1994

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Paull, Brett

http://hdl.handle.net/10026.1/1702

http://dx.doi.org/10.24382/3549
University of Plymouth

INVESTIGATION OF CHELATING DYE IMPREGNATED RESINS FOR THE ION CHROMATOGRAPHIC DETERMINATION OF TRACE METALS.

By

Brett Paull BSc (Hons), GRSC.

A thesis submitted to the University of Plymouth in partial fulfilment for the degree of

DOCTOR OF PHILOSOPHY

Department of Environmental Sciences,

Faculty of Science.

In collaboration with,

Dionex (U.K) Ltd.

November 1994.

ABSTRACT.

INVESTIGATION OF CHELATING DYE IMPREGNATED RESINS FOR THE ION CHROMATOGRAPHIC DETERMINATION OF TRACE METALS.

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A high-performance chelation ion chromatography (HPCIC) system using chelating dye impregnated resins for the determination of trace metals in various complex matrices is described.

The parameters involved in the production of dye impregnated chelating columns have been identified and optimised. A range of chelating dyes have been impregnated successfully into high-performance substrates, producing a number of chelating columns capable of both metal preconcentration and separations. Each of the chelating columns produced exhibited unique chelating properties. These columns have been classified in terms of metal retention characteristics, separation capability and efficiency, dye loading and column capacities.

A number of the above dye impregnated chelating columns have been employed successfully for the determination of trace metals in various sample types. This includes the determination of transition and heavy metals in seawater at low $\mu g\ dm^{-3}$ levels, the determination of aluminium in seawater, the determination of alkaline earth metals in oil-well brines and the determination of barium and strontium in calcium containing matrices such as mineral waters and milk powder.

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ACKNOWLEDGEMENTS.

I would like to offer my sincere gratitude to my Director of Studies Dr. Phil Jones. Dr. Jones has continually provided guidance and advice over the past three years and his support and supervision is deeply appreciated.

I would also like to thank Dr. Mike Foulkes, my second supervisor, for his contribution to this work.

For financial support of this work I would like to acknowledge the Engineering and Physical Sciences Research Council.

Thanks also to Mr. Alex Adam, my industrial supervisor, for his input into this research and Dionex (U.K) Ltd., for their sponsorship through the CASE award scheme. I am also grateful for the hospitality shown to me during my much enjoyed stay at the Leeds laboratory.

Finally, I would like to thank my mates in Plymouth and elsewhere for their bad influence over the past three years, and Annette Counsell for her good influence over the past five years (and also for her Building Society account). I dedicate this thesis to Annette.

Thank you.

At no time during the registration for the degree of Doctor of Philosophy has the author been registered for any other award.

The work described in this thesis has been entirely carried out by the author. A program of departmental research seminars were undertaken throughout the course and relevant scientific conferences attended at which this work was presented. Several papers detailing sections of this work have also been published.

This study was financed by the Engineering and Physical Sciences Research Council (EPSRC) in collaboration with Dionex (U.K) Ltd.

Signed .....

Date 5/JAN/95

CHAPTER 1. INTRODUCTION.

1.0. TRACE METAL ANALYSIS.

The implementation of an increasing number of regulations aimed at controlling the input into the environment of hazardous chemicals, demands efficient and sensitive methods of detection. The monitoring of trace metals is important for many reasons particularly in industrial and environmental samples. Industrial process streams and effluents require constant analysis to be able to control levels of trace metal contaminants both in the final product and being released into the environment. The routine determination of these metals in environmental samples is equally important both to establish natural concentrations, (natural fluxes in concentrations of each metal) and changes caused by anthropogenic activities. The analysis of environmental media such as natural waters, soils, the atmosphere and also biological samples not only provides information on the levels of these metals present, but helps to point out their source, their biological pathways and areas of eventual deposition. Speciation studies are now known to be very important in terms of monitoring toxicology and the environmental impact of releasing certain metals into the environment. Many metals exist as both toxic and non-toxic species and analytical techniques are required which are able to determine these species at natural and elevated concentrations. Atomic absorption spectrometry (AAS) is a well established technique in the analysis of trace metals, but can only determine one element at a time and does not have the ability to provide speciation information on its own. More advanced techniques such as inductively coupled plasma atomic emission spectrometry (ICP-AES) and inductively coupled plasma mass spectrometry (ICP-MS) are sensitive, multi-elemental detectors, but again are unable to provide speciation

information without some sort of sample pretreatment or additionally coupled technique such as liquid chromatography ie. LC-ICP-MS.

Modern chromatographic techniques, particularly liquid chromatography, which includes ion chromatography, have the capability of both multi-elemental determinations and speciation information. Modern instrumentation, improved substrates and sensitive detectors has enabled LC to rival more complex and expensive techniques, such as ICP-MS, in the analysis of trace metals in a large range of industrial and environmental samples. The relative simplicity of LC methodology enables LC to be easily automated and readily adapted to run on-line for continuous monitoring, which has led LC to become a commonly used analytical technique. A detailed review of the determination of trace metals and trace metal speciation using LC has been produced by Robards et al. [1] and covers the range of chromatographic techniques included in the phrase "liquid chromatography", the detectors used and the conditions required for the determination of various metal groups.

1.1. THEORETICAL ASPECTS OF LIQUID CHROMATOGRAPHY.

There are several parameters used to describe the quality of chromatographic separations, the processes controlling these separations and the ways in which the chromatography can be improved. These parameters make it possible to identify solute peaks, the degree of separation between the analytes of interest and the efficiencies of the processes involved.

Chromatographic separations are achieved through differences in the relative distributions of analytes in a sample between the stationary phase and the mobile phase. This distribution can be expressed as a ratio and is termed the partition coefficient (K) given by:

K = amount of solute in the stationary phase / amount of solute in the mobile phase

As the equation expresses the distribution of the components in the sample, it is also known as the mass distribution ratio. The retention of analytes, controlled by the above coefficients, is expressed as t_R and represents the time when the maximum concentration of the analyte is eluted. The retention times of each analyte can be used to provide more detailed descriptions of chromatographic separations, one of which is the capacity factor.

The capacity factor (k') is expressed in the following equation:

$$k' = (t_R - t_0) / t_0$$

In the above equation t_R is the retention time of the analyte peak and t_0 represents the retention time of the unretained peak (t_R and t_0 can be seen in Figure 1). Therefore the capacity factor expresses information as to where the analyte peaks elute relative to the

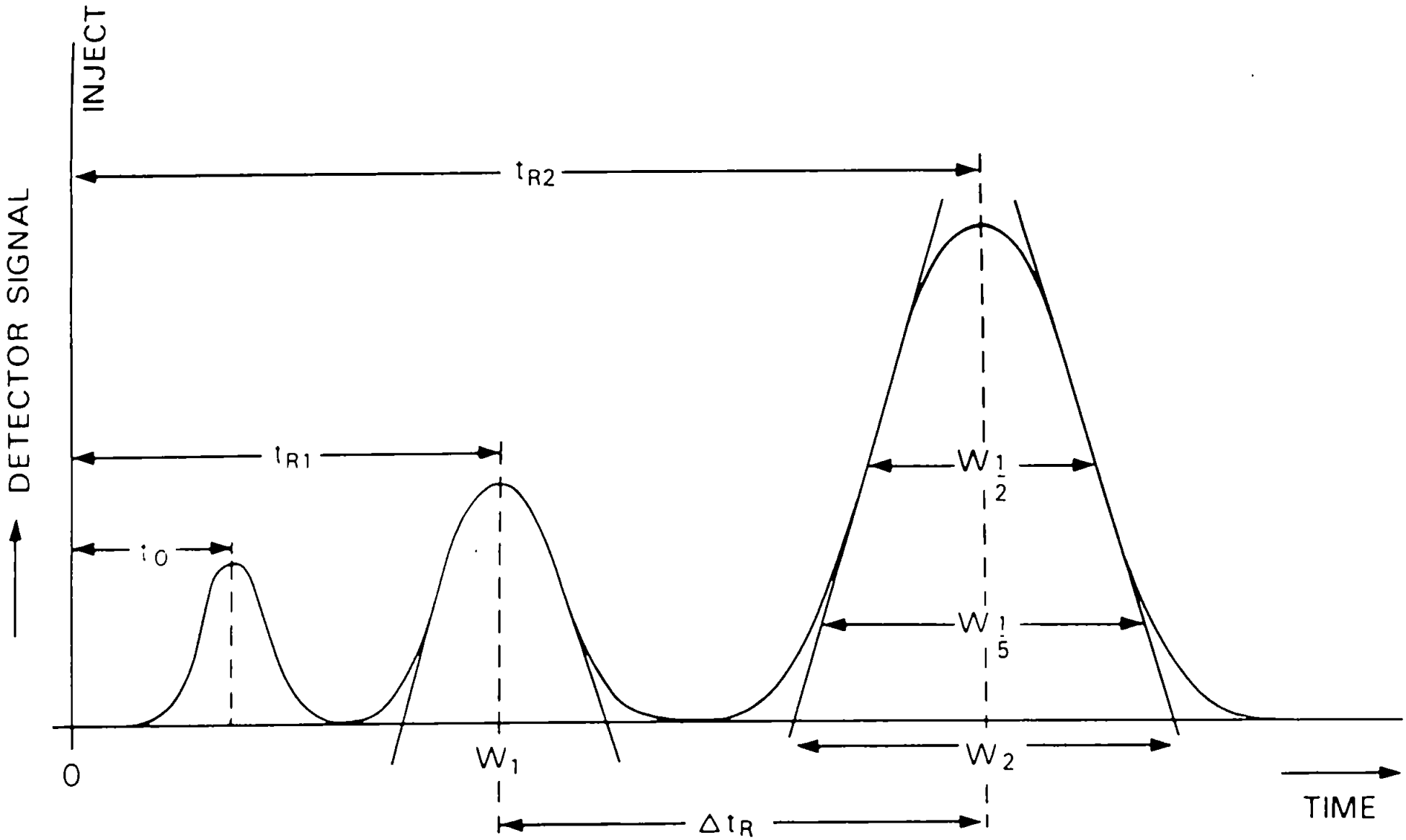


Figure 1. Parameters used to characterise a chromatographic separation [2].

unretained solvent peak. The expression used to describe the separation of two analyte peaks relative to each other is the selectivity or separation factor (α), which can be expressed as:

$$\alpha = k_2'/k_1' = (t_{R2}-t_0)/(t_{R1}-t_0)$$

From the above equation it can be seen that α can be obtained directly from the retention times of the two peaks or from their corresponding capacity factors.

The actual differences between retention times of two peaks can be expressed in terms of their resolution (R_s). The resolution is equal to the ratio of the distance between the peak maxima, to the mean width (W) of the analyte peaks, and can be written as:

$$R_s = (t_{R2}-t_{R1})/(0.5(W_1+W_2))$$

Figure 1 also shows the measurements required to express the resolution of two adjacent peaks.

All chromatographic techniques suffer the problem of peak broadening to some extent. The longer the solute remains in the system, a greater degree of dispersion will occur. The more efficient the column, the smaller this dispersion will be at a given point. Therefore more efficient columns produce sharper peaks compared with less efficient columns for the same retention times. Two expressions used to convey the efficiency of a LC column are plate height (H) and plate number (N). The chromatographic column can theoretically be said to consist of a series of narrow layers, lying perpendicular to the length of the column. These layers are termed theoretical plates.

The larger the number of theoretical plates, the more efficient the column. The plate number is defined as:

$$N = 5.54(t_R/W_h)^2$$

or

$$N = 16(t_R/W)^2$$

Where W_h is the peak width at half height. The plate height is achieved from the following:

$$H = L/N$$

Where L is equal to the column length. For comparative purposes the plate height is more appropriate as it expresses efficiency for unit column length. The plate number increases with column length. More detailed explanations of these chromatographic parameters can be found in books by Lindsay [3] and Heftmann [4].

The previous equations can be applied to all the liquid chromatographic techniques, which can be placed into four classes. Each depends upon a different mechanism to provide the selective interaction between solute and stationary phase required to produce a separation. These four classes are adsorption, partition, ion exchange and gel permeation chromatography. The general principles of each of these techniques is again detailed in the books by Lindsay and Heftmann.

For the separation and determination of ionic species, ion chromatography is predominantly used, which includes inorganic cation and anion exchange chromatography as

well as chelation ion exchange chromatography.

1.2. ION CHROMATOGRAPHY.

Ion chromatography (IC) is one of the most widely used and fastest growing chromatographic techniques. The term ion chromatography refers to the fast and efficient separation of all anions and cations. This generally involves the use of a high-performance anion or cation exchange column, although IC also covers other techniques such as ion-pair chromatography, ion exclusion chromatography and other chromatographic methods used for separating ionic species. Modern instrumentation allows detection of very low concentrations of ions (mg dm^{-3} to μg dm^{-3}). For the analysis of trace levels of anions in solution IC has few competitors. The determination of cations using IC has also become popular due to advances in types of substrate and more sensitive detection systems.

The basic principle of IC is the reversible interchange of ions between a solution (mobile phase) and a solid support (stationary phase). The stationary phase must carry fixed ionic functional groups which are generally covalently bonded to the support. These functional groups have exchangeable or mobile ions, held in place by electrostatic forces, which can be exchanged freely with ions within a sample. Both cation and anion exchange resins can be classed as either strong or weak, depending upon the nature of the bonded functional groups. Cation exchange resins with sulphonic acid groups, or anion exchange resins with quaternary amine groups, are highly ionised and called strong exchange resins. Resins with partially ionised bonded functional groups such as COOH, OH, PO_4^{3-} cation exchangers and NH_2 anion exchangers are termed weak exchange resins.

The following equations illustrate the exchange reactions which occur on cation exchange resins:

$$nResSO_3^-H^+ + M^{n+} \rightleftharpoons (ResSO_3)nM + nH^+$$

$$nResCOO^-H^+ + M^{n+} \rightleftharpoons (ResCOO)nM + nH^+$$

The same exchange reactions occur in anion exchange chromatography with the respective charges simply being reversed:

$$nResNR_3^+OH^- + A^{n-} \rightleftharpoons (ResNR_3)nA + nOH^-$$

$$nResNH_3^+OH^- + A^{n-} \rightleftharpoons (ResNH_3)nA + nOH^-$$

Where Res refers to the resin backbone. The selectivity orders of cations when using IC is dependent upon several controlling factors. When using sulphonic acid cation exchange resins the retention times for alkali and alkaline earth metal ions increases with increasing atomic weight, thus:

Ba(II) > Sr(II) > Ca(II) > Mg(II) and Cs(I) > Rb(I) > K(I) > Na(I) > Li(I).

Under similar conditions, certain transition metals and post-transition metal ions can be held more strongly than equally sized cations in the above series, for example Ag(I) and Tl(I) are retained for longer than Cs(I). The order of retention is also dependent upon charge. As a general rule tri-valent ions are retained longer than di-valent ions, which in turn are retained longer than mono-valent cations. It has also become clear that the ionic hydration of metal ions in solution influences the exchange selectivities. The ions which are the most strongly hydrated are those with the largest charge densities (ie. small and highly charged). In the above series of alkali and alkaline earth metals these are Li(I) and Mg(II). In the

alkaline earth metals, Mg(II) has a much larger charge density compared with Ba(II) which has a much greater atomic weight. Therefore Ba(II) is less hydrated in aqueous solution with its hydrated radius being smaller than that of Mg(II). This means the increase in size of the hydrated radius is opposite to the increase in size of the unhydrated ion and it is the less hydrated ions which are attracted the greatest to the ion-exchange sites.

As well as the basic ionic characteristics of the analyte cations, the selectivity and thus the degree of separation is also dependent upon the type of ionic group bonded to the stationary phase, the eluent used and any complexing agents employed within it, the size of the ion-exchange resin particles and the size of the column being used. The use of complexing agents within the eluent can greatly enhance the separations achieved and are generally employed to reduce retention times of the metal ions by forming complexes with the ions and speeding up the elution process. Several commonly used complexing agents include carboxylic acids, such as tartaric and citric acids which are classed as relatively strong complexing agents and weaker ligands which include acetic acid, lactic acid and succinic acid. An example of this approach is the use of the ethylenediammonium cation and either a tartrate or α-hydroxybutyrate anion, contained in the eluent. The latter two anions act as complexing ligands, L^{2-}, which partially complex with the metal ions and change the ion-exchange equilibrium, causing the metal to elute more rapidly. This can be illustrated in the following equation:-

$$\text{Res-M}^{2+} + \text{EnH}_2^{2+} \rightleftharpoons \text{Res-EnH}_2^{2+} + \text{M}^{2+}$$

$$\text{M}^{2+} \rightleftharpoons \text{ML} \quad (\text{with } L^{2-})$$

The degree of complexation is controlled through the pH of the eluent, with maximum complexation occurring when the molecule is in a fully ionized state. However, the degree of complexation should be kept as only partial, otherwise if the metal ions are complexed too strongly they will elute too rapidly and no separation will occur.

1.2.i. RECENT ADVANCES IN ION CHROMATOGRAPHY.

The advancement of ion chromatography over the years, from its early beginnings to its present day status, has been reviewed by a number of authors. Small [5] has produced a historical account of ion chromatography's development, including the emergence of efficient conductimetric detectors, new ion exchangers and the adaption of ion chromatography to new applications. Similar reviews have been produced by Fritz [6-7] and Haddad and Heckenberg [8]. The history of ion chromatography has also been discussed in books on the subject by Smith and Chang [9] and Gjerde and Fritz [10]. The development of the technique into an efficient analytical method for both anions and cations has led to a huge number of applications being published. An extremely comprehensive review of ion chromatography's role in the analysis of environmental samples including natural waters, soils, sediments, sludges, plant tissues, atmospheric samples and fossil fuels has been compiled by Frankenberger et al. [11]. The review also placed emphasis on the separation of different species of certain ions and the separation of less common ions which can be found in environmental samples.

The separations of various groups of metal ions using ion chromatography have been tabulated by Challenger [12]. The experimental conditions required for the separations of several metal ion groups are outlined with brief comments on their application and the results

achieved. The development of more efficient stationary phase supports has led to an increase in the number of metal ions which can be separated simultaneously using ion chromatography. The separating power of these resins can be optimised through modifications to the eluent.

The separation of ten mono- and di-valent cations in less than 20min has been illustrated by Reiffenstuhl and Bonn [13]. Using an eluent containing ethylenediamine and citric acid combined with a TSK IC cation exchange column, baseline separation of a mixture of ten alkali, transition and heavy metals was achieved and detected using conductivity.

Saraswati [14] described the separation of up to 22 transition and lanthanide metal ions in under 38min, using a 5μm ODS 1 separator column with gradient elution and an octanesulphonate and tartaric acid eluent. Detection was achieved using post column derivatization and visible detection and the technique was applied to the analysis of magnetic alloys.

The recent development of a novel weak cation exchange column (IonPac CS12, Dionex Corp.) has been reported by Jensen et al. [15]. The resin contains covalently bound carboxylate groups as the cation exchange sites, instead of the conventional sulphonated resins. The column exhibits much smaller differences in the ion exchange selectivities between the alkali and alkaline earth metals compared with sulphonated resins and therefore the baseline separation of eight alkali and alkaline earth metals in under 10min was possible using a methanesulphonic acid eluent and gradient elution. The support itself is an ethylvinylbenzene-divinylbenzene polymer and is highly cross-linked, which enables either aqueous or organic solvents to be used as the mobile phase.

Several recent publications have detailed the development of ion exchange systems capable of simultaneous anion and cation separations using a single column. Yan and Schwedt

[16] have reported the separation of inorganic and organic anions and alkaline earth metals simultaneously using silica-gel based anion exchangers with chelating agents. Various mixtures of up to 10 of the above analytes were separated in under 20min using ethylenediaminetetraacetic acid (EDTA) and 1,2-diaminocyclohexanetetraacetic acid (DCTA) eluents. Detection was achieved using a UV detector with metal ions causing a reversed negative signal upon elution. The technique was applied to the determination of certain organic and inorganic anions and cations in natural samples such as drinking water.

In a similar but more recent application, EDTA has again been used in the eluent for the separation of inorganic anions and cations by Goa et al. [17]. A silica based Shim-pack WAX-1 column was used for the separation of Ca(II), Mg(II), chloride and nitrate, using a phthalate/EDTA eluent. The separation was achieved in under 6min and detection achieved using indirect photometric techniques. The separation was applied to the determination of the above in natural and potable waters.

Increasing research into new substrates and modified eluents has enabled ion chromatography to be applied to the analysis of more and more complex samples. This is illustrated by Singh et al. [18] who have produced a technique for the simultaneous determination of Na(I), Mg(II), Ca(II) and Sr(II) in high salinity sub-surface waters using suppressed ion chromatography and an ethylenediamine-hydrochloric acid-Zinc(II) eluent. The sub-surface brine sample contained much larger amounts of Ca(II) than Sr(II). Previous cation exchange chromatographic methods resulted in Sr(II) eluting immediately after Ca(II). This would cause problems when analysing the above sample, as the Sr(II) would be masked by the larger Ca(II) peak. Altering the eluent to move Sr(II) away from the Ca(II) peak would cause the small Sr(II) peak to broaden and thus cause a loss of signal. Investigation into the above eluent has enabled the rapid separation of the four metals in under 8min with sufficient

resolution between Sr(II) and 20 times higher concentrations of Ca(II) and 125 times higher levels of Na(I).

Ion chromatography has also been used by Marr [19] in the analysis of oil-well brines. Here, suppressed ion chromatography was used for the analysis of sulphate in both the above brines and seawater, although substantial dilution of the samples was necessary to reduce the ionic strength effects caused by these complex sample matrices. It is in this area of complex sample matrices, including samples of high salt concentrations, that chelation ion chromatography exhibits several advantages over simple ion exchange techniques and this is discussed in more detail in the following section.

1.3. CHELATING ION EXCHANGE CHROMATOGRAPHY.

A major problem with conventional ion exchange chromatography is the inability to cope with samples of high ionic strength. Samples containing very high concentrations of salt ions can cause ion exchange sites to become "swamped" with these ions, resulting in large unwanted peaks and altered retention times. A large proportion of environmental, biological and industrial samples often contain high salt levels and so an answer to the above problem has been sought and found in chelation ion chromatography. The immobilisation of chelating functional groups onto stationary phase supports, either through chemical bonding or impregnation techniques, results in a chelating ion exchange material which exhibits retention of metal ions as a function of the conditional stability constants of each metal chelate and shows little or no affinity for monovalent salt ions. These conditional stability constants vary for each metal chelate and show large differences between mono-, di- and tri-valent metals. This means chelating ion exchange chromatography can be highly selective for particular

metal ions, which is an advantage in the analysis of trace metals in certain complex samples. However chelation ion exchange kinetics are slower than simple ion exchange and so high-performance separations of metal ions using chelation ion chromatography is more difficult, due to broader peaks. This probably accounts for the relative lack of publications involving high-performance chelation ion chromatography and the use of chelation ion chromatography mainly as a matrix elimination and metal preconcentration technique, often using large particle size stationary phases.

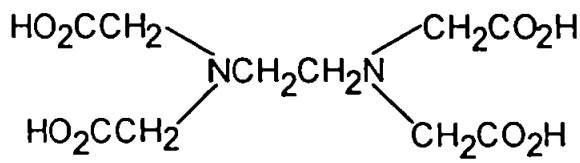
1.3.i. PRINCIPLES OF CHELATING ION EXCHANGE CHROMATOGRAPHY.

The branch of inorganic chemistry which is concerned with the behaviour of cations and ligands is termed coordination chemistry. A ligand is a species which is capable of donating an electron pair(s) to a metal ion. This means the ligand is acting as a Lewis base. In accepting electron pairs, the metal ion acts as a Lewis acid. The donation of the electron pair(s) from ligand to metal establishes a coordinate bond. Acids and bases can be characterised as either hard or soft. A metal ion is a hard acid if it is small, carries a high positive charge and is not easily polarised. A metal acts as a soft acid if it is the opposite of the above ie. is large, with a low charge and several easily excited outer electrons. A ligand acts as a hard base if the donor atom is of low polarizability, difficult to oxidise, has a high electronegativity and has high energy empty molecular orbitals. A soft base has the opposite of the above characteristics. It is a general rule that hard acids form stronger bonds with hard bases and likewise with soft acids coordinating with soft bases, although this is not always the case. Examples of metals which act as hard acids include alkali metals, alkaline earths, Al(III), Cr(III), Fe(III) and the rare earth metal ions. Soft acid metals include Hg(II), Cd(II),

Ag(I) and Au(III). Many of the transition metals including Zn(II), Cu(II), Ni(II), Co(II) and Fe(II) can be termed as exhibiting intermediate hardness. Hard bases include F^- and ligands containing oxygen ie. H_2O, CO_3^{2-}, NO_3^- or SO_4^{2-}. Soft bases generally have phosphorus or sulphur donor atoms such as SCN^- or $(RO)_3P$. Ligands containing nitrogen often show intermediate hardness, these include NH_3, NO_2^- and RNH_2.

The number of electron pairs complexed with a single metal ion is termed the coordination number. Coordination numbers can range from 2-12, but by far the most common is 6, followed by 4. The coordination number 2 is limited to the monovalent cations Cu(I), Ag(I) and Au(I), numbers 3 and 5 are rarely encountered and numbers above 6 are mainly experienced in the second and third series transition elements. Ligands can be classified as to the number of electron pairs available for donation to a metal ion. Ligands such as H_2O, NH_3, OH^- and CN^- have only a single electron pair which they can donate and are termed unidentate ligands. Those ligands capable of donating more than a single electron pair, from different atoms in the ligand are termed multidentate ligands or chelates. An example of a bidentate ligand ie. contains two donor atoms, is ethylenediamine (1,2-diaminoethane) (en), $NH_2CH_2CH_2NH_2$, which contains two nitrogens as the ligating atoms. An example of a hexadentate ligand is ethylenediaminetetraacetic acid (EDTA), which contains 4 oxygen and 2 nitrogen ligating atoms. The complexation of metal ions within these multidentate ligands often takes the form of a cyclic structure and is termed chelation. Ligands acting in this way are termed chelating agents and the complexes formed, chelates. Figure 2 illustrates some typical examples of chelating agents. The donor atoms of chelating agents may be part of an acidic or basic functional group. The acidic functional groups lose a proton when coordinating with a metal ion and are commonly, $-CO_2H$, $-SO_3H$, $PO(OH)_2$, -OH, =NOH and -SH. A basic group is one which contains an atom with a lone pair of

A



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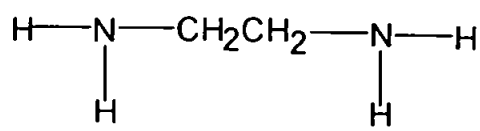

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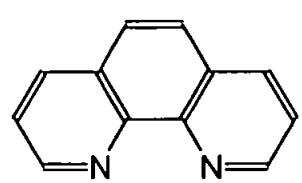


Figure 2. Typical examples of chelating agents, A) ethylenediaminetetraacetic acid (EDTA), B) ethylenediamine (1,2-diaminoethane) (en), C) 1,10-phenanthroline.

electrons which may interact with either a metal or a proton. Examples are $-NH_2$, -NH-, -N=, =O and -O-.

The coordination bonds formed between a chelating agent and a metal ion are more stable than those bonds formed with a non-chelating ligand. This rule is known as the chelate effect. A typical example is the reaction between hydrated Ni(II) ions and ammonia and a similar reaction between Ni(II) and ethylenediamine in solution:-

$$Ni[(H_2O)_6]^{2+} + 6NH_3 \rightleftharpoons Ni[(NH_3)_6]^{2+} + 6H_2O \qquad \log \beta_6 = 8.6$$

$$Ni[(H_2O)_6]^{2+} + 3en \rightleftharpoons Ni[en_3]^{2+} + 6H_2O \qquad \log \beta_3 = 18.3$$

From the previous two equations, and the stability of the resulting complexes expressed as their overall constants, it can be seen that ethylenediamine chelate is 10^{10} times more stable than the ammonia complex. The reason for this is based upon thermodynamic stability and the changes in enthalpy and entropy which occur during the reaction. In the above reaction both enthalpy and entropy favour the chelate formation. Yet the effect of enthalpy is very small and it is usual for entropy to play the major role in the formation of chelates. It is therefore considered that the chelate effect is essentially an entropy effect. The Ni(II) in the previous equations is initially coordinated by six H_2O molecules. With the addition of six ammonia molecules, the H_2O is replaced resulting in a single $Ni[(NH_3)_6]^{2+}$ complex and six H_2O molecules. The net change in the number of free molecules is zero. However with the addition of only three ethylene diamines, six H_2O molecules are still liberated resulting in an increase in the overall number of free molecules of 4 to 7. This net increase of 3 individual molecules causes a greater increase in disorder than the NH_3 reaction

and therefore an increased entropy which is directly related to the overall stability constants shown above.

Reactions occurring in solution between metal ions and complexing ligands can be expressed in terms of the equilibrium stability constants mentioned previously. A metal ion (M) and a monodentate ligand (L) in solution, can be described in the following way, provided no insoluble species or species containing more than one metal ion exist:

$$M + L = ML \qquad K_1 = [ML]/[M][L]$$

$$ML + L = ML_2 \qquad K_2 = [ML_2]/[ML][L]$$

$$ML_2 + L = ML_3 \qquad K_3 = [ML_3]/[ML_2][L]$$

$$\downarrow \qquad \downarrow \qquad \downarrow$$

$$ML_{n-1} + L = ML_n \qquad K_n = [ML_n]/[ML_{n-1}][L]$$

In the previous equations n represents the maximum coordination number of the metal ion, M, complexed with the ligand, L. The previous equations can also be expressed as follows for the formation of the product ML_n:

$$M + L = ML \qquad \beta_1 = [ML]/[M][L]$$

$$M + 2L = ML_2 \qquad \beta_2 = [ML_2]/[M][L]^2$$

$$M + 3L = ML_3 \qquad \beta_3 = [ML_3]/[M][L]^3$$

$$\downarrow \qquad \downarrow \qquad \downarrow$$

$$M + nL = ML_n \qquad \beta_n = [ML_n]/[M][L]^n$$

The relationship between β_n and K_n is $\beta_n = K_1K_2K_3....K_n$. K_n are known as stepwise

stability constants and β_n are overall stability constants. Information on the relative concentrations of various species can be gained from stepwise stability constants, whereas overall stability constants convey information on all the complexed species present combined. For the majority of systems the values of K, expressed as log K, decreases with each step. The following Cd(II)/CN^- system is a typical example:

$$Cd^{2+} + CN^- = [Cd(CN)]^+, \qquad \log K_1 = 5.48$$

$$[Cd(CN)]^+ + CN^- = [Cd(CN)_2], \qquad \log K_2 = 5.12$$

$$[Cd(CN)_2] + CN^- = [Cd(CN)_3]^-, \qquad \log K_3 = 4.63$$

$$[Cd(CN)_3]^- + CN^- = [Cd(CN)_4]^{2-}, \qquad \log K_4 = 3.55$$

The overall stability constant for the above system and the formation of $[Cd(CN)_4]^{2-}$ is the sum total of log K_1, log K_2, log K_3 and log K_4 which equals 18.78, therefore,

$$Cd^{2+} + 4CN^- = [Cd(CN)_4]^{2-}, \qquad \log \beta_4 = 18.78$$

In the calculation of the above stability constants, certain conditions are assumed to be constant, namely room temperature, pressure and zero or 0.1M ionic strength. It should be stated that the majority of these values are expressed in relation to concentrations and true stability constants can only be calculated using activities. A further assumption of stability constants is the presence of only a single ligand species in solution, existing in a 100% ionised form. This however is not the case for a large number of ligands and metals, which can exist as a number of species dependent upon the conditions in solution, principally pH. A change in pH can drastically alter the concentration of metal or ligand species in solution

which is in a reactive form. This would cause a change in the overall stability constant, to what is described as the conditional stability constant, or K'. It is the ability to control K', and therefore the stability of the complexes involved, through manipulation of pH, which enables the complexation of metals to be controlled on immobilised ligands or chelates. The expression for the conditional stability constant is as follows:

$$K'(ML) = K_{M'L'} = [ML]/[M'][L']$$

In the above equation the concentration terms [M'] and [L'] should include all species of metal ion and ligand which are not in the form ML. The principle of conditional stability constants is illustrated with the example of EDTA. EDTA can exist as any one of five species in solution depending upon the pH, this can be seen in Figure 3 . The five species of EDTA are H_4EDTA, H_3EDTA^-, H_2EDTA^{2-}, $HEDTA^{3-}$ and $EDTA^{4-}$. The EDTA species becomes more protonated as the pH increases in acidity. Thus in solution at pH 12 in the presence of no other species, 98.5% of the EDTA present is in the form $EDTA^{4-}$, at pH 8.1, 98.1% is in the form $HEDTA^{3-}$, pH 4.4 sees 96.2 as the H_2EDTA^{2-} species and at pH 2.3, 52.1% is present as the H_3EDTA^- species. Between pH 2 and 0 the fully protonated H_4EDTA rapidly becomes the most dominant species. Shown in Figure 4 are the conditional stability constants, log $K_{M'Y'}$, of various metal EDTA complexes as a function of pH. Maximum conditional stability constants for the majority of metals lies between pH 3 and 10. Below pH 3 the increasing concentration of non-complexing H_4EDTA causes the decrease in complexed metal ions and above pH 10 many metals form hydroxy species. To summarise, absolute stability constants are based upon the ligand, in this case EDTA being in a fully ionised form ie. $EDTA^{4-}$, and the metal being in a single ionic form. Conditional stability

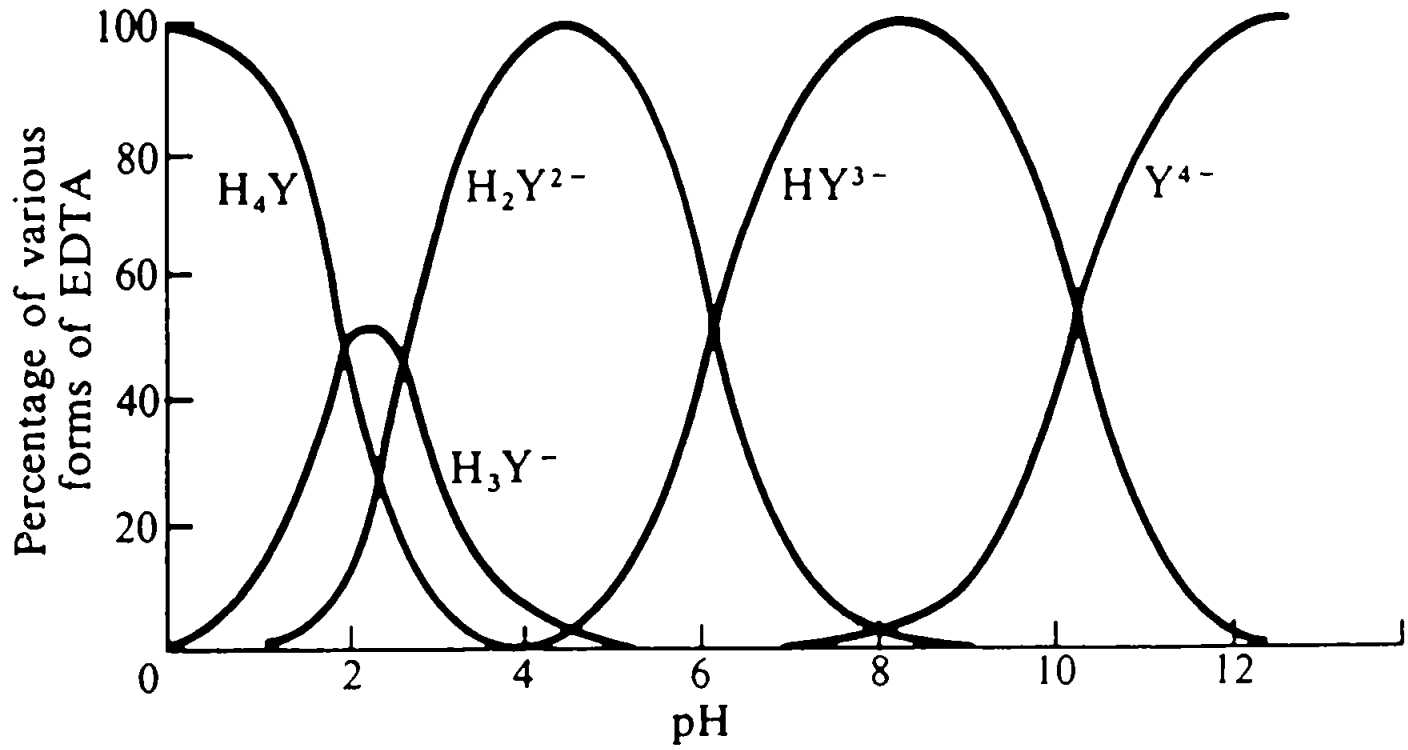


Figure 3. Variation with pH of the different forms of EDTA [20].

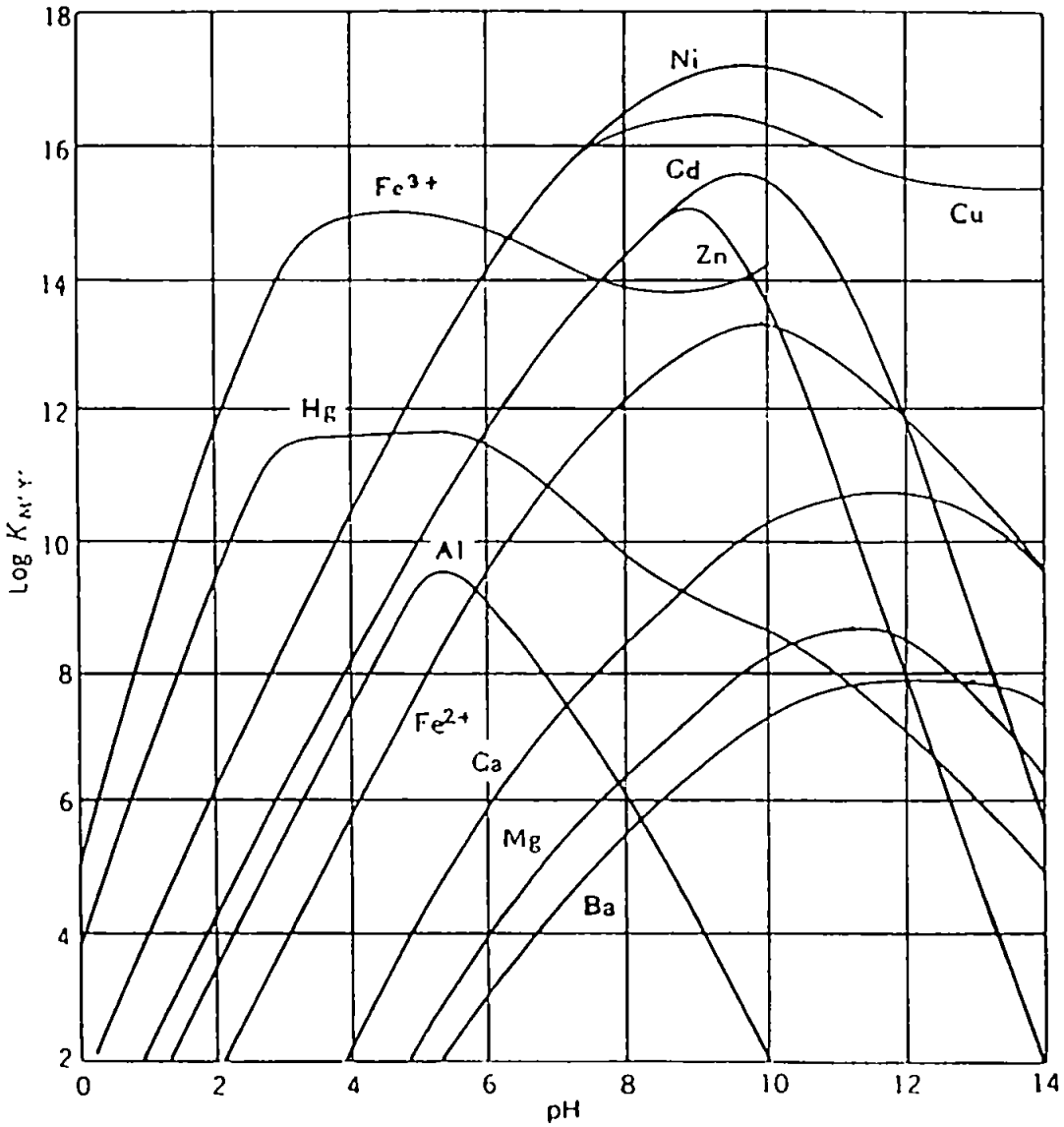


Figure 4. Conditional stability constants, log $K_{M'Y'}$, of various metal EDTA complexes as a function of pH [21].

constants however, take into account all the EDTA species present, L' being the sum of the concentrations of all the species, and M' takes into account all the metal species present. It is also necessary to consider the effects of ionic strength and to a lesser extent temperature, when applying stability constants to certain situations, for example in the analysis of seawater. Corrections to stability constants due to changes in temperature can be calculated if accurate values for the heat of reaction have been measured. Corrections for ionic strength are more difficult and certain estimations have to be made on the basis of trends in measured values. Table 1 shows the variation of stability constants, log K_1 and log β_2 due to changes in ionic strength (the changes are in relation to a solution of 0.1M ionic strength). It should be noted that the variation increases as a function of increasing charge on both the metal ion and the ligand. It is also clear that the largest variations occur between 0 and 0.1M and between 0.1M and 0.5M. Increasing the ionic strength above 0.5M appears to have a less drastic effect, with relatively small changes occurring between 0.5 and 3M. This can be important as many of the samples investigated using chelation techniques lie within or above these values.

1.3.ii. CHELATING RESINS.

Chelating ligands, the principles of which have been discussed in the previous section, immobilised onto stationary phase supports, maintain the ability to chelate with metal ions and thus remove them from solution. This process is termed chelating ion exchange chromatography. The selectivity of these chelating substrates can be controlled by altering the conditional stability constants of the chelates, through the pH of the eluent. This allows metals to be retained at high pH and eluted using an eluent with a low pH. Chelating supports

Ionic Strength		0.0	0.5	1.0	2.0	3.0
				M^{+}		
L^{-}	log K_1	+0.2	-0.1	-0.1	0.0	+0.2
	log β_2	+0.3	-0.1	0.0	+0.3	+0.6
L^{2-}	log K_1	+0.4	-0.2	-0.2	-0.1	0.0
	log β_2	+0.6	-0.4	-0.4	-0.3	0.0
L^{3-}	log K_1	+0.6	-0.3	-0.3	-0.3	-0.2
	log β_2	+1.0	-0.5	-0.5	[-0.4]	[-0.2]
				M^{2+}		
L^{-}	log K_1	+0.4	-0.2	-0.2	-0.1	0.0
	log β_2	+0.6	-0.4	-0.4	-0.3	0.0
L^{2-}	log K_1	+0.8	-0.4	-0.4	-0.4	[-0.3]
	log β_2	+1.2	-0.8	-0.8	[-0.7]	[-0.5]
L^{3-}	log K_1	+1.2	-0.6	-0.6	[-0.7]	[-0.6]
	log β_2	+1.8	[-1.0]	[-1.0]	[-1.0]	[-0.9]
				M^{+3}		
L^{-}	log K_1	+0.6	-0.3	-0.3	0.3	-0.2
	log β_2	+1.0	-0.5	-0.5	[-0.4]	[-0.2]
L^{2-}	log K_1	+1.2	-0.6	-0.6	[-0.7]	[-0.6]
	log β_2	+1.8	[-1.0]	[-1.0]	[-1.0]	[-0.9]
L^{3-}	log K_1	[+1.8]	[-0.9]	-0.9	[-1.1]	[-1.0]
	log β_2	[+2.6]	[-1.5]	[-1.5]	[-1.6]	[-1.5]
				M^{4+}		
L^{-}	log K_1	[+0.8]	[-0.4]	[-0.4]	[-0.5]	[-0.4]
	log β_2	[+1.6]	[-0.6]	[-0.6]	[-0.6]	[-0.5]
L^{2-}	log K_1	[+1.6]	[-0.8]	[-0.8]	[-1.0]	[-0.9]
	log β_2	[2.4]	[-1.4]	[-1.4]	[-1.5]	[-1.4]
L^{3-}	log K_1	[2.4]	[-1.2]	[-1.2]	[-1.5]	[-1.4]
	log β_2	[+3.4]	[-2.0]	[-2.0]	[-2.2]	[-2.1]

Table 1. Relative change in stability constants with ionic strength and charge. Values in [] are based upon trends [22].

can be divided into two groups based upon their manufacture. Firstly the physical covalent bonding of chelating functionalities to various supports and secondly the impregnation or 'coating' of stationary phase material with organic compounds which contain chelating groups, resulting in their permanent immobilisation under normal working conditions.

1.3.ii.a. CHELEX-100.

By far the largest proportion of chemically bonded chelating substrates, utilise the iminodiacetic acid (IDA) functional group, $NH(CH_2COOH)_2$. Some of the earliest substrates studied contained the IDA chelating group, including Dowex A-1, Chelex-100, Chelex-20 and Muramac A-1. The most commonly used of these, Chelex-100, has found wide application by numerous workers in the preconcentration of trace metals. Chelex-100 has the IDA functional group covalently bonded to a styrene-divinylbenzene polymer, which is only 1.5 to 2.0% cross-linked, resulting in substantial volume changes with changes in pH.

Chelex-100 was first investigated for the analysis of trace metals in seawater in 1968 by Riley and Taylor [23]. Since then many more detailed studies have been undertaken, investigating the suitability of Chelex-100 for trace metal preconcentration. Luttrell et al. [24] determined the effect of pH and ionic strength on both the ion exchange and chelating exchange properties of Chelex-100 with alkaline earth metals. Luttrell et al. found, using a column procedure, that Chelex-100 reacts with alkaline earth metals primarily by simple ion exchange at pH values below 4 and chelation becomes the dominant retention process at pH values above 6. Intermediate values between 4 and 6 result in both chelation and ion exchange. This phenomena was put forward as the reason for the elution curves showing double peaks. Both ion exchange and chelation are pH dependent, relating to the number of

ionic sites within the resin. Luttrell et al. quote the half neutralisation point (the point at which 50% dissociation of the diacetate groups occurs) at a pH of approximately 5.6 $\pm$ 0.2, which explains the rapid increase in chelation above this pH.

An early application of Chelex-100 for the analysis of trace metals was published by Olsen et al. [25]. Using a combination of flow injection analysis and FAAS, trace amounts of heavy metals in polluted seawater were determined, after being preconcentrated on-line with a micro-column of Chelex-100. Detection limits of 10μg dm^{-3} for Pb(II) and 1μg dm^{-3} Cd(II) and Zn(II) were obtained with a sampling rate of between 30 and 60 samples per hour, preconcentrating 150μl. A pH of 10 was required for the quantitative recovery of Cd(II), below this value non-linear responses were obtained.

Wada et al. [26] used Chelex-100 for the preconcentration and determination of Ca(II) and Mg(II) in brines, again using a flow injection system. Chelex-100, with the iminodiacetic acid functional group, was compared with a chelating resin with aminophosphate bonded groups (Duolite ES-467). Although it showed a greater adsorption of Ca(II) and Mg(II), it required more acidic eluents to elute the metal ions, therefore Chelex-100 was employed. Concentrations of 1-30μg dm^{-3} of Mg(II) and 8-120μg dm^{-3} of Ca(II) were determined in 2.5M sodium chloride by preconcentrating 9ml of sample at pH 10.5. Detection of metal ions was achieved using a spectrophotometric method based upon the formation of metal complexes with 1-(2-hydroxy-4-diethylamino-1-phenylazo)-2-hydroxynapthalene-3,6-disulphonic acid.

