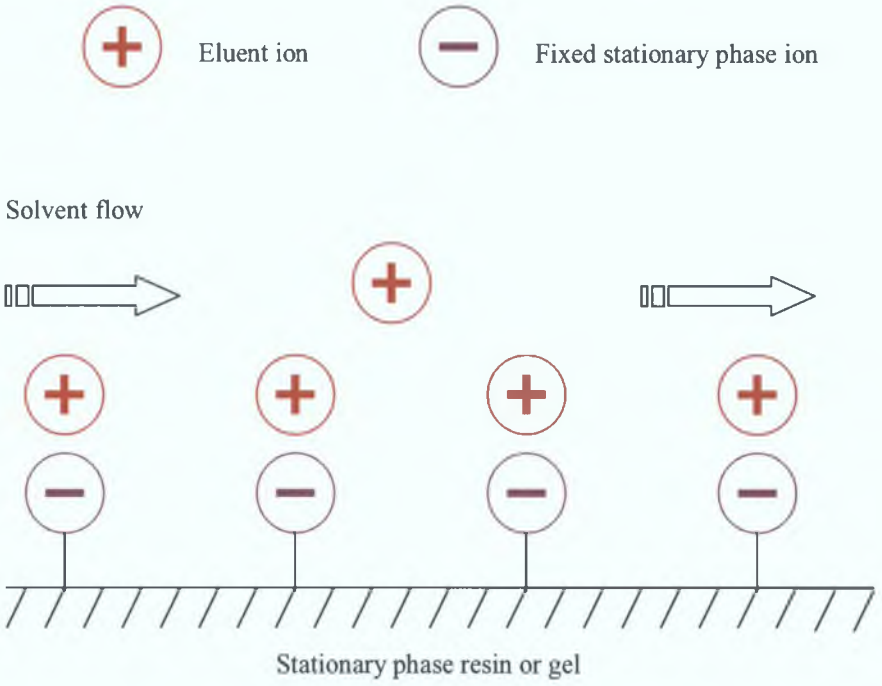


Figure 2.1. Formation of EDL in ion exchange chromatography.

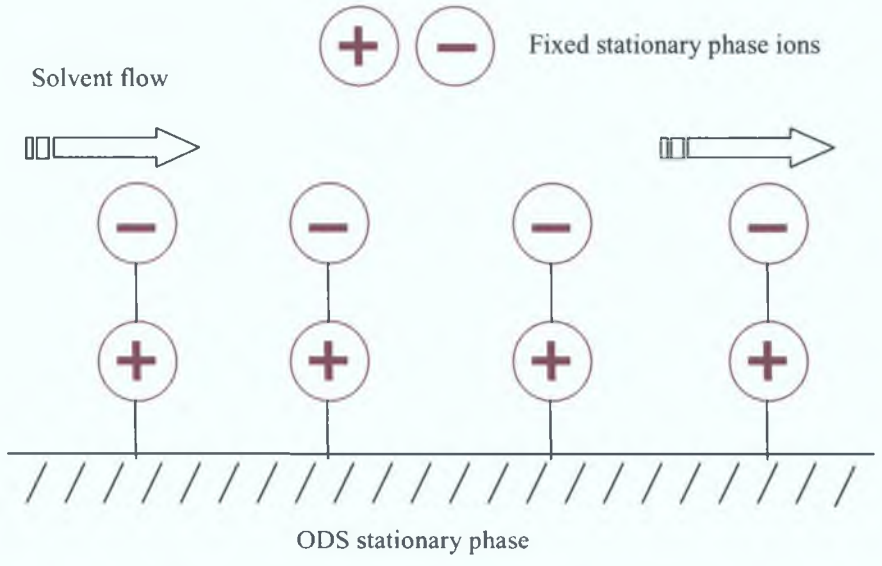


Figure 2.2. Zwitterionic coated stationary phase in EIC.

Upon introduction of an analyte, the cation is attracted to the negative site on the stationary phase and the anion is attracted to the positive site of the stationary phase, creating a zwitterionic electrical double layer (ZEDL) (Figure 2.3). Remaining ions distribute themselves between the diffuse layer and the bulk solution.

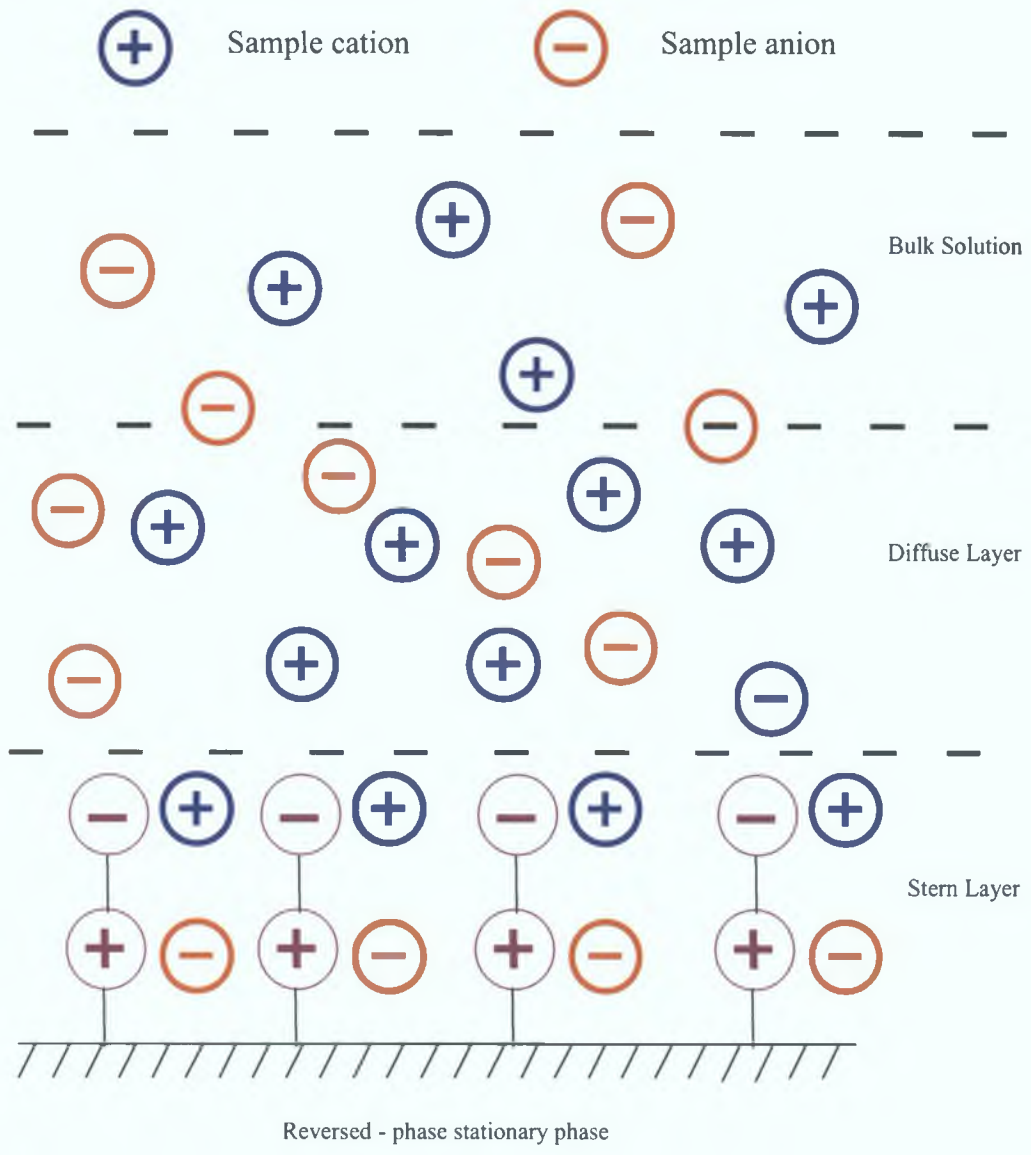


Figure 2.3. Formation of ZEDL upon addition of analyte ions in EIC.

However, when an ion is attracted to a fixed site on the stationary phase, it is simultaneously repulsed by the neighbouring fixed site of equal charge, and released into the bulk solution. In other words both analyte anions and cations are *retained*, by attraction between opposite charges and are *released to the mobile phase*, by repulsion between similar charges. No competing ion is required to release the ions into solution, and hence no electrolyte is needed. Distribution of the analyte ions between the stationary and mobile phases is possible using simply pure water as an eluent.

Both anions and cations are retained and released in the same manner However, the separation procedure is not so simple as to expect separation of all analyte cations and anions present A sample ion will be retained and released as explained above, but in order to keep the electroneutrality of the analyte band, any one anion will be eluted in combination with a cation, forming a weak 'ion - pair' When the term 'ion - pair' is used in relation to these ions, it does not mean 'ion - pair' in the normal sense of the word There is no strong association or bond between the two ions They are to be considered 'travelling partners' in that they migrate through the chromatographic system together Therefore, the detected peak, in anion analysis for example, is due to the anion in question, but also to the cation with which it is associated

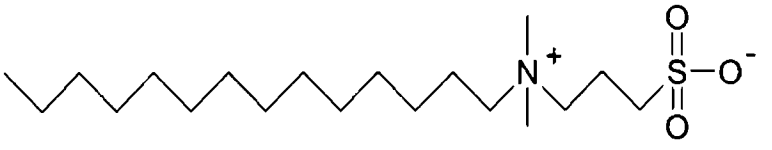
2 1 2 Zwitterionic Surfactants

The nature of the separation (cationic or anionic) is dependant on the nature of the zwitterionic surfactant used to functionalise the column There are many examples of surfactants used for the separation of anions, while only one surfactant for the separation of cations (N – dodecylphosphocholine) is commonly used The structure of the surfactants always contains two functional groups, one positive and one negative The position of these functional groups within the molecule determines whether the surfactant is suited in the separation of cations or of anions

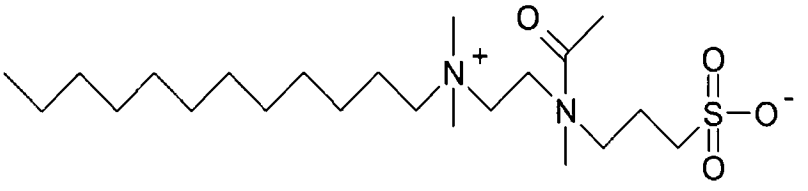
If the negative group is at the outermost end of the molecule, introduction of analyte ions will lead to the formation of a band of cations along the outer rim of the coating, along the stationary phase This means that only analyte anions can penetrate this layer and diffuse through to the stationary phase Hence it is the anions that are retained and

separated, then forming ion - pairs with the cations to keep the electric charge balance The opposite is the case when the outer functional group is positive The analyte anions are attracted to the outer layer, hence allowing only the distribution of analyte cations through to the stationary phase and therefore selectivity for cations is achieved

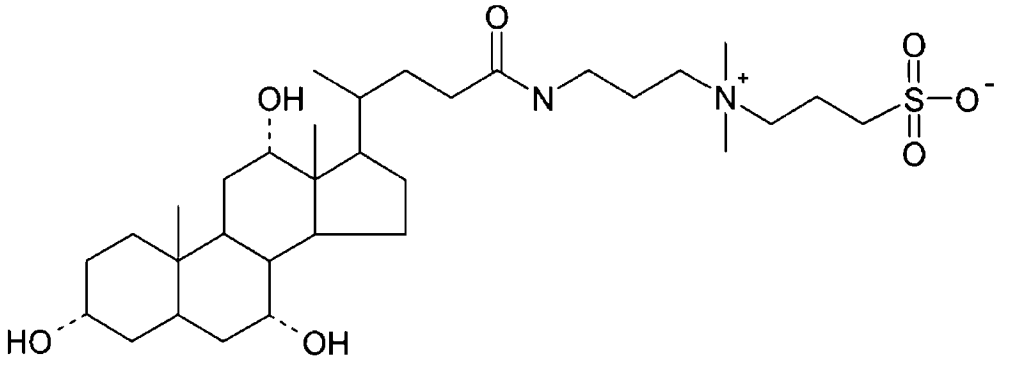
Work has been carried out by Hu *et al* [5] and Umemura *et al* [6] to investigate the different types of sulfobetaine-type surfactants used in anion separations by EIC Different surfactants show different separation efficiencies for the common inorganic anions with the hydrophobicity of the surfactant as well as its surface charge density being influential factors In general, as these properties increase, so too does the retention of the analytes Table 2 1 shows the molecular structures of several surfactants, with Zwittergent 3-14 being the most commonly used



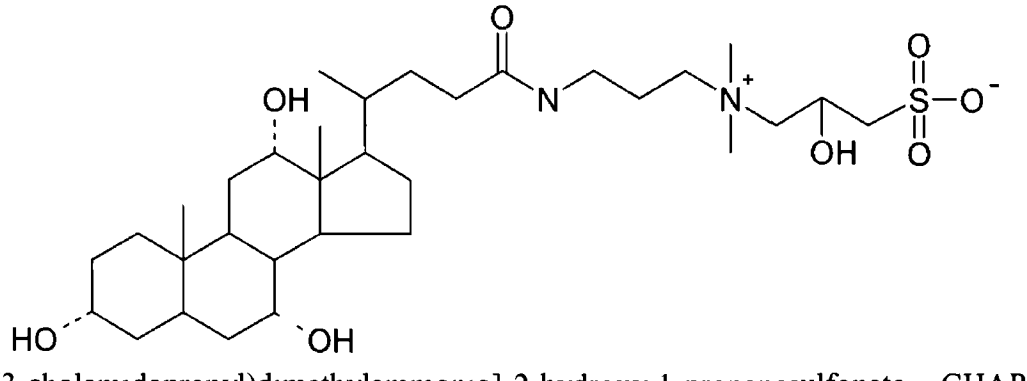
3-(N,N-dimethylmyristylammonio)propanesulphonate - Zwittergent 3-14



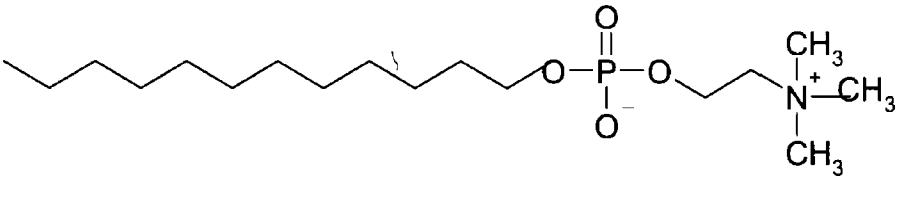
N-[2-acetyl(3-sulfopropyl)aminoethyl]-N,N-dimethyldodecanaminium hydroxide
Ammonium Sulfobetaine – 1



3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate - CHAPS



3-[(3-cholamidopropyl)dimethylammonio]-2-hydroxy-1-propanesulfonate - CHAPSO



N – Dodecylphosphocholine

Table 2 1 Molecular structures of zwitterionic reagents

2.1.3 Coating Technique

The coating technique first described by Hu *et al* [1] has been adopted in the majority of work done on EIC It is a simple procedure where a 30 mM solution of the surfactant is passed through the ODS analytical column, whereupon the zwitterionic reagent is dynamically coated onto the column Further work carried out by Umemura *et al* [6] shows that the concentration of the reagent does not actually matter Whether a concentrated solution is passed through the column for a short time or a more dilute solution is passed through for a longer time, the amount of surfactant adsorbed onto the column remains the same, once the saturation level has been reached Once a sufficient amount of reagent has been adsorbed, (as determined by the breakthrough method), any excess reagent present in the column is removed by rinsing of the column with water (A more detailed explanation of this procedure is described in section 3 2)

Deviations from this coating technique were employed by Hu *et al* [7] and Umemura *et al* [8] Here, small amounts of acetonitrile were in fact used in the coating solution This was carried out in the analysis of nucleosides and their bases, and the investigation of the reversed-phase properties of the zwitterionically coated stationary phases It was found that by changing the percentage of acetonitrile in the mobile phase and hence the amount of surfactant adsorbed, the reversed phase properties of the ODS column decreased with increasing adsorption of surfactant The surfactant 'shields' the reversed phase properties from the analytes "Hydrophobicity of reversed phase surfaces is controlled by manipulating the type and amount of the adsorbed surfactants" [8] With this method, efficient separation of nucleoside and their bases was achieved

2.2 TRENDS IN EIC

Now that the retention mechanism of EIC has been described, time must be spent describing the unique trends that are to be found with this new analytical technique Since the majority of the research carried out to date on EIC has pertained to the analysis of inorganic anions, the remainder of this review is being curtailed to this unless stated otherwise There are few comparisons to be made with conventional IC, and again, as with all new techniques, the mechanisms are not always understood

2 2 1 Elution order

In general the elution order of morganic anions is as follows

$$Fl^- < SO_4^{2-} < Cl^- < NO_2^- < Br^- < NO_3^- < ClO_3^- < I^- < SCN^-$$

This order correlates with the elution series for inorganic anions using ion exchange chromatography [9] with the exception of sulphate This unique arrangement for sulphate is convenient as in conventional ion exchange chromatography it generally elutes at a much later time For example using 15 mM sodium hydroxide at 1 ml/min, with a Dionex AS17 IonPac analytical column, fluoride, chloride, nitrite, bromide and nitrate all elute in under 5 minutes while sulphate elutes at just over 8 minutes As sulphate is an important anion in the analysis of environmental samples, this reduced retention time reduces the overall analysis time

2 2 1 1 Cation Contribution

The above elution order is maintained only if all the anions are 'paired' with cations of similar valencies Once there is a mixture of cation valencies, the elution order can vary It has been found [5 10] that although the separation and detector response is due to the anions in the samples, the cations play an important role in the retention behaviour of these same anions The retention time of a single anion e g NO_3 varies depending on its 'cation - pair' in such a way as that NO_3 paired with a monovalent cation is less retained than NO_3^- paired with a divalent cation (Figure 2 4)

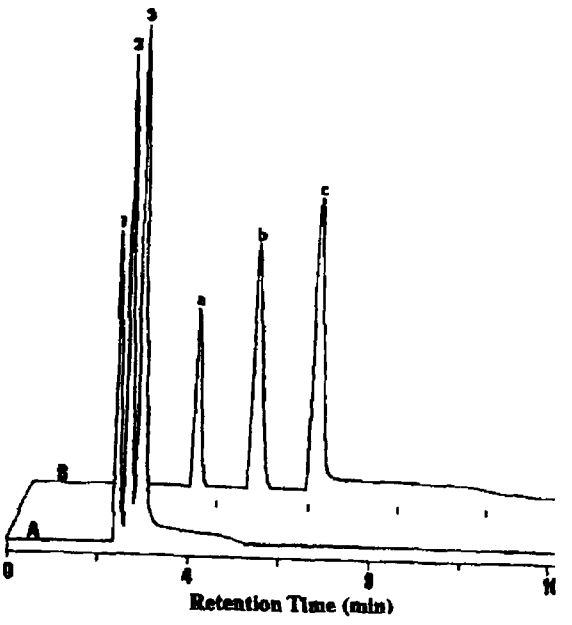


Figure 2 4 Chromatogram showing the different retention times of an anion depending on the cation with which it is paired [11] (A) Aqueous solution containing 2 0mM each of sodium, magnesium and cerium as the cation with chloride as the anion (B) Aqueous solution containing 2 0mM each of sodium magnesium and cerium as the cation with nitrate as the anion ODS – packed column (250 x 4 6mm I D) coated with Zwittergent 3-14 Pure water eluent, flow rate 1 0 ml/min Conductivity detection Peaks 1 = Na^+ Cl , 2 = Mg^{2+} 2Cl 3 = Ce^{3+} 3Cl , a = Na^+ NO_3 b = Mg^{2+} $2NO_3$ c = Ce^{3+} $3NO_3$

Therefore, the elution order holds for all of the above anions when paired with sodium, and at increased retention times, it also holds when they are all paired with

magnesium, but if there is a mixture of the two cations, then an overlap of elution times may occur

If an anion is paired with multiple cations, all having the same valency, then separation is not possible For example, if nitrate is paired with barium, magnesium and calcium, all divalent cations, then one peak will be observed Therefore it is possible to separate sodium nitrate from magnesium nitrate and from calcium nitrate, but not calcium nitrate from magnesium nitrate (Figure 2 5)

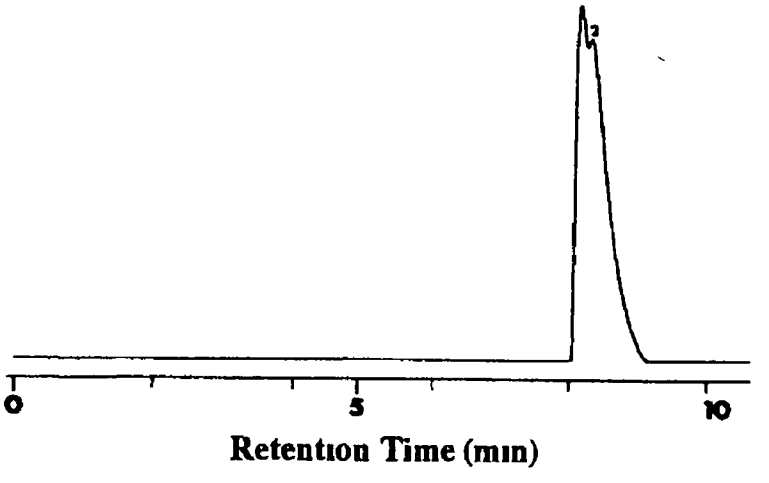


Figure 2 5 Chromatogram showing the separation of equivalent cations in EIC [5] ODS packed column (4 6 x 250 mm) coated with CHAPS – Zwittergent 3-14 mixed micelles Pure water eluent, flow rate 0 5ml/min Conductivity detection Peaks 1 = Mg^{2+} $2NO_3$ 2 = Ca^{2+} $2NO_3$

In order to investigate which cations are actually present in a sample, atomic absorption detection can been used [6 10] This method also permits the identification of 'ion - pairs' i e which cation is combined with which anion To sum up, the retention time of the anion (or in actual fact the 'ion - pair') is dependent on the species of the anion and the charge of the cation

2 2 2 Ion Redistribution

As explained before, if a mixture of anions having the same cation is introduced into a zwitterionic modified column, separation may be achieved However, if there are different cations present, the separation is complicated It is not only a matter of the anions having different retention times due to cations of differing valencies, but also due to the redistribution of the anions with the different cations For example, if a mixture of NaSCN and $BaCl_2$ is separated by IC we would get two peaks, one for SCN^- and one for Cl However, with separation by EIC, we get four peaks [10] Both anions are redistributed between the two cations, forming the following 'ion - pairs' Na^+ Cl , Ba^+ $2Cl^-$, Na^+ SCN and Ba^+ $2SCN^-$ (Figure 2 6)

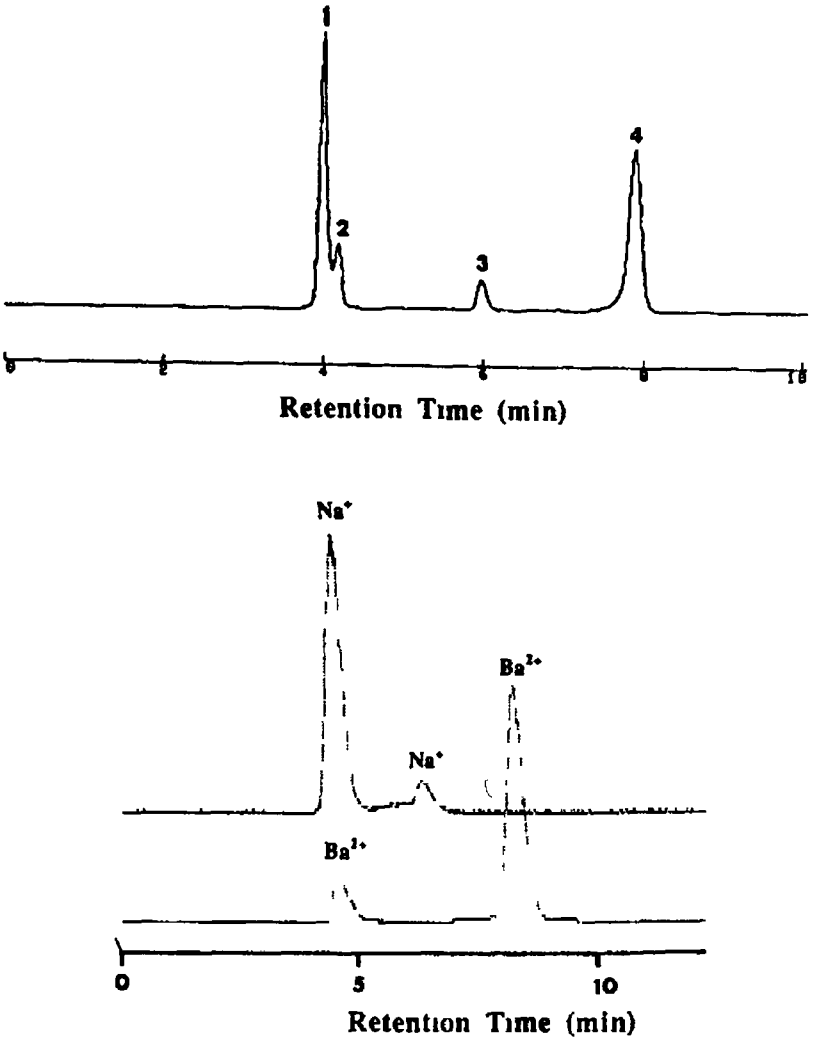


Figure 2 6 Chromatogram showing the redistribution of chloride in EIC [10] Aqueous solution containing 10mM NaSCN and 5mM $BaCl_2$ ODS packed column (4 6 x 250mm) coated with CHAPSO Pure water eluent, flow rate 0 7 ml/min Upper trace is conductivity detection, lower trace is ICP – AES detection Peaks 1 = Na^+ Cl , 2 = Ba^{2+} 2Cl , 3 = Na^+ SCN , 4 = Ba^{2+} 2SCN

The redistribution is not easily predicted and formation of the ion - pairs depends on the 'priority of formation', which is in turn dependant on the molal energies (ΔG) of the ions as well as their concentrations [11] Theories have been proposed allowing predictions of the ion - pairs that should be formed [5, 10-12], but the outcome is not always as expected In general, it is believed that for *m* amount of cations and *n* amount of anions, the number of ion - pairs expected is *m x n* The concentration of the ions is important as at lower concentrations, only the ion - pairs of highest priority of formation will be formed, with further ion - pairs forming once the concentration is increased

The number of ion - pairs formed however, does not equate to the number of identified peaks in a chromatogram If ion - pairs with the same anions (e g nitrate) but different cations having similar valencies (e g Na^+ and K^+) are formed, only one peak will be observed, since the retention time of any specific anion is dependant on the valency of the cation with which it is paired Without an elemental specific detection technique like atomic spectroscopy available to identify the cations present, it could be assumed that the observed peak was only due to the presence of one cation

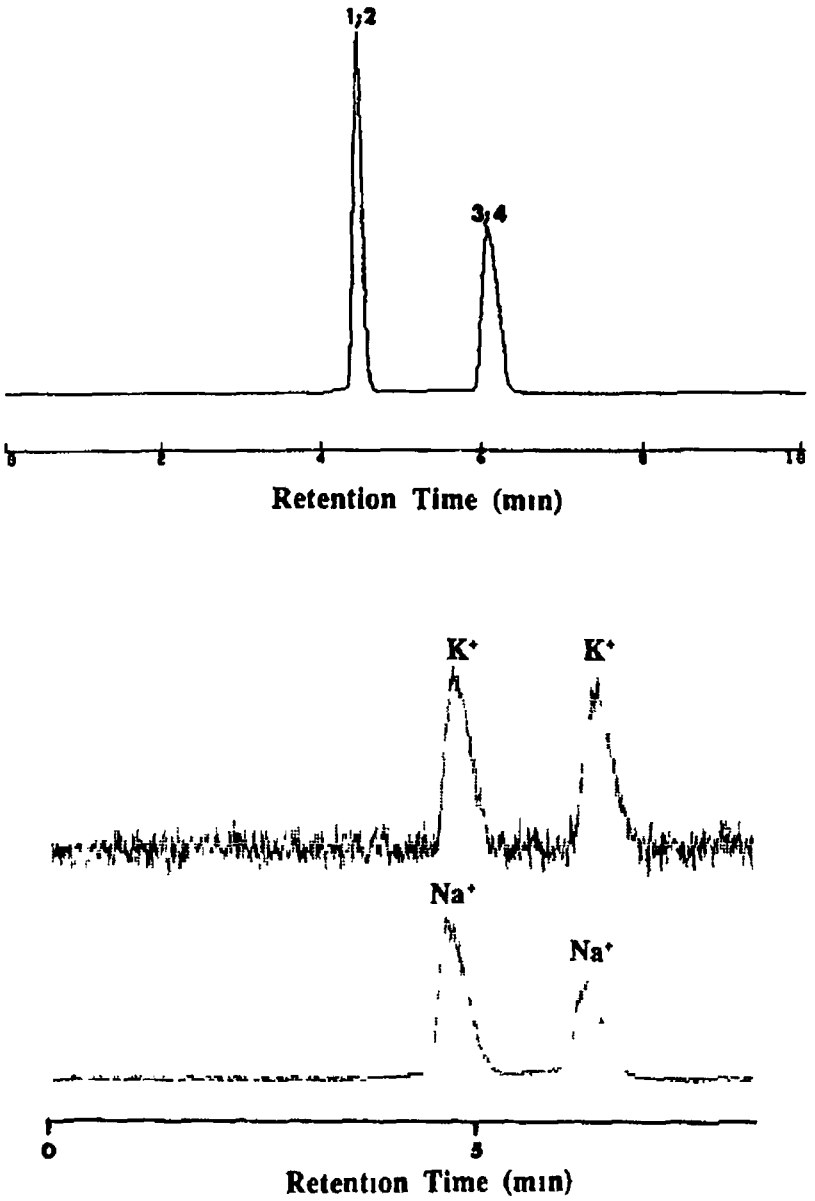


Figure 2 7 Chromatogram showing how atomic absorption helps in the identification of ion - pairs [10] Aqueous solution containing 10mM KBr and 10mM NaSCN ODS packed column (4 6 x 250mm) coated with CHAPSO Pure water eluent, flow rate 0 7 ml/min Upper trace is conductivity detection, lower trace is ICP – AES detection Peaks 1 = Na^+ Br , 2 = K^+ Br 3 = Na^+ SCN 4 = K^+ SCN

Despite these drawbacks, Hu *et al* [1] managed to successfully apply EIC to the determination of iodide and thiocyanate in human saliva samples However, in general, if quantitative work is to be carried out for multiple inorganic anions, all relevant ion - pairs must be identified, and all the corresponding peaks must be examined, leading to multiple

calibration curves and a time consuming analysis Therefore ion redistribution is a major drawback of EIC and much work has been carried out to try to eliminate it

2 2 2 1 Cation exchange

Different approaches have been undertaken to eliminate ion redistribution in anion analysis Among the first to be established was the introduction of an in-line cation exchange column prior to the analytical column, for the purposes of converting all cations to one suitable form [13,14] A resulting separation would contain only one ion - pair for each anion, resulting in a much simpler chromatogram Manipulation of the retention times and therefore the separation selectivity of the analytes was possible by changing the form of the exchanging cation, since as explained previously, the retention time of a given anion is dependant on the valency of the cation When the cation exchange column was in the magnesium (or any divalent cation) form, retention times of the resulting ion - pairs were longer than when the cation exchange column was in the sodium (or any monovalent cation) form (Figure 2 8 – 2 10)

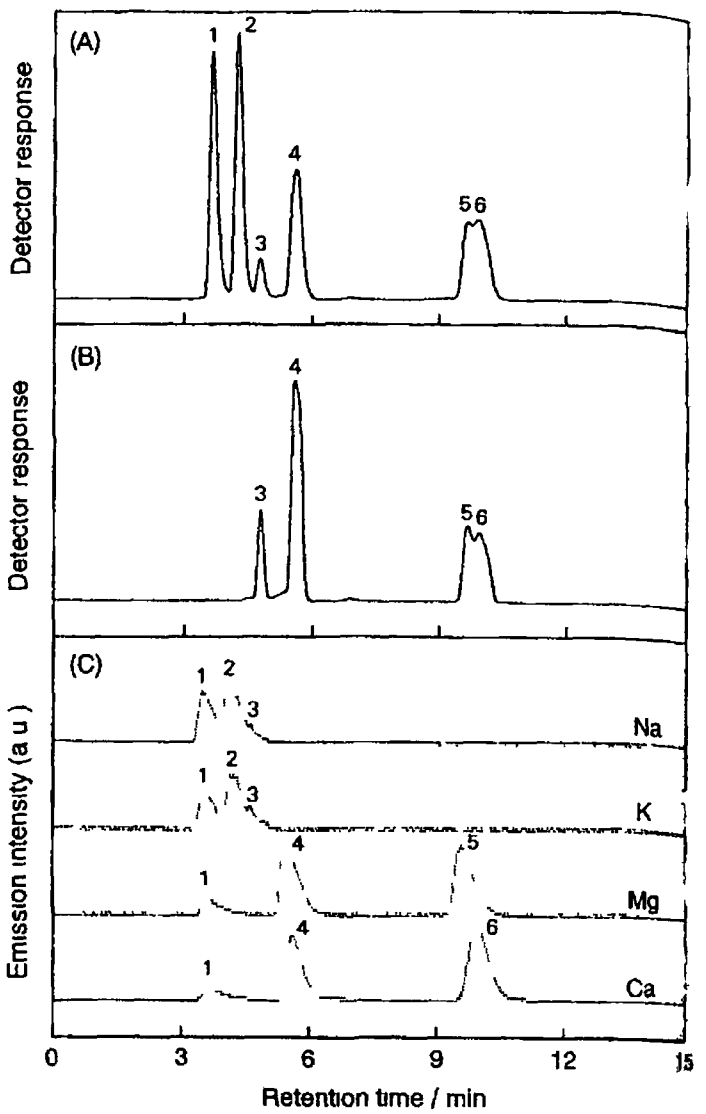


Figure 2 8 Chromatogram of a separation achieved without the aid of a preconditioning column [13] Aqueous solution containing 4 anions (5 mM SO_4^{2-}, 10 mM each of Cl NO_2 and NO_3) and 4 cations (10 mM each of Na^+ and K^+ 5 mM each of Mg^{2+} and Ca^{2+}) ODS packed column (4 6 x 250 mm) coated with Zwittergent 3-14 Pure water eluent flow rate 0 7 ml/min (A) Conductivity detection (B) UV absorption detection (230nm) (C) ICP – AES detection Peaks 1 = $2Na^+$ / $2K^+$ / Mg^{2+} / Ca^{2+} SO_4^{2-}, 2 = Na^+ / K^+ Cl , 3 = Na^+ / K^+ NO_2 4 = Mg^{2+} / Ca^{2+} $2NO_2$ 5 = Mg^{2+} NO_3, 6 = Ca^{2+} NO_3

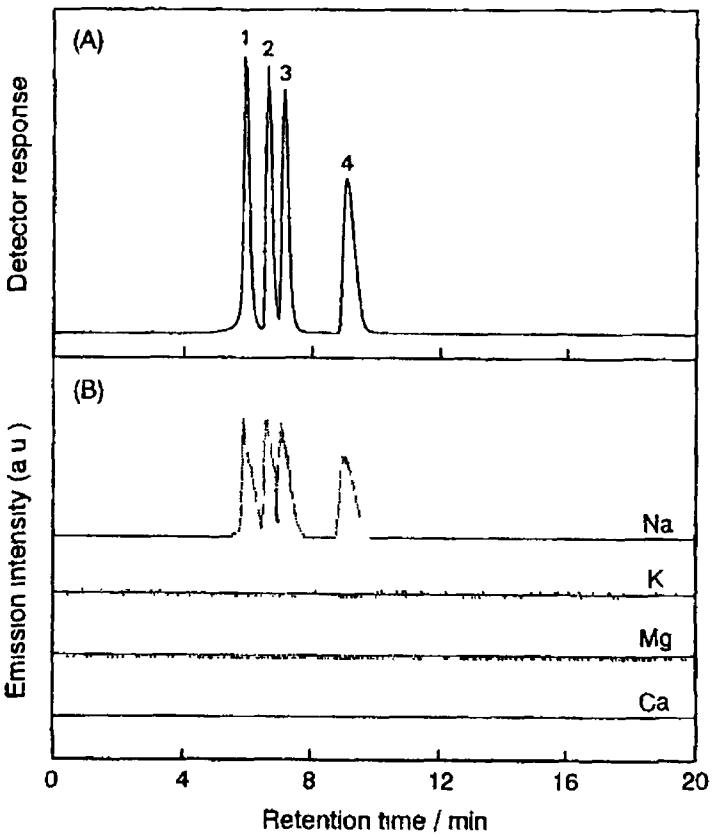


Figure 2 9 Chromatogram of a separation achieved with the aid of a sodium preconditioning column [13] Sample solution as above ODS packed column (4 6 x 250 mm) coated with Zwittergent 3-14 Pure water eluent flow rate 0 7 ml/min (A) Conductivity detection (B) ICP – AES detection Peaks 1 = $2Na^+ SO_4^{2-}$, 2 = $Na^+ Cl^-$, 3 = $Na^+ NO_2^-$ 4 = $Na^+ NO_3^-$

