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DEVELOPMENT OF COMPLEXATION ION CHROMATOGRAPHY FOR THE DETERMINATION OF METAL IONS.

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Development of Complexation Ion Chromatography for the Determination of Metal Ions.

By

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For my loving family!

I know I'm not wrong and the struggle is clean,
I'll keep pushing on and I'll never turn mean.
There aren't very many who can see how I've tried,
There's a lot that think they know but not deep down inside.
You're certain to win if you push right on through
And if you never give in your dream will come true.

Doug Hepburn

ABSTRACT

Development of Complexation Ion Chromatography for the Determination of Metal Ions.

By Wasim Bashir B.Sc. (Hons), M.Sc. AMRSC.

A simple ion chromatographic method was developed for the determination of Pb(II) in river and polluted water samples. The method was based upon the use of a colour-forming complexing eluent and direct visible detection of the eluting Pb(II) complex. Using the combination of a strong cation exchange column and an eluent consisting of 20 m*M* sodium acetate-acetic acid buffer and 0.2 m*M* xylenol orange (XO) (~pH = 4.2), Pb(II) was detected at 572 nm eluting in under 6.5 min. The developed method proved both sensitive and selective and eliminated the need for post-column reaction, replacing this with the novel approach of on-column complexation within the eluent.

This concept of on-column complexation was further investigated with the development of an ion-interaction reversed-phase liquid chromatographic method for the determination of Cd(II) at low μg/L concentrations in environmental water samples. Cd(II) and other matrix metals were separated through on-column complexation with 8-hydroxyquinoline sulphonate, using an octadecylsilica column and an eluent containing 15% acetonitrile, 10-13 m*M* tetrabutylammonium hydroxide (TBAOH), 5 m*M* 8-hydroxyquinoline 5-sulphonic acid (8-HQS) and 10 m*M* acetic acid-acetate buffer (pH 4.8-5.4). Detection was achieved using fluorescence, although once again the inclusion of the fluorescent complexing reagent within the eluent removed the requirement for post-column reaction.

Another form of complexation, in this case stationary phase complexation, was then investigated. The chromatographic behaviour of alkaline earth metals on a chelating/cation exchange high-performance stationary phase was studied using iminodiacetic acid (IDA) bonded silica. It was found that the ionic strength and pH of

the eluent greatly affected both retention time and selectivity by controlling the extent to which either simple cation exchange or surface complexation was responsible for retention. The effect of the ionic strength of the sample was also studied and it was found that utilising the highly selective nature of the IDA phase and by matching the eluent cation with that of the sample matrix, efficient separations of alkaline earth metals in 1.0 *M* NaCl and KCl brines could be obtained without matrix system peaks. The developed chelation ion chromatography was applied to the determination of trace levels of Ca(II) and Mg(II) in medicinal NaCl saline solution and laboratory-grade KCl, using post-column reaction with *o*-cresolphthalein complexone (*o*-CPC).

As a result of the success of the previously developed system, the highly selective nature of the IDA stationary phase was further utilised in the development of a chelation ion chromatographic method for the determination of trace Be(II) in water samples. The resolution of Be(II) from excess concentrations of alkali, alkaline earth and transition metal ions was possible when using the IDA phase through careful control of eluent parameters. The optimum eluent conditions were found to be 0.4 *M* KNO_3, adjusted to pH 2.5 using HNO_3, with detection of Be(II) achieved using visible detection at 590 nm following post-column reaction with 0.26 m*M* chrome azurol S (CAS), 2% Triton-X-100, 50 m*M* 2-(*N*-morpholino)ethanesulfonic acid (MES), pH 6.0. The concentration detection limit for Be(II) was found to be 3 μg/L in a standard solution and 4 μg/L in a tap water sample, using a 250 μl injection loop. The method was successfully applied to a number of fresh water samples of varying matrix complexity, including simulated seawater, and also to a natural freshwater certified reference material (NIST 1640).

Following the above work with alkaline earth metal ions, a further investigation into the selectivity of the same IDA-silica column for transition and heavy metal ions using non-chelating inorganic eluents was carried out. A number of parameters were investigated to determine the exact retention mechanism taking place when using such a chelating ion exchange phase for transition and heavy metal ions and to investigate the control of cation selectivity. The parameters studied were eluent ionic strength and the nature of the inorganic salt used, eluent pH and column temperature. A greater understanding of the above finally led to the development of an isocratic method using a 0.035 *M* KCl, 0.065 *M* KNO_3 (pH 2.5) eluent for the determination of Mn(II), Cd(II), Co(II) and Zn(II) at concentrations between 20 and 121 μg/L in a freshwater certified reference material (NIST 1640).

Finally, a new polymeric chelating ion exchange phase was investigated for the separation of alkaline earth and transition metal ions. The polymer phase was functionalised with a methylenesuccinic acid (itaconic acid) chelating ion exchange group, which like IDA showed a high degree of selectivity for divalent metals ions. As with previous work, the retention behaviour of transition, heavy and alkaline earth metals was investigated under a range of eluent conditions in an effort to evaluate the dominant retention mechanism. Mg(II), Ca(II), Mn(II), Cd(II), Zn(II) and Co(II) could be separated isocratically within 30 min using the itaconic acid phase, with results indicating stationary phase complexation and not ion exchange being responsible for retention of both sets of metal ions.

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ABBREVIATIONS

Capillary zone electrophoresis (CZE).

Chelation ion chromatography (CIC).

Flame atomic absorption spectroscopy (FAAS).

Flow injection analysis (FIA).

Flow injection analysis-inductively coupled plasma-atomic emission spectroscopy (FIA-ICP-AES).

Flow injection-graphite furnace atomic absorption spectroscopy (FI-GFAAS).

Gas chromatography (GC).

Gel permeation (exclusion) chromatography (GPC).

Graphite furnace atomic absorption spectroscopy (GF-AAS).

High performance chelation ion chromatography (HPCIC).

Hydrophobic interaction chromatography (HIC).

Inductively coupled plasma-atomic emission spectroscopy (ICP-AES).

Inductively coupled plasma-mass spectroscopy (ICP-MS).

Ion chromatography (IC).

Liquid chromatography (LC).

Liquid chromatography-inductively coupled plasma-mass spectroscopy (LC-ICP-MS).

Liquid-solid (adsorption) chromatography (LSC).

Reversed-phase high-performance liquid chromatography (RP-HPLC).

Supercritical fluid chromatography (SFC).

Thin layer chromatography (TLC).

Aminomethylphosphonic acid silica gel (AMPASG).

2-(5-Bromo-2-pyridylazo)-5-(diethylamino)phenol (5-Br-PADAP).

2-(5-Bromo-2-pyridylazo)-5-[N-propyl-N-(3-sulphopropyl)amino]phenol (5-Br-PAPS).

Cetyl-trimethyl-ammonium bromide (CTMABr).

2-(5-Chloro-2-hydroxyonaphthylazo)-1,8-dihydroxynaphthalene-3,6-phenylazo-disulphonic acid (Plasmacorinth B) (PCB).

Chrome azurol S (CAS).

1,2-Diaminocyclohexane-N,N,N,'N,'-tetraacetic acid (DCTA).

2-(3,5-Dibromo-2-pyridylazo)-5-diethylaminophenol (3,5-diBr-PADAP).

2-(3,5-Dibromo-2-pyridylazo)-5-[N-ethyl-N-(3-sulphopropyl)amino]phenol (3,5-diBr-PAESPAP).

7-Dodecenyl-8-quinolinol (DDQ).

Diphenylthiocarbazone silica gel (dithizone, DTSG).

Dipicolinic acid (DPA).

Eriochrome blue black R (EBBR).

Heptylamine-hydrochloride (HAHC).

8-hydroxyquinoline 5-sulphonic acid (8-HQS).

Iminodiacetic acid (IDA).

Ion-interaction reagent (IIR).

2-Methyl-8-quinolinol silica gel (MQSG).

2-(*N*-morpholino)ethanesulfonic acid (MES).

N-(1-Naphthyl)-ethylenediamine silica gel (NEDSG).

1-Nitro-2-naphthol-6-sodium sulphonate (126NNS).

2-Nitro-1-naphthol-6-sodium sulphonate (216NNS).

4-(5-Nitro-2-pyridylazo)-5-diethylamino phenol (5-NO_2-PADAP).

4-(5-Nitro-2-pyridylazo)resorcinol (5-NO_2-PAR).

o-Acetoacetylphenol silica gel (diketone, DKSG).

o-cresolphthalein complexone (*o*-CPC).

Porous graphitic carbon (PGC).

Post-column reaction (PCR).

Pyridine-2,6-dicarboxylic acid (PDCA).

1-(2-Pyridylazo)-2-naphthol (PAN).

4-(2-pyridylazo)-resorcinol (PAR).

3-(2-Pyridyl)-5,6-diphenyl-1,2,4-trazine (PDT).

Pyrocatechol violet (PCV).

Quinolin-8-ol (8-HQ).

2-(8-Quinolylazo)-5-(diethylamino)phenol (QADAP).

2-(8-Quinolylazo)-5-(dimethylamino)phenol (DMQAP).

Rare earth elements (REEs).

2-(4-Sulphophenylazo)-1,8-dihydroxynaphthalene-3,6-disulphonic acid (SPADNS).

Tetrabutyl ammonium hydroxide (TBAOH).

Tetraethylammonium (TEA).
Tetra-decyl-dimethyl ammonium bromide (TDDMABr).
Tetramethylammonium (TMA).
Tetraoctylammonium (TOA).
2-(2-Thiazolylazo)-5-diethylamino phenol (TADAP).
1-(2-Thiazolylazo)-2-naphthol (TAN).
Thenoyltrifluoroacetone silica gel (TTASG).
Tri-decyl-dimethyl-ammonium chloride (TDDMACl).
2,4,6-Tripyridyl-1,3,5-triazene (TPTZ).
Xylenol Orange (XO).

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AUTHORS DECLARATION

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Award, presentations and conferences.

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Publications

- Determination of trace cadmium in environmental water samples using ion interaction reversed-phase liquid chromatography with fluorescence detection. B. Paull, E. Twohill, W. Bashir, *Journal of Chromatography A*, **877**, (2000), p.123-132.

- Determination of trace alkaline earth metals in brines using chelation ion chromatography with an iminodiacetic acid bonded silica column. Wasim Bashir, Brett Paull, *Journal of Chromatography A*, **907**, (2001), p.191-200.

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Oral presentations

- The First Annual Conference in Analytical Sciences in Eire, NUIG, April 2000. Title: Separation of alkaline earth metals in iminodiacetic acid (IDA) bonded silica; Ionic strength and pH effects.

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Poster presentations

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- Royal Society of Chemistry, Analytical Research Topics Forum, Norwich, University of East Anglia, Norwich, UK, July, 2001.
  Title: Ionic strength, pH and temperature effects upon selectivity for transition and heavy metal ions when using chelation ion chromatography with an iminodiacetic acid bonded silica gel column and simple inorganic eluents.

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Signed Bashir

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Chapter 1.

Introduction.

Complexation Ion Chromatography.

1.0. Analysis of trace metals in the environment.

Most types of environmental contamination can be classified on the basis of the chemical nature of the contaminating material. Thus most environmental problems can be included in one of five broad categories;

- -Oil and oil waste related pollution.
- -Emergence of a biological oxygen demand from dispersion of organic wastes such as sewage effluents, sludge, etc.
- -Eutrophication of inland waters through soil nitrogen and phosphorus losses.
- -Environmental contamination by specific toxic compounds such as inorganic acids and alkalis, organic pesticides etc.
- -Environmental dispersion of individual elements including metals, whether alone or in combination.

Problems associated with environmental contaminations such as those included in the above categories have resulted in ever increasing concern for the environment and have been influential in changing attitudes to pollution control and monitoring. This has been demonstrated by provision in increased legislation, which has strengthened official demands for monitoring and surveillance schemes designed to control unwelcome emissions.

The term ‘trace’ element/metal refers to those elements which are present at concentrations between 1-100 mg/L (0.01%). ‘Ultra-trace’ refers to concentrations of < 1 mg/L, and the lesser used term ‘minor’ refers to concentrations between 0.01-1%. Certain trace metals require constant monitoring to establish their fate in the environment due to their potential toxicity when released from various anthropogenic sources. Increasing concentrations above natural levels for certain highly toxic elements can have devastating consequences for the local environment [1].

The continual monitoring and analysis of environmental samples not only provides information on the levels of these metals present but also helps to identify their initial source, environmental pathways and fate. For certain elements speciation studies are required to monitor the various forms of the contaminant in the environment and are vital in terms of monitoring the toxicological and environmental impact of the release of these metals into the environment.

For the determination of trace metals atomic absorption spectroscopy (AAS) is a well established and proven technique. However, atomic absorption spectroscopy, whether using a flame or furnace, can only determine one element at a time and no information on elemental speciation is provided. More complex techniques such as inductively coupled plasma-mass spectroscopy (ICP-MS) and inductively coupled plasma-atomic emission spectroscopy (ICP-AES) are both sensitive, multi-elemental analytical techniques. However, these techniques can be subject to mass and spectral interferences from sample matrices and also involve large capital and running costs. In addition, they also provide little information on elemental speciation, unless some form of pretreatment is carried out or hyphenated techniques such as liquid chromatography-inductively coupled plasma-mass spectroscopy (LC-ICP-MS) are used.

Liquid chromatography is an attractive alternative to the above techniques for the determination of metals, particularly in environmental samples. The technique combines the inherent selectivity of a chromatographic method with a large range of chromatographic options, in terms of the mode of separation employed and the type of detector used. A number of substantial reviews on the use of liquid chromatography in all its forms for the determination of metal ions and speciation have been published [2,3,4]. More specifically, advances in the field of ion chromatography (IC) have been summarised by Lucy [5]. This review shows clearly how IC has found wide application in the analysis of environmental samples for metal ions, an application of IC that has also been substantially reviewed by Frankenberger *et al.* [6].

The following Chapter provides an overview of the background and developments in the liquid chromatographic (LC) determination of trace metals in complex sample matrices. In particular, the Chapter details how metal ion complexation has been employed to control selectivity in both reversed-phase and ion exchange liquid chromatographic systems, whether this be via the pre-column formation of metal complexes, the formation of complexes within the mobile phase, or the use of complexing stationary phases.

1.1. Theoretical aspects of liquid chromatography.

Chromatography is a process for the separation of mixtures of substances. It is widely used for the identification and quantification of the components of mixtures. The principle of a liquid chromatographic separation centres upon the partition or differential adsorption/interaction of the individual components of a mixture between two phases. These phases are the mobile phase, in this case a liquid, and a stationary phase, which in most instances is composed of small uniform particles held stationary within the chromatographic column. Once a mixture is introduced into the mobile phase its components are subject to partition, adsorption or other interactions between the two phases as they progress through the column. Each component of the mixture exhibits a different affinity for the stationary phase and as a result they will migrate through the system at differing rates. The component showing the least affinity for the stationary phase will elute from the column first, whereas that which has the strongest affinity for the stationary phase elutes last. Detection of the individual components as they elute from the column allows the separation to be viewed in the form of a chromatogram.

Figure 1.1 shows a typical chromatogram for a mixture of 2 components. The time taken for each component (solute) to reach the detector from the time of injection into the chromatographic system is called the retention time and is given the symbol t_r. The time taken for unretained components of the mixture to pass through column, such as the mobile phase itself, is referred to as the void or dead time, t_m.

The average linear rate of analyte migration (ω) is defined as;

$$\omega = \frac{L}{t_r} \qquad \text{Eqn. 1.}$$

where, L is the length of the column packing.

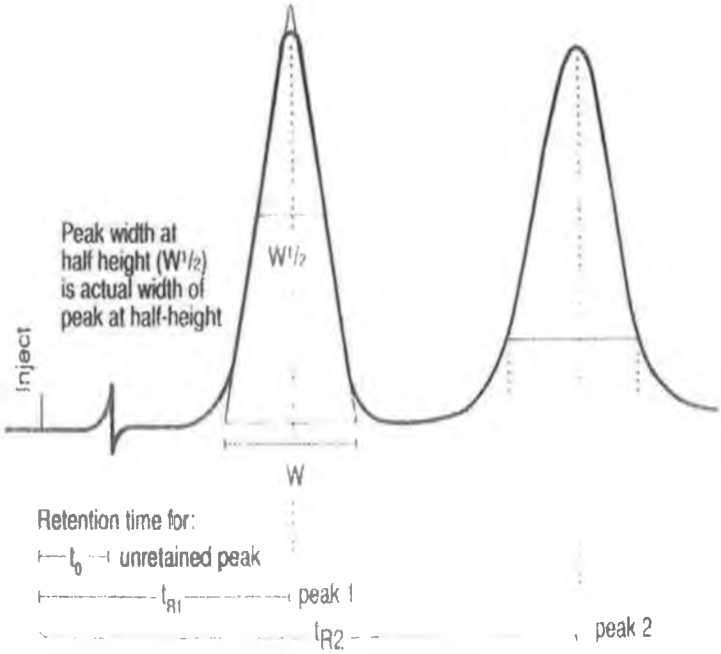


Figure1.1. Parameters used for the characterisation of a chromatographic separation [9].

Similarly, the average linear rate of movement (μ of the molecules of the mobile phase is defined as;

$$\mu = \frac{L}{t_m} \qquad \text{Eqn. 2.}$$

As mentioned previously when a solute enters a chromatographic system, it is instantaneously distributed between the mobile and stationary phase. The concentration of the solute in each phase is denoted by the thermodynamic partition coefficient or partition ratio and is defined as;

$$K = \frac{C_s}{C_m} \qquad \text{Eqn. 3.}$$

where, C_s is the concentration of solute in stationary phase and C_m is the concentration of solute in the mobile phase. If K=1, the solute is distributed equally between the two phases.

For a symmetrical peak, when the peak maximum appears at the column exit, half of the solute has eluted in the retention volume (V_r) and half remains distributed between the volume of mobile phase (V_m) and the volume of stationary phase (V_s).

Thus,

$$V_r C_m = V_m C_m + V_s C_s \qquad \text{Eqn. 4.}$$

or,

$$V_r - V_m = K V_s \qquad \text{Eqn. 5.}$$

The capacity factor (k') is an important descriptive parameter in the characterisation of any chromatographic process. The capacity factor relates the equilibrium distribution of the solute within the column to the thermodynamic properties of the column and to temperature. For a fixed set of operating parameters k' is a measure of the time spent in the stationary phase relative to the time spent in the mobile phase. It is defined as the ratio of the moles of a solute in the stationary phase to the moles in the mobile phase;

$$k' = \frac{C_s V_s}{C_m V_m} \qquad \text{Eqn. 6.}$$

or,

$$k' = K \frac{V_s}{V_m} \qquad \text{Eqn. 7.}$$

Thus, the capacity factor is the additional time a solute band takes to elute, as compared with an unretained solute (for which $k' = 0$) divided by the elution time of an unretained band;

$$k' = \frac{t_r - t_m}{t_m} \qquad \text{Eqn. 8.}$$

A second important descriptive characteristic of a chromatographic system is the separation efficiency. This basically describes the degree to which the migrating band of solute molecules increases in volume as it travels through the column and is expressed in terms of plate theory as the effective plate number (N_{eff}), which can be defined as;

$$N_{eff} = \frac{L}{H} = \left(\frac{t'_r}{\delta}\right)^2 \qquad \text{Eqn. 9.}$$

where, L = length of column, H = plate height, t'_r = adjusted retention time = (t_r-t_m) and δ = band variance.

When related to the measured width of the solute band, the following expression can be applied;

$$N_{eff} = \left(\frac{L}{N}\right) = 5.54\left(\frac{t'_r}{W_{1/2}}\right)^2 \qquad \text{Eqn. 10.}$$

where, $W_{1/2}$ is the peak width at half its height.

The relative selectivity of a chromatographic system for one sample component over another can be expressed in terms of the selectivity factor (α). The selectivity factor for the chromatographic separation of two solutes (t_{r1} and t_{r2}) can be defined as;

$$\alpha = \frac{k'_2}{k'_1} = \frac{t_{r2} - t_m}{t_{r1} - t_m} \qquad \text{Eqn. 11.}$$

where, k'_2 is the capacity factor for the greater retained solute and k'_1 is the capacity factor for the lesser retained solute.

Both of the above parameters, efficiency and selectivity, are important contributing factors to the degree of separation or resolution (R_s) of two adjacent solute bands. Resolution can be determined from a chromatogram by measuring the distance between band peaks (or centres) divided by the average baseline bandwidth (W);

$$R_s = \frac{t_{r2} - t_{r1}}{0.5(W_2 + W_1)}$$ Eqn. 12.

The Purnell equation relates resolution to three separate parameters, column selectivity factor (α), capacity factor (k'), and the theoretical plate number (N);

$$R_s = \frac{\sqrt{N}}{4}\left(\frac{\alpha - 1}{\alpha}\right)\left(\frac{k'}{k'_{av} + 1}\right)$$ Eqn. 13.

From the above expression it can be concluded that the greatest improvement in resolution is achieved through manipulation of column selectivity rather than column efficiency. Column selectivity is based upon the chemical interactions within the stationary and mobile phases, whereas efficiency is related to predominantly physical effects within the stationary phase. Increasing the capacity factor causes the solute to spend more time in the stationary phase and so emphasises differences in selectivity. However, increasing k' to improve resolution is limited by excessive run times [7].

1.2. Modes of liquid chromatography.

The above equations used to describe chromatographic separations can be applied to chromatograms obtained using each of the five modes of liquid chromatography. These being (a) liquid-solid (adsorption) chromatography (LSC), (b) liquid-liquid (partition) chromatography, (c) gel permeation (exclusion) chromatography (GPC), (d) ion exchange chromatography and (e) affinity chromatography. The principles of each of the above modes of liquid chromatography are briefly outlined in the following sections.

1.2.1. Liquid-solid (adsorption) chromatography.

This form of chromatography, which often is referred to as adsorption chromatography, essentially involves interaction between solute and fixed active sites that are either immobilised or part of the surface of a stationary phase. In column chromatography this stationary phase is predominantly a finely divided solid reversed-phase). One function of the adsorbent is to provide a high surface area, increasing the number of interactions occurring and maximising the selectivity of the phase. In most cases adsorption chromatography involves polar normal-phase stationary phases, with non-polar mobile phases such as hexane being frequently used. Selectivity can be manipulated through inclusion of small concentrations of more polar modifiers into the mobile phase such as propan-3-ol. The polar functional groups in the sample interact with the active sites on the polar stationary phase, active surface silanol groups in the case of silica gel (e.g. dipole-dipole interactions). The solute is displaced from the stationary phase by the polar components within the mobile phase, with the more polar molecules being more strongly adsorbed and hence being retained for longer within the column. Compounds that are best suited for separation by this technique are those that are soluble in organic solvents and are polar but non-ionic.

1.2.2. Liquid-liquid (partition) chromatography.

Liquid-liquid or partition chromatography is based upon the partitioning of the solute between two liquid phases, one mobile and one stationary. Selectivity is based upon the relative solubility of the solutes within each liquid phase. Partition chromatography can be classified according to the manner in which the stationary phase is immobilised within the chromatographic column. The liquid stationary phase can be (i) coated or (ii) bonded to an inert stationary phase support. With 'coated' partition chromatography, the stationary phase comprises of a liquid, which by virtue of physical adsorption adheres to the surface of the inert support. With a bonded phase the stationary phase is chemically attached (covalently bonded) to the surface of the support. In both techniques, separation is based on the relative polarities of the mobile and stationary phase. Initially, partition liquid chromatography involved the use of highly polar stationary phases immobilised on alumina and silica particles with non-polar mobile phases (normal phase). In complete contrast to normal phase chromatography, reversed-phase chromatography consists of a non-polar stationary phase and a relatively polar mobile phase. This was first investigated using non-polar liquids coated onto inert supports, although this was rapidly replaced with bonded phases. Reversed-phase liquid chromatography is discussed in more detail in Section 1.3.

1.2.3. Gel permeation (exclusion) chromatography (GPC).

Exclusion chromatographic methods allow the separation of solutes based upon differences in the molecular size and shape of the solutes. Therefore, this is a physical separation method as opposed to chemical interactions discussed above. The nature of the stationary phase is highly porous, with pore size predominantly determining which solutes are retained, as it is the pore size which governs which components of a sample will penetrate into the interior of the gel particles, this being the retention mechanism. Large molecules will be restricted from entering the gel network and pass through the column unretained. In contrast, small molecules can penetrate into the gel lattice and are retained. This technique can be utilised for the separation of both organic and inorganic substances, extending to studies of complex biochemical highly polymerised molecules [8].

1.2.4. Ion exchange chromatography.

Ion exchange chromatography is used extensively for the separation and determination of ionic species. It can be divided into cation and anion exchange chromatography depending on the nature of the stationary phase. Ion exchange chromatography is based on the exchange of ions of like charge between a liquid mobile phase and a charged stationary phase. The charged stationary phase contains active/counter ions that are capable of exchanging reversibly with ions of like charge in the surrounding mobile phase without any appreciable change occurring in the exchanger material. Most ion exchangers are polymeric in nature, although functionalised silica gels are also used. The ion exchangers are functionalised with either acidic or basic groups, resulting in either a positive or negative surface charge that is effectively neutralised by the charges of the counter ions. These counter ions are cations in a cation exchanger and anions in an anion exchanger.

Hence a cation exchange resin consists of an acidic group immobilised within a polymeric support and associated counter cations, while an anion exchange consists of a basic group immobilised within a polymeric support with associated counter anions. The mobile phase (eluent) consists of a weak aqueous solution of an electrolyte. If cations (M^{n+}) are to be separated, a column capable of exchanging cations will be used with an acidic eluent composed of mineral or organic acids. In such a system the counter cations are hydronium ions (represented below as simply H^+). The exchange reaction can be represented as follows;

$$[\text{Eluent}]M^{n+} + [\text{Resin-}SO_3^-]H^+ \rightarrow [\text{Eluent}]nH^+ + [\text{Resin-}SO_3^-]M^{n+} \qquad \text{Eqn.14.}$$

Similarly, if separation of anions (A^{n-}) is required, an anion exchange column with a basic eluent is used, such as dilute solutions of hydroxide or carbonate.

$$[\text{Eluent}]A^{n-} + [\text{Resin-}NR_3^+]OH^- \rightarrow [\text{Eluent}]nOH^- + [\text{ResinNR}_3^+]A^{n-} \qquad \text{Eqn.15.}$$

The distribution of inorganic ions between an ion exchange resin and solution is determined by a number of factors that affect the degree of electrostatic attraction between the ion and the immobilised ionic group. These being;

(1) Nature of exchanging ions;

(a) At low aqueous concentrations and room temperature the extent of exchange increases with increasing charge of exchanging ion i.e. Na(I) < Ca(II) < Al(III) < Th(IV).

(b) When the charge is constant and similar conditions used, for singly charged ions, the extent of exchange increases with decrease in size of hydrated cation, thus, Li < H <Na <NH_4 < K < Rb < Cs.

(c) For doubly charged ions the ionic size is an important factor but incomplete dissociation of salts or the formation of weak ion pairs also plays a part, thus, Cd(II) < Be(II) < Mn(II) =Zn(II) < Cu(II) = Ni(II) < Co(II) < Ca(II) < Sr(II) < Pb(II) < Ba(II).

(d) The extent of exchange for singly charged anions on strongly basic anion exchange resins varies with the size of the hydrated ion in a similar manner to that for cations.

(2) Nature of ion exchange resin;

The exchange of ions will depend upon the nature of the functional groups in the resin (i.e. strong or weak acid/basic groups) and the degree of cross-linking of the polymer itself [9,10].

1.2.5. Affinity chromatography.

Affinity chromatography is based upon the use of immobilised biochemicals as stationary phases which are often referred to as affinity ligands. These can be divided into two groups, specific and general. The specific ligands such as antibodies, bind to only one particular solute. General ligands, such as lectins, bind to certain groups of solutes.

The basic principle can be illustrated as follows; a sample containing enzymes is applied to the column which contains inhibitors in the presence of a mobile phase that has the correct pH, ionic strength and composition to allow solute-ligand binding. This solvent is referred to as the application buffer. The inhibitors are covalently bonded to an inert support material. As the sample progresses through the column, the enzyme molecules complementary to the affinity ligand molecules are adsorbed while the other components elute without retention. These adsorbed molecules are then eluted by means of an elution buffer.

Types of affinity chromatography include:

- Bioaffinity chromatography which employs a biological molecule as the affinity ligand which has been employed to purify enzymes.
- Immunoaffinity chromatography in which the ligand is an anti-body or antibody related reagent and is a popular tool for biological purification and analysis including methods for the isolation of antibodies, hormones viruses and sub-cellular supports.
- Dye-ligand affinity chromatography in which a synthetic triazine or triphenylmethane dye-ligand affinity is used as the immobilised ligand. This type of ligand is ideal for the purification of enzymes and proteins.

- Immobilised metal ion affinity chromatography whereby the ligand is a metal ion complexed with an immobilised chelating agent. This approach has been employed to separate proteins and peptides.
- Analytical affinity chromatography, which can be applied to study solute-ligand interactions.

Other methods related to this technique include:

- Hydrophobic interaction chromatography (HIC), which is based upon the interactions of peptides and proteins with short nonpolar chains on a support and,
- Thiophilic adsorption chromatography, also referred to as covalent or chemisorption chromatography which makes use of immobilised thiol groups for solute retention [11].

1.3. Reversed-phase high performance liquid chromatography (RP-HPLC).

High performance liquid chromatography is unquestionably one of the most extensively used analytical techniques due to its inherent selectivity, sensitivity and quantitative analytical performance. HPLC instrumentation is generally considered simple and robust, consisting of several components, each of which has an individual function critical to the overall result obtained. A schematic diagram of a generalised HPLC system is shown in Figure 1.2.

Reversed-phase HPLC employs a non-polar stationary phase operated with a polar, mixed aqueous/organic mobile phase (the opposite to normal phase chromatography), and is mainly used for analytes which readily dissolve in such eluents.
The most popular choice of stationary phase is that of the non-polar, hydrophobic organic species (e.g. octyl, octadecyl or phenyl groups) covalently bonded to the surface of a silica support. The mechanical strength of silica means the column can withstand high back pressures, and silica based columns generally exhibit good mass transfer characterisation. An additional merit of such silica supports is that they can be easily modified due to the reactive nature of the SiOH sites. However, such stationary phases are limited to operating within the pH range of approximately 2-8, (unlike

polymeric stationary phases), because of the possibility of hydrolysis of the bonds at the lower pH and the solubility of the silica at high pHs.

Chemically bonded silica stationary phases are now commonly used, with their synthesis optimised to reduce the number of surface silanol groups and metal impurities. Additionally manufacturers are improving the hydrolytic stability of the stationary phases in aqueous mobile phases and synthesising stationary phases with more predictable and controllable selectivities toward different types of analytes. Generally, in RP-HPLC, retention times increase with an increase in the number of C atoms present in the bonded stationary phase.

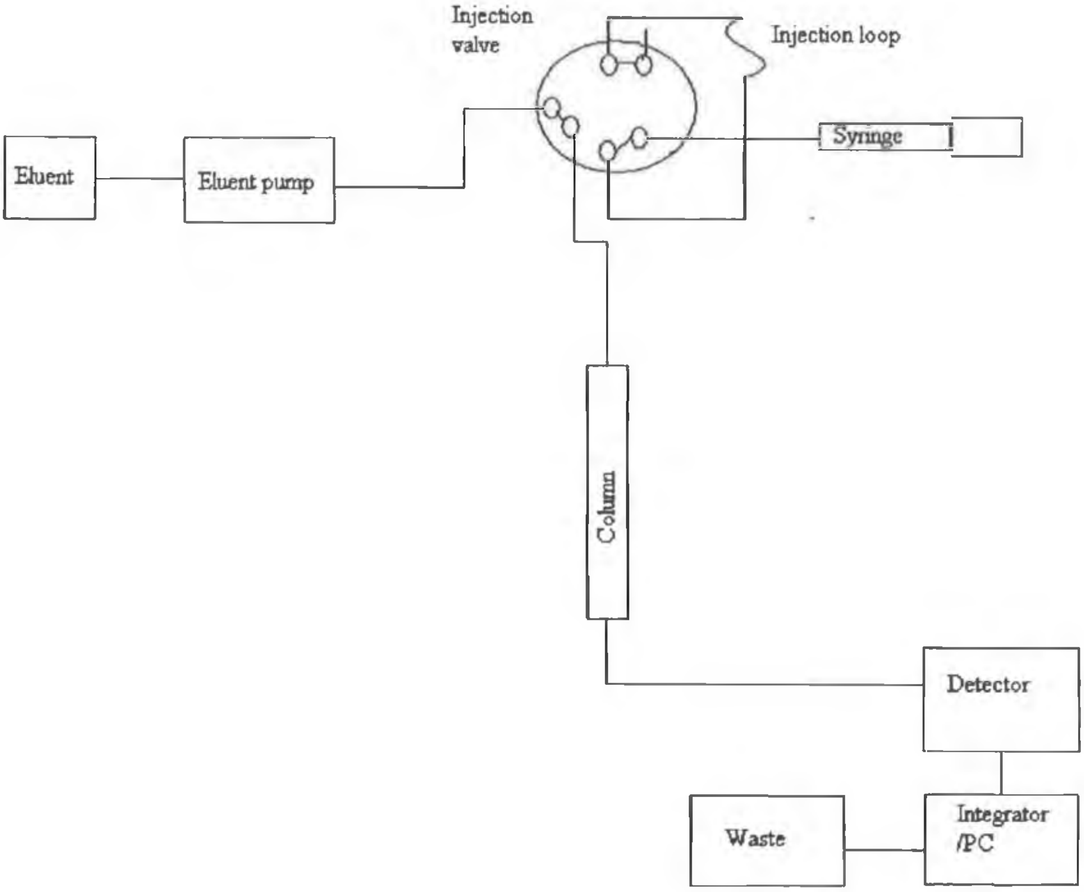


Figure 1.2. A schematic diagram of a generalised HPLC system [12].

The nature of the mobile phase is one of the most important parameters in RP-HPLC. The polarity of the mobile phase used will have a significant effect upon retention. It can also promote or suppress ionisation of the analyte molecules, and it also can shield accessible residual silanol or other active adsorption centres on the adsorbent surface. Proper selection of the mobile phase is the second most important step in the development of the separation method (the first being the type of adsorbent used). The main requirement for the mobile phase is that it must be able to dissolve the analytes up to concentrations suitable for detection. Variation of the eluent composition provides the greatest flexibility in the development of RP-HPLC separations.Variation of the eluent composition allows the chromatographer to adjust the retention times of target components in the mixture. Usually this adjustment is to keep the capacity factors between 1 and 10. In general, by changing the mobile phase polarity it is possible to change a components retention significantly; from elution within the dead volume, up to the retention in hours. At the same time, the selectivity of the separation will not necessarily change to such a great extent.

Organic modifiers frequently employed to fine tune retention are shown in Table 1.1. The eluotropic strength of these modifiers increases according to;

methanol < ethanol ~ acetonitrile < tetrahydrofurane

The analytes are eluted in order of polarity with the most polar eluting first. RP-HPLC has gained vast popularity for a number of reasons:

- Samples with a wide range of polarities can be separated.
- Ionic or ionisable compounds can be separated by using ion pairing techniques (as will be discussed in section 1.3.1).
- The columns used are extremely efficient and stable and therefore the variation in analysis from column-to-column or day-to-day is small.
- The method employs fairly inexpensive mobile phases with quick equilibration times for the column.
- It is generally easier, faster and more reproducible than other HPLC modes [12,13].

Table 1.1. The eluting strength of organic modifiers.

Methanol	Decreasing polarity
Acetonitrile	
Ethanol	
Isopropanol	↓
Dimethylformamide	
Propan-1-ol	
Dioxane	
Tetrahydrofurane	Increasing elution power

1.3.1. Reversed-phase ion-interaction liquid chromatography.

This technique is essentially a modification of RP-HPLC, which is used to effect the separation of ionised or ionisable species on reversed-phase columns. This technique overcomes problems associated with the retention of difficult solutes such as those, which are very polar, multiply ionised, and/or strongly basic or acidic.
A number of different names have been proposed for this technique including ion-interaction chromatography, soap chromatography, dynamic solvent generated ion-exchange chromatography, ion-pair RP-HPLC and ion-modified RP-HPLC [14].

Hydrophilic ionic solutes such as inorganic anions and cations exhibit very little affinity for lipophilic stationary phases when used with the usual RP-HPLC mobile phases. Addition to the mobile phase of a lipophilic reagent of opposite charge to the solute ion, facilitates retention and separation of the analyte components (see below). Ion-interaction chromatography can be performed on a number of stationary phases, including neutral polystyrene divinylbenzene polymers and bonded silica materials with C_8, C_{18}, phenyl and cyano groups as chemically bound functionalities. It is essential that the majority of ion-interaction reagents (IIRs) have;

- Appropriate charge which remains unaffected by eluent pH.
- Sufficient lipophillic properites to permit adsorption onto non-polar

stationary phases.
- Compatible nature with eluent components and detection systems.

Cation separation is affected using strong acid anions, such as the aliphatic sulfonate ion, as the IIRs. Anions are separated using strong base cations, such as tetraalkyl ammonium ions, as the IIR.

Three retention models have been proposed to describe the actual retention mechanism. These are;

- The Ion-pair model.
- The dynamic ion exchange model.
- The Ion-interaction model.

The ion-pair model essentially states that an ion-pair is formed between the solute ion and the IIR, as shown in Figure 1.3. This process predominantly occurs in the aqueous/organic mobile phase. A neutral ion-pair forms as a result of this interaction which is then capable of adsorbing onto the lipophilic stationary phase in a manner which resembles how a neutral molecule with some lipophilic character is retained in reversed-phase chromatography.

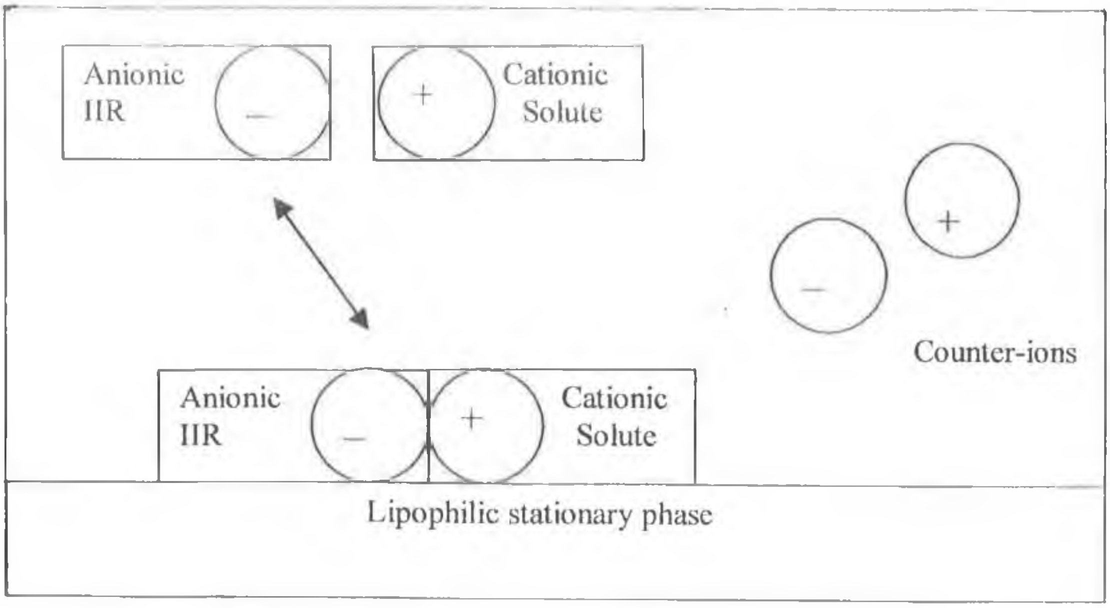


Figure 1.3. The ion-pair model [14].

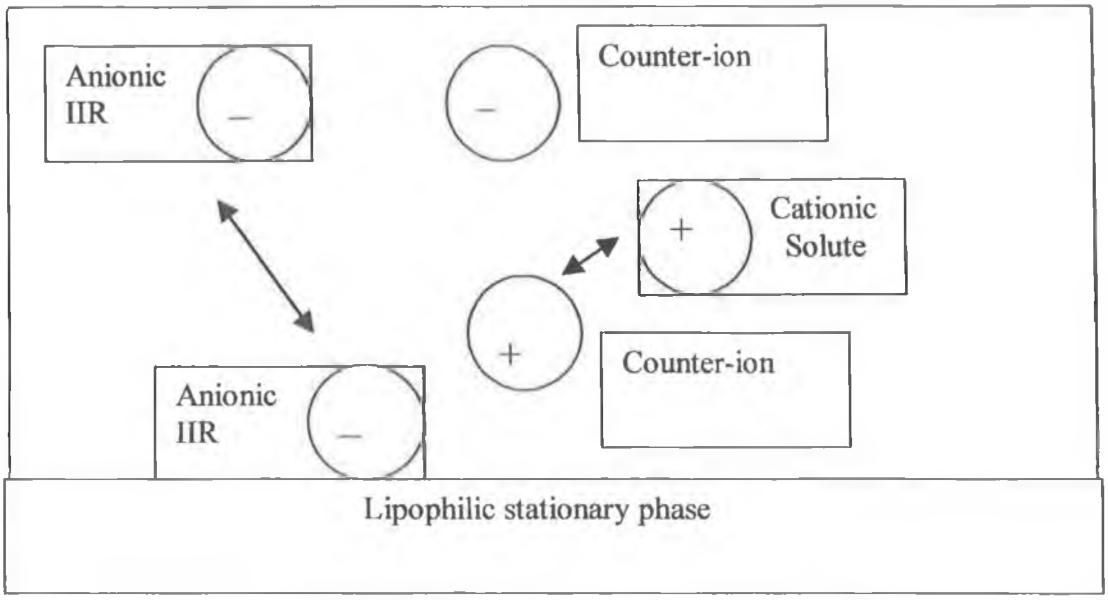


Figure 1.4. The dynamic ion exchange model [14].

(2) The dynamic ion exchange model, shown in Figure 1.4, on the other hand suggests the formation of a dynamic equilibrium between the IIR in the eluent (E) and the IIR adsorbed onto the stationary phase (S), as depicted by equation 16.

$$IIR^{+/-}\ (E) \longleftrightarrow IIR^{+/-}\ (S) \qquad \text{Eqn.16.}$$

The stationary phase now has a charge associated with its surface by virtue of the adsorbed IIR. The stationary phase is now capable of operating as an ion exchanger. The organic modifier content or the mobile phase determines the amount of IIR adsorbed, whereby lower percentages result in higher IIR concentrations on the stationary phase. There is a dynamic interchange of IIR occurring between the mobile phase and stationary phase and hence the model is termed as dynamic ion-exchange. Retention occurs by virtue of the conventional ion exchange mechanism. Solutes which have the same charge as the IIR are repelled from the charged stationary phase surface, while retention of neutral solute is unaffected by the IIR.

Therefore, the retention times of solute is dependent on;

- The dynamic ion exchange capacity of the column.
- The lipophilicity of the IIR.
- The % organic modifier in the eluent.

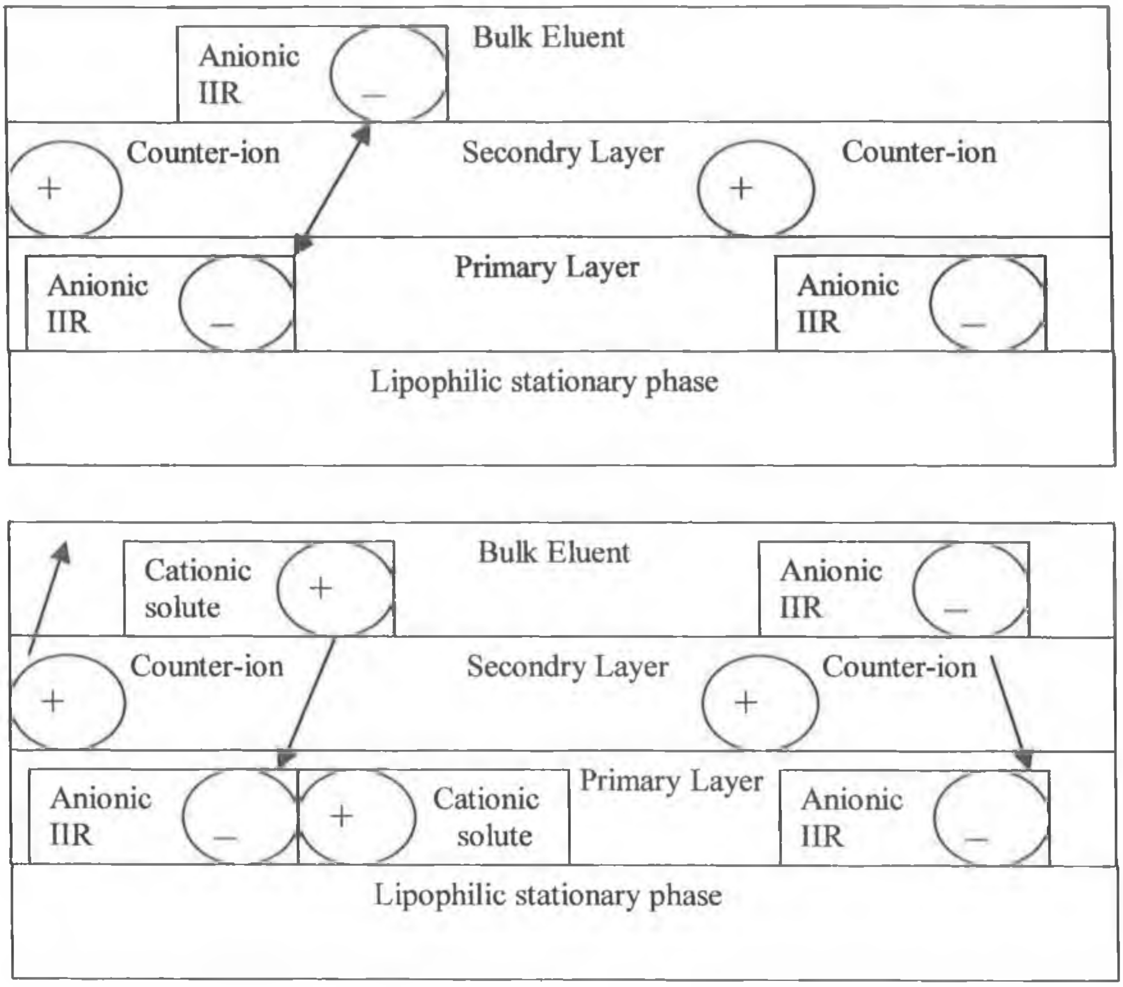


Figure 1.5. The ion interaction model [14].

The ion-interaction model, shown in Figure1.5, is an intermediary between the previously mentioned models and involves both electrostatic and adsorptive effects. An electrical double layer is formed at the stationary phase surface by virtue of the dynamic equilibrium mentioned above. Repulsion effects result in the IIR ions being uniformly adsorbed over the stationary phase. These adsorbed IIR ions result in a single layer of charge at the stationary phase surface. To this primary layer, a secondary layer of oppositely charged ions are attracted.

This secondary layer consists of ions of opposite charge (counterions) to the IIR ions. The resultant concentration of charge in these layers is dependent upon the following factors;

- Amount of IIR adsorbed.

- Lipophilicity of % IIR.
- Concentration of % organic modifier in eluent.

Electrostatic and solvophobic effects are responsible for the transfer of solutes to the stationary phase and hence effect separation. Stahlberg has proposed similar retention models in his review [15].

1.4. Ion Chromatography.

In 1975 Small and associates developed a novel strategy which permitted the separation and detection of cationic and anionic species using ion exchange chromatography combined with suppressed conductivity detection (see Section 1.5.1.i.) [16]. This technique was licensed to Dionex Corporation and was marketed under the terminology 'ion chromatography'.

Today ion chromatography (IC) is the phrase used to describe a wider variety of LC techniques applied to the determination of ionic solutes. These may be inorganic anions and cations, low molecular weight carboxylic acids and bases or ionic organometallic compounds.

Liquid chromatographic techniques which could be included in the wider definition of IC include;

- Ion exclusion chromatography.
- Ion exchange chromatography (discussed in section 1.2.4).
- Ion-interaction (or ion-pair RP-HPLC) chromatography, (discussed in section 1.3.1).
- miscellaneous separation methods involving the separation of ionic species.

Each of these above techniques can be coupled to one or more of the following detection methods, which will be discussed in section 1.5;

- Conductivity detection.

- Electrochemical and potentiometric detection.
- Spectroscopic/spectrophotometric detection.

Modern IC is applicable to a wide range of environmental samples [6], including rain water, seawater, brines, river and stream water, aerosols, soils and geological materials, all of which has been extensively reviewed by Haddad and Jackson [14].

1.5. Detection systems in IC.

A number of detection systems have been employed for use with IC depending on the analytes of interest. Efficient separations coupled to sensitive detectors are requisites for successful applications of IC.

1.5.1. Conductivity detection.

One of the most commonly used modes of detection in IC is conductivity, which is regarded as the classic IC detector and based on the simple principle of measuring the eluate conductivity, which is proprotional to ionic concentration. Its relative sensitivity decreases as the specific conductivity of the eluent increases. Conductivity can be used in either of two modes, direct or indirect. Direct conductivity detection is used mostly for separations of anions using low conductivity eluents such as potassium phthalate [17] and sodium benzoate [18]. Cations can also be determined in this way using organic bases. Indirect detection can be applied to anions using hydroxide eluents [19] and cations using mineral acids [20].

1.5.1.i. Suppressed conductivity detection.

In ordinary ion exchange chromatography, the eluent usually has a high background conductance making the sensitive detection of lower conducting ions difficult. This problem was overcome by employing a suppressor to reduce this eluent conductance. Initially this was achieved using a suppressor column, although today membrane based suppressors are commonly used.

For cation exchange, where the separator column is a strong acid cation exchanger, the suppressor column would be packed with a strong base anion exchanger, with a

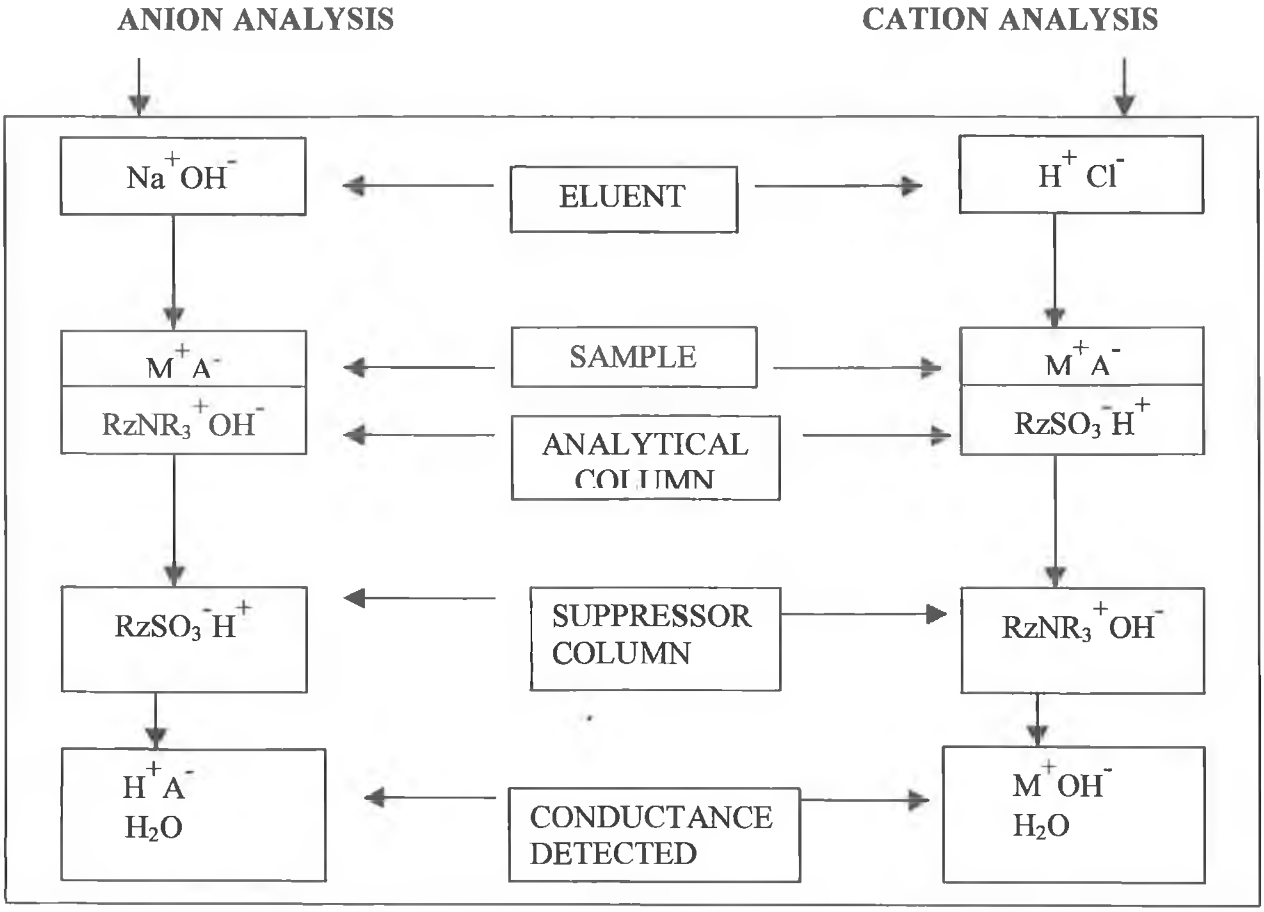


Figure 1.6. Principles of suppression in IC [21].

large anion exchange capacity, and charged in the OH^- form. The principles of suppression in IC are shown in Figure 1.6. The first column in which the separation takes place, called the separator column, is a cation exchange column.The eluent from this column passes directly into the second column, the suppressor column, where the conductivity of the eluent is reduced through an ion exchange process. Once this is accomplished, the eluate passes into the conductivity detector, and the conductance from each cation is determined. The successful suppression of the conductivity of the eluent depends upon the type of electrolyte used.

An example is shown below which considers the suppression of an HCl eluent. Eqn.18 shows how the solute cation, Na^+, passes through the suppressor unaffected;

$$\text{Resin-OH}^- + H^+ + Cl^- \longrightarrow \text{Resin-Cl}^- + H_2O \qquad \text{Eqn.17.}$$

$$\text{Resin-OH}^- + Na^+ + Cl^- \longrightarrow \text{Resin-Cl}^- + Na^+ + OH^- \qquad \text{Eqn. 18.}$$

The eluent is converted into water resulting in a sharp reduction of the eluent background, whilst the conductance of the sample is increased as a result of replacement of Cl^- ion by OH^- ions, because the hydroxide ion has an exceptionally high ionic equivalent conductance.

As mentioned above, direct conductivity detection can be quite insensitive because of limiting equivalent ionic conductances of certain ions compared to the high conductance of many eluents. This problem can be eliminated by means of suppressing the background conductance of the eluent as previously mentioned. Some examples of where suppressed ion chromatography has been used for the determination of anions and cations are given below.

Betti *et al.* developed a system for the determination of alkali and alkaline earth metal ions in sea water using suppressed IC. The procedure, which involved column switching with an eluent step change, allowed the simultaneous determination of Li(I) at low µg/L levels, K(I), Mg(II) and Ca(II) at mg/L levels (after a 50-fold dilution), and Na(I) separately after a 2000-fold dilution. A dynamic range of several orders of magnitude was obtained for all the metal ions investigated with % R.S.Ds lower than 1% [22].

Hodge *et al.* used a suppressed conductivity detection system for the determination of nine metal ions Li(I), Na(I), NH_4 (I), K(I), Rb(I), Cs(I), Mg(II), Ca(II), and Sr(II) in brine samples. A gradient of sulphuric acid (H_2SO_4), was required as the eluent (from 8.25 m*M* to 11 m*M*). No pretreatment was required and results compared well with complex and expensive instruments such as inductively coupled plasma-optical emission spectrometry (ICP-OES) [23].

Kaiser *et al.* used suppressed detection with high volume direct injection, which allowed sub µg/L levels of anions and cations to be detected without any significant loss in peak effeciency or resolution, even when a 1300 μl injection loop was used. The method was applied to amine treated waters [24].

Rohrer *et al.* determined low μg/L levels of Ca(II) and Mg(II) in brine (30% NaCl) using suppressed conductivity detection. Preconcentration of Ca(II) and Mg(II) was required on a MetPac chelating column and after Na(I) was rinsed from the column, the metal ions of interest were separated on a cation exchange column using methanesulphonic acid eluent. Limits of detection of 5 μg/L Ca(II) and Mg(II) were obtained in the brine samples, with R.S.Ds of below 2.5% obtained for peak areas [25].

Ohta and Tanaka employed suppressed conductivity detection for the simultaneous determination of common inorganic anions and some divalent cations which included Mg(II), Ca(II), Mn(II), Co(II), Ni(II), Zn(II) and Cd(II), usng 0.15 m*M* pyromellitate eluent at pH 5.4. The metal ions were eluted within 30 min with limits of detection between 0.73-2 μ*M* for the anions and 0.21-4.7 μ*M* for the cations. The system was applied to the river and rain water samples [26].

1.5.1.ii. Non-suppressed conductivity detection.

The non-suppressed conductivity detection means the sample components pass directly to the detector from the column and its configuration parallels that of traditional HPLC, whereby the analytical column is directly coupled to the detector. In non-suppressed IC, ion exchangers with low ion-exchange capacity are generally used in conjunction with dilute eluents. The eluent used should provide an eluting ion with a high-exchange selectivity and one which has a large difference in limited ionic equivalent conductance, compared with the analyte ion. The net effect of such eluents of dilute concentration and strong eluting ion, is a reduction in the background conductance yet reasonable elution times for the analyte ions.

With such systems it is quite possible that, when combined with the availability of a good quality detector with low internal volume, detection limits are often comparable to those obtained using suppression.

Ohta *et al.* developed one such indirect detection system for the analysis of mono- and divalent cations, Na(I), NH_4(I), K(I), Mg(II), Ca(II) in under 25 min. The method was applied to a range of natural water samples of differing pH values, including acid rain, brown forest soil water and natural lake waters using a tartaric acid and methanol eluent at acidic pH. Limits of detection for monovalents were 0.1 μM and 0.05 μM for divalents [27].

1.5.2. Electrochemical and potentiometric detection.

Electrochemical detection describes a range of techniques which involve the application of an electrical potential via suitable electrodes to a sample solution, followed by measuring of the resultant current. Both direct and indirect modes are applicable. Techniques used in this area include voltammetry, amperometry and coulometry which have the common characteristic of a chemical reaction taking place during the measurement. Voltammetry is a current-voltage technique. The working electrode potential is varied and the resultant current is measured as a function of the applied potential. With amperometry a fixed potential is applied to a working electrode and the resulting current from either oxidation or reduction reactions occuring at the working electrode is measured. Coulometry or high-efficieny amperometry is carried out on working electrodes of larger surface area (as opposed to a small surface area in amperometry). Electrochemical detection has been applied in situations where extreme sensitivity and selectivity is required. Transition metals have been determined by Girard [28], Hsi and Johnson [29] and Rivello *et al.* [30] in standards and sea water matrices using indirect coulometry, reverse pulse polarography and indirect amperometry respectively.

Potentiometric detection has some distinguishing features which renders it as more suitable as a chromatographic detector. It has been applied to the determination of ionic species through the use of ion selective electrodes. The principle is based on a change in potential at the indicator electrode being measured with respect to a reference electrode under a zero current flow. The potential of the indicator electrode changes with the concentration of a particular ion in solution contacting the electrode, thereby providing a means for the determination of the ion concentration. Isildat and Covington have reported the application of such ion-selective electrodes for potentiometric detection in ion chromatography [31]. Haddad *et al.* [32,33] have looked at transition metal detection using potentiometry, with limits of detection between 1-10 nmol [34].

1.5.3. Spectroscopic detection.

Spectroscopic detection can be divided into molecular or atomic spectroscopic techniques. Molecular spectroscopy includes UV-visible spectrophotometry, refractive index measurements and photoluminescent techniques. Atomic spectroscopy includes atomic emission and absorption spectroscopy.

Most of the above methods can operate in direct and indirect modes. With direct UV-Vis spectrophotometry simple inorganic cations are not detectable with great sensitivity because they possess small molar absorptivities and hence they are often separated as complexes with suitable ligands such as EDTA [35]. Haddad and Foley employed non-suppressed IC using simultaneous direct conductivity and indirect UV absorption detection. The authors investigated a range of protonated aromatic bases for the separation of alkali metal ions and ammonium, and the system was applied to the separation of Ca(II) and Mg(II) in seawater. Low μg/L detection limits were obtained using both detection modes [36]. Indirect detection is based on the principle that the eluent ion has higher molar absorptivity than the solute ion when both are univalent.

The coupling of ICP-MS to IC has become a powerful technique for the analysis of environmental samples and various other matrices due to its selectivity and excellent sensitivity. Seubert has reviewed on line coupling of IC with ICP-AES and ICP-MS and has compared applications of the techniques [37].
Atwood and Wallwey coupled IC with inductively coupled argon plasma spectroscopy (ICAPES), and were successful in determining alkaline earth metal ions at 100 μg/L in scaler dissolver samples which contained excess Na(I) and K(I) [38].

Williams *et al.* have used IC-ICP-MS to analyse soil and sediment samples for As(III) and V(V) [39]. Mannion and Pantsar-Kallio further developed this methodology for Cr(III) and Cr(VI) in waste-water samples [40]. Haddad and Jackson have comprehensively reviewed this technique with numerous applications detailed [14]. Finally, Buchberger has reviewed the detection techniques used with IC in detail [41].

1.5.4. Post-column detection.

Post column detection is also used extensively with IC, which involves the chemical reaction of solutes as they are eluted from the column prior to passage to the detector. The chief aims are to enhance the specificty and sensitivity of the detection so as to allow low concentrations of analytes to be detected, or selectivity detected if present in high concentrations of interferences. Requirements of the post column reagent include; rapid reaction between the metal ion and the reagent, so as to maximize the product concentration and minimise extra column band broadening; the product of the reaction should be strongly absorbing ($E_{max} > 10,000$) at a wavelength at which the uncomplexed reagent does not absorb; it must be miscible with the eluent without the formation of a precipitate. If a complexing agent is employed within the eluent, then the post-column reaction (PCR) reagent must form a stronger complex in order to displace the eluent ligand from the metal. Dasgupta has critically reviewed post-column reaction systems used by various workers [42]. A schematic diagram of a post column reaction system is shown in Figure 1.7.

1.5.4.i. Spectrophotometric Post-column detection.

One of the most widely used reagents for post-column reaction detection of metal ions is 4-(2-pyridylazo)-resorcinol (PAR), structure shown in Figure 1.8(a), which at alkaline pH forms a red-coloured complex with the majority of metal ions, absorbing over the wavelength range of 450-550 nm. The metal ions form mostly1:2 complexes with this reagent according to the following equation;

$$M(II) + 2PAR^{-} \longleftrightarrow M(PAR)_2 \qquad \text{Eqn. 19.}$$

The reason for the widespread use of this reagent is due to its sensitivity and versatility. Fritz and Arguello compared a number of reagents including Methylthymol blue, Sulphonazo(III), Eriochrome Black T, PAR, PAR/ZnEDTA, Titan Yellow, Murexide, and Arsenazo(I) and (III) [43].

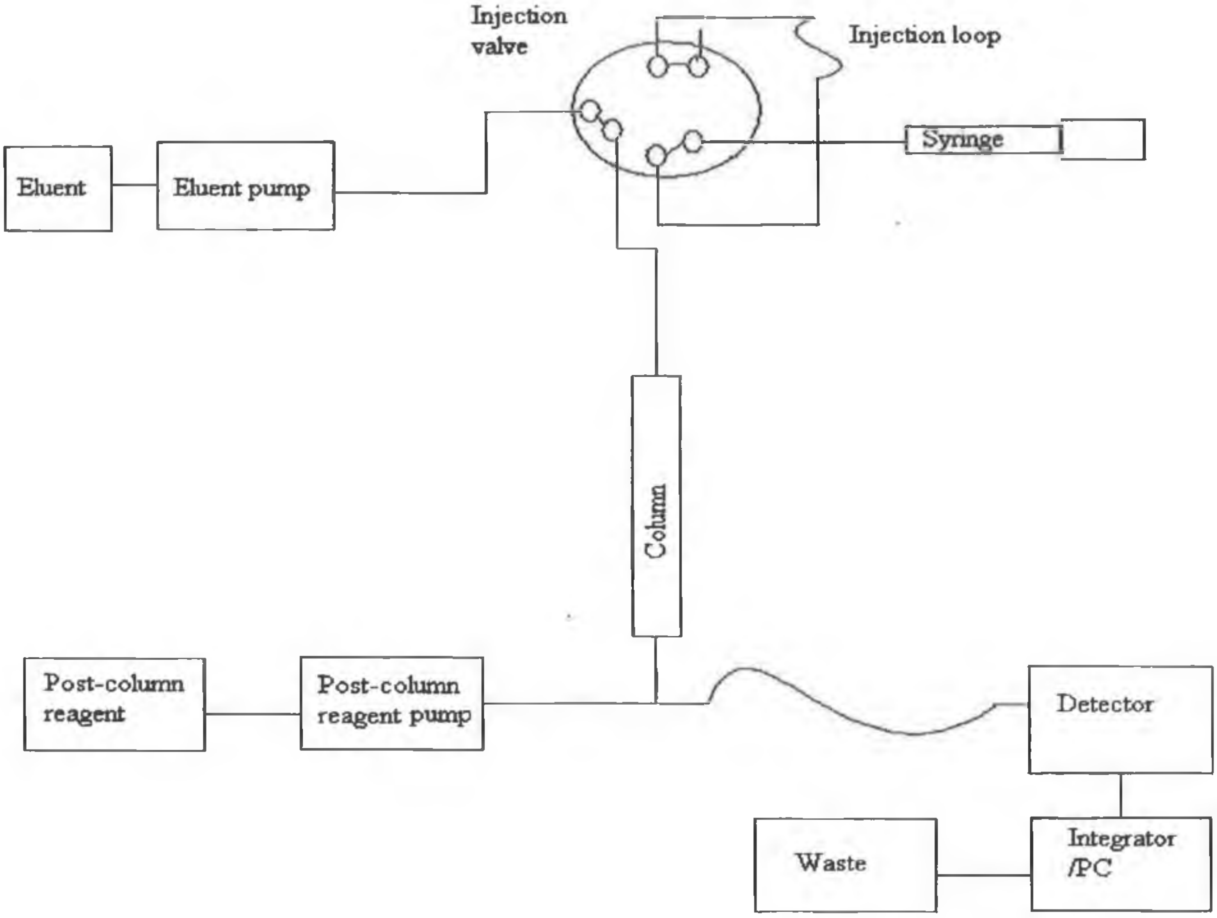


Figure 1.7. A schematic diagram of an HPLC PCR system [12].

PAR/ZnEDTA was ideal for the detection of metal ions which were not very sensitive using PAR alone, such as Ca(II) and Mg(II), this being based on the metal displacement reaction shown below;

$$\text{Mg(II)} + \text{ZnEDTA} + \text{PAR} \longleftrightarrow \text{Mg(II)EDTA} + \text{ZnPAR} \qquad \text{Eqn. 20.}$$

Similar work has been performed by Lucy and Dinh, who carried out an in study into the kinetics and equilibria of the above reaction. They discovered that the kinetics and sensitivity of this system were dependent upon the auxiliary complexing agents employed. Ethylenediamine and ammonia were investigated, with the conclusion that ethylenediamine was more effective than ammonia for the determination of alkaline earth metal ions. The choice of this complexing agent resulted in a 2 to 9 fold improvement in sensitivity, and kinetics for the reactions were much faster [44].

Freiser and Jezorek compared the responses and sensitivities of PAR and PAR/ZnEDTA for fourteen metal ions. They reported a 12 to 32 fold increase in sensitivity for the determination of alkaline earth metals when replacing PAR with PAR/ZnEDTA. When applied to the determination of heavy and transition metals there was a decrease in sensitivity for Zn(II), Ni(II), Cu(II), Al(III), and Fe(III) whereas for Pb(II) and Mn(II) responses were considerably increased [45].

Work by Co *et al.* was based on altering the selectivity of PAR with the addition of complexing agents to the PCR, which masked the response to one or more metal ions. With the addition of EDTA the reaction between the PAR and certain metal ions was completely suppressed. Nitrilotriacetic acid (NTA) was also investigated and was found to lower the response to specific transition metal ions and under certain conditions completely suppress the response to the lanthanides. With the addition of this ligand a competitive equilibrium was established, as shown below;

$$ML_n + 2PAR^- \longleftrightarrow M(PAR)_2 + n(L) \qquad \text{Eqn. 21.}$$

The selectivity of the PCR now becomes dependent upon the relative stabilities of the metal ions with the ligand and with PAR [46].

O' Connell *et al.* have looked at lead using on-line preconcentration and analysis with a porous graphite column using acidified aqueous eluents and post column derivatisation with PAR. The method was applied to river water analysis with Pb(II) eluting after 20 min and results comparing well with FAAS. Excellent linearity was obtained over the range 0.3 to 4 mg/L [47].

Heberling *et al.* separated rare earth elements (REEs) on an pellicular latex agglomerated resin based ion exchanger with PAR as PCR. However, the sensitivities decreased and the rare earth elements were not completely resolved even when a gradient of oxalic and diglycolic acids was applied [48]. In subsequent work Jones and Hemmings separated rare earth elements using ion-interaction liquid chromatography. The sensitivities of the REEs was increased and individual REEs were completely resolved [49].

Vera-Avila and Camacho separated REEs on a reversed-phase column with octanesulfonate, lactic acid and acetonitrile as the eluent, and PAR as PCR. 13 lanthanides were well separated within 25 min using a pH gradient from 2.80 to 3.50, with the disadvantage being that europium and samarium co-eluted. There is also significant interference by transition metals as they reacted with the PCR, which meant that there was a considerable problem with the method when applied to lanthanides at low concentrations [50].

Hwang *et al.* used an Arsenazo(III) based post-column detection system, structure shown in Figure 1.8(b), for the determination of rare earth metals in sand samples and high purity rare earth metal oxides [51].

Fritz and Smith have separated Mg(II) and Ca(II) within 3 min in hard water with detection using Arsenazo(I) as the PCR. The selective nature of the ligand meant the method was relatively free from interferences under to the conditions employed. The method was very sensitive with limits of detection of 2.3 and 6.3 ng for Mg(II) and Ca(II) using a 20 μl loop [52].