Pai et al. [27] has investigated the efficiency of Chelex-100 resin for the preconcentration of heavy metals, again from a seawater matrix. Batch equilibrium and breakthrough experiments showed the metal chelating efficiency of Chelex-100 was greater for freshwater than seawater. The reasons for this are the complicated stable metal species

present in seawater and the high concentrations of Ca(II) and Mg(II) present which act as competitors to the heavy metals of interest. Pai concluded that the salt matrix has a major effect upon the optimum pH values for preconcentration and careful choice of operational conditions is necessary to avoid loss of certain metal analytes of interest, especially Mn(II) and Cd(II). Pai also suggested the pH of the seawater sample be adjusted to pH 6-7, as this produced the best performance of the column, however pH values above this cause rapid formation of hydroxide species. Detection of the metal ions was carried out using AAS. A second publication by Pai [28] detailed a plate simulation method to determine the distribution of heavy metals on a Chelex-100 column and the optimum column conditions for maximum preconcentration efficiency. A series of ten Chelex-100 mini-columns containing 0.4g of resin were used to preconcentrate spiked levels of heavy metals in a seawater sample. From the distribution of the metals over the string of mini-columns, the size of column needed to remove 100% of the metal ions of interest can be determined. It was found that a column containing 2g of resin was suitable for the recovery of Cd(II), Cu(II), Co(II), Mn(II), Ni(II), Pb(II) and Zn(II) from seawater, at pH 6.5, at a flow rate of 4 ml min^{-1}, although Fe(III) and Cr(III) were not able to be preconcentrated in this way and Mn(II) and Cd(II) were not quantitatively recovered at pH values below 6.5. Samples of seawater were analysed using the Chelex-100 preconcentration method and GFAAS used as the detection system. Detection limits of 1-100ng dm^{-3} were achieved, preconcentrating 500ml of sample.

Chelex-100 has been used by Liu and Ingle [29] for the automated on-line preconcentration of trace metals from river water with FAAS detection. The studies revealed retention of metal ions on Chelex-100 is improved with lower sample loading flow rates, longer columns and larger inner diameter columns. However, lower loading rates increases sampling time and larger column bed volumes cause peak spreading and results in a higher

detection limit. The resin size was also found to affect the uptake of metal ions due to active surface area differences. The investigation concluded the smallest Chelex-100 column which could retain 500μg dm^{-3} Cu(II) completely at a flow rate of up to 15 ml min^{-1} was 3mm id x 50mm long with a particle size of 150-300μm. Liu and Ingle also removed the need for strong acid eluents (which can cause contraction of the resin) to be used to elute the metal ions by employing the complexing ligand EDTA in the eluent. The method was used for the determination of Cu(II) in a river water sample at levels of 0.44 $\pm$ 0.08μg dm^{-3}. Liu and Ingle have also used Chelex-100 as part of an automated two column ion exchange system for trace metal speciation studies in natural waters [30]. Flame AAS was used to detect metal ions which had been preconcentrated by sequential columns of Chelex-100 and AG MP-1 macroporous strongly basic anion exchange resin. The method divided the dissolved trace metal species into three fractions. Hydrated labile metal ions and metal ions dissociated during passage through the Chelex-100 are retained upon the resin and form fraction 1. Non-labile, strongly complexed metal ions pass through the Chelex-100 column and the negatively charged species, including anionic humic materials, are retained on the AG MP-1 column and form fraction 2. Neutral species and species with molecular weight greater than 75,000 pass through both columns and make up fraction 3. This method was used for the determination of Cu(II), Cd(II) and Zn(II) species in river and ditch water samples. The results showed as much as 85% of the Cu(II) in river water and 93% in ditch water exists as non-labile, complexed negatively charged species. Results for Cd(II) and Zn(II) in the same samples showed these values to be much less, ranging from undetectable amounts to 5%. The above results for Cu(II) species would indicate the need for sample pretreatment such as acid digestion or UV photolysis, if Chelex-100 is to be used for total Cu(II) determinations in natural water samples.

Comber [31] has used Chelex-100 as part of an ion chromatography system for the analysis of trace metals in saline waters. Comber outlined two problems with commercially available chelating resins being used as part of ion chromatography systems. Firstly, the inadequate selectivity of many resins which results in the retention of a proportion of the unwanted major ions, Mg(II) and Ca(II), and secondly, the need for very acidic mobile phases to elute complexed metal ions on certain strongly chelating materials. Comber claimed to have overcome these problems by using a two column preconcentration step, with initial enrichment using Chelex-100. Calcium(II) and Mg(II) were removed from the Chelex-100 using ammonium acetate and the strong acid required to elute the analyte metals from the resin was removed using a strong cation exchange column which retained the metal ions from the acid plug. These were then swept off the second column using a pyridine-2,6-dicarboxylic acid eluent and separated on a Dionex CS5 analytical column prior to post column reaction visible detection. Analysis of Cu(II), Ni(II), Zn(II), Co(II) and Mn(II) in estuarine water samples was carried out at low $\mu g\ dm^{-3}$ levels, with results comparing well to GFAAS, except Cu(II) which again appeared lower using the chelation technique.

The preconcentration of trace levels of transition metals and rare earth elements in highly saline solutions using Chelex-100 has been investigated by Strachan et al. [32]. Quantitative recovery of Ce(III), Co(III), Eu(III), Fe(III), Gd(III), Y(III) and Zn(II) from saturated brines was achieved. The preconcentration of Mn(II) was found to be adversely affected by the ionic strength of the sample and the quantitative recovery of Cr(III) was possible but its total elution caused several problems.

A recent paper by Reimer and Miyazaki [33] has combined the preconcentration of Pb(II) from seawater using Chelex-100 with hydride generation and detection using ICP-AES. Pb(II) was quantitatively preconcentrated at pH 6.0 with a flow rate of 2.0 ml min^{-1}.

Calcium(II) and Mg(II) which cause spectral interferences when using ICP-AES were removed using ammonium acetate. Levels of 40-50ng dm^{-3} Pb(II) were determined in open seawater using this method and a detection limit of 6ng dm^{-3} was quoted when preconcentrating 1litre of sample.

A summary of the metals preconcentrated using Chelex-100 is shown in Table 2.

1.3.ii.b. OTHER IMINODIACETIC ACID BASED CHELATING RESINS.

Other chelating substrates have been produced from chemically bonding iminodiacetic acid (IDA) to stationary phase supports. A highly cross-linked macroporous resin, MetPac CC-1 (Dionex Corp.), has been produced from chemically bonding IDA functional groups to a polystyrene divinylbenzene copolymer support. The higher percentage of cross-linking of the MetPac CC-1 resin compared with that of Chelex-100, means that the problem of volume changes, arising from changes in ionic form, is no longer a problem. Cardellicchio et al. [35] used the MetPac CC-1 column as part of a chelation ion chromatography system to determine Cd(II) and Pb(II) at $\mu g\ dm^{-3}$ levels in aqueous matrices. The system contained a combination of both chelating and analytical cation ion exchange columns with complex switching arrangements.

Seawater was preconcentrated onto the MetPac CC-1 column and the alkali and alkaline earth metals removed with ammonium acetate. The preconcentrated metals were then eluted and separated on a cation exchange column with detection using post column derivatisation with 4-(2-pyridylazo) resorcinol (PAR). Detection limits were sub-$\mu g\ dm^{-3}$ for Cd(II) and Pb(II). A gradient elution programme allowed the elution of Fe(II), Co(II), Ni(II), Zn(II), Cu(II) and Mn(II) between the two analyte metal peaks and so no interference was

Preconcentration of Metal Ions By Chelex 100

Metal ions	Soln pH	Wash soln	Eluent
U	3.0—4.0	0.1 *M* NH_4Ac	—
Cd, Zn, Pb, Cu, Fe, Mn, Co, Cr, Ni	5.4	0.1 *M* NH_4Ac	2.5 *M* HNO_3
U	4.0	0.1 *M* NH_4Ac	2.0 *M* HNO_3
Ni, Mn, Zn, Cu, Pb	5.0—5.5	1.0 *M* NH_4Ac	2.5 *M* HNO_3
Al, Cd, Co, Cr, Cu, Eu, Fe, Mn, Mo, Ni, Se, Sn Th, Ti, U, V, Zn	5.2—5.7	1.0 *M* NH_4Ac	—
Mo	5.0—5.5	Water	4 *M* NH_4OH
Al, Ba, Be, Cd, Co, Cu, Mn, Ni, Pb, Zn	9	0.5 *M* NH_4Ac	2.0 *M* HNO_3
Cr, Ti, V, Fe, Al	3.8	0.5 *M* NH_4Ac	2.0 *M* HNO_3
Al, Cd, Co, Cu, Fe, Mn, Mo, Ni, Pb, Ti, V, Zn	5.5—6.0	1.0 *M* NH_4Ac	2.0 *M* HNO_3
Fe, Mn, Cu, Ni, Cd, Pb, Zn	5.4	1.0 *M* NH_4Ac	2.0 *M* HNO_3
Al, As, Au, Ba, Br, Ca, Ce, Cl, Cr, Cu, Fe, Hg, K, La, Mg, Mn, Na, Sb, Sc, Se, Sm, Ti, V, Zn	5.0	—	—
Mn	8.0	—	3.0 *M* HNO_3
Bi	0.7	0.25 *M* HNO_3	2.0 *M* HNO_3
Al, Ti, V, Mn, Fe, Co, Ni, Cu, Zn, Y, Mo, Cd, Pb	6	1.0 *M* NH_4Ac	2.0 *M* HNO_3
Cd, Cu, Co, Mn, Cr, Zn, Ni, Pb, Fe	6.0—7.0	—	—
Cd, Cu, Cr, Fe, Mn, Ni, Pb, Zn	6.5	1.0 *M* NH_4Ac	2.0 *M* HNO_3
Cd, Co, Cu, Mn, Pb, Zn	5.7	0.4 *M* NH_4Ac 0.4 *M* NH_4NO_3	2.0 *M* HNO_3
Cd, Zn, Cu, Mn, Pb Fe(III), Cr(III)	3.0—5.0	—	2.0 *M* HNO_3
Cu, Cd, Mn, Pb, Zn	5.2	—	0.01 *M* Cys, pH 10 0.025 *M* EDTA, pH 8
Cu	6.8	—	0.02 *M* Cys
Cd			0.1 *M* EDTA
Zn			2.0 *M* HNO_3
Al	5.5	—	2.5 *M* NaOH

Note: NH_4Ac, ammonium acetate; Cys, L-cysteine.

Table 2. Preconcentration of metal ions with Chelex-100 [34].

experienced. A similar application is presented by Caprioli and Torcini [36] who employed the same system as above, using the MetPac CC-1 column, for the determination of Cu(II), Ni(II), Zn(II), Co(II) and Mn(II) in seawater. Detection limits for the above metals ranged from 0.05μg dm^{-3} to 0.5μg dm^{-3}, and results obtained for a seawater sample compared with a solvent extraction-AAS method were in good agreement. Caprioli and Torcini and Cardellicchio et al. point out the metals being determined using chelation ion chromatography are the dissolved labile fraction and not total levels. The IDA function is also found in the Tosoh TSK-Gel Chelate 5-PW column, a commercially available chelating column. The particle size of the Dionex MetPac CC-1 material is not stated, but is likely to be relatively large. The Tosoh resin is a 10μm polystyrene based material and exhibits sufficient efficiencies to enable the high-performance separation of certain metal ions. Applications of this column are detailed in section 1.3.iv.

Several other types of stationary phase have been used to support the IDA functional group. One such example is given by Horvath et al. [37]. Ethylcellulose has been chemically bonded with IDA and the resulting material investigated for the preconcentration of metal ions. The modified cellulose was applied to the analysis of trace metals in citrate or acetate solutions and the determination of Bi(III), Cu(II), Co(II), Cd(II) and Fe(III) in magnesium nitrate solution. Detection was achieved using ICP-AES. Complete elution of certain metal ions using 1.6M HNO_3 ie. Cr(III) and Al(III) was not possible, although 99% recovery of Cr(III) could be achieved through ashing of the stationary phase.

1.3.ii.c. OTHER TYPES OF CHELATING RESINS.

A large number of chelating resins have been prepared from chemically bonding other types of chelating functionalities to stationary phases. Leyden and Luttrell [38] have immobilised ethylenediamine and certain dithiocarbamates by reacting silica gel with various silylating reagents. The modified silica was used to preconcentrate metal ions which were then determined directly on the substrate using x-ray fluorescence. The resins were said to be easily prepared, with reasonable stability and have a possible application for trace metal determinations as part of a HPLC system. Dithiocarbamate resins are often used for the preconcentration of soft metal ions such as Hg(II). Commercial forms of poly(dithiocarbamate) (PDTC) resin exists as Sumichelate Q-10 (Sumitomo Chemicals, Japan) and ALM-125 (Nippon Soda Co. Ltd.). The Sumichelate Q-10 resin has been used by Minagawa and Takizawa [39] for the preconcentration of both organic and inorganic Hg(II) from natural waters. Hg(II) was preconcentrated at pH 2 at up to 30ml min^{-1} and eluted using 5% thiourea in 0.06M HCl. Detection was achieved using cold vapour AAS. Yamagami et al. [40] employed the ALM-125 chelating resin for the uptake of Hg(II) from natural waters. The resin was adjusted to pH 2-3 with 2M HCl prior to preconcentration. Recovery of the Hg(II) required wet digestion of the resin with concentrated HNO_3.

Another group of resins commonly employed for trace metal preconcentrations are the sulphur based resins which incorporate Dithizone functional groups. Grote and Kettrup [41] used a sulphur bonded dehydrodithizone chelation exchange resin for the preconcentration and separation of Au(III) and Pt(IV) group metals from large amounts of base metals and salts. The preconcentration of noble metals has been achieved by Huang et al. [42] using a Dithizone bonded polystyrene based resin. Platinum(IV), Ir(IV) and Au(III)

were removed from HCl rock digests at pH 1.5-2.0. The resin was ashed prior to analysis using neutron activation analysis.

Jonas et al. [43] have bonded 8-hydroxyquinoline to a gel type polystyrene divinylbenzene copolymer allowing certain separations of metal ions to be achieved, details of which are given in section 1.3.iv. Chambaz and Haerdi [44] have also used 8-hydroxyquinoline, bonded to silica gel, as a chelating substrate for the on-line preconcentration of trace metals prior to their separation using ion-pair chromatography followed by UV detection. Preconcentration volumes of 20ml resulted in detection limits of 5×10^{-6} to 5×10^{-8}M for certain transition metals. The sensitivity of the method was said to be reduced due to substantial blanks resulting from ultra-trace levels of metals in the reagents used. Chambaz and Haerdi have also investigated trace metal preconcentration on a N,N,N',N'-tetra(2-aminoethyl) ethylenediamine (PENTEN) bonded silica [45]. Cobolt(II), Ni(II), Cu(II), Zn(II), Cd(II) and Pb(II) were preconcentrated from various natural water samples and separated using ion chromatography with post column reaction detection. Detection limits (preconc. vol.=20ml) for all the above metals was 3×10^{-9}M and reproducibility (RSD, n=5) ranged from 1.8 to 3.6%.

Hutchinson et al. [46] have used sythetic macrocycles, known as calixarene tetrahydroxamates, chemically bonded to octadecylsilica and XAD-4 resins, for the preconcentration of transition metal ions from aqueous solutions. The chelating resins were packed into micro-columns and used for the quantitative preconcentration of trace levels of Cu(II), Zn(II) and Mn(II) from tap water prior to separation using ion chromatography.

In a recent paper by Blain et al. [47] a new commercially available chelating resin, Chelamine, based upon the bonding of 1,4,7,10,13-pentaazatridecane to an organic polymer, has been investigated for the preconcentration of trace metals from seawater. Blain et al.

stated how certain problems experienced when using IDA bound chelating resins to preconcentrate metals from seawater, including partial recovery of Mn(II) and Cd(II) and the loss of Mn(II) during the elution of Mg(II) and Ca(II), are due to the relatively poor selectivity of IDA. As transition metals are typically intermediate to soft acids and the alkali and alkaline earth metals are classed as hard acids, to maximise selectivity in favour of the transition metals a chelating agent containing only soft base groups should be chosen, in this case Chelamine (the IDA function contains oxygen atoms as part of the hard base group).

1.3.ii.d. COMPARISON OF CHELATING RESINS.

Several papers have been published on comparing various chelating resins for trace metal applications. Fang et al. [48] compared Chelex-100, azo-immobilised 8-quinolinol on controlled pore glass and a phenol formaldehyde based resin with bonded salicylate functional groups, for their preconcentration characteristics with special emphasis on interferences encountered during seawater analysis. The columns were investigated for their potentials as part of an on-line flow injection-AAS technique. Several general observations were made, firstly, the 8-quinolinol substrate gave the best performance for samples of low ionic strength. Secondly, Chelex-100 proved more successful in complex matrices such as seawater but experienced up to 40% loss of sensitivity because of unquantitative recoveries of certain metals, especially Cd(II). The salicylate chelating resin proved very similar to Chelex-100, without the troublesome swelling problems and was suggested as the best material of the three for seawater analysis, provided Cd(II) is not required.

Ryan and Weber [49] compared three chelating agents bonded onto porous glass silica beads with Chelex-100 for the removal and preconcentration of trace Cu(II). The

immobilised chelating agents included N-propylethylenediamine, it's bis-(dithiocarbamate) (DTC) and also 8-hydroxyquinoline (8-HQ). The addition of organic matter to the test Cu(II) solutions, at levels similar to natural freshwater caused reductions in the results achieved for all the substrates investigated. The DTC column proved most successful, removing 98% of the Cu(II) present, however the Cu(II) could not easily be eluted from the column without using strong acids. Chelex-100 was the poorest of the four chelating resins, only removing between 62 and 75% of the Cu(II). Overall the 8-HQ bound silica was found to be the most suitable for this particular application, although it was found longer columns and slower flow rates improved the results for all the substrates being studied.

Chelex-100 has been compared with several other chelating resins for the preconcentration of trace metals from concentrated salt solutions by Knapp et al. [50]. 8-Hydroxyquinoline bonded cellulose, CPPI chelating ion exchange resin (containing aminoacetic and iminodiacetic acid functional groups) and Hyphan chelating ion exchange cellulose were investigated for their suitability in the above area. Several disadvantages to each material were outlined. Chelex-100 and the Hyphan cellulose shrink considerably when in contact with acid eluents and the Hyphan substrate only complexes with Cu(II) to any appreciable extent ie. 80-90%. It was also noticed Al(III) and Ni(II) were not completely eluted from the 8-HQ cellulose and the CPPI resin using 2M HNO_3. It was concluded that all the chelating materials investigated, apart from the Hyphan cellulose, gave recoveries of between 90 and 103% for the following metals, Cd(II), Cu(II), Pb(II) and Zn(II), from concentrated salt solutions and no additional specific advantages were noticed for any of the chelating substrates, within the framework of the study.

Siriraks et al. [51] compared the Dionex MetPac CC-1 chelating ion exchange resin with Chelex-100 for the uptake of transition and heavy metals from seawater. The Chelex-100

showed several disadvantages when compared with the Dionex column. Firstly, the characteristic swelling of Chelex-100 caused problems when the resin was used in a packed column. The higher degree of cross-linking of the Dionex material solved this problem. A second disadvantage was the comparatively low recoveries of certain metal ions ie. Cd(II) and Mn(II) achieved using the Chelex-100 resin which was said to be a result of the physical degradation of the resin at pressures greater than 100psi. Again, the MetPac CC-1 did not suffer from this problem, being stable at much higher pressures.

In a recent paper by Baffi et al. [52] Chelex-100 was compared with Lewatit TP 207, a weakly complexing macroporous resin, for the preconcentration of Mn(II), Cu(II) and Cr(III) from seawater. The results showed the Lewatit TP 207 resin to be more successful for the preconcentration of Cr(III), being able to recover both colloidally bound and labile Cr(III) species. However, due to its generally weak chelation properties it produced lower values for Cu(II) compared with the Chelex-100 resin. The recoveries for Mn(II) were similar for both materials. Baffi et al. suggest the use of both chelating columns in sequence would provide information of Cu(II) speciation, due to organic Cu(II) species and colloidal species not being retained on the Lewatit TP 207 resin, but being retained by the Chelex-100 column. However, as many of the previous investigations have concluded, Chelex-100 is also unable to preconcentrate Cu(II) quantitatively in the presence of certain organic and colloidal species, so this is a questionable suggestion.

1.3.iii. IMPREGNATED SUBSTRATES.

An alternative to chemically bonding functional groups to supporting substrates, which can prove difficult, time consuming and costly, can be found in the impregnation of organic chelating compounds onto the surfaces and into the pore spaces of certain substrates, commonly macroporous polystyrene based resins or silica gels. The resulting immobilised chelating groups which usually contain ion exchange functionalities as well as chelating groups can then be used in a variety of applications such as matrix elimination, preconcentration, separation or a combination of the above. The advantages this approach has over chemically bonded substrates include the relatively low cost, ease of manufacture and the large range of complexes available which can be used for this purpose.

1.3.iii.a. IMPREGNATED ION EXCHANGE RESINS.

Impregnated substrates have been used for both anion and cation applications, using organic compounds containing ion exchange functional groups such as SO_3H. Golombek and Schwedt [53] impregnated a polystyrene-divinylbenzene (PRP-1) column with Methyl Green to produce an anion exchange column with absorbed quaternary ammonium groups. The baseline separation of eight common anions was achieved isocratically in under 15 minutes and the column was said to exhibit efficiency comparable to a commercial chemically bonded substrate, namely PRP-X 100. The inclusion of Methyl Green to the eluent was said to act both as a competing agent and to increase the lifetime of the column by continually replacing lost anion exchange sites. Muller and Meisch [54] investigated the impregnation of the silica gel RP-18 with Methylene Blue, Methyl Green and Crystal Violet for the separation of

inorganic anions. Efficient separation of up to seven anions was possible using a Crystal Violet anion exchange column without using organic modifiers or dyestuffs in the mobile phase. The working lifetime of these impregnated silica gels was quoted as being only 65h, this being mainly a consequence of using an eluent with an alkaline pH which rapidly degrades the silica substrate, a disadvantage silica gels concede to the polystyrene based polymers, such as the type used by Golombek and Schwedt. Work by Walker [55] in this area has involved Ethyl Violet both to impregnate 5 and 10μm polystyrene-divinylbenzene resins and 5μm ODS silica based packing material. Walker also used Ethyl Violet in the mobile phase allowing indirect visible detection of the eluting anions. Up to eight anions were separated in 30min. Three advantages were quoted for the use of polymer based substrates compared with silica. Firstly, the dye can easily be stripped from the polymer substrate using acetone and the material re-used, this was not so easy to do with silica. Secondly, the peak shapes were noticably poorer with the ODS substrate using similarly sized particles and reproducibility not as good as that achieved using polymeric supports. Thirdly, as mentioned by Miller and Meisch, polymeric supports have a much larger stability range, pH 1-13, unlike silica.

Walker [56] also used both silica based ODS substrates and polymer type resins as stationary phases to support Thymol Blue (TB), which contains cation exchange groups. Again high-performance grade substrates were used, the polymer based resins were 10μm polystyrene-divinylbenzene (PVB) PRP-1 Hamilton material and 5μm PVB PLRP-S Polymer Laboratories resin. The silica substrate used was 5μm B&J ODS silica gel. Walker also used TB in the mobile phase which enabled indirect UV detection to be used for the determination of metal ions. Separations of alkaline earth metals and transition metals were achieved using both types of impregnated substrate although in this case sharper peaks resulted from silica

columns. However the shorter lifetime of this type of column compared with polymer based supports meant the latter proved more successful for this particular application.

1.3.iii.b. IMPREGNATED CHELATING ION EXCHANGE RESINS.

By far the largest number of applications of impregnated supports have involved metals and unlike the previous high-performance anion separations, are generally large particle size substrates which are used mainly to preconcentrate metals with immobilised chelating ligands. Lundgren and Schilt [57] impregnated Amberlite XAD-2 with 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine (PDT), 1,10-phenanthroline, 2,2'bipyridine and 2,4,6-tripyridyl-1,3,5-triazine (TPTZ) and investigated retention capacities for various transition metals. The analysis of seawater and various reagent grade chemicals for trace levels of Fe(II), Co(II), Ni(II), Cu(II) and Zn(II) was achieved using a PDT impregnated support prior to detection using AAS. Lundgren and Schilt explain that adsorption of highly aromatic compounds such as 1,10-phenanthroline onto XAD-2 and similar substrates is a consequence of π-electron overlap between styrene moieties and the adsorbate molecules which produces a very stable immobilised complex. Amberlite XAD-4 was used by Chwastowska and Mozer [58] as a support for the immobilisation of 1-(2-pyridylazo)-2-naphthol (PAN). The resulting chelating resin was used for the preconcentration of Cu(II), Zn(II), Fe(II), Cd(II), Ni(II) and Pb(II) and applied to the analysis of river water prior to detection using AAS. Detection limits of low $\mu g\ dm^{-3}$ were achieved by preconcentrating up to 2 dm^3 of sample. Brajter and Sleszynska [59] impregnated Amberlyst A-26 macroreticular anion exchange resin with Xylenol Orange. The dependence of retention on pH was determined using a batch process and the selectivity differences observed made possible the separation of the following mixtures, Al(III)-In(III),

Ga(III)-In(III), Zn(II)-In(III), Cu(II)-Mn(II). The method was applied to the separation of Ga(III) and In(III) in a Ga(III)-In(III) alloy, again prior to analysis using AAS. In a later paper by Brajter and Zlotorzynska [60] three chelating compounds were investigated as to their properties when immobilised on the macroporous anion exchangers, Amberlyst A-26 and Varion AT-400. 2-(p-Sulphonylazo)-1,8-dihydroxy-3,6-naphthalene-disulphonic acid (SPADNS), p-(2-hydroxy-1-naphthylazo)benzene sulphonic acid (Orange II) and 1-nitroso-2-naphthol-3,6-disulphonate (Nitroso-R) were used for the selective separation of certain metal ions. The SPADNS impregnated Amberlyst resin was used in the determination of Bi(III) in $Pb(NO_3)_2$. Lead(II) was not retained at pH 3 and below, Bi(III) being retained completely and requiring 1M HNO_3 to elute, upon which it was determined using AAS without the interference that would have resulted from the excess of Pb(II). A second application involved the separation of Cd(II) from a range of other metals which cause interferences in the analysis of Cd(II) using AAS. A mixed bed resin impregnated with both SPADNS and Nitroso-R was used. The SPADNS allowed the separation of Cd(II) from Pb(II), Cu(II) and Fe(III) but not from Co(II) and Ni(II), therefore Nitroso-R which retained Co(II) and Ni(II) but not Cd(II) was also included. Together both resins retained all the above metals at pH 6.3-7.0 except Cd(II). A comprehensive review of similar early studies into the impregnation of anionic and non-ionic resins with organic ligands has been published by Marina et al. [61]. The influence of certain parameters ie. the nature of the immobilised ligands, the kind and chemical form of the impregnated resin and the pH of the aqueous solution on the separation characteristics and applications of these modified resins was discussed. In a more recent paper by Tanaka et al. [62] two applications were discussed. Firstly, the separation and preconcentration of Hg(II) using Dithizone sulphonic acid (DzS) and azothiopyrinesulphonic acid (ATPS) impregnated anion exchange resins and secondly, the separation and determination of Se(IV)

using a Bismuthiol-II modified substrate. Tanaka et al. noted the highly selective complexation of Hg(II) at pH 1-7 using the ATPS impregnated resin and observed no leakage from the columns of ATPS or DzS when using 1M NaCl, 0.5M HCl and 1M HCl. A 10mg dm^{-3} solution of Hg(II) can be reduced to 2ng dm^{-3} using the ATPS resin, which itself can be regenerated by elution of the Hg(II) with 10% thiourea solution in 0.1M perchloric acid. Tanaka et al. claimed commercially available resins for the collection of Hg(II) are unregenerative and require incineration for disposal. This technique for the collection of Hg(II) is suggested for the clean-up of waste water and the preconcentration of Hg(II) for trace analysis. The use of the Bismuthiol-II impregnated resin allowed the complete collection of Se(IV) below pH 2, at this pH most other metals are unable to form chelates. Again no leakage was observed and total elution of Se(IV) was possible using a penicillamine solution. With fluorometric detection, levels of 0.015-0.09μg dm^{-3} Se(IV) were detected in environmental samples, including seawater.

7-Dodecenyl-8-quinolinol (DDQ) has been used to impregnate Amberlite XAD-4 by Isshiki et al. [63]. The extraction behaviour of the resin was compared with solvent extraction techniques for Ag(I), Al(III), Bi(III), Co(II), Cd(II), Cu(II), Fe(III), Cr(III), Ga(III), Mn(II), Ni(II), Pb(II) and Ti(IV). Quantitative recoveries of all of the above except Ag(I), Cd(II) and Cr(III), at a flow rate of 3ml min^{-1}, was achieved from artificial seawater at pH 8. Preconcentration factors of 500 fold in seawater were achieved prior to elution with 2M HCl and determination using GFAAS. The stability of the impregnated complex was illustrated by the observed bleed from the column which was only 0.01% of the total impregnated amount per one analytical cycle. Brajter et al. [64] has illustrated the impregnation of XAD-2 with Pyrocatechol Violet (PCV) and the application of the resulting resin to the preconcentration and separation of certain metal ions. Brajter et al. described retention as

decreasing in the following order, Bi(III), In(III), Pb(II), Cu(II), Fe(III), Co(II), Ni(II). The sequence is in rough agreement with the stability constants of the complexes formed between PCV and the metal ions in solution and indicates these stability constants remain in the same order when the ligand is immobilised upon the support. Using the PCV-XAD-2 resin Bi(III) and In(III) were separated from large excesses of Cu(II) and Ni(II). The resin was also applied to the preconcentration of Pb(II) from tap water prior to detection using AAS and anodic stripping voltammetry. Levels of between 2.6-3.0μg dm^{-3} Pb(II) in tap water were detected using this method with reproducibility between 11 and 13%.

A well documented chelating agent applied in the analysis of trace metals is 8-hydroxyquinoline (8HQ). Abollino et al. [65] used both 8HQ and 8-hydroxyquinoline-5-sulphonic acid (8HQS) to impregnate Amberlite XAD-2 and an anion exchange resin (Bio-Rad AG MP-1), which were then used for investigation into the uptake and enrichment of Ca(II), Cd(II), Cu(II), Mg(II), Mn(II), Ni(II), Pb(II) and Zn(II). Recoveries of 100% were not achieved for all the metals studied but 100 fold enrichment factors were obtained and the metals detected using ICP-AES. An application to the analysis of trace metals in the concentrated salt solutions used for haemodialysis was also suggested.

An unusual paper by Blain et al. [66] detailed the column preconcentration of trace metals from seawater using macroporous resins impregnated with lipophilic tetraaza macrocycles. Again XAD-4 and XAD-7 were used as adsorbents for several of these compounds with the best results achieved using 1,4,8,12-tetraazacyclopentadecane. An average recovery of 98$\pm$8% was reported for the uptake of Cd(II), Mn(II), Pb(II) and Zn(II) from de-ionised water and seawater. Sub-μg dm^{-3} detection limits were possible for both Cd(II) and Pb(II) using AAS detection. The method was validated by the analysis of a certified seawater standard (NASS-2) and a precision quoted of better than 15%.

An industrial application of impregnated resins is given by Handley et al. [67] who used Xylenol Orange to impregnate the anion exchange resin, Dowex 1-X8, which was then used to remove the matrix and preconcentrate trace levels of alkaline earth metals from 30% m/v NaCl saturated brines. This resin was used as part of an on-line chromatographic system monitoring actual brine feedstock in a chlorine plant, successfully determining Mg(II), Ca(II), Sr(II) and Ba(II) in the range 2-100μg dm^{-3}. Handley et al. stated three processes enabling the immobilisation of chelating compounds on the substrates. Firstly, the strong ionic interaction of the $-SO_3^-$ groups, which many of the chelating ligands contain, and the tertiary ammonium groups of polymer type resins. Secondly, certain non-polar-non-polar attractions and finally physical trapping of complexes with the pore spaces of the resin backbone.

Similar dyes have been used by Naghmush et al. [68] to impregnate Amberlite XAD-2. Pyrocatechol Violet (PCV), 4-(2-pyridylazo) resorcinol (PAR) and Eriochrome Blue Black R were initially investigated with PCV being chosen to use as a preconcentration column for Cu(II) in a FI-AAS system, monitoring μg dm^{-3} levels in natural waters. Quantitative recovery of Cu(II) was possible in the presence of many other metals at greater concentrations except Fe(III), the effect of which could be reduced with the addition of fluoride to the sample. The results obtained for Cu(II) in three natural water samples using FI-AAS compared favourably with results achieved using electrothermal AAS. This indicates the strength of the chelation of Cu(II) with the immobilised PCV is sufficient to disassociate any organically bound Cu(II) present in the water samples. Singh and Dhingra [69] also used sulphonephthalein chelating dyes, namely PCV and Xylenol Orange (XO) to impregnate the anion exchange resin, Dowex-2. These modified resins were investigated for the preconcentration of Cu(II) and Cd(II) prior to their determination by AAS. Loadings of the two dyes were between 23-26mg per 1g of resin, impregnated between pH 7.0 and 8.0. Quantitative recoveries of both metals

were achieved from solutions pH 5.0 to 10.0 with levels above 0.01mg dm^{-3}. Concentrations below this figure produced non-reproducible and non-quantitative results. Elution of the preconcentrated Cd(II) and Cu(II) was achieved using 1M HNO_3 and 1M HCl respectively. The two resins exhibited similar capacities, approximately 100μg dm^{-3} of metal per 100mg of resin. The above technique using both modified resins in comparison was successfully applied to the analysis of Cd(II) and Cu(II), at levels ranging from 48-87μg dm^{-3}, in river water samples.

A recent paper by Kocjan [70] used silica gel as a support for the immobilisation of a mixture of Titan Yellow and Aliquat 336, with the aim of preconcentrating trace amounts of alkali and alkaline earth metal salts. The retention of 12 metals, Ca(II), Mg(II), Al(III), Cu(II), Fe(III), Ni(II), Co(II), Cd(II), Zn(II), Pb(II), Hg(II) and Cr(III), between pH 1.0 and 9.0 was investigated. All the metals were retained throughout this pH range, except Ca(II) and Mg(II) which needed more alkaline conditions for complete retention. Elution of the metal ions was achieved using mixtures of HCl and $HClO_4$ without any noticeable bleed of the immobilised complexes, allowing continued use of the chelating column. The column was applied to the separation of trace levels Cu(II), Pb(II), Cd(II) and Zn(II) from solutions of analytical grade alkali metal salts commonly used as supporting electrolytes in anodic stripping voltammetry and for the separation of certain metal ion mixtures by column extraction chromatography. Ryan et al. [71] also used silica impregnated with carboxymethyl and hydroxamate dextrans for the on-line preconcentration of metal ions prior to separation and detection using ion chromatography. The metal ions Cu(II) and Fe(III) were preconcentrated onto the columns at pH values of 6.0 for the hydroxamic acid dextran (HAD) column and 2.5 for the carboxymethyl dextran (CMD) column. By preconcentrating 1ml of a natural river water at pH 7.0 using the HAD column detection limits of 5-10μg dm^{-3} for

Cu(II), Mn(II), Co(II), Zn(II) and Ni(II) and 100μg dm^{-3} for Pb(II) and Cd(II) were quoted using a post column reaction visible detector.

1-(2-Thiazolylazo)-2-naphthol (TAN) has been used by Porta et al. [72] to impregnate a microcolumn of XAD-2 and preconcentrate trace metals in seawater prior to detection using inductively coupled plasma atomic emission spectrometry. The chelating resin was able to retain transition metals such as Cu(II), Ni(II), Zn(II), Cd(II), Fe(II) and Co(II) selectively, while other ions, Mg(II) or Ca(II), were not retained. Extremely sensitive detection limits of between 2 and 40ng dm^{-3} were possible from 50ml injection volumes and good agreement between the impregnated chelating column technique and electrothermal AAS was achieved for the majority of the metals studied, except Cu(II) and Cd(II). These were noticeably lower using chelation preconcentration, possibly a result of stable organo-Cu(II) complexes and stable chloro-Cd(II) species, not being retained on the column.

Chwastowska et al. [73] has produced a chelating resin based upon the immobilisation of 2-mercapto-N-2-naphthylacetamide (Thionalide) on the macroporous resin Bio-Beads SM-7, an acrylic ester polymer. The average loading of Thionalide on the resin was 44mg g^{-1}. The loading on the column did not decrease for 32 days but was reduced to 88% after 52 days of use. After 90 days the loading had reduced to only 16% of the initial value, although this still produced a quantitative recovery from a solution of 15μg of Sb(III) from 1M HCl. The Thionalide column was investigated for the preconcentration and separation of various elements, including the uptake of As(III), Sb(III) and Pb(II) from 1M HCl, the preconcentration of Pb(II) and Cd(II) at pH 6-7 and the preconcentration of Zn(II), Co(II) and Ni(II) at pH 7-8. Alkali and alkaline earth metals were completely unretained and so the column was suggested for use in the analysis of trace metals in biological materials and natural waters. Table 3 illustrates a range of substrates which have been impregnated with

Preconcentration with Chelate Impregnated Resin

Resin/chelate	Elements	Soln. pH	Eluent
Amberlite IRA-400/DzS	Hg(II)	1—5.4	Conc HCl
Amberlite IRA-400/ATPS	Hg(II)	1	—
Amberlite IRA-400/TPPS	Hg(II)	2.4	—
	Cu(II)	1—8	
Diaion SA 100/oxine sulfonic acid	Co, Ni	4—6	—
Amberlite IRA-400/ATPDS	Hg(II)	1—7	10% thiourea/1 *M*
	Cu(II)	4.5—7	$HClO_4$
	Cd(II)	6—7	
Bio-Rad AG MP1/Pyrocatechol Violet	Al(III)	7—9	?
Amberlyst A 26/Xylenol Orange	Al, Cr, Mn, Fe, Co, Ni, Cu, Zn, Ga, Cd, Ir, Pb	2—10	0.001—0.1 *M* HCl 0.03—0.2 *M* H_2SO_4
Diaion SA#100/oxine sulfonic acid	Hg, Cu, Co, Mn, As	7	—
Diaion Sa#100/Zincon	Hg, Cu	5.5	—
Dowex 1×4/Bathocuproine	Hg	1	—
Dowex 1×2/oxine sulfonic acid	Ag, As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Sb, Se, Sc, W, Zn	7	7 *M* HNO_3
Amberlite IRA-400/Bimuthiol-II	Se(IV)	<2	8—13 *M* HNO_3
Dowex 1×8/nitroso-R salt	Co	8	0.01 *M* $TiCl_3$/4 *M* HCl
Amberlite IRA-400/Chromoazurol S	Al	8	NaOH
Amberlite IRA-400/SPADNS	Cu, Fe, Pb	6.9	0.014 *M* HNO_3
Amberlite XAD-4/DDQ	Ag, Al, Bi, Cd, Cu, Fe, Ga, Mn, Ni, Pb, Tl	8	2 *M* HCl
Amberlite XAD/8HQ DXHQ MHQ	Cu, Cd, Ni, Zn, Pb, Hg, Fe	2—9	3—5 *M* HCl
Amberlite XAD-2/PAR	Ag	7.2—7.7	0.2 *M* HNO_3/thiourea
Dowex 1×8/T-azo-R T-azo-C	Cu, Ni	1—6.7	
Amberlite XAD-2/Pyrocatechol Violet	Pb, In	3—8	1—1.5 *M* HNO_3
Amberlite XAD-2/Pyrocatechol Violet	Zn, Cd	7.2—8.2	4 *M* HCl, HNO_3
Bio-Rad AG1×8/Chromotrope 2B	Al	6	0.01 *M* HCl

Note: DzS, *p*-hydrazenebenzenesulfonic acid; ATPS, aziothiopyrine sulfonic acid; TPPS, tetra-phenylporphine-trisulphonic acid; ATPDS, azo-thiopyrine disulfonic acid; Pyrocatechol Violet, 3,3',4'-trihydroxyfuchsone-2''-sulfonic acid; oxine sulfonic acid, 5-sulfo-8-quinolinol, Zincon, o-(2-[δ-(2-hydroxy-5-sulfophenylazo)-benzylidene]-hydrazino)benzoic acid; Bathocuproine,(4,7-diphenyl-2,9-dimethyl-1,10-phenanthroline di-sulfonic acid, sodium salt); nitroso-R salt, sodium 1-nitroso-2-naphthol-3,6-disulfonate; SPADNS, trisodium salt of 2-(*p*-Sulfenylazo)-1,8-dihydroxy-naphthalene-3,6-disulfonic acid; DDQ, 7-dodecenyl-8-quinolinol; 8HQ, 8-hydroxyquinoline; DXHQ, 5,7-dihalo-8-hydroxyquinoline; MHQ, 2-methyl-8-hydroxyquinoline; PAR, 4-(2-pyridylazo)resorcinol; T-azo-R, [1-(tetrazolylazo)-2-hydroxynaphthalene-3,6-disulfonic acid]; T-azo-C, [2-(tetrazolylazo)-1,8-dihydroxynaphthalene-3,6-disulfonic acid]; Chromotrope B, 1,8-dihydroxy-2-(*p*-nitrophenylazo)-naphthalene-3,6-disulfonic acid.

Table 3. Preconcentration of metal ions with chelate impregnated resins [74].

various chelating compounds, the elements preconcentrated, the solution pH and the eluent used to elute the complexed metals.

The previous applications have involved the use of chelating resins for matrix elimination and metal preconcentration applications. Publications detailing high-performance separations using chelating ligand impregnated resins are a lot less common. The majority of work has been carried out by Jones and co-workers [75-79] using azo and triphenylmethane dye impregnated polystyrene based resins. This work is discussed in the next section.

1.3.iv. HIGH-PERFORMANCE CHELATION ION CHROMATOGRAPHY.

High-performance chelation ion chromatography (HPCIC) is a relatively new area of analytical chemistry. Very little work has been carried out on the production of chelating resins with the ability both to preconcentrate metal ions and produce high efficiency chromatographic separations. Faltynski et al.[80] used silica bound azo-coupled chelating stationary phases for the high-performance separation of metal ions. The elution order of metal ions on 2-methyl-8-quinolinol (MQ) and N-(1-naphthyl)-ethyl-enediamine (NED) silica gels were in the order of known homogeneous solution stability constants, indicating chelation to be the dominant process occurring within the column. The MQ silica gel column was used to separate Mn(II), Zn(II), Cd(II) and Pb(II) isocratically at pH 2.75, although the efficiency was poor. Faltynski also used Dithizone bonded silica gel to achieve a six metal separation. However the order of elution was reversed and similar to simple ion exchange indicating chelation is not the only process occurring.

Toei [81] illustrated the use of a commercially available high-performance chelating column, (Tosoh TSK-GEL chelate 5-PW) in the separation of alkaline earth metal

cations with a modified post column procedure where a chelating agent, o-Cresol Phthalein Complexone (Phthalein Purple), was contained in the eluent. This is an iminodiacetic acid covalently bound polymer gel column and was used to separate Mg(II), Sr(II) and Ca(II), in the order stated. The elution order of the metals varied from the predicted order using known iminodiacetic acid stability constants and illustrated a decrease in retention time as a function of increased eluent salt concentrations which again indicates some degree of ion exchange taking place within the resin. The pH of the eluent used was only 5.3, which may have been too low to allow 100% chelation of the alkaline earth metals to occur. The presence of a chelating agent in the eluent will also have an effect upon the order of elution. Toei applied the above method to the separation and determination of Mg(II) and Ca(II) in seawater.

The rapid separation of Zn(II) and Cu(II) on a 8-hydroxyquinoline bonded polystyrene divinylbenzene copolymer matrix has been carried out by Jonas et al. [43]. A separation of four metal ions in the following order, Zn(II), Co(II), Ni(II) and Cu(II) was also shown. The efficiency however is poor and rapid peak broadening occurs, although the order of elution indicates the beginnings of true HPCIC are being illustrated.

Bonn et al. [82] used covalently bound iminodiacetic acid porous silica gel to achieve a separation of five metals, Mg(II), Fe(II), Co(II), Cd(II) and Zn(II) with particular reference to seawater analysis. Eluents containing carboxylic acids and stronger complexing agents such as dipicolinic acid were investigated as to their effect upon the elution mechanisms of alkali, alkaline earth and transition metals. The elution orders achieved were consistant with those expected for chelation exchange processes and a successful separation of spiked levels of Co(II), Zn(II) and Cd(II) in a seawater sample was illustrated.

Recent work by Jones and co-workers [75-79] has focused upon the impregnation of high-performance grade polystyrene divinylbenzene polymers with chelating dyes to

produce stable high-performance chelating columns. Initial studies by Jones et al. [75] illustrated the ability of a Chrome Azurol S impregnated Benson BPI-10 10μm particle size neutral resin to separate the divalent metals Mg(II), Mn(II) and Zn(II) isocratically, in a 1M KNO_3 eluent containing 0.05M lactic acid as a competing complexing agent, adjusted to pH 6.5. The use of a gradient elution programme enabled the separation of four divalent metals in the following order, characteristic of chelation ion chromatography, Mg(II), Mn(II), Zn(II) and Cu(II). Jones et al. illustrated the ability of the column not only to separate metals but to preconcentrate and separate groups of metals on a single column. The same Chrome Azurol S resin was used to produce a separation of Al(III), In(III) and Ga(III) in 1M KNO_3 at pH 2.25, with a column temperature increased to 60°C to compensate for slower exchange kinetics experienced with trivalent metals. The quantitative preconcentration of Al(III) was also achieved from deionised water. The work carried out by Jones et al. using dye impregnated techniques produced results, which although showed a relatively poor efficiency, indicated the potential of the technique and were therefore the basis of further studies.

Challenger et al. [76] investigated the impregnation of large particle size substrates, Dowex 1-X8 anion exchange resin, Amberlite XAD-2 and XAD-4 neutral resin with three chelating dyes, Xylenol Orange, Calmagite and Chrome Azurol S. From these preliminary studies Xylenol Orange and Chrome Azurol S were used to impregnate HPLC grade Polymer laboratories PLRP-S neutral 8μm polystyrene divinylbenzene resin. The high-performance Xylenol Orange impregnated column was used in comparison with a commercially available chelating column, the TOSOH TSK-GEL chelate-5PW column, in the separation of alkaline earth metals and achieved efficiencies approaching those of the commercial column.

Jones et al. [77] reported the extension of this work to include the separation of transition and heavy metals using both the commercial TOSOH column and dye impregnated

columns. A separation of Mn(II), Cd(II), Zn(II) and Pb(II) in 1M KNO_3 was carried out using the TOSOH column and the separation of Cd(II), Pb(II) and Cu(II) in 1M KNO_3 and 1000mg dm^{-3} Mg(II) using a Chrome Azurol S chelating column was also illustrated.

