Novel Preconcentration Techniques for the determination of Trace Metal Ions in Environmental Matrices

by

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Declaration

I hereby certify that this material, which I now submit for assessment on the programme of study leading to the award of Doctor of Philosophy is entirely my own work and has not been taken from the work of others save and to the extent that such work has been cited and acknowledged within the text of my work.

Signed: Eva Ryan ID No. 89700775

Date: 23/09/1993

To my parents for their never-ending support

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Abstract :

The analysis of single or multiple metal ions in trace amounts in environmental matrices is a general problem. As a result methods for the selective preconcentration of trace metals are continually investigated. Successful development and application of four novel preconcentration techniques for the determination of trace metal ions are described in the bulk of this thesis.

In chapter 1 traditional and novel preconcentration approaches including their inherent advantages and disadvantages are described.

A column switching RPHPLC technique coupled with UV/VIS detection for trace level determination of Cu(II), Fe(III) and Al(III) in waste water and beer samples is described in chapter 2. Metal precomplexation with 8-hydroxyquinoline enabled spectrophotometric detection at 400 nm. Limits of detection of 5 ppb for Al(III) and 40 ppb for Cu(II) and Fe(III) were obtained.

The use of ligand coated stationary phases incorporating a novel CTAB/DTC ion pair for copper determination in mine waste water is described in chapter 3. The CTAB-DTC precolumn has been used for on-site sample loading. Direct incorporation of the loaded precolumn back into the switching system decreased overall sample analysis time considerably. Precolumn preparation was extremely reproducible with < 3 % variation for a batch size of 50.

Preconcentration of Cu(II) using microbial biomass is described in chapter 4. Off-line copper determination using FAAS and on-line determination using HPLC coupled to a UV/VIS spectrophotometer was possible using this novel stationary phase. Rhizopus arrhizus (unlyophilized) packing material, particle size < 25 μm exhibited the highest overall % recovery of Cu(II), > 90 % recovery was achieved.

Surfactant enhanced adsorptive stripping voltammetry for trace level determination of aluminium in water and soil samples is reported in chapter 5. This electroanalytical preconcentration method allowed the determination of aluminium as its Solochrome Violet RS (SVRS) complex in the presence of a cationic surfactant, CTAB.

Chapter 1

Trace metal determination using traditional and novel preconcentration techniques

1.1 INTRODUCTION

The analysis of trace amounts of a single, or a mixture of, metal ions in environmental matrices poses particular problems. Owing to the toxicity of many trace metals, a simple, multi-element method of determination is required. A large number of established methods are available for quantitative trace metal analyses, including atomic absorption spectrometry [1], plasma emission spectrometry and colorimetry [2], as well as electrochemical methods including polarography and voltammetry [3]. However, with these more traditional methods there are associated drawbacks, including lack of selectivity, which makes them time consuming, and the necessity for expensive instrumentation. With these direct methods there is also the possibility of interferences on the analytical signal due to matrix effects or concomitant elements within the sample.

Chromatographic methods, such as thin layer chromatography [4], gas liquid chromatography and classical (gravity feed) column chromatography have also been used for trace metal analyses. However, these methods are quite limited and are therefore, generally not used for routine analysis. Liquid Chromatography, and in particular high performance liquid chromatography (HPLC), possesses excellent capabilities as a multi-element technique for inorganic compounds. It is one of the most suitable methods for separation and simultaneous determination of metal ions that have similar chemical properties, and determination of metal ions as inorganic metal complexes using HPLC is well documented [5]. Huber et al. [6] reported the first separation of metal complexes in 1967, where six metal acetylacetonates were determined in 25 minutes. Far more rapid determinations are now possible [7]; however the separation and identification processes are essentially unchanged. Separation and identification are normally achieved

using HPLC coupled to UV/VIS or fluorescence detection [8, 9]. Two options are widely recognised for this technique:

a. the metal ions of interest can be complexed with an organic ligand and the metal ligand complexes can be passed down a chromatographic column on which they are separated, or

b. "in-situ" complexation, whereby the metal ions are injected into the mobile phase containing the ligand and effective on-column complex formation is achieved.

Ligands such as quinolines [10], sulphonated heterocyclic dithiocarbamates [11], substituted naphthols [12, 13] and acetlyacetonates [14] have all been found suitable for this type of analysis.

Ion pair chromatography is another widely used technique for the separation of metal ions. Frequently, the mobile phase incorporates an ionic species which interacts with the ionic sample, therefore effecting its separation. A recent publication by Siren et al. [15] indicates the advances that have taken place in ion pair chromatography. They examined the effect of ion pair modifiers on the separation of Co(II), Cu(II), Fe(III) and Pd(II) by precolumn derivatisation and HPLC, with organic alkylammonium salts being used as the ion pairing reagents. The two options of precolumn and on-column complexation were examined in this study, the ligand and ion pair reagent being present in the eluent in the latter case and only the ion pair reagent in the first case.

Traditional single column HPLC is a powerful instrumental method for inorganic analysis. It's advantages include low detection limits, wide linear ranges and simultaneous qualitative and quantitative analysis. However, considering the complexity of environmental samples and the

ultra trace level of metals often present, preconcentration and separation of the analyte prior to actual determination are now recognised as indispensable steps in the analysis. Development of effective preconcentration techniques which enhance both sensitivity and selectivity of established procedures are now of paramount importance.

The bulk of this thesis investigates the use of novel on-line precolumn sample handling techniques which considerably increase the sensitivity of metal ion detection by HPLC. Selection of precolumn packing materials and/or precolumn derivatisation procedures which can distinctly enhance selectivity, thereby making demands on the final detection step less stringent are discussed. Rapid multi-element determinations have been achieved using the column switching approaches adopted. A novel electroanalytical method for the preconcentration of Al(III), an element with poor electrochemical properties is also outlined.

1.2 PRECONCENTRATION TECHNIQUES

The reason for developing column switching as a preconcentration tool for metal ion analysis in preference to other methods becomes clear if the methodology of other methods is examined. Both traditional and novel preconcentration techniques with obvious advantages and disadvantages are outlined.

1.2.1 Traditional Techniques

Traditional approaches to trace metal preconcentration based on solvent extraction [16], coprecipitation [17], and ion exchange [18] continue to receive attention particularly when high preconcentration factors are required by the user. However, solvent extraction, probably the most widely used of these techniques has only limited concentration ability due to the batch type nature of the process where trace enrichment of large sample volumes is difficult due to the single stage separations involved.

Coprecipitation requires judicious choice of a collector and precipitant, and is most effective when the precipitation process attains equilibrium very slowly. Obviously for an analyst requiring a fast, effective preconcentration step this would not be feasible. Incorporation of this preconcentration method on-line with a detection system would be extremely difficult. Accurate determination of total metal concentrations using ion exchange techniques is often unreliable as anthropogenic ligands in natural waters have high complexing capabilities and compete unfavourably with ion exchangers.

1.2.2 Novel Techniques

1.2.2.1 Use of Novel Preconcentration Supports

Many novel trace enrichment techniques have been reported, and can be defined by the different mechanisms by which metal ion uptake is achieved. Mechanisms which facilitate metal uptake include adsorption [19], ion exchange [20], partition [21], ion-pair interaction [22] and chelation [23 - 25]. Chelation techniques are the most widely documented. The organic ligands used have been immobilised on different solid sorbents; namely polymers, ion exchangers, reversed-phase octadecyl silica and controlled pore glass.

The ligands can be chemically bonded to or physically supported on the various substrates. The most frequently reported substrates are silica gel [26 - 28], resins [29 - 31], naphthalene [32 - 34] and polymers [35]. Active carbon has also been used to adsorb metal chelates [36, 37]. The use of these various adsorbents to concentrate and/or separate metal ions has gained popularity due to their high preconcentration ability and ease of operation. They are described in considerable detail in the following sections because of their importance in the area of trace metal preconcentration.

1.2.2.1.1 Silica Gel

Among the different substrates used for immobilisation, silica gel is of particular interest because it does not swell or strain, can undergo heat treatment and has good mechanical strength [38]. Several authors have reported the use of ligand functionalised chelating silicas for the preconcentration of metal ions, followed by ion chromatography (on-line, in some cases) and AAS or spectrophotometric detection.

Chambaz et al. [39] described the on-line preconcentration of Cu(II) and Ni(II) on 8-hydroxyquinoline bonded silica gel followed by ion chromatographic separation. Metal desorption from the preconcentration column was effected using 0.1 M potassium cyanide solution pH 8.5, and separation by ion pairing was achieved using tetrabutylammonium ions. Limits of detection of the order 10^{-7} M or less were attainable, however, only Cu(II) and Ni(II) could be determined using this method, and diode array UV detection was necessitated as the cyano metal complexes absorbed at two different wavelengths.

In a subsequent paper Chambaz et al. [40] described the use of an ethylenediamine triacetate (ED3A) bonded chelating silica for the preconcentration of divalent metal ions including Mn(II), Co(II), Ni(II), Cu(II), Zn(II), Cd(II) and Pb(II). The cyano eluent was replaced by 0.1 M nitric acid which allowed quantitative metal desorption from the precolumn. Unfortunately nitric acid was not found suitable for classical cation exchange chromatography (the method of separation chosen by these authors) so post precolumn eluent modification was effected using tartrate / sodium nitrate buffer (pH 3).

Following chromatographic separation on a cation exchange column the eluted metals were detected by post column reaction with 4-(2-pyridylazo)-resorcinol (PAR) and determined at 500 nm by diode

array-UV/VIS detection. Limits of detection were extended to metal concentrations of 3 x 10^{-9} M, a 100 fold improvement on the earlier method. Concentration factors of 1000 fold were therefore achieved, and 100 % metal recovery was possible in most cases. Disadvantages of this method appear few, however, it can be noted that because the neutralised eluent following post precolumn modification has a very high ionic strength, a high capacity cation exchanger would always be required for metal ion separation. Use of a more sensitive post column reagent (than PAR) would undoubtedly extend the detection limit. The method was applied to metal determination in river water and the authors report that free and labile metal complexes could be determined successfully.

The stability of ligand bonded silica adsorbents is an obvious advantage; however it doesn't compensate for the fact that the preparation of the silica gel with immobilised ligands can often be time consuming, and the number of ligands that can actually bond to the silica gel are limited.

Terada et al. [41] have described an alternative to the ligand bonded silica gel adsorbents, where they used a chelating agent supporting silica gel prepared by simple refluxing of the appropriate chelating agent with the activated silica gel. Simply by changing the loading of chelating agents a high selectivity for different metals could be achieved. Using this technique they separated Cu(II), Cd(II), Zn(II), Pd(II), Co(II), Au(I) and Ag(I) by selective preconcentration using ligands such as thioalide [42] and mercaptobenzothiazole [43]. No observable effects due to changes in the volume of the sample solution have been noted by authors using this technique (up to a volume of 1 litre). This is an obvious advantage over traditional preconcentration techniques such as solvent extraction.

The idea of supporting organic reagents on silica gel has been popularly received as the adsorbent can be prepared rapidly and simply. Tong and co-workers [44] preconcentrated indium on 1-phenyl-3-methyl-4-

stearoyl-5-pyrazolone supporting silica gel (C_{18}/SG) where C_{18} denotes the number of carbon atoms in the acyl chain. The compound was hydrophobic, extremely reactive and formed stable complexes with several metal ions at low pH. Indium was quantitatively retained on a C_{18}/SG column (1 g loading of packing material) even at flow rates as high as 16 cm^3 min^{-1}. Retained metals were desorbed using 1 M hydrochloric acid and analysed by flame AAS. The maximum volume of test solution used was 1 litre and the normal elution volume was 25 cm^3, therefore concentration factors were of the order of 40. In (III) was selectively retained in the presence of Ni(II), Co(II) and Zn(II), and only Al(III) was found to inhibit In(III) recovery.

In a second paper Tong et al. [45] reported the application of the preconcentration method to Cu(II), Co(II) and Ni(II) determination in sodium chloride solution and tap water. The sited metals were quantitatively retained above pH 4 and eluted as before with 1 M HCL. Detection limits of 60, 40 and 70 ppb for Cu(II), Co(II) and Ni(II) respectively were attained and the authors reported that much lower levels of detection were possible by increasing the loading volume.

A recent report by Kocjan [46] described the use of a Titan Yellow chelating silica which was prepared by impregnating the silica gel with a mixture of Aliquat 336 (methyltricaprylammonium chloride) and a sulphonated chelating reagent namely Titan Yellow. The Titan Yellow was actually immobilised within the gel via ion pair formation with Aliquat 336. The high stability of this ion pair made precolumn reuse extremely feasible as reagent release from the precolumn was very slow. 12 different metals including Ca(II), Mg(II), Al(III), Cu(II), Fe(III), Ni(II), Co(II), Cd (II), Zn(II), Pb(II), Hg(II) and Cr(III) were preconcentrated from alkali earth or alkali earth metal salts and could be desorbed using dilute perchloric (> 0.05 M) or hydrochloric (> 0.5 M) acids. Metals were

determined by AAS or UV/VIS spectrometry. Only Ca(II) and Mg(II) were not retained from acidic or neutral aqueous solutions which makes this method useful for the preconcentration of traces of heavy metals from aqueous solutions e.g., river or sea water. Matrix effects arising from alkaline and alkaline earth metals were therefore negated.

Chelating silicas with immobilised ligands of biological origin show considerable potential for use in the area of trace metal preconcentration. Hydroxamic acids are ligands of particular note as they are known to form stable complexes with a wide range of metals [47]. Recently, the use of chelating silicas with grafted hydroxamic acid groups for trace metal preconcentration has been reported by several authors.

Fadeeva et al. [48] synthesised propanohydroxamic acid and salicylhydroxamic acid chelating silicas and monitored the sorption behaviour of 13 metal cations onto these silicas as a function of pH. Propanohydroxamic acid chelating silica (PHA-SG) was easier to synthesise than the salicylhydroxamic acid, and in a batch study was found to exhibit higher distribution coefficients, although the sorption patterns were almost identical in the acidic pH range. Therefore, PHA-SG was used for all subsequent column studies by these authors. Columns were loaded with 0.2 - 0.5 g of PHA-SG and conditioned to the same acidity as the metal samples of interest. Zr(IV), Hf(IV) and Mo(VI) were quantitatively retained in the acid range 0.1 - 4 M HCL, Th(IV), Sc(III) and V(V) were retained at a pH 1 and Fe(III), Al(III), La(III), Yt(III), Co(II) and Ni(II) were retained at a pH 2. Metal elution was effected using different eluents e.g. 0.1 M oxalic acid in the case of Zr(IV) and 1 M H_2SO_4 in the case of Mo(VI). The method was successfully applied to Zr(IV) determination in titanium based alloys.

Glennon and Srijaranai [49] have studied the complexation and preconcentration capacities of various hydroxamic acid chelating

silicas including N-methylhydroxamic acid, Desferrioxamine as well as the unsubstituted hydroxamic acid silica using batch and cartridge preconcentration methods. Unsubstituted hydroxamic acid silica exhibited good stability, high metal ion retention with pH dependent binding providing a selective means of preconcentration. Fe(III) was preconcentrated at pH 2 - 4, Cu(II) at pH 4 - 7 and Zn (II) at pH 7 - 8. Cu(II) and Zn (II) could be eluted from the packing with acidified water pH 2, and Fe(III) with 0.08 M EDTA. Preconcentration factors of 200 fold were attained for a loading volume of 1 litre. Cu(II) and Fe(III) were selectively preconcentrated from sea water using this method and could be separated and quantified using the correct eluents.

1.2.2.1.2 Resins

Several authors have investigated the use of resins with chelating and/or ion exchange functionality's for the preconcentration of trace metal ions [50-51]. The general mechanism of operation involves passing a controlled sample volume through a column containing a cation, chelating resin or chelating groups immobilised on glass beads, which then retain the metal ions via an ion interaction or chelation mechanism. As in the case of chelating silicas, the ligands can be chemically bonded to, or physically supported on the resin material. Ligands may also be incorporated into the material via ligand impregnation procedures. Metal complexes (as opposed to free metal ions) have also been retained on resin packed columns after complexation with the ligand in aqueous solution.

Chelating resins possessing 8-hydroxyquinoline functionality's are well documented although recently, there has been an increasing number of reports on sample precomplexation with 8-hydroxyquinoline prior to the enrichment step; followed by preconcentration of the metal complex on activated resin materials. Several authors report the use of Amberlite XAD-2 resins [56] which have been activated with organic solvent mixtures e. g. methanol/acetonitrile and act as reversed-phase resin materials for trace enrichment of metal-8-hydroxyquinolate complexes.

Abollino et al. [52] have described a transiently bound ligand-resin system where immobilised 8-hydroxyquinoline units were used for the uptake of metal ions. The sorption of 8-hydroxyquinoline and 8-hydroxyquinoline 5-sulphonic acid on a polystyrene-divinyl benzene resin (Amberlite XAD-2) and on an anion exchange resin (BioRad AG-MP-1) was investigated. The system was used for the uptake and enrichment of several divalent metal ions including Cd(II), Ca(II), Cu(II), Mg(II) and

Mn(II) with maximum retention achieved for a metal to ligand ratio of 1 : 20. It was possible to load sample solutions over a wide pH range 2 - 9, which facilitated analysis of saline and natural water solutions. Hydrochloric acid (2 M, 50^{o}C) was used for metal ion desorption from the preconcentration column in all cases, except Cu(II), where desorption with 1 M HCL was sufficient. Uptake and recovery of metal ions was determined by ICP AES. A 100 fold enrichment factor was achieved and limits of detection of the order 0.1 ppb were achieved.

Persaud and Cantwell [53] prepared a non polar chelating resin with 8-hydroxyquinoline covalently attached to a macroporous styrene divinylbenzene copolymer Amberlite XAD-2. Determination of free Mn(II) in aqueous solution was possible although the method was found to be Mn(II) selective in the presence of hydrophilic (EDTA and oxalate), but not hydrophobic (picolinate) complexes.

Isshiki et al. [54] preconcentrated trace metals from sea water with 7-dodecyl-8-hydroxyquinoline (DDQ) impregnated macroporous resin (DDQ resin). Extraction behaviour of the DDQ resin compared with solvent extraction with DDQ was examined. Trace metal impurities in the DDQ resin were removed during the impregnation stage as DDQ was loaded from acid solution. Both XAD-7 and XAD-4 resins were used in this study though XAD-4 exhibited a higher metal ion capacity possibility due to the difference in hydrophobicity of the polymer surfaces. Metals were desorbed with 2 M HCL (normal elution volume 8 cm^{3}) and determined with GFAAS. Concentration factors of the order of 500 were attainable.

The method was extremely sensitive with limits of detection in the ppt range. Cd(II), Cu(II), Fe(III), Mg(II), Ni(II) and Pb(II) were successfully determined in sea water using this technique. GFAAS and FAAS were the detection methods employed. Column reuse was an added bonus with this system, as less than 0.01 % bleed of DDQ from the resin

was observed following multiple sample loadings and no apparent loss in complexation capacity was noted. Unlike the resin prepared by Persaud et al. [53], a negative effect was shown with this resin, on the recovery of metals, in the presence of EDTA.

Isshiki et al. [55] have also reported the preconcentration of Co(II) by precomplexation with various ligands, followed by sorption of the metal-ligand complexes on macroporous resins. 14 different ligands were investigated and 2 resin types, with XAD-4 again exhibiting maximum metal capacity. Of the 11 ligands investigated only 5 exhibited efficient Co(II) retention over a wide pH range, 8-hydroxyquinoline, 4-(2-thiazolylazo) resorcinol (TAR), 4-(2-pyridylazo) resorcinol (PAR), 2-(2-thiazolyazo)-p-cresol (TAC) and 2-(2-thiazolyazo)-5-(dimethylamino) phenol (TAM) and the complexes formed were not dissociated under acidic conditions. Complexes of coexisting metals which were retained on the precolumn were eluted with dilute acid whereas Co(II) was eluted with a chloroform/methanol mixture (1 + 1) using a backflush method. The combination of TAR and XAD-4 gave the most satisfactory results and the method was successfully applied to Co(II) determination in sea water. It was possible to determine Co(II) at low ppt levels (0.4 ppt).

Porta and Sarzanini [56] described an on-line preconcentration manifold for trace enrichment of Cu(II), Cd(II), Fe(III), Mn(II), Ni(II) and Zn(II). Off-line precomplexation with 8-hydroxyquinoline was again favoured, with subsequent metal-complex enrichment on an Amberlite XAD-2 resin, before acid release towards a plasma torch for ICP AES detection. Samples were loaded on to the precolumn at 6 cm^3 min^{-1} with a peristaltic pump, and eluted with 2 M HCL/ 0.1 M HNO_3 mixture at 1 cm^3 min^{-1}. Metals were detected at low ppt levels, and analysis of antartic sea water was possible without interference from alkali metal ions.

The emphasis placed on the use of polymeric chelating ion exchange resins for trace metal preconcentration is of particular note [57-60]. The resins most often contain ligands with nitrogen or oxygen donor atoms on a polymeric matrix capable of forming metal complexes, thereby incorporating the metal atom into the polymeric material upon complexation. Commercially available Chelex-100, is probably the best known of this type of resin and has been widely used for trace enrichment of heavy metals. It has a polystyrene backbone and contains an imino diacetate functional group.

Baffi et al. [61] examined the use of H^+ forms of Chelex-100 and Lewatit TP 207 for trace enrichment of Cr(III), Cu(II) and Mn(II) using a batch technique, where 0.15 g of the resin was equilibrated with 300 cm^3 of sea water for 30 hours. These authors reported that the buffer capacity of the sea water could be used to retain the metals on the resin providing prolonged contact was allowed between the two phases. The resin was then separated from the solution by decantation, washed with deionised water, and dried for 24 hours at 40°C. Retained metals were eluted with 2 cm^3 of 1 M HNO_3 for 1 hour and analysed by GFAAS.

Criticisms on the use of Chelex-100, and in particular the H^+ form are many [62 - 64]; Florence and Batley [63] reported that the H^+ form could chelate heavy metals only after the passage of 1 litre of sea water which resulted in a loss of about 10 % of the metal. They also recommended a column pretreatment step with sodium acetate to buffer the resin to an appropriate pH before sample loading, a step that Baffi et al. [61] reported as unnecessary.

Blain et al. [64] agreed with the criticisms of Florence et al. [63], admitting that although Chelex-100 was useful for trace metal preconcentration, only partial recovery of some traces e.g. Mn(II) and Cd(II) was attainable. They attributed some of these losses to the non

conditioning of the resin with a buffer solution before the preconcentration step. They speculated that metal losses were also due to the poor selectivity of the imino diacetate chelating agent, and therefore, proposed the use of Chelamine, a pentamine ligand (1, 4, 7, 10, 13-pentaazatridecane or tetren) immobilised on an organic polymer. The resin was conditioned before use with an appropriate pH buffer and samples were mixed with 1 M Tris / 2 M ammonia solution before preconcentration. Elution was effected using a small volume (4 cm^3) of 2 M HNO_3 and metals were determined by electrothermal atomic absorption spectrometry (ETAAS). Selective preconcentration was enabled using Chelamine by judicious choice of loading pH, pH 2 - 4 for Cu(II) complexation and pH 6.5 or higher for Pb(II), Zn(II), Ni(II) and Cd(II).

Other problems associated with Chelex-100 include shrinkage in its ionic form; and swelling as it changes from the hydrogen to the monovalent salt form. Polyamine-polyurea resins have been synthesised to overcome some of the aforementioned problems associated with Chelex-100. The use of resins containing polyethyleneimine in the polymer backbone is of particular note as several authors have reported the use of this resin type for the preconcentration of metal ions [65, 66]. These resins are stable over a wide pH range and high preconcentration factors are achievable.

Initial work by Hackett and Siggia [67] indicated that a poly(dithiocarbamate) chelating resin (PDTC) could be applied to trace level metal determination in natural waters. High preconcentration factors and complete separation from complex matrices were possible by judicious choice of pH as well as some other exchange conditions. PDTC had some inherent disadvantages, however, as not all the preconcentrated metals were recoverable by mineral acid elution. Resin digestion was necessary to recover some of the more strongly bound metals e.g. PDTC can therefore

be thought of as a non-reversible chelating resin which would not lend itself readily to reuse.

Horvath and Barnes [68] have modified the initial PDTC resin by imparting a slightly different functionality on the resin while retaining the original polymer backbone; they synthesised a carboxymethylated poly (ethyleneimine)-poly(methylene poly phenylene) isocyanate (CPPI) resin which was capable of preconcentrating metal ions for subsequent detection by inductively coupled plasma (ICP) spectrometry. Uptake of Cu(II), Cd(II), Pb(II) and Zn(II) was quantitative even in the presence of high concentrations of NH_4^+, Ca(II), Mg(II), K(I), Na(I) ions and acetate and citrate salts. Metals were desorbed from the resin using strong acids and volume changes associated with ionic form changes were not observed; this meant that the resin could be used for preconcentrating metals from high salt matrices such as sea water. The resin was used over a wide pH range and preconcentration factors up to 40 were attainable. Limits of detection were low using this method with typical values of 6, 2, 100 and 10 ppb achievable for Cu(II), Cd(II), Pb(II) and Zn(II) respectively.

Wang and Barnes [69] examined the use of CPPI and PDTC with respect to metal ion preconcentration using a flow injection on-line chelation system with ICP AES detection. Over 22 metals were effectively preconcentrated using these resins with pH conditions dictating which resin was most appropriate for a given sample. As many authors have pointed out resin preparation can be time consuming and the eluents are best suited to AAS or AES analysis. To ensure total metal recovery it is necessary to use mineral acids for resin elution and in the case of PDTC, as already stated, an acid digestion step , an added unwanted step leading to increased analysis time.

Several authors report the use of polymeric resins with hydroxamic acid chelating functions for the separation and

preconcentration of metal ions. Shah and Devi [70] examined the effect of electrophilic and nucleophilic substitution at the nitrogen in poly (hydroxamic acid) ion exchangers, and its corresponding effect on heavy metal ion uptake by the substituted resins. 7 different resins were prepared including substituted N-Phenyl, p-Chlorophenyl, m-Chlorophenyl, p-Tolyl, m-Tolyl and 3-Chloro-4-tolyl hydroxamic acids, as well as a control unsubstituted hydroxamic acid. Ion exchange columns were prepared by packing 5 g quantities of the resins (H^+ form) into glass columns, and following a resin conditioning step, the metals were loaded at a flow rate of 1 cm^3 min^{-1}. Both Pb(II) and Cu(II) were quantitatively retained by the resins at pH 4 - 5. Cu(II) was desorbed using 1 N HNO_3 and Pb(II) using 6 N NaCl, and could be determined spectrophotometrically or complexometrically. An attractive feature of these resins is their high stability and therefore their potential for re-use.

Selective preconcentration and separation of Be(II) and Al(III) from other elements using an ion exchange resin functionalised with N-benzoyl-phenylhydroxylamine (BPHA) has been reported by Das and Pobi [71]. Metal sorption patterns on this resin were investigated as a function of pH, Be(II) was adsorbed over a wide pH range 1 - 7, Fe(III) pH 2, Al(III) pH 2-5, Cu(II) pH 6.5 and Co(II) pH 5.5. Conditioning the column with 1 M sodium sulphate and then washing with 0.1 M glycine-sodium hydroxide buffer (pH 6.5) enabled selective adsorption of Al(III) and Be(II). Quantitative separation of the metals was achieved using different eluting reagents, 2 M sulphuric acid for Be(II) and 0.1 M Na_2EDTA for Al(III) elution, with metal concentrations determined by atomic absorption spectrometry (AAS). The resin was found to be extremely stable under acid, alkali and heat conditions, and Be (II) and Al(III) were successfully pre-concentrated from beryl and synthetic alloys.

Mendez et al. [72] have also described preconcentration and

separation of metal ions on an N-phenyl hydroxamic acid resin. Ten metal ions were extracted as a function of their pH including Cu(II), Fe(II), Ni(II), Co(II), Zn(II), Pb(II), Mn(II) and some more highly charged ions including Mo(VI), U(VI) and Cr(III). Detection was by UV spectrometry and flame atomic absorption spectrometry (FAAS).

Advantages of on-line ion exchange trace enrichment techniques compared with conventional off-line techniques in which the enrichment and separation step are separated include:

a. attainment of higher enrichment factors with smaller sample enrichment volumes, e.g. many mini columns of less than 1 cm^3 bed volume have been used;

b. sample throughput is greater as higher sample loading and elution flow rates are used;

c. faster analysis times.

Resins also have some associated disadvantages, including the use of mineral acids for resin elution, although stronger complexing agents can be used as an alternative. Liu et al. [29] noted that to improve recovery and to ensure that the total concentration of a given metal was measured, all chemical forms of the metal of interest should be converted to forms totally retained by the resin. This generally requires acid digestion, an added, unwanted extra step. Caution must be exercised when immobilising ligands onto polymeric surfaces as residual cationic groups should not be left exposed on the polymer surface. If 8-hydroxyquinoline is immobilised on XAD resins via an azo linkage, residual amine groups are often left exposed. Immobilisation via a methylene linkage reduces this problem [53].

1.2.2.1.3 Naphthalenes

Solid chelating materials, such as naphthalene supported ligands, have been reported as effective, rapid and highly selective as a means of preconcentration for metal ions. The materials, unlike ion exchangers, Chelex-100 and activated carbon, do not require regeneration since the solid mass consisting of the metal complex and naphthalene is dissolved with a suitable solvent such as dimethylformamide [33] from the column and determined directly by AAS. The quantities of naphthalene used for metal preconcentration are extremely small (less than 0.4 g in some cases [73]) making the method extremely economical.

Initial work using naphthalene for trace metal preconcentration was reported by Fujinaga and co-workers [73] who described a novel solid-liquid separation subsequent to a liquid-liquid extraction where naphthalene was used as the extractant. The method was applied to metal ions that form complexes with the complexing reagent at high temperatures, where a normal liquid-liquid extraction method could not be directly employed. Only thermally stable complexes could be determined using this method, so a second method carried out at room temperature was developed by Satake et al. [74], involving a solid-liquid separation after adsorption on a microcrystalline naphthalene surface. However, very small amounts of naphthalene were used which often resulted in errors in the determinations.

Nagahiro et al. have published several papers on the use of naphthalene supported chelating agents for trace metal enrichment from sea water [75, 76], and alloy and hair samples [77]. They have described the chromatographic preconcentraion of iron with 1, 10 phenanthroline and tetraphenylborate supported on naphthalene [75]. Chromatographic columns were packed with 0.5 g of the 1, 10 phenanthroline tetraphenyl-

borate ion-pair complex on naphthalene, and washed with pH 4 buffer solution before the sample was applied. All samples (normal loading volume 14 cm^3), were mixed with 1 cm^3 of 10 % hydroxylammonium chloride, and 5 cm^3 of buffer before precolumn application. Following sample application, the precolumns were washed with water and eluted with acetonitrile. Fe(II) concentrations were determined using spectrophotometric detection at 508 nm. The method was applied to iron determination in sea water and alloys and had a dynamic linear range of 200 ppb to 3.2 ppm.

In a subsequent report [76], Nagahiro et al. described the use of a neocuproine tetraphenylborate ion pair (on naphthalene), for the preconcentration and separation of microgram amounts of Cu(II), in the presence of Mg(II), Mn(II), Al(III), Cr(III), Fe(III), Co(II), Ni(II), Zn(II) and Cd(II). Sample pretreatment was similar to their earlier report [75], with the test solution being treated with hydroxylammonium chloride and buffer solution pH 4.5, however, Cu(II) concentrations were determined by dissolving the 'loaded' sorbent in 5 cm^3 of propylene carbonate and measuring the absorbance at 456 nm using UV spectrometry.

Satake and Nagahiro [78], described the solid phase extraction of Zn(II) on microcrystalline naphthalene, and its subsequent determination by AAS. The method was similar to one reported earlier by Satake et al. [74], where sample solutions containing 5 - 50 mg of Zn(II) were mixed with an excess of 8-hydroxyquinoline solution in acetate buffer pH 6.5, resulting in the formation of 1 : 2 metal-ligand complexes. Complexes were then adsorbed on to the naphthalene surface. The solid phase was then isolated from the solution and dissolved in a xylene / HNO_3 (1 : 15) mixture. Zn(II) concentrations were determined at 213 nm by AAS. The linear range was 100 ppb to 1 ppm and the method could be applied to Zn(II) determinations in Spring and River water.

Miura and Satake have recently published a series of papers on the use of chelating naphthalenes for trace metal determination in alloy materials. Fe(III) has been preconcentrated from alloys and biological materials, on columns packed with 2-naphthol-1-nitroso 3, 6 disulphonate and benzyldimethyl tetradecyl ammonium perchlorate adsorbent supported on naphthalene [79]. Quantitative Fe(III) retention on the sorbent was observed in the pH range 3.5 - 7.5. The solid naphthalene phase was dissolved out of the column with DMF, and Fe(III) was determined at 248 nm by AAS. Using this solid phase preconcentration technique, iron could be detected at levels as low as 19.6 ppb, however, ammonium citrate, Na_2EDTA, Pd(II) and Co(II) were all found to interfere with Fe(III) preconcentration, and therefore diminish iron recovery.

Cobalt has been preconcentrated by these authors from alloys and steels, using ion pair combinations such as [4-hydroxy-3 nitroso naphthalene - 1 - sulphonic acid] - tetradecyldimethylbenzyl ammonium [80] and tetradecyldimethylbenzyl ammonium thiocyanate [81] adsorbents, supported on naphthalene. In the former case, the aqueous solution of cobalt was mixed with ammonium citrate and buffered to pH 9.5 and applied to the column. Water insoluble Co(II) and Co(III) complexes were formed, and retained strongly on the column. In the latter case, cobalt was treated with a nitroso R salt solution, and the solution pH adjusted to 3.5 before sample introduction on to the cited adsorbent. DMF was used as before to dissolve the solid material from the column and Co(II) was determined at 241 nm by GFAAS.

Selective preconcentration of iron in beverages and water samples using a 2, 4, 6 -tri-2-pyridyl-1, 3, 5 triazine tetraphenylborate naphthalene packed column was outlined by Puri et al. [82]. Samples were pretreated with ascorbic and acetic acids, and buffered to pH 4.5 with ammonium acetate. Fe(II) was quantitatively retained in the pH range

3.3 - 7.0. DMF was again found suitable by these authors, for elution of the solid phase from the column with subsequent spectrophotometric analysis at 597 nm. EDTA and potassium were major interferents on this method, though they could be masked effectively using thiourea or sodium tartrate.

1.2.2.1.4 'Water Soluble' Polymers

High capacities and rapid metal separations have been achieved with gels which retain the chelating capabilities of the chosen ligand while remaining soluble in water [83]. Geckler et al. [84] described the synthesis of a poly (ethylene-imine)-based 8-hydroxyquinoline (PEQ) polymer, which was used to retain metal ions in aqueous solution. The retention capability could be altered by simply changing the pH. Lower pH's favoured the retention of highly charged ions, e.g. Zr(VI) and Nb(VI) while at higher pH's, preconcentration of many metal ions was possible due to the formation of stable quinolinolates. Convenient detection by AAS or ICP AES was possible since the pre-separated elements remained in the aqueous phase owing to the hydrophilic nature of the polymer complex in solution.

Resing and Mottl [85] have determined Mn(II) in sea water using flow injection analysis (FIA) with on-line preconcentration and spectrophotometric detection. Mn(II) was preconcentrated with 8-hydroxyquinoline immobilised on a vinyl polymer gel. Mn(II) could be desorbed from the preconcentration gel with acid eluents, and its concentration determined by spectrophotometric detection of the malachite green formed from the reaction of leucomalachite green and potassium periodate with Mn(II) acting as a catalyst. Compared with other spectrophotometric detection methods, kinetic catalytic methods are extremely sensitive, as each analyte ion produces more than one colour molecule. The authors have achieved a limit of detection of 0.036 nM using this technique, for a preconcentration volume of 15 cm^3 of sea water, although further study indicated that by increasing the sample loading volume more precise measurements would be possible at trace levels. FIA has been shown by these authors to lend reproducibility to both reagent

addition and reaction times and was shown to be ideally suited for catalytic methods.

One of the main problems associated with polymer use is their lack of rigidity, which means that use in a column without some kind of solid support is not possible. Glass beads are the most common kind of support used, and several authors have reported separation using polymer coated glass bead systems [86].

1.3 Conclusions

Considering the complexity of environmental samples and the ultra trace level of metals often present, preconcentration and separation of the analyte prior to actual determination are now recognised as indispensable steps in the analysis. As a result, many methods for the preconcentration of metal ions have been developed which increase the sensitivity and selectivity of established procedures. Sample preconcentration is increasingly carried out by means of so called solid-phase extraction (i.e., solid-liquid sorption) on relatively small precolumns, containing novel sorbent materials characterised by high concentrating abilities. In these solid-phase extraction techniques organic ligands have been chemically bonded to, or physically supported on, various substrates including silica gel, resins, naphthalenes and polymers.

The stability of silica gel makes it a particularly attractive sorbent material; it does not swell or strain, can undergo heat treatment and has good mechanical strength. High concentration factors (1000 fold) and excellent metal recoveries (100 %) are possible using functionalised silica gels. Disadvantages of this sorbent material are few, however, preparation of ligand immobilised silica can be time consuming and the number of ligands that can actually be bonded to silica are limited.

The use of resin materials with chelating and / or ion exchange functionalities is popular as high enrichment factors are attainable with small sample enrichment volumes, e.g. use of mini columns. Sample throughput is greater as higher sample loading and elution flow rates are used. A particularly attractive aspect of some resins was highlighted by Baffi et al. [61] who reported that the buffering capacity of sea water could be used to retain metal ions present in the sea water sample on the column. Unlike silica gel, resin stability is sometimes

questionable and shrinkage has been observed in several cases. Many resins require elution with mineral acids although stronger complexing agents can be used if necessary. Caution must also be exercised when immobilising ligands onto resin surfaces, as residual cationic groups left exposed on the polymeric surface may reduce metal uptake.

Naphthalene supported ligands are effective, rapid and highly selective for the preconcentration of trace metals. Regeneration is not normally required as the naphthalene/metal-complexes can be desorbed directly from the column and determined by FAAS or GFAAS. The method is extremely economical as very small quantities of naphthalene are required, however, the use of such small quantities is also the downfall of this method as errors in determinations often occur.

Polymer sorbent materials offer the user high metal capacities and rapid metal separations. The most obvious advantage of these functionalised gels is that they can retain the chelating ability of the chosen ligand while remaining soluble in water. Polymers, however, quite often lack rigidity and may need to be supported on solid support materials. Glass bead supports are most commonly used, but these too have inherent disadvantages including instability at high pH's. Ionisation of surface hydroxyl groups on the glass beads imparts a negative surface charge which can lead to a reduction in overall metal recovery.