Jones *et al.* developed an ion-interaction method for the determination of REEs, using Arsenazo(III) as the PCR, with elution using oxalic acid and tetrabutyl ammonium hydroxide (TBAOH) as the IIR. This resulted in the formation of anionic metal oxalic acid complexes which were separated on a C_{18} reversed phase column. These anionic complexes eluted in reverse order to ion-exchange which allows the separation and determination of traces of REEs on either side of large matrix or individual REE peaks. The elution order was from La to Lu in 30 min. Eventhough transition metals did not interfere with the separation and determination of the REEs, there was considerable interference by large amounts of alkaline earth metals with reduction in resolution and also with resultant peaks becoming much broader. Limits of detection were in the order of 0.01 mg/L [53].

Oguma *et al.* separated REEs in silicate rocks by preconcentration on a cation exchange column and separation on a C_{18} column using gradient elution with glycolate. The IIR used in this incident was 1-octanesulfonate with post column detection using Arsenazo(III) at 650 nm. The % recoveries were very high between 93 and 96% with absolute limits of detection between 6-10 ng [54].

1.5.4.ii. Chemiluminescence Post-column detection.

Post-column chemiluminescence detection can also be applied to trace analysis of various metal ions based on the luminol-hydrogen peroxide system. Neary *et al.* applied this technique for the detection of Cu(II) and Co(II) with a luminol post column reagent, with sensitive limits of detection for both metals [55].

Williams *et al.* later developed a more complex system which required two separation columns and four reagent pumps. However, sub µg/L detection limits were obtained for Cr(III) and Cr(VI) [56].

This system was then simplified by Jones and Beere to three pumps and only one column [57]. Jones *et al.* also developed a system for Co(II) determination in rice certified reference material. A limit of detection of 0.5 ng/l was obtained using a 200 µl loop, without any preconcentration being required [58]. Gammelgaard *et al.* used a similar method for Cr(III) and Cr(VI) with detection limits of about 0.1 µg/L [59].

1.5.4.iii. Fluorescence Post-column detection.

Fluorescence detection also allows extremely sensitive detection of particular metal ions without preconcentration being required. Limits of detection are often 10-20 times lower then those achieved using absorbance detection systems. This high sensitivity results from the difference in wavelengths between the excitation and emission radiation [60].

Jones and co-workers have used the ligand 8-hydroxyquinoline-5-sulphonic acid (8-HQS) for the sensitive detection of Al(III) Ga(III), In(III), Cd(II), Mg(II), and Sn(II). The method resulted in a limit of detection of 1 μg/L for Ga(III) [61].

The above technique was applied to Al(III) in tap water and certified reference material monel alloy. Excellent linearity was obtained over the range 5-10000 μg/L, with limits of detection of 1 μg/L, with no reported interferences [62].

This work was later advanced by to allow speciation of Al(III) to be carried out in both natural and potable waters at μg/L levels. Organically bound Al(III) fluoro-species and labile monomeric Al(III) species could also be determined [63].

The above method was then applid by Jones and Paull to the speciation of Al(III) in potable waters. In this case labile hydroxy Al(III) was determined in tap water between 29-96 μg/L [64].

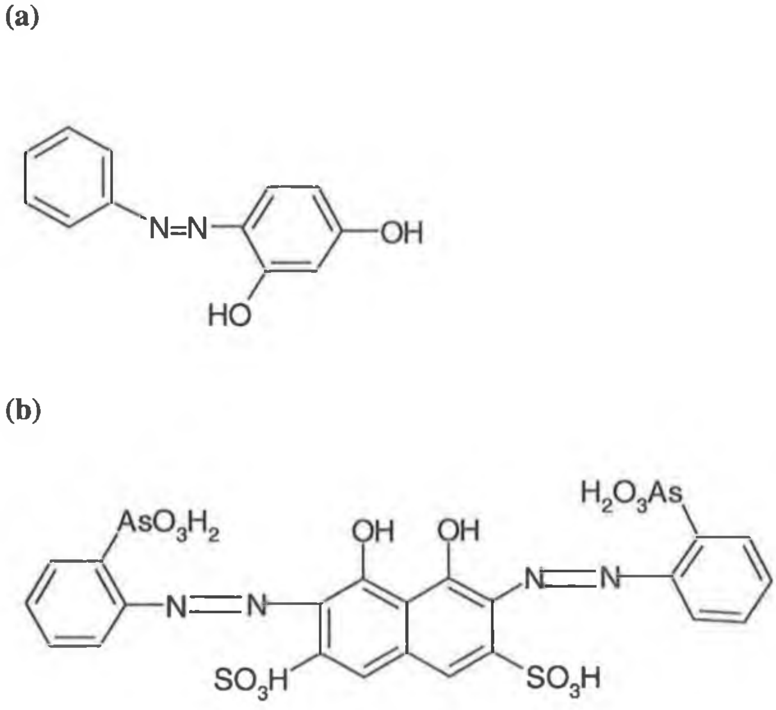


Figure 1.8. Structures of (a) 4-(2-pyridylazo)-resorcinol (PAR) and (b) Arsenazo III.

Lucy and Ye have further extended this idea to fluorescence detection based on a metal ion displacing Mg(II) from the $Mg(CDTA)^{2-}$ complex. This liberated Mg(II) which reacted with 8-hydroxyquinoline-5-sulphonic acid (8-HQS) to form an intensely fluorescent complex [65].

Finally, Williams and Barnett have looked in more detail at displacement reactions as discussed previously. Separation of metal ions was followed by mixing of the eluting ions with 8-HQS/MgEDTA complex. This allowed complexation of Mg(II) with 8-HQS, which is then detected. Linearity covered at least 2 orders of magnitude with sensitive limits of detection in the µg/L region. A number of alkaline earth, transitions, heavy metals and lanthanides were detected using this method [66].

1.6. Metal complexation and stability.

1.6.1. Principles of metal ion complexation.

Most metal atoms have the ability to serve as a central atom in a metal ligand complex, although some metals do this more readily than others. When the central atom is bound to neighbouring atoms by the covalent bonds, a complex is formed as a result of the acceptance of an electron pair by the metal ion from each non-metal atom. The bond between the co-ordinating atom and the metal atom is called a co-ordinate bond. Some metal atoms have the ability to contribute electrons to the bond as in π bonding. In some cases ligands can bind to the metal atom by one or more donor atoms to form a heterocyclic ring referred to as a chelate ring. The molecule or ion from which it is formed is known as a chelating agent. The process of forming a chelate ring is known as chelation. Any metal complex in which one or more chelating agents are present is defined as a metal chelate.

The nature and oxidation state of the central metal atom in a metal chelate governs the properties of the complex. The oxidation number influences the structure and properties of the complexes formed by the metal. In a covalent compound of known structure the oxidation number of each atom is the charge remaining on the atom when each shared electron pair is assigned completely to the more electronegative of the two atoms sharing it. A pair shared by two atoms of the same element is split between them. The co-ordination number has been defined as the number of donor or ligand atoms directly bound to a central metal atom and is dependent upon the nature of the donor atom; at other times on the oxidation state of the metal. The higher the co-ordination number the more of a chance it is associated with cationic complexes with lower numbers often resulting in anionic complexes. In the case of light metals the maximum co-ordination number is 4. However it rises to 6 in the second row e.g. $[Mg(H_20)_6](II)$ ion.

Two conditions are required for a molecule being able to behave as a chelating agent: Firstly, it must possess at least two donor atoms that are capable of combining with a metal atom by donating a pair of electrons. These electrons may originate from basic coordinating groups such as NH_2, or acidic groups that have lost a proton. Typical acidic groups that complex with metal atoms by replacement of hydrogen are;

$-COOH$, $-SO_3H$, $-OH$.

Some co-ordinating basic groups include;

$-NH$, $-NH_2$, $=NOH$, $-NH$, $-N=$, $-S-$

Secondly, these functional groups must be situated in the molecule such that they permit the formation of a partial ring structure with a metal as the enclosed atom. In certain cases steric factors influence chelation. For example one chelating atom may form a bond with a metal atom readily enough, but the formation of a second or third coordinate bond may be sterically hindered [67]. The simplest types of chelating molecules are those which coordinate metal atoms via 2 donor or ligand atoms. To describe this type of molecule the term bidentate chelating ligand is used. Thus, in forming a metal chelate a multidentate chelating agent may not involve all its donor atoms. A more detailed classification of chelating agents is based on the nature of the functional groups, which are described as either basic or acidic. Bidentate ligands are thus divided into three groups, namely those with;

(i) Two acidic groups – e.g. oxalic acid, shown in Figure 1.9(a).

(ii) Two basic groups – e.g. ethylenediamine shown in Figure 1.9(b).

(iii) One acidic and one basic group – e.g. glycine.

Other ways of classifying chelating molecules includes rigid (e.g. oxalic acid, 1,10-phenanthroline) and flexible (e.g. EDTA), which is an essential characteristic of all sexadentate chelating molecules. Bidentate chelating molecules may also be grouped on the basis of their donor atoms, O,O O,N and N,O.

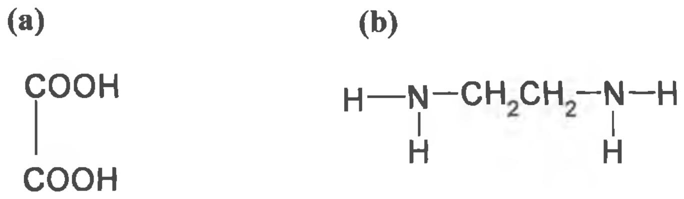


Figure 1.9. Structures of (a) oxalic acid and (b) ethylenediamine.

1.6.2. The stability of metal chelates.

The solution stability of a metal complex has an equilibrium of the type;

$$M + nL \leftrightarrow ML_n \qquad \text{Eqn. 22.}$$

where M is the metal ion and L is the ligand.

By applying the law of mass action to the above equation, an equilibrium constant shown below is obtained;

$$\beta_n{}^{o} = \frac{\{ML_n\}}{\{M\}\{L\}^n} \qquad \text{Eqn. 23.}$$

where $\beta_n{}^{o}$ is the overall thermodynamic stability constant for the reaction and $\{M\}$ is the activity of the species M.

Eqn. 22 almost always takes place in a stepwise manner shown ;

$$M + L \leftrightarrow ML \qquad \text{Eqn. 24.}$$

$$ML + L \leftrightarrow ML_2 \qquad \text{Eqn. 25.}$$

$$ML_2 + L \leftrightarrow ML_3 \qquad \text{Eqn. 26.}$$

$$ML_{(n-1)} + L \leftrightarrow ML_n \qquad \text{Eqn. 27.}$$

The step stability constant $Kn°$ for the last step is;

$$Kn° = \frac{\{ML_n\}}{\{ML_{(n-1)}\}\{L\}} \qquad \text{Eqn. 28.}$$

The constant that is determined experimentally is usually the concentration or stoichiometric stability constant ($\beta_{n°}$) in which concentrations of the species [ ] replace activities. Thus;

$$\beta n = \frac{[ML_n]}{[M][L]^n} \qquad \text{Eqn. 29.}$$

By applying the law of mass action to eqns 24 to 27 we obtain the following step constants;

$$K_1 = \frac{[ML]}{[M][L]} \qquad \text{Eqn. 30.}$$

$$K_2 = \frac{[ML_2]}{[ML][L]} \qquad \text{Eqn. 31.}$$

$$K_n = \frac{[ML_n]}{[ML_{n-1}][L]} \qquad \text{Eqn. 32.}$$

$$\beta n = K_1 K_2 K_3 .....K_n \qquad \text{Eqn. 33.}$$

The reciprocal of β_n is the instability constant and is occasionally used in describing metal complexes. Metal complexes may be roughly classified on the basis of speed with which equilibrium between metal ion and ligands is attained. The stability constant involves the ratio of velocity constants, one for the forward and one for the reverse reaction. The importance of stability constants is that they give an understanding of the behaviour of metal chelates in solution. Information about concentrations of different species of a metal complexes in equilibrium mixtures is invaluable in the study of analytical separations such as ion-exchange chromatography.

The stability constant refers to a system in equilibrium, it may be used to calculate the free energy change (ΔG) and other thermodynamic functions of the reaction involved by means of the equation;

$$-RT\ln K = \Delta G = \Delta H - T\Delta S \qquad \text{Eqn. 34.}$$

where R is a constant, T is temperature, ΔH is enthalpy change, ΔS is change in entropy. Experimentally enthalpy change (ΔH) can be determined by measuring the stability constant at a series of temperatures [68].

Either expression is useful, K_n providing information concerning the relative concentrations of the various species, whereas β_n conveys information on all the complexed species present combined. An assumption of the above stability constants is the presence of only a single ligand species in solution and that it is existing in a 100% ionised form. This however is not the case for a number of ligands and metals, which can exist as a number of species dependent upon the conditions "conditional" in solution, usually being pH. A change in slight pH can alter the concentration of the ligand in its complexing form in solution, by changing the degree of ionisation of the ligand. This would cause a change in the overall stability constant (K_n) also referred to as the conditional stability constant K'_n.

The expression for the conditional stability constant can be defined using:

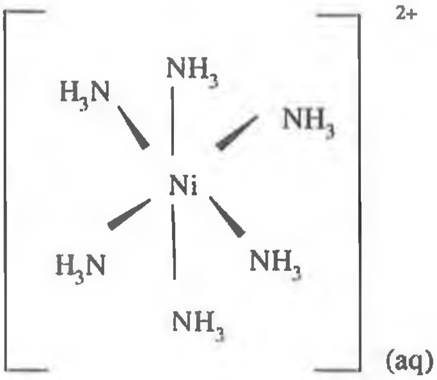
$$K'_n\,(ML) = K'_{M'L'} = \frac{[ML]}{[M'][L']} \qquad \text{Eqn. 35.}$$

where M' is the concentration of all the metal in solution that has not reacted with the ligand, and not just the concentration of free metal ions present. Similarly, L' represents the concentration of all species of the complexing agent not bound to the metal. Therefore, K'_n is not only governed by thermodynamics, as the experimental conditions play a vital role, with the solution pH determining these K'_n values of the metal chelates.

Metal chelates are inherently more stable than closely related non-chelate complexes. This describes the chelate effect, which can be illustrated using the following two compounds; For example, consider the reaction of Ni(II) with (i) six molecules of ammonia, shown by Eqn. 36 and (ii) with three molecules of ethylenediamine, (en), shown by Eqn. 37, where the latter is the more favourable reaction. By combining these two reactions together we get Eqn. 38. We know that $K=10^{9.7}$ and using Eqn. 34 above we calculate that $\Delta G = -RT\ln K = -67$kJ/mol $= \Delta H - T\Delta S$. Therefore, we can calculate that $\Delta H = -12$ kJ/mol and hence $-T\Delta S = -55$ kJ/mol. Both entropy and enthalpy favour the chelate complex, but it should be noted that the entropy contribution is of greater importance.

It has been noted that the chelate effect is essentially an entropy effect for the following reason. In solution the Ni(II) ion co-ordinates to six water molecules. Once the N ligands are involved in the co-ordination the result is that 6 water molecules are liberated. In one case six NH_3 molecules lose their freedom and there is no change in the number of free molecules whereas in the other situation, only three en molecules lose their freedom, and thus there is an increase of 3 free individual molecules in solution. There is a greater degree of disorder with the reaction of three en molecules than with the six ammonia molecules and hence ΔS is more positive and more favourable in the former case than the latter case [69].

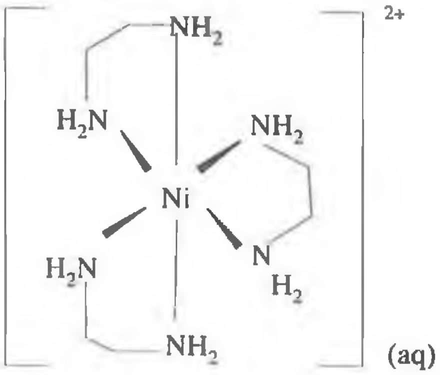
$Ni^{2+}(aq) + 6NH_3(aq) \rightleftharpoons$



Eqn. 36.

$\beta = 10^{8.6}$

$Ni^{2+}(aq) + 3H_2NCH_2CH_2NH_2(aq) \rightleftharpoons$



Eqn. 37.

$\beta = 10^{18.3}$

$$Ni(NH_3)_6^{2+}(aq) + 3(en)(aq) \rightleftharpoons Ni(en)_3^{2+}(aq) + 6NH_3\ (aq)$$

Eqn. 38.

$K = 10^{9.7}$

1.7. Chelating Resins for trace metal preconcentration.

The simplest way of dealing with interfering species in the determination of metal ions is to remove them from the sample. This allows in most cases quantitation to be performed of the metal of interest, provided a sufficiently sensitive and selective detector is used. One of the commonest approaches to metal preconcentration and matrix removal is the use of chelating resins. These function on the basis of the formation of a co-ordination complex between the metal ion and an immobilised chelating functional group. Selectivity of such groups towards metal ions can be controlled by manipulation of pH which alters the conditional stability constants of the metal-liquid complex formed.

1.7.1. Applications of iminodiacetate (IDA) based resins.

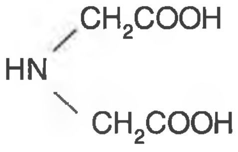


Figure 1.10. Structure of iminodiacetic acid (IDA).

One of the commonest chelating functional groups used in chelating resins is that of IDA, (shown above in Figure 1.10). The most widely used IDA resin is Chelex-100 due to its strong preference for transition metals over alkali and alkaline earth metals. At pH>4 maximum cation chelation occurs. At pH< 2 the resin displays anion exchange properties. However, swelling and contraction of the resin at high and low pH due to its low % crosslinking is often a problem.

The first published application of this resin was by Riley and Taylor [70], which triggered many further investigations, including Oslen *et al.* who employed a flow injection analysis (FIA) system coupled to flame atomic absorption spectrometry (FAAS) for determination of trace levels of heavy metals in sea-water, using Chelex-100 for preconcentration [71]. In this study, Oslen *et al.* developed a very reliable and rapid technique for analysing a large number of sea-water samples for Pb(II), Cd(II), Zn(II) and Cu(II). Limits of detection were quoted as 10 µg/L for Pb(II) and 1 µg/L for Cd(II) and Zn(II) . However, there was a problem of non-linear responses obtained for Cd(II), probably due to the formation of cadmium-chloro-complexes in the seawater matrix.

Wada *et al.* [72] compared the preconcentration of metal ions on IDA and aminophosphonate bonded resins, employing Chelex-100 and Duolite ES-467 respectively. The method was applied to Ca(II) and Mg(II) determination in brines using FIA. Chelex-100 required only dilute eluents for the elution of the ions and hence this was the more suitable resin. Determination was based upon spectrophotometry after complex formation with 1-(2-hydroxy-4-diethylamino-1-phenylazo)-2-hydroxynapthalene-3,6-disulphonic acid. Preconcentration of 9 mls of 2.5 *M* NaCl allowed 1-30 µg/L Mg(II) and 8-120 µg/L Ca(II) to be determined.

Moller *et al.* preconcentrated rare earth elements on Chelex-100 from sea-water with detection achieved using ICP-MS. Deep sea water samples were analysed with quantitative recoveries ranging between 85-110% for all metal ions studied [73].

Fernandez *et al.* analysed sea-water for Cd(II) and Pb(II). An automatic preconcentration step/system was coupled to a GF-AAS where the preconcentration step was performed using Chelex-100 immobilised within the injection tip of the autosampler. Limits of detection were 1 and 8 µg/L for Cd(II) and Pb(II) respectively. Analysis of the certifed reference material CASS-3 showed good agreement with certified values [74].

Noresson *et al.* developed an alternative low capacity chelating phase, based on IDA and a cross-linked agarose. The material named Novarose, was used for the preconcentration of Cu(II) and Cd(II), followed by ICP-AES detection. High and moderate affinities were observed for the above metal ions respectively. The advantage of this low capacity material was that easy removal of matrix interferences was possible. Cu(II) resulted in 100% recovery, whereas with Cd(II) the recovery was dependent upon the matrix composition, adsorbent capacity and flow rate [75].

Hashemi and Olin further extended work with Novarose, studying equilibrium and kinetic properties through its incorporation in a FIA-ICP-AES system. They discovered that it was about 50 times faster than Chelex-100 for the preconcentration of Ni(II), Cu(II), Cd(II), and Ca(II). Extremely fast flow rates of up to 110 ml/min allowed quantitative recoveries of trace amounts Cd(II) and Cu(II) in tap water [76].

The on-line preconcentration and separation of Mn(II), Pb(II), Ni(II), Co(II), Cd(II) and Cu(II) from pond and sea-water on an iminodiacetic acid-ethylcellulose (IDAEC) membrane using FI-ICP-AES detection has also been performed. The % recoveries were between 87 and 101%, which showed that matrix interferences were minor with this membrane sampling system. This, along with the advantage of a high sample throughput of 30 samples per hour showed how successfully a IDA membrane could be applied to metal ion preconcentration [77].

Horvath investigated the preconcentration of Cd(II), Cu(II), Co(II) and Pb(II) on the same membrane and compared it to Chelex-100 and carboxymethylated polyethyleneimine-polymethylenepolyphenylene isocyanate (CPP1). The IDAEC was most successful in terms of efficiency and long-term performance when analysing water, sea-water and urine samples by FIA-ICP-AES. Detection limits were improved considerably in comparison to the work performed by Olsen *et al.*[72]. Cd(II) was detectable as low as 0.25 μg/L and Pb(II) at 1.15 μg/L, which was an improvement of at least one order of magnitude [78].

Many more papers have been published comparing various chelating resins with chelex-100 for trace metal applications. Ryan and Weber [79] compared Chelex 100 with three alternative chelating agents bonded onto porous glass silica beads for the preconcentration and removal of Cu(II). The immobilised agents consisted of quinolin-8-ol (8-HQ), bis(dithiocarbamate) (DTC) and N-propylethylenediamine. In the presence of organic matter the DTC performed well, removing about 98% of the Cu(II). However, this Cu(II) was not readily recovered from the substrate. Chelex-100 gave the poorest results with only 62-75% Cu(II) being removed, with 8-HQ producing the best.

Table 1.2. Application of iminodiacetate (IDA) based resins [82].

Metals	Sample	Column	Detection	L.O.D.	Reference
Cd(II),Cu(II), Pb(II),Zn(II)	Seawater	Chelex-100	FI-FAAS	Pb(II) 10 µg/L Cd(II),Zn(II) 1 µg/L	83
Al(III),Ba(II),Be(II), Cd(II),Fe(II),Cu(II), Co(II),Mn(II),Ni(II), Pb(II),Zn(II)	Aqueous standard	Chelex-100	FI-ICP-AES	0.008-120 µg/L	83
Al(III),Cr(III),Fe(II), V(V)	Aqueous standards	Muramac A-1	FI-ICP-AES	Not reported	84
Cd(II),Co(II),Cu(II), Mn(II),Ni(II),Pb(II), Zn(II),	Seawater	Chelex-100	FAAS	Not reported	85
Cd(II),Cr(III),Cu(II), Fe(II),Mn(II),Pb(II), Zn(II)	Environmental samples	Muramac A-1	FI-FAAS, GF-AAS	0.002-0.72 µg/L	86
Cu(II),Cd(II),Fe(II)	Seawater	Chelex-100	FAAS	0.002-0.072 µg/L	87
Cd(II),Co(II),Cu(II), Fe(II),Mn(II),Ni(II), Pb(II),V(V)	Synthetic matrices and seawater	Chelex-100	FI-ICP-MS	0.0004 –1 µg/L	88
Cd(II),Cu(II),Co(II), Pb(II)	Water,seawater and urine	IDA-ethyl cellulose	FI-ICP-AES	0.022-0.5 µg/L	89
Cr(III),Cu(II),Mn(II)	Seawater	Chelex-100 and Lewatit TP 207	GF-AAS	Not reported	81
Pb(II)	Seawater	Chelex-100 and hydride generation	ICP-AES	6 ng/L	91
Pb(II)	Seawater	Chelex-100	ICP-MS	0.8 ng/L	92
Al(III),Cd(II),Co(II), Cu(II),Fe(II),Mn(II), Ni(II),Pb(II),Zn(II)	Fresh water and sea-water	Chelex-100, Sep Pak C18 and Fractogel DEAE	ICP-MS	0.001-3 µg/L	93
Alkaline earth and transition metals	Concentrated brines	MetPac CC-1	FI-ICP-MS	Not reported	94
Co(II),Cu(II),Mn(II), Ni(II),Zn(II)	Seawater	MetPac CC-1	Spectrophotometry, GF-AAS	0.05-0.15 µg/L	95
Cd(II),Pb(II)	Seawater	IDA resin	Spectrophotometry	Cd(II) 2ng Pb(II) 6ng	96
Co(II),Cu(II),Mn(II),	Seawater	MetPac CC-1	FI-ICP-MS	Not reported	97
Cr(III),Cr(VI)	Aqueous standards	Cellex P, Cellex CM, Chelex-100, Varion KS and Cellex T	FI-FAAS	Cr(III) 0.78 µg/L Cr(VI) 1.4 µg/L	98
Al(III),Zn(II)	River water and snow	Chelex-100	ICP-MS GF-AAS	Not reported	99

Siriraks *et al.* compared Chelex-100 with a Dionex Metpac CC1 chelating ion exchange resin, both of which contain the IDA functional group, for uptake of transition and heavy metals from sea water. As previously mentioned Chelex -100 suffered from swelling under certain conditions which meant that Metpac CC1, due to its higher degree of cross linkage, gave more reliable results [80].

Finally, Baffi *et al.* compared Chelex -100 with Lewatit-TP 207 for the preconcentration of Mn(II), Cu(II) and Cr(III) from sea water with detection using AAS. Lewatit TP 207 gave better results for Cr(III) preconcentration, being able to recover colloidally bound and labile species. However, much lower recoveries for Cu(II) in comparison with Chelex 100 were obtained. Chelex-100 was unsuitable for the quantitative preconcentration of Cu(II) in presence of organic and colloidal species, whereas for Mn(II) determinations the resins performed similarly [81].

Other applications of IDA are shown in Table 1.2.

1.7.2. Applications of Quinolin-8-ol (8-HQ) resins.

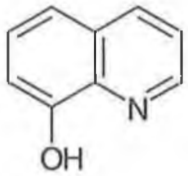


Figure 1.11. Structure of Quinolin-8-ol (8-HQ).

Quinolin-8-ol (8-HQ), (shown above in Figure 1.11.) can form chelates with over 60 metal ions under specific pH conditions. Malamus *et al.* used columns containing 8-HQ chemically immobilised on porous glass for the preconcentration of metal ions [100]. Marshell and Mottola critically studied the use of 8-HQ bonded silica gel. The ion-exchange capacity and reproducibility was very high with results for determination of Cu(II) in water samples determined at ng/ml levels [101]. Wantanabe *et al.* [102] employed the same chelating phase for the analysis of Cd(II), Co(II), Fe(II), Mn(II), Ni(II), Pb(II) and Zn(II) in sea-water using ICP-AES. A very sensitive method was developed which resulted in limits of detection < 0.1 µg/L for all the metal ions mentioned.

Orians and Boyle [103] have used a quinolin-8-ol chelating ion exchange resin for the determination of Ga(III), In(III) and Ti(IV) in sea water with ICP-MS detection. The extreme sensitivity of this method allowed limits of detection below µg/L to be achieved. Later work by Azeredo *et al.* reported the preconcentration of Cd(II), Cu(II), Fe(II), Mn(II), Ni(II), Pb(II) and Zn(II) from seawater on a silica-immobilised 8-hydroxyquinoline mini-column using FIA-GFAAS system. Quantitative recoveries were obtained for all the metal ions with an analysis rate of 10-20 samples per hour. Absolute limits of detection were between 0.3 and 10.2 pg for the above metal ions [104].

The majority of methods utilising 8-HQ involve direct chelation of trace metals onto a column of the immobilised chelating agent. However it is often also possible to complex the analytes directly in solution with 8-HQ followed by pre-concentration of the complex itself onto a suitable substrate e.g. bonded silica [105] or Amberlite [106].

Other applications involving 8-HQ are shown in Table 1.3.

1.7.3. Applications of Dithiocarbamate and Dithizone resins.

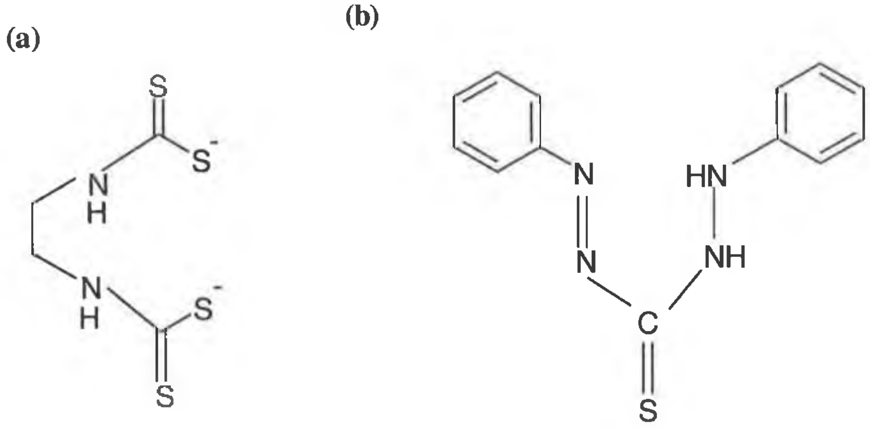


Figure 1.12. Structures of (a) ethylene-(bis)-dithiocarbamate and (b) dithizone.

Table 1.3. Applications of quinolin-8-ol (8-HQ) resins [82].

Metals	**Sample**	**Column**	**Detection**	**L.O.D.**	**Reference**
Cd(II),Cu(II),Pb(II), Zn(II),Fe(II),Mn(II), Ni(II)	Seawater	Complexation with Quinolin-8-ol and adsorption on C_{18} bonded silica gel.	ICP-AES	0.02-0.1 ng/L	107
Cu(II),Co(II),Fe(II), Ni(II).Ca(II)	Aqueous standard	Silica immobilised quinolin-8-ol.	FIA-FAAS	Not reported	108
Cd(II),Co(II),Cu(II), Mn(II),Ni(II),Pb(II)	Seawater	Quinolin-8-ol.	FIA-ICP-MS	0.03-0.2 ng/L	109
Bi(III),Cd(II),Cu(II), In(II),Pb(II),Zn(II)	Seawater	Immobilised quinolin-8-ol on silica gel.	FIA-anodic stripping voltammetry	Not reported	110
Cd(II),Cu(II),Ni(II), Fe(II),Mn(II),Zn(II)	Seawater	Precomplexation with quinolin-8-ol and retention on a XAD-2 column.	FIA-ICP-AES	4-60 ng/L	111
Mn(II)	Seawater	Quinolin-8-ol immobilised onto a vinyl polymer gel.	FIA-spectrophotometry	Not reported	112
Al(III),Cu(II),Fe(II)	Wastewaters	Complexation with quinolin-8-ol and separation by RPHPLC.	FIA-spectrophotometry	Al(III),5 ng/L Cu(II)40 ng/L Fe(II)40 ng/L	113
Cd(II),Cu(II),Fe(II), Mn(II),Ni(II),Pb(II), Zn(II)	Seawater	Silica immobilised quinolin-8-ol.	Discontinuous FIA-GF-AAS	0.3-10.2 pg	114
Ga(III),In(III),Ti(III)	Seawater	Quinolin-8-ol chelating ion exchange resin.	ICP-MS	Ga(III) 0.02 ppt, In(III) 0.01 ppt, Ti(III) 0.2 ppt	115
Al(III),Ga(III),Ti(III)	River water	Quinolin-8-ol immobilised on controlled pore glass	FIA-ICP-AES	3.0 ng/L	116
Cd(II),Cu(II),Ni(II)	Open ocean seawater	Quinolin-8-ol immobilised on silica.	FIA-ICP-AES with continuous flow ultrasonic nebulisation	0.016 -0.7 pg/mls.	117
Rare earth elements	Natural waters	Silica immobilised quinolin-8-ol.	ID-ICP-MS	Yb 0.23 pg/mls La 0.56 pg/mls.	118
Cu(II)	Seawater	Immobilised quinolin-8-ol.	FIA-chemilume-nescence	Not reported	119
Zn(II)	Seawater	Quinolin-8-ol immobilised on fractogel.	FIA-fluorimetry	Not reported	120

Dithiocarbamate, shown in Figure 1.12(a), is a type of complexing agent in which the resultant metal complexes are often neutrally charged and can readily be adsorbed onto numerous substrates. The most commonly used dithiocarbamate ligand is the diethylammonium form [121], but bis(carboxymethyl) [122] and tetramethylene [123] dithiocarbamates have also been reported. Fang *et al.* [124] and Sperling *et al.* [125] used solution chelation of trace metals with diethyldithiocarbamate, followed by their sorption onto a micro-column packed with C_{18} bonded silica reversed phase sorbent.

However, the approach was less than ideal as quantitative recoveries were not possible. The limits of detection were poor in comparison to those obtained by Azeredo *et al.* for the same group of metals [104] using 8-HQ as discussed in the previous section.

Sulphur based resins such as these incorpating dithizone groups, shown in Figure 1.12(b), have been used by Grott and Kettrup for the preconcentration and separation of Au(III) and Pt(IV) group metals from base metals and salts [126]. Chwastowska and Kosiarska investigated the analytical characteristics of a polystyrene-divinylbenzene loaded dithizone sorbent for the determination of Ag(I), Cu(II), Cd(II), Pb(II), Ni(II), Co(II), and Zn(II) by AAS. Cu(II) and Pb(II) were determined in river water and electrolytic Cu(II). Repeat analysis of a river water sample gave a R.S.D. of 1.5% for Cu(II) and Pb(II) at 5.3 and 7.8 μg/L levels. Results for electrolytic copper were in good agreement with other methods [127].

A review by Ebdon *et al.* also gives details of such applications [128].
Further applications involving dithizone and various other resins are shown in Table. 1.4.

1.7.4. Alternative chelating ion-exchange resins.

A number of additional chelating resins have been investigated often based upon chelating dyes. Lundgren and Schilt impregnated Amberlite XAD 2 with various ligands including 2,4,6-tripyridyl-1,3,5-triazene (TPTZ), 3-(2-pyridyl)-5,6-diphenyl-1,2,4-trazine (PTD), 1-10 phenanthroline and 2,2, bipyridine for the preconcentration of transition metals Fe(II), Co(II), Cu(II), Ni(II) and Zn(II) from sea water and various reagent grade chemicals [139].

Chwastowska and Mozer made use of Amberlite XAD-4 for the immobilisation of 1-(2-pyridylazo)-2-naphthol, which allowed the preconcentration of Cu(II) Zn(II), Fe(II), Cd(II), Ni(II), Pb(II) from river water prior to determination by AAS [140].

Isshiki *et al.* impregnated Amberlite XAD-4 with 7-dodecenyl-8-quinolinol (DDQ) for the preconcentration of Ag(I), Al(III), Bi(III), Co(II), Cd(II), Cu(II), Fe(III),

Table 1.4. Applications of dithiocarbamate and dithizone based resins [82].

Metals	Sample	Method	Detection	L.O.D.	Reference
Bi(III),Co(II), Cr(III),Cu(II), Hg(II),Ni(II),V(V)	Seawater and urine	Complexation with bis(carboxymethyl)-dithiocarbamate and adsorption onto a PS-DVB column in acidic conditions.	FIA-ICP-MS	8-80 ng/L	129
Co(II)	Seawater	Complexation with diethylammonium diethyldithiocarbamate and preconcentration with a bonded silca reverse-phase sorbent with octadecyl functional groups.	FIA-GF-AAS	1.7 ng/L	130
Cd(II),Cu(II), Pb(II)	Natural waters and waste waters	Complexation with diethylammonium diethyldithiocarbamate and and sorbent extraction onto bonded silica.	FAAS	Cd(II) 0.3 ng/L, Cu(II) 0.2 ng/L, Pb(II) 3.0 ng/L	131
Cd(II),Cu(II)	Seawater	Chelation with ammonium tetramethylenedithiocarbamate immobilised on a C_{18} column.	GF-AAS	Cd(II) 0.178 ng/L, Cu(II) 2.4 ng/L	132
Cd(II),Pb(II)	Potable water, soil, river sediment, tea leaves, hair.	Formation of the morpholine-4-carbodithioate complexes of the analytes and adsorption onto microcrystalline naphthalene..	Differential-pulse polarography	Cd(II) 0.014 mg/L, Pb(II) 0.14 mg/L	133
Cr(III),Cr(VI)	Sea and riverine waters	On-line determination of Cr(VI) and the oxidation product of potassium peroxydisulphate and complexation with sodium diethyldithiocarbamate with a C_{18} column.	FIA-GF-AAS	Cr(VI) 16 ng/L Total Cr 18 ng/L	134
Cd(II),Co(II), Cu(II),Fe(II), Mn(II),Ni(II), Pb(II),Zn(II)	Aqueous standards	Complexation with alkylene bisdithiocarbamates.	FAAS	3-90 ng/L	135
Cd(II),Cu(II)	Seawater	On-line preconcentration by complexation with ammonium tetramethylenedithiocarbamate on a silica gel C_{18} microcolumn.	FIA-GF-AAS	Cd(II) 1.26 ng/L Cu(II) 6.5 ng/L	136
Cd(II),Cu(II), Pb(II),Zn(II)	Seawater	Solvent extraction with dithiocarbamate followed by back extraction with a solution of Hg(II).	Differential-pulse anodic stripping voltammetry	Cd(II) 0.002 ng/L, Cu(II) 0.003 ng/L, Pb(II) 0.002 ng/L	137
Cu(II)	Potable water and seawater	Copper diethyldithiocarbamate chelate adsorbed on the walls of a PTFE knotted reactor.	FIA-FAAS	0.2 ng/L	138

Cr(III), Ga(III), Mn(II), Ni(II), Pb(II) and Ti(IV). Quantitation recoveries from artificial sea-water was possible with all metal ions except Ag(I), Cd(II), and Cr(III). Detection was achieved using GF-AAS [141].

Brajter *et al.* immobilised pyrocatechol violet (PCV), shown in Figure 1.13, on Amberlite XAD-2 for the preconcentration of Pb(II) in tap water followed by AAS detection. Very high preconcentration factors were obtained, which allowed levels of Pb(II) to be detected at very low μg/L levels (2.8 μg/L) [142]. Singh and Rita used the same ligand loaded onto Amberlite A-26 for the determination of Cd(II) and Pb(II) in river water [143].

Trojanowicz *et al.* compared a number of ligands loaded onto Amberlite XAD-2 including PAR, Eriochrome Blue Black R (EBBR) and PCV shown in Figure 1.13. PCV chelating sorbent gave superior results for Cu(II). Determination of Cu(II) by FIA-AAS in natural waters at μg/L levels was shown although severe interference by Ca(II) occurred when present in concentrations above 50 mg/L. Cu(II) had a small linear range of only 0-2 μg/L above which curvature resulted. The highlight of this resin was that it had sufficient affinity to retain Cu(II) even if it was complexed with weaker ligands present in water [144].

The on-line preconcentration of alkaline earth and transition metal ions from NaCl brines and sea-water using XO immobilised on Dowex 1-X8 anion exchange column was investigated with comparison to MetPac CC-1 by Koklu *et al.* Better recoveries were obtained for transition metals at high pHs with the XO column. Eventhough both columns have the same functional group they behaved differently from one another. The XO column gave poor recoveries of the first row transition metal ions. MetPac CC-1 on the other hand retained all metal ions studied with good linearity and precision with the exception of Al(III) and Ti(IV). Sea water reference material was analysed with results obtained agreeing well with certified values [145].

1-(2-thiazoylazo)-2-(naphthol) immobilised onto XAD-2 resin has been used for the determination of Cd(II), Cu(II), Fe(II), Mn(II), Ni(II) and Zn(II) in river and Antarctic sea water using FIA-ICP-AES. Limits of detection were at low ng/l levels with

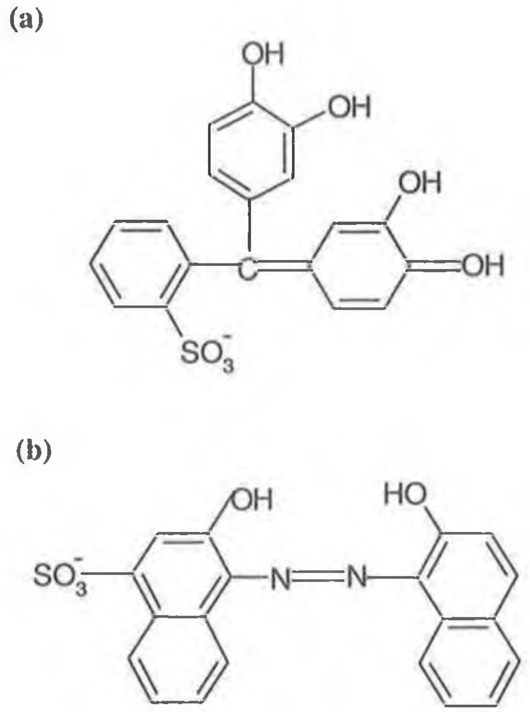


Figure 1.13. Structures of (a) Eriochrome Blue Black R and (b) Pyrocatechol Violet.

excellent precision but the method was not ideal for the determination of metal ions such as Cd(II) and Mn(II) at low concentrations [146].

Table 1.5 shows the variety of alternative chelating resins applied to metal ion determinations.

Various workers have extensively reviewed other applications using numerous other preconcentration resins [147-149]. In one such review Alexandratos and Beauvais [150] have dealt with polymer supported reagents for the selective complexation of metal ions. Marina *et al.* published a comprehensive review of impregnated anionic and non-ionic resins with organic ligands [151].

Table 1.5. Applications of alternative chelating ion-exchange resins [82].

Metals	Sample	Method	Detection	L.O.D.	Reference
Alkaline earth and transition metals	Concentrated brines	Preconcentration with xylenol orange immobilised on a Dowex 1-X8 column.	FIA-ICP-MS	Not reported	94
Cd(II),Co(II), Cu(II),Fe(II), Ni(II),Pb(II)	Seawater	Complexation with pyrolidin-1-yl dithioformate and preconcentration onto a C_{18} column.	FIA-GF-AAS	0.4 ng/L – 25 ng/L	152
Cd(II),Co(II), Hg(II),Zn(II), Cu(II)	Natural waters	Chelation with poly-(acrylamidoxime) resin.	NAA and AAS	NAA 0.0003-10 ng/L AAS 0.1-0.5 ng/L	153
Cd(II),Cu(II), Ni(II),Mn(II), Pb(II),Zn(II)	Seawater	Preconcentration with XAD-4 and XAD-7 macroporous resins and various lipophilic tetraaza macrocycles.	GF-AAS	0.75-7.5 ng	154
Fe(II)	Highly purified waters	Preconcentration on column of AG1X-8 with the eluent mixed with 4,7-diphenyl-1,10-phenenthrolin-edisulfonate (DPPS).	FIA-spectrophotometry	0.01 mg/L	155
Cd(II),Cu(II), Fe(II),Mn(II), Ni(II),Zn(II)	Natural waters	Chelation onto a column XAD-2 resin functionalised with 1-(2-thiazolylazo)-2-naphthol.	FIA-ICP-AES	2-40 ng/L	156
Cd(II),Cu(II), Ni(II),Pb(II), Zn(II)	Concentrated brine and seawater	Chelation ion chromatography with step gradient as a preconcen-tration and separ-ation technique using columns with XO and CAS.	Spectrophotometry	1-3 μg/L	157
Cd(II),Mn(II), Cu(II),Ni(II), Pb(II),Zn(II)	Sea water	Using chelamine containing a pentamine ligand as a preconcentrating agent agent for the study of trace elements in sea water.	GF-AAS	2.3-33 ng/L	158
Cd(II),Cu(II), Co(II),Ni(II), Pb(II)	Natural waters	Preconcentration of elements by complexation with butane-2,3-dione bis)N-pyridinoacetylhydrazone) and retention on a column packed with XAD-4 resin.	FIA-ICP-MS	2-10 ng/L	159
Cd(II),Cu(II), Co(II),Mn(II), Ni(II),Pb(II), Zn(II)	Aqueous standards	Trace metal chelation on a silica column coated with carboxymethyl Dextran followed by IC separation on a CS-5 column.	FIA-spectrophotometry	5-100 ng/L	160

Table 1.5. continued. Applications of alternative chelating ion-exchange resins [82].

Metals	Sample	Method	Detection	L.O.D.	Reference
Pd(IV),Pt(II)	Fresh waters	Preconcentration onto charcoal, with nebulisation and electrothermal vapourisation as sample introduction for ICP-MS.	ICP-MS	0.3-0.8 ng/L	161
Al(III),Ca(II), Cd(II),Co(II), Cu(II),Cr(III), Fe(II),K(I), Mn(II),Na(I), Ni(II),Pb(II), Zn(II)	Aqueous standards	Preconcentration of the metal species onto a silica gel modified with zincon and elution into $HClO_4$	FAAS	Not reported	162
Cu(II),Ni(II), Pb(II),Zn(II)	Seawater	HPLC with a XO impregnated resin.	FIA-spectral array detection	Not reported	163
Cd(II),Cu(II) Fe(II),Ni(II), Pb(II),Zn(II)	Rain water	Cobolt-ammonium pyrrolidin-1-ylidithio-formate with collection on PTFE membrane.	FIA-ICP-AES	0.2-1 μg/l.	164
Cd(II),Ni(II), Pb(II),Zn(II)	Well waters	Chelation with alizarin red S immobilised on Amberlite XAD-2 resin.	FAAS	Not reported	165
Cd(II), Cu(II), Hg(II), Pb(II), Pd(IV)	Standard solutions	Preconcentration onto XAD-2 resin coated with dimethylglyoxal bis(4-phenyl-3-thiosemicarbazone) with separation by HPLC.	Spectrophoto-metry	Not reported	166
Cd(II),Co(II), Pb(II),Zn(II)	Natural and waste waters	Chelation of analytes with polystyrene-azo-3-arsonophenol and elution into 1M HNO_3	FAAS	Not reported	167
Cd(II),Co(II), Cu(II),Mn(II), Ni(II),Pb(II)	Natural waters	Preconcentration on a chelated column of SO_3-quinolin-8-ol CM cellulose.	FIA-ICP-MS	Not reported	168

1.8. Determination of metal ions by complexation chromatography.

The term 'complexation chromatography' can be applied to any form of chromatography where the separation of metal ions is facilitated by their complexation with suitable ligands. The versatility of complexation chromatography is due in part to its suitability in all areas of chromatography including gas chromatography (GC), thin layer chromatography (TLC), HPLC and supercritical fluid chromatography (SFC). Other reasons include;

- Vast range of lewis acids, bases and complexing ligands available.
- Different ways of incorporating the complexation into the chromatographic system.
- The fact that many cases conventional chromatographic columns and or packings may be utilized.

Liquid chromatography of complexed metal ions has become a valuable analytical technique not only due its versatility but also because of specificity and sensitivity combined with the capacity for simultaneous determinations. Ligands utilised for the separation and determination of these metals should ideally fulfill requirements such as forming neutral or charged complexes with a large number of metals, if multiple metals are to be determined, or a small number if selectivity is the main requirement. Additional needs include the formation of well defined co-ordinately saturated complexes with high stability to minimize baseline drift and noise in the chromatographic system, reaction time with metals should be instantaneous, complexes should have sufficient solubility in the mobile phase and have high molar absorptivity if spectrophotometric detection is employed. The ligand must not be too voluminous, so that the element specific properties of central the atom are maintained thus avoiding leveling of the chromatographic behavior of the complexes.

There are a number of different ways to introduce such ligands for complexation into the chromatographic system. These include pre-column, on-column and post-column complexation. The success of pre-column derivatisation depends on ligand stability, thermodynamic and kinetic stability of complexes, and components of the chromatographic system i.e. stationary and mobile phases. The inclusion of complexation within the actual separation principle can be used to eliminate interference from environmental matrices, whilst by varying complex-forming conditions selectivity for particular metal ions can be increased [169].

1.8.1. Pre-column complexation of metal ions.

Nikitin *et al.* investigated the separation of Pd(II), Cu(II), Co(II) and Ni(II) with 1-(2-pyridylazo)-2-naphthol (PAN), (shown in Figure 1.14), as the precolumn derivatising agent on a silica gel (10 μm) column with detection at a wavelength of 620 nm [170].

A number of aqueous-organic mobile phases were used, with acetone giving very broad peaks and poor resolution. Chloroform resulted in the retention of Co(II) with poor resolution for Ni(II) and Pd(II). However, a mixture of benzene and propan-2-ol within the mobile phase allowed separation of all four metal complexes within 32 min, eventhough peak shapes were poor.

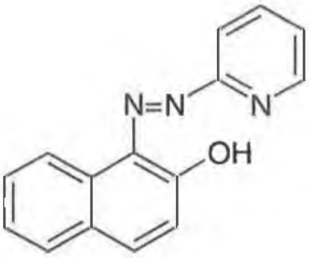


Figure 1.14. Structure of 1-(2-pyridylazo)-2-napthol (PAN).

Yasui *et al.* observed the behaviour of Co(II), Cu(II), Ni(II) and Fe(II) chelates with PAN on alkyl bonded silica gel stationary phase, using a mobile phase of 70:30 acetonitrile: water [171]. Results were compared using different kinds of alkyl-bonded commercially available silica gel columns and it was discovered that the elution order was affected by the hydrophobicity of the stationary phase, especially for Co(II). When adding organic and inorganic modifiers to the mobile phase, peaks for $[Cu(PAN)]^+$and $[Co(PAN)_2]^+$ which were adsorbed onto the stationary phase as a result of interaction with residual silanol groups, were detected as sharp peaks.

Niwa *et al.* also investigated six 2-quinolylazo based precolumn derivatising ligands using an ODS column for the separation of Fe(II) ,Co(II), Ni(II) and Cu(II) complexes [172]. The mobile phase consisted of an organic modifier-water mixture. When replacing acetonitrile with acetone the elution order changed from Cu(II), Co(II), HL, Ni(II), Fe(II) to Co(II), Cu(II), Ni(II), Fe(II), HL. The system developed had the advantage that Fe(II) did not interfere with the separation and determination of the other metal ions.

Miura has investigated the use of numerous alternative azo dyes as pre-column derivatising agents.These including 2-(8-quinolylazo)-5-(diethylamino)phenol (QADAP), 2-(8-quinolylazo)-5-(dimethylamino)phenol (DMQAP), 2-(5-bromo-2-pyridylazo)-5-(diethylamino)phenol (5-Br-PADAP), PAN, and 1-(2-thiazolylazo)-2-naphthol (TAN). The eluent composition consisted of acetonitrile-water with an IIR, such as tetraethylammonium (TEA), tetraoctylammonium (TOA) or tetramethylammonium (TMA) added. The only efficient separation obtained was with TBAOH which gave excellent peak resolution and short retention times. The method was applied to seawater and rain water and coal fly ash. Best results were obtained with DMQAP as the derivatising reagent. Spiking the above samples and a NIST certified reference material yielded high % recoveries between 95-100% for V(V). Eventhough the concentration of V(V) in the reference material was very high (300 mg/L), even higher concentrations of Al(III) and Fe(III) did not interfere with the separation [173].

Wang and Yuan separated V(V), Zr, Pd(II), Fe(II), Co(II), Cu(II), and Ni(II) utilising pre-column derivatisation with 2-(3,5-dibromo-2-pyridylazo)-5-diethylaminophenol (3,5-diBr-PADAP) on a C_{18} column. A ternary eluent composition was used containing TBAOH as an IIR. The method was very sensitive, with limits of detection between 1.1 and 3.6 µg/L reported. The order of elution was V(V) < Cu(II) < Zr(IV) < Pd(II) < Fe(II) < Ni(II) < Co(II), with a number of other metals investigated not interfering with the analysis [174].

Siren has studied 1-nitro-2-naphthol-6-sodium sulphonate (126NNS) and 2-nitro-1-naphthol-6-sodium sulphonate (216NNS) as pre-column derivatising agents for the determination of Co(II), Cu(II) and Fe(III). Ion-interaction RP-HPLC was again used with cetyl-trimethyl-ammonium bromide (CTAB), tetra-decyl-dimethyl ammonium bromide (TDDAB) and tri-decyl-dimethyl-ammonium chloride (TDDAC), with Uv-Vis detection at 400 nm. Both pre-column and on-column derivatisation were investigated, with elution order being consistent with Cu(II),< Pd(II),< Co(II),< Fe(III). The separations were carried out on silica based C_{18} and polymeric columns with the latter giving the best separation with CTAB as IIR [175].

Ohtsuka *et al.* employed 2-(3,5-dibromo-2-pyridylazo)-5-[N-ethyl-N-(3-sulphopropyl)amino]phenol (3,5-diBr-PAESPAP) and 2-(5-bromo-2-pyridylazo)-5-[N-propyl-N-(3-sulphopropyl)amino]phenol (5-Br-PAPS) as pre-column complexing agents for the separation of Cu(II), Co(II), Ni(II), Fe(III) and Zn(II) on several types of columns. An ion-exchange column showed significant tailing of the peaks in comparison to other silica and polymer gels chemically bonded with C_{18}, which gave superior resolution and selectivity of the chelates with excellent reproducibility [176].

Fritz and Qian synthesised a new thiohydrazone complexing reagent for the pre-column complexation of Bi(III), Ag(I), Cu(II), Ni(II), Pd(II), and Hg(II). The separations on reversed-phase column were moderate in comparison to a 50 mm polymeric resin column which produced a superior separation within 12 min. The IIR in the eluent was tetra-ethyl ammonium bromide (TEAB). Detection was possible at 474 nm. With a 250 mm polymeric column the above metal columns were separated within 21 min. The silica based column allowed the separation of In(III), Cu(II) and Hg(II) in under 12 min when using TBAOH as the IIR [177].

Jen and Chen separated chelates of Pb(II), Ni(II), Cd(II), Fe(II) and Cu(II) with EDTA on a C_8 column within 11 min. They used an eluent composition of acetonitrile-water (8:92), and TBAOH as IIR, which was applied to electroplating wastewater samples with limits of detection at the low ng/ml level [178].

Basova *et al.* studied the retention of Cu(II), Ni(II), Co(II), Fe(III), Zn(II), Pb(II), Cd(II) and Hg(II) chelates with 8-HQS on a C_{18} column. Three different IIRs were examined namely, CTAB, TBAOH and heptylamine-hydrochloride (HAHC). In terms of selectivity CTAB gave the best results but poor peak shapes were obtained with no baseline resolution for Co(II) and Fe(III), with Pb(II), Zn(II), Hg(II) and Cd(II) co-eluting with the reagent. The metal ions eluted in the order 8-HQS < Co(II) < Fe (III) < Ni(II). The retention mechanism was not clearly understood by the authors [179]. Several attempts have been made at pre-column complexation of Nb(V), and Ta(V), with various ligands such as PAR [180], Br-PADAP [181], 2-(2-thiazolylazo)-5-diethylamino phenol, (TADAP) [182], 4-(5-nitro-2-pyridylazo)-5-diethylamino phenol, (5-NO_2-PADAP) [183] and 4-(5-nitro-2-pyridylazo)resorcinol, (5-NO_2-PAR) [184]. However, all of the RP-HPLC methods gave broad peaks for Ta(V) complex and/or incomplete separation of Ta(V) and Nb(V) complexes. Split peaks were also obtained under certain conditions. Poor peak shapes were obtained with all methods and hence limits of detection were not confirmed.

This led to the work by Vachirapatama *et al.* Their work was based on pre-column formation of anionic metal-PAR-citrate ternary complexes of Nb(V) and Ta(V) and separation on a C_{18} column with an eluent consisting of TBAOH, citrate buffer, acetate, methanol-water at pH = 6.5. Detection was at 540 nm. The method was applied to geological rock samples and in comparison to the above mentioned techniques, high separation selectivity and lower limits of detection were achieved. Excellent baseline separation was achieved for Nb(V) and Ta(V) within 12 min, with the elution of all matrix ions in 40 min. When applied to the certified reference material results were in good agreement with certified values [185].

Wang *et al.* have investigated the separation of Nb(V), V(V), Co(II), Fe(III), Ni(II) and Pd(II) chelates with 5-NO_2-PAR by reversed-phase HPLC on a C_{18} column. The eluent of methanol-water, acetate buffer and TBAOH allowed the elution of all peaks in under 30 min. Peak shapes were good but as expected Pd(II) which eluted last showed significant tailing. However, there was serious interference from Rh(III) (which had two peaks) with the determination of Nb(V) and Co(II). The % recoveries in rain water samples was very good between 97.5 and 102.5% [186].

Finally, Sarzanini *et al.* developed an electrochemical detection system for the detection of Cu(II), Ni(II),V(V), Co(II) and Fe(III) chelates following their chromatographic separation. A number of sulphonated azo ligands such as 2-(4-Sulphophenylazo)-1,8-dihydroxynaphthalene-3,6-disulphonic acid (SPADNS), 4-hydroxy-3-(2-hydroxonaphthylazo)-benzenesulphonic acid (Acid Alizarin Violet N) and 2-(5-Chloro-2-hydroxyonaphthylazo)-1,8-dihydroxynaphthalene-3,6-phenylazo-disulphonic acid (Plasmacorinth B) (PCB), shown in Figure 1.15, were used as derivatising agents. This system had the advantage of high sensitivity and good selectivity capabilities which allowed the determination of Cu(II) and Fe(II) in natural waters. PCB was deemed as the ideal reagent. The eluent composition of methanol-water, acetic acid, NaCl and TBAOH allowed the simultaneous determination of

Cu(II), Ni(II),V(V), Co(II) and Fe(III) in a lake water sample without severe matrix interference. Results compared well with ICP-AES [187].

1.8.2. Mobile phase complexation of metal ions.

On-column complexation chromatography generally refers to a form of chromatography where the mobile phase contains a complexing or chelating ligand. The separation principle involved maybe ion exchange or reversed-phase interactions with the complex formed and the stationary phase.
With on-column mobile phase complexation, there is the advantage of avoiding the previous formation of the complex, which can reduce analysis times. This also avoids the decomposition of some complexes if left for any time prior to injection.

According to a review by Timerbaev and Bonn [188] complexing agents can be divided broadly into two classes;

(i) Weak complexing agents (e.g. oxalic, citric and tartaric acid).
(ii) Strong complexing agent (e.g. picolinic acid and dipicolinic acid (DPA), (pyridine-2,6-dicarboxylic acid) (PDCA), (shown in Figure 1.16).

1.8.2.i. Weak and strong complexing agents.

Voloschik *et al.* developed a multi-dimensional LC system for the separation of some transition and heavy metals in seawaters, natural and waste waters [189]. A complex setup was employed as it consisted of two integrated IC systems. The metal ions were determined as anionic complexes with oxalate. With this method Fe(II) interferences were completely eliminated allowing application to sea, natural and wastewaters. Macro amounts of Mg(II), and Ca(II) did not interfere with the separation of Co(II), Zn(II), Pb(II) and Cd(II).

Merly *et al.* as previously mentioned, investigated the retention of Pb(II) and Cu(II) on a porous graphitic carbon (PGC) column using an oxalic acid eluent [190]. Cu(II)

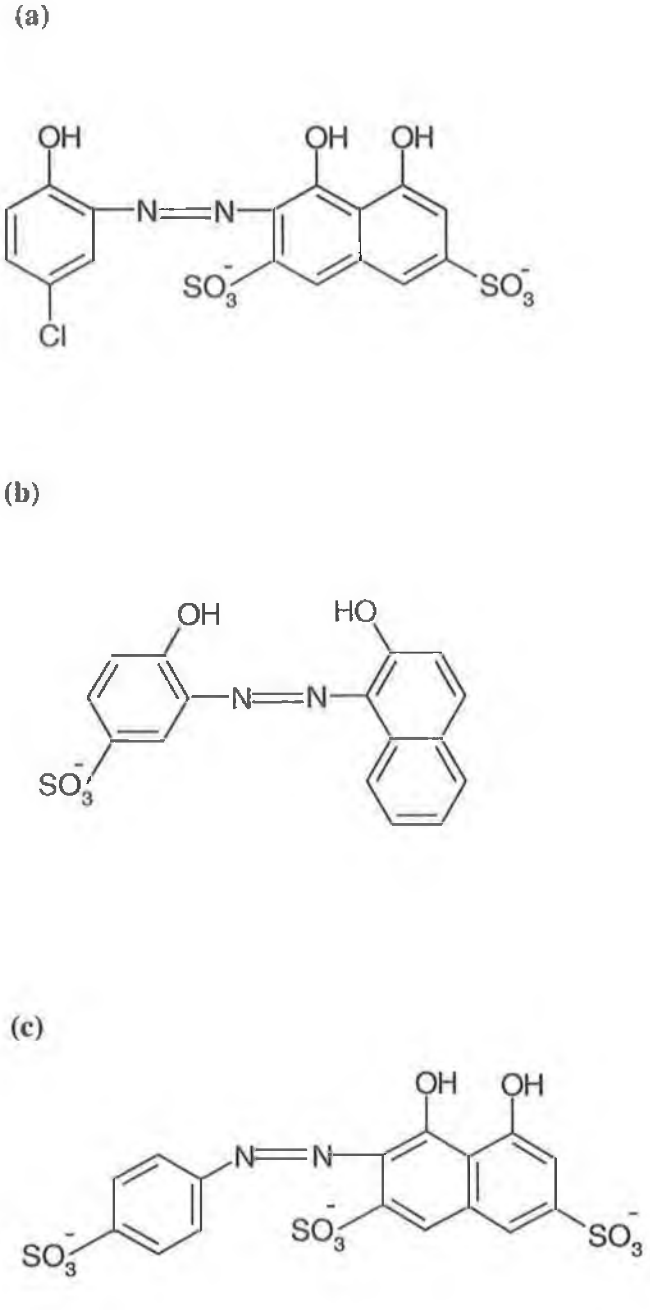


Figure 1.15. Structures of (a) 2-(4-Sulphophenylazo)-1,8-dihydroxynaphthalene-3,6-disulphonic acid (SPADNS), (b) 4-hydroxy-3-(2-hydroxonaphthylazo)-benzenesulphonic acid (Acid Alizarin Violet N) (c) 2-(5-Chloro-2-hydroxyonaphthylazo)-1,8-dihydroxynaphthalene-3,6-phenylazo-disulphonic acid (Plasmacorinth B) (PCB).

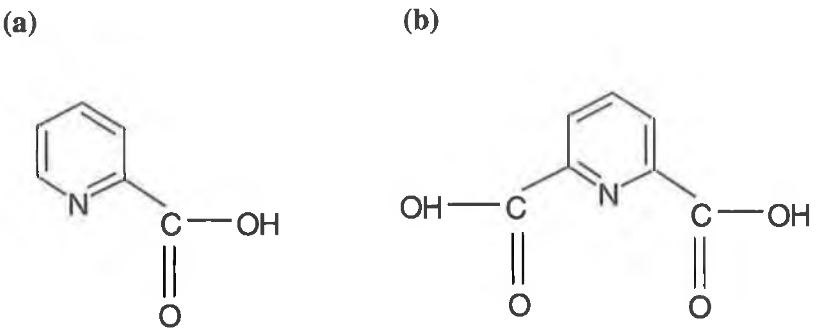


Figure 1.16. Structures of (a) Picolinic acid and (b) Dipicolinic acid (DPA), (pyridine-2,6-dicarboxylic acid) (PDCA).

had the highest affinity for the graphite surface and the largest stability constant for oxalate (Log $\beta_1 = 6.2$, Log $\beta_2 = 10.2$). Therefore, Cu(II) eluted only at high oxalate concentrations where the $CuOx_2^{2-}$ species dominates. Using 0.45 *M* oxalic acid at pH = 1.6, a separation of Ni(II), Cd(II), Pb(II) and Cu(II) was possible within 20 min. The selectivity of the column for these metals was assumed to be through the electronic interaction between the electronic cloud of the graphite and orbitals of the metal ion centers. Modification of the PGC surface with the oxalic acid allowed an increased range of metal ions to be determined.

Schwedt and Schaper separated alkali, alkaline earths, Zn(II) and Mn(II) on a reversed-phase column employing n-hexylsuccinic acid as the IIR and an oxalic acid eluent, followed by conductivity detection. The method was applied to mineral and wine samples with results comparing well with AAS. Linearity was excellent with $R^2 > 0.999$, eventhough the linear range was very small [191].

Walker has separated rare earth elements in under 12 min using Phenol Red as the IIR, which also served the dual purpose of allowing indirect detection at 490nm. A number of complexing eluents were added to the eluent to evaluate their effect. Citrate and tartrate gave very little differences in selectivities and resolution was very poor. Hence α-hydroxyisobutyric (α-HIBA) acid was used. However, the limits of detection were very poor with 25 mg/L being the average detection limit [192].

Marina *et al.* applied ion-interaction RP-HPLC to the determination of Ni(II), Co(II), Cu(II), Fe(III), Pb(II), Cr(III) and Hg(II), with on-column complexation using EDTA, and TBAOH as the IIR. The separation of 7 metal ions was possible in under 8 min. Absolute detection limits were particularly low ranging from 0.3 ng to 35 ng for Fe(III) and Cr(III) respectively [193].

Liu *et al.* employed a system for the analysis of complex matrices such as seawater and biological samples [194]. Preconcentration of 25 mls of the sample on to an γ-aminobutyrohydroxamate resin column allowed the selective removal of alkali and alkaline earth metals using KNO_3. The metals of interest were eluted using oxalic acid and separated on a cation exchange column followed by post-column detection with PAR at 495 nm. Other eluents such as citrate and tartrate resulted in excessive run times, particularly with citrate. The elution order observed was not anticipated and was related to the relative strength of complexation between oxalic acid and the metals, which decreased in the following order, Fe(II) >Zn(II) >Mn(II) >Cd(II). The resin was capable of eliminating alkali and alkaline earth metals efficiently, therefore the method was ideal for seawater analysis, as it showed high selectivity for transition metals in complex matrices. Linearity was very good between 0.5 to 10 ng/ml and limits of detection were between 0.3-1.2 ng/ml. However, serious overlapping occurred with Mn(II) and Cd(II) and Co(II) and Cu(II) in the analysis of a mussel sample which indicated the necessity for improvement of the separation selectivity of the system. No such problems occurred with sea water analysis.

Moteillier *et al.* later extended this work using a new mixed bed ion exchange column with on-line preconcentration and post-column reaction detection with PAR. They used a PDCA as eluent for the quantitative analysis of 6 transition metals [195]. This was applied to spring and mineral waters with % recoveries ~ 90% when 35 mls of sample was preconcentrated. Poor Ni(II) and Fe(II) recoveries of < 80% were attributed to slow kinetics during desorption. With such a system only waters of low ionic strength could be analysed successfully, because with high ionic strength samples several interferences occurred, such as Fe(II) co-eluting with Ca(II) and divalent metals with low affinity for the chelator such as Mn(II), not being quantitatively retained under the conditions used.

Ohta *et al.* developed a system for rain and river water analysis using a silica gel column and conductimetric detection [196]. Using an acidic HNO_3 eluent Na(I), NH_4(I), K(I), Mg(II) and Ca(II) were only weakly retained on the column therefore the addition of PDCA was investigated and resulted in an improved separation of the metal ions within 12 min. The results of this method compared well with non-suppressed IC for the monovalent metal ions and ICP-AES for the divalent metals, with the only problem in the developed system being that Mn(II) overlapped with K(I).

Kondratjonak and Schwedt have investigated several complexing agents as eluents such as tartaric acid, citric acid, PDCA, phthalic acid and benzoic acid, for the separation of up to 18 metal ions including alkali and alkaline earth metals on a polymer coated cation exchange column [197]. Drinking water and wine samples were analysed using conductivity detection with PDCA and citric acid eluent.

Litvina *et al.* also investigated the effects of adding a number of complexing agents to the eluent using a C_{18} column dynamically coated with dodecylbenzenesulphonic acid. Conductivity detection was used with and without the addition of an IIR to the eluent. Twelve alkaline earths, transitions and heavy metals were separated in under 20 min with good resolution. Similarly nine transition and heavy metals were separated using ion-interaction RP-HPLC using an oxalate-pyridinedicarboxylic acid-TBAOH eluent. This eluent allowed low limits of detection for transition metals to be obtained in the region of 0.005 mg/L. Due to the selective and sensitive nature of the developed method it was possible to determine the above metals in natural water samples without preconcentration.[198].

Zappoli *et al.* separated Fe(III), Cu(II), Cd(II), Pb(II), Co(II), Zn(II), Ni(II) and Mn(II) using an eluent composition of tartaric acid, octanesulfonate and an organic modifier such as methanol or acetonitrile on a C_{18} column. The eight metal ions were resolved to baseline with excellent separation within 50 min. Peak symmetry was good and the method was hence applicable to natural water samples such as rain and tap-water [199].

Nesterenko *et al.* separated a number of transition metals using picolinic acid as an on-column chelating agent with the separation performed on a reversed-phase column followed by UV-detection [200]. Three organic modifiers were investigated as a component of the mobile phase, yet it was acetonitrile which gave the best results in term of peak symmetry. The optimum mobile phase conditions were acetonitrile-water (6:94), 0.002 *M* picolinic acid, pH = 7. This allowed the determination of Ni(II), Co(II), Zn(II), Cd(II), Mn(II), Fe(II) and Cu(II) in under 45 min, with not only very sharp peaks for the first four metal ions, but also good agreement between retention found and known stability constants for the metal picolinic acid complexes. The method was applied to galvanic bath waste water with no additional pretreatment being required, and the results compared well with ICP-AES. Absolute limits of detection were in the low ng region.

Cardellicchio *et al.* determined Pb(II) and Cd(II) in seawater samples following their preconcentration onto an IDA chelating resin and the selective removal of alkali and alkaline earth metal ions with an ammonium acetate buffer. The metals were eluted and separated on a cation-exchange column using a non-complexing, H_2SO_4 – K_2SO_4 eluent. However other transition metals could not be determined and trivalents Fe(III) and Al(III) could not be selectivily eliminated while trace elements were being concentrated [201]. This work was therefore further investigated by Cardellicchio *et al.* in an attempt to simultaneously determine Fe(II), Fe(III), Cu(II), Ni(II), Zn(II), Co(II), Cd(II), Mn(II) and Pb(II) at μg/L levels in environmental samples using a mixed bed ion exchange column. In this case two complexing eluents, oxalic acid and PDCA, and two post column reagents 5-Br-PADAP and PAR were studied [202]. With PDCA, the metals were separated as anionic complexes, whereas, with the oxalic acid eluent the transition metals were separated as both anion and cationic complexes. When employing a gradient system for the separation of transition metals the elution order was observed to be inversely related to the stability constants. The gradient program of oxalic acid-HCl-NaCl-$NaNO_3$ resulted in separation of a number of metals, and a reduction in the of tailing of Pb(II) and Cd(II) resulting in improved peak shapes. 5-Br-PADAP increased sensitivity for Cu(II), Cd(II), Zn(II) and Mn(II) but the system was not very sensitive for heavy metals such as Pb(II). Up to 200 mg/L Mg(II) and Ca(II) did not interfere with the separation and quantitative analysis of metals.The method was applied to natural water and marine sediments with results comparing well with certified values.

