Development of fast ion chromatography.

by

Damian Connolly B.Sc. (Hons), AMRSC.

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

DOCTOR OF PHILOSOPHY

Dublin City University.
School of Chemical Sciences.
Supervisor: Dr. Brett Paull.

February 2005

Volume 2 of 2.

Contents **Page**

9 Overall conclusions.

List of figures.

List of tables.

5 Fast ion chromatography of common inorganic anions on a short ODS column permanently coated with didodecyldimethylammonium bromide.

5.1 Introduction.

5.1.1 Permanent coating of reversed phase columns with IIR's.

Since the efficiency of conventional anion exchange columns continues to be lower than that of reversed phase C_{18} columns, coating a C_{18} column with a hydrophobic ionic surfactant to convert a highly efficient HPLC column into an ion exchange column of comparable efficiency is a viable option. Permanent coating of reversed phase stationary phases was discussed in more detail in Chapter 1, Section 1-7.

The procedures for permanent coating have been well established. Mullins [1] coated a 250 X 5.0 mm I.D., 5 μm Spherisorb ODS-2 column with 10 m*M* hexadecyltrimethylammonium bromide (CTAB) in 10 % MeOH at 1.5 ml/min, and showed that the concentration of adsorbed CTAB decreased as the concentration of methanol in the CTAB coating solution was increased. Also it was shown by Mullins that depending on coating conditions, the surface coverage for CTAB approached that of the chemically bonded groups themselves. Unfortunately there was a need to remove the surfactant from the column with acetonitrile after each series of experiments and recoat with the same coating solution for the next set of experiments.

Michigami *et al.* [2] used 10 m*M* cetylpyridinium chloride in 10 % MeOH to coat a 50 X 4.6 mm I.D. TSK gel ODS-80T_M column. Again, the coated column allowed for the analysis of about 70 samples after which regeneration of the column was carried out by washing with methanol, followed by a fresh coating of cetylpyridinium chloride.

Ito *et al.* [3] permanently coated both a Capcellpak C_{18} column and a TSK gel ODS-80T_M column with 1 m*M* CTAC in (80:20) H_2O / MeOH. Again during coating, the amount of sorbed CTA^+ decreased with an increase in methanol concentration due to the decrease in hydrophobic interactions between the cetyl group and the octadecyl group on the silica surface.

Cetyldimethylamine was used by Michigami *et al.* [4] to coat a 50 mm X 4.6 mm Capcellpak C_{18} column. These workers used a 10 m*M* cetyldimethylamine solution in 1 m*M* HCl containing 10 % MeOH, and found that the coated column allowed the analysis of about 60 samples before peak tailing was observed and the column needed to be regenerated and recoated. Interestingly, Michigami *et al.* also examined the effect of chain length of the coating reagent upon the retention of thiocyanate using a 1 m*M* citrate eluent at pH 7.0. Increasing the chain length and therefore the hydrophobicity from cetylamine through cetyldimethylamine, cetyltrimethylammonium bromide to cetylethyldimethylammonium bromide caused an increase in the retention of thiocyanate.

Jun *et al.* [5] coated a Waters Novapak C_{18} (4 μm) column (150 X 3.9 mm I.D.) with 5 m*M* 1,12-diaminododecane in 10 % MeOH for the rapid (< 2.5 minutes) determination of sulphate with a 30 μ*M* $K_3[Fe(CN)_6]$ eluent. The advantage of using 1,12-diaminododecane as coating agent was that it resulted in a weak anion exchange group on the column, which in combination with the strong eluting power of the multi-charged hexacyanoferrate (III) anion allowed the rapid elution of sulphate, with the other common inorganic anions eluting in the void.

Later, Jun *et al.* [6] used n-cetylpyridinium chloride to coat a Waters Novapak C_{18} (4 μm) column (150 X 3.9 mm I.D.) column. A solution of 10 m*M* n-cetylpyridinium chloride in 10 % MeOH was used. Jun *et al.* found that the coated column could be used continuously for 4 months with no adverse effect upon retention time or peak efficiency. Also, satisfactory retention times were obtained for five replicate coatings of the same column and for a column to column coating study.

The majority of workers have reported the use of methanol in the coating solution as a means of regulating the amount of coating reagent ultimately coating the column. In this current work, methanol was removed from the coating solution in order to obtain the highest possible anion exchange capacity on the column. It was felt that a high exchange capacity would be necessary to achieve separations of the common group of inorganic anions, given the columns decreased length (3.0 cm) and therefore decreased resolving power. In addition, didodecyldimethylammonium bromide (DDAB) was chosen as the coating reagent. DDAB is a double chained cationic surfactant which had previously been used by Melanson and Lucy [7] and Baryla and Lucy [8] in capillary electrophoresis to coat fused silica capillaries, generating a reversal of electro-osmotic flow (EOF).

5.1.2 Indirect UV detection using phthalate eluents.

A disadvantage of the chromatographic separations discussed in Chapters 2 and 3 is that only UV absorbing anions could be detected. The use of indirect UV detection in the work described herein allowed such anions as chloride, sulphate and phosphate to also be determined along with other UV absorbing anions (nitrate, nitrite, bromate, bromide etc).

Indirect UV detection is discussed more fully in Chapter 1, Section 1-9. A range of UV absorbing eluents have been used for the analysis of common inorganic anions. Jun *et al.* [5] used an eluent of potassium hexacyanoferrate as a eluent for the rapid determination of sulphate based on its UV active absorption at 205 nm and its strong eluting power at low concentration which is necessary to obtain a low limit of detection for indirect UV detection. The detection limit for sulphate, defined as 4.65 times the standard deviation of six replicate analyses of a standard containing 0.08 mg/*L* sulphate was 34 μg/*L*.

The majority of eluent probes used for indirect UV detection are aromatic organic acids. Bidlingmeyer *et al.* [9] used 0.4 m*M* salicylate in an eluent also containing TBA as an ion-interaction reagent. A separation of phosphate, chloride, nitrite, bromide, nitrate, iodide, sulphate and thiosulphate was achieved in < 25 minutes on a 100 mm X 8 mm Resolve C_{18} Radial-Pak 5 μm column. Detection limits by indirect UV detection were as low as 120 μg/*L* for chloride.

The aromatic organic acid chosen for this work was phthalate. Much work has been reported using phthalate as an eluent for indirect UV detection because of its high molar absorptivity in the UV region [1,6,10,11]. Mullins [1] used a 5 m*M* phthalate eluent at pH 4.19 on a 250 X 5 mm Spherisorb ODS column (5 µm) permanently coated with CTAB. With a flow rate of 2 ml/min, an injection volume of 20 µL and indirect UV detection at 300 nm, iodate, phosphate, bromate, chloride, nitrite, bromide, nitrate and chlorate were separated in ~ 12 minutes.

Zou *et al.* [10] separated chloride, nitrite, bromide, nitrate, chlorate, iodide, phosphate and sulphate on a 100 X 4.6 mm I.D. TSK gel ODS-80 T_M (5 µm) column and a mobile phase of 0.5 m*M* phthalate and 0.5 m*M* TBA in 5 % MeOH, pH 6.4. With a flow rate of 1.0 ml/min, the total runtime was again ~ 12 minutes.

Jun *et al.* [6] used a 0.4 m*M* potassium hydrogen phthalate eluent at pH 4.87 to resolve a mixture of lactic acid, chloride, nitrite, bromide, nitrate, succinic acid, malonic acid, malic acid, maleic acid, tartaric acid and sulphate in ~ 21 minutes. The column used was a Nova-pak C_{18} (150 mm X 3.9 mm I.D.) of particle size 4 µm, coated with cetylpyridinium chloride. The flow rate was 2.0 ml/min, the injection volume was 70 µL and detection was at 254 nm. Detection limits were 0.05 mg/*L* (chloride), 0.09 mg/*L* (nitrite), 0.10 mg/*L* (bromide), 0.29 mg/*L* (nitrate) and 0.10 mg/*L* for sulphate.

In this chapter, the coating of a short 3 µm C_{18} column with didodecyldimethylammonium bromide to produce a high capacity anion exchange column is reported. The use of a phthalate eluent for indirect UV detection of common inorganic anions in a range of freshwater and sea water samples is demonstrated.

5.2 Experimental.

5.2.1 Equipment.

The ion chromatograph and analytical columns described in Chapter 4 were also used in this work. The injection loop used was 25 µL during optimisation studies, and 50 µL for analysis of real samples. Detection was by indirect UV at 282 nm during optimisation studies, and 279 nm for analysis of real samples.

5.2.2 Reagents and chromatographic conditions.

The water used for mobile phase and standard preparation was obtained from a Millipore Milli-Q water purification system (Millipore, Bedford, MA, USA). Before coating of the column, the new column was conditioned with 50 % MeOH, supplied by Labscan, (Labscan Ltd., Stillorgan, Dublin, Ireland) at 1 ml/min for ~1 hour, followed by de-ionised water. The DDAB used for permanent coating of the column, was supplied by Aldrich, (Aldrich, Milwaukee, WI, USA). The coating solution of 10 m*M* DDAB was filtered using 0.45 µm nylon membrane filters from Gelman Laboratories (Michigan, USA). The flow rate during column coating was 1 ml/min for 30 minutes with the eluent absorbance monitored at 190 nm to determine breakthrough. The optimised eluent for all separations was 5 m*M* phthalate adjusted to pH 7.5 with dilute NaOH. The eluent was prepared from potassium hydrogen phthalate, (Aldrich). The mobile phase was degassed and filtered before use using 0.45 µm nylon membrane filters from Gelman Laboratories (Michigan, USA). The flow rate used was 2.0 mL/min with column temperature set at 45 °C for all separations.

Standard solutions were prepared as described in Chapter 2 with the exception of phosphate standards which were prepared from their respective potassium salts (Aldrich) and fluoride, carbonate, chloride and sulphate which were prepared from their respective sodium salts (Aldrich). Seawater and tap water samples were collected and stored at 4 °C until use. Seawater samples were diluted with de-ionised water and syringe filtered (0.45 µm nylon membrane filter from

Gelman Laboratories (Michigan, USA)) prior to injection, with tap water samples requiring only a filtration step. River water samples were collected and filtered at source, and analysed within 24 hr.

5.3 Results and discussion.

5.3.1 Coating the column with DDAB.

The IIR chosen to coat the column was didodecyldimethylammonium bromide, $[CH_3(CH_2)_{11}]_2N(CH_3)_2Br$, which has two C_{12} chains resulting in a very high anion exchange capacity coating. A 10 m*M* solution of didodecyldimethylammonium bromide (DDAB) was prepared in de-ionised water, with a sonic bath used to aid dissolution. Due to the nature of this surfactant solution, excessive shaking and/or agitation of the solution was avoided, to prevent generation of foam.

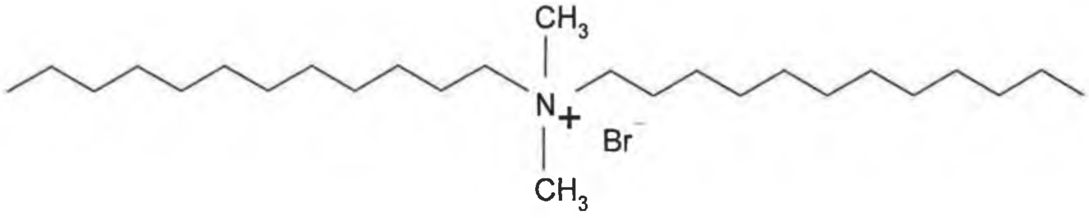

Figure 5-1. *Structure of didodecyldimethylammonium bromide.*

This surfactant solution was pumped through the 3 cm C_{18} column at 1 ml/min, with the column effluent directed through the AD20 absorbance detector. This facilitated the use of the breakthrough method, in which the absorbance of the eluate was monitored at 190 nm (a wavelength at which DDAB was known to absorb). During this experiment, the column was coated for 30 minutes, with the "break-through" plateau reached after approximately 12 minutes, representing ~0.12 mmol (~ 46 mg) coated DDA^+ corresponding to a maximum coating capacity of 120 μequiv, which is high compared to a conventional Dionex 250 mm x 4 mm AS14 column, (65 μequiv).

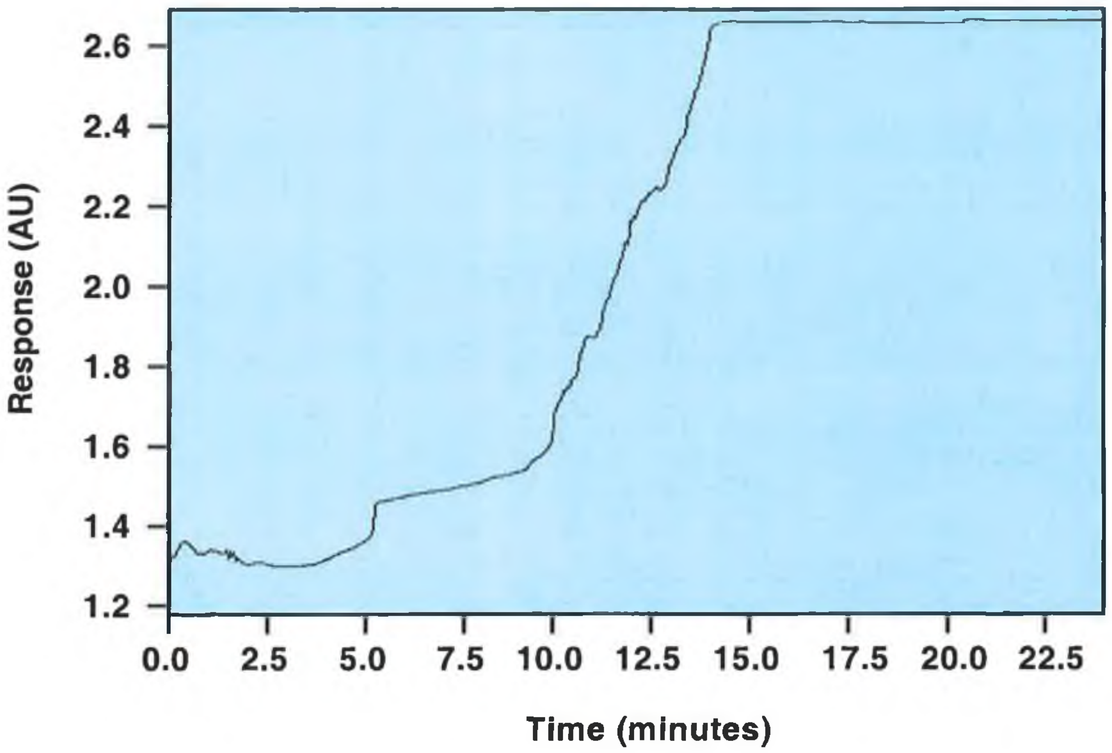

Figure 5-2. *Coating of the Phenomenex Hypersil, (3 μm) 30 mm x 4.6 mm I.D. column with 10 mM didodecyldimethylammonium bromide at 1.0 ml/min with "breakthrough" monitored at 190 nm*

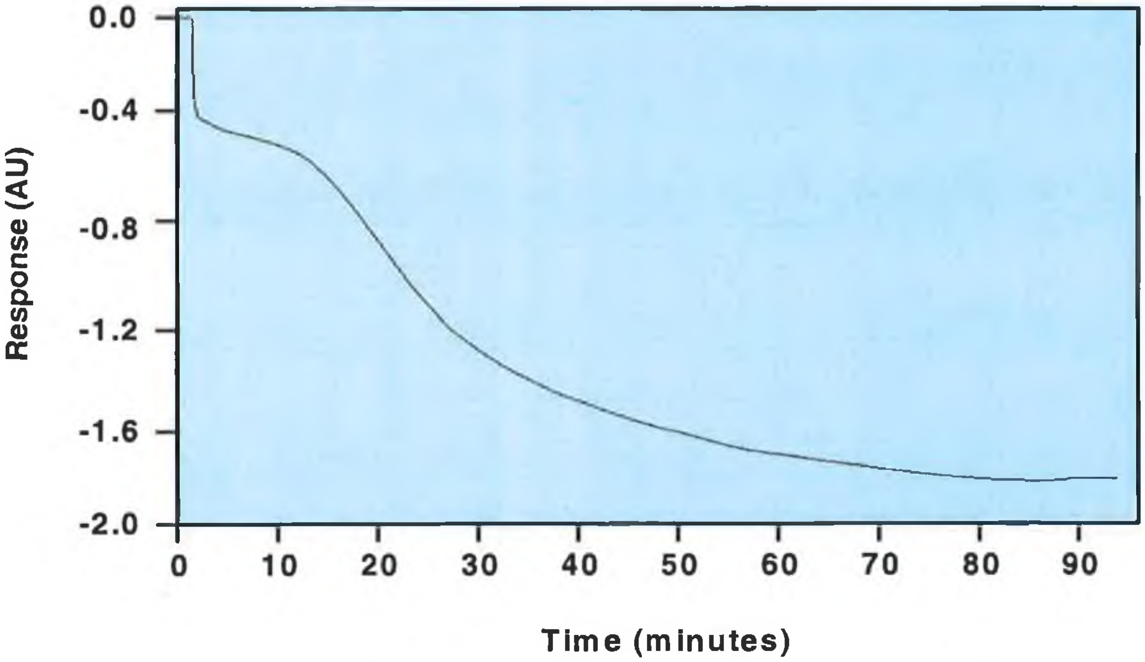

Figure 5-3. *Washing of unbound IIR from the Phenomenex Hypersil, (3 μm) 30 mm x 4.6 mm I.D. column with water at 1.0 ml/min for 100 minutes, subsequent to coating procedure.*

The column was subsequently washed with de-ionised water to remove unbound DDA$^+$ from the column. The remaining bound DDAB effectively converts the hydrophobic C_{18} stationary phase to a stable relatively high capacity anion exchange surface, due to the positively charged ammonium functionality present at the stationary phase/mobile phase interface. This allows the use of a simple anion exchange eluent, in this case a UV absorbing eluent to facilitate indirect UV detection. At the end of each day, the column was washed with water subsequent to the use of phthalate eluents, and stored as such throughout the course of this work.

5.3.2 Preliminary optimisation of eluent concentration.

The group of test anions chosen for this study were those that are typically routinely determined in both river and potable water samples, namely, fluoride, carbonate, phosphate, chloride, bromate, nitrite, sulphate, bromide and nitrate. A major advantage of the chromatography described herein is that non UV-absorbing anions such as chloride, phosphate and sulphate may be determined in real samples by the use of a UV absorbing probe in the eluent, and subsequent indirect UV detection. Phthalate was chosen as the UV absorbing eluent probe in this study, because of its relatively strong eluting strength and high absorbance in the UV region of the electromagnetic spectrum.

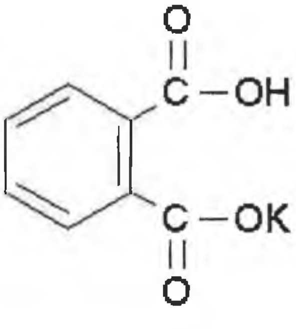

Figure 5-4. *Structure of potassium hydrogen phthalate.*

Eluent concentration was varied from 1 m*M* to 4 m*M* with eluents initially adjusted to pH 6.0 to ensure phthalate was ionised ($pK_1 = 2.95$ and $pK_2 = 5.41$). Throughout the optimisation of mobile phase, chromatographic conditions were held constant at a flow rate of 1 ml/min, column temperature at ambient (~22 °C), loop size of 25 μL and detection wavelength at 282 nm.

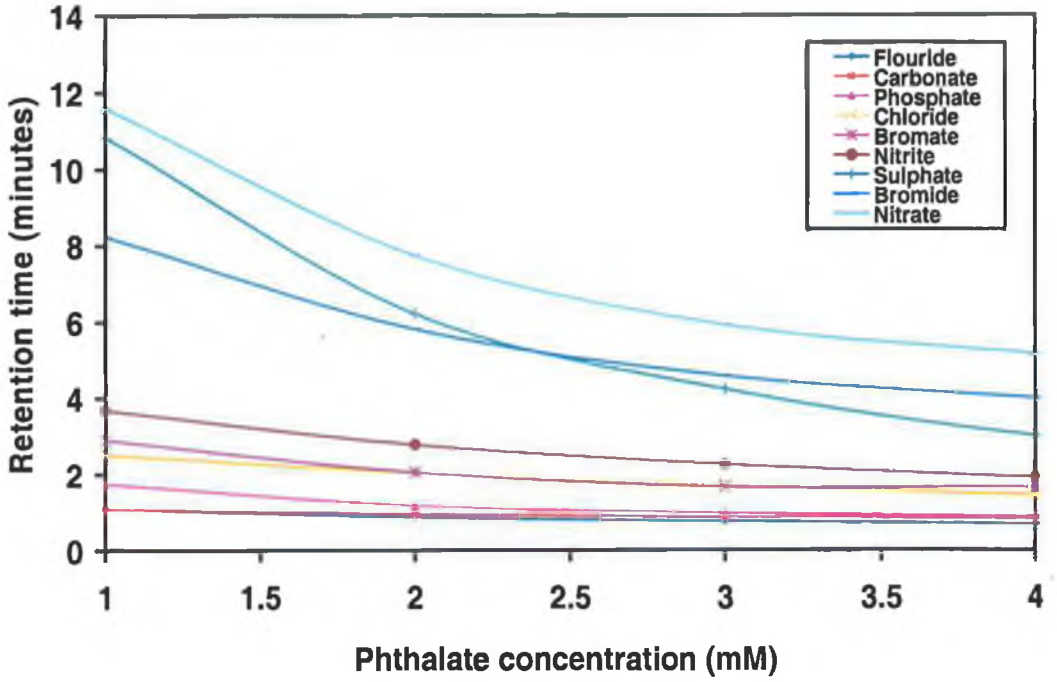

Figure 5-5. *Effect of phthalate eluent concentration on the retention of selected anions. Chromatographic conditions: Flow rate: 1.0 ml/min, Injection volume: 25 µL, Column temperature: ~ 22 °C, Detection wavelength: 282 nm.*

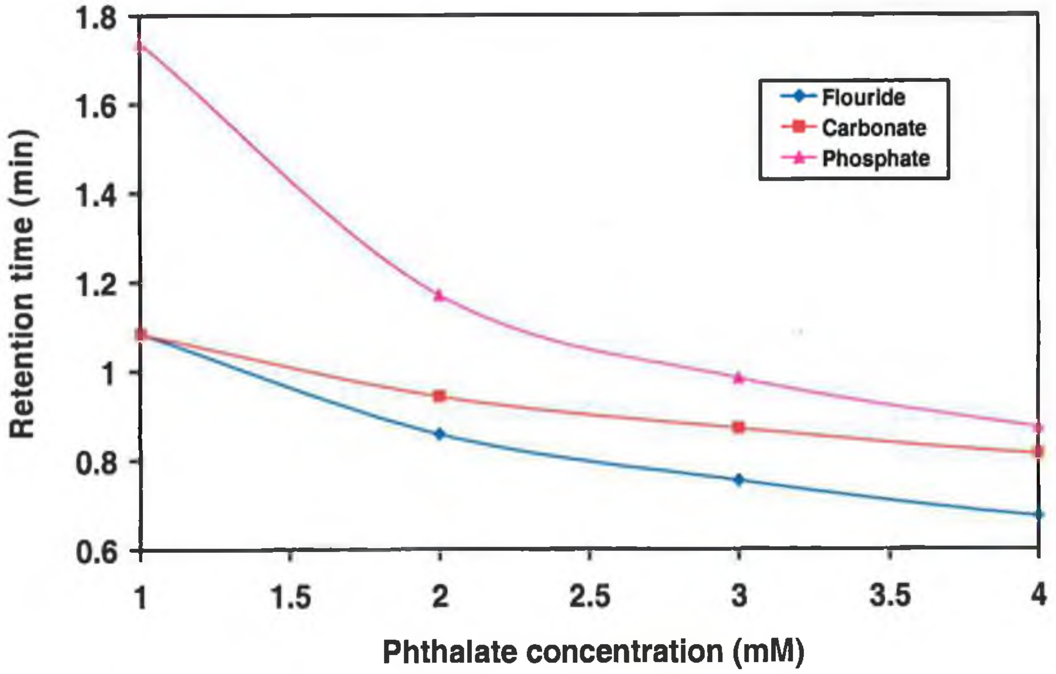

Figure 5-6. *Effect of phthalate eluent concentration on the retention of weakly retained anions. Chromatographic conditions as in Figure 5-5.*

As expected, retention times decreased with an increase in eluent concentration, with 4 m*M* phthalate initially chosen as optimum, based on a compromise between the resolution of the test anion mix achieved, and the runtime. Elution order[1] was fluoride (0.67 min), carbonate (0.81 min), phosphate (0.87 min), chloride (1.4 min), bromate (1.6 min), nitrite (1.9 min), sulphate (2.9 min), bromide (3.9 min) and nitrate (5.1 min). The unusual elution order observed (relative to a conventional anion exchange resin of equivalent capacity) was presumably due to the highly hydrophobic nature of the column coating, (C = 26), which resulted in the weak retention of hydrophilic anions such as fluoride, phosphate etc, and the much longer retention of the less hydrophilic anions bromide and nitrate. In fact this hydrophobic affect was such that at low eluent concentration (< 2 m*M*) sulphate eluted between bromide and nitrate, with the subsequent increase in eluent concentration causing a change in elution order as both bromide and nitrate were less affected by eluent concentration than sulphate.

5.3.3 Optimisation of eluent pH.

Due to the nature of the silica stationary phase, extremes of pH were avoided since low pH's would reduce the eluting strength of the weak acid eluent and also can destabilise the silica-C_{18} bond, and high pH's could result in the dissolution of the silica itself. Therefore, the pH of the eluent (4 m*M*) was simply varied from pH 6.0 to pH 7.5 to determine if pH had a significant affect upon retention over this pH range; and the retention data from the resulting chromatograms generated, was plotted against pH.

---

[1] The void volume time was 0.4 minutes.

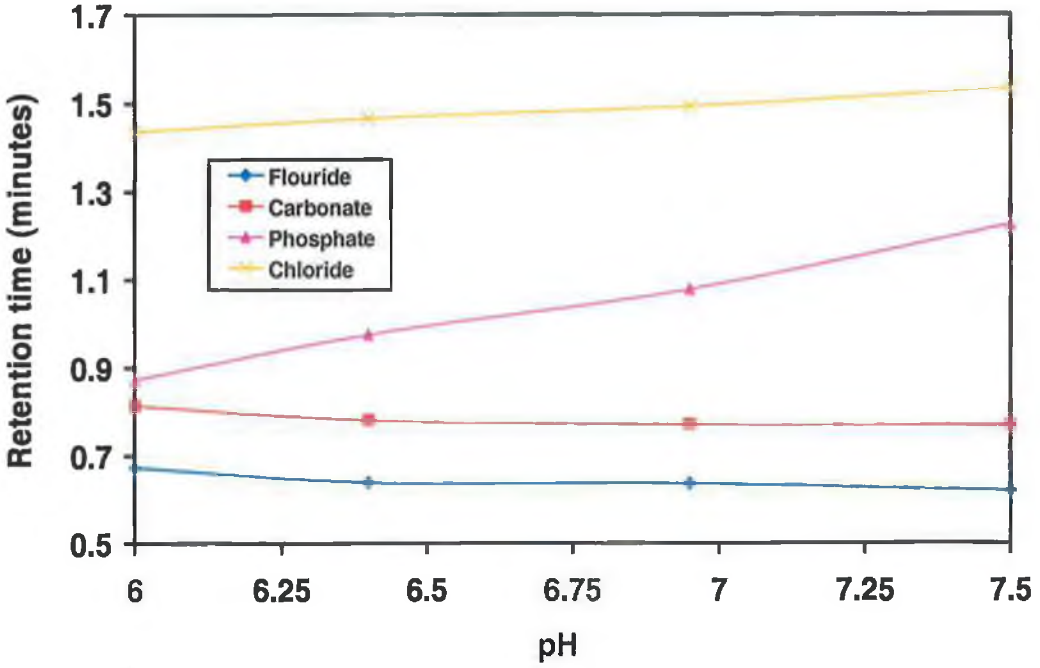

Figure 5-7. *Effect of eluent pH on the retention of weakly retained anions. Chromatographic conditions: Flow rate: 1.0 ml/min, Injection volume: 25 µL, Column temperature: ~ 22 °C, Detection wavelength: 282 nm. Eluent concentration: 4 mM phthalate.*

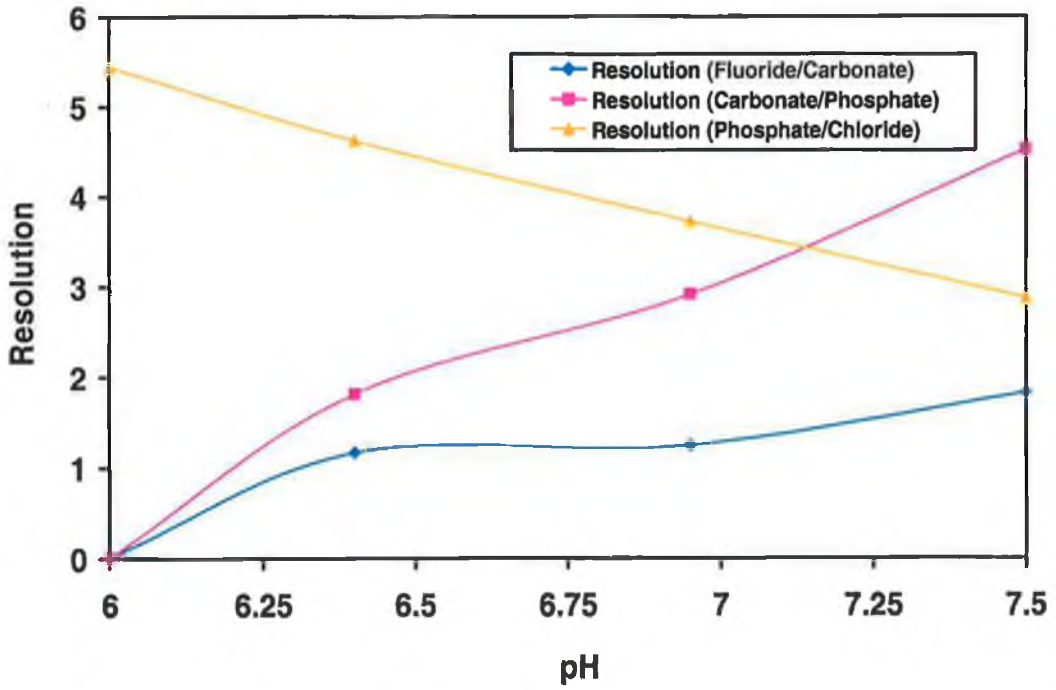

Figure 5-8. *Effect of eluent pH on the resolution between weakly retained anions. Chromatographic conditions as in Figure 5-7.*

At pH 6.0 the elution order of the first 4 eluting anions was fluoride, carbonate, phosphate and chloride with carbonate eluting closely to phosphate at 0.9 minutes. Increasing the eluent pH did not significantly affect the retention of fluoride, carbonate and chloride (or indeed the later eluting anions) but phosphate could be readily removed from carbonate by an increase in eluent pH to 6.5 or above. This is due to dissociation of phosphate over this pH range leading to an increase in negative charge of phosphate thus leading to an increase in retention[2]. An eluent pH of 7.5 was found to produce the best resolution of the above anions, with phosphate resolved from both carbonate and chloride, which could otherwise interfere with phosphate determinations in real samples. Indeed, with phosphate eluting before chloride, it was anticipated that samples containing relatively high levels of chloride, would not interfere with phosphate determination; as opposed to the situation where phosphate were to elute on the tailing side of a large chloride sample peak.

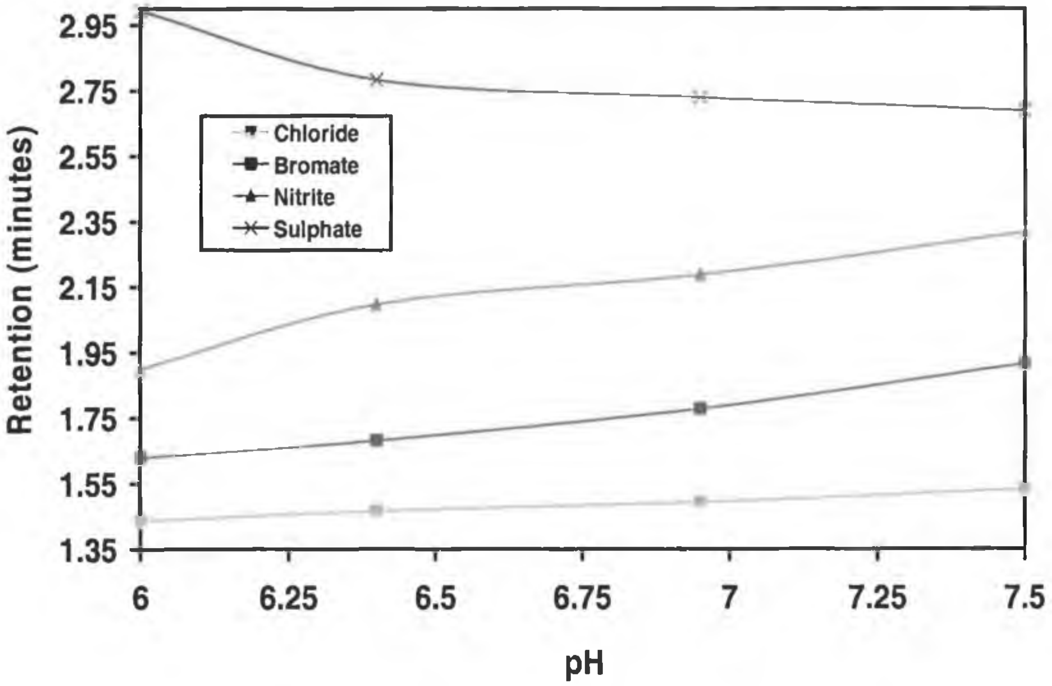

Figure 5-9. *Effect of eluent pH upon the retention of moderately retained anions. Chromatographic conditions as in Figure 5-7.*

---

[2] $pKa_1 = 2.16$, $pKa_2 = 7.21$ and $pKa_3 = 12.32$.

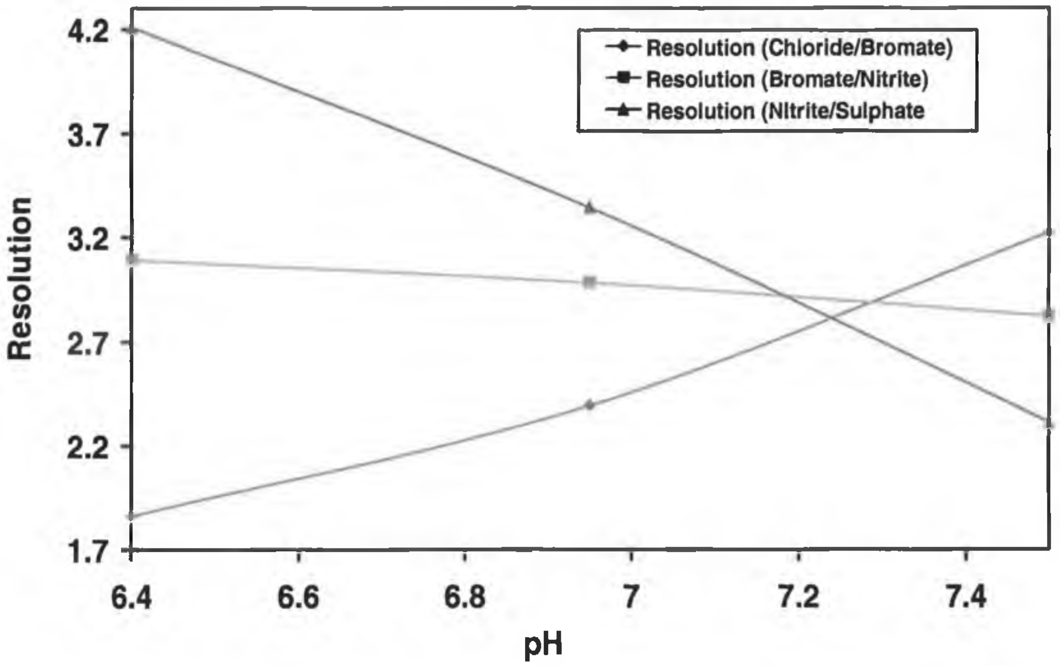

Figure 5-10. *Effect of eluent pH upon the resolution between the moderately retained anions. Chromatographic conditions as in Figure 5-7.*

Of the moderately retained anions, the greatest effect of increasing pH was on the separation of nitrite and sulphate peaks, with resolution decreasing from ~4.2 to 2.3 at pH 7.5. Of the strongly retained anions, there was a marked increase in retention for both bromide and nitrate (see Figure 5-11) relative to more weakly retained anions such as chloride. This effect of increased pH in a phthalate eluent on bromide and nitrate was also noted by Jun *et al.* [6] and Haddad and Jackson [12].

The optimum pH of 7.5 was chosen to reflect the optimised resolution of the carbonate/phosphate/chloride triplet, and also, higher pH values were avoided since runtimes would be too long based on longer nitrate retention times.

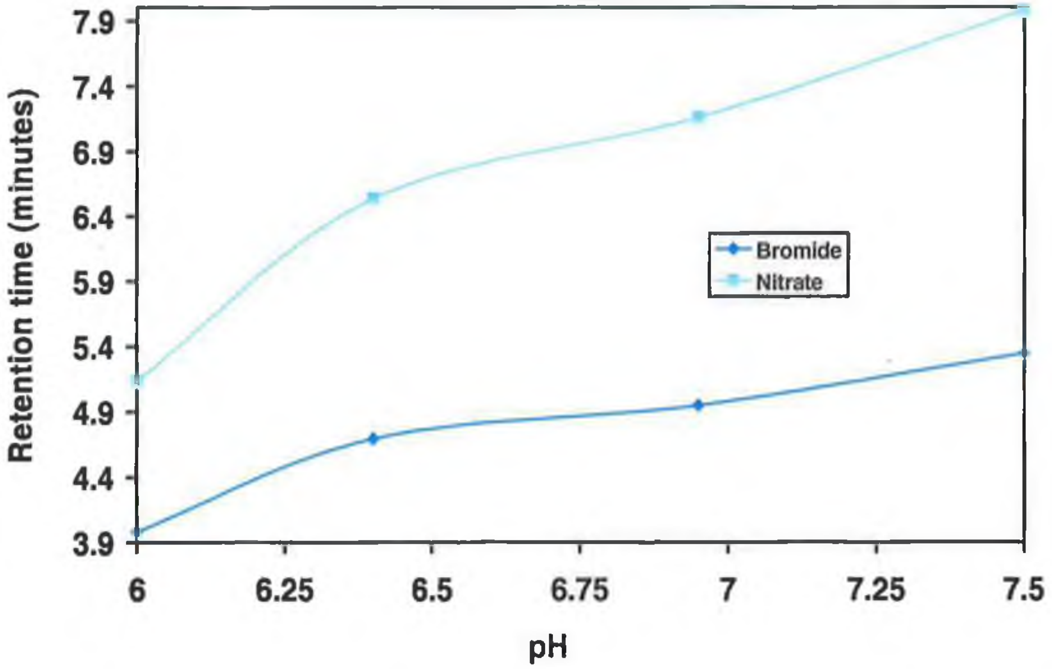

Figure 5-11. *Effect of eluent pH on the retention of strongly retained anions. Chromatographic conditions as in Figure 5-7.*

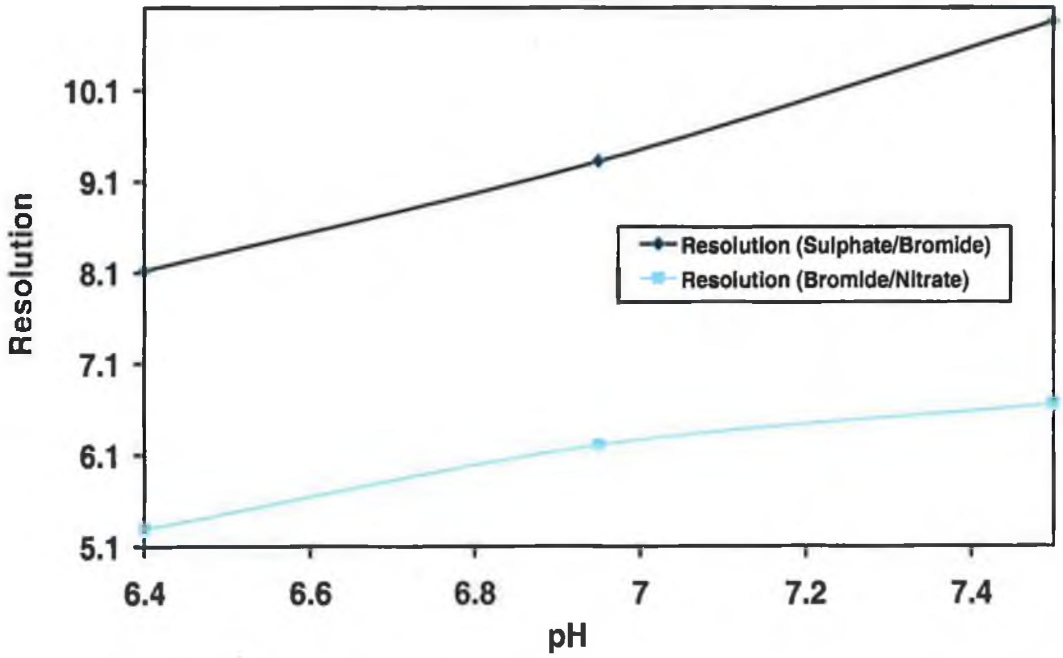

Figure 5-12. *Effect of eluent pH on the resolution between strongly retained anions. Chromatographic conditions as in Figure 5-7.*

5.3.4 Reoptimised eluent composition for fast anion separations.

From the above studies it was clear a 4 m*M* phthalate eluent of pH 7.5 resulted in the complete resolution of the test anion mixture. After subsequent analysis of the data generated both by eluent and pH optimisation, it became clear that resolution between carbonate and phosphate was excessive at 4.50.

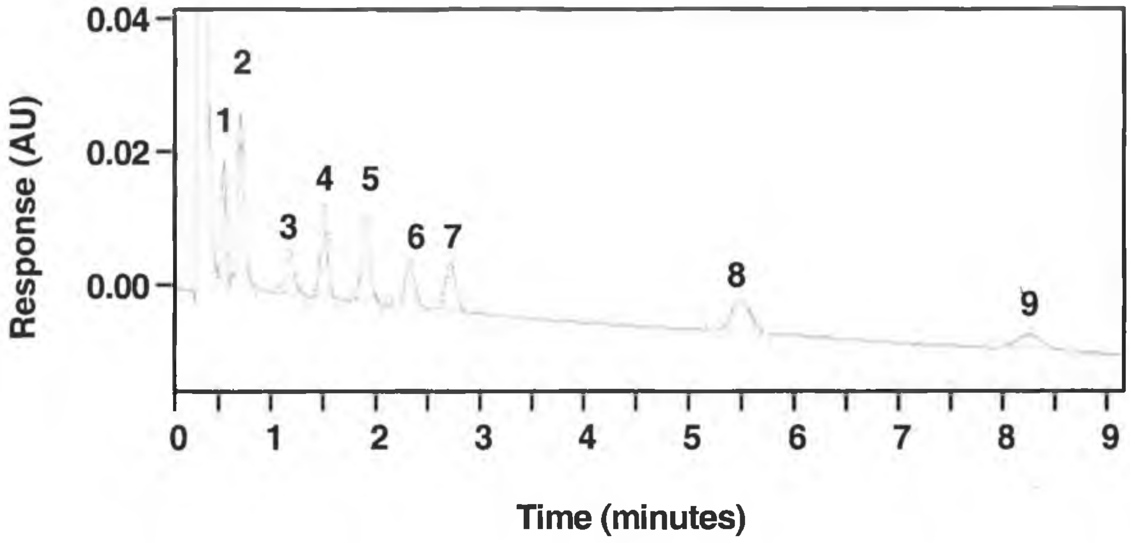

Figure 5-13. *Preliminary separation of selected anions on a short 3 cm 3 µm column coated with DDAB. Column: Phenomenex Hypersil, 3 µm, 30 mm x 4.6 mm I.D. Mobile phase: 4 mM phthalate pH 7.5, Column temperature: ~ 22 °C, Flow-rate: 1.0 ml/min, Loop volume: 25 µL, Detection: indirect UV at 282 nm. Peaks[3]: [1] fluoride (0.6 mins), [2] carbonate (0.8 mins), [3] phosphate (1.2 mins), [4] chloride (1.5 mins), [5] bromate (1.9 mins), [6] nitrite (2.4 mins), [7] sulphate (2.7 mins), [8] bromide (5.3 mins), [9] nitrate (8.0 mins).*

It was found that retention times could be further reduced by increasing the eluent concentration to 5 m*M* at pH 7.5, without significantly compromising resolution or increasing background noise. Resolution of carbonate/phosphate and phosphate/chloride was not compromised, particularly with phosphate adequately resolved from carbonate and the solvent peak (water). The retention time of the late

---

[3] At this early stage in method development, the "test mix" of anions was very crudely prepared, solely with a view to the gathering of retention time data. Concentrations of test anions lie in the approximate range of 5 mg/*L* to 25 mg/*L*.

eluting nitrate peak was reduced by one minute, which will be shown later to be a significant reduction in overall runtime given the rapid runtimes ultimately achieved.

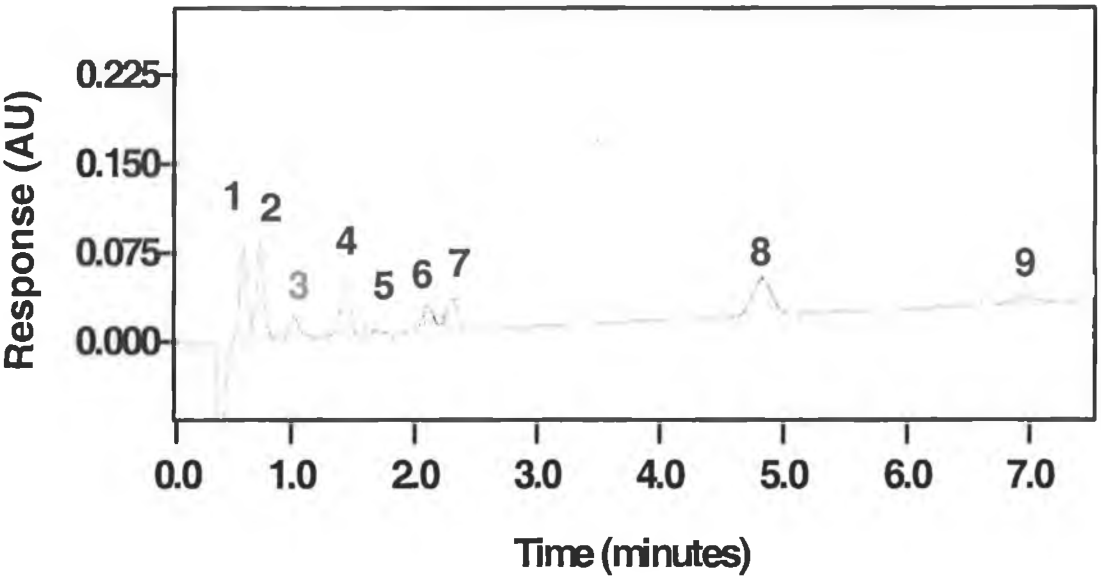

Figure 5-14. *Separation of selected anions with a re-optimised eluent on a short 3 cm 3 μm column coated with DDAB. Conditions as in Figure 5-13 except, Mobile phase: 5 mM phthalate pH 7.5. Peaks[4]: [1] fluoride (0.69 mins), [2] carbonate (0.74 mins), [3] phosphate (1.0 mins), [4] chloride (1.4 mins), [5] bromate (1.7 mins), [6] nitrite (2.1 mins), [7] sulphate (2.3 mins), [8] bromide (4.9 mins), [9] nitrate (7.0 mins).*

5.3.5 Optimisation of column temperature.

With a given mobile phase, (in this case 3 m*M* phthalate, pH 6.0) the column temperature was increased from ambient (22 °C) to 45 °C (the temperature maximum of the Dionex LC25 column compartment used). Retention[5] of each anion was plotted versus temperature, illustrating that an increase in temperature had little or no effect on the retention of most of the test mixture of anions except bromide and nitrate. Here there was a marked decrease in retention with the increase in column

---

[4] This test mix of anions differs quantitatively from that in Figure 4-13, because the initial test mix was re-prepared - again, solely with a view to collecting retention time data.

[5] The void volume time was 0.4 minutes.

temperature. This again is a clear indicator that the retention mechanism of these two anions differs to that of the remaining test mixture, with non-electrostatic interactions obviously playing a role. This may be attributed to the less hydrophilic nature of these anions relative to the remainder of the test anions selected for this study. This selective reduction in retention was, however, most advantageous as it allowed the excessive retention of bromide and nitrate to be reduced without affecting the resolution of the earlier eluting anions. As discussed in Chapter 1, Section 1.6.1, Hatsis and Lucy [13] reported that when temperature is varied during anion exchange separations on AS11 or AS14 columns, anions can be grouped together in terms of the extent to which their retention is affected by increasing temperature. Within a group, the temperature behaviours are approximately the same and therefore there is little effect on the selectivity between these anions. However, temperature can cause selectivity changes between anions from different groups. Inspection of Figure 5-15 shows that this current work is in good agreement with what was observed by Hatsis and Lucy on conventional anion exchange columns. If the anions in Figure 5-15 are grouped together as {fluoride, carbonate, phosphate, chloride, bromate, nitrite and sulphate} and {bromide and nitrate}, it is clear that the selectivity between the first group and the second group has changed, but that within each group, there is no selectivity change.

The retention data obtained for nitrate and bromide, with respect to increased column temperature, suggests that increasing the operating temperature past 45 °C could have reduced runtimes still further. However, the HPLC instrumentation was kept less complex, by avoiding the use of an external column compartment (eg: water bath). As discussed in other studies [14,15] increased column temperatures causes the viscosity of the mobile phase to decrease. In this work there was a resulting reduction in column backpressure, which was reduced by ~ 22 % from 930 psi to 730 psi when column temperature was increased from 22 °C to 45 °C. In addition, it was interesting to note that as the retention of these other anions (fluoride, carbonate, phosphate, chloride, bromate, nitrite and sulphate) was not affected by an increase in temperature, the stability of the column coating, even at elevated temperatures was likely being maintained.

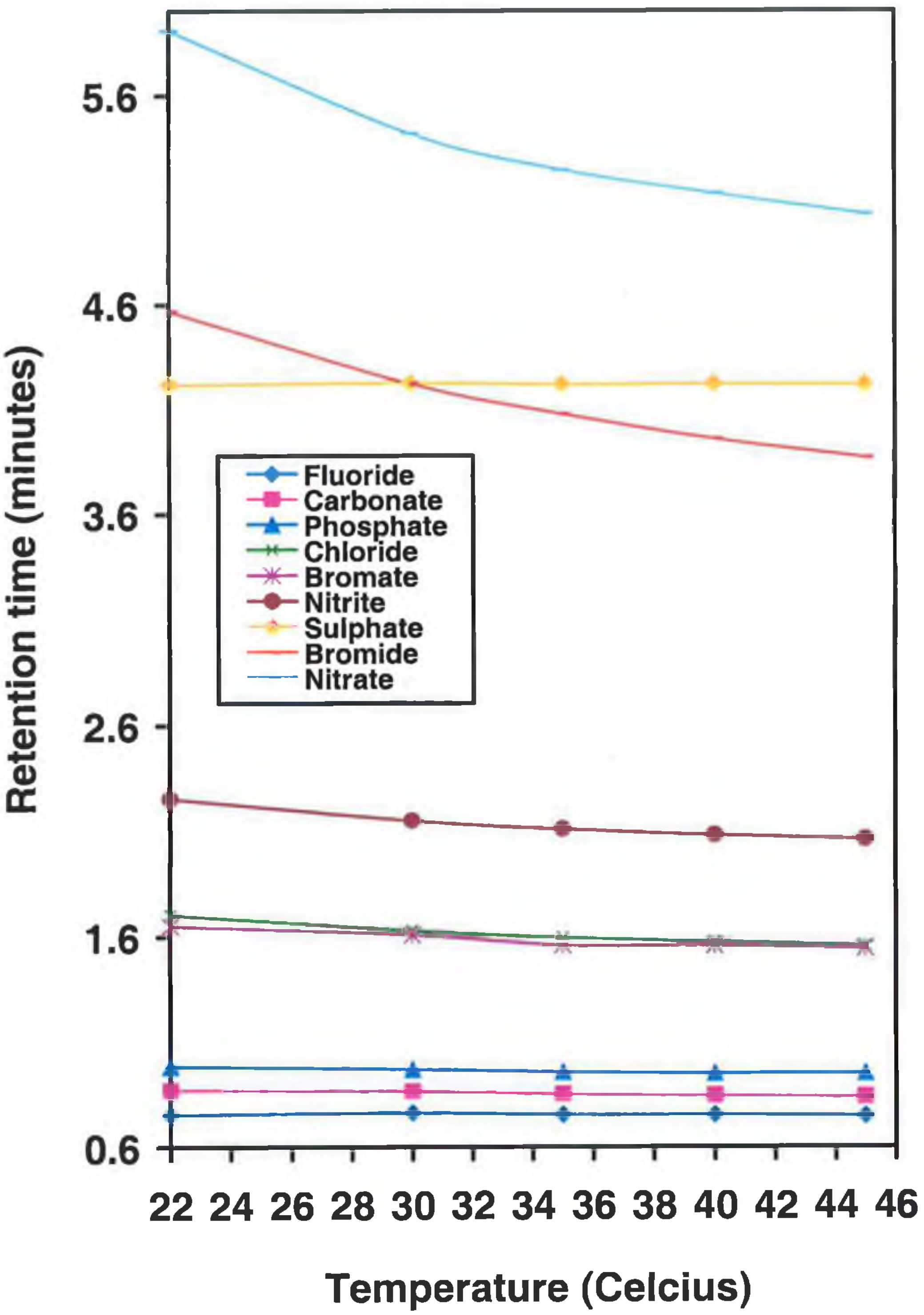

Figure 5-15. *Effect of column temperature upon the retention of selected anions on a short 3 cm 3 µm column coated with DDAB. Conditions as in Figure 5-13 except, Mobile phase: 3 mM phthalate pH 6.0.*

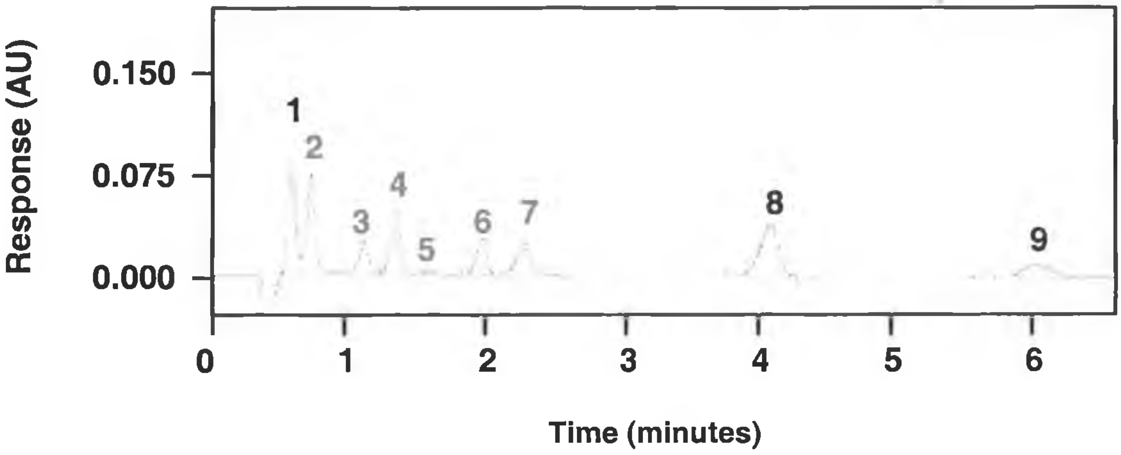

Figure 5-16. *Separation of anions with an optimised eluent at an elevated column temperature of 45 °C. Conditions as in Figure 5-14 except for a column temperature of 45 °C. In comparison with Figure 5-14, the runtime has been reduced by a further minute. Peaks: [1] fluoride (0.60 mins), [2] carbonate (0.75 mins), [3] phosphate (1.1 mins), [4] chloride (1.4 mins), [5] bromate (1.6 mins), [6] nitrite (2.0 mins), [7] sulphate (2.3 mins), [8] bromide (4.0 mins), [9] nitrate (6.0 mins).*

5.3.6 Optimisation of flow rate.

Initially, for the purposes of mobile phase and column temperature optimisation, a flow rate of 1.0 ml/min was used. The next step was to increase the flow rate to 2 mL/min to obtain the final conditions. The increase in flow rate was plotted against the change in resolution between adjacent anions. The limiting factor was the resolution of the peaks for nitrite and sulphate, which decreased steadily with increased flow rates, although at a flow rate of 2 mL/min both were still completely baseline resolved. Due to the short nature of the analytical column backpressures were < 1500 p.s.i. at this flow rate and so the column and analytical pump were not exposed to undue stress.

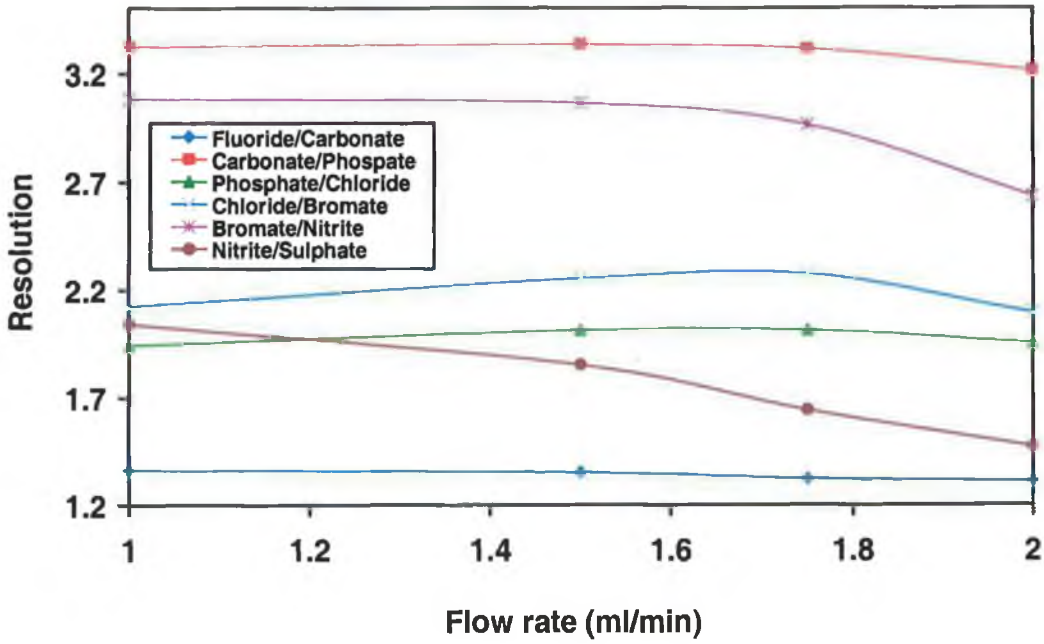

Figure 5-17. *Effect of flow rate on upon the resolution of selected anions on a short 3 cm 3 µm column coated with DDAB. Conditions as in Figure 5-16.*

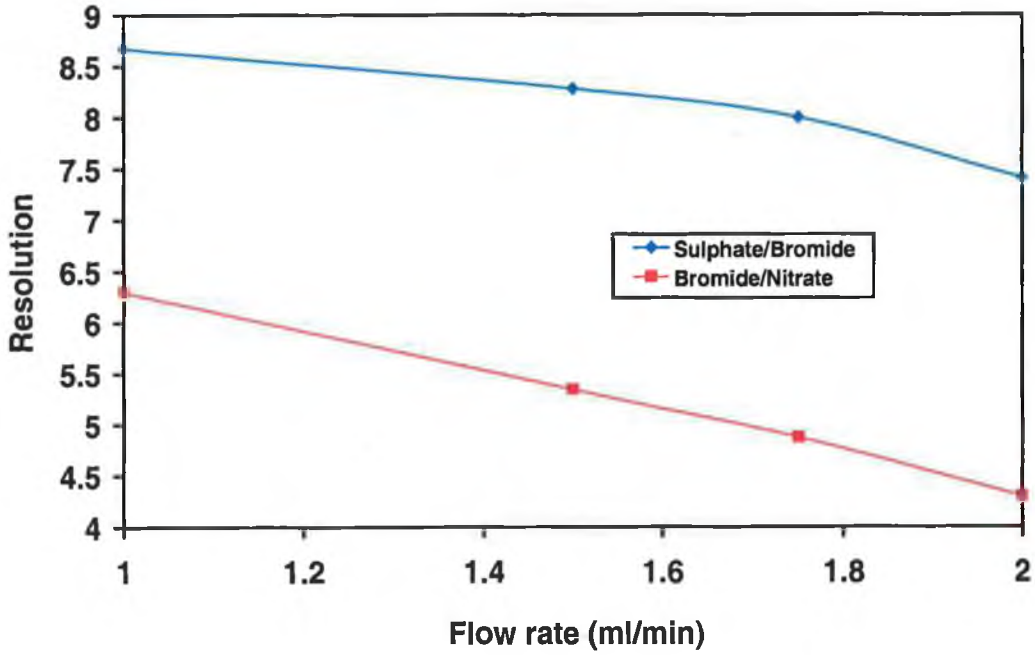

Figure 5-18. *Effect of flow rate upon the resolution of late eluting peaks on a short 3 cm 3 µm column coated with DDAB. Conditions as in Figure 5-16.*

5.3.7 Optimisation of detector wavelength.

Under optimised chromatographic conditions, a 5 mg/*L* phosphate standard was injected at detection wavelengths over the range, 214 nm to 285 nm. By plotting peak height against wavelength, an optimum of 279 nm was reached.

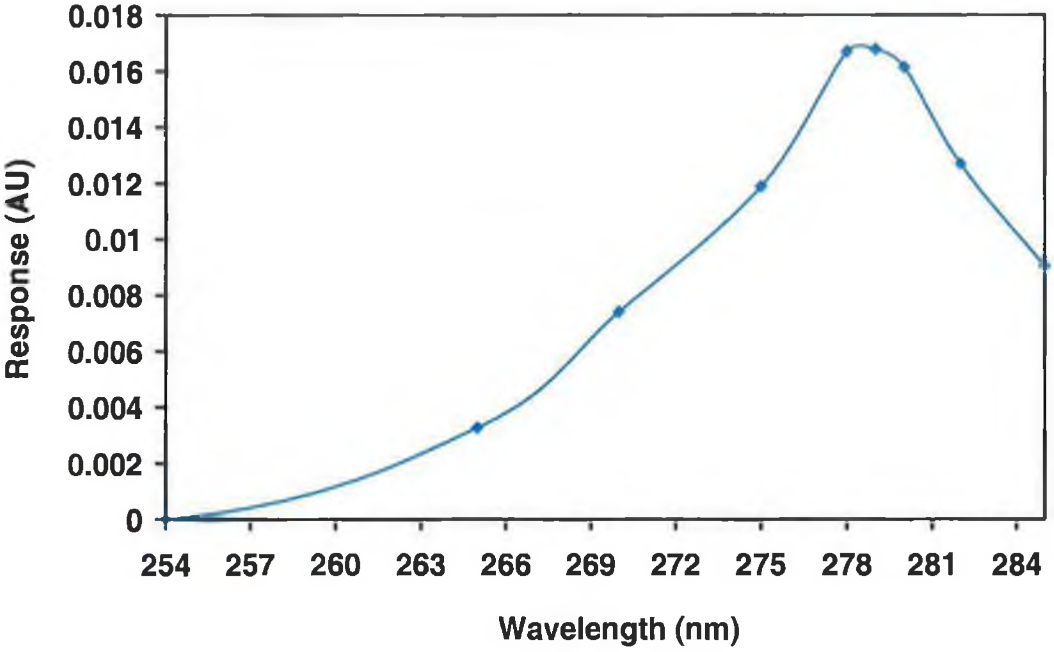

Figure 5-19. *Optimisation of detector wavelength with a 5 mg/L phosphate standard on a short 3 cm 3 μm column coated with DDAB. Conditions as in Figure 5-16, with a flow rate of 2.0 ml/min.*

5.3.8 Optimisation of injection volume.

During optimisation of chromatographic conditions, an injection volume of 25 μL was used. Three loop sizes were subsequently fitted, of size 25, 50 and 75 μL, and a 5 mg/*L* phosphate standard injected in triplicate for each loop size. As expected, peak heights increased for the test peak, with an optimum reached at 75 μL. Interestingly, the peak efficiency, measured in plates/metre showed no signs of decreasing with the use of the larger injection volume (from 60,522 with 25 μL inj., to 71,633 with 75 μL inj.) which illustrated the benefit of using a column with such a high capacity. However, an injection volume of 50 μL was chosen for use with real samples, in order to minimise possible column overload due to injection of samples of high ionic strength onto such a short column. For the optimised separation of the mixed anion standard run under the conditions described above see Figure 5-20.

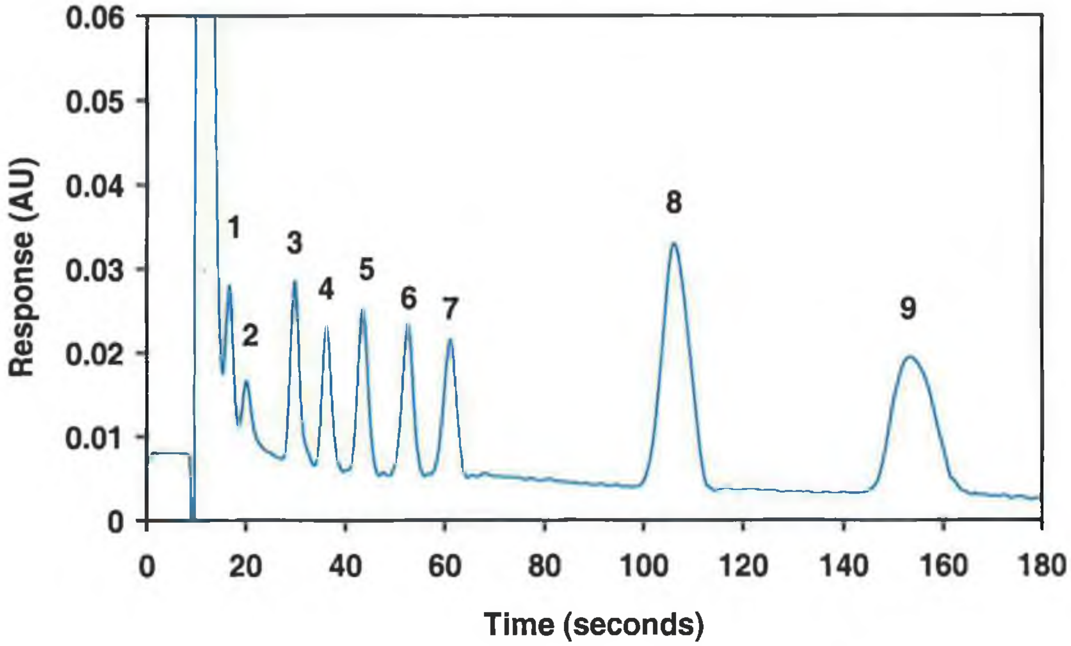

Figure 5-20. *Optimised rapid separation of nine anions on a short 3 cm 3 μm column coated with DDAB. Chromatographic conditions: Column: Phenomenex Hypersil, 3 μm 30 mm x 4.6 mm I.D. Mobile phase: 5 mM phthalate pH 7.5. Flow rate: 2 mL/min. Column temperature: 45 °C. Injection volume: 50 μL. Detection: indirect UV at 279 nm. Peaks: [1] fluoride (5 mg/L), [2] carbonate (12 mg/L), [3] phosphate (12 mg/L), [4] chloride (9 mg/L), [5] bromate (85 mg/L), [6] nitrite (15 mg/L), [7] sulphate (14 mg/L), [8] bromide (44 mg/L), [9] nitrate (56 mg/L).*

Column peak efficiency ranged from 23,700 plates/metre for fluoride, to 90,767 plates/metre for nitrite measured using the equation shown in Equation 5-1.

$$N = 16\left(\frac{t_{ret}}{W_{base}}\right)^2 \qquad \text{Equation 5-1.}$$

Figure 5-21 emphasises the excellent separation of the first seven common inorganic anions in < 70 seconds. With phosphate ion well resolved from carbonate and chloride in only 30 seconds, quantification of phosphate in real samples would be greatly accelerated, with standard curves constructed in a matter of minutes. Indeed, were a real sample to be passed though a reducing column prior to injection, nitrate (~155 seconds) would be reduced to nitrite (~53 seconds), thus allowing analysis of freshwater samples for important anions in < 70 seconds.

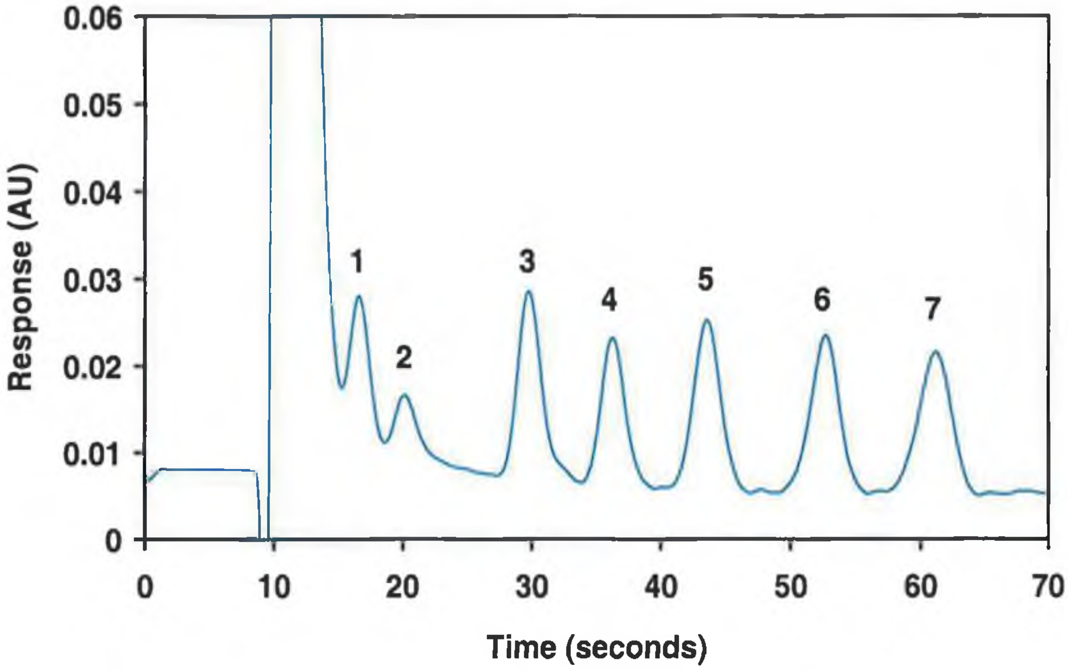

Figure 5-21. *Optimised rapid separation of the first seven anions on a short 3 cm 3 µm column coated with DDAB. Chromatographic conditions as in Figure 5-20. Peaks: [1] fluoride (5 mg/L), [2] carbonate (12 mg/L), [3] phosphate (12 mg/L), [4] chloride (9 mg/L), [5] bromate (85 mg/L), [6] nitrite (15 mg/L), [7] sulphate (14 mg/L).*

5.3.9 Analysis of real samples.

Typically drinking water samples were collected from an in-house laboratory supply. The sample required no other pre-treatment except a simple filtration step prior to injection, to remove particulate matter. The sample was ran bracketed by a standard and a blank in the order; standard, blank, sample, blank, standard. The resultant chromatogram, overlaid with a blank and a standard mix, is shown as Figure 5-22.

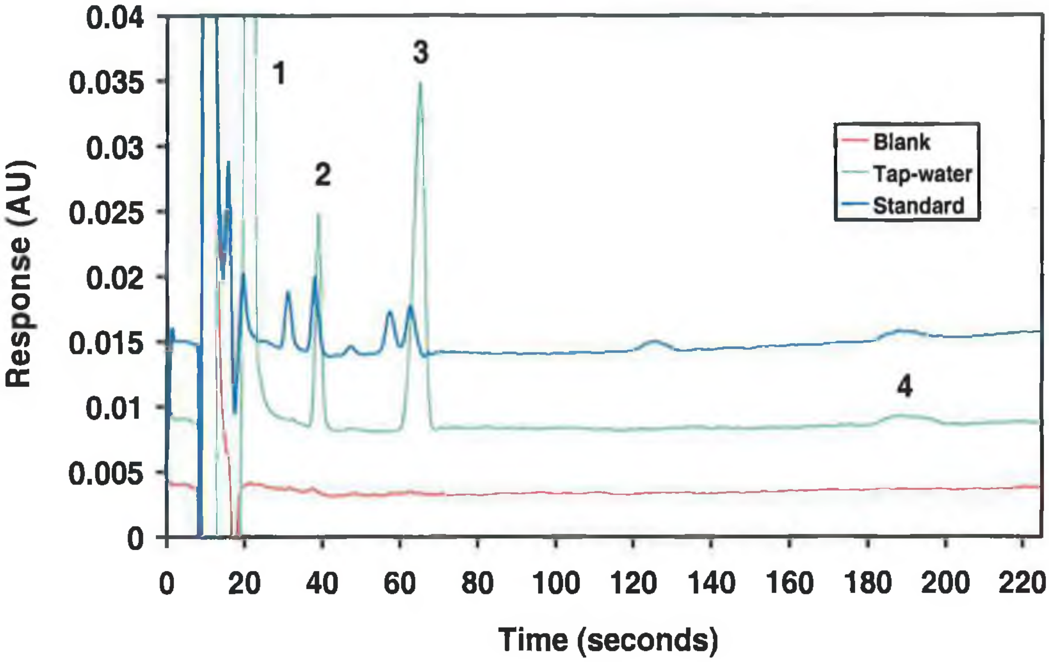

Figure 5-22. *Rapid qualitative analysis of tap water on a short 3 cm 3 µm column coated with DDAB. Chromatographic conditions as in Figure 5-20. Peaks: [1] carbonate, [2] chloride, [3] sulphate, [4] nitrate.*

The presence of carbonate, chloride, sulphate and nitrate can readily be seen. This figure clearly illustrates the potential use of this method in rapid screening (< 3 minutes) of multiple samples to determine sudden changes in sample composition. Retention times are slightly later than in Figure 5-20 (particularly for bromide and nitrate), possibly due to a slightly higher pH for this particular mobile phase preparation. Between-day reproducibility for retention times was strongly dependent upon mobile phase pH, such that a comparison between standards ran with separate mobile phase preparations show a slight variance. Nevertheless, each mobile phase preparation resulted in an adequate resolution of each adjacent peak in the standard solution. Also, as will be discussed in Section 5.3.11, the retention time reproducibility for a given mobile phase preparation over 200 injections was excellent, as illustrated in Figure 5-31.

