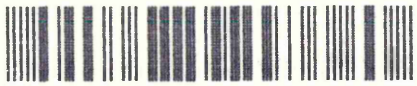


DETERMINATION OF TRANSITION METALS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

BY

LAILI RUSMAH MOHD NOOR BSc.

A thesis submitted to the Council for National Academic Awards in partial fulfilment of the requirements for the degree of Master of Philosophy

Sponsoring Establishment : School of Science
Sheffield City Polytechnic Division of Chemistry

September 1990

Determination of transition metals by high performance liquid chromatography

L. R. Mohd Noor

ABSTRACT

A simple chromatographic analysis system was developed based on dynamic ion-pairing using long chain anionic modifiers with a silica reversed phase based column. The aim was to carry out simultaneous multielement quantitative as well as qualitative analysis of samples containing mixtures of transition metal ions.

Among the problems encountered and solved were: metal contamination of the eluent, finding a suitable pump for the system, minimising the noise and reducing the elution times.

Detection of the separated metal ions was enabled by post-column derivatisation prior to introduction to the spectrophotometric detector. This introduced many problems as the eluent and post-column reagent stream must be properly mixed and reacted to ensure successful detection. The design and orientation of the device used for this purpose is also critical and this was studied in detail.

The role played by the various constituents of the eluent in the chromatographic separation was investigated thoroughly to enable modifications to it to be made so that the chromatographic analysis could be optimised. It was discovered that retention times were greatly affected by variations in concentration and type of anionic modifier, concentration and composition of the complexing agent in the eluent and variations in the pH of the eluent.

This enabled better understanding of the retention mechanism and a retention mechanism model for a dynamically coated column was proposed.

Separation of copper, lead, zinc, nickel, cobalt, cadmium and manganese in seven minutes was achieved using a mobile phase containing sodium hexanesulphate as anionic modifier, hydrogen tartrate as complexing agent at pH 3.1, with 4-(2-pyridylazo)resorcinol (PAR) detection monitored at 510 nm. Under these conditions, limits of detection in the range of 2 - 20 p.p.b. were achieved for 20 µL injections.

CONTENTS

		PAGE
CHAPTER 1 THEORY		
1.1	Introduction	1
1.2	Chromatography of heavy metal ions	5
	1.21 Pre-column derivatisation	6
	1.22 On-column derivatisation	7
1.3	Reversed-phase chromatography	11
	1.31 The stationary phase	13
	1.32 The mobile phase	15
1.4	Reversed-phase Ion-pair chromatography	15
1.5	Mechanism of reversed-phase ion-pair chromatography	16
	1.51 The ion-pair model	17
	1.52 The ion-exchange model	18
	1.53 The ion-interaction model	18
1.6	The detector	20
	1.61 Detector noise	22
	1.62 Limit of detection	23
	1.63 The linear range	24
	1.64 UV\Vis detectors	24
	1.641 Multi-channel detectors	27
1.7	Detection of separated metal ions or chelates	30
	1.71 Other methods of detection	31
	1.72 Post-column derivatisation	38

1.8 Post-column derivatising agent 40

1.81 General properties of 4-(2-pyridylazo)resorcinol 41

1.82 PAR Solution stability 49

1.9 Detection after post-column derivatisation 51

1.10 Aims of this work 53

CHAPTER 2 EXPERIMENTAL

2.1 Reagents 54

2.2 Columns 55

2.3 Equipment 56

2.31 Sample injector 56

2.32 Pumps 57

2.33 Mixing device 59

2.34 Detector 59

2.35 pH measurement 60

2.36 UV/Vis spectrophotometer 60

CHAPTER 3 PRELIMINARY INVESTIGATIONS

3.1 Introduction 61

3.2 Pumping systems for reagent addition 62

3.21 System A 62

3.22 System B 66

3.23 System C 67

3.3 Extra-column effects 67

3.4 Effect of tee-piece design and orientation 68

3.41 Design 68

3.42 Bore size 69

3.43 Orientation 70

3.5 Modifying the column 71

3.51 Advantages of C_6 over C_{12} as the ion-pairing agent 72

3.52 Effect of varying C_{12} concentration 72

3.6 Stability of the PAR reagent 73

3.7 Choice of monitoring wavelength 75

3.8 Effect of variation of monitoring wavelength 76

3.9 Initial detection 78

3.10 Preliminary separation of copper and cobalt 80

3.11 Preliminary calibration of copper and cobalt 82

CHAPTER 4 OPTIMISING SEPARATIONS

4.1 Introduction 84

4.2 Eluent Strength 85

4.3 Multielement capability 87

4.31 Quantitative determinations 90

4.32 Variations in eluent flow rate 92

4.4 Effect of variations in the pH of the mobile phase on the retention behaviour 94

4.41 Method I 95

4.42 Method II 96

4.5 Effect of variations in the concentration of sodium 98

4.6 Model for fixed site ion-exchangers 99

4.61 pH effect 101

4.7 Model for a dynamically coated column 103

CHAPTER 5 CAPABILITY OF CHROMATOGRAPHIC METHOD

5.1 Introduction 106

5.2 Determination of trace amounts of metal ions 106

5.3 Linear range 110

5.4 Limit of detection 113

CHAPTER 6 CONCLUSION 116

CHAPTER ONE

THEORY

1.1 INTRODUCTION

The most popular technique for quantifying trace metals is atomic absorption/emission spectroscopy and especially the inductively coupled plasma. Yet, when several elements have to be determined separately in many samples, essentially single element techniques eg. atomic absorption spectroscopy become very time consuming and multi-element techniques eg. the inductively coupled plasma are very capital intensive.

The advantages of the chromatographic method of analysis are well known :

speed - where multielement analysis per sample is often accomplished in less than an hour.

resolution- which can always be compromised for speed and gives the researcher the ability to resolve interferences

and finally,

automation- which is straightforward and adds another dimension to problem-solving efficiency where routine operations are performed.

In addition, it is uniquely powerful in solving problems involving ions in solution where very high sensitivity is required and where matrix problems cause interferences for other analysis techniques because it physically separates compounds prior to detection.

It is also possible to chromatographically separate, detect and identify ions of the same element but which exist in the sample in different oxidation states eg. Cr III/Cr VI, Fe II/Fe III.

Most metallic compounds or moieties as a direct result of their ionic nature (in the solid or liquid phase) cannot be directly volatilized at the temperatures used in commercially available gas chromatograph. Although a lot of work has been done in this field, early studies of the chromatographic behaviour of metals were mainly centered on liquid phase techniques such as adsorption, partition or ion-exchange modes (either in the planar or column forms) [1].

However, thin layer chromatography and the 'classical' column chromatography involve long elution times and tend to lack quantitative capability.

The first paper on ion chromatography which appeared in 1975 was on work done by Small et al [2]. They developed a patented apparatus capable of routine qualitative and quantitative determination of a range of alkali and alkaline earth ions (eluent; hydrochloric acid) and anions (eluent; sodium hydroxide or sodium phenate) in a diversity of background.

The set up consisted of two ion-exchange columns; the first separates ions into discrete bands and the second suppresses the background electronic signal from the eluent as well as enhancing the electronic signal of the separated bands thus enabling detection by monitoring the conductivity of the column effluent. Elution time was between 1.0 to 3.0 minutes per ion. The two significant aspects resulting from their work were :

A The development of a reproducible, low capacity high efficiency anion-exchange resin with selectivities identical to totally aminated materials but with better permeabilities thus permitting the use of higher flow rates. (The

cation-exchange resin used having been previously described by Small [3].)

B The development of post-column techniques (suppression) which permit the use of conductivity detectors under ideal conditions.

The major disadvantage with this method is the need to periodically regenerate the suppressor column. There will also be variations in retention time of some peaks with suppressor exhaustion. In addition some metal ions may be precipitated by the hydroxide form of the suppressor column hence limiting its application to metal ions and especially transition metal ions.

Subsequently Gjerde, Fritz and Schmuckler [4,5] introduced the one-column method for the analysis of anions. The careful choice of separation column and eluent (must have low background conductivity hence a weak acid is preferred eg. phthalic acid rather than hydrochloric acid) allows the elimination of the suppressor column. The pH of the eluent needs to be kept within a specified range so that the eluent is sufficiently ionised to perform the separation as well as to keep the concentration of H^+ and OH^- ions low (since these ions have unusually high conductance).

The drawback with this system is thus the limited choice of the phase system and the need to keep the temperature constant since conductivity varies with temperature. There will also be a loss in sensitivity due to matrix effects since the ion is being measured in a more conductive background.

This method of analysis is also not suitable for heavy metals eg. transition metal ions which due to their strong binding to ion exchange sites require stronger acids or else will take too long to elute. Work was therefore almost entirely focussed on anions and alkali and alkaline earth cations.

1.2 CHROMATOGRAPHY OF HEAVY METAL IONS

Chromatographic analysis of heavy metal ions have been carried out in a variety of different ways. Most ,tend to employ chelating or complexing agents either to overcome the strong attraction between cations and the resin, to keep metal ions in solution and/or to improve the selectivity of the chromatographic method. Its popularity is reflected in the rapidly increasing number of publications and appearance

of reviews [6] and books [7,8] in this area.

Metal ion derivatisation may be performed either before injection of sample (ie. pre-column) or complexing agents may be incorporated into the eluents and complexation occurs in-situ (ie. on-column).

Metal complexation reactions tend to occur more efficiently in an organic media hence organic mobile phases are preferred. In both instances, the concentration of the reagent has no effect on retention times, indicating that the process is effectively derivatisation rather than an equilibrium ion-pair formation [9,10,11].

1.21 Pre-column Derivatisation

Guided by the number of reports employing chromatographic separation of metal chelates, this method seems to be more popular than on-column derivatisation. This may be due to problems which may be encountered by the latter method, namely those associated with the rate of formation of the chelate/complex ie. the kinetics of the reaction and amount and/or concentration of derivatising agent to be

incorporated in the eluent. In this mode it is possible to work with a large excess of reagent as there is no difficulty in eliminating the excess.

HPLC of metal chelates formed prior to introduction to column has been extensively reviewed by Willeford and Veening [12].The main drawback of this method is that it requires multiple steps before analysis and hence tend to be time consuming and inconvenient.

1.22 On-column derivatisation

It seemed that it should be possible to carry out an on-column reaction to give the same products by injecting an aqueous solution of metal ions into an eluent containing the appropriate reagent. This method has similarities to ion-pair chromatography but the equilibrium strongly favours the complex.

Fritz et al [13,14] suggests the use of organic eluents with low equivalent conductance (for example ethylene diamine for ion exchange competition) with the addition of complexing organic anions (eg. tartrates or hydroxoisobutyrate for complex formation) to the

eluents in conjunction with a cation-exchange column. As a result of which the sharpness of separations were improved.

Tartrate was found to be a better complexing agent than hydroxoisobutyrate. As the concentration of the tartrate anion in the eluent was increased, retention times was observed to decrease. Also, eluents containing tartrate but no ethylenediammonium salt were ineffective for elution of the transition metal ions. This indicates that the elution mechanism is a combination of mass action 'pushing' effect of the ethylenediammonium cation and weakly complexing or 'pulling' effect of the tartrate anion.

As the pH of the eluent was increased, retention times decreased due to the greater complexing ability of the tartrate. Complexing ability of the tartrate was lost at a pH value of approximately 3. This is thus the lower limit of the practical pH range.

This particular method however still utilises conductimetric detection. Secondly, it employs ion-exchange chromatography which suffers from the following shortcomings :

1. The organic supports used are not pressure resistant and tend to swell in certain organic eluents.

2. Column efficiencies are low due to the large particle size.

Bond and Wallace [15] investigated a chromatographic method for simultaneous as well as automated determination of metal ions involving the on-column formation of dithiocarbamate complexes, employing either spectrophotometric or electrochemical detection. Separations were achieved on a silica based C_{18} reversed-phase column and mobile phase consisting of an aqueous solution containing various amount of methanol and /or acetonitrile.

Limits of detection of 1 ng per 10 µL injected were achieved with spectrophotometric detection. This was improved when an ion-exchange based suppressor column was added to remove excess dithiocarbamate ligand prior to detection but is deleterious for determination of lead and cadmium and decreases the period of time over which the automated instrument can be run unattended.

It would therefore, be more desirable and advantageous to develop single column (suppressorless) methods because :

1. Decreased complexity of instrumentation would result in increased reliability. This is especially important for the development of an automated and relatively maintenance-free chromatographic method.

2. Elimination of the suppressor column will reduce the dead volume hence result in faster analysis and enhanced resolution.

Smith and Yankey [16] described a reversed-phase chromatographic method whereby dithiocarbamates were incorporated in the methanol-water eluent. The transition metal ions were then detected spectrometrically at the optimum wavelength for each complex (320 - 450 nm). This method is recommended for metal ions in the 1-10 ppm range.

1.3 REVERSED-PHASE CHROMATOGRAPHY

In reversed-phase chromatography, the stationary phase is by definition less polar than the mobile phase. The mobile phase is normally water, with or without added salts or modifiers and the stationary phase typically,is hydrocarbonaceous in nature (octadecylsilane (ODS or C_{18}) or octylsilane (C_8)). These are relatively more stable in contact with aqueous eluents having pH values less than 8 compared to covalently bound polar functions. This is because it results in a thin greasy film being covalently attached to the silica backbone. The oily layer would not be soluble in aqueous or mixed aqueous eluents even in the absence of covalent bonds to the surface. As a result of this it is capable of shielding the silica from water and hence protects it from hydrolytic degradation to a much greater extent than do other functional groups.

Reversed-phase chromatography is thus preferred for the following reasons :

1 Its applicability to the separation of ionic or ionisable compounds by manipulating secondary chemical equilibria such as ionisation control and ion-pairing in the aqueous mobile phase.

2 The general rapidity of mobile phase - column equilibration as a result of weak surface attraction energies of the non-polar stationary phase.

3 Its general ease of use.

4 Elution order is reproducible and often predictable as retention time usually increases as the hydrophobic character of the solute increases.

5 Aqueous eluents having high optical transparency can be used. This is especially important when detection involves spectroscopic monitoring of the column effluent.

Its limitations are :

1 The bonded phase columns are only usable over a limited pH range ie between 2 to 7.5. However most separations can be achieved within this pH range hence this is not a serious handicap.

2 The presence of unreacted accessible silanols on the silica surface which in addition to causing

chemical instability, can also result in poor peak shape and irreproducibility of retention times between columns due to solute adsorption. This undesirable effect can, in many cases, be overcome by endcapping.

1.31 The stationary phase

Polymer columns have high chemical stability and can withstand extremes of pH but have not gained wide acceptance because efficiency of separations are often low and the softness of the material make them difficult to handle [17].

Polystyrene-divinylbenzene beads are more rigid and surface sulphonation of the resin produces cation-exchange resins. Separations of polar samples on such columns would be expected to tail less since the surface of the polymer is free from silanophilic interactions. Such resins would also have the added advantage of faster mass transfer. It is however more difficult to pack stable column beds with small particle resins [18] and the particle size of commercially available material is large. The capacity

of these resins also tend to be low. Elchuk and Cassidy [19] compared the performance of bonded phase strong acid ion-exchangers with that of polystyrene-divinylbenzene for the separation of lanthanides and concluded that bonded phase resins were slightly more efficient than the polystyrene-divinylbenzene resins even at room temperatures.

Silica gel is probably the best known aerogel and the technology of controlling pore size and pore size distribution in a wide range is available. Silica can be produced in bulk and subsequently crushed and size graded to obtain uniformly sized, irregularly shaped particles. On the other hand, microspheres of silica gel having controlled pore size can be grown to obtain a quasi-monodisperse product which may not require size grading.

Column efficiency, to a great extent, depends on the particle size and size distribution as well as on the pore structure and surface properties of the stationary phase. Silica based hydrocarbonaceous bonded phases therefore have the greatest potential in RPHPLC [20].

1.32 The mobile phase

Generally the higher the polarity the lower the strength of the eluent at a given set of conditions. Therefore neat aqueous solutions without organic solvent have the lowest eluent strength. Hence polar mixtures are usually separated in this medium. A buffer is normally incorporated to the eluent in order to maintain ionic strength and constant pH. The pH of the eluent affects the degree of ionisation of a solute and hence the retention times (ion suppression).

The retention behaviour can thus be manipulated and selectivity enhanced by varying the eluent composition and/or pH.

1.4 REVERSED-PHASE ION-PAIR CHROMATOGRAPHY

For reversed-phase ion-pair separations, anionic or cationic molecules containing hydrophobic functionalities are added to the mobile phase of a conventional reversed-phase separation. Anionic modifiers such as alkylsulphates and alkylsulphonates are used for separation of cations and

cationic modifiers such as quaternary ammonium salts and tertiary amines for anions. The observed effect often is a dramatic retention for otherwise unretained species due to the formation of an electrically neutral ion-pair. The interaction of solutes with simple counter-ions is basically by electrostatic forces and is relatively non-specific in nature. The separation selectivity obtained in these systems are essentially due to differences in the solute themselves.

Ion-pair chromatography is based on a liquid-liquid partition technique in combination with ion-exchange chromatography. Its application in many procedures for the isolation and determination of organic compounds especially drug extraction (Soap Chromatography) is well known. Its rapid acceptance as a new HPLC method owes much to the work of Schill and coworkers [22,23] and to its unique advantages. In recent years, it has also become an important technique for the systematic control of the separation of inorganic ions for example metal ions [21,24].

1.5 MECHANISM OF REVERSED-PHASE ION-PAIR CHROMATOGRAPHY

The exact mechanism to describe the ion-pair phenomenon is

still uncertain. There are three popular hypotheses. Two models propose extreme situations and each covers a substantial amount of chromatographic data. These two proposals are the ion-pair model and the dynamic ion-exchange model [25]. A third view, which is broader in scope than the previous two concepts accommodates both extreme views without combining the two models. This is the ion-interaction model.

1.51 The ion-pair model

The ion-pair postulate stipulates that the formation of an ion-pair occurs in aqueous mobile phase before it is adsorbed onto the bonded,hydrophobic stationary phase [26,27]. Retention is governed by the amount of non-polarity of the 'ion-pair' which determines the affinity to the stationary phase. A longer alkyl chain on the pairing agent simply makes a less polar ion-pair and the retention of the pair increases as a result of its greater affinity for the stationary phase.

1.52 The ion-exchange model

A second view stipulates an ion-exchange mechanism [28,29]. In this hypothesis, it is unpaired lipophilic alkyl ions that adsorb onto the non-polar surface causing the column to behave as an ion-exchanger. The longer the chain length of the ion-pairing agent the greater the surface coverage of the 'ion-exchanger' the longer the retention of the ionic sample.

1.53 The ion-interaction model

More recently an ion-interaction mechanism has been proposed by Bidlingmeyer et al [30] which is less restrictive than the two previous models. This is based upon conductance measurements which show that ion-pairs do not form in the mobile phase. Neither the ion-pairing nor the ion-exchange models can explain the data in a consistent way. Instead, the results suggest a retention mechanism that is broader in scope and is best described as one of ion-interaction. The ion-interaction mechanism does not require ion-pair formation in either phase neither is it based on classical ion-exchange chromatography. It assumes

dynamic equilibrium of the lipophilic ion resulting in an electrical double layer forming on the surface. The retention (or lack thereof) results from an electrostatic force due to the surface charge density provided by the reagent ion and from an additional 'sorption' effect onto the non-polar surface.

The mechanism of ion-pair separations is further complicated by the rearrangements of the alkyl bonded stationary phases commonly employed for RPHPLC. In water, C_{18} bonded phases assume a folded configuration in which the alkyl chains preferentially associate with each other rather than with the aqueous phase. However in less polar solvents, the alkyl chains are solvated and adopt a 'bristle' configuration in which the alkyl chains extend out into the solvent. Hence the counter-ions used for ion-pair separations may also influence the structure of the surface in addition to interacting with the ions being separated.

Recent fluorescence study of ion-pair interactions on hydrocarbon bonded surfaces by Dowling and Seitz [31] has provided direct evidence for surface charge density effects and surface rearrangements which affect the extent of lipophilic interactions between an organic

solute ion and a hydrocarbon bonded surface. The results show that the ion-pair and dynamic ion-exchange models are oversimplifications and to confirm that the more complex ion-interaction approach is required to adequately explain the mechanism of reversed-phase ion-pair liquid chromatography.

The debate as to the exact model to describe the ion-pair phenomenon will no doubt continue. Difficulties in devising a model originate from conflicting conclusions based upon a large amount of experimental data. However, it is vital to stress that theory guides experimentation. Therefore the significance of having a model is to perceive the factors that control chromatographic retention and thus aid the speedy and logical development of separations.

1.6 THE DETECTOR

The function of any detector employed in HPLC is to monitor accurately the concentration or amount of the sample components eluted from the column.

Generally the following requirements are deemed necessary:

1. No remixing of components as they pass the detector.

2. Fast response time to accurately record rapidly eluting peaks. Separating bands as seen on the recorder can be significantly broader (and peak height reduced) relative to those actually observed as a result of slow detector response.

3. Low noise and drift level to enable detection of small amounts of solute.

4. Insensitive to flow rate changes, pulsations and temperature variations. This is especially required to enable precise quantitative analysis.

5. Detector response should increase linearly with the amount of solute and have a wide linear range.

6. Have high sensitivity and the same predictable response.

7. Respond independently of the mobile phase.

8. Not contribute to extra-column broadening. This

relates to the dynamic relationship of the column and the detector. Detector cell cavities should be kept smooth with no unswept volumes (dead volumes) that can cause band tailing. It is often desirable that detector cells be able to operate under moderate pressures (5 - 10 atm). Fittings on the detector and in the high pressure flow system should be airtight to prevent diffusion of air into the mobile phase.

9. Easy to operate and reliable.

1.61 Detector Noise

This is defined as any disturbance in detector output that is not related to an eluted solute. Short term noise produces a 'fuzzy' baseline as a result of signal fluctuations. It arises from the electronics associated with the detector or recorder or due to the pump pulsations. The measurement of short term noise is important because it is used to define the limit of detection of a system.

Long term noise is due to random and low frequency

variations of the output signal. The resulting baseline is erratic and the signals formed by this type of noise cannot be differentiated from a component peak of similar amplitude. It is caused by temperature and pressure fluctuations or impurities.

Drift results in a continuing increase or decrease in detector signal and is due to changes in mobile phase or temperature.

However many problems with apparent detector noise and drift are actually a function of the total LC system (ie. solvent impurities, temperature variations etc...) rather than being inherent detector limitations.

1.62 Limit of Detection

This is the minimum amount of solute that can be detected. The limit of detection is measured with respects to short term noise level and is often defined as the amount of solute that will produce a signal equivalent to twice the short term noise level.

The concentration of solute that produces a signal

equal to the noise level is the noise equivalent concentration.

