TRACE METAL DETERMINATIONS IN AQUEOUS ENVIRONMENTS

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by

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I hereby certify that this material, which I now submit for assessment on the programme of study leading to the award of PhD 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: Fiona Regan **Date:** 5 October 1994.

Fiona Regan

To my brother
Francis

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TABLE OF CONTENTS

TRACE METAL DETERMINATIONS IN AQUEOUS ENVIRONMENTS.

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Abstract.

The accurate determination of metal ions at trace levels in environmental matrices is a complex problem and as a result, methods for determination and preconcentration of trace metals are continuously under investigation. In this thesis a number of approaches for metal determination and preconcentration are described.

Chapter 1 looks at trace metal speciation methods. Traditional and novel methods of metal determination and preconcentration are described including their advantages and disadvantages.

Chapters 2 and 3 describe the use of column-switching techniques for preconcentration of Cr(VI) and Cr(III) respectively. An ion-exchange chromatographic technique with post column derivitization detection is described for determination of hexavalent chromium, while a reversed-phase chromatographic technique, using complexation with 8-hydroxyquinoline, is reported for the trivalent species.

The use of dye-coated stationary phases for determination of Cr(VI) anion is described in chapter 4 in which exhausted reversed-phase HPLC columns are utilized to support methylene blue and crystal violet dyestuffs.

Determination of metal ions by capillary electrophoresis (CE) using on-column complexation with 4,2-pyridylazo resorcinol (PAR) followed by peak stacking is described in chapters 5 and 6. This method of trace enrichment achieved a limit of detection of 1.10^{-8} M for Co(II), Zn(II) and Fe(II). For Pb(II) determination, described in chapter 6, a 100 fold improvement in sensitivity was achieved using on-column complexation as opposed to precolumn derivitization.

Speciation of Cr(III) and Cr(VI) using two separation techniques is reported in chapter 7. Comparison of an ion-exchange chromatographic technique using post column derivitization detection, and a capillary electrophoretic separation technique is described. Both of these procedures allowed simultaneous determination of the two chromium species.

Chapter 1.0

Trace Metal Analysis in the Environment

1.1 TRACE METAL DETERMINATIONS USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY.

1.1.1 Introduction

The separation of metal species using HPLC is an area which has seen significant interest in recent years [1]. Interest has arisen in the application of HPLC and ion-chromatography methods for performing trace metal cation/anion, organometal and metal chelate type separations in complex environmental samples [2]. True metal speciation, calls for good chromatographic separations, together with element specific or element selective type detectors, notably spectrochemical type detectors. Techniques such as, flame atomic absorption (FAA), graphite furnace atomic absorption (GFAA), direct current plasma (DCP), inductively coupled plasma (ICP) and atomic fluorescence, interfaced with HPLC have been the interest of many researchers [3]. Considering the complete analytical procedure, however, preconcentration and separation of the analyte, prove frequently to be an indispensable step prior to actual determination [2]. Three distinct approaches have been applied to the determination of trace metal species, notably, ion-chromatography, ion-interaction chromatography and complexation chromatography.

1.1.2 Ion chromatography in trace metal analysis

Ion-chromatography (IC) is generally utilized in the non-suppressed mode utilizing high efficiency cation-exchange columns and indirect photometric detection after post column derivatization [4].

Spectrophotometric detection is frequently the detection method of choice by researchers using ion chromatography for trace metal analysis [5-11]. Detection is usually in the direct mode, but indirect photometric detection has been reported [11]. Pietrzyk et al. [11] utilized indirect detection for ion-exchange separation of anions and cations utilizing a mixed bed column. Chromophoric counterions (both anions and cations), notably benzyltrimethyl ammonium chloride and *p*-toluene sulfonic acid, were used as the eluent, with separation being achieved on Dionex HPIC-CS5 and HPIC-AS7 columns.

Post column reaction (PCR) detection is frequently employed for the determination of separated species [9]. A colour forming complexing reagent, which generates coloured complexes with high molar absorptivities at a specific wavelength in the visible region, is introduced following separation of the analytes. This enables photometric detection giving high sensitivity and selectivity [5,8,9]. PCR detection has been widely used in trace metal determinations using HPLC. Commonly utilized PCR reactions are those based on metal interaction with 4-(2-pyridylazo) resorcinol [7,12], diphenylcarbazide [9] and eriochrome black T [13].

When Small et al. [14] introduced ion chromatography for inorganic ion analysis, the detection mode used was conductimetric. Few recent determinations of metal ions have been reported using this method. Dionex [9] determined chromium concentrations in plating bath solutions by suppressed ion-chromatography with conductivity detection. Jones and Tarter [15] also utilized a Dionex ion-exchange and suppressor column to enable detection of six transition metal cations using conductivity detection. Fritz et al. [16] developed a method which enabled cation separations on a cation-exchange column of very low capacity using a very dilute eluent. This enabled detection by the conductimetric mode placed immediately after the separation column.

Detection by GFAAS, FAAS, or hydride generation atomic absorption spectrometry (HGAAS), [17-19] are widely used following separation by ion-chromatography. In conjunction with a preconcentration method prior to ion-exchange separation on anion and cation-exchange columns, Johnson [17] reported sensitivities in the low ppb range utilizing GFAA detection for Cr(VI) and Cr(III) species. A Dowex 1X8 ion-exchange resin was utilized for preconcentration and separation of inorganic selenium prior to detection by HGAAS [18]. These resins are based on crosslinked polystyrene and adsorb dissolved organic matter (DOM) from natural waters which would cause interferences in the selenium determination. An ion chromatographic separation of Cr(III) and Cr(VI) using DCP as an element selective detector was described by Urasa and Nam [20]. By using an anion or cation exchange column coupled with direct current plasma atomic emission spectrometry (DCP AES), separation and quantification of the two species was achieved without species conversion.

1.1.3 Ion-interaction chromatography

Recent investigations in the trace determination of metal ions have included studies involving ion-pair chromatography [21-23]. Ion-interaction chromatography (IIC) of complexed metal ions (cations or anions) is carried out on reversed-phase or normal-phase columns, employing an ion-pairing reagent, and photometric detection with post column derivitisation [1]. This is a very appealing procedure as it is analogous to IC but utilizes common RP columns.

Schuster and Hampel [21] utilized reversed-phase ion-pair separation with an eluent containing tartaric acid and sodium octane sulfonate. Post column reaction with pyridylazo resorcinol (PAR) for spectrophotometric detection of Fe(II), Mn(II), and Zn(II) in citric acid fermentation broth was reported. High levels of fermentation media constituents such as sugars and citric acid were not found to interfere with the determination. However interference arose due to high concentrations of Mg(II) and Ca(II) when the column capacity was exceeded. This caused problems for detection of Fe(II) and Mn(II) ions at the μgdm^{-3} range.

Trojanowicz et al.[22], investigated a high performance ion-pair chromatographic method for Cr(VI) and Cr(III) separation, using an eluent containing phosphate buffer with tetrabutylammonium bromide. This procedure involved the oxidation of Cr(III) to Cr(VI) using cerium(IV) sulphate, and detection was based on post column derivitization of the Cr(VI) species with diphenylcarbazide, for spectrophotometric detection at 560nm. Detection limits of 7 ng cm^{-3} for Cr(III) and 13 ng cm^{-3} for Cr(VI) were achieved.

Determination of Cr(III) and Cr(VI) with reversed-phase HPLC was described by Jen et al. [23]. Ion-pair chromatography utilizing an eluent containing acetonitrile and the tetrabutylammonium ion was described, with UV-visible spectrophotometric detection. Cr(III) was chelated with ethylenediaminetetraacetic acid (EDTA) prior to the analysis. As this reagent complexes readily with other metals to form anionic complexes, these complexes, as well as other anions might form ion-pairs and be retained on the column during elution. However on investigation, no interferences were found to occur. Detection limits were in the region of 0.4ng for Cr(III) and 1.6ng for Cr(VI) with a 20µl injection volume.

1.1.4 Chromatography of metal complexes

Chromatographic methods have been established for the ultra trace (ppb-ppt) determination and speciation of a number of metal ions. These applications often involve precolumn (or on-column) derivitization of metal species to form neutral complexes which are extracted and determined by reversed-phase liquid chromatography (RPLC) or normal phase liquid chromatography (NPLC) using photometric detection.

Three distinct roles for complexation can be identified:

(1) complexation in the mobile phase or stationary phase to effect partition coefficients and thereby to control retention times and resolution [24];

(2) the formation and chromatographic separation of discrete metal complex derivatives. The complex may be formed precolumn (either on-line or off-line) or on-column. Highly stable, non-labile complexes are desirable [25];

(3) post column derivitization as a means of metal ion detection as commonly used in ion chromatography [9].

Complexation of metal ions prior to HPLC separation is often desirable in order to improve column performance and selectivity, to increase the number of applicable detection systems, and to improve detection limits. In addition HPLC provides a closed environment which is important for complex stability. However factors such as insolubility of the metal chelates, lack of stability on column and decomposition of chelates during separation make separation of metal chelates by chromatographic methods difficult. No single ligand has been found to be suitable for all metallic ions. However a wide range of derivatizing reagents have been examined, of these β-diketonates [26,27], 8-hydroxyquinoline [28-30], dithiocarbamates [31-35] and PAR [36-38] have been most frequently used.

1.1.4.1 *Selection of a suitable ligand*

There are a number of important considerations when selecting a suitable ligand:

(1) The ligand should form neutral complexes with the metals of interest.

(2) The ligand should be stable and readily available in high purity.

(3) Complexes should be non-labile.

(4) Complexes should have a high detection sensitivity for example by

UV, visible, fluorescence or electrochemical detection [39].

Dithiocarbamate complexes constitute the most exhaustively studied group of ligands used in the chromatographic separation of metallic species [31-35, 40-45]. Common dithiocarbamates have low solubility in aqueous eluents. This limitation has been overcome by the addition of organic solvents to the mobile phase [43] or by modification of the dithiocarbamate structure [32-34] to enhance water solubility. This eliminates the need for solvent extraction prior to analysis. Aqueous mobile phases (typically of methanol or acetonitrile) present a problem with labile complexes. In such mobile phases inclusion of free ligand improves the performance [25]. Park and Hardy [43] used a reversed-phase liquid chromatographic technique for the separation of metal benzylpropionitrile dithiocarbamate (BPDTC) complexes. This method allowed the simultaneous determination of Sb(III), Cd(II), Cr(III), Cr(VI), Co(III), Cu(II), Pb(II), Hg(II), Ni(II), Se(IV), Ti(I) and Zn(II) species. In this case dichloromethane addition to the mobile phase was found to increase solubility and stability of the complex. In addition, free ligand was included in the eluent in order to reduce on-column dissociation of the complexes.

Bond and Wallace [24] utilized RPLC with electrochemical detection for the simultaneous determination of Cu(II), Ni(II), Co(II), Cr(III) and Cr(VI). These metals were precomplexed prior to HPLC separation, and subsequent electrochemical detection at gold, platinum and glassy carbon electrodes. In a subsequent report [33], ligand was included in the eluent, which was found to achieve quantitative complex formation.

Following on from those reports by Bond and Wallace [32], the same authors performed *in situ* formation of DTC complexes for simultaneous determinations of Cd(II), Hg(II), Co(II), Ni(II), and Cu(II). Excess ligand, was removed following separation by an ion-exchange suppressor column, to achieve improved sensitivity. Bond and Wallace [32] investigated three methods of analysis, notably, liquid-liquid extraction, direct complex formation in a suitable solvent and precolumn complex formation in which the complex is deposited onto a SepPak cartridge and then eluted for chromatography. Precolumn formation was found to be the preferred method [34].

4-(2-Pyridylazo) resorcinol (PAR) is a very sensitive chromophore, and chelates with a variety of metal ions [38], forming water soluble chelates with high molar absorptivities. As a result the ligand has seen widespread use for metal ion determinations in liquid chromatography. Roston [37] investigated the determination of the metal ions Cu(II), Co(II), Ni(II) and Fe(II) as PAR chelates, achieving a ppb level of sensitivity. The precolumn chelation mode of complex formation was utilized. V(V) and V(IV) speciation as PAR chelates by RPLC was reported in a recent publication by Tsai et al.[38], incorporating spectrophotometric detection.

Over 60 metal ions react with 8-hydroxyquinoline to form complexes. Use of 8-hydroxyquinoline and its derivatives in metal complexation chromatography has been widely reported [28-30,36,39,46,47]. This ligand has been immobilised on various resins and sorbents for metal ion concentration and separation and will be discussed at a later stage in relation to its importance in trace metal enrichment experiments. In a study by Jezorek and Freiser [36], 8-hydroxyquinoline was immobilised on porasil and its chromatographic use in separating divalent and trivalent metals was evaluated. The behaviour of Co(II), Cr(III) and V(V) chelates with 8-hydroxyquinoline was studied by Lajunen et al. [28] on a variety of stationary phases including reversed-phase columns. Complexes were prepared by mixing the ligand and metal, heating with subsequent extraction by chloroform. Eijarvi [29] utilized this ligand to determine Cr(III) and Cr(VI) species simultanouusly by HPLC with UV detection. Precolumn chelation with 8-hydroxyquinoline for determination of Zn(II), Al(III), Co(III), Cr(III), Cu(II), Ga(III), In(III) and Fe(III) by RPLC was examined by Baiocchi et al. [30]. UV absorption was utilized for on-line detection of the metal chelates.

Chelation of Cr(III) with 8-hydroxyquinoline in methyl alcohol, and subsequent extraction with isobutyl methyl ketone and electrothermal-AAS (ETAAS) was reported recently [46]. Heating of the metal / ligand solution was necessary due to the slow kinetics of complex formation, a factor which had also been reported by Lopez et al. [47] for Cr(III) complexation with this same ligand. Determination of Al(II), Cu(II), Fe(II), and Mn(II) as 8-quinolinol complexes by HPLC, with electrochemical and photometric detection was reported by Bond and Nagosa [39]. Direct formation and separation of the complexes was performed on-column, with the ligand contained in the eluent. It was found that direct injection experiments were

unfavourable for the determination of Mn(II) and thus an external complex formation method was applied.

8-Hydroxyquinoline ligands are the the most frequently utilized for metal determinations by complexation chromatography. However, continuing research in this area has led to other less well documented ligands. Most recently, the separation of ions by RPLC using complexation with 2-pyridinecarboxylic acid was reported [48]. This ligand has not seen wide use in this area, however it does have the attraction of forming stable water-soluble complexes with transition metals. Nesterenko et al. [48] demonstrated the applicability of this ligand in LC separation of Mn(II), Cu(II), Ni(II), Co(II), Cd(II), and Zn(II) with UV detection of the resultant complexes.

1.1.4.2 *Detection methods in complexation chromatography*

The most commonly employed detection method used in conjunction with complexation chromatography involves spectrophotometry, either by UV detection of the metal/ligand complexes or visible detection due to the formation of coloured complexes. For the most part metal DTC complexes are detected by UV-visible spectrometry [24,35,45]. Bond and Wallace [31] have also carried out electrochemical detection of the DTC complexes. As this ligand can form complexes in a wide range of oxidation states, it has been suggested that both oxidation and reduction processes are available for direct electrochemical detection in a non aqueous solvent [31].

Detection of PAR chelates is achieved predominantly by visible spectrometry at ~540nm [38]. However Roston [37], has carried out oxidative thin layer amperometric detection and fixed wavelength UV detection. This electrochemical property results from the resorcinol portion of the PAR molecule, which is oxidizable. This ligand has the advantage of forming water soluble chelates with a variety of metals.

Metal oxinate complexes have been detected using visible spectrometry at 400nm [25,39,47], or UV detection at 254nm [28,29]. Fluorimetric detection of Cr(VI) with 8-hydroxyquinoline-5-sulphonic acid was reported by Jie and Jiang [49]. Cr(VI) is a strong oxidising agent and can oxidise 8-hydroxyquinoline-5-sulphonic acid to give a fluorescent species. Bond and Nagosa [39] reported electrochemical detection of metal/oxinate complexes. Detection limits for Cu(II) and Fe(III) were 2.0 and 1.0 ng respectively for a 20 µl injection volume.

1.2 TRACE METAL SPECIATION

1.2.1 Why Speciate?

In contrast to a variety of environmentally important organics, metals have the particular property of not being degradable. Of particular importance for the transfer of metals in the environment are all types of natural waters rivers, lakes, ground water and sea as well as the atmosphere where precipitation plays a vital role [50]. In all types of natural waters toxic heavy metals are found normally at trace or ultra trace levels. Despite their occurrence at only trace levels, toxic metals constitute a very important group among the many chemicals of environmental significance in natural waters.

Most environmental studies dealing with toxic metals only quantify the pollutants as their total inorganic form following a total determination method such as AAS or AES [51]. The aim of speciation studies is to both identify and quantify the many species that together comprise the total trace element concentration. By definition, speciation of an element is the determination of the individual physico-chemical forms of that element which together make up its total concentration in a sample [52]. Measurement of the total concentration of a trace element provides no information about its bioavailability. Once metals have entered the environment, their potential toxicity is controlled largely by their physico-chemical form [52]. Some elements are essential to life, but for all these trace elements there exists a narrow concentration window between the essential and toxic levels. An element which is indispensable for normal body functions, may be highly toxic when present at higher concentrations, (for example selenium and vanadium) [52]. Arsenic containing compounds are a good example to illustrate the need for elemental speciation. The toxicity of As(III), As(V), monometalarsonate (MMA), and dimethylarsinate (DMA) has been shown to be significant, whereas there are also non-toxic forms such as arsenobetaine (ASB) and arsenocholine (ASC). Species of tin, lead, mercury and chromium also exhibit varied toxicities and have received considerable attention.

The various physico-chemical forms of a trace element present in a water sample are not necessarily in equilibrium with one another, but even if they are, any procedure applied to the sample may disturb the equilibria and

hence alter the speciation. An increasing awareness of species behaviour over the past few decades has triggered the development of new analytical methods.

1.2.2 Natural waters

The dissolved phase of natural waters can contain significant amounts of dissolved organic matter (DOM). The source of DOM is predominantly of natural origin. In polluted waters however a number of anthropogenic organic compounds can also contribute to DOM [50]. DOM components can significantly effect the speciation pattern of dissolved heavy metals in natural waters. Studies of organic complexation of trace metals in natural waters have revealed consistently that a large proportion of several trace metals occur complexed by dissolved organic material [53]. Metal ions interact in various ways with inorganic and organic dissolved components and particles in natural waters (river, lake and sea water). The relative importance of each of these interactions will change from one sample and locality to another. Special attention has been focused on dissolved organic compounds, in particular those termed humic substances, because of their potential effect on the bioavailability of toxic metals [54]. It is generally assumed that free metal ions are more toxic to aquatic biota than metal ions bound to large organic molecules like the humic substances. However in waters with a lowered pH due to acid precipitation, there is likely to be an increase of the inorganic fraction of trace metal species. This is of environmental importance because the inorganic fraction is the most active fraction in biological systems [55]. A metal can either have toxic effects or function as a nutrient [52]. In both cases, changes in the metal species present can have significant impact on a biological system.

1.2.3 *In situ* analytical methods for trace analysis

The collection treatment and preservation of samples for qualitative and quantitative analysis of species of one or several elements requires careful consideration and planning. The collection of a natural water sample, even surface water, for trace metal analysis runs the risk of contamination. The type of sampling bottle used must be considered. The most frequently used is the linear-polyethylene bottles, and treatments such as acidification and freezing affect the speciation. Speciation measurements have to be performed quickly

upon sampling, because of a possible change in the equilibrium during storage and transport of the samples.

In situ measurement of trace components in natural waters has proved an elusive goal of the analyst. Davison and Zhang [56], developed a simple technique for measuring trace metal concentrations *in situ* in water. The technique incorporates an ion-exchange resin separated from the solution by an ion-permeable gel membrane. Mass transport through the gel is diffusion controlled and thus well defined, making it possible to obtain quantitative data on concentration and speciation over relatively short time periods (from one hour to several weeks). Electrochemical methods are very suitable for the study of speciation because the electrochemical response is species specific [56]. Several studies on metal speciation, using electrochemical techniques have been reported [31-34,52]. Electrochemical techniques have a great advantage over most techniques since they can cause minimal perturbation of the aquatic medium during measurement. According to Mackey [57], ion-selective electrodes provide a method which is close to the ideal, as they do not appreciably consume or alter the sample, but detect an equilibrium potential produced by the interaction of the electrode surface and the active components in the solution. The main disadvantage of this technique is the lack of sensitivity in trace metal analysis [52]. Voltammetric techniques, such as ASV also do not greatly disturb the bulk sample and thus are very useful for *in situ* speciation.

Benson et al.[58] described the design and operation of an automated field monitor for the determination of dissolved aluminium in potable and treated waters using spectrophotometric detection with pyrocatechol violet. The authors reported a technique of flow injection analysis, in conjunction with solid state spectrophotometric detection. The on-line instrumentation used, had the attractions of rapid response and low cost, in addition to providing a technique with modular construction and easy on-site maintenance and calibration. Interferences from Fe(III) in potable and treated waters was overcome by reduction of this iron species to Fe(II), followed by complexation with 1,10-phenanthroline.

Achterberg and van den Berg [59] developed an automated voltammetric system for trace metal analysis in sea water. The system developed enables on-board measurement of samples using a voltammeter interfaced with a personal computer, a sample chamber, peristaltic pumps, a motor burette and a hanging mercury drop electrode [59]. The method enables

fully automated metal determinations, and speciation of Cr(VI) and Cr(III) in the mediterranean sea is described. Differentiation between tri- and hexavalent chromium was effected by adsorbing Cr(III) species on silica particles, following which the species was oxidised to Cr(VI) by UV irradiation and subsequently desorbed. A robust *in situ* field sampling technique was developed by Cox and McLeod [60], for chromium speciation in rivers. Microcolumns of activated alumina were utilized to retain the desired species, and on returning to the laboratory were analysed using flow injection-ICP-emission spectrometry.

1.2.4 Methods for measurement of trace metal species

Some of the first analytical techniques used for trace metal speciation were electrochemical methods which differentiated ionic from complexed metal species. Further refinements lead to "speciation schemes" in which metal species were classified according to their size, charge, polarity or chromatographic retention times. Quantitation was either by electrochemical methods or atomic absorption spectrometry (AAS). At present the two main approaches to trace metal speciation are computer modelling and combined experimental procedures.

1.2.4.1 *Computer modelling*

This approach to trace metal speciation in waters involves the use of published stability constant data, together with known concentrations of various ions and suspended solids in water to compute the equilibrium concentrations of the various species [61]. The main obstacle to the successful use of computer modelling for trace metal speciation in natural waters is the lack of reliable thermodynamic data. Reliable data are not available for metal ion interactions with the natural occurring ligands in waters [61,62], thus there is need to identify the important ligands in natural waters and measure the stability constants. Quinn and Taylor [63] proposed a chemical speciation program for personal computers, which would simulate chemical speciation in aquatic systems. The program developed, encorporates thermodynamic data, with formation constants corrected for ionic strength effects while simulating the aqueous speciation.

1.2.4.2 *Experimental methods*

In view of the limitations of chemical models, there is a need for development of reliable experimental methods for speciation. The method employed for measuring speciation must not modify the chemical composition or physical structure of the medium and should allow measurements to be made *in situ.* In addition, the method must be sensitive and selective, capable of determining concentrations between $10^{-10}M$ and $10^{-6}M$, which are typical concentrations of metals in natural waters [61]. Because of the low concentrations of metal ions in natural waters, experimental determination of the trace metals is a difficult task. The procedures used most frequently to study trace metal speciation in natural waters are anodic stripping voltammetry (ASV) and ion-exchange chromatography.

1.2.4.2.1 *Anodic stripping voltammetry (ASV)*

ASV is the main tool used in speciation schemes, such as those of Florence and Batley [64], and Figura and McDuffie [65]. ASV is one of the few analytical techniques that is sufficiently sensitive for direct determination of heavy metals in natural waters. A number of studies have shown the relationship between the ASV-labile fraction of the metal to the biological uptake by organisms [52], in turn giving an estimation of the potential metal detoxification capacity of the water sample (ie., complexation capacity) [50,62,66]. By contrast with the techniques such as atomic absorption and inductively coupled plasma emission spectrometry, which can determine only total metal in solution, ASV can determine not only total metal, but also ASV labile metal.

The ASV process however can be affected by sample solutes, thus affecting the correlation between ASV-labile metal and the toxic fraction. Florence [67], among other authors [68,69], found that the presence of detergent in a sample decreased the electrochemical response, but this did not correspond to a decrease in the toxic metal fraction. To overcome this, Florence utilized a double acidification technique, and upon using this method, good agreement was obtained between information on the ASV labile metal, and results from algal assays [67]. Cheng et al. [68], utilized ASV for the determination of the labile fraction of copper, lead and cadmium in rain water samples. Plavsic and Cosovic [69] also investigated the influence of surface active substances on metal speciation in natural aquatic environments. The effect of the non-ionic surfactant, Triton-X and humic acid was

investigated, and an inhibitory effect was observed in the presence of the surfactant.

Differential pulse anodic stripping voltammetric (DPASV) determination of zinc speciation in lake water was presented recently [70]. The technique involved exchange of the ligand, EDTA, with natural organic ligands, and measurement of the labile fraction by DPASV. Esteban et al. [71], presented the use of voltammetric techniques for the determination of chromium and arsenic species. Speciation schemes for both species were proposed. Voltammetry provided the possibility of distinguishing between total chromium and Cr(VI) (Cr(III) was converted to Cr(VI) by UV irradiation), and also between As(III) and As(V) as the latter is electroinactive, and is reduced prior to determination. In a report by Donat et al. [72], an approach using competitive ligand equilibration-cathodic stripping voltammetry was presented for Cu(II) and Ni(II) determination in a marine environment. The ligands, 8-hydroxyquinoline and dimethylglyoxime were used to compete with the naturally occurring ligands, and metal complex concentrations were determined by titration and cathodic stripping voltammetry (CSV) measurements.

1.2.4.2.2 *Ion-exchange chromatography*

Ion-exchange chromatography has been widely applied to the separation of ionic and non-ionic species. Non polar compounds can be resolved by ion-exchange chromatography by the formation of ionic complexes or by ligand exchange reactions. Haraldsson [55] advocated the use of a speciation scheme based on fractionation of metal species on three adsorbents, Chelex-100, SepPak C18 and Fractogel DEAE, which distinguished between labile complexes, non-polar organic adsorbable matter and ion-exchangable substances. Larsen et al. [73], utilized cation-exchange HPLC to separate six cationic arsenic species in sea food samples.

By far the greatest application of ion-exchange to speciation has been in the use of chelating resins. Although originally proposed for the total preconcentration of trace metals, their use in speciation studies expanded rapidly following the findings of Florence and Batley [64] that the resin was selective in the species it adsorbed. Chelex-100 is a styrene divinylbenzene (STDVB) copolymer resin incorporating iminodiacetate chelating groups. Conventional cation- and anion-exchange resins have been used for trace metal speciation [74], with Chelex-100 (an iminodiacetate containing resin)

being the most frequently utilized [75]. This resin binds ionic metals strongly, but excludes large molecules and colloidal particles, thus providing a simple, rapid and almost contamination free method for separation of ionic and colloidally associated metal [52]. This latter point is notable as it is these two fractions which represent the main classes of toxic and non-toxic metal in natural waters [52].

1.2.5 Speciation schemes

In formulating an approach to speciation measurements, it is important to consider the aims of the study and whether the planned measurement will provide meaningful data with these aims. The overriding justification for all speciation measurements is to identify those species which are likely to have adverse effects on biota and this includes bioavailability and toxicity studies [76].

Because there is no instrumental method available that is truly selective towards individual metal species at the concentration levels normally encountered in natural waters, various schemes have been developed which allow different operationally defined groups of metal species to be identified. Traditionally the basic speciation scheme has been to pass the natural water through a 0.45 μm filter. This separates the particulate from the dissolved fraction [57]. In most simple schemes, a distinction is primarily made between free metal ions and metal ions bound in inert complexes [76]. In the case of copper, lead and cadmium, the concentration of free metal ions is usually determined directly by anodic stripping voltammetry (ASV), while the total metal concentration is determined after digestion of the sample. The amount of bound metal is then calculated by a difference measurement [52]. The more elaborate speciation schemes may include a combination of separation methods, which fractionate according to size, charge, complexation or extractability. Separations based on chelating- and cation-exchange resins, and dialysis and ultrafiltration membranes are among the more frequently used methods [52]. The total metal concentration in each fraction is preferably measured by an instrumental technique which has low detection limits such as ASV.

The Batley and Florence [64] scheme for the determination of copper, lead and cadmium species involves four sample treatments, filtration, passage

through a Chelex-100 column, irradiation with UV light and chelex separation. For each treatment, labile metal is measured by direct ASV, and total metal by ASV after digestion. Later, Florence also included in his scheme other types of resins, and extraction with hexane-butanol to account for lipid soluble metal complexes. A scheme developed by Figura and MacDuffie [65] involved treatment of sample with chelex-100 in both column and batch modes. In this way they distinguished between labile, moderately labile and slowly labile species. Hart and Davies [77] combined Chelex and dialysis separations in their speciation scheme, with final measurement of copper, lead and cadmium by ASV while other metals were determined by AAS.

The most recently reported speciation scheme was that of Cheng et al. [68]. The scheme encorporated filtration and ultrafiltration of the sample, with graphite furnace AAS detection of the particulate and soluble phase fractions. Differential pulse ASV and chelex column and batch methods were used to determine the labile fractions. The scheme obtained information on the lability of species in rain water samples, ranging from labile, moderately labile, slowly labile and inert species. Chelex-100 resin provides not only suitable material for differentiation of metal complexes based on the affinity between the metal ion and the iminodiacetic acid chelating groups, but also a rapid method for separation of small metal species from macromolecular colloidally associated metal species [68].

1.2.6 Hyphenated analytical techniques.

Speciation techniques address only a fraction of the total metal present in the sample and therefore require ultra sensitive methods (ng dm^{-3}). Contamination free sampling is of the greatest importance since levels to be detected are extremely low. The stability of the chemical species during sample storage is a very critical step for environmental speciation analysis. In most cases, analytical speciation schemes rely on the combination of four basic stages, notably:

(1) analyte preconcentration;

(2) separation (chromatographic);

(3) selective detection (single or multielement);

(4) and their interfacing design at the instrumental level [78].

The demands for more reliable techniques in trace metal speciation have sparked improvements in instrumentation performance and design. One very successful approach to speciation has been to couple a chromatographic step with a detection system which is both very highly sensitive and selective for the element of interest [79]. Two chromatographic approaches are popular gas chromatography and high performance liquid chromatography. HPLC offers the possibility of separating a much broader range of analytes than GC but has a lower resolving power and is more difficult to interface to most detectors [78]. In order to be successful gas chromatography requires species to be volatile and thermally stable under temperature programmes designed for the analysis [3]. Liquid chromatography is more amenable to the separation of non-volatile, high relative molecular mass compounds, provided that suitable column packings and eluents compatible with the sample components can be found [3].

There has been a growth in hybrid chromatographic-atomic spectroscopic instrumentation for trace metal speciation [80]. A breakthrough in liquid chromatography interfacing with atomic absorption was achieved by Ebdon et al. [81]. Requirements of a suitable metal-specific detector include, high selectivity, sensitivity, simplicity and compatability with existing equipment [80]. The different options developed for speciation are the hyphenated techniques which use on-line sample pretreatment and concentration as opposed to analytical methods where sample pretreatment and concentration are performed off-line from the instrument set-up [3].

The major concern in the development of coupled techniques has been the interface [72,73,78,81-83]. A wide variety of applications involving coupled techniques are shown in the literature [3,83]. Coupled flow injection methods have been reported for speciation of Cr(VI) and Cr(III), Fe(III) and Fe(II), As(III) and As(V) and Se(IV) and Se(VI). However hyphenated chromatographic techniques are more widely documented [3]. Atomic absorption spectrometry is an attractive metal speciation technique due to its specificity when coupled to a chromatographic separation technique [82]. A novel approach to coupling HPLC-flame AAS was proposed by Hill et al. [83]. Three different interfacing techniques were described, notably, direct nebuliser coupling, a coupled hydride system and a direct transport system. The latter involves a novel transport system to take the eluate from the HPLC

directly into the flame. A similar hyphenated technique, utilizing direct inject nebulization was employed by Shum et al. [84], for arsenic and tin speciation.

There are many applications of HPLC coupled with flame AAS in speciation of organometallic compounds [82-83] which achieves poor sensitivity for environmental studies. However, sensitivity enhancement can be achieved by interfacing with atomic emission detectors such as DCP and ICP [79], and coupling is easily achieved using aqueous eluents.

Arsenic speciation using coupled techniques is by far the most widely documented [73,78,79,81,84-85]. Arsenic speciation was described using coupled chromatography-flame AAS, ICP-AES and flame fluorescence spectrometry [81]. The compatability of the liquid flow rate from liquid chromatography with the traditional sampling devices of ICP makes it relatively easy to interface ICP-MS according to Vela and Caruso [78]. These authors concluded from their studies, that chromatographic methods which introduce samples as gases, (using a spray chamber), such as supercritical fluid chromatography, provide the best levels of detection with ICP-MS.

Interface free coupling of HPLC and atomic spectrometry for trace metal speciation was demonstrated by Weber and Bernt [86] in a recent publication. They proposed a high performance flow, hydraulic high pressure nebulization (HPF/HHPN) for coupling HPLC / AAS. The principle of this procedure as described by the authors, is that, following elution from the HPLC column, the high pressure liquid stream is forced through a special nebulization nozzle ~10 - 20 μm. The authors reported an order of magnitude improvement in sensitivity using this form of coupling.

Because of the increasing demands of legislation on the levels of toxic substances in the environment, including heavy metals [87], and the inability of the most widely available detection instruments to reach these requirements, preconcentration / enrichment techniques have come to the fore, in order to enable detection of these ions at the levels at which they exist in the environment. The following section will deal with traditional and modern methods of trace metal preconcentration. Particular emphasis is placed on solid-phase techniques as these methods were employed in this study.

1.3 TRACE METAL PRECONCENTRATION.

1.3.1 Introduction

Trace metal preconcentration in the past involved the use of techniques such as coprecipitation, solvent extraction and ion-exchange. These techniques are still widely used and are well documented in the literature [88-96].

Solvent extraction usually involves a batch extraction procedure with repeated additions of solvent, the best results using this technique are obtained by a relatively large number of extractions with small volumes of solvent [88]. As a result this technique proves to be time consuming and prone to contamination, however, it has seen continued use even recently [88-93]. Solvent extraction techniques also suffer from several other limitations, including relatively low concentration factors (20-30 fold), possible emulsion formation and increased sample manipulation leading to increased errors [94].

Co-precipitation has also been successfully applied to the preconcentration of metallic species. Recently an on-line coprecipitation technique for trace enrichment of selenium was reported using lanthanum hydroxide as the precipitant, coupled with on-line flow injection hydride generation AAS [95]. Prior to this report precipitation was carried out off-line [96], which involved time consuming batch assays with lengthy equilibration conditions, a factor which, until this recent report, made coprecipitation an unattractive option for trace metal analysis [95].

Ion-exchange columns have long been used for preconcentration in trace metal determinations. Traditionally cation-exchange resins were used for enrichment of trace metals from aqueous solutions. However, as enrichment is based on non selective electrostatic interactions between cationic analytes and negative sites on the resin the major cations in surface water, eg., calcium and magnesium, occupy most of the exchange sites during preconcentration, causing only a small factor of enrichment for other metals of interest. As a result of this disadvantage in the area of trace metal enrichment, developments have taken the direction of more selective and diverse preconcentration supports. Chelex-100, an iminodiacetate-containing resin, is one of the most commonly employed chelating resins for the removal and preconcentration of trace metals [52,75,95]. However it is a time consuming

technique which needs careful washing procedures for the removal of certain cations.

1.3.2 Column trace enrichment techniques

1.3.2 *Principle*

A large volume of the sample is passed through a precolumn (approximately 10-100 cm^3) containing a cation chelating resin or chelating groups immobilized on glass beads, under conditions where the sample does not elute, but is adsorbed on the column. After concentration, the analytes are desorbed from the precolumn with a different solvent and the eluate is analysed. The success of sample preconcentration is dependent on reproducible and quantitative retention of solute ions by the concentrator column.

In liquid-solid partition the following equilibrium exists:

$$[A]_L \rightleftharpoons [A]_S$$

where $[A]_L$ and $[A]_S$ are the concentration of analytes in the liquid and solid phases respectively. By careful selection of a solid phase and solvent it is possible to either achieve, total retention of an analyte by driving the equilibrium towards the solid phase, or, total elution by forcing the equilibrium to the liquid phase [97]. Concentration methods based on the reversed-phase adsorption of complexed metals onto a small adsorbent column have shown promise in overcoming the limitations of solvent extraction [25,94]. This technique has the advantages of, (a) relatively high concentration factors and, (b) the ability to treat large volume samples in a closed system thus minimizing the risk of contamination [98].