Two recent papers by Challenger et al. [78] and Jones et al. [79] detailed the most recent advances in high-performance chelation ion exchange using dye impregnation techniques. The majority of the work has been carried out using a Xylenol Orange impregnated column. Challenger et al. separated nine di-valent metal ions successfully in under 40min, in 1M KNO_3 using step gradient elution in the following order, Ba(II), Sr(II), Mg(II), Ca(II), Mn(II), Cd(II), Zn(II), Ni(II) and Cu(II). Several applications for high-performance chelation ion chromatography have been carried out by Challenger et al. with promising results. The preconcentration and separation of alkaline earth metals in concentrated KCl and NaCl brines was accomplished with linear and quantitative results with detection limits ranging from 0.5 to 4μg dm^{-3}. The use of a step gradient programme allowed the preconcentration and separation of nine divalent metal ion in KCl and NaCl brines. Low μg dm^{-3} detection limits were achieved. The same system was also used for the detection of trace levels of metal impurities in laboratory chemicals and to carry out preliminary investigations into the analysis of trace metals in seawater. The separation of five metals in a spiked seawater sample was shown by Challenger et al. and the semi-quantitative determination of Zn(II) and Cu(II) in a local seawater sample achieved. The results achieved by Challenger et al. illustrate the potential for high-performance chelation ion chromatography for the determination of trace metals in these complex sample types.

1.4. DETECTION SYSTEMS IN ION CHROMATOGRAPHY.

The usefulness of ion chromatography as an analytical technique not only depends upon the ability to produce efficient separations but also upon sensitive detection systems. Original definitions of ion chromatography frequently include conductivity as the stated detection system. Modern ion chromatography often employs a variety of detection systems commonly used in other HPLC techniques. Conductimetric detection is just one of a range of detection methods used in modern ion chromatography. These include electrochemical detection methods such as amperometric, potentiometric and coulometric techniques and occasionally ion selective electrodes. An alternative approach to electrochemical detection is optical detection techniques, which describes photometric (UV-visible absorbance), photometric following post-column derivatisation, indirect photometric, fluorescence, chemiluminescence and refractive index detection methods.

1.4.i. CONDUCTIVITY AND ELECTROCHEMICAL DETECTION.

The development of conductimetric methodology provided ion chromatography with a suitable detection system for both inorganic and organic ions, based upon the fact all ions in solution exhibit electrical conductivity. However it was not until background conductivity, caused by electrolytes being used as eluents, could be reduced with the invention of eluent suppression technology, that conductivity detection could evolve into the sensitive and widely used technique applied in modern ion chromatography. Jensen et al. [15] used suppressed conductivity to detect eight alkali and alkaline earth metals separated on an IonPac CS12 column with gradient elution and achieved a detection limit of 100μg dm^{-3} for Ba(II) which

eluted after 13min. Small [5] has produced a review of the last twenty years of ion chromatography, with special emphasis on the evolution of conductimetric detection, from historic beginnings to modern applications.

The use of other electrochemical detection techniques in the ion chromatography of metals have been developed but their application is relatively rare. Haddad et al. [83] used indirect potentiometric detection for a wide range of metals separated using ion chromatography. The use of amperometric detection for the determination of Cu(II), Ni(II), Cr(III) and Cr(VI) has been reported by Bond and Wallace [84]. In a recent paper, Isildat and Covington [85] discuss the application of ion-selective electrode potentiometric detection in ion chromatography. The range of detection methods used in ion chromatography has been reviewed by Rocklin [86] which covers the "spectrum" of both organic and inorganic ion determinations, including electrochemical detection methodology.

1.4.ii. DIRECT SPECTROPHOTOMETRIC DETECTION.

Direct spectrophotometric detection of the eluent using ultraviolet and visible absorption methods is widely used in ion chromatography although its use in the detection of metal ions is limited due to the low molar extinction coefficients of most metal ions. An example of the determination of metal ions using photometric detection is described by Jones and Schwedt [87] who detected gold and platinum group metals utilising their absorbance in the UV region as chloro-complexes. By far the greatest use of direct spectrophotometric techniques has been in the detection of organic ions such as aromatics, heterocyclic acids and amines. The majority of which have one or more wavelengths of maximum absorbance within the UV region and high molar extinction coefficients of between 1000 and 100,000, allowing very

sensitive detection.

Indirect photometric detection is based upon the measurement of a negative absorbance signal produced as the analyte, typically non-UV-absorbing inorganic and organic ions, displaces a UV-active component in the mobile phase. An added feature of indirect photometric detection is the ability to use complexing ligands as the UV-active complexes in the mobile phase allowing increased selectivity and novel separations. Goa et al. [17] used a phthalate/EDTA eluent for the simultaneous determination of inorganic ions and cations by ion chromatography with indirect photometric detection. Jones et al. [88-89] used indirect photometric detection, based upon Eriochrome Black T, for nine metal ions separated using HPLC. A number of similar applications using this method as the detection system for inorganic ions have been published [90-94]. A disadvantage of indirect photometry is the high level of background noise caused by the eluent.

The use of colour forming reagents contained within the eluent has also been applied to the detection of metal ions. The method is based upon the on-column chelation of the metal ions with the colour forming ligands within the eluent and monitoring the maximum absorbance wavelengths of the eluting metal complexes. Zenki [95] and Toei [96-100] have illustrated the separation and determination of both alkaline earth metals and some heavy metals with the use of colour forming complexes in the mobile phase. Toei used o-cresolphthalein in the eluent for detection of alkaline earth metals separated on a TOSOH high-performance chelating column [100]. Toei has also investigated Xylenol Orange for the determination of transition metals [97] and Arsenazo III [98] for alkaline earth metals. Smith and Yankey [101] detected several transition metals using dithiocarbamates included in the mobile phase and DiNunzio et al. [102] used the PAR chelates of four transition metals as the absorbing complex. A recent publication by Wada et al. [103] compared the use of

Arsenazo III, Chlorophosphonazo III, Carboxyarsenazo, Sulphonazo III and Dimethylsulphonazo as complexing agents incorporated within the eluent. The ion chromatography of alkaline earth and heavy metal ions was carried out and Chlorophosphonazo III found to provide the most sensitive detection.

The relatively recent development of efficient interfaces for use in coupled techniques has allowed the use of a wide range of detection systems for metal ion analysis following HPLC and IC separations. Both element specific and multi-element detectors such as AAS, ICP-OES, MS and ICP-MS are becoming increasingly common as detectors coupled to modern chromatography systems. A common problem in coupling IC and HPLC to atomic spectrometric techniques is the incompatibility of the mobile phase with the detection instrumentation and chemical processes. A review on efforts to solve these problems is given by Ebdon et al. [104]. The coupling of ion chromatography to both ICP-OES and ICP-MS has been carried out by Riviello et al. [105]. The use of sensitive techniques allows detection limits of low $\mu g\ dm^{-3}$ for large numbers of elements. A large number of applications involving ion chromatography and preconcentration techniques coupled to the above detectors have involved the determination of trace metals in seawater (see Chapter 4). The coupling of HPLC systems to ICP-MS, in particular, has also led to the rapid development of metal speciation techniques [106].

1.4.iii. POST COLUMN REACTION DETECTION.

1.4.iii.a. SPECTROPHOTOMETRIC DETECTION.

This method of detection has become widely used in ion chromatography. The most commonly used post column reactions are based upon the principle of reacting eluting ions with complexing ligands to form highly absorbing coloured complexes ($\in$max typically between 10,000 and 40,000). The use of chelating agents as post column reagents has several advantages. The majority of complexes used absorb strongly in the visible region where absorbance from eluent reagents is generally minor. Chelating agents can also be used as both multi-element and highly selective detectors with reaction times generally fast, reducing the need for large reaction coils and reducing peak broadening. A further advantage is the large number of chelating photometric reagents available. Post column reaction detection can be applied to both organic ions and inorganic anions and cations. Al-Nadjafi et al. [107] used Ninhydrin as a colour forming post column reagent in the detection of amino acids and the monitoring of carboxylic acids can also be achieved at 424nm by post column reaction with potassium dichromate [108]. An example of an anionic application is the detection of fluoride in ion chromatography using zirconyl Xylenol Orange as the post column reagent which has been reported by Barnett et al. [109]. However the largest number of applications of post column reaction techniques has involved the detection of metal ions.

One of the first chelating complexes to be used as a post column reagent was 4-(2-pyridylazo) resorcinol (PAR). PAR dissolved in a high pH ammonium buffer, will form a red-coloured visible light absorbing complex with the majority of transition metals and the lanthanide series, with a λ max ranging between 450 and 550nm. Initial work by Kawazu and

Fritz [110] illustrated the use of PAR as a sensitive detector for Cd(II), Zn(II), Fe(III), Pb(II), Cu(II), Co(II) and Mn(II), separated on a cation exchange column. This early work was investigated further by Fritz and Story [111] who compared PAR with Arsenazo I and Arsenazo III as post column reagents for a range of metals. PAR was found to be the most sensitive and versatile of the three for most of the metals investigated although, Arsenazo I proved more sensitive to Ca(II) and Mg(II). Photometric reagents for Ca(II) and Mg(II) were developed by Arguello and Fritz [112], who compared Arsenazo I, Arsenazo III, Sulphonazo III, Methlythymol Blue, Titan Yellow, Murexide, sodium Rhodizonate, Eriochrome Black T, Chlorophosphonazo, PAR and PAR/ZnEDTA (zinc-ethylenediamine-tetraacetic acid). PAR/ZnEDTA was used to detect metals to which PAR alone was insensitive and proved to be the most sensitive for Ca(II) and Mg(II) of all the above reagents. The detection of metal ions using PAR/ZnEDTA is achieved as a result of a metal displacement reaction :-

$$M(II) + ZnEDTA + PAR \rightarrow M(II)EDTA + ZnPAR$$

The ZnEDTA complex is weakened by the presence of an auxiliary complexing agent, generally NH_3. Metal ions present displace the Zn(II) which forms a stronger complex with PAR and an increase in absorbance is measured in the visible region close to 500nm, due to the ZnPAR complex. Arguello and Fritz found a 2- to 10-fold increase in sensitivity using PAR/ZnEDTA for the alkaline earth metals compared with Arsenazo I. Jezorek and Freiser [113] compared the relative responses of PAR and PAR/ZnEDTA based detection systems for fourteen metal ions. The results showed between a 12- and 32-fold increase in sensitivity in the determination of alkaline earth metals when using PAR/ZnEDTA compared with PAR. There were also considerable increases in response to Pb(II) and Mn(II). However PAR/ZnEDTA was noticably less sensitive to Zn(II), Ni(II), Cu(II), Al(III) and Fe(III) with Co(II) being equal. A recent paper by Lucy and Dinh [114] reports the kinetics and sensitivity

of PAR/ZnEDTA to be strongly dependent upon the nature and concentration of auxiliary complexing agents. Comparison of NH_3 and ethylenediamine for the detection of alkaline earth metals showed both a decrease in reaction time ($t_{1/2}$ =0.7s for ethylenediamine, $t_{1/2}$ =3.6s for NH_3 for Mg(II)), and a 2- to 9-fold improvement in sensitivity for the latter.

A similar system to the PAR/ZnEDTA detector has been investigated by Handley et al.[67,115]. A Calmagite/MgEDTA system based upon the displacement of Mg(II) by eluting metal ions, which then complexes with the Calmagite causing a decrease in absorbance at 610nm. The system was used for the determination of Al(III), Zn(II) and the alkaline earth metals in concentrated brine solutions. This enabled the determination of Ba(II) and Sr(II), to which Calmagite alone is very insensitive.

By far the largest number of recent applications involving post column reaction detection have involved the use of PAR as the sole complexing agent. The Dionex Corporation was the first company to produce a commercial post column reaction ion chromatography system, using a pneumatic pump to deliver the post column reagent. A number of technical notes [116-119] have been produced describing various applications based upon the use of PAR. The recent production of a commercial chelation ion chromatography system (CIC) by Dionex [120] which uses PAR as the post column reagent, has led to the publication of several applications involving the determination of trace metals [31,35-36,45,51], the majority of which have involved the analysis of seawater. Caprioli and Torcini [36] obtained detection limits for Zn(II), Cu(II), Ni(II), Cd(II) and Mn(II) ranging between 0.05 and 0.5μg dm^{-3}, after preconcentration using the Dionex CIC system with PAR PCR detection. Cardellicchio et al. [35] used the same system to determine Cd(II) and Pd(II) in seawater and quoted similar detection limits for the determination of Cd(II) and Pb(II).

A recent paper by Saraswati [14] used PAR and Arsenazo III as post column

reagents in the simultaneous determination of transition and rare earth metals in Nd-Fe-B material after separation using ion chromatography. The effect of various complexing agents in the eluent such as tartrate, oxalate and citrate on the absorbance of these chelates was studied and tartrate found to produce increased sensitivity for metal-PAR chelates, with a maximum absorbance at tartrate concentrations of 2.3 x 10^{-2}M.

The use of Arsenazo I and Arsenazo III as post column reagents is also common to trace metal determinations. Hwang et al. [121] used an Arsenazo III PCR based detector for the determination of rare earth metals in monazite sands and high purity rare earth metal oxides. The same detector was used by Hayakawa et al. [122] for detecting lanthanides, who reported sensitive detection limits and linear calibrations over two orders of magnitude. An overview of the application of an Arsenazo III PCR detector to the determination of lanthanide and actinide metals in radioactive samples is given by Cassidy et al. [123]. Arsenazo I has been used by Elchuk and Cassidy [124], again for detection of lanthanide metals. Using on-column preconcentration techniques, detection limits of 10ng dm^{-3} were possible. Wang et al. [125] also reported a similar application.

Several other complexing agents have been investigated for metal ion detection. Bertsch and Anderson [126] used 4,5-dihydroxy-m-benzylenedisulphonic acid (Tiron) for the derivatisation and detection of Al(III) and a method for the determination of Cr(VI) has been developed using post column reaction with 1,5-diphenylcarbohydrazide [127]. Chenggucing and Bencheng [128] used bis-(2-hydroxyethyl) dithiocarbamate for the detection of Cu(II), Zn(II), Ni(II), Co(II) and Fe(II) in environmental samples. Absorbance was measured at 405nm and detection limits quoted in the ng dm^{-3} range using preconcentration techniques. Xylenol Orange has been used by Hirose et al. [129] for the detection of rare earth metals and Saitoh and Oikawa [130] developed bathophenanthroline disulphonic acid as a highly

selective detector for Fe(II) and Fe(III). The determination of Mn(II) in seawater has been carried out by Resing and Mottl [131] using an eluent containing both Leucomalachite Green and potassium periodate. Manganese(II) eluting from the column acted as a catalyst for the production of Malachite Green which was measured photometrically. Jones and Schwedt [75] used three PCR reagents, for the detection of divalent and trivalent metal ions separated using ion exchange and chelation ion exchange chromatography. Pyrocatechol Violet (PCV) was used to detect Al(III), Ga(III), In(III), Bi(III) and Fe(III), with an increase in absorbance measured at 580nm. A Chrome Azurol S post column detector was used for the detection of Be(II) but could also be used for the detection of Al(III), Ga(III) and Fe(III). For the detection of divalent metals, except Be(II), a Calmagite based post column detector was used which produced a decrease in absorbance at 610nm when complexed with metal ions.

1.4.iii.b. FLUORESCENCE DETECTION

The use of fluorescence in ion chromatography allows very sensitive detection of certain metal ions without the need for preconcentration techniques. Based upon a similar principle as photometric post column reactors, fluorescence detectors convert eluting metal ions into fluorescent derivatives allowing detection limits often over ten times lower than those achieved using absorbance detection systems. However the number of metal ions which can be detected this way is limited due to fluorescent quenching, which is a property of certain transition and heavy metals. Beckett and Nelson [132] were one of the first to produce a fluorescent PCR technique for the determination of trace metals. Metal ions were separated as 4-aminophenylethylene diaminetetraacetic chelates and converted into fluorescent species using Fluorescamine. The technique showed linear responses in the range 5×10^{-11}g to 5 x

10^{-7}g of metal ion injected and gave detection limits of 80pg for Pb(II) and Cd(II) and 60pg for Zn(II). A larger range of metal ions could not be detected using this method because of the chemical similarity and instability of the metal chelates used in the separation phase. In a series of papers by Jones and co-workers [89,133-135], a PCR system based upon 8-hydroxquinoline-5-sulphonate (8-HQS) has been developed. Jones et al. [89] achieved sensitive fluorescence for Al(III), Ga(III), In(III), Mg(II), Cd(II) and Sn(II) using 8-HQS, with a linear response for Ga(II) from 10 to 1000 μg dm^{-3}, and a detection limit of 1μg dm^{-3} using a 100μl injection. Jones et al. later applied the above technique to the determination of trace amounts of Al(III) both in tap water and in a certified reference material (monel alloy) [133]. An excellent linear range of 5 to 10,000μg dm^{-3} was possible and a detection limit of 1μg dm^{-3} quoted, with no interferences from other commonly occurring metals. This work was advanced by Jones [134] who improved the chromatography of the system allowing Al(III) speciation studies to be carried out in both natural and potable waters. Fluorescence detection of organically bound Al(III), fluoro-species and the labile monomeric Al(III) species was possible at low μg dm^{-3} concentrations. This method was used by Jones and Paull [135] for a brief study of Al(III) speciation in potable waters from the southwest of England. Labile hydroxy Al(III) was determined in Plymouth tap water in the range 29-96μg dm^{-3}.

A recent paper by Williams and Barnett [136] described a fluorescence PCR system based upon the displacement methods discussed in section 1.4.iii.a. Following the separation of metal ions using ion exchange chromatography, the analyte ions are mixed with a 8-HQS/MgEDTA PCR. The metal ions displace Mg(II) from the MgEDTA complex, which then reacts with 8-HQS producing the fluorescent species. A large range of metals were detected using this method including alkaline earths, transition and heavy metals and the lanthanide series. Linear calibrations for all the analytes covered at least two orders of

magnitude and detection limits ranged between 3.3 x 10^{-7} and 1.4 x 10^{-6}M.

1.4.iii.c. CHEMILUMINESCENCE DETECTION.

A number of metal ions have the ability to catalyse the oxidation of luminol to its excited state form, which during decay via photon emission, produces luminescence proportional to the concentration of the metal ions in solution. This allows chemiluminescence to be used as an extremely sensitive PCR detector. One of the first to apply this technique to ion chromatography was Neary et al. [137] who used a luminol PCR to detect Cu(II) and Co(II). Detection limits of 1 x 10^{-6}mol dm^{-3} for Cu(II) and 1 x 10^{-7}mol dm^{-3} for Co(II) were possible and emphasis was placed on the need for totally inert chromatography systems to reduce background chemiluminescence. Williams et al. [138] achieved sub-μg dm^{-3} detection limits for Cr(III) and Cr(VI), separated using ion chromatography with chemiluminescence detection. A reducing agent was used to reduce Cr(VI) to Cr(III) as the hexavalent species does not cause chemiluminescence with the luminol PCR. The HPLC system used was however complicated, requiring two columns and four reagent pumps. This was later simplified by Beere and Jones [139] to a single column, three pump system. The determination of Co(II) at picogram levels has been achieved by Jones et al. [140] using a chemiluminescence detector. A detection limit of 0.5ng dm^{-3} was possible without preconcentration using a 200μl sample loop. The system was used to detect Co(II) in rice flour certified reference material. Further work by Jones et al. [141] used the Co(II)-luminol chemiluminescent reaction as the basis of a novel multi-element detection system. A luminol/CoEDTA PCR was employed based upon the displacement of Co(II) by eluting metal ions and its subsequent reaction with the luminol present. Jones et al. determined Mg(II),

Ca(II), Sr(II), Ba(II), Fe(II), Fe(III), Co(II), Ni(II), Cu(II), Zn(II), Pb(II), the lanthanides, Tl(III), Al(III), Ga(III), In(III) and Bi(III), with detection limits ranging between 2 and 100μg dm^{-3}. A recent application of chemiluminescence is given by Obata et al. [142] who quoted a detection limit of 0.05nmol dm^{-3} when determining Fe(III) in seawater, using a luminol/ammonia/hydrogen peroxide PCR.

1.5. AIMS AND OBJECTIVES.

The work discussed in section 1.3.iv. by Jones and co-workers [75-79] has involved the technique of impregnating HPLC grade polystyrene divinylbenzene based substrates with various chelating dyes to produce high-performance chelating columns capable of both metal ion preconcentrations and separations. This work aims to advance the above technique in terms of efficiency and range of these chelating columns and to apply the technique to a wider variety of metals in analytically challenging and complex samples to achieve quantitative results.

The initial aims of this work were to determine the reproducibility of previous results and assess the factors controlling the degree of dye impregnation including properties of the resins themselves ie. pore size and particle diameter. The effect of the degree of dye loading upon separations was also to be investigated and studies into other parameters such as column length and temperature also carried out.

A major section of this work is the investigation of various new types of chelating dye and the production of a variety of impregnated chelating columns using these dyes, which will contain various chelating functional groups. This work aims to characterise these columns in terms of chelating strength and capacity, loading stability and separating efficiency.

The application of the most promising of these chelating columns to two particular samples of interest, namely alkaline earth metals in formation water brines and transition and heavy metals in seawater, is also a major aim of this work. Successful quantitative analysis of the above two samples will preceed investigations into the application of HPCIC for trace metal determinations in a variety of other complex samples.

The optimization of post column reaction detection systems for each particular application will also be carried out.

CHAPTER 2. EXPERIMENTAL.

2.0. INSTRUMENTATION.

The bulk of the work for the determination of trace metals was carried out using a high-performance chelation ion chromatography (HPCIC) post column reaction system. The system was modified to enable direct injection applications as well as those requiring preconcentration.

2.0.i. DIRECT INJECTION APPLICATIONS.

A schematic representation of the system used is shown in Figure 5. The instrumentation consisted of a titanium LKB 2150 HPLC pump (Bromma, Sweden) to deliver the eluent and a Constametric model III pump (Laboratory data control, Riviera Beach, FL, USA) as the post column reagent pump. Injection of the sample was via a PEEK lined Rheodyne 9010 six port injector valve (Rheodyne, Cotati, CA, USA) connected to PEEK sample loops ranging from 5-200μl depending upon the application. Post column detection was achieved with the mixing of the eluent with the post column reagent (PCR) at a zero dead volume T-piece followed by a 1.4m x 0.3mm I.D. PTFE reaction coil. A Dionex spectral array detector (Dionex (U.K) Ltd., Camberley, Surrey, U.K) was used to detect changes in absorbance caused by the resulting metal complexes and a chart recorder (Labdata, Surrey, U.K) was employed to record the chromatograms.

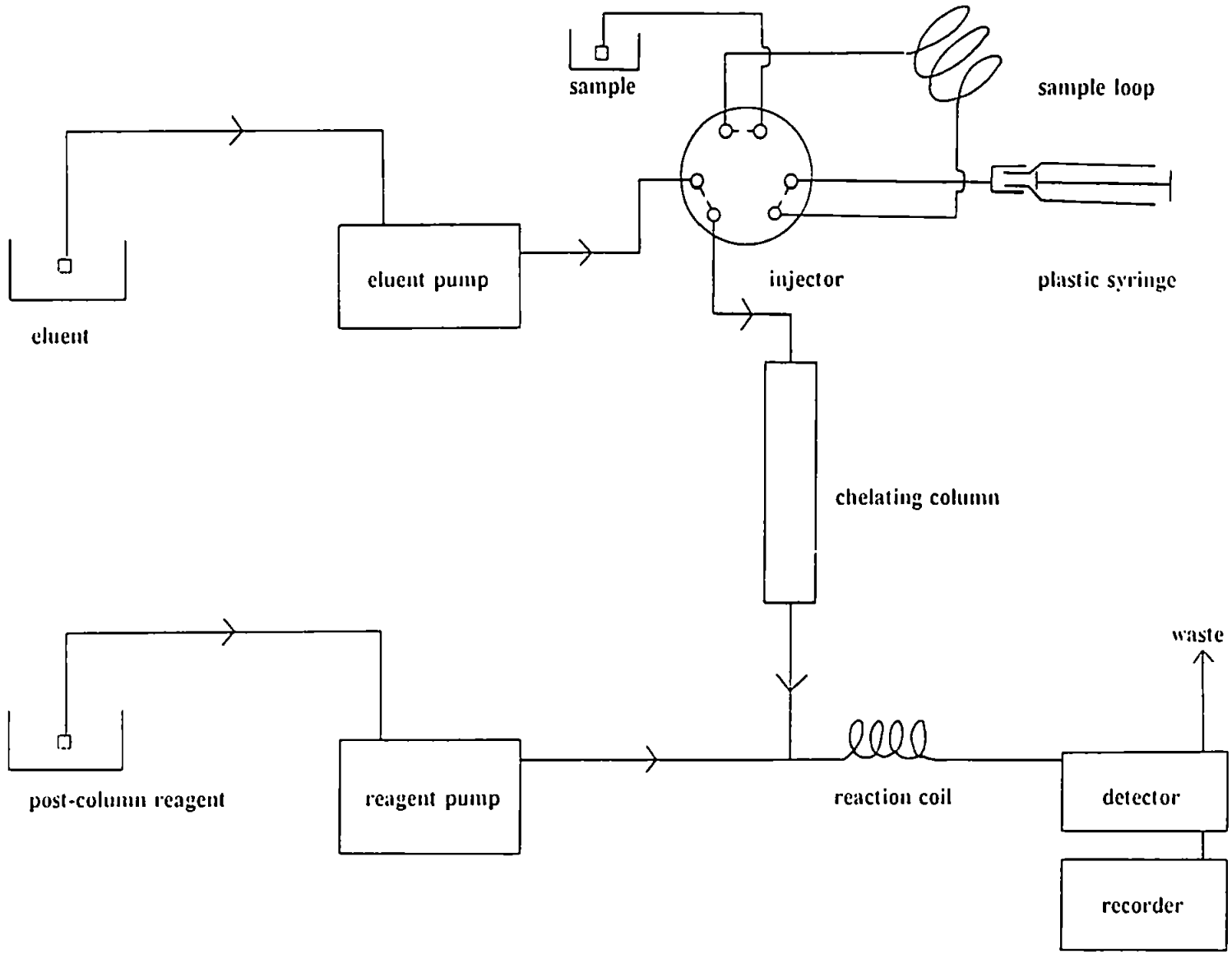


Figure 5. Schematic representation of the basic HPLC post column reaction system used.

2.0.ii. PRECONCENTRATION APPLICATIONS.

The addition of an Eldex laboratories model AA pump with a PTFE lined pump head (Eldex laboratories, Menlo Park, CA, USA) combined with a second inert six port injector (Valco, Schenkon, Switzerland) enabled the system to be used for both direct injection and preconcentration studies. The modified system is represented in Figure 6.

2.0.iii. INDUCTIVELY COUPLED PLASMA OPTICAL EMISSION SPECTROMETRY.

A Varian Liberty 200 inductively coupled plasma optical emission spectrometry (ICP-OES) system (Melbourne, Australia) was used to compare results with the HPCIC technique for a variety of samples.

2.1. COLUMN PREPARATION.

A range of resins were obtained from two manufacturers and investigated to establish the most efficient substrate for analytical applications. Polymer laboratories (Church Stretton, Shropshire, U.K) PLRP-S 10μm polystyrene-divinylbenzene resins with pore sizes of 100, 300, 1000 and 4000Å were compared for the degree of dye impregnation and separation capabilities. Dionex (U.K) Ltd. also supplied two samples of their hydrophobic macroporous polystyrene-divinylbenzene based resins, one of which was 8.8μm, 120Å pore size substrate, the second being 7.0μm, 300Å pore size material.

The resins were slurry packed into 10 x 0.46cm PEEK metal free HPLC columns (Alltech Chromatography, Carnforth, Lancashire, U.K) using a Shandon column packer

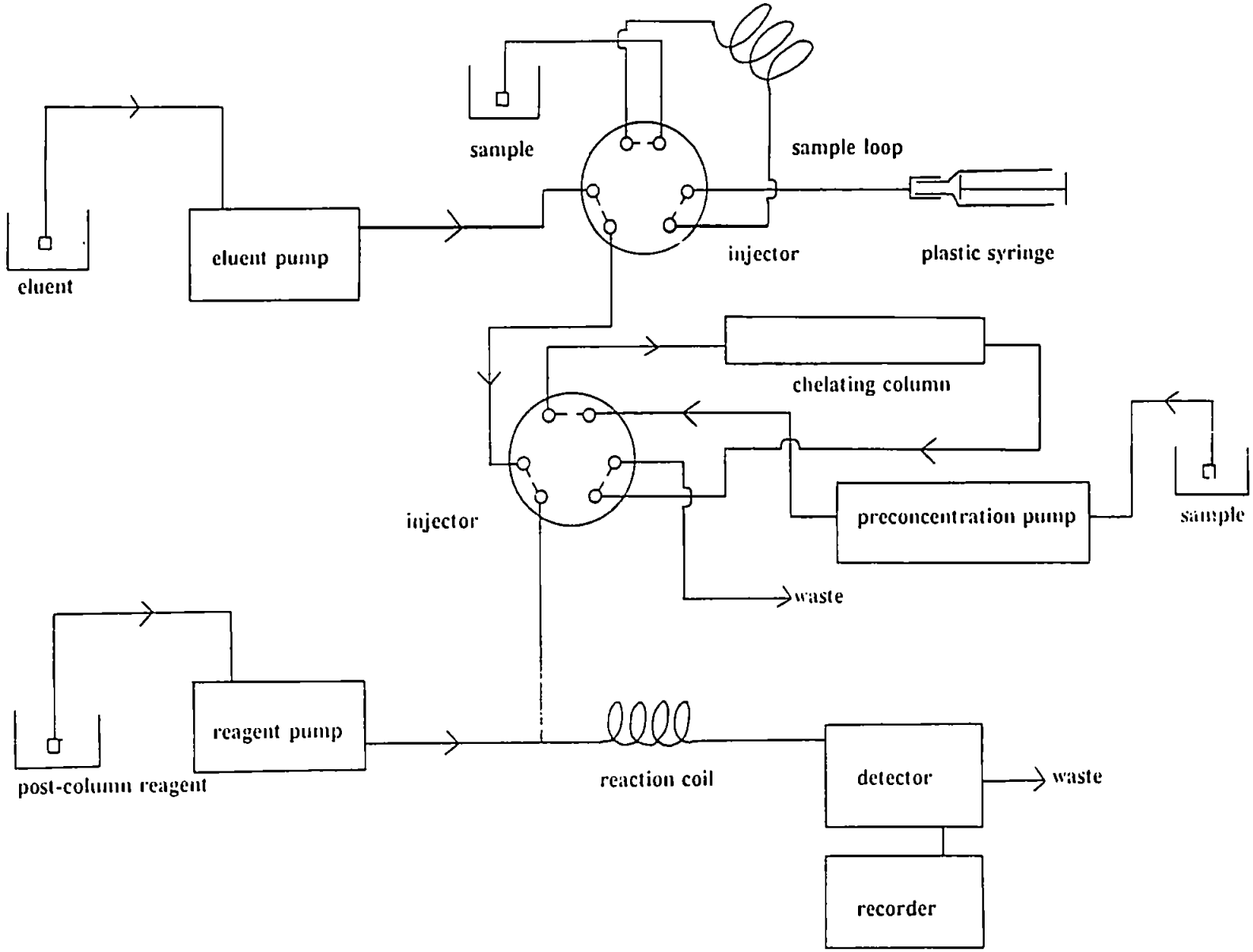


Figure 6. Schematic representation of the HPLC post column reaction system, modified for preconcentration applications.

(Shandon Southern Products Ltd. Cheshire, U.K). The slurries were prepared in 30% methanol/deionised water (DW) and sonicated using a sonic bath for 5min to thoroughly wet and produce a homogenous suspension of the resin particles. The slurry was made up to approximately $30cm^3$ and packed at 2000psi.

2.1.i. RESIN IMPREGNATION.

The impregnation of the resins was carried out using packed columns and recycling a dye solution through the column over night at 1ml/min. The dye solutions were made up to 100ml using 1:10v/v methanol and DW to produce a dye concentration of 5000mg dm^{-3}. The pH of the solution was adjusted to 5.0 prior to impregnation for the majority of the dyes studied. A short investigation, discussed in section 3.1, suggested pH 5.0 to be a good compromise pH to achieve maximum stable dye loading. More alkaline solutions resulted in a lighter loading and acidic solutions produced a high but less stable degree of impregnation. A 100 fold dilution of the dye solution was adjusted to a known pH and analysed for absorbance using a Perkin Elmer Lambda 7 UV Visible Spectrophotometer (Beaconsfield, Bucks, U.K). An identical dilution of the dye solution, after impregnation of the column, was taken, adjusted to the same pH and re-analysed to provide a value for the percentage reduction in absorbance which was used to calculate the level of dye in mg impregnated into the resin before conditioning of the column. To remove the unstable proportion of the dye remaining on the resin a program of conditioning was carried out involving passing DW, 0.1M HNO_3, DW, 0.1M NH_3, DW and 0.1M CH_3COOH through the column in the above order. Each solution was pumped through the column until visible signs of dye bleeding from the column had stopped. The total volume of conditioning solutions now containing the chelating dye

from the column, was made up to a known volume with DW and diluted 1:100v/v again using DW. This solution was adjusted to the same pH as the diluted dye solutions previously analysed and the absorbance determined as before to give a value from which the level of dye present can be determined. Subtracting this figure from the value of dye impregnated before conditioning gave a precise value of the stable loading of dye within the column.

2.1.ii. CLEAN-UP COLUMNS.

For seawater analysis where preconcentration was required, it was necessary to use a clean-up column to remove trace metals present in the KNO_3 eluent, thus reducing the procedural blank levels of these metals. Amberlite XAD-2 (Aldrich, Gillingham, Dorset, U.K) was gently shaken overnight in a 2000mg dm^{-3} solution of Xylenol Orange at pH 5.0 to impregnate the substrate. After conditioning as with high-performance resins, the substrate was slurry packed into a 25 x 2.0cm glass column. The column was buffered to pH 8.0 using 0.1M ammonium nitrate and the eluent gravity-fed through the column overnight at approximately 2ml/min. The preconcentrated metals were swept from the column after 1litre of the eluent had been purified, using 0.2M HNO_3. For the determination of Al(III) in seawater, a high-performance Chrome Azurol S chelating column was used both to clean-up the eluent and as an analytical column. The column was adjusted to pH 6.0 using a 0.1M ammonium acetate buffer and the 0.5M KNO_3 eluent (pH 6.0) pumped through the column overnight at 0.5ml min. The column was then swept through with 0.2M HNO_3 to remove the preconcentrated metal ions.

2.2. DYE PURITY STUDIES.

An important consideration when using chelating dye impregnation techniques is the purity of the dyes being used. Commercially available dyes can contain as little as 55% of the required dye, the remaining 45% consisting of impurities, non-chelating compounds and inorganic salts. It is necessary to ascertain the exact percentage of impurities in dyes from various manufacturers in order that the purest available be used (greater than 90% was considered acceptable). Certain chelating dyes are not easily available commercially and it becomes necessary to synthesise the required compound. A method was therefore required which could not only determine levels of impurities on an analytical scale but could be enlarged to a semi-preparative system to remove impurities from synthesised stock solutions.

2.2.i. XYLENOL AND SEMI-XYLENOL ORANGE.

Commercial sources of Xylenol Orange (XO) dye contain significant levels of Semi-Xylenol Orange (SXO), a chelating by-product of the XO production process. Several techniques were investigated with the aim of determining the purity of commercial sources of XO and isolating the SXO fraction. Cellulose column techniques reported by Olson et al. [143] and Murakami et al. [144] involving elution using organic solvents proved time consuming and inefficient. Similar problems were inherent in liquid/liquid extraction methods published by Kiciak [145-147]. A liquid chromatographic procedure detailed by Smedes et al. [148] proved to be the most efficient method of those investigated and was used to survey a range of XO dyes from various sources. The system was easily adapted to a semi-preparative scale. This enabled the production of pure SXO (which is not available commercially) from a crude synthesised

product achieved following a procedure reported by Nakayama et al. [149]. The basic LC system consisted of a solvent pump (Waters Chromatography, Middlesex, U.K) an injection valve (Waters Chromatography), the column and a spectral array detector (Dionex (U.K) Ltd., Camberley, Surrey, U.K). For analytical separations a 10 x 0.46cm ODS Hypersil stainless steel column was used. This was replaced with a 12 x 1.0cm column for semi-preparatory work. The sample loop, 20μl for analytical work, was replaced with a 1000μl loop. Exact experimental conditions are listed by Smedes et al. [148]. Approximately 60mg of pure SXO was obtained using the above methodology.

2.2.ii. CAPILLARY ELECTROPHORETIC DYE ANALYSIS.

A recent paper by Burkinshaw et al. [150] illustrated the use of capillary electrophoresis for the analysis of several dye classes. Following the methods outlined in the above paper, a Dionex CES-1 capillary electrophoresis system (Dionex (U.K) Ltd., Camberley, Surrey, U.K) was used as a rapid method to assess the purity of a large range of dyes from several classes.

2.3. REAGENTS.

Reagents were obtained from BDH (Poole, Dorset, U.K) except PAR and Zn-EDTA which were supplied by Fluka (Glossop, Derbyshire, U.K). All reagents were analytical grade unless stated otherwise. Solutions were prepared using DW obtained using a Milli-Q ultra pure water system (Millipore, MASS, USA) and degasssed using helium. Chelating dyes were obtained from a variety of suppliers which are discussed in section 3.0.i. of the results.

2.3.i. ELUENTS.

1M KNO_3 was used as the eluent for the majority of HPCIC applications. This was reduced to 0.5M KNO_3 for samples requiring preconcentration to reduce high blanks, which were a result of trace metals present in 'AnalaR' KNO_3. The eluent contained 0.05M lactic acid and was pH adjusted using a dilute solution of HNO_3 and a dilute solution of NH_3.

2.3.ii. POST COLUMN REAGENTS.

Several post column reagents were used throughout this work depending upon the analyte of interest. The majority of work, including the testing of each new column for metal separation efficiencies, used PAR/Zn-EDTA. The solution consisted of 30ml of stock 4 x 10^{-3}M PAR solution, 50ml of 4 x 10^{-3}M Zn-EDTA and 110ml of 35% NH_3 made up to 1litre with DW. Absorbance was measured at 490nm. For seawater analysis of divalent metals PAR alone was used as this is very insensitive to Ca(II) and Mg(II). The PAR solution was made up using 30ml of stock 4 x 10^{-3}M PAR, 192ml of 35% NH_3, 54ml of 70% HNO_3 and DW. The addition of HNO_3 facilitates an ammonium nitrate buffering capacity to reduce baseline changes resulting from gradient elution programs used. Absorbance was measured as above. The detection of trivalent metals was achieved using Pyrochatechol Violet (PCV). A 0.004% solution was buffered to pH 6.0 using 2M hexamine. An increase in absorbance was monitored at 580nm.

2.3.iii. SAMPLES.

Metal standards were prepared from 1000mg dm^{-3} 'SpectrosoL' solutions (BDH, Poole, Dorset, U.K) using the appropriate dilution with DW and acidifying with dilute HNO_3. All reagents, standards and samples were contained in acid washed (10% HNO_3) polyethylene containers (BDH).

Seawater samples were collected in conditioned sample bottles and usually analysed within 24h. It was therefore decided not to acidify the sample as this may introduce contamination. The sample was filtered using a 0.45μm filter and adjusted to pH 6.0 using dilute HNO_3 prior to the determination of divalent metals. This was reduced to pH 4.0 for the determination of Al(III) in seawater.

For the determination of Sr(II) in skimmed milk powder, an acid digestion of the sample was carried out in a nickel crucible. 1g of the sample was ashed using a high temperature Mecher burner and boiled in 10ml of 0.1M HNO_3 until the solution appeared clear. This was then cooled and neutralised using dilute NH_3. The solution was made up to 20.0ml with DW and immediately analysed.

The remaining samples used throughout this work were not pre-treated except for varying dilutions using DW. These dilution factors are stated within the appropriate sections of each results chapter. Standard addition techniques were used for all applications. The addition of metal spikes to samples were carried out immediately prior to analysis. All samples were thoroughly degassed with helium before being analysed.

2.3.iv. PRECONCENTRATION PROCEDURE.

Prior to the determination of divalent trace metals in seawater, any metals remaining on the column from previous work were swept off the column using a 0.5M KNO_3 eluent adjusted to pH 1.2 with HNO_3. When determining Al(III) in seawater the column was swept through with 0.5M KNO_3 which was previously adjusted down to pH 0.8. The columns were then switched to the preconcentration pump, which was used to deliver 10ml of a 0.1M ammonium acetate buffer solution adjusted to pH 6.0, for the divalent determinations, or 5ml of a 0.1M sodium acetate buffer solution adjusted to pH 4.0 for the Al(III) determinations. This was followed by a measured volume of sample adjusted to the same pH. A small volume of DW was then pumped through the column to remove the excess of NaCl and the bulk of the alkaline earth metals, which were not retained on either column at these pH values. The columns were then switched back on-line with the eluent pump and the appropriate step gradient programmes initiated to elute the preconcentrated metals.

CHAPTER 3. RESULTS AND DISCUSSION.

3.0. DYE PURITY STUDIES.

As mentioned in section 2.2., the purity of a chelating dye is an important consideration when producing dye impregnated chelating columns, especially if efficient and reproducible columns are required. Several techniques were investigated (details are given in section 2.2.i.), two of which were used to provide quick indications of the levels of impurities present in the dyes being used.

3.0.i. LIQUID CHROMATOGRAPHIC ANALYSIS.

Two major impurities in commercially available Xylenol Orange (XO) dyes are Semi-Xylenol Orange (SXO) and Cresol Red (CR), by-products of the production process. A liquid chromatographic (LC) technique detailed by Smedes et al. [148] uses 5μm c18-bonded silica, with a perchloric acid and acetone eluent to produce chromatographic separations of these and other impurities in various XO dyes. The technique was employed to survey XO dyes from a range of suppliers. Figure 7 shows the chromatogram produced from the injection of a solution of XO dye obtained from BDH (Poole, Dorset, U.K.), compared with a less pure XO dye supplied by Fluka (Glossop, U.K.). The presence of a significant amount of SXO is clearly visible in the sample supplied by Fluka. SXO is structurally very similar to XO, but contains only a single iminodiacetic acid functional group. The presence of chelating impurities such as SXO within a XO dye could have noticeable affects upon the efficiency and retention characteristics of chelating columns produced using the XO dye.

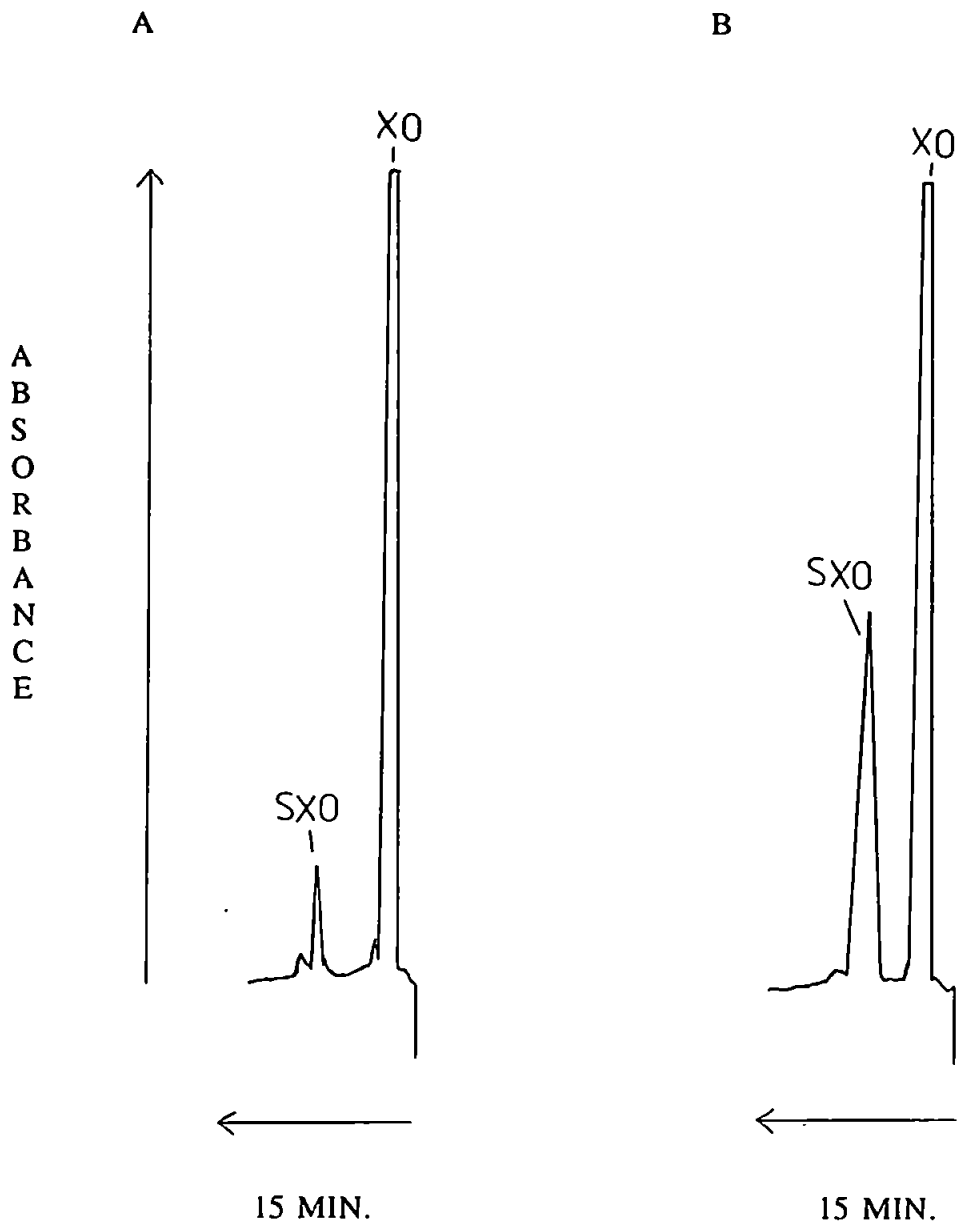


Figure 7. Chromatograms showing the Semi-Xylenol Orange (SXO) impurity present in A) Xylenol Orange (XO) dye obtained from BDH and B) XO dye obtained from Fluka.

From the investigation into the purity of commercial sources of XO, the dye supplied from BDH proved to be the purest ($\approx$93%) and so was used for the production of XO impregnated chelating columns.

SXO can also be used, in a pure form, to produce a chelating column. This would exhibit differing chelating characteristics to that of a XO impregnated column. In order to obtain a pure supply of SXO, the dye has to be synthesised and purified in the laboratory, this is due to the dye being unavailable commercially. A stock solution containing 70mg of SXO was synthesised using a procedure reported by Nakayami et al. [149] and purified using a scaled-up semi-preparative version of the above LC technique. Figure 8 shows a chromatogram of the synthesised stock solution, containing large amounts of XO, SXO and CR, compared to a chromatogram of the purified product. This pure SXO product was used to produce a SXO impregnated chelating column, which is discussed in section 3.2.ii.

The above technique proved very successful for the analysis and purification of XO and SXO dyes. The method could also be applied to similarly structured triphenylmethane-based dyes, such as Methylthymol Blue (MTB). However the LC technique was not suitable for the analysis of other dye types, such as azo dyes and so a capillary electrophoretic method was investigated.

3.0.ii. CAPILLARY ELECTROPHORETIC ANALYSIS.