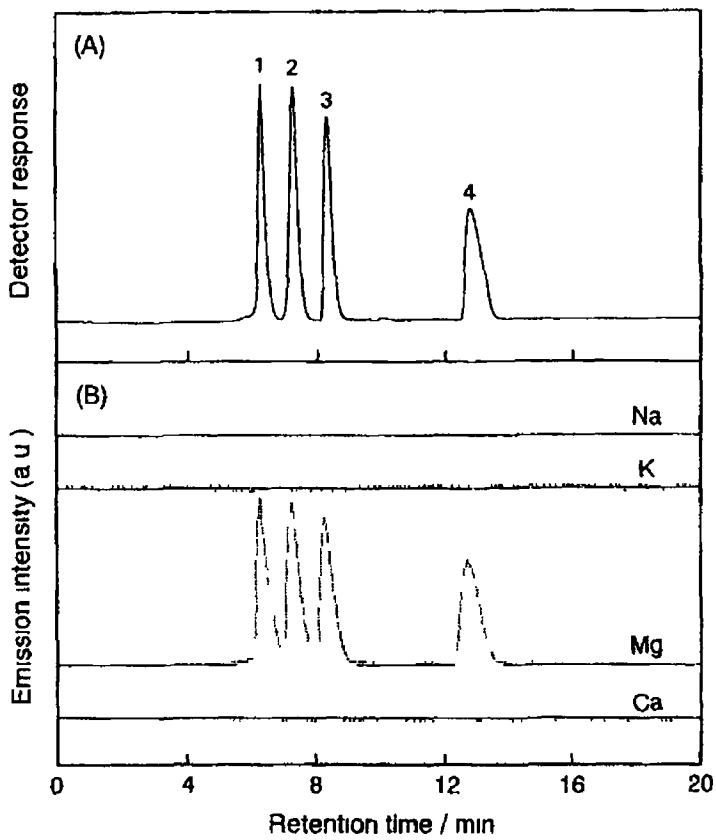


Figure 2 10 Chromatogram of a separation achieved with the aid of a magnesium preconditioning column [13] Sample solution as above ODS packed column (4 6 x 250 mm) coated with Zwittergent 3-14 Pure water eluent, flow rate 0 7 ml/min (A) Conductivity detection (B) ICP – AES detection Peaks 1 = Mg^{2+} SO_4^{2} 2 = Mg^{2+} 2Cl 3 = Mg^{2+} $2NO_2$, 4 = Mg^{2+} $2NO_3$

This technique was successfully applied to the analysis of inorganic anions in radish juice [13] using both a sodium cation exchange column and a magnesium cation exchange column It has also been applied to the analysis of inorganic anions in an underground water sample [14], using a cation exchange column in the sodium form Sodium was chosen here as the separation efficiency was adequate and the resulting elution times and therefore the overall analysis time was shorter with the monovalent cation

Although the problem of ion redistribution was successfully overcome with this method, a significant increase in retention times of the anions, and therefore in analysis

time was noted Although anions should be quickly eluted from a cation exchange column on the basis of the Donnan exclusion theory [13], the increase in the elution path still amounts to an increase in their elution times Umemura *et al* [13] witnessed an increase of over two minutes in the retention times of the ion - pairs due to the presence of the cation exchange column in their system For example, $NaSO_4$ was eluted at 3 79 minutes when no exchange column was used and at 5 92 minutes when an exchange column in the sodium form was used The retention time of $NaNO_3$ increased from 6 91 minutes to 9 14 minutes So although this method was successful in its attempts to eliminate ion redistribution, it had some limitations of its own

2 2 2 2 Decoy Analyte

Another method that has been developed to deal with ion redistribution tends towards controlling the situation rather than elimination of the problem [15] It has been previously noted that ion - pair formation varies depending on the molal energies of the ions leading to 'priority of formation' of the ion - pairs If a cation, which has a high 'priority of formation', is introduced into a sample containing inorganic anions, then the formation of ion - pairs tends to lean towards the pairing of the anions with this 'introduced' cation If the cation is of a high enough concentration, then all subsequent ion - pairs will consist of this cation alone, therefore controlling the partitioning of the ions

The initial cations present in the sample still need to be paired with an anion, so if an anion is introduced into the sample that has a high 'priority of formation' with the sample cations, then the subsequent ion - pairs formed will contain the sample cations and the

'introduced anion', further encouraging all the sample anions to pair with the introduced cation The introduced anion and cation are known as the 'decoy anion' and 'decoy cation' and together are known as the 'decoy analyte'

The selection process for this decoy analyte is important The decoy cation must lead to preferential pairing with the sample anions so that they may be analysed in the decoy cation form The decoy anion must pair with all the different cationic forms in the sample, thus removing them from the equation and preventing ion - pair formation of the sample cations and sample anions and therefore leading to the simple analysis of the anions Also, the concentration of the decoy analyte is important, and must be of a sufficient level so as to completely pair with the corresponding sample ions

It has been found [15] that in the analysis of the common inorganic anions such as chloride, bromide, nitrate and nitrite, a decoy analyte which satisfies these conditions contains the following

- A monovalent cation combined with an anion that has a longer retention time than the analyte anions of interest e g NaI
- A divalent cation combined with an anion that has a shorter retention time than the analyte anions of interest e g $MgSO_4$

When an excess amount of NaI is introduced into a sample containing chloride, bromide, nitrate, nitrite and various unknown cations, the anions pair with sodium and the cations pair with iodide The resulting chromatogram contains separated peaks for the

four sample anions plus additional peaks corresponding to the iodide, paired with the monovalent and divalent sample cations These iodide peaks have much longer retention times than the analytes of interest and so do not interfere with the analysis (Figure 2 11)

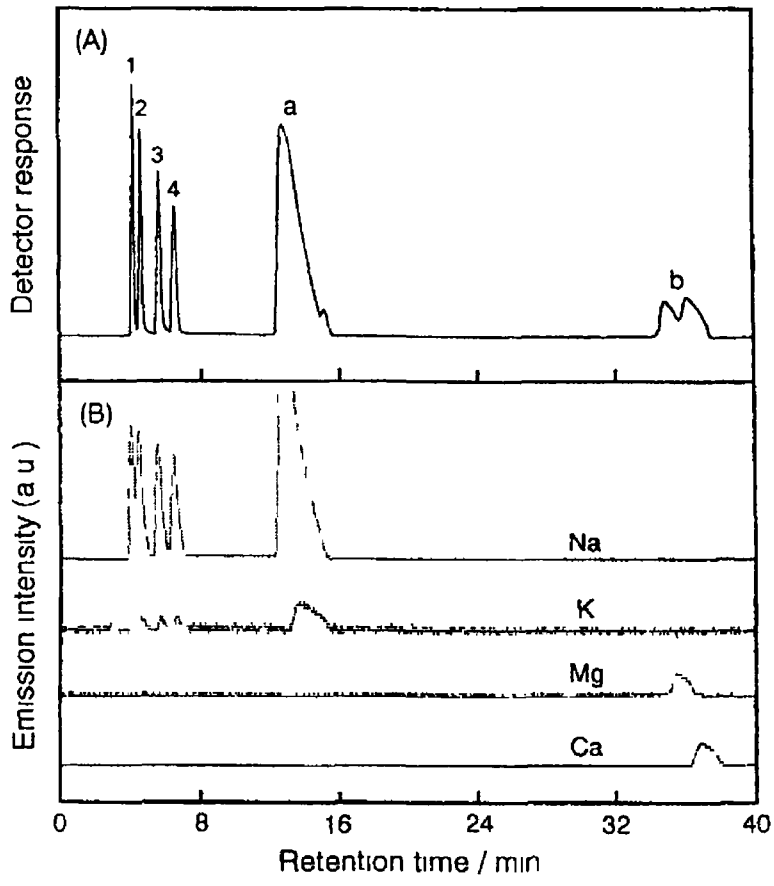


Figure 2 11 Chromatogram of a separation achieved using NaI as decoy analyte [15] Aqueous solution containing 4 anions (10 mM each of Cl , NO_2 Br and NO_3) and 4 cations (10 mM each of Na^+ and K^+ 5 mM each of Mg^{2+} and Ca^{2+}) with addition of 100 mM NaI ODS packed column (4 6 x 250 mm) coated with Zwittergent 3-14 Pure water eluent, flow rate 0 7 ml/min (A) Conductivity detection (B) ICP – AES detection Peaks 1 = Na^+ Cl 2 = Na^+ NO_2 3 = Na^+ Br 4 = Na^+ NO_3 a = Na^+ / K^+ I b = Mg^{2+} / Ca^{2+} 2I Peaks 1 – 4 include a small amount of potassium

Similar results were achieved with $MgSO_4$ In this instance, the sample anions paired with magnesium, and were eluted at the later retention times that correspond with the pairing of a divalent cation The sample cations paired with sulphate, and were eluted early, as sulphate is only slightly retained in EIC (Figure 2 12) This technique of ion

partitioning control has been successfully applied to the analysis of radish juice for inorganic anion content [15], and when compared with a conventional IC method, the results were very promising

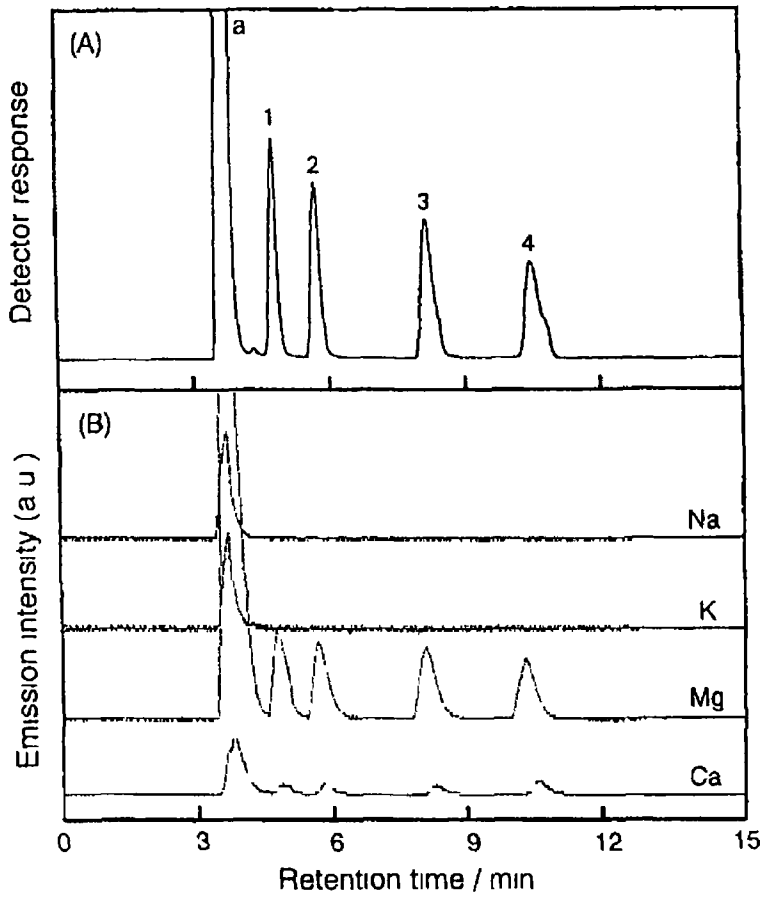


Figure 2 12 Chromatogram of a separation achieved using $MgSO_4$ as decoy analyte [15] Aqueous solution containing 4 anions (10 mM each of Cl , NO_2 Br and NO_3) and 4 cations (10 mM each of Na^+ and K^+, 5 mM each of Mg^{2+} and Ca^{2+}) with addition of 50 mM $MgSO_4$ ODS packed column (4 6 x 250 mm) coated with Zwittergent 3-14 Pure water eluent, flow rate 0 7 ml/min (A) Conductivity detection (B) ICP – AES detection Peaks 1 = Mg^{2+} 2Cl , 2 = Mg^{2+} $2NO_2$, 3 = Mg^{2+} 2Br , 4 = Mg^{2+} $2NO_3$ Peak a = $2Na^+$ / $2K^+$ / Mg^{2+} / Ca^{2+} SO_4^{2} Peaks 1 – 4 include a small amount of calcium

This method of introducing a decoy analyte was also used in the determination of bromide in seawater [16] Here, a mixture of $MgCl_2$ and $CaCl_2$ was introduced into the sample Both compounds satisfy the selection process of a decoy analyte as they contain a

divalent cation combined with an anion that has a shorter retention time than the analyte anions of interest The resulting chromatogram contained a peak corresponding to bromide paired with Mg^{2+} and Ca^{2+}, which was sufficiently separated from the large matrix of NaCl

The problem of ion redistribution has been successfully controlled with the introduction of a decoy analyte However, the simplicity of EIC is being somewhat undermined by having to introduce foreign elements into the sample of interest Sample preparation can be time consuming and tedious, and is a drawback of this method Also the formation of a large peak due to the decoy anion is produced If the anion is a strongly retained anion such as iodide, analysis time is further increased, especially if there are divalent or trivalent cations present Iodide, when paired with a divalent cation can take over half an hour to elute If the decoy anion is sulphate, a large chromatographic peak could result in the overlapping of chloride or fluoride peaks, therefore hindering the analysis of these anions Therefore, while this method of introducing a decoy analyte does solve some of the problems of ion redistribution, it also has many drawbacks

2 2 3 Trace Analysis

As described earlier, a ZEDL is formed in the analytical column when an analyte solution is introduced (Figure 2 3) Also to be noted is that a Stern layer and a diffuse layer are also created Hu *et al* [17] discovered that the Stern layer is influential to retention times at low concentrations When an analyte solution of low concentration (1 0 μM NaCl and $CaCl_2$) was introduced into the column, two peaks were observed for each ion - pair (Figure 2 13) Further investigation lead to the conclusion that two elution times

occur for each analyte in EIC, one corresponding to elution from the diffuse layer and one from the Stern layer The later eluting peak for each ion - pair was due to the portion of the ion - pair that had been retained in the Stern Layer

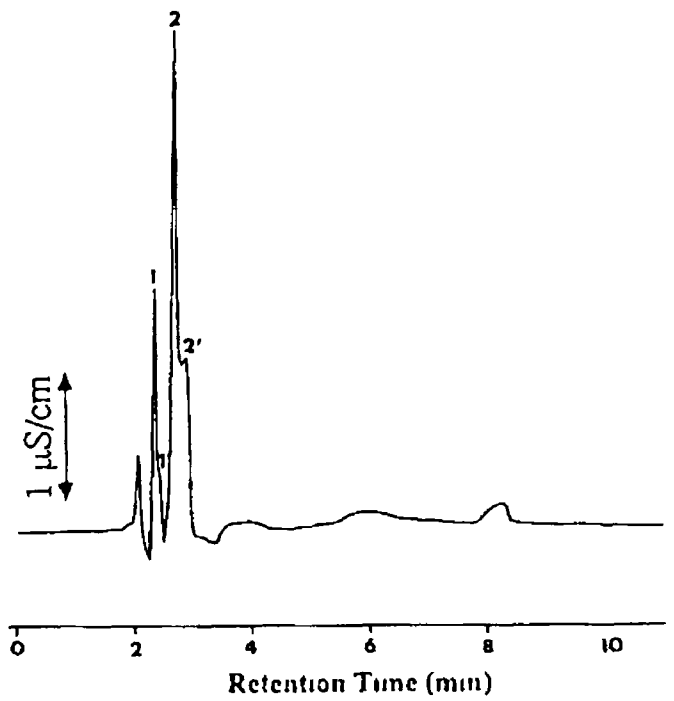


Figure 2 13 Chromatogram showing elution from the Stern layer and the diffuse layer [17] Aqueous solution containing 1 0 µM each of $CaCl_2$ and NaCl ODS packed column (4 6 x 250 mm) coated with Zwittergent 3-14 Pure water eluent, flow rate 1 0 ml/min Sample injection 100 µL conductivity detection
Peaks 1 1'=Na^+ Cl^- 2, 2 = Ca^{2+} 2Cl

This phenomenon always occurs in EIC, irrespective of the concentration of the analyte The Stern layer will only retain a certain amount of the analyte, and therefore the stern peak reaches its maximum at an extremely low level At higher concentrations, the diffuse layer peak is so large that the Stern layer peak goes unnoticed or is swamped by the diffuse peak As the concentration of the analyte decreases, the influence of the Stern peak becomes more evident when eventually peak splitting begins and two retention times

are noted for each anion At ultra low concentrations (trace levels), the diffuse layer peak may actually disappear, and all the analyte is eluted from the Stern layer

This dual elution is a drawback in the analysis of small amount of inorganic anions Both peaks would have to be integrated in order to carry out quantitative analysis To overcome this, Hu *et al* [17] developed a method in which a 'sacrifice' anion is introduced into the sample This 'sacrifice' anion replaces the sample anions in the Stern layer thus releasing them to the diffuse layer, and eliminating the formation of the second peak The 'sacrifice' anion must have a longer retention time than the analyte anions, as a longer retention time denotes a higher affinity for the stationary phase Iodide is a suitable 'sacrifice' anion, and by adding iodide to a solution, Hu *et al* [17] successfully separated and detected 0 1 μM $CaBr_2$ and $Ca(NO_3)_2$ using UV detection Addition of a 'sacrifice' anion is not usually necessary at trace levels, as the analyte anion may already be eluting from the Stern layer only

One of the many benefits of using water as a mobile phase is that detection sensitivity is excellent Conductivity detection and UV absorption detection are the two main techniques employed in the analysis of inorganic anions Since there is no electrolyte in the mobile phase, there is no need for a suppressor with conductivity detection UV absorption detection has also been shown to be highly sensitive when used with EIC and detection limits of both methods are in the low ppb range for many anions Hu *et al* [1] quoted detection limits of 0 3 μM for iodide using a 20 μL injection loop with UV detection at 230 nm With or without the need for a sacrificial anion, EIC is well suited to trace analysis of inorganic anions [18]

2.2.4 Cation Analysis

As described previously, there is one zwitterionic reagent being commonly used for the analysis of cations – N–dodecylphosphocholine. As is evident from Table 2.1, the position of the two charged groups is reversed in comparison to the reagents used for anion analysis. It is suggested by Hu *et al.* [19] that when the outer functional group of the reagent is positive (as is the case here), analyte anions are bound to the positive group, and therefore only analyte cations may distribute effectively into the stationary phase. The opposite is said to be true for sulfobetaine type reagents, where only anions may effectively distribute into the stationary phase.

Further work carried out [19] using N–dodecylphosphocholine as the coating on the column resulted in the following:

- Cations and anions were retained
- 'Ion - pairs' were formed
- Cations of the same charge could be separated
- Ion redistribution occurred when multiple anions were present in the analyte solution
- Introduction of a 'decoy analyte' resulted in single peaks for each cation
- Transition earth metals were completely separated from alkaline earth metals

The elution order obtained was $Ba^{2+} < Mg^{2+} < Ca^{2+}$ which differs from that observed using conventional cation exchange chromatography [9], proving that a retention mechanism other than ion exchange was taking place. The proposed mechanism was the

same as that assumed for anion separation, i e simultaneous attraction and repulsion effects with separation taking place between ions having different 'ion - pair' formations

2.3 ELECTROLYTIC ELUENTS

While ion redistribution has been somewhat eliminated or controlled by the addition of an exchange column or the introduction into the sample of a decoy analyte, there are several drawbacks to both methods A method was needed where the sample anions would be exclusively paired to one cation, without inserting an exchange column into the chromatographic system and without additional sample preparation Hu and Haddad together developed a method [20] which maintained the unusual selectivity of EIC but required the addition of a small amount of electrolyte to the eluent When the concentration was low, the sensitive detection that is inherent with EIC with a water eluent was still possible Addition of the electrolyte led to a single ion - pair for each anion, and therefore elimination of all sample cations from the resulting chromatogram (Figure 2 14) Different electrolytes have been investigated for their potential as eluents in EIC with differing selectivity obtained with each type

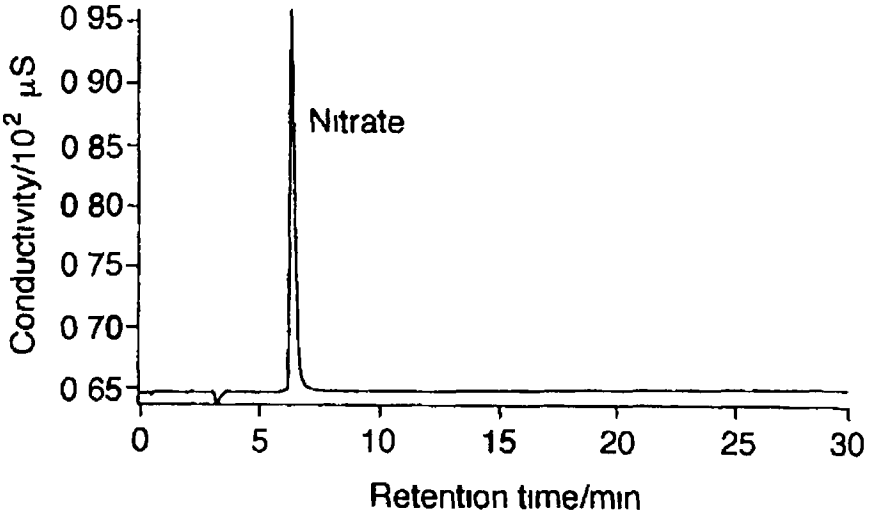


Figure 2 14 Chromatogram showing elimination of ion distribution due to the addition of an electrolyte to the eluent[20] Aqueous solution containing sodium nitrate and magnesium nitrate ODS packed column (4 6 x 250 mm) coated with Zwittergent 3-14 10 mM $NaHCO_3$ eluent, flow rate 1 0 ml/min Suppressed conductivity detection

2 3 1 Monovalent Electrolyte

When a monovalent eluent is used in EIC e g NaCl, $NaHCO_3$ or Na_2SO_4, an initial increase in retention times is noted for all anions Unlike IC, in EIC, as the concentration of the electrolyte is increased, the retention times of the analyte ions also increase These slight increases in retention times are noted until a certain level of electrolyte concentration is reached, after which no further changes in retention time occur No difference is noted in the elution order of the anions when compared with EIC using only pure water as a mobile phase This method was successfully applied to the analysis of iodide in seawater [21] NaCl was used as electrolyte with an ammonium sulfobetaine – 1 modified column Iodide was well separated form all matrix anions A detection limit of 0 82 ppb (100 µL loop) was determined for iodide using UV detection at 210 nm showing that no loss in detection sensitivity had occurred

Similar results are experienced when EIC is carried out with a phosphocholine modified stationary phase with a dilute electrolyte in the mobile phase [22] Single peaks were observed for all cations, and no retention was observed for any of the sample anions In fact it can be said that upon the addition of an electrolyte into the eluent, the sulfobetaine modified stationary phase lost its capability to distinguish between different cations and the phosphocholine modified stationary phase lost its ability to distinguish between different anions

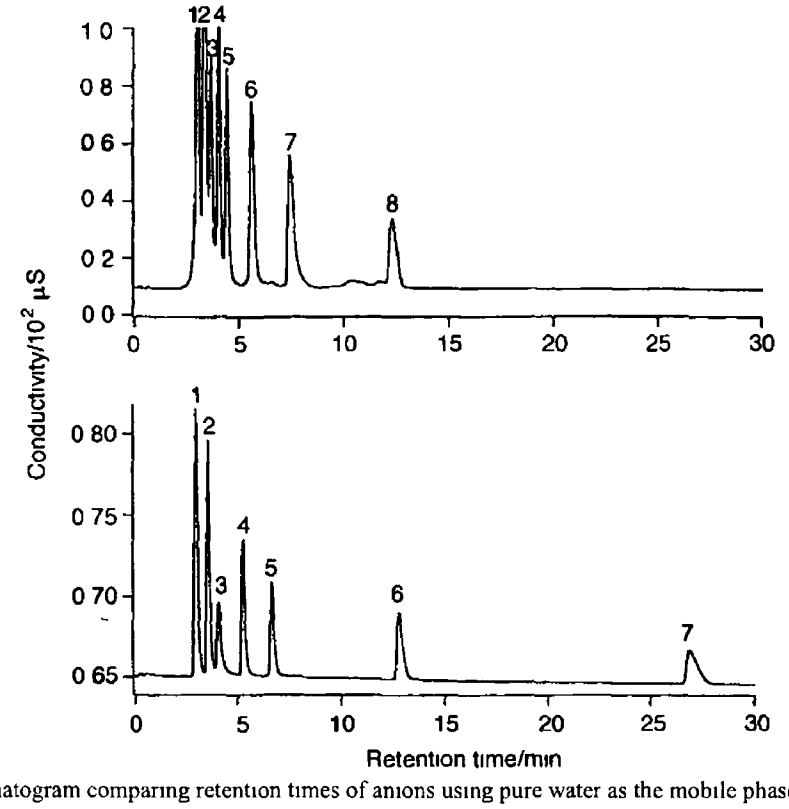


Figure 2 15 Chromatogram comparing retention times of anions using pure water as the mobile phase and an electrolyte as the mobile phase [20] ODS packed column (4 6 x 250 mm) coated with Zwittergent 3-14 Pure water eluent (upper trace) and 10 mM $NaHCO_3$ eluent (lower trace) flow rate 1 0 ml/min Suppressed conductivity detection Peaks 1 = sulphate 2 = chloride, 3 = nitrite, 4 = bromide, 5 = nitrate 6 = chlorate, 7 = iodide, 8 = thiocyanate The peak due to thiocyanate in the lower trace was eluted at 378 minutes

2 3 2 Divalent Electrolyte

When a divalent eluent was used e g $MgSO_4$ or $CaSO_4$, the elution trends differed somewhat [23] It was now noted that an increase in electrolyte concentration resulted in a decrease in retention times, as is found in ion exchange chromatography The proposed mechanism for the retention of anions using any electrolyte as eluent is schematically shown in Figure 2 16

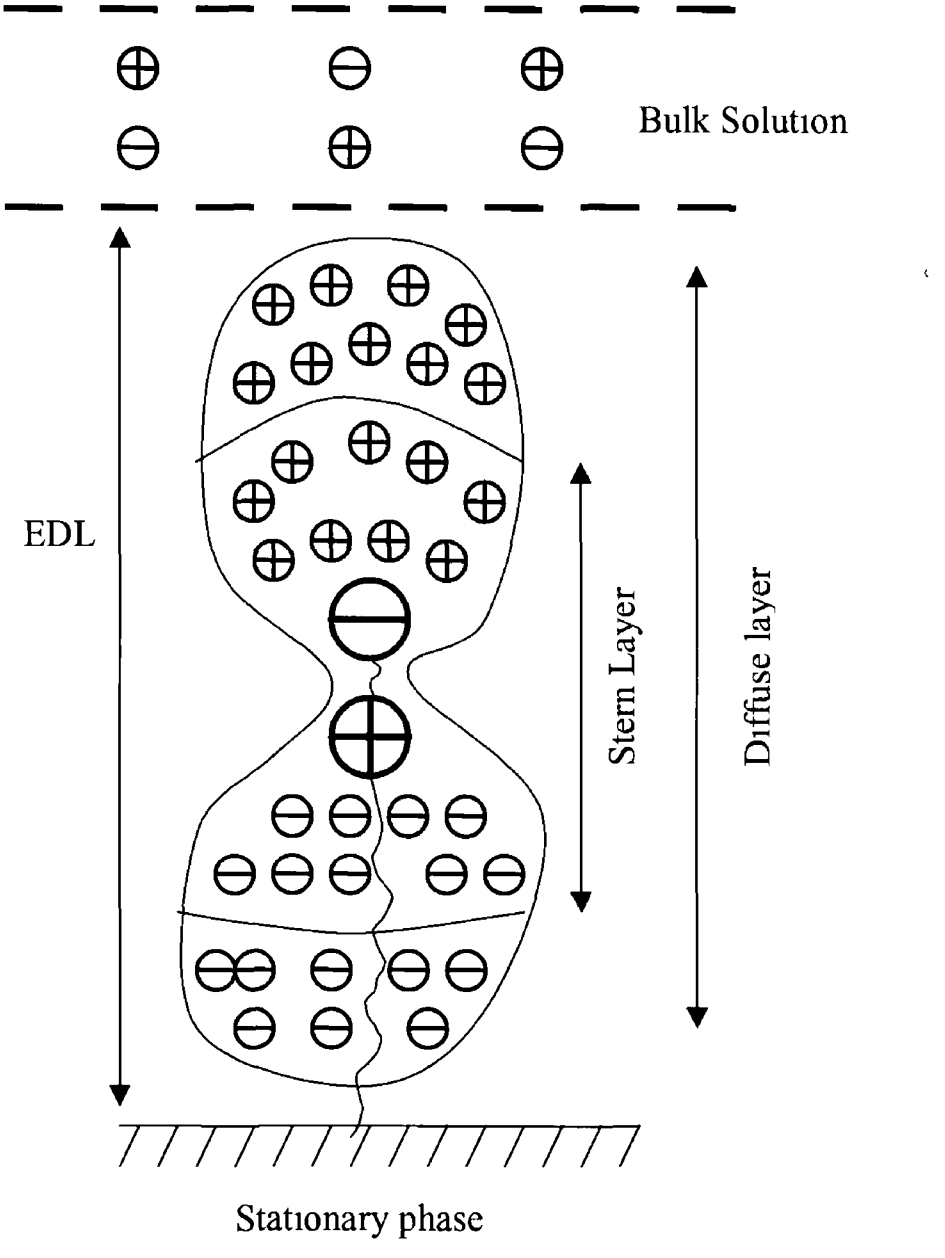


Figure 2 16 Schematic diagram of the adsorbed zwitterionic molecule and the binary EDL formed by the eluent cations and anions [23]

The proposed mechanism states that a binary EDL is formed at both functional group sites of the zwitterionic molecule The eluent cations and anions form an EDL with the outer negative group and the inner positive group Analyte anions are attracted by the cationic portion of the EDL and repulsed by the anionic portion Analyte cations are repulsed by the outer cationic layer of the EDL and hence not retained Analyte anions that have the highest priority of formation with the eluent cations are more strongly retained

In the case of the monovalent electrolyte, retention times of the analyte anions increased until a certain concentration of the electrolyte had been reached This concentration level is that which is required to saturate the EDL, after which, any further increase in electrolyte concentration does not affect the EDL and hence does not affect retention times of the analyte anions

With a divalent electrolyte, retention times decrease with increased concentration of the electrolyte While this is not fully understood, it is suggested that the sulphate in the eluent, which has a high propensity to form ion - pairs with divalent cations, competes with the analyte anions and reduces their retention times Therefore it is being suggested that the retention times of these anions are due to a complicated mixture of ion exchange as well as electrostatic attraction and repulsion

Manipulation of the retention times of sample anions can now be achieved by changing the electrolyte concentration This method has been successfully applied to the analysis of bromide, nitrate and iodide in a seawater sample [23] using artificial seawater as

the eluent Detection limits were found to be 0 75 ppb for bromide, 0 52 ppb for nitrate and 0 8 ppb for iodide using UV detection at 210 nm

2 3 3 Hydroxide Electrolyte

When a hydroxide electrolyte was used as the eluent in EIC, retention selectivity differed once more from that experienced when either of the two previous types of electrolytes (mono or divalent) were used [24] An *increase* in the concentration of the hydroxide electrolyte in the eluent caused an *increase* in the retention times of the analyte anions The proposed mechanism states that since hydroxide ions have an extremely low affinity for the quaternary ammonium group, the number of hydroxide ions in the EDL will be considerably less than what would be present with a different electrolyte, such as HCO_3 or Cl Since electroneutrality must be maintained, the cation layer of the EDL would also be less dense This results in shorter retention times for anions than those achieved with a different electrolyte [24] An increase in the concentration of the hydroxide eluent led to an increase in the density of the EDL and hence an increase in the retention times of the analyte anions As with other electrolytes such as HCO_3 or Cl , the increasing concentration reached a saturation point, from which point on, the retention times no longer increased For electrolytes such as HCO_3^- or Cl , this saturation point was very quickly reached With the hydroxide eluent, this saturation level could only be reached in theory, as the required concentration would be so high as to be impractical in liquid chromatography

Since ion - pair formation was still ongoing, the counter cation in the hydroxide eluent also played an influential part in the retention times of the analyte anions For

example, calcium hydroxide resulted in longer retention times than lithium hydroxide as a divalent cation always results in longer retention times for anions than a monovalent cation Therefore, adjustment of the electrolyte concentration and the choice of counter cation in the eluent can achieve manipulation of the retention times of the anions and subsequently the separation selectivity Detection sensitivity was maintained in this case by using suppressed conductivity detection Detection limits were quoted in the sub µM range for sulphate, fluoride, chloride, nitrate, bromide and nitrite with a 100 µL injection loop and 1 0 mM $Ca(OH)_2$ eluent

2 3 4 Tetraborate

Tetraborate has found limited use as an eluent in ion exchange chromatography as it has a weak eluting power for most analyte anions In EIC, it is not needed for its eluting ability, and hence works well as an eluent Its properties as an eluent in EIC are the same as those of the hydroxide electrolyte Retention times of the analyte anions increase with increasing concentration of tetraborate as it also has a very weak affinity for quaternary ammonium sites The use of tetraborate as an eluent has been successfully applied to the analysis of inorganic anions in snow and rainwater samples [25] Detection was suppressed conductivity and detection limits were similar to those quoted using hydroxide as the eluent

2 3 5 Mixed Micelles

Even though separation of analyte anions can now be manipulated by adjustment of the eluent electrolyte, it has proven impossible to separate fluoride, phosphate and sulphate as all three anions are eluted so closely to the void time Since these anions are

so important in inorganic analysis, it has been the aim of much research to achieve their rapid separation This has been successfully accomplished by the use of mixed micelles in the coating of the stationary phase [26]

Using a mixture of Zwittergent 3-14 and the cationic surfactant myristyltrimethylammonium (MTA) with a tetraborate eluent, the anions were successfully separated with the following elution order

fluoride < phosphate < sulphate < chloride < nitrite < bromide < nitrate

The early elution of phosphate and sulphate proves unique to this method The proposed retention mechanism explained how the retention of the anions was due to two different factors Firstly, the zwitterionic surfactant separates the anions by the conventional EIC mechanism of simultaneous attraction and repulsion Secondly, the cationic surfactant separates the anions by a conventional anion exchange mechanism The net result provides a unique separation that is deemed to have a 'dual' retention mechanism (Fihure 2 17)

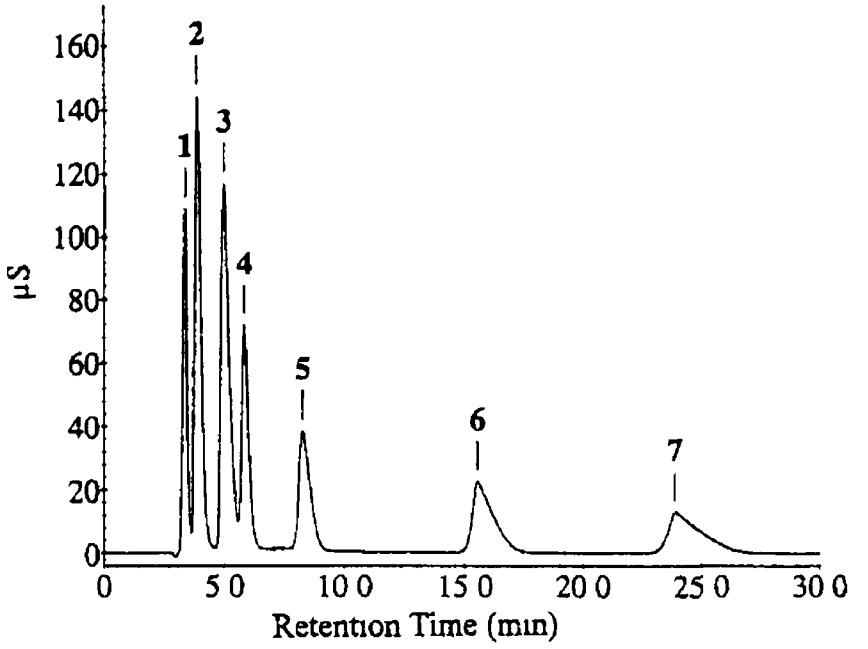


Figure 2 17 Chromatogram showing the separation achieved when using mixed micelles as the stationary phase in EIC [26] Aqueous solution containing 1 mM each of seven different anions ODS packed column (4 6 x 250 mm) coated with mixed micelles of Zwittergent 3-14/ MTA (20 mM / 2 mM) 5 mM sodium tetraborate eluent, flow rate 1 0 ml/min Suppressed conductivity detection Peaks 1 = fluoride, 2 = phosphate, 3 = sulphate, 4 = chloride, 5 = nitrite, 6 = bromide, 7 = nitrate

In this study, retention manipulation could not only be achieved by adjustment of the eluent concentration, but also by controlling the ratio of zwitterionic surfactant to cationic surfactant [27] As the mole ratio of cationic surfactant was increased, so too did the propensity for the anions to engage in ion exchange In this manner, retention times of the anions were modified and actual changes in the elution order of the anions could be achieved