1.3.3 Chelating sorbents

Column concentration sorbents which have been applied to the preconcentration of trace metals involve three types of methods, notably:

(1) a column packed with ligand immobilized material (ie. chelating resin) [99-102];

(2) a column packed with ligand impregnated sorbent [98-103];

(3) a method involving collection of a preformed metal complex with a sorbent packed column [104,105].

One of the most commonly used methods for preliminary concentration of metals from aqueous solution is based on the utilization of chelating sorbents. Many such materials containing different chelating groups have been proposed [75].

Chelating sorbents, also known as chelate resins and chelating ion-exchangers have been known for a long time [75]. Concentration with the help of chelating sorbents is characterised by a high selectivity which assures an increase in sensitivity and reliability of the determination. Several authors report the use of chelating sorbents with chelating and / or ion-exchange properties for trace metal preconcentration [100,101,105]. Chelate groups are usually capable of reacting with a large number of elements, but the stability of the formed compexes differs and depends on sorption conditions. This difference is used for selective concentration and separation of elements [75]. Immobilization of suitable chelating functional groups on polymeric supports results in chelating resins that are potentially more selective than cation exchange resins [74].

The majority of reports in the literature concern the use of chelation techniques for metal enrichment [98-105]. These techniques involve immobilization of sorbents such as polymers, reversed-phase ODS, ion-exchangers and controlled pore glass with a wide variety of organic ligands (particularly 8-hydroxyquinoline and dithiocarbamates) [75]. A number of authors have reported the use of resins based on crosslinked polystyrene with immobilized 8-hydroxyquinoline functionalities [100]. In a wide variety of applications this ligand has been immobilized on the sorbent [99,106], and in other instances the metal ion has been precomplexed with the ligand and subsequently enriched as the quinolate complex [25,98,107]. Advantage can

be taken of the difference in formation constants with different metal ions which may result in a more effective separation of trace metals from various kinds of sample matrices.

The chelating properties and selectivity of 8-hydroxyquinoline are well known, and it has been immobilized on several different substrates [75]. Abollino [108] investigated the immobilization of this ligand on Amberlite XAD-2 and an anion-exchange resin for enrichment of Ca(II), Cd(II), Cu(II), Mg(II), Mn(II), Ni(II), Pb(II) and Zn(II). Comparatively Isshiki et al. [98], reported Cr(III) preconcentration by precomplexation with 8-hydroxyquinoline and subsequent adsorption on a macroporous resin. Early reports of the use of chelating resins containing quinolin-8-ol groups have shown limitations of low exchange capacity and low chemical stability. Parrish [100] investigated possibilities of improving such properties by controlling the curing conditions. Immobilized quinolin-8-ol is another effective chelate. Preconcentration of lead was effected on resins synthesised from quinolin-8-ol and resorcinol with furfuraldehyde, formaldehyde or benzaldehyde as cross linking agents [106].

The most widely used techniques include resin chelation or sorption on inorganic and organic collectors (eg. SiO_2, modified silica, cellulose, cellulose derivatives, polyurethane foam, naphthalene and alumina). These supports will be discussed in the following section as their use in metal ion trace enrichment is a major feature in the literature [109-132].

1.3.4 Sorption on inorganic and organic collectors

More recently the use of new types of adsorbents for the concentration of metal ions has gained popularity because of their high concentrating abilities and overall simple operation.

1.3.4.1 *Poly (styrene -divinylbenzene) copolymers*

Amberlite XAD resins have been widely used as an adsorbent because of their high affinity for organic materials owing to their large surface area and macroporous structure [98]. Blain et al. [109] reported the use of XAD-4 and -7 impregnated with lipophilic macrocycles (for example, 1,4,7,10-tetraazacyclododecane) for enrichment of Cd(II), Cu(II), Mn(II), Ni(II), Pb(II)

and Zn(II) from deionized water and sea water. The highly crosslinked nature of the organic polymer matrix enables high exchange rates and improved complexation rates [109]. Following precomplexation of metal ions (Co(II), Cu(II). Hg(II) and Ni(II)) using sodium bis (2-hydroxyethyl)dithiocarbamate (NaHEDC) to form neutral complexes, enrichment was effected using a small column containing XAD-4 resin [105]. Similarly preconcentration of trace metals was achieved from sea water using the macroporous resin XAD-7 immobilized with 7-dodecenyl-8-quinolinol [98].

1.3.4.2 *Cellulose*

Some reports on the use of cellulose based sorbents have appeared in the literature [110-112]. Spherical cellolose sorbent material with chemically bound quinolin-8-ol was utilized for preconcentration of copper ions. Preconcentration was effected using a minicolumn packed with the spherical cellulose adsorbent [110]. Recently a cellulose based membrane filter was encorporated for trace enrichment from water samples. The procedure was treated as a form of solvent extraction in that the membrane filter acts as one of the solvents for the technique involving the distribution of a ligand 2-(2-pyridylazo)-5-diethylaminophenol (PADAP) / ion associate, between the membrane filter and the aqueous phase [111]. Enrichment of chromium species by selective preconcentration on cellulose sorbents was reported by Nagmush et al. [112]. Cellulose with sulphonic acid exchange groups was found to be effective for Cr(III) preconcentration whereas, a cellulose derivative with quaternary amine groups enabled Cr(VI) preconcentration.

1.3.4.3 *Naphthalene*

Naphthalene has been reported for use in preconcentration of cobalt in alloys and pepperbrush matrices by Satake and coworkers [113]. An ion-pair produced from an ammonium cation and tetraphenyl borate (TPB) anion was supported on naphthalene. Cobalt, complexed with a ligand 2-(5-bromo-2pyridylazo)-5-diethylamino phenol (5-Br-PADAP) was retained as a water insoluble Co-5-Br-PADAP-TPB complex on the surface of the naphthalene. Using dimethylformamide the solid mass was stripped from the column and detected directly by AAS. Detection sensitivity was in the low $\mu g\ dm^{-3}$ range. A solid chelating material, 2-mercaptobenzothiazole (2-MBT) on naphthalene

was utilized for the preconcentration of copper from natural water samples. This method was reported as being rapid, sensitive and economical due to the small quantities of naphthalene required (~ 0.4 g). It is particularly suitable for metal complexes insoluble in nonaqueous solvents [114]. No washing or elution of the material containing the adsorbed analytes is required as it is dissolved using a suitable solvent (eg. diethylformaldehyde). Satake et al. [115], also reported the preconcentration of titanium using an ion-pair supported on naphthalene with AAS detection.

The most recent report regarding the use of naphthalene for metal preconcentration involved complexation of the metal ion (Cd(II)) with quinolin-8-olate and adsorption of the complex on microcrystalline naphthalene. In this case the complex was desorbed using hydrochloric acid and determined by differential-pulse polarography following desorption [116].

1.3.4.4 *Polyurethane foam*

Polyurethane foam is well established in analytical chemistry for the extraction and preconcentration of various inorganic ions in their determination in environmental samples. The use of this organic collector was demonstrated for Fe(II) precomplexation [117]. The investigation concerned the use of a polyether type of polyurethane foam in the extraction and preconcentration of iron(II)-1,10-phenanthroline complex, from ceramic materials. A solid phase extraction procedure using a solid dithiocarbamate derivative, mixed with polyurethane foam was applied to the preconcentration of trace quantities of Cd(II), Co(II), Cr(VI), Cu(II), Fe(III), Hg(II), Mn(II), Ni(II) and Pb(II) [118] from analytical grade sodium salts. Analysis was carried out using atomic absorption spectrometry.

1.3.4.5 *Reversed-phase ODS*

The use of C18 bonded silica reversed-phase sorbent as an enrichment column material has been widely reported [25,119-121]. The interactions may take the form of ion-pair associations [103,120,121], or in many cases adsorption of metal ion chelates, precomplexed prior to concentration [25,102,119,122]. The majority of reports involve complexation of metal ions of interest with a dithiocarbamate ligand. Differences in procedures lie mainly

in the dithiocarbamate derivatives used which may lead to greater water solubility [122] or selectivity [102]. Ryan and Meaney [25] utilized a minicolumn of C18 reversed-phase sorbent for preconcentration of Al(III), Cu(II) and Fe(III) as their 8-hydroxyquinolate complexes. The breakthrough curves illustrated the selectivity of the procedure for Cu(II) and Fe(III) over Al(III). A similar approach using metal dithiocarbamate chelates enriched on a C18 column packing was proposed by Ichinoki et al. [104]. Due to the instability of these metal dithiocarbamate chelates it was necessary to include the ligand in the eluent, thus causing baseline disturbances.

Faltynski and Jezorek [101] described the chromatographic behaviour of several chelating agents covalently bonded by silica with an azo linkage. Their studies showed evidence of leaching of ligands from the surface of the silica substrates by the aqueous mobile phases used. Also it was shown that exchangers with silica bound ligands have low capacities, which, unlike those of exchangers with polymer supported ligands cannot be reproducibly varied by control of conditions during synthesis.

Ion-pair interactions involve the loading of an ion-pair, notably cetrimide-dithiocarbamate [103] ion-pair, prior to loading of the metal ion on the C18-bonded silica. The resulting non-polar complexes are retained on the sorbent. Determination of trace levels of gold(I) as its cyano complex by ion-interaction reversed-phase LC and preconcentration was described by Haddad and Rochester [120]. A similar approach by Irth et al. [123], utilized a cetrimide-dithiophosphate ion-pair, with dithiophosphate ligand for selective preconcentration of As(III), Sb(III) and Bi(III). Interference from metals present in the metallic parts of the HPLC system such as Ni(II) and Co(II) was not found to be a problem in the determination of As(III), Sb(III) and Bi(III). This was due to the instability of DTP chelates of Ni(II) and Co(II) under the acidic conditions used.

1.3.4.6 *Modified silica gel*

Many effective chelating agents, which are chemically bonded to or physically supported on various substrates have been employed for metals. Among the different immobilizing substrates silica gel is of particular interest, because it does not swell or strain, has good mechanical strength and can undergo heat treatment [124]. The ligand bonded silica gel adsorbents have

the advantage of stability but their preparation is complicated and time consuming. Further chelating agents which can bond to silica gel are limited [125]. Although resins loaded with silica bound chelating agents exhibit favourable kinetics, they are unstable at high pH values [125].

Use of chelating silicas was documented by Chambaz and co-workers [99] for the enrichment of Mn(II), Co(II), Ni(II), Cu(II), Zn(II), Cd(II) and Pb(II). Ethylenediamine triacetate-bonded silica was utilised for enrichment of these elements from sea water. Tong et al. [126], proposed the use of the ligand, 3-methyl-1-phenyl-4-stearoyl-5-pyrazolone as a chelating agent supported on silica gel for preconcentration of Cu(II), Co(II), and Ni(II). The method was applied to trace metal enrichment from sodium chloride solution and tap water. Using a $1dm^3$ sample volume, an enrichment factor of 40 was obtained with off-line AAS detection. A Calcon-modified silica gel sorbent was reported for preconcentration and/or elimination of up to 33 metal ions. The calcon functionality is more hydrophobic than some of the more commonly used molecules for modifying silica, thus it overcame the problem of elution from the sorbent, often a problem with other modifiers [127].

Other examples of the use of modified silica gel include, trace determination of Cr(VI) using a microcolumn containing Zr(IV) modified silica gel [128] and silica gel modified with titan yellow for preconcentration of 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) [128]. Silica gel immobilized with quinolin-8-ol was utilized for preconcentration of Cu(II), Pb(II), Cd(II), and Zn(II) from sea water. It was found that trace elements in sea water can be selectively preconcentrated and interferences eliminated using this chelating sorbent [129].

A noteworthy novel approach to utilizing silica gel as a support for trace metal preconcentration was reported recently by Mahan and Holcombe [130]. In their investigation algal cells were immobilized on silica gel and this substrate was utilized for lead enrichment. The results obtained, showed good reproducibility for the immobilization procedure. However the adsorption capacity of the algal resin declined by 15 % over 20 adsorption / elution cycles. Variability in results was observed with growth patterns of the algae and thus control of the adsorption capacity decline was difficult. However use of algal biomass immobilization on silica was shown to enhance analytical sensitivity significantly (ppt range).

1.3.4.7 *Activated alumina*

Alumina has been widely used in the past as a traditional inorganic ion-exchanger. Alumina can function either as a cation or an anion exchanger depending on the pH. It is used widely in the basic form for metal ion concentration as under basic conditions activated alumina displays a high affinity for a wide range of cationic species [131,132]. The use of this material as a sorbent for trace metal concentration has been demonstrated by a number of groups.

Basic alumina was utilized for enrichment of Cr(III) from urine [59]. Adsorption was effected using an alkaline carrier stream and desorption by nitric acid. Singh et al. [133] investigated the use of basic alumina containing sorbed tetracycline hydrochloride for concentration of eleven metal ions. The retention of the tetracycline hydrochloride on this substrate was found to be strong and stable, thus signifying the possibility of metal ion application. Breakthrough curves illustrated the selectivity of this sorbent for some metal ions particularly the selectivity of the method for Ni(II) over Cu(II). A similar approach was encorporated by Singh [133] for preconcentration of Pt (IV) and Cr(III). On this occasion alumina immobilized diethylethene-triaminepentacetic acid was utilized. Zhang and coworkers [131] investigated the use of basic alumina for enrichment of lead from potable waters. Using a 25 cm^3 sample volume, a detection limit in the ppb range was obtained.

McLeod and co workers [134] incorporated a minicolumn of activated alumina for analyte enrichment and matrix removal in an FIA-ICP AES system for the detection of trace phosphorus levels in steels. The manifold devised for the purpose incorporated a microcolumn of activated alumina with an analyte enrichment/matrix removal scheme. The authors illustrated the effective use of this sorbent as the phosphorus analyte of interest was retained on the acidic alumina, while interfering matrix cations such as Fe were unretained.

The most recent report involving preconcentration utilising alumina was reported by Dadfarnia et al. [132]. On-line preconcentration of lead using fibrous alumina was reported. The fibrous form of alumina displays similar characteristics to the powdered form, and was used as an on-line trace enrichment medium for Pb determination in drinking water. Very satisfactory limit of detection was achieved using a 15 cm^3 sample volume, the LOD was 0.7 μgdm^{-3}.

1.3.5 Ion-exchange materials for preconcentration

The most frequently used sorbent for trace enrichment is the commercially available Chelex-100 [75]. This resin has a polystyrene backbone and contains iminodiactate groups [75]. Problems associated with Chelex-100 include its slow exchange rates and, swelling and contraction with variation in the ionic strength of the solutions [6]. In addition to Chelex-100, recently reported [135] is the commercially available chelating resin chelamine for preconcentration of Cd(II), Cu(II), Mn(II), Ni(II), Pb(II) and Zn(II) from deionized and sea waters. The authors emphasised the high selectivity of the resin in the presence of alkali and alkaline earth metal ions. Caroli et al. [136] reported the use of a novel iminodiacetate resin encorporating ethylcellulose to serve as a chelating resin for preconcentration of Cd(II), Co(II), Cu(II) and Pb(II) from matrices such as sea water.

Olsen [137] described the use of a commercial ion-exchange resin for preconcentration of Cd(II), Pb(II), Cu(II), and Zn(II). Later ion exchange preconcentration of trace amounts of heavy metals was reported by Fang et al.[122], utilising a Chelex-100 column, and an ion-exchange resin of a salicylic acid functional group on a phenol-formaldehyde copolymer base. Hirate and coworkers [138], utilized a commercially available ion-exchange resin, Muromac A-1, which was found to overcome problems associated with the use of Chelex-100 resin, with the elements which form hydroxides easily at alkaline pH. The latter resin enabled preconcentration at acidic pH thus overcoming that hurdle.

Most recent uses of such commercial ion-exchangers for concentration involve an investigation by Ornemark and Olin [18]. Preconcentration of inorganic Se(IV) was achieved utilizing the anion-exchange resin, Dowex 1X8, with subsequent detection using hydride generation AAS. An important advantage of using the ion-exchange resin for this application, is that resins based on cross linked polystyrene adsorb dissolved organic matter from natural waters. Such organic matter can cause interferences in the determination of selenium (or any other species). Provided that the interferences do not co-elute with the analyte, use of such an ion-exchanger leads to samples less prone to contamination effects [18].

Jen et al. [139], exploited the differences in stability constants for selective retention of Cu(II), Ni(II), Co(II), Pb(II) and Zn(II) over calcium and magnesium, using an anion-exchange resin. Cation-exchange resins had been

used traditionally for enrichment of trace metal ions. However their use suffered due to the occupation of sites on the resin by cations such as Ca(II) and Mg(II) which occur in surface water samples. Thus by complexing the cations with a ligand, such as EDTA, forming anions with different conditional formation constants, it was possible to selectively adsorb the ions of interest and eliminate interfering ones.

An effective example of sample enrichment was reported by Ebdon et al., when analysing biological sample matrices [140]. A minicolumn of iminodiacetate based chelating resin was used to retain a number of metal ions (vanadium, manganese, copper, zinc, cadmium and lead) with simultaneous removal of interfering species, for example, sulphate, chloride, phosphate and sodium, with detection by ICP-MS.

1.3.6 On-line preconcentration

Some of the procedures described above involve off-line preconcentration techniques encorporating the use of mini-columns or plastic syringes containing sorbent. The analytes are desorbed and collected manually, prior to instrumental analysis off-line, generally by UV-visible spectrometry [114], atomic absorption [141] or ICP-AES [60,108].

1.3.6.1 *Reasons for on-line enrichment methods*

An alternative to using an adsorbent off-line, is the on-line incorporation of the adsorbent into the flow scheme of a liquid chromatograph as a pre-column, in advance of the analytical column. In this way concentration and clean up of samples become part of the chromatographic operation and are accomplished via switching of valves. Preliminary sample handling can therefore be minimized.

On-line preconcentration systems have proved to be the most interesting and effective way to improve the performance of AAS in trace ion determinations. ICP has a significant drawback in its inadequate detection power in the trace region. As a result techniques encorporating flow injection analysis (FIA) have been developed [107,136]. On-line solid phase extraction

systems are dominated by two processes, notably, that of on-line flow injection and on-line column switching techniques.

1.3.6.2 *On-line flow-injection methods*

Combining flow injection sorbent extraction preconcentration and separation on-line with ETAAS or HGAAS detection has been shown to provide effective separation of the analyte from the matrix, low contamination and low sample consumption, high sample throughput and very satisfactory detection limits [102,106,132,140]. The FI manifold for on-line preconcentration consists primarily of minicolumns containing chelating resins, a peristaltic pump, an injection valve, multi direction tap and detector (FAAS or ICPAES).

Among the different automated techniques for the determination of trace metals, flow injection analysis has attracted increasing attention because of its high precision, high sampling rate and the possibility of on-line sample pretreatment [132]. Continuous flow methods play a key role in process analyser design for the monitoring of chemical parameters, and flow injection methodology has been recognised as a valuable tool for this purpose [142]. The versatility, simplicity and sturdiness of systems based on this technique permit the construction of process analysers with very low instrumental and maintenance costs. These advantages were demonstrated by Benson et al [58] who developed an on-line FIA system for determination of residual aluminium in potable and treated waters.

The lack of sensitivity of these systems however, prevents their use with samples with large fluctuations of analyte concentrations. To solve this problem a FI procedure based on the sandwich technique [142] has been proposed. The sample was inserted at a slow flow rate between two 1,5-diphenylcarbazide solutions of different concentrations and which also had different flow rates. A slightly faster flow rate at the rear enabled concentration of the analytes.

1.3.6.3 *On-line solid-phase extraction with column-switching*

1.3.6.3.1 *Introduction*

Methods of liquid chromatography without column switching can be classified into two categories.

(a) Single-step methods, in which a small volume of the sample medium, diluted if necessary, is directly injected into the analytical column after centrifugation or dilution.

(b) Multi-step methods, in which several purification steps are carried out before chromatography (extraction, back extraction, cartridge purification etc.).

Switching devices permit off-line multistep methods for sample treatment to be transformed into single step procedures by the on-line purification and enrichment of samples. These systems should allow, firstly the injection of a large volume of sample to achieve adequate sensitivity and secondly, good reproducibility of the chromatographic steps [143]. When large volumes are injected, good reproducibility may be obtained if the columns are not over loaded, ie., if the linear capacity of the column is not exceeded. This means that the first column should be filled with some high-load-capacity packing material. Such materials are usually totally porous materials, stable and highly bonded with an average particle diameter of 10-40 μm.

1.3.6.3.2 *Principle of column-switching*

Because of the inherent disadvantage of off-line solid-phase isolations ie. relatively limited reproducibility, and laborious sample handling, the application of on-line automated column-switching techniques has proven to be very advantageous to the analyst. In general, column switching is a method of directing chromatographic eluents to different paths within the on-line system, by means of valves. When combined with automatic switching of pumps, samplers, detectors, and other apparatus, valve switching allows the automation of those areas of chromatography that can be time consuming. Some applications of valve switching include the following: sample clean up,

trace enrichment, sample identification, boxcar chromatography, multicolumn chromatography, and semipreparative chromatography.

Direct injection methods utilizing column switching have a number of advantages, notably:

*reduced analysis time;

*increased sample throughput;

*improved accuracy in quantification, due to minimized risks of chemical changes in the analytes, because of the reduced number of steps necessary. The fewer steps result in conditions precedent for improved precision.

Column-switching procedures have been applied widely in gas chromatography during the past few years [143,144]. Apart from its protective function, a guard or precolumn, as is often used in column-switching HPLC, may also act as an extraction column. By placing a switching valve between the precolumn and the analytical column the eluate from the precolumn may be either diverted to waste or into the analytical column, depending on the position in which the valve is set [97]. Any predetermined portion of the eluate may then be allowed to enter the analytical column for analysis. The analyte is first concentrated on the precolumn. The valve is switched to connect the precolumn with the analytical column, and an appropriate mobile phase is used to elute the analyte into the analytical column for analysis. At the end of the analysis the valve is again switched to the position which diverts the eluate to waste. An eluent containing a high proportion of organic modifier (in the case of reversed-phase material) is then used to clean the precolumn for subsequent operation.

1.3.6.3.3 *Column-switching apparatus*

Column-switching systems typically consist of a solvent delivery pump(s), switching valves and a precolumn manifold. Figure 1.1. shows a typical schematic of a 6-port 2-way valve column-switching system, as it is the valve system utilized in these studies. These valves are available commercially from manufacturers such as Valco and Rheodyne, and are the most widely documented in the literature for a variety of applications [145].

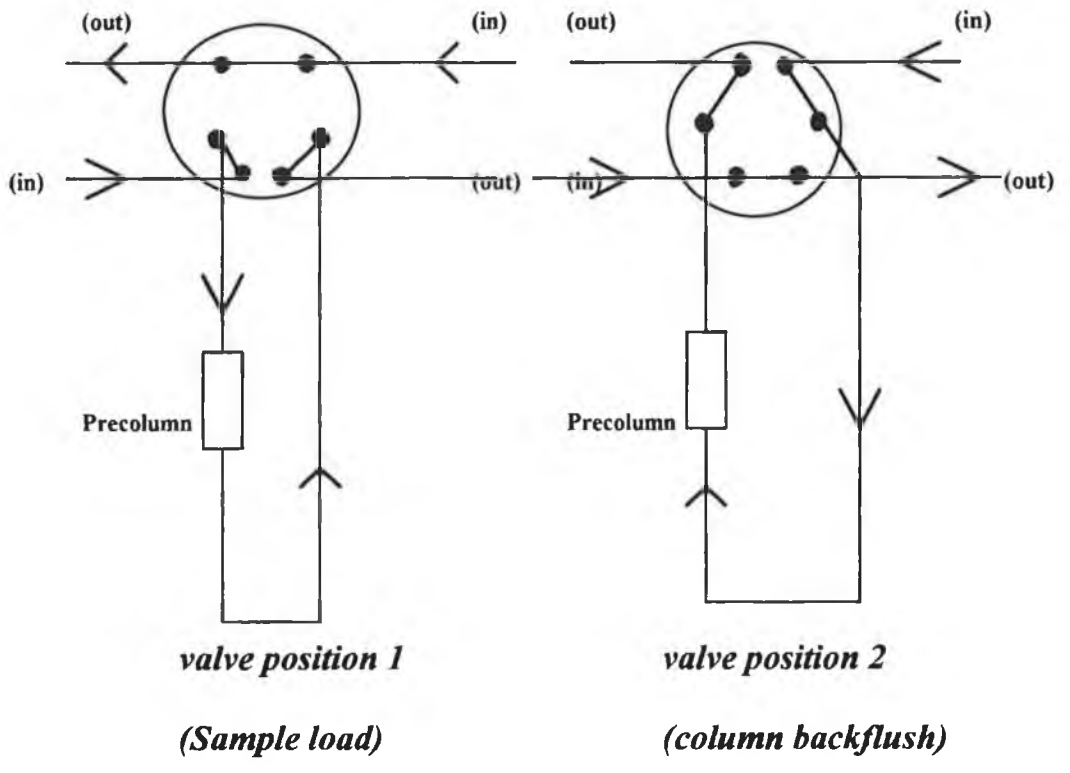

Figure 1.1 ***Diagram of a Six-Port 2-Way Column Switching Apparatus***

1.3.6.3.4 *Column backflushing*

Backflusing is a technique in which the sample components are eluted from the concentrator column in the opposite direction to that in which they were loaded. The sample ions are bound as a narrow band close to the input end of the concentrator column, allowing sample ions to be stripped in the opposite direction to which they were loaded. As a consequence, back flushing

results in sharp easily quantitated peaks. Backflushing can be used for sample elution and/or precolumn wash procedures. In the latter, it is an essential step, particularly in instances when a complex matrix is injected and forward flush would create clogging of the precolumn. This mode of column-switching is the most widely used for biological applications in sample clean-up.

Ascalone and Dalbo [146] demonstrated precolumn cleanup for removal of proteins and salts from plasma samples, using the backflush mode of column-switching. Owing to the cleanup procedure, the precolumn has a long life, and replacement of the column was unnecessary below 100 plasma injections.

1.3.6.3.5 *Sample cleanup*

Probably the most important application of column switching is sample cleanup. Sample cleanup is essentially a multicolumn technique and can utilize a wide variety of separation systems such as size exclusion, ion-pair, ion-exchange, adsorption and reversed-phase. The principles of sample cleanup are such that the zone of interest is selected and all other zones are directed to waste. This has been demonstrated by Little et al. [145], utilizing a large number of valve systems and columns for cleanup of a complex sample matrix. The potential of column switching for automatic sample cleanup can be demonstrated using three applications which cover a wide range of uses, notably front, heart and end zone cutting. Column-switching utilizing a front cut technique, enables direct analysis of samples without contamination of the analytical column. By using multi column separation of a sample [147] a heart cut can be taken from one column and transferred to a column of different stationary phase where, after a solvent change, analysis can take place.

Accurate determination of the heart-cut timing interval is essential in this technique. Standards are injected and the time for the onset of the analyte peak and complete elution of the analyte peak are noted. This time interval is the heart-cut. The entire system is then equilibrated, and a sequence of events is carried out as follows:

(1) diversion of the initial portion of the chromatogram to waste;

(2) the heart-cut is introduced to the analytical column and detector;

(3) clean-up of the precolumn.

The selection of the cut depends on estimation based on previous chromatographic separation and on inspection of the chromatogram obtained on the first column. A difference of several seconds in taking the fractions can result in a significant shift in the concentrations of the individual compounds in the fractions separated from the relevant heart-cuts [147].

Hotter et al. [148], proposed an on-line sample clean-up for urine samples for the analysis of prostanoids, with sample extraction and purification using column-switching. The automated system described consists of four programmable six-port valves. Villasenor [149] discussed the use of heart-cut column-switching techniques for matrix elimination for determination of anions in concentrated hydrochloric acid. The principle of the technique is such that the bulk of the matrix is diverted to waste and only a heart-cut of the analyte of interest is transferred to the separator column. The heart-cut column-switching technique can be extremely effective in either eliminating or at least simplifying sample preparation.

Asakawa et al. [150], observed problems of mobile phase compatibility associated with non-volatile mobile phases with a liquid-chromatographic-mass spectrometry (LC-MS) system. These hurdles were overcome by encorporating a "heart-cut" technique to separate the peak of interest from the efluent from the analytical column. A complex valve switching system was used, incorporating three analytical HPLC instruments, each running independently. Column 1 served to separate the components of interest, 2-served to trap the species and the third to pass the compounds to the MS system after final separation. This allowed the authors to overcome limitations of LC-MS interfaces so that optimum mobile phases (containing non-volatile buffers) could be used to separate the compound of interest.

1.3.6.4 *Traditional applications using column switching techniques*

Much of the research to date involving on-line column switching has concentrated on biological applications. Column switching systems previously applied in liquid chromatography are of particular interest when one or several minor components are to be quantified in a complex matrix [151]. One such application is the determination of drugs in biological fluids, where the drug and possible metabolites are present at low concentrations in a complex medium.

Determination of drugs in complex biological samples like blood plasma by gas or liquid chromatography frequently involves sample clean up steps, such as solvent extraction and protein precipitation, prior to injection onto the chromatograph. This is not only because of the large number of potentially interfering chromatographic peaks obtained from the plasma, but also because of high molecular weight components of plasma which may seriously contaminate or even plug the chromatographic column. In addition determination of trace levels of drugs generally requires prior concentration of the drug in order to raise its signal to noise ratio to enable accurate quantification.

Werkhoven-Goewie et al. [151], utilized a column-switching apparatus consisting of 3- and 10-cm cartridges containing CN-bonded silica packing material. A 10 port switching valve was mounted with a $1cm^3$ injection loop and a precolumn. A backflush step was used as a column wash procedure. The entire automated sequence of events for preconcentration and desorption / analysis of secoverine in blood samples is presented.

Hux et al. [152] advocated the use of precolumns of Amberlite XAD-2 (macroporous resin) for direct injection and determination of Methaqualone in blood plasma using LC. Column-switching was encorporated utilizing a six-port switching valve. The analyte was eluted from the precolumn in the backflush mode following the removal of plasma matrix components.

The characteristic reduction in analysis times achievable using column switching is illustrated for the analysis of the diuretic, ethacrynic acid in urine by Campins-Falco and co workers [153]. By encorporating a six-port switching valve and a precolumn of reversed-phase C18 material, effective retention of the analyte was achieved in addition to thorough sample cleanup. Applications by the same author demonstrated a similar utilisation of column

switching for screening other diuretics in urine samples. In these, different times for flushing the precolumn were investigated in order to optimize the selectivity and sensitivity. The backflush mode was preferred to minimize the dispersion of the sample into the chromatographic system. Thus no peak broadening was observed in comparison to direct injection of the samples onto the analytical column.

Elrod et al. [154] demonstrated on-line column-switching for the determination of benzalkonium chloride in eye care products. A 1 cm pre-column containing reversed-phase C18 material was utilized incorporating a 10-port electrically activated high pressure switching valve. The retained analytes were backflushed onto the analytical column for separation.

1.3.6.5 *Column-switching techniques for combination separations*

The principle of two-column technology is based on the combination of two identical columns and two different mobile phases or the combination of one mobile phase and two different columns. A fraction containing the analyte can be isolated from the first column and analysed on the second. The advantages of this technique are that three different methods (eg. front-, heart- and tail cutting) can be used to isolate the analyte from the interfering matrix components. This methodology allows a clean-up of the sample together with a concentration of the analyte in one step. The reproducibility of these techniques is therefore relatively high. Tjaden et al. [155] employed two-column reversed-phase LC separation using a heart-cutting technique, with two different columns and one mobile phase for the automated determination of chloramphenicol in kidney tissue homogenates.

Lecallion et al. [156], investigated three different column-switching systems, in the quantitative assay of drugs of low, medium and high polarity. It was found that a variety of components can be quantified provided that their retention characteristics are different on two columns. Compounds of high polarity show no retention on reversed-phase columns and thus normal phase columns should be used. The compounds to be assayed are first concentrated on a polar column (NH_2 or $N(CH_3)_2$ bonded phase) and then eluted. The selected heart-cut of the eluate is then separated on another, more polar column.

Chamkasem et al. [157] utilized a column-switching technique to shorten analysis times in the HPLC determination of intermediates of anaerobic degradation of toluene in ground-water. The column-switching method involved a six-port automated switching valve, and a reversed-phase C18 (3μm particle size) precolumn. Judicious selection of switching times was necessary to effect adequate separation of the sample components. Use of column-switching as an alternative to gradient elution, permitted the determination of PBC in the presence of non-polar components, particularly toluene, in a very short time. This eliminated the need for re-equilibration of the analytical column, and produced a constant spectral background. The procedure demonstrated an effective approach for analysis of polar compounds in the presence of non-polar interferents in an aqueous sample [157].

Precolumn technology was incorporated by Nielen et al. [158], for analysis of industrial wastewater samples. Industrial waste water represents a very complex matrix containing organic pollutants with a wide range of polarity. The complexity of such samples demands a high resolution chromatographic system incorporating sample pretreatment. Utilizing on-line precolumns (C18 and ion-exchange) for trace enrichment, with high pressure switching valve separation and enrichment, a number of pollutants in effluents were analysed. Ogan and Katz [159], coupled reversed-phase chromatography with size exclusion chromatography to generate a multidimensional chromatographic method for the analysis of complex samples. Two-six port valves were encorporated to effect sample diversion and matrix elimination in the analysis of coal liquids and oils for polyaromatic hydrocarbons. Goewie and co-workers [160] advocated the use of an automated preconcentration and liquid chromatographic analysis, consisting of a column switching apparatus for determination of herbicides in water. A six-port selector valve was encorporated for delivery of sorption and desorption solvents.

1.3.6.6 *Column switching systems in trace metal analysis*

The use of on-line column switching for metal ion preconcentration is becoming widely recognised. The advantages of the technique, particularly in the area of sample cleanup, for biological samples has been well documented. Similar characteristics are exploited for metal ion concentration, for cleanup from complex environmental matrices (for example, effluents, process wastes, mine water wastes etc.), and enrichment of trace concentrations from tap waters, and mineral waters. The reasons for development in this area stems largely from the inability of element selective detectors (ie, AAS, ICP) to detect sufficiently low concentrations. Thus by encorporating on-line precolumn enrichment these detection limitations are overcome, while maintaining satisfactory selectivity and resolution.

Olsen et al. [137] encorporated a single-line two-way valve FIA system for enrichment of 16 metal ions. The precolumn consisted of Chelex-100 ion exchange material, which demonstrated different selectivities for each cation. On-line preconcentration ion-exchange for enrichment of Al(III), Cr(III) and Fe(III), was presented by Hirata and coworkers [138]. A six-way valve was utilized to effect the concentration of the ions and subsequent elution by diverting the appropriate solvent onto the precolumn.

Irth and coworkers [123] loaded a precolumn, packed with C18 material, with a dithiocarbamate / CTAB ion pair. The metal complexes were formed by injection of the sample containing the metal ions onto the derivitization column. Trace enrichment was effected by loading a large volume of sample onto the column. By employing a six-port switching valve, it was possible to load the ion-pairing reagent onto the pre-column, load the sample ion at which stage derivitization occurs, and subsequently elute the complex by switching the valve thus diverting the eluent via the precolumn onto the separation column. As final separation takes place column cleanup occurred thus preparing the precolumn for the following sample injection. Chambaz et al. [99] reported a difference of up to 4% in standard deviation between off-line and on-line enrichment of metal ions (Cu(II), Pb(II), Zn(II), Ni(II), Co(II), Cu(II), and Mn(II)), with on-line being the more reproducible technique.

Ichinoki and Yamazaki [104] demonstrated the enrichment of Cd(II), Ni(II), Cu(II), Hg(II), and Co(II) as their dithiocarbamate chelates on reversed-phase C18 sorbent material, utilising an on-line column-switching procedure.

The switching system involved a six-port valve to effect sample loading ($2cm^3$) and subsequent elution by actuating the valve to divert the elution solvent. Metal chelates were loaded using a solvent with a low organic modifier content to enable complete retention on the hydrophobic stationary phase, and elution was achieved with an organic rich solvent.

