INVESTIGATION OF HIGH PERFORMANCE CHELATION ION CHROMATOGRAPHY FOR TRACE METAL DETERMINATIONS.

by

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ABSTRACT.

INVESTIGATION OF HIGH PERFORMANCE CHELATION ION CHROMATOGRAPHY FOR TRACE METAL DETERMINATIONS.

by Owen Challenger BSc (Hons), GRSC.

A high performance chelation ion chromatography system using chelating dye-coated columns for the determination of trace metals in several high ionic strength matrices is described. Spectrophotometric detection using 4-(2-pyridylazo)resorcinol (PAR) / zinc-EDTA and Pyrocatechol Violet post-column reagents was employed for the detection of divalent and trivalent metal ions respectively.

Initial investigations using various large particle size dye-coated columns showed that selective adsorption of metal ions was possible, with chelating ability dependent on substrate type and chelating dye. To obtain highly efficient columns capable of separating groups of metal ions, Xylenol Orange and Chrome Azurol S were coated onto a high performance (8μm) substrate. These two columns were distinctly different, with Xylenol Orange showing much stronger retention properties than Chrome Azurol S. Isocratic separations of alkaline earth, transition, heavy metals and trivalent metal ions were achieved. The efficiency of these dye-coated columns approached that of the commercial TOSOH chelating column also studied for comparison.

Several gradient and pH step gradient elution programmes were developed to enable the separation of a larger number of metal ions, where up to nine metals could be separated in a single injection. To determine trace metals at low ppb levels, combined preconcentration and separation was successfully achieved using a single column. Highly efficient separations were obtained. Using the Xylenol Orange column, quantitative studies involved the analysis of saturated sodium chloride (5.1M) and potassium chloride brines for alkaline earth and transition metals. Calibrations proved to be linear and reproducible with detection down to low ppb levels for 10cm^3 preconcentration volume. Further work involving the analysis of both synthetic and actual sea water samples, together with some laboratory chemicals, was also carried out and gave very good results for selected transition metals.

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CHAPTER ONE.

INTRODUCTION.

1.1. TRACE METAL ANALYSIS.

There is an increasing need to analyze for trace metals present in a wide range of samples. Trace levels of metals are normally considered to be less than 0.01% in concentration. In industry, trace metal levels in brine process streams, nuclear fuel reprocessing streams, plating baths, alloys and semi-conductors etc., can greatly affect the cost of production and purity of the final product. Foodstuffs such as milk, wine and fruit juices contain trace metals, as can biological fluids such as blood, and it is extremely important to be able to monitor these types of sample, particularly for toxic metals. In addition, there is an ever increasing awareness of the effects of often long term pollution of the environment. Many trace metals are toxic and their effect can be devastating. One example is the pollution of natural waters which can have a severe effect on aquatic life. Toxic metals can ultimately enter the food chain. Industrial effluents also need to be monitored for trace metals, as well as determining trace metals in environmental samples such as natural waters, rain, soil and rocks.

The determination of metal ions in solution is a common problem, and until fairly recently the analysis has principally been carried out using atomic absorption spectrometry. This has a major disadvantage of only being capable of single element determinations, and on its own cannot be used for speciation studies. More recent advances in instrumentation such as inductively coupled plasma-mass spectrometry (ICP-MS) can achieve multi-element determinations with high cost, but still not speciation information.

Chromatography, particularly liquid chromatography, is very versatile for the determination, separation and speciation of metal ions. High performance liquid chromatography (HPLC) is an inexpensive and relatively simple method of analysis, and lends itself easily to automation and continuous on-line monitoring. A review of the determination of trace metals by chromatography is given by Nickless (1), and a recent review by Robards et al. (2), highlights the advances in metal determination and speciation using liquid chromatographic methods over the past decade. There are several types of HPLC approach including the reverse phase of preformed chelates or ion exchange, now called ion chromatography.

1.2. CHROMATOGRAPHIC PARAMETERS.

There are several fundamental parameters and factors which are useful in chromatography to determine the performance of chromatographic columns and for comparison purposes. Some of these are outlined below, but a more detailed description can be found in chromatography books such as Hamilton (3) and Simpson (4).

Chromatography involves the separation of components in a sample by their distribution between two phases, a mobile phase and stationary phase. There are four modes of liquid chromatography, these being adsorption, partition, gel permeation and ion-exchange. Ion-exchange is principally used.

A fundamental parameter is the partition coefficient denoted by K, which gives the ratio of the concentration of solute in the stationary and mobile phase :-

$$K = \frac{C_S}{C_M}$$

where C_S=concentration of solute in the stationary phase, and C_M=concentration of solute in the mobile phase. During a chromatographic separation, different components of a mixture will have different partition coefficients, and are thus retained to varying degrees. The time taken for a component to elute to its maximum concentration is known as the retention time, denoted by t_R. This is illustrated in Figure 1.

A chromatographic column may be considered to compose of a series of narrow horizontal layers known as theoretical plates. For a solute passing through the column, there is an equilibrium of solute between the mobile and stationary phase at each plate. The number of theoretical plates is related to the column efficiency, where efficiency increases with an increasing number of theoretical plates. Overall, a more efficient column will have better separating power. The number of theoretical plates is denoted by N, which is a measure of column efficiency :-

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

where L=length of column packing, and H=height equilivalent of theoretical plate. Experimentally,

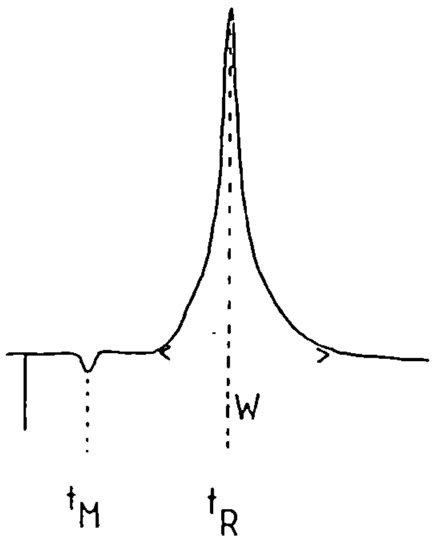
$$N = 16\left(\frac{t_R}{W}\right)^2$$

where W=peak width.

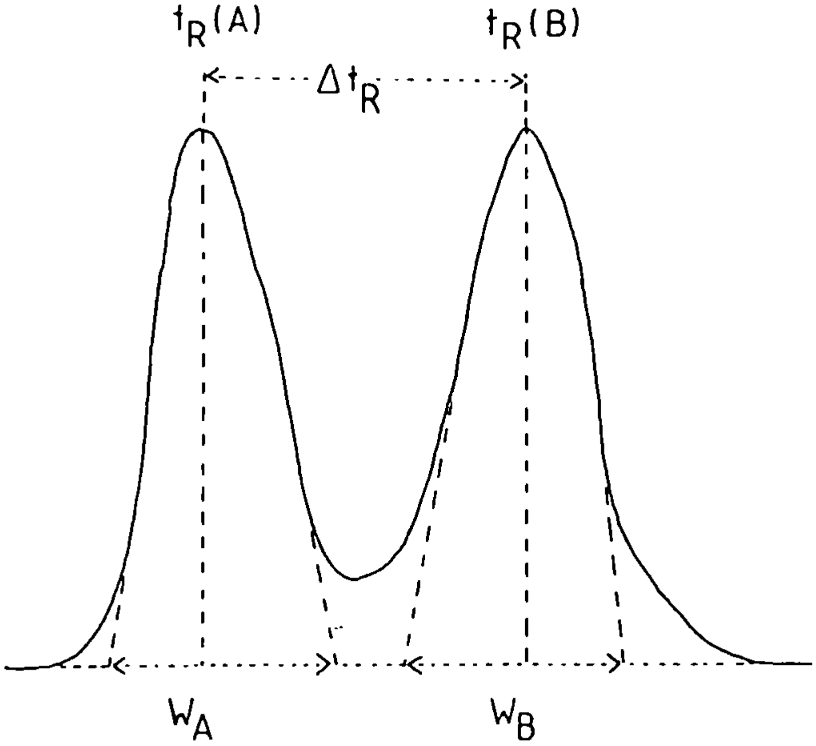
Sometimes it can be difficult to resolve two components, and the resolution is a measure of the ability of a column to resolve two solutes. It is denoted by R_S, and is experimentally defined as the difference in retention times of two peaks, divided by the average peak width :-

$$R_S = \frac{2(t_B - t_A)}{W_A + W_B}$$

This is illustrated in Figure 1.



RETENTION TIME.



RESOLUTION.

Figure 1. Illustration of parameters for retention time and resolution.

An important constant, which is related to the migration rate of the solute, is the capacity factor, denoted by k'. Experimentally,

$$k' = \frac{t_R - t_M}{t_M}$$

The selectivity factor, denoted by α, is the relative magnitude of partition coefficients for two solutes, for example, A and B, where,

$$\alpha = \frac{K_B}{K_A}$$

K_B is the partition coefficient for the more strongly retained solute, and K_A the solute which is less strongly held, and thus moves more rapidly through the column. Experimentally,

$$\alpha = \frac{(t_R)_B - t_M}{(t_R)_A - t_M}$$

1.3. ION CHROMATOGRAPHY.

Ion chromatography (IC) has become the most common HPLC technique for the analysis of metal ions in solution. Ion-exchange has been used since the 1950's for the separation of organic and inorganic substances and in biological analysis, for example, the separation of amino acids. The term ion chromatography was originally used for anion determinations using HPLC type equipment, but now this term refers to the liquid chromatography of all types of ions using high performance substrates. Ion chromatography basically involves an exchange of ions between a stationary and mobile phase.

1.3.1. PRINCIPLES OF ION CHROMATOGRAPHY.

Ion chromatography basically involves an exchange of ions on the stationary phase. Ion-exchange can occur naturally on materials such as clays and zeolites, but for HPLC the stationary phase is mainly either silica or polystyrene polymer to which various ionic functional groups are attached by covalent bonding. In general, there are four distinct types of ion-exchange resins, strong and weak cation-exchange, and strong and weak anion-exchange. Cation-exchange resins contain acidic functional groups, which can either be strong or weak. Strong cation-exchange resins contain fully ionized groups such as sulphonic acid, SO_3^-, whilst weak cation-exchange resins contain only partially ionized functional groups such as carboxylic acid, COO^-, and phosphate, PO_4^{3-}. The protons in these groups can exchange with other cations in the mobile phase :-

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

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

where Res=Resin, and n=charge of the metal cation, M.

Anion-exchange resins contain basic functional groups, which can also be strong or weak. A strong anion-exchange resin can contain for example, quaternary ammonium functional groups, whilst a weak anion-exchange resin can contain amine functional groups :-

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

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

where Res=Resin, and n=charge of the anion, A.

Strong ion-exchangers compared with weak ion-exchangers tend to be less influenced by pH.

When considering cation-exchange, monovalent cations such as the alkali metals are attracted to ion-exchange sites less strongly than divalent metal cations, which are held less strongly in comparison with trivalent cations. This is related to the size and charge of the cation. In aqueous solution cations are hydrated, and surrounded by water molecules. The strength of attraction between these water molecules and the metal cation increases with increasing charge density, so that small highly charged cations are more strongly hydrated. Within a group of metal cations with the same charge, for example, the alkali metals, the larger cations have a lower charge density, so are less hydrated in solution. This means that the change in hydrated radii is opposite to that of the bare ions. These are attracted more strongly to ion-exchange sites on a resin, so the elution order for alkali metals is Li^+, Na^+, K^+, Rb^+, with Cs^+ eluting last.

For certain metal cations the addition of complexing groups to the mobile phase speeds up the elution process and increases selectivity. These groups act as competing ligands, by forming complexes with the metal cations. Lactic acid is a good example, however, the proportion of the ionized form has to be quite high to complex with a metal ion and this occurs between pH3 and pH4. Thus retention can be controlled by varying the pH, concentration of complexing agent or by using a different complexing acid, for example, tartaric acid which forms stronger complexes than lactic acid.

1.3.2. ADVANCES IN ION CHROMATOGRAPHY.

Originally ion-exchange involved large particle size resins packed into glass columns, and the mobile phase flowed through by gravity. Detection involved the manual collection of various fractions. In the late 1960's and early 1970's there was a period of rapid development. The eluent was forced through the column under pressure and small particle size substrates of uniform particle size were developed, enabling a much more efficient column to be produced. Automatic detectors, principally on-line conductivity detectors were also introduced. These developments enabled a constant eluent flow rate using a small volume of sample, leading to an overall faster analysis time. Small has recently published a review (5) containing an historical account of the early developments of ion chromatography and conductimetric detection. Early ion chromatographic separations of metal ions with conductivity detection, involved a suppressor column to eliminate the background conductance of the eluent. This provided a sensitive method for detection of cations and anions.

Ion chromatography was first demonstrated by Small et al. (6) in 1975, where a rapid separation of cations including alkali metals, divalent metals, organic amines and quaternary ammonium compounds, was achieved. Anion separations including halides and chloroacetates were also achieved. Although conductimetric detection was principally used, other detection systems including post-column detection using colorimetric reagents, and coulometric detection were developed. Ion-exchange substrates were based on polystyrene-divinylbenzene where for example, this base material could be sulphonated to produce a strong cation-exchange resin. Fritz and Story (7) demonstrated the separation of various divalent metal ions, together with a separation of zirconium and lanthanum from thorium, using forced flow chromatography and colorimetric detection involving a post-column reagent. The substrate used for the separation was a low capacity, partially sulphonated macroporous cation-exchange resin. Takata and Fujita (8) demonstrated the high speed separation of six heavy metal ions using a coulometric detector, and found that for a high resolution separation, a higher concentration of eluent and a longer column were required. Arguello and Fritz (9) separated calcium and magnesium together with several other divalent metal ions, on a low capacity macroporous resin, similar to that used earlier (7). They investigated several colorimetric reagents for automatic post-column detection, which provided an easier and simpler detection method than suppressed conductimetric detection.

Further advances occurred when Elchuk and Cassidy (10) separated fourteen lanthanides, using several high performance commercial bonded phase packings (5 and 10μm particle size), with post-column detection. These separations were achieved in less than twenty minutes and on-line preconcentration proved to be successful with some of the columns. A comparison was made with conventional polystyrene-divinylbenzene ion-exchange resins, and generally ion-exchange silica substrates were advantageous, but they had a low capacity. Small and Miller (11) introduced indirect photometric chromatography, where the sample ions do not absorb light, but the eluent ions do at the monitoring wavelength. Several anions together with mono and divalent metal ions were separated, and detected as troughs in the baseline.

Improvements in detection have led to the more widespread use of ion chromatography for trace metal analysis, particularly now more highly efficient packings have become commercially available. Several divalent metals were separated by Jones et. al. (12,13), using inverse photometric detection. The determination of trace metals in permanent magnetic alloy and monel alloy were successfully achieved (12). Chromatography was improved by the use of polyfunctional carboxylic acids, such as lactic and tartaric acids in the eluent. This shortened the retention times and improved resolution. Separation depended upon the stability of the various metal complexes formed, and also the concentration in the eluent. Five metal cations were separated on a low capacity column using a lactate eluent, and a separation of nine metal ions using a tartrate eluent on a high capacity column (13). Here, these systems were applied to the determination of cobalt in simulated pressurised water reactor primary coolant. An on-line preconcentration column was later used to increase sensitivity, enabling detection to the sub-ppb level (14). Yan and Schwedt (15) successfully separated thirteen metal ions within thirty-four minutes, using complexing mixtures of ethylenediamine and tartaric, oxalic and citric acids as eluents, and applied this to trace metal determination in red wine and oatmeal.

Ion chromatography has increasingly been used for the determination of a wide range of metal ions in a variety of samples. Metal cations and anions can be determined simultaneously using ion chromatography and some metals can be detected as anions or complexes. This is particularly useful where the simple metal cation is not stable, and principally forms an anionic or complex species in solution. Some examples of trace metal determinations by ion chromatography are shown in Table 1.

Determination of trace metals in complex matrices such as sea waters and brines by ion chromatography is difficult due to the high salt content. Samples can be diluted before analysis but this could lead to errors during dilution and mixing, as brines are denser than water. Marr (16) has recently analyzed sea water and oil well brines (formation waters), for the determination of sulphate using a standard ion chromatography system, and involved a hundred-fold dilution of the sample. Concentrated brines from the chloralkali industry have been investigated for alkaline earth metals by Handley et al. (17). Here, a chelating ion exchange column was used for preconcentration and matrix elimination before separation of trace metals by conventional ion chromatography. This proved to be successful for on-line analysis of concentrated brines. This

METAL IONS.	EXPERIMENTAL CONDITIONS.	RESULTS / APPLICATIONS.	REFERENCE.
11 common heavy metals and alkaline earth metals.	Tartaric acid eluent. Pre-column concentration.	Complete separation in 24 minutes. Detection limit sub-ppt (ngl^{-1}). Applied to demineralised water.	Yan and Schwedt (18).
Alkaline earth, d-block, trivalent and lanthanide metals.	Chemiluminescence detection.	Determination of cobalt in simulated pressurised water reactant primary coolant with detection limit 0.5ppt. Detection limits from 2 to 100ppb for other metal ions.	Jones et al. (19,20).
Alkaline earth and transition metals.	Ethylenediamine / tartaric acid or α-hydroxyisobutyric acid eluent (HIBA). Post-column detection using Eriochrome Black T - MgEDTA.	Analysis of multivitamin tablet.	Bowles et al. (21).
Transition metals, lanthanides and actinides.	Mandelic acid eluent.	Dynamic ion-exchange process. Many multi-element separations.	Elchuk et al. (22) .

Table 1. Examples of trace metal determinations in various samples by ion chromatography.

METAL IONS.	EXPERIMENTAL CONDITIONS.	RESULTS / APPLICATIONS.	REFERENCE.
Rare earth elements.	Cation-exchange microcolumn. Spectrophotometric detection. Micro-HPLC technique.	Separation of 15 rare earth elements.	Hirose et al. (23).
Thorium in plutonium.	Hydroxyisobutyric acid (HIBA) eluent.	Other actinide impurities in eluent and possible interferences separated.	Hamilton et al. (24).
Major and minor constituents of steels.	Post-column detection with PAR or Arsenazo III.	Minor trace constituents of a low alloy steel sample e.g. cerium and zirconium. Minor trace constituents of a stainless steel sample e.g. tantalum and niobium.	Saraswati et al. (25,26).

Table 1 continued.

METAL IONS.	EXPERIMENTAL CONDITIONS.	RESULTS / APPLICATIONS.	REFERENCE.
Many trace metals.	Post-column detection. Dionex ion-exchange columns.	Analysis of natural lakewater, biological fluid, lanthanides, lead in beer, and the determination of chromium (III), gallium (III) and mercury (II).	Heberling and Riviello (27).
Trace metals in the environment.	Dionex ion-exchange and chelation column.	Chromium (VI) in wastewaters. Trace metals in sea waters with ICP detection.	Joyce and Schein (28).

Table 1 continued.

IONS.	EXPERIMENTAL CONDITIONS.	RESULTS / APPLICATIONS.	REFERENCE.
Cyanide, transition metals and lanthanides.	Two slightly different separation systems. A complimentary technique.	Separation of 9 transition metals. 14 lanthanides separated in less than 30 minutes.	Wang et al. (29).
Transition metals, chloride, sulphate and nitrate.	Two columns and a suppressor column to remove transition metals after detection and before anion determination.	Multi-vitamin extracts.	Jones and Tartar (30).
Inorganic and organic anions and alkaline earth metals.	Single anion-exchange column (silica based).	Mineral water, brine and spinach juice.	Yan and Schwedt (31).

Table 1 continued.

METAL SPECIES.	EXPERIMENTAL CONDITIONS.	RESULTS / APPLICATIONS.	REFERENCE.
Chloro-complexes of noble metals.	Silica anion-exchange resin.	Separation of platinum (IV), iridium (IV) and osmium (IV) With ion-interaction, only palladium (IV) and gold (III) gave stable chromatography. Rhenium (III) and ruthenium (III) were unstable.	Jones and Schwedt (32).
Oxo-anions of arsenic (V), germanium (IV), phosphorus (V) and silicon (IV).	Anion-exchange columns. Molybdenum blue post-column detector.	Rapid separation of germanium (IV), phosphorus (V) and arsenic (V).	Jones et al. (33).

Table 1 continued.

two column system was further developed to determine on-line, aluminium and zinc (34), as well as alkaline earth metals in concentrated brines. The use of a chelating exchange column avoided the need for sample dilution, and preconcentration enabled lower detection limits to be obtained. Chelating exchange has many advantages in the analysis of complex matrices, and ultimately can be used for trace metal separation and determination, which will be discussed in detail later (Section 1.5.).

1.3.2.1. SPECIATION STUDIES BY ION CHROMATOGRAPHY.

Ion chromatography can also be used for metal speciation studies, which involves the separation of distinctly different chemical forms of a particular metal. Trace metal speciation is extremely important, particularly in environmental studies, e.g. natural and potable waters, since different metal species can vary greatly in toxicity. Heberling and Riviello (27) have shown that vanadium(IV) and (V), and tin(II) and (IV) can be separated using commercial ion-exchange columns. Williams et al. (35) have separated and determined chromium(III) and (VI) at ultra-trace levels using a chemiluminescence detection system. Chromium(VI) is a much more toxic species than chromium(III) in the environment. This system proved to be simple, involving no sample pretreatment. It was highly sensitive with detection limits being 0.1ppb for chromium(III) and 0.3ppb for chromium(VI).

An ion chromatography system to detect aluminium using fluorescence detection was investigated by Jones et al. (36), and enabled sensitive detection of aluminium in monel alloy and tap water. The system was later developed to speciate aluminium, and an intensive study of aluminium species in natural and potable waters was carried out (37). Separation of inorganic (Al^{3+}) and organic aluminium, together with various hydroxy and fluoro-species of aluminium were obtained. An important finding is that there was a major change in aluminium speciation after passage through a potable water treatment plant, which involves addition of aluminium sulphate, suggesting that there was supersaturation of inorganic aluminium in drinking water. The separation of fluoro-species provides useful information as drinking waters are fluorinated in some areas of the UK. Further study of aluminium speciation in potable waters from the south west of England by Jones and Paull (38), concluded that soft waters contain higher concentrations of hydroxy, $Al(OH)_x^{(3-x)}$ species compared to harder water. Untreated water contained mainly organically bound aluminium. Further examples of metal speciation studies by ion chromatography in a wide range of samples are presented in a review by Robards et al. (2).

1.4. TYPES OF SUBSTRATE.

The substrate or stationary phase is the most important part of a chromatographic system, and determines the separation. The most common substrate for ion-exchange has been polystyrene or polystyrene-divinylbenzene resins, which have been used for several decades. Silica is not very suitable as a base material for ion-exchange as it dissolves above pH8.

Some of the earlier polystyrene-divinylbenzene co-polymer beads had the disadvantage of swelling when in solution, which severely affected the efficiency of a column. These tended to be lightly cross-linked, and collapsed under high pressure as they are easily compressed. In general, for use in HPLC, the degree of cross-linking in the polymeric beads has to be greater than 8%. A review of the earlier developments of stationary phases for ion chromatography is given by Small (5).

There are three general types of polymeric substrates. Microporous resin particles contain small molecular size pores and are cross-linked. However, a major disadvantage is that a drastic change in the eluent will reduce the chromatographic performance, as the bead diameter will change significantly. The shrinking and swelling of the beads can be overcome by using a macroporous resin, which is highly cross-linked. These contain macropores (over 300Å in diameter), as well as micropores, where the macropores are accessible to large molecules. Another type is a pellicular resin. This consists of an inert core surrounded by a stationary phase film. The core is spherical, and typically 10μm in diameter, composed of glass or polymer. The surface film can be based on for example, of silica, alumina or ion-exchange coating.

Polystyrene-divinylbenzene resins are prepared by suspension polymerization of styrene to produce small spherical particles of polystyrene. Varying concentrations of divinylbenzene, depending on the final requirement, are added to provide cross-linking between the polystyrene chains. Macroporous resins are prepared in a similar way. A typical reaction mixture contains polystyrene monomers, cross-linking divinylbenzene monomer, a polymerization initiator and a suitable porogen in a water organic solution. The porogen, which is a compound soluble in monomer but insoluble in polymer, can be varied depending on the required resin structure. The reaction mixture is stirred rapidly with water, and gradually droplets of the required particle size form in the organic phase. Polymerization occurs in the organic phase, where polymer chains precipitate out within the droplet. Ultimately, this produces a rigid 3-D structure with the cross-linking monomer, where the porogen voids produce the macropores. After drying, the resin can be accurately sorted into various particle sizes.

A range of macroporous co-polymers are now commercially available, for example, a commercial UK company, Polymer Laboratories have developed rigid macroporous co-polymers of styrene-divinylbenzene in a range of pore sizes. Lloyd (39) has recently produced a review of these substrates. They show high mechanical stability, high efficiency and can operate at high pressures and flow rates. The unmodified resin is produced in pore sizes of 100Å, 300Å, 1000Å and 4000Å. These are important for bioseparations for example, the small 100Å pore size substrate can be used for fingerprint peptide mapping, and the very large 4000Å pore size substrate for biological macromolecule separations. These substrates tend to have a much higher surface area than the equivalent silica packing materials, and contain micropores of less than 40Å diameter, which is suggested, are present in the network of cross-linked polymer chains surrounding the macropores (39). Surface area decreases with increasing pore size, as can be seen in Table 2. The PLRP-S 100Å substrate with a particle size of 8μm has been used as a base material for this study (see Section 3.). This substrate can be modified in a similar way to silica, by derivatization or by coating to produce ion-exchange resins which prove to be as stable as the unmodified substrate.

1.5. CHELATION ION CHROMATOGRAPHY.

The development of ion chromatography for the analysis of trace metals as described above has enabled high performance separations and determination of many groups of metal ions to be achieved. Although ion chromatography has been applied to the analysis of a wide range of samples, the technique is

ADSORBENT.	PORE SIZE. (Å)	SURFACE AREA. (m^2g)
PLRP-S	100	414
PLRP-S	300	384
PLRP-S	1000	267
PLRP-S	4000	139

Table 2. Pore size and surface area of Polymer Laboratories reverse phase unmodified PS-DVB substrate, determined by nitrogen adsorption isotherms (39).

generally limited to samples of low ionic strength. Many important environmental and industrial samples such as sea waters and industrial process streams are of high ionic strength and direct analysis by conventional ion chromatography becomes very difficult, as the ion-exchange sites become 'swamped' with salt ions. This can be overcome by using chelating exchange groups on the stationary phase substrate, instead of ion-exchange groups. Metal ion separations are then much less affected by ionic strength, and depend mainly on the conditional stability constants of each metal chelate, as discussed in the next section.

1.5.1. THEORETICAL ASPECTS OF CHELATING ION EXCHANGE.

Co-ordination chemistry is the branch of chemistry involving the formation of metal complexes. A metal complex is formed by an association between a metal ion and another species, called a ligand, which can be an anion or a polar molecule. A ligand is described by Lewis as an electron pair donor, and a metal ion as an electron pair acceptor. There is a tendency for positive ions such as metal ions to seek areas of high electron density, such as electron pairs. Metal ions therefore can be considered as Lewis acids, and the ligand as a Lewis base.

If a metal ion M^{m+}, approaches close enough to a ligand L^-, then a co-ordinate bond is established, and a metal complex is formed. For a number of ligands nL^- we have :-

$$M^{m+} + nL^- \rightleftharpoons [ML_n]^{(m-n)+}$$

Lewis Lewis Complex
Acid Base

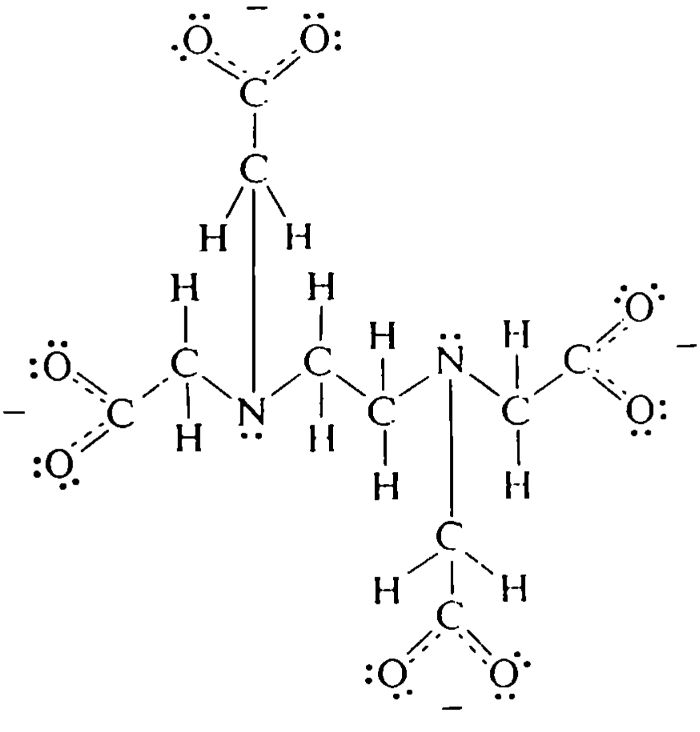
There are essentially two extremes of acid/base behaviour, 'hard' and 'soft'. A 'hard' acid or base retains valence electrons very strongly, whilst 'soft' acids or bases are generally large and easily polarised, with the electrons not being held very strongly. Examples of 'hard' acids include H^+, and hard bases NH_3, H_2O and SO_4^{2-}. Soft acids include copper and zinc cations, Ag^+, Au^+, Cd^{2+}, Hg^{2+}, and ligands containing phosphorus and sulphur donor atoms are examples of soft bases. However, some metals including many of the transition and alkaline earth metals show intermediate behaviour. In general, the strongest complexes are formed between metals and ligands of similar hardness or softness. However, there are a large number of acids and bases of intermediate hardness and softness, such as some transition metal cations, so prediction of complex stability is not quite so straightforward.

The number of ligands associated with a central metal ion is called the co-ordination number. Common co-ordination numbers are four, which generally give tetrahedral complexes, for example, $[Zn(NH_3)_4]^{2+}$, and six which generally give octahedral complexes, for example, $[Co(NH_3)_6]^{2+}$. The number of electron pairs that are donated classifies the type of ligand. Above, NH_3 donates one electron pair and is classified as a monodentate ligand, further examples being NO_3^-, SO_4^{2-}, H_2O, CN^- and OH^-. Ligands which donate two or three electron pairs are termed bidentate and tridentate respectively. In general, ligands which donate more than one electron pair are termed multidentate ligands. Multidentate ligands which donate more than one electron pair to the same central metal ion form a cyclic or ring structure. These type of complexes are termed chelates, and the process called chelation. An example is ethylenediamine which is a bidentate chelating ligand and EDTA which is hexadentate, and their structures are shown in Figure 2.

Chelating compounds usually contain replaceable hydrogens, where the hydrogen can be replaced with a metal ion under

$H_2\ddot{N}CH_2CH_2\ddot{N}H_2$

ETHYLENEDIAMINE (en).



EDTA^{4-}.

Figure 2. Structures of ethylenediamine (bidentate ligand) and EDTA^{4-} (hexadentate ligand) showing electron pairs.

appropriate conditions. Typical groups where this can occur are -OH, -COOH, $-NH_2$, =NOH, -SH and $-PO_3H_2$. The metal ion is co-ordinately bonded to a functional group, which is normally basic, and contains one or more electron pairs for bonding, to form the metal chelate. Common co-ordinating groups within a chelating compound are =O, $-NH_2$, -NH-, =N- and =S. Some typical examples are shown in Figure 3. Chelate complexes are much more stable than non-chelate complexes. This has been shown thermodynamically and is termed the chelate effect. Although there is little change in enthalpy, there is a more positive change in entropy. This is due to the increase in the number of unbound ligands present in the system. This can be seen in the example below for the formation of a complex with nickel and ammonia, and the formation of a chelate with nickel and ethylenediamine (en). In aqueous solution the Ni^{2+} ion is hydrated :-

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

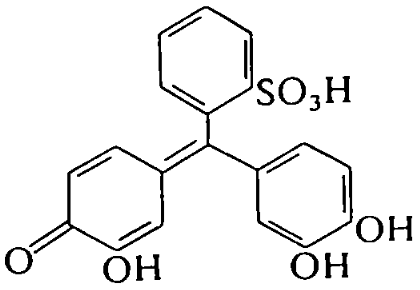
$$Ni[(H_2O)_6]^{2+} + 3en \rightleftharpoons Ni[en_3]^{2+} + 6H_2O \qquad \log \beta_3 = 18.3$$

The chelate formed with ethylenediamine is 10^{10} times more stable, as can be seen from the overall stability constant β. With the formation of the ammonia complex there is no net change in the number of molecules, however, in the formation of the chelate, there is an increase of three moles of individual molecules (from four to seven), which dramatically increases the entropy.

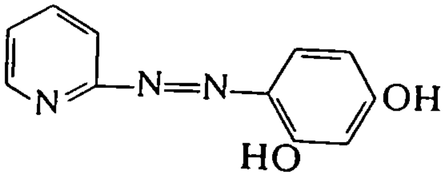
During the formation of metal complexes, various equilibria are established depending on the concentration of metal ion and ligand. For a monodentate ligand L, and metal M, in solution :-

$$M + L \rightleftharpoons ML , \qquad K_1 = \frac{[ML]}{[M][L]}$$

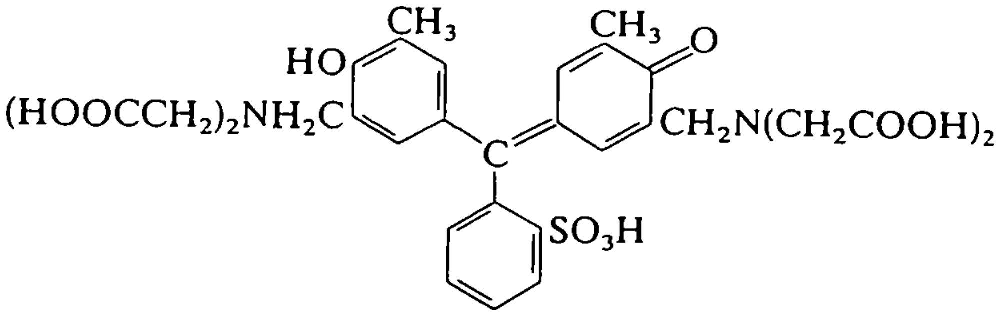
$$ML + L \rightleftharpoons ML_2 , \qquad K_2 = \frac{[ML_2]}{[ML][L]}$$



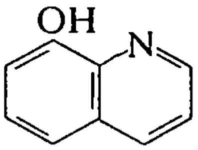
PYROCATECHOL VIOLET.



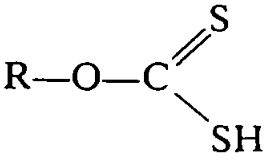
4-(2-PYRIDYLAZO)RESORCINOL (PAR).



XYLENOL ORANGE.

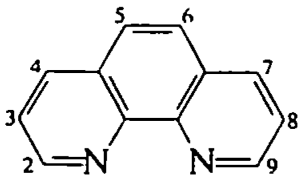


8-HYDROXYQUINOLINE.

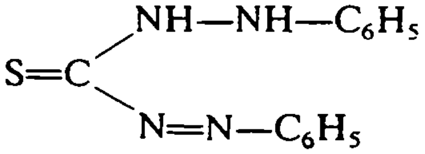


(as alkali metal salt)

XANTHATES.



1,10-PHENANTHROLINE.



(thione form)

DITHIZONE.

Figure 3. Some typical examples of a range of chelating compounds.

$$ML_2 + L \rightleftharpoons ML_3 \,, \qquad K_3 = \frac{[ML_3]}{[ML_2][L]}$$

$$\vdots \qquad \vdots$$

$$ML_{N-1} + L \rightleftharpoons ML_N \,, \qquad K_N = \frac{[ML_N]}{[ML_{N-1}][L]}$$

where N is the maximum co-ordination number of metal ion M for ligand L.

Alternatively for the formation of ML_N, the equation can be written :-

$$M + L \rightleftharpoons ML \,, \qquad \beta_1 = \frac{[ML]}{[M][L]}$$

$$M + 2L \rightleftharpoons ML_2 \,, \qquad \beta_2 = \frac{[ML_2]}{[M][L]^2}$$

$$M + 3L \rightleftharpoons ML_3 \,, \qquad \beta_3 = \frac{[ML_3]}{[M][L]^3}$$

$$\vdots \qquad \vdots$$

$$M + NL \rightleftharpoons ML_N \,, \qquad \beta_N = \frac{[ML_N]}{[M][L]^N}$$

The constants K_N are called stepwise formation or stability constants. β_N constants are the overall formation or stability constants.

The values of K_N and β_N are related, the overall stability constant, for example, $\beta_3 = K_1K_2K_3$. As the values of stepwise stability constants are often given as log K values, the overall stability constant for the formation of a particular species is the sum of the individual log K values. Stability constants are a measure of affinity of a ligand for a metal ion in solution. For example, for the formation of complexes between Co^{2+} and ammonia, NH_3 :-

$$Co^{2+} + NH_3 \rightleftharpoons [Co(NH_3)]^{2+} \,, \qquad \log K_1 = 2.0$$

$$[Co(NH_3)]^{2+} + NH_3 \rightleftharpoons [Co(NH_3)_2]^{2+} \,, \qquad \log K_2 = 1.5$$

$$[Co(NH_3)_2]^{2+} + NH_3 \rightleftharpoons [Co(NH_3)_3]^{2+} \,, \qquad \log K_3 = 0.9$$

$$[Co(NH_3)_3]^{2+} + NH_3 \rightleftharpoons [Co(NH_3)_4]^{2+} \,, \qquad \log K_4 = 0.6$$

The overall stability constant for the formation of $[Co(NH_3)_4]^{2+}$

complex, log $\beta_4 = \log K_1 + \log K_2 + \log K_3 + \log K_4 = 5.0$. This is for the reaction :-

$$Co^{2+} + 4NH_3 \rightleftharpoons [Co(NH_3)_4]^{2+} \quad , \beta_4 = 5.0$$

The values of stability constants normally quoted are based on many assumptions. In the above example, the values given are for zero ionic strength and a temperature of 25°C, and it assumes that the complex species formed is the only species present.

In general,

$$M + L \rightleftharpoons ML \quad , \quad K=K_{ML} = \frac{[ML]}{[M][L]}$$

The metal and ligand are assumed to be 100% ionized, and activity coefficients are not taken into consideration. More importantly, many metal ions and ligands are present as different species depending on the pH of the solution. Depending on the pH, the concentrations of reacting metal ion and/or ligand could be different. If this happens the overall value of K will appear to change. These stability constants are known as conditional stability constants, and are the key to chelating exchange, as this can be controlled by pH, which alters the conditional stability constants of the metal chelates.

Conditional stability constants are often denoted by K', with the equilibrium for the formation of the complex ML becoming :-

$$M + L \rightleftharpoons ML \quad , \quad K' = K_{M'L'} = \frac{[ML]}{[M'][L']}$$

The value of [ML] gives the concentration of metal complex, whilst $[M']$ and $[L']$ give the total concentration of uncomplexed metal ion and ligand regardless of the number of chemical forms present. This varies depending on the experimental conditions, such as pH, giving rise to the conditional stability constant $K_{M'L'}$.

A typical illustration of the change in conditional stability constants with pH, is the various metal-EDTA complexes, and this is shown in Figure 4 (40). As the pH increases from acidic solution, the conditional stability constants increase, due to increased dissociation of EDTA. However, many start to decrease again in alkaline solution. This is mainly due to the formation of metal hydroxide species or even mixed EDTA/hydroxy species.

EDTA itself is present in solution as different species depending on the pH. Essentially there are five species, H_4EDTA, H_3EDTA^-, H_2EDTA^{2-}, $HEDTA^{3-}$ and $EDTA^{4-}$. However, the absolute stability constant only considers the free metal ion and the fully ionized form of EDTA, as shown in the example below for a divalent metal ion :-

$$M^{2+} + EDTA^{4-} \rightleftharpoons MEDTA^{2-}$$

Thus the proportion of $EDTA^{4-}$ in the total EDTA concentration present will depend on pH. H_4EDTA predominates in highly acidic media, whilst H_2EDTA^{2-} generally predominates between pH3 and pH6. $EDTA^{4-}$ predominates above pH10. In the calculation of the conditional stability constant, the concentration of all these species at the particular pH is taken in consideration, and $[L']$ in the equation would be the sum of all these concentrations. A similar calculation is carried out for $[M']$ which represents the total concentration of all the metal, M, species present at that particular pH.

Stability constants also change with changing ionic strength. This change although small, is significant, and is related to the charge on the ligand and metal ion. Table 3 shows the relative change in stability constant with ionic strength and charge, relative to 0.1M ionic strength (41). From this table, it can be seen that this variation increases with an increasing charge on the metal cation, and increasing charge on the

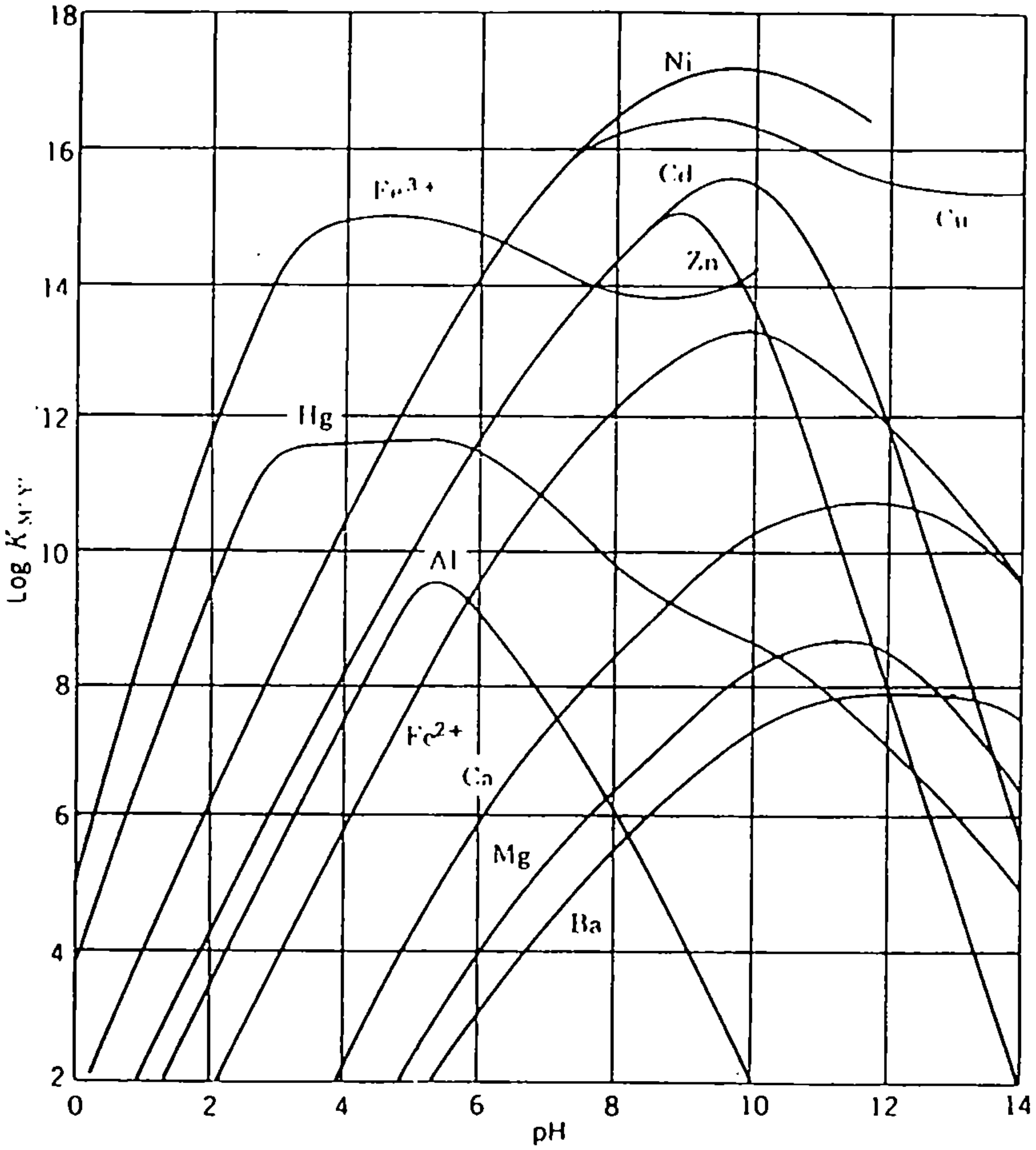


Figure 4. Conditional stability constants, log $K_{M'Y'}$, of various metal EDTA complexes as functions of pH (40).