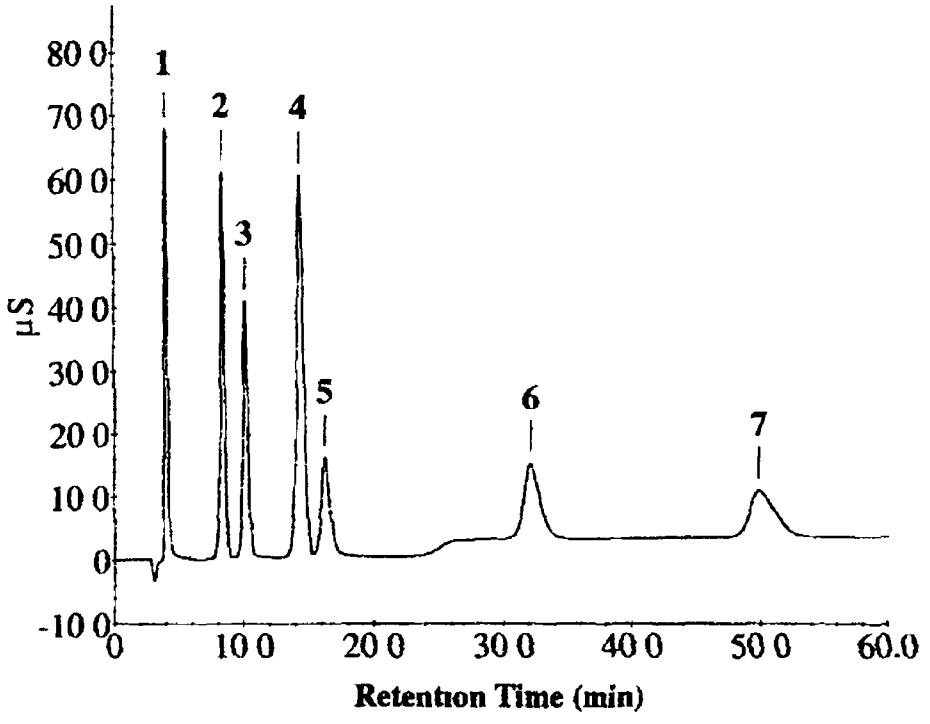


Figure 2 18 Chromatogram showing the separation manipulation possible when using mixed micelles as the stationary phase in EIC [27] Aqueous solution containing 0 1 mM each of seven different anions ODS packed column (4 6 x 250 mm) coated with mixed micelles of Zwittergent 3-14/ tetradecyltrimethylammonium (10 mM / 10 mM) 20 mM Na_2CO_3 eluent, flow rate 1 0 ml/min Suppressed conductivity detection Peaks 1 = fluoride 2 = phosphate 3 = chloride, 4 = sulphate, 5 = nitrite, 6 = bromide, 7 = nitrate

Application of this new method to the analysis of inorganic anions in a tap water sample was shown [26] Three anions, namely fluoride, sulphate and chloride, were successfully separated and quantified As tetraborate was used as the eluent, with suppressed conductivity detection, detection limits were again extremely low, with levels being quoted from 1 to 10 ng/L for the seven common inorganic anions, as listed in Table 2 2

Analyte	Detection limit (μM)	Detection limit (ng/L)	Precision (% relative standard deviation, n = 13)		
			Retention time	Peak height	Peak area
F^-	0 08	1 52	0 6	0 8	1 1
HPO_4^{2-}	0 11	10 56	0 6	0 8	2 1
SO_4^{2-}	0 09	8 64	0 7	0 7	1 9
Cl^-	0 07	2 49	0 7	0 9	2 3
NO_2^-	0 07	3 22	0 7	0 9	2 5
Br^-	0 07	5 59	0 8	0 6	1 1
NO_3^-	0 09	5 58	0 9	1 0	1 7

Table 2 2 Analytical performance data for the separation of common anions on an ODS column coated with mixed Zwittergent 3-14 / MTA micelles [26]

2.4 CONCLUSIONS

Although electrostatic ion chromatography is a relatively new technique, since its beginning in 1993, it has developed in many ways, from a separating system consisting of just a zwitterionically modified stationary phase and pure water as the eluent to one with a dual separating mechanism and an electrolyte as the eluent Despite the problems of ion redistribution and poor reproducibility, it is possible to successfully separate most important inorganic anions without much difficulty

EIC has been successfully applied to the analysis of a wide array of samples, ranging from biological samples such as saliva to seawater samples with their complex matrices Due to this ability to handle complex samples, and also due to the excellent detection limits achievable with both conductivity and UV detection, EIC is particularly suited to the trace analysis of inorganic anions, especially in those samples with concentrated matrix ions that prove unsuitable to conventional ion chromatographic methods

BIBLIOGRAPHY

1 W Hu, T Takeuchi and H Haraguchi, Analytical Chemistry (**65**), 2204 – 2208, 1993

2 W Hu and P Haddad, Trends in Analytical Chemistry, (**17**), no 2, 73 – 79, 1998

3 E Blasius, K P Janzen, W Adrian, G Klautke, R Lorscheider, P G Maurer, V B Nguyen, T Nguyen Tien, G Scholten and J Z Stockemer, Analytical Chemistry, (**284**), 337, 1977

4 H Small, M E Soderquist and J W Pischke, U S Patent 4,732,686, 1988

5 W Hu, H Tao, M Tominaga, A Miyazaki and H Haraguchi, Analytica Chimica Acta, (**299**), 249 – 256, 1994

6 T Umemura, S Kamiya, A Itoh, K Chiba and H Haraguchi, Analytica Chimica Acta, (**349**), 231 – 238, 1997

7 W Hu, K Hasabe, D Reynolds and H Haraguchi, Analytica Chimica Acta, (**353**), 143 – 149, 1997

8 T Umemura, K Tsunoda, A Koide, T Oshima, N Watanabe, K Chibe and H Haraguchi, Analytica Chimica Acta, (**419**), 87 – 92, 2000

9 Ion Chromatography, by Paul R Haddad and Peter E Jackson Journal of Chromatography Library – volume 46 Published by Elsevier

10 W Hu, H Tao and H Haraguchi, Analytical Chemistry, (**66**), 2514 – 2520, 1994

11 W Hu and H Haraguchi, Journal of Chromatography A, (**723**), 251 – 258, 1996

12 T Umemura, S Kamiya, R Kitaguchi and H Haraguchi, Chemistry Letters, 755 – 756, 1997

13 T Umemura, R Kitaguchi and H Haraguchi, Analytical Chemistry, (**70**), 936 – 942, 1998

14 K Hasabe, T Sakuraba and W Hu, J Liq Chrom & Rel Technol , (**22**), 4, 561 – 569, 1999

15 T Umemura, S Kamiya and H Haraguchi, Analytica Chimica Acta, (**379**), 23 – 32, 1999

16 W Hu, S Cao, M, Tominaga and A Miyazaki, Analytica Chimica Acta, (**322**), 43 – 47, 1996

17 W Hu, A Miyazaki, H Tao, A Itoh, T Umemura and H Haraguchi, Analytical Chemistry, (**67**), 3713 – 3716, 1995

18 W Hu, K Hasabe, D Reynolds, T Umemura, S Kamiya, A Itoh and H Haraguchi, J Liq Chrom & Rel Technol , (**20**), 12, 1903 – 1919, 1997

19 W Hu, P Haddad, K Hasabe and K Tanaka, Analytical Communications, (**36**), 97 – 100, 1999

20 W Hu and P Haddad, Analytical Communications, (**35**), 317 – 320, 1998

21 W Hu, K Hasabe, K Tanaka and P Haddad, Journal of Chromatography A, (**850**), 161 – 166, 1999

22 W Hu, Langmuir, (**15**), 7168 – 7171, 1999

23 W Hu, P Haddad, K Hasabe, K Tanaka, P Tong and C Khoo, Analytical Chemistry, (**71**), 1617 – 1620, 1999

24 W Hu, P Haddad, K Hasabe and K Tanaka, Analytical Communications, (**36**), 309 – 312, 1999

25 W Hu, K Tanaka, P Haddad and K Hasabe, Journal of Chromatography A, (**884**), 161 – 165, 2000

26 W Hu, P Haddad, K Hasabe, H Cook and J Fritz, Fresenius J Anal Chem, (**367**), 641 – 644, 2000

27 W Hu, P Haddad, H Cook, H Yamamoto, K Hasebe, K Tanaka and J Fritz, Journal of Chromatography A, (**920**), 95 – 100, 2001

CHAPTER THREE

SEPARATION OF INORGANIC ANIONS USING ELECTROSTATIC ION CHROMATOGRAPHY

Electrostatic ion chromatography has been under investigation as an analytical technique for the separation and analysis of inorganic anions and cations in a variety of sample matrices Many different approaches have been developed to eliminate ion redistribution and hence improve separation efficiency One approach incorporated the addition of low amounts of an electrolyte into the eluent However, EIC was first developed as a branch of WMP – IC, due to the simplicity of using pure water as an eluent As it was felt that the full potential of utilising water as an eluent had not been fully investigated, it was decided to avoid electrolytic eluents and further investigate the simpler situation of pure water as the eluent

It was also decided to concentrate solely on the separation of anions, particularly those found in environmental samples such as natural waters Zwittergent 3-14 was the zwitterionic reagent chosen to modify the column, as it is suited to the separation of anions and was readily available The phenomenon of ion redistribution was investigated and in doing so, led to the investigation of a simple off-line cation exchange method for the separation of inorganic ions This method employed a cation exchange resin in the sodium form, to quantitatively exchange the various mono- and divalent cations present in the samples to sodium forms The beneficial effect this had upon peak retention, resolution and efficiency will be described and explained

3.1 Apparatus and Instrumentation

A Dionex DX-100 Chromatograph (Dionex (U K) Ltd , Surrey, England) provided the pump and the conductivity detector used throughout This system was also equipped with a thermal stabiliser module (model TS-2) to control variations in temperature that

would otherwise affect the conductivity, and a high-pressure pulse damper that stabilised the conductivity baseline Nitrogen gas was used to keep the eluent reservoir under pressure and the also to operate the Rheodyne injection valve

The separating column was a Waters Spherisorb ODS 2, 5µm analytical column (250 mm x 4 6mm, Supelco Inc , Sigma-Aldrich Ltd , Airton Road, Tallaght, Dublin 24) coated with Zwittergent 3-14 surfactant A separate pump (Gilson, pump model 302 and manometric module, model 802c) was used to clean and coat the column The detector was connected to a PC via an ADC–16 high-resolution data logger (Pico Technology Ltd , Cambridgeshire, England) Integration and retention time analysis was carried out using Microsoft Excel and Origin software packages The cation exchange cartridges were Supelclean LC-SCX 1 mL tubes (Supelco Inc , Sigma-Aldrich Ltd , Airton Road, Tallaght, Dublin 24)

All chemicals used (analytes, solvents and Zwittergent 3-14) were analytical reagent grade and were manufactured by Fluka, Aldrich or Riedel-de Haen (supplied by Sigma-Aldrich, Airton Road, Tallaght, Dublin 24) All standard solutions were made up using pure water, prepared by a Milli-Q deionisation system, (Millipore (U K) Ltd , Walford, England) The pure water that was used as the eluent was also obtained using this system

3.2 Retention Data and Elution Order

Before any injections could be carried out, the column had to be prepared The coating technique employed was as outlined in the first paper published on electrostatic ion chromatography [1] and was quite simple A 30 mM solution of Zwittergent 3-14 was

passed through a clean reversed – phase column at 0 7 ml/mın, for 75 mın This was followed by rınsıng the column through wıth pure water for at least 40 mınutes, also at 0 7 ml/mın, to remove any excess surfactant The column was then left to equılıbrate usıng pure water as the mobıle phase Accordıng to Umemura *et al* [2], the amount of surfactant adsorbed onto the column durıng coatıng ıs the same regardless of the concentratıon of the surfactant supplıed That ıs, ıf a hıghly concentrated solutıon of Zwıttergent 3-14 was passed through the column for a short tıme or a solutıon of low concentratıon passed through for a long tıme, the total amount adsorbed would not change, once the column had become saturated

Several common ınorganıc anıons wıth varyıng counter catıons were ınjected onto the column, to determıne theır retentıon tımes and the elutıon order As all the components (both catıons and anıons) of an ınjected sample elute together ın dıfferent ıon – paırs ın EIC, there are no unretaıned elements and therefore no solvent or water dıp when usıng a water eluent to help calculate the 'dead volume' of the system Therefore ın order to calculate the capacıty factors of the anıons $(k' = (t_R - t_o) / t_o)$, ıt was decıded to use the retentıon tıme of sodıum thıosulfate as the 'dead tıme', as ıt was the least retaıned of all the analytes ınvestıgated Table 3 1 outlınes the ınıtıal results found

Analyte	Retention Time (min)	Capacity factor (k')
$Na_2S_2O_3$	2 27	0
Na_2SO_4	2 42	0 07
$FeSO_4$	2 53	0 11
$CuSO_4$	2 54	0 12
KCl	2 7	0 19
NaCl	2 71	0 19
$CdCl_2$	2 75	0 21
$BaCl_2$	2 77	0 22
$MnCl_2$	2 78	0 22
$PbCl_2$	2 79	0 23
KBr	2 8	0 23
$NaNO_2$	3 03	0 33
KNO_2	3 02	0 33
$NaNO_3$	3 38	0 49
$Na(CH_3COO)$	3 39	0 49
$Pb(CH_3COO)_2$	4 07	0 79
$KHCO_3$	6 45	1 84
$Pb(NO_3)_2$	8 32	2 67

Table 3 1 Retention times of several inorganic anions with varying countercations, in EIC Aqueous solutions at 1 mM each Pure water eluent, flow rate 0 7 ml/min conductivity detection

The elution order of the anions, when paired with a monovalent cation, was as follows

$$S_2O_3^{2} < SO_4^{2} < Cl < Br < NO_2 < NO_3 < CH_3COO < HCO_3$$

This elution order is the same as that quoted in the literature review The pattern of the retention times was on average as expected As explained in Chapter Two, anions with

equivalent counter cations show similar retention times while anions with counter cations of differing valencies show different retention times Following this trend, KCl and NaCl showed similar retention times, as did $PbCl_2$, $MnCl_2$, $CdCl_2$ and $BaCl_2$, while there was an increase in retention time between the two groups This trend was also evident for sulphate, nitrite, nitrate and acetate, with an increase in retention time between the monovalent and divalent counter cations

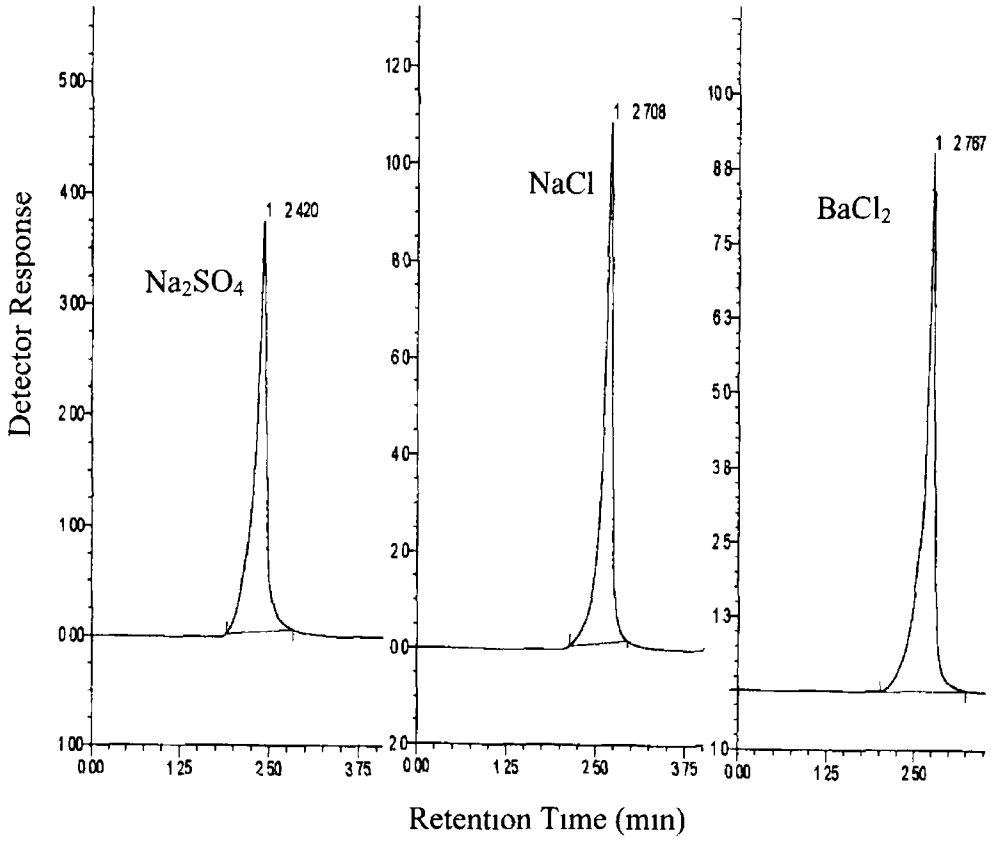


Figure 3 1 Chromatograms of sodium sulphate, sodium chloride and barium chloride using EIC Aqueous 1 mM solutions pure water eluent, flow rate 0 7 ml/min Conductivity detection

Generally when using a pure water eluent peak shapes were found to be rather poor, with many of the eluted peaks being over 1 minute wide As shown in Figure 3 1, peak fronting was also observed in many cases It was decided to investigate the addition of a small concentration of Zwittergent 3-14 to the mobile phase to see how this would affect peak shape and also retention times Eluents of 2 mM and 5 mM Zwittergent 3-14 were made up in Milli-Q water and once each had been allowed to equilibrate through the column, the same analytes were again injected onto the column Varying concentrations of sodium nitrate were injected to see if this affected the peak shape, but as can be seen from Figure 3 2, this wasn't the case Even at the lower concentrations, the peak shape was poor The retention times and capacity factors of the different anions can be seen in Table 3 2 The capacity factors were again calculated using the retention time of sodium thiosulphate, with pure water as the eluent, as the 'dead time'

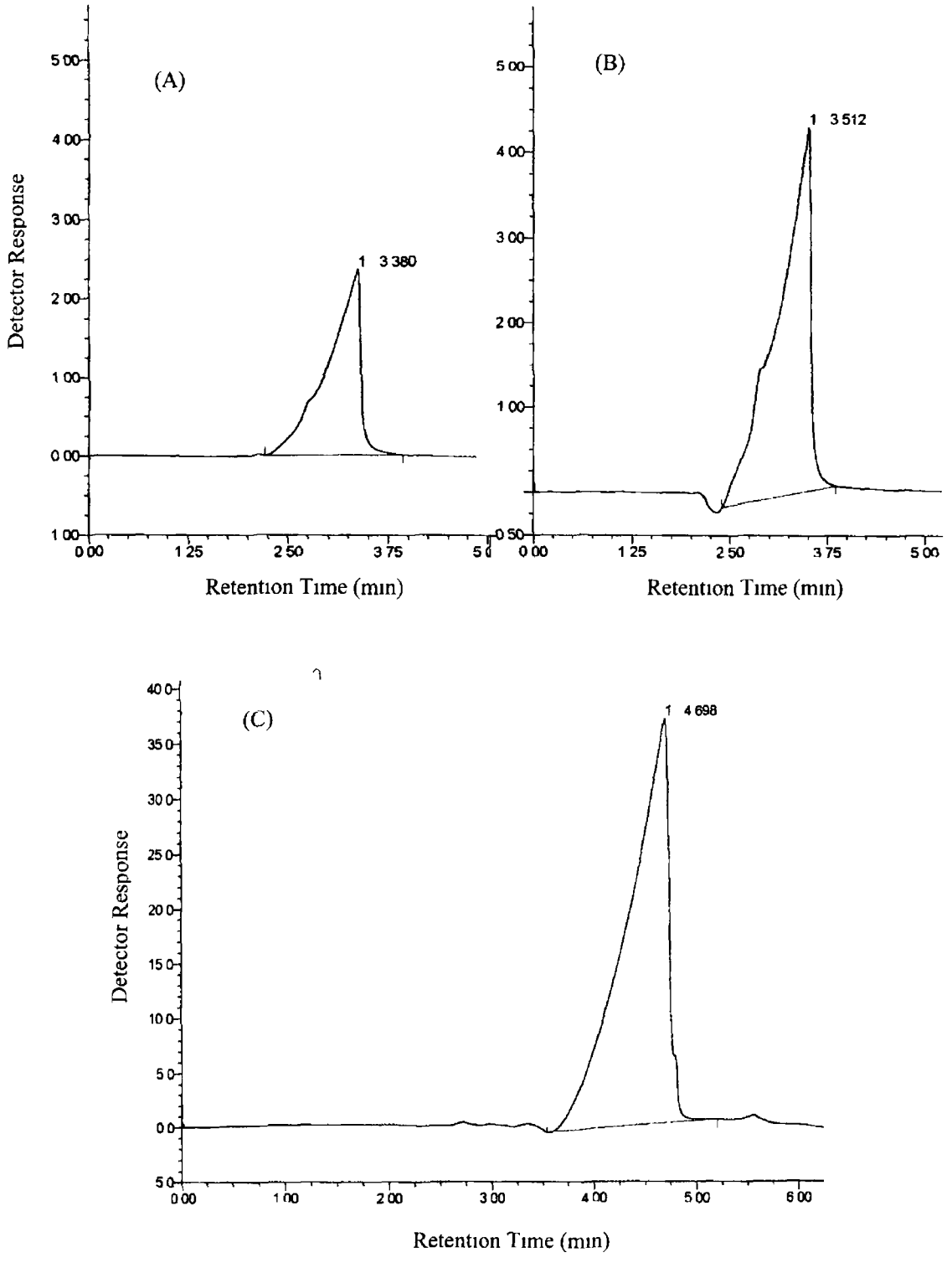


Figure 3.2 Chromatograms of sodium nitrate with varying amounts of Zwittergent 3-14 in the eluent. Aqueous solutions, flow rate 0.7 ml/min, conductivity detection. (A) 10 ppm sodium nitrate, pure water eluent. (B) 20 ppm sodium nitrate, 2 mM Zwittergent 3-14 as the eluent. (C) 100 ppm sodium nitrate, 5 mM Zwittergent 3-14 as the eluent.

Analyte	Concentration of Zwittergent 3-14 in Mobile phase					
	0 mM		2 mM		5 mM	
	Rt (min)	*k'*	*Rt* (min)	*k'*	*Rt* (min)	*k'*
$Na_2S_2O_3$	2 27	0	2 3	0 01	2 89	0 27
Na_2SO_4	2 42	0 07	2 4	0 06	2 9	0 28
$FeSO_4$	2 53	0 11	2 48	0 09	2 96	0 3
$CuSO_4$	2 54	0 12	2 48	0 09	2 95	0 3
KCl	2 7	0 19	2 67	0 18	3 32	0 46
NaCl	2 71	0 19	2 71	0 19	3 36	0 48
$CdCl_2$	2 75	0 21	2 79	0 23	3 46	0 52
$BaCl_2$	2 77	0 22	2 8	0 23	3 45	0 52
$MnCl_2$	2 78	0 22	2 8	0 23	3 46	0 52
$PbCl_2$	2 79	0 23	2 81	0 24	3 47	0 53
KBr	2 8	0 23	2 95	0 30	3 98	0 75
$NaNO_2$	3 03	0 33	3 1	0 37	4 2	0 85
KNO_2	3 02	0 33	3 09	0 36	4 21	0 85
$NaNO_3$	3 38	0 49	3 51	0 55	4 7	1 07
$KHCO_3$	6 45	1 84	6 4	1 82	6 5	1 86
$Pb(NO_3)_2$	8 32	2 67	8 4	2 70	9 1	3 01

Table 3 2 Retention times of anions with varying concentrations of Zwittergent 3-14 in the eluent

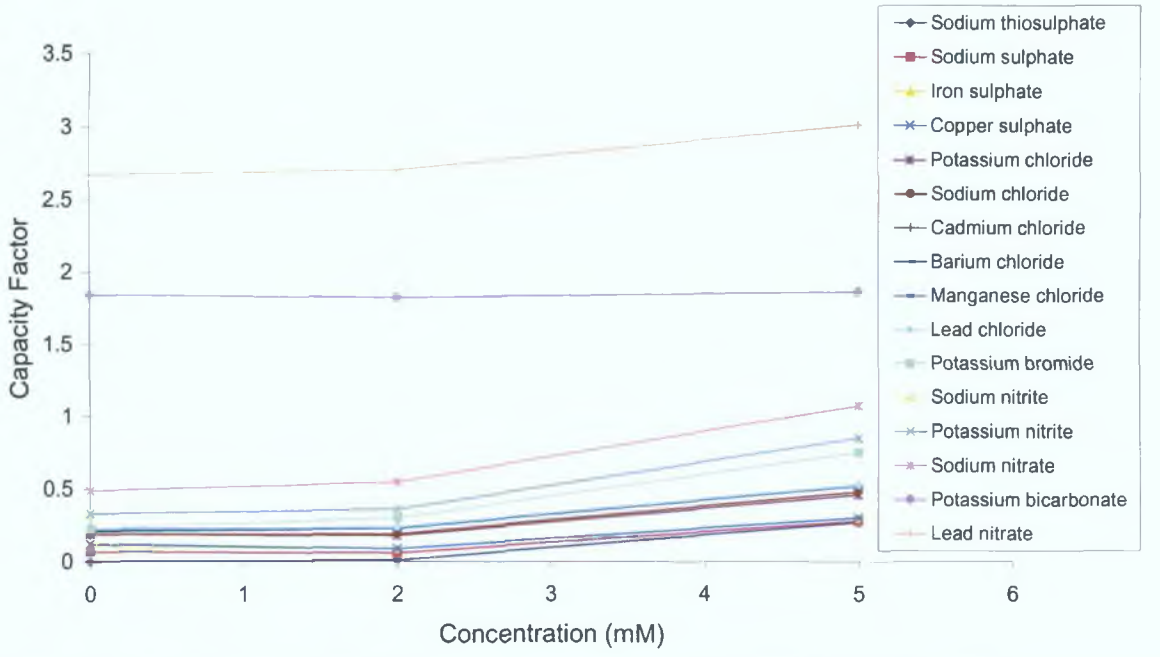


Figure 3.3. Plot showing the relationship between the concentration of Zwittergent 3-14 and the capacity factor, for the different analytes tested.

Increasing the concentration of the surfactant in the eluent from 0 to 2 mM seemed to only marginally affect retention of the test group of analytes. The greatest increase noted was for KBr, whose retention time increased from 2.8 minutes to 2.95 minutes. Peak shape did not noticeably change, with peaks still remaining broad. Upon increase to 5 mM Zwittergent 3-14, a further and more significant increase in the retention times was noted. The retention time of KBr, for example, had now increased to 3.98 minutes. There was no evidence to suggest that peak shapes were affected by addition of the Zwittergent 3-14 to the eluent, as they remained broad, with a baseline width of over one minute in most cases, and occasional peak fronting or peak tailing also evident.

In conventional IC, it is occasionally possible to improve peak shapes of less hydrophilic anions by the addition of organic modifiers such as acetonitrile or methanol. This is not possible in EIC when using pre-coated zwitterionic columns, as the addition of

these substances to the eluent would lead to the increased 'bleeding' of the zwitterionic coating from the column. Therefore, it was not possible to manipulate peak shapes here using organic solvents.

Even though the addition of Zwittergent 3-14 to the eluent increased retention times, as it did not seem to improve peak shapes, it was decided at this stage of the investigation to return to using pure water, to improve selectivity.

3.3 Initial Separations Using Water Eluents

Keeping in mind that a key application of EIC would be in the analysis of water samples, and that some of the main inorganic anions of interest to be found in water are sulphate, chloride and nitrate, it was decided to try to separate a mixture of these three anions (Figure 3.4).

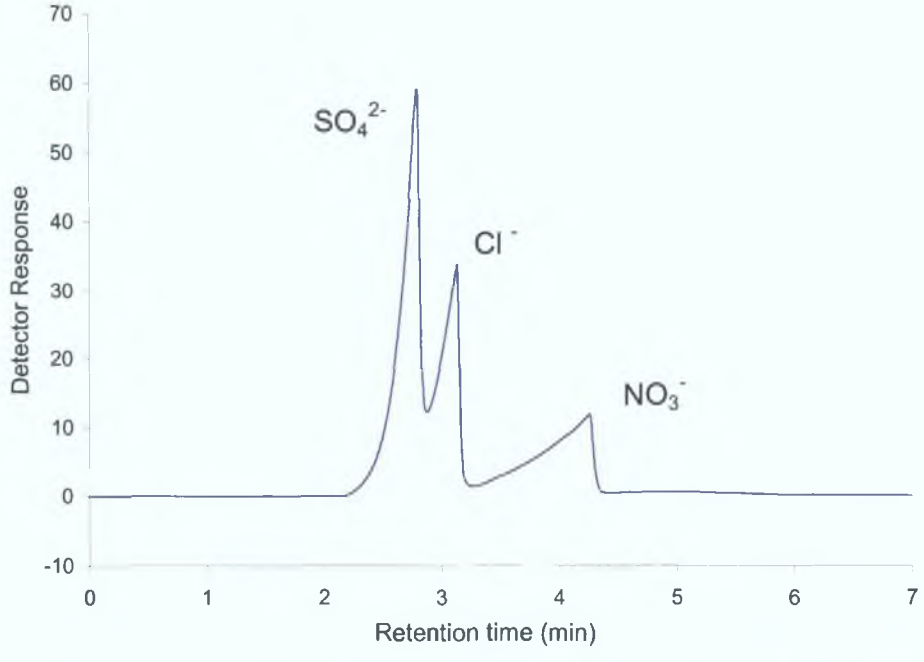


Figure 3.4. Chromatogram showing the separation of sulphate, chloride and nitrate using EIC. Aqueous solution containing all anions as their sodium salts at 1 mM. Pure water eluent, flow rate 0.7 ml/min. Conductivity detection.

As can be seen from Figure 3.4, sulphate and chloride were not baseline separated from each other but still showed adequate resolution for identification purposes. While nitrate was adequately resolved from chloride, its peak shape was poor, with significant peak fronting. Figure 3.5 demonstrates the separation achieved upon injection of a mixture of nitrite and nitrate onto the column.

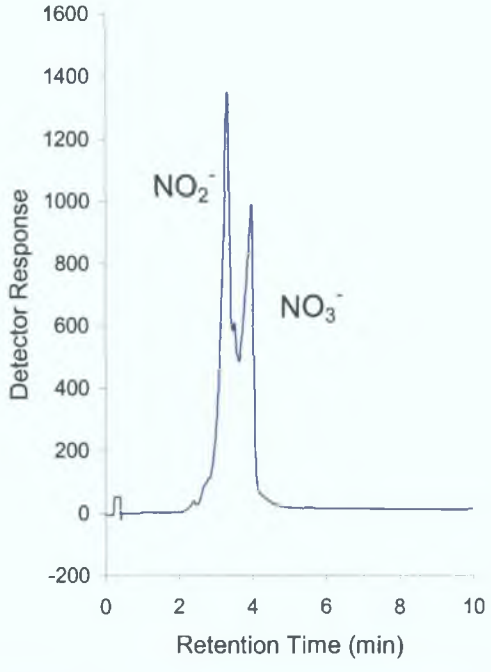


Figure 3.5. Chromatogram showing the separation of nitrite and nitrate with EIC. Aqueous solution containing both anions as their sodium salts at 1 mM. Pure water eluent, flow rate 0.7 ml/min. Conductivity detection.

Again, although the peaks were sufficiently resolved for identification purposes, they were not baseline separated. A mixture of seven different anions was injected onto the column. Figure 3.6 shows the resulting separations and the differing detector response for each anion. As can be seen, the early eluting peaks were not baseline separated, although good resolution was achieved for the highly polarizable anions, chlorate, iodide and thiocyanate, which exhibited the longer retention times.

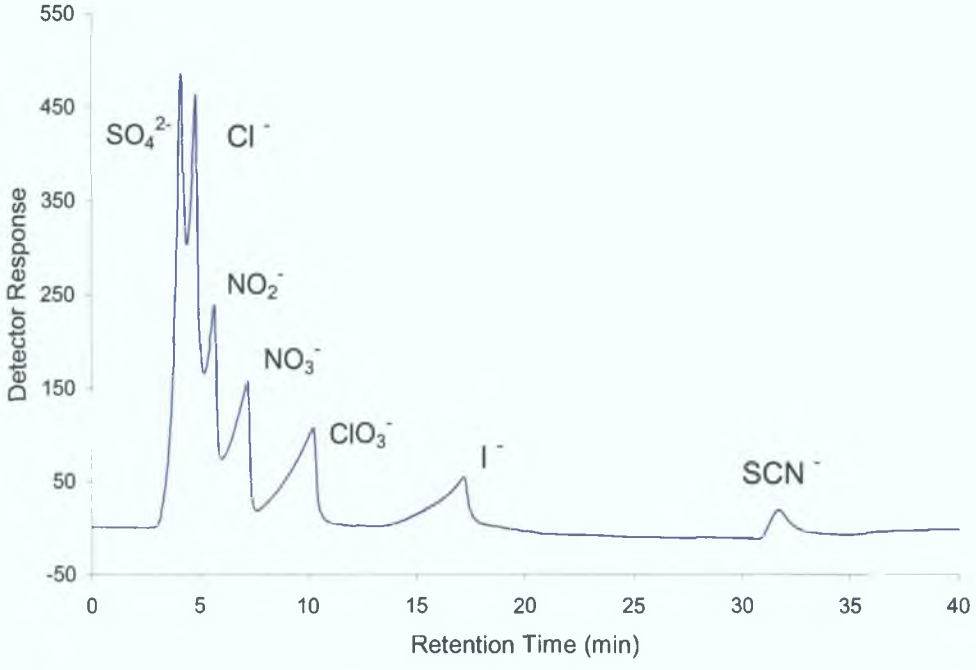


Figure 3.6. Chromatogram showing the separation of seven common inorganic anions using EIC. Aqueous solution containing all anions as their sodium salts at 0.1 mM. Pure water eluent, flow rate 0.7 ml/min. Conductivity detection.

To investigate the selectivity of the system with a real sample, a tap water sample was injected onto the column. The resulting peaks were identified by sequentially spiking the sample with sulphate, chloride, nitrate, nitrite, fluoride and hydrogen carbonate. Qualitatively, it was found that the tap water sample contained sulphate, chloride, nitrate and hydrogen carbonate. It was found that as the resultant spiked peak for fluoride co-eluted with the sulphate peak, and the nitrite peak eluted between the chloride and nitrate peaks, it could not be determined whether or not they were present in the sample analysed. They could have been present at low levels and not detectable due to inadequate resolution. Figure 3.7 shows the chromatogram achieved upon injection of the tap water sample.

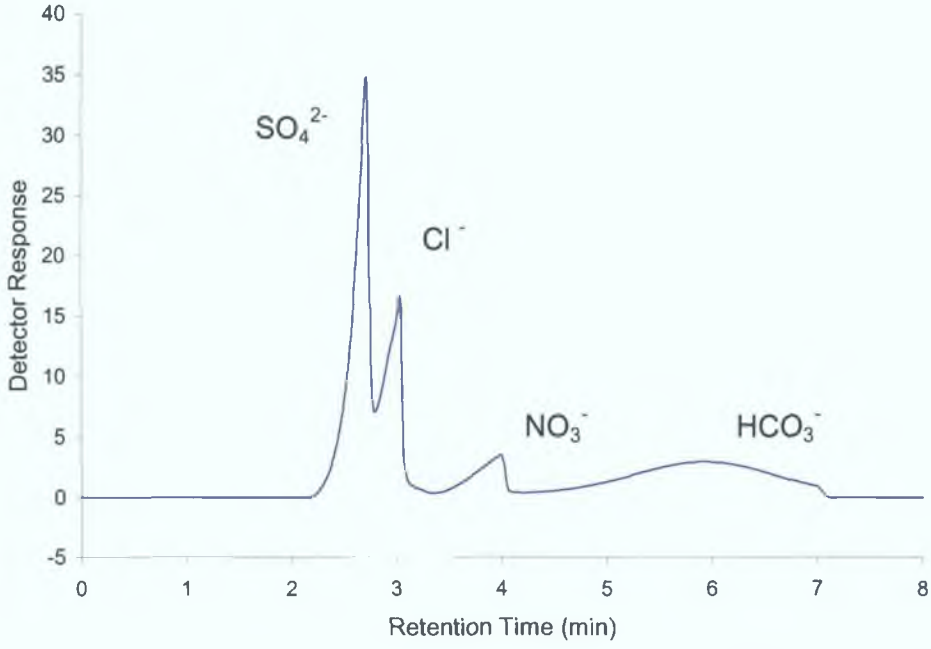


Figure 3.7. Chromatogram showing a tap water sample, analysed using EIC. Pure water eluent, flow rate 0.7 ml/min. Conductivity detection.