To illustrate the high capacity of the short column, Dublin Bay seawater samples were collected, diluted with de-ionised water by an appropriate factor, and syringe-filtered before injection. Depending on location and sampling depth (i.e. surface or sea-bed), chloride and sulphate levels can be 240 and 14 times higher than bromide ion respectively [16]. Fukushi *et al.* [16] used a tenfold-diluted artificial seawater as the buffer solution for the determination of bromide in seawater by capillary electrophoresis to prevent interference from high concentrations of chloride ion in seawater samples. The total runtime was five minutes but the capillary was also washed with 0.1 *M* sodium hydroxide between each run, which would have added to the total analysis time. Also, because of the nature of the buffer used, and with a detection wavelength of 200 nm, the analysis was limited to that of bromide, nitrate and nitrite.

With conventional anion exchange chromatographic techniques, some complex sample preparation may be necessary to avoid interference with bromide ion determinations. In addition, runtimes can be relatively long without the use of gradient systems to shorten the retention of the late-eluting sulphate and phosphate peaks. This latter disadvantage is true of the separation developed by Salhi and von Gunten [17] who used a 25 cm X 4.6 mm AS9HC anion exchange column with a 9 m*M* Na_2CO_3 eluent at 1.0 ml/min to determine bromide in sea water samples diluted 60 fold. Bromide retention time was ~ 10 minutes, but the analysis of samples was ran to 20 minutes due to the late eluting sulphate peak at ~ 17 minutes.

However, with the chromatography developed in this work, bromide ion can be rapidly determined (~130 seconds) and quantified in the presence of high levels of chloride and sulphate, using an isocratic system, with minimal sample preparation. Figure 5-23 shows a sea water sample diluted by a factor of 1/100, overlaid with a standard.

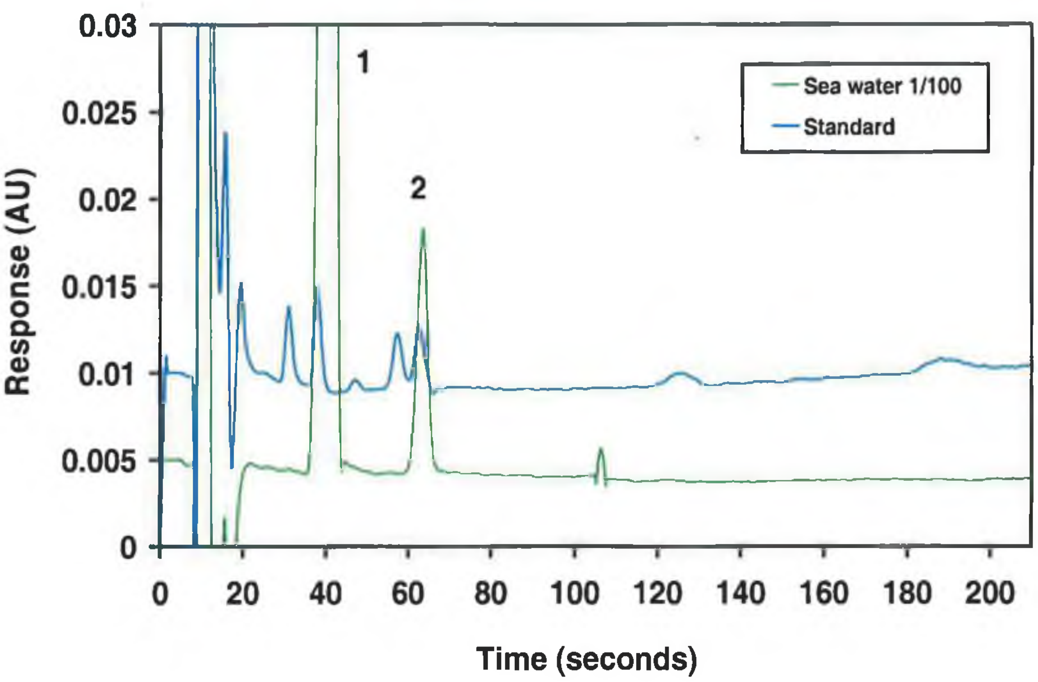

Figure 5-23. *Rapid analysis of seawater (diluted 1/100) for bromide ion on a short 3 cm 3 µm column coated with DDAB. Chromatographic conditions as in Figure 5-20. Peaks: [1] chloride, [2] sulphate.*

Chloride and sulphate exhibit good peak symmetry with excellent correlation of retention times relative to the standard. Figure 5-24 is an overlay of a sea water sample diluted only by a factor of 1/10, overlaid with the same standard. Despite the high ionic strength of the sample, bromide retention matches that in a standard very well, with no expected shift in retention due to excess chloride ion occupying anion exchange sites on the column. This indicates the unusually high exchange capacity of the column, due to the strong affinity of the ion-interaction reagent for the C_{18} stationary phase. Bromide was crudely quantified at a level of ~48 mg/*L*, against a one-point calibration with the standard solution. Nevertheless, this result, correlates quite well with literature values for bromide in seawater [16], of 39 – 56 mg/*L*, and indicates the advantage of small high capacity columns for rapid analysis of brine samples.

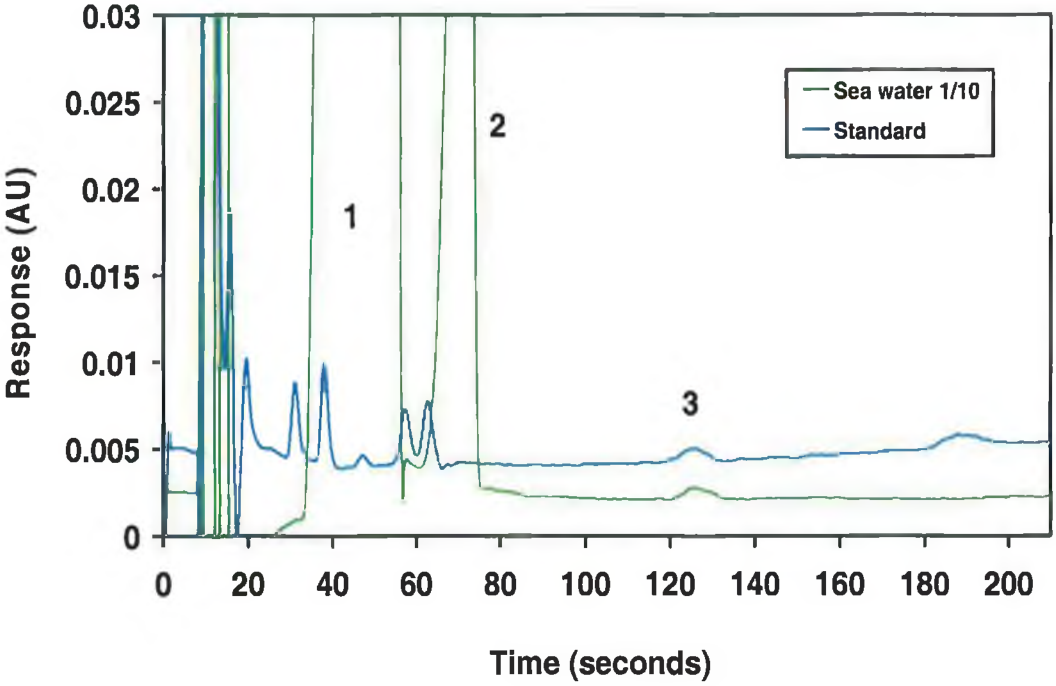

Figure 5-24. *Rapid analysis of seawater (diluted 1/10) for bromide ion on a short 3 cm 3 μm column coated with DDAB. Chromatographic conditions as in Figure 5-20. Peaks: [1] chloride, [2] sulphate, [3] bromide.*

Finally, the rapid chromatography developed in this work was used to analyse river water from a sampling point known to contain significant levels of phosphate (River Liffey, Liexlip, Co. Kildare, Ireland). With a phosphate retention time of only 30 seconds, linearity and system precision were determined in minutes rather than hours, permitting the rapid construction of standard curves for system calibration (6 point standard curve for phosphate, injecting each standard in triplicate, could be constructed in just 10 minutes).

The method quantification limit for phosphate was calculated to be of the order 0.5 mg/*L* which was again suitable for the rapid screening of river water samples. The method was linear from 0.5 mg/*L* up to at least 100 mg/*L* (R^2 = 0.9997), with a system precision determined at % RSD = 0.98 % for n=8 consecutive injections of a 5 mg/*L* phosphate standard. Nine consecutive replicate injections were made of a sample, and phosphate concentrations determined from a standard curve prepared fresh on the same day of analysis. (range: 0.5 mg/*L* to 50 mg/*L*, (n = 5), R^2 = 0.9999) Average phosphate levels in the river water sample (n=9) were 0.64 mg/*L*.

Figure 5-28 shows an overlay of the river water sample with a blank and a standard. Phosphate in the sample is well resolved from the large carbonate peak and from chloride.

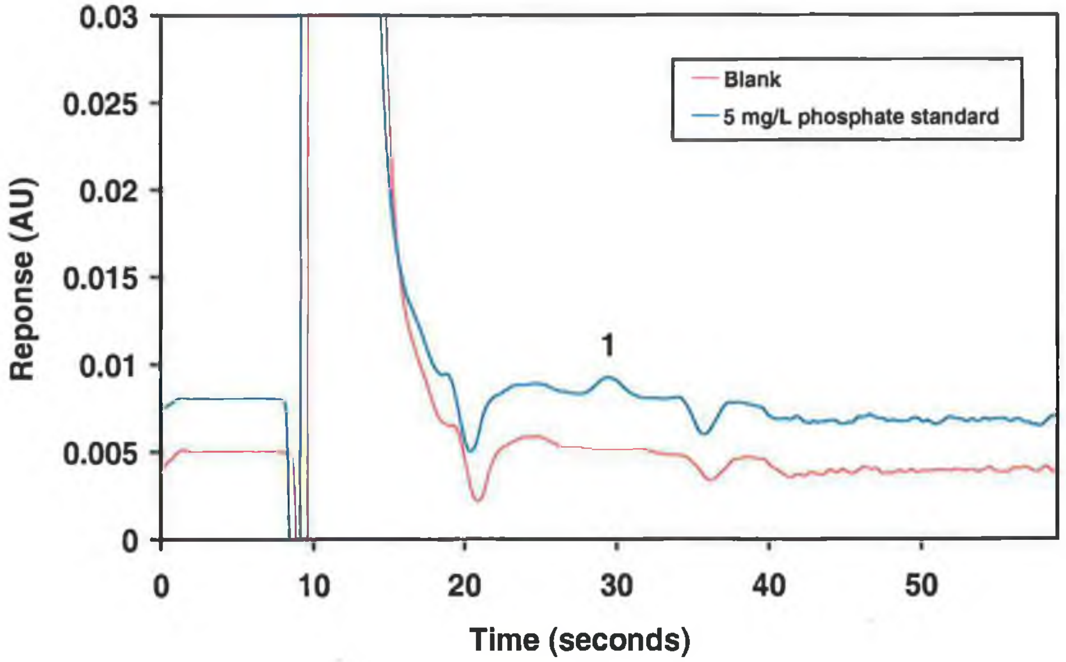

Figure 5-25. *Sensitivity study for the determination of phosphate on a short 3 cm 3 μm column coated with DDAB. Chromatographic conditions as in Figure 5-20. Peaks: [1] phosphate.*

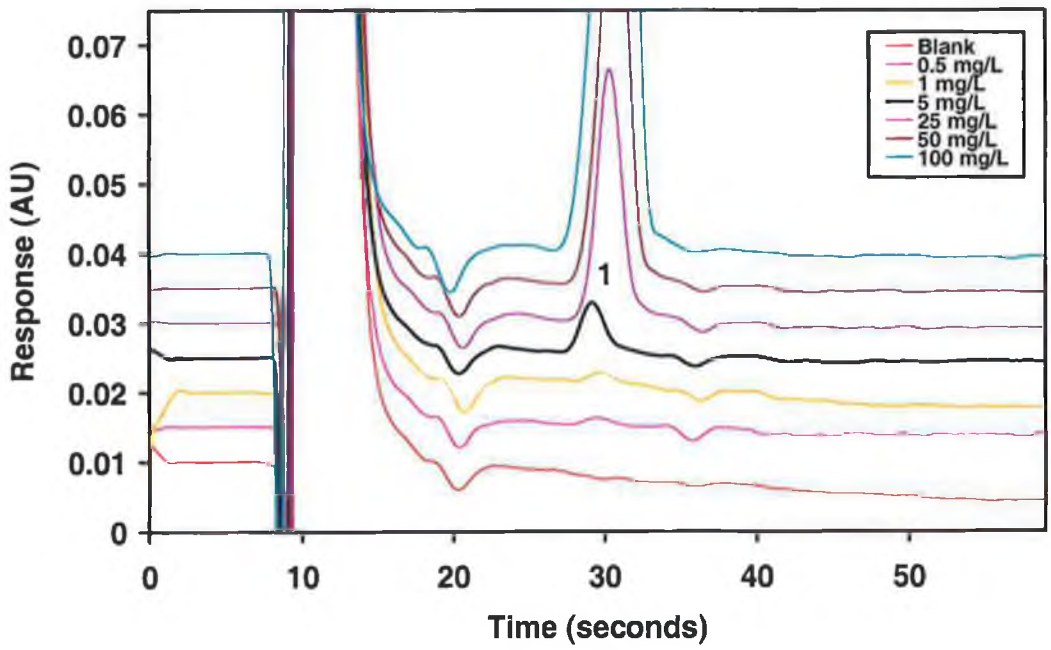

Figure 5-26. *Overlay of phosphate linearity standards on a short 3 cm 3 μm column coated with DDAB. Chromatographic conditions as in Figure 5-20. Peaks: [1] phosphate.*

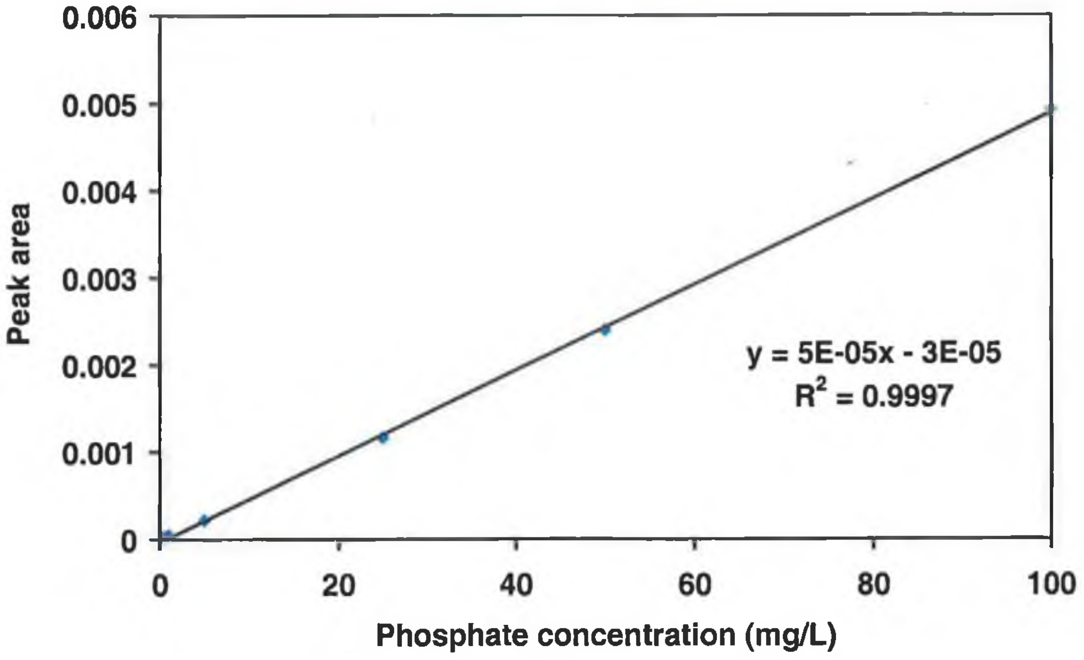

Figure 5-27. *Linearity study for phosphate on a short 3 cm 3 µm column coated with DDAB. Chromatographic conditions as in Figure 5-20.*

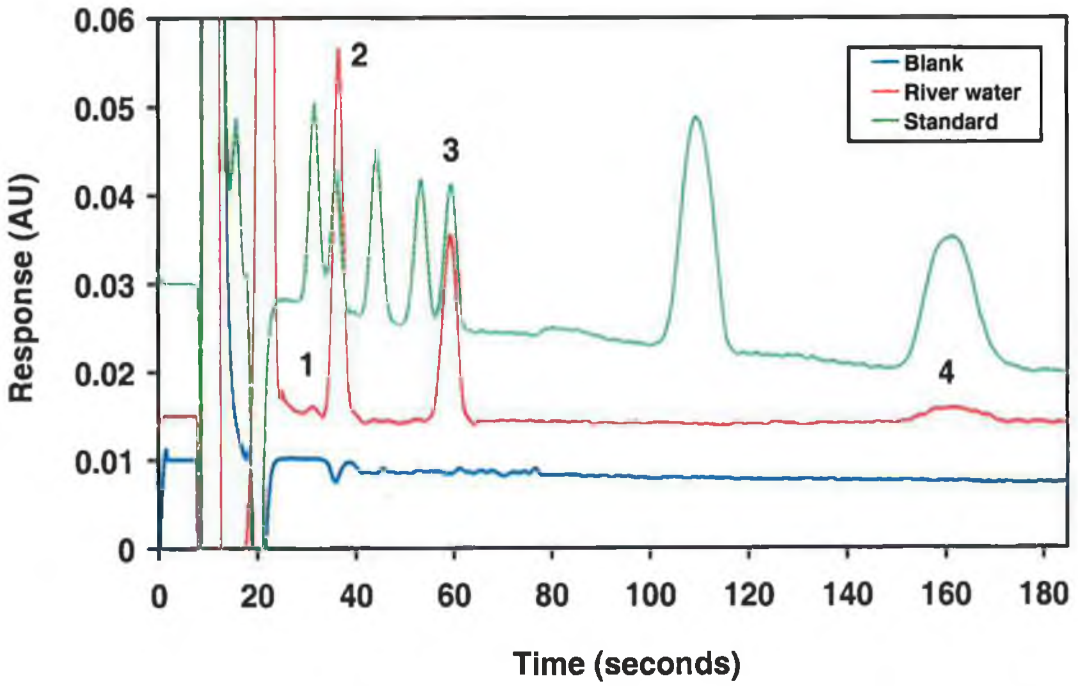

Figure 5-28. *Rapid analysis of river water for phosphate on a short 3 cm 3 µm column coated with DDAB. Chromatographic conditions as in Figure 5-20. Peaks: [1] phosphate, [2] chloride, [3] sulphate, [4] nitrate.*

5.3.10 Decreasing the anion exchange capacity of the column.

The analysis of the sea water sample for bromide ion, clearly demonstrates the advantage of using a column with a high anion exchange capacity. Despite the relatively high ionic strength of the sample matrix, bromide ion could be readily determined, with only a dilution factor of ten required to negate the effect of high concentrations of chloride and sulphate ions in the sample matrix.

However, the high exchange capacity of the column necessitates that the eluent strength be relatively high (with this work, 5 m*M*) in order to elute the analytes within a suitable timescale. When compared to typical phthalate eluent strengths for indirect UV detection of 0.1 m*M* to 0.5 m*M*, the eluent strength used in this work is an order of magnitude higher. This results in a much higher absorbance background, due to the higher concentration of phthalate directly affecting sensitivity by increasing background noise. The analysis of phosphate in river water is an example of the relatively high limits of detection achieved in this work, making the developed method unsuitable for analysis of freshwater samples containing trace levels of phosphate.

With eluent strengths of 5 m*M*, indirect detection at 254 nm resulted in very poor sensitivity due to the prohibitively high background absorbance. Figure 5-19 demonstrates that detection is only possible at higher wavelengths in this work where the background absorbance is less, resulting in improved signal/noise ratios.

However, since limits of detection would be highest at λ_{max} for the eluent anion (phthalate), lower concentrations of phthalate (eg: 0.1 to 0.5 m*M*) are necessary to allow detection at λ_{max} and therefore increase sensitivity. For example, Zou *et al.* [10] used 0.5 m*M* phthalate in their eluent with a detection wavelength of 257 nm, Jun *et al.* [6] used 0.4 m*M* phthalate at 254 nm and Hatsis and Lucy [11] used 1.1 m*M* phthalate at 255 nm.

Therefore, there was a need to reduce the capacity of the column (by approximately a factor of ten) to allow the use of lower eluent concentrations. The column was washed with a solution of 60:40 methanol/water at 1 ml/min for two hours, and then equilibrated with water at 1 ml/min for ~ 45 minutes. A coating solution of 0.1 m*M* DDAB was prepared, and the column was coated for 30 minutes at 1 ml/min. The column was subsequently washed with water at 0.5 ml/min overnight (approximately 15 hours).

To confirm that the column now had a lower anion exchange capacity than the initial coating, a 5 m*M* phthalate eluent was prepared (eluent pH not adjusted), and an injection was made of the test mix. Injections of phosphate and nitrate standards were made (representing both weakly and strongly retained anions). As expected, both anions eluted in the void, confirming that the exchange capacity of the column was now considerably lower.

Therefore, an eluent of more suitable concentration, given the lower exchange capacity of the column was prepared (0.25 m*M* phthalate (eluent pH not adjusted)) but the subsequent injection of the anion test mix showed that none of the anions were retained. It was concluded that the column capacity was too low to effect a separation, or even to retain analytes. The column was then washed with 70:30 methanol/water, with a view to coating the column again, with a higher concentration of DDAB. The column was then coated with 1 m*M* DDAB at 1 ml/min for 30 minutes. Unbound DDAB was washed from the column with water overnight. With an eluent of 1 m*M* phthalate (not pH adjusted), it was found that the peaks were badly split, and peak shape actually became worse, after the void was filled.

It is clear from the above discussion that in attempting to reduce the amount of sorbed DDAB during the coating step, the only parameter that was adjusted was the concentration of DDAB in 100 % water. It would appear that in the case of DDAB, merely decreasing its concentration in a 100 % aqueous solution is not sufficient to effect a decrease in the amount coated onto the stationary phase. This may be due to the more hydrophobic nature of DDAB compared to other surfactants previously used. It is clear from previous studies performed by other workers that increasing the concentration of an organic modifier (methanol or acetonitrile) is more effective in decreasing the amount of surfactant sorbed onto the column, thereby decreasing the exchange capacity [1,3,5,18].

Alternatively, Baryla and Lucy [8] recently reported the use of 1,2-dilauroyl-*sn*-phosphatidylcholine (DLPC) mixed with DDAB to produce a semi-permanent coating on fused silica capillaries to control EOF reversal in CE. They initially found that by coating the capillary with DDAB alone, the EOF produced was too fast to obtain baseline resolution of all anions, and polarisable anions such as iodide and thiocyanate strongly interacted with the wall coating leading to long migration times and poor peak shape. However, the EOF could be finely tuned by the addition of DLPC to the coating solution. Since this zwitterionic surfactant has a net charge of zero, it effectively dilutes the positive charge at the capillary wall when mixed with DDAB. Adjusting the DDAB/DPLC ratio in the coating solution caused the EOF to be modified from near zero (100 % DPLC) to fully reversed (100 % DDAB).

More recently, Fritz *et al.* [19] applied a similar principle to the coating of reversed phase columns with mixtures of DDAB and non-ionic surfactants. It was found that coating a reversed phase column first with a non-ionic relatively polar surfactant such as Tween, followed by a second coating over this first coated layer with DDAB, reduced retention times of organic anions such as benzoate and phthalate, and greatly improved efficiency. The presence of the non-ionic polar surfactant dramatically reduced the hydrophobic interaction between these anions and the stationary phase. It was demonstrated that ion-exchange retention behaviour is determined by both the lower neutral surfactant layer and the upper charged surfactant layer acting in concert, and not by the upper layer alone.

Attempts to reduce the column exchange capacity during coating in this work were not pursued further, and instead it was decided to coat a new column with the original coating of 10 m*M* DDAB, in order to examine to stability of the column coating over time, by making a large number of consecutive injections. The column chosen for this study was a Phenomenex Kingsorb C_{18} 30 X 4.6 mm, 3 µm column. The Kingsorb column was coated in the same manner as described in Section 5.3.1.

During the coating stability experiment discussed below in Section 5.3.11, it was observed that the chromatography obtained on the new coated Kingsorb column, was not completely comparable with the optimised separation achieved in earlier work with the Hypersil column. It was found that with an eluent of 5 m*M* phthalate, the pH had to be adjusted to pH 7.0 (instead of pH 7.5) to resolve phosphate from chloride. Figure 5-29 shows a comparison of retention times between the optimized separation on the Hypersil column and the optimized separation on the Kingsorb column. The retention times of the anions are all reduced on the Kingsorb column relative to the Hypersil column, (phosphate: 30 sec to 23 sec, chloride: 35 sec to 27 sec, sulphate: 60 sec to 44 sec, nitrate; 155 sec to 85 sec) with the greatest effect on the late eluting nitrate peak. This can be explained in part, by the effect of pH values lower than pH 7.5 on the retention time of nitrate. Figure 5-11 shows that the retention of nitrate is significantly reduced (relative to other anions in the test mix) with only a slight decrease in eluent pH of 0.5 units. However, since the retention time of nitrate is reduced almost by half on the Kingsorb column, this reduction cannot simply be due to the reduced pH alone, and so the difference in the stationary phases is also presumed to have played a part.

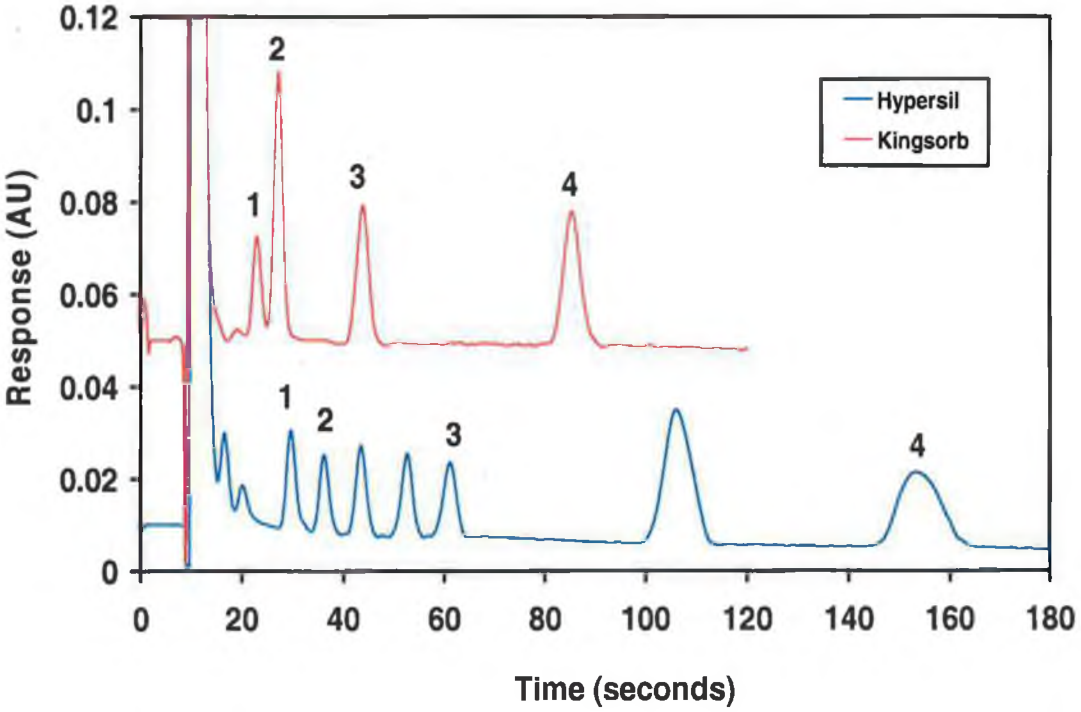

Figure 5-29. *Comparison of retention times of selected anions between 3 µm Hypersil and Kingsorb stationary phases coated with DDAB. All chromatographic conditions are as in Figure 5-20 except for the mobile phases for each column. The lower trace represents a separation of nine anions on the Hypersil column with a mobile phase of 5 mM phthalate pH 7.5 as shown in Figure 5-20. The upper trace represents a separation of phosphate, chloride, sulphate and nitrate on a Kingsorb column with a mobile phase of 5 mM phthalate pH 7.0. Peaks: [1] phosphate, [2] chloride, [3] sulphate, [4] nitrate.*

A similar shift to earlier retention times between Kingsorb and Hypersil stationary phases was previously observed and discussed in Chapter 4, Section 4.3.6. As was proposed in Section 4.3.6, and illustrated in Figure 4-17, a possible reason for the increased retention on the Hypersil phase could be that this phase has more exposed silanol groups than the Kingsorb column by virtue of its lower carbon loading. It is proposed that in this work, during permanent coating with DDAB, the surfactant is hydrophobically bound to the C_{18} chains of each phase to the same extent. However, in the case of the Hypersil stationary phase which has more exposed silanol groups, extra DDAB could also coat the column via electrostatic interactions with the silanol groups through two possible mechanisms. As illustrated in Figure 5-30, micelles of DDAB could also interact with charged silanol groups resulting in increased exchange capacity and therefore increased retention on the Hypersil column.

Figure 5-30 also shows that monomeric DDAB could be coating to the excess silanol groups in a manner similar to what occurs when fused silica capillaries are semi-permanently coated with DDAB in CE. Two layers form, the first being electrostatically bound to the silica via the positively charged head-group, and the second being hydrophobically bound to the first layer such that the charged groups face out into the bulk eluent. However, as no attempts were made to verify this proposed difference in coating mechanisms, it should be emphasised that these considerations are merely speculative.

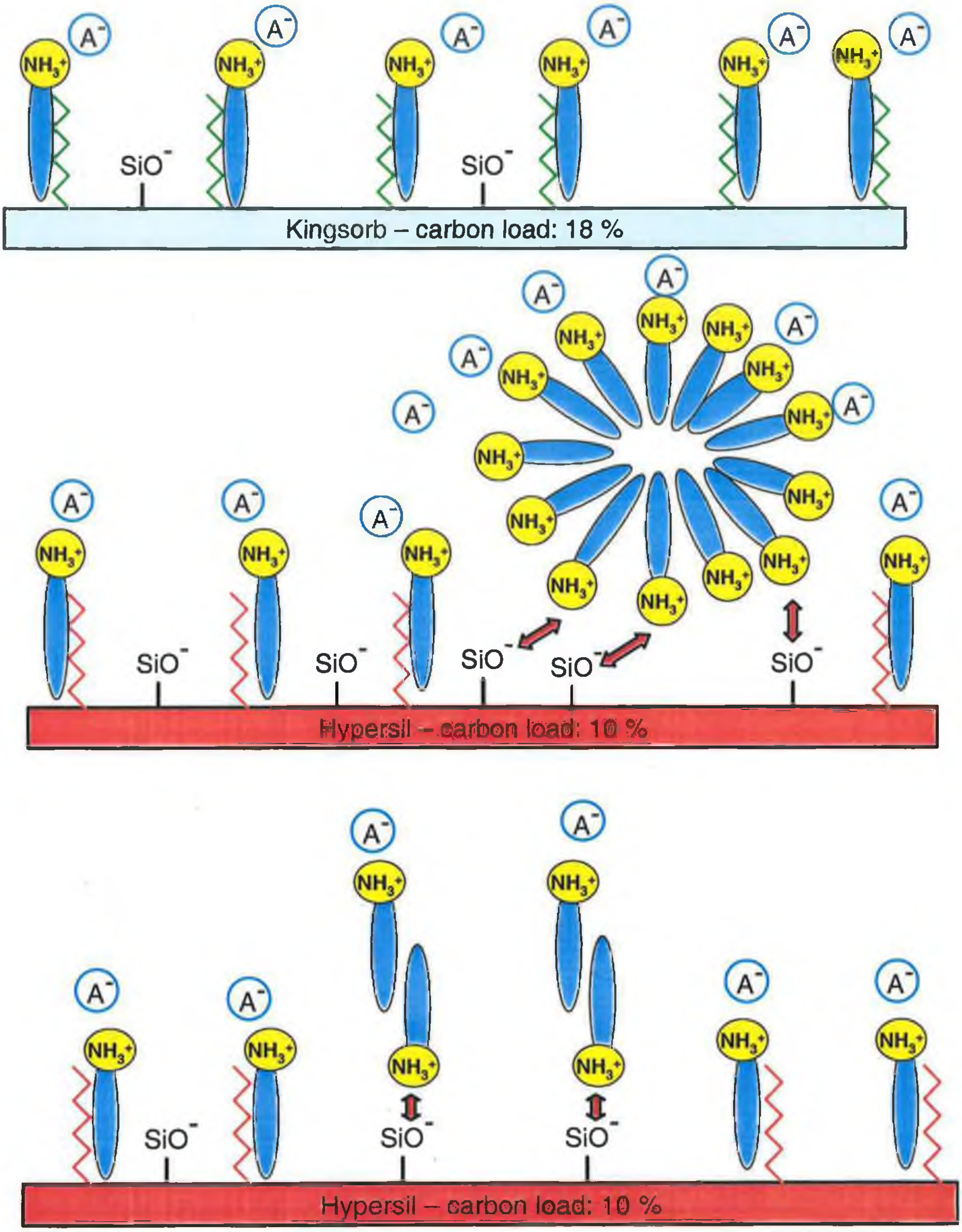

Figure 5-30. *Proposed mechanism resulting in increased retention on a 3 µm Hypersil stationary phase relative to a 3 µm Kingsorb stationary phase using DDAB as IIR. The upper figure represents the Kingsorb silica phase (in green), with IIR (two hydrophobic chains represented by blue oval) hydrophobically bound to the C_{18} chains (in green). Analyte anions (A^-) are retained by electrostatic interactions with the charged ammonium group of DDAB (in yellow). The middle figure represents the*

Hypersil silica phase (in red), with IIR (in blue) hydrophobically bound to the C_{18} *chains (in red). Despite the fact that there are less* C_{18} *chains, micelles of IIR are also electrostatically bound to exposed silanol groups, increasing the overall dynamic exchange capacity. The lower figure shows that the Hypersil silica phase could also electrostatically bind a double layer of monomeric DDAB via the exposed silanol groups such that the charged head-groups face out into the bulk aqueous phase.*

5.3.11 Column coating stability.

To examine the stability of the column coating over time, 400 consecutive injections were made of a standard containing 25 mg/*L* phosphate, chloride, sulphate, and 50 mg/*L* nitrate, using the optimised mobile phase. There was no appreciable shift in retention time for the analyte peaks despite the column conditions including an elevated temperature of 45 °C. There was also no observed shift in plate number for the analyte peaks, with average plate numbers of 26,906 plates/m for phosphate, 36,892 plates/m for chloride, 50,207 plates/m for sulphate, and 94,572 plates/m for nitrate. During the experiment, over 5300 column volumes of mobile phase were passed through the column (given an approximate column volume of 0.3 ml). The high sample throughput of the method is clearly demonstrated by this experiment, given that the 400 injections were made unattended in one night (less than 14 hours).

Figure 5-32 to Figure 5-46 illustrate how a number of standard parameters were used to evaluate the chromatography, namely; retention time, peak area, peak efficiency, and in the case of nitrate and sulphate, peak shape (asymmetry). The relative standard deviation was calculated for the data points for each injection in all of the figures.

However, in some cases, for example, Figure 5-33, which shows the peak area stability for phosphate over the 400 injections, the slope of the data points results in the % RSD being quite high (11.04 %). Given that the 400 injections were made from a single vessel containing the test mix of anions, in a manner previously described in Chapter 3, it was considered that the sample solution stability was possibly deteriorating over time. Therefore, this slope in the data points was not considered to be attributable to deteriorating chromatography over time.

A linear trend line was therefore fitted through the data points and the linear regression equation was determined. Microsoft Excel was used to determine the "standard error in the slope" of this trend line, which is expressed as a percentage. The % RSD discussed previously, is simply a measure of the deviation of each data point from the mean; whereas the "standard error in the slope" is a calculation of % RSD, while also correcting for the slope in the data. It is essentially a measure of % RSD, as if there were no slope in the data.

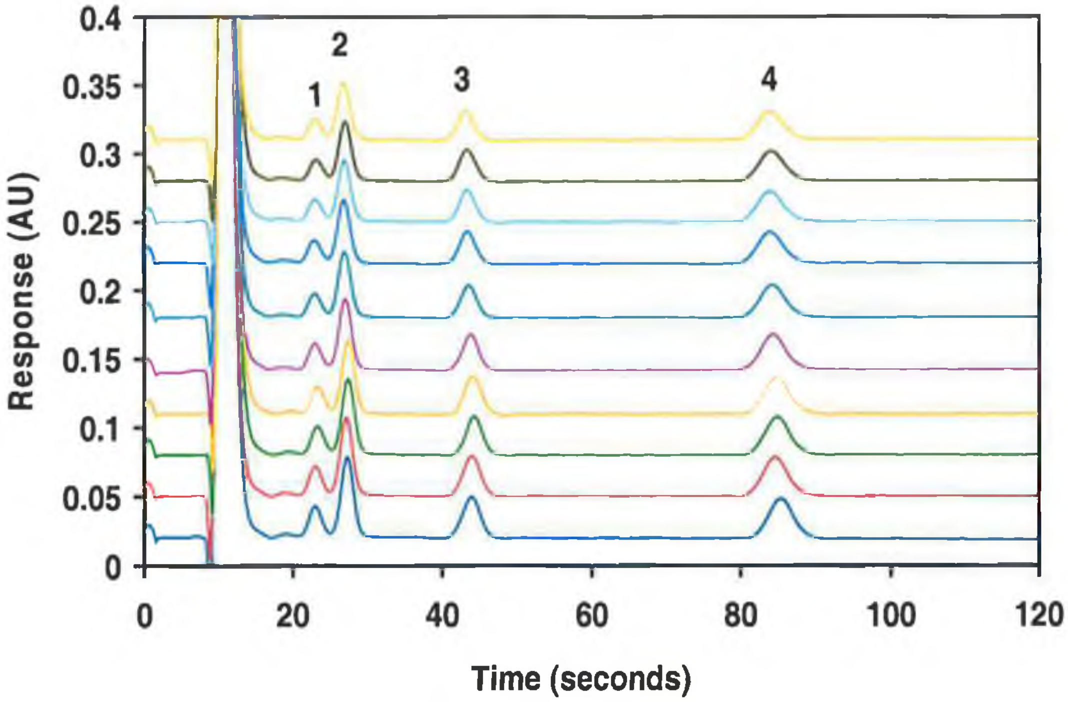

Figure 5-31. *Column coating stability (overlay of every 40th injection) of a short 3 cm 3 µm Kingsorb column coated with DDAB. The lowest trace is the first injection, the uppermost trace is the 400th injection. Chromatographic conditions as in Figure 5-20, except Column: Phenomenex Kingsorb, 3 µm particle size, 30 mm x 4.6 mm i.d. Mobile phase: 5 mM phthalate, pH 7.0. Peaks: [1] phosphate (23 seconds), [2] chloride (27 seconds), [3] sulphate (44 seconds), [4] nitrate (85 seconds).*

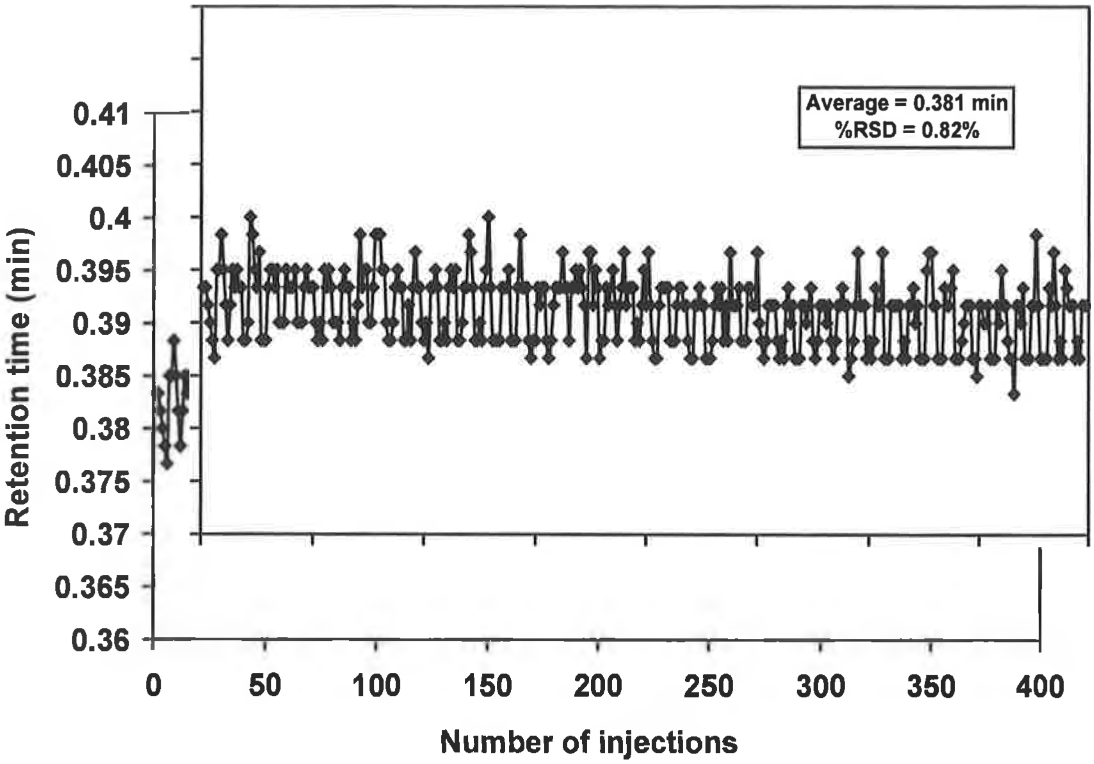

Figure 5-32. *Retention time stability study for phosphate on a short 3 cm 3 μm column coated with DDAB. Chromatographic conditions as in Figure 5-31.*

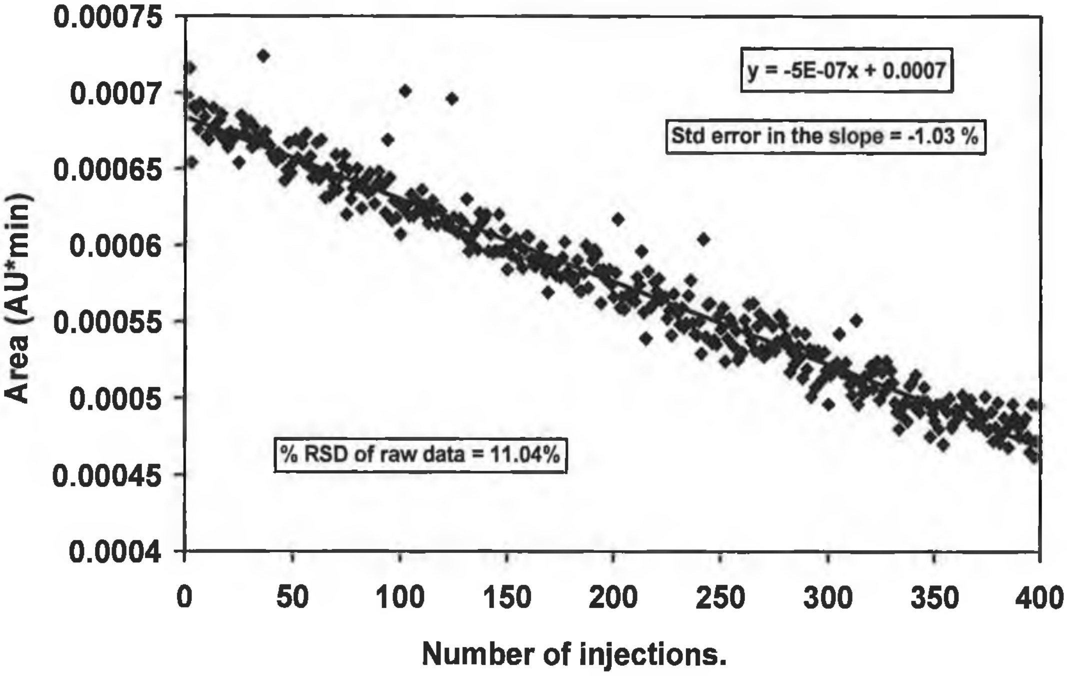

Figure 5-33. *Peak area stability study for phosphate on a short 3 cm 3 μm column coated with DDAB. Chromatographic conditions as in Figure 5-31.*

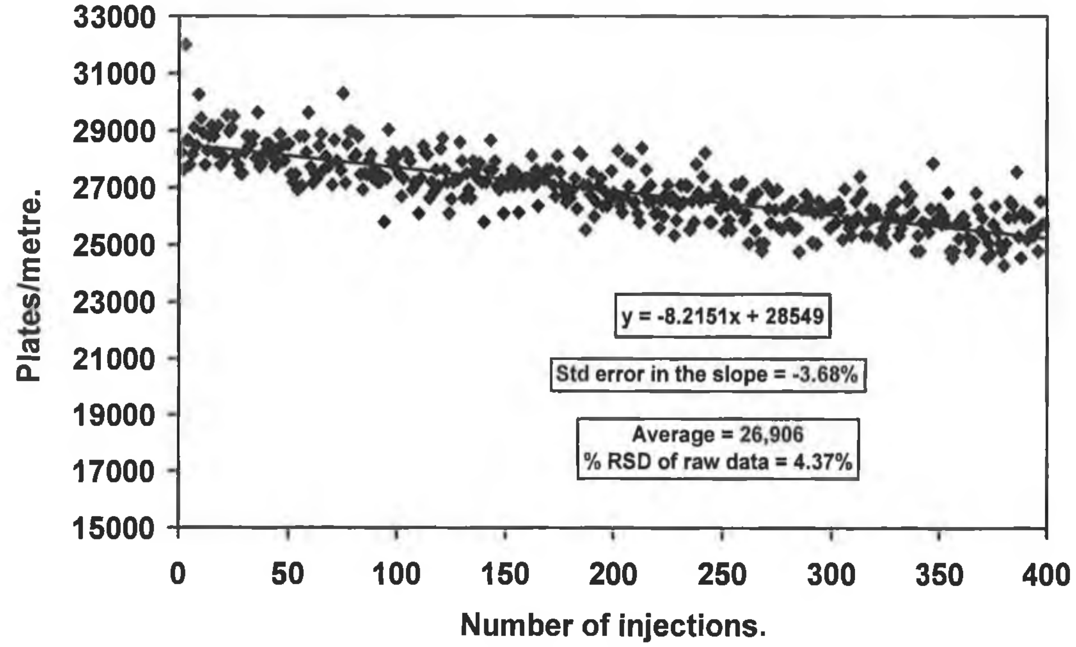

Figure 5-34. *Peak efficiency study over 400 injections for phosphate on a short 3 cm 3 μm column coated with DDAB. Chromatographic conditions as in Figure 5-31.*

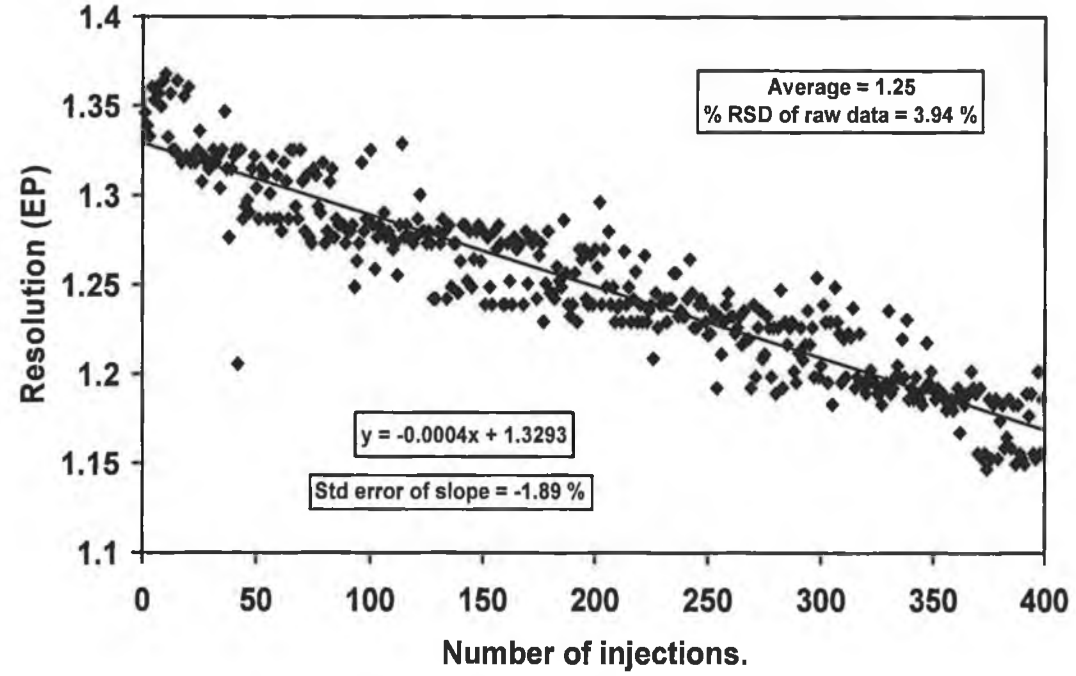

Figure 5-35. *Resolution between phosphate and chloride over 400 injections on a short 3 cm 3 μm column coated with DDAB. Chromatographic conditions as in Figure 5-31.*

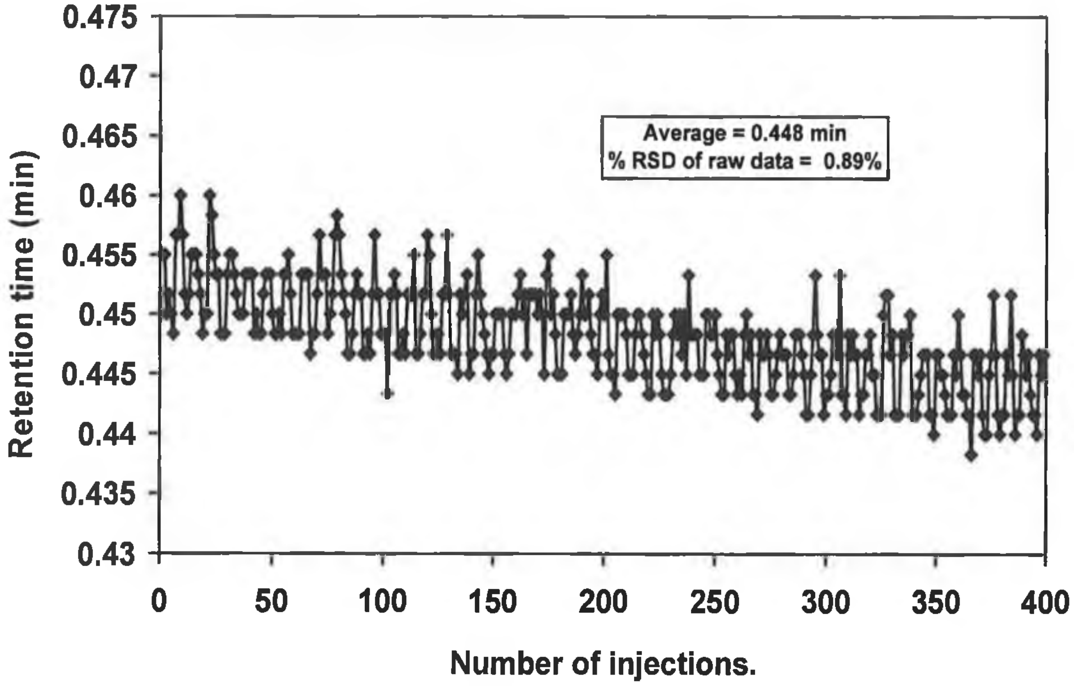

Figure 5-36. *Retention time stability study for chloride on a short 3 cm 3 μm column coated with DDAB. Chromatographic conditions as in Figure 5-31.*

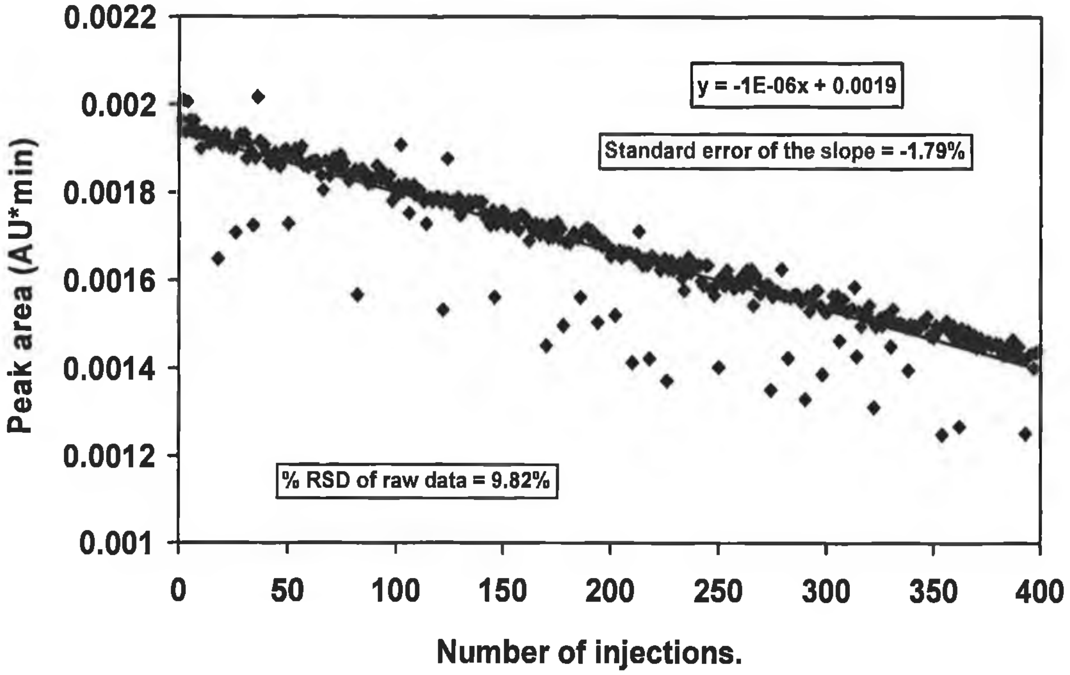

Figure 5-37. *Peak area stability study for chloride on a short 3 cm 3 μm column coated with DDAB. Chromatographic conditions as in Figure 5-31.*

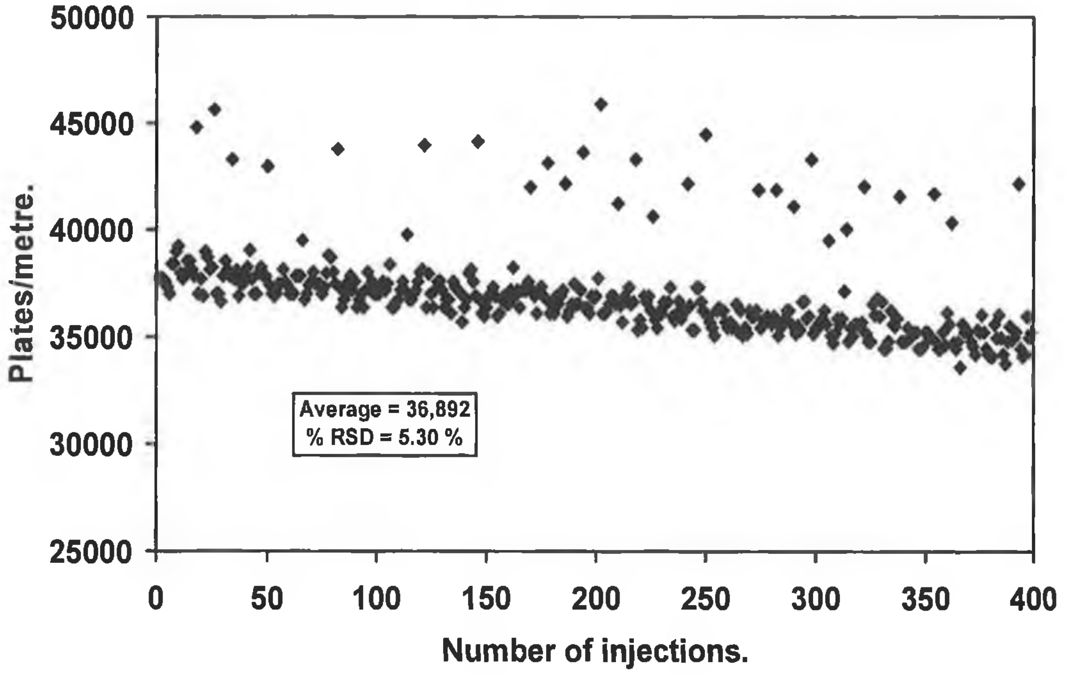

Figure 5-38. *Peak efficiency study over 400 injections for chloride on a short 3 cm 3 μm column coated with DDAB. Chromatographic conditions as in Figure 5-31.*

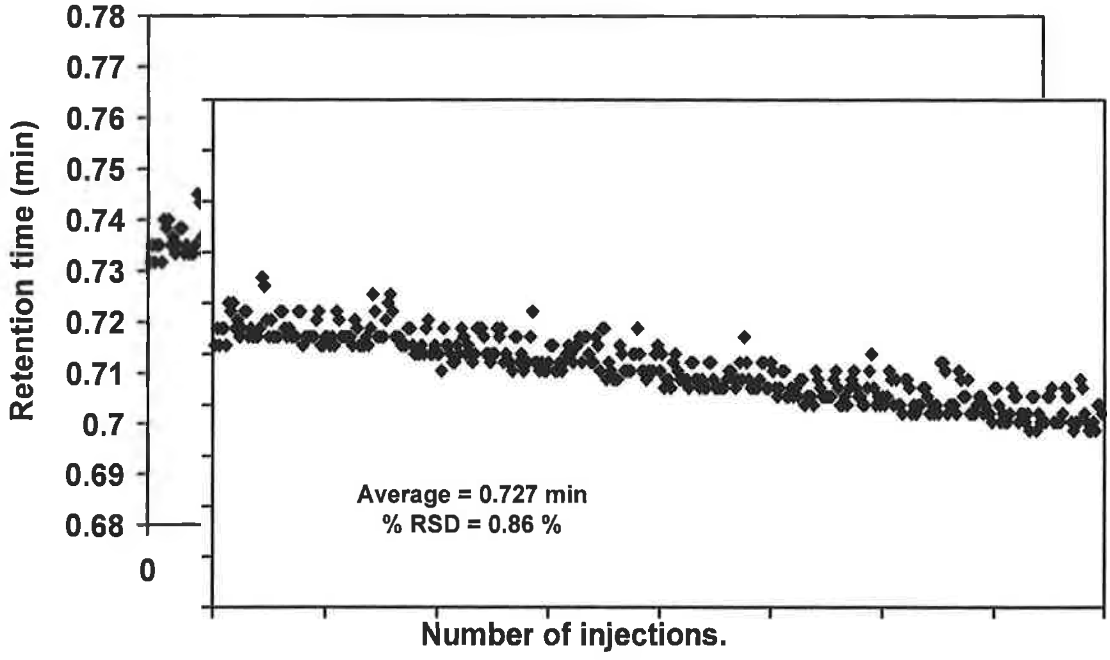

Figure 5-39. *Retention time stability study for sulphate on a short 3 cm 3 μm column coated with DDAB. Chromatographic conditions as in Figure 5-31.*

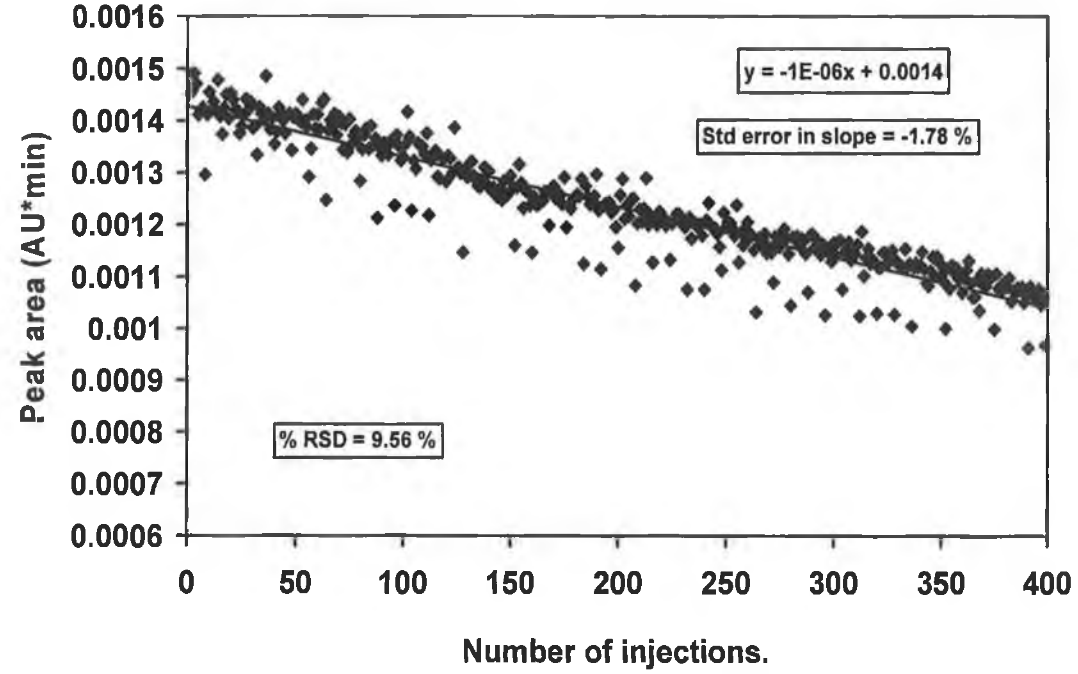

Figure 5-40. *Peak area stability study for sulphate on a short 3 cm 3 µm column coated with DDAB. Chromatographic conditions as in Figure 5-31.*

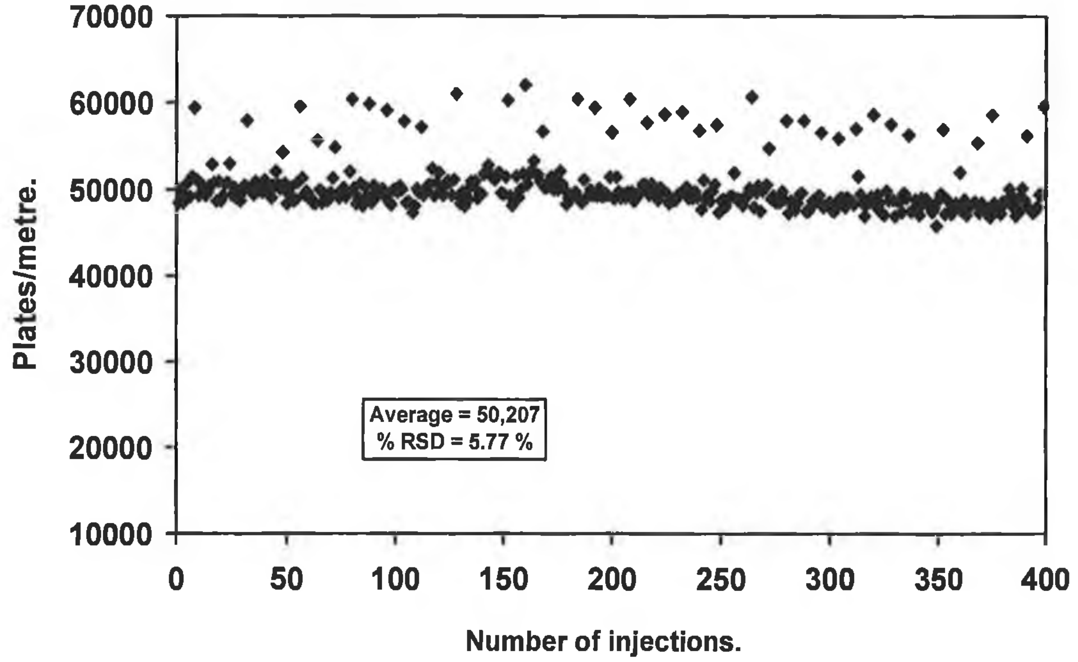

Figure 5-41. *Peak efficiency study over 400 injections for sulphate on a short 3 cm 3 µm column coated with DDAB. Chromatographic conditions as in Figure 5-31.*

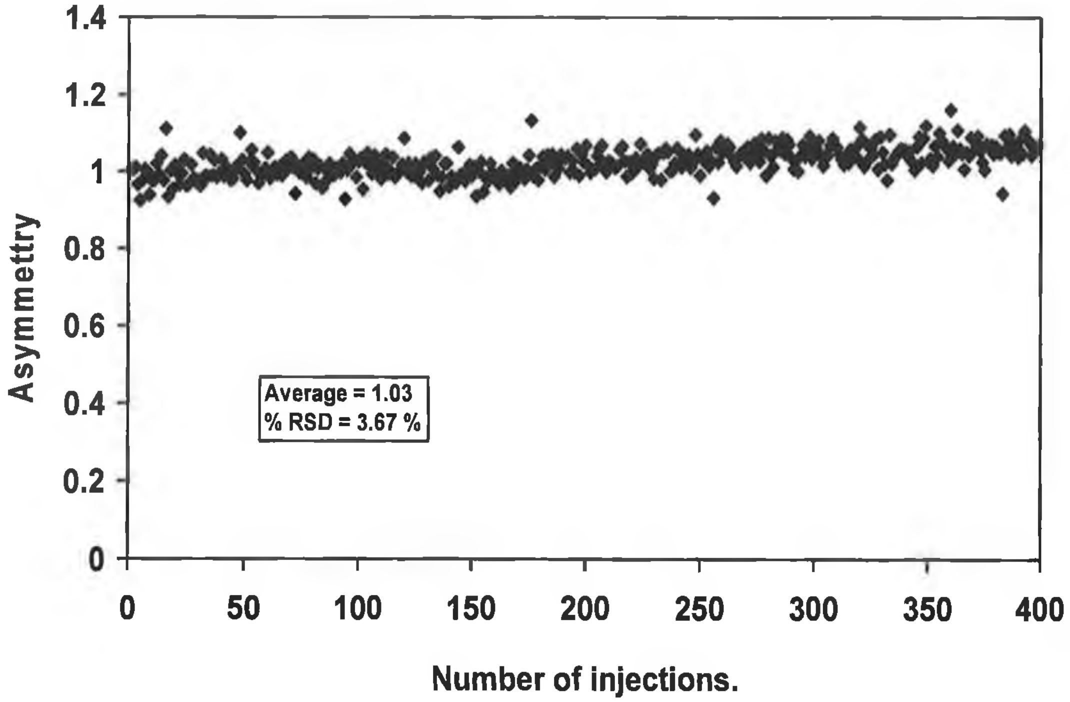

Figure 5-42. *Peak shape stability study for sulphate on a short 3 cm 3 μm column coated with DDAB. Chromatographic conditions as in Figure 5-31.*

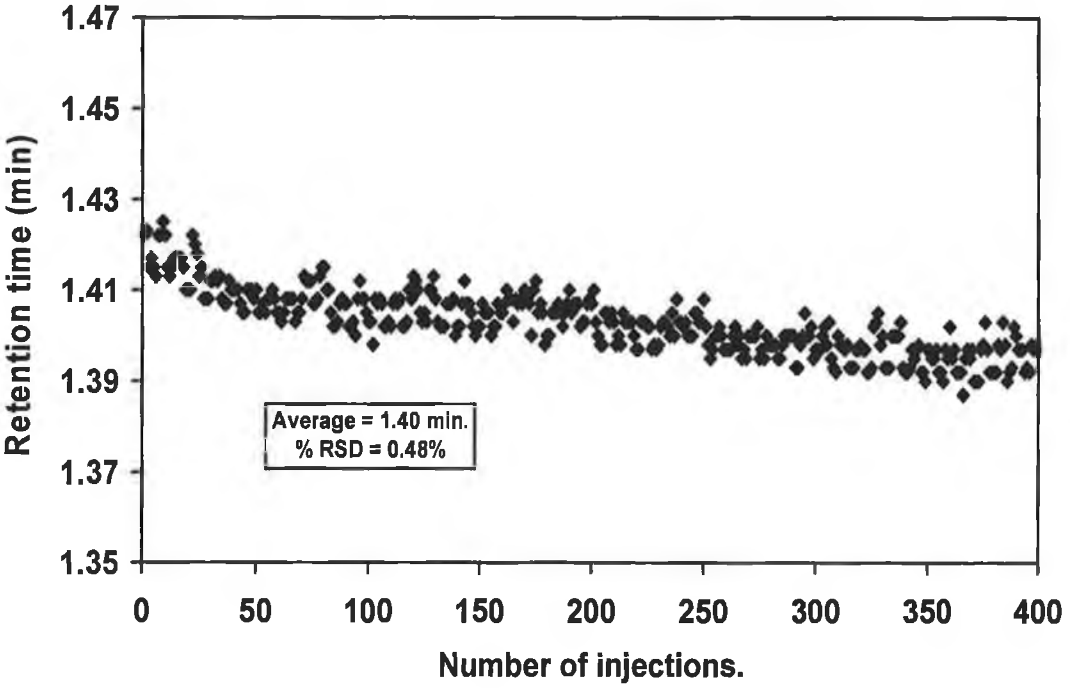

Figure 5-43. *Retention time stability study for nitrate on a short 3 cm 3 μm column coated with DDAB. Chromatographic conditions as in Figure 5-31.*

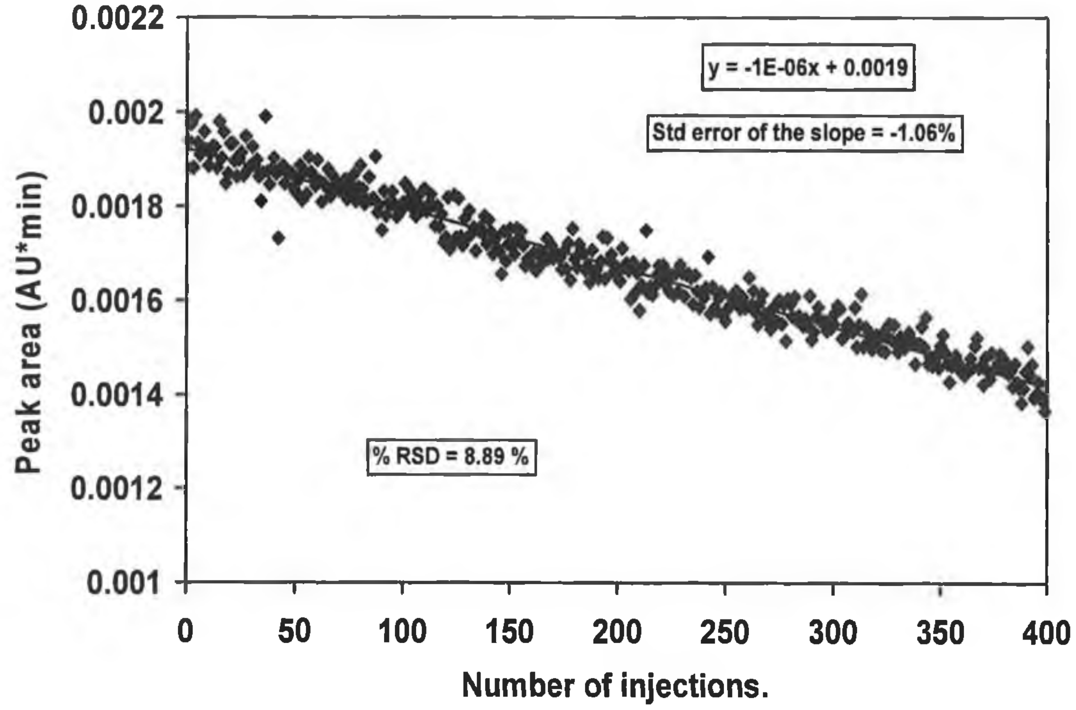

Figure 5-44. *Peak area stability study for nitrate on a short 3 cm 3 μm column coated with DDAB. Chromatographic conditions as in Figure 5-31.*

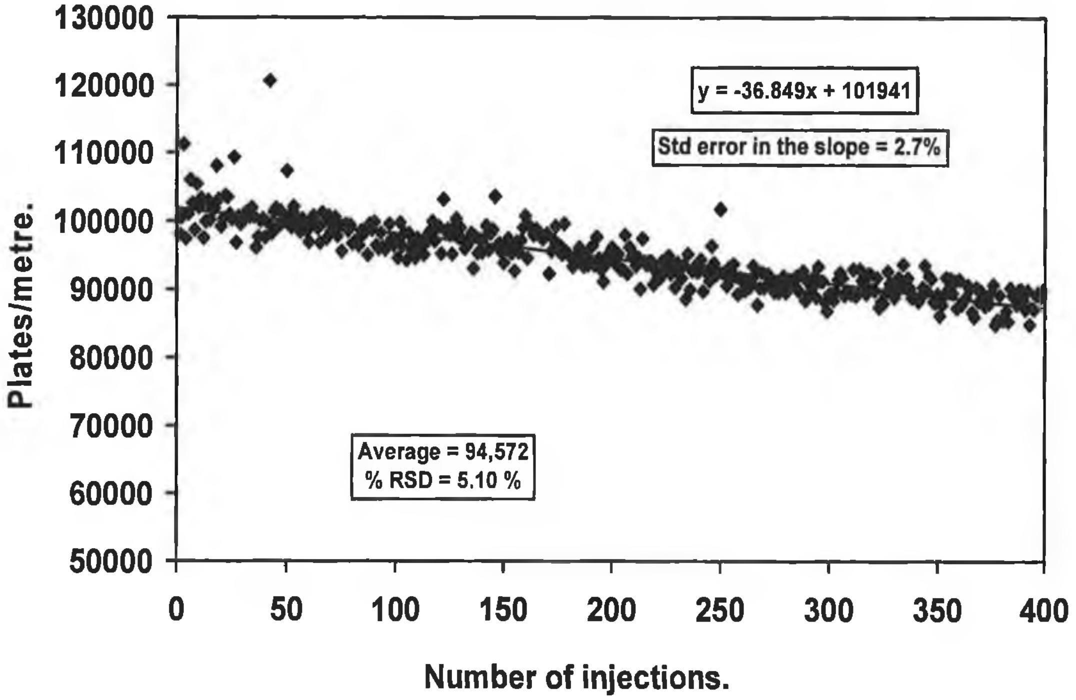

Figure 5-45. *Peak efficiency study over 400 injections for nitrate on a short 3 cm 3 μm column coated with DDAB. Chromatographic conditions as in Figure 5-31.*

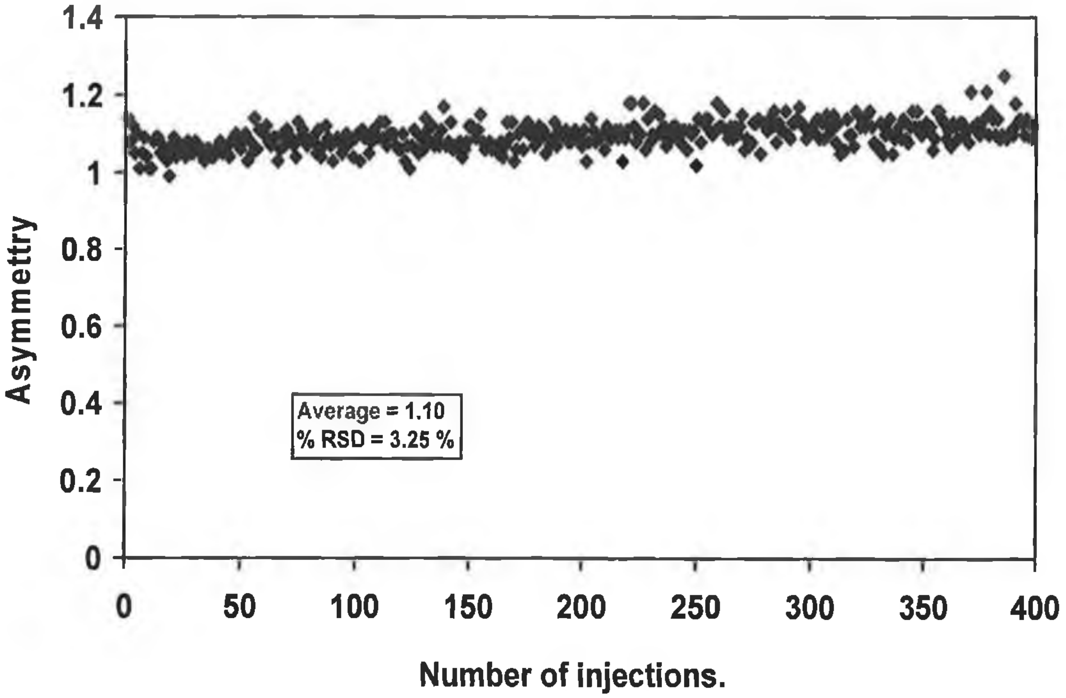

Figure 5-46. *Peak shape stability study for nitrate on a short 3 cm 3 μm column coated with DDAB. Chromatographic conditions as in Figure 5-31.*

5.4 Conclusion.

A rapid chromatographic method has been described for analysis of nine common anions in < 180 seconds, (with the first seven anions, including phosphate, chloride and sulphate, separated within 65 seconds) using a 3 cm C_{18} analytical column permanently coated with didodecyldimethylammonium bromide, and a simple phthalate eluent. Indirect detection at 279 nm was used to allow the detection of non-UV absorbing anions (chloride, phosphate and sulphate) along with those that absorb. The use of complex gradient systems was avoided, and non-specialised conventional HPLC equipment used throughout. Due to the higher surface area of the stationary phase particles, the excellent mass transfer characteristics of the column allowed for good peak shape and separation efficiency (up to 91,000 plates/metre). The applications of this method have been illustrated with analysis of a number of water samples, with the column performing particularly well with samples of considerable ionic strength, due to its high exchange capacity. This allowed for the analysis of complex sample matrices such as diluted seawater for bromide ion. The high capacity, highly hydrophobic ion exchange coating demonstrated excellent stability over time, with many injections possible before re-coating was necessary, even at elevated temperatures (45 °C).