Recently column switching was utilized to demonstrate the preconcentration of Cu(II), Al(III) and Fe(III) as their 8-hydroxyquinoline complexes, on a mini-column of C18 reversed-phase material [25]. The column-switching system consisted of a six-port, two-way switching valve, and two HPLC pumps (pump 1, for delivery of the sample and pump 2 for elution of the sorbed components with the desorption solvent). The retained analytes were desorbed using the backflush mode of column switching, whereby the components retained on the top of the precolumn were removed in the opposite direction to which they were loaded. This step ensured a minimum of dispersion of sample through the enrichment column, thus reducing the risk of band broadening. As a result of the on-line switching system, it was possible to load a large volume of sample onto the precolumn (up to 12 cm^3). This does not cause overloading of the analytical column, as the loading effluent is vented to waste by actuation of the valve.

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Chapter 2.0

Cr(VI) determination using high performance liquid chromatography with spectrophotometric detection and on-line preconcentration using column-switching techniques

2.1 INTRODUCTION

2.1.1 Sources of Cr (VI)

The oxidation state of chromium compounds is the most important feature discriminating between the different properties of the metal. The valence state distribution of dissolved chromium in natural waters is said to depend on :

*the oxygen content and redox potential of the water;

*the presence of dissolved or particulate organic matter;

*the presence of suspended inorganic matter [1].

The hexavalent form is almost always linked to oxygen and is a strong oxidizing agent. Cr(VI) is readily reduced to Cr(III) by contact with organic matter [2]. In aqueous systems Cr(VI) exists as CrO_4^{2-} or $Cr_2O_7^{2-}$ depending on the pH [3]. At pH values greater than 6 the former predominates, and between pH 2 and 6 the latter is present [4]. Cr(VI) has been found to be present at a range of concentrations, 0.1-0.5 μgdm^{-3} in sea waters and 200μgdm^{-3} in ground waters.

Industrially chromium is found in 3 main oxidation states, zero (metal), three and six. The major industrial use of chromium six (Cr(VI)) is in chromic acid which is used extensively in metal finishing processes, such as chromium plating. Cr(VI) is also commonly found in the fumes produced during stainless steel welding [5,6]. Protection of workers in such environments requires design and control, to ensure that the airborne concentrations of chromium compounds are below the current exposure limits. The limits vary depending on the form of chromium and reflect the differing toxicities eg limit for Cr(VI) = 0.05mg m^{-3} [7].

Four different methods were approved by the US EPA (1983) for chromium determination.

(i) Total chromium measurement by atomic absorption spectrometry.

(ii) Determination of Cr(VI) using coprecipitation followed by subsequent AA determination.

(iii) Photometric measurement of Cr(VI) by 1,5-diphenylcarbazide (DPC).

(iv) Complexation of Cr(VI) with ammonium pyrolidine dithiocarbamate followed by solvent extraction and AA determination.[8]

2.1.2 Cr(VI) toxicity

Toxicity of chromium is well known to be dependent on its oxidation state. Cr(III) has proven to be biologically essential [9], however dissolved Cr(VI) species is toxic. The mechanism of toxicity of Cr(VI) is not yet fully understood, but it appears to interfere with the enzymatic sulphur uptake of the cell. Airborne particulates containing Cr(VI) can be carcinogenic having an affect on the lungs, liver and kidneys [8,9].

2.1.3 Cr(VI) determination in the environment

In environmental samples chromium is present at very low levels, therefore accurate determination of trace amounts of Cr(VI) using a simple and rapid method is of great importance [10]. The preferred approach is one which physically separates the individual species present followed by direct quantitation [11].

With the development of HPLC and other chromatographic methods, a variety of separation methods have been employed [12,13] to separate metal ions, followed by colorimetric [13] or electrochemical detection [12]. Very often analysis of Cr(VI) requires conversion to Cr(III) followed by formation of a coloured complex which can be measured spectrophotometrically [8].

2.1.3.1 *Ion-chromatography*

Ion chromatography has become widely used in the analysis of cations and anions [14] in a variety of matrices. Separation of metal ions is carried

out on columns packed with cation exchangers based on poly (styrene divinylbenzene) copolymers or porous silica of low capacity [15]. The metal ions are eluted with buffers containing complexing agents such as pyridine dicarboxylic acid [16]. Ion chromatography has been widely employed in Cr(VI) determination [11,16-20]. Collins et al. [18], developed a method for Cr(VI) determination, whereby a small glass column containing 1 cm^3 of cation exchange material was utilized. The procedure was used both in the fraction collection mode, with radiometric detection of Cr-51-labelled species, and in the on-line mode, with spectrophotometric detection of Cr(VI) at 370nm.

For determination of the Cr(VI) anion, quaternary ammonium salts are used as ion-pairing reagents. Detection is usually carried out using post column derivitization with spectrophotometric monitoring of the analyte [19] or direct analysis using atomic absorption spectrometry [13]. The Ion-pairing reversed-phase HPLC procedure described by Milicac [13] reported a LOD of 0.3µg cm^{-3}, for Cr(VI) using this procedure. This author effected Cr(VI) determination by reversed-phase chromatography by the formation of an ion pair with the reagent tetrabutylammonium phosphate. This procedure was applied to Cr(VI) determination in soil samples, and detection was carried out off-line by electrothermal atomic absorption.

Ion-chromatographic analysis of Cr(VI) with various methods of detection have been reported notably using conductimetric [16,17], electro thermal atomic absorption [13], radiometric [18], and spectrophotometric detection [10,16,21,22]. Saleh et al. [17], coupled an ion-chromatographic method with a conductivity detector, for the determination of Cr(VI) in aquatic samples. A low capacity anion-exchange column was utilized with a borate buffer solution as an eluent. By using a 20 µl loop, a detection limit of only 0.5 $mgdm^{-3}$ was obtained, which would be unsuitable for determination of trace levels in the environment.

The majority of these techniques have detection limits in the μgdm^{-3} range, and detection is carried out off line. Procedures where detection is carried out on-line are more desirable. Dionex have developed a method encorporating an ion-chromatographic separation of the Cr(VI) and Cr(III) species, with on-line post column reaction for spectrophotometric detection of the analytes. On-line spectrophotometric detection [16] is the most widely used method encorporating the diphenylcarbazide (DPC) reagent for selective

Cr(VI) determination [23]. This technique is advantageous in that a highly coloured species is formed, enabling visible wavelength detection.

Another common method for the analysis of Cr(VI) involves reduction of this species to the trivalent form with subsequent separation and detection [22] Conversion of metal species from one form to another can have serious drawbacks, including, incomplete conversion, introduction of contaminants, interference from other metals present, and generally a complex sample pretreatment procedure [11].

Among all the methods of Cr(VI) determination reported, the most commonly documented method uses spectrophometry with 1,5-diphenyl carbazide as the colorimetric reagent [13, 16,24]. DPC is a reagent which reacts specifically with Cr(VI) to form a coloured complex. The technique has been used for about 75 years, and continued use has been made of this reagent in the analysis of Cr(VI) [23]. Much of the work involves separation using ion-exchange chromatography coupled with post-column derivitization using DPC [16,19].

2.1.3.1.1 *Post-column derivitization*

Post column reactor systems, although adding to the complexity of the apparatus, allow a tremendous range of metals to be analysed. The main systems under investigation are based on ion-exchange separation followed by photometric detection. The photometric detectors make use of post-column reactors incorporating a reagent, which produces a change in adsorption or fluorescence when mixed with metal species as they elute from the column. A number of post-column derivitization systems have been described in the determination of trace metals using HPLC [16,25-27].

The chief aims of this approach are to enhance the specificity and sensitivity of the detection method, so that detection can be achieved at low concentrations of analyte or in the presence of high concentrations of interferences [26]. Desirable features of derivitizing reagents used in these post column techniques are:

(i) the reagent must give a suitable chemical reaction with the analytes to provide suitable detection. Typically the reagent contains a strong fluorophore [10] or chromophore [16];

(ii) the reagent should be stable in order to minimise baseline drift and noise in the detector;

(iii) the reagent must be miscible with the eluent;

(iv) the reaction time between the analyte and the added reagents should be very short (seconds);

(v) the reagent should have similar detection properties to that of the eluent;

(vi) the reagent must not cause corrosion of the detector [27].

There have been two approaches to post-column derivitization methods, (a) on-line and (b) off-line. Advantages of the post-column on-line derivitization approach to the HPLC analysis are that artefact formation is not critical and it is not necessary for the reaction to be complete or well defined, provided it is reproducible [26]. In a typical post column procedure a dilute solution of chromogenic or fluorogenic reagent is mixed with the column eluent in a post column reactor and the coloured derivatives formed are detected at a suitable wavelength. The application of a post column reaction detector using a colour forming, complexing reagent and photometric detection gives high selectivity and sensitivity when analysing metal ions [25-27].

Post-column chemiluminescence reaction with luminol, following reduction of Cr(VI) to Cr(III) was described by Williams [22], and a similar procedure using luminol was also examined by Marino [21]. Post column reaction using DPC and spectrophotometric detection was utilized by Dionex [16] in the determination of Cr(VI). Using 250 µl injection volumes a limit of detection of 1ppb was reported.

2.1.4 Cr(VI) preconcentration

2.1.4.1 *Introduction*

There are very few reports describing the on-line preconcentration of Cr(VI) using minicolumns. Much of the work to date involves off line preconcentration [28,29] and/or reduction of Cr(VI) to Cr(III) with subsequent preconcentration and detection [30].

The application of pre-column techniques for sample concentration allows enrichment of the sample and thus, depending on the matrix, to inject up to 250 times the standard injection volume. The determination of trace metal ions ie concentrations of less than $10^{-7}M$, in natural waters by ion-chromatography requires a preconcentration step. On-line chromatography following preconcentration would be preferable as it would minimize the risk of contamination and at the same time attain high concentration factors [31]. Fong et al. [8], described a method using ion-chromatography-AAS with an on-line preconcentration unit. Cr(VI) was pretreated with EDTA and subsequently injected into an on-line preconcentration column consisting of anion-exchange material, followed by subsequent separation from other ions using ion-chromatography, with selective flame atomic absorption as a detector. A limit of detection of $0.5\mu gdm^{-3}$ for Cr(VI) was reported using this system. A 5cm preconcentration column containing modified C18 material was used [32]. Preconcentration of Cr(VI) was reported by Johnson [33], in which case a pre-column of anion exchange material was employed for off-line concentration, with subsequent detection using graphite furnace atomic absorption. The limit of detection using this off-line technique, with a 200 cm^3 sample volume was 0.019 μgdm^{-3} [33].

2.1.4.2 *Recent advances in trace metal preconcentration*

Recent studies on preconcentration, reported, an on-line column switching technique for metal ion analysis [34]. A six way switching valve was incorporated to initially effect the preconcentration of the analyte onto a mini column containing sorbent material. Removal of unwanted matrix material was achieved by switching the valve to effect a washing step, while retained analytes were unaffected. Elution of the retained analytes using the

backflush method occurred when the valve position was again manipulated, thus enabling the eluate to be directed to the separation column [34].

2.1.4.3 *Precolumn selection*

Anion-exchange resins have been used to preconcentrate cations, in which case cations are first complexed to an anionic ligand to form negative complexes and then enriched on the anion exchange resin [20]. Desorption of the enriched metals from the ion-exchange resin is important. A high recovery from a small volume of effluent is required in trace enrichment, and is favourable for the following detection step.

The use of on-line precolumn sample handling techniques considerably increases sensitivity and simplifies the overall procedure. This makes routine monitoring of large numbers of samples and automation readily attainable. Proper selection of precolumn packing material or alternatively of precolumn derivitization steps can distinctly enhance selectivity, thereby making demands on the final step less stringent [35].

In the literature a number of sorbents have been utilized for Cr(VI) preconcentration [29,36]. Preconcentration by column solid phase extraction using a dithiocarbamate derivative mixed with polyurethane foam has been shown to provide a means of enriching Cr(VI) [29]. The use of a solid chelate reagent such as ammonium hexamethylene dithiocarbamate, enabled preconcentration of a number of metal ions including Cr(VI). The preconcentration columns consisted of disposable syringes packed with polyurethane foam coated with the ligand. Detection was carried out off-line with a limit of detection of 0.015 μgdm^{-3}. A liquid anion exchanger supported on silica gel as a sorption system for preconcentration of anionic Cr(VI) from natural waters of high ionic strength was reported. Enrichment factors of 40 were achieved with a limit of detection of 2ng cm^{-3}. [36]. Recently a flow injection system with a microcolumn of Zr(IV) oxide was examined as a means of Cr(VI) enrichment [37]. A limit of detection of 2ppb was achieved using spectrophotometric detection with diphenylcarbazide.

Much of the work involving Cr(VI) analysis in sea water involved reduction to Cr(III) with adsorption of the trivalent species onto various sorbents [30]. Adsorption of Cr(VI) without being reduced to Cr(III) was

investigated in a study by Demirata [28]. In this investigation, modified melamine formaldehyde resin was used in the adsorption of the Cr(VI) anion. A detection limit of 0.1 μgcm^{-3} was reported, using up to 3000 cm^3 of solution. Atomic absorption was used off-line for detection.

In many of the investigations involving Cr(VI) preconcentration, minicolumns containing sorbent materials have been encorporated. With the preconcentration of large volumes, limits of detection in the low $\mu g\ dm^{-3}$ range have been achieved with good reproducibility. The sorbents used in these studies, notably ion-exchange and chelating sorbents have shown good enrichment and recovery characteristics. However, many of the techniques reported have involved off-line detection methods such as atomic absorption, which is an undesirable step when analysing large numbers of samples for trace quantities of metal ions.

2.1.5 Cr(VI) preconcentration using column switching

In this investigation an on-line column switching method has been developed to preconcentrate anionic Cr(VI) species. Column switching has been reported previously as a method of on-line sample cleanup and / or preconcentration [38]. This technique has the advantages of improved sample throughput, good separation efficiency and excellent preconcentration capabilities [34]. Ease of on-line transfer of the concentrated analytes is characteristic of this approach. In addition increased speed of analysis, reduced sample preparation time and the ability to use higher sample loadings, is achieved.

Special attention was given to precolumn sorbent selection and optimisation of the conditions necessary to enhance Cr(VI) preconcentration. Boundary conditions notably, loadability, flow rate, breakthrough volume, and elution volumes were investigated and optimised. A preconcentration sequence was developed which enabled the loading of up to $50cm^3$ of aqueous solution with a resulting enrichment factor of 100 using PCR detection at 520nm.

2.1.6 Aims and Objectives

The aim of this investigation was to develop a trace enrichment technique suitable for Cr(VI) preconcentration using on-line column switching.

The objectives include:

*selection of a suitable sorbent material for trace enrichment;

*optimisation of preconcentration boundary conditions, namely, loadability, wash volumes, and limit of detection.

2.2 EXPERIMENTAL

2.2.1 Apparatus

2.2.1.1 *On-line preconcentration*

The chromatographic equipment consisted of two Waters 501 HPLC pumps. Pump A was employed for on-line enrichment of the sample onto the pre-column (10 x 1.5 mm i.d.) housed in a cartridge. Loading could be achieved by pumping the sample (in the case of large sample volumes) or by injection of the sample via the injection valve with a $2cm^3$ fixed volume loop (Rheodyne 7125) onto the concentration column. Pump B delivered solvent to the analytical column and subsequently to the concentrator column in the back flush mode. A six-port two-way switching valve (Rheodyne 7000) was used, and the direction of eluent flow was controlled by switching the valve positions.

Reversed phase separations were carried out on a C18 column LC18 DB (25cm x 4.6 mm i.d., 5mm, supplier Supelco). A guard column packed with nucleosil C18 was mounted prior to the analytical column.

The ion-chromatographic system consisted of a guard (HPIC-CG5) column and analytical column (Dionex HPIC-CS5) [16], followed by post-column reaction with 1,5-diphenylcarbazide to form a coloured complex. A third Waters 501 HPLC pump was encorporated to deliver the post column reagent. A mixing tee was included following the separator column. Spectrophotometric detection was carried out using a Shimadzu SPD-6A variable wavelength detector.

2.2.1.2 *Off-line preconcentration*

Preconcentration was achieved using $2cm^3$ plastic syringes which were packed manually with 0.1g of sorbent material. The sample was loaded onto the column and subsequently eluted using a suitable eluent. The eluate was then collected and injected into a chromatographic system, consisting of a Waters 501 HPLC pump equipped with a 50 µl sample loop. A reversed-phase C18 column (LC 18DB, 25 x 4.6mm i.d.) was utilized, and detection was achieved using a Shimadzu SPD-6A variable wavelength detector.

2.2.2 Reagents

All of the chemicals used were of analytical reagent grade. Acetonitrile and methanol were supplied by Merck. LC-quality water was prepared by purifying water in a Milli Q filtration system (Millipore, Bedford, MA, USA). Analytical grade 1,5-diphenylcarbazide (Aldrich Chemicals) was used without purification. The Cr(VI) standard solutions were prepared from the solid salts K_2CrO_4 . 96% Spectrophotometric grade sulphuric acid was used in the preparation of the post column reagent. The ion-chromatographic separations encorporated a PDCA mobile phase containing 2m*M* PDCA (pyridine dicarboxylic acid), 2m*M* Na_2HPO_4, 10m*M* NaI, 50mM $CH_3CO_2NH_4$ and 2.8 mM LiOH all of which were analytical grade.

2.2.3 Procedure

2.2.3.1 *Standard preparation*

Cr(VI) standards were prepared by dissolving the (K_2CrO_4) potassium chromate salt in milli Q water .

2.2.3.2 *Buffer preparation*

A 0.02*M* acetate buffer solution was prepared by dissolving the appropriate quantity of ammonium acetate in water, and adjusting the pH using acetic acid or NaOH.

2.2.3.3 *Chromatographic procedures*

2.2.3.3.1 *Off-line procedures*

Off-line procedures were carried out in order to select suitable packing materials, concentration solvents and desorption solvents which were then applied to the on-line preconcentration system. Vydac 301 SC anion (30-40μm) packing material was selected for Cr(VI) enrichment. A $2cm^3$ syringe containing 0.1g of the sorbent was washed initially with $5cm^3$ of acetate buffer, followed by $2cm^3$ of water prior to concentration of the chromium

species. Cr(VI) samples up to volumes of 15 cm^3, were concentrated using the activated sorbent. The metal anion was desorbed using 2 cm^3 of acetate buffer (0.02*M*, pH 6.0). An eluent containing PDCA was also investigated in the desorption of Cr(VI) as an alternative to acetate buffer. The eluent from the syringe was collected and was then injected into the reversed-phase system using a 50µl loop size. Detection was carried out at 400nm .

2.2.3.3.2 *On-line procedures*

Prior to the introduction of the sample onto the concentration column, both concentration and analytical columns were allowed to equilibrate with the appropriate eluents. Standards were prepared in a similar manner to the off-line procedure.

(i) Reversed-phase HPLC

After optimisation the mobile phase selected for the separation of Cr(VI) from interfering species was (50:50) acetonitrile: acetate buffer (pH 6.0, 0.02*M* acetate, 0.1*M* KNO_3). This mobile phase was adapted from work carried out by Ryan [34] whereby Cu^{+2}, Fe^{+3} and Al^{+3} were separated as oxinate complexes using a similar eluent.

(ii) Ion exchange chromatography

As Cr(VI) is found in the anionic form, it was possible to use an ion exchange column following preconcentration for its analysis. A post-column derivitization step with 1,5-diphenylcarbazide was carried out to form a coloured complex, which was detected at 540 nm. A PDCA eluent was used in the separation on the ion exchange column and elution of the chromate anion from the concentrator column [16].

2.2.3.4 *Column Switching*

The backflush mode for column switching was operated, whereby components which were retained by the enrichment column were backflushed onto the analytical column, ie the desorption eluent was diverted, from direct flow onto the analytical column via the preconcentration column in the opposite direction to which concentration took place. In doing this, components of interest were backflushed onto the analytical column and subsequently separated. The switching of flows to enable backflush was achieved by switching the 6-port valve to position 2 (Figure 2.1). Switching back to position 1 enabled preconcentration and simultaneous equilibration of the analytical column.

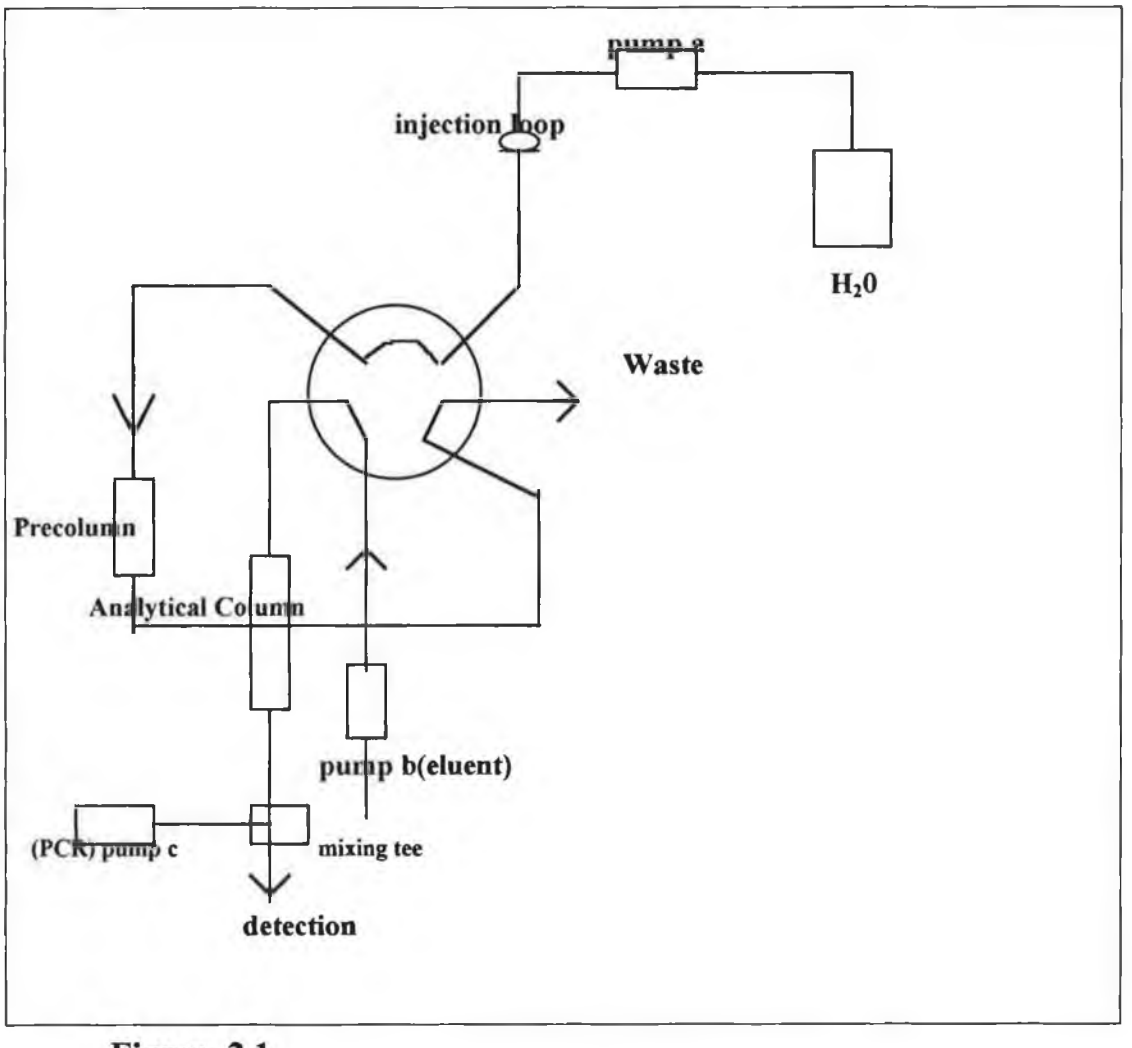

Figure. 2.1

Schematic Diagram of HPLC Column Switching System.

2.3 RESULTS AND DISCUSSION

2.3.1 Introduction

The use of on-line pre-column preconcentration techniques in HPLC considerably increases sensitivity and simplifies the sample preparation procedure, as demonstrated by Ryan at al. [34], in the analysis of beverage samples. Proper selection of the pre-column packing material can also enhance selectivity. It is, however, necessary to select compatible eluents of different elutrophic strengths, one to concentrate the sample onto the precolumn and the second to elute the components of interest. The solvents used to concentrate the analytes onto the precolumn must have poor elution capacities for the analytes in order to ensure complete and maximum preconcentration [38]. In this work initial studies involved selection of suitable eluents and sorbents for preconcentration using off-line techniques. The optimised conditions were subsequently transferred to the on-line chromatographic system which was found to be less time consuming and more reproducible.

2.3.2 Optimisation of preconcentration off-line

2.3.2.1 *Precolumn selection*

Cr(VI) as the chromate anion was preconcentrated on an anion exchange resin Vydac 301 SC 30-40 μm packing. Only two anion packings were investigated but as Vydac showed excellent retention and elution characteristics no further investigations were necessary. This material was selected for on-line work. The Cr(VI) anion was preconcentrated from water.

2.3.2.2 *Eluent Selection*

Eluents which contain metal complexing acids such as pyridine dicarboxylic acid (PDCA), can be used to enhance separation when using ion-exchange chromatography. The result of the addition of these acids is the formation of anionic metal complexes, which can compete with the organic acid anion (PDCA) for the exchange site of the resin. A PDCA eluent was used to analyse Cr(VI) on an ion exchange column in a procedure reported by

Dionex [16] and was subsequently incorporated as the analysis step in this preconcentration procedure. This eluent was found to have good elution characteristics, achieving an average of 113% recovery of the concentrated Cr(VI) from the anion packing, for 6 replicate analyses.

In the reversed-phase procedure an acetate buffer was used as the eluent. The addition of 50% acetonitrile to the buffer had no adverse effects on elution of the Cr(VI), and facilitated the use of this eluent mixture as a mobile phase for chromatographic separation. A recovery of 93% from the preconcentration column was achieved using acetate buffer (pH 6.0, 0.02*M*, acetate 0.1*M* KNO_3).

The maximum loadable concentration of Cr(VI) onto the Vydac packing, after which breakthrough occurred was found to be 10ppm (using both reversed-phase and ion-exchange analytical columns). The % coefficient of variation (C.V) for six sample loadings was found to be 4.9 %.

2.3.3 On-line preconcentration

Following the selection of the precolumn packing and eluants, using the off-line studies, online concentration was investigated. Optimisation of the column-switching preconcentration procedure involved the selection of boundary conditions which gave the most satisfactory detection and operating conditions for the system available. Boundary conditions which were investigated included: pre-column wash volumes, loadability, breakthrough volumes and the effect of flow rate on preconcentration.

Concentrations of 10ppb and 100ppb Cr(VI) were used for the initial study. A 100ppb concentration was chosen for the investigation of most boundary conditions initially, in order to observe the results more clearly, and to avoid using long analysis times for preliminary work. When boundary conditions were optimised for the 100ppb level, lower concentrations and higher loading volumes were then investigated in order to obtain the limit of detection and the linear range.

2.3.4 Boundary conditions

Cr(VI) when preconcentrated from water onto the VYDAC 301 SC anion exchange packing material was found to be strongly retained. Following the preconcentration of the anion, the boundary conditions were investigated. It was found that a maximum flow rate of 1cm³/min was necessary to concentrate the chromate anion (Figure 2.2), an increase in flow rate above this caused a rapid decrease in the efficiency of concentration, as the metal anions were being coeluted with the solvent. Figure 2.3 illustrates that a minimum volume of 2·0 cm^3 of appropriate eluent is required to quantitatively elute the sample components from the packing material.

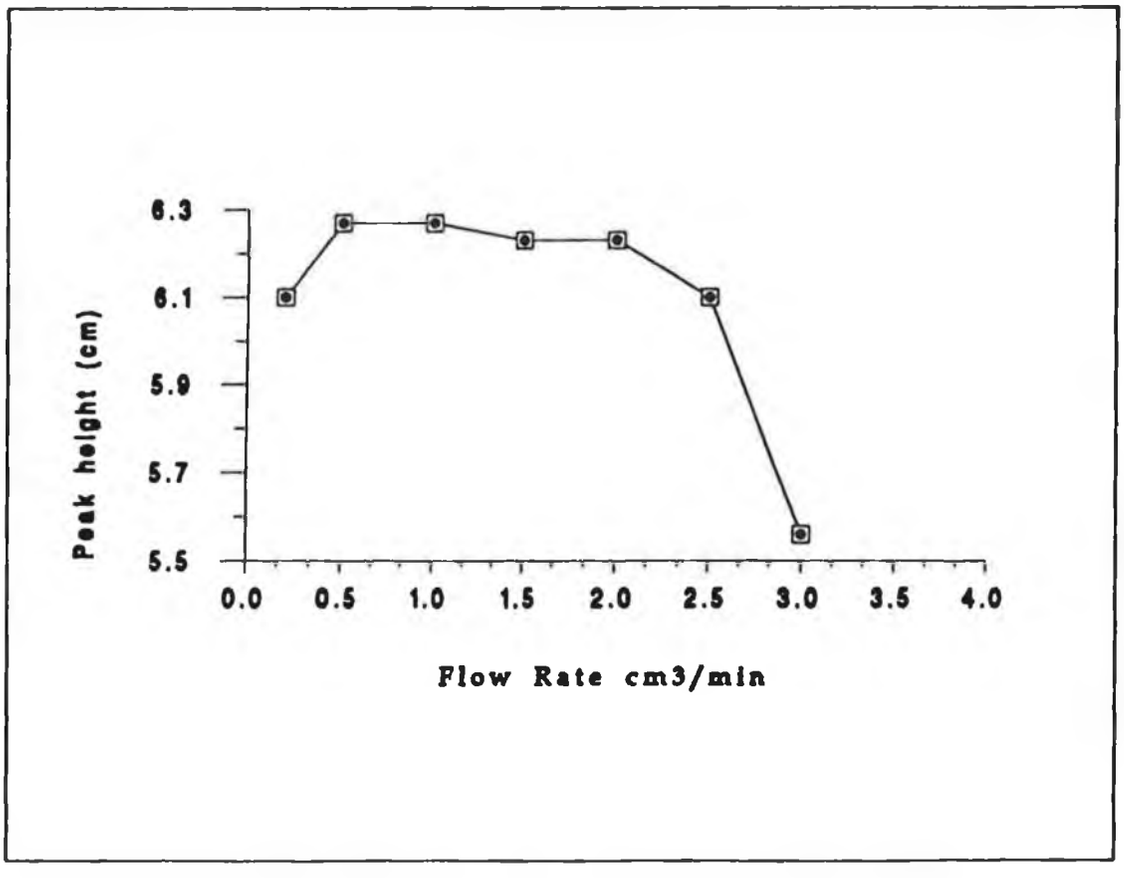

Figure 2.2

The effect of Eluent Flow Rate on Cr(VI) Preconcentration. Conditions; [Cr(VI)]=100ppb, Preconcentration solvent = water; Elution solvent = 0.02 M Acetate

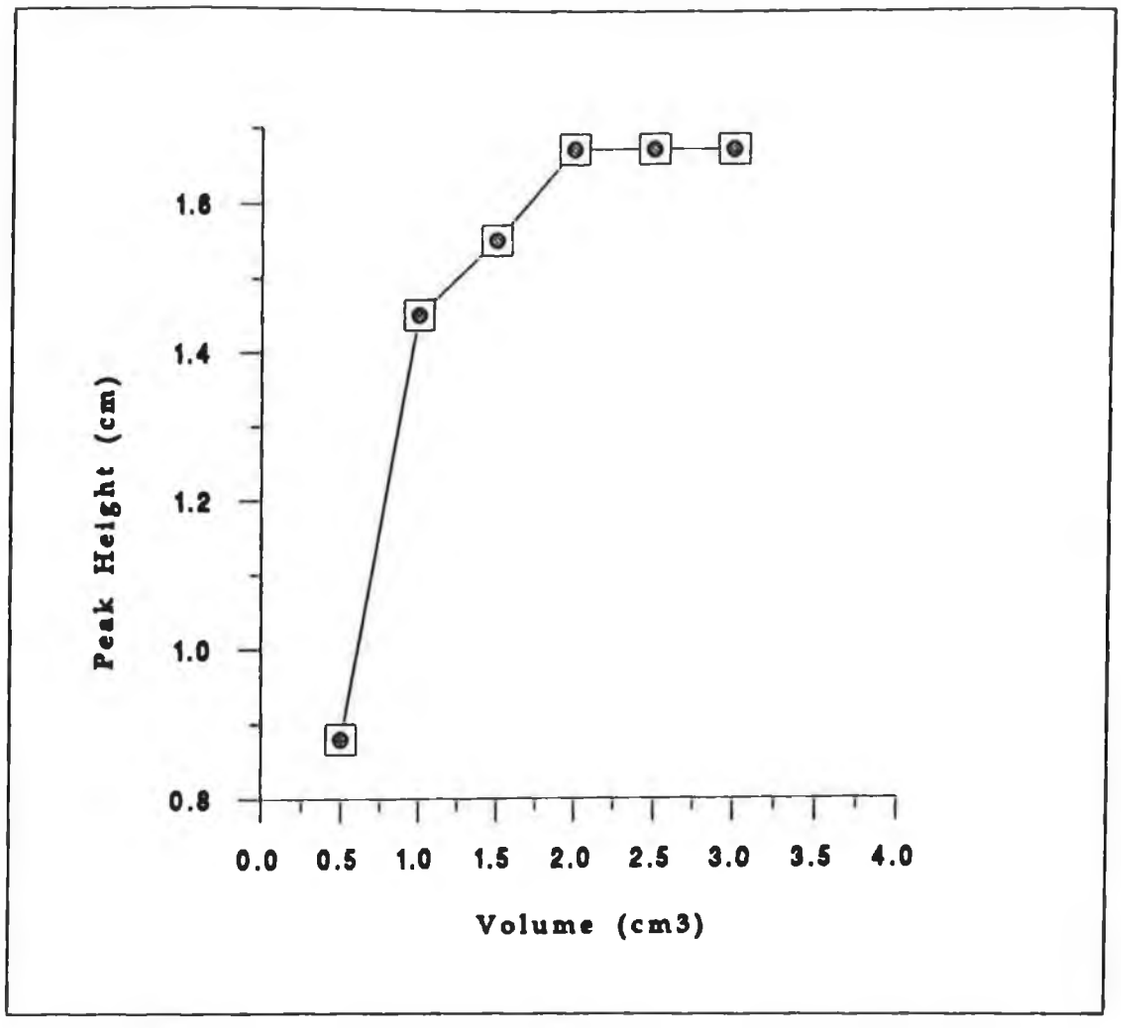

Figure 2.3.

Volume of eluent necessary to elute Cr(VI) from the precolumn. Conditions; [Cr(VI)]=100ppb, Preconcentration solvent = water; Elution solvent = 0.02 M Acetate

An additional equilibrium wash volume of 2.0cm^3 was necessary ie. the volume of mobile phase A (water) required to re-equilibrate the precolumn following elution of the retained analytes using mobile phase B before it could be used again. Figure 2.4 shows the effect of inadequate equilibrium washing resulting in a decrease in peak height for the subsequent analysis.

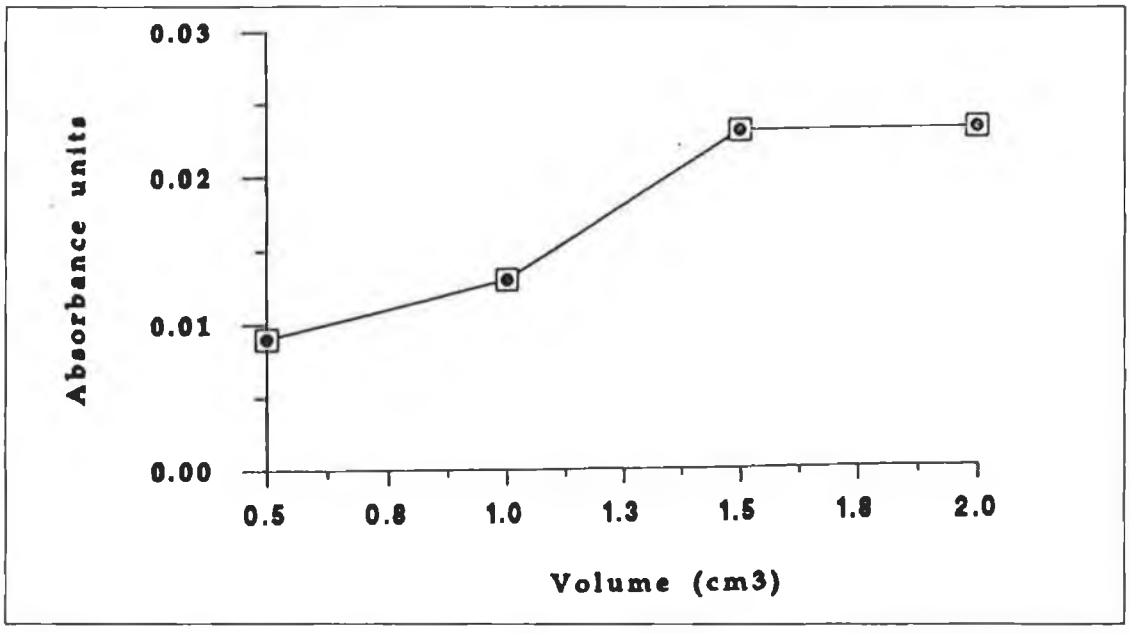

Figure 2.4.