As can be seen from the chromatogram shown in Figure 3.7 the main anionic components of the sample are clearly identified, although the peak shape for carbonate was particularly broad. In order to determine the linearity of the method, standards of varying concentrations (0.2 mM to 1 mM) were made up for the anions sulphate, chloride and nitrate, as their sodium salts. Calibration curves were then prepared and linearity proved to be satisfactory with R^2 values greater than 0.98 for all three curves (Figure 3.8 – 3.10). However, it was not possible to quantitatively analyse the water sample for its anion content, since it was not known which counter-cations were present in the sample. The peak due to sulphate for example could have been due to sodium sulphate, calcium sulphate, magnesium sulphate, etc. Since the peak size when using conductimetric detection is related to the sum of the ionic conductances of both the anion and the cation components, different cations would produce different size peaks. Since quantitative

analysis was based on the size of the peak, it was not possible as both components of the peak were not known

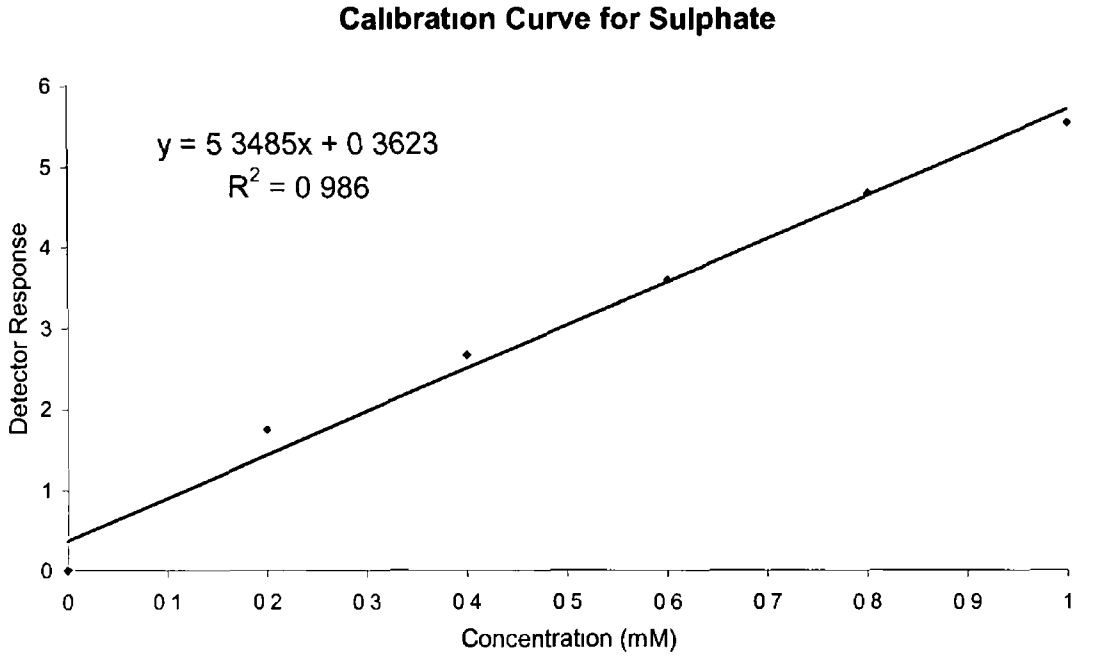


Figure 3 8 Calibration curve for sulphate Pure water eluent, flow rate 0 7 ml/min Conductivity detection

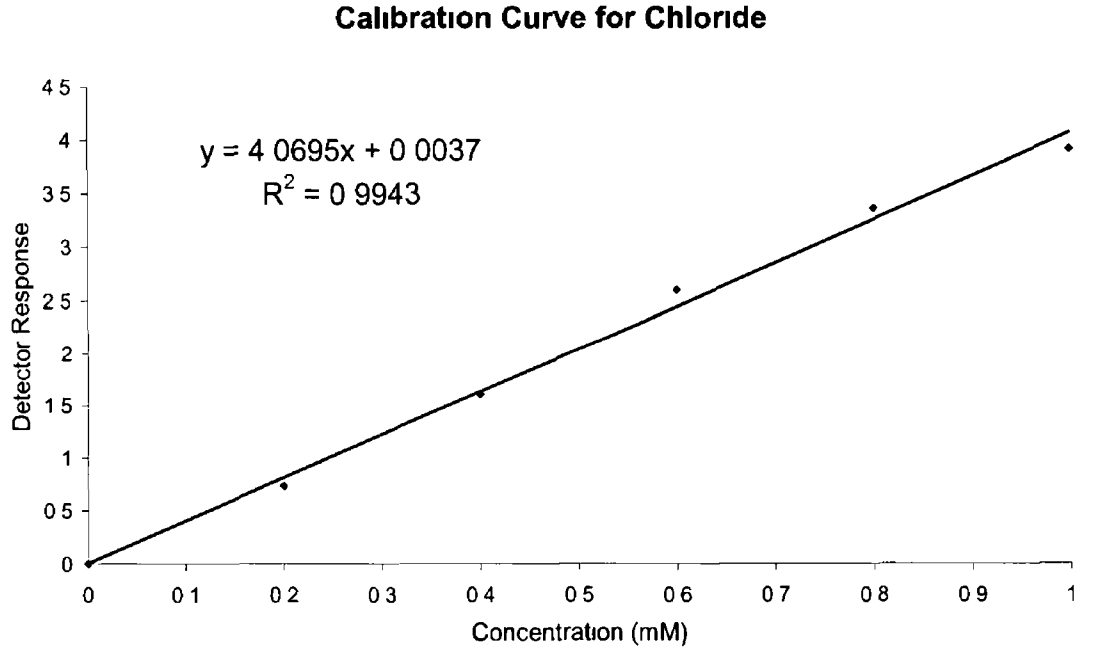


Figure 3 9 Calibration curve for chloride Pure water eluent flow rate 0 7 ml/min Conductivity detection

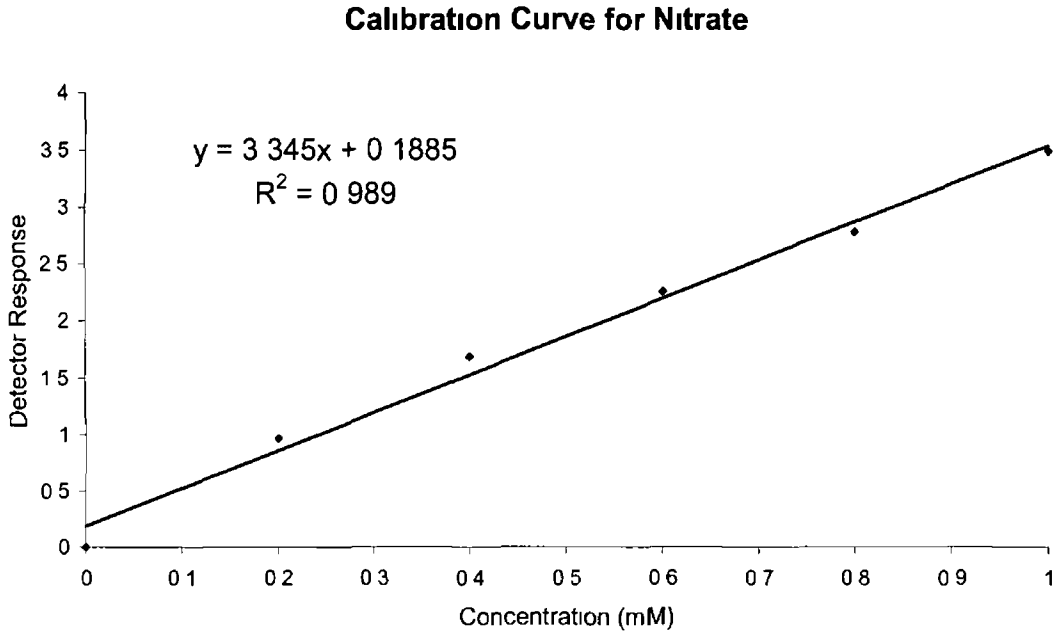


Figure 3 10 Calibration curve for nitrate Pure water eluent, flow rate 0 7 ml/min Conductivity detection

3.4 Two Step Modification of the Column

Zwittergent 3-14 is a linear surfactant with a 14 C hydrocarbon chain Therefore an ODS C_{18} column, when coated with Zwittergent 3-14, still exhibits some reversed-phase activity [3] This helps to explain the longer retention times of less hydrophilic polarizable anions such as iodide and thiocyanate CHAPS on the other hand, is a steroidal surfactant and does not lead to reverse-phase activity It has been shown by Hu *et al* [3] that the retention times of these anions are shorter with a CHAPS modified column than with a Zwittergent 3-14 modified column It was decided to investigate the possibility of reducing the retention times of the polarizable anions while maintaining the resolution of the faster eluting anions by coating the column with a mixture of both reagents The column was first coated with a 30 mM solution of CHAPS, before coating with a 30 mM solution of Zwittergent 3-14, at 0 7 ml/min for 75 minutes, a procedure described previously in the above study by Hu *et al* [3]

The same standard mixtures were again injected onto the column. The following chromatograms outline the results achieved using this two step modification process. As can be seen in Figure 3.11, the retention times of iodide and thiocyanate are indeed noticeably shorter, although this was the case for the earlier eluting anions also. Retention times have decreased from 17.28 minutes to 11 minutes for iodide and from 31.5 minutes to just under 20 minutes for thiocyanate.

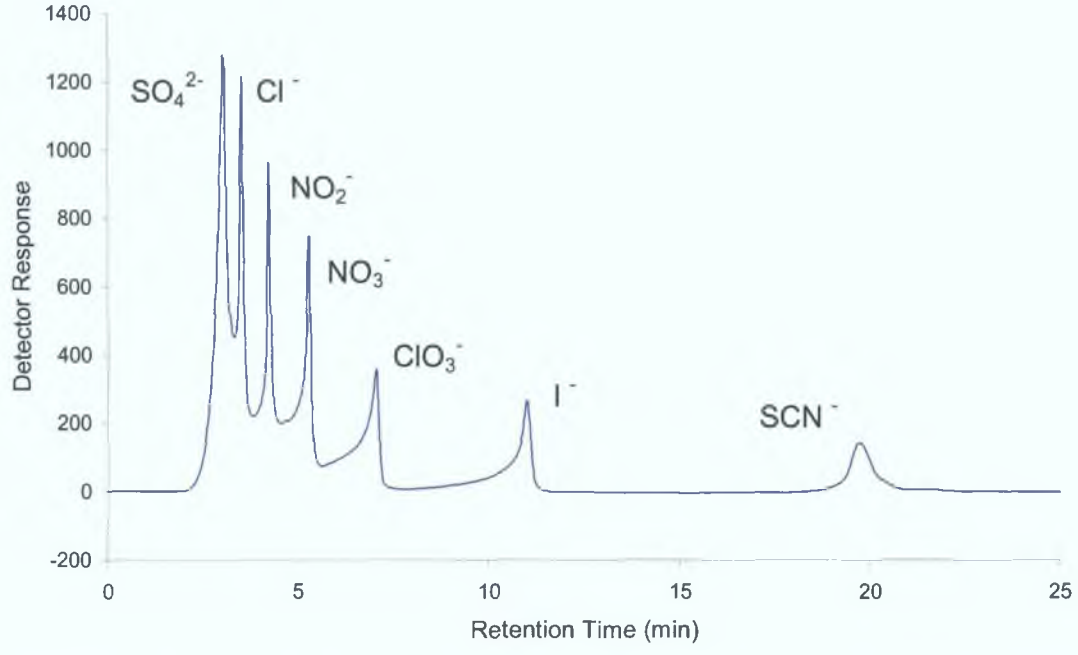


Figure 3.11. Chromatogram showing the separation of seven common inorganic anions using both Zwittergent 3-14 and CHAPS in the coating technique. Aqueous solution containing all anions as their sodium salts at 0.4 mM. Pure water eluent, flow rate 0.7 ml/min. Conductivity detection.

Capacity factors were calculated for each of the injected anions, to compare the two coating methods. The retention time of sodium thiosulphate (2.27 minutes) was once again used to calculate the dead time of the system.

Analyte	Zwittergent 3-14		Zwittergent 3-14 + CHAPS	
	Retention Time (min)	*k'*	Retention Time (min)	*k'*
Na_2SO_4	4 17	0 84	3 03	0 33
NaCl	4 83	1 13	3 52	0 55
$NaNO_2$	5 70	1 51	4 23	0 86
$NaNO_3$	7 23	2 19	5 30	1 33
$NaClO_3$	10 30	3 54	7 08	2 12
NaI	17 28	6 61	11 03	3 86
NaSCN	31 85	13 03	19 80	7 72

Table3 3 Comparison of capacity factors between the two coating methods

As can be seen from the above table, the capacity factors were significantly lower when the column was coated with CHAPS and Zwittergent 3-14 However, an improvement in the separation between nitrate and nitrite was noted (Figure 3 12) principally due to an improvement in peak shape for these two anions

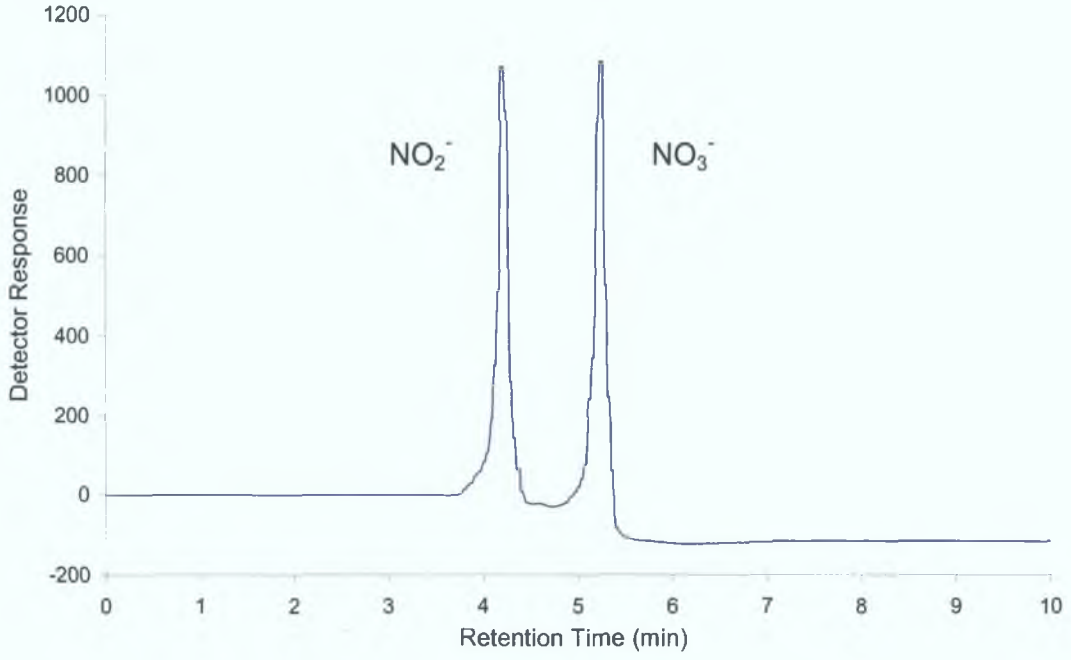


Figure 3.12. Chromatogram showing the separation of nitrite and nitrate using both Zwittergent 3-14 and CHAPS in the coating technique. Aqueous solution containing both anions as their sodium salts at 1 mM. Pure water eluent, flow rate 0.7 ml/min. Conductivity detection.

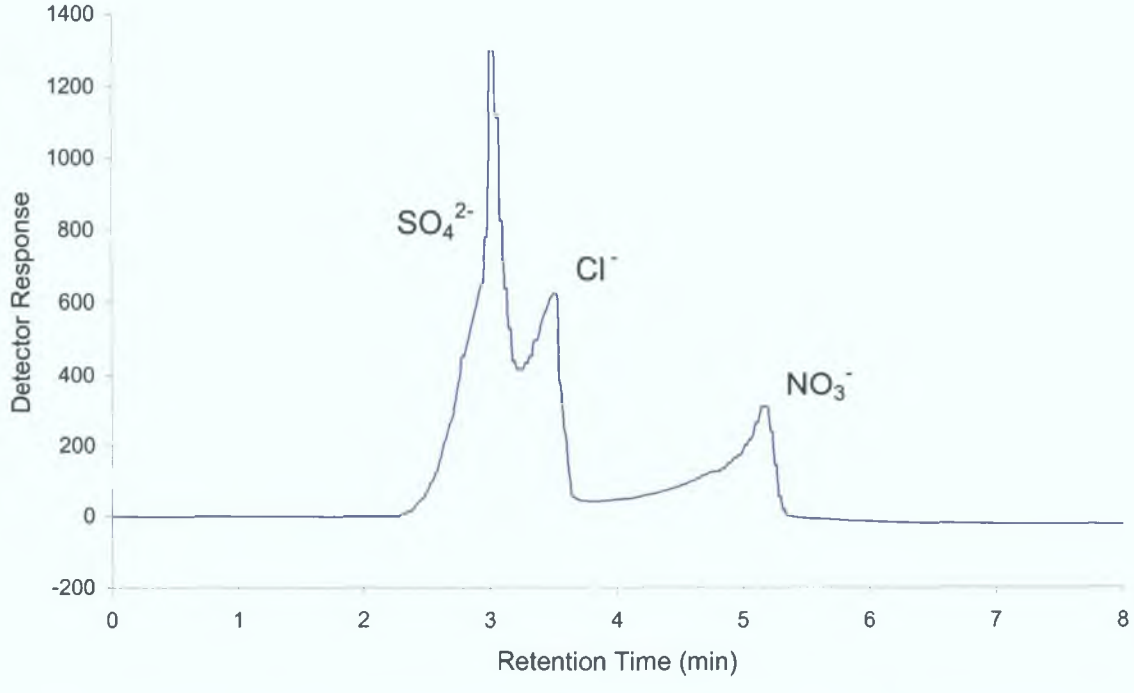


Figure 3.13. Chromatogram showing the separation of sulphate, chloride and nitrate using both Zwittergent 3-14 and CHAPS in the coating technique. Aqueous solution containing all anions as their sodium salts at 1 mM. Pure water eluent, flow rate 0.7 ml/min. Conductivity detection.

When the mixture of sodium sulphate, chloride and nitrate was again injected onto the column (Figure 3 13), a decrease in resolution was noticed when compared to that achieved when using just Zwittergent 3-14 as the coating agent Peak shape had also deteriorated, especially for sulphate Therefore, although the two step modification method showed a decrease in retention times for iodide and thiocyanate, and increased resolution between nitrite and nitrate, it resulted in a decrease in the resolution between chloride and sulphate Since they are two of the main components of natural water samples, it was decided to return to the original coating solution of just Zwittergent 3-14 In addition, it was found that the less hydrophobic CHAPS formed a less stable coating on the ODS column which meant the column coating needed constant regeneration, making the system both less practical and reducing long term precision

3.5 Ion Redistribution

To investigate the phenomenon of ion redistribution, the separation of a mixture of cations associated with a single counter-anion was investigated Nitrate was chosen as the counter-anion with sodium, lead and cerium chosen as the target cations These three cations each have a different valency, and so when all are associated with the same anion, peaks for each cation should be seen As can be seen from Figure 3 14, with retention times of 9 3 and 8 7 minutes respectively, cerium and lead nitrate were well resolved from sodium nitrate, which showed a retention time of 5 6 minutes The peak shape of sodium nitrate was superior to those of both lead nitrate and cerium nitrate, which were rather poor and not completely resolved At this point it is interesting to note how the sample components have a major affect upon analyte peak shapes, as here the nitrate peak has now become clearly tailed, instead of the fronting seen previously

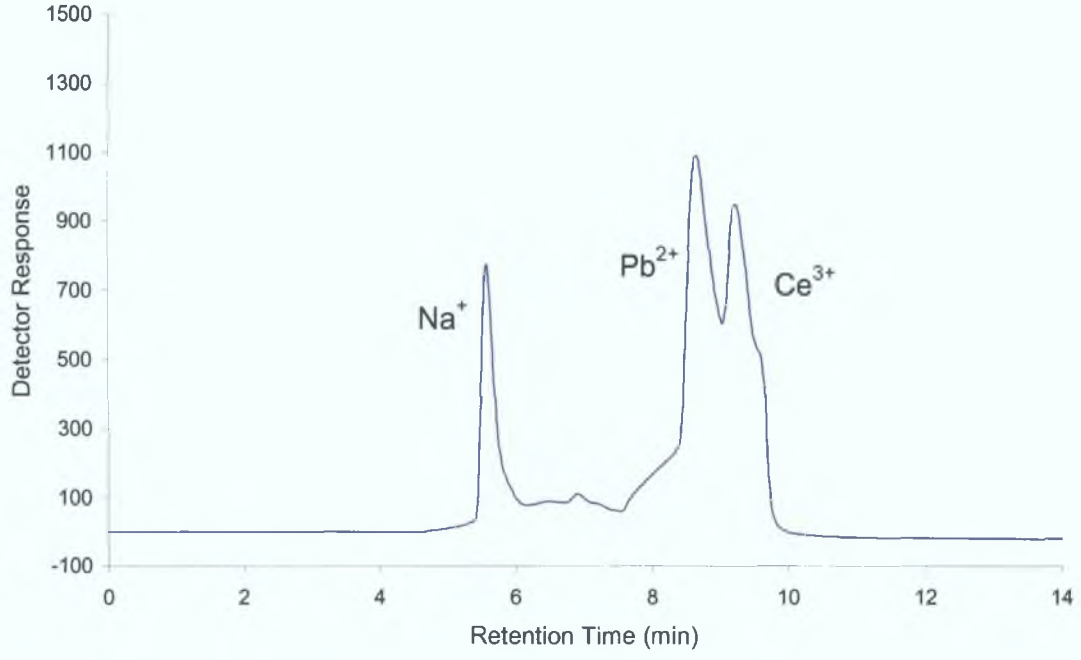


Figure 3.14. Chromatogram showing the separation of $NaNO_3$, $Pb(NO_3)_2$ and $Ce(NO_3)_3$ with EIC. Aqueous solution containing all analytes at 1 mM. Pure water eluent, flow rate 0.7 ml/min. Conductivity detection.

The effect of adding alternative anions to the above standard mixture was then investigated, using chloride and sulphate. Upon addition of sodium sulphate to the above mixture, several observations were made. Firstly, the peaks corresponding to lead nitrate and cerium nitrate were noticeably smaller. Secondly, the peak corresponding to sodium nitrate was larger, and thirdly, the retention time of the sulphate peak was longer than expected for sodium sulphate. When injected separately, sodium sulphate showed a retention time of 3 minutes, while the sulphate peak in the above chromatogram is at 3.5 minutes, suggesting that sodium is not the cation present in the observed peak. Ion redistribution was occurring. It is suggested that the later sulphate peak was due to the co-elution of lead and cerium sulphate. Since some of the lead and cerium was now associated with sulphate, there was a decrease in size of the lead and cerium nitrate peaks. Also, the sodium cations that were associated with the sulphate were now pairing with the excess nitrate, released from the lead and cerium, hence increasing the size of the sodium

nitrate peak. The simple experiment had clearly shown the problem of ion redistribution in action. However, despite the occurrence of ion redistribution, sulphate and nitrate in the test mixture remained baseline separated, peak shape was good and both peaks were well separated from the later eluting nitrate peaks. Figure 3.15 shows the chromatogram obtained from the above experiment.

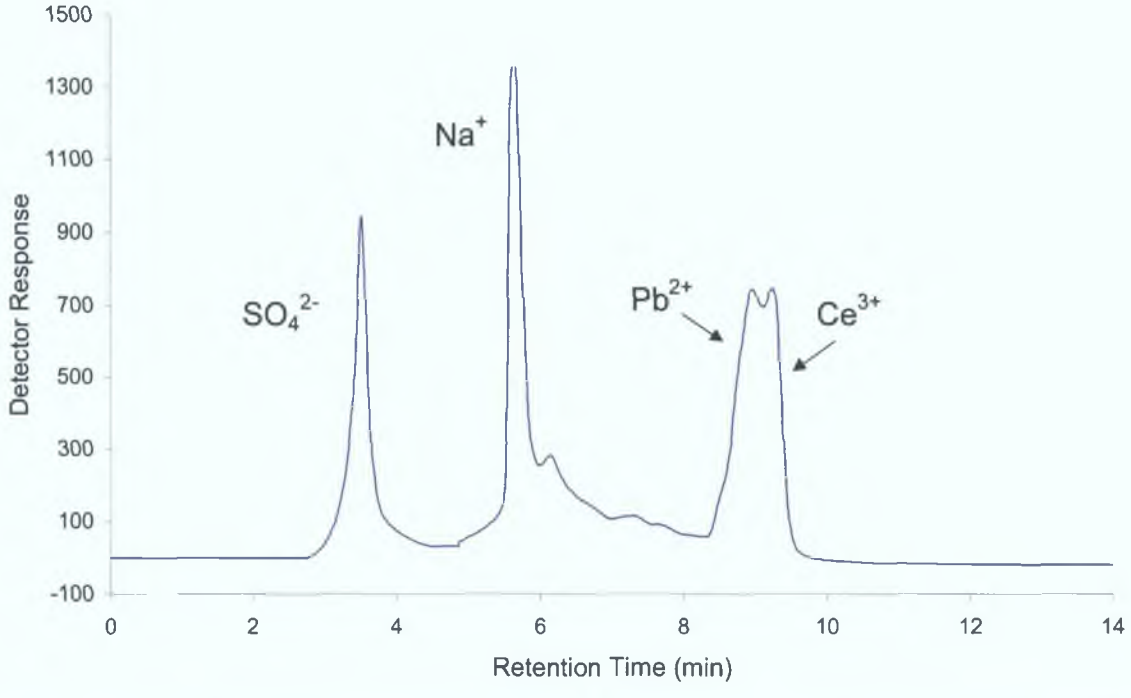


Figure 3.15. Chromatogram showing the separation of $NaSO_4$ from $NaNO_3$, $Pb(NO_3)_2$ and $Ce(NO_3)_3$ with EIC. Aqueous solution containing all analytes at 1 mM. Pure water eluent, flow rate 0.7 ml/min. Conductivity detection.

Upon addition of chloride to the above mixture, several observations were again made. The resultant chloride peak was observed at a retention time longer than expected, 4.1 minutes as opposed to 3.7 minutes when injected separately as sodium chloride. This indicated that the cation with which the chloride was associated was one other then sodium, this being either lead or cerium or a co-elution of both. The sodium nitrate peak had increased in size again, beyond the scale of the detector response. This indicated that

the nitrate liberated from either lead or cerium was again associating itself with the sodium liberated by the introduced sodium chloride.

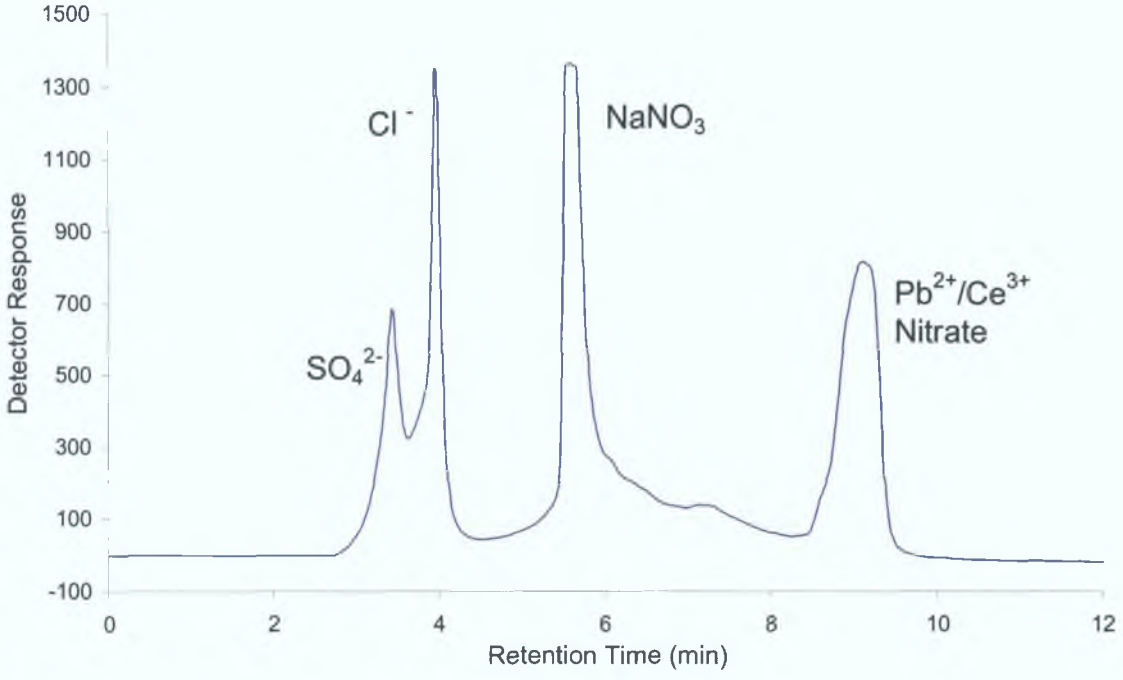


Figure 3.16. Chromatogram showing the separation of NaCl and $NaSO_4$ from $NaNO_3$, $Pb(NO_3)_2$ and $Ce(NO_3)_3$ with EIC. Aqueous solution containing all analytes at 1 mM. Pure water eluent, flow rate 0.7 ml/min. Conductivity detection.

As can be seen from the chromatogram shown in Figure 3.16, lead and cerium were now no longer partially separated. This suggested that one of them had a higher priority of formation with the anions than the other, and was now at too low a concentration level to be identifiable as a separate peak. By examination of the retention time (9.2 minutes), the remaining peak corresponded more closely to that of cerium nitrate, indicating that it was lead that had the higher priority of formation with the introduced anions. In order to test this assumption, a 1 mM solution of sodium sulphate and lead nitrate were injected onto the column. The resulting peaks corresponded solely to lead sulphate and sodium nitrate (Figure 3.17). The observed sodium nitrate peak was much larger as:

$$Na_2SO_4 + Pb(NO_3)_2 \longrightarrow PbSO_4 + 2NaNO_3$$

These trends suggest that sodium has a higher priority to form ion - pairs with nitrate than with sulphate while lead has a higher priority to form ion - pairs with sulphate than with nitrate. Lead nitrate should have appeared at approximately 8.7 minutes, but as can be seen from Figure 3.17, nitrate was only present as its sodium form.

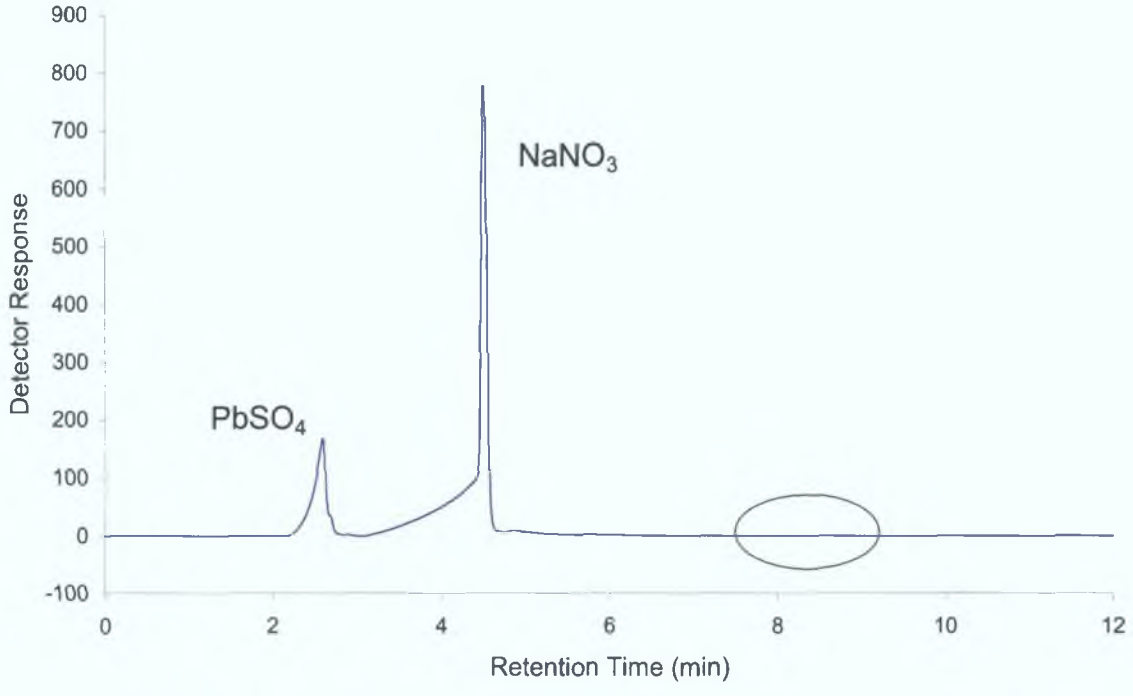


Figure 3.17. Chromatogram showing the ion redistribution of Na_2SO_4 and $Pb(NO_3)_2$ with EIC. Aqueous solution containing all analytes at 1 mM. Pure water eluent, flow rate 0.7 ml/min. Conductivity detection.

The experiments have shown how ion redistribution can prove to be a problem in the analysis of sulphate, chloride and nitrate, if there are different cations present within the sample. As this is the case in a typical water sample such as tap water or mineral water, ion distribution would have to be eliminated before quantitative analysis could be carried out.

3.6 Off Line Cation Exchange

3 6 1 Method Development

There are several ways in which to control or eliminate ion redistribution in EIC These include the use of a cation exchange column placed in-line, prior to the separation column, and also the introduction of a decoy analyte to the sample solution, to forcibly control the redistribution of ions Both of these techniques, while managing to solve the problem of ion redistribution, have their own inherent drawbacks The cation exchange column can increase the retention times of the analytes by over two minutes [4] and therefore increases the analysis time In addition to this, ion-exclusion effects have also been shown, which again can affect analyte retention The introduction of a decoy analyte into the sample means additional time spent preparing complicated standardised solutions and also an increase in the run-time, especially if sodium iodide is used as the decoy analyte, due to the late eluting divalent cation - iodide peak [5] (Figure 2 11) Of course, a third means of eliminating ion redistribution is the addition of small amounts of an electrolyte into the mobile phase However, here it was decided to remain with pure water as the mobile phase as the potential of its separating power had yet to be fully explored

Obviously an ideal solution to the problem of ion redistribution would not produce increased retention times, not involve adding anything to the sample, and should still allow the use of pure water as an eluent In order to achieve this here, a simple offline cation exchange procedure was developed This procedure utilised disposable cation - exchange cartridges in the sodium form to quantitatively exchange the sample cations with sodium and so eliminate the possibility of ion redistribution The conversion was simple, quick and did not result in the loss of any of the sample

The cartridges were in the form of 1 mL syringes, and the sample solutions were simply passed through the cartridge, during injection of the sample onto the analytical column. Therefore, sample preparation was limited and very quick, without the introduction of any extra components to the sample and hence without the need to make up complex standardised solutions. Previous work had shown that peak shape was poor when divalent or trivalent cations were present, and retention times longer. Therefore, sodium was chosen as the exchange cation as it led to shorter retention times, better peak shape and also is commonly the cation present in the highest concentrations in most natural water samples, from tap water to sea water.

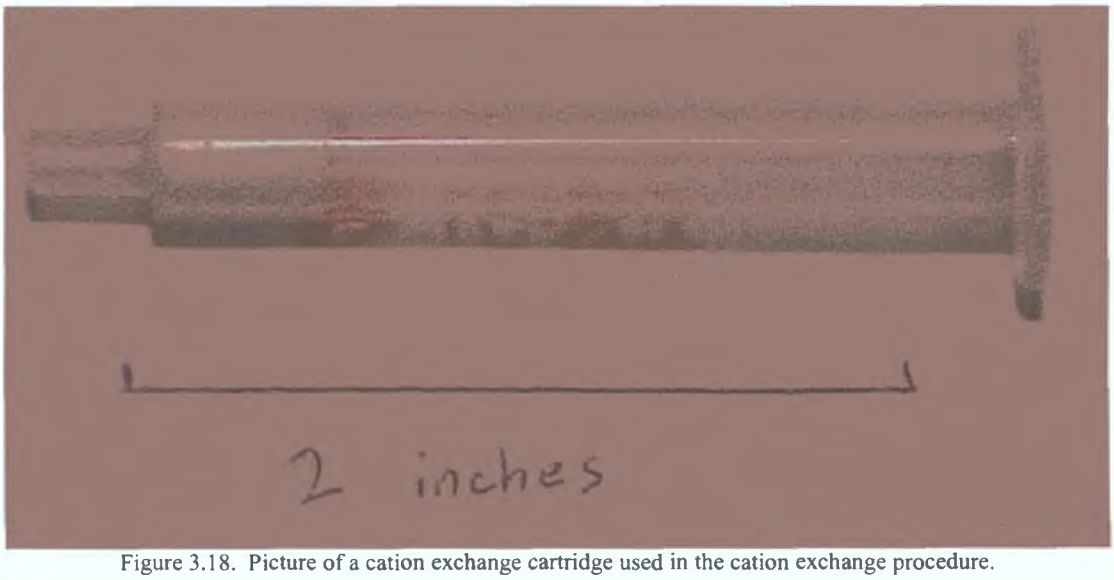


Figure 3.18. Picture of a cation exchange cartridge used in the cation exchange procedure.

The disposable cartridges, if in the acid form, could be converted using a washing step (2 mL) with a suitable solution of a sodium salt, followed by a wash with Milli-Q water (2 mL).

3.6.2 Initial Results

This method was tested with the injection of individual standards, containing the anions sulphate, chloride and nitrate, (prepared form divalent cationic salts) onto the column, via passage through the sodium cation exchange cartridge. When an anion, paired with a divalent cation is subjected to the off-line exchange procedure, the resulting chromatographic peak should correspond to that of the anion - paired with sodium. For example, when a standard of $Ca(NO_3)_2$ was passed through the cation exchange cartridge, the calcium was quantitatively converted to sodium, as can be seen from Figure 3.19.

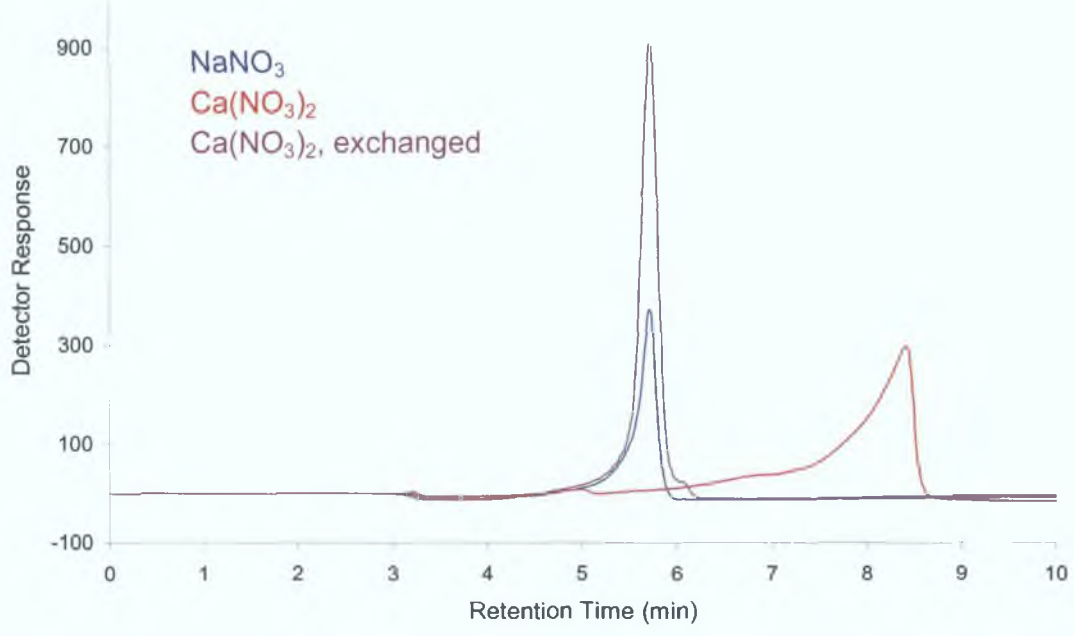


Figure 3.19. Chromatogram showing the conversion of calcium nitrate to sodium nitrate using the cation exchange procedure. Aqueous solutions containing 0.6 mM $NaNO_3$, $Ca(NO_3)_2$ and $Ca(NO_3)_2$ after subjection to the ion exchange procedure. Pure water eluent, flow rate 0.7 ml/min. Conductivity detection.