5.5 References.

[1]. F.G.P. Mullins, Analyst 112 (1987) 665.

[2]. Y. Michigami, Y. Yamamoto, K. Ueda, Analyst 114 (1989) 1201.

[3]. K. Ito, Y. Ariyoshi, F. Tanabiki, H. Sunahara, Anal. Chem. 63 (1991) 273.

[4]. Y. Michigami, K. Fujii, K. Ueda, Y. Yamamoto, Analyst 117 (1992) 1855.

[5]. X. Jun, J.L.F.C. Lima, M.C.B.S.M. Montenegro, Analyst 120 (1995) 2469.

[6]. X. Jun, J.L.F.C. Lima, M.C.B.S.M. Montenegro, Anal. Chim. Acta. 321 (1996) 263.

[7]. J.E. Melanson, C.A. Lucy, J. Chromatogr. A 884 (2000) 311.

[8]. N.E. Baryla, C.A. Lucy, J. Chromatogr. A 956 (2002) 271.

[9]. B.A. Bidlingmeyer, C.T. Santasania, F.V. Warren Jr., Anal. Chem. 59 (1987) 1843.

[10]. J. Zou, S. Motomizu, H. Fukutomi, Analyst 116 (1991) 1399.

[11]. P. Hatsis, C.A. Lucy, Analyst 127 (2002) 451.

[12]. P.R.Haddad, P.E.Jackson, Ion Chromatography: Principles and Applications, J. Chromatogr. Library, Vol.46, Elsevier, Amsterdam, 1990.

[13]. P. Hatsis, C.A. Lucy, J. Chromatogr. A 920 (2001) 3.

[14]. B. Yan, J. Zhao, J.S. Brown, J. Blackwell, P.W. Carr, Anal. Chem. 72 (2000) 1253.

[15]. J. Chong, P. Hatsis, C.A. Lucy, J. Chromatogr. A 997 (2003) 161.

[16]. K. Fukushi, K. Watanabe, S. Takeda, S. Wakida, M. Yamane, K. Higashi, K. Hiiro, J. Chromatogr. A 802 (1998) 211.

[17]. E. Salhi, U. von Gunten, Wat. Res. 33 (1999) 3239.

[18]. P. Hatsis, C.A. Lucy, Anal. Chem. 75 (2003) 995.

[19]. J.S. Fritz, Z. Yan, P.R.Haddad, J. Chromatogr. A 985 (2003) 359.

6 Simultaneous determination of anions and cations on a 3 µm C_{18} column.

6.1 Introduction.

Benzenepolycarboxylates have been used for IC with indirect UV detection due to their strong absorbance in the UV and also due to the multiple charges which they carry when fully dissociated. This means that benzenepolycarboxylates such as *o*-phthalate (1,2-benzenedicarboxylate) [1-4], trimellitate (1,2,4-benzenetricarboxylate) [5], trimesite (1,3,5-benzenetricarboxylate) and pyromellitate (1,2,4,5-benzenetetracarboxylate) [6-8], are also strong eluting anions. Benzenepolycarboxylates are also useful for the simultaneous determination of common inorganic anions along with calcium and magnesium, since they are known to form UV absorbing complexes with these anions.

The use of phthalate eluents in IC has been discussed previously in Chapter 5. Trimellitic acid (1,2,4-benzenetricarboxylic acid) has been used by Ohta and Tanaka [5] for the simultaneous determination of chloride, nitrate, nitrite, sulphate, carbonate, calcium and magnesium. Using an anion exchange column (150 mm X 4.6 mm (5 µm) Tosoh TSKguardgel QAE-SW) and an eluent of 0.5 m*M* trimellitic acid – 0.25 m*M* EDTA, pH 6.0 with a flow rate of 1.0 ml/min all analytes were separated within 15 minutes. The addition of EDTA to the eluent resulted in the formation of stable complexes with calcium and magnesium. The influence of eluent pH upon the stability of these complexes and upon eluent strength due to increased dissociation of trimellitate and EDTA was investigated.

Ohta *et al.* [6] used an eluent of 0.15 m*M* pyromellitate – 22.5 % methanol/water, pH 6.25 at 1.0 ml/min with an anion exchange column Tosoh TSKguardgel QAE-SW 250 X 4.6 mm (5 µm) to separate carbonate, chloride, nitrate, nitrite, sulphate, calcium and magnesium within 20 minutes. The influence of eluent pH upon retention was investigated and it was found that the retention of the calcium and magnesium complexes increased with an increase in pH. Ohta *et al.* concluded that the stability of the complexes increased leading to stronger retention on the column. The retention of the calcium and magnesium complexes also increased drastically with an increase in methanol content in the eluent. Several other divalent metals were studied and found to also form complexes with pyromellitate, (Sr^{2+}, Ba^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, Cd^{2+}).

Later, Ohta and Tanaka [7] used the same column with a mobile phase of 0.15 m*M* pyromellitate at pH 5.4 to separate the same inorganic anions, but extended their studies to also include manganese, cobalt, nickel, zinc and cadmium as well as calcium and magnesium. At a flow rate of 1.0 ml/min, all of the above cations as well as chloride, nitrate, iodide, sulphate, thiocyanate and thiosulphate were resolved within 30 minutes. The use of suppressed conductivity detection as a detection mode with pyromellitate eluents was also studied.

Pyridine-2,6-dicarboxylic acid (dipicolinic acid) is another good complexing agent for divalent metals such as calcium and magnesium and so has been used as an eluent for the simultaneous determination of anions and cations. Ding *et al.* [9] used ion-exclusion chromatography with a dipicolinic acid/18-crown-6 eluent for the simultaneous determination of inorganic anions (Cl^-, NO_3^- and SO_4^{2-}) and cations (Na^+, NH_4^+, K^+, Mg^{2+} and Ca^{2+}). Using a 150 X 7.8 mm (5 μm) TSKgel OApak-A weakly acidic cation exchange column and an eluent of 1.6 m*M* dipicolinate/8 m*M* 18-crown-6, all eight analytes were resolved within 25 minutes at a flow rate of 1.0 ml/min.

Dipicolinic acid has also been used as a chelating agent for the chromatographic determination of selected transition metals [10-14]. A mixed bed ion-exchange column such as the Dionex IonPac CS5A, allows separation of transition metals by cation or anion exchange depending on the complexing agent chosen and so improved selectivity control is a distinct advantage. Janvion *et al.* [11] used a mixed bed ion-exchange stationary phase and found that with an acetate buffer / dipicolinate eluent, the analytes were separated as their ML_2^{2-} complexes (for divalent cations Cu^{2+}, Ni^{2+}, Co^{2+} and Zn^{2+}) and as an ML_2^- complex for Fe^{3+}. Motellier and Pitsch [12] also used a mixed bed ion-exchange column with an acetate buffer / dipicolinate eluent for the determination of Mn^{2+}, Co^{2+}, Zn^{2+}, Ni^{2+}, Cu^{2+} and

Fe^{3+}, but performed additional studies upon the interference of calcium in the separation.

Cardellicchio *et al.* [13] using a mixed bed ion-exchange column with an eluent of 7.0 m*M* dipicolinate, 66 m*M* potassium hydroxide, 5.6 m*M* potassium sulphate and 74 m*M* formic acid, separated Mn^{2+}, Co^{2+}, Zn^{2+}, Ni^{2+}, Cu^{2+}, Cd^{2+} and Fe^{3+} within 17 minutes at a flow rate of 1.0 ml/min. The influence of Mg^{2+} and Ca^{2+} as interferants was studied and it was concluded that the formation of ML_2^{2-} complexes with Mg^{2+} and Ca^{2+} could overload the column at high levels of these cations in the sample.

In this work, pyromellitate and later, dipicolinate, were investigated as eluents on a short 3 μm column permanently coated with didodecyldimethylammonium bromide with a view to the simultaneous determination of calcium, magnesium and common inorganic anions. The dipicolinate eluent was further used to determine these ions in a range of samples including river and sea water samples.

6.2 Experimental.

6.2.1 Equipment.

The ion chromatograph described in Chapter 2 was also used in this work. The analytical column used was a Phenomenex Kingsorb, 3 μm particle size, 30 mm x 4.6 mm I.D. column (Macclesfield, Cheshire, U.K). During optimisation studies, a loop volume of 25 μL was used, with a 50 μL loop used for sample analysis. Detection was by indirect UV at 266 nm for pyromellitic acid studies, and indirect UV at 286 nm for dipicolinic acid studies.

6.2.2 Reagents and chromatographic conditions.

The water used for mobile phase and for preparation of column coating and standard solutions, was obtained from a Millipore Milli-Q water purification system

(Millipore, Bedford, MA, USA). The analytical column was coated with DDAB in a similar manner as described in Chapter 4. The pyromellitic acid and dipicolinic acid used for eluent preparation were supplied by Aldrich, (Aldrich, Milwaukee, WI, USA). Eluent pH was adjusted using a dilute solution of NaOH, from a stock 50 % w/w solution supplied by Aldrich. The flow rate used during method development work with both pyromellitate and dipicolinate eluents was 1.0 ml/min. The flow rate used during sample analysis with dipicolinate eluents was 2.5 ml/min. Column temperature was set at ambient (~ 22 °C) for all pyromellitic acid work, and 45 °C for dipicolinic acid work. Standard solutions were prepared as described in Chapter 5, with the exception of calcium and magnesium standards which were prepared from their respective sodium salts (Aldrich). Sample pre-treatment was minimal, with samples treated only to filtration using a 0.45 μm nylon membrane filter from Gelman Laboratories (Michigan, USA).

6.3 Results and discussion.

6.3.1 Coating of the column.

Based on the comparison made between the separations obtained on the Kingsorb column relative the Hypersil column (discussed in Chapter 5) it was found that the Kingsorb column resulted in a lower overall anion exchange capacity after coating. This resulted in shorter retention times for a given mobile phase, an obvious advantage in "rapid" chromatography. Therefore, following on from the work described in Chapter 5, the same Kingsorb column was used in the work described herein.

Despite the fact that the coated Kingsorb column gave a good separation of chosen anions with a phthalate eluent, shown at the end of Chapter 5, the column had to be re-conditioned, and a fresh column coating established. The column was therefore washed with 100 % acetonitrile to remove any remaining DDAB from the column. The column was then conditioned with 95:5 water/acetonitrile at 1 ml/min for thirty minutes. In an effort to reduce the exchange capacity of the column, a coating solution of 5 m*M* DDAB in 5 % acetonitrile was used instead of 10 m*M*

DDAB in water, which was used previously. It was anticipated that the 5 % acetonitrile in the coating solution would act as a "wetting" agent to ensure a more uniform and reproducible coating. In addition, it would shift the equilibrium away from the hydrophobic stationary phase resulting in a lower anion exchange capacity after coating. As before, the column eluate was directed through a UV absorbance detector set at 214 nm, in an effort to determine the point at which the stationary phase was saturated with DDAB. The coating profile is shown as Figure 6-1.

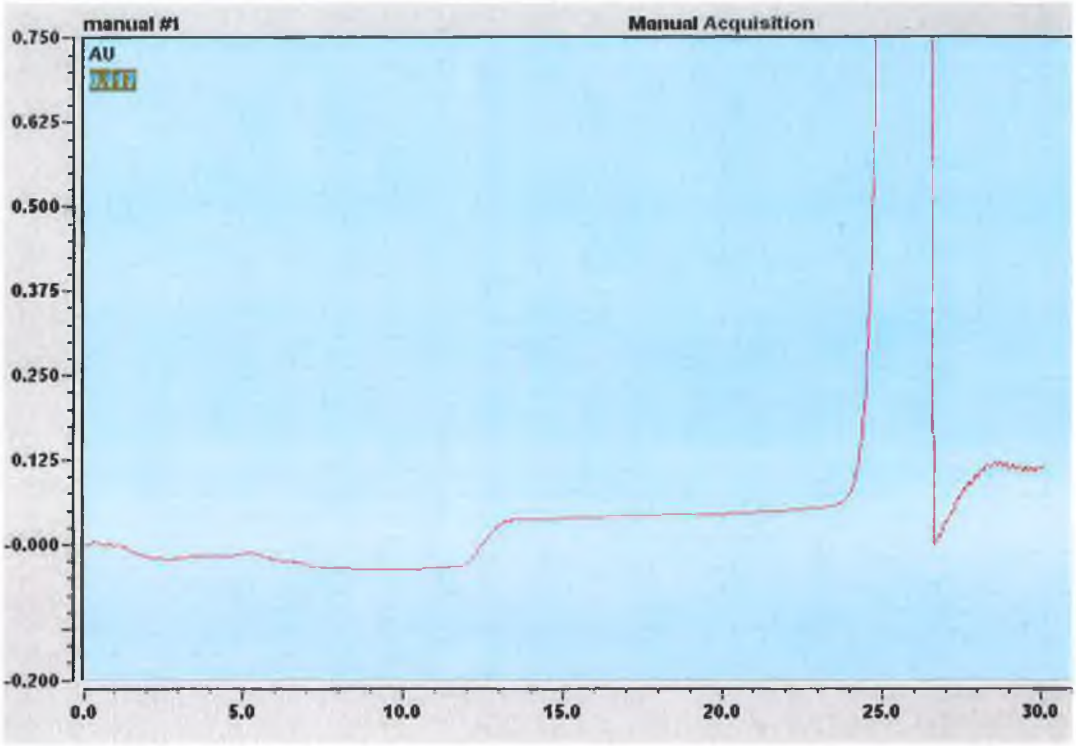

Figure 6-1. *Coating profile of a 3 cm 3 µm* C_{18} *column coated with 5 mM DDAB in 5 % ACN.*

Figure 6-1 illustrates that from time zero to twenty four minutes, the DDAB was coating the stationary phase such that only the 5 % acetonitrile solution was reaching the detector cell, resulting in the relatively low background. After ~ 24 minutes, the stationary phase was completely coated with DDAB such that unbound DDAB then began to reach the detector cell, giving a response at 214 nm.

6.3.2 Mobile phase development.

Pyromellitic acid was chosen as an eluent in this work, since unlike divalent phthalic acid, it is tetravalent, and therefore is a stronger eluent than phthalate, when fully dissociated. In addition, it is a good complexing agent, forming anionic complexes with calcium and magnesium, allowing them to be determined simultaneously with simple inorganic anions.

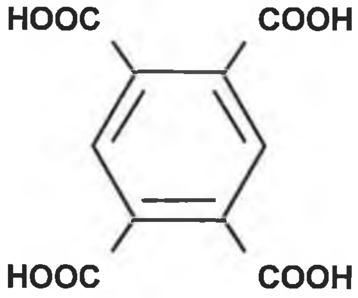

Figure 6-2. *Structure of pyromellitic acid (1,2,4,5-tetracarboxylic acid). pKa$_1$: 1.92, pKa$_2$: 2.82, pKa$_3$: 4.49, pKa$_4$: 5.64. [7]*

The test anions and cations chosen for study were phosphate, chloride, nitrate, nitrite, sulphate, calcium and magnesium. The two mobile phase parameters which would affect selectivity were the concentration of pyromellitic acid, and the pH of the eluent. It was anticipated that the optimum eluent pH would be ~ 1 pH unit higher than pKa$_4$, ie: 5.64. At these pH values, pyromellitic acid would be fully dissociated, with an overall charge of -4, and would therefore be a relatively strong eluting anion. Eluent concentrations were varied between 0.1 m*M* and 1 m*M*. Unlike the phthalate eluent described in previous work (requiring a concentration of 5 m*M*), the manner in which this column was coated, combined with the tetravalent nature of the eluting anion, would allow for lower eluent concentrations leading to improved sensitivity.

This effect is illustrated in Figure 6-3, in which a 100 mg/*L* nitrate standard was injected onto the column. A 0.5 m*M* pyromellitate eluent results in a peak height three times greater than that obtained with a 1 m*M* eluent, with all other parameters remaining constant.

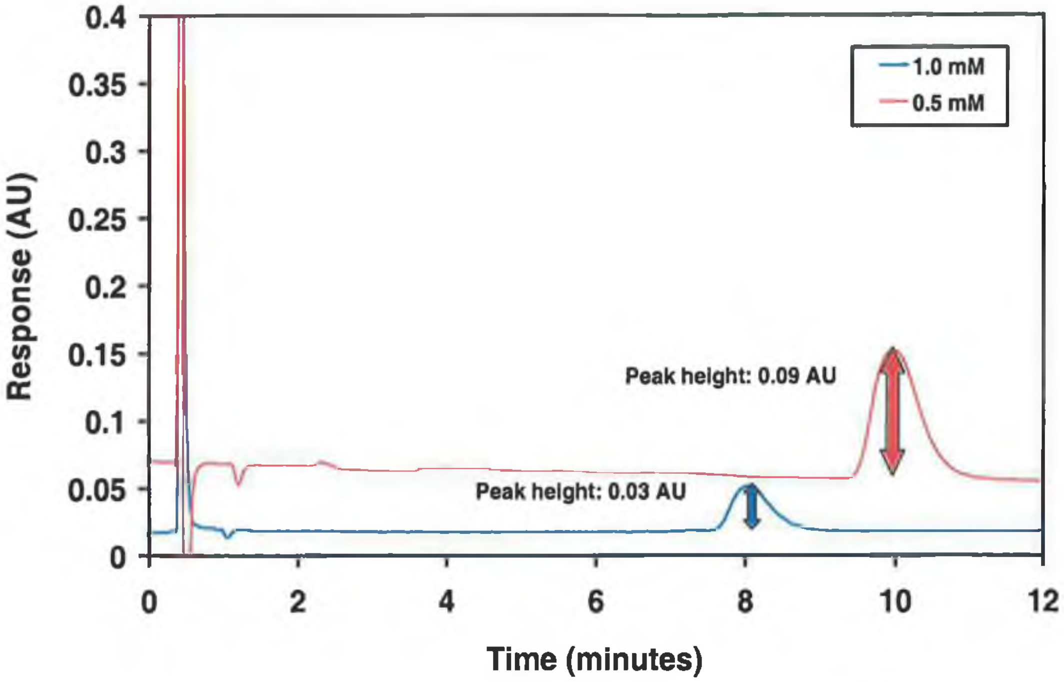

Figure 6-3. *Effect of pyromellitate eluent concentration upon the sensitivity of a nitrate determination on a short 3 µm DDAB coated column. The 0.5 mM pyromellitate eluent is offset on the Y-axis by 0.07 AU. Chromatographic conditions: Column: Phenomenex Kingsorb 3 µm, 30 mm x 4.6 mm I.D. column, Eluent: 0.5 mM pyromellitic acid, pH 6.53 and 1 mM pyromellitic acid, pH 6.48, Flow rate: 1.0 ml/min., Loop size: 50 µL, Detection wavelength: 266 nm.*

6.3.3 Comparison of eluting strength of pyromellitate versus phthalate.

A study was carried out to investigate whether a pyromellitic acid eluent would result in a faster separation compared to a phthalate eluent, as discussed previously. Both eluents were prepared at concentrations of 1 m*M* and pH 6.1. Results of this study are illustrated in Figure 6-4, Figure 6-5 and Table 6-1.

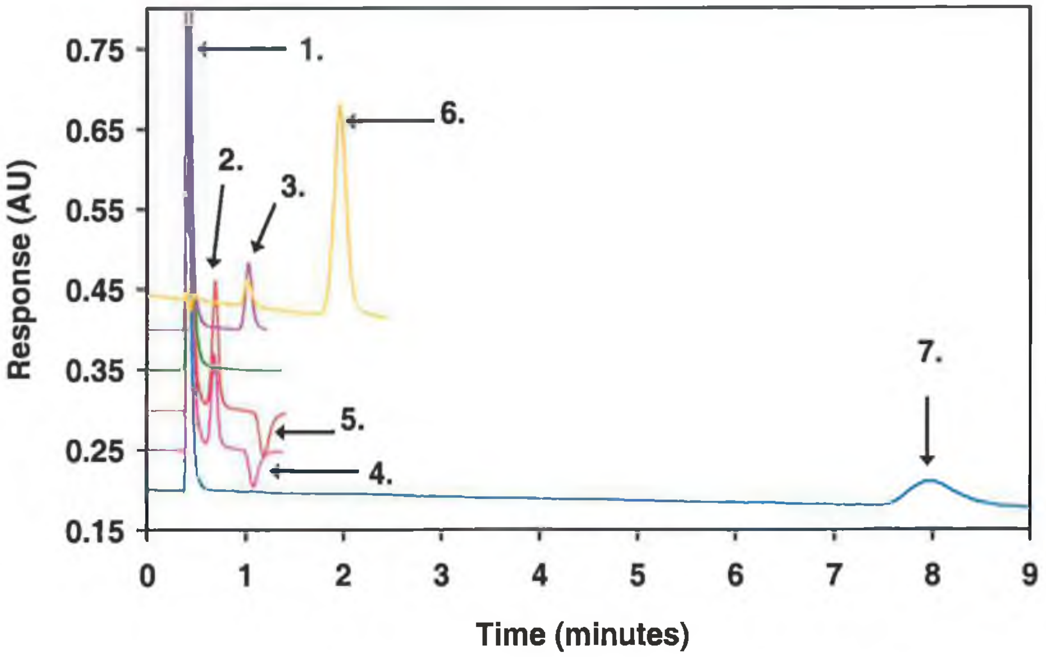

Figure 6-4. *Separation of inorganic anions with an eluent of 1 mM pyromellitate, pH 6.1 on a short 3 µm DDAB coated column. Chromatographic conditions as in Figure 6-3 except: Eluent: 1 mM pyromellitic acid pH 6.1. Loop size: 25 µL. Peaks: [1] phosphate (0.43 minutes), [2] sulphate (0.71 minutes), [3] chloride (1.04 minutes), [4] magnesium (1.1 minutes), [5] calcium (1.20 minutes), [6] nitrite*[1] *(1.98 minutes), [7] nitrate (8.06 minutes).*

---

[1] The nitrite standard was inadvertently contaminated with chloride, accounting for the extra peak in the nitrite standard, which co-elutes with chloride.

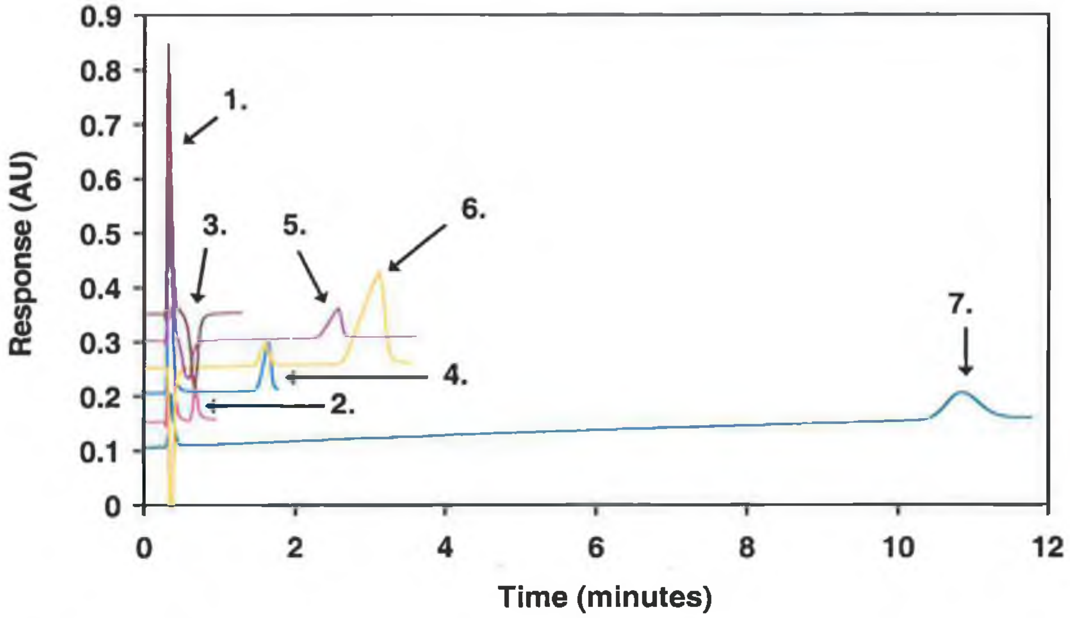

Figure 6-5. *Separation of inorganic anions with an eluent of 1 mM phthalate, pH 6.1 on a short 3 µm DDAB coated column. Chromatographic conditions as in Figure 6-3except: Eluent: 1 mM phthalic acid pH 6.1. Loop size: 25 µL. Peaks: [1] magnesium (0.61 minutes), [2] phosphate (0.68 minutes), [3] calcium*[2] *(0.69 minutes), [4] chloride (1.65 minutes), [5] sulphate (2.60 minutes), [6] nitrite*[3] *(3.14 minutes), [7] nitrate (10.9 minutes).*

Table 6-1. *Comparison of the eluting strength of pyromellitate versus phthalate eluents for selected anions on a short 3 µm DDAB coated column.*

Anion	**Retention time (minutes)**	
	1 m*M* pyromellitic acid, pH6.1.	**1 m*M* phthalic acid, pH 6.1.**
Phosphate	0.43 min.	0.68 min.
Sulphate	0.70 min.	2.60 min.
Chloride	1.04 min.	1.65 min.
Magnesium	0.61 min.	1.08 min.
Calcium	0.69 min.	1.20 min.
Nitrite	1.98 min.	3.14 min.
Nitrate	8.06 min.	10.90 min.

---

[2] The calcium standard was prepared from the sulphate salt. The data acquisition subsequent to injection of this standard was cut short (uppermost trace) which accounts for the lack of a sulphate peak at 2.60 minutes.

[3] Again, the nitrite standard was inadvertently contaminated with chloride, accounting for the extra peak in the nitrite standard, which co-elutes with chloride.

keeping with basic ion-exchange chromatography theory. All anionic analytes are retained on the column for a shorter time with the pyromellitic acid eluent; because at pH 6.1, pyromellitic acid has a higher overall charge relative to phthalic acid. Effects are most pronounced for nitrate and sulphate, with nitrate retention shortened by almost three minutes and sulphate retention by almost two minutes using a pyromellitic acid eluent.

In the case of calcium and magnesium, both are retained by forming anionic complexes with the eluent anion. In the case of pyromellitic acid, (with calcium and magnesium both having a charge of M^{2+} and pyromellitate having a charge of L^{4-} at pH 6.1) the complex is likely to have an overall charge of ML^{2-}. However, in the case of phthalic acid which would have a charge of -2 at pH 6.1, an ML complex, being neutral, would not be retained on the anion-exchange column Therefore the resulting complex is proposed to have a metal:ligand ratio of 1:2 such that an anionic complex (ML_2^{2-}) is formed. This could be possible due to the high concentration of ligand (phthalate) present at 1 m*M* relative to the concentration of metal ion. Calcium and magnesium pyromellitate complexes were retained longer than phthalate complexes, possibly due to larger formation constants for pyromellitic acid relative to phthalic acid. In any case, pyromellitic acid was found to be a better eluent than phthalate because it results in shorter retention times for inorganic anions, yet it offers a better separation of calcium and magnesium because of greater retention of calcium and magnesium complexes on the stationary phase.

6.3.4 Effect of eluent concentration on retention.

The effect of pyromellitate concentration on retention and resolution was investigated by preparation of 1 m*M* and 0.5 m*M* eluents, with resulting chromatograms shown in Figure 6-6, Figure 6-7 and Table 6-2.

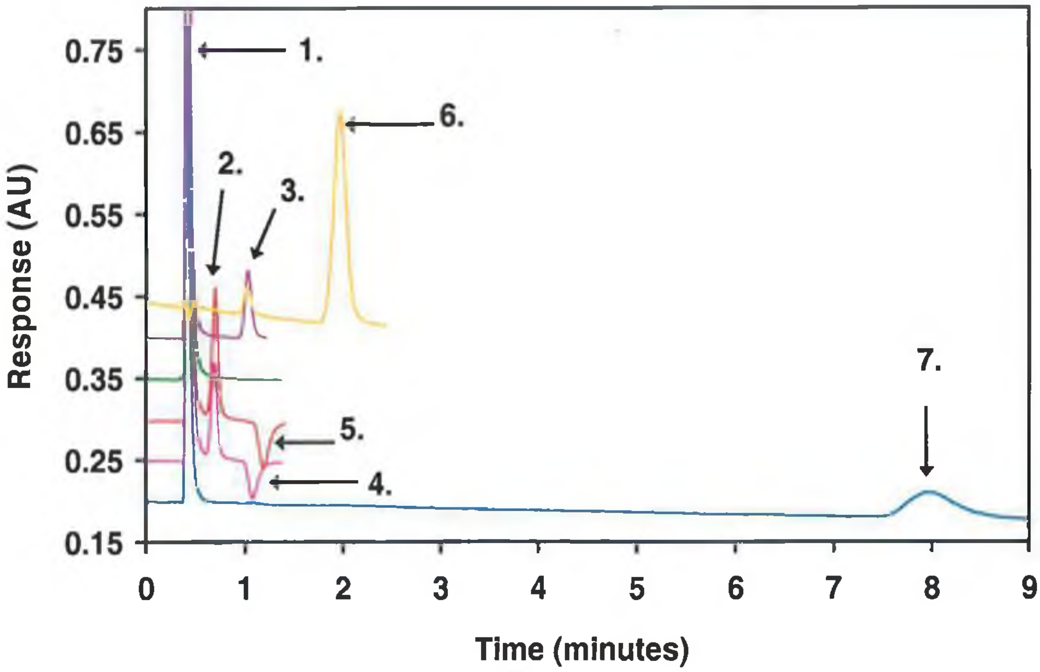

Figure 6-6. *Separation of inorganic anions with an eluent of 1 mM pyromellitic acid, pH 6.1 on a short 3 µm DDAB coated column. Chromatographic conditions as in Figure 6-3 except: Eluent: 1 mM pyromellitic acid pH 6.1. Loop size: 25 µL. Peaks: [1] phosphate (0.43 minutes), [2] sulphate (0.71 minutes), [3] chloride (1.04 minutes), [4] magnesium (1.1 minutes), [5] calcium (1.20 minutes), [6] nitrite (1.98 minutes), [7] nitrate (8.06 minutes).*

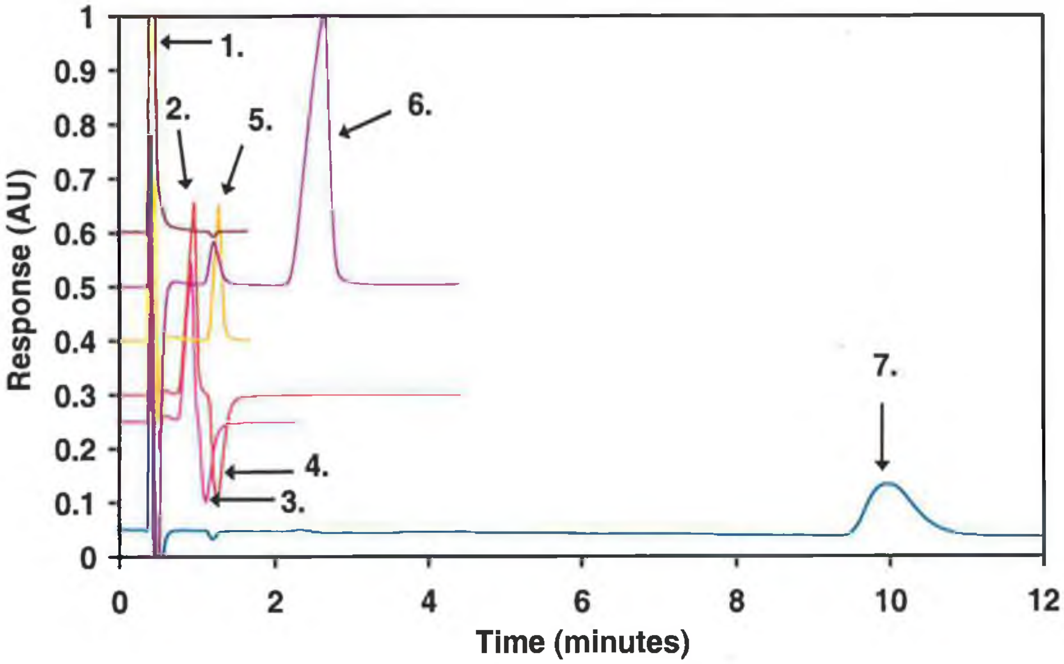

Figure 6-7. *Separation of inorganic anions with an eluent of 0.5 mM pyromellitic acid, pH 6.53 on a short 3 µm DDAB coated column. Chromatographic conditions as in Figure 6-3 except: Eluent: 0.5 mM pyromellitic acid pH 6.53. Peaks: [1] phosphate (0.42 minutes), [2] sulphate (0.98 minutes), [3] magnesium (1.13 minutes), [4] calcium (1.27 minutes), [5] chloride (1.29 minutes), [6] nitrite (2.66 minutes), [7] nitrate (10.03 minutes).*

Table 6-2. *Retention time comparison for a 0.5 mM pyromellitate eluent relative to a 1.0 mM pyromellitate eluent.*

Anion	**Retention time (minutes)**	
	0.5 m*M* pyromellitic acid, pH 6.53	**1.0 m*M* pyromellitic acid, pH 6.10**
Phosphate	0.42 min.	0.43 min.
Sulphate	0.98 min.	0.71 min.
Chloride	1.29 min.	1.04 min.
Magnesium	1.13 min.	1.10 min.
Calcium	1.27 min.	1.20 min.
Nitrite	2.66 min.	1.98 min.
Nitrate	10.03 min.	8.06 min.

As expected, retention times increased with a decrease in pyromellitate concentration, with the biggest increase noticed for the more strongly retained nitrite and nitrate. However, resolution of early eluting peaks (phosphate, sulphate, chloride, magnesium and calcium) was still difficult to achieve, and the strong retention of nitrate resulted in prohibitively long runtimes.

The anion exchange capacity of the column was considered to be a parameter which could influence the separation. With a higher anion exchange capacity, anions would spend relatively more time in the charged stationary phase than before, allowing the column to demonstrate a greater degree of selectivity. The exchange capacity of the column was therefore increased by re-coating the column, such that more DDAB was hydrophobically bound to the C_{18} phase. (One anticipated disadvantage of this action was that the late eluting nitrate peak would be later still, and would be the limiting factor in terms of overall runtimes. At this stage, a pyromellitate concentration gradient was considered as a possible solution if necessary, although throughout this work, gradient systems were avoided in order to keep instrumentation as simple as possible)

In an attempt to increase the columns exchange capacity, the column was recoated as follows: The column was washed with 100 % acetonitrile for two hours at 1.0 ml/min to remove the initial coating. The column was then conditioned with 100 % water for one hour at 1.0 ml/min. A coating solution of 10 m*M* DDAB in 100 at 1.0 ml/min to remove the initial coating. The column was then conditioned with 100 % water for one hour at 1.0 ml/min. A coating solution of 10 m*M* DDAB in 100 % water was then pumped through the column at 1 ml/min for thirty minutes. The column was finally washed with water overnight, to remove any unbound DDAB.

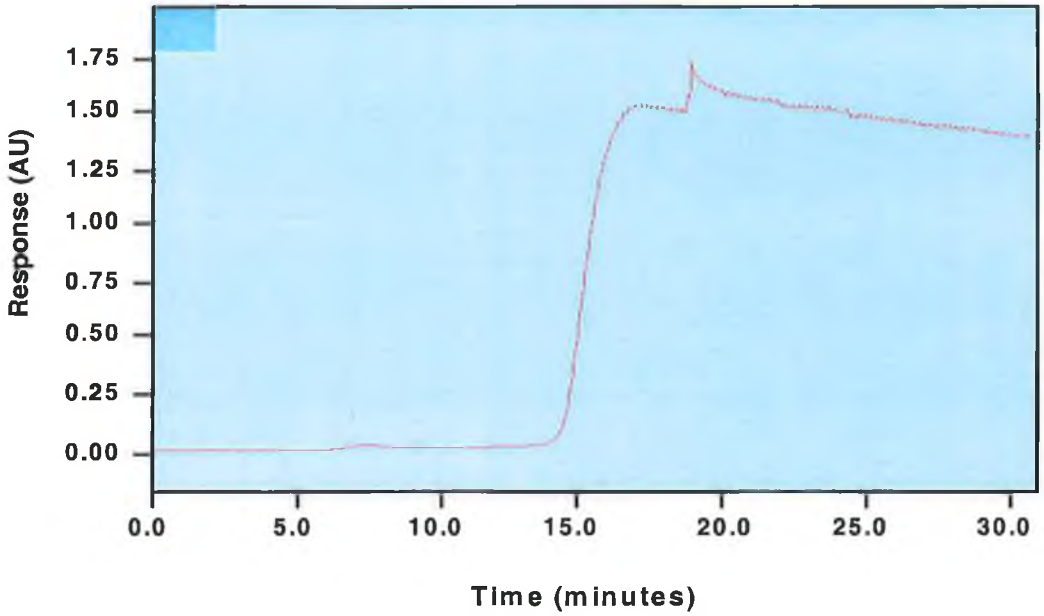

Figure 6-8. *Re-coating of the 3 μm column with 10 mM DDAB in 100 % water.*

Figure 6-8 illustrates that from time zero to approximately fifteen minutes, the DDAB was coating the stationary phase such that only the 100 % water diluent was reaching the detector cell, resulting in the relatively low background. After ~ 15 minutes, the stationary phase was completely coated with DDAB such that unbound DDAB then began to reach the detector cell, giving a response at 214 nm.

After coating this column, the anion exchange capacity was tested by preparing a 1 m*M* pyromellitate eluent, pH 5.7. The separation subsequently achieved (Figure 6-9) was not what had been expected. It had been anticipated that by coating the column with a more concentrated solution of DDAB in 100 % H_20 rather than in 5 % acetonitrile, that the column exchange capacity would then be increased, relative to the initial column coating. A separation of the test group of ions with a 1 m*M* eluent would then exhibit longer retention times relative to Figure 6-4. However in Figure 6-9 the opposite effect was observed. Retention times were considerably shorter for all analytes, particularly with nitrate eluting over 3.5 minutes earlier.

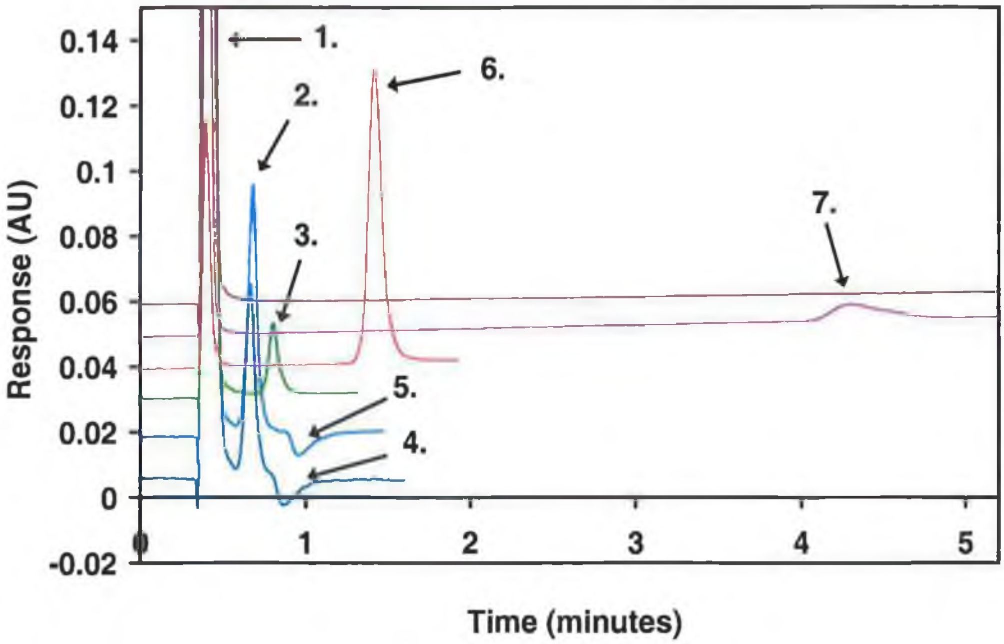

Figure 6-9. *Separation of inorganic anions on a 3 µm column coated with 10 mM DDAB in 100 % H_2O with a pyromellitate eluent. Chromatographic conditions as in Figure 6-3 except: Eluent: 1 mM pyromellitic acid pH 5.7. Loop size: 25 µL. Peaks: [1] phosphate (0.40 minutes), [2] sulphate (0.68 minutes), [3] chloride (0.81 minutes), [4] magnesium (0.88 minutes), [5] calcium (0.99 minutes), [6] nitrite (1.42 minutes), [7] nitrate (4.30 minutes).*

It is possible that since the initial column coating procedure included 5 % acetonitrile in the coating solution, this may have aided in "wetting" the stationary phase, thereby allowing more DDAB to coat the column. This is consistent with the fact that the breakthrough of DDAB occurs later during the initial coating (Figure 6-1) than during the second coating (Figure 6-8) using 100 % H_2O in the coating solution.

It was subsequently decided to reject pyromellitic acid as a candidate eluent for rapid ion chromatography in this work. With pyromellitate being a strong eluting anion due to its four dissociable hydrogen's, rapid chromatography was indeed achieved relative to phthalate, but at the expense of resolution of the weakly retained analytes. When attempts were made to change the column exchange capacity, it was noted that adjusting this parameter was difficult to do with any degree of accuracy.

6.3.5 Investigation of dipicolinic acid as an eluent.

Dipicolinic acid (2,6-pyridinedicarboxylic acid) was next considered as a possible eluent. Dipicolinic acid forms anionic complexes with a number of divalent metals, with particular selectivity for calcium. Shaw *et al.* [10] compared and contrasted three complexing pyridine carboxylic acids and noted that picolinic acid is a N,O chelator such that metals complex with the nitrogen of the pyridine ring and the oxygen of the carboxyl group. Dipicolinic acid with its second carboxyl group is therefore a N,O,O chelator.

Its pKa values are 2.10 and 4.68 [11], and therefore the target pH of the optimised eluent was > pH 5.7, to ensure that dipicolinate was fully dissociated such that its eluting strength was maximised.

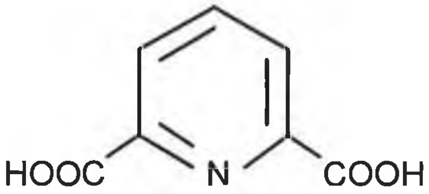

Figure 6-10. *Structure of dipicolinic acid.*

Again, the test mix of analytes comprised of phosphate, carbonate, chloride, nitrate, sulphate, nitrite, calcium and magnesium. During initial method development work, the chromatographic conditions included a flow rate of 1.0 ml/min, a loop size of 25 μL, ambient column temperature and indirect UV detection at 285 nm.

Preliminary studies with an eluent of 1.5 m*M* dipicolinic acid pH 6.0, gave an excellent separation of the test mix of analytes in under 12 minutes, as shown in Figure 6-11. Calcium was retained significantly longer than magnesium, by virtue of dipicolinate (PDCA) as a complexing ligand being more selective for calcium than magnesium (log $K_{MgPDCA}{}^{2-}$ = 2.32 and log $K_{CaPDCA}{}^{2-}$ = 4.60) [15]. Ding *et al.* [9] obtained similar results but found that calcium eluted earlier than magnesium because their studies involved the use of a cation-exchange resin. They concluded that dipicolinate was selectively complexing calcium as an anionic complex so that the interaction of uncomplexed calcium with the stationary phase was reduced relative to magnesium, resulting in the lower retention of calcium.

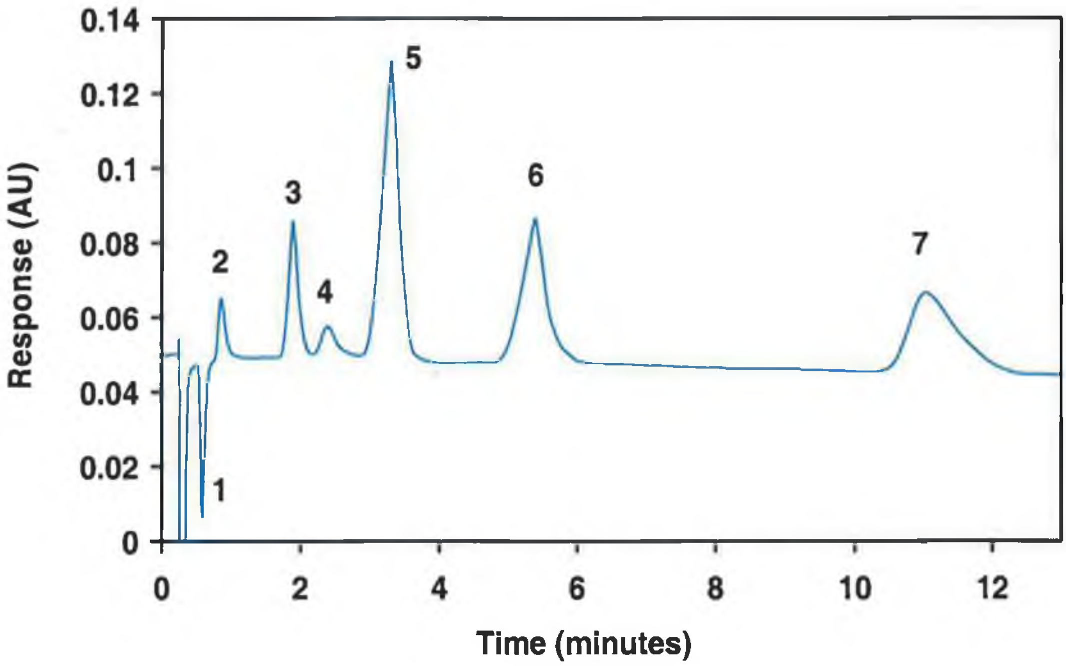

Figure 6-11. *Preliminary separation of anions and cations with dipicolinic acid on a short 3 µm DDAB coated column. Chromatographic conditions: Column: Phenomenex Kingsorb 3 µm, 30 mm x 4.6 mm I.D. column, Eluent: 1.5 mM dipicolinic acid pH 6.0, Flow rate: 1.0 ml/min., Loop size: 25 µL, Detection wavelength: 285 nm. Column temperature: ambient. Peaks: [1] 5 mg/L magnesium, [2] 10 mg/L phosphate, [3] 10 mg/L chloride, [4] 10 mg/L calcium, [5] 100 mg/L nitrite, [6] 44 mg/L sulphate, [7] 100 mg/L nitrate.*

6.3.6 Effect of column temperature and flow rate upon retention.

To study the effects of temperature on the above separation, the column temperature was increased from ambient (~22 °C) to 45 °C. It was noted that at this elevated temperature, the column backpressure was reduced from 610 psi to 494 psi, allowing higher flow rates to be subsequently used without causing excessively high backpressures. The effect of increased column temperature was to decrease the retention of all peaks, with the greatest retention time decrease noted for nitrate (~1.5 minutes). The retention time of sulphate, however, increased (~1.0 minutes) with an increase in column temperature. The endothermic retention behaviour of sulphate (that is, an increasing retention with increasing temperature) has previously been studied by Hatsis and Lucy [16].

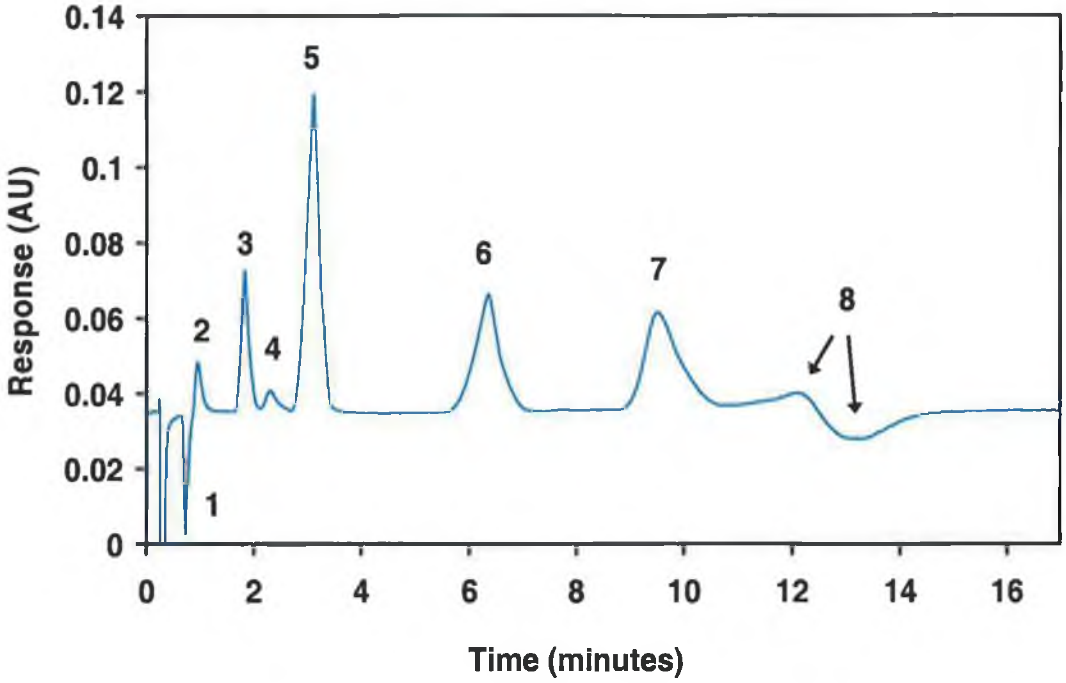

Figure 6-12. *Separation of anions and cations on a short 3 μm DDAB coated column at an elevated column temperature of 45 °C with a dipicolinate eluent. Chromatographic conditions as in Figure 6-11 except: Column temperature: 45 °C. Peaks: [1] 5 mg/L magnesium, [2] 10 mg/L phosphate, [3] 10 mg/L chloride, [4] 10 mg/L calcium, [5] 100 mg/L nitrite, [6] 44 mg/L sulphate, [7] 100 mg/L nitrate, [8] system peaks.*

The above separation was then run at 2.0 ml/min (with a resulting column backpressure of 989 psi, relative to 494 psi at 1.0 ml/min (see Figure 6-13)).

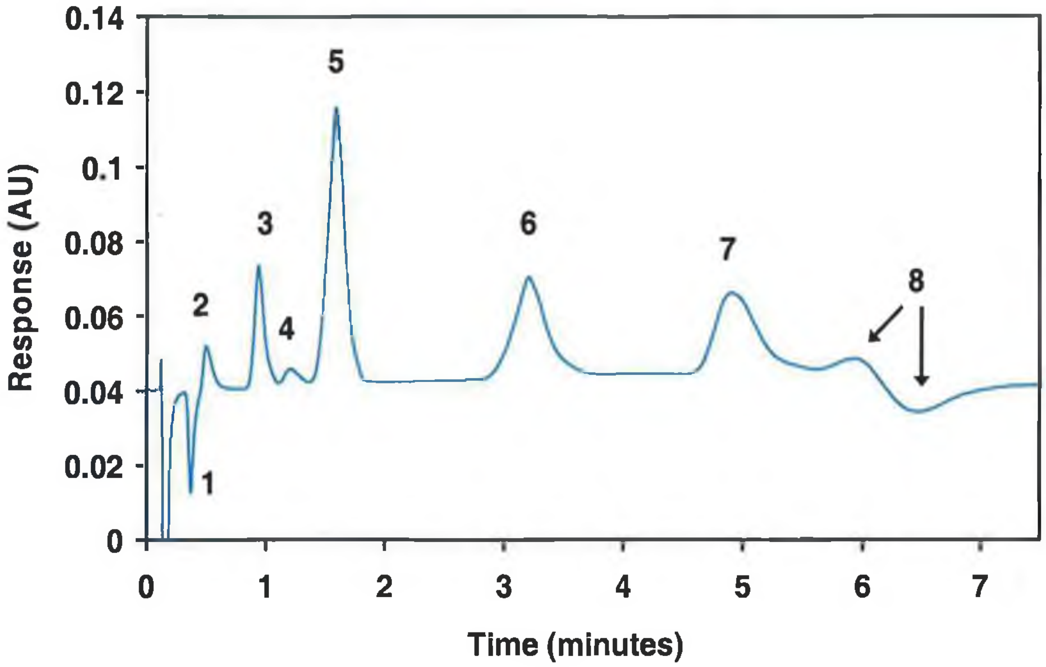

Figure 6-13. *Separation of anions and cations on a short 3 μm DDAB coated column at an increased flow rate of 2.0 ml/min with a dipicolinate eluent. Chromatographic conditions as in Figure 6-12 except: Flow rate: 2.0 ml/min. Peaks: [1] 5 mg/L magnesium, [2] 10 mg/L phosphate, [3] 10 mg/L chloride, [4] 10 mg/L calcium, [5] 100 mg/L nitrite, [6] 44 mg/L sulphate, [7] 100 mg/L nitrate, [8] system peaks.*

6.3.7 Effect of dipicolinic acid concentration on retention.

With promising results obtained in Figure 6-11, Figure 6-12 and Figure 6-13, a more methodical study of mobile phase development was initiated. With the mobile phase pH first held constant at pH 6.2, the dipicolinic acid concentration was varied across the range 1 m*M* to 4 m*M*. The capacity factor of each analyte in the test mix was plotted against increasing dipicolinate concentration, and an overall decrease in retention was noted and is illustrated in Figure 6-14 and Figure 6-15. The optimum eluent concentration was chosen as 4 m*M* dipicolinic acid based on the poor resolution of calcium and nitrite at dipicolinate concentrations < 3 m*M*. In addition, higher concentrations of dipicolinate resulted in significant decreases in nitrate retention, nitrate being the limiting factor in terms of achieving a faster separation.

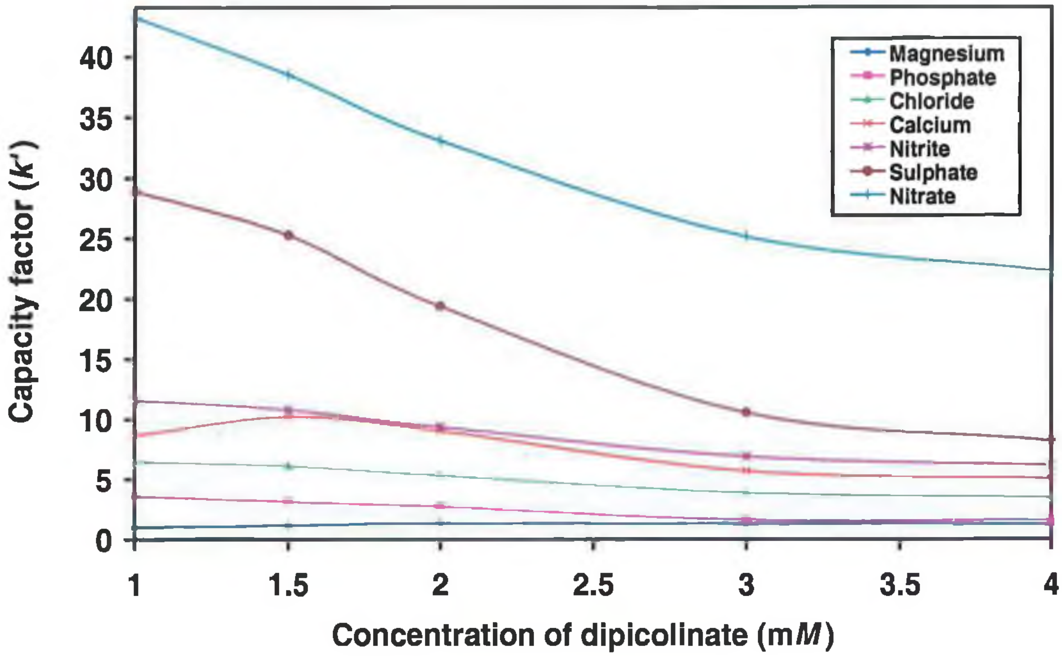

Figure 6-14. *Effect of dipicolinic acid concentration on analyte capacity factor on a short 3 µm DDAB coated column. Chromatographic conditions as in Figure 6-12. Eluent pH was maintained at pH 6.2. Analyte concentrations: 25 mg/L magnesium, 50 mg/L calcium, 10 mg/L phosphate, 10 mg/L chloride, 100 mg/L nitrate, 50 mg/L nitrite and 220 mg/L sulphate.*

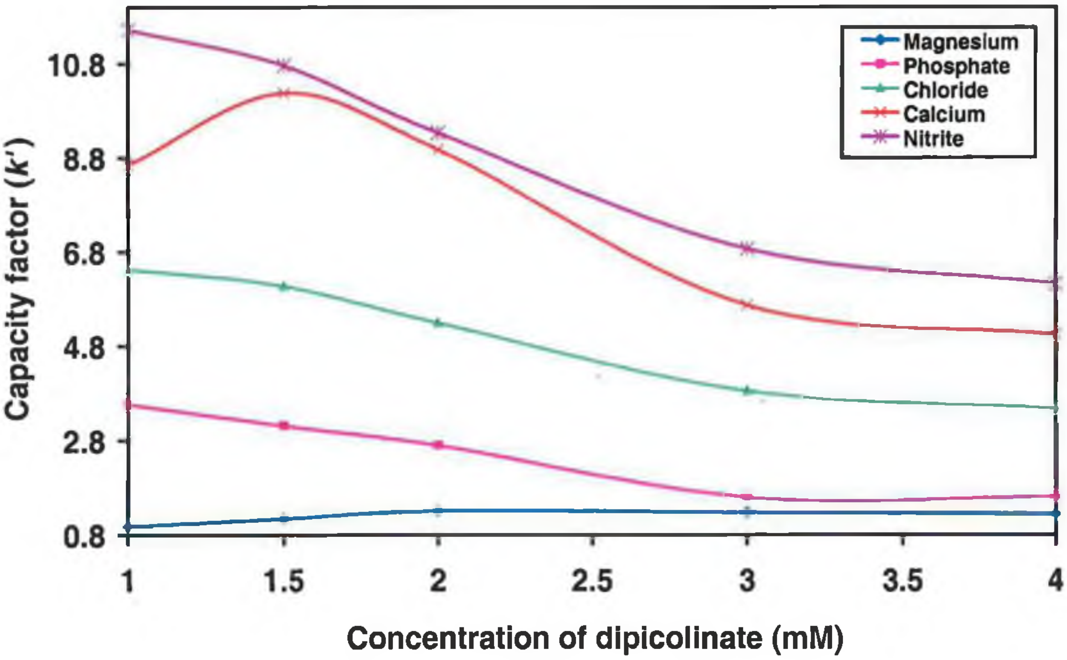

Figure 6-15. *Effect of dipicolinic acid concentration on the capacity factor of weakly retained analytes. Chromatographic conditions as in Figure 6-14.*

Classic ion chromatography theory predicts that the slope of log [capacity factor] versus log [eluent ion concentration] will be x/y, where x is the charge of the analyte anion and y is the charge of the eluent anion. Since divalent dipicolinic acid is fully dissociated at pH 6.2, it has a valence of -2. In Figure 6-16 to Figure 6-22 this relationship is shown, demonstrating that the log [capacity factor] versus log [eluent ion concentration] plot for monovalent anions (chloride, nitrite and nitrate), has a slope of ~0.5 as expected. In the case of divalent sulphate, the slope is ~1.0 as expected. Phosphate is a slightly different case, since, with pKa values of 2.16, 7.21 and 12.32, it exists in the eluent in two forms at pH 6.2; $H_2PO_4^-$ and HPO_4^{2-}, with HPO_4^{2-} likely to be the predominant species, but yet phosphate is not exclusively divalent. This would explain the slope of ~0.66 for phosphate. However, viewed overall, Figure 6-16 to Figure 6-20, show that the anions in the test group are retained by an ion-exchange mechanism, since the slope of each respective plot is equal to x/y.

In contrast to this group, are magnesium and calcium. Both slopes have correlation coefficients well below 0.92, a correlation coefficient shown previously by the anions (excluding phosphate). This would indicate that the retention mechanism for both cations is one other than simple ion-exchange. In order for calcium and magnesium to be retained at all on the column, they must form anionic complexes. The results are in agreement with those of Janvion *et al.* [11] and Cardellicchio *et al.* [13] both of whom proposed that an ML_2^{2-} complex is formed for calcium and magnesium cations giving the resulting complex an overall negative charge.

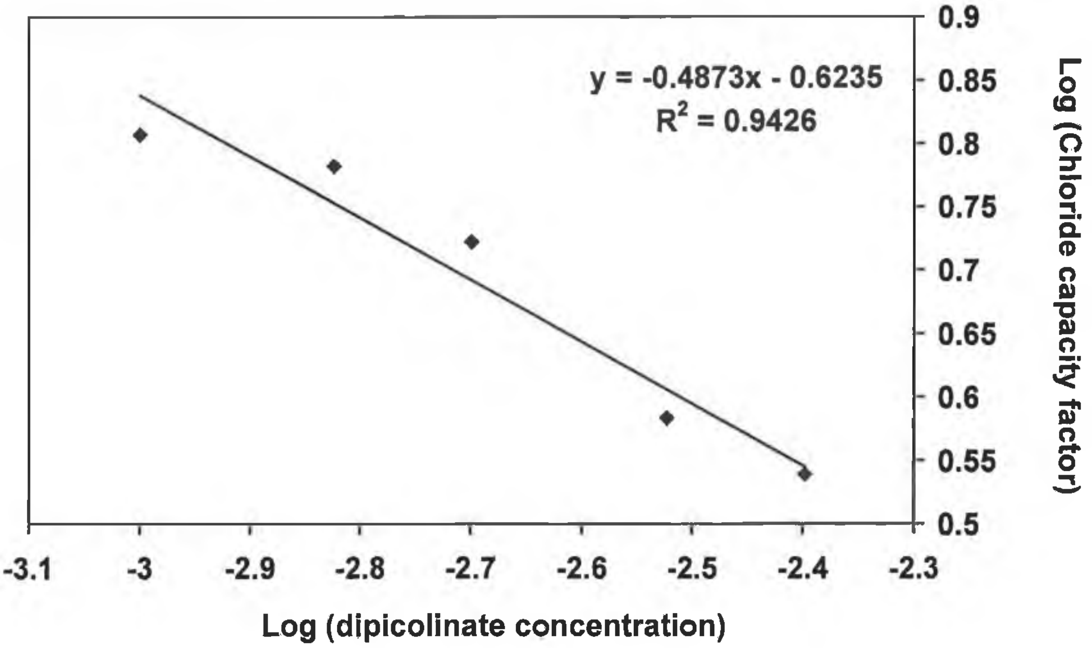

Figure 6-16. *Plot of log [chloride capacity factor] versus log [dipicolinate concentration] on a short 3 μm DDAB coated column.*

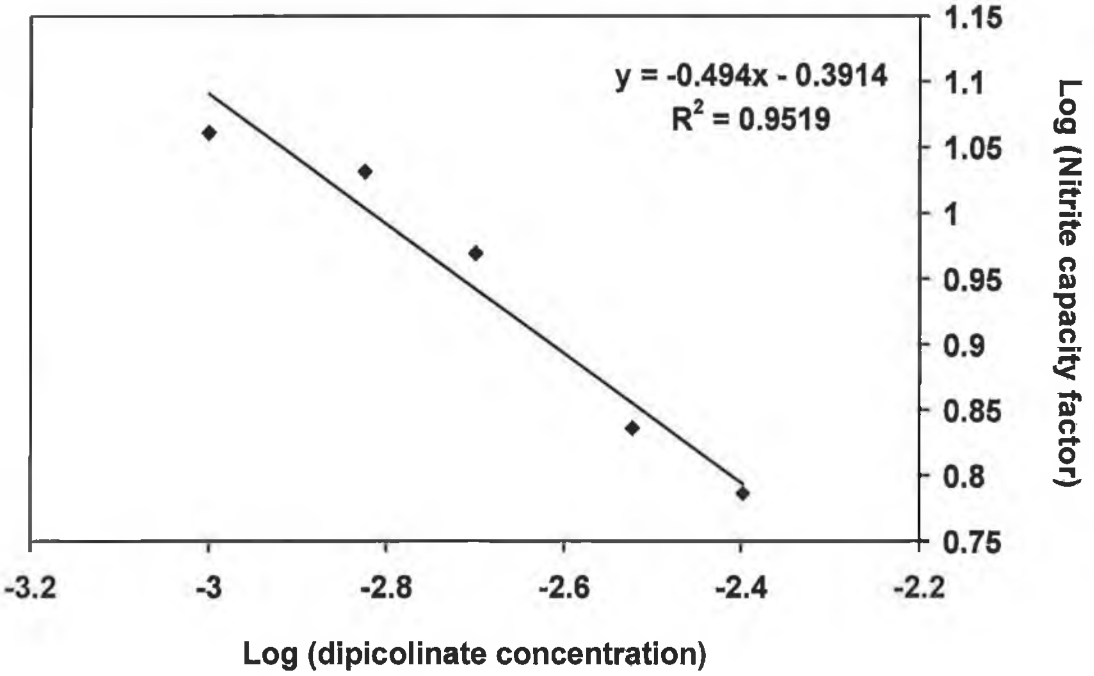

Figure 6-17. *Plot of log [nitrite capacity factor] versus log [dipicolinate concentration] on a short 3 μm DDAB coated column.*

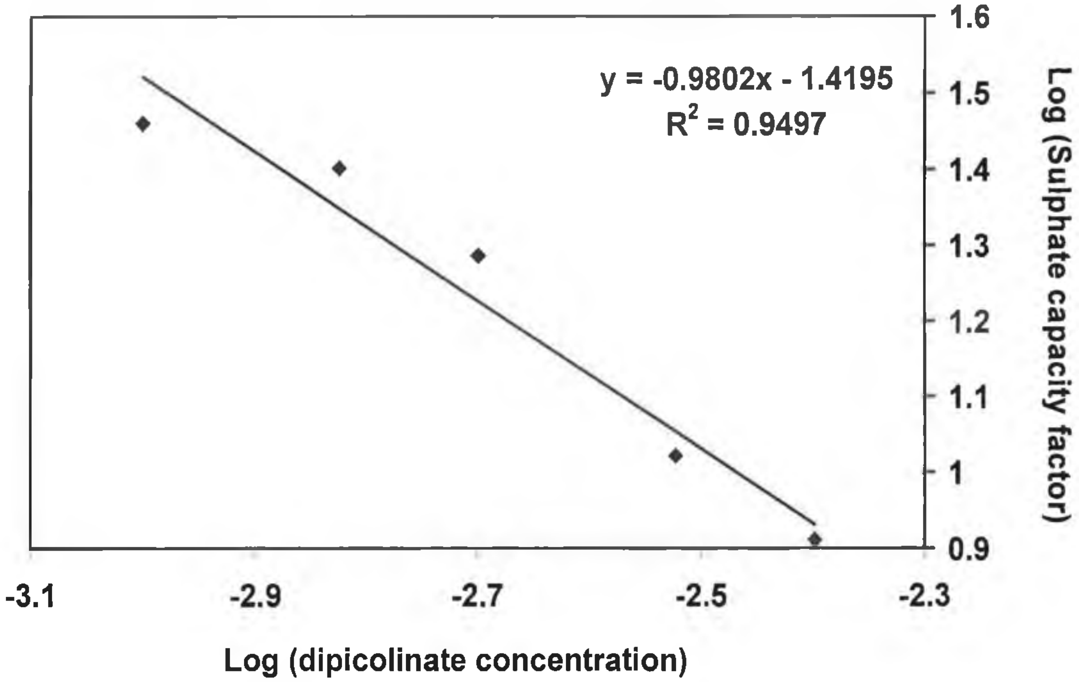

Figure 6-18. *Plot of log [sulphate capacity factor] versus log [dipicolinate concentration] on a short 3 μm DDAB coated column.*

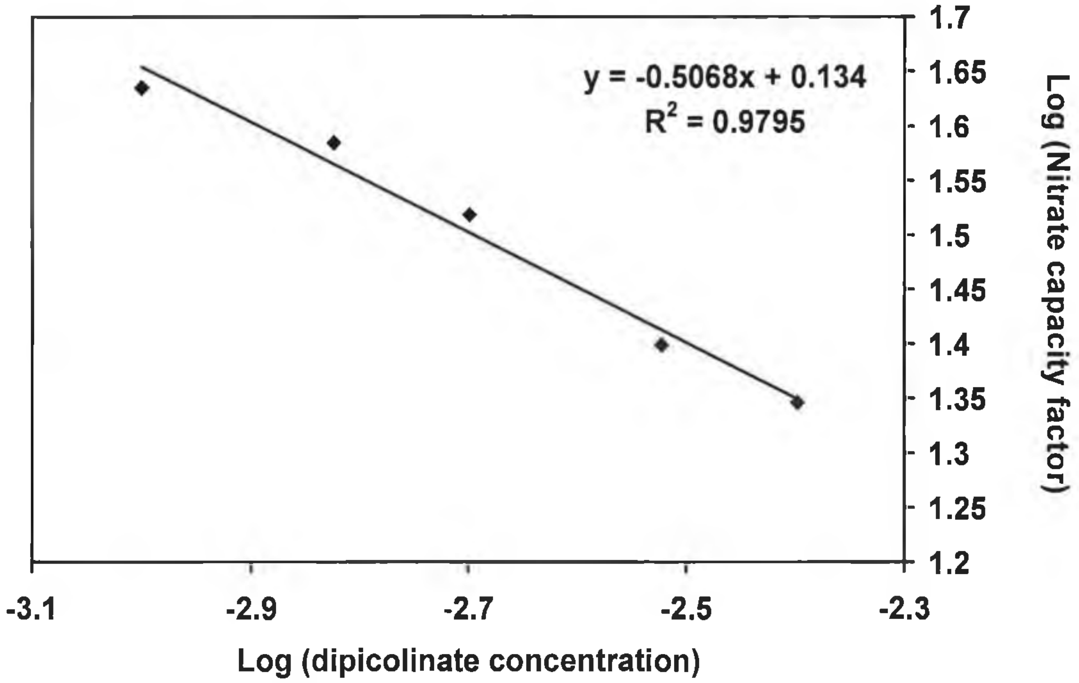

Figure 6-19. *Plot of log [nitrate capacity factor] versus log [dipicolinate concentration] on a short 3 μm DDAB coated column.*

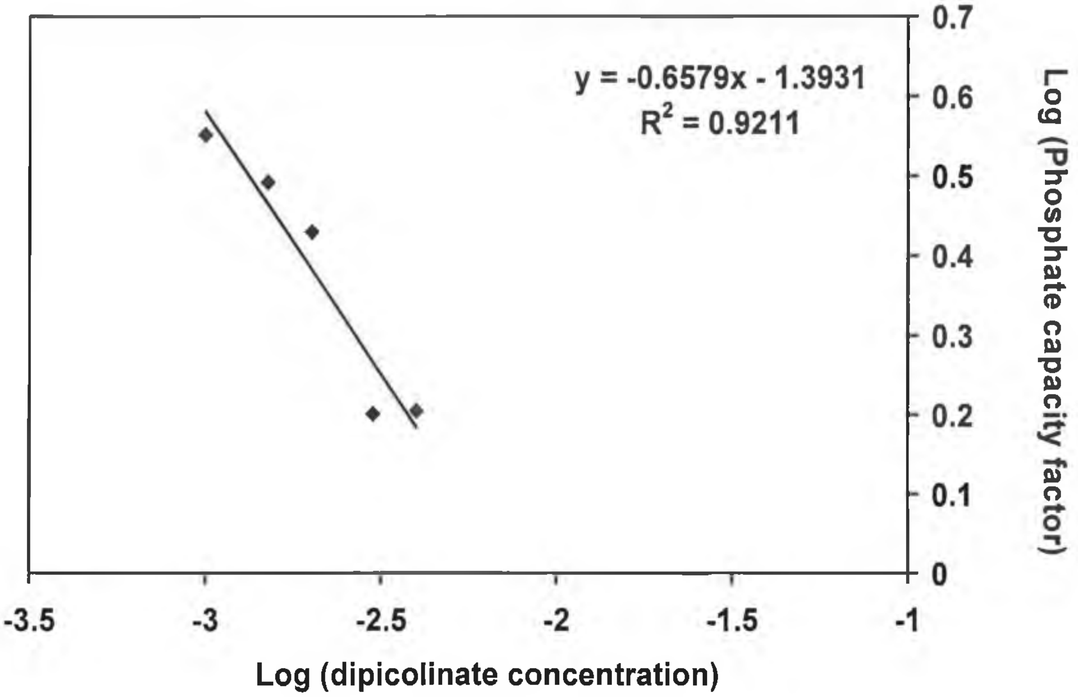

Figure 6-20. *Plot of log [phosphate capacity factor] versus log [dipicolinate concentration] on a short 3 µm DDAB coated column.*

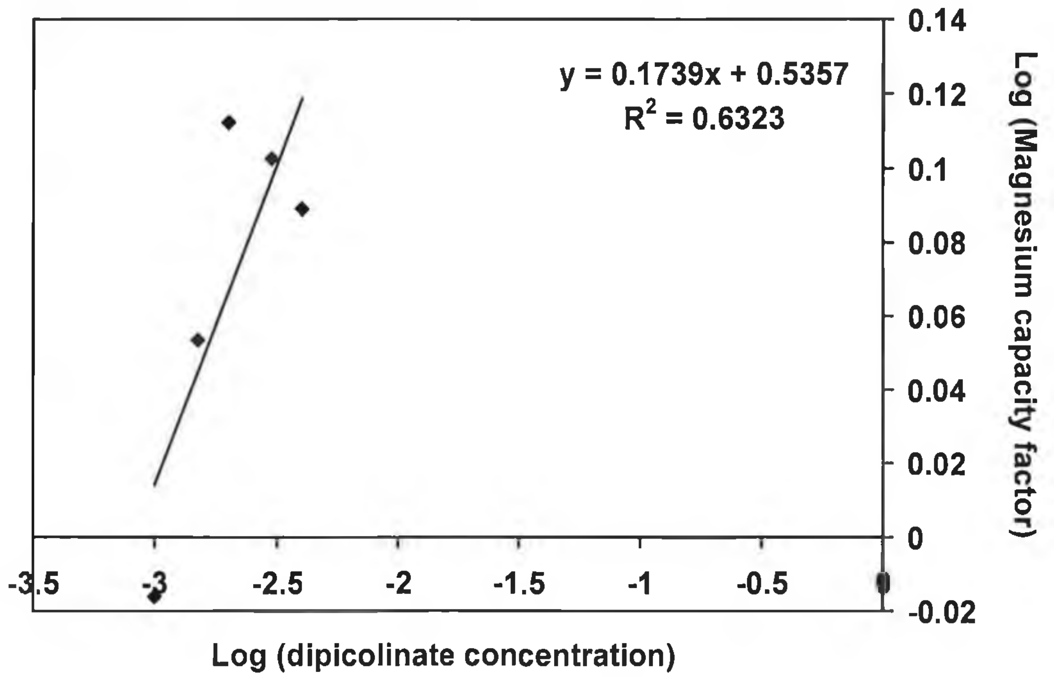

Figure 6-21. *Plot of log [magnesium capacity factor] versus log [dipicolinate concentration] on a short 3 µm DDAB coated column.*

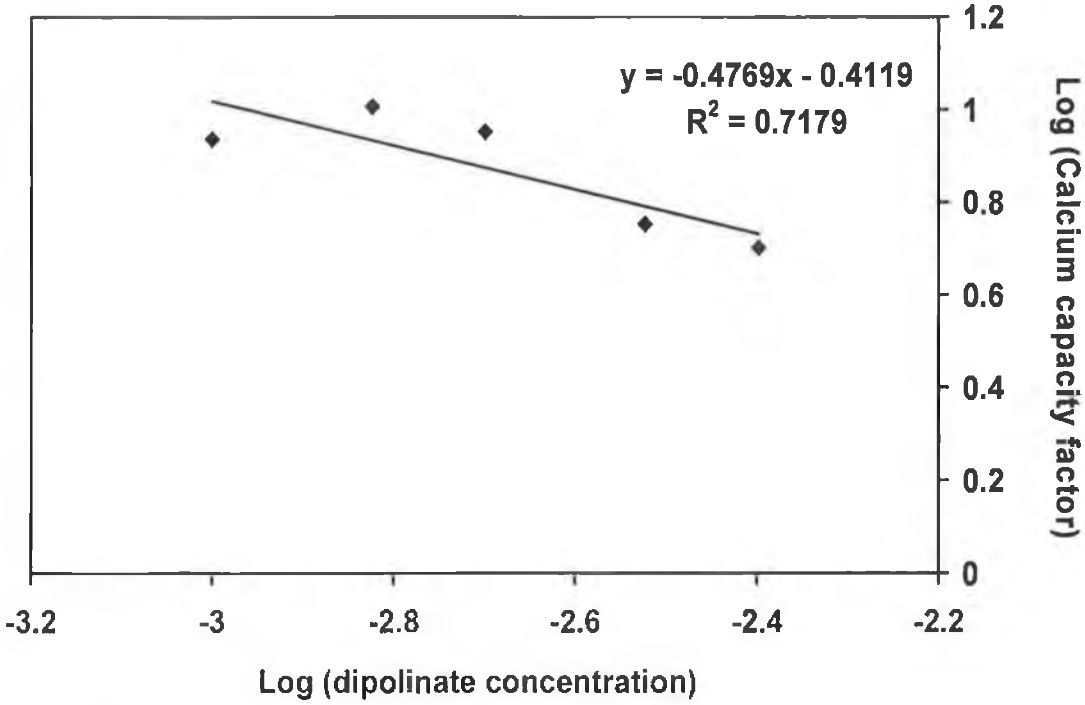

Figure 6-22. *Plot of log [calcium capacity factor] versus log [dipicolinate concentration] on a short 3 μm DDAB coated column.*

6.3.8 Effect of eluent pH on retention.

The pH of the optimum eluent was varied across the range 5.0 to 7.0. Retention time decreased with an increase in eluent pH as expected, since dipicolinic acid is dissociated to a greater extent. An eluent pH of 6.5 was chosen as optimum.