1.63 The Linear Range

The linearity is determined by plotting the log of the detector response against log of the concentration of a typical solute. The gradient of such a plot would be exactly unity for a perfectly linear detector response. Knox [32] defined the useful linear range of the detector as the range of solute concentration over which the response index lies between 0.98 and 1.02.

1.64 UV/VIS Detectors

The UV detector was one of the earliest HPLC detectors and still is the most popular, partly because any solute with a UV absorption can be monitored.

The solutes are detected by measuring the amount of light that is absorbed as it passes through the flow cell. The concentration of the solute is determined from Beer's Law which states that for monochromatic

light, the fraction of radiation absorbed is proportional to the number of absorbing molecules.

$$A = \log I_o/I = \varepsilon c l$$

where

A = Absorbance

I_o= Incident light intensity

I = Transmitted light intensity

ε = Molar absorptivity [m^2 mol^{-1}]

c = Solute concentration [mol m^{-3}]

l = Path length of flow cell [m]

Therefore the sensitivity as defined above is proportional to the path length of the flow cell, provided noise is independent of the path length. The design of the flow cell is thus of extreme importance as turbulence, band dispersion and cell volume all affect the overall performance of the detector.

Fixed wavelength detectors were among the first type to be developed commercially and are among the cheapest forms of UV detectors currently available. Low pressure mercury lamps gave a range of discrete wavelengths and the 254 nm line is the most predominant. This serves as

an excellent source for a HPLC detector because a very large number of organic molecules absorb at this particular wavelength.

The more recent fixed wavelength detectors use deuterium lamps which provide a continuum of radiation rather than a line source. When used with a good quality filter, any solute absorbing in the wavelength range 180 - 400 nm can be detected.

Next came the variable wavelength instruments. The desired wavelength is obtained by manual operation of a diffraction grating and a deuterium lamp generally provides the source of radiation. The advantage here is that the selectivity can be enhanced by choosing the wavelength at which the solute exhibits maximum absorption.

However it has recently been shown that fixed wavelength detectors even when operated at a wavelength that does not coincide with the absorption maximum of a solute will give greater sensitivity than a variable wavelength detector because they produce less background noise and they also achieve baseline stability faster [33].

Detectors that operate solely in the visible wavelength range of 400 - 800 nm are available, but generally manufacturers tend to produce instruments that cover the UV and VIS range. The sensitivity of these detectors can be enhanced by switching to a tungsten source.

A number of manufacturers fit a heat exchanger (ie. a length of narrow bore steel tubing) to the flow cell to minimise the temperature variations. The heat exchanger is fitted to the inlet side of a flow cell and this effectively increases the cell volume thus a decrease in flow cell sensitivity and detection efficiency results but there will be less drift and lower noise levels.

A useful property of the UV/VIS detector is that it is non-destructive and therefore can be coupled together in series with any other monitoring device.

1.641 Multi-channel Detectors

The attractiveness of UV/VIS detectors can be increased manyfold if the analyst is not limited

to one wavelength and if the entire spectrum of the solute can be obtained. For modern high-efficient liquid chromatography this requires a fast scanning spectrometer.

An excellent review of multielement detectors has been published by Talmi [34]. The development of two rapid scanning spectrometer for use as liquid chromatographic detector systems has been described by Dessy et al [35]. Denton and coworkers [36] gave an early demonstration of the greater flexibility offered by the rapid scanning detector in HPLC. Using a commercial oscillating galvanometer mirror with grating dispersion optics and fast response photomultipliers, three-dimensional plots of A,t & λ chromatograms for model systems were obtained, while the normal elution chromatograms could be presented at stationary wavelengths optimised for each component.

McDowell and Pardue [37] described application of a silicon target vidicon tube, which uses a grating polychromator to disperse the eluate transmission spectrum across the front surface of a silicon

intensified vidicon detector, as a multiwavelength detector and describes [38] the system and its performance characteristics in more detail. Some applications illustrating the advantages of multiwavelength detectors in general are discussed.

Fell [39] outlines the mode of operation of vidicon detectors. A major advantage of the vidicon tube device is their ability to integrate radiation intensity with time, thanks to their charge storage characteristics.

These examples however involve the use of extensive computer facilities and expertise thus limiting the more general use of vidicon detectors outside research establishments. This is not so with linear diode array (LDA) detector.

Until recently, the LDA required cooling for acceptable operation; however the recent commercial LDA detectors not only operates at ambient temperatures but also does not require external computer resources and relies on microprocessor for control and data processing.

The LDA detector is particularly useful when chromatographic bands cannot be efficiently resolved as it permits deconvolution of the overlapping bands by applying the principle of "over-determination" and least mean squares maximum probability statistics. This technique involves finding the best statistical fit of linear combinations of each component spectrum stored in a reference archive, using all spectral datum points within a specified wavelength interval.

It does however, pre-suppose that each component is known and available in purified form for storage in the spectral archive.

1.7 DETECTION OF SEPARATED METAL IONS OR CHELATES

Traditionally UV/VIS detectors have been used for the analysis of absorbing species. Direct photometric detection of most inorganic species is difficult because of the low wavelengths at which they absorb. However, recently developed techniques has enabled greater exploitation of

this detector and extended its use to ionic non-absorbing species; notably by the use of pre-, on- and post-column derivatisation techniques employing colourimetric reagents.

The first of these methods, reported by Denkert et al [40] was based on the detection of non-absorbing ions by forming ion-pairs with a UV absorbing component dissolved in the eluent.

Small and Miller [41] introduced a method named indirect photometric chromatography (IPC) in which a small amount of UV absorbing compound for example a phthalate is dissolved in the eluent and any non-absorbing ion eluted from the ion-exchange column will produce a negative peak owing to the displacement of the UV absorbing ions from the eluent. This technique has become the basis for the development of other techniques for the detection of a wide variety of inorganic anions and cations [42-46].

1.71 Other methods of detection

Yakata and Muto [47] have developed a flow coulometric detector which has been shown to be suitable for inorganic and organic ions, including metal ions. Radiometric detectors were applied by Sisson et al [48]

and Huber et al [49] and conductometric detection has been applied by Fritz and coworkers [50] and Small et al [2] for the analysis of heavy metal ions.

Comparison of spectrophotometric and electrochemical detectors for the determination of nickel and copper by Bond and Wallace [10] showed that electrochemical detectors were generally more sensitive and by application of the right waveform, was more selective. However less maintenance was associated with spectrophotometric detectors. With electrochemical detectors, suppressor columns need to be cleaned regularly. Day to day reproducibility was also higher with spectrometric detection.

Fluorescence detectors are becoming more popular because of their selectivity and sensitivity. Selectivity means that the compounds of interest can be readily distinguished from a complicated matrix of compounds which do not exhibit fluorescence.

Fluorescence is a luminescence phenomenon that occurs when a compound absorbs radiation then emits it at a longer wavelength. Hence it is possible to irradiate a fluorophor (a solute that will produce fluorescence)

that absorbs strongly in the UV region and observe the fluorescence in the visible region. As the irradiating light can easily be removed, the measurement of fluorescence is obtained, in theory, against a zero background. This is different to UV monitors which measure small differences between the incident and transmitted light intensities. Therefore fluorescence detectors are two to three orders of magnitude more sensitive. Sensitivity has been shown to be 10^{-11} g or lower parts per billion.Both fixed wavelength and scanning fluorescence units are offered.

The choice of solvent used in the separation process is very important when using fluorescence detection. Fluorescence is very sensitive to certain deactivating species, or quenchers. Eluents containing highly polar solvents, buffers or halide ions should be avoided as they promote fluorescence quenching. Further, molecular collisions of the solute also quench fluorescence, high temperatures should not be used, and eluents with high viscosities are preferred in order to reduce such collisions.

Beckett and Nelson [51] described a detector that improved sensitivity based on pre-column derivatisation

of Pd, Cd and Zn with an ethylene-diaminetetraacetic acid (EDTA) analogue and the separated metal-ligand species was reacted with fluorescamine in the post-column reactor to give fluorescent detection in the sub-pico molar range. Separation was affected in acetate media.

A paper by DiCesare and Ettre [52] describes new techniques which enhance the applicability of the fluorescence detector by improving its selectivity. These techniques involve post-column acid-base manipulations, wavelength selection and the use of synchronous scanning for qualitative verification.

Some application of these methods of detection to heavy metal samples have been tabulated under the following headings :

TABLE 1 Methods employing pre-column derivatisation

TABLE 2 Methods employing post-column derivatisation

TABLE 3 Methods not employing post- or pre-column derivatisation

TABLE 1

PRE-COLUMN DERIVATISATION

SAMPLE	DERIVATISING AGENT	DETECTION	REFERENCE
Co, Ni & Fe	α- PAN - 4S	Spectrometric	H.Wada, S.Nezu, T.Ozawa & G.Nakagawa. Journal of Chromatography 295 [1984] 413
Transition Metals & Lanthanides	4-(2-Pyridylazoresorcinol)	Amperometric	D.A.Roston Analytical Chemistry 56 [1984] 241
Hg, Cu, Ni Co & Pb	Dithiocarbamate & Dithizone	Spectrometric	E.B.Edward - Inatimi Journal of Chromatogrphy 256 [1983] 253
Mn, Co, Cr, Be, Rh, Ru, Pd & Pt	β -diketone	Spectrometric	R.C.Gurira, P.W.Carr J. of Chromatographic Sc. 20 [1982] 461
Ni, Fe, Cu, Co & Hg	Diethyldithiocarbamate	Spectrometric	Y.T.Shih, P.W.Carr Anal. Chim. Acta 142 [1982] 55
Transition Metals	Diethyldithiocarbamate & Dithizone	Spectrometric	J.W.O'Laughlin, T.P.O'Brien Anal. Letters A11 10 [1978] 829
Hg, Cu, Cd, Pb, Cr, Ni, Bi & Co	Diethyldithiocarbamate	Spectrometric	M.Moriyasu, Y.Hashimoto Anal. Letters A11 10 [1978] 593
Pb, Cd & Zn	Flourescamine	Flourescence	J.R.Beckett, D.A.Nelson Analytical Chemistry 53 [1981] 909
Transition Metals	Dithiocarbamic acid	Spectrometric	O Liska, J.Lehotay, E.Brandseterova, G.Guiochon, H.Colin Journal of Chromatography 172 [1979] 384
Transition Metals	β-betoamines	Spectrometric	E.Gaetani, C.F.Laureri, A.Mangia, G.Parolari Analytical Chemistry 48 [1976] 1725

TABLE 2

POST-COLUMN DERIVATISATION

SAMPLE	DERIVATISING AGENT	DETECTION	REFERENCE
Transition Metals	4-(2-Pyridylazo)resorcinol	Spectrometric	R.M.Cassidy, S.Elchuk Analytical Chemistry 54 [1982] 1558
Cu, Zn, Pb, Fe & Mn	4-(2-Pyridylazo)resorcinol	Spectrometric	R.M.Cassidy, S.Elchuk J. of Chromatographic Sc. 18 [1980] 217
Transition Metals	4-(2-Pyridylazo)resorcinol	Spectrometric	G.J.Schmidt, R.P.W.Scott Analyst 109 [1984] 997
Transition Metals	4-(2-Pyridylazo)resorcinol	Spectrometric	J.S.Fritz, J.N.Story Analytical Chemistry 46 [1974] 825
Transition Metals	4-(2-Pyridylazo)resorcinol	Spectrometric	K.Kawazu Journal of Chromatography 137 [1977] 381
Lanthanides	4-(2-Pyridylazo)resorcinol Alizarine Red S & Arsenazo I	Spectrometric	R.M.Cassidy, S.Elchuk Analytical Chemistry 51 [1979] 1434
Transition Metals	Eriochrome Black T	Spectrometric	P.Jones, P.J.Hobbs, L.Ebdon Analyst 109 [1984] 703
Transition Metals	Eriochrome Black T & Dithizone	Spectrometric	P.Jones, P.J.Hobbs, L.Ebdon Analytical Proc. 20 [1983] 612
Transition Metals	4-(2-Pyridylazo)resorcinol & 1-(2-Pyridylazo)-2-napthol	Spectrometric	K.Kawazu, J.S.Fritz Journal of Chromatography 77 [1973] 397
Transition Metals	Dithizone	Spectrometric	P.Jones, P.J.Hobbs, L.Ebdon Anal. Chim. Acta 149 [1983] 39
Lanthanides	4-(2-Pyridylazo)resorcinol	Spectrometric	J.M.Hwang, J.S.Shih, Y.C.Yeh, S.C.Wu Analyst 106 [1981] 869

TABLE 3

NEITHER PRE- NOR POST-COLUMN

SAMPLE	NOTES	DETECTION	REFERENCE
Nb from others	Column effluent collected, treated then reacted with PAR	Spectrometric	A,Mazzuatelli, K.Frache, A.Dadone, F.Bafti Analyst 102 [1977] 825
Pb, Cd, Hg, Co, Ni & Cu	In-situ complexation with Dithiocarbamate	Spectrometric & Electrochemical	A.M.Bond, C.G.Wallace Analytical Chemistry 56 [1984] 2085
Transition Metals		Coulometric	Y.Takata, K.Fujita Journal of Chromatography 256 [1983] 253
Rare Earth	Mixed cation-anion exchange resin	Coulometric	T.Yanabe, T.Hayashi Journal of Chromatogrphy 76 [1973] 213
Cu, Ni & Co	In-situ complexation with Dithiocarbamate	Spectrometric & Elctrochemical	A.M.Bond, C.G.Wallace Analytical Chemistry 55 [1983] 718
Divalent metal ions & trivalent lanthanides	Complexing anion in the eluent	Conductivity	G.T.Sevenich, J.S.Fritz Analytical Chemistry 55 [1983] 12
Transition Metals		Coulometric	J.E.Girard Analytical Chemistry 51 [1979] 836
Transition Metals	In-situ Dithiocarbamate complexation	Electrochemical & Spectrometric	R.M.Smith, L.E.Yankey Analyst 107 [1982] 744
Transition Metals & Alkaline Earths	Electrolyte added post-column	Coulometric	Y.Takata, G.Muto Analytical Chemistry 45 [1973] 1864
Transition Metals	Tartaric acid eluent	Coulometric	Y.Takata, K.Fujita Journal of Chromatography 108 [1975] 255

1.72 Post-column Derivatisation

There has been a rapid increase in the use of post-column reaction detection systems. Although experimentally a more complicated system than pre-column derivatisation,it enables greater freedom and flexibility in the choice of reaction and chromatographic conditions. Bearing in mind that most environmental samples are dynamic in nature, injection without sample pre-treatment has distinct and obvious advantages. Secondly such systems are readily automated.

The major problem with post-column systems is avoiding excessive band broadening which generally occurs in the mixing device during addition of reagent and/or the reactor coil which has been included in to enable the reaction to complete (ie. to provide a time delay between the column and the detector).

Various devices [6] have been used to provide this time delay, the simplest being a reactor coil consisting of a length of tubing of the same i.d. as used elsewhere in the chromatographic system [46]. The design of the mixing unit and the reactor is ofutmost importance to

avoid substantial decreases in chromatographic resolution due to band broadening.

Schimdt and Scott [53] describes a post-column reactor exhibiting high sensitivity and low dispersion for the analysis of transition metals in the 50 -100 parts per billion range employing sodium tartrate to elute and PAR as the colorimetric agent.

With zero dead volume fittings and flow rates in the range of 1 -2 mL min^{-1}, typical hold-up times between column outlet and the detector are approximately 1 ms. Elchuk and Cassidy [21] found that the complexation reactions on mixing of PAR and the column effluent were complete even in the absence of a reactor coil since the sensitivities obtained for detection after post-column were in good agreement with those calculated from measured values of molar absorptivities.

Thus a time delay between column outlet and detector inlet is not always necessary and really depends on the rate of reaction of the post-column derivatising agent.

1.8 POST-COLUMN DERIVATISING AGENT

Desirable colourimetric reagents should act quickly and their formation constants should be high. They should form a complex of high molar absorptivity at the wavelength of the complex and the reaction should occur in solution in which close pH control is not necessary to eliminate the need for the presence of large amounts of buffer or close control of reagent flow rate. The colour formed should be stable with time and the absorbance of the solution should not decrease on standing.

Many heterocyclic azo dyestuffs have been synthesised and orthohydroxy dyes have assumed importance in analytical chemistry as metallochromic indicators [54]. Pyridylazo and thiazolylazo derivatives of napthols and resorcinols are the four main classes of reagents that have found wide applications. In recent years, 1-(2-pyridylazo)-2-napthol (PAN) and 4-(2-pyridylazo)resorcinol (PAR) have received considerable attention in analytical determinations.

PAN was first introduced by Cheng and Bray in 1955 as a metallochromic indicator for the complexometric titration of Cu(II), Zn(II) and In(II). In 1957, Wehber recommended the use of PAR in complexometric titrations claiming it to be

superior to PAN because of the solubility in water of the dye and its chelates and because of its sharper end-points.

Pollard, Hanson and Geary [55] used it as a spectrophotometric reagent for Co(II), U(IV) and Pb(II). No solvent extraction was required with this method and PAR was found to be the most sensitive reagent for Co(II), the most water-soluble reagent for U(IV) and the first water-soluble reagent for Pb(II).

It was also noticed that PAR gave a uniform reddish colour as opposed to the variety of shades produced by PAN. This makes it easier to monitor the metal chelates spectrophotometrically. PAR also reacts with most heavy metals ie. is less selective unlike PAN. PAR does not react with alkali metals, chromium(VI), antimony(III), molybdenum(VI), tungsten(VI) and arsenic(III) and (V).

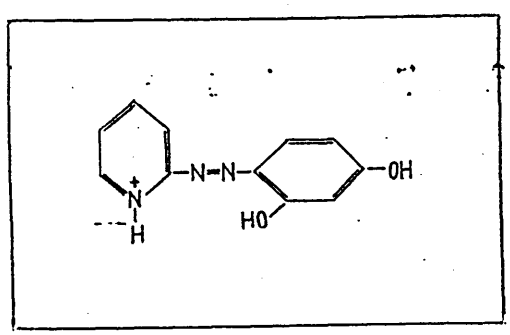
1.81 General properties of 4-(2-pyridylazo)resorcinol

PAR was first prepared by Chichibabin by coupling resorcinol with sodium 2-pyridyldiazotate. The sodium salts are more water soluble than the free dye itself and in analysis are used in preference for this reason.

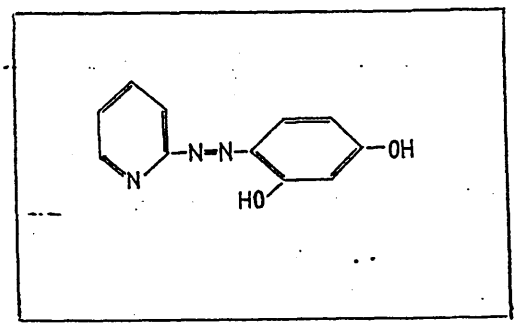
It is soluble in both acid and alkaline aqueous solutions and to a lesser extent in alcohol but not in ether. The aqueous solutions are orange in colour.

The formation of the metal-PAR complexes is very pH dependent. Hnilickova and Sommer [56] have investigated the formation and stoichiometry of some metal-PAR complexes as a function of pH and have shown that in acid solutions M(PAR)H was formed whereas in alkaline solutions, $M(PAR)_2$ forms. Other types are rare but $Th(PAR)_4$ and $Ga(PAR)_5$ have been reported.

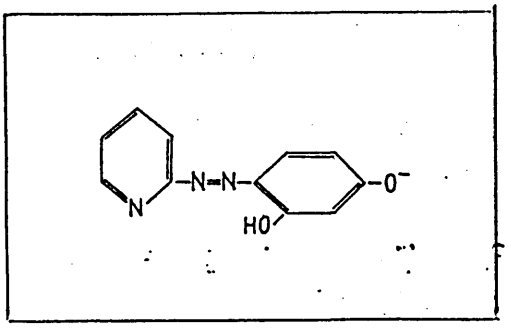
To explain its high sensitivity, a study of the structural properties of this reagent and of the metal complexes has been made by Geary and coworkers [57]. Four chromophoric species of the reagent were identified and their physical properties has been tabulated below:



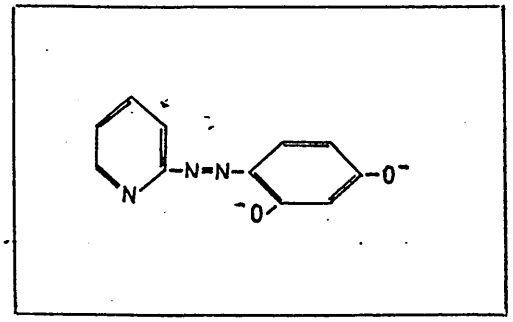
A



B



C



D

Species	λ_{max} (nm)	ε (m^2 mol^{-1})	pH
A	395	1550	1.3, 1.7 & 2.4
B	383	1570	3.6
C	415	2590	5.9 - 12.5
D	485	1730	all pH values

Table 4 Physical properties of the 4 chromophoric species of the PAR reagent.

The most dominant form is thus the mono-ionic species C. Confirmation that the reagent were infact these chromophoric forms was obtained by potentiometric titration from which the average number of hydrogen ion bound per ligand could be calculated.

The reagent used in this work has λ_{max} at 415 nm and molar extinction coefficient ε = 2900 m^2 mol^{-1} at pH 9.8 ie. species C.

The red colouration obtained with PAR is explained by the presence of a pseudo-phenanthroline system and an o-o'-disubstituted azo system. PAR chelates with metals through the pyridine nitrogen atom, the azo-nitrogen furthest away from the heterocyclic ring and the o'-hydroxyl group. PAR thus acts as a terdentate ligand forming two stable 5-membered chelate rings.