Carroli and Torcini, used a similar method to Cardellicchio *et al.* to determine Cu(II), Ni(II), Zn(II), Co(II) and Mn(II) in seawater. Again Pb(II) and Cd(II) could not be determined sensitively, because the Pb(II)-PDCA complex formed in the eluent was more stable than corresponding complex with PAR. Also Cd(II) was difficult to separate from Co(II) [203].

Problems encountered first by Cardellicchio *et al.* of Fe(III)/Al(III) not being eliminated during preconcentrated of transition metals on the IDA resin were overcome by Lu *et al.* who developed a method for the simultaneous determination of Pb(II), Cu(II), Cd(II), Co(II) and Ni(II) in drinking water, with Al(III)/Fe(III) being eliminated prior to separation using pyrophosphoric acid [204]. A concentration gradient of oxalic acid and $NaNO_3$ eluents was applied, coupled with post-column spectrophotometric detection with 5-Br-PADAP at 560 nm. Oxalic acid caused problems with the elution of Fe(III) and Al(III), but PDCA allowed heavy and transition metals to be separated at the expense of reduced sensitivities, as metal PDCA complexes were more stable than metal-oxalate complexes. Adding sodium nitrate ($NaNO_3$) to the eluent reduced tailing and improved peaks of Pb(II) and Cd(II). Drinking water spiked with the above metal ions gave % recoveries between 96-104%, with detection limits below μg/L levels.

Ding *et al.* later further improved this method by performing isocratic separation of the above metal ions using a PDCA-$Na_2C_2O_4$-LiOH-NaCl eluent followed by spectrophotometric detection at 565 nm with 5-Br-PADAP [205]. Mussel tissue and seawater samples were analysed once they had been digested and acidified with nitric acid, with satisfactory results obtained. Sodium oxalate ($Na_2C_2O_4$) was used to prevent any Fe(III) and Al(III) interferences. However, a problem with this method for sea water analysis was that only Mn(II) and Cu(II) could be determined, because Ni(II), Pb(II), Zn(II), Co(II) and Cd(II) were present in the blank. A certified reference material was analysed and the results were in good agreement with certified values.

Yan and Schwedt separated both monovalent and divalent cations on a weak cation exchange column (polymer coated silica with polybutadiene-maleic acid) with conductivity detection. In comparison to indirect UV detection they discovered that conductivity was 10 times more sensitive [206]. The organic complexing agents used alone could not give complete separation of alkali, alkaline earths, transitions and heavy metals, therefore mixed eluent systems were examined. Citric acid-PDCA allowed the separation of 10 metal ions, including alkali and alkaline earth metals in under 12 min. A mix of tartaric acid and oxalic acid allowed the separation of 13 metals including alkali, alkaline earths and transition metals in 35 min. Use of an EDTA-PDCA eluent allowed trace amounts of Na(I), K(I) and Ca(II) to be separated from a Mg(II) containing matrix, where the ratio of the metal ions to Mg(II) was 1:1000, which was not possible using AAS. This allowed the method to be applied to water and brines as well as deionised, drinking, mineral and wastewaters.

Laubli *et al.* used a similar column for the determination of Na(I), K(I), Ca(II) and Mg(II) in drinking water using isocratic separation with a tartaric acid and PDCA eluent [207].

Later, Ohta *et al.* separated alkali, alkaline earths and transition metal ions on unmodified silica gel using a number of mixed eluents with conductimetric and UV-visible spectrophotometric detection [208]. PDCA and HNO_3 were ideal for the separation of alkali and alkaline earths, whereas oxalic acid gave the best separation for alkali, alkaline earths and transition metal ions. The best separation of alkaline earths and transition metals was achieved with a combined eluent mix of oxalic acid and ethylenediamine.

Liu *et al.* used complexing eluents to determine lanthanides in complex matrices. The method was based on the use of a cation exchange column with an oxalic acid-diglycolic acid eluent, followed by post-column reaction with PAR/ZnEDTA. Prior to separation, sample preconcentration was performed on a γ-aminobutyrohydroxamate resin [209]. The elution order was Er < Eu < Sm < La. However, applying a gradient elution of 0.06 *M* oxalic acid to 0.02 *M* oxalic acid to 0.01 *M* diglycolic acid, resulted in a separation in 25 min with a surprising change in elution order, La < Ce < Pr < Nd

< Eu < Sm < Gd < Tb < Dy < Ho < Er. This was accounted for by these metals were separated negatively charged complexes. Under the conditions poor separation of Eu, Sm, Gd, Tb, Dy, Ho and Er was obtained in sea water and La, Ce, Pr and Nd could not be separated. The technique was very sensitive in that limits of detection of 0.0023 mg/L for a preconcentration volume of 25 mls were obtained from both pure and sea water.

Santoyo *et al.* have looked at anionic, cationic and bifunctional ion exchangers for the determination of heavy metals at trace levels in ground water [210]. The cation exchange column, tested with two eluents – oxalic acid-citric acid and tartaric acid-citric acid, gave detection limits that were too high. When using oxalic acid as the eluent with the anion exchange column, again poor sensitivity and high limits of detection were obtained. Using an oxalic acid-LiOH eluent, the bifunctional column gave the lowest limits of detection from 0.6-2.7 μg/L. The PDCA eluent with the same column gave limits between 0.9-1.3 μg/L. The system showed no interference or matrix problems with the ground water samples.

1.8.2.ii. Crown ether complexes.

Other materials, which have been used for complexing purposes in the chromatography of metal ions are crown ethers, which are macrocyclic ligands with hydrophilic interior cavities and hydrophobic exteriors. They form stable complexes by co-ordinating metal cations within their cavities. Selectivity of these cyclic polyethers for cations depends upon the size of the crown ether cavity and the metal ion diameter. They can be used in the mobile phase, applied through physical adsorption or as a coating on a solid support, or covalently bonded to silica or polymeric packings. For example, Laubli and Kampus separated Li(I), Na(I), NH_4(I), K(I), Ca(II), and Mg(II) using crown ethers as eluent additives [211]. With only a tartaric acid and PDCA eluent, the above metal ions were eluted within 10 min. However, adding 18-crown-6 (1,4,7,10,13,16-hexaoxocyclooctadecone) to this eluent eluted K(I) last at 12 min, which improved the separation of Na(I) and NH_4(I).

Similar work by Ohta using indirect photometric detection allowed the determination of Li(I), Na(I), NH_4(I), K(I), Mg(II) and Ca(II) on a cation exchange column [212].

Initially a tyramine and oxalic acid eluent gave incomplete separation of the monovalent metal ions and complete separation of divalent metal ions. The addition of 18-crown-6 to the eluent resulted in an excellent separation for all metals within 17 min. No interferences were observed from Pb(II), Cs(II), Ni(II), Pb(II), Zn(II), Co(II), Fe(II), Fe(III), Mn(II), Cd(II), and Cu(II). Linearity was investigated between 0.005 m*M* and 1 m*M* for all cations with regression co-effecients > 0.99.

Ohta and Tanaka separated Na(I), NH_4(I), K(I), Mg(II) and Ca(II) on an unmodified silica gel column with application to various natural water samples [213]. A number of complexing agents were investigated within the eluents, including malonic, tartaric, citric, malic and succinic acid. All gave incomplete separation of the monovalent and divalent metal ions. However, oxalic acid improved the separation for the divalents but not for the monovalent metal ions and therefore various crown ethers were added to this eluent to determine their effect on the separations. Three crown ethers were investigated, 18-crown-6 which gave complete separation of monovalent and divalent metal ions, as opposed to 12-crown-4 which had no effect due to low stability of the complexes formed. Finally, 15-crown-5 resulted in overlapping peaks of Na(I) and NH_4 (I), aswell as disagreement with the predicted stability constants of these complexes. Low limits of detection were obtained using a 20 μl injection loop in the range of 0.6 μg/L for Li(I) to 5 μg/L for Ca(II) and 28 μg/L for K(I). The order of elution was in agreement with predicted stability constants of complexes formed between the cations and the 18-crown-6;

$$\mathrm{Li(I)} < \mathrm{Na(I)} < \mathrm{NH_4\,(I)} < \mathrm{Cs(I)} < \mathrm{Rb(I)} < \mathrm{K(I)} \quad \text{and;}$$

$$\mathrm{Mg(II)} < \mathrm{Ca(II)} < \mathrm{Sr(II)} < \mathrm{Ba(II)}$$

The method was applied to rain and river water samples, with results in agreement with alternative IC methods.

Finally, Huber and Haidekker have used an IDA silica column for the isocratic separation of transitions, alkali metals and ammonium ion with conductivity detection [214]. The eluent of 18-crown-6, PDCA and HNO_3 was ideal for the analysis of different water samples with detection limits at low μg/L levels with excellent linearity. An investigation into the separation mechanism occurring when using the IDA column and crown ether eluent lead to the conclusion that (i) ion-exchange was occurring involving the carboxylic functions of IDA, (ii) complexation upon adsorption of 18-crown-6 and (iii) competing complex formation between IDA stationary phase and PDCA in the eluent, which determined the retention of the transition metal ions. Stability in the case of crown ether alkali metal complexes depended, as previously mentioned, on the size of the hydrophilic cavity of the macrocyclic ligand and the diameter of the metal ion. The multi-modal separation mechanism allowed the separation of monovalent and polyvalent metal ions under isocratic separation conditions. Drinking water, rain water and river water were analysed for Na(I), NH_4 (I) and K(I) with excellent linearity, $R^2 > 0.999$ over the range 0.5-100 ng.

1.8.2.iii. Colour forming complexing agents.

The ability to use reagents within eluents to form highly coloured and stable chelates with metals not offers alternative selectivities but can also eliminate the need for post-column reaction detection. Detection limits can be surprisingly low because extra dispersion of chromatographic zones is not introduced by post-column mixing procedures and hence the use of colour forming ligands within the eluent has been investigated by a number of workers for the determination of selected metal ions. The principle is based on complex formation within the eluent with the metal and monitoring the absorbance of the metal complexes as they elute. With metallochromic ligands present in the eluent the system functions through partial or complete complexation of the metal ion within the eluent itself. Retention can be due to ion exchange and/or to some extent surface adsorption.

Zenki was the first to investigate such eluents where upon he separated alkaline earth metals using a colour forming eluent containing chlorophosfonazo (III), (shown in Figure 1.17(a)), at pH = 7.5, on a cation exchange column [215]. Excellent separations were achieved within 15 min and stepwise elution or gradient work was not necessary. This method did not require a post-column reaction system and therefore no mixing tee-piece or additional pump was required. When applied to the determination of Ca(II) in a number of natural water samples, results agreed well with AAS. When used with a sulfosalicylic acid eluent, chlorophosfonazo(III) formed a ternary complex, sulfosalicylic acid-metal ion-chlorophosfonazo(III), with low absolute limits of detection between 2 ng for Ca(II) and 50 ng for Mg(II). However, Ni(II) and rare earth elements formed a stable complex with chlorophosfonazo(III) and gave a large peak causing interference with Ca(II) determinations when present.

Later work by Hutchinson *et al.* separated trace levels of Fe(II), Zn(II), Ni(II), and Co(II) by on-column complexation with PAR on a reversed-phase column with detection at 525 nm [216]. At pH < 4 few other metals formed stable complexes and hence this pH was ideal for good analytical selectivity. The results were compared with C_8 and C_2 columns with the order of elution on the latter two phases paralleling the order of the stability of the metal cations, which supports the concept that separation was controlled by the degree of formation of the metal complex. However, with the C_{18} column there was a noticeable change in elution order, in that Co(II) eluted last as opposed to second on the other two columns. The peak shapes for Ni(II) and Co(II) using PAR within the eluent were very sharp in comparison to those obtained by Wallace *et al.* who used dithiocarbamate ligands for the same purpose [217].

Wallace and Heneghan went on to investigate Cu(II), Ni(II), Co(II), Fe(II) and Hg(II) on a C_{18} column using mixed ethylxanthate (Etxan) and 1,10 Phenanthroline eluent with UV detection [218]. Ni(II), Cu(II), Co(II), and Hg(II) eluted within 9 min, with poor separation of two of the peaks. The limits of detection were reasonable with 0.5 mg/L for Ni(II), Cu(II) and Co(II), 0.05 mg/L for Fe(II) and 0.2 mg/L for Hg(II) using a 10 μl loop. However there was no application of the developed method to real samples.

Toei and Baba investigated alkaline earth metals on a chemically bonded carboxylic acid type silica using *o*-cresolphthalein complexone (*o*-CPC), shown in Figure 1.17(b) as a colour forming eluent and ammonium hydroxide buffer as the post-column reagent [219]. The separation was achieved in 25 min. Both isocratic and gradient analysis was performed, with the elution order being Mg(II), Sr(II), Ca(II) and Ba(II).

The gradient work shortened the analysis time to 16 min using elevated temperature and a step up in KCl concentration from 0.1 to 0.4 *M*. As eluent concentration increased retention times decreased, which showed that some degree of ion exchange was occuring. However, this system required an extra pump for delivering the post column buffer. This work was extended using a chemically bonded sulphonic acid type cation exchange column by Toei [220]. As these gel columns could not be used with alkaline eluents, post-column buffering was again required. A Ca(II) and Mg(II) mix was spiked with 0.5 and 1.0 *M* NaCl and no interference was observed, which indicated that high ionic strength samples such as brines could be analysed. The main separation mechanism was a combination of ion exchange between cation exchange groups in the gels and metal ions and chelating interactions between the eluent ligand and metal ions.

Toei has also looked at the separation of these metal ions using Arsenazo(III) as a component of the eluent, where no post-column buffer system was required. The separation was achieved within 10 min with excellent resolution on a TSK gel chemically bonded sulfonic acid cation exchange column [221].

Similar work was performed on a short non-porous gel column with Mg(II) and Ca(II) eluting within 55 seconds, with sharp peak shapes for Ca(II), Mg(II) and Sr(II) and full resolution for all four alkaline earth metals. The system was applied to industrial waters with results comparing well to AAS [222].

Toei has also separated Ni(II) and Zn(II) as anionic complexes using XO, shown in Figure 1.17(c), as a component of the eluent, again with no post-column derivatisation required [223]. The retention was based on anion exchange although the exact mechanism was not established. Partial separation was shown of Ni(II), Co(II), Zn(II) and Cu(II) using a 0.1 m*M* XO eluent and a phosphate buffer (pH=5.20) at $5O^{O}C$. However, the separation effeciency was rather poor making the developed method unsuitable for real sample analysis.

Alkaline earth metals were separated on a chelating column by Teoi in which *o*-CPC was again employed as the ligand in the eluent [224]. The previous work with the sulphonic acid cation exchanger did not allow selective determination of alkaline earth metals because of the low selectivity of the column. However, this was not the case with the carboxylic acid based chelating column, as it exhibited high selectivity for alkaline earth metals over alklali metals. Chelating interaction between gel and metal cations and chelating interaction between chelating agent and the metal cations controlled selectivity. 0.5 *N* K(I), Na (I) and Li (I) did not interfere with Ca(II) and Mg(II) determinations hence this method was applied to sea-water analysis, with no interference from transition metals. The limits of detection were 200 μg/L using a 20 μl loop.

Dasgupta *et al.* employed 8-HQS as a component of the eluent using cation exchange chromatography with fluorescence detection. Attention was focused on alkaline earth metals along with selected other divalent and trivalent metal ions. The exact retention mechanism was the subject of debate, as a combination of ion-exchange and adsorption of 8-HQS onto the stationary phase was suggested [225].

Takeuchi *et al.* also used fluorimetric detection in a method developed for Al(III) and Mg(II) determination. He compared on- column and post-column derivatisation with oxine using a laboratory made micro-column prepared from fused silica tubing [226]. On-column complexation gave the most sensitive results. With oxine in the eluent Mg(II) and Al(III) were detected using emission and excitation wavelengths of 516 nm and 370 nm. Without oxine, Al(III) was detected only which implied that samples with high Mg(II) matrices could be examined.

Wada *et al.* compared a number of colour-forming ligands as eluents on a sulphonated PS-DVB gel (10 μm) and sulphonated silica gel (5 μm) for the determination of alkaline earths and transition metals in tap water [227]. The ligands were of the bisazochromotropic acid type. Chlorophosphonazo(III) was the most sensitive ligand tested and the PS-DVB gave the best results with excellent separation of Ca(II), Sr(II) and Ba(II) in just 5 min.

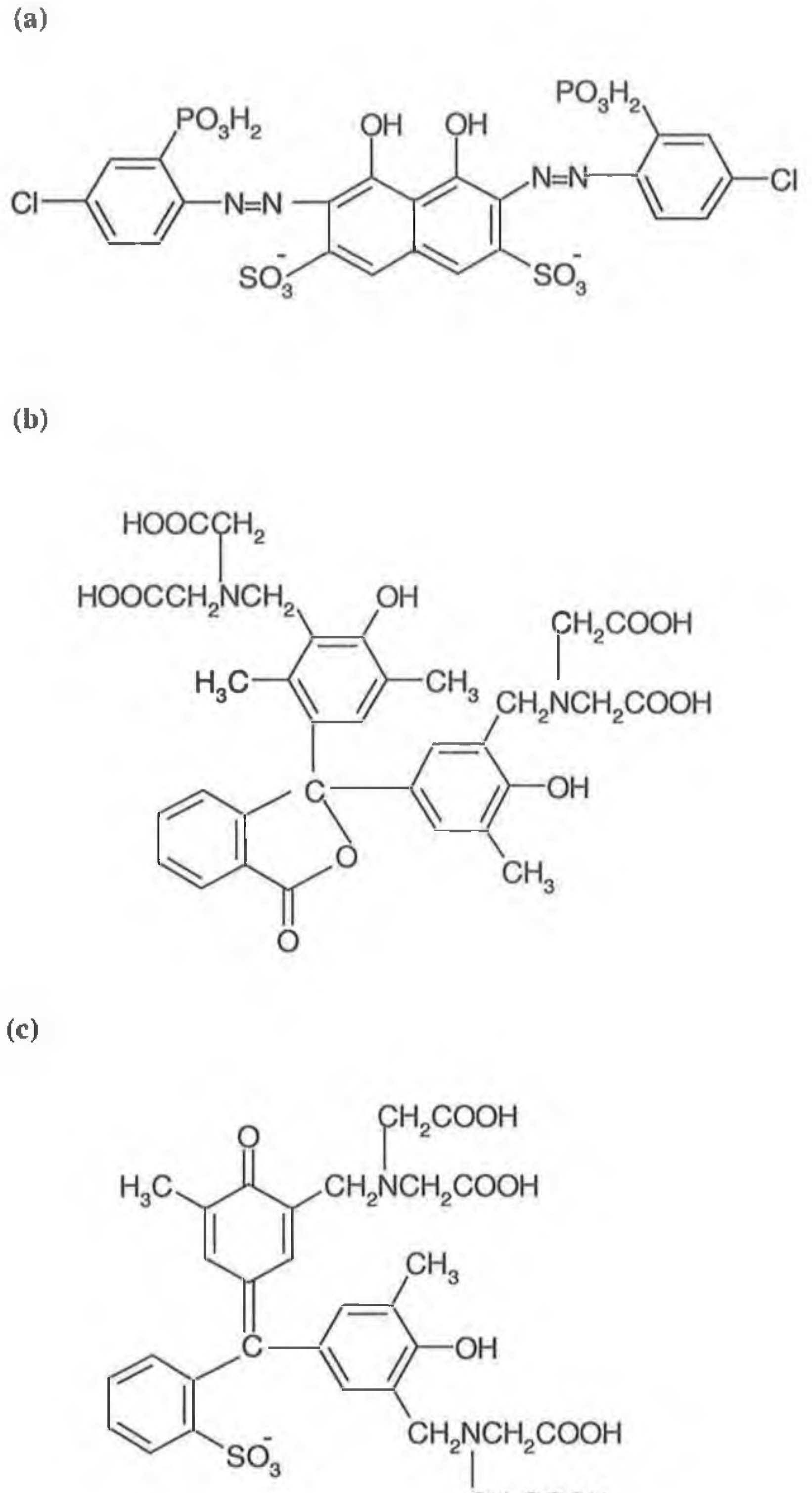


Figure 1.17. Structures of (a) Chlorophosphonazo (III), (b) *o*-Cresolphthalein complexone (*o*-CPC) and (c) Xylenol Orange (XO).

However, an irregular elution sequence was observed in that Mg(II) eluted last at 30 min which may have been due to its smaller stability constant with the eluting ligand. The results of several tap water samples compared well with AAS and EDTA titrations.

Ion-interaction or ion- pair LC methods have also been developed using colour-forming ligands as components of the mobile phase. For example, Fritz and Main used 2-acetylpyridine-4-ethyl-3-thiosemicarbazone (Hapet), shown in Figure 1.18(a), as a colour forming agent and $NaClO_4^-$ as the inorganic IIR for the separation and determination of Co(II), Fe(III), Cd(II), Zn(II) and In(III) on a polymeric column with UV detection at 380 nm. Other metal ions were also investigated and Hg(II), Pb(II), and Bi(III) gave good separations, yet those with Ni(II), Ga(III), and In(III) gave particular broad peak responses. When applied to a complex sample matrix for the determination of Ni(II), Co(II), Fe(III), Ga(III) and In(III), a 1000 fold molar ratio of Na(I) and Ca(II), 100 fold molar ratio of Al(III) and a 10 fold molar ratio of Mn(II), Pb(II), Ce(III) and Bi(III) gave no interference on analysis carried out above [228].

Sarzanini *et al.* have used the disulphonated azo dye PCB as an on-column complexing agent in the presence of TBAOH using both 5 and 10 μm reversed-phase columns for the analysis of Co(II), Cu(II), Fe(III), Ga(III), In(III), Ni(III), V(V) and Zr(IV). The method was applied to natural water samples with results comparing well with GF-AAS. The 10 μm column showed better performance in terms of resolution of peaks at shorter retention times. However, the 5 μm was more efficient for the trivalent metals. The system proved highly sensitive with limits of detection between 15 and 150 μg/L [229].

Sarzanini *et al.* also employed the azosulphonated ligand Calcion, (shown in Figure 1.18(b)), with TBAOH, as eluent components for the determination of Ni(II), Cu(II), Fe(III) and Al(III) in tap water. The application of a gradient showed a sufficient decrease in analysis times without any loss of selectivity. pH was a crucial parameter for controlling selectivity and thus avoiding interferences by certain metal ions [230].

Padarauskas and Schwedt performed the simultaneous determination of inorganic anions and cations using ion-interaction RP-HPLC and UV-detection. A number of ligands were investigated for their on-column derivatisation properties. Twelve anions and cations were determined using an eluent containing 1,2-diaminocyclohexane-N,N,N,'N,'-tetraacetic acid (DCTA) and TBAOH. Excellent separation was obtained within 30 min with very good resolution between peaks. Fourteen anions and cations were simultaneously separated within 30 min with excellent separation and baseline resolution between all peaks [231].

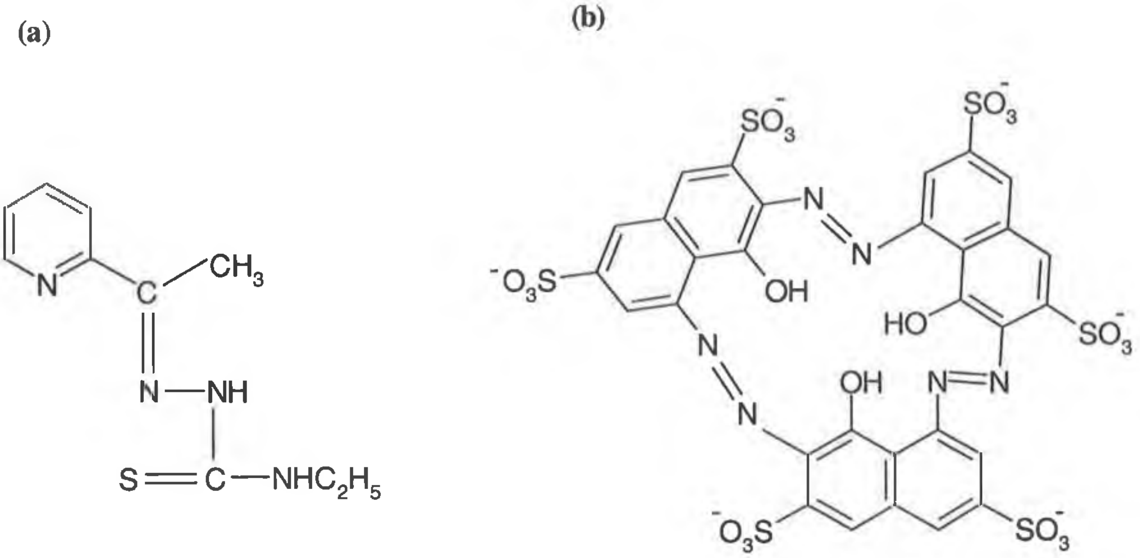


Figure 1.18. Structures of (a) 2-acetylpyridine-4-ethyl-3-thiosemicarbazone (Hapet) and (b) Calcion.

O' Laughlin [232] and Timerbaev *et al.* [233] have both published reviews dealing with the separation of various metal chelates using LC, although most examples are pre-column complexation. Further examples of the use of colour-forming eluents can be found in the review by Marina *et al.* [234].

1.9. High-performance Chelation Ion Chromatography (HPCIC).

Chelating resins have mainly been employed for matrix elimination and preconcentration applications as discussed in Section 1.7. However, more recent work has been based on using such chelating substrates for high-performance separations. This technique has been referred to as high-performance chelation ion chromatography (HPCIC) and has been comprehensively reviewed by Nesterenko and Jones [235]. The technique is differentiated from chelation ion chromatography (CIC), as developed by the Dionex corporation in that only one chelating column is used for high-performance separations and in some cases preconcentration also. The main advantage of this technique over standard IC is that it has the ability to separate metal ions in samples of high ionic strength. Such ionic strength samples can 'swamp' out the ion-exchange sites on standard ion-exchange phases, a process known as self-elution, leading to a reduction in the capacity of the column.

A disadvantage of HPCIC is peak broadening due to the slower reaction kinetics. As the retention of the metal ions is based upon the formation and dissociation of a coordinate bond, rather than simple electrostatic interaction, mass transfer is slower in HPCIC than simple IC:

$$M + nL \underset{K_b}{\overset{K_f}{\longleftrightarrow}} ML_n \qquad K_{stab} = \frac{K_f}{K_b} \qquad \text{Eqn. 38.}$$

If the velocity of the forward reaction, (K_f), is much larger than the backward reaction, (K_b), then K_{stab} is large which implies that chelation of the metal ion will occur quickly but release it slowly, which results in tailing of the peak. The solutes with the smaller conditional constants will have quick dissociation kinetics, as opposed to those with larger conditional stability constants which will have longer retention times and will have much slower dissociating kinetics. Either increasing the column capacity or reducing the particle size can partially overcome the problem. This is because once the capacity of the column is increased, a lower eluent pH can be employed, which in turn decreases the conditional stability constant of the complexes and hence sharper solute peaks.

There are three mechanisms available to prepare a chelating sorbent. The first is to impregnate a chelating ligand into the pores on onto the surface of a suitable substrate, which can be either polymeric or silica based. The ligands used in this way have mostly been from the triphenylmethane group of synthetic dyes often with attached IDA groups, although a number of azo based ligands have also been studied. The applications of such systems will be mentioned in Section 1.9.1.

Paull and Jones have compared the metal selective properties of a number of dyes impregnated on high efficiency resins. Studies indicated that triphenylmethane based dyes with IDA functionality provided the most stable coatings and column capacities [236].

The second approach is to dynamically modify the substrate with a chelating ligand added to the mobile phase where the substrate initially has no chelating surface. The applications of such dynamic systems will be discussed in Section 1.9.2. In this approach equilibrium is established between the chelating compound in the mobile phase and the sorbed monolayer of the chelating compound on the substrate. This is referred to as dynamic modification because when the chelating agent is removed from the eluent, the sorbed layer leaches off. In this case the separation is mainly controlled by the chelating groups on the substrates surface because the dynamically sorbed layer is much higher in concentration than the amount in the mobile phase. This approach has been reviewed by Paull and Haddad [237].

The final approach is to covalently bond chelating groups to high efficiency sorbents. The applications of such bonded phases will be discussed in Section 1.9.3.

1.9.1. Permanently coated stationary phases.

One of the earliest publications on the use of permanently coated chelating stationary phases was that produced by Yamazaki *et al.,* who coated a reversed-phase column with N-n-dodecyliminodiacetic acid for the separation of alkaline earth metals ions. The ions were detected using post-column reactions with either colour-forming or fluorescent reagents. Ca(II), Mg(II), Sr(II) and Ba(II) were separated within 8 min with excellent resolution using tartaric acid as the eluent. The method was applied to seawater analysis using *o*-CPC as the post-column derivatising reagent. The levels of Ca(II) and Mg(II) determined were 10.8 and 39.8 m*M* respectively [238].

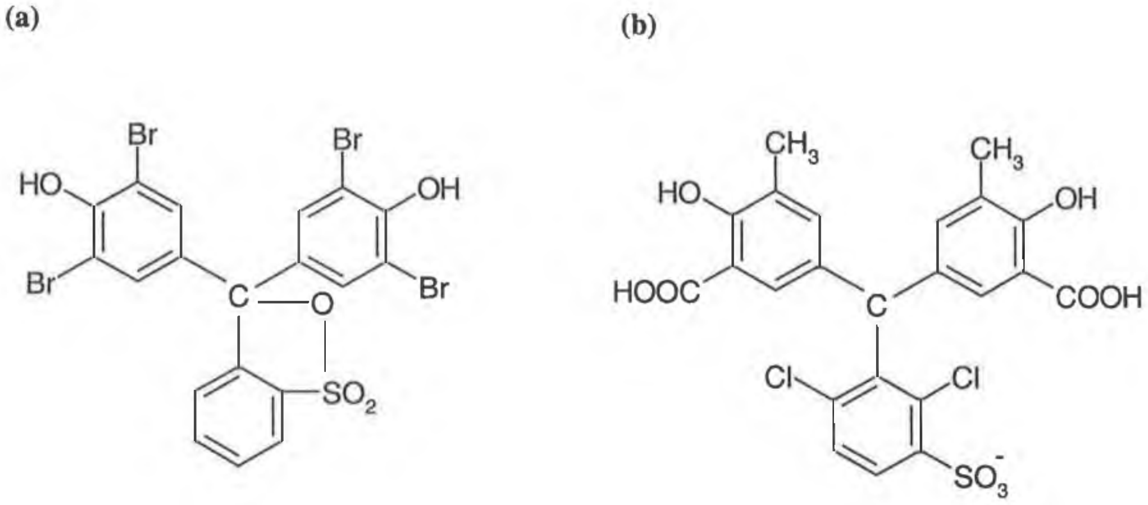


Figure 1.19. Structures of (a) Bromophenol blue (b) Chrome azurol S (CAS).

Jones and Schwedt were the first to permanantly coat HPLC grade neutral polystyrene resins, in this case using triphenylmethane type dyes. The preconcentration and separation of divalent and trivalent metal ions was studied. Bromophenol blue, shown in Figure 1.19(a), produced an ion exchange coating similar to a low capacity sulphonated resin but with much lower efficiency. Chrome azurol S (CAS) shown in Figure 1.19(b), resulted in a chelating exchange coating which allowed the isocratic separation of Mg(II), Mn(II) and Zn(II). Eventhough only sufficient resolution was obtained and the peaks were broad, the relative insensitivity to ionic strength was ideal for samples with high salt levels. Relatively large volumes of samples were injected showing how preconcentration and separation could be performed on the same column. Three post-column reagents were employed for detection which included CAS for Be(II), Al(III), Ga(III), Fe(III); PCV for detecting Al(III), Ga(III), In(III), Fe(III), and Calmagite for other divalent metal ions [239].

This work was further extended by Jones *et al.* who investigated CAS, Calmagite shown in Figure 1.20(a), and XO as chelating dyes for coating Dowex 1-X8 anion exchange resins, Amberlite neutral XAD-2 and XAD-4 resins and a smaller particle size HPLC PS-DVB substrate. The resultant separations were compared with those obtained on a Tosoh TSK-GEL, a commercially available chelating column. The metal ions studied were Mn(II), Mg(II), Co(II) and Zn(II). Eventhough the relative chelating ability of each dye varied, the metals were retained in the same order i.e. Zn(II)>Co(II)>Mn(II)>Mg(II). The XAD-2 dye coated columns showed the strongest retention for the metal ions. The XAD-4 showed the weakest retention whereas, Dowex 1-X8 showed retention characteristics inbetween these two. Due to the large particle size these columns showed limited separating power and hence smaller particle size HPLC grade PS-DVB substrates were investigated. When coated with XO and CAS their performance compared well with that of the commercially available column. A pH gradient was applied to the separation of Cd(II), Pb(II) and Cu(II), which were completely separated from high concentrations of alkaline earth metals. This was of special interest when dealing with samples such as seawater [240].

Later Jones *et al.* using similar HPLC grade PS-DVB chelating columns and investigated the separation of both divalent and trivalent metal ions, using a common 1 *M* KNO_3 eluent and different post-column reagents. In this case 2.4 *M* KCl and 5.1 *M* NaCl brines were analysed. Various gradient systems were applied, including (i) a single step gradient from pH 11 to 6 for the preconcentration and separation of alkaline earth metals in KCl brine, (ii) a three step gradient for the preconcentration and separation of nine cations in KCl brine and, (iii) the preconcentration and separation of alkaline earth metals in NaCl brine. The exceptional results obtained in the determination of these trace metal ions in such concentrated brines encouraged further work with other matrices such as laboratory chemicals, 1 *M* KNO_3, 1 *M*

Na_2SO_4 and 1 *M* CsI. The only problem encountered was in quantifying Cu(II) in CsI due to interference from iodide. A CAS coated column was not as efficient as a XO column when applied to the separation of trace metal ions in seawater. The results from these studies showed how the combined preconcentration and separation of metal ions in various high ionic strength media could be achieved using a single chelating column. However the work was limited to the brief semi-quantitative study of Zn(II) and Cu(II) [241].

Jones *et al.* then went on to extend this work to the analysis of Ba(II) and Sr(II) in mineral water and milk powder samples. The determination of Sr(II) in skimmed milk powder was possible eventhough the ratio of Sr(II):Ca(II) was 1:2000, which would have caused problems with other techniques. The determinations were carried out on a PS-DVB column coated with (i) Methylthymol blue (MTB), shown in Figure 1.20(b), for Ba(II) and Sr(II) analysis in mineral water and (ii) *o*-CPC for Sr(II) determination in skimmed milk. 1 *M* KNO_3 was used as the eluent, suppressing any ion-exchange interactions, this resulting in Ba(II) eluting first ahead of high levels of Ca(II) and Mg(II), which would have interferred with the analysis if ion exchange was the dominant mechanism. A step-down in eluent pH was used after the elution of Ba(II) and Sr(II) to sweep off Ca(II) and Mg(II), thus reducing analysis time. A simulated river water certified reference material IAEA/W-4 containing a ratio of Ba(II) and Sr(II) to Ca(II) of 1:200 was also analysed [242].

Jones *et al.* applied the same system for the determination of alkaline earth metals in offshore oil well brines using a MTB impregnated PS-DVB neutral resin. A limit of detection of 1 mg/L Ba(II) was obtained in samples containing 1600 mg/L Ca(II) and 3% sodium using a 5 μl injection loop. As previously encountered, the high eluent concentration of 0.5 *M* KNO_3 allowed suppression of ion-exchange interactions and very little affinity for monovalent salt ions such as Na(I) or K(I) was exhibited, resulting in the ionic strength of the sample having little or no effect on the chromatographic separation. Upon dilution of a 3 *M* NaCl brine sample, containing 700 mg/L Mg(II) and 350 mg/L Ca(II), a limit of detection of 8 mg/L was obtained for Sr(II). The results compared well with alternative analytical methods such as ICP-OES [243].

A XO impregnated resin was also applied by Jones *et al.* to the determination of Zn(II), Pb(II), Ni(II) and Cu(II) in sea water samples and the certified reference material CASS-2. As in the previous case both preconcentration and separation of the metal ions was possible using the same column.Two seawater samples were analysed with Mn(II) and Cd(II) co-eluting and hence not being determined in the samples.

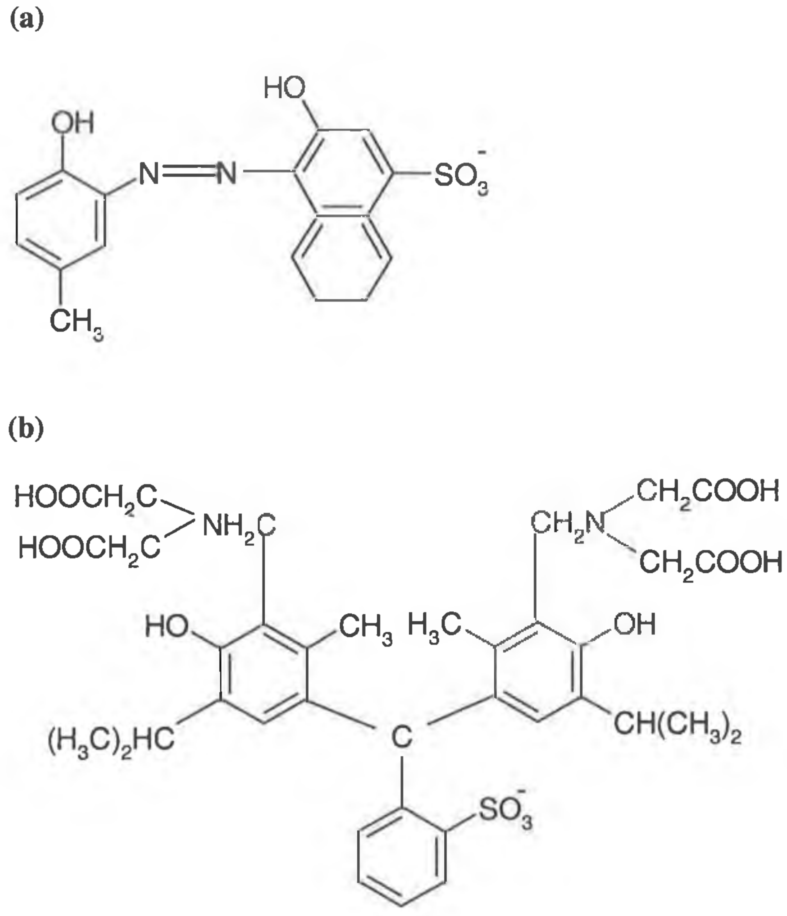


Figure 1.20. Structures of (a) Clamagite (b) Methyl thymol blue (MTB).

Zn(II), Pb(II), Ni(II) and Cu(II) were determined using standard addition. The second sample required a step gradient to remove Pb(II) away from Zn(II). Excellent linearity was shown for Pb(II), Ni(II), Cu(II) and Zn(II). Pb(II) and Ni(II) were below the limits of detection when applied to the certified reference material yet other results compared well with certified values [244].

Jones *et al.* also looked at alternative substrates to PS-DVB in an extension of the above work, to compare their chelating exchange abilities. PAR was used to impregnate Amberlite IRA904, Amberlite XAD-2, Purolite MN100, Purolite MN200 and cellulose. The selectivity order was as expected for each of the impregnated substrates, being Cu(II) > Zn(II) > Pb(II) > Mn(II) > Cd(II) > Mg(II), with especially strong affinity being shown for Cu(II). XAD-2 showed the highest dye loading but did not posses the highest capacity for the metal ions. The MN100 and MN200 resins had lower dye loading than XAD-2 but much greater capacities for metal ions. Amberlite IRA-904 showed both low dye loading and capacity for metal ions. The MN100 and MN200 was considered as the most suitable resins as they were totally neutral, which resulted in the elimination of any interactions with the functional groups in the resin backbone [245].

Paull and Haddad applied the columns developed by Jones *et al.* to the determination of trace levels of uranyl in highly saline samples. A MTB impregnated polymer column was used with KNO_3 as the eluent, followed by post-column detection with Arsenazo(III). Less than 1 μg/L uranyl could be detected in brine samples using a step gradient from 6 mM HNO_3 to 60 mM HNO_3, following large sample volume injection (2 mLs). The method was applied to a saturated saline Antarctic lake sample containing 3.1 M Na(I), 0.1 M K(I), 0.6 M Mg(II) and 0.05 M Ca(II). The results were compared satisfactorally with ICP-MS. Under the isocratic conditions employed alkali and alkaline earth metals were unretained on the column when using acidic eluents. Mn(II), Fe(II), Cd(II), Zn(II), Co(II), Pb(II), Ni(II) and the lanthanide series also showed no retention. Spiking 0.1 M and 1.0 M NaCl with uranyl and Cu(II) showed no significant salinity effects on retention time, peak shape and area, and no detector response was shown for the NaCl. Under the optimum isocratic conditions the limit of detection was 8 μg/L. This work again showed how such hyper-saline samples could be injected directly without sample pretreatment, allowing the separation of low μg/L levels of uranyl from excessive matrix metals [246].

Finally, Nesterenko and Jones compared three chelating ion-exchangers having ligands containing IDA functional groups immobilised at the surface of different substrates namely silica gel, a hydrophilic polymer and a hydrophobic polymer for the separation of alkaline earths and transition metals. These columns were further compared with commercially available column, Diasorb IDA silica, Tosoh TSK gel chelate 5PW and a polystyrene divinylbenzene substrate coated with *o*-CPC. The main drawback of the hydrophobic polymeric substrate was swelling and shrinking due to the change in eluent pH and ionic strength due to its low % crosslinking. However, the hydrophilic polymer had no tendency for change in volume in the various eluents. The affinity of the various columns for the metal ions studied was in the order IDA-silica > TSK gel chelate 5PW > *o*-CPC column.The elution order for Zn(II) and Cd(II) was different for the TSK gel chelate 5PW and the IDA-silica columns. Further investigations led to the conclusion that the IDA-silica showed the strongest retention and the *o*-CPC column showed the weakest retention. The IDA-silica and TSK gel chelate 5PW columns were ideal for isocratic analysis of the transition metals, yet the latter column was restricted by dissolution in acid media [247].

The above studies have been summarised in Table 1.6.

An advantage of these dye immobilised columns is the inter-group selectivity between transition and trivalent metal ions and the alkaline earth metals, which could facilitate the use of these columns to determine trace metals in concentrated brines and highly mineralised samples. A possible disadvantage is the poor intra-group selectivity exhibited by many columns, but increasing column length, column capacity and using pH gradient elution programs could possibly overcome this.

A significant drawback of the pre-coated substrates is the relatively poor efficiency in comparison with covalently bonded high-performance chelating columns. The use of high efficiency small particle size substrates allows no more than 5 metal ions to be separated isocratically, due to the slow exchange kinetics causing rapid peak broadening, meaning that the slower eluting peaks exhibit significant tailing. Also a change in the eluent pH, in some cases can cause bleeding of the ligand from the column resulting in an overall reduction in the capacity of the column.

1.9.2. Dynamically coated stationary phases.

The dynamic coating approach is one in which some of the previously mentioned problems with pre-coated columns can be eliminated. With the ligand in the eluent several functions can be served. Not only does it result in creating a chelating surface similar to that of the pre-coated columns but it also acts as a complexing agent within the eluent. The selectivity of various metal ions can be manipulated and the fact that a colour forming ligand is used in most cases can be utilised in such a way that no post-column reagent is required.

Paull *et al.* dynamically coated a porous graphitic carbon reversed-phase column with *o*-CPC for the determination of Ca(II) and Mg(II) in seawater samples. Seawater was selected due to its complex matrix, which illustrated the selectivity of the method. The extremely high level of sodium did not complex with the ligand and therefore passed through the column. The fact that a pH tolerant column was used meant that post-column reaction detection was not required. Baseline separation of Mg(II) and Ca(II) was obtained within 10 min using 5% methanol, 0.4 m*M* *o*-CPC at pH 10.5 as the eluent [248].

Paull *et al.* further extended this method to the analysis of other environmental water samples including saturated saline Antarctic lake samples, again using the graphitic carbon stationary phase and *o*-CPC as the ligand for dynamic coating. Detection limits of 0.05 mg/L and 0.1 mg/L were obtained for Mg(II) and Ca(II) respectively in samples containing excess of 2,300 mg/L Na(I). No interference was observed from 50 fold excess K(I), Ba(II) or Sr(II). Sr(II) did however tail severly and its sensitivity was 10 times less than for Ca(II) or Mg(II) due to its low stability constant with *o*-CPC. A number of other samples were analysed including NATA certified reference material and a mine process water with results comparing very well with other analytical techniques such as AAS, capillary zone electrophoresis (CZE) and ICP-MS [249].

Paull *et al.* repeated similar work on a PS-DVB reversed phase column for the determination of Sr(II) in a highly concentrated brine sample from an Antarctic lake. Other alkaline earth metals were also investigated. The fact that the order of elution was reversed in comparison to simple ion exchange meant that Ba(II) and Sr(II) eluted prior to Ca(II) and Mg(II) and hence interference from excess concentrations of these metals was eliminated. The Antarctic lake brine sample contained 3.23 *M* Na(I), 0.101 *M* K(I), 0.573 *M* Mg(II) and 0.054 *M* Ca(II). Sample dilution of 1:15 was required to prevent overloading of the column. The limits of detection were 10 μ*M* for both Ba(II) and Sr(II) [250].

Paull *et al.* also compared a precoated MTB and dynamically coated MTB reversed-phase column for the isocratic separation of 12 metals. Their investigation led to the conclusion that the dynamically coated MTB column gave much better efficiencies in comparison to a pre-coated version. This was clearly evident in the case of Pb(II) which gave a broad peak on the pre-coated column as opposed to a sharper peak using the dynamic system, although Cu(II) was broader than expected with both methods, due to the high stability constant for the Cu-MTB complex. The determination of twelve metal ions including selected lanthanides was possible using a 0.5 *M* KNO_3, 2 m*M* MTB eluent at pH 2.1 at 65°C in under 40 min. Retention was dependent upon mobile phase pH and the ligand concentration. The limits of detection were in the range of 1 μ*M* for Zn(II) and Pb(II) in 1 *M* NaCl using a 100 μl loop [251].

Later work by Hill *et al.* also applied the dynamic approach when they investigated the separation of uranium from multi-elemental mixtures using HPCIC. They prepared a range of different chelating columns some pre-coated and some dynamically coated. These included two neutral polystyrene substrates, a hyper-cross linked macronet and a low cross linked polymeric column, which were impregnated with Calmagite, PAR and PDCA. Studies showed that different retention orders were observed on on three columns. The pre-coated calmagite produced a uranium peak which was particularly broad eventhough it eluted quickly. A PAR coated column showed stronger affinity for uranium than the calmagite column. The retention order was reversed compared to the calmagite column, with Th(IV) eluting close to the solvent front. Eventhough promising selectivity was obtained using the PAR column, bleed from the column resulted at acidic pH. The best separation was obtained on the macronet resin dynamically coated with PDCA, which produced good peak symmetry which was well resolved from Th(IV) and Fe(III). The elution order was the same as

Table 1.6. continued. High-performance separation of metal ions on chromatographic columns pre-coated with chelating dyes.

Chelating agent	Eluent	Metal ions	Detection	Application	Ref.
MTB.	0.5 *M* KNO_3, pH 1.2.	UO_2, Cu(II).	PCR with PAR.	UO_2 in saline lake sample.	243
MTB	0.5 *M* KNO_3, pH 1.4.	Ca(II), Mg(II), UO_2	PCR with ammonium acetate pH 5.9.		243
PAR	0.5 *M* KNO_3, 0.05 *M* lactic acid, pH 10	Alkaline earth metals ions	PCR with Zn/EDTA		253
N-dodecyl-iminodiacetic acid	Tartrate buffer, pH 5.5	Alkaline earth metals ions	PCR with *o*-CPC	Coastal seawater	246
Chrome Azurol S (CAS).	Two step pH gradient from 2.2 to 1.0 in 1 *M* KNO_3.	Al(III), Ga(III), In(III), Fe(III)	PCR with PCV		243
CAS	1 *M* KNO_3, pH 4.0.	Pb(II), Cd(II)	PCR with PAR-Zn/EDTA		248
CAS	2-step pH gradient from7 - 4 and 4 -1.5 in 1 *M* KNO_3.	Mg(II), Cd(II), Pb(II), Cu(II)	PCR with PAR-Zn/EDTA	KCl / NaCl brines	243
o-Cresol-phthalein complexone (*o*-CPC)	1 *M* KNO_3, pH 3.7.	Mn(II), Zn(II), Cd(II), Pb(II)			243
(*o*-CPC)	1 *M* KNO_3, pH 9.8.	Ba(II), Sr(II), Ca(II)	PCR with ZnEDTA/ PAR	Sr(II) in milk powder	250
(*o*-CPC)	1 *M* KNO_3, pH 8.3.	Alkaline earth metals ions	Direct detection		243
Glycine Cresol Red	1 *M* KNO_3, pH 9.0.	Alkaline earth metals ions	PCR with PAR-Zn/EDTA		243
Calmagite	1 *M* KNO_3 pH 5.2.	Mn(II), Cd(II), Pb(II), Zn(II)	PCR with PAR-Zn/EDTA		243

Jones *et al.* extended this concept, by employing 3 complexing pyridine carboxylic acids, picolinic acid, PDCA and quinaldic acid for dynamically coating both PS-DVB and silica C_{18} columns. Their work showed the potential to use small organic acids as opposed to relatively large metallochromic ligands, for dynamically coating the columns for the separation of Pb(II), Cd(II), Co(II), Mn(II), Ni(II) and Zn(II). As in most cases a high eluent concentration was used, 1 *M* KNO_3, and post-column reaction detection with PAR was required. Using picolinic acid many metals eluted close to the solvent front with some split peaks were evident. The elution order for the divalents was strikingly different to PDCA and similar to picolinic acid when using quinaldic acid, with the exception of Pb(II) and Cd(II), which clearly showed the varying chelating properties of the mono and dicarboxylic acids. The peaks were slightly broader for the silica gel column. A surprising outcome was that Ni(II) produced a symmetrical peak, which was unusual when compared to alternative chelating phases such as those incorporating IDA [253].

Similar work was repeated by Jones *et al.*using a neutral PS-DVB column, this time with 4-chloro-dipicolinic acid for the separation of transition and heavy metal ions. A particularly strong affinity was displayed for Cd(II) and it was retained even longer than Cu(II), which allowed the system to be exploited for its selective determination. As previously encountered, Ni(II) showed a good symmetrical peak shape, which was again unusual [254].

The above preliminary work [254], showed how the 4-chloro-dipicolinic dynamic system produced a special selectivity for uranium, which led to the use of the column for the determination of uranium in selected complex samples. Isocratic elution of uranium within 10 min, away from matrix interferences, was achieved using a 1 *M* KNO_3 and 0.1 m*M* PDCA eluent with post-column detection using Arsenazo(III). Mineral water, seawater, sediment and certified reference materials were analysed. A limit of detection of 20 μg/L was obtained. Increasing the loop size decreased detection limits threefold without noticeably broadening the peaks. Certified reference materials for soft water and seawater samples were analysed for U(VI) with results agreeing well with the certified values [255].

The success of such small organic ligands for dynamic coating led to the development of a method by Cowan *et al.* for the separation of high valence metal cations over the pH range 0-3 on a neutral polystyrene resin column dynamically coated with PDCA. High valency metal ions are troublesome when using non-complexing acid media for separation purposes. However, this problem was overcome in the present work. The retention order was Th(IV) < V(V) < Bi(III) < U(VI) < Hf(IV) < Zr(IV). Ti(IV), Sn(IV), V(V) and Fe(III) showed no retention. A feature of this system was the high selectivity for U(VI) and Hf(IV), with both metal ions being resolved within 15 min. Two post-column reagents were used, PCV for detection of V(IV) and V(V); Arsenazo(III) for the others. The insensitivity to ionic strength, which has become a notable feature of chelation systems, made this method suitable for the determination of high valence metal ions in high ionic strength environmental and biological samples after acid digestion [256].

The above applications have been summarised in Table 1.7.

1.9.3. Chemically bonded stationary phases.

An alternative to the above impregnated/pre-coated and dynamically coated techniques is the chemically bonded stationary phases which have become increasingly available in the past number of years. Both silica and polystyrene based chelating columns have been developed, with silica being the more popular, as will be discussed further.

Jezorek and Faltynski separated a number of metal ions on several silica bound azo-coupled chelating stationary phases. The stationary phases used were 2-methyl-8-quinolinol silica gel (MQSG), N-(1-naphthyl)-ethylenediamine silica gel (NEDSG), *o*-acetoacetylphenol silica gel (diketone, DKSG), thenoyltrifluoroacetone silica gel (TTASG) and diphenylthiocarbazone silica gel (dithizone, DTSG). The various resins gave different retention behaviours and times with two retention mechanisms occuring, namely ion exchange and chelation exchange. Excellent separation of 6 transition metal ions was obtained using the dithizone column [257].

Table 1.7. High-performance separation of metal ions on chromatographic columns dynamically coated with chelating dyes.

Chelating agent	Eluent	Metal ions	Detection	Application	Ref.
Methyl Thymol Blue (MTB)	0.5 *M* KNO_3, 0.2 m*M* MTB, pH 1.2	Mg(II), Mn(II), Zn(II), Cd(II), Pb(II).	Direct detection 600 nm	Zn(II) in industrial gypsum	259
MTB	0.5 *M* KNO_3, 0.4 m*M* MTB, pH 0.9	Pb(II), Cd(II).	Direct detection 600 nm		259
MTB	0.5 *M* KNO_3, 0.2 m*M* MTB, pH 2.1	Mg(II), Mn(II), Zn(II), Cd(II), Pb(II), La,Ce, Nd,Sm, Tb, Tm,Lu.	Direct detection 600 nm		259
o-Cresol-phthalein complex-one (*o*-CPC)	58% Methanol, 0.4 m*M* *o*-CPC, pH 10.5	Mg(II), Ca(II).	Direct detection 572 nm	Seawater	256
o-CPC	45% Methanol, 0.2 *M* NaCl, 0.4 m*M* *o*-CPC, pH 10.0	Na excess, Mg(II), Ca(II).	Direct detection 572 nm	Saline lake samples	257
o-CPC	25% Methanol, 0.5 *M* KNO_3, 0.4 m*M* *o*-CPC, pH 10.0	Mg(II), Ca(II).	Direct detection 572 nm		258
o-CPC	0.5 *M* KNO_3, 0.2 m*M* *o*-CPC, pH 9.5	Ba(II), Sr(II).	Direct detection 572 nm	Sr(II) in saline lake sample	258
o-CPC	0.5 *M* KNO_3, 0.2 m*M* *o*-CPC, pH gradient	Ba(II), Sr(II), Ca(II), Mg(II).	Direct detection 572 nm		258
Picolinic acid, PDCA and quinaldic acid	1 *M* KNO_3	Pb(II), Cd(II), Co(II), Mn(II), Zn(II).	PCR with PAR	Not reported	253
4-chloro-dipicolinic acid	1 *M* KNO_3	Transition and heavy metals.	PCR with PAR	Not reported	254
4-chloro-dipicolinic acid	1 *M* KNO_3, 0.1 m*M* PDCA	U(VI).	PCR with PAR and Arsenazo(III)	Environmental water and sediment samples	255
PDCA	1 *M* KNO_3 0.1 m*M* PDCA	Th(IV), V(V), Bi(III),U(VI), Hf(IV),Zr(IV).	PCR with PCV and Arsenazo(III)	Not reported	256

1.9.3.1 Iminodiacetic acid (IDA) bonded silica column.

Bonn *et al.* first investigated the separation of a number of metal ions on an IDA bonded silica stationary phase, shown in Figure 1.21. A number of eluents were investigated in order to observe the elution mechanism of alkali, alkaline earths and transition metal ions. Citric acid and tartaric acid both gave similar elution characteristics but the retention times with citric acid were slightly longer owing to its lower degree of dissociation. PDCA was very useful as an eluent because its anion exhibited a uniquely high degree of complexation which becomes advantageous in acidic solution. The retention of Na(I) and Mg(II) showed a strong dependence on eluent pH whereas transition metal ions showed extremely strong dependence on both eluent pH and PDCA concentration. The order of retention was Cd(II)< Co(II)< Zn(II)< Cu(II). The strong affinity of the IDA silica phase towards the transition metal ions was a great advantage for the determination of trace transition metal ions in complex matrices such as sea-water [258].

Elefterov *et al.* studied the behaviour of Cd(II), Co(II), Zn(II) and Pb(II) on a similar silica IDA column, again using a PDCA eluent, this tme with indirect spectrophotometric detection at 295 nm. The limits of detection were relatively poor, being in the region of 0.1-1 μg/ml and the method was applied to industrial wastewaters. The fact that PDCA forms highly stable complexes with transition metal ions allowed a low eluent concentration to be used and this increased the efficiency of the separation and lowered the limits of detection. The method showed that a 50-fold molar excess of alkali and alkaline earth metal ions did not interfere with the separation as they eluted within the dead volume. Hg(II), Cu(II), Mn(II), Ni(II) and Fe(II) did not interfere with the analysis either. Results of the analysis of low mineralised waters agreed well with those obtained by FAAS [259].

Later work by Voloschik *et al.* separated Be(II) and alkaline earth metals on an IDA bonded silica gel with indirect conductimetric detection. As previously shown PDCA was an ideal eluent and hence this eluent was further investigated. Limits of detection of 10 μg/L were obtained, with preconcentration used to further decreased detection limits to sub μg/L levels. Work on a sulphonated cation exchanger resulted in Be(II) eluting as a broad peak within 3 min before Mg(II), Ca(II), Sr(II) and Ba(II) and samples containing transition and heavy metal ions interferred with Be(II) analysis. This present work with the IDA-silica column produced both better selectivity and sensitivity. A change in elution order was observed with Be(II) eluting after the alkaline earth metals. The Be(II) peak was a symmetrically sharp peak. Waste water samples were also analysed and 1 μg/L Be(II) was determined. No interference was observed from alkaline earth, transition or heavy metal ions at the levels found. However, as Be(II) eluted after the alkaline earth metal ions, samples containing high levels of these metal ions would have swamped or interferred with the Be(II) peak [260].

Jones and Nesterenko further investigated preconcentration and separation of trace transition metal ions on an IDA-silica column, followed by post-column reaction detection using PAR. As a result of the strong complexing ability, in combination with excellent kinetics and ion-exchange selectivity of the IDA functional groups, preconcentration of Mn(II), Cd(II), Co(II), Zn(II), Ni(II) and Cu(II) from high salinity waters, and efficient separations on the single IDA column was possible. Baseline separations were obtained using a three step gradient scheme. Acidic eluents were used for eluting purposes, which not only illustrated the strong complexing ability of the IDA-silica column but also its stability [261]. Previous work with polymeric resins showed lower efficiency separations in comparison to the above silica based column. A four-step concentration gradient was required for the satisfactory separation of nine alkaline earth and transition metal ions [241].

The above work [241] led to development of a simple method for the separation of 14 lanthanides and yttrium in under 70 min by Jones and Nesterenko using the IDA-silica column with potassium nitrate as an eluent. In comparison to previously mentioned polymeric columns, the main advantage of this column was higher efficiency and better thermostability of the silica matrix [262].

Later a more detailed study into the effect of nitric acid concentration, eluent ionic strength, temperature and various complexing eluents such as diglycolic and maleic acid was carried out with the IDA-silica column for the separation of lanthanides. Maleic acid formed weak complexes with little effect on the selectivity of separation. Diglycolic acid however formed more stable complexes with the lanthanides. A good separation was obtained for both light and heavy lanthanides but the peaks of 7 lanthanides and Yb were poorly resolved. Optimum separation was obtained using 0.5 *M* KNO_3, 65°C, 0.0016 *M* HNO_3 for 14 lanthanides and yttrium in 65 min, which was unusual because of the relatively small differences in stability constants with IDA between individual members of the lanthanide series [263].

These studies have been summarised in Table 1.8.

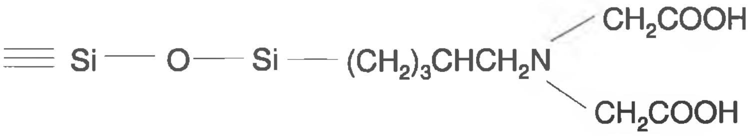


Figure 1.21. Structure of IDA chemically bonded to silica.

Table 1.8. Applications of IDA-silica bonded column.

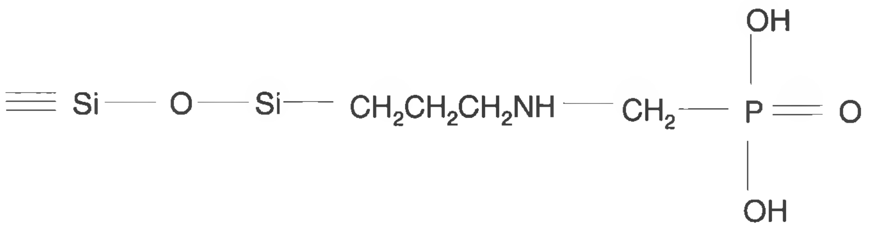
Metal ions	Eluent	Detection	Sample	L.O.D.	Ref.
Co(II), Zn(II), Cd(II),	Tartaric/ citric acid or PDCA	Conductivity	Seawater		258
Co(II), Cd(II), Zn(II), Cu(II), Pb(II)	0.001 *M* PDCA	Indirect spectro-photometric	Industrial waste water	0.1-1 μg/mls.	259
Be(II)	PDCA and HNO_3	Indirect conduct-imetric	Rock and waste water	10 μg/L	260
Alkaline earths and transition metals	Tartaric/ oxalic acid	PCR with *o*-CPC or PAR/ ZnEDTA			261
Transition metals	Gradient elution with KCl-tartaric acid-DPA	PCR with PAR	High saline samples		241
Lanthanides and yttrium	0.5 *M* KNO_3	PCR with Arsenazo(III)			262

1.9.3.2 Aminomethylphosphonic acid (AMPA) bonded silica column.

Nesterenko *et al.* investigated the retention behaviour of Cu(II), Mn(II), Co(II), Zn(II), Ni(II), Pb(II) and Cd(II) using a new chelating substrate, aminomethylphosphonic acid (AMPA) functionalised silica column [264] shown in Figure 1.22.

The AMPA chelating group behaves as a tridentate ligand having a co-ordination site at the secondary nitrogen atom and two bonding sites at the phosphonic group, resulting in a N,O,O chelating system. At low pH it is considered to behave as a bidentate ligand with O,O chelation existing, due to protonation of the amino group. Studies of capacity factor vs pH showed that there was a change in retention order, suggesting that there was a change in co-ordination of metal ions from O,O to N,O,O chelation as shown by s-shaped curves obtained for the retention of Zn(II) and Mg(II). A similar effect was observed with Pb(II) and Cu(II) when the eluent pH was increased. Such characteristics signified that this particular chelating substrate was potentially more useful and versatile than IDA phases. There was also an increased retention for Mn(II) at low pH, which is quite unusual as with most chelating groups, including IDA, Mn(II) is only weakly retained.

Very high selectivity between the alkaline earth metals and divalent transitions and heavy metals at high pH was shown, while good resolution between individual members of each group was obtained at selected pH. Using either nitric acid or strong KNO_3 eluents there was a similarity in retention orders implying that even in the absence of suppression of ion exchange interactions between cations and the



$$\equiv Si-O-Si-CH_2CH_2CH_2NH-CH_2-P(=O)(OH)_2$$

Figure 1.22. Structure of AMPA-bonded to silica.

Table 1.9. Applications of AMPA-bonded to silica.

Metal ions	**Eluent**	**Detection**	**Applications**	**L.O.D**	**Ref.**
Cu(II),Mn(II), Co(II),Zn(II),Ni(II), Pb(II),Cd(II)	1 *M* KNO_3 and 100 m*M* HNO_3	PCR with PAR	Not Given	Not given	264
Be(II)	1 *M* KNO_3, 0.5 *M* HNO_3 and 0.08 *M* ascorbic acid	PCR with CAS, Hexamine and EDTA	Soil Certified reference material	35 µg/L	265

negatively charged phosphonic groups in diluted eluents, the complexation at the surface of the AMPA still remains the main separation mechanism.

Further work on the same column by Shaw *et al.* was carried out, investigating the selectivity of the AMPA silica for Be(II). The isocratic elution of Be(II) was shown using a 1 *M* KNO_3, 0.08 *M* ascorbic acid and 0.5 *M* HNO_3 eluent in under 6 min in a sample containing >800 mg/L matrix alkali and alkaline earth metal ions. A limit of detection of 35 µg/L was obtained, although this detection limit was higher than expected due to the broad peak shape which resulted from the strong affinity of the stationary phase for Be(II). The method was applied to a certified reference material, GBWO7311, with results agreeing well with the certified values. Injecting a Ni(II), Zn(II), Cd(II) and Mn(II) standard and a mix prepared in 2 *M* KNO_3 showed no change in retention times and the peaks were still as sharp as in the standard, which clearly illustrated the insensitivity of the stationary phase to changes in ionic strength when ion exchange interactions have been suppressed. Be(II) showed very strong retention in comparison to other divalent metal ions, including Cu(II) which is usually the strongest retained. Up to 50 mg/L fluoride showed no change or reduction in the Be(II) signal [265].

The above work has been summarised in Table 1.9.

Chapter 2.

Determination of Lead in water samples using ion chromatography with a xylenol orange containing eluent.

2.0. Introduction.

There is currently a strong interest in the use of liquid chromatographic methods for the determination of metal ions, particularly as a simple alternative to atomic spectroscopic methods. The latest developments in this area have been recently reviewed by Sarzanini [3], who identified improvements in detection and the application to complex sample types as key areas of interest. When using ion chromatography (IC) for the determination of transition and heavy metals (and to a lesser extent alkaline earth metals), detection has generally involved post-column reaction (PCR) systems, using suitable reagents such PAR for visible detection, 8-HQS for fluorescence detection. The use of PCR systems is predominantly due to the limited sensitivity and non-selective nature of conductimetric detection for the above metal ions.

However, in the past IC combined with PCR detection has been perceived as rather complicated and troublesome for everyday use, requiring complex reaction and mixing coils and suffering from noisy and unsteady baselines. Therefore, many workers have opted for atomic spectroscopic methods when presented with the choice. With this in mind, simple chromatographic methods are still required which provide sensitive and/or selective detection for the above metals, and which do not require complex PCR detection. One approach to the detection of transition and heavy metals following their separation using IC is to use colour-forming eluents. These are eluents which contain a metallochromic ligand of the kind used in PCR systems, and which operate through partial or complete complexation of the metal ion within the eluent itself. Retention can be due to ion-exchange of either the metal ions or metal-ligand complexes and/or a degree of surface adsorption. However, in such systems sufficient eluent phase complexation must occur (this being dependent upon pH) to permit sensitive visible detection.

A number of workers have previously investigated the use of colour-forming eluents in IC. Zenki was the first to investigate such eluents as far back at 1981 using a chlorosulfonazo III containing eluent for the determination of alkaline earth metal ions [215]. Toei and co-workers [219-224] also studied this approach some years ago.

A number of colour-forming metallochromic ligands were investigated, namely *o*-CPC [219, 220, 224], Arsenazo III [221, 222] and XO [223]. Of the above work, the majority focused on the detection of alkaline earth metals [215, 219, 222, 224], with only XO being investigated as a colour-forming eluent for the detection of transition and heavy metals [223]. In this study Toei used an anion exchange separation column with a XO containing eluent. Injection of metal ions resulted in the on-column formation of anionic metal-XO complexes and anion exchange was said to be predominantly responsible for retention, although the exact retention mechanism was not established. The partial separation of Ni(II), Co(II), Zn(II) and Cu(II) was shown using an eluent of 0.1 m*M* XO with 50 m*M* phosphate buffer (pH 5.2) at 50 °C. However, the separation efficiency was rather poor making the developed method unsuitable for the analysis of real samples.

Later work by Dasgupta *et al.* [225] investigated the use of 8-HQS as a component of the eluent using cation exchange chromatography for the separation and fluorescent detection of metal ions. Attention was again focused on the alkaline earth metals, although retention data was determined for many other divalent and trivalent metal ions. As with the above studies, the exact retention mechanism in Dasgupta's system was a subject for debate, with a combination of ion exchange and adsorption of the 8-HQS onto the stationary phase being suggested. The method was successfully applied to the determination of Ca(II) and Mg(II) in several water samples. Also, Wada *et al.* [227] used a 0.2 m*M* Chlorophosphonazo III eluent (buffered with 25 m*M* MOPS/NaOH) with a sulfonated cation exchange column, once again for the determination of Ca(II) and Mg(II), this time in tap water. With detection at 665 nm, the responses for the two metals were shown to be linear over the range 10 – 80 μ*M*.

2.1. Aims of this study.

One of the key heavy metals of concern today, in both environmental samples and also potable water supplies, is Pb(II). No simple, direct detection ion chromatographic methods, that is methods which do not require PCR detection or sample derivatisation, have been developed which are suitable for the routine determination of Pb(II) in these types of samples. Therefore, the following work investigated the possibility of developing a simple, selective ion chromatographic method, based upon the use of a colour-forming eluent, for the above purpose. Methylthymol blue (MTB) and XO were two metallochromic ligands chosen for study as their complexing abilities are well known and they are available as relatively pure and inexpensive sodium salts. Eluent conditions were investigated with the aim of eluting Pb(II) in a reasonable time and as a relatively sharp peak, and also to resolve Pb(II) from other metal ions which may also complex with the eluent ligand. The determination of Pb(II) in complex sample types such as spiked river water and polluted drainage sample would illustrate the selectivity of the developed method.

2.2. Experimental.

2.2.1. Instrumentation.

The basic IC system used for this work consisted of a Waters Model 510 pump (Milford, MA, USA) and a Rheodyne injector (Cotati, CA, USA) fitted with a 100 μL injection loop. A Model SPD-6AV Shimadzu UV/Vis detector (Kyoto, Japan) was used at 572 nm, and chromatograms were recorded using a Shimadzu CR-3A Chromatopac intergrator. The analytical column used was a HiChrom P10SCX sulfonated silica strong cation exchanger. The flow rate used throughout was 1.5 mL/min.

2.2.2. Reagents.

The eluent was prepared using de-ionised water from a Millipore Milli-Q Water Purification System (Bedford, MA, USA). Methanol (HPLC grade) was purchased from Labscan (Labscan Ltd., Co. Dublin, Ireland). The colour forming ligands, MTB and XO, were obtained from Sigma-Aldrich (Gillingham, Dorset, UK) and used without further purification. Sodium acetate and acetic acid was obtained from BDH Chemicals (Poole, Dorset, UK). Final eluent conditions used for the analysis of samples were 20 m*M* sodium acetate-acetic acid buffer, 0.2 m*M* XO. The pH of each eluent was adjusted using dilute solutions of NaOH and HNO_3. All solutions prepared were filtered through a 0.45 μm filter and degassed using sonication. Low level standard solutions were generally prepared freshly each day from stock solutions (1000 mg/L) prepared in 0.1% nitric acid.