A capillary electrophoretic technique published by Burkinshaw et al. [150], illustrates the analysis of various types of dye, including azo and triphenylmethane dyes. This technique was applied to the analysis of the majority of chelating dyes being used in this study. Figure 9 shows the capillary electrophoretogram achieved from the injection of a solution of XO dye

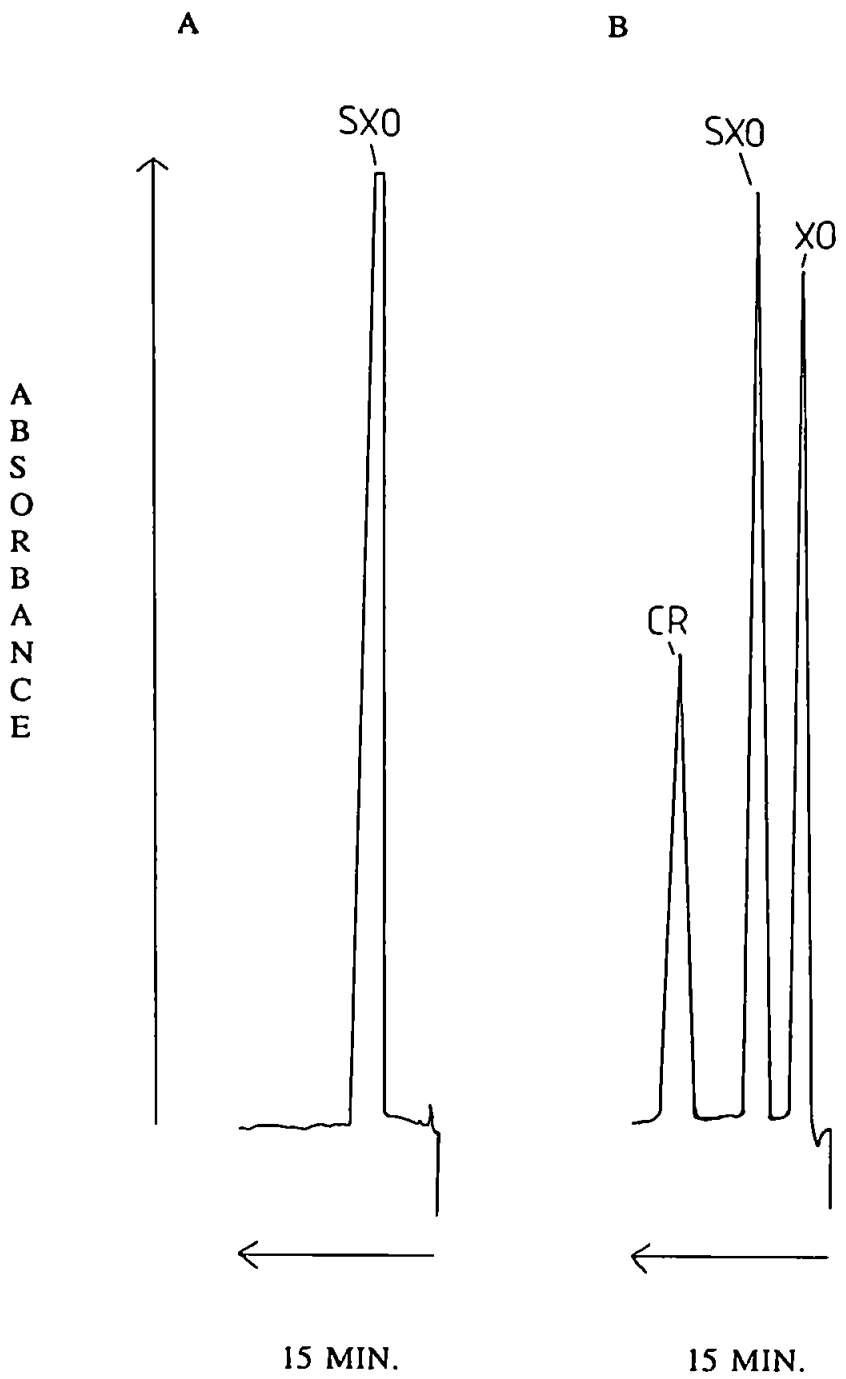


Figure 8. Chromatograms showing A) purified Semi-Xylenol Orange obtained from B) a synthesised stock mixture containing Xylenol Orange (XO), Semi-Xylenol Orange (SXO) and Cresol Red (CR).

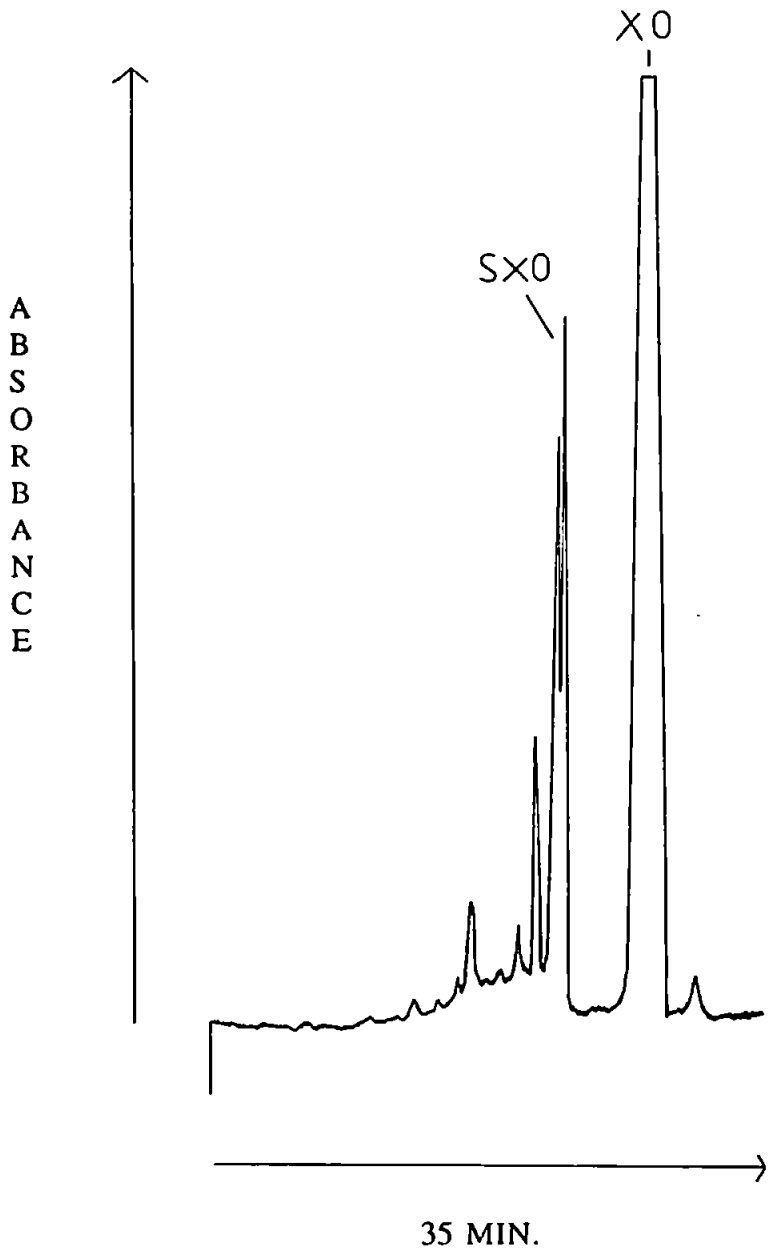


Figure 9. Capillary electrophoretogram showing the Semi-Xylenol Orange (SXO) impurity present in Xylenol Orange (XO) dye obtained from BDH.

obtained from BDH. The analysis of these chelating dyes using capillary electrophoresis and liquid chromatography generally served to verify the claims of the manufacturers, in terms of percentage dye content. Where possible, dyes were chosen which contained over 90% dye content. Some commercial sources of these types of dyes are reluctant to supply information on purity and the above techniques provided relatively simple ways of assessing the quality of certain dyes.

3.1. PRODUCTION OF DYE IMPREGNATED COLUMNS.

The ability to easily reproduce chelating columns, which exhibit essentially the same chelating characteristics and separating efficiencies, is very important if the technique is to become an accepted analytical method. The two major parameters which affect the reproducibility of these columns are, firstly, the purity of the dye, which the previous techniques help to determine, and secondly, the ability to achieve similar loadings of the impregnated dye.

Early attempts to reproduce a XO impregnated 10μm, 100Å PLRP-S column used by Jones and co-workers [76-79], which exhibited the same separation efficiency, proved unsuccessful. Using the same coating technique and the same resin, it proved difficult to achieve similar dye loadings. The column used by the above workers had a loading of approximately 48mg of dye per 0.9g of resin. Several attempts to reproduce this level of impregnation failed.

The efficiency of columns was compared via the ability to separate Mn(II), Cd(II), Zn(II) and Pb(II) at a low pH, and Ba(II), Sr(II), Mg(II) and Ca(II) at a higher pH. Loadings of between 17 and 28mg XO, although achieving efficient separations of the first set of metals at low pH values of between 3.5-4.2, failed to separate Ca(II) and Mg(II) in the higher pH

ranges, 8.0-10.0. These lower loaded XO columns were all capable of separating Ba(II) and Sr(II), from each other and from a combined Ca(II) and Mg(II) peak, but Ca(II) and Mg(II) remained as a single peak at pH values as high as 9.0-10.0. Figure 10 shows the chromatograms of separations achieved using a XO column loaded with only 28mg of dye.

A small study into the affect of the pH of the dye upon the level of loading, was carried out by adjusting the pH of a XO dye solution during impregnation of PLRP-S resin and monitoring the rate of removal of dye from the solution. It was noticed that lowering the pH of the dye solution increased the level of the dye being impregnated into the resin. Previous attempts at impregnating the resin used dye solutions at pH values of between 6.0 and 8.5. Reducing this to pH 5.0 appeared to cause an increase in the loading achieved. Reducing the pH further also increased the loading, although the increased loading was unstable and bled off the column during conditioning. For this reason an optimum pH of 5.0 was used for the impregnation of the majority of dyes investigated, provided they were suitably soluble at this pH.

Another variation on the impregnating procedure used by previous workers, was to increase the concentration of dye in solution from 0.2% to 0.5%. This increase in concentration was aimed to help force increased numbers of dye molecules into the pores of the resin. Using the above methods it was possible to produce XO chelating columns, which exhibit almost identical separation efficiencies to the column used by Jones and co-workers. The level of loading achieved was increased to close to 40mg, which was enough to allow the complete separation of all the alkaline earth metals (illustrated in section 3.2.i.).

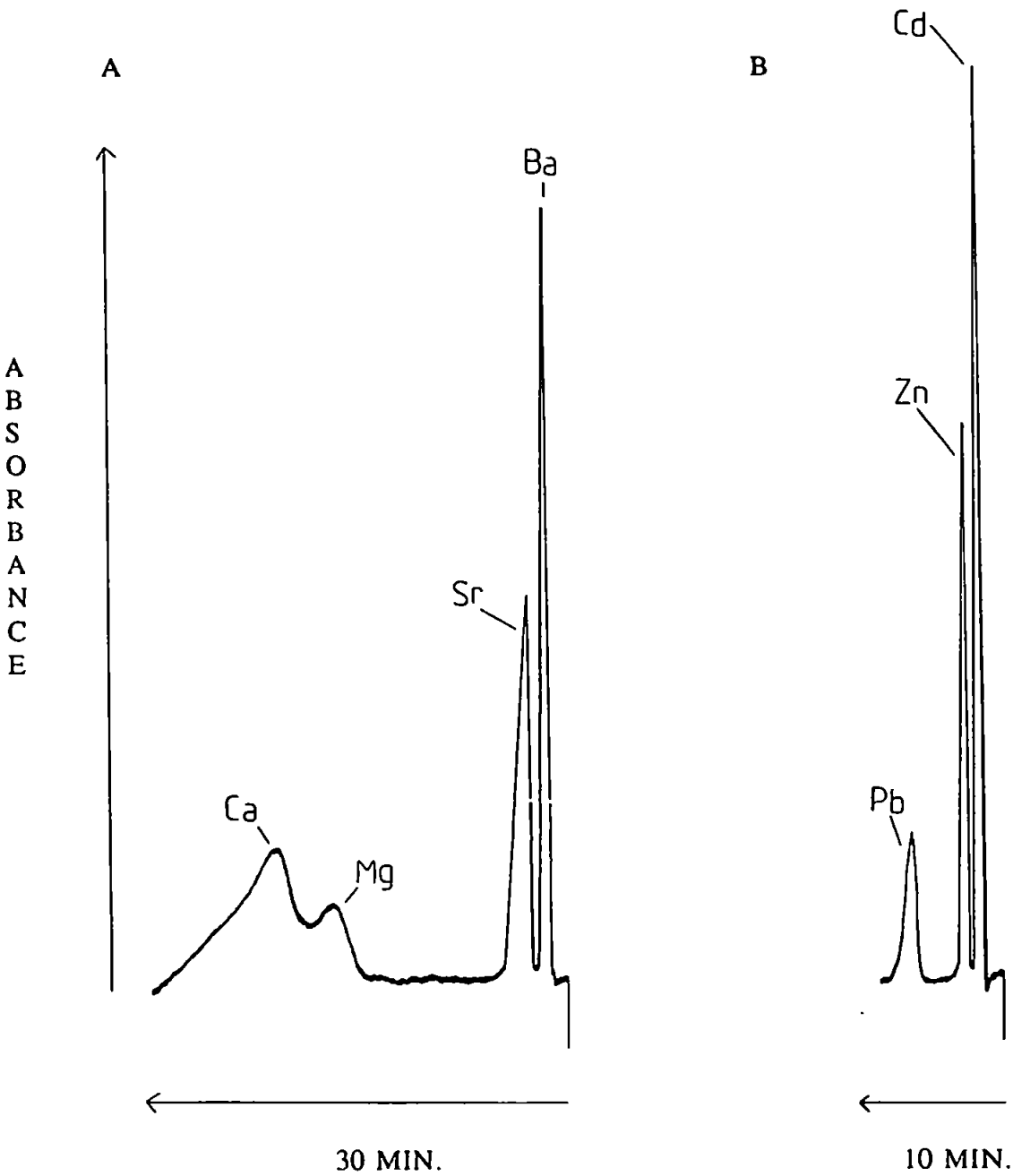


Figure 10. Chromatograms showing the separation of A) Ba(II), Sr(II), Mg(II) and Ca(II) at pH 9.0 and B) Cd(II), Zn(II) and Pb(II) at pH 3.5 in 1M KNO_3 using a lower loaded (28mg) PLRP-S impregnated Xylenol Orange column.

3.1.i. THE EFFECTS OF RESIN CHARACTERISTICS.

The effect the pore size of a resin has upon the level of dye impregnated was also investigated. The efficiency of chelating columns produced from impregnating varying resins with XO dye were compared via their ability to separate a range of metal ions. Two sets of similar hydrophobic polystyrene-divinylbenzene (PS-DVB) neutral resins were obtained, exhibiting a range of pore sizes. The first, obtained from Polymer Laboratories, was 10μm PLRP-S resin with pore sizes of 100, 300, 1000 and 4000Å. The second, which was supplied by Dionex (U.K.) Ltd., was 8.8μm, 120Å pore size substrate and also 7.0μm, 300Å pore size material.

Initial investigations into the impregnation of the range of PLRP-S resins with XO dye were carried out before optimum impregnating procedures had been determined, therefore the loadings of dye are likely to be less than could be achieved, although certain trends do become apparent. Table 4A shows the loadings of XO dye achieved on PLRP-S resins of differing pore sizes. The results show a significant increase in dye loading with the resins containing the smaller pore sizes of 300Å and 100Å, approximately 30% higher than the loadings of the much larger pores of 1000Å and 4000Å. Possible explanations for this include a straightforward surface area affect. As can be seen from Table 4A, surface area increases with decreasing pore size, therefore there is simply more surface area onto which the dye can become adsorbed. Another possible reason is the physical trapping of dye molecules within the smaller pore spaces. The larger pores may be too large to retain the dye molecules which simply pass straight through. The increased loading on the 100Å PLRP-S resin, achieved using optimised impregnated procedures, can be seen in Table 4A.

The impregnation of the two Dionex resins resulted in lower loadings than the

A) Polymer Laboratories PLRP-S resin.

Pore size (Å).	Surface area (m^2/g).	Particle size (μm).	Amount impregnated (mg dye per 0.9g resin).
4000[a]	139[c]	10	19
1000[a]	267[c]	10	19
300[a]	384[c]	10	28
100[a]	414[c]	10	28
100[b]	414[c]	10	39

B) Dionex macroporous neutral resin.

Pore size (Å).	Surface area (m^2/g).	Particle size (μm).	Amount impregnated (mg dye per 1.1g resin).
300[b]	60	7.0	24
120[b]	470	8.8	28

[a] Dye impregnated using procedure detailed by Jones and co-workers.

[b] Dye impregnated using optimised procedure.

[c] Ref. [171].

Table 4. A) Loadings of Xylenol Orange achieved on PLRP-S resins of varying pore size.

B) Loadings of Xylenol Orange achieved on Dionex Resins of varying pore and particle size.

PLRP-S resins, using optimised impregnating procedures. This can be seen in Table 4B. However, the 120Å, 8.8μm impregnated Dionex resin, although having a loading of only 28mg, exhibited separating efficiencies equal to the PLRP-S 100Å, 10μm resin with a loading of 39mg. As was the trend with the PLRP-S resins, the larger pore size Dionex substrate, 300Å, showed a lower loading than the 120Å material. The surface areas of the two Dionex substrates differ greatly and it could be assumed that if surface area was the only factor affecting dye loading, that the differences in loading achieved between the two resins would be much greater.

Of the impregnated resins investigated and shown in Tables 4A and 4B, only the higher loaded 100Å PLRP-S material and the Dionex 120Å material were efficient enough to successfully separate fully the alkaline earth metals, although all the impregnated resins showed similar efficiencies in the separation of transition and heavy metals. As the difference in loading between the two Dionex substrates is small, the difference in efficiency may be caused by the metal ions which pass through large pore spaces, having less contact with the immobilised chelating functional groups, than they would with smaller enclosed pore spaces. From the results achieved for all the resins investigated, exact conclusions as to the effects of pore size upon dye loading and column efficiency are difficult to make. However it does seem clear that a pore size close to 100Å, with a high surface area appears to produce high loadings and the most efficient separations. For the remaining investigations into the separating efficiencies of other impregnated chelating dyes, the Dionex 8.8μm, 120Å substrate was used.

3.1.ii. LOADINGS, ACTIVITIES AND COLUMN CAPACITIES.

The loading values discussed in the previous section refer to the total amount of dye impregnated into the resin. However the capacity of the impregnated column is dependent upon the percentage of this dye which is actively chelating. Due to steric effects within the column a percentage of the impregnated dye is unable to complex with the metal ions present. This percentage of inactive dye varies depending upon the type and structure of the impregnated chelating dye.

The method used to determine these percentage activities and column capacities involves pumping a solution of Zn(II) through each column, at a pH significantly higher than required for the column to fully retain the metal. For all the dyes investigated pH 8.0 was sufficient. When all the active chelation sites are complexed with Zn(II) ions, the metal will begin to elute from the column. By calculating the exact amount of Zn(II) in moles required to fill all the chelation sites, and using the number of moles of dye known to be impregnated into the resin, it is possible to determine the percentage of this dye which is complexing with the metal ion. A problem with this approach is the release of hydrogen ions from the chelating functional groups when forming a chelate with a metal ion. This increase in acidity will reduce the effective capacity of the column. For this reason the solution of Zn(II) used, was buffered to help keep the column pH close to 8.0 during the loading of the metal. Table 5 shows the loadings achieved for a range of chelating dyes. Also shown is the percentage of this dye which is actively chelating and the total capacity of the columns expressed as mg Zn(II).

A large variation in all three of the above parameters is evident, although some conclusions can be made. Firstly, with the exception of calmagite (CAL), five of the six dyes

Chelating dye.	Amount impregnated (mg dye per 1.1g resin).	% Activity.	Active dye (mg dye per 1.1g resin).	Capacity (mg Zn(II) per 1.1g resin).
XO	28	28.0	7.8	0.74
SXO	16*	—	—	—
MTB	40	26.0	10.4	0.95
PP	110	14.0	16.0	1.6
GCR	60	6.6	4.0	0.48
CAS	23	6.0	1.4	0.23
CAL	11	25.0	2.8	0.36
PAR	103	4.4	4.5	1.36
OII	43	1.5	0.6	0.12
SPPD	15	4.2	0.6	0.1

* amount impregnated in mg dye per 0.9g PLRP-S resin.

Table 5. Loadings, percentage activity and capacity of various chelating dye impregnated resins.

with the highest percentage activities are all triphenylmethane dyes, namely, Xylenol Orange (XO), Methylthymol Blue (MTB), Phthalein Purple (PP), Glycine Cresol Red (GCR) and Chrome Azurol S (CAS). A possible reason for this is the above four dyes all contain two chelating functional groups, as opposed to the one active chelating site of the remaining dyes. Therefore if one of the sites is unable to chelate with metal ions due to steric effects, the other site may still be able to do so. It is also noticeable that of the five dyes mentioned, the three with the highest activities and therefore capacities are the three iminodiacetic acid containing dyes, these being XO, MTB and PP.

With the remaining azo type dyes any trends are difficult to determine. The results which do stand out include the high loading of 4-(2-pyridylazo) resorcinol (PAR), which, although having a low percentage activity, had one of the highest total capacities. Also the activity of the CAL impregnated column was significantly higher than the remaining azo based dyes. The affect these parameters have upon the separation characteristics of each dye will be discussed in the next section.

3.2. METAL SEPARATIONS.

The following section details the metal separations achieved using a range of chelating dye impregnated columns. As mentioned previously the separating efficiency of these chelating columns was determined using the ability to separate the alkaline earth metals, a number of transition and heavy metals and for selected dyes, the ability to separate a number of trivalent metals. The resulting separation profiles of these metals and the pH required to achieve the separations, provides information on the relative efficiencies and chelating strength of each column. The physical structures of the ten dyes investigated are shown in Figures 11-15.

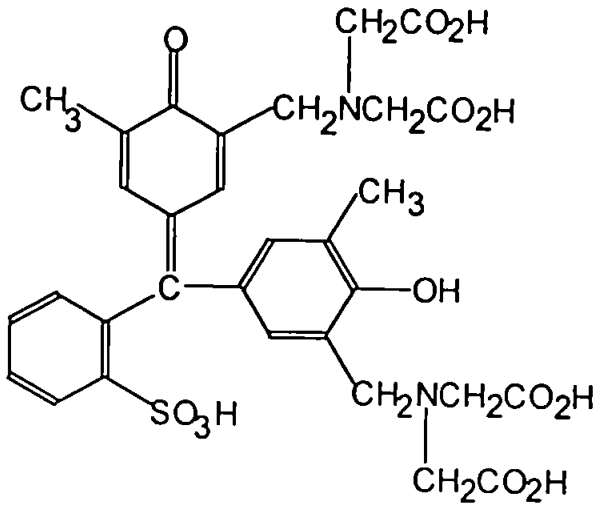
Figures 11-13, show the triphenylmethane class of dyes, Figures 14-15, illustrate the azo type dyes investigated. All the following separations, bar SXO, are carried out in 1M KNO_3, using 10cm impregnated columns, containing Dionex 8.8μm, 120Å PS-DVB resin. Separations using SXO were achieved using PLRP-S 10μm, 100Å PS-DVB impregnated resin. The mixed metal standards used to produce the separations shown in the following sections were all in the range of 1 to 50mg dm^3.

3.2.i. XYLENOL ORANGE (XO).

Figure 16A, is a chromatogram showing the high-performance separation of Ba(II), Sr(II), Mg(II) and Ca(II), eluting in the above order, achieved using a XO impregnated column. The efficient separation of the four metals is complete in under 15min. The pH required to produce the separation was 7.7. The elution order of the four metals is reversed to that of simple ion exchange, although, as shall be seen with a number of the chelating dyes used, the retention order of Mg(II) and Ca(II) can alternate depending upon the dye. Here, Mg(II) elutes before Ca(II). The rapid broadening of peaks eluting after the first 8-10min, in this case Ca(II), is a result of the relatively slow kinetics involved in chelation reactions. Because of this, the number of metals which can be separated isocratically is usually limited to 3 or 4. The use of step gradient elution can increase this number (this is illustrated in Chapter 4, with the analysis of trace metals in seawater).

Figure 16B, shows the chromatogram produced from the injection of a mixture of Mn(II), Cd(II) and Zn(II) at pH 3.2. The peaks are sharp with very little tailing, producing an efficient separation. At pH 3.2, Pb(II) is retained for over 30min, reducing the pH to 3.0 allows the separation of Cd(II), Zn(II) and Pb(II), shown in Figure 16C.

A



B

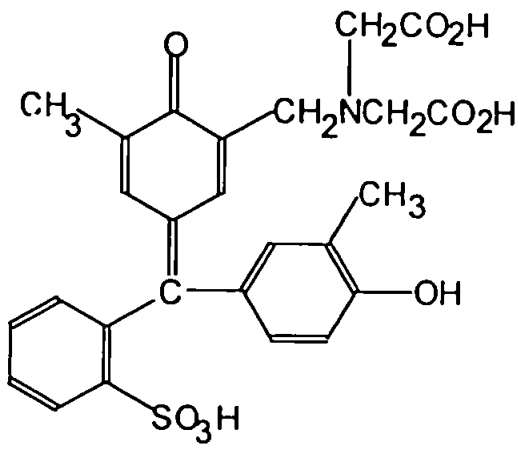
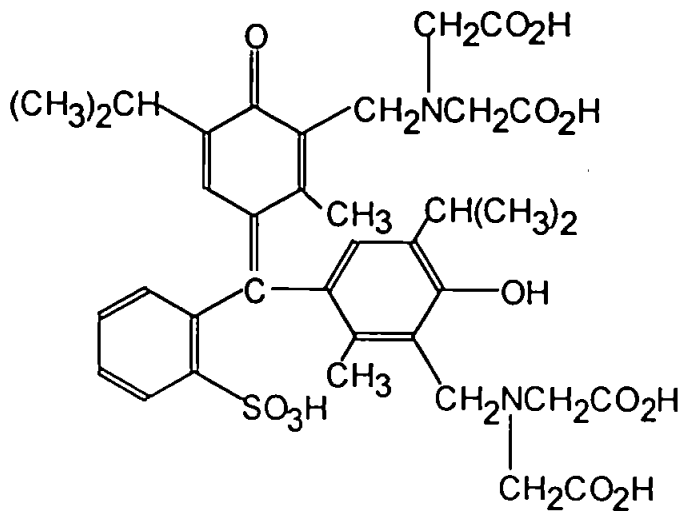


Figure 11. Structures of A) Xylenol Orange, B) Semi-Xylenol Orange.

A



B

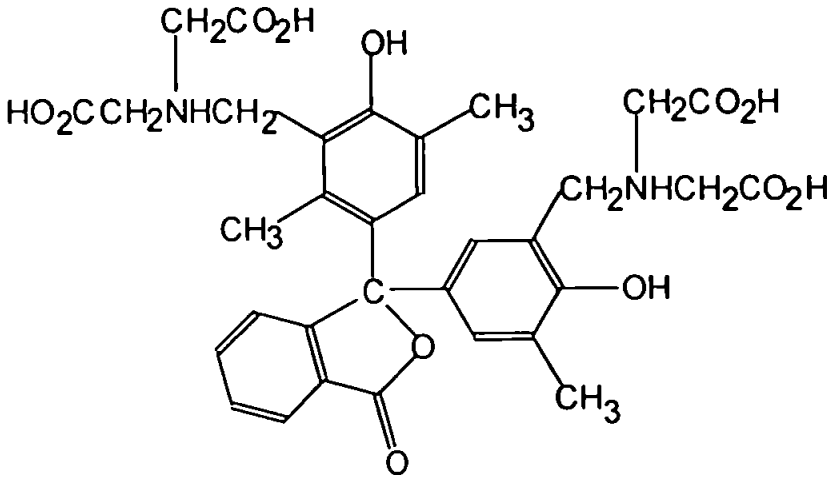
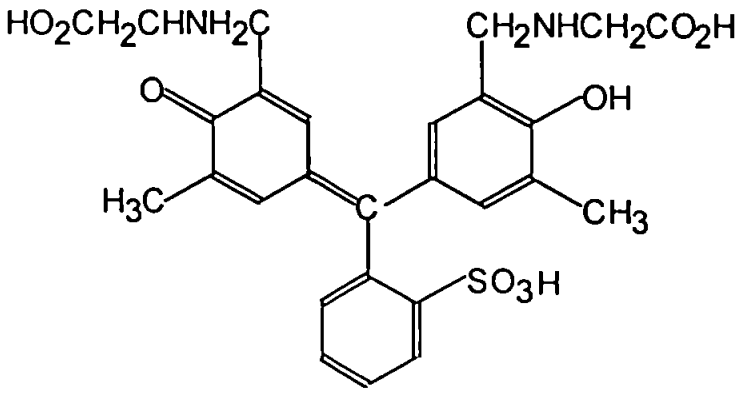


Figure 12. Structures of A) Methylthymol Blue, B) Phthalein Purple.

A



B

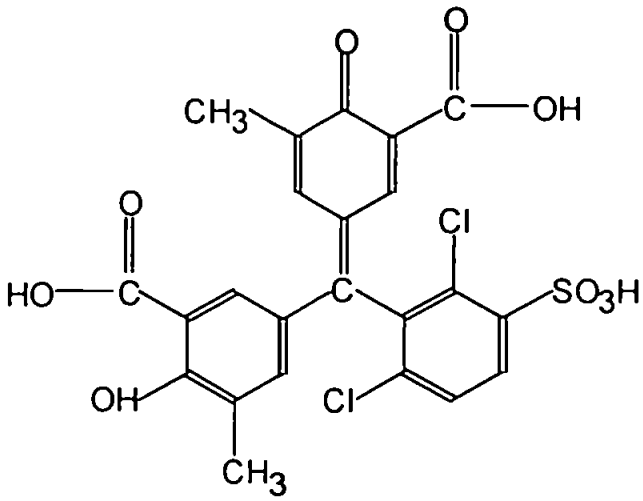
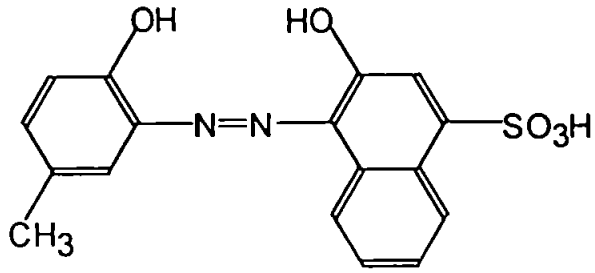


Figure 13. Structures of A) Glycine Cresol Red, B) Chrome Azurol S.

A



B

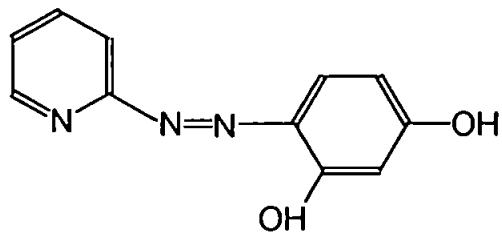
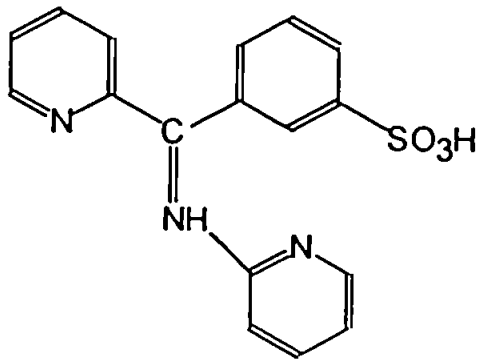


Figure 14. Structures of A) Calmagite, B) 4-(2-pyridylazo) resorcinol.

A



B

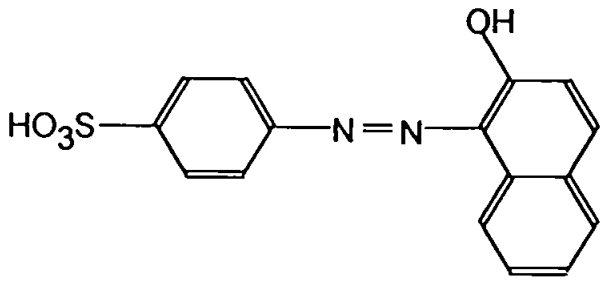


Figure 15. Structures of A) 2-(3-sulphobenzoyl) pyridine 2-pyridylhydrazone dihydrate, B) Orange II.

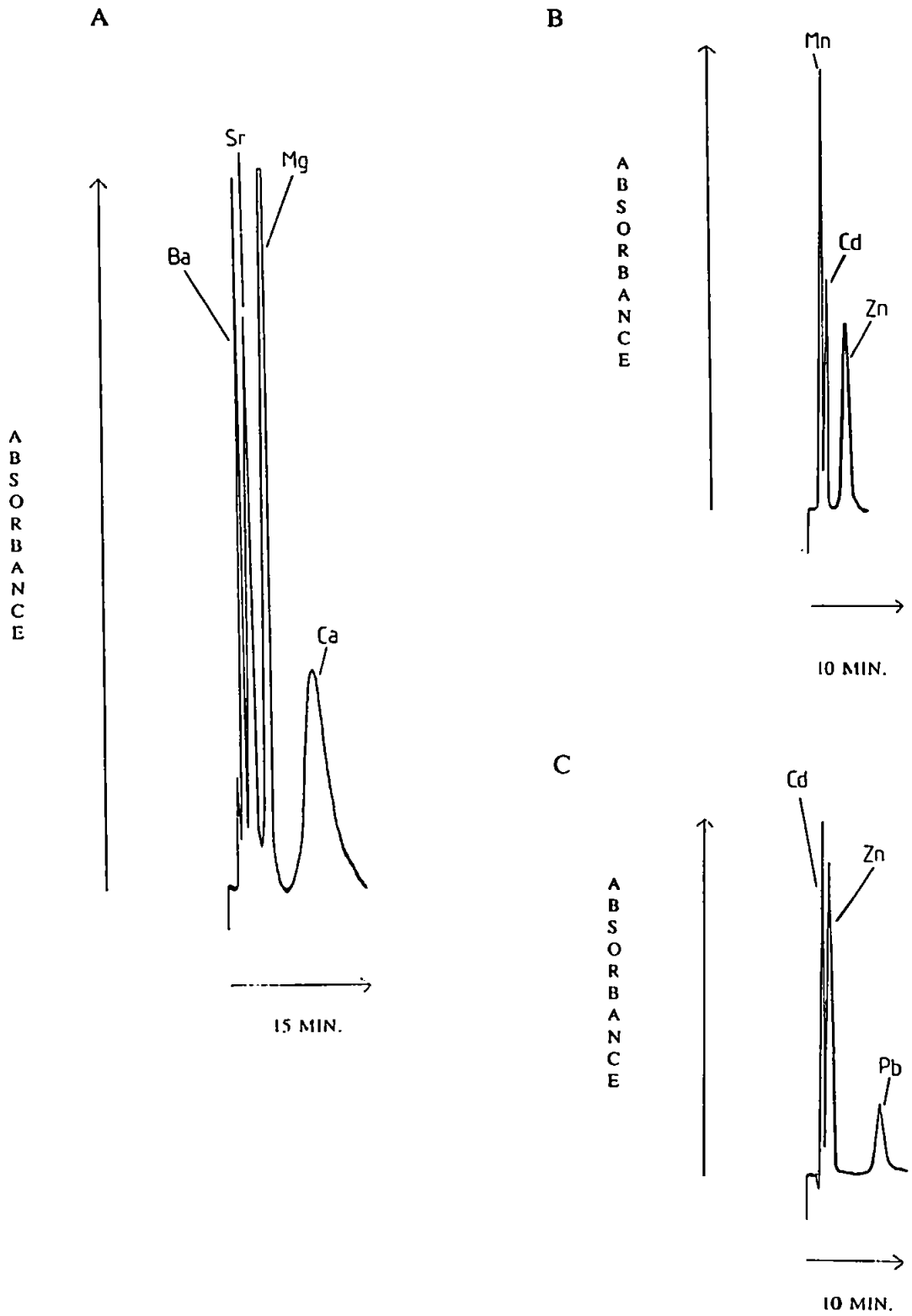


Figure 16. Chromatograms showing the separation of A) Ba(II), Sr(II), Mg(II) and Ca(II) at pH 7.7, B) Mn(II), Cd(II), and Zn(II) at pH 3.2 and C) Cd(II), Zn(II) and Pb(II) at pH 3.0 in 1M KNO_3 using a Xylenol Orange impregnated column.

The percentage activity of the XO impregnated column was the highest of the dyes investigated, 28%, resulting in a relatively high concentration of actively chelating XO dye on the resin, approximately 8mg. This amount of active sites may be required to sufficiently separate the Ca(II) and Mg(II) peaks in the alkaline earth mixture, as these co-elute when using lower loaded XO columns.

3.2.ii. SEMI-XYLENOL ORANGE (SXO).

Figure 17A, shows the chromatogram produced from the injection of an alkaline earth metal standard mixture using a SXO impregnated column. The column exhibits a much lower efficiency than the XO impregnated column, this is illustrated with the inability of the column to produce a separation of Ca(II) and Mg(II) and the asymmetrical tailing shape of the co-eluting Ca(II) and Mg(II) peak. The combination of the presence of only a single iminodiacetic acid chelating group on the SXO molecule (as opposed to two on the XO dye), and a low loading, only 16mg, means the SXO impregnated column exhibits weaker chelating properties than the previous XO column. This is reflected in the high pH of 9.7 required to obtain the partial separation of the alkaline earth metals.

The separation of Cd(II), Zn(II) and Pb(II) using the SXO column is shown in Figure 17B. Again, the pH required to produce the separation, 4.0, is higher than that required using the XO impregnated column. The separation profile of the above three metals using the SXO column is similar to that of the XO column, although the resolution between Pb(II) and Zn(II) appears slightly greater with the SXO column.

The SXO was initially intended to be used for the preconcentration of trace metals from seawater, replacing the XO column used by Challenger et al. [78]. Due to the weaker

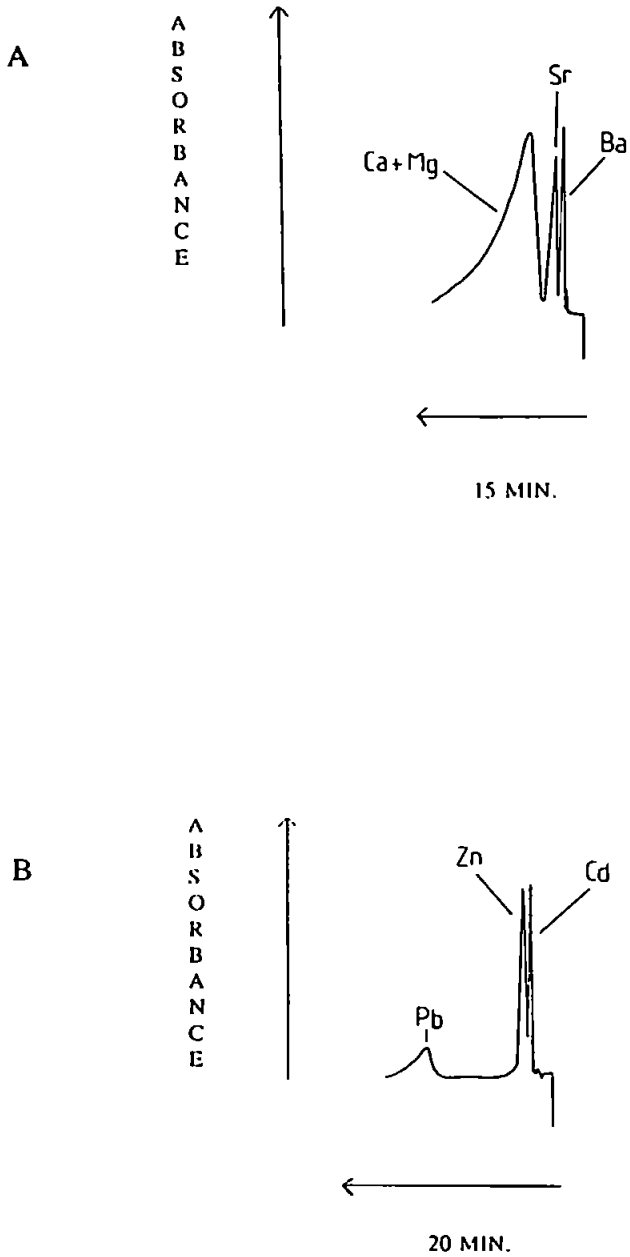


Figure 17. Chromatograms showing the separation of A) Ba(II), Sr(II), Mg(II) and Ca(II) at pH 9.7 and B) Cd(II), Zn(II) and Pb(II) at pH 4.0 in 1M KNO_3 using a Semi-Xylenol Orange impregnated column.

chelating nature of the SXO column, it would allow the preconcentration of the analyte metals at pH 8, without retaining the alkaline earth metals present. This would therefore remove the need to adjust the pH of the seawater, which can lead to contamination of the sample. However during early investigations into this application using the SXO column it was noticed the efficiency of the column was slowly deteriorating and rapid changes in ionic strength caused by the preconcentration program caused substantial bleeding of the dye from the column. Therefore XO was chosen for use in this particular application.

3.2.iii. METHYLTHYMOL BLUE (MTB).

As can be seen from Figures 11 and 12, the physical structure of MTB is very similar to that of XO. The loadings and capacities achieved for the two dyes are also comparable. It is therefore not surprising that these similarities are reflected in the resulting metal separations. Figure 18A shows the separation of a standard mixture of alkaline earth metals. Several similarities between this separation and that achieved using the XO column are clear. The order of elution for the above metals is the same and the pH required for the complete separation is close to 8.0 for both columns, MTB being slightly the weaker of the two columns and requiring a pH of 8.1. The profile of the separation does vary between the two columns, in particular, the resolution between Ca(II) and Mg(II), which is a little greater when using the MTB column. This can be important in certain applications as shall be seen in Chapter 5, with the determination of Ba(II) and Sr(II) in mineral waters. The exponential broadening of the peaks with increasing retention time, when carrying out isocratic separations, can clearly be seen in Figure 18A. This is an unfortunate characteristic of the slow kinetics involved in chelation ion chromatography when in isocratic mode.

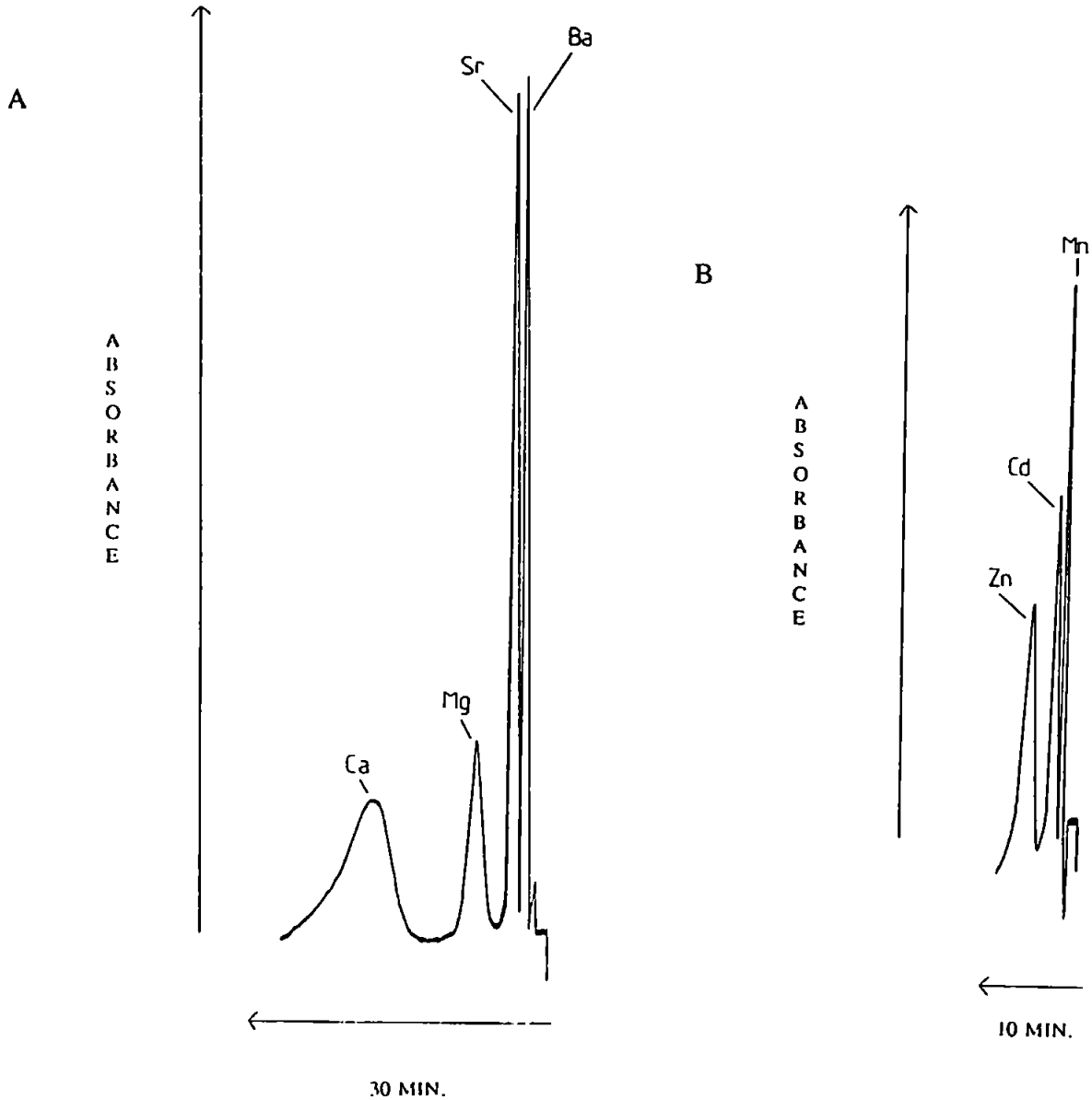
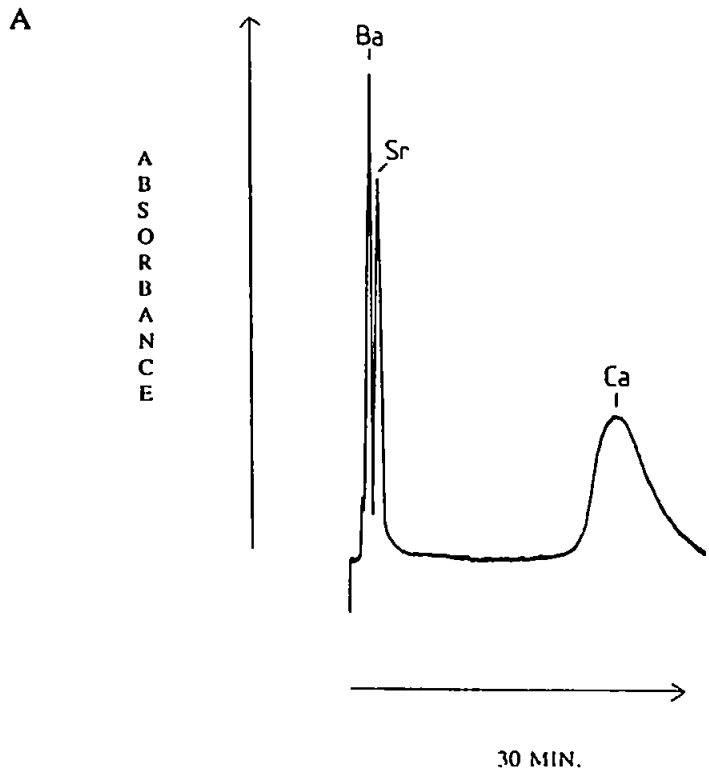


Figure 18. Chromatograms showing the separation of A) Ba(II), Sr(II), Mg(II) and Ca(II) at pH 8.1 and B) Mn(II), Cd(II), and Zn(II) at pH 3.5 in 1M KNO_3 using a Methylthymol Blue impregnated column.