IONIC STRENGTH.	0.0	0.5	1.0	2.0	3.0
M^+.					
L^-	+0.3	-0.1	0.0	+0.3	+0.6
L^{2-}	+0.6	-0.4	-0.4	-0.3	0.0
L^{3-}	+1.0	-0.5	-0.5	[-0.4]	[-0.2]
M^{2+}.					
L^-	+0.6	-0.4	-0.4	-0.3	0.0
L^{2-}	+1.2	-0.8	-0.8	[-0.7]	[-0.5]
L^{3-}	+1.8	[-1.0]	[-1.0]	[-1.0]	[-0.9]
M^{3+}.					
L^-	+1.0	-0.5	-0.5	[-0.4]	[-0.2]
L^{2-}	+1.8	[-1.0]	[-1.0]	[-1.0]	[-0.9]
L^{3-}	[+2.6]	[-1.5]	[-1.5]	[-1.6]	[-1.5]

[ ] - Based on trends.

Table 3. Variation of overall stability constant, log β_2, with ionic strength and charge relative to 0.1M ionic strength (41).

ligand. The greatest change occurs between ionic strength of zero and 0.5, but interestingly, above this there is little further change, and if anything, the variation is less when the ionic strength is higher such as 2.0 and 3.0. This is important as samples such as concentrated brines are of very high ionic strength, with saturated sodium chloride brine being over 5M.

1.5.2. DEVELOPMENT OF CHELATING ION EXCHANGE IN ION CHROMATOGRAPHY.

Erlenmeyer and Dahn (42) observed in 1939, that mixtures of various cations could be chromatographed on a column of powdered 8-hydroxyquinoline. The metal ions were detected following the formation of coloured complexes with 8-hydroxyquinoline, resulting in bands at various positions along the column. Later, Meinhard published a review of the development and advances in chromatography (43), and suggested that the potential problem of the 8-hydroxyquinoline being displaced from the column used by Erlenmeyer and Dahn, could be overcome by 'fixing' the complexing agent irreversibly on another solid, such as silica. This led to the eventual development of covalently bonded substrates for chelating ion exchange.

Chelating ion exchange chromatography employs a chromatographic column where the chelating groups are actually attached to the substrate, and thus the metal ions in the mobile phase form chelates depending on the conditions. Most chelating groups are covalently bonded to a substrate, such as silica or polystyrene-divinylbenzene, but relatively few are coated onto a substrate. These chelating columns have principally been used for sample preconcentration and clean-up or matrix elimination before analysis by another technique. Very few have been used for the high performance separation of metal ions. Since the development of chelating exchange has involved both covalently bonded substrates and coated substrates, these two groups will be examined separately.

1.5.2.1. COVALENTLY BONDED SUBSTRATES.

Many functional chelating groups have been covalently or chemically bonded to various substrates where often they have been synthesized beforehand by research workers. There are also commercially available chelating substrates. However, as stated previously, the majority of their applications in trace metal analysis has been for the preconcentration of metal ions and not separation.

One of the earliest commercially available chelating resins was Chelex-100 (or Dowex A-1). This contains chemically bonded iminodiacetic acid chelating groups. However, it has the disadvantage of swelling and contracting with changing pH. Christell et al. (44) in 1961, demonstrated its use in nuclear chemistry. The resin was saturated with copper(II), and the exchange of ions with lanthanum(III) which forms a stronger chelate was shown. Dowex A-1 was also used by Imoto (45) to retain manganese in concentrated sodium chloride and caustic soda. Above pH3, manganese was 100% retained on this resin. For the analysis the sample was adjusted to pH6-7, to retain all the manganese which was then eluted off with 1-2M sulphuric acid, after agitation and filtration of the sample. The manganese was determined photometrically after oxidation to MnO_4^-. A similar resin was used by Kuehn and Hering (46) to reduce the concentrations of a wide range of metal ions in sodium chloride to below the limits of spectrographic detection. Van Willigen (47) investigated and determined stability constants of metal-resin complexes for Dowex A-1 chelating resin, and from the results obtained he suggested that metal ion separations using this resin would be very difficult. The difference in stability constants were not enough to produce any efficient separation. Only a partial separation of calcium and copper was obtained, however, this was at a high temperature of 70°C.

Riley and Taylor (48) in 1968, were one of the first to study the uptake and recovery of a large range of metal ions in sea water using Chelex-100. Five metal ions in sea water, zinc, copper, nickel, cobalt and cadmium were then investigated in detail. The final determination was by atomic absorption spectrophotometry (AAS). Some metal ions were not retained on the resin, but in general, the percentage recovery of metal ions that were strongly retained was around 100%, except silver which was 90%. More recent work has also used Chelex-100 for preconcentration of metal ions. Muruganan et al. (49) removed trace impurities from phosphoric acid, and subsequently these were determined by atomic absorption, using either flame or graphite furnace atomization techniques. The Chelex-100 containing iminodiacetic acid functional groups proved to be more efficient at removing impurities than a Dowex-50 resin containing sulphonic acid functional groups. Preconcentration of radionuclides in nearly saturated brines using Chelex-100 was achieved by Strachan et al. (50). Eight of the ten radionuclides studied were eluted off quantitatively, and recoveries ranged from 98 to 103%, using a γ-spectrometer for detection. Some of the brines were diluted before analysis, but this method proved to be very useful for the analysis of nuclear wastes which are stored in salt repositories.

Chelating functional groups other than iminodiacetic acid have been prepared and covalently bonded to various substrates for the preconcentration of metal ions. 8-hydroxyquinoline has been immobilized onto silica gel, and used for the preconcentration of eight metals from sea water (51). This method enabled large enrichment factors to be attained, and quantitative recovery, using electrothermal atomization atomic absorption spectrophotometry (ETAAS) for determination. Chambaz and Haerdi (52) used an 8-quinolinol bonded chelating silica for the on-line preconcentration of transition metal ions. These metals were then determined by ion-pair chromatography, after desorption using potassium cyanide. Problems were encountered as the detection limit was limited to 10^{-8}M due to high background, and the method limited to the determination of copper and nickel. The prepared bonded silica also tended to decompose with time. An on-line preconcentration system has recently been used by Mohammad et al. (53), utilizing 8-hydroxyquinoline immobilized onto controlled pore glass for the preconcentration of aluminium. The optimum pH for preconcentration is 8, however, an anionic buffer had to be added to avoid precipitation of $Al(OH)_3$, or formation of the aluminate, $[Al(OH)_4]^-$. The effect of several buffers were investigated, but a malonate buffer for preconcentration in the range pH9.2 to pH10 was found to be optimum. The system was applied to river and sea water samples, where the detection was by atomic absorption spectrophotometry.

Gennaro et al. (54) preconcentrated a range of divalent metal ions using a cellulose filter to which methyliminodiacetic acid had been immobilized. The percentage uptake with changing pH was investigated, and the retained metal ions eluted using 1M hydrochloric acid. For all the metal ions studied, the maximum uptake was found to be greater than 90% around pH3. However, mercury (II) was poorly retained, and the maximum uptake achieved was only 50%. Methyliminodiacetic acid forms more stable metal complexes than iminodiacetic acid alone, although when bound to the cellulose, the uptake of metal ions is extremely similar, even for magnesium and calcium which form less stable chelates. A thioglycolate chelating resin has been prepared by Howard et al. (55), and used for the preconcentration of cadmium, zinc, lead and nickel from artificial sea waters. The substrate used was the large particle size Amberlite XAD-4. However, the recovery of nickel and zinc was poor, but for cadmium and lead the recovery reached nearly 100%, although for an equilibrium to be achieved between the chelating resin and the metal ions took up to ninety minutes.

Glennon et al. (56) prepared a biochelation cartridge for the uptake of metal ions utilising hydroxamate complexation. Later this type of cartridge was successfully used for on-line trace metal preconcentration (57), before separation and detection by ion chromatography. A separation of six metal ions was achieved, with preconcentration of trace metals from a sample of tap water demonstrated. Recently, some biopolymers were prepared and investigated for the uptake of lead and copper (58). The biopolymers which were found to be the most efficient at removing these metals from various natural and waste waters, were those containing hydroxamic acid derivatives. It was suggested that conventional ion-exchange could be used to clean up high concentrations of lead and copper from polluted waters, where the chelation ion-exchange step could be used for removing lower concentrations remaining. This complimentary technique would remove heavy metal ions to sub-ppb concentrations.

Aluminium and beryllium were separated from other ions in beryl and some synthetic alloys by Das and Pobi (59), using a

N-benzoylphenylhydroxylamine chelating resin. The functional groups were bonded onto styrene-divinylbenzene polymer beads, and a batch technique used to determine the metal uptake. A glass column containing this chelating resin was subsequently used for preconcentration of various metal ions, which were selectively eluted by using different eluting agents. Detection was by atomic absorption spectrophotometry. Chambaz et al. (60) used an ethylenediamine triacetate chemically bonded silica for the preconcentration of several divalent metal ions, before separation and detection by standard ion chromatography. This method was applied to the analysis of trace metals in river waters.

Several chelating columns have recently been commercially available for the preconcentration of trace metals from complex matrices. Siriraks et al. (61) used a chelating column with iminodiacetic acid functional groups for trace element analysis in complex environmental and biological samples. This column, the MetPac CC-1 developed by Dionex, was only used for preconcentration. Separation was by conventional ion chromatography, utilizing a rather complex multi-valve system. The wide range of samples that can be analyzed include sea waters, brines, biological, botanical and geological materials. This substrate is macroporous and designed to form strong chelates with transition metals, with alkaline earth metals more weakly bound. The optimum pH for retention of transition metals and lanthanides is pH5 to 6. The full details of the method of chelation ion chromatography using this column, together with examples of separations is described by Dionex (62). Joyce and Schein (28) have illustrated further chelation concentration for ion chromatographic analysis of environmental samples such as sea waters using the MetPac column for preconcentration. This column has been coupled to inductively coupled plasma-optical emission spectrometry (ICP-OES), and inductively coupled plasma-mass spectrometry (ICP-MS), to enhance their performance (63). A range of samples were analyzed including sea waters, brines and biological samples, where in general, using the MetPac column for preconcentration, detection limits were in the sub-ppb range.

A chelating cation exchange resin with diphosphonic acid functional groups bonded onto a styrene based polymer matrix has been used for the treatment of industrial wastewaters (64), and is commercially available as Diphonix. It forms strong chelates with transition metals, post-transition metals and radionuclides, and is claimed to reduce metal ion concentrations by four orders of magnitude from highly salted solutions. It can be used as a clean-up column for waste streams such as the removal of actinides, or as a preconcentration column for the analysis of metal ions.

A review by Kantipuly et al. (65) highlights many chelating polymers, including commercially available polymers, used for the preconcentration and separation of trace metals from natural waters. The majority of these chelating polymers are used for the preconcentration of a wide range of metal ions, and functional groups are all chemically bonded onto the substrate. Figure 5 shows some commercially available chelating resins and the functional groups involved in chelation. A recent review by Terada (66) describes preconcentration of trace elements by sorption, using a variety of supports. Silica was principally used and modified as a supporting material, but other supports such as activated carbon, porous polymers and natural complex forming polymers, such as chitin and chitosan, are described.

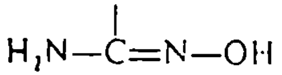
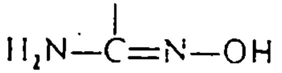
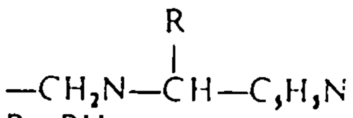
Trade name	Functional group	Nature of functional group	Donor atoms	Company
Chelex-100	$CH_2N(CH_2CO_2H)_2$	Iminodiacetate	N, O, O	Bio-rad
Duolite ES-466	$CH_2N(CH_2CO_2H)_2$	Iminodiacetate	N,O, O	Duolite
Not named		8-Hydroxyquinoline	N, O	Seakem
Duolite ES-467	$CH_2NCH_2PO(OH)_2$	Aminophosphonic acid	N, O, O	Duolite
Chelite-P	$CH_2NCH_2PO(OH)_2$	Aminophosphonic acid	N, O, O	Duolite
Duolite ES-465	$-SH$	Mercapto	S	Duolite
Chelite-S	$-SH$	Mercapto	S	Duolite
Duolite ES-346	 $H_2N-\overset{\vert}{C}=N-OH$	Polyacrylate with amidoxime	O, N	Duolite
Chelite-N	 $H_2N-\overset{\vert}{C}=N-OH$	Macroporous polymer with amidoxime	O, N	Duolite
MISSO ALM	NCS_2H	Dithiocarbamic acid	N, S, S	Nippon Soda
KRUPTOFIX 221B	Cryptand	Cryptand	N, O	Parish
CR20	$CH_2N(CH_2CH_2N)_n-H$	Polyamine	N	Mitsubishi
XFS4195	 $-CH_2N-\overset{R}{\overset{\vert}{C}}H-C_5H_4N$	Weak base	N, N	Dow Chemicals
Amborane 345	$P-BH_3$	Amine-borane	—	Rohm and Haas
Amborane 355				

Figure 5. Some examples of commercially available chelating resins (65).

Some of the functional groups were covalently bonded onto the support, but many were loaded and absorbed onto the support.

There are a large number of covalently bonded substrates used for preconcentration of metal ions, but relatively few have been employed for the separation of metal ions. Fritz (67) has prepared many covalently bonded substrates and demonstrated their potential for the separation of metal ions, although the efficiencies were low. The majority of chelating functional groups have been bonded onto a silica support, but a number have been bonded to styrene-divinylbenzene copolymer. Faltynski and Jezorek (68) prepared several silica bound chelating stationary phases for the separation of some transition and heavy metals. These are illustrated in Figure 6. A separation of six metal ions was achieved using a dithizone bonded phase (DTSG), however, as with several other of the separations, the retention order was the opposite to that expected for chelating exchange. This may be due to the presence of tartaric acid which is complexing, in the mobile phase. Separations illustrated where the metal ions eluted in the expected order for chelating exchange utilised the MQSG and NEDSG bonded silicas (Figure 6). Simonzadeh and Schilt (69) prepared a silica immobilized 2-pyridine-carboxy- aldehyde-phenylhydrazone bonded phase and applied it to the separation of transition and heavy metals. Although metal ion separations were achieved, the peaks tended to broaden badly with increasing retention time. Some metals co-eluted, but various anions such as perchlorate and oxalate, acting as a buffer, were added to the mobile phase to improve separation, and alter selectivity. The broad peaks may be due to the slow kinetics of the chelation reaction, as isocratic conditions were used.

Bonn et al. (70) covalently bound iminodiacetic acid to a

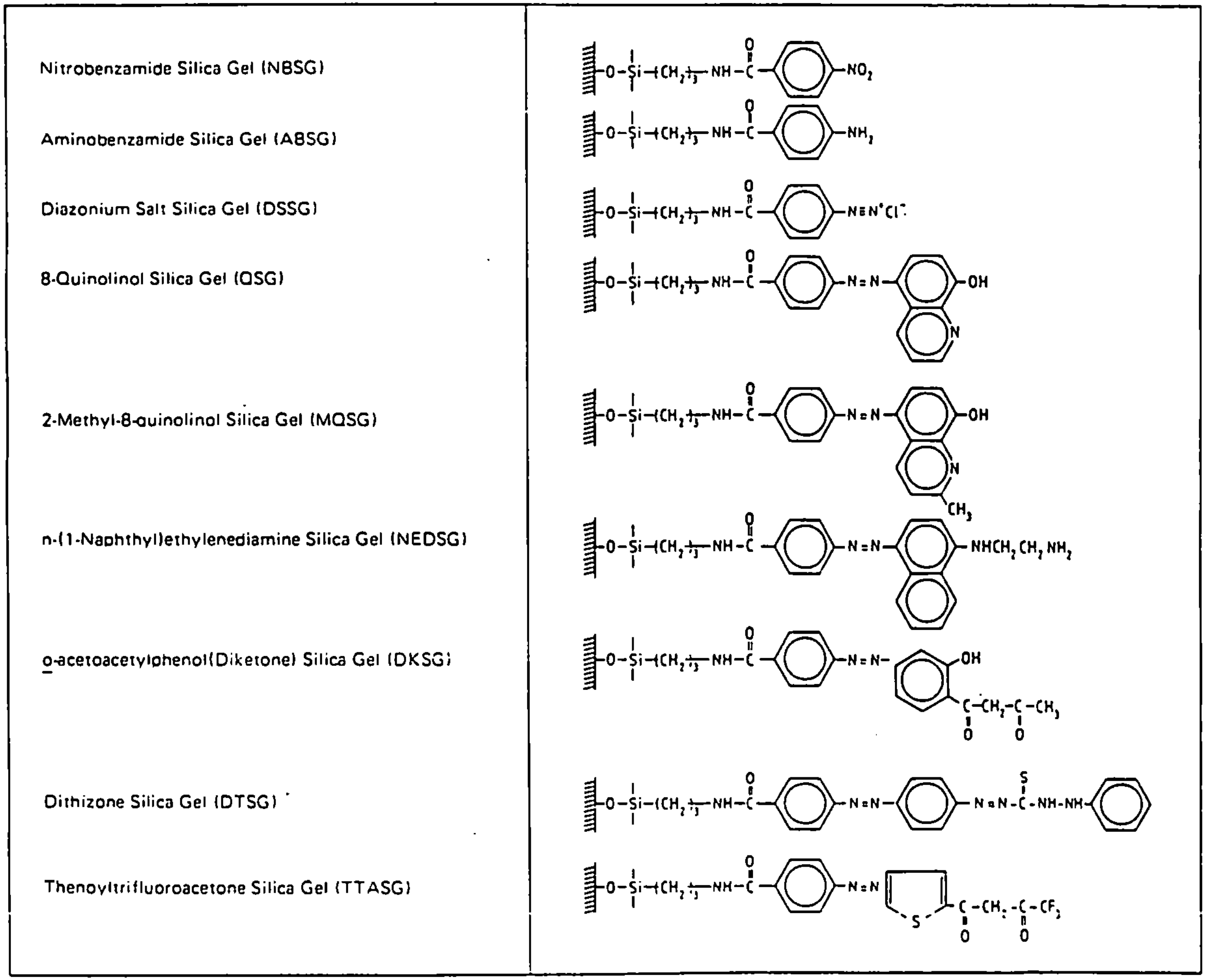


Figure 6. Silica bound chelating stationary phases used by Faltynski and Jezorek (68).

porous silica support to separate transition metal ions, particularly from sea waters. Various carboxylic acids and complexing agents in the eluent were used to study elution mechanisms of metal ions. Using organic acid eluents such as citric, tartaric and dipicolinic acid, several metal ion separations were achieved, where they eluted in the expected order for a chelating exchange process. Dipicolinic acid proved to be particularly useful, as it is strongly complexing in acidic solutions and has little affinity for alkaline earth metal ions. The retention of monovalent metal ions and alkaline earth metals showed a strong dependence on pH, however, for transition metal ions a dependence on both pH and dipicolinic acid concentration was observed. By controlling the pH, the optimum conditions where complexation of metal ions by the iminodiacetic acid on the stationary phase and dipicolinic acid in the eluent are favoured, can be chosen. A separation of cobalt, zinc and cadmium in sea water was achieved using a tartaric acid eluent, and the ease of separation demonstrated the strong complexing ability of iminodiacetic acid towards transition metals.

Cations and anions can be separated using bonded silicas. Lauth and Gramain (71) used benzo-18-crown-6-modified silica to separate alkali and alkaline earth metal chlorides and some anions using water as an eluent. A bonded 8-quinolinol stationary phase has been used by Thompson and Jezorek (72) to separate transition metals, neutral organics (phenols and anilines), and inorganic anions in a single injection.

Functional chelating groups have been bonded onto polystyrene-divinylbenzene substrates. Jonas et al. (73) immobilized 8-hydroxyquinoline onto this type of substrate and rapidly separated copper and zinc. By reducing the flow rate, zinc, cobalt, nickel and copper could be separated, and elute in the correct order for chelating exchange, but peaks broaden with increasing retention time.

A recent review by Liu (74) highlights the synthesis and characterisation of chelating ion-exchange resins. A number of metal ion separations are illustrated including the speciation and separation of selenium (IV) and (VI) using a N-(hydroxymethyl) thioamide resin column, and the separation of platinum and gold from other transition metals. This was achieved using the same column, and a ß-hydroxydithiocinnamate resin column. This review also describes the use of chelating resins in inorganic and organic analysis in analytical chemistry.

Toei (75) investigated the commercially available TOSOH TSK-GEL chelating column for the separation of magnesium and calcium in sea waters using a colour-forming agent in the eluent (o-cresolphthalein complexone). This column contains covalently bonded iminodiacetic acid groups attached to a polymer based gel. Retention times for magnesium, calcium and strontium decreased with increasing salt concentration and on increasing pH and column temperature, retention times increased. A separation of magnesium, strontium and calcium standards, and magnesium and calcium in sea water was achieved, however, there is some doubt whether the mechanism is totally chelating exchange. The chelating colour-forming reagent in the eluent will also affect the separation, and retention order.

1.5.2.2. COATED SUBSTRATES.

Many chemically bonded substrates have a limited lifetime, as the functional groups are gradually released from the substrate during use, resulting in loss in efficiency. Chemically bonding functional groups to a substrate can be a very time consuming and difficult procedure, where often the functional groups are synthesized beforehand by a complex series of steps. An alternative method is to coat or impregnate the substrate, often using a chelating dye. Substrates can be coated in situ by mixing with the dye, or dynamically coated by passing or pumping the chelating dye through a column containing the substrate. The dyes tend to be impregnated into the pores of the substrate. For good results, the ligands should be chemically stable, have a low solubility in aqueous solution, and strongly adsorbed onto the substrate.

As with chemically bonded substrates, these coated substrates have principally been used for preconcentration of metal ions, and little work has been carried out to separate metal ions. A review by Marina et al. (76) describes many coated resins which have been used for separation and preconcentration of metal ions, but the majority have involved coatings on anion-exchange resins and used for preconcentration. More recently, Terada (66) reviewed some silica substrates that had been impregnated or loaded with a complexing reagent for the preconcentration of a range of metal ions. There are a wide range of chelating dyes that can be chosen to coat substrates, and aimed at a particular group of metal ions, as can be seen in a book on photometric reagents by Sandell and Onishi (77). An extensive review of heterocyclic azo dyestuffs by Anderson and Nickless (78) highlights their use in analytical chemistry, with PAR (4-(2-pyridylazo)resorcinol) and PAN (1-(2-pyridylazo)-2-naphthol) studied in detail. At the time of this review in 1967, these dyes were principally used for spectrophotometric detection of various metal ions, but their potential for other new uses in analytical chemistry were recognised.

Coated columns for the preconcentration of metal ions have employed large particle size substrates. The Amberlite XAD type cross-linked polystyrene resins have been used extensively for this purpose. Mackey (79) showed that copper, iron and zinc are readily adsorbed onto Amberlite XAD-1 and XAD-2 resins, and suggests that they are unsuitable for quantitative studies of metal speciation in natural waters. The adsorption of copper and zinc onto XAD-2 is not strongly dependent on pH, and methanol or acetonitrile do not remove these ions. In fact, XAD-2 can remove copper and zinc from acetonitrile. The columns become saturated with copper and zinc at less than 5μg, but by methylation of the resin, the adsorption can be reduced.

The results obtained by Mackey above, interestingly are for uncoated and unmodified resin. Lundgren and Schilt (80) coated XAD-2 with four different ferroin type chromogens, and found that of these four, 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine (PDT) proved to be most effectively adsorbed. Initial studies involving the adsorption of their iron(II) chelates showed that adsorption was 100% after equilibrium had been reached for PDT. Retention increased with increasing pH, and was very efficient for the retention of copper(I). This column was applied to the removal of trace metal ions from reagents and for preconcentration for analysis of laboratory reagents and sea water. The metal ions studied were iron(II), cobalt, nickel, copper(I) and copper (II) and quantitatively removed even in the presence of large concentrations of more weakly chelating metal ions. The potential of these coated columns for use in purification and preconcentration for ultra-trace determinations was clearly recognised. Howard and Arbab-Zavar (81) coated dithizone onto XAD-2 and used for the preconcentration of mercury and methylmercury from natural waters. The uptake was 100% for hydrochloric acid concentrations up to 3M, with the mercury and methylmercury eluted with 9M hydrochloric acid before analysis by cold vapour atomic absorption spectrophotometry. The optimum conditions for preconcentration were pH1 to 2, and a flow rate of less than 5ml min^{-1}. Pyrocatechol Violet has been coated onto XAD-2 by Brajter et al. (82), and used for the preconcentration of lead and indium. The optimum pH for sorption of the dye was pH2, and differences in retention of various metal ions were found. Lead, indium, bismuth and copper were retained at pH2, whilst nickel, cobalt and iron(III) were only partially retained, where maximum retention was pH4. The selectivity order on this coated resin was found to be Bi ≥ In ≥ Pb > Cu > Fe > Co > Ni, at pH1. This resin showed strong retention of indium and lead with the possible separation of lead and bismuth from other metal ions. High preconcentration factors could be obtained, and applied to the analysis of lead in tap water. Recently, Naghmush et al. (83) used a Pyrocatechol Violet coated XAD-2 column in a flow-injection system, for the preconcentration of copper from natural waters before determination by AAS. Detection down to the 1ppb level was achieved.

Abollino et al. (84) immobilized 8-hydroxyquinoline and 8-hydroxyquinoline-5-sulphonic acid onto XAD-2 and an anion-exchange resin respectively. A range of divalent metal ions were used to study the uptake, and metal enrichment factors of 100 from environmental samples were obtained, although the elution process was not entirely quantitative for some metals. After elution, the metal ions were detected by inductively coupled plasma-atomic emission spectrometry (ICP-AES). An on-line chelating exchange microcolumn containing 1-(2-thiazoylazo)-2-naphthol (TAN) coated XAD-2 has been recently used by Porta et al. (85) to preconcentrate cadmium, copper, iron, manganese, nickel and zinc from river water and sea water. Selective retention of transition metals from alkaline earth metal ions was obtained, and after elution, detection was by ICP-AES. To avoid contamination from impurities in the buffer solution, a column containing Chelex-100 was used. These coated columns prove to be one of the best methods of sample pretreatment in atomic spectrometry.

Amberlite XAD-4 has also been used as a substrate for coating with functional chelating groups. Chwastowska and Mozer (86) coated XAD-4 with PAN under static and dynamic conditions. The prepared columns were very stable, with a high total sorption capacity. Under static conditions, equilibrium with various metal ions was not reached until over one hour, and only 80-85% of lead, nickel, cadmium and zinc were sorbed. Dynamically coated resin gave a more efficient overall sorption, and this was used to sorb metal ions from synthetic water, and river water, before determination by AAS. At the optimum pH8.0, some magnesium and calcium was retained, however at higher pH values, these tended to reduce the sorption of transition metals, as they begin to compete for the chelating sites. An N-benzoyl-N-phenylhydroxylamine coated XAD-4 chelating resin has been prepared by Chwastowska and Przygoda (87), and used to sorb a range of divalent metal ions from natural waters. The resin was coated under static and dynamic conditions, and a pH of 9 was found to be optimum for copper, lead, zinc, cadmium and cobalt sorption, which was over 90%. Magnesium and calcium are not sorbed much until above pH11, which is an advantage when analyzing natural waters. Isshiki et al. (88) impregnated XAD-4 resin with 7-dodecenyl-8-quinolinol for the preconcentration of trace metals from sea water. A wide range of metal ions from monovalent to tetravalent were investigated for their uptake. Titanium, gallium, iron, copper and bismuth were strongly retained at pH1 to 2, and good recoveries were obtained. Other metal ions such as silver, aluminium, cadmium, manganese, nickel and lead were recovered at a higher pH, whilst magnesium was virtually not retained at all. Metal ions were extracted from the coated resin for analysis, however, there was a slight bleed of the chelate with each run, but did not interfere. Lipophilic tetraaza macrocycles have been impregnated into XAD-4 and XAD-7 macroporous resins by Blain et al. (89), for the preconcentration of trace metals from sea water. Six metal ions were studied, these being cadmium, copper, manganese, nickel, lead and zinc, and recoveries were around 98%. These tetraaza macrocycles are very selective for transition metals compared with alkali and alkaline earth metals, and are therefore ideal for sea water analysis. The optimum pH for uptake was pH8, but total complexation with manganese was not achieved even after six hours. Copper formed the strongest chelate, being retained at the lowest pH of around 4, whilst only a small quantity of calcium is retained above pH7. Good results were obtained for the preconcentration of manganese, cadmium, zinc and lead in sea water, but all of the copper was not desorbed using dilute nitric acid, and the column had to be regenerated using potassium cyanide after about twenty samples, to elute this remaining copper and any other contaminants. Elci et al. (90) adsorbed 8-hydroxyquinoline and ammonium pyrrolidine dithiocarbamidate (APDC) onto XAD-4 resin for the preconcentration of trace metals from river waters. In general, on increasing pH, recoveries increased, and APDC formed stronger chelates than 8-hydroxyquinoline. The optimum pH for retention of a range of transition and heavy metals was pH6 for APDC and pH8 for 8-hydroxyquinoline. Low recoveries for calcium and magnesium were obtained, showing that these columns can be used for analysis of natural waters. The metal chelates were formed before the mixture is passed through a column containing XAD-4, with detection by AAS after elution using 1M nitric acid in acetone.

Chelating agents have also been coated onto anion-exchange resins. Chikuma et al. (91) modified Amberlite IRA-400 anion-exchange resin (chloride form) with several chelating reagents. The resin prepared from the sulphonic acid derivative of dithizone gave the best results, and may be held by physical adsorption and ion-attraction between $-SO_3^-$ on the dye and $-NR_3^+$ on the resin. The release of chloride from the anion-exchange resins by the complexing agents was measured, to give an indication of the number of ion-exchange groups present, as well as investigating the loading. The stability of these resins in 1M sodium chloride was shown, where the dithizone derivative and zincon showed high stability, as they are mainly held by physical adsorption. Others which proved to be unstable in 1M sodium chloride were resins modified with Sulphonazo III, Arsenazo III, thiosalicylic acid and p-mercaptobenzenesulphonic acid. Using the dithizone derivative in estuarine water, it is completely taken up by the resin. The distribution coefficients of several of these resins with mercury(II) and copper(II) are shown. Hernandez et al. (92) later used this procedure to coat an anion-exchange resin with Chrome Azurol S, to preconcentrate aluminium from haemodialysis fluids and human serum. This column was part of a flow injection system, with detection by AAS. Results were linear in the range 25 to 200ppb for a 1ml sample, with a detection limit of 14.8ppb, which is comparable with ETAAS. Handley et al. (17) used Xylenol Orange which was dynamically coated onto Dowex 1-X8(Cl) resin to preconcentrate alkaline earth metals from concentrated brines, as part of an automated HPLC system. This system was tested on-line on a fully automated chlorine plant, and worked successfully over the thirty-six hour period studied, with detection limits in the low ppb levels. Further work using this dye-coated column for preconcentration of aluminium and zinc as well as alkaline earth metals, was carried out (34). Quantitative results, and elimination of the saturated sodium chloride matrix were achieved, with separation by conventional ion chromatography. This system was also developed for on-line analysis of concentrated brines, and capable of analyzing six samples per hour. Xylenol Orange and Pyrocatechol Violet were loaded onto Dowex-2 anion-exchange resin by Singh and Dhingra (93), to investigate the preconcentration of copper and cadmium. With the Pyrocatechol Violet loaded resin, copper was completely sorbed above pH5, and cadmium above pH9.5, and with the Xylenol Orange loaded resin, complete sorption for copper occurred above pH6, and for cadmium above pH8. For 1g of resin, 23-26mg of both dyes were adsorbed onto the resin at pH7-8, however, below 0.01ppm, sorption of copper and cadmium was not quantitative or reproducible. These two dye-coated resins were used for the determination of copper and cadmium in river water, where it was found that copper is adsorbed more strongly as expected, with the Xylenol Orange coated resin having a greater sorbing capacity than the Pyrocatechol Violet coated resin.

Silica gel has been modified with chelating dyes. Kocjan (94) modified silica gel with Titan Yellow for the preconcentration of trace amounts of heavy metals from alkali or alkaline earth metal salts. Twelve metal ions were investigated, where in slightly acidic or neutral conditions, all metals except calcium and magnesium were retained. This impregnated silica showed high selectivity towards heavy metals, where calcium and magnesium were not retained unless the mixture was pH9 or higher. Aluminium and chromium formed complexes very slowly (1 hour), however, they were retained quantitatively when the flow rate is relatively high (4ml/min). Separations were achieved by eluting the various groups of metal ions off the column using several mixtures of hydrochloric acid/perchloric acid. Detection was by AAS, spectrophotometry, or voltammetric determination. The Titan Yellow impregnated silica can be utilized for the purification of electrolytes and for preconcentration and matrix elimination, for the determination of heavy metals by atomic absorption.

Brajter and Dabek-Zlotorzynska (95) modified porous aluminium oxide with seven chelating dyes. The dyes were immobilized onto the surface of the alumina by sorption, but the capacity for sorption decreased with increasing molecular size. Nitroso-R-salt (NRS) was chosen for further studies, where the retention order of metal ions with increasing pH agrees with the expected order for chelating exchange. At a low pH, alumina itself acts as a cation-exchanger, but without any complexing agent, metal hydroxides would be precipitated. Below pH2, only cobalt, copper and iron are retained, however, cobalt is retained more strongly than copper possibly due to the formation of cobalt(III), and the fact that the cobalt-NRS complex is very stable in acidic media. Retention in general decreased again in alkaline media for the more strongly complexing metal ions such as cobalt, copper, nickel and chromium. Retention of cobalt decreases dramatically, as it forms as 3:1 complex with NRS, but there is insufficient NRS sorbed on the alumina for its formation. The NRS-alumina column has several applications. In acidic media below pH2, only cobalt, copper and iron are retained, so under these conditions, they can be selectively preconcentrated from other metal ions. Cobalt(II) was preconcentrated from a tap water sample and determined by atomic absorption, with 98% recovery from spiked samples achieved. Palladium was preconcentrated and separated from mixtures of rhodium and platinum, with the palladium content of two rhodium compounds determined.

There are very few coated columns that have been used for the actual separation of metal ions. Little work has been carried out using coated high performance grade substrates for the separation of various metal ions. Some separations of anions and metal ions have been achieved using dye-coated columns, but the mechanism was ion-interaction or ion-pair chromatography. DiNunzio and Freiser (96) coated silica and controlled pore glass with Brilliant Green and a separation of octanoic, pentanoic and butanoic acid was achieved. Golombek and Schwedt (97) coated a polystyrene-divinylbenzene column with Methyl Green to separate a range of anions. In a similar way, Müller and Meisch (98) coated silica gel with methyl green, methylene blue and crystal violet to separate fluoride, chloride, bromide, nitrite, nitrate, sulphate and phosphate. Walker (99) coated ethyl violet onto both silica and polystyrene-divinylbenzene substrate (PLRP-S), for the separation of inorganic anions. Recently, Walker (100) coated the same two substrates with Thymol Blue for the separation of inorganic and organic cations.

Toei (75,101,102) has investigated several colour forming reagents in the mobile phase for trace metal separations. The mechanism appears to be a combination of ion exchange and chelating ion exchange. Xylenol Orange has been used for the separation and determination of nickel and zinc (101). A TOSOH TSK-GEL anion-exchange column was utilized for the separation. The effect of ionic strength, column temperature, Xylenol Orange and organic solvent concentration, and pH on the retention time were studied. Nickel and zinc were successfully separated, and the elution order suggests that the mechanism may principally be ion-exchange, as the salt concentration was only 0.05M potassium chloride. A major problem with the actual stability of the column occurred, as the separation capacity decreased during the studies, and needed to be completely replaced within a week of use. Only one pump is required however, as the Xylenol Orange complexes can be directly detected. Alkaline earth metals were separated using a TOSOH non-porous gel column involving Arsenazo III in the mobile phase (102). A similar set of experiments to find the optimum conditions were carried out. Magnesium and calcium could be rapidly separated in less than one minute, however, magnesium was much less sensitive. Strontium and barium were also included, but because these metals react with Arsenazo III, the chromatographic conditions had to be changed by lowering the ionic strength. All four metals were separated, but the sensitivity for magnesium, strontium and barium, compared to calcium, was poor. The elution order suggests that the mechanism is again principally ion-exchange. This column was more stable having not deteriorated for over two hundred injections.

Dynamically loaded chelating exchange, where the complexing groups are present in the eluent and can be adsorbed onto the column resin, has been utilised by Elchuk et al. (22) who used mandelic acid as an eluent for the separation of transition metals, lanthanides and actinides. A number of good separations were achieved, and for the transition metals the elution order corresponded to the order expected for chelating exchange. The retention of copper varied markedly with change in pH, and increasing the mandelic acid concentration improved peak symmetry, whilst calcium was not retained as strongly as manganese, which forms weak chelates. Increasing the concentration of mandelic acid also significantly decreased retention times, but the opposite effect than would normally be expected occurs when the pH is increased. Retention times increased with increasing pH, which suggests that the mechanism is not due to the formation of anionic metal-ligand complexes with the actinides.

Large particle size resins have been coated with chelating agents, and some separations of two or three metal ions can be achieved. Cheng and Guh (103) investigated ligand sorption and chromatographic separation of metal ions on XAD-2 resin. Chelating agents were immobilized onto the resin. They demonstrated their use for the preconcentration and separation of metal ions, and for purification. Separations were achieved by selective elution of metal ions using various eluting mixtures. Detection of the fractions was obtained using different colorimetric reagents. It was found that the retention of metal ions, and the actual adsorption of chelating agents were greatly influenced by pH. Retention behaviour at pH6 with different coated columns showed that their selectivity was very different. 8-hydroxyquinoline adsorbed a range of metal ions and would be suitable for separation of metal ions in polluted waters. 5-chlorobenzotriazole would be highly selective for silver in the presence of EDTA, whilst with 8,8'-

(ethylenediimino)diquinoline, at pH6, only copper is retained on the column. Breakthrough curves for 1,10-phenanthroline showed that many metal ions could possibly be separated from mixtures. No selectivity was observed with tetraphenylboron, but it tends to decompose in acidic media. These breakthrough studies enabled some separations to be achieved. Magnesium was separated from copper using the 8-hydroxyquinoline column, whilst lead, copper and silver could be separated using 5-chlorobenzotriazole resin. Lead could be separated from copper using the 8,8′-(ethylenediimino)diquinoline resin. These separations were not isocratic, and depended on the complexing agents used for the selective elution.

Chao and Suzuki (104) studied the adsorption behaviour of scandium, yttrium, cerium and uranium on a Xylenol Orange coated anion-exchange resin. The maximum exchange capacity was observed in the pH range of 2.7-3.1. The adsorption of the metal complexes was very dependent on pH. Little adsorption occurred at pH1 or less due to the instability of the complexes, but on reaching pH4-5, virtually all of the metal complexes are adsorbed. By reducing the pH of the eluent during a run, cerium could be separated from scandium, and yttrium from scandium. Cerium and yttrium could be separated isocratically at pH2.75. Xylenol Orange was coated onto Amberlyst A-26 macroreticular anion-exchange resin by Brajter and Olbrych-Śleszyńska (105) and the uptake of a wide range of metal ions investigated. There was a strong dependence of retention time with pH, where it was possible to separate some ions. Separations of aluminium and zinc from indium were achieved, together with a separation of manganese and copper. The eluent, however, was changed to elute each metal ion in turn. Copper was retained very strongly on this coated column, with 1M hydrochloric acid necessary for elution.

Sharma and Misra (106) impregnated silica gel-G layers with dimethyl sulphoxide for separating cadmium, tungsten and zirconium from transition metal ions by thin layer chromatography. Using various solvent systems, some of these metal ions could be separated from over twenty other metals.

Very little work has been carried out to investigate coated high performance grade substrates for the chelating exchange of metal ions. Yamazaki et al. (107) coated a reverse phase silica with N-n-dodecyliminodiacetic acid to separate alkaline earth metal ions. Four alkaline earth metals were separated, and also magnesium and calcium in sea water, however, the elution order suggested that the process was ion-exchange and not chelating exchange. The elution order was not consistent with the stability constants for iminodiacetic acid, but tartaric acid used as an eluent may have an effect as it is a complexing acid. One of the first more detailed studies into dye-coated chelation columns using high performance grade substrates was carried out by Jones and Schwedt (108). A neutral Benson BPI-10 10μm particle size neutral polystyrene based resin was coated with Chrome Azurol S. This dye gave the deepest coating in preliminary coating experiments using XAD-4 coated with ten different chelating dyes. Separations of divalent and trivalent metal ions in 1M potassium nitrate were achieved using this column, but the efficiency was relatively low. The preconcentration and separation of several divalent metal ions was also demonstrated using only one column. To improve peak shapes, and separate a wider range of metal ions, step gradient elution was employed. Slower exchange kinetics were observed for trivalent metal ions, so the column temperature was increased to 60°C, where aluminium, indium and gallium were completely separated at pH2.25. Preconcentration and determination of aluminium proved to be quantitative. These extremely encouraging results were the basis for this further study.

1.6. DETECTION SYSTEMS.

There is a range of detection methods for detecting metal ions after they have been separated by ion chromatography. Detection in the early days of ion chromatography involved the collection of fractions as they eluted off the column. When commercial instrumentation began to be developed for detection, the obvious choice for ion chromatography was conductimetric detection, as this is a universal property of ionic solutions and is a function of concentration. Small et al. (6) developed the method of 'modern' ion chromatography utilizing suppressed conductimetric detection. By using a combination of resins, the background conductivity could be neutralised, enabling the conductivity detector to become much more sensitive, as eluent conductivity is virtually eliminated. Separations of a variety of organic and inorganic cations and anions could be determined. An historical account of the evolution of conductimetric detection from its early days to recent developments, has been produced by Small (5).

Electrochemical methods such as amperometric or coulometric detection have been used. Other detection methods include ion selective electrodes, together with refractive index detectors, however, these are rarely used in ion chromatography.

Spectrophotometric methods such as ultra-violet and visible absorption have been widely used. The absorbance is directly related to the concentration through the Beer-Lambert law, which states that :-

$$\text{Absorbance} = \log_{10}\frac{I_0}{I} = \epsilon c l$$

where I=intensity of transmitted light, I_0=intensity of incident light, ϵ=molar extinction coefficient, l=path length and c=concentration of absorbing species. Direct UV is used principally for aromatic and heterocyclic acids and amines, as these absorb in the UV range. Jones and Schwedt (32) detected the chloro complexes of the platinum group metals and gold using UV detection. Indirect photometric detection has been developed by Small and Miller (11), where a decrease in absorbance of eluent ions during elution is measured, however, a disadvantage is that there is a high background level.

A review of detection methods in ion chromatography has been produced by Rocklin (109). Further examples of the development of detection in ion chromatography, particularly spectrophotometric methods, can be seen in reviews by Nickless (1) and Robards et al. (2).

