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The Determination of Trace Metals by High Performance Liquid Chromatography with Photometric Detection

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A thesis entitled

The Determination of Trace Metals by High Performance Liquid Chromatography with Photometric Detection

Presented by Philip John Hobbs, M.Sc., C.Chem, M.R.S.C.

In part fulfilment of the requirements

for the degree of

DOCTOR of PHILOSOPHY

of the

COUNCIL for NATIONAL ACADEMIC AWARDS

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<u>Trace Metal Analysis by High Performance Liquid</u> Chromatography.

by Hobbs P.J.

Abstract.

The importance of trace metals and their species is reviewed and it is recognised that improved methods of determination are required. High performance liquid chromatography has grown in importance as a technique, especially since the synthesis of better column packings. The adaptability of reverse phase and ion chromatography with new post-column reagents for photometric detection for the determination of transition metal cations, is discussed.

Separation of divalent transition metal by high performance chromatography was improved by eluents used in column ion chromatography, new eluents were also assessed. Lactate eluents were particularly effective and tartrate, oxalate and thiomalic buffers also showed promise.

Two photometric reagents, dithizone and eriochrome black T were used as post-column reagents, sensitivity and calibration characteristics are determined. A novel method of photometric detection, observes the decrease in absorbance of the metallochromic indicator used.

Using lactate eluent with the Eriochrome Black T post-column detector, linear calibrations from 10 µg ml to 5 ng ml for cadmium, cobalt, copper, indium, iron(III), iron(II), lead, magnesium, manganese, nickel and zinc were achieved with limits of detection

between 0.5 and 5 ng.

The dithizone post-column reactor achieved quantitative analysis from 10 ng ml to 10 µg ml for cadmium, cobalt, copper, indium, lead, nickel and zinc with limits of detection for the lactate eluent between 1 and 8 ng except for lead(45 ng).

Applications of the eriochrome black T detector are shown for the speciation of iron and the determination of transition metals in river water and the analysis of alloys.

The separation of oxyanions is discussed with detection by a pyrocatechol violet ternary complex. The possibility of fluorescent metal detection with 8-hydroxyquinoline-5-sulphonic acid and photometric detection with a water soluble derivative of dithizone is shown. CONTENTS.

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1 Introduction.

1.1 The Role of Trace Metals.

Over the last few decades the importance of trace analysis for metals has been recognised. The impact of a trace quantity can cause significant effects especially regarding health and malignant diseases(1). Physical defects can also occur in manufactured solids because of the presence of trace contaminants. This is critical in high technology industries that require a high degree of purity, especially in the production of semi-conductors and fibre optics. The chemical industry is dependant upon the catalysis of chemical reactions where for of the d-block chemical versatility example the transition metals make their complexes ideal for organic synthesis. A quick and accurate assessment of catalyst lifetime and the degree of poisoning is an aid for lower energy requirements and higher yields. Major areas of trace analysis are raw materials and for concern quality control of chemicals, especially production since the introduction of recent E.E.C. legislation.

Recently more concern has been shown in the investigation of trace quantities especially relating to their impact as different species. As the role of metal species has become evident, medical and clinical studies, and the monitoring of pollutants has become

increasingly important. A trace element may play a special role in health and disease as:

i)an essential nutrient to higher mammals, such as cobalt, copper, chromium, iron, manganese, zinc and molybdenum(2);

ii) a causative agent, such as mercury, cadmium,nickel and lead in toxicology;

iii) therapeutic agents such as gold and platinum.

Trace element pathways and cycles in the environment have become increasing important as more instances of pollution have occurred. Also for a better understanding of the eco-system and the geological processes we need to follow the pathways between the various environments, and the dynamic relationships of the constituent components of each.

Thus trace metals can have significant effect in a wide variety of fields and the development of scientific models in fundamental research is often dependant upon the ability to measure low concentrations of a metal or metallic species.

1.2 Importance of Trace Metal Species.

The speciation, or chemical form, of an element can have a dramatic effect on its toxicity and availability as an essential trace element. For example, chromium an essential element, which maintains normal glucose metabolism(3), is much more toxic in the hexavalent state, as a carcinogen, than in the trivalent state. Chromium essential element activity is limited to the trivalent state, however radioactive tracer work indicates that chromium(VI) is absorbed better than chromium(III), readily passing through the red blood cells to become adsorped to the globulin fraction of the haemoglobin(4). This is one of numerous examples and it is evident that we need to know more than the quantity of metal present. It may be necessary to know:

i)the oxidation state(s);

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ii) the degree and type of complexation;

iii) the presence of organo-metallic forms.

Organo-metallic species can have many times the toxicity of the purely ionic species. The solubility of the organo-metallic species enhances the toxicity and lipid solubility leads to biological reconcentration in the fatty tissue. So the dilution effect of for instance the sea, maybe countered by this reconcentration effect.

For higher animals the form and consequent availability of an element is important. Hence for one element it is possible to have a situation of severe deficiency through a range of adequacy, to

pharmacological effects, to acute toxicity(5). To assess the long term toxicity and observe indicators a need to study the sub-clinical levels arises. Such studies require more sensitive means of detection in terms of concentration and speciation.

The toxicity of cadmium, copper, mercury and lead are well documented by authors and government mercury publications(6-11). form Lead and organo-metallic compounds that have a greater toxicity than the element. The toxicity of organo-lead compounds to the brain and liver, and the effect organo-mercurials on genetic mutation make them the subjects of intensive study(12).

So there is increasing need for methods capable of multi-element and speciation determinations on the same sample. One field of research has been to develop chromatographic techniques using modern high performance liquid chromatography(HPLC). Primarily the concern of this thesis is with transition metal cationic species ion chromatography with and their separation by detection by photometric reagents. It is hoped to show that it is possible to determine cadmium, chromium, copper, indium, iron, lead, manganese, cobalt, magnesium, molybdenum, nickel, tungsten, vanadium and zinc and to deleiging them in environmental as well as These elements are given good samples. industrial coverage in the literature for their environmental,

geological and biological roles(13-15).

1.3 Review of Trace Metal Analysis.

The range of trace analysis and ultra-trace analysis can be defined as concentrations of 1 to 100 $\mu g g^{-1}$ less than 1 $\mu g g^{-1}$, respectively. Because of the and low concentrations there are inherent problems; such as matrix removal. A topological problem, involved with for example, small areas of corrosion may require a special probe so the choice of method is normally determined by the problem. Often the trace element plays a vital role and analysis may involve determining a rare, toxic, or unstable species. Sampling, and pretreatment of a sample may affect(and often does affect) the observation, and has to be taken into account. The major associated contamination, those of; problems(16) are interference(s), preparation of an accurate standard and sample or standard during the analytical loss of adsorption occurs on a container procedure, where surface. Contamination may occur from the air, the using a sample container that is not analyst or suitable.

Early quantitative elemental determinations which were the precursors of instrumental techniques were micro-analytical methods(17), chiefly concerned with gravimetric and titrimetric procedures. Colourimetry and

nephelometry were the first to be measured with electrical assistance(18). The general lack of selectivity and sensitivity of these methods led to the development of other techniques such as those based on atomic spectroscopy and voltammetry.

1.4.Present_Methods.

There are many solution and solid techniques available; a few are compared in Table 1. The number of elements determinable with limits of detection of 10 p.p.b. or less are quoted in Table.1, absolute weights are used to give a better comparison. Flame spectrophotometry approaches the picogram level of detection for about ten elements. ۰.

Comparison of range of Detection Limits for Different Analytical Methods.

Method.

Range of Detection

Limit / µg

Molecular Absorption	1-0.1
Molecular Fluorescence	0.01-0.001
Flame Atomic Absorption	0.1-0.0001
Electrothermal Atomic Absorption	0.001-0.000001
Flame Emission	1-0.1
Plasma Emission	0.01-0.001
ICP-MS	0.01-0.000001
Neutron Activation	0.0001-0.00001
Pulse Polarography	0.01-0.001
Anodic Stripping Voltammetry:	
Mercury drop	0.01-0.0001
Thin Film	0.0001-0.00001

There are over 20 methods of electrochemical analysis some of which have p.p.b. detection limits.

Emission techniques are commonly used for solid samples, spark source mass spectrometry has a limit of detection of 100 picograms for over 70 elements. Activation analysis approaches the same sensitivity, but for fewer elements. X-ray analysis, often of solid surfaces and solid samples has limits of detection around 0.1 μ g.g⁻¹.

1.5.Multi-element and Speciation Analysis.

Many analytical investigations now require the determination of several elements in an analyte, excessive time is involved if the elements have to be quantified individually. Because of this multi-element methods have become popular, but either the number of elements determined is limited or the instrumentation is relatively expensive due to the high degree of sophistication necessary.

Atomic emission and inductively coupled plasma optical emission spectroscopy(ICP-OES) instruments with simultaneous multi-element capabilities are now available; ICP-OES can determine some seventy elements, bettering the sensitivity of other emission techniques for some elements by orders of magnitude.

Anodic stripping voltammetry(ASV) or chronopotentiometry, effects the sequential polarographic stripping of metals by reversing the polarity after deposition. A liquid mercury electrode, which has the voltage reversed for analysis, prevents the trapping of minor constituents. It is one of the most sensitive methods because of the concentration step

and can determine elements in the pg ml⁻¹ concentration range, but is usually limited to 3 to 6 elements in a particular determination.

Although ICP-OES and ASV are very sensitive they have limited speciation capability, especially ICP-OES. The area of speciation requires particular attention and this role as well as having multi-element capability may be filled with HPLC methods, especially with the recent rapid development of column materials.

increasing demand for sensitive multi-element An determinations is evident in high technology. E.E.C. implemented stringent limits for legislation has concentrations of certain toxic elements, active pharmaceutical ingredients and food additives. Research alternatives with also requires less investment, offering chemical speciation and multi-element analysis. Liquid chromatography(LC), can play a role in filling this analytical niche. Early LC techniques were slow, but forced-flow and HPLC have illustrated that more metals can be determined with quicker elution times. The HPLC of metals was not previously seriously considered, because of the dominance, in terms of selectivity and sensitivity of electrochemical and atomic spectroscopic techniques.

Metal determinations and speciation studies with gas chromatography(GC) coupled to atomic absorption detectors(19) have the disadvantage that only the

volatile metal derivatives are separable. LC can be used to separate volatile and non-volatile metallic species. It is hoped to show that this is possible with modern ion exchange and neutral liquid column chromatography materials, for the separation of cations and transition metal anions.

<u>1.6.Liquid</u> Chromatographic Determination of Trace Metals.

Chromatography(20,21) physically separates chemically different substances as a dynamic process. The varying attractions of a substance for the chromatographic substrate in an eluent flow are responsible for the separation. Choice of column packing material and variation of the eluent composition are the main variables for resolving mixtures.

The notable pioneer of LC techniques was Mikhail Semenovich Tsvet who in 1903 separated plant pigments using a silica substrate(22). However, research reveals that as early as 1850, Way experimented with the ion exchange ability of a column of soil(21). Thin layer chromatography has considerable usage, but is not considered further in this thesis.

Since the late 1960's better determinations and separations have been developed. To understand LC and HPLC processes and recent improvements, a brief

discussion of chromatographic parameters is necessary.

1.7.Chromatographic Parameters(20,21).

Retention of an elute, neglecting peak broadening and non-linearity, is measured as the retention factor or capacity factor(k);

$$k = \frac{(t_r - t_o)}{t_o} = \frac{t_r}{t_o}$$

volume of where the retention time or the elute(t_r) is given a dimensionless value using the column void parameter of the same dimensions(t_0). This is an allowance for the chromatographic delay within the column. A non-interactive substance that can enter the interstitial and intraparticulate spaces occupied by the mobile phase is used to determine the void quantity. This has been in debate recently, as the supposedly non-interacting deuterated solvents were giving false values(23). The adjusted retention quantity has important practical and theoretical significance, equations normally express these fundamental relationships as volumes.

$$V_r = V_0(1+k)$$

Where V_{r} and V_{o} are the retention and void volumes respectively. There is a direct relationship to thermodynamic values between the stationary(V_{s}) and mobile phase volumes in the column for the eluted species,

$$V_r = V_o + KV_s$$

providing that a linear relationship is observed between the concentration of the component on the substrate(C_s) and the mobile phase(C_m)(21). Linear elution sorption chromatography is a mathematical model and the column is considered a Goussian operator on the introduced sample giving a symmetrical peak. Langmuir and anti-Langmuir sorption isotherms of C_s versus C_m demonstrate tailing and fronting of the chromatographic peak, respectively. Column overloading frequent in ion chromatography is usually because of low capacities and is indicated by peak fronting.

1.8.Resolution.

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The effectiveness of the separation may be measured as the resolution R_{s} . Consider two peaks of adjusted retention time t_r, and t_r. Then:

$$R_{s} = \frac{t_{r} - t_{r'}}{0.5(w + w')}$$

Where 0.5(w+w') is the mean band width at the base of the peaks. If a Goussian chromatographic peak is observed the mean peak width is expressed as 4 standard deviations. The resolution of a column is a function of selectivity, retention and efficiency of the column. Selectivity(a) is a function of the ratio of the thermodynamic equilibrium constants associated with the retention and should be greater than 1, this may be expressed as a relative retention function to an ascribed elute, which should also be greater than 1 for separation.

Column efficiency is measured as the relative narrowness of peaks, by the ratio of the retention time to peak width. Efficiency units are expressed in terms of theoretical plates(N). The required number of plates in terms of column length for a separation can be calculated.

The square root of the required plates for the separation of two peaks is expressed as:

$$N^{1/2} = (4(a)(k+1)R_{s})$$

The efficiency of the column has an inverse geometric relationship with particle size. Band broadening is related to uniformity and size of the particle. Major contributors being eddy diffusion, molecular diffusion and mass transfer(24). Although other models exist the defined above for adsorption relationships chromatography may also be applied to classical as well exchange performance liquid ion high as diameter spherical chromatography(HPLIEC). Small better rapid transfer and give mass particles resolution. Classical column chromatography used large diameter particles and it was not until the technology involving high pressure pumps and small diameter column materials was available that an improvement in column efficiency transpired, which considerably increased the separating power and speed of analysis.

1.9. Development of HPLC Column Materials.

Early HPLC materials were of silica and used chiefly for organic separations. The particles were usually greater than 20 μ m in diameter and the subsequent slow

diffusion in the porous stationary phase was to the detriment of resolution. Resolution was improved for microporous materials by using an inert non-porous core, known as a pellicular phase or alternatively by using a smaller particle. The surface of pellicular particles can have various groups attached to form reverse phase or ion exchange stationary phases. A pellicular ion exchanger was developed in the late 1960's for the separation of nucleotides(25), but large particles gave that contributed diffusion term large eddy band broadening. Better HPLIEC is considerably to smaller sized bonded ion stationary with obtained phases.

The transition to HPLC from LC was easier for silica stationary phase substrates since ion exchange resins have problems of swelling and slow mass transport rates. developed for liquid channelled particle was Α chromatography, known as macroreticular ion exchanger, which increased the mass transfer as a result of smaller length micropores. Some macroporous resins are available for HPLIEC, but as yet have proven unsuccessful. HPLIEC partially sulphonated materials of typical uses capacities between 0.005 and 0.1 meq.g⁻¹. Resins can withstand concentrated acid, alkali or a decimolar EDTA solution making them easy to regenerate after poisoning and giving a virtual unlimited choice of eluent. Silica exchangers operate between pH 2 to 7 and are almost

impossible to regenerate. Swelling of the resin in an organic solvent. was a problem, but is minimised with a higher degree of cross-linking.

A good cation exchanger should have a low capacity, 0.005 to 0.05 meq.g⁻¹ ;pH stability over a wide range with a wide range of selectivities.

Anion exchange resins are manufactured as a rigid core of styrene divinyl benzene polymer converted to a cation exchange resin by sulphonation and then an aminated latex is agglomerated to the sulphonated bead, to form a quaternary anionic exchanger. However developments in the manufacture of polymer based ion exchange materials remain behind silica-based materials in terms of column efficiency.

The HPLC of trace metals is dependant upon sensitive multi-element detection, however metal separation had to be improved before the technique could be considered a viable alternative to spectroscopic and electrochemical techniques.

1.10 Review of HPLC of Trace Metals.

1.11 Partition and Silica Separations.

Numerous liquid chromatographic techniques on different substrates were developed since Tsvet, but primarily, adsorption chromatography using silica and alumina substrates was adapted for HPLC, because of their previous successes with column chromatography. The HPLC of trace metals at that time was not considered a serious contender to atomic spectroscopy and electrochemical methods.

early HPLC techniques showed flow and Forced considerable possibilities as in reviews by Schwedt(26) and Fritz(27) where the primary concern was with the separation of metals as their complexes. Veening also HPLC of organo-metallic species and metal reviewed but mostly for synthetic chemistry, complex complexes(28).

One of the earliest HPLC separations involved metals as their acetylacetonates. This was achieved with an isocratic liquid / liquid separation, using diatomaceous earth particles(5 to 10 µm diameter) as the substrate for the aqueous phase and an ethanol 2,2,4-trimethylpentane mixture as the mobile phase(29). Tollinche(30) reported on the separation of di- and trivalent metals as their beta-diketonates on a silica substrate.

Early separations mostly performed on silica required the pretreatment of samples, which may include a preconcentration step using organic solvent(s). Derivatised complexes were then injected onto the column top, usually into an organic eluent. Those chelating agents that gave the greater number of complexes and of

absorptivity were found to be particularly highest dithizonates and successful, especially diethyldithiocarbamates(31). Cobalt, copper, mercury, and nickel dithizonates were separated on a 30 µm silica gel column(32). Lohmuller also reported retention data for more metals with numerous other solvents. Glass lined stainless steel columns have been used with polar modifiers such as acetic acid to avoid using aromatic Heizmann(34) the metal eluents(33). investigated agents 1,2-diketobisthiosemicarbazone, complexing and dialkyldithiocarbamate

metal complexes 1.2-diketobisthiobenzhydrazone. Four were separated in forty minutes and this was improved to 10 minutes by gradient elution. Nanogram levels were detectable. Different chelating agent substituents were found to alter the retention time. Schwedt(35) reported transition metals separated by adsorption chromatography and cyclopentadienyl acetylacetonates, as triphenylphosphine complexes, quoting references to the separation and detection techniques. Schwedt drew an TLC and the HPLC of parallel between obvious state that practical dithiocarbamates and went on to analytical problems have yet to be solved by HPLC thoroughly separations had been although some 0'Liska(37) demonstrated the best investigated(36). separation of nine diethyl dithiocarbamates on a 10 µm silica in 20 minutes. The elution sequence of the

substituted dithiodiethylcarbamates was stated for various chloroform cyclohexane mixtures. Chromatographic separations of mixtures of bis-dialkyldithiocarbamates illustrated that ligand exchange reactions occurred with ease during the separation process(38).

1.12 Reverse Phase Separations.

A non-polar stationary phase of hydrocarbonaceous ligates attached to a silica base using a siloxane bond is known as a reverse phase substrate. This type of packing has C_2 to C_{18} alkyl chain length capped sites on the silica substrate and can exhibit higher efficiencies than silica substrates.

Separation depends upon the varying degree of polarization of the molecules to be separated, a more polar eluent encourages the hydrophobic complexes to be retained. Medium to high polarity compounds may also be separated if part of the molecule is hydrophobic.

The diethyldithiocarbamates of chromium(III), cobalt, copper, lead, mercury, nickel, selenium, and zinc were investigated. Lead and copper co-eluted, a variable wavelength was necessary for detection(39). Cadmium, cobalt, copper, lead, mercury, nickel and zinc were separated as their tetramethylenedithiocarbamates(40). Pre-column complexation and phase separation of an aqueous analyte was avoided, with a direct injection

onto a Hypersil ODS column and a 0.5% m/v sodium diethyldithiocarbamate eluent(41). Such time saving allows dynamically unstable samples to be determined.

Some difficulty may be encountered with bonded phases because of chemisorption on the uncapped sites as for example, in the determination of beta-diketonates(30).

1.13 Reverse Phase Ion Pair Separations.

A charged species can form an ion pair with a compound that has a hydrophobic fraction which is attached to the reverse phase. The attraction of the reverse phase by this eluent molecule is the basis for differential retention. Normally a quaternary ammonium salt is used for anions and a long chain sulphonate or a sulphate for cations.

A related technique uses dynamically coated columns for the separation of metal ions and anions by paired ion pairing agent, chromatography(42,43). The ion usually a surfactant with a hydrophobic carbon chain, present in the eluent can be passed through the column solvent keep а constant to organic using an concentration on the solid phase, higher chain members maybe deposited on the column prior to separation. Columns coated with a carbon chain containing 12 atoms gave the same efficiency as a carbon chain containing 20 atoms, however the former was not retained on the

Peak broadening can occur because of the column. accumulation of the exchanger at the column top. The reproducibility for anionic separations was investigated by Cassidy(44), he concluded that reverse phase dynamic coating is more versatile than bonded ion exchangers and more reproducible. It was thought that higher chain to C₁₈) exhibited members(C₈ surface ion exchange properties and those below about C8 form ion pairs. A more complex explanation states that a dynamic equilibrium exists between the species present and the substrate and is described as ion hydrophobic interaction chromatography(45).

Tartrate has been used in conjunction with a C_6 sulphonate to separate cadmium, cobalt, copper, manganese, nickel, lead and zinc. An improved efficiency of 50 to 90 % is obtained with C_8 sulphonate over the long chain sulphates. Whether it is due to the chain length or the functional group was unclear(46).

1.14 Speciation Analysis by Reverse Phase.

Any molecule or fraction of a molecule that is hydrophobic lends itself to reverse phase separation. This could be useful for organo-metallic speciation studies. Brinkmann compared UV detection with flameless AAS after separation by HPLC of alkyl and aromatic organo-mercurials(47). The determination of arsenic, lead, mercury, tin and their organo-metallic species by HPLC and a coupled technique are described. Numerous synthetic organo-metallic compounds have been separated. But few environmentally important species have been determined. A technique for the determination of alkyl and aromatic mercury compounds has been assessed(47,48).

<u>1.15 Review of High Performance Ion Exchange</u> Chromatography.

Before the discussion of HPLIEC, LC ion exchange has to be mentioned because it has considerable bearing on the column materials and eluents chosen, also all metals mentioned further in this thesis are _: divalent unless otherwise stated.

In the 1930's column chromatography was beginning to be recognised as an analytical technique. Ion exchange chromatography was brought to the attention of the scientific world in 1935 after the synthesis of a sulphonated phenol-formaldehyde resin. This rapidly developed because of great interest in lanthanoid and actinoid fission products during the 1940's which were separated as their citrates(21).

Mineral acids, especially hydrochloric acid enabled the separation of transition metals, but as their anionic complexes. Gradient elution was necessary as iron(III) is held on almost a 100 times stronger than

zinc(20). Hydrochloric acid eluent was employed with a sulphonated column to separate transition metals(49). An acetone/hydrochloric acid mixture was also effective(50).

Organic acids eluents gave good separations on anionic and cation exchange materials, thus avoiding the use of corrosive mineral acids. Numerous papers revealed that the combination of cationic columns and organic acid resolved many transition metals, e.g. eluents citrate(51) and malonate(52) eluents for alkali earths separations. Acetate and ammonium chloride eluent was assessed for cerium(III), lanthanum(III), indium(III), found to give quantitative and mercury and was separations(53). Succinate(54) and malate(55) eluents for transition metal separations have been investigated. merits of alpha-hydroxy-isobutyrate(HIBA) as an The eluent were recognised in the cationic displacement chromatography for the separation of barium, calcium, cerium(III) and strontium(56,57).

Although low pH obtained by mineral acids are normally separate the highly charged cations, used to vanadium(V), iron(III), nobium(V), and titanium(IV), uranyl(II), the possibility of separation was investigated for formic, oxalic, tartaric and citric distribution coefficients were also acid eluents, determined(58).

The advantage in terms of column efficiency of forced
flow elution with smaller diameter ion exchangers was realised. A low capacity macroreticular resin was investigated for cationic separations under forced flow conditions(59). A similar work used hydrochloric acid and an acetone mixture with more success(60).

Anionic exchange column chromatography was investigated to separate uranium and thorium with a citrate eluent(61). Tartrate showed the most promise for transition metals, achieving the separation of cobalt, iron, manganese, molybdenum and nickel(62). Pitstick also separated cobalt, copper, iron(III), manganese and zinc with a tartrate eluent(63).

It was not until the development of pellicular ion exchangers which have surface only exchange sites that a higher efficiency was evident(64). Pellicular materials can also withstand a higher column pressure gradient. Early HPLIEC was initially performed on sulphonated silica substrates. Resins were used at a later stage because of the temporary advantages of forced flow conditions. Polar eluents are necessary for HPLIEC separations because the method relies upon differential especially attractions. Mineral acids and ionic hydrochloric acid have been used at varying strengths, these could now be used with resin exchangers. Although mineral used acids, chromatography early column difficulty was experienced in transferring to HPLIEC the corrosion of the metal systems, because of

components within the HPLIEC system. Some hydrochloric acid separations were used. However, at the outset of LC less corrodible eluting agents were investigated. Organic acids gave good separations and as a consequence a preference for column packing materials of lower capacities was shown.

Takata(65) illustrated and thoroughly investigated the use of a superior strong cation exchange(SCX) resin Hitachi No.2611 with a tartrate eluent. The effect of the degree of cross-linking, sodium chloride concentration, pH of the tartrate, flow velocity through the column and the effect of temperature were shown. The separation of six metals using a 40 by 6 mm column with a 5-8 μ m resin in two minutes illustrates HPLIEC capabilities.

Sevenich(66) illustrates the advantages of adding ethylene diamine to the tartrate eluent to sharpen the peaks and increase the number of metals that can be separated. The improved chromatography obtained by using the ethylene diammonium ion was reviewed by Fritz(67).

Succinic acid eluent was studied for the cation exchange separation of cadmium, cobalt, iron(III), and zinc(68).

Hwang <u>et al.(69)</u> studied eluents on a lightly sulphonated SCX Vidac column showing a comparison of retention times of citrate, tartrate, lactate and HIBA for bismuth, cadmium, cobalt, copper, iron(II) & (III),

lead, manganese, nickel, and zinc. Another thorough study by Takata(70) using lactate eluent separated sixteen rare earths using coulometric detection. Some of the rare earths could be separated without a complexing agent present in the eluent. A comparison of column SCX materials is made with HIBA eluent for Partisil, Nucleosil and Aminex A5, for the lanthanoids.

The effects of sodium chloride, ethanol and a mixture of two SCX materials has also been investigated(71). Ethanol broadened the peaks and gave a longer retention time. Sodium chloride decreased the retention except for cadmium.

Few organo-metallic separations have been performed on a cation exchange column, however, methyl mercury and the trialkyl tins have been separated on a Partisil SCX column with a 60% v/v methanol and 0.042 M ammonium acetate eluent at pH 5.3(72).

Anion exchange(73,44) has found little application in HPLIEC metal separations and has not had the success of cation exchange chromatography. Organic and inorganic acids have been used as anionic complexing agents. A low capacity anion exchanger was used for a notable separation of chromate, molybdate, tungstate and arsenate anions, with a sodium carbonate, potassium hydroxide eluent(74).

Some advantages are offered by microbore chromatography using 1 to 2 mm I.D. columns.

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Considerably less solvent and packing material are required. However smaller particle size materials can give up to a five times increase in sensitivity, but low volume precision pumps and detection cells are required.

Ishii and co-workers(75) performed a micro-scale HPLIEC separation of 16 rare earths using HIBA eluent and a 6 µm diameter resin-based sulphonated exchanger in a 0.5 by 75 mm column with an HIBA eluent. A micro-feeder, which consisted of a small synchronous motor, gears and screws was used as a delivery pump with <u>+</u> 0.01 microlitre accuracy. A Hitachi No.2610 SCX resin w.a.s used by the same author to separate the radionuclides of sodium, potassium, rubidium and caesium(76).

1.16 High Performance Liquid Chromatography Detectors.

There are numerous techniques that can be adapted to detect an analyte in a continuous flow by physical and chemical means. Methods adapted for HPLC detection by post-column(PC) reaction are analogous to flow injection analysis and automatic analysis. All may offer possibilities of investigating kinetic parameters. Most post-column reactor(PCR) detector requirements are virtually the same. Primarily, detectors should have streamline flow, low volume, wide linear response range

and an insensitivity to flow rate and temperature fluctuations. Detector baseline instability does occur and compensating measures are necessary for lower limits of detection. This may be termed noise and is a major problem for low levels of detection and is chiefly associated with the electronic signal, pump pulsation and to a lesser extent data assimilation.