The calcium nitrate had been completely converted to sodium sulphate, resulting in a shorter retention time and an improvement in peak shape, as the peaks due to sodium proved to be sharper then the peak due to calcium. An increase in peak area was also

observed between the two sodium nitrate peaks, one which represented 0 6 mM $NaNO_3$ and one which represented 0 6 mM $Ca(NO_3)_2$ which has been converted to 1 2 mM $NaNO_3$ Due to the fact that divalent cations associate with two equivalents of a monovalent anion, when a divalent cation is exchanged with sodium, the peak area of the resulting peak should be twice that of an equal concentration of an anion prepared from a monovalent cation containing salt For example, when 1 mM $ZnCl_2$ is converted to NaCl using this ion - exchange procedure, the resulting peak has twice the area that 1 mM of NaCl would have since there is twice the number of moles of NaCl present

$$ZnCl_2 + \text{excess } Na^+ \longrightarrow 2NaCl + Zn^{2+}$$

The results obtained from applying the procedure to a calcium chloride standard can be seen in Figure 3 20 Upon application of the exchange procedure to calcium chloride, the retention time again decreased, and peak shape also improved, as the peak due to sodium was sharper and more symmetrical The twofold increase in peak area compared to a sodium chloride standard was also clearly evident indicating quantitative exchange had taken place

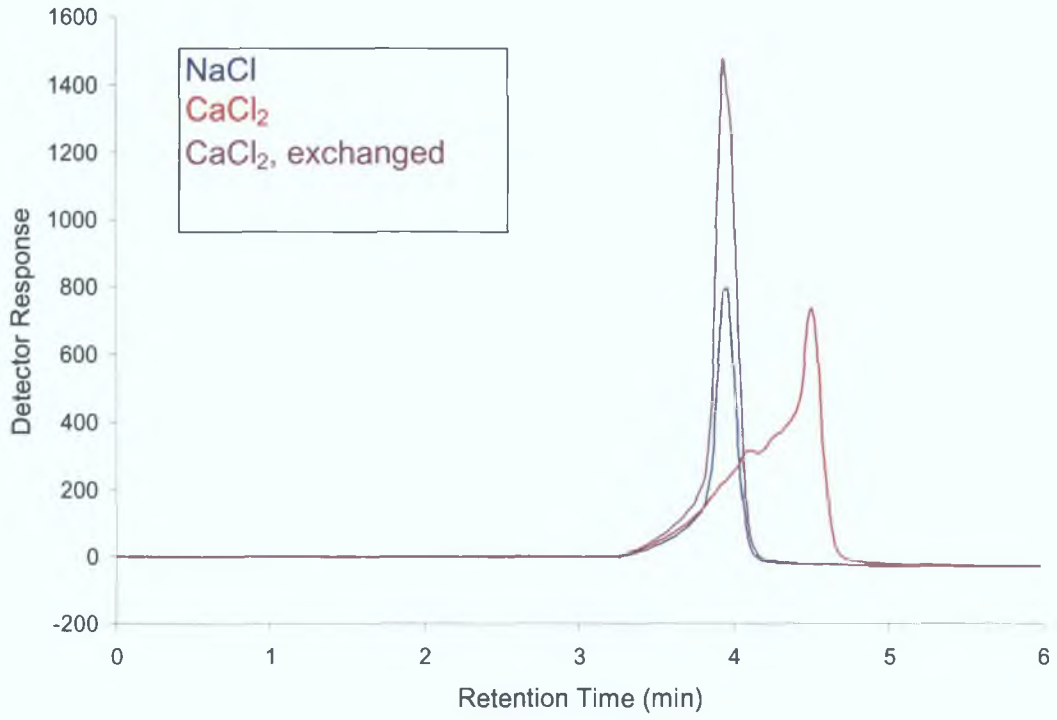


Figure 3.20. Chromatogram showing the conversion of calcium chloride to sodium chloride using the cation exchange procedure. Aqueous solutions containing 1.0 mM NaCl, $CaCl_2$ and $CaCl_2$ after subjection to the ion exchange procedure. Pure water eluent, flow rate 0.7 ml/min. Conductivity detection.

Sulphate, however, is a divalent anion, and the peak area of sodium sulphate after conversion from a divalent cation is equivalent to that of the anion prepared from a monovalent cation containing salt. The number of mmoles of the sulphate cation pair does not change during the exchange procedure and therefore, the peak area of sulphate does not change (Figure 3.21). Upon application to the exchange procedure, the retention time of sulphate was however decreased showing exchange had occurred, with peak shape also improved as the resulting sodium sulphate peak was sharper.

$$ZnSO_4 + \text{excess } Na^+ \longrightarrow Na_2SO_4 + Zn^{2+}$$

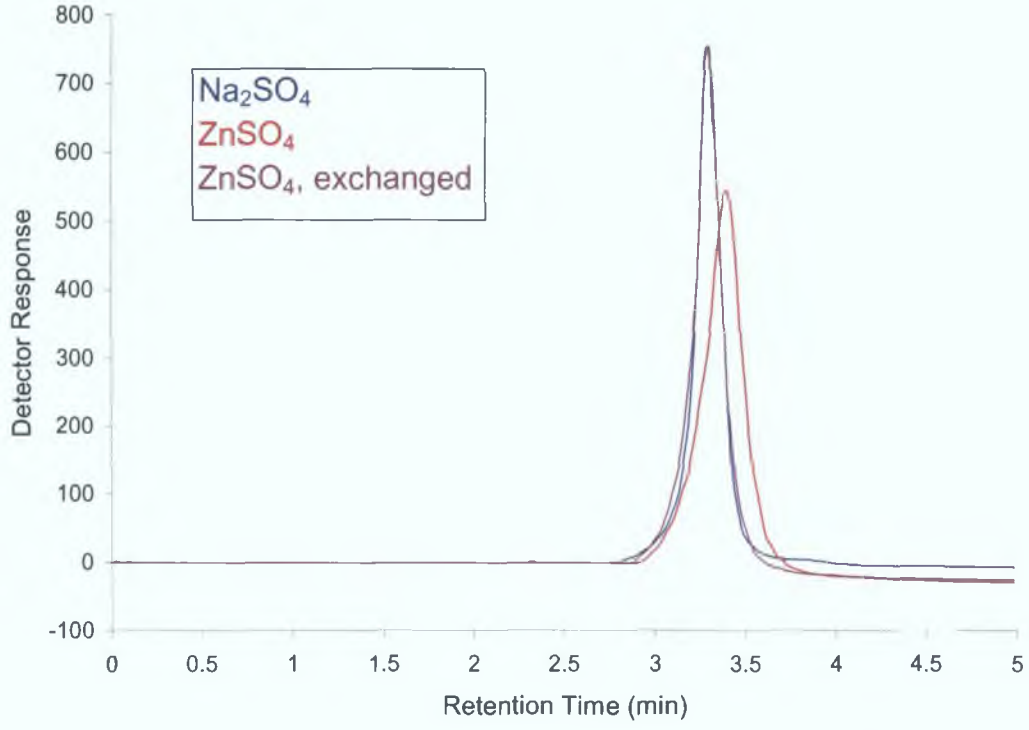


Figure 3.21. Chromatogram showing the conversion of zinc sulphate to sodium sulphate using the cation exchange procedure. Aqueous solutions containing 0.8 mM Na_2SO_4, $ZnSO_4$ and $ZnSO_4$ after subjection to the ion exchange procedure. Pure water eluent, flow rate 0.7 ml/min. Conductivity detection.

3.6.3 Calibration Curves

To ensure that there was complete exchange occurring between the divalent cation and sodium, calibration curves were prepared. In the case of chloride or nitrate, if the divalent cation was quantitatively exchanged, then the slope of the resulting calibration curve should be twice that of the calibration curve prepared for sodium. In the case of sulphate, the slopes of the two curves should be the same. Standard solutions covering the range of 0.2 mM to 1 mM were prepared for each anion both in the sodium form and in a divalent cationic form. The sodium standards were injected onto the column, followed by the divalent form, after having been subjected to the ion exchange procedure. As can be seen from the resulting calibration curves, linearity proved to be greater then $R^2 = 0.98$ for all six curves.

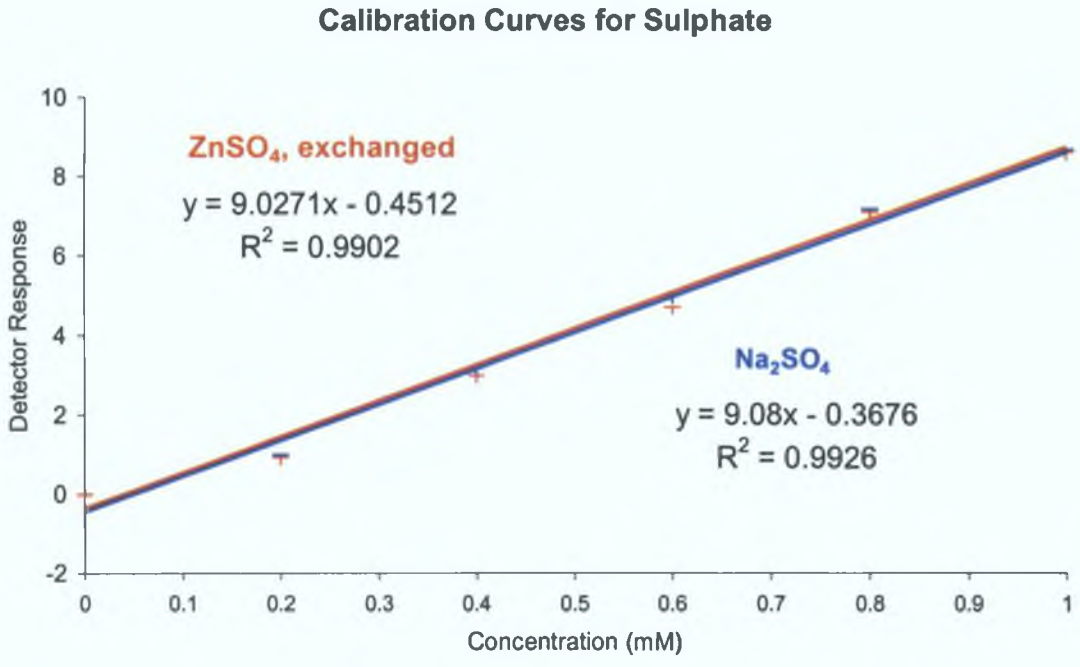


Figure 3.22 Calibration curves for sulphate, before and after application to the cation exchange procedure. Pure water eluent, flow rate 0.7 ml/min, conductivity detection.

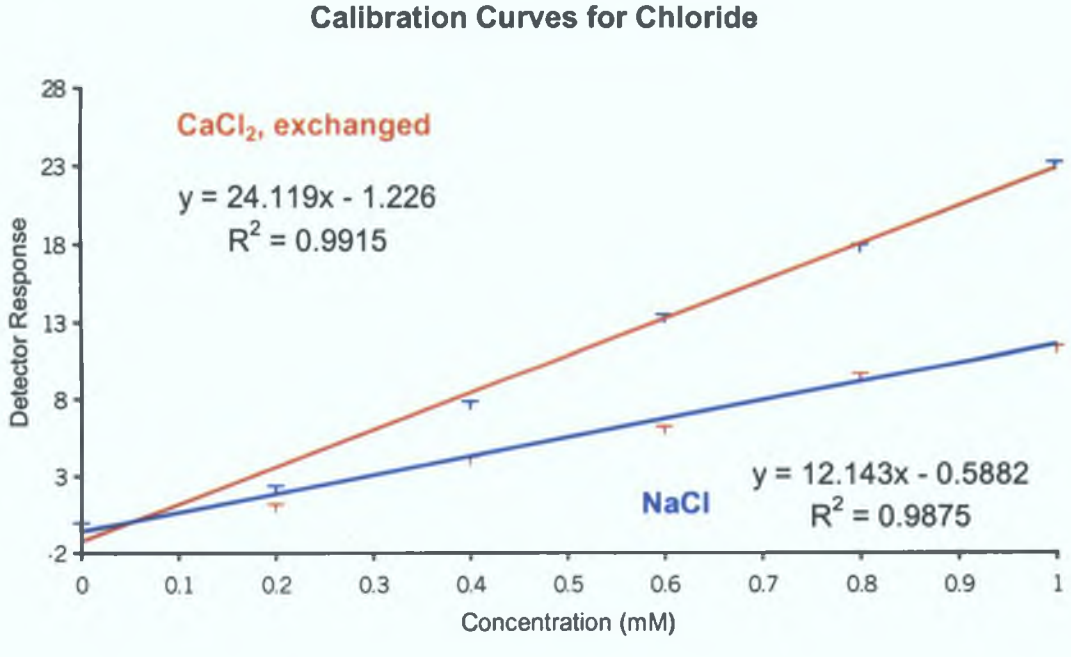


Figure 3.23 Calibration curves for chloride, before and after application to the cation exchange procedure. Pure water eluent, flow rate 0.7 ml/min, conductivity detection.

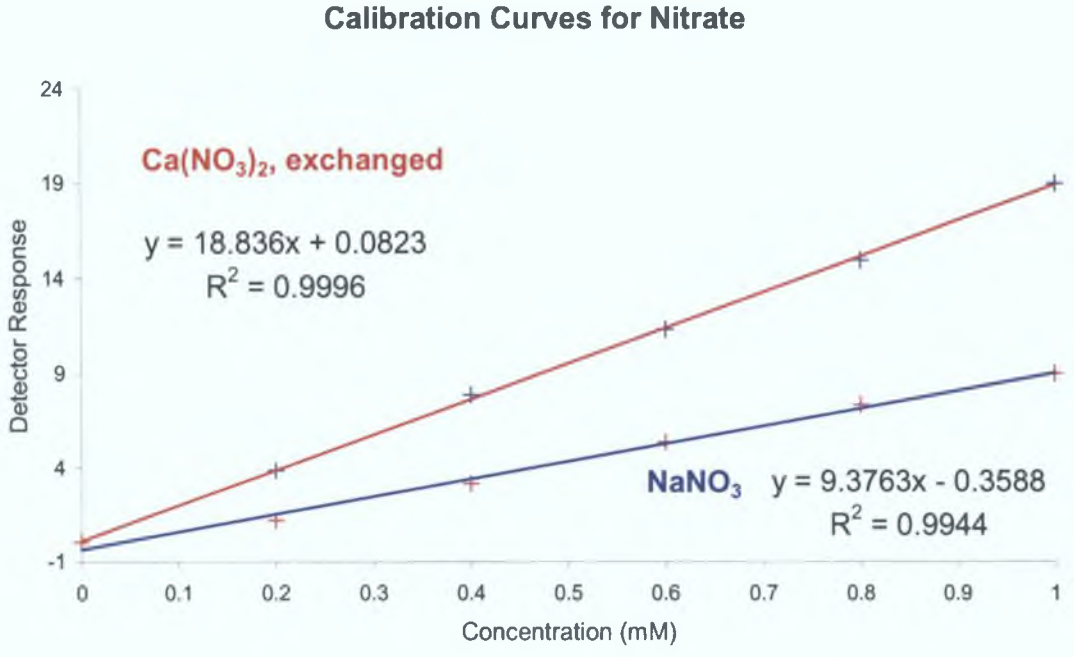


Figure 3.24 Calibration curves for nitrate, before and after application to the cation exchange procedure. Pure water eluent, flow rate 0.7 ml/min, conductivity detection.

Analyte	Slopes of calibration curves for the different cations	
	Sodium	Divalent cation (after ion exchange)
Sulphate	9.080	9.027
Chloride	12.143	24.119
Nitrate	9.376	18.836

Table 3.4. Comparison of the slopes of the different calibration curves prepared using the cation exchange procedure.

As can be seen from the figures in Table 3.4, the slope of the calibration curve for the divalent cation after ion exchange was twice that of the slope of the curve for the anion in the sodium form, for chloride and nitrate. In the case of sulphate, the slopes were equal. This indicates that complete quantitative exchange has taken place. The capacity of the exchange cartridges was high. Samples containing over 10 mM of cations were

successfully exchanged with no evidence to suggest that the cartridges were saturated. As it was deemed unlikely that samples with total analyte concentration levels higher then 10 mM were going to be analysed, no further testing was carried out to calculate the total capacity of the cartridges.

3.6.4 Mixtures

To test the ion exchange procedure on a mixture, a solution containing three different forms of nitrate (1 mM each of sodium, zinc and cerium nitrate) was subjected to the procedure and injected straight onto the column. The retention time of the analyte anion had significantly decreased, as all of the nitrate was now present in the faster eluting sodium form. The resulting peak of $NaNO_3$ was now six times as large as that corresponding to 1 mM $NaNO_3$ due to the accumulation of the nitrate from the original cationic forms (Figure 3.25).

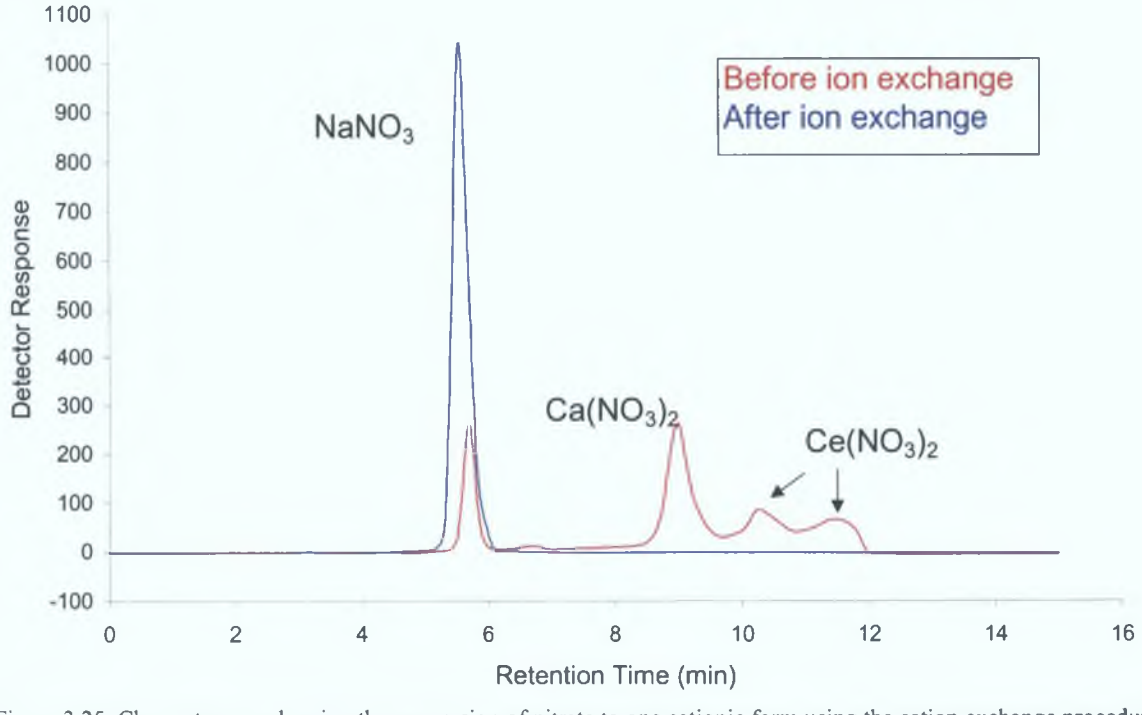


Figure 3.25. Chromatogram showing the conversion of nitrate to one cationic form using the cation exchange procedure. Aqueous solution containing 1.0 mM $NaNO_3$, $Zn(NO_3)_2$, and $Ce(NO_3)_3$ before and after subjection to the ion exchange procedure. Pure water eluent, flow rate 0.7 ml/min. Conductivity detection.

Complete exchange took place, with no visible presence of any zinc or cerium nitrate A second mixture containing three forms of nitrates (sodium, calcium and cerium) was then tested, this time also containing sodium chloride and sodium sulphate When the sample was injected without subjection to the ion exchange procedure, five peaks were present in the chromatogram, with co-elution occurring between the sodium and calcium nitrate peaks Upon application to the exchange procedure, only three peaks were present, as the nitrate was now represented by one individual peak (Figure 3 26) Once again an increase in peak size was noted for nitrate, which was six times as large as that corresponding to 1 mM $NaNO_3$ There was again no visible evidence to suggest the presence of any calcium or cerium in the mixture, indicating that complete quantitative exchange took place Therefore, ion redistribution has been successfully eliminated from a mixture of anion and cations in EIC by application of a novel method incorporating off-line cation exchange

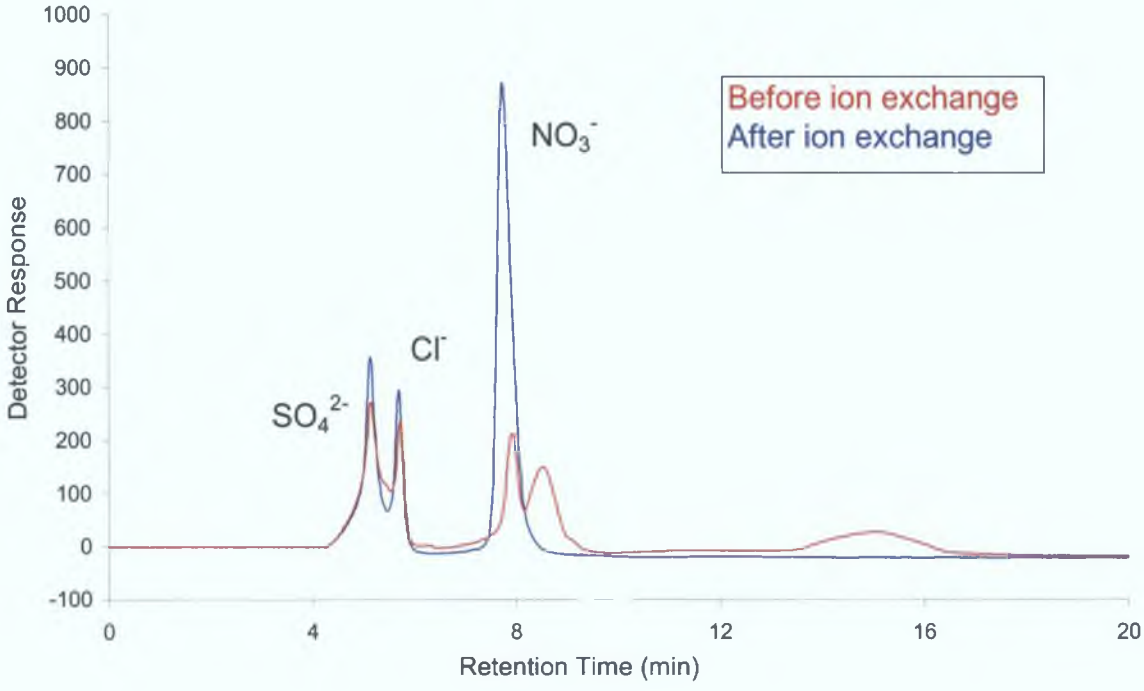


Figure 3.26. Chromatogram showing the elimination of ion redistribution in EIC by using the cation exchange procedure. Aqueous solution containing 1.0 mM NaCl, Na_2SO_4, $NaNO_3$, $Ca(NO_3)_2$, and $Ce(NO_3)_3$ before and after subjection to the ion exchange procedure. Pure water eluent, flow rate 0.5 ml/min. Conductivity detection.

3.7 Reproducibility

Despite the successful elimination of the problem of ion redistribution, the technique still suffered from poor reproducibility due to the gradual bleeding of the zwitterionic reagent from the stationary phase over time. As the method involved the pre - coating of the zwitterionic surfactant onto the column, the coating was gradually being washed off with time, with the average life span of the coating being approximately 3 weeks before significantly reduced retention times were evident. The result of this was that elution times would vary depending on the amount of surfactant in the column at any one time. Therefore, day to day retention time reproducibility was not acceptable. Table 3.5 shows values for the reproducibility of retention times of several anions covering a period of 1 month, injected under exactly the same conditions. It was therefore decided that an

alternative to pre-coating the stationary phase was required which would lead to a more stable column capacity This next stage of the research is detailed in Chapter 4

Analyte	Retention Time % RSD	*N*
Sulphate	11 28	10
Chloride	13 92	10
Nitrate	15 15	10

Table 3 5 Reproducibility figures for sulphate chloride and nitrate in EIC

3.8 CONCLUSIONS

The separation of selected inorganic anions has been investigated using EIC with a water only eluent Coating a reversed – phase analytical column with a zwitterionic surfactant reagent such as Zwittergent 3-14 facilitated the separation of common anions such as sulphate, chloride and nitrate among others, although efficiency was rather poor The problem of ion redistribution has been overcome by the development of a simple off-line procedure that uses an ion exchange cartridge to replace all sample cations with one designated cation of choice

The developed ion exchange procedure was quick and simple The required volume of sample was simply passed manually through the cartridge and then injected straight onto the column With a cation exchange cartridge in the sodium form, all sample cations were quantitatively exchanged, and all eluting anions were therefore in the sodium form

This led to reduced retention times, improved peak shape and thus improved detection limits

BIBLIOGRAPHY

1 W Hu, T Takeuchi and H Haraguchi, Analytical Chemistry (**65**), 2204 – 2208, 1993

2 T Umemura, S Kamiya, A Itoh, K Chiba and H Haraguchi, Analytica Chimica Acta, (**349**), 231 – 238, 1997

3 W Hu, H Tao, M Tominaga, A Miyazaki and H Haraguchi, Analytica Chimica Acta, (**299**), 249 – 256, 1994

4 T Umemura, R Kitaguchi and H Haraguchi, Analytical Chemistry (**70**), 936 – 942, 1998

5 T Umemura, S Kamiya and H Haraguchi, Analytica Chimica Acta, (**349**), 23 – 32, 1999

CHAPTER FOUR

DEVELOPMENT OF A RECYCLING SYSTEM IN EIC

4.1 INTRODUCTION

As mentioned in the previous chapter, one of the disadvantages of pre-coated EIC is the need for constant regeneration of the zwitterionic stationary phase The number of times a column had to be regenerated was dependent upon the flow rate of the mobile phase and the amount of time the column had been in use since the previous coating On average, this regeneration was necessary every three to four weeks Regeneration was time consuming as the column must first be stripped of all the old surfactant, by washing the column thoroughly with acetonitrile, then methanol, then Milli-Q, before the new coating could be applied Once the column was coated, any excess Zwittergent 3-14 was then washed off, the whole process normally taking a full working day to complete

The only way to combat the problem of column regeneration is to stabilise the column coating, so that column capacity remains fixed over time One way to do this would be to constantly re-introduce any lost coating due to column bleed back onto the column again, thus keeping the level of surfactant on the column constant However, re-introduction of the lost coating would mean re-introducing the eluent waste back into the system, which would also bring with it any sample ions injected onto the column, which over a short period of time would begin to affect analyte retention and background conductivity The solution to this problem lay in the nature of the zwitterionic reagent itself Unlike sample anions and cations, the zwitteriomc reagent would be unretained on standard anion and cation exchange resins Therefore, it would be possible to remove these sample ions from the column effluent before it re-entered the eluent reservoir, without removing the zwitterionic eluent To do this a cation exchanger (acid form) and an anion exchanger (hydroxide form) were inserted in series, after the detector, to remove all

analyte ions from the water eluent In this way, only water and the desorbed Zwittergent 3-14 would re-enter the eluent reservoir, successfully recycling the surfactant, and keeping the coating of the column at the required constant level In doing this, a novel eluent recycling system had been developed, that constantly compensated for column bleed and therefore negated the need for regular regeneration

4 1 1 Apparatus and Instrumentation

The instrumentation used in the recycling system was the same as that used in the previous chapter with the exception of a UV detector (LDC Monitor III, model 1204A) placed in series after the conductivity detector This detector was followed by two ion exchangers, one for cations and one for anions The cation exchanger comprised of a cation exchange suppressor cartridge and holder (both from Alltech Associates, Lancashire, England) The anion exchanger comprises of a similar cartridge and holder, but the cartridge contained 'Amberlite' IRA 400 resin, (BDH Chemicals Ltd, Poole, England)

The cation exchanger resin was coloured yellow when in the acid form and pink when it contained metal cations, i e sodium, magnesium, etc In this way, it was possible to tell in advance when the cartridge needed regeneration, as the colour change slowly spreads down through the resin For this reason, the cation exchanger was placed first in the series and acted as an indicator to the level of exchange that was taking place, and therefore warned when regeneration of the cartridges was required As these ion exchangers were both of a very high capacity and were only exchanging small concentrations of injected sample ions, regeneration was only occasionally required This

was a simple procedure involving pumping 1 M nitric acid or sodium hydroxide through each cartridge for a short time. Figure 4.1 demonstrates the system set-up.

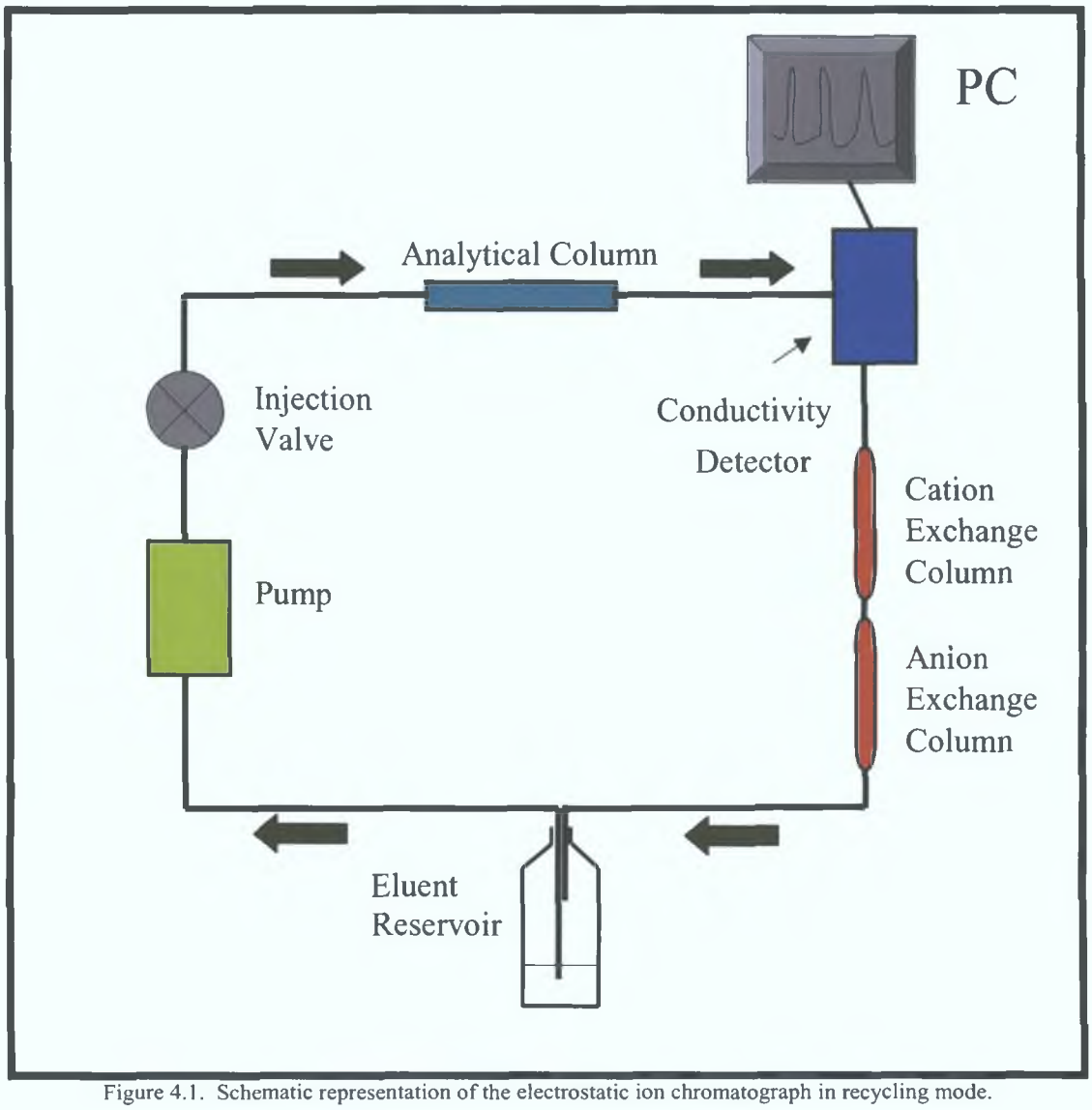


Figure 4.1. Schematic representation of the electrostatic ion chromatograph in recycling mode.

4.2 Development of the System

4.2.1 Comparison with the Non – Recycled System

With the non – recycled system, the retention times and peak shapes of the analytes varied considerably, depending on whether the column had just been re-coated or was in need of re-coating. This variation in time could be as much as a 15 % deviation in

retention times, as shown in Chapter 3. Here, the column was first regenerated as before, then the freshly coated column was immediately included into the recycling EIC system. Below are chromatograms showing a separation of a standard test mixture, carried out with the non – recycled system and the new recycling system.

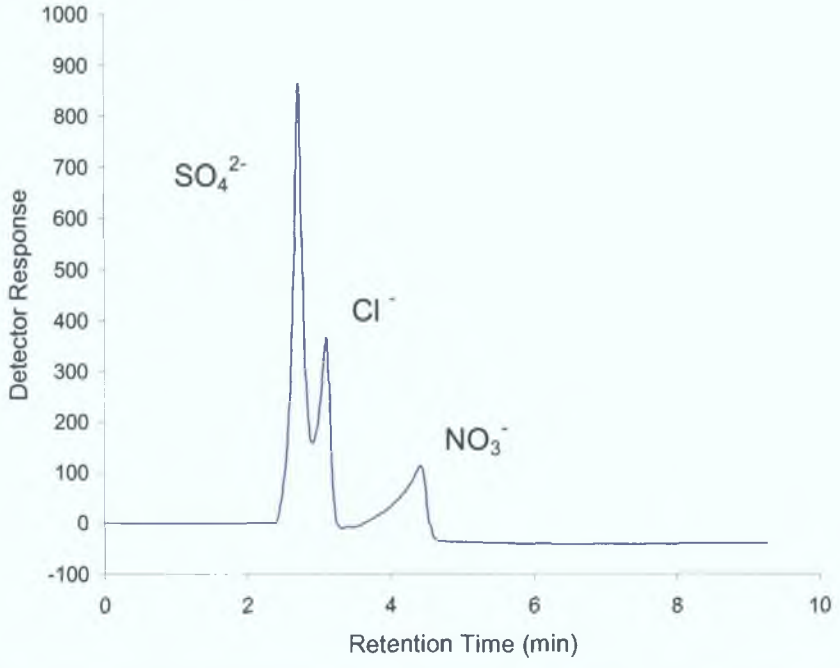


Figure 4.2. Chromatogram of a standard test mixture, non – recycled mode. Aqueous solutions containing each anion at 1 mM each. Pure water eluent, flow rate 0.7 ml/min, conductivity detection.

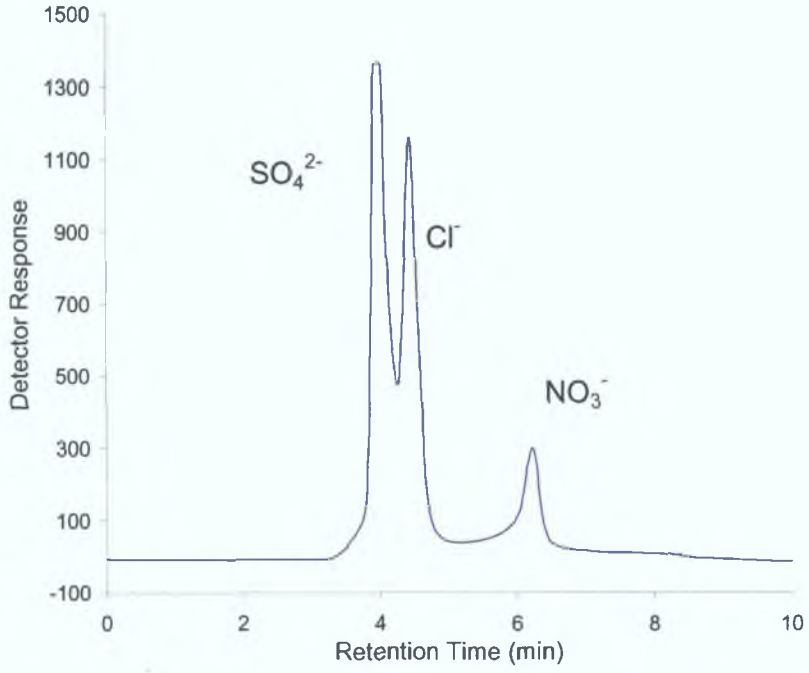


Figure 4.3. Chromatogram of a standard test mixture, recycled mode. Aqueous solutions containing each anion at 1 mM each. Pure water eluent, flow rate 0.7 ml/min, conductivity detection.