Different major analytical techniques can be connected via a suitable interface. These coupled techniques have now been widely used. An ion chromatography system can be coupled to element specific detectors such as AAS, ICP-MS, and ICP-AES. Recently, Riviello et al. (63) improved the performance of ICP-OES and ICP-MS by coupling to an ion chromatography system. Ion chromatography also was used to modify the sample so it was suitable to be introduced into the spectrometer. Detection of over sixty elements, which can be quantified simultaneously, with detection limits in the mid to low ppb levels could be obtained, however, interferences are common and have to be overcome. In many cases these coupled techniques are used for the detection of metal ions after preconcentration using a chelating exchange column. Porta et al. (110) coupled an on-line preconcentration system to ICP-AES for the detection of a range of divalent metal ions in Antarctic sea water. Atomic fluorescence detection has been used by Mackey (79) to study interactions of trace metals on XAD-1 and XAD-2.

Colour forming complexing agents can be added to the eluent to enable on-column chelation with metal ions and visible detection of their chelates after separation. This system only requires a single pump. Smith and Yankey (111) incorporated dithiocarbamates in the eluent for the determination of several transition metals. DiNunzio et al. (112) separated the PAR chelates of zinc, iron, nickel and cobalt by on-column chelation. Toei (75,101,102) has used several colour forming reagents for the detection of transition and alkaline earth metals. Xylenol Orange has been used for the detection of nickel and zinc (101). Alkaline earth metals have been detected by the use of Arsenazo III (102), and o-cresolphthalein complexone (75) in the eluent.

The most versatile approach for the detection of metals is probably the use of post-column reagents as discussed in the next section.

1.6.1. POST-COLUMN DETECTION.

Post-column detection involves the use of a post-column reagent, where after the eluent has passed through the analytical column, these two reagent streams are mixed. Metal ions eluting off the column after separation are detected by a change in a particular physical or chemical property when mixed with the post-column reagent. Takata and Fujita (8) developed a flow coulometric detector for the detection of heavy metal ions after separation. The response of this coulometric detector was sufficiently rapid for high speed separations. The most popular post-column reagent involves the formation and absorbance of highly coloured complexes. These require a rapid reaction and a constant low background level. The reagent itself should be highly stable. There are many photometric reagents that can be used as seen in a book by Sandell and Onishi (77).

Post-column reagents can be used for the detection of organic molecules, for example, Al-Najafi et al. (113) determined six amino acids using ninhydrin as the colour forming post-column reagent. This system utilized computer-assisted optimisation of six and seven experimental variables, producing a significant improvement in separation, sensitivity and analysis time. However, post-column reagents are also used extensively for the detection of metal ions as described in detail below.

One of the first general post-column reagents used for the detection of metal ions was PAR. Anderson and Nickless (78) carried out a complete investigation into heterocyclic azo dyestuffs, including PAR, and reviewed the many uses of PAR as a complexometric and spectrophotometric reagent. PAR reacts with a wide range of metal ions, but does not react with the alkali metals, chromium(VI), antimony(III), molybdenum(VI), tungsten(VI) and arsenic(III) or (V). The formation of the various metal-PAR complexes is dependent on pH.

A flow system using PAR was used by Fritz and Story (7) in the early studies of ion chromatography separations. PAR does not react very much with alkaline earth metals, however, by adding ZnEDTA which is a weak complex, a metal displacement reaction occurs, and the signal measured is due to ZnPAR :-

$$M^{n+} + ZnEDTA \rightleftharpoons MEDTA + Zn^{2+}$$

$$\text{then} \qquad Zn^{2+} + PAR \rightleftharpoons ZnPAR$$

Arguello and Fritz (9) separated calcium and magnesium from several other metals, and found that the PAR-ZnEDTA gave a much superior sensitivity compared with other post-column reagents used. Jezorek and Freiser (114) studied the PAR-ZnEDTA system used in a continuous metal ion detection system. Thirteen metal ions were investigated and their responses with PAR and PAR-ZnEDTA measured and compared. Using PAR-ZnEDTA improved responses with alkaline earth metals, lead and manganese significantly, especially the alkaline earth metals. Reduced response was obtained however, with zinc, aluminium and iron(III) and particularly mercury(II). There was little change for nickel, copper and cobalt. If stored in the dark in plastic containers, the PAR-ZnEDTA proved to be very stable, with little or no change after five weeks. This reagent was also used to look at contamination in the various eluents used, by measuring the absorption at 510nm. Yan and Schwedt (15,18) later used the PAR-ZnEDTA system for multi-element analysis by ion chromatography of transition, heavy and alkaline earth metals, with separations of up to thirteen metal ions achieved. Wang et al. (29) obtained several separations of transition metals using PAR and PAR-ZnEDTA post-column reagents, with a comparison for nine metal ions made. Sensitivity for lead and manganese was much improved with PAR-ZnEDTA, whilst for iron(III), iron(II) and cadmium a reduction in sensitivity occurred compared with PAR alone. Chambaz et al. (60) recently used PAR-ZnEDTA for the detection of trace metals in river water, however, the linear range for lead was low, due to the low concentration of ZnEDTA used to minimize the increase in background signal. PAR and PAR-ZnEDTA prove to be very sensitive spectrophotometric reagents, with low ppb level detection limits achieved with preconcentration.

A wide range of metal ion separations with speciation has been achieved by Heberling and Riviello (27) using PAR. Metals are determined colorimetrically using PAR either in the pH range 3 to 6, or 9 to 11, or both. Overall, forty-eight different metals can be determined, principally transition, heavy and lanthanide metals. PAR was used as a post-column reagent by Saraswati et al. (25,26) in the analysis of low alloy stainless steel samples. Stable complex formation with PAR was obtained with vanadium(V), manganese, cobalt, nickel, copper, zirconium(IV), tantalum(V), niobium(V), lead and cerium(III). Other examples of the use of PAR as a post-column reagent for transition metal detection has been shown by Faltynski and Jezorek (68), Simonzadeh and Schilt (69), and Elchuk et al. (22).

Recently, Gomez et al. (115) simultaneously determined metal-PAR complexes using a diode array spectrophotometer and a multiple linear regression program to analyze the spectra. No separation of metal ions was necessary, and the wavelength range was from 450-600nm. However, a compromise PAR concentration had to be used to achieve a good response for seven metal ions. Metal ions with low molar absorptivities such as lead, could not be minor components of mixtures, and there was a problem with accuracy with five metal mixtures. This method was successfully applied to analysis of brass and coin alloys. However, the results obtained were only approximate, though estimates could be made very fast with just one measurement.

Two other post-column reagents which have been used for some time are Arsenazo I and Arsenazo III. Fritz and Story (7) investigated these in their study of early ion chromatography, together with PAR. Arsenazo I is a sensitive reagent for the determination of magnesium and calcium, and does not react with many other metal ions. Arsenazo III reacts with a good range of metal ions, notably aluminium, chromium(III), calcium, strontium and barium, which do not react with PAR, as well as some divalent metal ions, lanthanides and tetravalent metal ions.

Arsenazo I was used by Arguello and Fritz (9) for the separation and determination of calcium and magnesium by ion chromatography, with good sensitivity. Much higher molar absorptivities, and thus sensitivities were achieved using the PAR-ZnEDTA post-column reagent. Elchuk and Cassidy (10) used Arsenazo I for the detection of all the lanthanides after separation by ion chromatography. Using on-column preconcentration of 100ml of sample, detection of metal ions to 10ppt could be possible. Wang et al. (29) also used this reagent for the detection of lanthanides after their separation.

Arsenazo III was employed as a post-column reagent by Hamilton et al. (24) for the quantitative analysis of thorium in plutonium. Interferences were studied, as actinides, UO_2^{2+}, NpO_2^{+}, Pu^{3+}, Pu^{4+} and Am^{3+} together with yttrium(III), iron(III) and lead react with Arsenazo III. A separation of some of these species was also achieved, but most eluted before the thorium(IV) peak and did not interfere. Saraswati et al. (25,26) used Arsenazo III for the detection of zirconium, chromium(III), cerium(III), copper, aluminium and lead in stainless steels. These metal ions gave better results compared with PAR, although aluminium and chromium(III) do not react with PAR. Molybdenum could not be studied in stainless steels as it does not react with PAR, Arsenazo I or III. Elchuk et al. (22) also used Arsenazo III for the detection of the lanthanides, thorium, uranium and other actinides, after separation.

Eriochrome Black T, and its more stable analogue Calmagite has been developed by Jones et al. (12,13,14) for trace metal detection. A decrease in absorbance is measured (inverse photometric detection), so reagent background levels are higher. However, these reagents proved to be good general metal detection systems. Eriochrome Black T was used to detect a range of metal ions in magnetic and monel alloy, with good quantitative performance (12). Later, the analysis of trace metals, particularly cobalt in simulated pressurized water reactor primary coolant was demonstrated using preconcentration and the Eriochrome Black T detector, with detection limits as low as 10ppt for cobalt (14). Ten metal ions could be separated by ion chromatography utilizing on-line preconcentration (14).

Calmagite (CAL) is a more stable analogue of Eriochrome Black T. Eriochrome Black T does tend to be oxidised with time, and has to be made up fresh every day. Calmagite was used by Jones and Schwedt (108) to detect some divalent metals in aqueous and 1M potassium nitrate media using dye-coated columns. Handley et al. (17,34), used a Calmagite-MgEDTA post-column reagent for the detection of alkaline earth metals together with aluminium and zinc, in concentrated brines. This metal displacement reaction works in a similar way to the PAR-ZnEDTA post-column system, where magnesium is displaced :-

$$M^{n+} + MgEDTA \rightleftharpoons MEDTA + Mg^{2+}$$

$$\text{then} \qquad Mg^{2+} + CAL \rightleftharpoons MgCAL$$

This enabled strontium and barium to be detected, which do not react with Calmagite alone. Bowles et al. (21) employed a similar detection system using Eriochrome Black T and MgEDTA for the detection of magnesium, calcium and strontium and several other transition metals.

Other post-column reagents have been employed for trace metal detection, many of which are more selective and aimed at a particular group of metal ions. Xylenol Orange has been used by Hirose et al. (23) in a micro-HPLC technique for the separation and detection of rare earth metals. Dithizone was employed by Jones et al. (116) as a multi-element detection system, measuring a decrease in absorbance, with some transition and heavy metals. A problem occurred with precipitation as dithizone is more soluble in acetone than water. However, an 80% acetone:water mixture was used as the eluent in which dithizone readily dissolves. Yamazaki et al. (107) used o-cresolphthalein complexone for the detection of alkaline earth metal ions, and it proved to be more suitable than PAR or Arsenazo I due to its low absorbance without metal ions present. In the chelating-exchange studies by Jones and Schwedt (108) using dye-coated columns, Pyrocatechol Violet was employed to detect aluminium, gallium, indium, bismuth and iron, and Chrome Azurol S for the detection of beryllium. Jones et al. (33) also developed a molybdenum blue post-column reagent for the detection of arsenic, germanium, phosphorus and silicon oxoanions.

1.6.1.1. FLUORESCENCE AND CHEMILUMINESCENCE DETECTION.

For lower detection limits to be achieved with direct injection of the sample, more highly sensitive detection methods can be used. Fluorescence detection, using a fluorophor-forming post-column reagent is more sensitive than UV-visible detection. For the detection of alkaline earth metals, Yamazaki et al. (107) used ethanolamine containing 8-hydroxyquinoline-5-sulphonic acid and also MgEDTA in the mixture, for the detection of calcium. Jones et al. (13) showed that 8-hydroxyquinoline-5-sulphonate gave good fluorescence with aluminium, gallium, indium, magnesium, cadmium and tin(II), with sensitivities up to ten times greater than with conventional UV-visible detection. Later, this post-column reaction system was used for the determination of trace amounts of aluminium (36), and applied to the analysis of aluminium in monel alloy and tap water. The linear range was over nearly four orders of magnitude from 5 to 10000ppb, and the detection limit was 1ppb aluminium. This system was developed further by Jones (37) for the detection of several aluminium species, including aluminium fluoro species, in natural and potable waters. This reagent was also used recently by Jones and Paull (38), for the study of aluminium speciation of potable waters in the South West of England.

An even more sensitive detection method with detection limits in the sub-ppb range is chemiluminescence. Williams et al. (35) determined chromium(III) and chromium(VI) at ultra-trace levels using a post-column reaction with luminol, with detection limits 0.1ppb and 0.3ppb respectively. The linear range was over three orders of magnitude from 1ppb to 1ppm. Detection limits could be improved with purer reagents. Jones et al. (19) used this luminol chemiluminescence reaction for the determination of cobalt at picogram levels, with good agreement with a certified reference material. The sensitivity of this chemiluminescence detection system for cobalt was better than graphite furnace atomic absorbance spectrometry and ICP-MS. Jones et al. (20) developed this detection further to enable multi-element determinations after separation by ion chromatography. This was achieved by modifying the luminol-peroxide post-column reagent with the addition of CoEDTA, so that the cobalt is displaced by other metal ions eluting off the column, before reacting with the luminol. The general metal displacement reaction that takes place is shown below :-

$$(CoEDTA)^{2-} + M^{n+} \rightleftharpoons Co^{2+} + (MEDTA)^{(4-n)-}$$

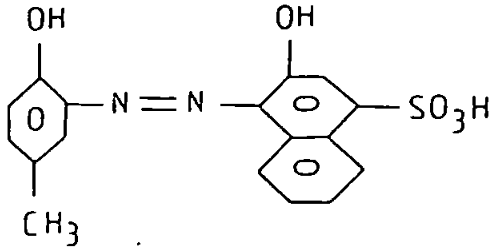
This detection system was non-selective and detected a large range of metal ions including alkaline earth, transition and lanthanide metals. Some calibrations were however non-linear, producing a negative curvature, but this is probably due to kinetic and thermodynamic factors of the metal displacement reaction. Many separations could be achieved with this system, with detection limits ranging from 2 to 100ppb, depending on the metal ion, and even the calibrations which showed some non-linear nature could still be useful for quantitative analysis.

1.7. AIMS AND OBJECTIVES.

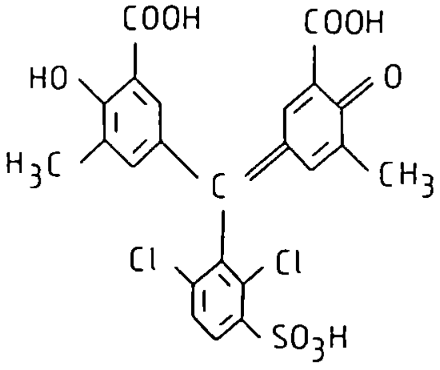
As can be seen in the literature review (Section 1.5.2.2.), little work has been carried out to investigate high performance separations of metal ions in high ionic strength matrices using chelating dye coated substrates. The encouraging results from the work by Jones and Schwedt (108) formed the basis for this research. More detailed studies on the effect of dye type and substrate characteristics needed to be carried out, and this will form the initial investigations of this work. This involved studying the retention properties of metal ions with various dye coated columns, and the effect of parameters such as pH and ionic strength. Three chelating dyes will be chosen with a range of chelating ability, namely, Calmagite, Chrome Azurol S, and Xylenol Orange, and their structures are shown in Figure 7.

After these initial studies, it was clear that particular emphasis needed to be placed on separations in high ionic strength media using high performance dye coated substrates. Detailed investigations of the effect of mobile phase conditions on separation will be carried out and optimized for selected groups of metal ions. The main aim of this research is to combine the preconcentration and high performance separation of trace metals in various high ionic strength matrices using a single dye-coated HPLC grade chelation exchange column. Finally, the most promising columns will be applied to the quantitative analysis of samples such as concentrated brines and sea waters.

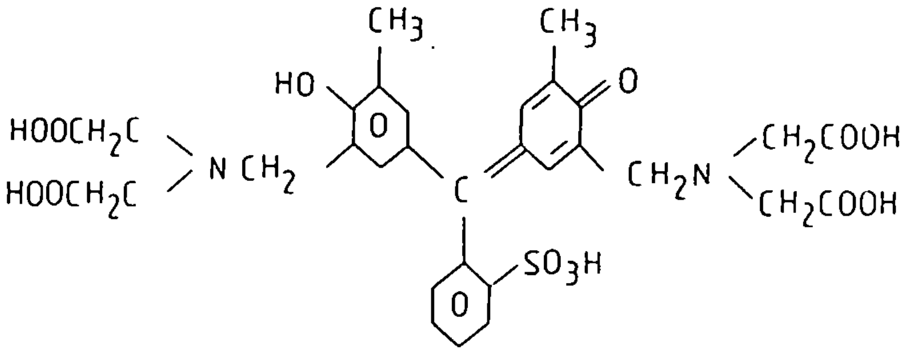
The post-column detection systems reported previously also needed to be improved, and this study was carried out in conjunction with the above investigations. This was considered important since lower detection limits would be achieved, and the detection of a wider range of metal ions facilited.



CALMAGITE.



CHROME AZUROL S.



XYLENOL ORANGE.

Figure 7. Structures of Calmagite, Chrome Azurol S and Xylenol Orange.

CHAPTER TWO.

EXPERIMENTAL.

2.1. INSTRUMENTATION.

The instrumentation involved for the determination of trace metals employed a HPLC post-column reaction system. In the initial studies involving separation and detection of metal ions by direct injection, a basic HPLC system was used. Later this was modified by the addition of a preconcentration cycle to determine trace metals in the low ppb range.

2.1.1. BASIC CHELATION ION CHROMATOGRAPHY POST-COLUMN REACTION SYSTEM.

A basic HPLC post-column reaction system was employed for direct injection of samples onto the column. This system is shown in Figure 8. It consists of two pumps, the mobile phase (eluent) pump and the post-column reagent pump. The eluent is pumped through a six port switching valve and through the analytical column to mix with the post-column reagent stream at a zero dead volume T-piece. When the switching valve is in the load position, the sample can be drawn through the sample loop. On injection, the sample in the sample loop passes onto the analytical column. Metal ions eluting off the column mix with the post-column reagent at the T-piece, then the mixture passes through a reaction coil before reaching the UV-Visible detector. Detection is achieved due to a change in absorbance, and this measurement is in the form of an electrical signal which can be passed to a chart recorder or integrator to obtain a chromatogram.

The instrumentation consisted of two ConstaMetric Model III pumps (Laboratory Data Control, Riviera Beach, FL, USA.) for the eluent and post-column reagent. A steel injector with 100μl sample loop (Rheodyne, Cotati, CA, USA.) was used for direct

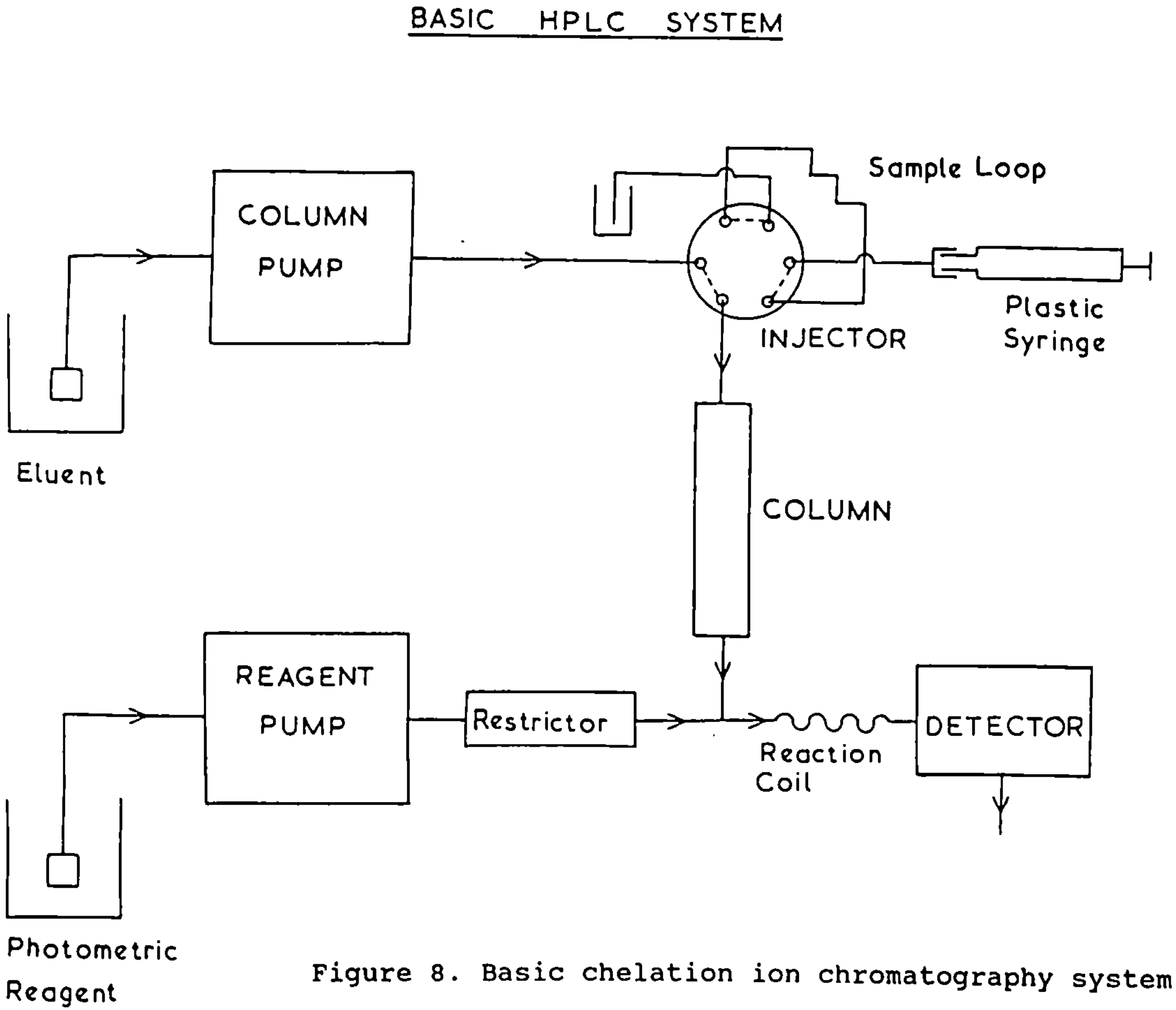


Figure 8. Basic chelation ion chromatography system.

injections, and the UV-Visible detector was a Spectroflow Monitor SF770 (Schoeffel Instrument Corporation, Westwood, NJ, USA.). Some of the later isocratic separations and gradient elution work employed a Spectral Array Detector (Dionex, Sunnyvale, CA, USA.).

2.1.2. CHELATION ION CHROMATOGRAPHY POST-COLUMN REACTION SYSTEM FOR PRECONCENTRATION AND SEPARATION OF METAL IONS.

The basic chelation ion chromatography system was modified to include a preconcentration cycle to enable low ppb levels of trace metals to be detected. This system is shown in Figure 9. Two injection valves were used to enable either preconcentration or a direct injection onto the column. When the preconcentration valve is in the load position, the sample can be pumped through the column and the required volume preconcentrated before injection.

The preconcentration system was composed of entirely inert materials which comprised an Eldex Laboratories Model AA pump with PTFE lined pump heads and check valves (Eldex Laboratories, Menlo Park, CA, USA.), and a titanium injector (Valco, Schenkon, Switzerland). All connecting tubing and junctions were composed of PTFE.

2.2. CHELATING EXCHANGE COLUMNS.

For the initial investigations into chelating dye-coated columns, three chelating dyes coated onto three large particle size substrates were studied. The chelating dyes were Xylenol Orange (BDH, Poole, Dorset, UK.) and Calmagite and Chrome Azurol S (Sigma Chemicals, St Louis, MO, USA.). Large particle size resins investigated were Dowex 1-X8(Cl) anion exchange resin, and Amberlite neutral XAD-2 and XAD-4 resins (BDH.).

The Dowex anion exchange resin contained quaternary ammonium

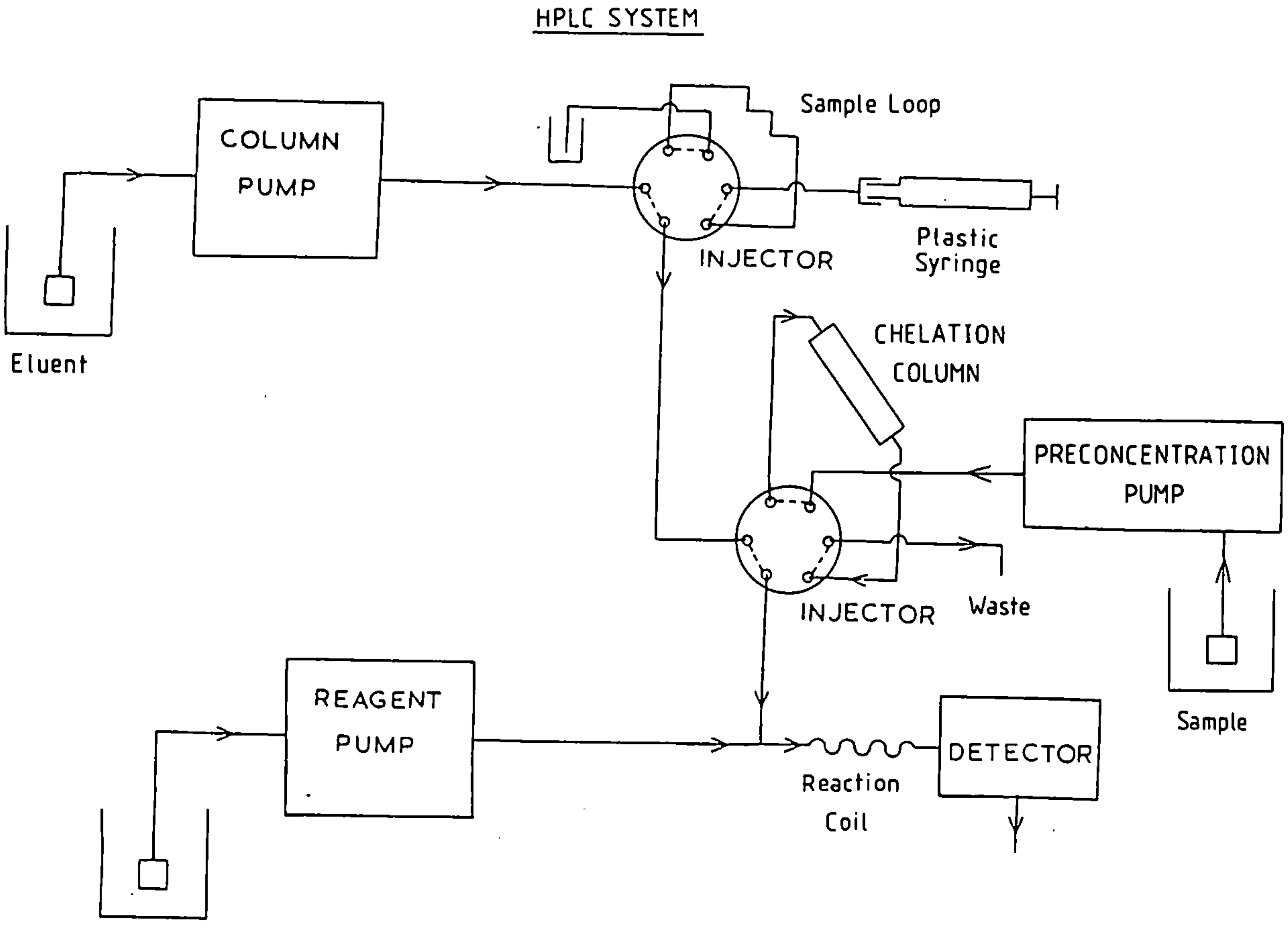


Figure 9. Chelation ion chromatography system.

groups bonded to cross-linked polystyrene-divinylbenzene, with particle sizes ranging from 0.075 to 0.150mm in diameter. The neutral XAD-2 and XAD-4 resins were crossed-linked polystyrene absorbent beads with no ionic groups attached and with particle sizes of between 0.3 and 0.78mm. These dye-coated resins were packed into 10cm x 4.6mm i.d. stainless steel columns.

The majority of the work involved high efficiency studies. Xylenol Orange and Chrome Azurol S were coated onto small particle size HPLC grade resin. This was a PLRP-S neutral 8μm, 100Å macroporous polystyrene-divinylbenzene substrate (Polymer Laboratories, Church Stretton, Shropshire, UK.). This was packed into a 10cm x 4.6mm i.d. inert metal free PEEK (polyether ether ketone) column (Alltech Chromatography, Carnforth, Lancashire, UK.).

Two commercially available chelating columns were also studied. A TOSOH TSK-GEL Chelate-5PW column (7.5cm x 7.5mm i.d.)(TOSOH, Tokyo, Japan.), was investigated for retention and separation of trace metal ions. Also, a Dionex MetPac CC-1 column (5cm)(Dionex, Sunnyvale, CA, USA.) was investigated for the retention of metal ions.

2.2.1. COATING AND PACKING OF LARGE PARTICLE SIZE DYE-COATED COLUMNS.

The dye-coated resins in each case were prepared by dissolving 0.1g of dye in 50cm^3 of 20% methanol:water mixture, to give a 0.2% dye solution. Enough resin was added to pack a 10cm column, and the mixture stirred overnight on a magnetic stirrer, to enable an even coating to be obtained.

Before packing the column, the end fittings, frits and the column itself were cleaned in 10% methanol:water mixture in an ultra-sonic bath for around an hour. The dye coated resin was then packed into the column by using a micro-spatula, soaking up excess liquid. When the column appeared fully packed, it was connected up to a HPLC pump, and DDW pumped through at $3cm^3$/min to compress the packing material, before opening up and filling any void with more dye-coated resin. This process was repeated until there were no voids. All these large particle size coated columns were conditioned before use, by eluting through (a) distilled deionized water (DDW), (b) dilute ammonia, until eluent is colourless, (c) dilute nitric acid, to wash any metal impurities off the column, (d) dilute acetic acid, and (e) DDW.

2.2.2. PACKING AND COATING OF SMALL PARTICLE SIZE DYE-COATED COLUMNS.

The resin chosen for high efficiency studies was Polymer Laboratories 8μm, 100Å polystyrene-divinylbenzene resin (PLRP-S). Before coating, a 10cm PEEK column, which is completely metal free and inert, was packed with this resin using the column packer. Column, end fittings, and frits were cleaned in 10% methanol:water in the ultra-sonic bath for around an hour. 1g of resin was weighed out into a glass vial, and a slurry made with approximately $30cm^3$ of methanol:water mixture (10:20); the methanol required to thoroughly wet the resin. To obtain an evenly dispersed mixture of particle sizes, the slurried resin was placed in the ultra-sonic bath for around five minutes. Using the packing adapter for the PEEK column, the column was packed on the column packer (Shandon), at a pressure of 2000psi. This resin may collapse at pressures above 3000psi, although it has high mechanical stability. $80cm^3$ of the 1M potassium nitrate reservoir was passed through the column, before turning the column around and collecting another $80cm^3$ of potassium nitrate. The compressed air cylinder was turned off to allow the pressure to slowly drop to atmospheric pressure when packing was complete, or else a sudden pressure drop would cause air bubbles to form in the column. Any voids were filled, and the end of the column made slightly concave with a microspatula, before placing the frit on top and sealing the column.

The packed PLRP-S column was coated by recycling 100cm^3 of a 0.2% solution of the dye, dissolved in 10% methanol. Before coating began, a 1cm^3 sample of the dye solution was taken and diluted to 100cm^3 with DDW in a volumetric flask. An absorption spectrum, using a Perkin-Elmer Lambda 7 UV-Visible spectrophotometer, was obtained of this solution. The dye solution was pumped through the column at 1cm^3/min, and left for around twenty hours to obtain a good even coating. An absorption spectrum of the remaining dye solution was then obtained, using the same procedure as above. The difference in absorption at the wavelength of maximum absorption, enables the quantity of dye coated onto the resin to be calculated. The pH of these solutions had to be the same, and a slight adjustment was made if necessary.

The dye-coated column was conditioned with (a) DDW, (b) dilute ammonia, until there was no further bleed, (c) dilute nitric acid, to elute any metal impurities, (d) dilute acetic acid and (e) DDW, and any bleed collected. Another absorption spectrum of the bleed was obtained, after dilution to the appropriate volume, and any adjustment in pH made, so that it was the same as the dye-coating solution, before measurement. This enabled the amount of dye which remained coated on the resin to be calculated.

2.2.3. CLEAN-UP COLUMNS.

There was a need for a clean-up column when using gradient elution, as metal impurities in the eluent tend to be preconcentrated at the starting pH. An online system was investigated by using the Xylenol Orange coated XAD-2 dye-coated column, which was placed before the injection valve. During the analytical separation this was switched offline.

Several offline clean up systems were also investigated. Chelex 100 (Sigma Chemicals) and Duolite ES467 (obtained from ICI Chemicals & Polymers, Runcorn, UK.) resins were slurry packed into 25cm^3 burettes. These columns were gravity fed, with an adjustable tap to control the flow rate on the inlet tube. Before the eluent could be cleaned up, 0.1M borate buffer, adjusted to pH10 was passed through the column, until the outlet pH was 10. Around 50cm^3 of eluent adjusted to pH10, was then passed through the column, before collection of the cleaned eluent began. The retained metal impurities were later eluted off the column by passing through 0.2M nitric acid. It was often necessary to backflush the column with water, as these resins, especially Chelex-100 contract and swell with changing pH. These columns were protected with black card to avoid any decay due to ultra-violet light.

The Xylenol Orange coated XAD-2 column was used in an offline clean-up system. This column was connected to an Eldex model AA pump. Before cleaning, around 20cm^3 of pH10, 0.1M borate buffer were pumped through. This was followed by around 20cm^3 of eluent or buffer, before collecting. The flow rate was approximately 1cm^3/min, and the metal impurities later eluted off the column with 0.2M nitric acid. When cleaning large volumes, the metal impurities on the column had to be eluted off after cleaning around 300cm^3, or the column may become saturated with impurities.

2.2.4. PRECONCENTRATION PROCEDURE.

The sample was adjusted to an appropriate pH using dilute nitric acid or dilute ammonia, depending on the metals of interest. Before preconcentration it was necessary to equilibrate the column to the same pH as the sample by pumping 10cm^3 of the appropriate buffer (0.1M borate for pH10 or pH11, and 0.1M hexamine for pH6). This was followed by 10cm^3 of the sample, and finally 3cm^3 of distilled deionized water. This was all carried out with the potassium nitrate eluent bypassing the column as shown in Figure 9. The gradient program was initiated by switching the titanium injector to allow the potassium nitrate eluent to pass through the preconcentration column. The pH of the 1M potassium nitrate eluent was then changed in a series of steps at specified times as described in Chapter 3.

2.3. REAGENTS.

All reagents in general were obtained from BDH. PAR, ZnEDTA and sodium sulphate were obtained from Fluka (Fluka, Buchs, Switzerland.), and Calmagite and CAS from Sigma Chemicals. All reagents and working standards were prepared using distilled deionized water obtained from a MilliQ system (Millipore, Bedford, MA, USA.), and were degassed using helium before use, to remove any air bubbles. The eluent and post-column reagent were pumped at a flow rate of 1cm^3/min.

2.3.1. ELUENTS.

The eluent used throughout this work was potassium nitrate. This was either 0.2M or 1M in concentration for initial studies, but generally 1M potassium nitrate was used, containing 0.05M lactic acid for divalent metal ion studies. For trivalent metal ions, 1M potassium nitrate alone was used as the eluent. When using step gradient elution, several eluents at a different pH were prepared, and switched at the appropriate time in the gradient or step gradient program. The eluents were adjusted to the appropriate pH using either dilute nitric acid or dilute ammonia.

2.3.2. POST-COLUMN REAGENTS.

Several post-column reagents were employed for the detection of trace metal ions. Calmagite with a final concentration of 2.5×10^{-4}M was prepared by dilution of a $4gl^{-1}$ stock solution, and made up in 1M ammonia. A decrease in absorbance was measured at 610nm. Later, 1.2×10^{-4}M PAR / 2×10^{-4}M ZnEDTA post-column reagent was used for the detection of divalent metal ions, particularly for alkaline earth metals. These were prepared by dilution of 4×10^{-3}M stock solutions and made up in 2M ammonia. An increase in absorbance was measured at 490nm.

For the detection of trivalent metal ions, two post-column reagents were investigated. A 0.008% solution of CAS in 0.5M hexamine at pH5.6 was used, and an increase in absorbance at 546.5nm measured. Also, a 0.004% of Pyrocatechol Violet (PCV) in 0.5M hexamine at pH6, was used for the detection of trivalent metal ions. An increase in absorbance was measured at 580nm.

2.3.3. SAMPLES.

All metal standards were prepared by appropriate dilution from 1000ppm analytical grade standards, using distilled deionized water. Sodium chloride and potassium chloride brines were obtained from ICI Chemicals & Polymers Ltd., Runcorn, UK. Synthetic sea water was prepared by dissolving the appropriate quantity of magnesium and calcium nitrate in approximately 0.5M sodium chloride (ten fold dilution of ICI sodium chloride brine), to give 1270ppm Mg^{2+} and 400ppm Ca^{2+}. A real sea water sample was obtained from Plymouth Sound. Laboratory chemical samples were 1M in concentration.

For injections, the samples were adjusted to the same pH as the eluent, or in the case of gradient elution, adjusted to the starting pH. To avoid any possible precipitation when preparing spiked samples in concentrated brines and sea waters, the sample itself was adjusted to the appropriate pH, before the metal ions were added.

2.4. PURIFICATION OF CHROME AZUROL S.

In general, commercially available dyestuffs can be very impure, and impurity levels vary greatly depending on the manufacturer. Commercially available CAS is often only up to 65% pure. One of the two columns coated with CAS was coated with purified CAS.

A batch of CAS was purified by dissolving 1g of impure CAS in 100cm^3 of 0.1M sodium hydroxide, to convert it into the sodium form. To precipitate purer CAS, 50cm^3 of concentrated hydrochloric acid producing around 5M HCl in solution, was added. The mixture was then centrifuged for one hour to completely separate the precipitate from solution and left to settle, before decanting the liquid layer. Cleaning the precipitate was done by adding a little 1M HCl, and centrifuging for about twenty minutes. The solid was then dried on a watchglass in an oven at 40°C.

2.5. DETERMINATION OF THE CONCENTRATION OF CONCENTRATED SODIUM AND POTASSIUM CHLORIDE BRINE SAMPLES.

This was carried out using Mohr's method for the determination of chloride by titration. A 0.1M solution of silver nitrate was accurately prepared. The sodium and potassium chloride brines were diluted by a factor of 50 using graduated flasks, to be in the concentration range for the titration.

$25cm^3$ portions of these diluted brines were pipetted into conical flasks, and a little solid sodium hydrogen carbonate added until effervescence ceased, as the optimum pH for titration is neutral to slightly alkaline. Then $2cm^3$ of 5% potassium chromate indicator (K_2CrO_4) was added, and the mixture titrated against 0.1M silver nitrate until the first signs of a brick red/buff precipitate occurred.

Silver chloride is less soluble than silver chromate (Ag_2CrO_4) so silver chromate precipitation is inhibited in these conditions, until no more silver chloride can precipitate out i.e. when all chloride ions have been used up, at the end point (117).

CHAPTER THREE.

RESULTS AND DISCUSSION.

PART A.

3.1. INITIAL INVESTIGATIONS USING LARGE PARTICLE SIZE DYE-COATED COLUMNS.

Preliminary studies involved large particle size dye-coated substrates packed into glass columns. These showed that metal ions were being retained and were eluted by a reduction in eluent pH. As an initial investigation into dye-stuff coated columns, three chelating dyes were coated onto three different substrates in turn, and used in a HPLC system with post-column reaction detection (Figure 8). The chelating dyes were Calmagite, Chrome Azurol S and Xylenol Orange, and the substrates chosen were Dowex 1-X8(Cl) anion-exchange resin, used in the preliminary investigations work, and two neutral resins, Amberlite XAD-2 and XAD-4. These XAD resins are crossed-linked polystyrene absorbent beads. XAD-2 has an average pore diameter of 90Å, with a surface area of 330m^2/g, whilst XAD-4 has a smaller pore size averaging 40Å, but a much higher surface area of 725m^2/g.

Their chelating performance was compared by determining the pH at which a particular metal ion was completely retained on the column. Four metal ions were chosen, namely magnesium, manganese, cobalt and zinc, so as to cover a wide range of complexing ability. Calmagite was used as the post-column reagent.

Results obtained for all these dye-coated columns are shown in Tables 4 to 6. The relative chelating ability of each dye for the four metal ions can be seen, where Xylenol Orange forms the strongest chelates, and CAS forming weaker chelates. As expected, the degree of retention is in the order $Zn^{2+} > Co^{2+} >$

STANDARD.	CALMAGITE.	CHROME AZUROL S.	XYLENOL ORANGE.
Mg^{2+}	>9	>11	>10
Mn^{2+}	9	9	8
Co^{2+}	9	9	7
Zn^{2+}	8	8	5

Table 4. pH required for complete retention of 10ppm metal standard in 1M KNO_3, using dye coated Dowex 1-X8(Cl) anion-exchange resin columns.

STANDARD.	CALMAGITE.	CHROME AZUROL S.	XYLENOL ORANGE.
Mg^{2+}	10	>9	9
Mn^{2+}	9	9	7
Co^{2+}	6	9	4
Zn^{2+}	6	8	4

Table 5. pH required for complete retention of 10ppm metal standard in 1M KNO_3, using dye coated Amberlite XAD-2 neutral resin columns.

STANDARD.	CALMAGITE.	CHROME AZUROL S.	XYLENOL ORANGE.
Mg^{2+}	>10	>10	10
Mn^{2+}	10	>10	8
Co^{2+}	10	>10	6
Zn^{2+}	8	9	6

Table 6. pH required for complete retention of 10ppm metal standard in 1M KNO_3, using dye coated Amberlite XAD-4 neutral resin columns.

$Mn^{2+} > Mg^{2+}$. This order was found for all the chelating dye-coated columns tried.

With the XAD-2 dye-coated columns, the column bled badly for Calmagite and CAS coated columns when conditioned with dilute ammonia. However, when the bleeding stopped this substrate showed the strongest retention of metal ions. The XAD-2 dye-coated column results, especially for Xylenol Orange show good retention properties. With the Xylenol Orange coated column, all transition metals would be adsorbed onto the coated resin at pH8.

With the XAD-4 dye-coated columns, there was no bleeding of the dye with the Calmagite coated column, but there was with the CAS coated column when cleaned with dilute ammonia. From Table 6 it can be seen that little chelation occurred with the CAS coated column, with only the Xylenol Orange coating showing significant metal chelation.

These differences in retention properties between XAD-2 and XAD-4 coated columns could be due to stereochemical effects as XAD-2 and XAD-4 vary in pore size. XAD-2 has a larger pore size of 90Å, compared with 40Å for XAD-4. It is possible that with larger pores there is less steric hindrance for surface chelation. In preliminary experiments, XAD-4 gave a lighter coating with Calmagite than XAD-2, although the coating appeared more even with XAD-4. There may be a variation in the total chelation capacity due to the small pore size and large surface area, which may account for the poorer chelation properties of XAD-4 dye-coated columns.

3.2. SMALL PARTICLE SIZE DYE-COATED COLUMNS.

The work on large particle size dye-coated columns clearly showed that the selective adsorption of metal ions was possible. However, they are limited in separating power due to their large particle size. In order to produce a much more efficient column, capable of separating groups of metal ions, small particle size HPLC grade substrates were coated and investigated. The substrate chosen for coating was obtained from Polymer Laboratories and was 8μm diameter, 100Å pore size, HPLC grade macroporous polystyrene-divinylbenzene resin (PLRP-S). Xylenol Orange and CAS were chosen to coat this substrate as they showed a significant difference in chelating strength.

Initial investigations were carried out in a similar way to the large particle size dye-coated columns, but a larger number of metal ions was studied, and the effect of ionic strength investigated. After this the separating power of these columns were studied in detail.