Figure 18B shows the chromatogram produced from the injection of Mn(II), Cd(II) and Zn(II). Again the separation is very similar to the XO column. The two differences which are apparent, are firstly, the increased pH required to separate the three metals using MTB and secondly, the slightly broader peaks resulting from the MTB column, indicating a slightly less efficient column.

3.2.iv. PHTHALEIN PURPLE (PP).

As with the previous three chelating dyes, PP is also a triphenylmethane based dye. It contains two iminodiacetic acid chelating groups, as does XO and MTB. However the structure of PP does differ from that of the above two chelating dyes. The SO_3H group of XO and MTB has been replaced with CO_2H in PP. This makes the dye molecule less soluble, which may be one reason for the higher loading achieved for PP compared to the above dyes. The differences in the basic structure of PP and the above dyes can be seen in Figures 11 and 12. The column exhibits very different chelation characteristics to the previous columns discussed. Figure 19A, shows the separation of Ba(II), Sr(II) and Ca(II) at pH 9.8 using the PP column. At this pH Mg(II) is completely retained. This shows a complete reversal in the elution order of Ca(II) and Mg(II) compared to XO and MTB. The greater resolution between Ca(II) and the Ba(II) and Sr(II) peaks is immediately apparent with the PP column. The degree of separation between Ca(II) and Mg(II) is equally large. These large differences in retention times are utilised in an application determining Sr(II) in milk powder, discussed in Chapter 5. From the separation shown in Figure 19A, it is clear the conditional stability constants for the chelates of alkaline earth metals and the immobilised PP are more varied than those of the previously discussed chelating dyes. They are also lower, as the pH required



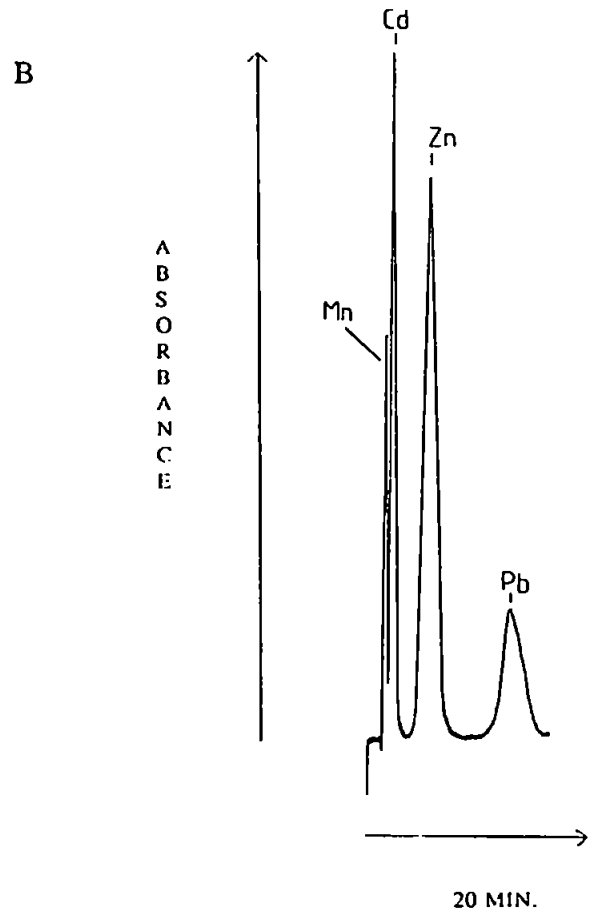


Figure 19. Chromatograms showing the separation of A) Ba(II), Sr(II) and Ca(II) at pH 9.8 and B) Mn(II), Cd(II), Zn(II) and Pb(II) at pH 3.7 in 1M KNO_3 using a Phthalein Purple impregnated column.

to produce the separation of the alkaline earth metals is substantially higher than that required by the XO or MTB columns. This shows PP produces an overall weaker chelating column.

The separation of Mn(II), Cd(II), Zn(II) and Pb(II) using the PP impregnated column is shown in Figure 19B. The separation is achieved at pH 3.7, again higher than the two columns mentioned previously. The ability to separate all the four metals above, isocratically, shows their conditional stability constants are quite similar, this being opposite to those of the alkaline earth metals. At the pH required to produce a complete separation of Mn(II), Cd(II) and Zn(II) with the previous three columns, Pb(II) is retained. The high efficiency of the column is also clear from the still relatively sharp peak obtained for Pb(II), eluting after 11min.

3.2.v. GLYCINE CRESOL RED (GCR).

Like the previous four chelating dyes discussed, GCR is a triphenylmethane based dye. However, unlike the previous dyes it does not contain iminodiacetic acid functional groups. Instead the dye molecule contains iminoacetic acid groups. Therefore GCR acts as a nitrogen/oxygen chelator, unlike the nitrogen/oxygen/oxygen chelating nature of the iminodiacetic acid containing dyes. For the above reason GCR was expected to exhibit weaker chelating characteristics than the previous chelating dyes investigated.

Like PP, the impregnation of the Dionex resin with GCR produced a high loading, approximately 60mg. The percentage activity of this immobilised dye was however only 6.6%, thus reducing greatly the capacity of the column. The separation of the alkaline earth metals using the GCR column is shown in Figure 20A. The column is able to separate the four metals at pH 9.0, although Ba(II) and Sr(II) are not quite baseline resolved. As with the

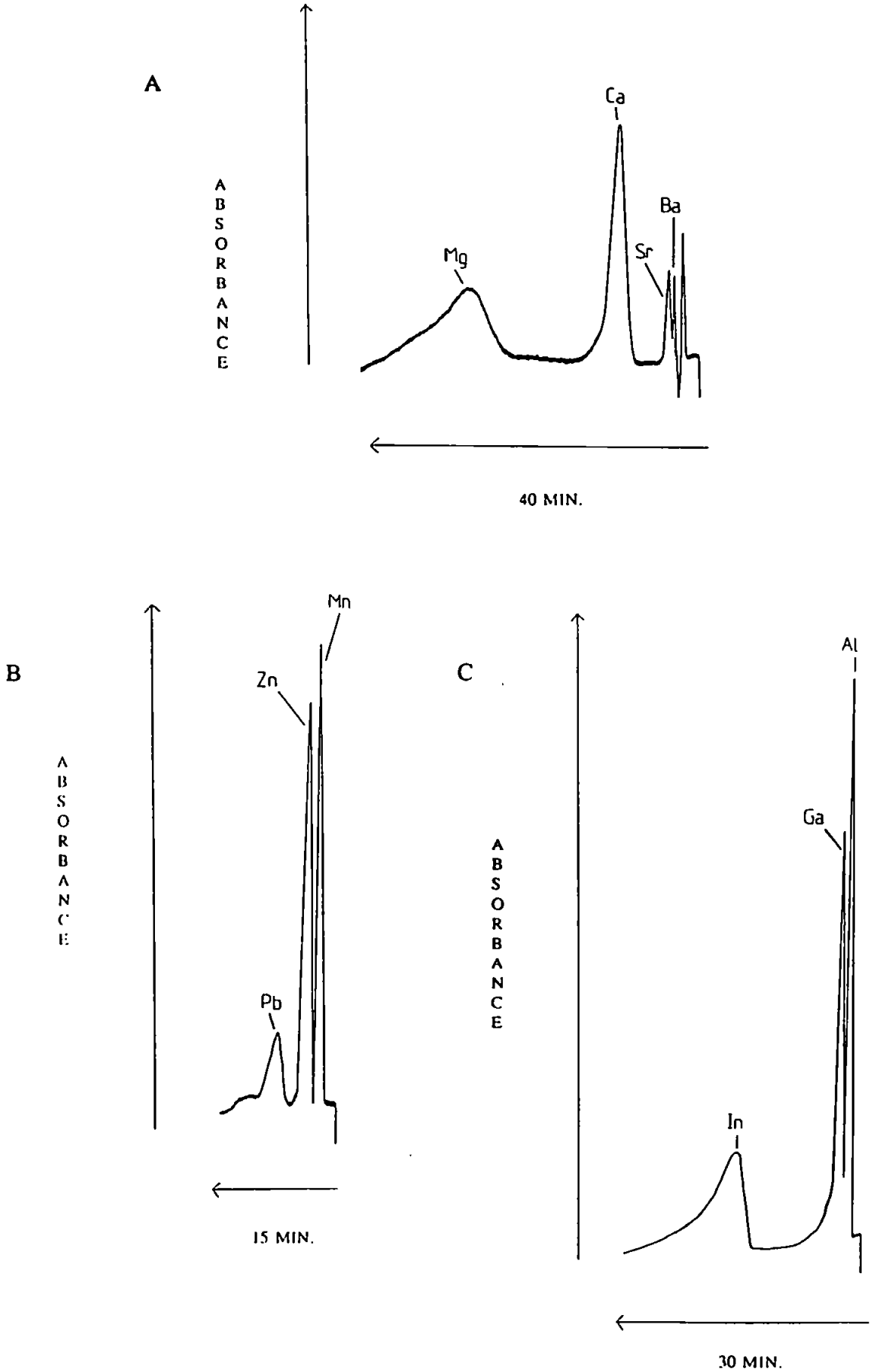


Figure 20. Chromatograms showing the separation of A) Ba(II), Sr(II), Ca(II) and Mg(II) at pH 9.0, B) Mn(II), Zn(II) and Pb(II) at pH 4.8 and C) Al(III), Ga(III) and In(III) at pH 2.4 in 1M KNO_3 using a Glycine Cresol Red impregnated column.

PP column, Mg(II) elutes last and there is a high degree of resolution between the Ca(II) and Mg(II) peaks. The tailing of the last two peaks indicate the efficiency of the column is not quite as good as some of the previous columns.

Figure 20B shows the separation of Mn(II), Zn(II) and Pb(II) using the GCR column. This is carried out at pH 4.8, showing the column is quite weakly chelating, exhibiting similar retention characteristics to the SXO column. Zn(II) and Cd(II) co-elute with the GCR column under the conditions used, although the use of modified eluents or gradient elution may resolve the two peaks.

As GCR was shown to be a significantly weaker chelator than those dyes examined so far, it was possible to investigate the separation of some trivalent metals. With many of the previous columns which exhibit strong chelation characteristics, the elution of trivalent metals would require very acidic eluents. This could have caused corrosion of the apparatus used or adversely affected the columns themselves. Figure 20C shows the separation of Al(III), Ga(III) and In(III) using the GCR column at pH 2.4. The kinetics involved with the chelation of trivalent metals are generally slower than those of divalent metal ions. This leads to even more increased peak broadening and tailing. This can be seen with the tailing of the Ga(III) and In(III) peaks. The retention of Al(III) using the GCR column was not possible. This may be caused by the size of the ionic radii of Al(III), which being the smallest of the metals investigated, may not be able to chelate effectively with the immobilised dye.

3.2.vi. CHROME AZUROL S (CAS).

CAS is a triphenylmethane based dye but differs from all the preceding chelating dyes as the iminodiacetic acid or iminoacetic acid functional groups have been replaced with carboxylic acid groups. This means the dye acts as a oxygen/oxygen chelator and should generally exhibit weaker chelating characteristics than the previous dyes. The loading and percentage activity of the dye was quite low, resulting in a low capacity. This would also reduce the chelating strength of the column. The separation of the alkaline earth metals achieved using the CAS column is shown in Figure 21A. As can be seen from the chromatogram the column is unable to separate Sr(II) and Ca(II) at pH 9.5. For the retention of the alkaline earth metals the CAS column is noticeably weaker than the XO, MTB and to a lesser extent GCR columns. It exhibits a similar strength to PP and SXO.

For the retention of Mn(II), Cd(II), Zn(II) and Pb(II), CAS exhibits weaker retention characteristics than any of the previous dyes. The separation of Mn(II), Zn(II) and Cd(II) was carried out at pH 5.3, and is shown in Figure 21B. Pb(II) is retained at this pH. The chromatogram shows how sharp the peaks remain when eluting within the first 5min. The sharpness of the peaks illustrate how the column is able to produce efficient separations even though it is has a low capacity and is a weakly chelating column. The retention order of the above metals differs from the previous dye impregnated columns. As can be seen from Figure 15B the elution order of Cd(II) and Zn(II) is reversed. With the CAS column Zn(II) elutes between Mn(II) and Cd(II).

The separation of four trivalent metals using the CAS impregnated column is shown in Figure 21C. The separation of Al(III), Ga(III) and In(III) was achieved at pH 2.2, however a simple step gradient down to pH 1.0 after 10min elutes Fe(III). The degree of tailing for

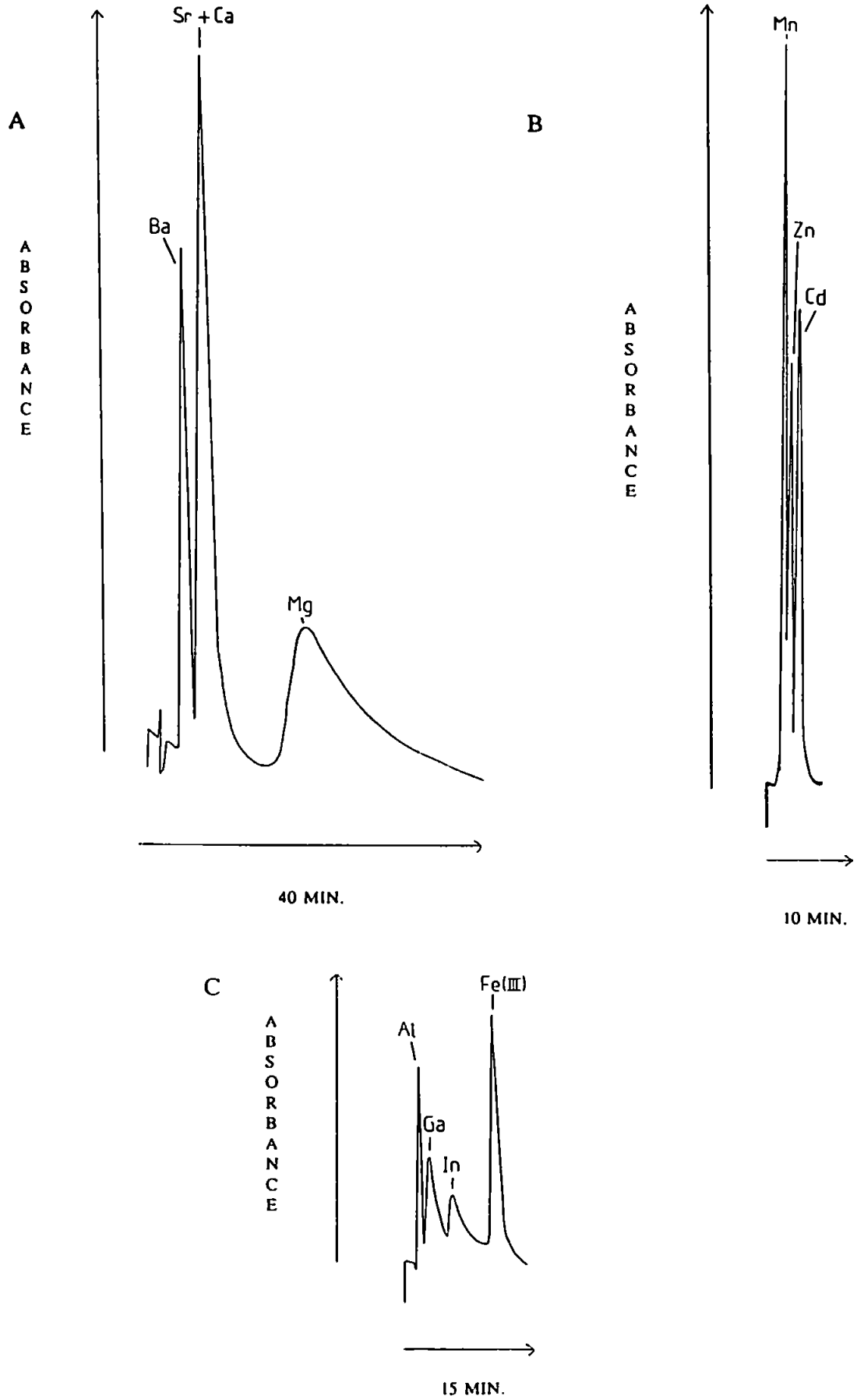


Figure 21. Chromatograms showing the separation of A) Ba(II), Sr(II), Ca(II) and Mg(II) at pH 9.5, B) Mn(II), Zn(II) and Cd(II) at pH 5.3 and C) Al(III), Ga(III), In(III) and Fe(III) at pH 2.2 stepped down to pH 1.0 after 10min., in 1M KNO_3 using a Chrome Azurol S impregnated column.

the Ga(III) and In(III) peaks is greater than that of the peak for Fe(III). This illustrates how the peak tailing can be decreased and the peaks sharpened using gradient elution.

3.2.vii. CALMAGITE (CAL).

CAL is the first of the azo type dyes to be discussed, exhibiting the nitrogen/nitrogen azo linkage. CAL is however similar to the first five dyes discussed in that it is a nitrogen/oxygen/oxygen chelator. The loading achieved for CAL was low at only 11mg but the activity of the immobilised dye was high, resulting in a higher capacity than the previous CAS column. Several early Calmagite columns were prepared using an impure source of the dye which resulted in much higher loadings. However attempts to determine the capacity of these columns produced irregular results and have been dismissed. This emphasizes the need to use only pure sources of dye. The separation achieved for the alkaline earth metals is shown in Figure 22A. At pH 8.5 the column is unable to separate Ba(II) and Sr(II), although Ca(II) and Mg(II) are well resolved. The CAL column exhibits quite a strong affinity for the alkaline earth metals compared to the preceding dyes, comparable with the XO and MTB impregnated columns.

The high efficiency of the CAL column is illustrated with the separation of Mn(II), Cd(II), Zn(II) and Pb(II), shown as Figure 22B. Although the separation is not quite baseline resolved, the sharpness of the peaks within the first 5min is again evident. The pH at which the above separation was achieved was 5.2. This shows the CAL column exhibits a similar strength to CAS for the chelation of these particular metals. It is somewhat supprising that although the CAL column shows quite a strong affinity for the alkaline earth metals, it also exhibits a weakly chelating nature for the complexation of several of the transition and heavy

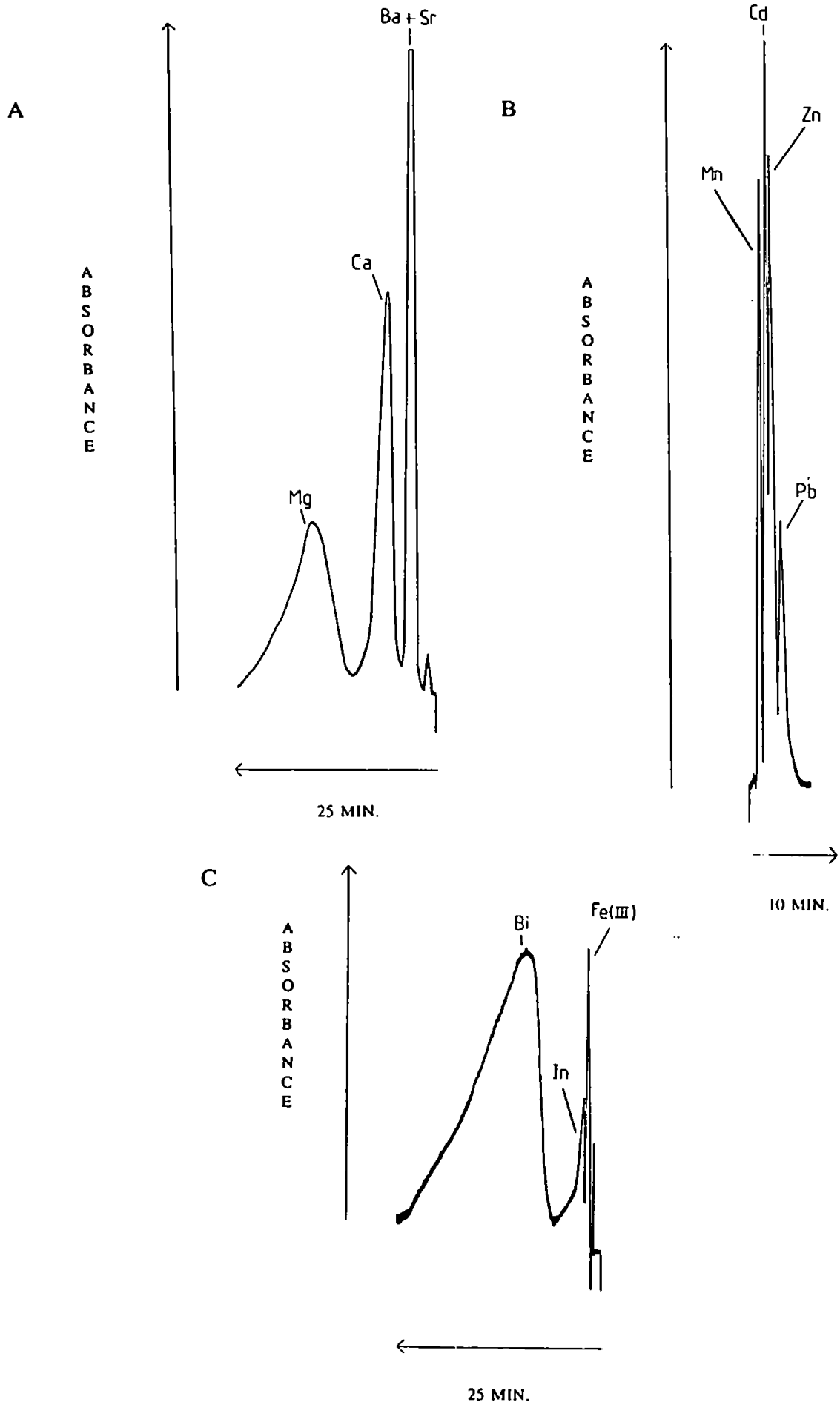


Figure 22. Chromatograms showing the separation of A) Ba(II), Sr(II), Ca(II) and Mg(II) at pH 8.5, B) Mn(II), Cd(II), Zn(II) and Pb(II) at pH 5.2 and C) Fe(III), In(III) and Bi(III) at pH 1.3 in 1M KNO_3 using a Calmagite impregnated column.

metals. This illustrates the unique selectivity of chelation ion chromatography, particularly when using immobilised chelating dyes. They may chelate strongly with some metals and weakly or not at all with other similarly charged metals.

The separation of certain trivalent metals was investigated using the CAL column, although the resulting peak shapes were particularly poor. Once again Al(III) was not retained on the column, the reasons for which are unclear but are again likely to be related to the ionic radii of the Al(III) ion. Figure 22C shows the separation of Fe(III), In(III) and Bi(III) at pH 1.3. The chromatogram shows the tailing of the In(III) peak and an extremely broad and asymmetrical peak achieved for Bi(III).

3.2.viii. 4-(2-PYRIDYLAZO) RESORCINOL (PAR).

PAR is similar to the previous CAL dye in that it is also an azo based chelating dye. However the nature of chelation is different, as PAR is a nitrogen/nitrogen/oxygen chelator. The ligating atoms are the nitrogen of the pyridine ring, one of the nitrogens in the azo linkage and a phenolic oxygen. The high loading achieved with PAR, despite a low percentage activity, resulted in a high capacity. The capacity achieved was far higher than any of the other azo dyes investigated, due in part to the lower molecular weight of the PAR molecule, and explains the strongly chelating nature of the PAR column. The chromatogram showing the separation of the alkaline earth metals is shown in Figure 23A. This was carried out at pH 5.8, which is significantly lower than any of the other columns investigated. It is also noticeable that the elution order has changed, with Mg(II) eluting first before the co-eluting Ba(II) and Sr(II) peak.

The PAR column exhibited an equally strong affinity for some of the transition and

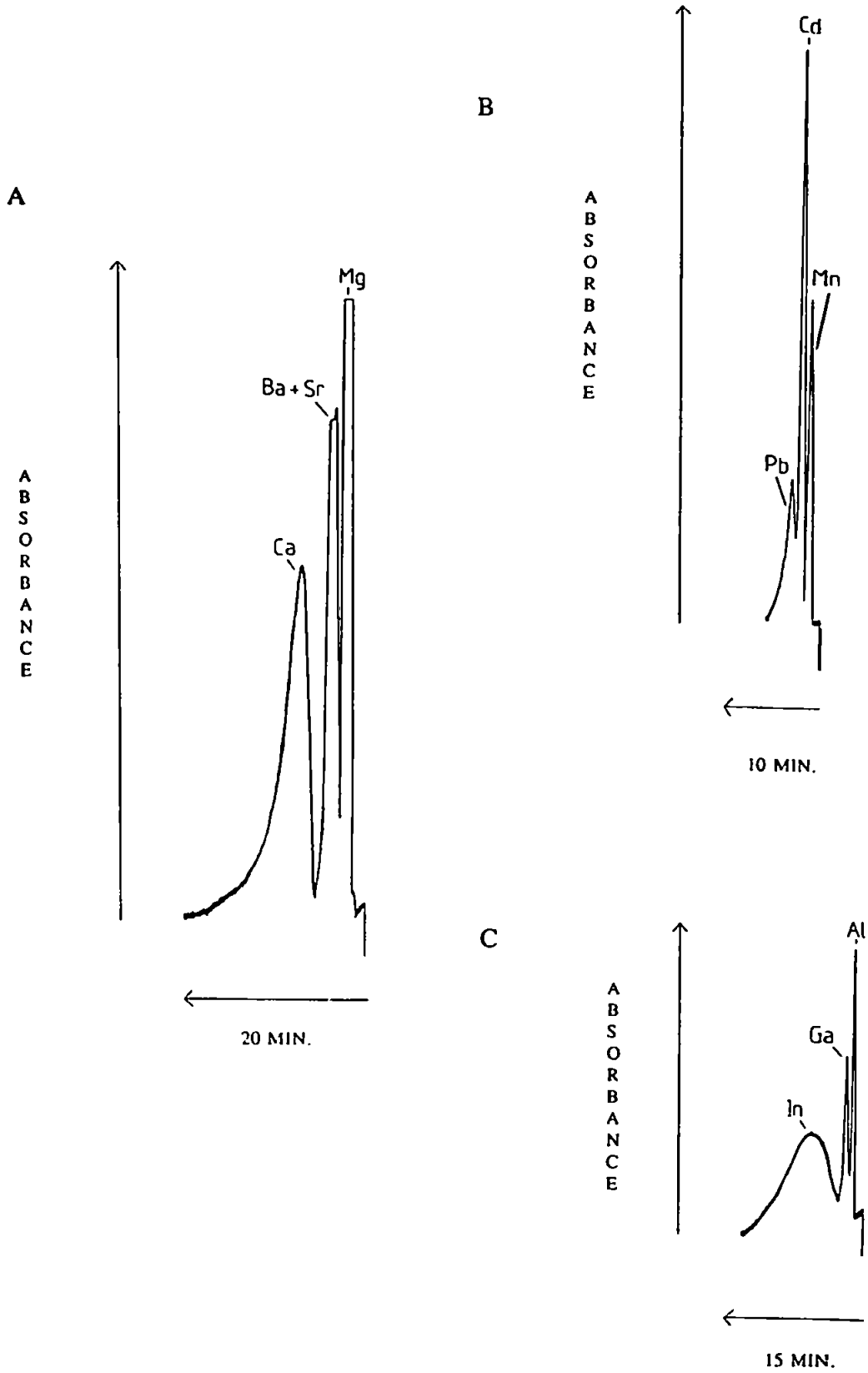


Figure 23. Chromatograms showing the separation of A) Mg(II), Sr(II), Ba(II) and Ca(II) at pH 5.8, B) Mn(II), Cd(II) and Pb(II) at pH 1.8 and C) Al(III), Ga(III) and In(III) at pH 0.9 in 1M KNO_3 using a 4-(2-pyridylazo) resorcinol impregnated column.

heavy metals. The separation of Mn(II), Cd(II) and Pb(II), shown in Figure 23B, was achieved at pH 1.8. The separation shown is not quite baseline resolved and it was not possible to separate Zn(II) and Cd(II) which co-elute.

The strength of the PAR column for the trivalent metals was also greater than the previous columns although not to such an extent as the divalent metals. The separations of trivalent metals using the PAR column, produced particularly poor peak shapes, similar to those of the CAL column. Figure 23C shows the separation of Al(III), Ga(III) and In(III), carried out at pH 0.9. The unusual separation profile illustrates how the rapid broadening of the In(III) peak is more of a kinetic affect, than normal band broadening occurring within the column.

3.2.ix. ORANGE II (OII).

OII is also an azo based dye, however unlike CAL and PAR there are only two ligating atoms. These are the nitrogen from the azo linkage and a phenolic oxygen. For this reason the OII column should be one of the weakest chelating columns investigated so far. The loading achieved for the dye was 43mg, however the percentage activity of the dye was very poor, only 1.5%, resulting in a low capacity. The column itself exhibited a very low affinity for the alkaline earth metals. This was expected due to the nature of the ligating atoms involved (alkaline earth metals are classed as "hard acids" and nitrogen forms stronger complexes with "intermediate and soft acids" such as many of the transition metals). The low capacity also acted to reduce the chelating strength of the column. It was possible to separate Ba(II) and Ca(II) using the OII column at pH 10.0, this is shown in Figure 24A. At this pH Sr(II) and Mg(II) co-elute with the above two metals. Increasing the pH further does not help

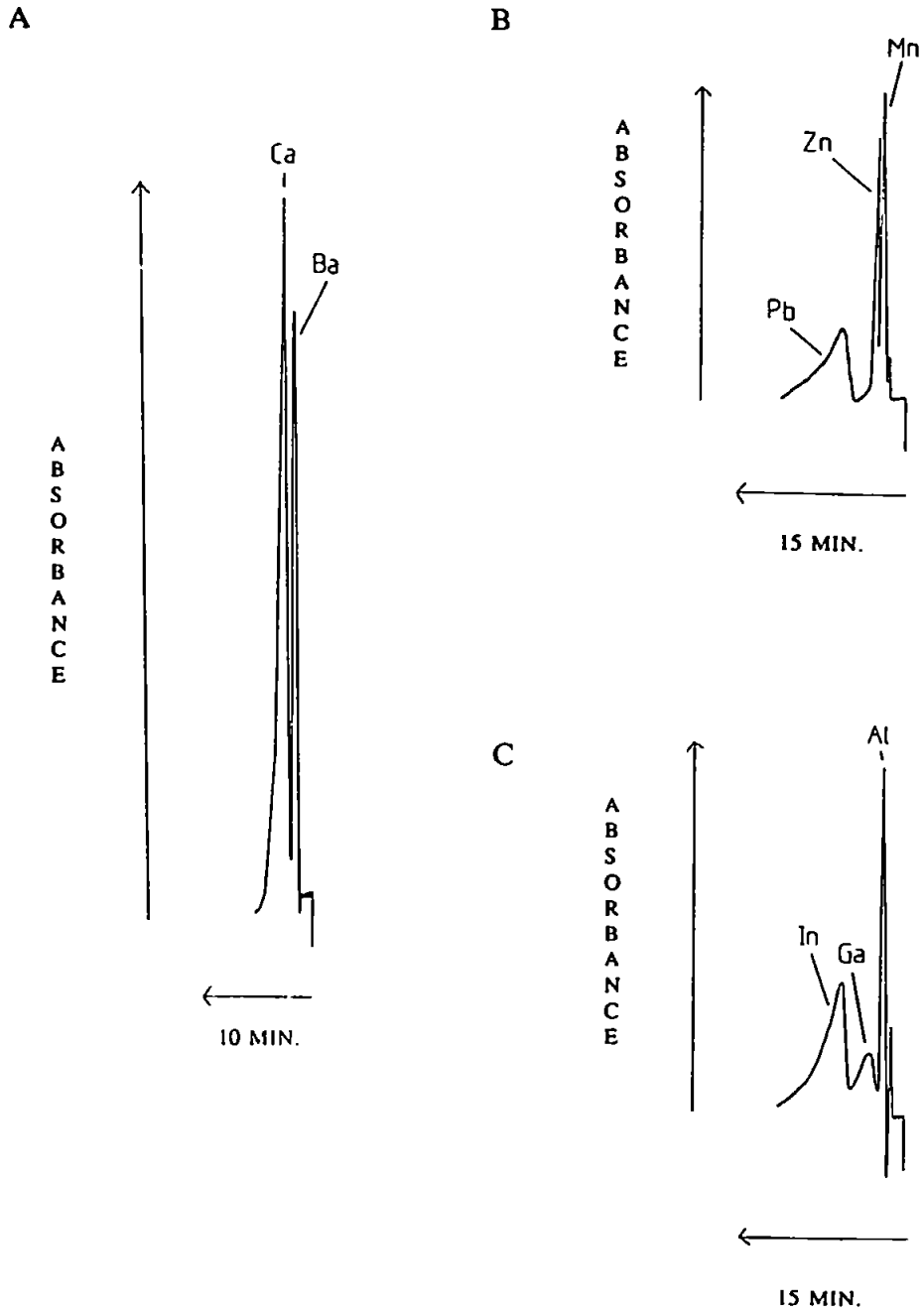


Figure 24. Chromatograms showing the separation of A) Ba(II) and Ca(II) at pH 10.0, B) Mn(II), Zn(II) and Pb(II) at pH 5.0 and C) Al(III), Ga(III) and In(III) at pH 2.9 in 1M KNO_3 using an Orange II impregnated column.

to separate the metals.

The OII column exhibited a similar strength to the CAL and CAS columns for several of the transition and heavy metals. The column showed signs of poor efficiency and this can be seen in the separation of Mn(II), Zn(II) and Pb(II), where the peak for Pb(II) tails quite severely. This is shown as Figure 24B. The separation was achieved at pH 5.0. With the OII impregnated column Cd(II) co-eluted with Zn(II), again increasing the pH did not help to separate the two metals.

Figure 24C shows the separation of Al(III), Ga(III) and In(III) using the OII column. The separation profile is very similar to that achieved using the CAS impregnated column. The pH required to produce the separation was higher at pH 2.9, showing the column is weaker for the trivalent metals than the CAS column and all the other dyes investigated for these group of metals.

3.2.x. 2-(3-SULPHOBENZOYL) PYRIDINE 2-PYRIDYLHYDRAZONE DIHYDRATE (SPPD).

SPPD is different to the previous chelating dyes investigated as it does not contain any oxygen ligating atoms. Instead it acts as a nitrogen/nitrogen chelating dye, and as such should show little affinity for the alkaline earth metals. This was found to be the case with the impregnated dye, which showed no signs of retention for any of the alkaline earth metals up to pH 11.

The loading of the SPPD dye was low at only 15mg and a low percentage activity resulted in a very low capacity, the lowest of all the dyes investigated. However the column did chelate quite strongly with several of the transition and heavy metals. Figure 25 shows

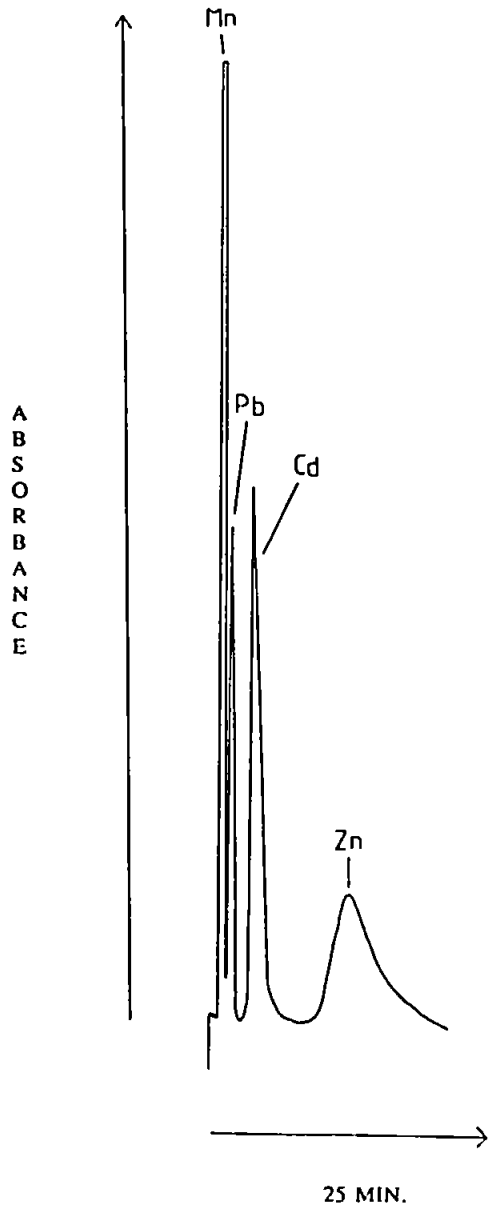


Figure 25. Chromatogram showing the separation of Mn(II), Pb(II), Cd(II) and Zn(II) at pH 3.6 in 1M KNO_3 using a 2-(3-sulphobenzoyl) pyridine 2-pyridylhydrazone dihydrate impregnated column.

the separation of Mn(II), Pb(II), Cd(II) and Zn(II), achieved at pH 3.6. As can be seen from the figure, the usual elution order for these metals has altered. Using the SPPD impregnated column Pb(II) elutes before Cd(II) and Zn(II), this is the opposite to all the other impregnated dyes. The column also produced a much greater resolution between Cd(II) and Zn(II) than has been achieved previously, therefore the column had the potential to be used in the determination of Pb(II) and Cd(II) in samples containing high concentrations of Zn(II). However this could not be investigated further due to the column showing signs of deteriorating with time, which was found to be a result of dye bleeding from the column.

3.3. METAL RETENTION CHARACTERISTICS.

Table 6 shows the pH required for the retention of a range of metals using the various dye impregnated columns. The values are quoted to the nearest 0.5 of a pH unit and refer to the pH which results in a retention time greater than approximately 45min. The values preceeded by a greater than symbol (>) indicate a pH at which no sign of retention was noticed and a much higher pH would be required to facilitate some degree of retention. It was not possible to determine the pH values which result in the retention of Fe(II) by the various impregnated dyes. This was due to the inability to produce stable peaks for the above metal, which may have been due to oxidation to Fe(III), either on-column or somewhere within the system. The plotting of these values for the alkaline earth metals, and some of the transition and heavy metals, reveals more clearly the general trends in the retention orders experienced when using chelation ion chromatography. These plots can be seen as Figures 26 and 27. The dyes investigated have been divided into two groups, to simplify the figures. These are the triphenylmethane based dyes and the azo dyes, to which SPPD has been included. As can be

Dye	Ba	Sr	Ca	Mg	Mn	Cd	Zn	Co	Fe(II)	Pb	Ni	Cu	Al	Ga	In	Fe(III)	Bi
XO	10.0	10.0	8.5	9.0	5.5	4.5	4.0	4.0	—	3.5	3.0	2.0	2.0	—	—	1.5	—
SXO	>11.0	>11.0	10.5	10.5	>5.5	5.5	5.0	—	—	4.5	4.0	2.5	—	—	—	—	—
MTB	10.0	10.0	8.5	9.0	6.0	5.0	4.5	4.0	—	3.5	3.5	2.0	2.0	—	—	1.5	1.5
PP	>11.0	11.0	10.0	9.5	6.0	5.0	4.5	4.5	—	4.0	3.5	1.5	—	—	—	1.5	—
GCR	>10.0	>10.0	10.0	9.5	6.5	6.0	6.0	—	—	5.5	4.0	2.5	>4.0	3.5	2.5	2.0	1.5
CAS	>11.0	11.0	11.0	10.5	7.5	6.5	6.5	6.5	—	5.5	5.5	4.5	3.5	3.0	3.0	2.0	2.0
CAL	>11.0	>11.0	10.5	9.5	8.0	7.0	6.5	—	—	6.5	6.0	3.0	>5.0	2.5	2.5	3.0	2.0
PAR	7.5	7.5	6.5	8.5	5.0	3.0	3.0	2.5	—	2.5	2.5	1.5	>3.0	1.5	1.5	1.5	1.0
OII	>11.0	>11.0	>11.0	>11.0	7.5	6.5	6.5	6.5	—	5.5	>5.0	4.0	>3.5	3.5	3.5	3.5	3.0
SPPD	>11.0	>11.0	>11.0	>11.0	7.5	4.5	4.0	4.0	—	5.5	>5.0	1.5	—	—	—	—	—

Table 6. pH required for the complete retention of a range of metals using various chelating dye impregnated resins.

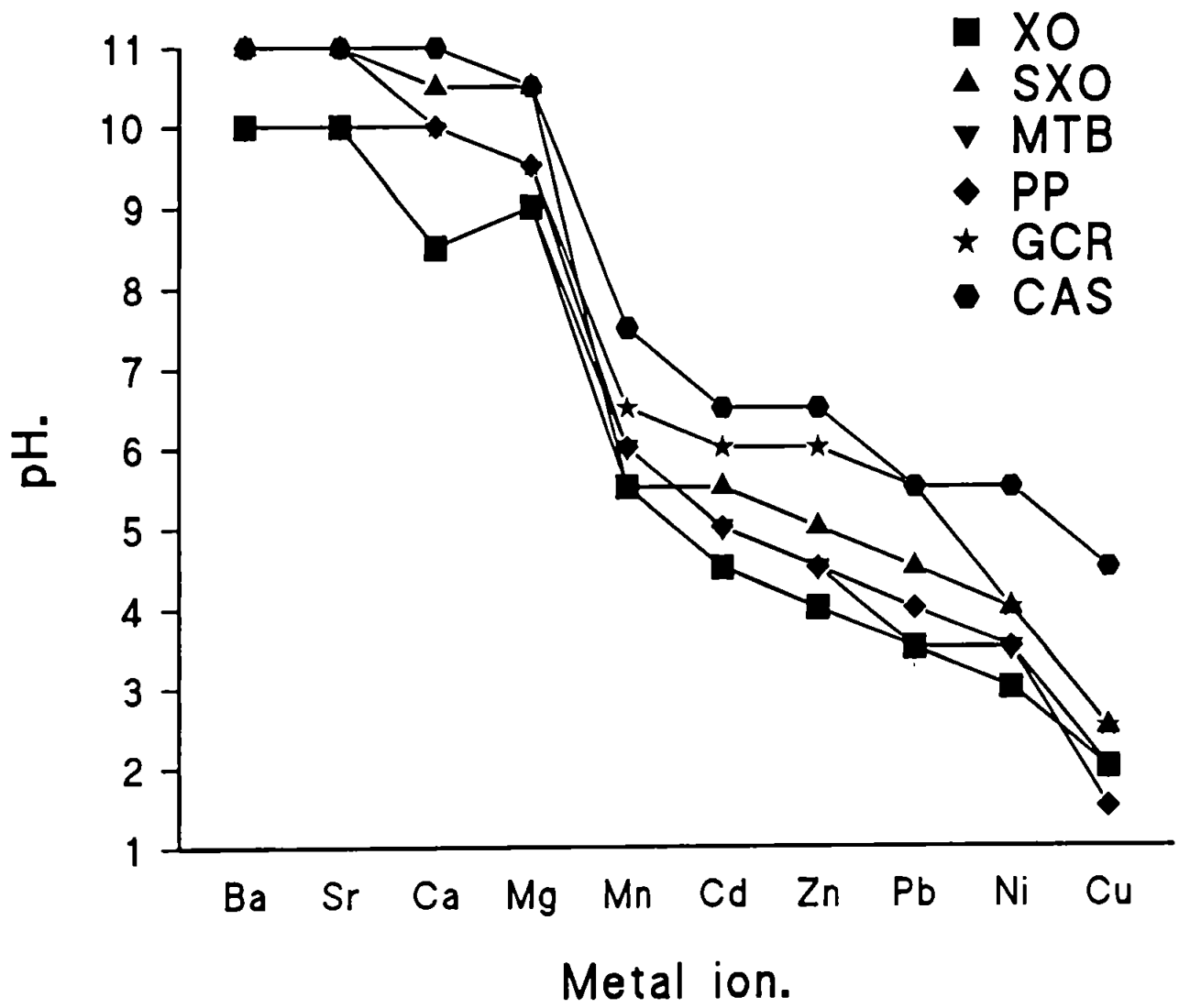


Figure 26. pH required to completely retain a range of metals using various resin impregnated chelating dyes.

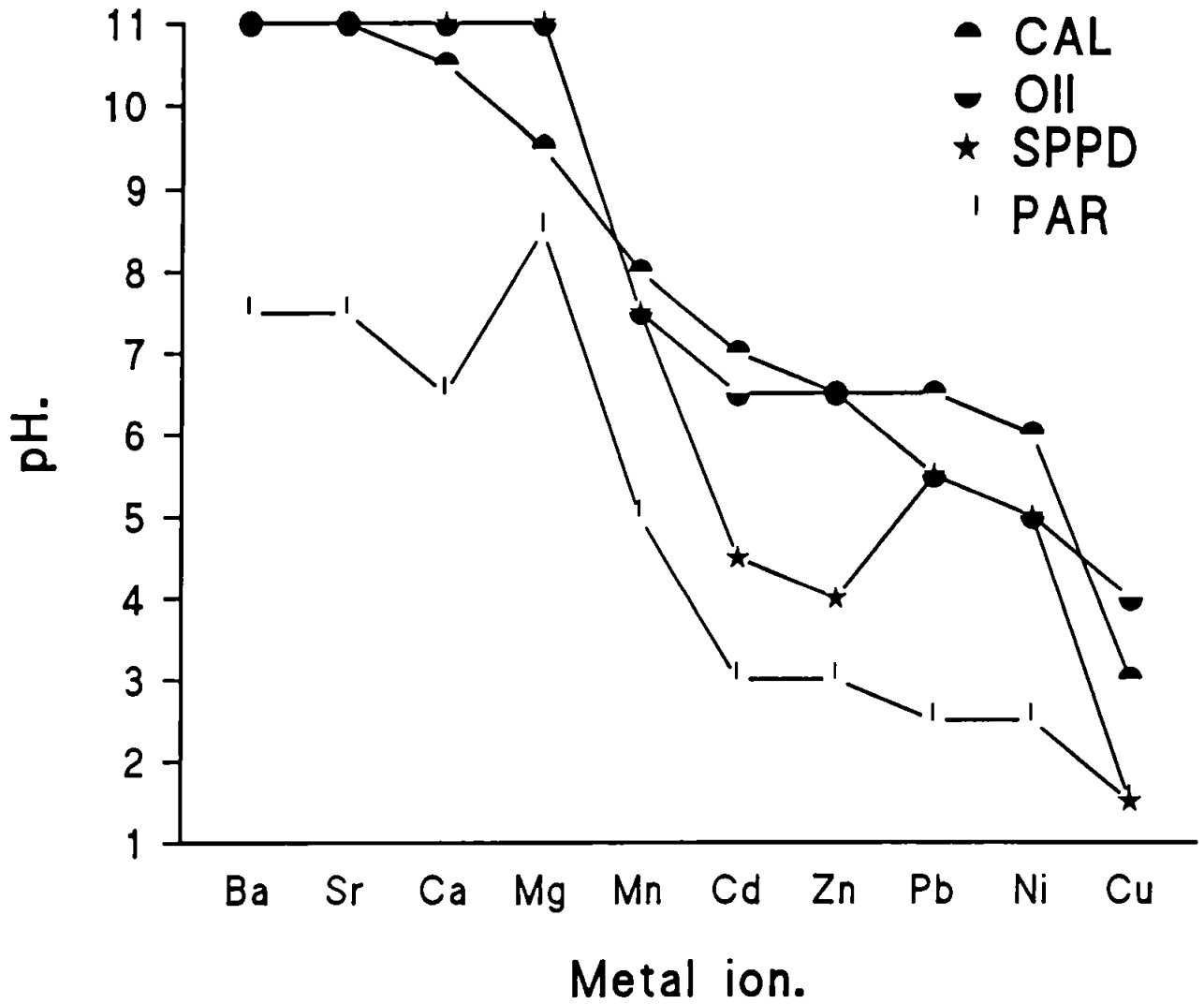


Figure 27. pH required to completely retain a range of metals using various resin impregnated chelating dyes.

seen from Figures 26 and 27, and Table 6, the overall retention order is very similar for all the dyes investigated. The deviations from the expected order mainly involve Mg(II), although the SPPD column also showed a deviation from the order for Pb(II) and Ni(II). All the dyes investigated showed a much greater affinity for the transition and heavy metals than the alkaline earths, which was expected. However this does illustrate the obvious advantages chelation techniques have in the analysis of these metals in samples containing high levels of alkaline earth metals, such as seawater.