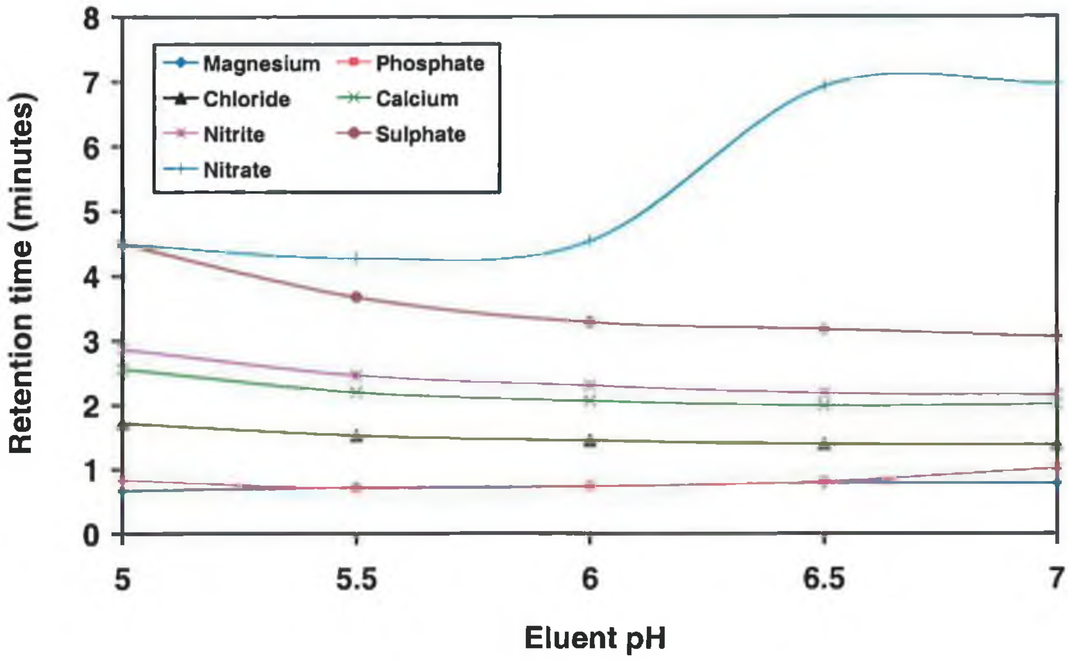

Figure 6-23. *Effect of dipicolinate pH upon analyte retention on a short 3 μm DDAB coated column. Chromatographic conditions as in Figure 6-12. Eluent concentration was maintained at 4 mM. Analyte concentrations: 25 mg/L magnesium, 50 mg/L calcium, 10 mg/L phosphate, 10 mg/L chloride, 100 mg/L nitrate, 50 mg/L nitrite and 220 mg/L sulphate.*

At this pH, phosphate co-elutes with magnesium, but given that phosphate is present in freshwater samples at very low concentrations (often less than 0.1 mg/*L*), this method was likely not to be sensitive enough for phosphate determinations in any case. Also at this pH, nitrite partially co-elutes with calcium as seen in Figure 6-24 and so was subsequently removed from the test group of analytes, since calcium is likely to be present at a significantly higher concentration in samples.

At this point, carbonate was also added to the test group of analytes at a concentration likely to be present in environmental samples. Figure 6-24 shows that high levels of carbonate present in samples yields two peaks. H_2CO_3 is a weak acid ($pKa_1 = 6.35$ and $pKa_2 = 10.33$) [6]. Figure 6-25 represents the separation at the optimised flow rate of 2.5 ml/min, increasing column backpressure from 494 psi at 1.0 ml/min to 1220 psi at 2.5 ml/min.

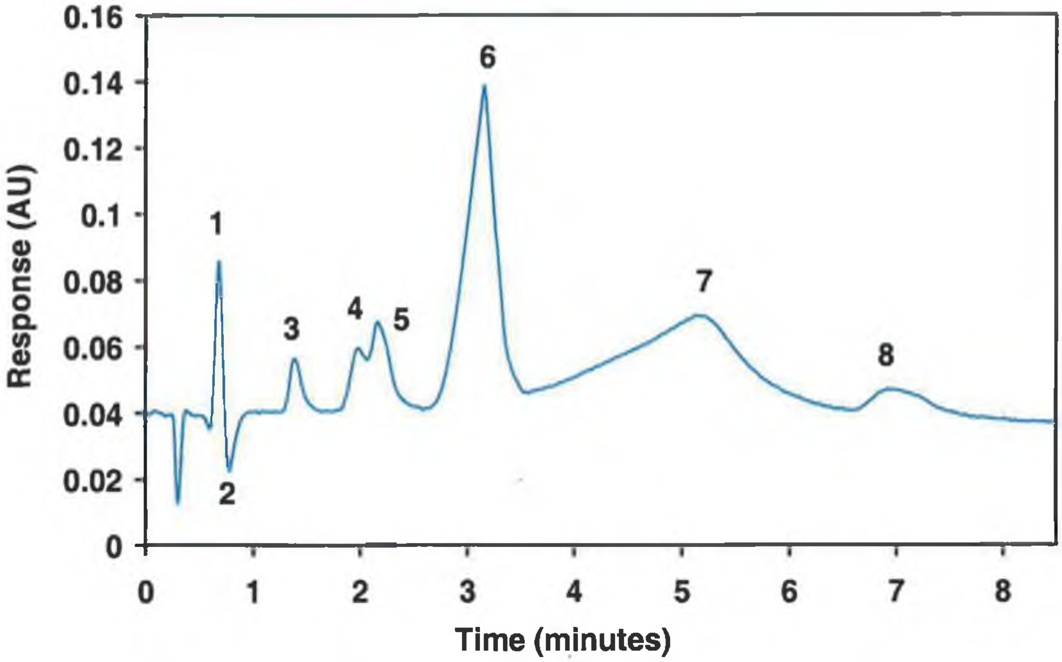

Figure 6-24. *Optimised dipicolinate eluent for the determination of anions and cations on a short 3 μm DDAB coated column. Chromatographic conditions: Column: Phenomenex Kingsorb 3 μm, 30 mm x 4.6 mm I.D. column, Eluent: 4.0 mM dipicolinic acid pH 6.5, Flow rate: 1.0 ml/min., Loop size: 25 μL, Detection wavelength: 285 nm. Column temperature: 45 °C. Peaks: [1] 100 mg/L carbonate, [2] 25 mg/L magnesium / 10 mg/L phosphate, [3] 10 mg/L chloride, [4] 50 mg/L calcium, [5] 50 mg/L nitrite, [6] 220 mg/L sulphate, [7] 100 mg/L carbonate, [8] 100 mg/L nitrate.*

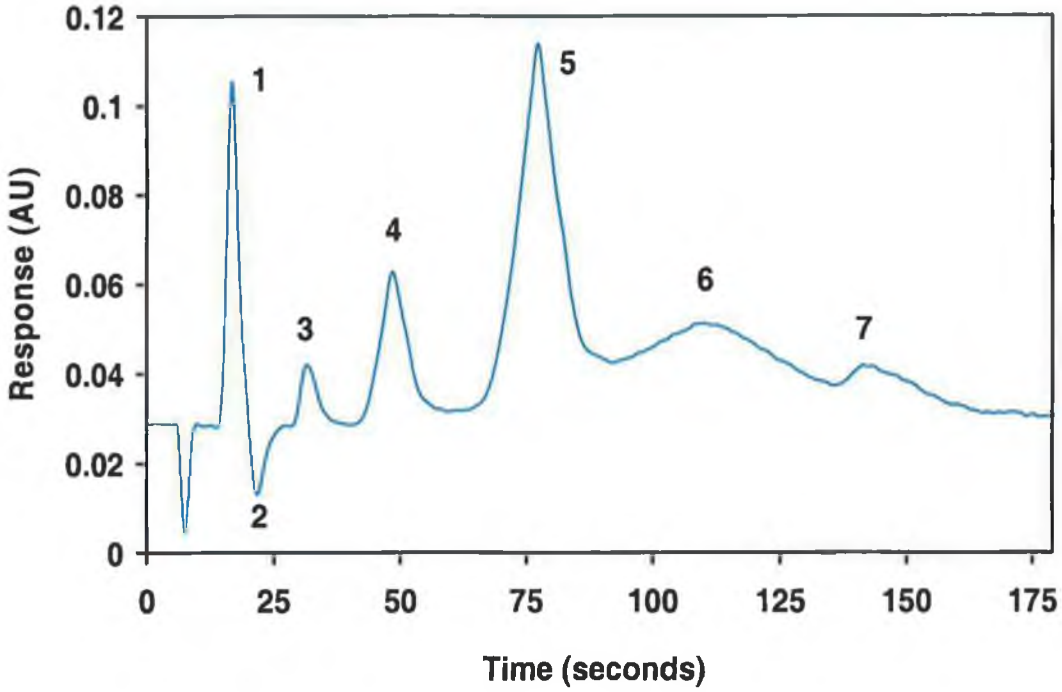

Figure 6-25. *Optimised rapid simultaneous determination of anions and cations on a short 3 μm DDAB coated column with a dipicolinate eluent*[4]. *Chromatographic conditions as in Figure 6-24 except Flow rate: 2.5 ml/min. Peaks: [1] 100 mg/L carbonate (17 seconds), [2] 25 mg/L magnesium (22.2 seconds), [3] 10 mg/L chloride (32.3 seconds), [4] 50 mg/L calcium (49 seconds), [5] 220 mg/L sulphate (77.7 seconds), [6] 100 mg/L carbonate (111.1 seconds), [7] 100 mg/L nitrate (142 seconds).*

6.3.9 Wavelength optimisation.

Using a 10 mg/*L* chloride standard, peak height was determined over a range of detector wavelengths from 240 nm to 310 nm. A detector wavelength of 286 nm was chosen as optimum, with this wavelength being used in all subsequent analyses.

---

[4] Nitrite and phosphate have been removed from this test group.

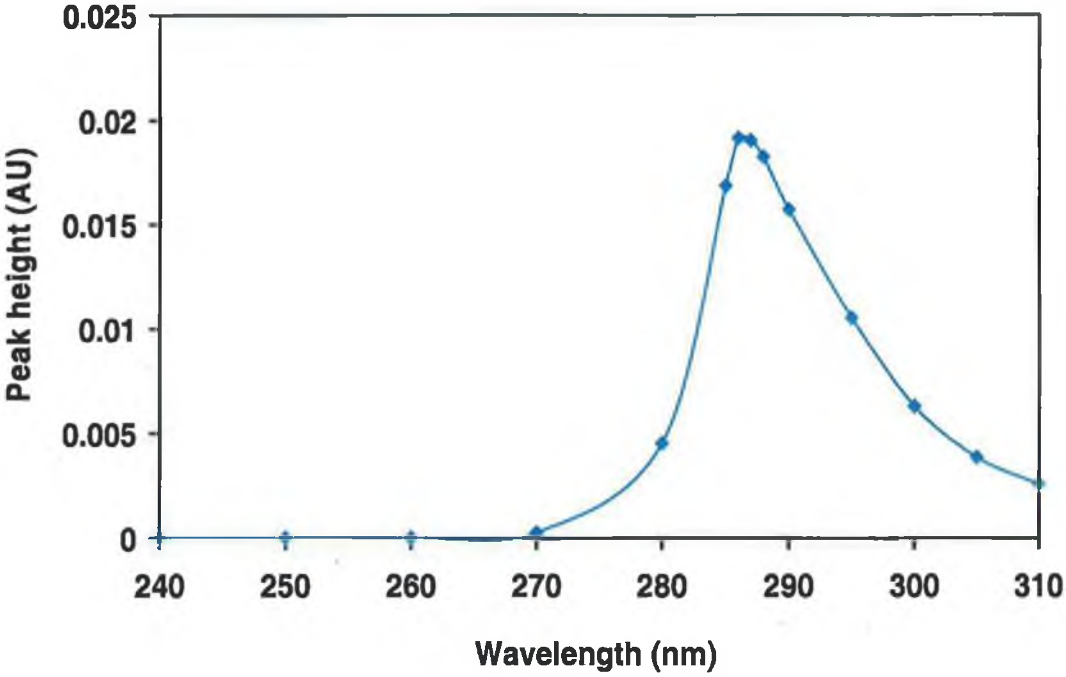

Figure 6-26. *Optimisation of detector wavelength for a chloride standard using a dipicolinate eluent. Chromatographic conditions as in Figure 6-25. Analyte: 10 mg/L chloride.*

6.3.10 Injection volume optimisation.

Using the optimum chromatographic conditions previously established, the test group of analytes was injected onto the column using an injection volume of 25 μL and 50 μL. Since there was no observed detrimental effect upon resolution or peak efficiency at the higher injection volume as shown in Figure 6-27, the 50 μL loop size was chosen as optimum.

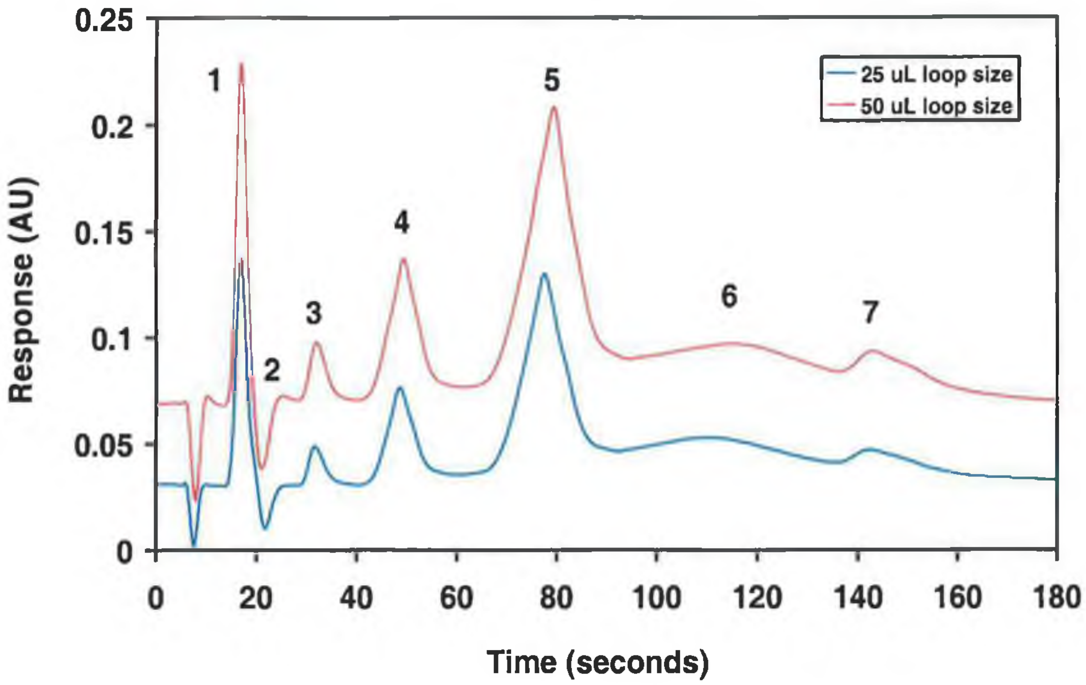

Figure 6-27. *Effect of increased injection volume upon the separation of anions and cations on a short 3 μm DDAB coated column with a dipicolinate eluent. Chromatographic conditions as in Figure 6-25. Peaks: [1] 100 mg/L carbonate (17 seconds), [2] 25 mg/L magnesium (22.2 seconds), [3] 10 mg/L chloride (32.3 seconds), [4] 50 mg/L calcium (49 seconds), [5] 220 mg/L sulphate (77.7 seconds), [6] 100 mg/L carbonate (111.1 seconds), [7] 100 mg/L nitrate (142 seconds).*

6.3.11 Analysis of real samples.

Samples were collected from a wide range of sources, to demonstrate the applicability of this method for the determination of common inorganic anions and cations. Sample pre-treatment was minimal, with samples treated only to filtration using a 0.45 μm nylon membrane filter. The sea water sample was also diluted by an appropriate factor using deionised water. The developed method was used for qualitative screening of samples only. It was clear that the main disadvantage of this method was its poor sensitivity, particularly noticeable in relation to determination of nitrate in the samples (Peak 7). Therefore, further method validation was not carried out (sensitivity, linearity etc).

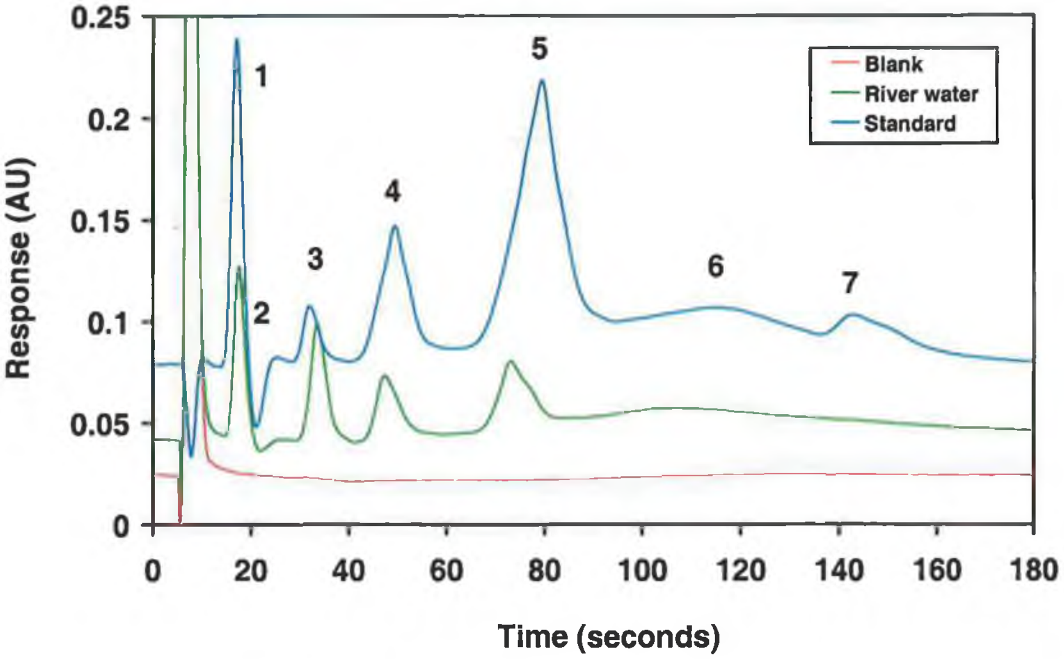

Figure 6-28. *Analysis of river water with a dipicolinate eluent on a short 3 µm DDAB coated column. Chromatographic conditions: Column: Phenomenex Kingsorb 3 µm, 30 mm x 4.6 mm I.D. column, Eluent: 4.0 mM dipicolinic acid pH 6.5, Flow rate: 2.5 ml/min., Loop size: 50 µL, Detection wavelength: 286 nm. Column temperature: 45 °C. Peaks: [1] 100 mg/L carbonate (17 seconds), [2] 25 mg/L magnesium (22.2 seconds), [3] 10 mg/L chloride (32.3 seconds), [4] 50 mg/L calcium (49 seconds), [5] 220 mg/L sulphate (77.7 seconds), [6] 100 mg/L carbonate (111.1 seconds), [7] 100 mg/L nitrate (142 seconds).*

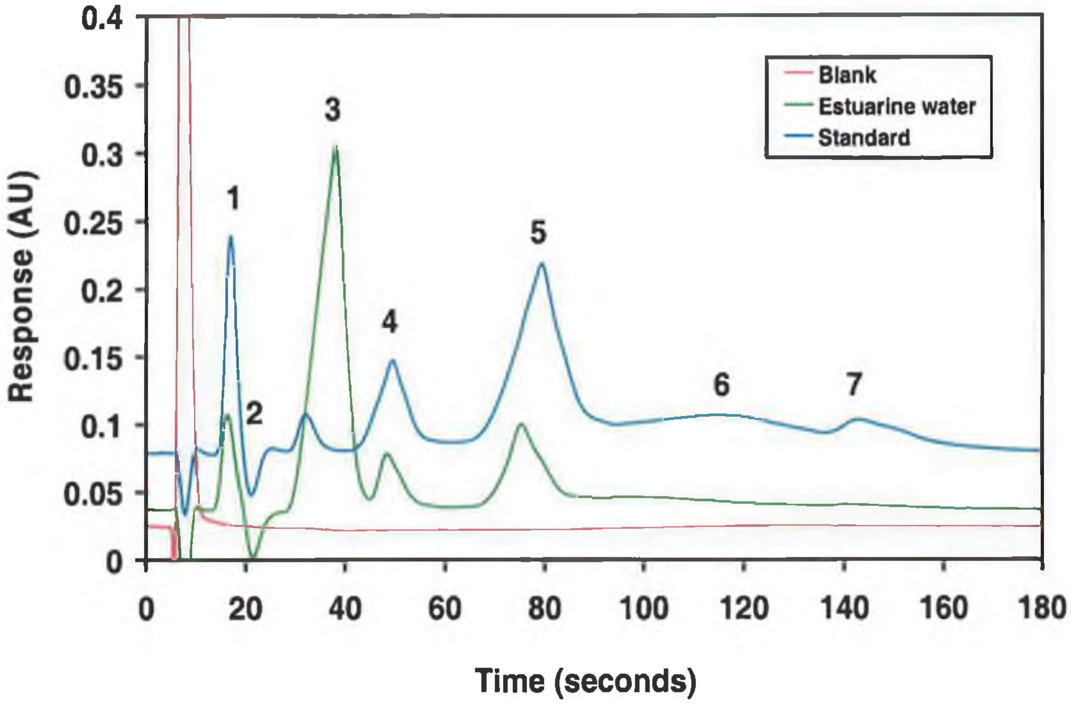

Figure 6-29. *Analysis of estuarine water with a dipicolinate eluent on a short 3 µm DDAB coated column. Chromatographic conditions: Column: Phenomenex Kingsorb 3 µm, 30 mm x 4.6 mm I.D. column, Eluent: 4.0 mM dipicolinic acid pH 6.5, Flow rate: 2.5 ml/min., Loop size: 50 µL, Detection wavelength: 286 nm. Column temperature: 45 °C. Peaks: [1] 100 mg/L carbonate (17 seconds), [2] 25 mg/L magnesium (22.2 seconds), [3] 10 mg/L chloride (32.3 seconds), [4] 50 mg/L calcium (49 seconds), [5] 220 mg/L sulphate (77.7 seconds), [6] 100 mg/L carbonate (111.1 seconds), [7] 100 mg/L nitrate (142 seconds).*

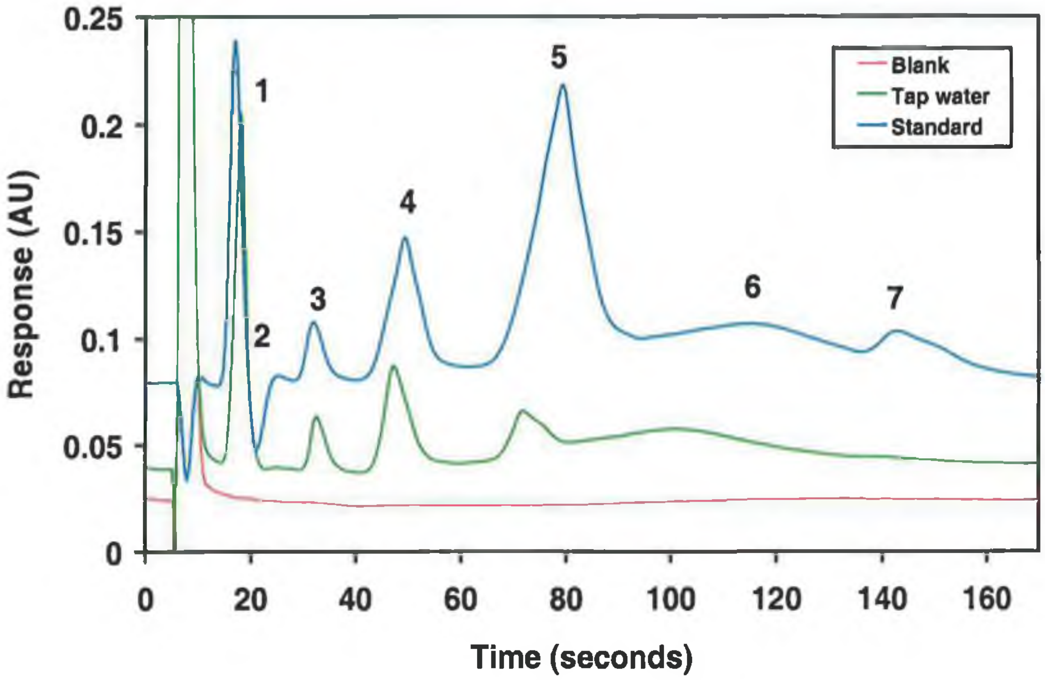

Figure 6-30. *Analysis of tap water with a dipicolinate eluent on a short 3 μm DDAB coated column. Chromatographic conditions: Column: Phenomenex Kingsorb 3 μm, 30 mm x 4.6 mm I.D. column, Eluent: 4.0 mM dipicolinic acid pH 6.5, Flow rate: 2.5 ml/min., Loop size: 50 μL, Detection wavelength: 286 nm. Column temperature: 45 °C. Peaks: [1] 100 mg/L carbonate (17 seconds), [2] 25 mg/L magnesium (22.2 seconds), [3] 10 mg/L chloride (32.3 seconds), [4] 50 mg/L calcium (49 seconds), [5] 220 mg/L sulphate (77.7 seconds), [6] 100 mg/L carbonate (111.1 seconds), [7] 100 mg/L nitrate (142 seconds).*

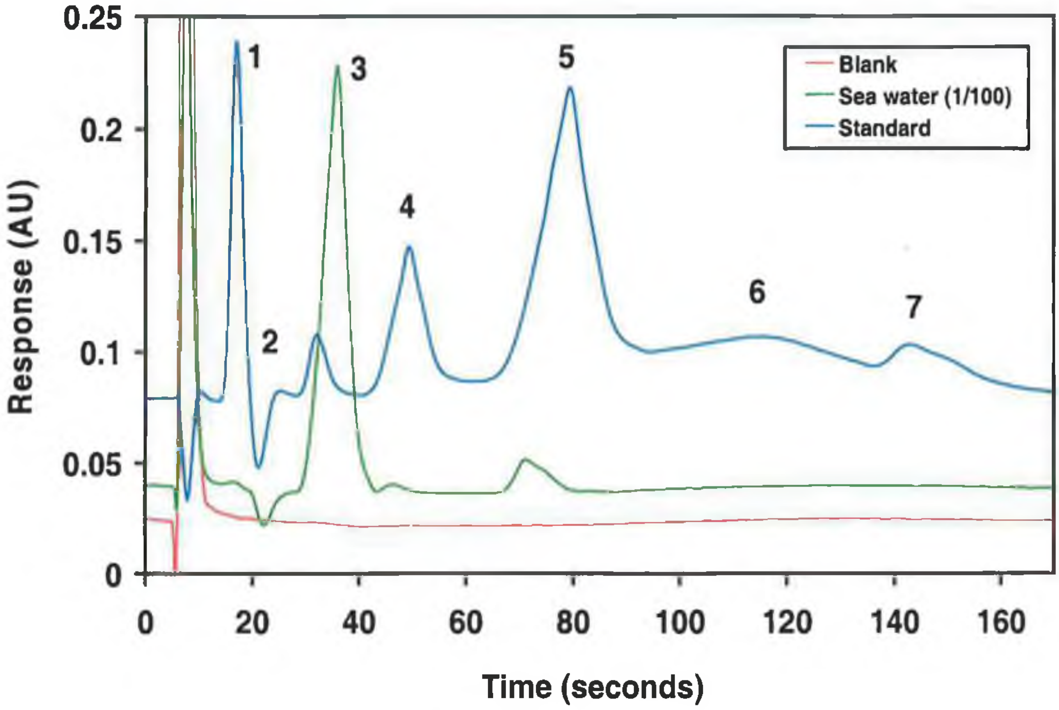

Figure 6-31. *Analysis of sea water (diluted 1/100) with a dipicolinate eluent on a short 3 μm DDAB coated column. Chromatographic conditions: Column: Phenomenex Kingsorb 3 μm, 30 mm x 4.6 mm I.D. column, Eluent: 4.0 mM dipicolinic acid pH 6.5, Flow rate: 2.5 ml/min., Loop size: 50 μL, Detection wavelength: 286 nm. Column temperature: 45 °C. Peaks: [1] 100 mg/L carbonate (17 seconds), [2] 25 mg/L magnesium (22.2 seconds), [3] 10 mg/L chloride (32.3 seconds), [4] 50 mg/L calcium (49 seconds), [5] 220 mg/L sulphate (77.7 seconds), [6] 100 mg/L carbonate (111.1 seconds), [7] 100 mg/L nitrate (142 seconds).*

6.3.12 Determination of column efficiency.

A standard of 10 mg/*L* chloride, 25 mg/*L* magnesium and calcium, and 160 mg/*L* sulphate was prepared and chromatographed using the optimum conditions. The separation efficiency is shown in Figure 6-32, expressed as plates/metre. It was noted that the separation efficiency was considerably lower than that which was achieved in previous phthalic acid work on the same column, which had been coated in the same manner. For example, the efficiency of a 25 mg/*L* chloride standard was 38,133 plates/metre, with the phthalic acid eluent, but only 16,267 plates/metre for a 10 mg/*L* standard with the dipicolinic acid eluent.

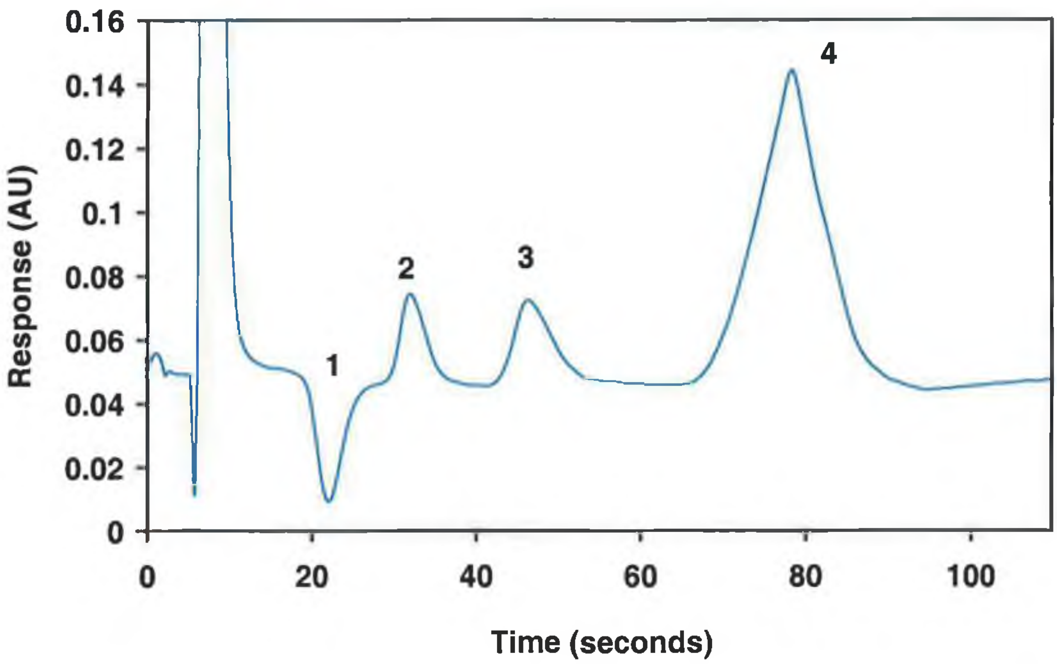

Figure 6-32. *Determination of separation efficiency for the short 3 µm Kingsorb column coated with DDAB, using a dipicolinate eluent. Chromatographic conditions: Column: Phenomenex Kingsorb 3 µm, 30 mm x 4.6 mm I.D. column, Eluent: 4.0 mM dipicolinic acid pH 6.5, Flow rate: 2.5 ml/min., Loop size: 50 µL, Detection wavelength: 286 nm. Column temperature: 45 °C. Peaks: [1] 25 mg/L magnesium (7,767 plates/metre), [2] 10 mg/L chloride (16,267 plates/metre), [3] 25 mg/L calcium (15,833 plates/metre), [4] 160 mg/L sulphate (14,533 plates/metre).*

Given that a comparison between separation efficiencies for two separate mobile phases was being made, a study was performed in which the mobile phase was held constant, at 5 m*M* phthalate pH 7.0. A test group of anions was injected onto the current column, and the separation efficiencies directly compared with an identical study which had previously been performed on the same column, and reported in Chapter 4. With all chromatographic conditions now being identical between the two studies, the only parameter which had been subjected to change, was that the column had been washed and re-coated (albeit using the same coating procedure), between the time that the first study was performed, and the time that this second comparative study was made.

Results are tabulated in Table 6-3 and illustrated in Figure 6-33. Since the "current coating" of the same column resulted in much reduced separation efficiencies, a second phthalate eluent was prepared on a second day, to account for any possible analyst error in mobile phase preparation. Results indicate that the poor efficiency of the dipicolinic acid work is likely to be due to either a stationary phase degradation of some kind, or a non –reproducible coating technique.

Table 6-3. *Reduction in column efficiency over time for a short 3 µm Kingsorb column coated with DDAB*[5].

Peak	Initial coating (N/m)	Current coating (day 1) (N/m)	Current coating(day 2) (N/m)	Current coating (Average) (N/m)
Chloride (25 mg/L)	**38,133**	18,700	18,933	**18,817**
Sulphate (25 mg/L)	**50,056**	20,633	22,000	**21,317**
Nitrate (50 mg/L)	**100,600**	18,333	18,800	**18,567**

---

[5] Chromatographic conditions: Column: Phenomenex Kingsorb, 3 µm 30 mm x 4.6 mm I.D. Mobile phase: 5 m*M* phthalate pH 7.0. Flow rate: 2 mL/min. Column temperature: 45 °C. Loop volume: 50 µL. Detection: 279 nm.

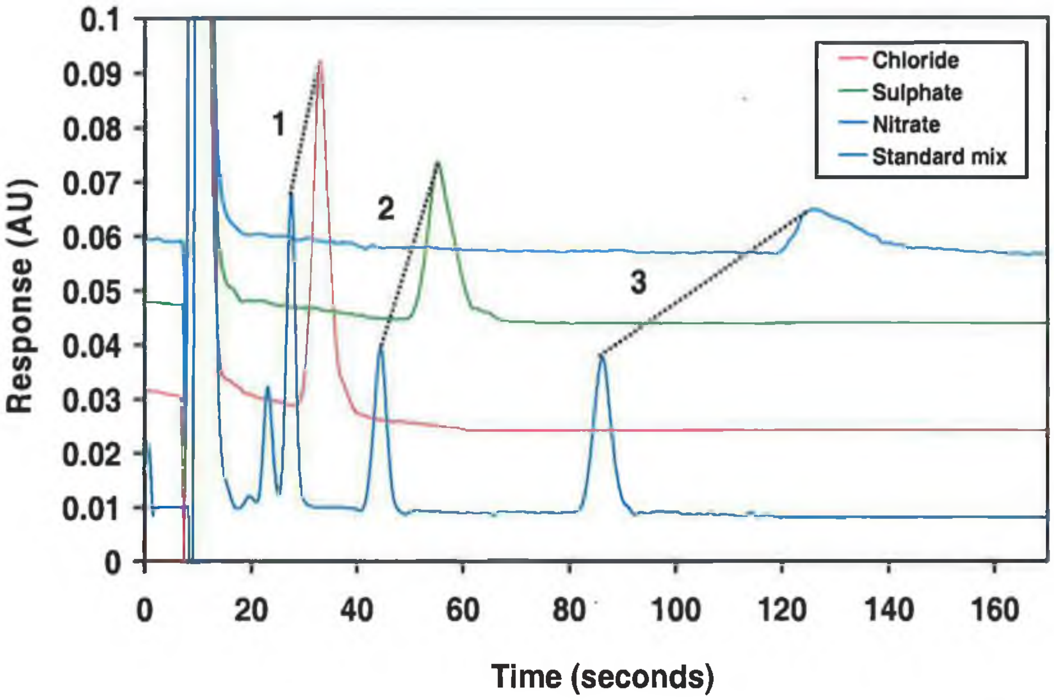

Figure 6-33. *Comparison of separation efficiency between the initial column coating and the current column coating. Chromatographic conditions: Column: Phenomenex Kingsorb, 3 µm 30 mm x 4.6 mm I.D. Mobile phase: 5 mM phthalate pH 7.0. Flow rate: 2 mL/min. Column temperature: 45 °C. Loop volume: 50 µL. Detection: 279 nm. Peaks: [1] 25 mg/L chloride (38,133 plates/metre to 18,817 plates/metre), [2] 25 mg/L sulphate (50,056 plates/metre to 21,317 plates/metre), [3] 50 mg/L nitrate (100,600 plates/metre to 18,567 plates/metre).*

6.4 Conclusion.

The use of complexing eluents was investigated with a view to the simultaneous determination of simple inorganic anions along with both calcium and magnesium, in a single isocratic run. An ion-exchange chromatographic separation was developed on a 30 mm X 4.6 mm C_{18} 3 μm analytical column permanently coated with didodecyldimethylammonium bromide (DDAB), using a pyromellitic acid eluent. The eluting strength of a tetravalent pyromellitic acid eluent was compared with a divalent phthalic acid eluent which had been used in previous work; demonstrating the advantage of multivalent eluents for rapid ion chromatography. Further studies involved the use of dipicolinic acid eluents, which allowed the simultaneous separation of chloride, sulphate, nitrate, carbonate, magnesium and calcium in less than 180 seconds in a single isocratic run on a C_{18} column coated with DDAB. A range of samples including river water and sea water were qualitatively screened for the test group of analytes, with minimal sample preparation necessary.

6.5 References.

[1]. J. Zou, S. Motomizu, H. Fukutomi, Analyst 116 (1991) 1399.

[2]. F.G.P. Mullins, Analyst 112 (1987) 665.

[3]. X. Jun, J.L.F.C. Lima, M.C.B.S.M. Montenegro, Anal. Chim. Acta. 321 (1996) 263.

[4]. P. Hatsis, C.A. Lucy, Analyst 127 (2002) 451.

[5]. K. Ohta, K. Tanaka, Anal. Chim. Acta. 373 (1998) 189.

[6]. K. Ohta, K. Tanaka, J.S. Fritz, J. Chromatogr. A 731 (1996) 179.

[7]. K. Ohta, K. Tanaka, J. Chromatogr. A 804 (1998) 87.

[8]. K. Ohta, J. Chromatogr. A 920 (2001) 181.

[9]. M.Y. Ding, K. Tanaka, W. Hu, K. Hasebe, P.R. Haddad, Analyst 126 (2001) 567.

[10]. M.J. Shaw, S.J. Hill, P. Jones, Anal. Chim. Acta. 401 (1999) 65.

[11]. P. Janvion, S. Motellier, H. Pitsch, J. Chromatogr. A 715 (1995) 105.

[12]. S. Motellier, H. Pitsch, J. Chromatogr. A 739 (1996) 119.

[13]. N. Cardellicchio, P. Ragone, S. Cavalli, J. Riviello, J. Chromatogr. A 770 (1997) 185.

[14]. K. Ohta, K. Tanaka, B. Paull, P.R. Haddad, J. Chromatogr. A 770 (1997) 219.

[15]. L.G. Sillen, A.E. Martell, Stability Constants of Metal-Ion Complexes, The Chemical Society, London, 1964.

[16]. P. Hatsis, C.A. Lucy, J. Chromatogr. A 920 (2001) 3.

7 Fast ion-interaction chromatography of common inorganic anions on short ODS monolithic columns.

7.1 Introduction.

In the past two years a new approach to the rapid separation of inorganic ions has emerged, involving the use of modified monolithic silica stationary phases. These monolithic phases have been discussed in detail in Chapter 1, Section 1-4. Ion chromatography on monolithic phases was first investigated by Hatsis and Lucy [1], who used an ion-interaction method for the separation of common anions on a 5.0 cm reversed-phase monolithic silica column. With a 1.5 m*M* tetrabutylammonium / 1.1 m*M* phthalate eluent, pH 5.5 with 5 % v/v acetonitrile, Hatsis and Lucy managed to separate eight anions[1] in just 15 seconds (albeit with an eluent flow rate of 16 ml/min). Either direct conductivity or indirect UV absorbance (255 nm) could be used for detection with this eluent. Hatsis and Lucy immediately proceeded to improve on the above work and subsequently developed a rapid suppressed ion chromatography method based on the same column, this time permanently coated with didodecyldimethylammonium bromide (DDAB) [2]. The eluent used in this case was 6 m*M* o-cyanophenol, pH 7.0 at a flow rate of 5.5 ml/min. This work resulted in ultra-fast separations (under 30 seconds for 7 anions) with detection limits in the low μg/ml range using suppressed conductivity detection.

Following on from the above work with anions, a number of workers have recently utilised monolithic phases for the rapid separation of cations. For example, Xu *et al.* [3] dynamically modified a 5.0 cm reversed phase monolithic column with lithium dodecylsulphate (Li-DS) to facilitate the separation of hydronium, lithium, sodium, calcium and magnesium. The eluent used contained 5 m*M* EDTA, dipotassium salt dihydrate (EDTA-2K) and 0.1 m*M* Li-DS (pH 4.8) and the method used direct conductivity detection. However, with the above conditions the peak shape for calcium was rather poor and magnesium was retained for over 20 minutes. To reduce the excessive retention of these divalent cations, Xu *et al.* went on to

---

[1] Phosphate, chloride, nitrite, bromide, nitrate, chlorate, iodide and sulphate.

further develop the method, this time using a 2 m*M* ethylenediamine / 0.1 m*M* Li-DS eluent (pH 6.0), which saw the elution of hydronium, calcium and magnesium in under 4 minutes [4].

Studies into bonded ion exchangers using bare silica monoliths as the supporting substrate have been less obvious in the literature. Sugrue *et al.* recently reported the application of an iminodiacetic acid functionalised silica monolith for the separation of alkaline earth metals in high ionic strength samples [5]. Utilising flow rates of up to 5.0 ml/min, with an eluent of 1 *M* KCl, trace levels of magnesium and calcium were determined in NaCl and KCl brine samples, with runtimes of under 40 seconds.

The work described in this chapter involves the use of a 5 cm monolithic C_{18} column for the determination of inorganic anions by ion interaction chromatography using a TBA-phthalate/acetonitrile eluent. The developed rapid separation was subsequently used for the determination of nitrate in a large number of freshwater samples.

7.2 Experimental.

7.2.1 Equipment.

The ion chromatograph described in Chapter 2 was also used in this work. The analytical columns used were two Merck Chromolith Flash RP-18e 2.5 cm X 4.6 mm columns (Merck KgaA, Damstadt, Germany) connected in series. The column used for comparative Van Deemter curve studies was a Phenomenex Kingsorb, 3 µm particle size, 30 mm x 4.6 mm I.D. column (Phenomenex, Macclesfield, Cheshire, U.K). The analytical column used for comparative analysis studies for nitrate analysis was a Dionex AS11 HC 250 mm X 2.0 mm column, (Dionex Corporation, Sunnyvale, CA, USA) with an AG11 HC 50 mm X 2.0 mm guard column. The injection loop used was 25 µL for the ion-interaction chromatography studies, and 25 µL for comparative analysis studies using the Dionex AS11 HC column. Detection was by direct conductivity for determination of anions using ion-interaction chromatography and by UV at 214 nm for comparative analysis studies using the Dionex AS11 HC column.

7.2.2 Reagents and chromatographic conditions.

The reagents used for mobile phase preparation were as described in Chapter 2 and Chapter 5. The optimised eluent for ion-interaction chromatography studies on the monolithic columns was (97:3) 1.54 m*M* tetrabutylammonium hydroxide, 0.8 m*M* phthalic acid, pH 7.25 / Acetonitrile. The mobile phase was adjusted to pH 7.25 with 0.5 m*M* NaOH and 5 m*M* phthalic acid. The eluent for comparative analysis studies using the Dionex AS11 HC column, was 30 m*M* NaOH. The flow rate used was 6.0 mL/min for ion-interaction chromatography on the monolithic columns, and 0.38 ml/min for comparative analysis studies using the Dionex AS11 HC column. Column temperature was set at 30 °C for all separations.

Standard solution were prepared as described in Chapter 6. Samples were diluted with Milli-Q water where necessary, and syringe filtered prior to injection using 0.45 μm swinny filters from Gelman Laboratories. All samples when collected, were filtered at source, and analysed within 24 hours.

7.3 Results and discussion.

7.3.1 Comparison of column backpressures between a particulate column and a monolithic column.

Column backpressures were determined and compared for a conventional 3 cm 3 μm particulate column, and a monolithic column of approximately comparable length (5 cm). The test mobile phase used was the same for both columns, as was the column temperature, such that differences mobile phase viscosity was not an issue. It is clear from Figure 7-2 that column backpressure on the conventional particulate column is five times higher than that of the monolithic column. This is despite the fact that the monolithic column used in this experiment is a factor of ~1.7 times longer than the particulate column. Also notable, is that with the monolithic column, significantly higher flow rates can be used compared to the particulate column, without reaching the backpressure limit as specified by the manufacturer. It is acknowledged that higher flow rates could have been achieved on a 5 μm or 10 μm particulate column because of the lower column backpressures due to the larger particle sizes. However, a 3 μm column was chosen, because the column efficiency more closely matches that of the monolithic stationary phase, as will be shown later.

Figure 7-1. *Short 2.5 cm monolithic ODS column. Merck Chromolith Flash RP-18e 25 mm X 4.6 mm column.*

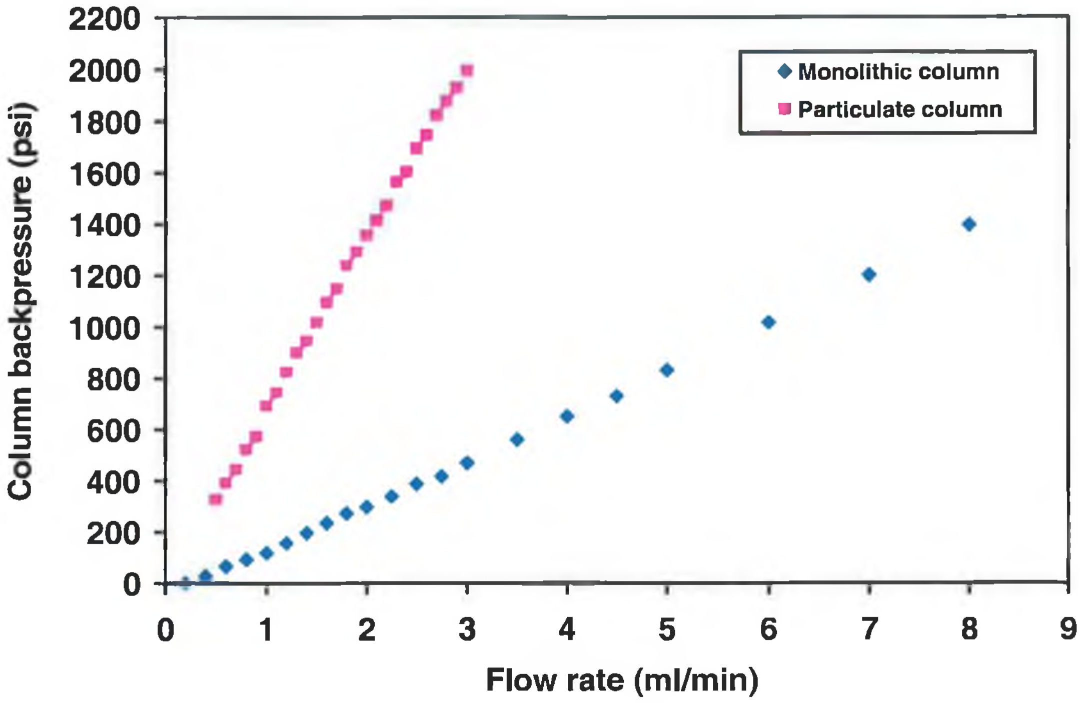

Figure 7-2. *Comparison of column backpressure between a particulate stationary phase (3 μm) and a monolithic stationary phase. Chromatographic conditions: Particulate column: Phenomenex Kingsorb C_{18} 3 μm 30 mm X 4.6 mm. Monolithic column: Two Merck Chromolith Flash RP-18e 25 mm X 4.6 mm columns connected in series. Mobile phase: (97:3) 1.54 mM TBA, 0.8 mM phthalic acid, pH 7.0 / Acetonitrile. Column temperature: 30 °C.*

7.3.2 Determination of suitable concentration ranges of mobile phase components during mobile phase optimisation.

The group of anions chosen for study was chloride, phosphate, sulphate, nitrate, nitrite and carbonate, since these small inorganic anions would typically be expected to be present in drinking water and fresh-water samples. The work done by Hatsis and Lucy [1] was used as a starting point for this work. A tetrabutylammonium-phthalic acid eluent with acetonitrile as organic modifier was chosen, since phthalate is very suitable for direct conductivity detection, being a weak acid. The approach adopted for mobile phase optimisation involved first holding the concentration of acetonitrile constant, and varying the concentrations of the tetrabutylammonium cation (TBA^+) and phthalic acid. During mobile phase optimisation a flow rate of 2 ml/min was used, with an injection volume of 25 μL, detection by direct conductivity, and column temperature at 30 °C.

The difficulty with the approach of modifying TBA and phthalate concentrations is that the eluent pH also becomes a factor affecting the separation, since TBA-OH was effectively titrated with phthalic acid during mobile phase preparation. Therefore, a three dimensional response surface was plotted showing the combined effects of TBA and phthalic acid concentration on pH during a TBA/phthalic acid titration. The surface was constructed on Sigma Plot Version 4.0, which allows the user to rotate the surface in 3 dimensions and view from different angles in order to examine the surface with respect to each axis. As illustrated in Figure 7-3, mobile phases were subsequently investigated within the range 0.8 m*M* to 1.3 m*M* phthalic acid, and 0.7 m*M* to 1.5 m*M* TBA. These concentration ranges resulted in mobile phase pH values which are well within the working pH range as suggested by the column manufacturer. Also, by preparing mobile phases in this manner, no other acid or base needs to be added to the mobile phase for pH adjustment, with the phthalate component of the eluent providing an adequate buffering capacity.

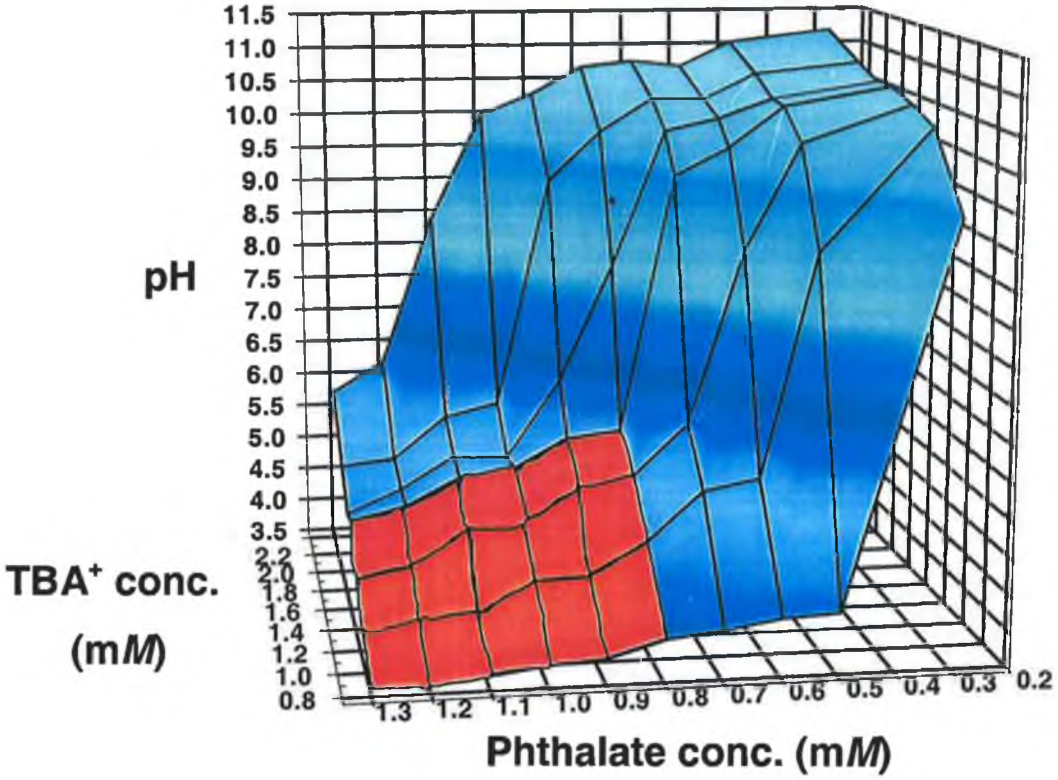

Figure 7-3. *Plot of TBA concentration (mM) versus phthalic acid concentration (mM) versus pH showing the optimum mobile phase pH range (shaded in red).*

7.3.3 Effect of TBA concentration upon retention of target anions.

To examine the effect of TBA concentration on retention, four mobile phases were prepared over the range 0.77 m*M* to 1.54 m*M*. Other mobile phase parameters were held constant at 0.8 m*M* phthalic acid, 3 % acetonitrile and pH 7.0. From Figure 7-4 it can be seen that the retention times of two system peaks also had to be considered, referred to hereafter as “system peak 1” and “system peak 2”. The retention times of all peaks were not strongly affected by TBA concentration, but there was a marked decrease in retention for system peak two. An optimum TBA concentration of 1.54 m*M* was chosen based on a shorter overall runtime with respect to system peak 2. At this concentration, the resolution between nitrate, phosphate and system peak 1 was also optimum.

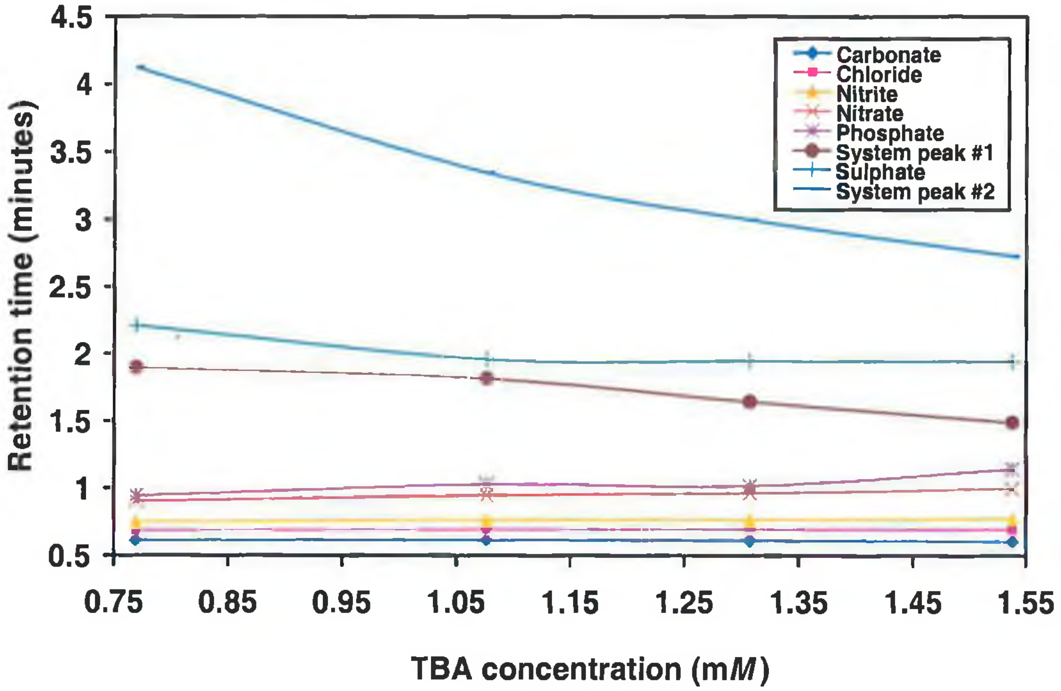

Figure 7-4. *Effect of TBA concentration on the retention of anions on a 5 cm monolithic column*[2]*. Chromatographic conditions: Column: Two Merck Chromolith Flash RP-18e 2.5 cm X 4.6 mm (connected in series). Mobile phase: Phthalic acid concentration held constant at 0.8 mM, and acetonitrile concentration at 3 %, with pH held at 7.0. Flow rate: 2.0 ml/min. Column temperature: 30 °C. Detection: direct conductivity. Loop volume: 25 μL. Analyte concentrations: 10 mg/L.*

7.3.4 Effect of phthalic acid concentration.

To examine the effect of phthalic acid concentration on retention, four mobile phases were prepared over the concentration range 0.8 m*M* to 1.5 m*M*. Other mobile phase parameters were held constant at 1.54 m*M* TBA^+, 3 % acetonitrile and pH 7.0. A phthalic acid concentration of 0.8 m*M* was considered optimum, based on the coelution of sulphate with system peak 1, and nitrate with phosphate at higher concentrations.

---

[2] Retention time of unretained solutes (t_o) was 0.35 minutes.

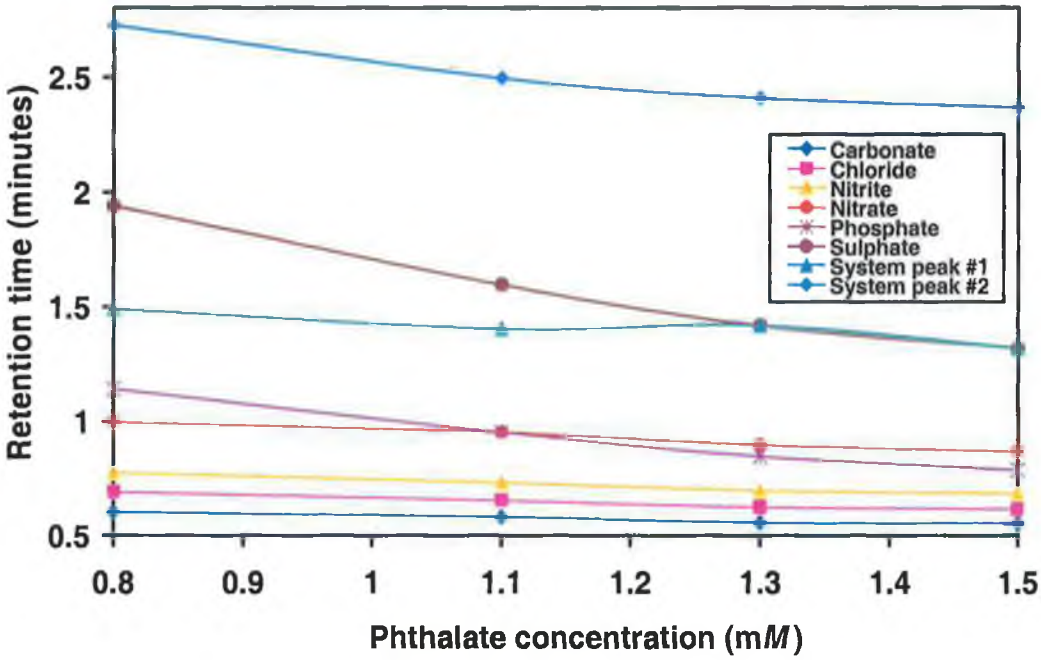

Figure 7-5. *Effect of phthalic acid concentration on the retention of anions on a 5 cm monolithic column* [3]*. Chromatographic conditions: Column: Two Merck Chromolith Flash RP-18e 2.5 cm X 4.6 mm (connected in series). Mobile phase: TBA$^+$ concentration held constant at 1.54 mM, and acetonitrile concentration at 3 %, with pH held at 7.0. Flow rate: 2.0 ml/min. Column temperature: 30 °C. Detection: direct conductivity. Loop volume: 25 µL. Analyte concentrations: 10 mg/L.*

7.3.5 Effect of pH on retention.

With TBA$^+$ and phthalic acid concentrations held constant at 1.54 m*M* and 0.8 m*M* respectively and acetonitrile at 3 %, the eluent pH was adjusted across the range 6.6 to 7.25. Lower pH mobile phases were not prepared, because, as far as was practical, the mobile phase pH was reached by direct titration of the mobile phase constituents themselves. Lower pH's would involve the addition of a stronger acid than phthalic acid, resulting in extra system peaks. Further addition of phthalic acid itself, as a means of reducing the pH, would make the exact concentration of phthalate difficult to control. Higher pH's were achieved with NaOH. Figure 7-6 shows that there was no significant effect on retention across the pH range, except

---

[3] Retention time of unretained solutes (t_o) was 0.35 minutes.

for phosphate, which increased steadily. An optimum pH of 7.25 was chosen to maximise the resolution of the nitrate/phosphate/system peak #1 triplet.

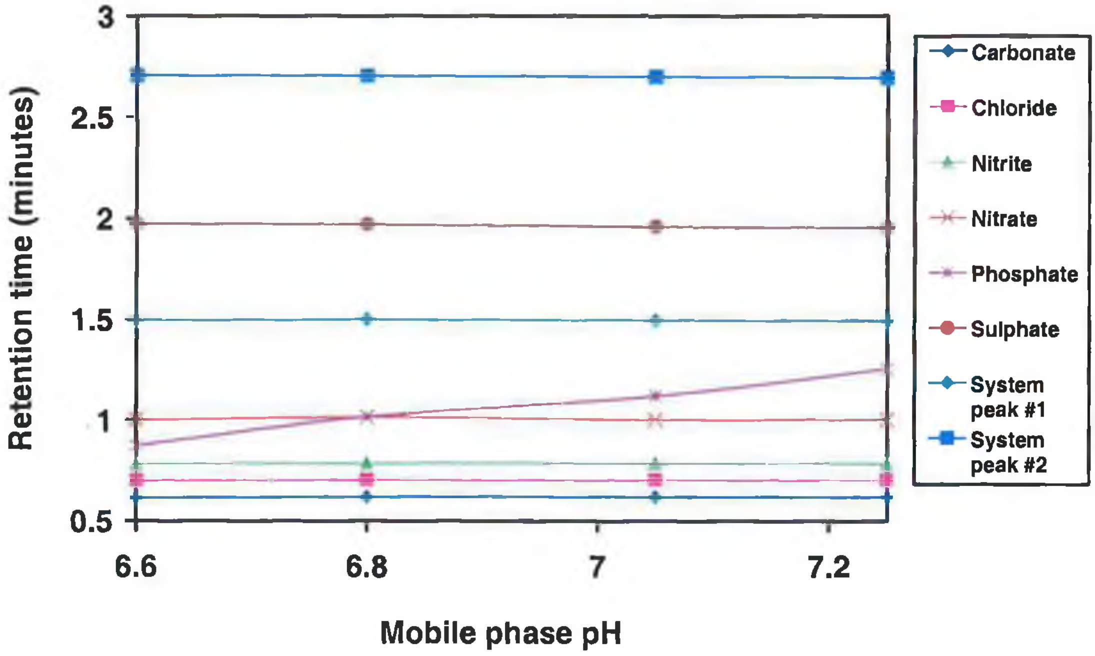

Figure 7-6. *Effect of eluent pH on the retention of anions on a 5 cm monolithic column*[4]*. Chromatographic conditions: Column: Two Merck Chromolith Flash RP-18e 2.5 cm X 4.6 mm (connected in series). Mobile phase: TBA$^+$ and phthalic acid concentrations held constant at 1.54 mM and 0.8 mM respectively with acetonitrile concentration at 3 %, Flow rate: 2.0 ml/min. Column temperature: 30 °C. Detection: direct conductivity. Loop volume: 25 µL. Analyte concentrations: 10 mg/L.*

7.3.6 Effect of acetonitrile on retention.

The concentration of acetonitrile was varied over the range 1 % to 5 %. TBA and phthalic acid concentration was held constant at 1.54 m*M* and 0.8 m*M* respectively, with mobile phase pH at 7.25. 3 % acetonitrile was chosen as optimum, such that the final optimized mobile phase was (97:3) 1.54 m*M* tetrabutylammonium hydroxide, 0.8 m*M* phthalic acid, pH 7.25 / acetonitrile.

---

[4] Retention time of unretained solutes (t_o) was 0.35 minutes.

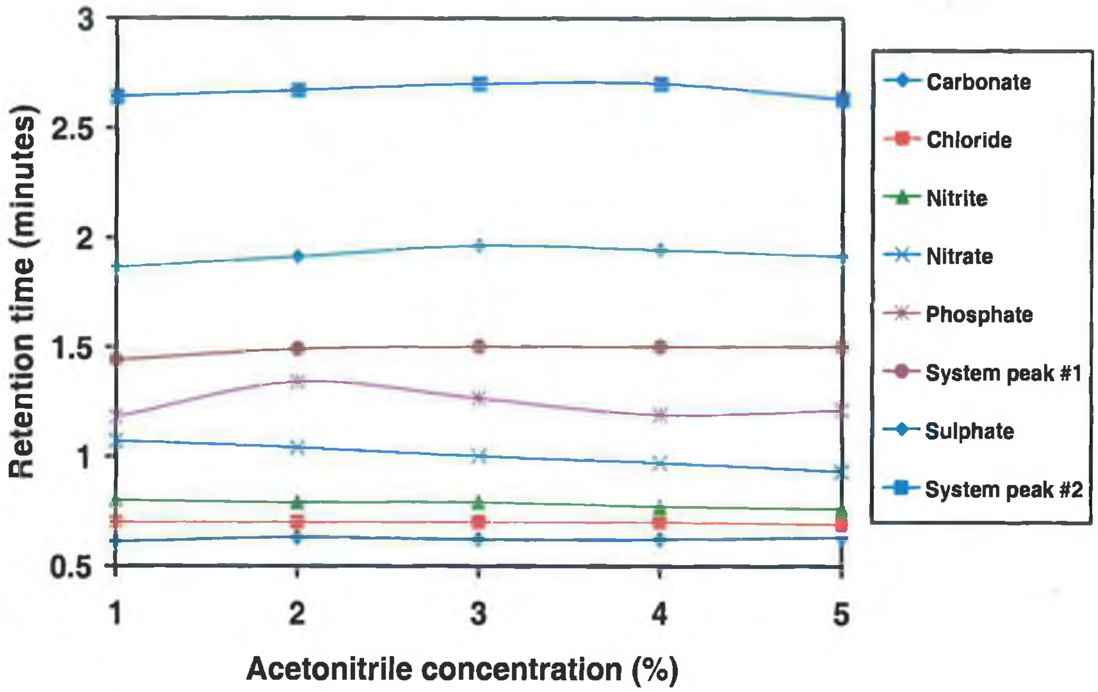

Figure 7-7. *Effect of acetonitrile concentration on the retention of anions on a 5 cm monolithic column*[5]*. Chromatographic conditions: Column: Two Merck Chromolith Flash RP-18e 2.5 cm X 4.6 mm (connected in series). Mobile phase: TBA*$^+$ *and phthalic acid concentrations held constant at 1.54 mM and 0.8 mM respectively with a pH of 7.25. Flow rate: 2.0 ml/min. Column temperature: 30 °C. Detection: direct conductivity. Loop volume: 25 µL. Analyte concentrations: 10 mg/L.*

7.3.7 Effect of column temperature.

The column temperature was varied from 27 °C to 45 °C. Since there was essentially no effect upon retention, the column compartment temperature was controlled at 30 °C, to protect the column from temperature fluctuations for the remainder of this study.

---

[5] Retention time of unretained solutes (t_o) was 0.35 minutes.

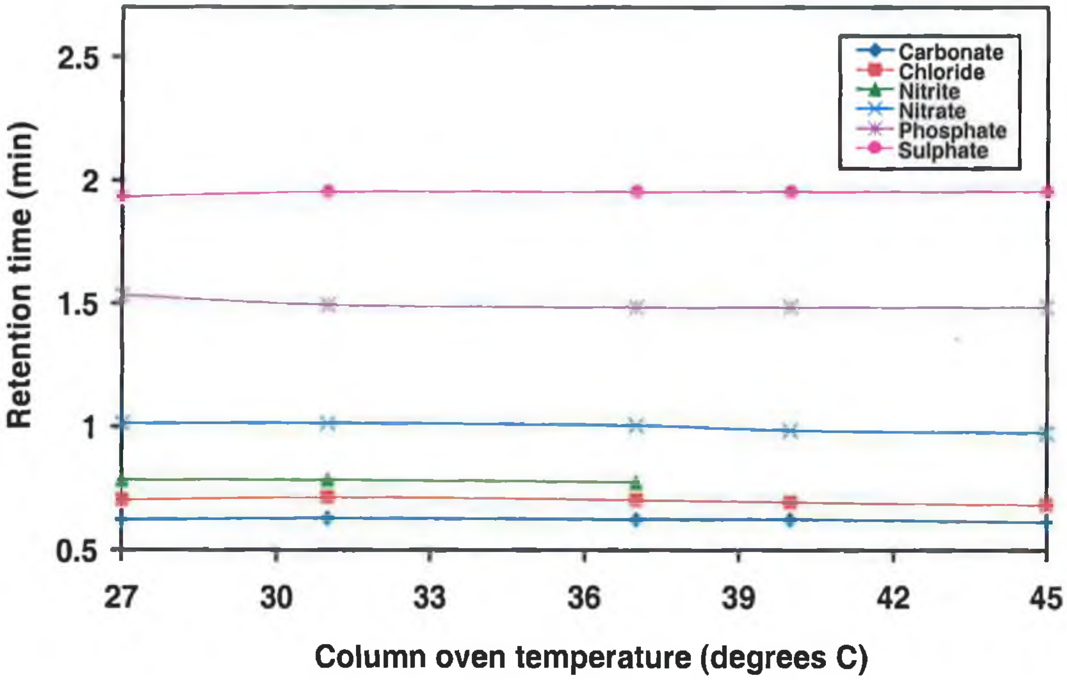

Figure 7-8. *Effect of column temperature on the retention of anions on a 5 cm monolithic column*[6]*. Chromatographic conditions: Column: Two Merck Chromolith Flash RP-18e 2.5 cm X 4.6 mm (connected in series). Mobile phase: (97:3) 1.54 mM tetrabutylammonium hydroxide, 0.8 mM phthalic acid, pH 7.25 / Acetonitrile. Flow rate: 2.0 ml/min. Detection: direct conductivity. Loop volume: 25 μL. Analyte concentrations: 10 mg/L.*

7.3.8 Comparison of conventional 3 µm particulate stationary phases and monolithic stationary phases using Van Deemter plots.