3.2.1. PRELIMINARY INVESTIGATIONS.

A small quantity (5-7mg) of the resin was added to 0.2% solutions in 20% methanol of Calmagite, CAS and Xylenol Orange respectively, and left to stir overnight. This did not prove very successful, as the resin is fairly hydrophobic and remained on the surface of the solution. The adsorption of the dyes was poor and uneven and only Calmagite was well adsorbed onto the substrate surface. Around 30% of the particles were coated with CAS, with little adsorption at all with Xylenol Orange. It was then decided to coat the substrate using vacuum filtration. Around 10mg of substrate was coated by filtration of a 0.2% dye solution in 5-10% methanol through a NuFlow 0.45μm membrane filter. The dye solution was recycled several times to obtain a good coating on the substrate, and then cleaned/conditioned with dilute ammonia, dilute nitric acid and deionized distilled water (DDW). When dried, the various coated

PLRP-S resins were examined under a microscope. The PLRP-S resin itself was in the form of spherical particles, however these appeared to have a range of particle sizes. Coatings obtained by vacuum filtration were much better as observed under the microscope. With CAS the surface layer appeared deep green, whilst underneath particles were deep orange. The variation in colour may be due to impurities in the CAS. Xylenol Orange also produced a stronger coating, though some particles adsorbed more dye than others.

From these results, it is clear the best coating was obtained when some force was applied, such as vacuum filtration, or pressure, to enable the dyes to impregnate the pores of the resin. It was decided to coat a column of PLRP-S resin using moderate pressure. This was achieved by recycling a 0.2% dye solution in 10% methanol through a column, pumping at 1cm^3/min for around twenty hours to achieve an even, stable coating.

3.2.2. INITIAL INVESTIGATIONS.

Two small particle size columns were prepared by coating the 8μm PLRP-S resin with Xylenol Orange and purified CAS obtained by recrystallising the commercial product. A similar set of experiments to those described for large particle size dye-coated columns were performed, but using a larger number of metal ions.

3.2.2.1. XYLENOL ORANGE DYE-COATED COLUMN.

0.87g of resin was used to pack the column, and from absorbance measurement of the dye solution before and after coating, 52mg of the total 200mg of Xylenol Orange had been adsorbed onto the PLRP-S resin. However, during conditioning 4.5mg bled off, leaving 47.5mg of Xylenol Orange coated onto the resin.

This Xylenol Orange coated column gave much sharper peaks than with large particle size dye-coated columns, with little tailing, showing that the efficiency was much higher. A comparison of the peak shapes obtained for manganese with large and small particle size substrates coated with Xylenol Orange is shown in Figure 10. It can be seen that particle size has a large effect on peak shape.

Using the PAR-ZnEDTA post-column reagent, and a 0.2M or 1M potassium nitrate eluent (containing 0.05M lactic acid), there was much more of a variation in retention time with pH, compared with the large particle size dye-coated columns. This enabled separations to be achieved. Figures 11 and 12 show graphs of the variation of retention time with increasing pH for ionic strength of 0.2M and 1M potassium nitrate respectively. Both Xylenol Orange and Chrome Azurol S contain SO_3^- groups which can act as ion-exchange sites. At lower ionic strength, where the pH is not too high, barium is eluting after magnesium, showing that some ion-exchange is taking place on the column. This has the most pronounced effect on barium as it is held strongly by ion-exchange. For chelating exchange, the observed elution order was Ba^{2+}, Sr^{2+}, Mg^{2+}, Ca^{2+}. This elution order is achieved with 0.2M ionic strength above pH7, where chelating exchange predominates over ion-exchange. However, from stability constant data for Xylenol Orange (Table 8) magnesium should elute after calcium. This may be due to the larger hydration shell of the Mg^{2+} ion. This retention order was not observed with the Xylenol Orange coated column. No retention anomalies are shown with 1M ionic strength eluent, as no ion-exchange should take place and only chelating exchange should predominate. This is because any ion-exchange sites are 'swamped' with K^+ ions from the eluent. Figure 12 shows that with 1M ionic strength, at all pH values, the elution order is

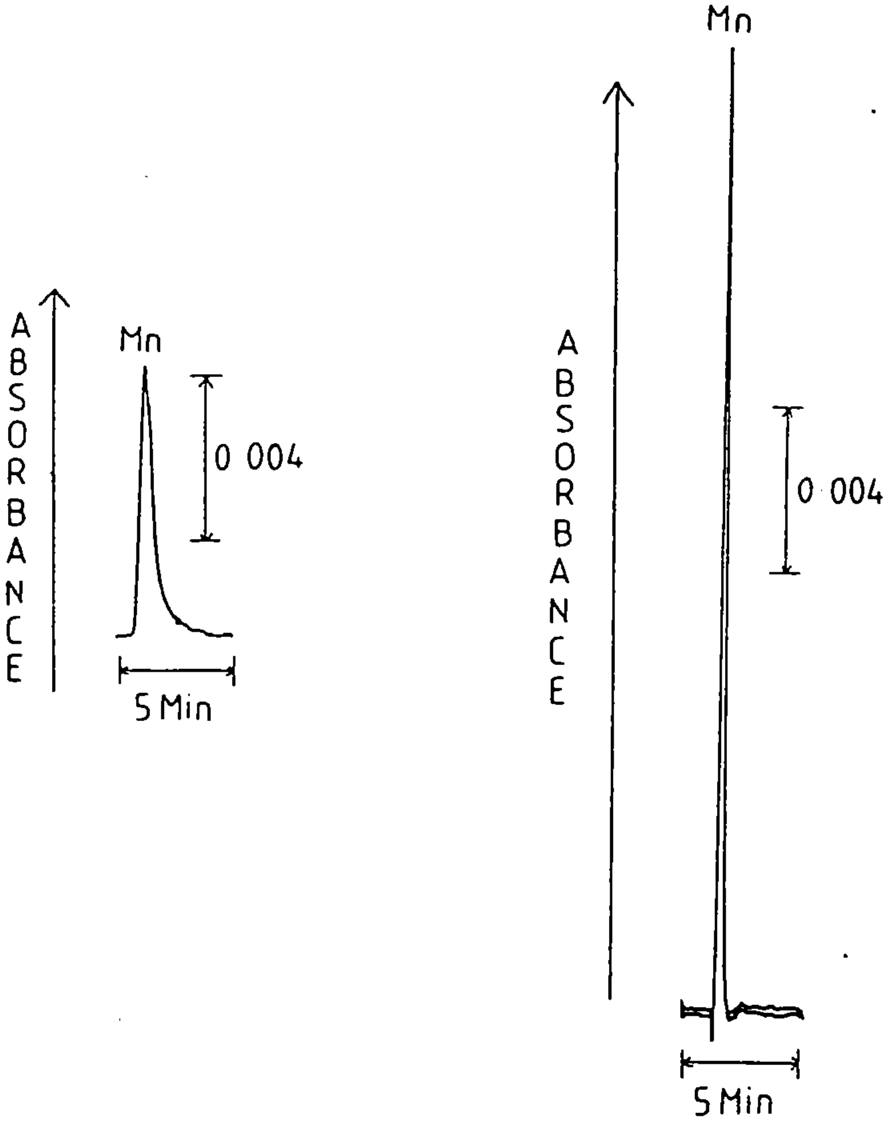


Figure 10. Comparison of peak shapes obtained for injection of 5ppm Mn^{2+} onto Xylenol Orange coated XAD-2 (left), and PLRP-S substrates at pH3 in 1M KNO_3.

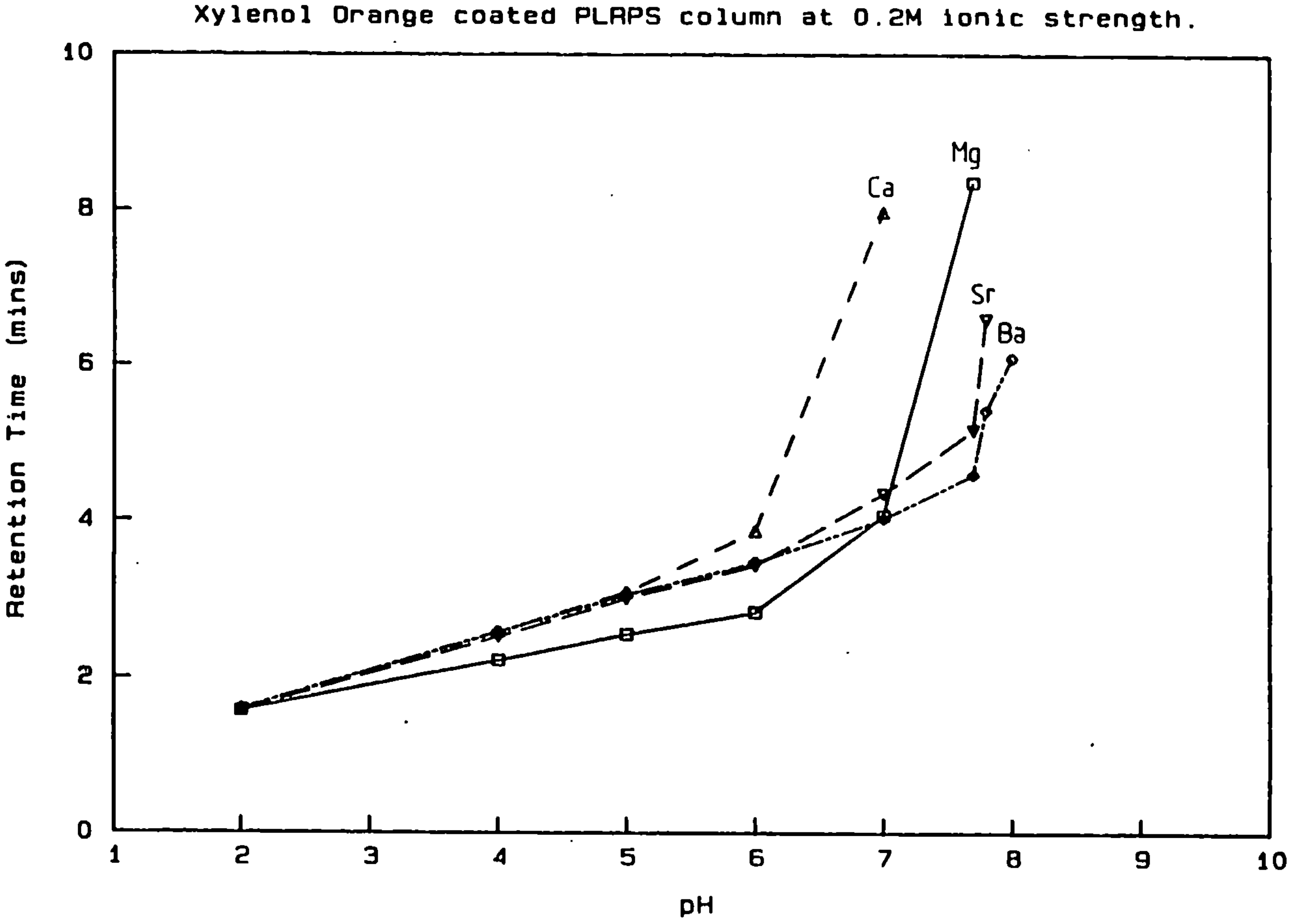


Figure 11. Variation of retention time with pH for the Xylenol Orange coated PLRP-S column at 0.2M ionic strength.

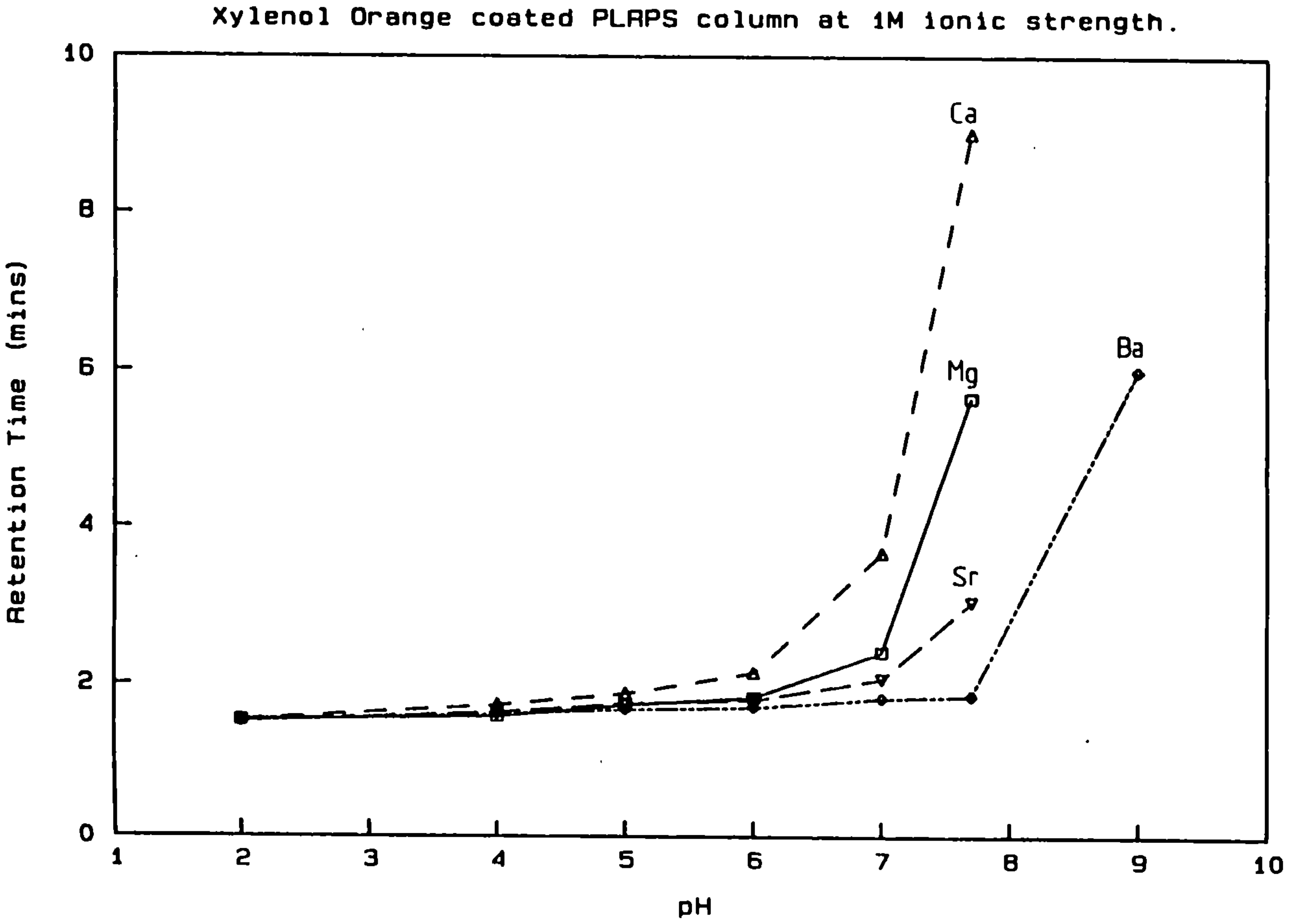


Figure 12. Variation of retention time with pH for the Xylenol Orange coated PLRP-S column at 1M ionic strength.

the same and so can assume only chelating exchange is operating. With ion-exchange the elution order is the opposite to the expected order for chelating exchange. For ion-exchange alkaline earth metals elute in the order Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}.

Table 7 shows the pH required for complete retention of a range of divalent metal ions. The Xylenol Orange column shows particularly strong chelation with metal ions, where all the alkaline earth and transition metals studied are completely retained at pH10. Even at a low eluent pH of 2, copper is still completely retained on this column.

3.2.2.2. CHROME AZUROL S DYE-COATED COLUMN.

Before this column could be packed and coated with CAS, it was decided to purify it by recrystallisation (Section 2.4.). Paper chromatography of CAS (Sigma Chemicals), showed that when a neutral spot of CAS dye was used, and dilute nitric acid used as the solvent, two distinct components showed up, only one which was sensitive to metal ions.

Using the purified CAS, a 10cm PEEK column was packed with PLRP-S resin and coated using the same procedure as for Xylenol Orange. This column showed a very high loading initially, but unfortunately, a lot of the CAS bled off during conditioning with dilute ammonia. The absorption measurements of the dye solution before and after coating showed that 82mg of CAS had been adsorbed onto the column. However, during cleaning 54mg bled off leaving 28mg of CAS coated onto the resin.

Again very sharp peaks were obtained with this column, showing high efficiency. Figure 13 shows a comparison of the peak shape with XAD-2 and PLRP-S substrates coated with CAS respectively. There was also a noticeable variation of retention times with pH, although this was not as good as for the Xylenol Orange coated PLRP-S column. The variation of

STANDARD.	XYLENOL ORANGE COATED PLRP-S.	CHROME AZUROL S COATED PLRP-S.
Ba^{2+}	10	>11
Mg^{2+}	9	11
Sr^{2+}	9	>11
Ca^{2+}	8	>11
Mn^{2+}	6	8
Co^{2+}	4	7
Zn^{2+}	4	7
Cd^{2+}	5	7
Pb^{2+}	4	6
Ni^{2+}	3	6
Cu^{2+}	2	5

Table 7. pH required for complete retention of 5ppm metal standards (10ppm for barium), on small particle size dye-coated columns, using 1M potassium nitrate mobile phase.

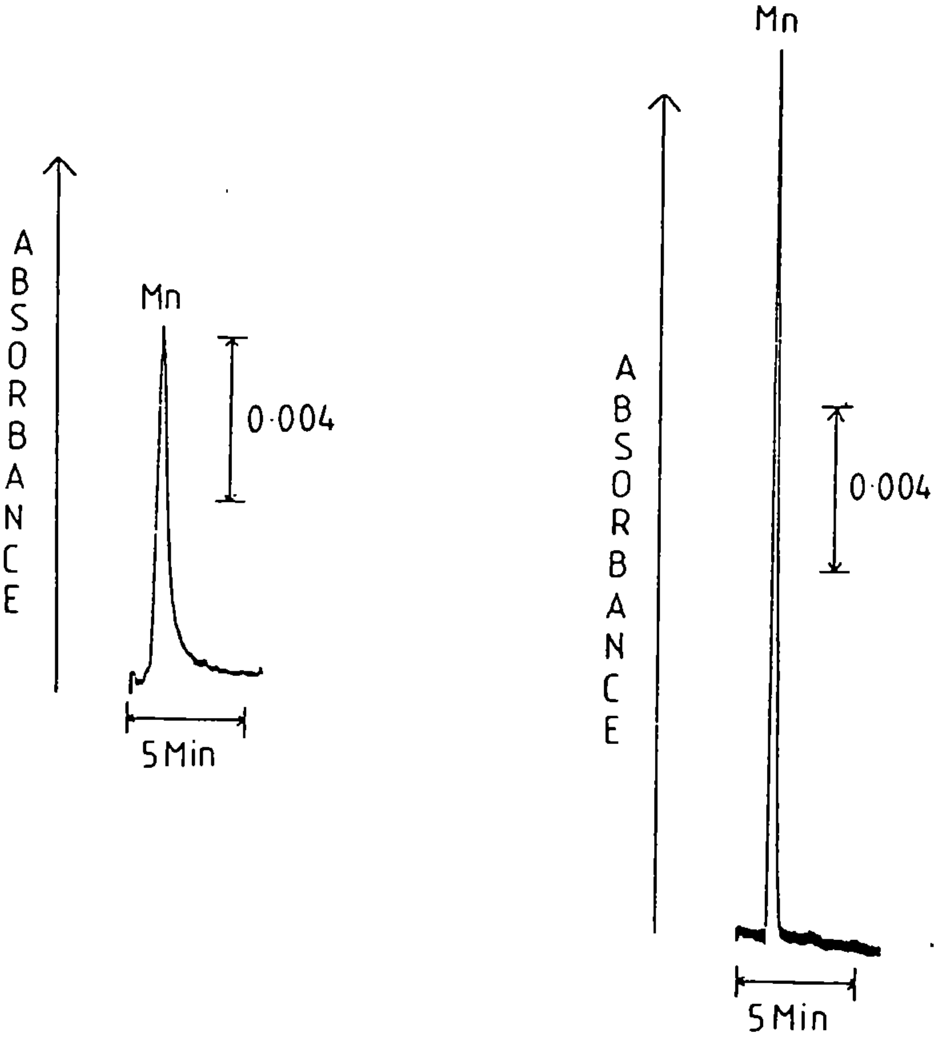


Figure 13. Comparison of peak shapes obtained for injection of 5ppm Mn^{2+} onto Chrome Azurol S coated XAD-2 (left), and PLRP-S substrates at pH3 in 1M KNO_3.

retention time with pH, at ionic strengths of 0.2M and 1M, are shown in Figures 14 and 15 respectively. At 0.2M ionic strength, the effects of ion-exchange at lower pH values can again be seen, with barium eluting after magnesium and strontium. The correct elution order for chelation exchange is not obtained at 0.2M ionic strength, even at pH10, where magnesium elutes correctly, but strontium is continuing to elute before barium. It seems likely that barium is still held on the column mainly by ion-exchange, as chelating exchange appears to be much weaker with this CAS coated column. At 1M ionic strength, the elution order is as expected as no ion-exchange should take place.

The pH required for the complete retention of various metal ions is shown in Table 7. The alkaline earth metals are not retained completely on this column until pH11 or above, showing that it is a much more weakly chelating dye in comparison with Xylenol Orange. From Table 7, it is clear that we have prepared two distinctly different chelating type columns that require very different pH values for retention.

3.2.3. ISOCRATIC SEPARATIONS OF METAL IONS.

The high efficiency coupled with the large variation of retention time with pH using small particle size dye-coated columns shows the potential for good separations of divalent metal ions. The separation properties of these columns were now investigated in more detail for various groups of metal ions.

3.2.3.1. XYLENOL ORANGE DYE-COATED COLUMN.

A complete separation of four alkaline earth metals could be achieved. This separation was possible in both 0.2M and 1M potassium nitrate. A separation in 0.2M potassium nitrate was possible at pH7.8, although the calcium peak is quite broad, as can be seen in Figure 16. Magnesium, calcium, strontium and

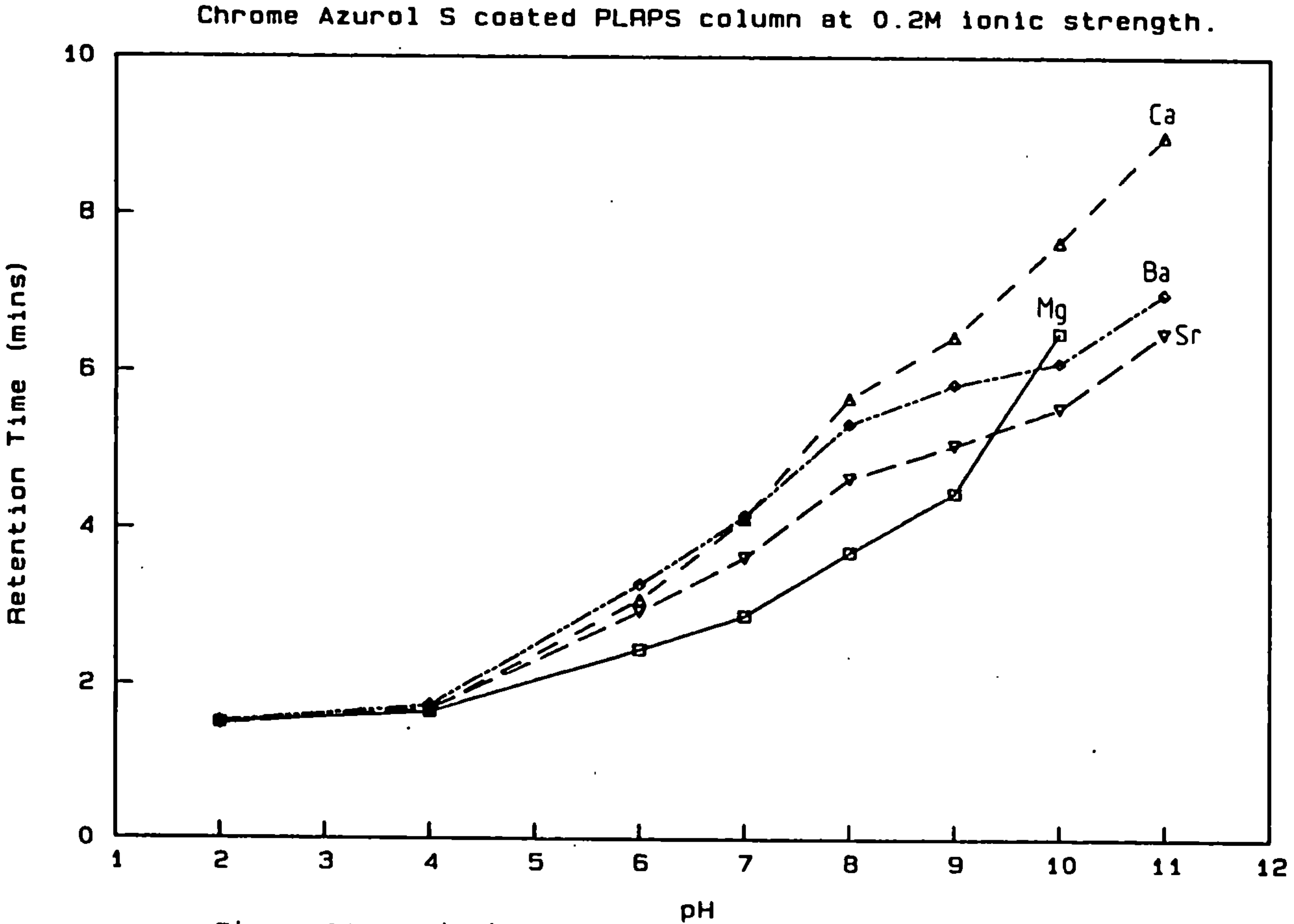


Figure 14. Variation of retention time with pH for the Chrome Azurol S coated PLRP-S column at 0.2M ionic strength.

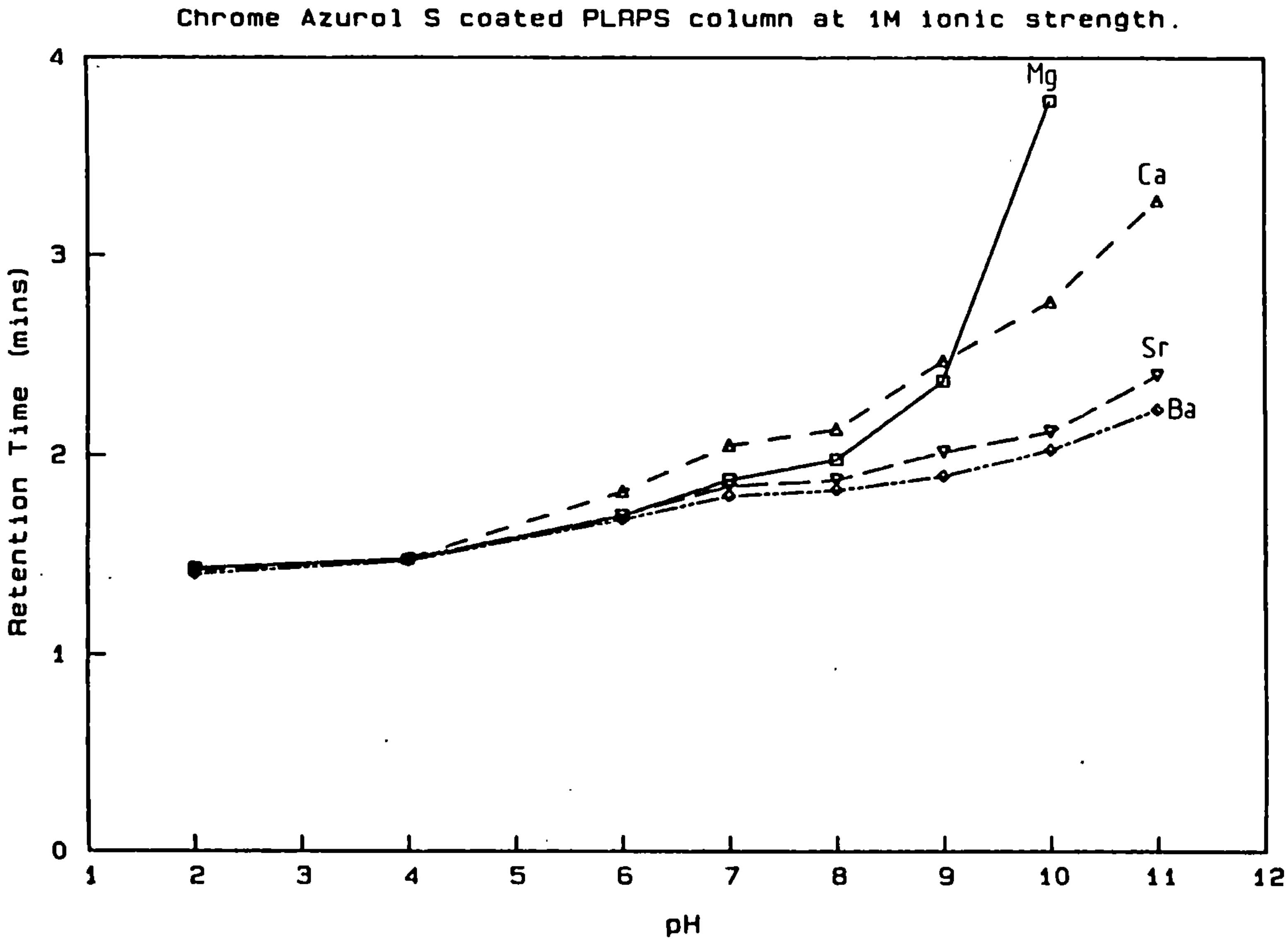


Figure 15. Variation of retention time with pH for the Chrome Azurol S coated PLRP-S column at 1M ionic strength.

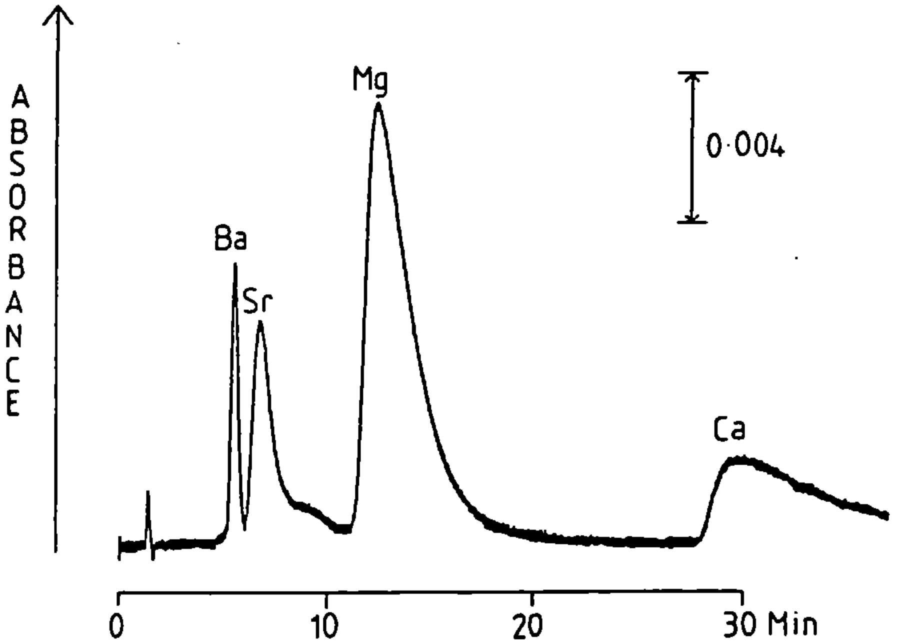


Figure 16. Chromatogram showing the isocratic separation of 10ppm Mg^{2+}, Ca^{2+}, Sr^{2+} and Ba^{2+} in 0.2M KNO_3 at pH7.8, using the Xylenol Orange coated column.

barium are clearly separated in 1M potassium nitrate at pH7.7, as shown in Figure 17. The effect of ionic strength can also be seen in comparison, where the retention times are much lower at the higher 1M ionic strength, due to the ion-exchange sites present containing K^+ from the eluent. In general, peaks become broader, and tailing occurs with increasing retention time. This is due to the kinetics of the dissociation of the metal chelates. In general, the formation of metal chelates are much faster than their dissociation depending on the equilibrium constants. Peaks broaden with retention time with ion chromatography. However, this effect is much more pronounced with chelating ion-exchange as reaction kinetics are slower in comparison.

The Xylenol Orange dye-coated column proved to be ideal for the separation of alkaline earth metals. From the literature so far, the separation illustrated (Figure 17) is the first published high performance separation on a dye-coated substrate. However, it can also be used to separate more strongly chelating metal ions by lowering the pH of the eluent. A complete separation of cadmium and lead at pH2.5 is shown in Figure 18. The peaks are sharp showing that the efficiency of this column remains high.

3.2.3.2. XYLENOL ORANGE DYE-COATED 10μm, 4000Å PLRP-S COLUMN.

This column was briefly investigated to see whether the pore size has any great effect on the chelating and separating performance. On coating, the loading was much lower compared with the 8μm, 100Å Xylenol Orange coated PLRP-S column. Between 20 and 30mg were adsorbed onto the resin, and 2 to 3mg bled off during the cleaning process.

With the alkaline earth metals, the best separation that could be achieved was at pH8.3, and is shown in Figure 19. It

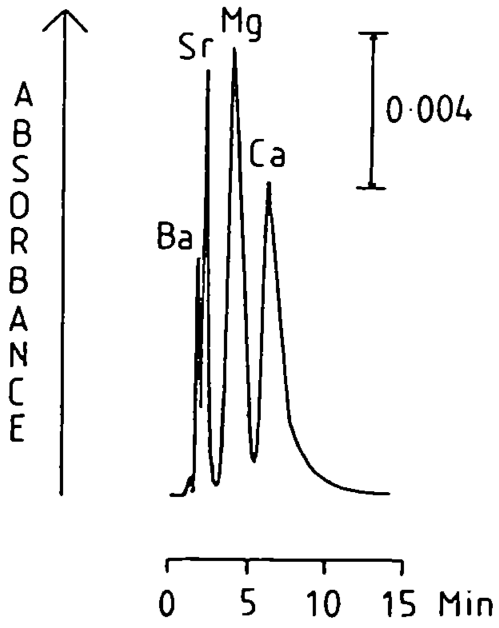


Figure 17. Chromatogram showing isocratic separation of 10ppm Mg^{2+}, Ca^{2+}, Sr^{2+}, and Ba^{2+} in 1M KNO_3 at pH7.7, using Xylenol Orange dye-coated column.

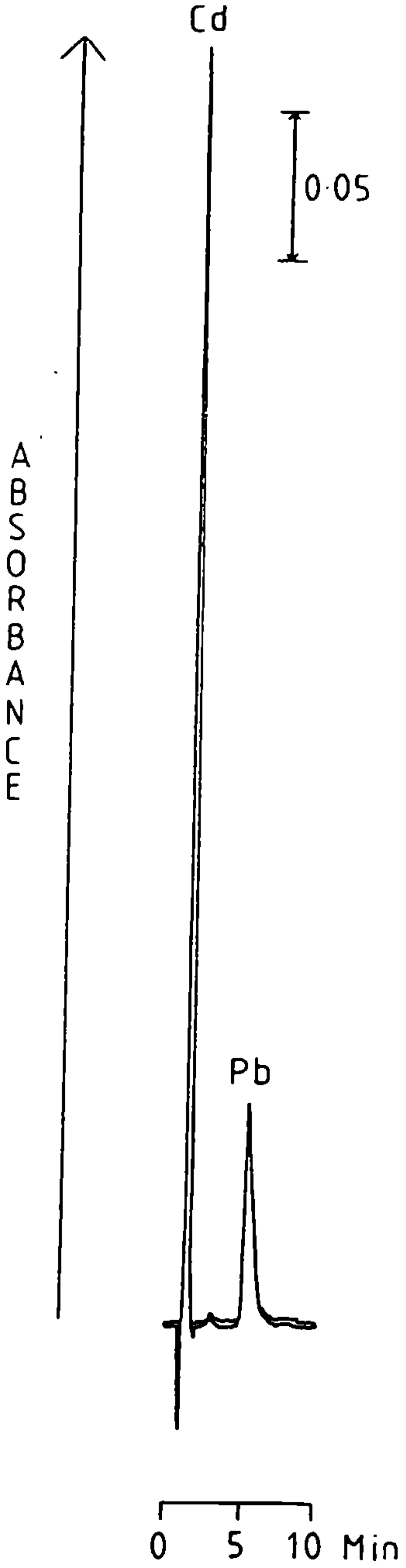


Figure 18. Chromatogram showing isocratic separation of 5ppm Cd^{2+} and 10ppm Pb^{2+} in 1M KNO_3 at pH2.5, using Xylenol Orange dye-coated column.

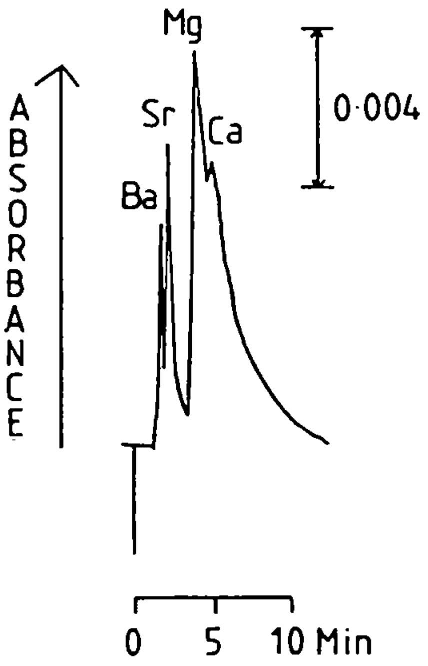


Figure 19. Chromatogram showing the best isocratic separation of four alkaline earth metals in 1M KNO_3 at pH8.3 using the Xylenol Orange coated 4000Å PLRP-S column. Concentrations were all 10ppm.

can be seen that barium and strontium are virtually separated, but magnesium and calcium could not be fully resolved, suggesting that the efficiency of this column is lower. For more strongly chelating metals, a complete separation of cadmium and lead at pH3 was achieved, as shown in Figure 20. Increasing the eluent pH slightly to pH3.2 enabled a separation of cadmium, zinc and lead to be obtained, as shown in Figure 21.

This column appears to be more weakly chelating than the 100Å column, as in all cases the pH required for a separation is higher. This could be due to the much larger pore size of 4000Å. Metal ions may easily pass in and out of these pores without reacting with chelating groups present on the pore surface. This could also be due to the lower loading, as well as the slightly larger particle size.

3.2.3.3. CHROME AZUROL S DYE-COATED COLUMN.

This column was coated with recrystallised CAS. The functional groups are more weakly chelating in comparison with Xylenol Orange. This can be seen in Table 8 which shows a selection of stability constants for complexes of some metal ions with Xylenol Orange, Chrome Azurol S and Calmagite. Literature values available for comparison are limited, however, it can be seen that Xylenol Orange forms a much stronger chelate with Fe^{3+} than Chrome Azurol S. Calmagite forms a stronger chelate with Cu^{2+} compared with Chrome Azurol S, but alkaline earth complexes are slightly weaker compared with Xylenol Orange. From the experimental results (Tables 4 to 7) involving the required pH for retention it is clear that the chelating power follows this pattern. It is interesting to note from the stability constant data that the expected order of retention for alkaline earth metals with Xylenol Orange is

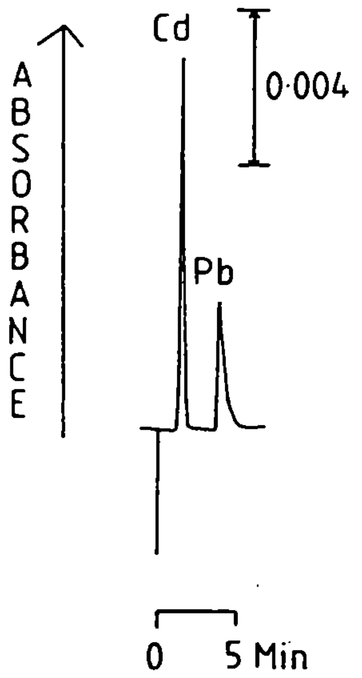


Figure 20. Chromatogram showing the isocratic separation of 5ppm Cd^{2+} and 10ppm Pb^{2+} in 1M KNO_3 at pH3 using the Xylenol Orange coated 4000Å PLRP-S column.

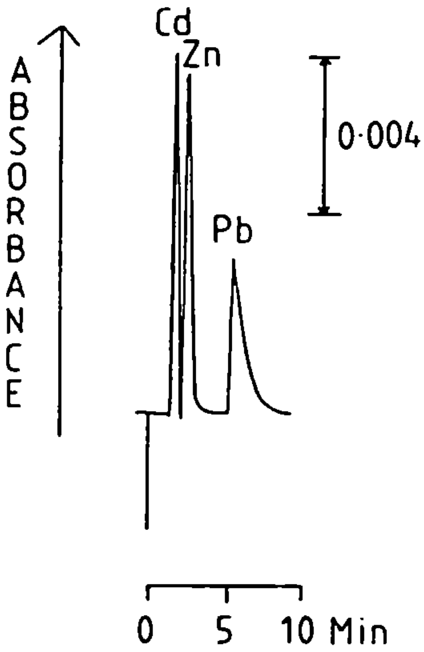


Figure 21. Chromatogram showing the isocratic separation of 5ppm Cd^{2+} and Zn^{2+} and 20ppm Pb^{2+} in 1M KNO_3 at pH3.2 using the Xylenol Orange coated 4000Å PLRP-S column.

METAL ION.	XYLENOL ORANGE. (L^{6-}).	CHROME AZUROL S. (L^{4-}).	CALMAGITE. (L^{3-}).
Mg^{2+}	9.02[1](118)	N.A.	8.1[3,5]
Ca^{2+}	8.65[1](118)	N.A.	6.1[3,5]
Sr^{2+}	7.71[1](118)	N.A.	N.A.
Ba^{2+}	6.67[1](118)	N.A.	N.A.
Cu^{2+}	N.A.	13.7[4](120)	21.70[3](121)
Fe^{3+}	39.80[2](119)	20.2[4](120)	N.A.
Zn^{2+}	N.A.	N.A.	12.52[3](121)
Pb^{2+}	N.A.	N.A.	12.90[3](121)
Ni^{2+}	N.A.	N.A.	21.63[3](121)
Co^{2+}	N.A.	N.A.	21.03[3](121)
Cd^{2+}	N.A.	N.A.	12.59[3](121)

Notes.

[1] - For the reaction $M^{2+} + L^{6-} \rightleftharpoons ML^{4-}$.

[2] - For the reaction $2Fe^{3+} + L^{6-} \rightleftharpoons Fe_2L$.

[3] - For the reaction $M^{2+} + L^{3-} \rightleftharpoons ML^-$.

[4] - For the reaction $2Cu^{2+} + L^{4-} \rightleftharpoons Cu_2L$,
and $2Fe^{3+} + L^{4-} \rightleftharpoons Fe_2L^{2+}$.

[5] - Unknown source.

N.A. - Not available.

Table 8. A selection of stability constants (log K) for metal complexes with Xylenol Orange, Chrome Azurol S and Calmagite.

different to those obtained experimentally. Magnesium should be retained more than calcium, however this did not occur. This could be due to the fact that the Mg^{2+} ion is larger in size due to its larger hydration shell. The values for Calmagite show the general trend, as metals such as nickel and copper form strong chelates and alkaline earth metals form weaker chelates in comparison.

This column proved to be too weakly chelating for any separation of four alkaline earth metals to be achieved. However, at pH10, calcium could be separated from barium as shown in Figure 22. Magnesium and strontium would not be fully resolved from calcium and barium at this pH. With more strongly chelating metals, a complete separation of cadmium and lead at pH4 could be achieved, as shown in Figure 23. A higher pH in comparison with Xylenol Orange is required for this separation as CAS contains a weaker chelating group. The peaks are very sharp, showing that the efficiency of this column is high.