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Chapter 2

On-line Sample Preconcentration of Cu(II), Al(III) and Fe(III) as their 8-hydroxyquinolate complexes using a column switching technique

2.1 Analysis of Trace Metal Ions using Chromatographic Techniques

2.1.1 Introduction

Substances dissolved in water can occur in a broad range of concentrations according to the type of water and its' genesis of origin. In this chapter the analysis of waste water from a mine, for copper, iron and aluminium content is described with a particular emphasis being placed on the concentration process employed. As waste water can contain a large amount of dissolved organic substances as well as hazardous trace elements, the concentration process was selected so as to minimise or eliminate interfering components in the water sample and to enhance the separation efficiency of the analysis procedure.

2.1.2 Determination of Trace Metal Ions.

Atomic absorption spectrometry is used routinely for the determination of Fe(III), Cu(II) and Al(III) in waste water. The water itself may be removed by evaporation or distillation; or conversely, interfering components may be eliminated by precipitation, adsorption or extraction techniques. Novel methods for the separation and enrichment of metal ions in water coupled with AAS detection have been described by several authors [1-3].

Wang et al. [4] have used a liquid ion exchange membrane for the enrichment of Fe(III) and eleven other metal ions from natural waters. The membrane comprised an organic external phase which contained surfactant, ion carrier and emulsion strengthening agent (dissolved in kerosene), and an acidic aqueous internal phase. Metal ions

were enriched by mixing the emulsion with the aqueous sample whose pH was controlled (external phase); the pH gradient between the external and internal solutions being the driving force of the trace enrichment process. Metals could be recovered from the membrane by membrane demulsification and determined by AAS. Fe(III), Co(II) and Ca(II) were successfully enriched in natural water samples with limits of detection in the range 0.1 - 1.0 ppb. 99.5 % recovery of enriched metals was possible with this method.

Recently Salacinski et al. [5] have reported a coupled flow-injection analysis-flame atomic absorption spectrometry approach for the quantitative determination of aluminium in beverage and water samples. The FIA analysis method incorporated an on-line cation exchange preconcentration column for both metal enrichment and matrix modification. All samples were adjusted with 5 % v/v nitric acid before injection into the flow system, to ensure that the $[Al\ (H_20)_6]^{3+}$ hydrolysed species was present in solution. The cation exchanger was used in the H^+ form, as the buffering capacity of the resin helped to maintain the presence of tripositive aluminium on the precolumn. Aluminium was eluted from the precolumn with 4 M HCl and determined by FAAS at concentrations as low as 75 ppb.

The main disadvantage associated with AAS is that each metal must be analysed individually, which can be very time consuming. Multi-element approaches which allow simultaneous determination of more than one element are increasingly reported. Flow injection methodologies used in conjunction with a spectrophotometric detection step have offered advantages for multi-element analysis in waste water samples.

Araujo et al. [6] have described the simultaneous determination of Fe(III) and Cr(VI) in waste water, using an FIA system

based on the sandwich technique, which involves injecting the water sample between two different carrier systems. They report the use of 1,5 diphenylcarbazide (carrier 1) for Cr(VI) determination, and 1,10 phenanthroline (carrier 2) for Fe(III) determination. Hydroxylamine-ammonia buffer present in carrier 1, ensured the reduction of any Fe(III) present to Fe(II), therefore, total iron content of the sample could be determined. Fe(III) and Cr(VI) were simultaneously detected at 526 nm, and the limits of detection were 180 ppb and 160 ppb, respectively.

Benson et al. [7] reported on-line determination of residual aluminium in potable and treated waters by FIA coupled with spectrophotometric detection at 580 nm. They have developed a portable, automated field monitor for aluminium determination, spectrophotometric detection was based on Al(III) complexation with pyrocatechol violet, to form a colloidal lake, maximum absorbance 580 nm. The analytical cycle duration was only 30 minutes, thereby enabling 48 results per day to be recorded. The limit of detection using this method was 45 ppb Al(III).

2.1.2.1 HPLC of metal chelates

2.1.2.1.1 Dithiocarbamates

Metal determination and metal speciation using liquid chromatography is also of particular note, and was recently reviewed by Robards and Starr [8]. Determination of metal chelate complexes by HPLC is particularly evident in the literature. Earlier authors favoured the use of dithiocarbamates in solvent extraction or on-line precomplexation procedures, with subsequent reversed-phase separation, followed by spectrophotometric or electrochemical detection of the metal chelates. Dithiocarbamate metal chelates are typically soluble in non polar solvents

such as chloroform, therefore small extraction volumes yielded high preconcentration factors. These ligands also form strong complexes, with high molar absorptivities, and were therefore suitable for photometric detection. Multi-element analysis of trace metals in some environmental samples by solvent extraction and HPLC of metal-diethyldithiocarbamate and -dithizone complexes was reported by Edward-Inatimi [9]. Trade effluents were analysed using this method and Cu(II), Ni(II), Hg(II), Pb (II), Co(II), Mn(II) and Bi(III) could be determined simultaneously as their dithiocarbamate complexes. This method emphasised the multi-element analysis aspect, rather than that of preconcentration.

2.1.2.1.2 8-hydroxyquinolates

8-hydroxyquinoline is a useful reagent for the extraction, separation and spectrophotometric detection of metal ions as it forms thermodynamically stable chelates which can be separated by various techniques e.g. TLC and HPLC on silica gel [10]. Baiocchi et al. [11] described the use of 8-hydroxyquinoline as a precolumn chelating agent for multi-element determination using a conventional reversed-phase HPLC system. Fixed wavelength UV absorption was employed for detection of the metal chelates and detection at the ppb level was feasible.

Bond and Nagaosa [12] have reported the simultaneous determination of Cu(II), Fe(III), Al(III) and Mn(II) in biological and water samples, by reversed-phase liquid chromatography of the metal-8-hydroxyquinoline complexes with subsequent electrochemical detection (at a glassy carbon working electrode, in a thin layer chemical cell) or spectrophotometric detection at 400 nm. They reported that the simplest method of separation was by direct formation of the metal-ligand chelates, followed by separation of the 8-hydroxyquinolate complexes on a column

with 1 : 1 acetonitrile : water containing 5×10^{-3} M 8-hydroxyquinoline, 0.4 M KNO_3 and 0.2 M acetate. A direct injection volume of 20 cm^3 of sample with this method allowed Cu(II) and Fe(III) to be detected at levels as low as 2 ppb and 1 ppb, respectively.

The concentration of Fe(III) in drinking water was too low to be determined by the direct injection method, so preconcentration of the Fe(III)-8-hydroxyquinolate chelate was attempted on a Sep-Pak C_{18} column. A dichloromethane extract containing the metal chelate was loaded on to the Sep-Pak column, chelate desorption was enabled with methanol elution, on to an analytical column. A 50 fold concentration factor was achieved compared to the direct injection method, and the method had good reproducibility.

In a subsequent report, Mooney et al. [13] described a chromatographic method for the determination of Cr(III), Zn(II), Cu(II) and Fe(III) as their 8-hydroxyquinolate complexes. In this method the complexes were injected onto a C_{18} reversed-phase column using a mobile phase of acetonitrile (made 1×10^{-2} M in 8-hydroxyquinoline) and 0.01 M acetate buffer, pH 6 (made 0.1 M in KNO_3) (1:1). Detection was at 400 nm using a spectrophotometric detector and detection limits of the order of 100 - 250 ppb for Cu(II) and 250 - 600 ppb for Fe(III) were achieved. Elution was of a 10 minute duration.

2.2 Column Switching Techniques

2.2.1 Introduction

Among the many novel preconcentration techniques reported recently which is of particular interest is "column switching", or multidimensional high performance liquid chromatography (MDHPLC). The term "column switching" includes in the widest sense all techniques by which the direction of flow of the mobile phase is changed by valves so that the eluent from a primary column is passed to a secondary column for a defined period of time [14]. Column switching has become extremely popular since the advent of the first high pressure, low dead volume valve in 1973 [15]. This method has traditionally been applied to the separation of drugs and biological substances; however renewed interest in it's use, this time as a preconcentration tool for inorganic analysis, is evident. The main objectives of column switching can be summarised as follows:

a. to increase chromatographic resolution and separation;

b. to enrich trace amounts of samples;

c. to protect sensitive detectors, e.g. electrochemical detectors, from contamination by coextracted material;

d. to prevent destabilisation of the chromatographic equilibrium of the column by coextracted material.

Column switching techniques offer the analyst greater separation power than was normally available with single column HPLC. Obviously, the essential features which govern HPLC are applicable to column switching, and as such have been described in detail by many authors [16]. However, the reasons for increased separation power and sensitivity can only be explained by examining the effects of the column switching procedure on the basic parameters which govern HPLC and are therefore described in brief in the following section.

2.2.1.1 Theory

Chromatography is essentially a physical separation method in which the components to be separated are distributed between the two phases, a stationary and a mobile phase, which can percolate through or over the stationary phase. Repeated sorption and desorption of the sample components as they move through or over the stationary phase, effect separation, as the sample components have different distribution coefficients K as they move through the system, where the distribution coefficient is a constant for a given system and can be determined from the relationship

$$Vr = Vm + Vs\,K \qquad \text{Equation 2.1}$$

where K is the distribution coefficient, Vs is the volume of the stationary phase, Vm, the column dead volume and Vr, the retention volume of a given solute. HPLC involves migration of samples in a liquid mobile phase through a column containing the stationary phase under a pressure gradient applied between the column ends. The chromatographic behaviour of a given solute is most frequently described in HPLC in terms of its retention volume, Vr and the retention ratio, R.

Vr is defined as the volume of mobile phase that must flow through the column for elution of a given component and can be obtained from the equation :

$$Vr = F \times t_r \qquad \text{Equation 2.2}$$

where F is the flow rate ($cm^3\ min^{-1}$) and t_r is the time required for solute elution [17]. R is the retention ratio which is the probability that a solute molecule will be found in the mobile phase at any given time. R can be defined as :

$$R = n_m / (n_m + n_s) \qquad \text{Equation 2.3}$$

n_m and n_s are the total number of moles of solute in the mobile and stationary phases respectively.

In column switching effective sequential separation takes place as two or more columns are used, which means that not one, but two separation mechanisms or modes are operating. The mode depends on the interactive relationship between the analyte, mobile phase and stationary phase, and is based on the nature of the stationary phase. By judicious choice of stationary phases, the advantages of column switching are clearly seen, i.e. increased separation capability, as the first stationary phase may be very different fom the second.

Freeman [18] has demonstrated mathematically the advantages of column switching or mode sequencing. Gidding's treatment [19] of the peak capacity for a single mode has been expanded by Freeman [18] and the following expression obtained, where ϕ represents the peak capacity and can be defined as "the maximum number of peaks which can be separated on a given column".

$$\phi = 1 + \frac{N^{1/2}}{m} \ln (1 + k_n') \qquad \text{Equation 2.4}$$

m = 4 implies unit resolution (4 σ separation)
N = number theoretical plates
k_n' = capacity factor for the last member of a series of peaks numbered from zero (non-retained) through n (last peak)

A sequence of independent modes each having a peak capacity ϕ_i can exhibit a multiplicative effect expressed as ;

$$\phi_i = \phi_1\phi_2\phi_3.....\phi_n = \Pi\phi_i \qquad \text{Equation 2.5}$$

When each mode has the same capacity a simple exponential results

$$\phi_r = \phi^n \qquad \text{Equation 2.6}$$

For comparisons sake, if one takes peak sharpness as the main goal, then a connection of n identical column units used in series to increase the overall column efficiency gives the following equation :

$$\phi_r = 1 + \left[\frac{(nN)^{1/2}}{m}\right] \ln (1 + k_n') \qquad \text{Equation 2.7}$$

The overall effect is then apparent because :

$$\phi_r \cong n^{1/2} \qquad \text{Equation 2.8}$$

If a ten-fold increase in relative peak bandwidth is required using a single mode system, obviously a 100 fold increase in column length would be required. Using column switching, in which two or more modes of separation are used, an exponential increase in peak capacity can be obtained, which means that control on the basis and quality of separation can be exerted by the chromatographer.

2.2.1.2 Hardware and Methodology

Recent reviews by Little et al. [20] have described the potential of column switching methodology in HPLC. They reported that the majority of published methodologies use a six-port, two-way valve, obtainable from several manufacturers including Valco and Rheodyne. The principal features of the valve are shown in Figure 2.1 below.

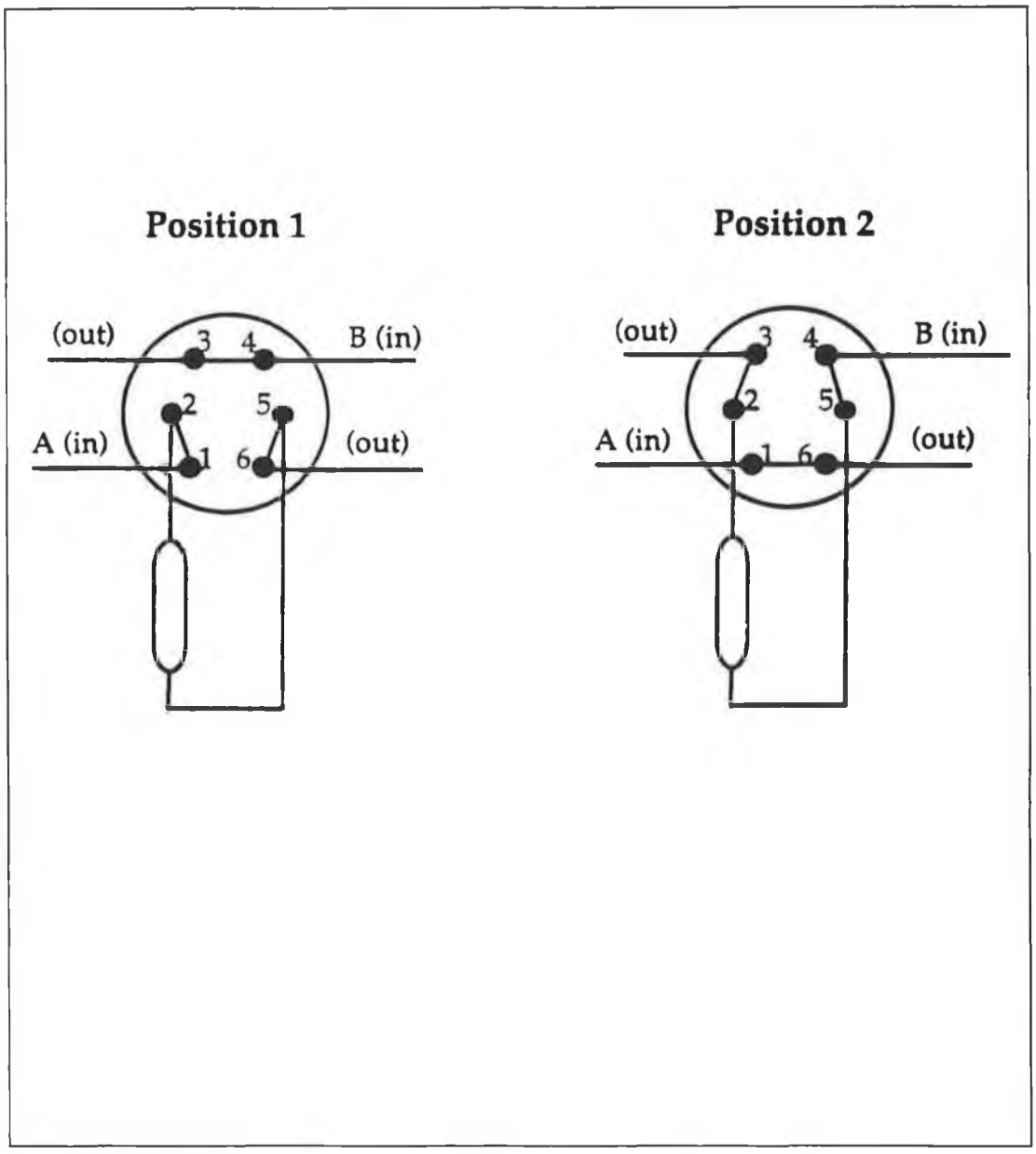

Figure 2.1 Principle features of six-port, two way valve.

2.2.1.2.1 Mechanism of Action

Several different modes of action are possible with six-port valves. These are :

a. two independent flow paths are available through the valve which enable simultaneous cleaning and equilibration of one column while the other is being used;

b. sample injection into the system can be in either solvent line (designated S' or S");

c. the second solvent line can be reversed in direction resulting in the ability to backflush the column.

2.2.1.2.2 Transfer Techniques and Switching Functions

Optimisation of chromatgraphic parameters can be achieved by judicious choice of transfer techniques and switching functions. Four basic techniques have been described for the transferal of a sample fraction from a primary to a secondary column [21]. The direction of the flow of the mobile phase during transferal determines whether the technique is designated as reversed or direct transferal.

Commonly used column switching functions are column selection, recycling chromatography and column back flushing. Column back flushing speeds up the analysis of complex mixtures without the use of gradient elution and it reduces band broadening considerably. It was chosen as the switching function for use in the enrichment system and is described briefly in the following section.

2.2.1.2.2.1 Column Backflushing

The eluent from the primary column is normally vented to waste, thus bypassing the secondary column and preventing its contamination by early or late eluting peaks. Removal of the strongly retained components from the primary column is possible using backflushing. Once the fraction of interest has eluted from the primary column for further separation on the secondary column, this technique reverses the flow of the primary column to waste. A backflushing transfer technique is shown in Figure 2.2.

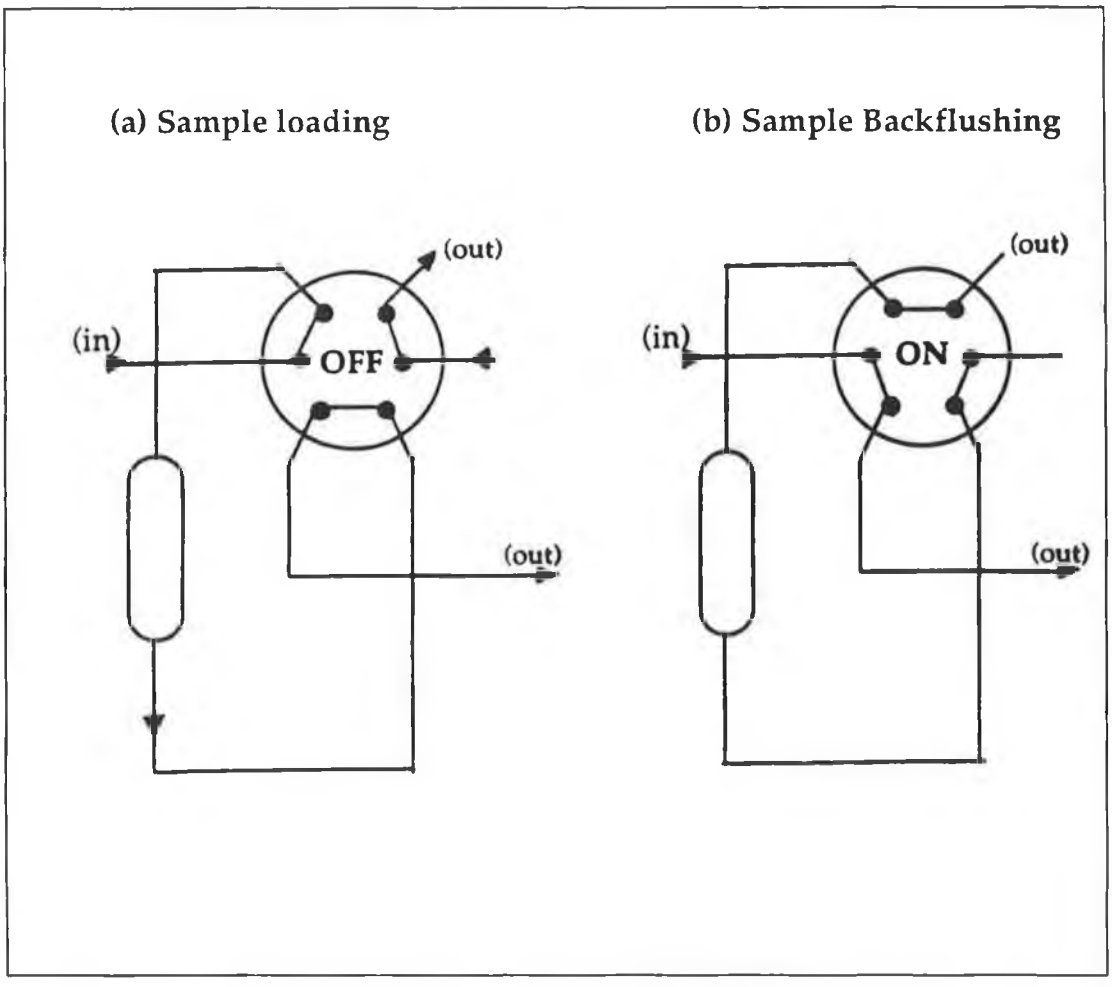

Figure 2.2 Backflush Sample Transfer Technique

2.2.2 Application of Column Switching to Sample Analysis

2.2.2.1 Trace Enrichment

Trace enrichment, or preconcentration using column switching, is based on the fact that the components of interest will be retained in a narrow zone on the top of the column when a large volume of sample is passed through the column. With an increase in injected sample volume, separation efficiency is further dependent on the nature of the solute and the composition of the mobile phase [22]. De Jong et al. [23] examined the relationship between column plate number, particle size and column loadability [24]. Poppe and Kraak used a one solute model to describe the sample capacity of chromatographic columns as a function of the amount of packing, the phase ratio and the plate number [25]. They found that column dimensions *per se* had no effect on peak detectability, and with respect to mass overload extra peak broadening was dependent only on the total mass of the solute per gram of stationary phase contained in one plate. The phase ratio, i.e. volume of solute in stationary phase relative to its volume in the mobile phase was found to strongly influence peak detectability. Detectability was found to be directly proportional to this ratio, as long as stationary phase overload was the critical factor.

Knox and Pyper [26] have studied volume and concentration overloading effects, to achieve maximum sample throughput. They observed that, good reproducibility could be obtained if the column was not overloaded, and if the capacity of the column was not exceeded. Trace enrichment of less strongly adsorbed components required a reduction in sample injection volumes, or an increase in column volume, to prevent sample breakthrough. Trace enrichment is readily achieved, when relatively non polar components from aqueous solutions are injected on to a reversed-phase column.

2.2.2.1.1 Traditional Applications

Trippel et al. [27] described the trace enrichment and HPLC analysis of chlorophenols in environmental samples using precolumn sample preconcentration and electrochemical detection. On-line preconcentration of total mono- and dichlorophenols was performed using a divinylbenzene-styrene copolymeric sorbent (PRP_1) as the precolumn packing material. Due to the presence of aromatic structures in the PRP_1 sorbent, retention of the chlorophenols was as expected, higher than that on C_{18} materials of comparable specific surface area, thus making it an effective means of preconcentration for polar compounds with organic moieties.

Aerts et al. [28] have reported the purification by on-line dialysis of aqueous extracts containing sulphonamides followed by trace enrichment on a short column containing silica or a polymer-based material. Using this combination of continuous flow and column switching techniques effective monitoring of veterinary drug residues was possible. Trace enrichment of the drugs on the preconcentration column was effected by the polarity of the drug, the eluent composition and the nature of the packing material.

2.2.2.1.2 Inorganic Applications

Few authors have reported on the use of column switching for trace enrichment of inorganic ions. Until recently, workers using this technique had applied it to the trace analysis of anions, but rarely cations. Robert et al. [29] successfully used an on-line concentration method with a concentration column, for anion analysis. Extremely dilute samples could be concentrated; however, there were drawbacks, including the need for an

extra pump. The concentration column required extensive washing after each sample as it was prone to contamination. Trace anion determination was seen to decrease in the presence of major elements.

The use of large injection volumes solved many of the problems experienced by earlier workers. When a large sample volume is injected on to the preconcentration column, solutes contained in the solution are compressed at the top of the column and sample bands do not move unless the eluent is passed through the column.

Okada et al. [30] reported trace anion analysis using a backflush method and large injection volume in ion chromatography. Fluoride, chloride, nitrate, nitrite, sulphate and bromate anions were separated; the concentration column was packed with an anion exchanger and could be switched on-line with the ion chromatograph Model HLC-601 by valve actuation. Heckenberg and Haddad [31] have also reported the use of column switching ion chromatography for the determination of ppb levels of inorganic anions namely chloride, nitrate and sulphate.

Trace metal preconcentration using column switching techniques has only been reported by a handful of authors in the last decade. Haring [32] reported the use of an on-line preconcentration system involving the introduction of dilute metal chelates onto an organic precolumn and retention of these chelates via lipophilic attraction with the packing material.

Drasch [33] advocated the use of column switching techniques for the on-line preconcentration and separation of heavy metals, in particular, Cd(II), Pb(II) and Hg(II). Off-line formation of dithiocarbamates was recommended followed by on-line preconcentration on a small precolumn. Elution of the metal chelates onto an analytical column for further separation was effected using a tertiary eluent of methanol/water/chloroform, 50/25/25 or 1 mM sodium diethyldithio-

carbamate in methanol/water, 70/30. Limits of detection of 1 ppb were achieved.

Trace analysis of cadmium, cobalt, mercury and nickel in water using a similar column switching technique to Drasch, was reported by Munder and Ballschmiter [34]. Off-line formation of metal dithiocarbamates with bis (ethoxyethyl) dithiocarbamate, with subsequent on-line enrichment of the lipophilic neutral metal chelates on a small (0.8 x 40 mm) phenyl modified silica precolumn was the basis of this preconcentration method. A quaternary solvent mixture with admixture of surfactant (methanol/acetonitrile/water diisopropylether 41-44/20/32-39/7, 1 % sodium dodecylsuphate (SDS)) was used to elute the metal chelates on to a C_{18} analytical column for separation. Metal concentrations were determined photometrically at 254 nm. Limits of detection in the low ppb range were obtained though these, according to the authors, may be further extended, by increasing the sample loading volume to 50 cm^3.

More recently Haddad et al. [35] have applied a column switching technique to trace level determination of precious metals. Gold was determined as its Au(I)-cyano complex by ion interaction reversed phase chromatography with an on-line sample preconcentration step incorporated before separation on an analytical column, from other trace metals in the samples analysed.

Preconcentration of heavy metals as their hexamethylene dithiocarbamate chelates was reported by Ichinoki et al. [36]. On-line enrichment of the chelates on a Capcell C_{18} mini column was described in detail. Metal samples (2 cm^3) were mixed with 0.4 cm^3 of 1 M ammonium citrate buffer pH 9 and 0.1 cm^3 of 0.01 M dithiocarbamate solution, and left to equilibrate for 20 minutes. 2 cm^3 aliquots of the metal solution were then injected onto the Capcell precolumn in a mobile phase solution containing methanol/water/ammonium chloride buffer (pH 9)/ ammonium citrate

and hexamethylene dithiocarbamate, 100/385/15/3/1. Metal chelates were eluted on to analytical column for further separation with a second mobile phase containing methanol/water/chloroform/ ammonium chloride buffer (pH 9.3) and dithiocarbamate solution (0.01 M), 360/75/15/1. Metal concentrations were determined by spectrophotometric detection at 260 nm. Multi element analysis of tap water was enabled using this technique and Cd(II), Cu(II) and Co(II) were easily determined in the concentration range 0.04 - 0.55 ppb.

Irth et al. [37] have also reported trace enrichment and separation of metal ions as dithiocarbamate complexes by a liquid chromatographic method incorporating a switching step. A small precolumn (packed with Spherisorb ODS, 5 μm) was loaded with a hexadecyltrimethylammonium bromide - diethyldithio carbamate ion pair. Metals were then loaded onto the derivatised precolumn and retained as their dithiocarbamate complexes; elution to an on-line analytical column was effected with a mixture of phosphate buffer (pH 6.8) and hexadecyltrimethylammonium bromide (in acetonitrile). Cu(II), Co(II), Pb(II), Hg(II), Ni(II), and Bi(III) were all determined at the sub ppb level using this technique.

In a second report, Irth et al. [38] described a modified column switching procedure which allowed trace enrichment of Al(III), Sb(III) and Bi(III) as their tris (diethyldithiophosphate) (DTP) complexes, and their separation by reversed-phase liquid chromatography. DTP complexes were formed on a PRP-1 precolumn, previously loaded with cetrimide DTP ion pair. The apolar DTP metal complexes were strongly retained by the PRP-1 packing but could be eluted on-line to a C_{18} analytical column for further separation with acetonitrile/water (85/15) containing 10 mM DTP ligand. Metal complexes were detected spectrophotometrically at 280 nm. Modifications on the original procedure

used by these authors [37] included, incorporation of the DTP ligand into the eluent mobile phase when multi metal separations were being performed, and the use of a second PRP-1 column to remove any apolar compounds which might interfere with the analytical separation.

2.2.2.2 Sample Cleanup

One of the problems associated with on-line trace enrichment is that it also concentrates impurities, i.e. sample components other than the analyte of interest at the head of the second column. As a consequence, cleanup may be inadequate and separation of components of interest from interfering substances may necessitate further on-line cleanup steps [39]. The principle of on-line sample cleanup is to analyse the fraction of interest while discarding all others [40]. Enhanced separation based on the reduction of the amount of interfering components relative to the amount of analyte can be achieved using the cleanup application of column switching.

Irth et al. [38] obviously recognised the benefits of a sample cleanup application, and therefore incorporated a second PRP-1 precolumn, placed before the DTP-CTAB derivatised precolumn into their switching system, for the removal of apolar complexes other than the metal complexes of interest. When urine samples were injected into this system, organic substances normally found in urine were retained by the PRP-1 precolumn, while metals remained in the mobile phase and were flushed on to second PRP-1 column, containing the ion pair where they were subsequently complexed and retained.

As the number of preconcentration techniques for trace metals featuring column switching methodologies are extremely limited, few cleanup procedures using this technique, for metal ion analysis, have been reported. Therefore, 'traditional' cleanup applications have been referenced briefly in the following section.

2.2.2.2.1 Traditional Applications

Traditionally the cleanup application of column switching has been applied to drug determination in complex biological samples, like urine, blood plasma and serum. Hux et al. [41] described the chromatographic determination of methaqualone in blood plasma using Amberlite XAD-2 precolumns which enabled the direct injection of undiluted plasma, thus permitting measurement of therapeutic levels of the drug. Plasma samples were concentrated on the precolumn which was then washed with ammonium chloride 0.1 M / ammonia 0.1 M buffer, pH 9.3. This caused elution of the plasma components while the drug component was retained on the column. The choice of a buffer of pH 9.3 was essential to the cleanup process for the following reasons :

a. most plasma proteins have a molecular pI below 7 and are thus negatively charged at pH 9.3; therefore they have a high solubility in water;

b. many of the smaller proteins will be negativey charged at this pH and will thus be unretained on an XAD-2 resin.

Lecallion et al. [42] examined the influence of solute polarity in column switching chromatography for drug assays in plasma and urine. They reported that if the polarity of the drug was low or medium then reversed-phase chromatography was preferable.

Automated determination of theophylline and it's metabolites in serum using a column switching sample cleanup technique was reported by Matsumoto et al. [43]. A modified reversed-phase precolumn (TSK BSA-ODS) was used in conjunction with a modified reversed-phase analytical

column (TSK gel ODS 80 TM). The serum samples were directly injected on to the precolumn which was then washed with 0.1 M NaH_2PO_4. Elution in a backflushing mode eliminated serum proteins from the sample. The flow direction was then reversed and the precolumn was subjected to gradient elution with 0.1 M NaH_2PO_4 and increasing concentrations of methanol. Theophylline and it's nine derivatives were gradually eluted in this way due to differences in their hydrophilic nature. Simultaneous determination of theophylline and all nine derivatives was possible without serum protein interferences, which was the object of this on-line sample cleaning technique.

2.2.2.3 Group Separations

Another important application of column switching is group separation. In the initial fractionation stage, the sample components are separated into groups based on some shared chromatographic characteristic; fractionation selection could be on the basis of molecular size, ionic characteristics or polarity. By judicious choice of the primary solvent system, only sample components of analytical interest will be transferred to the second column. The reduction of interferents using this method means that fewer peaks and increased resolution of the secondary analytical system compared with direct separation of the whole sample are obtained.

Ogan et al. [44] analysed complex sample mixtures using coupled column chromatography They used reversed-phase chromatography coupled with size-exclusion ion chromatography to generate a multi-dimensional chromatographic method for the determination of polycyclic aromatic hydrocarbons in coal liquids and oils. The first fractionation step was a low resolution version of the final step, and selected components with retention times comparable to those of compounds of analytical interest. The second fractionation step was size-exclusion chromatography, which selected components on the basis of their molecular size. The coal liquids and oils, which have much greater molecular size, had a much longer elution time, and were therefore separated from the hydrocarbons of interest.

Nielen et al. [45] have used small columns packed with different stationary phases for on-line group separation of industrial waste water samples. Non polar fractions were adsorbed on a C_{18} stationary phase, medium polarity fractions on PRP-1 (polystyrene-divinylbenzene)

and polar bases on cation exchangers. Individual components were then separated using C_{18} reversed-phase liquid chromatography.

2.3 Determination of trace level Cu(II), Al(III) and Fe(III) by Reversed - Phase Liquid Chromatography, using a Novel On-line Sample Preconcentration Technique.

2.3.1 EXPERIMENTAL

2.3.1.1 Apparatus

The instrument arrangement incorporating a six-port/two way switching valve (Rheodyne 7000) is shown in Figure 2.3. Two HPLC pumps (M45, Waters.Assocs.) were used in the switching system. Pump A was connected to the injection valve (Rheodyne 7125) with a 2 cm^3 fixed volume loop. The loop could be switched on-stream with the stainless steel precolumn (10 mm x 1.5 mm i.d.) housed in a SepPak cartridge. The analytical column LC 18 DB, (25 cm x 4.6 mm i.d., 5 micron) was supplied by Supelco. A guard column packed with Nucleosil C_{18} was mounted before the analytical column. Eluent flow direction was controlled by manual actuation of the switching valve. The analytical column was supplied with solvent by pump B and detection was achieved using a Shimadzu SPD-6A variable wavelength detector. Column switching was operated in a back flush mode [21]

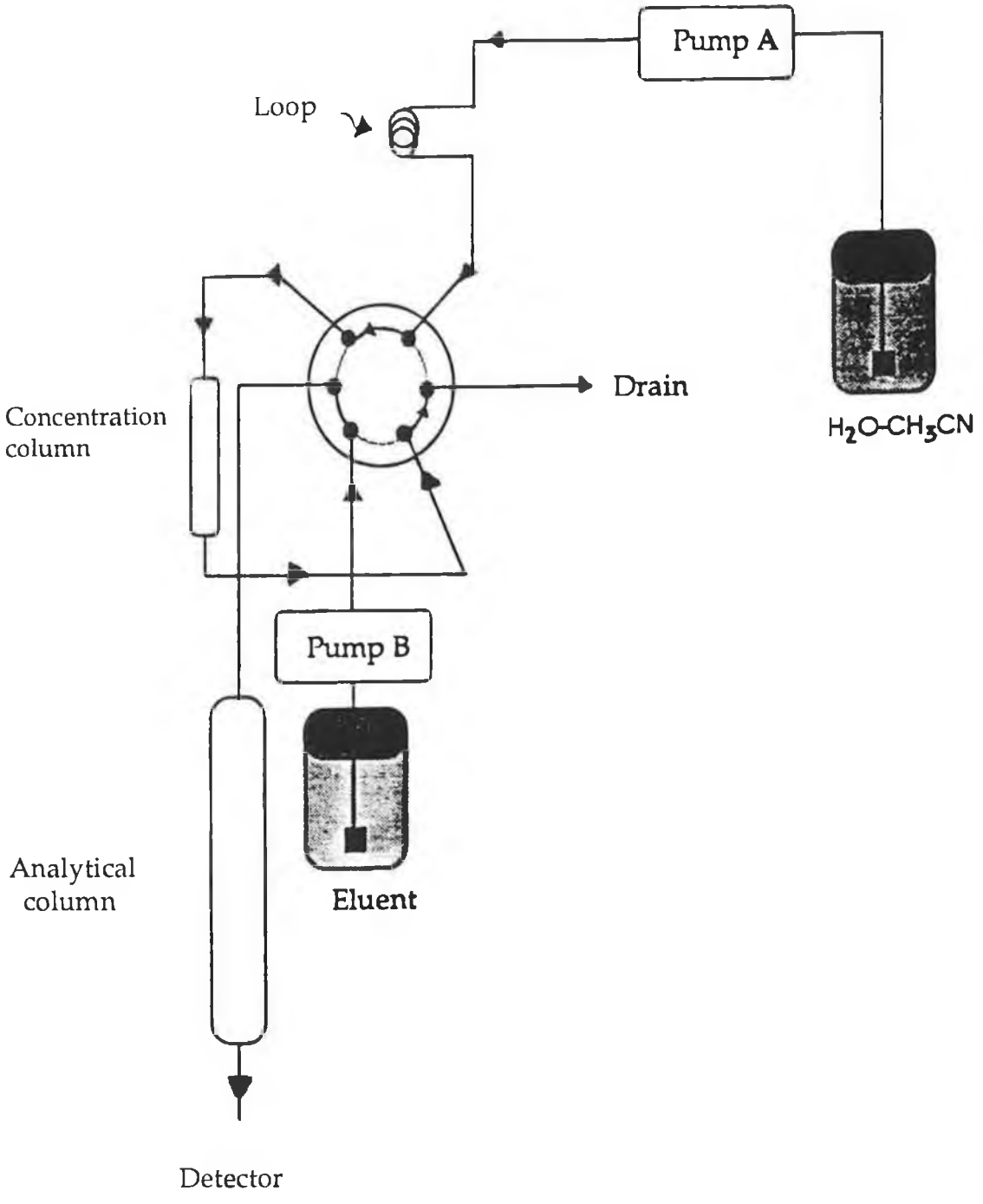

Figure 2.3 Column Switching Assembly

2.3.1.2 Materials

All chemicals used were of analytical grade. Acetonitrile (AN) was supplied by Merck. Deionised water (resistivity 18 M ohms) was obtained using a Millipore Milli-Q water purification system. Analytical grade 8-hydroxyquinoline (Carlo, Erba, Milan Italy) was used without purification. All metal salt solutions were prepared by dilution of the stock standard solutions (1000 ppm, Carlo Erba atomic absorbtion grade). Chelates were prepared by addition of the metal ion solutions (pH 4.0) to a 10 fold molar excess of 8-hydroxyquinoline and diluting the solution to 10 cm^3 with the mobile phase.

2.3.1.3 Methods

2.3.1.3.1 Sample Preparation

Minimal sample pretreatment was carried out on the waste water and beer samples analysed. The pretreatment steps involved acidification followed by filtration through a 0.45 μm filter. The samples were then mixed with appropriate volumes of mobile phase B, a 50:50 mixture gave best results, allowed to stand for 1 hour in the case of the beer sample to allow proteins to precipitate and were analysed using the column switching technique.