2.3. Results and discussion.

2.3.1. Choice of eluent.

It was clear from previous work [215, 219-224] that to obtain both good separation efficiency and a good detector signal to noise ratio that the colour-forming ligand should be present in the eluent at a relatively low concentration. Therefore, as the analytical column used in this work was a sulfonated cation exchanger, the eluent needed to include, in addition to the metallochromic ligand, a buffer to control retention and pH.

In this system several buffer systems were investigated as to their affect upon retention and detection of metal ions. Complexing buffers such as tartrate and citrate reduced detector sensitivity for Pb(II) when used with the colour-forming ligands, and phosphate, which was previously used by Toei [223], could produce insoluble precipitates with certain metal ions. Therefore, sodium acetate was used, as this had little affect upon detector response when present in the eluent at between 0 - 20 m*M*, and also was an ideal buffer between pH 4.5 and 5.5.

The two colour-forming ligands initially investigated were MTB and XO. These ligands are structurally very similar, each containing two IDA functional groups which are responsible for complexing metal ions, and both being triphenylmethane based reagents. They also exhibit very similar stability constants for most metal ions and so it was expected they would produce very similar separations when used in IC. When added to a 20 m*M* acetate eluent at a concentration of 0.2 m*M*, both eluents produced very similar retention data for a number of different metal ions. As expected, with both ligands there was no response for alkali metals and Ca(II) and Mg(II) were both unretained, giving only a response at the eluent dip when present in excess. Upon the injection of a Pb(II) standard, retention times were found to be similar for either ligand, although Pb(II) elutes slightly earlier with the XO containing eluent. Chromatograms from the injection of a 5 mg/L Pb(II) and Zn(II) mixed standard, achieved using a (a) XO containing eluent (pH 4.2) and (b) a MTB containing eluent (pH 4.1), under the above conditions, are shown in Figure 2.1. As can be seen from comparison of the two chromatograms, although retention of Pb(II) is very similar, Zn(II) is retained somewhat longer relative to Pb(II), using the XO eluent. As there was clearly very little difference between the two eluents, the remainder of the study focused on the development of just one, XO, for the determination of Pb(II).

2.3.2. Retention mechanism.

A brief study was undertaken to further understand the exact retention mechanism taking place within the system. A number of parameters were investigated, namely eluent pH, buffer concentration, ligand concentration and % organic modifier. Figure 2.2 shows (a) the effect upon the retention of Pb(II), Zn(II), Ni(II) and Cu(II) of varying eluent pH, (b) the effect upon the retention of Pb(II) and Zn(II) of varying the eluent buffer concentration, and (c) the effect upon the retention of Pb(II) of the addition of an organic modifier. It can be seen that both Figure 2.2(a) and 2.2(b) show typical responses expected from an ion-exchange retention mechanism with a complexing eluent. When the eluent pH is increased the concentration of the complexing form of XO is increased and so retention decreases. Also those metals which strongly complex with XO are retained very little, such as Cu(II) and Ni(II), and also, although not shown, Fe(III). As expected, increasing the buffer concentration also caused a reduction in retention, typical of an ion-exchange mechanism. However, the effect upon retention of increasing the concentration of sodium acetate above 12 - 15 m*M* was very small, with retention times for Pb(II) and Zn(II) remaining fairly stable. This indicated a second mechanism may also be playing some part in the retention of the above metal ions.

As mentioned above, previous work using by Dasgupta *et al.* [225] using a cation exchange column with an 8-HQS acid eluent, showed adsorption of the ligand or ligand-metal complex onto the stationary phase played a significant role in retention selectivity. To assess if XO or the XO-metal complexes were being adsorbed here, methanol (MeOH) was added to the eluent from 0 – 30%. Figure 2.2(c) shows how the retention of Pb(II) decreases substantially with the increase in MeOH concentration. This indicates that a dual retention mechanism is indeed taking place within the system. As to exactly what is being absorbed, an

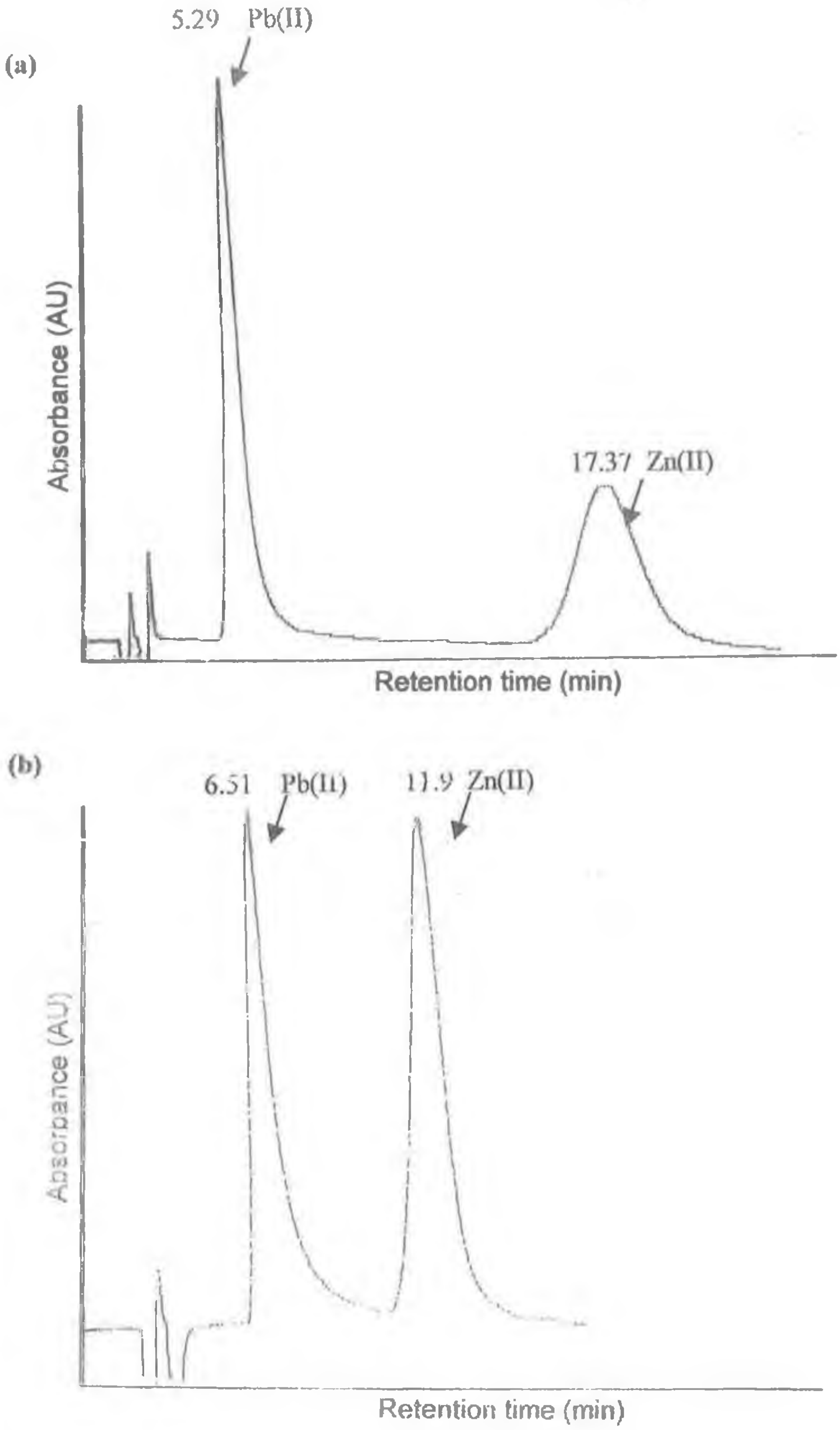


Figure 2.1. Chromatograms showing (a) the separation of 5 mg/L Pb(II) and Zn(II) using a XO eluent (conditions; 20 m*M* sodium acetate/acetic acid, 0.2 m*M* XO, pH 4.2), (b) the separation of 5 mg/L Pb(II) and Zn(II) using a MTB eluent (conditions; 20 m*M* sodium acetate/acetic acid, 0.2 m*M* MTB, pH 4.1).

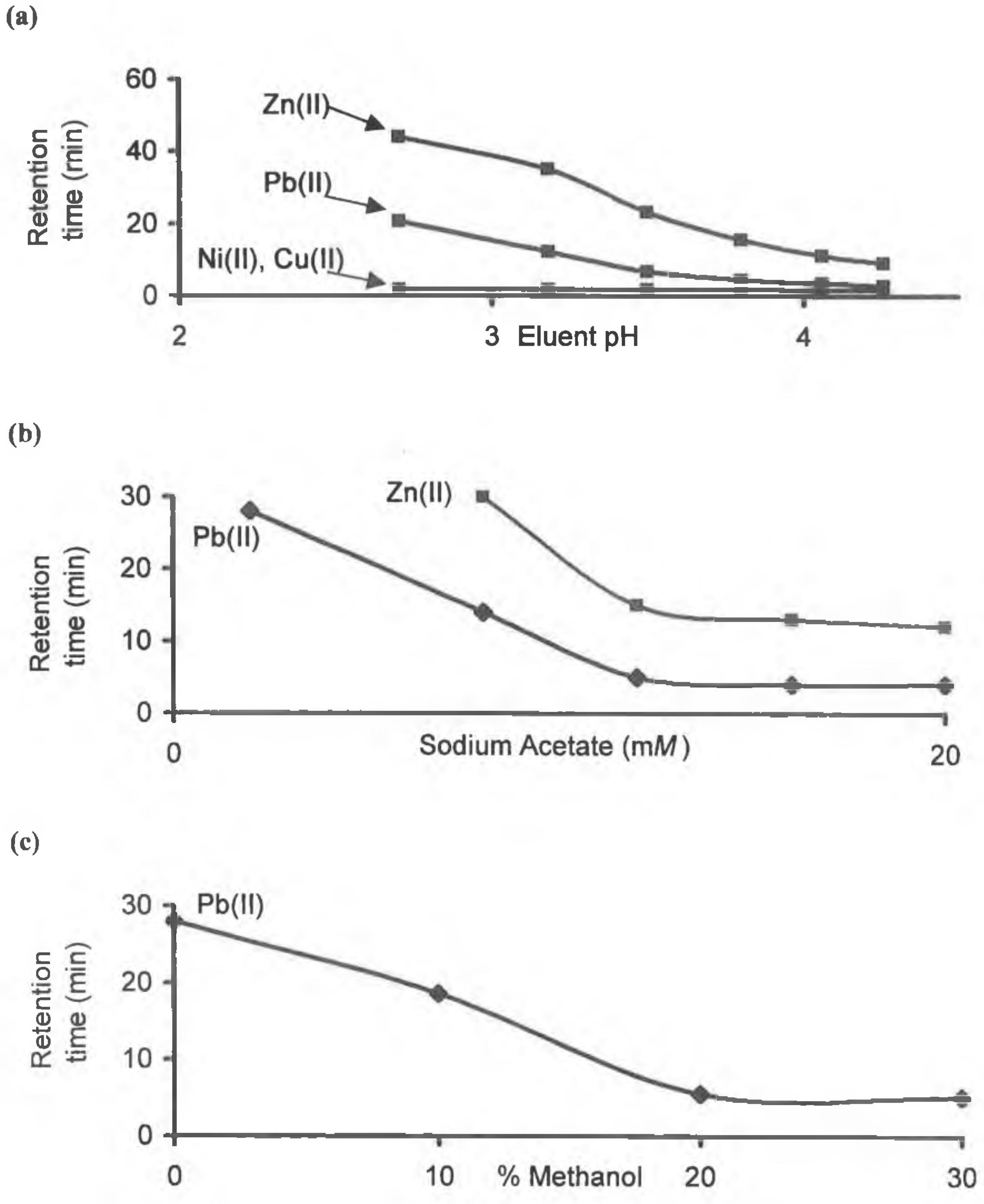


Figure 2.2. The effect of (a) eluent pH on the retention time of Pb(II), Zn(II), Ni(II) and Cu(II), (other conditions; 20 m*M* sodium acetate/acetic acid, 0.2 m*M* XO), (b) buffer concentration on the retention time of Pb(II) and Zn(II) (other conditions; 0.2 m*M* XO, pH 4.1), and (c) addition of MeOH on the retention of Pb(II) (other conditions; 2 m*M* sodium acetate/acetic acid, 0.2 m*M* XO, pH 4.2).

indication lies in the chromatograms shown in Figure 2.1. A negative system peak elutes close to the eluent dip that represents the retention of XO itself within the system. The magnitude of this system peak is proportional to the concentration of the metal ions injected, as it represents the amount of XO from the eluent involved with on-column complexation. As XO itself is clearly not being retained, it must be the metal-ligand complexes that are being retained through adsorption. This is of course as expected, as the ligand itself should have a greater overall negative charge and would thus be repelled by the sulfonated stationary phase.

This stationary phase adsorption affect was doubly confirmed by increasing the concentration of XO itself within the eluent. If only ion-exchange was responsible for retention, an increase in the ligand concentration would have seen a decrease in the retention of the analytes. However, over the range 0.2 to 1.0 m*M* there was no such response, with retention times remaining relatively unchanged over this range (for Pb(II) the retention time was 6.31 min at 0.2 m*M* XO and the retention time was 6.76 min at 0.8 m*M* XO, pH 4.0). Presumably an increase in eluent ligand concentration also lead to an increase in metal-ligand adsorption which cancelled out the expected decrease in retention.

Two other effects are worthy of note from the above studies. Firstly, there was no significant improvement in efficiency from adding an organic solvent to the eluent, with the tailing evident in Figure 2.1 remaining. Therefore, for the remainder of this study, the concentration of the buffer was used to control retention rather than MeOH. Secondly, increasing the eluent concentration of XO lead to an increased detector response. Over the range 0.2 to 0.8 m*M* XO the peak response for Pb(II) increased by approximately 60%. However, with this increased response there was also an increase in background noise, resulting in the signal to noise ratio remaining fairly constant. Therefore, there was no sensitivity advantage to be gained from increasing the concentration of XO in the eluent and so it was kept at 0.2 m*M*.

2.3.3. Selectivity.

From the above results it is clear that prediction of selectivity from a system where two retention mechanisms appear to be in operation is rather complex. Therefore it was important to investigate the retention of possible interfering metal ions using the XO eluent. A range of metals at varying concentrations were injected and their effect upon retention time and peak height for Pb(II) determined. As mentioned previously, alkali metals gave no detector response and injections of Pb(II) standards containing up to 100 fold excess Na(I) or K(I) showed no intererfering effects (see Figure 2.3(a)). The alkaline earth metals, Ca(II) and Mg(II), did produce a small detector response when present in excess of ~ 10 mg/L. However, these metals were unretained using the eluent conditions developed above and eluted as a sharp peak at the eluent dip, which again had no effect upon the peak for Pb(II), even at concentrations as high as 100 mg/L (see Figure 2.3(b)). A number of transition metals were also injected to determine their retention times and to ascertain if their presence in real samples would affect the determination of Pb(II). These were Zn(II), Cd(II), Cu(II), Mn(II), Co(II), Ni(II), and Fe(III). Injections were made of a standard containing 3 mg/L Pb(II) and 5 mg/L of each of the above. Those metal ions which were unretained using the 20 m*M* acetate/0.2 m*M* XO eluent were Cu(II), Ni(II), and Fe(III) with Cd(II) and Mn(II) not giving a significant detector response. Of these metals only Fe(III) caused a possible interference, as it showed severe tailing, which under the conditions used partially co-eluted with Pb(II). This tailing was likely to be due to the insolubility of Fe(III) at the eluent pH, even though here it would be partially complexed with XO. No such effect was evident with Cu(II), or Ni(II), which also eluted at the eluent dip and also strongly complex XO. However, this interference is not likely to be significant in water samples at pH above 3, where the concentration of dissolved labile Fe(III) is likely to be very low. Figures 2.4(a) and (b) show the injection of 3 mg/L Pb(II) with 5 mg/L Fe(III), and 3 mg/L Pb(II) with 5 mg/L Cu(II), respectively.

Of all the metal ions injected only Zn(II) and Co(II) exhibited greater retention than Pb(II). As shown in Figure 2.1(a), Zn(II) eluted between 15 and 18 min., at eluent pH 4 – 4.2. A further increase in eluent pH lead to a decrease in the retention of Zn(II) and a slight improvement in peak shape but caused Pb(II) to elute at the eluent dip. Cobalt(II) eluted as a rather poorly shaped peak at ~ 12 min.

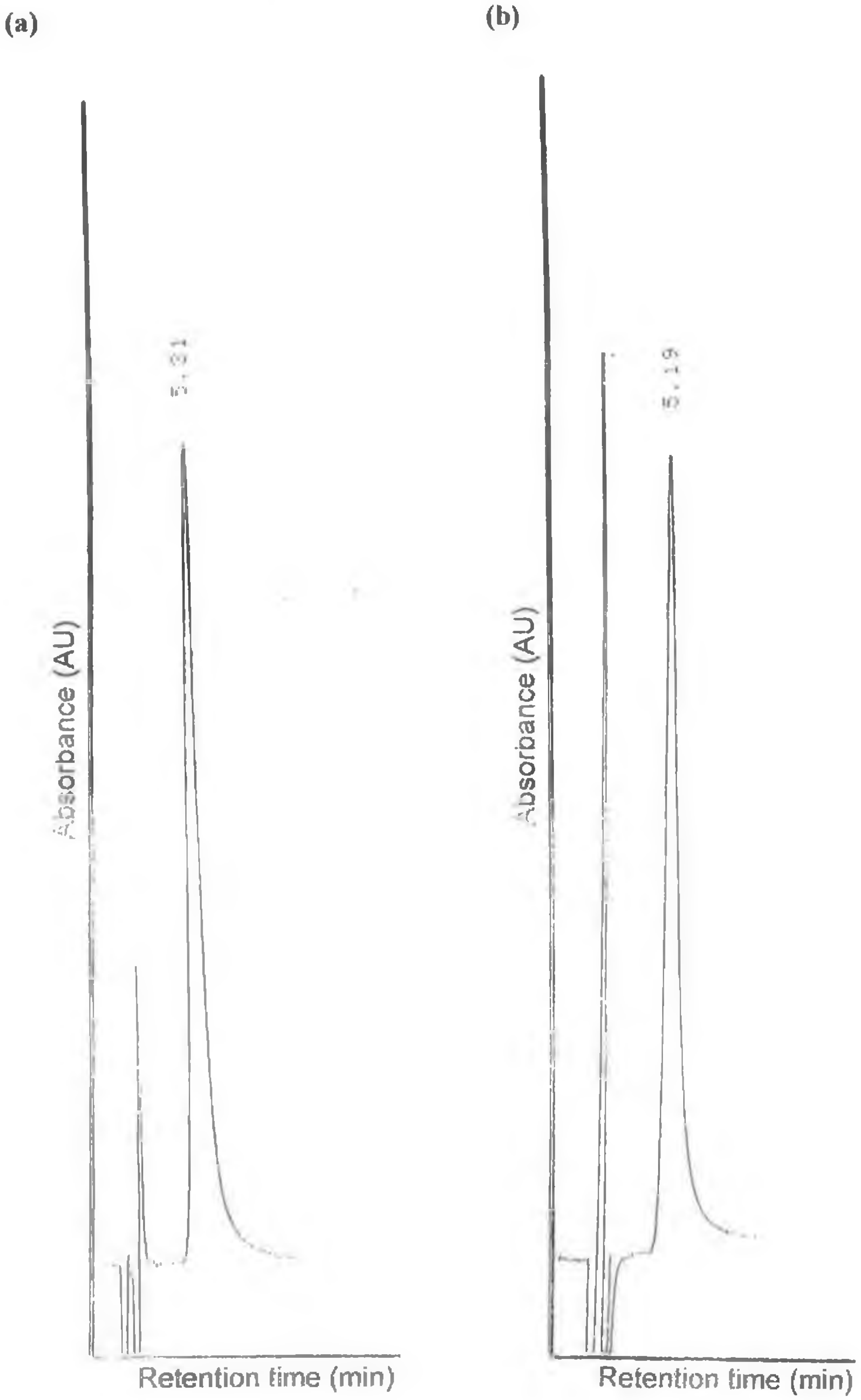


Figure 2.3. Chromatograms showing the separation of (a) 3 mg/L Pb(II) with 100 mg/L Na(I), (b) 3 mg/L Pb(II) with 100 mg/L Mg(II). Other conditions as in Figure 2.2(a).

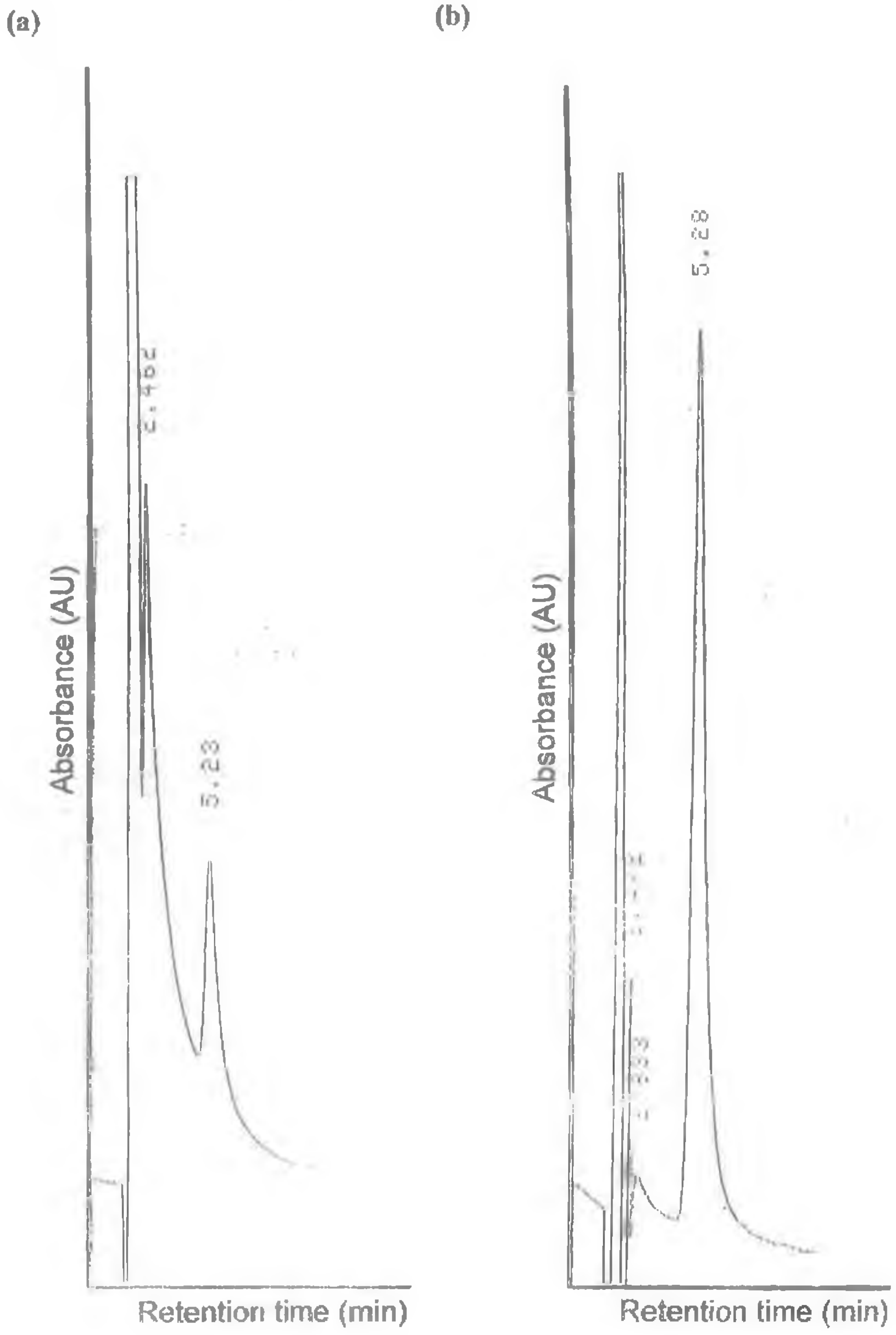


Figure 2.4. Chromatograms showing the separation of (a) 3 mg/L Pb(II) with 5 mg/L Fe(III) and (b) 3 mg/L Pb(II) with 5 mg/L Cu(II). Other conditions as in Figure 2.2(a).

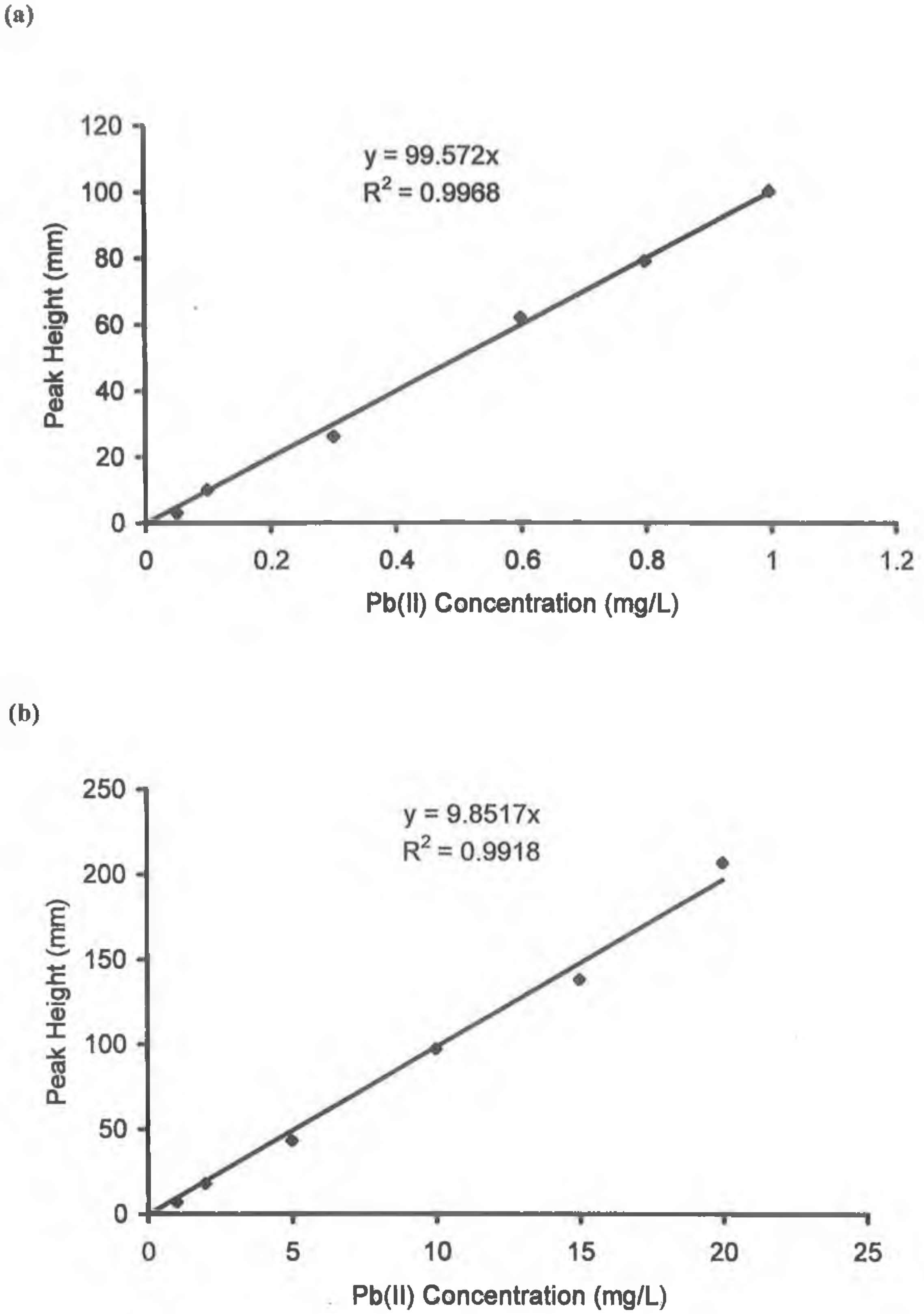


Figure 2.5. Standard calibration plots of Pb(II) in the region (a) 0.05 – 1 mg/L, and (b) 1.0 – 20 mg/L.

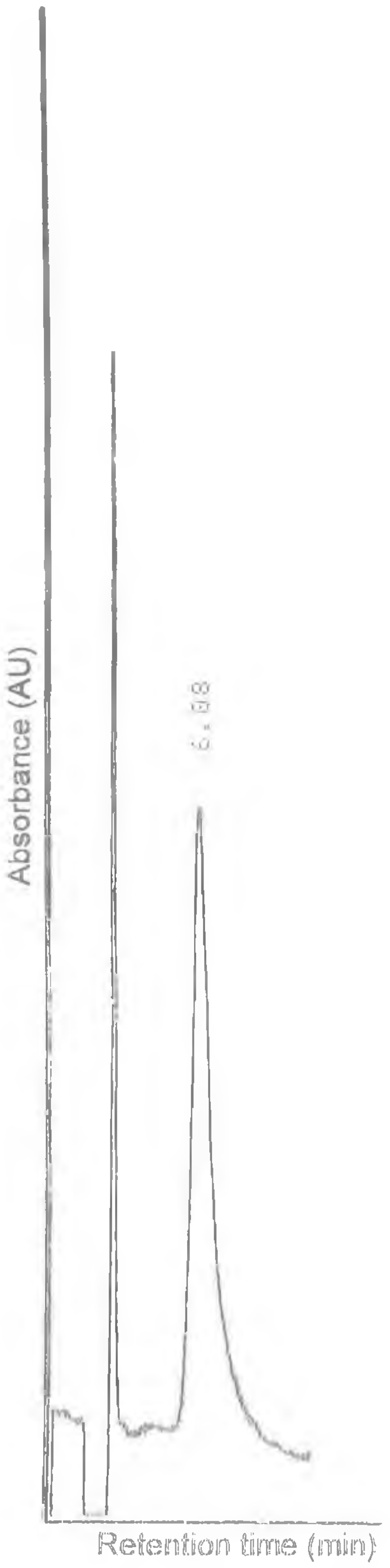


Figure 2.6. Chromatogram showing a river water sample spiked with 0.7 mg/L Pb(II). Other conditions as in figure 2.2(a), eluent pH 4.1.

2.3.4. Linearity, detection limit and reproducibility.

The analytical performance characteristics of the above method for the determination of Pb(II) were investigated. Linearity was determined over a low range (0.05 to 1.0 mg/L, n=6) and a high range (1.0 to 20.0 mg/L, n=6) of Pb(II) standards with corresponding standard calibration plots shown in Figure 2.5.

Correlation coefficients ranged from $R^2 = 0.992$ to 0.998 over the low concentration range and was $R^2 = 0.994$ for the higher concentration range.

The detection limit for Pb(II) was calculated using the 3 times the standard deviation of the background noise method. This was determined to be approximately 30 µg/L based on a 100 µL injection, which compares favourably with that which can be obtained using ion chromatography with post-column reaction with PAR.

Precision of method was evaluated by 8 repeat injections of a 3 mg/L Pb(II) standard. The method was found to be highly reproducible with % R.S.D values of 0.4 for retention time, 0.8 for peak height and 4.7 for peak area.

2.3.5. Analysis of water samples .

Two water samples were analysed for Pb(II) to illustrate the method could be applied to real samples. A river water sample and a polluted drainage sample were obtained as these presented relatively varied matrices and analysed using both the IC method developed here and also atomic absorption spectroscopy to validate the results. Upon injection of the river water sample it was clear that if Pb(II) was present it was below the detection limit of the method and so the sample was spiked with 0.7 mg/L Pb(II). Figure 2.6 shows the resultant chromatogram, from which it is clear that there were no interfering peaks resulting from the river water matrix. As expected the analysis of the polluted drainage sample showed a higher concentration of Pb(II) which could be easily determined and again no interferences were evident despite the complexity of the sample. Table 2.1 shows the results obtained for the two samples.

Table 2.1. Comparative Analyses of Water Samples.

		Ion chromatography		Atomic absorption spectroscopy	
Sample*	Metal ion	Conc. added	Conc. found	Conc. added	Conc. found
River water	Pb(II)	0.70	0.76 (±0.04)[1]	0.70	0.68
	Ca(II)	——	——	——	17.9
	Mg(II)	——	——	——	21.9
Drainage sample	Pb(II)	——	13.1 (±0.6)	——	13.0

*All samples analysed in triplicate, values in mg/L.

[1] (±RSD).

2.4. Conclusions.

A selective and sensitive ion chromatographic procedure for the determination of Pb(II) in water samples has been developed based on ion chromatography and on-column complexation with the colour-forming ligand, xylenol orange. The proposed method is both simple and robust, requiring no sample pretreatment or post-column reactions. The response for Pb(II) is linear over the range investigated, 0.05 to 20 mg/L, and the method is highly reproducible.

Chapter 3.

Determination of Trace Cadmium in Environmental Water Samples Using Ion-Interaction Reversed-Phase Liquid Chromatography With Fluorescence Detection.

3.0. Introduction.

Both 8-hydroxyquinoline (8-HQ) and its more soluble derivative 8-HQS have been used by several workers in differing ways in the liquid chromatography (LC) of metals ions. The reasons for using these ligands include their ability to rapidly form stable complexes with a large range of metals, and the fact that a number of these metal-ligand complexes are fluorescent. These ligands have generally been used in one of three ways, firstly, as post-column reagents, as in the post-column reaction detection system for aluminium species developed by Jones and co-workers [63, 65, 266]. Secondly, to form metal complexes 'pre-column' that are subsequently separated using reversed-phase chromatography, and thirdly as additions to the mobile phase, whereby a combination of 'pre-column' and 'on-column' complexation may be utilised. Some of the earliest work in this latter area was carried out by Dasgupta *et al.* [225]. In this work 8-HQS was added into the mobile phase and used with various types of stationary phases, including reversed-phase, anion and cation exchangers. Some promising separations were obtained using a surface sulphonated cation exchanger. This was particularly the case for Cd(II) and Zn(II), which were injected as uncomplexed ions, forming complexes 'on-column', allowing low μg/L detection limits using fluorescence at 470 nm. However, the peak shapes and resolution obtained using this approach were poor and not suitable for quantitative analysis of real samples. Later Soroka *et al.*[267], used the same system with gradient elution, achieving the partial separation of Zn(II), Cd(II), Mg(II) and Ca(II) in approximately 8 min.

Work by Meaney *et al.*[268, 269] employed a reversed-phase system for the separation of Fe(III) and Al(III) complexes of 8-HQ. Metal complexes were formed 'pre-column' and separated on an octadecylsilica column using an acetonitrile mobile phase containing 10 m*M* 8-HQ and 0.2 *M* KNO_3. The method was applied to various soil digests and water samples. However, the method did result in rather broad peaks, and as the detection method used was UV/Vis at 400nm, the detection limits were only between 10 and 50 μg/L.

Ion-interaction RP-HPLC of metal-8-HQS complexes has been carried out by several groups in recent years with varying degrees of success. Shijo *et al.* [270] successfully separated 8-HQS complexes (again formed pre-column) of Al(III), Cu(II), Ga(III) and Fe(III) by ion-interaction LC, using a mobile phase containing 8-HQS to suppress on-column dissociation and hexylamine hydrochloride as an ion-interaction reagent. However, once again UV/Vis detection was employed which resulted in detection limits of between only 0.05 and 2 mg/L, mainly due to the 8-HQS added to the mobile phase, which caused a significant increase in background absorbance. Similar studies were later carried out by Basova *et al.*[271] who investigated the use of several IIRs, namely TBA, hexadecyltrimethylammonium and heptylamine hydrochloride for the separation of pre-formed metal-8-HQS complexes, again using UV/Vis detection, although here 8-HQS was not added to the mobile phase to reduce background absorbance.

With fluorescence detection this is much less of a problem, due to 8-HQS not exhibiting fluorescence in its uncomplexed form. Feng *et al.* [272] illustrated this when they studied the retention behaviour of Zn(II), Cd(II), Al(III), Ga(III) and In(III) 8-HQS complexes on a octadecylsilica column with a mobile phase containing 0.5 - 4 m*M* 8-HQS, 1-10 m*M* TBA as the IIR, and 0.1 *M* of either phosphate or acetate buffer (made up in 20% acetonitrile). Feng *et al.* focused most of their attention on the retention behaviour of Al(III)-8-HQS complexes, which consistently eluted as two peaks when using a phosphate buffer in the mobile phase. The retention behaviour of Cd(II) and Zn(II) complexes was also investigated. It was stated that the peak for Cd(II) was not observed at a mobile phase pH of below 6 (said to be due to on-column complex dissociation) and that at a pH of above 7 the complex eluted as a strongly skewed peak. Chromatograms of the above metal complexes were not shown, although it is clear that the above mobile phase composition was again not suitable for the quantitative analysis of these metal ions in real samples.

3.1. Aims of this study.

This chapter reports on the development of a simple ion-interaction LC method suitable for the determination of trace levels of Cd(II) in environmental water samples. Few simple and sensitive chromatographic methods are available for Cd(II) determinations and the toxic and bio-accumulative nature of this particular heavy metal require such methods be developed. The following method is based upon 'on-column' formation and separation of metal-8-HQS complexes. The technique resulted in both reduced analysis time (through less sample preparation) and also in improved peak shapes, and therefore lower detection limits, compared to those obtained for complexes formed 'pre-column'. Mobile phase conditions which provided adequate retention and resolution of Cd(II) from other matrix metal ions present in excess, yet still allowing sensitive fluorescence detection, were developed. The method was applied to both a spiked river water sample and a drainage water sample from a disused copper mine.

3.2. Experimental.

3.2.1. Instrumentation.

The LC system used for the initial section of this study consisted of an Varian 9012 Solvent Delivery System (Walnut Creek, CA, USA) and a manual sample injector fitted with either a 100 or a 250 µL injection loop (Rheodyne, Cotati, CA, USA). A Millipore Waters 470 Scanning Fluorescence Detector (Milford, MA, USA) was used set at an excitation wavelength of 360 nm and an emission wavelength of 500 nm (excitation and emission slits set at 18 nm). The analytical column used was a Millipore Waters Bondapak C_{18} reversed-phase column (300 x 3.9 mm ID). The flow rate used was 1.5 mL/min. Later the above pump was replaced with a Dionex Model GPM2 Gradient Pump Module (Sunnyvale, CA, USA). Chromatograms were recorded using Dionex AI450 chromatographic software.

3.2.2. Reagents.

The mobile phase was prepared using de-ionised water from a Millipore Milli-Q Water Purification System (Bedford, MA, USA). HPLC grade Acetonitrile (MeCN) was purchased from Lab-Scan (Labscan Ltd., Co. Dublin, Ireland). The IIR tetrabutylammonium hydroxide (TBAOH), was purchased as a 40 wt % solution from Aldrich (Sigma-Aldrich, Dorset, UK), and 8-hydroxyquinoline 5-sulfonic acid hydrate

(98%) was also obtained from Aldrich (Sigma-Aldrich, Dorset, UK). Final mobile phase conditions used were 10 m*M* acetate/acetic acid buffer, 5 m*M* 8-HQS, 10 - 13 m*M* TBAOH, prepared in 15:85 acetonitrile:water (pH 4.6-5.4). All solutions prepared were filtered through a 0.45 µm filter and degassed using sonication. Low level standard solutions were generally prepared immediately prior to injection, although they remained stable for several days without sign of degradation. Where stated, 8-HQS was added to both standard and sample solutions to obtain a final concentration of 5 m*M* (see Section 3.3.2.).

3.3. Results and Discussion.

The aim of this work was to develop a simple, sensitive and selective method for Cd(II) determinations in environmental water samples. Ion-interaction LC was investigated as it required only simple LC instrumentation (no post-column reaction system required) and an inexpensive reversed-phase C_{18} column. As discussed in the introduction, Feng *et al.*[272] has shown the possibility of using ion-interaction LC to separate metal-8-HQS complexes followed by their detection using fluorimetry. As Cd(II) forms a strongly fluorescent complex with 8-HQS, this approach was chosen for further development and application to real samples.

In an ion-interaction LC system a number of mobile phase parameters exist which can be changed to control selectivity and analyte resolution. In addition to this, in cases where metal-ligand complexes are being separated, mobile phase conditions can also have an effect upon both peak shapes and detector sensitivity (e.g. the concentration of the ligand in the mobile phase and the mobile phase pH may increase or reduce on-column dissociation). Therefore, in this study it was necessary to understand and develop suitable mobile conditions that provided the desired selectivity, efficiency and sensitivity.

3.3.1. Development of the mobile phase.

In this study the concentration of 8-HQS added to the mobile phase was kept constant at 5 m*M*. The reason for this relatively high concentration (compared to previous work [226, 267, 270-272] was initially to try to maximise the formation of the ML_2^{2-}

complex and limit on-column dissociation to either ML or M^{n+}, as it was first thought this may have lead to poor peak shapes or even completely split peaks. This effect had been shown by Feng *et al.*, for Al(III) 8-HQS complexes. In this case, the second of two peaks obtained for the injected Al(III)-8-HQS standard increased relative to the first when the mobile phase 8-HQS concentration was increased, presumably due to the increased formation of the AlL_3^{3-} complex over the AlL_2^- form. Earlier studies into the chromatographic behaviour of Al(III) 8-HQS complexes [273, 274] had shown that such complexes are kinetically inert and so could indeed be separated chromatographically. Cadmium however forms much weaker and more labile complexes with 8-HQS than Al(III), that could not be individually separated under chromatographic conditions. Although, as mentioned previously, due to fast complexation kinetics, the equilibrium is highly labile and so the various Cd(II) 8-HQS complexes should pass through the column as a single sharp band.

In this present study, the above mobile phase conditions produced only single reasonably sharp peaks for Cd(II) and Zn(II), indicating the assumptions made above to be correct. As sharp peaks were being obtained, the mobile phase concentration of 8-HQS was kept at 5 m*M*.

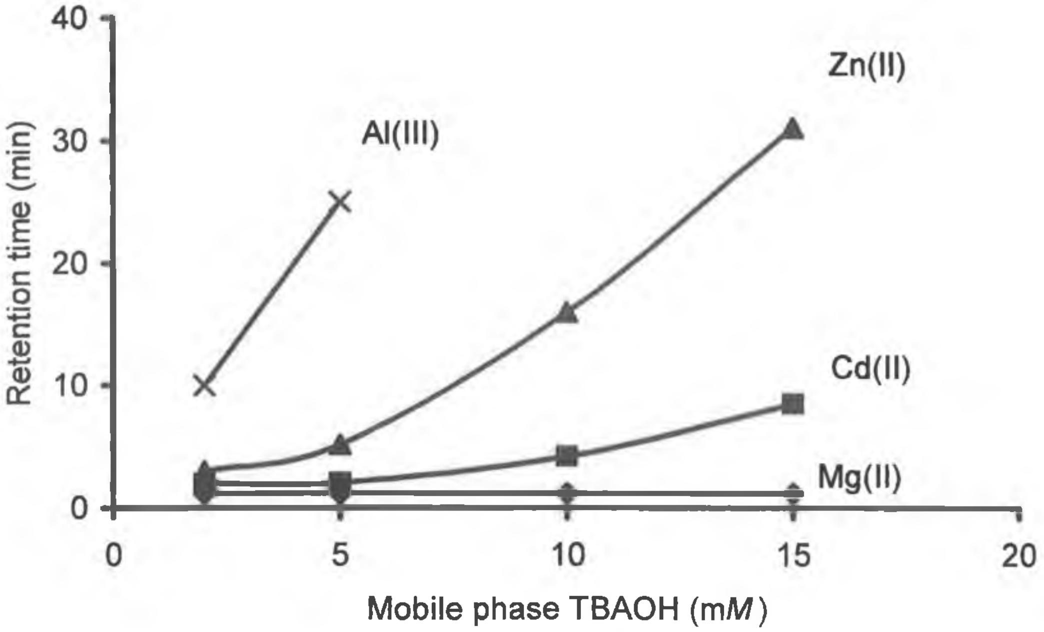
The concentration of acetate/acetic acid buffer added to the mobile phase was also kept constant at 10 m*M*. The addition of the buffer in this system was carried out not to control analyte retention, (through competition between the acetate and the negatively charged complex for the IIR) but to simply buffer the mobile phase pH. Therefore, the concentration was kept low as this would also limit any unfavourable equilibria, such as those shown once again by Feng *et al.*, who used 0.1 *M* acetate and phosphate buffer concentrations in their mobile phase. Such high concentrations of these anions can not only cause peak splitting but can also drastically reduce sensitivity due to competitive complexation and may even result in the formation of insoluble precipitates within the system, particularly when using phosphate.

The effect of varying the concentration of the IIR in the mobile phase is shown in Figure 3.1(a) (other mobile phase conditions: 10 m*M* buffer (pH 4.6), 5 m*M* 8-HQS, 15 % MeCN). The increase in retention is typical of an ion-interaction retention mechanism and also eludes to the type of metal-ligand complexes being formed/separated. From Figure 3.1(a) it is clear that Al(III) shows a very strong retention compared to the divalent metals. This indicates Al(III) could be being retained as the AlL_3^{3-} species and Cd(II) and Zn(II) may be predominantly forming ML_2^{2-} complexes. For Mg(II), retention does not increase with increasing IIR, indicating Mg(II) passes through the column as either a neutral or slightly cationic species. This is supported by stability constant data which suggests Mg(II) would be only very weakly complexed with 8-HQS at the mobile phase pH used [216]. From Figure 3.1(a) it is clear that the retention of Zn(II) increases more rapidly than that of Cd(II), and that Mg(II) is almost unaffected. Therefore, selectivity for these three metals in real samples can be manipulated through control of this parameter (see analysis of water samples).

However, having stated the above regarding the metal-ligand species likely to be being formed, it is clear that in any ion-interaction system the exact retention mechanism involved is difficult to ascertain. It has been assumed by previous workers [270-272] that the metal ions injected first form a negatively charged complex with the 8-HQS (as discussed above) (or are injected as pre-formed complexes), followed by the formation of an ion-pair with the positively charged IIR (which maybe predominantly present in the mobile phase or absorbed onto the stationary phase, or more likely a combination of both). However, in this case it is also possible that the 8-HQS first forms a neutral ion-pair with the IIR and this results in the formation of a dynamic coating on the stationary phase. This would then act as a dynamic chelating stationary phase, with the retention of metal ions being due to stationary phase complexation. In this situation, selectivity would be based upon relative stability constants (K_{STAB}) for each metal with 8-HQS, and here as expected retention of the above metal ions increased with increasing K_{STAB} (Mg(II) $\beta_1 = 4.1$, $\beta_2 = 7.6$, Cd(II) $\beta_1 = 6.9$, $\beta_2 = 13.4$, Zn(II) $\beta_1 = 7.5$, $\beta_2 = 14.3$, Fe(III) $\beta_1 = 11.6$, $\beta_2 = 22.8$) [275], (Al(III) not given in ref.). Of course mobile phase complexation must also be occurring, as the metal ions injected would not be detectable if they were not eluting from the column as complexes.

In practice, it is difficult to determine which of the above models is correct for this particular system and the most likely answer is that it may in fact be a combination of

(a)



(b)

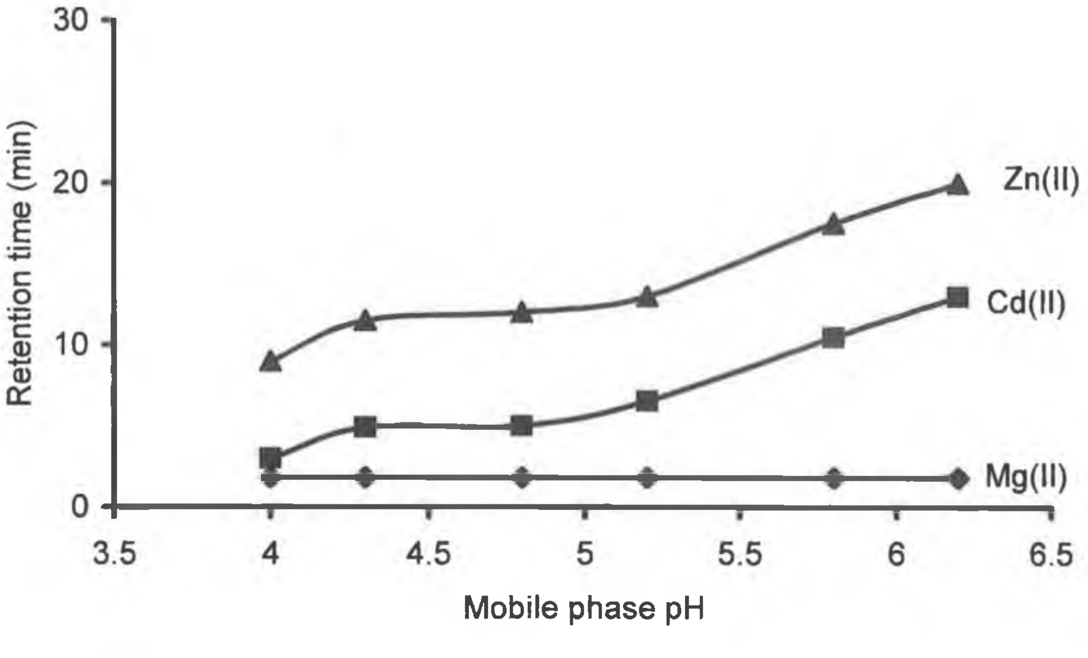


Figure 3.1. Effects of mobile phase (a) ion-interaction reagent concentration, (b) pH, on retention times for Al(III), Mg(II), Cd(II) and Zn(II). Other conditions see text.

both. Increasing the concentration of IIR would result in the observed increase in retention in both cases and so does not help to elucidate the exact mechanism. This is also the case with mobile phase pH.

The effect of mobile phase pH on both retention time and detector sensitivity was investigated over the range 4.0 and 6.1 (other mobile phase conditions: 10 m*M* buffer, 10 m*M* TBAOH, 5 m*M* 8-HQS, 15 % acetonitrile). The pH was not investigated above 6.1 due to solubility problems of both metals and other metals likely to be present in real samples. The results obtained are given in Figure 3.1(b), showing retention times and in Table 3.1, showing the effect upon peak areas. As can be seen Cd(II) and Zn(II) again behave very differently to Mg(II), which shows very little response to mobile phase pH, due to the reasons discussed above. Both Cd(II) and Zn(II) show a similar increase in retention over the pH range shown, with little change in resolution of the two metals. The trends shown can again be readily explained with the use of formation constants for each metal-8-HQS complex. Figure 3.2 shows the percentage species distribution diagrams for Mg(II), Cd(II) and Zn(II) with 8-HQS over the above pH range. The data is based upon a metal ion concentration of 0.01 m*M* in the presence of 5.0 m*M* 8-HQS and calculated using the formation constants given above (the effect of the presence of 10 m*M* acetate has been ignored). The response for Mg(II) in terms of both retention times and detector sensitivity is clearly (as mentioned above) due to the fact 8-HQS does not begin to complex Mg(II) until pH 5 (ML) and only begins to form the negatively charged species (ML_2^{2-}) at pH 6 and over. This explains why there is a definite rapid increase in detection sensitivity above pH 5 but little or no increase in retention. For Cd(II) and Zn(II), the retention and detector responses correspond closely to that expected considering the species distribution data shown in Figure 3.2.. At pH 4 it is only Zn(II) which exhibits significant retention as it is mostly present as either ML or ML_2^{2-}, whereas Cd(II) exists mainly as either M^{2+} or neutral ML. Above pH 4 retention of both metals increases as the percentage of each metal present as ML_2^{2-} increases. Although not investigated for reasons given above, it is reasonable to assume that the increase in retention shown in Figure 3.1(b) would level off above pH 6.1, as at this point practically all the Cd(II) and Zn(II) present should be present as the ML_2^{2-} species.

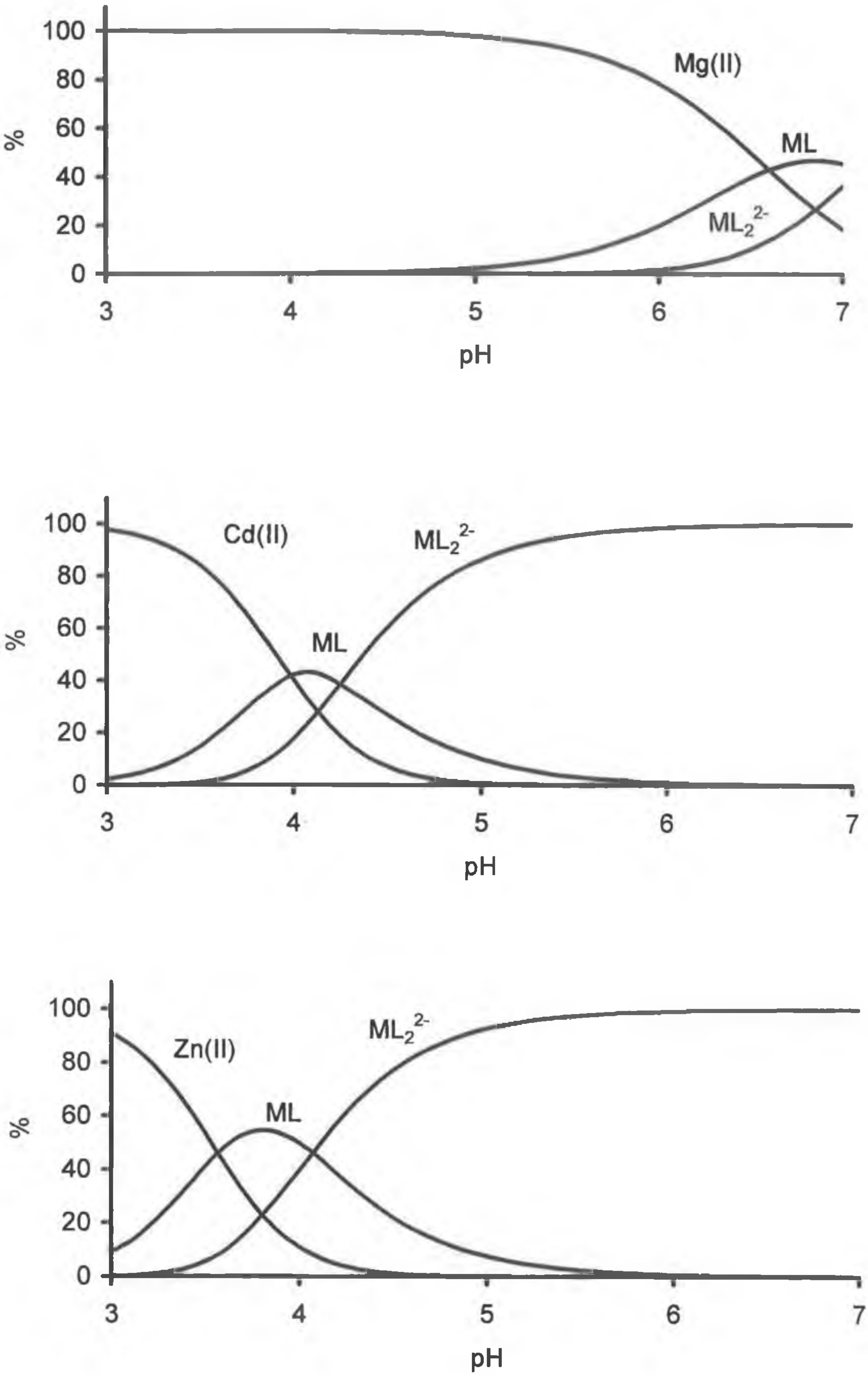


Figure 3.2. Percentage species distribution diagrams for Mg(II), Cd(II) and Zn(II) (0.01 m*M*) and 8-HQS (5.0 m*M*).

Table 3.1. Effect of mobile phase pH on peak area for Mg(II), Cd(II) and Zn(II).

	Relative Peak Area*		
Mobile phase pH	Mg(II)	Cd(II)	Zn(II)
4.0	0.18	0.42	0.64
4.4	0.44	0.77	0.85
4.8	1.00	1.00	1.00
5.1	1.98	1.22	1.16
5.4	3.16	1.41	1.17
5.8	_____	1.54	1.23
6.1	_____	1.52	1.23

*Relative to pH 4.8. Injected standard contained 0.5 mg/L Mg(II), Cd(II) and Zn(II).

There is also some correlation between the leveling off of the detector response shown in Table 3.1 for each metal and the pH at which the presence of the free metal (thus non-fluorescent species) becomes insignificant, approximately pH 5.0 for Zn(II) and 5.4 for Cd(II). These results match closely those obtained by Soroka *et al.*[267],who investigated fluorescence intensity as a function of pH for group II metal-8-HQS complexes.

From the above investigations a mobile phase pH of between 4.8 and 5.4 seemed optimum as this provided reasonable retention times and sensitive detection. Any further increases in peak areas above pH 5.4 did not greatly improve detection limits due to increased peak broadening, making accurate peak integration more difficult.

3.3.2. 'On-column' complexation.

As mentioned in the introduction, 8-HQS has found several uses in chromatographic systems due to it's relatively fast complexation kinetics. In this system, the effects of 'pre-forming' the metal-ligand complex by adding 8-HQS to the standard and sample solutions, and the effects of simply injecting the metal ions in uncomplexed form were investigated. Figures 3.3(a) and 3.3(b) show two chromatograms obtained from the injection of a 0.5 mg/L standards of Mg(II), Cd(II) and Zn(II). Chromatogram (a) was obtained from the injection of the above metals in water. Chromatogram (b) shows the chromatogram obtained from the injection of standards prepared with the addition of 5 m*M* 8-HQS. It is clear from the two chromatograms that although retention times were unaffected by the form of the injected metal ions, both peak efficiency and consequently peak height, were improved when Cd(II) was injected as the free metal ion. The reason for this is somewhat unclear, although one suggestion would be that, unlike Zn(II), the injected Cd(II) complex had insufficient time to re-equilibrate itself with mobile phase conditions as it passed through the column and so showed clear evidence of peak splitting. However, this clearly seems to contradict the idea that 8-HQS exhibits very favourable complexation kinetics, which must be the case for on-column complexation to be possible in the first place, without resulting in very broad peaks. This splitting effect was not seen with the peak for Zn(II), which eluted as a single well defined peak when injected as either the free ion or complex. The ability to inject the samples directly without the addition of 8-HQS to the sample is an obvious advantage to this system as it greatly simplified the method and reduced sample preparation and overall analysis time. As the above observations, however contradictory, were very reproducible, for all further work 8-HQS was not added to either standard or sample solutions prior to injection.

3.3.3. Analytical performance figures.

The linearity of the method for Cd(II) was determined using both the standard calibration method and the standard addition technique. Over the range 0 - 100 µg/L, Cd(II) standards (n = 4) produced a slightly negatively curved response ($Y = 0.003 X^2 + 1.57 X - 1.4$, $R^2 = 0.997$) as shown in Figure 3.4(a).

A river water sample containing Cd(II) below the method detection limit was spiked with Cd(II) at 25, 50 and 100 µg/L concentrations. The resultant standard addition curve showed a slightly reduced slope to the above standard curve ($Y = 0.001 X^2 + 1.25 X$, $R^2 = 0.999$) as shown in Figure 3.4(b), indicating the river water matrix has a

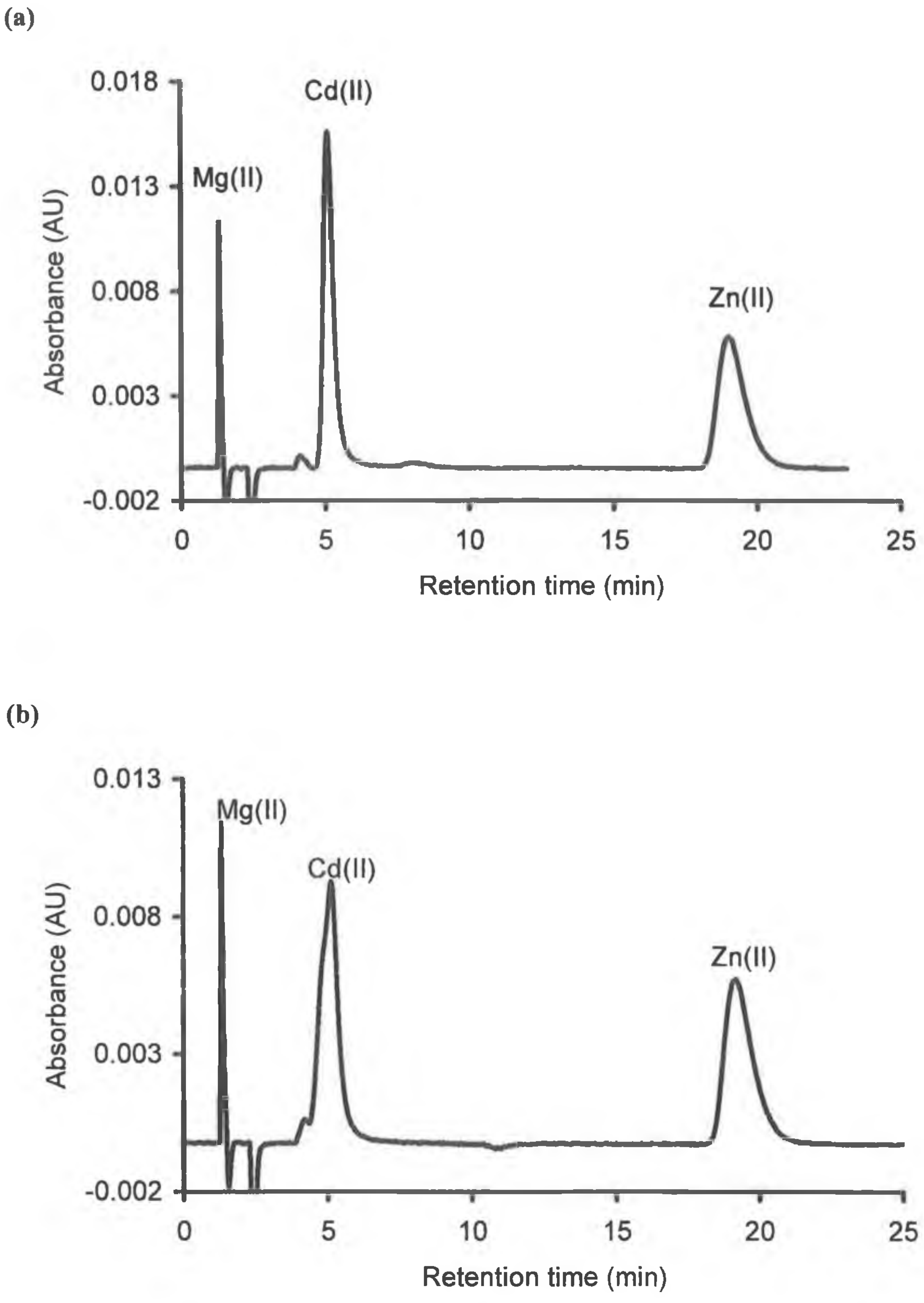
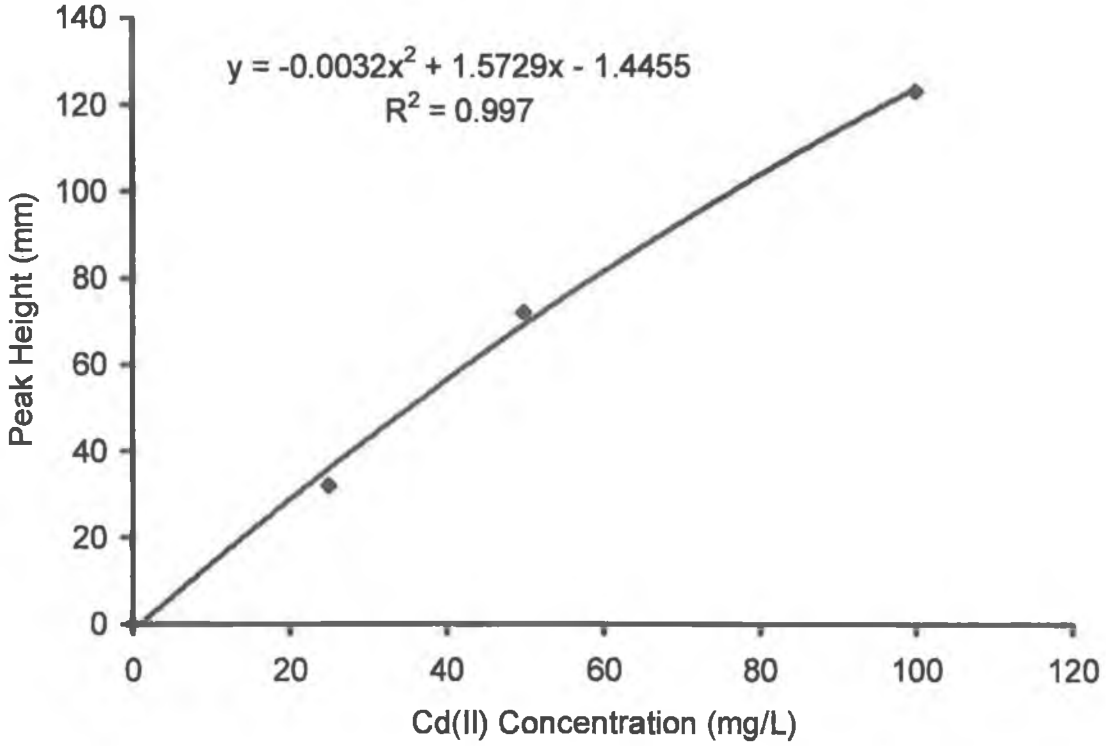


Figure 3.3. Chromatograms showing (a) the separation of Mg(II), Cd(II) and Zn(II) (0.5 mg/L) resulting from the injection of the metal ion standards and (b) metal-8-HQS complex standards.Other conditions: 10 m*M* buffer (pH 4.8), 12 m*M* TBAOH, 5 m*M* 8-HQS, 15 % acetonitrile.



(b)

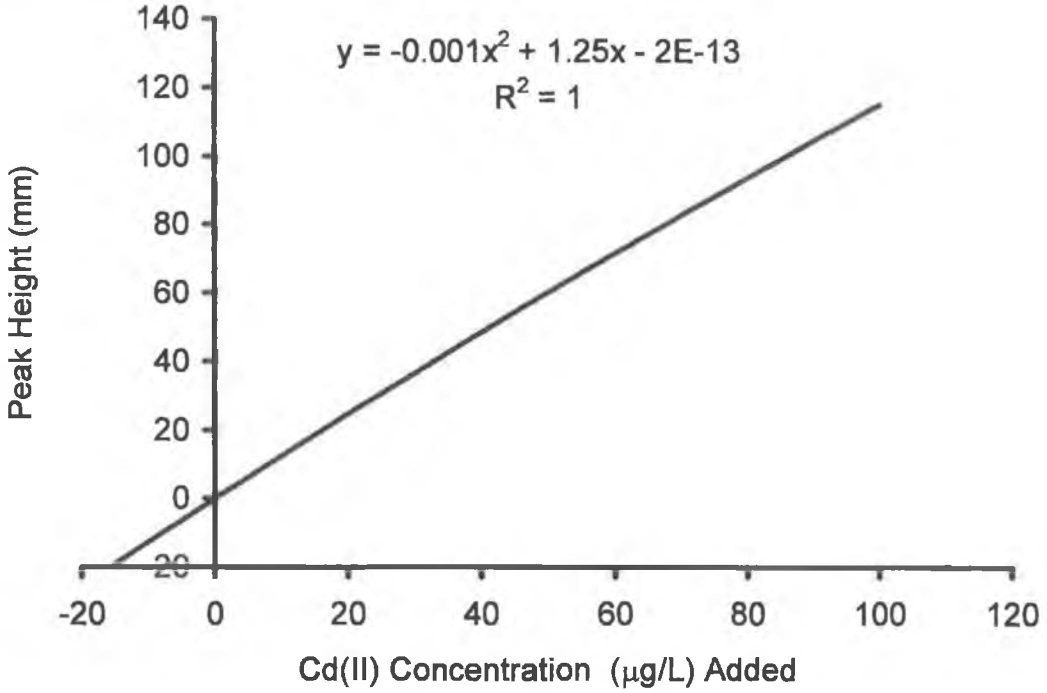


Figure 3.4. (a) Standard calibration and (b) standard addition plots for river water sample analysis.

slight negative (~20%) effect upon the response for Cd(II), probably due to the presence of unknown complexing or quenching agents (Soroko *et al.* [267] found Fe(III) to have a drastic quenching effect upon many fluorescent metal-8-HQS complexes and the above river water samples are known to be rich in Fe(III)).

The detection limit for the method was calculated using the signal equal to twice the peak to peak noise level of the baseline, using mobile phase conditions as in Figure 3.3. Based upon a 100 μL injection volume of a standard solution, a detection limit of 2 μg/L was obtained. This was decreased to 1 μg/L through the use of a 250 μL injection loop without significant broadening of the Cd(II) peak. It should be noted that the detection limit achievable using the larger injection loop would be sufficient to detect Cd(II) at concentrations below the US FDA/EPA maximum permitted levels of 0.01 mg/L for potable drinking waters.

Figure 3.5 shows the chromatogram obtained from the 100 μL injection of a 20 μg/L Cd(II) standard. The chromatogram shows how the peak for Cd(II) is well resolved from an unknown system peak eluting between 4 and 4.5 min.

Method precision was determined through 8 repeat injections of a 100 μg/L standard. Relative standard deviation (%) was determined as 2.2 for retention time, 2.8 for peak height and 3.2 for peak area.

The selectivity of the method was investigated through the injection of standards containing 0.5 mg/L of the following metal ions; alkali metals, Ca(II), Ba(II), Sr(II), Mn(II), Pb(II), Co(II), Ni(II), Cu(II), Fe(III), and Al(III). Under the mobile phase conditions used none of the above standards could be detected. The latter two trivalent metals were fully detected on the column and if necessary, could be removed from the column by using a lower mobile phase pH or IIR concentration or higher MeCN.