3.3. COMMERCIAL CHELATING COLUMNS.

Two commercially available columns were investigated for their chelating and separating ability towards divalent metal ions. These columns were the TOSOH TSK-GEL Chelate 5-PW, and the Dionex MetPac CC-1. Both of these column substrates are of small particle size and contain chemically bonded iminodiacetic acid functional groups, which are strongly chelating. Interestingly the TOSOH column is manufacturered for high performance affinity chromatography of biomolecules. Xylenol Orange used as a chelating dye, contains the same iminodiacetic acid functional groups as these commercial columns. The structure of Xylenol Orange is shown in Figure 7.

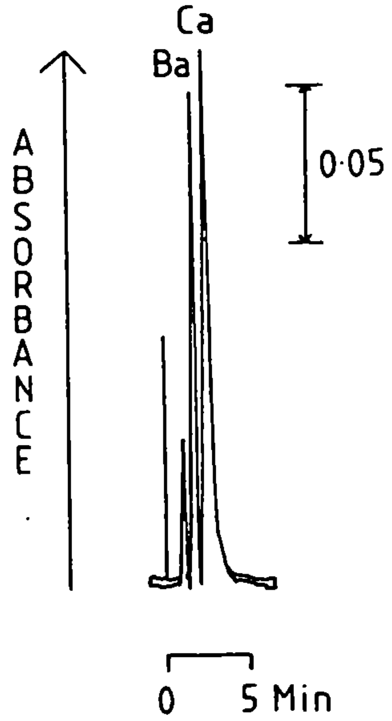


Figure 22. Chromatogram showing the isocratic separation of 2ppm Ca^{2+} and Ba^{2+} at pH10 using the Chrome Azurol S coated column.

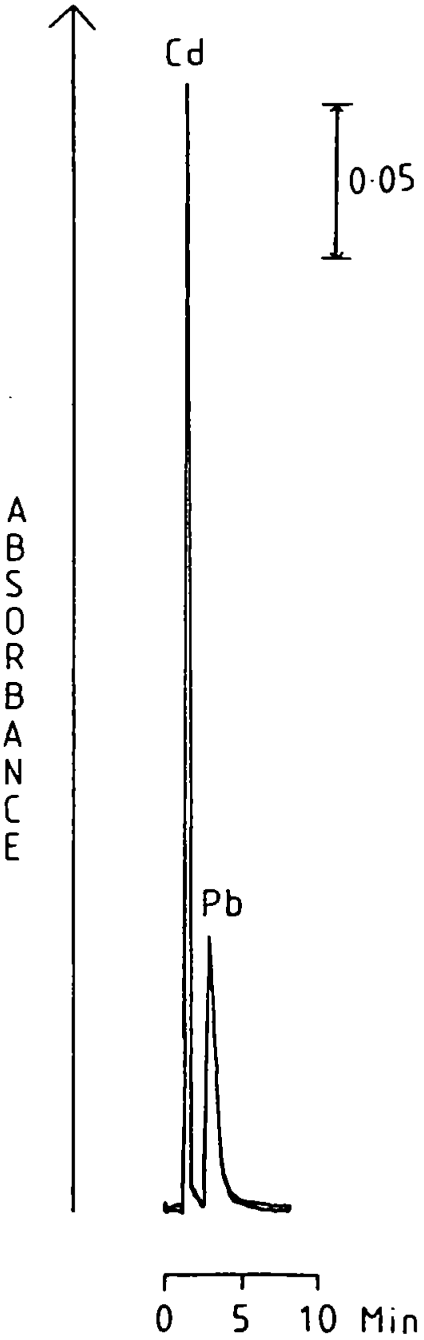


Figure 23. Chromatogram showing isocratic separation of 5ppm Cd^{2+} and 10ppm Pb^{2+} in 1M KNO_3 at pH4, using Chrome Azurol S dye-coated column.

3.3.1. TOSOH COLUMN.

The investigation involved an initial study to determine the chelating power, followed by a more detailed study into the separating ability, and finally a comparison was made with the chelating dye-coated columns.

3.3.1.1. INITIAL INVESTIGATIONS.

A similar set of experiments to those described for dye-coated columns was carried out. This involved determining the pH required to completely retain a range of metal ions. The results obtained are shown in Table 9. It can be seen that the TOSOH column and the Xylenol Orange dye-coated column, which contain the same iminodiacetic acid functional chelating groups, give similar, although not identical results. This encouraging result demonstrated that the metal chelation was as strong for the dye-coated column as for the commercial column.

3.3.1.2. ISOCRATIC SEPARATIONS.

As expected, due to the small particle size (10μm), peaks were much sharper, and efficiency higher than large particle size dye-coated columns, enabling some metal ion separations to be achieved. Using the Calmagite post-column reagent, and a 0.2M potassium nitrate eluent, Figure 24 shows a separation of manganese, cobalt and zinc at pH2.5. Increasing the eluent pH to 4 enabled a separation of magnesium and manganese to be obtained and this is shown in Figure 25. The iminodiacetic acid chelating groups are very strongly chelating towards metal ions, as copper is completely retained at pH2. Figure 26 shows a separation of nickel, iron(III) and copper at a low pH of 1. Here, even copper does not elute off the column until nearly ten minutes after injection.

To enable alkaline earth metals to be studied, the PAR-ZnEDTA post-column detection system was used. The TOSOH column used

STANDARD.	TOSOH COLUMN.	XYLENOL ORANGE COATED PLRP-S.	CHROME AZUROL S COATED PLRP-S.
Ba^{2+}	9	10	>11
Mg^{2+}	9	9	11
Sr^{2+}	8	9	>11
Ca^{2+}	7	8	>11
Mn^{2+}	6	6	8
Co^{2+}	4	4	7
Zn^{2+}	4	4	7
Cd^{2+}	4	5	7
Pb^{2+}	3	4	6
Ni^{2+}	3	3	6
Cu^{2+}	2	2	5

Table 9. pH required for complete retention of 5ppm metal standards (10ppm for barium), with small particle size columns, using 1M potassium nitrate mobile phase.

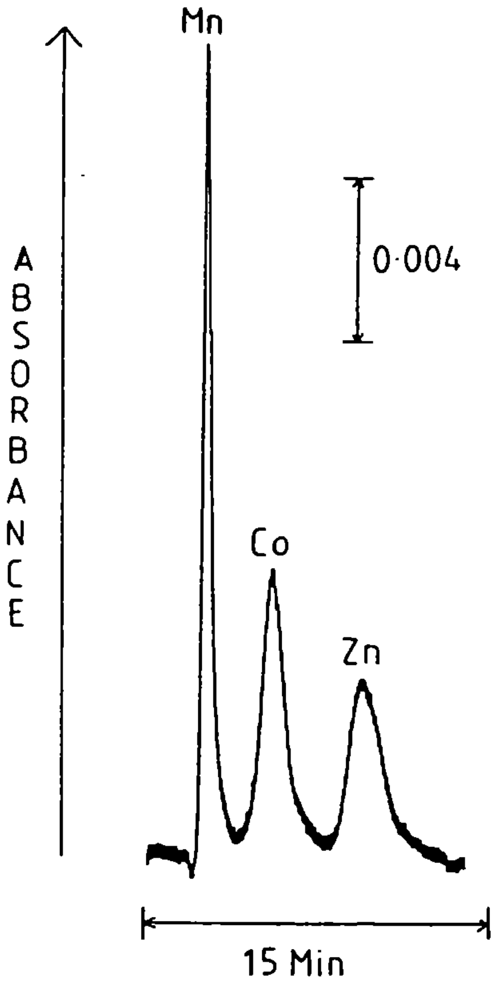


Figure 24. Chromatogram showing the isocratic separation of 10ppm Mn^{2+}, Co^{2+} and 20ppm Zn^{2+} in 0.2M KNO_3 at pH2.5, using the old TOSOH column with Calmagite post-column reagent.

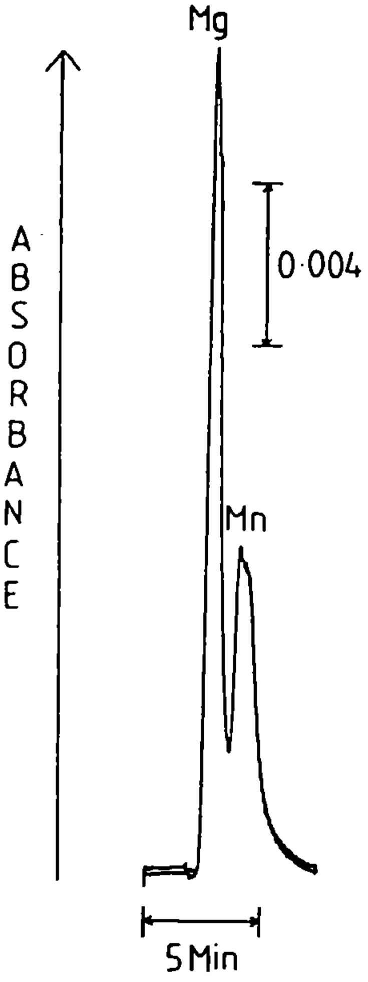


Figure 25. Chromatogram showing the isocratic separation of 10ppm Mg^{2+} and Mn^{2+} in 0.2M KNO_3 at pH4, using the old TOSOH column with Calmagite post-column reagent.

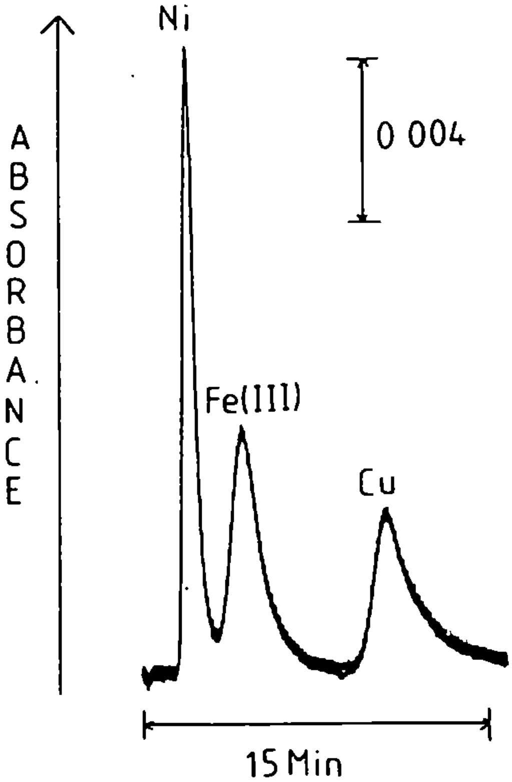


Figure 26. Chromatogram showing the isocratic separation of 10ppm Ni^{2+} and Fe^{3+} and 20ppm Cu^{2+} in 0.2M KNO_3 at pH1, using the old TOSOH column with Calmagite post-column reagent.

here was new, as the old column had seriously deteriorated from previous use with concentrated brines. This could be due to highly acidic or alkaline media slowly causing deterioration of the substrate. Figure 27 shows a separation of magnesium, strontium and barium in 1M potassium nitrate at pH7.3. However, due to the slightly stronger chelation with this column, compared to the Xylenol Orange coated column, calcium was completely retained at this pH. Also, the elution order is different, as strontium shows a longer retention time than magnesium. This indicates that surface chelation can produce unexpected retention behaviour when compared with the order of conditional stability constants in solution. The Mg^{2+} is a small ion in comparison once it has lost all or part of its hydration shell and may not be totally adsorbed into the large 1000Å pores.

By reducing the eluent pH, further separations of more strongly chelating metals could be obtained. Figure 28 shows an excellent separation of manganese, zinc, cadmium and lead in 1M potassium nitrate at pH2.2. Lead is clearly separated from zinc as it forms a stronger chelate. On comparison with the separations obtained with the Xylenol Orange column, it is particularly encouraging to find that the efficiency of the coated column approaches that of the commercial chelating column.

3.3.2. DIONEX MetPac CC-1 COLUMN.

Table 10 shows the pH required for complete retention of a range of metal ions on this column using Calmagite as the post-column reagent. It can be seen that the affinity of the column substrate towards metal ions is similar, and as strong in most cases as the TOSOH column. However, for magnesium, and some of the transition metals, the chelation appears to be stronger.

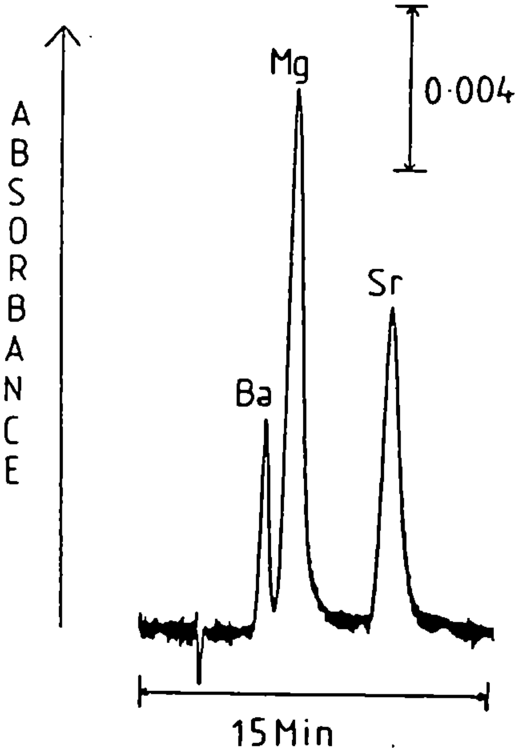


Figure 27. Chromatogram showing isocratic separation of 5ppm Mg^{2+}, and 10ppm Sr^{2+}, and Ba^{2+} in 1M KNO_3 at pH7.3, using the TOSOH column with PAR-ZnEDTA post-column reagent.

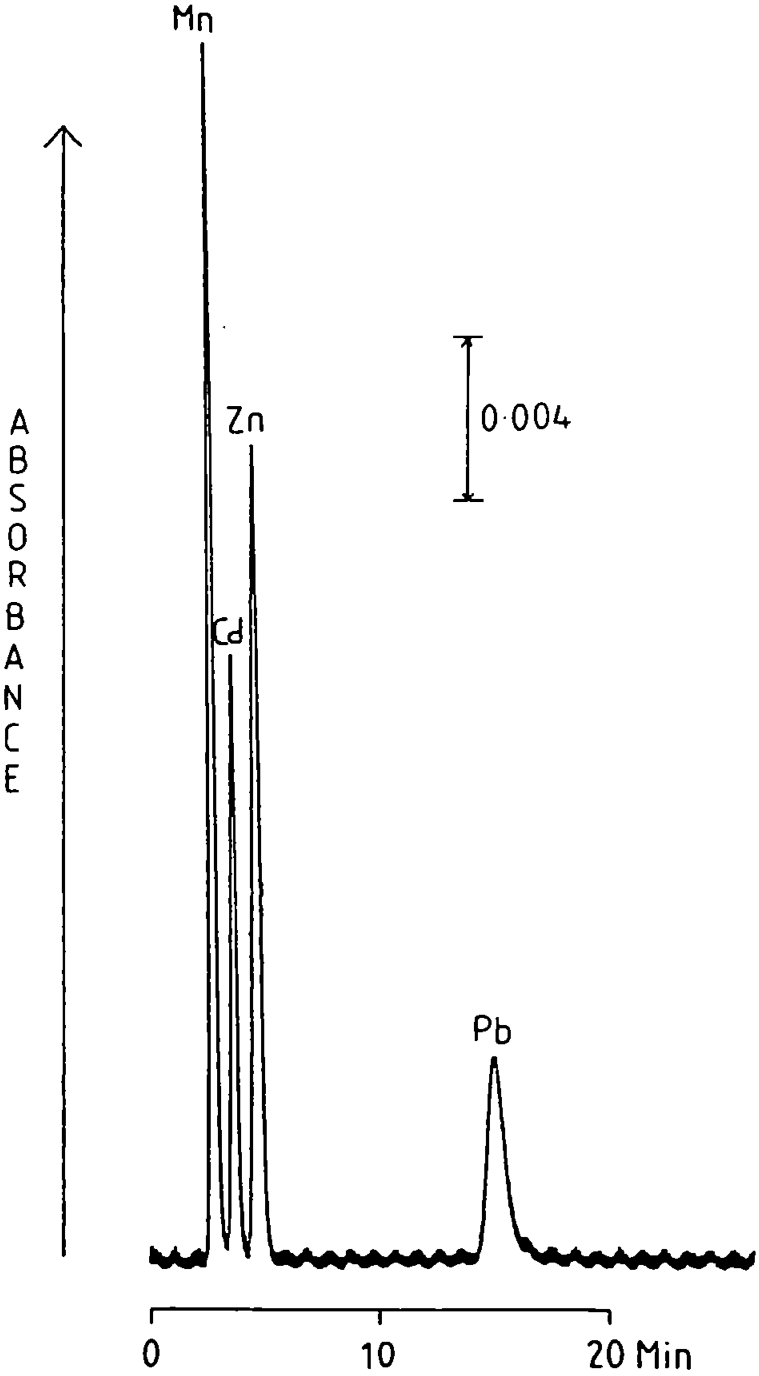


Figure 28. Chromatogram showing isocratic separation of 10ppm Mn^{2+}, Cd^{2+}, Zn^{2+}, and 20ppm Pb^{2+} in 1M KNO_3 at pH2.2, using the TOSOH column with PAR-ZnEDTA post-column reagent.

STANDARD.	TOSOH COLUMN.	MetPac COLUMN.
Mg^{2+}	9	6
Mn^{2+}	6	4
Co^{2+}	4	3
Zn^{2+}	4	3
Ni^{2+}	3	3
Cu^{2+}	2	2
Fe^{3+}	2	2

Table 10. pH required for complete retention of 10ppm standard on the commercial TOSOH and MetPac columns, using 1M KNO_3 mobile phase.

This column was investigated for the separation of alkaline earth metals using PAR-ZnEDTA post-column reagent, at 0.2M and 1M ionic strength. At the higher 1M ionic strength, peaks were sharper as expected. There was a slight difference in retention times, and thus the elution order could be seen. By pH4, magnesium elutes through very slightly, and calcium, strontium, and barium are virtually retained on the column. An interesting finding is that the elution order changes with ionic strength. From 0.2M to 1M potassium nitrate, the elution order changes from $Ba^{2+} > Ca^{2+} > Sr^{2+} > Mg^{2+}$, to $Ca^{2+} > Sr^{2+} > Ba^{2+} > Mg^{2+}$ respectively. It is considered that at a lower ionic strength barium is held on the column mainly by ion-exchange and this effect can be seen at pH3.5.

The MetPac column is specifically manufactured for the preconcentration of metal ions, as it has a very high chelation capacity of 0.7 mequil ml^{-1}. Analytical separations could not be achieved here mainly because of the short column length of 5cm, however, the actual particle size is unknown but it may be relatively large as it is only manufactured for preconcentration and not for the separation of metal ions.

3.4. POST-COLUMN DETECTION SYSTEMS.

In general, four post-column reagents were employed, depending on the group of metal ions that had to be detected. These reagents were prepared using previous recipes, except for the PAR-ZnEDTA post-column reagent system which was optimized. All these systems detected trace metal ions by a change in absorbance in the visible range, due to the formation of a complex with the metal ion being detected.

3.4.1. CALMAGITE.

Calmagite was used for the detection of divalent transition metals and also magnesium, however, it does not react with calcium, strontium and barium. A decrease in absorbance was monitored at 610nm. This tends to be a disadvantage as by measuring a decrease in absorbance, the baseline tends to be noisy, due to the high background levels. Figure 29 shows the absorbance spectrum of Calmagite, together with the absorbance spectrum of Calmagite-zinc complex. In this run the wavelength of maximum absorbance for Calmagite was 607nm, and when complexed with zinc, changed to 550.5nm.

3.4.2. PAR AND PAR-ZnEDTA.

The detection system using PAR or PAR-ZnEDTA is more sensitive than Calmagite, mainly because an increase in absorbance is being measured rather than a decrease, leading to a lower background noise level. PAR alone does not react well with the alkaline earth metals, however this is advantageous when determining trace metals in sea waters, which contain high levels of magnesium and calcium. Figure 30 shows the absorbance spectrum of PAR, together with the absorbance spectrum of PAR-zinc complex. In both cases there is a large absorbance at around 415nm, but when complexed with zinc the absorbance monitored at 490nm, increases. In this run the wavelength of maximum absorbance was 487nm.

The PAR-ZnEDTA post-column reaction system was investigated, to enable the alkaline earth metals to be studied. The addition of ZnEDTA to PAR enables a metal displacement reaction to occur as described in Section 1.6.1. Calmagite only reacts with magnesium, and although a Calmagite-MgEDTA system would enable the detection of all the alkaline earth metals, it would be less sensitive as a decrease in absorbance is being measured. It was decided to optimize the PAR-ZnEDTA detection system.

Using equal concentrations of PAR and ZnEDTA (4×10^{-5}M) gave

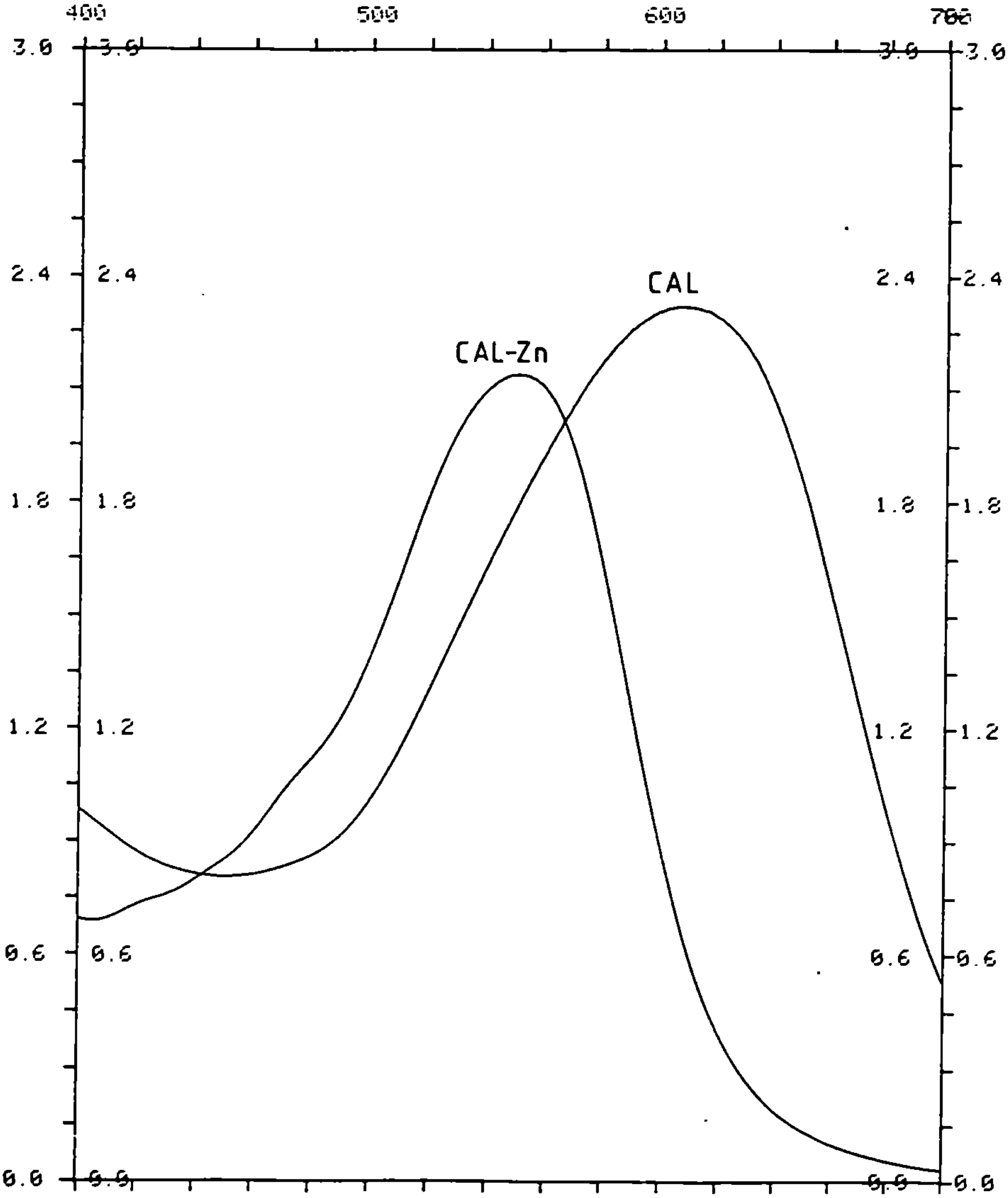


Figure 29. Absorbance spectra of Calmagite and Calmagite-zinc complex.

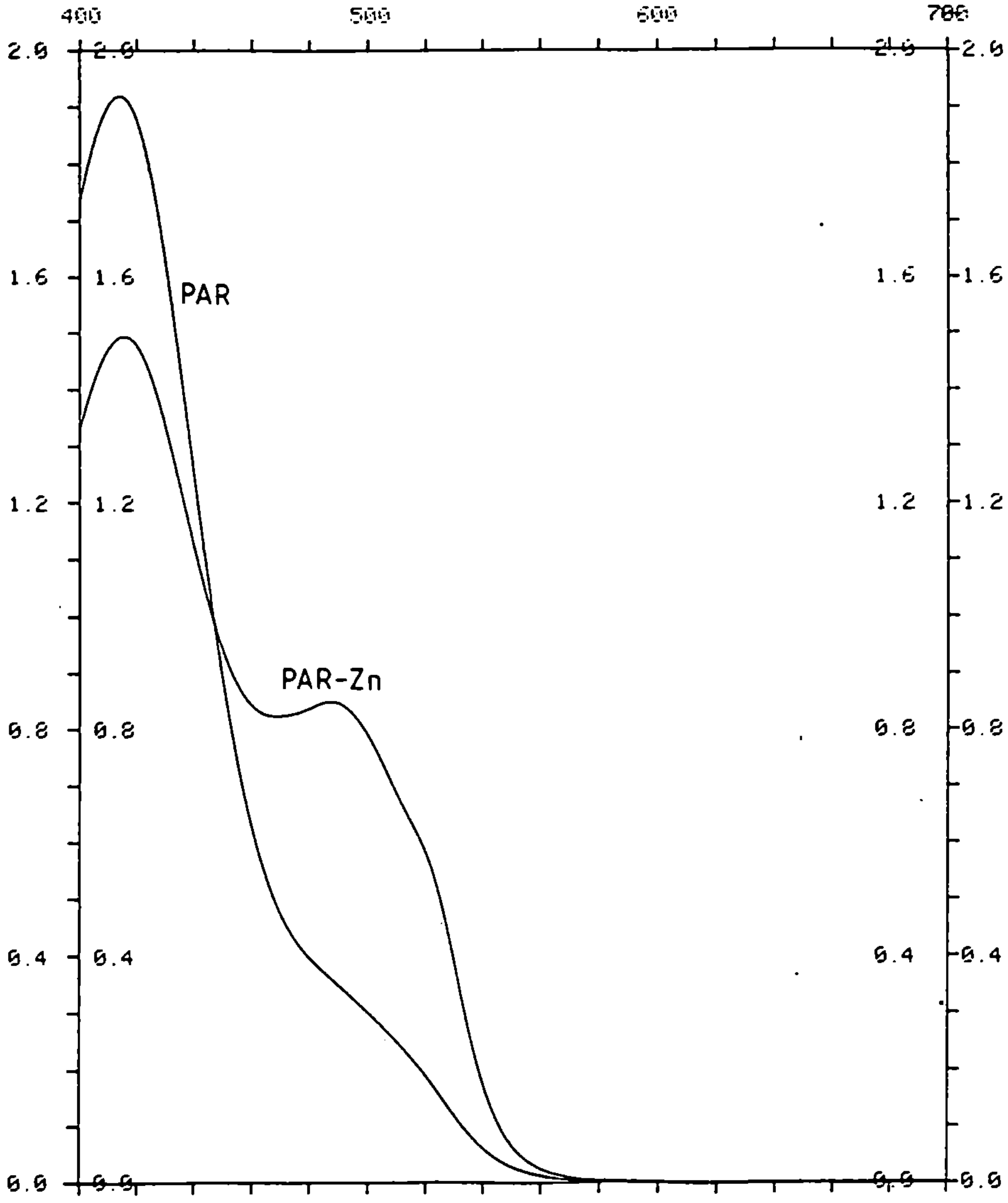


Figure 30. Absorbance spectra of PAR and PAR-zinc complex.

only a slight response for magnesium and calcium, and none for strontium and barium. On increasing the concentrations of PAR and ZnEDTA to 2 x 10^{-4}M and ammonia to 2M, with 0.2M potassium nitrate eluent, a colour change occurred at room temperature but the reaction rate was very slow. On heating, the colour fades completely, but returns on cooling. Various ratios of PAR, ZnEDTA and ammonia were then tried with all four metals. When a significant excess of ZnEDTA was used a fast reaction occurred at room temperature for magnesium and calcium, with strontium and barium taking around ten seconds to complete. On heating, the mixture faded very little. This post-column reagent mixture subsequently used (see Experimental Section 2.3.2.) was prepared using the acquired component ratio.

When this PAR-ZnEDTA detector was tested on the ion chromatography system very good response and sensitivity was obtained for all four alkaline earth metals at room temperature. Increasing the column temperature to 30°C improved sensitivity slightly, however on heating to 60°C, the response for strontium and barium was much poorer. The post-column reagent mixture is complex, and it seems that equilibrium constants are changing with temperature, as this is affecting the sensitivity, especially for strontium and barium. Colour changes were gradual, showing that the kinetics of the metal displacement reaction is slow. However, adding an excess of ZnEDTA in the post-column reagent, seems to greatly reduce this effect, as it could be acting as a catalyst. This has been found in other studies where some impurities, especially copper, can act as a catalyst for the metal displacement reaction (122).

The absorbance spectrum of PAR-ZnEDTA together with PAR-ZnEDTA-Ca^{2+} mixture is shown in Figure 31. Again as with PAR,

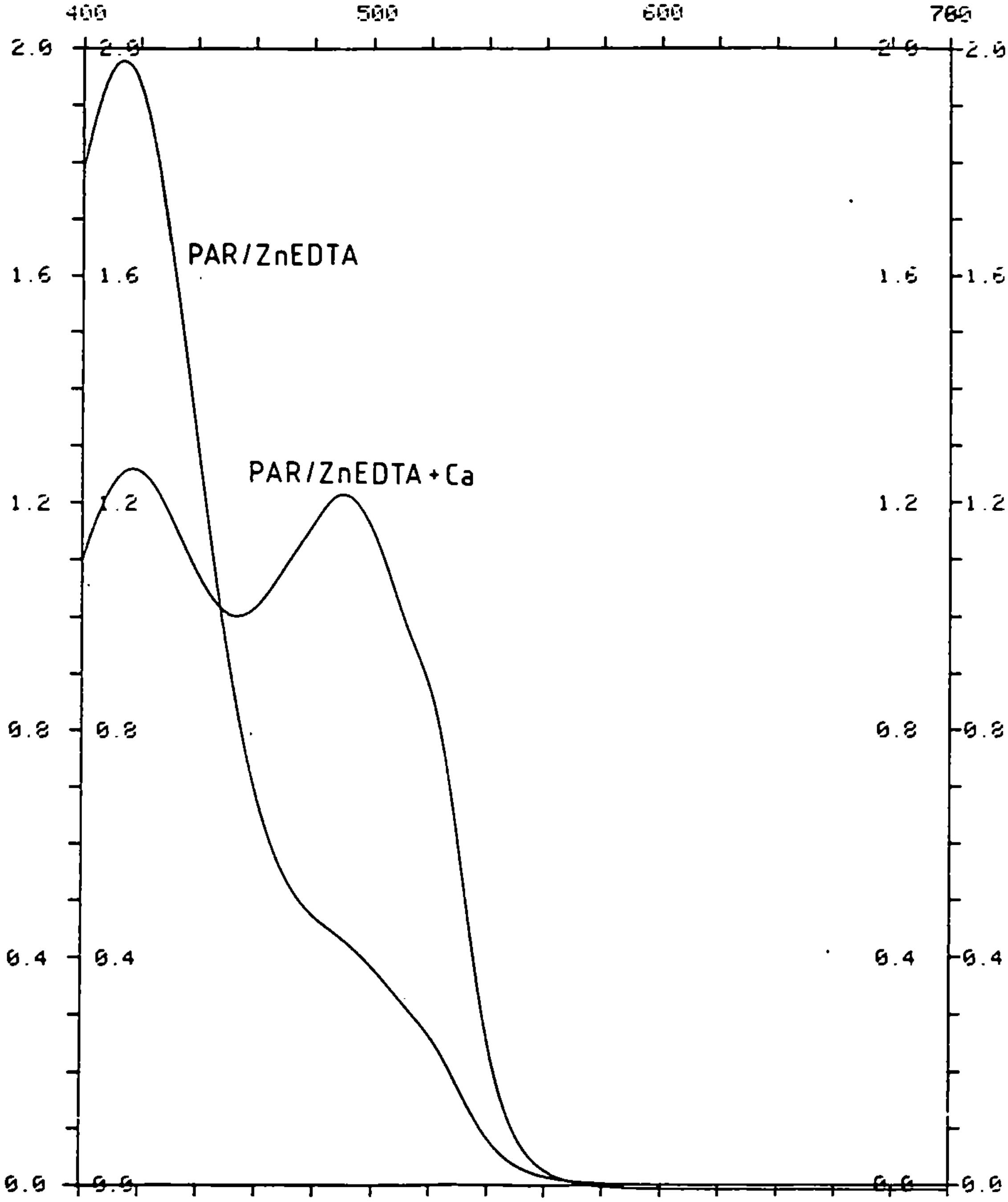


Figure 31. Absorbance spectra of PAR-ZnEDTA and PAR-ZnEDTA-calcium mixture.

there is a high absorbance around 415nm, but this is reduced when another metal ion is introduced. The wavelength at which maximum absorbance is reached when calcium is added is 489nm. This in effect is due to the formation of ZnPAR, via the metal displacement reaction. This is explained in Section 1.6.1.

Using the Dionex spectral array detector, and the CAS coated PLRP-S column, a range of metal ions were injected using PAR and PAR-ZnEDTA post-column reagents. These metal ions were injected at a pH where they elute through the column, and using the spectral array software, the absorbance spectra were obtained for each metal ion. Table 11 shows the wavelength of maximum absorbance with a range of metal ions, with both PAR and PAR-ZnEDTA post-column reagents. It can be seen that cobalt and copper appear not to react via the metal displacement reaction with PAR-ZnEDTA. With PAR alone, monitoring at 490nm, the sensitivity for lead, and particularly iron(III) would be reduced as they form a complex with PAR with maximum absorbance significantly higher than 490nm.

3.4.3. PYROCATECHOL VIOLET.

This post-column reagent was used for the detection of trivalent metal ions, and it is a very sensitive reagent for the detection of aluminium. An increase in absorbance was monitored at 580nm, as the wavelength of maximum absorbance for aluminium, gallium and indium were close to 580nm. Table 12 shows the wavelength of maximum absorbance with Pyrocatechol Violet post-column reagent for a range of trivalent metal ions, which were experimentally determined. Figure 32 shows the absorbance spectrum of Pyrocatechol Violet, together with Pyrocatechol Violet-aluminium complex. The wavelength of maximum absorbance for the Pyrocatechol Violet-aluminium complex was 582nm.

METAL ION.	PAR.	PAR-ZnEDTA.
Mg^{2+}	-	492
Ca^{2+}	-	492
Sr^{2+}	-	492
Ba^{2+}	-	492
Mn^{2+}	498	495
Co^{2+}	505	504
Zn^{2+}	492	491
Ni^{2+}	488	488
Cu^{2+}	505	503
Cd^{2+}	493	492
Pb^{2+}	510	490
Fe^{3+}	530	-

Table 11. Wavelength (nm) where maximum absorbance occurs for metal complexes with PAR and PAR-ZnEDTA post-column reagents.

METAL ION.	WAVELENGTH.
Al^{3+}	582
Ga^{3+}	581
In^{3+}	576
Fe^{3+}	606
Bi^{3+}	614

Table 12. Wavelength (nm) where maximum absorbance occurs for metal complexes with Pyrocatechol Violet post-column reagent.

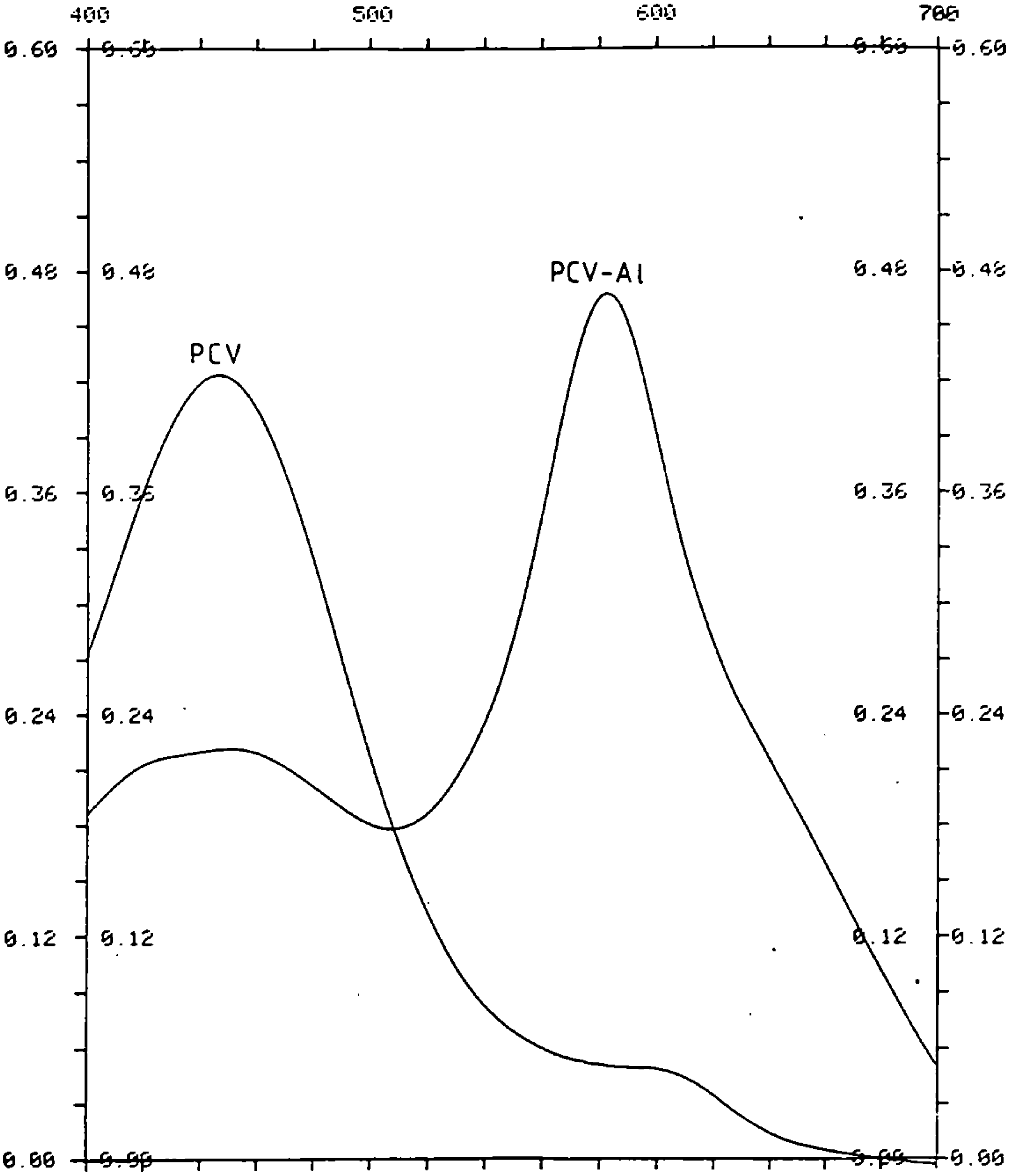


Figure 32. Absorbance spectra of Pyrocatechol Violet and Pyrocatechol Violet-aluminium complex.

3.4.4. CHROME AZUROL S.

This post-column reagent was investigated for its potential in the detection of trivalent metal ions. The post-column reagent mixture does not react much with copper, whilst copper does react significantly with Pyrocatechol Violet, leading to interference with trivalent metal ions, as copper forms strong chelates. A problem however, was that CAS tends to form a colloidal lake with some trivalent metals, and the tubing can become coated with CAS leading to contamination. An increase in absorbance was monitored at 546.5nm. Figure 33 shows the absorbance spectrum of CAS together with CAS-aluminium complex. The wavelength at which maximum absorbance occurred for the CAS-aluminium complex was 546.0nm.

3.5. GRADIENT AND STEP GRADIENT ELUTION.

Since pH is a major controlling factor on the stability of metal chelates, gradient elution should enable a larger range of metal ions to be separated in a single injection. With isocratic and gradient elution, the metal ions are eluted in the order of increasing chelate strength but retention times become very long after about four metals. By reducing the pH however, the conditional stability constants of the metal chelates will change. This will allow more strongly chelating metal ions such as copper to be separated and eluted much more quickly. Gradient elution also has the advantage that later eluting peaks become much sharper due to peak compression.

However, a problem arose when using a descending pH gradient with chelating columns as metal impurities tended to be preconcentrated at the starting pH. As the pH drops, these impurities eluted off the column causing large peaks for a blank run. This was largely overcome by using a clean-up

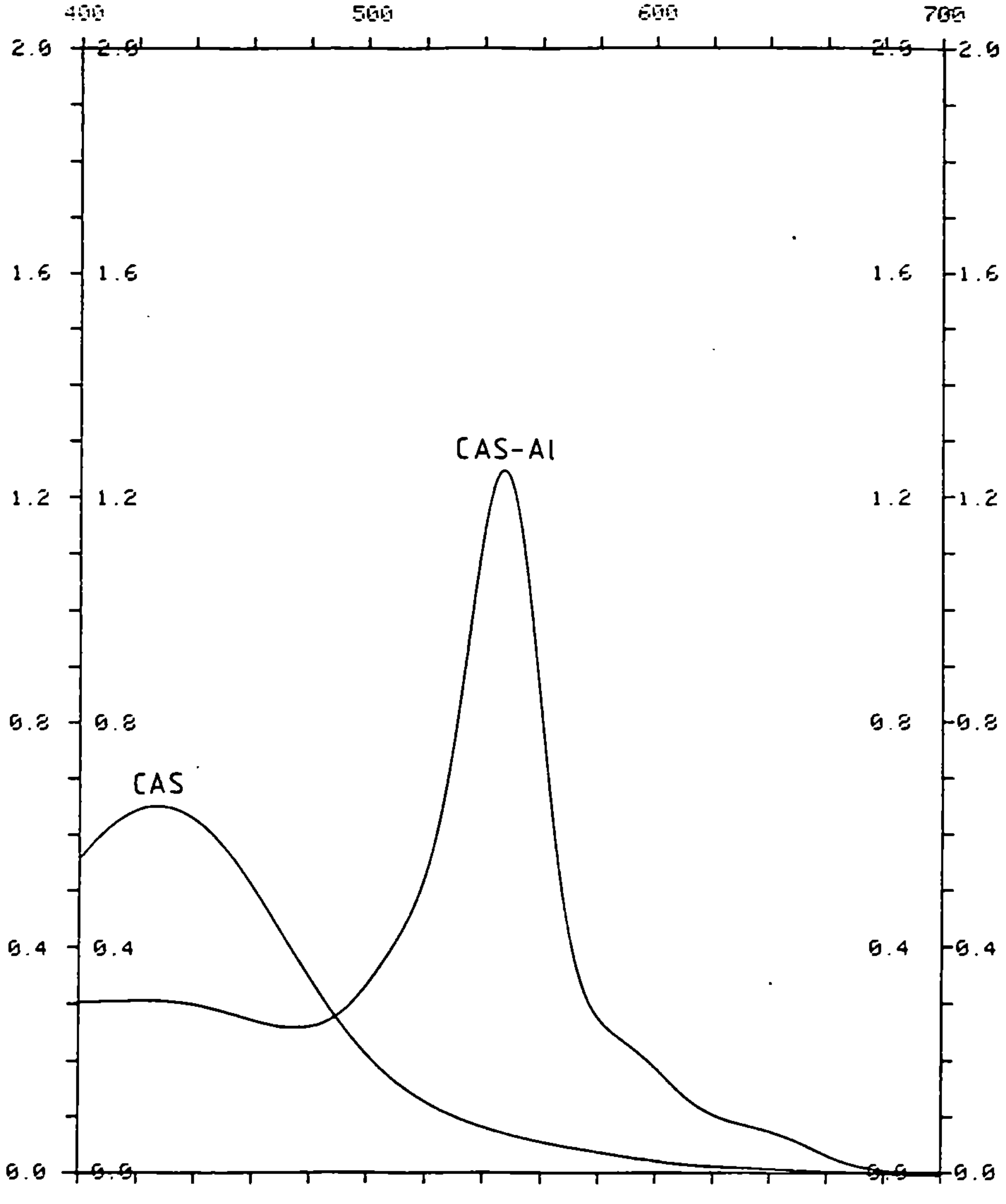


Figure 33. Absorbance spectra of Chrome Azurol S and Chrome Azurol S-aluminium complex.

column, either on-line or off-line. Metal ion separations using gradient or step gradient elution were investigated on both the dye-coated and commercial chelating column.