Equilibrium wash volume, flow rate = 1cm^3/min. Washing solvent = water.

Prior to elution of the retained analytes various volumes of the concentrator eluent (water) was washed through the packing This was carried out in order to observe the maximum volume of mobile phase A which could be used to wash the precolumn, without causing elution of the retained analytes. This volume is called the " breakthrough volume". Figure 2.5 shows that a maximum volume of 1.0cm^3 of mobile phase A can be washed through the column after which there is a significant decrease in peak height for Cr(VI). This is due to the washing effect of the mobile phase. This low breakthrough volume would suggest that the anions are not very strongly attached to the packing material. However, on investigation of loadability (Figure 2.6) it was found that up to 14 cm^3 of 500ppb Cr(VI) could be preconcentrated. The graph illustrates a clear linear response between volume and peak height up to a loadable volume of 14 cm^3. Beyond this there is a slight deviation from the linear line. This would suggest that the anions are in fact strongly retained on the packing material. The ability to preconcentrate such volumes greatly enhances the preconcentration technique, particularly in real samples where concentrations are usually too low to detect. It was also observed that by lowering the concentration of metal, the volume of solution which can be loaded increases.

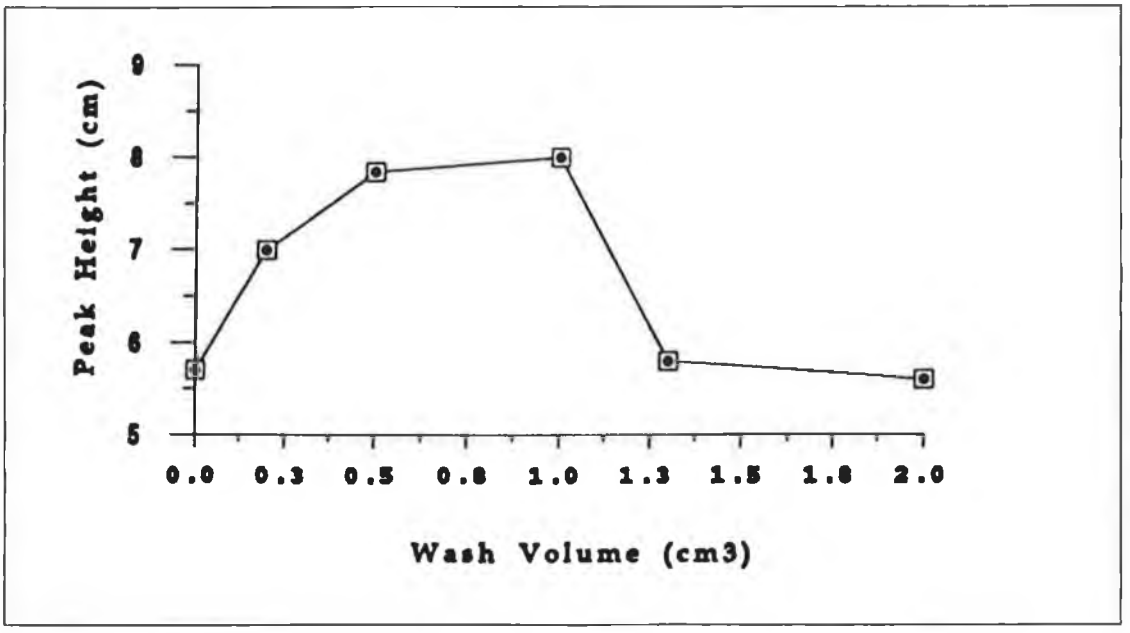

Figure 2.5

Breakthrough curve for Cr(VI) preconcentration, Flow rate=1 cm^3/min. Wash solvent=water. Eluting solvent = PDCA eluent (ion-exchange), 50:50 ACN:Acetate buffer (0.02M, pH 6) (reversed-phase)

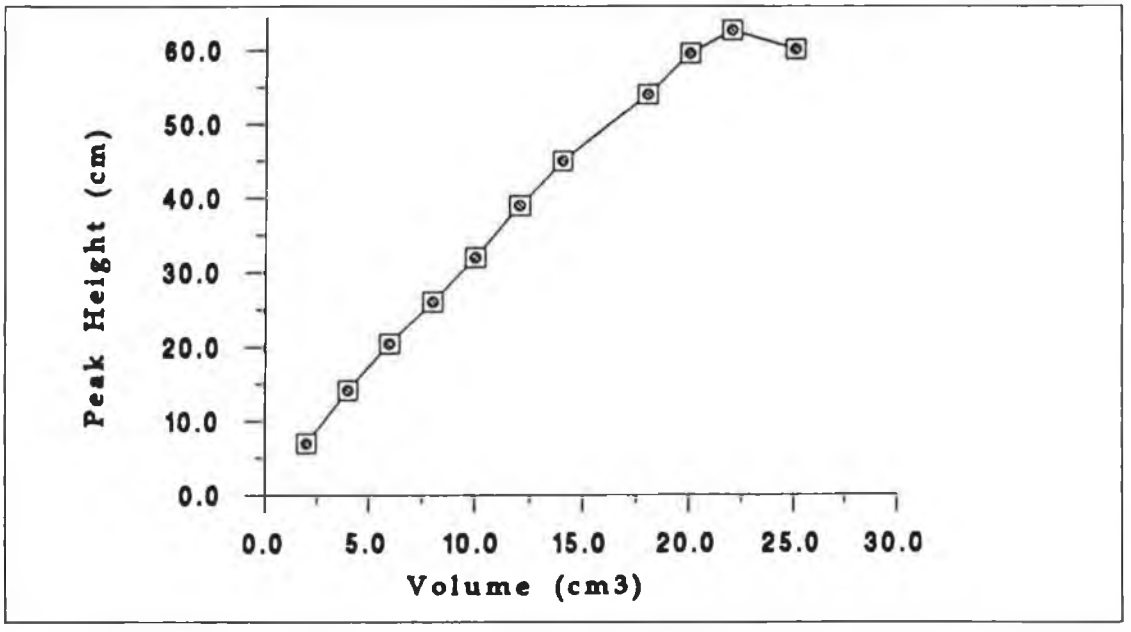

Figure 2.6

Loadability curve for Cr(VI) preconcentration. [Cr(VI)]=100ppb, loaded in water @ 1cm^3/min, Eluted in acetate buffer (0.02 M pH 6) @ 1cm^3/min.

The object of the preconcentration procedure is to enable the loading of a large volume of sample onto the packing material in order to concentrate low concentrations and thus enable detection at trace levels. This was achieved by loading a fixed volume of 50 cm^3 of dilute sample in the range, 10ppt to 100ppt, in order to obtain a calibration curve (using the ion-chromatographic analytical procedure) and limit of detection for this volume. Because of the high volume loaded the washing effect may have caused some of the metal to elute resulting in a decrease in efficiency. In this investigation a linear calibration curve was obtained (linear regression, r, = 0.9987) in the range mentioned above. It is expected that even higher volumes could be loaded to enable still lower concentrations to be detected. A 50 cm^3 volume was chosen for loading as this is an adequate sample volume for work in the field. Six replicate 50 cm^3 loadings of Cr(VI) standard solution had a %CV of approximately 3.3%. Table 2.1 shows a typical preconcentration sequence for Cr(VI).

Step	Mode	Duration	Solvent	Flow Rate cm^3/min	Volume Delivered
1	Sample Flush	1 min.	Sample	6.0	6cm^3
2	Sample Load.	2 min.	Sample	1.0	2cm^3
3	Water Flush	1 min.	Water	6.0	6cm^3
4	Precolumn Wash	1 min	Water	1.0	1cm^3
5	Sample Strip	2 min	Eluent B	1.0	2cm^3
6	Re-equilibrate	2 min	Eluent A	1.0	2cm^3

Table 2.1

Cr(VI) Preconcentration Sequence

Step one is a preparation step prior to loading the sample onto the preconcentration column. Sample flush involves allowing the Cr(VI) sample solution to flow through the solvent lines from pump A to waste (Figure 2.1). This ensures that upon actuation of the valve to preconcentrate, the sample goes directly onto the precolumn, without any interference from previous samples. Step 3 is a water flush of the solvent lines, again in preparation for the precolumn wash step. These two steps are carried out to ensure that quantitative preconcentration is achieved, as any residual sample or eluent in solvent lines would lead to erroneous observations.

2.3.5 Chromatographic separations and limits of detection

2.3.5.1 *Reversed-phase procedure*

Chromium in the anion form is not retained on the C18 analytical column, once eluted from the precolumn. This suggested that if interferences were present, or matrix components which may interfere, these would mask the chromium. However by taking advantage of selective sorbent material in the precolumn, the effect of column switching in washing extraneous components to waste, and by judicious use of a selective wavelength (400nm) for detection, should reduce the problem of interferences.

The removal of the C18 analytical column was investigated, as its purpose as a separator was not being utilized. However the column switching procedure ie. switching of the valve positions, had the effect of causing a pressure change accross the system, which was detected and gave a response and so it was not possible to obtain a calibration curve. A small mixing column containing glass beads was mounted on-line before the detector, in an attempt to dampen the noise, however it only eleviated the problem slightly. Thus the C18 column was necessary to dampen the noise due to pressure change. The choice of a selective precolumn and eluant was important in ensuring the elimination of any interference effects.

The mobile phase used was 50:50 ACN: acetate buffer and detection was carried out at 400 nm. Without preconcentration a detection limit of 5ppb was obtained for Cr(VI).Using preconcentration and a loading volume of 50cm^3 a linear calibration in the region 10ppt-100ppt was obtained with a limit of detection of 10ppt (Table 2.2). The criteria for limit of detection (LOD) was a

signal to noise ratio of 3:1. Reproducibility for six sample loadings onto the concentrator column was 3.23% CV.

	REVERSED-PHASE	ION-EXCHANGE
Volume Loaded	$50cm^3$	$50cm^3$
LOD	10ppt	30ppt
% RSD	3.23	3.30
n*	6	6
Linear Dynamic Range	10-100ppt	30-100ppt
Wavelength	400nm	540nm

* n = Number of sample replications

Table 2.2

Comparison of reversed-phase and ion exchange separations (S/N=3/1)

2.3.5.2 *Ion-exchange Post-column Derivitization procedure*

The preconcentration procedure applied here was similar to that used in the previous method, however, in this case, a PDCA eluent was utilized for sample desorption, as this was a more compatible eluant for separation on the ion exchange system [16]. This sytem encorporated a post column reagent (PCR) to facilitate detection. Once Cr(VI) was eluted from the analytical column it was mixed with 1,5-diphenylcarbazide [23], a reagent which has been used for many years in the selective detection of Cr(VI). This rapidly formed a coloured complex which was subsequently detected spectrophotometrically at 540 nm. The addition of the reagent delivery pump on-line, did increase the pressure somewhat, but it remained satisfactorily low to carry out the procedure. The selectivity of the reagent for Cr(VI) and also the selective wavelength for detection provided yet another means of eliminating the possibility of interfering effects. Without preconcentration a

limit of detection of 0.5ppb was obtained and by preconcentrating a volume of 50cm^3 a LOD of 30ppt was achieved. A linear calibration curve in the range 30ppt-100ppt was obtained and the reproducibility for six sample replications was 3.30% C.V (Table 2.2). From this investigation it appeared that preconcentration of larger volumes would enable detection of lower concentrations.

Figures 2.7a and 2.7b show typical chromatographic separations with 100ppb (2cm^3) loadings using the preconcentration technique. Both of these procedures showed a greater than 10 fold increase in detection limits with good reproducibility. Using larger volumes with lower concentrations, 50cm^3 and 66cm^3 loading volumes of 50ppt (Figure 2.8a & 2.8b) achieved up to a 100 fold reduction in LOD.

2.3.6 Interferences

Interference by a number of anions (Cl^-, NO_3^-, SO_4^{2-}) and cations (Fe^{2+}, Al^{3+}, Zn^{2+}, Cr^{3+}) was investigated, however they did not interfere as both systems involved selective preconcentration with chromatographic separation and detection in the visible range.

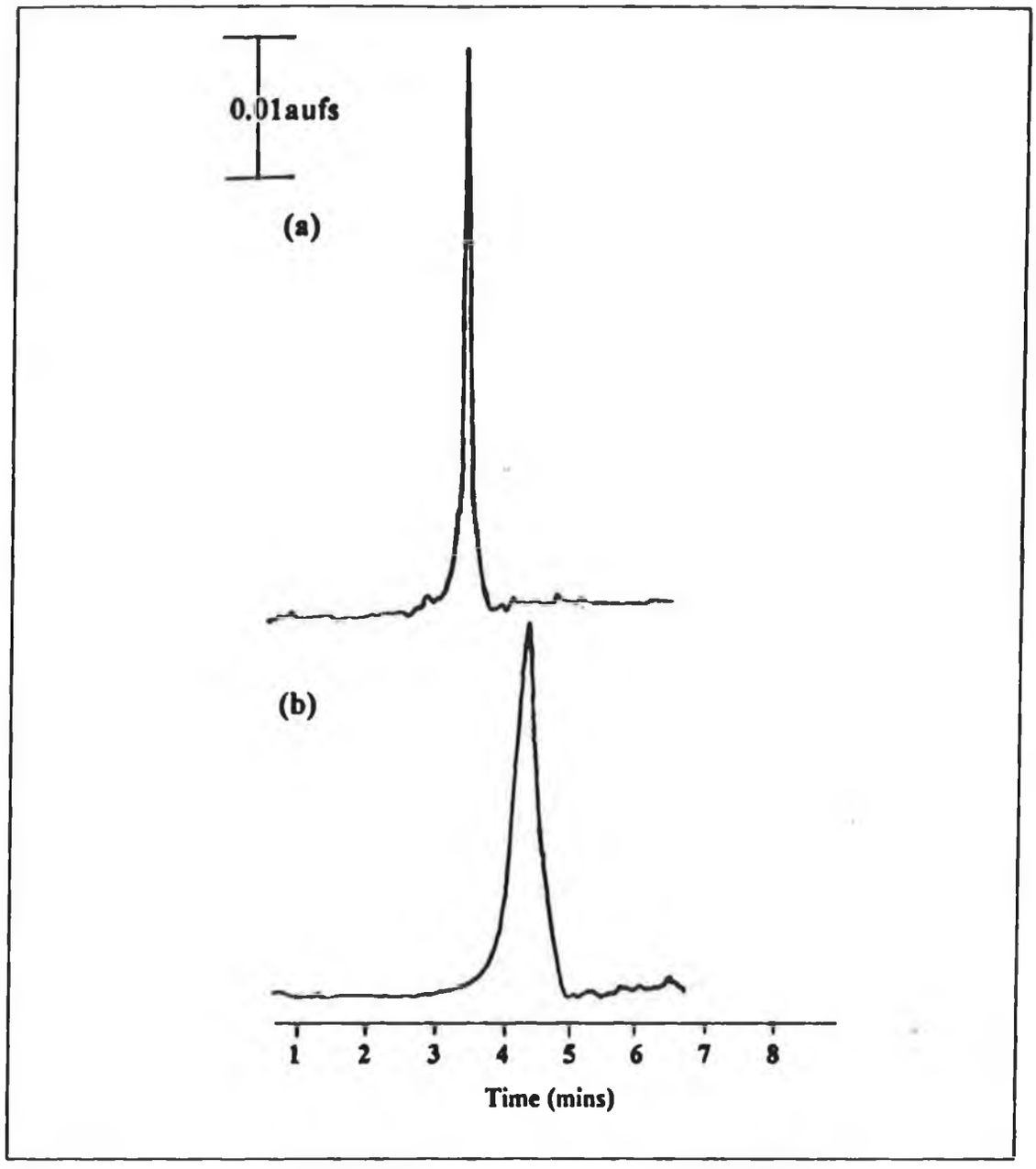

Figure. 2.7

(a) Chromatogram of Cr(VI) using column switching and reversed phase separation column. Eluent: 50:50 Acetonitrile: Buffer (pH 6.0, 0.02 mol dm^{-3} acetate, 0.1 mol dm^{-3} KNO_3). Detection at 400nm. [Cr(VI)]=100ppb.

(b) Chromatogram of Cr(VI) using preconcentration and ion-exchange separation with post column derivitization detection at 540nm. Eluent: 2mM PDCA. [Cr(VI)] = 100ppb

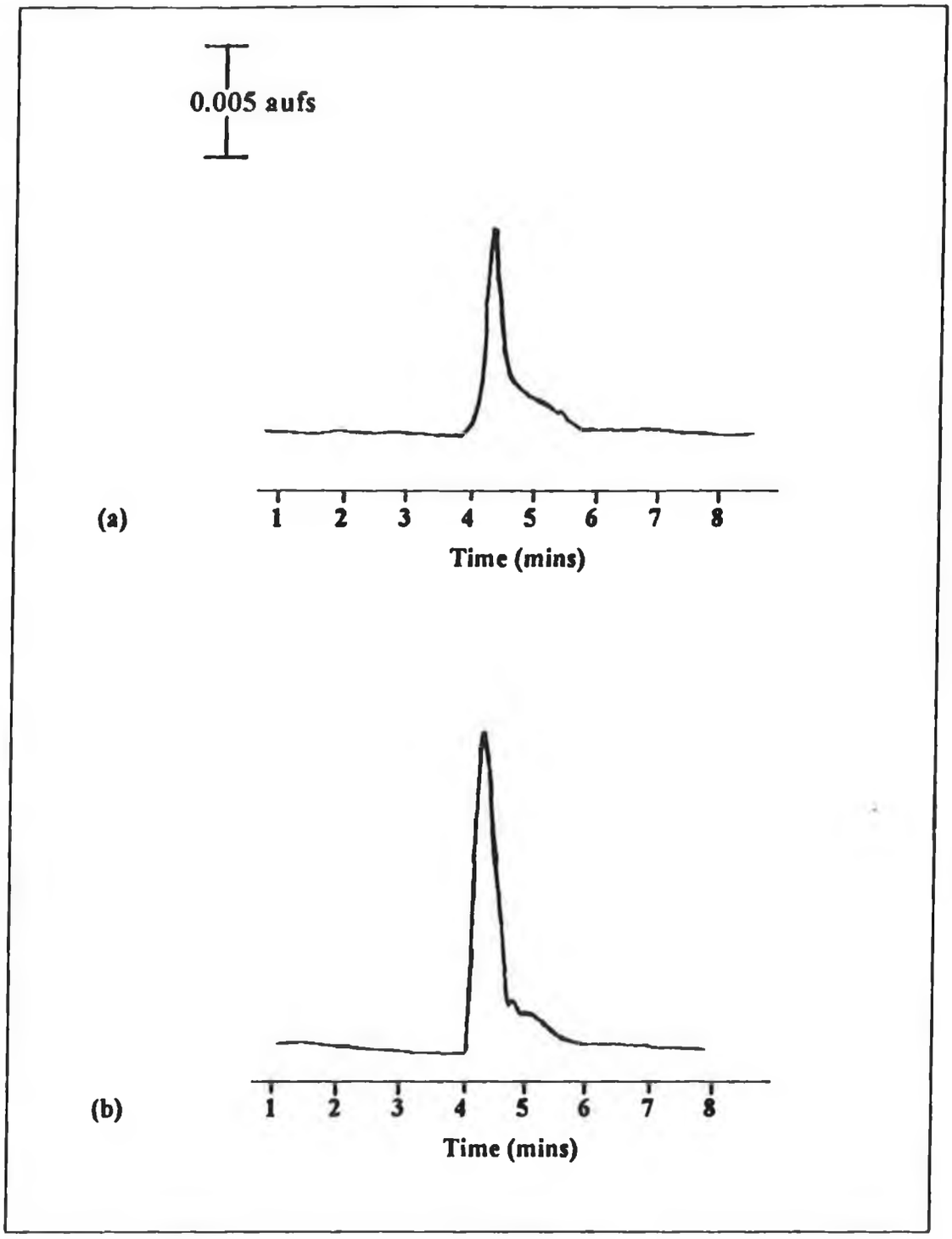

Figure. 2.8

Chromatograms showing Cr(VI) Preconcentration of large volumes. (a) 50cm^3 of 50ppt. (b) 66cm^3 of 50ppt; Eluent = 2mM PDCA; Preconcentration flow rate=1 cm^3/min; Elution flow rate =1 cm^3 /min

2.4 CONCLUSIONS

In this investigation it was our aim to use column switching to enhance both selectivity and sensitivity for Cr(VI) measurement. Choice of a suitable sorbent material obviously increases selectivity and as an anion-exchange packing material retains only anions, allowing all metal cations to elute to waste, an anion-exchange material was the obvious choice. Both reversed-phase and ion-exchange analytical columns provided similar results, with the C18 column providing a slight increase in sensitivity over the ion-exchange. Both methods show a 100 fold reduction in LOD, while maintaining separation efficiency, reproducibility and short analysis time. The levels of sensitivity and selectivity achieved here confirms the usefulness of this technique.

On-column enrichment in the determination of metal species, has advantages of achieving the detection of lower concentrations due to the use of higher sample loadings on the concentrator column, thus increasing the sensitivity of the method. From the investigation it was observed that preconcentration of large volumes up to 50 cm^3 was possible which reduced the LOD significantly. Higher loading volumes could also improve sensitivities further. However this may effect reproducibility due to the washing effect of the loading solvent, or cause over loading of the preconcentrator column. Interference from other anions or cations was not a problem due to the selectivity of the precolumn and the use of photometric detection.

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Chapter 3.0

Determination of Cr(III) by complexation with 8-hydroxyquinoline using reversed-phase liquid chromatography and trace enrichment by column-switching techniques.

3.1 INTRODUCTION

3.1.1 Sources of Cr(III)

Chromium dissolved in water is present as two different oxidation states, Cr(III) and Cr(VI) and the stable trivalent form is by far the most widespread species in the environment. In seawater, as reported by Boussemart et al. [1] the relative proportions of both species are governed by thermodynamic properties (pH and temperature) and also by kinetic stabilization of the Cr(III) species (hydration) which hinders the oxidation of Cr(III) to Cr(VI).

Trivalent chromium is considered to be an essential element to mammals for the maintenance of glucose, lipid and protein metabolism, whereas Cr(VI) is toxic to humans [2]. Chromium together with insulin, plays an important role in carbohydrate metabolism. The determination of Cr(III) in biological materials has been of great interest in clinical research and in elucidating the exact role of this element in human nutrition and health. A wide range of publications report chromium analysis and chromium speciation [3,4].

3.1.2 Chromatographic techniques for Cr(III) determination

3.1.2.1 *Chelation chromatography*

In recent years high performance liquid chromatography (HPLC) has been widely applied to the separation of many inorganic compounds. In 1972, Huber et al. [5] reported the separation of metal-β-diketonates by modern HPLC. Since then many groups have investigated the determination of metal chelates by HPLC [6-11], and many ligands have been found suitable for this type of analysis. These ligands include, dithiocarbamates [8,12], phenanthrolines [7] (pyridylazo) resorcinol [13] and 8-hydroxyquinolates [4,9,14].

Dithiocarbamates are useful reagents for extraction, separation and spectrophotometric detection of many metals due to their abilities to form

stable complexes [12,15]. Bond et al. [12] investigated the separation of Cu(II), Ni(II), Co(II), Cr(VI) and Cr(III) by reversed-phase HPLC with electrochemical detection using complex formation with dithiocarbamate. Both *in situ* and pre-column complexation was investigated [12].

A number of reports describe either precomplexation with the appropriate ligand [12] or on-column complexation, *in-situ*, very often with the ligand included in the mobile phase [16]. Addition of 8-hydroxyquinoline to the eluent in order to minimize complex dissociation has also been reported [17,18].

The first reported separation of metal oxinate complexes was in 1979 by Berthod et al. [19]. This is an ideal ligand for the separation of multielement mixtures because of its ability to complex with metal ions to produce neutral chelates [18]. New applications of oxine and its derivatives in modern analytical chemistry are continually being developed [4,20-23]. Developments using this ligand take advantage of the fluorescence properties [24] and absorption properties [18] of the resultant complexes. Baiocchi et al.[17], described the precolumn chelation of Cr(III) among other metal ions with oxine, and its analysis by reversed-phase HPLC. Lajunen et al. [9] described the separation behaviour of Cr(III) oxinate on silica gel, reversed-phase and size-exclusion columns. The uptake of metal ions using different solid sorbents (Amberlite XAD-2 and an anion exchange resin) immobilized with 8-hydroxyquinoline was investigated for the enrichment of trace metal from environmental samples [21]. Parrish [14] described the use of macroporous resins incorporating oxine, and Jezorek et al. [25], investigated the immobilization of oxine on silica, and with resultant separation of 8 metal ions.

Oxinate chelates are thermodynamically stable, chromatographically separable and absorb in the UV-vis region [19]. Several chromatographic procedures, based on different stationary and mobile phases, have been tested [4,9,17,22] for the separation of various mixtures of metal chelates. Cr(III) oxinate is known to have slow formation kinetics compared to that of other metal chelates, and some reports have used a heating step to speed up the complexation process [22].

3.1.2.2 *Ligand exchange chromatography*

Applications of ligand exchange chromatography include enrichment of trace concentrations of metal ions, to enable more effective measurements, and to achieve greater selectivity in multicomponent determinations [26]. Ligand exchange chromatography is a process in which complex forming compounds are separated through the breaking and formation of labile coordinate bonds to a central atom, coupled with partition between a mobile and stationary phase [27]. Use of immobilized ligands on solid supports to separate metal ions has received considerable attention in recent years [14,25,28].

The use of solid phases such as macroporous resins [14,20] silica gel [28], activated alumina [26] and naphthalene [29] have been reported as supports onto which organic ligands such as 8-hydroxyquinoline have been impregnated. King et al. [30], described the use of a small column of XAD-4 resin, for sorption of metal ions following precomplexation with a dithiocarbamate ligand. Detection was carried out off-line by atomic absorption spectrometry. An off-line preconcentration method using column solid phase extraction, encorporating polyurethane foam, mixed with a dithiocarbamate ligand was applied to the preconcentration of Cd(II), Co(II), Cu(II), Fe(II), Hg(II), Mn(II), Ni(II), and Pb(II), prior to analysis by AAS [31]. This procedure was applied to the preconcentration of trace metals from sea water. Concentration methods based upon the reversed-phase absorption of complexed metals onto a small adsorbent column has become more and more widely used [18,32-33]. This procedure is advantageous in that it overcomes the limitations of solvent extraction, notably, time consuming and tedious extractions, with several sample handling steps, which can lead to contamination problems. [27].

3.1.3 Preconcentration techniques

3.1.3.1 *Introduction*

An increasing need for sensitive and selective detection techniques to analyse trace metal concentrations in complex environmental matrices has been clearly recognised. Sample handling procedures have been developed which are more sophisticated than conventional liquid-liquid extraction [27]. A promising approach is to enrich trace compounds of interest on suitable sorbents, in order to isolate and preconcentrate them, before their separation and detection by means of a suitable chromatographic method [27].

Solvent extraction has been until recently one of the most popular methods of preconcentration of metal ions including Cr(III) [34]. However this method is inadequate for the concentration of trace amounts of ions in large sample volumes [28]. Many references report the preconcentration of trace metal using commercial chelating resins [35] and ion-exchange columns [36]. Chambaz et al. [36] developed a novel method of on-line preconcentration and separation of the trace metals Mn(II), Cu(II), Co(II), Pb(II), Ni(II) and Cd(II) on ethylenediamine triacetic acid-bonded silica. The procedure involved a post-precolumn modification of the eluent, to allow the separation of metals on an analytical column. Post column derivitization using PAR was encorporated with visible detection. In this investigation, both on-line and off-line preconcentration methods are studied, with the former exhibiting more satisfactory preconcentration results.

Many of the preconcentration techniques used today involve off-line enrichment on a minicolumn [16]. On-line preconcentration systems have proved to be the most interesting way of improving trace ion determinations [18,32-33,36-40]. On-line preconcentration manifolds have been coupled with a variety of detection systems mainly for atomic absorption spectrometry [37], inductively coupled plasma [23,40] and UV-vis spectrometry [32,38].

3.1.3.2 *Preconcentration of metal ions using 8-hydroxyquinoline*

Many preconcentration, trace enrichment techniques encorporating oxine describe its (or its derivatives) immobilization on solid phases [20,23,41]. An oxine derivative, 7-dodecenyl-8-quinolinol, impregnated onto macroporous resin was utilized for the enrichment of 11 metal ions, and the

technique was applied to sea water analysis, with spectrophotometric detection [20]. Silica gel impregnated with quinolin-8-ol was used as a chelating sorbent for the preconcentration of 6 metal ions from seawater. A 5-10 cm^3 volume of sample was typically concentrated, with a detection limit in the tens of ngl^{-1} (ppt) range [41].

Other reports describe precomplexation with the ligand and subsequent concentration of the neutral complex [16] on the solid sorbent material. Bond and Nagosa [16] reported off-line preconcentration of Al(III), Cu(II) and Mn(II) oxine complexes on Sep-pack cartridges, with photometric detection.

Recent advances in trace metal enrichment (including Cr(III)) involve complexation of the metal ion species with sulphonated azo dyes, and subsequent concentration on an ion exchange resin. In this study two different procedures were evaluated, ie., one where the ligand was in solution and the second where the ligand was immobilized on a resin [42].

Recently, 8-hydroxyquinoline has been employed in the trace enrichment of a number of metal ions (Cu(II), Al(III) and Fe(III)) using a novel column-switching technique. Limits of detection in the μgdm^{-3} range were achieved using 10-12 cm^3 of sample [18].

3.1.4 Cr (III) preconcentration

Several determinations of Cr(III) by reversed-phase liquid chromatography which encorporate trace enrichment techniques utilizing solid phases have been reported [13,28,44]. Determination of Cr(III) by chelation chromatography has been described using a variety of ligands, notably, dithiocarbamates [8,12], 8-hydroxyquinoline [4,9] and derivatives of these ligands. There are a number of reports in the literature concerning Cr(III) preconcentration. These methods include on-line [43-44] and off-line [16] techniques.

Preconcentration of Cr(III) by flow injection ICP-AES was described by Cox et al. [43]. In this procedure a manifold incorporating an activated alumina minicolumn was used. By preconcentrating a volume of $10cm^3$, a limit of detection of 0.05 $mgdm^{-3}$ was achieved. In this case Cr(III) was not oxidized to Cr(VI) for determination, which is an attractive feature of the technique, resulting in minimal sample pretreatment. In addition by using a

sorbent for preconcentration larger volumes of sample can be analysed. Cox et al [43], applied this method to Cr(III) analysis in urine samples with on-line flow injection enabling rapid and reproducible analysis.

Cr(III) preconcentration using an alumina-immobilized diethyenetriamine pentacetic acid sorbent has been described, whereby the selectivity behaviour of Cr(III) was utilized for its preconcentration in the presence of other metal ions [26]. In this study, Singh and Mehrotra, investigated the rate of sorption and breakthrough capacity of the metal ions on the ligand immobilized activated alumina. It was found that complexation of metal ions, including Cr(III), with immobilized ligand, bonded to an alumina matrix is quite rapid, and it exhibited an advantage over using untreated alumina. Cr(III) was found to be strongly adsorbed to the exchanger.

On-line trace enrichment of Cr(III) using an ion-exchange column, with ICP-AES detection was reported by Hirata et al. [44]. The ion-exchange resin was 0.3-1.2 μm pore size chelating resin and a styrene divinylbenzene copolymer on which metal uptake was monitored. A signal enhancement of up to 113 times using preconcentration was achieved. This procedure involved an on-line preconcentrating FIA-ICP system, which was connected via a six-port valve for on-line introduction of sample and elution solvents. A disadvantage of this procedure for Cr(III) determination was the interference from Mg(II), Mn(II) and Al(III) ions, which would make it unsuitable for analysis of environmental samples.

3.1.4.1 *Cr(III) preconcentration using column switching techniques*

This work involves optimisation of mobile phase conditions for complexation and separation of the Cr(III) oxinate complex using reversed-phase HPLC. An on-line column switching technique was employed to preconcentrate the Cr(III) oxinate complex on a minicolumn of C18 sorbent material prior to analysis on the C18 analytical column. The detection of Cu(II), Al(III) and Fe(II) as their oxine complexes, encorporating a 1:1 acetonitrile:buffer eluent mixture has been previously reported [18]. Adaptation of this method has been carried out here to optimise the procedure for the analysis of Cr(III). Once the separation conditions were optimised, particular emphasis was placed on optimisation of the precolumn selection and boundary conditions for enrichment of the Cr(III) species.

3.1.5 Aims and Objectives

The aim of this project was to apply an on-line preconcentration technique using column switching, to enhance detection sensitivity for Cr(III) determination.

The objectives include:

* optimisation of chromatographic conditions, notably, mobile phase and Cr(III) complex stability;

* optimisation of preconcentration boundary conditions (ie. flow rate, wash volumes and loadability) and selection of suitable sorbent material.

3.2 EXPERIMENTAL

3.2.1 Apparatus

3.2.1.1 *On-line preconcentration*

The chromatographic equipment consisted of two Waters 501 HPLC pumps. Pump A was employed for on-line enrichment of the sample onto the pre-column (10 x 1.5mm i.d.) housed in a cartridge. Loading could be achieved directly by pumping the sample (in the case of large sample volumes) or via the injection valve with a 50 μm fixed volume loop (Rheodyne 7125). Pump B delivered solvent to the analytical column and subsequently to the concentration column in the backflush mode. A six-port, two-way switching valve (Rheodyne 7000) was used, and the direction of eluent flow was controlled by switching valve positions.

3.2.1.2 *Off-line preconcentration.*

Preconcentration was achieved using $2cm^3$ plastic syringes, which were packed manually with 0.1g of sorbent material. The sample was loaded onto the column and subsequently eluted using a suitable eluent. The eluate was then collected and injected into the chromatographic system, consisting of a Waters 501 HPLC pump equipped with a 50μl sample loop.

3.2.1.3 *Reversed phase separations*

These were carried out using a C18 analytical column LC 18 DB (25cm X 4.6mm id., 5μm). A guard column packed with Nucleosil C18, 10μm was used to protect the analytical column. Detection was carried out using a Shimadzu SPD-6A variable wavelength detector.

3.2.2 Reagents

All of the chemicals used were of analytical reagent grade. Acetonitrile and methanol were supplied by Lab. Scan. LC quality water was obtained by purifying water in a Milli Q filtration system (Millipore, Bedford, MA, USA.). Analytical grade 8-hydroxyquinoline (Carlo Erba), was used without purification. The trivalent chromium standard solutions were prepared by dissolving $Cr(NO_3)_3.9H_2O$ in Milli Q water.

3.2.3 Procedure

3.2.3.1 *Standard preparation*

To achieve separation, Cr(III) required a precomplexation step with 8-hydroxyquinoline. The standards were prepared by dissolving the $Cr(NO_3)_3.9H_2O$ salt in a 50:50 acetonitrile : buffer solution (pH 6.0, 0.02 *M* acetate; 0.1 *M* KNO^3) containing a 10 fold molar excess of 8-hydroxyquinoline (oxine). The slow kinetics of ligand exchange for Cr(III) meant that solutions had to stand for a period of 6 hours prior to injection to ensure complete complexation had occurred.

3.2.3.2 *Chromatographic separations*

The mobile phase selected for separation following optimisation was a 50:50 acetonitrile: buffer (pH6.0, 0.02 *M* acetate & 0.1 *M* KNO_3) solution. The Cr(III) oxinate complex was injected onto the column using a 50µl injection loop. Detection was carried out at 400nm.

3.2.3.3 *Preconcentration of Cr(III) oxinate*

3.2.3.3.1 *Off-line procedures*

Off-line studies were carried out in order to select suitable packing materials, concentrator solvents and desorption eluents which were then applied to the on-line preconcentration system. The $2cm^3$ plastic syringes containing 0.1g of C18 packing material (37-50µm particle size) was

conditioned by passing 5cm^3 of mobile phase solution (50:50 ACN:Buffer) through the sorbent. An additional 2cm^3 of buffer solution was passed through prior to preconcentration of the metal complex. Volumes of the chromium standard solutions ranging from 2-10 cm^3 were preconcentrated. Following this step, the chromium oxinate complex was then desorbed using 2cm^3 of 50:50 ACN:Buffer solution. The eluate was then injected into the reversed phase system, and detection was carried out at 400nm.