The resolution of each of the above separations was calculated as follows (although this equation is not ideal for asymmetric peaks):

$$R = \frac{1.18\,(t_{R2} - t_{R1})}{(W_1{}^1\!/_2 + W_2{}^1\!/_2)}$$

Equation 4.1

R = resolution

t_R = retention time

$W^1\!/_2$ = width at half height

Analyte	Rt Time (min)	Resolution
Na_2SO_4	2.72	-
NaCl	3.1	1.29
$NaNO_3$	4.42	3.59

Non – recycled mode

Analyte	Rt Time (min)	Resolution
Na_2SO_4	4.02	-
NaCl	4.52	1.14
$NaNO_3$	6.25	3.72

Recycling system

Table 4.1. Comparison of resolutions for the non - recycled mode and the recycling system

When comparing the two separations, the retention times have slightly increased for the new recycling system, with little change in resolution In addition, the peak shapes, especially that of nitrate, have improved It was clear that by recycling the eluent in this way that the small reagent concentration in the water eluent from column bleed was sufficient to stabilise the initial column coating It was then necessary to assess how this novel recycling system improved reproducibility over the short and long term

4 2 2 Reproducibility of the Recycling System

4 2 2 1 Monitoring of the Baseline

Although the exchange columns inserted in-line after the detectors were assumed to work for protracted periods of use before requiring regeneration, it was decided to monitor the background conductance and absorbance to ensure that no anions or their counter cations were entering the eluent reservoir If this occurred, a slight but gradual rise would be expected in the baseline of both detectors To maximise any visible baseline change due to the unwanted introduction of sample ions, it was decided to have the lowest volume of water possible in the eluent reservoir In this way, any introduction of ions would be more noticeable, as they would be less diluted than if they had been introduced into a larger volume The smallest practical eluent volume was 200 mL as this was the level of water needed to just cover the filter at the bottom of the reservoir line

The baseline was continuously monitored over a three-week period, during which routine injections were continuously carried out No significant difference was noticed in the baseline of either detector during this period or during the future months of research Neither of the ion exchangers needed regenerating during this period, this being evident as

no colour change appeared in the resin of the cation exchanger To examine if the exchanger was indeed working, a solution of 1 M NaOH was passed through it (off – line) and the resin changed colour immediately

4 2 2 2 Reproducibility

Upon development of the recycling system, it was decided to investigate the effect that the constant regeneration had on reproducibility While the baseline was being monitored over a period of three weeks, standard solutions of the common test anions were injected onto the column, as both individual samples of 1 mM, and also in mixtures of varying concentrations The following chromatograms show a test mixture and an individual standard of sodium sulphate which were injected at the beginning and at the end of this three-week period The chromatograms have been overlaid to illustrate this long term reproducibility As can be seen, the reproducibility of peak shape, area and retention times was excellent

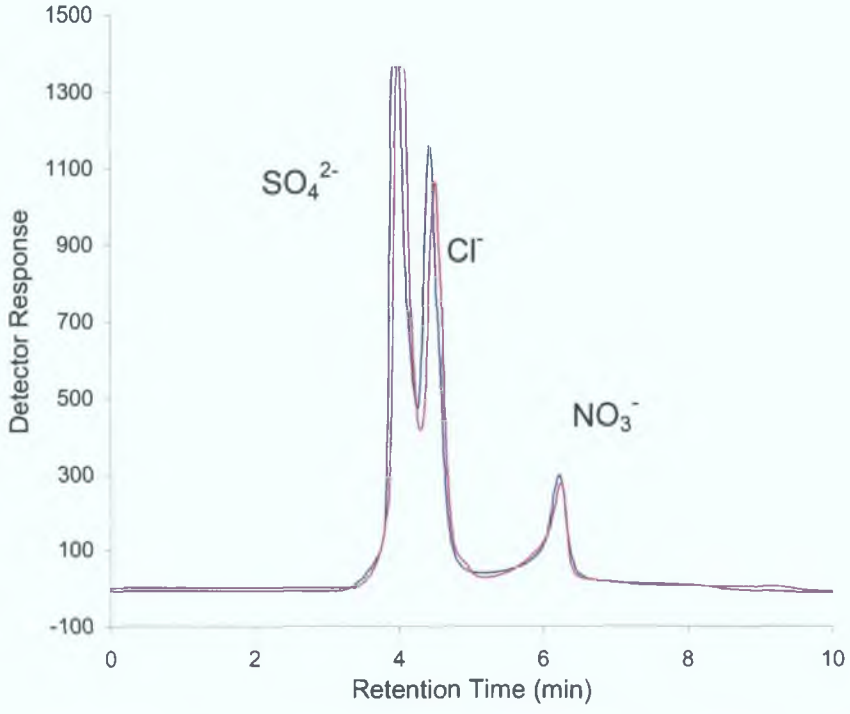


Figure 4.4. Chromatograms showing the reproducibility of the recycling system. Aqueous solutions, all analytes each at 1 mM. Pure water as the eluent, flow rate 0.7 ml/min. Conductivity detection.

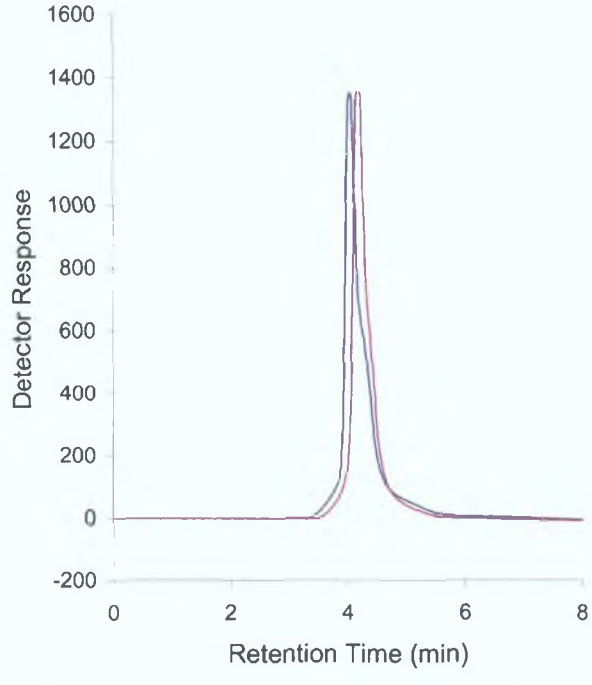


Figure 4.4. Chromatograms showing the reproducibility of the recycling system. Aqueous solutions of sodium sulphate at 1 mM. Pure water as the eluent, flow rate 0.7 ml/min. Conductivity detection.

4 2 2 3 Efficiency

The samples injected over this three-week period were also analysed to investigate the efficiency of the column in this recycling mode The tables below lists the efficiency (N/m plate number per metre of column) for each anion, as well as percentage relative standard deviations (% RSD) of peak area and retention times The % RSD of the retention times has decreased significantly when compared with that of the non-recycled mode of chromatography as described in Chapter 3

$$N = \frac{5.54\,(t_{R1} + t_{R2})}{(W_1{}^1\!/_2 + W_2{}^1\!/_2)}$$

Equation 4 2

N = plate number

t_R = retention time

W½ = width at half height

Analyte	Average Rt Time (min)	% RSD Rt Time	% RSD Peak Area	Efficiency, N/m	*n*
Na_2SO_4	4 027	2 12	9 07	5216	13
NaCl	4 468	2 24	12 55	7863	13
$NaNO_3$	6 307	2 35	11 06	12396	13

Table 4 2 Retention data for the recycling system (individual standards 1 mM each)

Analyte	Average Rt Time (min)	% RSD Rt Time	Efficiency, N/m	*n*
Na_2SO_4	4 040	1 56	5084	10
NaCl	4 495	1 43	6796	10
$NaNO_3$	6 302	2 27	9428	10

Table 4 3 Retention data for the recycling system (mixed standards, various concentrations)

Whether together in a mixed standard or injected as individual standards, the test anions showed low % RSD values for retention precision However, the peak area precision was improved but still could only be described as adequate The technique was now sufficiently robust to investigate its application to real samples

4 2 3 Application to Real Samples

The method was applied to several real water samples such as tap water, mineral water and river water Carrying out the off-line exchange procedure and in recycling EIC mode, peaks for sulphate, chloride and carbonate were always identifiable in each of the above sample types However, this was not the case for nitrate Due to the relatively high concentration of chloride in the samples, any nitrate present was being partially masked A reduction in eluent flow rate, improved the resolution of nitrate and chloride slightly The following chromatograms show a mineral water sample and the same sample spiked with a nitrate standard (approximately 1 mM)

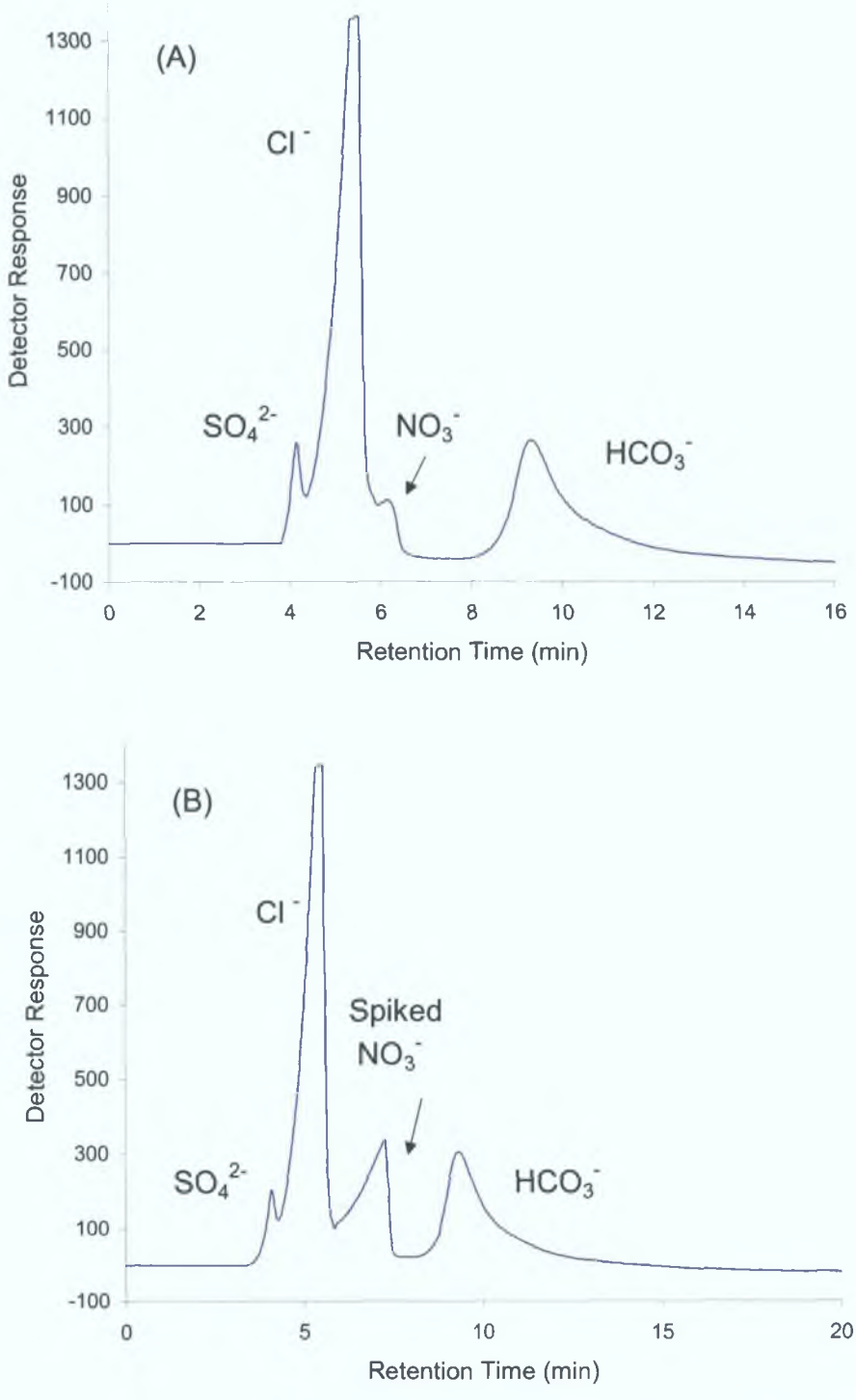


Figure 4.5.. Chromatogram showing application of the recycling system to a mineral water sample. Pure water as the eluent, flow rate 0.5 ml/min, conductivity detection. (A) Finches mineral water sample (B) Same sample spiked with $NaNO_3$

In the original water sample, the resolution between sulphate, chloride and nitrate was poor due to the high concentration of chloride, which was quoted at 49.9 ppm by the water suppliers, while sulphate was quoted at 10.8 ppm and nitrate at 14 ppm. At the original flow rate of 0.7 ml/min, the nitrate peak was masked by this large chloride peak, but when the flow rate was decreased to 0.5 ml/min, the peak began to appear. When the sample was spiked with approximately 1 mM sodium nitrate, the peak area increased and was therefore more clearly distinguishable from the chloride peak. It was again found that peak shapes were rather broad, especially carbonate and nitrate, whose peak showed considerable fronting.

While the nitrate peak was identifiable in the previous chromatogram, it was not quantifiable. In order to carry out quantitative nitrate analysis in water samples, the nitrate peak must be completely visible. Chloride does not absorb strongly in the UV range, while nitrate does. Therefore, if a UV absorption detector were used in place of the conductivity detector, an injection of the above water sample would produce a chromatogram containing only one significant peak for nitrate, which would be readily quantifiable. Therefore, in all subsequent work a UV detector was incorporated into the system, to facilitate the quantitative determination of nitrate (and indeed other UV absorbing ions), in the above water samples.

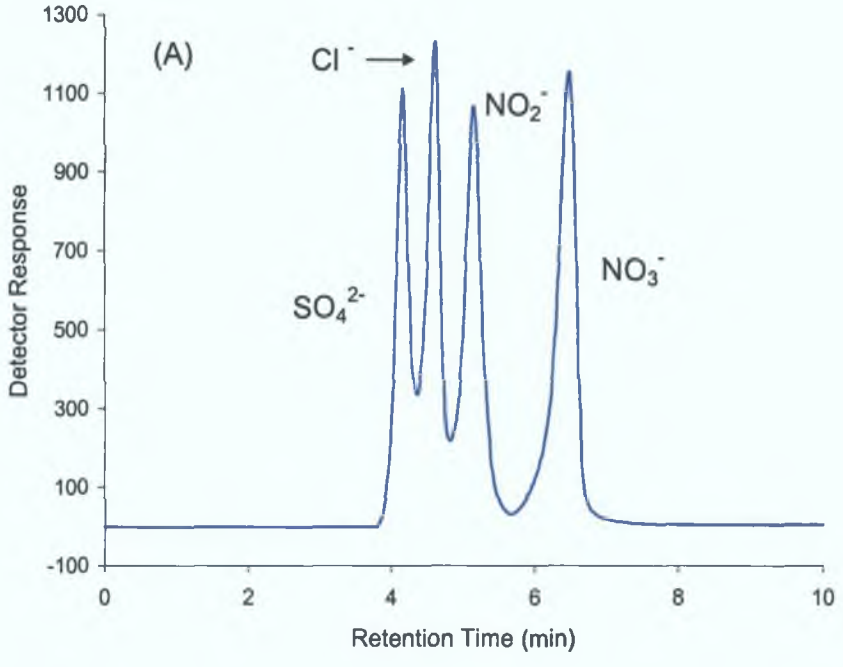
4.3 Dynamic Coating

In the recycling mode, any zwitterionic reagent bleeding from the column passed unretained through the post-detector ion exchangers and was re-adsorbed onto the analytical column, thus removing the need to re-coat the column at regular intervals.

However, the zwitterionic reagent could also be additionally added to the eluent at low concentrations, without affecting the eluent recycling or detection (Zwittergent 3-14 has very low background conductance and does not absorb in the UV above 200 nm) This additional reagent would again pass unretained through the post-detector exchange columns but would now effectively increase the capacity of the analytical column, as it would now have become a dynamically coated system It was decided to add 2 mM Zwittergent 3-14 into the eluent This concentration was chosen, as it has been noted before that at such levels of the surfactant, the retention times of the analyte ions had increased The effect this addition had upon peak shapes and also resolution of the anion mixtures tested, is described below

4 3 1 Improved Separations

A solution of 2 mM Zwittergent 3-14 was made up and transferred into the eluent reservoir The recycling system was left to equilibrate for several hours before any injections were carried out Once the system was equilibrated, a mixture containing four test anions, (sulphate, chloride, nitrate and nitrite) was injected onto the column and the resulting chromatogram was compared with one of the same mixture, analysed using only water as the eluent As the following chromatograms demonstrate, a rather unexpected decrease in retention times was noted, although without a subsequent reduction in resolution With the zwitterionic eluent, all four anions were now separated in under 4 minutes, while previously, it had taken almost 7 minutes



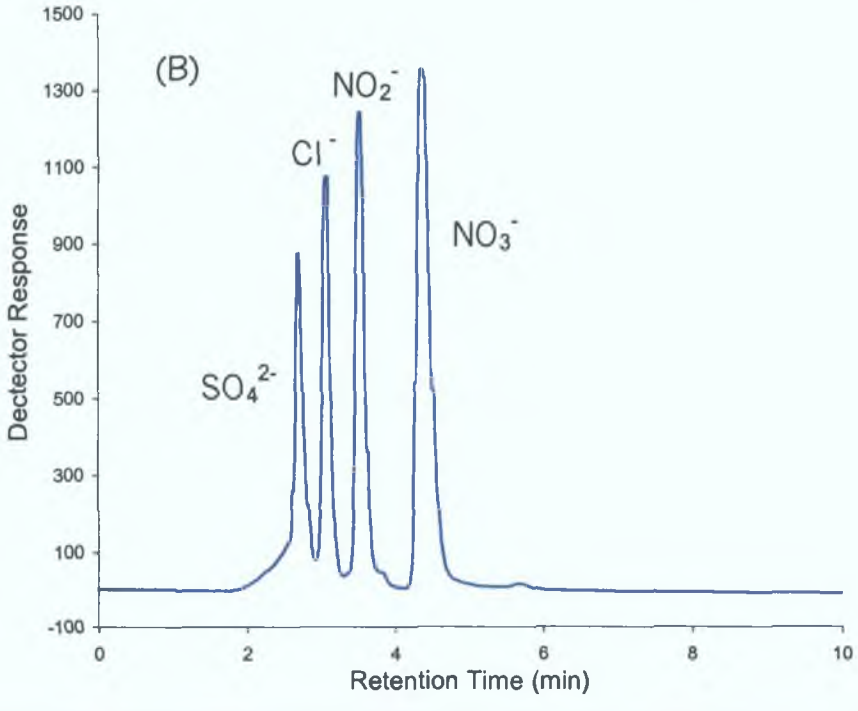


Figure 4.6. Chromatograms showing a standard mixture of the four test anions. Both with a flow rate of 0.7 ml/min and conductivity detection. (A) Pure water as the eluent, concentrations 0.5, 1.0, 1.5 and 2.5 mM respectively. (B) 2 mM Zwittergent 3-14 as the eluent, concentrations 0.4, 0.8, 1.2 and 2.0 mM respectively.

Despite the reduction in retention times, resolution actually increased between sulphate and chloride, and indeed chloride and nitrite, which were now baseline separated.

Peaks shapes were much improved, as the peaks were less broad and much sharper, resulting in a potential decrease in detection limits The following table gives the resolution values achieved under the different eluent conditions calculated according to equation 4 1

Analyte	Rt Time (min)	Resolution
Na_2SO_4	2 68	-
NaCl	3 05	1 85
$NaNO_2$	3 53	2 28
$NaNO_3$	4 35	2 89

2mM Zwittergent 3-14 as eluent

Analyte	Rt Time (min)	Resolution
Na_2SO_4	4 15	-
NaCl	4 6	1 33
$NaNO_2$	5 15	1 39
$NaNO_3$	6 48	2 95

Pure water as eluent

Table 4 4 Comparison of resolutions for the different eluents used in the recycling system

4 2 3 Efficiency and Reproducibility

Individual standards of each of the four test anions were made up at a concentration of 1 mM Repeat injections were carried out to investigate the reproducibility parameters of the new system, which proved to be even lower than the pure water system Figure 4 7 shows chromatograms of three sodium nitrite peaks, staggered so that the reproducibility of the peaks may be fully seen While there is still some peak fronting evident, the peaks are reasonably sharp Table 4 5 outlines the efficiency and percentage deviations in retention time and peak area found for the new system, with efficiency calculated according to equation 4 2

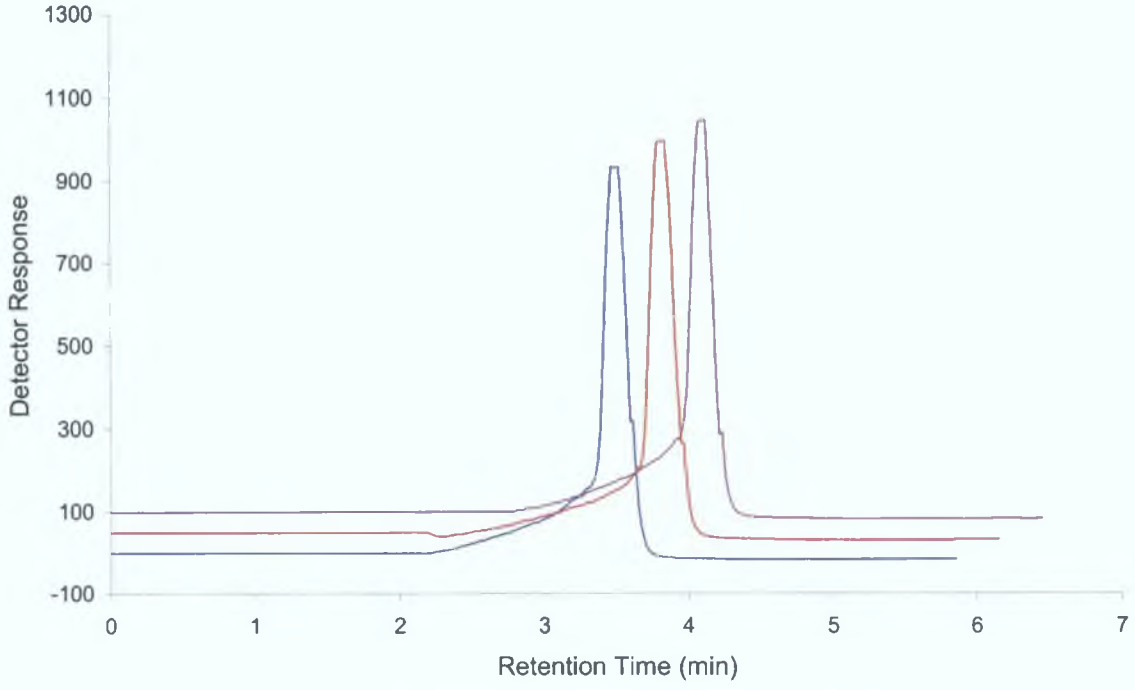


Figure 4.7. Chromatograms of three individual sodium nitrite peaks. Aqueous solutions of 1 mM each. 2 mM Zwittergent 3-14 as the eluent, flow rate 0.7 ml/min. Conductivity detection.

Analyte	% RSD Rt Time	% RSD Peak Area	Efficiency, N/m	*n*
Na_2SO_4	0.53	3.30	9684	10
NaCl	0.53	5.99	10652	10
$NaNO_2$	0.25	2.62	14279	10
$NaNO_3$	0.34	3.40	16188	10

Table 4.5. % RSD and efficiency figures for new recycling system.

When compared with Table 4.3 (pure water system) even though the retention times are lower, the efficiencies have greatly improved. Also, % RSD is very much decreased for both the retention time and the peak area, with the variation in retention time at less than 1 %. Overall, the addition of the Zwittergent 3-14 to the eluent has improved reproducibility when compared with using pure water as the eluent, as can be seen in

Table 4 6 (There are no values for nitrite using pure water as the eluent as repeat injections were not carried out for nitrite at this stage)

Analyte*	Pure water as eluent				Zwittergent 3-14 as eluent			
	Average Rt (min)	% RSD Rt Time	% RSD Pk Area	Eff N/m	Average Rt (min)	% RSD Rt Time	% RSD Pk Area	Eff N/m
Na_2SO_4	4 03	2 12	9 07	5216	2 96	0 53	3 30	9684
NaCl	4 47	2 24	12 55	7732	3 36	0 53	5 99	10652
$NaNO_2$	-	-	-	-	4 4	0 25	2 62	14279
$NaNO_3$	6 31	2 35	11 06	12396	4 86	0 34	3 40	16188

Table 4 6 Comparison of retention and efficiency data for the different eluents used in the recycling system

* All analytes were injected as individual standards of 1 mM, and injections were repeated 13 times with pure water as the eluent and 10 times with Zwittergent 3-14 as the eluent

4 2 4 Application to Real Samples

This method was again applied to various water sample including tap water, bottled mineral water and river water Due to the increased resolution between the samples' anionic components, nitrate was now clearly evident in the water samples, even when using conductivity detection Also, with the addition of the UV detector, nitrate was very readily quantifiable, even at trace levels

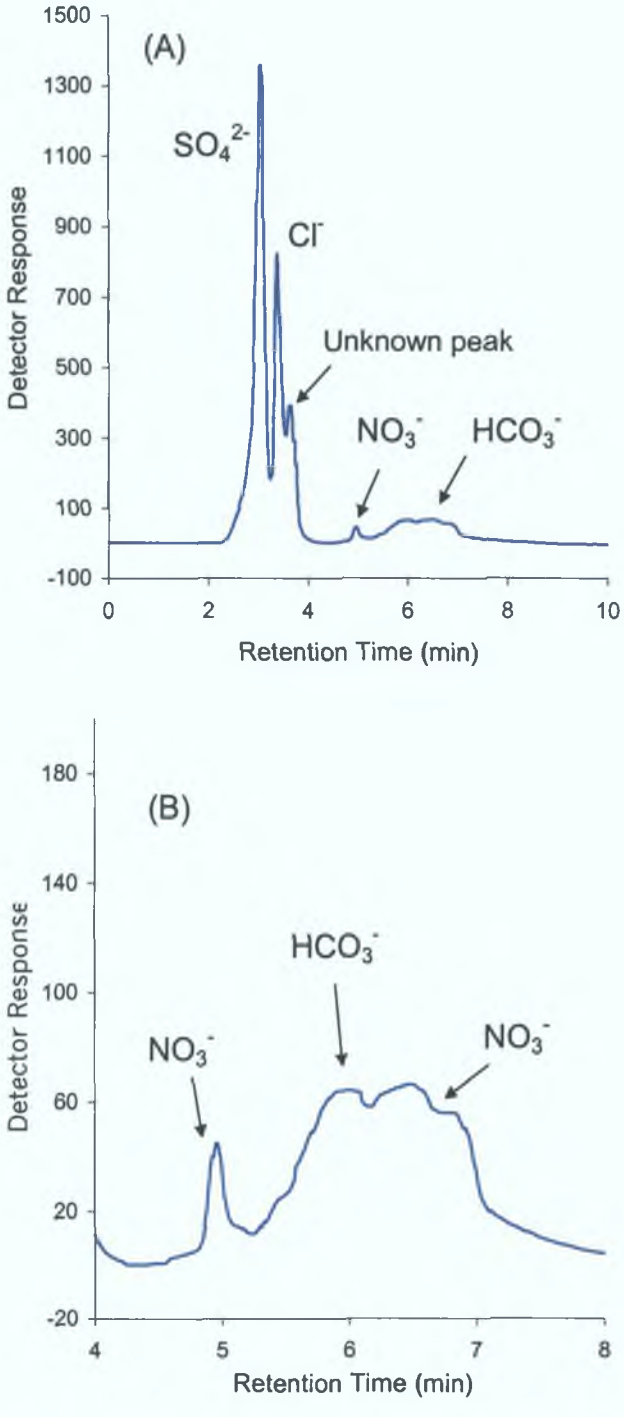


Figure 4.8. Chromatograms of a river water sample before passage through the exchange cartridge. 2 mM Zwittergent 3-14 as the eluent, flow rate 0.7 ml/min. (A) Conductivity detector (B) Conductivity detector – nitrate peaks scaled-up.

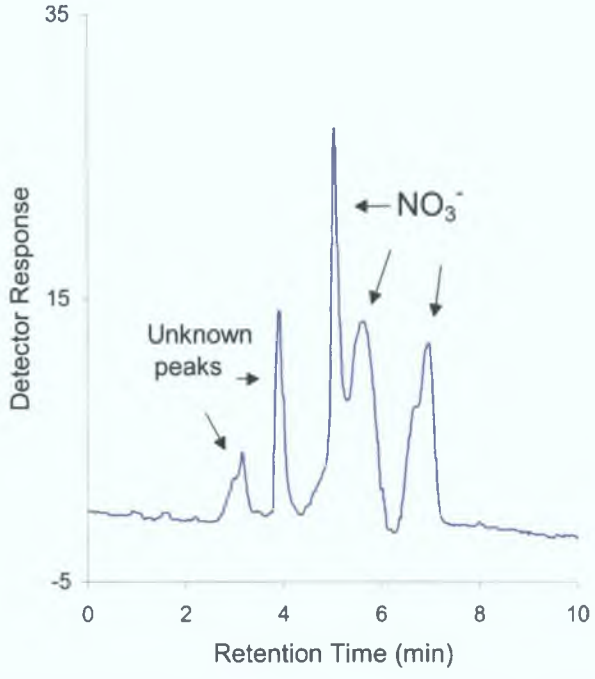


Figure 4.9. Chromatogram of a river water sample before passage through an exchange cartridge. 2 mM Zwittergent 3-14 as the eluent, flow rate 0.7 ml/min. UV detection.

In the above chromatograms, sulphate, chloride, nitrate and carbonate are identifiable, despite the presence of some unknown peaks. However, the presence of multiple peaks for nitrate, indicates the presence of divalent cations within the sample. This is of course as expected for a river water sample which is likely to contain both calcium and magnesium ions. Therefore, the same river water sample was again analysed, this time after subjection to the off-line exchange procedure. Using this procedure the multiple nitrate peaks were eliminated as the following chromatograms demonstrate.

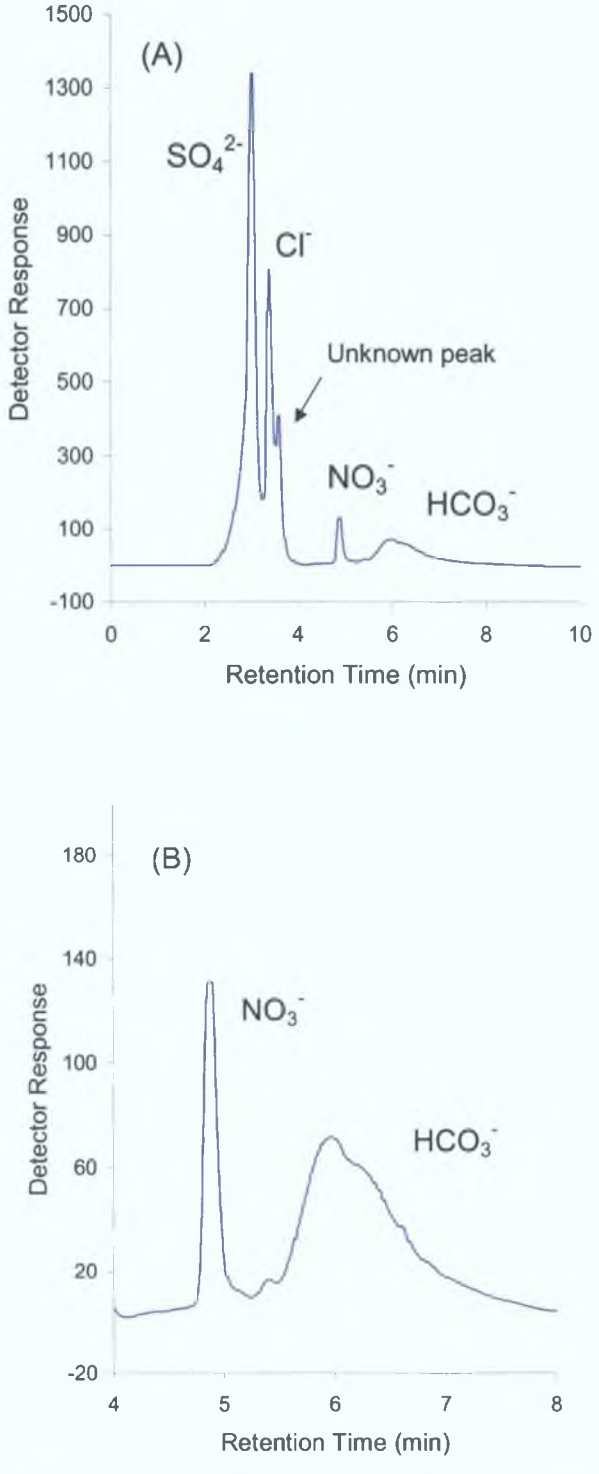


Figure 4.10. Chromatograms of a river water sample after passage through the exchange cartridge. 2 mM Zwittergent 3-14 as the eluent, flow rate 0.7 ml/min. (A) Conductivity detector (B) Conductivity detector – nitrate peaks scaled-up.

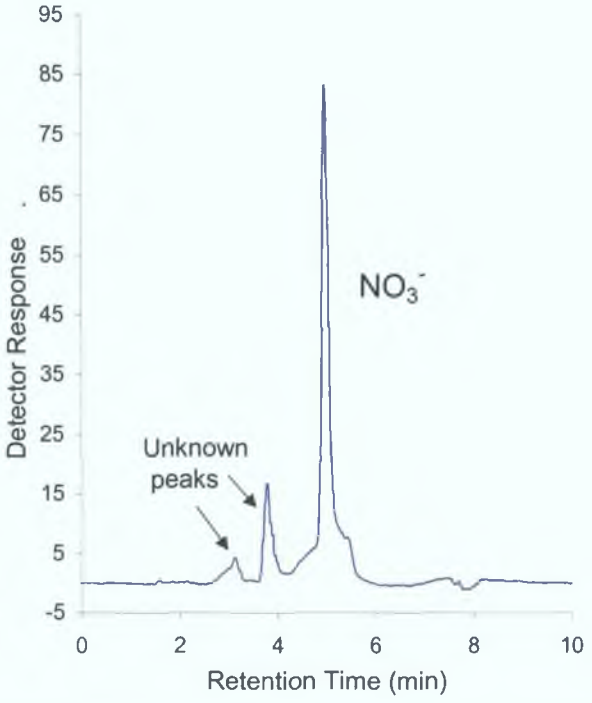
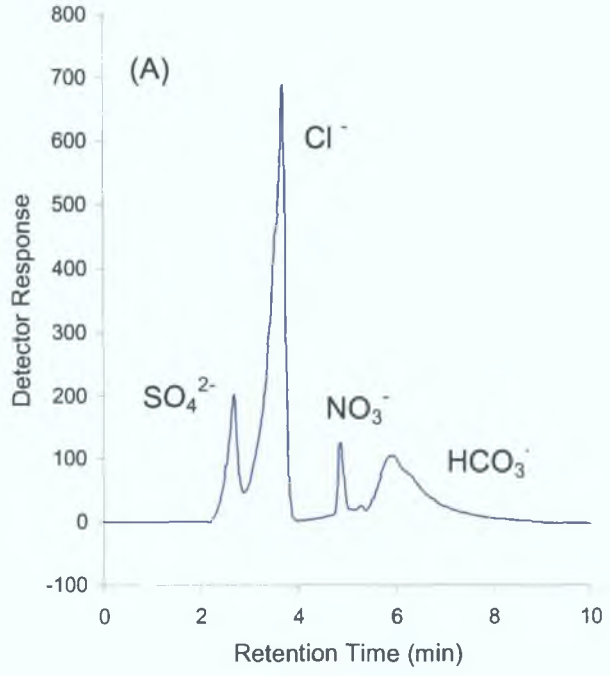


Figure 4.11. Chromatogram of a river water sample after passage through the exchange cartridge. 2 mM Zwittergent 3-14 as the eluent, flow rate 0.7 ml/min. UV detector.

The above water samples show the necessity of the off-line cation exchange method. The nitrate peak that was barely visible with conductivity detection before exchange is now clearly visible. As shown with the UV traces, the whole chromatogram is very much simplified. Before exchange, multiple peaks were evident due to the different cationic forms present in the river water, while subsequently just one large peak was seen, making the quantification of nitrate much easier. Similar results were found for the tap water and the mineral water samples, as is shown by the following chromatograms.