There are many types of detector, outlined in Table 1.2 measuring spectroscopic, electrochemical and physical properties of the column effluent. Some such as the mass, GC and mass spectrometric detectors require a phase change of the eluent.

Table 1.2.

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Property Measured

Spectrophotometric	Electrochemical	Physical
U.V. / visible Fluorescent Infrared A.A. methods Nuclear Magnetic Resonance Electron Spin Resonance	Permittivity Conductivity Potentiometric Voltammetric	Refractive Index Radioactivity Optical Activity Low angle Laser Light Scattering Mass spectrometric GC Mass

There are also other detectors for HPLC that are not used very much, these are discussed in comprehensive reviews by White(77) and others(26,27,78). They include radiochemical(75) and photoacoustic(78) detectors.

1.17 Electrochemical Detectors.

Electrochemical detectors can measure a change of one of the following; capacitance, resistance, voltage or current of the column effluent. Coulometric is the most popular and has μg ml⁻¹ to ng ml⁻¹ range of sensitivities for most analytes.

Ions can be detected conductometrically by observing a resistance of a flow cell. in the decrease Conductometric cells have been used since Small, Stevens and Baumann(79) developed a technique that excluded the HPLIEC ionic buffer in a suppressor column to allow an increase in sensitivity. Ethylene diammonium tartrate been used as it shows a lower eluent has also conductance than the detected polyvalent metal cations to give a better sensitivity(66).

Conductivity detectors detect in the µg ml⁻¹ range, but difficulty may be experienced in obtaining a steady baseline. An alternating current may be used, however, capacitance and heat effects are still noticeable. Molner(42) also reduced the limit of detection by an improved design.

Takata(65) introduced a coulometric detector for ions phenols and sugars. Takata concluded it was virtually flow rate independant and ng ml⁻¹ levels are easily detected. Coulometric cells were employed to detect rare earths(70) showing the applications of a controlled

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potential and virtual elimination of heavy metal interferants by using constant sodium chloride concentration. Cadmium, cobalt, copper, lead, mercury, nickel, were separated at concentrations in the 10⁻⁷ M range(65).

A dropping mercury electrode detector has recently been developed and was discussed in a review by White on Electrochemical and Spectroscopic detection in HPLC(77).

1.18 Spectrophotometric Detectors.

Spectroscopic measurements offer the largest range of detectors and include photometric, fluorescent and atomic spectroscopic techniques. Ultra violet, infrared, nuclear magnetic and electron spin resonance are well reviewed in other literature sources(77,78).

Flame atomic absorption spectroscopy(80,81) can be directly coupled for single element determinations as the aspiration rate is approximately compatible with HPLC effluent flow rate. Some metalloids require hydride production for low sensitivities(19). Analysis of toxic elements in the environment by atomic absorption methods were reviewed by Van Loon(82) and Fuwa(83). Nebulisation is also necessary for ICP-OES where the plasma produces an excited state of the analyte. An advanced optical system allows multi-element determinations. This was demonstrated in the investigation of the association and quantification of carbon, copper, iron, manganese,

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phosphorous and zinc to proteins using exclusion chromatography(78).

1.19 Photometric Post Column Reactors.

separations have brought Improved chromatographic exacting demands on detection and consequently a renewed interest in pre-column and PCR techniques, because of the improved analytical versatility and sensitivity. Pre-column derivatisation techniques, discussed earlier can give an enhanced response, but have disadvantages, possible 'ghost peaks' or artifact peak such as formation and often time consuming sample preparation. These limitations arise because the technique has to separate derivatised products which is often difficult, and this may be the reason that it has not achieved larger applicability.

An improvement of chromatographic fittings and design has produced a notable renewed interest in PCR detectors in recent years(84). Smaller quantities are required for analysis e.g. spinal fluid in which ultra-trace amounts have to be determined. Numerous tried and tested techniques for pre- and PC reactions prior to detection of organic analytes are discussed for the trace analysis of carbonyl, amines and amino acids in a review of HPLC trace analysis by Kirkland(85). Few studies of PCRs for trace metal ions have been undertaken because the

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separation of trace amounts was not possible until recently.

For fast PC reactions a highly efficient system can be because of minimal peak broadening, most designed inorganic reactions fit this description. However the PCR detector has some drawbacks. An eluent or a gradient elution may affect the kinetics of the reagent with the determinand, or render it irreproducible. A time delay and/or heating bath may be necessary to effect greater sensitivity, this may complicate detection. However there are many possibilities with PC reactants to obtain better selectivities. and higher sensitivities Interfering components may co-elute and a reagent can preferentially react with the determinand. A variable selectivity could also give the detector advantages. In practice very few problems seem to be encountered. Metal PC reaction detection systems have considerable scope for development. Such an approach appears to offer greater possibilities considering the large amount of information available on the photometric determination metals. Each metallochromic reagent will of trace require a different PCR design according to the kinetic and thermodynamic parameters.

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1.20 Post Column Reactor Design.

Metal cations separated on ion exchange material in an aqueous media will elute from the column as complexes with little or no absorbance in the visible region, which is the chief region of absorbance for metal complexes. Metal ions can be converted into highly absorbing complexes by introducing a photometric reagent to react with the effluent and be monitored at the specified wavelength. A PCR should extend the range and versatility of detection for a particular species which does not absorb or fluoresce at sufficient levels in the UV visible regions. Chromatographic resolution should be retained as far as possible for maximum sensitivity. The kinetics of a reaction will strongly influence the reactor design. Precise control of the flow rates for reagents becomes important as a the addition of concentration change of reagent or the determinands may result in a non-reproducible response. Heating the PCR to decrease the reaction time and reduce the length of PCR tubing may retain some chromatographic resolution the highest temperature should be used providing the and reaction products are not unstable or side reactions are not limiting. This would suggest a mobile phase with a high boiling point, it should also not quench the signal or slow the reaction.

There are three PCR designs adopted to minimise band

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broadening. These are divided according to the reaction time necessary. Narrow diameter tubing for a delay time 10 to 30 seconds, where classical flow dynamics are of observed. Here two phenomena are primarily responsible for a resulting Guassian concentration profile of a square shaped 'plug of' injection. The axial flow is not uniform and a cross section of a tubular reactor is considered laminar with the maximum flow rate occurring at the centre. This process would give considerable band broadening problems if it were not for the counter diffusion process that is radial in direction. Narrower tubing has a greater radial component. Band broadening becomes a considerable problem with a long straight tubing, this can be reduced by coiling the tubing, centrifugal force gives two а resulting because hemispherical secondary flow components which increase radial mixing. This was investigated with the intention of designing a PCR that has a spiralling tube containing the effluent within a larger tube(86). A column packed with small diameter particles can give a PC delay time 3 minutes(87). Classical to of 30 seconds chromatographic processes are obeyed, however difficulty can be experienced in finding a non-interacting column which does not reduce the detector signal. A long length of narrow tubing with air segmentation is necessary to minimise diffusion for reaction times greater than 4 minutes and can provide a reaction time of up to 20

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minutes(87). Air segmentation also allows the possibility of column effluent storage. The chief sources of diffusion here is related to the wetting of the tube surface. It was suggested that a non-wetting tubing arrangement should be implemented(84).

reaction-detector resident times require an Small effective mixing procedure. It is necessary for small quantities of reactant to be added to the column effluent to give the maximum response because then the elute can virtually retain its initial concentration, ensuring a better response. With a PC reagent solvent that has difficulty in mixing with the eluent a zero dead volume mixing unit is necessary. The angle of mixing was found to be important(84), although a 180 degree angle for the incoming flows with the outgoing effluent at 90 degrees was effective for easily miscible eluents, mixing at an acute angle was necessary for less miscible solvents. This was particularly so for the detection of organic analytes. For PC detection, these longer than metal ion reactions generally take colourimetric reactions.

1.21 Photometric Post Column Reaction Detectors for Metals.

The enhanced detection obtained by the complexation of metals with organic reagents has been studied

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thoroughly, because they have been used for many years as spectrophotometric reagents, colourimetric spot test reagents and as metallochromic indicators. However it is only recently that they have been utilised for PC reagents and it is necessary to summarise some of the spectrophotometric approaches.

Spectrophotometry is still a popular technique for some trace metal determinations, although a few of the methods have been superseded. However, there are many publications on organic reagents for metals(over 600 in 1975 according to Analytical Abstracts). Comprehensive coverage in books, by Sandell and Onishi(88), Snell(89) (who describes many practical applications) and Hopkins(90) are available for photometric reagents. The topic was also reviewed by Savvin(91) and Marczenko(92).

It has always been the aim to synthesize organic reagents that are element specific, these would have analytical and economic advantages. Due to the general nature of the coordinate bond this aim has been difficult and eliminative techniques were necessary to stop interference from competing elements. However chromatography would give the necessary selectivity because the elements can be separated.

Photometric quantification, whether it is by a photocell or a photocell in conjunction with a PCR depends upon measuring the relative intensity of electromagnetic radiation absorbed, usually at a

wavelength of maximum absorbance of the determinand. This is normally in the visible range for metal complexes. Infrared is relatively insensitive for trace determination purposes and is associated with the rotational and vibrational fine structure transitions.

Beer and Lambert showed that for many solutes in practically transparent solvents:

Abs =
$$\log_{10} \frac{Io}{I} = Elc$$

Where the log of the absorbance(Abs) was proportional to the concentration(c) expressed as molarity for a given path length(l) in cm(93). The extinction coefficient(here as \mathcal{E}) is often expressed in terms of molar absorptivity (l.mole⁻¹.cm⁻¹).

Deviations from the law can occur because of turbidity, light scattering and fluorescence within the solution being measured. Nevertheless only severe deviations would prohibit quantitative determinations.

The relative accuracy of quantifications for trace amounts is poorer than for higher concentrations. Trace determinations can have a 10% error and ultra-trace analysis, after pre-concentration can be around 30%(92).

Similarly precision suffers for trace quantities. Absorbance is normally less than 0.2. Photometric instruments give an electrical response that is directly proportional to the radiation falling on the photocell. The smallest detectable amount will be independent of

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the absolute value of that power of the signal. So small changes of absorbance at the lower absorbance range represents a large fraction of the total concentration. At a higher absorbance range a small change in absorbance represents a larger imprecision in concentration, a compromise is mathematically in the region of 0.43 absorbance(94).

A colour contrast between the complex and the organic reagent is necessary, if an overlap of reagent and complex colour is observed the range of response will be decreased, sensitivity is not affected. The photometric sensitivity is measured in terms of molar absorptivity ξ . Sensitive reagents have a resulting complex with ξ greater than 10,000 1.mole⁻¹.cm⁻¹. Using a 2 cm cell giving an absorbance of 0.05 with a M_r of 100 and ξ 50000 1.mole⁻¹.cm⁻¹ then the detected amount is 0.05ug ml⁻¹. According to quantum theory, ξ cannot exceed 150,000 1.mole⁻¹.cm⁻¹ for direct methods although in practice they rarely exceed 80,000 1.mole⁻¹.cm⁻¹. Some ion association methods, which are discussed later may exceed this value.

The selectivity of a trace photometric method is primarily controlled by the structure of an organic reagent, the pH, presence of masking agents(92) and, if necessary, extraction procedure(95). Consequently the prerequisites for photometric trace analysis are: high sensitivity of the reagent;

specificity of the organic reagent or selectivity of the reproducibility; stability of the resulting method; calibratable response; simplicity of complex; a reliable instrumentation. Except for operation and element selectivity these are also the demands imposed a PC reagent. Consequently selectivity is less on important for a PCR and this completely revises the the research of PC reagents since, approach to selectivity is not a major difficulty. Thus reagents that have been rejected in the past because of their poor selectivity, will have to be reconsidered.

There are various types of colourimetric systems for spectrophotometric determinations. A sensitive colourimetric response normally occurs with the addition of an organic reagent that has a chelating action, which will form a stable 5 or 6 membered ring complex. The molecule also has a chromophoric fraction such as an azo-dye, triphenyl methane or a p-quinoid group. The group should be electronically affected in the reaction with a metal to give a colour change. Photometric reagents normally form a binary complex which is quantitatively measured.

A constituent of the binary complex may have an ionic group that forms an ion-associate with the respective cationic or anionic surfactant to give a bathochromic response. The resulting ion associate is known as a ternary complex.

Indirect methods are often a means of analysing a difficult species(96). Some of these are amplification methods and can involve redox or an exchange reaction(97). However these have not yet been found adaptable as PC reagents.

HPLIEC effluents generally earlier discussed As contain organic acids that weakly complex the metal analyte. The weakly complexed ions present in the column buffer effluent, readily form coloured complexes with the organic reagents, and few problems are encountered such reagents as times with with response 4-(2-pyridylazo) resorcinol(PAR), which belongs to the does reagents as pyridyl-azo group of l(2-pyridylazo)-2-naphthol(PAN), these are shown in Figure 1.1 and have been used as PC reactants. Both are unselective, although highly sensitive. 4-(2-pyridylazo) resorcinol can be used as metallochromic indicator for cadmium, cobalt, copper, nickel and zinc with a back titration, normally using copper and can be used as a PC reagent, forming water soluble complexes with 20 metals to give a red or red-violet coloured complex(98). Limits of detection for PAR as a PC reagent with cadmium, cobalt, copper, iron(III), lead, magnesium, manganese, nickel, and zinc were found to be less than 10 ng, except for lead and cadmium, which were 15 and 20 ng respectively(69). A displacement reaction with a zinc EDTA complex at pH ll was used to detect

Figure 1.1

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<u>4-(2-Pyridylazo)-resorcinol</u>



<u>1-(2-Pyridylazo)-2-naphthol</u>



Figure 1.2

<u>Arsenazo(III)</u>



<u>Arsenazo(I)</u>

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the alkali earth metals barium, calcium, magnesium, and strontium which do not react with PAR alone and an improved response was also obtained for lead and manganese(99). However the authors noted a decrease of response for detection of some transition metals because of hydroxy species competition at high pH and suggest that a high PAR concentration would counter the low response. The applicability of PAR was demonstrated with the analysis of trace metals of nuclear materials, the uranium response with PAR was masked with the presence of 0.3M carbonate(100).

Superior detection abilities of PAR were compared with arsenazo I and III(59) which have one or two arsonic attached in the ortho position to the azo groups conjugate, respectively as shown in Figure 1.2. The PC reagents were introduced into the forced flow column assistance. The most air pressure effluent with versatile and convenient was found to be PAR, responding to most transition metal cations(69,101,102). It does not require precise acid pH control like arsenazo III. Although Cassidy and Elchuk(103) separated 14 rare earth metals with a gradient elution in 28 minutes using arsenazo I as the PC reagent. The baseline drifted a little, but it illustrated that it is possible to use a gradient elution with a PCR.

Fewer metals react with PAN than PAR and consequently is little used as a PC reagent, but can detect zinc and

cadmium(104).

Few reagents have been used as PC reagents although the detection by xylenol orange was reported for lanthanoids(105). Zincon was used by Frei(84) for aluminium(III), beryllium, cobalt, cadmium, chromium(III), copper, iron(III), manganese, molybdenum, nickel and titanium cations.

Zenki(106) adopted an unusual procedure, chlorophosphonazo (III), the photometric reagent, was added directly to the sulphosalicylic acid eluent, pH control was easier, but the reagent concentration should be less than 0.00005 M to allow the separation of metal ions.

If photometric reagents are to be used as PC reagents they should have a low absorptivity at the metal complexes wavelength of maximum absorbance. The complex should form quickly to reduce the complications of slow kinetics and the formation constant should be high for a resulting photometric complex to be stable. If possible the pH operating range should be large to minimise the complications of adding a buffer which may require precise flow rate control.

<u>1.22</u> Fluorescent Post Column Reaction Detection for metals.

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Although fluorescence is potentially a more sensitive technique there are limitations because of accuracy and precision errors, long associated with this technique. Only a few metals can be quantified by this approach. Such methods may offer a 100 times better sensitivity.

Metal ions as their complex with an aniline derivative of EDTA were detected with the PC reagent fluorescamine(107). The limits of detection were 60 to 80 pg for cadmium, lead and zinc. It was stated that sub-femtomole concentrations could be reached with laser induced fluorescence.

Chemiluminescence is the induction of fluorescence with chemical energy and requires no light source. Chemiluminescence reactions can be catalysed by transition metals and are known to be very sensitive, however, this enhanced luminescence procedure means that impurities constitute a considerable source of error. The addition of several PC reagents was found necessary. Notable reactants for PCRs are luminol and lucigenin with hydrogen peroxide or oxygen(26,108,109).

1.23 Direction of Research and Present Intentions for the Determination of Trace Metal Species by HPLC.

metal Increasing of important trace numbers determinations are required on materials, for reasons outlined earlier, which are present in a varying range of matrices. Fast quantitative knowledge of organic compounds has been shown to be feasible by HPLC, but not for metals and their species. HPLC is still in its infancy regarding this area, consequently potential for essential development in the areas of separation and detection has arisen. It is hoped firstly to improve the efficiency of ion exchange separations by using better column packings with organic acids eluents that have exchange liquid classical ion shown promise in chromatography. Chromatographic possibilities with neutral and anionic columns will be investigated.

The subsequent detection after separation of the metal ions may be effected with a different PC reagent for a more sensitive photometric response. There are a vast previously investigated metallochromic of number reagents to chose from, but only a few have been PC reagents. It is hoped to improve reported as detection by looking at this new area which has been left unexplored until the recent manufacture of more The possibility of materials. efficient HPLC considerable improvement of selectivity, sensitivity and

increased number of species is evident. It is also hoped to improve the PCR technique for metals so that more metals can be detected by improved PCR design and optimisation procedures.

2 Instrumentation and Experimental.

2.1 Introduction.

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There are numerous types of HPLC equipment available and a problem of choice confronts the experimenter. Because the new detection technique proposed depends in absorbance of the decrease measuring а upon post-column reagent rather than an increase, a major problem was pump pulsations. The mixing of two eluent streams has to be relatively pulse free as it is emphasised by the detector as the column effluent stream at wavelengths using virtually transparent is metallochromic reagents, consequently the design is orientated around minimising systematic noise.

A typical HPLC system was used, as in Figure 2.1. This shows two pumps one of which pumps the PCR reagent and other the mobile phase they are co-ordinately the operated with a microprocessor control unit(Control Command Module, Laboratory Data Control, Riviera Beach, data from the FL.33404). The accumulated spectrophotometric detector was temporarily stored and peak height or area. The be assessed as could chromatogram and the data were printed on a thermal printer plotter(Thermal Printer Plotter Model E6220-1, Houston Instrument, Austin, Texas, U.S.A.) with the option of storage on a IEEE-488 interfaced dual floppy

disc unit(Commodore 8050, Commodore Business Machines Inc., 3330 Scott Boulevard, Santa Clara, California, 95051) for possible further interpretation.

2.2 Pumps.

High pressure delivery pumps were used (Constametric III; L.D.C.), providing a constant volume at pressures below 5000 psi between 0.1 to 10.0 ml min⁻¹. A cam drives two positive displacement sapphire pistons 180 degrees apart. A phase locked loop controller gives a + repetitive flow-rate accuracy. The post-column 0.3% reactant was pumped via a dual piston system (Model AA Dual Piston Eldex pump; Eldex Laboratories, 3551 Haven Avenue, Menlo Park, C.A. 94025), through a pressure restrictor from the high sensitivity accessory of a pump(Waters Associates Inc., Milford, HPLC Waters Massachusetts, U.S.A.) to reduce the pump pulsations and then to the T junction with 2 m of 0.8mm I.D. PTFE tubing.

Both pumps have 5 µm filters on the eluent inlets to curtail pump wear and increase the column operational time.



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2.3 Injector System.

A Rheodyne injector valve 7125 (Rheodyne, Cotati, C.A.94928) that can be fitted with various volume loops from 5 to 200 μ l for different analyte concentrations was used. A 100 microlitre metal fitted S.G.E. syringe was used initially and changed later to an all plastic substitute to minimise contamination problems.

2.4 Analytical Column.

Two different dimension analytical columns were used; one for a silica based cation exchanger in a 25 by 0.46 cm column and another for resin-based materials in a 10 by 0.4 cm column. Both columns were manufactured in stainless steel and 2 µm pore stainless steel frits were placed at either end of the columns.

The silica based cation exchanger (Whatman Partisil 10 SCX, Whatman Ltd., Springfield Mill, Maidstone, Kent, ME142LE England.) has benzene sulphonated groups bonded to 10 µm silica particles.

Column packing conditions are critical for trace determinations. A Shandon Column Packing Instrument(Shandon Southern Instruments Inc., 515 Broad Street, Sewickley P.A., 15143 U.S.A.) was used to pack both materials. The silica substrates were packed as a 25 % slurry, after an half an hour vacuum or sonic

degassing, with a (1+3) methanol and isopropyl alcohol solvent, respectively. The inverted column,was packed with 150 ml of alcoholic solvent and then a 100 ml water, followed by 150 ml of the intended eluent at a pressure of 7-10,000 psi. The column has its top end placed upwards after 75ml to ensure a solid column top. Channelling is a major packing problem and if gravity is opposite to the flow this may be avoided, a more uniform particle size distribution is also assured.

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The resin-based materials should be degassed as an eluent slurry under vacuum for at least 2 hours. The eluent should be at its highest concentration and its lowest pH for the Aminex A-9 11 µm cation exchange (Bio-Rad copolymer sulphonated divinvl benzene Griffin, Richmond, California, 32 å Laboratories, U.S.A.) and the highest pH for the Benson 7-10 μm anion exchanger(Benson Company, P.O. Box 12812 Reno, Nevada 89510, U.S.A.). An initial pressure of 500 psi was used which minimised physical fracturing of the beads. After 50 ml the pressure was raised to 5-7,000 psi for a 200 ml volume; again column inversion ensured column top uniformity.

2.5 Post-Column Reactor.

A variety of PCR lengths were used depending on the desired reaction time which was usually less than

a minute. After preliminary investigations the best option was to use various lengths of 0.8 mm I.D. PTFE tubing. Zero dead volume fittings connected pumps, column and detector. Stainless steel tubing(0.3mm I.D.) prior to the column was substituted for 0.8mm I.D. PTFE tubing after the column. Where possible, PTFE tubing and ferrules were implemented at pressures up to 1,500 psi to minimise trace impurities.

The eluent was mixed with the post-column reactant at 180 degrees at a T junction. If necessary the resulting effluent tubing was placed in a thermostatically controlled water bath(Grant Instruments(Cambridge) Ltd., Barrington, Cambridge CB2 50Z, England.).

2.6 Detector.

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The post-column effluent was connected directly to the 0.3 mm I.D. tubing of a metal block that acts as a heat exchanger to reduce temperature fluctuations. The double spectrophotometric flow UV/visible beam monitor(SF770;Schoeffel Instrument Corporation, Westwood N.J.) has two 10 µl volume flow cells of 1 cm length, one of which was an air reference cell. The mechanism incorporated a chopper double channel system that against noise and unchopped stray discriminates radiation. Stop flow spectral scans are possible for wavelengths up to 700 nm and variable time constant

filters some output signal noise.

2.7 Reagents.

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Distilled deionised water was prepared in a glass still and the distillate passed through an Elgastat deioniser(Elga Products Ltd., Lane End, Bucks., England.) to give a conductivity less 4 micro-ohms cm⁻¹.

Analytical grade reagents(BDH, Poole, Dorset, England) were used throughout. Stock organic acid solutions(2M) were prepared by mixing the acid with distilled deionised water; at this concentration biological growths did not form. The required organic acid eluent was diluted and the pH adjusted with a mixture of 2 M sodium and ammonium hydroxides(2+3). The eluent was degassed with a helium bubbler or by placing in a sonic bath.

Stock Eriochrome Black T reagent was prepared as a 0.4g per 100 ml solution in 0.1M ammonium hydroxide.

Dithizone solution was prepared by dissolving sufficient in a (4+1) acetone and water solvent respectively, to give the required absorbance at 595 nm.

Spectrosol(BDH) metal solutions prepared in lN nitric acid.

A few 1000 μ g g⁻¹ stock metal solutions were prepared by dissolving 1 g of the metal in a (1+1) mixture

of concentrated nitric acid and distilled deionised water, diluted to l litre with distilled deionised water. Indium and bismuth were diluted with a 0.2M tartaric acid solution. Storage of metal solutions does pose problems due to evaporation, even in a sealed container as well as adsorption onto the container surface. To minimise this a polyethylene container with screw cap was used for stock solutions(16) and ml⁻¹ of any of lower 5 μg down to solutions concentrations were made fresh each day this alleviated any problems associated with the instability of dilute solutions. These dilutions were made using volumetric flasks and the same pipette after prolonged washing with the minimum distilled water so that deionised comparative error between the metal ions was introduced.

2.8 Contamination Control.

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Probably the greatest difficulties encountered when analysing ultra-trace quantities are those from contamination. This includes that from the laboratory atmosphere, the surfaces of containers, HPLC fittings, injection systems and sampling procedure.

To minimised these instrumentation was placed in a laboratory exclusively for trace HPLC work. The deioniser was only used for the purpose of preparing water for trace metal analysis solutions and organic

acid eluents. The same pipettes and glassware was used, with metals solutions being repeatedly prepared and stored in the same low density polyethylene container. Low density polyethylene is preferable to the high density product which is manufactured in a catalytic process which uses transition metal oxides(16).

PTFE tubing minimises contamination and was preferable to stainless steel, however at pressures greater than 1500 psi, PTFE was not strong enough for continuous use. Preconcentration work revealed that stainless steel released iron into the eluent or sample especially at lower pH. This problem also arose in the sampling loop as well as with the injection syringe. An aluminium needle is recommended to be used in conjunction with a disposable plastic syringe. Where outdoor sampling was necessary new polyethylene containers were used and the solutions were made acid by using a plastic tipped repeatable pipette that could deliver constant volumes of acid. Samples were filtered after repeated washings with the sample solution and placed immediately into the polyethylene containers. Samples were kept in a refrigerator for a minimal time until required.

3 Chromatography of Metal Ions.

3.1 Introduction.

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The rapid advancement of HPLIEC column packings has made further investigations into column eluent systems is often analysis Speed of chemical necessary. synonymous with inaccuracy and imprecision of results, but the opposite is true of HPLC determinations and this is a major point in favour of HPLC. One of the major aims of this thesis is to improve and adapt liquid chromatographic procedures that have been successful in the past, using present HPLC technology. Preliminary observations showed that the correct choice of column material was extremely important for good chromatography as there is a large variety of materials on the market. Various column packing materials and eluents that were found successful are discussed.

Although chromatography controls the number of metals analysed in one injection, the chief aim of this work is to investigate and develop sensitive PC metal detectors. It is impossible to consider detection in isolation from the chromatography, for example, considerable interference was found with highly conducting eluents when using conductometric detectors(43). Previous papers suggest that little or no interaction occurs with the eluent using PC reagent detection, but this is not

assumed here. The chromatography was developed first, detector response is then assessed at a later stage. Early development of the chromatography was considered the most logical approach. Although some detector response anomalies may occur because the system is not optimised as a whole complications arising from improving both the chromatography and the detector may be complex, but could be performed if necessary.

The chromatography was considered in theoretical terms to compare separations. Retention was used to calculate the column void time. As using capacity factors chapter one improved column in earlier discussed parameter determinations require the measurement of the column void using the solvent front and not a retention measurement from the chromatogram starting point. This measured accurately, normally using a be has tο calibrated eyepiece, as resulting large errors in calculated column parameters may occur. Although it was necessary to assess and optimise the chromatography, some variability occurred after a column was packed, making assessment more difficult. The exact conditions chosen depended upon the number of metal species to be separated and the capacity of the particular batch. Batch to batch variations in the manufacture of high still be ion-exchange materials can efficiency significant and it was found that a new batch sometimes required a different pH of organic acid buffer or even a

different nominal concentration to achieve the same separation as the previous batch.

It was not the intention here to manufacture new column materials, however, some new eluents will be compared in terms of speed, selectivity and sensitivity with a particular PC reagent.

Chromatographic effects are related to the eluents chelating properties, its concentration and pH. Adjustment of the pH was made by adding alkali, however the cation present in the alkali will compete with the analyte for ionic sites and is known as the counter ion. The effect of concentration of counter ion, ethanol and sodium chloride will also be assessed with the view to maximising column efficiency.

Selectivity for metal analyte ions in the presence of a complexing agent is partially dependant upon three factors:

i.the relative complexation of the metal,

ii.the competition of the counter ion;

iii. affinity for the stationary phase.

Ligand complexation is a major contributor to separation and is discussed with regards to quantifying
a relationship to elution order.