2.3.1.3.2 Chromatographic Procedures

Column switching was operated in the backflushing mode whereby the components of interest were retained on the precolumn, the effluent from this column being vented to waste bypassing the analytical column and therefore preventing its contamination. After switching the

six-port valve the fraction of interest was eluted from the precolumn and transferred for separation to the analytical column. Separation of metal ions using the system first proposed by Mooney et al [13] was investigated. Efficient separation of Cr(III), Zn (II), Cu(II) and Fe(III) was achieved with limits of detection of 500 ppb when using a 20 μL direct injection technique. The metal ions were precomplexed prior to injection using 8-hydroxyquinoline. This ligand forms neutral chelates with several divalent and trivalent metal cations and as a result it was also possible to detect these metal ions under similar conditions with comparable limits of detection.

The mobile phase required for separation contained acetonitrile made (1×10^{-2} M in 8-hydroxyquinoline) and 0.02 M acetate buffer (pH 6.0 made in 0.2 M potassium nitrate) (1:1). Optimisation of the complexation reaction was studied in detail by Bond and Nagaosa [12] with respect to ligand concentration. Our results are in agreement with these except for the optimum KNO_3 concentration which we found to be 0.2 M.

2.3.2 RESULTS AND DISCUSSION

2.3.2.1 Precolumn Selection

Our aim in this study was to use column switching to achieve both improved sensitivity and selectivity. When using HPLC to analyse trace metals selection of suitable precolumn-column combinations to achieve such aims has been the subject of considerable research [28]. The initial selection of the packing material is dependent on the development of the analytical separation. The chromatographic procedure described earlier gives the desired separation so it was our intention to select a precolumn packing suitable for the retention of the metal ions when injected as their 8-hydroxyquinolate complexes.

The precolumn packing should display high retention of the metal-8-hydroxyquinolate complexes during the preconcentration step, and have high loadability. Pellicular sorbents although investigated were not expected to exhibit high retention characteristics. Retention during the desorption step should be negligible in order to minimise extra-column band broadening during the elution step. If the ligand bound metals were injected in a predominantly aqueous phase they should be retained strongly by a reversed phase sorbent whose hydrophobic nature would attract the neutral chelates. Elution would then be possible when an eluent with much higher elutropic strength is introduced, i.e. one having a much higher organic content.

Commercially available reversed phase materials with particle sizes in the range 30-50 μm are generally well suited for use as enrichment column packings, however in our studies we found increased band broadening with these particle sizes. In Table 2.1 the materials investigated are shown with their characteristics in respect to retention of

the various metal chelates. On the basis of these results one material Nucleosil 10 C_{18} (10 micron) was evaluated further, with both standard and real samples.

Concentration Material	Manufacturer	Particle Size (μm)	Shape	Retention /Elution Characteristics
Nucleosil 10 C_{18}	Waters	10	Porous	Complete Retention / Complete Elution
Nucleosil 10 C_8	Waters	10	Porous	Breakthrough
Licrosorb C_{18}	Merck	10	Porous	Complete Retention / Incomplete Elution
Phenyl Hypersil CN	Shandon	10	Porous	Concentration column clogged
Bondapak Corasil C_{18}	Waters	37 - 50	Pellicular	Complete Retention / Incomplete Elution
Pellicular ODS	Whatman	37 - 53	Pellicular	Complete Retention / Complete Elution

Table 2.1 Reversed-phase materials as precolumn enrichment packings

Note: *Both Nucleosil 10 C_{18} and Pellicular ODS exhibit good retention and elution characteristics however Nucleosil 10 C_{18} shows a lesser degree of band broadening and was therefore chosen.*

2.3.2.2 Solvent Compatibility

Two compatible eluents of different elutropic strengths had to be selected, one to concentrate the sample onto the precolumn and the second to elute the components of interest from the precolumn onto the analytical column. The 8-hydroxyquinoline containing mobile phase had already been determined as a suitable analytical column eluent and was designated mobile phase B. The solvent selected for loading and concentration of the sample on the precolumn (designated mobile phase A) had to have poor elution capability for the components of interest in order to ensure maximum preconcentration. Nucleosil C_{18} the reversed phase packing used is a hydrophobic sorbent so the solvent of least elutropic strength is water, however water was found to be miscible to a very poor extent with mobile phase B.

The solvents investigated are shown in Table 2.2 with their characteristics in respect to compatibility with mobile phase B and the sample (containing metal 8-hydroxyquinolate complexes). Various mobile phase compositions with increasing organic content were evaluated and as expected the greater the compatibility of the two solvents the higher the sample loss on the precolumn. Precipitation of the samples due to incompatibility was observed when 100% aqueous phases were used and when the sodium acetate concentration of Mobile phase A was increased. On the basis of these results Water : Acetonitrile (90 :10) was chosen as mobile phase A as it is miscible with mobile phase B and its ability to elute the metal complexes from the precolumn was very poor therefore achieving maximum preconcentration.

Mobile Phase A Composition	Compatibility with Mobile phase	Sample
100 % H_2O	Very Poor	Precipitation
100 % NaAc / KNO_3	Very Poor	Precipitation
95 % H_2O / 5 % CH_3CN	Good	Some ppt.n
95 % NaAc / 5 % CH_3CN	Poor	Precolumn clogged
90 % H_2O / 10 % CH_3CN	Good	No ppt.n
90 % NaAc / 10 %CH_3CN	Poor	Precipitation
85 % H_2O / 15 % CH_3CN	Very Good	Sample loss
85 % NaAc /15% CH_3CN	Very Good	Precipitation
80 % H_20 / 20 % CH_3CN	Very Good	Sample loss
80 % NaAc /20 % CH_3CN	Good	Precipitation

Table 2.2 Optimisation of Mobile Phase A

Note: *For the determination of the optimum composition of mobile phase A a 250 µL sample of a 500 ppb Fe (III) standard was used for all studies.*

2.3.2.3 Metal Preconcentration

In order to determine the boundary conditions that could be used for application and desorption of the ligand-bound metals on the precolumn, the breakthrough volumes of the first and last eluting metal chelates were measured under different conditions. Preliminary studies indicated that the Cu(II) complex eluted first and the Fe(III) complex last. It was envisaged that the preconcentration step would be applied to metals in the concentration range 10 ppb - 10 ppm so a realistic concentration of 500 ppb of each metal complex was loaded onto the precolumn for all breakthrough studies.

The Cr(III) complex was found to be very unstable in the aqueous environment of the precolumn and therefore could not be detected. No retention of the Zn (II) complex on the precolumn was observed under the experimental conditions used. Consequently this preconcentration method offers a selective determination of Cu(II), Al(III) and Fe(III). Interference from other metals forming 8-hydroxyquinolate complexes was not found to be a problem during this study. However, if an interfering metal was to be present, separation from the peaks of interest could be efffected by varying the composition of mobile phase B.

2.3.2.3.1 Breakthrough Volume Determination

By varying the flow rate of mobile phase A the most suitable wash volumes could be determined i.e. the volume of mobile phase A with which it is possible to wash the precolumn without causing elution of the retained analytes due to the washing effect of the solvent. Increasing volume aliquots of the metal chelate solutions were individually loaded onto the preconcentration column, the retained chelates were then backflushed onto the analytical column and determined spectrometrically

at 400 nm. When the precolumn capacity was exceeded only a fraction of the loaded chelates were retained on the precolumn, indicating breakthrough of the loaded solutions at a certain loading volume. A typical breakthrough curve is shown in figure 2.4 for the simultaneous analysis of Cu(II)-, Al(III)- and Fe(III)- 8-hydroxyquinolate complexes. The Fe(III) complex when analysed individually has the largest optimium wash volume indicating that the breakthrough occurs at a higher volume than with either the Cu(II) or Al(III) complexes. This is probably due to the stability of the Fe(III) complex which binds strongly to the C_{18} sorbent and is difficult to displace even as a result of volume effects of the carrier stream of mobile phase A. The iron complex can withstand a wash volume of up to 5 cm^3 before breakthrough occurs.

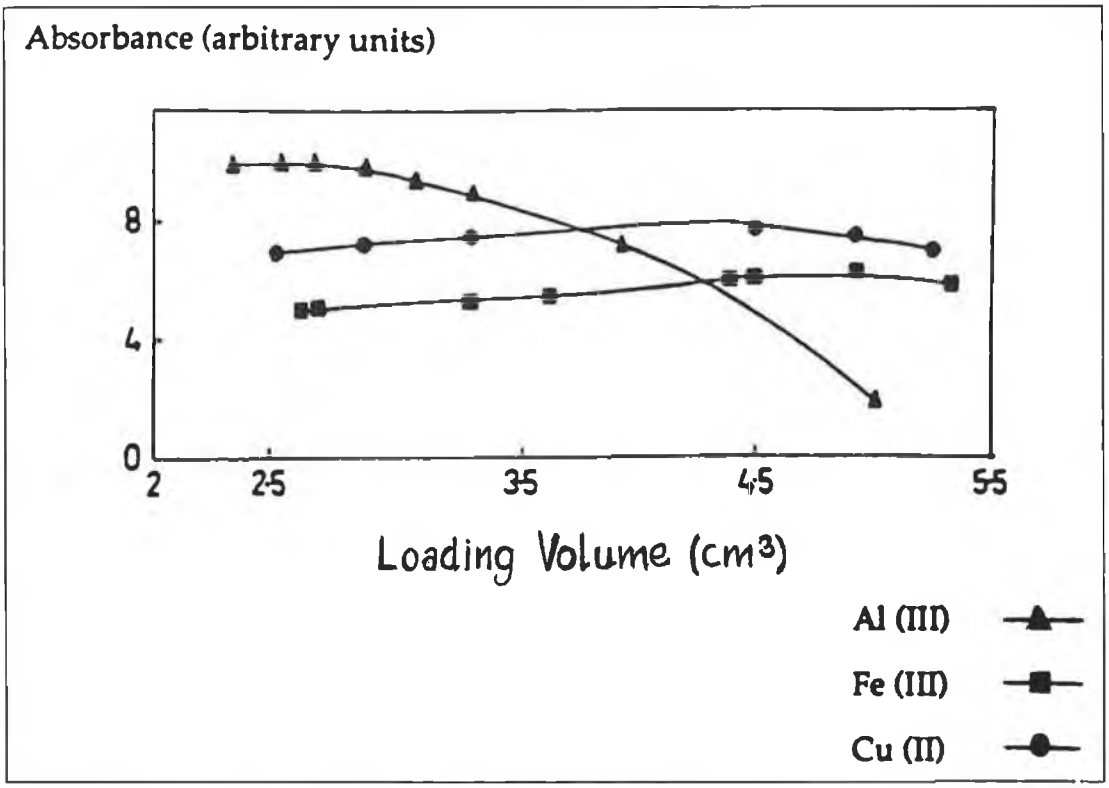

Figure 2.4 Breakthrough curves recorded for Cu(II), Al(III) and Fe(III) injected as their 8-hydroxyquinolate complexes using 50:50 acetonitrile (made 1 x 10^{-2} M in 8-hydroxyquinoline): 0.02 M acetate buffer (pH 6.0, made 0.2 M in KNO_3). Sample loading was at 1 cm^3 min^{-1} using acetonitrile-water (90:10).

2.3.2.3.2 Optimisation of Eluent Composition

Both aluminium and copper form stable complexes with 8-hydroxyquinoline. However, in the predominantly aqueous environment of the precolumn during the washing step, they become displaced from the precolumn quickly in comparison with the Fe(III)-8-hydroxyquinolate complex. The Cu(II)-8-hydroxyquinolate complex has an optimum wash volume of 2.85 cm^3 and the Al(III)-8-hydroxyquinolate complex 2.75 cm^3. The Cu(II) complex had a lower breakthrough volume when injected into a multi-element system; this was probably due to the instability of the Cu(II)-8-hydroxyquinolate complex in the aqueous precolumn environment in comparison with the Al(III) and Fe(III) complexes and also the reduction in active sites on the precolumn. Buffering of mobile phase A was investigated in an attempt to increase the stability of the Cu(II) complex on the precolumn; however, the buffers tended to precipitate out on the precolumn on elution with mobile phase B (see Table 2.2).

2.3.2.3.3 Optimisation of Wash Volume

The equilibrium wash volume for the precolumn was also investigated and can be defined as the volume of mobile phase A required to re-equilibrate the precolumn following elution of the retained analytes using the 8-hydroxyquinoline containing mobile phase B. In Fig. 2.5 a typical equilibrium wash volume curve is presented. It can be seen from this profile that the use of an inadequate volume of mobile phase A for re-equilibration will result in a decrease in peak height. The optimum re-equilibration volume of mobile phase A was found to be 0.5 cm^3.

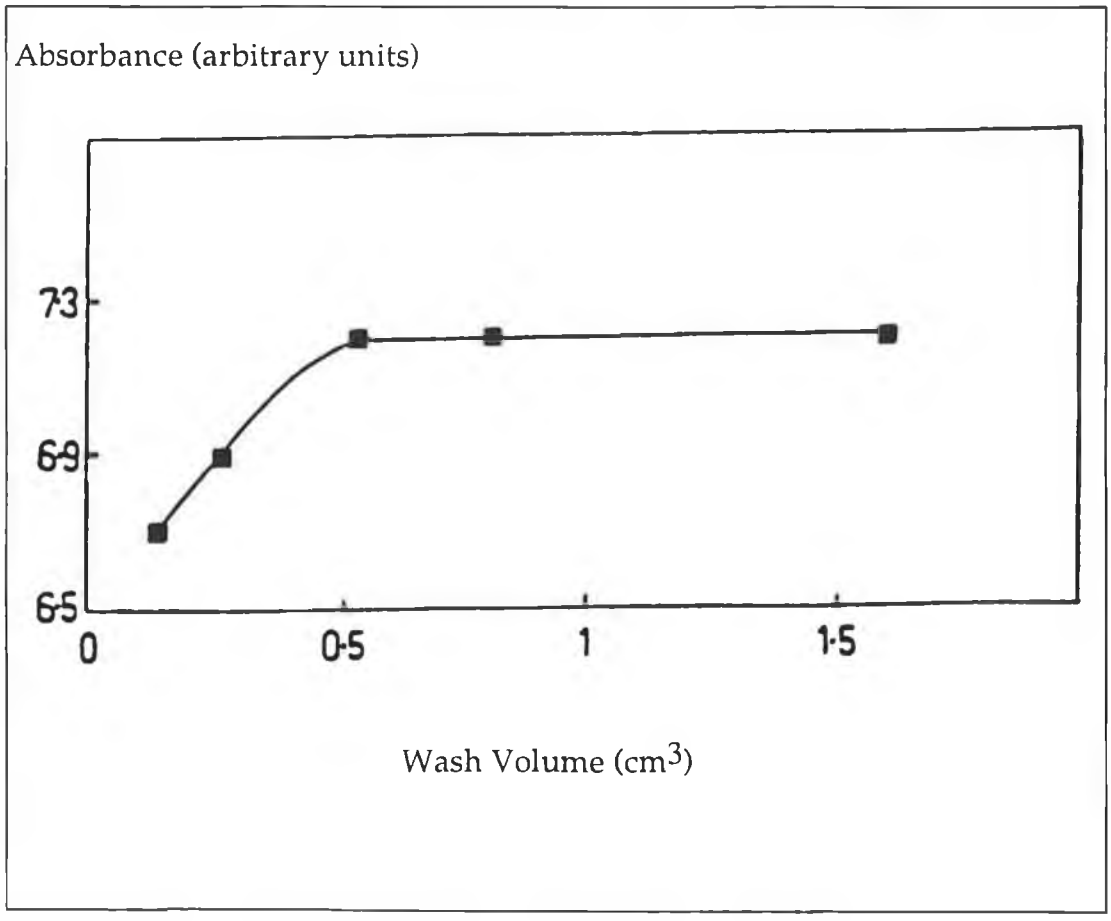

Figure 2.5 Variation of absorbance of Al (III)-8- hydroxyquinolate complex at 400 nm with increasing equilibrium wash volumes.

2.3.2.3.4 Mass Loadability

The problem of mass loadability was addressed with respect to the maximum sample concentration that can be loaded onto the precolumn. The effect of high loadings on trace enrichment and on the efficiency of the chromatographic separation was investigated. Fig. 2.6 shows typical loadability curves; it is obvious that the loadability of the Fe(III)-8-hydroxyquinolate complex is far greater than either that of the Cu(II)- or Al(III) 8-hydroxyquinolate complexes. An 800 µL volume of 500 ppb Fe(III) complex could be loaded without an observable decrease in the enrichment efficiency of the precolumn.

A maximum of 300 µL each of a 500 ppb solution of the Cu(II) and Al(III) complexes can be loaded. Above this volume the efficiency of the preconcentration decreases. However, as the concentration of the 8-hydroxyquinolate solution injected decreases, the volume of the sample that can be injected without loss in concentration efficiency increases.

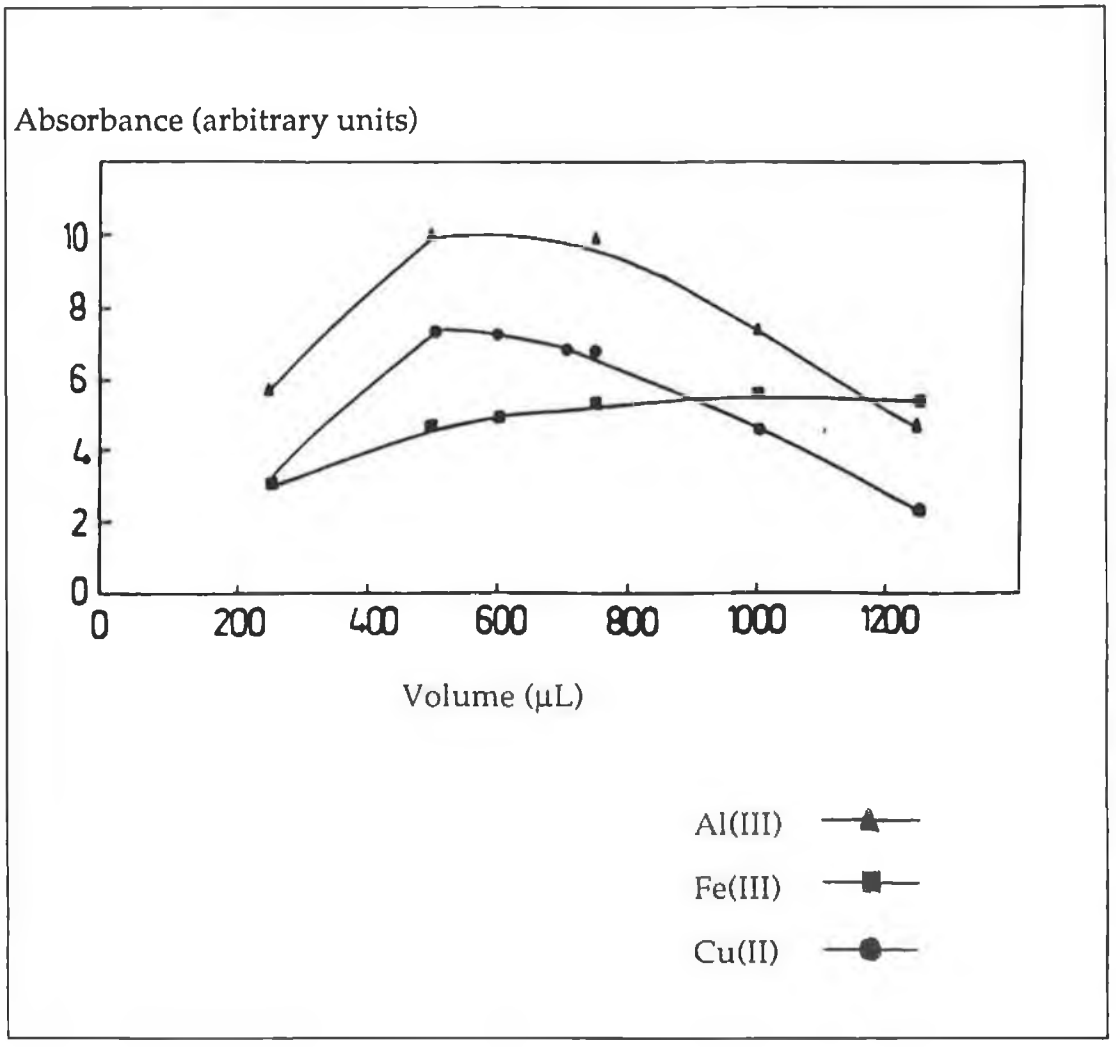

Figure 2.6 Loadability curves for 500 ppb Cu (II), Al (III) and Fe(III) standards : Flow rate of mobile phase A 1 cm^3 min^{-1}.

2.3.2.3.5 Chromatographic Separations

Evaluation of the enrichment procedure was performed using the optimum boundary conditions outlined; however, it was found that the resolution of the multi-metal system in the lower region of the concentration range required a slight change in the composition of mobile phase B. A mobile phase containing acetonitrile-acetate buffer was used but the proportion was changed from 50+50 to 47+53. Resolution of the trace metals could then be achieved. Fig. 2.7 shows typical chromatographic separations obtained with 250 µL injection volume using the direct injection technique [Fig. 5(a)] and trace enrichment [Fig. 5 (b)].

Elution was of a similar duration (approximately 8 min); however, the trace enrichment procedure enabled a 100 fold dilution of the standards to be loaded without appreciable loss in sensitivity. It was possible, using the switching technique to inject a standard at the ppb level at the same detector sensitivity as a ppm standard, and record its chromatogram with good resolution and similar elution characteristics. Resolution of the Co(II) and Al(III) was slightly more difficult using the enrichment system, and co-elution was evident if the flow rate of mobile phase B was too high. Considering the similar interactive chemistry of the two ligand-bound metals with the reversed phase sorbent, this co-elution possibility had been expected.

Co(II) if present at appreciable levels did not interfere with the determination of trace amounts of Al(III). However, if found to be present at high concentrations a slight alteration of the chromatographic conditions allowed the Co(II) and Al(III) complexes to be resolved.

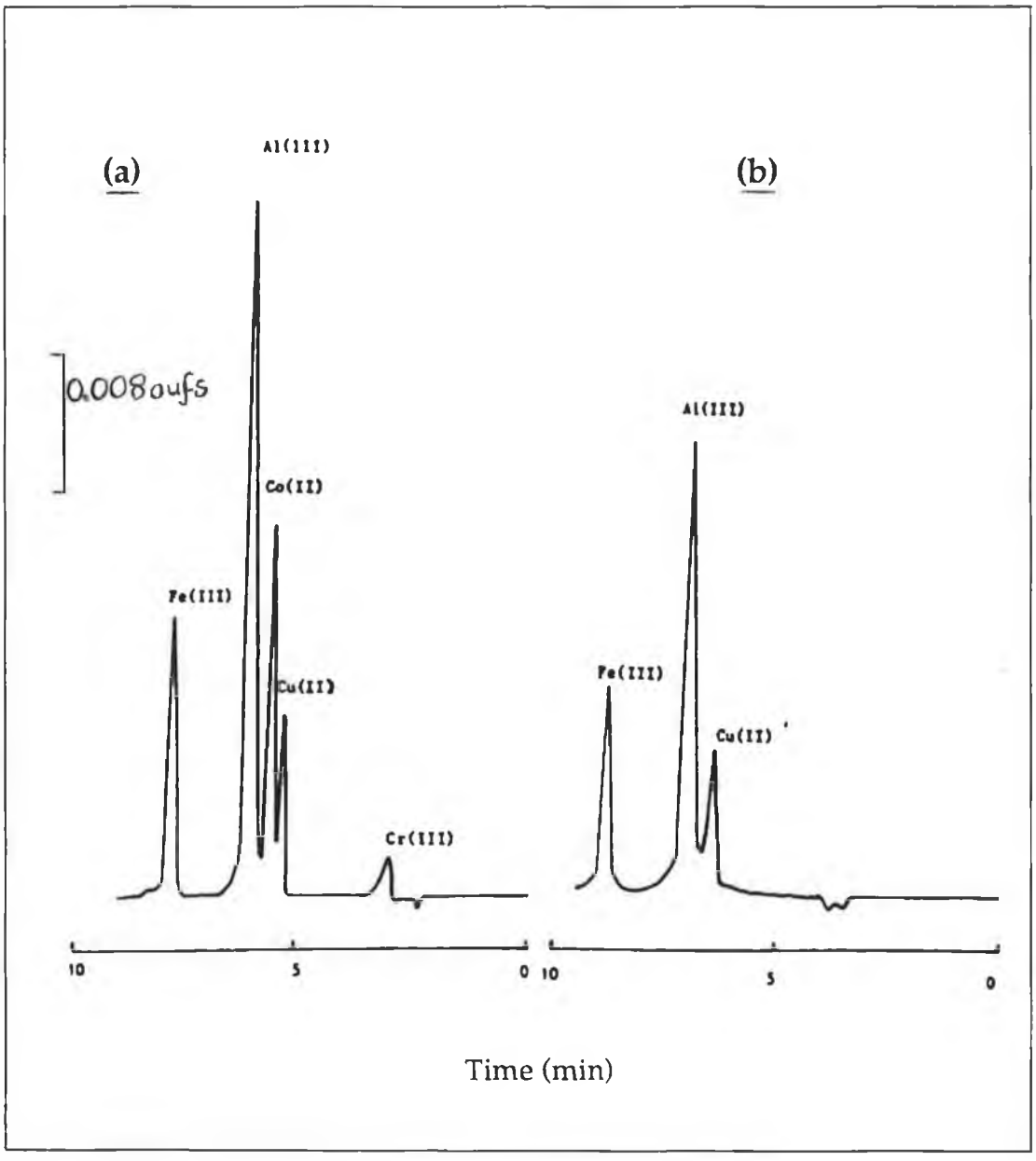

Figure 2.7 (a) HPLC separation of 1 ppm Cr(III), Cu(II), Co(II), Al(III) and Fe(III) using a direct injection technique. Mobile phase B acetonitrile-water (made 1×10^{-2} M in 8-hydroxyquinoline), flow rate 1.2 $cm^3\,min^{-1}$.

(b) HPLC separation of 10 ppb Cu(II), Al(III) and Fe(III) using the trace enrichment procedure. Mobile phase A water:acetonitrile 90:10 and Mobile phase B(as for 7 (a)). Flow rate A 0.8 $cm^3\,min^{-1}$, flow rate B 1.2 $cm^3\,min^{-1}$.

2.3.2.3.5.1 Trace Enrichment

On the basis of the chromatographic results it was decided to evaluate trace enrichment further for the Al(III)-, Cu(II)- and Fe(III)- 8-hydroxyquinolate complexes at low concentration levels. Calibration plots for the determination of Cu(II), Al(III) and Fe(III) were obtained under the optimum experimental conditions. Peak heights were proportional to the metal concentrations in the range from 50 ppb to 5 ppm. From the regression analyses the following relationships were obtained:

For Fe(III): (y(mm) = (5.122 x 10-2 +/- 0.68113 x 10-4)x + 2.09 +/- 0.7)
r = 0.9987

For Al(III): (y(mm) = (6.75 x 10-2 +/- 7.6376 x 10-4)x +0.305 +/-0.000245)
r = 0.9998

For Cu(II): (y(mm) = (3.67 x 10-2 +/- 4.9469 x 10-2)x + 0.206 +/- 0.11701)
r = 0.9994

Limits of detection of 5 ppb for Al(III) and 40 ppb for Cu(II) and Fe(III) were obtained. These limits of detection correspond to a signal to noise (background) ratio of 3:1. A zero intercept for the calibration plots indicated that no substantial decomposition of the metal complexes had occurred on the precolumn and/or the analytical column during elution. The limit of detection can also be found using the slope, intercept and error values determined using the method of least squares, obviously, there is an associated error with this value as it involves the use of the calculated mean which is also subject to errors and is only an estimate of the true mean value.

2.3.2.3.5.2 Application to Minewater-waste

In order to demonstrate the applicability of this trace enrichment technique to samples containing metals at the ppb level the Cu(II), Al(III) and Fe(III) content in waste water from a mine was examined. Representative samples were taken near the waste water outlet of the mine and further downstream from the outlet. These samples were designated upstream sample (D1) and downstream sample (D2), respectively. The samples were pretreated to ensure the metals present remained in solution. Owing to the extremely low level of metals present in the waste water sample, standard addition was chosen as the most suitable method for determination of the metallic species. Both Al(III) and Fe(III) were detected and their concentrations determined; however, although Cu(II) was also present, it could not be quantified as its concentration was lower than 20 ppb and outside the limit of detection of this method. The upstream sample was found to contain 98 ppb of Al(III) and 214 ppb of Fe(III). The correlation coefficients were 0.9996 and 0.9911 for Al(III) and Fe(III) respectively. The downstream sample was found to contain 8 ppb of Al(III) and 202 ppb of Fe(III), respectively; the correlation coefficients being 0.9993 and 0.9989 respectively.

Typical chromatographic separations achieved using this trace enrichment system are shown in Figures 2.8 (a-d).The results indicate a much higher Al(III) content near the waste water source than downstream as expected due to dilution effects as the waste travels downstream, the Fe(III) content of both upstream and downstream samples is virtually identical as, generally, river water has a high background level of Fe(III) present. Similar separation was attempted using the direct injection technique however no metals were detected.

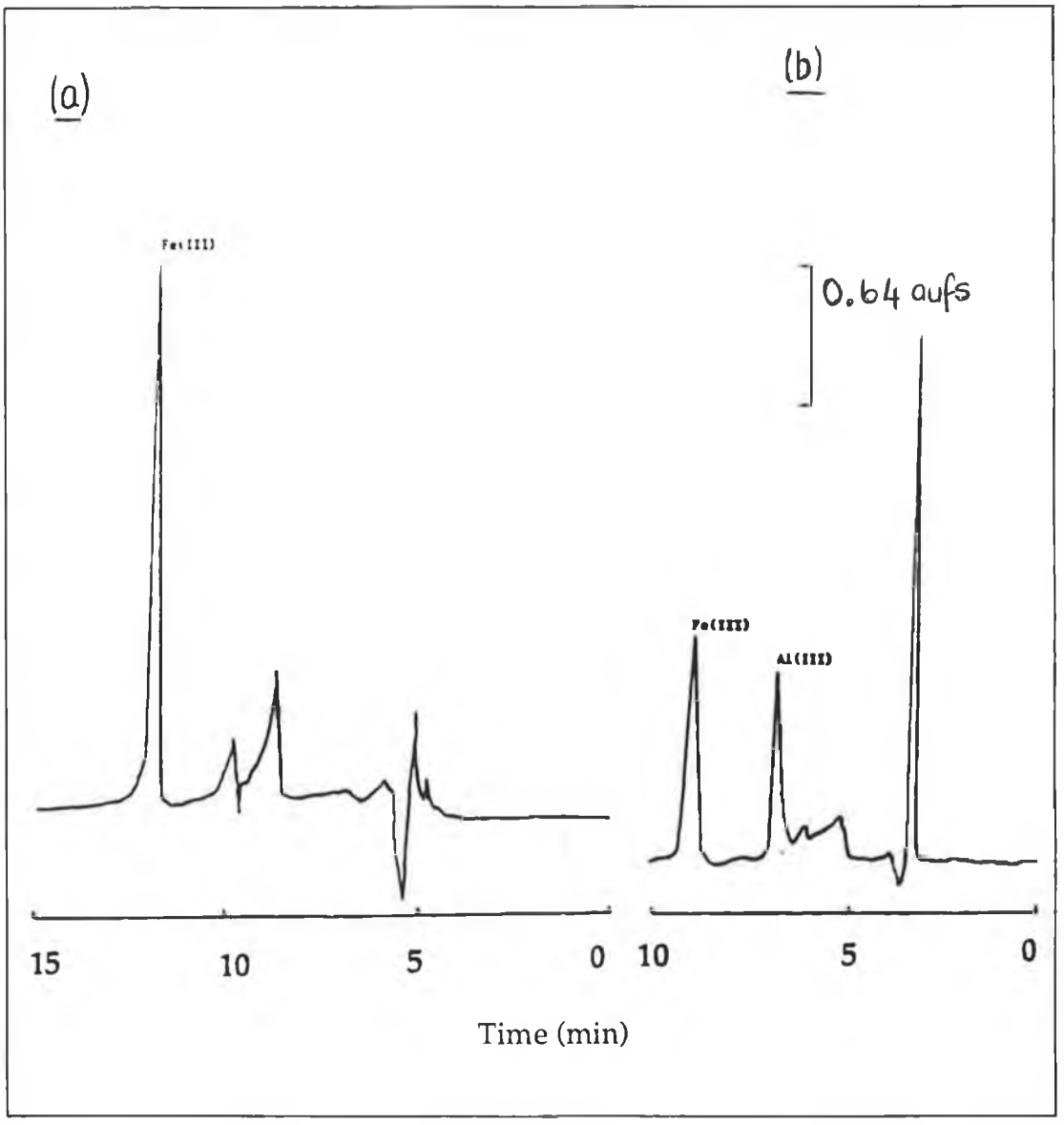

Figure 2.8 (a) Determination of Fe(III) in waste water using a standard addition method. Sample composition upstream sample (**D1**) - mobile phase B, 50:50; spiked with 100 ppb of Fe(III). Mobile phase A H_2O-CH_3CN (90:10), flow rate 0.8 cm^3 min^{-1}. Mobile phase B, CH_3CN (made 10^{-2} M in 8-hydroxyquinoline) acetate (pH 6.0, made 0.2 M in KNO_3), flow rate 1.2 cm^3 min^{-1}.

(b) (**D1**)-mobile phase B (50:50), spiked with 30 ppb of Al(III)

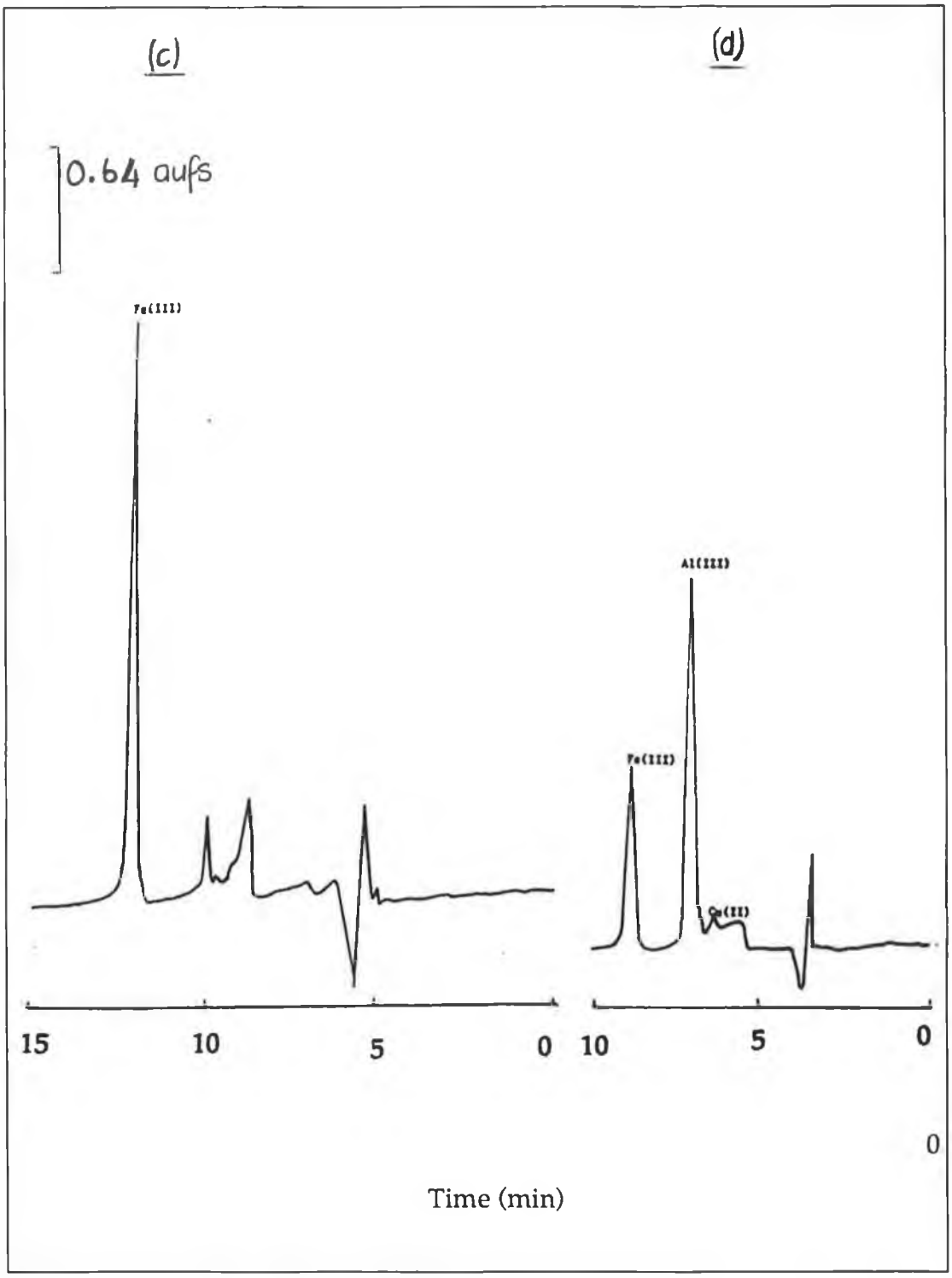

Figure 2.8 (c) (**D2**)-mobile phase B (50+50), spiked with 100 ppb of Fe(III). (d) (**D2**)-mobile phase B (50+50), spiked with 50 ppb of Al(III). Conditions (as for **2.8 (a)**).

2.3.2.3.5.3 Application to Beverage Samples

In order to demonstrate the selectivity enhancement obtainable using trace enrichment the column switching technique was also applied to beer samples. Beer is routinely tested by brewing companies using AAS, HPLC, TLC, FPLC and SCABA analysis techniques, generally these techniques are preceeded by an off-line sample cleanup step. Methods of sample preparation have included liquid/liquid extraction, precipitation and solid phase extraction. However, on-line cleanup approaches incorporating column switching methodologies have also been investigated [28].

Trace metal presence in beer is acceptable to certain permittable levels. Beers such as Guinness are renowned for their medicinal benefits due to the high iron content present, normally less than 1 ppm, which can be beneficial to anaemia sufferers. However, metal concentrations exceeding allowable levels may be detrimental to health and so must be quantified precisely to ensure the consumer of the quality of the beer. Metal ions are known to reduce beer clarity, therefore, from the brewers view point, metals present at high levels are highly undesirable. The beer samples tested were bought from local supermarkets in the canned form where the shelf life was guaranteed to be at least 5 months.

The beer was prepared for analysis by mixing it with mobile phase B at a ratio of 60:40; in this case no sample pretreatment was necessary but the mixed sample was allowed to stand for 1 hour before injection to allow the proteins to coagulate before injecting the clear supernatant. Figure 2.9 shows the chromatographic separation obtained. Fe(III) was the only metal quantified in the beer sample and its concentration could be read directly from a calibration curve. Iron was found to be present at a concentration of 875 ppb. It would be useful to

determine the accuracy of this method with a standard testing procedure used in the brewing industry such as SCABA to determine the efficacy of our method.