A comparison of Van Deemter plots[7] for the 5 cm monolithic column, and a 3 cm, 3 μm particulate column illustrates a clear reduction in the HETP for a nitrate standard, for the monolithic stationary phase. Due to high column backpressures for the particulate column, generating data at flow rates higher than 3 ml/min was not possible, as shown in Figure 7-9, and so Excel Solver was used to model the curve at higher flow rates. A Van Deemter curve was determined for flow rates from 0.2 ml/min up to 8 ml/min. for the monolithic column. The optimum theoretical flow rate μ_{optimum} was higher for the monolithic column, at 1.95 ml/min compared to the 3 μm particulate column which showed a μ_{optimum} of 1.31 ml/min. The monolithic column

---

[6] Retention time of unretained solutes (t_o) was 0.35 minutes.

[7] Van Deemter plots and plate theory are discussed in Chapter 1, Section 1-1.

also demonstrated a significantly flatter C-term of 0.75 relative to 1.34, resulting in much higher flow rates being possible on the monolithic column, without adversely affecting column efficiency to a significant extent.

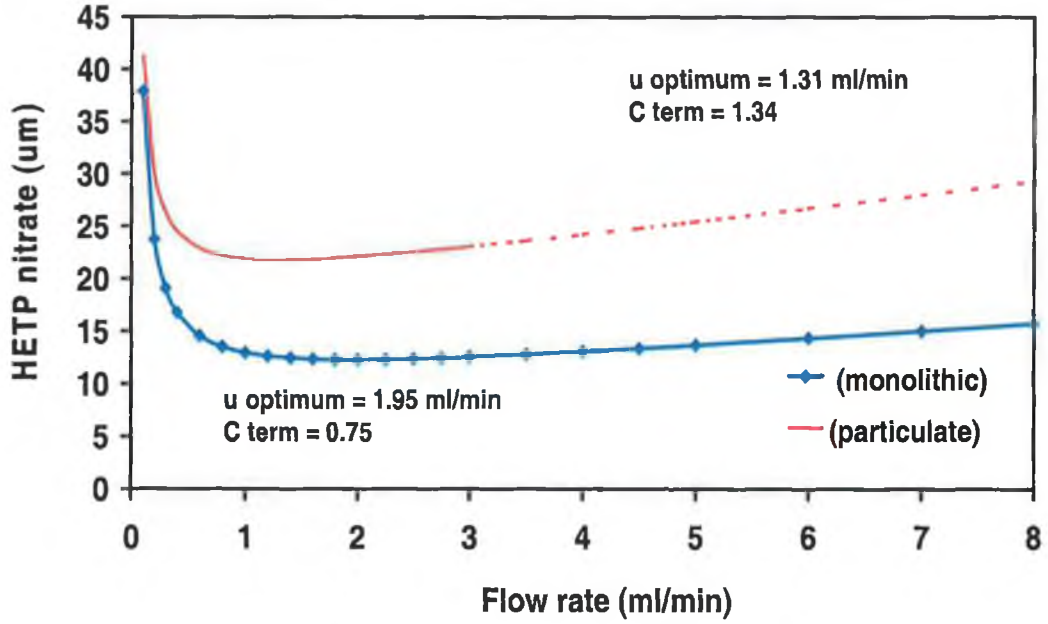

Figure 7-9. *Comparison of Van Deemter curves for a 3 µm particulate stationary phase versus a monolithic stationary phase for 10 mg/L nitrate standard. Chromatographic conditions: Columns: Two Merck Chromolith Flash RP-18e 2.5 cm X 4.6 mm (connected in series) and a Phenomenex Kingsorb, 3 µm particle size, 30 mm x 4.6 mm I.D. column, Mobile phase: (97:3) 1.54 mM tetrabutylammonium hydroxide, 0.8 mM phthalic acid, pH 7.25 / Acetonitrile. Column temperature: 30 °C. Detection: direct conductivity. Loop volume: 25 µL. A 10 mg/L NO_3 standard was used with flow rates of 0.2 ml/min to 8.0 ml/min.*

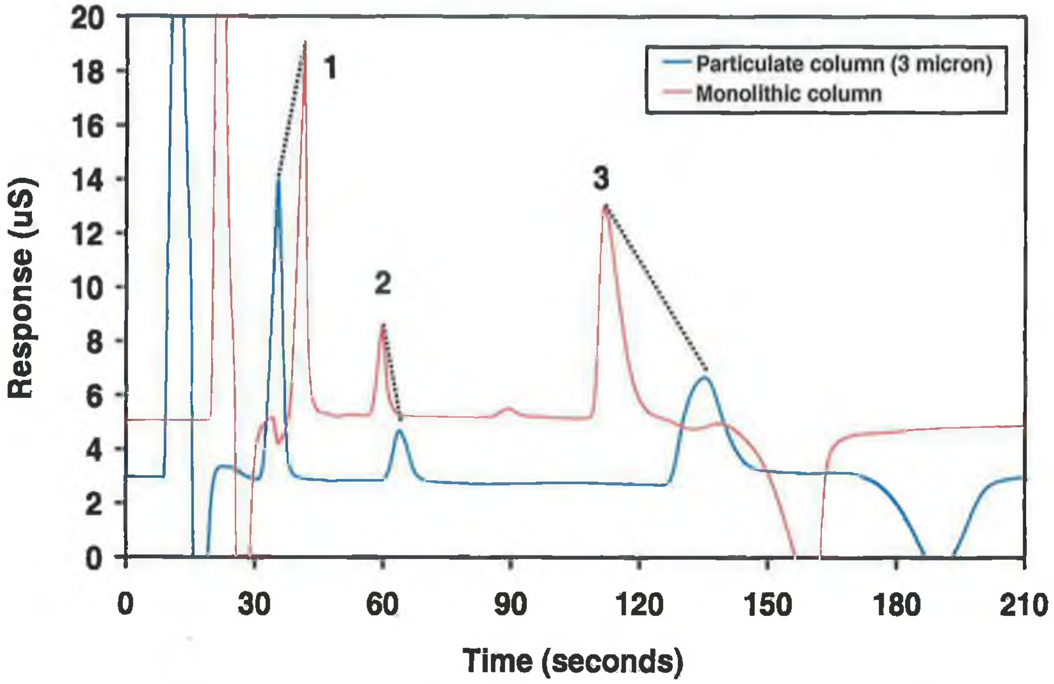

Figure 7-10. *Comparison of column efficiency on a 3 µm particulate column at 2.0 ml/min versus a monolithic column at 2.0 ml/min for selected anions. Chromatographic conditions as in Figure 7-9, except flow rate: 2.0 ml/min. Peaks: [1] 10 mg/L chloride, [2] 10 mg/L nitrate, [3] 10 mg/L sulphate.*

7.3.9 Selectivity studies.

Using a flow rate of 2.0 ml/min, a number of small inorganic and organic anions were injected onto the column to determine retention times. Other conditions included an injection volume of 25 µL, a column temperature of 30 °C, and direct conductivity detection.

Table 7-1. *Selectivity of the 5 cm monolithic column for anions using a TBA-phthalate/ACN eluent.*

Anion (elution order)	Retention time (min)
Unretained solutes, t_o	*0.35*
Citrate	*No response*
Perchlorate	*No response*
Iodate	*0.60*
Carbonate	***0.60***
Chloride	***0.67***
Fluoride	*0.68*
Acetate	*0.68*
Chlorite	*0.76*
Nitrite	***0.77***
Bromate	*0.78*
Bromide	*0.86*
Nitrate	***0.98***
Phosphate	***1.18***
Chlorate	*1.33*
System peak #1	***1.47***
Sulphate	***1.90***
Thiocyanate	*1.92*
Iodide	*1.95*
Thiosulphate	*2.43*
System peak #2	***2.63***
Benzoate	*6.61*

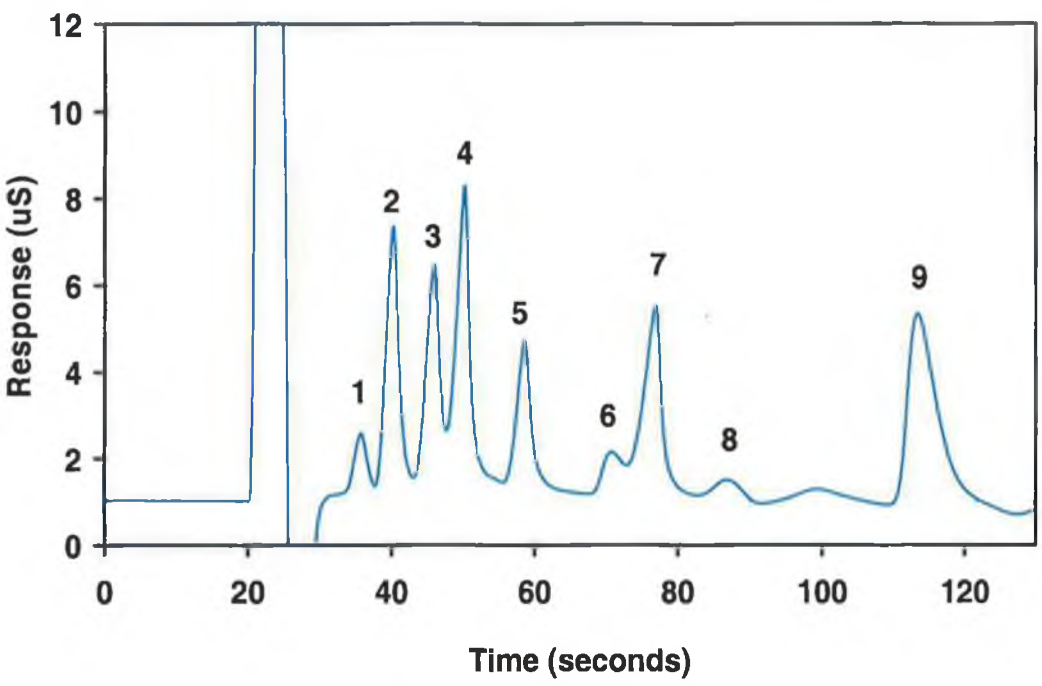

Figure 7-11. *Separation of selected anions including bromide and chlorate using the optimised eluent on a 5 cm monolithic column. Chromatographic conditions: Column: Two Merck Chromolith Flash RP-18e 2.5 cm X 4.6 mm (connected in series). Mobile phase: (97:3) 1.54 mM tetrabutylammonium hydroxide, 0.8 mM phthalic acid, pH 7.25 / Acetonitrile. Flow rate: 2.0 ml/min. Column temperature: 30 °C. Detection: direct conductivity. Loop volume: 25 μL. Analyte concentrations: 10 mg/L, Peaks: [1] carbonate, [2] chloride, [3] nitrite, [4] bromide, [5] nitrate, [6] phosphate, [7] chlorate, [8] system peak #1, [9] sulphate.*

7.3.10 Optimisation of flow rate.

Using the optimized mobile phase, a separation of a test mix of anions was ran at a number of flow rates from 2.0 ml/min to 8.0 ml/min. 6.0 ml/min was chosen as the optimum flow rate based on increased baseline noise at higher flow rates, resolution of earlier peaks, and higher system backpressures which could cause premature wear on the system (pump seals etc). Column efficiency was quite high, with peak efficiencies ranging from 34,860 plates/metre to 65,620 plates/metre for nitrate.

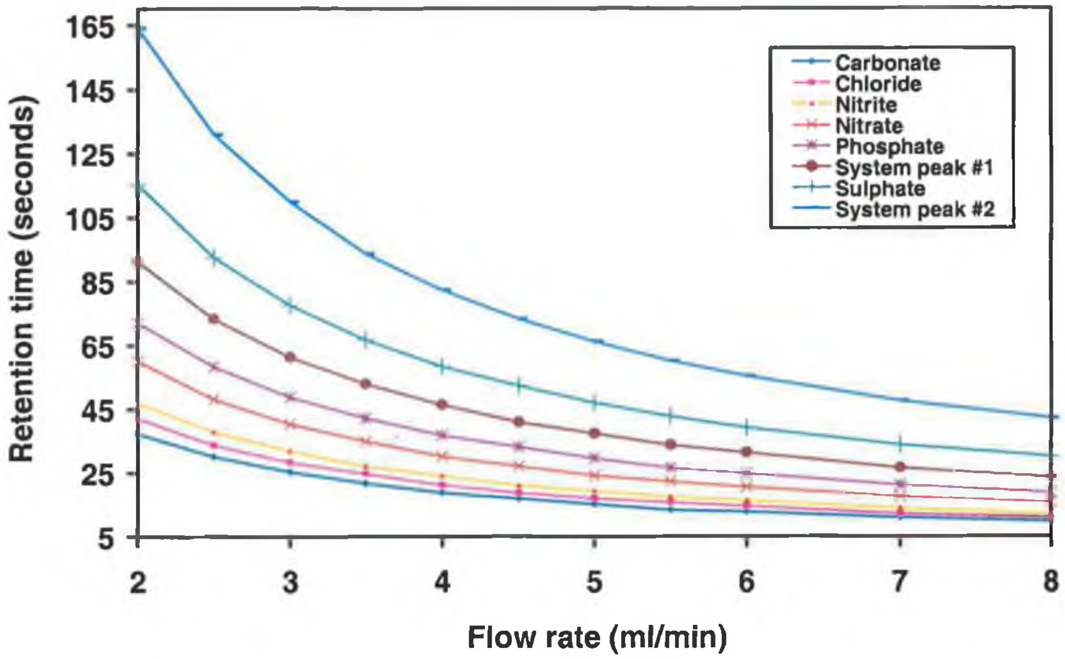

Figure 7-12. *Effect of flow rate upon the retention of anions on a 5 cm monolithic column. Chromatographic conditions as in Figure 7-11.*

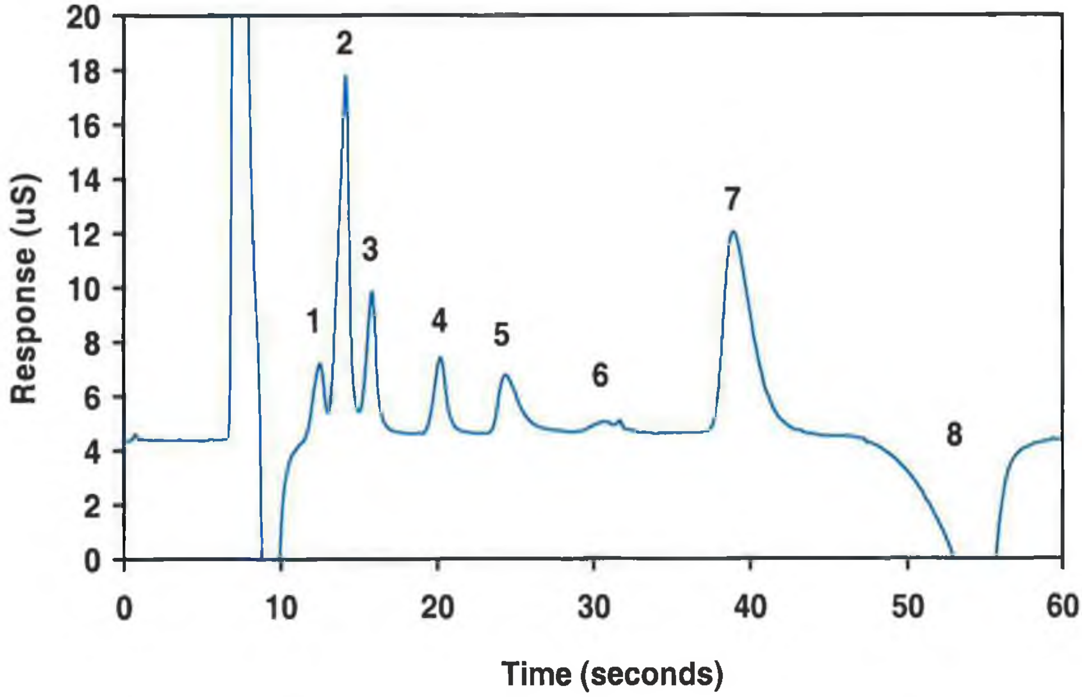

Figure 7-13. *Rapid separation of selected anions on a 5 cm monolithic column with a TBA-phthalate/ACN eluent. Chromatographic conditions as in Figure 7-11 except flow rate 6.0 ml/min. Peaks: [1] 10 mg/L carbonate (12.6 seconds), [2] 10 mg/L chloride (14.2 seconds), [3] 10 mg/L nitrite (15.9 seconds), [4] 10 mg/L nitrate (20.2 seconds), [5] 10 mg/L phosphate (24.5 seconds), [6] system peak #1 (30.9 seconds), [7] 10 mg/L sulphate (39.1 seconds), [8] system peak #2 (55.1 seconds).*

Table 7-2. *Separation efficiency of the 5 cm monolithic column under optimised chromatographic conditions*[8].

Anion	Plates/metre (N/m)
Chloride	37,680
Nitrite	62,400
Nitrate	65,620
Phosphate	34,860
Sulphate	44,100

7.3.11 Sensitivity, linearity, and system precision studies.

The test mix of anions (10 mg/*L* carbonate, chloride, nitrite, nitrate, phosphate and sulphate) were injected using loop volumes of 25 μL and 50 μL at a flow rate of 2.0 ml/min. Figure 7-14 illustrates why 25 μL was chosen as optimum, based on the adverse effect upon resolution of carbonate, chloride and nitrite at 50 μL.

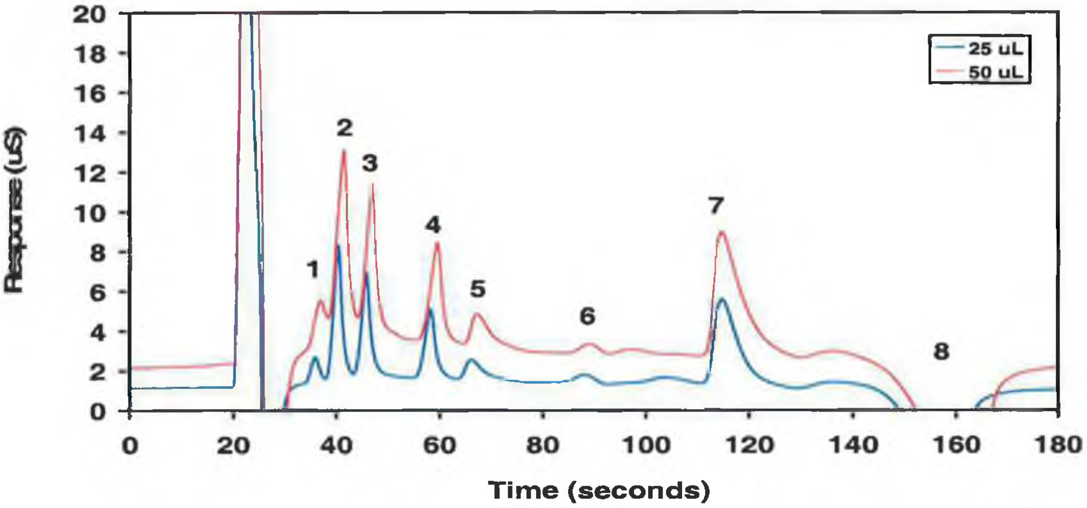

Figure 7-14. *Effect of injection volume upon the separation of target anions on a 5 cm monolithic column. Chromatographic conditions: Column: Two Merck Chromolith Flash RP-18e 2.5 cm X 4.6 mm (connected in series). Mobile phase: (97:3) 1.54 mM tetrabutylammonium hydroxide, 0.8 mM phthalic acid, pH 7.25 / Acetonitrile. Flow rate: 2.0 ml/min. Column temperature: 30 °C. Detection: direct conductivity. Analyte concentrations: 10 mg/L. Peaks: [1] carbonate, [2] chloride, [3] nitrite, [4] nitrate, [5] phosphate, [6] system peak #1, [7] sulphate, [8] system peak #2.*

---

[8] Chromatographic conditions as in Figure 7-13.

For sensitivity studies, standards were injected in triplicate and the average peak height determined. Injections of a blank (deionised water) were also made in triplicate, and an average noise value was determined over the retention time window of each respective standard anion. Limits of detection were expressed as a signal to noise ratio of 3. Figure 7-15 to Figure 7-19 show standards of the anions of interest at concentrations close to their limits of detection. Results are shown in Table 7-3.

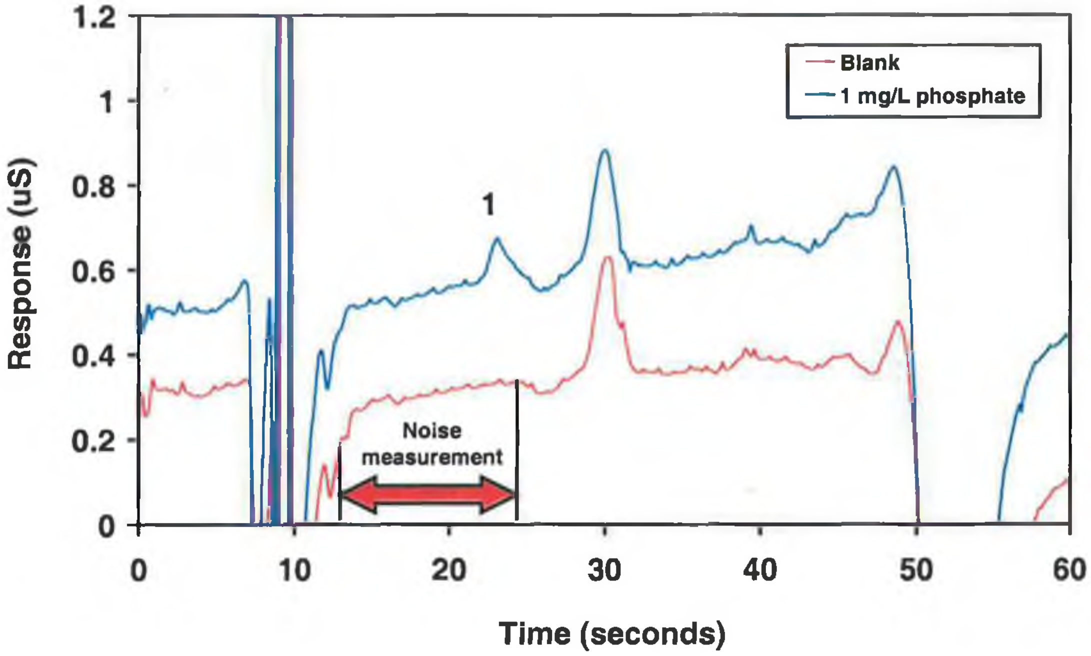

Figure 7-15. *Sensitivity study for phosphate on a 5 cm monolithic column with a TBA-phthalate/ACN eluent. Chromatographic conditions as in Figure 7-13. Peaks: [1] 1.0 mg/L phosphate.*

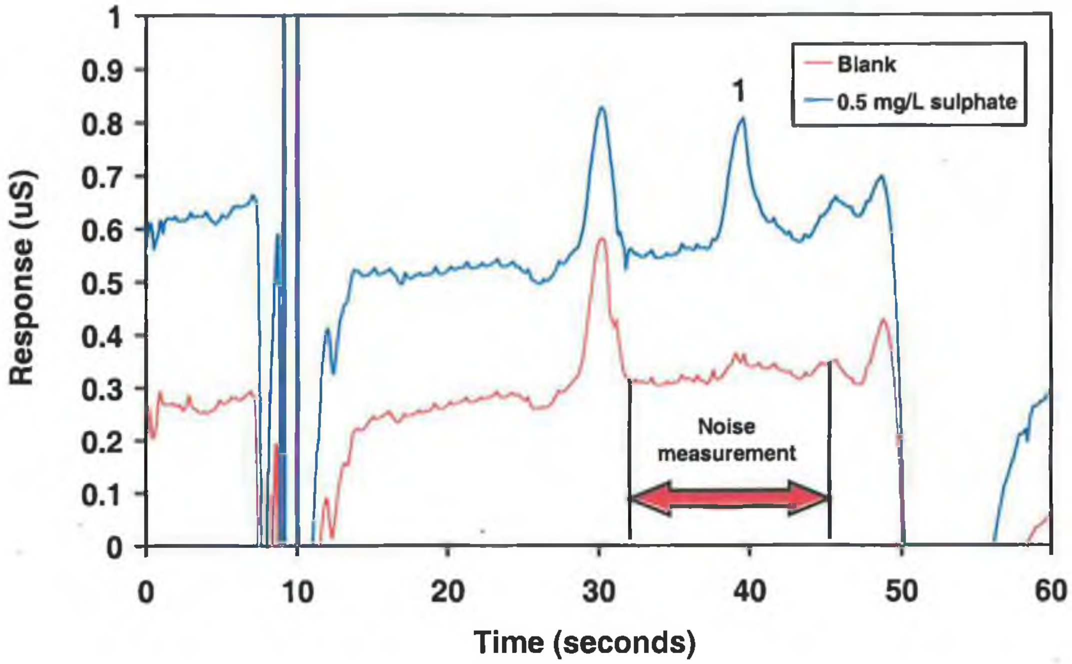

Figure 7-16. *Sensitivity study for sulphate on a 5 cm monolithic column with a TBA-phthalate/ACN eluent. Chromatographic conditions as in Figure 7-13. Peaks: [1] 0.5 mg/L sulphate.*

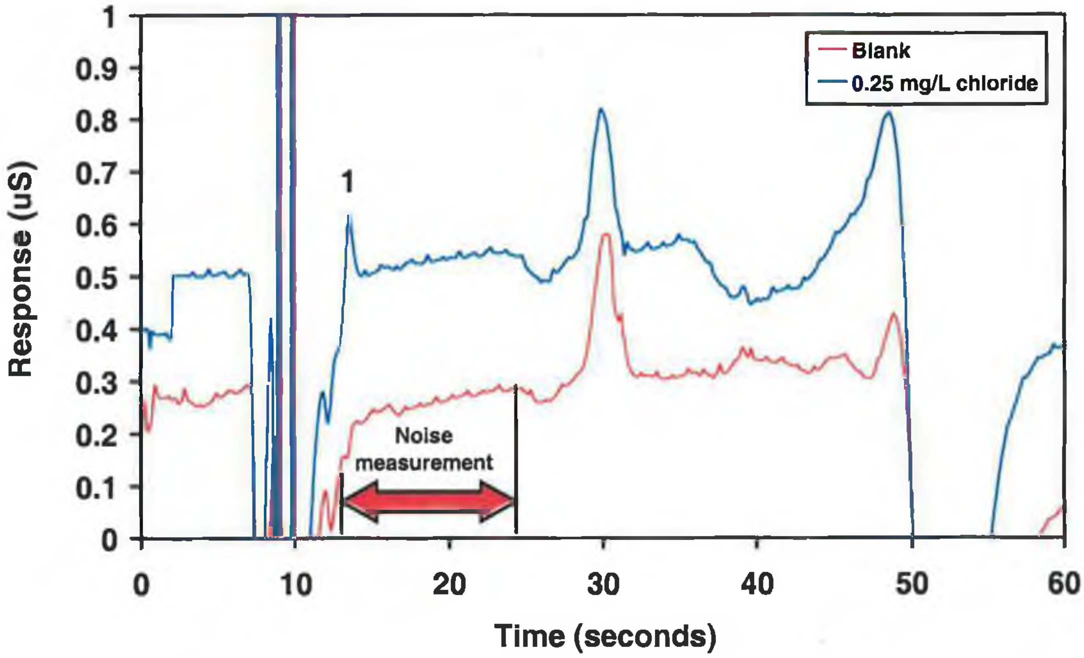

Figure 7-17. *Sensitivity study for chloride on a 5 cm monolithic column with a TBA-phthalate/ACN eluent. Chromatographic conditions as in Figure 7-13. Peaks: [1] 0.25 mg/L chloride.*

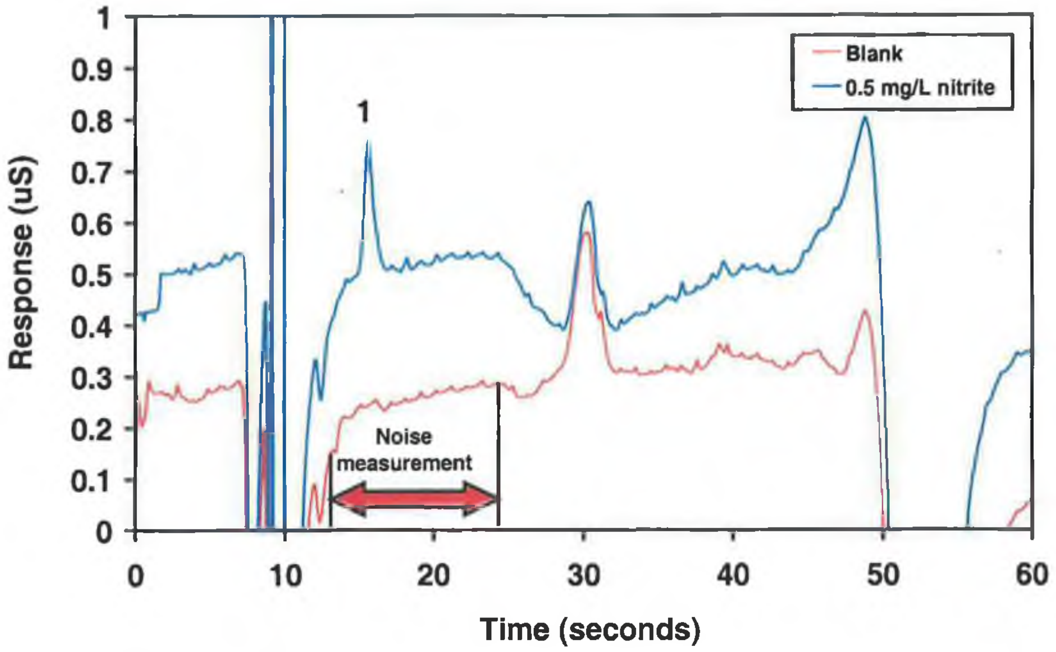

Figure 7-18. *Sensitivity study for nitrite on a 5 cm monolithic column with a TBA-phthalate/ACN eluent. Chromatographic conditions as in Figure 7-13. Peaks: [1] 0.5 mg/L nitrite.*

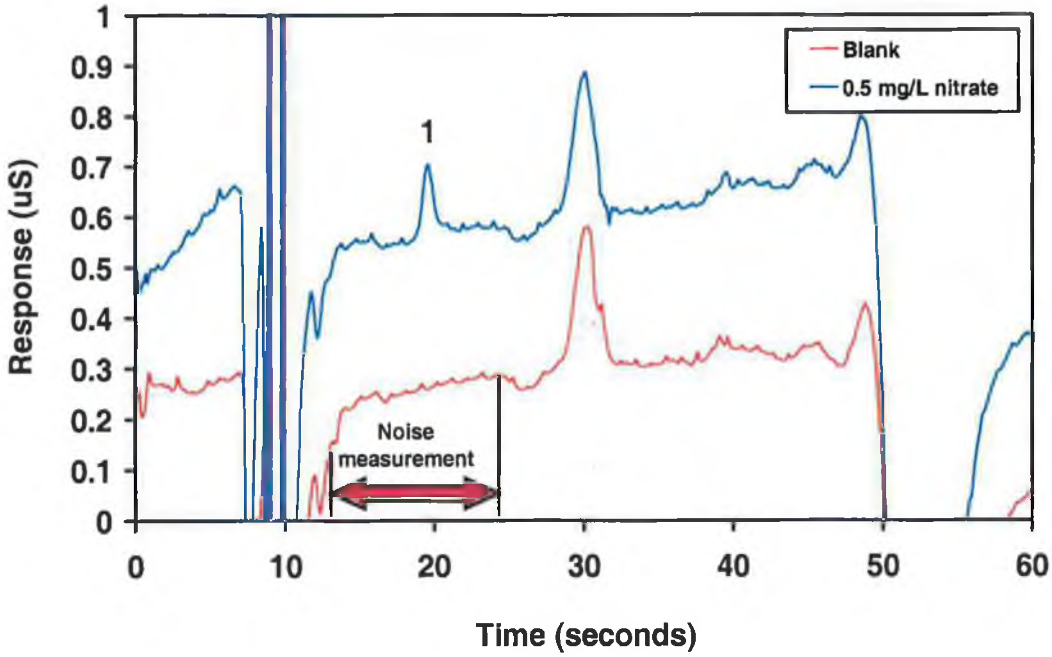

Figure 7-19. *Sensitivity study for nitrate on a 5 cm monolithic column with a TBA-phthalate/ACN eluent. Chromatographic conditions as in Figure 7-13. Peaks: [1] 0.5 mg/L nitrate.*

Table 7-3. *Limits of detection for target anions on a 5 cm monolithic column with a TBA-phthalate/ACN eluent.*

Anion	Limit of detection (S/N = 3)
Phosphate	1.79 mg/*L*
Sulphate	0.38 mg/*L*
Chloride	0.23 mg/*L*
Nitrate	0.31 mg/*L*
Nitrite	0.60 mg/*L*

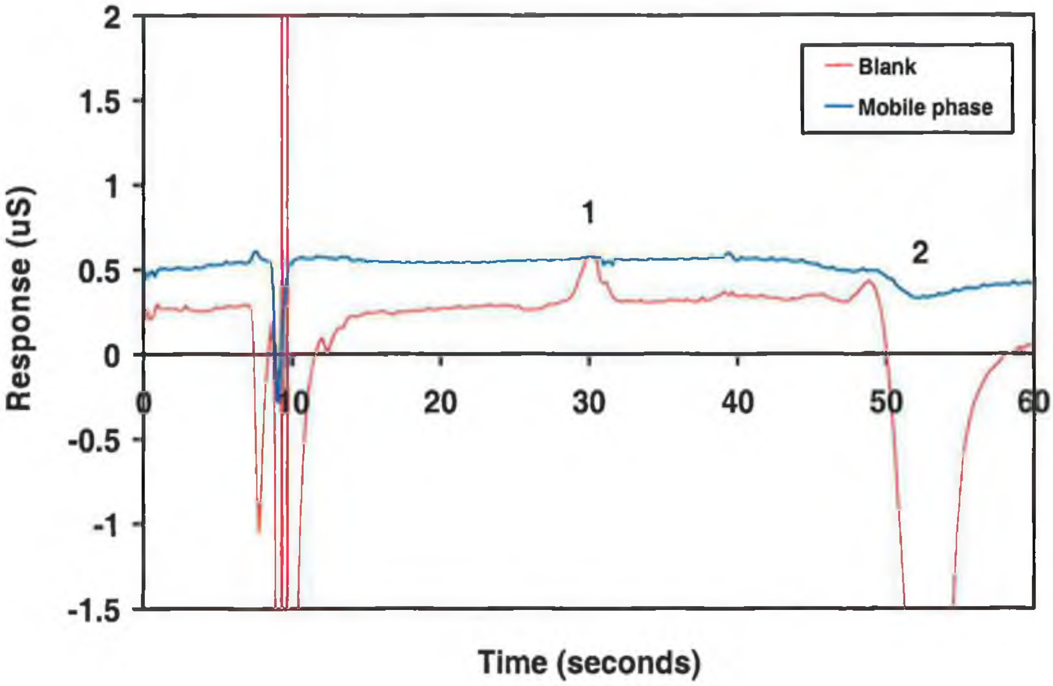

Figure 7-20. *System peak reduction by the injection of mobile phase on a 5 cm monolithic column with a TBA-phthalate/ACN eluent. Chromatographic conditions as in Figure 7-13. Peaks: [1] system peak #1, [2] system peak [2].*

During this work, all standards were prepared in deionised water. Injections of deionised water as a blank yielded two system peaks, “system peak #1” at 30 seconds and “system peak #2” at 53 seconds which are present in all standards and samples. An injection of mobile phase shown above, demonstrates that system peak #1 is no longer present, and system peak #2 is considerably reduced in size. While preparation of standards in mobile phase may not be practical, it is clear that any necessary dilution of samples, if performed using mobile phase as a diluent, would have minimised the effect of system peaks upon the chromatography.

Linearity standards were injected over the concentration range, 0.5 mg/*L* to 100 mg/*L* for chloride, sulphate and nitrite, 1.0 mg/*L* to 100 mg/*L* for nitrate and 10 mg/*L* to 100 mg/*L* for phosphate. To determine system precision, six consecutive injections of a 1.0 mg/*L* standard of each anion were made with the exception of nitrite, for which three consecutive injections of a 0.5 mg/*L* standard were made. Linearity and system precision data are tabulated in Table 7-4. Standard curves for phosphate, chloride, nitrate, nitrite and sulphate are shown in Figure 7-21 to Figure 7-25.

Table 7-4. *Linearity and system precision studies for selected anions on a 5 cm monolithic column with a TBA-phthalate/ACN eluent.*

Anion	**R^2 value**	**System precision (% RSD, n=6)**	**System precision (% RSD, n=3)**
Phosphate	0.9976	4.11 %	▨
Chloride	0.9972	2.35 %	▨
Nitrate	0.9987	2.45 %	▨
Nitrite	0.9998	▨	2.15 %
Sulphate	0.9998	2.35 %	▨

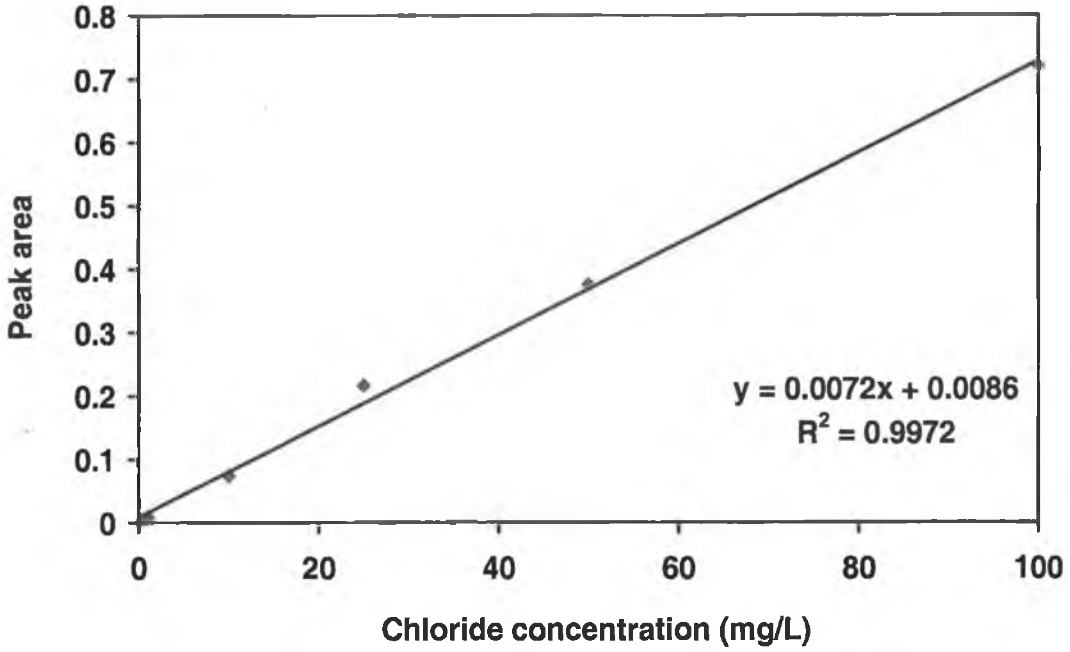

Figure 7-21. *Linearity study for chloride on a 5 cm monolithic column with a TBA-phthalate/ACN eluent. Chromatographic conditions as in Figure 7-13.*

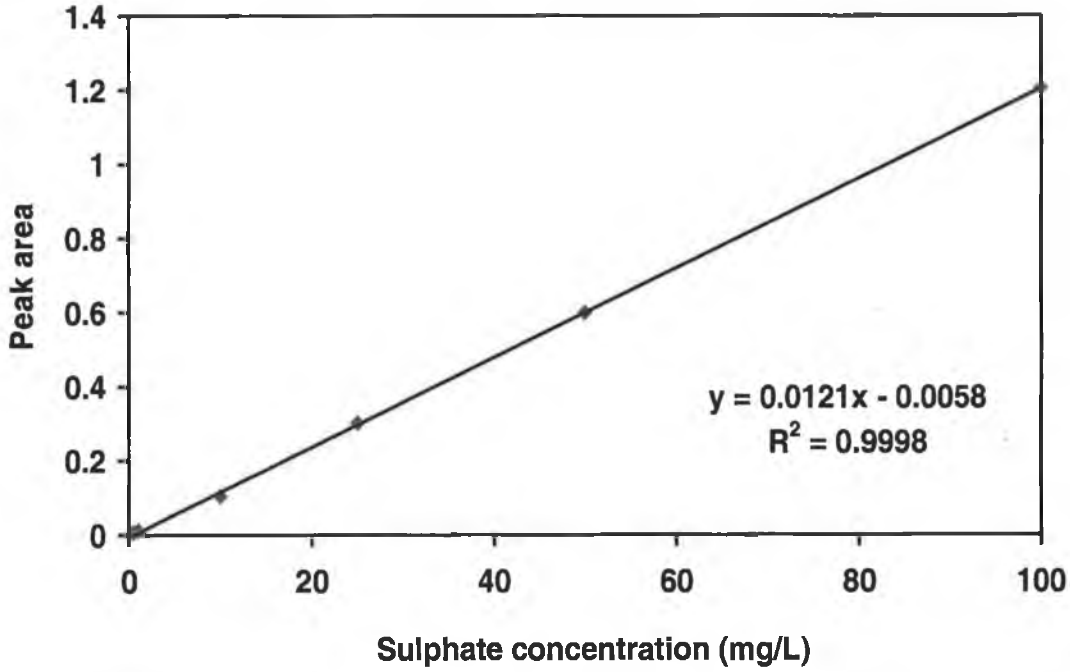

Figure 7-22. *Linearity study for sulphate on a 5 cm monolithic column with a TBA-phthalate/ACN eluent. Chromatographic conditions as in Figure 7-13.*

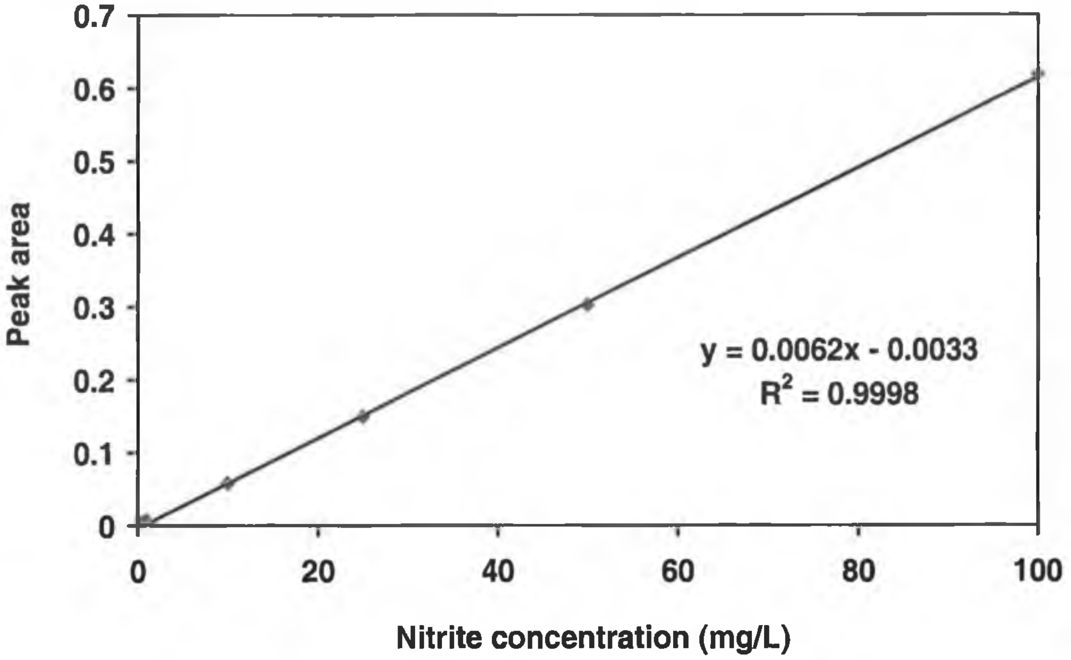

Figure 7-23. *Linearity study for nitrite on a 5 cm monolithic column with a TBA-phthalate/ACN eluent. Chromatographic conditions as in Figure 7-13.*

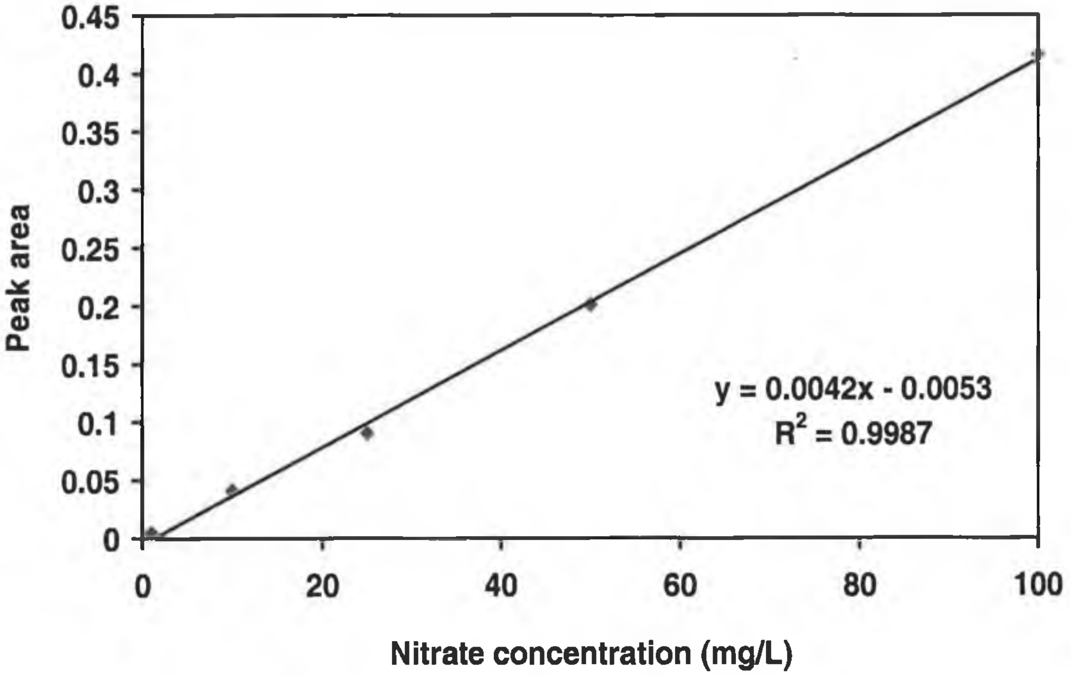

Figure 7-24. *Linearity study for nitrate on a 5 cm monolithic column with a TBA-phthalate/ACN eluent. Chromatographic conditions as in Figure 7-13.*

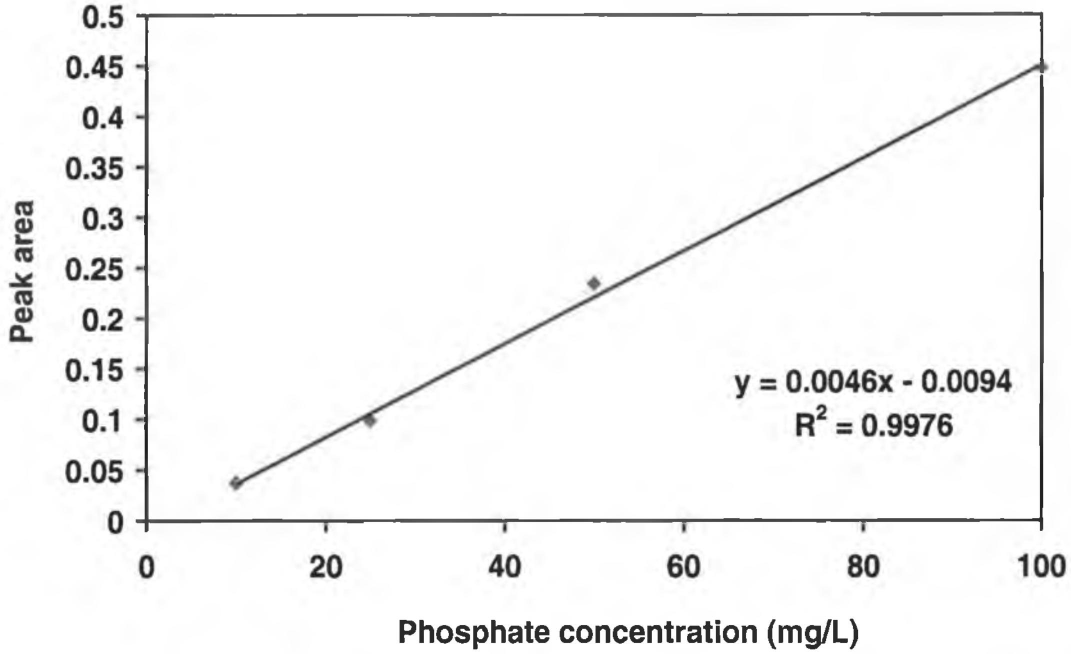

Figure 7-25. *Linearity study for phosphate on a 5 cm monolithic column with a TBA-phthalate/ACN eluent. Chromatographic conditions as in Figure 7-13.*

7.3.12 Analysis of real samples for nitrate.

The optimised rapid separation was used to test forty nine freshwater and estuarine samples for nitrate. Analysis of each sample took < 60 seconds, and standard curves were constructed in minutes, indicating the advantage of this method in the rapid screening of water samples. Sample preparation was rapid, involving a simple filtration step. Since a number of samples were estuarine in nature, high levels of chloride present also necessitated a dilution step. The nitrate linearity data previously discussed provided a standard curve for quantification of nitrate in the samples. Figure 7-26 shows that results compare very well with a separate validated anion exchange method using a conventional 25 cm microbore Dionex AS11 HC column.

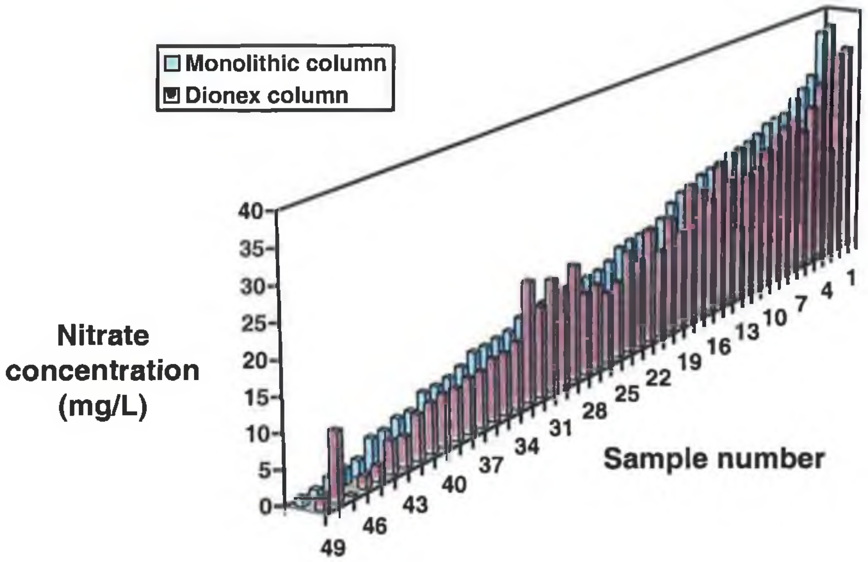

Figure 7-26. *Comparison of sample analysis results obtained on a 5 cm monolithic column by ion-interaction chromatography, with results obtained on a Dionex anion-exchange column.*

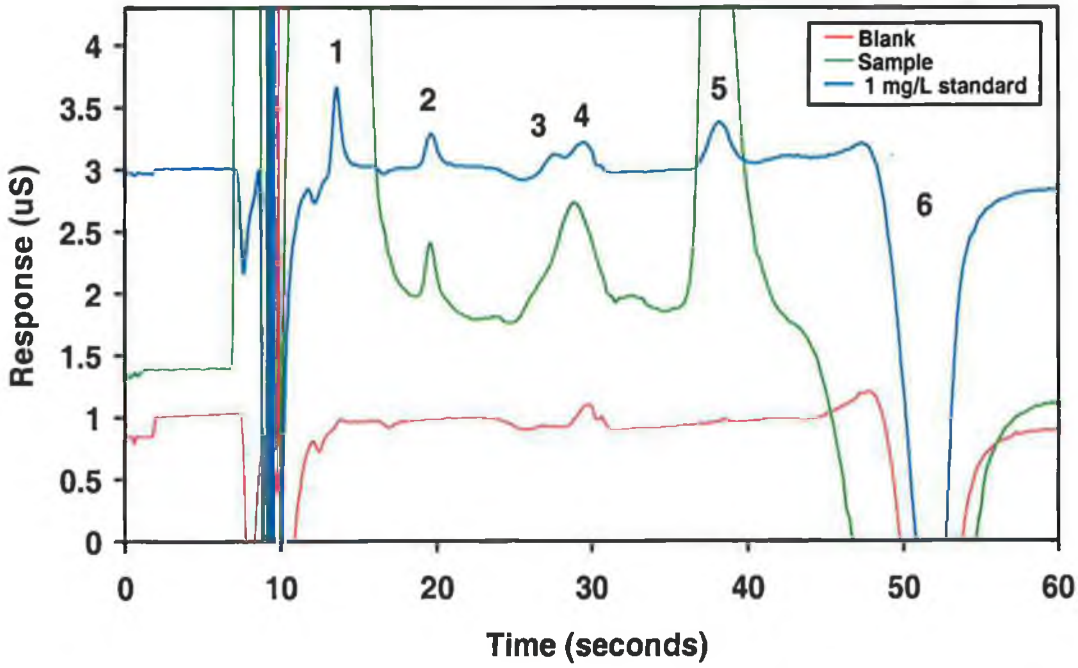

Figure 7-27. *Sample of a small stream flowing into an estuary, overlaid with a 1 mg/L chloride, nitrate, phosphate and sulphate standard as determined on a 5 cm monolithic column with a TBA-phthalate/ACN eluent. Chromatographic conditions as in Figure 7-13. Peaks: [1] chloride, [2] nitrate, [3] phosphate, [4] system peak #1, [5] sulphate, [6] system peak #2.*

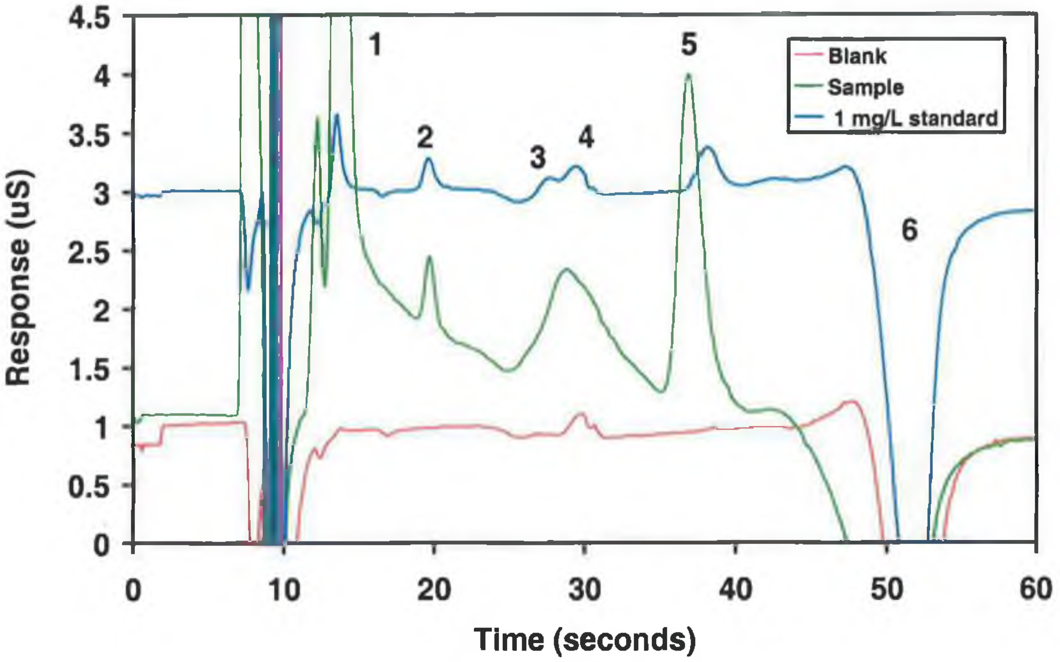

Figure 7-28. *River water, sampled ~150 metres from an estuary, overlaid with a 1 mg/L chloride, nitrate, phosphate and sulphate standard as determined on a 5 cm monolithic column with a TBA-phthalate/ACN eluent. Chromatographic conditions as in Figure 7-13. Peaks: [1] chloride, [2] nitrate, [3] phosphate, [4] system peak #1, [5] sulphate, [6] system peak #2.*

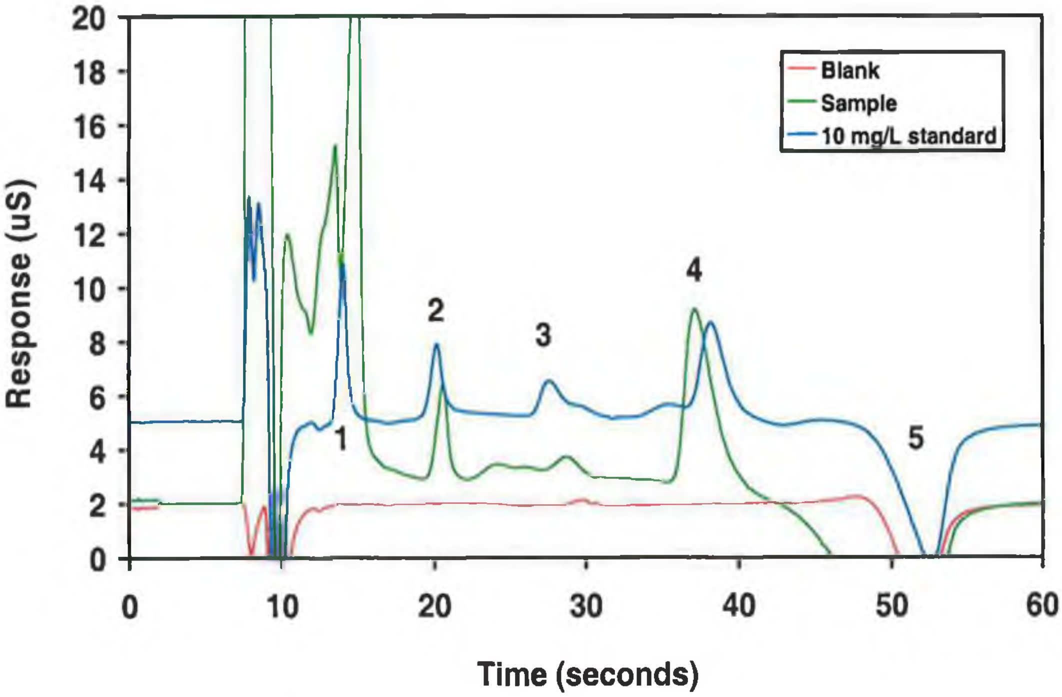

Figure 7-29. *River water sample, overlaid with a 10 mg/L chloride, nitrate, phosphate and sulphate standard as determined on a 5 cm monolithic column with a TBA-phthalate/ACN eluent. Chromatographic conditions as in Figure 7-13. Peaks: [1] chloride, [2] nitrate, [3] phosphate, [4] sulphate, [5] system peak #2.*

7.4 Conclusion.

A simple rapid ion-interaction chromatographic method has been described for analysis of six common inorganic anions in < 60 seconds, using a 5 cm monolithic column and a tetrabutylammonium-phthalate eluent for direct conductivity detection. The column efficiency was compared with a 3 µm particulate column of similar dimensions. The resulting Van Deemter plots illustrated that the HETP did not decrease significantly at high flow rates, compared with the particulate column. Reduced runtimes allowed the analysis of a large number of water samples for nitrate, indicating the advantage of this method in the rapid screening of water samples.

7.5 References.

[1]. P. Hatsis, C.A. Lucy, Analyst 127 (2002) 451.

[2]. P. Hatsis, C.A. Lucy, Anal. Chem. 75 (2003) 995.

[3]. Q. Xu, K. Tanaka, M. Mori, M.I.H. Helaheh, W. Hu, K. Hasebe, H. Toada, J. Chromatogr. A 997 (2003) 183.

[4]. Q. Xu, M. Mori, K. Tanaka, M. Ikedo, W. Hu, J. Chromatogr. A 1026 (2004) 191.

[5]. E. Sugrue, P.N. Nesterenko, B. Paull, Analyst 128 (2003) 417.

8 Simultaneous and rapid ion chromatography of common inorganic anions and cations on short permanently coated monolithic columns.

8.1 Introduction.

In order to fully analyze a sample for total anions and cations by IC, it is usually necessary to first perform an anion determination followed by a cation determination. This will generally involve the use of two separate columns and two separate eluents. Often the costs of running an anion determination on one system and a cation determination at the same time on a second system are prohibitive. The only alternative remains for a single system to be used first for anion analysis, followed by equilibration of the system with the second eluent for the cation determination; and in this instance the time taken for the total analysis of the sample for anions and cations can be very long.

In a recent review on the subject of the simultaneous separation of anions and cations using IC [1], *"simultaneous separation"* was defined as the analysis of anions and cations from a single injection of sample on one chromatographic system; a system which may incorporate more than one pump, eluent, column or detector and which may also include the use of switching valves to vary the direction of the eluent flow-path during the run. Table 8-1 presents a range of methodologies for the simultaneous determination of anions and cations, which are separated into subgroups based on the use of (1): specialist mobile phases, (2): specialist stationary phases and (3): the use of multi-column systems.

(1): Mobile phases containing complexing ligands which convert alkaline earth and transition metal ions into anionic complexes allow these complexes to be simultaneously determined along with common inorganic anions by anion exchange chromatography. The use of ligands such as pyromellitate and dipicolinate has been discussed previously in Chapter 6. Other ligands such as EDTA have been widely used, but a disadvantage with this methodology is that alkali metal ions are not complexed by these ligands and so cannot be determined in the same analysis.

Table 8-1. *Simultaneous determination of anions and cations by IC.*

(1): Anionic metal/ligand complex formation					
Column.	**Eluent / Flow rate.**	**Analytes / Total analysis time.**	**Detection mode.**	**Application.**	**Ref.**
150 X 4.6 mm I.D. (5 µm) TSKguardgel QAE-SW	0.5 m*M* trimellitic acid – 0.25 m*M* EDTA, pH 6.0. (1.0 ml/min).	SO_4^{2-}, Cl^-, NO_3^-, NO_2^- HCO_3^-, Mg^{2+}, Ca^{2+} (15 minutes)	Indirect UV at 270 nm, (anions), direct UV at 270 nm (cations).	Rain water, river water.	[2]
250 X 4.6 mm I.D. (5 µm) TSKguardgel QAE-SW	0.15 m*M* pyromellitate, pH 5.4 (1.0 ml/min)	SO_4^{2-}. Cl^-, NO_3^-, Mg^{2+}, Ca^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, I^-, Zn^{2+}, SCN^-, Cd^{2+}, $S_2O_3^{2-}$ (30 minutes)	Suppressed conductivity (Dionex AMMS-I with 1 *M* H_2SO_4)	Rain water, river water.	[3]
250 X 4.6 mm I.D. (5 µm) TSKguardgel QAE-SW	0.15 m*M* pyromellitate – 22.5 % methanol – water at pH 6.25. (1.0 ml/min)	SO_4^{2-}, Cl^-, NO_3^-, NO_2^- HCO_3^-, Mg^{2+}, Ca^{2+} (within 20 minutes)	Indirect UV at 262 nm (anions), direct UV at 262 nm (cations).	River water.	[4]
50 X 4 mm I.D. Dionex AG9 guard column and 250 X 4 mm I.D. Dionex AS9.	3.5 m*M* $NaHCO_3$ with a pH gradient of t=0 min, pH 9.75 t=10 min pH 10.5 (flow rate not given)	BrO_3^-, Cl^-, Pb^{2+}, Cd^{2+}, Ni^{2+}, NO_3^-, SeO_3^{2-}, Cu^{2+}, $HAsO_4^{2-}$, SeO_4^{2-}, SO_4^{2-}, WO_4^{2-}, MoO_4^{2-}, CrO_4^{2-}. (within 20 minutes)	Suppressed conductivity (Dionex AMMS-II with 25 m*M* H_2SO_4)	River water.	[5]
250 X 4 mm I.D. Nucleosil $5C_{18}$	Acetonitrile-water (2:98 v/v) containing 1 m*M* TBA, 0.5 m*M* *trans*-1.2-diaminecyclohexane-*N,N,N',N'*-tetraacetic acid, pH 6.5. (1.0 ml/min)	Cl^-, NO_2^-, Br^-, NO_3^-, CrO_4^{2-}, SO_4^{2-} MoO_4^{2-}, Cr(III) (within 14 minutes).	Direct and indirect UV detection at 210 nm.	Mortar and leather extracts.	[6]
150 X 3.0 mm I.D. (20 µm) MCI SCA-01 anion exchange column	0.5 m*M* $Na_2[Cu(edta)]$ + 0.05 m*M* Na_2H_2edta. (1.0 ml/min)	SO_4^{2-}, Cl^-, NO_3^-, Mg^{2+}, Ca^{2+} (within 20 minutes)	Direct and indirect UV detection at 290 nm.	Tap water.	[7]
50 X 4.6 mm I.D. (5 µm) TSK gel IC-Anion-SW	1 m*M* EDTA pH 6.0. (1.0 ml/min)	SO_4^{2-}. Cl^-, NO_3^-, NO_2^-, $H_2PO_4^-$, Mg^{2+}, Ca^{2+} (within 15 minutes)	Conductivity.	Tap water and diluted sea water.	[8]
(2): Ion exclusion chromatography					
Two 150 X 7.8 mm I.D. (5 µm) TSKgel OA-Pak-A weakly acidic cation exchange columns connected in series.	1.25 m*M* sulfosalicylic acid in methanol – water (7.5:92.5) (1.2 ml/min)	SO_4^{2-}, Cl^-, NO_3^-, Na^+, NH_4^-, K^+, Mg^{2+}, Ca^{2+}. (within 30 minutes)	Direct conductivity (anions), indirect conductivity (cations).	Acid rain.	[9]

Column.	Eluent / Flow rate.	Analytes / Total analysis time.	Detection mode.	Application.	Ref.
300 X 7.8 mm I.D. (5 µm) TSKgel OA-Pak-A weakly acidic cation exchange column.	5 m*M* tartaric acid / 6 m*M* 18-crown-6 / methanol – water (7.5:92.5) (1.2 ml/min)	SO_4^{2-}, Cl^-, NO_3^-, Na^+, NH_4^+, K^+, Mg^{2+}, Ca^{2+}. (within 30 minutes)	Direct conductivity (anions), indirect conductivity (cations).	Rain water, river water.	[10]
Two 150 X 7.8 mm I.D. (5 µm) TSKgel OA-Pak-A weakly acidic cation exchange columns connected in series.	1.5 m*M* sulfosalicylic acid – 6 m*M* 18-crown-6, pH 2.6 (1.5 ml/min)	SO_4^{2-}, Cl^-, NO_3^-, Na^+, NH_4^+, K^+, Mg^{2+}, Ca^{2+}. (within 14 minutes)	Direct conductivity (anions), indirect conductivity (cations).	Acid rain.	[11]
Two 150 X 7.8 mm I.D. (5 µm) TSKgel OA-Pak-A weakly acidic cation exchange columns connected in series.	6 m*M* tartaric acid in methanol – water (7.5:92.5) (1.2 ml/min).	SO_4^{2-}, Cl^-, NO_3^-, Na^-, NH_4^+, K^+, Mg^{2+}, Ca^{2+}. (30 minutes)	Direct conductivity (anions), indirect conductivity (cations).	Acid rain.	[12]
300 X 7.8 mm I.D. (5 µm) TSKgel OA-Pak-A weakly acidic cation exchange column.	5 m*M* DL-malic acid – 5 % methanol/water. (1.2 ml/min).	SO_4^{2-}, Cl^-, NO_3^-, F^-, Na^+, NH_4^+, K^+, Mg^{2+}, Ca^{2+} (30 minutes).	Direct conductivity (anions), indirect conductivity (cations).	Rain water.	[13]
150 X 7.8 mm I.D. (5 µm) TSKgel OA-Pak-A weakly acidic cation exchange column.	1.6 m*M* dipicolinic acid and 8.0 m*M* 18-crown-6. (1.0 ml/min)	SO_4^{2-}, Cl^-, NO_3^-, Na^+, NH_4^+, K^+, Mg^{2+}, Ca^{2+}. (within 25 minutes)	Direct conductivity (anions), indirect conductivity (cations).	Rain water, tap water, snow water.	[14]
150 X 6.0 mm I.D. (3 µm) TSKgel Super-IC-A/C weakly acidic cation exchange column.	15 m*M* tartaric acid and 2.5 m*M* 18-crown-6. (1.5 ml/min)	SO_4^{2-}, Cl^-, NO_3^-, Na^-, NH_4^+, K^+, Mg^{2+}, Ca^{2+}. (within 5 minutes)	Direct conductivity (anions), indirect conductivity (cations).	Rain water, river water.	[15]
(3): Zwitterionic stationary phases.					
200 X 4.6 mm I.D. (5 µm) PolyCAT A	0.3 m*M* H_2SO_4 and 0.2 m*M* Li_2SO_4 (1.0 ml/min)	Cl^-, NO_3^-, $H_2PO_4^-$, Na^-, K^+, Mg^{2+}, Ca^{2+}. (9 minutes)	Conductivity.	Tap water.	[16]
150 X 0.35 mm I.D. (5 µm) Develosil ODS-5 coated with sodium taurodeoxycholate.	5 m*M* $CuSO_4$ (2 µL/min)	$S_2O_3^{2-}$, NO_3^-, I^-, SCN^-, Na^+, K^+. (10 minutes)	Indirect UV detection at 210 nm.	-----	[17]
250 X 4.6 mm I.D. ODS column coated with Zwittergent-3-14 micelles.	Pure water (1.0 ml/min)	Na^+, Ca^{2+}, SCN^-, Cl^-. (within 10 minutes)	Conductivity.	-----	[18]
(4): Mixed bed stationary phases					
125 X 4.6 mm I.D. column packed with ICS-A23 anion exchange resin and CHI cation exchange resin.	0.8-2.0 m*M* oxalic acid. (1.0 ml/min)	Acetate, lactate, succinate, F^-, malic, NO_2^-, citrate, tartrate, Cl^-, Na^+, Br^-, NH_4^+, NO_3^-, K^+. (30 minutes)	Conductivity.	Wine and Japanese sake.	[19]

Column.	**Eluent / Flow rate.**	**Analytes / Total analysis time.**	**Detection mode.**	**Application.**	**Ref.**
70 X 4.1 mm I.D. column packed with 10 µm Hamilton PRP-X100 anion exchange resin and sulphonated Hamilton PRP-1 cation exchange resin.	A variety of mobile phases were studied at 1.0 ml/min: (acetate + LiOH), (phthalic acid + LiOH + LiCl), (phthalic acid + ethylenediamine)	Na^+, Cl^-, NO_3^-, K^+, F^-, Rb^+, Cs^+, SO_4^{2-}, Mg^{2+}. (within 20 minutes)	Conductivity.	-----	[20]
250 X 2.0 mm I.D. Dionex HPIC-CS5 (mixed bed)	2 m*M* $CuSO_4$ pH 5.40 (0.8 ml/min)	Cl^-, NO_2^-, benzoate, cyanate, Br^- NO_3^-, Na^+, NH_4^+, K^+, Rb^+, Cs^+. (within 10 minutes)	Two all solid-state contact tubular PVC-membrane electrodes selective for monovalent anions and cations in series.	River, sea and tap water	[21]
(5): Columns connected in series.					
50 X 4.6 mm TSKgel IC-Anion-SW and 150 X 4.6 mm TSKgel Super IC-Cation connected in series via two six-way switching valves.	1.0 m*M* H_2SO_4 and 0.1 m*M* L-Histidine (1.0 ml/min).	Na^+, NH_4^+, K^+, Mg^{2+}, Ca^{2+}, IO_3^-, BrO_3^-. Br^-, I^-, Cl^-, NO_3^- (within 30 minutes)	Indirect UV at 210 nm (cations), direct UV at 210 nm (anions) and direct conductivity (chloride).	Tap water, river water, pond water.	[22]
50 X 4.0 mm I.D. Hitachi IC 2710 (10 µm) strong anion exchange column and 50 X 4.0 mm I.D. Hitachi IC 2720 (10 µm) strong cation exchange column connected in series.	2.5 m*M* benzoic acid + 1.5 m*M* Tris. Flow gradient used via t-piece connection between both columns. (Total flow rate: 1.0 ml/min)	Cl^-, NO_2^-, NO_3^-, Br^-, Na^+, NH_4^+, K^+, Li^+. (20 minutes)	Conductivity.	-----	[23]
500 X 2.1 mm I.D. Du Pont Zipax SCX in series after a 500 X 2.1 mm I.D. Du Pont Zipax SAX.	0.25 m*M* copper *o*-sulfobenzoate, pH 4.3 to 4.4. (1.0 ml/min)	Cl^-, NO_2^-, NO_3^-, Br^-, Na^+, NH_4^+, K^+, SO_4^{2-}, (20-30 minutes)	Indirect UV at 240 nm and 270 nm.	-----	[24]
Dionex HPIC-AS7 column in series with two Dionex HPIC-CG2 guard columns	5 mM oxalic acid + 3.75 mM citric acid adjusted to pH 4.37. (1.0 ml/min)	Cl^-, SO_4^{2-}, Na^+, Zn^{2+}, Fe^{2+}, Co^{2+}, NO_3^-, K^+. (30 minutes)	Conductivity	Vitamin supplements	[25]

(2): Specialist stationary phases for simultaneous determination of anions and cations have been developed such as ion-exclusion stationary phases, mixed bed stationary phases and zwitterionic stationary phases. With regard to ion-exclusion stationary phases, the use of a weakly acidic cation exchange column based on poly-methacrylate resin has been used by a number of workers using dilute solutions of weak organic acids. Such stationary phases (TSKgel OA-Pak A in the H^+ form) retain cations by cation exchange and anions by an ion-exclusion mechanism.

Mixed bed columns involve the packing of a column with a mixture of anion exchange resin and cation exchange resin such that the column is both an anion exchanger and a cation exchanger. An eluent such as $CuSO_4$ will then allow the elution of cations by the Cu^{2+} counterion, and the elution of anions by the SO_4^{2-} counterion. The advantage of mixed bed columns is that the ion-exchange capacity of the column can be adjusted by varying the relative ratio of anion and cation exchange resins during the packing process.