Classical ion chromatography shows that the charge density of an eluting metal ion is proportional to the attraction for the stationary site, consequently size and charge governs the primary elution order. Highly charged ions may complicate the order by having a solvation layer which diffuses the charge density. Another important consideration is the effect of enthalpy change for the equilibrium reaction with the chelating eluent, which may have a large impact on the Gibb's free energy of the exchange reaction.

Table 3.1

Strong Cation Exchange Materials.

Name	Manufacturer	meq.g l dry mass.	Diameter /μm
Lichrosorb KAT	Merck	1.2	10
Aminex A4, A5, A6, A7,	Bio-Rad	2.0	variable within
A8, A9, Q155, Q150S.			defined limits
Benson BA-X10	Benson	1.6	7-10
Partisil 10	Whatman	Quoted as	10
		HETP 0.09	nm
Nucleosil SA	Merck	1.0	10 & 5
Vydac cation exchar	nge Applied Science	0.1	30 to 40

Table 3.2.

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Strong Anion Exchange Materials.

Name	Manufacturer	meq.g ⁻¹ D dry mass.	liameter /μm
Lichrosorb AN Aminex Al4,A25, A27 A28,A29.	Merck	0.55 1.2	10 as for SCX resins
Benson BC-X10 Partisil 10	Whatman	l.3 Quoted as HETP 0.045m	7-10 10 1m
Nucleosil SB Vydac anion exchan	Merck ge Applied Science	1.0 0.1	10 & 5 30 to 40

Obtained from reference (110)

3.2 Physical Properties of Ion Exchange Materials.

The most commonly used commercial high efficiency ion exchange materials are presented in Tables3.1 and 3.2. Virtually all HPLC exchange resins are polystyrene based materials with divinylbenzene cross-linking. The Vydac beads are based on a polymer layer on glass solid core sphere. Only the Whatman and Nucleosil exchangers have a an irregular porous Lichrosorb is base. silica silica-based material. Anion exchangerguse a quaternary ammonium group and the cation exchangers an aromatic sulphonated moiety. The HPLIEC resins can withstand temperatures up to 150 °C and the ionogenic groups can The structure tolerate very high and low eluent pH. and size of the resin has considerable impact upon the chromatography.

Although silica substrates are only stable at

has no noticeable intermediate рH the structure Resin-based materials have effects. chromatographic chromatographic effects dependant on the degree of cross-linking. Increased cross-linking diminishes the swelling of a resin and the water content. The pore size of the bead reduces to give a molecular sieve effect that partially increases selectivity(110). Takata(111) compared 8, 10 and 12 % cross-linked sulphonated resin separations with a tartrate eluent and illustrated that heavier metals, cadmium and lead, were increasingly compared to other metals, such that lead retained from nickel with % divinylbenzene 10 separates cross-linking.

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Perhaps the most distinguishing feature of resin-based exchanger from silica-based material is the greater kinetic rate of exchange and the lower capacity. Resins are sulphonated at the centre of the resin as well as the exterior, silica-based ion exchangers have surface-bonded benzene sulphonic acid functional groups.

Improved chromatography results from using small particle size stationary phases but will require higher pressures to maintain flow rates. A macroporous resin also has less substrate to diffuse through and should have a rapid rate of diffusion. A thin film present on the particle is less of a barrier with smaller particles, and its effect is similarly diminished with

temperature. A temperature increase from 25 to 50 °C doubles the diffusion coefficient which is normally the rate controlling process, hence doubling the exchange rate(112). A larger analyte ion gives a slower diffusion rate which is to some extent controlled by the degree of cross-linking. Highly charged ions also produce a slower rate of exchange. A high capacity ion exchanger would consequently have a greater affinity and slow the ion exchange reaction. Thus a higher capacity or degree of cross-linking does not mean that a better efficiency is obtained.

Diffusion is an important factor when considering ion exchange processes as it is often the rate limiting step, making the effect of column temperature important. The Partisil SCX and especially the Aminex A9 SCX columns have a considerable gain in efficiency with temperature. The Partisil 10 SCX column was operated at temperatures up to 56 °C, however the increase in increasing accompanied by an efficiency is This silica. problem may be solubilisation of circumvented by a sacrificial guard column of cheaper silica material, which saturates the eluent stream with silica. A compromise temperature of 40°C was used without a guard column present.

Styrene based SCX columns are stable until over 120 ^OC and are more efficient at high temperatures, because the cations can enter the bead at greater rates to reduce

peak broadening processes. For this work the silica column was used at 40° Cand the resin column at 60° C unless otherwise stated.

3.3 Choice of Cationic Chromatographic Substrate.

Initially it was intended to separate about ten trace metals as cationic species. Table 3.1 lists the choice of commercially available HPLC cation exchangers. Few metal separations by HPLIEC are known and at present there are few cation exchangers that can be considered to be of sufficient quality for the objectives of this thesis.

The ability to separate transition metals with Hitachi No.2611 & 2613 and the CK 10 and 12 resins(Mitsubishi Chemical Industries, Japan) has been well demonstrated by Takata(111). Takata also showed the separation of rare earths with a Hitachi 2611 15 µm diameter SCX resin(70). These resins were unobtainable here. American manufactured Dionex cation exchangers could only be purchased with the whole chromatographic system. It was readily available the decided to use therefore silica-based Partisil 10 SCX as a low capacity exchanger and the high capacity highly cross-linked Aminex A9 resin that offers higher efficiencies with a closely defined 11 ± 0.5 µm particle size. The lower capacity exchanger would naturally require a lower concentration

buffer for elution. This option covers the extremes of the buffer concentration range necessary with good separations. This wide range of buffer concentrations can then be used to assess detector performance which is discussed in the next two chapters. The resin and silica-based SCX materials will be used in columns with dimensions of 10 by 0.46 cm and 25 by 0.46 cm respectively, unless otherwise stated.

Table 3.3.

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	Silica S	SCX P10	Resin	SCX A9	Resin S	SCX Dionex
Metal	RT ^a /mir	RRT ^b	RT / m	RRT 1 n	RT / D	RRT nin
void	1.52		0.72		0.65	
Copper	2.90	1.00	3.84	1.00	3.29	1.00
Zinc	6.08	3.69	5.97	1.68	9.04	3.04
Nickel	6.86	3.88	7.63	2.21	9.70	3.42
Cobalt	8.02	4.72	13.0	3.94	17.1	6.23
Cadmium	9.77	5.99	20.2	6.14	25.4	9.37
Tartrat	e					
Conc. /	M 0.04		0.2		0.0)2
рН	3.66		4.5	2	4.5	5

Comparison of Silica and Resin Based SCX Materials.

f denotes the void time of the column unless otherwise stated. a retention time(RT) b relative retention time(RRT) The column dimensions for the lightly sulphonated Dionex resin SCX column was 25 by 0.46 cm.

> The retention times for all metal ions eluted are tabulated to the second decimal place of a minute unless otherwise stated, in practice a variation of \pm 0.01 minutes was observed. To allow for retention variation

within a chromatographic system the relative retention time(RRT) was used where the ratioed retention time(RT) against copper was calculated. In each ratio the column void time was subtracted from the copper and metal elute retention times.

3.4 Choice of Cation Column Eluent.

Classical cation exchange chromatography mainly used mineral acids to elute transition metals. Smaller particle size and therefore more efficient ion exchange materials for HPLIEC were manufactured that gave better separations with organic acid eluents. Although EDTA, nitric and sulphuric acids have been used, carboxylic acids were found to be more successful for cation chromatography(113). Cation exchange exchange chromatography of metal ions was considerably improved poly-functional carboxylic acid eluents. by using Retention times were much shorter and the separation of ions with similar size and charge was enhanced. This was normally performed with an isocratic rather than the elution, except for some step gradient familiar lanthanoid separations.

The capacity of the cation exchanger determined the eluent. A high capacity required carboxylic acids with larger metal stability constants to maintain short retention times. Thus cation exchange materials based on

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high capacity cross-linked polystyrene resins tend to use the more strongly chelating tartaric and citric acids.

The capacity of the Whatman silica SCX is considerably lower than the Aminex A9 resin and thus a weaker complexing acid or a strong complexing acid at a lower concentration can be used.

Carboxylic acids such as tartaric and citric eluents separations with classical column poor gave chromatography, however it may be possible to take better advantage of them for HPLIEC separations. If only a few metals are to be determined then it may be possible to use an eluent which only separates the necessary metals thus speeding up the analysis time as the remainder elute together and quickly. To separate difficult peaks or samples the possibility of mixing phases can combine the advantages of both mobile the chromatography more eluents, which may make efficient. Plots of inversely varying concentrations of eluents against retention could be used to ascertain the correct combination, these are known as window diagrams. For this work many acids were studied as a base, in the belief that such information may be useful for window diagrams(114). Window techniques could be developed not only for mobile phases, but also for combined stationary phases as they have for GC. Therefore it was thought prudent to study metal retention with the weakly

complexing acids, acetic, formic, lactic, maleic and succinic acids as well as the more familiar tartaric and citric acids using the Partisil 10 SCX column.

Other acids were considered as alternative eluents to assess their ability to separate and change the elution order, as well as their possible use in combined eluents. New eluents were chosen on the basis that the constants should be of the same metal stability magnitude as the carboxylic acids that were successful. Comparison of stability constants are shown in Appendix 1. For each eluent studied only those that gave good are illustrated, others are tabulated. separations Alternative eluents were chosen by adopting the following separation optimisation strategy:

i. a pH in the range 2 to 4.5 was used and the concentration of the organic acid eluent altered to achieve maximum separation;

ii. for a given total concentration of acid, precise control of elution speed was achieved by pH adjustment with the addition of a (3+2) mixture of 2M NH, OH:NaOH, respectively.

3.5 Citrate Elution.

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Citric acid is a trivalent carboxylic acid and forms strong complexes with most metals, especially the ferric

ion. This is reflected in a change of elution order when compared to sodium sulphate or weak organic acid eluent. Poor separation occurs even with the resin cation exchanger shown in Figure 3.1, because of the strong complexes formed. It is better when used with the Partisil 10 SCX column and Figure 3.2 shows the best resolution of peaks for the Partisil 10 SCX column with a 0.04M citrate eluent at pH 3.2 which gives indium(2.30 min) copper(2.57 min) eluting together as do zinc(5.26 min) and cobalt(5.79 min). Iron (8.78 min) and manganese(11.4 min) elute separately.

Table 3.4.

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Citrate	Elution	with a	Resin-Based	SCX	Column.
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Metal	Retention Time / min	
Void time	0.96	
Bismuth	1.11	
Copper	1.28	
Zinc	1.55	
Nickel	1.92	
Lead	3.12	
Cobalt	8.32	

Table 3.5.

Citrate Elution with a Silica-Based SCX Column.

Metal	Retention Time / min	
Void time	1.41	
Indium	2.30	
Copper	2.57	
Zinc	5.26	
Cobalt	5.79	
lron	8.78	
Manganese	11.4	



The separation of a 100 µl of 2 µg ml⁻¹ copper(1.42 min) and nickel(3.2 min) and 10 µg ml⁻¹ of zinc(7.49 min) using a 0.2M citrate eluent at pH 3.75 with an Aminex A9 SCX column. Detection was at 595 nm with an EBT reactant in .5M ammonia solution. Most metals co-elute with zinc. Cobalt(1.52 min) co-elutes with copper.



The separation of a 100 μ l of 3 μ g ml⁻¹

of indium(2.30 min) 1.2 µg ml⁻¹ of copper(2.57 min), zinc(5.26 min) cobalt(5.79 min), Fe(8.78 min) and manganese(11.4 min) using a 0.04M citrate eluent at pH 3.17 with a Partisil 10 SCX column. Detection was at 610 nm with EBT as a post-column reactant in a .4M ammonia solution and a F.S.D. of 0.2 absorbance. Lead(4.94 min) and nickel(4.33 min) co-elute.

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3.6 Tartrate Elution.

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This is a divalent organic acid that is a slightly weaker complexing agent than citrate and, as Appendix 1 demonstrates, has larger differences in metal stability constants. This was reflected in better chromatographic differentiating powers. It is amenable to use with Partisil 10 and Aminex A9 SCX columns. However the late elution of peaks decreases sensitivity because of peak broadening as Figure 3.3 shows for the Aminex SCX resin. To separate lead from nickel it was necessary to reduce the pH to 4.25, with cadmium eluting at 23.58 minutes. The metals elute with better efficiency using a silica based SCX column as shown in Figure 3.4

Table 3.6.

Tartrate Elution with a Resin-Based SCX Column.

Metal	Retention Time / min	
Void time	0.45	
Copper	3.37	
Zinc	6.93	
Nickel	8.88	
Lead	9.98	
Cobalt	15.73	
Cadmium	23.09	



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The separation of a 100 µl of l µg ml⁻¹ of copper(2.09 min), 2 µg ml⁻¹ of zinc(5.63 min) 4 µg ml⁻¹ of nickel(7.10 min), 3 µg ml⁻¹ of cobalt(12.31 min) and l µg ml⁻¹ of cadmium(19.68 min) using a 0.2M tartrate eluent at pH 4.32 using an Aminex column. Detection was at 595 nm with dithizone in acetone 80:20 water and l % acetic acid as the post-column reactant and the F.S.D. was 0.1 absorbance.



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The separation of a 100 µl of 2.5 µg ml^{-1} of indium(4.09 min), 3 µg ml^{-1} of zinc(5.49 min), 2.5 µg ml^{-1} of cadmium(7.62 min) and magnesium(11.23 min) using a 0.02M tartrate eluent at pH 3.3 with a Partisil 10 SCX column. Fluorescent detection was at 515 nm with excitation at 364 nm. 0.0002M 8-hydroxy-quinoline 5-sulphonic acid was the post-column reactant in water with 7 % 880 ammonia.

3.7 Oxalate Elution.

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This is another divalent acid which is comparable to tartrate for separations, but has the problem of forming insoluble calcium compounds at higher concentrations and was considered second to the tartrate system because calcium is present in most environmental samples.

0.02M concentration was necessary to effect a Α separation at pH 2.5 on a Partisil 10 SCX column. The peaks in Figure 3.5 illustrate a high efficiency and selectivity for this elution system. Table 3.7 shows it is possible to separate copper and iron (III) from nickel, lead, zinc and cadmium, cobalt, iron, manganese in 38 minutes as well as the late eluting magnesium and calcium in a single injection. However the large time span requires an investigation of gradient elution. An anomaly arises when the pH is increased to 3.5, where which previously eluted after zinc, nickel, cobalt lead now elutes before this series. and cadmium Preliminary studies showed that a more concentrated oxalate eluent may separate manganese, aluminium(III), magnesium and calcium.

Table 3.7.

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Oxalate Elution with a Silica-Based SCX Column.

Metal	Retention Time / min
Void time	2.03
Copper	2.55
Iron(III)	2.07
Nickel	7.18
Lead	7.84
Cadmium	8.98
Zinc	9.06
Cobalt	10.80
Iron (\hat{u})	19.97
Manganése	35.69

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The separation of a 100 µl of 6 µg ml⁻¹ of copper(2.55 min), 1 µg ml⁻¹ of Fe(III(2.09 min), 1.75 µg ml⁻¹ of zinc(9.07 min) 1.4 µg ml⁻¹ of nickel(7.18 min), 3 µg ml⁻¹ of lead(7.84 min), 1.75 µg ml⁻¹ of cobalt(10.80 min), 2.55 ug ml⁻¹ of Fe(19.97 min) and 1.4 µg ml⁻¹ of manganese(35.69 min) using a 0.02M oxalate eluent at pH 2.48 with a Partisil 10 SCX column. Detection was at 610 nm with EBT as a post-column reactant in a .4M ammonia solution and a F.S.D. of 0.2 absorbance. Cadmium(8.89 min) co-elutes with zinc. Nickel(3.83 min), manganese(4.95 min) and magnesium(8.5 min) elute with considerably better resolution under the same conditions except for a pH change to 3.15.

3.8 Lactate Elution.

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Lactic acid was found to give particularly good Partisil 10 SCX material. The results with the concentration of this monovalent acid produced the most interesting chromatographic effects with changing 3.8 shows that concentration concentration. Table affects elution time and therefore critically concentration can be used to optimise the chromatography, which contrasts with other acids investigated where the concentration effect was less pronounced. This was demonstrated in the chromatograms of Figures 3.6, 3.7 and 3.8. Cadmium and ferrous are distinguishable at 0.05M, but not so easily at 0.1M lactate. Early eluting metals are not distinguishable at 0.05M but are at 0.1M lactate concentrations. Also with the 0.05M lactate eluent nickel and lead elute before zinc, with a 0.1M lactate they elute before and the order was reversed again at the 0.04M lactate, zinc elutes before nickel.

Table 3.8.

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Comparison	of	Elution	Times	for	Different
Concentrations	of Lac	tate Buffe	r.		
Metal	Ret	ention			
	Tim	e / min			
Conc.of					
Lactate / M	0.04	0.05	0.1		
Void time	2.74	1.51	1.23		
Indium	21.10	2.16	4.14		
Copper	6.75	2.20	3.50		
Iron(III)	11.70	1.29	4.80		
Zinc	16.70	4.26	7.54		
Nickel	18.60	3.56	8.44		
Lead		3.90	8.48		
Cobalt		5.42	10.44		
Iron		8.23	11.40		
Cadmium		11.0	12.30		
Manganese		12.3	13.90		
Magnesium		16.0	15.40		
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Those that have larger retention times were not

recorded.



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The separation of a 100 µl of 0.9 µg $m1^{-1}$ of copper(6.75 min), 0.9 µg $m1^{-1}$ of Fe(111(11.70 min), 6 µg $m1^{-1}$ of zinc(16.70 min) 0.9 µg $m1^{-1}$ of nickel(18.60 min) and 2.2 µg $m1^{-1}$ of indium(21.10 min) using a: 0.04M lactate eluent at pH 3.55 with a Partisil 10 SCX column. Detection was at 610 nm with EBT as a post-column reactant in a .4M ammonia solution and a F.S.D. of 0.02 absorbance.:



The separation of a 100 ul of .0.75 ug ml⁻¹ of copper(2.20 min), iron (III)(2.29 min), nickel(3.56 min), zinc(4.26 min), cobalt(5.42 min), cadmium(11.0 min), iron(8.23 min), manganese(12.3 min) and magnesium(16.0 min) using a 0.05M lactate eluent at pH 3.50 with a Partisil 10 SCX column. Detection was at 610 nm with EBT a post-column reactant in a 0.4M ammonia solution and a F.S.D. of 0.2 absorbance. The metals bismuth(1.90 min), indium(2.16 min), copper and iron(III) virtually co-elute on the solvent front. elutes between nickel, which Lead(3.90 min) unpredictably elutes earlier than zinc.





The separation of a 100 µl of 1 µg ml⁻¹ of indium(4.14 min), copper(3.50 min), Fe(III(4.80 min), zinc(7.54 min) nickel(8.44 min), cobalt(10.44 min), manganese(13.90 min) and magnesium(15.40 min) using a: 0.1M lactate eluent at pH 3.5 with a column. Detection was at 610 nm with EBT as a post-column reactant in a .4M ammonia solution and a F.S.D. of 0.2 absorbance. Cadmium(12.30 min), and Fe(11.40 min) co-elute at 11.94 min. Lead also co-elutes with nickel. Traces of calcium are seen(16.85 min).

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3.9 Succinate Elution.

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Table 3.9 shows that a 0.1M succinate buffer at pH 4.35 gave a different order of retention from most indium(12.76 min) and organic acids in that bismuth(16.89 min) eluted later than most species on a Partisil 10 SCX column. Lead(4.74 min) and iron(6.89 eluted earlier than zinc(8.97 min) and min) also min). attempt to use lower nickel(8.38 An concentrations of succinate gave unstable chromatography and hence variable metal retention values.

Table 3.9.

Succinate Elution with a Silica-Based SCX Column. Retention Metal Time / min 2.13 Void time 16.89 Bismuth 12.76 Indium 4.55 Copper 8.38 Zinc 8.97 Nickel 4.74 Lead 9.69 Cobalt Cadmium 6.89

3.10.Maleate Elution.

Retention times are given in Table 3.10 for the optimum separation conditions for varying maleic acid concentrations and pH values using a Partisil 10 SCX column. The optimum conditions were at 0.04M maleate and pH 2.50. No distinctive separations are shown which would fulfil the initial interest.

A 0.1M maleate buffer at pH 3.63 shows poor chromatographic separation and a dilution to 0.04M, at pH 3.75, did not improve the situation. Metals did not elute at exactly the same times, but merely eluted at a later stage to give the same closely knit configuration.

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Table 3.10.

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Maleate Elution with a Silica-Based SCX Column.

Metal	Retention
	Time / min
Wedd bine	1 20
void time	1.38
Indium	9.32
Copper	5.72
Zinc	8.85
Nickel	9.12
Lead	10.20
Colbalt	9.40
<pre>Iron(II)</pre>	9.17
Cadmium	9.15
Manganese	9.27
Magnesium	9.12

3.11 Pyruvate Elution.

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This acid is closely related to lactic acid and changing the functional group in the alpha position to the carboxylate system may effect a different elution order. The optimum conditions for this acid using a Partisil 10 SCX column were 0.04M pyruvate at pH 3.05 Table 3.11 shows retentions for poorly separated metal species at two pH values with a 0.04M pyruvate eluent.

Table 3.11.

Pyruvate Elution with a Silica-Based SCX Column.

Metal	Retention Time / min	Retention Time / min
рН	3.05	3.41
Void time	1.64	1.64
Copper	3.72	4.75
Iron(III)	5.33	7.25
Zinc	4.45	6.38
Nickel	5.03	6.95
Colbalt	5.15	6.92
<pre>Iron(II)</pre>	5.33	
Cadmium	4.82	
Manganese	5.14	
Magnesium	5.05	

If the pH or the pyruvic acid concentration was decreased then the metal ions only increased their retention without improving the separation.

3.12 Formate Elution.

This is the weakest complexing organic acid in the series, Table 3.12 shows that the optimum conditions using a Partisil 10 SCX column were at pH 4.25 and 0.075M.

Table 3.12.

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Formate Elution with a Silica-Based SCX Column.

Metal	Retention Time / min	
Void time	1.50	
Copper	3.57	
Zinc	5.38	
Nickel	5.51	
<pre>Iron(II)</pre>	6.18	
Cadmium	4.67	
Manganese	5.62	

Again the acid has too weak a selectivity effect to be of any chromatographic use on its own. Copper eluted earlier and iron later, separating from zinc, nickel, manganese, lead and iron(III).

3.13 Effect of The Counter-Ion and The Counter-Ion Concentration.

Metal ions have a preferential order of complex formation with a ligand according to the Irving-Williams order, which is magnesium <manganese <iron <cobalt <nickel <copper > zinc. This is related to charge density and in the case of transition metals is also

related to the ligand-field stabilisation energy. It therefore follows that with ions of the same charge, elution order follows decreasing size. Elution is also dependant upon competition with a counter-ion for column ionic sites, it should therefore follow that the size and charge of the counter-ion will control the speed and possibly the degree of separation. A knowledge of the counter-ion will give a better comprehension of the organic acid eluent selectivity. Elution was therefore attempted without an organic complexing agent present. sulphate ion has relatively little complexing The ability so the sodium and hydrogen ions can completely control the separation. The di-sodium salt was used and the acidity was adjusted with sulphuric acid to a pH of 3.70.

Table 3.13.

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Sodium_	Sulphate	Elution	with	а	Silica-Based	SCX
Column.						
	Re	tention Ti	ime / m	in		
	Su	lphate Mol	larity			
Metal	0.0	0.02	l 0.	005		
Copper	2.	15 3.00) 6.	90		
Zinc	2.	14 3.04	47.	10		
Manganes	e 2.	25 3.17	7.	20		
Magnesiu	m 2.1	24 3.27	7.	54		•

The competing counter-ion causes an expected exponential decrease in retention with increasing concentration, Table 3.13 shows that retention relative to copper remains constant. Distinctive retention differentials are shown and although the peaks are not completely separated the expected elution order obtained was similar to that of a weak organic complexing acid, illustrating a minimal complexing effect for some organic acids which was only just sufficient for separation.

The counter-ion effect was further investigated because the nature of its action may be altered in the presence of a complexing agent. To assess this aspect a 0.04M succinate buffer at pH 3.70 was used. Its differential separating ability on a Partisil SCX column was found to be insufficient for the easier transition metal separations, allowing any effect of the counter-ion to be noticed.

Table 3.14.

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Succinate	Sodium	Sulphate	Elution	with a	Silica-Based
SCX Column.					
Sulphate Molarity	0.0	0.001 0	•002 0	•004	
Metal	Rete	ntion Time	/ min		
Copper Zinc Nickel Manganese	11.2 19.9 21.7 21.7	6.69 5 9.64 7 9.93 7 10.2 8	.02 3 .03 4 .75 4 .00 4	• 64 • 49 • 59 • 84	

As shown in Section 3.9 succinate gave peaks that were better separated, but the elution time was longer. An increase of sodium in the eluent as sodium sulphate decreases the retention time and shortens the separation between metal peaks. Decreasing retention relative to copper demonstrated a decline in the number of plates which is not due to sodium sulphate alone.

An investigation of different counter-ions was performed with dithizone as a PC reactant. An organic acid was buffered with sodium and ammonium hydroxides for the greatest concentrations of tartrate eluent. Therefore any chromatographic variations would be highlighted using a high concentration of buffer and hence alkali. The impact was assessed using the high capacity Aminex A9 SCX resin. A 0.2M tartrate eluent at pH 4.1 adjusted with each hydroxide was prepared, the chromatography was performed quickly as precipitation occurred with ammonium tartrate.

Table 3.15.

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Tartrate	Elution	with	Different	Alkali	using	a
Resin-Based	SCX Column	1.				
			Caldura			
	Ammoni	Lum	Sodium			
	Tartra	ate	Tartrate			
Metal	Retei	ntion T:	ime / min			
Copper	1.48	3	1.38			
Zinc	3.69)	3.53			
Nickel	4.68	3	4.56			
Cobalt	8.0	5	7.72			
Cadmium	12.5		12.6			

The ammonium and sodium ion show little observable differences in retention times achieved for the metals studied. 3.14 Effect of Sodium Chloride Concentration on Elution.

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The reason for choosing this particular salt was that unlike salts of sulphate and nitrate the chloride ion has a considerable complexing ability. This can alter the elution order such that the mercury cation can be eluted before copper with a tartrate eluent with a Hitachi SCX resin(65).

For this work sodium chloride was added to a slightly reduced concentration of eluent using a tartrate elution on a Aminex A9 SCX sulphonated resin. Figure 3.9 b) shows that 0.08M salt in a 0.18 M tartrate eluent at a pH of 4.6 causes cadmium(6.23 min) to elute more quickly than cobalt(7.12 min). Compared with cobalt(12.31 min) and cadmium(15.68 min) with a 0.2M tartrate eluent at pH 4.33 in Figure 3.9 a). The reduced elution times were for copper(1.62 min), zinc(3.01 min) and nickel(3.75 min).

A tartrate sodium chloride eluent was used to separate copper and mercury(65). However an attempted repetition of the separation with an Aminex A9 column was unsuccessful.

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Effect of Sodium Chloride





Figure 3.9 a) shows a chromatogram which no sodium chloride in the eluent and Figure 3.9 b) shows the quicker elution with 0.08 M sodium chloride present in the tartrate eluent as detailed in 3.14.

3.15 Effect of Ethanol on Elution.

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Ion exchange in mixtures of organic and aqueous solvents require partial ionic ability of that solvent. Organic solvents are known to reduce the ion exchange rate by orders of magnitude usually to the detriment of chromatography(110), especially as regards swelling effects upon resin-based exchangers, however, organic solvents might reduce organic contamination from samples and prevent bacterial growth. Miwi and Yamabe(71) investigated the effect on mixtures of resin-based cation exchangers and found ethanol reduced column efficiency.

Here a 0.05M lactate eluent at pH 3.5 was used in conjunction with a Partisil 10 SCX silica column with the intention of observing minimum chromatographic interferences that would allow a water insoluble PC reactant to be used. Peak broadening for increasing ethanol concentrations of 2.5, 5, and 10 % v/v ethanol eluents was observed. Only the ferric ion altered its relative retention by eluting nearer to copper. Kinetic ion exchange processes were demonstrated by quicker elution with a temperature rise to 40 °C from ambient temperatures.

Table 3.16.