Further evidence of the terdentate nature of PAR and the greater part in chelation of the azo nitrogen furthest from the heterocycle may be adduced from the decrease in stability on change from a 1:1 to a 2:1 complex. The structures of the 1:1 and 2:1 PAR chelates can be expressed as in the formulae below :

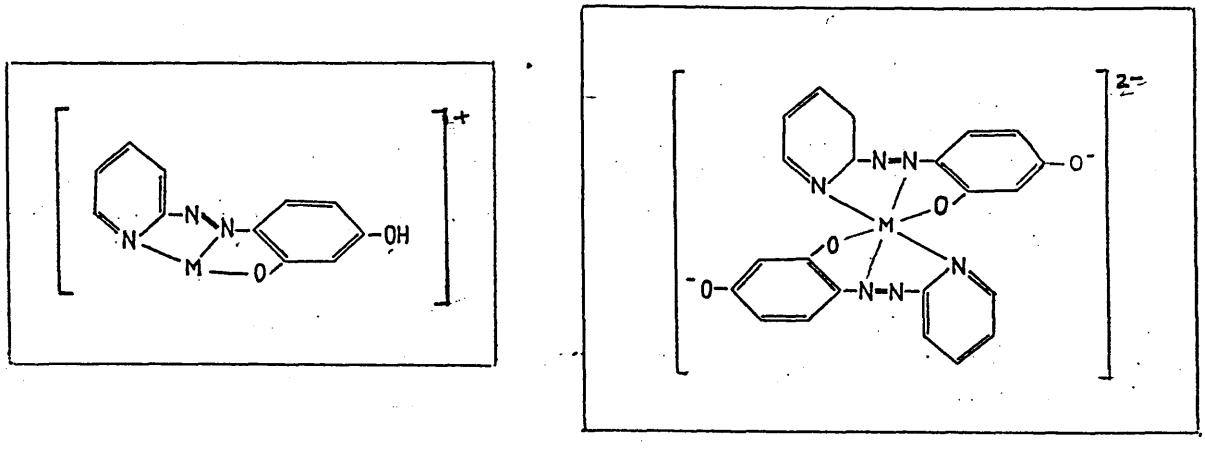


Fig. 1.81 1:1 and 2:1 PAR chelates

The absorbance curves of typical 1:1 and 2:1 complexes of PAR in water are shown in the figure below. In the visible region, the 2:1 complexes of PAR exhibit a

single absorbance maxima and the 1:1 complexes 2 absorbance maxima.

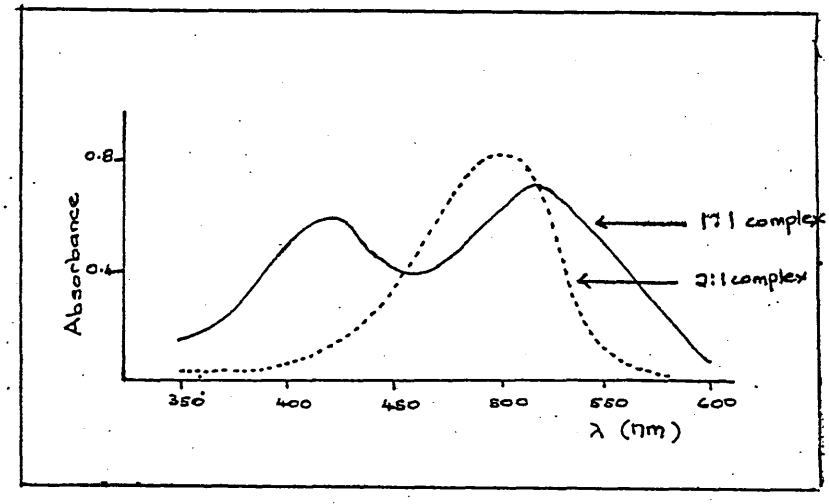


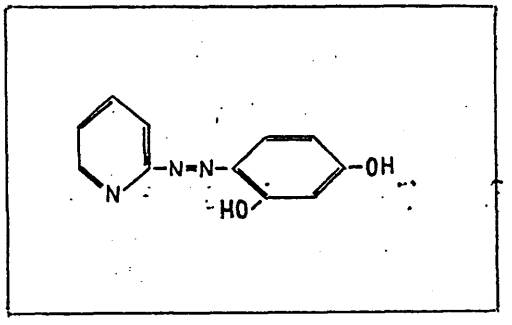
Fig. 1.82 Typical absorbance curves of 1:1 & 2:1 complexes of PAR in water.

Corsini and coworkers [58] studied the effect of chelation on the acid strength of the para hydroxyl group in the PAR chelates and found an increase in the following order :

$$Mn(II) < Zn(II) \sim Ni(II) < Cu(II) < Co(II)$$

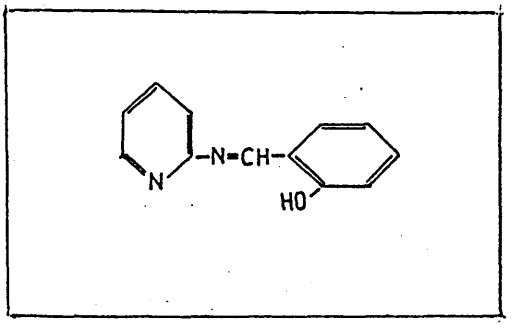
The order parallels the stability of the chelates for Mn, Zn and Ni. This acid strengthening effect of the metal ion in PAR chelates is probably transmitted to the para hydroxyl group via the bonding oxygen atom rather than through the azo group since inspection of the molecule indicates that in the 2:1 chelate the azo group does not lie in the plane of the resorcinol ring.

Further evidence that the azo nitrogen furthest from the heterocyclic ring is the one involved in the chelation is obtained by considering the following species.



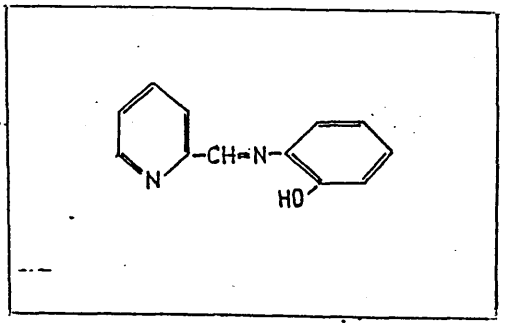
I

4-(2-pyridylazo)resorcinol



II

salicylidene-2-aminopyridine



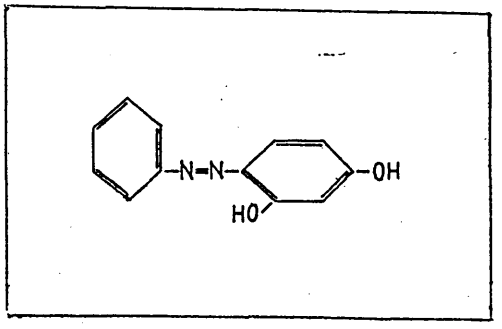
III

2-(o-hydroxy-phenyl-imino-methyl)-pyridine

In terdentate ligands I and III, the value of (log K_1 - log K_2) is large and almost equal. On the other hand, for the immine II, in which the other immine

nitrogen is involved and the bidentate arrangement is more favoured, the value of (log K_1 - log K_2) is much smaller.

The melting points of the copper(II) complexes of I,II,III and IV

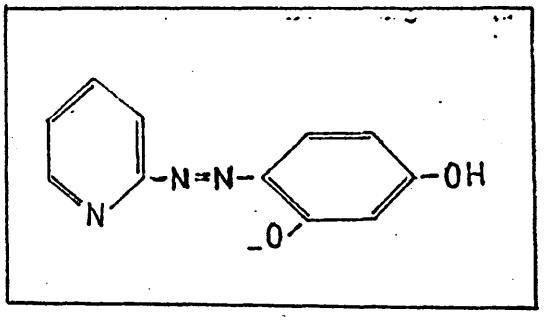


IV benzeneazoresorcinol

also form an interesting series [57] from which it can be concluded that both the azo nitrogen in PAR play an equal role in chelation ie. that the coordination should be considered as a function of the azo group as a whole rather than as one of the component nitrogen atoms.

The first hydroxyl ionization of PAR causes a shift in peak wavelength of 32 nm. This seems to confirm the view that in azo-resorcinol dyestuffs the p-hydroxyl group ionises first. The ionisation of this hydroxyl

group would be expected to produce less disruption of the chromophoric arrangement than would that of the o-hydroxyl groups, for the latter would require rupture of the hydrogen bonded ring formed by the O-hydroxyl group and the azo nitrogen nearest to the heterocycle. However on chelation, which often occurs at pH values lower than either pK_{OH}, protons are released preferentially from the o-hydroxyl group. this means that theoretically, PAR chelates in the thermodynamically unfavoured form ;



for which the dissociation constant is not known.

The role of the o-hydroxyl group as a ligand in PAR is fundamental to the high stability of the complexes. The p-hydroxyl group has a smaller stabilizing effect on the complex but is largely responsible for the visible spectrum of PAR and the high absorptivity shown by this

reagent and its complexes.

2-pyridylazo compounds are not selective either as chromogenic agents or as extractancts. However some measures may be taken to improve the situation. The most important ones are readjustment of the pH and the use of masking agents.

Hydroxonium ions competes with the metal ions for combination with the pyridylazo compounds. Consequently, the higher the stability of the metal complex, the lower the pH at which it can exist. Therefore, the lower the pH selected, the fewer the number of metals that are complexed. Secondly, the wavelength for the photometric determination can be varied.

1.82 PAR solution stability

The stability of various types of PAR solutions and their suitability as an indicator for various metals has been studied by Jezorek and Freiser [59]. They found that the storage of approximately 8 x 10^{-5} M PAR solutions either in the light or in glass bottles

caused deterioration overnight so that little or no detector response was obtained upon injection of a metal sample. This situation did not occur if plastic containers were used and the solutions stored in the dark.

Attempts to prepare 10^{-4} M or higher unbuffered PAR stock solutions for subsequent dilution also resulted in significant deterioration overnight, whether stored in glass or plastic containers, as evidenced by a 50% decrease in the absorbance at 415 nm (λ_{max} for PAR) overnight, and the appearance of a new band at 340 nm.

A 10^{-5} M solution prepared independently or immediately diluted from the above stock solution after preparation, and buffered at pH9 showed good stability over several days.

A 10^{-4} M stock solution buffered with NH_4^+/NH_3 at pH9 was found to be more stable than the unbuffered stock; and that buffered at pH11 even more so. These solutions could be kept for several days without significant deterioration.

1.9 DETECTION AFTER POST-COLUMN DERIVATISATION

The success of this detection depends on the efficiency of the mixing cell, rate of reaction between reagent and metal ions and to a great extent on the background absorbance and noise. The major underlying factor that determines the magnitude of the background noise is the background absorbance of the derivatising agent [19], namely PAR.

The absorbances of the eluent and the reagent are different. Thus, short term fluctuations in flow will cause corresponding short term changes in absorbance; the nett result is the detector noise. The magnitude of the noise will depend on :

1. the magnitude of the difference in absorbance between the reagent solution and the eluent.

2. the monitoring wavelength.

3. the magnitude of the fluctuations in the mixing cell.

The effect of these variables can be minimised if the concentration of the reagent is kept as small as possible

without limiting its ability to react with the metal ions over a reasonably wide concentration range in a sufficiently short time. Elchuk and Cassidy [19,60] found that the optimum reagent concentration was 2.0×10^{-4} mol L^{-1} PAR. With the HPLC system they used, these concentrations gave a linear working range from about 10 ng to 600 ng for most metal ions studied. The linear range can be extended if larger concentrations of reagents are used.

Since the reagent is the main source of the noise in this detection system, the signal to noise ratio is not necessarily maximised at the λ_{max} for the metal complex. Instead, the maximum signal to noise ratio will be obtained at the wavelength where the ratio of the absorptivity of the complex to the reagent is maximised. For PAR, this maximum is at 540 nm (λ_{max} for metal-PAR complexes lies between 500 - 520 nm).

To further minimise the noise from PAR, the pH of the reagent solution should be maintained at pH 9.7 (2 mol L^{-1} in ammonia and 1 mol L^{-1} in ammonium acetate). This pH maximises the acid dissociation of PAR thus minimising the background absorption of the reagent.

It has also been observed that the use of acid eluents (0.1 M for example) results in less noise than if solutions

closer to neutral were used. This may be the result of better mixing of the two liquid streams due to the heat released in neutralisation of the acid by the alkaline PAR solution.

1.10 AIMS OF THIS WORK

We have set out to assemble a simple, practical and reliable HPLC system capable of simultaneous multielement analysis of transition metals involving reversed phase columns with post-column derivatisation of the separated ionic species to enable spectrophotometric detection.

The influence of the mixing device and mobile phase composition on the chromatographic separation was studied and a suitable theory put forward to explain the observed behaviour.

The precision, limit of detection, linear range, speed and reliability of the system was also evaluated.

CHAPTER TWO

EXPERIMENTAL

2.1 REAGENTS

All reagents used were either HPLC or Analar grade.

The mobile phase was made up of aqueous solutions of :

A 0.3 mol L^{-1} tartaric acid and 2.5×10^{-4} mol L^{-1} dodecylsulphate [sodium salt]. These were made up by weighing accurately 22.51 g of tartaric acid and 0.033 g of dodecylsulphate and dissolving them in 0.5 litres of deionised water.

B 4.5×10^{-2} mol L^{-1} sodium tartrate and 1×10^{-4} mol L^{-1} hexanesulphate [sodium salt]. These were made up by accurately weighing 5.18 g of sodium tartrate and 0.009 g of hexanesulphate and dissolved in approximately 400 mL of deionised water. A few drops of orthophosphoric acid was added to the solution to adjust it to the desired

pH and deionised water was then added to make up to 0.5 litres.

Fresh mobile phase was prepared at the start of each working day and was degassed with helium prior to use. This prevents the formation of bubbles as the mobile phase exits the column (a region of high pressure) and enters the detector (a region of low pressure). Otherwise an extremely erratic and unusable baseline would be recorded.

The post-column reagent consisted of an aqueous solution containing 2.0×10^{-4} mol L^{-1} PAR (44×10^{-3} g L^{-1}), 1 Mol L^{-1} ammonium acetate (77 g L^{-1}) and ammonia solution (sp. gr. 0.88) (40 mL L^{-1}).

The reagent was stored in plastic containers in the dark (refer section 1.82).

2.2 Columns

The following C_{18} reversed-phases bonded to silica were used to enable chromatographic separation of the metal ions :

A Partisil PXS 10/25 ODS, Whatman.

B Partisil PXS 10/25 ODS-3, Whatman.

C Spherisorb 5 ODS.

To enable better control of the flow rate of the post-column derivatising agent, an empty 300 mm column half-filled with glass beads was connected to the gas coil pump used to pump in the reagent. Thus a slight backpressure was exerted on the system.

At the end of each day, the columns were rinsed out with distilled water (flow rate 1.0 mL min^{-1} for approximately 30 mins and the analytical column was stored overnight in methanol.

2.3 EQUIPMENT

2.31 Sample Injector

Injections were made using a Rheodyne injection valve (Model 7125) which had an externally located position for installing sample loops of various volumes. In this work, a 20 µL loop was used. This was usually filled

completely but there were occasions when it was only partially filled with a microsyringe. The schematic diagram below shows how the injector works. The needle port is built into the valve shaft and moves along with the rotor when the handle is turned. The diagram is a view from the front looking through the injector to the rotor-starter interface where flow switching occurs.

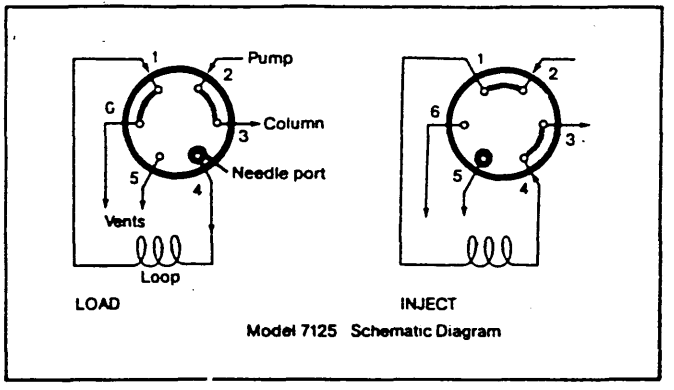


Fig. 2.31 Model 7125 schematic diagram

2.32 Pumps

A - Universal Metering Pump

The Gilson Model 302 is a single piston, constant

stroke, reciprocating pump. The piston stroke is driven by a cam, directly mounted on the shaft of a stepper motor. One revolution of the motor is subdivided into 1200 steps thus giving precise control and relatively pulse free flow.

B - Peristaltic Pump

A very basic peristaltic pump was used. The flow was regulated by varying the size of the peristaltic pump tube used.

C - Gas Coil Pump

The coil was filled with PAR prior to use. It was capable of holding about 250 mL of solution. Nitrogen was then used to push the reagent out. Reagent flow rate was regulated by adjusting the pressure exerted on the liquid by the nitrogen.

2.33 Mixing device

This device consisted of a tee-piece which was bored out on a perspex block (illustrated below). The internal diameter of the bore was 0.5 mm.

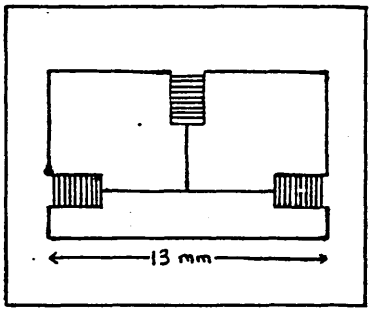


Fig. 2.33 Tee-piece mixing device

2.34 Detector

A variable wavelength (190 - 600 nm) absorbance detector (Gilson Model KM/HPLC UV-VIS Detector) with an illuminated volume of 11 µL was used. The sample cell was fitted with a heat exchanger and a Deuterium lamp provides the source of radiation. A filter blocks out UV light when operating in the visible range.

2.35 pH Measurement

A pH meter with a digital read-out which enabled pH determinations up to 2 decimal places was used. It was first zeroed with a pH 7 buffer than calibrated with a pH 4 or pH 9 buffer.

2.36 UV-VIS Spectrophotometer

A Pye Unicam SP 800 UV-Visible spectrophotometer was used to determine the absorption spectras.

CHAPTER THREE

PRELIMINARY INVESTIGATIONS

3.1 INTRODUCTION

This chromatographic set-up involves a large number of experimental variables namely : the column/mobile phase , post-column system and the detector system. At the outset it was important to find out what some of the key variables were as the analytical method can only be optimised when these have been ascertained and carefully studied.

Among the initial experiments carried out therefore were those involving :

(i) finding a suitable way of adding the post-column reagent and mixing it with the column effluent.

(ii) investigating the effect of the post-column reactor on peak dispersion (apparent column efficiency).

(iii) choice of ion-pair 'modifier' chain length.

(iv) effect of variation in mobile phase composition and flow rate on separation.

(v) choice of a suitable detector wavelength.

3.2 PUMPING SYSTEMS FOR REAGENT ADDITION

Pump A (ie. the Gilson pump) was used to pump in the mobile phase throughout the work. For the post-column reagent however, several pumps were tried out and the suitability of each was evaluated. Basically, however, the set-up of the chromatographic system remains unchanged; ie. as given overleaf (Fig 3.2).

3.21 System A

Pump A was used for mobile phase as well as post-column reagent addition. The solutions then went through two separate mixers (Gilson Model 802) which it was thought might dampen the liquid pulsations. A manometric module placed prior to the column enabled inlet pressure monitoring.

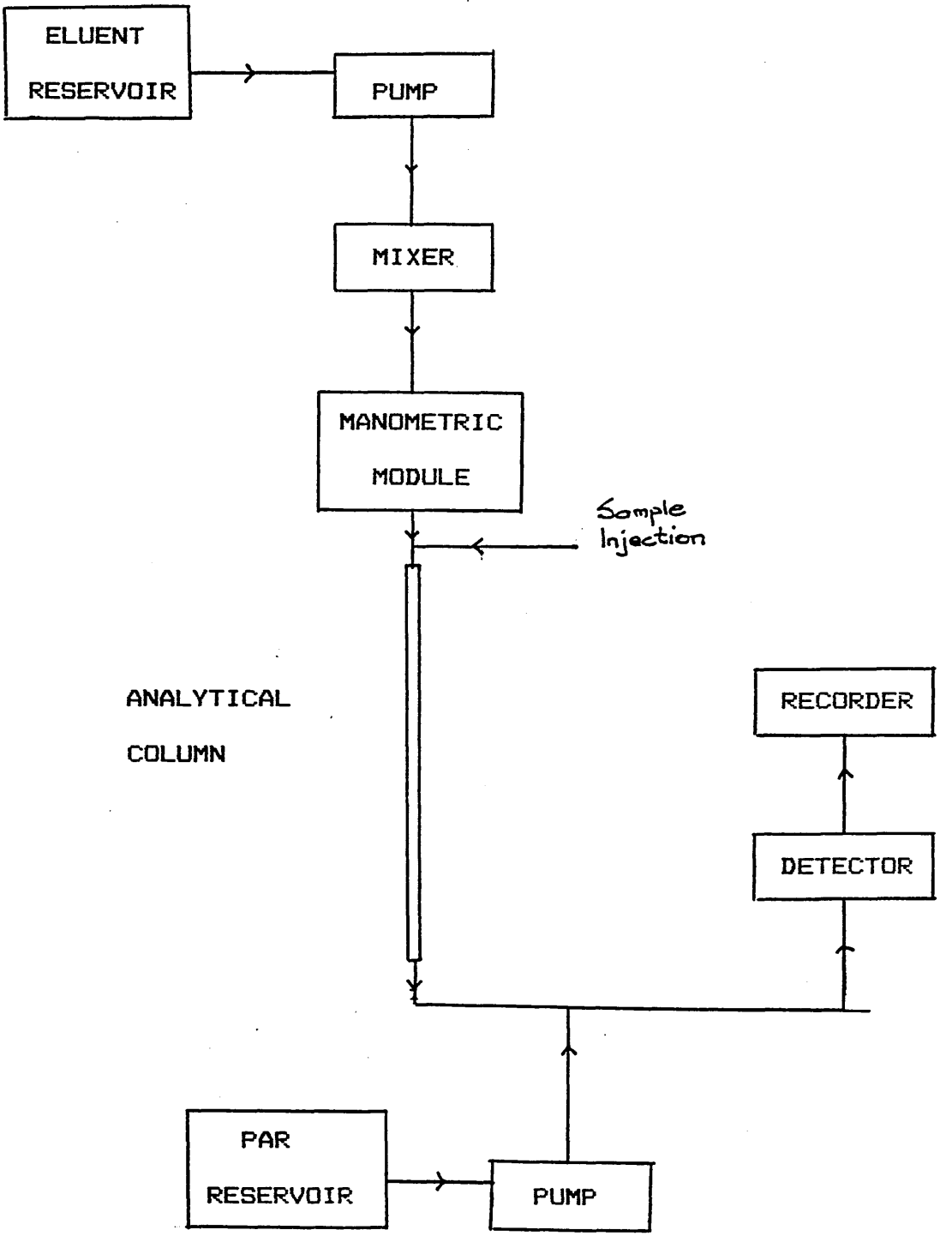


fIG. 3.2 Set-up of the chromatographic system used

This set-up was found to be unfeasible because a very high background resulted. This was most probably due to contamination of the solutions by metal surfaces in direct contact with it [61]. The two mixers were thus removed and wherever possible PTFE tubing were used in place of the stainless steel tubing hence minimising contact between the liquid phases and metallic surfaces [60].