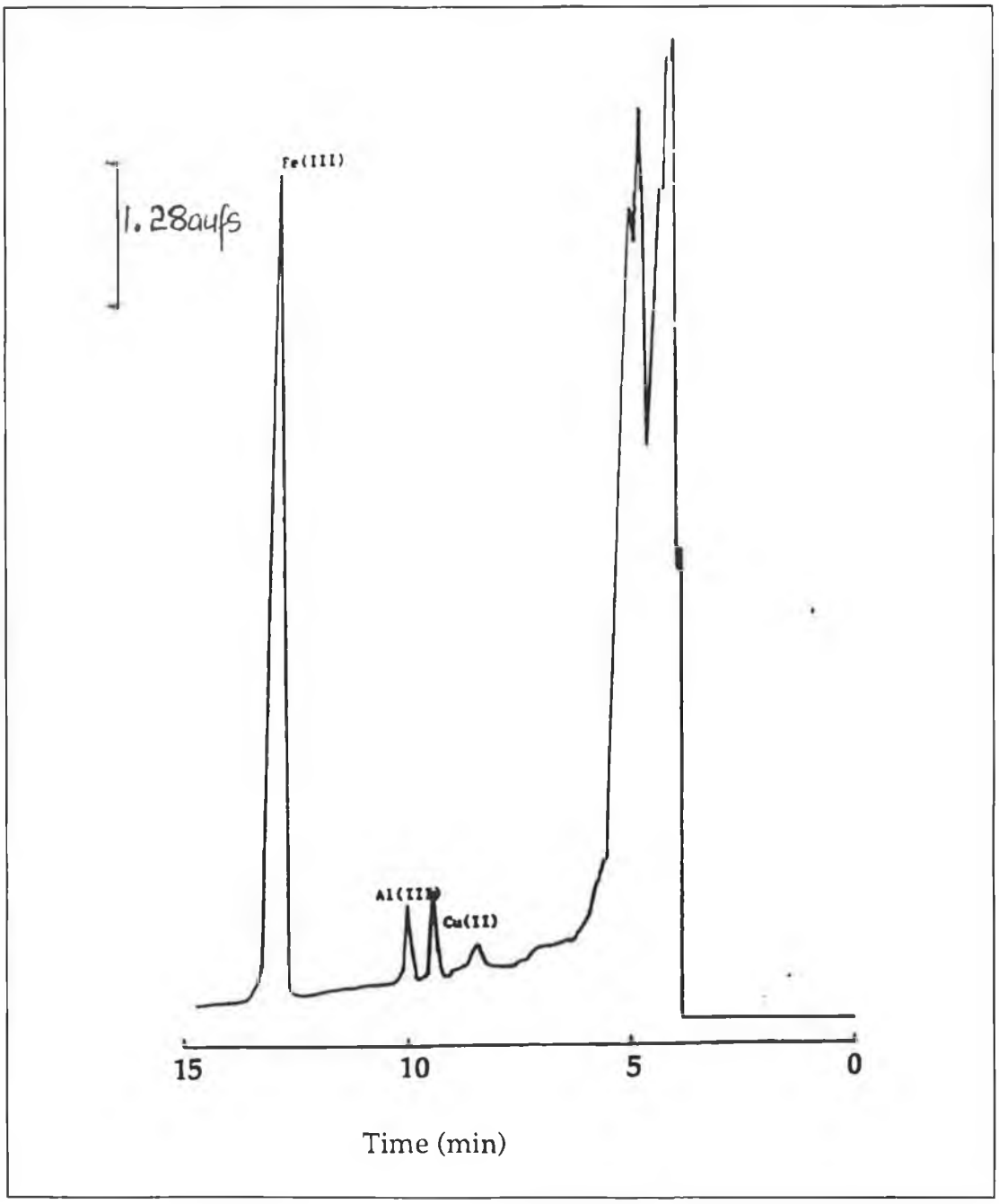

Figure 2.9 Determination of Fe(III) in beer using water-acetonitrile (90:10) as mobile phase A and CH_3CN (containing 10^{-2} M in 8-hyroxyquinoline) and acetate (47:53) as mobile phase B. Flow rate of : mobile phase A, 1 cm^3 min^{-1}; and mobile phase B , 0.9 cm^3 min^{-1}. Sample composition: beer-mobile phase B, (60:40).

2.3.3.3 Conclusions

Trace enrichment of metal ions using column switching is an effective tool for the enhancement of both sensitivity and selectivity when applied to the analysis of trace metals as their 8-hydroxyquinolate complexes. The sample preconcentration it affords to the user is a complex process and paramount to its success is the efficiency of sample loading. Judicious choice of the loading mobile phase and eluent is very important. The column switching technique described provides optimum efficiency for separation of the metals of interest, whilst simultaneously minimising analysis time by decreasing the time spent in sample preparation and in separating the components of the sample which are of no particular interest. The working range of the technique was extended considerably as a direct result of increased sensitivity, attainable with column switching through use of higher sample loadings on the precolumn.

The application of the method to the analysis of Al(III) and Fe(III) in real samples has been demonstrated, however, it should be noted that if samples containing a high concentration of particulate matter are to be analysed the use of a freshly packed precolumn is recommended after 30 - 40 injections, otherwise the column can normally be used at least 50 times without clogging. The use of a filtering system e.g. 'Swinny filtering' can also be used to alleviate the problem of precolumn clogging.

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Chapter 3

On-line Preconcentration of trace metals using CTAB/DTC ion pair mini cartridges

3.1 Selective Copper Analysis.

3.1.1 Introduction

Copper is often present in fresh waters at significantly high concentrations, generally as a result of copper sulphate use as an algicide. The presence of copper at high concentrations in, for example tap water may make the water unsuitable for many biological applications. Copper and/or zinc derived from piping or galvanised tanks may lead to toxicity in waters used for aquaria. Trace elemental analysis of water containing copper at high levels poses particular problems, as copper interferes in almost all chemical methods for the enrichment and determination of trace metals. Copper forms extremely stable complexes with several reagents which means that trace element determination is often, not entirely selective for the element of interest if high concentrations of copper are present. The development of a preconcentration technique which would allow the determination of copper in solution even at extremely low levels is therefore of considerable importance, once copper presence is known a suitable method of copper elimination treatment could be used on all samples prior to analysis.

Frigge et al. [1] have reported two methods for the removal of copper from solution to enable the determination of other trace metals present in the sample. Copper was precipitated as copper oxide (Cu_2O) from alkaline solutions and copper thiocyanide (CuSCN) from acidic solutions. Following copper precipitation and removal, other elements present in the sample were precipitated with a hexamethyleneammonium hexamethylenedithiocarbamate solution and the metal complexes determined by FAAS or GFAAS. Electrolytic copper separation methods have also been described which rely on the electropositive reduction

potential of copper + 0.34 V [2]. If, however, a selective preconcentration technique for copper could be developed that involved a column switching methodology, a copper selective precolumn could then be incorporated on-line into a HPLC system for trace element determination; copper interference could therefore be removed directly before analysis of other trace elements present in the sample or the direct analysis of copper would also be achievable.

3.1.1.1 Projected Aims of the Copper Preconcentration Technique.

In chapter 2 the selective preconcentration of Cu(II), Fe(III) and Al(III) as their 8-hydroxyquinolate complexes on a short precolumn, with subsequent separation by RPLC, using a column switching preconcentration methodology was outlined. The method was extremely sensitive and allowed Cu(II) to be determined at levels as low as 40 ppb. The primary aim of the work outlined in this chapter was the development of a preconcentration method that had increased copper sensitivity and allowed determination of Cu(II) at the ppt level; the enhanced sensitivity previously achieved using column switching through the use of higher sample loadings on the precolumn, prompted the use of a similar methodology. In addition, it was hoped to develop a preconcentration method that would be highly selective for copper in the presence of other elements.

No suitable ligand that would selectively remove copper in the presence of other ions, and that could be incorporated into a column switching preconcentration system was found in the literature; however, as complexation with dithiocarbamates [3-11] is routinely used for heavy metal determination, in particular cadmium, lead, mercury and copper the incorporation of these ligands into a column switching preconcentration

system was examined. The preconcentration method described in chapter 2 required preconcentration of metal chelates on the precolumn, as opposed to uncomplexed metal ions. Irreversible adsorption of dithiocarbamate metal chelates on container walls and/or column packing materials is commonly reported [12] and although this problem was not observed with 8-hydroxyquinolate-metal complexes, the possibility of traces being lost due to irreversible adsorption of metal-DTC complexes was noted. Therefore, a method which would allow injection of uncomplexed metal ions onto a precolumn, which would then selectively retain copper (possibly via complexation with a dithiocarbamate reagent) in the presence of other metals was examined. Several approaches which have allowed the injection of uncomplexed metal ions onto a precolumn, with subsequent metal ion preconcentration are outlined below.

3.2 Methods of Copper Preconcentration

Methods which involve the direct injection of metal ions on to a precolumn with subsequent on-column derivatisation using dithiocarbamate reagents in the mobile phase have been reported by several authors [13,14], however one of the associated drawbacks with this method is that preconcentration of cations as opposed to apolar metal dithiocarbamates is more difficult and the stationary phase must contain cation exchange groups or complexing ligands instead of a simple hydrophobic surface.

3.2.1 Ion Pair Techniques for Trace Metal Preconcentration

An alternative method for the preconcentration of metal ions is the use of ion interaction /or ion pair chromatography [15,16,17]. This

HPLC mode can be carried out in two different ways; the first involves pretreatment of the column packing (which can be a simple octadecylsilane packing) with a dilute solution of ion interaction reagent (IIR), usually a long chain cationic or anionic species which then adsorbs at the mobile phase/stationary phase interface. The IIR is not incorporated into the mobile phase for the subsequent separation step. The stationary phase is thought to retain a permanent coating of the IIR thence this variant of ion pair chromatography is known as permanent coating ion interaction chromatography (IIC) [15].

The second method which is more commonly used for cation analysis is known as dynamic coating IIC [17]. It involves the addition of a dilute solution of IIR (typically sodium hexane sulphonate or sodium octane sulphonate) to the mobile phase. The mobile phase in both methods of IIC generally contains an organic solvent, an acid-base buffer and/ or complexing agent in addition to the IIR. Complexing agents which are frequently used include phenanthroline, bipyridyl and carboxylate derivatives.

Preconcentration techniques involving ion pair / ion associate formation (another variant of IIC) have also attracted considerable interest in the area of trace metal analysis. According to published literature [18-21] metals converted into ion associates via metal complex formation include Zn(II), Fe(III), Co(II), Co(III), Cu(I), Cu(II), Cr(III), Cr(VI), Pd(II), Mg(II), Ca(II), Cd(II) and Ni(II). Frequently organic alkyl ammonium salts are used as the ion pairing reagents. Metal complex formation is routinely carried out on-line in an HPLC apparatus fitted with an extra pump for the ion pairing reagent [22]. Ion pairing techniques were originally developed to promote separation of ionic compounds in HPLC. In ion pair chromatographic separation (IPC) ionizable counter ions are added to the chromatographic system in order to convert metal complexes into ion

associates. As in the case of IIR, chromatographic conditions have to be strictly controlled; relative retention and capacity factors of metal ion associates are regulated by column material, type of ion pair former, pH of eluent, % of organic modifier, extraction coefficients of metal complexes and ionic strength. Examination of the literature indicated that copper had been successfully preconcentrated using ion pair/ ion associate techniques, however many of the methods were not sufficiently selective for copper and would not have been suitable for incorporation into the column switching system.

Yin [23] reported the preconcentration of Cu(I) from effluents and aluminium alloys as an ion pair compound, with subsequent spectrophotometric determination at 610 nm. Copper was loaded from effluent solutions under controlled acidic conditions onto a mercaptoacetoxycellulose column, and then eluted onto a second column with a pH 3 buffered eluent. Copper was then eluted from this second column with 1 M HCl and the resultant solution was evaporated to fumes with sulphuric acid. The cooled residue was then treated with a series of solutions, the final solution contained copper in the form of a Cu(I)-potassium iodide-butylrhodamine B-gelatin-Triton X-100 ion pair compound. The solution absorbance was directly proportional to the concentration of copper present, and copper was successfully determined at a level of 1.3 ppm in effluent solutions.

Gonzalez-Perez et al. [24] have described the potential of ion pairing reagents for the preconcentration of trace metal ions using a less elaborate scheme than Yin [23]. Several metals were precipitated as ion pair complexes with hexadecyltrimethyl ammoniun bromide in the presence of thiocyanide. The complexes formed were extractable with organic reagents and high preconcentration factors were achieved.

In a more recent paper Buchberger et al. [25] also described the use of hexadecyltrimethyl ammonium bromide (HDTMAB) as an ion pairing reagent for separation of Fe(III)-, Cu(II)- and Pb(II)-complexes of ethylenediaminetetraacetic acid by ion chromatography with UV and potentiometric detection. Samples (100 cm^3) were mixed with a solution containing 2 ppm EDTA and left to equilibrate. The complexed samples were then introduced onto a C_{18} Bondapak column and could be separated with a mobile phase containing 1% HDTMAB in 1.2 mM phosphate buffer (pH 7.2) - acetonitrile - methanol, 12 : 5 : 3. The method was applied to the analysis of river water and sediments for metal content. Limits of detection in the range 1.5 - 4 ng were obtained for a direct detection UV method at 250 nm.

Siren [22] examined the effect of ion pairing modifiers on the separation of Co(II), Cu(II), Fe(III) and Pd(II) by precolumn derivatisation and HPLC. The differences in metal-complex behaviour with 1-nitroso-2-naphthol-6-sodium sulphonate or 2-nitroso-1-naphthol-6-sodium sulphonate ion associated before injection, or during elution, with quaternary ammonium salts tetradecyltrimethylammonium bromide (TDTMABr), cetyltrimethylammonium bromide (CTMABr) and tridecyldimethylbenzylammonium chloride (TTDMBACl) were examined. On-column ion pair formation was shown to be a faster and more selective method of metal determination with elution orders of Cu(II) < Pd(II) < Co(II) < Fe(III) being achieved, using a gradient elution system of water / methanol, (2 : 8, v : v). Separation and resolution of metal complex anions was strongly influenced by the choice of cationic counter ion; CTMAB was indicated as the optimum counter ion for the system. Inclusion of a second counter ion (i.e. competing co-ion) namely trimethyloctylamine (TMOA) into the mobile phase was found to decrease retention times and increase resolution of ion associate metal complexes.

Lin et al. [26] determined Cu(I) after separation, by adsorption of its 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine tetra phenyl-borate ion associate complex on microcrystalline naphthalene. The Cu(I) ion-associate complex was then determined by AAS.

Satake et al. [27] reported the preconcentration of Cu(II) and Al(III) from biological samples with Alizarin Red S and cetyltrimethylammonium-perchlorate adsorbent supported on naphthalene. The ion pair of $CTMAB^+$ and ClO_4^- showed a high percentage adsorption of Alizarin Red S metal chelate anions, in particular Al(III), Cu(II) and Mo(II). The method was highly selective and no extensive sample pretreatment was necessary.

Irth et al. [28] have reported an on-line preconcentration technique involving the simultaneous formation of metal dithiocarbamate complexes, subsequent metal ion separation with reversed phase LC and UV/VIS diode array detection. A cetrimide-dithiocarbamate (CTAB-DTC) ion pair was formed off-line and pumped onto a C_{18} precolumn to effect ion pair adsorption. Metals were then injected onto the DTC loaded precolumn and retained as DTC-metal complexes. Gradient elution with a mobile phase containing 10 mM CTAB and 10 mM phosphate buffer (pH 6.8) was followed by UV detection at 254 nm. Enrichment factors of 2500 were achieved for a 25 cm^3 sample loading volume and detection limits were in the range 0.2 - 2.0 ng of metal.

3.2.2 Proposed Use of Ion Pairing for Copper Preconcentration.

The literature indicates the use of ion pairing techniques as an attractive means of preconcentration, however, many of the methods described involved elaborate complexation procedures for ion pair formation. Ion associate approaches involving metal complexes and

ligands necessitaed the use of ion pair systems with high solvating ability. Increased hydrophobicity and/or concentration of the counter ion [22] was observed to increase retention times and peak capacities. One of the most obvious disadvantages of the on-line complex-formation methodology was the slow formation of ion complexes during the elution process, and their correspondingly slow transferal to azeotropic eluent mixtures.

The approach adopted by Irth et al. [28] was very attractive for metal ion preconcentration as it allowed introduction of Cu(II) ions on to the precolumn, and very high preconcentration factors were achievable. The use of organic alkyl ammonium salts was again found to be beneficial in the switching system described by Irth.

In the bulk of this chapter, preliminary findings on the development of an on-line preconcentration technique involving the use of the CTAB-DTC ion pair for metal ion preconcentration are reported. The ion pair was formed on-line using a column switching system, and metal ions could be loaded immediately after ion pair formation. Preconcentration cartridges were prepared reproducibly, loaded with a sample in the field and brought back to the laboratory for analysis. Metal determination was achieved by RPLC and applicable to UV/VIS and/or AAS detection methods. An initial off-line study was performed to determine the recoverability of the loaded metal samples. Optimum loading volume and concentrations were also examined. Following off-line optimisation of the loading parameters the precolumn was switched on-line and the factors effecting on-line preconcentration were assessed. The effects of loading volume, loading concentration, wash time and pH on the preconcentration process were all examined. Metal recovery was greater than 95 % in all cases and Cu(II) and Fe(III) were effectively preconcentrated in this preliminary study.

3.3 Determination of Cu(II) using a CTAB /DTC Ion Pair Derivatised Precolumn

3.3.1 EXPERIMENTAL

3.3.1.1 Reagents

All of the chemicals used were of analytical-reagent grade. Sodium *N, N* - diethyl dithiocarbamate (Na (DTC)) and cetyltrimethyl ammonium bromide (CTAB, cetrimide) were obtained from BDH chemicals Ltd. (Poole, England). HPLC grade methanol was supplied by Labscan Ltd. (Stillorgan, Dublin). Water used in the preparation of aqueous solutions was deionised using a Millipore water purification system. All metal solutions were prepared by dilution of the 1000 ppm standard solutions (Carlo Erba, atomic absorption grade).

3.3.1.2 Instrumentation

The LC system consisted of two Waters HPLC pumps (Model 501) linked to a Shimadzu SPD-6A UV spectrophotometric detector and a Linseis L650 chart recorder. The on-line preconcentration system incorporated a Rheodyne model 7000, six port, two-way switching valve. The analytical column LC $_{18}$ DB, (250 x 4.6 mm i.d., 5 µm) was supplied by Supelco. The 10 x 2.1 mm i.d. stainless-steel precolumn was handpacked with Lichrosorb 10 C_{18} packing material. Copper presence in the off-line preconcentration eluent was detected by AAS, model IL451 AA/AE spectrophotometer (Instrumentation Laboratory Inc). Copper was determined in the on-line preconcentration system using the Shimadzu SPD-6A variable wavelength detector.

3.3.1.3 Methods

3.3.1.3.1 Preparation of CTAB and DTC reagents

A 0.02 M CTAB solution was prepared by dissolving 3.6446 g of the surfactant material in water. The solution pH was buffered to pH 5.5 by the addition of 0.01 M acetate buffer. A 0.02 M solution of Na (DTC) was prepared by dissolving 4.5062 g of the salt in 1000 dm^3. The solution pH was also adjusted to 5.5 by the addition of acetate buffer.

3.3.1.3.2 Overview of Cu(II) Preconcentration

0.02 M CTAB solution was pumped through the preconcentration column for a fixed time period. The long chain aliphatic moiety of this surfactant was expected to interact strongly with the reversed-phase precolumn packing resulting in CTAB retention on the precolumn. The precolumn was then washed with water to remove any unbound CTAB which could interfere in the DTC binding process. A 0.02 M DTC solution was then pumped onto the precolumn for a fixed time period. The precolumn was washed with water to remove any unbound DTC. DTC was retained on the precolumn in the form of an apolar ion pair with the column bound CTAB.

A small volume of copper standard was then pumped through the precolumn, again followed by a washing step, this step to remove excess/unbound copper or other interferents. DTC forms a non polar complex with the copper and therefore retains it on the precolumn. Desorption of the copper from the precolumn was effected using 100 % methanolic eluent (made 0.025 M in DTC). Elution of the copper as a Cu(II)-DTC complex was observed (confirmed by UV spectrum of a

standard solution of Cu(II)-DTC in methanol λ = 430 nm). Copper presence in the aqueous effluent obtained during copper loading on the precolumn was monitored to examine the retention efficiency of the CTAB-DTC packing material for copper. Assuming 100 % retention of copper had occured, no copper should have been present in the effluent. Percentage recovery of copper was determined by AAS analysis of the eluents. Following Cu(II)-DTC complex elution, the precolumn was washed with 100 % methanol and then water, to remove residual CTAB, DTC or copper complex remaining on the precolumn. The precolumn was then ready for loading of the CTAB and DTC for the next sample analysis.

Ideally the CTAB-DTC derivatised precolumn should be reusable and not require renewal of the CTAB-DTC ion pair after each sample preconcentration, however, decreased retention of Cu(II) was observed for each additional sample that was preconcentrated; therefore the precolumn was loaded with fresh CTAB-DTC for each new sample. It is quite probable that elution with methanol elutes both the complex and the reagents. Approximately 100 % retention and elution values were obtained for the copper sample so a brief preconcentration study was performed which indicated copper could be concentrated effectively from solution on the small precolumn.

Efficacy of the off-line uptake/recovery of copper prompted the incorporation of the CTAB-DTC loaded precolumn into a column switching system where on-line copper preconcentration followed by reversed-phase liquid chromatography (RPLC) separation from concomitant elements within the sample was possible. A schematic of the switching system is shown in Figure 3.1. Preconcentration was achieved via column backflushing (described in chapter 2).

During the conditioning and copper loading stages of the precolumn, the analytical column was disconnected from the switching

valve (this is only recommended for short time periods to protect the analytical column life). The analytical column was connected back on-line before Cu(II)-DTC complex elution from the precolumn. Valve actuation to position 2 switched the precolumn on-line with the analytical column. The analytical column was also disconnected during precolumn regeneration. Table 3.1 indicates the switching sequence for preconcentration of a single sample.

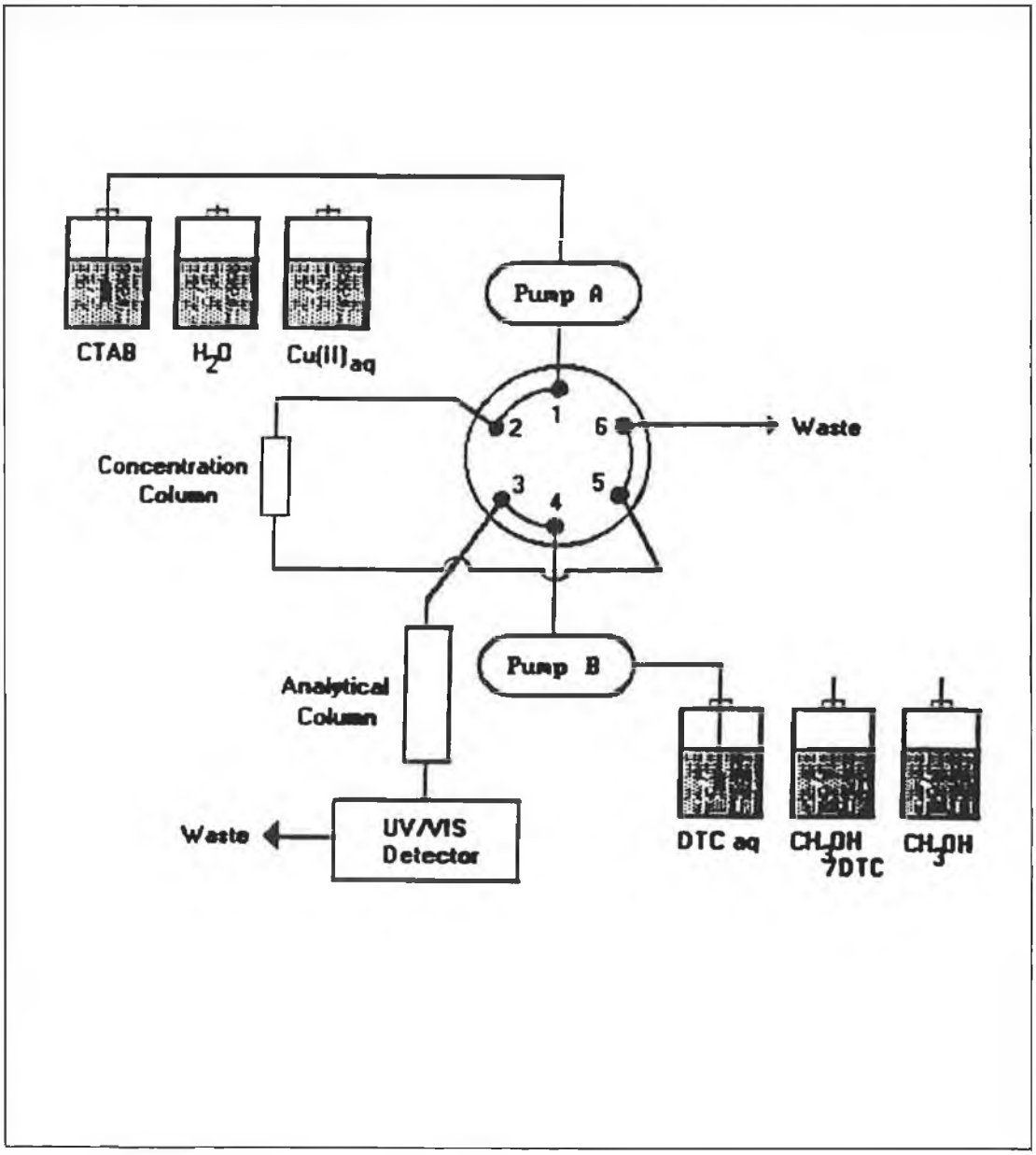

Figure 3.1 Column Switching System

Valve Position	Pump A	Pump B
2	Solvent lines filled with CTAB (0.02 M buffered solution	--
1	Conditioning of precolumn with CTAB solution	Solvent lines filled with (0.02 M) DTC_{aq} solution
2	Solvent lines filled with water	Precolumn conditioned with (0.02 M) DTC_{aq}
1	Precolumn washed with water	Solvent lines filled with (0.025 DTC_{meth}
2	Solvent lines filled with $Cu(II)_{aq}$ sample	--
1	Sample pumped through precolumn for fixed time period	Analytical column connected to port 3 of the switching valve and solvent flow initiated
2	--	Cu(II)/DTC complex eluted onto analytical column from precolumn
1	--	Analytical column disconnected from port 3 and solvent lines filled with methanol
2	Solvent lines filled with water	Methanol pumped through precolumn
1	Water pumped through precolumn to remove methanol residues	--

Table 3.1 Column Switching Sequence.

During the preliminary off-line preconcentration study copper solutions were loaded for a maximum of 5 minutes at a flow rate of 1 cm^3 min^{-1}. Both CTAB and DTC loading times were optimised to determine the optimum ion pair combination which facilitated maximum copper uptake. The wash times necessary to remove unbound CTAB or DTC were also optimised. A complete analysis run time of 30 minutes was necessary for initial experiments, though this was considerably reduced when all conditions had been optimised.

3.4 RESULTS AND DISCUSSION

3.4.1 Off-line Preconcentration

3.4.1.1 Optimisation DTC Loading Time

The loading time for DTC_{aq} (0.02 M) solution on the precolumn was varied from 30 - 0.5 minutes while all other parameters were kept constant. The CTAB loading time was 30 min at a flow rate of 1 cm^3 min^{-1} and the wash time following CTAB and DTC loading was set at 15 min, again at a flow rate of 1 cm^3 min^{-1}. A standard Cu(II) solution (3 ppm) was used to assess the effect of DTC loading time. Copper was loaded on to the precolumn for 5 min at 1 cm^3 min^{-1}. The resultant 5 cm^3 aqueous effluent was analysed to determine the efficacy of copper retention on the precolumn. The Cu(II)-DTC complex was desorbed from the column with 5 cm^3 of 0.025 M DTC_{meth} eluent. Assuming 100 % retention and recovery of copper, the collected eluent was expected to contain 3 ppm Cu(II).

Copper concentration in the eluent was determined from a copper standard curve. Table 3.2 shows the effect of DTC loading time on copper presence in the aqueous effluent, with overall % recovery of copper indicated by the eluent concentration of copper. Copper retention was not affected by a reduction in the DTC loading time, even at a minimum loading time of 0.5 minutes. Replicate copper samples were loaded onto the precolumn for various DTC loading times; irrespective of DTC loading time greater than 93 % copper could be recovered.

Little variation in the percentage recovery as a result of DTC loading time was observed therefore a short loading time of 1 minute was selected for our initial experiments. Variations in the % recovery of copper

were found to be random in nature, each % recovery result was an average of 5 replicate sample loadings. The % recovery obtained for a DTC loading time of 15 minutes was 92 %, quite low in comparison to the other results; again, this result was an average of five readings, as further reduction in loading time did not result in decreased % recovery of copper, the result was not considered significant.

DTC loading time (min)	$Effluent_{aq}$ [Cu(II)] (ppm)	R.S.D.	$Eluent_{meth}$ [Cu(II)] (ppm)	R.S.D	% recovery Cu(II)
30.0	< 0.1	9.74	2.9	6.81	98.4
15.0	< 0.1	12.13	2.7	3.36	92.9
7.5	< 0.1	10.45	2.9	4.83	99.2
5.0	< 0.1	11.64	2.9	4.40	98.9
4.0	< 0.1	12.75	3.0	5.40	102.5
3.0	< 0.1	13.24	2.5	6.65	96.6
2.0	< 0.1	6.13	3.0	7.250	100.4
1.0	< 0.1	5.40	2.0	4.97	98.0
0.5	< 0.1	14.08	3.1	6.17	105.5

Table 3.2 % recovery of Cu(II) in relation to DTC loading time.

3.4.1.2 Optimisation of Wash Time

The effect of varying the precolumn wash time following CTAB and/or DTC loading was assessed. Wash times were varied from 15 to 0.5 min. CTAB was loaded for a 30 min time period at a flow rate of 1 cm^3 min^{-1} and DTC was loaded for 1 min, again at a flow rate of 1 cm^3 min^{-1}. Copper samples were loaded onto the precolumn and the aqueous effluent and methanolic eluents were examined for Cu-DTC presence. The copper complex was desorbed in 5 cm^3 0.025 M DTC_{meth} eluent as before. It was obvious that shorter wash times did not effect copper retention or desorption down to the limiting value examined of 0.5 min, see Table 3.3

Wash time (min)	$Effluent_{aq}$ [Cu(II)] (ppm)	R.S.D.	$Eluent_{meth}$ [Cu(II)] (ppm)	R.S.D	% recovery Cu(II)
15.0	< 0.1	0.01	3.1	2.17	103.6
7.5	< 0.1	0.01	2.9	1.05	97.9
5.0	< 0.1	7.43	3.3	1.97	101.2
2.5	< 0.1	9.42	3.0	0.59	101.5
1.0	< 0.1	6.14	3.2	1.29	107.5
0.5	< 0.1	10.57	2.9	0.91	96.7

Table 3.3 Wash time effects

The percentage recovery of copper was greater than 96 % in all cases. It appears that both unbound CTAB and DTC can be readily washed from the column in small volumes of water. If excess DTC remained unbound on the precolumn copper loss would have occured,

with a corresponding decrease in % recovery as some of the copper would have complexed directly with the unbound DTC and eluted during the copper loading stage.

3.4.1.3 Optimisation of CTAB Loading Time

The effect of CTAB loading time on the recovery of Cu(II) was examined using the optimised DTC and wash times. The loading time was varied in the range 30 to 0.5 minutes. No significant effect of the loading time duration was observed on copper retention or desorption, a loading time of 1 minute was chosen for further studies. Table 3.4 outlines the results of the various loading times.

CTAB loading time (min)	$Effluent_{aq}$ [Cu(II)] (ppm)	R.S.D.	$Eluent_{meth}$ [Cu(II)] (ppm)	R.S.D	% recovery Cu(II)
30.0	< 0.1	7.74	2.9	4.34	98.5
15.0	< 0.1	5.39	2.9	3.31	98.0
7.5	< 0.1	3.95	2.9	4.26	95.7
5.0	< 0.1	16.20	3.1	5.80	103.5
4.0	< 0.1	2.51	2.8	3.10	94.1
3.0	< 0.1	8.10	2.9	3.17	98.0
2.0	< 0.1	5.35	3.0	4.74	101.2
1.0	< 0.1	7.49	3.0	2.93	101.9
0.5	< 0.1	5.93	3.0	3.81	100.7

Table 3.4 % recovery of Cu(II) in relation to CTAB loading time.

Following optimisation of the loading time for CTAB, the DTC loading time was rechecked as a precaution to ensure 1 minute was the optimum value. Again both aqueous and methanolic effluents were examined for copper content. No significant copper loss was observed and % recovery was > 95 %.

3.4.1.4 Off-line Concentration Effect

A preliminary examination of the preconcentration ability of the packing material was assessed by loading copper (3 ppm) onto the precolumn for 5 minutes at a flow rate of 1 cm^3 min^{-1} and eluting in decreasing volume aliquots. Copper was desorbed using the DTC-methanolic eluent previously optimised. Obviously the smaller the elution volume relative to the initial loading volume the greater the preconcentration factor attainable. The results of this preconcentration study are shown in figure 3.2 overleaf. Elution volumes smaller than 2.5 cm^3 did not result in the expected preconcentration factor which indicated that for the flow rate or the eluent composition used, complete elution of the retained copper was not possible in less than 2.5 cm^3. Therefore to achieve preconcentration, volumes of sample greater than 2.5 cm^3 should be loaded onto the precolumn.

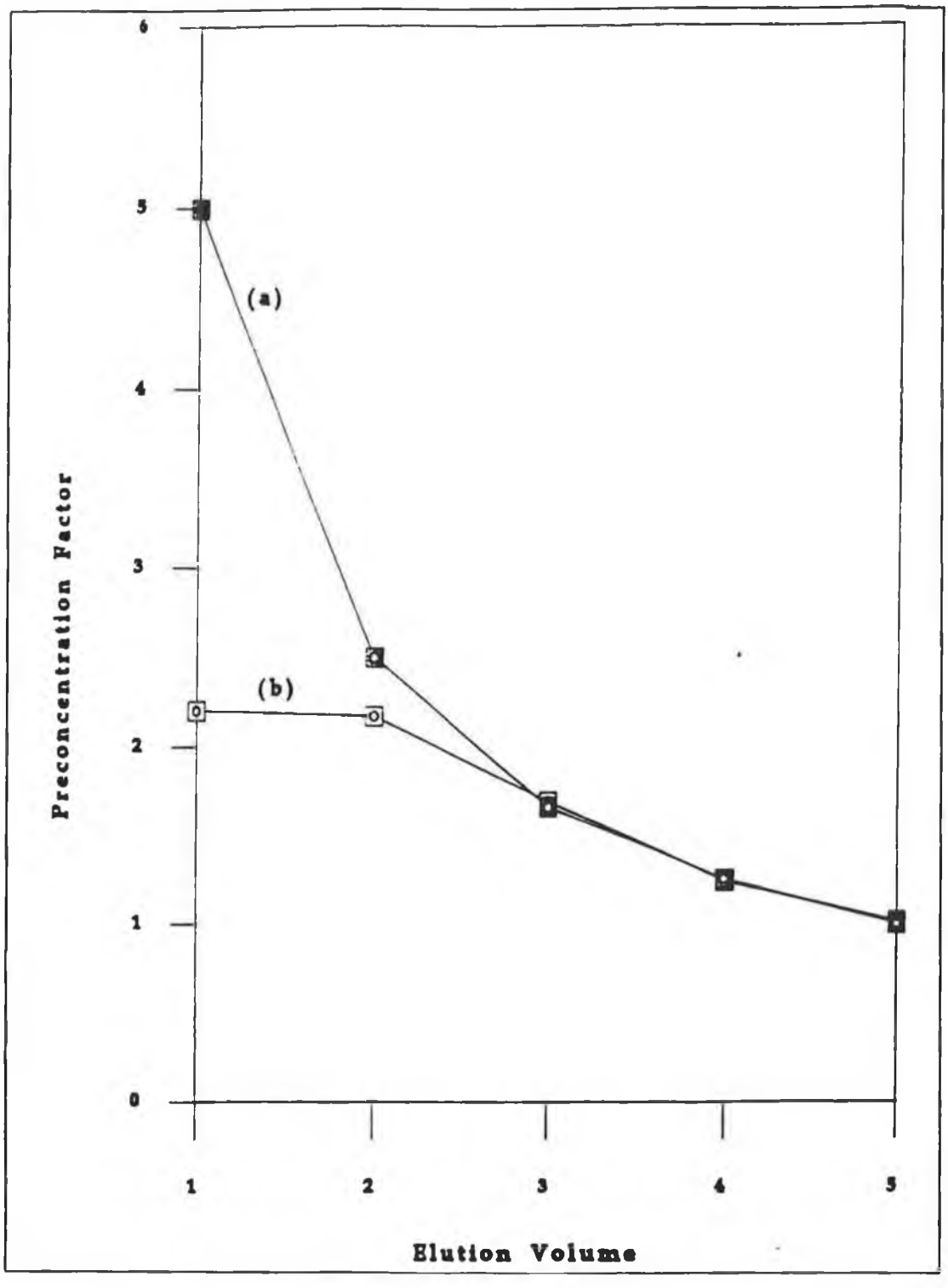

Figure 3.2 Comparison of theoretical (a) and experimental (b) preconcentration factors for an experimental loading concentration of 3 ppm. Loading flow rate 1 cm^3 min-1, loading volume 5 cm^3.

3.4.1.5 Effect of Eluent Composition on Copper Recovery

Preliminary results indicated that maximum copper recovery was possible using a 100 % methanolic eluent (made 0.025 M in DTC); approximately 100 % recovery was possible. However as the main objective of this work was the development of an on-line preconcentration system, the precolumn eluent should ideally be capable of metal complex separation on the analytical column (unless a gradient elution system was to be used). The elution strength of a more polar eluent was therefore assessed, by decreasing the methanol content, and increasing the aqueous content of the original eluent. A copper solution (3 ppm) was loaded on to the precolumn for 5 minutes at 1 cm^3 min^{-1}. Copper desorption was effected using 5 cm^3 of the modified eluents; following elution with these eluents the column was eluted with 100 % methanol (made 0.025 M in DTC) to desorb any copper not eluted by the eluent under examination. An eluent composition containing a methanol content of 85 % or higher was found necessary if copper was to be recovered in any sizeable amount. The effects of the eluent composition on copper desorption can be seen in Figure 3.3.