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Variati	on with	<u>Ethanol</u>	•
0	2.5	5.0	10
Retenti	on Time	e / min	
3.25	3.39	4.13	4.62
6.34	5.61	4.62	4.62
7.50	7.32	10.3	13.7
8.25	8.06	11.2	15.7
9.53	9.78	14.0	19.4
11.7	12.5	17.9	25.5
12.5	13.0	17.9	25.5
	Variati 0 Retenti 3.25 6.34 7.50 8.25 9.53 11.7 12.5	Variation with 0 2.5 Retention Time 3.25 3.39 6.34 5.61 7.50 7.32 8.25 8.06 9.53 9.78 11.7 12.5 12.5 13.0	Variation with Ethanol 0 2.5 5.0 Retention Time / min 3.25 3.39 4.13 6.34 5.61 4.62 7.50 7.32 10.3 8.25 8.06 11.2 9.53 9.78 14.0 11.7 12.5 17.9 12.5 13.0 17.9

3.16 Alternative Eluents.

There is always a search to find eluents that give a faster analysis time, separate difficult species or the range of metals species that can be extend separated. Various organic chelating agents were selected on the basis of their first metal stability $constants(K_1)$ because not all the values for higher complexes are known. These are shown in Appendix 1. Those with a value of $\log_{10} K_1$ for most metal complexes of less than 7 or 8 were found to offer promise. The basis for this choice is that these values are similar to those for the best organic acid eluents discussed previously and if a functional group of the chelator is different then the elution order may change. Normally carboxylic acids are used, so if we could use a additional group to this or a chelator with an completely new group then the elution order may change.

Unfortunately it is often only above K₁ values of 8 that other chelating groups which are stronger are found and difficulty was encountered finding alternative complexing eluents for this very reason. Many acids were scrutinised, but only the more successful elution systems are mentioned below.

3.17 Glycine Elution.

Table 3.17 shows the retentions obtained with a 0.04M glycine eluent used at varying pH with a Partisil 10 SCX column, to demonstrate that as the pH increased then the elution time increased rather than decreased as with eluting agents containing the carboxylic acid functional group alone.

Table 3.17.

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Glycine Elution with a Silica-Based SCX Column.

рН	2.22	2.64	3.03	5.70
Metal	Reter	ntion T	ime / min	
Copper	3.6	6.4	17.0	>50
Zinc	3.8	8.1	25.5	>50
Nickel	3.9	8.0		
Cobalt	4.0	8.0		
Manganese	4.0	6.8		
Lead	4.6	10.9		
Iron	4.2	8.2		

Later eluting peaks demonstrated considerable peak broadening and selectivity was not good, although magnesium(4.0 min), indium(7.1 min) and iron(III)(17.5 min) eluted in a different order to usual organic acid retentions. An interesting pattern emerged which reversed the usual increase of retention with decreasing pH associated with organic acid eluents. Zwitter ion formation steadily increases to an optimum with increasing pH to a specific pH value, presumably the cationic moiety assumed the dominant elution role.

3.18 Aspartic Elution.

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The investigation of this dibasic amino acid as an eluent may show it to have better chromatographic separating abilities than glycine. Table 3.18 shows a 0.04M aspartic acid eluent at pH 3.6 eluting seven metals, which were in two groups with a Partisil 10 SCX column.

Table 3.18.

Aspartate Elution with a Silica-Based SCX Column.

Metal	Retention Time / min
Copper	2.46
Iron(III)	3.90
Zinc	3.18
Indium	5.76
Cobalt	15.3
Manganese	16.4
Iron(II)	16.7

The peaks were of a very broad nature and cannot be considered for use alone although there may be
applications if used with another eluting agent.

3.19 Thiomalate Elution.

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Difficulty was experienced in eluting ions of a sulphophilic nature from cation exchange columns. A chosen sulphur containing chelating moiety should have the same order of magnitude of K, as an organic acid eluent. Thiomalic or mercaptosuccinic acid have the required values with relatively high stability constants for cadmium, lead, mercury, silver and zinc. When a 0.04M thiomalate eluent at pH 3.81 was used with a Partisil 10 SCX column a change in elution order was observed, with the more sulphophilic cadmium being eluted before zinc, nickel, and cobalt respectively, this is shown in Table 3.19. Figure 3.10 shows that the eluent can give high column efficiencies, dilution of the eluent does not produce any change in elution order. Mercury and silver are eluted on the solvent front for higher thiomalate concentrations than 0.1M but are not separated, or eluted at lower concentrations.



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The separation of a 100 μ l of 2.5 μ g g⁻¹ of zinc(4.60 min) nickel(8.00 min), cobalt(10.35 min) and cadmium(2.60 min) using a 0.04M thiomalate eluent at pH 3.93 with a Partisil 10 SCX column (25 by .46 cm) at 40 °C Detection was at 595 nm with dithizone as a post-column reactant in a acetone (4+1) water and 1 % acetic acid with a F.S.D. of 0.4 absorbance,

Table 3.19

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Thiomalate Elution with a Si	ilica-Based	SCX	Column.
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Metal	Retention Time / min			
Void time	1.16			
Copper	1.50			
Cadmium	2.49			
Zinc	5.48			
Nickel	10.71			
Cobalt	13.71			
<pre>Iron(II)</pre>	14.4			
Manganese	16.5			
Magnesium	16.6			

3.20 Anion Exchange Separation of Metals.

Anion exchange uses the differential attractions of different metal anionic complexes for the SAX column material. Higher complexation formation effects greater retention. It has had considerable success in the past with inorganic acid eluents, however the determination ug ml⁻¹ levels of metal in a solution by HPLIEC of difficult with a corrosive acid. Modern HPLIEC is demands a new approach to anion exchange separations with a less corrosive eluent media. Chapter one mentions the use of some organic acids for column chromatography. In preliminary studies with a Partisil 10 SAX column 25 by 0.46 cm few peaks separated with any organic acid and then with considerable peak broadening and consequently was not experimented with further. Only the 16 by 0.46cm resin-based Benson SAX was partially successful. Citric, lactic, tartaric and acetic acids were investigated and only tartrate eluents were capable of separating a few metals, they are listed in Table 3.20.

Table 3.20.

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Tartrate Elution with a Resin-Based SAX Column.

Tartrate Molarity	0.02	0.04	0.1	0.2
Metal	Retenti	on Time ,	/ min	
Copper Zinc Nickel Cobalt Cadmium	57.5 8.5 6.75 3.0 2.5	42.5 8.75 9.0 2.75 2.5	18 6.25 5.0 1.75 1.25	21 20 £ 3.5 2.75

£ denotes difficulty in obtaining reliable result.

Retentions times are at a minimum around 0.1 M. This would suggest that a complex formed at lower tartrate concentrations has less competition for the cationic functional group on the SAX resin. At higher concentrations higher retentions suggest that an anionic complex of greater charge is formed and therefore has a greater affinity for the SAX resin sites. The chromatographic equilibrium processes are more complex and this may be the reason for the considerable peak width. Better SAX column materials may overcome this problem.

3.21 Conclusion and Discussion.

is difficult not to discuss the quantitative It chromatography doubtless HPLIEC. Good of aspects contributes to statistically better results, however these aspects will be discussed in Chapters four and PCR response is closely linked with the five as preceding chromatography. With the objective of separation, the main PCR after assessing the chromatographic aspects are brought to a conclusion first in this chapter.

In the coming years microprocessor-controlled chromatography systems will be able to readily assess chromatographic parameters for optimisation. This may exchange eluents and gradient include mixing ion elution. Here the aforementioned strategy was adopted to assess each eluent. Incorrect values of theoretical plate numbers and capacity factors can be realistically assessed by using the relative retention of an eluting the representative of which is more metal, chromatographic processes. This overcomes the inaccurate determination of the void time by using a relative retention factor which has been introduced using copper as a marker. Copper elutes in most chromatograms and can be used as a calibrating retention because it usually retention time, because of a accurate gives an comparatively small peak width associated with an early

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elution and fast column processes.

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Guard columns can be used and such action has economic rewards, they are normally associated with pre-column concentration procedures in the injector loop, which are discussed in **C**hapter 6. The saturation of the eluent stream with silica can reduce column degradation due to dissolution. Silica saturation of the eluent can be achieved with a lesser grade material.

Figure 3.4 demonstrates that a better separation can achieved with а tartrate eluent using the be silica-based exchanger over the resin SCX materials shown in Figure 3.2. Whatman silica SCX generally performed better than the resin. A comparison of the silica and resin-based exchangers shows the advantages of using a lower capacity column. The resin-based Aminex capacity than the Dionex or Α9 has а greater silica-based Whatman Partisil 10 SCX materials. The Partisil 10 SCX gave the best separations especially for the lactate eluent. Although resin-based materials can be cleaned by a recommended procedure using strong acid and alkali they were not so physically durable as the silica-based SCX columns. Without a guard column the Whatman SCX column could endure three weeks continuous use with a lactate eluent. The resin-based material showed signs of compaction within the column making the impracticable. longer column lengths А use of microscopic examination revealed bead fragmentation had

occurred and was accompanied by higher column pressure gradients. Radially striated columns may stabilise the resin packing and reduce the physical stress on resin materials.

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Resins such as the highly commended Japanese materials used by Takata(65) may become available, but present studies reveal that lightly sulphonated resin beads used at low pressure gradients with weaker chelating agents offer the most promise.

Separation is dependant upon the varying relative attractions of the mobile and stationary phases. For ion exchange chromatography the sites of opposite charge are in competition for the metal ion with the anion that metal analyte is also in forms а complex. The competition with the positively charged counter-ion. The degree of complexation is dependant upon the metal constant values and the anionic ligand stability concentration. A concentration increase of the organic acid will give a shorter retention time for a SCX but elution time is not solely related to the column. organic acid present. An increase in pH increases the degree to which the organic acid is ionised and a larger anionic content results in a greater degree of complexation. To accurately assess the effect of organic acid anion concentration computer programs were developed, to calculate the anionic concentration for mono-, di- and tribasic organic acids at a stated

initial organic acid concentration and varying pH. Varying concentrations of anionic species can be immediately calculated and any correlation with the chromatography assessed.

Citric acid is the only trivalent carboxylic acid used stronger complexes with most metals, and forms ferric ion. The strength of the especially the complexing ability is reflected in the change of elution order when a comparison is made with sodium sulphate or a weak organic acid eluent. Poor separation was shown in Figure 3.1 with the resin cation exchanger, possibly because a lesser chromatographic abilities of citrate for the metals. Zinc has an earlier elution at higher pH between 3.7 and 4.3, suggesting at that critical concentration, a different complex stoichiometry may account for this erratic behaviour. The form and Partisil 10 SCX column in Figure 3.2 shows the best resolution of peaks using a 0.04M citrate eluent.

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The oxalate anion forms weaker complexes than the citrate anion, separating more metals with the silica SCX column. Although greater selectivity was demonstrated, the later peaks eluted more slowly suggesting gradient elution may be necessary for faster elution. Preliminary investigations suggest that it may offer novel separations at higher concentrations.

Tartrate elution separates copper, zinc, nickel, cobalt and cadmium well, but it was difficult to

separate lead from nickel and changing the pH or the tartrate concentration did not improve the situation. Tartrate elution also led to late eluting peaks for manganese and magnesium. However the complexing power of the tartrate eluent proved to be the best for the A9 SCX resin.

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Several generalisations can be made about the order of elution and separation regarding K_1 , shown in appendix 1. Primarily K_1 should not exceed 7 and elution order generally follows a decreasing value of K_1 . The greater the magnitude of the difference between K_1 for each metal the better the separation. Although lactic acid has smaller K_1 values than most it has the best separations for this reason. Generally the trivalent metal ions, of bismuth, indium and iron elute first (demonstrating that as aqueous ions there is a greater attraction to complex rather than attach to the cation exchange sites) and copper between them. Zinc nickel and lead are then followed by cobalt, cadmium, iron, manganese and magnesium although not necessarily in that order.

Other organic acid eluents were scanned. Succinic acid was found to elute indium(III) and bismuth(III) late, and well separated from other metals. Maleic acid eluted copper earlier followed by an unseparated mass of transition metals.

Weaker acids are used with the silica-based SCX

column, possibly because of the lower capacity. The lactate eluent was the most efficient mobile phase of acids, separating, in elution order, copper, the iron(III), indium(III), zinc, nickel, cobalt, cadmium, magnesium. Table 3.8 shows how the manganese and concentration was used to obtain an order of separation with the fine adjustment being made by pH change. However care has to be taken in identifying the peaks of lead, nickel and zinc from one another as well as co-workers and have Hwang cadmium from iron. demonstrated that the lactate eluent performs better than the closely related HIBA eluent(69). A Vydac column which is a superficially porous SCX resin was also demonstrated with a 0.12M lactate eluent.

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Changing physical effects upon the column were investigated. Raising the temperature was the most influential for both columns to give more Guassian shaped peaks as opposed to tailing peaks. Only iron(III) persisted in demonstrating a broad based tailing peak, especially with a lactate eluent. Iron(III) forms greater than 1 % hydroxo complexes above pH l and this may inhibit it passage through the column bed.

Increased concentrations of counter-ion were found to improve the efficiency, with quicker elution and consequently sharper peaks which could not be performed with increasing pH or decreased chelating agent concentration. The effect on the column of a salt eluent

was not enough alone to separate metal ions. Sodium chloride generally decreased retention times, having a greater effect on cadmium, eluting relatively earlier, which may be the result of a stronger complex formation Therefore column anion(65). with the chloride efficiencies may be increased by salt addition, but heed must be paid to the corrosive ability of sodium chloride advantages. An all plastic HPLC although it offers system may allow sodium chloride and hydrochloric acid to be used with a high pressure anion exchange column, thus taking advantage of previous successes with the chloride anion.

The temperature was raised whilst an ethanolic eluent was used, this countered the poor induced column efficiency and suggested that the effect of adding an alcoholic eluent was to reduce the kinetic rate of exchange for the column processes(110), which is the rate limiting step(112). Iron(III) was found to elute nearer to copper as a result of adding alcohol to the eluent.

Different chelating agents need to be explored to the advantages of high efficiencies fully exploit obtainable with the smaller particle sized columns which will become available. The carboxylic acid moiety in an eluting agent offers a basis and the additional adjacent of improving existing moieties means may be а from investigating differently Apart separations.

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structured organic acids those with different elements adjacent positions to the carboxylic acid were in investigated. A radical change in the chelating agents structure to a completely different molecule such as nitrilo-triacetic acid was found to give little or no stability constants(K,) with separation. Small partially-ionised chelating groups that are sensitive to adjustment offer the best alternatives. Careful pН consideration should be paid to the choice of acid regarding the magnitude and difference in magnitude of stability constants which implies any possible the success as an eluent.

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Thiomalic acid which has sulphophilic tendencies gave highly efficient separations and virtually reversed the elution order expected of a carboxylic acid. This eluent can offer a slightly reducing environment and may be able to separate the 'soft acid' metal ions. The successful separation of cadmium, zinc, nickel and copper suggests that further studies with thiomalic acid are necessary, possibly with anionic and neutral columns.

The simplest amino-acid, glycine, has like most amino-acids, a cationic ammonium group on the same molecule and offers new separation possibilities with the ammonium group and anionic carboxylate ion being directly opposed in chromatography effect, although within the same molecule. Elution of metal ions becomes

slower with increasing pH, which indicates a greater affinity for the column. There are two possible explanations, one where the attraction by the anionic component of the eluent appears to be dominant, and secondly where the ammonium molety of the glycine molecule is attracted to the sulphonate functional group whilst blocking the metal for that site.

Aspartic acid which is a dibasic amino-acid akin to succinic acid, causes better resolving power with broad peaks for the later eluting cobalt, iron and manganese.

It may be possible that the complexing abilities of many organic acids confuse the attempted separation by the SCX column because of preferential or differing complexing strength.

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The requirements of cation HPLIEC have recently focused on low capacity columns using an organic chelating acid eluent within a concentration of range 0.01 to 0.2M. Chromatography can only be improved with the advancement of column materials in conjunction with better knowledge of functional groups available. Many functional groups have been investigated but none seem capable of surpassing the capability of the sulphonate moiety, used with a chelating organic acid.

Although combined eluents were considered, chromatograms with the tartrate and lactate eluents allow isocratic elution to prevail giving steady conditions to test the PC reactants.

4 Eriochrome Black T Post Column Detector.

4.1 Introduction.

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reporting upon EBT and dithizone it is Before The outline why they were chosen. necessary to advantages of PCR systems have been outlined in chapter one. Many of the metal chromatography systems employing PC reagents use a wavelength which is not necessarily gives the greatest response for a the one that particular group of eluted metals. This is because absorb at different metal-complexes different wavelengths. A compromise may exclude some metals and give a wide range of sensitivities for the metals in an injected sample.

The problem required a new approach, if we are to take wavelength. The using one full advantage of metallochromic indicator molecule can be observed at one wavelength and a decrease of the uncomplexed molecule occurs when the metal is being complexed. Therefore the decrease of the absorbance of the indicator should be proportional to the concentration of the metal. This can with this detector by measuring the performed be absorbance decrease at the wavelength at which the metallochromic indicator absorbs. In this work with PCRs it is hoped to prove that the metal ion concentration is proportional to the decrease in absorbance of the

indicator. There are restricting qualifications placed on the choice of a reagent which must fulfil certain if the capability of the spectral conditions chromatographic detection is to be improved by using this inverse photometric method. Firstly the reagent must complex with metals to be determined. Secondly the molecule must absorb strongly at low reagent concentrations, and thirdly it must absorb at a wavelength that does not overlap too much with the spectra any of the analytes or any possible of interfering eluted species.

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There are a number of organic reagents that fit the requirements. Eriochrome Black T was chosen because it chelates through nitrogen and oxygen and therefore reacts with a wider range of metals. Similarly dithizone was chosen and is discussed in chapter five, dithizone reacts with the more sulphophilic metals and shows a highly sensitive photometric response to those metals, such as mercury and cadmium which have a high toxicity.

4.2 Properties of Eriochrome Black T.

Eriochrome Black T is sodium 1-(1-hydroxy-2-naphthylazo)-6-nitro-2-naphthol-4-sulphon--ate also known as Solochrome black, Mordant black II and C.I.No.14645. It is a brownish-black slightly granular solid that dissolves rapidly in mildly alkaline

solutions and slowly in water to give an ultramarine blue coloured solution. It can be used as a metallochromic or acid-base indicator(115).

the most commonly used of the It is of one dihydroxyazo dyes, mostly as a metallochromic indicator EDTA titrations. Eriochrome Black T can be for represented as H,D below pH 6.5, where D represents the neutral EBT molecule and H subscript indicates the number of ionisable protons attached to the molecule. The sulphonic group is ionised before pH 7. The pKa values of the two phenolic groups are 6.3 and 11.5 respectively(116). The two colour changes occur at pH 6.5 (wine-red to blue) and blue to orange at pH 11.5(116).

The addition of a reactive metal gives a wine-red colour by displacement of a proton, consequently the pH has to between 7 and 11, to give the reagent a contrary blue colour.

4.3 Response with Metals.

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Eriochrome Black T has been reported to react with numerous metals, these are aluminium(III), cadmium, cobalt, copper, calcium, gallium(III), indium(III), iron, iron(III), lanthanoids, lead, magnesium, manganese, mercury, titanium(IV), zinc and the platinum metals(115,116). Foster Dee Snell(89) reports that EBT

also reacts with molybdenum, the rare earths, thorium, tungsten, uranium and vanadium.

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Eriochrome Black T was first used by Schwarzenbach and Biedermann(117,118) as a metallochromic indicator for EDTA titrations for and calcium magnesium Several other metals give virtually determinations. identical colour responses. Cadmium, manganese, lead and zinc may also be titrated. Schwarzenbach quotes the mole ratios of 1:1 for magnesium and calcium and a 1:2 ratio zinc, some authors suggest otherwise(119,120). for Aluminium(III), copper and nickel form such strong complexes that they inhibit the indicator action.

Eriochrome Black T forms a 1:2:2 ternary complex with rare earths and diphenyl guanidine respectively, in an acetate buffer(89).

Eriochrome Black T is also sensitive to oxidation in conditions, manganese(IV) alkaline and cerium(IV) oxidise EBT under these conditions, resulting in rapid discolouration of the reagent. A solution of the dye has to be made immediately before use because it degrades in acid and alkali. In strongly acidic conditions the dye polymerises to a red-brown product and is rarely used below pH 6.5. Calmagite, a more stable analogue of EBT is considered to be an alternative to EBT, but is not regarded superior to EBT for titrimetric as purposes(90).



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Figure 4.2

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4.4 Eriochrome Black T as a Post Column Reactant.

To assess a PC reactant it was necessary to perform elementary experiments to obtain the best wavelength and solution conditions. A large hypsochromic effect is the best for observing a decrease in absorbance, although only spectral measurement will reveal if there is considerable residual absorption at the indicator absorbance peak maximum, which could affect the sensitivity or linearity of response. Greater responses were obtained at higher EBT concentrations although deterioration of the absorbance occurred with time. Metal-EBT complex spectra were plotted for the soluble metal salts in a solution buffered to between pH 9 and 10.5 with ammonia, those of copper, magnesium and iron are shown in Figures 4.1 to 4.3 respectively. Other spectra were recorded showing decreases at 610 nm with little absorbance for the metal-EBT complex and were similar to those in Figures 4.1.to 4.3. Figures 4.1 to 4.3 the extremes of spectral absorbance for show differing final concentrations of metal ions, from the relatively highly absorbing metal-EBT species such as those obtained with magnesium and ferrous ions to the virtually non-absorbing copper-EBT complex.

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The peak maxima for metal-EBT complexes are shown in Table 4.1 along with the observed decrease in absorbance at 610 nm for each metal for 0.1 μ g ml⁻¹. Lower

decreases would be observed for those metals with larger relative molecular masses.

Table 4.1

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١	Wavelength	Max	cima	of	Metal	Eriochrome	Black	T
Cor	mplexes.							
ł	Metal	Wave	elengtl	h	Response 0.1 µg ml	ef		
		- /	nm		/ absorban	ce at 610 nm		
	Aluminium(I)	[])	573		0.13			
(Copper	-	524		0.10			
-	Iron (III)		450		0.07			
-	Iron		530		0.12			
(Cobalt		576		0.09			
(Cadmium		470		0.03			
1	Lead		550		0.02			
2	Zinc		556		0.04			
1	Nickel		580		0.06			
1	Manganese		555		0.06			
1	Magnesium		558		0.12			

Eriochrome Black T reacts quickly with the above metals except aluminium(III). Many of the metal-complex absorption peaks recorded very little positive increase from the baseline and some none at all. Not only were responses recorded at 610 nm, but it was linear considerably more sensitive for a number of metals than measuring a wavelength increase as shown by the responses for 0.1 $\mu g g^{-1}$ of metal. Although slightly better negative responses could be obtained from 620 to 650 nm it was decided not to use absorbance differences resulting from the side of the peak but to observe the absorbance that should give better difference in precision for PCR detection. Consequently the difference was observed between the EBT maximum at 610 nm which has

a broad peak top and the background absorbance. The EBT spectra is overlapped a little with that of the metal-EBT complex at the peak maxima of EBT at 610 nm but the absorbance was much less. The partially absorbing metal-EBT complexes may account for any discrepancies in a calibration graph, but this appears not to be so and suggests that linear responses could be obtained for an EBT PC reactant.

Eriochrome Black T was investigated initially as a reagent in a flow injection analysis(FIA) system without the chromatographic column. Then a column was introduced and a comparison of responses at different wavelengths was performed, positive responses had to be recorded at shorter wavelengths. These were performed with the same column conditions and the responses are shown in Figures.4.4 to 4.8. Figure 4.4 and 4.5 show the minimal response at 420 and 460 nm. Comparisons of responses for metals after separation of a 100 μ l of 4 μ g g⁻¹ of copper(4.07 min), Fe (III)(5.67 min), indium (7.30 min), zinc(8.14 min) nickel(9.14 min), cobalt(11.40 min), cadmium(13.07 min) and manganese(15.60 min) are shown. Separation was performed using a 0.1M lactate eluent at

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pH 3.7 with a Partisil 10 SCX column 25 by 0.46 cm, preceded by a 3 by 0.46 cm guard column also packed with Partisil 10 SCX, both columns were at 40 $^{\circ}$ C. Detection was with EBT as a PC reactant in a 0.4M ammonia solution and a F.S.D. of 0.1 absorbance for those wavelengths

less than 610 nm and 0.2 absorbance at 610 nm. The same quantities of cations were injected for detection as shown in Figures 4.6 and 4.7 by positive absorbance at 500 and 540 nm. Figure 4.8 clearly demonstrates the advantages of observing a decrease in absorbance at 610 nm. The increase in response at 610 nm was attenuated by 2 for purposes of chart measurement. The response at 610 nm was double the increase over that obtained at 500 nm which gave the best response with positive absorbance(Figure 4.6).

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Figure 4.4 <u>Chromatogram with</u> EBT Detection at 420 nm

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Figure 4.5 <u>Chromatogram with</u> EBT Detection at 460 nm

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Figure 4.6 <u>Chromatogram with</u> EBT Detection at 500 nm

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4.5 Solvent and Instrumental Considerations.

No special attention for EBT PC reactant was necessary, except for daily preparation and ensuring that the glassware and pumping systems were relatively clear of metal contamination. An advantage of measuring a decrease of absorbance in the a PC reactant is that constant backgrounds of metals in the system can be tolerated, this may solve difficult contamination problems when applications of the detector are being considered.

A spectral scan of EBT with an associated eluent reveals that for organic acids a peak maximum of 610 nm is at an optimum absorbance in the pH range of 10 to 10.5.

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When observing a decrease in absorbance for metal the baseline was very sensitive to pump detection pulsations and to a lesser extent other fluctuations temperature and shot noise from the from photomultiplier. There are three general approaches to counter the largest source of noise which are associated with systematic HPLC pump pulsations. For each method it is necessary to consider which is the best pump to be conjunction with each approach. Several used in practical methods of tackling this problem were adopted.

i)The possibilities of using the PCR tubing as a pulse dampener was investigated. A 10 m length of 0.8 mm I.D. tubing before and after the T junction mixer had little impact, except to broaden the peaks when replacing a shorter length PCR.

Experiments with a buffer volume of air of 2 to 10 ml adjacent to the PCR tubing with a pressure restriction placed on the tubing did not give a quick enough response to pressure changes from the pump pulsations.

The reagent pump has very little back pressure and a Waters 500 in-line pressure restrictor accessory was the most effective of those considered. However, in an effort to reduce this further, various pumps were examined for pulsations. The Eldex model AA pump gave the least noise even though the coloured reagent pulses could be seen directly after the mixing T junction. The. quick pulses seem to escape the detection although the PCR emitted the effluent in spurts.

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ii)Electronically placing a choke device between the the recorder computer in the wire detector and connection. This approach appears the easiest, however it would be necessary to quench complex impedances resulting from placing a choke in series with the impedances require detector signal output, such considerable electronic expertise to counter.

signal can be electronically or computer iii)The manipulated to cancel baseline noise. Hydrodynamic pulse dampening requires large volumes and as it is necessary to minimise preinjection volume, Fourier transform analysis of the detector signal appears a better option. transforms a complex signal of Fourier analysis waveshapes which by nature are systematic, from the time to the frequency domain giving a resulting graph of versus frequency. The waveforms may be amplitude represented as a series of simple harmonic functions, the frequencies are integral multiples of the original signal. Once the major higher interfering frequencies are defined then they can be eliminated by computer or by filtering the signal electronically.

A paper by Weber(121) describes how to minimise flow associated noise in electrochemical detectors for HPLC. Detectors using PCR systems are sensitive to flow rate changes of an erratic or systematic nature. Fourier transforms have been performed using Fortran on a main frame computer with a fast routine technique(122).

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Another approach was to do a round averaging calculation in which a running average was carried through, where for example point 3 is the average of point 2,3, and 4. However there was a major drawback regarding the microprocessor here, which was that data points from the chromatogram could only be collected at 15 points \min^{-1} and this gave a vague and erratic

chromatographic reproduction on retrieval from the floppy disc. A better computing integrator may also reduce the periodic noise.

A PCR coil has an increasing noise reduction effect with increasing length. A typical effect is illustrated in Table 4.2 for a Aminex A9 SCX column which operated at a low pressure gradient of 400 psi and constant flow rate of 2 ml min⁻¹ with a constant PC reactant flow rate of 0.7ml min⁻¹.

Table.4.2

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Noise Recorded for Varying Post Column Reactor Lengths.

Tubing length	Noise			
/ m	/ mm			
0.5	3.5			
1.0	1.2			
1.5	0.4			
2.0	0.3			

There are several ways of overcoming noise and perhaps the best would be in the design of the pump, although for many of the pumps operating at low back pressure, incorporated feedback loops were ineffective and specially designed PCR systems may be necessary.