3.2.3.3.2 *On-line preconcentration using column switching*

Equilibration of the concentrator and analytical columns with the appropriate eluent was necessary prior to the introduction of the sample onto the precolumns. Standards were prepared in a similar manner to the off-line procedure. The mobile phase selected for the separation was the 50:50 ACN:Acetate buffer, pH 6.0 as described in section 3.2.3.2.

The backflush mode for column switching was operated, whereby components which were retained by the enrichment column (Figure 3.1), were backflushed onto the analytical column, ie the desorption eluent was diverted from direct flow onto the analytical column via the preconcentration column in the opposite direction to which concentration took place. In doing this, components of interest were backflushed onto the analytical column and subsequently separated. The switching of flows to enable backflush was achieved by switching the 6-port valve position. Switching back to the initial position enabled preconcentration and the simultaneous equilibration of the analytical column.

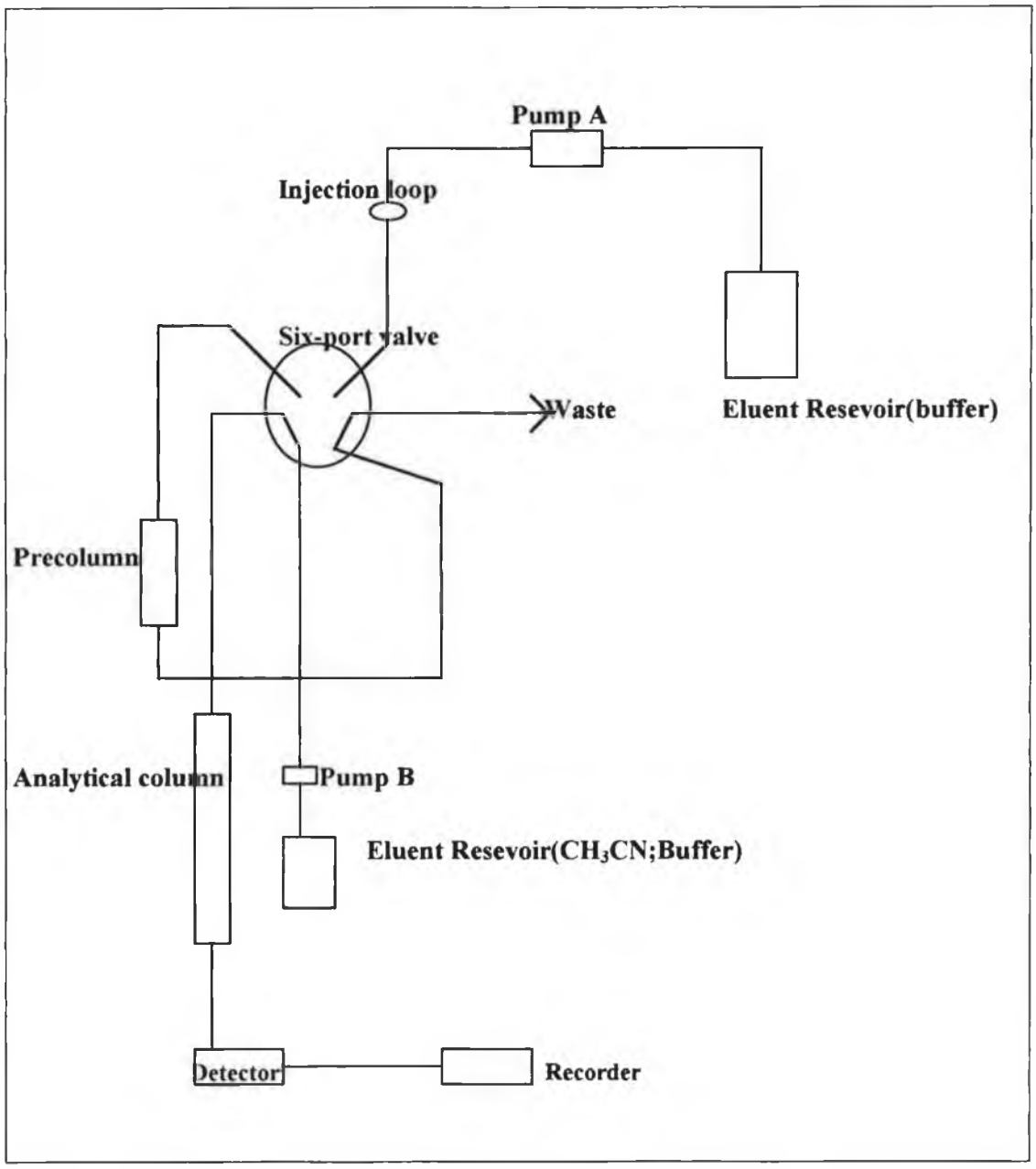

Figure. 3.1.
Schematic Diagram of On-Line Column-Switching System for Cr(III) Determination

Note: The switching valve above is shown in position 1 to enable preconcentration of the analytes; by changing the valve postion, flow will be diverted, thus enabling backflush of the analytes from the precolumn onto the analytical column

3.3 RESULTS AND DISCUSSION

3.3.1 Cr(III)-8-hydroxyquinoline complexation

In this project the method of Cr(III) analysis was based on the formation of stable metal oxinate chelates, and their separation on a C18 reversed-phase column by HPLC with UV-vis detection at 400nm. Due to the slow kinetics of Cr(III)oxinate chelate formation [22], it was necessary to allow the Cr(III) standards containing the ligand to stand for a period of time. The time necessary for complexation to be complete was investigated. Figure 3.2 shows a plot of Cr(III) oxinate formation versus time. From the study it was concluded, that it was necessary to allow the solutions to stand for a period of at least 6 hours before analysis. Beyond this time no further increase in signal was noted suggesting that equilibrium had been reached. However the standards were found to remain stable for at least 24 hours after formation. A similar drawback was reported by Lopez et al. [22], in which case heating of the complex was used to speed up complexation and maintain stability of the complex. Other researchers have reported the inclusion of the ligand in the mobile phase, in order to maintain complex stability [12,17].

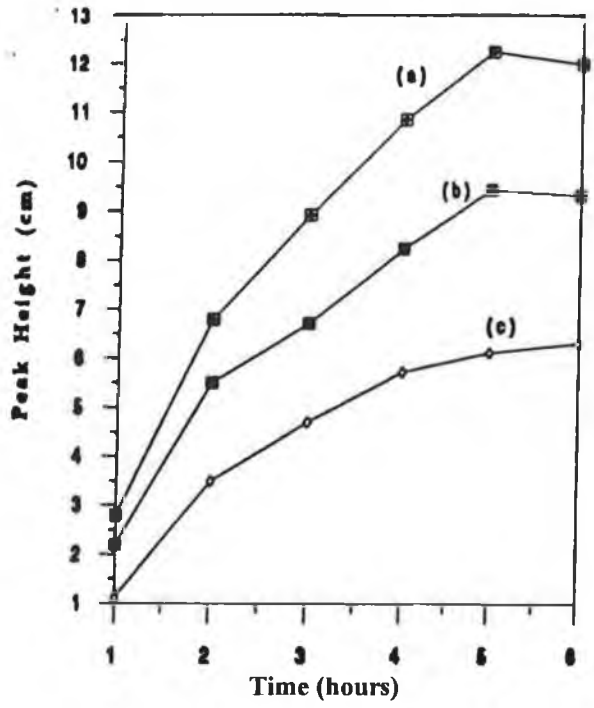

Figure. 3.2.

Formation of Cr(III)-8-hydroxyquinolate complexes. Cr(III) standards in $1 \cdot 10^{-3}$M oxine, buffer pH 6.0 (0.1M KNO_3; 0.02M acetate). Detection @ 400nm. [Cr(III)] a=0.08ppm, b=0.05ppm, c=0.03ppm.

A number of factors were investigated in order to optimise the formation of the Cr(III) oxinate complex. Figure 3.3 illustrates the effect of the composition of the diluent used for the preparation of the standards on complexation. This shows that chelation yield is influenced by the organic phase composition (% ACN), a factor also noted by Lopez et al. using this ligand [22]. The content of acetonitrile in the standard solutions was varied in the range 80-40%. It was evident from these results that the higher the organic content, the lower the complexation reaction efficiency. A 50:50 ACN : buffer solution was found to be most satisfactory. Bond and Nagosa [16] reported the importance of the inclusion of potassium nitrate to improve complex stability, thus the effect of its inclusion on the reaction efficiency of Cr(III) oxinate was investigated. Figure 3.4 illustrates that 0.1*M* KNO_3 was effective in giving maximum response. Higher concentrations caused the response to decrease.

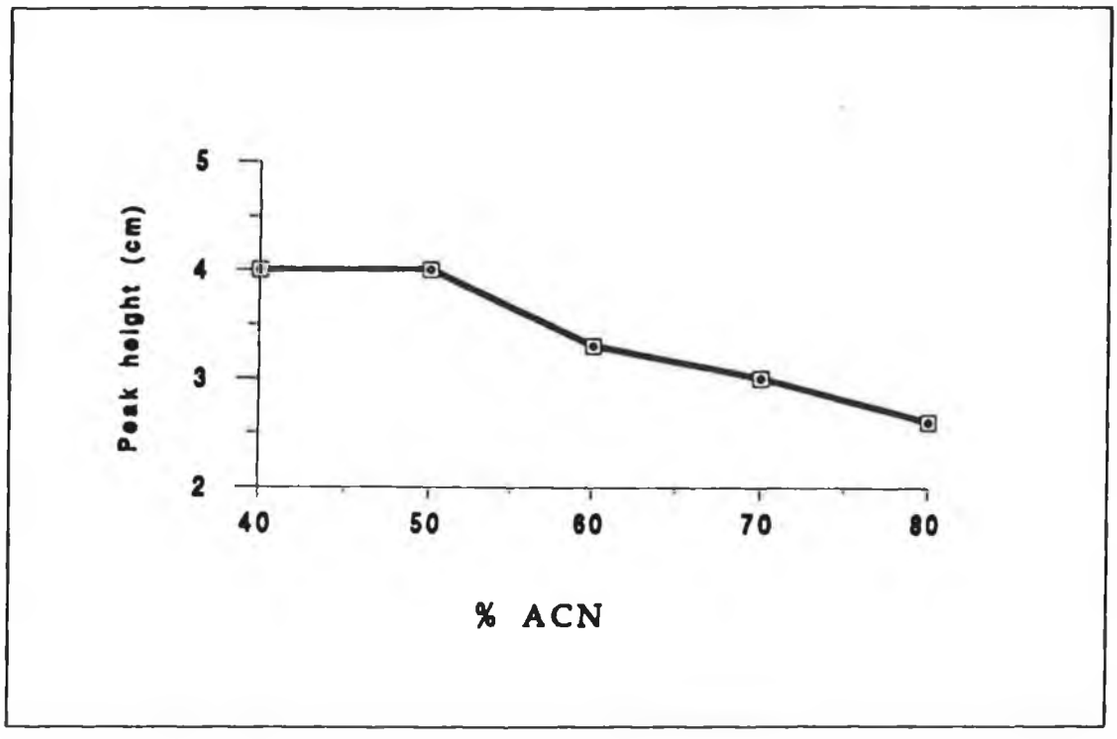

Figure. 3.3
Effect of diluent composition for Cr(III) complexes. [Cr(III)]=100ppb. [Oxine] = $1 \cdot 10^{-3}$ M

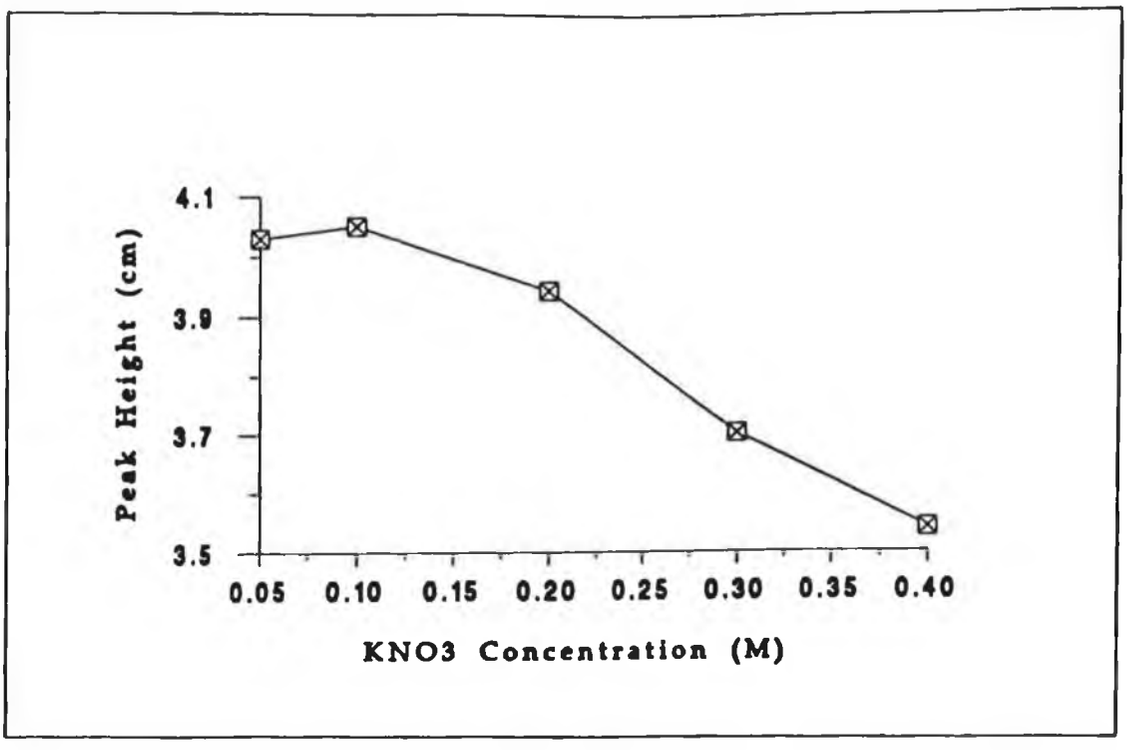

Figure. 3.4
Effect of KNO_3 concentration on complexation. [Cr(III)] = 100ppb; [Oxine] = $1 \cdot 10^{-3}$ M; Eluent =50:50 ACN;acetate buffer (0.02 M pH6)

3.3.2 Effect of mobile phase composition

The effect of varying the mobile phase composition can be seen in table 3.1. By lowering the organic content, better separation (from the free ligand) and longer retention times for the complex were noted. 50% ACN was selected as the optimum, giving the best resolution of Cr(III) from free ligand and optimum peak shape characteristics. It was found that ligand concentration itself played an important role in obtaining sufficiently low detection limits. The concentration of ligand required, depended on the concentration of metal present. In this investigation the maximum ligand concentration required for the analytes at two concentrations, 100ppb and 10ppb Cr(III), was studied. It was found that an optimum ligand concentration of $1 \cdot 10^{-3} M$ oxine is required. However in concentrations greater than 100ppb Cr(III), a higher ligand concentration is necessary. This study found that an excess of ligand greatly decreased the peak height, probably due to the high background absorbance of the ligand itself.

% CH_3CN	Retention Time (min)
80	2.5
70	2.7
60	2.8
50	3.2
40	4.1

Table 3.1

Effect of Solvent Composition on Cr(III) Retention Time [Cr(III)] = 0.1ppm, [oxine] = $1 \cdot 10^{-3}$M, Buffer pH 6.0 (0.1M KNO_3, 0.02M Acetate)

3.3.3 Off-line Preconcentration

3.3.3.1 *Precolumn selection*

The pre-column sorbent selection was initially investigated off-line, and subsequently suitable solvents were chosen for concentration and elution of the ions of interest. Cr(III) as its 8-hydroxyquinolate complex, forms a stable neutral chelate, which should be strongly retained on hydrophobic packing materials. A number of reversed-phase packings were investigated as sorbents as their hydrophobic nature would attract the neutral chelates when injected in a largely aqueous phase. The reversed-phase packing material C18, is a hydrophobic sorbent, thus a solvent of low elutrophic strength is required to preconcentrate the neutral species of interest. The solvent of least elutrophic strength is water, however this was not satisfactory as the oxinate complexes were found to be unstable in pure water, and required buffering. Acetate buffer was selected with no organic solvent included as it caused loss of efficiency in the preconcentration step. Off-line work investigated the preconcentration efficiency of different packing materials. Table 3.2 shows the range of packings which were investigated, and their retention characteristics for the Cr(III)oxinate complex. Nucleosil 10 C18, Techoprep C18 and Corasil C18, all exhibited good retention characteristics for the Cr(III) oxinate complex. However Corasil C18 was selected for further work as the

% recovery from this sorbent material was greatest. Techoprep shows only 50% recovery and a smaller particle size of Nucleosil created an increase in pressure in the chromatographic system.

Off-line work only enabled selection of the suitable packing and eluent. It was not possible to see from these studies the effect of particle size on band broadening, thus the three packings which showed the greatest retention ie., 1, 2 & 3 (Table 3.2) were investigated on-line. Corasil C18 was found to have the most satisfactory characteristics. Even though its larger particle size would suggest band broadening may occur, it did not. In fact the larger particle size eleviated the problem of increased pressure which was noted when using smaller packing sizes, eg. Nucleosil 10 C18, 10μm. A calibration graph prepared for the off-line procedure using Corasil C18, gave a linear response in the range 0.05-0.5ppm. Studies showed that pH was an important factor in the retention of the metal complex onto the reversed-phase sorbent. On investigation, pH 6.0 was found to be the optimum, lower pH values caused incomplete retention onto the packing.

Number	Packing Material	Particle Size(μm)	Cr(III) Retention.
1	Nucleosil 10 C18	10	Retained but caused pressure increase on-line
2	Techoprep C18	25-40	Retained but poor recovery
3	Corasil Bondapak C18	37-50	Completely retained (89-95% recovery +/- 5%)

Table 3.2
Assessment of Cr(III) complex retention behaviour on precolumn sorbents.

3.3.4 On-line preconcentration.

3.3.4.1 *Introduction*

The use of on-line pre-column sample handling techniques in HPLC considerably increases sensitivity and simplifies the overall extraction procedure [18]. Proper selection of pre-column packing material can enhance selectivity. It is also necessary to select compatible eluents of different elutrophic strengths, one to concentrate the sample onto the precolumn and the second to elute the components. The concentrator solvents must have poor elution capacities in order to ensure complete and maximum preconcentration.

The aim of the preconcentration procedure is to enable the detection of trace levels of Cr(III) by loading large volumes on-line. In order to do so, a suitable packing material is required to retain the species, and suitable eluents are required to concentrate and elute it. Following the preliminary work off-line involving the selection of three possible packing materials and suitable eluents, optimisation of the precolumn procedure was investigated. This procedure involved the selection of boundary conditions for the appropriate packing material.

3.3.4.2 *Effect of Flow Rate on Preconcentration*

Investigation of the effect of flow rate on enrichment found that 1.0 cm^3/min or 1.5 cm^3/min provided an optimum response (Figure 3.5). The latter was selected as it also appeared to enhance the chromatography. Flow rates above 1.5 cm^3/min had the effect of forcing the complex through the packing material, not allowing sufficient time for the complex to bind. Thus a drop in response is observed at higher flow rates.

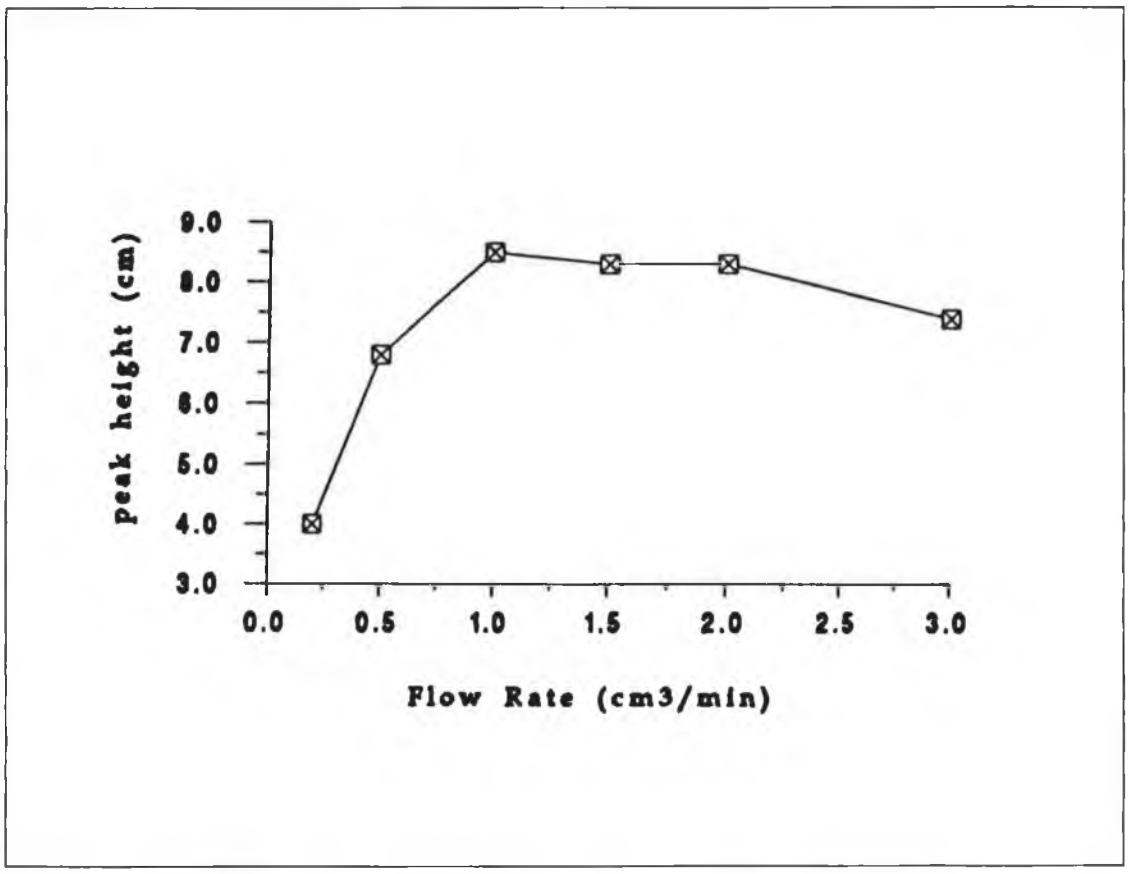

Figure 3.5

Effect of flow rate on Cr(III) preconcentration, Cr(III) loaded as Cr(III) oxinate complex. [Cr(III)] = 100ppb; [oxine] = $1 \cdot 10^{-3}$ M; Eluent = 50:50 ACN : acetate buffer (0.02M acetate, 0.1M KNO_3, pH 6)

3.3.4.3 *Boundary conditions*

The boundary conditions which were investigated included: precolumn washvolumes, loadability, breakthrough volumes and the effect of flow rate. The concentration of chromium in the natural environment is at very low levels, it is important therefore to detect these low levels. This can be done by preconcentrating higher volumes of low concentrations. In the analysis of boundary conditions however larger concentrations were chosen, ie., 10ppb-100ppb, in order to shorten analysis times for these preliminary studies. The use of lower concentrations at this stage would require longer analysis times and larger volumes to enable detection. It was observed that the trends obtained for boundary conditions using the higher concentrations corresponded to loading higher volumes of lower concentrations. When boundary conditions were obtained for 100ppb, lower concentrations and higher loading volumes were then investigated in order to obtain the limit of detection for Cr(III) as its oxinate complex.

3.3.4.3.1 *Elution volume*

The elution volume necessary was found to be 2.0 cm^3. This volume refers to the quantity of eluent required to remove all, or a reproducible quantity of the retained complex from the precolumn.

3.3.4.3.2 *Effect of pre-column washing*

In this study the equilibrium wash volume is defined as, the volume of mobile phase A required to re-eqilibrate the precolumn, following elution of the retained analytes, using mobile phase B. When investigated, it was found that 1.5 cm^3 of mobile phase A was necessary for re-equilibration in order to obtain a maximum response from the next injection. Volumes greater than 1.5 cm^3 appeared to have the effect of reducing the efficiency of the concentration process (Figure 3.6). This may be due to the sites being taken up by ACN in the buffer solution (mobile phase B).

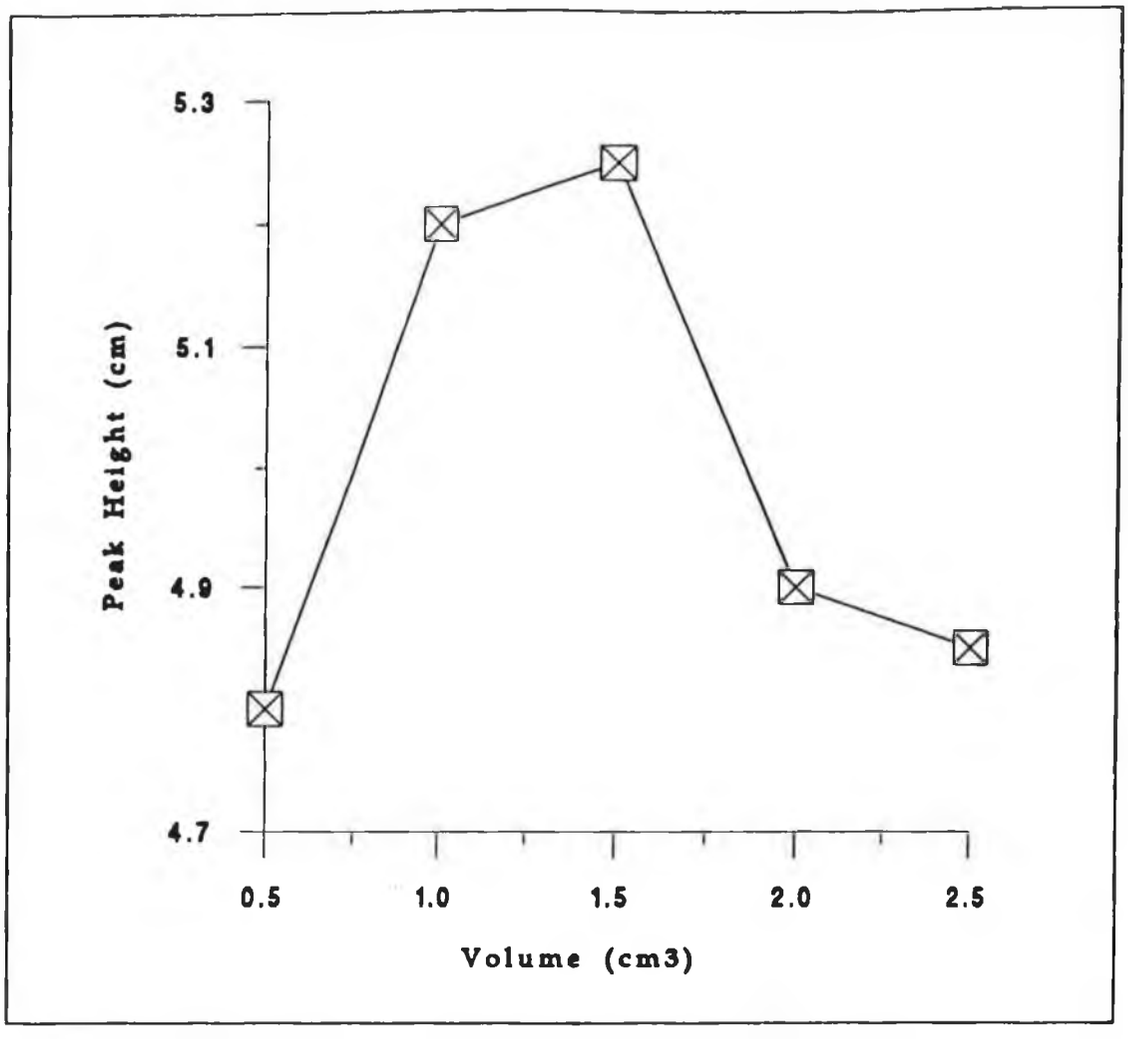

Figure. 3.6.

Equilibrium wash volume using a 50:50 CH_3CN:acetate buffer (0.1M KNO_3: 0.02M acetate.) eluent. Sample loading at 1.5 cm^3/min using acetate buffer pH 6.0.

3.3.4.3.3 Breakthrough

The term breakthrough volume is defined for these experiments as, the volume of mobile phase with which it is possible to wash the precolumn containing retained analytes, without causing elution of those analytes due to the washing effect of the solvent. Investigation of a breakthrough wash volume step in the preconcentration of the Cr(III) complex, showed that any degree of washing of the precolumn caused breakthrough of the analytes

(Figure 3.7). From the profile it would appear that the metal complex is not well retained on the reverse-phase packing. A reason for such a trend may be incomplete complex formation, which in turn would inhibit complete retention of the metal complex. In addition this low breakthrough volume, may be due to the dissociation of the Cr(III) complex in the predominantly aqueous environment during this washing step. From previous investigations it was found that a 6 hour waiting period is required to ensure complete complex formation as the kinetics of Cr(III) complex formation is slow. This intermediate washing step is important in cases where matrix or interfering ions are a problem. However in many cases it will be possible to carry out the preconcentration sequence without including this step.

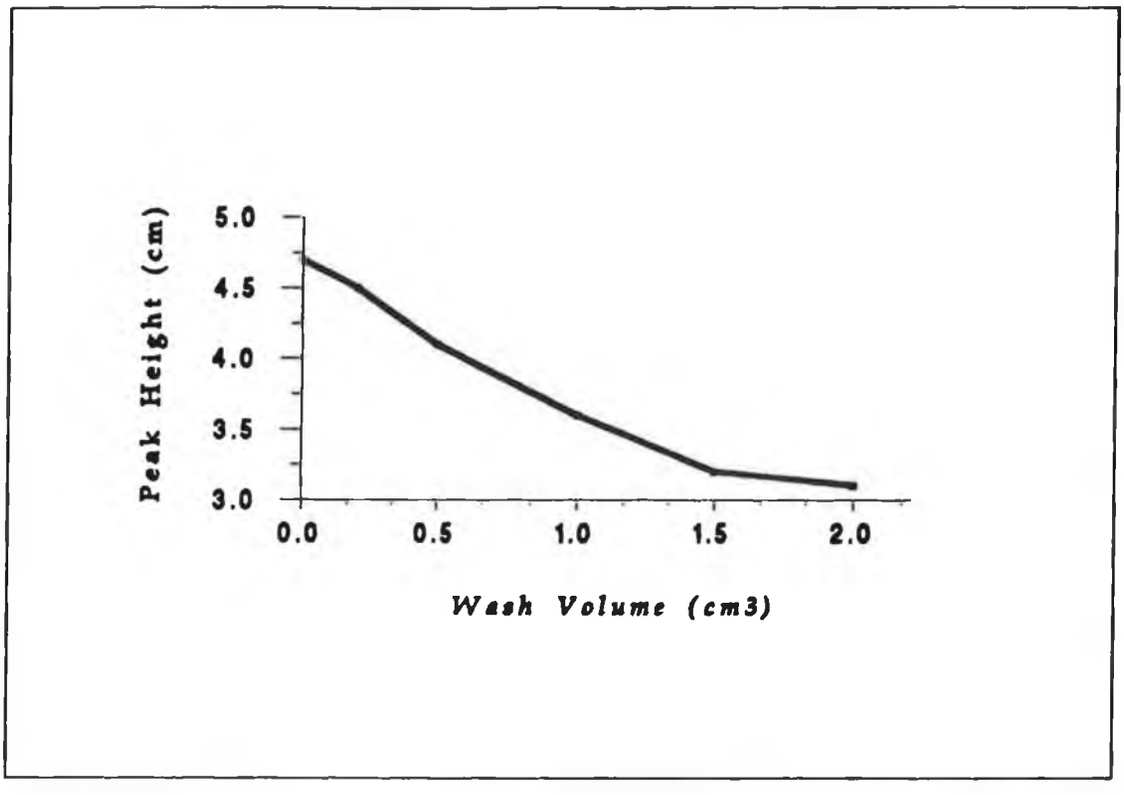

Figure. 3.7

Breakthrough wash volume. Cr(III) loaded as its Cr(III)-8-hydroxyquinolate complex, (1.10^{-3}M 8-HQ in 0.1M KNO_3; 0.02M acetate)[Cr(III)]=100ppb

3.3.4.4 *Loadability*

Results of studies shown in Figure 3.8 show that volumes of up to 10 cm^3 of 100ppb Cr(III)oxinate can be loaded without any significant deviation from linearity, thus enabling significant trace enrichment. Also higher volumes were investigated even though it was observed that the washing effect would cause a decrease in response. From this, it was observed that higher volumes (30 cm^3) of lower concentrations ie. ppt. level, enabled substantial enrichment on the precolumn while maintaining good reproducibility over a linear dynamic range of 0.05ppb-0.5ppb.

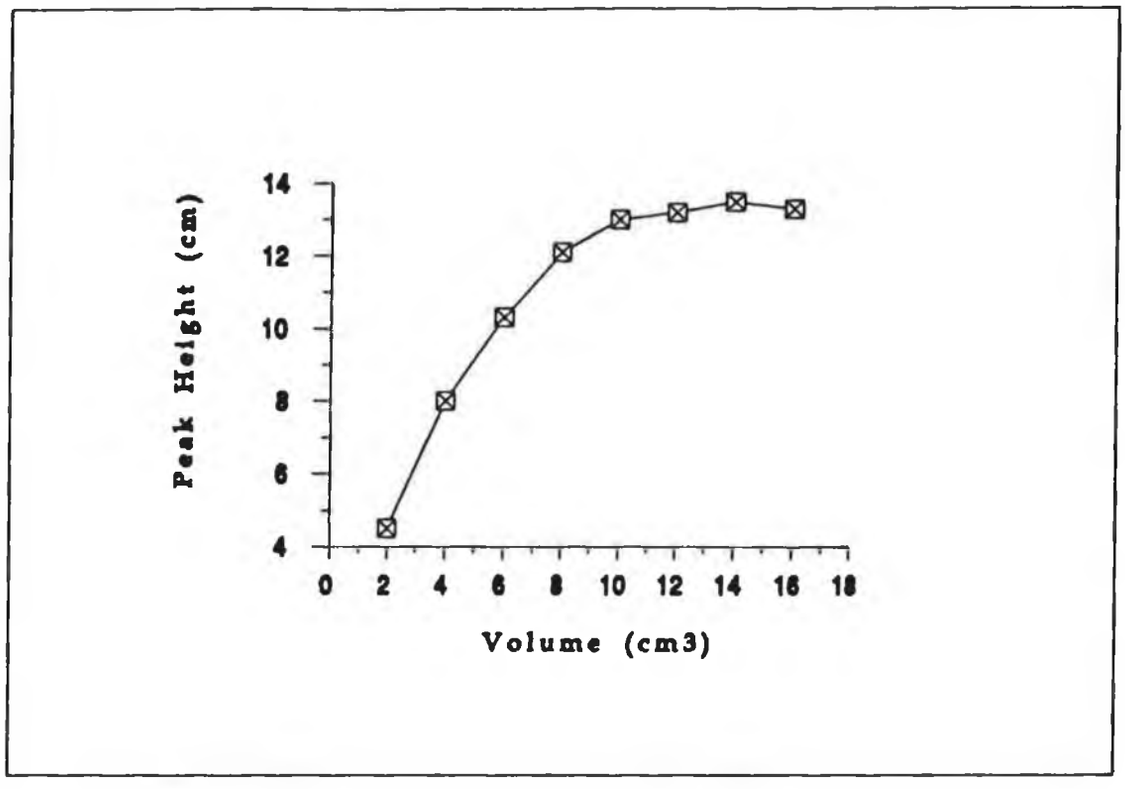

Figure. 3.8

Loadability curve for Cr(III) preconcentration. [Cr(III)]=100ppb in acetate buffer, $1 \cdot 10^{-3}$ M oxine. Flow rate = 1 cm^3/min.

3.3.4.5 *Chromatographic Separation*

The mobile phase for elution and analysis of the metal complex was a 50:50 ACN:buffer solution, using a C18 reversed-phase analytical column. The eluent mixture had the same composition as that reported in section 3.2.3.2.

3.3.5 Limits of Detection

Detection was carried out at 400nm and a LOD of 50ppt, using a 30cm^3 sample volume, was achieved. This can be compared to the detection limit of 10ppb when not using preconcentration. Reproducibility for 6 sample loadings was 4.3% CV (Table 3.3). Figure 3.9 shows a series of typical chromatograms using preconcentration of a 30cm^3 volume of Cr(III)oxine standard, and a typical preconcentration sequence is shown in Table 3.4. (Steps 1 and 3 were carried out in order to ensure no residual sample or solvents remained in the solvent delivery lines from previous runs.) It was observed from the investigation that higher loading volumes, ie., 10-30 cm^3 enable detection of lower concentrations of Cr(III). Figure 3.8 shows that even a very small volume of solvent has a washing effect as the complex is not very strongly retained. However a linear calibration graph in the range 0.05ppb (50ppt)-500ppt, using a 30 cm^3 loading was obtained with a regression of 0.9977 suggesting that higher loadings are possible and reproducible (Fig 3.10).