Copper that was inaffectually desorbed using the more polar eluents was successfully desorbed using the 100 % methanolic eluent. A slight increase in copper recovery could be achieved using the more polar eluents, simply by reducing the copper loading concentration; 70 % copper (loading concentration 800 ppb) recovery was possible using methanol / water, 75 / 25 as opposed to 33 % recovery when a sample with a higher copper concentration (3 ppm) was loaded.

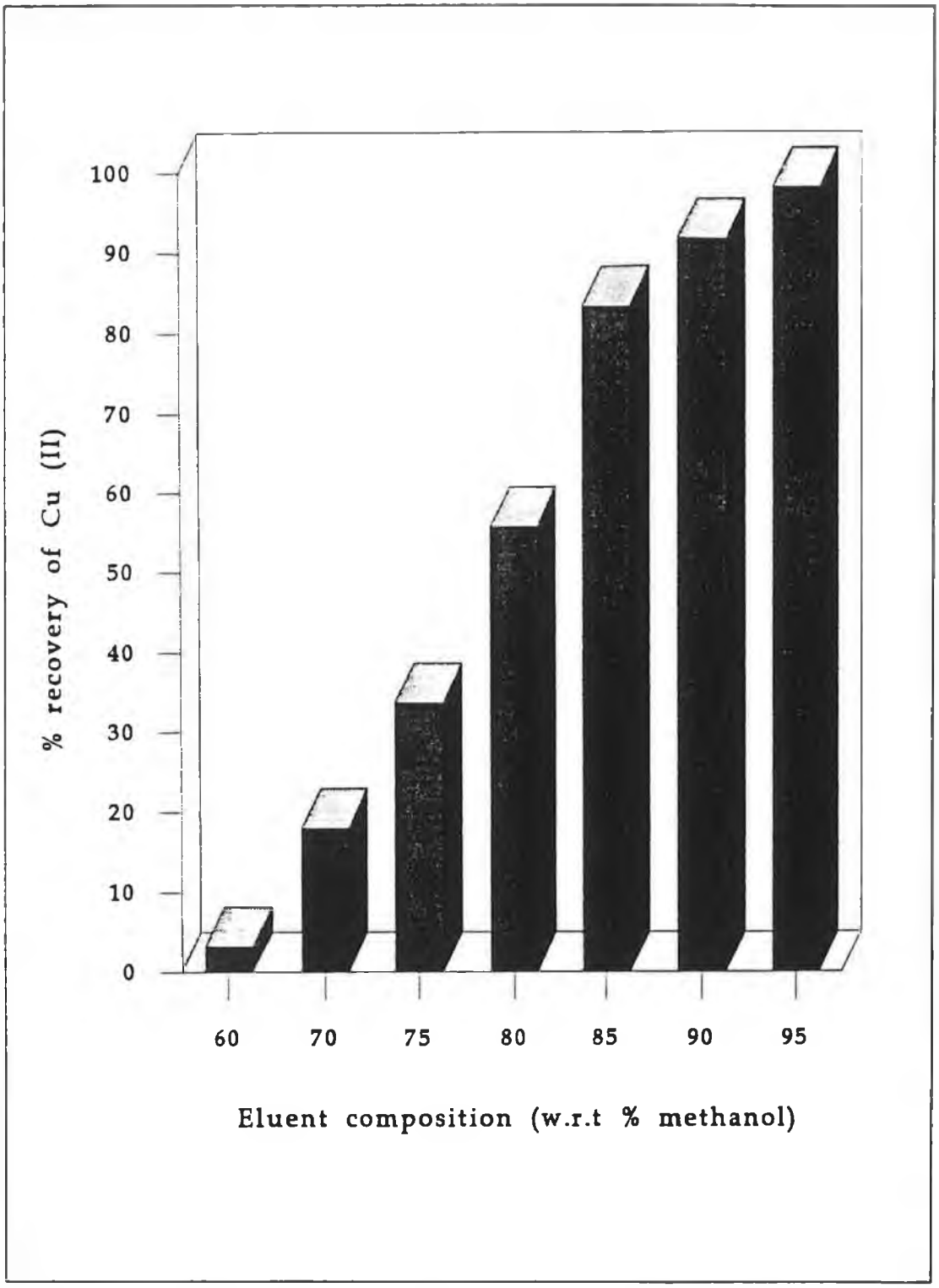

Figure 3.3 Effect of eluent composition on copper desorption. [Cu (II)] = 3ppm, elution and loading volumes 5 cm^3.

The elution profiles shown in figure 3.4 overleaf indicate the 'effectiveness' of the polar eluents for copper recovery in comparison to the

100 % methanolic eluent. The elution profile for the 75 / 25 eluent shows incomplete copper recovery however provided that the recovery is reproducible this eluent could be used as it should separate effectively metal complexes on the analytical column.

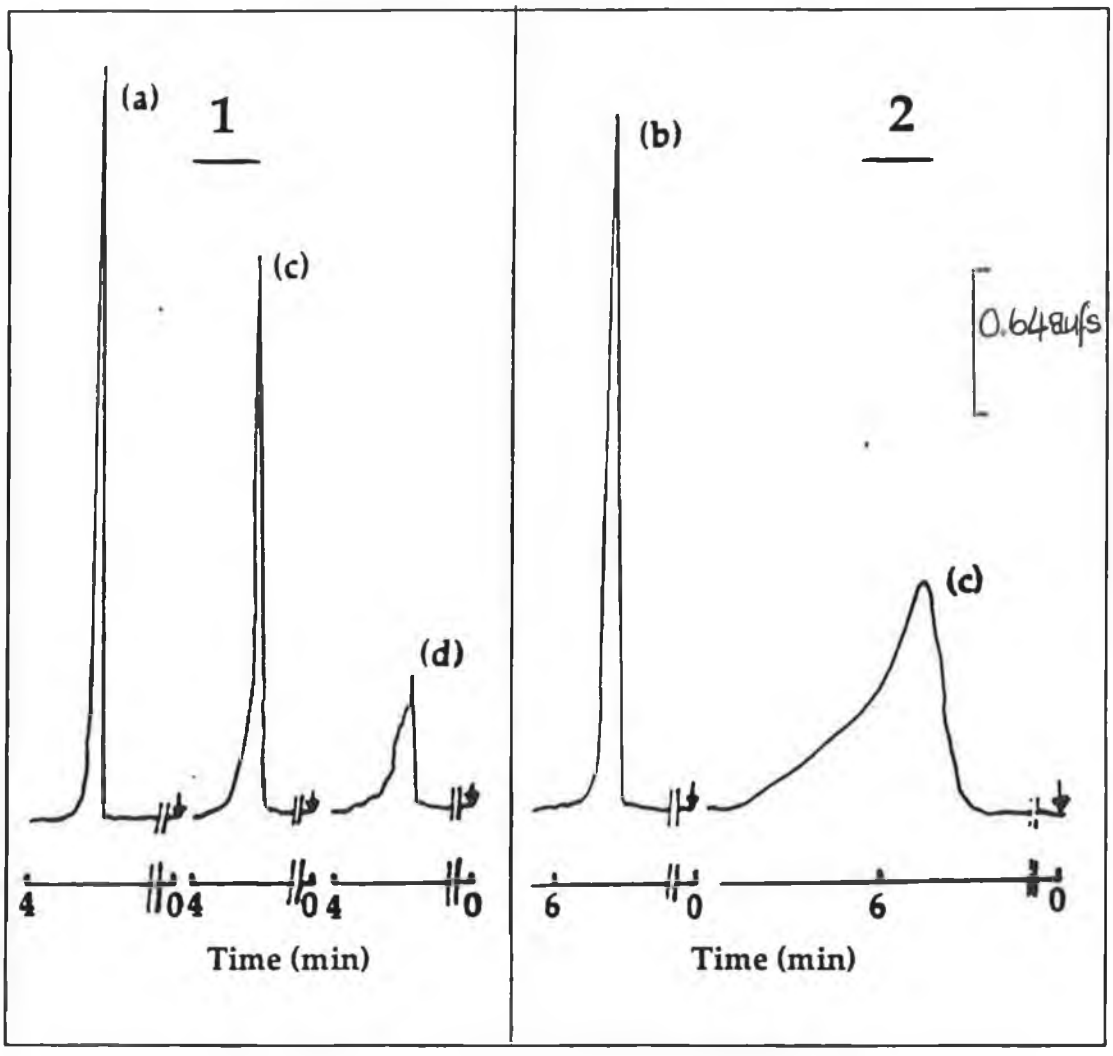

Figure 3.4 **Comparison of (1) off-line and (2) on-line effectiveness of methanolic eluents for copper desorption for a copper loading concentration of 3 ppm. Elution rate 1 cm^3 min^{-1}. (a) 100 % methanol*, (b) 90/10 methanol/ water*, (c) 75/25 methanol/water* and (d) 60/40 methanol/ water*. (*indicates that all eluents were 0.025 M in DTC).**

3.4.2 On-line Preconcentration

The development of an on-line preconcentration system was assessed by incorporating the CTAB-DTC derivatised precolumn on-line with the analytical column. The optimised preconcentration conditions determined during off-line analysis were used in initial on-line studies. Several eluents were tested for copper desorption from the precolumn on to the analytical column, however unlike the off-line system only eluents with greater than 90 % methanolic content were found to effectively elute the copper complex. The copper elution profile using methanol/water, 90/10 (made 0.025 M in DTC) eluent, resulted in a sharp peak, retention time 5.4 minutes. See Figure 3.4, (2), (b). The reproducibility of copper elution using this eluent was greater than 99 % ([Cu(II)] = 40 ppb, n = 10).

3.4.2.1 Stability of CTAB/DTC Derivatised Precolumn

The stability of the derivatised precolumn was assessed by flushing the column with different volume aliquots of water. No breakthrough of the CTAB/DTC ion pair was observed in the range 5 - 500 cm^3 of solution; indicating a possible loading volume of at least 500 cm^3 of aqueous sample containing copper without sample breakthrough, see Figure 3.5 for the stability of the derivatised precolumn for loading volumes of 5-50 cm^3. All copper samples that were examined were in the pH range 4 - 4.5, any pH adjustments necessary were made by the addition of a small volume of nitric acid, and no sample buffering was found to be necessary for these samples.

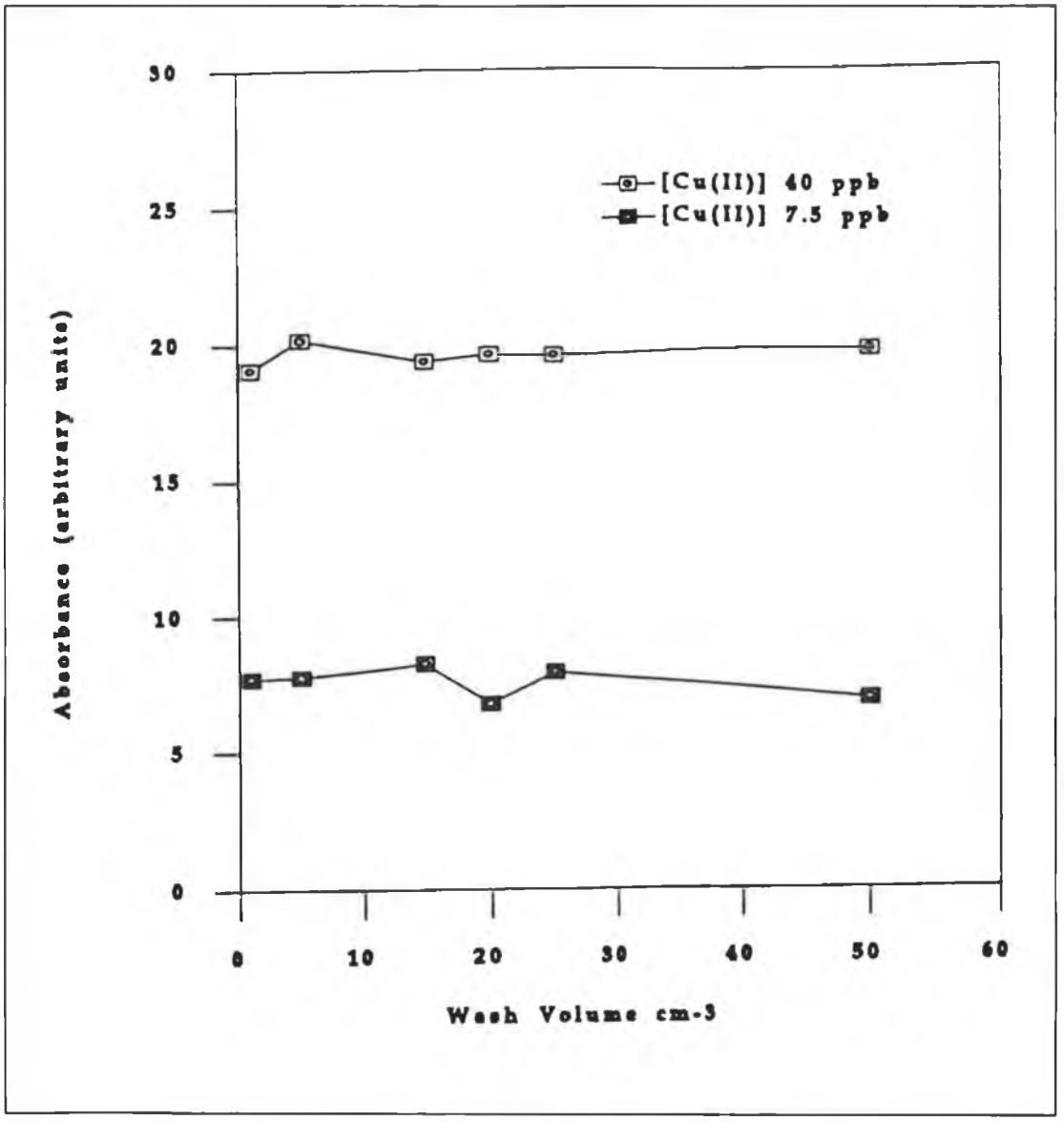

Figure 3.5 Stability of the CTAB/DTC ion pair derivatised precolumn.

The ability to preconcentrate multi-metal samples on the precolumn was also investigated; as many labile metals require a buffered environment the effect of buffers on the precolumn was assessed. The precolumn was flushed with acetate (0.5 - 0.01 M) and phosphate (0.1 - 0.01 M) buffers in the pH range 3 - 8 with no apparent breakthrough being observed over the volume range 5 - 50 cm^3.

3.4.2.2 Preconcentration / Loadability Potential of the Packing Material

The preconcentration ability of the CTAB-DTC packing was assessed by loading copper samples of increasing volume onto the precolumn. A linear increase in preconcentration with loading volume was observed for copper samples, (10 ppb Cu(II)) in the volume range 1 - 10 cm^3. For copper samples in the mid concentration range (100 ppb Cu(II)) a linear increase was observed up to 5 cm^3 loading, for higher loading volumes, a slight tailing of the response was noted. Copper samples in the concentration range 1 ppm or greater, showed an initial increase in preconcentration with loading volume but this preconcentration ability decreased dramatically as the loading volume was increased further. See figure 3.6 for comparison of the preconcentration curves obtained for the 10 ppb and 100 ppb Cu(II) samples.

Copper samples (5 cm^3 loading volume) were affectively preconcentrated in the range 10 ppb to 1 ppm, however samples in the lower concentration range showed a better linear response w.r.t loading volume, in comparison with samples in the higher concentration bracket. Regression values obtained indicated a value of r= 0.99989 (n=7) for 10 ppb Cu(II) and r=0.99791 (n=7) for samples in the concentration range 100 ppb to 1 ppm Cu(II). The limit of detection (3 S/N ratio) was determined to be 7.5 ppb of Cu(II) for a 5 cm^3 loading volume. By increasing the loading volume for samples in the low concentration range the limit of detection could be extended further, possibly as low as 60 ppt for a loading volume of 70 cm^3.

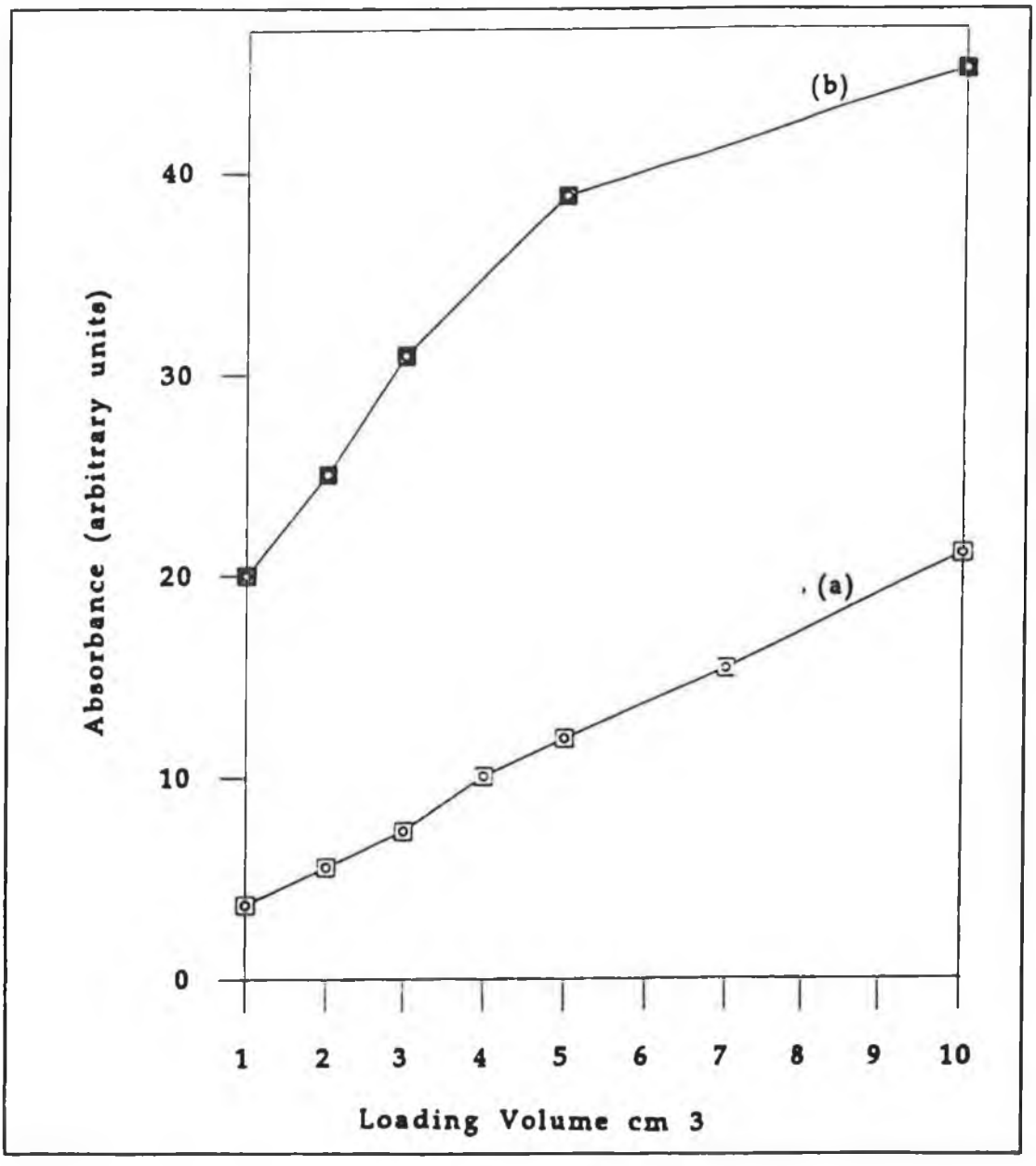

Figure 3.6 Relationship between copper loading volume and preconcentration ability. Two different copper concentrations were preconcentrated (a) 10 ppb and (b) 100 ppb. Loading flow rate was 1 cm^3 min^{-1}, eluent composition was 90/10 methanol/water (made 0.025 M in DTC) and elution volume was 5 cm^3.

3.4.2.3 Interference from Other Metals

Preconcentration of other metals on the derivatised precolumn was also investigated. Only copper, iron and nickel were effectively preconcentrated and subsequently separated on the analytical column. It was observed that for samples containing all three metals, that only copper and nickel could be determined simultaneously or copper and iron, but not all three metals in the same chromatographic run. The retention time of copper increased slightly when loaded from a multi metal sample; this may be as a result of competition between metals for complexation with the DTC in the eluent. In an attempt to overcome this increased retention time effect, the concentration of DTC in the eluent was increased to 0.04 M. The retention time of copper improved slightly from 6.2 min to 5.9 min but it was observed that a new peak appeared at a retention time of 2 minutes. This peak may be due to the CTAB-DTC ion pair being stripped from the precolumn as a direct result of increased DTC concentration in the eluent, see figure 3.7 overleaf.

Various concentrations of Cu(II) were loaded in the presence of either Ni(II) or Fe(III) onto the precolumn. The loading solution pH was maintained at pH 4. A 200 fold excess of nickel relative to copper was not found to decrease copper preconcentration even at copper concentrations as low as 30 ppb. A 400 fold excess of Fe(III) relative to Cu(II) (again 30 ppb) was not found to interfere with copper preconcentration. These results would seem to indicate the high selectivity of this method for Cu(II). Loading concentrations of Fe(III) > 15 ppm on the precolumn resulted in precolumn clogging and obvious difficulty in elution of retained metals. The possibility that excess acetate remaining on the precolumn following the ion pair formation step could be causing this clogging problem was addressed (iron tends to form iron acetates at high

iron concentrations). Use of phosphate as opposed to acetate buffer in the initial ion pair loading stage was not found to alleviate the problem. An initial AAS analysis of samples is recommended to determine iron levels, if suspected of being present at very high concentrations.

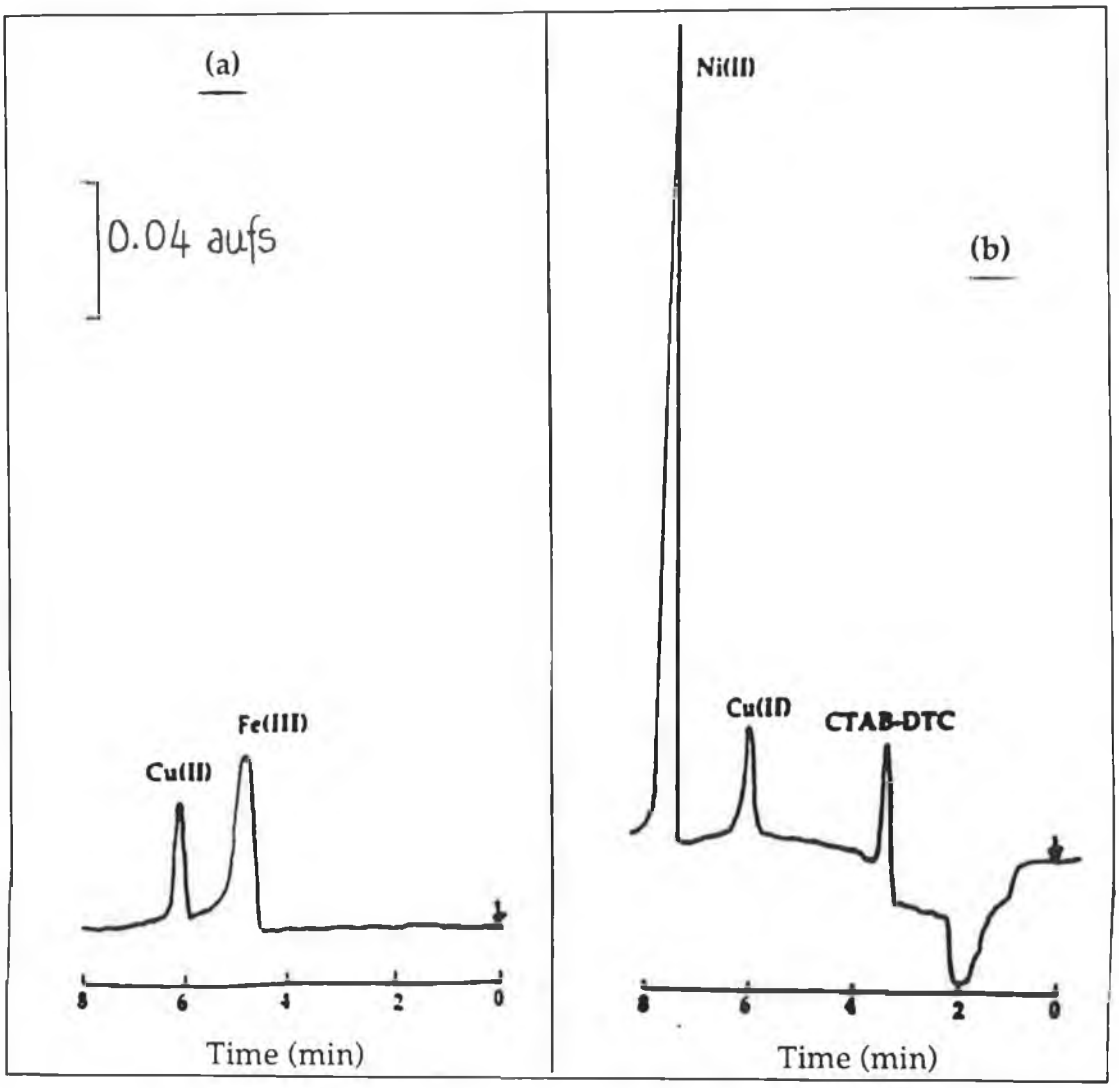

Figure 3.7 (a) Separation of Cu (II) and Fe(III), Loading [Cu(II)] = 10 ppb, [Fe(III)] = 250 ppb; loading volume 5 cm^3 and flow rate 1 $cm^3\ min^{-1}$. Eluent 90/10 methanol/water (made 0.025M in DTC). (b) Separation of Cu(II) and Ni (II), loading [Cu(II)] = 10 ppb, [Ni (II)] = 900 ppb, loading volume 5 cm^3, flow rate 1 $cm^3\ min^{-1}$. Eluent 90/10 methanol/ water (made 0.04 M in DTC)

3.4.2.4 Application to River Water Analysis

Samples taken from the Tolka River were filtered on site to remove any large particulate material. Various sample volumes were then loaded on to the CTAB-DTC precolumns using syringes (volume range 1 - 500 cm^3). Copper levels were determined directly from calibration curves and compared with standard addition results for spiked samples, see Figure 3.8. Copper was found to be present at a level of 115 ppb, this result was confirmed using AAS off-line analysis of the precolumns loaded with the same sample solutions. Although a peak (very poor shape) appeared at the retention time for iron, the amount of iron present in the sample was very difficult to quantify. Spiking the sample with Fe(III) did result in a much sharper peak though the resultant chromatograms were not completely reproducible.

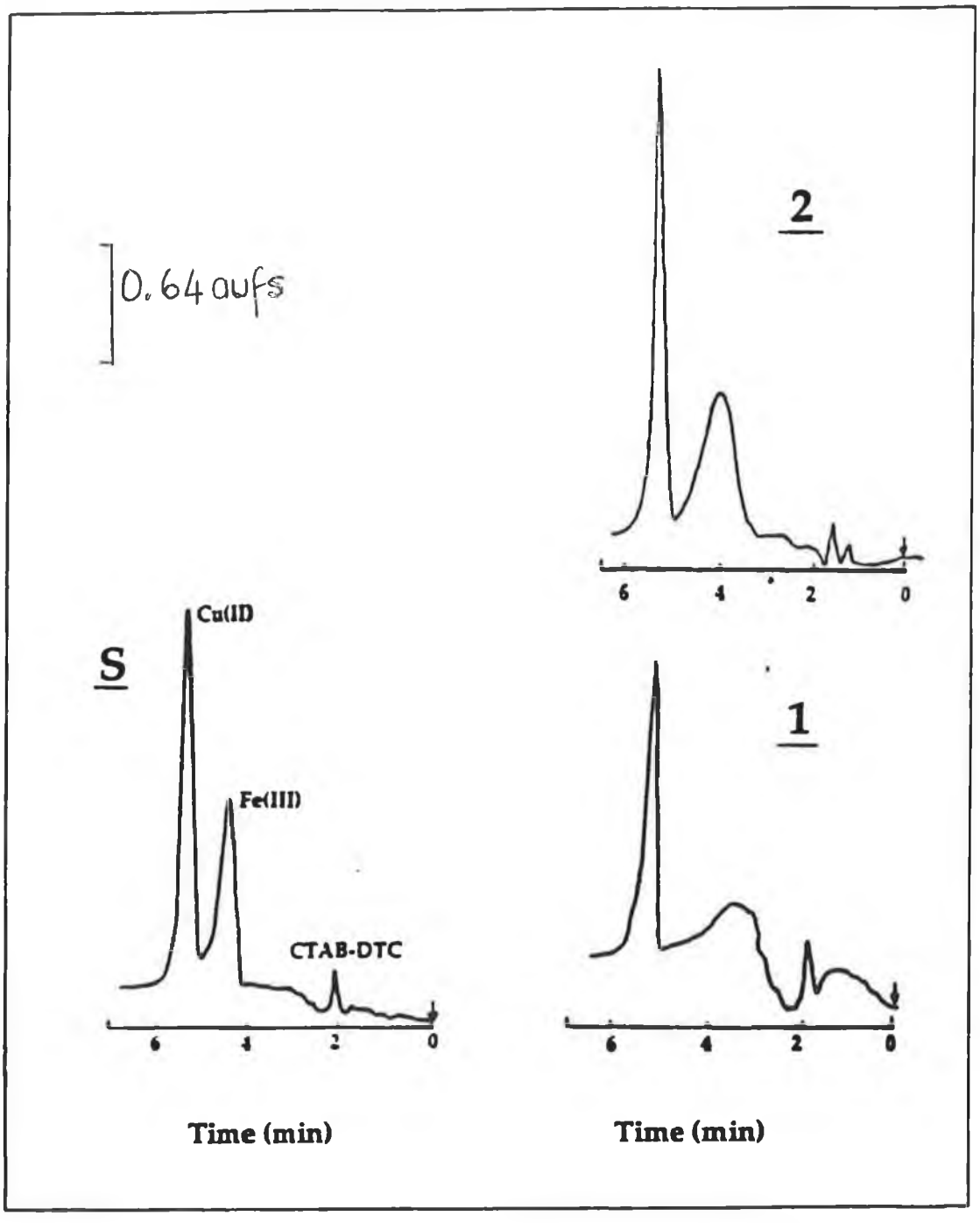

Figure 3.8 Determination of copper in a river water sample. (S) injection of a standard solution of copper and iron, [Cu (II)] = 140 ppb and [Fe (III)] = 600 ppb. Loading volume 5 cm^3, flow rate 1 cm^3 min^{-1}, eluent 90/10 methanol/water (made 0.025M in DTC). (1) Unspiked river water sample. (2) river water sample spiked with 65 ppb Cu (II) and 400 ppb Fe (III).

3.4.3 Conclusions

A column switching system for the selective preconcentration of Cu(II) has been developed which involves the initial on-line formation of a CTAB-DTC ion pair derivatised precolumn. Cu(II) was loaded on the precolumn and affectively retained as its Cu(II)-DTC complex. Quantitative copper desorption from the precolumn onto the analytical column was achieved using methanol / water, 90 / 10 (made 0.025 M in DTC). Copper was separated from other preconcentrated metals including Fe(III) and Ni(II) using RPLC coupled with UV/VIS detection at 430 nm.

The CTAB-DTC precolumn has been used for on-site sample loading. Direct incorporation of the loaded precolumn back into the switching system was possible which decreased overall sample analysis time considerably. This approach also allowed for direct sampling onto a solid phase, therefore, no liquid samples needed to be returned to the laboratory. It is also thought that such an approach would result in very little disturbance of speciation information which is vital in environmental analysis. Precolumn preparation was extremely reproducible with < 3 % variation for a batch size of 50. At present limits of detection for Cu(II) of the order 7.5 ppb have been achieved although reduction to 60 ppt is possible by increasing the sample loading volume.

Analysis of river water for low level copper presence was enabled using this technique. Following a routine filtering step samples could be loaded directly onto the derivatised mini cartridges. The loaded mini cartridges were then brought back to the laboratory where they were incorporated back into the column switching system for on-line analysis.

The benefits of this preconcentration system are obvious, faster sample analysis times and a very selective preconcentration system for copper. The possible use of CTAB-DTC derivatised preconcentration

column for sample cleanup and copper elimination by its incorporation into a HPLC system, before a second preconcentration column, or analytical column, selective for trace metals other than copper is currently under investigation and results look promising.

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Chapter 4

Preconcentration of Cu(II) using Microbial Biomass

4.1 Trace Enrichment of Metal Ions using Sorbent Materials of Biological Origin

4.1.1 Introduction

The increasing need for fast, low cost, selective analytical methods of metal determination at lower and lower levels has prompted the investigation of novel stationary phases in trace metal enrichment systems. Several authors have reported the use of sorbent materials of biological origin, and many forms of biomass have been shown as effective metal adsorbents [1 - 3].

Bioaccumulation of heavy metals has been well known for over 30 years, therefore, it is surprising that the potential of biomass technologies for use as an alternative or adjunct to conventional methods of trace enrichment have only recently been recognised. Microbial biomass has many potential applications and recently attention has focussed on its use in the area of metal 'winning' in metal bearing waste streams; several authors have shown particular interest in the use of fungal biomass for metal winning processes [4, 8, 11].

The use of microbial biomass as a biological sorbent material has been investigated in considerable detail, and the results are outlined in this chapter. Three different types of microbial biomass namely Saccharomyces cerevisiae (a yeast), Aspergillus niger and Rhizopus arrhizus (both are fungal species) have been assessed for their metal uptake ability. Comparison of the metal uptake ability of the microbial biomass packing material with a poly (dithiocarbamate) resin, (poly (ethyleneimine)-poly (methylenepolyphenylene) isocyanate, poly (DTC)) is discussed. Successful on-line preconcentration of trace metal ions with the poly (DTC) chelating resin was previously reported by Hackett and Siggia

[5], therefore, it was thought to be an ideal sorbent to compare with biomass for metal uptake and possible metal preconcentration ability. Factors affecting initial metal ion uptake and subsequent desorption from the packing material were evaluated including: loading pH, packing weight, optimum particle size, eluent composition and interfering ions. Some microorganisms have been observed to selectively bind metal ions [6-8] therefore, possible selective metal uptake by the biomass material was also monitored.

The reasons why sorbents of biological origin should prove an attractive alternative to chemical sorbent use for metal ion preconcentration are numerous, and have therefore been summarised in the following sections. Intrinsic to the preconcentration ability of these sorbents is the biosorption process which governs metal uptake, how it occurs and therefore the factors which can adversely or favourably influence this sorption process. The distinct processess of metal-microbe interaction, as well as the relevant literature which is pertinent to metal uptake using sorbents of biological origin are discussed below.

4.1.2 Why is Microbial Biomass Such An Attractive Sorbent Material ?

4.1.2.1 Biosorption/Processess of metal uptake

The process of metal, metalloid species, compound or particulate removal from solution, by biological material was designated 'biosorption' by Gadd [9]. Bacteria, fungi, algae and yeasts accumulate heavy metals and radionuclides from their external environment, often at levels as high as 25 % of their dry weight [10]. Uptake may be governed by

both adsorptive and metabolism driven processes in the case of living microbes, and adsorptive processes if the microbes are dead.

Several distinctive types of metal-microbe interaction are possible including 1. intracellular accumulation, which involves metal interaction with cell surface ligands, followed by slow transport into the cell (necessity for living cells, therefore, cannot be feasibly used as a precolumn packing material), 2. metal-siderophore interactions, 3. metal binding to specific functional groups in the cell wall and 4. interaction of metal ions with bacterial exopolysaccharides.

Processess 2, 3 and 4 have been the most widely exploited for purposes of metal ion removal and preconcentration from solution. However, metal binding to exopolysaccharides (4) is somewhat restrictive as it relies on the use of bacterial cell cultures or biomass material which may have to be cultured or grown solely for the purpose of metal uptake. Fungal and yeast biomass are often produced as waste products during industrial processes, and would therefore prove extremely economical if they were to be used as column packing materials, as they would not have to be grown specifically for use in a metal uptake process.

4.1.2.1.1 Uptake of Metals by Dead Microbial Cells

Microbial biomass is capable of adsorbing metal ions from aqueous solution even when the cells have been killed [6, 9, 11]; disruption of the cell membrane may actually result in enhanced metal uptake due to exposure of intracellular metal binding sites. If biomass is to be used as a sorbent for metal ion preconcentration on a commercial scale, it should be easy to handle and pack evenly into a preconcentration column or cartridge. Heat killing of the microbial culture followed by a freeze drying process would be the most likely treatment procedure required to obtain

biomass in a suitable physical state for precolumn packing, therefore, the fact that the metal binding sites remain intact after microbial death is an extremely important factor.

4.1.2.2 Economical Viability of Microbial Biomass

Biomass is inexpensively and easily produced by microorganisms [6, 9, 11, 13] and exhibits considerable metal ion uptake ability, therefore, it is an attractive alternative to chemical sorbent use for metal ion preconcentration. The development of a disposable preconcentration cartridge, containing fungal biomass for metal ion enrichment is extremely attractive from an economic viewpoint as it can be obtained at a minimal cost to be user. Waste fungal biomass can arise in quantity from a number of different industrial fermentations, waste biomass is produced in large quantities by the citric acid industry and economic uses should be sought [11]. Rhizopus arrhizus biomass material produced as a fermentation waste product has been used successfully by Tzesos et al. [12] for the recovery of uranium from waste streams. Macaskie and Dean [13] have reviewed many methods of waste treatment and report that biomass technologies are both economical and competitive with existing treatments, for metal overload in aqueous systems.

4.2 Types of Biological Sorbent Systems in Use

4.2.1 Siderophore-Based Systems

The use of siderophores for their metal uptake, and preconcentration ability, is becoming increasingly popular in analytical methods [14, 19], and both synthetic and naturally occuring siderophores have been investigated for this purpose. Siderophores are microbially generated chelating agents specifically secreted to increase the bioavailability of ferric iron, although they have also been known to bind copper and molybdenum, and some authors report the use of synthetically prepared siderophores for selective metal ion uptake [15]. Siderophores are generally low molecular organic compounds; two main types are known to dominate the iron scavenging process, hydroxamates and catecholates.

Hydroxamic acid siderophores have beeen found to be particularly useful in the area of trace metal analysis as they form stable chelates with a large number of metal ions generally through a bidentate chelate mechanism at the [-C(O)-N(OH)-] group, see figure 4.1. Hider [15] reported that the uptake of ferric iron by hydroxamates was a function of both the size and charge of the ion, and that ions with similar size/charge ratios would also be readily chelated by this ligand group.

Brink and Crumbliss [16] initially investigated the mechanisms, kinetics and thermodynamics involved in the chelation and dissociation of Fe(III) with hydroxamate ligands over a decade ago, and found that the carbon and nitrogen substituents strongly influenced the uptake of ferric iron. More recently [17, 18], siderophores have been synthetically prepared with specific functionalities to influence/increase the metal uptake ability of the ligand.