The range in chelating strength of the ten columns investigated is also illustrated by the above Figures. The pH required to facilitate the retention of each metal varies by approximately four pH units over the whole range of dyes. The strongest of the columns produced was the PAR impregnated column, with CAS, OII and CAL producing similarly weak chelating columns.

A further way of comparing the relative strengths of these ten impregnated dyes is shown in Table 7. Here the pH required to produce a retention time of 5min for four selected metals has been tabulated. Again this illustrates the varying resolution between these metals which can be achieved using these chelating dye impregnated columns.

3.4. EFFECTS OF COLUMN LENGTH AND PARTICLE SIZE.

To investigate the effects of the column length and particle size upon the resolution and efficiency of the separations discussed earlier, a 15cm Hamilton 5μm, 75Å PRP-1 column was impregnated with PP. PP was chosen as it had been shown to produce high loadings and efficient separations. A loading of 111mg was achieved, which was almost identical to that of the 10cm Dionex resin. However the separations achieved using the 15cm PP column were

Chelating dye.	Zn(II).	Pb(II).	Ca(II).	Mg(II).
XO	3.3	2.8	7.5	7.8
SXO	4.5	3.6	9.6	9.6
MTB	3.5	2.6	7.5	7.8
PP	3.5	3.2	9.2	8.2
GCR	5.1	4.6	8.7	8.4
CAS	5.8	4.5	9.3	9.1
CAL	5.4	5.2	8.3	8.1
PAR	2.0	1.9	5.5	7.0
SPPD	3.1	3.8	>11.0	>11.0
OII	5.8	4.9	>11.0	>11.0

Table 7. pH required to produce a retention time of approximately 5min for selected metals using various chelating dye impregnated resins.

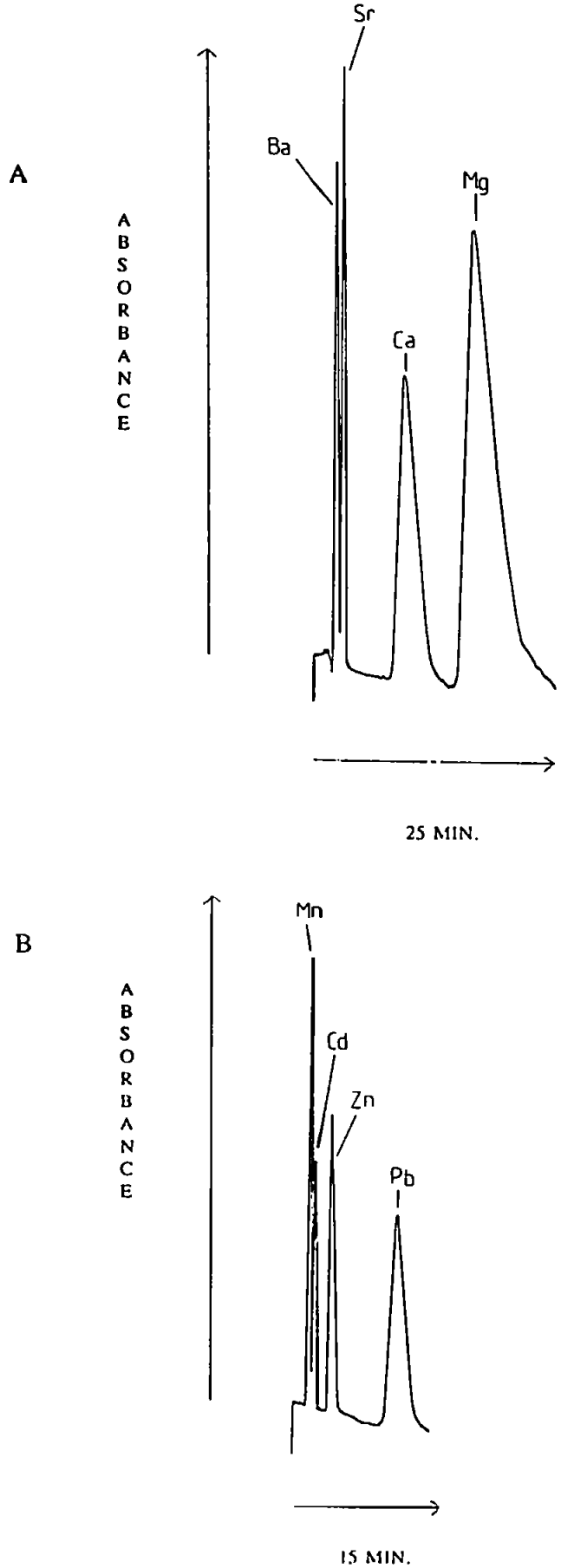


Figure 28. Chromatograms showing the separation of A) Ba(II), Sr(II), Ca(II) and Mg(II) at pH 8.3 and B) Mn(II), Cd(II), Zn(II) and Pb(II) at pH 3.2 in 1M KNO_3 using a Phthalein Purple 15cm, 5μm, 75Å Hamilton impregnated column.

noticeably more efficient. Figure 28A shows the separation of the alkaline earth metals using the 15cm 5μm PP column. The pH of 8.3 required to produce the above separation is substantially lower than the that required to produce the separation achieved with the 10cm 8.8μm PP column (Figure 19A). This is probably because the longer column has an increased number of theoretical plates enabling the resolution of the peaks to remain, despite reducing the retention times of the metals by lowering the eluent pH. Using the equations shown in section 1.1., the theoretical number of plates for the 15cm Hamilton column, calculated using the peak for Pb(II) was approximately 1,900. This compares to 1,600 for the 10cm Phthalein Purple column. Therefore it was possible to reduce the pH low enough to elute the Mg(II) peak and still have a baseline separation of Ba(II), Sr(II) and Ca(II). This was not possible with the shorter column. The smaller particle size will also have improved the efficiency of the separation, resulting in sharper peak shapes, which allows the separation of a larger number of metals in a shorter overall retention time. This can be seen in Figure 28B with the separation of Mn(II), Cd(II), Zn(II) and Pb(II). Again the pH required to produce the separation has been reduced from 3.7 to 3.2, also the four metals are separated in slightly less time than with the 10cm column (Figure 19B). It can also be seen that the peaks are marginally sharper.

3.5. SUMMARY.

The previous chapter has discussed the various parameters involved in producing dye impregnated high-performance chelation ion chromatography columns and the range of metal separations which can be achieved with these columns. Several conclusions can be made from the previous investigations which may indicate the general guidelines involved in producing

efficient chelating columns. Firstly, the amount of dye loaded on the resin appears dependent upon the nature of the resin involved, particularly the pore size. In the case of XO, the 100Å pore size produced the highest loadings of the range of resins investigated. Optimising the impregnating procedure can also lead to increased loadings, ie pH and concentration of the dye solution. The amount of dye impregnated into the resin governs the ability of the column to produce the required separations, this was shown with XO, which was only able to completely separate the alkaline earth metals with columns loaded with 30-40mg of dye. In general the dyes producing some of the most efficient separations were those with the higher capacities, therefore high loadings appear to produce better results.

For the separation of the alkaline earth metals, the dyes containing iminodiacetic acid functional groups produce the most efficient separations ie. XO, MTB and PP. The only other dye capable of producing a complete separation of the metals is GCR, which contains the iminoacetic acid function, although the separation is not as efficient as the above dyes. For the separation of the transition and heavy metals the range of dyes exhibit more diverse results. The three columns capable of producing a four metal separation were PP, CAL and SPPD, which are three very different chelating dyes. This shows the large range of dyes which are capable of being exploited for these types of separations. The slow kinetics involved in the separation of trivalent metals mean the efficiency of the separations produced is relatively poor. In general some of the weaker chelating columns produced the better separations, namely CAS, OII and GCR.

Increasing the column length and reducing the particle size, as with other types of LC, leads to more efficient separations in chelation ion chromatography. However due to the nature of chelation ion chromatography, which is unlikely to be able to separate more than 4 or 5 metals isocratically, even with these changes, it is questionable whether the relatively

minor improvements in efficiency warrant the increased cost of smaller particle size resins and longer columns. In practice 10cm 10μm columns are efficient enough to be successfully employed in a range of applications.

CHAPTER 4. RESULTS AND DISCUSSION.

4.0. DETERMINATION OF TRACE METALS IN SEAWATER.

Chelation ion exchange has been combined with numerous techniques in the determination of trace metals in seawater. Due to the interfering effects of the complex seawater matrix and the trace levels of the analyte, sample pre-treatment in the form of matrix elimination and preconcentration techniques is usually required. Solvent extraction preconcentration followed by detection using atomic absorption spectrometry (AAS) has been reported by Sturgeon et al. [151]. However this method can involve laborious sample pre-treatment and necessitate mixing of the sample with a range of reagents, increasing the risk of contamination.

Chelex-100 is well documented in the analysis of trace metals in seawater. Since the original investigation in this area by Riley and Taylor [23], it has been modified and developed by numerous workers as both on-line and off-line systems in the form of preconcentrator columns preceeding detection often using spectrophotometric techniques such as AAS [25,28,52] and inductively coupled plasma atomic emission spectrometry (ICP-AES) [33] or colorimetric methods often involving post column reaction using complexing reagents such as PAR [31]. Similarly covalently bonded chelating substrates are also commonly used in seawater trace metal determinations. Lewatit TP 207 and Chelamine are two commercially available large particle macroporous chelating resins which have been applied to seawater analysis [47,52]. Other covalently bound chelating agents applied in this area include N,N,N,N-tetra-(2-aminoethyl)ethylenediamine (PENTEN) bonded to silica gel [45] and iminodiacetic acid (IDA) bound to porous silica [82]. Dionex use IDA covalently bonded to polystyrene divinylbenzene resin to produce the Metpac CC-1 preconcentrator column used

in their chelation ion chromatography system which has been applied to seawater analysis by several workers [35-36,51].

In recent years there has been a large number of publications based upon the use of 8-hydroxyquinolinesulphonate (8-HQS) as a chelating agent in the analysis of seawater. It has been immobilised upon a variety of substrates ranging from silica gels [152-154] and vinyl polymers [131,142] to the preconcentration of Al(III) using 8-HQS immobilised on controlled pore glass beads [155]. Other dynamically coated chelating agents employed in this field include ammonium pyrrolidine dithiocarbamate (APDC) [156], sodium diethyldithiocarbamate (NaDDTC) [157], 1-(2-thiazolylazo)-2-naphthol (TAN) [72] and thioglycolate [158]. Kantipuly et al. [159] review the range of chelating polymers and functionalised supports with special emphasis on seawater analysis.

Natural chelating polymers, such as modified chitins, have also been investigated for the collection of metal ions from natural waters, including seawater [160].

The use of chelating resins in the analysis of trace metals in seawater is often a subject of debate. This is due to the fact these column methods are known to give lower values than techniques aimed at giving "total" concentrations. The latter often use sample pre-treatments, such as boiling acid digestion [161] or ultraviolet (UV) photolysis [162]. These complicated sample pre-treatments require extremely clean conditions and ultra-pure reagents to avoid contamination of the sample. Direct chelating column techniques, which are not aimed at determining "total" concentrations, do not employ the above sample pre-treatments, and are therefore more convenient. Also, because less additional reagents are used, the technique is less prone to contamination. A study carried out by Florence [163], who has been involved in several detailed investigations into trace metal speciation in natural waters, found chelating techniques predominantly measure the dissolved labile fraction of the metals

present. The sub-micron colloidally bound fraction passes straight through the column. Florence suggested it is the dissolved labile fraction which most closely represents the metal species available to organisms and thus chelation techniques can give important information on potential toxicology.

Recent advances in chelating techniques have led to the production of commercially available self-contained systems, which incorporate short chelating columns with on-line analytical separator columns, and use post-column derivatisation with PAR to detect the eluting metals. The Dionex Corporation have produced these complete systems and patented the technique as "chelation ion chromatography" (CIC). These systems have been successfully used in the analysis of seawater by several workers, details of which are given in section 1.3.ii.b. A disadvantage of the above systems is they require two or three columns and complex switching arrangements.

The early development of dye-impregnated high-performance chelation ion chromatography (HPCIC) by Jones and co-workers is detailed in section 1.3.iv. The feasibility of using these impregnated columns for both the preconcentration and separation of trace metals from seawater, using only a single column, has been illustrated by Challenger et al. [78]. The basic technique involves the loading of a known volume of pH adjusted seawater onto the chelating column and using step gradient elution to facilitate a separation of the preconcentrated metals, followed by post-column reaction detection. The brief semi-quantitative study carried out by Challenger et al., using a Xylenol Orange (XO) impregnated column, was limited to only two metals, Zn(II) and Cu(II), although the detection of these metals in actual seawater samples was possible at low $\mu g\ dm^{-3}$ levels. The following results were achieved from a more detailed study on the effectiveness of HPCIC for the determination of a wider range of metals, carried out after further improvements to the

chromatography and post-column reaction detection system used by Challenger et al.

Samples were obtained from two local coastal seawater sites to investigate the performance of the technique. The first was Plymouth Sound, which receives Cu(II)- and Pb(II)-rich run-off into the Tamar estuary. The Carnon estuary was the second location sampled, where there have been a number of pollution incidents involving the flooded Wheal Jane tin mine. The four metals which were determined in these samples were Zn(II), Pb(II), Ni(II) and Cu(II).

4.0.i. PERFORMANCE OF THE METHOD.

The fabrication and separation efficiency of a XO impregnated chelating column are discussed in section 3.2.i. The XO molecule contains two iminodiacetic acid functional groups and therefore the column has the ability to complex with a wide range of metal ions. The retention and selectivity of these metal ions is a function of the relative magnitude of the metal-complex conditional stability constants and can therefore be controlled through adjustment of the pH of the eluent. Step gradient elution can produce rapid reductions in these stability constants and cause groups of metals or individual metals to be eluted from the column with each step down in pH. The action of the eluent sweeping through the column at a reduced pH can be compared to a force pushing the metal ions from behind and compressing the band of metal ions within the column, resulting in sharper peaks, particularly when using short columns. The step gradient programme used can be altered to suit the metals being determined. Changing the gradient programme allows the relative retention times of various metal ions to be increased or decreased, allowing large interfering peaks to be eluted away from smaller peaks of interest. The sharp pH changes experienced when using

step gradient elution also helps to overcome the buffering capacity of the chelating column resulting from the structure of XO, which contains weak acid groups.

The complexation of alkaline earth metals is generally much weaker than most transition and heavy metals when using chelating resins. This means by adjusting the sample and chelating column to pH 6.0 before preconcentration, the high levels of Ca(II) and Mg(II) present in the sample will pass through the column unretained, however the stronger complexing transition and heavy metals remain on the column and can be swept off using gradient elution.

PAR was chosen as a post-column reagent (PCR) as it was insensitive to Ca(II) and Mg(II). It was noticed that changes in the pH of the eluent, caused by using gradient elution, results in a change in the pH of the combined PCR/eluent stream. This causes a change in the baseline absorbance, which results in a fluctuating baseline. To overcome this problem the PCR was heavily buffered with ammonium nitrate. However, the addition of a high concentration of the buffer to the PAR solution caused a slight decrease in sensitivity to certain transition metals.

4.0.ii. ANALYTICAL BLANKS.

A major problem with most trace metal determinations is the detection limit is often limited by levels of these metals in the procedural blank. Such is the nature of chelation ion chromatography that the column will always be preconcentrating metal impurities from the eluent even as the gradient elution programme is underway. To decrease the levels of metals present in the KNO_3 eluent, it was passed through a XO impregnated XAD-2 clean-up column, buffered to pH 6.0. This removed the majority of the metals present, although small

amounts of Zn(II) and Cu(II) still remained and were evident in the procedural blank chromatograms. It is possible some of the metals present resulted from leaching of the stainless steel eluent pump. Fe(III) was also detected in significant amounts, originating from the Aristar nitric acid used to adjust the eluent pH. However, as Fe(III) is strongly held by the XO column and elutes after all the metals being investigated, its presence did not cause any problems with the analysis.

As the samples were analysed within 24h of collection where possible, it was decided not to acidify the sample for preservation purposes, as this could introduce contamination. A short comparative study was carried out with acidified and unacidified samples, with the results indicating no significant differences between the two treatments.

4.0.iii. ANALYTICAL RESULTS.

Figure 29 shows the chromatogram produced from preconcentrating 20ml of Plymouth Sound coastal seawater compared with the chromatogram showing the procedural blank. The step gradient programme used was as follows, pH 6.0 to pH 4.0 (upon injection), to pH 2.45 (after 5min) to pH 1.35 (after 11.5min). Clear peaks for all four metals of interest are visible, substantially larger than the levels of metals shown in the procedural blank. In initial investigations, attempts were made to preconcentrate and separate Mn(II), Cd(II), Zn(II), Pb(II), Ni(II) and Cu(II) from coastal seawater samples. However, the separation of Mn(II) and Cd(II) proved difficult and increases in the signal for Cd(II) when carrying out standard additions were not linear. For these reasons, the above two metals were not quantitatively determined in the actual samples. The quantitative results for the four metals actually determined in samples taken from Plymouth Sound seawater are given in Table 8. Four

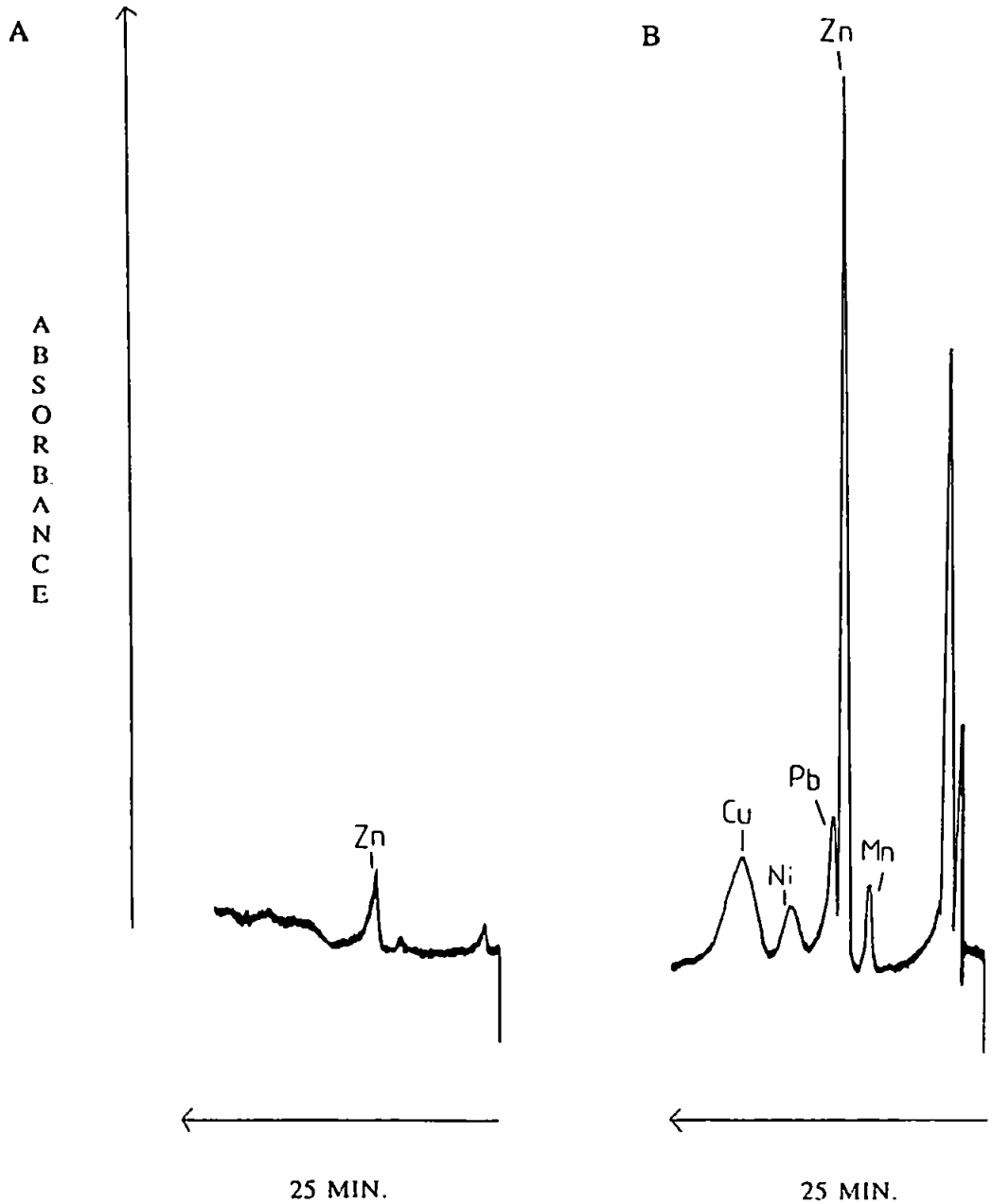


Figure 29. Chromatograms showing Zn(II) in A) procedural blank and B) Mn(II), Zn(II), Pb(II), Ni(II) and Cu(II) in Plymouth Sound seawater, preconcentrated and separated using a Xylenol Orange impregnated column. Preconcentration volume = 20ml.

Sample No.	Zn(II)	Pb(II)	Ni(II)	Cu(II)
1	11.9	0.7	0.8	2.9
2	7.1	1.3	0.8	5.0
3	—	0.7	0.3	1.2
4	15.2	1.0	0.2	3.7

Values in $\mu g\ dm^{-3}$.

Table 8. Concentrations of four metals determined in four samples of Plymouth Sound seawater using HPCIC.

samples were collected over a four week period and during varying tidal conditions. Samples were collected in seawater conditioned polyethylene bottles and filtered through 0.45μm filters prior to analysis.

Calibration was always achieved using standard addition techniques to take account of any matrix effects which may have adversely affected the slope of the calibration. Some typical examples of the calibration curves achieved for the addition of Zn(II), Pb(II), Ni(II) and Cu(II) to Plymouth Sound seawater are shown in Figure 30. The regression values for the standard addition of the above metals are as follows, Zn(II) r=0.991, Pb(II) r=0.993, Ni(II) r=0.994 and Cu(II) r=0.996.

The affect of increasing the preconcentration volume upon the peak height was also investigated, using a 5μg dm^{-3} mixed metal spike to a sample of Plymouth Sound seawater. The increase in response when preconcentrating 10, 30 and 60ml of the spiked sample was found to be linear, although Zn(II) showed a slightly larger degree of deviation than the others. The calibration curves achieved are shown in Figure 31. The regression values for the above calibrations are, Zn(II) r=0.985, Pb(II) r=0.998, Ni(II) r=0.996 and Cu(II) r=0.992. The reproducibility of the technique was calculated with six repeat determinations of Zn(II) and Cu(II) in the procedural blank and Zn(II), Pb(II), Ni(II) and Cu(II), in a seawater sample spiked with 20μm dm^{-3} of the above metals. The relative standard deviations (s_R) of all four metals studied were less than 10% and are shown in Table 9.

Three samples were collected from locations close to the mouth of the Carnon estuary, near Falmouth. Figure 32 shows the sampling sites, Nos. 1, 2 and 3, and the location of the Wheal Jane tin mine. Titration of the three samples resulted in salinity values ranging from 27-35‰. Sample No. 1 was collected midway along the length of the Carnon estuary, sample No.2 at the estuary mouth and sample No.3 approximately 5km along the Carrick

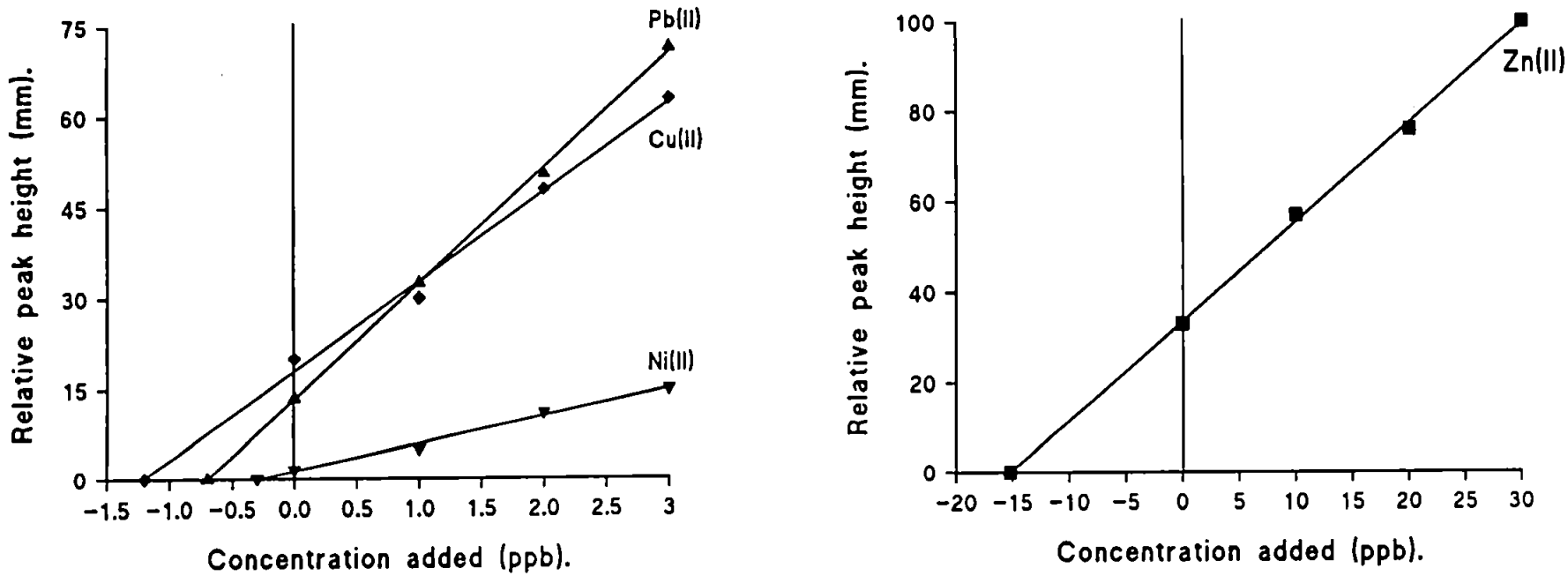


Figure 30. Typical calibration curves for the standard addition of Zn(II), Pb(II), Ni(II) and Cu(II) to Plymouth Sound seawater.

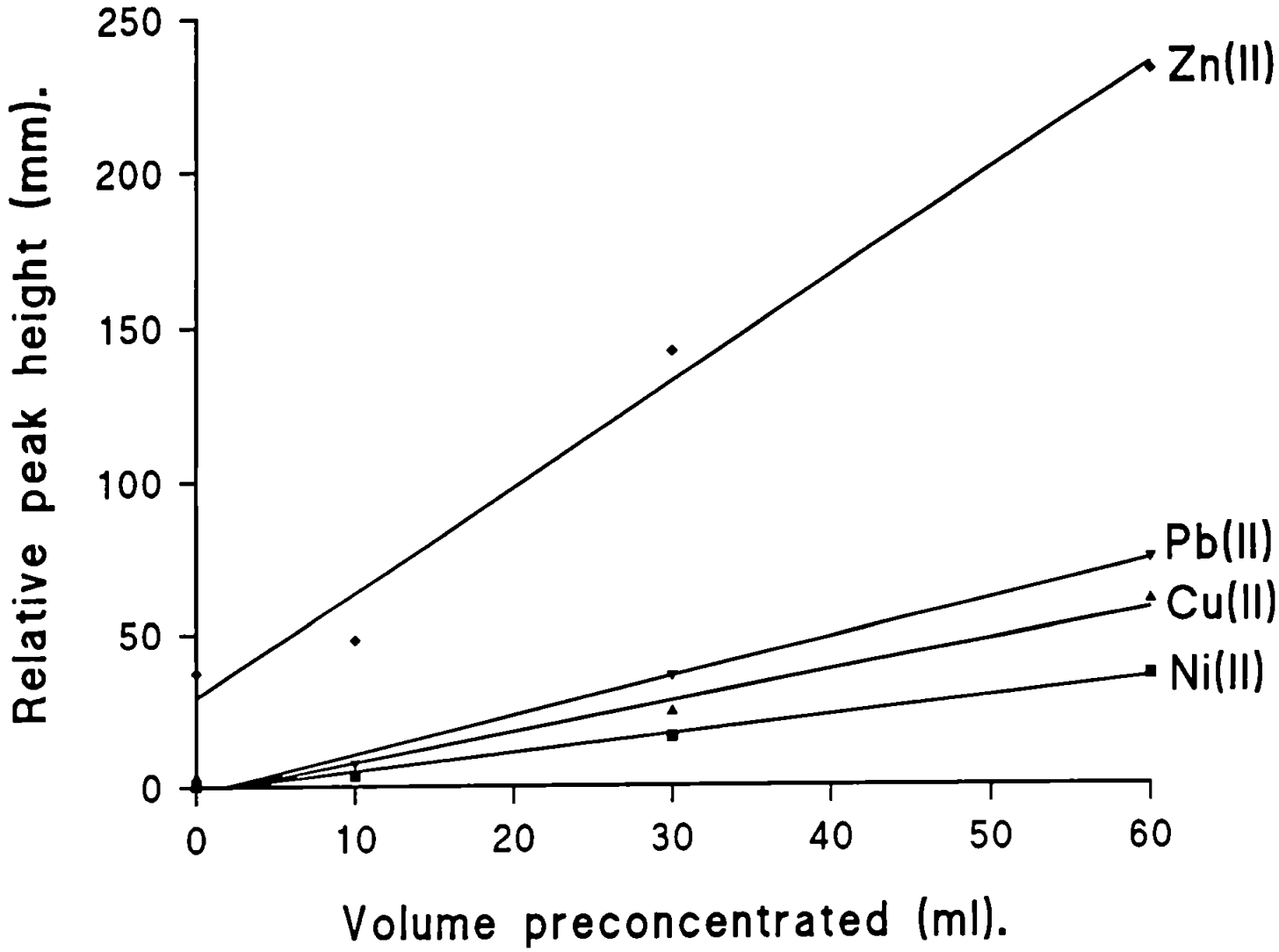


Figure 31. Calibration curves showing the increase in response with increasing preconcentration volume, for a 5μg dm^{-3} mixed metal spike added to Plymouth Sound seawater.

Sample	Zn(II)	Pb(II)	Ni(II)	Cu(II)
Blank	8.4	ND[a]	ND[a]	5.3
Spiked seawater	6.4	4.7	8.7	8.2

[a] Not detected.

Table 9. Relative standard deviations (s_R) of six replicate procedural blank and spiked seawater determinations (20μg dm^{-3} spike).

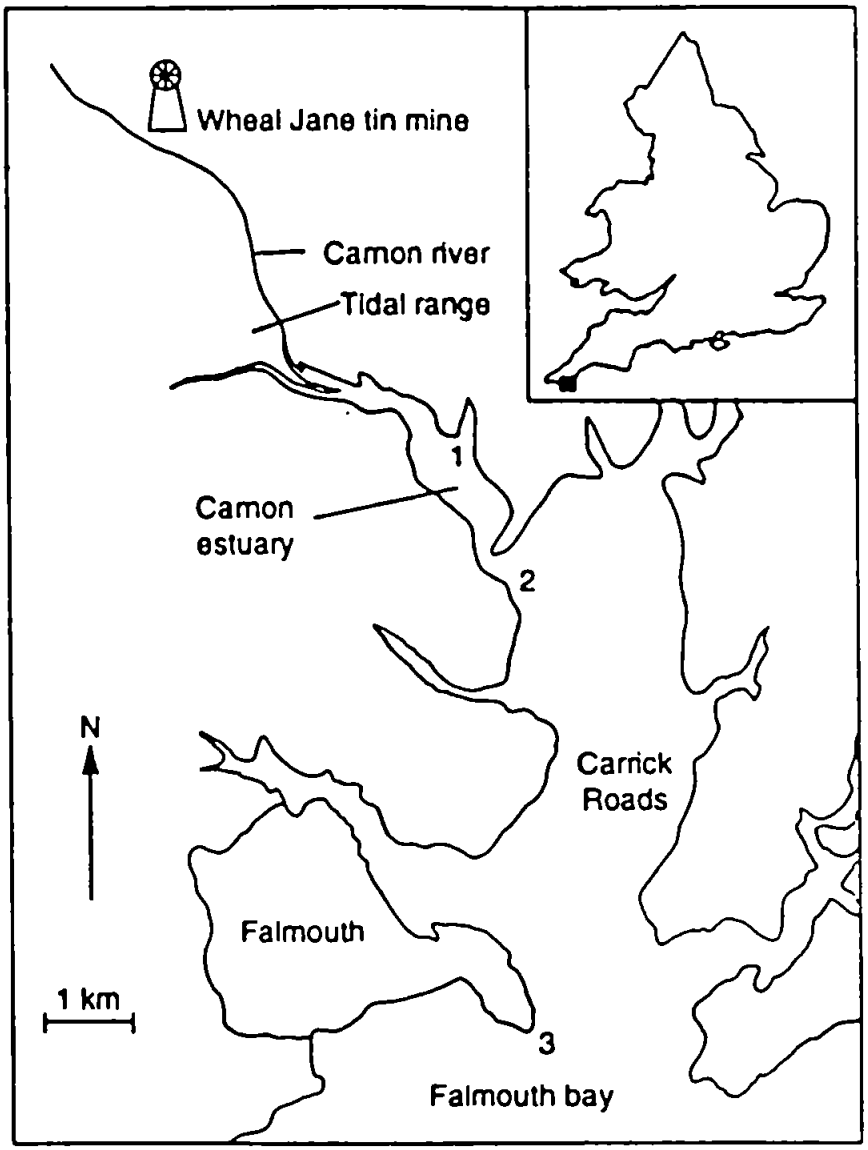


Figure 32. Sampling sites Nos. 1, 2 and 3, and the location of the Wheal Jane tin mine.

Roads from the estuary mouth. All the samples were collected one hour after high tide. The chromatogram produced from preconcentrating 6ml of sample No.1 is shown in Figure 33, the procedural blank is also shown. Due to the concentrations of the metals present in the Carnon estuary being higher than those in Plymouth Sound samples, only 6ml preconcentration volumes were required, as opposed to the 20ml required for the latter samples. These high levels were caused by the overflow from the flooded Wheal Jane tin mine, situated close to the beginning of the Carnon estuary. Although some water pre-treatment is being carried out before discharge into the Carnon river, decreasing the very high metal loading when first flooded, our results show that relatively large amounts of heavy metals are still entering the estuary. Elevated levels of Zn(II) in the samples produced the need for an adjustment of the step gradient programme, to elute the Pb(II) peak further away from the much larger Zn(II) peak. It was also possible to adjust the post-column detector to be less sensitive to Zn(II), by monitoring at a wavelength removed from the λmax of the Zn/PAR complex, 490nm. The wavelength used was 520nm, this did not cause a change in the sensitivity of Pb(II) or Cu(II), although Ni(II) was slightly less sensitive at this wavelength. The levels of the four metals determined in the three Carnon samples are shown in Table 10. Good linearity was again achieved for the standard addition of Pb(II), Ni(II) and Cu(II) to the samples. The very high levels of Zn(II) present in the samples meant that simple standard calibration techniques could be used to determine the actual concentrations. The s_R values calculated using six repeat Carnon estuary determinations are Zn(II) 7%, Pb(II) 13%, Ni(II) 9% and Cu(II) 3%.

A certified standard coastal seawater sample (CASS-2) from the chemistry division of the National Research Council of Canada was analysed for Mn(II), Zn(II) and Cu(II). The levels of Pb(II) and Ni(II) present in the reference material were too low to detect using these

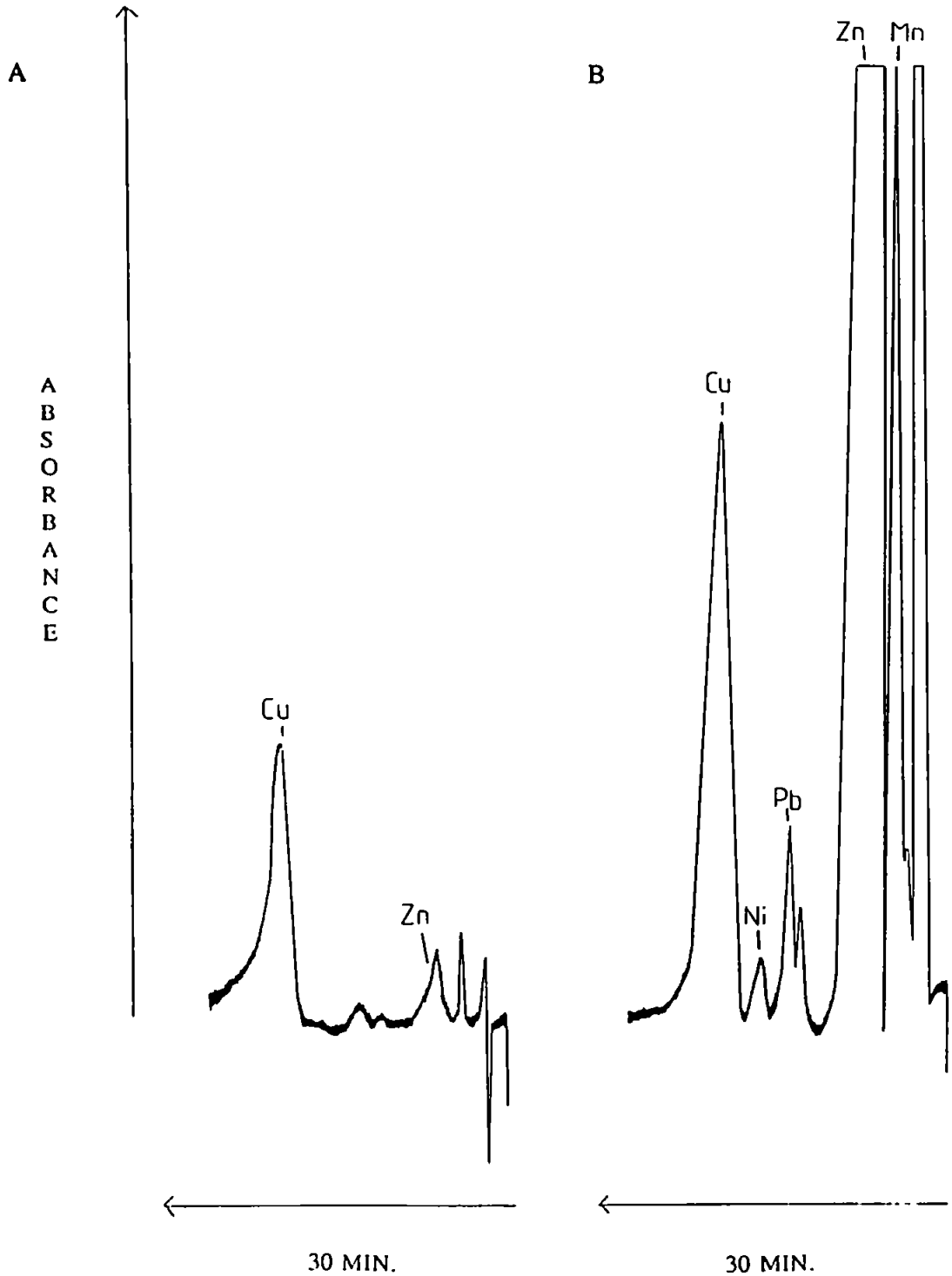


Figure 33. Chromatograms showing Zn(II) and Cu(II) in A) procedural blank and B) Mn(II), Zn(II), Pb(II), Ni(II) and Cu(II) in Carnon estuarine water, preconcentrated and separated using a Xylenol Orange impregnated column. Preconcentration volume = 6ml.

Sample No.	Zn(II)	Pb(II)	Ni(II)	Cu(II)
1	357	1.9	3.7	4.1
2	285	1.3	2.4	4.2
3	147	0.9	2.4	3.4

Values in $\mu g\ dm^{-3}$.

Table 10. Concentrations of four metals determined in three samples of Carnon estuarine water using HPCIC.

preconcentration volumes. However it was possible to determine Mn(II), as the levels of Cd(II), which would normally interfere, were also much too low to detect. This particular reference material was considered a suitable sample to evaluate the accuracy of the HPCIC technique, as the methods used to certify the sample included several chelating column and chelation solvent extraction techniques. Also the sample had not been pre-treated with methods such as boiling acid solutions or UV photolysis, to give "total" concentrations.

Two determinations were carried out on the CASS-2 sample, the first preconcentrating 30ml at pH 6.0, and the second at pH 7.0. The reason for carrying out two determinations at different pH values, was to determine whether an increase of one pH unit would affect the amount of each metal being preconcentrated onto the column. The results of the two determinations matched closely and were within 10% of the certified values, therefore pH 6.0 is sufficient to preconcentrate all the available Mn(II), Zn(II) and Cu(II) in the CASS-2 sample. The results achieved, together with the certified concentrations are shown in Table 11.

From the results obtained for the two sets of samples, certain observations can be made. The results for Plymouth Sound seawater show elevated levels of the four metals investigated. However this is expected in coastal waters, especially in areas which receive run-off from catchments which have had substantial past Cu(II) and Pb(II) mineralization. The variation noticed in the results for the Plymouth Sound samples are expected to be a result of differing tidal conditions during sampling. As mentioned earlier, the Carnon samples were collected simultaneously, just after high tide. The results show substantially elevated levels of Zn(II) in all three samples. Pb(II), Ni(II) and Cu(II) also show increased concentrations when compared to Plymouth Sound, which is a similarly used coastal port. Determination of the four metals in the three samples revealed a decrease in concentrations in relation to the

	Mn(II)	Zn(II)	Cu(II)
Determination No.1[a]	1.74	2.13	0.73
Determination No.2[b]	1.66	2.12	0.65
Certified values ± SD	1.99 ± 0.15	1.97 ± 0.12	0.68 ± 0.04

Values in $\mu g\ dm^{-3}$.

[a] metals preconcentrated at pH 6.0.

[b] metals preconcentrated at pH 7.0.

SD = Standard deviation.

Table 11. Concentrations of three transition metals determined in CASS-2 coastal seawater certified reference material using HPCIC, compared with certified values.

distance from the beginning of the Carnon estuary, pointing to the Wheal Jane tin mine as a continuing source of heavy metals into the surrounding coastal waters.

4.1. DETERMINATION OF ALUMINIUM IN SEAWATER.

The determination of Al(III) in seawater suffers the same matrix related problems as the determination of other trace metals in seawater when using traditional analytical techniques, which include spectroscopic, photometric and ion chromatographic methods. Chelating resins have in the past been successfully used for the preconcentration of Al(III) from seawater. One of the most recent determinations involving chelation has been illustrated by the Dionex Corporation, using their "Chelation Ion Chromatography" system [164]. The system has been discussed earlier with reference to the determination of other trace metals in seawater. The system for the above application comprises of two MetPac CC-1 chelating columns and an IonPac CS-5 ion exchange column in a complex switching arrangement. Al(III) is preconcentrated onto the chelating columns at pH 5.5, swept onto the separator column using 0.75M HCl, where it is separated from any Fe(III) present. A detection limit of 1μg dm^{-3} Al(III) in seawater is quoted, preconcentrating 20ml.

Jones and Schwedt [75] have illustrated the ability of a Chrome Azurol S (CAS) impregnated chelating column to preconcentrate Al(III) from distilled water. The separation of trivalent metals using a similar CAS chelating column has been discussed in section 3.2.vi. Due to the weaker chelating nature of the CAS column, again discussed in the above section, it is ideally suited for trivalent metal applications, such as the preconcentration and determination of Al(III) in seawater. The use of the Xylenol Orange column for example, would incur problems due to the highly acidic eluents required to elute the metal.

The principle techniques and procedures involved in using a CAS impregnated chelating column to preconcentrate Al(III) from seawater, are the same as those employed in the previous application for transition and heavy metals. The main differences in the two applications were the pH used to preconcentrate the metal, the step gradient programme and the PCR used. The column was buffered to pH 4.0 using a sodium acetate buffer solution. At this pH all alkali, alkaline earth and the majority of transition and heavy metals are not retained upon the column. However the trivalent metals are completely retained, allowing Al(III) to be preconcentrated. The gradient programme for the elution of Al(III) was as follows, pH 4.0 to 1.8 (upon injection), to pH 1.1 (after 10min). The post column reagent used was Pyrocatechol Violet (PCV), buffered to pH 6.0 using 2M hexamine. This was extremely insensitive to the majority of divalent metals and so reduced the risk of any interferences caused by the presence of these metals.

4.1.i. ANALYTICAL RESULTS.