The background level was hence reduced from 1.8 abs. units to 0.08 abs. units. The metal content of the effluent collected from the detector outlet was determined spectroscopically and was found to be less than 0.02 p.p.m. compared to 10 p.p.m. previously (mobile phase being 4.5×10^{-2} mol dm^{-3} sodium tartrate in each case). With constant daily usage of the system, the metal contamination level was expected to reduce even further.

However the noise level (in the form of regular oscillations) increased three fold to approximately 0.1 abs. units. The regular oscillations indicated that much of the noise was due to the pulsations of the liquid which resulted in poor mixing of the two reagents. Thus a second T-piece (1.7 mm i.d.) with a

sealed end was connected as illustrated below.

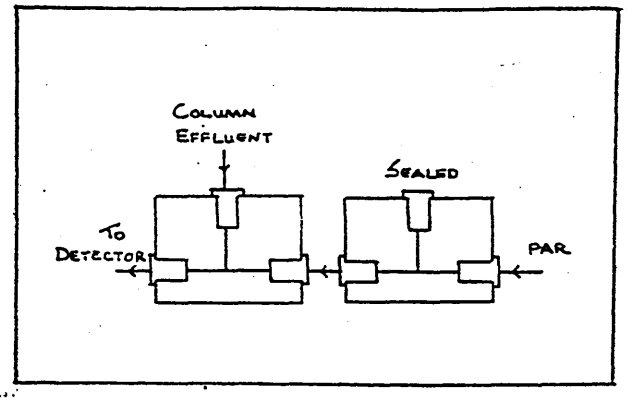


Fig. 3.21 Tee-piece connection to dampen liquid pulsations.

It was hoped that such an arrangement would dampen the pulsations of the post-column reagent prior to mixing with the column effluent thus leading to a reduction in the magnitude of the baseline noise. This was achieved but not to the extent hoped. The noise was reduced by about 20% only.

It was also found that the piston seal on at the pump head of the pump being used to pump in the post-column reagent kept giving way thus resulting in a leakage at the pump head. This was because it was unable to withstand the PAR solution used.

Pump A was thus found to be unsuitable for pumping in the PAR solution. In an effort to reduce the baseline noise so as to enable operation at a more sensitive attenuation, other pumps were tried out in place of the Gilson pump.

3.22 System B

The peristaltic pump ie. pump B was used for post-column reagent addition instead of pump A.

The main problem with this system is the inability of the peristaltic pump to function at the high operating pressures required for a reasonable eluent flow rate ie. approximately 1 mL min^{-1}. In fact the eluent flow rate had to be halved to lower the operating pressure before pump B could function effectively. Otherwise the post-column reagent was pushed out against the pumping action of the peristaltic pump by the column effluent.

This was therefore not a viable system either as the slower eluent flow rate meant longer retention times. Secondly, the PAR flow rate could not be accurately controlled.

3.23 System C

The gas-coil pump ie. pump C was used for post-column reagent addition.

Pump C unlike pump A is not a reciprocating piston pump hence was expected to produce a pulse-free liquid stream without the aid of a pulse dampener thus minimising baseline noise. This was indeed realised. The noise measured 0.01 abs. units only.

The optimum chromatographic conditions were therefore achieved with this system.

3.3 EXTRA-COLUMN EFFECTS

Using pumping system A, a 20 µL sample of 0.02 p.p.m. (0.4 ng) Cu was injected into the system. The eluent and PAR flow rates were 1.0 mL min^{-1} and the chart speed was set at 50 mm min^{-1}. The extra-column effect was calculated as below :

2.(Width at 1/2 peak height).(Flow rate).(1000)

= 2.(1.5 mm).(1mL ÷ 50 ').(1000) = 60 µL

3.4 EFFECT OF TEE-PIECE DESIGN AND ORIENTATION

The tee-piece despite being the simplest and smallest item in the system, plays a very important role. Its function is just to channel the two liquid streams together. However, a good tee-piece would also be able to mix the two so as to result in a homogenous stream being introduced to the detector. Hence baseline noise would be insignificant. To achieve this close attention need to be paid to the design and orientation of the tee-piece.

3.41 Design

Three different tee-piece designs , as drawn below, were assessed.

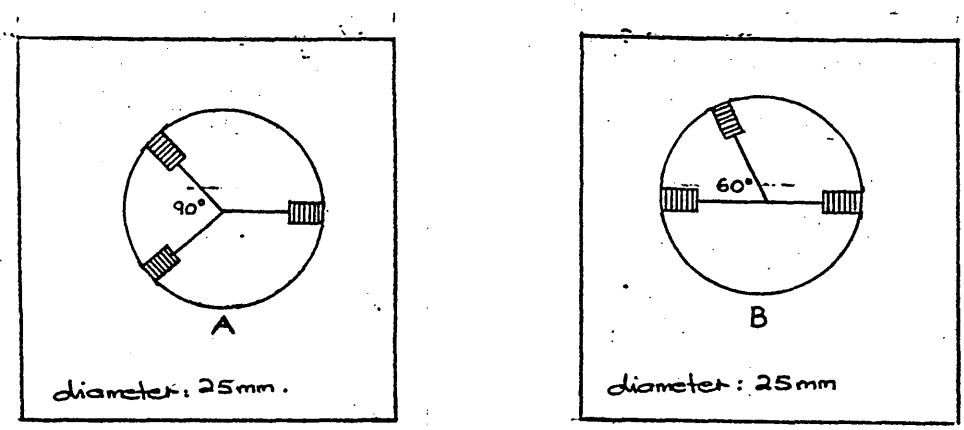


Fig. 3.41 A & B Tee-piece designs studied

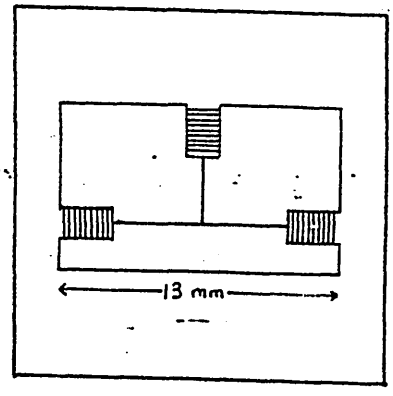


Fig. 3.41C Tee-piece design studied

In each case, the internal diameter was 1.7 mm. The magnitude of the baseline noise for each tee-piece was measured. Design C was found to be the best (noise: 0.02 abs. units) and B the worst, noise approximately triple that of C (0.07 abs. units). With tee-piece A, the noise measured 0.05 abs. units.

3.42 Bore size

Two tee-pieces which only differed in their bore size were tested and compared. One had an internal diameter of 1.7 mm and the other 0.5 mm.

The most significant effect was on the baseline noise. The narrower bore resulted in decreased noise ; 0.002

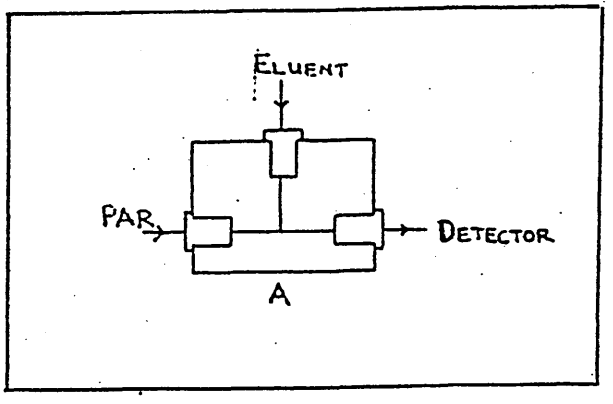
abs. units compared to 0.016 abs. units obtained with the wider bore tee-piece.

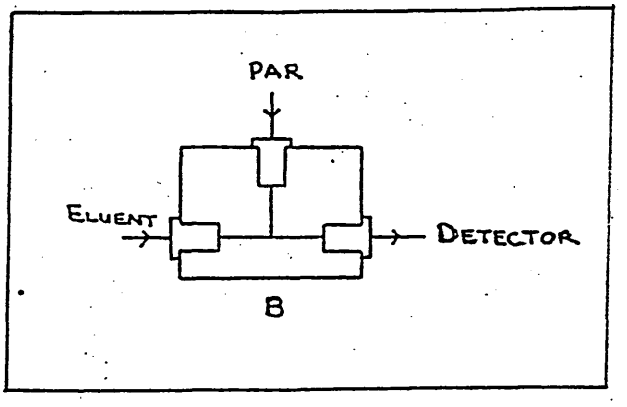
This is most probably because all tubing leading to the tee-piece have a 0.5 mm internal diameter. With the wider bore tee-piece, the liquid streams would be suddenly introduced to a much larger volume on entering the tee-piece. This would result in turbulent flow hence more noise.

Thus the tee-piece with a 0.5 mm internal diameter is preferred.

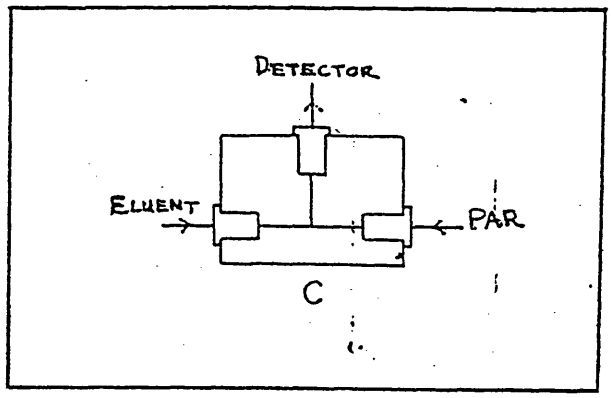
3.43 Orientation

The three different tee-piece orientations investigated were:





Figs. 3.43 A, B & C Various tee-piece orientations



Orientation C resulted in the least baseline noise (0.005 abs. units) and orientation B the worst (noise: 0.01 abs. units). With orientation A, noise measured 0.009 abs. units.

3.5 MODIFYING THE COLUMN

Initially, sodium dodecylsulphate ($C_{12}H_{25}SO_4Na$) was used as the modifier. Sodium eicocylsulphate was preferred [21] but was unavailable. The column coating procedure was as was done by Cassidy and Elchuk [21].

In later work, sodium hexanesulphate was used instead.

3.51 Advantages of C_6 over C_{12} as the ion-pairing agent

The main advantage of C_6 over C_{12} as a modifier is that there is no need to coat the column first. C_6 is just incorporated into the eluent being used. This is possible because C_6 being of much shorter chain is hence not retained by the column whereas C_{12} being of intermediate length is retained by the column but it is also easily washed off. Thus, the column has to be coated with C_{12} prior to use and some C_{12} still has to be incorporated in the eluent. The amount of C_{12} added to the eluent is very crucial. With the C_6 compound such problems are unlikely.

In this work, the amount of C_6 in the eluent was 0.010 mol dm^{-3} [21].

3.52 Effect of varying C_{12} concentration

Varying the C_{12} concentration in the eluent changes the retention times dramatically. For example, a reduction in the amount of C_{12} in the eluent from 1×10^{-4} mol dm^{-3} to 7.5×10^{-5} mol dm^{-3} resulted in very much shorter elution times; Copper and Cobalt were eluted in

less than 13 minutes compared to 25 minutes in the latter case. This is thus a very easy and convenient method of controlling the retention times.

However there is a limit to the extent to which the concentration of C_{12} in the eluent can be varied. Too much of it will lead to a build up of C_{12} in the column which may even result in a sharp increase in the operating pressure of the system. On the other hand, too little would result in poor retention and resolution due to insufficient exchange sites (small k').

3.6 STABILITY OF PAR REAGENT

There have been reports [59] of encountering problems associated with the stability of PAR solutions which had been stored for several days. An experiment was thus conducted to ascertain the viability of storing PAR solutions over a period of several days.

40 mL of the tartrate eluent was added to 10 mL of freshly prepared PAR solution and another 40 mL was added to 10 mL

of PAR solution which had been prepared ten days earlier and stored in a dark glass bottle in a dark cupboard. The PAR solutions were prepared as outlined in section 2.1. The absorbance of the two were then determined spectroscopically.

On comparison, the absorbance of the ten day old solution was found to be only 16% less than that of the freshly prepared PAR solution. Hence it can be concluded that buffered PAR solutions, as prepared in this work is stable. Nevertheless, in this work, PAR reagents are never stored for more than 2 days.

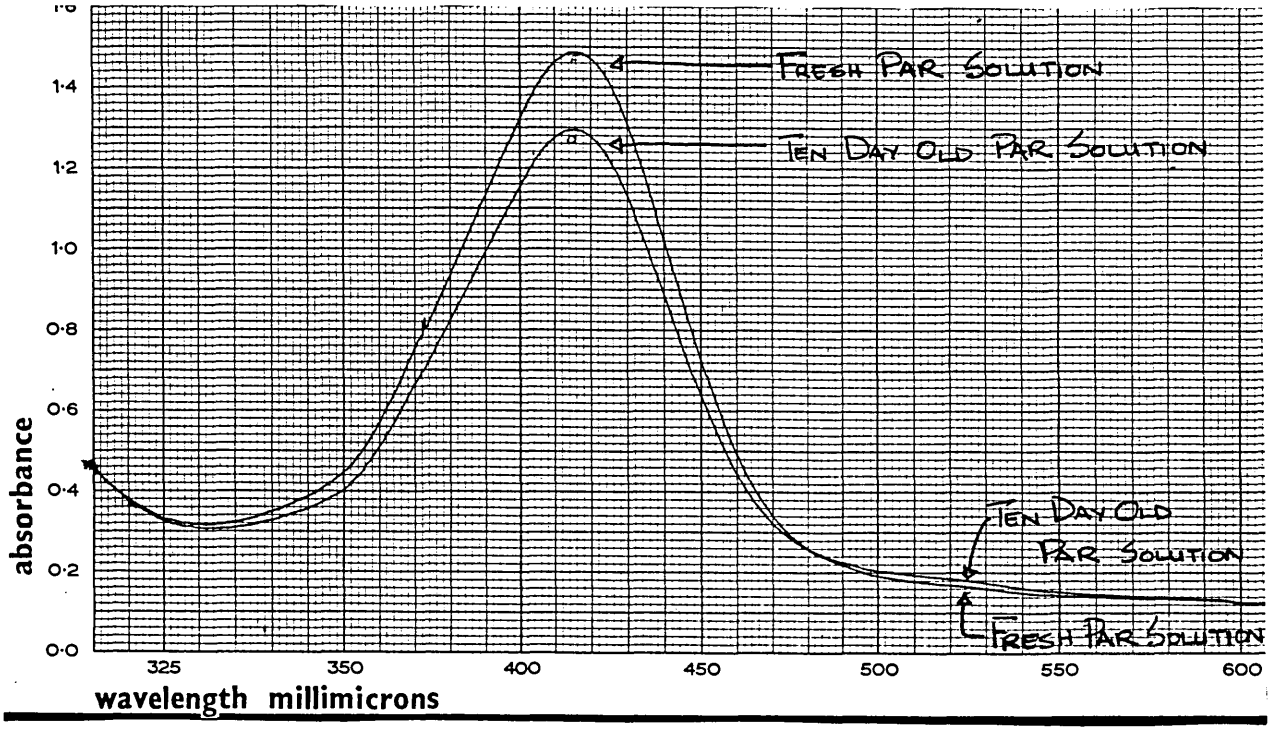


Fig. 3.6 Absorbance spectra of freshly prepared & 10 day old PAR solutions.

3.7 CHOICE OF MONITORING WAVELENGTH

The figure below shows the absorption spectra obtained for a sample of the PAR reagent used in this work. It exhibits a maxima at approximately 415 nm. A few drops of a solution containing some Cu^{2+} ions were then added to it. The new absorption spectra now contains two peaks ie. one for the PAR at 415 nm for which the peak height is now lower and another at approximately 510 nm corresponding to the PAR-metal complex.

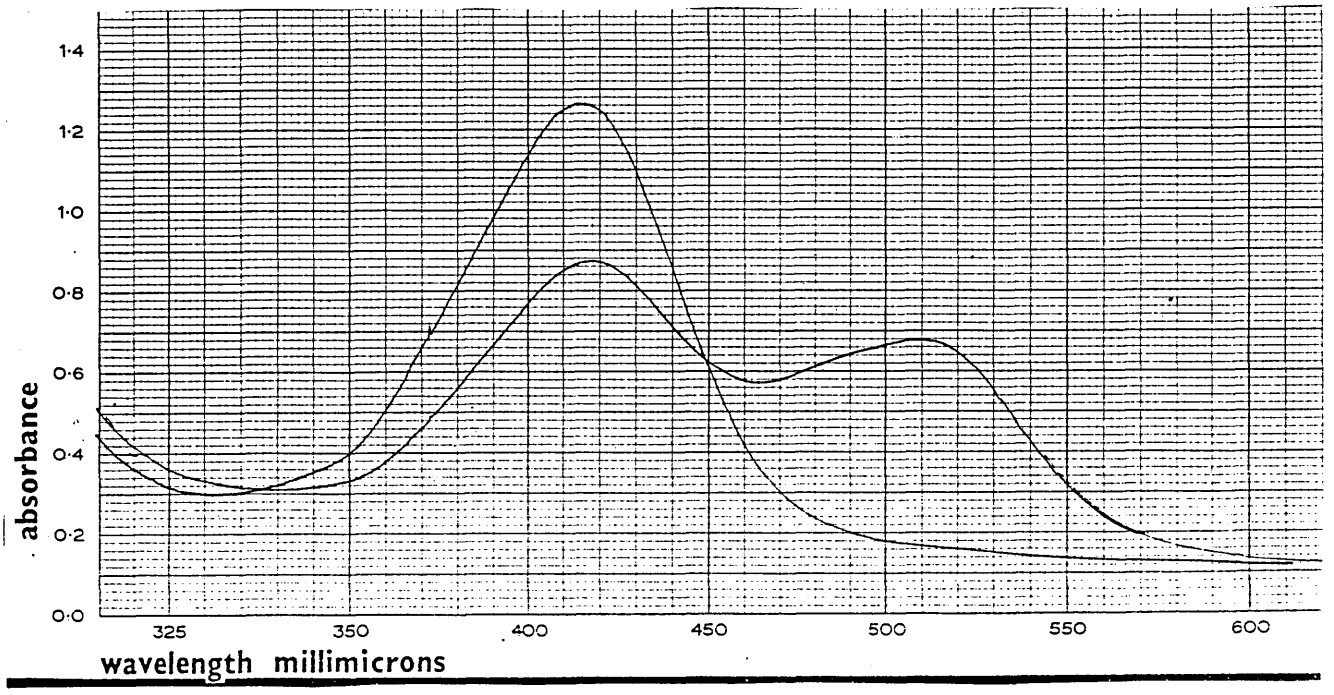


Fig. 3.7 Absorbtion spectra of the PAR Reagent and of the copper-PAR complex.

Generally the λ_{max} for the PAR-metal complex varies between 500 to 520 nm depending on the metal involved. This means that there is a large choice in possible monitoring wavelength and at any particular wavelength, the absorbance of some complexes will be enhanced and others diminished. The wavelength selected must be such that the reduction in peak height is minimal.

3.8 EFFECT OF VARIATION OF MONITORING WAVELENGTH

The aim here is to investigate the effect on peak height and baseline noise and hence decide the optimum monitoring wavelength.

The monitoring wavelength was varied between 500 nm and 540 nm and the absorption spectra for various PAR-metal complexes were obtained. At wavelengths greater than 540 nm, peak heights were expected to be lower because it greatly exceeds the λ_{max} of the PAR-metal complexes. At wavelengths below 500 nm, PAR absorption would be expected to increase but that of the PAR-metal complex to decrease thus enhancement of baseline noise and lower peak heights would result. Thus observations were only made within this range and the results tabulated overleaf.

Wavelength (nm)		500	505	510	520	530	540
Noise (abs. units)		0.009	0.009	0.008	0.008	0.005	0.004
	t (mm)	18.5	18.5	18.5	19	16.6	16.5
Cu	h (ab.u.)	0.072	0.076	0.072	0.056	0.04	0.032
	S/N	8	8.4	9	7	8	8
	t (mm)	21.5	22	21	21.5	19	18.5
Co	h (ab.u.)	0.28	0.276	0.26	0.206	0.114	0.08
	S/N	31.1	30.6	32.5	25.8	22.8	20
	t (mm)	25.5	26	25	25.5	22.5	21.5
Zn	h (ab.u.)	0.24	0.248	0.25	0.212	0.16	0.152
	S/N	26.7	27.6	31.3	26.5	32	38
	t (mm)	29.5	29.5	29	28.5	25.5	25
Fe(II)	h (ab.u.)	0.112	0.112	0.12	0.096	0.072	0.056
	S/N	12.4	12.4	15	12	14.4	14
	t (mm)	32	32.5	31.5	31.5	28	27.5
Mn	h (ab.u.)	0.276	0.27	0.266	0.212	0.168	0.116
	S/N	30.7	30	33.3	26.5	33.6	29

Table 5 Effect of variation of monitoring wavelength on the elution time (t), peak height (h) and signal to noise ratio (S/N)

For the metals studied, peak heights were found to be at its maximum when monitored between 500 and 510 nm. However baseline noise is also at its highest level here. These observations are consistent with the theory. The best wavelength at which to operate would thus be one at which the signal to noise ratio (S/N) for most of the complexes is highest. This occurs at 510 nm.

3.9 INITIAL DETECTION

Having dealt with the key variables in this set-up, the next logical step would be to test the system to determine whether samples would be detected.

For this purpose, the analytical column was substituted with 300 mm of stainless steel tubing (0.5 mm i.d.). Using pumping system A, the eluent (0.045 mol dm^{-3} tartaric acid) flow rate was set at 0.1 mL min^{-1} and the monitoring wavelength at 512 nm.

20 μL of standard copper solutions of 20, 10 and 5 p.p.m. (ie. 400, 200 and 100 ng) was injected into the system. The resulting traces are given overleaf.

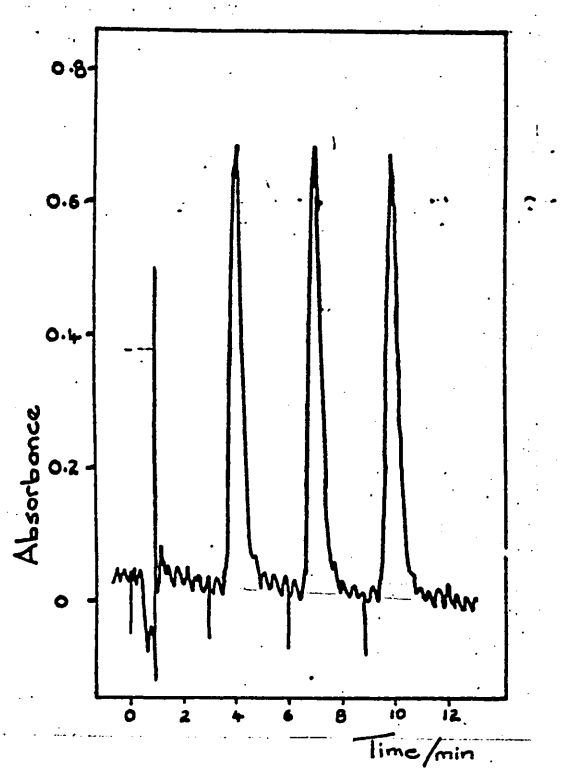


Fig. 3.9 A Chromatogram of a sample containing copper (20 p.p.m. ; 400 ng).