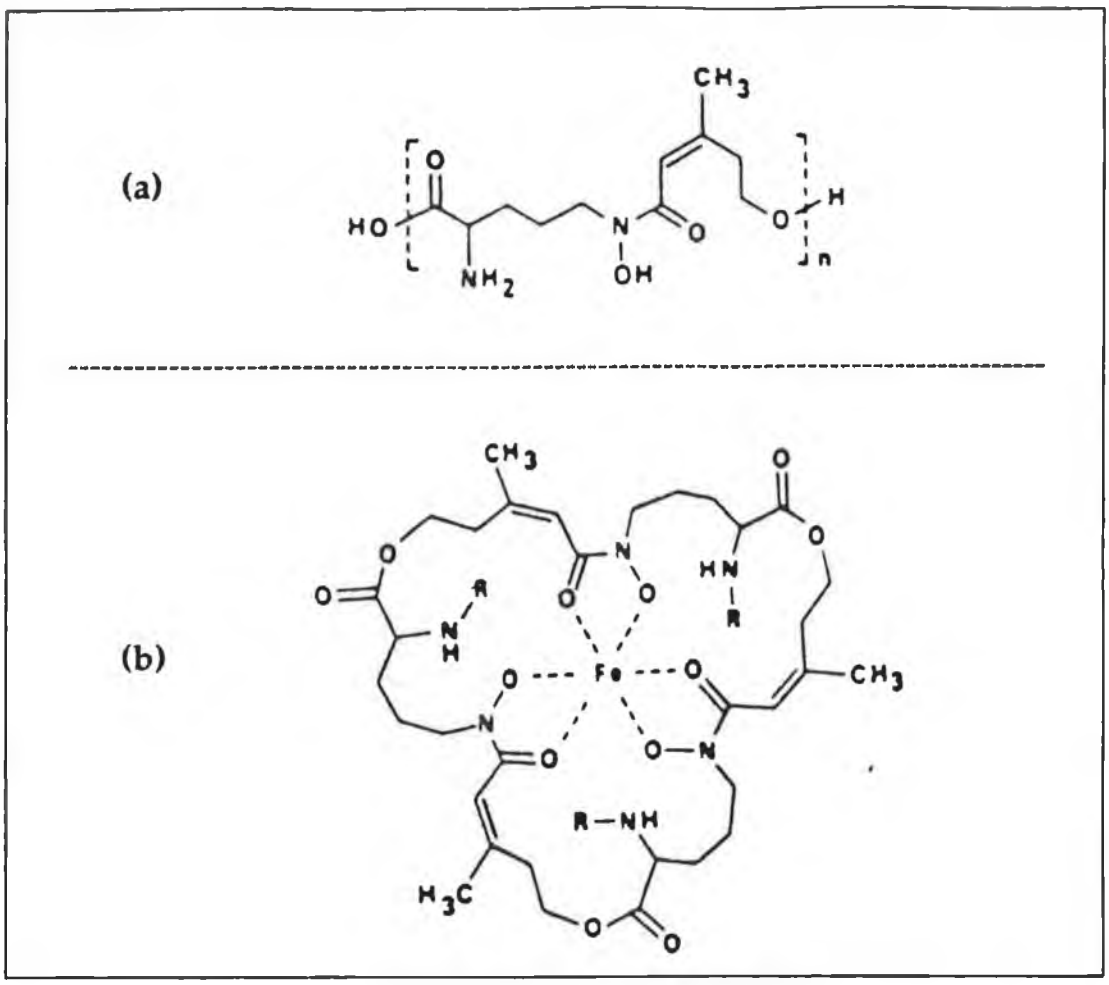

Figure 4.1 **(a) Basic structure of the Fusarinines (fungal siderophores) which contain naturally occuring monohydroxamic acids joined by ester groups. The functional siderophore is believed to be the cyclic trimer Fusarinine C, Figure 4.1 (b).**

Several authors have used hydroxamates in chromatographic methods for the determination of trace metal ions. Palmieri and Fritz [19] reported the determination of metal ions by HPLC separation of their hydroxamate chelates. The metals were complexed off-line with N-methylfurohydroxamic acid (NMFH), and then injected on to a PLRP S or a Zorbax C_8 column in an aqueous acetonitrile mobile phase, containing 1 mM NMFH and hydrogen perchlorate. The metal complexes were detected at 304 nm using UV spectrometry. Optimum complex formation

was observed at low pH (acidic) values, and the method was found particularly selective for Fe(III), as well as some other highly charged cations including Al(III), Zr(IV), Hf(IV), Nb(V) and Sb(III). Limits of detection were in the range 0.5 - 5.8 μM of metal.

Recently, a number of papers have been published on the use of polymeric resins containing hydroxamic acid chelating functionalities for the separation and trace enrichment of metal ions. Early work on these resins was reported by Vernon and Eccles [20-22] who prepared a divinylbenzene (DVB) cross-linked poly (hydroxamic acid) ion exchanger from poly (acrylonitrile) and successfully separated copper and iron from cobalt and nickel. Metal uptake from sea water was also reported and iron and uranium were quantitatively recovered.

In a later paper, Shah and Devi [23] examined the effect of substitution at the nitrogen on the poly (hydroxamic acid) resin. Synthesis involved co-polymerising acrylonitrile with divinylbenzene, and hydrolysis with sulphuric acid at 70 - 80°C; the resultant poly (acrylic acid) was then treated with various hydroxylamines. Both electrophilic and nucleophilic substituents were added at the nitrogen, however, the unsubstituted resin was found to have the highest metal exchange capacity. Lead and copper were successfully separated on all columns but results were more favourable for the unsubstituted resin.

Mendez and Pillai [24] have also investigated the use of substituted hydroxamic acid resins for the preconcentration and separation of metal ions. They prepared an N-phenylhydroxamic acid resin by attaching the N-phenylhydroxamic group to poly (styrene-co-maleic acid) crosslinked with divinylbenzene. The resin was used in the trace enrichment and chromatographic separation of Cu(II) and Fe(III), Ni(II) and Fe(III), Ni(II) and Cu(II) and Mn(II) and Cu(II); the metal ion mixtures were conveniently separated using different elution concentrations of

hydrochloric acid, and were determined by UV/VIS spectrometry or flame atomic absorption spectrometry FAAS.

Several authors have also examined the use of hydroxamates bound to substrate materials, other than polymeric resins, for the preconcentration of metal ions. Glennon et al. [25] described the use of a biochelation cartridge for the solid phase extraction of Cu(II), Ni(II), Co(II), Cd(II), Zn(II), Fe(III), Al(III), Au(III) and V(V) as a function of pH. Four chemically immobilised hydroxamic acid silicas were prepared, unsubstituted hydroxamic acid silica (HA-Si), N-methylhydroxamic acid silica (NMHA-Si), Desferrioxamine silica (DFA-Si) and carboxymethyl silica (CM-Si) and their metal complexation capacities were assessed. Using copper and iron as test ions, HA-Si was observed to have a higher metal complexation capacity than the other hydroxamate silicas. Fe(III), Cu(II) and Zn(II) were successfully preconcentrated from aqueous solution using the biocartridge, Fe(III) could be eluted with 0.08 M EDTA, and Cu(II) and Zn(II) with acidified water (pH 2.0). 5 cm^3 aliquots of the eluent were collected and metal presence was determined using GFAAS and spectrometry.

More recently Glennon et al. [26] have described the on-line preconcentration of trace metal ions using a biochelating silica cartridge, with subsequent metal ion separation using ion chromatography. Both silica and dextran coated silica were prepared with hydroxamic acid functionalities. Following preconcentration on the biocartridge (Tefzel column 5 x 0.4 cm), the metals were separated on a Dionex 4500i ion chromatographic system with a mobile phase of 0.05 M oxalic acid and 0.095 M LiOH. Post column derivatisation of the column eluent with 4-(2-pyridylazo) resorcinol (PAR) enabled spectrophotometric detection of the metal complexes at 520 nm. When the metals were preconcentrated from a multi-metal system, limits of detection as low as 5 and 10 ppb for Cu(II),

Co(II), Zn(II) and Ni(II) were obtained, with slightly higher levels for Pb(II) and Cd(II), greater than 100 ppb.

Trace enrichment of aluminium using desferrioxamine, a naturally occuring siderophore which has a very high binding constant for Al(III) was reported by Ljunggren et al. [27]. Aluminium was quantitatively preconcentrated in a flow injection system on a column containing desferrioxamine immobilised on porous glass. It was necessary to acidify samples with 0.001 M HNO_3 to prevent precipitation of metal hydroxides, though, the samples were neutralised with sodium hydroxide prior to column introduction. The carrier stream used in the FIA system was sodium acetate 0.1 M and calcium lactate 0.002 M. Aluminium was eluted from the preconcentration column with acid eluents and determined by GFAAS. Limits of detection were extremely low, 2 ppt aluminium for a sample loading volume of 3.2 cm^3.

However, Ljunggen et al. noted that aluminium binding by the immobilised desferrioxamine was lower than binding using the free desferrioxamine reagent. The immobilisation procedure involved activation of the controlled pore glass (CPG) with glutaraldehyde, the desferrioxamine was then mixed with the activated glass where covalent coupling of the siderophore to the CPG is thought to occur via salt formation. The reduction in aluminium binding observed by Ljunggen et al. [27] using the immobilised desferrioxamine is extremely important, as it highlights the problems that can occur using chemical immobilisation procedures for ligands of biological origin. Possible alteration or total denaturation of the binding site may occur if the immobilisation procedure is not carefully chosen. Use of mixed stationary phases where the biological material is mixed homogenously with another packing material and no chemical immobilisation procedure is required would be preferable.

4.2.2 Cell Wall Functional Group-Based Systems

There have been an increasing number of reports over the last decade on the use of biomass for metal ion uptake from aqueous solution. Although reports have mainly described batch type studies, there have been several advances in this area and some authors now report the use of column/chromatographic systems for the uptake and enrichment of trace metal ions [28 - 30].

Metal binding to specific functional groups is probably one of the most important processes of metal uptake as a multiplicity of metal uptake sites of differing affinities for various ions are present in biomass. In the case of fungal biomass, metal uptake occurs through a mechanism which is primarily a reversible association of the metal ions with different functional groups including phosphates, carboxylates and hydroxamates which probably participate to varying degrees in metal binding [30, 31, 35]. Primary interactions may be augmented by the presence of hydroxyl or proton groups, therefore, by judicious choice of pH, the uptake ability of the biomass material could in fact be altered to suit the needs of the user.

Bioaccumulation by bacterial cells, has been largely attributed to interaction of microbial cell wall constituents, polyanions, with cations in aqueous solution. Metal binding appears to be at least a two step process in which the first event is a stoichiometric interaction between metal and reactive chemical groups in the cell wall fabric. Inorganic deposition of increased amounts of metal would seem to be the second step [32 - 34].

Tobin et al. have published a series of papers [35 - 38] on the use of Rhizopus arrhizus fungal biomass for the uptake of trace metal ions. In a preliminary paper in 1984 [35], these authors proposed metal uptake to be primarily as a result of a complexation mechanism, involving sites on the biomass containing carboxylate, phosphate or other functional groups

and possibly to a much smaller extent, as a result of an electrostatic attraction with negatively charged functional groups. Tobin et al. [36] have recently confirmed their proposed uptake mechanism by denaturing carboxylate and phosphate binding sites with chemical reagents and observing the effects on metal uptake capacities. A 60 % reduction in metal binding was observed if the carboxyl and phosphate groups were denatured.

In their preliminary investigations in 1984 [35] adsorption studies were performed for ionic species of 17 different metals and significant metal uptake of UO_2(II), Pb(II), Cr(III), Ag(I), La(III), Ba(II), Zn(II) and Cu(II) was observed. The highest metal uptake was observed for the uranyl ion at pH 3.5, where 0.82 mM/g was adsorbed by the biomass material. A pH value of 4 was determined to be optimum for metal uptake by the biomass material; this pH also avoids the problem of metal precipitation at alkaline pH values. Tobin et al. found the degree of metal uptake to be directly related to the ionic radii of the metals. Although this study was carried out using batch type conditions, the method was found to have considerable potential as the Rhizopus arrhizus biomass material was not observed to adsorb alkali ions in any appreciable amount.

Another attractive phenomenom of biosorbents was highlighted by Zosim et al. [39] in their investigation of emulsan, a biopolymer, for uranyl uptake. It was observed that large cation excesses did not adversely affect the uptake of the uranyl ion. Tsezos et al. [40] had observed a similar 'non effect' of cation presence on uranyl uptake using Rhizopus arrhizus material although they noted that this effect was pH dependent.

Tobin et al. recently examined the effects of cation [37] and anion [38] competition, on metal uptake by Rhizopus arrhizus biomass material. A range of cations could be adsorbed from a multi metal system

with a maximum uptake of 0.82 mM/g being observed for the uranyl ion. La(III), Zn(II), Cd(II), UO_2(II) and Ag(I) were chosen to investigate the effects of cation competition on the metal uptake process. Two different methodologies were examined to determine the competition effects of selected cation pairs; these were (a) direct competition and (b) exchange competition. In the direct competition study, various cation pairs were equilibrated with the biomass material and the amount of metal uptake was monitored by FAAS. Below the saturation concentration of metals, it was observed that a certain fraction of the uptake sites preferentially bound specific cations. The trend was reversed at higher concentrations of the primary ion used, i.e. if any ion was present in excess the tendency for this ion to be bound was greater.

The results of the exchange competition study indicated that the biomass functional group binding process was completely reversible. Equimolar concentrations of the uranyl ion completely displaced adsorbed cadmium ions. Adsorbed cadmium ions were also completely displaced by Zn(II) ions, but at much higher molar excesses of the latter. The cation competition results are very interesting as they indicate selective binding of various cations by the biomass material. However, the selectivity can be altered to suit the needs of the user by increasing the concentration of the ion of interest. The results obtained by Tobin et al. [37] would seem to agree with those of Zosim and co-workers [39] as both authors found that only in the presence of extremely large cation excesses was uptake affected.

In contrast to the results obtained for cation competition effects on metal uptake, a marked effect was observed in the presence of anions. Uptake of La(III), Cd(II), UO_2(II) and Ag(I) by Rhizopus arrhizus was seriously inhibited by anion presence, with the degree of inhibition following the order EDTA >>> SO_4^{2-}> Cl > PO_4^{3-} and CO_3^{2-} > glutamate [38].

4.3 Choice of Copper for Metal Uptake Studies

Copper was chosen for uptake studies because very little has been reported on its accumulation by biomass materials. Over 15 % of the worlds primary copper production comes from the winning of copper from copper sulphide by chemolithoautotrophic bacteria in an ore leaching process [41], which indicates some kind of specific copper uptake mechanism by these bacteria.

It is also known to be required by bacteria for the stability and assembly of the plasma membrane, and for specific metal enzymes and structural components in fungi and yeasts. Therefore, the presence of specific functional groups on both fungal and yeast biomass material is possible, which makes copper an attractive metal for examination in biomass uptake studies.

4.4 Choice of Biomass Types for Metal Uptake Studies

It is obvious from the literature that the potential of biomass technologies for use as an alternative or adjunct to conventional methods of trace enrichment or metal recovery is considerable. Rhizopus arrhizus is particularly attractive as it adsorbs a wide variety of metal ions, has a high uptake capacity, and preliminary studies have indicated that its' selectivity can be altered by judicious choice of pH and limiting metal concentrations. Many authors [12, 37, 40, 42] have cited the use of Rhizopus arrhizus for uptake of uranyl ions, however, little has been reported on the uptake of copper by this biomass material.

In a review on the leaching of metal ions with fungi, Burgstaller [4] noted that the most commonly used metal leaching fungi

belonged to the genera Penicillium and Aspergillus. Aspergillus niger was investigated for copper uptake and preconcentration ability as it is more readily available; if it proved successful as a preconcentration material it could be obtained in large quantities as an industrial by-product [43].

The use of yeast biomass was also investigated for copper uptake and preconcentration ability. Saccharomyces cerevisiae has previously been shown to adsorb uranium, thorium and zinc in large quantities [9]. Huang [44], recently reported the uptake of Cu(II) from dilute aqueous solutions using Saccharomyces cerevisiae, therefore, it was thought to be an ideal biomass species for this study.

4.5 Preconcentration of Cu(II) using Microbial Biomass and Poly (DTC) Mini Cartridges.

4.5.1 EXPERIMENTAL

4.5.1.1 Reagents and Standard Solutions

Water used during the course of this work was purified with a Millipore Water Purification system. Stock metal solutions were prepared by dissolving the appropriate metal salt in water. The solutions were stabilised if necessary, with mineral acids as recommended by Koch, Koch-Dedic [45]. The metal ions studied included nitrate or sulphate salts of Cu(II), Fe(III), Co(II), Cd(II), Zn(II), Ni(II), Mn(II), Al(III) and Cr(III). Organic solvents and miscellaneous chemicals used were Analar grade unless otherwise stated. The eluent was prepared by dissolving sodium diethyldithiocarbamate trihydrate in Spectrograde methanol (Fisons Scientific Instruments, Loughborough, Great Britain). No eluent buffering was found necessary for metal ion desorption. The preparation of the poly (DTC) chelating resin which was used as a packing material is described in section **4.5.1.4.1**.

4.5.1.2 Biomass

Strains were kindly donated by the School of Biological Sciences, Dublin City University. Initial uptake experiments were performed with biomass material, particle size less than 25 μm, (unless otherwise stated). Three different microbial strains were examined for metal uptake activity including Saccharomyces cerevisiae, Aspergillus niger and both lyophilised and unlyophilised strains of Rhizopus arrhizus.

4.5.1.3 Instrumentation

A Model IL 451 AA/AE Spectrophotometer (Instrumentation Laboratory Inc.) was used for the determination of all metal ions. All copper measurements were made at λ = 324.8 nm. This wavelength was adjusted accordingly to measure any other metal present in the sample.

4.5.1.4 Methods

4.5.1.4.1 Poly (DTC) Resin Preparation

A poly (dithiocarbamate) resin, (poly (DTC)) of poly (ethyleneimine) - poly (methylene polyphenylene) isocyanate was synthesised according to the procedure described by Hackett and Siggia [6] with slight modifications. Approximately 3.56 g of poly(ethyleneimine) (PEI) was dissolved in 50 cm^3 of a 2 / 1 mixture of 1, 4 dioxane / ethylene glycol monomethylether. 1.45 g of poly [(methylene (polyphenylene/ isocyanate)] PAPI was dissolved in 50 cm^3 of 1,4-dioxane. The PAPI solution was added quantitatively to the PEI solution by dropwise addition using a dropping funnel. Rapid formation of a white precipitate was observed. 20 cm^3 of 1,4-dioxane was added slowly with constant stirring. The polymer obtained was filtered under vacuum and repeatedly washed with isopropyl alcohol and methanol to remove excess PEI.

To impart the dithiocarbamate functionality on the polymer 25 cm^3 of carbon disulphide and 15 cm^3 of concentrated ammonia were added and the mixture was stirred overnight. Maximum yield of poly (DTC) was obtained if the solution was stirred continuously for 20 hours. The poly (DTC) resin obtained was filtered under vacuum, washed with methanol and water and finally air dried to constant mass. The polymer

was ground manually, sieved and sorted according to particle size. Particle size diameter range obtained was 32-120 μm.

4.5.1.4.2 Syringe Preparation

Plastic syringes (7 mm diameter) were used for all experiments. The syringes were washed with equal volumes (5 cm^3) of dilute nitric acid (1 M) and water, before use. Silanised glass wool was packed into the base of these syringes (height 2 mm) to prevent leakage of packing material.

4.5.2 Off-line preconcentration

Syringes were packed manually with 0.1 g of either biomass or poly (DTC) resin, (height 3 mm). Silanised glass wool was packed into the base of these syringes (height 2 mm) to prevent leakage of packing material. Following packing, the syringes were repeatedly washed with water (10 cm^3) to ensure uniform settling of the packing (thus voids within the packing material itself were considerably reduced). The final step before loading the metal ions onto the packing material was to wash the packings with water (pH adjusted to 4) containing a small volume of dilute nitric acid (0.1 M). Aqueous metal solutions (pH 4) were pumped manually through the packing material at a rate of 0.5 cm^3 per minute. The effluent from the syringe following this initial loading step was collected in a teflon container. Metal desorption from the packing was achieved with a methanolic eluent (made 10^{-1} M in diethyldithiocarbamate). The eluent composition was optimised during the course of our experiments. The desorbed metal solutions were again collected in teflon containers. Sample

solutions could be stored for several days without metal loss. All solutions were analysed by FAAS.

Initial experiments involved monitoring the uptake / retention and elution characteristics of the packing materials for Cu(II). Metal samples were loaded in a 2 cm^3 volume and eluted in a 2 cm^3 volume to determine the exact % retention and % recovery of copper attainable. Having ascertained which of the biomass packings was the most efficient material for copper uptake, further preconcentration studies were performed on it in conjunction with the poly (DTC) material. Larger volumes were thus loaded on to the packing materials and desorbed with 5 cm^3 (unless otherwise stated) of eluent.

4.6 RESULTS AND DISCUSSION

Establishing the exact nature of the site of metal uptake is very difficult as the cell wall fabric of biomass material contains many potential ligands including phosphates, carboxylates and hydroxamates. A single uptake site could have several different functional groups all, to some degree contributing to metal binding [35]. Judicious choice of pH was therefore an important consideration when conditioning the biomass material before uptake experiments and obviously when loading aqueous metal ion solutions.

An acidic pH results in a high degree of functional group protonation in the cell wall. Acidic protons would be easily displaced by most metal ions, however some functional groups would remain protonated so maximum metal uptake would not be possible. At pH 4 amines and carboxylates would be positively charged, again metal displacement of these protons should be feasible. Phosphate groups are normally negatively charged above pH 3 [46, 47] so if metal uptake is occuring as a result of electrostatic attraction of the solvated ions, phosphate groups would play an important role in metal binding at pH 4. At pH's higher than 4 many of the amine groups become quaternized and these positive charges would repel metal ions. At higher pH values the possibility of metal oxide precipitation occuring is increased [47] so basic pH's were never considered for metal loading. Preliminary experiments were all performed at pH 4 on the assumption that maximum metal uptake woud result. To validate our assumption a complete pH study was performed.

During the investigation of biomass materials for their metal uptake properties Tobin et al [35] observed the complexation of metal ions to a small degree by buffers and noted buffer interference on the metal

uptake process. Therefore buffer inclusion for pH maintenance was not used in preliminary experiments. Solution pH's were instead adjusted with 0.1 M nitric acid.

4.6.1 Optimisation of Eluent Composition

Efficiency of Cu(II) uptake by each of the packing materials was assessed in order to determine which of the biomass cell types in conjunction with the poly (DTC) resin merited further investigation as a preconcentration material. An aqueous copper solution (2 ppm), pH 4, was loaded onto each of the packed syringes and the effluent was analysed by AAS for Cu(II) presence. Copper was retained by all of the packings to some degree; an eluent study was then performed to determine what % of the copper was recoverable from the packing materials. Various dilute mineral acids including nitric, hydrochloric and sulphuric acid (0.1 - 1.0 M range) and organic solvents including methanol, acetonitrile and chloroform were pumped through the biomass and poly (DTC) packings. Only methanol showed any appreciable elution of copper from the packings. The highest overall % recovery of copper was observed using 100 % methanol; 65 % of the loaded copper was quantitatively recovered from the Rhizopus arrhizus (unlyophilized) material. To increase copper desorption from the packing materials the effect of ligand inclusion in the methanolic eluent was assessed. Dithiocarbamates are known to complex a wide range of metal ions [48-52] including the metals of interest in this work, therefore a dithiocarbamate ligand was chosen for further investigation to enhance metal ion desorption. Sodium diethyldithiocarbamate was added to the methanolic eluent at various concentrations and its' effect on metal elution was evaluated. A methanolic solution (made 10^{-1} M in diethyldithiocarbamate (DEDTC)) enabled at

least 30 percent overall copper recovery for all of the packing materials. The highest overall recovery was again observed for the Rhizopus arrhizus material, greater than 93% recovery of Cu(II) was achieved using an eluent composition of 100 % methanol (made 10^{-1} M in dithiocarbamate), see Figure 4.1 below.

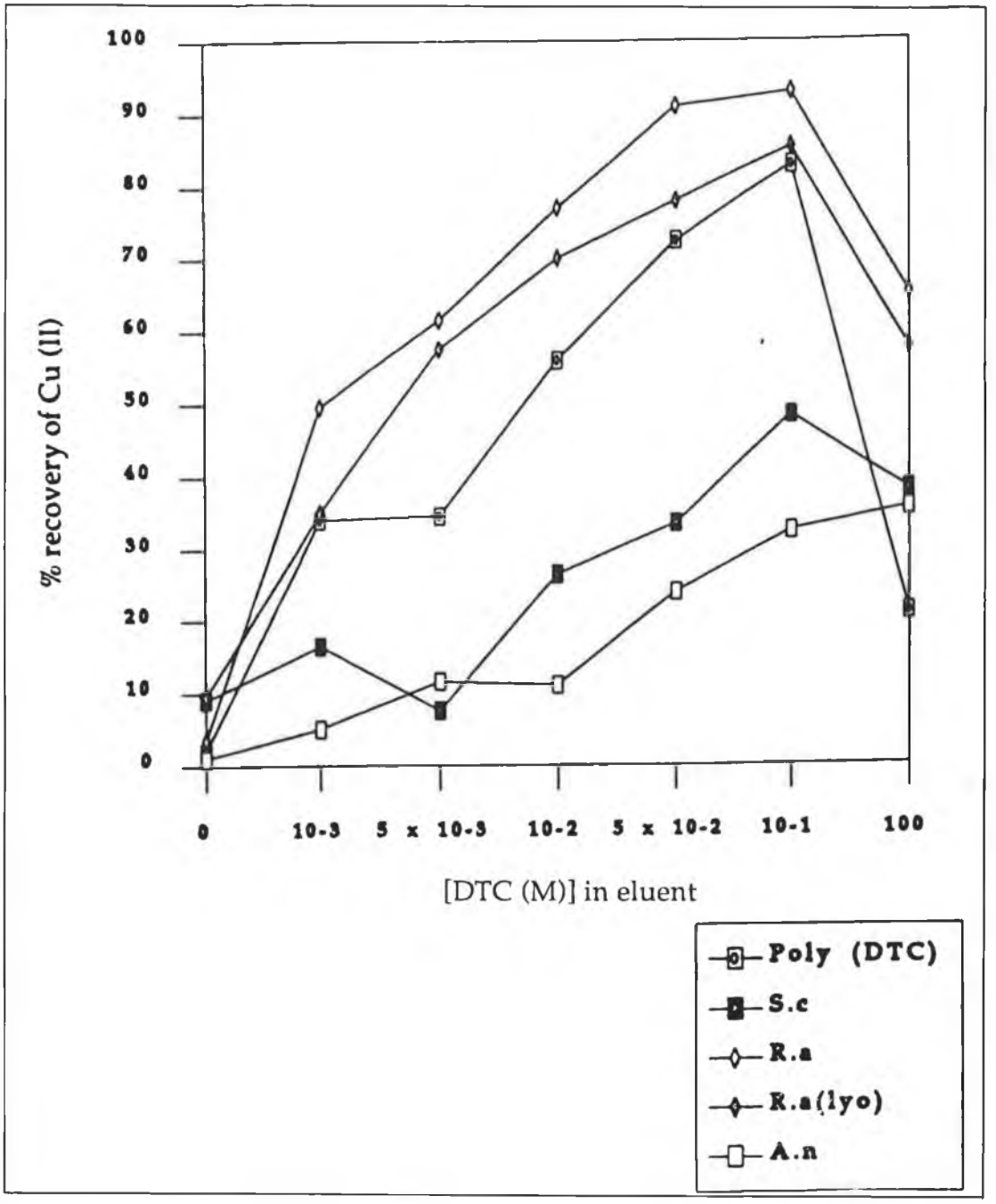

Figure 4.1 Optimisation of Eluent Composition

Extraction experiments have shown that the reactivity of dithiocarbamates towards metal ions is strongly pH dependent and that the dithiocarbamate ligand is unstable in acidic solution [53]. Increasing the pH of the eluent solution used in this study to 5 or 6 resulted in decreased copper desorption from the packings. Introduction of this eluent onto the packing material may have resulted in an increased number of negatively charged functional groups in the biomass material.

As already stated, a number of functional groups may participate in metal binding at any one uptake site therefore the degree of copper/biomass binding may actually have increased on the introduction of this neutral eluent thereby competing with DEDTC complexation of the copper ions. Use of the pH 5-6 eluent on the poly (DTC) resin was not observed to increase copper desorption, increased binding by the DEDTC ligand at this pH may have been off-set by an increase in copper ion precipitation on the packing material as copper exhibits a tendency to precipitate out of solution at neutral and basic pH's. This precipitation phenomenom may also have occured on the biomass material at pH 5 - 6.

4.6.2 Optimisation of Loading pH

The solution chemistries of many metals are quite complex [47] and dependent on several factors, one of which is pH. Careful pH adjustment was therefore necessary to stabilise the Cu(II) in solution. Metal uptake by the packing material may be directly affected by the pH of the loading solution as introduction of a very acidic metal solution could result in competition between H^+ ions and copper ions for binding sites. The loading solution pH was varied in the range 2 - 7. The percentage retention of copper was greatest for all packing materials at pH 4. Poly (DTC)

resin was the only packing material to retain 100 % of the loaded copper at this pH See Table 4.1 below.

pH	%Cu(II) retained				
	Poly (DTC)	**S. cerevisiae**	**R. arrhizus**	**R. arrhizus(lyo)**	**A. niger**
2	60.15	61.75	67.50	55.79	60.85
3	80.45	75.00	73.00	75.00	67.50
4	100.00	75.62	93.62	89.50	71.50
5	100.00	59.50	74.00	91.00	70.50
6	100.00	45.95	63.35	75.57	54.75
7	100.00	32.15	57.45	68.61	48.00

Table 4.1 Effect of loading pH on copper retention

Note: A standard copper solution (2 ppm) was loaded onto the precolumn for this pH study

Using the biomass packings Rhizopus arrhizus biomass material showed the highest retention of copper; approximately 94 % Cu(II) retention was observed at pH 4. Desorption of the retained copper from the biomass and poly (DTC) packings with methanol (made 0.1 M in DEDTC) indicated that the highest overall % recovery of copper could be obtained using Rhizopus arrhizus (unlyophilized) material, approximately 90 % recovery was observed. Overall recovery of copper from poly (DTC) was slightly lower at 88 %, indicating that elution from the packing is not

quite as efficient as that from Rhizopus arrhizus for an initial loading pH of 4, see Figure 4.2.

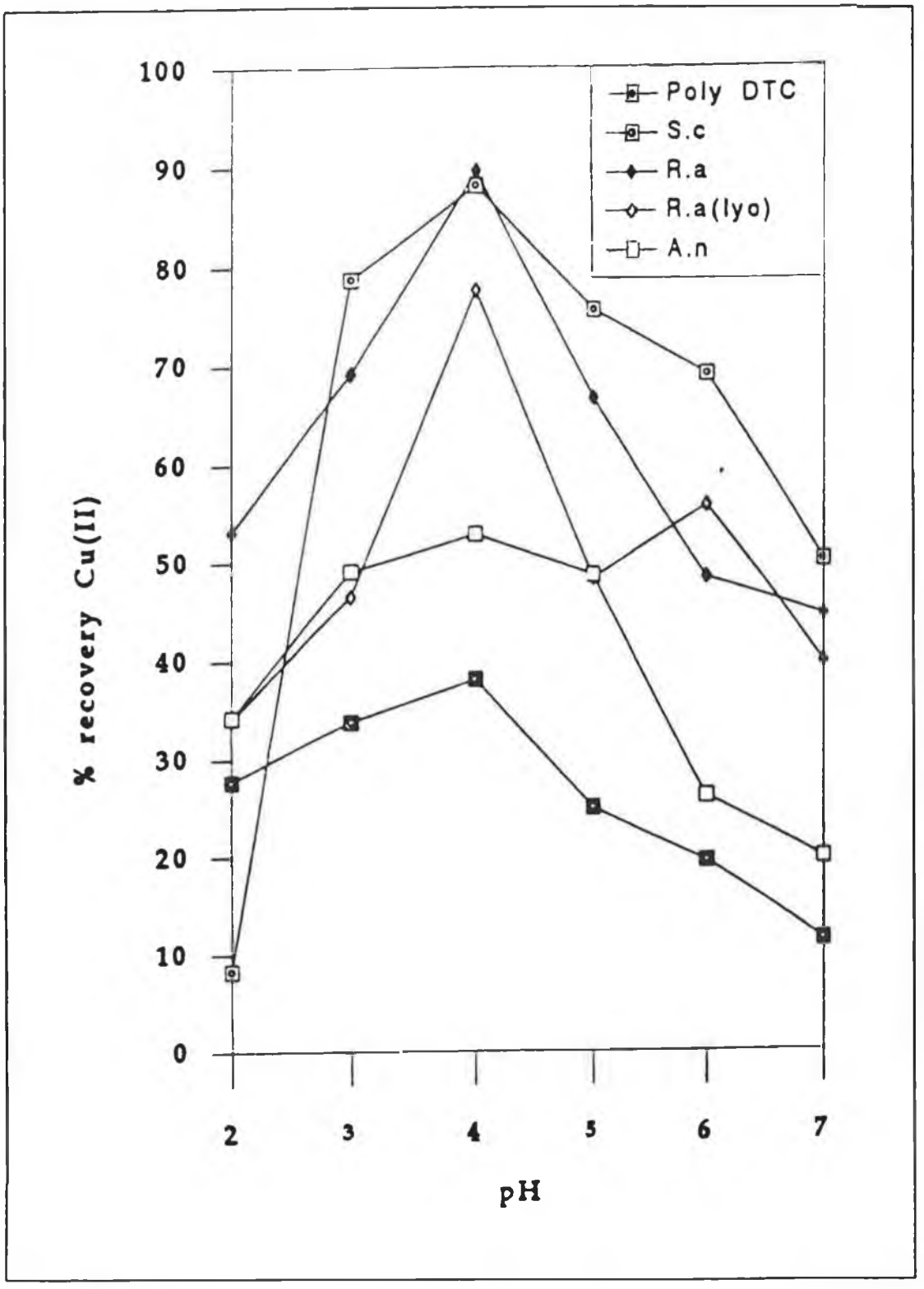

Figure 4.2 Optimisation of loading pH

4.6.3 Overall Recovery of Cu(II)

The results of the pH and eluent studies indicated Rhizopus arrhizus (unlyophilized) biomass as the most efficient packing material for uptake and recovery of copper. Poly (DTC) as expected showed high retention and elution of the loaded copper ions. On the basis of overall copper uptake and recovery efficiency results, both poly (DTC) and Rhizopus arrhizus were examined further with a view to their use as preconcentrating materials in mini portable systems for the determination of trace metal ions.

4.6.3.1 Optimum Particle Size

The effect of biomass particle size on the retention / recovery of a loaded copper solution was evaluated. Syringes were packed with 0.1 g of Rhizopus arhizus and coded according to particle size, see Table 4.2. Rhizopus arrhizus, particle size < 25 μm (coded R.a 1) exhibited the highest retention/recovery properties for loaded Cu(II). Approximately 90 % recovery of loaded Cu(II) was achieved see Figure 4.3 (As this was the particle size used in both the pH and the eluent study, high recovery had been expected).

R. arrhizus Code	Particle Size (μm)	Description
R. a 1	< 25	Powder
R. a 2	< 200	Fine
R. a 3	< 250	Fine
R. a 4	< 500	Ground
R. a 5	< 500	Coarse

Table 4.2 Rhizopus arrhizus 'particle size' code system

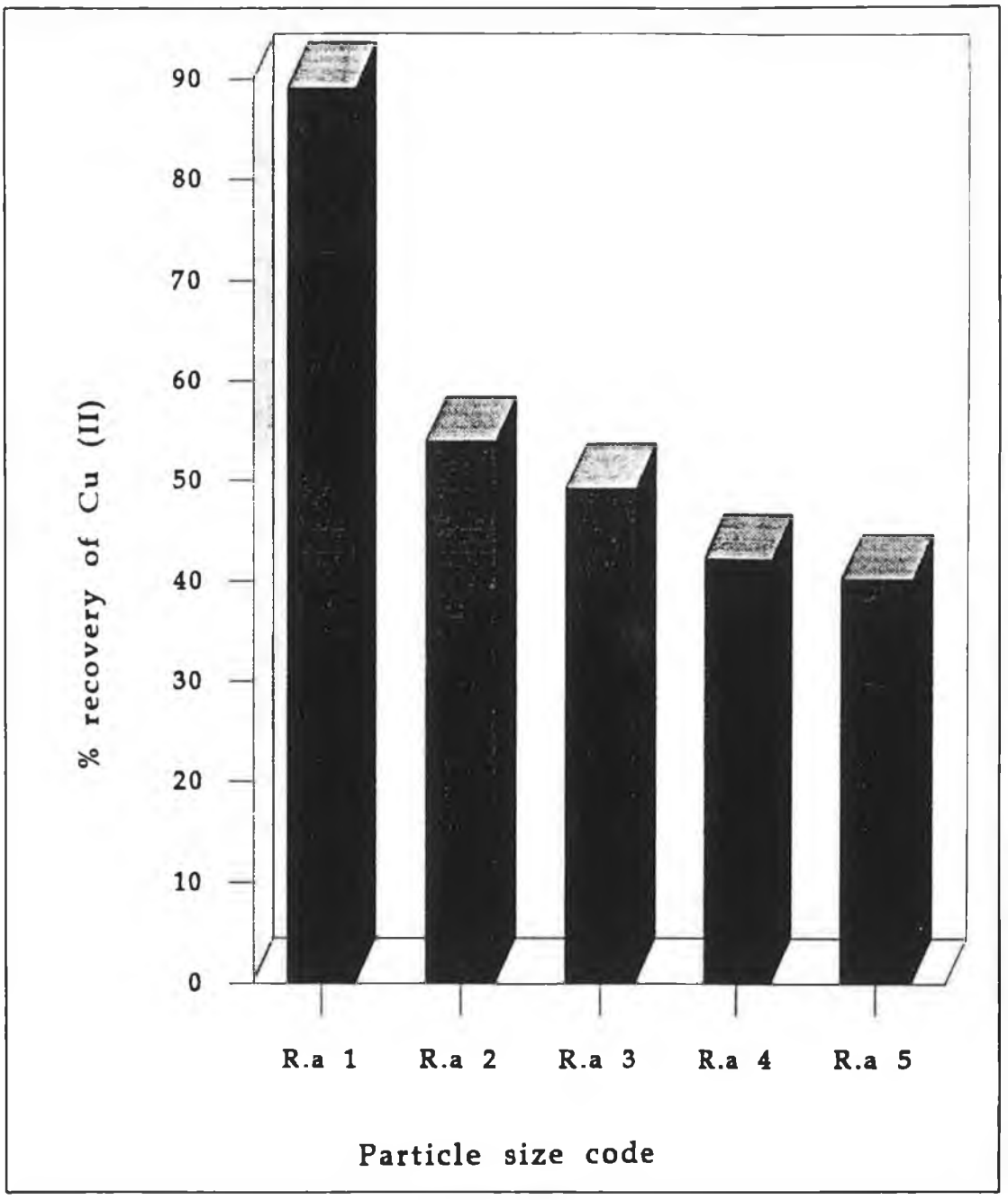

Figure 4.3 Optimisation of Rhizopus particle size

4.6.4 Off-line Preconcentration

Poly (DTC) and Rhizopus arrhizus (particle size < 25 μm) were examined with respect to preconcentration of metal ions. A fixed concentration of Cu(II) (0.05 ppm) was loaded onto the packed syringes in different loading volumes (volume range 5 - 1000 cm^3). The retained copper was desorbed in a 5 cm^3 volume of methanol (0.1 M DEDTC) eluent. The preconcentration factors obtained for different loading volumes were compared with expected theoretical values (i.e. load 25 cm^3 and elute in 5 cm^3, expect a 5 fold preconcentration factor) see Table 4.3.