3.3.4. Analysis of water samples.

A number of water samples were collected for the analysis from a local system (Avoca River, OS 199825). The river itself flows between two disused mine works

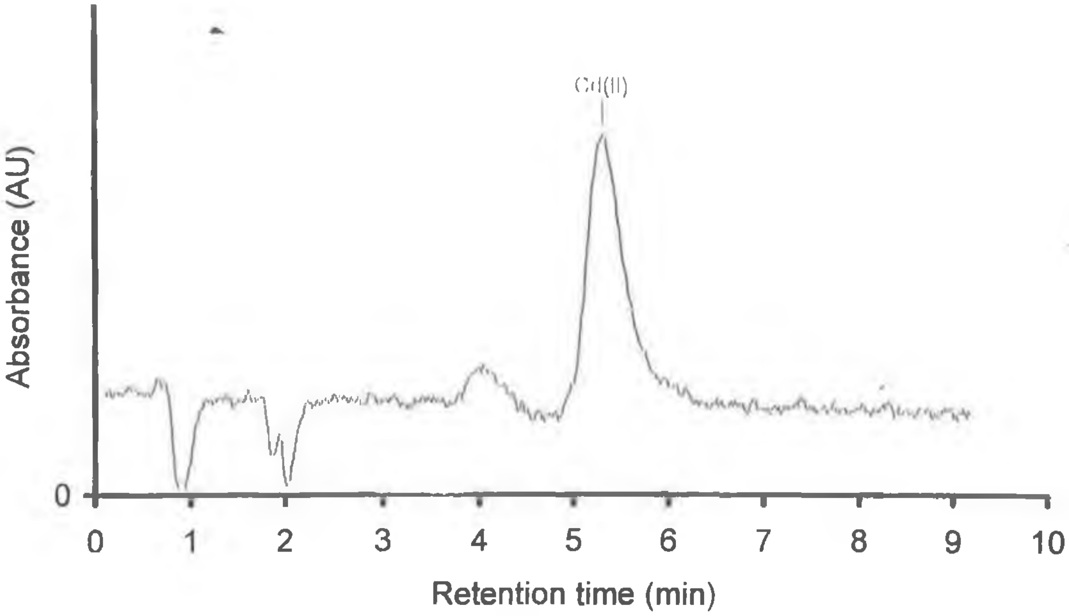


Figure 3.5. Chromatogram showing the injection of 20 µg/L Cd(II) standard. Other. conditions: 10 m*M* buffer (pH 4.8), 12 m*M* TBAOH, 5 m*M* 8-HQS, 15 % Acetonitrile.

where extensive lead, followed by copper extraction was carried out between 1750 to 1982. A sample of the mine drainage water was also collected, which continuously flows into the above river system. The area itself is a popular fishing area and leads to an estuary where shellfish are collected and so the monitoring of heavy metal input due to the mine is of great importance. Figure 3.6 shows chromatograms obtained from (a) the injection of a river water sample taken ~100 m downstream of the point of input of the mine drainage flow and (b) a similar sample spiked with 100 µg/L Cd(II). It can be seen that the concentration of what could be described as dissolved labile Cd(II) in the river sample is below the detection limit of the method (< 2 µg/L). However, it is also clear that between an elution time of 5 and 6 min., there were no interfering peaks arising from the river water matrix.

The chromatogram obtained for the actual mine drainage sample is shown in Figure 3.7. The drainage sample had a pH of 2.9 upon collection and was diluted ten times

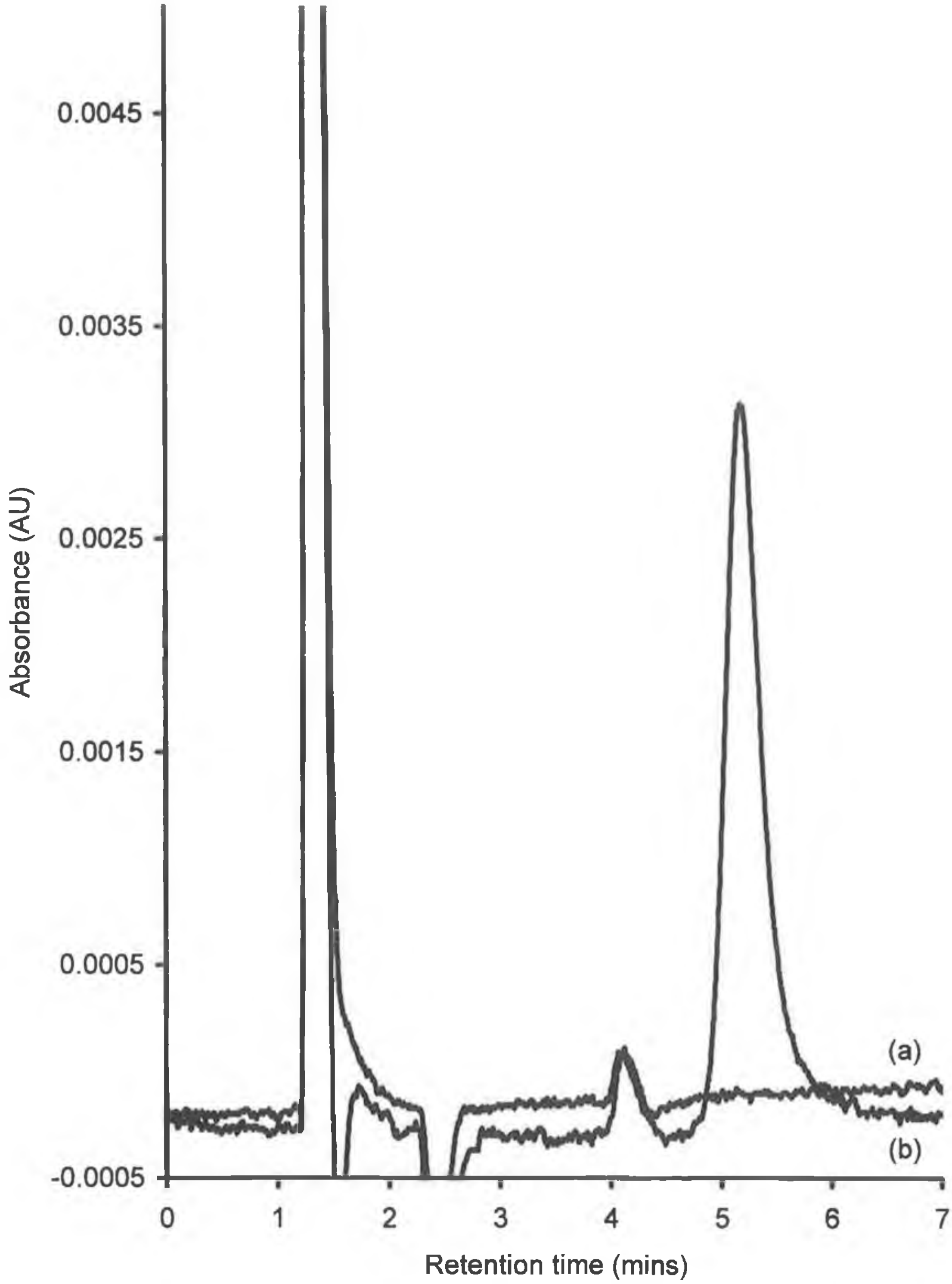


Figure 3.6. Chromatograms obtained from (a) the injection of an Avoca River 10 sample and (b) an Avoca River sample spiked with 100 µg/L Cd(II). Other conditions: m*M* buffer (pH 4.8), 13 m*M* TBAOH, 5 m*M* 8-HQS, 15 % acetonitrile.

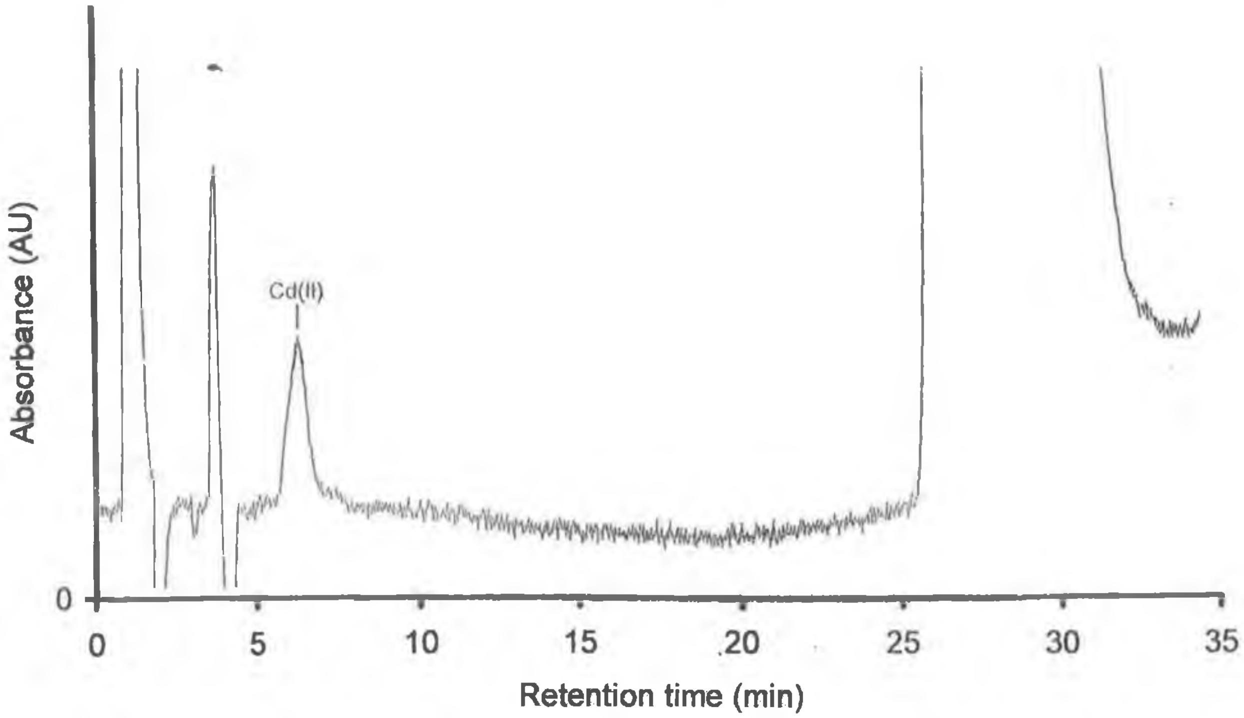


Figure 3.7. Chromatogram obtained from the injection of an Avoca Copper Mine drainage sample (diluted x 10). Other conditions: As in Figure 3.6.

Table 3.2. Concentration of Cd(II), Zn(II) and Mg(II) in mine drainage sample as determined by ion-interaction LC and atomic absorption spectroscopy.

Method	Ion-interaction LC		Atomic absorption spectroscopy	
Metal ion	Cd(II)	Cd(II)	Mg(II)	Zn(II)
Dilution required*	x 10	x 10	x 400	x 50
Concentration in undiluted mine drainage sample (mg/L)	0.190 (± 0.01)	0.174	127	56

*Dilution of sample in de-ionised water prior to analysis

Results based upon triplicate analysis

with de-ionised water prior to injection due to the high concentration of Mg(II) and Zn(II) in the sample.

The concentration of TBA added to the mobile phase was increased to 13 m*M* for the analysis of the river samples to increase the resolution of Cd(II) and Zn(II) and further remove the Cd(II) peak from the system peak which is shown eluting between 4 and 5 min. Analysis of the sample was carried out using standard addition calibration (0 – 100 μg/L, n = 3) resulting in a correlation coefficient of $R^2 = 0.999$. The sample was also analysed using atomic absorption spectroscopy (AAS) for Cd(II), Mg(II) and Zn(II).

Table 3.2 shows concentrations of the above metals present in the undiluted sample. The results show that the concentration of Cd(II) determined using the ion-interaction method matches closely (within 10%) that obtained using AAS. It is also clear from Table 3.2 and Figure 3.7 that Cd(II) can easily be determined at the low μg/L level in 500 – 1,000 fold excess concentrations of Mg(II) and Zn(II).

3.4. Conclusions.

A simple, sensitive and selective chromatographic method for the determination of Cd(II) in environmental water samples has been developed based on ion-interaction RP-LC combined with fluorescence detection. The method does not require any complicated post-column reactions or time consuming sample preparation. The response for Cd(II) is linear over the concentration range likely to be present in such samples and the method is highly reproducible. In addition, the selectivity of the developed method is such that Cd(II) can be detected at low μg/L concentrations in samples containing excess levels of matrix metals such as alkali and alkaline earths, and those metals which also form fluorescent complexes with 8-HQS, such as Zn(II) and Al(III).

Chapter 4.

Determination of Trace Alkaline Earth Metals in Brines Using Chelation Ion Chromatography with an Iminodiacetic Acid Bonded Silica Column.

4.0. Introduction.

The determination of trace metals in complex sample matrices using ion chromatographic methods has received considerable attention in recent years. Simple ion exchange chromatography is ideal for the determination of alkali and alkaline earth metal ions, but only if the sample is relatively low in ionic strength. If the ionic strength is too high, the matrix ions swamp the ion-exchange sites (a process known as self-elution), seriously affecting the separation efficiency. Thus the direct analysis of important samples, such as surface saline waters, biological/geological digests, and natural/industrial brines can be extremely difficult by conventional ion exchange chromatography, with many published methods requiring matrix elimination/preconcentration or large sample dilutions before injection.

One such system which has been developed by the Dionex Corporation, involves the preconcentration of trace metal ions onto a chelating mini-column, followed by their elution onto a strong ion exchange concentrator column, and finally their separation on a standard ion exchange analytical column [80, 201, 203, 204, 276-280]. This methodology has been applied to a range of high ionic strength samples such as seawater [80, 201, 203], biological fluids and tissue digests [80, 275], geological digests [276], drinking and surface waters [204, 278], inorganic reagents and organic solvents [279] and industrial brines [280]. However, despite the obvious versatility of the above system, it is evident that the equipment required is rather complex, involving a combination of a number of concentrator and separator columns, pumps and switching valves.

A better solution to the analysis of these types of samples would be the development of stationary phase materials which exhibit the correct selectivity to allow the injection of high ionic strength samples directly, without any on-line pre-treatment. One approach, which has been extensively developed over the previous decade, is the use of high-performance chelating stationary phases. These exhibit unique selectivity compared to simple ion exchange resins, showing little or no affinity for alkali metals, whilst at the same time being capable of producing high-performance separations of many di- and trivalent metal ions (depending upon the type of functional group). Two recent reviews on so-called ‘high-performance chelation ion chromatography’ have been compiled by Jones and Nesterenko [235] and Paull and Haddad [236], which detail the types of stationary phase materials available, how these materials have been produced and the applications which have been developed.

IDA-silica is one such material and has been investigated as a stationary phase for the separation of metal ions. Much of this work has focused on the separation of transition and heavy metal ions [247, 258, 259, 261] and more recently the lanthanide series metals [262, 263], although several workers have also investigated the potential of IDA-silica for the separation of both alkali and alkaline earth metals [260, 281, 282]. Elefterov *et al.* [281] studied the chromatographic behaviour of alkali and alkaline earth metal ions on IDA-silica using mineral and organic acid eluents. Retention of alkali metals was found to be predominantly due to simple ion exchange, whereas retention of the alkaline earth metals was thought to be due to a combination of ion exchange and complexation. A mixed eluent containing perchloric acid and a complexing organic acid was used to obtain a simultaneous separation of alkali and alkaline earth metals in a single run. Later work by Nesterenko *et al.* [282] studied the effect of temperature on the retention of alkali and alkaline earth metals on selected aminocarboxylic acid bonded silica columns. It was shown that IDA-silica behaved somewhat differently to the other polyfunctional ion exchangers studied, again indicating that a dual retention mechanism was responsible for the changes in selectivity shown.

Application of IDA-silica columns to the analysis of alkaline earth metals in complex sample types has been rather limited. Elefterov *et al.* [281] applied their developed method to the analysis of simple drinking and mineral waters, whilst Voloschik *et al.* [260] developed a method for the determination of Be(II) in rock digests using ion chromatography with an IDA-silica column.

4.1. Aims of this study.

This current chapter looks at the use of IDA-silica as a stationary phase for the separation of alkaline earth metals in high ionic strength samples, such as chloride brines. It also further investigates the true retention mechanism taking place within the system and illustrates the unique and versatile selectivity IDA-silica offers for the analysis of alkaline earth metal ions. A potential application of the method described is the analysis of trace levels of Ca(II) and Mg(II) in concentrated solutions of NaCl and KCl. This is of great interest to the chlor-alkali industry, where membrane poisoning in chlor-alkali cells can result from concentrations of Ca(II) and Mg(II) above 50 µg/L. A number of workers have developed methods for this particular analysis, however, each contains a preconcentration/matrix elimination step [241, 280, 283]. The method described here is for the determination of Ca(II) and Mg(II) in high ionic strength samples, through direct injection onto the analytical IDA-silica column with sensitive and selective post-column reaction detection.

4.2. Experimental.

4.2.1 Instrumentation.

A Dionex Model GPM2 Gradient Pump Module (Sunnyvale, CA, USA) was used to deliver the eluent (1.0 mL/min). A manual sample injection valve, model 7125, (Rheodyne, Cotati, CA, USA), fitted with a 100 µL injection loop was used for introduction of the samples. The IDA-silica packed analytical column (8 µm particle size, 130 µ pore size, 250 x 4.0 mm i.d) used was supplied by BioChemMack (Moscow, Russia). A pressure driven Dionex Reagent Delivery Module was used for introduction of the post column reagent (1.5 mL/min), which was mixed at room temperature with the eluent using a 0.5 m PEEK reaction coil (0.01” i.d.). A model SPD-6AV Shimadzu UV/Vis detector (Kyoto, Japan) was used at 572 nm to monitor the resultant chromatograms. These were recorded using Dionex AI450 chromatographic software.

4.2.2 Reagents.

The eluent and post column reagent (PCR) were prepared using deionised water from a Millipore Milli-Q Water Purification System (Bedford, MA, USA.). The PCR ligand, *o*-cresolphthalein complexone (*o*-CPC), was purchased from Sigma-Aldrich (Gillingham, Dorset, UK) and used without further purification. Potassium nitrate and boric acid was obtained from Merck (Darmstadt, Germany). Sodium nitrate was purchased from Riedel-De-Haen Ag (Sleeze-Hannover, Germany) and lithium chloride, sodium chloride and potassium chloride were all obtained from Fluka Chemie (Buchs, Switzerland). Final eluent conditions for the analysis of samples were 1.0 *M* KNO_3 or $NaNO_3$ adjusted to pH 4.9 using dilute HNO_3. The post-column reagent solution was 0.25 *M* boric acid, 0.3 m*M* *o*-CPC adjusted to pH 10.4 using NaOH. All solutions prepared were filtered through a 0.45 μm filter and degassed using sonication. Low level standard solutions were generally prepared freshly each day from stock solutions (1000 mg/L) stored in 1% nitric acid.

4.3. Results and Discussion.

4.3.1 The effect of eluent ionic strength.

With IDA functionalised stationary phases both the eluent pH and ionic strength are known to play vital roles in governing which is the dominant retention mechanism taking place within the system. In the case of silica based substrates it has been shown that when using low ionic strength acidic eluents such as mineral acids, ion exchange is the dominant retention mechanism for both alkali and alkaline earth metal ions [281, 282]. This ion exchange capacity originates from both residual silanols and other active hydroxy groups, as well as the attached carboxylic acid groups. At low pH (< pH 4 - 5) complexation of alkaline earth metal ions plays only a minor role in retention and this can be clearly seen from the selectivity obtained by Nesterenko and co-workers [281, 282] when using simple perchloric acid eluents, namely Mg(II) < Ca(II) < Sr(II) < Ba(II), which is the same as that obtained on weak carboxylic acid cation exchange resins [14].

If IDA-silica is to be applied to high ionic strength samples such ion exchange effects have to be minimised so that complexation is the dominant retention mechanism, as it is complexation that offers the desired selectivity. In previous work using IDA based chelating stationary phases KNO_3 has been found to be a suitable eluent and ionic strength modifier due to its relative high purity and low cost [235]. Therefore, the effect of KNO_3 concentration upon selectivity for alkaline earth metals was investigated here.

The results obtained from varying the concentration of KNO_3 over the range 0.1 to 1.0 *M* are plotted in Figure 4.1, with a number of the resultant chromatograms shown in Figure 4.2 and 4.3 (for simplicity, during the following experiments eluents were adjusted to pH 4.2 using dilute HNO_3, although it is recognised that at this pH such an eluent would have very little buffering capacity for real samples). From the retention data obtained a number of conclusions can be drawn. Firstly, at 0.1 *M* KNO_3 the selectivity shown is not typical of either simple ion exchange or chelation, but in fact a combination of both. However, it is clear that ion exchange is the dominant retention mechanism under these conditions, as the selectivity shown matches quite closely to that expected from simple ion exchange on carboxylic acid exchangers, here Mg(II)< Sr(II) <Ca(II) <Ba(II). This is supported by the known stability constant data available for IDA-alkaline earth metal complexes (0.1 *M* KNO_3, 25 °C, Mg(II) K_{STAB1}=2.94, Ca(II), K_{STAB1}=2.59, Sr(II) K_{STAB1}=2.23, Ba(II) K_{STAB1}=1.67) which indicates interaction should only be due to ion exchange and not complexation at pH < 5 – 5.5 [258, 284]. However, as can be seen in Figures 4.2 and 4.3, the retention of Ca(II) is unusually high, indicating that some degree of complexation may be responsible for this increased retention of Ca(II) compared to the remaining alkaline earth metal ions. This would appear to contradict the above stability constant data, however, as can be seen from Figures 4.2 (b), (c) and 4.3 (a-c), Ca(II) elutes last under higher ionic strength eluent conditions indicating the strongest degree of complexation. This is supported through comparison of the slopes of linear regression lines fitted to the retention data shown in Figure 4.1. The slopes were as follows; Ca(II) = -1.20, Mg(II) = -1.21, Sr(II) = -1.28 and Ba(II) = -1.38.

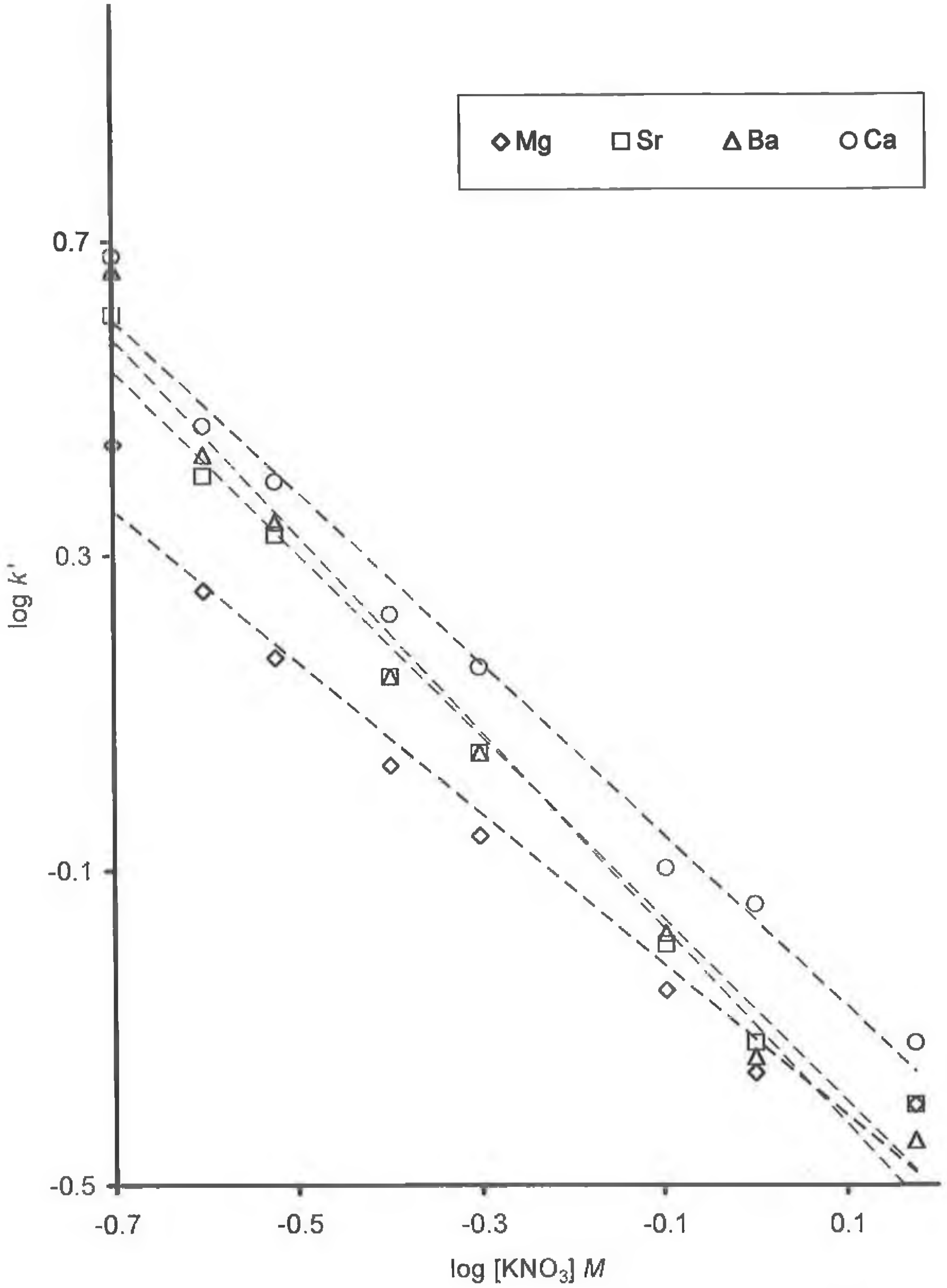


Figure 4.1. Plot of log *k′* vs. log [KNO_3] for alkaline earth metal ions on IDA-silica column. Other conditions; Eluent pH = 4.2 (HNO_3).

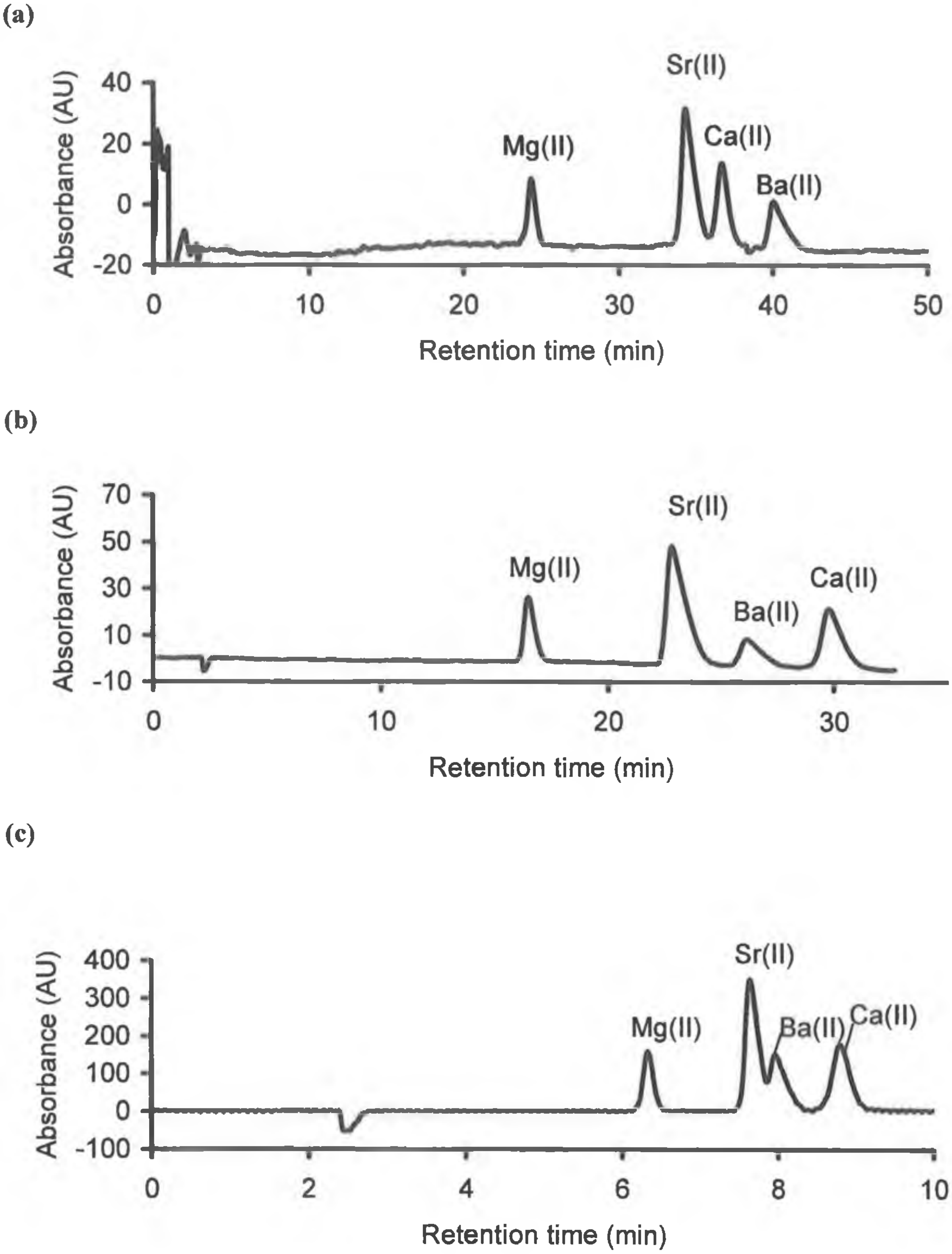


Figure 4.2. Separations of alkaline earth metal ions using IDA-silica column with (a) 0.1 *M*, (b) 0.2 *M*, and (c) 0.3 *M*, KNO_3 eluent. Other conditions: Eluent pH = 4.2 (HNO_3).

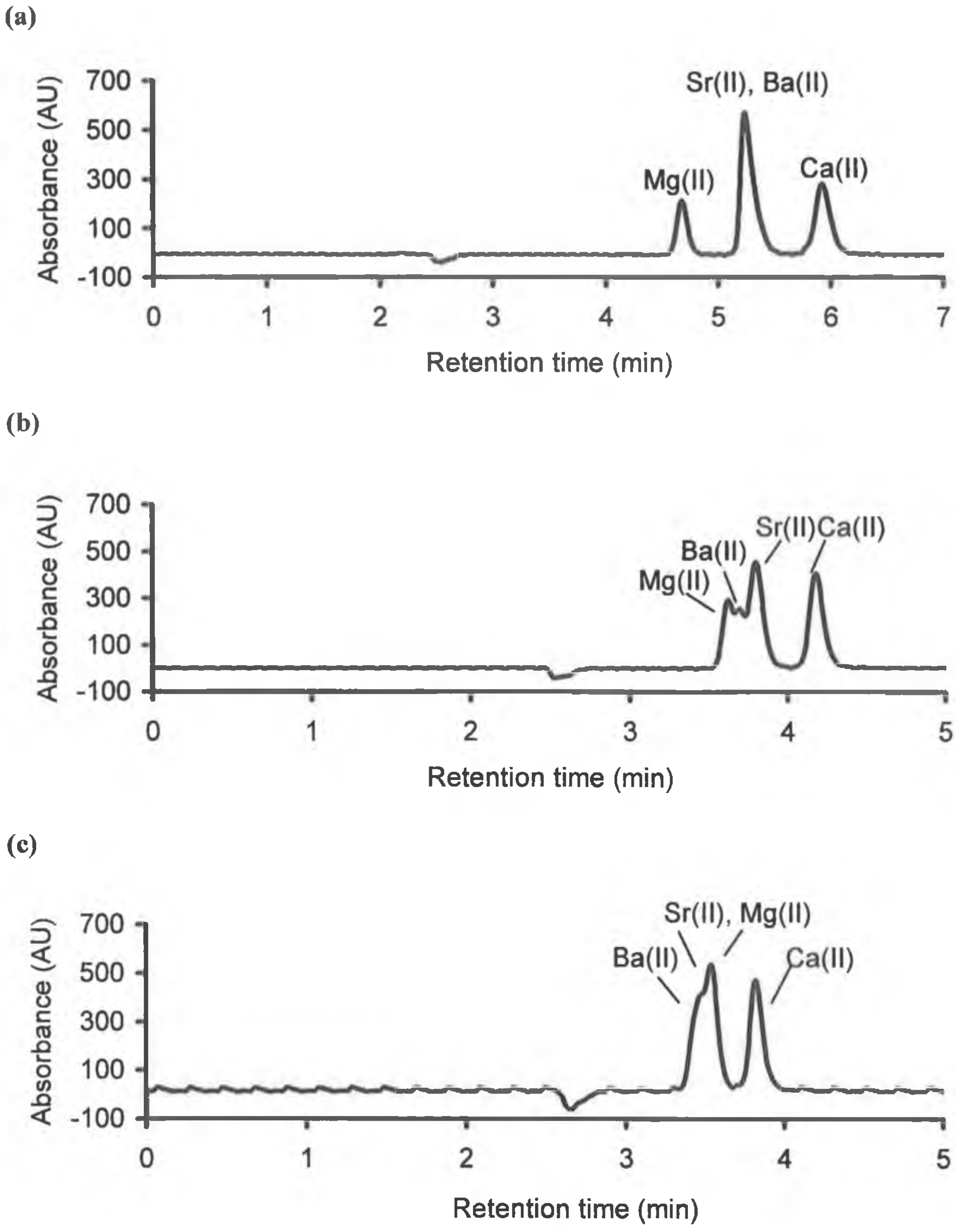


Figure 4.3. Separations of alkaline earth metal ions using IDA-silica column with (a) 0.5 *M*, (b) 1.0 *M*, and (c) 1.5 *M* KNO_3 eluent. Other conditions: Eluent pH = 4.2 (HNO_3).

It is clear from these results that Ca(II) is least affected by eluent ionic strength, indicating complexation is the dominant mechanism, whereas Ba(II) is most affected by ionic strength, thus indicating ion exchange is dominant. The quite dramatic transition from last eluting ion to first eluting ion shown by Ba(II) is a clear illustration of how the ion exchange capacity of IDA-silica can be effectively 'switched off' through use of high ionic strength eluents.

4.3.2 The effect of eluent pH.

The effects of increasing the eluent pH using both a (a) 1 *M* KNO_3 eluent and (b) a 0.5 *M* KNO_3 eluent are shown in Figure 4.4. Similar responses were obtained using both eluents, although as expected retention times were greater with the 0.5 *M* eluent due to the added contribution to retention from ion exchange. Once again Ca(II) exhibited differing behaviour to the remaining alkaline earth metal ions, showing a rapid increase in retention at approximately pH 5 and above. It is unclear why Ca(II) should show such differing selectivity to Mg(II) when using non-complexing eluents, particularly when considering the similar stability constant data for the IDA complexes in solution, where in fact the Mg(II) complex is slightly higher [284]. However, it is clear from Figure 4.4 that with both eluents complexation begins to dominate retention at > pH 5 for Ca(II) and > pH 6 for the remaining metal ions (eluent conditions were kept below pH 6.8 as the stationary phase was silica based). This rather unusual selectivity is illustrated in Figure 4.5., where the chromatogram shows the baseline separation of Mg(II), Ba(II) and Sr(II) in under 7 min., whilst Ca(II) is retained for 25 min. The chromatogram was obtained using a 0.5 *M* KNO_3 eluent (pH 5.9). Taking Figure 4.5 together with figure 4.2(a) and 4.2(b), it can be seen that three different baseline separations of the 4 alkaline earth metals can be achieved, each exhibiting unique selectivity, without using a complexing eluent. This shows the clear advantages to be had from using the IDA-silica column for the separation of this group of metal ions compared to a simple cation exchanger.

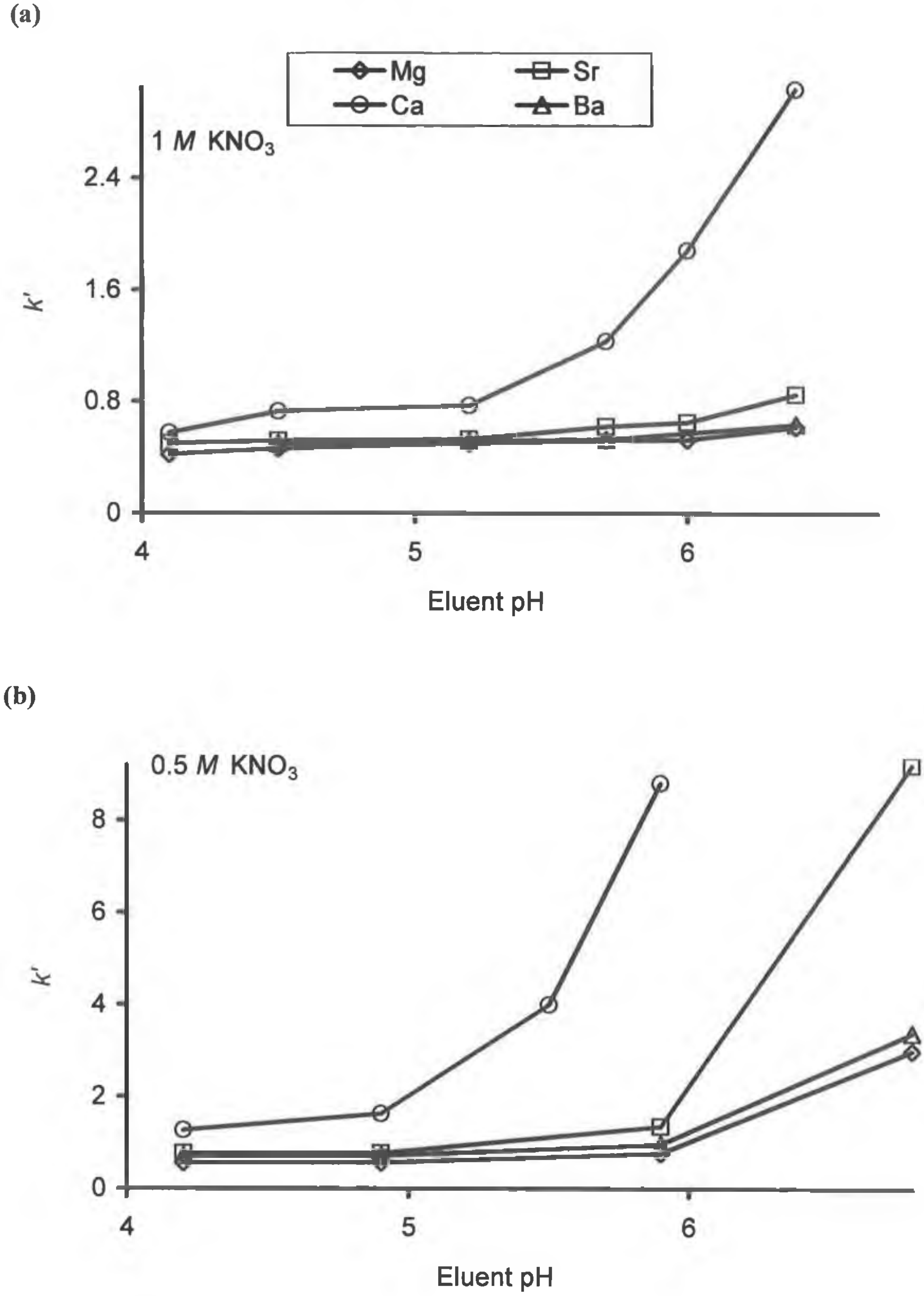


Figure 4.4. Plot of (a) *k′* vs eluent pH for alkaline earth metal ions on IDA-silica column using a 1.0 *M* KNO_3 eluent, and (b) *k'* vs eluent pH for alkaline earth metal ions on IDA-silica using a 0.5 *M* KNO_3 eluent.

4.3.3. The effect of column temperature.

Column temperature is known to affect ion exchange chromatography in a somewhat unpredictable manner, depending upon the type of ion exchange material used. Increased column temperature can lead to increased diffusion rates into the ion exchange matrix and thus increased efficiency and stationary phase interaction [14]. In pure ion exchange this effect is relatively poorly documented, with temperature having little effect upon selectivity for metal ions when using sulphonated cation exchangers [260]. However, carboxylic acid type ion exchangers have been shown to behave differently due to their complexation properties. In their recent review, Jones and Nesterenko state that as the "chelate effect" is predominantly an entropy effect, surface complexation will result in a positive increase in entropy and so the change in Gibb's free energy with temperature will be negative. If temperature is increased, the change in Gibb's free energy will become even more negative and so the equilibrium constant for the complex being formed will become bigger, hence an increase in retention. Jones and Nesterenko state that with this in mind, if complexation is purely responsible for retention, an increase in temperature should result in an increase in retention [234].

With IDA-silica the effect of temperature upon selectivity is still somewhat unclear. It's effect will be dependent upon the exact combination of retention mechanisms taking place, which itself is dependent upon the ionic strength, pH and complexing or non-complexing nature of the eluent. Kolpachnikova *et al.* [282] looked at the effects of temperature upon the retention af alkali and alkaline earth metals on IDA-silica and found that under acidic non-complexing conditions, retention of alkaline earth metals actually decreased with an increase in column temperature. However, at the pH of the eluent used in the Kolpachnikova *et al.* study, namely pH 2.7, it was clear that complexation was not playing a role in retention. Here, the effect of temperature was investigated using a 0.1 *M* and a 1.0 *M* KNO_3 eluent at pH 4.1. Figure 4.6. shows the results obtained with respect to the retention of Mg(II) and Ca(II). It is evident from the results that temperature effects upon selectivity are dependent upon eluent conditions, here the eluent ionic strength.

There is a clear increase in retention of both Mg(II) and Ca(II) with temperature when using the 0.1 *M* KNO_3 eluent. This in itself is the opposite effect to that shown by Kolpachnikova *et al.* However, even more unusual is that when using the 1.0 *M* KNO_3 eluent, where complexation should play a more dominant role in retention, there is an apparent decrease in the retention of Ca(II), whereas Mg(II) remains unaffected. Once more this is the opposite response to that expected, although it again indicates that the exact retention mechanism responsible for the retention of Ca(II) differs considerably to that for Mg(II).

From the above results it is noticeable that there is a distinct change in retention mechanism taking place with increasing ionic strength. However, the responses to changes in column temperature do not correspond to those expected from either pure ion exchange or pure chelation. It seems clear, as mentioned previously, that under this range of eluent conditions a dual retention mechanism is indeed taking place with the dominance of ion exchange or chelation being dependent upon pH and/or ionic strength. Therefore, it can only be concluded here that the effects shown are characteristic of IDA-silica at this particular pH with KNO_3 eluents. Further work is obviously required to fully understand the full effects of temperature upon such a system. However, here it was clear that although temperature could be used to change retention times slightly, it did not change elution orders or greatly improve efficiency and so was neglected for the remainder of the study.

4.3.4. The effect of sample matrix.

As this study aimed to develop simple methodologies for the analysis of alkaline earth metal ions in high ionic strength samples, it was important to determine the real effects of the sample matrix upon the separation achieved and the correct choice of eluents to use

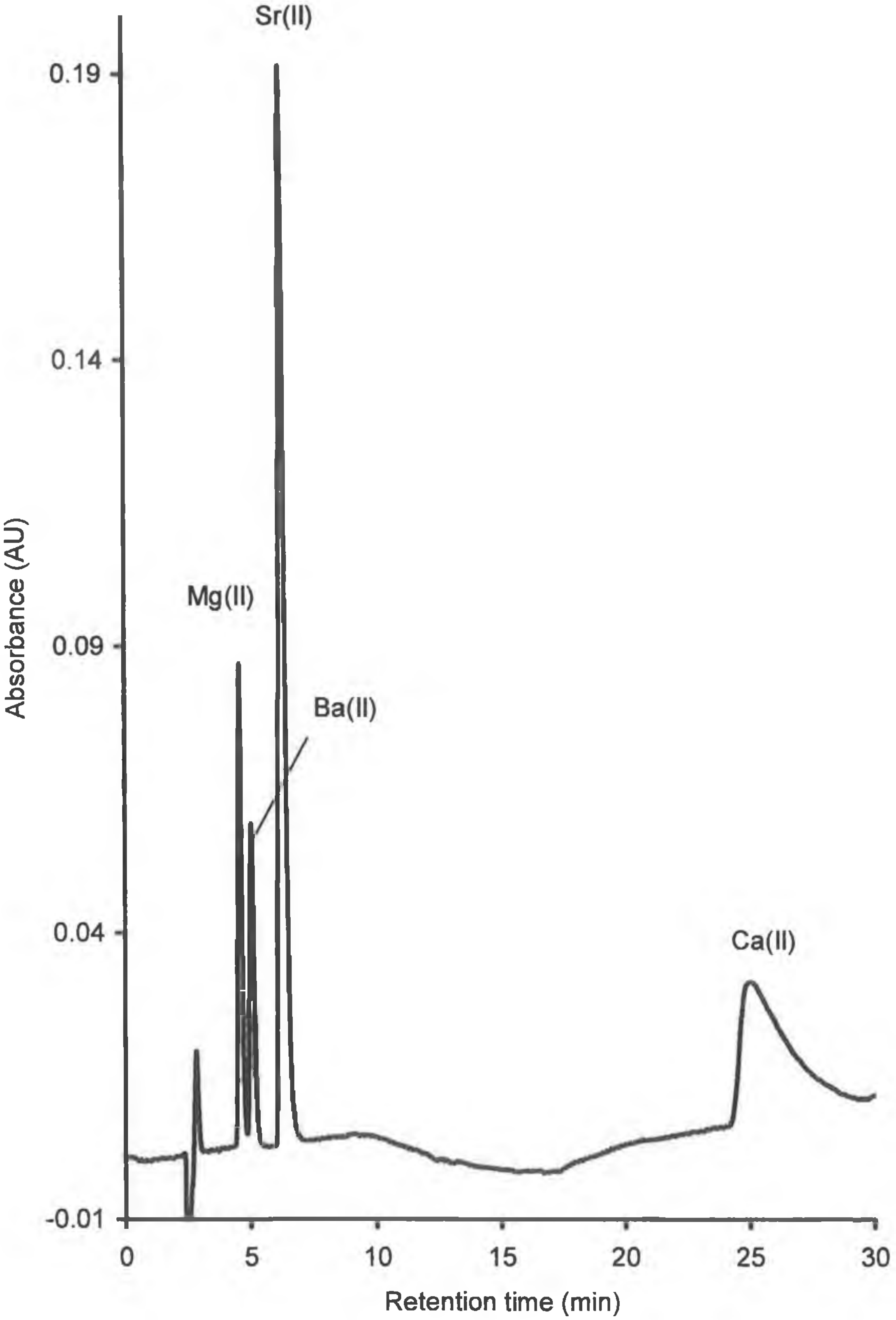


Figure 4.5. Separation of alkaline earth metal ions on IDA-silica column using a 0.5 *M* KNO_3 eluent, adjusted to pH 5.9 using HNO_3.

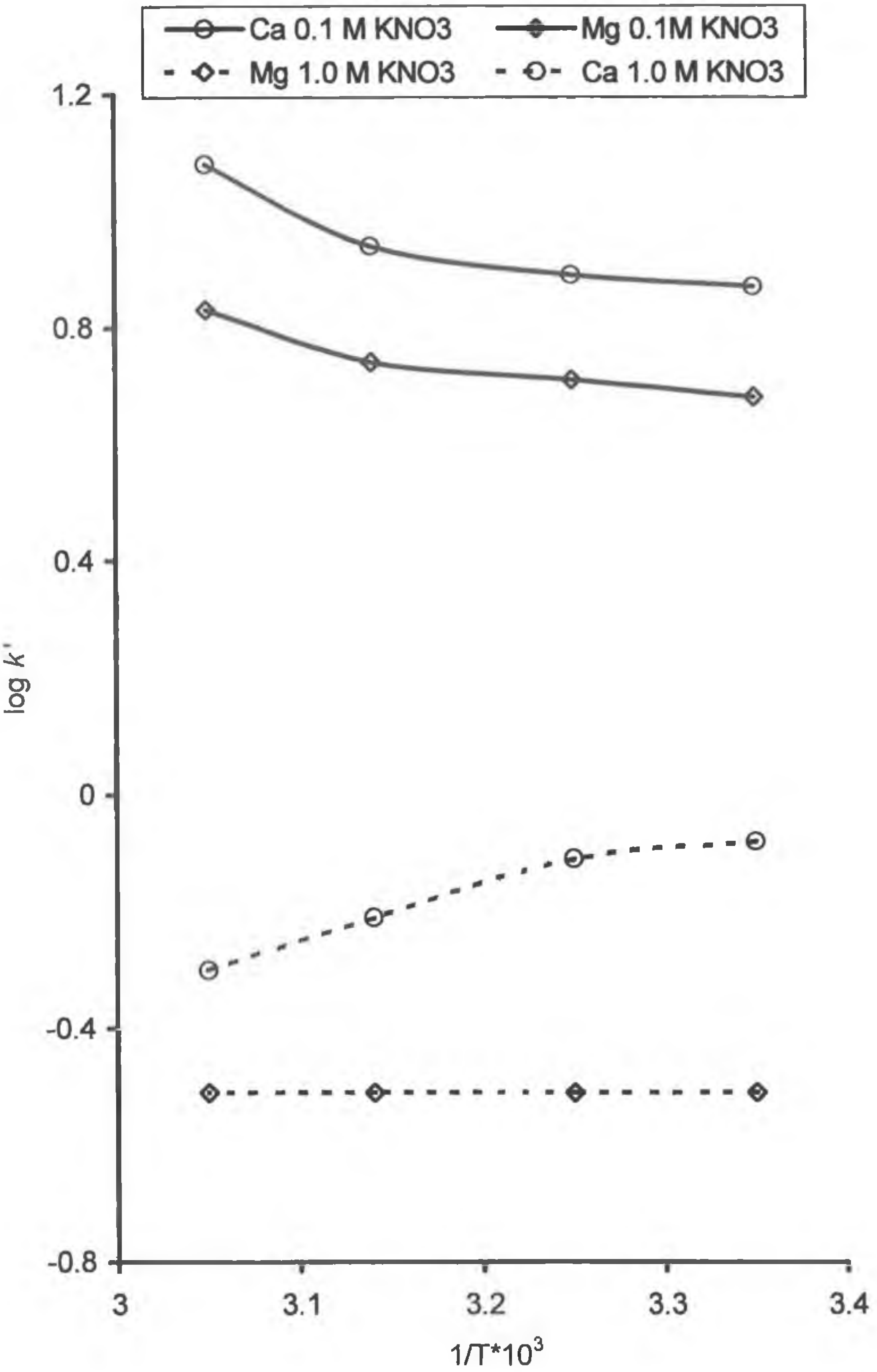


Figure 4.6. Plot of log *k′* vs 1/T*10^3 for Ca(II) and Mg(II) on IDA-silica column using 1.0 *M* and 0.1 *M* KNO_3 eluents. Other conditions; Eluent pH = 4.1

with each particular sample. If the contribution to retention from ion exchange is minimised through the use of a high ionic strength eluent, the ionic strength of the sample will have less effect upon retention and efficiency. However, even with a high ionic strength eluent, there remains a noticeable matrix effect upon the separation of Mg(II) and Ca(II) when using the IDA-silica column. Figure 4.7 shows the overlaid separations of 1 mg/L Mg(II) and Ca(II) standards prepared in pure water and 1.0 *M* KCl using a 1.0 *M* KNO_3 eluent, adjusted to pH 4.9. Two observations can be made from Figure 4.7. Firstly, there is a lack of any system peak from the KCl matrix, a point that is discussed below. Secondly, the retention times of the two peaks are only marginally affected, and it is only the peak for Mg(II) which shows a significant loss in efficiency. It would appear that as chelation is playing a greater role in the retention of Ca(II) than in the retention of Mg(II), it is Ca(II) which is less affected by sample ionic strength, despite the fact that here the ionic strength of the sample matches that of the eluent.

Two other chloride salt matrices were also investigated, namely NaCl and LiCl. The separation of 1 mg/L Mg(II) and Ca(II) in 1.0 *M* solutions of the above two salts is shown in Figures 4.8(a) and (b) (eluent conditions as in Figure 4.7). In both cases there was a detector disturbance due to the matrix eluting at the eluent dip. In the case of 1.0 *M* LiCl the disturbance was quite significant. This was due to the fact Li(I) behaves differently to other alkali metals, namely K(I) and Na(I), in that is it known to form very weak complexes with certain metallochromic ligands, including *o*-CPC [286]. As no such system disturbance was observed when analysing a KCl containing sample using a KNO_3 eluent, the disturbance from the NaCl containing sample was assumed to be due to the Na cation. To eliminate these system disturbances it was clear the eluent cation had to match that of the sample. To illustrate this, Figure 4.8(c) shows the separation of 1 mg/L Mg(II) and Ca(II) in 1.0 *M* NaCl using a 1.0 *M* $NaNO_3$ eluent. As can be seen from Figure 4.8(c), by matching the eluent cation with that of the sample, the system peak can be eliminated completely. Using either a KNO_3 or $NaNO_3$ eluent has little effect upon selectivity for the two metal ions, except for the slightly increased retention of Ca(II) with the $NaNO_3$ eluent. Increasing the ionic strength of the sample further led to the reappearance of these system peaks, although it was possible, using a 1.0 *M* $NaNO_3$

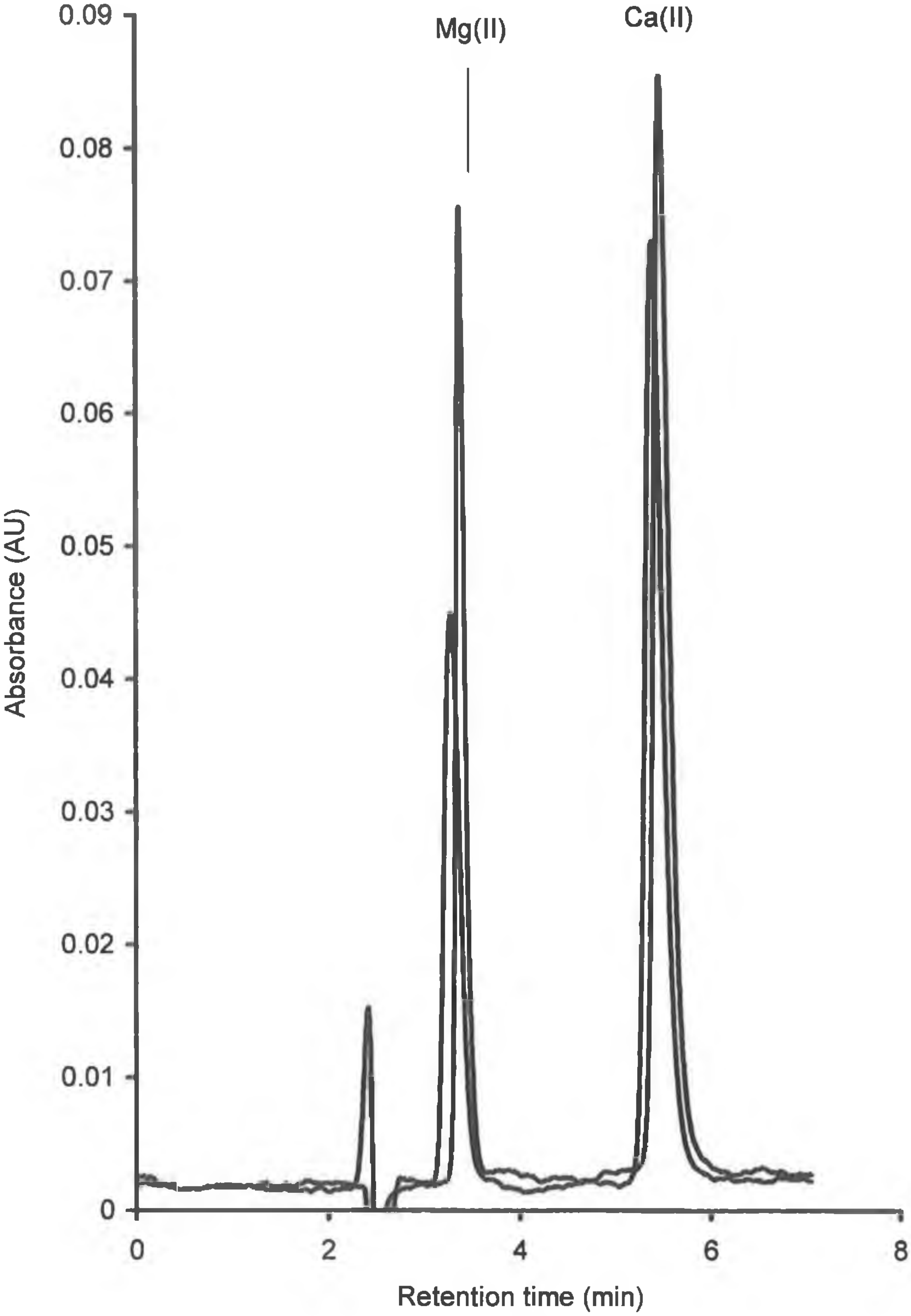


Figure 4.7. Overlaid chromatograms showing the separation of 1 mg/L Mg(II) and Ca(II) standards prepared in water and in 1.0 *M* KCl on an IDA-silica column with a 1.0 *M* KNO_3 eluent, adjusted to pH 4.9 using HNO_3.

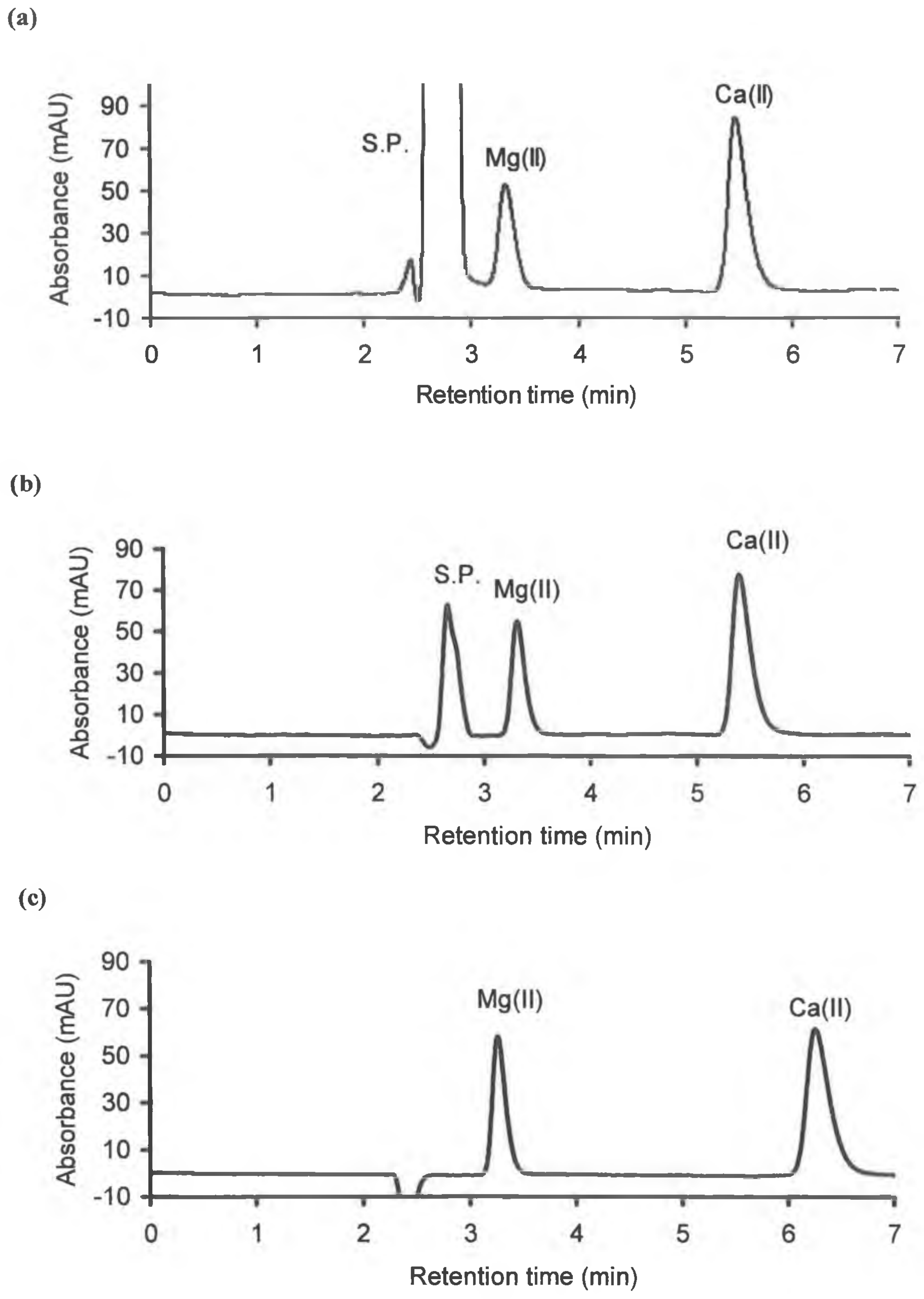


Figure 4.8. Chromatograms showing the separation on an IDA-silica column of 1 mg/L Mg(II) and Ca(II) in (a) 1.0 *M* LiCl, (b) 1.0 *M* NaCl, with a 1.0 *M* KNO_3 eluent (pH 5.6), and (c) the separation of 1 mg/L Mg(II) and Ca(II) in 1.0 *M* NaCl with a 1.0 *M* $NaNO_3$ eluent (pH 4.9). S.P = system peak.

eluent, to inject samples containing up to 3.0 *M* NaCl without the system peak interfering with peaks for Mg(II) and Ca(II).

4.3.5. Analytical performance data.

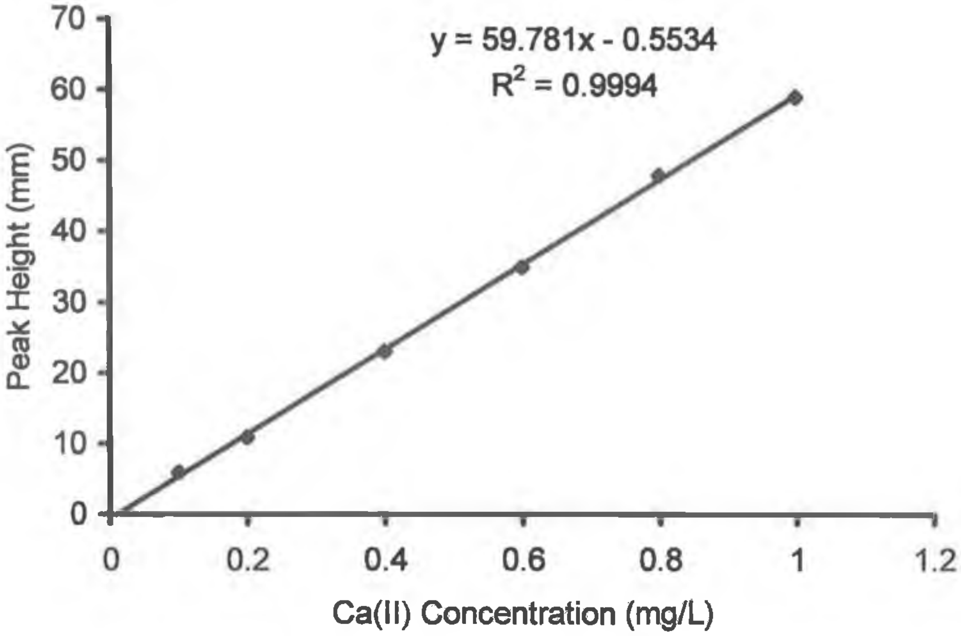
Before applying the developed method to the analysis of real samples the usual analytical performance criteria were investigated. Table 4.1 shows the results obtained for linearity over high and low analyte concentration ranges (with corresponding graphs shown in Figure 4.9 and 4.10.), detection limits in water and 1.0 *M* NaCl, and peak reproducibility. The above parameters were determined under an analytical routine whereby at the end of each day the analytical column was flushed through with ~ 50 mL of a 0.1 % HNO_3 solution, to remove any build-up on the column of transition metals or trivalent metal ions originating from the eluent/sample. The build-up of these highly retained metals would otherwise eventually (over a period of several days) lead to a reduction in column capacity and thus reduce retention times and separation efficiency.

From Table 4.1 it can be seen that the post-column reaction with *o*-CPC is particularly sensitive to Ca(II) and Mg(II). This is due to the uncomplexed ligand being almost colourless at pH 10.0 (thus producing relatively little background noise), but forming a dark purple coloured complex with the above two metal ions. However, *o*-CPC is not particularly sensitive to Ba(II) and Sr(II). Therefore, if trace determinations of these two alkaline earth metals are required, an alternative ligand such as Sulfonazo III may be preferable, this being one of the most sensitive metallochromic ligands available for the determination of Ba(II) [286].

4.3.6. Analysis of NaCl and KCl brine solutions.

The developed method was applied to the determination of Mg(II) and Ca(II) in a 0.9% NaCl medical eyewash saline solution using standard addition over the range 0.1 to 1

(a)



(b)

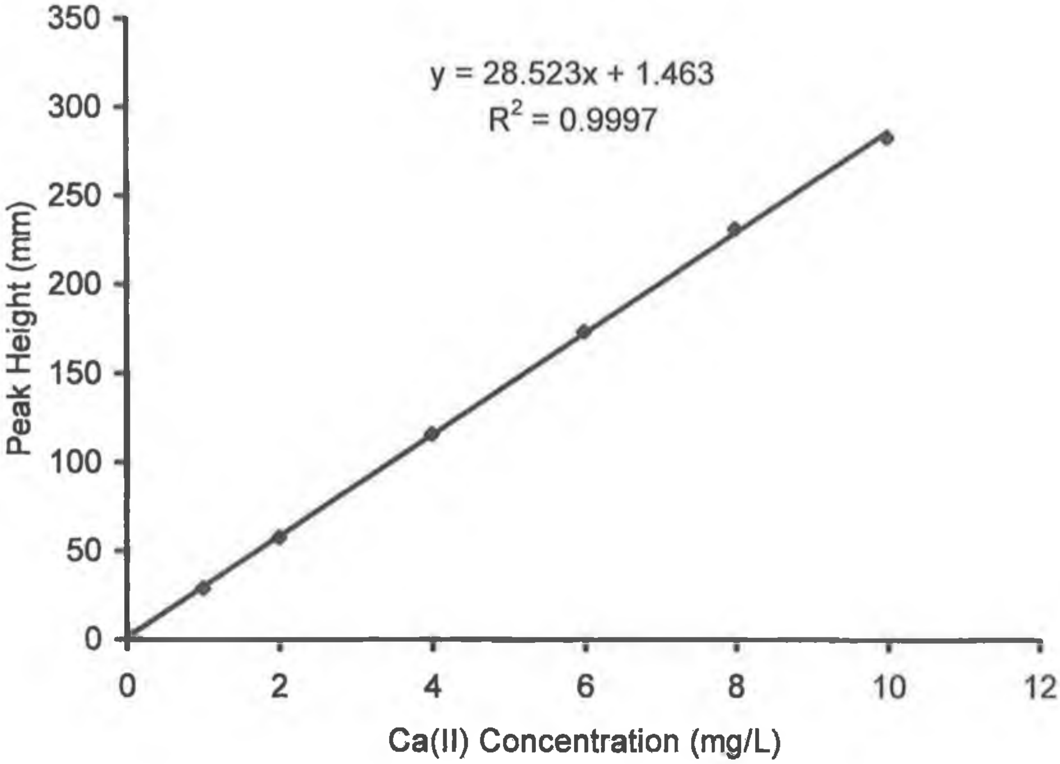


Figure 4.9. Standard calibration plots of Ca(II) in the region (a) 0.1-1 mg/L and (b) 1-10 mg/L.

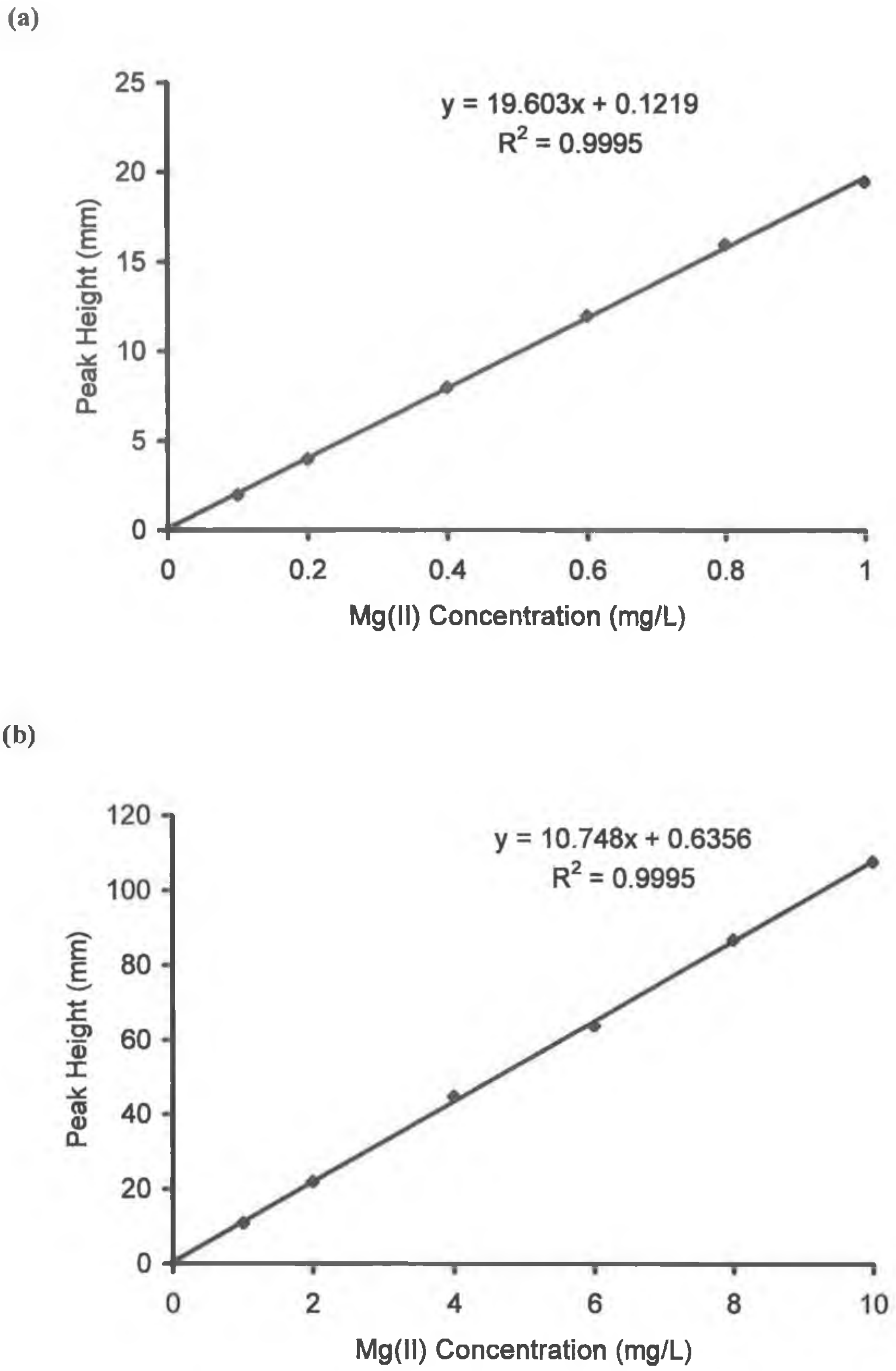


Figure 4.10. Standard calibration plots of Mg(II) in the region (a) 0.1-1.0 mg/L, and (b) 1-10 mg/L.

Table 4.1. Analytical performance data.