Figure 34 shows the chromatogram produced from the preconcentration of 20ml of Plymouth Sound seawater, compared to the procedural blank. Both Al(III) and Fe(III) are clearly present and well resolved in under 20min. In order to quantitatively determine the concentrations of both the metals present, standard addition calibrations were carried out. However, due to the insoluble nature of Fe(III) at pH 4.0, it was not possible to produce a linear response for the Fe(III) peak. This was because adding more Fe(III) to the sample resulted in immediate hydroxide formation and subsequent deposition, although using standard calibration techniques an estimate of 2-3μg dm^{-3} Fe(III) in the Plymouth Sound sample was possible. Unlike Fe(III), Al(III) remains soluble enough at this pH and at these low μg dm^{-3}

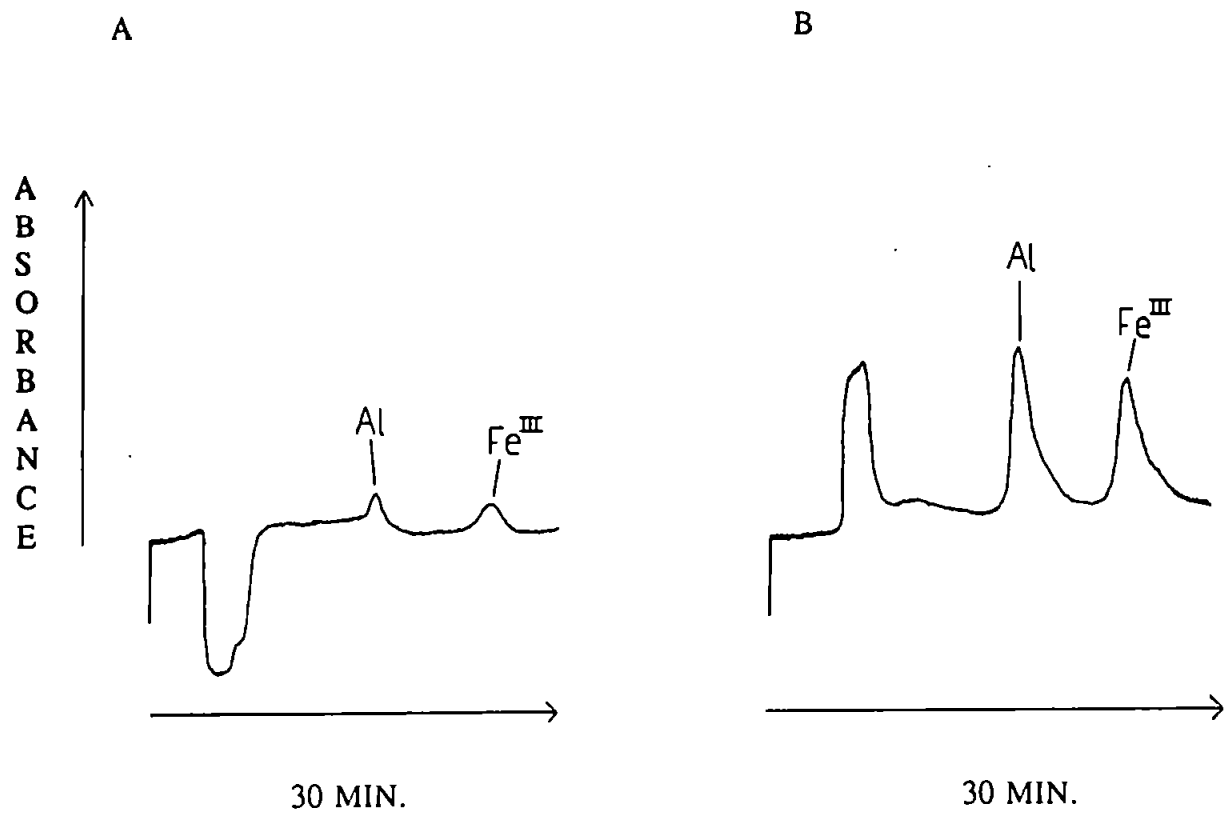


Figure 34. Chromatograms showing Al(III) and Fe(III) in A) procedural blank B) Plymouth Sound seawater, preconcentrated and separated using a Chrome Azurol S impregnated column. Preconcentration volume = 20ml.

levels to enable standard addition to be used. Four standard additions of 10, 20, 30 and 40μg dm^{-3} Al(III) were added to the sample resulting in the calibration curve illustrated in Figure 35, calculated using peak height. A negative curve resulted from the additions of Al(III) to the seawater sample making the determination of the level of Al(III) in the actual sample more difficult. However using curved calibration techniques a value of approximately 6.0μg dm^{-3} Al(III) was calculated. The affect of increasing the preconcentration volume upon peak height was also investigated by preconcentrating 5, 10, 15 and 20ml of the Plymouth Sound sample. Figure 36 shows the linear calibration curves achieved for both Al(III) and Fe(III) in the seawater sample. The regression values for the two metals are, Al(III) r=0.995 and Fe(III) r=0.996. The linear response for Fe(III) illustrates how the concentration of Fe(III) which is present in the sample is in a stable soluble form, altering this equilibrium by adding standard additions results in a non-linear response, as discussed earlier. The reproducibility of the technique was calculated using six repeat determinations of the seawater sample, resulting in s_R values of 6.0% for Al(III) and 13.2% for Fe(III). Again these figures were calculated using peak height.

4.2. THE EFFECT OF UV RADIATION UPON TRACE METAL DETERMINATIONS.

A short study was carried out comparing the levels of certain trace metals determined in seawater samples, some of which had been pre-treated with UV radiation and some which had not. A seawater sample was collected from Plymouth Sound and acidified to pH 1.0 with nitric acid. The sample was then divided into two aliquots, one of which was treated with UV radiation for a period of four hours. The samples were analysed for both Al(III) and divalent trace metals using the techniques previously outlined. No major differences were noticed for

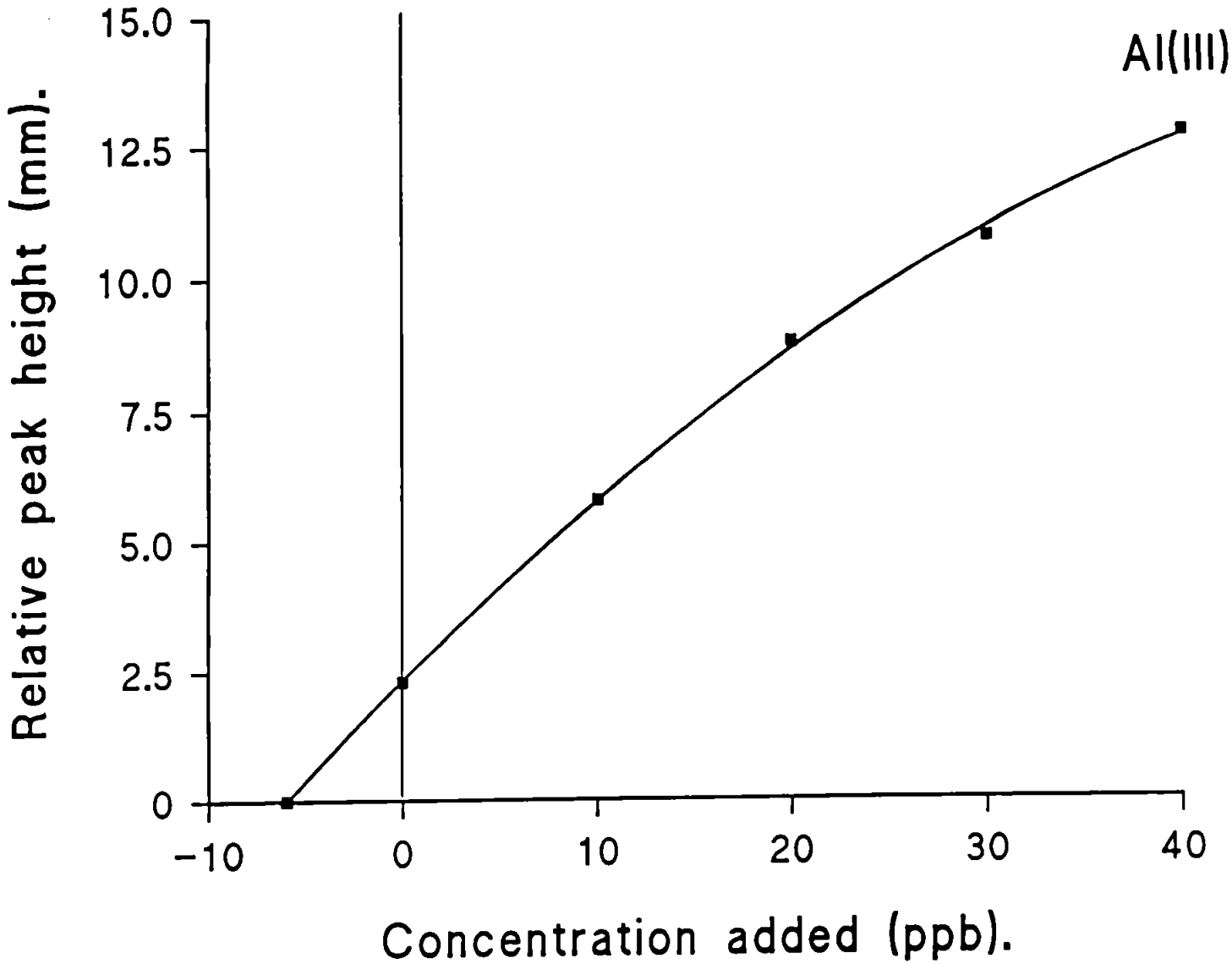


Figure 35. Calibration curve for the standard addition of Al(III) to Plymouth Sound Seawater.

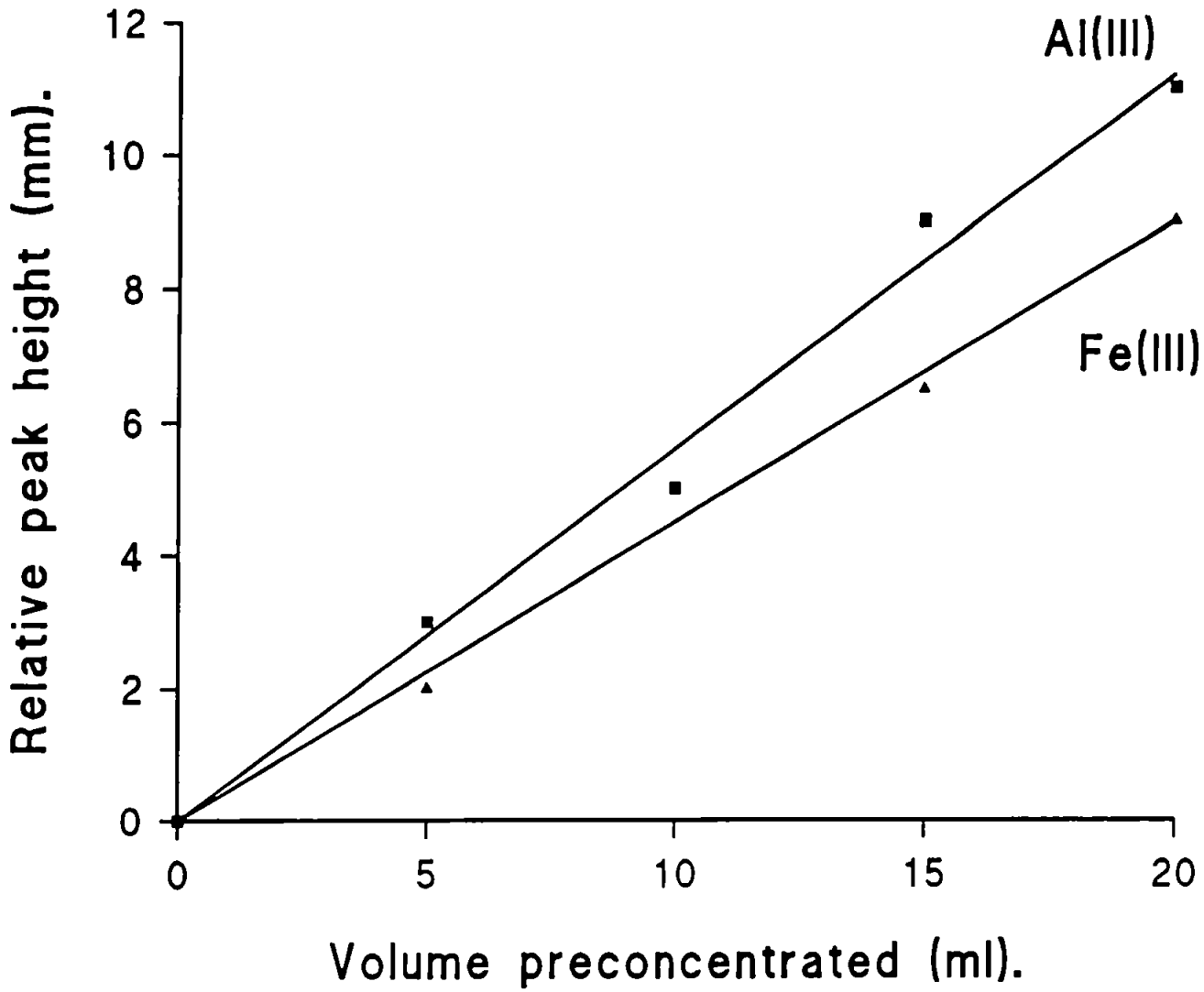


Figure 36. Calibration curves showing the increase in response with increasing preconcentration volume for Al(III) and Fe(III) in Plymouth Sound seawater.

the majority of the metals investigated, although a significant increase in the signal for Cu(II) was noticed. This was approximately an increase of about 40%.

4.3. SUMMARY.

The previous applications have illustrated that dye-impregnated HPCIC can provide a simple solution to the analysis of seawater for a number of trace metals, especially in samples containing elevated levels of these metals, such as coastal samples. The results achieved for the two applications could be greatly improved with the use of totally inert gradient system. This would reduce blank levels further, and together with increasing the preconcentration volumes, would reduce the detection limits and allow the technique to be used for analysis of off-shore samples of interest, such as the North Sea. The result of the UV photolysis investigation supports the widely accepted theory that chelation techniques measure only the "dissolved labile" metal fraction. Cu(II) is known to form extremely stable organic complexes and the increased signal resulting from UV photolysis of the sample indicates the presence of these complexes within the sample studied. A similar problem exists for the determination of Cd(II) in seawater due to the formation of stable chloride complexes, $CdCl^{y-}_{x}$. Cd(II) also elutes very close to the peak for Mn(II) when using the XO impregnated column. This, together with the above chloride problem meant the above two metals could not be studied in the actual seawater samples. A possible solution to this problem is the use of a different chelating column which exhibits a greater resolution between Cd(II) and Mn(II). The CAS impregnated resin produces this required resolution, (shown in section 3.2.vi.) and could be employed for this particular application.

CHAPTER 5. RESULTS AND DISCUSSION.

5.0. DETERMINATION OF BARIUM AND STRONTIUM IN COMPLEX MATRICES.

One of the major advantages of high-performance chelation ion chromatography (HPCIC) is the variation in the retention order of various groups of metal ions compared to simple cation exchange chromatography (CEC). As mentioned earlier, the retention of metal ions when using HPCIC is a function of the relative magnitude of conditional stability constants and the elution order of many groups of metals is completely reversed. The separation of the alkaline earth metals using HPCIC, as shown and discussed in section 3.2, exhibits the following retention order, Ca(II) > Mg(II) > Sr(II) > Ba(II), although Ca(II) and Mg(II) show changes in order depending upon the type of chelating dye impregnated into the resin, again this is discussed in Section 3.2. This reversed order of elution for the alkaline earth metals allows HPCIC to be employed for the analysis of a range of samples which could cause serious problems if being analysed using certain other techniques. The determination of Ba(II) and Sr(II) in samples containing substantially higher levels of other alkaline earth and alkali metals using CEC suffers two major problems. Firstly, high levels of alkali metals cause swamping of the ion exchange sites, seriously affecting the chromatography. Secondly, as Sr(II) and Ba(II) elute after Ca(II) and Mg(II) when using CEC, high levels of these two metals would cause the Sr(II) and Ba(II) to elute on the tail of two larger pre-eluting peaks. It should also be noted that the width of peak increases with increasing retention time due to dispersion within the column, and thus when using simple CEC, the peaks for Sr(II) and Ba(II) tend to be broader than those for Mg(II) and Ca(II). Therefore the detection of these broader peaks becomes less sensitive. With HPCIC, Ba(II) elutes first before Sr(II), followed

by Mg(II) and Ca(II). Because Ba(II) and Sr(II) elute close to the solvent front, HPCIC produces not only very sharp peaks, allowing for much greater sensitivity for these two metals but also baseline resolution from larger amounts of Ca(II) and Mg(II) which are retained for longer on the column. Mono-valent salt ions such as Na(I) and K(I) have very little affinity for the chelation sites of these impregnated chelating resins and are unretained, therefore the ionic strength of the sample does not cause as great an effect upon the separation when using HPCIC as occurs with CEC.

The determination of Ba(II) and Sr(II) using other analytical techniques can also suffer problems, often in the form of interferences due to higher levels of alkali and other alkaline earth metals. AAS has been used by Jaber and El-Issa [165] for the determination of Ba(II) in Ca(II) containing matrices and reported large interferences resulting from the formation of the CaOH molecular species. Direct current plasma atomic emission spectroscopy has been used to determine Ba(II) in excess levels of alkali and alkaline earth metals by Jerrow et al. [166]. Here the interfering effects of Ca(II) were said to be reduced by the addition of, or by the presence of Mg(II) in the sample, although strict guidelines of matrix matching were suggested if accurate results were to be obtained. Manna et al. [167] reported a spectrophotometric method for the determination of Ba(II) using the complexing agent Sulphonazo III. However, once again the presence of either Ca(II) or Sr(II) in the samples studied resulted in major interferences.

5.1. DETERMINATION OF BARIUM AND STRONTIUM IN MINERAL WATERS.

New proposed EC limits [168] for levels of Ba(II) in mineral waters of 0.7mg dm^{-3} require a rapid and sensitive method of analysis, which would ideally be able to detect concentrations ten times lower than the above levels. Mineral waters often contain up to 300mg dm^{-3} Ca(II), combined with high levels of Mg(II) and to a lesser extent, Na(I) and K(I). The above ratio of Ba(II) to Ca(II) would make it extremely difficult to determine Ba(II) using cation exchange chromatography for the reasons discussed above.

5.1.i. CHOICE OF CHELATING COLUMN.

From the investigations into the range of characteristics of dye-impregnated chelating columns, discussed in section 3.2, several different separation profiles of the alkaline earth metals were produced. The most efficient separations achieved for this particular group of metals resulted from columns impregnated with members of the triphenylmethane group of chelating dyes. These were Xylenol Orange (XO), Methylthymol Blue (MTB) and Phthalein Purple (PP). Each of these dyes contain two iminodiacetic acid functional groups which act as the active chelation sites when the dye is immobilised upon the resin. Although these three dyes contain the same functional groups, when immobilised upon the resin significant differences in the resolution of the four metals can be seen and changes can even be seen in the retention order of Ca(II) and Mg(II). A probable explanation for these differences is small changes in the relative basicity of the iminodiacetic acid groups which will have a drastic affect on the conditional stability constants of the individual metal chelates. It is these subtle differences in the separation profiles of groups of metals when using different dye-

impregnated resins, which allows the analyst to choose the type of chelating column best suited to the analyte and sample under investigation. For the determination of Ba(II) in mineral waters, a MTB impregnated column was employed as it displayed several advantages over the other triphenylmethane dyes for this particular application. With the MTB column the resolution of the four metals achieved was slightly greater than that resulting from using the XO column. This greater resolution means the degree of separation between Ca(II) and Mg(II) and the peaks for Ba(II) and Sr(II) was larger with the MTB column. This is an obvious benefit in samples which contain several orders of magnitude higher levels of Ca(II) and Mg(II) than the levels present of the analyte ions, Ba(II) and Sr(II). The separation of the alkaline earth metals using the PP impregnated column showed an even greater peak resolution than that of the previous two columns. However the total elution time of all four metals using the PP column was much longer, therefore MTB was more appropriate as it facilitated a faster sample turnover. The PP column was employed in an application which required an even greater separation between Sr(II) and Ca(II), this is discussed in section 5.2.

5.1.ii. PERFORMANCE OF THE METHOD AND RESULTS.

A selection of eight bottled mineral waters were analysed, three of which were found to contain detectable concentrations of both Ba(II) and Sr(II) and also relatively high levels of Ca(II), and thus were chosen for quantitative determinations. The three brands of bottled water investigated were Evian, Highland Spring and Buxton mineral water. Figure 37 shows the chromatograms produced from the injections of each of these samples and the exact conditions used. As shown in the figure, a step down in the pH of the eluent was used, afthe elution of Ba(II) and Sr(II), to sweep off the Ca(II) and Mg(II) rapidly and therefore reduce

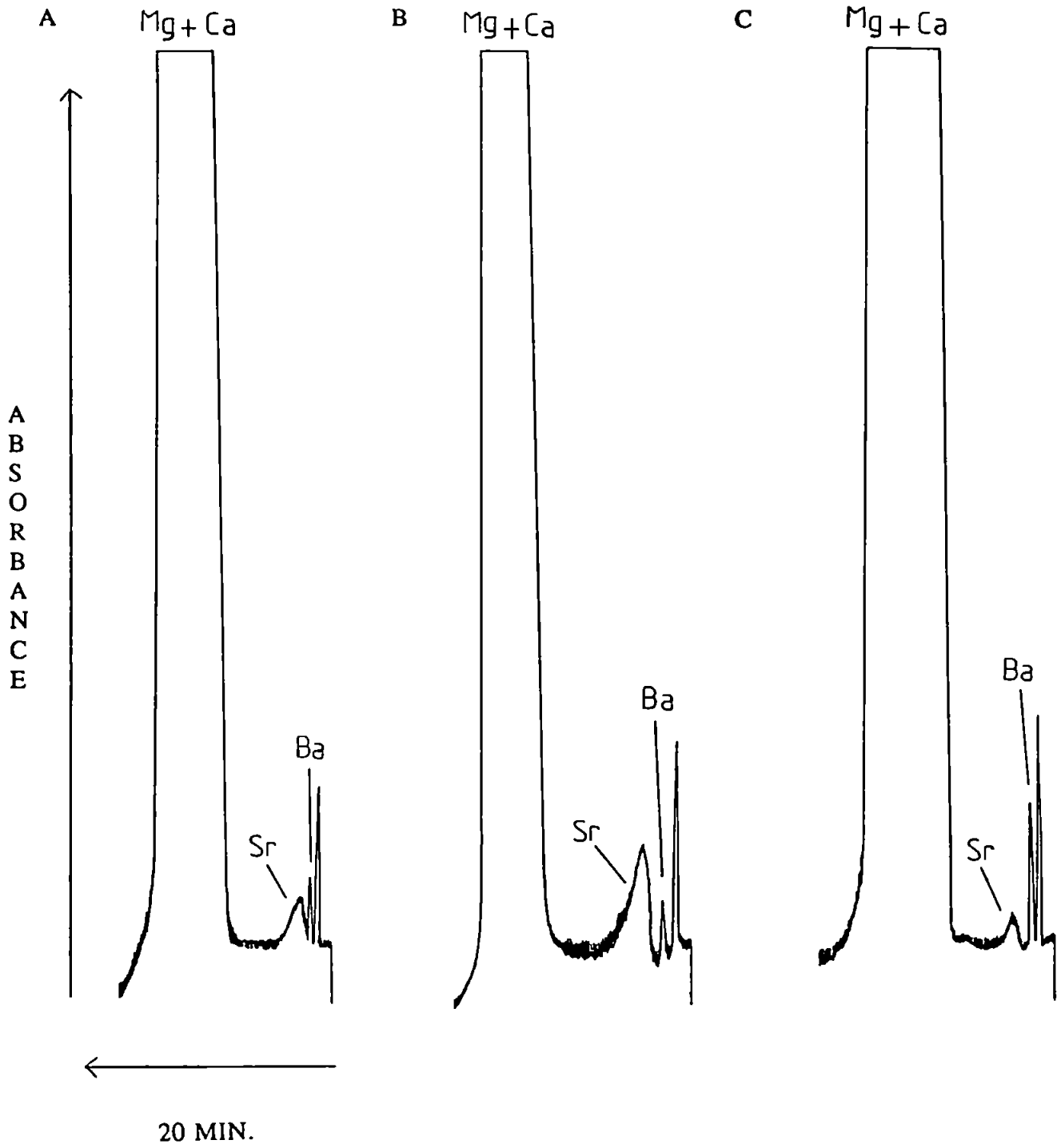


Figure 37. Chromatograms showing the separation of Ba(II), Sr(II), Mg(II) and Ca(II) in A) Evian mineral water, B) Buxton mineral water and C) Highland Spring mineral water using a Methylthymol Blue impregnated column. pH 8.5 stepped down to pH 5.0 after 5min.

analysis time.

Of the samples analysed, all fell well below the new proposed limits. Highland Spring had the highest levels of Ba(II), which at 0.33mg dm^{-3} was approximately half the proposed maximum level. To take into account any matrix interferences which could adversely affect the slope of the calibrations, standard addition techniques were used throughout this application. For each determination five mixed metal standard additions were used. These ranged from 50 to 1000μg dm^{-3} for Ba(II) and 100 to 1000μg dm^{-3} for Sr(II). Dilution of the original sample by the addition of metal spikes was kept to negligible levels by keeping the overall volume of the spikes as small as possible. For example, to produce a 500μg dm^{-3} Sr(II) standard addition, 100mm^3 of a 500mg dm^{-3} Sr(II) standard was added to 100cm^3 of sample. The calibrations achieved for both metals in all three samples are shown in Figure 38. Linear responses were achieved measuring peak heights, with typical regression values of r=0.999 for Ba(II) and r=0.998 for Sr(II). Table 12 shows the concentrations of the two metals determined in the three samples and the corresponding results obtained using ICP-OES. Overall good agreement between the two techniques was achieved although, as might be expected the standard deviation of the results gained using HPCIC are higher than those of the ICP-OES technique.

The precision of the method was determined by repeating eight injections of a mixed metal spike in a sample of Buxton mineral water. The spike contained 0.4mg dm^{-3} Ba(II) and 0.8mg dm^{-3} Sr(II). The concentrations of Ca(II) and Mg(II) present in the sample were 50 and 20mg dm^{-3} respectively. The relative standard deviation (s_R) values calculated from the above, were 3% for Ba(II) and 10% for Sr(II). The analysis of a certified reference material was carried out to help validate the technique. The river water certified reference material IAEA/W-4 was chosen, this has a very similar matrix to the bottled waters and contained low

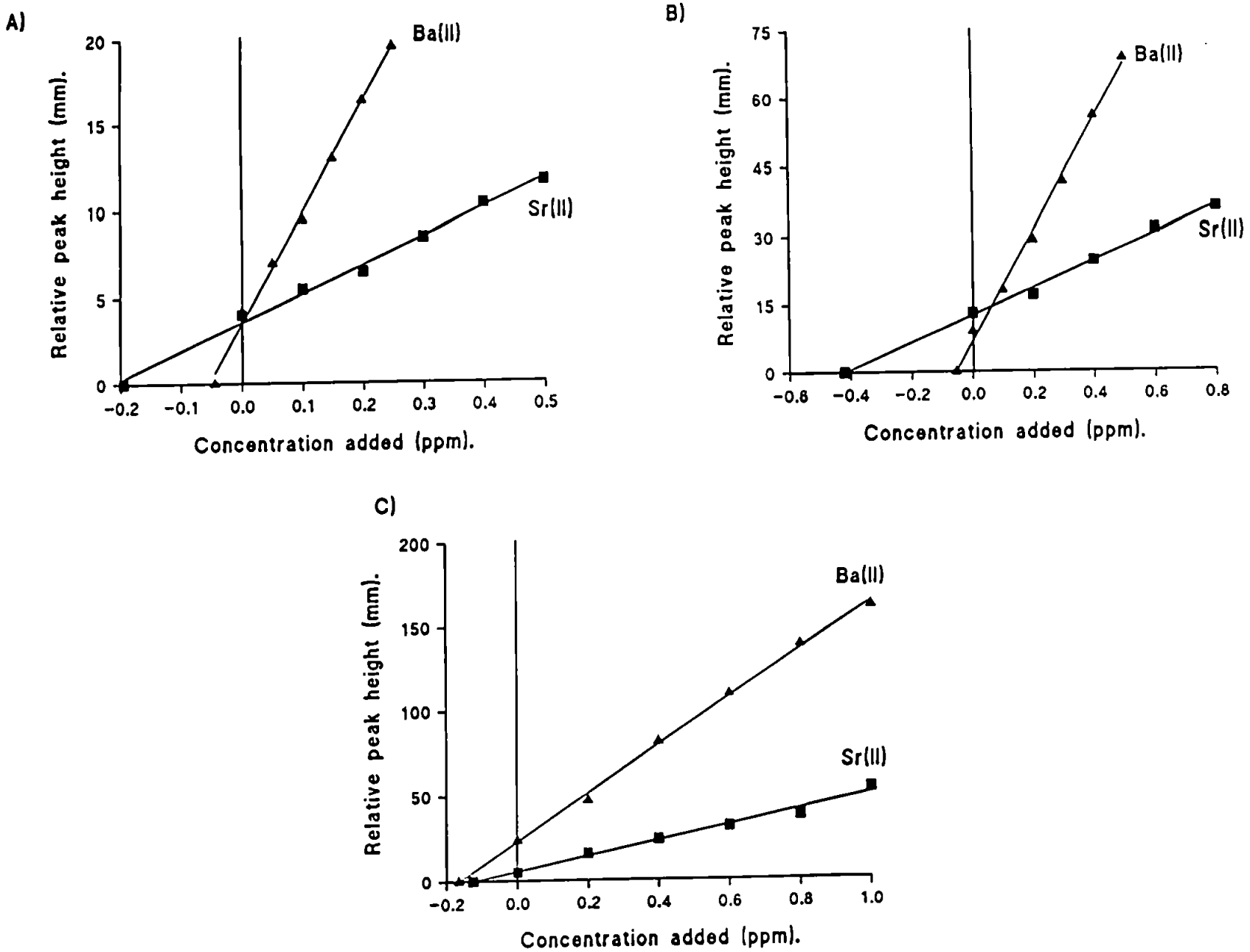


Figure 38. Calibration curves for the standard addition of Ba(II) and Sr(II) to A) Evian, B) Buxton and C) Highland Spring mineral water (Dil.1:1 v/v with DW).

Mineral Water	Metal ion	HPCIC	ICP-OES
Evian	Ba(II)	0.09 (0.01)	0.078 (0.003)
	Sr(II)	0.39 (0.01)	0.370 (0.005)
Buxton	Ba(II)	0.11 (0.02)	0.137 (0.015)
	Sr(II)	0.84 (0.07)	0.585 (0.023)
Highland Spring	Ba(II)	0.33 (0.04)	0.364 (0.016)
	Sr(II)	0.25 (0.08)	0.268 (0.010)

Values in mg dm^{-3} ($\pm$ S.D.).

Table 12. Levels of Ba(II) and Sr(II) determined in three bottled mineral waters using HPCIC compared with results achieved using ICP-OES.

levels of both Ba(II) and Sr(II) (0.052mg dm^{-3} and 0.050mg dm^{-3} respectively) combined with concentrations of 10mg dm^{-3} Ca(II) and 4mg dm^{-3} Mg(II). The above ratio of Ba(II) and Sr(II) to Ca(II), approximately 1:200 is very similar to the average ratios found in bottled mineral water samples. Figure 39 shows the chromatogram produced from the injection of the river water standard. Once again the Ca(II) and Mg(II) are swept from the column as a single peak using an acid eluent to reduce analysis time. As is illustrated by the chromatogram, the levels of Ba(II) and Sr(II) present are very close to their detection limits, using the conditions shown. The results gained from the analysis of the above reference material are shown in Table 13. It can be seen from the table that the results achieved using HPCIC compare very well with the certified values. ICP-OES was again used in comparison with the chelation technique producing a fair agreement. The relatively high standard deviation of the HPCIC results are due to the fact that the limits of detection are being approached. Using a 200μl sample loop, limits of detection of approximately 0.03mg dm^{-3} for both Ba(II) and Sr(II) were achieved, calculated as twice the baseline noise.

5.2. DETERMINATION OF STRONTIUM IN POWDERED MILK.

Powdered milk and similar milk products contain extremely high concentrations of Ca(II). Determination of Sr(II) in these samples usually requires laborious sample pre-treatment, often in the form of column chelation techniques to remove the excess of Ca(II) from the sample matrix. The determination of Sr(II) in milk is routinely carried out to monitor levels of radioactive isotopes such as Sr^{90}. In a recent publication [169], the overall analysis time for a single Sr(II) determination, using the chelating resin Chelite P to remove the Sr(II) from the milk followed by numerous extraction techniques, was between five and eight hours. This

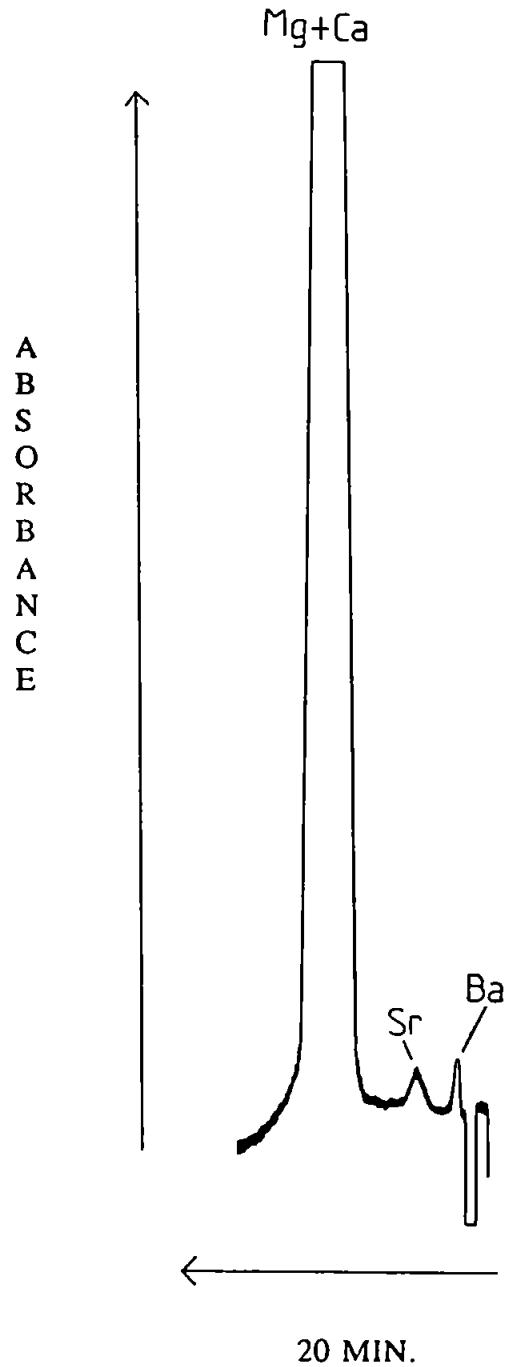


Figure 39. Chromatogram showing the separation of Ba(II), Sr(II), Mg(II) and Ca(II) in river water certified reference material IAEA/W-4 at pH 8.5 stepped down to pH 5.0 after 5min., using a Methylthymol Blue impregnated column.

Metal ion	HPCIC	ICP-OES	Certified values
Ba(II)	0.05 (0.01)	0.041 (0.002)	0.052 (0.002)
Sr(II)	0.05 (0.01)	0.053 (0.003)	0.050 (0.003)

Values in mg dm^{-3} ($\pm$ S.D.).

Table 13. Determination of Ba(II) and Sr(II) in simulated fresh water IAEA/W-4 certified standard reference material using HPCIC and ICP-OES.

clearly illustrates the need for investigation into simpler and much more rapid separation techniques.

Milk powder contains approximately 12,000mg dm^{-3} Ca(II) and only trace amounts of Sr(II), typically less than 10mg dm^{-3}. The ashing and acid digestion of the sample prior to analysis, detailed in section 2.3.iii., produced a clear digest, which when made up to 20ml with DW resulted in an overall dilution factor of 20. As can be seen from the values above, the ratio of Sr(II) to Ca(II) in this particular sample (often greater than 1:1,200), is even more pronounced than those experienced in the previous section, investigating Ba(II) and Sr(II) in mineral water. Therefore, a column capable of producing a large degree of resolution between Sr(II) and Ca(II) was required for the analysis of this particular sample. The column which resulted in the greatest difference in retention times for these two metals was the PP impregnated column. The particular separation characteristics of the PP column are illustrated and discussed in section 3.2.iv.

Figure 40 shows the chromatogram produced from the injection of 100μl of the milk powder digest using the PP impregnated column. In practice the massive peak obtained for Ca(II) could be rapidly swept from the column in only a few minutes using an acid wash. In this case the Ca(II) peak has been allowed to elute isocratically to illustrate the huge differences in sizes of the two peaks. Figure 40 clearly illustrates the problems that this type of sample would cause simple cation exchange chromatography, where the two metals would elute in a reverse order. The resolution between Sr(II) and the large Ca(II) peak shown in Figure 40 is less than that which would be produced from an injection of these metals at lower concentrations. The reason for this apparent loss of resolution between Sr(II) and Ca(II) is the massive excess levels of Ca(II) in the sample filling the most of the chelation sites at the beginning of the column, this effectively reduces the capacity of the column and thus

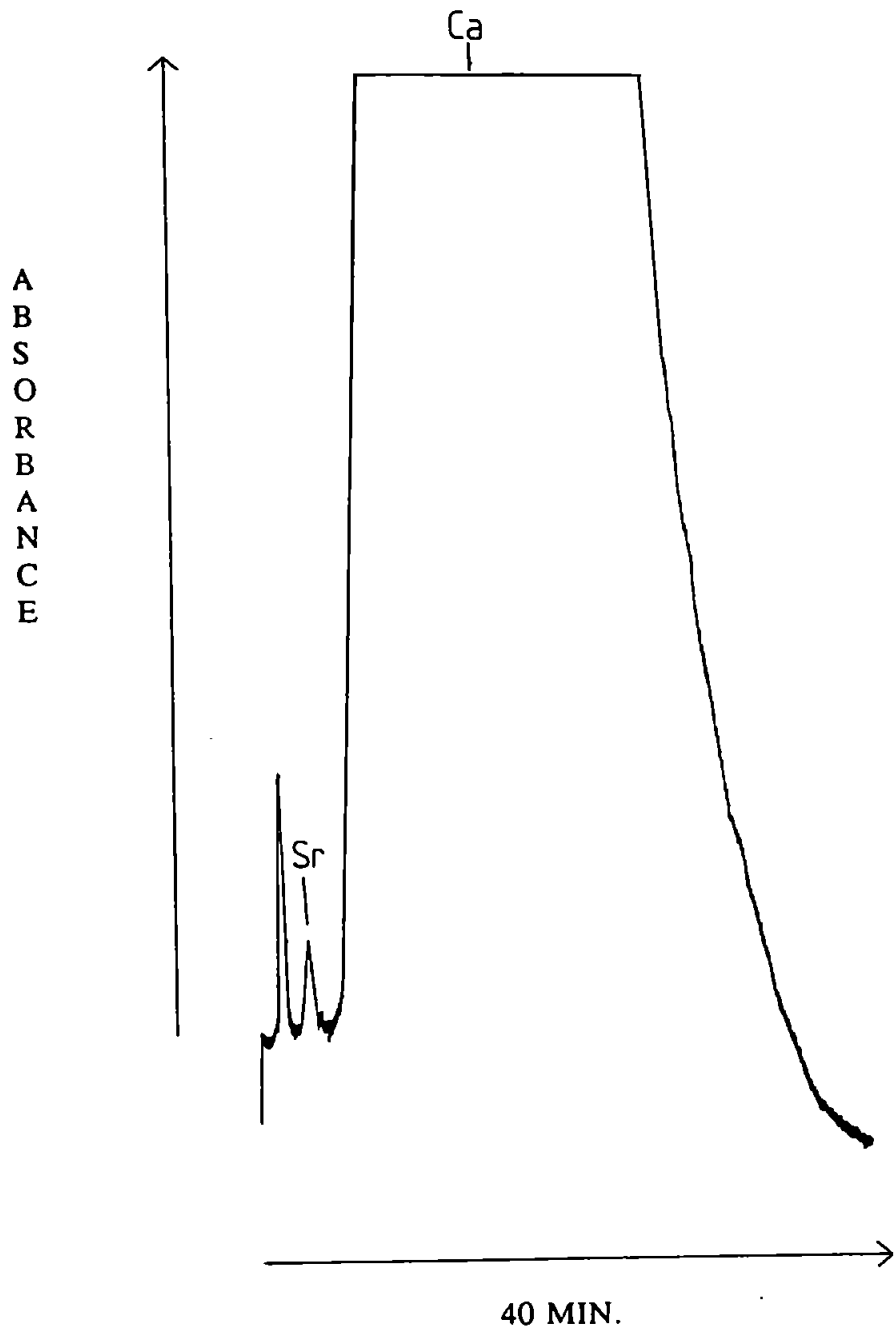


Figure 40. Chromatogram showing the separation of Sr(II) and Ca(II) in milk powder digest using a Phthalein Purple impregnated column.

speeds up the elution of the metal ions. To help compensate for this, increasing the pH of the eluent will increase the retention of the metal ions and ensure Sr(II) elutes away from the solvent front. Close matrix matching and standard addition techniques take into account any such matrix affects and were therefore used throughout this analysis. Four standard additions, ranging from 0.3mg dm^{-3} to 1.2mg dm^{-3} Sr(II) were added to the sample digest resulting in a linear calibration with a regression value of r=0.998. Figure 41 shows the calibration curve achieved for the previous standard additions. The level of Sr(II) found in the actual digest was 0.15mg dm^{-3}. This corresponds to 3.0mg dm^{-3} Sr(II) in the milk powder itself. Ba(II) was not quantitatively determined using the HPCIC technique, as after the 20 fold dilution of the sample the levels present fell below the working detection limit using a 100μl sample loop. ICP-OES was again used as a comparative method, the results of which are shown in Table 14. Ba(II) was determined using the ICP-OES at levels of 0.027mg dm^{-3} in the digest and thus 0.54mg dm^{-3} in the actual milk powder. Good agreement between the two methods was achieved for the determination of Sr(II), although the standard deviation of the result gained using the chelation technique was higher than that of the ICP-OES analysis. This however is a reflection of the closeness of the signal to the detection limit of the HPCIC method compared to ICP-OES.

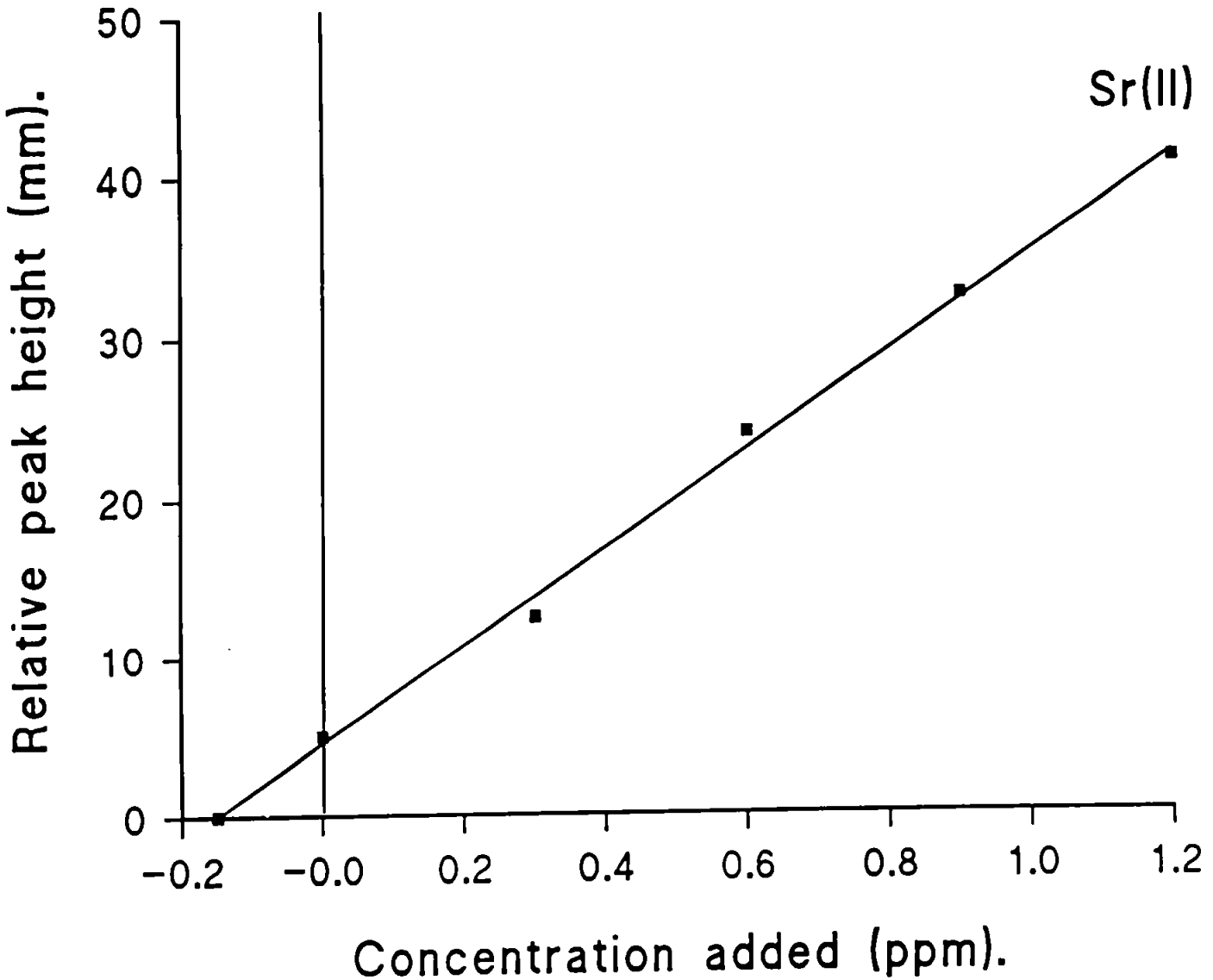


Figure 41. Calibration curve for the standard addition of Sr(II) to milk powder digest.

Metal ion	HPCIC	ICP-OES
Ba(II)	ND[a]	0.027 (0.002)
Sr(II)	0.15 (0.03)	0.125 (0.005)

Values in mg dm^{-3} ($\pm$ S.D.).

[a] Below detection limit.

Table 14. Determination of Sr(II) in milk powder digest using HPCIC compared with results achieved using ICP-OES.

5.3. DETERMINATION OF ALKALINE EARTH METALS IN OFFSHORE OIL-WELL BRINES.

The quantitative determination of the alkaline earth metals in sub-surface formation waters also known as oil-well brines is extremely important in the drilling operations of the petroleum industry. Of particular interest is the need for the determination of Ba(II) in excess levels of alkali and other alkaline earth metals. While drilling for oil, seawater is pumped downhole to replace the oil being removed. This maintains the hydrostatic pressure in the oil-well and helps to force the oil out of the well. The mixing of this seawater, which can contain high levels of sulphate, with sub-surface formation waters, which often contain elevated levels of alkaline earth metals, can result in the rapid precipitation of insoluble alkaline earth sulphates into the pore spaces of the rocks. Worse still is the precipitation of these sulphates into drilling equipment which requires the use of expensive remedies. It is the rapid formation and deposition of $BaSO_4$ in particular, which leads to the need for monitoring of Ba(II) in these complex samples.

The analysis of formation waters using traditional cation exchange chromatography (CEC) suffers two major problems. Firstly, many of these samples can contain up to 30% salt ions, which as mentioned previously, can cause severe problems due to "swamping" of the ion exchange sites, when using the above technique. Secondly, the main analyte of interest is Ba(II), which often exists at concentrations several orders of magnitude lower than the other alkaline earth metals present. Therefore as with the previous two applications discussed, a technique which allows Ba(II) to elute first, rather than last as is the case of CEC, has obvious benefits. This point is illustrated by Singh et al. [18] who has published a technique for the analysis of alkaline earth metals in formation waters using CEC, discussed in section

1.2.ii. Singh was unable to determine Ba(II) in the samples studied for precisely the reasons outlined above.

HPCIC has the potential to be used for the routine monitoring of alkaline earth metals in these complex samples, as it does not suffer with the above problems. Three typical formation water samples were obtained from a well known oil company to evaluate the advantages HPCIC has to offer in the analysis of these rather complex brines.

5.3.i. PROCEDURES.