	Without Preconcentration	Preconcentration
LOD	**10ppb**	**50ppt**
Regression	**0.9994**	**0.9977**
Linear Dynamic Range	**0.01-0.1ppm**	**0.05-0.5ppb**
% CV	**2.2%**	**4.3%**
n	**6**	**6**
Sample Volume Loaded	**50 µl**	**30cm^3**

n= number of sample replications

Table 3.3

Comparison of Cr(III) Data With and Without Preconcentration

Oxine concentration $1 \cdot 10^{-3}$M in standard solutions. Eluent 50:50 ACN:Buffer (0.1 M KNO_3, 0.02 M acetate, pH 6.0) Detection @ 400nm.

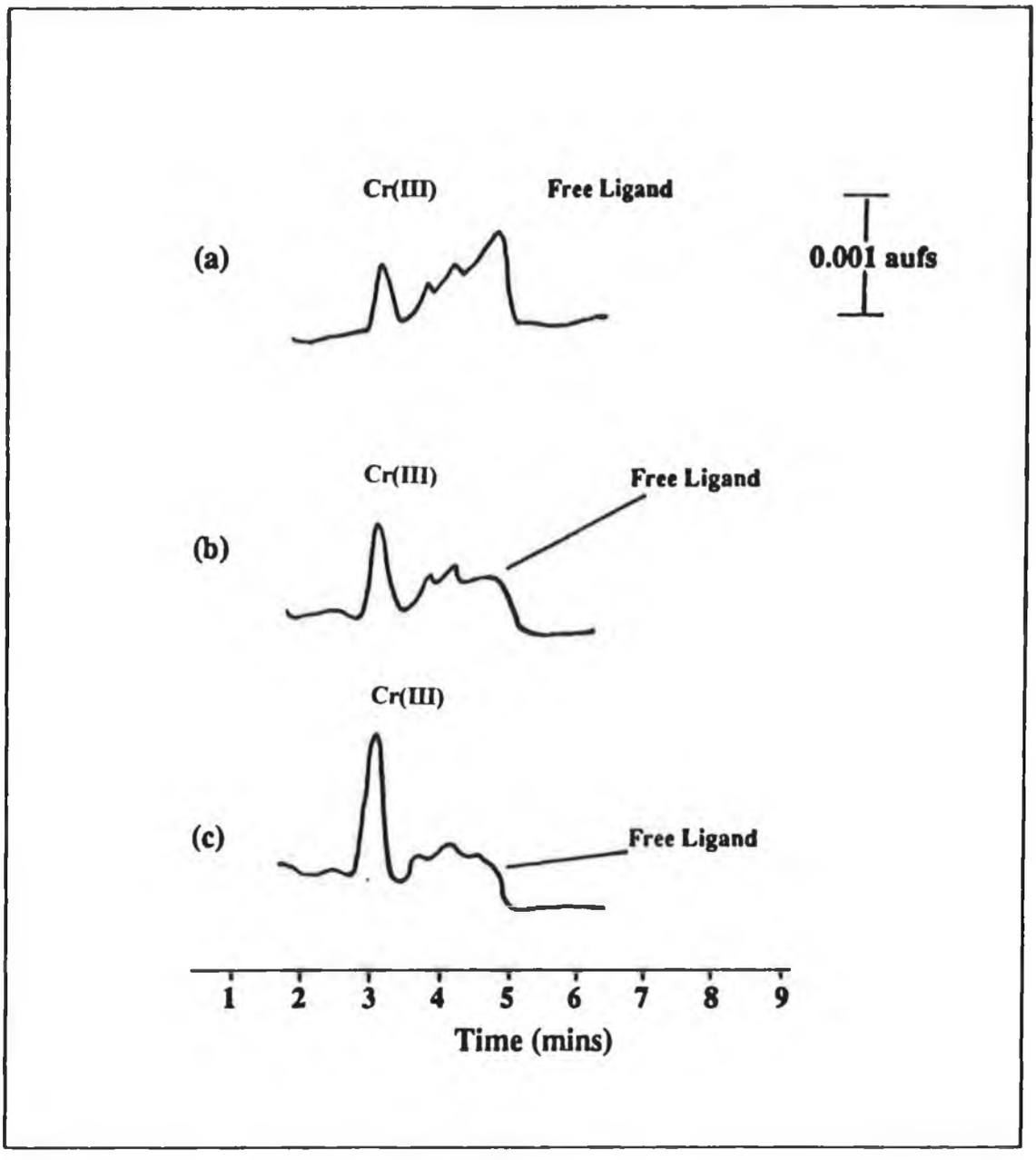

Figure. 3.9

Reversed-phase liquid chromatography separation of Cr(III) complex following preconcentration of 30cm^3. (a) 50ppt (b) 80ppt (c) 100ppt. (0.001 aufs).
Loading flow rate 1cm^3/min (Cr(III) loaded as its Cr(III)-8-hydroxyquinolate complex), 1·10^{-3}M 8-HQ in 0.1M KNO_3; 0.02M acetate. Elution flow rate 1.5 cm^3/min, using a 50:50 CH_3CN:acetate buffer.

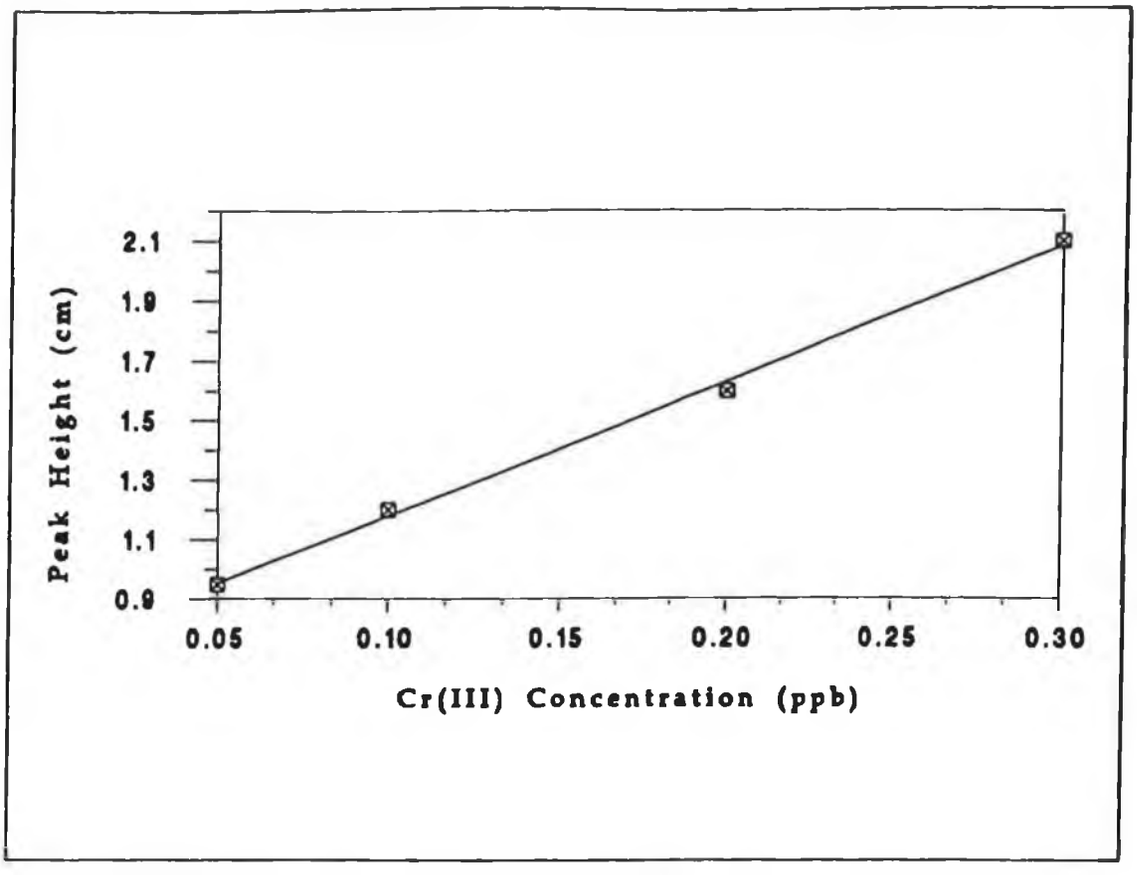

Figure 3.10

Calibration plot using Cr(III) preconcentration.
Volume preconcentrated = 30cm^3; Concentration solvent = acetate buffer (0.02M); desorption and chromatographic solvent = 50:50 ACN:Buffer pH6.

3.3.6 Interferences

No interferences were found to occur (Cu, Fe, Al, Ni, Zn, were investigated), but variations in mobile phase composition could eliminate the problems should they occur. Use of this complexing agent, although it can complex with over 60 metal species, confers selectivity for the various metal species when suitable optimised chromatographic and detection conditions are employed (ie. eluent pH and ionic strength, detection wavelength).

Step	Mode	Duration	Solvent	Flow Rate cm^3/min	Volume Delivered cm^3
1	Sample flush	1 min	Sample	6.0	6.0
2	Sample Load	2 min	Sample	1.5	2.0
3	Buffer Flush	1 min	Buffer	6.0	6.0
4	Precolumn Wash	30 secs	Buffer	1.0	0.5
5	Sample Strip	2 mins	Eluent B	1.0	2.0
6	Re-equilibrate	1 min	Eluant B	1.0	1.0

Table 3.4

Cr(III) Preconcentration Sequence

3.4 CONCLUSION

On-column enrichment for the determination of metal species, has the advantage of achieving the detection of lower concentrations due to the use of higher sample loadings, thus increasing the sensitivity of the method. From this investigation it was observed that preconcentration of large volumes, ie., > $30cm^3$ was possible. This resulted in a reduction in the LOD significantly. The limit of detection achieved by preconcentrating 30 cm^3 of Cr(III) standard was 50ppt, and reproducibility for six repeated preconcentrations was 4.3% ($30cm^3$ of 50ppt Cr(III)). An important factor of preconcentration is the selection of a suitable sorbent material, concentrator solvent and eluting solvent. Interferences were not found to pose a problem, and where resolution between interfering peaks (ie. free ligand) became a problem, alteration of the mobile phase enabled satisfactory separation.

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Chapter 4.0

Development of dyestuff-coated stationary phases using anion active organic dyes for the determination of Cr(VI) species.

4.1 INTRODUCTION

4.1.1 Ion chromatography

Since its introduction in 1975 [1] ion-chromatography has evolved into a major analytical technique. The original reason for its success was the ability to rapidly and simultaneously determine several inorganic anions at high sensitivity with minimal sample pre-treatment. The need to determine metal ion concentrations at low levels in complex matrices in the health and environmental areas is increasing. Use of high performance liquid chromatography (HPLC) for metal ion analysis in these types of samples is well documented [2].

Three main approaches can be identified in the literature reports on HPLC separations of metal ions.

(i) The formation of metal ligand complexes prior to injection or *in situ* in the mobile phase followed by separation on a non-polar stationary phase in the reversed-phase mode [3,4].

(ii) Use of an ion-exchange column and aqueous mobile phases with conductivity or spectrophotometric detection with post column reaction [5].

(iii) An approach whereby stationary phases are immobilized or impregnated with complexing agents. These have shown considerable promise as selective materials for metal ion separations [6-8].

4.1.1.1 *Ion-exchange materials*

The ion-exchange materials used in ion-chromatography fall into three general classes:

(a) polymeric ion-exchangers;

(b) silica based ion-exchangers;

(c) coated materials.

The exchange capacity of these materials must be low so that a dilute eluent can be used. Poly (styrene divinylbenzene) ion exchangers are probably the most rugged and dependable [9]. Efficiency is improving as spherical resins of uniform particle size become available. Cation exchangers are usually lightly sulfonated so that the exchange sites are in a thin zone at the outer perimeter of the beads. Anion exchangers predominantly have quaternary ammonium functional groups, which are most likely to be near the outside of the resin beads [10].

Due to the limited selectivity of ion-exchange materials the addition of a complexing agent to the mobile phase is often required in order to produce metal complexing species with a range of effective charges [11]. Alternatively, binding a complexing agent to the stationary phase itself can directly impart selectivity to the column. This would enable the use of common buffers to adjust mobile phase pH and hence control the effective formation constant of the metal with the bound ligand [8]. A major approach is to convert the sample ions to organic metal chelates which can then be separated on reverse-phase columns. The most popular chelating agents include, diethyldithiocarbamate, (pyridylazo)resorcinol, and 8-hydroxyquinoline. These reagents form strong complexes with a large number of metal ions and the complexes can be detected spectrophotometrically. Most of these seprations have been carried out on silica based supports [7].

Many publications dealing with the determination of trace metals by HPLC have concentrated on ion-exchange techniques [2]. Although ion-exchange separations of metal ions have been used in a number of important applications [12-14] there are still several problems associated with the approach. One problem is the column disturbance due to the ionic strength of the sample solution. Very high ionic strength can cause changes in the column capacity, destroying the separation. This results in the need for further sample handling to remove the bulk matrix before injection [15]. The determination of trace levels of metal ions in complex biological and environmental matrices has never been simple. Many environmental and industrial samples such as sea waters, concentrated brines, and effluent streams are of high ionic strength [16] and suffer badly from the problems mentioned above. Selective preconcentration of analytes is difficult because of the influence of alkali and alkaline earth metals in real samples. Siriraks [17], developed chelation ion chromatography as a solution to the problem, using a macroporous, poly

(styrene divinylbenzene) copolymer column with iminodiacetate functionalities to preconcentrate metals and remove Ca(II) and Mg(II).

4.1.2 Chemically bonded stationary phases

There are a number of publications describing metal separations on high performance liquid chromatographic (HPLC) grade chelating stationary phases [9,18-20]. Nearly all of these publications describe substrates with chemically bonded chelating groups. Concentration and separation with the help of chelating sorbents is characterised by a high selectivity which assures an increase in sensitivity and reliability in the subsequent determination of metal ions [9]. Chelating exchange materials have been used principally for sample clean up and preconcentration. However, an increasing number of publications are appearing in which chelation chromatography is being used for high performance separations with the chelating ligand usually present in the stationary phase [6-8 21,22].

Immobilization of suitable chelating functional groups on polymeric supports results in chelating stationary phases that are potentially more selective than ordinary cation exchange resins. Among the grafted ligands, 8-hydroxyquinoline (8HQ) has been extensively used in different forms especially grafted to controlled pore glass [6] or adsorbed on octa decyl reverse phase silica [6,8]. The chelating properties and selectivity of 8-hydroxyquinoline on polystyrene divinylbenzene copolymers has been investigated [23-25]. Although these resins are quite stable at extremes of pH their overall exchange rates are slow particularly at low pH's [25]. Risner and Jezorek [20] described a study of 8HQ-bonded silica gel with the finding that a very lightly loaded column gave the best separations. Higher ligand coverage may be sought for metal preconcentration purposes, but this is not necessarily desirable for liquid chromatographic purposes as band broadening can occur. These authors reported the possibility of making 8-quinolinol silica gel phases of the desired capacity, with high capacity for weakly interacting ions, lower coverage for strongly interacting ions and intermediate coverage for general purpose columns. Chambaz and Haerdi [19] use a similar 8-HQ bonded silica gel column to study the preconcentration and elution of a range of divalent metals (Cu, Ni, Co, and Fe). The authors found that the analytical column underwent slow decomposition. In addition a high background absorbance was a problem which meant that sensitivities could not be extended lower than 10^{-8} *M*.

4.1.2.1. *Stationary Phase Matrices*

Different solid sorbents, namely polymers, anion exchangers, reverse-phase ODS and controlled pore glass, have been used for the immobilization of organic ligands. Such substrates have been used for the uptake of metal ion present at trace levels from aqueous samples [21]. Most widely used are the sorbents based on copolymers of styrene divinylbenzene. Of great practical importance today are the styrene copolymers with macroporous structure, which compared with gel structure, provide for better permeability of the matrix. Styrene copolymers with divinylbenzene have been used to synthesise numerous chelating sorbents with iminodiacetate groups, notably, Chelex 100, Dowex A-1 and many others [9]. The sorbents based on styrene copolymers are usually characterized by a high stability in acid and alkaline solutions [22,26]. Silica bound chelating agents exhibit favourable kinetics and specificity for transition metal ions, however they are unstable at alkaline pH's [9]. A similar disadvantage was reported using controlled pore glass [22].

4.1.2.2 *Separation of metal species*

Chelating sorbents are usually capable of interacting with a large number of metals, but the stability of the formed complexes differ and depends on sorption conditions.This difference is used for selective concentration and separation of metals [9]. The difference in the selectivity of chelating sorbents with respect to certain metal ions is used for their separation. Sorbent selectivity is determined by the nature of the chelating groups and depends on the experimental conditions. Under specific conditions the sorption capacity of sorbents with respect to certain ions can differ substantially. The distribution coefficients may vary within several orders of magnitude.

Metals exhibiting a high affinity for the chelating groups in the sorbent phase can be separated by successive elution of sorbed metals with acids and complexing agents. Numerous mixtures can be separated by this technique owing to the different stability of complexes formed by the metal ions in the sorbent phase [27]. Many of the methods for sorption and separation of ions have not been employed on-line, therefore requiring off-line detection methods following the sorption step. Bonn et al.[27], described the effect of eluents containing carboxylic acids either alone or in combination with stronger complexing agents in order to study the elution of alkali, alkaline earth, and

transition metal ions from sea water. The study demonstrated the occurrence of both complexing and ion-exchange mechanisms, the rate of which, was governed by the stability of the metal complexes.

4.1.3 Coating of stationary phases

Although a number of bonded chelating groups have shown some interesting results, a greater range needs to be investigated in order to ascertain the full potential of chelating-exchange HPLC substrates. One way of achieving this without resorting to lengthy and perhaps difficult synthesis of chemically bonded groups is to coat a particular substrate with selected compounds. Modifying stationary phases by coating with specific compounds is a well known technique in ion-chromatography [15], and mainly concerns the formation of dynamic ion-exchange coatings using quaternary ammonium or alkyl sulphonate-based compounds. Resins coated with dyes are easily prepared and offer the potential for a number of novel separations.

4.1.3.1 *Developments in coated stationary phases*

The use of dye-stuff coated stationary phases offers a promising and effective means of metal ion analysis. A number of publications report the preconcentration and/or separation of inorganic anions on dye-coated stationary phases [28-33]. The majority of the reports deal with the preconcentration, with the need for detection methods off-line [28,29].

Few workers have investigated dyestuff coatings on HPLC grade materials. Recently Dowex-2, an anion-exchange polystyrene based resin was coated with sulfonephthalein dyestuff for the preconcentration of Cu(II) and Cd(II) with subsequent detection by atomic absorption spectrometry [28]. These authors reported a more rapid sorption of the cadmium ion on the dye-coated resins, than on common ion-exchange resins, and the optimum pH range for sorption of Cu(II) was wider than for Cd(II). In addition the pyrochatecol violet (PV) coated resin offered a wider pH range for Cu(II) sorption than a xylenol orange coated resin, and XO coated resin showed a greater sorbing capacity than Dowex-2 coated with PV. The % recovery for Cd and Cu for both dye-coated columns was of the order 96.7-98.3%. However a % RSD of greater than 5%, for river samples analysed, was

obtained with a slight improvement when using a standard addition method. A LOD of 10ppb was achieved, but quantitative and reproducible determination of either Cd(II) or Cu(II) was not possible at lower concentrations.

4.1.3.2 *Separations on dye-stuff coated materials*

An ion chromatographic technique commonly reported is ion pair or ion interaction chromatography [26]. Ion interaction columns are generally produced by sorption of an organic, hydrophobic and/or ionic molecule on the surface of the stationary phase. In ion interaction chromatography the hydrophobic counterion is sorbed onto the stationary phase and forms a double layer. The analyte ions of interest are then separated on the diffuse secondary layer. Silica based reversed-phase columns and neutral poly (styrene divinylbenzene) copolymer columns have been used as supports for the ion interaction reagent. Dyestuff molecules have been used as counter ions in ion-pair chromatography [30].

Golombek and Schwedt [33] recently showed that it was possible to achieve excellent high-speed separations of common anions using a dynamic coating of dyestuff (methyl green) on a neutral polystyrene based resin. Eluents consisted of aqueous solutions of 2,4-hydroxybenzoic acid or 4-hydroxybenzoic acid with potassium hydroxide and small amounts of dyestuffs as eluents. A similar approach by Walker [30] reported the separation of seven inorganic analyte anions on an ethyl violet coated stationary phase. A variety of stationary phases were investigated, including a polymer-based packing and a silica-based ODS packing. Emphasis was placed on mobile phase variables affecting analyte retention such as pH, ionic strength and counterion type and concentration. In this, the dye (ethyl violet), included in the mobile phase, acts as an ion interaction reagent (IIR) which enables indirect visible detection, due to the chromophoric nature of the ethyl violet.

Jones and Schwedt [15] encorporated the idea of dyestuff coatings to produce HPLC grade cation exchange and chelating exchange substrates for the separation of metals (Mg(II), Cu(II), Zn(II)). The separation and preconcentration of divalent and trivalent species is described in which the effect of eluent pH and ionic strength is investigated. It was found that eluent pH had a strong influence on separation and preconcentration, while ionic strength causes little effect. By using a pH gradient the authors found it possible to separate and preconcentrate the metal ions (Mg(II), Mn(II), Zn(II),

and Cu(II)) on a single column. The dyes chosen, bromophenol blue and chrome azurol S, both gave permanent coatings on a neutral polystyrene resin. The insensitivity to ionic strength was an attractive feature, particularly when applied to analysis of samples of high salt concentration.

Development of anion exchange columns by coating RP-18 stationary phases with methylene blue, methyl green and crystal violet was reported recently [29]. Retention behaviour of fluoride, chloride, bromide, nitrate, phosphate and sulphate anions was investigated, and the influence of ionic strength and pH on the separation was discussed. A permanent anion exchange coating of crystal violet was obtained, enabling efficient separation of the anions.

4.1.3.3 *Cr(VI) determination using dye-stuff coated stationary phases*

Studies encorporating dye coated stationary phases for metal ion analysis have used the approach of coating dyestuff onto large particle size resins. Resins such as Dowex anion exchange resin, neutral XAD-2 and XAD-4 resins, and small particle size HPLC grade resin have been coated, with post column reaction detection. The results obtained compared well with those of commercially available chelating resins. Particular emphasis was placed on separations in high ionic strength media [31,32].

To date no reference has been made including the determination of Cr(VI) species using dyestuff coated columns. Much of the work to date involved the use of chemically bonded stationary phases, both silica based and ST-DVB based phases encorporating the immobilization of an appropriate ligand. The most commonly applied ligand was 8-hydroxyquinoline and its derivatives.

In this investigation a similar approach is used to achieve permanently coated anion exchange columns for the analysis of Cr(VI) species. Exhausted reverse-phase C18 columns are employed in the study, whereby the used columns are coated with two anion active organic dyes, methylene blue and crystal violet. Permanent coatings and efficient, reproducible analysis was achieved. Long column lifetimes were characteristic of the approach.

As a comparison to coating HPLC grade material, nonionic styrene-divinyl benzene copolymer resins (XAD-2 and -4) were also investigated. These resins have been utilized in a number of studies in the separation and preconcentration of metal ions [21,23]. In this investigation a variety of dye coating techniques are investigated in order to demonstrate their applicability in Cr(VI) analysis with comparison to the HPLC grade substrate.

4.1.4 Aims and Objectives

The aims of the investigation include.

* Study of the effect of coating exhausted reverse-phase HPLC columns and neutral poly (ST-DVB) copolymer resins with anion active organic dyes in order to effect Cr(VI) anion analysis.

* To assess the use of the dye coated columns as an alternative to using commercial ion-exchange columns, to achieve adequate efficiency, reproducibility and limits of detection for the analysis of Cr(VI).

4.2 EXPERIMENTAL

4.2.1 Apparatus

A high performance liquid chromatograph consisting of, a Waters (M 501) HPLC pump, a model 7125 injector (Rheodyne) with a 20 µl loop, a tunable absorbance detector (Waters 486) and a computing integrator (Waters 746 Data Module) was used. For post-column reagent (PCR) delivery a second Waters (M 501) HPLC pump was used.

A column containing small glass beads (10 cm x 4.6 mm) was required as a mixing column, effecting mixing of the eluent from the dye-coated analytical column and the post column reagent, 1,5-diphenylcarbazide, prior to detection. Additional work involved the use of empty stainless steel columns of various lengths and sizes packed with neutral resins, coated with methylene blue dyestuff, to assess their use for Cr(VI) analysis.

4.2.2 Reagents

The chemicals used were analytical-reagent grade. Methanol was supplied by Lab. Scan. Liquid chromatography quality water was obtained by purifying water in a milli Q filtration system (Millipore, Bedford, MA, USA). Post-column reagent, 1,5-diphenylcarbazide (1,5-DPC) (Aldrich chemicals), methylene blue (BDH Chemicals), crystal violet (Gurr, Microscopy materials, BDH. Ltd), and p-hydroxybenzoic acid (BDH Ltd.) were all used without purification. Cr(VI) standard solutions were prepared by dissolving the potassium chromate salt (K_2CrO_4) in deionised water.

4.2.3 Columns

Preliminary work concentrated on using disguarded reverse-phase C18 columns of various lengths and packings. Methylene blue was coated onto a Whatman Partasil C18 (25cm x 4.6mm i.d.) and a Waters Bondapac C18 (30cm x 4.6mm i.d.) column. A Microsorb C18 column (15cm x 4.6mm) was used with crystal violet. The columns employed here had previously been used for chemical and biological analyses but had lost efficiency and could no longer be used.

4.2.4 Resins

The large particle size resins employed for this study were Amberlite XAD-2 and -4, poly(styrene divinylbenzene) copolymer resins.

4.2.5 Mobile phase

The appropriate quantity of *p*-hydroxybenzoic acid (*p*-HBA) was dissolved in water, and sonicated to aid dissolution. The pH was adjusted by adding 0.1 *M* KOH until the required pH was reached.

4.2.6 Post Column Reagent

1,5-diphenylcarbazide reagent (2m*M* 1,5-DPC, 10% MeOH, 1N H_2SO_4) was prepared by dissolving the appropriate quantity of 1,5-DPC in methanol and subsequently adding sulphuric acid in milli Q water. This reagent must be stored in a light tight container.

4.2.7 Column Coating Procedure

The initial investigations involved the use of disguarded commercial HPLC C18 columns. The two step procedure for coating the dyes, methylene blue and crystal violet, was carried out similarly to that described by Muller et al. [29]. 1m*M* aqueous methylene blue (pH 9.0) was loaded onto the column at a flow rate of $1cm^3$/min, until, the dyestyuff appeared at the column exit. Then methylene blue (1.0 m*M*) dissolved in an aqueous solution of 3m*M* *p*-HBA, pH 9.0 was loaded onto the column at a flow rate of 1.0 cm^3/min, until breakthrough occurred. In the case of crystal violet, the first coating involved 0.6m*M* aqueous dye solution (pH 9.0) and then 0.05m*M* solution of the dye in 3m*M* *p*-HBA.

Following the coating procedure, the chromatographic equipment (except columns) was washed with methanol, then water, and then the mobile phase. The columns were then equilibrated with mobile phase for 30 mins prior to injection of the sample.

4.2.8 XAD-Resin coating

In the case of large particle neutral resins on-line and off-line coating procedures were investigated. The dye-stuff used in these studies was methylene blue.

4.2.8.1 *On-line coating.*

A 10 cm stainless steel column was packed with Amberlite XAD-2 resin and coated using a variety of procedures summarised in Table 4.1. Prior to coating the column under a new set of conditions, the dye was removed using 100% methanol and then the packing was washed with water.

4.2.8.2 *Off-line coating.*

10g of the resin was stirred in 1m*M* methylene blue solution prepared in 20% methanol (pH 4.6-4.8, ie., no pH adjustment) for 24 hours. The coated resin was filtered and dried in an oven overnight (70^0C) and packed dry into the column. Following packing with the resin, 1m*M* dye in 3m*M* *p*-HBA, pH 4.8 was pumped through the column at a flow rate of 0.5 cm^3/min for 60 mins in order to equilibrate the column. Alternatively the wet resin (prior to drying) was packed as a slurry, and the columns equilibrated in a similar manner.

PROCEDURE		COATING CONDITIONS	OBSERVATION
1	(a)	1m*M* methylene blue (MB)	No retention
	(b)	1m*M* (MB) in 3m*M* *p*-HBA pH 9.0	Poor coating
2	(a)	1m*M* MB in 20% methanol pH 9.0	No retention
	(b)	1m*M* MB in 3m*M* *p*-HBA pH 9.0	Well coated
3	(a)	1m*M* MB in 20% methanol pH 4.7	Slight retention
	(b)	1m*M* MB in 3m*M* *p*-HBA pH 9.0	Well coated
4	(a)	1m*M* MB in 20% methanol pH 7.0	Slight retention
	(b)	1m*M* MB in 20% methanol and 3m*M* *p*-HBA	Well coated.

Table 4.1

On-line dye coating steps carried out on Amberlite XAD-2 and XAD-4 neutral resins.

4.3 RESULTS AND DISCUSSION

4.3.1 C18 reversed-phase columns

4.3.1.1 *Column coating.*

The columns used in this investigation were disguarded reversed-phase HPLC columns. These disguarded columns, whose separation efficiencies when used in reversed-phase chromatography had diminished, were used to investigate the analysis of Cr(VI), following coating with anion active organic dyes.

The organic dyes investigated, were methylene blue and crystal violet (Figure 4.1). The columns were coated by using a two step procedure, by firstly pumping aqueous solutions of the dyes in water and then in a 3m*M* aqueous solution of *p*-hydroxybenzoic acid (*p*-HBA). Alkaline solutions of the dye were coated, as it was found that coating at low pH tended to reduce the hydrophobicity of the dyes, and thus decrease the adsorption rate of the dye [29]. The addition of *p*-HBA to the dye solutions resulted in greater adsorption, but it also limited the pH values and the dyestuff concentrations which could be used for coating by reducing the solubility of the dyes [29]. It was found that the addition of mobile phase containing *p*-HBA to the coating dye solution assisted column equilibration following the coating procedure and reduced the effect of breakthrough which was evident when the *p*-HBA reagent was not encorporated in the coating procedure.

The amount of the dye-stuff adsorbed by the column packing was determined by the breakthrough method. Breakthrough is defined for these set of experiments, as the quantity of dyestuff which was pumped through the column until the dyestuff appeared at the column exit. The first column coated was a (Whatman) Partasil C18 column (25cm x 4.6mm i.d.) on which a total of 123.87 mg of methylene blue was adsorbed following both coating steps. On the crystal violet coated Microsorb C18 column (15cm x 4.6mm i.d.) 50.9 mg adsorbed before breakthrough occurred. Maximum absorption of the dye was achieved by inclusion of 3m*M p*-HBA in the dye solution to be loaded.

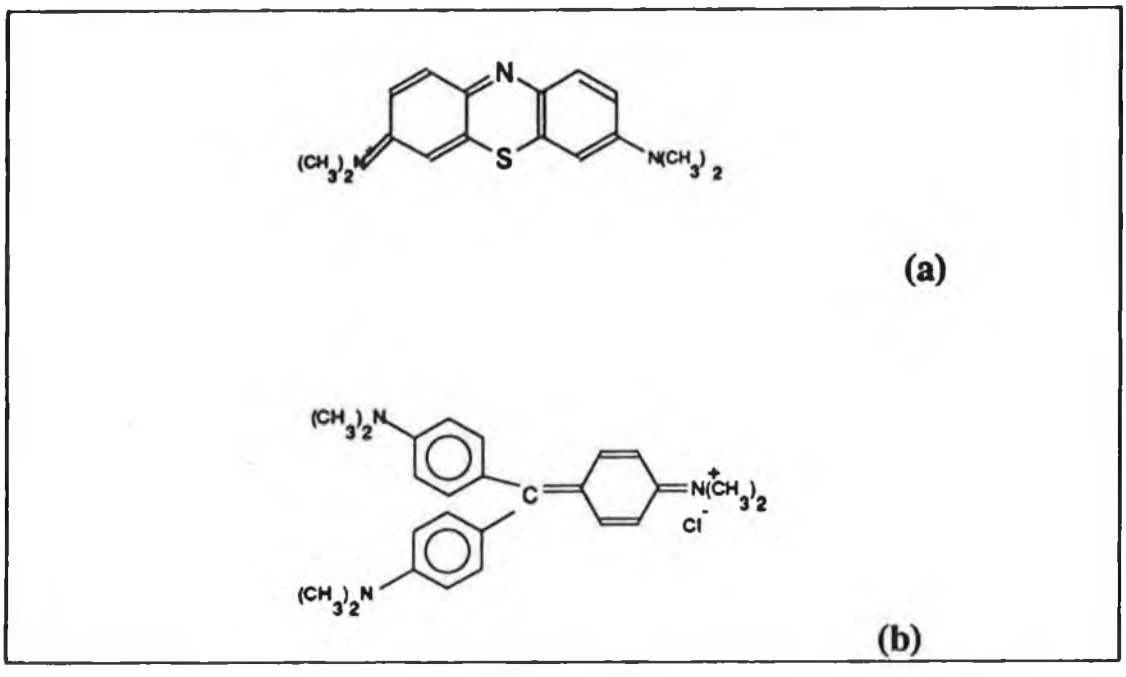

Figure 4.1

Chemical structures of (a) methylene blue and (b) crystal violet.

4.3.1.2 *Effect of mobile phase characteristics*

From the investigations carried out it was evident that pH and concentration of the mobile phase played a dominant role in the retention characteristics of the Cr(VI) anion. In order to assess the anion exchange properties of the new column, aqueous Cr(VI) 1ppm standard solutions were injected directly onto the column. Mobile phase characteristics were varied to achieve optimum retention and elution characteristics. Figure 4.2a illustrates a chromatogram of Cr(VI) analysed on the methylene blue coated column and Figure 4.2b, Cr(VI) on the crystal violet column using the optimum conditions selected. Following coating of the columns and adequate equilibration (approx. 30 mins.) with *p*-HBA pH 8.2, none of the dye appeared to leach from the column. However on a column where the dye broke through, the addition of a small quantity (5 x 10^{-3}m*M*) of dye to the mobile phase enabled stabilisation of the baseline.

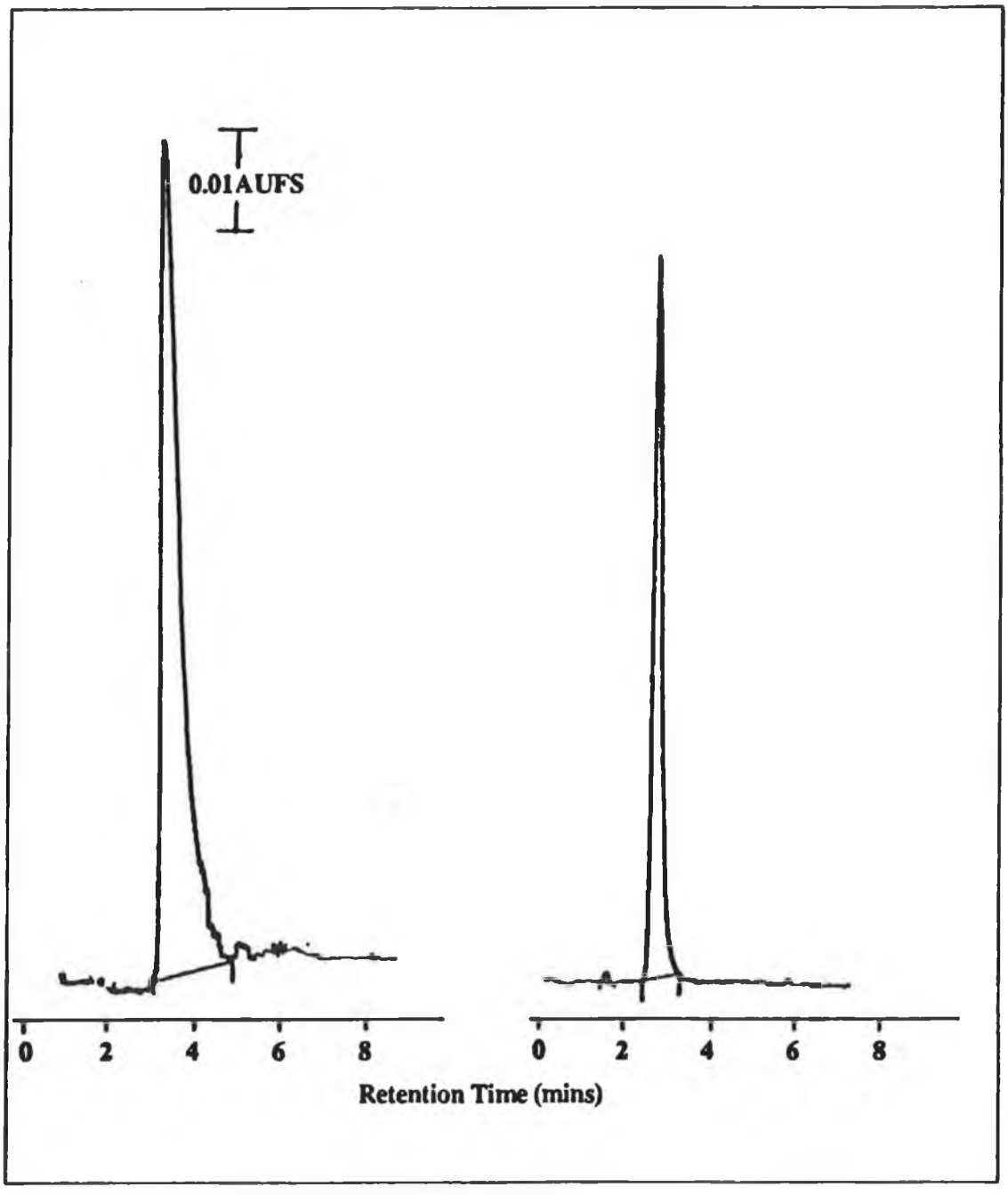

Figure 4.2

Chromatograms showing Cr(VI) determination on (a) methylene blue coated and (b) crystal violet coated C18 columns.