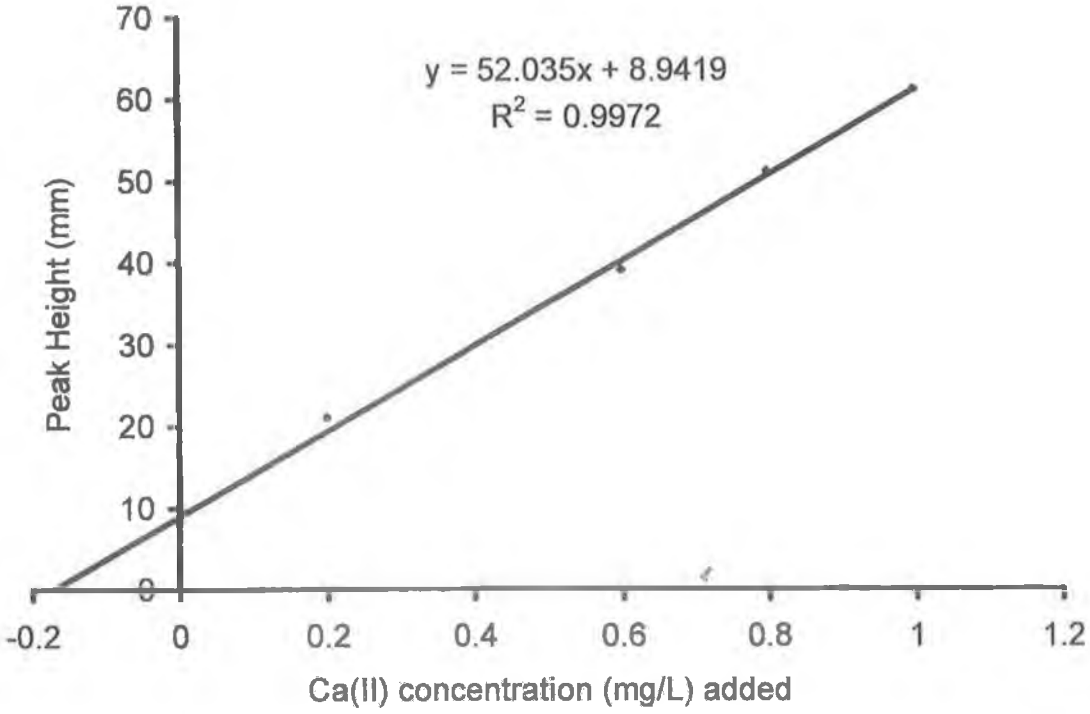
Linearity (n=6)	Mg(II)	Ca(II)	Sr(II)	Ba(II)
- 1.0 mg/L	R^2=0.999	R^2=0.999	___	___
1.0 - 10.0 mg/L	R^2=0.999	R^2=0.999	___	___
*Absolute detection limits**				
In water	0.8 (±0.1) ng	0.7(±0.1) ng	4.8 (±0.5) ng	29.0 (±3.0) ng
In 1.0 *M* NaCl	1.6 (±0.2) ng	1.4(±0.2) ng	___	___
Reproducibility %RSD (n=11)[1]				
Retention time	0.7	1.0	___	___
Peak height	2.5	1.4	___	___
Peak area	0.3	0.3	___	___

* calculated as twice the peak to peak baseline noise, 100 μL inj. volume.

[1] Based on 1.0 mg/L standards

mg/L (n=4) with the corresponding plot shown in Figure 4.11. Under the conditions used in Figure 4.8(c), namely a 1.0 *M* $NaNO_3$ eluent, the saline sample was found to contain 180 μg/L Ca(II), with the Mg(II) concentration below the detection limit (16 μg/L).

Overlaid chromatograms of the saline solution itself and the same sample spiked with 200 μg/L Mg(II) and Ca(II) are shown as Figure 4.12. The chromatograms show how trace amounts of Ca(II) were easily detectable in an approximately 20,000 fold excess concentration of Na(I) through using the correct choice of eluent, without any large matrix peaks or significant affect upon selectivity or peak efficiency.



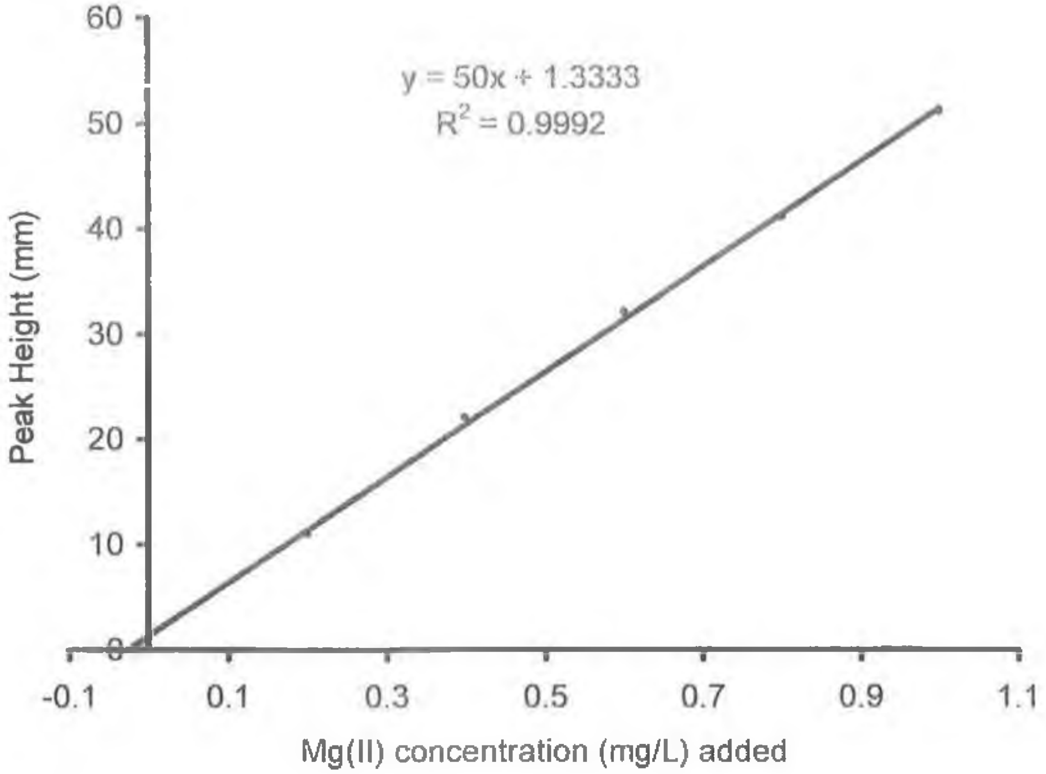


Figure 4.11. Standard addition plots for spiked 0.9% NaCl medical eyewash saline solution.

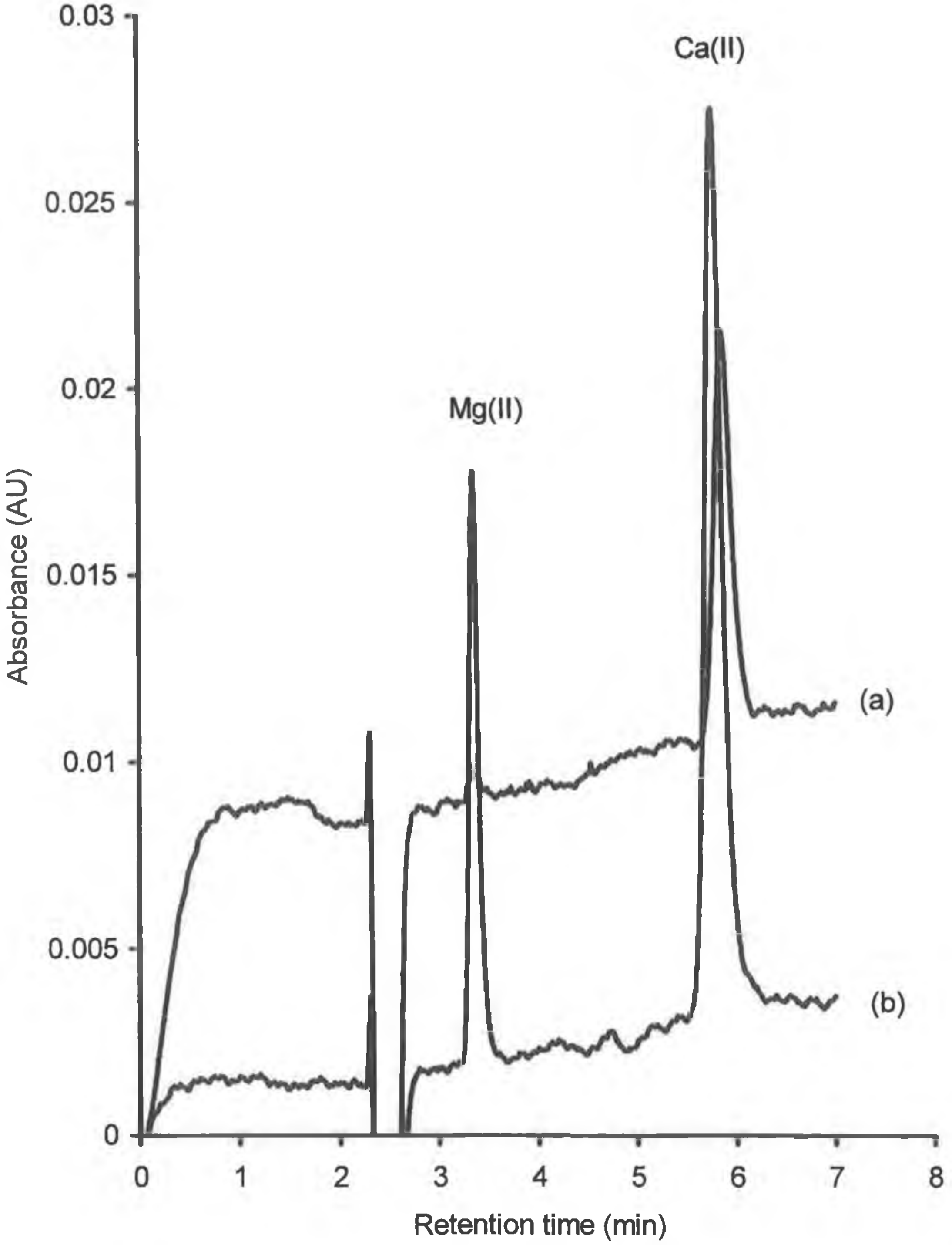


Figure 4.12. Chromatograms showing (a) saline eyewash sample (0.9% NaCl) overlaid with (b) spiked 200 μg/L Mg(II) and Ca(II). Other conditions as in Figure 4.7(c).

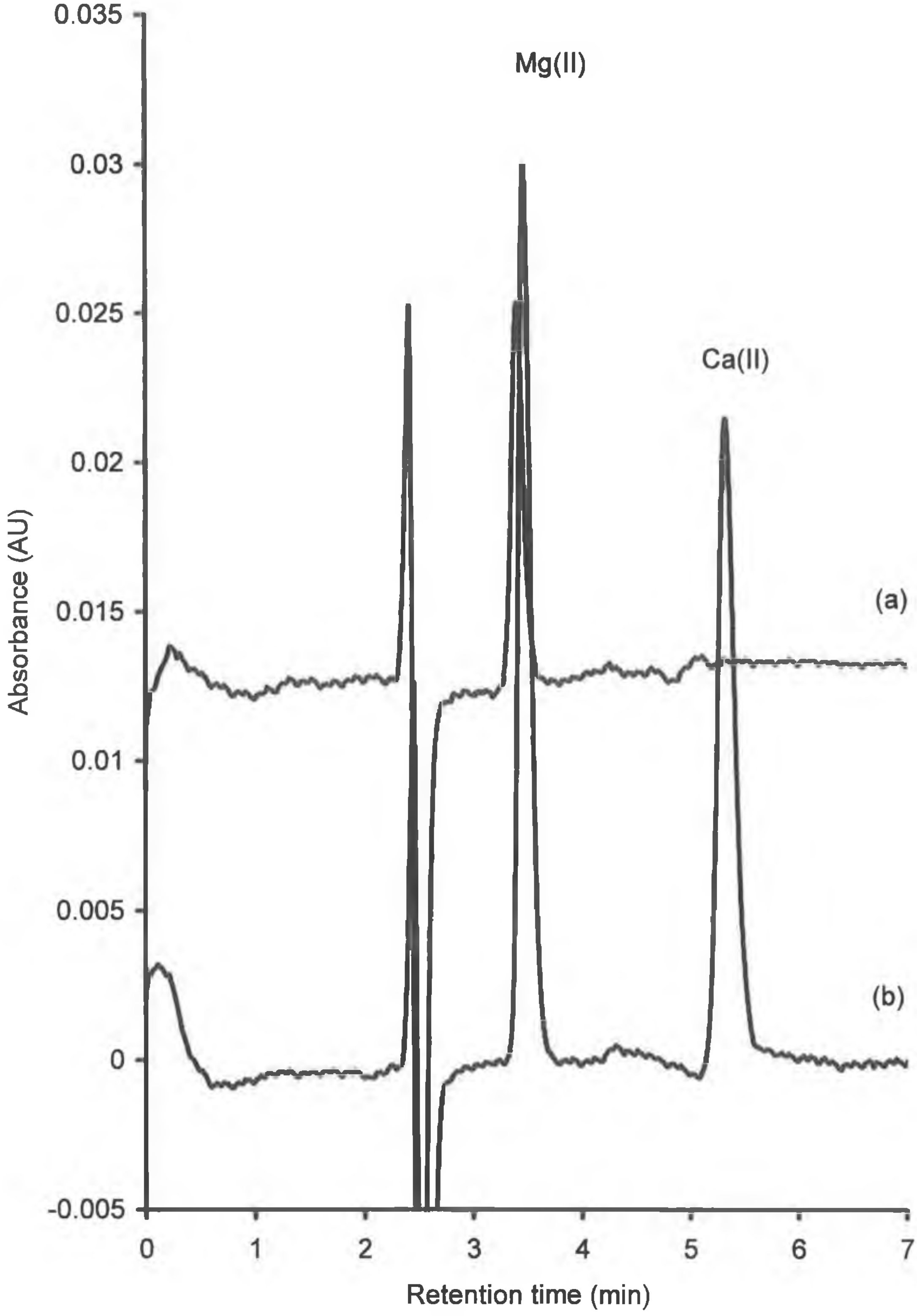


Figure 4.13. Chromatograms showing (a) 0.5 *M* KCl overlaid with (b) spiked 300 μg/L Ca(II) and Mg(II) sample. Other conditions as in Figure 4.8.(c).

The method was also applied to the determination of Mg(II) and Ca(II) in a 0.5 *M* solution of laboratory grade KCl (*Fluka >99%*), this time using a 1.0 *M* KNO_3 eluent. Once again standard addition was used and the sample was found to contain a Mg(II) concentration of 210 µg/L Mg and a Ca(II) concentration which fell below the method detection limit (14 µg/L).

The overlaid chromatograms of the 0.5 *M* KCl sample and the same sample spiked with 300 µg/L Mg(II) and Ca(II) are shown as Figure 4.13. As above, through the correct choice of eluent conditions, no matrix peak was observed and Mg(II) and Ca(II) both elute as sharp well resolved peaks in under 6 min (although not the case with the above two samples, when analysing real samples which contain increased concentrations of acidic or basic components it may be necessary to buffer the eluent with a suitable non-complexing buffer to reduce changes in retention times due to high or low sample pH).

4.4. Conclusions.

The ion chromatographic separation of alkaline earth metals has been investigated using an IDA bonded silica column. Results clearly indicate a mixed mode retention mechanism over the range of eluent conditions investigated with the dominance of ion exchange or chelation being dependent upon eluent ionic strength and pH. The correct choice of eluent allows the efficient and well resolved separation of alkaline earth metal ions in samples of significant ionic strength without large matrix peaks or signs of 'self elution'. Trace concentrations of Mg(II) and Ca(II) at the low µg/L level could be easily detected in NaCl and KCl brine samples by using sensitive post-column reaction detection with *o*-CPC.

Chapter 5.

Sensitive and Selective Chelation Ion Chromatographic Method for the Determination of Trace Beryllium in Water Samples.

5.0. Introduction

Beryllium is one of the most toxic non-radioactive elements to be found at trace levels in natural and industrial waste waters. Beryllium acts as an insidious carcinogenic poison, affecting cellular membranes and binding specific regulatory proteins in cells, with detectable excretory amounts present in urine for up to 10 years after initial exposure [287, 288].

The monitoring of trace levels of Be(II) in natural waters is of interest as this indicates the extent of environmental pollution from anthropogenic sources such as the nuclear, aeroneutical and metallurgical industries. Developed methods need to be both selective and sensitive as concentrations of Be(II) in natural and waste waters generally range from 0.1 to 500 μg/L, with concentrations exceeding 0.2 μg/L constituting an environmental hazard [260].

Over recent years numerous spectroscopic analytical techniques have been employed in the detection of Be(II) in water samples. These include spectrophotometric methods [289-291], spectrofluorimetric methods [292-294], FAAS, [289, 294], ET-AAS, [294-296], ICP-AES, [298], and ICP-MS, [299]. Of these methods, ICP-MS is perhaps the most ideal due to the combination of a relative lack of susceptibility to matrix interferences, and sensitive mass detection, thus allowing most samples to be analysed without preconcentration or extraction steps. However, this method does require the purchase of highly complex and expensive instrumentation and so is not readily available to a great number of analytical laboratories. Of the remaining techniques mentioned above, spectroscopic interferences can be a problem when looking at real samples and so many published methods require the incorporation of some sort of separation/extraction methodology prior to analysis.

Such problems are not encountered when applying chromatographic methods to the determination of Be(II), particularly with the correct stationary phase selectivity. However, chromatographic methods for the determination of Be(II) are surprisingly few and far between considering the environmental interest in the monitoring of this toxic element. Several methods have been developed which are based upon either

RP- HPLC of strongly absorbing Be(II) chelates [300, 301], or cation exchange chromatography [42, 302, 304]. Of the above, those methods based upon the pre-column formation of metal-ligand chelates have obvious disadvantages in terms of complex sample pretreatment and analysis time and so are not suited for routine monitoring. Those methods based upon cation exchange chromatography are more suited to such an application but lack the desired stationary phase selectivity (or indeed detector selectivity if using conductivity) required for the analysis of anything other than relatively simple samples types such as drinking water and other low ionic strength samples.

Alternative complexing or chelating stationary phases for use in the ion chromatographic determination of Be(II) have been investigated by Voloschik *et al.* [260] and more recently by Shaw *et al.* [265]. Voloschik *et al.* used an iminodiacetic acid (IDA) functionalised silica gel column combined with a 5 m*M* HNO_3 - 2 m*M* dipicolinic acid eluent. Under these conditions Be(II) was well separated from common transition and heavy metals which were unretained, but eluted immediately after other alkaline earth metals which meant the method was limited to samples with a relatively low Ca(II)/Mg(II) to Be(II) ratio, particularly as the detection method used was indirect conductivity. Shaw *et al.* developed a method for the determination of Be(II) in a stream sediment which employed an aminomethylphosphonic acid functionalised silica gel stationary phase with a 1.0 *M* KNO_3, 0.5 *M* HNO_3 and 0.08 *M* ascorbic acid eluent. This substrate exhibited unique selectivity toward Be(II) based upon the coordination of the Be(II) ion with up to three O atoms of the methylphosphonic acid functional group. When used with the above strongly acidic eluent Be(II) could be separated from large excesses of common alkaline earth and transition metal ions which were unretained. In this study by Shaw *et al.*, post column reaction detection was used for added selectivity. The post-column reagent solution used consisted of 0.008 % chrome azurol S, 10 m*M* EDTA, 1.0 *M* hexamine (pH 6). However, the sensitivity of the method was reduced, due to the rather broad peak shape obtained for Be(II), which resulted from its strong affinity for the stationary phase. A detection limit of 35 μg/L was obtained for Be(II) in stream sediment digest.

5.1. Aims of this study.

The following study details the development of a ion chromatographic method based upon the use of an IDA functionalised silica gel column with a high ionic strength eluent for the selective determination of trace Be(II) in various water samples. Development of the eluent conditions required for optimal selectivity when analysing complex natural and waste water matrices has been carried out, coupled with further development of sensitive post-column reaction detection. The final method was applied to the analysis of spiked potable and natural water samples, including simulated seawater, and a certified reference freshwater sample (NIST 1640).

5.2. Experimental.

5.2.1. Instrumentation.

A Dionex model GPM2 gradient pump module (Sunnyvale, CA, USA) was used to deliver the eluent at 1.0 mL/min. A manual sample injection valve, model 7125 (Rheodyne, Cotati, CA, USA), fitted with a 100 or 250 μL injection loop, was used for sample introduction. The dimensions of the IDA-silica gel analytical column used were 250 x 4.0 mm i.d., with an 8 μm average particle size (BioChemMack, Moscow, Russia). A pressure driven Dionex reagent delivery module was used for introduction of the post-column reagent solution at 1.5 mL/min, which was mixed with the eluent using a 0.5 m x 0.3 mm i.d. PEEK reaction coil. A model SPD-6AV Shimadzu UV/Vis detector (Kyoto, Japan) was used at 590 nm to monitor the resultant chromatograms. These were recorded using Dionex A1-450 chromatographic software.

5.2.2. Reagents.

The eluent and post-column reagent (PCR) were prepared using deionised water from a Millipore Milli-Q water purification system (Bedford, MA, USA). Chrome azurol S (CAS) 65 % sodium salt, 2-(N-morpholino) ethanesulphonic acid (MES) and Triton

X-100 were purchased from Sigma-Aldrich (Gillingham, Dorset, UK) and used without further purification. Potassium nitrate was obtained from Merck (Darmstadt, Germany). Final eluent conditions for the analysis of samples were 0.4 *M* KNO_3, adjusted to pH 2.5 using dilute HNO_3. The post-column reagent solution was 0.26 m*M* CAS, 2 % Triton X-100, 50 m*M* 2-(N-Morpholino) ethanesulphonic acid (MES), adjusted to pH 6.0 using dilute NaOH (after mixing of the eluent and PCR, the final solution pH at the detector was 5.9). All solutions were filtered through a 0.45 µm filter and degassed using sonication. Low-level standard solutions were generally prepared freshly each day from stock solutions (1000 mg/L), stored in PTFE containers and acidified using dilute nitric acid.

5.3. Results and discussion.

5.3.1. Stationary phase selectivity.

The use of chelating stationary phases for the high-performance separation of metal ions has been extensively investigated over the past ten years and a number of comprehensive reviews have been compiled on the technique known as 'chelation ion chromatography' [235,237]. Retention of metal ions on chelating stationary phases is dependent upon the conditional stability constants of the metal ion and the immobilised ligand, which are governed by the nature of the immobilised ligand, namely the number, position and type of coordination sites. As mentioned previously, the aminomethylphosphonic acid functionalised chelating stationary phase used by Shaw *et al.* [265], coordinates with the Be(II) ion through two or three O donator atoms. Iminodiacetic acid, as used here and previously by Voloschik *et al.* [260] exhibits a lesser affinity for Be(II), coordinating with the metal ion though single N and O donator atoms. This means that when using a simple low ionic strength non-complexing eluent the column exhibits similar selectivity for Be(II) as for other alkaline earth metal ions. This was shown by Voloschik *et al.*, who, with a 7 m*M* HNO_3 eluent, obtained retention times of between 4 and 6 min for five alkaline earth metal ions (Mg(II), Ca(II), Sr(II), Ba(II), Be(II)), and retention times between 5 and 8 min when the eluent contained 2 m*M* dipicolinic acid.

In the previous chapter, we have shown how retention of the alkaline earth metals Mg(II), Ca(II), Sr(II) and Ba(II) on IDA functionalised silica gel was based upon a mixed mode retention mechanism of surface complexation and simple ion exchange [305]. It was shown how exploitation of this dual retention mechanism, through control of eluent pH and ionic strength, could result in large individual changes in selectivity for the alkaline earth metal ions, Ca(II), Mg(II), Ba(II) and Sr(II). Using differing eluent combinations of ionic strength and pH, the above metals could be baseline separated in three different retention orders. In this current study, it was once again important to understand how eluent ionic strength and pH altered selectivity for Be(II), as this would provide information on the exact mechanism responsible for retention.

It is known that of the alkaline earth metals, Be(II) is the most weakly retained when ion exchange alone is responsible for retention [42, 302-304]. Therefore, it was expected that on the IDA-silica column, retention of Be(II) would be predominantly due to surface complexation and so less affected by eluent ionic strength than the above metals.

Figure 5.1. shows a plot of *log capacity factor, k '*, against *log [eluent]* for both Be(II) and the remaining alkaline earth metals, using KNO_3 as the ionic strength modifier. The data for Be(II) was obtained using an eluent pH of 2.5. The data for the remaining alkaline earth metals was obtained using a pH of 4.2 (as Mg(II), Ca(II), Sr(II) and Ba(II) were unretained at pH 2.5, and Be(II) was completely retained at pH >3). From the graph it can be seen that Mg(II), Ca(II), Sr(II) and Ba(II) showed a greater reduction in capacity factor as a function of eluent ionic strength than Be(II), corresponding to ion exchange playing a more significant role in their retention. This is as expected, as at pH 4.2, one carboxylic acid of the iminodiacetic acid is almost fully dissociated (pK_{a1} value for IDA is approx. 2.6 [259]), therefore the stationary phase has the properties of a weak cation exchanger. However, at pH 2.5 the ion exchange capacity of the stationary phase is greatly reduced, which together with the lower affinity of Be(II) for simple cation exchangers, indicates that Be(II) is predominantly being retained through surface complexation. Comparison of the linear regression slopes for the two sets of data clearly illustrate this difference in analyte ion retention mechanism, for Mg(II), Ca(II), Sr(II) and Ba(II), slopes range from

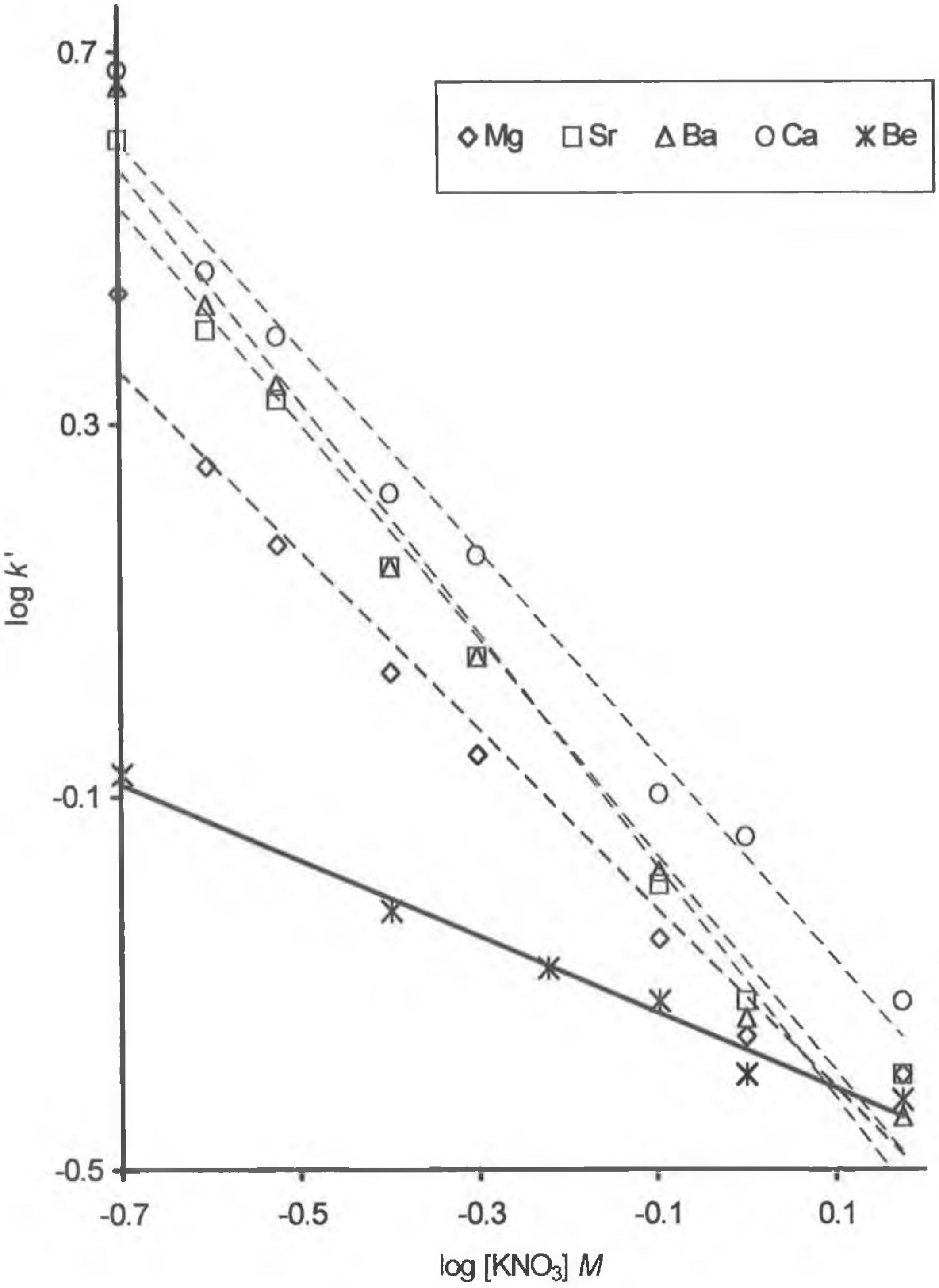


Figure 5.1. Plot of log of the capacity factor, k', against log [KNO$_3$] for Be(II) at pH 2.5 and Mg(II), Sr(II), Ba(II), Ca(II) at pH 4.2.

-1.20 to –1.38, for Be(II) the slope is only –0.37, which in practical terms corresponds to a small reduction in retention time from 4 min with a 0.4 *M* KNO_3 eluent, to 3.5 min with a 1.5 *M* KNO_3 eluent. This high selectivity for Be(II) makes the IDA-silica column ideal for the determination of the metal ion in high ionic strength samples (*see Section 5.3.3*), which is indeed one of the main advantages of chelation ion chromatography in general. Thus, for the remainder of this study, 0.4 *M* KNO_3 was used as the eluent, as this was a sufficiently high enough concentration to suppress any retention due to ion exchange for both Be(II) and other alkaline earth metals, and also resulted in an ideal retention time for Be(II) of between 4 and 5 min (pH 2.5). The effects of eluent pH and column temperature were also studied. Figure 5.2. shows the effects upon retention of Be(II) of (a) increasing eluent pH and (b) eluent temperature, with corresponding chromatograms shown in Figure 5.3(a) and (b). Both sets of results were typical of surface chelation being the dominant retention mechanism, although retention through simple ion exchange would also increase with an increase in eluent pH, due to an increase in the concentration of deprotonated carboxylate groups. An increase in eluent pH effectively results in an increase in the conditional stability constants (K'_{stab}) for the Be(II)-IDA complex. Therefore, large increases in retention over relatively small changes in pH are typical. Here the peak shape for Be(II) also showed signs of rapidly broadening as the pH was increased above 3. This is again typical of surface chelation and is thought to be due to the relatively slow kinetics of complexation compared to those of ion exchange, particularly if the stability constants for the chelate are high [235]. An additional factor to consider here is the formation of $Be(OH)^+$ ions at pH above 3, which could also play a role in peak broadening. This however was not a significant problem when using an eluent at pH < 3 and so an eluent pH of 2.5 with 0.4 *M* KNO_3 was considered optimum. Figure 5.2(b) shows the effect of temperature upon the retention of Be(II), investigated using the above eluent. As the temperature of the column was increased from 25-55 ^oC, the retention time of Be(II) also increased. This effect, which as mentioned above, is typical of complexation, has been convincingly explained by Jones and Nesterenko [234]. They stated that as the “chelate effect” was predominantly an entropy effect, surface complexation would result in a positive increase in entropy and so the change in Gibb’s free energy with temperature would be negative. If temperature was increased, the change in Gibb’s free energy would become even more negative and so the equilibrium constant for the complex being 2

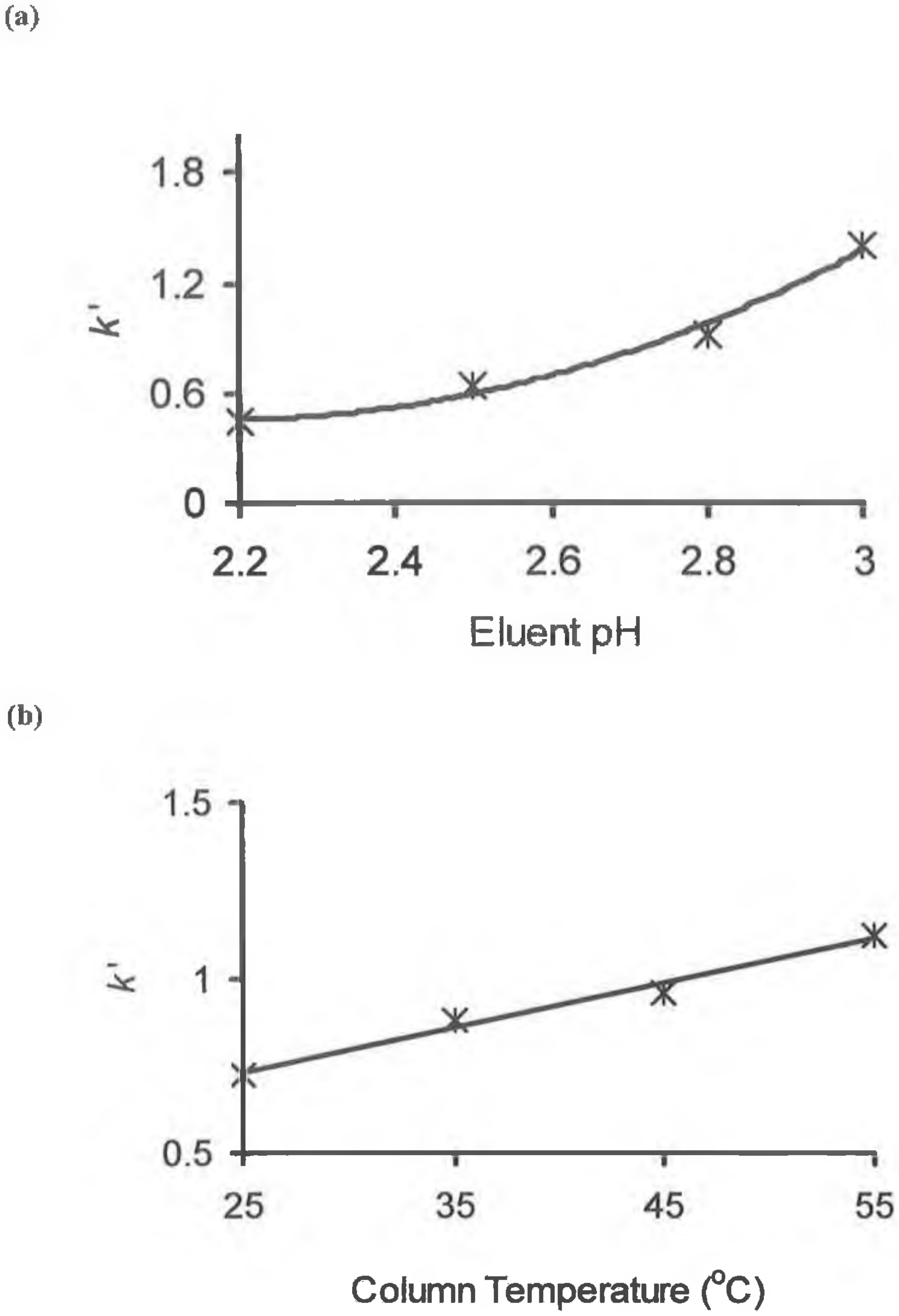
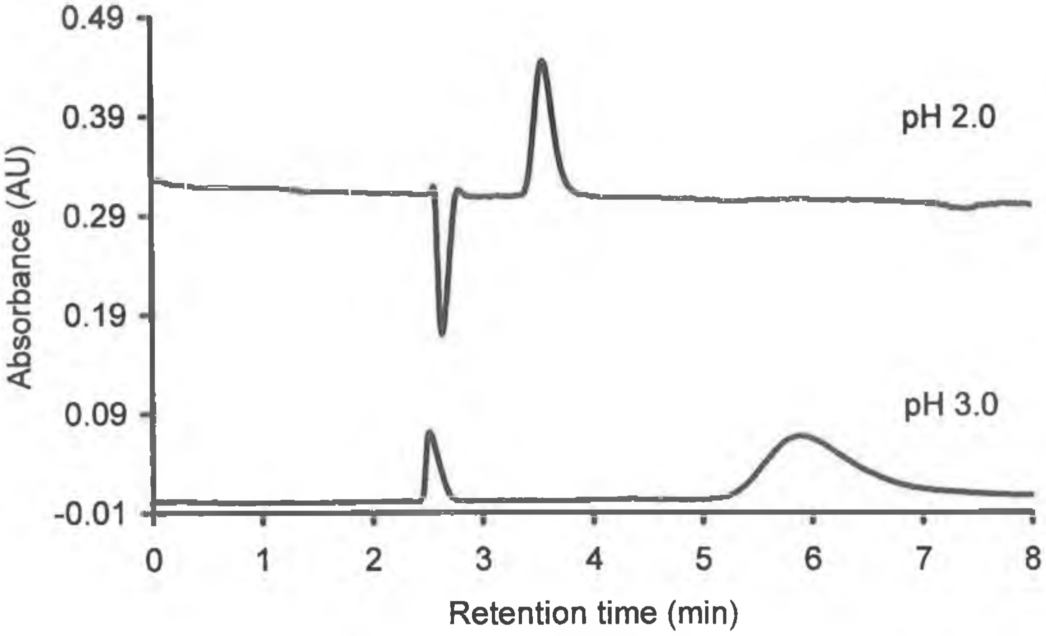


Figure 5.2. Effects upon retention of Be(II) of (a) increasing eluent pH (other conditions: 0.4 *M* KNO_3), and (b) eluent temperature (other conditions 0.4 *M* KNO_3, pH 2.5).

(a)



(b)

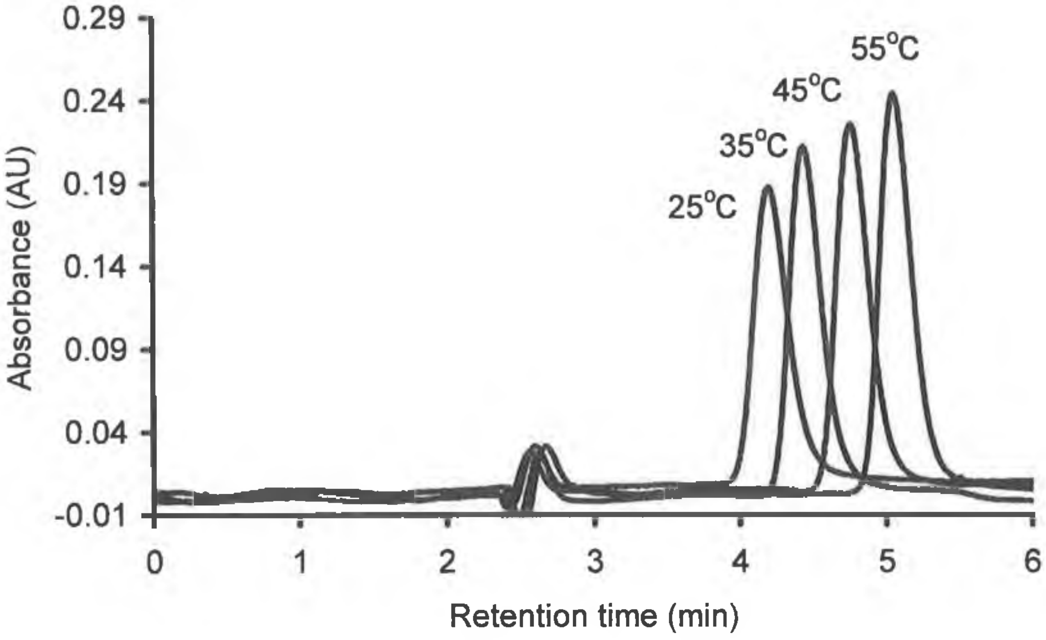


Figure 5.3. Chromatograms showing the effect of (a) increasing eluent pH (other conditions: 0.4 *M* KNO_3), and (b) eluent temperature (other conditions: 0.4 *M* KNO_3, pH 2.5).

formed would become bigger, hence an increase in retention. Jones and Nesterenko state that with this in mind, if complexation was purely responsible for retention, an increase in temperature should result in an increase in retention. Therefore, from results obtained here we can again conclude that the retention of Be(II) was likely to be predominantly due to surface chelation. However, despite the above effect, temperature did not noticeably improve peak efficiency or other method performance indicators and so for simplicity reasons was neglected for the remainder of this study.

5.3.2. Post-column reaction detection.

For improved selectivity and sensitivity in the chromatographic determination of metal ions, UV/Vis detection based on post-column reaction is often used. In their recent study, Shaw *et al.* [265] had shown CAS to be a sensitive reagent for the determination of Be(II). CAS is also a readily available and inexpensive reagent, and was therefore again employed in this work. A further advantage to using this reagent was that under the conditions detailed below, CAS did not produce a significant detector response for matrix metals such as alkali and alkaline earth metal ions, which are likely to be present in excess in most natural, industrial and potable water samples.

Optimisation of the post-column reagent conditions involved investigating the effect of CAS concentration, pH and addition of the surfactant, Triton X-100. From it's visible spectrum it was clear that the 1:1 Be(II)-CAS complex absorbed strongly over a broad region between 560 and 610 nm. Injections of a 2 mg/L Be(II) standard solution at 10 nm intervals between the above two wavelengths revealed 590 nm to be the optimum wavelength in terms of peak signal to noise ratio.

The effect of varying the CAS concentration over the range 0.065 m*M* to 0.65 m*M*, had a great influence on the peak height of Be(II) as shown in Figure 5.4. Increasing the concentration of CAS initially led to an increase in the peak height for Be(II). However, as the CAS concentration approached 0.65 m*M*, the peak height of Be(II) began to level off and the background absorbance was substantially increased, resulting in a decreased signal-to-noise ratio. From the above results 0.26 m*M* CAS was found to be most appropriate.

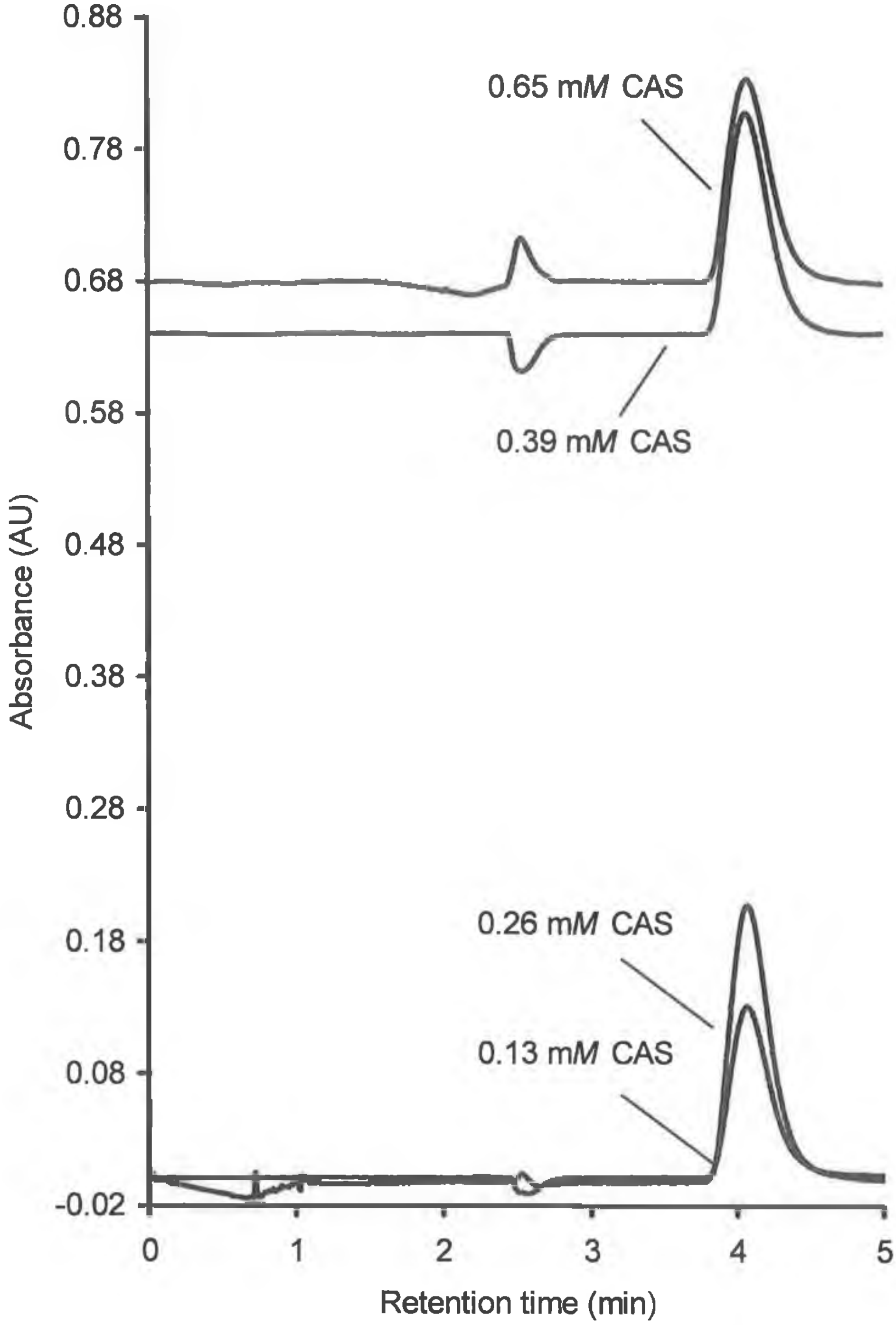


Figure 5.4. Chromatograms showing the effect of increasing chrome azurol S (CAS) in the post-column reaction system (other conditions: eluent: 0.4 *M* KNO_3, pH 2.5 and PCR: 50 m*M* MES, 2% Triton-X-100, pH 6.0).

Post-column reagent pH was investigated using repeat injections of a standard solution over the pH range of 4-7.5. On the basis of signal-to-noise ratio a pH of 6.0 was found to be most sensitive. 2-(N-Morpholino) ethanesulphonic acid (MES) at a concentration of 50 m*M* was used to buffer the post-column reagent to pH 6.0, which when combined with an eluent composed of 0.4 *M* KNO_3 at pH 2.5, resulted in a final pH of 5.9.

Finally, the effect of adding the surfactant Triton X-100 to the post-column reagent solution over the concentration range 0-4 % was investigated, with corresponding chromatograms shown in Figure 5.5. Triton X-100 has been shown previously to be an ideal surface active agent in such colour-development reactions, as it improves the solubilisation, dispersion and stabilisation of the metal complexes in aqueous media [205,306].

Increasing the % surfactant added to the post-column reagent solution over the range 0 – 4 % resulted in a steady increase in the detector response for Be(II) over the range 0 – 2 %, such that, at the 2 % concentration, the peak obtained for 0.1 mg/L Be(II) was increased by a factor of 3. However, above 2 % there was no further significant improvement in sensitivity due to an increase in background noise, and thus on the basis of highest signal-to-noise ratio, 2 % Triton X-100 was found to be the optimum concentration.

Figure 5.6(a) shows the chromatogram obtained for a 2 mg/L Be(II) standard, obtained using the above optimised conditions. As can be seen, the peak for Be(II) is both sharp and symmetrical (particularly for chelation ion chromatography, where peak shapes can be significantly broader than those obtained using simple ion exchange chromatography, even after short retention times), reflecting the relatively fast complexation/dissociation kinetics of the IDA functional group under acidic conditions.

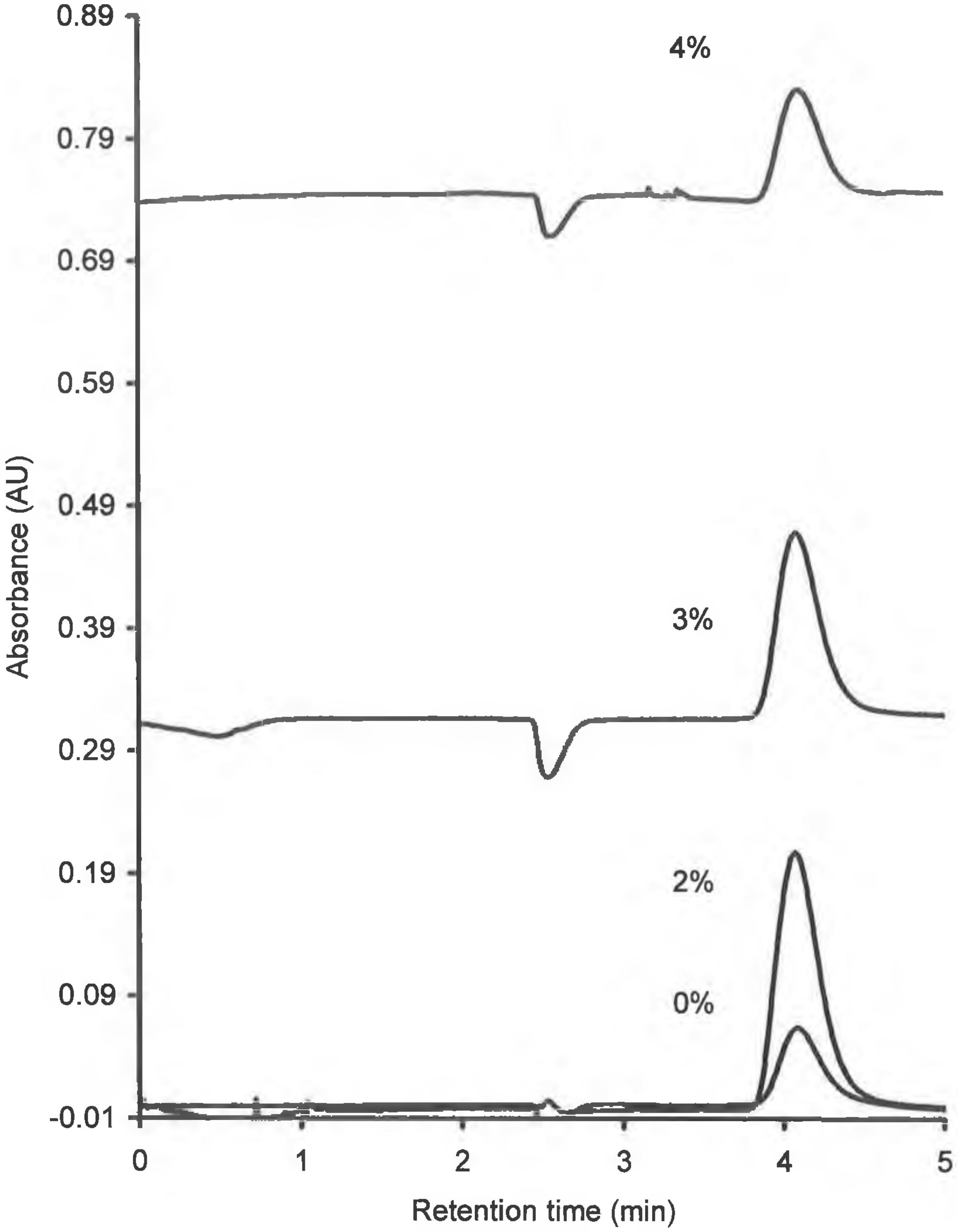


Figure 5.5. Chromatograms showing the effect of increasing Triton-X-100 in the post-column reaction system (other conditions: eluent: 0.4 *M* KNO_3, pH 2.5 and PCR: 50 m*M* MES, 0.26 m*M* CAS, pH 6.0).

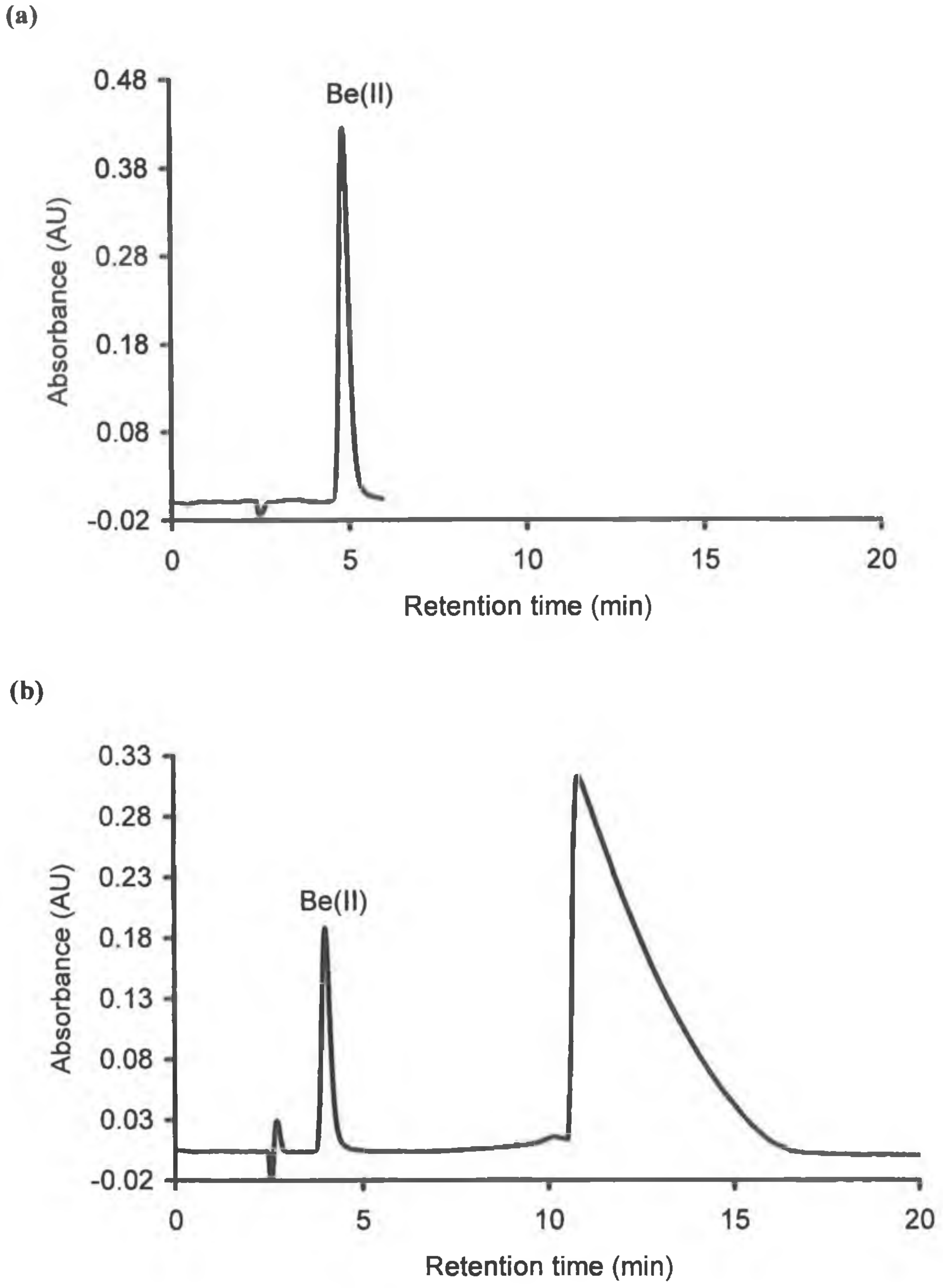


Figure 5.6. Chromatograms showing (a) the Be(II) peak for a 2 mg/L standard, and (b) the peak for 1 mg/L Be(II) in the presence of 5 mg/L Mn(II), Co(II), Zn(II), Cd(II), Ni(II), Pb(II), Cu(II), Fe(III) and Al(III). Eluent conditions: 0.4 *M* KNO_3, pH 2.5.

5.3.3. Linearity, reproducibility, detection limits and interferences.

Using the optimised conditions the system exhibited excellent linearity over three orders of magnitude. Over the standard concentration ranges 0.01 - 0.1 mg/L, 0.1 - 1.0 mg/L and 1.0 - 10 mg/L (n=5) regression coefficients of $R^2 = 0.998$, 0.999 and 0.999 were obtained respectively. Linearity was also calculated using standard addition to a number of water matrices such as tap water (0.01 - 0.05 mg/L, n=5, R^2=0.997), river water (0.1 - 1.0 mg/L, n=5, R^2=0.998) and simulated seawater (*see below*) (0.02 - 0.1 mg/L, n=5, R^2=0.996). The corresponding graphs are shown in Figures 5.7. and 5.8. Table 5.1 shows the system reproducibility and absolute detection limits obtained in various sample matrices. As can be seen from Table 5.1, reproducibility was investigated with the repeat injection of both high and low standard solutions, with % RSD's of less than 1% obtained for retention time, peak height and peak area. Absolute detection limits (250 µL inj.vol.) were also calculated for Be(II) in various sample matrices, namely, de-ionised water, tap water, wastewater and simulated seawater.

It was found that the ionic strength of the sample matrix caused a slight broadening of the peak shape for Be(II) at concentrations close to the detection limits. However, peak areas were unaffected. Figure 5.9. illustrates this effect at the 10 µg/L Be(II) level in (a) de-ionised water and (b) spiked tap water. The peak areas for the two injections were 37599 and 37389 respectively, less than 1 % difference.

As mentioned previously, both the selectivity of the stationary phase and post-column reaction detection is suited for the determination of Be(II) in the presence of excess concentrations of alkali and alkaline earth metals. To illustrate this selectivity, a simulated seawater matrix was prepared, consisting of 0.52 *M* NaCl, 1,300 mg/L Mg(II) and 400 mg/L Ca(II). The simulated sample was spiked with trace levels of Be(II) at concentrations of between 0.02 and 2 mg/L. The eluent pH was increased to 3.0 to increase the retention of Be(II) and so resolve the Be(II) peak from a large negative peak at the eluent front resulting from the non-retained matrix ions. In the resultant chromatograms Be(II) was eluted as a reasonably sharp, well resolved peak at a retention time of 5.5 min. Figure 5.9. shows the overlaid chromatograms of the

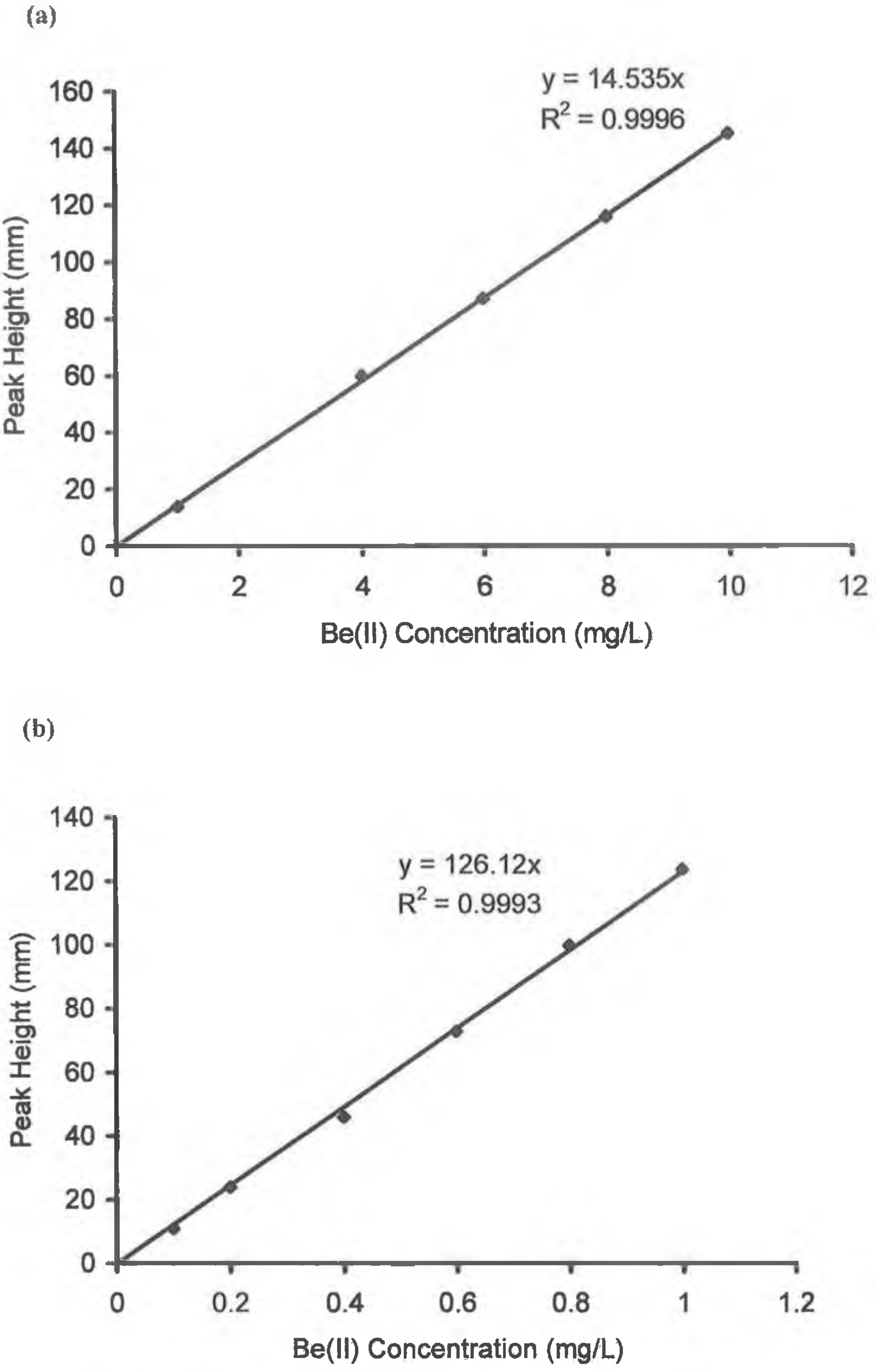
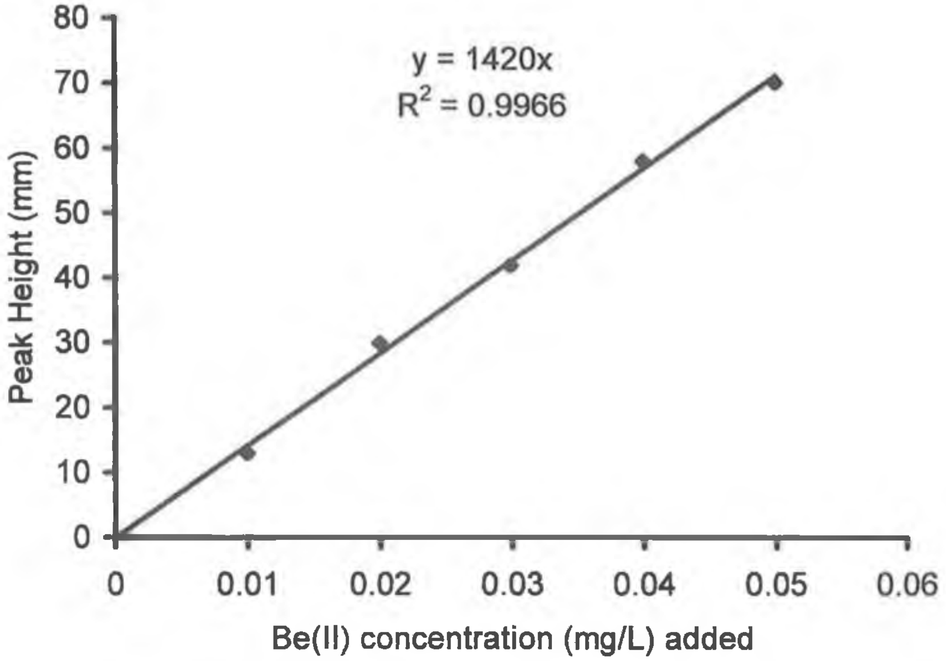


Figure 5.7. Standard calibration graphs of (a) 1 mg/L - 10 mg/L, and (b) 0.1 mg/L – 1 mg/L Be(II).

(a)



(b)

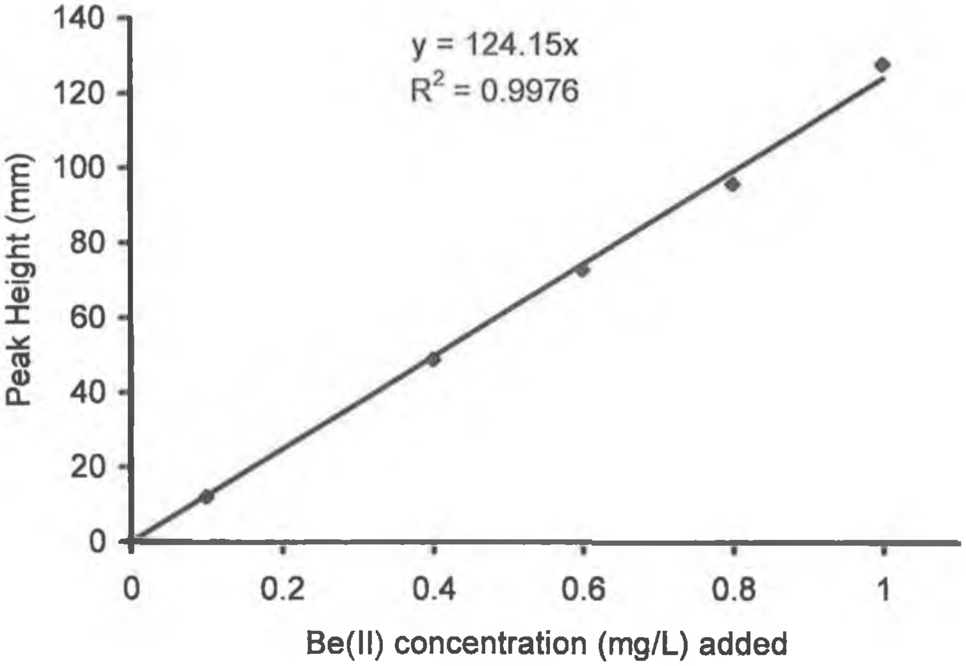


Figure 5.8. Standard addition plots of (a) tap-water, and (b) river-water.

Table 5.1. Reproducibility and detection limits.

Reproducibility % RSD (n=8)

Concentration mg/L Be(II)	Retention time	Peak height	Peak area
2.0	0.55	0.08	0.49
0.05	0.45	0.22	0.85

Sample matrix	Absolute detection limits* ng Be(II)
Milli-Q water	0.75 (± 0.1 ng)
Tap water	1.0 (± 0.1 ng)
Waste water	1.0 (± 0.1 ng)
Seawater**	2.75 (± 0.5 ng)

* Calculated as twice the peak to peak baseline noise, 250 µL inj.

** Seawater composition = 1,300 mg/L Mg(II), 400 mg/L Ca(II) and 0.5 M NaCl.

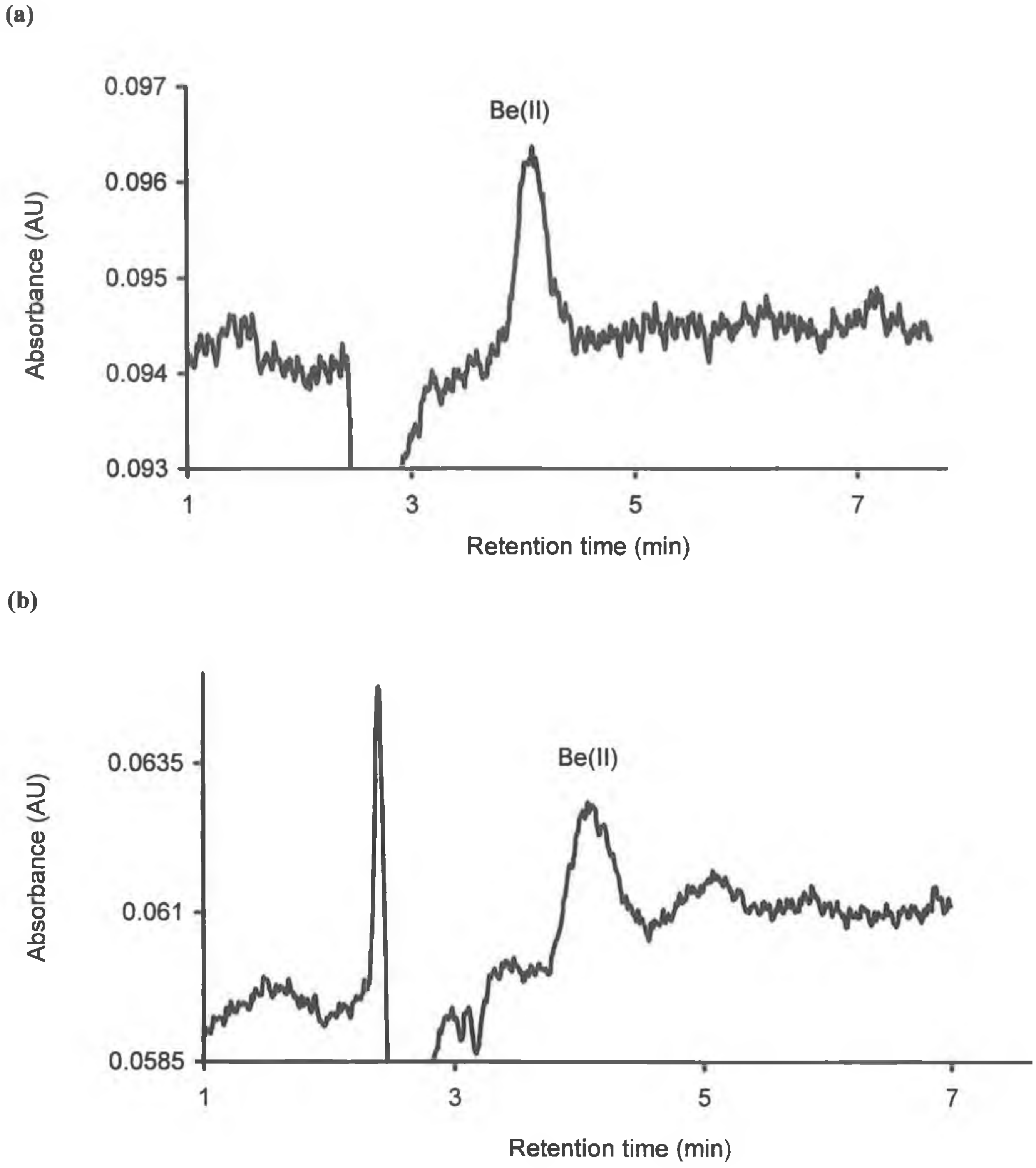


Figure 5.9. Chromatograms showing (a) 10 μg/l Be(II) standard in deionised water, and (b) 10 μg/l Be(II) spiked into tap water sample. Eluent conditions: 0.4 *M* KNO_3, pH 2.5.

simulated seawater itself and the simulated seawater spiked with 0.04 mg/L and 0.08 mg/L Be(II). Such a complex sample matrix truly represents a challenge to most analytical techniques with with ratios of Be(II) to Na, Mg(II) and Ca(II) of 1:300000, 1:32500 and 1:10000 respectively.