For reactions that take time, flow-splitting has been suggested, this approach could also be used as a controlled leak which may allow pulses to be contained. Splitting the PCR effluent and passing one stream through the reference cell of a double beam instrument may considerably reduce the noise(123). However to date the best and quickest approach is the one adopted in this thesis, using an Eldex pump and a pressure restrictor. For future development Fritz's(59) approach which uses air pressure to supply the PC reagent may work for low level detection as this has no pulsing input, however it may be prone to baseline instability. A pressurised container which allows the reactant to diffuse through a membrane into the column effluent may also stabilise the baseline noise.

The nature of reverse mode photometric detection makes baseline noise a considerable problem for highly sensitive work, but this should be quickly overcome with the advent of better pumps and PC delivery systems.

4.6 Optimisation Data.

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are numerous methods available to optimise There instrumental methods in chemical analysis, some, such as simplex optimisation(19) are mathematical. This method developed for organic reaction optimisation to was produce the maximum yield from a set of reactants. This technique depends upon a geometrical representation of the system under study. Performance results are used and projected parameters are tested. The new parameters the projected on the basis of the cumulative expression are the cycle is repeated until there is no advantage to and gained from continuing. This technique was considered be

and rejected here because initial investigation revealed that the response for most metals was not critically dependant upon any of the PCR parameters. Also difficulty would be encountered in obtaining numerous pieces of varying lengths of tubing. To fully evaluate a detection technique requires that it should be operated at the extremes of operation. Consequently a set of considered parameters were assessed for each eluting acid, these were:

> i.PCR residence time; ii. PCR temperature; iii. the pH range; iv. concentration of the PC reactant, expressed as absorbance; v. the calibration range; vi. as a final coordinated check, PC reactant flow rate.

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The strategy was to test them in the above sequence and early data showed that PCR time effects upon the response had to be considered. The pH range of the PC effluent was studied. Although a rough estimate of the middle of the range was used for the reaction studies, it is known that temperature can influence the pH. Consequently the observed pH for the cooled PCR effluent

was not necessarily the one in the PCR system and the value was only used as a means of obtaining the optimised condition for pH. The concentration of EBT that gave a PC effluent absorbance range of between 0.2 and 1.4 absorbance was investigated by measuring the PCR effluent absorbance at 610 nm for the preceding optimisation studies. This was followed by a check on the response by changing the PC reactant flow rate which simultaneously affects most of the variables that contribute to the optimisation.

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4.7 Responses for a Citrate Eluent.

The maximum interference for the formation of the metal-EBT complex was found for a high concentration of citrate, which was used as an eluent with the Aminex A9 SCX column. Lower capacity columns would not perform the separations at a 0.2M concentration with such a strong chelating agent. This strong complexing agent does not separate many metals and two chromatographic runs were necessary, one for copper, nickel and zinc; the other for cobalt and iron(III).

4.8 Results with a Citrate Eluent.

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The graphs of response calculated from peak area, against varying conditions of; pH, PCR residence time, EBT concentration as absorbance, PCR temperature and finally the calibration are presented in Figures 4.9-18.

Table 4.3 summarises the best conditions for each metal. Aliquots of a 100 μ l containing 2 μ g ml⁻¹ of iron(III) and cobalt in one and copper, nickel and 10 μ g ml⁻¹ of zinc in the other were injected.

A plot of PC reactant flow rate versus response confirms the data obtained from changing one parameter and any deviations from the expected can be further assessed by comparison of graphs of subsequent decrease in metal eluate concentration with increasing EBT concentration obtained with increased PC reactant flow rate as in Figure 4.19-20. The nickel response to PC reactant flow rate is not presented here as there was no change of response.

Linear responses using peak area were plotted for all the metals examined in Figures 4.16-18, however no limits of detection were calculated. In practice little difference was observed between recording response in terms of peak area or peak height.

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EBT Detection with a Citrate Eluent

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Figure 4.18

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Table 4.3

Optimum conditions for Eriochrome Black T PC Reactant with Citrate Eluent.

Parameter

	PCR time	Effluent pH	EBT* conc	PCR temp	PC reactant flow rate
metal	/ min			∕°c	$/ ml min^{-1}$
Copper	0.2	9.5-10.5	>0.4	30-50	>2.0
Iron(III)	0.2	9.5-11	>0.7	30-50	1.2
Cobalt	>0.1	9.8-10.3	>0.7	<10	1-1.75
Zinc	0.2	10.5-10.7	>0.7	<10	>1
Nickel	>0.2	10.4-11	>1.4	>80	>0.5

* as absorbance.

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<u>4.9 Discussion of Optimisation Data with the Citrate</u> Eluent.

Before discussing the data there are some important points that emerge from the studies that have already been performed on ligand exchange reactions. These have to be considered here because the metal ion is involved in an exchange between the resultant eluting organic acid, the hydroxide ion complex or complexes and the PC reactant. The exchange rates for the hydroxy ligand are considered fast for all metals eluted. Larger ions exchange hydroxyl groups more quickly. Lesser charged ions also have a higher exchange rate. There are numerous factors to be considered for the rate of a reaction for a metal-complex(124). Excluding the effect of catalysis and temperature, the most important factors are:

i. the nature of the central metal ion;
ii. the nature of the leaving ligand;
iii.the nature of the entering ligand;
iv. the nature of other ligands attached to the central metal ion;

v. the charge on the complex;

vi. the nature of the solvent;

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vii. the presence of other metal ions or ligands in the solvent.

Some difficulty was encountered with nickel for the dithizone PC reactant, this is discussed in chapter five, however it was thought that an ammonium buffered eluent would quicken the reaction rate for most metals, as was the case for the dithizone PC reactant, and a respective (3+2) 2M ammonium and sodium hydroxide mixture for pH adjustment of the organic acid was used. The same mixture was used for the EBT detector for comparison.

Before the discussion of each metal response it is necessary to mention some general trends. Figure 4.4 to 4.8 show that EBT has an absorbance maximum at 610 nm between pH 8 and 11. The chromatographic response does not necessarily reflect the true response, that would be better obtained on а FIA system, because the chromatographic separation gives broader peaks as

retention time increases, which means poorer sensitivities for the later eluting metals.

The temperature change gave a gentle response curve for copper and iron(III) with a maximum value in the range 30 to 50 °C. Nickel, cobalt and zinc gave a linear response with either a negative or positive slope with temperature as shown in Figure 4.9. It is difficult to draw conclusions about kinetic and thermodynamic reaction parameters as they are interactive with PCR temperature, pH and residence time, and as the study of them is not the intention of this work only comparisons will be made with other PCR systems.

Figure 4.10 shows an increase of response for copper with an increased PC reactant flow rate, which dilutes the citrate concentration and lessens the strength of the complex, whilst increasing the EBT concentration as shown in Figure 4.19. However there comes a point where the analyte is diluted considerably and the dilution effect no longer aids response as in the case for iron(III) in Figure 4.10. Zinc response shows a slight interference and then the signal diminishes with increased PC reactant flow rate. Copper and cobalt were not plotted as they gave a similar curve to zinc.

Figure 4.11 shows the variable response with differing EBT absorbances of the PCR effluent. Cobalt, nickel and zinc show curves that peak to a plateau, this suggests the possible formation of higher complexes, which are

known to occur for zinc. The ferric ion gives variable response at different EBT concentrations (although not plotted). This was also the case for the PCR residence time response curve for iron(III) suggesting that multiple complex formation may occur. However for a set of given conditions a linear response curve was recorded for iron(III) as in Figure 4.16.

Figure 4.12 shows a very slight increase for cobalt response concerning PCR time and the expected decrease in response associated with an increase in PC reactant flow rate confirms this. A negative linear response for cobalt is shown in Figure 4.9 against temperature where a half response was achieved over 86 °C, however it is difficult to draw any thermodynamic conclusions.

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Zinc similarly has a fast response as in Figure 4.12 this is evident in Figure 4.10 where the increased PC reactant flow rate revealed the expected decrease in response at greater PC reactant flow rates. Figure 4.20 shows the expected decrease of analyte concentration which should be consistent with response for no interference. An increased response shown in Figure 4.9 by a factor of two occurs with 54 °C lowering of temperature.

Nickel response increases considerably with temperature as in Figure 4.9, compared to other metals investigated. The response to nickel was increased by a factor of four over a 45 ^oC rise, also an unusual 3 times greater response was recorded at pH 10.6 over pH 10 as in Figure 4.13. Cobalt and nickel show a better response as in Figure 4.14 at pH 10 to 10.5. Copper and iron(III) show in Figure 4.15 an extended optimisation range to pH lower than 10.

conditions the calibration curves in At optimum Figures 4.15-18 confirm that quantitative data can be obtained for 100 µl injections of the above mentioned rough observation of equal molar metal ions. Α reactivity is shown given that some variation should be expected for ions that have differing retention times. This is with the exception of iron(III) which gave a larger response and again, suggests the formation of a higher complex. An advantage of reverse mode photometric detection is that the upper range of detection is not so as in the positive increase of absorbance limited because higher concentrations of EBT could be used, to for small absorbances more accurate measure determinations.

4.10 Metal Responses for a Lactate Eluent.

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Lactic acid is a much weaker complexing agent, but has been shown to be the best eluent separating more metals as shown in chapter three and publications by the author(125,126 see appendix 2). The same PCR investigative technique mentioned above was adopted for

detection with this eluent. The metals cadmium, cobalt, copper, iron(III), lead, magnesium, manganese and zinc are all separated with 0.1M lactate on a Partisil 10 SCX column in one injection.

4.11 Results and Discussion.

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Table 4.4 records the optimum conditions for metals, calcium elutes after magnesium, but the response was approximately 20 times less and was not considered here trace analysis by HPLIEC. Indium and iron(II) for co-elute with iron(III) and cadmium respectively, but the data for the calibration curves (Figures 4.25 to injections. The 4.27) was recorded in separate co-eluting pairs mentioned above require a lower concentration of lactate for separation and further response data for them was therefore not considered for this reason. Nine metals should give sufficient data to optimise the detector as the overall best response was the objective of the work. Only those responses that are different or representative of a set of metals are graphically illustrated here.



Figure 4.21



Figure 4.22



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Figure 4.26

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Table 4.4

Optimum	n Res	sponse	Conditions	for	Lactate	effluent	with	the	Eriochrome
Black T P	ost (Column	Reactor.						

rarameter	PCR	Effluent pH	.EBT* absorbance	PCR temp	PC reactant flow rate
metal	/ min	·		/ ° c	/ ml min. ⁻¹
Cadmium	>0.1	9.5-11	>0.2	50-70	1-1.4
Cobalt	>0.3	8-10.5	>0.5	40-70	0.5-1.2
Copper	>0.1	8-10	>0.2	0-80	0.5-2.0
Iron(III)	>0.1	8-10	>0.4	30-70	>0.5
Lead	0.3	9.5-10.5	>0.4	0-10	0.5-1.5
Magnesium	0.25-0.4	10-11	>0.8	20-60	1.5-2
Manganese	0.3-0.4	8.5-10	>0.4	40-70	1.3-1.7
Nickel	<0.3	8.10.5	>1	<40	1-1.8
Zinc	0.05	8-10	>0.9	<10	0.7-1.3

* measured as absorbance.

Cadmium, iron(III) and copper show virtually no response to an EBT concentration increase as in Figure 4.21, suggesting a different reaction mechanism or stoichiometry for those metals i.e. that more than one higher order complex may be formed.

Only a high pH appears to inhibit the copper response in Figure 4.22, which is similar to that of iron(III) and zinc pH response curve. Nickel, cobalt and manganese show a gentle curvature optimising around pH 9 to 10. Magnesium and lead show in Figure 4.22 more acute curvatures, as does manganese. Only cadmium has an increase above pH 11.

Nickel, zinc and lead show a decrease of response with temperature in Figure 4.23. Only copper shows no effect of response with temperature. Other metals give a variation of response with an optimum temperature value.

By comparing response plots for different parameters general trends for each metal can be ascertained. The response of cobalt was quick with a relatively lower dependence on EBT concentration and better responses were obtained with PCR residence times greater than 0.3 minutes. A negative slope against temperature was observed for those metals in Figure 4.23, indicating either degradation of EBT, or a reaction which has preference at higher temperatures, however for most metals a gentle curve showing an optimum at 40 to 70 °C or a linear response was observed.

With the lactate eluent only a 20 % change occurred for response with differing reactor response times from 0.1 to 0.4 minutes, with the exception of nickel which fell by 50 % from 0.3 to 0.4 minutes.

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The majority of the graphs of response versus PC reactant flow rate show an increase due to the EBT concentration increase followed by a decrease as the metal eluate is diluted according to Figure 4.20. The optimum for most metals occurs around 1.5 ml.min^{-1} , with the exception of those shown in Figure 4.24. Copper reacts readily with no parameter causing a noteable change of response, however the PC reactant flow rate causes a slight decrease in response for copper and the ferric ion.

Another factor to be considered is the ratio of PC

reagent to the column eluent flow rate and its effect on the detector performance. Increasing the PC reagent will dilute the organic acid eluent and so reduce any limiting effect by the organic acid on the metal-EBT response. If the percentage addition becomes too large this beneficial effect will be offset by increased dilution of the metal peaks eluting from the column, countering effects are show in Figure 4.24.

Although a high EBT concentration is preferred, too concentration gives high an EBT а baseline absorbance(say above 1.5 absorbance) that would result in very low intensity radiation falling on the photocell and consequently an increased baseline noise and poorer sensitivity as outlined in chapter one. However, before this baseline absorbance was reached a more serious noise problem arose from pump pulsations. This showed itself as a complex sine wave superimposed on the baseline. becoming particularly evident above 0.9 absorbance.

If high sensitivities are required they could be achieved by reducing EBT concentration. This was indeed found to be the case for most metals down to a baseline absorbance of 0.2. Below this absorbance, linearity and detection limits start to deteriorate as metal response decreased.

A possible explanation is that if the EBT concentration is too low then the lactate will begin to

effectively for the metal ion, thus compete more reducing the sensitivity of the EBT reaction, even though the stability constants of metal-EBT complexes are much greater. In many respects an increasing EBT concentration in the PCR system demonstrates a similar response to that obtained in the Methods of Mole Ratios, ligand concentration is increased with where the constant metal ion concentration to give a coloured complex, this is used to graphically determine solution metal ion and a complexing equilibria between а ligand(127). However for a PCR system a time limit is placed upon the response and consequently no conclusive constants can be obtained, although a well defined edge a plateau indicating complex formation shows a to strongly formed complex and an apparent variation in this region at the plateau edge indicates a higher complexes may be formed with other species absorbing in the same region. Here the implication is the same even though a reverse photometric mode at 610 nm was used.

As Table 4.4 shows the average optimum conditions for the metals was 0.3 min retention time; a PCR at 50 $^{\circ}$ C; a pH between 10 to 10.5; a PC reactant flow rate of 1.5 ml min⁻¹ and an EBT PC reactant absorbance of 0.5 to 0.9 at 610 nm. These conditions were used to determine the sensitivity and calibration curve of each metal. Calibration curves were obtained for the metals mentioned. Only cadmium and cobalt gave slightly curved

responses, which cannot be explained in terms of overlapping spectral peaks as their metal-EBT spectra show low absorbance compared to other metals such as that of zinc. The limits of detection are shown in Table 4.5.

Table 4.5

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Metal	Limits of Detection / ng
Indium	5.8
Copper	6.5
Zinc	3.1
Ferric	5.1
Nickel	4.6
Lead	5.1
Cobalt	1.2
Ferrous	3.7
Cadmium	5.1
Manganese	0.7
Magnesium	2.2

The limits of detection were calculated as two standard deviations. The silica SCX column was new and the limits quoted are approaching the best available, given the current difficulties of pump pulsation noise.

4.12 Conclusion.

The objective was to develop a sensitive HPLC detector for transition metals with linear calibration curves. This was achieved for various eluents with lactate being the best. The optimisation data for the lactate EBT PCR system are shown in Table 4.4. Indium and iron(II), although not optimised gave good linear ranges at the optimum conditions of the other metals bringing the total number determinable to eleven. Although limits of detection were not calculated for citrate eluent, calibration curves in Figures 4.16 to 4.18 were plotted. Radical changes of response were not found for this method measuring a decrease in absorbance so extending the range of operation. Quantitative detection has therefore proved effective for both strong and weak chelating eluents.

The EBT PC reactant has been shown to be sensitive from pH 9.8 to 10.8 without a significant decrease in the EBT absorbance at 610 nm over this pH range. To responses for greater metal eluate obtain EBT are concentrations, higher concentrations of necessary. High absorbances however gave greater signal to noise ratio, although it was not sufficient to counter the accuracy and the expected decrease of the coefficient of variation was obtained.

Lactate was the best eluent with linear calibrations from 10 μ g ml⁻¹to 5 ng ml⁻¹ for cadmium, cobalt, copper, indium, iron(III), iron, lead, magnesium, manganese, nickel and zinc. Limits of detection were in the range of 0.5 to 5 ng and as expected appear dependant upon the speed of elution, because rapidly eluted peaks have a smaller peak width.

In chapter six applications of the lactate EBT PCR

system show the determination of cobalt, copper, iron, manganese and nickel in two standard alloys. Together with ng ml⁻¹ concentrations in estuarine environmental samples and simulated primary coolant of a pressurised water nuclear reactor.

In practical terms the EBT PCR was simple to set up and very stable in operation. Eriochrome Black T does deteriorate slowly in solution due to oxidation, but preparation of the PC reactant solution was daily sufficient to maintain detector response. Calmagite, an analogue of EBT without the nitro group is much more stable and could possibly be used in its place where long term stability is required, although initial trials showed that by observing a decrease in response it has approximately half the sensitivity of EBT for the better responses of cobalt and nickel. A comparison with a PAR an increase in the where PCR detection system, wavelength was observed at 540 nm shows that although good responses are achieved for nickel and cobalt on a lower optical F.S.D. setting because of less baseline noise, there was a four fold sensitivity improvement with EBT. Other metals show a considerably greater improvement.

The quantitative performance of the EBT PCR system has been shown to be sensitive and easy to use, for the determination of trace metals, proving that the determination of metal eluate by monitoring the decrease

in absorbance of the reagent is a useful technique and could be of more general application with other water indicators(128) metallochromic or by soluble especially for PCR detection. A synthesising some particular advantage of this inverse photometric system metals which react with the same is that a11 stoichiometry will give approximately the same molar response, if the PCR parameters of each metal are similar and most are, with the exception of aluminium, which is slow to react. The EBT PCR system should be capable of determining more metal species than those investigated including aluminium(III), titanium(I) and the platinum metals. Naturally this will depend on the development of other chromatographic systems.

A disadvantage of this detection system was that increased baseline noise, tended to amplify pump pulsations. However this noise could be further reduced by using better pulse free non-reciprocating pumps, or very fast reciprocating pumps, such as the Eldex type, where the frequency of the pulse is too fast for detection.

5 The Dithizone Post Column Reactor System.

5.1 Introduction.

Of the many organometallic reagents dithizone is one of the most used and well documented(129-131). The reactions and properties of dithizone and its analogues have been extensively reported since being introduced to trace metal analysis by Fischer in 1925(132). It is used for trace metal determinations mostly as an extractant from aqueous media, which may involve a preconcentration stage. The absorbance can then be measured in the organic solvent in which the dithizone is dissolved.

5.2 Properties of Dithizone.

Dithizone (Dz) also known as diphenylthiocarbazone is a black or black-purple crystalline powder that is water and acid insoluble; sparingly soluble in ethanol, and soluble in alkali, carbon disulphide, carbon tetrachloride, chloroform and hexane. Enol-keto tautomerism is exhibited.

Dithizone is a dibasic acid with a weak secondary dissociation constant. Solutions are green in acid media and yellow above pH 5.5.
5.3 Response with Metals.

Primary and secondary dithizonates are formed when one or two hydrogens are replaced, respectively. All metals that react form primary dithizonates, with only a few forming the secondary structure. Metal ions initially react to form primary dithizonates for silver(I), gold(I) & (III), arsenic(III), bismuth(III), cadmium, cobalt(II) & (III), copper(I) & (II), gallium(III), mercury(I) & (II), indium(III), nickel, lead, palladium, polonium, platinum, selenium(IV), tellurium(IV), thallium(I) and zinc(129-131).

In excess metal media secondary dithizonates are formed with silver(I), cadmium, copper(I), mercury(I) & (II), nickel, osmium(MI), lead and zinc.

Others such as iron, manganese and tin also form dithizonates(129), but are either unstable in air due to oxidation or give a poor spectral response.

5.4 Dithizone Response with Organometallic Compounds.

A dithizonate complex is formed when an organometallic species has at least one positive charge. The tetra-organic substituted metal compounds such as those of lead and tin do not react, although dithizone has been used as a spray reagent in T.L.C. to distinguish such compounds(133).

Dithizone reacts with di- and triethyl tin to give yellow and orange complexes respectively, tetrachloro and tetraethyl give no reaction. Dibutyl tin also reacts but wavelength maximum depended the upon pН, concentration and the composition of the buffer employed(134) this was subsequently explained(135) as (Butyl,Sn),Dz, forming in the phosphate buffer and a mono-substituted dithizonate being formed in the chloracetate buffer. Both alkyl and phenyl tins react after separation(129).

Organo-lead dithizonates are formed. The tetra-derivatives do not react but may be found by difference after adding iodine in an ammonia buffer to decompose the tetraorgano-lead. This technique is applicable alkyl and phenyl substituted to organo-leads(129,131).

formed from the Organo-mercury dithizonates are mono-substituted organo-mercurials. Irving(129) quotes for the determination of a mixture of two Kiwan mono-substituted organo-mercury species and divalent inorganic mercury by extracting from a known amount of dithizonated solvent. The excess of dithizone is determined at 620 nm to find the total mercury, then reading off a mixed calibration curve to determine the organo-mercury content.

The incomplete organically substituted compounds of thallium, gallium and indium also form dithizonates.

5.5 Dithizone as a Post Column Reactant.

Dithizone is light sensitive and requires provisions be made for use as a PC reactant because of to insolubility in aqueous solvents. Dithizone undergoes photodecomposition and is slowly oxidized to form diphenylthiocarbadiazone and related oxidation products to give a yellow brown solution. Dithizonates are also photochemically degraded in diffuse light and the rate is increased with temperature. Despite these problems dithizone has been used successfully to detect alkyl and aryl mono-substituted mercurials(136), but this was in special considerations for organic solvents and dithizone used as a metal PC reactant for aqueous eluents are necessary. Degassing the PC reactant solvent and keeping the dithizone solution in an opaque container reduces the degradation process. A plastic container is recommended as a glass container increases PC reactant should be degradation. The dithizone replaced daily.

Several organic solvents were considered for the dissolution of the PC reactant. They also have to be easily miscible with an aqueous organic acid salt. Acetone, one of the more polar and easily water miscible solvents was investigated in various mixtures with water. With PCR mixtures of less than 80 % v/v acetone

metal dithizonates precipitated and formed a lining inside the PCR tube. Any mixtures with less than 20 % v/v water gave precipitation of tartrate salts. Consequently an acetone water mixture was used as a (4+1) mixture respectively.

Initial work showed that ammonia was better for pH adjustment because of its buffering abilities. Also it has been found that the addition of a nitrogen base speeds the reaction of water complexed metals by dehydrating the aquo-complex(137). The slow response of nickel was considerably reduced and was sufficient for the purposes of PCR detection when using an ammonia tartrate buffer at pH 3, whereas an acetic acid media revealed a half life of over 8 minutes. However an ammonium tartrate buffer eventually gave precipitation problems obstructing the PC reactant flow. Sodium tartrate has a higher solubility in water and was investigated in conjunction with ammonia for the of using a mixture to find the highest intention of ammonia for which permissible content no precipitation occurs. The best ratio for 2M ammonium and sodium hydroxide was as a (3+2) mixture respectively.

Deterioration of the residual dithizone occurred quickly during test tube experiments. The response at fast PCR residence times showed little deterioration as baseline drift. No responses were recorded for iron(III), iron, manganese, magnesium and calcium

although their extraction is documented. Spectrophotometric response for these metals with the exception of calcium and magnesium have been noted but in organic solvent media.

The nature of metal dithizonate spectra show that the same arguments for using a reverse mode detection with EBT as a PC reagent as stated in chapter four can be applied to dithizone.

reveal that some metal dithizonate peaks Spectra overlap with the dithizone peak and although the peak maxima of metal dithizonates are given in a monograph by Irving(129) it was necessary to verify if any peak shift occurred in the (1+1) v/v acetone water mixture. Table shows the peak maxima recorded for the metal 5.1 dithizonate complexes and shows little deviation from organic media. Little positive those reported in increase from the baseline was recorded and some gave no absorbance at 595 nm demonstrating the possibility of decrease in absorbance for metal observing а Early work shows that linear responses determination. were recorded at 595 nm, which was by far the more sensitive wavelength, consequently the difference in absorbance was observed at 595 nm.

Table 5.1.

Peak Maxima for Metal Dithizonate Complexes.

Metal	Dithizonate Maxima / nm		
Bismuth	486		
Cadmium	440		
Cobalt	558		
Lead	485		
Copper	438		
Indium	510		
Nickel	500		
Mercury	450		
Silver	495		
Zinc	510		

5.6 Optimisation Data for the Dithizone Post Column Reactor.

The same methodology for the EBT PCR detection parameters in chapter four was adopted. Detector response was assessed, firstly for the weaker complexing lactate eluent for which a lower concentration of 0.1M was favoured, because it decreases possibility of precipitation, and then with tartaric rather than citric acid, because more metals can be eluted from the Aminex SCX column.

A new eluent thiomalic acid was found to elute metals in a different order with a preference for sulphophilic metals. This required a stronger complexing PC reactant as thiomalic acid(a sulphophilic derivative of succinic acid) should compete more for the sulphophilic metals that react with dithizone. A concentration of dithizone that gave a PC effluent absorbance range of 0.2 and 1.4 absorbance at 595 nm was used for optimisation studies and the pH of the PC effluent was reduced by adding acetic acid to the PC reactant.

5.7 Response with a Lactate Eluent.

A 0.1M lactate eluent at pH 3.4 was used with the Partisil SCX column. Two solutions for injection were prepared each of a 100 μ l aliquot, one containing 2.5 μ g ml⁻¹ of cobalt, cadmium, nickel and zinc. The other solution contains 10 μ g ml⁻¹ of lead and 2.5 μ g ml⁻¹ of copper.

5.8 Optimisation Results with a Lactate Eluent.

The reaction parameters are graphically illustrated in Figures 5.1 to 5.4. Table 5.2 summarises the optimum conditions assessed from the graphs for each metal. Table 5.2.

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Optimum Conditions for Lactate Elution with the Dithizone Post Column Reactor.

	PCR Time	рН	Dithizone Conc*	PCR Temp	PC reactant Flow Rate
metal	/ min			/ °c	/ ml min ⁻¹
Cadmium	0.4	3.5-3.8	>0.1	20-40	1.0-1.5
Copper	0.1-0.4	3.5-4.5	>0.5	<20	2.0-3.0
Cobalt	0.4	4.2-4.6	>0.7	None	1.7-2.1
Lead	0.3-0.4	3.8-4.7	1-1.5	<20	1.2-1.7
Nickel	0.4	4.2-4.4	>1.2	60-90	1.5-2.2
Zinc	0.4	4.2-4.7	>0.7	20-40	1.0-2.0

* expressed as absorbance

The sensitivity expressed as 2 standard deviations using Bessels correction factor for samples of low frequency are as shown in Table 5.3

Table 5.3.

Limits of Detection for a Lactate Eluent.

Metal	Limits of Detection / ng
Copper Zinc Nickel Lead Cobalt	5.2 2.8 5.1 45 2.1
Cadmium	8.0



Figure 5.1



Dithizone Detection with a Lactate Eluent











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5.9 Response with a Tartrate Eluent.

Tartaric acid is a stronger complexing agent and it was used in conjunction with a Aminex A9 column at a 0.2M concentration as shown by the author in a paper(138) demonstrating the dithizone PCR. Data reveals parameters are similar to those obtained for a lactate eluent and therefore only a brief outline of the investigation is given. An injected solution consisting of a 100 μ l aliquot containing 2.5 μ g ml⁻¹ of cobalt, cadmium, copper, nickel and zinc was used.

Reaction parameters are not illustrated but feasibility of the system was assessed in terms of calibration curve response in Figure 5.6 and the sensitivity.

5.10 Results with a Tartrate Eluent.

For both tartrate and lactate eluents dithizone changes from light blue through grey-blue to a grey-brown from pH 4.0 to 5.3. Absorbance at 595 nm was reduced with the accompanying change in colour. A pink colour is produced by reaction with a metal.

Metals gave different responses at varying pH. Three pH values of 4.3, 4.8 and 5.2 were used, any lower pH resulted in precipitation of the ammonium tartrate salt. The overall sensitivity was better at pH 4.8 to 5.0. Nickel and lead showed little response at the lower pH. The PC reactant flow rate was optimised to 1.5 times the column flow rate.