Eluent: 5mM p-HBA, pH 8.2. Post column derivitization using 2mM 1,5-diphenylcarbazide reagent. Spectrophotometric detection @ 540nm. [Cr(VI)]=1ppm

4.3.1.2.1 *Effect of pH*

The influence of pH of the mobile phase on the elution times is illustrated in Figure 4.3. The study showed that a reduction in the pH caused increased retention, resulting in broader peak shapes. The optimum pH for both dye-coated systems was pH 8.0-8.4. At lower pH's the elution times were too long and higher pH values caused the Cr(VI) species to be eluted with the solvent front. The investigations showed that dye coated columns were very sensitive to pH in that even a small change in pH, caused a substantial variation in retention times.

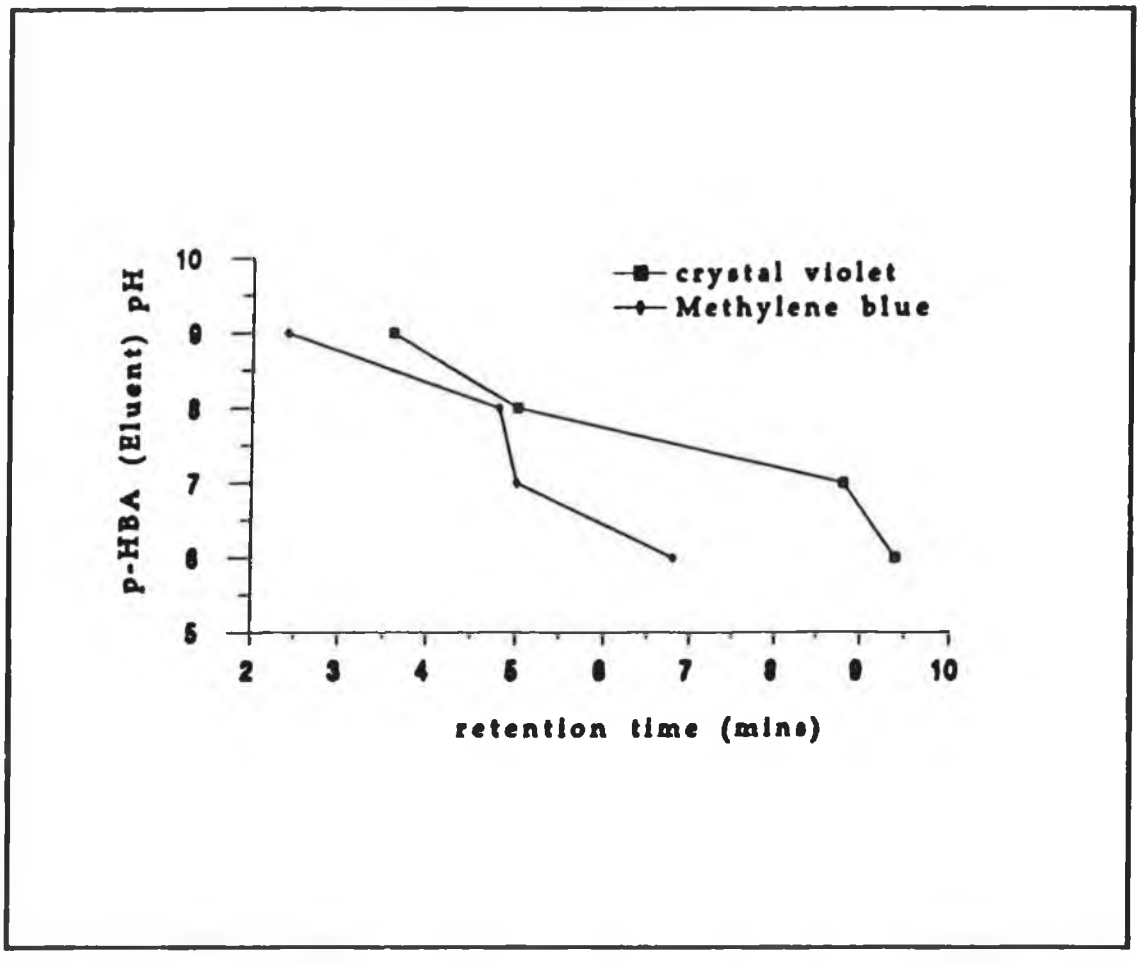

Figure 4.3

Effect of mobile phase pH on Cr(VI) retention on methylene blue and crystal violet coated C18 columns. [Cr(VI)]=1ppm,[p-HBA]=5mM. PCR; 2mM diphenylcarbazide(DPC), 540nm detection.

4.3.1.2.2 *Effect of mobile phase ionic strength*

Figure 4.4 illustrates the influence of *p*-HBA concentration on the elution time of the Cr(VI) species. Investigations showed that an increase in concentration of *p*-HBA had the effect of reducing the elution times, in addition to improving peak shape. A combination of adjusting pH and concentration of p-HBA provided the optimum condition for Cr(VI) elution and suitable peak symmetry was achieved. A pH of 8.2 and a concentration of 5.0m*M* p-HBA was found to provide the optimum separation for this system.

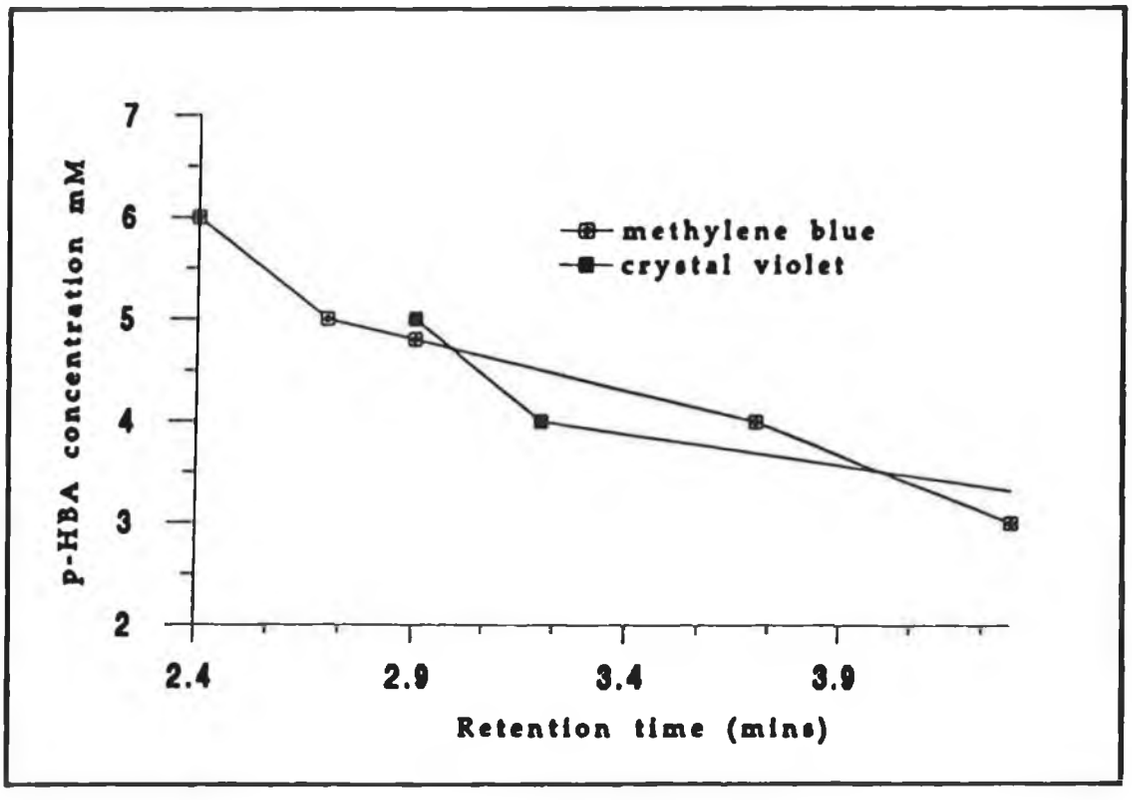

Figure 4.4

Effect of eluent p-HBA concentration.

[Cr(VI)]=1ppm, pH 8.2;PCR detection, 2mM DPC at 540nm.

4.3.1.3 *Cr(VI) detection*

Detection of Cr(VI) species was carried out spectrophotometrically. Cr(VI) can be detected directly at 400nm [34]. In this investigation a detection limit of 50ppb was achieved using this wavelength for both dye coated columns. The ligand 1,5-diphenylcarbazide has been widely used as an indicator specific for Cr(VI) determination [35]. By encorporation of this ligand for post column derivitization, with detection at 540nm, a LOD of 5ppb was achieved. This improved sensitivity was confirmed by comparing calibration curves prepared for detection at 400nm and 540nm. It was found that the slope of the curve for 540nm detection was 20 times greater than for direct detection at 400nm.

In cases of dye leaching from the column, post column derivitization was not possible due to the high noise level created as a result of interactions between the dye and PCR. In this study however the dye did not leach when adequate equilibration of the columns was carried out, thus post column detection was possible.

4.3.1.4. *Interferences*

The encorporation of post column derivitization with 1,5-DPC enabled selective Cr(VI) detection at 540nm. At this wavelength, interferences from NO_3^{2-}, NO_2^-, SO_4^{2-} and, Cl^- were not found to be a problem. These ions were also investigated using detection at 400nm again showing no interference. However the mobile phase pH value and *p*-HBA concentration could be varied, to combat interference, should it occur.

4.3.1.5 *Column lifetime*

Following daily use of the column, it was washed with milli-Q water, to remove any remaining salts. This ensured good reproducibility the following day. The efficiency of the column did not appear to diminish greatly during a period of two months. Following any reduction in efficiency (ie., drop in peak area, or broadening of peaks), the column was stripped of the dye using 100% methanol, and subsequently recoated in an identical manner to the initial

coating procedure. As a result of the second coating the retention times changed and mobile phase conditions had to be re-optimised. However similar limits of detection and quality of chromatography was achieved. Thus it was evident from this study that columns could be re-used following a reduction in efficiencies, and column lifetimes were substantial. A drop in efficiency may be a consequence of the sensitivity of the silica based columns towards alkaline pH. It was also observed that repeated strippings and coatings of the column had deleterious effects. It was found that the columns could be stripped and recoated up to 5 times (variable, depending on each individual column), beyond this their efficiencies diminished. Intra-repeatability of the procedure was assessed using methylene blue dye, in that a second column (Bondapack C18, 30cm x 4.6mm) was coated in a similar manner. The results obtained were compared to those of the first column coated. Similar detection limits and efficiencies were observed, indicating that the procedure is repeatable with other used C18 columns.

4.3.1.6 *Efficiency and Reproducibility*

In order to assess the reproducibility of both crystal violet and methylene blue coated columns, 1ppm solutions of Cr(VI) were injected repeatedly. Table 4.2 shows that the results for the methylene blue coated column, using PCR detection had a % CV of 1.2% for 6 injections of 1ppm. For the crystal violet system with PCR detection, the CV was 3.7% for six replicate injections. In addition to peak height reproducibility, retention times were also noted, having coefficients of variation of 0.3 % and 0.4% for methylene blue and crystal violet respectively (n=6). These results indicate that this is a reproducible procedure for Cr(VI) determination.

4.3.1.7 *Column efficiencies*

A Microsorb C18 column (15cm x 4.6mm), coated with crystal violet showed greater efficiency than the Partasil (Whatman) C18 column (25cm x 4.6mm) coated with methylene blue. In order to compare these columns of different lengths, the efficiencies are expressed as Height Equivalent to a Theoretical Plate (HETP or H). A value of 4.39 (H) for the crystal violet column as compared to 29.5 for the methylene blue column confirms that the

former has approx. 7 times greater efficiency than the latter. The reason for these differences in efficiencies, may however be due to the history of the column usage and also its degradation due to such high pH's [29].

	Methylene Blue	**Crystal Violet**
Cr(VI) Retention time (mins)	2.89	3.73
Limit of Detection	5ppb	5ppb
Linear dynamic range	5ppb-1ppm	5ppb-1ppm
N	846	3410
HETP	29.5	4.39
% CV	1.2	3.6

N = number of theoretical plates.

Results based on post column derivitization detection.

TABLE 4.2.
Efficiency of dye-stuff coated C18 columns in the analysis of Cr(VI).

4.3.1.8 *Calibration Curves*

Calibration curves using a 20µl injection over a linear dynamic range of 5ppb-1ppm on both columns were obtained. Correlation coefficients of 0.999 were achieved for both columns, and the limit of detection for chromate, using post-column derivitization with detection at 540nm, was found to be 5ppb. Spectrophotometric detection at 400nm achieved a limit of detection which was 10 times higher ie. 50ppb. Limit of detection was based on the criteria of a signal to noise ratio of 3:1. Figure 4.5 illustrates calibration curves for Cr(VI) standards injected onto both methylene blue and crystal violet coated reversed-phase columns. The curves illustrate clearly that the most sensitive system is the crystal violet one, as the slope of the curve is much steeper than that for methylene blue.

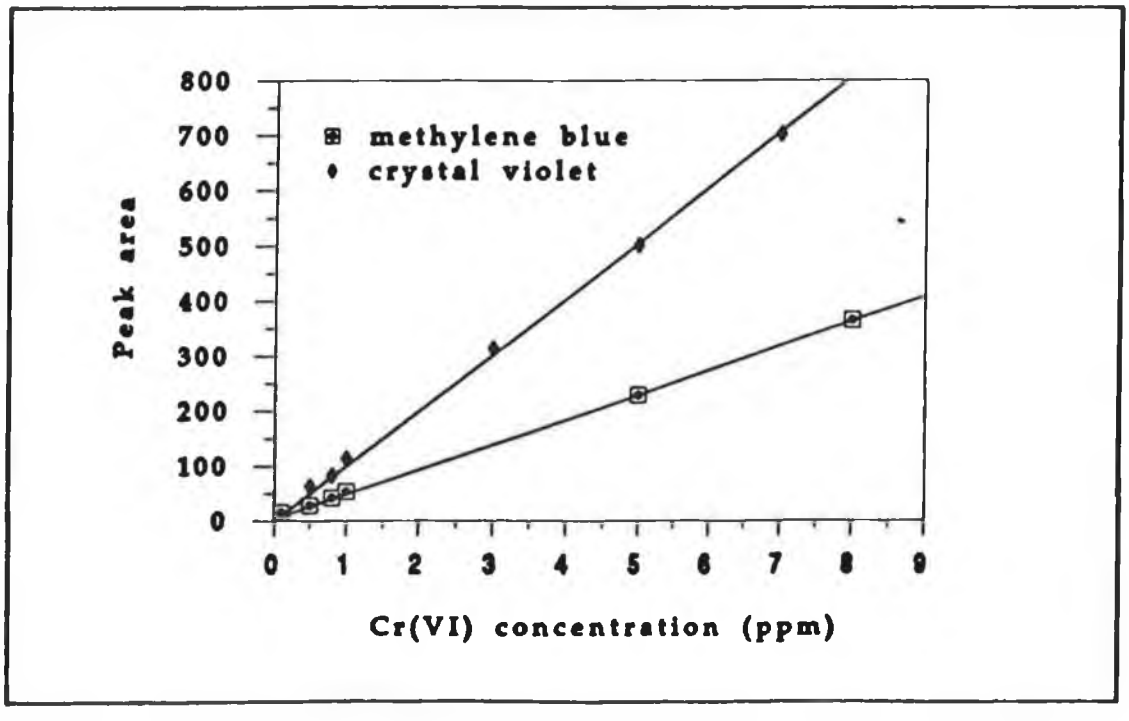

Figure 4.5

Calibration curve for Cr(VI) determination using methylene blue and crystal violet coated columns.

Eluent: 5mM p-HBA, pH 8.2. PCR detection: 2mM DPC @ 540nm.

4.3.2 Non-ionic poly(styrene-divinylbenzene) copolymer resins

For investigations involving the neutral resins only methylene blue dye was used.

4.3.2.1 *On-line coating*

Table 4.1 outlines a variety of coating combinations investigated for the XAD resins. Step 1 demonstrated that no retention of Cr(VI) occurs with the absence of methanol in the coating solutions. Encorporation of methanol was shown previously to aid coating of chelating dyes onto XAD resins [33]. Step 4 however, encorporates 20% methanol with the loading dye solution without pH adjustment, and slight retention characteristics were noted using this sequence. A further coating step encorporating *p*-HBA (mobile phase) in the dye to be loaded was employed as it was thought to aid coating and equilibration, a factor noted previously in the coating of C18 silica based columns [29]. The chromatograms (Figure 4.6a & b) obtained using this technique show a broad slightly retained peak for Cr(VI), demonstrating the poor coating characteristics, ie uneven and inadequate coating using the on-line procedure.

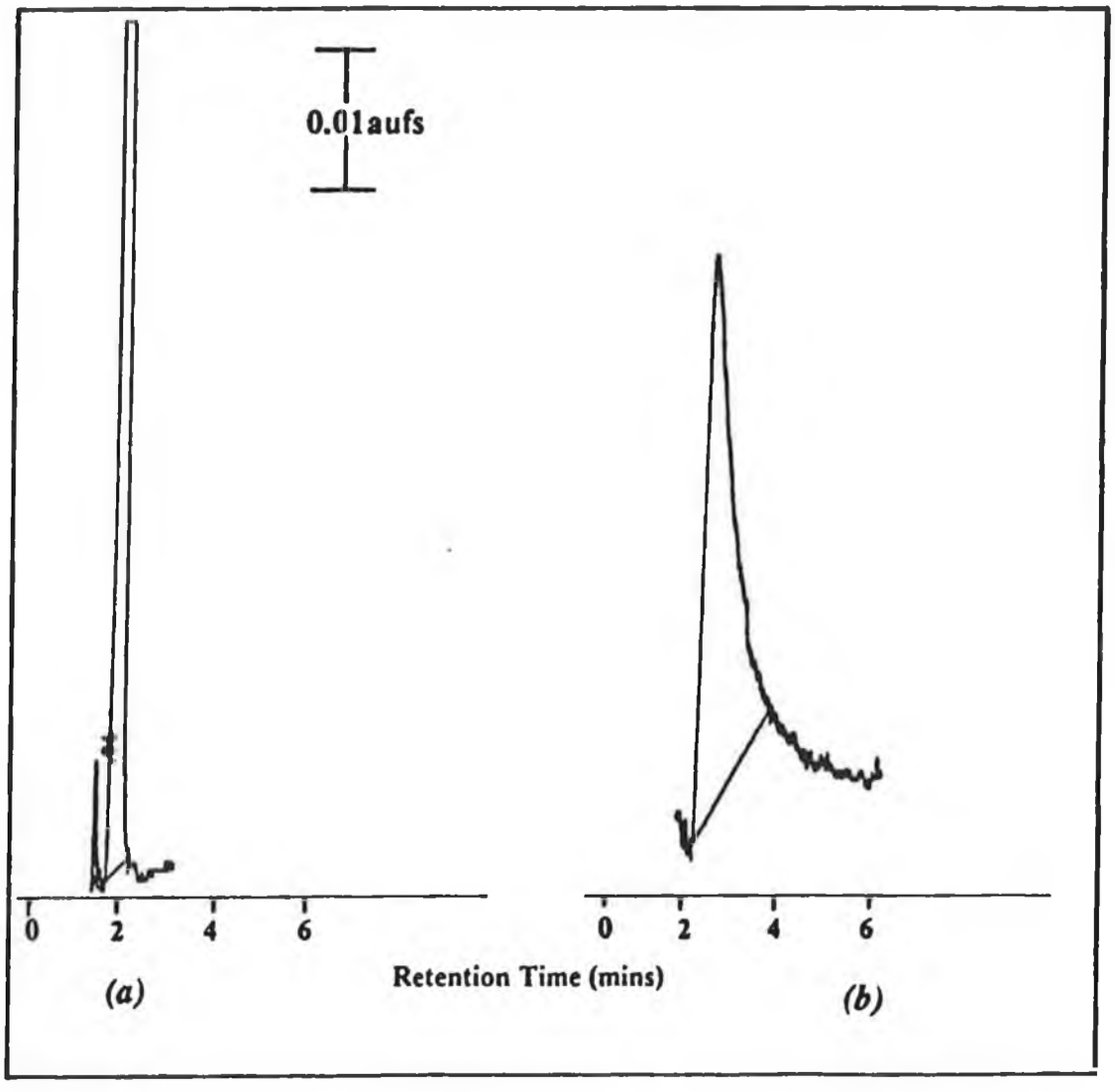

Figure 4.6

Chromatograms showing the effect of on-line coating of Amberlite XAD-2 neutral resin.

Coating sequences: (a) 1mM methylene blue, 1mM methylene blue in 3mM p-HBA, pH 9.0. (b) 1mM methylene blue in 20% methanol, pH 7.0, 1mM methylene blue in 20% methanol and 3mM p-HBA.

(Breakthrough method was used when coating.)

4.3.2.2 *Packing of the resin slurry*

Off-line coating of the resins demonstrated better retention characteristics and provided better peak symmetry for the analysis of Cr(VI). Variation of mobile phase conditions, pH and ionic strength (*p*-HBA concentration), had significant effects on retention and elution characteristics. Packing of the resin as a slurry in a solution containing dye and 20% methanol proved to be a time consuming operation. Following packing, the mobile phase containing a low concentration of dye was passed through the column to aid equilibration prior to analysis. Injection of 1ppm Cr(VI) standards using the optimum elution mobile phase of pH 7.0, 6m*M* *p*-HBA provided sharp peaks, but slight tailing was noted (Figure 4.7a). A linear dynamic range for the calibration was achieved in the range 10ppb-1ppm, with an LOD of 10ppb using a 15cm column and visible detection at 400nm. Reproducibility for six replicate sample injections of 1ppm was 2.9%.

4.3.2.3 *Packing of dried resin*

A lesser degree of band broadening was observed using a column which was packed with dried resin and which had been coated with methylene blue dyestuff. However problems associated with packing columns of such lengths (15cm), with dried coated resin include, the occurrence of voids along the column length as opposed to at the column ends. However very satisfactory reproducibility of 1.09% for six replicate injections of 1ppm Cr(VI) was achieved using a mobile phase of 5m*M* *p*-HBA pH 9.0 (Figure 4.7b).

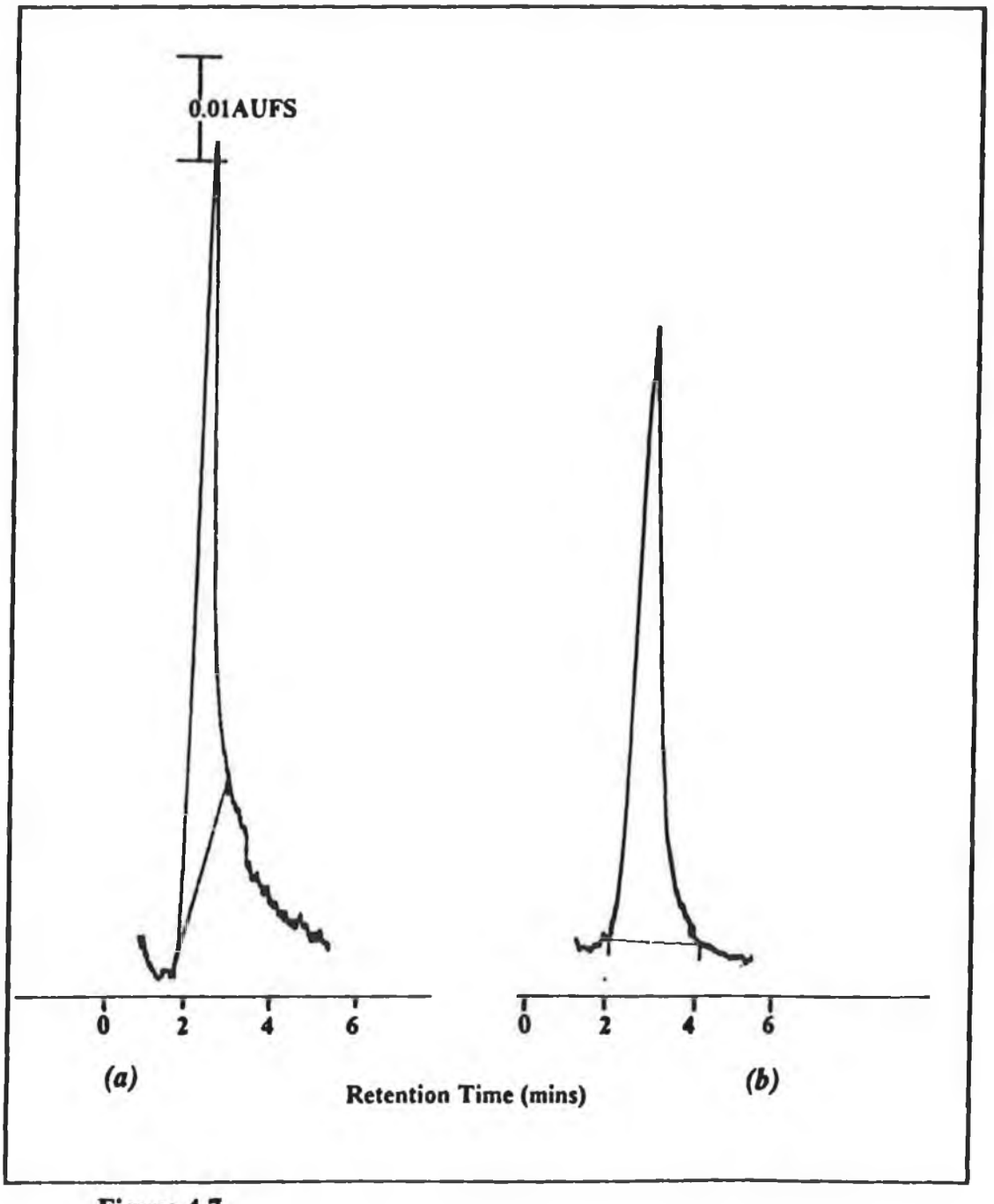

Figure 4.7

Chromatograms showing the effect of off-line coating of XAD-2 resin.

(a) Packing of the column with the resin slurry

Eluent:6mM p-HBA, pH 7.0

(b) Packing the column with dry coated resin.

Eluent: 5mM p-HBA, pH 9.0

4.3.2.4 *Effect of particle size*

Investigations carried out on both XAD-4 and -2 demonstrated shorter retention times for the smaller XAD-4 resin than -2. Increased retention due to the smaller particle size of XAD-4 was expected. However the larger particle size of XAD-2 may have allowed greater adsorption of the dye, thus creating more ion-exchange sites which in turn enhanced retention. This variability of retention between these two resins was also noted by Jones et al. [15] where the effect was described as being due to the stereochemical effects with the varying pore sizes of the two resins. It is possible that with larger pore sizes there is less steric hindrance for surface interaction. Investigation of these resins shows limited use in the area of Cr(VI) determination. The LOD and linear dynamic range is satisfactory when compared to commercial low capacity ion-exchangers [5], however efficiencies are very unsatisfactory. Interferences could pose a problem as the species elutes close to the solvent front. However this may be eleviated with the use of specific detection using post column derivitisation with 1,5-DPC.

Problems associated with developing a reproducible coating and column packing procedure for these resins should be overcome in order to produce columns that would be acceptable for analytical work. Production of a more efficient column using HPLC grade substrates is a much more desirable approach.

4.4 CONCLUSION

The investigations carried out, showed that by coating used C18 columns with anion-active organic dyes, it is possible to achieve a permanently coated anion exchange column on which Cr(VI) can be analysed, without the addition of dye-stuff or organic modifier to the eluent. The efficiencies for these dye coated columns were much greater than commercial ion exchange columns and limits of detection of these dye-coated columns, are comparible to commercial anion exchange columns, eg., Dionex Ion Pac columns [5]. In addition to providing a reproducible procedure, the use of dye-coated columns, provides a rapid, sensitive, selective and cost effective means for Cr(VI) determination.

Coating of neutral poly(styrene divinylbenzene) copolymer resins on the other hand had proved to be unsatisfactory for Cr(VI) anion determination. The notable problems include achievement of adequate coating with dye-stuff, and obtaining uniform packing of the columns with the coated resins. There is greater potential for analysis when using off-line coating as opposed to coating the resin on-line. Off-line coating enabled greater interaction of the dye with the stationary phase particles, therefore providing a more homogeneously coated stationary phase for analysis. On-line the dyestuff is not given sufficient time for interaction with the particles. Additional investigations are required for selection of more suitable dyes and mobile phases for better interaction of the analyte using these polymeric resins.

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Chapter 5.0

Determination of metal ions by capillary electrophoresis using on-column complexation with PAR following trace enrichment by peak stacking.

5.1 INTRODUCTION

5.1.1 History of Capillary Electrophoresis

Electrophoresis has been defined as the differential movement of charged species (ions) by attraction or repulsion in an electric field [1]. Historically electrophoresis was used as a separation technique using anticonvective media, such as polyacrylamide gels or agarose gels, in the slab or tube form [1]. These were used in size dependent separations of biological macromolecules, such as nucleic acids and proteins. Since the introduction of high performance capillary electrophoresis (HPCE), electrophoresis is no longer limited to the separation of macromolecules and can also be used to separate cations, anions and neutrals in a single analysis [2]. HPCE can be considered as an instrumental approach to electrophoresis. The development of electrophoresis in capillary tubes offers several exciting methods for fast, highly efficient separations of ionic species, separations of macromolecules important in the area of analytical biotechnology, and development of small volume separations-based sensors [2]. Hjerten [3] provided the earliest demonstration of the use of high electric field strength in free solution electrophoresis in 3mm i.d. capillaries in 1967. Mikkers et al.[4] adapted instrumentation from isotachophoresis to perform capillary electrophoresis, and used 200 μm i.d Teflon tubes obtaining separations with plate heights less than 10 μm. The most widely accepted initial demonstration of the power of capillary zone electrophoresis was that by Jorgenson and Lukacs [5], in which he provided the first demonstration of high separation efficiency (plate heights < 1μm for protein analyses) with high field strength in narrow (<100 μm i.d.) capillaries. Since then development in the area of capillary electrophoresis has continued to grow at a rapid rate.

5.1.2 Capillary electophoresis (CE) in the determination of inorganic ions

While a wide variety of chromatographic modes are available for the determination of inorganic ions in environmental samples, there is always the necessity to discover new modes of their determination. Only in the last 3-4 years has capillary electrophoresis started to emerge as an alternative to ion chromatography for the analysis of ionic solutes [6]. In view of the evident capabilities of capillary electrophoresis for analysis of biological and

pharmaceutical matrices, this technique has been introduced into the area of inorganic ion analysis. Investigations have already shown, that efficiencies are far exceeding those of liquid chromatography, but the challenge lies in achieving adequate sensitivity. Capillary electrophoresis has been utilised in these studies to assess its ability in metal ion determinations in comparison to liquid chromatography (LC), with emphasis placed on achieving comparable limits of detection to LC.

From the perspective of an alternative analytical technique, CE offers several advantages, including:

*simplicity;

*reduced matrix dependence;

*enhanced separation efficiency and

*separation selectivity. [1].

5.1.3 Modes of Capillary Electrophoresis

The versatility of high performance capillary electrophoresis is partially derived from its numerous modes of operation. The most frequently used modes of CE have been capillary zone electrophoresis (CZE), isotachophoresis, capillary electrokinetic chromatography, capillary gel electrophoresis and isoelectric focussing (IEF).

CZE is the most widely used mode due to its simplicity of operation and versatility and it will be discussed in more detail in this chapter, as it was the mode used in these investigations. Apart from this form of capillary electrophoresis, (CZE) in which the separation depends only on mobility differences, numerous variations have been introduced. Uncharged molecules can be separated by means of micellar electrokinetic chromatography (MEC). When detergents are added to the buffer, the neutral molecules are distributed between the buffer and the micelles according to their hydrophobic properties. Since a distribution process is involved this is a chromatographic technique. The separation is due to the mobility of the micelles, which are generally negatively charged.

In the case of IEF the separation is carried out in a pH gradient, which is formed in the electric field when ampholytes are added to the buffer. Isotachophoresis (ITP), which is the oldest capillary separation technique, has become more important due to its use in CE for sample concentration. Capillary gel electrophoresis is an area of capillary electrophoresis with great potential for protein separations. Separation is accomplished with the use of gel-filled capillary columns. Gels are potentially useful for electrophoretic separations because they are an anticonvective media; they minimize solute diffusion, which contributes to zone broadening; they prevent solute adsorption to the capillary walls; and they eliminate electroosmosis, allowing maximum resolution in short lengths of column [7].

5.1.4 Principles of Capillary Zone Electrophoresis (CZE)

The separation mechanism in capillary zone electrophoesis is the same as that in conventional electrophoresis. Differential migration into discrete zones is due to differences in electrophoretic mobilities, which in turn are related to the mass to charge ratio of the solutes [8]. Zone electrophoresis in capillaries is analogous to elution chromatography techniques in that a narrow plug of solute is introduced into a potential field. In CZE (or CE as it is now referred to) as in all electrophoretic methods, separation is based on the different mobilities of charged molecules in an electric field. A 20-200-μm i.d. fused silica capillary is filled with buffer, and each end of the capillary is immersed in a reservoir containing buffer and a platinum electrode. One of the electrodes is connected to a high voltage dc power supply and the other to the ground. Sample is injected into the capillary either by electromigration or by hydrostatic or pneumatic methods. Voltage is then applied to begin the electrophoretic separation, which is monitored by an on-column UV-vis detector at the end of the column opposite the sample inlet. Ionic solutes differentially migrate in a homogenous buffer to provide discrete moving zones. Because the small dimensions of the capillary column provide efficient dissipation of joule heat, which allows relatively high voltages to be used, separations can be performed rapidly. In addition zone broadening caused by molecular diffusion and convection currents in the electrophoretic medium, is minimized. Using 1-m capillaries and voltages of 30,000 V, high separation efficiencies and rapid analyses can be achieved for small molecules such as

fluorescent labelled amino acids and peptides [9]. Efficiencies of hundreds to thousands of plates and analysis times of 20 min can easily be obtained.

5.1.4.1 *Components of the CE Apparatus*

The basic instrumental configuration for CE is relatively simple. All that is required is a fused silica capillary with an optical viewing window, a controllable high voltage power supply, two electrode assemblies, two buffer resevoirs, and an ultraviolet (UV) detector. Quartz capillaries of the type used in GLC make it possible to use detectors for the on-line detection of the separated substances within the capillary. The basic construction of a CE apparatus is shown schematically in Figure 5.1 The thin fused silica capillary (20 -100 μm in diameter) 20 to 100 cm in length, bridges the two buffer vessels, between which a voltage of up to 30,000 volts is applied.