Selectivity as regards other transition and heavy metal ions was also investigated, as these may also be present at higher concentrations in natural and industrial waste water samples. To investigate these possible interferences, injections of a 1 mg/L Be(II) standard spiked with 5 mg/L of each of the following metal ions were carried out; Mn(II), Co(II), Zn(II), Cd(II), Ni(II), Pb(II), Cu(II), Fe(III) and Al(III). All of the above metal ions, under the eluent conditions developed above, eluted between 10 and 15 min and did not interfere with the determination of Be(II). When a combined standard was prepared containing all of the above metals at 5 mg/L and Be(II) at 1 mg/L, a single large tailed peak resulted, but this was again well resolved from the earlier eluting Be(II) peak. This chromatogram is shown next to the standard Be(II) chromatogram in Figure 5.6.

5.3.4. Analysis of a certified reference wastewater.

The developed method was applied to the determination of trace Be(II) in a certified reference freshwater sample NIST 1640. The sample contained excess concentrations of Na, Ca(II) and Mg(II) at concentrations of between 6 and 29 mg/L, as well as Sr(II), Ba(II), Cd(II), Co(II), Pb(II), Cu(II), Ni(II), Zn(II), Al(III) and Fe(III) at concentrations between 20 and 150 μg/L. The sample itself was analysed using a standard calibration technique and standard addition (n=4) to check for possible matrix effects. Analysis of the sample and standards were carried out in triplicate, using a 250 μL injection loop and an eluent pH of 2.5. The two calibration methods resulted in mean values for Be(II) of 35.3 (± 0.5) and 34.0 (± 0.5) μg/L respectively, which compared extremely well with the certified value of 34.94 ± 0.41 μg/L. Figure 5.11. shows the overlaid chromatograms of the tested reference material and the same sample spiked with 20, 40, 60 μg/L Be(II). Once again the chromatogram illustrates the totally unique selectivity the IDA column exhibits for Be(II), with the peak being

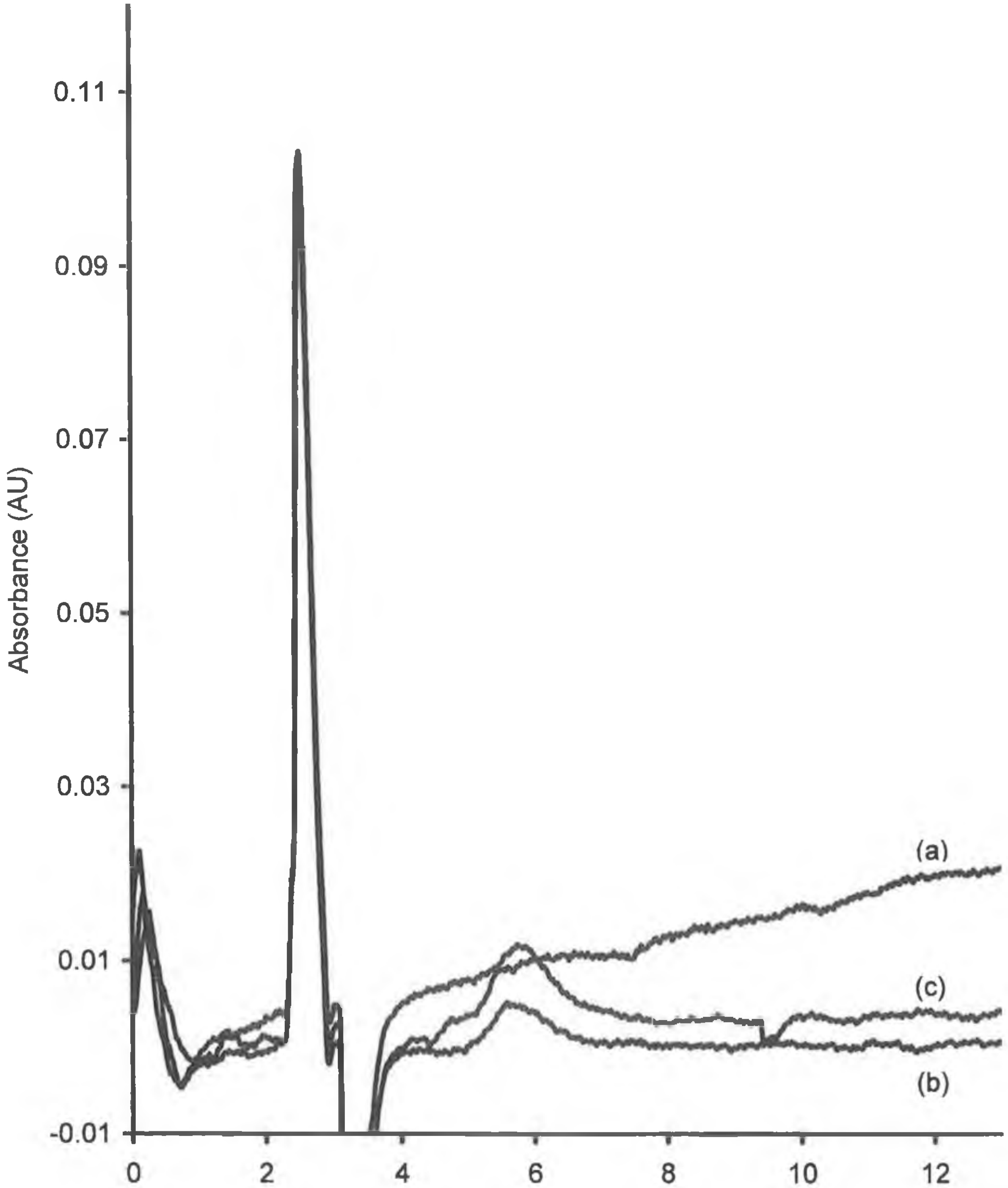
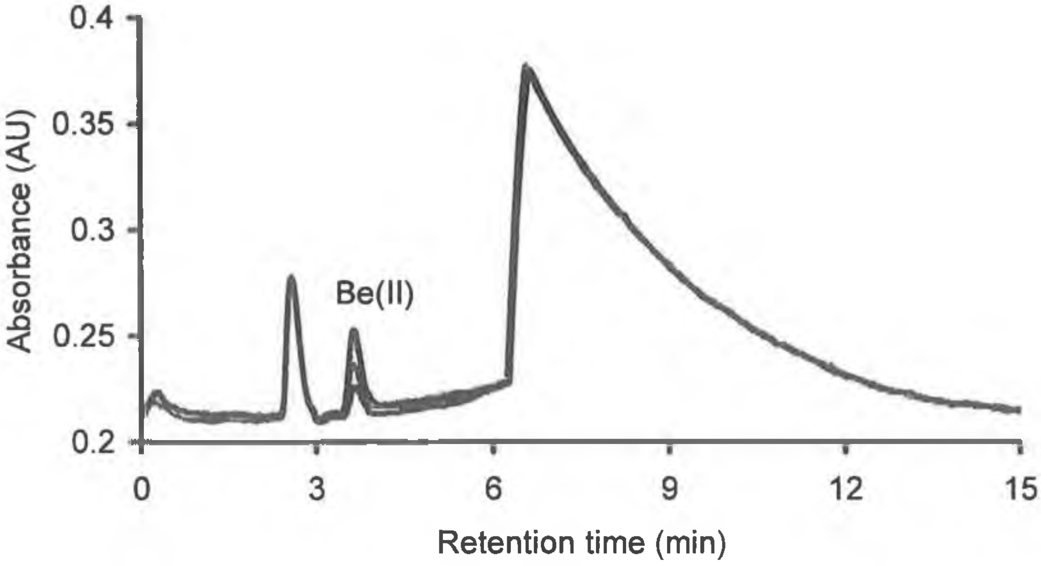


Figure 5.10. Overlaid chromatograms of (a) simulated seawater and simulated seawater spiked with (b) 0.04 mg/L and (c) 0.08 mg/L Be(II). Eluent conditions: 0.4 *M* KNO_3, pH 3.0.

(a)



(b)

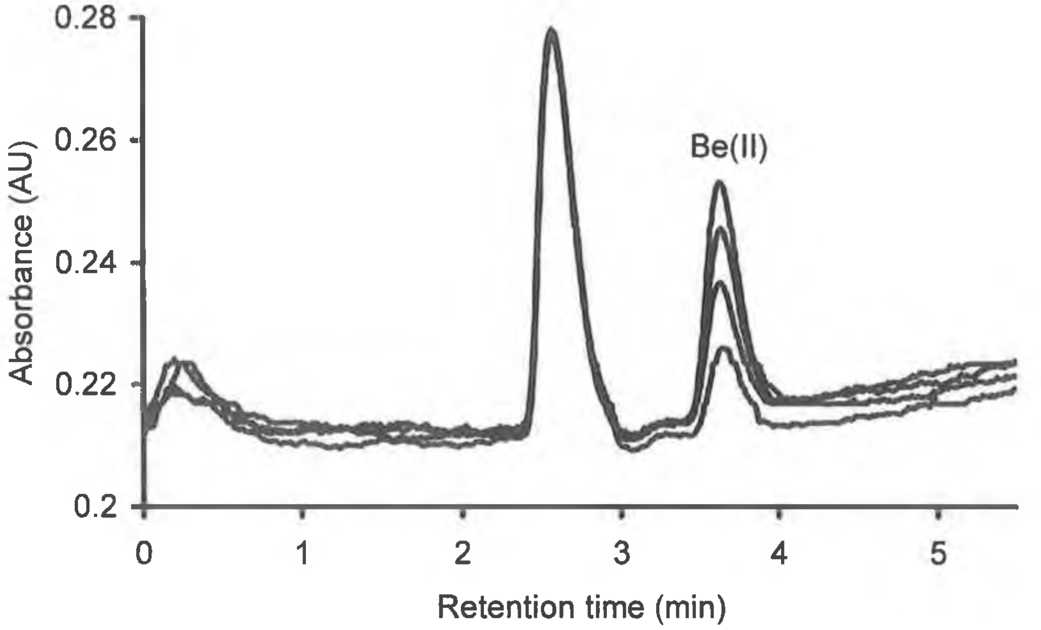


Figure 5.11. Chromatograms of (a) Certified Reference Material NIST 1640 and the same sample spiked with 20, 40, 60 μg/L Be(II). (b) Close up of Be(II) peaks. Eluent conditions: 0.4 *M* KNO_3, pH 2.5.

well resolved from the other unretained alkaline earth metals and also the large combined peak for the common transition and trivalent metal ions.

5.4. Conclusions.

A sensitive and selective chromatographic method for the determination of Be(II) in differing water samples has been developed, that represents a simple alternative to complex atomic spectroscopic methods. The method requires only simple chromatographic instrumentation but is both linear over several orders of magnitude and highly reproducible. The selectivity obtained from the IDA-silica column is such that Be(II) can be detected at low µg/L concentrations in samples containing excess levels of common matrix metal ions without interference. In addition, the fact that retention is predominantly obtained through stationary phase complexation and not ion exchange means sample ionic strength has only little effect upon the chromatography of Be(II), allowing samples containing up to 0.5 *M* NaCl to be injected directly.

Chapter 6.

Ionic Strength, pH and Temperature Effects Upon Selectivity for Transition and Heavy Metal Ions When Using Chelation Ion Chromatography With an Iminodiacetic Acid Bonded Silica Gel Column and Simple Inorganic Eluents.

6.0. Introduction

The chelation ion chromatography (CIC) of metal ions using high-performance grade chelating stationary phases is a specialised ion chromatographic technique that offers alternative selectivities to those achievable when using simple sulphonated or carboxylated ion exchange stationary phases. As metal ion retention is based upon the relative conditional stability constants of each metal and the immobilised chelating ligand, retention and selectivity is predominantly controlled through eluent pH, although, as in simple ion exchange chromatography, a range of eluent parameters can be used to obtain desired changes in selectivity. In addition to this, CIC has the added advantage of there being a large and varied number of chelating ligands available to functionalise the stationary phase, each offering unique metal ion selectivity. Two approaches to the production of chelating stationary phases have been investigated in recent years, namely, (a) permanently or dynamically coating polymeric and silica based reversed-phase phases with hydrophobic chelating ligands, and (b) co-valently bonding chelating groups to either polymeric resins or silica gels. A number of review and comparative articles on the principles, various modes and applications of CIC have been compiled which discuss in detail the above approaches and highlight the versatility of this ion chromatographic technique [235-237, 247, 253].

Of all the studies carried out to-date on the CIC of metal ions (with the exception of some recent work using aminophosphonic acid functionalised silica [264]), those using stationary phases functionalised with carboxylic acid containing ligands have proven to be the most successful in terms of selectivity, efficiency and number of applications. This would appear to be the case whether the stationary phase contained co-valently bonded chelating groups [258, 261, 263, 305, 307], was permanently coated with chelating ligands [241, 243], or dynamically coated using an eluent containing such a ligand [251, 253]. Results from the above studies have shown one ligand, namely iminodiacetic acid, to be particularly suitable for this specific mode of ion chromatography, due mainly to the following two factors; Firstly, stability constants for alkaline earth metal ions and common transition/heavy metal ions with the IDA ligand are not prohibatively high as to require excessively strong eluents. Secondly, the complexation/dissociation kinetics of the IDA-metal ion complex seem to be sufficiently rapid to produce separation efficiencies comparable with simple ion exchange.

Several studies have been carried out using iminodiacetic acid bonded silica gel stationary phases for the separation of metal ions. These include the separation of alkali and alkaline earth metals [260, 281, 282, 305, 307], lanthanide series metal ions [263] and transition and heavy metal ions [247, 258, 259, 261]. However, the separation of the latter group of metal ions using IDA-silica has received little attention when compared with the number of studies carried out with this group of metal ions on simple ion-exchange stationary phases. Bonn *et al.* [258] were amongst the first to investigate the use of IDA-silica for the separation of alkaline earth and transition metal ions. Using organic chelating eluents such as citric, tartaric and dipicolinic acid the study showed how under acidic conditions a separation of Mg(II), Fe(II), Co(II), Cd(II) and Zn(II) could be achieved in just under 30 min. A separation of Co(II), Cd(II) and Zn(II) spiked into a seawater matrix was also shown, although due to the strong complexing nature of the eluent and the use of conductivity detection, sensitivity was poor. Later work by Elefterov *et al.* [259] also employed dipicolinic acid as an eluent with an IDA-silica column, this time using direct UV absorbance of the eluting metal-ligand complexes for detection. A separation of Zn(II), Pb(II), Co(II) and Cd(II) was shown, although once again sensitivity was relatively poor with detection limits ranging from 0.4 to 1.2 mg/L. Nesterenko and Jones [247, 261] further investigated the use of various complexing eluents for both the isocratic and gradient separations of alkaline earth and transition metal ions. Using a three step gradient, beginning with a KCl/HNO_3 eluent, followed by tartaric acid and finally picolinic acid, the separation of Mg(II), Ca(II), Mn(II), Cd(II), Co(II), Zn(II), Ni(II) and Cu(II) was obtained, although the overall run time was excessively long at just under 50 min.

6.1. Aims of this study.

The following study describes a more detailed study into the retention behaviour of common transition metal ions on IDA-silica using simple inorganic salts as eluents. The omission of chelating ligands such as tartaric and picolinic from the eluents used in this study allowed the true selectivity of the IDA-silica gel to be observed. Optimum conditions for the isocratic separation of Mg(II), Ca(II), Mn(II), Cd(II), Co(II) and Zn(II) were investigated, together with a pH step gradient method which allowed the separation of Mn(II), Cd(II), Co(II), Zn(II) and Pb(II) in under 10 min. Due to the non-chelating nature of the eluent, sensitive post-column reaction detection with 4-(2-pyridylazo) resorcinol (PAR) was possible, resulting in detection limits for of between 4 and 10 µg/L for the above transition metal ions in a reference freshwater sample.

6.2. Experimental.

6.2.1. Instrumentation.

A Dionex Model GPM2 Gradient Pump Module (Sunnyvale, CA, USA) was used to deliver the eluent (1.5 mL/min). A manual sample injection valve, model 7125, (Rheodyne, Cotati, CA, USA), fitted with a 250 µL injection loop was used for introduction of the samples. The IDA-silica packed analytical column (8 µm particle size, 130 Å pore size, 250 x 4.0 mm i.d) used was supplied by BioChemMack (Moscow, Russia). A pressure driven Dionex Reagent Delivery Module (RDM) was used for introduction of the post column reagent (1.5 mL/min), which was mixed at room temperature with the eluent using a 0.5 m PEEK reaction coil (0.01" i.d.). A model SPD-6AV Shimadzu UV/Vis detector (Kyoto, Japan) was used at 495 nm to monitor the resultant chromatograms. These were recorded using Dionex AI-450 chromatographic software. For the determination of column outlet pH, the flow from the RDM was turned off and discrete samples taken from the detector outlet every minute. Each sample pH was then measured using a standard laboratory pH meter.

6.2.2. Reagents.

The eluent and post column reagent (PCR) were prepared using deionised water from a Millipore Milli-Q water purification system (Bedford, MA, USA.). The PCR ligand, 4-(2-pyridylazo) resorcinol (PAR), and sodium hydroxide were purchased from Sigma-Aldrich (Gillingham, Dorset, UK) and used without further purification. Potassium nitrate was obtained from Merck (Darmstadt, Germany). Sodium nitrate was purchased from Riedel-De-Haen Ag (Sleeze-Hannover, Germany) and sodium chloride and potassium chloride were obtained from Fluka Chemie (Buchs, Switzerland). Final eluent conditions for the isocratic analysis of samples were 0.065 M KNO_3-0.035 M KCl adjusted to pH 2.5 using dilute HNO_3. The post-column reagent solution was 0.5 M Ammonia, 0.4 mM PAR, adjusted to pH 10.5. The pH of the NIST reference standard material was increased to pH 2.5 using measured quantities of dilute NaOH prior to injection. All solutions prepared were filtered through a 0.45 µm filter and degassed using sonication. Low level standard solutions were generally prepared freshly each day from stock solutions (1000 mg/L) stored in 1% nitric acid.

6.3. Results and discussion.

6.3.1. Stationary phase selectivity

In previous studies involving IDA-silica stationary phases it has been shown that considerable retention of alkali and alkaline earth metal ions can be achieved through simple ion-exchange interactions with the dissociated carboxylic acid groups of the IDA ligand ($pK_{a1} = 1.76$, $pK_{a2} = 2.70$ [282]). For the alkaline earth metal ions this ion-exchange interaction is the predominant means of retention between pH's 2 and 5, as complexation under such acid conditions has been calculated previously to be insignificant [258]. However, within the above pH range any ion-exchange interactions can be minimised through the use of high ionic strength eluents. In this work the retention behaviour alkaline earth and transition metal ions was firstly evaluated with simple 0.5 and 1.0 M KNO_3, $NaNO_3$, KCl and NaCl eluents. The pH was controlled with the addition of HNO_3 between the investigated pH ranges of 2 to 3. Under all conditions studied Cu(II) and Fe(III) were completed retained on the column. As expected Ca(II) and Mg(II) were unretained under the above conditions as complexation was not significant and ion-exchange interactions were suppressed. The retention behaviour observed for Mn(II), Cd(II), Co(II), Zn(II) and Pb(II) is shown in Figure 6.1. It was clear from the results obtained that there was very little difference in selectivity when using either the sodium or potassium form of either the nitrate (Figure 6.1(a) and Figure 6.1 (b)) or chloride salt (Figure 6.1(c) and Figure. 6.1(e)). However, there were significant differences between the selectivity shown with the nitrate eluent and that of the chloride (e.g. Figure 6.1(a) and Figure 6.1(c)). With the nitrate eluent the retention of Pb(II) rapidly increased above pH 2.3, such that it was completely retained (>100 min) at pH 2.6. With the chloride eluents the retention of Pb(II) was significantly reduced. This was also noticeable with Cd(II), which co-eluted with Co(II) when using a nitrate eluent, irrespective of eluent pH, and which co-eluted with the slightly retained peak for Mn(II) when using the chloride eluents. The above observation was as expected when considering the stronger tendency of chloride ions to form chloro- complexes with both Pb(II) and Cd(II) relative to the remaining metal ions ($K_{STAB1(ZnL)} = 0.11$, $K_{STAB1(PbL)} = 1.18$, $K_{STAB1(CdL)} = 1.38$, medium = 1 *M* $NaClO_4$, 25 °C [308]). Increasing the concentration of the chloride eluent from 0.5 to 1.0 *M* resulted in a further decrease in the retention of Pb(II) in relation to Zn(II), Co(II) and Mn(II), such that Pb(II) and Zn(II) co-eluted at pH < 2.3. As Zn(II) and Co(II) showed little decrease in retention when the concentration of the eluent was increased, it can be assumed that retention for these metal ions by ion exchange interactions (under these conditions) was not significant. Using the 1.0 *M* chloride eluent both Mn(II) and Cd(II) were unretained at pH< 2.5.

Figures 6.2(a) and 6.2(b) show chromatograms resulting from the injection of a 1 mg/L mixture of Mn(II), Cd(II), Co(II), Zn(II) and Pb(II) (5 mg/L) when using a 0.5 *M* KNO_3 eluent (Figure 6.2(a)) and a 0.5 *M* NaCl eluent (Figure 6.2(b)), both adjusted to pH 2.0 with HNO_3. As can be seen from the two chromatograms shown, the isocratic separation of all five metals was not possible, with either Cd(II) and Co(II), or Cd(II) and Mn(II) co-eluting. However, the above conditions resulted in excellent selectivity for Pb(II) which was well resolved from all the other metal ions injected and eluted as a reasonably sharp

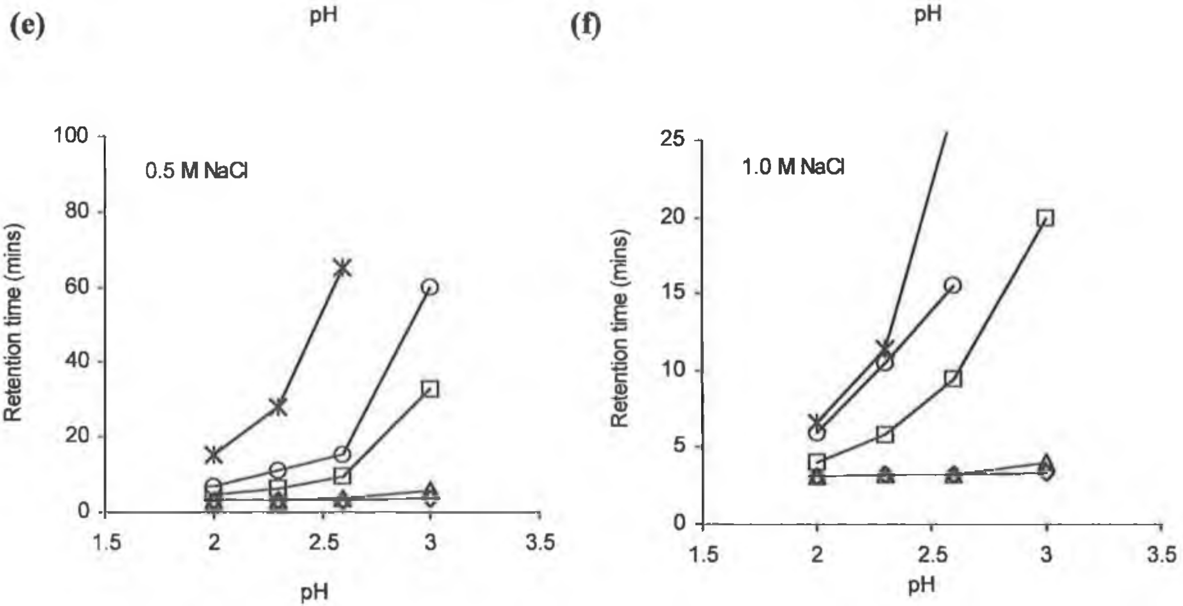
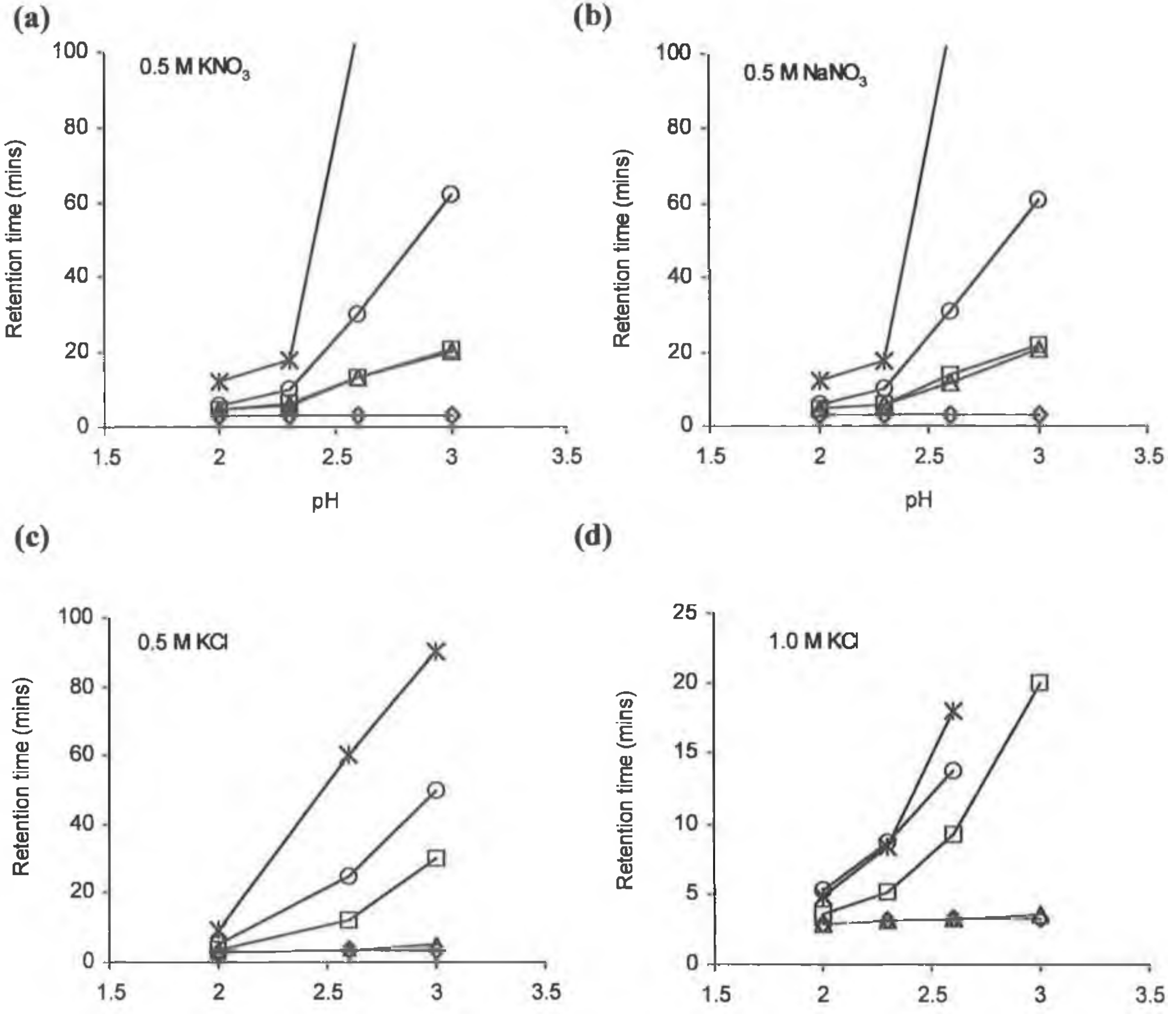


Figure 6.1. Effect of pH upon retention of Mn(II) ◇, Cd(II) △, Co(II) □, Zn(II) ○ and Pb(II) ∗ in 0.5 and 1.0 *M* KNO_3, $NaNO_3$, KCl and NaCl eluents.


(a)
0.5 M KNO$_3$
Retention time (mins)
0
20
40
60
80
100
1.5
2
2.5
3
3.5
pH
(b)
0.5 M NaNO$_3$
Retention time (mins)
0
20
40
60
80
100
1.5
2
2.5
3
3.5
pH
(c)
0.5 M KCl
Retention time (mins)
0
20
40
60
80
100
1.5
2
2.5
3
3.5
(d)
1.0 M KCl
Retention time (mins)
0
5
10
15
20
25
1.5
2
2.5
3
3.5

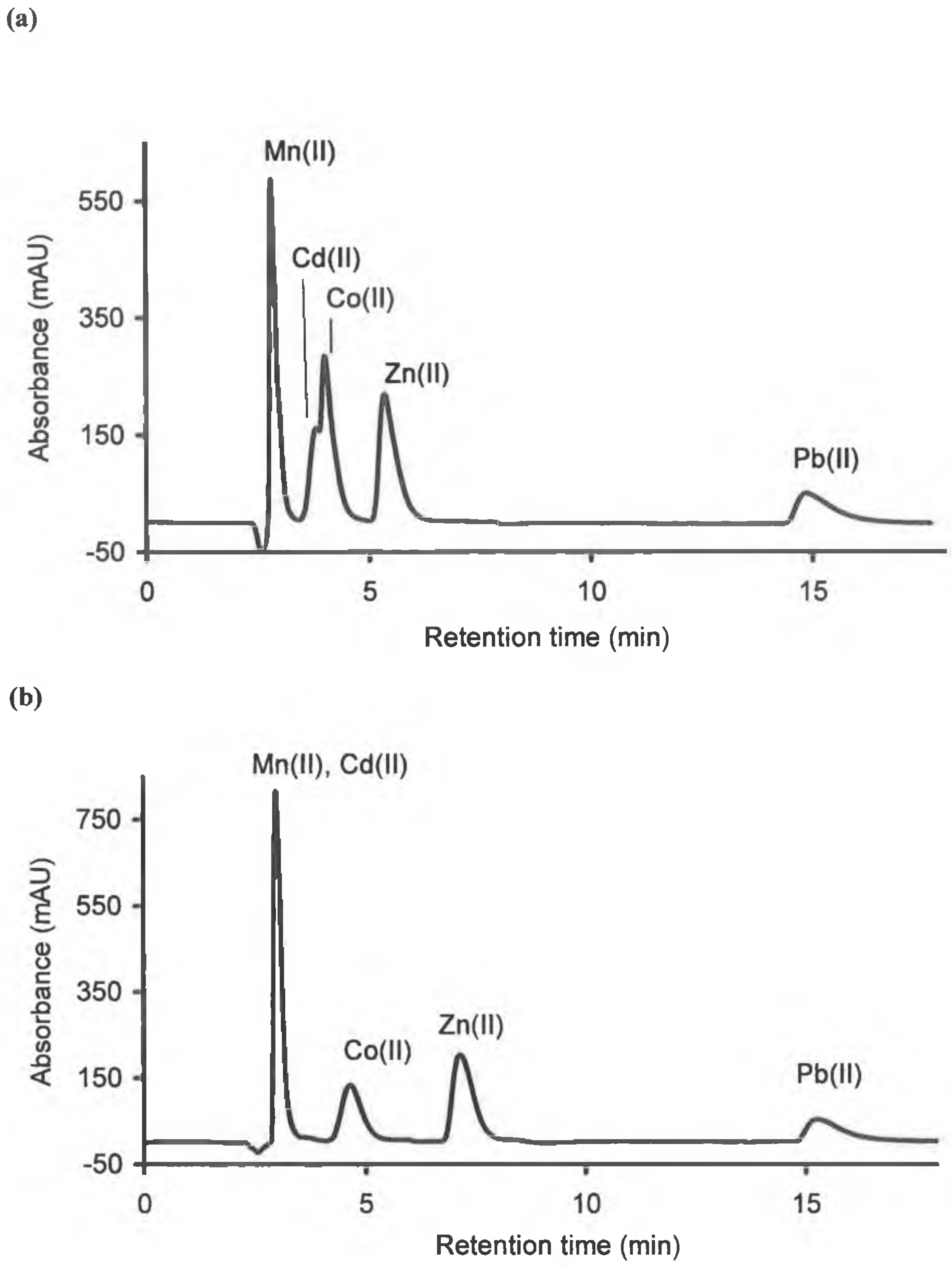


Figure 6.2. Chromatogram showing (a) the separation of Mn(II), Cd(II), Co(II), Zn(II) and Pb(II), using a 0.5 *M* KNO_3 eluent (pH 2.0), and (b) the separation of the same metal ions using a 0.5 *M* NaCl eluent (pH 2.0) Standard conc. = 1 mg/L for Mn(II), Cd(II), Co(II) and Zn(II), 5 mg/L for Pb(II).

peak giving a sensitive detector response. By further increasing the pH of the chloride eluents to 2.6 - 3.0, Mn(II) and Cd(II) could be separated but eluted too close to the void to be of use in real samples. In addition, the retention of Zn(II) and Pb(II) was too great (>40 min) at the increased pH.

As mentioned above, reducing the concentration of the eluent below 0.5 *M* would result in the unwanted retention through ion-exchange of alkaline earth metals, and also the excess retention of Pb(II) (unless very acidic conditions were used, pH < 2.0). To investigate this a 0.1 *M* KCl eluent (pH 2.0) was prepared and retention times for above transition metal ions and Ca(II) and Mg(II) were determined. It was found that under these conditions Ca(II) and Mg(II) were indeed beginning to exhibit retention and that Mn(II) partially co-eluted with Ca(II). Increasing the pH to 2.6 resulted in the improved resolution of Ca(II) and Mn(II), but also increased the retention of Zn(II) to >25 minutes. In addition the peak for Cd(II) eluted closely to that of Ca(II) and Mn(II) when using the chloride eluent, which would interfere with the determination of Cd(II) in real samples where Ca(II) was present in excess. To remedy this the concentration of KCl in the eluent was reduced to 0.035 *M* and replaced with KNO_3 at 0.065 *M*, keeping the overall concentration of the eluent at 0.1 *M*. This resulted in an increase in the retention of Cd(II) relative to the remaining metal ions, meaning it now eluted free from possible interferences from excess alkaline earth metals. A slight reduction in eluent pH to 2.5 resulted in the optimum isocratic separation of the above alkaline earth and transition metals as shown in Figure 6.3.

6.3.2. Temperature effects.

In previous work with IDA-silica stationary phases the effect of temperature upon the retention of alkali and alkaline earth metal ions [282, 307] and the lanthanide series metals [263] has been studied and explained. It is generally agreed that temperature can be used to improve selectivity depending upon the nature of the dominant retention mechanism acting upon the analyte ions. For example, alkali and alkaline earth metal ions are predominantly retained through simple ion-exchange interactions with both carboxylate groups and to a lesser extent uncapped surface silanol groups. It would therefore be expected that the retention of these metals would be affected differently by a change in column temperature than transition metal ions, which under the conditions used in this work, were likely to be exhibiting retention due to a combination of ion-exchange and complexation, with the latter being dominant. It has been shown previously that where the main retention mechanism is complexation, an increase in column temperature results in a clear increase in analyte retention [251, 263, 305]. Therefore, the effect of column temperature upon the retention of both alkaline earth metals (Ca(II) and Mg(II)) and transition metal ions (Mn(II), Cd(II), Co(II) and Zn(II)) was investigated. The temperature of the column was varied from 15 to 50 ^{o}C (other conditions as in Figure 6.3), with the results shown as Figure 6.4. As can be seen from Figure 6.4, there appears to be a clear correlation between the degree of retention (and thus complexation) and the effect of temperature. Both Zn(II) and Co(II), which were quite strongly retained by the IDA-silica column, exhibited similarly rapid increases in retention as the column temperature was increased. With Cd(II) and Mn(II) the effect of temperature was less pronounced, although a clear increase in retention was observed (in the case of Cd(II), it may be expected that the affect of temperature would have been more significant if a non-chloride containing eluent were used). With the alkaline earth metal ions, Mg(II) was totally unaffected and Ca(II) actually showed a slight decrease in retention. These results indicated that even though Mn(II) eluted close to both Ca(II) and Mg(II) the mode of retention was different, in that unlike Mg(II) and Ca(II), Mn(II) showed retention behaviour which suggested that under the conditions used complexation was indeed playing a significant role in retention.

To illustrate how the above difference in behaviour could be exploited when analysing a real sample, a certified reference freshwater sample (NIST 1640) was obtained which contained trace amounts of Mn(II) (0.121 μg/L) and excess Mg(II) and Ca(II) (5.82 and 7.05 mg/L respectively). The sample was injected over the temperature range shown in Figure 6.4 and the resultant chromatograms (at 15, 20, 25 and 50 ^{o}C) are show as Figure 6.5 and 6.6. As can be seen, by using an elevated column temperature, Mn(II) can be readily resolved and thus quantified in a real sample containing excess Ca(II) and Mg(II).

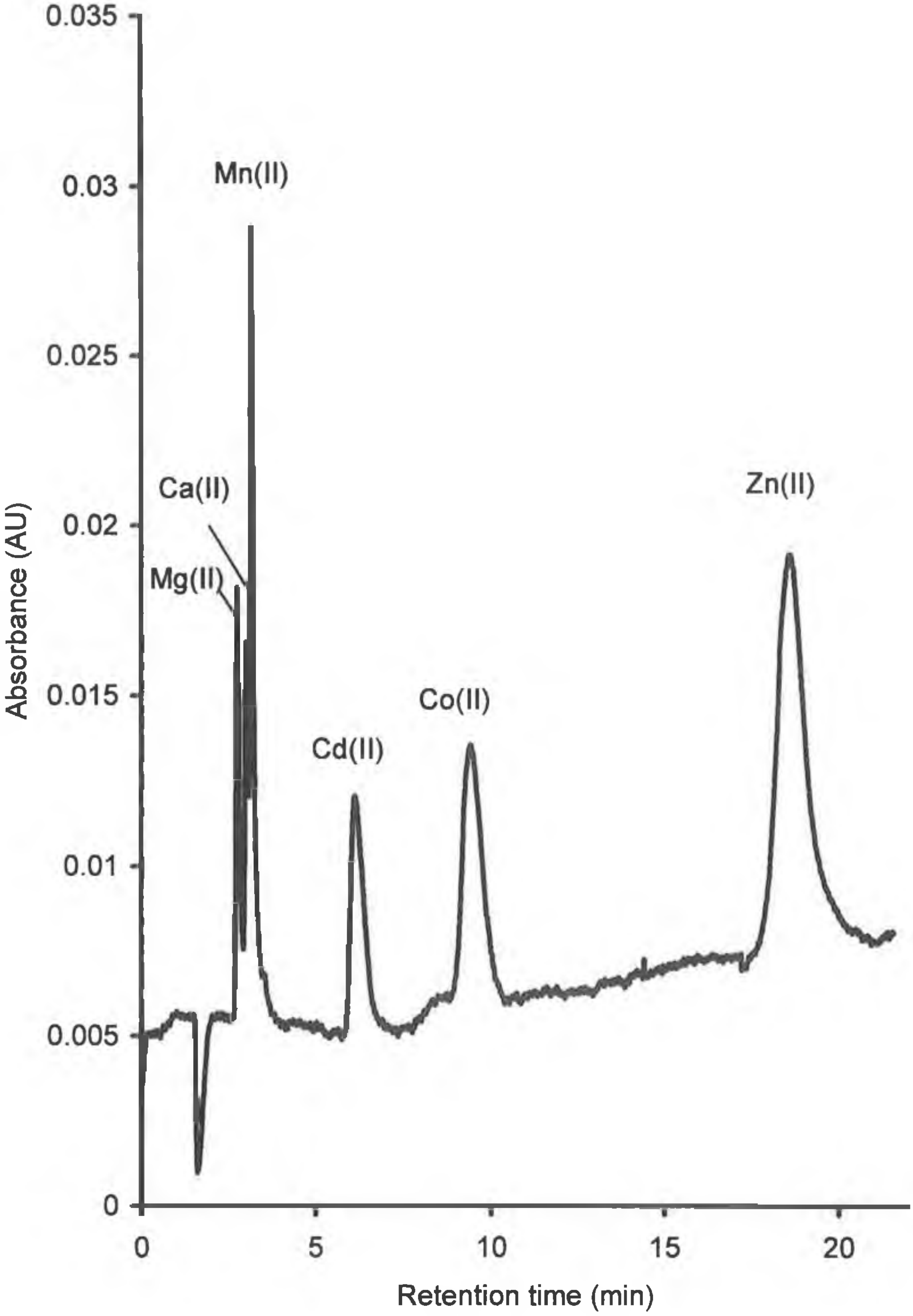


Figure 6.3. Chromatogram showing the optimised separation of Mg(II), Ca(II), Mn(II),Cd(II), Co(II) and Zn(II). Eluent conditions: 0.035 *M* KCl, 0.065 *M* KNO_3 (pH 2.5). Standard conc. = 0.3 mg/L for Mg(II) and Ca(II), 0.05 mg/L for Cd(II), Co(II) and Zn(II) and 0.02 mg/L Mn(II).

6.3.3. Gradient elution.

From the above results it was clear that both the use of lower ionic strength eluents and elevated column temperatures led to long retention times for ions such as Zn(II) and more importantly Pb(II). When using any eluent which resulted in the resolution of Mn(II) and Cd(II) (either as in Figure 6.3 or simply 0.1 *M* KCl at pH 2.6-3.0), the retention of Zn(II) was greater than desired and Pb(II) was totally retained. Therefore, in order to obtain the resolution of Mn(II) and Cd(II) from each other and from the void, and also to decrease the retention time of Zn(II) and Pb(II), the investigation of concentration and pH gradients was the next logical step. Initially a simple concentration gradient was investigated rapidly increasing from 0.1 *M* to 1.0 *M* NaCl (pH 2.3) following the elution of Co(II) at 10 minutes, to speed the elution of Zn(II) and Pb(II). However, as shown previously, when using 1.0 *M* NaCl under isocratic conditions, Pb(II) and Zn(II) were retained for over 10 minutes. Therefore, the above gradient was not strong enough, even at 1.0 *M* NaCl, to facilitate the fast elution of Zn(II) and Pb(II) from the column, and this resulted in Zn(II) eluting after 18 minutes and Pb(II) eluting as a broad peak after approximately 35 minutes. It was therefore necessary to investigate a pH gradient to obtain the desired separation of all five metal ions. As in previous studies using chelation ion chromatography [241,244,261], a pH step gradient was used to attempt the rapid elution of Zn(II) and Pb(II). To achieve this, a 0.1 *M* NaCl eluent (pH 2.6) was switched to 0.1 *M* NaCl (pH 1.6) following the elution of Mn(II), Cd(II) and Co(II) (10 min). At pH 1.6 under isocratic conditions both Zn(II) and Pb(II) were only marginally retained, and so when using the above gradient both metals should have been rapidly eluted from the column as the lower pH eluent moved through the system. However, it was found that the step decrease in eluent pH did not result in the rapid elution of Zn(II) and Pb(II) from the column, which instead eluted from the column after > 25 min. It was clear from this result that the IDA itself was buffering the sudden pH change within the column and that for a pH gradient to be successful the buffering capacity of the column would have to determined and overcome.

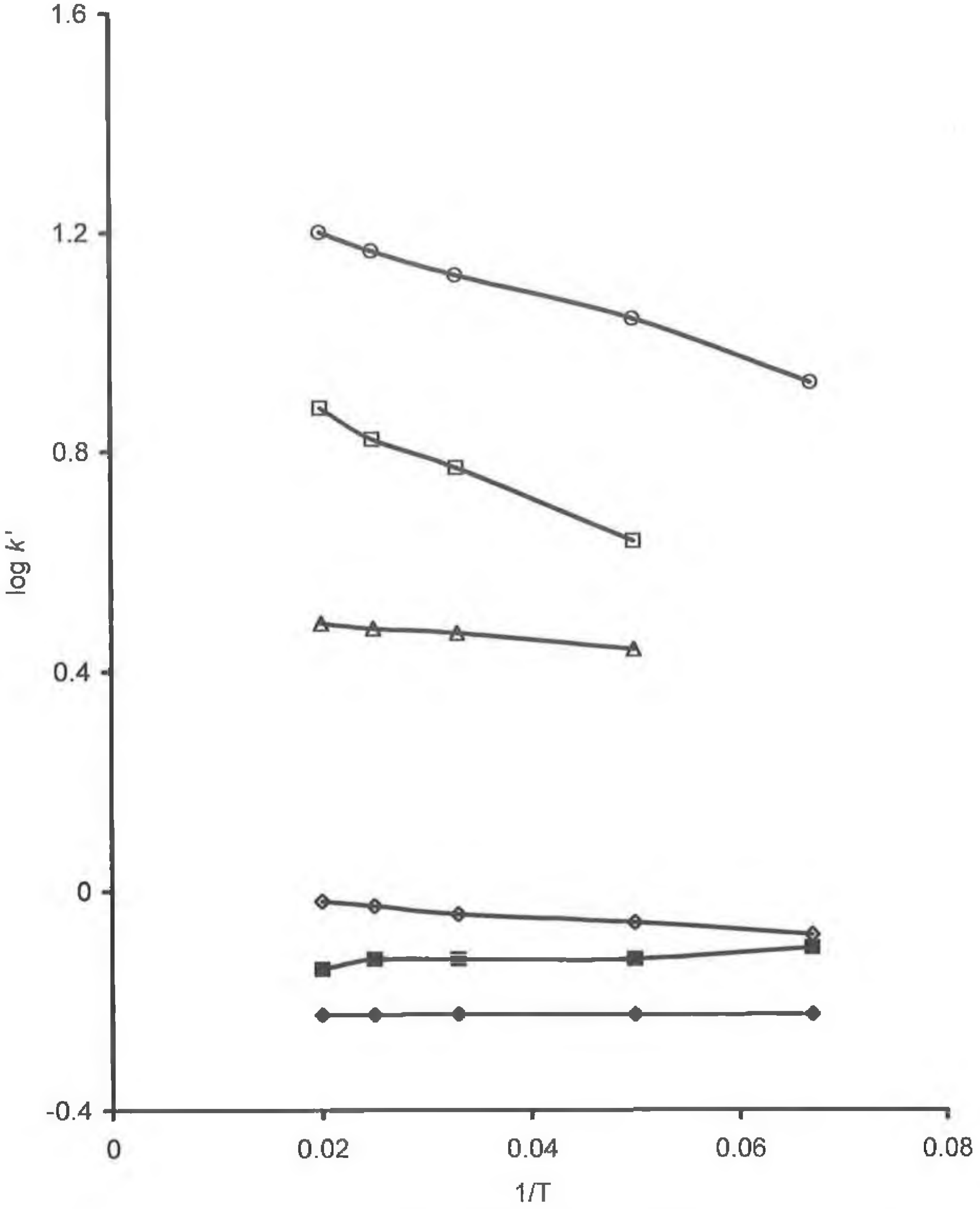
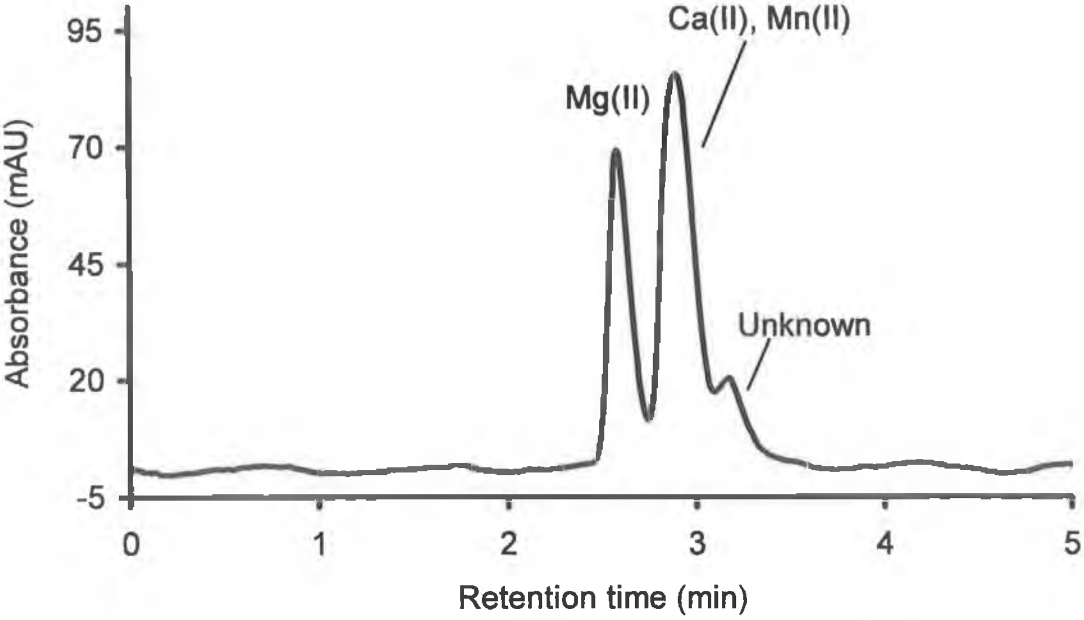


Figure 6.4. Effect of temperature upon retention of alkaline earth and transition metal ions. Ca(II) ■, Mg(II) ◆, Mn(II) ◇, Cd(II) △, Co(II) □, Zn(II) ○. Other eluent conditions as in Figure 6.3.

(a)



(b)

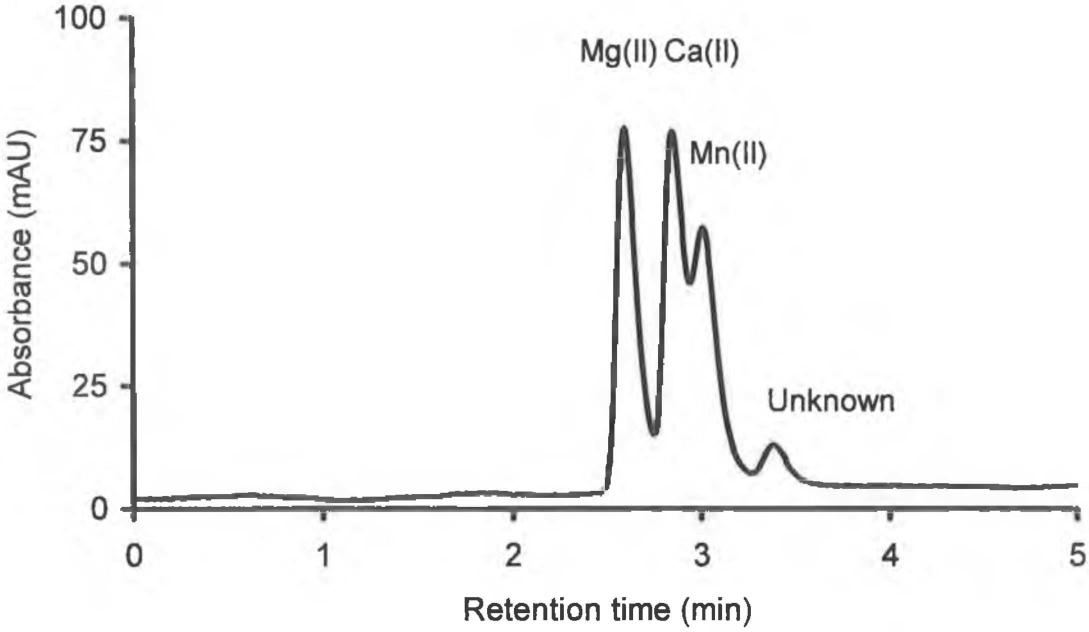
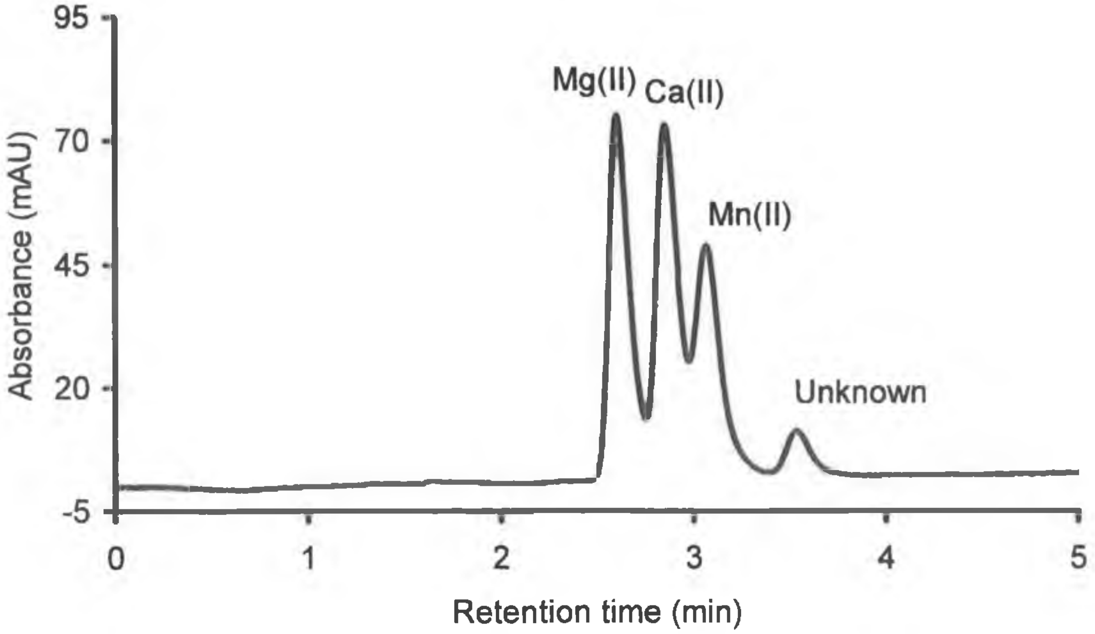


Figure 6.5. Chromatograms showing the effect of column temperature on the resolution of Mg(II), Ca(II) and Mn(II) at (a) 15 and (b) 20°C. Other eluent conditions as in Figure 6.3.

(a)



(b)

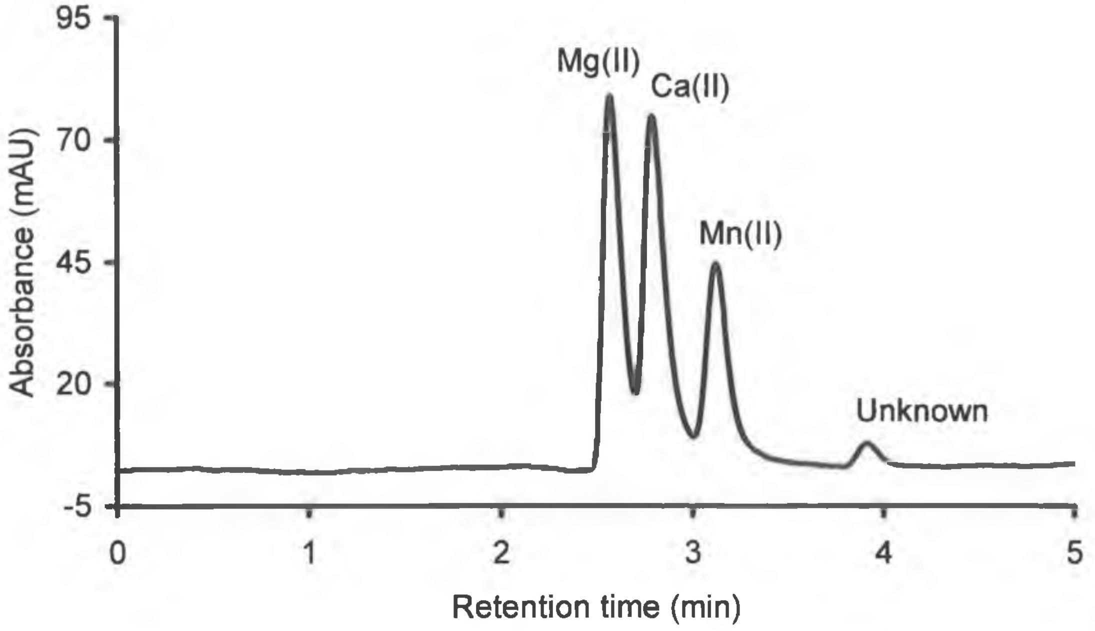


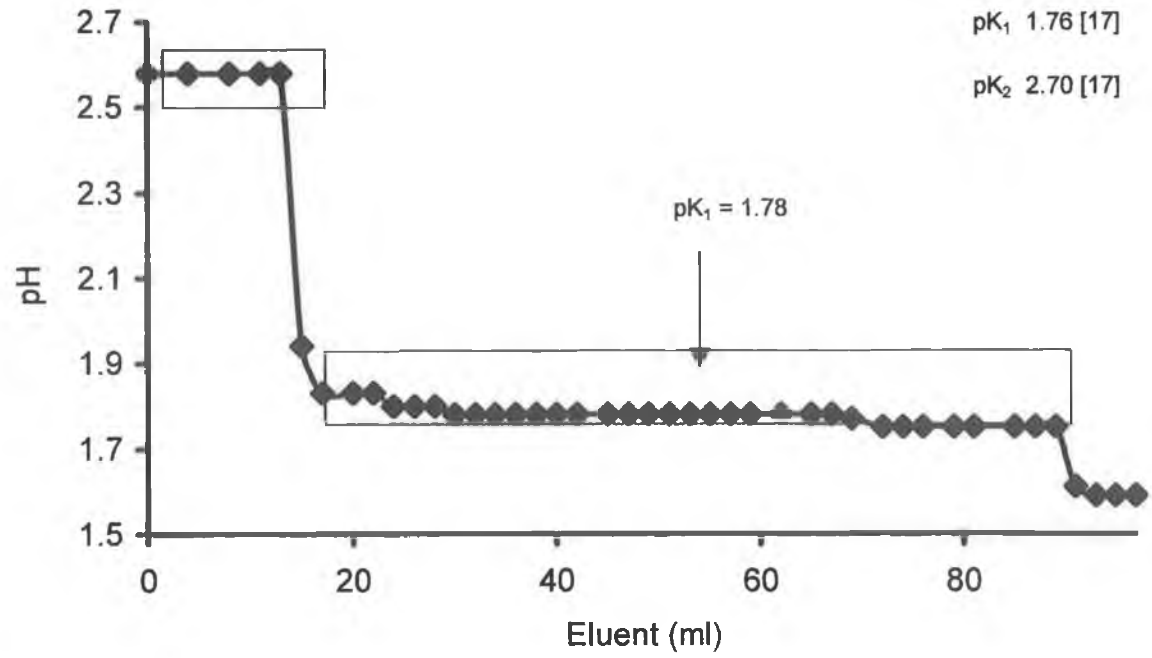
Figure 6.6. Chromatograms showing the effect of column temperature on the resolution of Mg(II), Ca(II) and Mn(II) at (a) 25 and (b) 50 °C. Other eluent conditions as in Figure 6.3.

To graphically illustrate the strong buffering capacity of the IDA-silica column, the column outlet pH was monitored during a typical pH step gradient. Figure 6.7(a) shows the column outlet pH against eluent volume for an IDA-silica column (V_o =1.1 ml) previously equilibrated with an eluent of pH 2.6, and switched at the column inlet to an eluent of pH 1.6 at a flow rate of 1.5 ml/min (volume = 0). As can be seen from the figure, two distinct buffering regions are evident, corresponding to the pK_{a1} and pK_{a2} of IDA, which under the conditions investigated meant the outlet pH only reached that of the eluent after 90 ml (or 60 min). The results shown were used to calculate the approximate capacity of the column, which was found to be ~185 μmol of 'active' IDA (which corresponds closely to the 130 μmol/g reported earlier by Nesterenko and Jones a similar IDA-silica [247]).

However, despite the strong buffering capacity of the column, the pK_{a1} value for IDA was sufficiently acidic (1.78) to allow the elution of Zn(II) and Pb(II) from the column and so the gradient could be used provided the timing of the drop in eluent pH was correct. With a column equilibrated to pH 2.6 it was found that switching to the lower eluent 3 minutes prior to sample injection caused a drop in column pH approximately 5 minutes after injection and eluted both Zn(II) and Pb(II) as well resolved and relatively sharp peaks (again using a flow rate of 1.5 ml/min). Figure 6.7(b) shows the resultant chromatogram, showing all five metals well resolved, removed from the void and eluted in under 10 minutes. It was found that the developed gradient method was reproducible provided the column was fully equilibrated with the higher pH eluent at the start.

Following each gradient run the length of this re-equilibrium stage was dependent upon the length of time the column was exposed to the pH 1.6 eluent. Therefore, it was vital to monitor the outlet pH after each gradient run to ensure the column had fully re-equilibrated to the starting pH before the next injection could be made. However, it was found that this added considerably to the total analysis time and seriously limited the practicality of the gradient method.

(a)



(b)

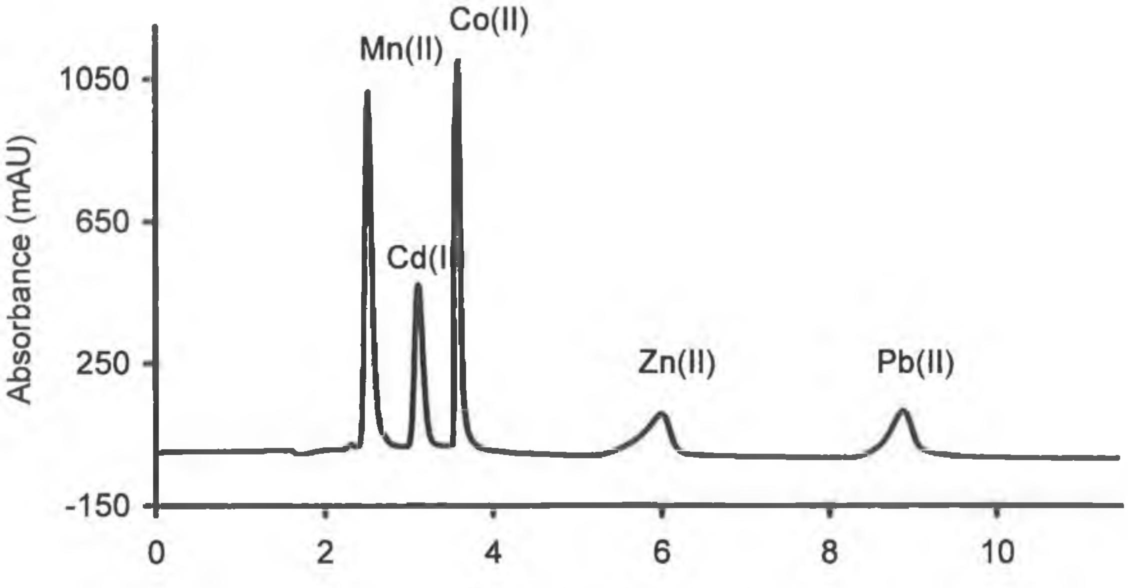


Figure 6.7. (a) Graph showing column outlet pH against eluent volume following a pH step gradient from pH 2.6 to pH 1.6 at V=0. (b) Step gradient (pH 2.6) separation of Mn(II), Cd(II), Co(II), Zn(II) and Pb(II). Eluent conditions: 0.1 *M* NaCl switched to 0.1 *M* NaCl (pH 2.6) at time = 3 minutes prior to standard injection.

6.3.4. Applications.

To illustrate that the unique selectivity exhibited by chelation ion chromatography can indeed be utilised in the analysis of real samples, a certified reference freshwater sample (NIST 1640) was analysed for trace levels of Mn(II), Cd(II), Co(II) and Zn(II). As mentioned above the CRM sample consisted of a typical freshwater matrix containing Mg(II) and Ca(II) at ~ 6 and 7 mg/L, and K(I) and Na(I) at ~ 1 and 30 mg/L respectively. Standard analytical performance indicators (linearity, precision, accuracy and detection limits) were determined using the sample itself under the isocratic eluent conditions optimised in Section 6.3.1. Under these conditions it was not possible to determine Pb(II) in the CRM sample, this being an unfortunate disadvantage of the developed method.

All four metal ions gave a linear response ($R^2 > 0.999$) over the investigated range (0 – 250 µg/L). For Cd(II) both a standard calibration and a standard addition calibration were carried out. This resulted in an R^2 value of 0.9999 for the standard calibration ($n=5$, 50 – 250 µg/L) and an R^2 value of 0.9993 for the standard addition calibration ($n=3$, 20 – 100 µg/L). Precision was determined using 5 repeat injections of the CRM and calculated for retention time, peak area and peak height. The method proved highly reproducible with %RSD values for the retention times of all 6 metals ranging between 0.7 to 2.9. The same precision was reflected in the %RSD values for peak areas (1.1 – 2.0) and peak heights (0.6 – 1.1). Detection limits for Mn(II), Cd(II), Co(II) and Zn(II), determined using a 250 µL injection of the CRM and calculated using 3 X baseline noise, were found to be 4.2 (±1) µg/L for Co(II), 4.6 (±1) µg/L for Mn(II), 5.4 (±1) µg/L for Cd(II), and 9.0 (±2) µg/L for Zn(II). The excellent sensitivity exhibited by the method was in part due to the absence of any complexing ligands within the eluent that would otherwise compete with the PCR and reduce detector response. Finally, accuracy was assessed with the determination of the above 4 metal ions in the CRM. The results obtained using standard addition are shown in Table 6.1, calculated using both peak area and peak height. As can be seen from the results, considering the trace concentrations of the 4 metals, the accuracy of the method was found to be satisfactory. The chromatogram obtained for the NIST 1640 sample is shown overlaid with a spiked NIST sample in Figure 6.8.

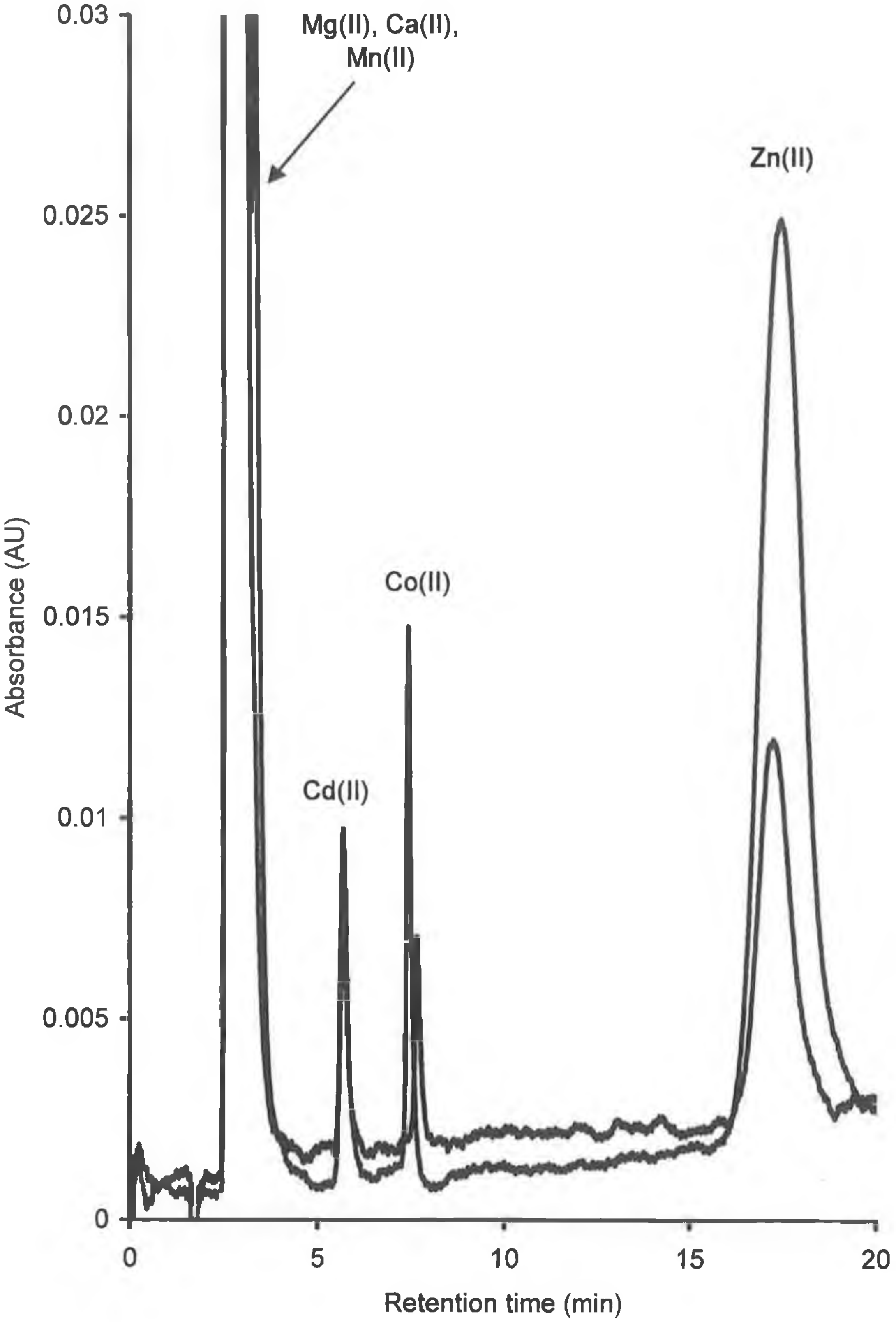
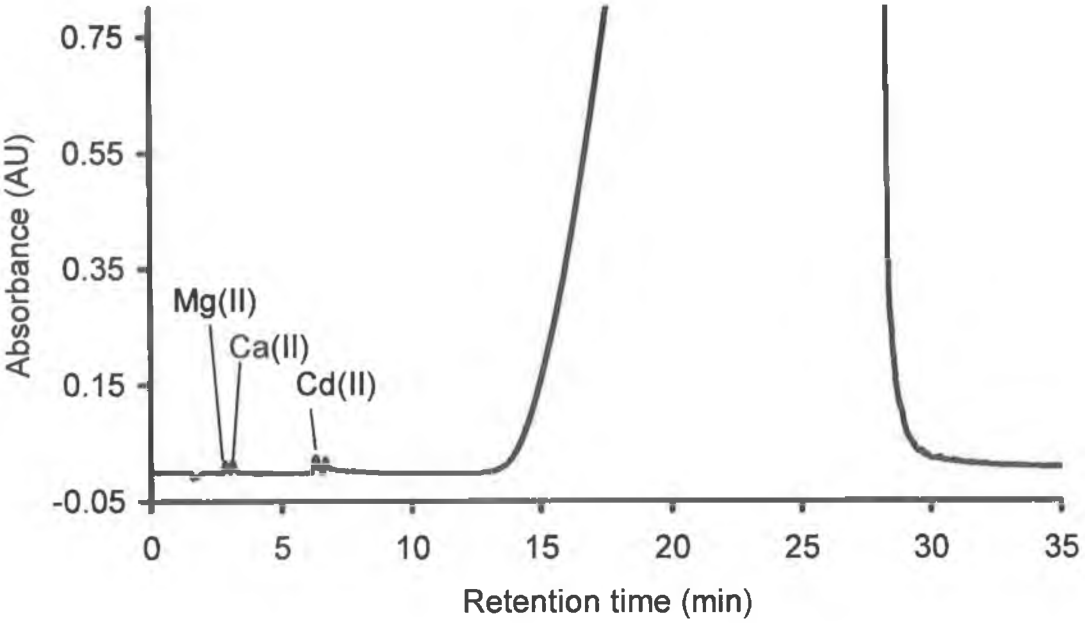


Figure 6.8. Chromatogram of NIST 1640 freshwater sample overlaid with spiked sample. Eluent conditions as in Figure 6.3.