3.5.1. CLEAN-UP COLUMNS.

Several clean-up columns were investigated for removing metal impurities from eluents and buffers. An on-line clean-up column, which could be switched off-line during the analytical separation was studied. This was the Xylenol Orange coated XAD-2 column employed in the initial studies, and placed before the injection valve. This on-line system was partially successful in removing metal impurities in the eluent. Some problems arose in that during the analytical separation the column had to be switched offline, and from this time some metal impurities, particularly strongly chelating metals such as copper, can build up on the column. Both the clean-up column and the analytical column act as a pH buffer. This is particularly pronounced when the pH is increased before another injection. This buffering effect is due to the SO_3^- groups to some extent, but mostly due to the carboxyl, COO^-, groups which are weakly acidic. To completely equilibrate both columns to the required pH could take a long period of time, especially if the eluent is close to pH7.

An off-line clean-up system was then investigated. Two burettes were packed with Chelex-100 chelating resin which contains iminodiacetic acid chelating groups, and Duolite ES467 ion-exchange resin which contains aminophosphonic acid complexing groups, and fed by gravity. These columns successfully removed virtually all common metal impurities from the eluent, found by blank runs and by injecting the eluent as the sample on the chelation ion chromatography system. However when the cleaned eluents were used, drastic changes in baseline level occurred with different pH eluents. Some form of contamination had occurred, as the PAR-ZnEDTA post-column reagent became saturated, producing an extremely high absorbance at 490nm. It seems likely that some of the chelating groups attached to the substrate have become detached and entered the cleaned eluent, as all the contamination could not just be due to high levels of metal ions. Chelating groups in the cleaned eluent would significantly affect the PAR-ZnEDTA post-column reagent, as this is a complex system, containing many different equilibria.

The most successful clean-up system involved pumping the eluent through a Xylenol Orange coated XAD-2 column offline. Virtually all the metal impurities were removed from the eluent, and also the baseline proved to be very stable. There was however, a slight bleeding of the dye, which was noticeable due to the purple tinge in alkaline conditions, although this did not seem to interfere with the analysis. This clean-up system was used to remove metal impurities from eluents and buffers. The clean-up column was reconditioned by eluting with 0.2M nitric acid after around $300cm^3$ had been processed, to ensure the column does not become saturated with adsorbed metal ions.

3.5.2. GRADIENT ELUTION.

Using a Dionex gradient pump and the CAS coated PLRP-S column, the potential of gradient elution to separate trace metal ions was investigated. A separation of cadmium, lead and copper in 1M potassium nitrate was achieved and is shown in Figure 34. This separation utilized a gradient from pH7 to pH4 over ten minutes to elute cadmium, then a gradient to pH1.5 over a further ten minutes to elute and separate lead and copper. Using gradient elution, copper, which forms strong

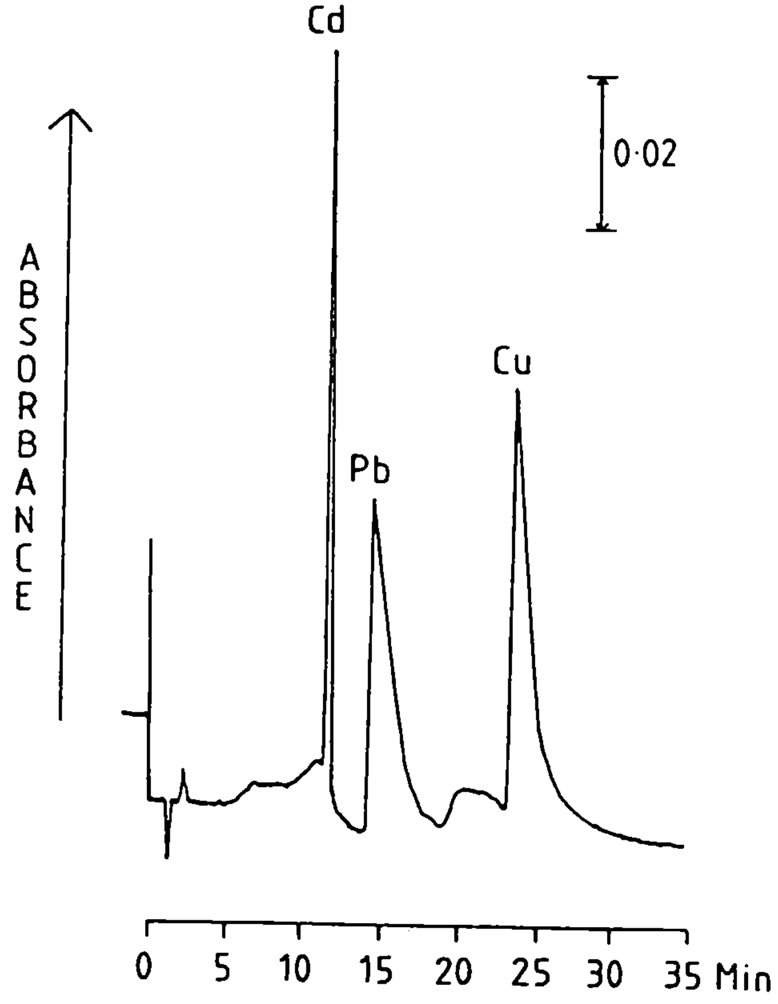


Figure 34. Chromatogram showing separation of 5ppm Cd^{2+}, Cu^{2+} and 10ppm Pb^{2+} in 1M KNO_3 using the Chrome Azurol S coated column and gradient elution.

chelates can be separated from metals such as cadmium which form weaker chelates in comparison. Figure 35 shows the same separation, but in a solution also containing 1000ppm magnesium in the sample. These metals were completely separated from high concentrations of alkaline earth metals, which form the weakest chelates and elute at or near the solvent front. This is of particular interest for samples such as sea water which contain high concentrations of magnesium and calcium.

3.5.3. STEP GRADIENT ELUTION.

Step gradient elution was investigated in more detail for the separation of various groups of metal ions. Eluents of differing pH were prepared, and switched at the appropriate time in the analytical run. A step gradient produces a sharper drop in pH than gradual gradients and is more effective at overcoming the buffering action of the column. Step gradient elution was investigated with both of the chelating dye-coated columns and the commercial TOSOH column.

3.5.3.1. CHROME AZUROL S DYE-COATED COLUMN.

Figure 36 shows a separation of cadmium, copper and lead in 1M potassium nitrate using two step gradients. The sample was injected into pH7 eluent, and immediately after injection, stepped to pH4.5 eluent to elute cadmium and lead. After ten minutes a further step to pH1.5 enabled copper to be eluted off the column. However, a large blank peak occurred at fifteen minutes, which could be due to zinc impurities, or nickel coming from the stainless steel pump tubing. A successful separation of these three metal ions in a sample also containing 1000ppm magnesium and 400ppm calcium is shown in Figure 37. This separation clearly shows the potential of this column for sea water analysis.

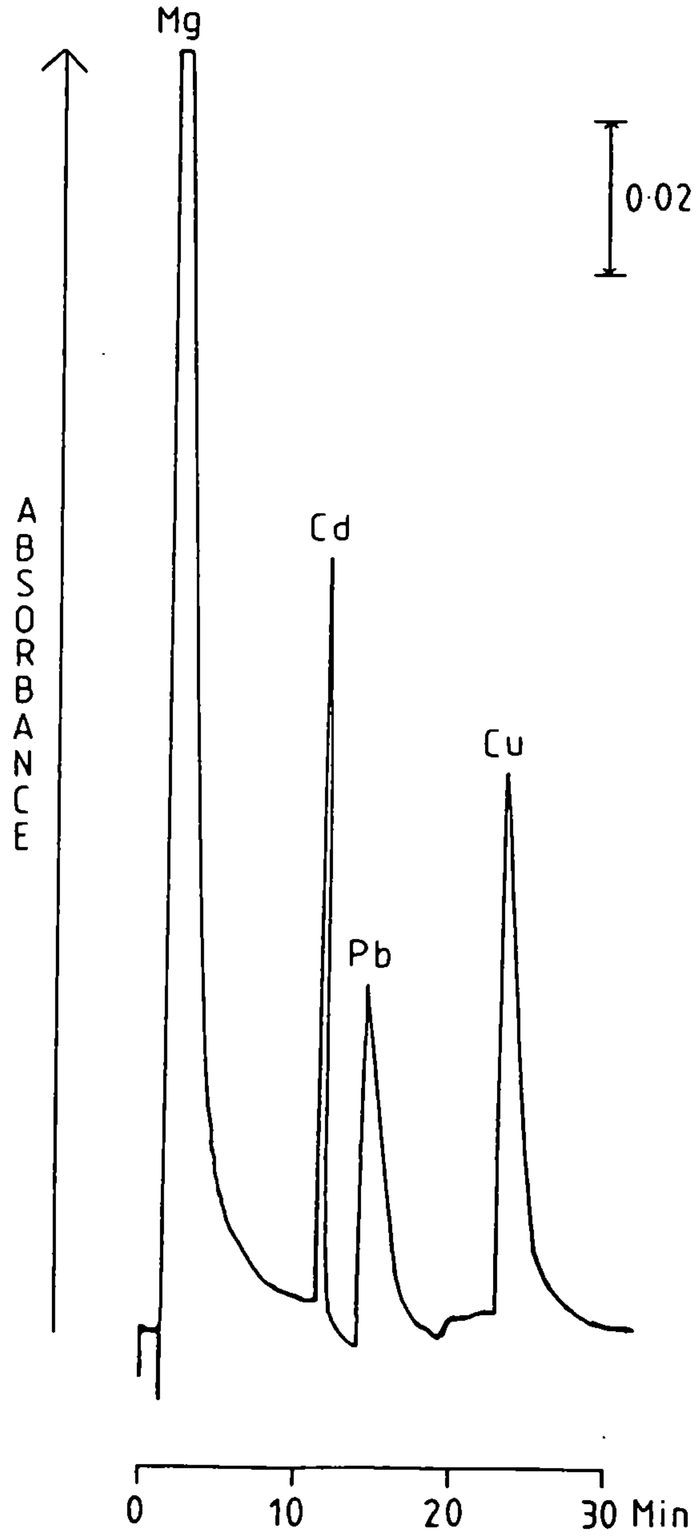


Figure 35. Chromatogram showing separation of 5ppm Cd^{2+}, Cu^{2+}, and 10ppm Pb^{2+} in 1M KNO_3, and 1000ppm Mg^{2+} with Chrome Azurol S dye-coated column, using gradient elution.

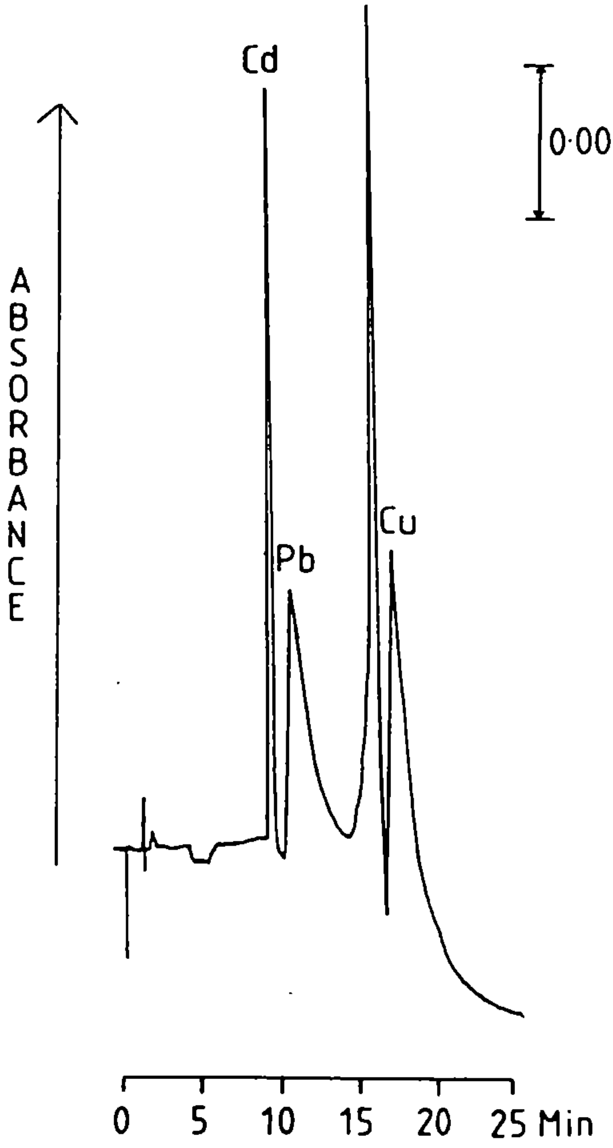


Figure 36. Chromatogram showing the separation of 5ppm Cd^{2+} and Cu^{2+} and 10ppm Pb^{2+} in 1M KNO_3 using the Chrome Azurol S coated column and step gradient elution.

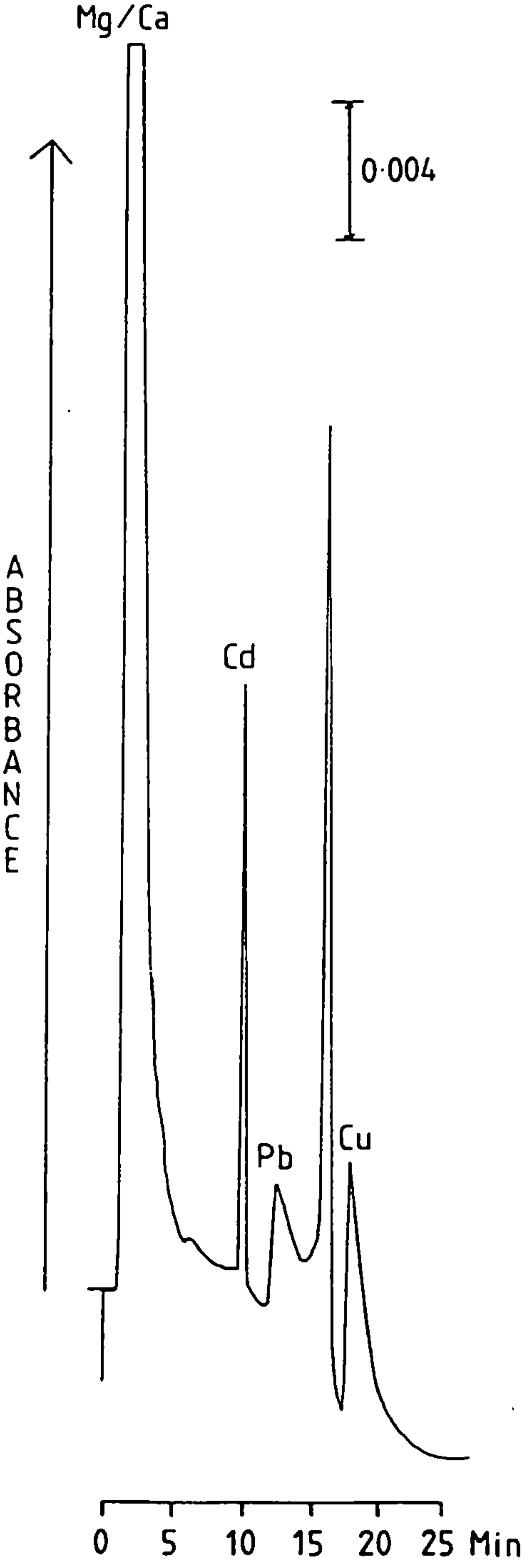


Figure 37. Chromatogram showing the separation of 5ppm Cd^{2+} and Cu^{2+} and 10ppm Pb^{2+} in 1M KNO_3 also containing 1000ppm Mg^{2+} and 400ppm Ca^{2+} using the Chrome Azurol S coated column and step gradient elution.

3.5.3.2. XYLENOL ORANGE DYE-COATED COLUMN.

This proved to be extremely successful for the complete separation of a range of metal ions using step gradient elution, as it appears to be more efficient than the CAS coated column. A separation of manganese, cadmium, zinc, nickel and copper in 1M potassium nitrate using two step gradients is shown in Figure 38. The first step from pH6 to pH3 at injection eluted manganese, cadmium and zinc, whilst a further step after ten minutes eluted nickel and copper. As Xylenol Orange forms stronger chelates than CAS, it can be used to separate alkaline earth metals as well. The complete separation of nine metals with this Xylenol Orange coated column using three step gradients was achieved, and this is shown in Figure 39. The metal mixture was injected at pH10 where every metal would be retained on the column. By stepping to pH6.5 the alkaline earth metals were eluted, then pH3 after fifteen minutes eluted manganese, cadmium and zinc, and a final step to pH0.5 after thirty minutes to elute nickel and copper which form the strongest chelates.

3.5.3.3. TOSOH COLUMN.

This commercial column proved to be as strongly chelating as the Xylenol Orange coated column and capable of isocratic separations of both alkaline earth and other divalent metal ions. Figures 40 and 41 show the separation of six metal ions with PAR-ZnEDTA and PAR post-column detection respectively. The mixture was injected at pH6, and eluent pH stepped to pH2.1 at injection. After fifteen minutes there was a further step to pH0.5 to elute nickel and copper. The run using PAR (Figure 41) used a final step at twenty minutes after injection to separate lead better from nickel. Using PAR-ZnEDTA post-column reagent (Figure 40), a large peak due to alkaline earth metal

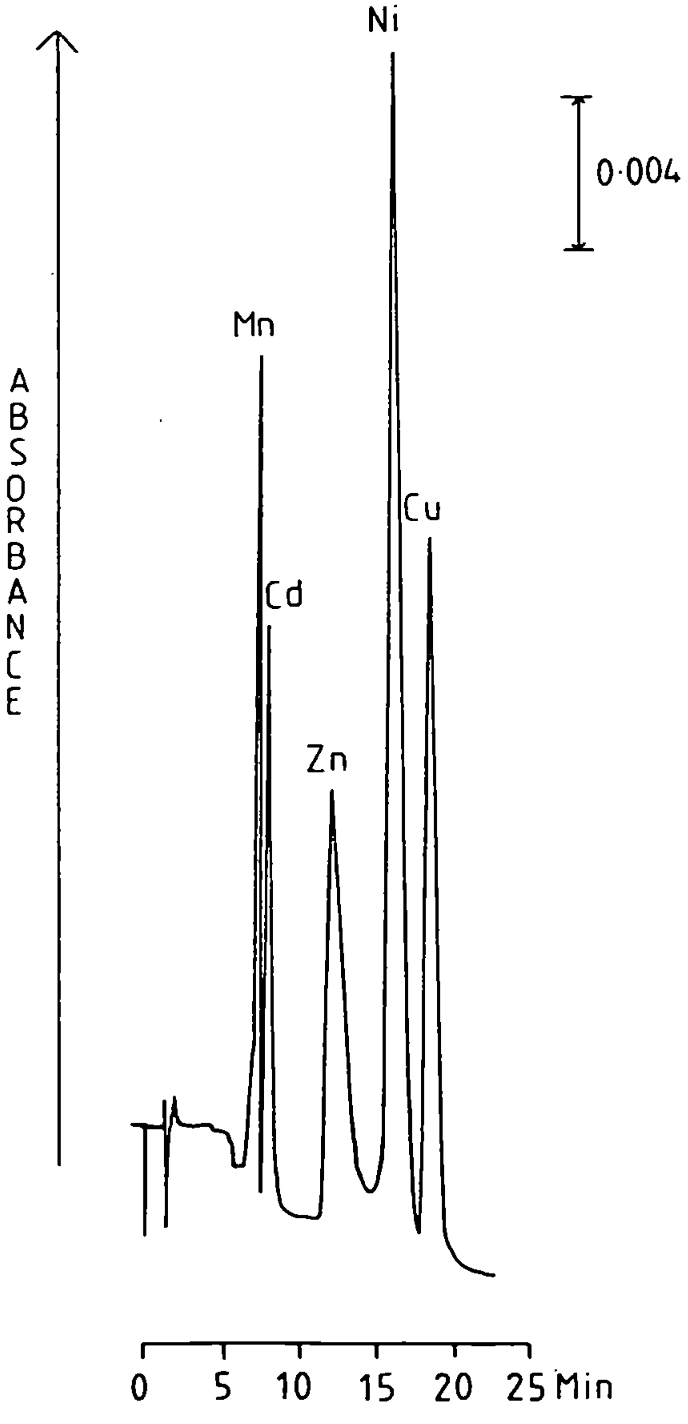


Figure 38. Chromatogram showing the separation of five metal ions in 1M KNO_3 using the Xylenol Orange coated column and step gradient elution. The concentrations were 5ppm Mn^{2+}, 10ppm Cd^{2+}, Zn^{2+}, Cu^{2+} and 30ppm Ni^{2+}.

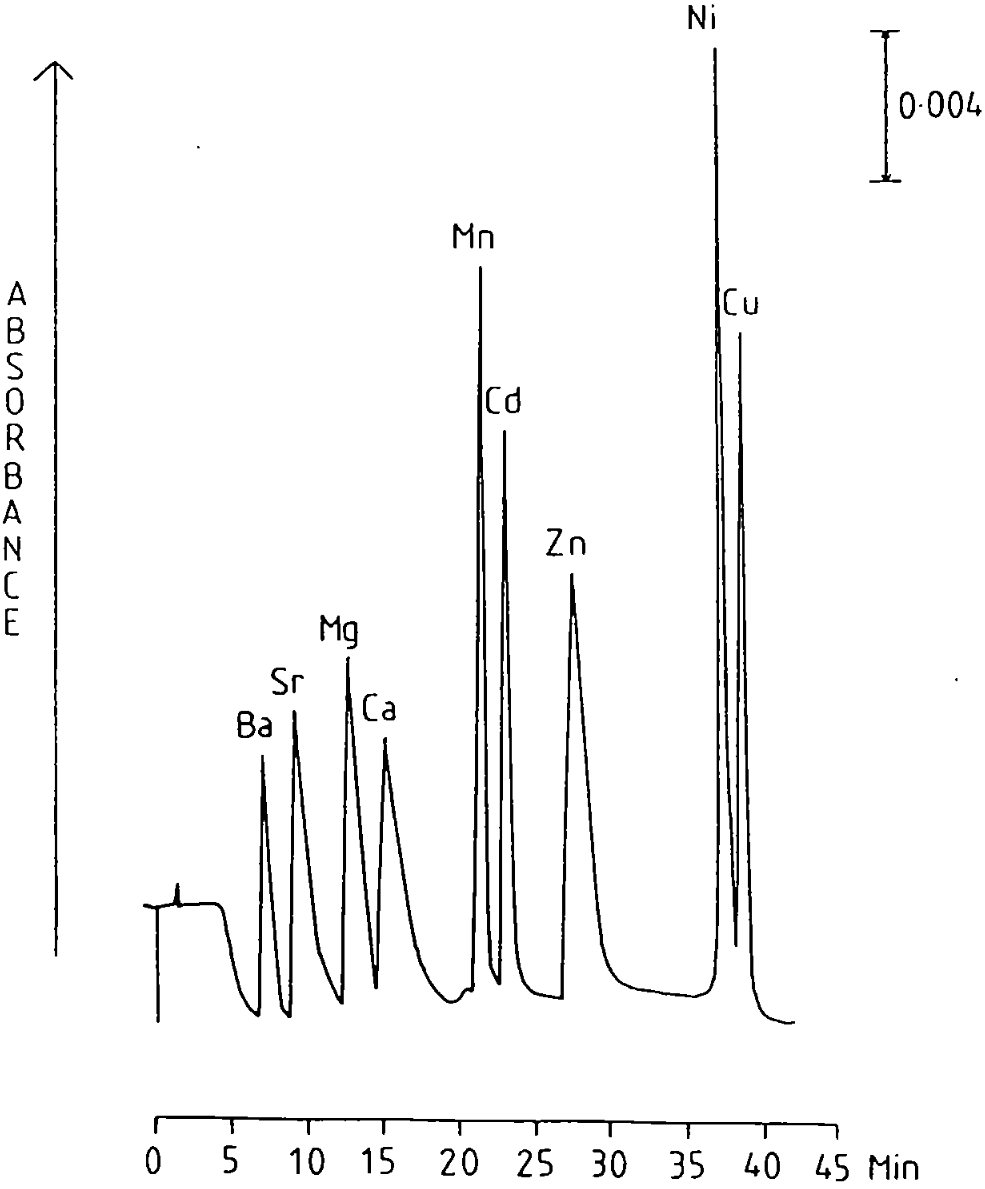


Figure 39. Chromatogram showing separation of nine metals in 1M KNO_3 with Xylenol Orange dye-coated column, using three step gradients. The concentrations were 5ppm Mg^{2+}, Ca^{2+}, Mn^{2+}, Ni^{2+}, Cu^{2+}, 10ppm Cd^{2+} and Zn^{2+}, and 20ppm Ba^{2+} and Sr^{2+}.

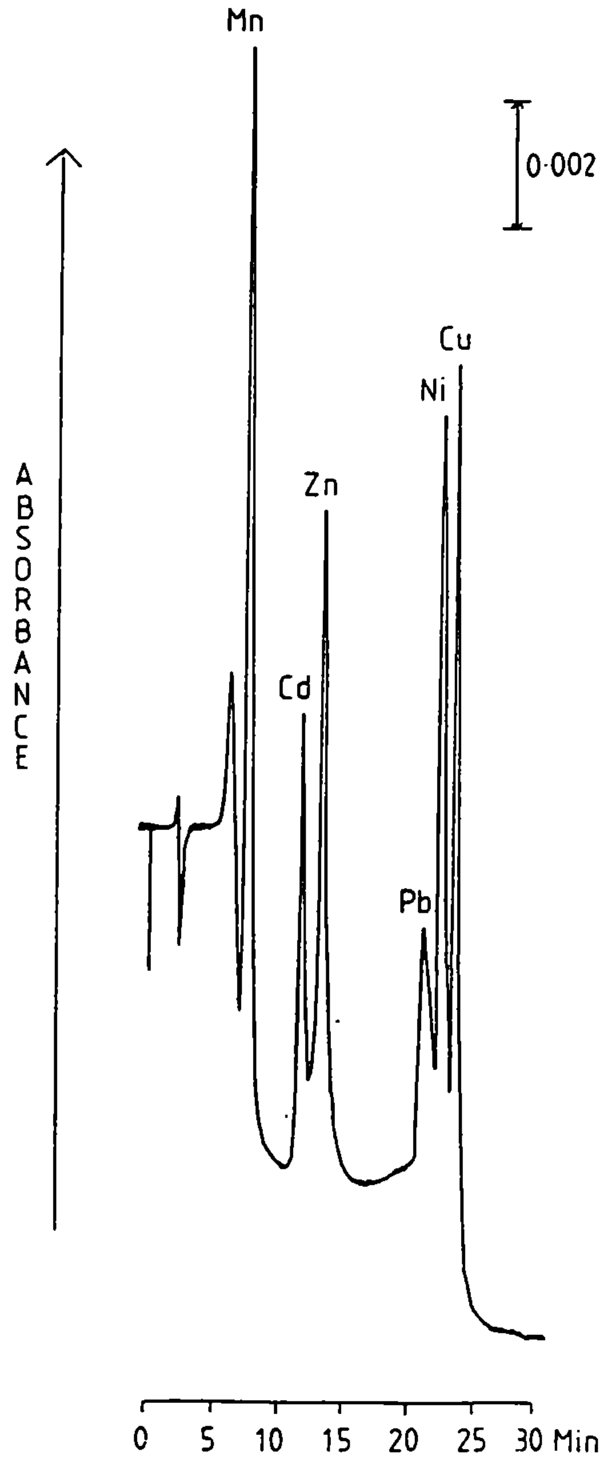


Figure 40. Chromatogram showing the separation of six metal ions in 1M KNO_3 using the TOSOH column and PAR-ZnEDTA post-column reagent with step gradient elution. The concentrations were 5ppm Cu^{2+}, 10ppm Mn^{2+}, Cd^{2+}, Zn^{2+}, Ni^{2+} and 20ppm Pb^{2+}.

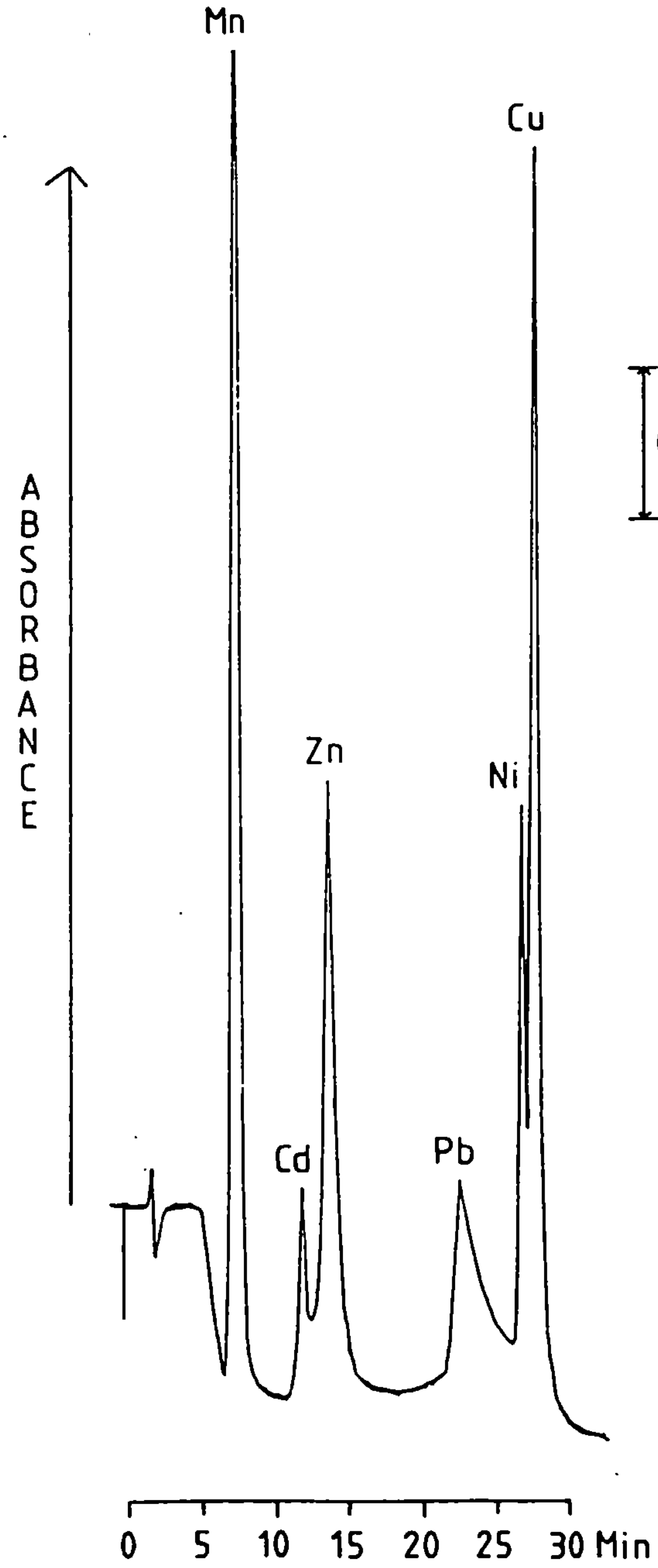


Figure 41. Chromatogram showing the separation of six metal ions in 1M KNO_3 using the TOSOH column and PAR post-column reagent with step gradient elution. The concentrations were 5ppm Cu^{2+}, 10ppm Mn^{2+}, Cd^{2+}, Zn^{2+}, Ni^{2+} and 20ppm Pb^{2+}.

impurities occurs before manganese, and the baseline drift is greater than when PAR alone is used. When PAR was used instead, the sensitivity for cadmium and lead is reduced. Using a step from pH10 to pH6.5, a separation of the four alkaline earth metals could not be obtained, as barium and magnesium co-eluted. By stepping to a pH of 7.4, the peaks merged to form two large peaks.

3.6. SUMMARY.

Initial investigations involving large particle size dye-coated columns showed that the retention of metal ions is dependent on the eluent pH. An increase in retention occurs with an increase in pH. The chelating ability changes markedly with different substrates and chelating dyes. Xylenol Orange coated columns showed the strongest retention of metal ions, and Chrome Azurol S the weakest retention. This is due to the fact that Xylenol Orange contains more strongly chelating groups in comparison with Chrome Azurol S.

Coating Xylenol Orange and Chrome Azurol S onto small particle size ($8\mu m$) HPLC grade substrates produced much more efficient columns capable of separating groups of metal ions. After conditioning, 47.5mg of Xylenol Orange remained coated onto the substrate, with a lower loading of 28mg for the Chrome Azurol S dye-coated column. The effect of ionic strength was important as at 0.2M some ion-exchange was still taking place for alkaline earth metals, however, little or no ion-exchange occurred at 1M ionic strength. With the Xylenol Orange coated column all alkaline earth and transition metals are retained at pH10, with all transition metals retained at pH6. The Chrome Azurol S dye-coated column shows weaker retention characteristics and does not retain all alkaline earth metals, but at pH8 all transition metals are completely retained.

An isocratic separation of barium, strontium, magnesium and calcium was achieved using the Xylenol Orange coated column, together with a separation of cadmium and lead. The Chrome Azurol S coated column proved to be more weakly chelating, however, some isocratic separations of transition and heavy metals were achieved. The dye-coated columns, compared well with the commercial TOSOH chelating column showing similar efficiencies. This TOSOH column and the Xylenol Orange coated column contain the same iminodiacetic acid functional chelating groups and show similar retention and separating properties. Separations of both alkaline earth and transition metals were obtained using the TOSOH column.

A larger number of metal ions can be separated by employing gradient and step gradient elution. Strongly chelating metals such as copper can then be separated from metals such as manganese and zinc which form much weaker chelates. This exploits the fact that the conditional stability constants change with changing pH. However, metal impurities in the eluent tend to be preconcentrated at the starting pH causing a contamination problem. This was overcome by cleaning up the eluent before use by pumping through a Xylenol Orange coated XAD-2 column used in the initial investigations.

The use of gradient and step gradient elution produced some very encouraging results. Transition metals could be selectively eluted and separated from alkaline earth metals with both the Xylenol Orange and Chrome Azurol S coated columns. Using a series of step gradients, a complete separation of nine metal ions in 1M potassium nitrate was also achieved using the Xylenol Orange coated column.

The PAR-ZnEDTA post-column reagent system gave good sensitivity for the detection of alkaline earth and transition metals. It was superior to the Calmagite post-column reagent used initially, as an increase in absorbance was being measured resulting in a lower background noise level. Also, Calmagite only reacts with magnesium out of the alkaline earth metals. Some degree of optimization of PAR and ZnEDTA concentrations had to be carried out as the kinetics of the metal displacement reaction particularly with the alkaline earth metals can be very slow.

PART B.

4.1. PRECONCENTRATION AND SEPARATION SYSTEM FOR THE DETERMINATION OF TRACE METALS IN CONCENTRATED BRINES.

The initial studies, even using large particle size dye-coated columns showed that metal ions could be completely retained if the pH was sufficiently high. Using high performance grade dye-coated substrates, which showed a higher efficiency, metal ion separations could be achieved. In the investigations with gradient and step gradient elution using direct injection, metal impurities in the eluent were being effectively preconcentrated on the column. This problem was overcome by cleaning up the eluent. However, this shows the potential for these columns to retain metal ions from a high ionic strength matrix. Step gradient elution was successful in the separation of a larger range of metal ions, producing high performance separations. If the dye-coated column was used in place of the sample loop, the sample could be pumped through at a pH where the particular metal ions of interest would be retained. Separation could then be achieved using a series of step pH gradients. This modified system is shown in Figure 9. Metal ions would be preconcentrated near the top of the column and then, after a drop in pH, they would be separated further down the column, before elution. This single column technique for preconcentration and separation of metal ions would allow very low detection limits down to sub-ppb, depending on the volume of sample preconcentrated.

The Xylenol Orange dye-coated column clearly shows the potential for the separation of a large number of metal ions in a single injection using pH step gradients. These separations were in a 1M potassium nitrate matrix, but to fully evaluate this column for the separation of trace metals as well as quantitative performance, trace metals in concentrated brines were investigated in detail.

As explained above in order to investigate samples containing metals at low ppb levels, a preconcentration step was used, prior to the analytical separation. This was all achieved using a single column. Actual industrial samples of concentrated sodium and potassium chloride brines used in the chloralkali industry, obtained from ICI, were analyzed for trace metal content using the Xylenol Orange coated column. In all cases blank runs were carried out using the same step gradient program but omitting the sample. All quantitative data was blank corrected if necessary.

4.1.1. TRACE METALS IN SATURATED SODIUM CHLORIDE BRINE.

The actual concentration of this brine determined by titration was 5.1M. This brine was analyzed for alkaline earth metals by employing a single step gradient from pH11 to pH6. Figure 42 shows a typical separation of four alkaline earth metals in saturated sodium chloride brine. A sample and spiked sample are shown. Using this step gradient a calibration series for alkaline earth metals in sodium chloride brine was obtained by the method of standard additions. Figure 43 shows the calibration for barium, and Figure 44 shows the calibration results for magnesium, calcium and strontium. Good linear results were obtained with correlation coefficient, r, for magnesium, calcium, strontium, and barium being 0.994, 0.994, 0.990 and 0.996 respectively. The actual concentrations of alkaline earth metals determined from these calibrations are shown in Table 13. It can be seen that there is a high level of strontium, but this is expected due to its natural occurrence in the source of rock salt.

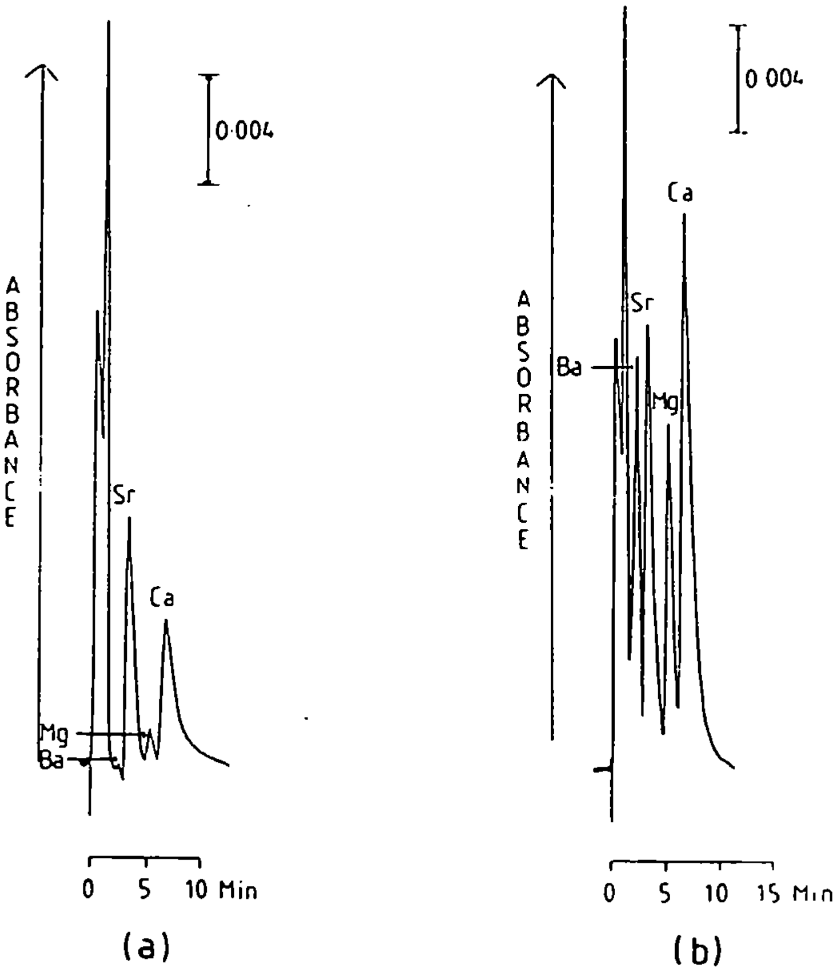


Figure 42. Chromatograms showing preconcentration and separation of alkaline earth metals in saturated (5.1M) NaCl brine using the Xylenol Orange coated column. (a) NaCl brine sample, (b) NaCl brine spiked with 25ppb Mg^{2+}, 75ppb Ca^{2+}, Sr^{2+} and 0.75ppm Ba^{2+}.

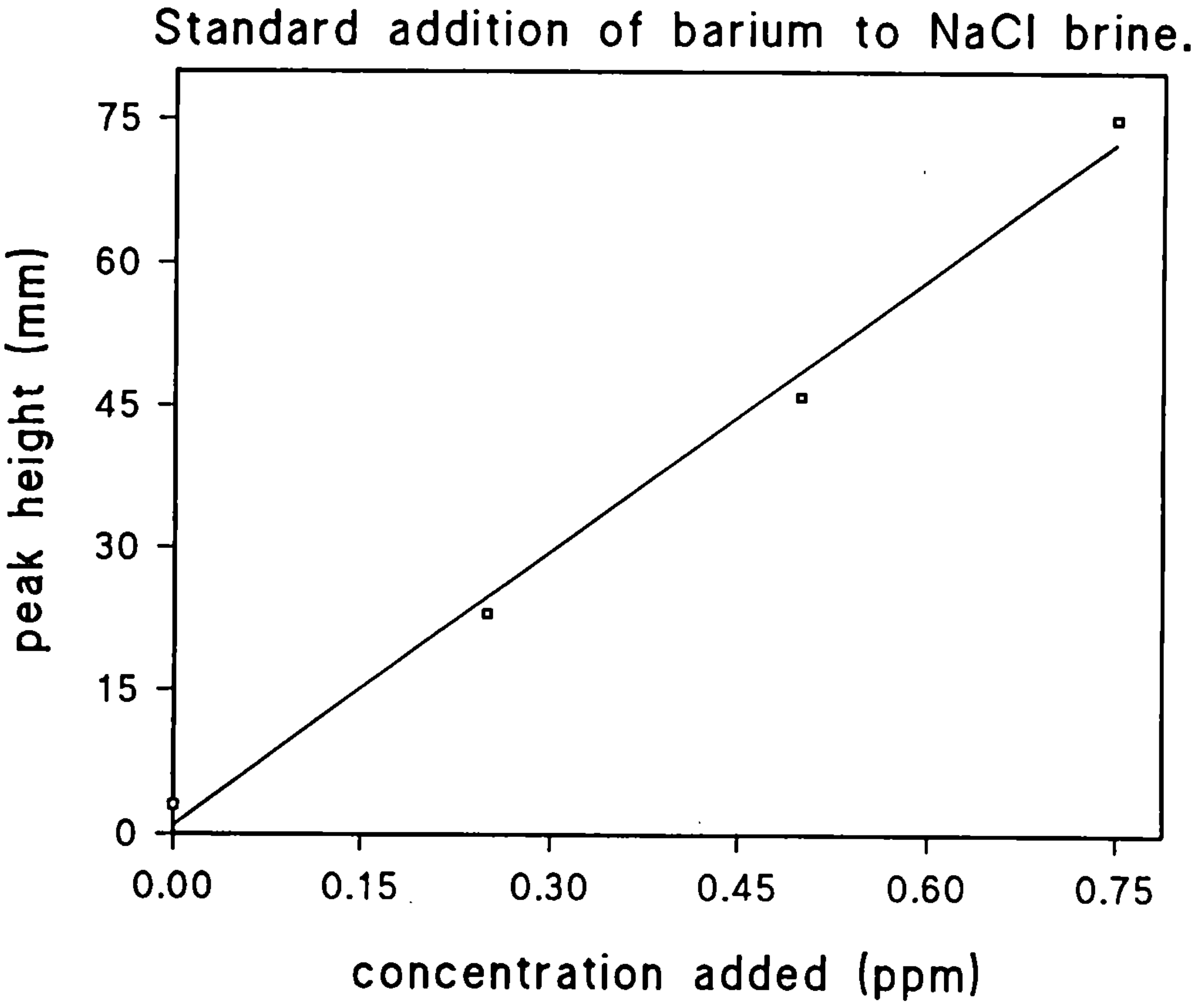


Figure 43. Calibration of barium in sodium chloride brine using the method of standard addition.