Zwitterionic stationary phases contain both negative and positive charges in close proximity to each other such that anions and cations traverse the column as an "ion-pair" due to simultaneous attractive and repulsive electrostatic forces. Zwitterionic stationary phases are generally reversed phase columns coated with a zwitterionic reagent such as Zwittergent 3-14 which incorporates a C_{14} chain with an ammonium group separated from a sulphonate group by a C_3 moiety. Despite the fact that pure water can be used as an eluent, thereby greatly increasing the sensitivity of conductivity detection, a disadvantage of this mode of IC, (also known as *"electrostatic ion chromatography"*) is that peak identification is often quite complex because injected anions and cations in a sample elute as ion-pairs. Therefore for example, if a mixture of sodium nitrate and calcium chloride is injected using pure water as a mobile phase, four peaks will be obtained for sodium nitrate, sodium chloride, calcium chloride and calcium nitrate.

(3): In Nesterenko's review on simultaneous separation and detections of anions and cations by IC [1] it was stated that the approach of using anion and cation exchange columns in parallel or in series had not received much attention due to the complexity of the instrumentation required, namely multiple pumps, switching valves and detectors. One of the main disadvantages of multi-column systems is the high column backpressure generated by the combination of two columns, which can damage the detector flow cell if it is not resistant to high backpressures. A possible remedy is to increase the particle size of the columns such that backpressure is reduced, but separation efficiency will then also be compromised.

In this work, with the advent of monolithic columns which have been shown to result in substantially lower backpressures (see Figure 7-2) than conventional particulate columns of comparable efficiency, the possibility of using monolithic columns in parallel for simultaneous determination of anions and cations is investigated.

8.2 Experimental.

8.2.1 Equipment.

The ion chromatograph and analytical columns used were as described in Chapter 7, with the addition of a Merck Chromolith SpeedROD RP-18e 5.0 cm X 4.6 mm column (Merck KgaA, Damstadt, Germany). During method development work, an injection volume of 25 μL was used. For the analysis of real samples, the injection loop used was 25 μL for the analysis of anions, 50 μL for analysis of cations, and 100 μL for the simultaneous determination of anions and cations. Detection was by direct conductivity for anions, and by indirect conductivity for cations. For the work involving the simultaneous use of both monolithic columns, the instrumental setup is shown later as Figure 8-3.

8.2.2 Reagents and chromatographic conditions.

The water used for mobile phase and standard preparation was obtained from a Millipore Milli-Q water purification system (Millipore, Bedford, MA, USA). The optimised eluent for both anion and cation separations on the modified monolithic columns was 2.54 m*M* phthalic acid and 1.47 m*M* ethylenediamine, pH 4.5. The ethylenediamine and phthalic acid were both supplied by Aldrich (Aldrich, Milwaukee, WI, USA). All mobile phases were degassed and filtered before use using 0.45 µm nylon membrane filters from Gelman Laboratories (Gelman Laboratories, Michigan, USA). The flow rate used was 4.0 ml/min for the individual determination of anions. Flow rates of 5.0 ml/min and 8.0 ml/min were used for determination of cations. For the simultaneous determination of anions and cations, a flow rate of 3.0 ml/min was used. Column temperature set at 30 °C for all separations.

Standard solutions were prepared as described in Chapter 6 with the exception of calcium and barium standards which were prepared from their chloride salts (Aldrich), while magnesium and copper standards were prepared from their sulphate salts (Aldrich). Strontium standards were prepared from a 1000 mg/*L* strontium AAS standard (Aldrich). All samples were stored at 4 °C until use. Samples were diluted with Milli-Q water where necessary, and syringe filtered prior to injection. River water samples were collected and filtered at source, and analysed within 24 hr.

8.2.3 Column coating procedures.

The didodecyldimethylammonium bromide (DDAB) and the dioctylsulphosuccinnate sodium salt (DOSS) used for permanent coating, was supplied by Aldrich, (Aldrich, Milwaukee, WI, USA). Before coating of the columns, the new columns were washed and then conditioned with aqueous acetonitrile solutions, using acetonitrile supplied by Labscan, (Labscan Ltd., Stillorgan, Dublin, Ireland). All coating solutions were filtered using 0.45 µm nylon membrane filters from Gelman Laboratories (Michigan, USA). The flow rate during column coating was 5 ml/min for 30 minutes.

8.2.3.1 Permanent coating for anion exchange chromatography.

In this work, two Merck Chromolith Flash RP-18e 2.5 cm X 4.6 mm columns connected in series were first washed with 5 % acetonitrile for 30 mins at 5 ml/min, then coated with a solution of 1 m*M* DDAB, by pumping the solution through the column at 5 ml/min for 30 minutes. This coating procedure effectively converts the C_{18} stationary phase to an anion exchanger. The columns were then washed with 100 % water for 30 mins at 5 ml/min to remove unbound IIR. As discussed later, in most of the following work, only one of the 2.5 cm coated columns was actually used for anion analysis in order to reduce runtimes.

8.2.3.2 Permanent coating for cation exchange chromatography.

A Merck Chromolith Speed ROD RP-18e 5.0 cm X 4.6 mm column was first washed with 100 % acetonitrile for 30 mins at 3 ml/min, then coated with an aqueous solution of 1 m*M* dioctylsulphosuccinnate in 10 % ACN by pumping the solution through the column at 5 ml/min for 30 minutes. This coating procedure effectively converts the C_{18} stationary phase to a cation exchanger. The column was then washed with 100 % water to remove unbound IIR for 30 mins at 5 ml/min.

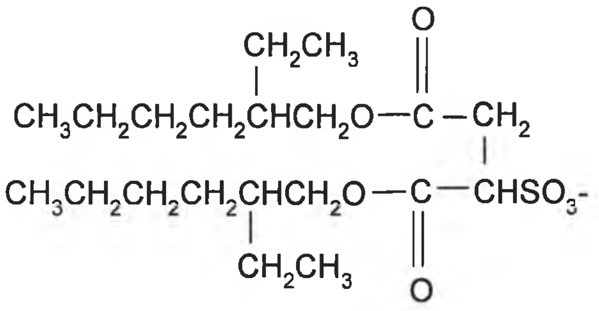

Figure 8-1. *Structure of dioctylsulphosuccinnate (DOSS).*

8.3 Results and discussion.

8.3.1 Optimisation of mobile phase and ion-exchange capacities of the cation and anion exchange columns.

8.3.1.1 Eluent design.

In developing a mobile phase for the simultaneous determination of anions and cations, a number of factors were considered. It was necessary for the single mobile phase to consist of two oppositely charged eluting ions, one being anionic for the determination of anions, and the other being cationic for the determination of cations. Also, in keeping with the objective of achieving rapid separations, eluting ions having a valency of two or greater were considered. The pH of the mobile phase could then be controlled such that the charge on both eluting ions was maximised, resulting in shorter analyte retention times due to the eluting strength of the mobile phase being maximised. Also, the mobile phase components were chosen such that the pH could be controlled by the inherent buffering capacity of the two components themselves, rather than adding a separate acid or base (eg phosphoric acid, acetic acid, NaOH etc), which would introduce further interfering anions/cations to the eluent. Therefore, anionic organic acids were considered which could be directly titrated with a cationic organic base to the desired pH.

A further consideration was the mode of detection to be used for this work. Detection modes that were considered possible for determination of anions, were direct and indirect UV detection, and also direct conductivity detection. For determination of cations, indirect UV detection was considered, as well as direct/indirect conductivity detection. (In the case of both anions and cations, suppressed conductivity detection was avoided, such that the chromatographic conditions were kept as simple as possible). Wherever practical, eluent components were considered which lent the most flexibility in terms of possible modes of detection. A copper-phthalate eluent was considered as a possible option. Both components are divalent, resulting in faster separations. This eluent would also offer a range of detection possibilities; indirect UV for cations (copper being a UV absorbing cation), and also indirect UV for anions (since phthalate has a benzene ring chromophore). Direct conductivity could also be used for determination of anions, since phthalate, being an organic acid, would also yield suitably low background conductivity. However, this eluent was rejected due to the prohibitive cost (~ €71 per 50 milligrams of copper phthalate).

The mobile phase ultimately chosen for this work was ethylenediamine/phthalate. Ethylenediamine is a divalent cationic organic base, which can be directly titrated with phthalic acid, a diprotic organic acid, to the desired pH. Ethylenediamine has a charge of +2 at pH values less than 6.99 (pKa_2 for ethylenediamine), and phthalate has a charge of -2 at pH values greater than 5.51

($pKa_1 = 2.89$, $pKa_2 = 5.51$). Therefore, in order to maximise the eluting strength of the mobile phase with respect to simultaneous anion and cation determinations, the optimum mobile phase pH would ideally lie between pH 5.51 and pH 6.99. Over this pH range the eluent would also exhibit a reasonable buffering capacity, negating the need for addition of any other buffering species, an important requirement if indirect modes of detection are used. A further advantage of this ethylenediamine/phthalate mobile phase was the range of detection modes which it afforded. As discussed earlier, phthalate allows the use of indirect UV, as well as direct conductivity. Ethylenediamine is suitable for indirect conductivity detection of metals.

An ethylenediamine/phthalic acid eluent has previously been used by Pietrzyk and Brown [20], who used an eluent of 0.954 m*M* ethylenediamine, 1.00 m*M* phthalic acid, pH 5.82 at a flow rate of 1.0 ml/min to separate sulphate, calcium and magnesium within 20 minutes. A 70 X 4.1 mm I.D. mixed bed column packed with 10 µm Hamilton PRP-X100 anion exchange resin and sulphonated Hamilton PRP-1 cation exchange resin was used.

Fritz *et al.* [26] separated magnesium, calcium, strontium and barium on a 340 mm X 2 mm I.D. column packed with 20 µm BN-X4 polystyrene-DVB cation exchange resin. The separation was complete within 15 minutes using an eluent of 1 m*M* ethylenediammonium dinitrate, pH 6.1 at a flow rate of 1.0 ml/min.

Ohta *et al.* [27] used an eluent of 0.5 m*M* ethylenediamine at pH 5.5 (flow rate: 1.0 ml/min) to separate nickel, cobalt, iron (II), manganese, magnesium, calcium, strontium and barium within 15 minutes using a 150 X 4.6 mm I.D. (5 µm) Develosil 30-5 column.

Xu *et al.* [28] coated a 5.0 cm monolithic column with lithium dodecylsulphate and used a 2 m*M* ethylenediamine / 0.1 m*M* Li-DS eluent (pH 6.0), to elute hydronium, calcium and magnesium in under 4 minutes with a flow rate of 4.0 ml/min. Xu *et al.* went on to demonstrate that because at pH 6.0, the predominant form of ethylenediamine was divalent cations, (EnH_2^{2+}) [29], the separation was performed by cation exchange. This was demonstrated by plotting log [EnH_2^{2+}]

against log *k'* of H^+, Mg^{2+} and Ca^{2+} and obtaining slopes of -0.4851 for H^+, -0.9916 for Mg^{2+} and -0.9921 for Ca^{2+}.

In the work described herein, with a single ethylenediamine/phthalate eluent delivered using a single isocratic pump, two modes of detection were considered. One possibility was to run the anion exchange column in parallel with the cation exchange column, with the column for anions routed through a UV detector, and the detection of cations via a separate conductivity detector. In this manner (Figure 8-2), two chromatograms would be simultaneously acquired using a common data acquisition system. Cations would not cause interference in the anion separation, since they would not be retained on the anion exchange column and would elute in the void. Similar reasoning follows in the case of the cation determination using the conductivity detector.

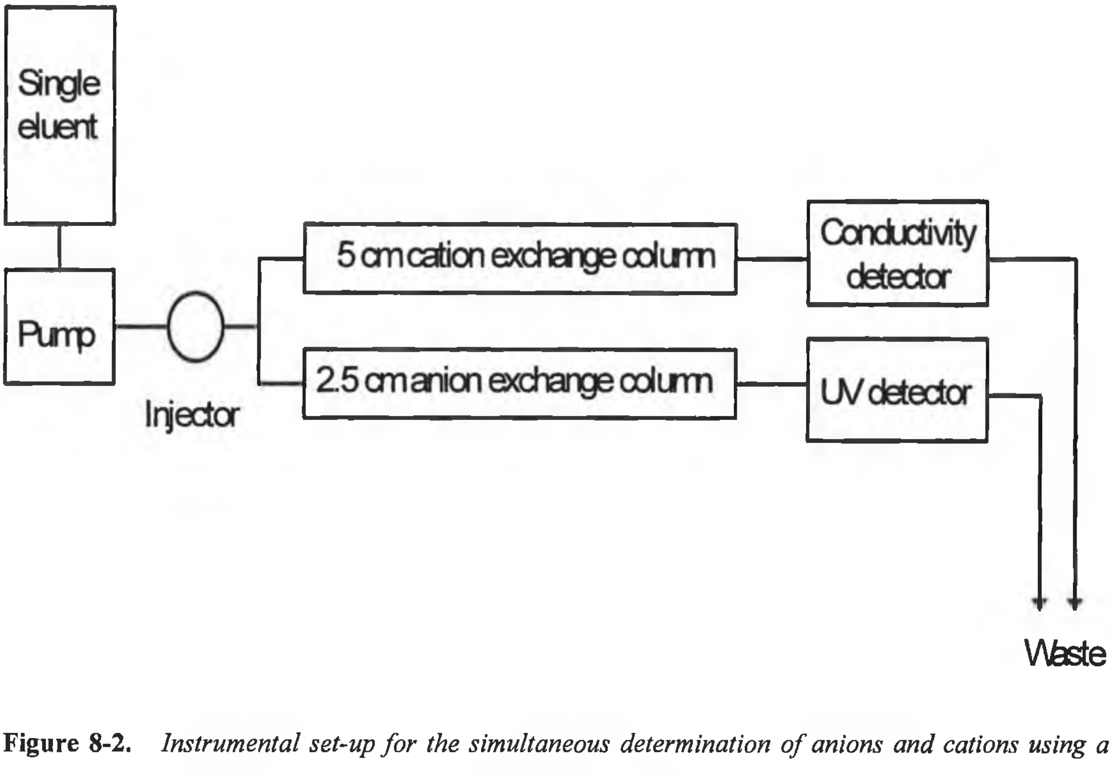

Figure 8-2. *Instrumental set-up for the simultaneous determination of anions and cations using a single eluent with two ion-exchange columns in parallel and two detectors.*

However, in this work, the mode of detection which was ultimately chosen was conductivity detection, both for anion and cation determinations. Again, using a single eluent, the flow of which was split by a tee-piece placed immediately after the injector, both columns were placed in parallel, and then routed through a single conductivity detector (Figure 8-3). With this setup, a single chromatogram was collected. The advantage of using the chromatographic setup shown in Figure 8-3 relative to that in Figure 8-2 is one of cost, since only one detector is used. Method development is also simplified since only one chromatogram is acquired. This setup could also be easily reconfigured allowing for one of the columns to be removed, such that the rapid individual determination of either anions or cations was possible. With this in mind, method development was initiated in this "single column" configuration, before finally being set up as in Figure 8-3 to allow for the simultaneous analysis of anions and cations.

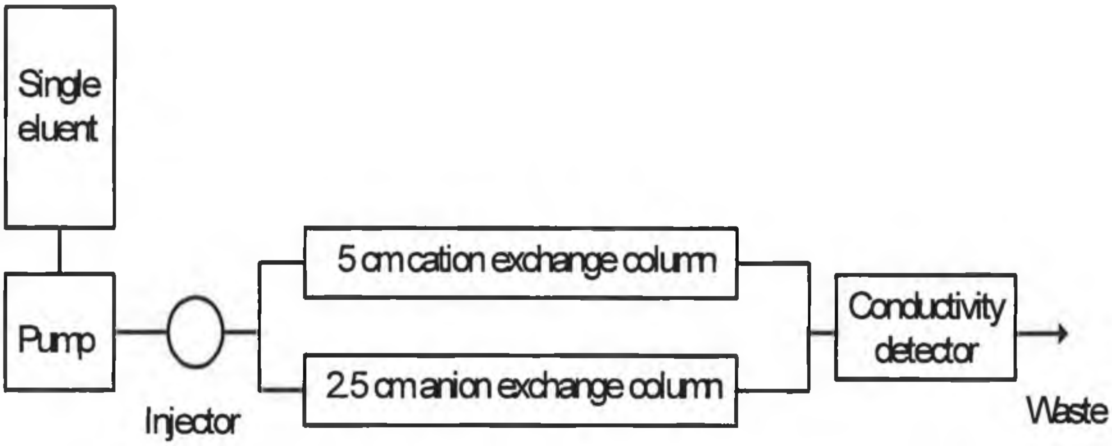

Figure 8-3. *Instrumental set-up for the simultaneous determination of anions and cations using a single eluent with two ion-exchange columns in parallel and a single detector.*

8.3.1.2 Optimisation of anion exchange column length.

An initial set of common anions were chosen for evaluation of the DDAB coated monolithic columns. These included chloride, phosphate, nitrate, nitrite, bromide, chlorate and sulphate. Mobile phases were initially prepared by titrating a solution of ethylenediamine with a solution of phthalic acid to pH 6.2, such that both ethylenediamine and phthalate ions were fully charged, yielding a mobile phase with a maximized eluting strength, as previously discussed. In this respect, an ethylenediamine solution was titrated with phthalic acid yielding a solution of 1.28

m*M* ethylenediamine / 1.23 m*M* phthalic acid, pH 6.2. Figure 8-4 illustrates a preliminary separation achieved on the permanently coated 5.0 cm anion exchange monolithic column.

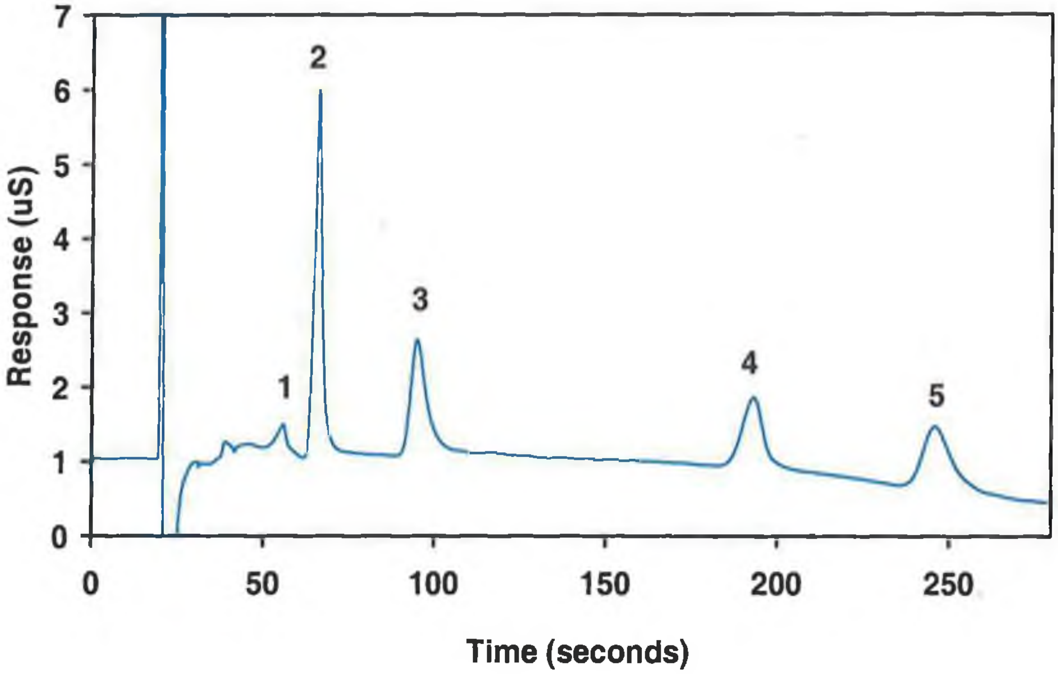

Figure 8-4. *Preliminary anion exchange separation on a 5.0 cm monolithic C_{18} column coated with DDAB. Chromatographic conditions: Column: Two Merck Chromolith Flash RP-18e 2.5 cm X 4.6 mm columns connected in series (permanently coated with DDAB), Mobile phase: 1.23 mM phthalic acid, 1.28 mM ethylenediamine, pH 6.2, Flow rate: 2.0 ml/min, Column temperature: 30 °C, Detection: direct conductivity, Loop volume: 25 μL, Peaks: [1] 10 mg/L phosphate, [2] 10 mg/L chloride, [3] 10 mg/L nitrite, [4] 10 mg/L nitrate, [5] 10 mg/L sulphate.*

As can be seen from Figure 8-4, the resolution of the five anions was very acceptable and peak shapes were good, with very little sign of tailing. However, the detector response for phosphate was rather poor. Since this 5.0 cm column was actually two 2.5 cm columns connected in series, the above separation was repeated on one of the 2.5 cm columns, to study the effect of halving the column length, upon the separation. A comparison of both column lengths in Table 8-2 shows that resolution is only adversely affected for phosphate and chloride when the column length is halved. Peak efficiency is actually improved for all peaks except nitrate using the shorter column. Separation of the five anions could be obtained in under 1.8 minutes on the 2.5 cm column compared to 4.1 minutes on the 5.0 cm column using a flow rate of only 2.0 ml/min in both instances. It should be noted that at this flow rate on the 2.5 cm monolith, the pressure drop across the column was only in the order of 160 – 180 p.s.i. In the case of anion analysis, method development was therefore continued using the 2.5 cm DDAB coated monolith.

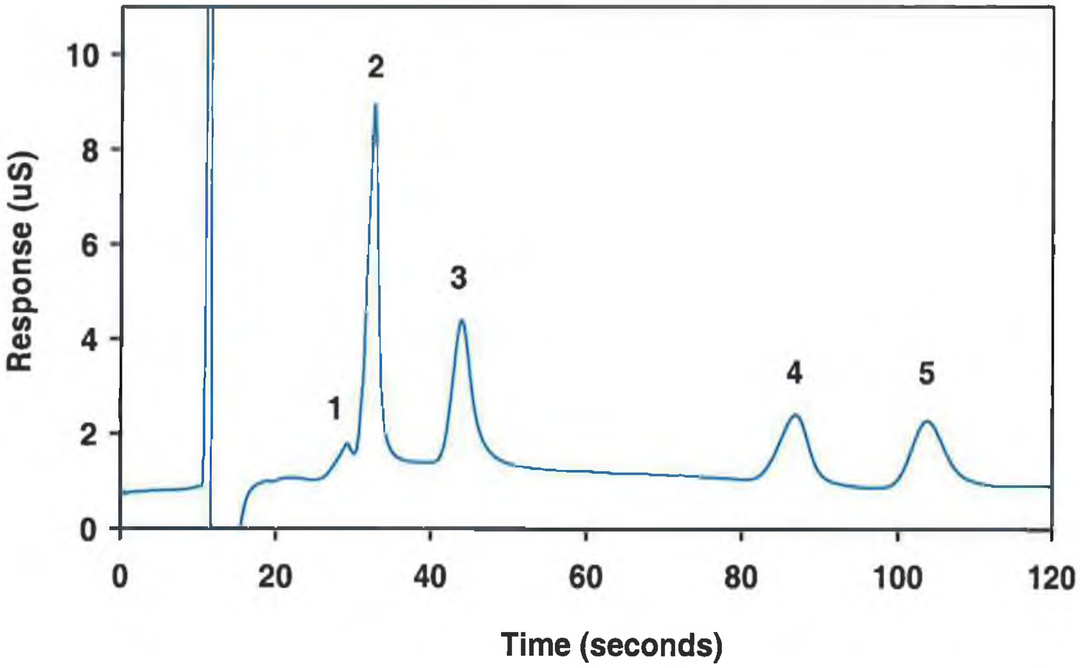

Figure 8-5. *Preliminary anion exchange separation on a 2.5 cm C_{18} monolithic column coated with DDAB. Conditions as in Figure 8-4 except for the column used. (Merck Chromolith Flash RP-18e 2.5 cm X 4.6 mm column, permanently coated with DDAB).*

Table 8-2. *Effect of monolithic column length upon the separation of anions.*

Anion	**Peak efficiency (N/m)**		**Resolution**	
	5.0 cm column	**2.5 cm column**	**5.0 cm column**	**2.5 cm column**
Phosphate	37,820	N/A	2.31	N/A
Chloride	91,800	93,720	4.79	3.18
Nitrite	43,580	65,160	10.36	7.52
Nitrate	100,980	96,000	4.07	2.25
Sulphate	85,280	108,320	N/A	N/A

8.3.1.3 Optimisation of cation exchange column length.

Cations chosen for study included calcium, magnesium, strontium, barium and copper. As described in Section 0, a 5.0 reversed phase silica monolith was coated with a solution of 1 m*M* DOSS in 10 % acetonitrile. In order to investigate the effect of column length upon cation determinations, a shorter 2.5 cm reversed phase silica monolith was also coated with the same solution, in the same manner. A mobile phase of 2.5 m*M* phthalate/1.47 m*M* ethylenediamine pH 4.5 was used to study the effect of halving the column length upon separations of selected cations. Figure 8-6 shows a preliminary separation of copper, magnesium, calcium, strontium and barium on both columns with this given mobile phase and demonstrates that resolution is markedly affected by the decrease in column length. Total runtime is reduced by a factor of ~ 2.5, but at the expense of resolution as illustrated in Table 8-2. Since the five cations were separated within a very narrow window on the shorter 2.5 cm DOSS coated column, it was decided to continue this work with the longer column which, it was anticipated would allow for greater flexibility with regard to mobile phase development and analysis of complex sample matrices, due to the much improved resolution.

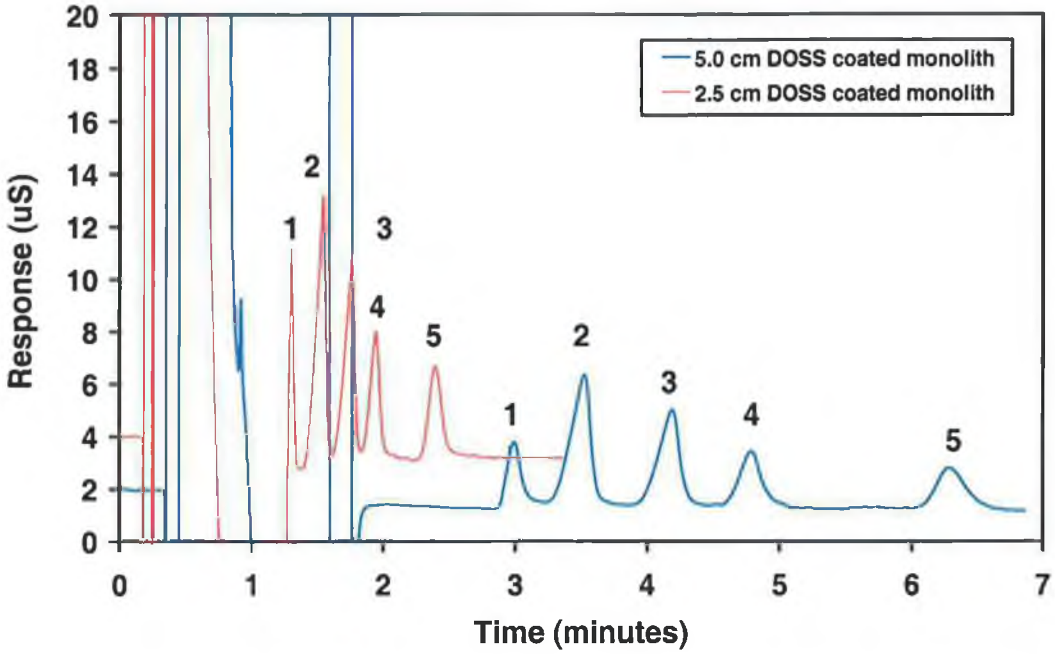

Figure 8-6. *Effect of column length upon the separation of copper, magnesium, calcium, strontium and barium by cation exchange chromatography on a monolithic column coated with DOSS. Chromatographic conditions: Columns: Merck Chromolith Flash RP-18e 2.5 cm X 4.6 mm column and Merck Chromolith SpeedROD RP-18e 5.0 cm X 4.6 mm column (both permanently coated with DOSS), Mobile phase: 2.5 mM phthalic acid, 1.47 mM ethylenediamine, pH 4.5. Flow rate: 2.0 ml/min, Column temperature: 30 °C, Detection: indirect conductivity, Injection volume: 25 µL, Peaks: [1] 25 mg/L copper, [2] 25 mg/L magnesium, [3] 50 mg/L calcium, [4] 50 mg/L strontium, [5] 100 mg/L barium.*

Table 8-3. *Effect of monolithic column length upon the separation of cations.*

Cation	**Resolution**	
	5.0 cm column	**2.5 cm column**
Copper	2.24	2.59
Magnesium	2.34	1.59
Calcium	2.07	1.36
Strontium	4.26	2.85
Barium	N/A	N/A

8.3.1.4 Optimisation of ion exchange capacity for the DOSS coated monolith.

As with anions, the rapid separation of common cations is also a challenge that has recently begun to receive attention [30]. With this in mind, a short 5.0 cm reversed-phase silica monolith was coated with a double chained anionic surfactant (DOSS) to produce a strongly acidic monolithic cation exchanger. The choice of DOSS to modify the surface of the reversed-phase monolith was made due to the structural similarity between DOSS and DDAB, but with DOSS containing a strongly acidic sulfonic acid group in place of the quaternary ammonium group (both double chained surfactants, DDAB = 2 x C_{12}, DOSS = 2 x C_8). DDAB has been shown to result in very stable coatings under aqueous conditions and DOSS was found here to be equally stable.

Despite the advantage of a stable column coating due to the very hydrophobic nature of the surfactant, a disadvantage of using a double-chained surfactant as a coating agent is that the exchange capacity of the coated column is often quite high. This results in long run times for strongly retained ions, such that rapid chromatography is difficult to achieve without the use of high eluent concentrations, which in turn leads to sensitivity difficulties when indirect detection is used.

One way to modify the ion exchange capacity of a permanently coated reversed-phase column is by changing the concentration of organic solvent present in the coating solution. Higher levels of organic solvent will shift the equilibrium away from the C_{18} stationary phase, resulting in less surfactant being coated onto the column, and thereby decreasing the final cation exchange capacity of the column. This approach has been used by Hatsis and Lucy [31] with a monolithic column, albeit with reference to the reduction of anion exchange capacity using DDAB, but the principle should also apply in the case of reducing cation exchange capacity using DOSS. Hatsis and Lucy used 0 % to 30 % acetonitrile concentrations in a 1 m*M* DDAB coating solution, and showed that column capacity could be controlled in a linear fashion over this range with a correlation coefficient of > 0.999. The exchange capacity could be reduced by a factor of up to 3.5 using 30 % acetonitrile in the coating solution compared with a fully aqueous coating solution.

A similar approach was investigated by coating the 5.0 cm monolith with a solution of 1 m*M* DOSS in 100 % H_20, and comparing the resulting retention with the retention achieved on the same 5.0 cm monolith coated with 1 m*M* DOSS in 10 % acetonitrile. Figure 8-7 illustrates that 10 % acetonitrile in the coating solution (relative to the coating solution comprising 100 % water) caused the retention times of magnesium and calcium to be decreased (by 0.5 minutes in the case of calcium) with a given mobile phase. This indicates that the exchange capacity of the column had indeed been decreased. Resolution of magnesium and calcium was reduced from 2.11 to 1.93. There was no appreciable difference in peak efficiency noted. Further decreases in the cation exchange capacity of the DOSS coated column were not attempted, since the resolution of magnesium and calcium would likely be compromised at earlier retention times. Also, at this stage in these studies, it was anticipated that simultaneous separations of anions and cations would necessitate that magnesium and calcium retention be longer relative to anion retention, to avoid overlap of anions and cations (see Section 8.3.4.1) Therefore, further method development for all cation determinations was performed on a 5.0 cm reversed-phase silica monolith coated with 1 m*M* DOSS in 10 % acetonitrile.

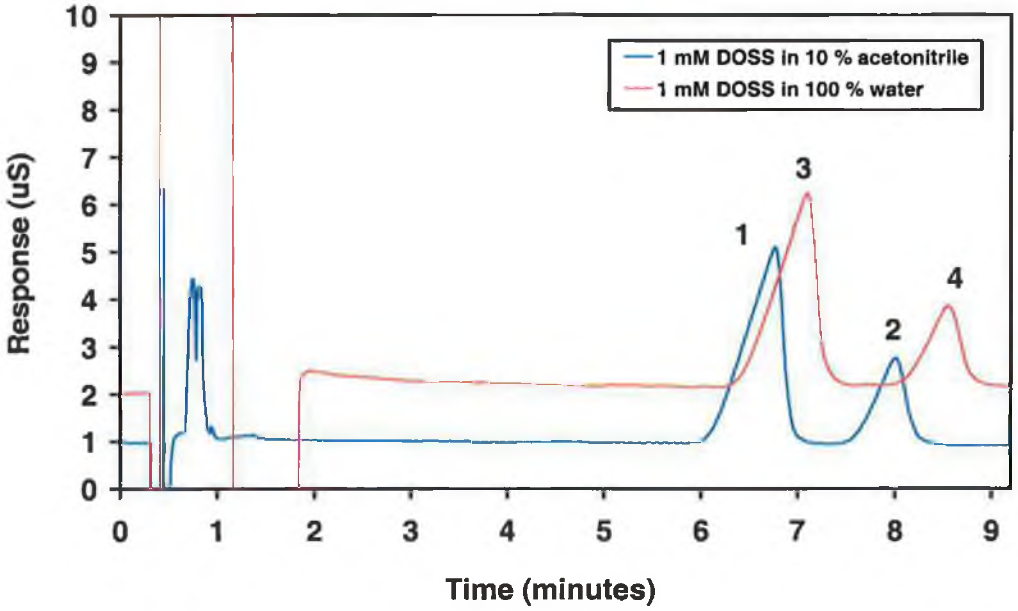

Figure 8-7. *Effect of DOSS coating solution composition upon the cation exchange capacity of a 5 cm* C_{18} *monolithic column. Chromatographic conditions as in Figure 8-6 except: Column: Merck Chromolith SpeedROD RP-18e 5.0 cm X 4.6 mm column (both permanently coated with DOSS), Mobile phase: 1.25 mM phthalate and 0.735 mM EDA, pH 4.5, Peaks: [1] 50 mg/L magnesium: 6.79 min, [2] 50 mg/L calcium: 8.01 min, [3] 50 mg/L magnesium: 7.12 min, [4] 50 mg/L calcium: 8.60 min.*

8.3.1.5 Effect of mobile phase concentration upon retention of anions and cations.

Mobile phases were prepared by titrating a phthalic acid stock solution with an ethylenediamine stock solution to the desired pH. The advantage of this method was that no other buffering species needed to be added to the mobile phase for pH adjustment, eliminating any possible interferants with the indirect modes of detection used. However, this made the independent adjustment of the concentration of one of the components relative to the other impossible, without a resultant change in mobile phase pH. For example, increasing the concentration of phthalic acid (to reduce anion retention) while holding the concentration of ethylenediamine constant, would result in a corresponding decrease in mobile phase pH. As will be discussed in Section 8.3.1.7 the mobile phase pH had a considerable effect upon the separation selectivity of both columns. Therefore, in order to adjust the concentration of mobile phases, the concentration of both components were adjusted to the same extent, such that the concentration ratio, and therefore the mobile phase pH remained constant.

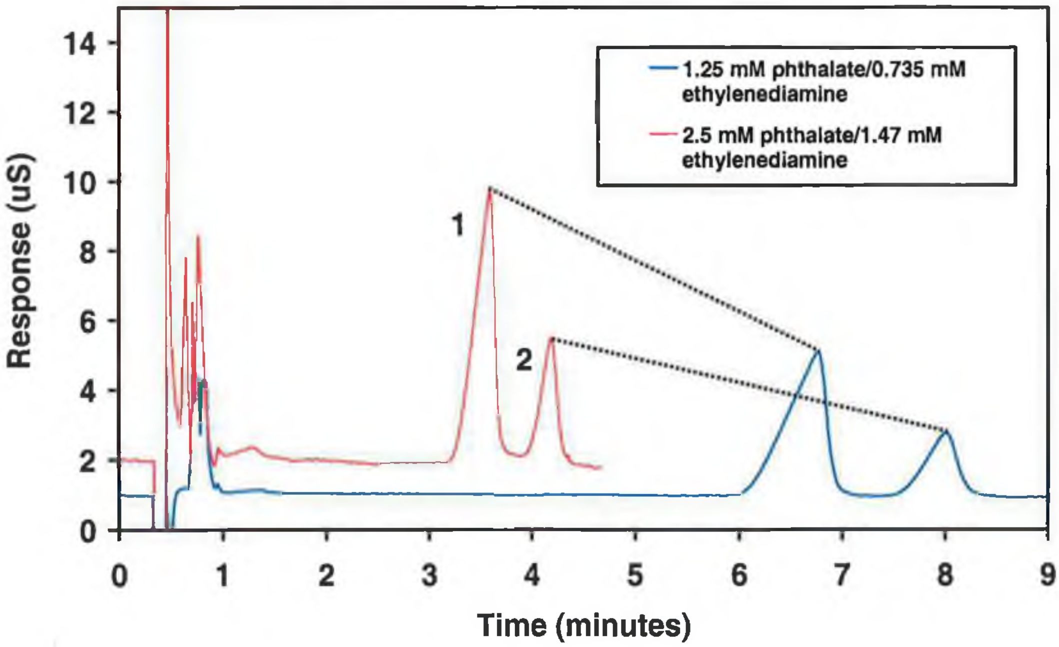

Figure 8-8. *Effect of mobile phase concentration upon the retention of cations on a DOSS coated monolithic column. Chromatographic conditions as in Figure 8-7 except: Mobile phase (1): 1.25 mM phthalate/0.735 mM EDA, pH 4.5, Mobile phase (2): 2.5 mM phthalate/1.47 mM EDA, pH 4.5, Peaks: [1] 50 mg/L magnesium, [2] 50 mg/L calcium.*

Figure 8-8 illustrates the effect of doubling the mobile phase ionic strength upon resolution of cations. Both mobile phases had a pH of 4.5, since the concentration ratio of both components remained constant. With the mobile phase concentration doubled, (to 2.5 m*M* phthalate/1.47 m*M* ethylenediamine, pH 4.5) retention of magnesium was reduced by over three minutes, with retention of calcium reduced by over 3.5 minutes. Both peaks were still well resolved, with a noticeable improvement in peak efficiency (Table 8-4).

Table 8-4. *Effect of mobile phase concentration on peak efficiency and resolution for a separation of cations on a DOSS coated monolithic column.*

Cation	**Peak efficiency (N/m)**		**Resolution**	
	Mobile phase (1)	**Mobile phase (2)**	**Mobile phase (1)**	**Mobile phase (2)**
Magnesium	27,840	30,660	1.93	1.84
Calcium	64,520	69,600	N/A	N/A

Given that a single eluent was to be used for both cation and anion analysis, the mobile phase concentration was not increased further, since this would have had an adverse effect upon the separation of anions on the DDAB coated monolith, particularly upon the resolution of early eluting peaks. Figure 8-9 and Table 8-4 both show the effect of doubling the mobile phase concentration upon resolution of anions. The retention times of all anions were reduced, with considerable reductions in retention time noted for the later eluting peaks, nitrate and sulphate. The retention time of phosphate was reduced by 0.11 minutes, chloride by 0.17 minutes, nitrite by 0.21 minutes, nitrate by 0.42 minutes and sulphate by 2.32 minutes. Peak efficiency was improved for all anions except nitrite. Peak shape and peak efficiency was much improved for sulphate. However, the resolution between chloride and nitrite was reduced at the higher mobile phase concentration (2.5 m*M* phthalate/1.47 m*M* ethylenediamine, pH 4.5). As will be discussed in Section 8.3.1.6, the anion exchange capacity of the DDAB coated monolith was subsequently adjusted to improve the overall resolution of the anion test mix (particularly that of chloride and nitrite) and so for the remainder of this work, the optimum mobile phase concentration of 2.5 m*M* phthalate/1.47 m*M* ethylenediamine, pH 4.5 was used.

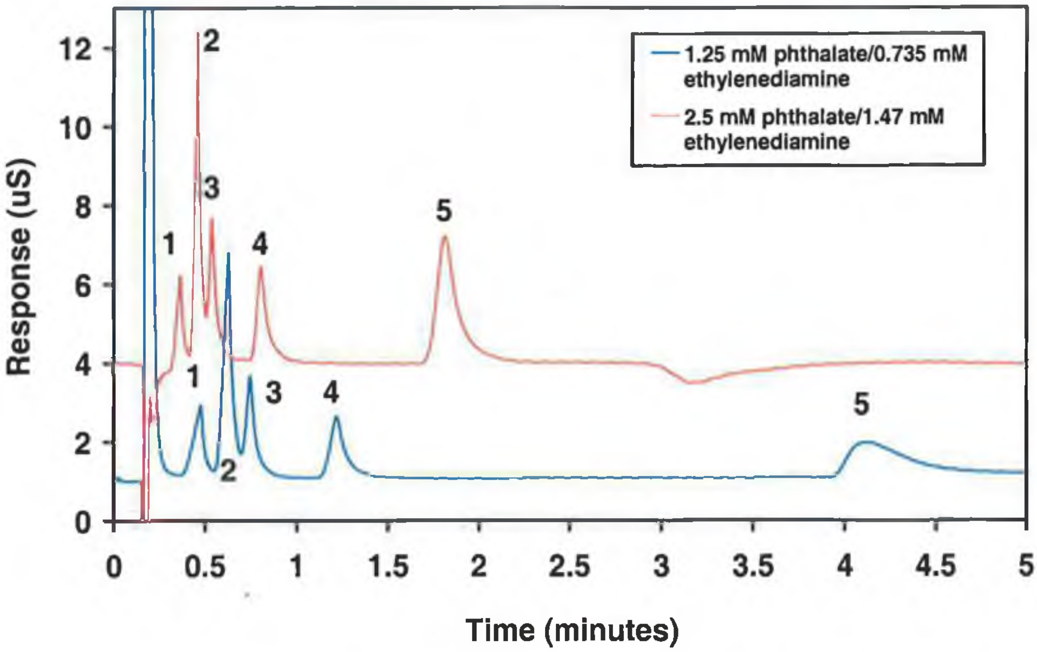

Figure 8-9. *Effect of mobile phase concentration upon the retention of anions on a DDAB coated monolithic column. Chromatographic conditions: Column: Merck Chromolith Flash RP-18e 2.5 cm X 4.6 mm column (permanently coated with DDAB), Mobile phase (1): 1.25 mM phthalate/0.735 mM EDA, pH 4.5, Mobile phase (2): 2.5 mM phthalate/1.47 mM EDA, pH 4.5, Flow rate: 2.0 ml/min, Column temperature: 30 °C, Detection: direct conductivity, Loop volume: 25 µL, Peaks: [1] 10 mg/L phosphate, [2] 10 mg/L chloride, [3] 10 mg/L nitrite, [4] 10 mg/L nitrate, [5] 10 mg/L sulphate.*

Table 8-5. *Effect of mobile phase concentration on peak efficiency and resolution for a separation of anions on a DDAB coated monolithic column.*

Anion	**Peak efficiency (N/m)**		**Resolution**	
	Mobile phase (1)	**Mobile phase (2)**	**Mobile phase (1)**	**Mobile phase (2)**
Phosphate	15,400	21,960	1.74	1.72
Chloride	40,400	53,080	1.48	1.35
Nitrite	54,600	49,720	4.50	3.73
Nitrate	57,120	58,440	8.34	7.55
Sulphate	33,520	62,840	N/A	N/A

8.3.1.6 Optimisation of ion exchange capacity for the DDAB coated monolith.

The reduction in resolution between chloride and nitrite shown in Figure 8-9 is the limiting factor in the overall evaluation of the separation resulting from the higher ionic strength eluent. The column used in Figure 8-9 was a 2.5 cm reversed-phase silica monolith, coated with 1 m*M* DDAB in 5 % acetonitrile. As discussed previously, coating the column in 5 % acetonitrile limits the eventual anion exchange capacity of the coated column. By re-coating the column using 1 m*M* DDAB in 100 % H_2O, the anion exchange capacity of the coated column would be increased. An increased number of anion exchange sites on the modified stationary phase would result in improved resolution between the chloride/nitrite pair. This was investigated by washing the column (initially coated in 1 m*M* DDAB in 5 % acetonitrile) with 100 % acetonitrile, and re-coating it with 1 m*M* DDAB in 100 % water. In Figure 8-10 and Table 8-6 the separation of the anion test mix on the re-coated column is compared to the separation achieved on the initial column of lower exchange capacity, using the mobile phase of 2.5 m*M* phthalate/1.47 m*M* ethylenediamine, pH 4.5. As expected, the retention time of all anions is increased on the column with the higher exchange capacity. However, the increase in retention times does not greatly increase the overall runtime, with the biggest increase in retention being for sulphate. Peak efficiency was improved for all anions as was resolution of chloride and nitrite with the increase in anion exchange capacity. Therefore, for the remainder of this study, the 2.5 cm reversed-phase monolith coated with 1 m*M* DDAB in 100 % water was used for all anion determinations.

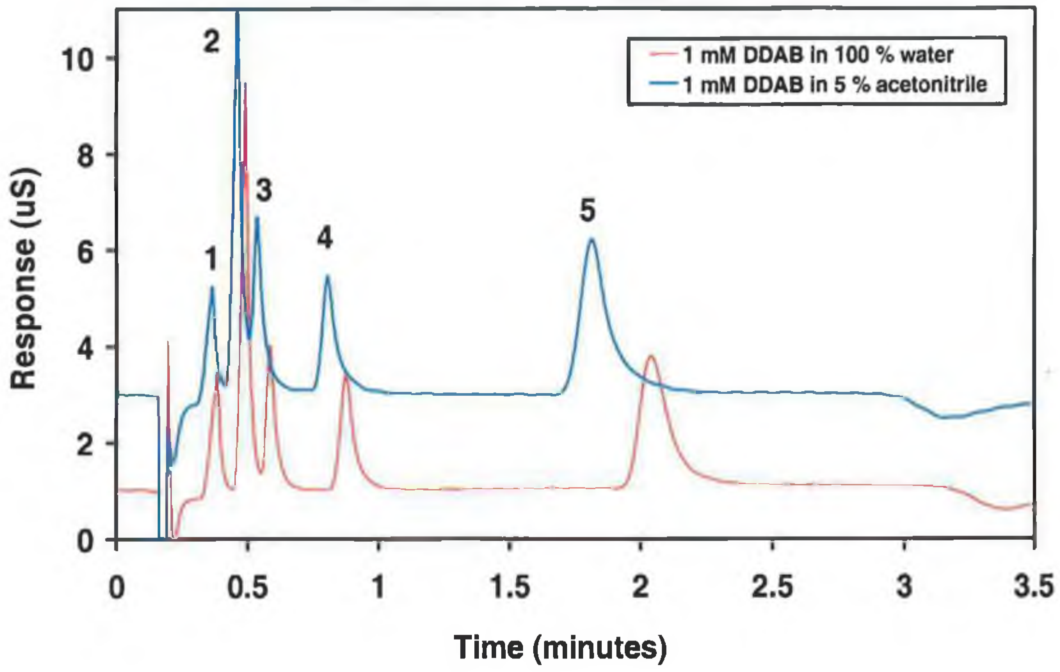

Figure 8-10. *Effect of increased anion exchange capacity upon the separation of selected anions on a DDAB coated monolithic column. Chromatographic conditions as in Figure 8-9 except: Mobile phase: 2.5 mM phthalate/1.47 mM EDA, pH 4.5, Peaks: [1] 10 mg/L phosphate, [2] 10 mg/L chloride, [3] 10 mg/L nitrite, [4] 10 mg/L nitrate, [5] 10 mg/L sulphate.*

Table 8-6. *Effect of anion exchange capacity upon peak efficiency, retention time and resolution for the separation of selected anions on a DDAB coated monolithic column.*

Anion	Retention time (minutes)		Peak efficiency (N/m)		Resolution	
	Initial coating	**Second coating**	**Initial coating**	**Second coating**	**Initial coating**	**Second coating**
Phosphate	0.365	0.383	21,960	26,080	1.72	1.99
Chloride	0.462	0.492	53,080	63,680	1.35	1.73
Nitrite	0.537	0.585	49,720	62,520	3.73	4.03
Nitrate	0.807	0.875	58,440	67,880	7.55	8.31
Sulphate	1.813	2.040	62,840	69,120	N/A	N/A

8.3.1.7 Effect of pH on retention of anions and cations.

To optimise the separation conditions, a number of mobile phases were subsequently prepared across a range of pH values (pH 3.5 to 7.5) by varying the concentration ratios of phthalic acid and ethylenediamine. Of the anions typically expected to be present at detectable levels in freshwater samples, (phosphate, chloride, nitrate and nitrite), there was no appreciable effect upon retention. However, the retention time of sulphate increased markedly when using eluents below pH 5.0.

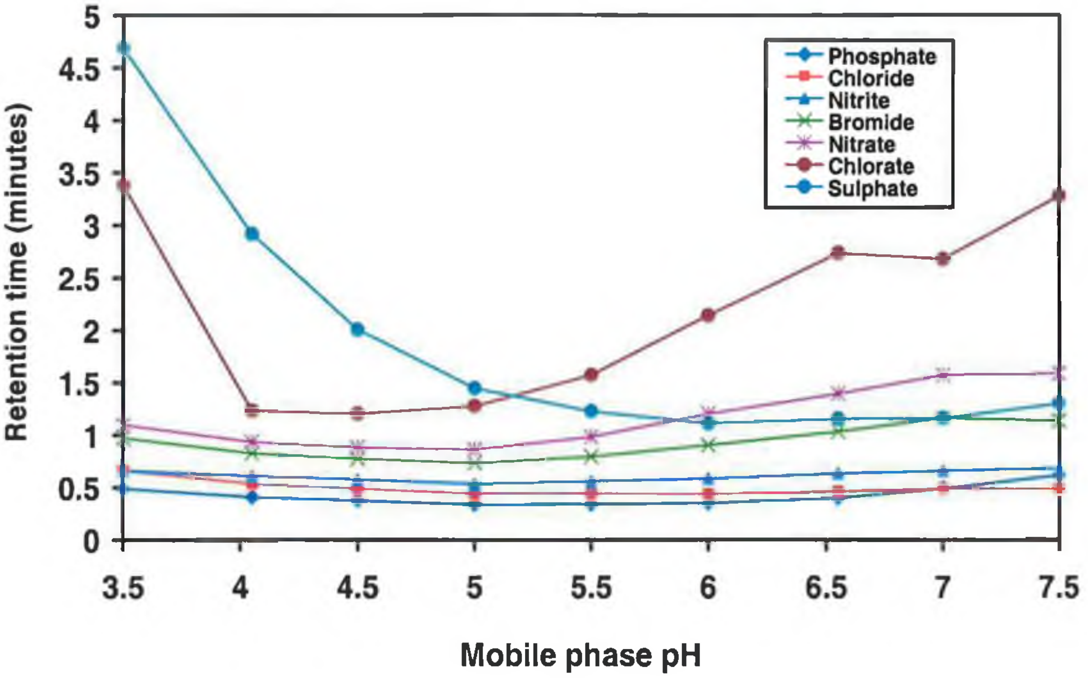

Figure 8-11. *Effect of pH on the retention of anions on a DDAB coated monolithic column. Chromatographic conditions as in Figure 8-10 except: Column: Merck Chromolith Flash RP-18e 2.5 cm X 4.6 mm column (permanently coated with DDAB). Analyte concentrations: 10 mg/L.*

Figure 8-11 shows these trends. Optimal resolution of the test mixture of anions used whilst keeping total runtimes below 2 minutes, was found to lie between pH 5.0 and 5.5. However, since this mobile phase was also being optimised with respect to cation determinations on the cation exchange column, this pH study was repeated for the alkaline earth metal cations on the DOSS coated column. As shown in Figure 8-12, above pH 7.0, there is a rapid increase in retention of target cations due to the deprotonation of the base. Also shown is the effect of pH upon the retention of copper, the peak for which is no longer visible above pH 4.5. This is presumably due to strong complexation with phthalate above this pH, forming a monovalent $CuHL^+$ complex with phthalate as HL^-, causing copper to elute within the void due to the reduced charge of the complex [32]. The stability constant of the phthalate complex of copper relative to calcium is $K_1 = 3.14$ for copper and $K_1 = 2.41$ for calcium [32]. Therefore, the single eluent chosen as optimum for both the individual analysis of anions and cations was 2.54 m*M* phthalate/1.47 m*M* ethylenediamine, pH 4.5.

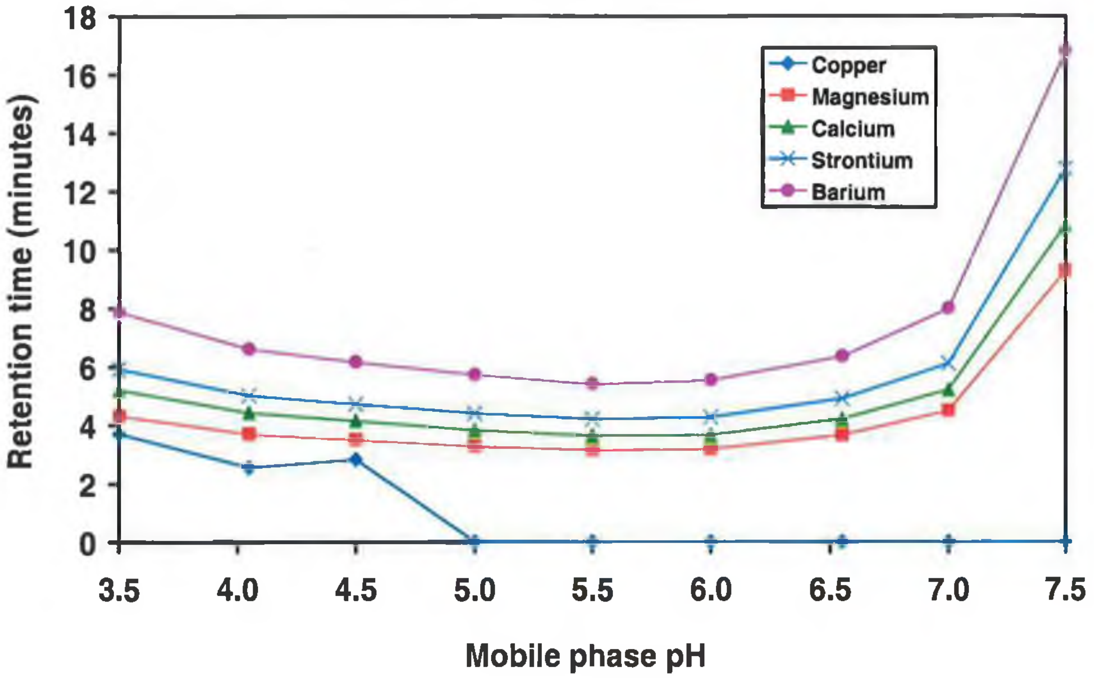

Figure 8-12. *Effect of pH on the retention of cations on a DOSS coated monolithic column. Chromatographic conditions: Column: Merck Chromolith SpeedROD RP-18e 50 mm X 4.6 mm (permanently coated with DOSS). Flow rate: 2.0 ml/min, Column temperature: 30 °C, Detection: indirect conductivity, Loop volume: 25 μL, Analyte concentrations: 25 mg/L copper, 25 mg/L magnesium, 50 mg/L calcium, 50 mg/L strontium and 100 mg/L barium.*

8.3.2 Cation analysis on DOSS coated monolith.

8.3.2.1 Selectivity studies on DOSS coated cation exchange column.

As mentioned above, in this study ethylenediamine was chosen as a suitable eluent cation as it can be used for the indirect conductimetric detection of metal ions. Using the optimized 2.5 m*M* phthalate/1.47 m*M* ethylenediamine (pH 4.5) eluent, the retention of a number of metal ions on the 5.0 cm DOSS coated cation exchange monolithic column was investigated. The metal ions investigated included some transition metals and heavy metals as shown in Table 8-7. The selectivity of the DOSS column for alkaline earth metals was very good, however, using the ethylenediamine/phthalate containing eluent all alkali metal ions eluted within the sample void and could not be detected. However, as mentioned, the short cation exchange monolithic column did exhibit good selectivity for the alkaline earth metal ions, with the baseline separation of magnesium, calcium, strontium and barium (along with transition metal copper) possible without adjustment to the above eluent conditions.

Table 8-7. *Selectivity of the DOSS coated monolithic column for cations.*

Cation	Retention time (minutes)	Relative retention time[1] (relative to Mg)
Lithium	< 0.5	N/A
Sodium	< 0.5	N/A
Potassium	<0.5	N/A
Cesium	<0.5	N/A
Beryllium	1.93	0.54
Cadmium	3.12	0.87
Copper	3.27	0.91
Zinc	3.34	0.93
Nickel	3.43	0.96
Magnesium	3.58	1.00
Cobalt	3.60	1.01
Manganese	3.64	1.02
Calcium	4.17	1.16
Strontium	4.88	1.36
Aluminium	5.89	1.65
Barium	6.30	1.76

Chromatographic conditions: Column: Merck Chromolith SpeedROD RP-18e 5.0 cm X 4.6 mm column (permanently coated with DOSS), Mobile phase: 2.5 mM phthalate/1.47 mM EDA, pH 4.5, Flow rate: 2.0 ml/min, Column temperature: 30 °C, Detection: indirect conductivity, Loop volume: 25 µL.

8.3.2.2 Optimisation of flow rate for cation determinations.

During mobile phase development, flow rates of 2.0 ml/min were routinely used. Elevated flow rates were then investigated to see the effect upon resolution of the test mix of cations (copper, magnesium, calcium, strontium and barium). It was found that separation of all five metals could still be maintained at a flow rate of 8.0

---

[1] Relative retention times are calculated for the purposes of optimising flow ratios in Section 8.3.4.1.

ml/min. This gave retention times ranging from only 45 to 100 seconds, with a column backpressure of 1030 p.s.i. The separation obtained is shown as Figure 8-13. Overlaid with the standard shown in Figure 8-13 is a tap water sample showing the presence of magnesium and calcium. The efficiencies of the peaks shown in the cation standard, calculated as N/m were found to be 37,000 for copper, 43,000 for magnesium, 51,000 for calcium, 58,000 for strontium and 53,000 for barium.

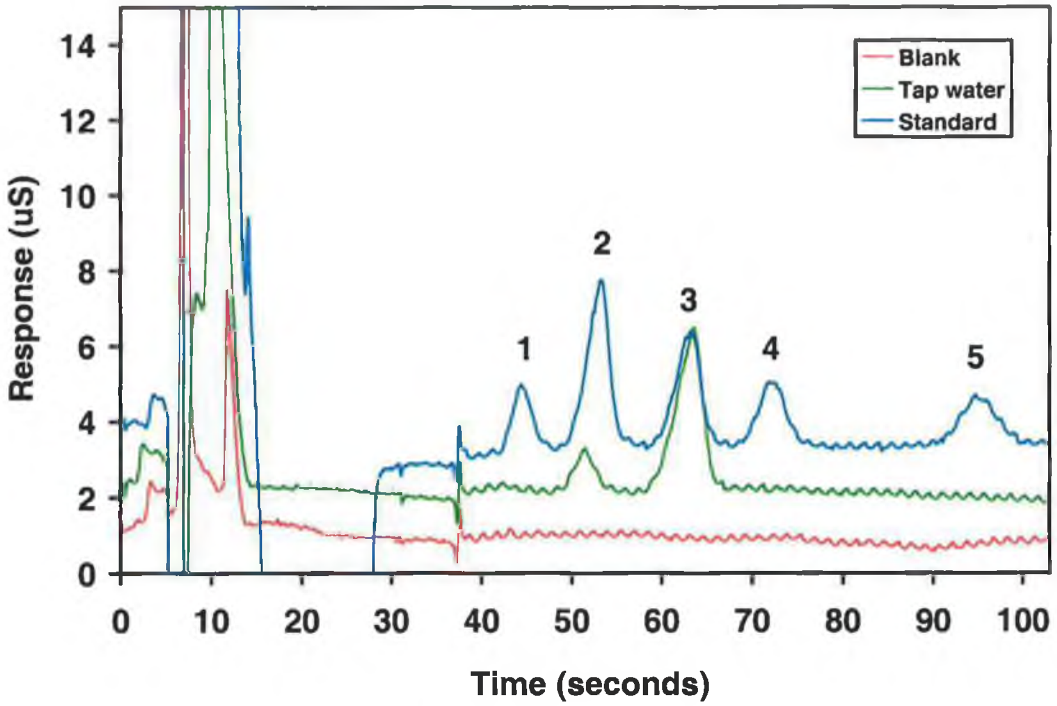

Figure 8-13. *Rapid separation of five cations on a DOSS coated monolithic column at 8.0 ml/min. Chromatographic conditions as in Figure 8-12 except: Mobile phase: 2.5 mM phthalate/1.47 mM EDA, pH 4.5, Flow rate: 8.0 ml/min. Analyte concentrations as in Figure 8-12.*

From Figure 8-13, it is clear that the very fast flow rate of 8.0 ml/min, has resulted in considerable baseline noise, likely to be due to the HPLC pump used. A lower flow rate of 5.0 ml/min was ultimately used for analysis of real samples, since sensitivity was improved at this slower flow rate, (as shown in Figure 8-14) and it was anticipated that the later eluting strontium and barium would not be present at detectable levels in real samples. At a flow rate of 5.0 ml/min, the column backpressure was reduced to 650 p.s.i. In addition, peak efficiency was improved for all five cations at the lower flow rate (Table 8-8).

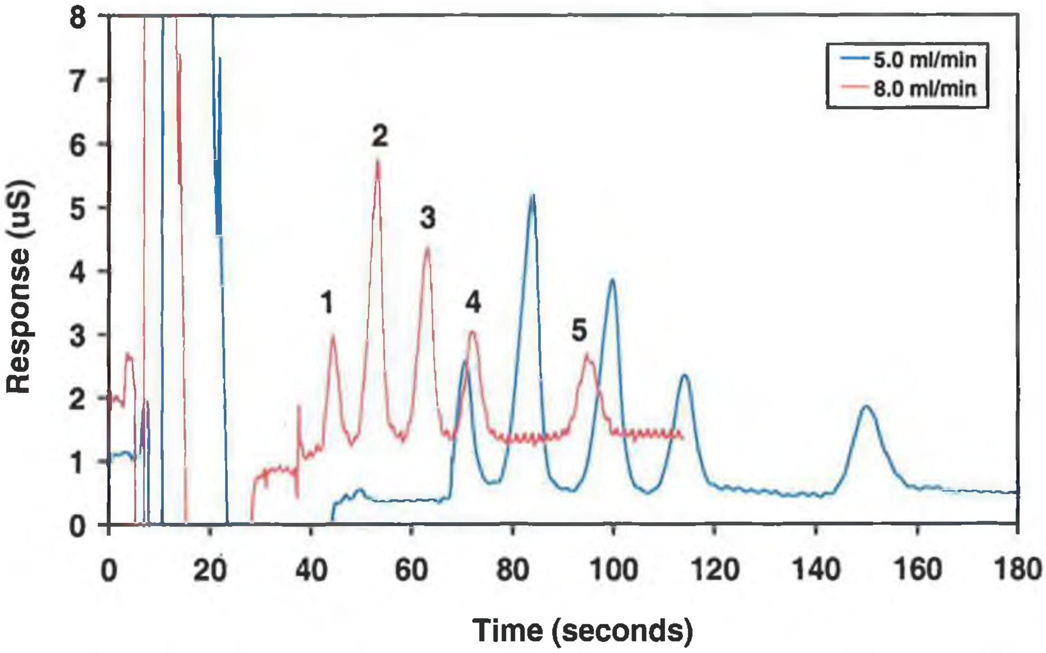

Figure 8-14. *Effect of two different flow rates (5.0 ml/min and 8.0 ml/min) upon the separation time for selected cations on a DOSS coated monolithic column. Chromatographic conditions as in Figure 8-13 except: Flow rates: 5.0 ml/min and 8.0 ml/min. Analyte concentrations as in Figure 8-12.*

Table 8-8. *Effect of flow rate upon peak efficiency and resolution for a separation of cations on a DOSS coated monolithic column.*

Cation	Peak efficiency (N/m)		Resolution	
	8 ml/min	5 ml/min	8 ml/min	5 ml/min
Copper	36,680	45,860	2.04	2.05
Magnesium	43,180	47,900	2.07	2.22
Calcium	50,860	57,900	1.67	1.86
Strontium	57,940	67,000	3.62	3.88
Barium	53,240	62,900	N/A	N/A

8.3.2.3 Optimisation of sample injection volume for cation determinations.

Using the optimum flow rate for determination of cations in real samples (5.0 ml/min), injections of 25 mg/*L* copper, magnesium and calcium, were made with loop volumes of 25 μL and 50 μL. The peaks were still well resolved with a sample loop volume of 50 μL, which was chosen as optimum for further analysis of real samples. Sample loop volumes greater than 50 μL were not considered, because of the complex nature of the sample matrices.

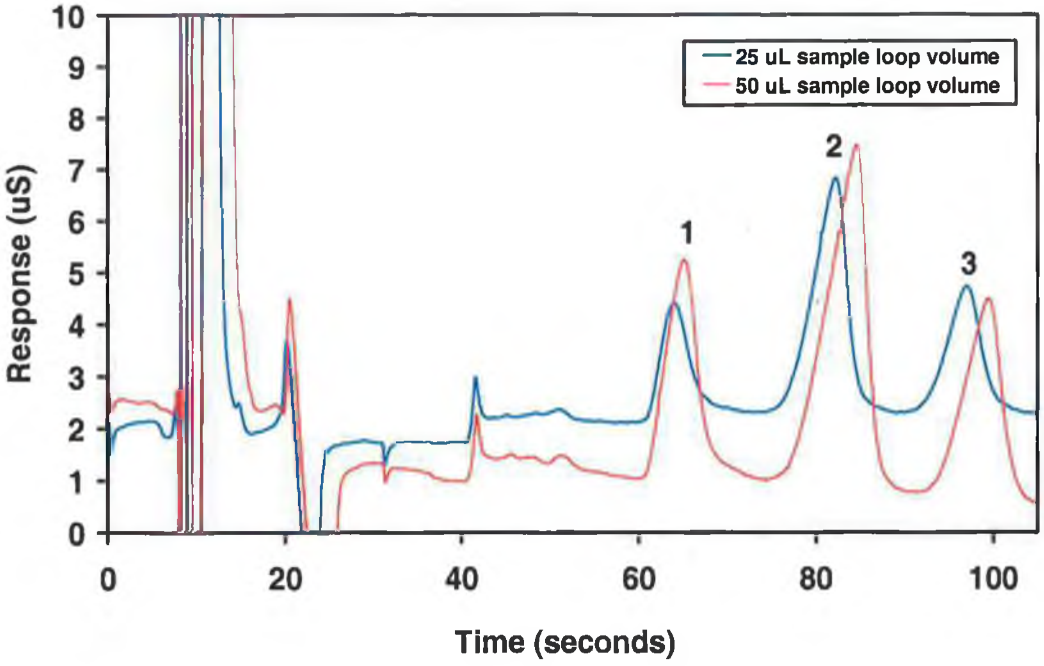

Figure 8-15. *Effect of increased injection volume upon the separation of selected cations on a DOSS coated monolithic column. Chromatographic conditions: Column: Merck Chromolith SpeedROD RP-18e 50 mm X 4.6 mm (permanently coated with DOSS), Mobile phase: 2.5 mM phthalate/1.47 mM EDA, pH 4.5, Flow rate: 5.0 ml/min, Column temperature: 30 °C, Detection: indirect conductivity, Loop volumes: 25 μL and 50 μL, Peaks: [1] 25 mg/L copper, [2] 25 mg/L magnesium, [3] 25 mg/L calcium.*

8.3.2.4 Sensitivity studies for the cation separation.

Sensitivity was determined for copper, magnesium and calcium, using the optimized flow rate of 5.0 ml/min. Sensitivity was not determined for strontium or barium at this optimized flow rate, since as discussed previously, it was anticipated that strontium and barium would be unlikely to be present at detectable levels in real samples. For the purposes of limit of detection (L.O.D.) determinations, the signal to noise ratio was defined as two. Signal to noise ratios close to the L.O.D. for copper, magnesium and calcium, are shown in Table 8-9 and depicted in Figure 8-16 and Figure 8-17.

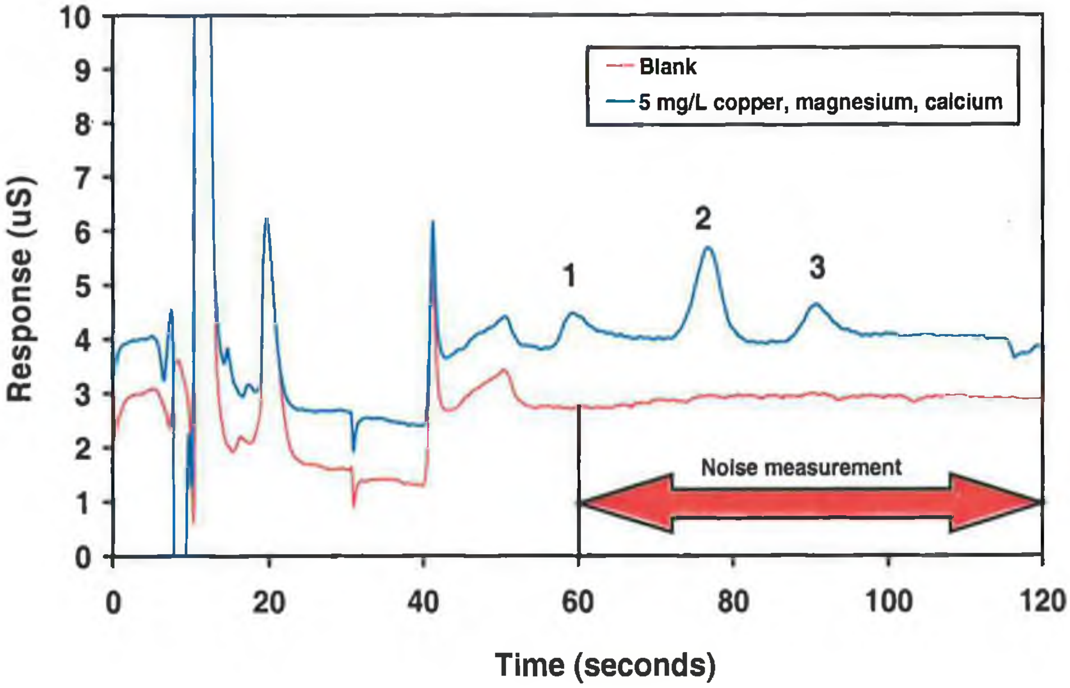

Figure 8-16. *Sensitivity study for copper and calcium separated on a DOSS coated monolithic column at 5.0 ml/min. Chromatographic conditions as in Figure 8-15 except sample loop volume: 50 µL, Peaks: [1] 5 mg/L copper, [2] 5 mg/L magnesium, [3] 5 mg/L calcium.*

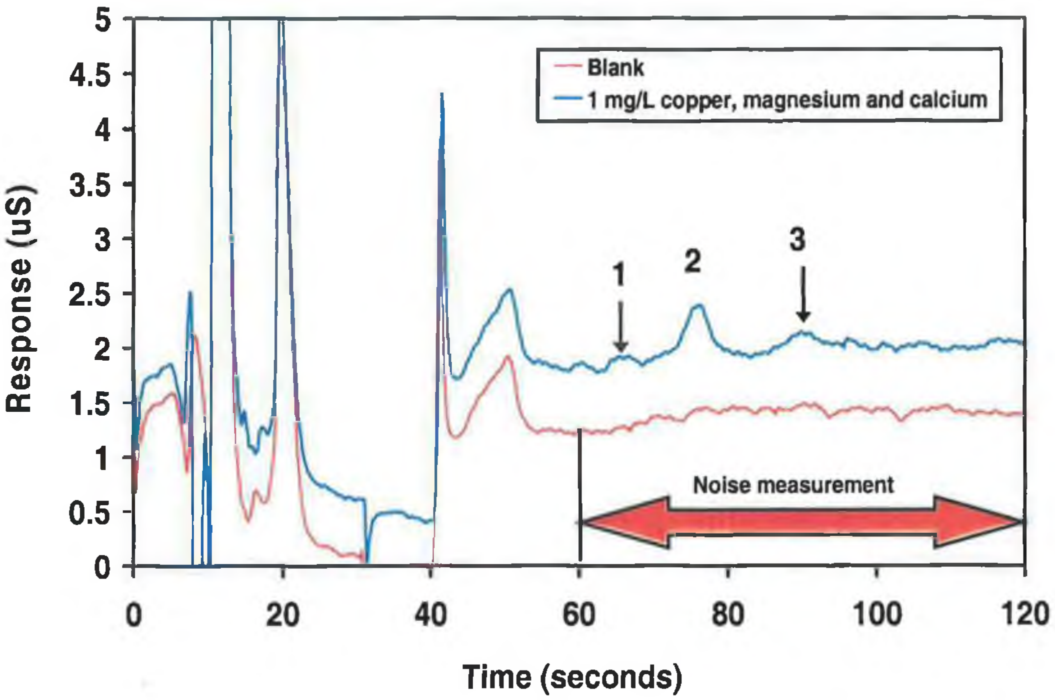

Figure 8-17. *Sensitivity study for the determination of magnesium on a DOSS coated monolithic column at 5.0 ml/min. Chromatographic conditions as in Figure 8-16. Peaks: [1] 1 mg/L copper, [2] 1 mg/L magnesium, [3] 1 mg/L calcium.*

For all signal to noise determinations, the noise was measured in a blank (deionised water injection), over a time window approximating the retention time window of copper, magnesium and calcium. Noise was measured over three injections of blank and averaged. Peak heights of copper, magnesium and calcium were also determined in triplicate and averaged. Figure 8-18 illustrates the advantage of using the slower flow rate of 5.0 ml/min (relative to 8.0 ml/min) for cation determinations, in terms of achieving improved sensitivity. As discussed previously, copper, magnesium, calcium, strontium and barium could be separated in less than 110 seconds at an elevated flow rate of 8.0 ml/min. Figure 8-18 demonstrates that at a lower flow rate of 5.0 ml/min, baseline noise due to the pump is significantly reduced. At 8.0 ml/min baseline noise measured over 60 seconds was 0.5451, whereas at 5.0 ml/min it was reduced by over 2.5 times to 0.2014. To further illustrate the improved sensitivity at 5.0 ml/min, magnesium and calcium L.O.D's were determined at 8.0 ml/min also such that a direct comparison could be made using Table 8-9. An increase in baseline noise at high flow rates (> 3.0 ml/min) was also noted by Gerber *et al.* [33]. The analysis of mixtures of pharmaceuticals on monolithic columns was found to be more sensitive at high flow rates depending upon the type of detector used. Gerber *et al.* used Agilent HP1100 systems throughout their studies and investigated the effects of the type of pump and detector used. They found that the increase in baseline noise was independent of the type of pump that they used (Agilent HP1100 binary and quaternary pumps), but found a marked difference between HP1100 variable wavelength detectors from HP1100 diode array detectors. A significant difference in the flow cell geometries was noticed between the two detector types when the detector characteristics were compared, and it was theorised that an inadequate geometry of the cell could generate an interruption of the laminar flow and cause turbulence due to mechanical agitation, thereby increasing baseline noise at higher flow rates.