(a)



(b)

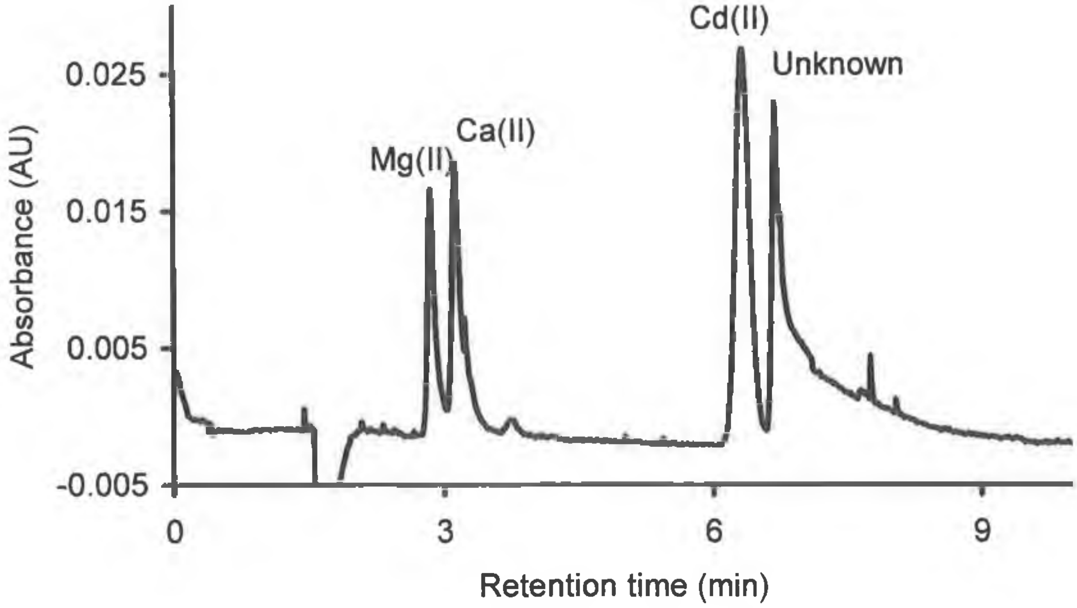


Figure 6.9. Chromatogram of (a) 800 mg/L Zn(II) spiked with 0.5 mg/L Mg(II) and Ca(II) and 100 μg/L Cd(II). (b) Close up of Mg(II), Ca(II) and Cd(II). Eluent conditions as in Figure 6.3.

Table 6.1. Analysis of NIST 1640 standard reference material.

Analysis of NIST 1640 standard reference material

Metal Ion	Certified Value (mg/L)	*Amount determined (mg/L)	**Amount determined (mg/L)
Mn(II)***	0.121 ± 0.001	0.095	0.093
Cd(II)	0.022 ± 0.001	0.028	0.028
Co(II)	0.020 ± 0.003	0.018	0.016
Zn(II)	0.053 ± 0.001	0.065	0.063

* Calculated using peak height plot.

** Calculated using peak area plot.

*** Analysis carried out at 50 ^{O}C.

The excellent selectivity for Cd(II) is clear from the chromatograms shown, making the developed method suitable for the determination of Cd(II) in samples containing excess alkali/alkaline earth metals and common transition metals such as Zn(II).

This prompted a further application to the analysis of Cd(II) in zinc chloride salt 800 mg/L Zn(II) was spiked with 0.5 mg/L Ca(II) and Mg(II) and 100 μg/L Cd(II), the resultant chromatograms shown in Figure 6.9. As clearly illustrated a sharp peak for Cd(II) was obtained, further emphasising the fact the the column exhibits unique selectivity for the metal ion.

6.4. Conclusions.

Iminodiacetic acid bonded silica gel offers a viable alternative to simple cation exchange resins for the determination of trace transition metal ions in real samples. Its unique selectivity can be manipulated to produce well resolved separations of several alkaline earth and transition metal ions when using only simple inorganic eluents, with the resultant chromatograms exhibiting peak efficiencies comparable to those obtainable with modern cation-exchange resins.

Chapter 7.

Retention of Alkali, Alkaline Earth and Transition Metals on an Itaconic Acid Cation Exchange Column: Eluent pH, Ionic Strength and Temperature Effects Upon Selectivity.

7.0. Introduction

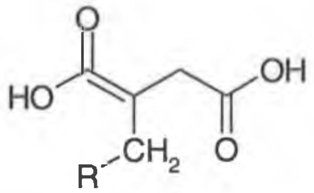
The use of ion chromatography for the determination of alkali, alkaline earth and transition metal ions in water samples presents a useful alternative to atomic spectroscopic techniques and as such is often the method of choice in many analytical laboratories. With this in mind it is not surprising that a great deal of research effort has focused on the development of new stationary phases that can offer useful selectivities for these and other less common metal ions. Most of these recent developments in stationary phase technology for the separation and determination of metal ions using various modes of ion chromatography is detailed in recent review articles by Pohl and Jackson [310, 311] and Sarzanini *et al.* [309].

In it's early stages ion chromatography was very much seen as purely being based upon the use of simple ion exchangers as the stationary phase. For cations these were primarily either carboxylated or sulphonated resins or silica gels. However, the selectivity exhibited by these cation exchange phases was limited and so new phases with more than one type of ion exchange group were developed, such as carboxylic/phosphonic acid functionalised columns (Ionpac CS12A), which showed improved selectivity for the simultaneous isocratic separation of alkali and alkaline earth metal ions.

More recently however, it has become clear that even greater selectivity, can be obtained through the inclusion of alternative retention mechanisms or solute interactions within the system, in particular stationary phase complexation or chelation has received much attention. For example, Ionpac CS15 contains crown ethers in addition to carboxylic/phosponic acid groups, which acts to alter the selectivity of the column for those cations that can be complexed by the ligand. Alternative functional groups have also been investigated which possess the ability to retain the metal ion through both a simple ion exchange mechanism and also through chelation/complexation. The extent to which either of these two retention mechanisms occurs is dependent upon eluent conditions such as pH, ionic strength and temperature. Examples of functional groups which have this chelation/ion exchange capability are IDA, aminophosphonic acid (IPA) and glutamic acid, all of which have been investigated regarding their application to the separation of alkali, alkaline earth and transition metal ions in earlier studies [264, 305, 307, 312, 313]. In each case unusual selectivities could be obtained due to what could be described as a dual retention mechanism. The use of such complexing stationary phases for the separation of metal ions in ion chromatography is the subject of a review by Jones and Nesterenko [235].

7.1. Aims of this study.

In this current study a methylene succinic acid (itaconic acid) functionalised polymeric stationary phase, shown in Figure 7.1, was evaluated for it's selectivity for alkali, alkaline earth and common transition metal ions. To the author's knowledge there has been no published studies into the selectivity of an itaconic acid resin for the above range of metal ions and so this selectivity was investigated here. As itaconic acid contains two carboxylate groups potential exists for complexation to play a role in retention, namely coordination of the metal ion with two oxygen donator atoms. The high degree of selectivity shown by the resin for certain transition metals over alkaline earth metals certainly indicates this to be the case. However, as shown in this study, eluent pH, ionic strength and manipulation of column temperature, can be used to control the degree of each retention mechanism occurring and thus achieve several useful separations of metal ions from each of the above groups.



R is the polymer to which the itaconic acid is attached.

Figure 7.1. Structure of itaconic acid functionalised polymeric stationary phase.

7.2. Experimental.

7.2.1. Instrumentation.

A Dionex model GPM2 Gradient Pump Module (Sunnyvale, CA, USA) was used to deliver the eluent (1.5 mL/min). A manual sample injection valve, model 7125, (Rheodyne, Cotati, CA, USA), fitted with a 250 μL injection loop was used for introduction of the samples. The analytical column was a 250 x 4.0 mm i.d. PRP-X800 cation exchange column (particle size = 7 μm) supplied by the Hamilton Company (Reno, Navada, USA). A pressure driven Dionex Reagent Delivery Module was used for introduction of the post column reagent (1.5 mL/min), which was mixed with the eluent post-column using a 0.5 m PEEK reaction coil (0.01" i.d.). A model SPD-6AV Shimadzu UV/Vis detector (Kyoto, Japan) was used to monitor the resultant chromatograms at 510 nm or 570 nm (see below). These were recorded using Dionex AI450 chromatographic software. For the work on the effect of eluent temperature upon the retention of alkali metal ions a Dionex DX-120 ion chromatograph was used employing indirect conductivity detection.

7.2.2. Reagents.

The eluent and post column reagent (PCR) were prepared using deionised water from a Millipore Milli-Q water purification system (Bedford, MA, USA). The post-column reagents used, 4-(2-pyridylazo) resorcinol (PAR) and *o*-cresolphthalein complexone (*o*-CPC), and sodium hydroxide were purchased from Sigma-Aldrich (Gillingham, Dorset, UK) and used without further purification. Potassium nitrate was obtained from Merck (Darmstadt, Germany) and potassium chloride from Fluka Chemie (Buchs, Switzerland). For the detection of transition metal ions the post-column reagent solution was 0.5 *M* ammonia, 0.4 m*M* PAR, adjusted to pH 10.5 (monitored at 510 nm). For the detection of alkaline earth metals this was 0.4 m*M* *o*-CPC, 0.25 *M* boric acid adjusted to pH 10.5 using NaOH (monitored at 570 nm). All standard solutions prepared were filtered through a 0.45 μm filter and degassed using sonication. Low level standard solutions were generally prepared freshly each day from stock solutions (1000 mg/L) stored in 1% nitric acid.

7.3. Results and discussion.

7.3.1. Inorganic eluents.

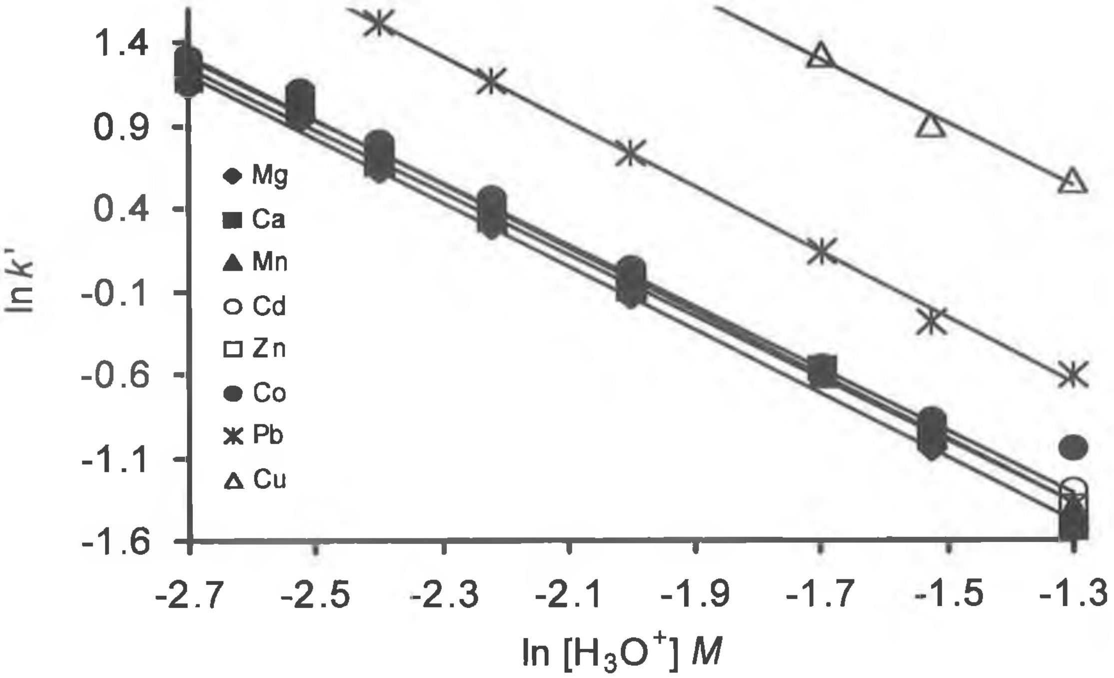
In this work the retention behaviour of alkaline earth and common transition and heavy metal ions on the itaconic acid stationary phase was evaluated using simple inorganic eluents. Initially, three eluents were investigated, these being HNO_3, KNO_3 and KCl. As itaconic acid contains two weak carboxylic acid groups ($pKa_1 = 3.85$, $pKa_2 = 5.45$ [314]), eluent pH will have a large affect upon metal ion retention due to the strong affinity of H^+ for the carboxylate groups. With simple dilute HNO_3 eluents the itaconic acid stationary phase exhibited similar selectivity to other dicarboxylate stationary phases, such as IDA [305, 311]. With HNO_3 eluents the stationary phase selectivity was determined as follows; Li < Na < NH_4 < K < Cs < Mg(II) < Ca(II) ≤ Sr(II) < Mn(II) ≤ Ba(II) < Cd(II) < Zn(II) < Co(II) << Pb(II) << Cu(II). For the alkali and alkaline earth metal ions the retention order shown was close to that expected if only ion exchange were responsible for retention, as would be the case on a sulphonated cation exchanger. However, for the transition and heavy metals investigated the selectivity was close to the reverse of that expected if only ion exchange were taking place. In particular, the itaconic acid stationary phase showed strong selectivity towards Pb(II) and Cu(II) ions over the reminder of the above metals, with strongly acidic eluents (up to 50 m*M* HNO_3) being required for their elution from the column. Under such acidic conditions all the other metal ions tested were unretained, such that it was possible to selectivity separate and determine Cu(II) in samples containing a mixture of all of the above metal ions. Figure 7.2(a) shows the strong affect of pH (HNO_3 eluent) upon retention of the alkaline earth and transition metal ions using the itaconic acid column. The retention slopes obtained for each of the metals injected were all very similar, ranging from -1.802 for Mn(II) to -1.978 for Cu(II). Figure 7.2(b) demonstrates the unusual selectivity for Cu(II) with overlaid chromatograms of Ca(II), Pb(II) and Cu(II) standards, obtained using a 30 m*M* HNO_3 eluent.

As can be seen from Figure 7.2(b) it was evident that peak shapes rapidly became broad with relatively small increases in retention. This effect is often seen in chromatographic systems where more than one retention mechanism is taking place and is a strong indication of stationary phase chelation occurring, as complex formation/dissociation kinetics are generally much slower than the kinetics of simple ion exchange [235]. The peak broadening shown was independent of standard concentration, with lower concentration standards being equally broad, this then eliminating column overload as a possible cause of the rather poor peak shape. In comparison with our previous work using dicarboxylate chelating ligands, namely IDA bonded 8 µm silica gels [305, 307, 312], it can be qualitatively stated that the efficiency of the itaconic acid resin was clearly poorer, this being an obvious disadvantage of the new polymer phase.

Where stationary phase complexation/chelation is contributing to retention it is expected that the effect of eluent ionic strength upon retention will be less than that seen if simple cation exchange were the only retention mechanism. Therefore, the effect of eluent ionic strength upon retention was investigated here using varying ionic strength eluents prepared from nitrate and chloride potassium salts.

The pH of all the eluents investigated was kept constant and slightly acidic at pH 3.3 to minimise error and ensure capacity factors were under 20 for the weakest eluents used. The effect of ionic strength was investigated over the range 0.02 to 1.0 *M* KNO_3 and KCl. The resultant log retention graphs are shown as Figure 7.3(a) and (b) respectively. It can be seen that as expected the selectivity of itaconic acid for K(I) is much lower than for H^+. Slopes obtained for the KNO_3 eluent ranged from -0.607 for Mg(II) to -0.948 for Zn(II). The eluents prepared using KCl proved to be the stronger eluents due to the added weak complexing ability of the chloride ions, here slopes ranged from -0.969 for Mg(II) to -1.346 for Co(II). Under all the eluent conditions tested Cu(II) was fully retained, even with the 1 *M* KCl eluent. The retention of Pb(II) was also considerable with retention times of $>$ 50 minutes when using a 0.1 *M* KCl eluent. Even with a 0.5 *M* KCl eluent (at pH 3.3) Pb(II) was retained for 8 minutes, illustrating the unusually selective properties of the itaconic acid functional group.

(a)



(b)

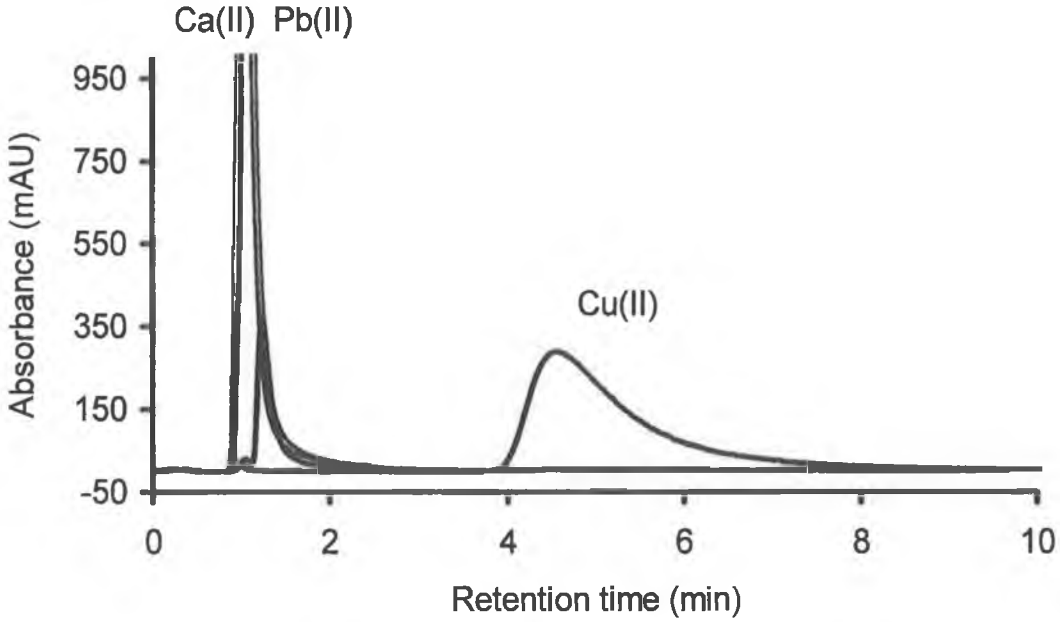


Figure 7.2. (a). Graph showing effect of eluent pH on capacity factors (*k'*) of alkaline earth and transition metals. (b). Overlaid chromatograms of Ca(II) (5 mg/L), Pb(II) (2 mg/L) and Cu(II) (2 mg/L) standards obtained using a 30 m*M* HNO_3 eluent. Detection using PCR with PAR.

7.3.2. Metal separations.

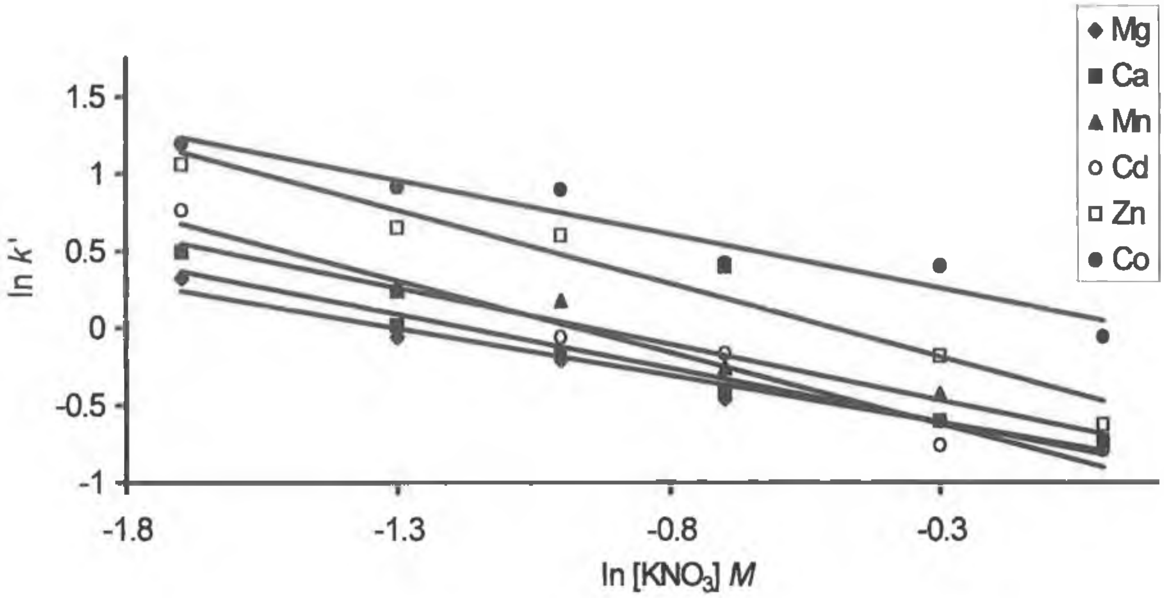
Using only dilute HNO_3 (0.6 - 2 m*M*) eluents separations of Mg(II), Ca(II), Mn(II), Cd(II), Zn(II) and Co(II) could be obtained using the itaconic acid column, with Pb(II) and Cu(II) totally retained. However, under these conditions run times were excessive (up to 30 minutes), as peak shapes rather poor, particularly for the later eluting metal ions. More acidic eluents resulted in co-elution of the early eluting peaks of Mg(II), Ca(II) and Mn(II). Addition of either KNO_3 or KCl (20 m*M*) to the above eluents was investigated to see if peak shapes improved. As expected this led to a reduction in retention times and a slight improvement in peak shapes. In both cases the resolution of Mn(II) from the early eluting peaks of Mg(II) and Ca(II) was improved. However, the additions also caused the co-elution of Cd(II) and Zn(II) with the KNO_3 eluent and Zn(II) and Co(II) with the KCl eluent. Typical chromatograms obtained using the above eluents are shown in Figures 7.4 and 7.5. To facilitate the elution of Pb(II) and Cu(II) from the column both pH and ionic strength gradients were investigated. However, it was found that the high buffering capacity of the column due to the itaconic acid groups made using pH gradients difficult, resulting in poor reproducibility, attributed to long column re-equilibration times following each gradient run. The use of an ionic strength gradient whilst keeping the pH uniform proved more successful in terms of reproducibility and could be used to obtain a separation of Mg(II), Ca(II), Mn(II), Cd(II), Zn(II), Co(II) and Pb(II), although even when using a final eluent strength of 1 *M* KCl, Cu(II) was still retained. Figure 7.5(b) shows the chromatogram obtained using a KCl gradient.

Finally, in an attempt to obtain the optimum resolution of the above metal ions the eluent pH was increased to 3.5 under isocratic conditions. The optimum conditions found were 20 m*M* KCl (pH 3.5), which resulted in the chromatogram shown as Figure 7.6.

7.3.3. Temperature effects.

The unusual selectivity exhibited by the itaconic acid column prompted an investigation into the effect of column temperature upon the retention of alkaline earth and transition

(a)



(b)

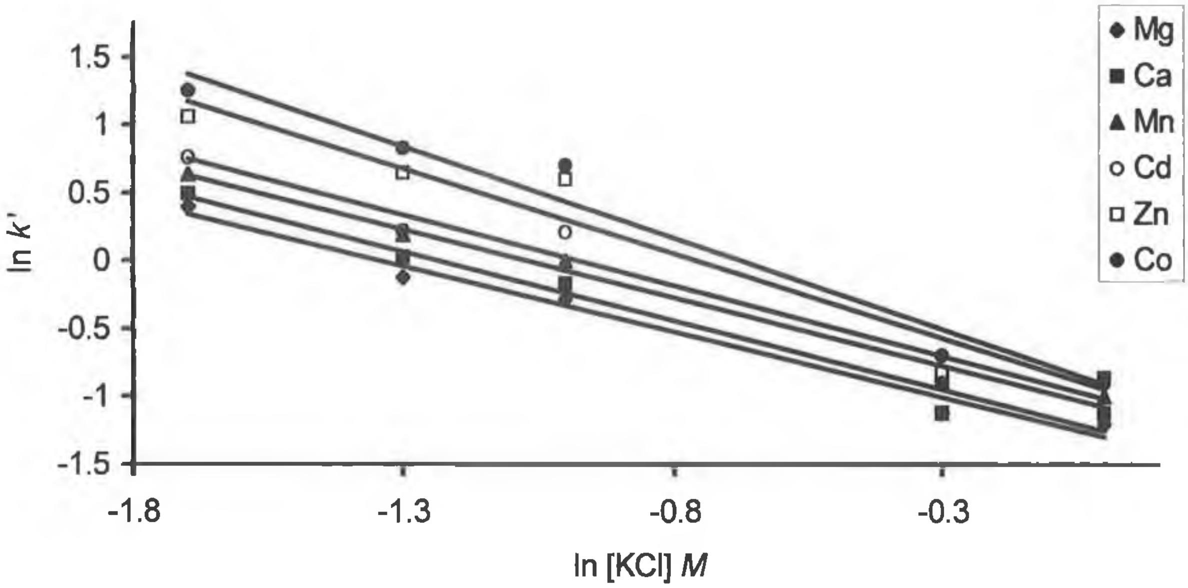
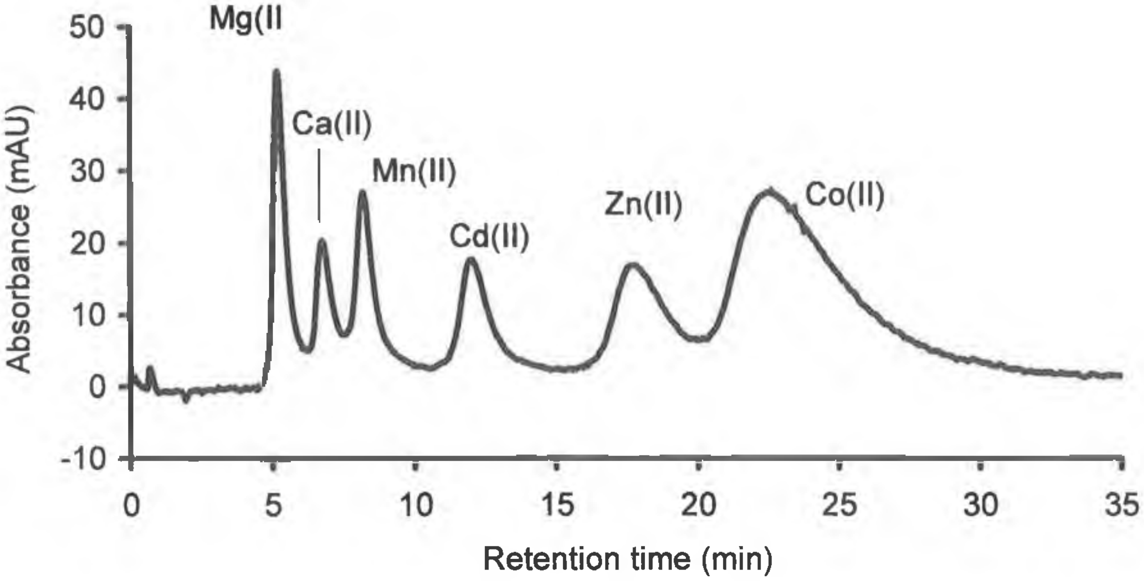


Figure 7.3. Effect of eluent ionic strength on retention using a (a) KNO_3 eluent, and (b) KCl eluent (pH adjusted to 3.3 using dilute HNO_3). Standard concentration = 2 mg/L.

(a)



(b)

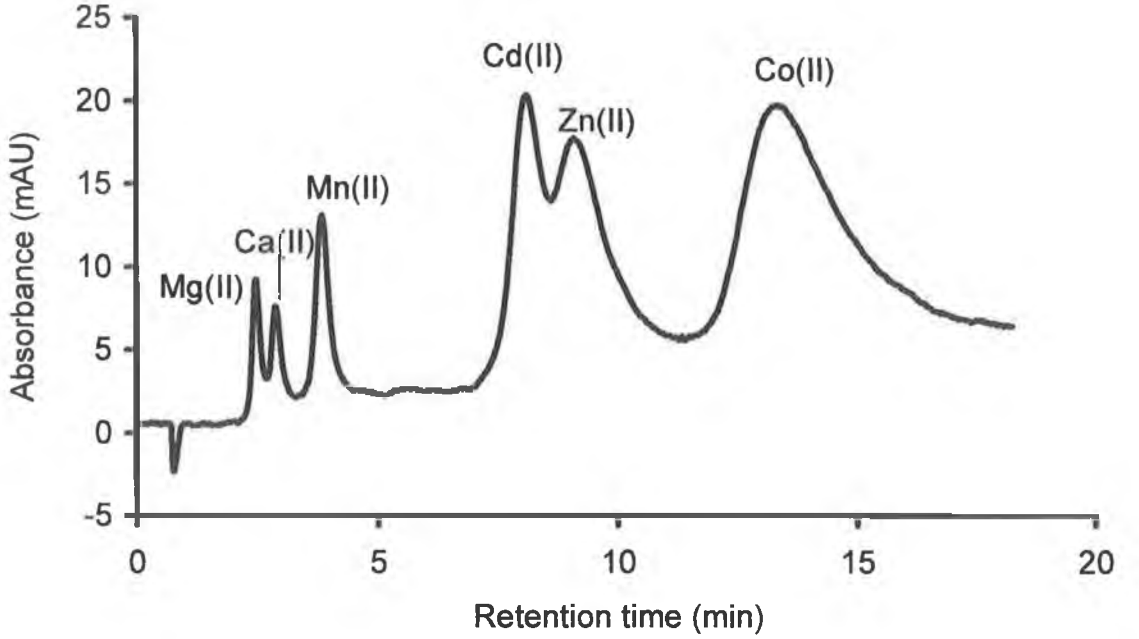


Figure 7.4. Chromatograms showing the separation of Mg(II), Ca(II), Mn(II), Cd(II), Co(II) and Zn(II) using (a) a HNO_3 eluent (pH 2.7), and (b) a 20 m*M* KNO_3 eluent (pH 3.3). Detection using PCR with PAR. Standard concentrations between 0.5 – 5 mg/L.

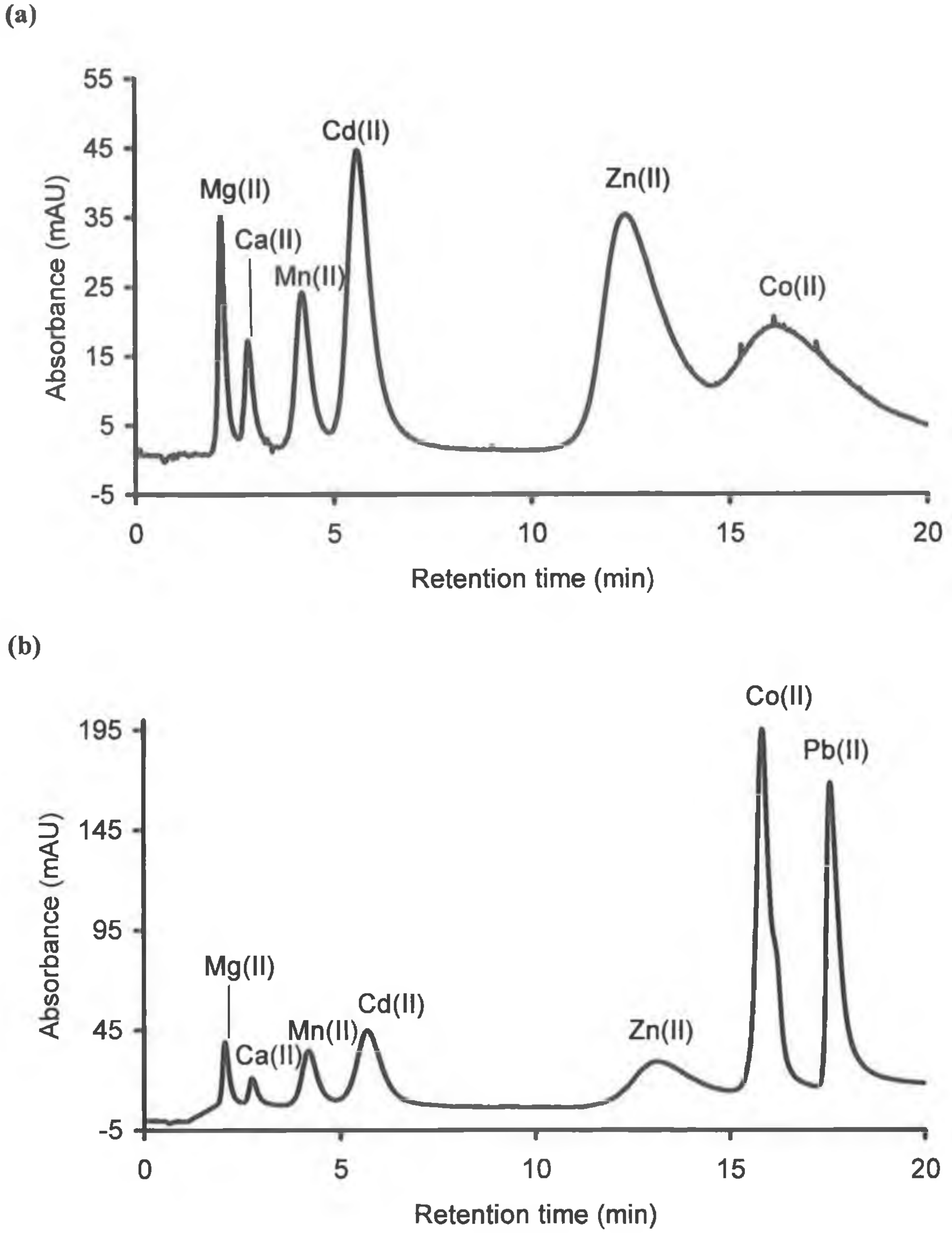


Figure 7.5. Chromatograms showing the separation of Mg(II), Ca(II), Mn(II), Cd(II), Co(II) and Zn(II) using (a) a 20 m*M* KCl eluent (pH 3.3), and (b) a 20 m*M* KCl eluent (pH 3.4) increased to 1 *M* KCl (pH 3.4) between 11 and 12 minutes. Detection using PCR with PAR. Standard concentrations between 0.5 – 5 mg/L.

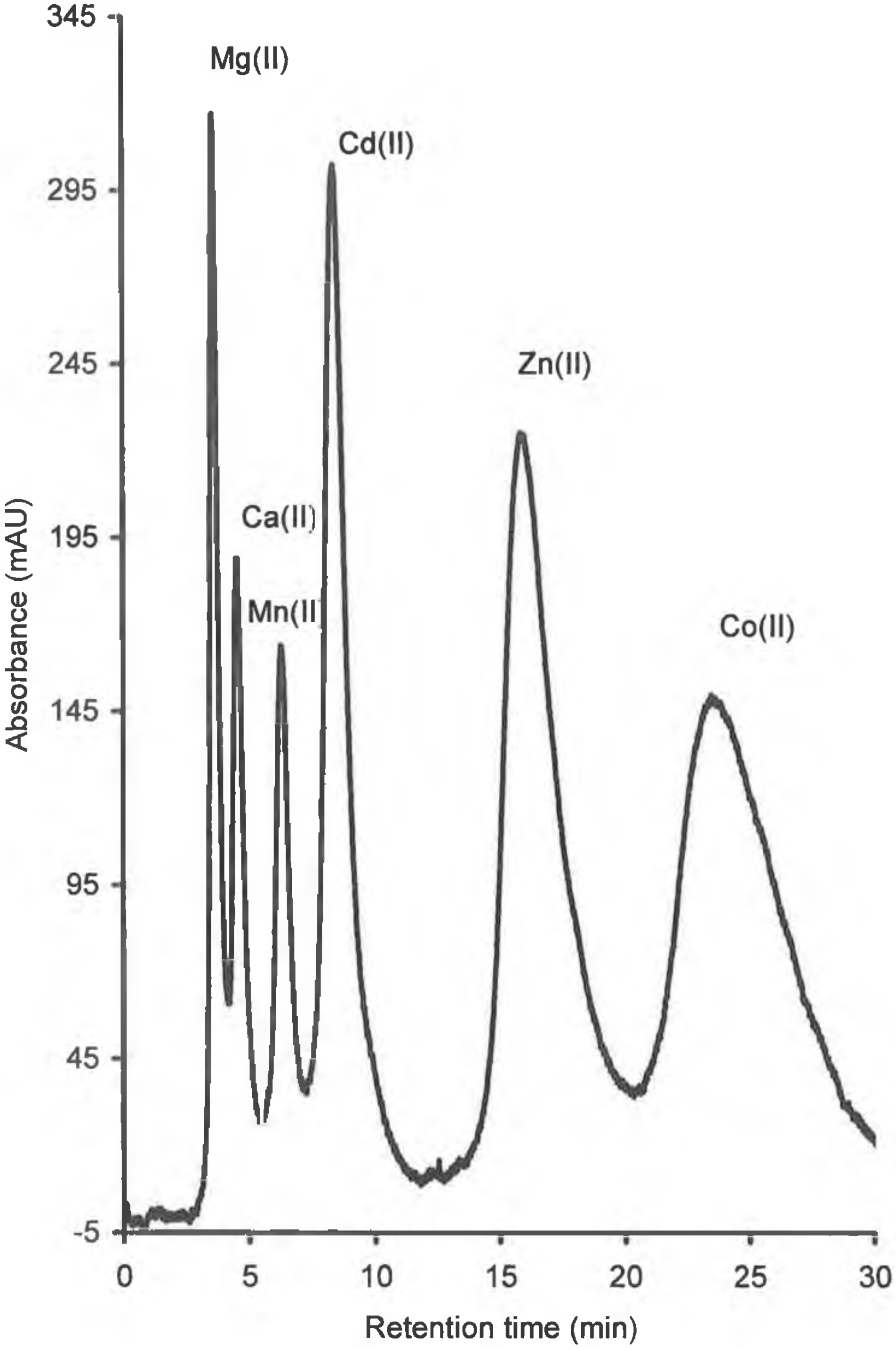


Figure 7.6. Chromatogram showing the optimised separation of Mg(II), Ca(II), Mn(II), Cd(II), Zn(II) and Co(II) obtained using an eluent of 20 m*M* KCl (pH 3.5). Standard composition: Mg(II), Ca(II), (10 mg/L) Cd(II), Co(II) (2 mg/L), Zn(II) (1 mg/L), Mn(II) (0.2 mg/L). Detection using PCR with PAR.

metal ions. It has been shown in several previous studies that temperature can have a very different affect upon the retention of metal ions depending upon the exact mode of retention. Where only cation exchange is responsible for retention, temperature is known to have only a small predominantly negative affect upon retention. Fortier and Fritz [315] first investigated this effect using a strong cation exchange resin with a perchloric acid eluent. The results showed retention times of divalent metal ions decreased with an increase in system temperature. Much more recently, work by Rey and Pohl [316] and Hatsis and Lucy [317] showed similar trends when using a carboxylate/phosphonate stationary phase used with a methanesulphonic acid eluent. However, where retention of metal ions is the result of stationary phase complexation (or indeed a combination of both ion exchange and stationary phase complexation) the above negative effect of temperature is no longer seen. This has been illustrated in a number of studies on the separation of alkaline earth [304], transition metals [313] and the lanthanide metals [263] using iminodiacetic acid functionalised silica columns, where in each case under the eluent conditions studied, an increase in retention resulted from an increase in column temperature.

Fortier and Fritz [315] proposed the following equation to express the effect of column temperature upon retention ($\ln k'$) in ion exchange;

$$\ln k' = -\Delta H^o/RT + \Delta S^o/R + \ln \varphi \qquad (1)$$

Where, ΔH^o = the enthalpy of the sorption process, ΔS^o = the change in entropy and φ = the phase ratio. Where the sorption process exhibits exothermic behaviour (negative values of ΔH^o), the retention of the analyte ion will decrease with increasing column temperature, as shown recently by Hatsis and Lucy [317]. This will present itself graphically as a positive slope in a Van 't Hoff plot ($\ln k'$ v's 1/T). However, studies on stationary phases capable of metal ion complexation have shown much higher values of ΔH^o, particularly under eluent conditions which favour stationary phase complexation over ion exchange (high ionic strength and increased pH) [4]. Under these conditions, the sorption process exhibits endothermic behaviour, resulting in a negative slope in a Van 't Hoff plot.

The plots shown in Figure 7.7. depict the effect column temperature has upon retention of alkali, alkaline earth and transition metal ions using the itaconic acid stationary phase under various eluent conditions. Using simple 1 and 2 m*M* HNO_3 eluents, the column was first tested for retention of alkali metal ions through simple ion exchange. The results obtained were similar to those shown by Hatsis and Lucy [317] with all (bar lithium with 1 m*M* HNO_3 eluent) exhibiting a decrease in retention with increased column temperature when using either eluent. With the stronger 2 m*M* HNO_3 eluent it was possible to elute Ca(II) and Mg(II) from the itaconic acid column. With this low ionic strength eluent the effect of temperature upon retention of these alkaline earth metals was clearly different to the effect upon the alkali metals. Only a very slight decrease in retention of Mg(II) could be seen, with the retention of Ca(II) remaining unaffected by temperature.

However, as shown in Figure 7.8, when using an eluent of higher ionic strength and pH (20 m*M* KCl eluent adjusted to pH 3.5 using HNO_3), both Ca(II) and Mg(II) behave very differently, and now like the transitions metals shown, exhibit an increase in retention with an increase in column temperature. Under these conditions it would be expected that both ion exchange and complexation would be contributing to the retention of the alkaline earth and transition metals. As complete stability constant data sets for itaconic acid complexes of alkaline earth and transition metals are not readily available, it was not possible to predict the exact contribution to retention of stationary phase complexation. However, if itaconic acid behaves similarly to other dicarboxylate ligands, it should be expected that complexation was playing a greater role in the retention of the transition metal ions than the alkaline earth metals. The slopes obtained from the data shown in Figure 7.8 (listed in Table 7.1) support this assumption, with temperature having significantly less effect upon the retention of the alkaline earth metals compared to the transition metals. This non-uniform response to temperature means column temperature can be used as an extra means to manipulate selectivity. For example, under the eluent condition used in Figure 7.8, the baseline resolution of Mn(II) from Ca(II) at room temperature was not possible. However, an increase in column up to 50 °C caused a greater increase in the retention of Mn(II) relative to Mg(II) and Ca(II), resulting in their improved resolution (see Figure 7.9).

From the above results it was predicted that if eluent conditions were such that ion exchange interactions were minimised and complexation was the dominant retention mechanism, the effect of temperature upon the retention of alkaline earth metals would be much more pronounced. Therefore a 100 m*M* KCl eluent was prepared and adjusted to pH 5.7. The effect column temperature had upon the retention of alkaline earth metal ions using this eluent is shown as Figure 7.10. From the results obtained it is clear that under these conditions the effect of temperature is indeed more pronounced with considerable increases in retention for all four metals. The slopes obtained (see Table 7.1) are now even greater than even those obtained for the transition metal ions using the previous 20 m*M* KCl eluent. However, it is also interesting to note here that relative retention is not reflected in response to temperature, as Ba(II) being retained for the longest shows the least effect and Mg(II), which is the least retained of the four ions, shows the greatest effect. This may be a reflection upon the ion exchange selectivity of the column. As the selectivity of most cation exchangers, including carboxylated resins, is Ba(II) > Sr(II) > Ca(II) > Mg(II), it could be that even with the 100 m*M* eluent, ion exchange was playing a minor role in the retention of Ba(II) and this was being reflected in resulting slope. For Mg(II), the opposite is true, with any ion exchange interactions likely to have been predominantly suppressed, resulting in the ion showing the greatest response to temperature.

However, this hypothesis does not explain the response of Sr(II), which is almost equal to that of Mg(II). Therefore, the only absolute conclusions that can be drawn from these observations are that clearly an increase in all four alkaline earth metal ions results from an increase in column temperature and that under these eluent conditions stationary phase complexation was likely to be the dominant retention mechanism. As before, it was clear that non-uniform responses to changes in temperature could be used to improve resolution. At room temperature, the itaconic acid column showed poor resolution of

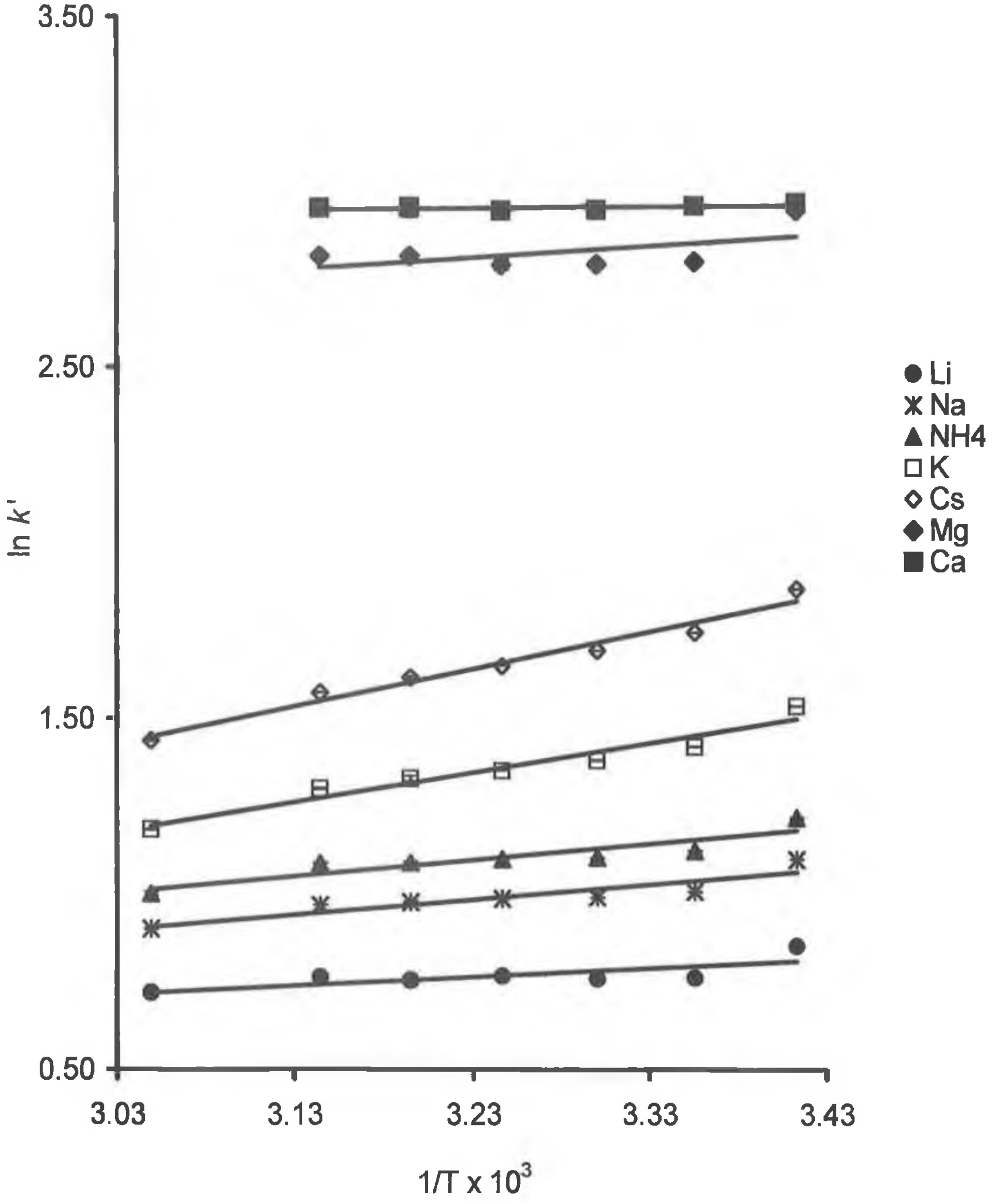


Figure 7.7. Van 't Hoff plot of metal retention v's column temperature. Eluent conditions: 2 m*M* HNO_3.

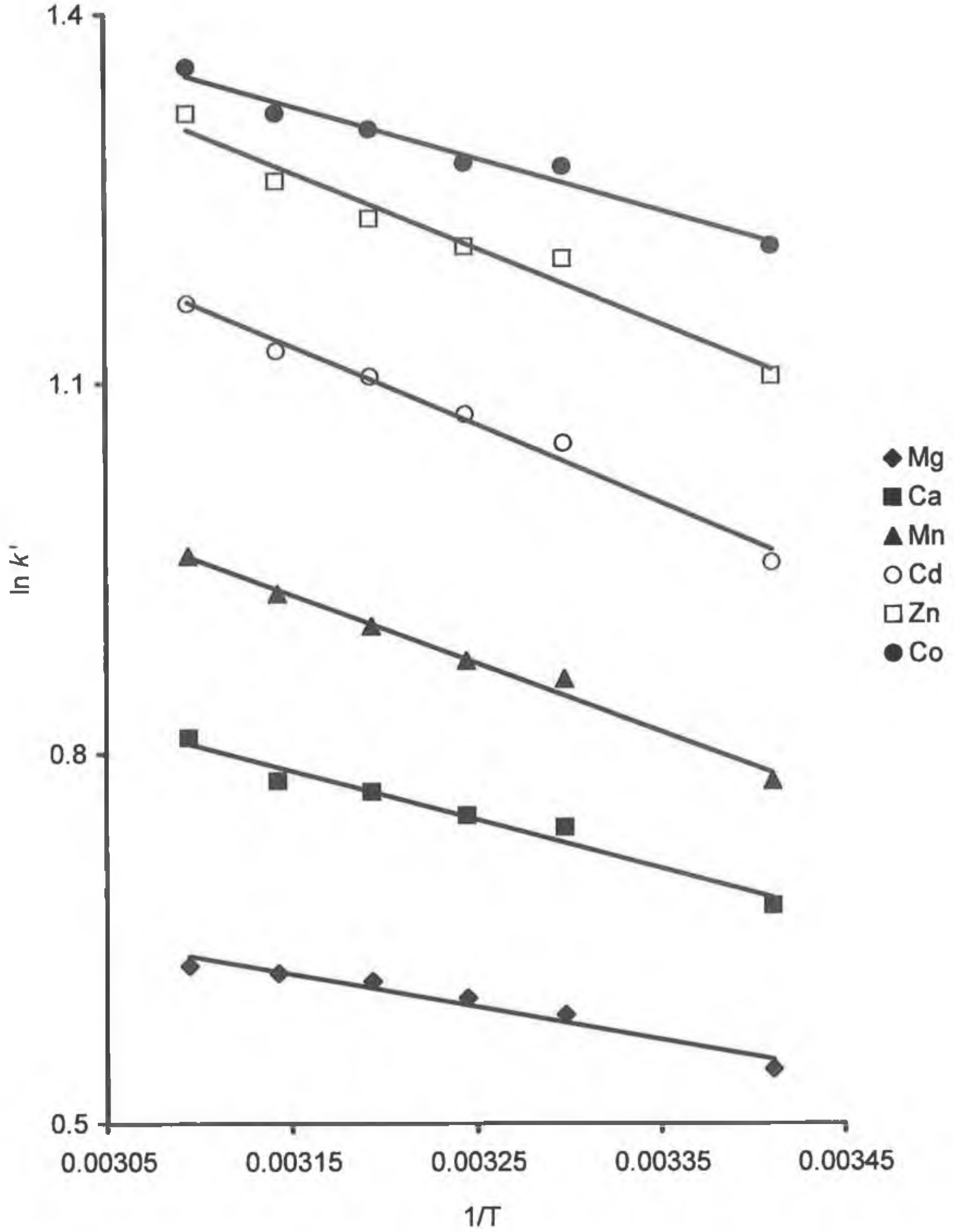


Figure 7.8. Van 't Hoff plot of metal retention v's column temperature. Eluent conditions: 20 m*M* KCl (pH 3.5).

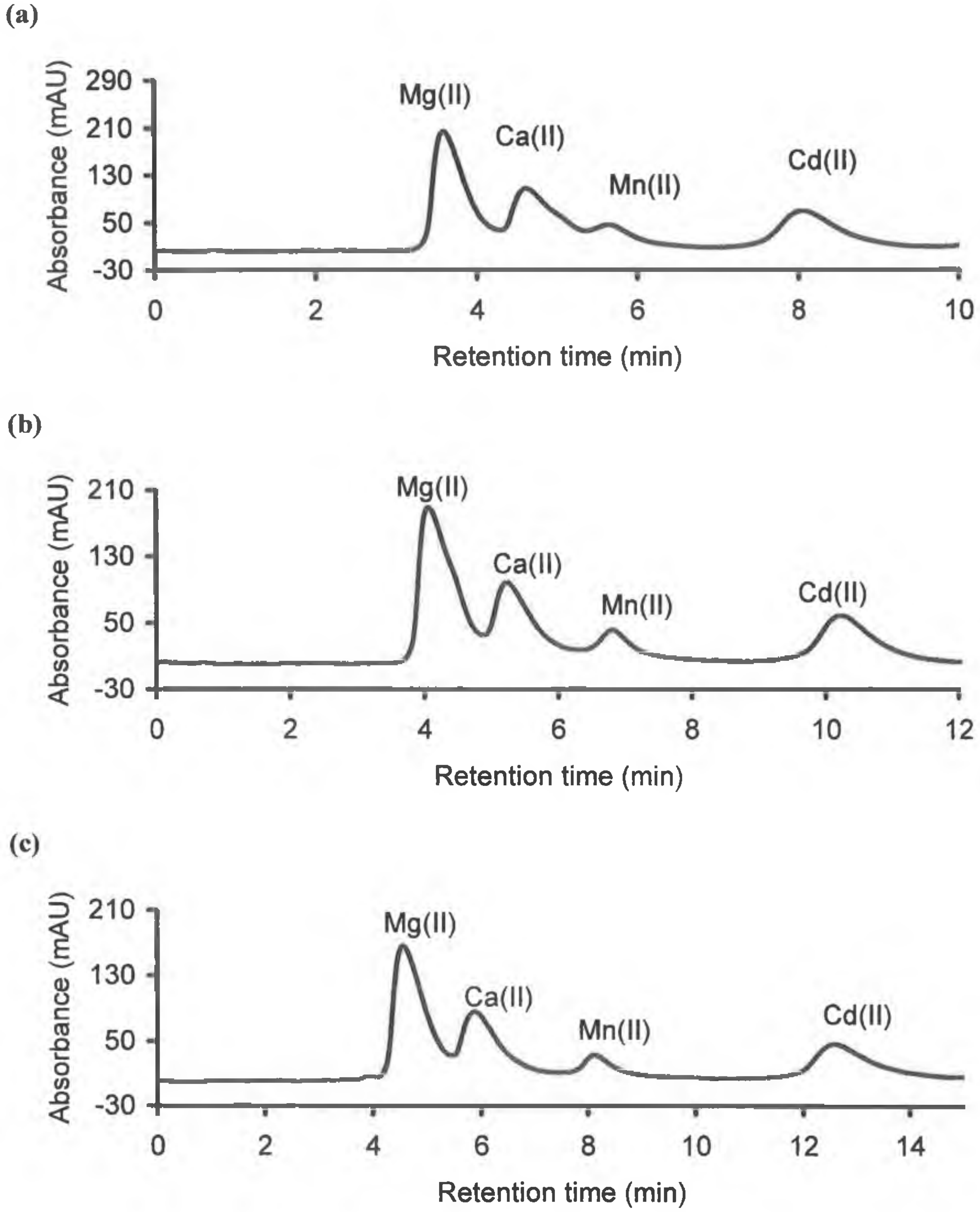


Figure 7.9. Chromatograms showing the resolution of Mg(II), Ca(II), Mn(II) and Cd(II) at (a) 19, (b) 40 and (c) 50 °C. Eluent conditions: 20 m*M* KCl (pH 3.5). Standard composition: Mg(II) (5 mg/L) Ca(II) (2 mg/L), Cd(II) (1 mg/L) and Mn(II) (0.2 mg/L). Detection using PCR with PAR.

Table 7.1. Slopes and correlation coefficients from Van 't Hoff plots of alkali, alkaline earth and transition metals.

Eluent – 1 m*M* HNO_3[a]		
	Slope	r^2
Lithium	-0.1219	0.896
Sodium	0.1012	0.843
Ammonium	0.1213	0.837
Potassium	0.5033	0.997
Cesium	0.7155	0.998
Eluent – 2 m*M* HNO_3[b]		
Magnesium	0.3356	0.319
Calcium	0.0485	0.326
Lithium	0.2517	0.596
Sodium	0.4290	0.832
Ammonium	0.4641	0.832
Potassium	0.8380	0.945
Cesium	1.0661	0.969
Eluent – 20 m*M* KCl at pH 3.5[c]		
Magnesium	-0.265	0.952
Calcium	-0.395	0.967
Manganese	-0.553	0.988
Cadmium	-0.637	0.984
Zinc	-0.617	0.965
Cobalt	-0.427	0.970
Eluent – 100 m*M* KCl at pH 5.7[d]		
Magnesium	-0.905	0.969
Calcium	-0.711	0.990
Strontium	-0.874	0.982
Barium	-0.628	0.974

ab – temperature range 20-60 °C

c – temperature range 20-50 °C

d – temperature range 19-65 °C

Ca(II) and Sr(II) under most eluent conditions tested. However, as can be seen from the chromatograms shown in Figure 7.11, under the eluent conditions described above, temperature could be used to resolve these two metal ions, without leading to the excessive retention of Ba(II).

7.3.4. Sample ionic strength.

Finally, the unique selectivity of the itaconic acid phase was investigated in relation to the effect of sample ionic strength. If retention was predominantly due to complexation not ion exchange, sample ionic strength should have a less detrimental effect upon the separation of the alkaline earth metals. Therefore, two alkaline earth metal mixed standards were prepared. To each standard mix Li was added as a marker for the elution of alkali metals (Li is the only alkali metal to show significant detector response when using PCR with *o*-CPC). As mentioned above the alkali metals did not show an increased retention at elevated column temperatures and were only marginally retained with the 100 m*M* KCl eluent.

To one of the standard mixes 0.1 *M* NaCl was added (2.3 g/L Na). The resulting chromatograms for the two mixed standards are shown overlaid as Figure 7.12. Two observations can be made from the chromatograms obtained. Firstly, the usual loss in efficiency associated with high ionic strength samples can be seen with the peak for Li(I), with the peak shape dramatically poorer. Secondly, although a slight decrease in retention can be seen for the alkaline earth metals, their overall resolution and individual peak efficiencies were unaffected. This experiment illustrates a considerable advantage of the use of such complexing stationary phases in the analysis of real samples, which are often of high ionic strength, and shows how the itaconic acid phase is suitable for the determination of alkaline earth metals in samples containing excess concentrations of alkali metals.

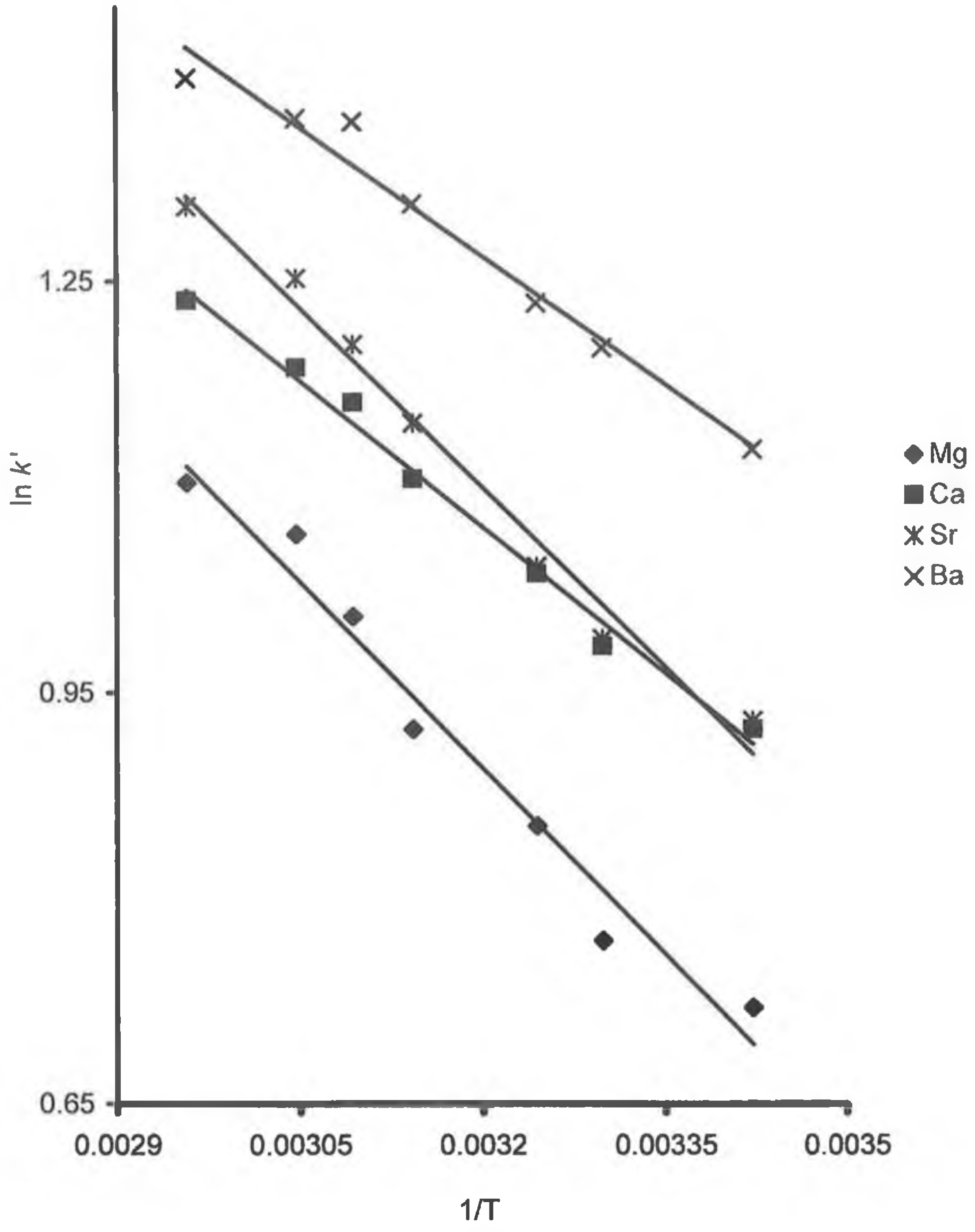


Figure 7.10. Van 't Hoff plot of metal retention v's column temperature. Eluent conditions: 100 m*M* KCl (pH 5.7).

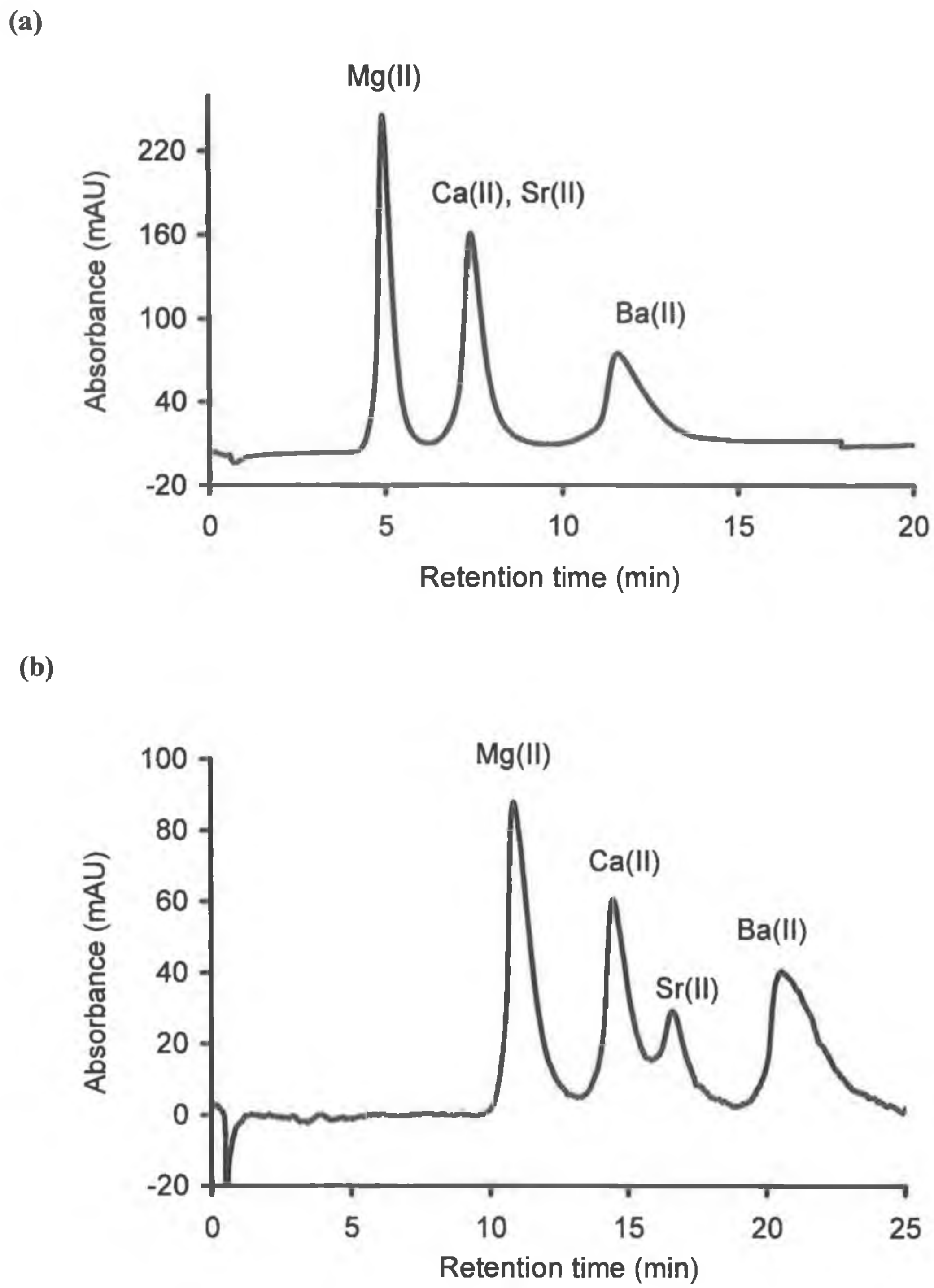


Figure 7.11. Chromatograms showing the resolution of Mg(II), Ca(II), Sr(II) and Ba(II) at (a) 19, and (b) 65 °C. Eluent conditions: 100 m*M* KCl (pH 5.1). Standard composition: Ba(II) (50 mg/L), Sr(II) (10 mg/L), Mg(II) (5 mg/L) and Ca(II) (2 mg/L). Detection using PCR with *o*-CPC.

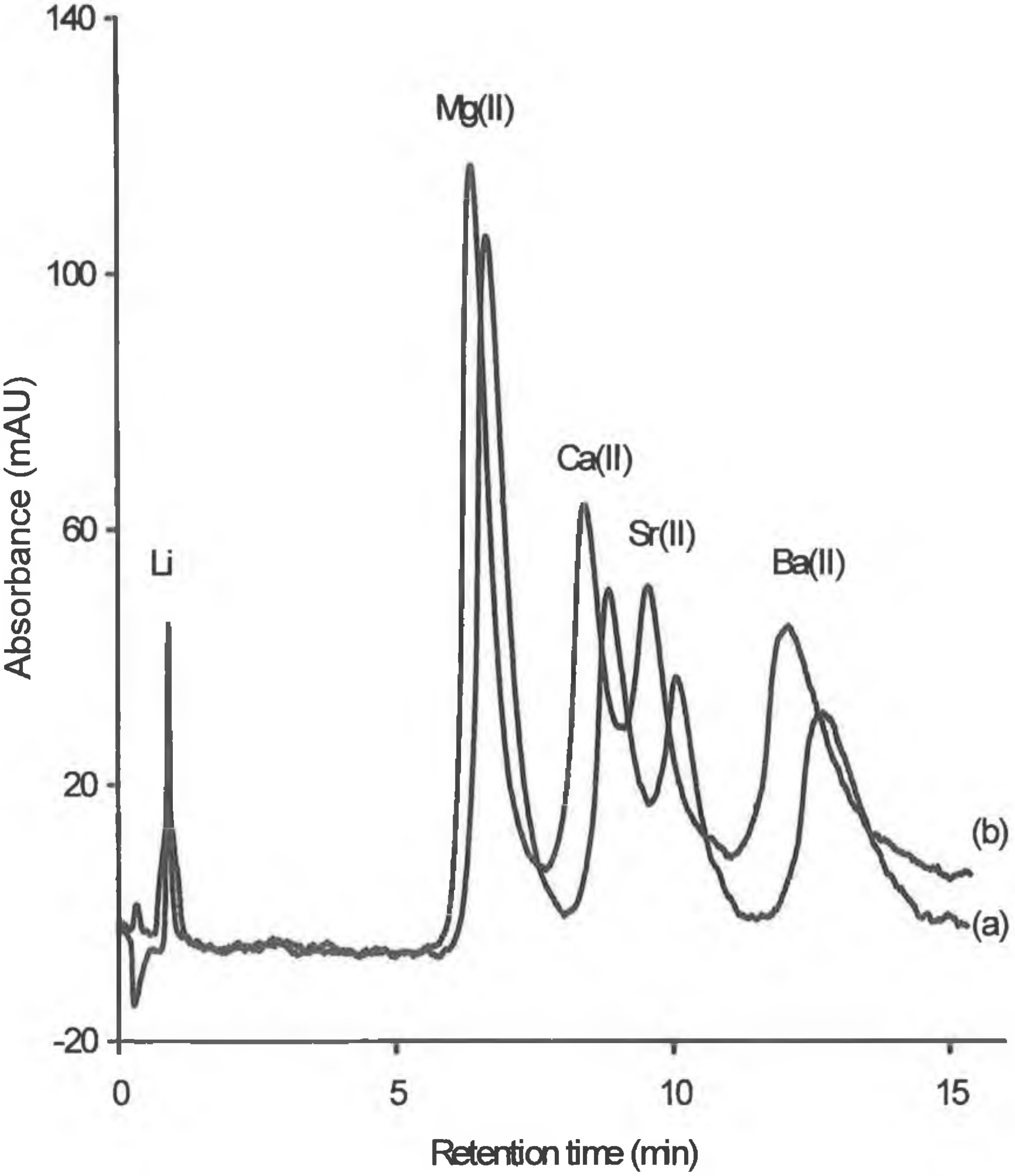


Figure 7.12. Overlaid chromatograms of alkaline earth metal mixed standard solutions. Standard composition: (a) Li (70 mg/L), Ba(II) (50 mg/L), Sr(II) (10 mg/L), Mg(II) (4 mg/L) and Ca(II) (1 mg/L). (b) as (a) plus 2.3 g/L Na. Eluent conditions: as Figure 7.7. Column temperature: 65 °C. Flow rate: 2.5 ml/min. Detection using PCR with *o*-CPC

7.4. Conclusions.

The results have shown the itaconic acid phase exhibits unusual and useful selectivity for alkaline earth and transition/heavy metal ions resulting from a combination of both ion exchange and complexation interactions. Control of eluent pH was the most critical parameter in manipulating cation selectivity. Column temperature was shown to also affect selectivity, with an increase in column temperature causing an increase in the retention of all alkaline earth and transition/heavy metals tested, this being the opposite case to that expected if only ion exchange were taking place.

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