The linear range of the detector was established using different values of dithizone concentration, which gave absorbances of 0.2 to 0.7 at 595 nm, this extended the range up to 20 µg injectable quantities for zinc, nickel, cobalt and cadmium.

Table.5.4 shows the sensitivity expressed as 2 standard deviations, again using Bessels correction factor.

Table 5.4.

Limits	of	Detection	for	а	Tartrate	Eluent.
Metal			Limi	its	sof	
Detect			tion / ng			
Copper				1	. 4	
Zinc				6	.5	
Nickel				8	• 2	
Cobalt				4.	.3	
Cadmiu	m			4.	•7	
Lead			10	00		



5.11 Response with Thiomalate Eluent.

The use of thiomalic acid as eluting agent has not been reported before, its interaction with the dithizone PC reactant may differ from other organic acids. Dithizone responds to many more metals in a thiomalic acid media than those mentioned, such as mercury and silver, only cobalt, cadmium, zinc and nickel were evaluated because they were easily separated.

5.12 Results with a Thiomalate Eluent.

When the PC reactant is mixed with a thiomalic acid eluent the green colouration of dithizone was extended from pH 4.4 to 4.6 and was greener in colour than that obtained with previously mentioned organic acids.

Table 5.5 and Figures 5.7 to 5.11 show the optimum conditions for the thiomalic acid dithizone system.





















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Table 5.5.

Optimum Response Conditions for the Thiomalic Eluent.

	PCR Time	рH	Dithizone Conc*	PCR Temp	PC reactant Flow Rate
Metal	/ min			∕°c	/ ml min ⁻¹
Cadmium	>0.1	3.0-4.0	>0.4	10-70	1.0-1.5
Cobalt	0.4	4.0-4.25	>0.6	20-40	1.0-2.0
Nickel	0.35-0.45	3.75-4.5	>0.6	>30	1.0-1.5
Zinc	>0.1	4.0-4.25	0.6-0.9	>30	1.0-1.7

* expressed as absorbance

Table 5.6.

Limits of Detection for Thiomalate Eluent.

Metal	Limits of Detection / ng
Cobalt	23.6
Cadmium	7.9
Nickel	17.4
Zinc	4.5

The limits of detection shown in Table 5.5 were again calculated on the basis of two standard deviations using Bessels correction factor.

5.13 Discussion.

The capability of the dithizone PC reactant has been shown using three organic acid eluents. The lactate eluent, as expected has a lesser interfering effect. Figures 5.1 to 5.4 demonstrate the response showing graphs for PCR time, temperature pH, PC reactant flow rate and dithizone concentration for the lactate eluent. A slight decrease of response was recorded with PCR temperature for cobalt and cadmium similar to that obtained for zinc, however dithizone is known to degrade quickly and probably does so in a heated PCR. The rate of degradation may be effected by the metal analyte. Conclusions as to the effect of temperature should therefore be treated with caution.

Lead shows a similar increase of response as for nickel and zinc shown in Figure 5.2, with a increase of dithizone concentration using a lactate eluent. Other metal responses level off with the exception of cadmium which shows an independance of response with increasing a levelling at 0.6 after concentration dithizone absorbance. Zinc and nickel show an optimal response at pH 4.3 as does cobalt in Figure 5.3. Copper and cadmium also give a plateau of response down to pH 3.5, with only lead showing a decrease of response with decreasing pH. Lower pH effluents were not investigated because of the risk of precipitation, however the best overall response for the metals in question was at a pH of 4.3 to 4.5. No plots of PCR residence time versus response are shown, in most cases the best response was between 0.3 to 0.4 seconds. An increasing PC reactant flow rate gave an increased dithizone absorbance from 0.16 at 1 ml min⁻¹ to 0.8 at 2.2 ml min⁻¹ Figure 5.4 shows only cadmium decreasing in response, presumably due to dithizone response upon non-dependance of the the independance of confirms concentration, which

response obtained in Figure 5.2. Lead and copper give response curves similar to that for zinc with increasing PC reactant flow rate.

The optimum conditions for the lactate eluent with a dithizone PCR system is a 0.4 second residence time; a dithizone absorbance at 595 nm of greater than 0.5 absorbance; a PC reagent flow rate of 1.5 ml min⁻¹; a PCR temperature of 40 $^{\circ}$ C and a pH of 4.4.

Figure 5.5 shows only nickel to have a non-linear calibration response curve and suggests that nickel degrades dithizone. Lead gives a linear response from 4 ng to 20 μ g ml⁻¹, having one third the sensitivity of zinc. Calibration graphs are shown for 100 μ l injections for cadmium, copper, cobalt, nickel and zinc from 1 ng to 10 ng.

Tartrate elution does not effect metal calibration, except to give a slight curvature for cobalt as in Figure 5.6. The limits of detection reveal deteriorated values for the less efficient Aminex A9 SCX column. However at such high concentrations of tartrate precipitation difficulties were encountered for low pH effluents. However low pH eluents gave decreasing responses for all metals, especially for nickel below pH 4.3. A general decrease was observed for metal responses above pH 5.0, copper was not detectable at pH 5.2.

Figure 5.7 shows an optimum response for cadmium, cobalt, nickel and zinc at pH 3.8 to 4.2 for thiomalic

acid, this contrasts with that obtained for the lactate eluent which almost has level response curves. Cadmium shows a similar response to nickel, cobalt was also similar to zinc in curvature for increasing dithizone absorbance at constant pH, as in Figure 5.8. An optimum temperature of 40 to 0 60 C was recorded in Figure 5.9 for the four metals. Thiomalic acid eluent demonstrates more clearly defined optimum PCR parameters and this was especially so for PC reactant flow rate, with an optimum value of 1.5 ml min⁻¹. However the calibration curves in Figure 5.11 reveal downward curvatures for zinc and cobalt above 100 µl injection concentrations of 8 µg ml⁻¹.

Thiomalic acid does not inhibit the response of nickel, which gave a better response than cadmium with a tartrate eluent. Zinc has a decrease of response with temperature increase, inferring that some other reaction mechanism or parameter is operating. The limits of detection vary more than for other organic acid eluents and this is reflected in variable calibration curves. A continued green colour with increasing pH to 5.6 may extend the pH range of the detector.

Bismuth and indium also respond but were not considered for calibration or optimisation, because bismuth elutes on the solvent front and indium only elutes on the silica SCX column with a lactate eluent.

5.14 Conclusion.

A simple PC reactor based on dithizone has been demonstrated to determine trace quantities of copper, zinc, nickel, lead, cobalt and cadmium after separation on a Partisil 10 SCX column with thiomalate and lactate buffers. and also with a tartrate buffer using a Aminex A9 column. This demonstrates that varying strengths of different organic acid eluents can be used with the dithizone PCR detector. The PCR pH can be adjusted to selectively react with metals that may co-elute, especially for a tartrate eluent. Quantitative analysis of all eluted metals was achieved with three eluents with calibrations up to 10 μ g ml⁻¹ for cadmium, cobalt, copper, indium, lead, nickel and zinc with limits of detection in the range of 1 to 10 ng except for lead with a 100 ng limit.

The ability of dithizone to react with many metals and may organometallic derivatives widen their the PCR detector. Organometallic of this versatility derivatives(129-131) that respond to dithizone such as those of gallium, indium, lead, mercury, thallium and tin were not investigated, because although they respond it was the intention of this work to primarily separate species. A completely new approach would be ionic necessary for the separation of organometallic species using organic solvents and neutrally charged column packings.

Other metals of note such as indium and gallium react are used extensively in the semiconductor industry. and Manganese and iron(III) were shown to react slightly with dithizone, probably because of the oxidation of dithizone(129). Decomposition reactions may also be used to detect species as they similarly give a decrease in dithizone absorbance at 595 nm. Of dithizone and EBT the latter is more stable, however one of the advantages of PCR detection is that there is insufficient time for degradation of reagents because of fast detection within an enclosed PCR system and if they are rapidly oxidised it does not appear to effect quantification. For some samples detection of overlapping eluting metals may prevent assay of the sample. The dithizone PCR pH can be adjusted to a lower pH thus eliminating the response of copper or nickel making determinations of other closely eluting metals possible.

This detector has a great number of applicable analytical possibilities, because of the wide spectrum of metal ions and organometallic species with which it responds. However, solubility limitations inhibit development for metal ion detection for concentrations of organic acids greater than 0.1M and the synthesis of a water soluble derivative would enhance the range of eluents that could perform with this detector.

<u>6 Applications of the Eriochrome Black T Post Column</u> Detector.

6.1 Introduction.

There are many reports of metal analysis by HPLC, however few determinations of real samples are evident although rare earths have been determined in Monazite sand and rare earth oxides by HPLIEC(139). In this chapter it is hoped to outline the versatility of HPLIEC with EBT PC detection in the industrial sphere, with the of Magnetic and Monel alloys, which were analysis supplied with certified values. Environmental samples from the Carnon river estuary were analysed and the data from conventional atomic with that compared spectroscopy.

For reasons mentioned in chapter one lower limits of detection are always being sought. Thus preconcentration of the analyte was investigated for HPLIEC by using a column of ion exchange material through which large volumes of sample can be passed. This may lead to low concentrations of analyte being accurately determined. The U.K.A.E.A., (Winfrith) are interested in ultra-trace metals present in the primary coolant of a nuclear pressurised water reactor(PWR) by this technique.

6.2 Analysis of Monel and Magnetic Alloys.

The EBT PCR system was used in conjunction with the Partisil 10 SCX column to determine cobalt, copper, manganese and nickel in Monel and Magnetic alloys. iron. microprocessor was preprogrammed to adjust the The for the minor metal the signal of attenuation Sample diluted where solutions were components. necessary for a more accurate assay.

6.3 Preparation of Samples and Standards.

The sample solutions were prepared by dissolving lg of the reference material (from the Bureau of Analysed Samples Ltd, Newham Hall, Middlesborough, England.) in a (1+1) mixture of distilled water and AnalaR nitric acid. The resulting mixture was then made up to 1 litre in a volumetric flask to give a lM nitric acid concentration. Mixed metal standard solutions were prepared to give similar ratios to those in the certificate of analysis. Working standards were prepared as required.

6.4 Analysis of Hycomax III Permanent Magnetic Alloy(BSC No.384).

This was analysed with two series of injections, one at 100 times dilution to give a 10 μ g ml⁻¹ nominal

solution for the major constituents, copper, iron(III), nickel and cobalt, and one at 20 times dilution to give a 50 µg ml⁻¹ nominal solution for the determination of manganese. The composition was obtained by comparison with those chromatograms obtained from mixed metal standards diluted to give similar concentrations. The microprocessor was programmed to change the amplification at 8 minutes, immediately after the cobalt peak was eluted.

6.5 Analysis of Monel Alloy 400(BSC No.363/1).

This was analysed in a similar way to the above with injections of the stock solution diluted to a 10 µg ml⁻¹ nominal solution. More concentrated solutions were not necessary to determine cobalt, a minor constituent, as sufficient sensitivity was available. The microprocessor was programmed to change the amplification at 6.2 minutes, just before the cobalt peak was eluted.

6.6 Results of Alloy Analysis.

Chromatograms shown in Figures 6.1 and 6.2 are those obtained from the analysis of Monel and Magnetic alloys, respectively, using 0.1 ml injections of 10 μ g ml⁻¹ nominal solutions.

A 0.1M lactate eluent at pH 3.5 and a flow rate of 1.5 $ml min^{-1}$ through a Partisil 10 SCX column at a temperature of 40 $^{\circ}$ C was used.

Conditions for the analysis of the Magnetic alloy were such that the PC addition of 0.5 ml min⁻¹ gave an absorbance of 0.77 at 610 nm and a chart F.S.D. of 0.2 absorbance. The manganese content was too low for accurate determination and a separate injection was performed with a five times greater concentration of alloy in solution. The signal amplification was increased to 0.05 F.S.D. for this minor metal constituent.

For the Monel alloy the PCR conditions gave a resultant absorbance of 0.54 at 610 nm, the scale change was from 0.5 to 0.05 F.S.D. for cobalt and manganese determinations.





Figure 6.2



Chromatogram of Magnetic Alloy Analysis

Table 6.1.

Analysis	of Hycomax	III Permanent	t Magnetic Alloy(BSC
No.384).			
Metal	Found	Coefficient\$	Certified Value
	/ % m/m	of Variation	/ % m/m
Cobalt	33.7	1.3	33.7
Copper	3.06	5.2	3.06
Iron	34.6	2.4	*
Manganese	0.10	11.5	0.10
Nickel	14.8	4.4	14.6

\$ % R.S.D. of replicate injections.

* Iron has no certified value, but the value calculated by difference was 35.6 % m/m.

Table 6.2.

Analysis of Monel Alloy 400(BSC No.363/1)

Metal	Found / % m/m	Coefficient £ of Variation	Certified Value / % m/m
Cobalt	0.033	8.3	0.032
Copper	31.5	3.0	31.9
Iron	1.73	9.2	1.86
Manganese	1.17	9.4	1.26
Nickel	64.2	1.6	64.7

£ % R.S.D. of replicate injections.

6.7 Discussion of Alloy Analysis.

After the appropriate dilution, up to 10 replicate injections were carried out with mixed metal standards being injected on every fourth analysis. The average assay was then calculated for each element together with the coefficient of variation. The results of the Magnetic and Monel alloy analysis are shown on Tables 6.1 and 6.2. The composition agrees very closely with the certificate values and applies to both major and

minor elements whose concentration range vary by up to three orders of magnitude. No certificate value for iron was quoted so a value was calculated by difference. A lower value was found, but this may be possible since impurities in the alloy may not be accounted for in the certified analysis. The precision for each element is shown by the coefficients of variation which as expected becomes progressively poorer for the minor components and reveals that the results for the Monel alloy are not as close to the certificate values as obtained for the Magnetic alloy. However this study shows the capability of HPLIEC with an EBT PC quantitative detection system where reasonable accuracy and precision can be obtained for some transition metals which have concentration ranges over three orders of magnitude.

6.8 Analysis of The Carnon River Estuary Samples for Trace Metals.

The site of sampling was a tributary of the Falmouth Estuary, South Cornwall, which extends from the Carnon river and contains water from the Carnon Downs. This is a metalliferous area of high iron and transition metal content, because of this it was thought a good area for initial studies. A high iron content should make it easier to obtain the iron(II), iron(III) ratio which may act as an indicator of the oxidation state of the salt
wedge.

The analysis was performed by both HPLIEC and atomic absorption spectroscopy for ferrous, ferric, copper, zinc and manganese cations(only the total for iron can be obtained with AAS). A photometric method using ferrozine for iron(II) and (III) was also compared.

6.9 Sampling Technique.

samples are by nature dynamic and Environmental analyse. Possible reactions that the difficult to may undergo must be considered before the analyte sampling is performed so that adequate provisions can be made to stabilize the sample. For example where the primary concern is the preservation of the iron species, a low pH is necessary. Low pH should also keep other divalent cations in solution. A routine procedure was for the environmental samples all adopted for preservation of sample integrity by adding 1 ml of 6 % v/v concentrated hydrochloric acid in distilled water to a 20 ml sample. The sample was filtered through a Swinex filter of 0.45 µm pore diameter. A 0.22 µm filter was used for some selected samples to check if particle sizes between 0.45 and 0.22 µm play a role in the water chemistry. Filtering should remove particulates, which may dissolve on acidification. Colloidal iron and iron attached to particulate matter should be retained. This

should not affect the measurement of the acidifiable dissolved iron species ratio which should reflect the oxidation state of the sample, because dissolved species are, in thermodynamic terms, relatively isolated from the solid state. Stability studies of an acidified environmental sample revealed that the sample remains stable over a two week period whilst stored at 5 °C. methods were performed HPLIEC Photometric and simultaneously. The AAS analysis was performed within the next 2 days. In all instances the same standards were used with each analysis for direct comparison of techniques.

6.10 Methods.

<u>6.11 High Performance Liquid Ion Exchange</u> Chromatographic Method.

Separation by HPLC was performed with the SCX Partisil 10 column and detection by the EBT PCR as previously described.

Many environmental samples contained low quantities of metal ions that cannot be determined accurately by a 0.1 ml injection, various approaches have been adopted. Preconcentration methods by packing a column with the same SCX material has been suggested by Cassidy and Elchuk(101) for large volumes where small concentrations are to be determined. Another approach is to increase the injector loop volume, a 2 ml injection can be accommodated on a Partisil 10 SCX column without any appreciable deterioration of chromatographic resolution. Preconcentration was not necessary here because of the relatively high metal content. 6.12 Flame Atomic Absorption Spectrophotometric Method.

The analysis was performed using an IL 151 atomic absorption spectrophotometer (Instrumentation Laboratory (UK) Plc., Birchwood, Warrington, Cheshire, England. WA3 7PB). Standard conditions for metal determinations were adopted, as quoted in the instrument method book. A deuterium continuum light source was used for background correction. The recommended AAS conditions are as follows;

Table 6.3.

Atomic Absorption Spectrophotometer Settings.

Element	Wavelength / nm	Background Correction	Comments
Cadmium	228.8	yes A	An air-acetylene flame Ised unless stated.
Copper	324.7	no	-
Iron	248.3	yes	-
Manganese	279.5	yes	-
Zinc	213.9	yes	-

6.13 Photometric Method.

Ferrozine was used to determine the ferrous content in an aqueous sample in a method devised by Gibbs(140) who further suggested adding a reducing agent; hydroxylamine hydrochloride to reduce the ferric ion to give total iron content, this technique was adopted.

To determine the total iron content 5 ml of sample was added to 0.8 ml of 5M hydrochloric acid and 2 ml of 10 % m/v hydroxylamine hydrochloride in 0.01M hydrochloric acid. After 2 minutes 2 ml of 0.075 % m/v ferrozine solution also in 0.01M hydrochloric acid was added, which was again left for 2 minutes, followed by 5 ml of acetate buffer. The acetate buffer contained 473g of sodium acetate trihydrate plus 115 ml of glacial acetic acid, made up to l litre with distilled water.

The ferrous content was determined by not adding the reducing agent. Ferric content can be calculated by difference.

6.14 Comparison of Results.

A lactate eluent makes the separation and determination of 10 metals possible. However as Figure 6.3 shows only copper, iron(II), iron (III), manganese, zinc magnesium and calcium were found by HPLIEC in a more concentrated sample, denoted as Al. Sample volumes of 0.1 ml were used. Those of salinity greater than 10 parts per thousand were injected as 20 µl aliquots to stop distortion of the chromatography by the high salt content. Table 6.4 shows samples presented in increasing order of salinity in a cross section of the salt wedge.

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Table.6.4.

lethods.						
Element	t C	opper	Manga	anese	Zin	c@
Method	HPLC	AAS	HPLC	AAS	HPLC	AAS
Sample		Metal	found /	µg m1 ⁻¹ .		
18	0.50	0.47	2.7	2.6	10.0	8.0
C12	0.50	0.78	2.8	2.7	8.3	7.9
Al	0.47	0.56	2.8	2.7	8.1	8.0
9	0.50	0.60	2.7	2.8	10.3	7.9
C8	0.60	0.63	*	n	9.7	7.2
3	0.00	0.48	0.4	2.5	4.7	6.3
A5	0.30	0.45	*	2.3	6.5	6.0
1	0.00	0.56	0.77	2.2	1.4	n
2	0.17	0.37	*	1.7	4.3	6.2
4	0.14	0.30	0.84	1.1	2.4	0.0
5	-	0.1	0.65	0.64	0.80	n
6	-	n	*	0.37	n	0.0
7	-	0.11	*	0.17	2.2	n

Comparison of High Performance Liquid Ion Exchange Chromatography and Atomic Absorption Spectrophotometric Methods.

@ diluted tenfold for analysis.

none detected.

* manganese co-eluted with magnesium, this masked manganese in samples of high magnesium content. n not determined.

The observed content of the Carnon for zinc, manganese and copper is graphically illustrated in Figures 6.4 to 6.6, where metal concentration detected is plotted against salinity. Manganese levels are particularly variable. This may be due to the high magnesium content making chromatographic peak evaluation, as shown in Figure 6.3. difficult. Other metals such as nickel, cobalt and cadmium were below the limits of detection for HPLIEC and many were not detected by AAS. Little difference was observed between samples from the same sampling site using the two different sized filters mentioned in the sampling technique.

6.15 Iron Speciation in the River Carnon.

To ascertain any possible deviation or difference between HPLIEC and photometric methods a comparison was carried out using acidified deionised water solutions containing varying concentrations of iron(II) and (III), inversely varying concentrations of the two species in ml⁻¹ were prepared and μg are graphically illustrated in Figures 6.7 and 6.8 which shows of a plot of increasing ferric concentration with decreasing ferrous concentration. The graphs reveal good correlation for the inversely varying concentrations, although Figure 6.7 revealed that the ferrozine method

recorded a zero response below 1.7 µg ml⁻¹ of iron(II).

Carnon samples analysed by AAS, HPLIEC and photometric methods are compared in Table 6.5. A standard addition of iron by AAS, HPLIEC and the ferrozine method was also performed, a linear increase of response for all the techniques was obtained.

Table 6.5.

Comparison of Iron Speciation Analysis.

Metho	d		HPL	С	Ferro	ozine		AAS
Iron	Species	(11)	(111) Tot*	(11)	(111)	Tot*	Tot*
Iron found µg ml ⁻¹								
Sampl	Salinity e ‰							
18 C12	1.8	8.8 9.9	.9	9.7 10.2	8.3 8.0	0.1 0.8	8.5 8.8	8.6 8.7
Al	1.9	10.0	.3	10.3	7.8	1.0	8.8	8.7
9	2.0	9.0	•3	9.3	8.9	-ve	8.9	9.2
C8	2.6	8.6	• 3	8.9	8.3	0.5	8.8	8.5
3	5.4	6.8	-	6.8	7.2	-ve	7.2	8.3
A5	5.5	4.8	• 3	5.1	6.0	0.1	6.1	5.7
1	7.8	7.8	•0	7.8	5.0	0.1	5.1	4.5
2	14.5	8.4	• 1	8.5	3.0	-ve	3.0	2.3
4	21.6	9.1	•1	9.2	0.5	-ve	0.5	0.3
5	26.9	1.2	•0	1.2	0.01	-ve	0.01	0.0
6	30.0	0.0	•0	0.0	0.1	-ve	0.1	0.0
/	34.1	0+2	•0	0•2	0.0	-ve	0.0	0.0

- not determined.

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-ve indicates that the blank gave a higher absorbance than the sample.

* cumulative concentration total of the two species.

Figure 6.3

Chromatogram from Carnon River Sample





Figure 6.4



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Figure 6.5



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Figure 6.7



6.18 Analysis of a Simulated Pressure Water Reactor Primary Coolant.

Much of the piping in the heat transfer system of a reactor is made of minimally corroding stainless steel tubing. The coolant contains 1500 μg ml⁻¹ boron as borate added as a neutron moderator. Although boron decays to lithium and helium after neutron bombardment in a reactor the coolant also has $1.5 \ \mu g \ ml^{-1}$ of lithium added to the coolant. Although a reducing atmosphere of hydrogen is introduced wherever possible, a number of metal species are found in the primary coolant at very low concentrations. These are in turn exposed to high levels of nuclear radiation which form radioactive nuclides. Some have a short half life and emit radioactivity strongly. Cobalt 60 is a gamma ray emitter and must be kept to a minimum concentration to reduce operator exposure. Concern was also expressed by A.E.E. Winfrith about the nuclides of iron, nickel, manganese copper and chromium, although corrosion was the main reason for wanting to determine the iron species within the primary coolant. A major problem in the analysis was in situ sampling of this superheated water at 300 °C. By a construction of high pressure tubing it was hoped to overcome this problem and obtain Α list of approximate concentrations. direct concentrations for the above mentioned metals are

tabulated in Table 6.6. A borate placebo primary coolant solution that was not irradiated and contains virtually no transition metals was obtained from Winfrith. Simulated solutions were prepared and used to estimate the feasibility of a HPLIEC method before testing the method with radioactive samples. Such radionuclides pose obvious threats and an enclosed liquid system would prevent their escape into the atmosphere unlike for example with AAS.

Table 6.6.

Estimated Trace Metal in Pressurised Water Reactor Coolant.

Metal	concentration ng	ml ⁻¹
Chromium	0.5	
Cobalt	0.05	
Iron(II) & (III)	0.5	
Manganese	0.5	
Nickel	0.5	

The concentration of the metal species was below that which can be detected with injections up to 2 ml and therefore determination was attempted by concentrating ions. By passing the sample through a the metal preconcentration column containing the same exchange material as the separating column it may be possible to the same eluent. There are elute them using chromatographic problems presented by this approach, such as the choice of preconcentration column dimensions and the cation load imposed upon it.

6.19 Method of Preconcentration.

For the sake of ease of elution the same material as in the analyte column, Aminex SCX A9 resin, was initially used in the preconcentration column. Metals are best separated with a tartrate buffer. A 5 by 0.46 cm column was placed within the injection loop. The back pressure was too large to pump a syringe sample through, therefore a L.D.C. HPLC pump was used at a set flow rate and its operating time recorded with the microprocessor. Two disadvantages are, firstly the metal contamination from piping and pumping sources and secondly, the inaccuracy of analyte volume measurement, because HPLC pumps do not have constant flow rates at low back pressures.

A procedure was adopted with the sample solution containing 10 ng ml⁻¹ nickel and iron(II) and 1 ng ml⁻¹ cobalt a; 1.5 ml min⁻¹. Detection was with the EBT PCR as described in chapter four.

6.20 Results of the Analysis of a Simulated Coolant of the Pressurised Water Reactor.

A chromatogram of low concentration analytes in the simulated coolant is shown in Figure 6.9. Iron and nickel were added as 10 ng ml^{-1} and cobalt as 1 ng

ml⁻¹. Figure 6.10 shows graphs of metal detected versus time of flow of sample onto the preconcentration column for the above concentrations. An upward curvature is noted for nickel and ferrous ions. Cobalt gave a more variable response for increased preconcentration times although linear preconcentration response was achievable at a low concentration of 1 ng ml⁻¹. Data obtained for concentrations less than 10 pg ml⁻¹ were subject to considerable variation and subsequent investigations revealed that contamination from the sample delivery system and HPLIEC fittings was comparatively large.





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6.21 Discussion and Conclusion.

Samples that can contain small particulates or colloidal material may escape filtration. Such particles may adhere to the HPLC column material and not be detected. Although HPLIEC and AAS methods can be compared, results may differ because AAS results may include a contribution from the analyte ions bound to any suspended material. Chromatography may therefore offer a similar defined classification of ions in solution to electroanalytical techniques.

Estuarine samples were compared using AAS and HPLIEC techniques for copper, zinc and manganese. Samples analysed are graphically illustrated in Figure 6.4 to 6.6. Standard addition for the AAS method with zinc, manganese and copper reveal no discrepancies and corroborate the AAS data obtained. Good agreement was shown between the two methods for samples of low salinity. Figure 6.5 revealed that samples of intermediate salinity show agreement for manganese determination between HPLIEC and AAS methods. At high salinities HPLC data varied, possibly because of a smaller injected volume. Higher magnesium content and higher ionic strength of the highly saline samples may upset the chromatographic stability for manganese.

Little agreement between the methods is shown for zinc, although a sharp decrease of zinc content was

noticed for AAS at salinities greater than 20 to levels below the limit of detection. A gradual decrease was noted in Figure 6.6 showing the HPLIEC data which is consistent with the metal decrease expected in salt wedge samples. This suggests there may be interference from the dissolution of metal from the HPLIEC tubing effected by salt in the estuarine samples.

Perhaps the most distinguishing feature from the zinc data, was that when results are copper and compared, AAS has higher results. Possibly because of complexation of the metal within the organic humic content or because of a chemisorption or physical trapping on a particulate in the river water, which may have escaped the filter. Consequently when a sample containing particulates is placed in a flame the structure breaks up or the chelate is broken down, releasing metal for detection. This may be a way of distinguishing particulate attached species; and those that are chelated to the organic content of the water from that which will more readily take part in exchange processes, dynamic or otherwise within estuarine waters.

Analysis of pore waters from a lake with laminated sediment, Loe Pool, showed the possibility of measuring the degree of erosion by calculating the ratio of calcium to magnesium; a higher ratio of calcium indicates a greater degree of erosion according to Mackerbeth(141). Mackerbeth also stated that the

manganese / iron ratio in sediment can record the redox state as manganese is more easily transported in reducing conditions.