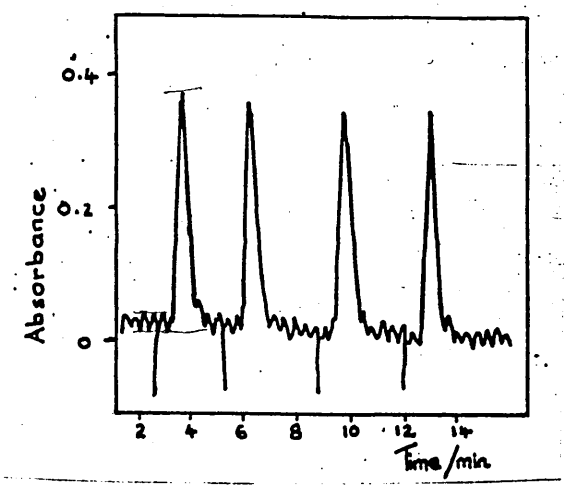


Fig. 3.9 B Chromatogram of a sample containing copper (10 p.p.m.; 200 ng).

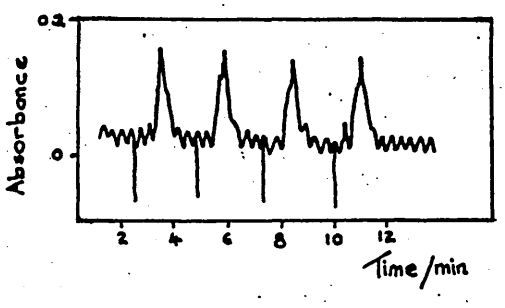


Fig. 3.9 C Chromatogram of a sample containing copper (5 p.p.m. ; 100 ng).

Note that the peaks obtained were relatively symmetrical and the peaks heights vary proportionally with the amount of sample injected.

This indicates that the post-column reaction between the PAR and the column effluent works well.

3.10 PRELIMINARY SEPARATION OF COPPER AND COBALT

The analytical column was connected back in place (ie. set-up as was given in 3.2). Samples containing 50 p.p.m. Cu and

50 p.p.m. Co (ie. 1000 ng of each) were injected onto the column and the first successful separation of metal ions was obtained on a partisil PXS 10/25 ODS column employing a 0.25 mol dm^{-3} tartaric acid eluent containing 1 x 10^{-4} mol dm^{-3} sodium dodecylsulphate flowing at 0.40 mL min^{-1} (pumping system B).

The time taken to elute the samples were however very long, just under 1 hour. This can very easily be reduced by increasing the eluent flow rate and adjusting the eluent content and concentrations.

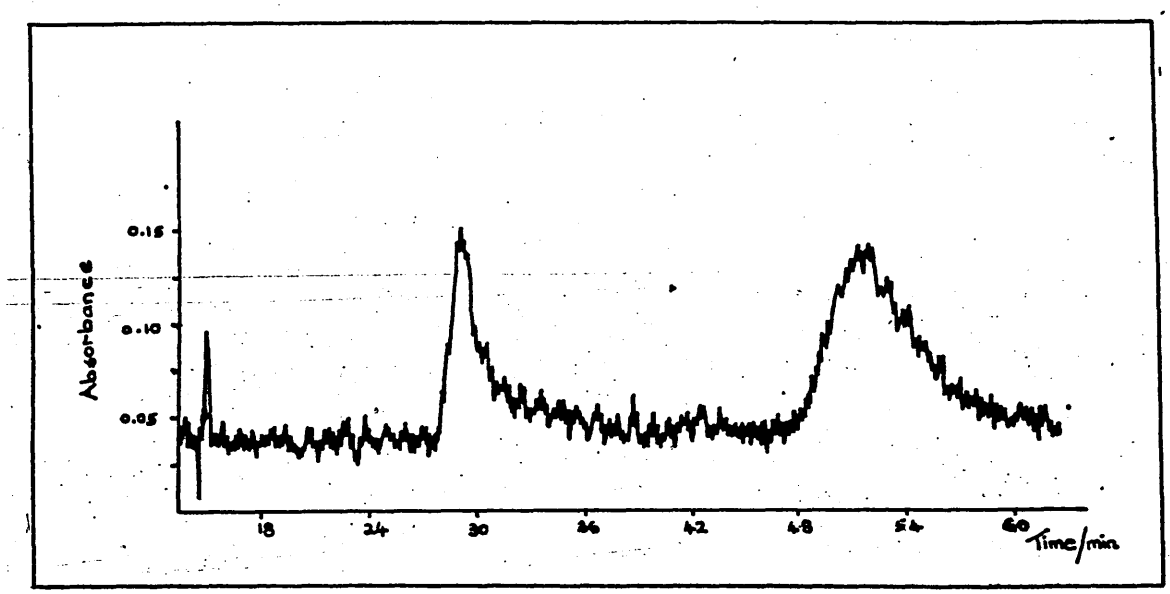
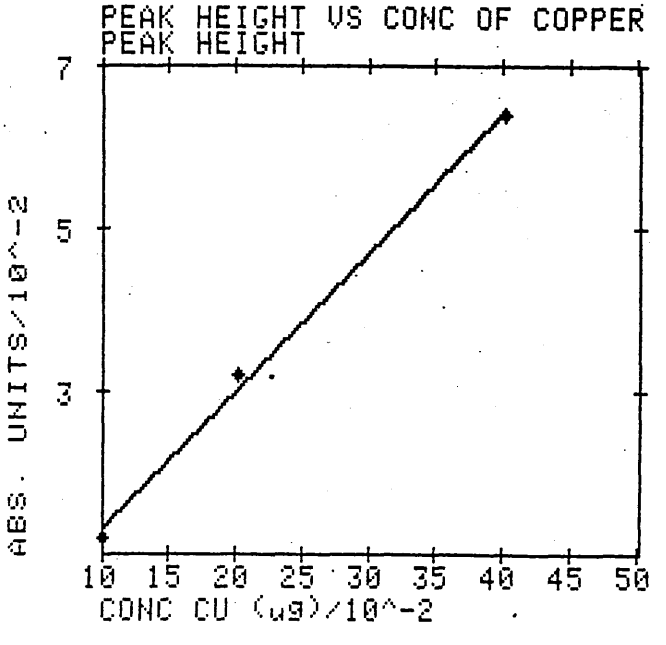


Fig. 3.10 Preliminary separation of samples containing 50 p.p.m. each of Cu^{2+} and Co^{2+}

3.11 PRELIMINARY CALIBRATION OF COPPER AND COBALT

To obtain calibration graphs for samples containing Cu and Co, 20 uL of solutions containing 50, 60 70 80 and 100 p.p.m.(mg L^{-1}) each of copper and cobalt (ie. 1000 ng-2000 ng each of Cu and Co) were injected onto the Partisil PXS 10/25 ODS column. The 0.30 mol dm^{-3} tartaric acid eluent containing 7.5 x 10 $^{-5}$ mol dm^{-3} sodium dodecylsulphate flowed at 1.40 mL min^{-1} and the post-column reagent (PAR) at 0.30 mL min^{-1} (pumping system B).

Samples were eluted much faster ie. just under 25 minutes. This is due to the higher tartaric acid concentration in the eluent plus the faster eluent flow rate. The resulting linear plots (given below) with intercepts very close to the origin were very encouraging.



GRADIENT : .171428572
INTERCEPT : -4.00000002E-03
REGRESSION COEFFICIENT : .998337488

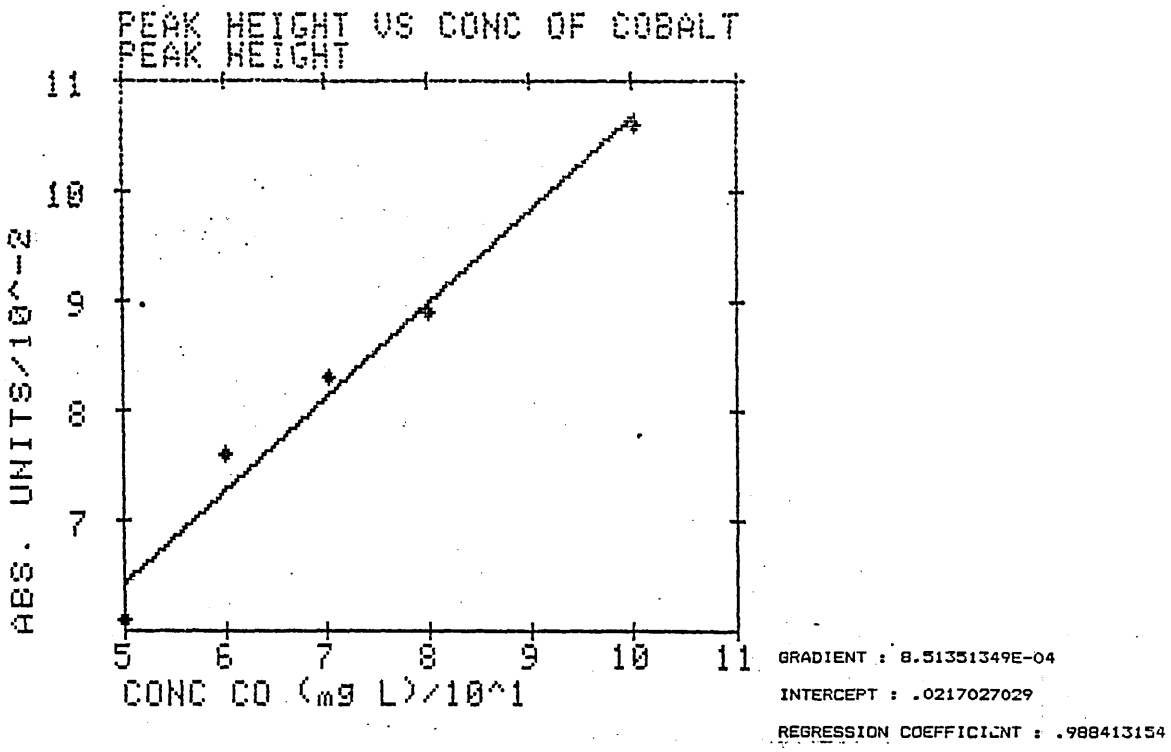


Fig. 3.10 Preliminary calibration graphs for sample solutions containing various amounts of copper and cobalt ions

CHAPTER FOUR

OPTIMISING SEPARATIONS

4.1 INTRODUCTION

The chromatographic method developed is capable of qualitative as well as quantitative determinations of samples of aqueous solutions containing copper and cobalt. However the time taken to elute the ions is still too long. The detected peaks are thus broad and peak heights low. Secondly, baseline noise is high thus preventing analysis at more sensitive attenuations. This in turn makes it impossible to analyse samples containing trace amounts of metal ions.

Further investigations are hence required to :

1) reduce the elution times
2) reduce baseline noise

this would result in an increase in the signal to noise ratio thus enabling qualitative as well as quantitative determination of a much larger number of metal ions per injection and to analyse samples containing trace amounts of metal ions.

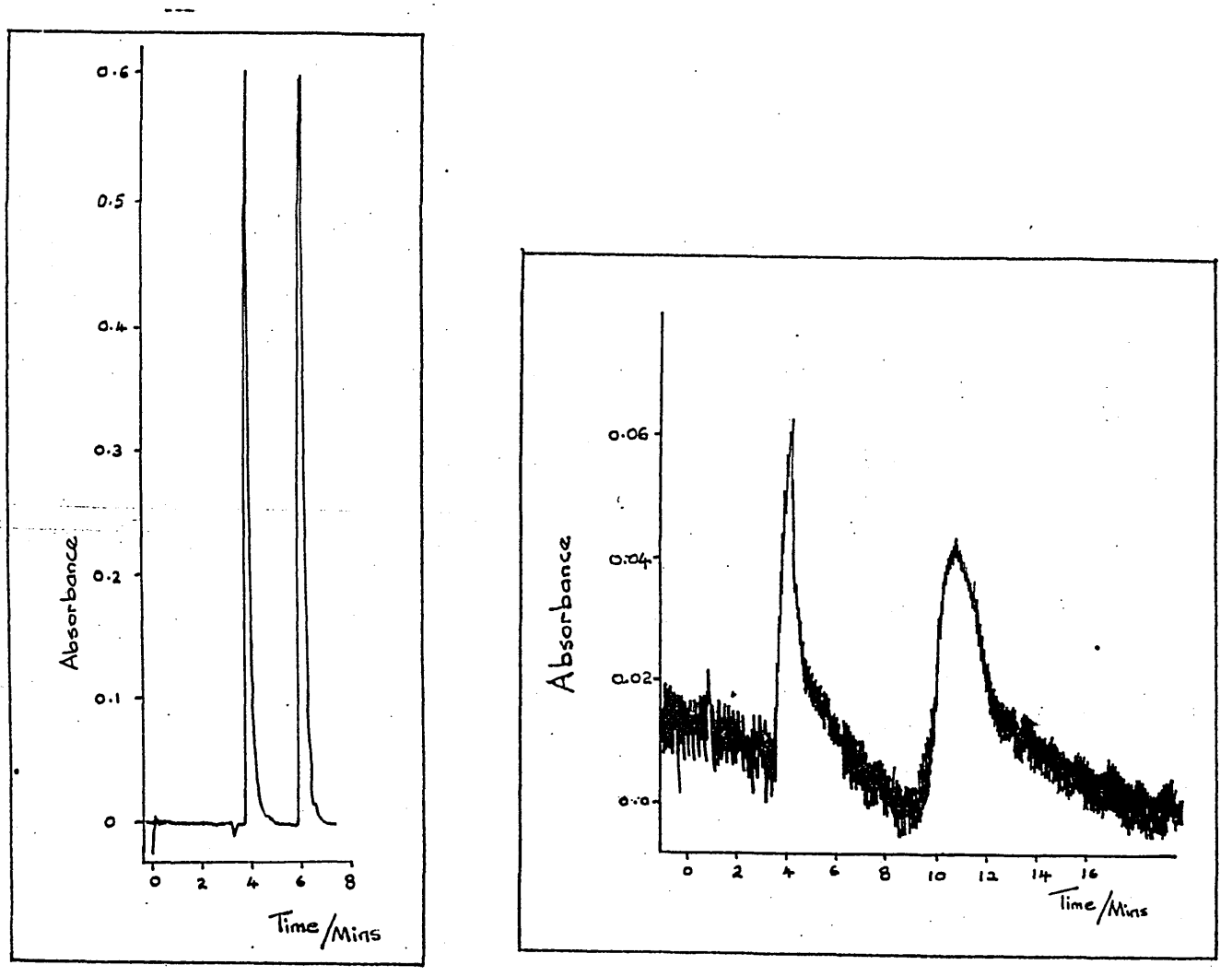
4.2 ELUENT STRENGTH

The pH of the tartaric acid eluent was 1.9. Tartaric acid is a weak acid. This means that the extent of dissociation of the acid molecule is very much dependent on the pH of the solution. At low pH, dissociation is suppressed because the concentration of hydrogen ions in solution is already high. Concentration of tartrate anions which is required to elute metal ions from column will thus also be low. Hence elution times will be longer.

Sodium tartrate on the other hand is an ionic salt. The tartrate ion concentration would thus be high. Chromatographing samples with such solutions would thus be expected to complete in a much shorter time.

20 μL samples of solutions containing equal amounts of copper and cobalt in the range of 8 to 15 p.p.m.(mg L^{-1}) were thus chromatographed on a Partisil PXS 10/25 ODS-3 column using a 0.045 mol dm^{-3} disodium tartrate solution containing 0.01 mol dm^{-3} Sodium hexanesulphate. The pH of the eluent was adjusted to 3.1 by adding a few drops of phosphoric acid.

A sample of the traces obtained is given below. Note the tremendous improvement when compared to the traces obtained previously,



A B

Fig. 4.2 Comparison of separations obtained with sodium tartrate eluent (A) and tartaric acid eluent (B).

and with reference to the tabulated data.

ELUENT	SIGNAL (10 ppm Cu)	NOISE	S/N	ELUTION TIME
0.30 mol/dm^3 Tartaric acid	0.064 abs. units	0.01 a.u.	6.4	15 mins
0.045 mol/dm^3 Sod.tartrate	0.470 abs. units	0.004 a.u.	117	7 mins

Table 6 Summary of data from fig. 4.2.

Part of this great improvement can be attributed to the greater complexing ability of tartrate at the higher pH. The higher concentration of sodium ions in the eluent may also have contributed to it. The role of sodium, if any, will be investigated and determined later.

4.3 MULTIELEMENT CAPABILITY

The aim of this work is to develop an analytical method capable of simultaneous determinations. The success and applicability of this method would thus be enhanced if the number of metal ions determined per injection were increased.

20 μL samples containing 10 p.p.m.(mg L^{-1}) ie. 200 ng each of Copper, Zinc, Cobalt and Iron were injected onto the Partisil PXS 10/25 ODS-3 column and eluted with an eluent made up of 0.045 M sodium tartrate and 0.01 M sodium hexane sulphate ; pH 3. Eluent flow rate 1.0 mL min^{-1}.

The resulting traces gave four very well resolved symmetrical peaks with negligible baseline noise. Retention times and elution order were reproducible and predictable.

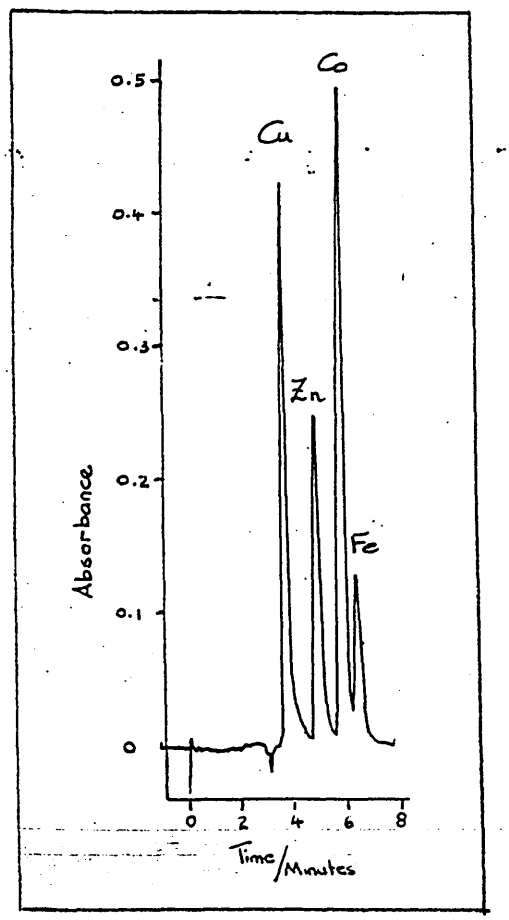


Fig. 4.3 A.
Chromatogram of sample containing 10 p.p.m. each of copper, zinc, cobalt and iron.

With such good resolution it seemed possible that a greater

number of ions may be analysed per injection.

20 µL samples containing seven metal ions ie. 12.5 p.p.m. (250 ng) each of copper, zinc and cobalt and 25 p.p.m. (500 ng) each of lead, nickel, cadmium and manganese, was chromatographed (experimental conditions : Eluent - 0.045 M sodium tartrate + 0.010 M hexane sulphate at 1.0 mL min^{-1} and pH 3.1 ; Detector wavelength - 510 nm ; Column- Speherisorb 5 ODS).

In fact, seven well resolved symmetrical peaks were recorded in less than seven minutes.

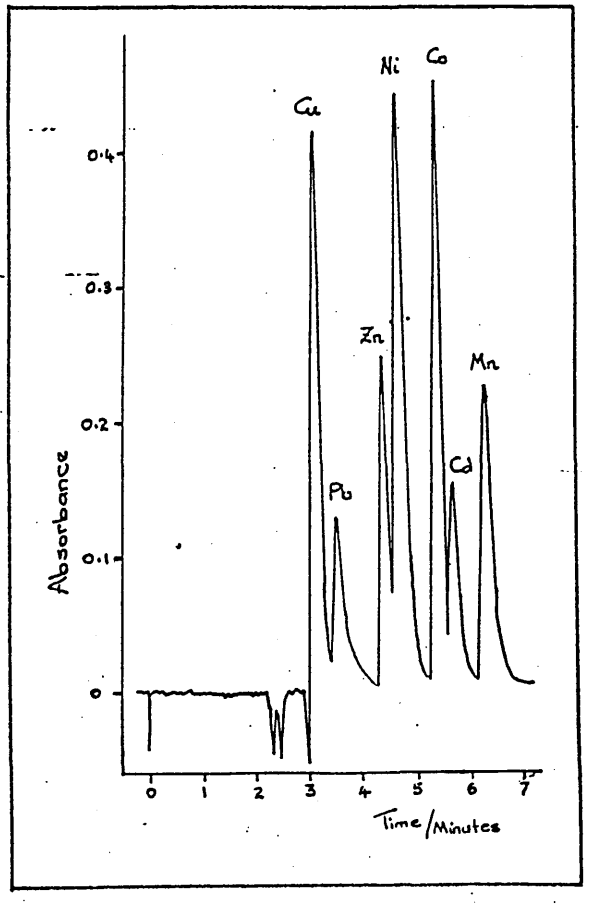
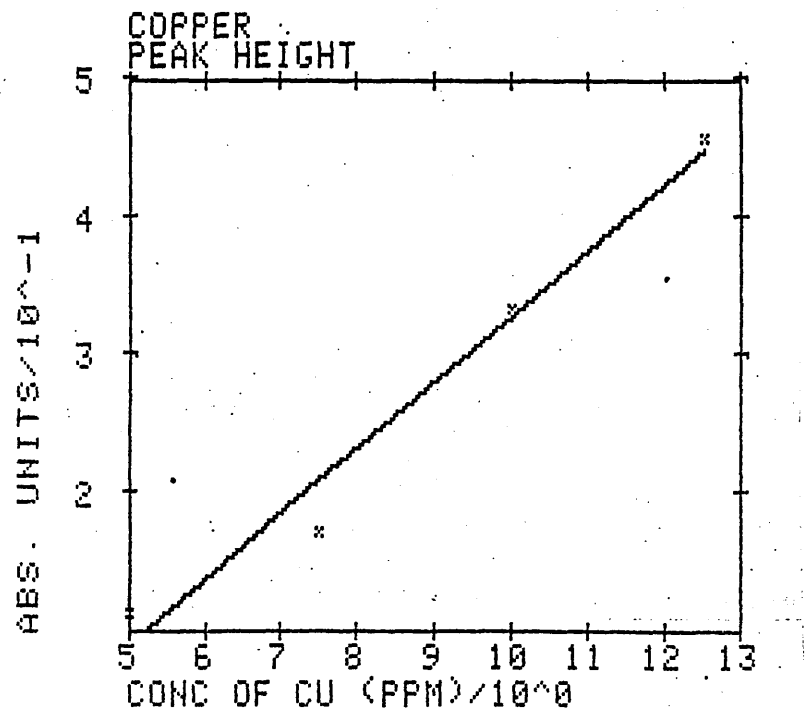


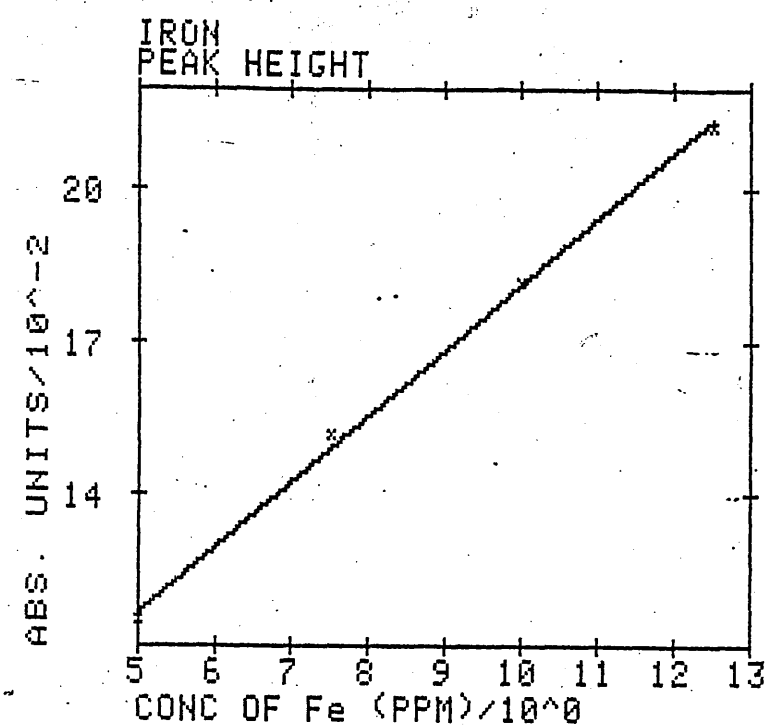
Fig. 4.3 B Chromatogram of sample containing a mixture of 7 metal ions.