[Cu(II)] 0.05 ppm	**Packing Material**				**Preconcentration Factor x fold**		
Volume (cm^3)	Poly (DTC) [Cu (II)] ppm		R.arrhizus [Cu(II)] ppm		Poly (DTC)	R.arrhizus	Theoretical
	F1	F2	F1	F2	-	-	-
5	0.00	0.00	0.00	0.00	0.00	0.00	0
25	0.00	0.16	0.04	0.20	3.20	4.00	5
50	0.08	0.29	0.14	0.340	5.80	6.80	10
75	0.00	0.55	0.09	0.59	11.00	11.80	15
100	0.00	0.80	0.16	0.86	16.00	17.20	20
200	0.20	1.50	0.28	1.70	30.00	34.00	40
400	0.30	3.18	0.48	3.46	63.60	69.20	80
600	0.50	5.10	0.55	5.17	102.00	103.40	120
1000	0.99	5.60	1.26	7.94	112.00	158.80	200

Table 4.3 Relationship between loading volume and preconcentration effect

Copper was concentrated on both poly (DTC) and Rhizopus arrhizus packing materials. Neither of the packings preconcentrated copper to quite the expected theoretical value. Rhizopus arrhizus preconcentrated copper more efficiently than the poly (DTC) resin. A preconcentration factor of 17.2x was obtained when 100 cm^3 of copper solution was loaded onto the Rhizopus arrhizus material and eluted in a 5 cm^3 volume. This value is only slightly lower than the 20x preconcentration factor expected. A 1000 cm^3 loading volume of copper solution should result in a preconcentration factor of 200x however the values obtained were somewhat lower 158x and 112x for Rhizopus arrhizus and poly (DTC) respectively. The volume of sample loaded may have exceeded the breakthrough capacity of the 0.1 g of packing material used, resulting in copper loss solely due to volume effects. It would only require an increase in the amount of packing used, to increase the preconcentration factor .

It is obvious from the results that for a packing weight of 0.1 g, a volume of 100 cm^3 can be efficiently loaded without inherent preconcentration loss due to volume overloading. Using a fixed volume (100 cm^3) of copper solution various concentrations of Cu(II) (range 10 - 750 ppb) were loaded onto the packed syringes. Copper was desorbed with a 5 cm^3 volume of eluent. The theoretical preconcentration factor is 20x for this loading/eluent volume ratio. At low concentration values the preconcentration factor was slightly lower than expected. At copper concentrations in the range 50 - 250 ppb approximately a preconcentration value very close to the theoretical value was obtained using both poly (DTC) and Rhizopus arrhizus. At concentrations above 250 ppb a significant decrease in preconcentration was observed. Again overloading of the packing material is the most probable cause of reduction in the preconcentration factor obtainable, see Table 4.4.

Volume 100 cm^3	Packing		Material		Preconcentration Factor x fold	
Loading [Cu(II)] ppm	Poly (DTC) [Cu(II)] ppm		R.arrhizus [Cu(II)] ppm		Poly (DTC)	R.arrhizus
	F1	F2	F1	F2	-	-
0.010	0.00	0.07	0.00	0.15	7.07	15.00
0.050	0.00	0.70	0.00	0.79	14.00	15.80
0.075	0.00	1.85	0.00	1.49	24.66	19.86
0.100	0.00	2.10	0.04	1.98	21.00	19.80
0.250	0.00	2.04	0.10	4.47	8.16	17.88
0.500	0.06	2.25	0.10	4.00	4.50	8.00
0.750	0.14	3.60	0.20	4.40	4.80	5.86

Table 4.4 Relationship between loading copper concentration and preconcentration effect

An extended series of copper standards (100 cm^3 sample volume) were loaded onto the biomass concentration column to determine the exact cut off point for overloading of a 0.1 g packing material. The preconcentration response was linear up to a copper concentration value of 300 ppb, beyond this concentration the response decreased sharply indicating column overloading as can be seen in Figure 4.4. Regression values for the linear part of the graph indicated an r value of 0.999. The working range of this method could be improved to allow preconcentration of higher copper/metal concentrations if the amount of packing material was increased.

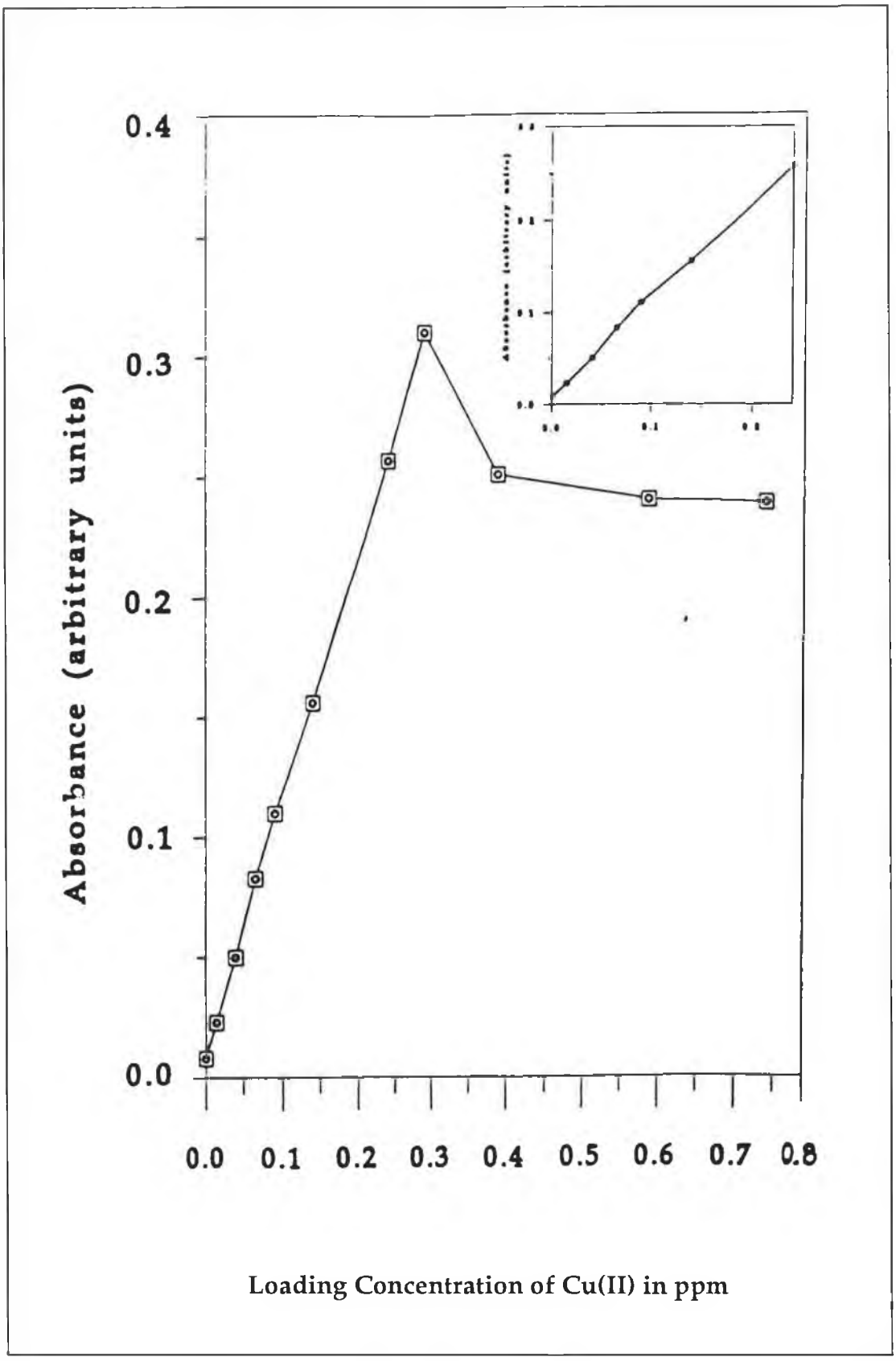

Figure 4.4 Standard curve for the preconcentration of a fixed volume Cu(II) sample on a 0.1 g biomass column.

4.6.5 Preconcentration of Other Metals

4.6.5.1 Preliminary Uptake Studies

The results indicated that poly (DTC) was less effective as a preconcentating material than Rhizopus arrhizus (using the experimental conditions outlined) therefore the possibility of preconcentrating other metals was examined using the Rhizopus packing material. Preliminary studies examined the retention/elution efficiencies of the Rhizopus packing for individually loaded metals (as in the copper study). Initially metals were loaded and eluted in the same volume (2 cm^3) to ascertain whether uptake and subsequent quantitative elution were in fact possible. The retention/elution behaviour of 8 metal ions including Fe(III), Zn(II), Ni(II), Co(II), Cd(II), Mn(II), Al(III), Na(I) and Cr(III) was assessed. Eluent and pH conditions were unchanged from the previous study. All of the metals were retained to some extent by the biomass material. The overall % recovery of loaded metals is shown in Table 4.5 in decreasing order of recoverable metal. Appreciable recovery (> 50 %) was only possible for Mn(II) and Cd(II). Chromium although retained by the packing material was found to be un-recoverable using the DEDTC eluent.

Metal cation	Overall % recovery
Cu(II)	89.62
Cd(II)	67.44
Mn(II)	62.80
Ni(II)	37.68
Zn(II)	34.66
Co(II)	21.97
Na(I)	4.12
Al(III)	-
Cr(III)	-

Table 4.5 Recovery of individually loaded metals from Rhizopus arrhizus packing material

4.6.5.1.1 Synergistic Metal Uptake

Each metal with the exception of chromium was loaded onto the packing material from a solution also containing copper. Initially equivalent concentrations (< 150 ppb) of the copper /metal solution were loaded onto 0.1 g of packing to ensure that concentration overloading of the packing did not occur. Copper presence in solution resulted in an overall decrease in the % recovery of the other metals. Nickel was the only exception with an increase in % recovery of nickel from 37.68 % to 51.94 % being observed, see Table 4.6. A corresponding decrease in copper recovery was seen in the presence of nickel from 90 % ca. to 55.75 %. Both retention and elution values improved for nickel in the presence of copper

possibly indicating synergistic absorption of nickel with copper. Copper elution was decreased by over 30 % perhaps as a result of selective complexation by DEDTC of nickel in preference to copper and hence selective elution of the Ni-DEDTC complex. Copper retention was not significantly reduced which indicated that reduction in overall recovery occured at the elution stage.

Metal	% Retained	% Eluted	Overall % Recovered
Cu(II)	**95.00**	**98.00**	**93.10**
Cu(II)	93.49	88.16	82.42
Cd(II)	76.36	83.03	63.40 (67.44)
Cu(II)	85.00	94.96	80.71
Mn(II)	79.00	59.50	47.00 (62.80)
Cu(II)	91.90	60.66	55.75
Ni(II)	53.00	98.00	51.94 (37.68)
Cu(II)	92.40	94.53	87.35
Zn(II)	59.66	24.46	14.60 (34.66)
Cu(II)	93.15	96.62	90.00
Co(II)	39.35	51.20	20.15 (21.97)
Cu(II)	93.45	99.62	93.09
Na(I)	3.60	-	- (4.12)
Cu(II)	93.00	99.31	92.35
Al(III)	-	-	-
Cu(II)	90.00	94.00	84.60
Cr(III)	-	-	-

Table 4.6 % recovery of copper in the presence of one other metal

Note: ( ) indicates the recovery values for individually loaded metals

4.6.5.1.2 Preconcentration of Copper from a Multi-Metal System

Copper was loaded onto 0.1 g of biomass material from a solution containing all 8 of the other metals mentioned previously. The results again indicated selective binding of copper however a reduction in overall recovery values was noted for all metals. This was obviously as a direct result of overloading of the biomass material. Using a larger volume of packing material 0.5 g, the mixed metal solution was again loaded onto the biomass material. The results confirmed selective binding of copper in the presence of other metals, see Table 4.7. Copper, nickel and manganese were selectively retained by the biomass material, a decrease in the retention values for all other metals was noted. Elution was observed in the order Ni > Cu > Cd > Mn.

Metal	% Retained	% Eluted	Overall % Recovered
Cu (II)	95.00	86.00	94.70
Ni (II)	51.48	98.15	56.52
Mn (II)	74.35	59.00	45.86
Cd (II)	66.36	83.03	60.09
Zn (II)	59.00	31.46	18.56
Co (II)	20.30	31.00	5.29
Na (I)	-	-	-
Al (III)	-	-	-
Cr (III)	-	-	-

Table 4.7 Preconcentration of Cu(II) from a multi-metal sample

The retention data obtained for Na (I) was of particular note; loaded as a single metal cation 4 % of the sodium loaded was retained however in the presence of any other metal cation no retention was noted. This was of particular interest as many authors have reported the interference of sodium on FAAS [54]. The selective retention of other metals in preference to sodium on our small biomass packed syringe is therefore an obvious advantage as the analysis of sea water containing high levels of sodium would be simplified considerably.

4.7 Conclusions

An off-line preconcentration method for the determination of Cu(II) in the presence of 8 other trace metals is reported. Microbial biomass and a poly (dithiocarbamate) resin were evaluated as packing materials for Cu(II) uptake from aqueous solution. 3 types of microbial biomass were examined w. r. t metal ion uptake; Saccharomyces cerevisiae, Aspergillus niger and Rhizopus arrhizus (both lyophilized and unlyophilized cells were evaluated). A methanol (10^{-1} M diethyldithiocarbamate) eluent facilitated metal ion desorption from the packed syringes. Metal ion presence in the eluent was determined with FAAS. Rhizopus arrhizus (unlyophilized) packing material, particle size < 25 μm exhibited the highest overall % recovery of Cu(II). > 90% Cu(II) recovery was achieved when the aqueous solution only contained Cu(II). When copper was preconcentrated from a multi cation sample the overall % recovery value increased indicating selective retention and recovery of Cu(II).

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Chapter 5

Surfactant enhanced adsorptive stripping voltammetry of Al(III) as its SVRS complex

5.1 Introduction

Considerable interest in the behaviour of aluminium in environmental and biological systems has developed since the significance of its' toxicity, which is now widely recognised, became known. The possibility of a link between aluminium and the causation of Alzheimer's disease is of particular concern, and several authors have reported the presence of aluminium in senile plaques [1] and neurofibrillary tangle bearing neurons [2] in brains of patients who suffered from the disease. Martyn et al. [3] in a regional study within England and Wales, found the risk of Alzheimers disease to be 1.5 times higher in districts where the average aluminium concentration was higher than 110 ppb, compared with districts where the concentrations were circa 10 ppb.

The current European Community Directive specifies a maximum allowable aluminium concentration of 200 ppb, however, a 'safe' acceptable level of 50 ppb is now required by several of the Member states. Obviously there is a need for a sensitive method of aluminium determination which allows measurement of aluminium at low levels, possibly at ppb or ppt concentrations. Numerous methods of aluminium determination are available, however, spectrophotometric [4 - 9], fluorometric [10 - 14] and atomic absorption (GFAAS [15], FAAS [16, 17] and ETAAS [18]) methods are most frequently reported. The presence of natural interferents e.g. humic and fulvic acids in biological samples, and organic ligands in soils, makes the direct determination of aluminium in its native environment quite difficult; therefore, many authors have developed selective preconcentration methods for aluminium and coupled them to conventional detection modes [19 - 26].

A survey of the literature however, reveals the lack of suitable electroanalytical methods for low level aluminium detection; this is due to

the poor electrochemical behaviour of aluminium which makes its polarographic or voltammetric quantitation very difficult. Aluminium is reduced at -1.75 V vs. S.C.E., yielding an irreversible wave distorted by the hydrogen evolution background current. This is also very close to the reduction potentials of sodium, barium and potassium, so their presence in high concentrations interfers strongly during aluminium determinations.

Recently, advances have been made in the application of cathodic stripping voltammetry (CSV) to the determination of trace elements in the environment. In CSV, the preconcentration step may consist of preconcentration of an insoluble salt, such as the hydroxide Tl(III) [27] or adsorption of a surface active complex prior to reduction of the deposited material. The latter method, adsorptive cathodic stripping voltammetry (AdCSV) of electrochemically active complexes has allowed the quantitation of several metals not normally amenable to detection by conventional stripping methods [28-30]. Complexation of the metal of interest with an electrochemically active ligand, controlled accumulation of this complex at the electrode surface and a subsequent stripping step is the method generally employed. Several authors have reported the determination of aluminium in trace amounts using solochrome violet RS (SVRS) [42, 45-47, 51-54], a ligand with good electrochemical behaviour which forms an adsorptive complex with the metal making it suitable for CSV detection. The majority of these authors however, have used AdCSV of the aluminium complex at a mercury type electrode.

In this chapter, the results of adsorptive cathodic stripping voltammetry of the Al(III)/SVRS-complex at a carbon paste working electrode have been reported. A controversial area in adsorptive stripping voltammetry is the inclusion of surfactants to enhance the adsorption process, therefore, the influence of surfactant presence on the adsorptive process of the Al(III)/SVRS-complex has also been assessed. The

quantitative utility of electroanalytical preconcentration methods, in general, have been outlined briefly in the following sections, as well as the more relevant literature pertaining to the adsorptive stripping behaviour of the Al(III)/SVRS-complex.

5.1.1 Preconcentration using Electroanalytical Methods

5.1.1.1 Anodic Stripping Voltammetry (ASV)

The limit of detection of any analytical method is governed by the ratio of the signal from the test component to the background which involves the noise generated in the solution and in the apparatus. By preconcentrating the component of interest the signal to noise ratio may be improved. Preconcentration is most commonly achieved by accumulating the test substance electrolytically on the working electrode, while selectivity is controlled by judicious choice of the solution composition and the pre-electrolysis potential. The preconcentrated component is then electrolytically stripped into the solution. The analytical signal then increases as a direct result of the concentration procedure.

Anodic stripping voltammetry (ASV) was for a long time the only electrochemical technique by which concentrations of trace elements in aqueous samples, e.g. brine, could be determined. Trace analysis, mainly of heavy metal ions using ASV is popular due to its low limit of determination (sub ppb levels), accuracy, precision, and the low cost of instrumentation involved. ASV is based on the previous electrolytic accumulation of the compound of interest on the working electrode followed by oxidation of the reduced substance formed [31]. Some limitations are unfortunately inherent in ASV, such as the requirement that the element is reduced to the elemental state, as this is normally the least

soluble state at lower valencies, and that the elemental state is soluble in mercury. Only four metal ions Cd(II), Pb(II), Cu(II) and Zn(II) have been determined frequently in polluted seawater with ASV [32]. Other elements are present in too low concentrations or are subject to interferences arising from the formation of intermetallic compounds in the mercury.

5.1.1.2 Cathodic Stripping Voltammetry (CSV)

The use of cathodic stripping voltammery (CSV) techniques for the determination of trace elements in the environment is increasingly reported [33, 34]. Unlike the preconcentration step in ASV where the element is deposited in its elemental state, in CSV the preconcentration step may consist of preconcentration of an insoluble salt or adsorption of a surface active complex prior to reduction of the preconcentrated material. CSV is particularly useful for the determination of trace metals, as it results in the formation of a monomolecular layer of complexed species at the electrode surface. The reduction current is independent of the diffusion of the reactant [35], therefore, the response of AdCSV is directly related to the concentration of the adsorbed metal-ligand complex. Following the preconcentration step, the reduction current is measured by scanning to more negative potentials. The high sensitivity for the analyte is a result of the complete electrochemical reduction of the monomolecular layer of the adsorbed material.

5.1.1.2.1 Surfactant Use to Enhance the Adsorption Process

A somewhat contradictory area in adsorptive stripping voltammetry is the inclusion of surfactants to enhance the adsorption process. Competitive adsorption by other surface-active materials and/or free ligand present in the sample has been considered the main interferent to the AdCSV signal response [36], therefore, the enhanced adsorption sometimes observed in surfactant presence is unexpected.

Several authors have reported surfactant enhanced adsorption processes. Addition of the anionic surfactant sodium dodecylbenzene sulphonate (SDBS) was observed to improve the adsorption of the DMG-Pd complex at the mercury drop electrode [37]. Mlakar et al. [38] have examined the synergistic adsorption of mixed ligand complexes at a mercury surface; determination of a uranyl mixed complex with salicyclic acid and tri-n-butyl phosphate in aqueous solution [38] and voltammetric determination of U(VI) with tri-n-butyl phosphate addition [39] have been reported. Several direct polarographic methods with surfactant enhanced signal response have also been described [40, 41]. Subbaraman and Shetty [40] noted that the polarographic reduction of Cu(II), Pb(II), Cd(II) and Fe(III) was enhanced by the addition of camphor which shifted an interfering phoshate wave to more negative potentials. Surfactant enhanced adsorption may in some cases arise from a fast liquid-liquid exchange where a non polar surfactant layer is formed on for example the HMDE which then rapidly adsorbs non polar molecules.

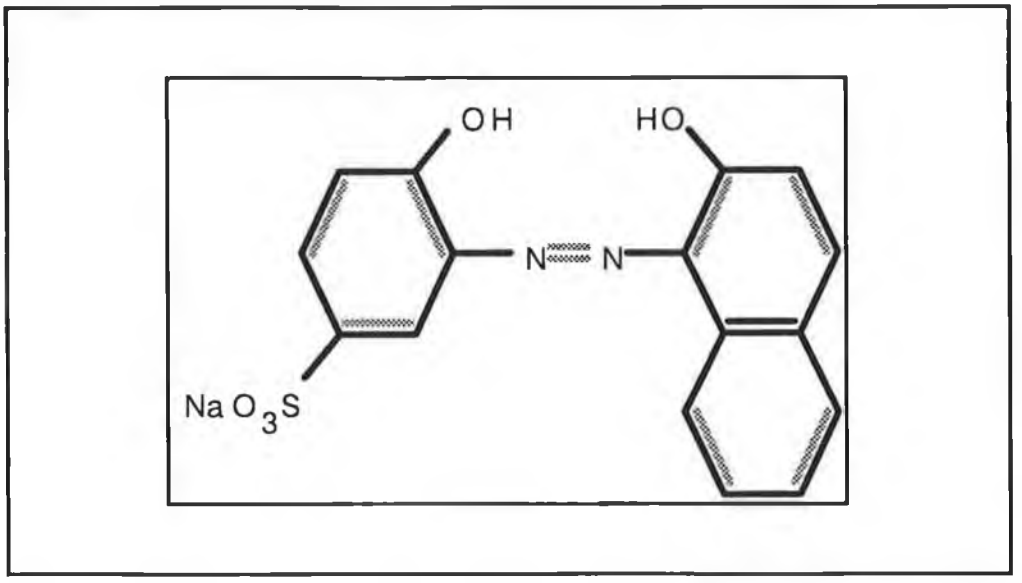
The mechanism of reduction of the Al(III)/SVRS-complex is well documented [42-44] and many polarographic and voltammetric methods have been employed for its detection [45 - 47, 51 - 54] (see section 5.1.2). The Al(III)/SVRS-complex is thought to have an overall positive charge, therefore, enhancement of the adsorptive process via ion pair

formation of the metal complex with an anionic surfactant at the electrode surface was examined. The influence of both cationic and non-ionic surfactant presence in solution on the Al(III)/SVRS-complex adsorption process was also examined.

5.2 Determination of Al(III) with SVRS

5.2.1 General Mechanism

The ability of certain *o*, *o'*-dihydroxyazo or di-*o*-hydroxyazo dyes to form discrete polarographic reduction waves in the presence of metal ions was first reported by Willard and Dean [42] who found that they could determine aluminium in trace amounts using solochrome violet RS, see structure 5.1.



Structure 5.1 Solochrome Violet RS

In the presence of an excess of the dye two peaks were obtained, the first more positive peak is due to the unreacted dye and the second one is related to the reduction of the dye complexed by aluminium. The half-wave reduction potentials are more positive than either the reduction potential of Al(III) or that of hydrogen evolution. The height of the complex wave, which is about 0.2 V more cathodic than that of the free dye was shown to be proportional to the aluminium concentration. The

electroanalytical response was shown to be enhanced by the adsorption of the complex at the dropping mercury electrode (DME) and thus enabled the detection of Al(III) at the 10 - 100 ppb level.

The mechanism of reduction of the di-*o*-hydroxyazo compound at the mercury electrode has been established with reasonable certainty by Florence and Belew [43]. The reduction path involves a potential-determining two electron transfer step which yields an unstable hydrazo intermediate which disproportionates within the lifetime of the drop. The original azo compound and the amines are produced during disproportionation, this leads to a total value of n = 4.

$$R\text{-}N{=}N\text{-}R' + 2H^+ + 2e^- \text{----}> R\text{-}NH\text{-}NH\text{-}R' \qquad \textbf{Equation 5.1}$$

$$2\ R\text{-}NH\text{-}NH\text{-}R' \text{----}> R\text{-}N{=}N\text{-}R' + RNH_2 + R'NH_2 \qquad \textbf{Equation 5.2}$$

Postulations as to why the metal complex of SVRS is reduced at more negative potentials than the free dye are numerous. Willard and Dean [42], suggested that the potential shift was due to the the stabilisation of one of the geometric isomers of the dye. Dean and Bryan [44] reported that the rigidity of the metal complex formed affected the $E_{1/2}$ value, i.e. ionic radius effect of the metal ion.

Florence et al. [43] attempted an interpretation of the polarographic behaviour of the metal/ligand complex using kinetic studies results. The reaction rates indicated that the metal remains complexed by

the dye after reduction, and the $E_{1/2}$ depends only on the ratio of the relevant stability constants and acid dissociation constants. The potential-determining step in the reduction of the SVRS involves a two electron reduction to the hydrazo derivative. In acid media, the reaction in equation 5.3 occurs:

$$H_2D + 2H^+ + 2e^- \text{ ----> } H_2D^* \qquad \textbf{Equation 5.3}$$

The azo group in the metal/SVRS-complex is reduced, but the metal remains complexed to the hydrazo derivative as in equation 5.4., where D^* is the hydrazo derivative, and D^i represents SVRS with both phenolic hydrogens ionised.

$$MD^i + 2H^+ + 2e^- \text{ ----> } MD^* \qquad \textbf{Equation 5.4}$$

The Al(III)/SVRS-complex is thought to have an overall positive charge [42], the main reaction being:

$$Al(OH)_2^+ + H_2D \text{ ----> } AlD^+ + 2H_2O \qquad \textbf{Equation 5.5}$$

5.2.2 Preconcentration Techniques for the Determination of Al(III) with SVRS and Related Ligands

Low level aluminium detection in the ppb - ppt concentration range requires a sensitive electrochemical technique, and therefore, recently reported methods have favoured adsorptive stripping techniques for aluminium determination. Adsorptive stripping, accumulation and oxidation (+ 0.7 V) of the Al(III)/SVRS- complex at a carbon paste electrode was reported by Specker et al. [45], however, with a poor detection limit of 100 ppb, less than was originally achieved with straight foward polarography.

Florence et al. [46] have also reported an oxidative mode for the detection of the Al(III)/SVRS-complex however a pyrolytic graphite electrode was used. Similar detection limits to those reported by Willard and Dean were obtained by Florence et al.

Wang et al. [47] have described a very sensitive voltammetric method using an electrochemical stripping procedure, which involves controlled adsorption of the Al(III)/SVRS-complex at a static mercury drop electrode (SMDE), i.e. the accumulation step, followed by a linear scan stripping procedure in the cathodic direction. The reduction response was typified by two waves, the first at -0.49V and the second at -0.61V versus a Ag/AgCl reference electrode arising from free SVRS reduction and Al(III)/SVRS-complex reduction respectively. A linear calibration was found over the 5 - 30 ppb concentration range for a 1 minute accumulation time. The limit of detection was extended to 150 ppt using an accumulation time of 10 minutes. The main disadvantage of this method was the long heating time required to effect Al(III)/SVRS-complex formation (10 minutes at 90^{o}C. A subsequent 15 minute cooling step was then required prior to analysis.

Downard et al. [48, 49, 51] have recently published a series of papers describing novel improved methods for the indirect electrochemical determination of Al(III). Aluminium has been voltammetrically determined using an alizarin modified electrode, which was simply prepared by dip-coating a high density graphite electrode in an *N, N*-dimethylformamide solution of alizarin [48]. Optimum experimental conditions included a solution pH of 8.4 +/- 0.2, an accumulation time of 1 minute and a differential pulse mode of measurement for the oxidative stripping peak signal of the Al(III)/ alizarin complex. The detection limit was 4 ppb aluminium, and the response was linear up to 270 ppb. One disadvantage of this method, however, was the 'single use' nature of this chemically modified electrode (CME) which meant that a new electrode had to be prepared for each measurement.

Downard et al. [49] have also determined the Al(III)/DASA (dihydroxyanthraquinone-3-sulphonic acid)-complex using differential pulse CSV. A solution pH of 8.8 was found optimum to effect Al(III)/DASA-complex formation. The authors emphasised that at this pH the reactive "aluminium fraction" approximated the total dissolved aluminium in solution. Van den Berg et al. [50] have also reported the accumulation of the Al(III)/DASA-complex followed by DPCSV measurement of the stripping signal, however, the solution pH was 7.1. The limit of detection was determined to be 27 ppt for an accumulation time of 45 seconds.

The pH used by Van den Berg et al. for initial complex formation, and in all subsequent solutions for analysis was 7.1, unfortunately, this is very close to the pH for minimum solubility of hydrated aluminium oxide. Downard et al. [51] have proposed that at this pH (7.1) buffer presence leads to rapid initial polymerisation of Al(III) in

natural waters, modifying the speciation and leading to a reactive aluminium fraction which is poorly defined.

Downard et al. [51] have recently reported a modification of the solochrome violet RS method for aluminium described by Wang et al. [47]. Room temperature complex formation was effected by maintaining the solution pH at 8.8 which eliminates the heating and cooling steps required at pH 4.5. The overall analysis time was reduced considerably as complex formation occured during the degassing stage, and an accumulation time of 1 minute as opposed to 10 minutes was sufficient to yield a limit of detection of 148 ppt. Linear sweep and stripping voltammograms were obtained using a static mercury drop electrode (SMDE) by scanning from -0.2 to -0.7 V. The optimum accumulation potential of -0.2 V differed significantly from that reported by Wang (-0.45 V) though this difference has been attributed to the use of different reference electrode potentials and SVRS solutions. The modifications described by Downard et al. have afforded a simpler and faster method of analysis; the 10 minute complexation reaction effected during the degassing step at room temperature is a considerable improvement.

Romero et al. [52-54] have described the determination of total soluble aluminium in water samples, particularly haemodialysis water (used for the preparation of dialysis fluids for chronic renal failure patients) using SVRS and metal-complex reduction at the static mercury drop electrode. In their most recent paper [54], two alternative sample mineralisation procedures coupled with a differential pulse polarographic-based detection method which have permitted determination of total soluble aluminium in haemodialysis water have been described.

Interferences from organic matter in the sample were negated by the use of closed-vessel microwave or high pressure digestion procedures. Following digestion samples were heated to 60^{o}C for 15

minutes and then cooled to ambient temperature. Optimised polarographic conditions included a potential scan rate of 5 mV s^{-1}, drop time of 0.5 s and a purge time of 2 minutes. The optimum solution pH was determined to be 5.3. The detection limits for aluminium were 6 and 3 ppb for microwave and high pressure mineralisation respectively.

5.3 Determination of Al(III) with Solochrome Violet RS using Surfactant Enhanced Adsorptive Stripping Voltammetry

5.3.1 EXPERIMENTAL

5.3.1.1 Reagents

All solutions were prepared from deionised water. A 1000 ppm aluminium stock solution was prepared by dissolving 1 g of aluminium metal in 20 cm^3 of hydrochloric acid and diluting to 1000 cm^3 with water. The SVRS (Aldrich Chemical Co.) stock solution 1 x 10^{-4} M was prepared daily. The supporting electrolyte was 0.2 M acetate buffer pH 4.5, prepared by mixing ammonium acetate and perchloric acid.

A stock solution (1 x 10^{-3} M) of the surfactant under investigation was prepared and appropriate dilutions were made. Surfactant solutions were degassed with nitrogen for 5 minutes before addition to either background electrolyte and/or sample solutions.

5.3.1.2 Instrumentation

Stripping and cyclic voltammograms were obtained with an EG & G Princeton Applied Research Model 264A Polarographic Analyzer/Stripping Voltammeter in conjunction with the conventional three electrode potentiostatic system. A home-made carbon paste electrode with a geometric area (0.031 $cm^{2)}$ was used as the working electrode, while a platinum wire served as the auxillary electrode. Potentials were measured versus an Ag/AgCl reference electrode. Voltammograms were measured on a J. J. Instruments PL4 Recorder. The cell was covered with

aluminium foil to prevent photodecomposition of the photo-labile SVRS. A magnetic stirrer (1 cm long, 2 cm thick) provided convective transport during the accumulation step.

5.3.1.3 General Procedures

5.3.1.3.1 Complex Formation

Microwave heating allowed for rapid Al(III)/SVRS-complex formation. Samples were prepared in 20 cm^3 test-tubes containing 10 cm^3 of supporting electrolyte (acetate pH 4.5 buffer), SVRS at a concentration of 6×10^{-5} M and various aluminium concentrations. The test tubes which were housed in a dark walled container were placed in a microwave (Husqvarna QN 1231) for 40 s, at setting 2 (240 W) and heated to 70°C. The temperature of each test solution was then measured to ensure that uniform heating to 70°C had occured. The solutions were allowed to cool to room temperature, 25°C for 15 minutes. Samples were transferred to the electrochemical cell where they were degassed with nitrogen for 5 minutes. The accumulation potential used was -0.4 V which was applied to the electrode under stirred conditions. A 5 s quiescent period was allowed between cessation of stirring and scanning to allow equilibration of the solution. Voltammograms were recorded by applying a negative potential scan to the electrode at 50 mV s^{-1}. The scan was terminated at -0.8 V and the adsorptive stripping cycle was repeated following a "cleaning scan.".

5.3.1.3.2 Soil Analysis

1 g of soil was shaken with 20 cm^3 of 66% HCl for 24 hrs. The sample was centrifuged and the resultant supernatant diluted with 19 parts deionised water. The pH was adjusted to 2.0 with 3 M NaOH (for storage purposes). Serial fold dilutions of the extracted soil samples were made with electrolyte before addition to test solutions containing SVRS (6×10^{-5} M). Microwave assisted complex formation was used and the aluminium content was determined by comparison with a standard curve.

5.4 RESULTS AND DISCUSSION

A preliminary investigation of the voltammetric behaviour of SVRS at the carbon paste electrode was performed. Repetitive cyclic voltammograms were recorded for a 5 x 10^{-6} M solution of SVRS in acetate buffer (pH 4.5) solution. Stirring the solution at -0.1 V prior to the scan resulted in one cathodic peak due to the reduction of the adsorbed dye (at -0.49 V), no other peaks were observed. A second scan directly after the completion of the first scan resulted in no cathodic peaks, which indicated rapid desorption of the dye from the electrode surface. This second scan was found to be an effective cleaning scan and no additional electrode surface renewal step was required prior to the next analysis. Electrode re-use was therefore possible, and passivation of the electrode surface was not observed during subsequent experiments. The relative standard deviation of the SVRS stripping current signal using this cleaning procedure was less than 1% for replicate scans, and the signal was extremely reproducible for an SVRS concentration of 1 x 10^{-5} M. No peaks were observed in the anodic branch of the voltammogram.

Using the same conditions, a sample containing aluminium at a concentration of 27 ppb (1 x 10^{-6} M) was also studied. No new cathodic peaks resulted and no shift in the SVRS peak potential was observed, however a slight increase in the dye peak magnitude could be observed by variation of its concentration relative to that of aluminium, this suggested that a different accumulation potential might be required. In a similar experiment Wang et al. [47] reported four cathodic peaks (at -0.39, -0.49, -0.61 and-0.75 V) when using an SMDE, the peak at -0.61 V was found to be due to reduction of the adsorbed Al(III)/SVRS-complex. They reported that when using an accumulation potential of -0.45 V only one cathodic peak at -0.61 V caused by the reduction of the adsorbed complex was

present. In preliminary experiments, variation of the accumulation potential indicated that -0.4 V was the optimum accumulation potential as signals due to the free ligand and the Al(III)/SVRS-complex were both evident. The second wave corresponding to the Al(III)/SVRS-complex appeared at -0.61 V but was very small and the peak profile was poor. Increased accumulation times did not improve the signal, however, the first peak at -0.49 V arising from the reduction of uncomplexed SVRS was shown to increase linearly with accumulation time.

The effect of surfactant presence on the Al(III)/SVRS-complex adsorption process was evaluated. Repetitive linear scans were recorded for the complex in the presence of (a) an anionic surfactant, sodium lauryl sulphate (SLS), (b) a cationic surfactant, cetyltrimethylammonium (CTA); both the chloride and bromide forms were tested (CTAC and CTAB) and (c) a non ionic surfactant, Triton-X-100. Both CTAB and CTAC enhanced the cathodic stripping signal but no beneficial effects were observed due to the presence of either SLS or Triton-X-100. Figure 5.1 (a) shows the preconcentration of the Al(III)/SVRS-complex in the presence and absence of CTAB. An experiment was performed to determine the optimum addition order for CTAB i.e. before complex formation, in the sample preparation stage or after complex formation to the cooled sample solution prior to analysis. Addition before complex formation did not enhance the signal to the same extent that addition after complex formation did (Figure 5.1(b)).