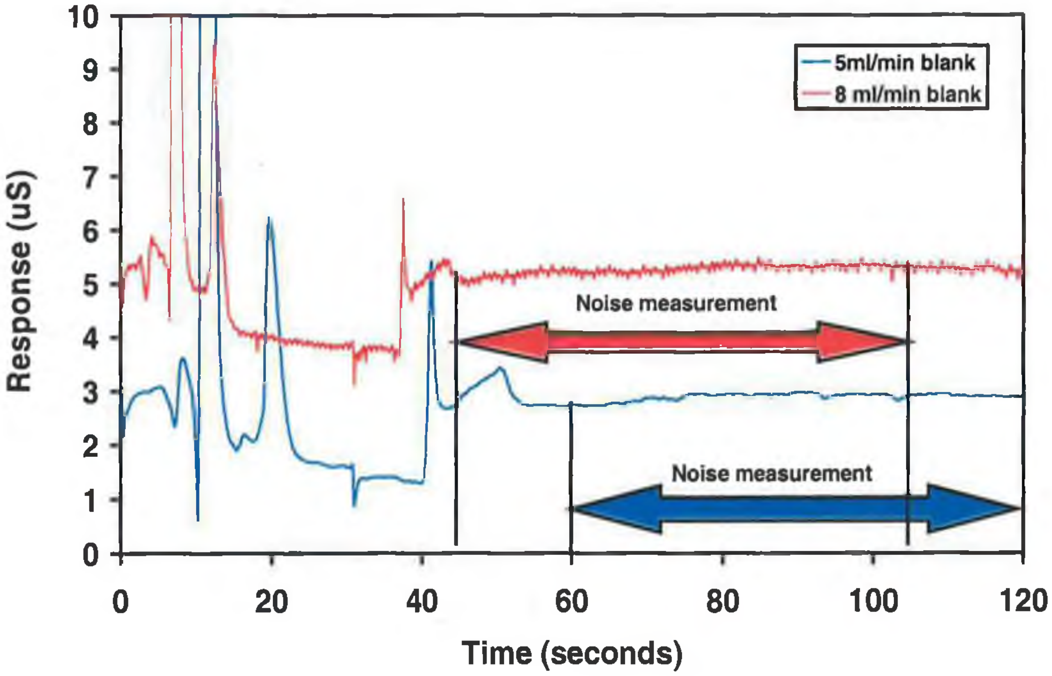

Figure 8-18. *Comparison of baseline noise at the optimum flow rate of 5.0 ml/min Vs an elevated flow rate of 8.0 ml/min on a DOSS coated monolithic column. Chromatographic conditions as in Figure 8-16 except flow rates: 5.0 ml/min and 8.0 ml/min.*

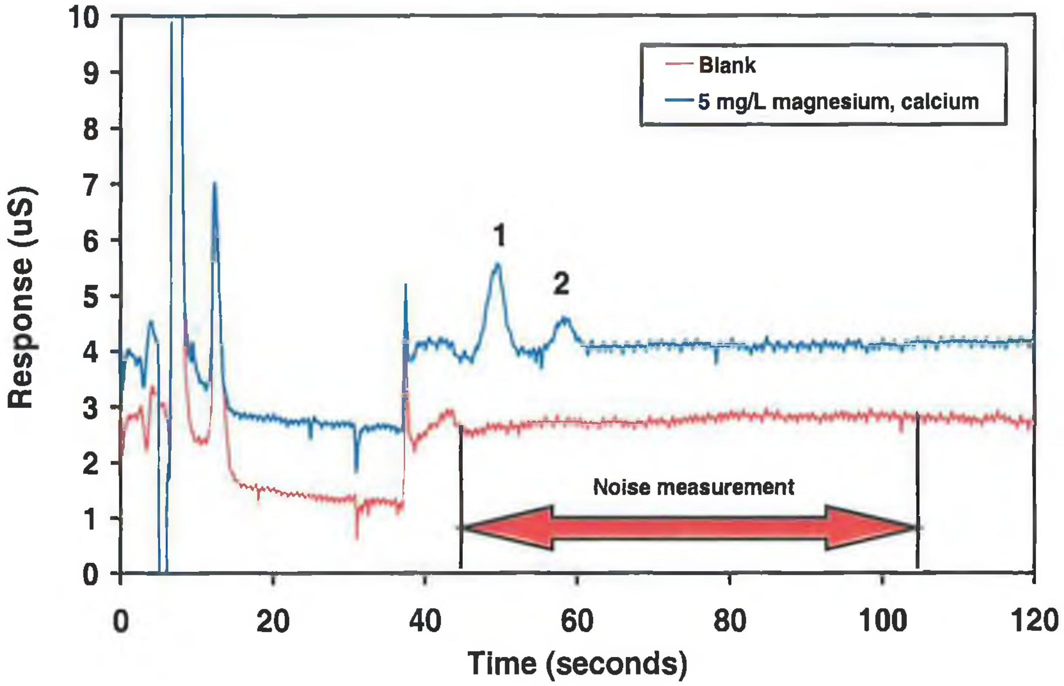

Figure 8-19. *Sensitivity study for the determination of magnesium on a DOSS coated monolithic column at an elevated flow rate of 8.0 ml/min. Chromatographic conditions as in Figure 8-16 except flow rate: 8.0 ml/min, Peaks: [1] 5 mg/L magnesium, [2] 5 mg/L calcium.*

Table 8-9. *Comparison of sensitivity for the analysis of cations on a DOSS coated monolithic column at 5.0 ml/min and 8.0 ml/min.*

Cations at 5 ml/min flow rate with 50 μL injection volume.		
Cation	**S/N ratio**[2]	**S/N ratio (5 mg/*L*)**
Copper		2.7, (5 mg/*L*)
Magnesium	2.1, (1 mg/*L*)	8.5, (5 mg/*L*)
Calcium		3.1, (5 mg/*L*)
Cations at 8 ml/min flow rate with 50 μL injection volume		
Cation	**S/N ratio**[3]	**S/N ratio**
Magnesium	3.1, (5 mg/*L*)	
Calcium	1.2, (5 mg/*L*)	2.1, (10 mg/*L*)

---

[2] For noise determinations, values in the blank were measured in triplicate over the time range 60 seconds to 120 seconds.

[3] For noise determinations, values in the blank were measured in triplicate over the time range 45 seconds to 105 seconds.

8.3.2.5 Linearity studies for the cation separation.

The linearity of copper, magnesium and calcium was determined at the optimum flow rate of 5.0 ml/min. Peak areas were found to be linear over the range 5 mg/*L* to 100 mg/*L* for copper and calcium, and 1 mg/*L* to 100 mg/*L* for magnesium. Each standard was injected in duplicate. Correlation coefficients were > 0.99 for each cation.

A comparative linearity study was also performed at 8.0 ml/min, with magnesium found to be linear from 5 mg/*L* to 100 mg/*L* (5, 10, 50 and 100 mg/*L* standards injected) and calcium found to be linear from 10 mg/L to 100 mg/*L* (10, 50 100 mg/*L* standards injected). For this linearity study at 8.0 ml/min, all standards were injected in triplicate.

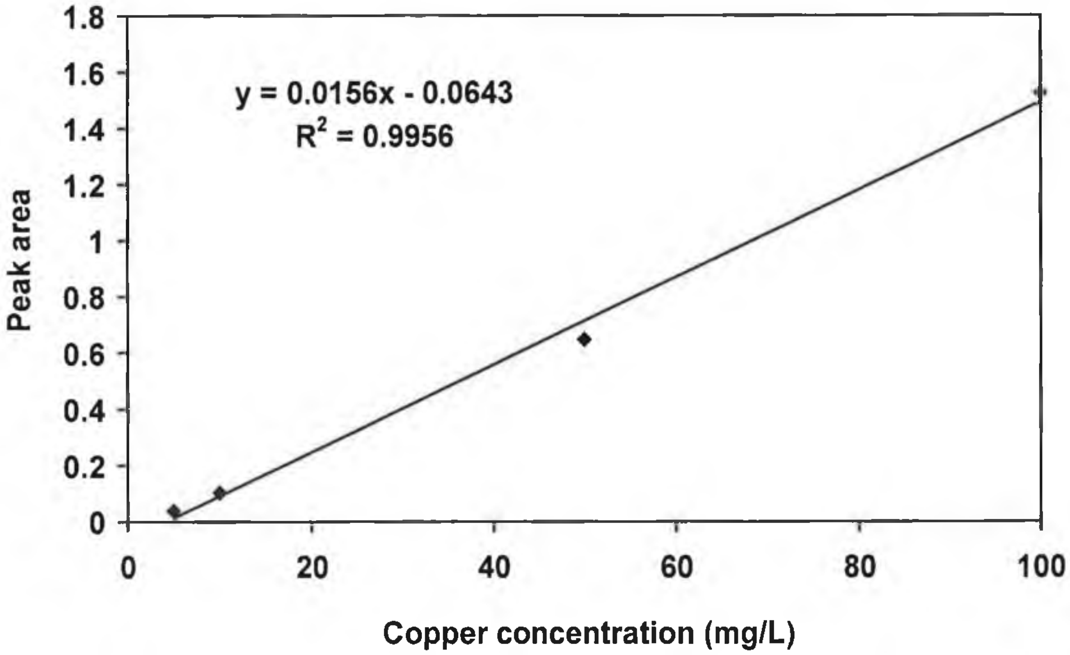

Figure 8-20. *Linearity study for copper on a DOSS coated monolithic column at 5.0 ml/min. Chromatographic conditions as in Figure 8-16.*

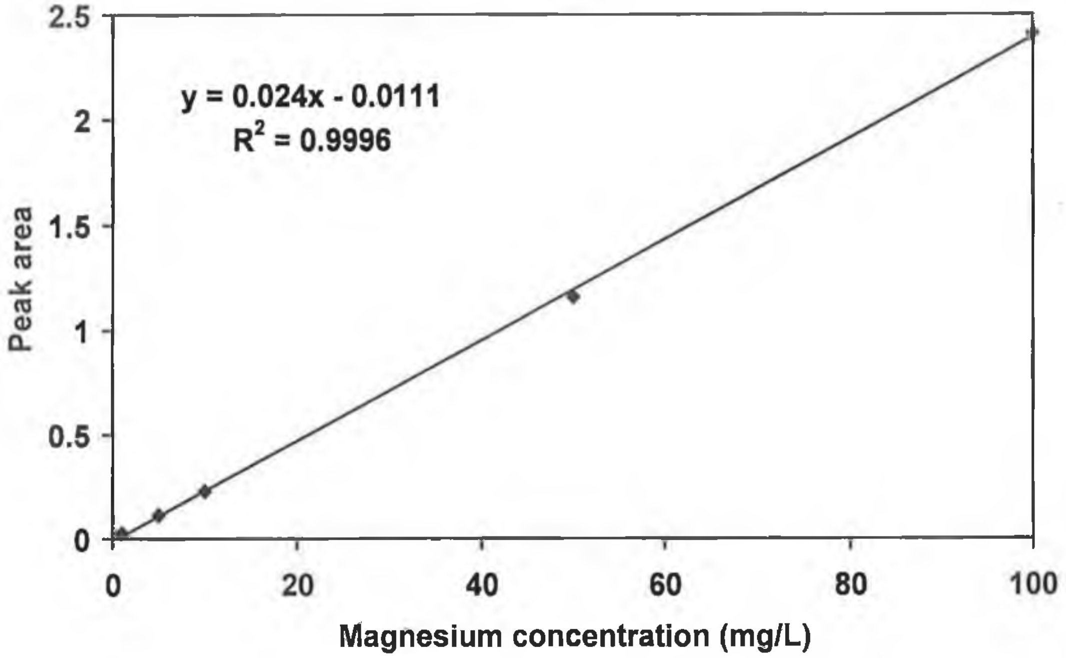

Figure 8-21. *Linearity study for magnesium on a DOSS coated monolithic column at 5.0 ml/min. Chromatographic conditions as in Figure 8-16.*

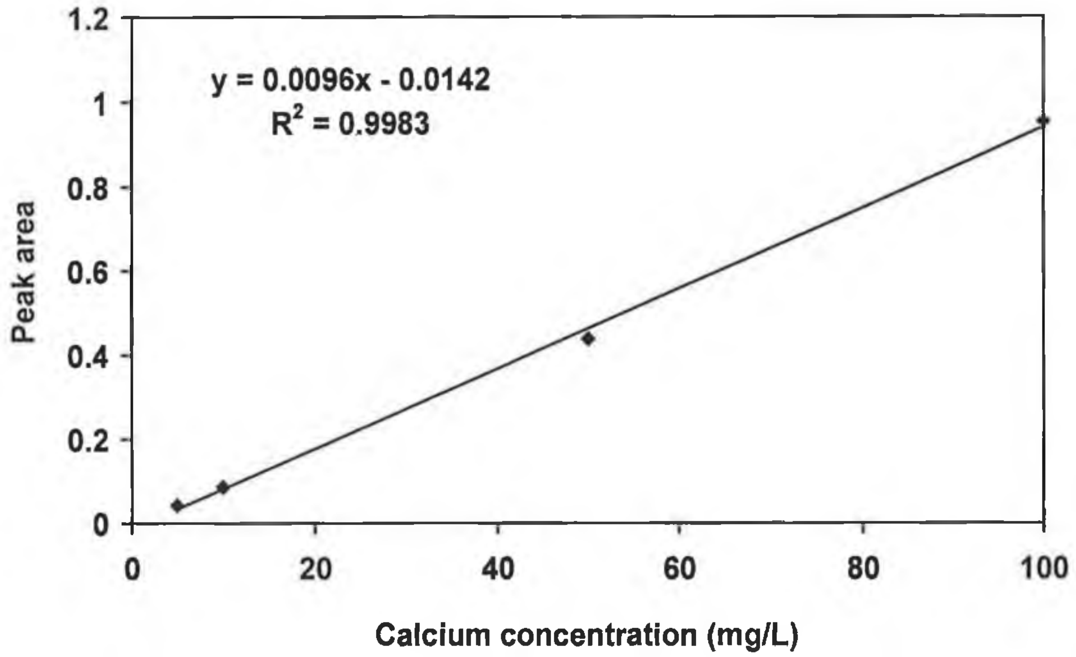

Figure 8-22. *Linearity study for calcium on a DOSS coated monolithic column at 5.0 ml/min. Chromatographic conditions as in Figure 8-16.*

8.3.2.6 System precision studies for the cation separation.

Six consecutive injections of a 10 mg/*L* copper, magnesium and calcium standard were made at the optimum flow rate of 5.0 ml/min. Peak area precision and retention time precision were calculated and are tabulated in Table 8-10. A comparative study was made at 8.0 ml/min, with results also shown in Table 8-10. It is interesting to note that the peak area, and retention time precision results at 8.0 ml/min are similar to that at 5.0 ml/min, particularly with no appreciable loss in retention time precision at 8.0 ml/min despite the higher flow rate.

Table 8-10. *System precision studies for the analysis of selected cations on a DOSS coated monolithic column at 5.0 ml/min and 8.0 ml/min.*

Cations at 5 ml/min flow rate with 50 µL injection volume		
Cation	**Peak area precision % RSD, n=6 (10 mg/L standard)**	**Retention time precision % RSD, n=6 (10 mg/L standard)**
Copper	4.33 %, (10 mg/*L*)	0.21 %, (10 mg/*L*)
Magnesium	2.49 %, (10 mg/*L*)	0.26 %, (10 mg/*L*)
Calcium	3.76 %, (10 mg/*L*)	0.26 %, (10 mg/*L*)
Cations at 8 ml/min flow rate with 50 µL injection volume		
Cation	**Peak area precision % RSD, n=6**	**Retention time precision % RSD, n=6**
Magnesium	1.79 %, (10 mg/*L*)	0.56 %, (10 mg/*L*)
Calcium	3.10 %, (10 mg/*L*)	0.66 %, (10 mg/*L*)

8.3.2.7 Determination of cations in real samples.

As described in Section 8.2.2, samples were collected and treated only to filtration using a 0.45 µm filter. The sea water sample was diluted by a factor of twenty with deionised water, due to the complex nature of the sample matrix. All samples were qualitatively and rapidly screened for copper, magnesium and calcium using the optimized conditions described above, resulting in total runtimes of 120 seconds.

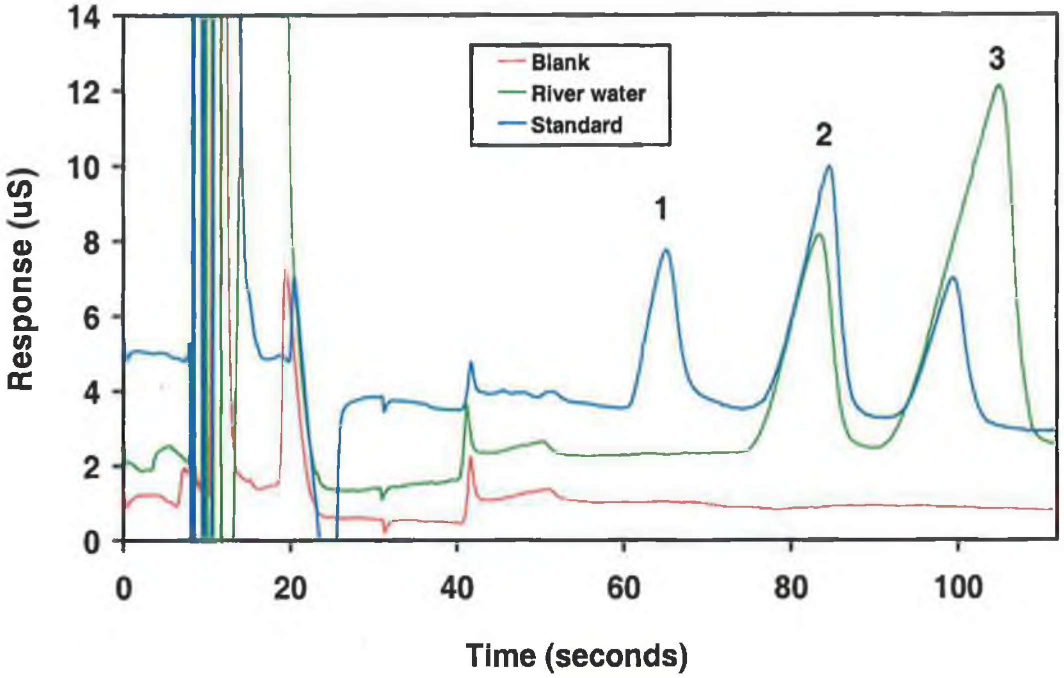

Figure 8-23. *Rapid analysis of river water for cations on a DOSS coated monolithic column at 5.0 ml/min. Chromatographic conditions as in Figure 8-16. Peaks: [1] 25 mg/L copper, [2] 25 mg/L magnesium, [3] 25 mg/L calcium.*

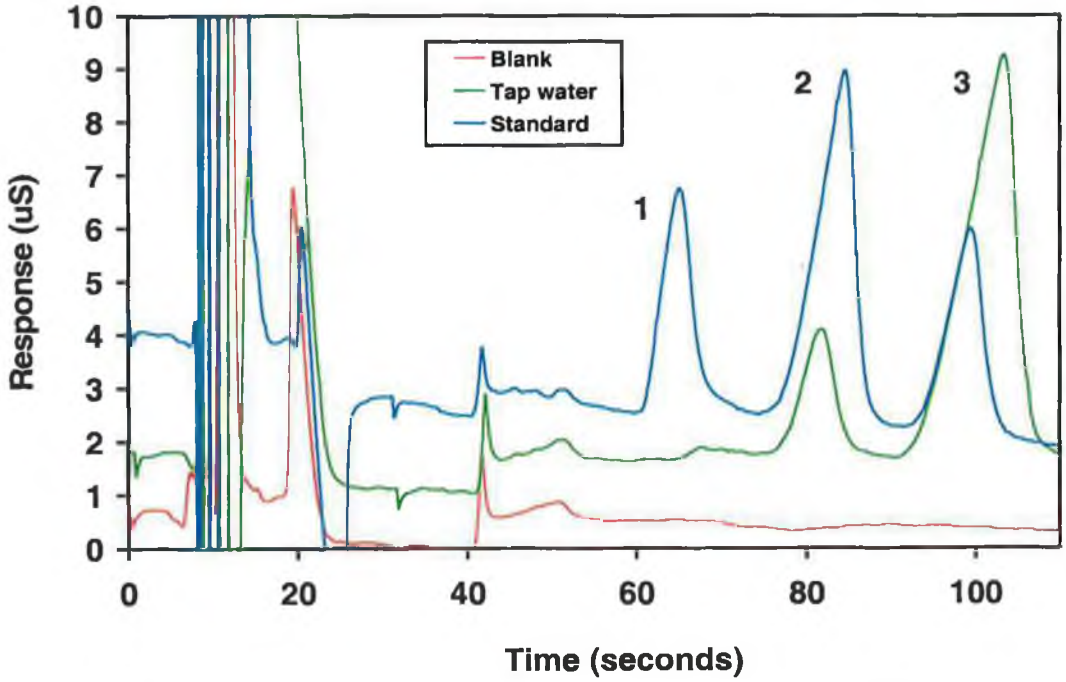

Figure 8-24. *Rapid analysis of drinking water for cations on a DOSS coated monolithic column at 5.0 ml/min. Chromatographic conditions as in Figure 8-16. Peaks: [1] 25 mg/L copper, [2] 25 mg/L magnesium, [3] 25 mg/L calcium.*

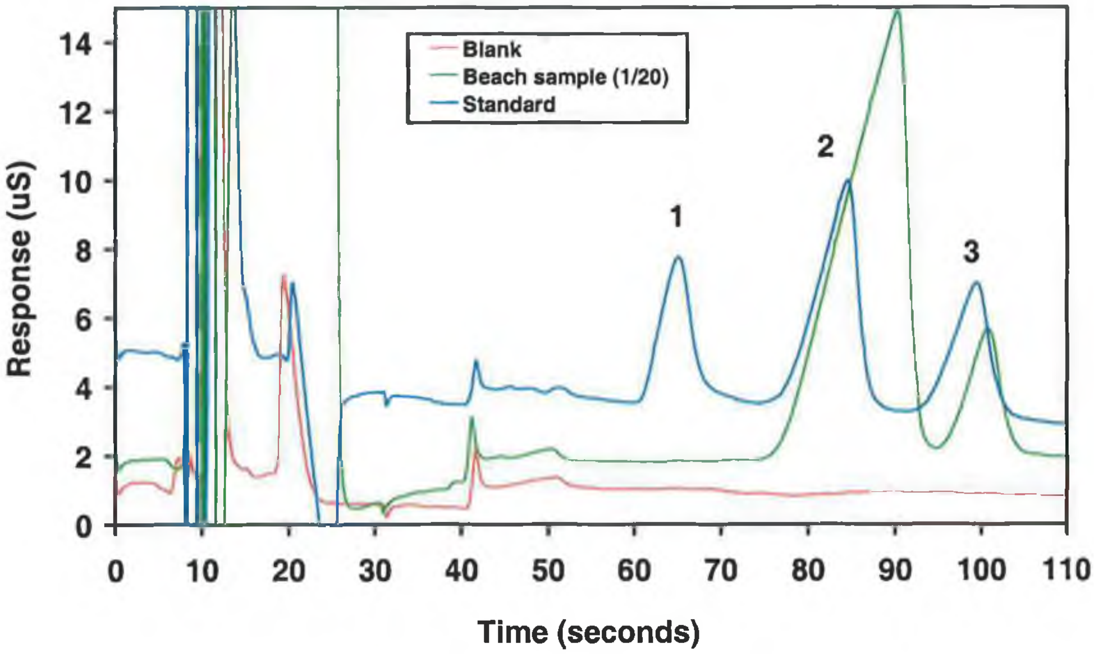

Figure 8-25. *Rapid analysis of seawater for cations on a DOSS coated monolithic column at 5.0 ml/min. Chromatographic conditions as in Figure 8-16. Peaks: [1] 25 mg/L copper, [2] 25 mg/L magnesium, [3] 25 mg/L calcium.*

8.3.3 Anion analysis on DDAB coated monolith.

8.3.3.1 Effect of column temperature on anion retention.

Using the optimized mobile phase of 2.54 m*M* phthalic acid, 1.47 m*M* ethylenediamine, pH 4.5 the anion mix was separated on the anion exchange column at 30 °C and 45 °C. There was no appreciable improvement in the separation at higher temperatures, (although the retention of sulphate increased slightly (~ 5 %) at the higher temperature) and so for further work the column oven temperature was maintained at 30 °C, to protect to the column from unexpected temperature fluctuations.

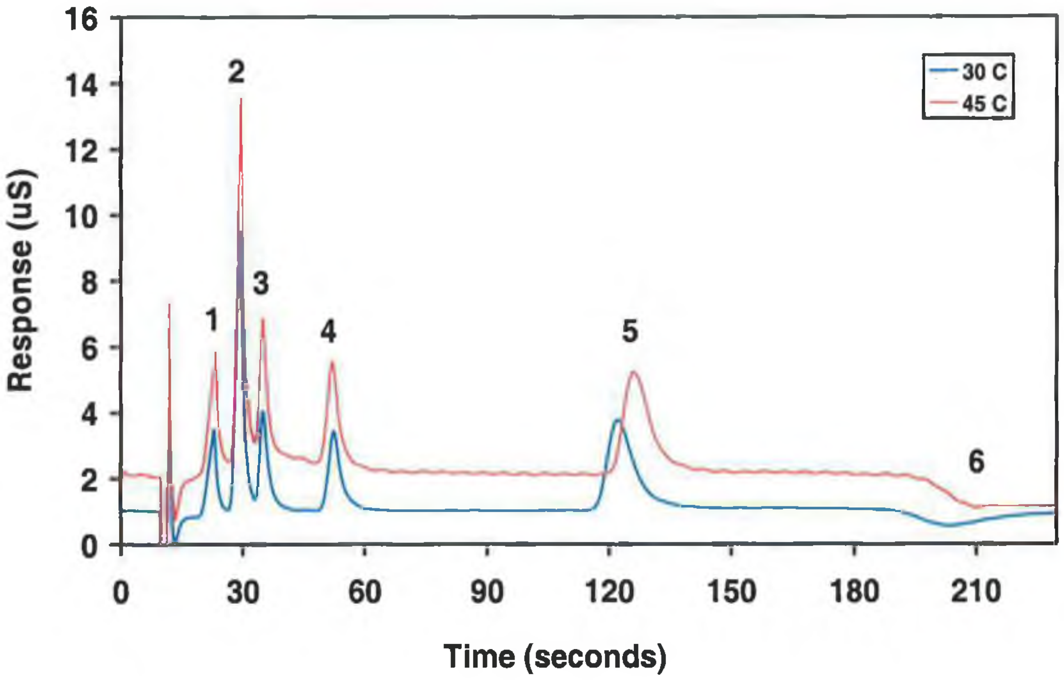

Figure 8-26. *Effect of column temperature upon the retention of anions on a DDAB coated monolithic column. Chromatographic conditions: Column: Merck Chromolith Flash RP-18e 25 mm X 4.6 mm (permanently coated with DDAB), Mobile phase: 2.5 mM phthalate/1.47 mM EDA, pH 4.5, Flow rate: 2.0 ml/min, Column temperatures: 30 °C and 45 °C, Detection: direct conductivity, Loop volume: 25 µL, Peaks: [1] 10 mg/L phosphate, [2] 10 mg/L chloride, [3] 10 mg/L nitrite, [4] 10 mg/L nitrate, [5] 10 mg/L sulphate, [6] system peak.*

8.3.3.2 Selectivity studies on the DDAB coated anion exchange column.

A number of small inorganic and organic anions were injected onto the anion exchange column, and retention times at a flow rate of 2.0 ml/min (the flow rate used throughout method development) were determined and tabulated in Table 8-11 below.

Table 8-11. *Selectivity study for small inorganic and organic anions on a DDAB coated monolithic column.*

Anion	**Retention time (minutes)**
Carbonate	0.19
Acetate	0.35
Iodate	0.37
Phosphate	0.38
Chloride	0.49
Bromate	0.52
Nitrite	0.59
Bromide	0.77
Nitrate	0.88
Chlorite	0.90
Chlorate	1.28
Sulphate	2.05
Thiocyanate	2.06
Thiosulphate	2.10
Sulphite	2.12
Iodide	3.09
System peak (phthalate)	3.42

Taking the retention data in Table 8-11 into account, the optimum separation of anions possible on the 2.5 cm DDAB coated monolith is shown as Figure 8-27. The flow rate used for the chromatogram shown was 4.0 ml/min, which is acceptable and well within the capabilities of most conventional HPLC pumps. Higher flow rates compromised the resolution of early peaks. At the optimised flow rate of 4.0 ml/min, eight small inorganic anions are separated in under 120 seconds. Peak efficiency, measured in plates/metre (N/m) ranged from 23,000 plates/metre for phosphate, to 62,000 plates/metre for chlorate.

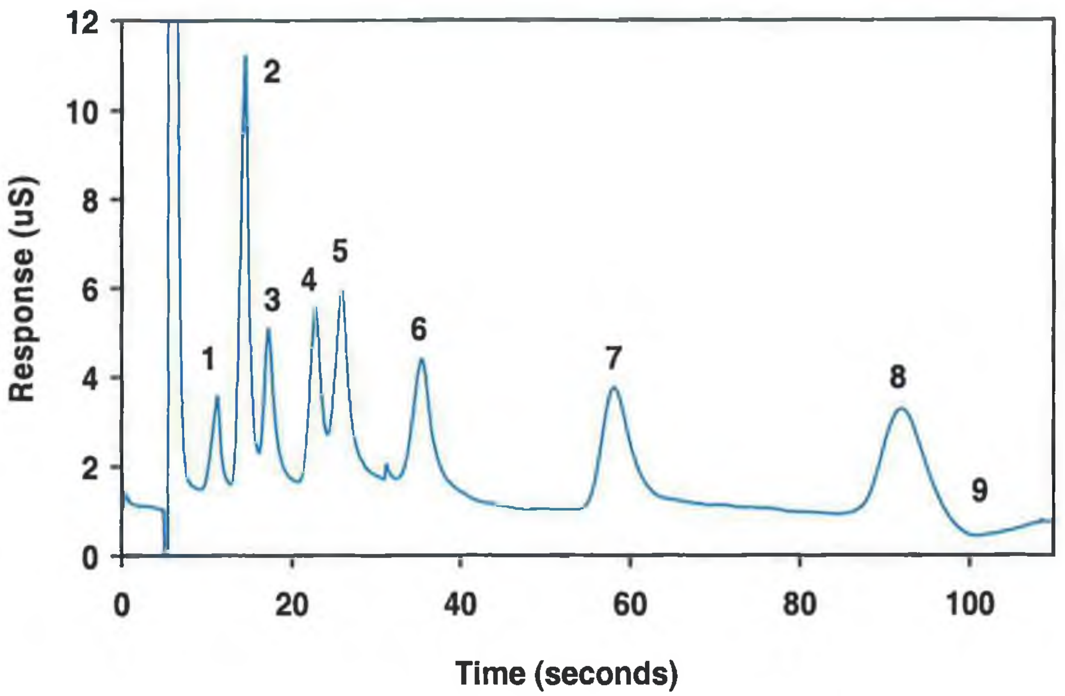

Figure 8-27. *Optimised fast separation of anions on a DDAB coated monolithic column. Chromatographic conditions: Column: Merck Chromolith Flash RP-18e 2.5 cm X 4.6 mm column (permanently coated with DDAB). Mobile phase: 2.5 mM phthalate/1.47 mM ethylenediamine pH 4.5, Flow rate: 4.0 ml/min, Detection: direct conductivity. Column temperature: 30 °C, Loop volume: 25 μL. Peaks: [1] 100 mg/L phosphate, [2] 10 mg/L chloride, [3] 10mg/L nitrite, [4] 20 mg/L bromide [5] 20 mg/L nitrate [6] 30 mg/L chlorate, [7] 40 mg/L sulphate, [8] 50 mg/L iodide, [9] system peak (phthalate).*

8.3.3.3 Optimisation of sample loop volume for anion determinations.

With regard to the analysis of real samples for the target anions, obviously the column capacity must be considered. The effect of increasing sample loop size was investigated using the mixed standard of eight anions as in Figure 8-27. It was found that under these conditions, the maximum injection volume possible without significantly affecting peak efficiency or compromising the resolution of chloride/nitrite, and bromide/nitrate was 25 μL.

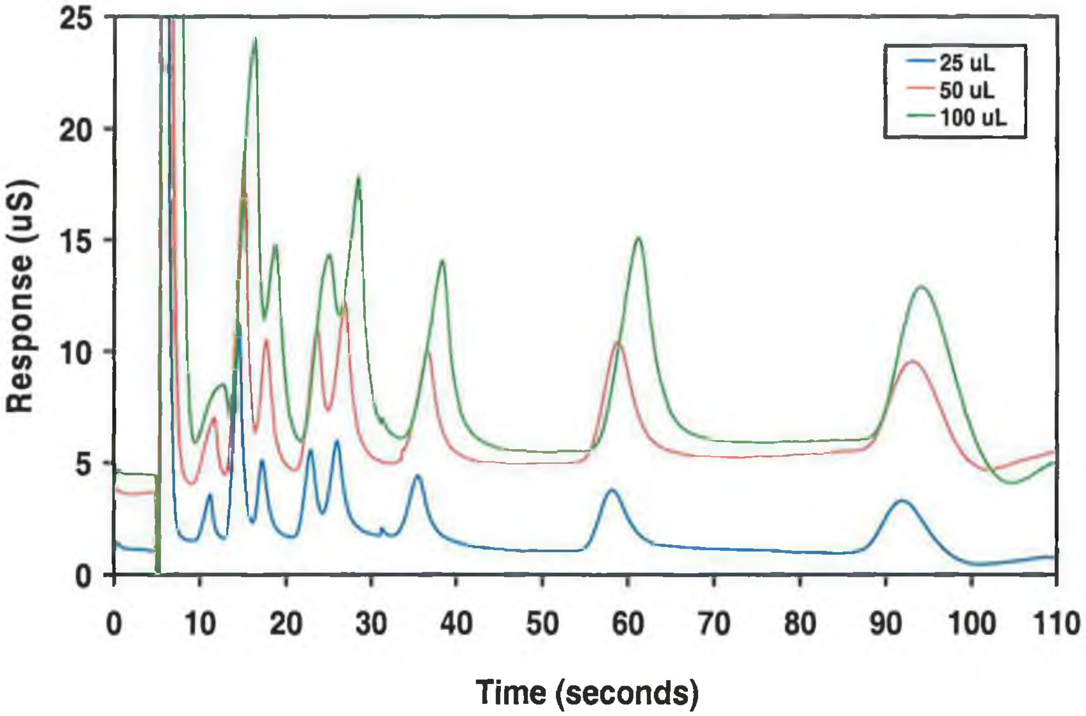

Figure 8-28. *Effect of increased injection volume upon the separation of anions on a DDAB coated monolithic column. Chromatographic conditions as in Figure 8-27 except loop volume: 25 µL, 50 µL and 100 µL.*

Table 8-12. *Effect of injection volume upon the peak efficiency and resolution for the determination of anions on a DDAB coated monolithic column.*

Anion	**Peak efficiency (N/m)**			**Resolution**		
	25 µL	**50 µL**	**100 µL**	**25 µL**	**50 µL**	**100 µL**
Phosphate	23,840	11,560	5,160	1.92	1.34	0.91
Chloride	46,320	26,520	12,760	1.38	1.08	N/C
Nitrite	44,760	32,120	N/C	2.49	2.09	N/C
Bromide	55,560	35,680	N/C	1.22	1.01	N/C
Nitrate	55,040	40,920	26,240	2.91	2.58	2.11
Chlorate	57,720	49,440	39,480	4.60	4.29	3.93
Sulphate	57,720	57,200	51,600	4.52	4.19	3.72
Iodide	68,440	53,600	47,320	N/A	N/A	N/A

8.3.3.4 Sensitivity studies for the anion separation.

Sensitivity was determined for bromide, phosphate, nitrate, nitrite, chloride and sulphate using the optimized flow rate of 4.0 ml/min. Again, for the purposes of limit of detection (L.O.D.) determinations, the signal to noise ratio was defined as two. Signal to noise ratios close to the L.O.D., for the test anions are shown in Table 8-13, and depicted in Figure 8-29 to Figure 8-34.

For all signal to noise determinations, the noise was measured in triplicate using injections of deionised water as a blank. For all anions, noise was measured over a time window of 24 seconds to 60 seconds. However, it should be noted that the retention times of phosphate, chloride, nitrite and bromide all lie outside this retention time window. Due to the steep slope of the baseline in the region of ~10 seconds to ~30 seconds, measured noise values in this region would have been very high, with resulting poor S/N ratios not fully reflecting the fact that peaks were observed to be discernable over the steep baseline slope.

It should also be noted that the second named "peak" in the phosphate and bromide chromatograms below, is due to a backpressure spike on the baseline, due to the automatic rheodyne sampling valve switching back into the "load" position after 30 seconds in the "inject" position. Sensitivity studies were momentarily paused, and the software controlling the rheodyne valve was re-configured such that the valve would switch back to "inject" after 5 seconds.

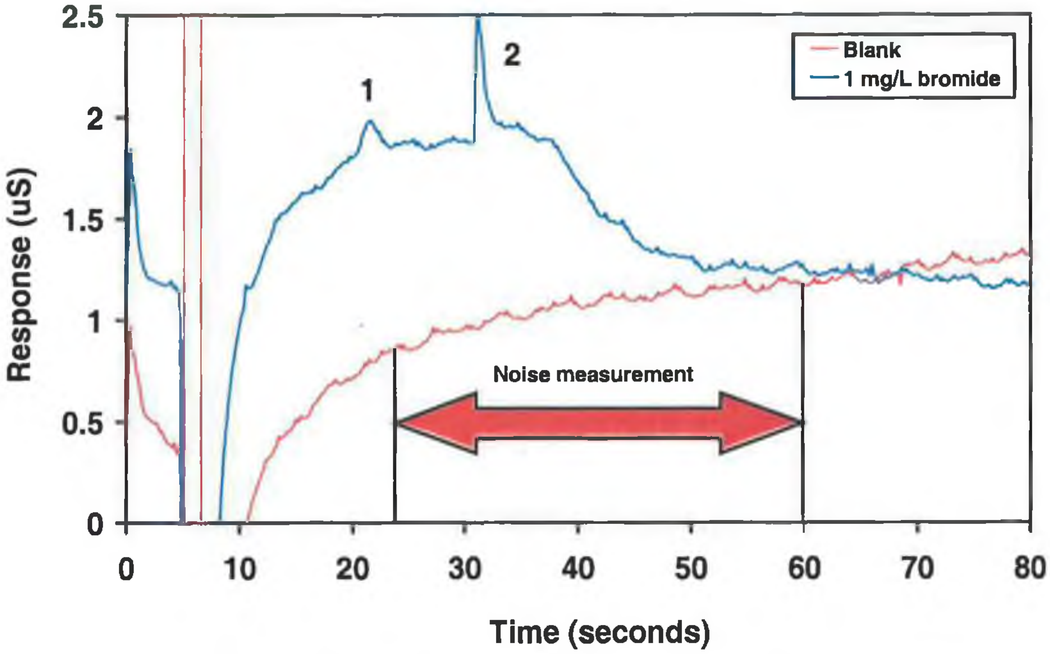

Figure 8-29. *Sensitivity study for the determination of bromide on a DDAB coated monolithic column. Chromatographic conditions as in Figure 8-27. Peaks: [1] 1.0 mg/L bromide, [2] rheodyne valve spike.*

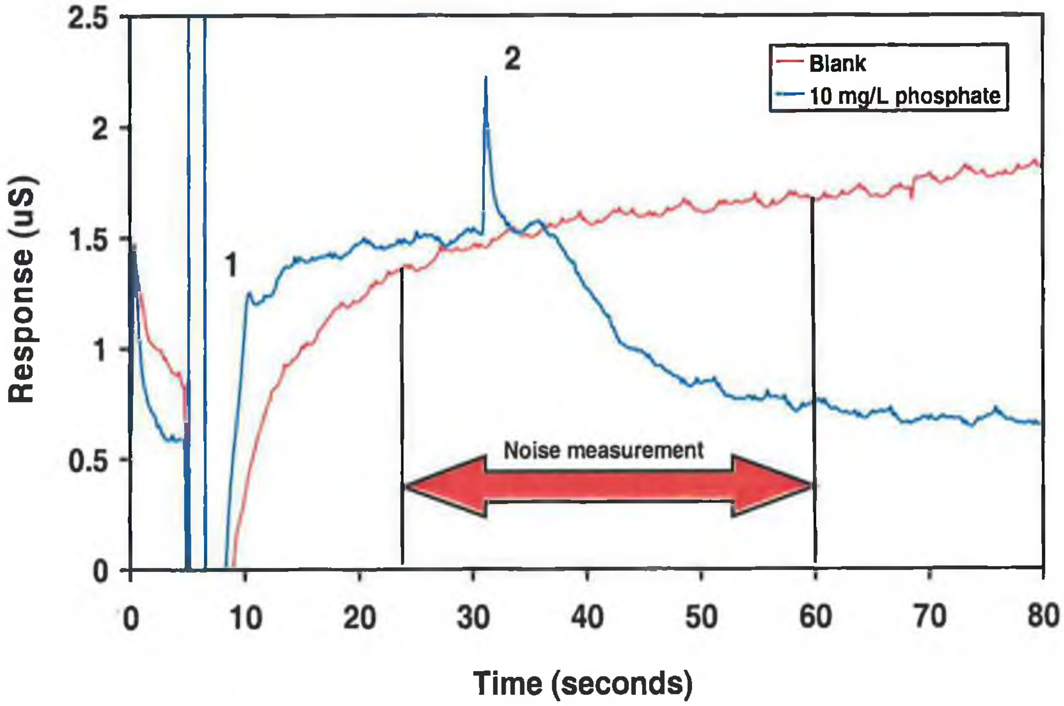

Figure 8-30. *Sensitivity study for the determination of phosphate on a DDAB coated monolithic column. Chromatographic conditions as in Figure 8-27. Peaks: [1] 10 mg/L phosphate, [2] rheodyne valve spike.*

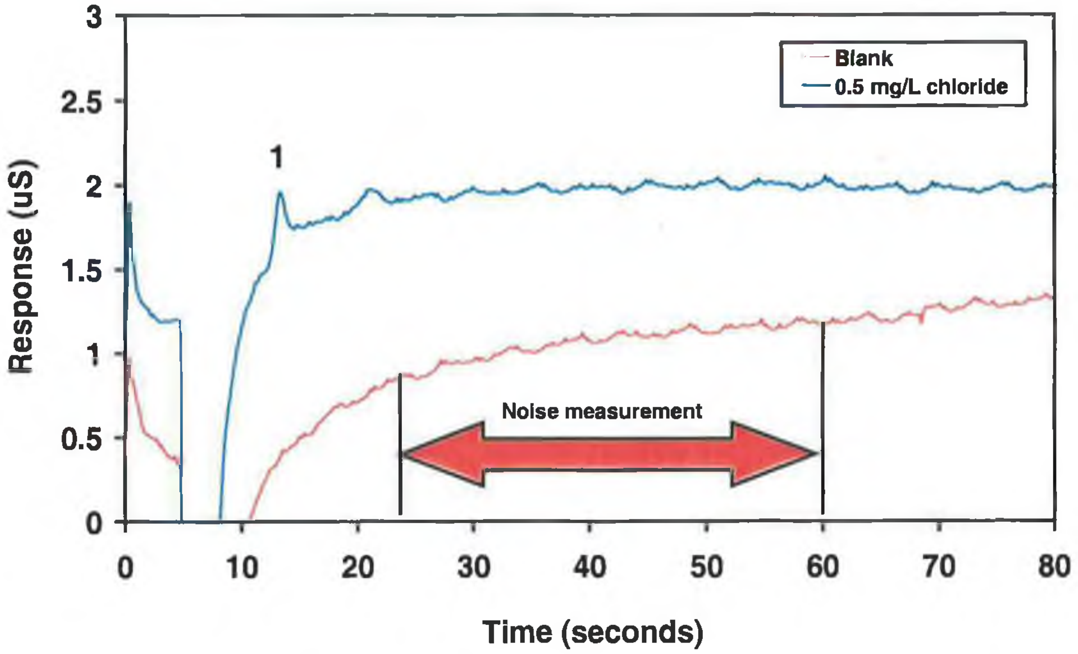

Figure 8-31. *Sensitivity study for the determination of chloride on a DDAB coated monolithic column. Chromatographic conditions as in Figure 8-27. Peaks: [1] 0.5 mg/L chloride.*

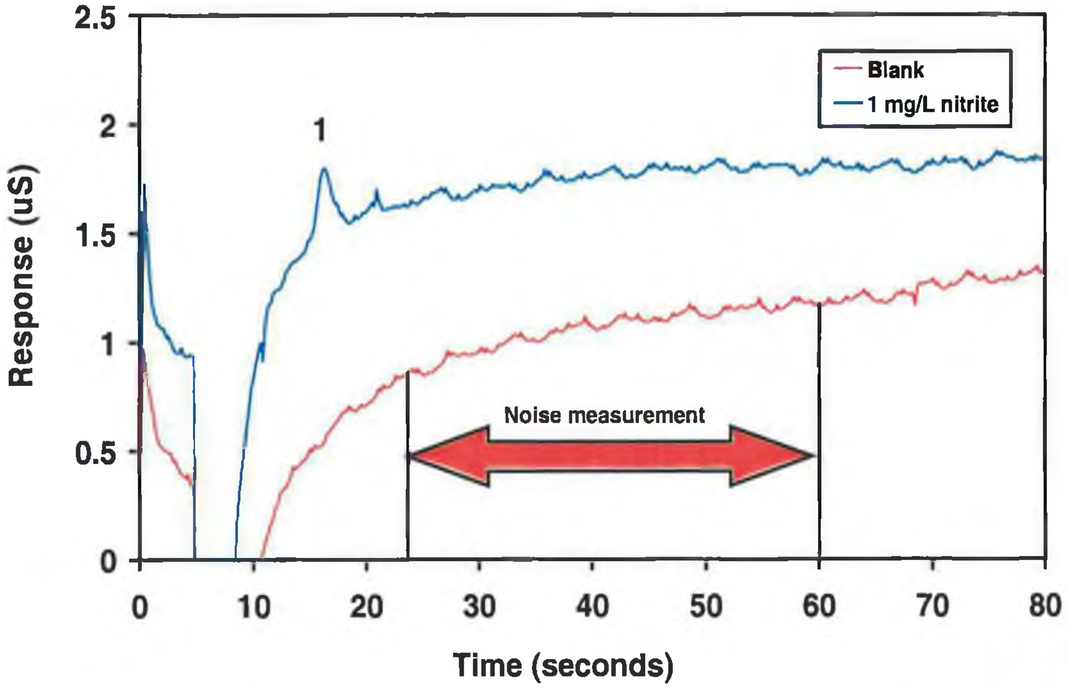

Figure 8-32. *Sensitivity study for the determination of nitrite on a DDAB coated monolithic column. Chromatographic conditions as in Figure 8-27. Peaks: [1] 1.0 mg/L nitrite.*

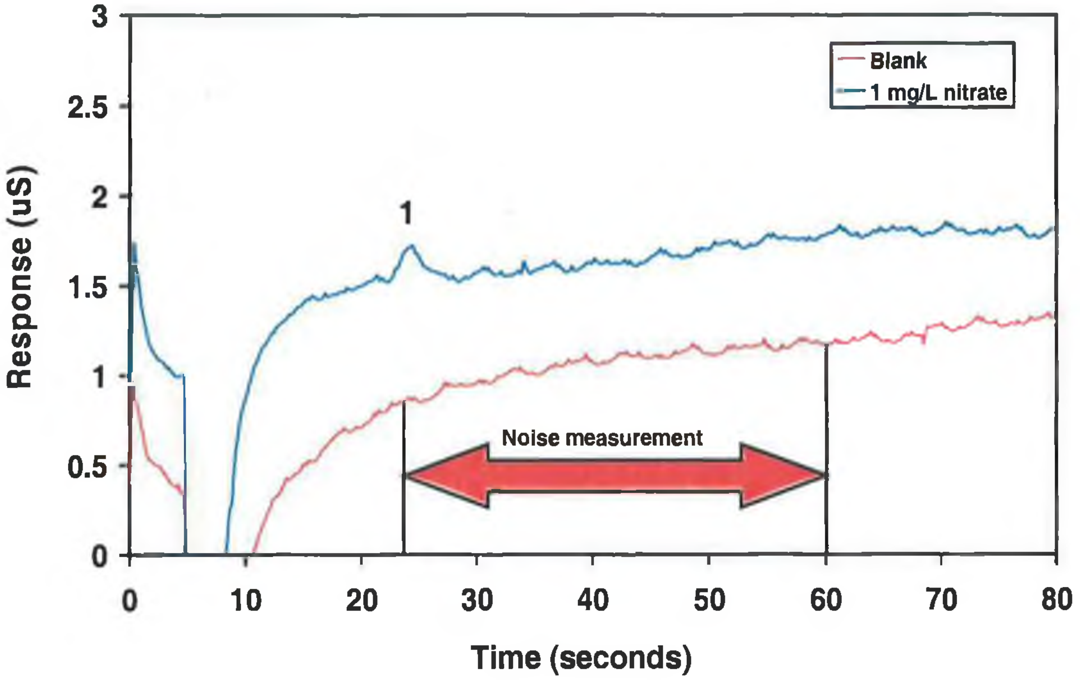

Figure 8-33. *Sensitivity study for the determination of nitrate on a DDAB coated monolithic column. Chromatographic conditions as in Figure 8-27. Peaks: [1] 1.0 mg/L nitrate.*

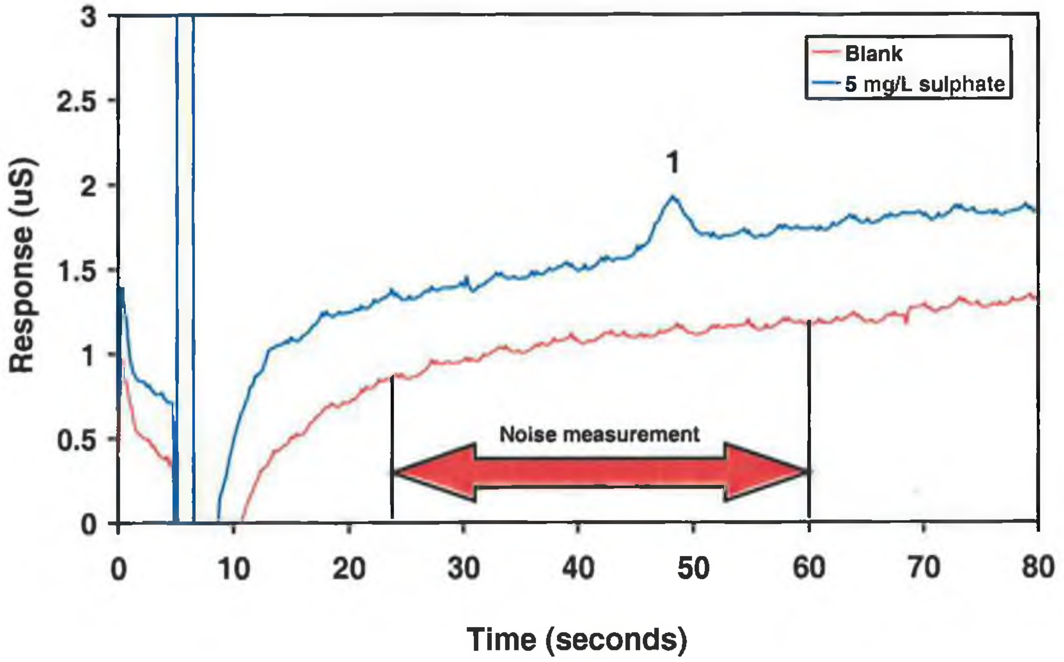

Figure 8-34. *Sensitivity study for the determination of sulphate on a DDAB coated monolithic column. Chromatographic conditions as in Figure 8-27. Peaks: [1] 5.0 mg/L sulphate.*

Table 8-13. *Sensitivity studies for the determination of selected anions on a DDAB coated monolith.*

Anions at 4 ml/min flow rate with 25 µL injection volume	
Anion	**S/N ratio**
Phosphate	2.1, (10 mg/*L*)
Chloride	2.8, (0.5 mg/*L*)
Nitrite	2.4, (1 mg/*L*)
Bromide	7.9, (5 mg/*L*)
Nitrate	6.8, (5 mg/*L*)
Sulphate	2.2, (5 mg/*L*)

8.3.3.5 Linearity studies for the anion separation.

The linearity of phosphate, chloride, nitrite, bromide, nitrate and sulphate was determined at the optimum flow rate of 4.0 ml/min. Peak areas were found to be linear over the range 10 mg/*L* to 100 mg/*L* for phosphate, 0.5 mg/*L* to 100 mg/L for chloride, 1 mg/*L* to 100 mg/*L* for nitrite, and 5 mg/*L* to 100 mg/*L* for bromide, nitrate and sulphate. Correlation coefficients were > 0.999 for phosphate, chloride, nitrite, bromide, nitrate and > 0.998 for sulphate.

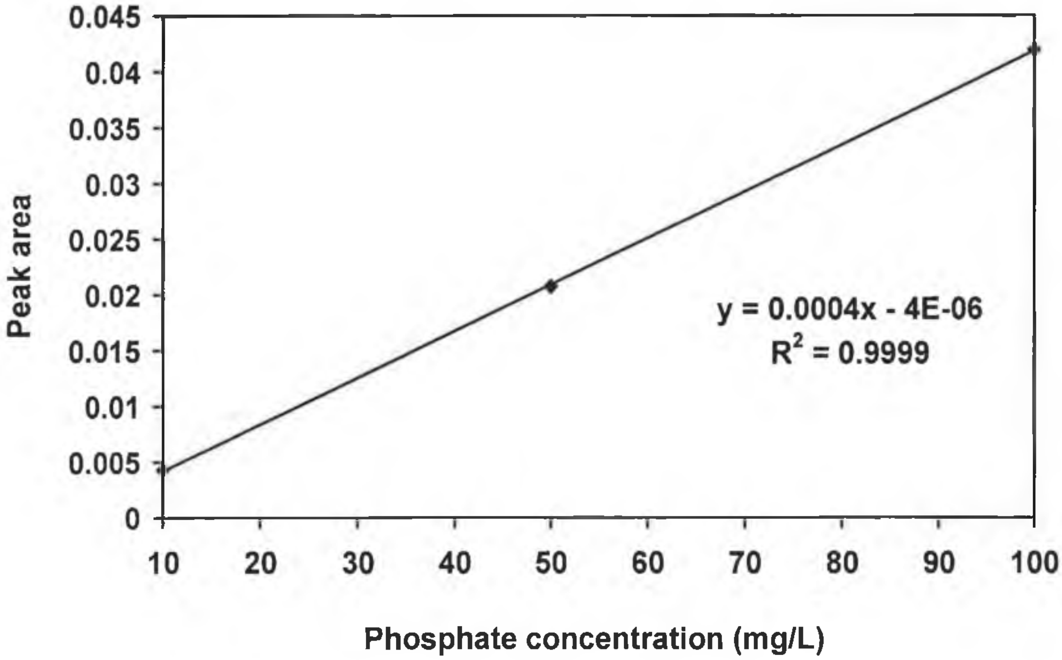

Figure 8-35. *Linearity study for the determination of phosphate on a DDAB coated monolithic column. Chromatographic conditions as in Figure 8-27.*

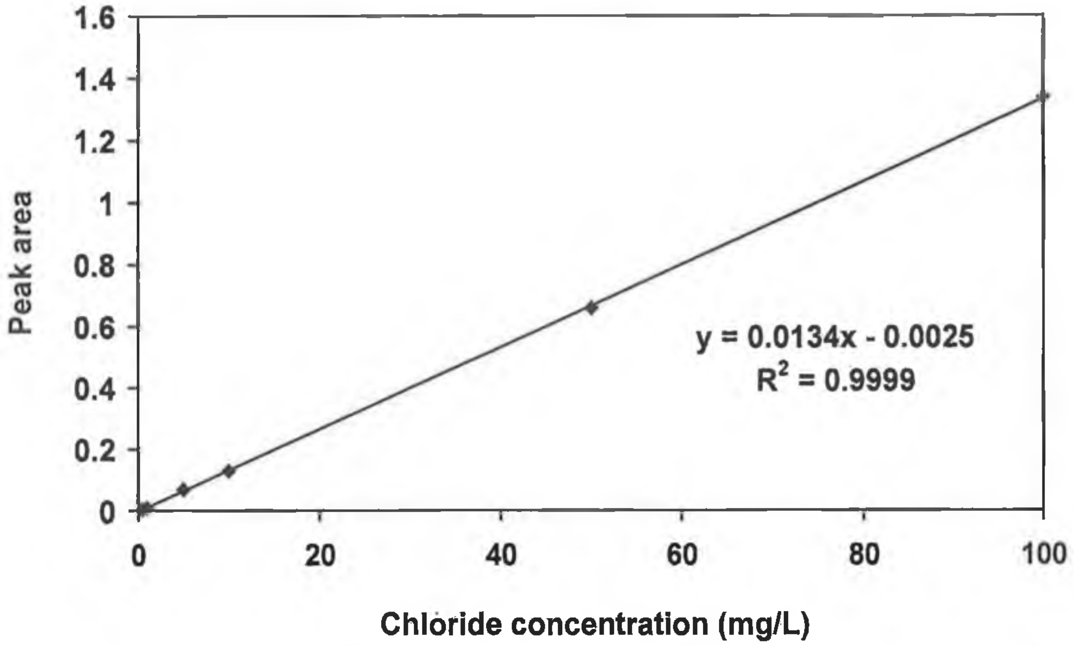

Figure 8-36. *Linearity study for the determination of chloride on a DDAB coated monolithic column. Chromatographic conditions as in Figure 8-27.*

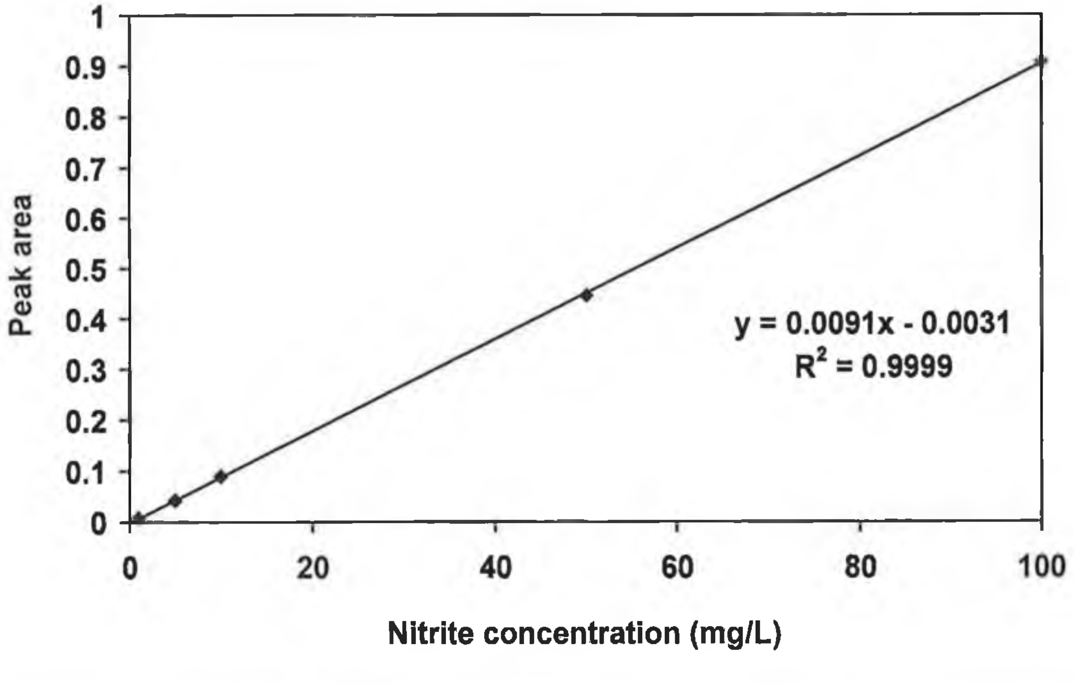

Figure 8-37. *Linearity study for the determination of nitrite on a DDAB coated monolithic column. Chromatographic conditions as in Figure 8-27.*

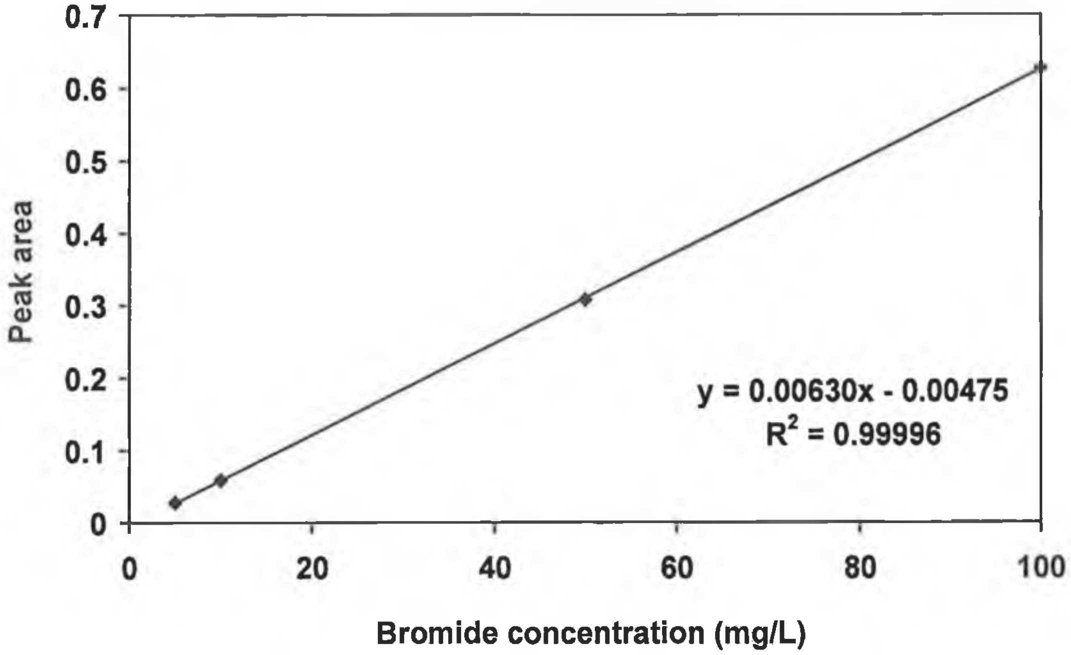

Figure 8-38. *Linearity study for the determination of bromide on a DDAB coated monolithic column. Chromatographic conditions as in Figure 8-27.*

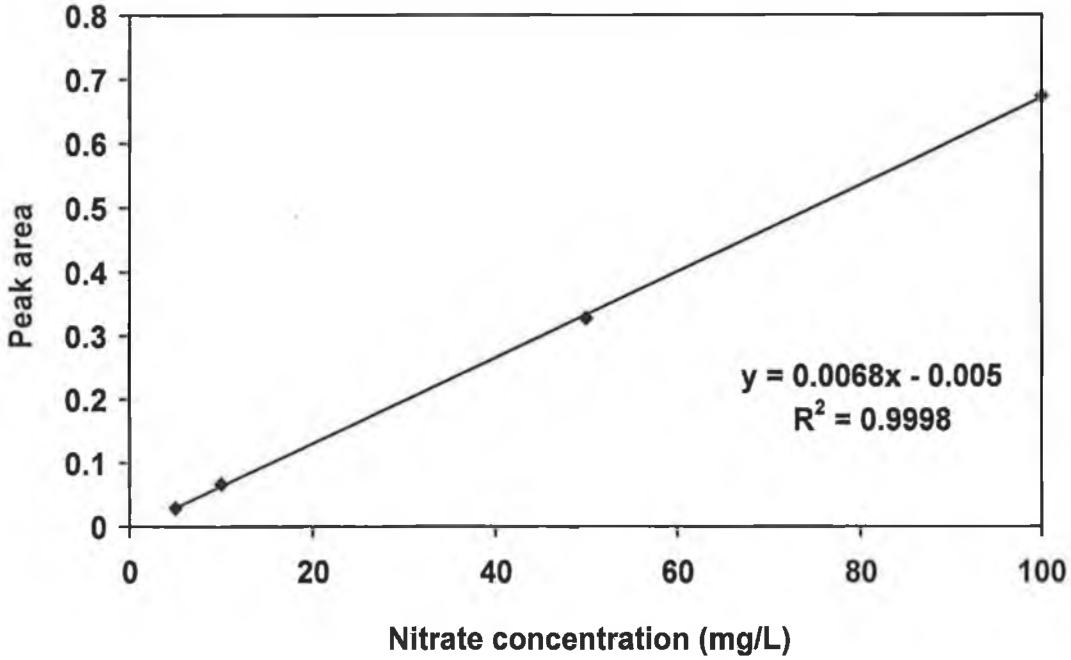

Figure 8-39. *Linearity study for the determination of nitrate on a DDAB coated monolithic column. Chromatographic conditions as in Figure 8-27.*

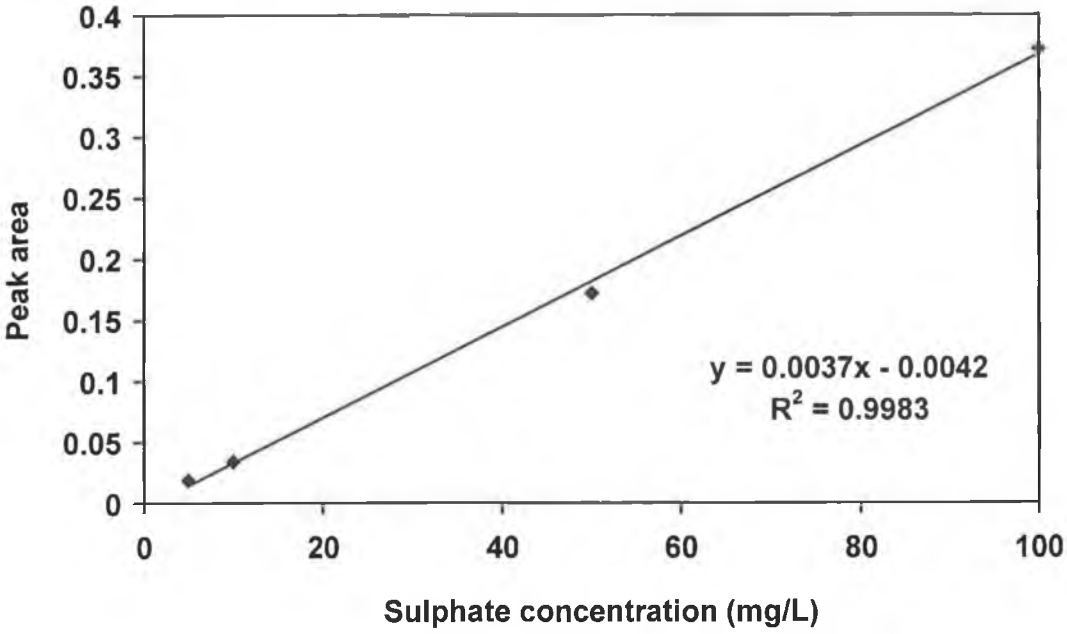

Figure 8-40. *Linearity study for the determination of sulphate on a DDAB coated monolithic column. Chromatographic conditions as in Figure 8-27.*

8.3.3.6 System precision studies for the anion separation.

Six consecutive injections of a 10 mg/*L* standard for chloride, nitrite, bromide, nitrate and sulphate were made at the optimum flow rate of 4.0 ml/min. In the case of phosphate a 50 mg/*L* standard was used. Peak area precision and retention time precision were calculated and are tabulated in Table 8-14.

Table 8-14. *System precision studies for selected anions on a DDAB coated monolithic column.*

Anions at 4 ml/min flow rate with 25 μL injection volume		
Anion	**Peak area precision % RSD, n=6**	**Retention time precision % RSD, n=6**
Phosphate	3.70 %, (50 mg/*L*)	0.72 %, (50 mg/*L*)
Chloride	1.39 %, (10 mg/*L*)	0.59 %, (10 mg/*L*)
Nitrite	1.73 %, (10 mg/*L*)	0.73 %, (10 mg/*L*)
Bromide	1.81 %, (10 mg/*L*)	0.74 %, (10 mg/*L*)
Nitrate	2.69 %, (10 mg/*L*)	0.45 %, (10 mg/*L*)
Sulphate	6.76 %, (10 mg/*L*)	0.39 %, (10 mg/*L*)

8.3.3.7 Investigation of coating stability of anion exchange monolith.

In order to examine the stability of the DDAB coating over time, 200 consecutive injections of a standard containing 10 mg/*L* chloride, 25 mg/*L* nitrate and 25 mg/*L* sulphate were made. The column coating stability over time was evaluated by plotting the retention time, peak area and peak efficiency versus the total number of injections made, for chloride, nitrate and sulphate, as shown in Figure 8-41 to Figure 8-50. Figure 8-41 is a plot of every twentieth injection of standard on the column. The results of this study indicate that the column coating exhibited excellent stability over time, with no appreciable drift in retention for the test anions. Peak efficiency was also maintained throughout.