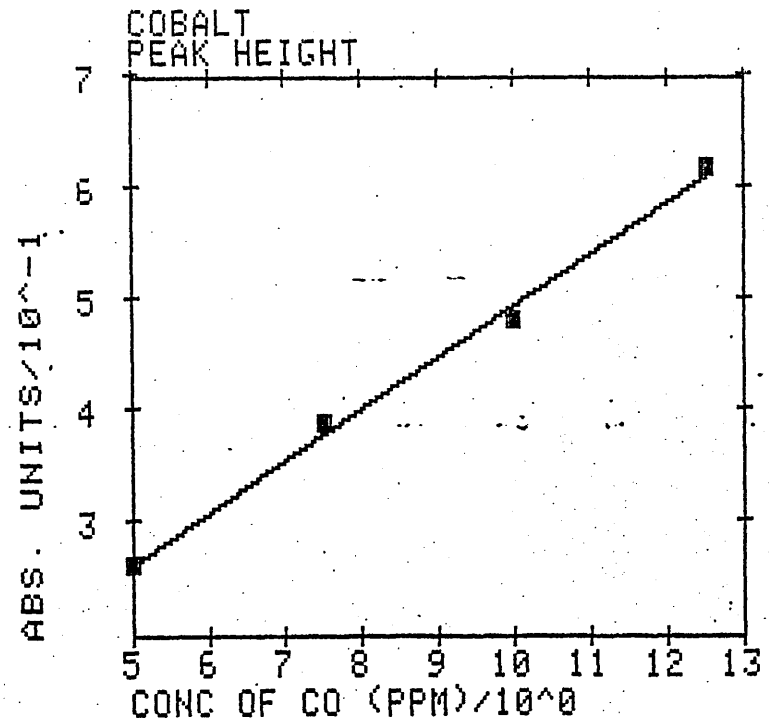
4.32 QUANTITATIVE DETERMINATION

20 µL of samples containing between 12.5 p.p.m (500 ng) to 5 p.p.m (100 ng) each of copper, zinc, cobalt and iron was analysed on the Partisil PXS 10/25 ODS column; experimental conditions were as detailed above.

It is obvious from the graphs plotted that this method enables quantitative determinations of metal ions in the part per million range. The results also compares very favourably to those obtained by others [21,24].




IRON
PEAK HEIGHT
ABS. UNITS/10^-2
20
17
14
5 6 7 8 9 10 11 12 13
CONC OF Fe (PPM)/10^0


COBALT
PEAK HEIGHT
ABS. UNITS/10^-1
7
6
5
4
3
5 6 7 8 9 10 11 12 13
CONC OF CO (PPM)/10^0

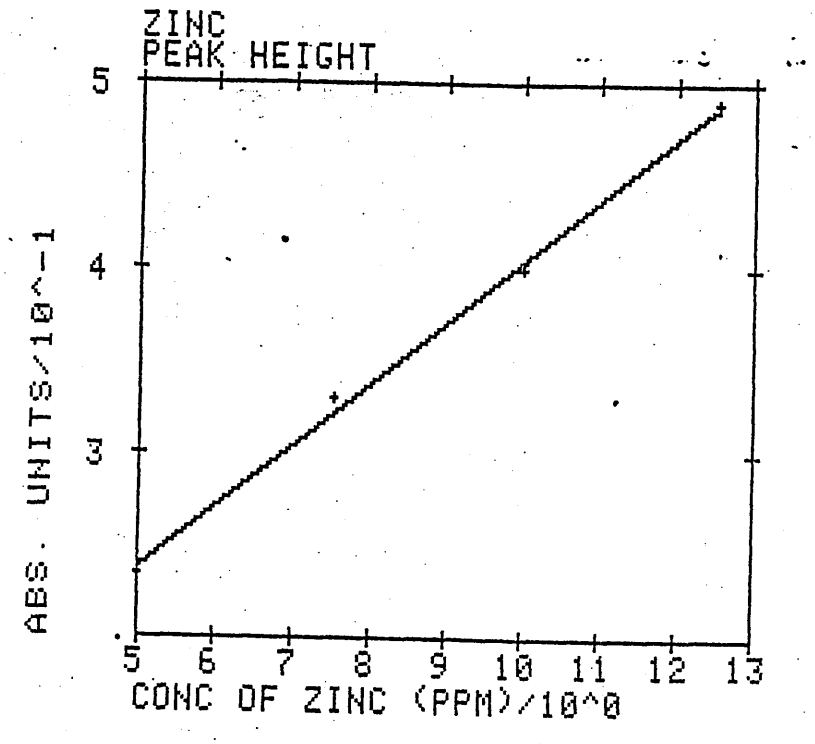


Fig. 4.31 Graphs obtained from the chromatographic analysis of samples containing various amounts of copper, iron, cobalt & zinc.

4.32 VARIATIONS IN ELUENT FLOW RATE

In practice it may sometimes be difficult to maintain a constant eluent flow rate and/or to control the flow rate precisely. It is therefore important to determine

whether minor changes in the eluent flow rate will have any adverse effects on the separation.

The sample containing the four metal ions were thus chromatographed under identical experimental conditions except for the eluent flow rate which was varied between 1.2 mL min^{-1} and 0.9 mL min^{-1}.

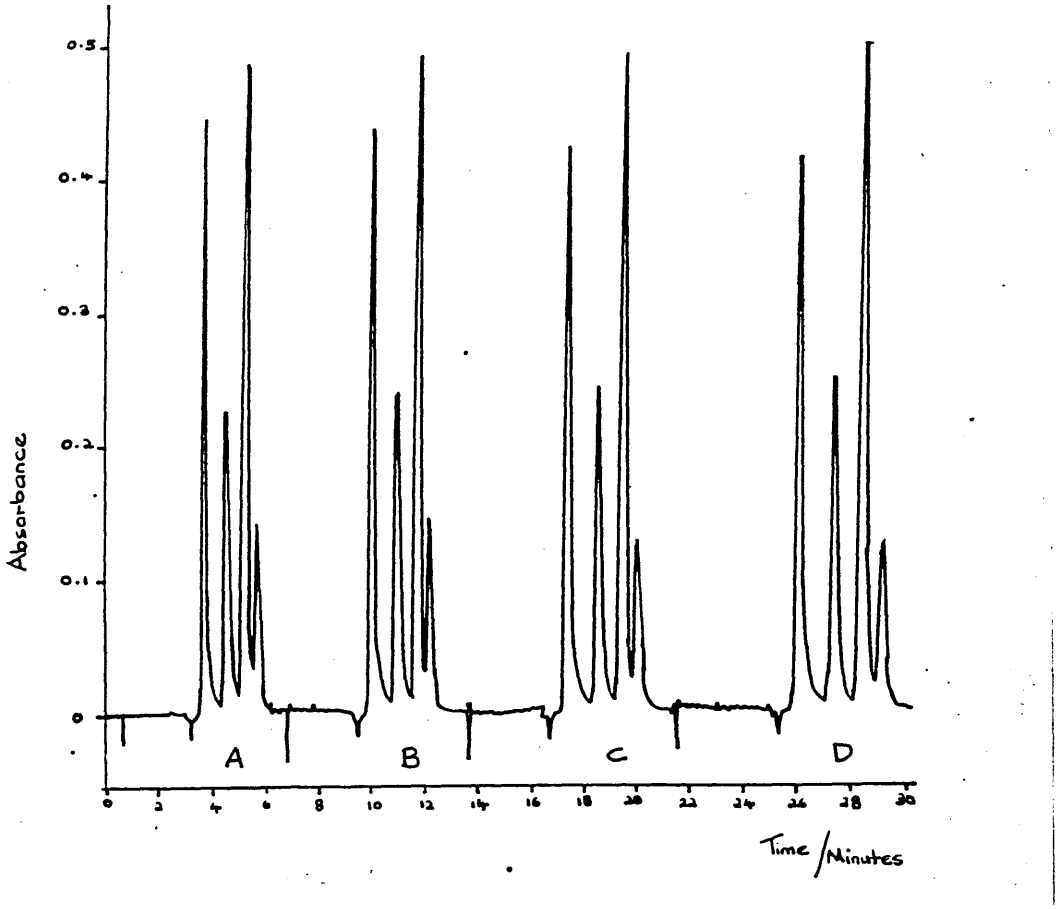


Fig. 4.32 Effect of minor variations in eluent flow rate on the chromatographic separation.

A - Flow rate 1.2 mL min^{-1} B - Flow rate 1.1 mL min^{-1}

C - Flow rate 1.0 mL min^{-1} D - Flow rate 0.9 mL min^{-1}

From the traces obtained it was clear that the chromatographic separation was relatively unaffected by small variations in the eluent flow rate.

4.4 EFFECT OF VARIATIONS IN THE pH OF THE MOBILE PHASE ON RETENTION BEHAVIOUR

It is obvious that the retention times varied significantly when the pH of the mobile phase was altered in section 4.2. However it is not clear whether this was due to changes in mobile phase composition only and not due to change in pH or whether just due to pH change or a bit of both.

In this section we set out to investigate this phenomenon and thus attempt to explain the retention mechanism.

The pH of the eluent may be altered in two ways :

Method I By keeping the amount of sodium tartrate and tartaric acid fixed then adding a

few drops of phosphoric acid to vary the pH

OR

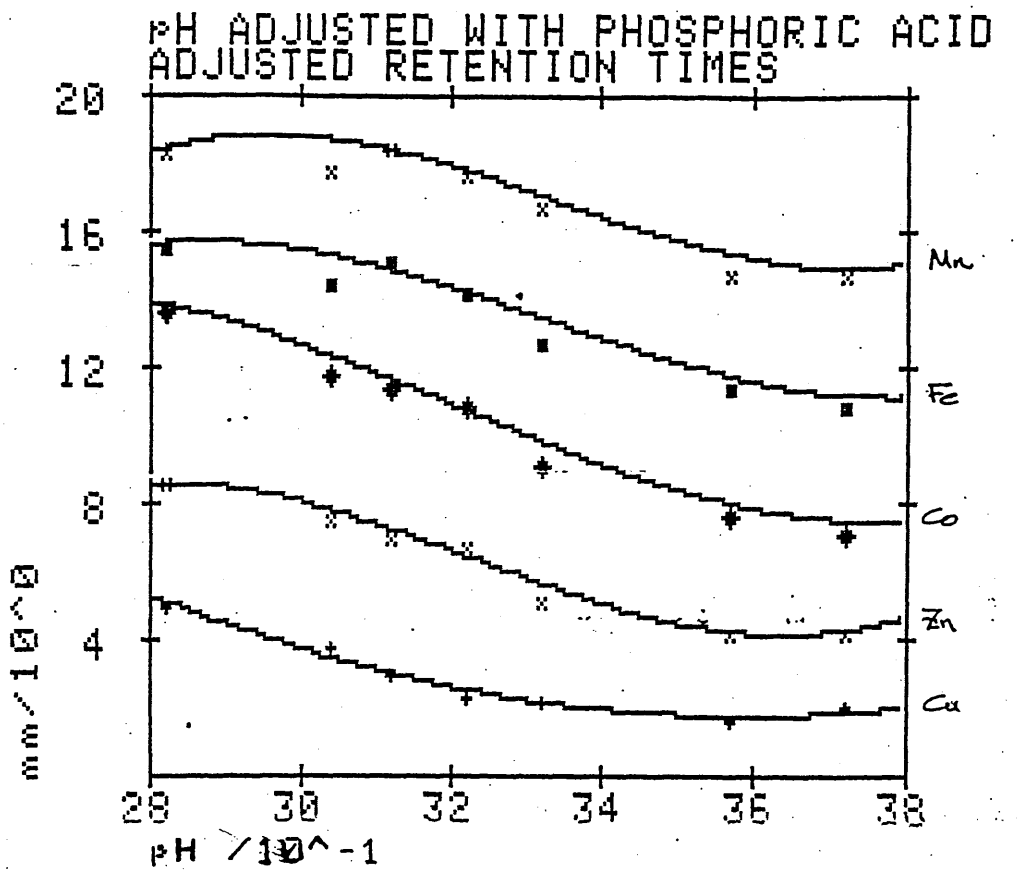
Method II By varying the ratio of sodium tartrate to tartaric acid and hence vary the pH but keeping the total tartrate concentration constant and equal to that in Method I.

The pH of the mobile phase was determined as outlined in section 2.35.

4.41 METHOD I

Data was collected over the pH range of 2.82 to 3.72 and the graph of adjusted retention times against pH of mobile phase was plotted (given overleaf) from which it can be concluded that as the pH is increased, retention times decreases.

eluent was varied by the addition of phosphoric acid



4.42 METHOD II

Data was collected over a smaller pH range ie from pH 2.83 to 3.56. This is because of the very poor resolution at either end of the pH range. This effect was not observed in section 4.41 , whereby resolution was good over the entire pH range investigated. Therefore this is most probably due to the change in sodium ion concentration - too much or too little has adverse effects on the chromatographic process.

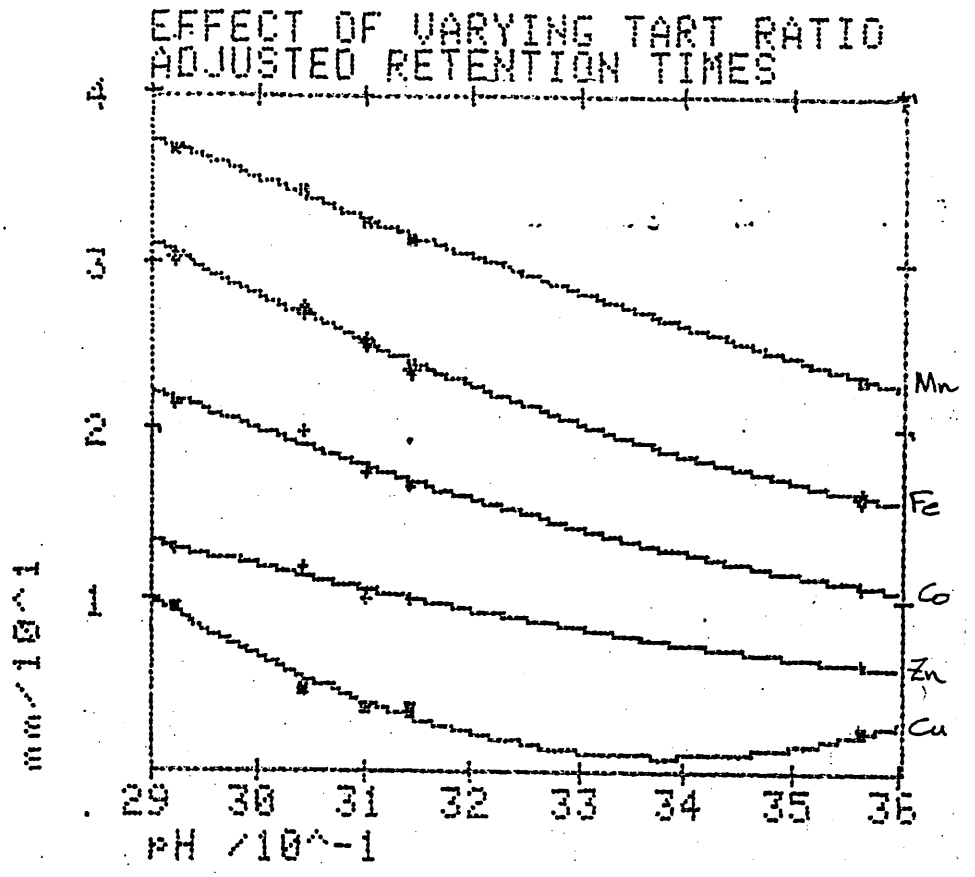


Fig. 4.42. Variation in retention times as eluent pH was varied by varying the sodium tartrate : tartaric acid ratio.

As with method I retention times is observed to decrease as pH increases however for the same pH value the corresponding retention times were found to be much longer in this case. This is most probably due mainly to the lower complexing ability of the tartrate since not all of the tartaric acid would have ionised. The ~~tartrate anion concentration would thus be lower even though the total tartrate content in both cases is identical~~.

tartrate anion concentration would thus be lower even though the total tartrate content in both cases is identical.

Therefore it may be concluded that ionic strength is also an important influencing factor when considering retention times and hence retention mechanisms.

4.5 EFFECT OF VARIATIONS IN THE CONCENTRATION OF SODIUM

In order to determine the effect of sodium ions on the separation, a set of experiments were conducted whereby the same sample was eluted by an aqueous solution of 0.025 mol dm^{-3} sodium tartrate , 0.020 mol dm^{-3} tartaric acid and 0.010 mol dm^{-3} sodium hexanesulphate for which the pH was 3.1.

Varying amounts of sodium nitrate were dissolved in the eluent to vary the amount of sodium ions. The graph overleaf summarizes the results obtained. (Each point plotted was an average of six readings.)

The graph clearly indicates that an increase in the sodium ion concentration reduces the retention times.

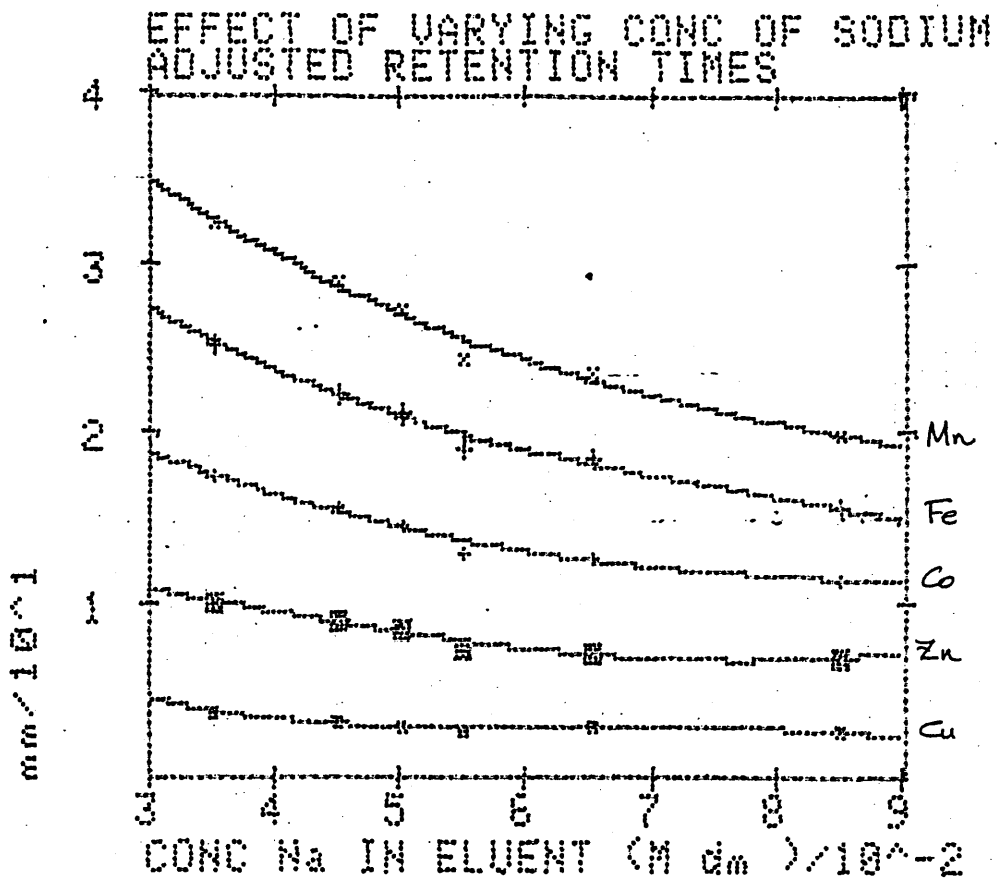


Fig 4.5 Variation in retention times as the concentration of sodium ions in the eluent is varied.

4.6 MODEL FOR FIXED SITE ION-EXCHANGERS

The ion-exchange process for fixed site ion exchangers may be represented by the equation

$$P^-,C^+ + M^+ \rightleftharpoons P^-,M^+ + C^+ \qquad (1)$$

where

P^-,C^+ represents an ion-exchange site.

P^- represents group which is fixed to the matrix.