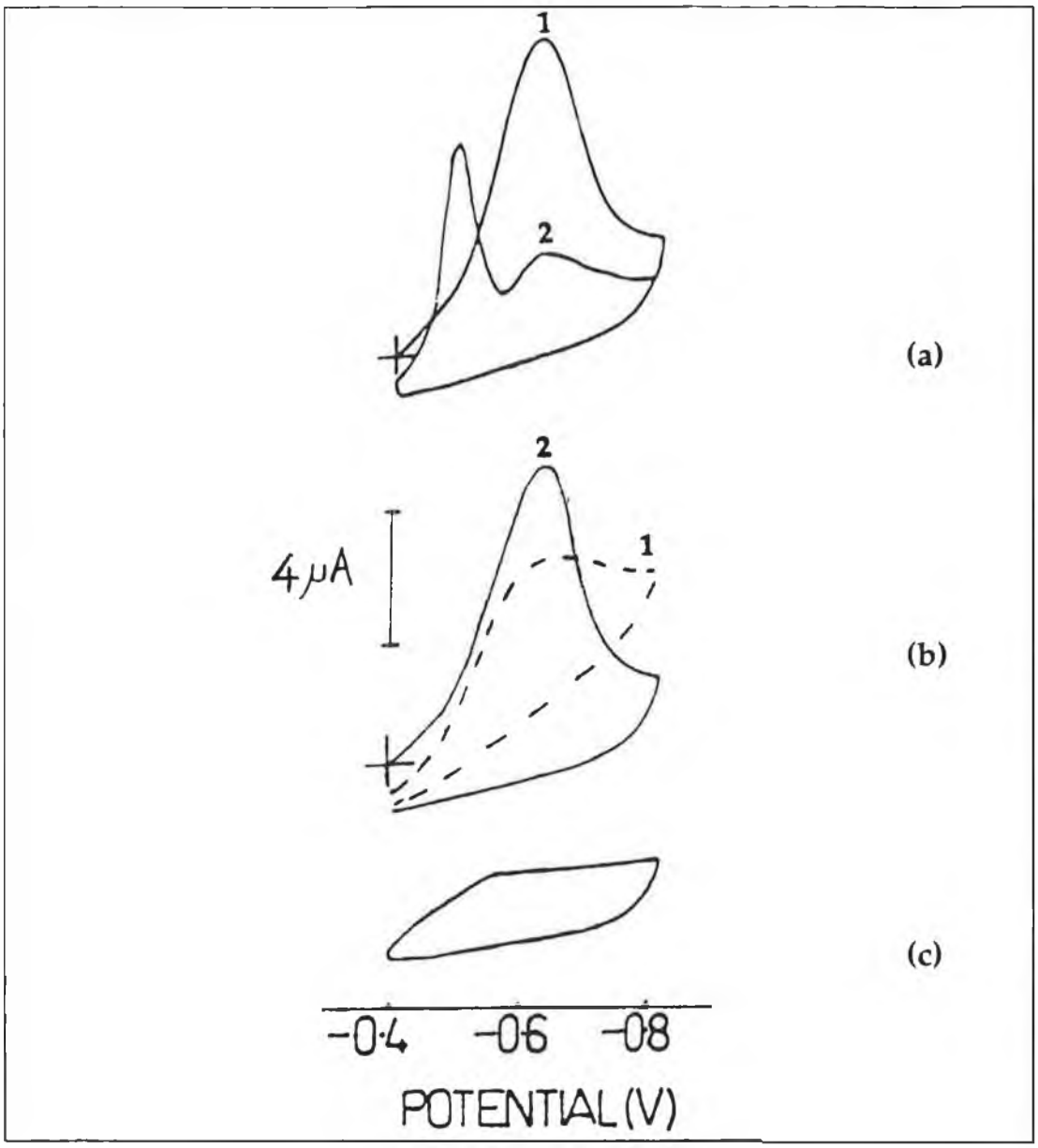


Figure 5.1 (a) Cyclic voltammograms for Al(III) / SVRS complex preconcentration in the presence (1) and absence (2) of CTAB for an aluminium concentration of 486 ppb (1.8 x 10^{-5} M) in acetate buffer solution (pH 4.5), containing 1 x 10^{-4} M SVRS and 1 x 10^{-4} M CTAB. Accumulation potential -0.4 V and scan rate 50 mV s^{-1}.

(b) Optimisation of CTAB addition order, (1) before complex formation and (2) after complex formation. Sample composition as in Figure 5.1(a).

(c) Background

Following optimisation experiments, the procedure adopted for CTAB enhanced complex adsorption for all subsequent experiments involved addition of CTAB to all samples at a concentration of 1×10^{-4} M. (Note: this concentration is below the critical micelle concentration for this surfactant (CMC = 9.2×10^{-4} M at 25°C)). Sample solutions were stirred at a rate of 500 rpm for 1 minute prior to adsorptive accumulation at the electrode surface. New electrodes were conditioned by immersion in a CTAB/acetate electrolyte solution prior to use and stirring at a rate of 500 rpm for 1 minute with no applied potential. Variation of the conditioning time above 1 minute did not increase the signal response.

Cyclic scans were recorded in the presence and absence of CTAB following accumulation at (a) -0.1 V to examine the resolution of the uncomplexed dye and complexed dye peaks and at (b) -0.4 V to examine the effect on the Al(III)/SVRS peak. The effect can be seen in Figure 5.2 for a -0.1 V accumulation potential. In the presence of CTAB, two peaks one at -0.455 V and the second at -0.61 V (well defined) were obtained. In the absence of CTAB only one peak not resolved from the uncomplexed SVRS species was obtained.

Accumulation at -0.4 V yielded a single peak at -0.61 V for the Al(III)/SVRS-complex, and no interference from the uncomplexed dye was present (Figure 5.1(a)). The SVRS concentration was 1×10^{-4} M, CTAB 1×10^{-4} M and aluminium 486 ppb (1.8×10^{-5}M). In the presence of CTAB an anodic shift in potential of the excess uncomplexed dye was noted (from -0.49 V to -0.455 V) which meant that the resolution of the Al(III)/SVRS-complex peak and the uncomplexed SVRS peak improved, thus the peak profile of the Al(III)/SVRS peak also improved.

Another advantage of CTAB inclusion is the increase in magnitude of the peak signal indicating enhanced preconcentration of the Al(III)/SVRS-complex at the electrode surface. Effects of accumulation

time, applied potential, stirring rate, scan rate, CTAB concentration, SVRS concentration and interferents on the Al(III)/SVRS adsorption process were all evaluated.

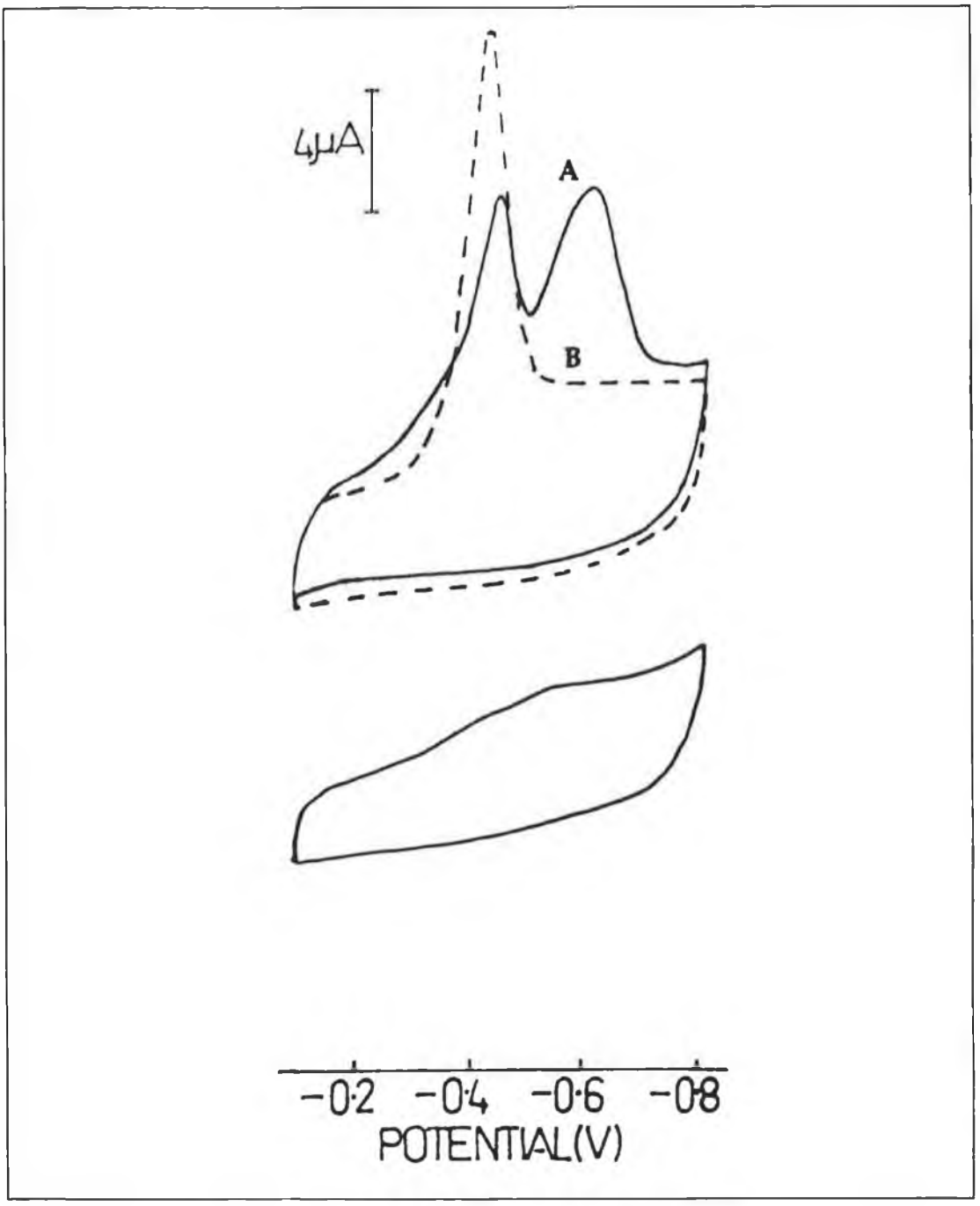


Figure 5.2 **Cyclic voltammograms for 486 ppb aluminium (1.8×10^{-5} M) in acetate buffer (pH 4.5), containing 1×10^{-4} M SVRS after 3 minutes stirring at 0.1 V in the presence (A) and absence (B) of CTAB.**

Note: *A cleaning scan following each analysis resulted in a constant background signal indicated at the base of figure 5.1 and figure 5.2.*

5.4.1 Optimisation Studies

5.4.1.1 Accumulation Time

The dependence of the adsorption stripping peak current on the accumulation time was examined following electrode conditioning for 1 minute in the background electrolyte solution. Longer accumulation times resulted in larger peak currents as more of the Al(III)/SVRS complex adsorbed on to the electrode surface. Two concentrations of aluminium were evaluated, both showed an initial linear dependence on time, however, for accumulation times greater than 120 s the linearity ceased. Electrode surface saturation by the complex at higher accumulation times was the most probable cause of deviation from linearity, see Figure 5.3. An accumulation time of 120 s was chosen for all further stripping measurements which allowed substantial complex preconcentration (10 - fold peak current enhancement compared with a 0 s preconcentration time in quiescent solution), while not adding significantly to the analysis time.

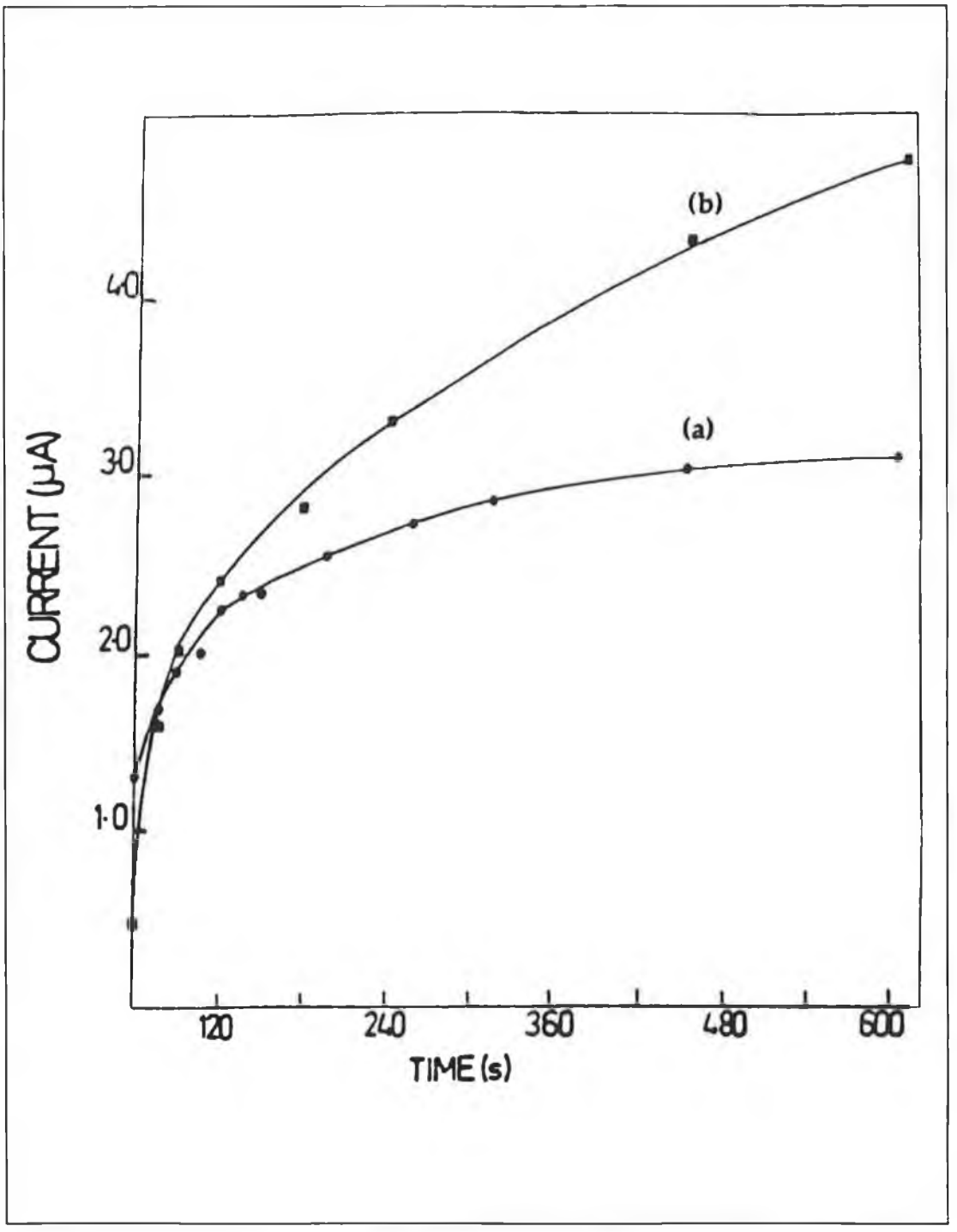


Figure 5.3 Dependence of the stripping peak current on accumulation time for [Al (III)] = (a) 27 ppb (1×10^{-6} M) and (b) 216 ppb (8×10^{-6} M), [SVRS] 5×10^{-5} M, and CTAB 1×10^{-4} M.

5.4.1.2 Stirring Rate / Scan Rate

Forced convection increases the rate of analyte transport to the electrode surface, therefore, the effect of stirring rate on the preconcentration process at the electrode surface was evaluated. The optimum stirring rate was determined to be 500 rpm. The effect of scan rate on the peak current stripping signal was also evaluated. The optimum scan rate was found to be 50 mV s^{-1}, higher scan rates did not increase the signal while lower scan rates only caused a slight decrease in signal magnitude. Variation of the scan rate did not cause any shift in the peak potential.

5.4.1.3 Accumulation Potential

The relationship between stripping peak current and accumulation potential was examined in the potential range -0.275 to -0.45 V. The cathodic wave of the uncomplexed SVRS was present until accumulation potentials more negative than -0.2 V were applied. The magnitude of the uncomplexed SVRS signal decreased significantly when these more negative potentials were employed. The magnitude of the Al(III)/SVRS signal was identical when accumulation potentials of -0.375 V and -0.4 V were used, however preconcentration at -0.45 V caused a 25% reduction in the magnitude of the signal. The uncomplexed SVRS wave was no longer visible in the cathodic scan when these potentials were used for accumulation.

If large aluminium concentrations were to be determined some experiments would necessitate the use of higher SVRS

concentrations so the more negative accumulation potential was chosen as the optimum for subsequent experiments, as this potential negates interference from the broad peak observed for free SVRS which was found to infringe on the complex peak if the SVRS concentration was high. Accumulation at -0.4 V destroys the free SVRS peak, leaving the peak of interest intact. Figure 5.4 indicates the preconcentration potential/peak current response relationship.

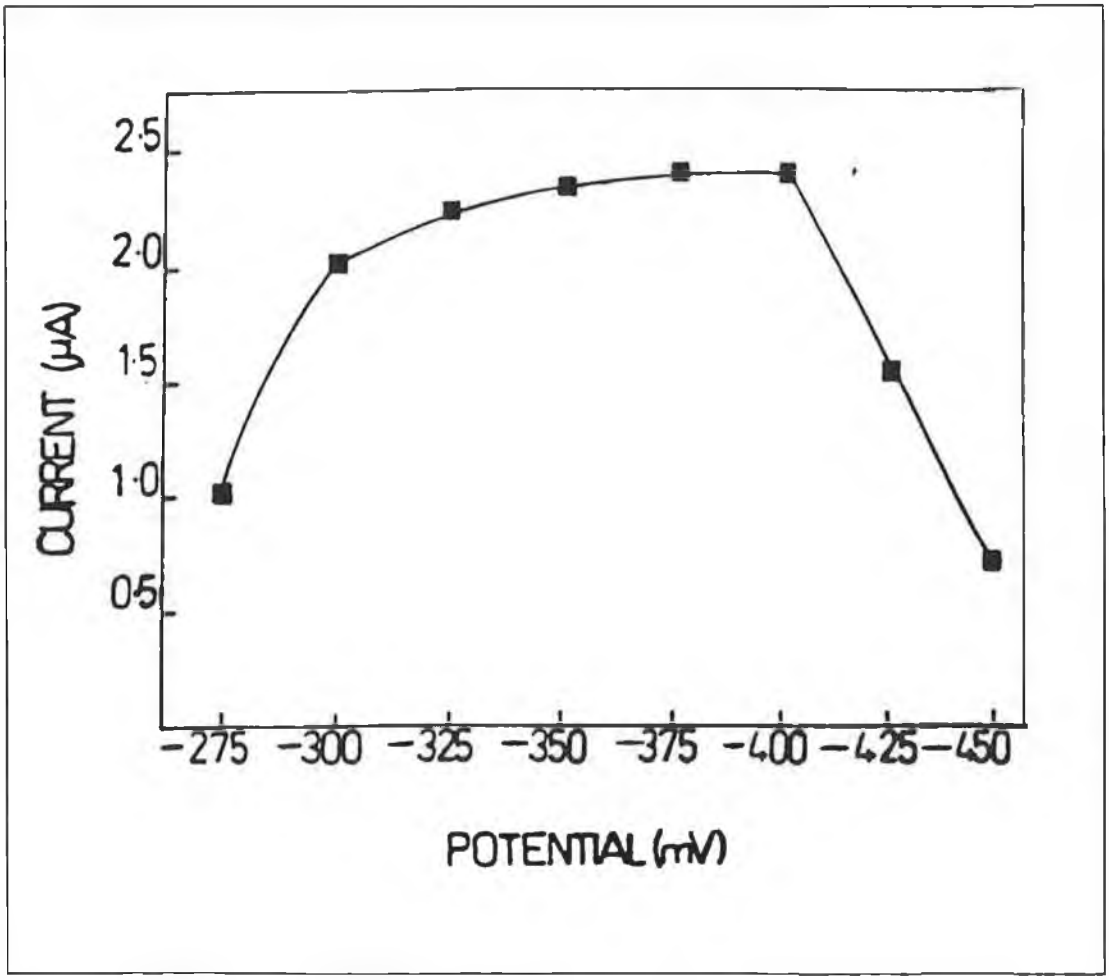


Figure 5.4 Dependence of the stripping peak current on the preconcentration potential applied. [Al(III)] = 216 ppb (8×10^{-6} M). Other conditions as for figure 5.3.

5.4.1.4 *SVRS Concentration*

The dependence of the stripping current on the SVRS concentration was examined. A peak maximum in the concentration versus current profile was obtained for an SVRS concentration of 6 x 10^{-5} M and an aluminium concentration of 216 ppb (8 x 10^{-6} M). The signal magnitude decreased significantly on either side of this optimum value. Linear dependence of the stripping current on SVRS concentration was only evident in a very small SVRS concentration window, 4 x 10^{-5} M to 6 x 10^{-5} M. Wang et al. [47] reported linearity between 1.5 x 10^{-7} M and 1 x 10^{-6} M SVRS. Such low concentrations were not feasible for our system. The non linear nature of our current versus concentration profile indicates that the adsorption process was affected by CTAB presence.

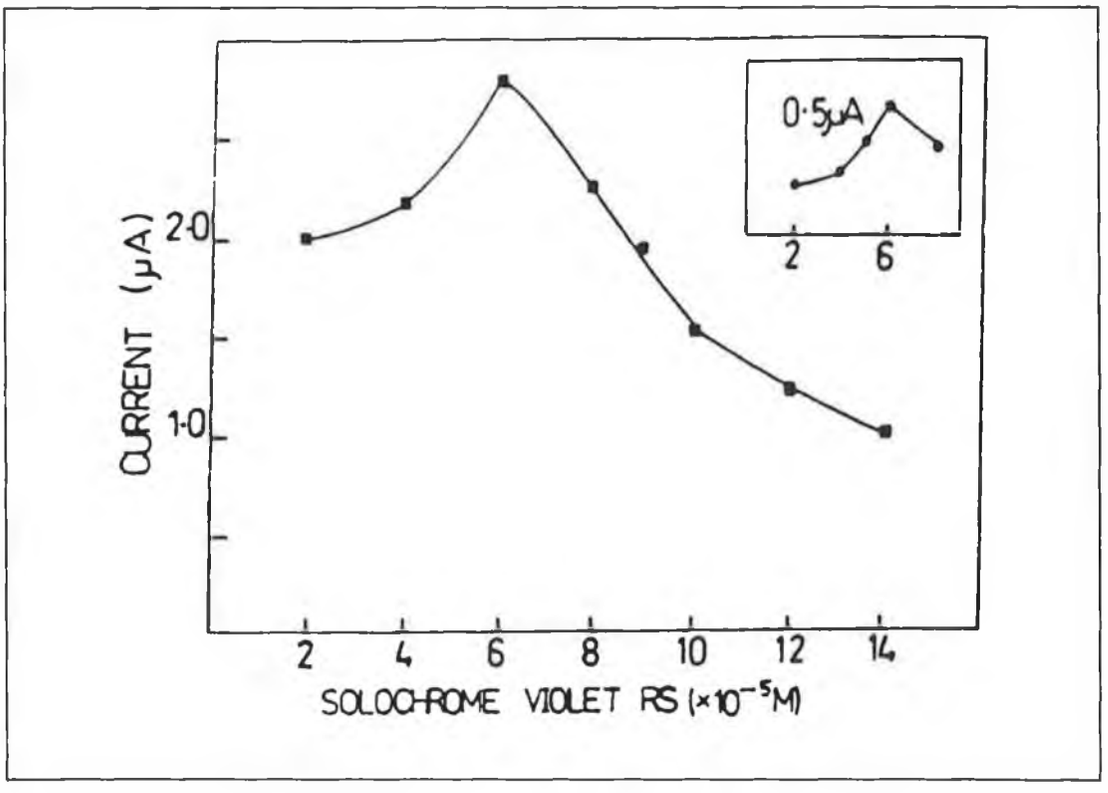


Figure 5.5 Relationship between SVRS concentration and stripping peak magnitude. [Al (III)] = 135 ppb (5 x 10^{-6} M)

5.4.1.5 *CTAB Concentration*

Improved resolution of the Al(III)/SVRS-complex peak and the uncomplexed SVRS peak arising from surfactant inclusion in the sample solution was described previously. The dependence of the stripping peak current on CTAB concentration was evaluated to determine an optimum concentration for experimental use. The response was found to be linear in the range 6 x 10^{-5} M to 1 x 10^{-4} M for CTAB and an aluminium concentration of 216 ppb (8 x 10^{-6} M) respectively. At concentrations greater than 1 x 10^{-4} M CTAB, the peak signal decreased significantly. A concentration of 1 x 10^{-4} M was found to be optimum for the system described.

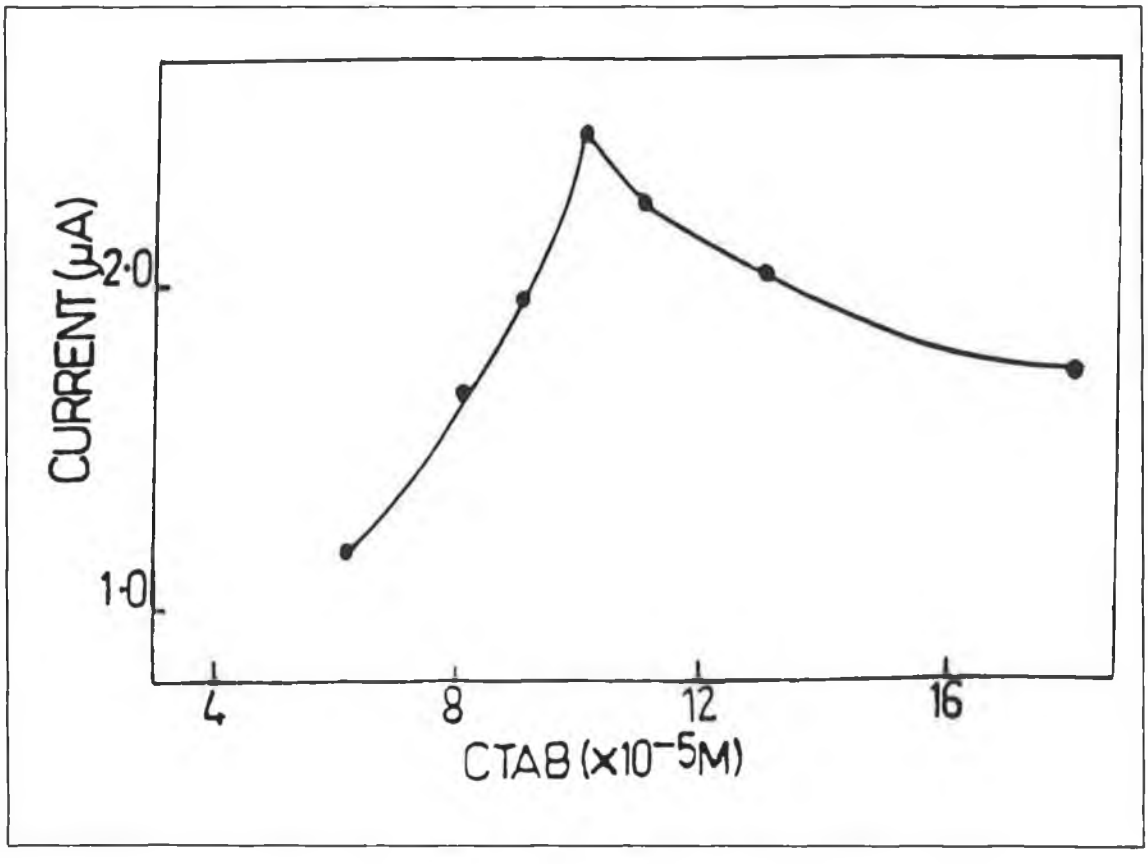


Figure 5.6 Effect of CTAB inclusion in the sample on the stripping peak current. [Al (III)] = 216 ppb, (8 x 10^{-6} M).

Variation of the stripping response for different aluminium concentrations, with increasing accumulation times with CTAB and SVRS present at their optimum levels indicates the true benefit of CTAB inclusion in the sample solutions. For samples not containing CTAB no response was obtained as can be seen in Figure 5.7. CTAB enhanced the adsorption process as is evident by the enhanced signal response. Higher concentrations of aluminium yielded greater current signal magnitudes for the same CTAB concentration.

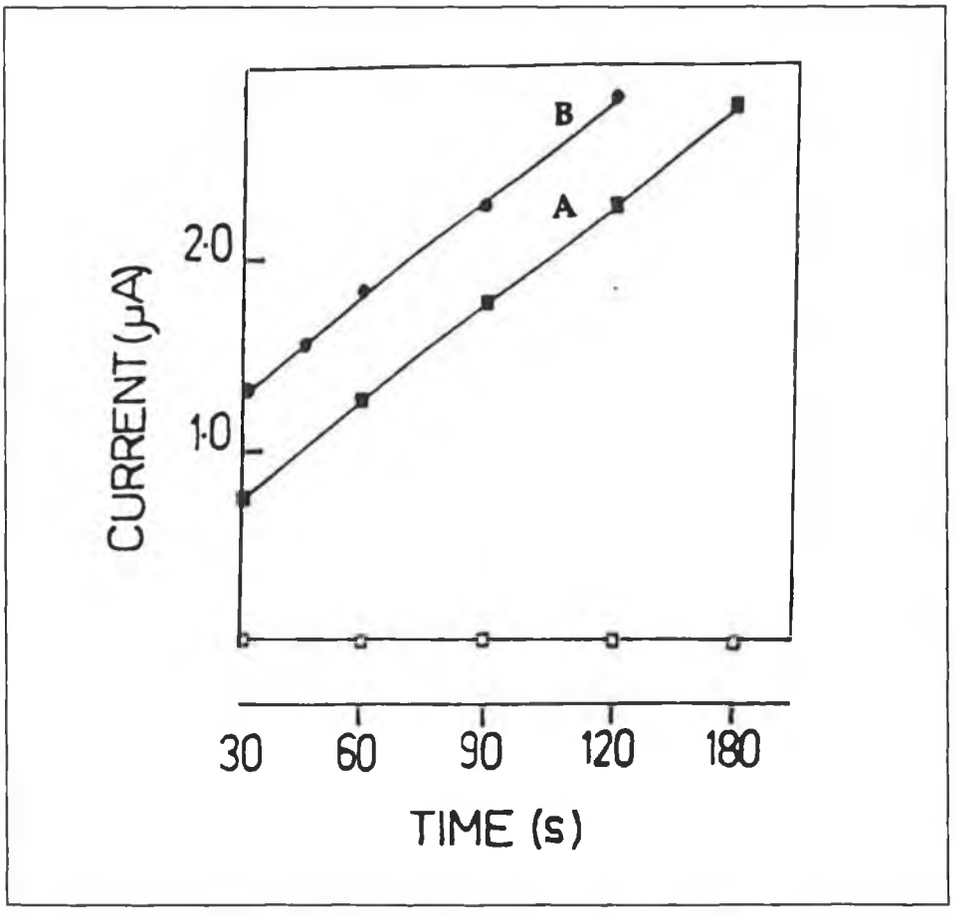


Figure 5.7 Dependence of the stripping peak magnitude on CTAB inclusion for different aluminium concentrations, (A) 27 ppb, (1 x 10^{-6} M) and (B) 216 ppb, (8 x 10^{-6} M).

Note: *The ---○--- plot indicates the response obtained for both aluminium concentrations in the absence of CTAB.*

5.4.2 Aluminium(III) Response

The quantitative utility of the method was assessed following the successful optimisation of experimental parameters. Cyclic voltammograms for increasing aluminium concentrations in the range 13.5 to 675 ppb (5×10^{-7} M - 2.5×10^{-5} M) were recorded following a 120 s accumulation period. The stripping peak current increased linearly with aluminium concentration and no deviation from linearity was observed in the range investigated ($r = 0.9999$, $n = 8$). At concentrations above 675 ppb (2×10^{-5} M), deviation from linearity was observed arising from electrode surface saturation. The accuracy of the method and the reproducibility of the signal for replicate electrode preparations was examined for an aluminium sample of 216 ppb (8×10^{-6} M). 10 consecutive analyses yielded a mean of 214.86 ppb (7.95×10^{-6} M) with a standard deviation of less than 2%. Standard deviation for replicate electrode preparations was 1.27%. The limit of detection was 13.5 ppb (5×10^{-7}M) for an accumulation time of 3 minutes.

5.4.3 Interferences

Interferences on the cathodic stripping signal were from two main sources, 1. uncomplexed SVRS adsorption at the electrode surface and 2. complexation by SVRS of metals other than aluminium in the sample, with subsequent reduction of these interferent complexes at the electrode surface. Use of an accumulation potential of -0.4 V reduced interference from uncomplexed SVRS to a large extent as did the addition of CTAB, so the main source of interference if present was assumed to be from other metal ions present in solution. However, the electrolyte pH of 4.5 alleviated the problem of complex formation by SVRS with many elements, most of which favour a more alkaline pH. Elements most likely to interfere were assessed, see Table 5.1 overleaf.

Cobalt was the main interferent, both the Al(III)/SVRS-complex peak and the uncomplexed SVRS peak were destroyed when Co(II) was added at a concentration of 295 ppb, (5×10^{-6} M). Interference from cobalt could be alleviated if the cobalt present was oxidized to the Co(III) form.

Ni(II) addition at a concentration of 29 ppm, (5×10^{-4} M) produced the most interesting result; the Al(III)/SVRS-complex peak was destroyed and a new peak appeared at -0.71 V. Chromium and iron when present interfered with the adsorption process to a lesser extent than Co(II). Reduction of Fe(III) to Fe(II) completely negated the interference on the signal from iron.

Cation	Concentration (ppm)	(moles/L)
Cu(II)	3.17	5×10^{-5} *
Co(II)	0.29	5×10^{-6}
Cr(III)	0.26	5×10^{-6}
Ca(II)	20.04	5×10^{-4} *
Fe(III)	0.28	5×10^{-6}
Ni(II)	29.34	5×10^{-4}
Zn(II)	32.69	5×10^{-4} *
Na(I)	22.98	1×10^{-3}

Table 5.1 Cation concentration causing a 25 % reduction in stripping signal

Note: *Above this concentration* the equilibrium of the SVRS complex was affected and the reaction was pushed in the direction of the free ligand. The complex peak was no longer visible in the CV and only the uncomplexed SVRS peak was evident.*

5.4.4 Soil Analysis

The possibility of applying the stripping procedure to soil samples for aluminium quantitation was evaluated. Both dissolution and digestion procedures were evaluated for metal ion extraction from the soil samples, however, digestion with HCL allowed higher concentrations of metal to be extracted. Various aliquots of the extracted sample were added to the SVRS/electrolyte solution and heated to 70°C as before. Fe(III) was the main interferent but could be eliminated by reduction to Fe(II) with ascorbic acid. Determination of aluminium using a comparative HPLC method yielded similar results, see Table 5.2.

Soil Type	Analytical Method forAl(II) determination (ppm)	
	CSV	HPLC
A	1900 ± 20	2000 ± 20
B	1790 ± 20	1710 ± 20

Table 5.2 Comparison of soil analysis techniques

5.4.5 Interpretation of Results

The Al(III)/SVRS-complex is considered to exist in the 1:1 form as reported by Florence et al. [43] who performed thermodynamic stability constant measurements for Al(III)/SVRS complexes in acetate buffer pH 4.3 and concluded that only 1 : 1 complexes were formed. Kinetic studies to gain further insight into the nature of the complex indicated that the reaction of Al(III) with SVRS proceeded via a hydroxy intermediate, the main reaction being given below.

$$Al(OH)_2^+ + H_2D^i \longrightarrow AlD^+ + 2H_2O \qquad \textbf{Equation 5.5}$$

D^i is the SVRS molecule with both hydroxy groups ionised and the charge on the sulphonic group neglected. How then does CTAB inclusion enhance the adsorption process for the Al(III)/SVRS complex ? Two possible mechanisms are proposed.

1. CTAB binds uncomplexed SVRS in solution and prevents its co-adsorption with the aluminium complex at the electrode surface; the positive ammonium group of the CTAB molecule attracts the negative sulphonic moiety of the SVRS forming an ion pair in solution. The aluminium complex which probably has a residual positive charge if 1:1 Al(III)/SVRS-complex formation is assumed repels the positively charged CTAB molecule and thus only uncomplexed SVRS is bound. This may explain why addition of the surfactant before complex formation does not enhance complex adsorption at the electrode as the CTAB may compete with the aluminium for SVRS complexation and reduce the effective number of SVRS molecules available to aluminium for binding. Addition

of the surfactant after complexation will only leave free SVRS molecules available to bind with CTAB.

2. Inclusion of surfactants has resulted in the formation of mixed micelles and/or ternary complexes as reported in several spectroscopic methods [5, 7]. Possible micelle formation in our sample solutions may have resulted in complex stabilisation which could enhance the adsorption process at the electrode surface.

A third possibility that the electrode surface was being coated with the surfactant and an ion pair mechanism was responsible for enhanced adsorption was thought initially to be the reason for the enhanced magnitude of the analyte signal. However medium exchange studies in which the electrode was removed from the test solution and placed in a surfactant free electrolyte followed by re-immersion in the sample solution was found not to reduce the signal magnitude indicating that CTAB enhancement takes place in the sample solution and not at the electrode surface. Considering the negatively charged nature of the SVRS molecule in acidic to neutral media it was thought that CTAB might also enhance the signal for uncomplexed SVRS if the ion pair formation was responsible for enhanced adsorption. As expected the peak signal for SVRS remained essentially unchanged in CTAB presence, unlike that of Al(III)/SVRS which was definitely enhanced. CTAB inclusion has therefore alleviated the problem of co-adsorption of free ligand at the electrode surface suggesting that the first mechanism proposed, is the one which is occuring whereby the free ligand is complexed in solution.

5.5 Conclusions

The stripping voltammetry of aluminium based on adsorptive accumulation of its SVRS complex at the carbon paste electrode in the presence of CTAB is a rapid method for aluminium determination. Surfactant presence significantly increases the stripping signal for the complex. The detection limit obtained was 13.5 ppb Al(III), the response was linear up to 675 ppb and the relative standard deviation for replicate preparations of the electrode [at 21.62 ppb Al(III)] was 1.27 %. The detection limit could be improved if the carbon paste working electrode was replaced by a carbon fibre electrode, this is under current investigation.

The use of the microwave for rapid and reproducible heating to effect complex formation significantly reduces overall analysis time. The renewable nature of the carbon paste electrode is an obvious advantage and it does not require an extensive re-activation procedure as do many mercury electrode systems. Successful application of this stripping method to the determination of Al(III) in soil extracts confirms its use for the analysis of real samples.

5.6 REFERENCES

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Appendix

1 *High performance liquid chromatographic determination of some trace metal ions using a novel column switching technique.* Ryan, E. and Meaney, M., Anal. Proc., Feb., 1991, 28.

2 *Determination of trace level Cu(II), Al(III) and Fe(III) by reversed phase liquid chromatography techniques.* Ryan, E. and Meaney, M., Analyst, Sept., 1992, 117, 1435.

3 *Surfactant enhanced adsorptive stripping voltammetry of Al(III) as its SVRS complex.* Ryan, E. and Meaney, M., manuscript submitted to Anal. Chim. Acta. for publication.

4 *Preconcentration of Cu(II) using Microbial Biomass.* Ryan, E. and Meaney, M., manuscript submitted for publication.

5 *On-line Preconcentration of trace metals using CTAB/DTC ion pair mini cartridges.* Ryan, E. and Meaney, M., manuscript in preparation.