Perhaps the greatest problem encountered with environmental samples is to comprehend the complex mechanisms. With an easily oxidisable and hydrolysable metal such as iron, which also adsorps other transition metals(142), considerable care has to be taken when drawing any conclusions. The determination of iron species is fraught with difficulties, mostly because of hydroxy formation which begins above pH 1. With saline samples of high pH, around 8, precipitation of the iron could cause complications. Generally iron concentrations obtained by HPLIEC for the estuary waters were higher than those obtained by the ferrozine or AAS methods. High HPLIEC results may be due to corrosion of the steel fittings in the system to give, initially iron(II). A saline sample left in the sample loop confirmed this hypothesis. Higher iron results are shown for the photometric method compared to the AAS method. The low pН obtained with the ferrozine method by adding hydrochloric acid may explain the presence of more iron for the relatively higher results. Ferrozine and AAS methods compare favourably and although the HPLIEC data was erratic the trend of the slope was the same. Better correlation was observed for iron(III) between HPLIEC and the ferrozine method. The poor correlation of the

iron(II) suggested that salinity may affect HPLIEC results for the reason stated above. To minimise the high salinity effect upon the chromatography a 20 μ l volume was used instead of a 0.1 ml injection. However the concentrations found varied, although some variation was expected with a smaller injection.

Preconcentration of simulated PWR primary coolant water showed that low concentrations can be determined in a borate buffered background down to 1 ng ml⁻¹, although the sample was acidified by the addition of metal solutions. In situ assessment of the coolant by monitoring stainless steel corrosion may be difficult because at pH 6 the coolant may contain colloidal or particulate matter. Employing a preconcentration column gave better calibrations down to 1 ng ml⁻¹

better calibrations gave concentrations. However the problem that gave the curved calibration should first be resolved. Quantifiable below this concentration require that measurements studies should be performed because of further variability obtained from contamination sources of the Schmidt and Scott(143) HPLIEC fittings pump. and contamination from ferrules, tubing iron reported connections and sample loop. Cassidy and Elchuk used Teflon, polypropylene or Kel-F fittings on all wetted surfaces(100,101). An all non-metal system may also overcome sample contamination problems whilst introducing the sample, although some saline samples may

still leach residual metal contamination from the surface.

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7 Initial Studies of Alternative Systems, Suggestions for Further Work and Conclusions.

7.1 Initial Studies of Alternative Systems.

When initiating the design of a new PC reactor, detection of toxic or environmentally harmful metal species is normally experimented with to obtain lower limits of detection. Many metallochromic indicators are available, but only those that offer the advantages of high sensitivity and react with a broad spectrum of metals can be considered, unless speciation studies of a selected metal are intended. In the following discussion it is hoped to outline work showing the way forward by using different photometric techniques. Primarily a very sensitive reagent such as dithizone was taken advantage by the afore mentioned novel approach, however of dithizone is water insoluble and this limitation for dithizone and others(128) may be improved by using water soluble derivatives. The synthetic preparation of a non-specific PCR organometallic reagent may be possible. This may lead to a higher extinction coefficient and response with many more metals. Previous reagents were early organometallic indicators, but most researchers concerned with the synthesis of specific are now reagents(91).

Although EBT has been shown to react with many metals

in the inverse mode of photometric detection, some reagents react with metals. more Pyrocatechol violet(PCV) is such a compound and reacts with highly charged cations as well as oxyanions of transition metals and can be used with a cationic surfactant to form a ternary complex. Such a ternary system has the advantage of forming complexes that absorb at virtually the same wavelength, which is normally over 600 nm and is distant from the parent and metal-complex spectra of the binary complex. Fluorescence is the most sensitive method of detection, however transition metals and most of their complexes do not fluorescence and as with many techniques of high sensitivity, contamination problems considerable difficulties. pose Either derivative complexing agents or substitution reactions have to be used tο overcome non-fluorescing metals or metal-complexes.

Perhaps the greatest difficulty is that separation from a matrix may prevent the detection of a species and consequently any properties that can be attributed to it are not quantifiable. This may be especially so for transition metal oxyanions species which are pH dependant. In this work a chromatographic technique for the separation of oxyanions is proposed and initial work reveals that low limits of detection may be possible with a pyrocatechol violet(PCV) PC reagent.

Figure.7.1



7.2 Dithizone-S Post Column Reactor System.

A major difficulty with the dithizone PCR as discussed previously is the insolubility of the dithizonates and organic acid salts in the PC reactant mixture. The immediate answer is to make a water soluble derivative of dithizone. A recent paper by Zhang, et al. (144) shows that a sulphonated molecule can be prepared which water soluble and is known as dithizone-S. A is colourimetric response was recorded for silver(I), mercury(I) & (II), lead, nickel, manganese, iron(III) chromium(111). The suggested and synthesis using sulphanilic acid in a diazotisation reaction was attempted and was confirmed successful as spectroscopically. Elementary PC detection in Figure 7.1 shows a positive absorbance response at 580 nm to metals from a 100 µl injection of 0.4 µg ml⁻¹ of copper, zinc, nickel and 0.6 μ g ml⁻¹ of cobalt. The PCR pH was adjusted to 9.5 by the addition of ammonia to the dithizone-S reagent solution, in these conditions secondary dithizonates are probably formed with peak maxima between 575 and 590 nm and may have higher coefficients of extinction, because of two dithizone-S molecules in the complex. Good responses are shown for copper, nickel and especially cobalt with a 0.4 F.S.D. Separation was performed on a Partisil SCX column at 40 ^OC with a 0.1M lactate eluent at pH 3.5. The dithizone-S

used was sufficiently concentrated to give a PCR absorbance for the metal-complexes of greater than 0.5 absorbance at 580 nm at the unoptimised PC reactant flow rate of 1.4 ml min⁻¹.

7.3 Pyrocatechol Violet as a Detector for Anions and Cations.

Of the organic chelating agents surveyed PCV appears to offer considerable promise for the sensitive detection of divalent cations and transition metal oxyanions. It is a dark red solid, triphenylmethane dye, containing four phenolic and two sulphonic acid groups. A 0.1% m/v solution is stable indefinitely. It is suitable for a wide range of metallic titrations, especially with EDTA in acidic or alkali media for aluminium(III), bismuth(III), nickel, manganese, thorium, cobalt and cadmium metals. It is considered inferior to EBT for titration in alkaline media, but it is not blocked by heavy metals(145). Like the majority of the phenolic type organic reagents there is a greater selectivity for the highly charged oxyanion species at low pH. PCV changes colour according to the pH;

Red pH 1.5 < Yellow pH 6 < Violet

A blue complex is obtained with bismuth(III),

iron(III), gallium(III), MoO2²⁺, niobium(V)(146), tin(IV), thorium(IV) and ZrO²⁺(147). Responses are also recorded with chromate, molybdate, tungstate and vanadate(148) oxyanions well as antimony(III), as aluminium(III)(149,150) and others(151); which total over thirty metals. Spectrophotometric activity is due to the p-quinoid group. Subsequent ternary complexes are formed from the binary metal PCV complex after the addition of an ionic surfactant to form an ion associate. Such ion associates are normally insoluble in aqueous media and are extracted into an organic phase before measuring the absorbance. The resultant ion has a hyperchromic effect on the binary associate complex and a large extinction coefficient due to size making it one of the most sensitive spectrophotometric methods 150,000 with approaching 1.mole⁻¹.cm⁻¹. A bathochromic effect gives a peak maximum around 650 nm.

For cationic associates the critical micelle concentration does not have to be reached before the ion associate can be formed, however Fogg <u>et al.</u> (150) recommend that a larger concentration of surfactant be used for a better response.

Examples and applications of this type of sensitised reactions are numerous. Aluminium is detected with eriochrome cyanine R and cetyl trimethyl ammonium chloride(152). Rare earths with xylenol orange and cetyl

pyridinium chloride(153). Metals may form mixed ligand complexes with triphenylmethane dyes. In some instances this enhances the sensitivity and selectivity(151).

Cetyl trimethyl ammonium bromide (CAB) and cetyl pyridinium bromide (CPB) are the most used cation surfactants. The determination of tin in steel with PCV and CAB for example, uses a 1:2 complex with PCV and then a CAB molecule attaches to each of the two PCV molecules to give an extinction coefficient of 94,000 $1.mole^{-1}cm^{-1}$ and a peak maximum at 662 nm(150). Further details of these reactions and their mechanism may be found in reviews by Marczenko(92) and Savvin(91).

7.4 Pyrocatechol Violet as a Post Column Reactant.

The criterion for a good PCR are exhibited by PCV if an ion associate complex is formed. Many PCV cationic divalent metal ternary systems absorb light at the same wavelength of 650 nm, this should eliminate any variation in detection limits because of different peak maxima, as observed for binary metal PCV species and give a detection system that responses with more metals. PCV was investigated for the detection of highly charged oxyanions of transition metals. This would greatly reduce the noise problems associated with measuring a decrease in absorbance.

Figure 7.2

<u>Chromatogram with Pyrocatacohol</u> <u>Violet Post Column Detection</u>



7.5 Solvent_and Instrumental Considerations.

The PCV solution and surfactant have to be mixed at a T junction prior to being mixed with the eluent stream because a better reproducibility and response can be obtained. A different solution of surfactant has to be prepared for anion and cation detection to give the correct PCR pH, which was more critical for divalent The response was relatively quick and no cations. special PCR apparatus was necessary, although basic optimisation procedures were used. The operating wavelength were 700 and 640 nm for both cationic and anionic species respectively and although 700 nm is near most detectors' response range, many the end of instruments can now be purchased that go beyond these limits.

7.6 Results and Discussion.

Feasibility studies performed in test tubes and by FIA for both cationic and anionic PCR detection show that it is possible to have a very sensitive PCV PCR detection system. The flow rates of PC reactant solutions were optimised, these solutions contained ammonia or acetic acid in the surfactant to buffer the pH which prevents the PCV degrading. Figure 7.2 shows the chromatogram of a 100 µl injection of 4 µg ml⁻¹ of copper iron(III),

indium, zinc, nickel, cobalt and 8 μ ml⁻¹ of cadmium and manganese. A 0.1M lactate eluent at pH 3.35 was used with a Partisil 10 SCX column at 40 $^{\circ}$ C. The best operating conditions were with the flow rate of a 0.1% w/v PCV solution at 0.4 ml min⁻¹ and a 0.2% w/v CAB solution buffered with ammonia to give a 2M alkali solution at 0.5 ml min^{-1} , this gave a resulting PCR pH of 9.3, good responses were achieved for copper iron, indium, zinc, cobalt and manganese with a F.S.D. of 0.2 absorbance at 700 nm. An 8 μ g ml solution was necessary to obtain a response from cadmium, which gave no response when the PC reactant flow rates were increased, possibly due to a reaction parameter factor. No reference in classical texts to the response of cobalt has been made but a response of some sensitivity was found if the pH was controlled carefully, so that the pH does not exceed 10, with a peak maxima at 750 nm for the ternary complex. A comparison with the EBT detector shows a three times greater response for copper and zinc. Cobalt and nickel gave similar responses to those for an EBT PCR. The calibration curve appears to be linear in the 1 to 10 μ g ml⁻¹ range.

Work on initial optimisation data for both systems yielded good sensitivities (responses are illustrated in Figure 7.3) for anionic detection. Because of the ternary nature of the complex it is recommended to use a simplex optimisation program that includes an overall

chromatographic response factor for those metals of poor detection. This introduces a limitation of minimal response for any one metal so as not to bias the optimisation in favour of others.

7.8 Chromatographic Separation of Oxyanions.

Many metals can exist as an oxy or hydroxy anionic complex and for better representation of their effect on the environment it is better to analyse for such species rather than obtain a total assay, as for example with To separate those metals which form anionic species AAS. requires an anion exchange column, however many separations have considerable differences in retention time and consequently give mg sensitivity. Zolotov et al. (154) separated oxyanions of arsenic, molybdenum, chromium, selenium and tungsten in 48 minutes. A reverse phase separation of chromium and vanadium 8-hydroxyquinolates has also performed by Lajunen(155).

For higher sensitivities and shorter analysis times, another approach was necessary. Ion pair chromatography(42,44,45,46,156), where a cationic moiety is dissolved in the eluent to form an ion pair with the anionic analyte was attempted.

Although good separations have been achieved with longer quaternary ammonium carbon chain lengths over C_{16} where the cationic surfactant was deposited on a

hydrophobic column with partially aqueous solvent prior to separation(46), here the surfactant CAB, was at a constant concentration. Figure 7.3 shows how the oxyanions of tungsten, vanadium, chromium and molybdenum as tungstate, vanadate, chromate and molybdate can be separated on a divinyl benzene cross-linked polystyrene polymer resin material, using an alkali solution of CAB. An alkali eluent at pH 10 simplifies the separation because a variety of polymeric and other oxyanion species are formed in acidic media to give broad peaks. Conditions for this separation were as follows.

Column eluent A: 0.28 g CAB 0.13g sodium sulphate and 8 ml of 2M ammonia in 1 litre of deionised distilled water.

Column eluent B: Deionised distilled water.

A Hamilton PRP-1 material in a 10 by 0.46 cm column at 60 °C was used with a 2ml min⁻¹ flow rate.

PC reactant 1; 0.05g of PCV in 1 litre of deionised distilled water.

PC reactant 2; 0.5g of CAB in 1 litre of 15 % glacial acetic acid in distilled deionised water.

The PC solution gave a resultant pH of 3.5.
Figure 7.3 <u>Chromatogram with Pyrocatacohol</u> Violet Post Column Detection



Table 7.1

Column Gradient for Oxyanion Chromatogram.

Time	% flow		
min.	pump B		
0	5		
3.5	5		
4.5	80		
6.5	60		
8.8	5		

As conditions reveal in Table 7.1 separation was complex and found to be sensitive to pH change. Peak integrity alters with pH, molybdate split into two peaks at lower pH. If the CAB eluent is not increased then the chromate peak elutes very late and broad. Any lower initial concentration of CAB merges vanadate and tungstate peaks, with the vanadate peak becoming considerably broader and like molybdate showing a disregard for usual chromatographic rules concerning peak broadening and column separation.

The method appeared not to give very stable retention times and suspicions were aroused when responses varied. However this may be due to chromatographic instability because of non-equilibrium conditions between the column and eluent. Decomposition of the injected anions could also have occurred. The appearance of the column top suggested that the deep red purple colour was due to the ferrate complex $[FeO_4]^{2-}$ which is stable in basic solution and may be the result of oxidation by the chromate anion of steel. This strong oxidising agent appears to attack the column top, because it had to be periodically replaced. However work showed promise, with these four difficult species eluting quickly and with high efficiency.

7.9 Fluorimetric Trace High Performance Liquid Chromatography Determinations.

In a search for lower detection limits fluorescence has to be considered for it can be over a 100 times more sensitive than conventional photometric techniques. This is especially so with the emergence of laser excited fluorescence. However this sensitive method cannot be applied directly to metal detection as most metals do not fluoresce on their own, notable exceptions are some lanthaniods and uranium. Paramagnetism inhibits transition metal ions from fluorescing, however, they can be quantified by their quenching effect(157). Some metal-complexes do fluoresce and they may be very sensitive for HPLIEC metal detection. 8-Hydroxyquinoline(8HQ) is known to form complexes with many metals which fluoresces in alkaline conditions and may therefore be a good PC reagent, forming complexes with a metal by bonding through the oxygen and nitrogen atoms.

Although 8HQ has been used as an extracting agent it

has a low extinction coefficient (for chromium(III) and vanadium(VI) does not exceed 10,000 l.mole⁻¹ cm⁻¹) and is of little use in trace determinations by absorption. However, many complexes do fluoresce, of which eleven can be determined analytically(158) they are; aluminium(III), gallium(III), indium(III)(159); cadmium(160), hafnium, magnesium(161), scandium, tin(II), yttrium, zinc and zirconium(162).

However 8HQ is not water soluble and complicates the PCR procedure. This may be overcome by using an analogue, 8-Hydroxyquinoline 5 sulphonic acid(8HQ5SA), which has been used for the fluorescent determination of cadmium and magnesium(163,164). Tin also fluoresces strongly in aqueous solution with 8HQ5SA in the di- or tetravalent state (165).

7.10 Fluorescent Post Column Reactor Detection with 8-Hydroxyquinoline-5-Sulphonic Acid.

This water soluble compound forms many complexes and it is hoped to show that fluorescence detection of indium, zinc, cadmium and magnesium can be performed with HPLIEC and a 8HQ5SA PC reactant solution, which is buffered to an alkaline pH. The chromatogram in Figure 3.4 (page 82) demonstrates the detection of fluorescent species of four metals with good sensitivity, certainly below the μg ml⁻¹ levels. The background, although slightly noisy here is considerably better than that achieved in conventional photocell measurements, because the often large background light levels are eliminated. A 0.04M tartrate buffer at pH 3.3 with a 1 ml min⁻¹ flow rate has little effect on detection. A 1 ml min⁻¹ PC reactant mixture of a 0.002M 8HQ5SA solution in an aqueous 7 % v/v solution of 0.88 ammonia gave a PCR pH of 8.2. The fluorescent excitation and emission wavelengths were averaged and the excitation was at 364 nm and emission at 515 nm.

The wide linear response range of the fluorescence method makes it an attractive option for metal detection with high sensitivity. However many metals do not fluoresce. Some authors have overcome this problem by a displacement reaction where a kinetically quick cation of high thermodynamic stability replaces а non-fluorescing cation of lesser complexing ability. A good example is given by Beckett and Nelson(107) where sensitivities are be achieved. The authors better further suggest that greater sensitivities can be obtained with laser excitation.

7.11 Conclusions as to Future Work.

As more knowledge of speciation is required lower limits of detection are necessary. The above work shows the possibilities of improved detection and separation

of oxyanion species. However the chromatography requires further investigation to obtain more reproducible results. The detection of oxyanions and other highly charged species by the PCV PC reactant shows promise although optimisation may be more difficult than for a binary system.

Similarly the fluorescent detector may offer considerably improved detection limits for some metals, but chromatographic systems should also be developed to separate other metals that respond with 8HQ5SA.

7.12 General Conclusions.

New methods of transition metal cation separation and detection are outlined with two new PC reactants. A novel approach was adopted to engage the use of well known organic reagents dithizone(125,126) and EBT(126,138). A reverse mode of photometric detection for which a decrease of absorbance was found to be proportional to the metal quantity detected was used. The applicability and adaptability of HPLIEC with detection by these reactants has been demonstrated for trace analysis. The analysis of metal alloys(125) and saline samples illustrated good agreement with certified values and those determined by conventional atomic spectroscopy techniques.

Although the above mentioned detectors show low levels

of detection an improvement especially for the inverse mode of detection by reducing the noise would lower the detection limits further beyond those of other photometric detectors. Improvement of HPLIEC naturally involves better sensitivity, separation and reliability. When considering ways of obtaining better sensitivity, column efficiency and lower detection limits are intimately linked. As column particles of lower regular diameter are being manufactured better detection limits should follow.

Appendix I.

Stability Constants for Metal Complexes.

The lst stability constant expressed as Log₁₀ for metal eluting agent complexes are shown below and are in order of that for most eluting agents to assimilate comparison.

Ligand	Citrate	Tartrate	Oxalate
Metal			
Hydrogen K	5.7	4.5	3.8
Ion K	4.3	2.9	
к	3 2.8		
Bismuth			
Indium	6.2	4.5	3.1
Copper	5.9	2.6	6.3
Iron III	11.4	6.9	9.4
Zinc	5.0	2.7	4.9
Nickel	5.4		5.3
Lead	4.3	2.9	
Cobalt	5.0	2.8	3.7
Cadmium	3.7	3.7	3.7
Iron	4.4	2.2	4.7
Manganese	2.8	1.4	3.9
Magnesium	3.7	1.9	3.4
Calcium	3.4	2.1	3.0
Mercury	10.9		
Silver			2.4

Ligand	Thiomalate		Succinate	Lactate	
Metal Hydrogen	ĸ	9.9	5.3	3.8	
Ion	к2	3.5	4.0		
	к ₃	3.2			
Bismuth					
Indium					
Copper			2.9	2.5	
Iron III			6.9		
Zinc		8.3	1.7	1.6	
Nickel		7.0		1.6	
Lead		8.5		1.7	
Cobalt		5.8	1.7	1.3	
Cadmium				1.2	
Iron					
Manganese	9	4.4		0.9	
Magnesium	Ċ			0.7	
Mercury		9.9			
Silver		7.0			

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Ligand	l	Maleate	Formate	Glycine	Aspartic Acid
Metal Hydrog	gen				
	ĸı	5.8	3.7	9.7	10.6
ion	К2	1.9		2.5	3.7
	Кз				1.9
Bismut	h				
Indium	1	5.0			
Copper	•	3.4	1.9	8.6	
Iron I	11		3.0		
Zinc			• 6	5.9	
Nickel				6.5	7.1
Lead		•7	•8	5.1	5.9
Cobalt				5.3	6.0
Iron I	I				
Cadmiu	m	2.2		6.0	
Mangan	ese			3.2	
Magnes	ium				
Calciu	m		•8		
Mercur	У				
Silver				3.6	

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Analytica Chimica Acta, 149 (1983) 39-46 Elsevier Science Publishers B.V., Amsterdam - Printed in The Netherlands

A DITHIZONE POST-COLUMN DETECTOR FOR THE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC DETERMINATION OF TRACE METALS

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(Received 4th November 1982)

SUMMARY

A multi-element post-column high-performance liquid chromatographic detector based on dithizone as a complex-forming reagent is described. The separation was achieved in aqueous media on a sulphonated 10% cross-linked polystyrene resin. Cadmium(11), cobalt(11), copper(11), lead(11), nickel(11) and zinc(11) ions were detected down to nanogram levels. Working ranges up to the microgram level were possible. The use of this heavy metal detector which may be tuned by pH contro¹ is discussed. An advantage of the system for multi-element determinations is that the decrease in the dithizone absorbance at 590 nm is measured rather than increased absorbances at different wavelengths particular to each metal dithizonate.

Considerable attention has been directed in recent years towards the detection and determination of trace metals. Perhaps the most popular technique for quantifying trace metals is atomic absorption spectrometry. Yet, when several elements have to be determined separately in many samples, essentially single-element techniques such as atomic absorption spectrometry may become very time-consuming and may use excessive sample. Rapid multi-element techniques for quantifying trace metals have become of increasing interest, but several of these multi-element approaches, e.g., inductively-coupled plasma emission spectrometry, are very capital-intensive. The multi-element technique discussed in this paper requires only equipment now available in many laboratories, i.e., high-performance liquid chromatography (h.p.l.c.).

The aim of this study was to separate trace metals by h.p.l.c. followed by sequential spectrophotometric detection at a single wavelength by using a simple post-column reactor system. Such an investigation is timely because of the availability of novel separation materials, particularly small particle size resins. Many previous h.p.l.c. separations of trace metals have involved the formation of metal complexes prior to injection onto the column. This may prevent some species from being eluted and normally involves using quantities of organic solvents. Liska et al. [1] separated nine metals as their

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diethyldithiocarbamates, using LiChrosorb Si-60 (10 μ m) and 10% chloroform in cyclohexane as the eluent. Similarly, strong complexing reagents such as dithizone [2] and 8-mercaptoquinoline [3] have been used with pre-column preparation. Further examples of this approach have been discussed in a comprehensive review by Schwedt [4] of inorganic trace analysis by chromatography. Many environmental samples are dynamic in nature and injection without sample pretreatment has obvious advantages.

Takata and Muto [5] separated six metals on a cation-exchange resin column with tartaric acid as the eluting agent. The effluent was passed through a coulometric detector with the addition of electrolytes to stabilize the detector signal. While few laboratories have access to coulometric detectors, most h.p.l.c. systems incorporate a spectrophotometric detector. Thus the systems employing separation and then reaction with a colour-forming reagent perhaps offer more ready applicability. Careful choice of the reagent and wavelength should allow either selective or general detection with acceptable sensitivity. Other advantages of post-column derivatisation are that it is readily automated and that defined products and completed reactions are not essential. Significantly for environmental applications, the separation is now based upon the parent species.

Fritz and Story [6] found that arsenazo-I formed coloured species in a post-column reactor with calcium, magnesium and several other ions. Arsenazo-III gave coloured species with eleven metals and the lanthanides, whereas 4-(2-pyridylazo)-resorcinol (PAR) formed coloured species with sixteen metals [6]; PAR has also been used by Cassidy and Elchuk [7]. Kawazu [8] separated ten metals with a macroreticular resin (Amberlite 200) and again PAR was used to detect these species after a 35-min separation. Polystyrene cross-linked resins are increasingly being used because of their stability between pH 1 and 13. Kawazu and Fritz [9] separated cadmium and zinc on a macroreticular resin, using 1-(2-pyridylazo)-2-naphthol as a post-column reactant.

Beckett and Nelson [10] described a detector that offered improved sensitivity based on derivatisation with an ethylenediaminetetraacetic acid (EDTA) analogue; the EDTA molecule had an aniline moiety attached and the separated metal-ligand species reacted with fluorescamine in the postcolumn reactor to give fluorescent detection in the sub-picomolar range. However, pre-column derivatisation was necessary and the column separation was effected in acetate media.

Chromatographic systems also offer considerable potential for the investigation of metal speciation. Interest in differentiating inorganic and organic mercury in environmental samples led Gast and Kraak [11] to develop a method whereby the mercury species were separated on a reversed-phase column with acetonitrile and 0.1 M sodium bromide as the mobile phase and then detected with a dithizone post-column reaction system set at 480 nm.

In the work described here, a general detector was based on monitoring the decrease of absorbance of unreacted dithizone. This report is concerned with the separation of cadmium, cobalt, copper, lead, nickel and zinc and their detection by a post-column reaction. The current limits of detection and linear working ranges are reported and the possibilities of the method are discussed. The most successful approach was to separate the analyte species on a polystyrene-based cation-exchange resin with an aqueous tartaric acid eluent. Dithizone dissolved in acetone was added to the column effluent which then proceeded into a reactor coil prior to the detector. The decrease in absorbance at 590 nm was used to quantify the trace metal levels.

EXPERIMENTAL

Apparatus

A schematic diagram of the main components is shown in Fig. 1. A doublebeam u.v./visible spectrophotometric flow monitor (SF 770; Schoeffel Instrument Corporation, Westwood, NJ) was equipped with a tungsten lamp and the wavelength was set to 590 nm. A 1-cm cell was used with a 10- μ l volume. The pumps (Constametric III; Laboratory Data Control, Riviera Beach, FL 33404) were operated from a central microprocessor control unit (Control Command Module, L.D.C.). Samples were injected on to the cation-exchange resin (Aminex A9, 11.0 ± 0.5 μ m, Bio-Rad) in a 90 mm \times 5 mm column, with an injection valve (Rheodyne Model 7125; Rheodyne, Cotati, CA 94928) and a 100- μ l sampling loop.

The cation-exchange column was prepared by degassing the beads in a vacuum whilst in a beaker containing the eluent at the highest concentration to be used to separate the metal species. The eluent has also to be at the lowest pH at which it will be required to function. A standard packing pro-



Fig. 1. Schematic diagram of the apparatus. Solid lines represent liquid flows; dashed lines represent electrical connections. The ions are separated on column D (Aminex A9 cation-exchange resin) with the tartrate eluent pumped by pump A. The post-column reactant (dithizone) is pumped by pump B to meet the effluent in reactor coil E. Column C packed with silica gel is inserted into the dithizone line to provide back-pressure.

cedure was used except that the initial pressure was 500 psi which was raised to 5000 psi after 5 min, until 150 ml of eluent had passed through. In this instance the eluent was 0.4 M tartaric acid at its natural pH of approximately 2.4.

The eluent was met by a flow of dithizone as post-column reactant in a T-junction. A back-pressure was induced in the dithizone flow by a 100 mm \times 4 mm column packed with 5- μ m silica gel prior to the T-junction. A reactor coil (1.5 m \times 0.3 mm) was connected to the detector which incorporated a heat sink.

Reagents

Glass-distilled water was used throughout and all the chemicals were of analytical-reagent grade (BDH Chemicals). Stock solutions of 1000 ppm cadmium, copper, cobalt, lead, nickel and zinc were diluted as necessary for working standards. A typical standard solution of 10 ppm copper, 20 ppm cadmium and zinc, 30 ppm cobalt and 30 ppm nickel was prepared and dilutions of this mixture were injected. A separate solution was prepared for the separation of lead and nickel.

A stock solution of 0.4 M tartaric acid was diluted to 0.2 M with distilled water and an alkaline solution was added to give the required pH. The alkaline solution contained a 40:60 mixture of 2 M sodium hydroxide and 2 M ammonia solution, respectively. The post-column reagents were made up as needed for each experiment, but in all instances the dithizone, sufficient to give a required absorbance, was dissolved in acetone (80 ml) and diluted with 20 ml of distilled water. Solutions were degassed with helium.