C^+ is the associated counter ion in the liquid phase.

The counterion can be displaced by the solute (metal) ion M^+ to form (P^-,M^+) which results in retention of the solute ion.

For effective chromatography, the above displacement reaction must be at equilibrium. Retention for the reaction represented by expression (1) is therefore controlled by the equilibrium constant K_{IE} which is

$$K_{IE} = \frac{[C^+]\ [P^-,M^+]}{[M^+]\ [P^-,C^+]}$$

The capacity ratio k' is related to the distribution coefficient D_{IE} by

$$k' = \frac{C_S\ V_S}{C_M\ V_M} = D_{IE}\ \frac{V_S}{V_M}$$

where

C_S = Equilibrium concentration of solute in stationary phase.

C_M = Equilibrium concentration of solute in mobile phase.

and

V_S & V_M = volume of stationary and mobile phase respectively.

Therefore,

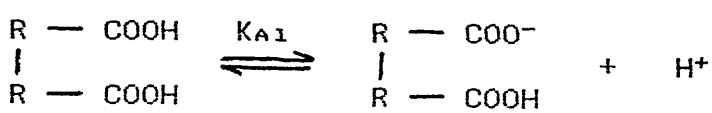
$$k' \propto D_{IE} = \frac{[P^-,M^+]}{[M^+]} = K_{IE}\frac{[P^-,C^+]}{[C^+]}$$

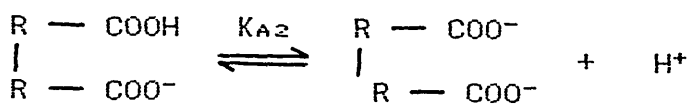
The concentration of ion exchange sites ie. $[P^-,C^+]$, when present in large excess, is constant and fixed by the structure of the matrix. Thus,

$$k' \propto 1/[C^+]$$

4.61 pH EFFECT

For a dibasic acid like tartaric acid for instance, the following equilibria exist ;





$$K_{A1} = \frac{[HOOC\text{-}R\text{-}R\text{-}COO^-]\ [H^+]}{[HOOC\text{-}R\text{-}R\text{-}COOH]}$$

Thus,

$$[HOOC\text{-}R\text{-}R\text{-}COO^-] = \frac{K_{A1}\ [HOOC\text{-}R\text{-}R\text{-}COOH]}{[H^+]}$$

$$\text{Fraction ionised} = \frac{[HOOC\text{-}R\text{-}RCOO^-]}{[HOOC\text{-}R\text{-}R\text{-}COOH] + [HOOC\text{-}R\text{-}R\text{-}COO^-]}$$

$$= \frac{K_{A1}\ [HOOC\text{-}R\text{-}R\text{-}COOH]/[H^+]}{[HOOC\text{-}R\text{-}R\text{-}COOH] + K_{A1}\ [HOOC\text{-}R\text{-}R\text{-}COOH]/[H^+]}$$

$$= \frac{K_{A1}}{K_{A1} + [H^+]}$$

For tartaric acid, pK_{A1} is 2.9 and pK_{A2} is 4.1 [62].

The pH of the eluent affects the fraction of tartaric acid ionised. When the pH is high (ie. $[H^+]$ low) the fraction of tartaric acid ionised will be high and vice versa.

Assuming that the unionised form is not absorbed by the matrix, then, for a given $[P^-]$ an increase in the pH

value would thus result in lower retention times. This agrees with the experimental data obtained.

The fraction ionised will also affect C_s and C_m values. Hence the pH of the eluent indirectly affects k' through the fraction of tartrate ionised.

4.7 MODEL FOR A DYNAMICALLY COATED COLUMN

In reversed-phase ion-pair chromatography, the organic phase forms the stationary phase and the aqueous phase is the eluent. The partition equilibrium can be represented by

$$M^+_{(aq)} + P^-_{(aq)} \overset{E_1}{\rightleftharpoons} (M^+,P^-)_{(aq)} \overset{E_2}{\rightleftharpoons} (M^+,P^-)_{(org)} \quad (2)$$

In this case the pairing ion P^- is present in relatively high concentration compared to the solute ion M^+ and is associated with a counterion C^+.

The extraction equilibrium E is defined by

$$E = E_1 . E_2 = \frac{[M^+,P^-_{(aq)}]}{[M^+_{(aq)}][P^-_{(aq)}]} \times \frac{[M^+,P^-_{(org)}]}{[M^+,P^-_{(aq)}]}$$

$$= \frac{[M^+,P^-_{(org)}]}{[M^+_{(aq)}][P^-_{(aq)}]}$$

Thus

$$D_{M+} = \frac{[M^+,P^-_{(org)}]}{[M^+_{(aq)}]} = E\,[P^-_{(aq)}]$$

Since k' is proportional to D_{M+}, k' will also be proportional to the pairing ion concentration. As such increasing the pairing ion concentration will increase retention. (See section 3.52).

If the pairing ion is very hydrophobic, then it will itself be strongly extracted into the organic phase along with its normal counterion C^+. We then have to consider the additional equilibrium

$$P^-_{(aq)} + C^+_{(aq)} \rightleftharpoons [P^-,C^+_{(org)}] \qquad (3)$$

In fixed site ion-exchangers, the concentration $[P^-,C^+_{(org)}]$ is fixed. In ion-pair systems however, this is not so.

Subtracting (3) from (2) gives

$$[P^-,C^+_{(org)}] + [M^+_{(aq)}] \rightleftharpoons [P^-,M^+_{(org)} + C^+_{(aq)}]$$

which is similar to the equation obtained for fixed site ion-exchangers.

The equilibrium constant for the reaction is

$$K_{IE} = \frac{[P^-,M^+_{(org)}][C^+_{(aq)}]}{[M^+_{(aq)}][P^-,C^+_{(org)}]}$$

for which the distribution coefficient for M^+ can be written

$$D_{M+} = \frac{[P^-,M^+_{(org)}]}{[M^+_{(aq)}]} = K_{IE}\frac{[P^-,C^+_{(org)}]}{[C^+_{(aq)}]}$$

The ratio $[P^-,C^+_{(org)}] / [C^+_{(aq)}]$ is determined by the equilibrium represented by equation (3).

Under conditions where the pairing ion P^- is very dilute in both phases, this ratio will be constant for any given $[P^-_{(aq)}]$, as is the case considered above where $[P^-_{(aq)}]$ is used directly to control the distribution and retention.

CHAPTER FIVE

CAPABILITY OF CHROMATOGRAPHIC METHOD

5.1 INTRODUCTION

We have now established a chromatographic method of analysis which is able to detect and identify up to seven metal ions per injection in less than seven minutes.

We now set out to determine the extent of the capabilities of this method. The experimental conditions for these investigations are as follows :

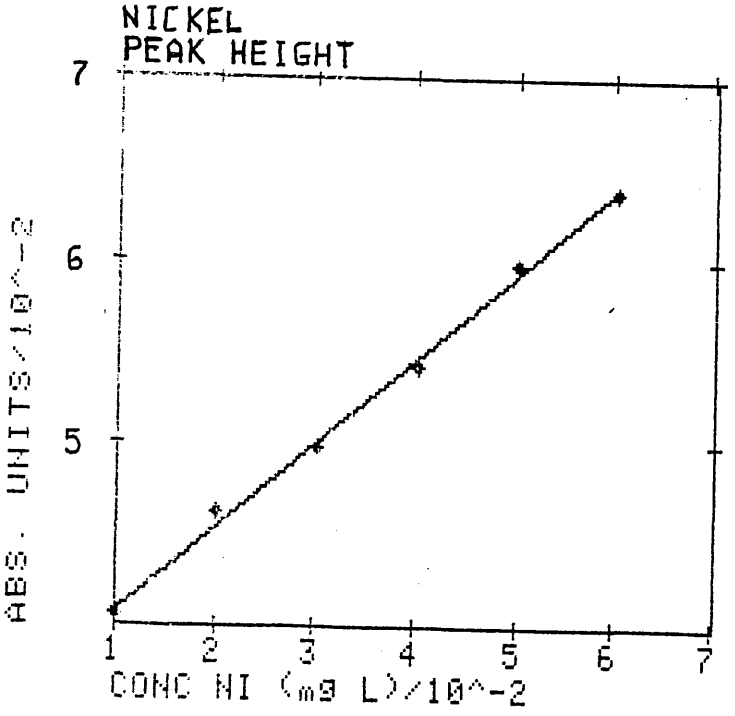
Eluent	:	0.045 M sodium tartrate + 0.010 M sodium hexanesulphonate; pH 3.2
Flow Rate	:	0.80 mL/min
Column	:	Spherisorb 5 ODS
Detector	:	510 nm

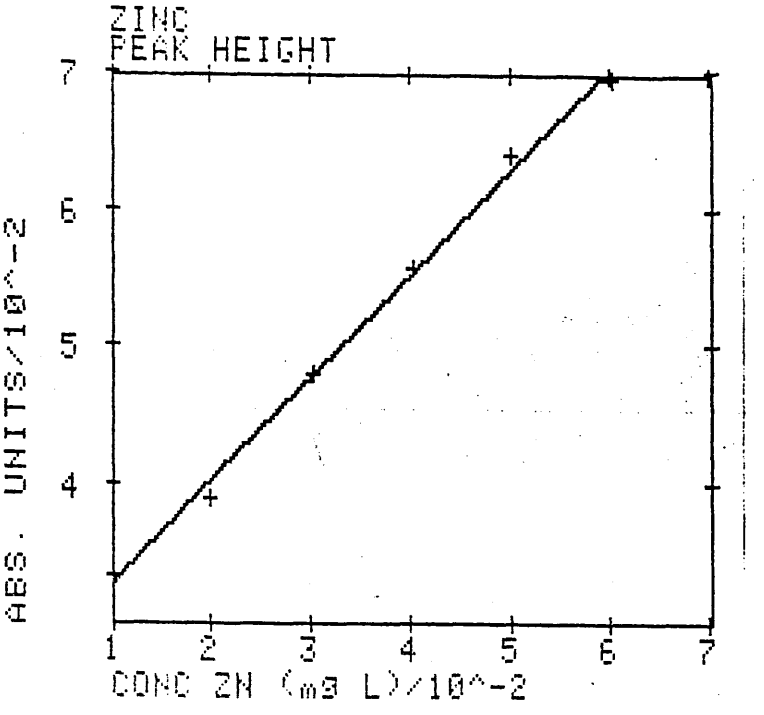
5.2 DETERMINATION OF TRACE AMOUNTS OF METAL IONS

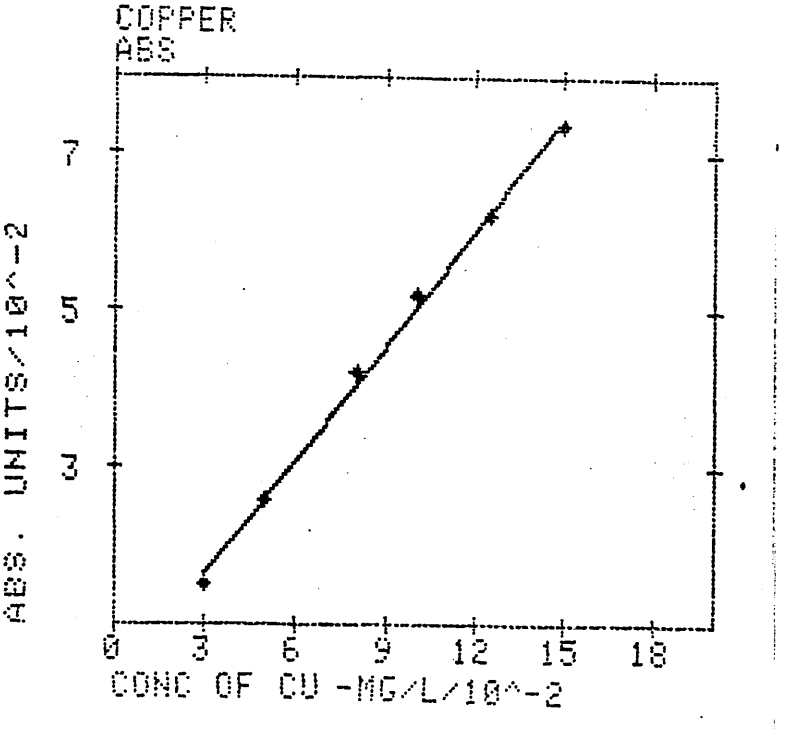
20 µL samples containing the following amounts of metal ions were analysed :

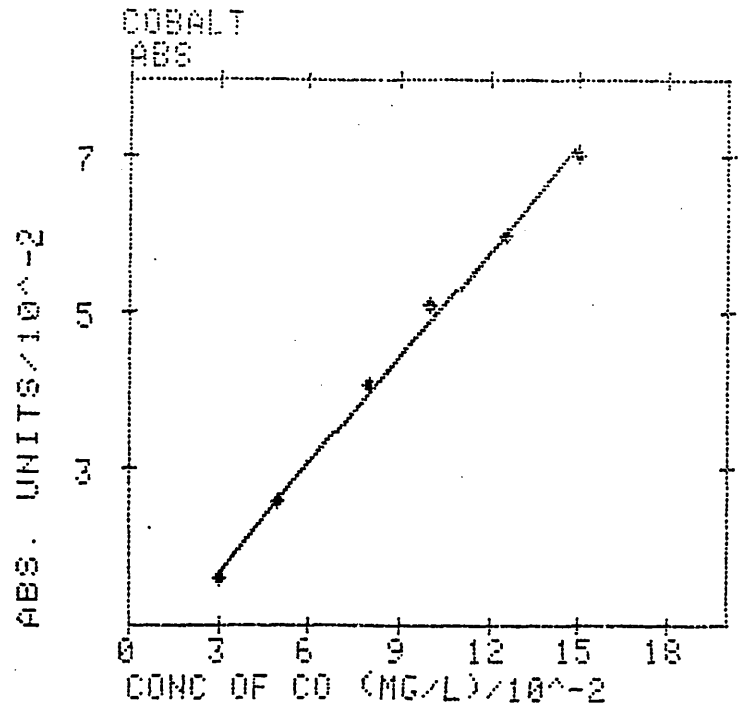
ION	CONC (ppm)	CONC (ppb)	Amount (ng)
Cu	0.030 - 0.120	30 - 120	0.60 - 2.4
Zn	0.010 - 0.060	10 - 60	0.20 - 1.2
Ni	0.010 - 0.060	10 - 60	0.20 - 1.2
Co	0.030 - 0.120	30 - 120	0.60 - 2.4
Mn	0.040 - 0.240	40 - 240	0.80 - 4.8

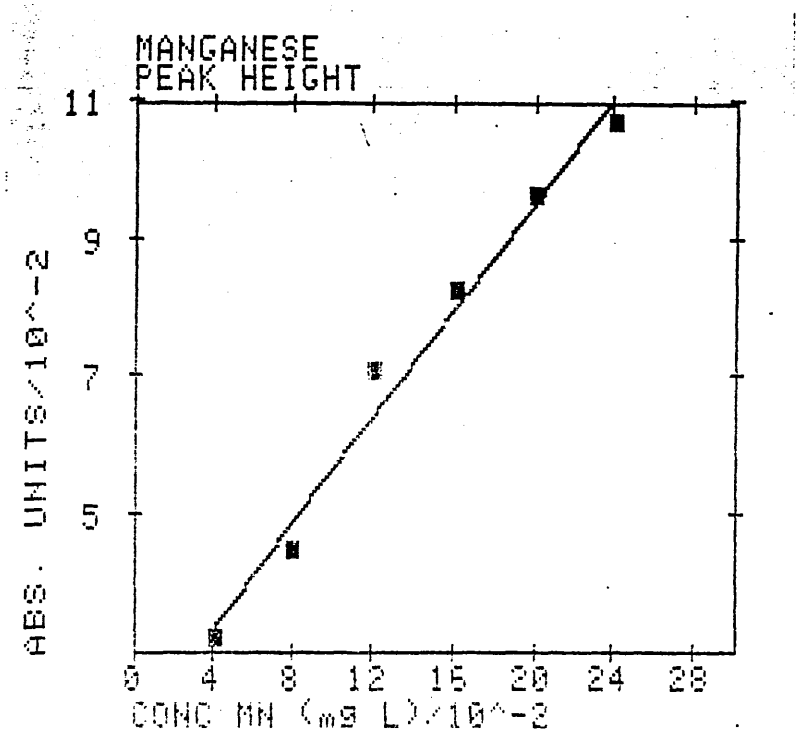
The results have been plotted in the following graphs.




ZINC
PEAK HEIGHT
ABS. UNITS/10^-2
4
5
6
7
1
2
3
4
5
6
7
CONC ZN (mg L)/10^-2


COPPER
ABS
ABS. UNITS/10^-2
3
5
7
0
3
6
9
12
15
18
CONC OF CU -MG/L/10^-2





It may thus be concluded that trace amounts of metal ions can be successfully analysed via this method.

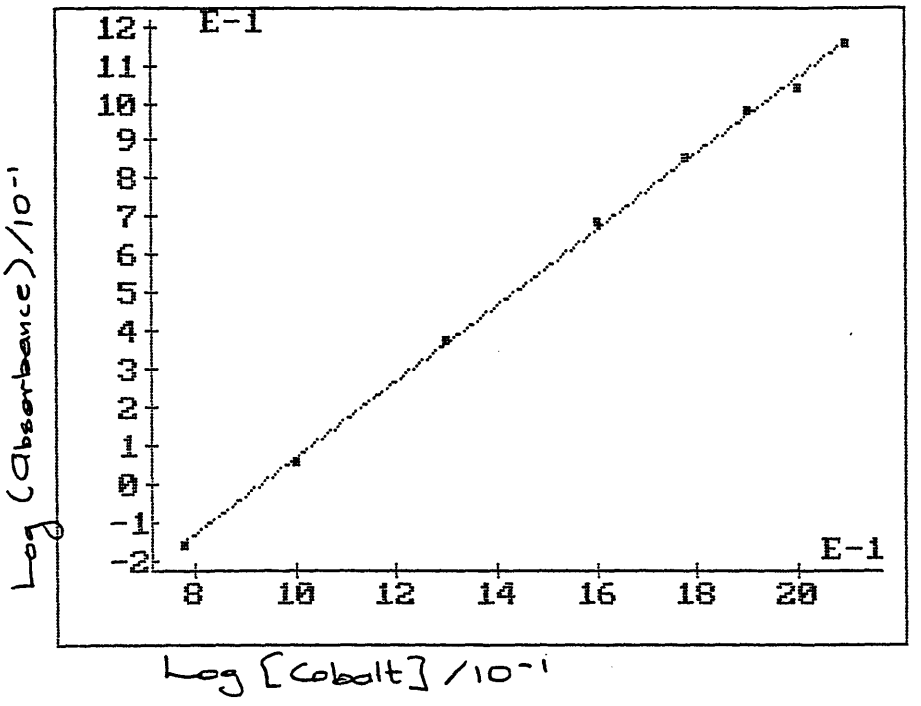
5.3 LINEAR RANGE

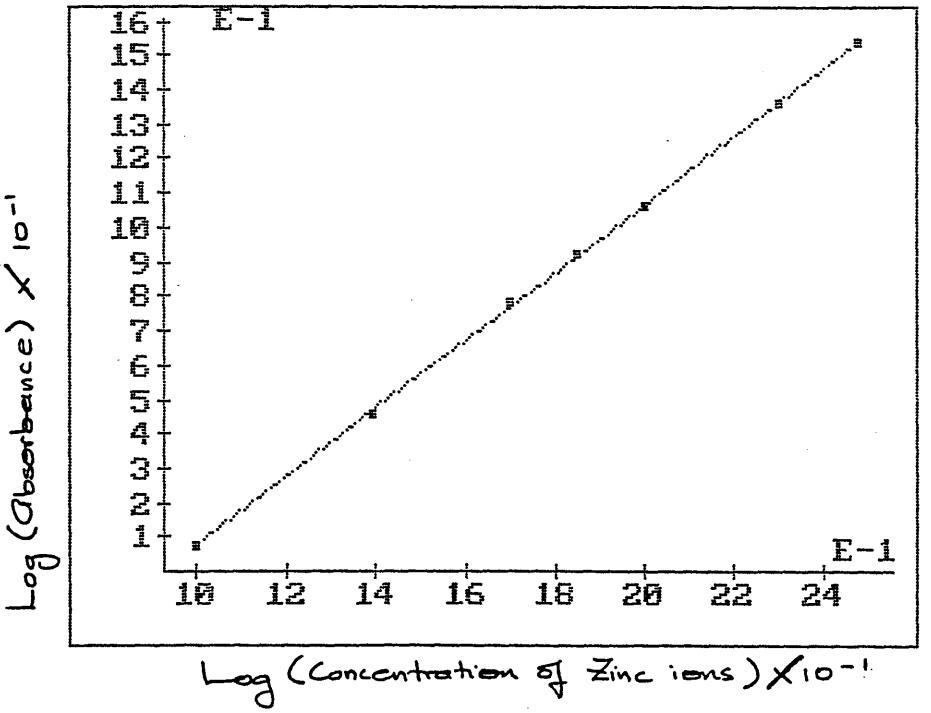
To determine the linear range 5 μL samples containing the following metal ion concentrations were analysed and the graph of log of peak height against log of concentration of metal ion in the tabulated range was plotted.