The analysis of the three samples was carried out using a Methylthymol Blue (MTB) impregnated column. Methylthymol blue was chosen as it produced a well resolved separation of all four alkaline earth metals in a relatively short time, less than 20min. Two separate determinations were carried out for each sample. This was to take into account the large differences in the concentrations of Ba(II) and the remaining alkaline earth metals. For the determination of Ba(II) the sample was diluted 1:1v/v with DW, this avoided overloading of the column. For the determination of the Sr(II), Mg(II) and Ca(II) the dilution used was 1:100v/v with DW. Standard addition techniques were used in all determinations to avoid any possible matrix effects. For the determination of Ba(II) the pH of the eluent was adjusted to 9.2. When analysing a more dilute aliquot of the sample for the analysis of the remaining alkaline earth metals, this was adjusted down to pH 8.0. The reason for this is discussed in the next section. As the sample volume being injected was very small, only 5μl, it was not necessary to adjust the pH of the sample, as this small amount would not affect the pH of the column upon injection.

5.3.ii. PERFORMANCE OF THE METHOD AND RESULTS.

As was the case in the analysis of Sr(II) in milk powder, discussed in section 5.2, injections of large amounts of metal ions onto the column can cause the chelation sites at the beginning of the column to become filled, resulting in a reduction in retention time of each metal. The separation of alkaline earth metals using the MTB column, which is illustrated and discussed in section 3.2.iii, was achieved with an eluent pH of 8.1. When injecting one of the formation water samples for the determination of Ba(II), the eluent pH was increased to 9.2. This increased pH, increases the individual stability constants of the metal chelates and so increases retention times, thus compensating for the effects of partially overloading the column.

Figure 42 shows the chromatogram obtained from the injection of sample 1. The figure illustrates the separation of Ba(II) from a large Sr(II) peak and an extremely large peak representing the combined levels of Ca(II) and Mg(II). Once again the large peaks following the elution of Ba(II) have been allowed to elute isocratically. This is to illustrate the massive differences in the concentrations present, and the obvious difficulties simple CEC would experience in the analysis of such a sample. In practice, as it is only Ba(II) which is being determined quantitatively at this point, the Sr(II), Ca(II) and Mg(II) could be swept off rapidly using an acid sweep, thus greatly reducing analysis time. Standard addition of Ba(II), using four additions ranging from 2.5mg dm^{-3} to 10.0mg dm^{-3}, to the brine samples resulted in a linear calibration with a regression value of $r=0.999$. This was achieved measuring peak height. The calibration curve showing the standard addition of Ba(II) to sample 1 is shown as Figure 43. The reproducibility of the method, for the determination of Ba(II) in the brine samples, was calculated using six repeat injections of a sample, spiked with 6mg dm^{-3}

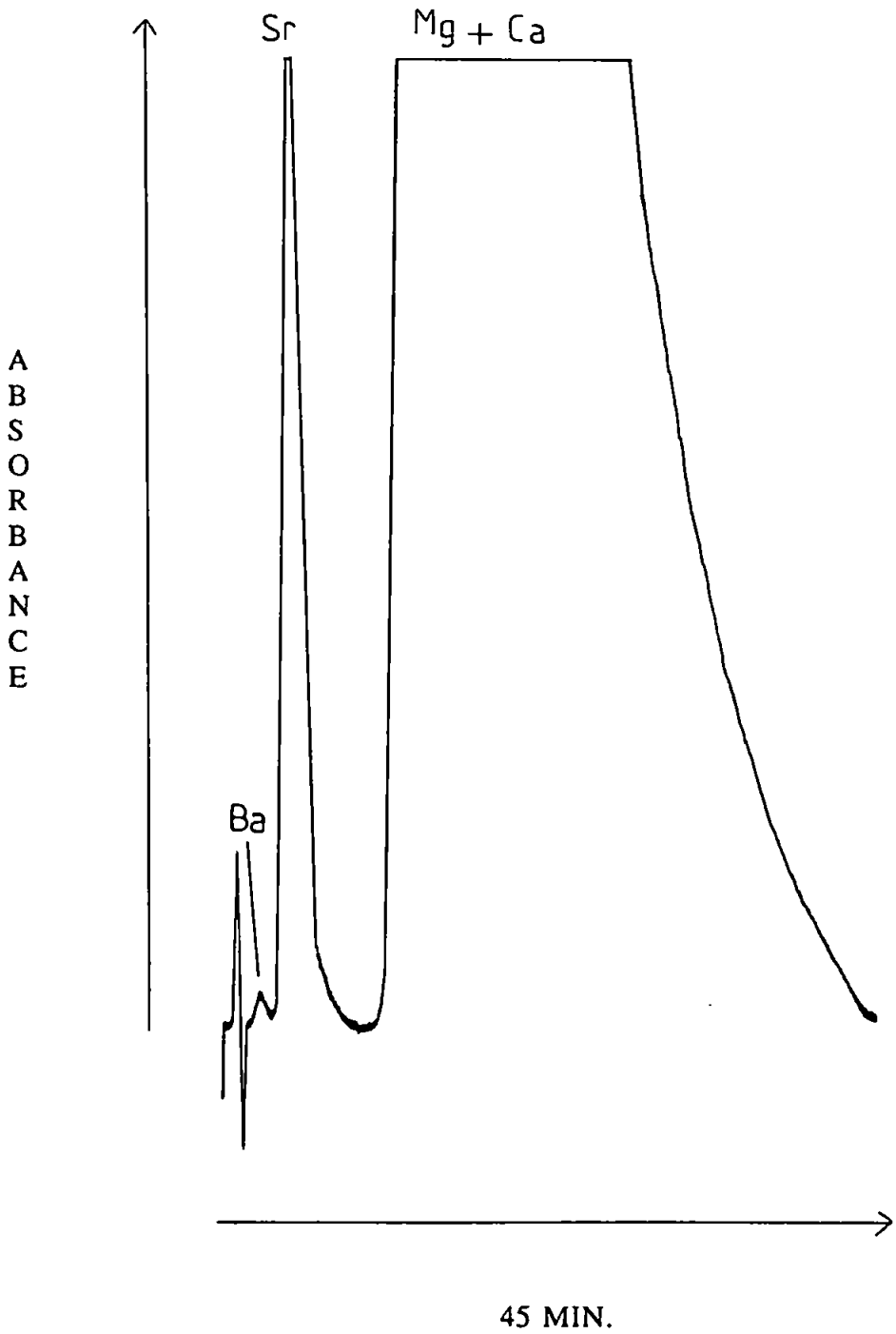


Figure 42. Chromatogram showing the separation of Ba(II), Sr(II), Mg(II) and Ca(II) in oil-well brine sample No.1 (Dil. 1:1 v/v with DW) using a Methylthymol Blue impregnated column.

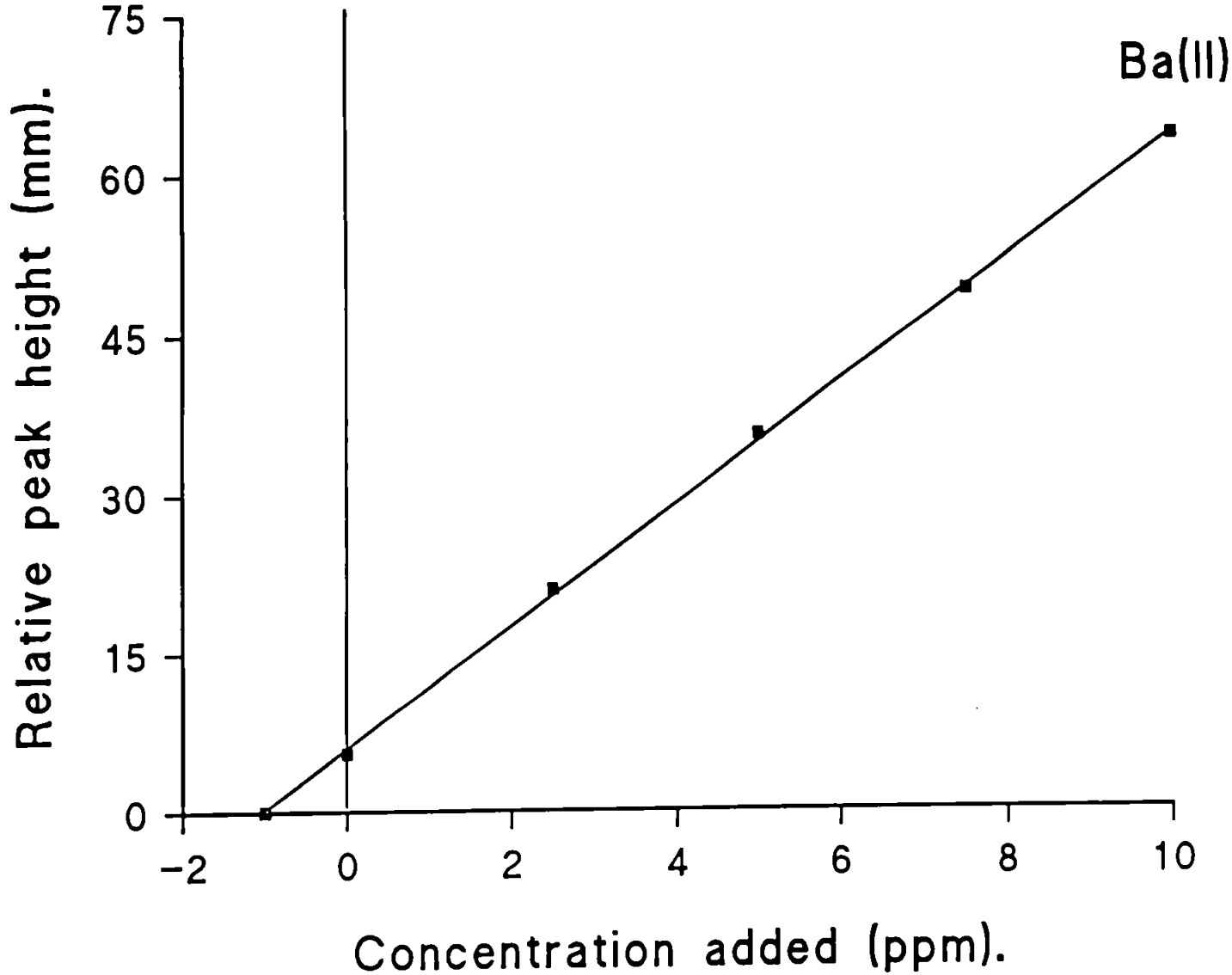


Figure 43. Calibration curve for the standard addition of Ba(II) to oil well brine sample No.1.

of Ba(II). Again using peak height, the relative standard deviation, s_R, was found to be 8.3%. The detection limit for Ba(II), using a 5μl sample loop, was calculated as twice the baseline noise and found to be approximately 1mg dm^{-3} in samples containing total levels of over 2,200mg dm^{-3} of Sr(II), Ca(II) and Mg(II) and up to 16,000mg dm^{-3} of Na(I).

For the determination of the remaining alkaline earth metals, the samples were diluted 100-fold using DW. This was due to the massive amounts of the metals present in the samples, particularly Ca(II) and Mg(II). The chromatogram produced from the injection of diluted sample 3 is shown in Figure 44. The detection limit calculated for Sr(II), in the diluted samples, was approximately 8mg dm^{-3}. This was achieved in samples containing over 700mg dm^{-3} of Mg(II) and 350mg dm^{-3} Ca(II). Standard addition techniques were again used for the determination of all three metals in the three brine samples. Linear calibration curves were achieved for the addition of Sr(II), Ca(II) and Mg(II), with regression values ranging from r=0.994 to r=0.999. Figure 45 shows typical calibration curves obtained for the addition of the above metals to a formation water sample. Reproducibility was again determined using a series of six repeat injections. The relative standard deviations (s_R) of six repeat injections of a 100-fold dilution of sample 1 were 6.1% for Sr(II), 7.3% for Mg(II) and 6.1% for Ca(II). These s_R values were calculated using peak area.

The results obtained for all four alkaline earth metals in the three brine samples are shown in Table 15. The values determined using the HPCIC technique were again compared to ICP-OES and were generally in good agreement. As with the two previous applications discussed in sections 5.1 and 5.2, the s_R values achieved using HPCIC are higher than those calculated using ICP-OES. However the s_R values for all four metals remain below 10% in this particular application, even for Ba(II) at levels close to the working detection limits. As can be seen from Table 15, the levels of Ba(II) present in samples 2 and 3 fell below the

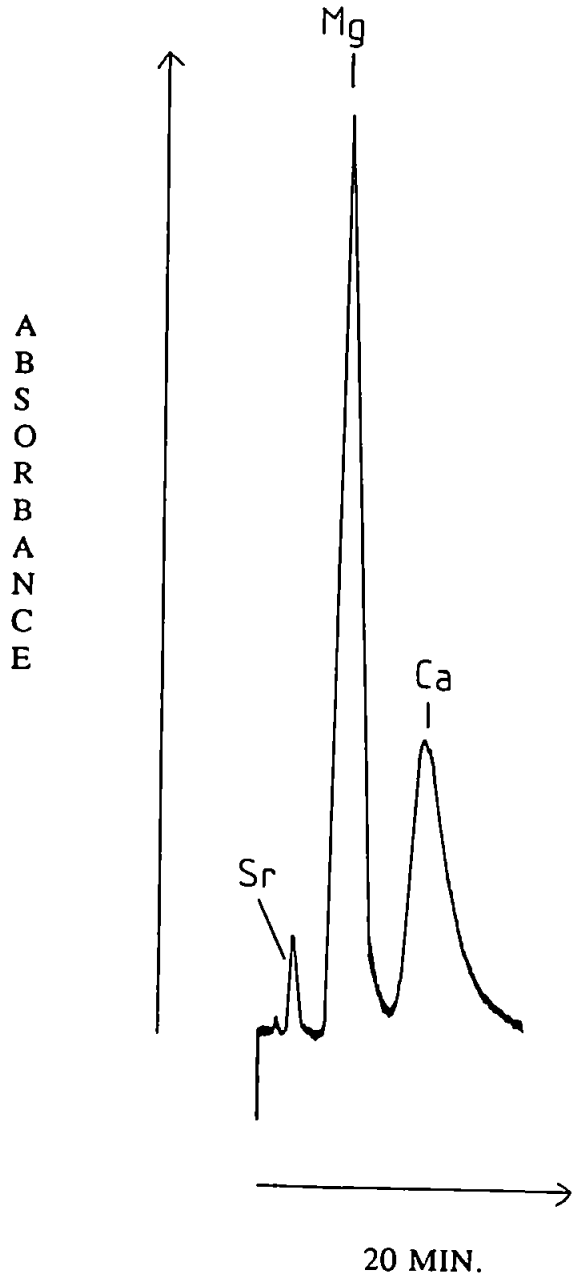


Figure 44. Chromatogram showing the separation of Sr(II), Mg(II) and Ca(II) in oil-well brine sample No.3 (Dil. 1:100 v/v with DW) using a Methylthymol Blue impregnated column.

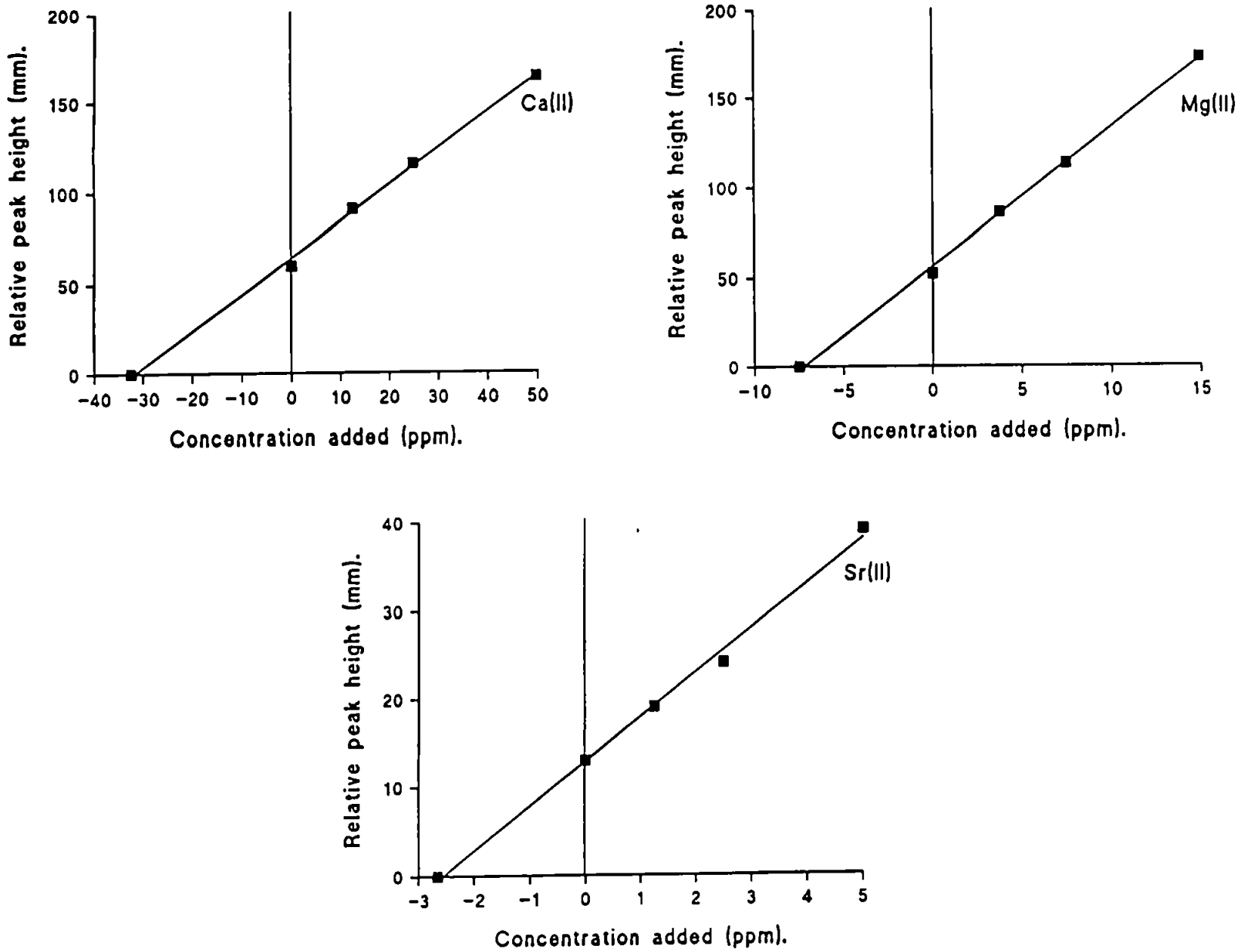


Figure 45. Calibration curves for the standard addition of Ca(II), Mg(II) and Sr(II) to a diluted (Dil.1:100 v/v with DW) oil-well brine.

Sample		Ba(II)	Sr(II)	Mg(II)	Ca(II)
1	HPCIC	2.0 (7.5)	265 (6.0)	750 (7.2)	3250 (6.1)
	ICP-OES	1.95 (1.0)	253 (1.4)	741 (1.0)	2790 (1.0)
2	HPCIC	ND[a]	19 (6.3)	680 (7.4)	350 (6.0)
	ICP-OES	ND[a]	18 (0.6)	750 (0.7)	342 (1.0)
3	HPCIC	ND[a]	31 (6.1)	432 (7.4)	408 (6.1)
	ICP-OES	ND[a]	31 (0.8)	410 (1.0)	348 (1.0)

Values in mg dm^{-3} ($\pm$ s_R).

[a] Below detection limit.

Table 15. Levels of alkaline earth metals determined in three oil-well brine samples using HPCIC compared with results achieved using ICP-OES.

detection limit of the HPCIC technique. For analysis using ICP-OES the three samples were diluted 100 times, this caused the levels of Ba(II) present in samples 2 and 3 to fall below the detection limit of the ICP-OES instrument.

5.4. DETERMINATION OF CALCIUM AND MAGNESIUM IN NATURAL WATERS.

The determination of Ca(II) and Mg(II) in natural waters is frequently monitored for its important role in biological and ecological systems. Usual methods employed for determining the levels of the above two metals in natural and potable waters (the latter being analysed to determine water hardness) often involve titrimetric techniques, which can be time consuming and often inaccurate. A simple, rapid and inexpensive technique has been investigated, designed to accurately determine the concentrations of Ca(II) and Mg(II) in a range of water samples.

The investigation was based upon the combination of the simplistic approach and low-cost benefits associated with flow injection analysis (FIA) and the unique separation capabilities of chelating exchange resins. The separation of the alkaline earth metals achieved using a Calmagite impregnated column is illustrated and discussed in section 3.2.vii. From the investigations into the metal retention characteristics of this particular impregnated chelating dye, a large difference in the retention time of Ca(II) and Mg(II) was noticed. This large degree of resolution between the two metals was achieved using a 10cm Calmagite impregnated column. The ability to separate Ca(II) and Mg(II) using a much shorter Calmagite column, of only 1-2cm, which should produce very little back pressure, would allow the column be used as part of a FIA system for the simultaneous and rapid determination of the two metals. Due to the nature of the peristaltic pumps used in FIA

systems the back pressure of any column used much be kept to a minimum, therefore if high-performance grade material is used, the column must be as short as possible.

A 1.5 x 0.46cm PEEK HPLC mini-column was slurry packed manually, using a micro-spatula, with the same Calmagite impregnated resin used for the separations discussed and illustrated in section 3.2.vii. This mini-column was initially used in the place of the usual 10cm chelating columns, as part of the chelation ion chromatography system shown as Figure 5, to determine if such a short column would still have enough capacity to separate Ca(II) and Mg(II). Figure 46 illustrates the separation of Ca(II) and Mg(II) achieved using the 1.5cm Calmagite impregnated column, using an eluent adjusted to pH 9.4. The chromatogram shows how the two metals are completely baseline resolved and removed from the solvent front. Using a 10cm Calmagite column both Ca(II) and Mg(II) would be retained for well over 20min at this pH. The reduction in capacity caused by shortening the column, approximately 1/7th of the larger column, means the retention times of the two metals are greatly reduced. However the differences in the conditional stability constants of the two metal chelates are great enough for a clear separation to occur.

Although the mini-column illustrated efficient separation characteristics, the combination of the column with a FIA system proved less successful due to the back pressure of the column still being too great for use with peristaltic pumps. However, even though the initial application of this Calmagite impregnated mini-column proved unsuccessful, the column still exhibited several unique characteristics and certain advantages, when used as part of an HPLC system. The easily produced and low cost column is capable of separating Ca(II) and Mg(II) in under 6min. It is also almost totally unaffected by the ionic strength of the sample. The determination of Ca(II) and Mg(II) in tap water using CEC has been carried out by Fritz et al. [170]. The length of the column required to produce a baseline separation in

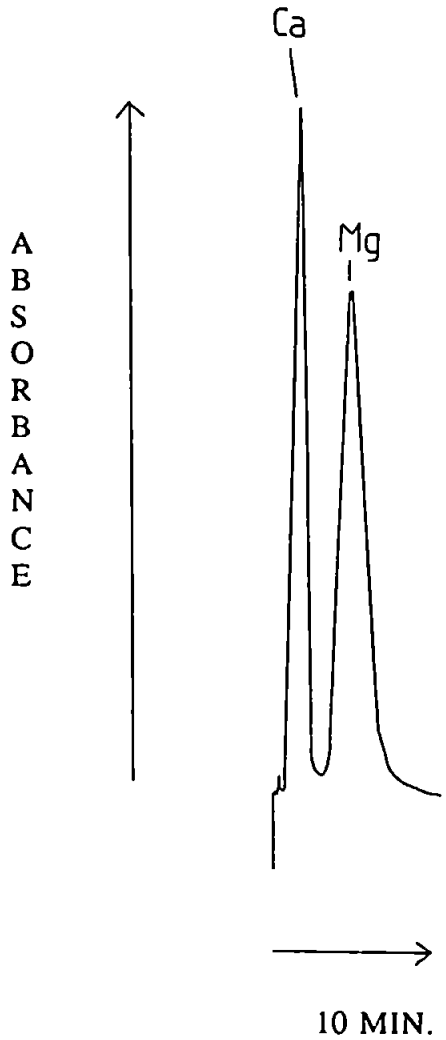


Figure 46. Chromatogram showing the separation of Ca(II) and Mg(II) at pH 9.4 in 1M KNO_3 using a 1.5cm Calmagite impregnated column.

under 5min, removed from the solvent front was 34cm, illustrating the unique separating efficiency of the chelating mini-column.

Several samples were chosen to investigate the potential of this mini-column, namely drinking water, bottled mineral waters and seawater. Figure 47 shows the separations of Ca(II) and Mg(II) in Plymouth tap water and a range of bottled mineral waters. Typical standard calibration curves achieved using the mini-column are shown in Figure 48. Linear regression values of r=0.999 for Ca(II) and r=0.997 for Mg(II) were obtained for standards ranging from 0.5 - 40mg dm^{-3} and 0.5 - 20mg dm^{-3} respectively. The reproducibility of the technique was determined with six repeat injections of a Plymouth tap water sample. Using peak height s_R values of 1.1% for Ca(II) and 1.2% for Mg(II) were calculated.

The separation of Ca(II) and Mg(II) in seawater, diluted 25-fold v/v with DW, is shown in Figure 49. The figure shows how the separation of the two metals is unaffected by the high levels of salt ions present in the sample, $\approx$0.02M. The concentrations calculated for Ca(II) and Mg(II) in all of the previous samples are shown in Table 16. Analysis of the samples using ICP-OES compared very well with the chelation technique for the majority of the samples analysed.

5.5. SUMMARY.

The previous chapter has presented and discussed the results of four applications of dye-impregnated high-performance chelation ion chromatography, in the analysis of various alkaline earth metals in a range of sample types. The advantages HPCIC exhibit in the analysis of these samples over certain other analytical techniques has been demonstrated.

In the determination of Ba(II) and Sr(II) in Ca(II) and Mg(II) containing matrices

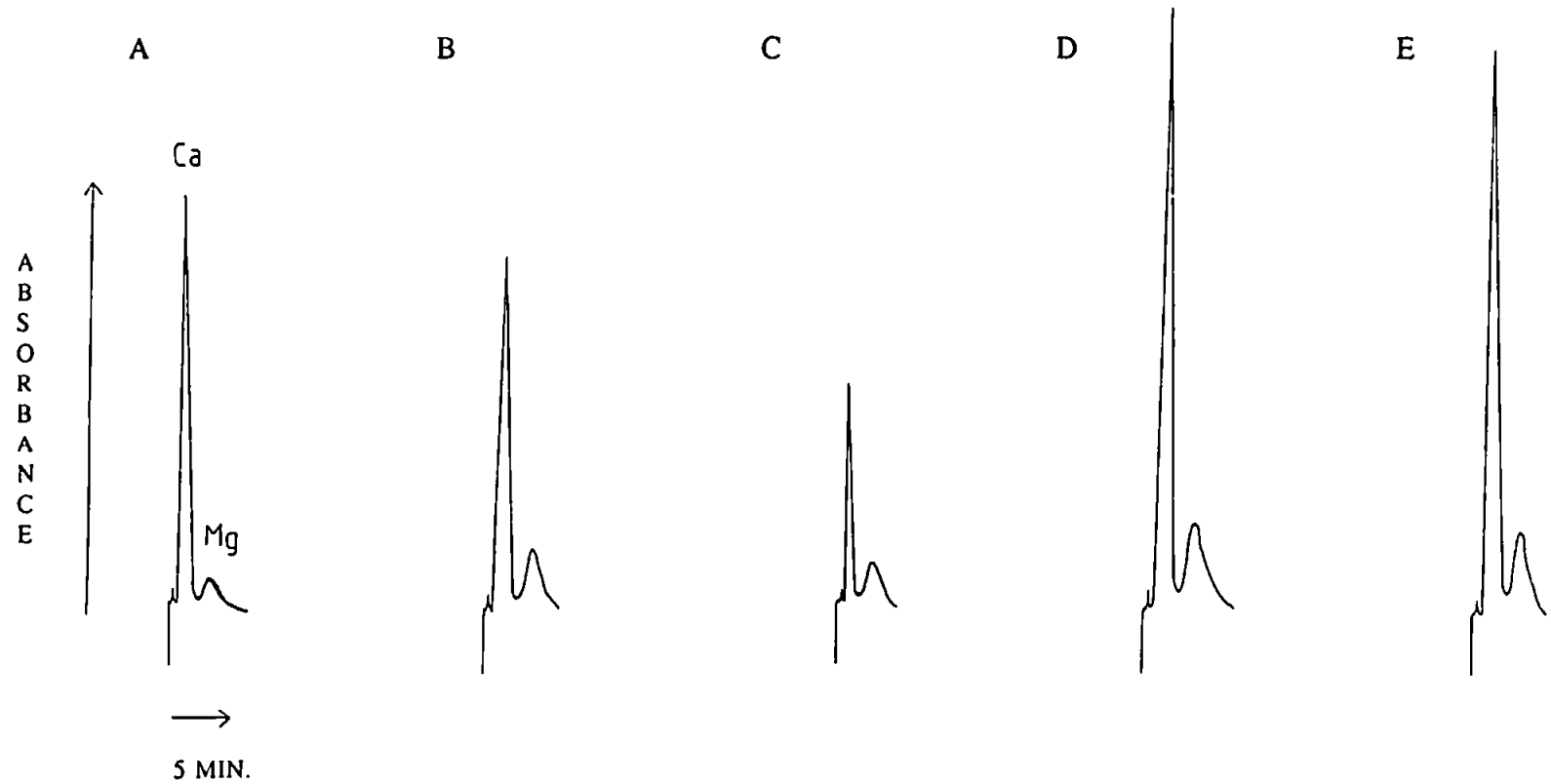


Figure 47. Chromatograms showing the separation of Ca(II) and Mg(II) in A) Plymouth tap water, B) Buxton mineral water, C) Highland Spring mineral water, D) Evian mineral water and E) Monistere mineral water, using a 1.5cm Calmagite impregnated column.

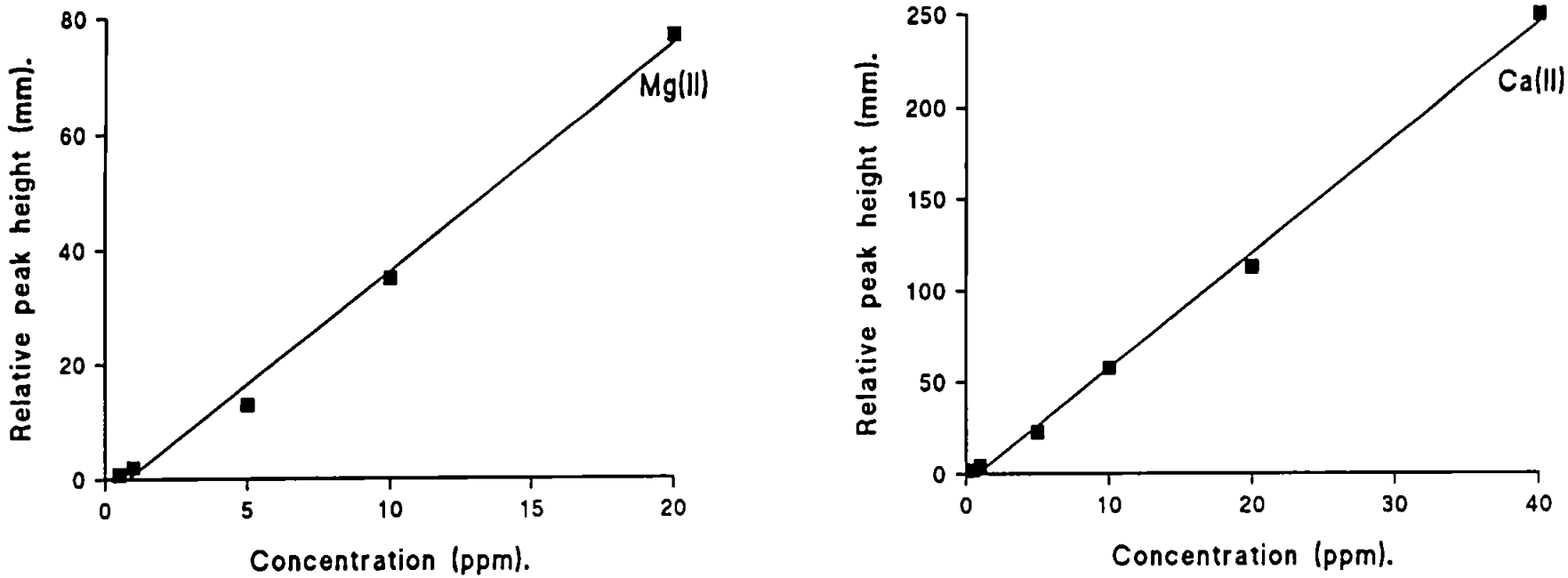


Figure 48. Typical calibration curves for Mg(II) and Ca(II) using a 1.5cm Calmagite impregnated column.

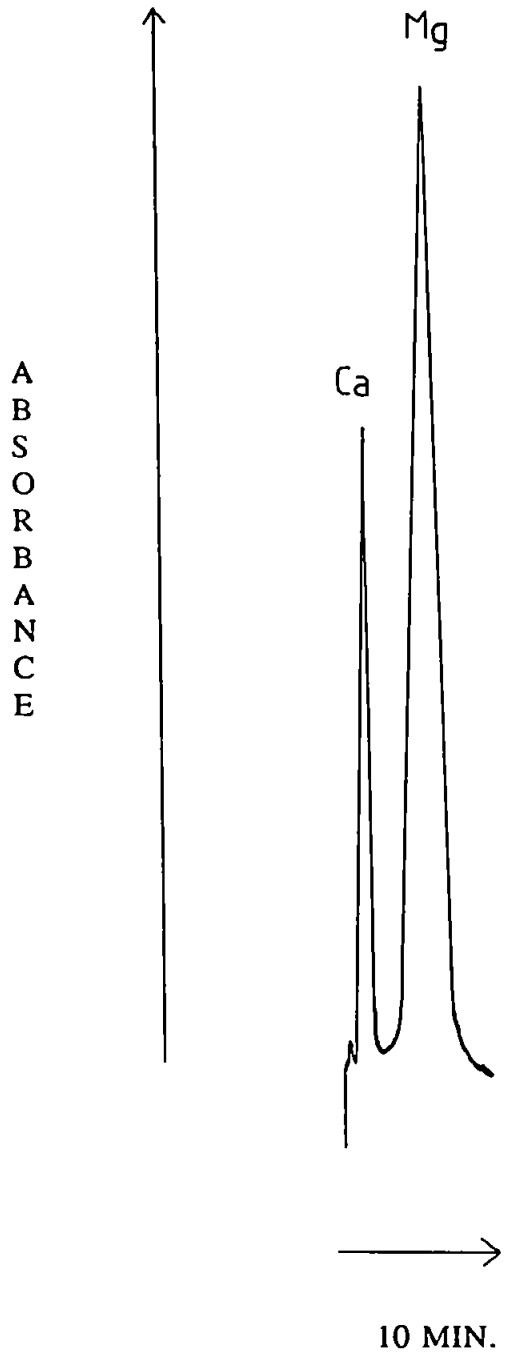


Figure 49. Chromatogram showing the separation of Ca(II) and Mg(II) in seawater (Dil.1:25 v/v with DW) using a 1.5cm Calmagite impregnated column.

Sample	Metal ion	HPCIC	ICP-OES
Tap water	Ca(II)	8.7 (1.1)	9.5 (1.0)
	Mg(II)	1.1 (5.5)	1.2 (0.8)
Seawater	Ca(II)	345 (4.9)	370 (1.2)
	Mg(II)	1125 (2.3)	1190 (0.4)
Highland Spring	Ca(II)	27.0 (1.1)	28.2 (1.0)
	Mg(II)	11.7 (3.4)	12.6 (0.7)
Buxton	Ca(II)	38.5 (1.3)	43.3 (0.9)
	Mg(II)	16.7 (2.9)	16.8 (1.1)
Evian	Ca(II)	57.5 (1.7)	61.5 (0.3)
	Mg(II)	22.3 (3.1)	21.3 (0.9)
Monistere	Ca(II)	61.0 (1.1)	59.5 (0.2)
	Mg(II)	23.0 (3.0)	21.9 (0.4)

Values in mg dm^{-3} ($\pm$ s_R).

Table 16. Levels of Ca(II) and Mg(II) determined in various water samples using HPCIC compared with results achieved using ICP-OES.

HPCIC provides a quick and inexpensive solution to an analytically troublesome matrix. Successful comparison of the results with more complex analytical techniques, such as ICP-OES, illustrates the potential of HPCIC as a valid analytical technique. The production of dye-impregnated chelating columns is relatively simple and a large range of dye types exist which have the potential to be used in such a way.

The analysis of alkaline earth metals in formation water samples is an equally difficult problem due to differences in concentrations of the metals present and the high ionic strength of the sample itself. HPCIC has successfully been applied to this application and resolved the problems which inhibit simple CEC. The need for dilution of the sample could be removed totally with the use of micro-injectors fitted with 0.2-0.5μl internal sample loops.

The separating strength of dye-impregnated resins has been illustrated by the high-performance separation of Ca(II) and Mg(II), using only a 1.5cm chelating column. The ability to successfully determine these metals in a range of samples using such a small amount of dye-impregnated resin again illustrates the efficiency, simplicity and potential of these techniques.

HPCIC also shows a ready adaptability to automated process analysis, which makes the technique a particularly attractive alternative to atomic spectrophotometric methods, particularly for the analysis of alkaline earth metals in complex samples.

CHAPTER 6. CONCLUSIONS AND IDEAS FOR FURTHER WORK.

6.0. CONCLUSIONS.

The previous work has investigated the use of dye impregnated high-performance chelation ion chromatography for the analysis of trace metals in a variety of complex matrices, with very encouraging results. Early investigations into the factors controlling the production of dye impregnated columns have led to the formulation of general guidelines for the production of efficient and reproducible chelating columns. Firstly, the purity of the dyes obtained commercially should be as high as possible, and both liquid chromatographic and capillary electrophoretic techniques have been successfully employed to investigate this for the majority of chelating dyes used in this study. Secondly, the amount of dye impregnated varies with the type of dye used and also with certain resin characteristics, such as pore size and thus surface area. This variation can be as much as ten fold between certain dyes impregnated into similar resins, the highest loadings being achieved with smaller pore size materials, which have greater surface areas. The amount of dye impregnated into the resin has also been increased through optimizing the impregnating procedures. Significant increases in loading, up to 30%, can be achieved through controlling the pH and concentration of the dye solutions. In the case of XO, a minimum amount of impregnated dye was required to be able to fully separate the alkaline earth metals. This was close to 40mg on the PLRP-S 10μm 100Å resin and 30mg for the Dionex 8.8μm 120Å material. Interestingly, the ability to separate a range of transition and heavy metals was less dependent upon the level of impregnated dye, with the lower loaded XO columns exhibiting similar separations to the higher loaded columns.

The above optimised impregnation techniques were used to produce a range of dye

impregnated chelating columns, which exhibited a variety of retention and separation characteristics. The ten chelating columns produced were all unique in terms of active loadings, overall capacity and separating efficiency. All the chelating columns were capable of producing some examples of metal separations, although the quality of these separations varied greatly. As the work progressed it became clear that the range of chelating columns which had been produced and the subsequent variety of separation profiles, which often showed deviations from the general retention order, allowed certain columns to be matched to suit the particular requirements of certain applications.

Some generalisations can be concluded about the chelating columns produced. Firstly, the most efficient separations for the alkaline earth metals were produced using the columns containing the iminodiacetic acid (IDA) functional group. These were the XO, MTB and PP impregnated columns. These columns also produced some of the highest percentage activities, some of the highest overall capacities and exhibited an overall strong chelating nature for the range of metals investigated. For the separation of the transition and heavy metals, some of the weaker chelating columns, with lower capacities, namely CAL, CAS and SPPD, produced separations which exhibited efficiencies similar to those of the IDA containing columns. This is particularly encouraging as these three columns show little affinity for the alkaline earth metals and could therefore be successfully employed for the determination of transition and heavy metals in seawater, with the ability to preconcentrate these metals at the natural pH of seawater without retaining the alkaline earth metals. Separations of several trivalent metals, although showing significant tailing and poor efficiency due to the slow kinetics involved, were possible using some of the weaker natured chelating dyes. The use of a single step gradient allowed the separation of four trivalent metals using the CAS impregnated column.

It is possible to improve on the efficiency of the separations achieved using these chelating columns by impregnating smaller particle size resins and lengthening the columns. This was shown using a PP impregnated 15cm 5μm column which did exhibit sharper peaks, although the improvement was relatively small.

The application of dye impregnated high-performance chelation ion chromatography has been demonstrated with a number of complex sample types. The XO impregnated column was successfully used for the preconcentration and separation of transition and heavy metals at low μg dm^{-3} levels from two samples of coastal seawater. The determination of trace metals in a seawater standard reference material resulted in a good agreement between the certified results and the chelation technique. UV photolysis of a seawater sample increased the response for Cu(II) by up to 40%, which verified the common contention that many chelating techniques only measure the "labile dissolved" fraction of the metals present.

In a similar application a weaker chelating column, impregnated with CAS, was used to determine low μg dm^{-3} levels of Al(III) and Fe(III) in coastal seawater samples. Due to the low solubility of the above two metals, especially Fe(III), problems were experienced obtaining linear calibrations for this particular application although approximate concentrations for the two metals were determined.

A number of applications involving the determination of alkaline earth metals in a variety of matrices was also carried out. The MTB impregnated column was successfully used for the determination of Ba(II) and Sr(II) at sub mg dm^{-3} levels in mineral water samples containing several orders of magnitude higher concentrations of Ca(II) and Mg(II). The method was verified using river water standard reference material and also by comparing results to those achieved using ICP-OES. Both of the above matched closely with results gained using chelation.

The MTB column was also used for the determination of alkaline earth metals in oil-well brine samples. The ratio of these metals in the samples, combined with their high ionic strength, make these samples particularly difficult to analyse. However using the MTB column it was possible to directly determine Ba(II) at a level of 1mg dm^{-3} in a sample containing combined levels of over 2,200mg dm^{-3} Sr(II), Ca(II) and Mg(II) and up to 16,000mg dm^{-3} Na(I). All of the above alkaline earth metals were quantitatively determined in three samples and the results were successfully compared with ICP-OES.

The determination of Sr(II) in milk powder digest, which contained Ca(II) at a concentration ratio of 1,200:1, was achieved using a PP impregnated column. Quantitative results were obtained and once again a good agreement with ICP-OES was possible.

The use of a 1.5cm CAL impregnated column, originally intended for use in a flow injection system, illustrated the separating power of these impregnated chelating resins. The column was applied to the separation and determination of Ca(II) and Mg(II) in a number of natural and potable water samples. The column produced a baseline separation of the two metals in all the samples analysed and as with the other alkaline earth applications compared well with ICP-OES.

The work detailed in this study has involved the investigation into, and production of a number of chelating dye impregnated chelating columns capable of both metal preconcentration and high-performance separations. After column conditioning the majority of these columns have remained stable under the working conditions described, without visible signs of deterioration or loss of efficiency. These columns have been very successfully applied to a diverse range of complicated samples for the determination of a variety of trace metals. An advantage of the dye impregnation approach is the large number of dyes which can be used in this way. This study has shown the potential of a small number and many

more exist which could form the basis of substantial further studies. The following are some ideas for further investigations within this area.

6.1. FURTHER WORK.

As mentioned above a large number of dyes are available for further studies, however a particular class of chelating dyes which were not studied in this work were those dyes containing sulphur ligating atoms. These dyes could be used for the separation of certain soft metals, such as Hg(II), Ag(I) or Au(III). An interest exists in the determination of Hg(II) in environmental samples, particularly seawater. Dye impregnated chelating columns could be developed for this purpose. Further investigation is also needed into chelating dyes which are suitable for a more efficient separations of trivalent metals.

The seawater studies carried out in this program of work could be improved in several key areas. The use of a totally inert system would reduce the contribution to blank levels from the metal components and allow lower detection limits. Detection limits could also be reduced by both increasing the preconcentration volumes, which would not increase the blank levels, and the continuing development of more sensitive post column reagents. An inert gradient pump could be employed to remove the need for step gradient elution and also deliver more accurate preconcentration volumes, which would improve the reproducibility of the technique. The use of a chelating column which exhibits a greater resolution between Mn(II), Cd(II) and Zn(II), may allow the first two metals to be determined in seawater samples, although the chelation of Cd(II) and the effect of certain chloro species needs to be fully understood. Other speciation studies require further investigation, particularly organo-Cu(II) complexes. The use of on-line UV photolysis would allow a more detailed comparison

of total and "labile dissolved" metal concentrations.

A number of applications have been suggested to which high-performance chelation ion chromatography may offer some advantages over presently used techniques. The first of these is preconcentration of trace levels of metals in groundwater. The presence of higher levels of Ca(II) and Mg(II) in these waters leads to the overloading of ion exchange resins used to preconcentrate the analyte metals. Chelating dyes which show little affinity for Ca(II) and Mg(II) would remove this problem and the preconcentrated metals could be swept off and determined in a similar way to the seawater studies. The second possibility is the use of chelating dye impregnated resins for the separation of Sr(II) from Ca(II), with the aim of isolating the Sr(II) peak for isotope studies using mass spectrometry. Wine has been suggested as one matrix where this type of separation is required to identify the source of the grape used in its production.

Other possible applications include the determination of trace metals in the presence of massively higher levels of other metals, ie. trace metal impurities in copper or iron alloys. Copper for example, could be retained upon the column while certain metal impurities present could be separated and determined, with the copper being swept off afterwards.

These chelating columns could also be used for the monitoring of trace metals in salts, such as CsI, which is needed in a very pure form for liquid crystal manufacture. The determination of metals in salt solutions in general is also a potential use of high-performance chelating columns. The on-line monitoring of trace metals in high ionic strength industrial effluents would be relatively simple using chelation ion exchange chromatography and would provide a method for determining the amount of trace metals being released into the environment.

High-performance chelation ion chromatography is a simple and inexpensive

technique for the determination of trace metals. Although this study has illustrated the numerous benefits of this approach to certain analytically challenging problems, the potential exists both for improvements in the separation and determination of these metals and in the development of many new applications.

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PRESENTATIONS AND CONFERENCES ATTENDED.

Oral presentation entitled "The determination of trace metals in complex matrices using high-performance chelation ion chromatography" presented at "Research and Development Topics in Analytical Chemistry" meeting held at the University of Hertfordshire on July the 18th and 19th 1994.

Poster presentation entitled "Determination of trace metals in high ionic strength samples using high-performance chelation ion chromatography" presented at the "24th International Symposium on Environmental Analytical Chemistry" meeting held at Charlton University, Ottawa, Canada, on May the 16th to 19th 1994.

Poster presentation entitled "Determination of trace metals in high ionic strength samples using dye-coated chelation ion chromatography" presented at "Research and Development Topics in Analytical Chemistry" meeting held at the University of Bradford on July 13th and 14th 1993.

Attended "Research and Development Topics in Analytical Chemistry" meeting held at the University of Birmingham on July 7th and 8th 1992.

Attended and presented current research at weekly departmental research seminars at the University of Plymouth.

PUBLICATIONS.

Study of aluminium speciation in potable waters from the southwest of England using ion chromatography. Phil Jones and Brett Paull, Analytical Proceedings, 1992, 29, 402-404.

High-performance chelation ion chromatographic determination of trace metals in coastal sea-water using dye-impregnated resins. B.Paull, M.Foulkes and P.Jones, The Analyst, 1994, 119, 937-941.

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