RESULTS AND DISCUSSION

Preliminary considerations

Most h.p.l.c. methods have been concerned with the separation of organic species, and it is only in the last few years with the production of the necessary materials that h.p.l.c. has become an important area of study for inorganic species. Thus some of the more recent papers have been concerned with metal ions, which have been separated in several ways. Some of these involved separations on silica or reversed-phase systems, and the use of organic solvents and precolumn derivatisation was often necessary. The aqueous ion-exchange systems were preferred here.

Separations could potentially be performed on either a cation- or an anion-exchange resin normally in conjunction with a low-molecular-weight polycarboxylic acid. A cation-exchange column was chosen, as high-quality resins of small particle size are easily obtainable and adequate separations had already been demonstrated [5, 12]. Of the polycarboxylic acids considered, tartaric acid gave the best chromatographic performance with the Aminex A9 resin, producing relatively fast elution times with sharp peaks of good symmetry for all metals investigated, except for copper which always showed significant tailing. To obtain optimum separation, pH adjustment was required. Initially this was achieved with sodium hydroxide solution, but subsequent work showed ammonia to be a better base because nickel gave an increased response. Further investigations showed this to be a kinetic problem and that the reaction went to completion after a period of time. Unfortunately, however, after the addition of this post-column reactant, precipitation occurred and it was necessary to reduce the dilute ammonia solution to 60% of the neutralizing agent, the other 40% being sodium hydroxide. This compromise mixture of bases was sufficient to prevent precipitation up to a maximum eluent concentration of 0.2 M tartrate.

As dithizone is more soluble in acetone, which is easily miscible with water, it was decided to use this as the solvent for dithizone. The optimum conditions for the reactions with dithizone involved the following variables. At low percentage additions of the acetonic dithizone solution to the postcolumn reactor, the high water content of the cation-exchange column effluent caused precipitation of the metal dithizonates. At high additions of the reagent solution to the column effluent, the greater dilution resulted in a lower detection signal. Maximum response was found when, after the post-column addition, the absorbance at 590 nm was between 0.15 and 1.0 absorbance units in a 1-cm path length cell. At high absorbance, the noise contributed a large part of the signal when set to detect low quantities of metal ions. At low absorbance, the sensitivity was less. The pH also had an effect on the sensitivity and the greatest response was achieved when the final pH of the mixed reactant and column effluent was between 4.85 and 5.0, except for lead and nickel where the maximum response occurred at pH 5.2. Noise came from many sources and was high with dithizone solution additions less than 25% of the total flow. To reduce the noise from pump pulsing, a back-pressure was introduced by placing a column before the Tjunction in the post-column reactant system. The addition of 20% water reduced the compressibility of the acetone and increased the back-pressure, PTFE tubing also aided the buffering of the pulses from the pumps. Figure 2 shows the maximum sensitivity for four metals obtained by optimising the response in terms of pH, elution time, dithizone concentration and ratio of post-column reactant to column effluent .

Selection of working pH

Dithizone dissolved in aqueous acetone gradually changes from light blue through blue-grey to grey-brown from pH 4.0 to 5.3. This is accompanied by a decrease in the molar absorptivity of dithizone at 590 nm especially above pH 4.5. The colour changes to pink with the addition of a metal. A different pH is optimal for different metal complexes and three pH levels were therefore studied, each at high and low absorbances (about 0.8 and 0.25, respectively) of dithizone. The linearity of response, the working range and detection limits were then calculated. The usual chromatographic problem of a decrease in sensitivity by peak broadening with increasing



Fig. 2. Chromatogram showing the detection of low quantities at pH 5.2: F, 0.02 μ g of zinc; H, 0.03 μ g of nickel; I, 0.03 μ g of cobalt; J, 0.02 μ g of cadmium; no peak was obtained from 0.01 μ g of copper.

Fig. 3. Chromatogram showing the separation of: A, $0.5 \ \mu g$ of copper; B, $1.0 \ \mu g$ of zinc; C, $1.5 \ \mu g$ of nickel; D, $1.5 \ \mu g$ of cobalt; E, $1.0 \ \mu g$ of cadmium. Sample (100 μ l) injected on Aminex A9 (90 mm x 50 mm) at 60°C; 0.2 M tartrate eluent at pH 4.25; post-column eluent pH 4.8, with absorbance 0.613.

retention time being opposed by the need to retain resolution, was further complicated in this case, because the sensitivity of the detector and the retention times depended on the pH of the eluate. It was difficult to lower the pH by post-column addition, as metals were extracted by acid from the surfaces of the pumps and tubing. The dithizone also decomposed in the polythene container and therefore pH manipulation via the post-column reactant solution was considered too difficult. The variability of response for individual metals may offer the possibility of selective detection and a greater sensitivity for a required species but the concern here was for multi-element determinations. Therefore three pH values, 4.3, 4.8 and 5.2, were chosen for study in the post-column detector. At pH 4.3, only a 20% addition of the dithizone solution could be made to the analytical column eluate; more caused precipitation of the ammonium tartrate, which is less soluble in acetone than in water, and thus blocked the post-column reactor. Also the rhythmic pulsing of the pumps at this low percentage addition prevented the detection of smaller quantities of the metals. Consequently, the linear ranges possible when the post-column mixture gave an unreacted absorbance

of 0.25 at 590 nm were from 200 ng to 3 μ g for cobalt, copper and zinc, and from 200 ng to 1 μ g for cadmium. At this low pH the sensitivity to nickel is poor and lead does not respond.

The post-column reactor was at its most sensitive at pH 4.8–5.0. By adjustment of the dithizone concentration in the post-column reactant mixture, the working range could be extended. When the analytical column effluent was mixed in a 3:2 ratio with the dithizone solution in the post-column reactor and when this ratio was varied so that the absorbance of the final unreacted effluent at 590 nm was 0.2 at one extreme and 0.6 at the other, a working range for the five metals of 10 ng-20 μ g was obtained. The limits of detection (2 σ) in the order of their elution were 1 ng of copper, 6 ng of zinc, 8 ng of nickel, 4 ng of cobalt and 5 ng of cadmium.

Analytical performance

A comparison of the responses at pH 4.8 and 5.2 is shown in Figs. 3 and 4, where the absence of a copper peak and the generally decreased response at



Fig. 4. Chromatogram of the same solution as in Fig. 3 under the same conditions except that the 0.2 M tartrate eluent was at pH 4.55 and the post-column pH was 5.2, with absorbance of 0.49. Response to copper is negligible.

Fig. 5. Chromatogram under the same conditions as Fig. 3: K, 0.06 μ g of zinc; L, 0.093 μ g of nickel; M, 0.5 μ g of lead.

pH 5.2 can be noted (Fig. 4). At the higher pH, the linear working range was from 20 ng to 2 μ g for all metals except lead, with a lower limit of 100 ng and zinc with an upper limit of 1 μ g. Figure 5 shows the difficult yet quantifiable separation of lead from nickel. In contrast to the other metals, the response for lead and nickel was at an optimum at a post-column pH of 5.2.

An anomaly that occurred throughout the pH range was the slight negative curvature of the cobalt calibration graphs irrespective of the quantity injected. Copper gives a broad peak although it emerges first and this is more pronounced when the column is not heated, suggesting a kinetic interaction with the cross-linked sulphonated resin.

The ability of dithizone to react with many metals is well known and investigation into the detection of other metals is in progress. Bismuth, gold, indium, mercury, palladium, platinum, silver and tin react with dithizone, as do some of their organic derivatives [13]. Bismuth and indium were eluted on the solvent front from the column used at all pH values in the present study. Manganese and iron species reacted but with little sensitivity. In the case of iron(III), the response was probably due to loss of dithizone by oxidation. While manganese(II) may form a complex of low stability, the complex is susceptible to oxidation and decomposition at the pH used. The loss of dithizone by oxidation or by the formation of unstable complexes may be exploited by monitoring the decreased dithizone absorption as in this detector. The instability of some metal complexes may also be countered by the rapid air-free detection system reported here. The detector may also be adapted to the specific requirements of an operator, by pH adjustment or by altering the dithizone absorbance in the final eluted mixture.

Work is continuing on this promising approach to the h.p.l.c. of metal ions with a view to both improving the separation by studying other chromatographic systems and to exploiting the utility of this versatile dithizone post-column detector.

We thank the S.E.R.C. for the award of a grant towards equipment used in this work.

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Inverse Photometric Detector, Based on Eriochrome Black T, for Trace Metal Determination by High-performance Liquid Chromatography

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A simple post-column reactor based on Eriochrome Black T (EBT) has been developed to determine trace metals after separation on a silica-based cation-exchange column. Quantitative detection is performed by measuring the decrease in the absorbance of the post-column reactant. Quantitative analysis of nine metals in 16 min can be achieved. Linear calibrations up to 10 p.p.m. for cadmium, cobalt, copper, indium, iron(III), iron(III), magnesium, manganese, nickel and zinc are possible with limits of detection of 2-5 ng. The relationship of the cation ion-exchange chromatography and the organic acid eluent to the post-column reactor is discussed. The system was used to determine cobalt, copper, iron, manganese and nickel in standard alloys and the accuracy of the method is shown to be very good with coefficients of variation in the range 1-11%.

Keywords: Inverse photometric detection; trace metal determination; Eriochrome Black T; high-performance liquid chromatography; alloy analysis

A large number of investigations now require the determination of several elements in a single sample. In many instances the sample analysis time will be excessive if the elements have to be quantified individually. Thus there has been an increasing demand for sensitive techniques capable of rapid multi-element determinations. Atomic-emission spectroscopic techniques have always been capable of simultaneous multi-element analysis and highly sophisticated inductively coupled plasma (ICP) instruments are now available for the determination of as many as 80 elements. An alternative approach, involving much less capital cost and offering more scope for the investigation of chemical species is the use of liquid chromatographic techniques for rapid sequential multielement determination.

Until recently little attention had been focused on this area, particularly for the determination of groups of metals, as classical liquid chromatography processes using columns were very slow and no convenient sensitive on-line metal detection systems were available. The development of highly efficient column packing materials for use in high-performance liquid chromatography (HPLC) systems has considerably increased the speed of liquid chromatographic analysis and lately several workers have demonstrated the feasibility of using HPLC instrumentation for the rapid sequential analysis of groups of metal cations.

Most papers describe the use of ion-exchange systems to separate metal ions in aqueous media using polyfunctional carboxylic acids as eluting agents. Takata and Fujita¹ described the separation of seven metals on small particle size polystyrene-based cation-exchange resins using tartaric acid as the eluting system. Cassidy and Elchuk² used a citrate eluent to compare column packing materials for the separation of metal ions and their subsequent applicability to trace enrichment methods.

Metal detection systems still allow considerable scope for development and two approaches in particular have been successful. One involves electrochemical detection of metal complexes using coulometric principles³ and the other photometric detection of metal complexes.⁴ The latter approach appears to offer the most versatility, considering the large amount of information available on the photometric determination of trace metals. Metal cations separated on ionexchange material in aqueous media will elute from the column generally as carboxylic acid complexes with little or no absorbance in the UV - visible region. These metal species can be converted into highly absorbing complexes by introducing a

chromophoric reagent at the column outlet to mix with the effluent. After allowing sufficient time for reaction, the mixture is passed through the detector and the absorbance of the metal complexes monitored at a specified wavelength. Post-column reactor systems although adding to the complexity of the apparatus, allow a tremendous range of reagent type and conditions to be used and the determination of most metals, if chromatographic systems can be developed to separate them. Many of the systems reported in the literature measure the absorbances of the metal complexes formed in the post-column reactor. Unfortunately, for a particular group of metal complexes the wavelength maximum can vary over a wide range and it is difficult to choose a compromise wavelength to achieve optimum sensitivity for every metal. Another more novel approach is to monitor the decrease in the absorbance of the reagent as each metal reacts with it. This procedure was pioneered in our laboratories and a dithizone post-column reactor was reported that gave a good quantitative performance for six separated metal species.4 For good sensitivity and low detection limits monitoring based on measurement of the decrease in absorbance of the photometric reagent requires that the reagent itself absorbs very strongly and dithizone is ideal for this purpose. Dithizone is particularly suitable for the determination of the sulphophilic metals, but reacts slightly or not at all with many of the more oxophilic metals. It was, therefore, considered that a more general metal detection system was needed and a number of chelating agents were investigated for use in post-column reactors. Eriochrome Black T (EBT) looked particularly promising as it reacts with a wide range of oxophilic and

sulphophilic metals and is itself strongly absorbent at 610 nm. This paper describes the performance of an EBT postcolumn reaction system when used for the determination of a group of metals separated by cation exchange. Two certified reference metal alloy samples were analysed and the accuracy and precision of the detector assessed for both the major and minor constituents.

Experimental

Apparatus

A typical isocratic high-performance liquid chromatographic system was used (Fig. 1). A high-pressure pump (Constametric III, Laboratory Data Control, Riviera Beach, FL, USA) at a constant flow-rate was connected via an injection valve (Rheodyne, Model 7125, Rheodyne, Cotati, CA, USA) i



Fig. 1. Schematic diagram of the inverse photometric system, showing A, analytical column pump; B, post-ordiumn reagent pump; C, microprocessor: D, the pressure restrictor; and E, post-column reactor, coil. Solid lines represent liquid flows and dotted lines electrical connections

fitted with a 100-µl loop to an analytical column (250 mm \times 4.6 mm i.d. stainless-steel tubing).

The effluent from the analytical cation-exchange column (Whatman Partisil 10 SCX, Whatman Ltd., Maidstone, Kent) mixed with the post-column reactant at 180°C with the greater outlet flow adjacent to the inlets. The reactant was pumped (Model AA Dual Piston Eldex pump, Eldex Laboratories, Mento Park, CA, USA) through a pressure restrictor to reduce the pump pulsations. A reactor coil of 0.6 ml capacity and 0.5 mm i.d. connected

the T-junction to the double-beam UV - visible spectro-photometric flow monitor (Model SF770, Schoeffel Instrument Corp., Westwood, NJ, USA). The 1-cm length flow cell of 10 µl capacity was monitored at 610 nm.

Reagents

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Analytical-reagent grade reagents (BDH Chemicals, Poole,

Dorset) were used throughout. Stock Eriochrome Black T solution, 0.4 g per 100 ml of 0.1 M ammonia solution

Stock lactic acid solution, 2 M. Prepared by mixing the acid with distilled water at this concentration so that biological growths did not form.

Sodium hydroxide (2 M) - ammonia (2 M) solution (2 + 3). Used for pH adjustment.

Stock metal solutions. AnalaR grade metal (1 g) was dissolved in nitric acid to give a resultant 1000 p.p.m. solution in a 1 м acidity.

Preparation of Samples and Standards

The sample solutions were prepared by dissolving 1 g of the reference material (Bureau of Analysed Samples Ltd., Middlesborough) in a mixture of distilled water and AnalaR nitric acid (1 + 1). The resulting mixture was then made up to I I in a calibrated flask with sufficient nitric acid to give a 1 m concentration

Mixed metal standard solutions were prepared to give metal concentrations in the ratio similar to that given in the certificates of analysis. Dilutions were prepared from these standards as required.

Analysis of Magnetic Alloy

This was analysed in two series of injections, one at 100-fold dilution (10 p.p.m. nominal) for the major constituents copper, iron(111), nickel and cobalt and one at 20-fold dilution (50 p.p.m. nominal) for the manganese determination. The resultant chromatograms were compared with those obtained from mixed metal standards diluted to give similar sized peaks. The microprocessor was programmed to change the amplification immediately after the cobalt peak was eluted (8 min).

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Analysis of Monel Alloy

This was analysed in a similar way to the above with injections of the stock solution diluted 100-fold. Injections of more concentrated solutions were not necessary to determine cohalt, the minor constituent, as sufficient sensitivity was available. The microprocessor was programmed to change the amplification just before the cobalt peak was cluted (6.2 min).

Results and Discussion

Chromatography

Cation-exchange chromatography was considerably improved by the use of polyfunctional carboxylic acids in aqueous solution as eluents. Retention times were much shorter and the separation of ions with similar size and charge was enhanced. The speed of elution depended upon the fraction of the metal cation complexed with the carboxylic acid, which in turn depended on the magnitude of the metal stability constants, and the concentration of the carboxylate anion in the eluent.

The capacity of the cation-exchange material was also of prime consideration: high-capacity phases need carboxylic acids with larger metal stability constants to maintain short retention times. Thus cation-exchange materials based on high-capacity cross-linked polystyrene resins tend to be used with the more strongly chelating tartaric and citric acids.^{1,2}

For this work maximum efficiency was needed to try and separate up to ten metal species in as short a time as possible. Therefore, silica-based phases were investigated as they are more efficient than the resin-based for a similar particle size. Partisil 10 SCX is a 10-µm particle size silica phase with surface-bonded strong cation-exchange groups. The capacity of this silica phase is much lower than the Aminex resin used in previous work4 and so carboxylic acids such as tartaric and citric acids gave elution times that were too short and close to the solvent front to be of any value. A number of weaker chelating acids were studied, namely acetic, formic, lactic and succinic acids. Lactic acid was found to give particularly good results with the Partial 10 SCX material. The concentration of the lactate ion critically affects the elution time and, therefore, the concentration can be used to optimise the chromatography, in contrast to the other acids investigated where the concentration effect is smaller. Like most carboxylic acids. lactic acid is a weak acid and the concentration of the lactic anion depends on the pH. Therefore, for a given total concentration of acid, precise control of elution speed was achieved by pH adjustment. For this work, two nominal concentrations were used, namely, 0.05 and 0.1 M, and the pH was adjusted to between 3 and 4. The actual conditions chosen depended on the number of metal species to be separated and the capacity of the particular batch of Partisil 10 SCX. Batch to batch variations in the manufacture of high efficiency ionexchange materials can still be significant and it was found that a new batch sometimes required a different pH or even a different nominal concentration of lactic acid to achieve the same separation as the previous batch. The temperature of the chromatography column was a further consideration, as, in general, temperatures above ambient improved column ef-ficiency and peak symmetry. A temperature of 40 °C proved a reasonable compromise for the Partisil 10 SCX column. because although higher temperatures would increase efficiency a little more, this would be at the expense of reduced column life resulting from the increased solubility of the silica matrix.

Fig. 2 shows a chromatogram for nine metals obtained in 16 min, using 0.05 m lactic acid as eluent at a pH of 3.6. The column flow-rate was 1.5 ml min-1. The pH of the reactant mixture flowing through the detector cell was 9.9 and the absorbance 0.75 at 610 nm. Figs. 3 and 4 show the chromatograms obtained from the analysis of Magnetic and Monel Alloys, respectively, using 100-µl injections of 10 p.p.m.



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Time min Fig. 2. Chromatogram obtained wing a lactate elucin with a 10-bit injection of a solution containing 0.75 p.p.m. of cobdt, copper, iron(111), iron(11), magnesum, manganes, nickel and zine and 4 p.p.m. of cadmum, Column temperature, 40°C; postcolumn reagent flow-rate, 0.5 ml min 1; reactor coil, 6 ml; and full-scale deflection, 0.2 absorbance units

Fig. 3. Analysis of the Permanent Magnetic Allos BCS No. 384 Hycomas III. 0.1 st lactate cluent, pH 3.5, flowrate 1.5 ml min 4; a postcolumn addition, 0.7 ml min 4; absorbance, 0.77 at 610 nm, and full-scale deflection, 0.2 absorbance units amplified to 0.05 tull-scale deflection for manganese

nominal solutions. The conditions were the same as described in Fig. 2 except that the factic acid concentration was increased to 0.1 w to allow for the higher capacity of a new batch of Partosil 10 SCN. The detector sensitivity was set initially to 0.1 foll-scale deflection for the Monel Alloy and changed to 0.05 absorbance using the microprocessor, just before the cobah and manganese were eluted. Similar sensitivity changes were also used for the Magnetic Alloy with amplification before the manganese was eluted, but this time the manganese peak was too close to the background noise for accurate results; therefore, a separate manganese determination was carried out using a 50 p. p. m. nominal alloy solution (Fig. 3).

Fig. 4. Chromatogram of the Monel Alloy 400 using the same conditions as in Fig. 3. Absorbance, 0.54 at $610~\rm{nm}$

Detector Performance

Eriochrome Black T is one of the most frequently used metallochromic indicators for EDTA titrations and is known to react with aluminum, cadmium, cobalt, copper, indium, iron(II), iron(III), lead, magnesium, manganese, mercury, ittanium, zinc and the platinum metals. Within the pH range 7–11 the above metal ions produce a colour change from blue to red. Preliminary investigations revealed that although the indicator reacted with many of the above metals, both the molar absorptivities and the wavelength maxima of the resulting complexes varied over a wide range and so, as discussed previously, the cluted metals were detected by measuring the decrease in the absorbance of the EBT reagent. The most important parameters affecting detector performance were found to be the EBT concentration, lactic acid concentration and pH

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whereby low detection limits were achieved without excessive mainte the shore factors into account the following conditions were found to be a resonable condition were found to be a reasonable construction.

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pataadka se pue appendaaae si uonenes jo suuaianjaoa ap be accounted for in the certificate. The precision as shown we ton year zolle odi ai sommungai odi lla osusood gaizingine ton zi tion ranges vary over nearly three orders of magnitude. There was no certificate value tor iron and so the value was calculated by difference. The value found was lower, but this calculated by difference. applies to both major and minor elements whose concentuait se guigemoone vincluonted et sich bon voules onorhineo Hycomax III Permanent Magnetic Alloy analysis are shown in Table 1. The values found agree very closely with the together with the coefficient of variation. The results for the concentration (a mim) was then calculated for each clement out interspersed with mixed metal standards. The average For each alloy a 1-g sample was dissolved and, after

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Determination of Trace Metals by High-performance Liquid Chromatographic Separation and Spectrophotometric Detection

P. J. Hobbs, P. Jones and L. Ebdon

Department of Environmental Sciences, Plymouth Polytechnic, Plymouth, Devon, PLA 8AA

The recent renewed interest in the determination of metal species by liquid-chromatographic techniques is due, in part, to the availability of more efficient chromatographic materials and to the development of sensitive on-line metal detection systems. A number of approaches have been reported for the efficient separation of groups of metal ions including the separation of metal chelates in non-aqueous media using silica columns.¹ Because the majority of sample preparation methods result in aqueous solutions of the metals, it was considered that ion exchange might be a more generally applicable separation medium. Several sensitive metal detector systems have recently been described, some utilising electrochemical properties such as coulometry² and some utilising the spectrophometric properties of metal chelates.³ Many of these photometric systems are based on the addition of a reagent to the column effluent (post-column reactors) to produce strongly absorbing metal complexes, which are then detected at a specified wavelength. The optimum absorbance of metal chelates can vary significantly from metal to metal and in many instances it is difficult to choose a compromise

wavelength for the detector. A more novel approach outlined in this work is to measure the decrease in absorbance of the photometric reagent as a quantitative measure of the metal ion present. This paper describes the performance of two photometric post-column reaction systems coupled to an ion-exchange high-performance liquid chromatography (HPLC) system for the determination of metal ions in aqueous media.

Apparatus

A schematic diagram of the apparatus is shown in Fig. 1. The isocratic HPLC system consisted of a Laboratory Data Control (LDC) Constametric III pump, a Rheodyne injector valve with a 100- μ l loop, a steel column and a Kratos variable wavelength UV - visible spectro-photometric detector with a 10- μ l flow cell. A post-column pump was needed to supply the photometric reagent solution to mix with the column effluent. A short reaction coil followed the mixing T-junction to allow a sufficient time for the metal complexes to form before flowing through the detector cell. It was advisable to include a restrictor in the post-column pump outlet to reduce pump pulsations.



Fig. 1. HPLC apparatus with post-column reactor.

Chromatographic System

The factors controlling the separation of metal ions on cation-exchange media are reasonably well understood and, in instances where the metal ions have similar size and charge, the presence of relatively weak complexing agents in the elution system can considerably improve the separation. Using these principles Takata and Fujita? demonstrated the fast and efficient HPLC separation of six metal cations on small particle size cation-exchange resins using tartaric acid as the complexing agent. For this work two types of cation-exchange material were used (BioRad Aminex A9, 11 μ m polystyrene based resin, and Partisil 10 SCX, 10 μ m surface bonded silica based particles), and their performance was compared when used with a number of selected carboxylic acids. The chromatographic behaviour of the two materials was found to be similar, except that the silica based material had a much lower exchange capacity. To summarise, the elution order of the divalent metal species was essentially the same regardless of the eluting acid used (assuming -COOH and -OH were the only functional groups present) and retention times decreased with increase in pH or concentration of the acid. As a consequence of the higher exchange capacity of the Aminex A9 resin compared with the Partisil 10 SCX material, higher concentrations of more strongly chelating acids were needed to obtain similar retention times.

Detection System

Dithizone Post-column Reactor

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Dithizone is a well known photometric reagent reacting sensitively with up to 20 metals including a number of organometallic species. The reagent itself absorbs very strongly and was therefore considered particularly suitable for use in a post-column reactor system where the decrease in absorbance of the reagent was used as a quantitative measure of the separated metal ions. The main problem was the insolubility of dithizone and its complexes in aqueous media. This was overcome by using acetone - water mixtures in the post-column system. For

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most determinations the solution of dithizone in acctone was mixed with the column effluent to give a water to acctone ratio of approximately 4:1, the exact ratio depending on the concentration of the acid and the pH of the elution solvent.⁴ The dithizone concentration was adjusted so that on dilution with the effluent it would give a base-line absorbance at 590 nm of between 0.1 and 1. Any metal reacting with the dithizone would then produce a negative absorbance peak on the recording system. The sensitivity of the metals varies with pH and a reasonable compromise was found to be pH 4.9. Of the metals investigated, namely, Bi, Cd, Cu, Co, In, Ni, Pb and Zn, all gave good linear calibrations for up to 10 p.p.m. except cobalt, which showed a slight curvature under all conditions. For 100- μ l injections absolute detection limits were between 2 and 10 ng except for lead, which was 100 ng. An example of the separation of five metals followed by dithizone post-column detection is shown in Fig. 2 and was achieved on an Aminex A9 column (100 - 4.6 mm) using 0.2 M tartaric acid at a pH of 4.9 as the eluting agent.

Eriochrome Black T Post-column Reactor

Although dithizone is a particularly good reagent for the sulphophilic metals it reacts with few of the more oxyphilic metals. Other reagents were investigated to extend the range of metals that can be determined by this technique and Eriochrome Black T(EBT) was particuharly promising. Using the principle of measuring a decrease in the absorbance of the reagent as a means of detection, EBT was found to react with a wide range of metals including magnesium, manganese and iron. The post-column reagent, an aqueous ammonia solution of EBT, was mixed with the column effluent in the usual way. The concentration of EBT was



Fig. 2 Separation of five metals on Annuex A9 using dithizone postcolumn detection.



Fig. 3. Separation of nine metals on Partisil 10 SCX using EBT post-column detection and a lactate eluent.

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adjusted to give a background absorbance, at 640 nm, of between 0.6 and 0.9 at a pH of 10, For the metals Cu, Co, Cd, Fe(II), Fe(III), Mg, Mu, Ni, Pb and Zn the detection limits (100 μ 1 injections) were close to 10 ng except for magnesium and manganese, which were nearer 2 ng. The linearity of calibrations up to 10 p.p.m. was good. Fig. 3 shows the separation of Fe(11), Cu, Ni, Zu, Co, Fe(11), Cd, Mn and Mg at the parts

per million level on a Partisil 10 SCX column (250 - 4.6 mm) using factic acid as the eluting agent.

Conclusion

The dithizone detector is particularly suitable as a very sensitive system for the more sulphophilic metals. The EBT detector, although generally less sensitive, reacts with a wider range of metals including the more oxyphilic types and shows potential as a more general metal detector. To exploit this the chromatography will be investigated further to include some of the anionic metal species.

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Acknowledgements.

I would like to thank the following;

Dr P. Jones for his valuable supervision.

Dr L. Ebdon for his enthusiasm.

British Drug House(Poole) for acting as the collaborating establishment.

Dr E. Newman for his advice and travels to Plymouth.

Mr R. Evens and Dr L. Brown.

Mr T. Ingram for providing a quick computer repair service.

 $\ensuremath{\texttt{Mr}^{\ensuremath{\texttt{K}}\ensuremath{\texttt{C}}\ensuremath{\texttt{P}}\ensuremath{\texttt{e}}\ensuremath{\texttt{A}}\xspace$, Tonkin for help with reagent synthesis.

S.E.R.C. for a grant for which this work would not have been possible.

Devon L.E.A. for my salary.