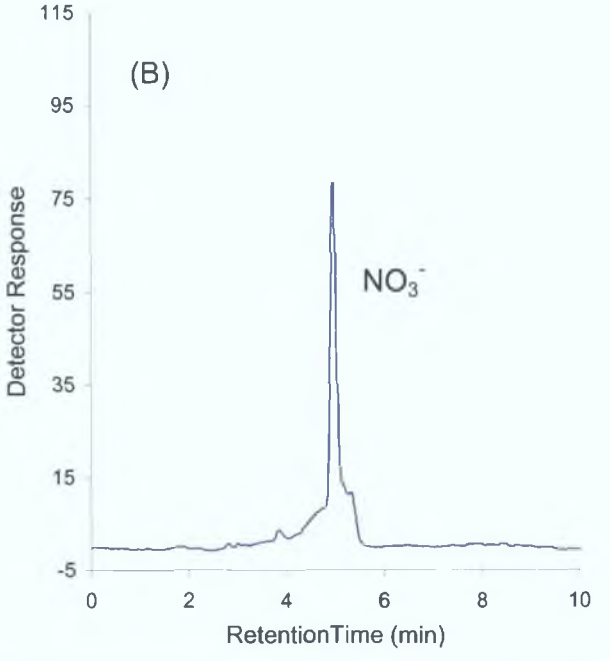
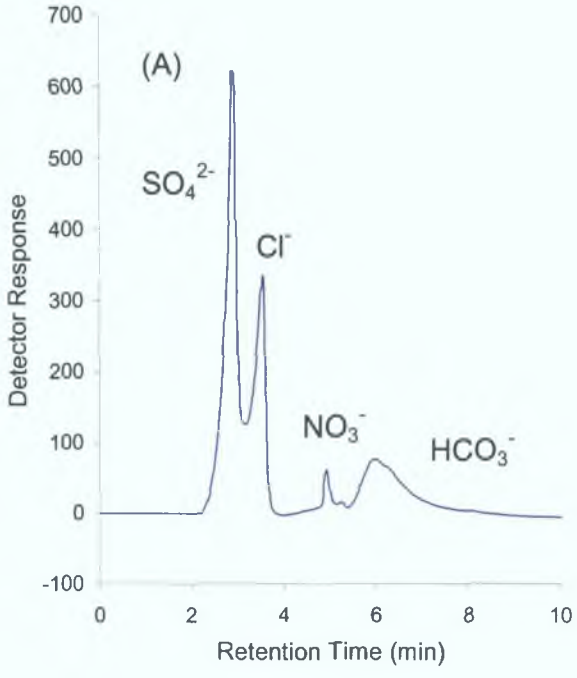


Figure 4.12. Chromatogram of a mineral water sample after passage through the exchange cartridge. 2 mM Zwittergent 3-14 as the eluent, flow rate 0.7 ml/min. (A) Conductivity detection (B) UV detection.

Figure 4 12 shows a chromatogram of the same mineral water that was analysed using pure water as the mobile phase in the previous section (Figure 4 5) In that case, sulphate and chloride were not well resolved, while the nitrate peak was masked by the larger chloride peak at a flowrate of 0 7 ml/min, and was only just visible at a flow rate of 0 5 ml/min In this case, sulphate and chloride are almost baseline separated, while chloride and nitrate are totally baseline separated Since the water sample was subjected to the cation exchange procedure, nitrate was seen as one sharp peak, which was clearly visible with the conductivity detector and even more so with the UV detector Quantification of each of the three analytes of interest was now possible

The tap water sample contained a higher concentration of sulphate than chloride, but both peaks were still separated and readily identifiable Peak shape was good, and nitrate was again well separated and clearly visible as a single peak due to the cation exchange procedure In general, the addition of Zwittergent 3-14 to the eluent considerably improved the separation efficiency of the recycling system Analysis and quantification was now possible for real samples



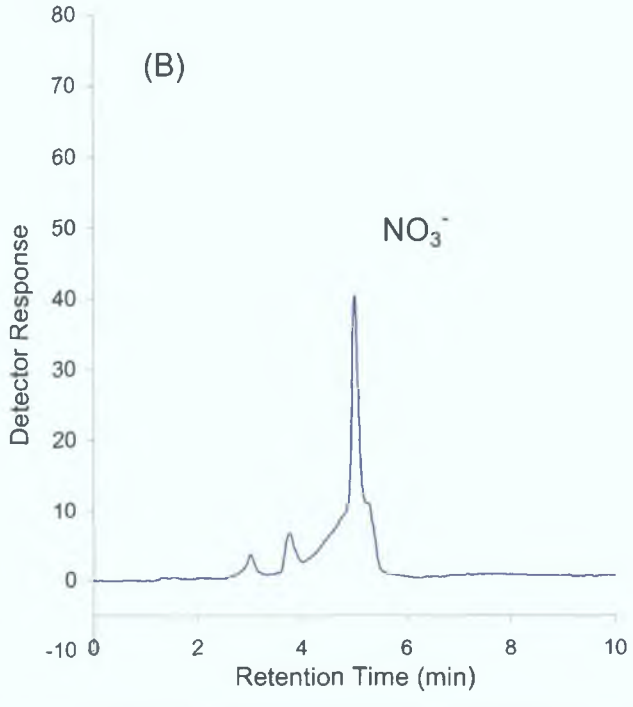


Figure 4.13. Chromatograms of a tap water sample after passage through the exchange cartridge. 2 mM Zwittergent 3-14 as the eluent, flow rate 0.7 ml/min. (A) Conductivity detection (B) UV detection.

4 2 5 Calibrations and Quantification

In order to determine the quantity of the analyte anions in the water samples, a range of standards (from 0 2 to 1 mM) was made up in Milli-Q water for each anion involved and injected repeatedly ($n \geq 3$) onto the column Calibration curves were then produced for sulphate, chloride and nitrate and all curves were found to be linear with R^2 values greater than 0 98 for all analytes

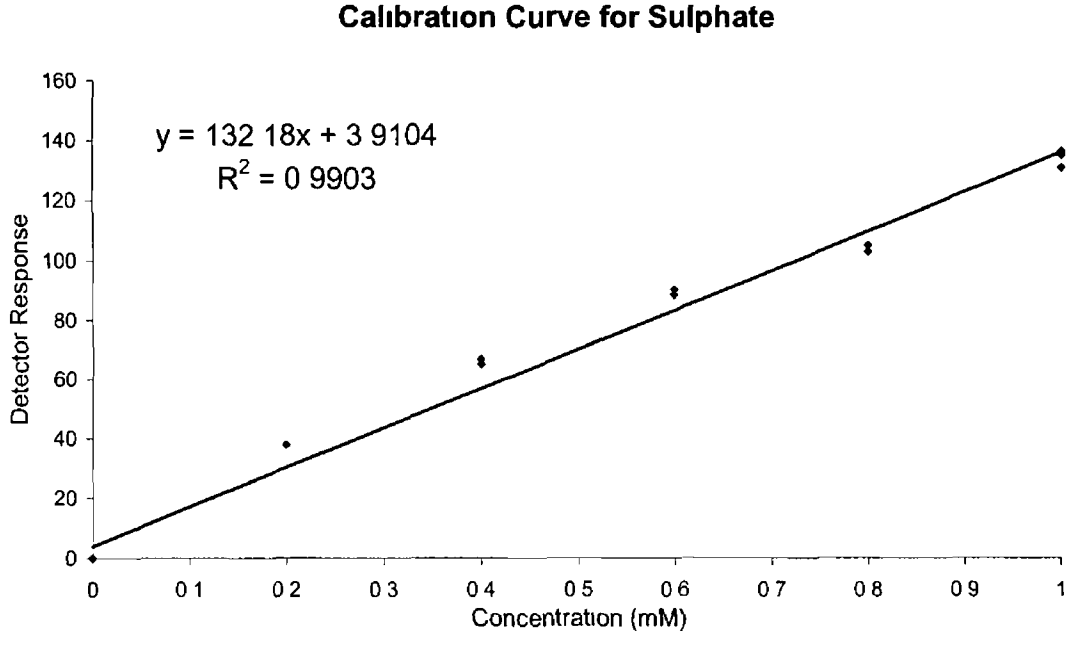


Figure 4 14 Calibration curve for sulphate Aqueous solutions, 2 mM Zwittergent 3-14 as the eluent, flow rate 0 7 ml/min Conductivity detection

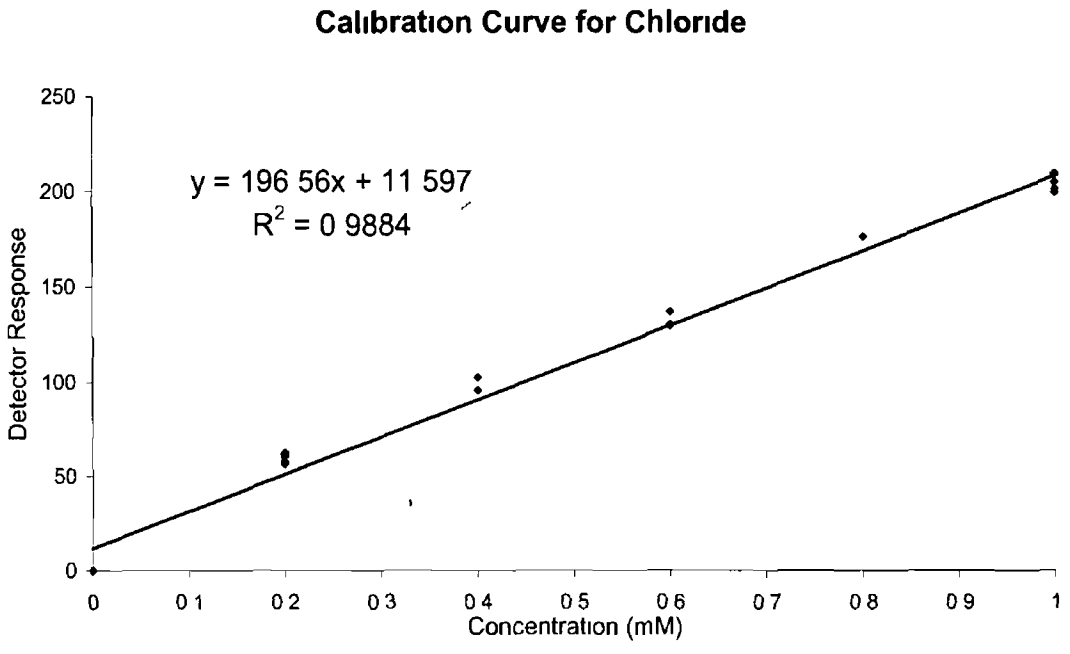


Figure 4 15 Calibration curve for chloride Aqueous solutions, 2 mM Zwittergent 3-14 as the eluent, flow rate 0 7 ml/min Conductivity detection

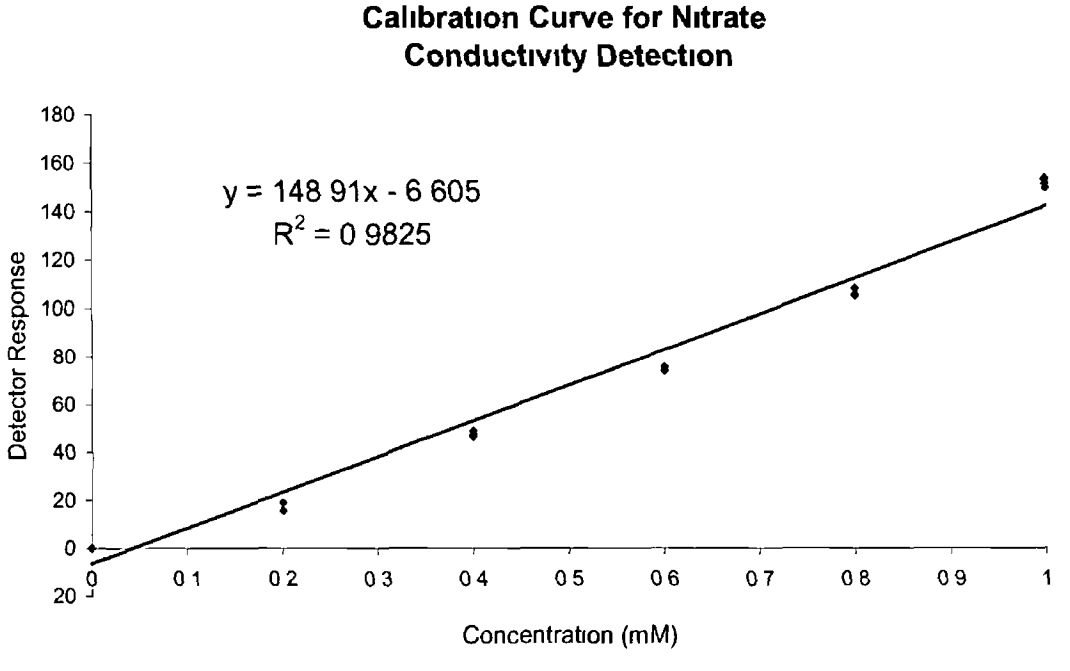


Figure 4 16 Calibration curve for nitrate Aqueous solutions, 2 mM Zwittergent 3-14 as the eluent, flow rate 0 7 ml/min Conductivity detection

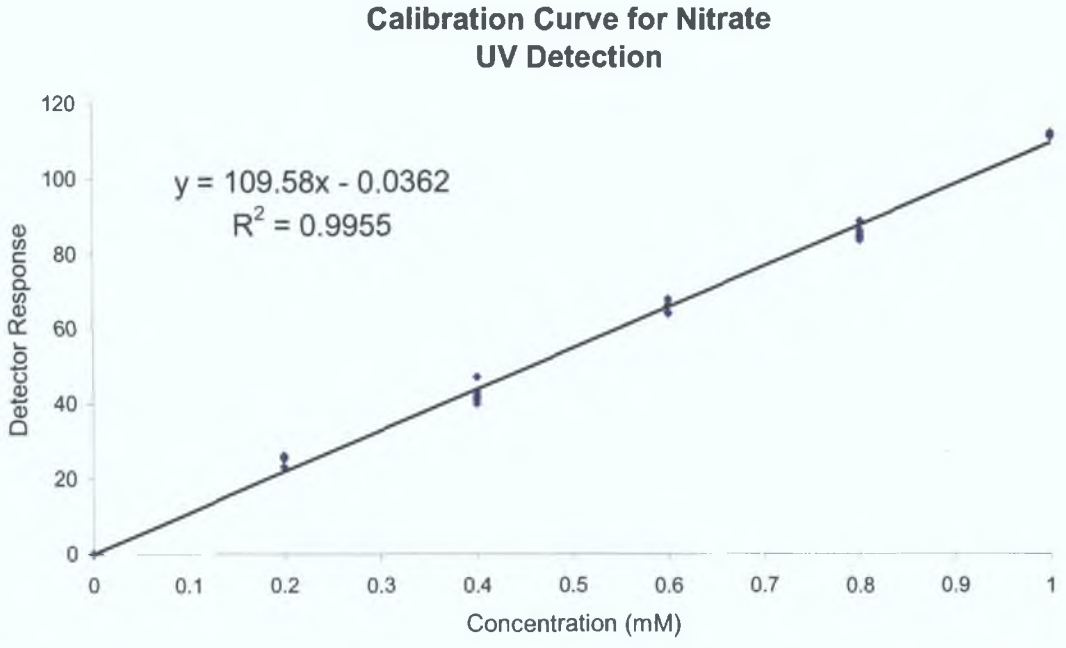


Figure 4.17. Calibration curve for nitrate. Aqueous solutions, 2 mM Zwittergent 3-14 as the eluent, flow rate 0.7 ml/min. UV detection.

The following table outlines the results found for each sample, including the response found for nitrate using both the conductivity detector and the UV detector.

Analyte	Tap water		River water		Mineral water	
Sulphate	1.38 mM	132 ppm	2.41 mM	231 ppm	0.11 mM	11 ppm
Chloride	0.46 mM	16 ppm	0.68 mM	24 ppm	1.18 mM	42 ppm
Nitrate – Cond.	0.1 mM	6 ppm	0.16 mM	9.9 ppm	0.18 mM	11 ppm
Nitrate – UV	0.1 mM	6 ppm	0.15 mM	9.3 ppm	0.18 mM	11 ppm

Table 4.7. Concentrations of the different anions found in the analysed water samples.

According to the EU directive 91/271/EEC on surface water regulations, total nitrogen content of river or drinking water must not exceed 10 ppm. Total nitrogen content includes organic nitrogen, nitrate and nitrite. The samples were spiked with small

amounts of nitrite to ascertain if any unknown peaks were due to nitrite, which they were not Therefore at 6 ppm nitrate, the tap water analysed was within EU limits for total nitrogen content The river water on the other hand, at over 9 ppm, was almost at the maximum level As the most common form of nitrate pollution is from agricultural waste such as fertilisers, this high nitrate level was not surprising The river sampled passes through many farms before entering Dublin city, where the sample was taken, while tap water undergoes water purification before it is distributed into the local water supply However, the surprising result is the mineral water At 11 ppm it is in fact higher than EU regulations allow for drinking water Even more interesting to note is that the bottle's label quotes the nitrate at an even higher level of 14 ppm

Sulphate and chloride are not as strictly monitored as nitrate Chlorine is in fact the main component used in the purification of water, so levels are expected to be high Even though the levels of sulphate and chloride are not enforced, they are monitored and it is recommended that they remain below 250 ppm each The above results comply with these expected values

4 2 6 Standard Additions

Since the Finches mineral water had a 'table of mineral content' quoting the concentration of sulphate, chloride and nitrate (among others) to be found in the water, it was decided to construct a standard addition curve, to further verify the method's accuracy A range of standards was again made up (from 0 2 to 1 mM) this time in the mineral water rather than with Milli-Q water, and injected repeatedly (n = 3) onto the column

All curves were again linear with R^2 values greater than 0 97

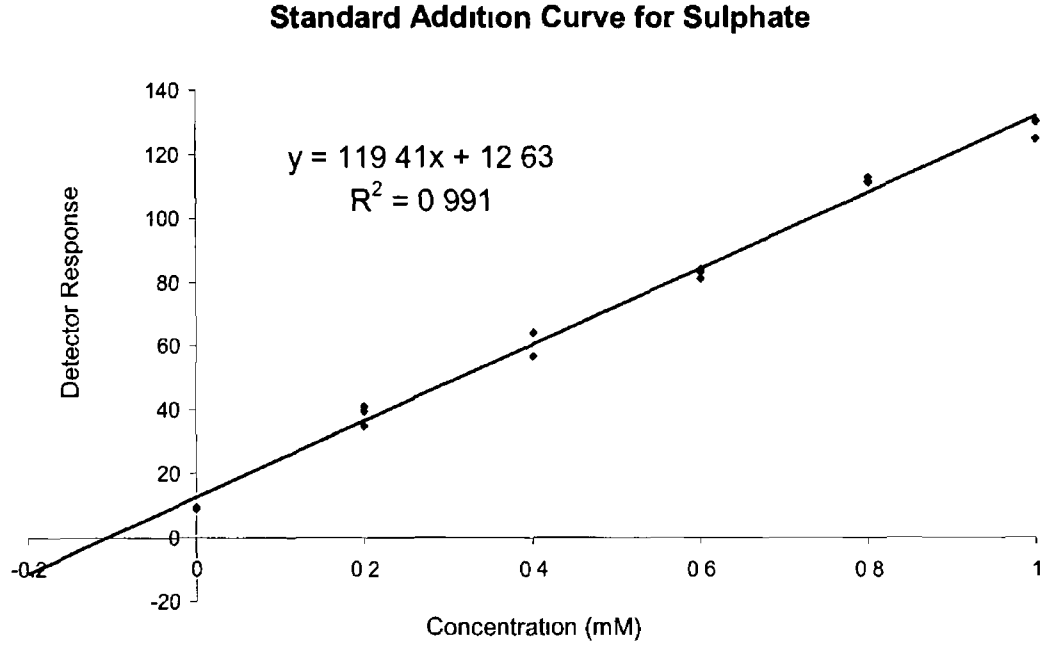


Figure 4 18 Standard addition curve for sulphate Aqueous solutions made up in mineral water, 2 mM Zwittergent 3-14 as the eluent, flow rate 0 7 ml/min Conductivity detection

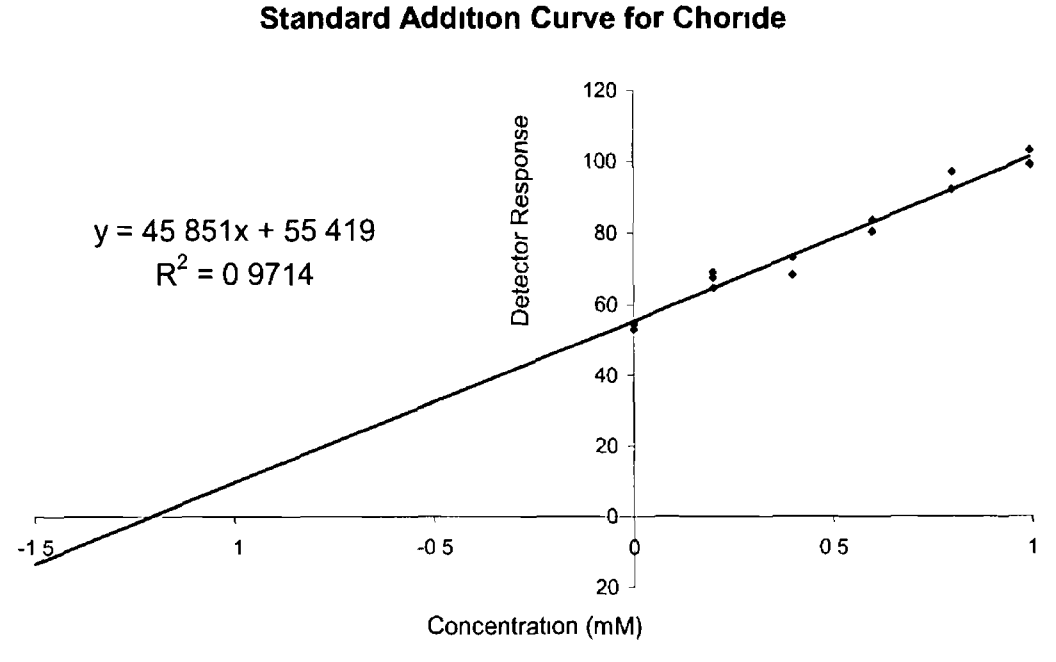


Figure 4 19 Standard addition curve for chloride Aqueous solutions made up in mineral water, 2 mM Zwittergent 3-14 as the eluent, flow rate 0 7 ml/min Conductivity detection

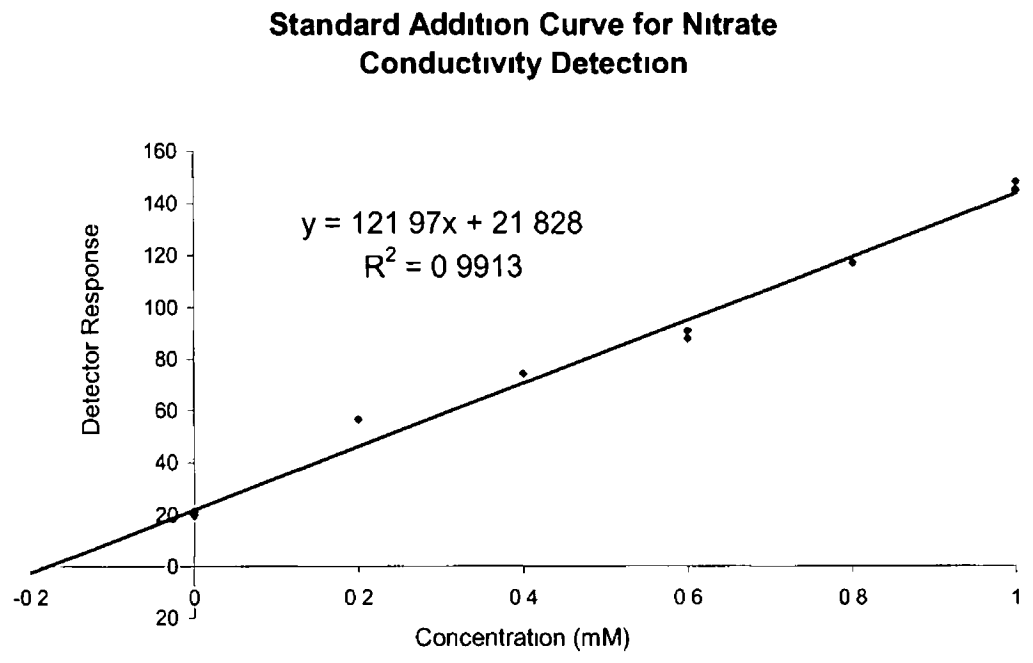


Figure 4 20 Standard addition curve for nitrate Aqueous solutions made up in mineral water 2 mM Zwittergent 3-14 as the eluent, flow rate 0 7 ml/min Conductivity detection

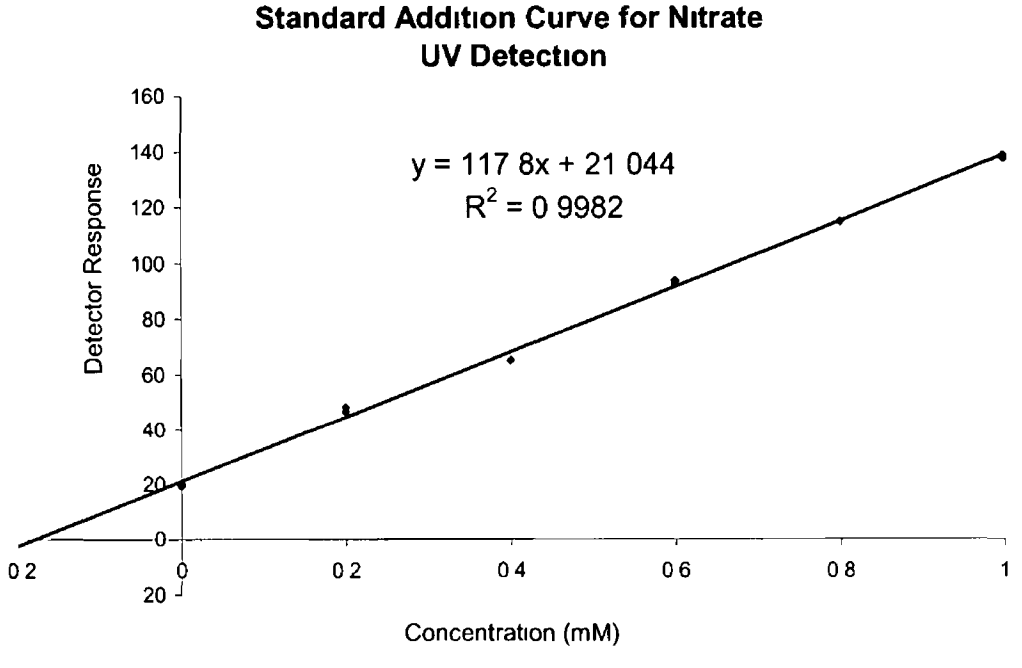


Figure 4 21 Standard addition curve for nitrate Aqueous solutions made up in mineral water, 2 mM Zwittergent 3 14 as the eluent, flow rate 0 7 ml/min UV detection

The following table outlines the results found for each ion, including the response found for nitrate using both conductivity and UV detection

Analyte	Conc (mM) Calibration Curve	Conc (mM) Standard Addition Curve	Conc (ppm) Standard Addition Curve	Conc (ppm) Finches bottle
Sulphate	0 11	0 11	10 56	10 8
Chloride	1 18	1 21	42 96	49 9
Nitrate – Cond	0 18	0 18	11 16	14
Nitrate – UV	0 18	0 18	11 16	-

Table 4 8 Concentrations of the anions found in the analysed mineral water sample compared with those quoted on the bottle

As can be seen from this table, the standard addition results agreed well with those found using a normal calibration curve, and they also were close to those listed in the table of contents, for the mineral water

4.4 CONCLUSIONS

The newly developed recycling system showed greatly improved reproducibility over the non – recycled mode due to the constant recycling of the water eluent, and therefore reduction in any loss of Zwittergent 3-14 from the stationary phase With this system, the column was coated only once, and then could be almost run indefinitely on a fixed volume of eluent, (as low as 200 ml) without the need for regular column regeneration Even though this system showed slight increased retention when compared with the non – recycled method, this was not considered a drawback or a disadvantage

However, poor peak shapes, particularly for real samples was a disadvantage of the method

The addition of Zwittergent 3-14 to the eluent in the recycling system has also proven to be very successful This approach reduced retention times, and therefore analysis time, but did so without any loss of resolution between the main anions of interest In fact it actually improved resolution and allowed the baseline separation of chloride and nitrite Peak shapes were improved overall, with all peaks found to be sharper leading to an increase in efficiency In addition, both peak retention time and area precision was considerably improved Although the addition of the reagent to the eluent removed the simplicity of using simply water, the zwitterionic nature of the reagent meant detector sensitivity was unaffected, and as the eluent was being recycled, eluent preparation only had to be carried out once during the many months of running time

Calibration curves and standard addition curves were constructed and linearity was found to be greater than $R^2 = 0\ 98$ for all curves The method was successfully applied to real water samples including Finches mineral water All results found for this mineral water closely agreed with concentrations quoted on the bottle's 'table of contents' The method has proven to be both accurate and reproducible, however, further validation and application is detailed in the final chapter

CHAPTER FIVE

METHOD VALIDATION

The newly developed method of using a Zwittergent 3-14 eluent with the recycling system had proven to be both accurate and reproducible To complete the development of this novel method, further work was carried out Included in this was the determination of an elution series for the common inorganic anions, determination of the detection limits of several of these anions, comparison with another validated technique (IC), and finally the method was further applied to a saline sample for the analysis of iodide

5.1 Elution Series

1 mM standards were prepared for each anion and all injections were carried out with the recycling system using 2 mM Zwittergent 3-14 as the eluent and a flow rate of 0 7 ml/min The elution order found correlated with that quoted by Umemura *et al* who used a simple water eluent [1] Table 5 4 shows that the elution order for anions having the same cationic counter - ion (e g sodium) is as follows

$$S_2O_3^{2-} < SO_4^{2-} < H_2PO_4^- < Cl^- < NO_2^- < C_2H_3O_2^- < Br^- < NO_3^-$$

$$< HCO_3^- < C_2O_4^{2-} < ClO_3^- < I^- < SCN^-$$

Other trends to be recognised in the table are that anions with different monovalent counter cations (e g Na^+ and K^+) have similar retention times As was expected, divalent counter cations increased the retention times of the anions but upon exchange with sodium, these times returned to those similar to their monovalent counterparts

Analyte	Retention Time (min)
NaS_2O_3	2 97
Na_2SO_4	3 03
$CaSO_4$	3 05
$CaSO_4$, exchanged with sodium	3 03
KH_2PO_4	3 20
KH_2PO_4, exchanged with sodium	3 17
NaCl	3 45
KCl	3 48
KCl, exchanged with sodium	3 48
$CaCl_2$	3 68
$CaCl_2$, exchanged with sodium	3 45
$NaNO_2$	4 03
$NaC_2H_3O_2$	4 28
KBr	4 37
KBr, exchanged with sodium	4 40
$NaNO_3$	5 15
KNO_3	5 30
KNO_3, exchanged with sodium	5 17
$CaNO_3$	6 15
$CaNO_3$, exchanged with sodium	5 17
$KHCO_3$	6 73
$KHCO_3$, exchanged with sodium	6 75
$Na_2C_2O_4$	6 87
$NaClO_3$	6 98
NaI	10 52
NaSCN	17 12

Table 5 1 Elution order for the different anions, and their various counter cations

The chromatogram below in Figure 5.1 shows the separation of seven different anions using this new system. When compared with the separation achieved for the same mixture in chapter three, peak shape has improved, as has resolution. This again underlines the improvements that the recycling system and the addition of the Zwittergent 3-14 have had on this technique.

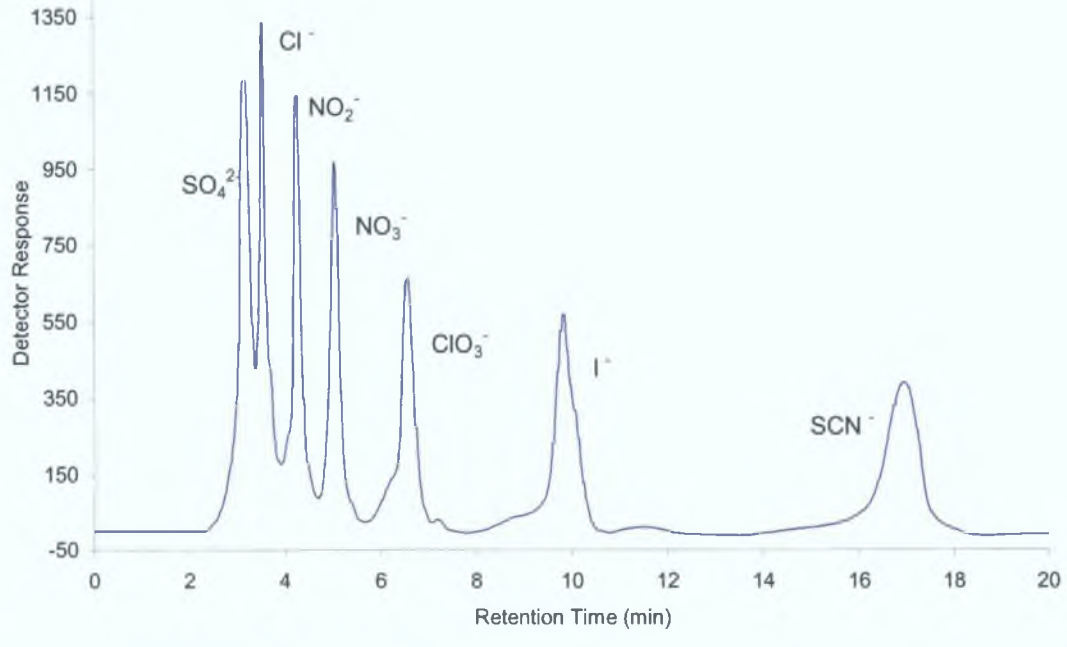


Figure 5.1. Chromatogram showing a separation of seven of the anions listed above, in their sodium form. Recycling system, 2 mM eluent, flow rate 0.7 ml/min and conductivity detection. Concentrations are 1.5, 2, 2.5, 2.5, 3, 4, 5 mM respectively.

5.2 Detection Limits

An investigation into the limits of detection of the technique for several common anions was carried out. To do this, dilute standards were made up and further diluted down until the analyte peaks were no longer visible. Detection limits were then calculated as the concentration of each anion that was equivalent to three times the signal to noise ratio. To increase the peak signal for the analytes the injection loop was increased to a 50

μL loop. At the beginning of this section, a sample of Milli-Q water was injected onto the column to investigate the presence of any interfering anions. As can be seen from the following chromatographs (Figures 5.2 and 5.3), there are indeed several anions present in the Milli-Q water. Although they were not of a high enough concentration to significantly interfere in the calibration work done previously, they would have interfered with analyte ions in the evaluation of detection limits.

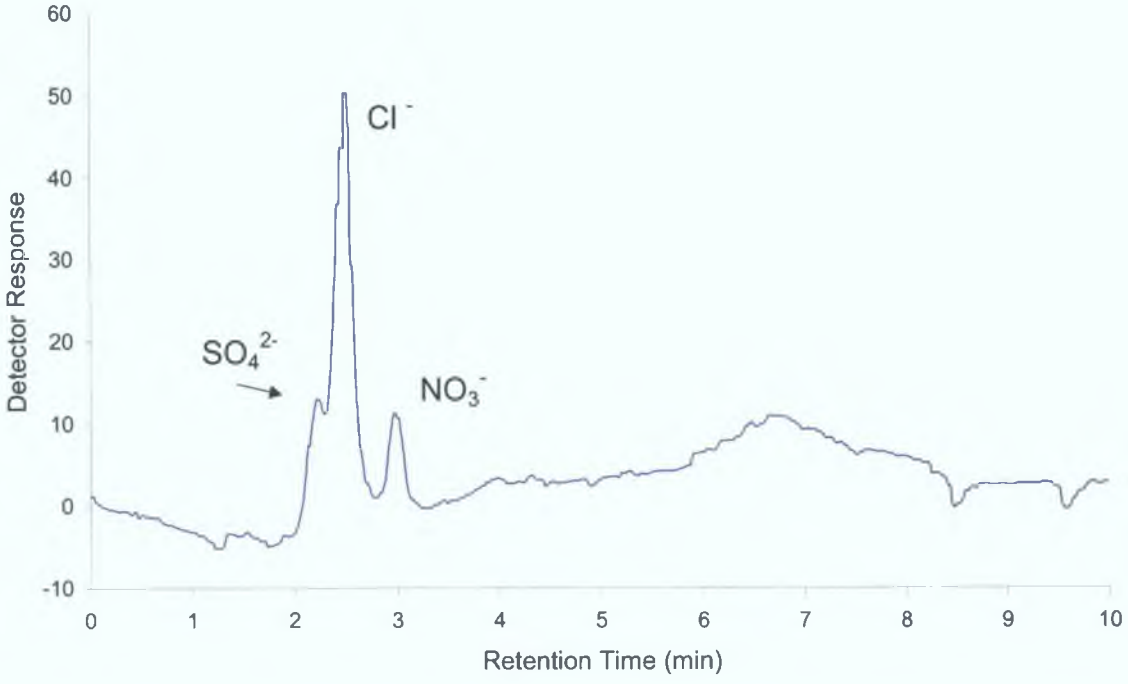


Figure 5.2 Chromatogram showing the presence of anions in Milli-Q water. 2 mM Zwittergent 3-14 as the eluent, flow rate 0.7 ml/min, conductivity detection.