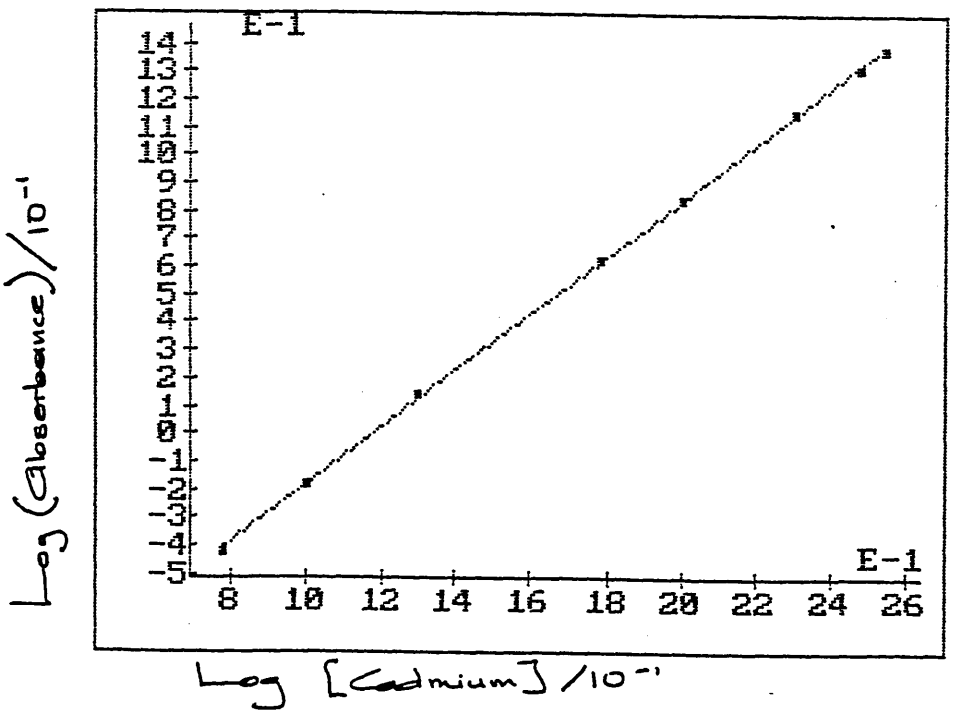
ION	CONC (ppm)	A.U.F.S.
Zn	700 - 10	1.0
Ni	400 - 10	0.5
Co	250 - 6	1.0
Cd	400 - 6	0.5
Mn	200 - 6	1.0

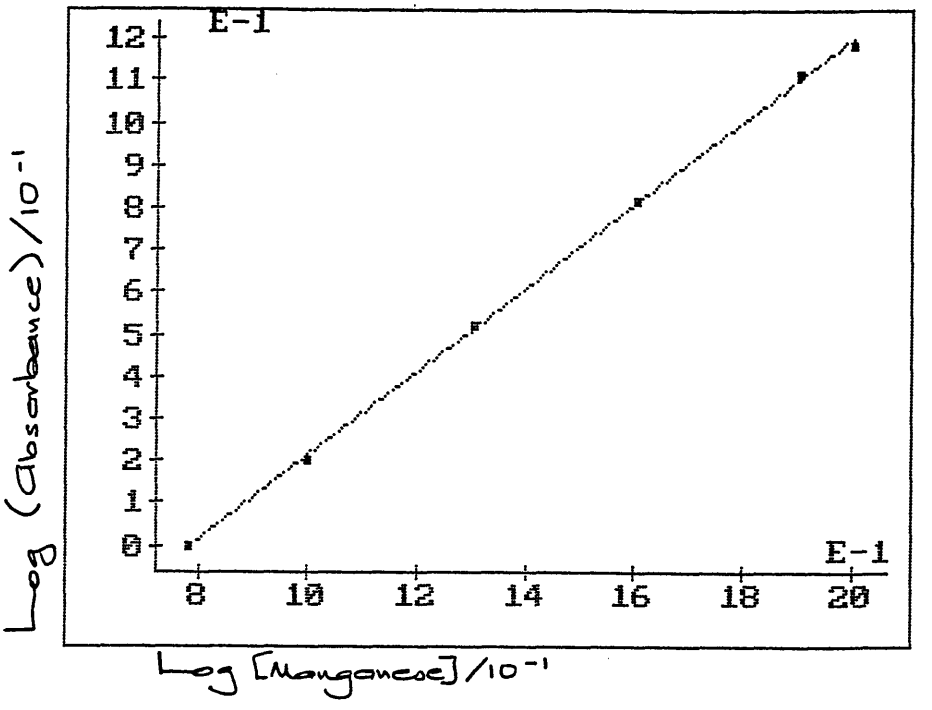
With reference to section 1.63 the acceptable gradient value for linear range is between 1.02 and 0.98. To comply to this requirement a narrower concentration would have to be used, resulting in the following linear ranges for the metal ions

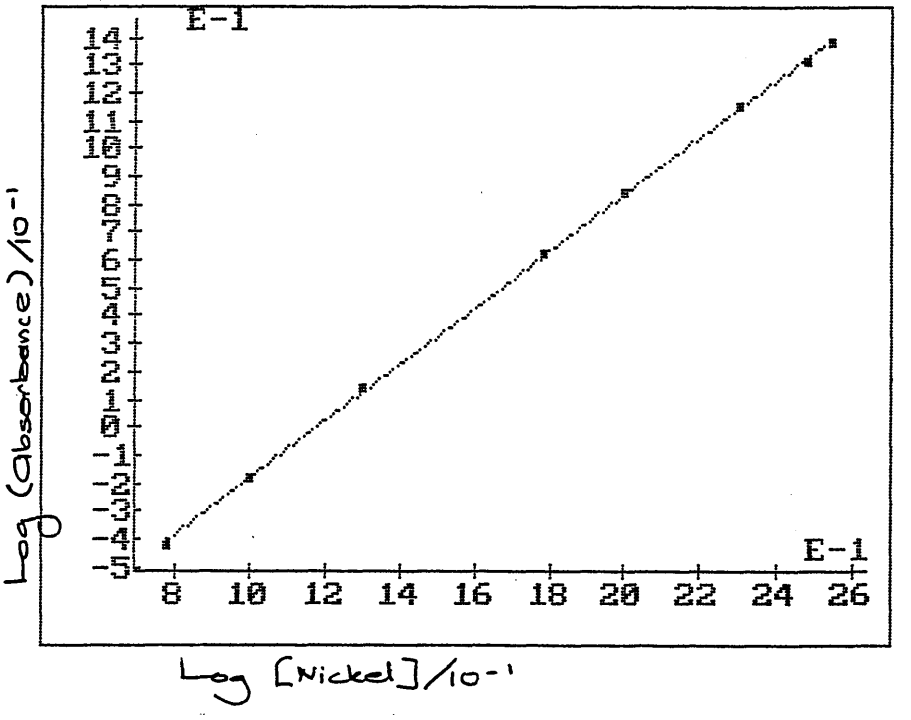
Zinc	300 - 10 ppm
Nickel	350 - 10 ppm
Cobalt	125 - 6 ppm
Cadmium	300 - 6 ppm
Manganese	100 - 6 ppm


E-1
12
11
10
9
8
7
6
5
4
3
2
1
0
-1
-2
E-1
8
10
12
14
16
18
20
Log (Absorbance)/10⁻¹
Log [Cobalt] /10⁻¹


E-1
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
E-1
10
12
14
16
18
20
22
24
Log (Absorbance) X 10⁻¹
Log (Concentration of Zinc ions) X10⁻¹


E-1
E-1
8
10
12
14
16
18
20
22
24
26
Log (absorbance)/10⁻¹
Log [cadmium]/10⁻¹





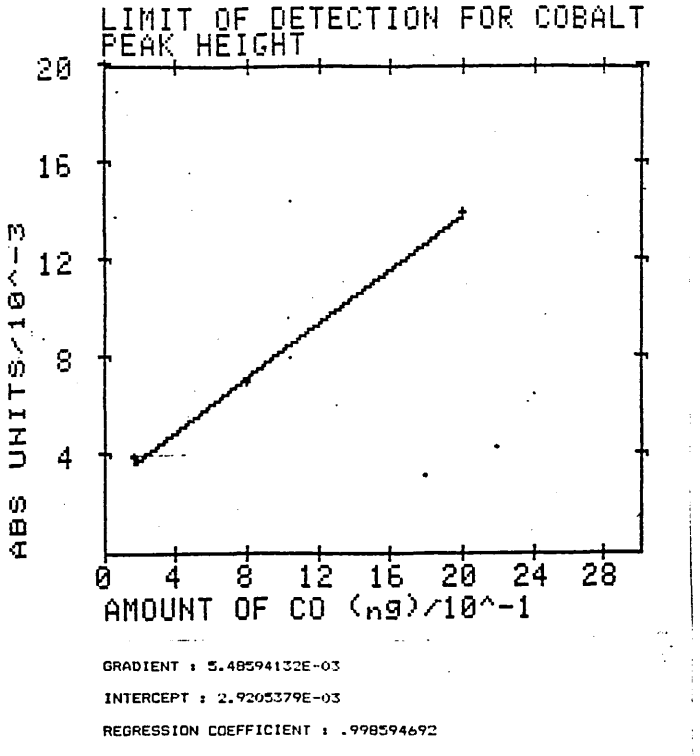
Figs. 5.3 A, B, C, D & E Linear range for the various metal ions

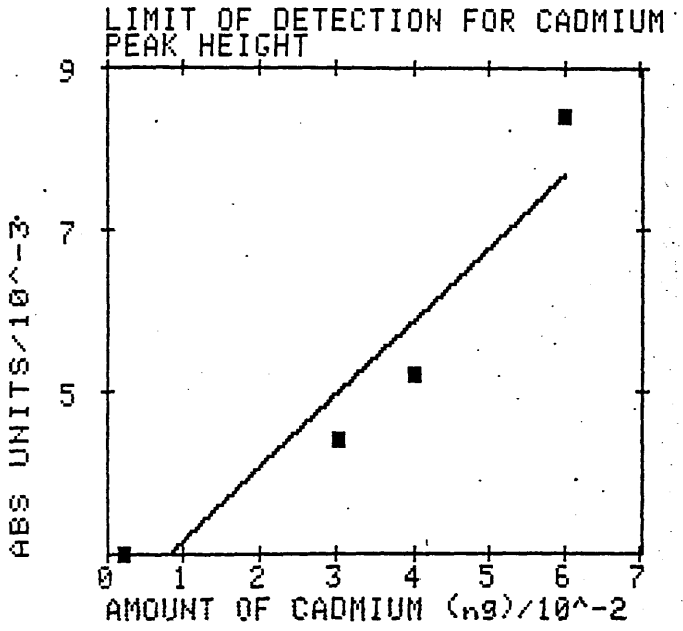
The relatively wide linear range enhances the applicability of this method of analysis.

5.4 LIMIT OF DETECTION

It is important to determine the limits of detection of an analytical method which is to be employed to detect trace amounts of metal ions. For this purpose 20 μL samples containing the following metal ion concentrations were analysed.

ION	CONC (ppm)	AMOUNT (ng)	NOISE (a.u.)
Zn	0.005 - 0.04	0.10 - 0.80	5×10^{-3}
Ni	0.003 - 0.009	0.06 - 0.18	4×10^{-3}
Co	0.008 - 0.10	0.16 - 2.0	2×10^{-3}
Cd	0.001 - 0.003	0.02 - 0.06	3×10^{-3}

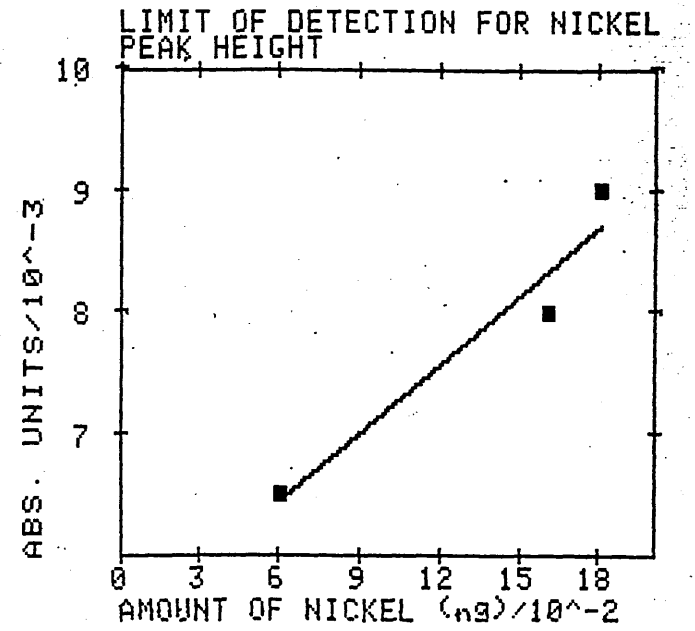




GRADIENT : .0898169336

INTERCEPT : 2.28604119E-03

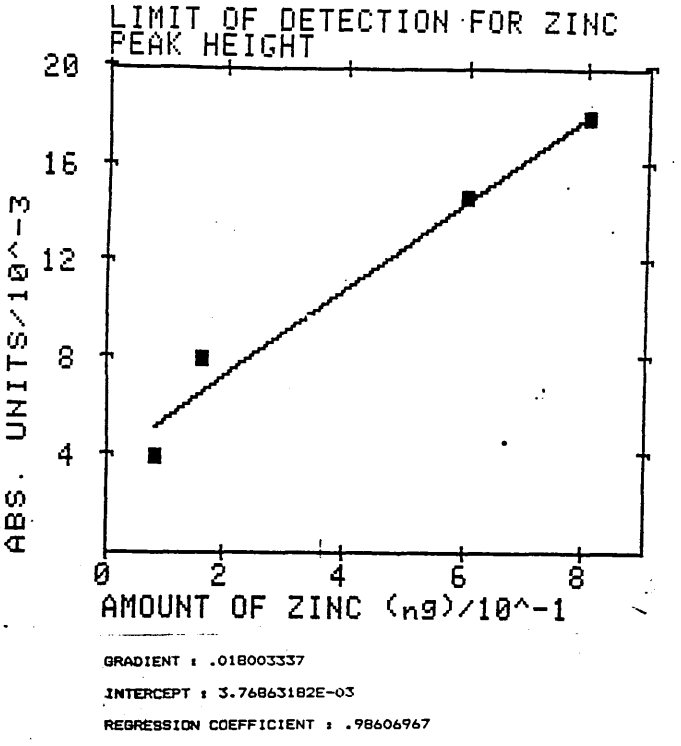
REGRESSION COEFFICIENT : .947416286



GRADIENT : .0197516129

INTERCEPT : [illegible]

REGRESSION COEFFICIENT : [illegible]



With reference to section 1.62, the limit of detection for the metal ions will therefore be :

0.40 ng (20 ppb) for zinc

0.15 ng (7.5 ppb) for nickel

0.25 ng (12.5 ppb) for cobalt

0.045 ng (2.5 ppb) for cadmium.

CHAPTER SIX

CONCLUSIONS

The development of a chromatographic system for analytical work does not involve a heavy investment. However, care must be taken to ensure that the items of equipment selected are compatible. In this work, for example, it was discovered that the reciprocating pump which was ideal for delivery of the mobile phase was not suitable for the post-column reagent. This is because the stream of post-column reagent should as far as possible be pulse-free to minimise the noise levels. Minor flutuations in the eluent flow rate howver did not adversely affect the separation.

The best chromatograms were also achieved when the eluent and post-column reagent flows were similar and when all tubings and internal bores (of the mixing device for instance) were kept as equal as possible.

The mixing device, although small, plays a critical role and close study of its design and orientation is required.

4-(2-pyridylazo)resorcinol (PAR) as a post-column derivatising agent to enable spectrophotometric detection of the separated metal ions was found to be very satisfactory, easy and convenient to handle and work with. This is because it reacts with a very wide range of metal ions (non-selective) almost instantaneously and the λ_{max} for the metal-PAR complexes lies within a narrow range and well away from the λ_{max} of the unreacted PAR. This means that detection of the chromatographed metal ions may be done at a fixed wavelength (in this case 510 nm was found to be the best) without any interference from the unreacted PAR even though a large excess of PAR is used.

The mobile phase was found to be a powerful tool in ensuring good chromatograms are achieved. It is an aqueous solution containing an anionic modifier (eg sodium hexanesulphate) which dynamically coats the stationary phase and hence enables retention of the ions and a complexing agent (eg. sodium tartrate) which by controlling the concentration of free metal ions in solution enables its elution from the column.

Increasing the concentration of the anionic modifier results in longer retention of the ions. On the other hand, the ions are eluted faster if the concentration of the tartrate is increased.

Sodium hexanesulphate was found to be a better anionic modifier than sodium dodecylsulphate. This is because with the former there is no need to coat the column first prior to the chromatographic run. this not only saves time and also means that it is easier to vary the modifier concentration to suit the experimental conditions.

The separations were also affected by the pH of the eluent. Generally the retention times decreased as the pH of the eluent was increased. The same effect was also observed when the concentration of sodium ions in the eluent was increased.

Based on these observed data a model for fixed site ion-exchangers and for a dynamically coated column was put forward.

The success of this analytical procedure is evidenced by the fact that using a mobile phase containing 0.045 M sodium tartrate and 0.010 M sodium hexanesulphate at an eluent pH of 3.1, enabled the separation of up to seven metal ions ie. copper, lead, zinc, nickel,cobalt,cadmium and manganese, in seven minutes, detected at 510 nm after a post-column reaction with PAR. Under these conditions, detection limits in the range of 2 - 20 p.p.b. were achieved.

Unfortunately, this analytical method was not applied to real samples eg. water samples. It would have been interesting to study the modifications which need to be made to ensure the success of such determinations.

REFERENCES

1 U.A.Brinkman, G.deDries, R.Kuroda
Journal of Chromatography 85 (1973) 187

2 H.Small, T.S.Stevens, W.C.Bauman
Analytical Chemistry 47 (1975) 1801

3 H.Small
Journal of Inorganic Nuclear Chem. 18 (1961) 2322

4 D.T.Gjerde, J.S.Fritz, G.Schmuckler
Journal of Chromatography 186 (1979) 509

5 D.T.Gjerde, J.S.Fritz, G.Schmuckler
Journal of Chromatography 187 (1980) 35

6 R.W.Frie
Journal of Chromatography 165 (1979) 75

7 J.F.Lawrence, R.W.Frie
Chemical Derivatisation for Chromatography
Elsevier (1976)

8 S. Shibata [Editors: Flaschka, H.A; Barnard, A.J.]
Chelates in Analytical Chemistry, Vol 1.
Marcel Decker, New York, 1969

9 A.M.Bond, C.G.Wallace
Analytical Chemistry 53 (1981) 1209

10 A.M.Bond, C.G.Wallace
Analytical Chemistry 54 (1982) 1706

11 A.M. Bond, C.G.Wallace
Analytical Chemistry 55 (1983) 718

12 B.R.Willeford, H.Veening
High-Performance Liquid Chromatography: Applications to Organometallic and Metal Coordination Compounds.
J. Chromatography 251 (1982) 61

13 J.S.Fritz, D.T.Gjerde, R.M.Becker
Analytical Chemistry 52 (1980) 1519

14 G.J.Sevenich, J.S.Fritz
Analytical Chemistry 55 (1983) 12

15 A.M.Bond, C.G.Wallace
Analytical Chemistry 56 (1984) 2085

16 R.M.Smith, L.E.Yankey
Analyst 107 (1982) 744

17 R.E.Majors
Journal of Chromatographic Sc. 18 (1980) 488

18 W.H.Delphin, E.P.Horwitz
Analytical Chemistry 50 (1978) 843

19 S.Elchuk, R.M.Cassidy
Analytical Chemistry 51 (1979) 1434

20 B.L.Karger, R.W.Glese
Analytical Chemistry 50 : 1048A (1978)

21 S.Elchuk, R.M.Cassidy
Analytical Chemistry 54 (1982) 1558

22 S.Eksborg, P.O.Lagerstrom, R.Modin, G.Schill
Journal of Chromatography 83 (1973) 99

23 I.M.Johansson, K-G Wahlund, G.Schill
Journal of Chromatography 149 (1978) 281

24 R.M.Smith
Analytical Proceeding 21 (1984) 73

25 HPLC Advances and Perspectives Volume I
Editor: Csaba Horvath, Page: 100 , 1980
Academic Press .

26 C.Horvath, W.Melander, I.Molnar, P.Molnar
Journal of Chromatography 125 (1976) 129

27 C.Horvath, W.Melander, I.Molnar
Analytical Chemistry 49 (1977) 2295

28 J.C.Kraak, K.M.Jonker, J.F.K.Huber
Journal of Chromatography 142 (1977) 671

29 P.T.Kissinger,
Analytical Chemistry 49 (1977) 883

30 B.A.Bidlingmeyer, S.K.Dening, W.P.Price, B.Sachok, M.Petruzek
Journal of Chromatography 186 (1979) 419

31 S.D.Dowling, W.R.Seitz
Analytical Chemistry 57 (1985) 602

32 J.H.Knox (Editor)
High Performance Liquid Chromatography
Edinburgh University Press, 1978

33 J,F.Lawrence
Journal of Chromatography 211 (1981) 144

34 Y.Talmi
Analytical Chemistry 47 (1975) 658A

35 R.E.Dessy, W.G.Nunn, C.A.Titus
J. of Chromatographic Sc. 14 (1976) 195

36 M.S.Denton, T.P.DeAngelis, A.M.Yacynych,
W.R.Heinemann, T.W.Gilbert.
Analytical Chemistry 48 (1976) 20

37 E.McDowell, H.L.Pardue
Analytical Chemistry 48 (1976) 1815

38 E.McDowell, H.L.Pardue
Analytical Chemistry 49 (1977) 1171

39 A.F.Fell
Analytical Proceeding 17 (1980) 266

40 M.Denkert, L.Hackzell, G.Schill, E.Sjogren
Journal of Chromatography 218 (1981) 31

41 H.Small, T.E.Miller Jr.
Analytical Chemistry 52 (1982) 462

42 R.A.Cochrane, D.E.Hillman
Journal of Chromatography 241 (1982) 392

43 J.R.Larson, C.D.Pfieffer
Journal of Chromatography 259 (1983) 519

44 J.R.Larson, C.D.Pfieffer
Analytical Chemistry 55 (1983) 393

45 M.H.Gaffney, M.Cooke
Analytical Procedings 21 (1984) 440

46 P.Jones, P.J.Hobbs, L.Ebdon
Analyst 109 (1984) 703

47 Y. Yakata, G.Muto
Analytical Chemistry 45 (1973) 1864

48 D.H.Sisson, V.A.Mode, D.O.Campbell
Journal of Chromatography 66 (1972) 129

49 J.F.K.Huber, A.M.Van Urk-Schoen
Analitica Chimica Acta 58 (1972) 395

50 J.S.Fritz, D.T.Gjerde, R.M.Becker
Analytical Chemistry 52 (1980) 1519

51 J.R.Beckett, D.A.Nelson
Analytical Chemistry 53 (1981) 909

52 E. Di Cesare
Journal of Chromatography 251 (1982) 1

53 G.J.Schimdt, R.P.W.Scott
Analyst 109 (1984) 997

54 W.J.Geary, G.Nickless, F.H.Pollard
Analitica Chimica Acta 26 (1962) 575

55 F.H.Pollard, P.Hanson, W.J.Geary
Analitica Chimica Acta 20 (1959) 26

56 M. Hnilickova, L. Sommer
Collect. Czech. Chem. Commun. 26 (1961) 2189

57 W.J.Geary, G.Nickless, F.H.Pollard
Analitica Chimica Acta 27 (1962) 71

58 A.Corsini, I.M-L.Yih, Q.Fernando, H.Freiser
Analytical Chemistry 34 (1962) 1090

59 J.R.Jezorek, H.Freiser
Analytical Chemistry 51 (1979) 373

60 R.M.Cassidy, S.Elchuk
J. of Chromatographic Science 18 (1980) 217

61 G.O.Franklin
International Lab. July/August (1985) 56

62 D.D.Perrin
Masking and Demasking of Chemical Reactions.
John Wiley & Sons Inc. 1970