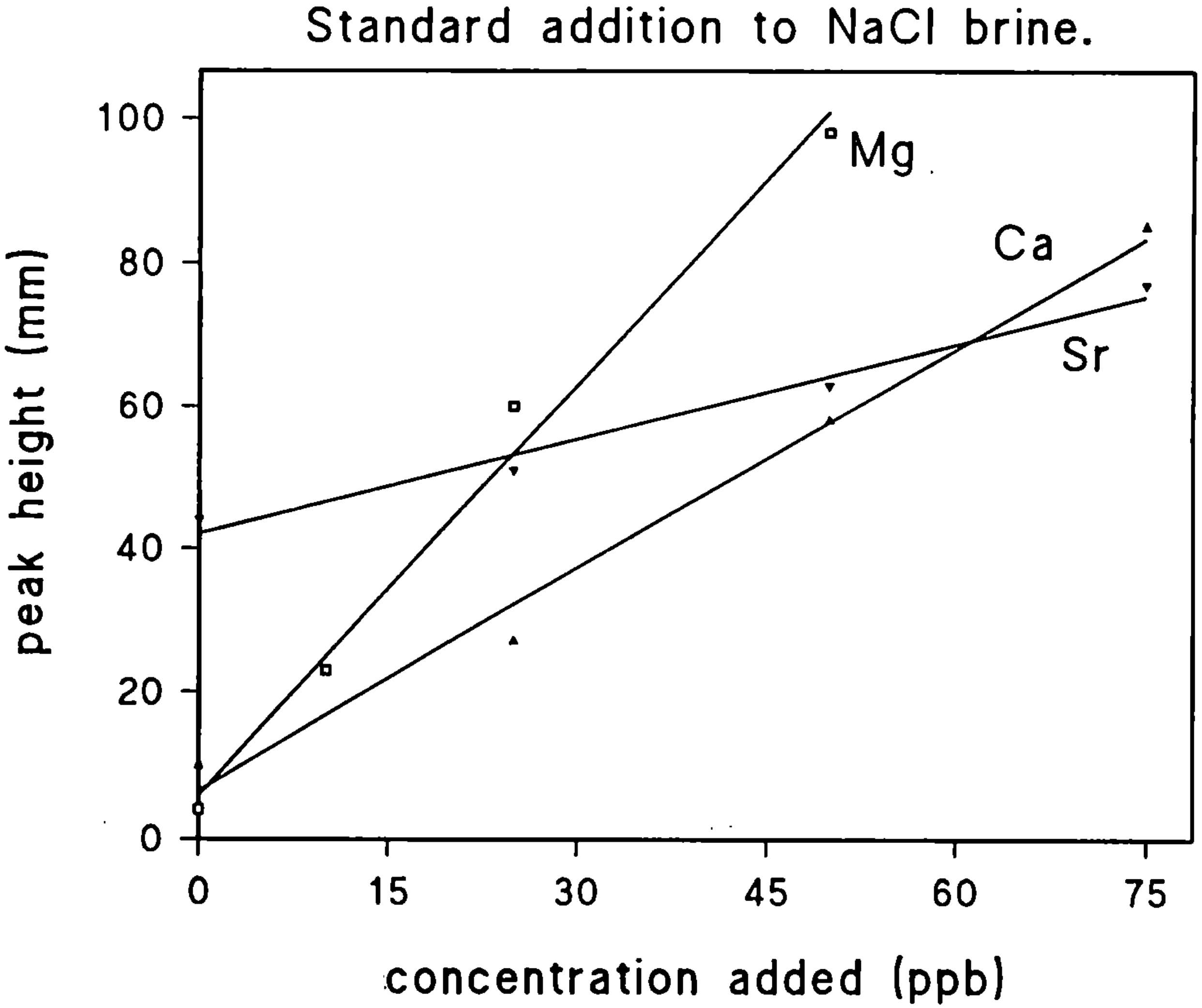


Figure 44. Calibration of magnesium, calcium and strontium in sodium chloride brine using the method of standard addition.

METAL ION.	CONCENTRATION.
Mg^{2+}	3
Ca^{2+}	6
Sr^{2+}	95
Ba^{2+}	9

Table 13. Concentration of alkaline earth metals in NaCl brine in ppb.

4.1.2. TRACE METALS IN POTASSIUM CHLORIDE BRINE.

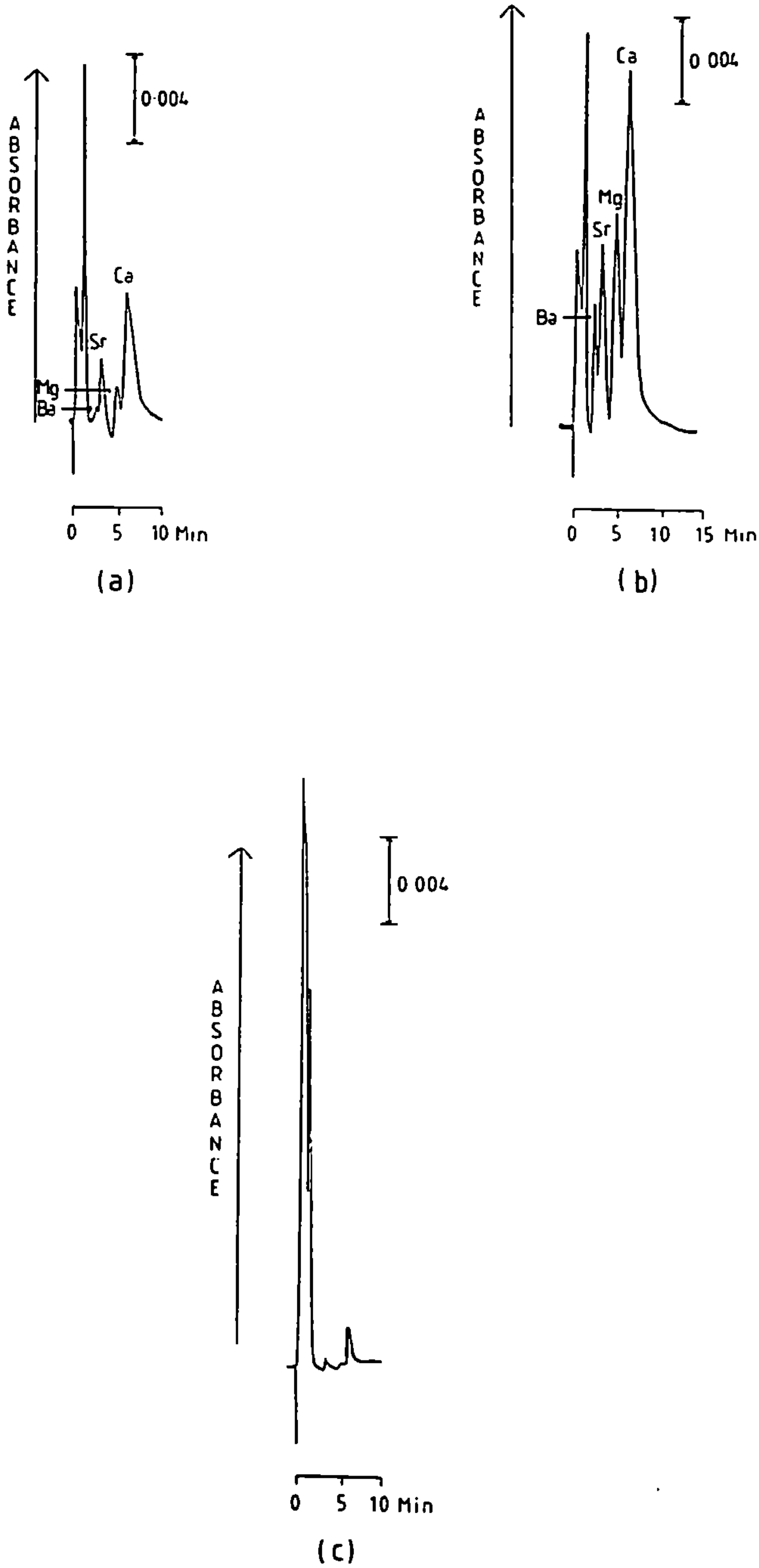
The actual concentration of this brine determined by titration was 2.4M. This brine was investigated for alkaline earth metals, and some transition metals. By combining the step gradient programs for these two groups of metal ions, a separation of a large number of metal ions in a single injection could be achieved.

4.1.2.1. ALKALINE EARTH METALS IN POTASSIUM CHLORIDE BRINE.

Using the same step gradient from pH11 to pH6, as used for the sodium chloride brine, alkaline earth metals could be separated. Figure 45 shows a typical separation of four alkaline earth metals in potassium chloride brine. A sample and spiked sample are shown, together with a blank which shows only very low levels of eluent impurities are present. Using the method of standard addition, a calibration series for these alkaline earth metals in potassium chloride brine was obtained as shown in Figure 46. Good linear results were again obtained with correlation coefficient, r, for magnesium, calcium, strontium and barium being 0.998, 0.991, 0.992 and 0.991 respectively. A series of reproducibility trials for the sample, and a spiked sample, yielded a relative standard deviation for each peak of between 3 and 5%, for six consecutive runs. The actual concentrations of alkaline earth metals in potassium chloride brine determined from the calibrations are shown in Table 14. On comparison with sodium chloride brine (Table 13), it can be seen that the potassium chloride brine contains higher levels of calcium, but much lower levels of strontium.

4.1.2.2. TRANSITION METALS IN POTASSIUM CHLORIDE BRINE.

Some degree of selectivity is possible by changing the starting pH of the gradient. By employing a step gradient from



(a)

(b)

(c)

Figure 45. Chromatograms showing preconcentration and separation of alkaline earth metals in KCl brine using the Xylenol Orange coated column. (a) KCl brine sample, (b) KCl brine spiked with 25ppb Mg^{2+}, 50ppb Ca^{2+}, Sr^{2+}, and Ba^{2+}, (c) blank run.

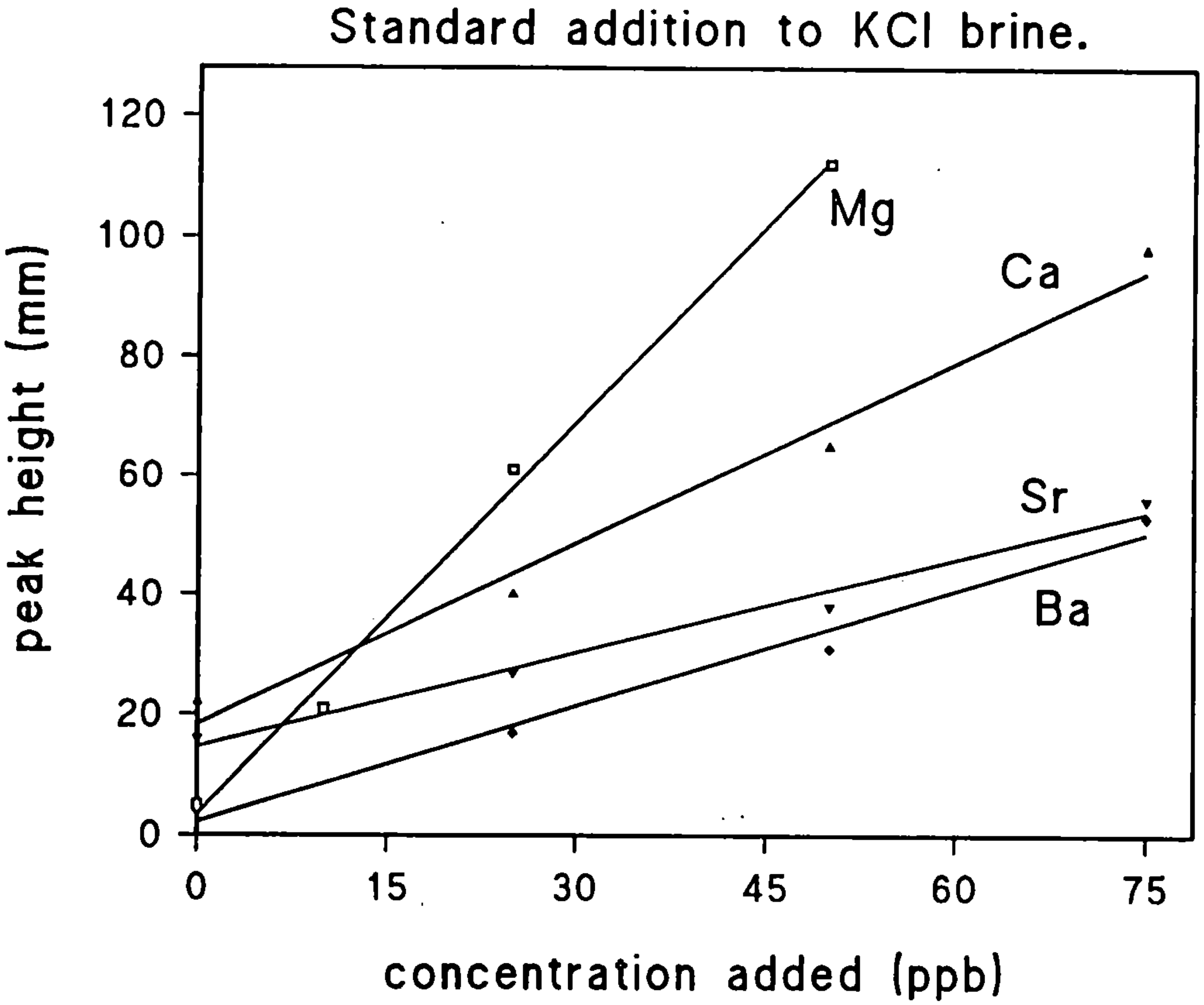


Figure 46. Calibration of alkaline earth metals in potassium chloride brine using the method of standard addition.

METAL ION.	CONCENTRATION.
Mg^{2+}	1
Ca^{2+}	18
Sr^{2+}	28
Ba^{2+}	3
Mn^{2+}	8
Zn^{2+}	17
Ni^{2+}	4
Cu^{2+}	<1

Table 14. Concentration of trace metals in KCl brine in ppb.

pH6 to pH3, then to pH0.7 after five minutes enabled a separation of some transition metals in potassium chloride brine to be achieved. A separation of manganese, zinc, nickel and copper in potassium chloride brine is illustrated in Figure 47. Again a sample and spiked sample are shown. The change in baseline level when copper elutes is due to a disturbance of the post-column reagent mixture. The more acidic eluent when mixed with the post-column reagent causes a slight change in overall pH (up to 0.4 pH units), which changes the background absorbance slightly. A calibration series for these four metal ions, obtained by using the method of standard addition is shown in Figure 48. Again, good linear results were achieved with the correlation coefficient, r, for manganese, zinc, nickel and copper being 0.998, 0.999, 0.999 and 0.998 respectively. A series of reproducibility trials using six consecutive runs of the sample and a spiked sample yielded a relative standard deviation for each peak of between 2 and 5%. Table 14 shows the actual concentrations of these four metals in potassium chloride brine, determined from the calibrations. It can be seen that there is a significant level of zinc, however the brine is virtually free of any copper.

4.1.2.3. SEPARATIONS OF A RANGE OF DIVALENT METAL IONS IN POTASSIUM CHLORIDE BRINE.

If a greater range of metal ions needed to be studied and determined in one operation, then a three step gradient system is possible, for the separation of both alkaline earth metals and transition metals in a single injection. Figure 49 shows a complete separation of nine metal ions in potassium chloride brine. A step gradient from pH10 to pH6 eluted the alkaline earth metals, followed by a step to pH3 after seven minutes to elute manganese, cadmium and zinc, then to elute nickel and

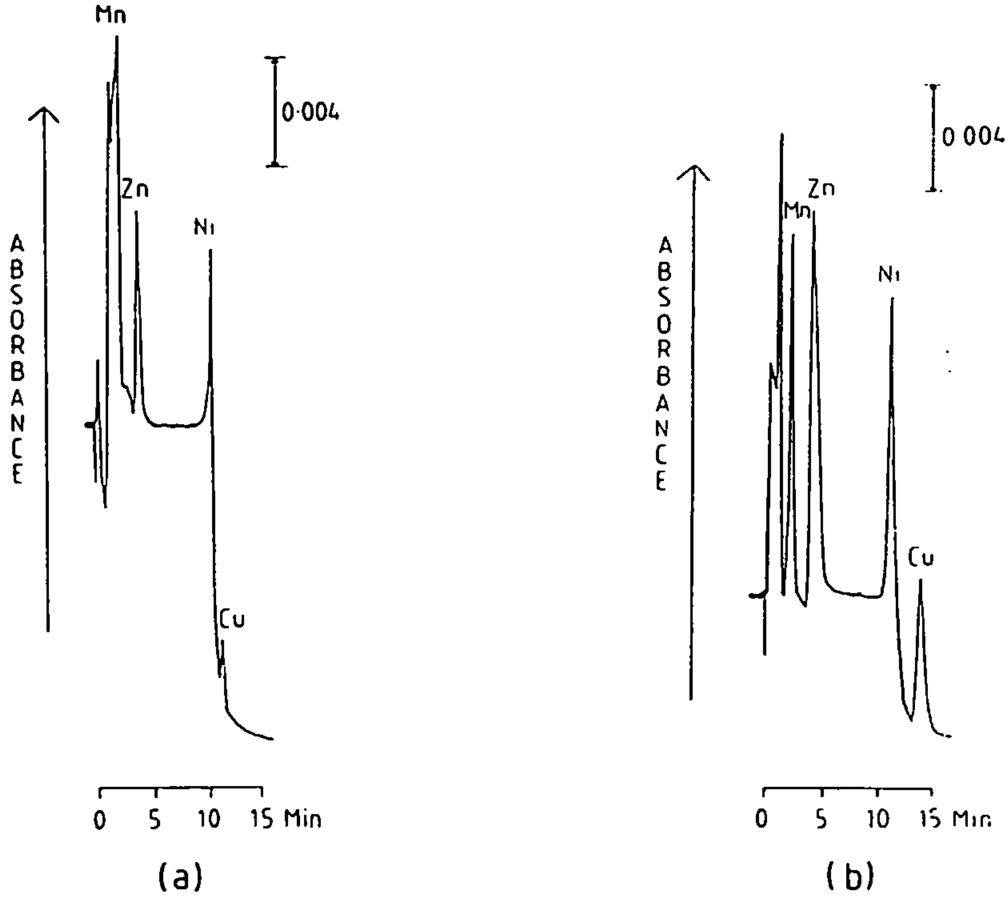


Figure 47. Chromatograms showing preconcentration and separation of some transition metals in KCl brine using the Xylenol Orange coated column. (a) KCl brine sample, (b) KCl brine spiked with 10ppb Mn^{2+}, 40ppb Zn^{2+}, Ni^{2+} and Cu^{2+}.

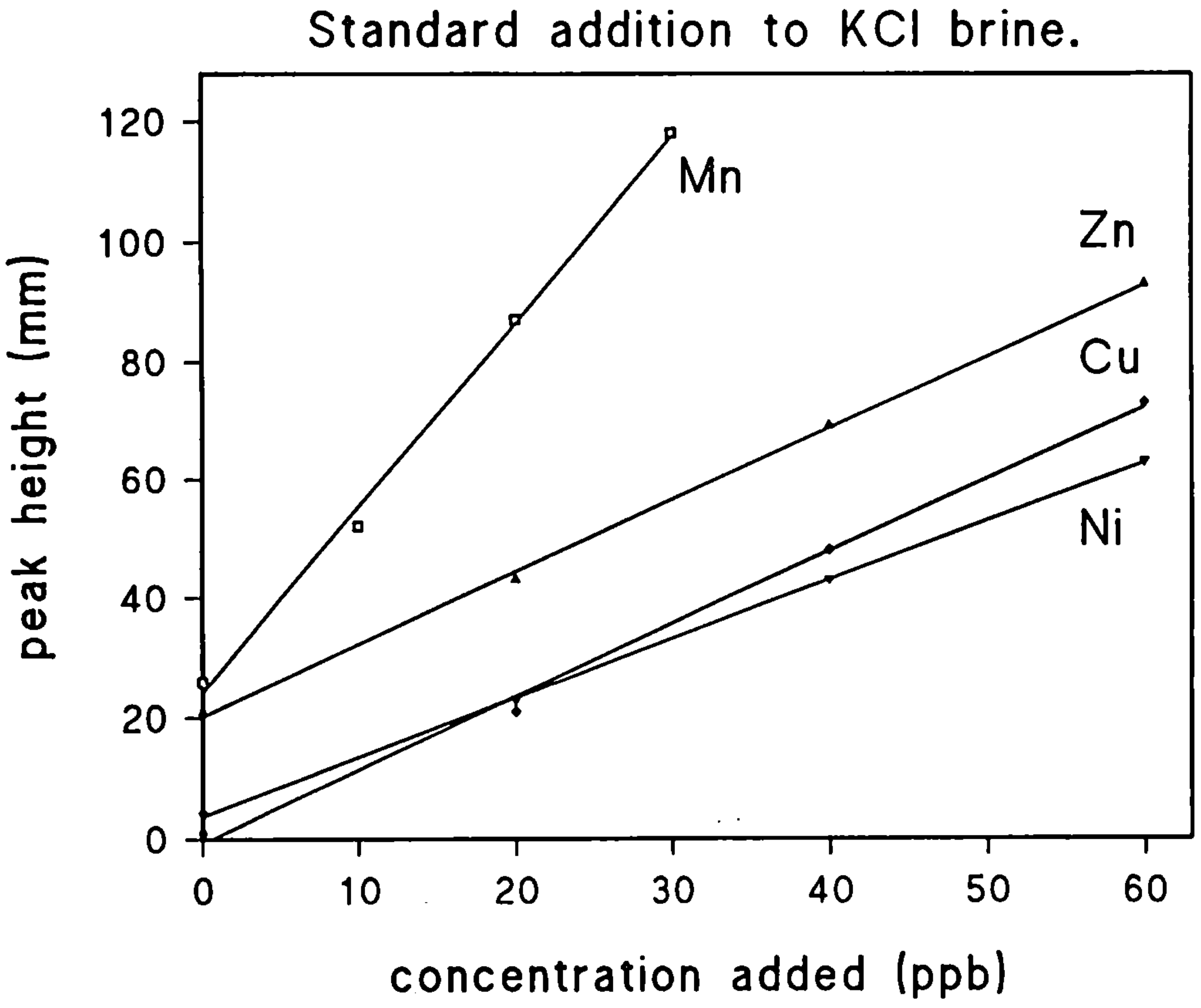


Figure 48. Calibration of some transition metals in potassium chloride brine using the method of standard addition.

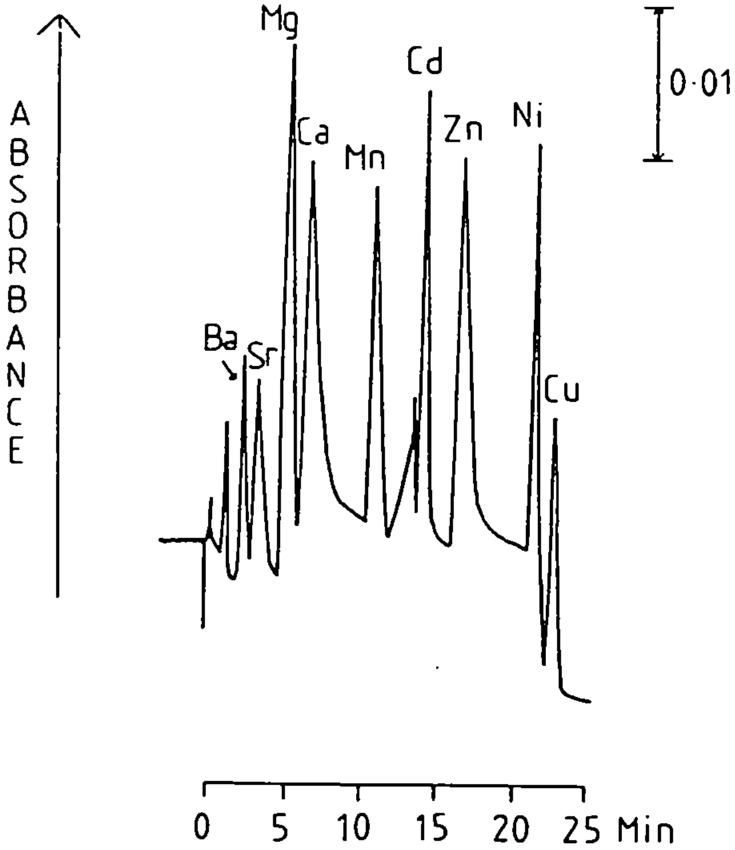


Figure 49. Chromatogram showing preconcentration and separation of nine metal ions in KCl brine using the Xylenol Orange coated column, with three step gradients. Spiked metal concentrations were 50ppb Mg^{2+}, Ca^{2+}, Zn^{2+}, Ni^{2+}, Cu^{2+} and 0.1ppm Sr^{2+}, Ba^{2+}, Mn^{2+} and Cd^{2+}.

copper, a final step to pH0.7 after fifteen minutes.

Another separation of divalent metals employed three step gradients. A step gradient from pH6 to pH3.7 eluted manganese and cadmium, then a step to pH2 after one minute eluted zinc and lead, followed by a final step to pH0.5 after six minutes to elute nickel, copper and iron(II). This separation of seven metal ions is shown in Figure 50. As iron(II) eluted after copper, it is likely that it may have been oxidised to iron(III) during the analytical separation by atmospheric oxygen.

These studies show that selectivity can be controlled by starting at a different pH. By starting at pH10 or pH11, alkaline earth and transition metals would be retained. After which, using a single step gradient, a complete separation of four alkaline earth metals could be achieved without any transition metals being eluted. The step gradient process can be continued further to separate transition metals as well. However, by starting at pH6, the transition metals can be separated, whilst the alkaline earth metals elute on or near the solvent front, and do not interfere with the separation. Essentially, three different gradient programs have been developed, to selectively separate groups of metal ions or a complete set of metal ions.

4.2. FURTHER APPLICATIONS OF CHELATION ION CHROMATOGRAPHY.

The successful use of chelating dye-coated columns in the separation and determination of trace metals in 1M potassium nitrate and concentrated brines encouraged further work with other matrices. Impurities in some laboratory chemicals were investigated, as it has already been shown that impurities in our eluent can be detected and separated. The Chrome Azurol S

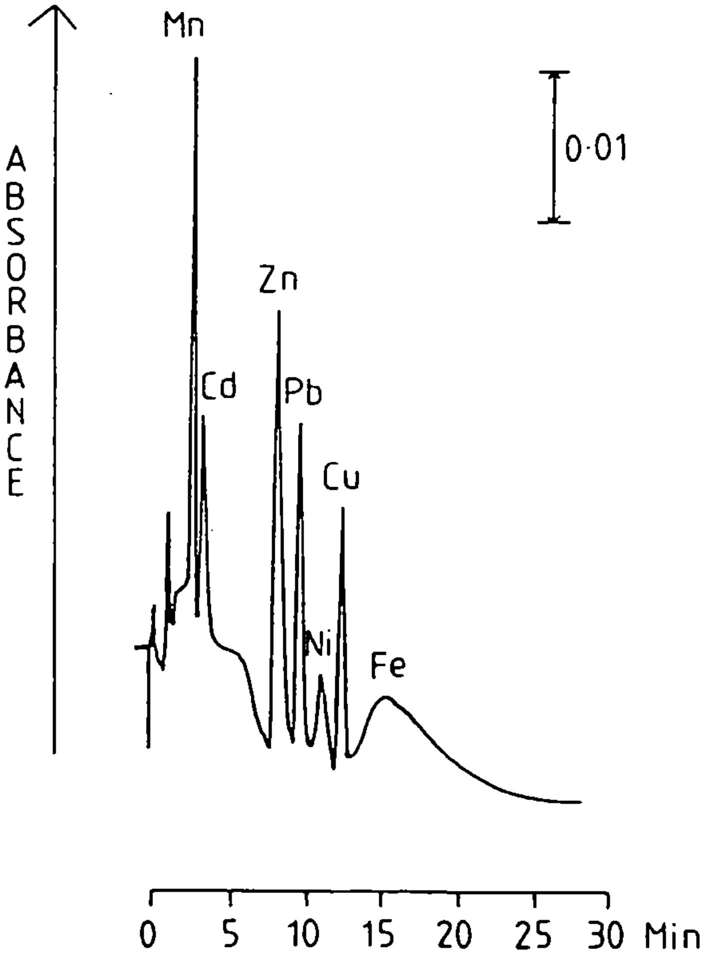


Figure 50. Chromatogram showing the preconcentration and separation of seven metal ions in KCl brine using the Xylenol Orange coated column and three step gradients. The spiked metal concentrations were 50ppb Mn^{2+}, Zn^{2+}, Ni^{2+}, Cu^{2+}, 0.1ppm Cd^{2+}, Pb^{2+} and 0.5ppm Fe^{2+}.

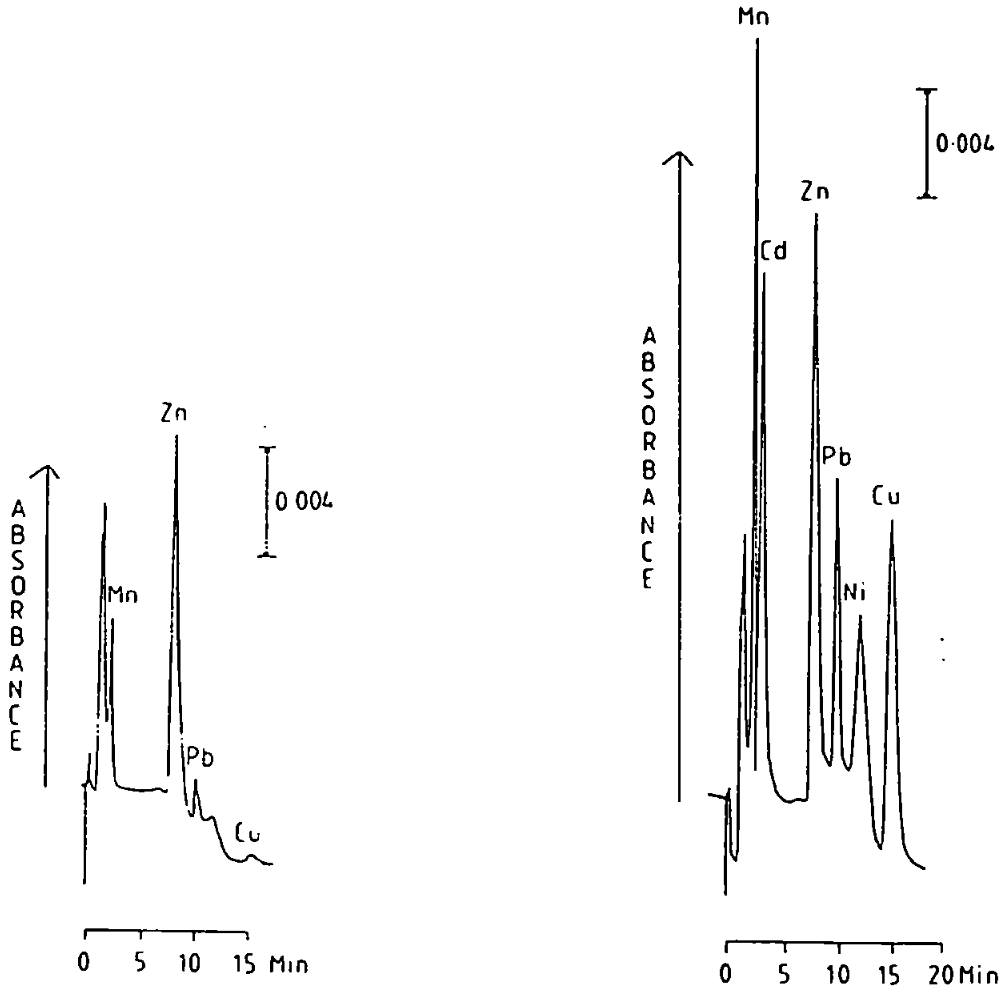
coated column was chosen for the study of some trivalent metal ions.

4.2.1. IMPURITIES IN LABORATORY CHEMICALS.

Three laboratory chemicals were selected for the determination of some divalent metal ion impurities. These were potassium nitrate, sodium sulphate and caesium iodide, made up as 1M solutions for analysis. Using a step gradient from pH6 to pH3.7, then pH2 after one minute, and pH0.7 after six minutes, six metal impurities could be separated. Figures 51, 52 and 53 show a complete separation of manganese, cadmium, zinc, lead, nickel and copper in these laboratory chemicals. Approximate impurity levels in all three chemicals are shown in Table 15. It can be seen that the potassium nitrate, used as our main eluent, contains relatively high levels of zinc, and shows the necessity for clean-up of this reagent as described in the experimental (Section 2.2.3.). The sodium sulphate contains significantly higher levels of manganese, whilst there were some problems in quantifying copper in caesium iodide, probably due to interference of iodide which is known to reduce copper(II) to copper(I).

4.2.2. ANALYSIS OF TRACE METALS IN SEA WATERS.

The concentrations of major and minor metal constituents of sea water are shown in Table 16. The main constituents are Na^+ and Mg^{2+}, however the total ionic strength of sea water is approximately 0.7M as Mg^{2+} and Ca^{2+} have a pronounced effect. At the natural pH of sea water many metals are not present as simple ionic species. They are in the form of a complex or precipitate. Most of the Na^+, K^+, Mg^{2+} and Ca^{2+} is uncomplexed, however many other transition and heavy metals are present as carbonato, chloro or hydroxo complexes as well as organically bound. The analysis of sea waters usually only takes into



(a) (b)

Figure 51. Chromatograms showing preconcentration and separation of metal ions in 1M KNO_3 using the Xylenol Orange coated column. (a) 1M KNO_3 sample and (b) 1M KNO_3 spiked with 25ppb Mn^{2+}, Zn^{2+} and 0.1ppm Cd^{2+}, Pb^{2+}, Ni^{2+} and Cu^{2+}.

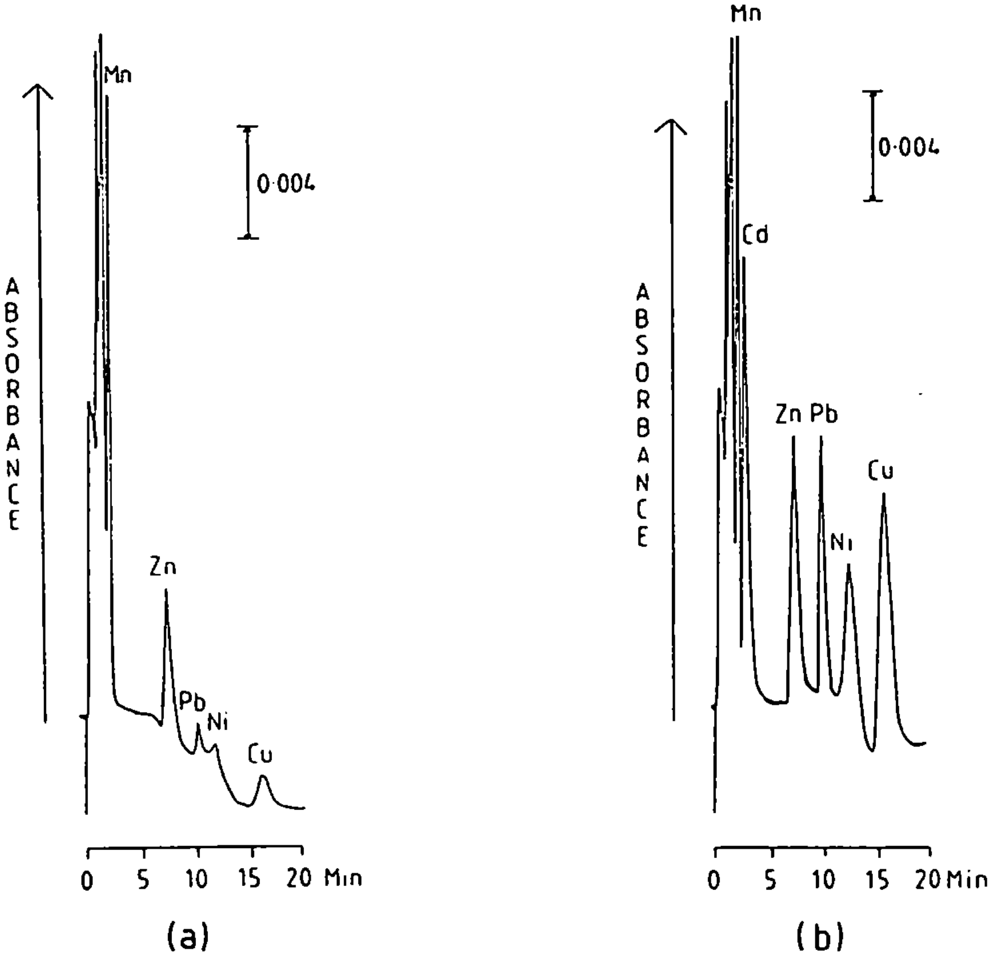
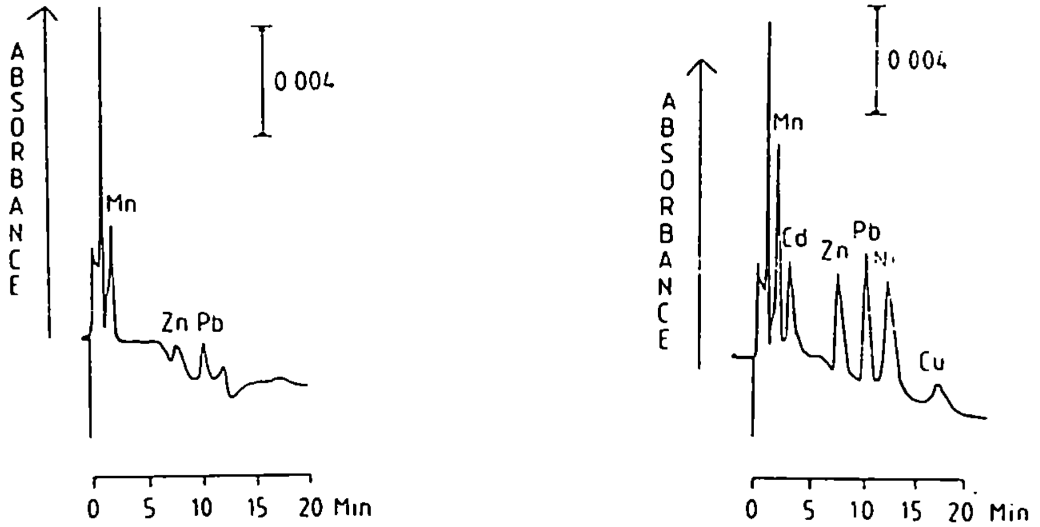


Figure 52. Chromatograms showing preconcentration and separation of metal ions in 1M Na_2SO_4 using the Xylenol Orange coated column. (a) 1M Na_2SO_4 sample and (b) 1M Na_2SO_4 spiked with 10ppb Mn^{2+}, 25ppb Zn^{2+} and 0.1ppm Cd^{2+}, Pb^{2+}, Ni^{2+} and Cu^{2+}.



(a) (b)

Figure 53. Chromatograms showing preconcentration and separation of metal ions in 1M CsI using the Xylenol Orange coated column. (a) 1M CsI sample and (b) 1M CsI spiked with 5ppb Mn^{2+}, 10ppb Zn^{2+} and 50ppb Cd^{2+}, Pb^{2+}, Ni^{2+} and Cu^{2+}.

	1M KNO_3	1M Na_2SO_4	1M CsI
Mn^{2+}	5	28	4
Cd^{2+}	-	-	-
Zn^{2+}	60	21	2
Pb^{2+}	10	10	15
Ni^{2+}	-	5	-
Cu^{2+}	3	12	X

X - Not quantified (see text).

Table 15. Approximate impurity levels in three laboratory chemicals in ppb.

MAJOR ION.	CONCENTRATION.	TRACE ELEMENT.	CONCENTRATION.
Na^+	11000ppm	Mn	0.2ppb
Mg^{2+}	1325ppm	Fe	0.45ppb
Ca^{2+}	421ppm	Ni	0.3ppb
K^+	407ppm	Cu	0.25ppb
		Zn	0.3ppb
		Cd	11ppt
		Hg	4ppt
		Pb	10ppt
		U	3ppb

Table 16. The major and some minor metal constituents of sea water (41). (ppm = mgl^{-1}, ppb = μgl^{-1}, and ppt = ngl^{-1}).

account the total dissolved trace metal content.

The CAS coated column has been shown to be suitable for sea water analysis because of its weak affinity for calcium and magnesium. The fact that all transition metals are retained at pH8, makes this column particularly attractive as the natural pH of sea water is pH8.1. No adjustment of sample pH will be needed before injection. However, after several studies it was found not to be as efficient as the Xylenol Orange coated column and could not separate all of the metals investigated. The Xylenol Orange coated column was investigated for its suitability.. Its stronger affinity for magnesium and calcium was overcome by preconcentrating sea water at pH6, rather than the natural pH of 8.1, and thus most of the magnesium and calcium present will not be retained. Also by using PAR alone as the post-column reagent, which does not react well with alkaline earth metals, the magnesium/calcium peak at the solvent front could be further reduced. Synthetic sea water samples, together with sea water samples were studied for their trace metal content using the Xylenol Orange coated column, and a comparison made with the commercial TOSOH column.

4.2.2.1. SYNTHETIC SEA WATER.

To investigate the separating properties of the Xylenol Orange coated column for use in sea water analysis, synthetic sea water was prepared by ten fold dilution of a saturated sodium chloride brine sample, with the addition of appropriate concentrations of magnesium and calcium. This was then spiked with various metal ions for analysis. PAR-ZnEDTA was however used for the post-column reagent.

Preconcentrating at pH6, then stepping to pH3.7 after ten minutes, pH2 after fifteen minutes, then finally pH0.7 after twenty minutes, enabled a separation of five metals in synthetic sea water. Figure 54 shows a separation of cadmium, zinc, lead, nickel and copper, with varying sensitivities for each metal ion.

A separation of cadmium, copper and lead from a synthetic sea water sample also containing 200ppm zinc was achieved using a similar step gradient program, where each step occurs five minutes earlier in the run. Figure 55 shows this separation, and shows the potential for the analysis of mining wastes draining into estuaries which can contain high levels of cadmium, zinc, copper and lead.

Similar experiments with the commercial TOSOH column showed the metal ions were more strongly chelated to the substrate, as a sharper step gradient was required for a separation of a range of metal ions. Using a step gradient from pH6 to pH2 after five minutes, followed by a final step to pH0.7 after twenty-five minutes, enabled a separation of manganese, cadmium, zinc, lead, nickel and copper in synthetic sea water to be achieved. This separation is shown in Figure 56, where a large peak also occurs before manganese which is due to alkaline earth metals. Iron(III) is also detected as a small peak between nickel and copper. However, when used for the preconcentration of sea waters, this column proved to be too strongly chelating for the alkaline earth metals, since even thirty minutes after injection, magnesium and calcium were still eluting off the column.