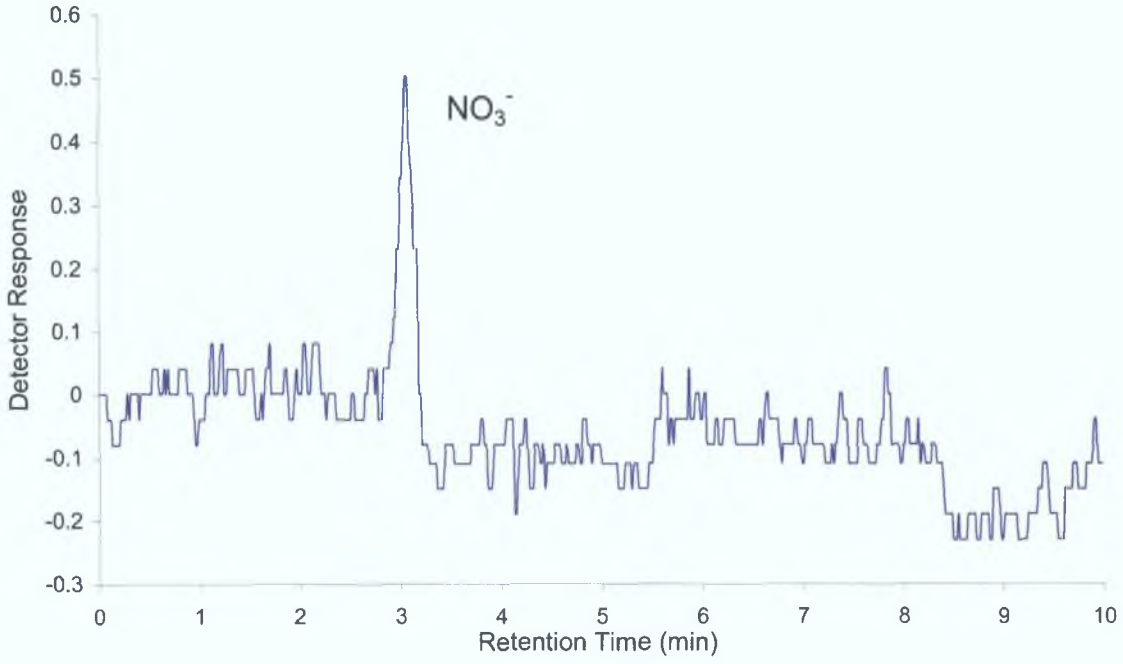


Figure 5.3. Chromatogram showing the presence of anions in Milli-Q water. 2 mM Zwittergent 3-14 as the eluent, flow rate 0.7 ml/min. UV detection.

Even though the eluent is prepared form Milli-Q water, it was possible to see these anions at trace levels when a blank was injected onto the column, as they had been continuously removed from the eluent due to the recycling exchange cartridges. The eluent has effectively been cleaned up by these cartridges and was purer than the Milli- Q water from which it was prepared. For this reason, it was decided to use certified ultra pure water, (Water G, CHROMASOLV®, Riedel-de Haën, supplied by Sigma-Aldrich Ltd., Airton Road, Tallaght, Dublin 24) in which to make up the standard solutions. When a sample of this ultra pure water was injected onto the column, no anions were noticeably present with either UV detection or conductivity detection (Figure 5.4).

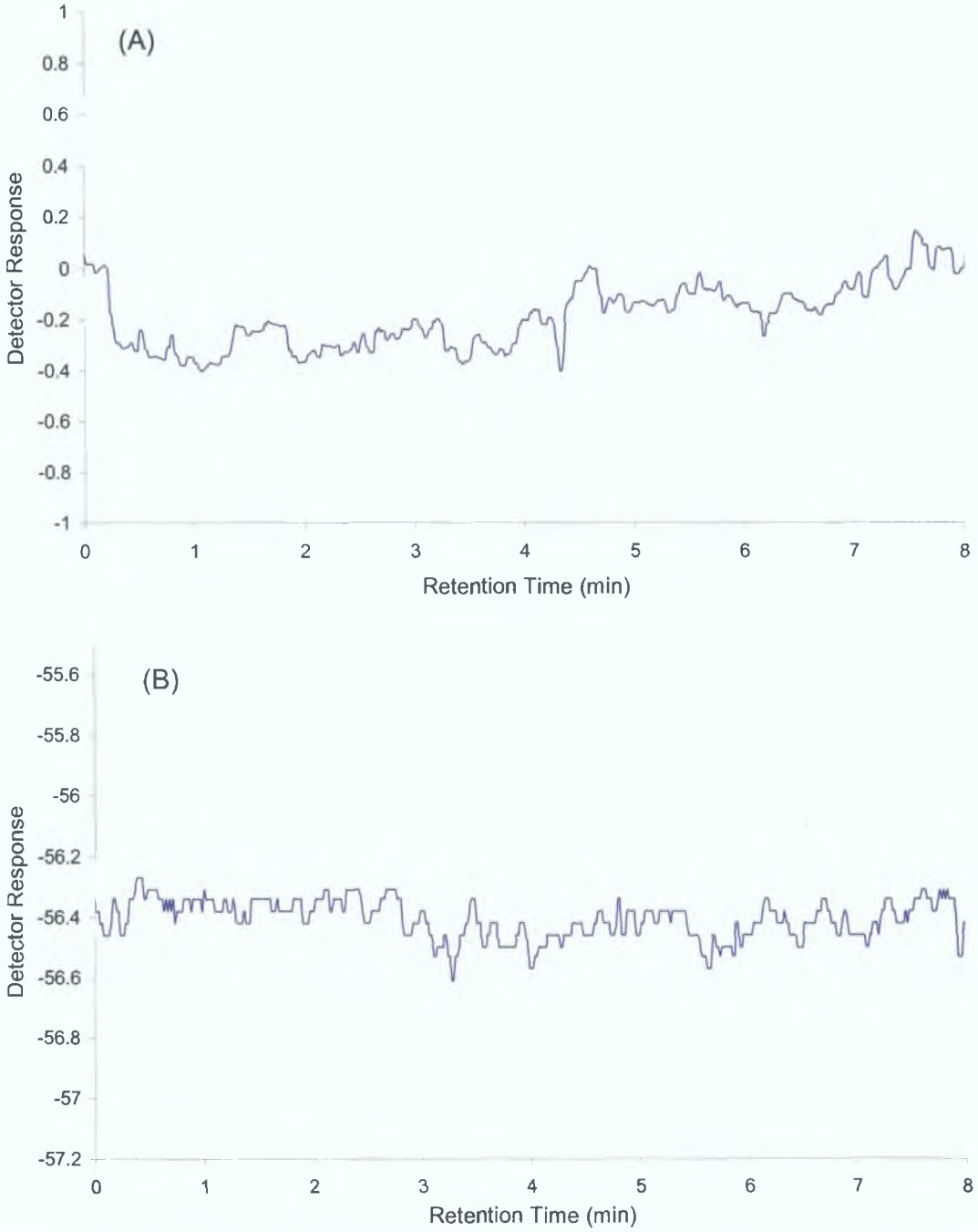


Figure 5.4. Chromatograms showing the absence of interfering anions in Chromasolv ultra pure water. 2 mM Zwittergent 3-14 as the eluent, flow rate 0.7 ml/min. (A) Conductivity detection (B) UV detection.

All standards used in the determination of the detection limits were therefore prepared using this Chromasolv water Serial dilutions were made using a microlitre pipette, to ensure accuracy at the lower levels The following results were found

Analyte	Lowest Injected Standard	S/N Ratio	Detection Limit	Detector
Sulphate	20 ppb	6 3	9 ppb	Cond
Chloride	20 ppb	4	15 ppb	Cond
Nitrite	20 ppb	3 2	18 ppb	UV
Nitrate	20 ppb	3 5	17 ppb	UV

Table 5 2 Detection limits for the new recycling system with 2 mM Zwittergent 3-14 as the eluent

As expected with when using a zwitterionic eluent, the background conductivity was very low and therefore the technique proved to be very sensitive, comparable to that obtained using the alternative technique of suppressed ion chromatography However the UV detector baseline was less noisy at these low levels and proved more sensitive for nitrate and nitrite than the conductivity detector The following chromatograms show the lowest injected standards for each of the four analytes

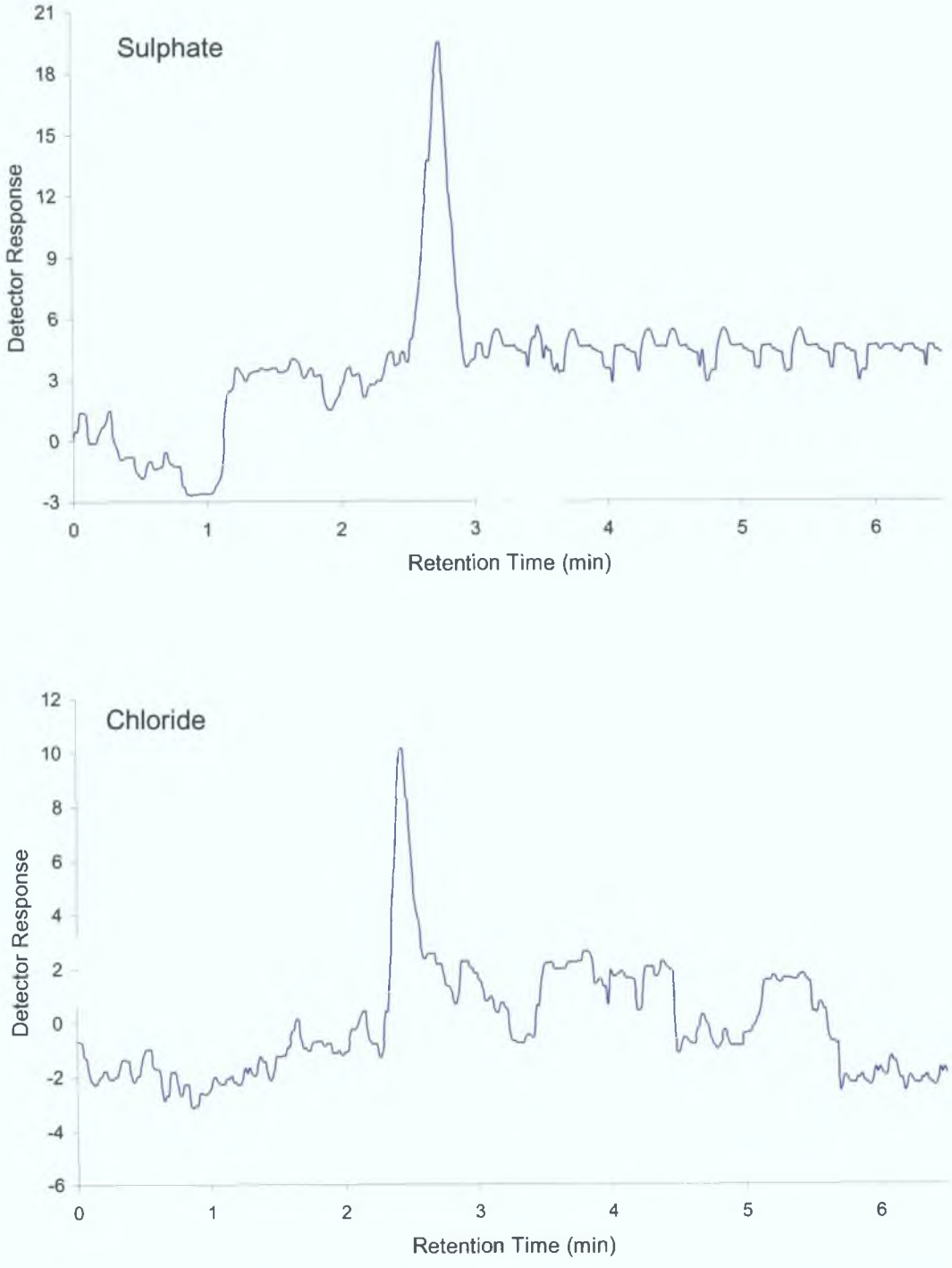


Figure 5.5. Chromatograms of sulphate and chloride at 20 ppb. Recycling system, 2 mM Zwittergent 3-14 as the eluent, flow rate 0.7 ml/min and conductivity detection.

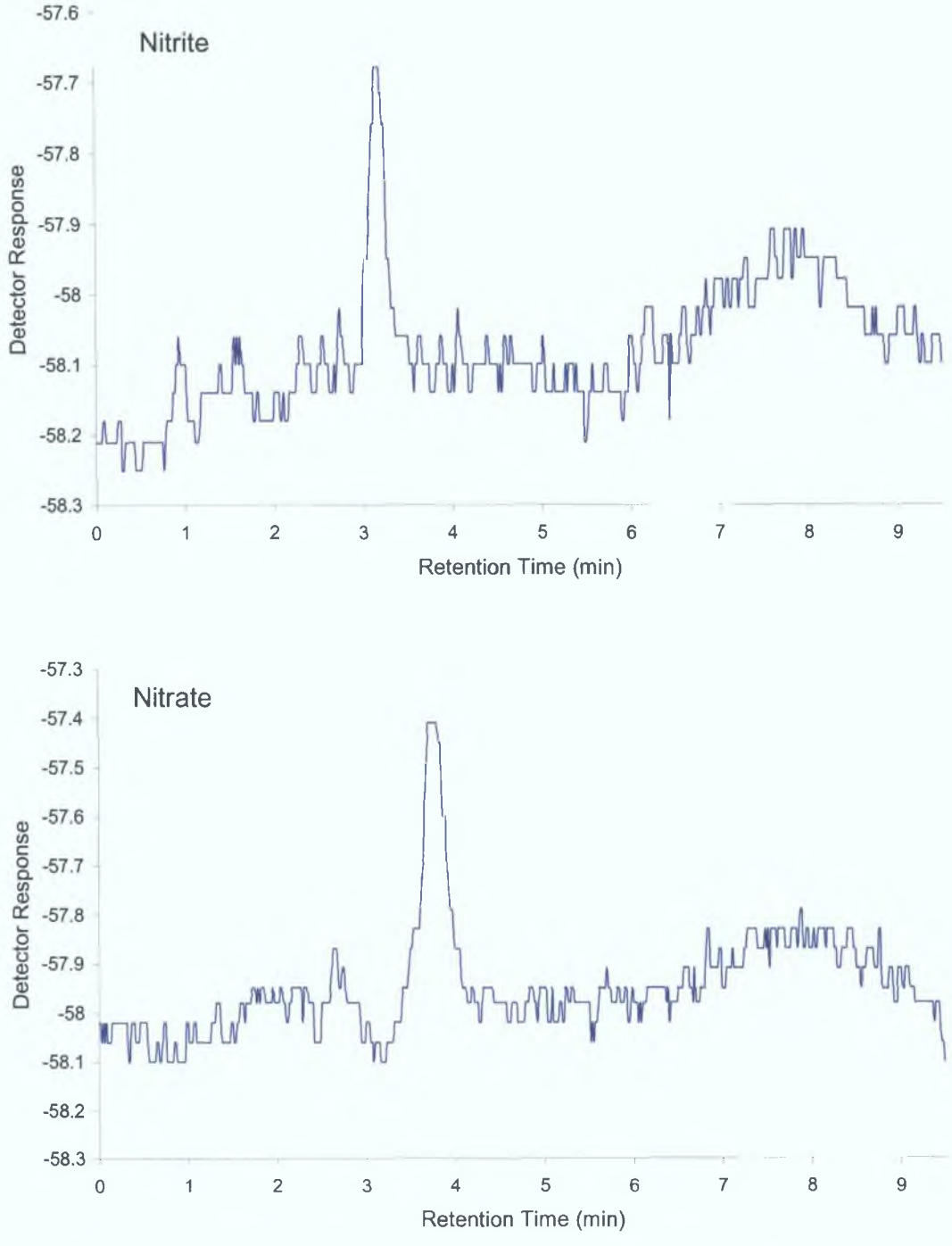


Figure 5.6. Chromatograms of nitrite and nitrate at 20 ppb. Recycling system, 2 mM Zwittergent 3-14 as the eluent, flow rate 0.7 ml/min and UV detection.

Obviously the detection limits of these standards do not necessarily equate to detection limits possible with real life samples, where matrix peaks could affect the detection limits, especially with the non-selective conductivity detector. However, the UV detector still

proved to be very sensitive for nitrate, nitrite and other UV absorbing analytes, as was evident in the water samples analysed previously.

5.3 Ion Exchange Chromatography

To date, it has been shown that this novel recycling system has the potential to be applied to the analysis of water samples. To further verify this potential, it was decided to compare the recycling system to an existing validated analytical technique, such as suppressed ion exchange chromatography.

5.3.1 Apparatus and Instrumentation

To compare electrostatic ion chromatography with a conventional ion exchange method, it was decided to use an AS17 25 cm analytical column and an AG17 guard column (Dionex (U.K.) Ltd., Surrey, England) with a 45 mM NaOH eluent (NaOH 50 % solution in water, Aldrich, Sigma –Aldrich Ltd., Airton Road, Tallaght, Dublin 24). The suppressor used was a CARS ^{TM} (Continuous Anion Regeneration System manufactured by SeQuant, Umeå, Sweden) which exchanged the Na^+ ions of the eluent with H^+ ions. In order to further compare the methods, the flow rate was kept at 0.7 ml/min. UV detection was carried out at 210 nm.

5.3.2 Reproducibility

Injections were carried out in triplicate and percentage relative standard deviations of the retention times were calculated and compared with those found using EIC. As can be seen from the following table, the % RSD's for both techniques were lower than 1 %.

Analyte	% RSD Rt Time EIC	% RSD Rt Time IC
Sulphate	0 30	0 20
Chloride	0 53	0 25
Nitrate	0 34	0 89
Nitrite	0 25	0 23

Table 5 3 Comparisons of %RSD in retention times in IC and EIC

5 3 3 Calibrations

With the separation conditions chosen, the three test anions (nitrate, sulphate and chloride) were not successfully separated Sulphate and nitrate were not adequately resolved However, despite this fact, sulphate does not absorb at 210 nm, therefore, nitrate could be successfully quantified with the UV detector Therefore, it was possible to analyse the mineral water for its chloride and nitrate content, but not its sulphate content

Standard solutions of nitrate were injected onto the column, ranging from 0 2 mM to 1 mM, and calibration curves for both detectors were prepared Both showed good linearity, similar to that found with EIC Table 5 2 outlines the results found for nitrate, using the UV detector, and also compares them to those found with EIC

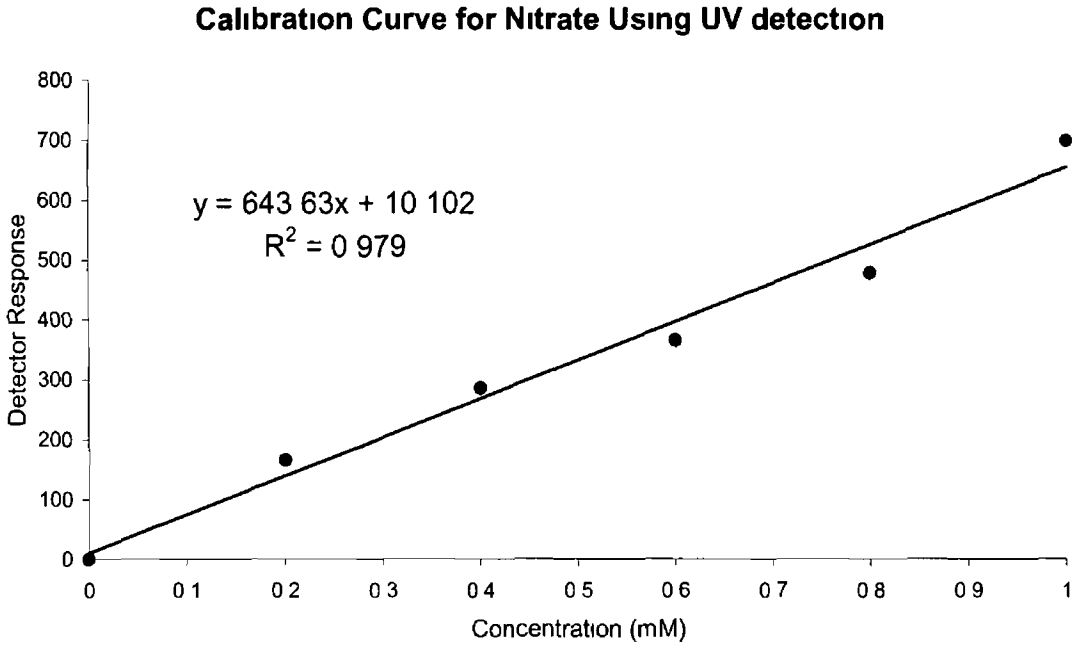


Figure 5 7 Calibration curve for nitrate with IC Aqueous solutions, 45 mM NaOH as the eluent, flow rate 0 7 ml/min UV detection at 210 nm

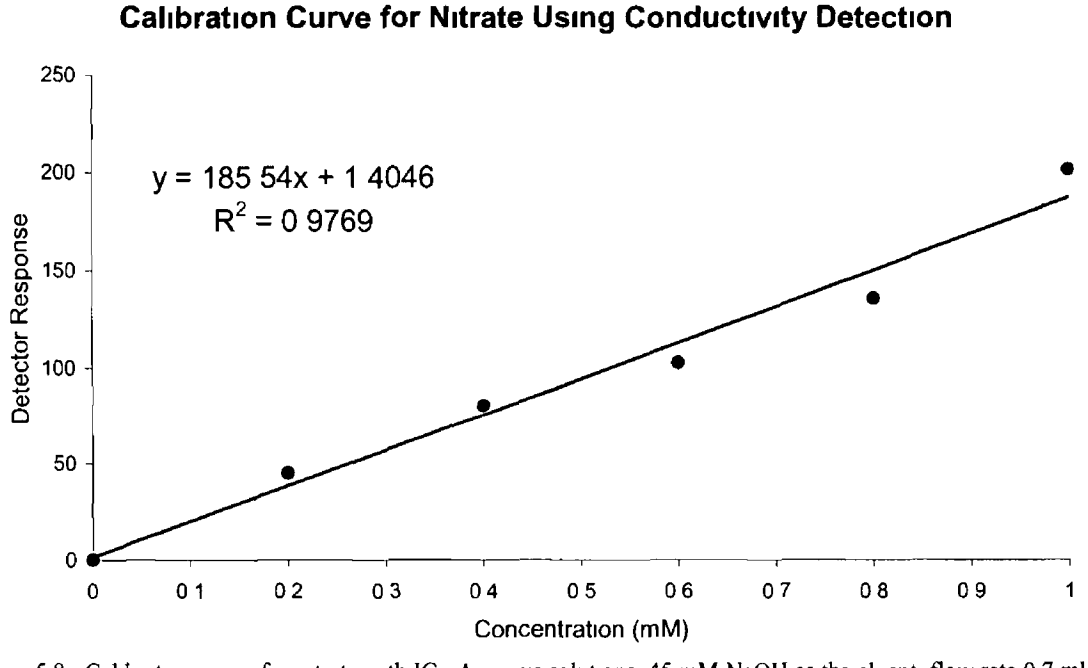


Figure 5 8 Calibration curve for nitrate with IC Aqueous solutions, 45 mM NaOH as the eluent, flow rate 0 7 ml/min Suppressed conductivity detection

A Finches bottled water sample was injected onto the column and analysed for its nitrate content The result was found to match exactly that found using EIC

Source	Concentration (mM)
IC calibration curve (UV)	0 18
EIC calibration curve	0 18
EIC standard addition curve	0 18

Table 5 2 Comparison of results found for nitrate analysed with IC and EIC

Standard solutions of chloride were also injected onto the column, ranging from 0 2 mM to 1 mM A calibration curve was prepared, which showed good linearity, with an R^2 figure greater than 0 98, similar to that found with EIC Table 5 2 outlines the results found for chloride, using suppressed conductivity detection, and also compares them to those found with EIC

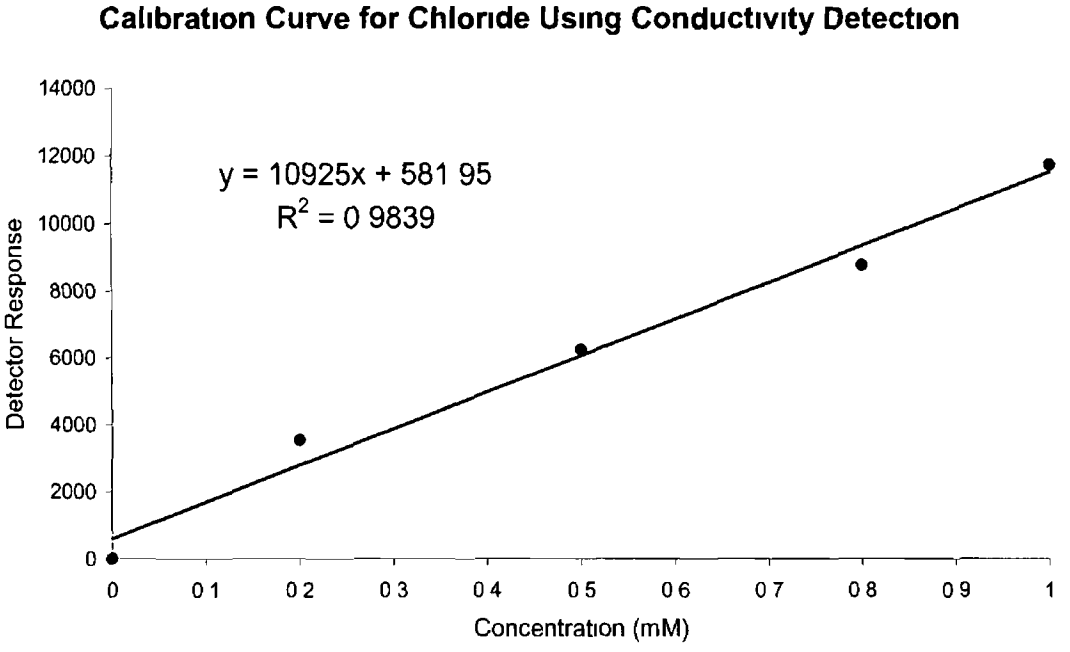


Figure 5 9 Calibration curve for chloride with IC Aqueous solutions 45 mM NaOH as the eluent, flow rate 0 7 ml/min Suppressed conductivity detection

Source	Concentration (mM)
IC calibration curve	1 17
EIC calibration curve	1 18
EIC standard addition curve	1 21

Table 5 3 Comparison of results found for chloride, analysed with IC and EIC

As can be seen from the Table 5 2 and Table 5 3, the level of chloride and nitrate found in the mineral water, using IC was very much in agreement with that found using EIC

5.3 4 Conclusions

With the conditions chosen for this comparison, the three test anions were not separated as well as they could have been using the chosen ion exchange column Despite this, results from the IC analysis, which is an accepted accurate and precise technique, corresponded closely to those obtained using the developed EIC method Analysis of the same water sample using both methods showed excellent correlation with results of 0 18 mM and 1 2 mM for nitrate and chloride respectively Since the developed EIC method compared well with ion exchange chromatography, it could now be stated that this newly developed recycling system was both reproducible and accurate

5.4 Application of EIC to a Saline Sample

The developed method had proven to be accurate when applied to simple water samples such as the mineral water sample for the determination of chloride, sulphate and nitrate However, a further advantage of EIC is that retention of analyte ions is less affected by sample ionic strength than simple IC To illustrate this advantage, it was decided to apply the method to another type of water sample Since iodide was very well separated from all other anions, it was decided to analyse a saline solution of an iodised table salt for its iodide content The main component of any table salt is sodium chloride Upon inspection of Figure 5 1, it can be seen that chloride and iodide were well separated (by 7 minutes) However, upon injection of a relatively concentrated saline sample, the peak representing NaCl was so large that it swamped the iodide peak when using conductivity detection However, this was not the case with UV detection as chloride only absorbs strongly below 200 nm, thus it was possible to perform quantitative analysis of trace iodide, even in the presence of huge excesses of sodium chloride

Iodide is an important constituent of a healthy diet To ensure that the pathological deficiency state (endemic goitre) does not readily occur, potassium iodide can be added to table salt to make up for the modern day dietary lacking of fresh iodine sources, such as fresh fish Even so, it is added in low quantities, in the microgram range per 100 grams of salt The brand analysed here was 'SAXA Iodised Table Salt' (RHM Food Limited, Cheshire, England)

5 4 1 Experimental

A 20 g/L solution of the table salt was made up in Milli-Q water and spiked with 20 mg of potassium iodide salt, resulting in a 0 1 % solution of iodide to chloride A range of standards were then made from this solution, containing from 0 1 to 1 ppm potassium iodide, using a stock 20g/L solution of the table salt A standard addition curve was then constructed to determine the iodide content of the table salt Linearity was excellent with R^2 greater then 0 99 ($n = 6$)

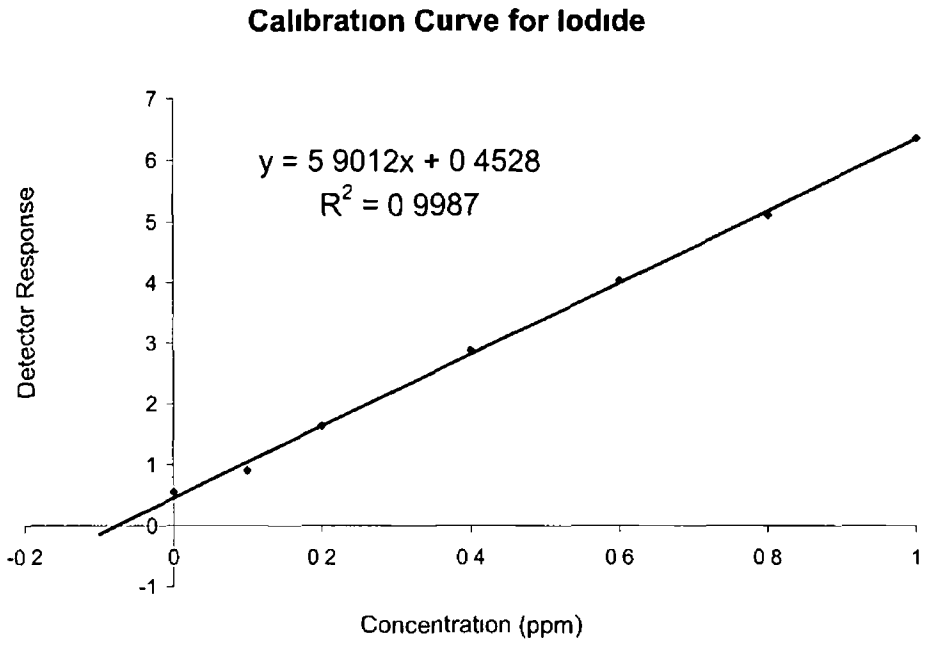


Figure 5 10 Calibration curve for iodide Aqueous solutions prepared with 20 g/L saline solution, 2 mM Zwittergent 3-14 as mobile phase flow rate 0 7 ml/min UV detection at 225 nm

5 4 2 Results

The iodide content in the salt was found to be 77 µg/L in a 20 g/L solution This equates to 3 85 x 10^{-4} % of iodide to chloride in the salt or 385 µg of iodide per 100 g of table salt Although the exact amount of iodide was not quoted on the manufacturer's label, when contacted, they quoted the level of iodide as being in the range of µg/100g of iodide to chloride This agrees with the result found using the recycling system

The following chromatograms represent the separation achieved with this technique plus the excellent response for iodide using UV detection even at µg/L levels Figure 5 12 shows how EIC unlike IC can withstand extreme sample ionic strengths Here the sample contains 100 g/L of NaCl, and the peak for iodide (< 0 1 mg/L) is still strongly retained

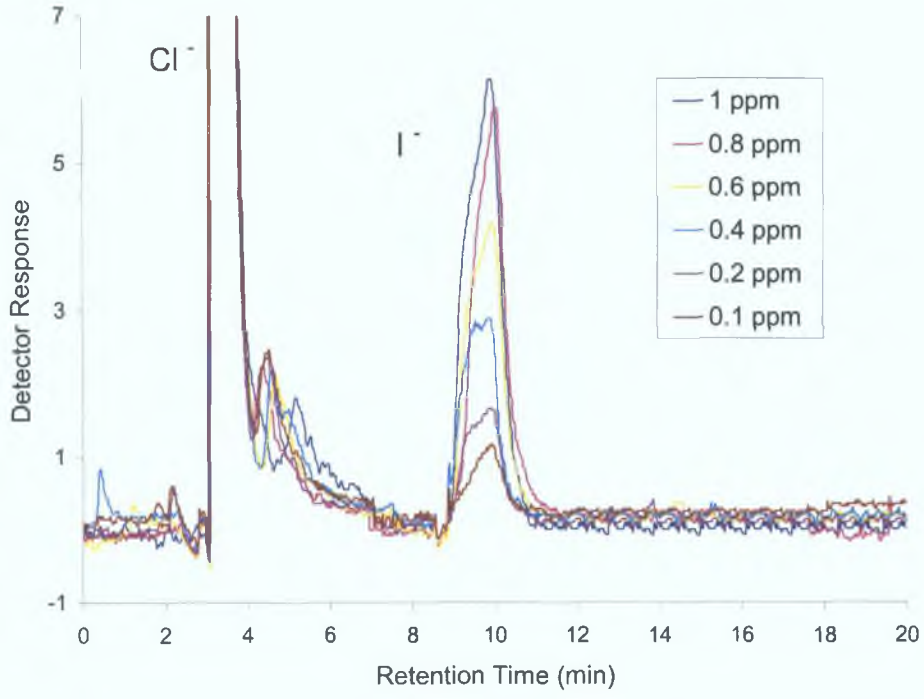


Figure 5.11. Chromatogram of iodised SAXA table salt 20 g/L containing spikes of 0.1 to 1 ppm potassium iodide. Recycling system, 2mM Zwittergent 3-14 as the eluent, flow rate 0.7 ml/min, UV detection at 225 nm.

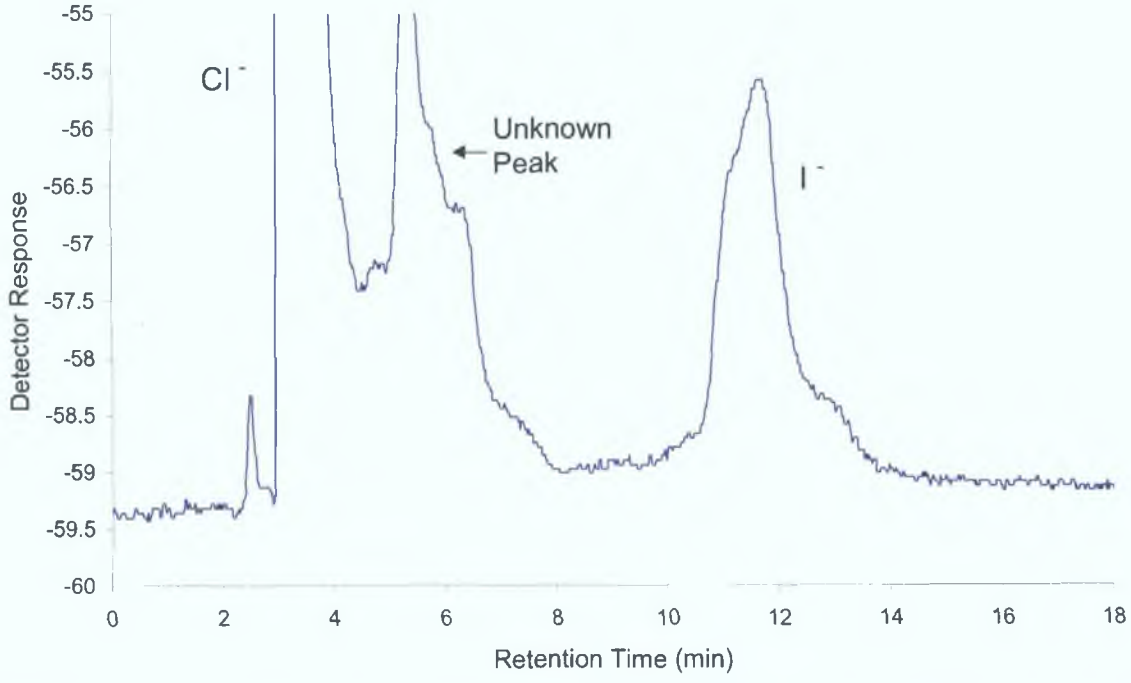


Figure 5.12. Chromatogram showing iodide in a 100 g/L solution of iodised SAXA table salt. Recycling system, Zwittergent 3-14 as the eluent, flow rate 0.7 ml/min, UV detection at 225 nm.

Even though chloride is not considered a UV absorbing anion, at the enormous concentration present in the above sample, chloride does absorb slightly at 225 nm Despite this, the chloride and iodide peaks were still adequately resolved The unknown peak present after chloride was probably due to the caking agent (magnesium carbonate) added to the salt, as described on the container

5 4 3 Conclusions

The recycling system using Zwittergent 3-14 as the eluent proved to be successful in the analysis of iodide in table salt, due to the unique selectivity and sensitivity of the method Despite the extremely low levels of iodide in the salt, and the presence of huge excesses of chloride, the concentration of iodide was found to be 77 µg/L in a 20 g/L solution of table salt Another example of the analytical potential of this technique has been successfully demonstrated

5.5 FINAL REMARKS

A novel analytical technique has been developed that can be successfully applied to the analysis of inorganic anions in water samples When compared with a conventional chromatographic technique, such as ion exchange chromatography, analysis of the same water sample using both methods showed excellent correlation, with results of 0 18 mM and 1 2 mM for nitrate and chloride respectively Reproducibility figures were also compared for the four test anions (sulphate, chloride, nitrite and nitrate) with percentage relative standard deviations of the retention times under 1 % for both methods

Detection limits of the four test anions using this new method were calculated and found to be less than 20 ppb for all four anions The method was also applied to the analysis of trace amounts of iodide in an iodised saline sample The method was well suited to trace analysis due to the very low background noise produced by the eluent Iodide levels in the iodised table salt were found to be 77 µg/L in a 20 g/L saline solution, which agreed with the range quoted by the manufacturers The novel analytical method has proven to be accurate, reproducible and sensitive

Future work that might be carried out in this area could include the use of different surfactants with the recycling system Zwittergent 3-12 or Zwittergent 3-16 are also commercially available and could be used to investigate the possibilities of retention manipulation The use of mixed micelles is also a possibility, as was carried out by Hu *et al* [2] A cationic surfactant such as tetradecyltrimethylammonium could be used along with Zwittergent 3-14 as a mixed coating with the recycling system In this way, it might

prove possible to separate and analyse phosphate and fluoride along with the already separated anions such as chloride, sulphate, nitrite and nitrate

BIBLIOGRAPHY

1 T Umemura, S Kamiya, A Itoh, K Chiba and H Haraguchi, Analytica Chimica Acta, (**349**), 231 – 238, 1997

2 W Hu, P Haddad, K Hasabe, H Cook and J Fritz, Fresenius J Anal Chem, (**367**), 641 – 644, 2000