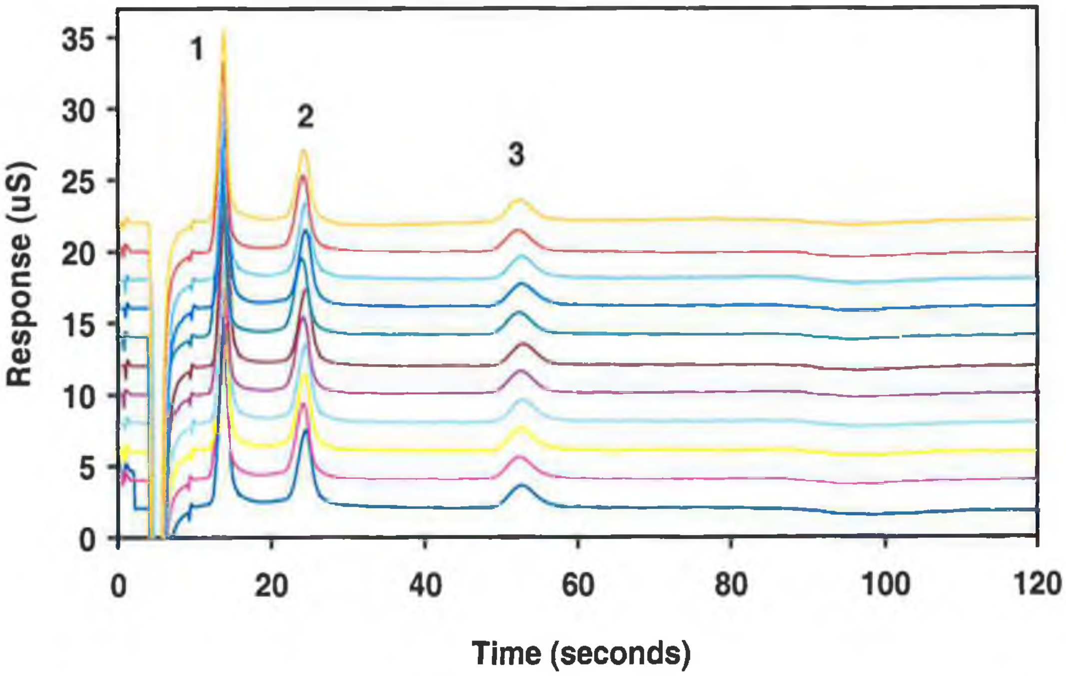

Figure 8-41. *Coating stability study for the DDAB coated monolithic column. Chromatographic conditions as in Figure 8-27.*

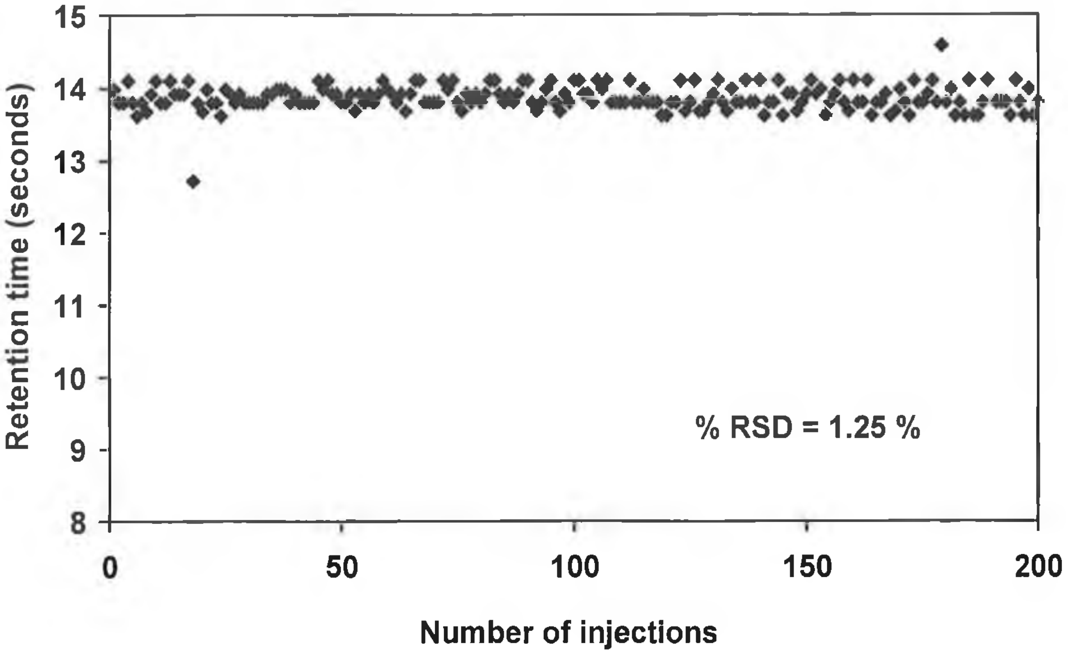

Figure 8-42. *Retention time stability study for the determination of chloride over 200 consecutive injections on a DDAB coated monolithic column. Chromatographic conditions as in Figure 8-27.*

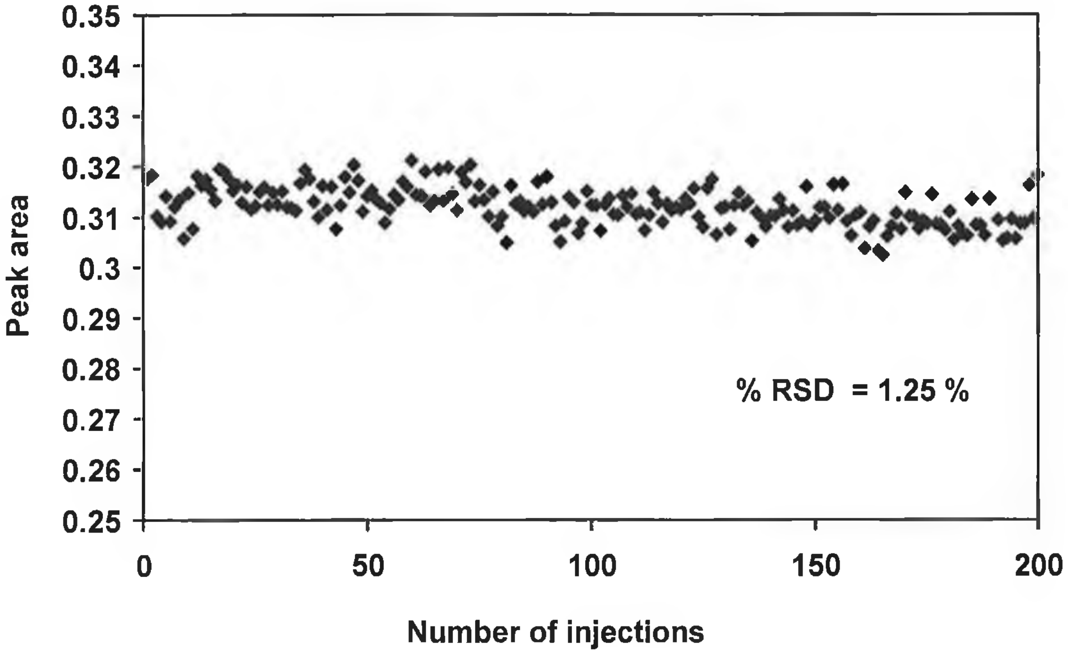

Figure 8-43. *Peak area stability study for the determination of chloride over 200 consecutive injections on a DDAB coated monolithic column. Chromatographic conditions as in Figure 8-27.*

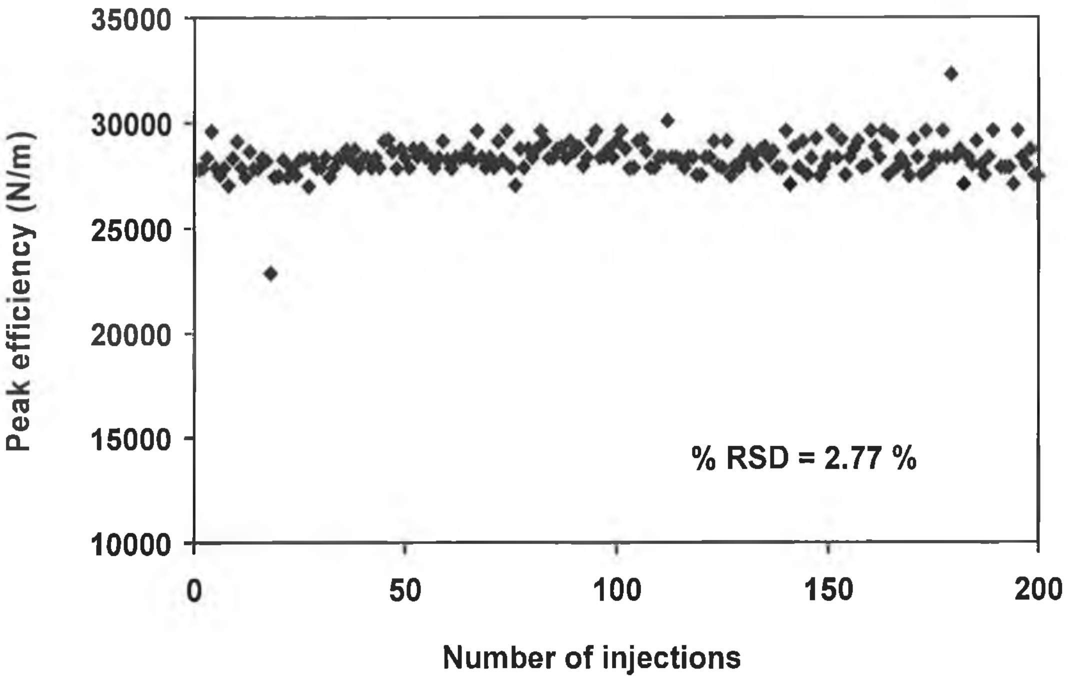

Figure 8-44. *Peak efficiency stability study for the determination of chloride over 200 consecutive injections on a DDAB coated monolithic column. Chromatographic conditions as in Figure 8-27.*

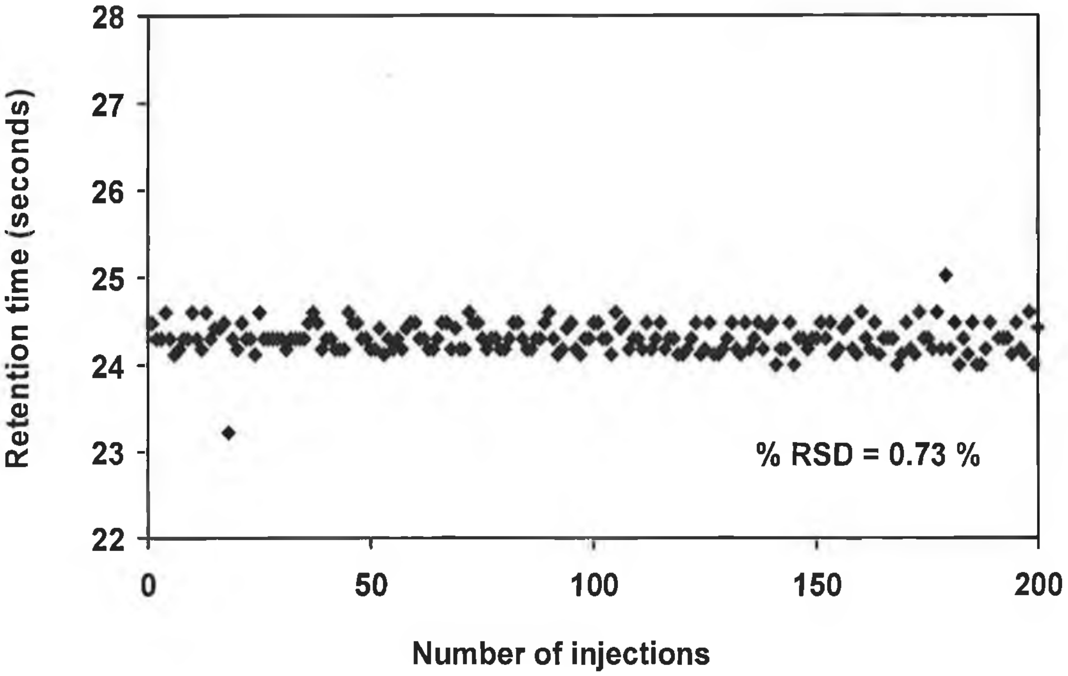

Figure 8-45. *Retention time stability study for the determination of nitrate over 200 consecutive injections on a DDAB coated monolithic column. Chromatographic conditions as in Figure 8-27.*

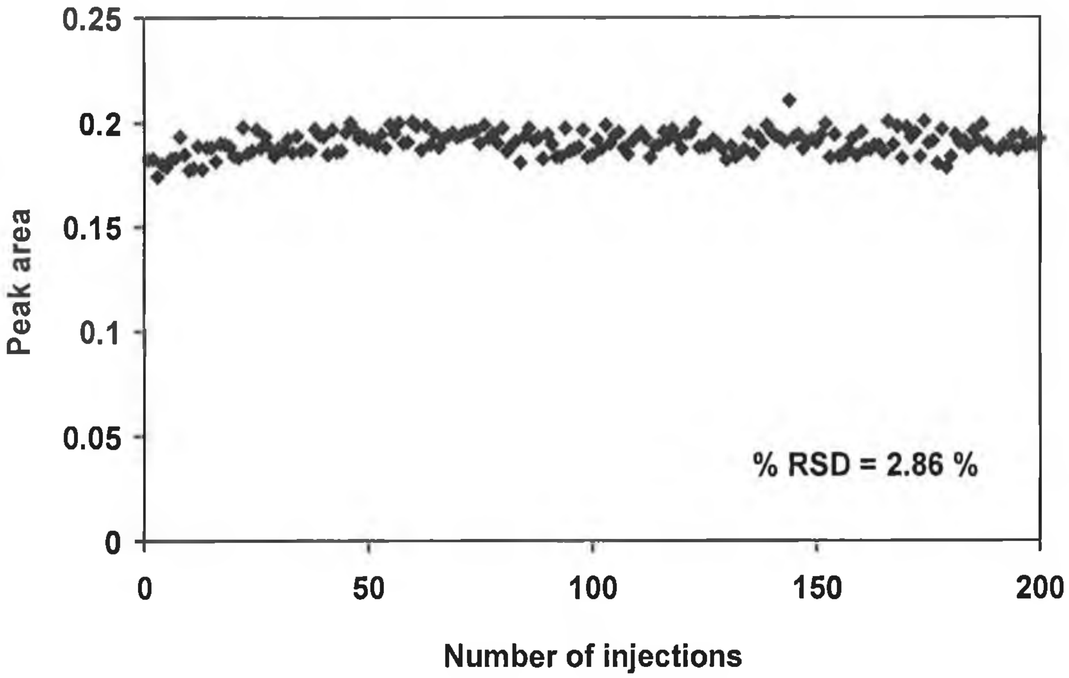

Figure 8-46. *Peak area stability study for the determination of nitrate over 200 consecutive injections on a DDAB coated monolithic column. Chromatographic conditions as in Figure 8-27.*

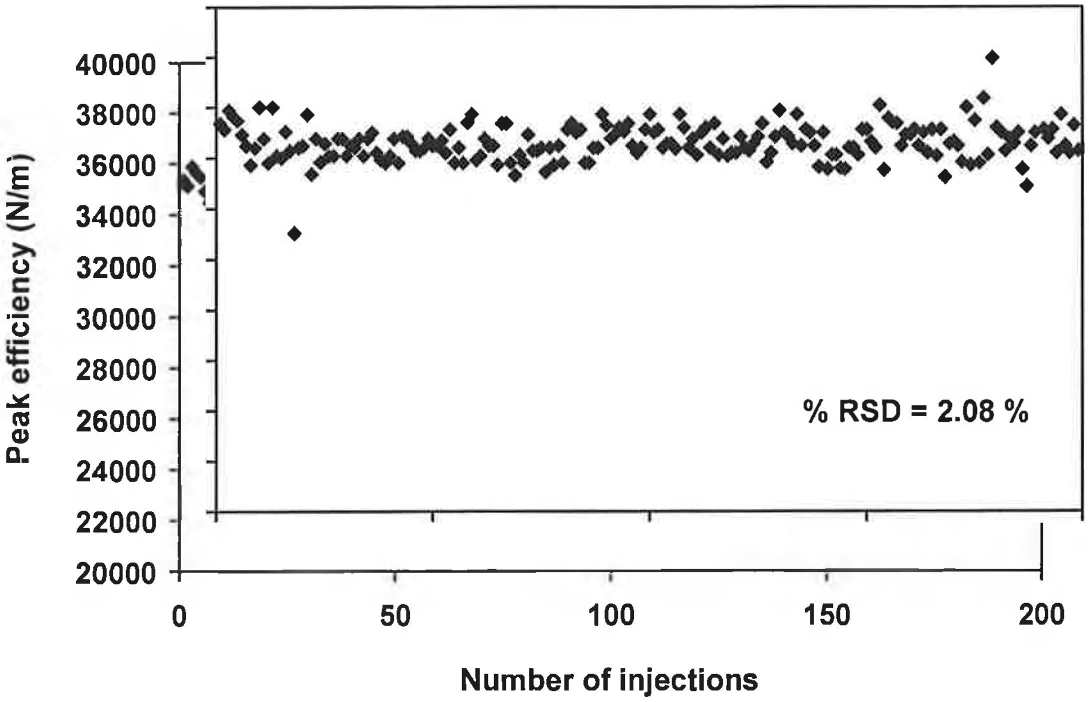

Figure 8-47. *Peak efficiency stability study for the determination of nitrate over 200 consecutive injections on a DDAB coated monolithic column. Chromatographic conditions as in Figure 8-27.*

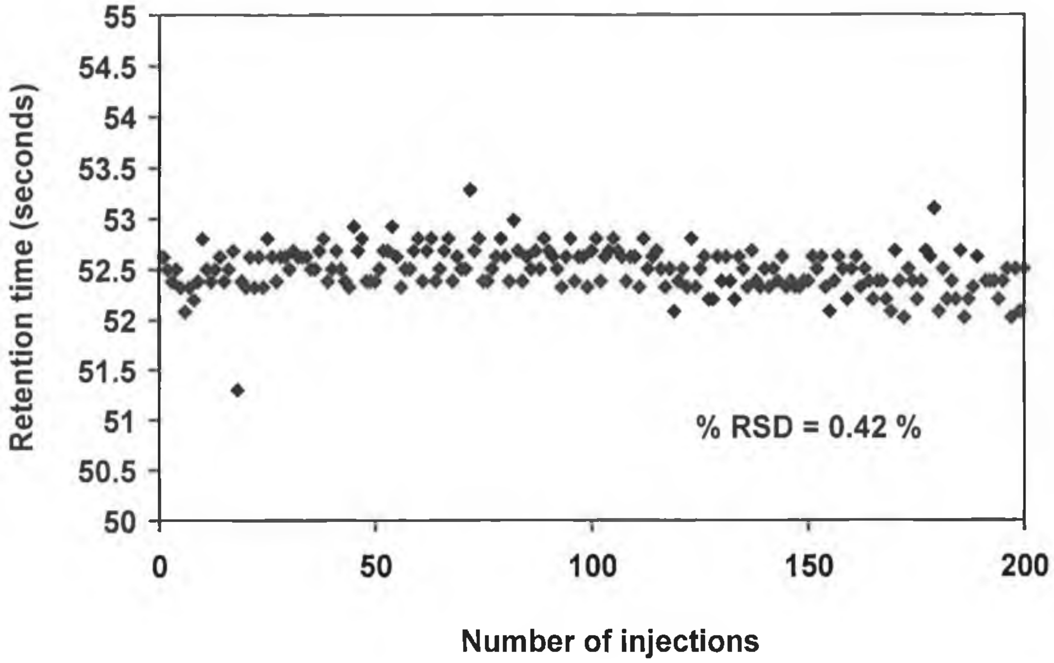

Figure 8-48. *Retention time stability study for the determination of sulphate over 200 consecutive injections on a DDAB coated monolithic column. Chromatographic conditions as in Figure 8-27.*

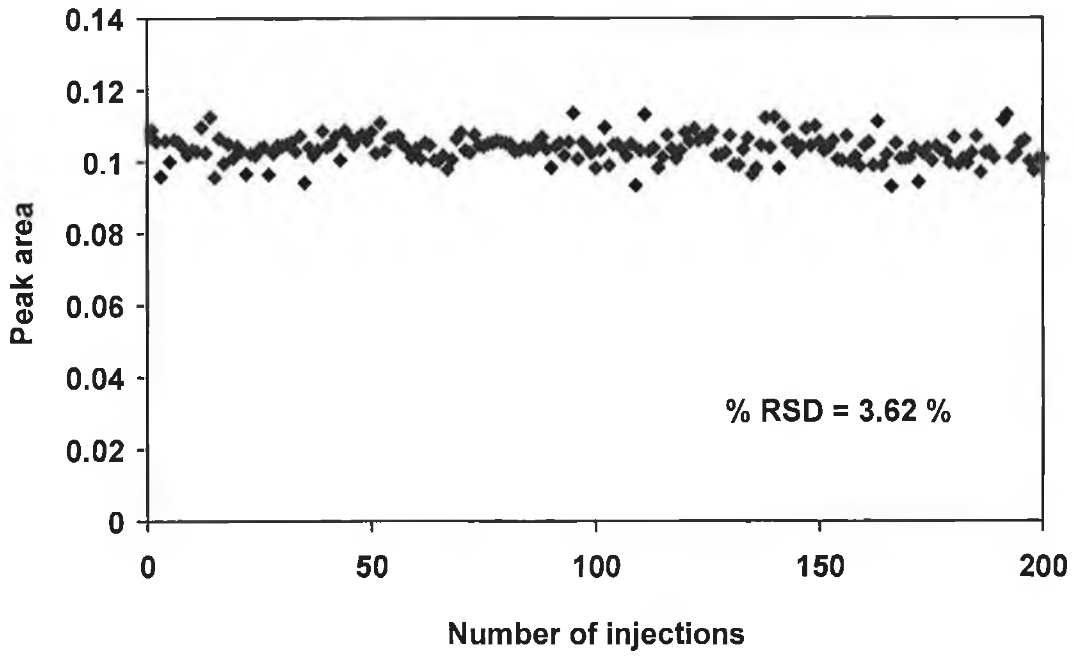

Figure 8-49. *Peak area stability study for the determination of sulphate over 200 consecutive injections on a DDAB coated monolithic column. Chromatographic conditions as in Figure 8-27.*

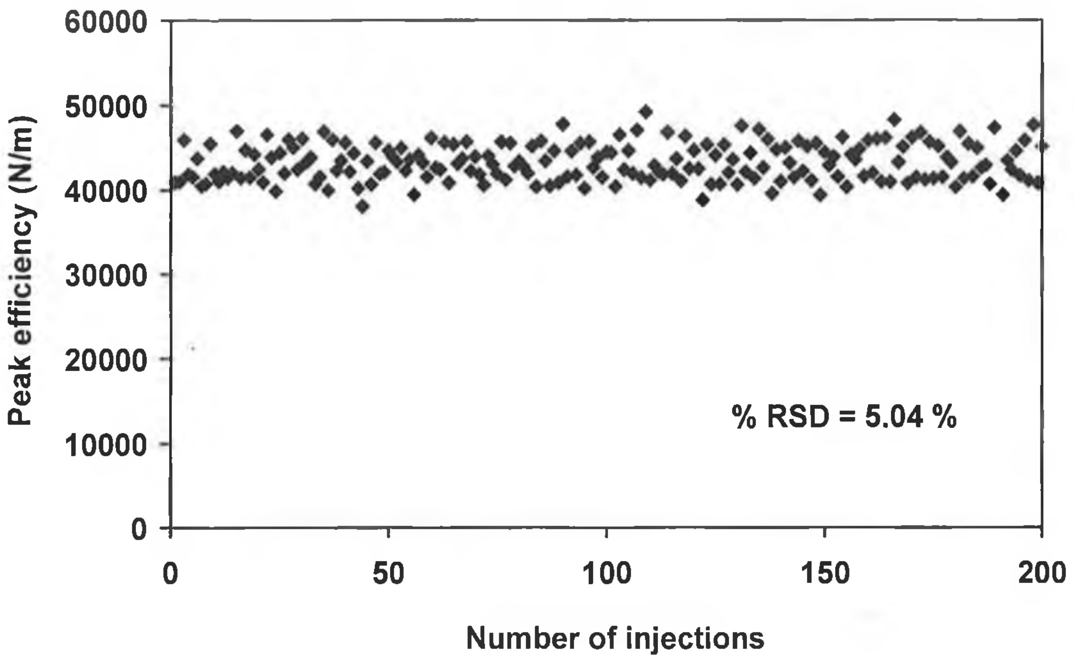

Figure 8-50. *Peak efficiency stability study for the determination of sulphate over 200 consecutive injections on a DDAB coated monolithic column. Chromatographic conditions as in Figure 8-27.*

8.3.3.8 Determination of anions in real samples.

Drinking water, river water and sea water samples were collected, filtered and analysed. The sea water sample required dilution of 1 in 100. The resultant chromatograms are shown in Figure 8-51 to Figure 8-53 respectively. Overlaid with the sample chromatograms are standard chromatograms and blanks to illustrate that the column capacity (and eluent buffering capacity) was sufficiently high to cope with the differing sample matrices. The detection of bromide eluting immediately after the large chloride peak in the diluted sea water sample illustrates the high capacity of the short monolithic anions exchanger.

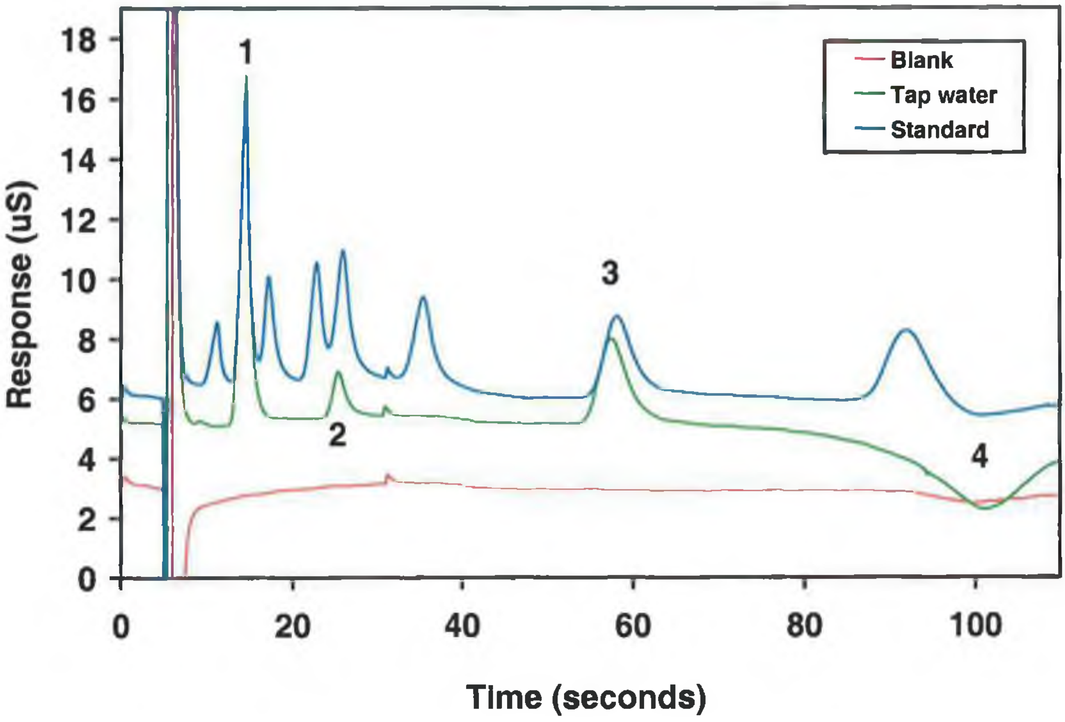

Figure 8-51. *Rapid analysis of drinking water for anions on a DDAB coated monolithic column. Chromatographic conditions as in Figure 8-27. Peaks: [1] chloride, [2] nitrate, [3] sulphate, [4] system peak (phthalate).*

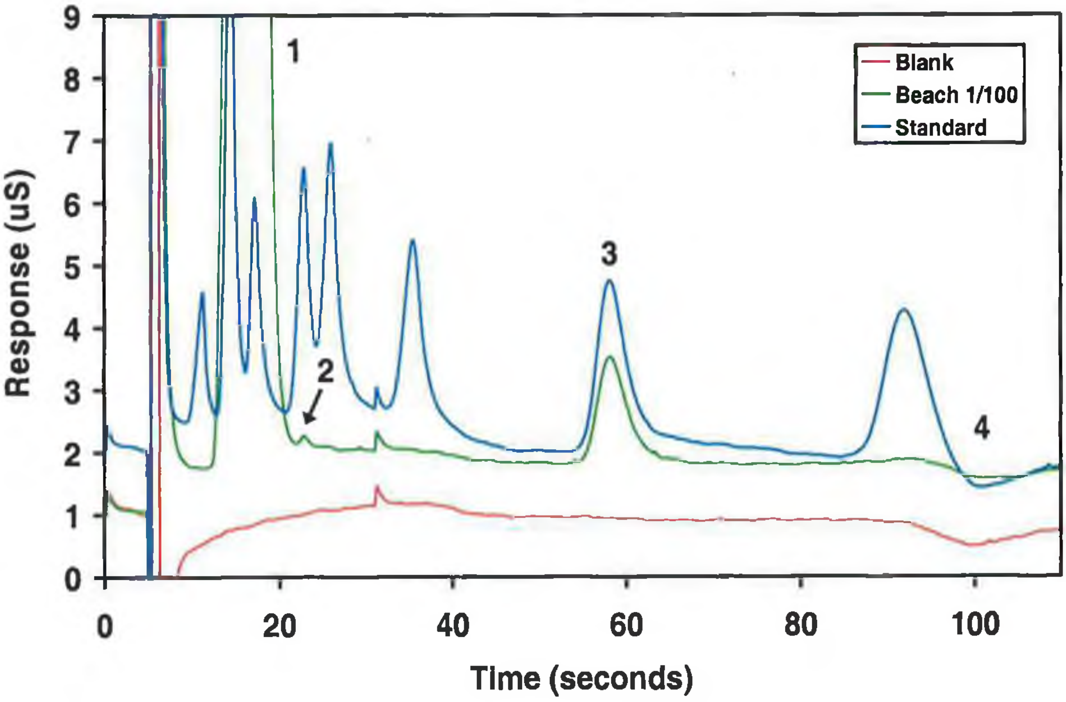

Figure 8-52. *Rapid analysis of seawater for anions on a DDAB coated monolithic column. Chromatographic conditions as in Figure 8-27. Peaks: [1] chloride, [2] bromide, [3] sulphate, [4] system peak (phthalate).*

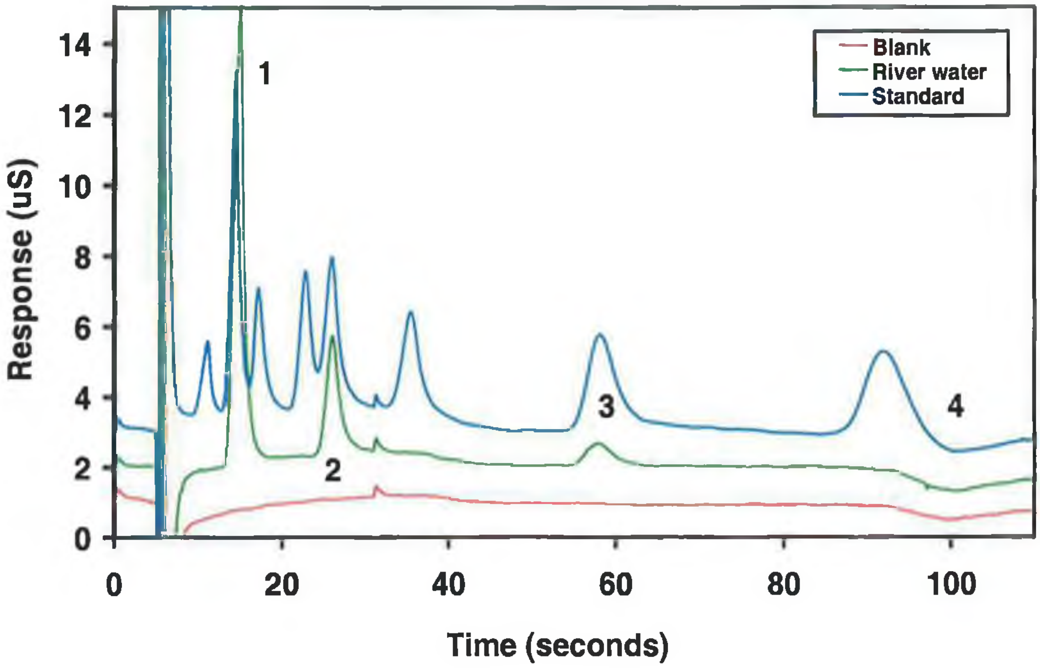

Figure 8-53. *Rapid analysis of river water for anions on a DDAB coated monolithic column. Chromatographic conditions as in Figure 8-27. Peaks: [1] chloride, [2] nitrate, [3] sulphate, [4] system peak (phthalate).*

8.3.4 Simultaneous determination of common anions and cations.

8.3.4.1 Optimisation of column lengths and relative flow rates.

The unique characteristics of the two monolithic exchangers described above, most notably high peak efficiencies at elevated flow rates and low column back-pressures, offered obvious potential for rapid separations using multi-column systems. In this study there was also the added advantage of using a single eluent, which facilitated simultaneous conductivity detection (direct and indirect) for both anionic and cationic analytes, which made the use of a single conductivity detector possible.

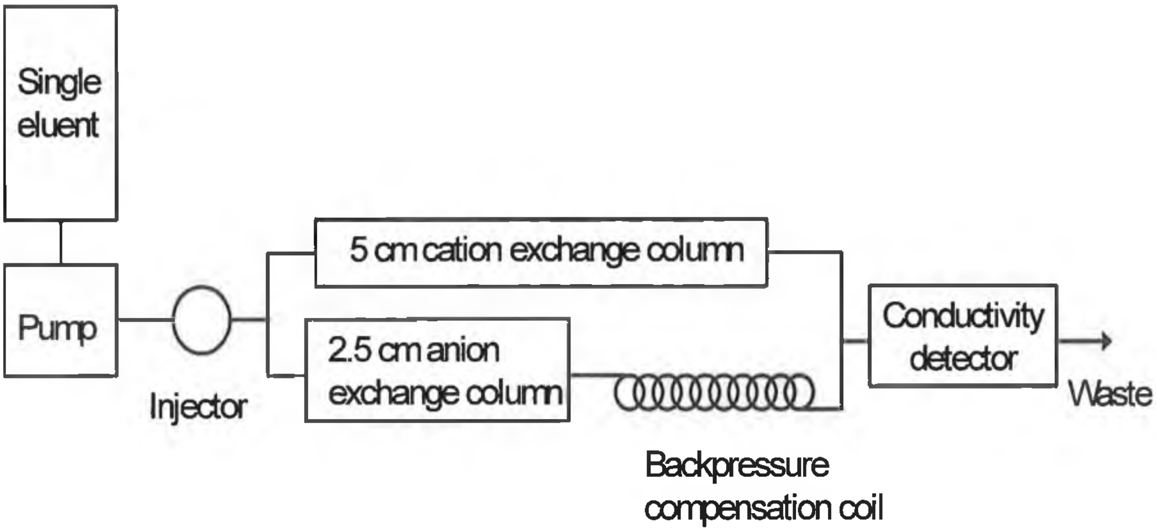

Figure 8-1. *Instrumental setup for the simultaneous analysis of anions and cations with two ion exchange columns in parallel and one detector.*

With this in mind, the IC system was configured as shown in Figure 8-54. Both columns were connected in parallel, receiving eluent from a single pump via a 50:50 flow splitter, located after a single sample injector such that anions and cations could be determined using the same single eluent, in a single injection. Eluent flows from both columns were then recombined using a second 50:50 flow splitter and passed through a single conductivity detector.

Since both columns were running in parallel from the same pump, the flow of mobile phase was split at the first tee-piece proportionately, according to the relative backpressures of each "channel", ie the anion channel and the cation channel. In the case where all tubing dimensions were equal for each channel, (in all cases, PEEK tubing with an internal diameter of 0.005 inches was used) the only remaining parameter affecting backpressure (and therefore the flow rate through each channel), was the backpressures associated with each column. In the case where both columns were of equal length (for example, a 2.5 cm DDAB coated column and a 2.5 cm DOSS coated column (as discussed in Section 8.3.1.3), then the backpressure of both "channels" would be equal. Therefore, in this instance, the flow rate through each column would be equal. Running a separation in this configuration with a single detector is equivalent to overlaying the anion chromatogram with the cation chromatogram, each obtained individually in the "single column mode". The chromatogram that would be obtained by running an anion exchange column in parallel with a cation exchange column of equal length was simulated by running both separations individually at 2.0 ml/min and overlaying the resulting chromatograms[4]. The overlaid chromatograms are shown in Figure 8-55. Two large system peaks in the cation separation co-elute with phosphate, chloride and nitrite in the anion separation and the resolution between magnesium and calcium is quite low (as discussed in Section 7.3.1.3). Therefore this column configuration was not used further in this work. Column lengths of 2.5 cm for anions and 5.0 cm for cations were used for further studies.

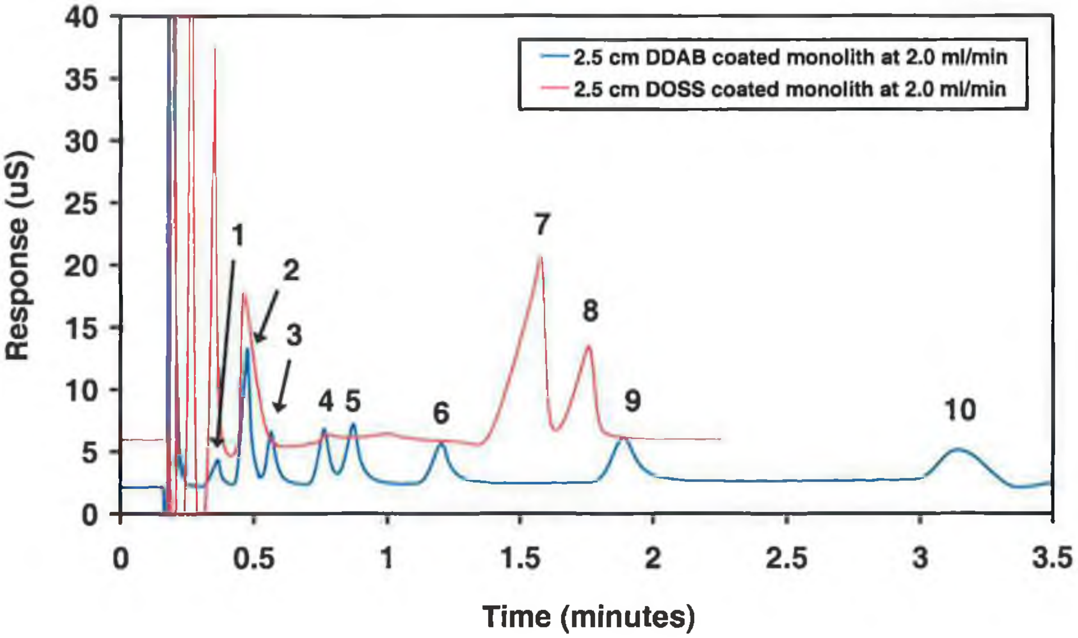

Figure 8-55. *Overlay of anion and cation separations on a DDAB coated monolithic column and a DOSS coated monolithic column of equal length at 2.0 ml/min. Chromatographic conditions: Columns: Merck Chromolith Flash RP-18e 2.5 cm X 4.6 mm column (permanently coated with DDAB) and Merck Chromolith Flash RP-18e 2.5 cm X 4.6 mm column (permanently coated with DOSS). Mobile phase: 2.5 mM phthalate/1.47 mM ethylenediamine pH 4.5, Flow rate: 2.0 ml/min, Detection: direct conductivity (anions) and indirect conductivity (cations). Column temperature: 30 °C, Loop volume: 25 µL. Peaks: [1] 100 mg/L phosphate, [2] 10 mg/L chloride, [3] 10mg/L nitrite, [4] 20 mg/L bromide [5] 20 mg/L nitrate [6] 30 mg/L chlorate, [7] 50 mg/L magnesium, [8] 50 mg/L calcium, [9] 40 mg/L sulphate, [10] 50 mg/L iodide.*

---

[4] Given that both columns are of equal length (2.5 cm) a flow rate of 2.0 ml/min through each column individually, is equivalent to an overall pump flow rate of 4.0 ml/min if the columns had been ran in parallel.

If a 2.5 cm column was used for anion separations, and a 5.0 cm column used for cation separations, the flow rate would be faster through the anion column by virtue of the lower backpressure. For example, with an initial pump flow rate of 1.0 ml/min, the flow rates become 0.67 ml/min through the 2.5 cm anion column, and 0.33 ml/min through the 5.0 cm cation column.

In order for neither separation to interfere with the other in terms of coelution, either of analyte peaks or of void peaks with analytes, the relative flow rates through each column were optimised in the "single column mode" first, by running the anion separation, followed by the cation separation. Figure 8-56 shows that a relative flow ratio of 0.75/1.25 (anion column/cation column) results in co-elution of two system peaks from the cation separation with phosphate and chloride, and partial co-elution of magnesium with sulphate.

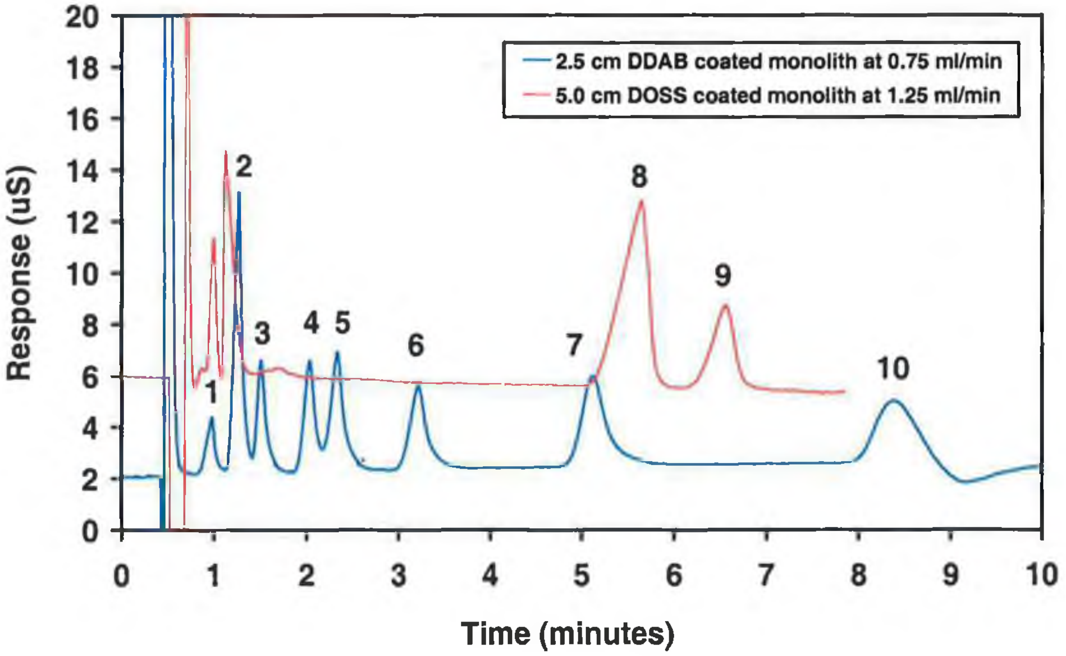

Figure 8-56. *Overlay of an anion separation on a 2.5 cm DDAB coated monolithic column at 0.75 ml/min with a cation separation on a 5.0 cm DOSS coated monolithic column at 1.25 ml/min. Chromatographic conditions as in Figure 8-55 except Columns: Merck Chromolith Flash RP-18e 2.5 cm X 4.6 mm column (permanently coated with DDAB) and Merck Chromolith SpeedROD RP-18e 5.0 cm X 4.6 mm column (permanently coated with DOSS). Flow rates: 0.75 ml/min (anions) and 1.25 ml/min (cations). Peaks: [1] 100 mg/L phosphate, [2] 10 mg/L chloride, [3] 10mg/L nitrite, [4] 20 mg/L bromide [5] 20 mg/L nitrate [6] 30 mg/L chlorate, [7] 40 mg/L sulphate, [8] 50 mg/L magnesium, [9] 50 mg/L calcium, [10] 50 mg/L iodide.*

Figure 8-57 shows that a relative flow ratio of 0.66/1.33 (anion column/cation column) results in co-elution of a system peak from the cation separation with phosphate, and poor resolution of magnesium, sulphate and calcium.

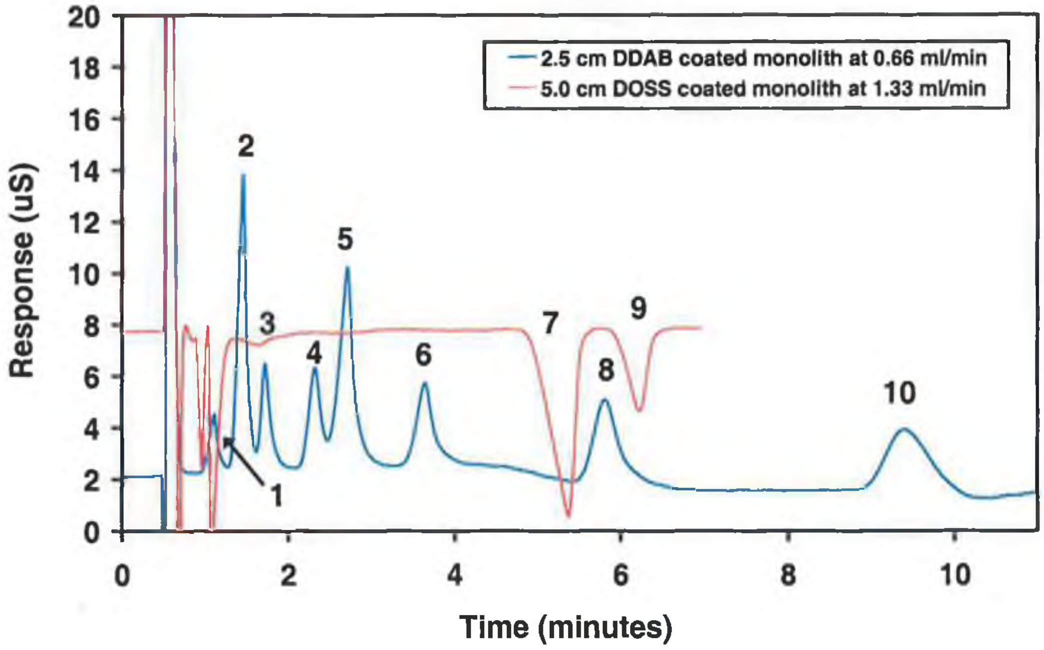

Figure 8-57. *Overlay of an anion separation on a 2.5 cm DDAB coated monolithic column at 0.66 ml/min with a cation separation on a 5.0 cm DOSS coated monolithic column at 1.33 ml/min. Chromatographic conditions as in Figure 8-56 except Flow rates: 0.66 ml/min (anions) and 1.33 ml/min (cations). Peaks: [1] 100 mg/L phosphate, [2] 10 mg/L chloride, [3] 10mg/L nitrite, [4] 20 mg/L bromide [5] 20 mg/L nitrate [6] 30 mg/L chlorate, [7] 50 mg/L magnesium, [8] 40 mg/L sulphate, [9] 50 mg/L calcium, [10] 50 mg/L iodide.*

Ultimately it was found that a flow ratio of 0.50/1.5 (anion column/cation column) resulted in neither separation interfering with the other in terms of coelution, either of analyte peaks, or of void peaks with analytes. The overlaid chromatograms are shown in Figure 8-58. The early eluting system peaks in the cation separation do not interfere with the determination of phosphate. This allowed the simultaneous separation of phosphate, chloride, nitrite, bromide, nitrate, copper, magnesium, calcium, strontium and sulphate. With chlorate co-eluting with magnesium, it was subsequently removed from further retention time markers, since it was unlikely to be present in freshwater samples at appreciable levels. Strontium and barium were also subsequently removed from the standard preparation for similar reasons. Barium was found to partially co-elute with sulphate under the conditions shown[5].

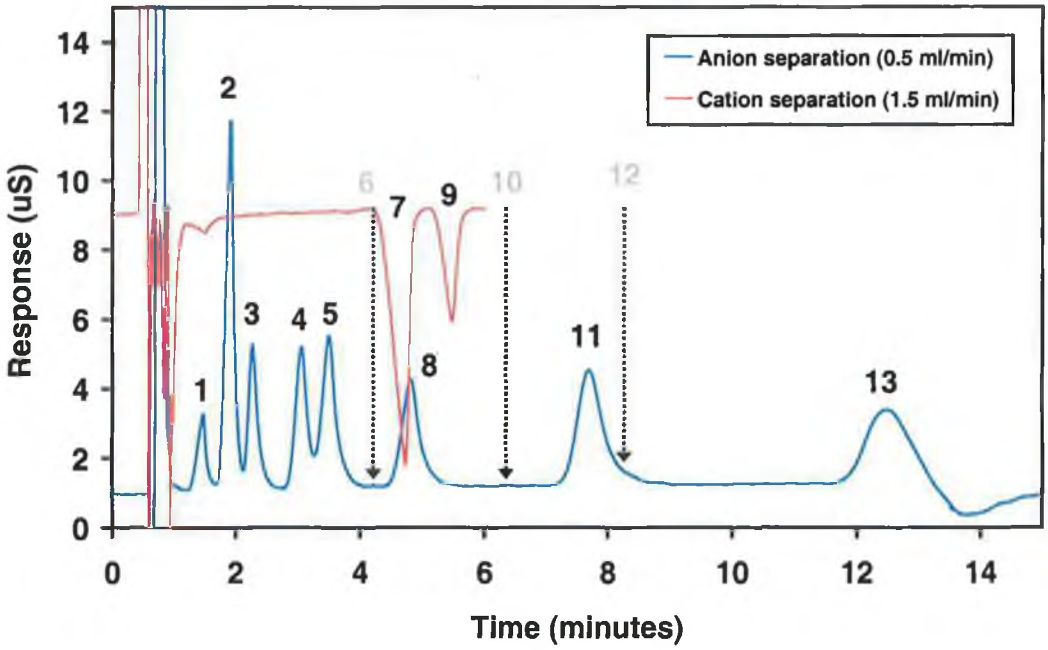

Figure 8-58. *Optimised flow rate ratio for the simultaneous determination of anions and cations on a DDAB coated monolithic column in parallel with a DOSS coated monolithic column. Chromatographic conditions as in Figure 8-56 except Flow rates: 0.50 ml/min (anions) and 1.50 ml/min (cations). Peaks: [1] 100 mg/L phosphate, [2] 10 mg/L chloride, [3] 10mg/L nitrite, [4] 20 mg/L bromide, [5] 20 mg/L nitrate, [6] copper, [7] 50 mg/L magnesium, [8] 30 mg/L chlorate, [9] 50 mg/L calcium, [10] strontium, [11] 40 mg/L sulphate, [12] barium, [13] 50 mg/L iodide.*

Because a single pump was used, the flow would normally be split proportionately such that with a flow rate of 2.0 ml/min for example, the actual flow rates through the 2.5 cm anion column and the 5.0 cm cation would be 1.33 ml/min and 0.66 ml/min respectively (Figure 8-57). In order to achieve a relative flow ratio of 0.50/1.5 (anion column/cation column), the flow rate had to be reduced through the anion exchange column by added a large backpressure compensation coil (216 cm) after the column (See Figure 8-60).

---

[5] Since copper, strontium and barium were not present in the cation test mix shown in Figure 8-58, relative retention times (calculated earlier in Table 8-7) were used to approximate their retention times.

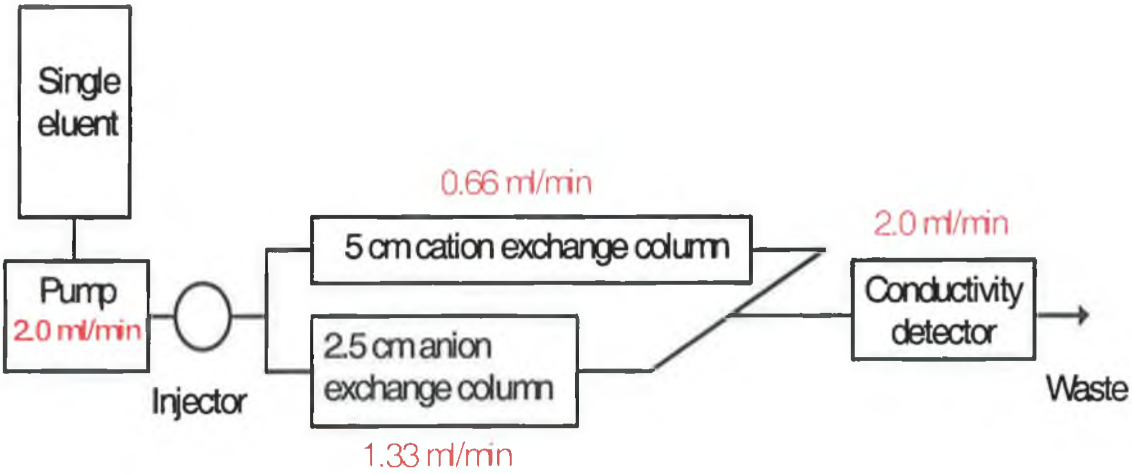

Figure 8-59. *Initial instrumental setup resulting in a non-optimum flow ratio of 1.33/0.66 (anion/cation).*

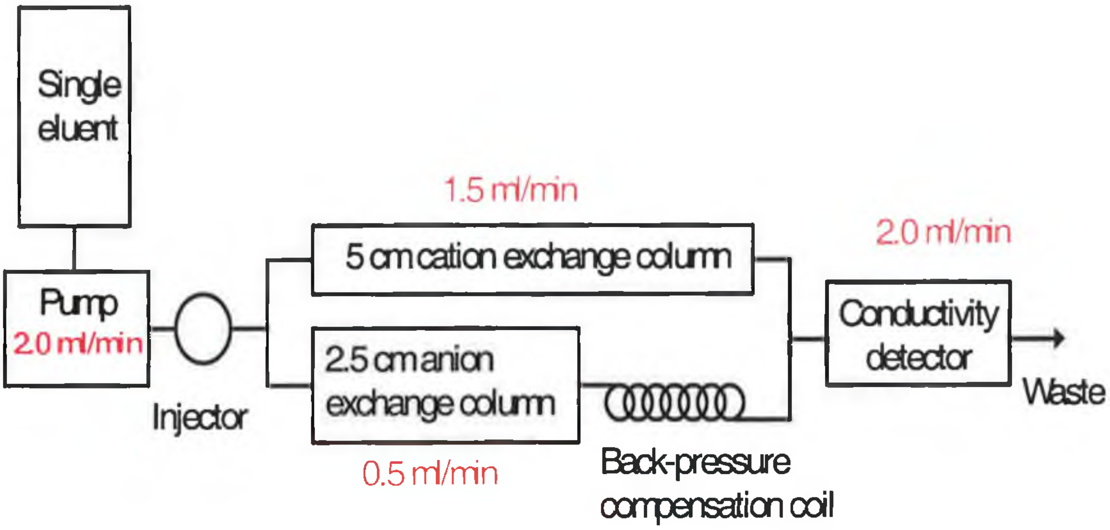

Figure 8-60. *Final instrumental setup including backpressure compensation coil, resulting in an optimum flow ratio of 0.5/1.5 (anion/cation).*

Figure 8-61 shows an example of a typical ion chromatogram of an anion and cation standard obtained using the dual column system. The final eluent flow rate used was 3.0 ml/min, which resulted in a flow of 0.75 ml/min through the anion exchange column and 2.25 ml/min through the cation exchange column. Overlaid with the simultaneous determination of anions and cations, is the individual anion separation at 0.75 ml/min and the individual cation determination at 2.25 ml/min.

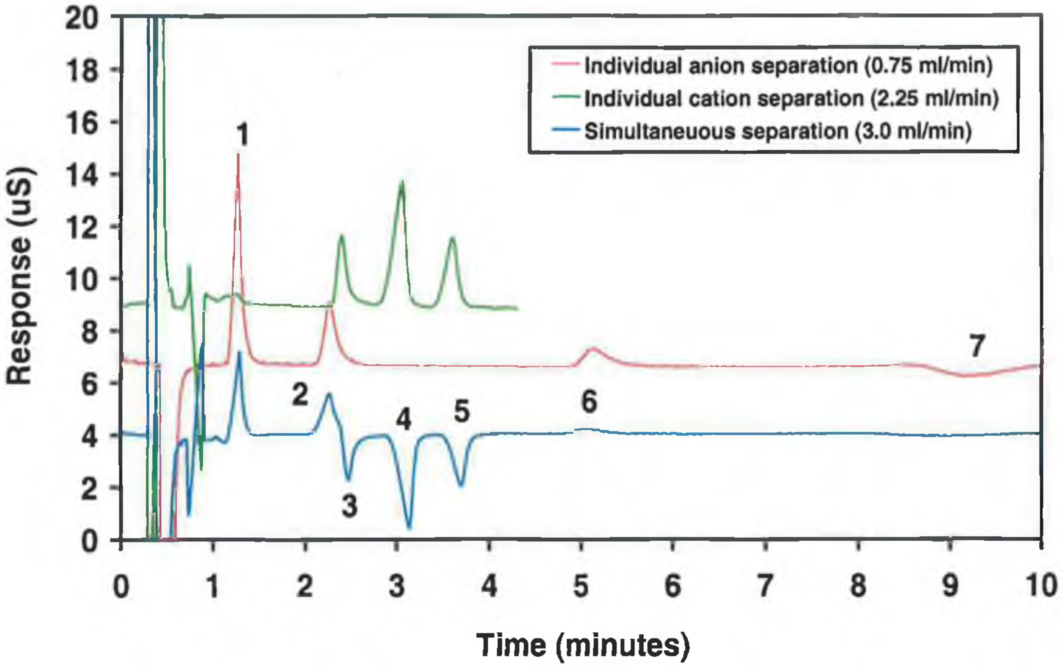

Figure 8-61. *Optimised simultaneous separation of anions and cations on a DDAB coated monolithic column in parallel with a DOSS coated monolithic column. Chromatographic conditions: Columns: Merck Chromolith Flash RP-18e 2.5 cm X 4.6 mm column (permanently coated with DDAB) and Merck Chromolith SpeedROD RP-18e 5.0 cm X 4.6 mm column (permanently coated with DOSS). Mobile phase: 2.5 mM phthalate/1.47 mM ethylenediamine pH 4.5, Flow rate: 3.0 ml/min (0.75 ml/min / 1.25 ml/min anion/cation), Detection: direct conductivity (anions) and indirect conductivity (cations). Column temperature: 30 °C, Loop volume: 100 µL. (25 µL for individual anion and cation separations), Peaks: [1] chloride, [2] nitrate, [3] copper, [4] magnesium, [5] calcium, [6] sulphate, [7] system peak (phthalate).*

The sample loop volume used was 100 µL. Since the eluent flow was being split at the flow splitter by a ratio of 0.5/1.5 (anion/cation), it can be assumed that the 100 µL of sample is not split evenly between the columns, and that the cation column receives a higher sample loading than the anion column (approximately 25 µL/75 µL; anion/cation). This assumption, however, was not investigated further. Future work with this study would include a full method validation such as sensitivity, precision and linearity.

8.3.4.2 Simultaneous determination of anions and cations in real samples.

As before, the method was briefly applied to the qualitative screening of three type of water sample, namely (a) drinking water, (b) river water and (c) diluted seawater. All samples were treated to filtration using 0.45 μm syringe filters, with river and seawater samples also requiring an additional dilution step prior to injection. Figure 8-62 to Figure 8-65 illustrates how several common inorganic anions and cations could be easily identified in each of the three water samples, with a run time of under ten minutes when using only a moderate flow rate.

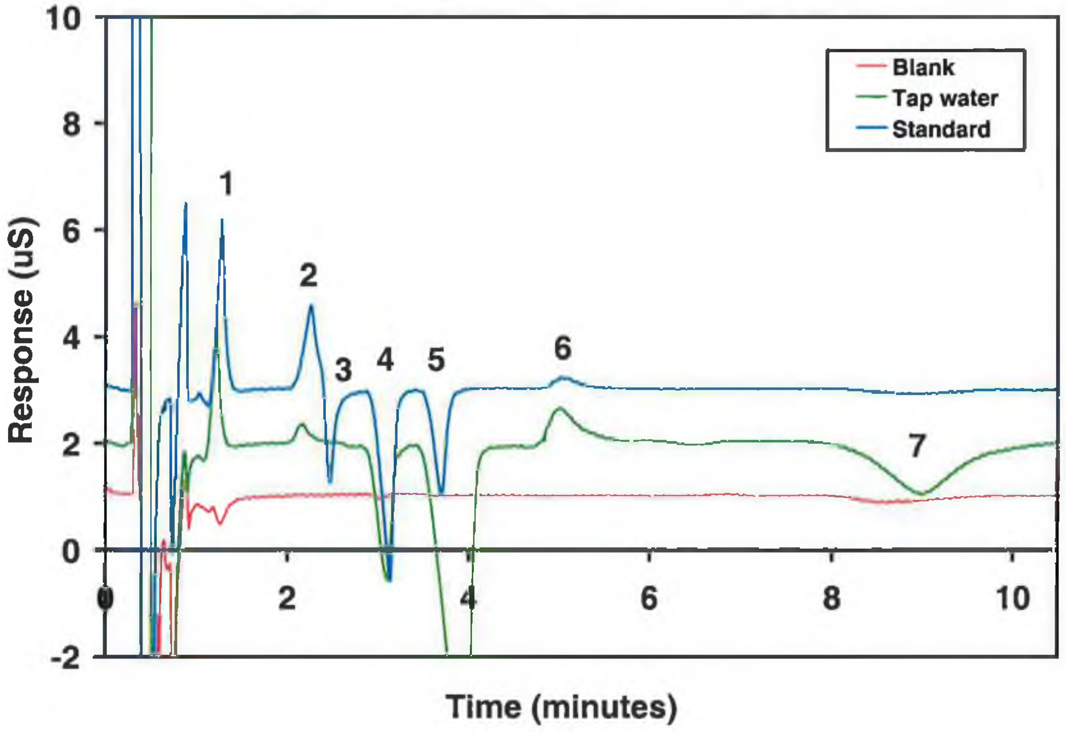

Figure 8-62. *Simultaneous analysis of drinking water for anions and cations on a DDAB coated monolithic column in parallel with a DOSS coated monolithic column. Chromatographic conditions as in Figure 8-61. Peaks: [1] chloride, [2] nitrate, [3] copper, [4] magnesium, [5] calcium, [6] sulphate, [7] system peak (phthalate).*

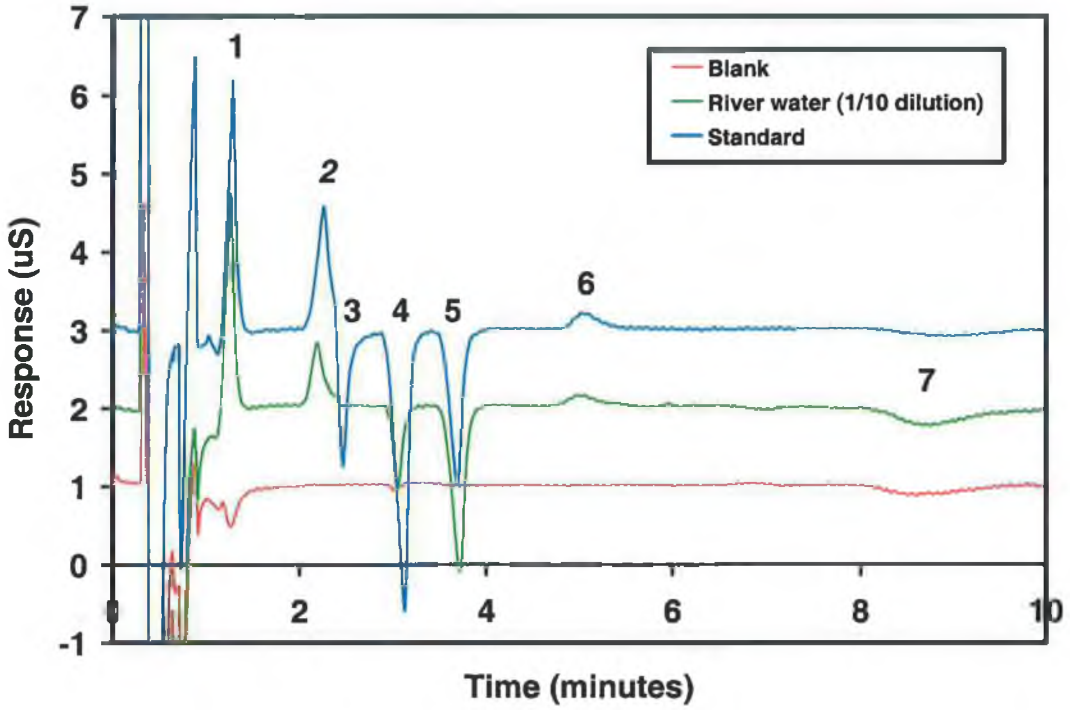

Figure 8-63. *Simultaneous analysis of river water for anions and cations on a DDAB coated monolithic column in parallel with a DOSS coated monolithic column. Chromatographic conditions as in Figure 8-61. Peaks: [1] chloride, [2] nitrate, [3] copper, [4] magnesium, [5] calcium, [6] sulphate, [7] system peak (phthalate).*

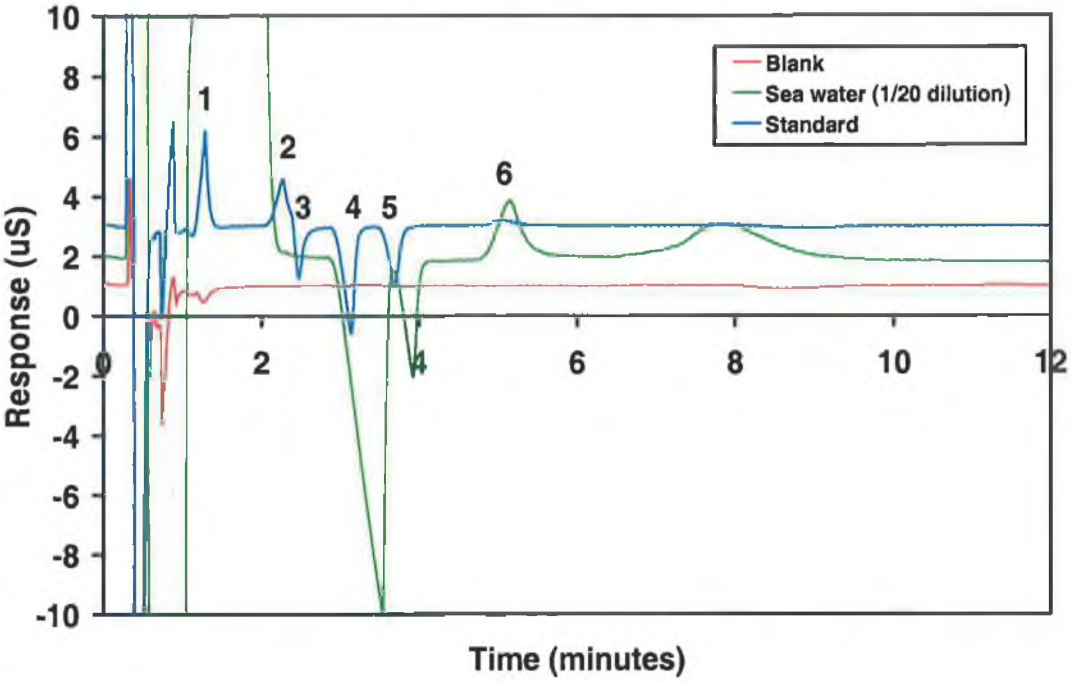

Figure 8-64. *Simultaneous analysis of seawater (diluted 1/20) for anions and cations on a DDAB coated monolithic column in parallel with a DOSS coated monolithic column. Chromatographic conditions as in Figure 8-61. Peaks: [1] chloride, [2] nitrate, [3] copper, [4] magnesium, [5] calcium, [6] sulphate.*

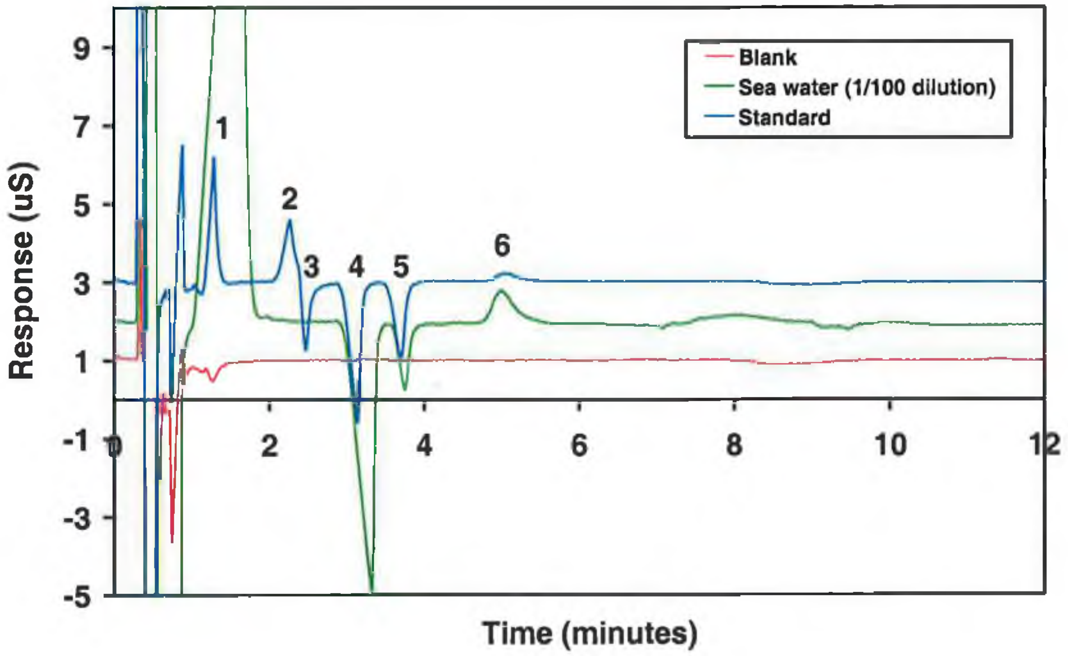

Figure 8-65. *Simultaneous analysis of seawater (diluted 1/100) for anions and cations on a DDAB coated monolithic column in parallel with a DOSS coated monolithic column. Chromatographic conditions as in Figure 8-61. Peaks: [1] chloride, [2] nitrate, [3] copper, [4] magnesium, [5] calcium, [6] sulphate.*

8.4 Conclusion.

The results from this work illustrate the practical potential of short monolithic anion and cation ion exchangers for rapid yet efficient separations of common inorganic anions and cations. For routine monitoring purposes, the potential benefits of such an approach are particularly significant. In addition, the ability to develop multi-column analytical systems based upon short monolithic columns is an approach that will see more development in the near future, particularly in the area of simultaneous and multi-dimensional separations.

8.5 References.

[1]. P.N. Nesterenko, Trends Anal. Chem. 20 (2001) 311.

[2]. K. Ohta, K. Tanaka, Anal. Chim. Acta. 373 (1998) 189.

[3]. K. Ohta, K. Tanaka, J. Chromatogr. A 804 (1998) 87.

[4]. K. Ohta, K. Tanaka, J.S. Fritz, J. Chromatogr. A 731 (1996) 179.

[5]. M.C. Bruzzoniti, E. Mentasti, C. Sarzanini, Anal. Chim. Acta. 382 (1999) 291.

[6]. A. Padarauskas, G. Schwedt, Talanta 42 (1995) 693.

[7]. K. Hayakawa, T. Sawada, K. Shimbo, M. Miyazaki, Anal. Chem. 59 (1987) 2241.

[8]. M. Yamamoto, H. Yamamoto, Y. Yamamoto, S. Matsushita, N. Baba, T. Ikushige, Anal. Chem. 56 (1984) 832.

[9]. K. Tanaka, K. Ohta, P.R. Haddad, J.S. Fritz, A. Miyanaga, W. Hu, K. Hasebe, J. Chromatogr. A 884 (2000) 167.

[10]. S.M. Kwon, K.P. Lee, K. Tanaka, K, Ohta, J. Chromatogr. A 850 (1999) 79.

[11]. K. Tanaka, K. Ohta, P.R. Haddad, J.S. Fritz, A. Miyanaga, W. Hu, K. Hasebe, K.P. Lee, C. Sarzanini, J. Chromatogr. A 920 (2001) 239.

[12]. K. Tanaka, K. Ohta, P.R. Haddad, J.S. Fritz, K.P. Lee, K. Hasebe, A. Ieuji, A. Miyanaga, J. Chromatogr. A 850 (1999) 311.

[13]. Q. Xu, W. Zhang, C. Xu, L. Jin, Analyst 125 (2000) 1065.

[14]. M.Y. Ding, K. Tanaka, W. Hu, K. Hasebe, P.R. Haddad, Analyst 126 (2001) 567.

[15]. M. Mori, K. Tanaka, M.I.H. Helaleh, Q. Xu, M. Ikedo, Y. Ogura, S. Sato, W. Hu, K. Hasebe, P.R. Haddad, J. Chromatogr. A 997 (2003) 219.

[16]. M.G. Kiseleva, P.A. Kebets, P.N. Nesterenko, Analyst 126 (2001) 2119.

[17]. W. Hu, H. Haraguchi, Anal. Chem. 66 (1994) 765.

[18]. W. Hu, P.R. Haddad, TRAC 17 (1998) 73.

[19]. M.Y. Ding, Y. Suzuki, H. Koizumi, Analyst 120 (1995) 1773.

[20]. D.J. Pietrzyk, D.M. Brown, Anal. Chem. 58 (1986) 2554.

[21]. I. Isildak, A. Asan, Talanta 48 (1999) 967.

[22]. K.J.B.A. Karim, J.Y. Jin, T. Takeuchi, J. Chromatogr. A 995 (2003) 153.

[23]. K. Deguchi, K. Kohda, M. Ito, J. Chromatogr. A 845 (1999) 165.

[24]. Z. Iskandarani, T.E. Miller Jr., Anal. Chem. 57 (1985) 1591.

[25]. V.K. Jones, J.G. Tarter, Analyst 113 (1988) 183.

[26]. J.S. Fritz, D.T. Gjerde, R.M. Becker, Anal. Chem. 52 (1980) 1519.

[27]. K. Ohta, K. Tanaka, B. Paull, P.R. Haddad, J. Chromatogr. A 770 (1997) 219.

[28]. Q. Xu, M. Mori, K. Tanaka, M. Ikedo, W. Hu, J. Chromatogr. A 1026 (2004) 191.

[29]. P. Hajos, J. Chromatogr. A 955 (2002) 1.

[30]. J. Chong, P. Hatsis, C.A. Lucy, J. Chromatogr. A 997 (2003) 161.

[31]. P. Hatsis, C.A. Lucy, Anal. Chem. 75 (2003) 995.

[32]. IUPAC Stability constants of metal-ion complexes: Part B: Organic ligands. D.P. Perrin, Pergamon Press, 1979.

[33]. F. Gerber, M. Krummen, H. Potgeter, A. Roth, C. Siffrin, C. Spoendlin, J. Chromatogr. A 1036 (2004) 127.

9 Overall conclusions.

In this work, fast-chromatography, previously predominantly confined to reversed-phase separations, has been successfully applied to ion analysis using ion-interaction chromatography and ion-exchange chromatography on short (30 x 4.6 mm) 3 μm ODS columns. The separation efficiencies (> 50,000 plates/m) and resolution achieved compare very favourably with similar separations performed on columns of more traditional length and analysis times have been significantly reduced. For example, the use of an optimised mobile phase containing 20 m*M* TBA-Cl and 20 % methanol resulted in the baseline separation of five important anions (iodate, bromate, nitrite, bromide and nitrate) in a separation window of just 28 seconds, with a total analysis time of < 50 seconds. Applications included rapid screening of fresh water samples for particular UV absorbing anions (for example, nitrate and nitrite in river water) without interference from other common matrix anions such as chloride, sulphate and phosphate.

The fast ion-interaction chromatography thus developed was also briefly applied to the rapid analysis of river water and tap water samples for nitrate and nitrite determinations. With the addition of a peristaltic pump and in-line filter, the developed method was subsequently configured for continuous monitoring of nitrate and nitrite concentrations in tap water. Up to 60 analyses per hour could be carried out unattended using the on-line system, which matches the analysis rate possible with traditional FIA based methods. Results obtained using the fast ion-interaction method compared well to those obtained using a conventional ion chromatographic method.

The fast separations achieved above utilised ion-interaction chromatography, involving the inclusion of an ion-interaction reagent with an organic modifier in the mobile phase. Alternatively, by first coating the column with an ion-interaction reagent, such that it could subsequently be removed from the eluent and replaced by a simple anion exchange counter-ion, fast ion-exchange chromatography was shown to be possible on short 3 μm ODS columns. A rapid chromatographic method was described for analysis of nine common anions in < 180 seconds, (with the first seven anions, including phosphate, chloride and sulphate, separated within 65 seconds) using a 3 cm C_{18} analytical column permanently coated with didodecyldimethylammonium bromide, and a simple phthalate eluent. Indirect detection at 279 nm was used to allow the detection of non-UV absorbing anions (chloride, phosphate and sulphate) along with those that absorb. The use of complex gradient systems was avoided, and non-specialised conventional HPLC equipment used throughout. Due to the higher surface area of the stationary phase particles, the excellent mass transfer characteristics of the column allowed for good peak shape and separation efficiency (up to 91,000 plates/metre). The applications of this method were illustrated with analysis of a number of water samples, with the column performing particularly well with samples of considerable ionic strength, due to its high exchange capacity. This allowed for the analysis of complex sample matrices such as diluted seawater for bromide ion. The high capacity, highly hydrophobic ion exchange coating demonstrated excellent stability over time, with many injections possible before re-coating was necessary, even at elevated temperatures (45 ^{o}C).

The use of complexing eluents was investigated with a view to the simultaneous determination of simple inorganic anions along with both calcium and magnesium, in a single isocratic run. An ion-exchange chromatographic separation was developed on a 30 mm X 4.6 mm C_{18} 3 µm analytical column permanently coated with didodecyldimethylammonium bromide (DDAB), using a pyromellitic acid eluent. The eluting strength of a tetravalent pyromellitic acid eluent was compared with a divalent phthalic acid eluent which had been used in previous work; demonstrating the advantage of multivalent eluents for rapid ion chromatography. Further studies involved the use of dipicolinic acid eluents, which allowed the simultaneous separation of chloride, sulphate, nitrate, carbonate, magnesium and calcium in less than 180 seconds in a single isocratic run on a C_{18} column coated with DDAB. A range of samples including river water and sea water were qualitatively screened for the test group of analytes, with minimal sample preparation necessary.

Finally, the advantages afforded by the use of recently developed monolithic stationary phases were investigated for fast ion chromatography. One of the limitations of conventional particulate columns is that the maximum flow rate which can be used is limited to about 2.5 to 3.0 ml/min, which obviously places a limit upon the speed and analysis in "fast LC". Indeed, it was noted that continual use of high flow rates caused a gradual compaction of the stationary phase bed, which impacted negatively upon chromatographic efficiency. The use of monolithic stationary phases eliminated such problems due to the much increased permeability of these phases. In this work, flow rates up to 8 ml/min could be used without exceeding backpressure limits, and columns could be connected in series to increase the number of achievable theoretical plates.

A simple rapid ion-interaction chromatographic method was thus described for the analysis of six common inorganic anions in < 60 seconds, using a 5 cm monolithic column and a tetrabutylammonium-phthalate eluent for direct conductivity detection. The column efficiency was compared with a 3 µm particulate column of similar dimensions. The resulting Van Deemter plots illustrated that the HETP did not decrease significantly at high flow rates, compared with the particulate column. Reduced runtimes allowed the analysis of a large number of water samples for nitrate, indicating the advantage of this method in the rapid screening of water samples.

Finally, two short C_{18} monoliths were separately coated with DDAB and DOSS and individually used to separate eight anions in 100 seconds and five cations in 100 seconds using a common phthalate/ethylenediamine eluent. By subsequently coupling the columns in parallel, with the eluent delivered using a flow splitter from a single isocratic pump, the simultaneous analysis of anions and cations was also possible, based on a single conductivity detector.

The advantages of the use of monolithic columns over conventional particulate columns for fast ion chromatography have been clearly illustrated. There is considerable potential for such chromatographic systems particularly for the routine rapid monitoring of multiple samples for common inorganic anions and cations. The advantages of fast reversed phase separations have been shown to be also applicable to fast ion chromatography, and with the advent of monolithic stationary phases, either functionalised with ODS or with an anion or cation exchange group, a paradigm shift in the timescale of ion chromatographic analysis has been achieved.