4.2.2.2. PLYMOUTH SOUND SEA WATER.

Sea water from Plymouth Sound, which had been filtered through a 0.45μm filter before use to remove particulate matter, was investigated for traces of cadmium, zinc, lead, nickel and copper. This separation was achieved with a step gradient from pH6 to pH3.7, then to pH2 after five minutes, and

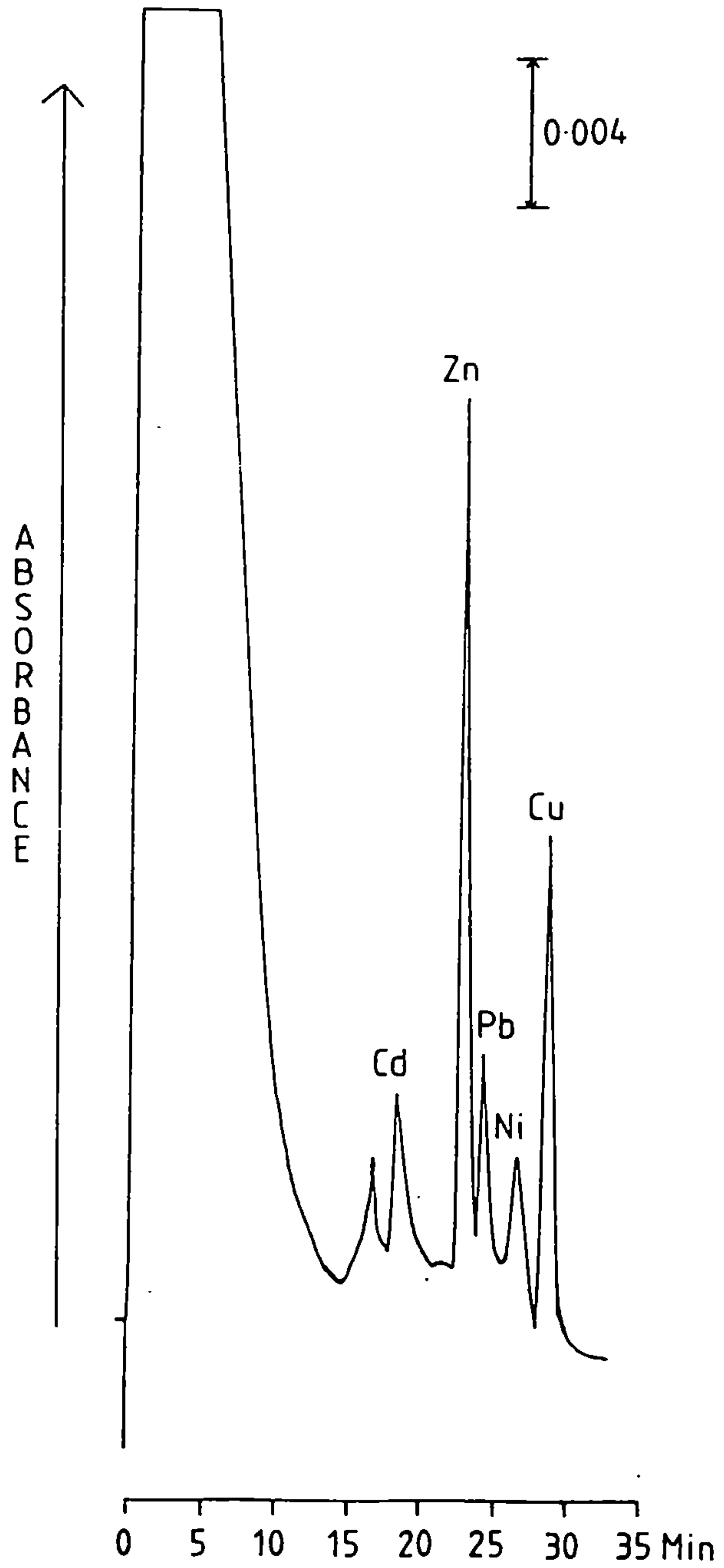


Figure 54. Chromatogram showing the preconcentration and separation of five metal ions in artificial sea water using the Xylenol Orange coated column. The spiked metal concentrations were 50ppb Cd^{2+}, Zn^{2+}, Pb^{2+}, Ni^{2+} and Cu^{2+}.

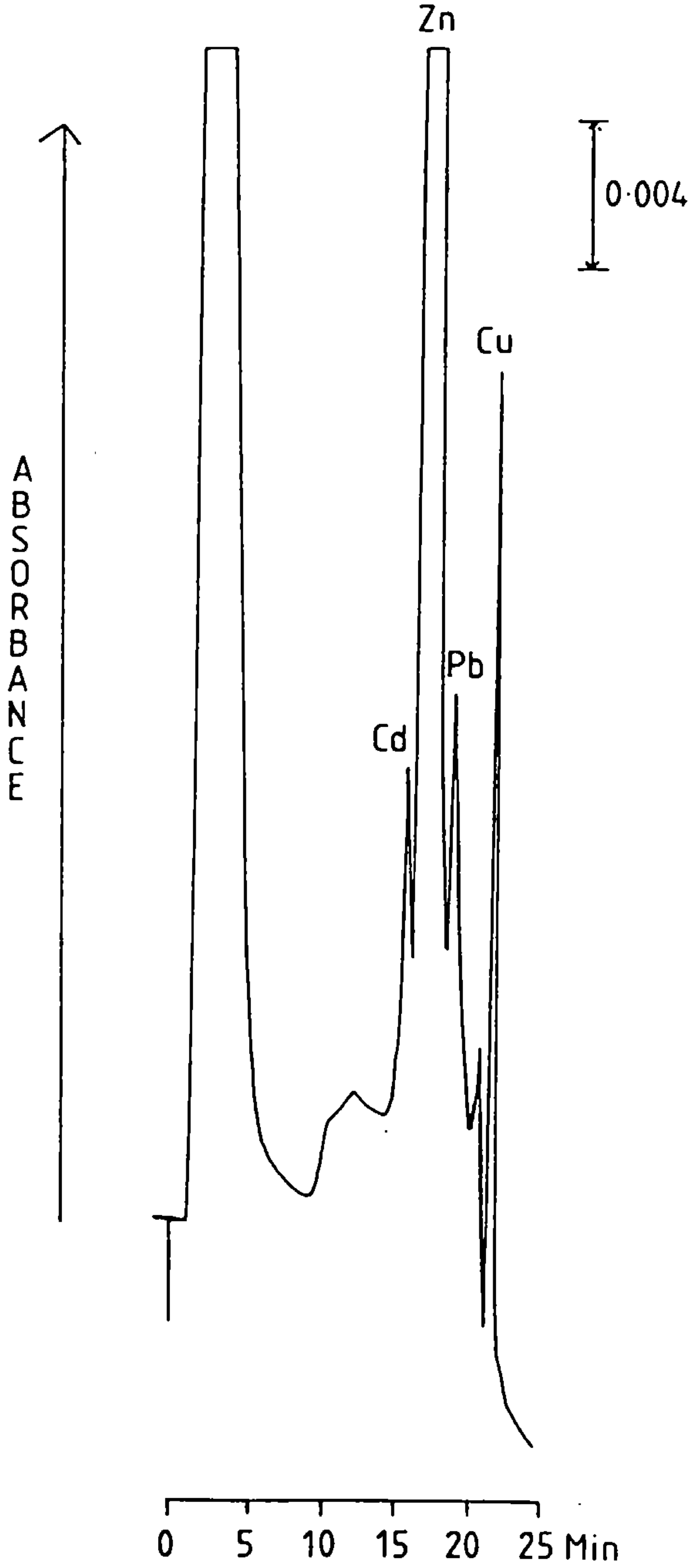


Figure 55. Chromatogram showing separation of 5ppm Cd^{2+}, Pb^{2+} and Cu^{2+} in artificial sea water also containing 200ppm Zn^{2+} using the Xylenol Orange coated column.

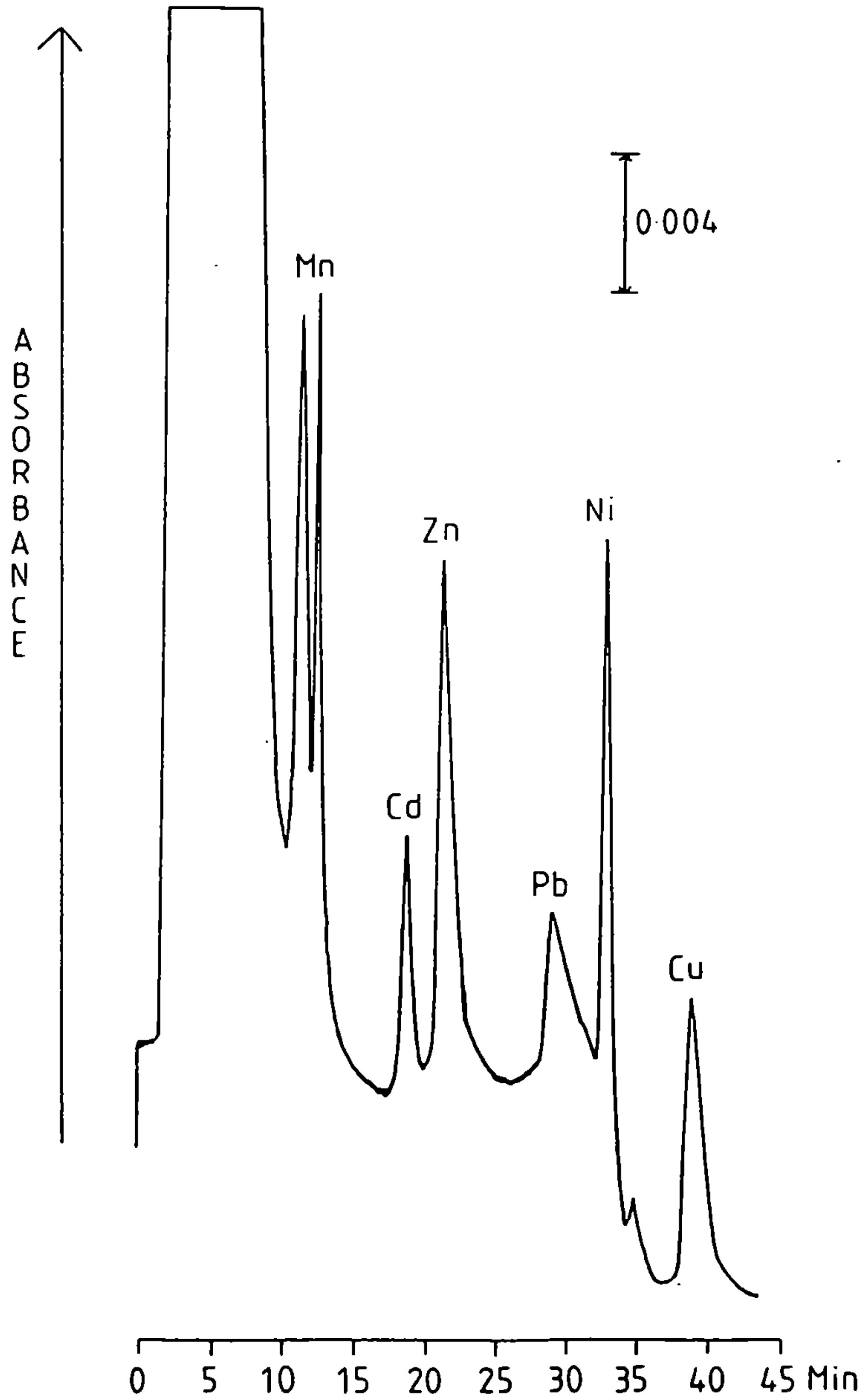


Figure 56. Chromatogram showing the separation of six metal ions in artificial sea water using the TOSOH column. The spiked metal concentrations were 5ppm Mn^{2+}, Zn^{2+}, 10ppm Cd^{2+}, Ni^{2+}, Cu^{2+} and 20ppm Pb^{2+}.

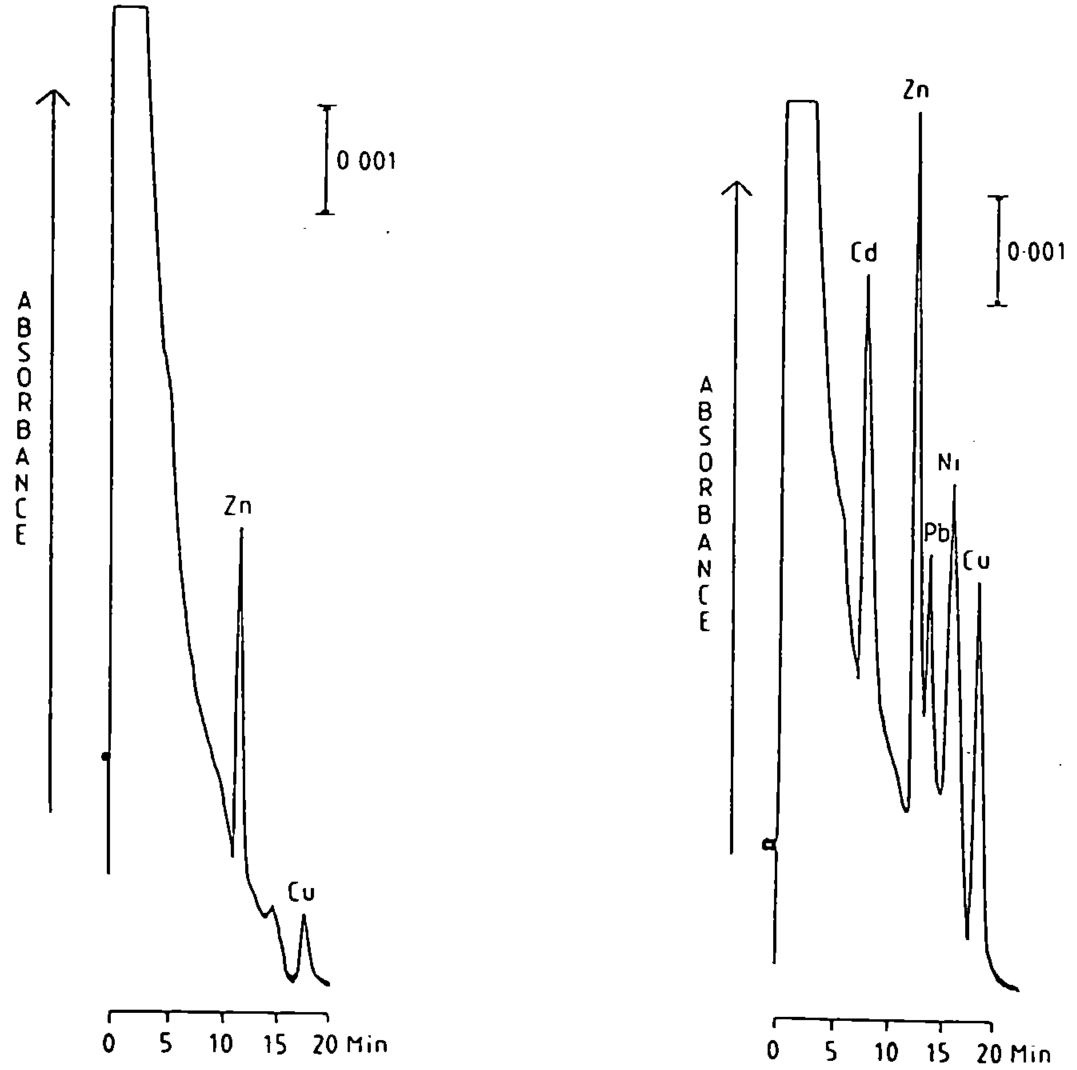
pH0.7 after ten minutes. Figure 57a shows the results from a sample of Plymouth Sound sea water, whilst Figure 57b shows a similar spiked sample. A large peak due to magnesium and calcium still occurs at the solvent front as PAR does react slightly with these ions (114). Zinc and copper were found to be present at approximately 1ppb and 3ppb respectively, with a slight hint that lead may be present in the sea water. Higher preconcentration volumes will be needed to quantify this. The level of copper present is significantly higher than the normally expected concentration of 0.25ppb (Table 16). This is because several rivers feeding into Plymouth Sound are enriched with copper due to the local geology.

4.2.2.3. FORMATION WATER.

A sample of formation water used as oil-well brines, which contains high levels of alkaline earth metals in a concentrated brine media, was investigated for trace metals. A series of step gradients from pH6 to pH3.7 after ten minutes, followed by a step to pH2 after fifteen minutes and finally pH0.7 after twenty minutes, enabled five metal ions to be studied. Figure 58 shows a Statfjord brine sample from the North Sea, where zinc and lead were detected at concentrations of approximately 13ppb and 11ppb respectively. This result is very encouraging as formation waters can be difficult to analyze directly, due to both the high salt content and alkaline earth metal content.

4.2.3. TRIVALENT METAL IONS.

The work so far demonstrates the suitability of the chelation chromatography approach for the determination of divalent metal ions. It would be useful to investigate the performance of these columns for trivalent ions. However, trivalent metal ions in general form much stronger chelates than divalent metal ions. The Xylenol Orange coated column would require much lower



(a)

(b)

Figure 57. Chromatograms of trace metals in Plymouth Sound sea water. Preconcentration and separation using the Xylenol Orange coated column. (a) Plymouth Sound sea water showing Zn^{2+} and Cu^{2+} present in the sample, (b) Plymouth Sound sea water spiked with 5ppb Zn^{2+}, 20ppb Cu^{2+} and 50ppb Cd^{2+}, Pb^{2+} and Ni^{2+}.

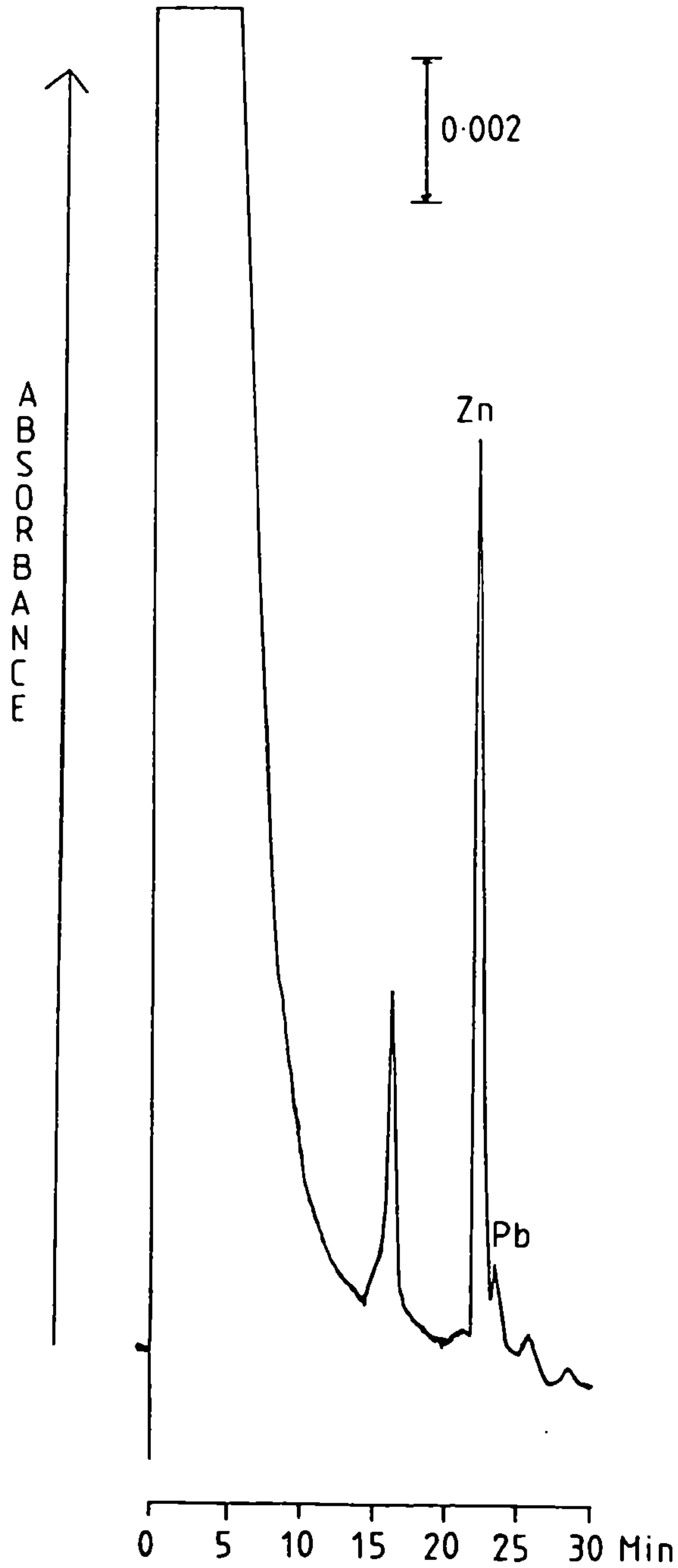


Figure 58. Chromatogram of trace metals in Statfjord brine formation water showing Zn^{2+} and Pb^{2+} present in the sample. Preconcentration and separation using the Xylenol Orange coated column.

pH eluents for desorption, therefore it was decided to investigate trivalent ions using the weaker chelating Chrome Azurol S column. Two Chrome Azurol S columns were prepared using purified and unpurified CAS dye for coating. The results were not identical for the two columns.

4.2.3.1. SEPARATIONS WITH THE CHROME AZUROL S COATED COLUMN.

This column was coated using purified CAS dye. It proved to be successful for the isocratic separation of aluminium, gallium and indium. Figure 59 shows an isocratic separation of aluminium, gallium and indium in 1M potassium nitrate, achieved at pH2.2, however because of slow exchange kinetics, the column was set at an elevated temperature of 60°C. The separation profile is similar to previous work (108), but the elution order is different, gallium eluting before indium. Many commercial sources of CAS are impure and this might have affected the chelating properties.

4.2.3.2. SEPARATIONS WITH THE NEW CHROME AZUROL S COATED COLUMN.

This column was coated using unpurified CAS dye, however it only had a slightly lower loading after cleaning of 23mg compared with the original column. A separation of aluminium, gallium and indium at pH2.2 with the column temperature at 60°C in a water bath, was not as good as the original column, as gallium and indium could not be fully resolved. This is shown in Figure 60. On decreasing the pH, peaks merge, whilst on increasing the pH, they rapidly broaden. However, by increasing the pH to 2.6, and the column kept at room temperature, enabled a complete separation of all three metals to be achieved. This separation is shown in Figure 61.

Iron(III) and bismuth form stronger chelates than aluminium, gallium and indium, and a lower pH is required for any

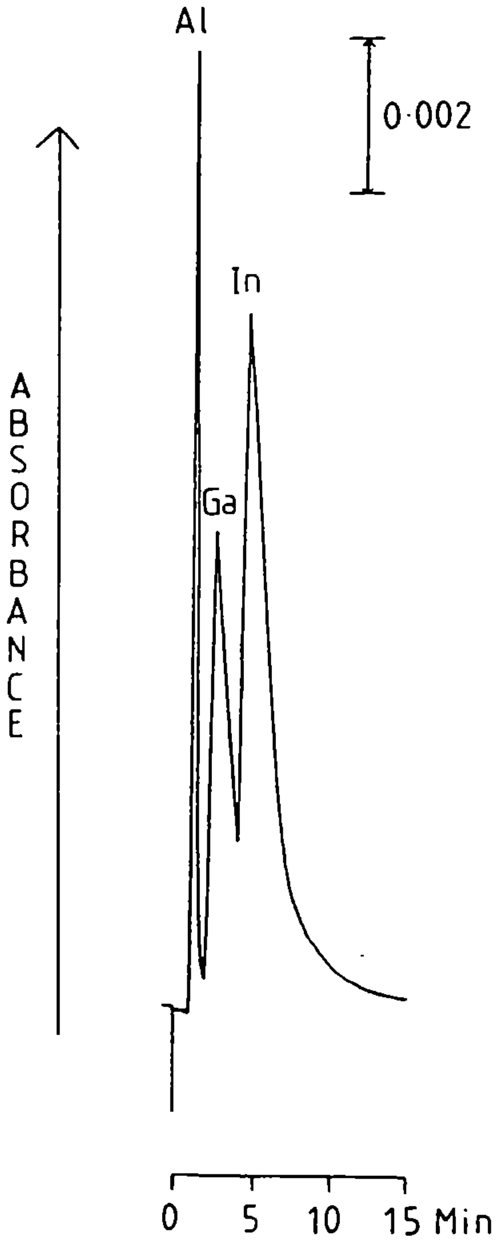


Figure 59. Chromatogram showing isocratic separation of 2ppm Al^{3+}, 10ppm Ga^{3+} and 20ppm In^{3+} in 1M KNO_3, at pH2.2 using the Chrome Azurol S coated column at 60°C.

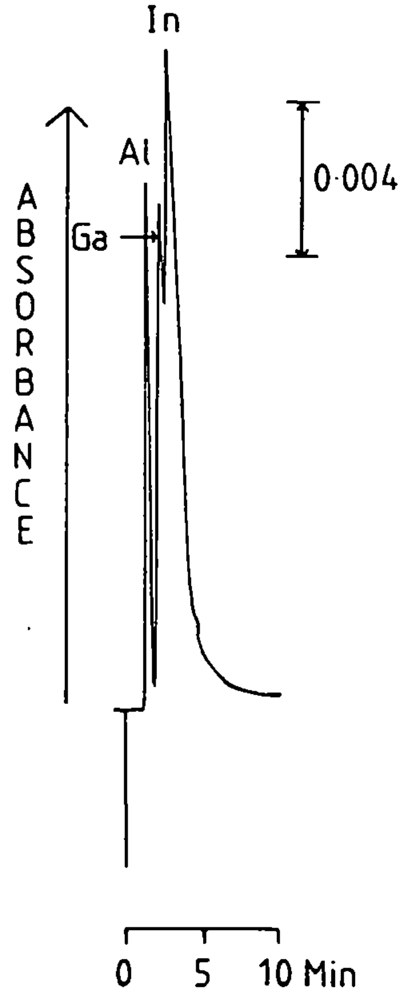


Figure 60. Chromatogram showing the best isocratic separation of 2ppm Al^{3+}, 10ppm Ga^{3+} and 20ppm In^{3+} in 1M KNO_3 at pH2.2 using the new Chrome Azurol S coated column at 60°C.

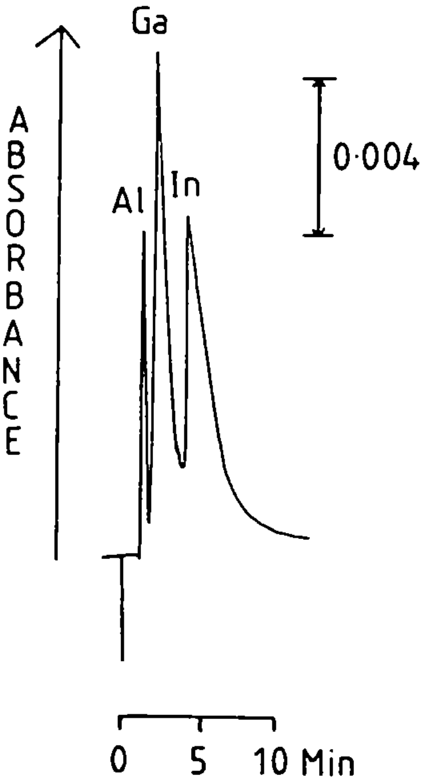


Figure 61. Chromatogram showing the isocratic separation of 2ppm Al^{3+}, 10ppm Ga^{3+} and 20ppm In^{3+} in 1M KNO_3 at pH2.6 using the new Chrome Azurol S coated column at room temperature.

separation. To separate iron and bismuth, a step gradient from pH2.5 was used, however a complete separation could not be achieved, probably due to the poorer efficiency of the column. Figure 62 shows a partial separation of iron and bismuth using a step gradient from pH2.5 to pH0.8, with a column temperature of 60°C.

The kinetics of the complex formation and dissociation are much slower with trivalent metal ions compared with divalent metal ions. This can be improved by increasing the column temperature. Trivalent metal ions tend to be extensively hydrolysed even in weakly acidic solutions and problems with possible precipitation could occur. However, there is clear potential for the use of dye-coated columns for the analysis of trivalent metal ions. Iron(III) and bismuth are retained at pH2.5 and so they could be preconcentrated before separation. Aluminium, gallium and indium are completely retained at pH4. Using preconcentration and a step gradient, separation of low ppb levels of these metals in various high ionic strength media could be achieved.

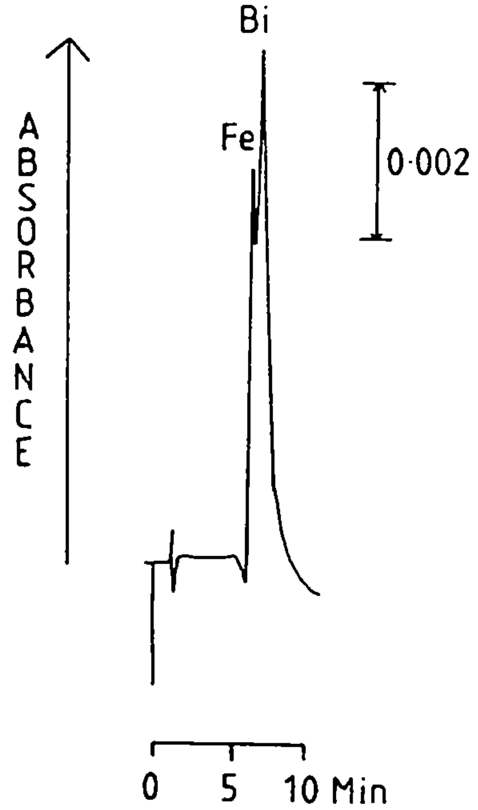


Figure 62. Chromatogram showing the partial separation of 5ppm Fe^{3+} and 50ppm Bi^{3+} in 1M KNO_3 using the new Chrome Azurol S coated column at 60°C with a single step gradient.

4.3. SUMMARY.

The use of step gradient elution enabled high performance separations of a larger number of metal ions in various high ionic strength matrices. By replacing the sample loop with a dye-coated column, metal ions can be preconcentrated prior to the analytical separation using the same column. This will allow detection down to low ppb or sub-ppb levels to be achieved. Using pH step gradient programmes with preconcentration, the selectivity can be controlled. With the Xylenol Orange coated column the trace metal content of saturated sodium chloride brine (5.1M) and potassium chloride brine (2.4M) was determined. Alkaline earth metals were separated using a single step from pH11 to pH6, with transition metals separated using two step gradients from pH6. In order to separate both groups in a single injection, a three step gradient system preconcentrating at pH10 was used. A complete separation of nine metals and seven transition and heavy metals in potassium chloride brine was achieved using these systems. Results were quantitative and reproducible with calibrations for eight metal ions in potassium chloride brine producing correlation coefficients, r, all between 0.990 and 0.999.

Results using chelating dye-coated columns for the preconcentration and separation of trace metals in concentrated brines were very encouraging, so further work involving other high ionic strength matrices was investigated. The Chrome Azurol S dye-coated column would be ideal for sea water analysis as all transition metals would be retained at pH8, but it proved to be less efficient than the Xylenol Orange coated column. However, because of its more weakly chelating functional groups, this column proved to be successful for the isocratic separation of some trivalent metal ions, namely aluminium, gallium and indium. Using the Xylenol Orange coated column with preconcentation at pH6 with a series of pH step gradients, allowed transition and heavy metals in sea waters to be investigated. Modification of post-column reagent using PAR instead of PAR-ZnEDTA significantly reduced the magnesium/calcium peak. Good separations of five metals in synthetic and actual sea water samples were achieved. A sample of Plymouth Sound sea water was found to contain approximately 1ppb zinc and 3ppb copper. To determine other metals present at sub-ppb levels, higher preconcentration volumes would be required.

This Xylenol Orange coated column was also successfully applied to the analysis of North Sea formation water using the same step gradient program as for sea waters. A separation of cadmium, lead and copper in a synthetic sea water solution containing high levels of zinc was also achieved, and this shows the potential for the analysis of mining wastes for polluted coastal/estuarine waters e.g. originating from acid mine waste. Another important application involved the analysis of impurities in various laboratory chemicals, including the potassium nitrate eluent used. Again, using a similar step gradient program, detection down to low ppb levels could easily be achieved. It was found that the potassium nitrate eluent contained relatively high levels (60ppb) of zinc.

CHAPTER FOUR.

CONCLUSIONS AND SUGGESTIONS FOR FUTURE WORK.

This work shows that chelation ion chromatography using dye-coated columns demonstrates clear potential for the determination of trace metals in high ionic strength media. The large particle size dye-coated substrates used in the initial investigations showed that the chelating ability changes markedly with different substrates and chelating dyes. Selective adsorption of metal ions was possible as the controlling factor for retention and separation is pH. This is because the conditional stability constants of each metal chelate alter with changing pH. From these studies, the column which gave the best performance was the Xylenol Orange coated XAD-2 column, which retained all transition metals studied at pH6. This column subsequently proved successful as a clean-up column, to remove metal impurities from eluents and buffers in later work.

Investigations involving dye-coated 8μm HPLC grade PLRP-S substrate have been particularly encouraging as they show high efficiencies. The ionic strength has to be kept quite high, to make sure any ion-exchange sites are 'swamped' with salt ions. Some ion-exchange did occur at 0.2M, particularly with alkaline earth metals which are held strongly by ion-exchange, especially barium. The high efficiency enabled several isocratic separations to be achieved. With the Xylenol Orange coated column, barium, strontium, magnesium and calcium in 1M potassium nitrate could be separated at pH7.7, and by reducing the pH to 2.5, cadmium and lead were separated. Chrome Azurol S was more weakly chelating than Xylenol Orange, as expected, and did not retain alkaline earth metals, but cadmium and lead were separated. This column proved to be more ideal for trivalent metal ions, which form stronger chelates. A complete separation of aluminium, gallium and indium was possible, but much further work is necessary to fully evaluate the potential for the determination of trivalent metal ions.

The efficiency of the prepared dye-coated columns approached that of the commercial TOSOH column studied. This column in initial studies, showed that its chelation ability was as strong as the Xylenol Orange coated column, which contains the same iminodiacetic acid functional chelating groups. Good separations of alkaline earth and transition metals were achieved with the TOSOH column. The Dionex MetPac column although showing similar chelating ability, showed little separating power, mainly due to its short length of 5cm and probable larger particle size.

Dye-coated chelating columns can be used to separate selective groups of metal ions, or a larger range using gradient elution. With the use of gradient elution, the more strongly chelating metal ions were separated from high concentrations (over 1000ppm) of alkaline earth metals, using both the Chrome Azurol S and Xylenol Orange coated column. A complete separation of nine metals (barium, strontium, magnesium, calcium, manganese, cadmium, zinc, nickel and copper) in 1M potassium nitrate was successfully achieved using three step gradients.

The main aim of combining preconcentration and separation of trace metals in various high ionic strength media using a single chelating column, has been achieved. Alkaline earth metals, transition metals, or both groups combined in a single run can be analyzed using step gradients. High ionic strength samples involved were concentrated potassium chloride brine and sodium chloride brine, which is virtually saturated at 5.1M, together with sea waters and laboratory chemicals. Standard addition used for calibration gave good linear results, with detection in the low ppb range. Separations of a range of metal ions including a separation of nine metals (alkaline earth and transition metals) in potassium chloride brine, and seven transition metals in potassium chloride brine were successfully obtained.

Both the dye-coated columns were suitable for sea water analysis. With the Xylenol Orange coated column alkaline earth metals are little adsorbed at pH6 where all transition metals were completely retained. This selectivity enabled five metals namely cadmium, zinc, lead, nickel and copper to be separated from artificial and actual sea water samples. The Chrome Azurol S coated column proved to be not as efficient as the Xylenol Orange coated column, as only a few metal ions could be separated. However, this column is more suitable for sea water analysis as all transition metals are completely retained at pH8, which is approximately the natural pH of sea water.

Chelating dye-coated columns are much easier to prepare than chemically bonded chelating columns. They can be used for a wide range of applications, such as preconcentration and separation of trace metal ions in complex matrices, for example, concentrated brines and sea waters. They can also be used for the purification of reagents, and for the actual analysis of impurities in samples and laboratory reagents. The Xylenol Orange coated column has proved to be very stable, having been used for over one and a half years without any apparent loss in efficiency. Overall this column has been used for well over 600 injections, many involving preconcentration of concentrated brines and sea waters. However, with the Chrome Azurol S coated column, after several months use, a noticeable drop in efficiency was seen, and on inspection, the dye coating had changed colour from orange to deep blue/green. This may be due to the fact that commercial sources of Chrome Azurol S can be very impure, although the purified Chrome Azurol S coated column did deteriorate in a similar way. The dye could be slowly oxidised, or decompose in some way over time.

The PAR-ZnEDTA post-column detection system, although not fully optimized, proved successful for the detection of alkaline earth metals, with sensitivity for other metals increased compared with the Calmagite system. Further optimization is needed to maximise sensitivity and achieve lower detection limits. With the analysis of sea waters, PAR alone can be used, which reacts only slightly with alkaline earth metals. For the detection of trivalent metal ions, Pyrocatechol Violet proved to be ideal, as it is very sensitive, particularly for aluminium. Problems were encountered with Chrome Azurol S, as it tended to coat the tubing, leading to contamination problems.

The full potential of chelation ion chromatography using chelating dye-coated columns has yet to be fully exploited. Many improvements, together with further investigations will need to be carried out. Some examples of these are discussed below. Other high ionic strength samples could be studied, as the separation of trace metals in formation water using the Xylenol Orange coated column has already been shown to be successful. Dye-coated columns have also been used in the analysis of anions, with some success, and this area could be developed further. For dye-coated columns to be more suitable for commercial development, it might be advantageous to develop a method for both cation and anion analysis.

The instrumentation used in this research has been well used, with some parts over ten years old. The pumps contained stainless steel parts, which can easily lead to contamination problems, and higher background levels. The use of inert pumps, with a constant flow rate would reduce these potential problems. The step gradients and preconcentration procedure, which was done manually, could be improved by using a gradient pump. Step gradient programs, once optimized could be programmed, and called up, depending on the analysis. Using an accurate pump, the preconcentration volume could be precisely controlled, leading to better quantitative results. Ultimately, the entire process could easily be automated, for the batch analysis of samples, on-line or off-line.

To improve detection limits, the post-column reagent system needs to be optimized further, as well as the investigation of other post-column reagents. Using the present system, detection limits could easily be improved by preconcentrating a larger volume of sample. Blank levels due to any impurities should not have any greater effect. By packing a large particle size dye-coated resin into a micro-burette, for example, the Xylenol Orange coated XAD-2 used, may work as well for an off-line clean-up process as pumping through a packed HPLC column.

The dyes used for coating were commercially available, and used without any further purification. Commercially available dyes can be very impure, with impurities having a marked effect on the chromatography. Commercial Xylenol Orange is known to be very impure, and in fact contains semi-Xylenol Orange, which only has one iminodiacetic acid functional group instead of two, together with some Cresol Red, which is not chelating.

The Xylenol Orange column prepared contains both Xylenol Orange and semi-Xylenol Orange, which are both chelating. In order to understand better the chelating-exchange process, semi-Xylenol Orange and Xylenol Orange have to be isolated and coated in turn, to determine their chelating ability. To increase the efficiency further and improve separations, chelating dyestuffs could be coated onto smaller particle size substrates, such as 5μm particle size. This should improve chromatographic performance further. The actual coating procedure has not been optimized. Effects such as pH, dye concentration and coating time need to be studied, in order to maximise the loading, and efficiency of the coated column. Other chelating dyestuffs should be investigated. Calmagite used in initial studies, gave a good coating with the 8μm PLRP-S substrate, but was not studied further. It should produce a chelating column which is more strongly chelating than Chrome Azurol S, but more weakly chelating than Xylenol Orange. There are many other chelating dyes which could be coated onto high performance substrates to study different groups of metal ions such as trivalent and tetravalent metals. Dyes that are very selective, which do not react with magnesium and calcium, could prove useful for sea water analysis. Highly selective dyes could be used for the determination of a single, or small group of metal ions.

Chelation ion chromatography should develop further in the future as a relatively simple and inexpensive method of directly determining trace metals in various high ionic strength media.

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PUBLICATIONS.

1. O.J. Challenger, S.J. Hill, P. Jones and N.W. Barnett.
   Application of Chelating Exchange Ion Chromatography for the Determination of Trace Metals in High Ionic Strength Media.
   Anal.Proc., 1992, 29, 91-93.

2. P. Jones, O.J. Challenger, S.J. Hill and N.W. Barnett.
   Advances in Chelating Exchange Ion Chromatography for the Determination of Trace Metals using dye-coated columns.
   Analyst., 1992, 117, 1447-1450.

3. O.J. Challenger, S.J. Hill and P. Jones.
   Separation and Determination of Trace Metals in Concentrated Salt Solutions using Chelation Ion Chromatography.
   J.Chromatogr., accepted for publication.

4. P. Jones, O.J. Challenger and S.J. Hill.
   Recent Advances in High Performance Chelation Ion Chromatography for Trace Metal Determinations.
   To be published in the conference proceedings of IonEx'93 (Royal Society of Chemistry publication).

PRESENTATIONS GIVEN AND MEETINGS ATTENDED.

Presentations.

1. Presentation entitled 'High Performance Chelation Chromatography of Trace Metals - Separations in Media of High Ionic Strength.', presented at 'Research Topics in Chromatography' meeting held at the Two Rivers Hotel, Chepstow on 31st May 1991.

2. Poster presentation entitled 'Determination of Trace Metals in High Ionic Strength Media using Chelation Ion Exchange Chromatography.', presented at 'Research and Development Topics in Analytical Chemistry' meeting held at University of Aberdeen on July 9th and 10th 1991.

3. Poster presentation entitled 'Separation of Trace Metals in High Ionic Strength Media using Chelation Ion Exchange Chromatography.', presented at HPLC'92 - Sixteenth International Symposium on Column Liquid Chromatography, held at Baltimore Convention Center, Baltimore, Maryland, U.S.A., from June 14th to 19th 1992.

4. Poster presentation entitled 'Chelation Ion Chromatography using chelating dye-coated columns for the Separation and Determination of Trace Metals in Concentrated Salt Solutions.', presented at 'Research and Development Topics in Analytical Chemistry' meeting held at University of Birmingham on July 7th and 8th 1992.

5. Poster presentation entitled 'Separation and Determination of Trace Metals in Concentrated Salt Solutions using Chelation Ion Chromatography.', presented at SAC92 International Conference on Analytical Chemistry, held at University of Reading from September 20th to 26th 1992.

Meetings attended.

1. 'Research and Development Topics in Analytical Chemistry' meeting held at ICI Chemicals and Polymers Ltd., The Heath, Runcorn on July 16th and 17th 1990.

2. Royal Society of Chemistry Analytical Division, Western Region Annual General Meeting, held at University of Bristol on 25th January 1991.

3. 'Research Topics in Chromatography' meeting held at the Two Rivers Hotel, Chepstow on 31st May 1991.

4. 'Research and Development Topics in Analytical Chemistry' meeting held at University of Aberdeen on July 9th and 10th 1991.

5. 'Speciation in Natural Waters' meeting held at Polytechnic South West, Plymouth on 17th September 1991.

6. 'Research Topics in Chromatography' meeting held at the Two Rivers Hotel, Chepstow on 14th May 1992.

7. HPLC'92 - Sixteenth International Symposium on Column Liquid Chromatography, held at Baltimore Convention Center, Baltimore, Maryland, U.S.A., from June 14th to 19th 1992.

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

9. SAC92 International Conference on Analytical Chemistry, held at University of Reading from September 20th to 26th 1992.

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