5.1.4.1.1 *CZE Capillaries*

Polyimide coated quartz capillaries, 25 to 100 μm in diameter are generally used in CZE. This polyimide coating protects the otherwise fragile (thin-walled & flexible) glass tubing. The polyimide layer of the quartz capillaries must be removed at the site of the detector, either mechanically or by burning off before the capillary is used. Untreated or unmodified capillaries are used for most applications. It is advisable to treat new capillaries with 1 *M* NaOH prior to their initial use in order to obtain hydroxylation of the surface, an important step on which the electroosmotic flow (EOF) depends [7].

5.1.4.1.2 *Voltage source*

The voltage should be adjustable within the range -30kV to +30 kV, and should remain constant at the value required. Precautions must be taken when an apparatus is constructed in the laboratory. Commercial instruments possess a high voltage safety shut-off system, for when the analytical system is open, to prevent accidents due to high voltage.

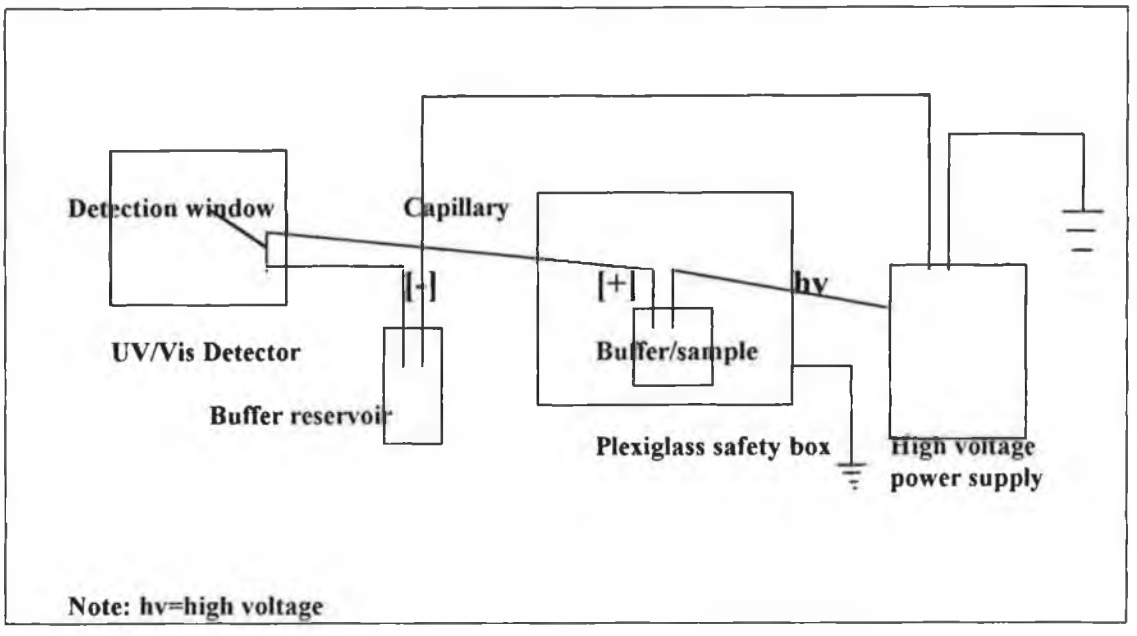

Figure 5.1

Schematic Diagram of the Capillary Electrophoresis System

5.1.4.2 *Sample Introduction*

In HPCE only minute volumes of sample are loaded into the capillary in order to maintain high efficiency [10]. A small plug of sample (nl volumes) is introduced at the anodic end of the capillary. This is done by replacing the buffer vessel by a sample vial, ie the end of the capillary is dipped into the sample solution. The migration of the sample is induced by the applied voltage. The most difficult problem in capillary electrophoresis is reproducible sample introduction, an important requirement for quantitative analysis. The sample zone must be kept small in order to avoid band broadening, this requires injection of very small sample volumes, ie. 5-50 nl. Very large sample volumes result in peak distortions and loss of resolution. Quantitative sample injection can be accomplished by a number of methods. The two most common methods are hydrodynamic and electrokinetec injection.

5.1.4.2.1 *Hydrodynamic injection*

This method of injection is accomplished by application of pressure at the injection end of the capillary, or by siphoning action obtained by elevating the injection resevoir relative to the exit resevoir. With hydrodynamic injection, the quantity of sample loaded is nearly independent of the sample matrix. The volume of sample loaded will be a function of the capillary dimensions, the viscosity of the buffer in the capillary, the applied pressure and the time.

5.1.4.2.2 *Electrokinetic injection.*

The electrokinetic injection method arose from the finding that electroosmosis acts as a pump. Electrokinetic or electromigration, injection is performed by replacing the injection-end resevoir with the sample vial, and applying the voltage. Usually a field strength 3 to 5 times lower than that used for separation is applied. In eletrokinetic injection, analyte enters the capillary by both migration and pumping action of the EOF, and the quantity loaded is dependent on the electrophoretic mobility of the individual solutes [11].

Sample loading is dependent on the EOF, sample concentration, and sample mobility. Variations in conductivity, which can be due to matrix effects such as a large quantity of sodium or chloride ion, result in differences in voltage drop and quantity loaded [10]. As a result electrokinetic injection is generally not as reproducible as hydrodynamic injection. Despite this limitation, electrokinetic injection is very simple and requires no additional instrumentation [11].

Two important features relating to the injection of analytes must be kept in mind.

(1) Because the amount of material injected is a function of several parameters that can be hard to control, it is difficult to get a high degree of reproducibility for sample injection over the course of analyses.

(2) The amount of each sample component loaded onto the capillary will vary as a function of the mobility of each sample species.

To preserve high resolution, samples must be introduced into the capillary column with the minimum volume in a very short time. The small volume of material injected into the column makes it difficult to detect low concentrations of the material [10,12].

5.1.5 Theory of Operation of Capillary Electrophoresis

5.1.5.1 *Electrophoretic migration*

Separation by electrophoresis is based on differences in migration velocity of the charged analytes in an electric field. The applied electric field (V/cm) is a function of the applied voltage and capillary length. An increase in voltage and consequently an increase in the field strength, ***E***, leads to an increase in the electrophoretic migration velocity μ of the ions and thus to a faster analysis. The electrophoretic mobility for a given ion in a medium is characteristic of that ion. Small, highly charged species have high mobilities, whereas large, minimally charged species have low mobilities [7].

5.1.5.2 *Electroosmotic flow (EOF)*

One of the most distinguishing properties of CE is the electroosmotic flow, or electroendoosmotic flow. EOF is the bulk flow of liquid in the capillary and is a consequence of the surface charge on the interior capillary wall (Figure 5.2). The EOF results from the effect of the applied electric field on the solution double layer at the wall. The fused silica capillaries that are typically used for separations have ionizable silanol groups in contact with the buffer contained within the capillary. The pI of fused silica is about 1.5 [13], and the degree of ionization is controlled mainly by the pH of the buffer. EOF becomes significant above pH 4. The surface charges are balanced by corresponding opposite charges in the buffer [2,7,14].

Figure 5.2. shows a diagram of the charge distribution at the capillary surface. Under normal aqueous conditions the solid surface of the fused silica capillary has an excess of anionic charge resulting from ionization of surface functional groups. Counterions to these anions are in the stagnant double layer adjacent to the capillary walls. The cationic nature extends into the diffuse layer, which is mobile. The potential this creates across the layers is termed the zeta potential.

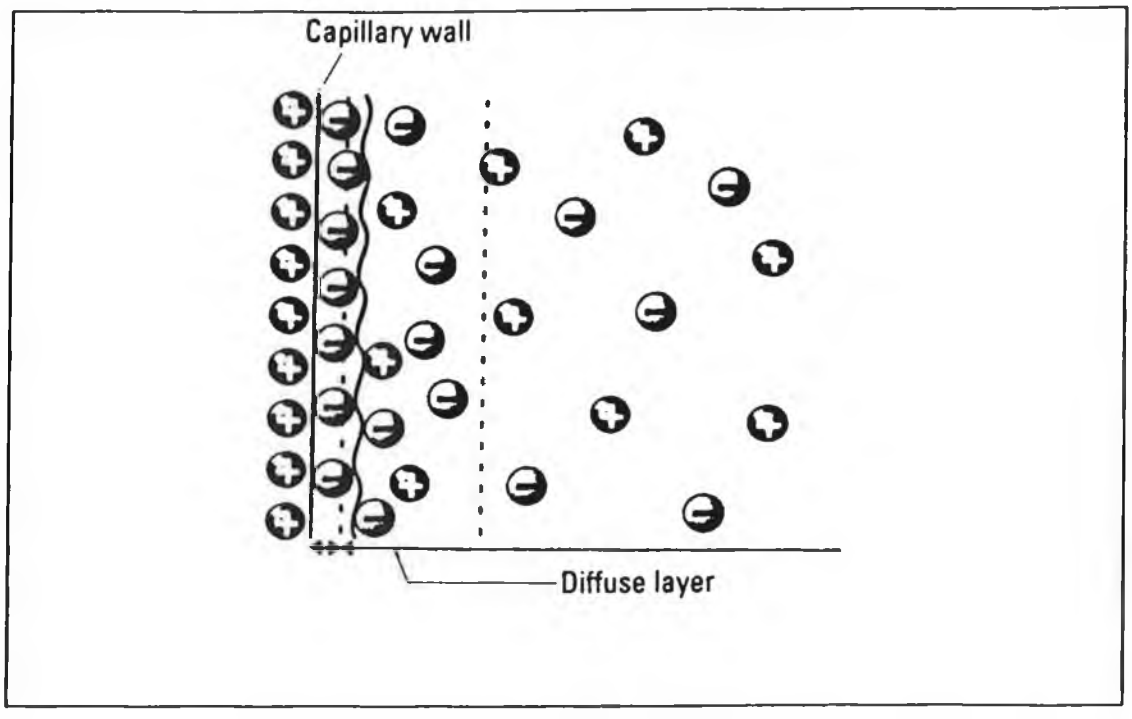

Figure 5.2

Representation of the double layer at the capillary wall.

The zeta potential is essentially determined by the surface charge on the capillary wall. Since this charge is strongly pH dependent, the magnitude of the EOF varies with pH. At high pH, where silanol groups are predominantly deprotonated, the EOF is significantly greater than at low pH where they become protonated. The zeta potential is also dependent on the ionic strength of the buffer, as described by the double layer theory, ie., increased ionic strength results in double-layer compression, decreased zeta potential and reduced EOF. If a field is applied parallel to the surface, the counterions in the mobile layer are pulled parallel to its axis along with the liquid in the capillary. Thus for fused silica capillaries the induced EOF is in the direction of the cathode. The cationic counterions in the diffuse layer, migrate toward the cathode, and, because these ions are solvated, they drag solvent with them. The extent of potential drop accross the double layer governs the rate of flow.

5.1.5.2.1 *Electroosmotic flow profile*

The double layer thickness ranges from 3 to 300 nm for electrolyte concentrations of 10^{-2} to 10^{-6} *M*, respectively [7]. The extremely small size of this double layer leads to flow that originates at the walls of the capillary, resulting in a flat flow profile. Since the driving force of flow is uniformly distributed along the capillary (ie. at the walls), there is no pressure drop within the capillary, and the flow is nearly uniform throughout. In capillary electrophoresis, a flat flow profile and lack of need for a stationary phase result in a system of extremely high efficiency [2,7,13].

5.1.5.3 *Order of analyte migration*

EOF makes possible the simultaneous analysis of cations, anions and neutral species in a single analysis. The order of migration is cations, neutrals and anions. A benefit of the EOF is that it causes movement of nearly all species, regardless of charge, in the same direction. Under normal conditions (ie. negatively charged surface), the flow is from the anode to the cathode. Anions will be flushed towards the cathode since the magnitude of the flow is greater than their electrophoretic mobilities (ie. in the direction of the anode). Thus cations, neutrals and anions can be electrophoresed in a single run, since they all migrate in the same direction. Cations migrate fastest, since the electrophoretic attraction towards the cathode and the EOF are in the same direction. Neutrals are all carried at the velocity of the EOF but are not separated from each other, and anions migrate slowest since there are attracted to the anode, but are still carried by the electroosmotic flow toward the cathode. The electrophoretic migration is always accompanied by an electroosmotic flow (EOF) of a certain magnitude, which contributes in a passive manner to the transport of the sample zones but not their separation.

5.1.6 Detection Methods in Capillary Electrophoresis

Detection in CE is a significant challenge as a result of the small dimensions of the capillary. A number of detection methods have been used in CE to meet the challenge, many of which are similar to those employed in liquid column chromatography. Detection methods such as UV-visible absorbance [15,16], electrochemical [17], mass spectrometric measurements [18], conductivity [19], and indirect fluorescence have been employed [20].

The vast majority of CE applications relating to the separation of ionic solutes utilize UV absorbance detection, typically on-line with the capillary itself (after removal of the polyimide coating) acting as a detector cell. While this is a very straight forward method of detection, its sensitivity is limited in that the capillary diameter (25-100 μm) limits the path length. The simplest photometric detection mode is direct UV spectrophotometric absorption which has been used for the determination of UV absorbing solutes such a metallocyanide complexes [15] and, metal cations [16]. Photometric detection was employed by Iki and coworkers [16] in the detection of metal chelates following separation by CE. Weston et al. [21], reported the determination of group IA, group IIA, transition metal and lanthanide cation mixtures with indirect UV determination using a highly UV absorbing amine. Indirect UV absorbance is the preferred approach when a universal detection mode is required. In this approach, a UV absorbing electrolyte or detection probe compound is employed and the analyte ions are monitored as a decrease in the background absorbance. Fluorescence detection of ionic solutes in CE is operated almost exclusively in the indirect mode. In this case the electrolyte contains a fluorophore and fluorescence detection is used. This detection approach offers the advantage of greater sensitivity over UV absorption, as it does not depend on path length [22]. Detection limits are typically lower than those achieved with UV absorption detection methods, particularly when laser light is used as the exitation source [20]. Fluorescence detection has been used for the direct detection of metal cations following on-capillary complexation with 8-hydroxyquinoline-5-sulfonic acid [23].

Recently CE has been used with mass spectometry for the determination of inorganic ions [18]. However CE/MS analysis of an anion mixture (containing 11 anions) was complicated by interfering ion current signals from the cluster ions formed between the additives (electrophoretic buffer) and the analytes. Thus only four anions were satisfactorily detected.

Lu and Cassidy [24] demonstrated the detection of 14 metal ions using amperometric detection at a mercury-film ultra microelectrode. Although detection limits were in the micromolar range for most metals, problems with long term stability of the electrode restricted the usefulness of this method.

5.1.7 Sample Concentration Techniques in Capillary Electrophoresis

5.1.7.1 *Isotachophoresis (ITP)*

Generating an isotachophoretic system is one method of sample concentration. Isotachophoresis can be used prior to CE by performing a concentration step in one column and then using the appropriate plumbing to transfer the concentrated sample plug to the separation column [10]. This technique has been reported to yield an increase in sensitivity of up to three orders of magnitude [12]. Alternatively judicious choice of buffers in a discontinuous system allows the use of this method with current CE systems [10]. Capillary isotachophoresis is a moving boundary electrophoretic technique. In ITP a combination of two buffer systems is used to create a state in which the separated zones all move at the same velocity (isotacho). The zones remain sandwiched between so called leading and terminating electrolytes. For anion analysis for example, the buffer must be selected so that the leading electrolyte contains an anion with an effective mobility that is higher than that of the solutes. Similarly the terminating anion must have a lower mobility than that of the solute [7,25]. A number of publications report the use of ITP and signal enhancement for inorganic species including metal ions [26-28].

Zelensky and co-workers [26] investigated the use of capillary isotachophoresis for metal cation analysis. The study involved utilization of xylenol orange as a complexing co-counter ionic constituent in the cationic mode of ITP, ie. the leading and terminating electrolytes containing cations NH_4^+ and H^+ respectively. The leading electrolyte which contained xylenol orange as a co-counter ion, mixed with the analyte metal ions forming a photometrically detectable species. Under optimized ITP conditions, the analysis of Mn^{2+} and Cd^{2+} with detection limits of $10^{-8}M$ for a 30µl sample volume were reported.

In another report, use of crown ethers in the isotachophoretic determination of metal ions was described [27]. In this study crown ethers were used to adjust the effective mobilities of analyte ions to effect isotachophoretic separation of the ions which have similar ionic mobilities. Separation of twenty metal cations was effected using an acetic acid buffer and an α-hydroxyisobutyric acid (HIB) complexing system [28]. This system was used to study the recovery, migration order and separation efficiency when using a complexing agent such as HIB for ITP. Again the leading electrolyte used was NH_4^+ and acetate acted as a counter ion.

5.1.7.2 *Field amplified sample stacking*

On-column sample concentration in a single continuous support buffer, also known as sample stacking was first used in CZE by Mikkers et al. [4]. In conventional electroinjection in HPCE samples are prepared in a buffer solution which has the same concentration as that inside the capillary column. The amount of sample injected into the column using this method is limited [29-31]. Several techniques have been described to enhance sensitivity by on-capillary sample concentration during or just following sample injection. These methods are based on field strength differences between the sample zone and the running buffer, and are called "stacking" [31].

This method of sample stacking is effected when the conductivity of the sample is significantly lower than that of the running buffer. Upon application of the voltage, a proportionally greater field will develop accross the sample zone causing the analyte ions to migrate at a faster rate. Once the ions reach the running buffer boundary, the field decreases and they migrate at a slower rate. This continues until all the ions in the sample zone reach the boundary which causes the sample to become concentrated into a smaller zone. At this point the field becomes homogeneous in the zone and normal electrophoresis begins [31,32].

The simplest way to perform a stacking experiment is to dissolve the sample in water or low conductivity buffer (100 to 1000 times lower than that of the running buffer) and inject normally either hydrodynamically or electrokinetically. Under these conditions stacking will occur automatically. If the conductivity of the sample and the running buffer are equivalent, stacking can be induced by injecting a short plug of water before sample introduction

[7]. The lower the buffer concentration in the sample, the higher the analyte concentration that is injected after stacking.

Vinther and co-workers [32] noticed the effect of a baseline disturbance due to the water plug during sample stacking. The results obtained revealed that during CZE with stacking conditions, the buffer zone prior to the neutral plug is not identical with the buffer zone after the plug. Hence, zone electrophoresis of the analytes is not the only process taking place in the capillary. It was reported that a pH shift occurred as a result of isotachophoresis occurring at the top of the CZE separations [32]. By switching the column directly from the high conductivity buffer resevoir to the low conductivity sample solution, the buffer at the end of the column is disturbed and the electric field at the injection point might not be amplified properly. By injecting a short plug of water before sample introduction, a high electric field strength from the begining of the injection can be achieved, resulting in a several hundred-fold enhancement in the amount injected [29].

Stacking of extremely large injection volumes in HPCE was reported by Chien and Burgi [33]. The principle is based on the fact that the local electrophoretic velocity of the ions inside the sample buffer is much faster than the bulk electroosmotic velocity of the solution. By applying a high voltage, with reversed polarity after loading the sample, sample buffer can be removed prior to the separation of the analytes. Since a source of peak broadening (the buffer) is removed from the system, better resolution can be achieved for a large sample volume. When large sample volumes are loaded onto the column, signal enhancements of several hundred fold may be obtained [33].

Recently it was proposed that a short plug of water injected into the column prior to sample introduction, enabled longer injection times and higher injection voltages (~30KV as opposed to 5KV). This allows a significant enhancement in LOD (~10^{-8} *M*) without serious reduction in efficiency [29]. This effect is due to the fact that in field amplified sample injection at high voltage, a tremendous amount of sample ions move into the running buffer and try to "stack up" in front of the water boundary. As the sample concentration becomes higher, the local conductivity of the stacking region will increase, which causes a further decrease in the electric field strength. Consequently the leading edge of the sample region will slow down more and further enhance the stacking effect [29].

A method of concentrating negatively charged ions when injected into the capillary was reported recently [30]. The effect was aided by use of polarity switching of the electrodes at a correct time, thus enabling not only the concentration of positively charged ions but also negative ions. In the case of field-amplified injection, where samples are prepared in a low-conductivity buffer, and injected electrically into the capillary, the number of positive ions injected is proportional to the field enhancement factor at the injection point. The negative ions will not be enhanced, but will be pushed away from the column by this high field strength. However, since the electroosmotic velocity of the bulk solution is much slower than the electrophoretic velocity of the sample under the enhanced field, one can inject and concentrate both positive and negative ions into the column by switching the polarity of the electrodes at the appropriate time [30].

5.1.8 Recent Developments in Sensitivity Enhancement in Capillary Electrophoresis.

Several research groups have developed their own highly sensitive CZE detectors, based on laser induced fluorescence [2], mass spectrometry [18] and UV absorbance with extended path lengths [2].

According to Beers Law, the optical absorbance of a sample is directly proportional to the optical pathlength through which the absorbance measurement is performed [7]. Therefore the extension of the optical path should lead to increases in sensitivity. However simply increasing the diameter of the capillary is not always an attractive alternative because joule heating can result [13], leading to loss of resolution from increased peak widths.

The high efficiency observed in HPCE is due in part to on-capillary detection. Since the optical window is directly in the capillary there is no zone broadening as a result of dead-volume or component mixing. However it is this short path length which is the factor that limits sensitivity in HPCE. Due to the curvature of the capillary, the actual pathlength in the capillary is less than the inner diameter, since only a fraction of the light passes directly through the center. Emphasis has been placed on the injection of large sample volumes using a field amplified sample stacking method [31,32].

5.1.8.1 *Methods of Sensitivity Enhancement*

Other attempts at on-line sample enrichment have included extended path length of a Z cell [13] or bubble [7]. The optical path in the case of the former, can be extended by bending the capillary, forming what is known as a Z cell, and illuminating through the bend. However, eventhough the path length may be increased by up to 40 fold, only about 5 fold sensitivity enhancement occurs, because of increased background noise levels from poor efficiency in light throughput [12]. A recent approach involved a design consisting of a switching valve containing a precolumn for on-line sample enrichment [34]. This method involved loading of samples onto a precolumn with a micro liquid chromatographic pump, and desorption was effected by the electroosmotic flow. Zone cutting was used (by means of valve switching) to prevent band broadening and migration time shifts.

Solid phase extraction inside the capillary has recently been shown to effect enrichment. Waters Corporation [35] have developed a system involving the use of a short plug of reversed-phase material at the beginning of the capillary. The disadvantage of using such solid phases inside the capillary, as opposed to off-line solid phase extraction, are that raw environmental samples cannot be introduced directly due to the risk of plugging the system and severe contamination. The sample must therefore be cleaned or partially cleaned prior to introduction into the capillary [25].

5.1.9 Separation of Metal Chelates using Capillary Electrophoresis

Currently, the most utilized technique for separation and determination of metal ions as their chelates is reversed-phase liquid chromatography (RPLC) with spectrophotometric detection [27]. Several chelating agents have been employed as derivatizing reagents in metal ion analysis, including dithiocarbamic acids, 8-hydroxyquinoline and β-diketones [36]. 4-(2-pyridylazo) resorcinol (PAR) has been employed as a chelating agent for metal ion determinations using HPLC, with both pre-column [36-38] and post-column [39-41] derivatizations.

5.1.9.1 *4-(2-Pyridylazo) resorcinol as a chelating agent*

PAR is reported [37] as being an ideal ligand for HPLC studies, as it is an unselective ligand which forms water soluble chelates with the vast majority of transition metals. PAR chelates are reported as being amenable to two detection modes. In addition to UV-visible absorption, PAR chelates can be detected by oxidative thin layer amperometry [42], because the resorcinol portion of the PAR molecule is oxidizable at potentials accessible in aqueous media at solid electrodes. PAR is considered to be a tridentate ligand, with the pyridine nitrogen, azo nitrogen and the orthohydroxy oxygen as the donor atoms [43].

Separation of PAR-metal chelates by micellar electrokinetic chromatography was described by Saitoh and co-workers [38]. In this procedure a small quantity of PAR was also added to the eluent to prevent dissociation of relatively unstable chelates such as Zn(II), Cd(II) and Cu(II) during the separation. Hydrodynamic injection was employed, and theoretical

plate numbers for the chelates were in the range 105,000 - 120,000 per 60 cm. It was found that addition of the micelle, SDS, achieved better resolution of the metal chelates.

5.1.10 The Challenges to CE in Trace Metal Analysis

The use of CE in the area of inorganic ion analysis, is only at a very early stage of development. However the potential of CE for use in environmental analyses is great. This is due to the simplicity of instrumentation, small sample and solvent volumes, rapidity of analysis, and very high efficiencies. The major hurdle of this technique for metal ion analysis, is in the area of detection. Due to the low levels, eg., 10^{-7}-10^{-8} M, at which metals exist in the environment, detection methods are necessary to reach these limits. As of yet, very few reports have occurred regarding satisfactory detection limits. However it is the aim of future research, and the studies reported here, to look at ways of improving both sensitivity and selectivity for trace metal determinations using capillary electrophoresis.

The achievements in CE during the last few years have made it perhaps the most successful separation method of the last decade, particularly in the area of pharmaceutical sciences [8]. Femtogram to picogram sample amounts have been handled owing to improved injection methods and the low dilution involved in this separation process as compared with HPLC [44]. The general consensus among separation scientists appears to be that this new analytical technique will not replace existing techniques, but instead play a complementary role. Continued efforts aimed at improvements in separation capabilities, detection sensitivity and reliable quantitation will encourage acceptance of this technique.

5.2 EXPERIMENTAL

5.2.1 Apparatus

Fused silica capillaries, 50 μm I.D. x 300 μm O.D., were obtained from Polymicro Technologies (Phoenix, AZ). Prior to use, these capillaries were treated with 0.1*M* NaOH. The high voltage power supply was a Glassman MJ30P400 power supply (Glassman High Voltage, Whitehouse Station, NJ), the input from which was placed in a plexiglas box with an interlock in the access door for safety. UV-visible on-column detection with a C4 capillary electrophoresis absorbance detector (ISCO, Lincoln, NE) was carried out at 500 nm. The total length of the capillary was 85 cm and the length to the detector was 75 cm.

5.2.2 Reagents

The stock metal ion solutions were prepared by dissolving metal salts of nitrates (Fe^{+2} and Co^{+2}), sulfate (Cu^{+2}) or acetate (Zn^{+2}) (Aldrich, Milwaukee, WI) in Nanopure water (Sybron-Barnstead, Boston, MA). An electrophoretic buffer of 10 m*M* N-tris[hydroxymethyl]methyl-3-aminopropane sulfonic acid (TAPS) (Sigma, St. Louis, MO) was used throughout the investigation. The pH of this buffer was adjusted using 0.1*M* NaOH. The working PAR solution was prepared by dissolving the reagent in electrophoretic buffer at pH 8.4.

5.2.3 Procedure

5.2.3.1 *Precolumn complexation*

For precolumn formation of PAR metal chelates, an appropriate quantity of metal ion stock solution was added to 10 m*M* TAPS buffer, pH 8.4, containing $1 \cdot 10^{-3}$ *M* PAR. A 10-fold excess of reagent was used to ensure complete complexation. Chelates of all metal ions formed immediately at room temperature, and were injected within 30 min of preparation. A lower concentration of PAR ($1 \cdot 10^{-4}$ *M*) was added to the electrophoretic buffer to prevent the dissociation of relatively unstable chelates such as those of Zn^{+2}

and Cu^{+2}. The applied voltage was 30 kV, resulting in a current of 5 μA. Solutions were injected onto the capillary electrokinetically at 10 kV for 10 s.

5.2.3.2 *On-column complexation*

On column complexation involved a three-step sequence. In the first step, a 1 m*M* solution of the ligand was injected onto the capillary for a period of 10 s at 10 kV. The sample solution containing the metal ions was then injected electrokinetically for 15 s at 10 kV. Lastly, the anodic end was once again placed in the run buffer (containing 0.1 m*M* PAR) and 10 kV was applied for a 5 s period (pause time). This ensured adequate complexation of the ligand and the metal ion. Subsequently, the voltage was ramped to 30 kV to effect the separation. For sample stacking, samples were injected in Nanopure water, employing the three-step sequence previously described, and complexation occurred on column.

5.2.4 Sample preparation

Commercial vitamin supplements in tablet form (Prenatal Formula with Zinc, Nature Made Nutritional Products, Los Angeles, CA) were dissolved in water and filtered using a 0.2 μm filter. Subsequently, a volume of the sample filtrate was added to the PAR solution; this solution was then injected electrokinetically onto the capillary.

The water samples were obtained from a small pond in Lawrence, KS. They were spiked with $1 \cdot 10^{-7}$ *M* Fe^{+2} and Zn^{+2} and acidified with HCl to remove organic matter. They were then injected directly into the CE.

5.3 RESULTS AND DISCUSSION

5.3.1 The effect of pH on complex stability

PAR complexes exhibit greatest stability at alkaline pH. Under acidic conditions (pH < 5), the PAR reagent degrades completely, thus inhibiting complexation. The pH range 8–10 provides greatest chelate stability; however, resolution of the different metal chelates is compromised at very high pH due to the high electroosmotic flow. Therefore, a pH of 8.4 was chosen for the separations. This provided adequate resolution and good ligand stability.

5.3.2 Electrophoretic buffer composition

Phosphate, borate, and zwitterionic TAPS were investigated for separation of the metal complexes. Higher currents were evident with the use of phosphate and borate, and this resulted in lower efficiencies due to joule heating. In contrast, the low conductivity characteristic of TAPS provided much smaller currents (5 μA) and enabled more efficient separations.

Because some of the metal/PAR chelates *e.g.*, Zn^{+2} and Cu^{+2}, were unstable [16,38], a low concentration of PAR ($1{\cdot}10^{-4}$ *M*) was added to the electrophoretic buffer to enhance the separation. Table 5.1 illustrates that higher efficiencies are obtained when PAR was used in the run buffer, than when it was absent. As a result of this increase in efficiency, the metal ions were much better resolved with the inclusion of PAR in the electrolyte. The presence of PAR in the run buffer at this concentration did not result in higher background absorbance, and therefore did not reduce detection sensitivity.

5.3.3 Metal ion separations

Figure 5.3 illustrates the separation of Co^{+2}, Cu^{+2}, Fe^{+2}, and Zn^{+2} and free PAR following precomplexation, with electrokinetic injection of the chelate. This separation is an improvement over that reported by Iki et al., in that Cu^{+2} is resolved from the PAR reagent [16]. The efficiencies are lower, but this was probably due to the longer column employed for the separation. Calibration curves of all metal ion chelates showed linear dynamic ranges from $1 \cdot 10^{-6}$ to $1 \cdot 10^{-4}$ *M*, with an average correlation coefficient of 0.9996. Detection limits (S/N = 2) of $1 \cdot 10^{-6}$ *M* for Zn^{+2}, Fe^{+2} and Co^{+2} and $3 \cdot 10^{-6}$ *M* for Cu^{+2} were achieved, with reproducibility for 6 injections averaging 1.8%.

The poor limits of detection obtainable with this method are partly attributable to the small pathlengths characteristic of capillary columns. Incorporation of a Z-cell or bubble cell could increase the effective pathlength and enhance the detection sensitivity. An additional factor contributing to the poor LODs reported here is the poor quality of the lamp in this detector. For these studies a deuterium lamp was employed which has little power output at 500 nm. A lamp or laser providing more power at 500 nm would greatly increase the sensitivity of the method. The average wavelength selected for detection of the metal ions was 500 nm. Selection of specific wavelength maxima for each individual species, *e.g.*, Co^{+2} (500 nm), Cu^{+2} (506 nm), Fe^{+2} (540 nm), and Zn^{+2} (490 nm), could also improve the sensitivity somewhat.

	PAR present		PAR absent	
Metal	N[1]	R[2]	N	R
Co^{+2}	58564	6.0	27777	1.9
Cu^{+2}	34348	1.2	1038	0.3
Fe^{+2}	99575	1.16	14208	0.4
Zn^{+2}	106711	1.16	3696	0.4

[1]Number of theoretical plates

[2]Resolution between neighbouring peaks

Table 5.1
Effect of inclusion of PAR in the Electrophoretic Buffer on Separation Efficiency and Resolution

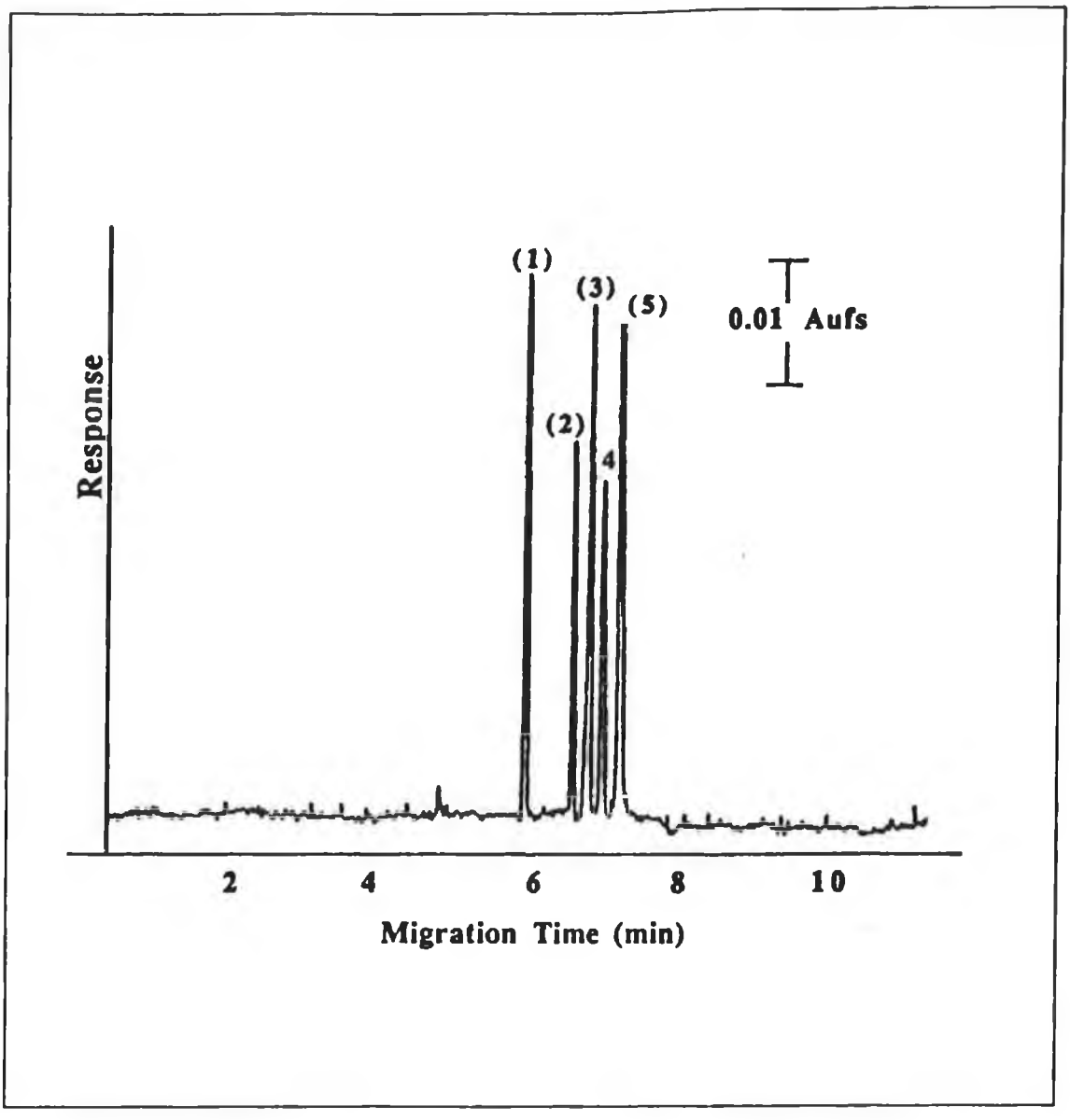

Figure 5.3

Separation of metal/PAR chelates with precomplexation. Peak identification: (1) Co^{+2}, (2) free PAR, (3) Cu^{+2}, (4) Fe^{+2}, (5) Zn^{+2}. Sample pH, 8.4; applied voltage, 30 kV; [M] = $4 \cdot 10^{-5}$ M; [PAR] $1 \cdot 10^{-3}$ M; Electrokinetic injection at 10 kV, 10 s.

5.3.4 Sample analysis

The applicability of the precomplexation procedure to real samples was confirmed through the analysis of a commercial vitamin supplement (Figure. 5.4). The correlation between expected and observed results was very good, for the six samples analyzed. The values obtained were 286 $mgdm^{-3}$ for Fe^{+2} and 118 $mgdm^{-3}$ for Zn^{+2}. These were only slightly lower than the expected values based on the product label, which were 300 $mgdm^{-3}$ and 125 $mgdm^{-3}$, respectively. The R.S.D. for six vitamin samples was 2.0 and 2.6% for Fe^{+2} and Zn^{+2}, respectively.

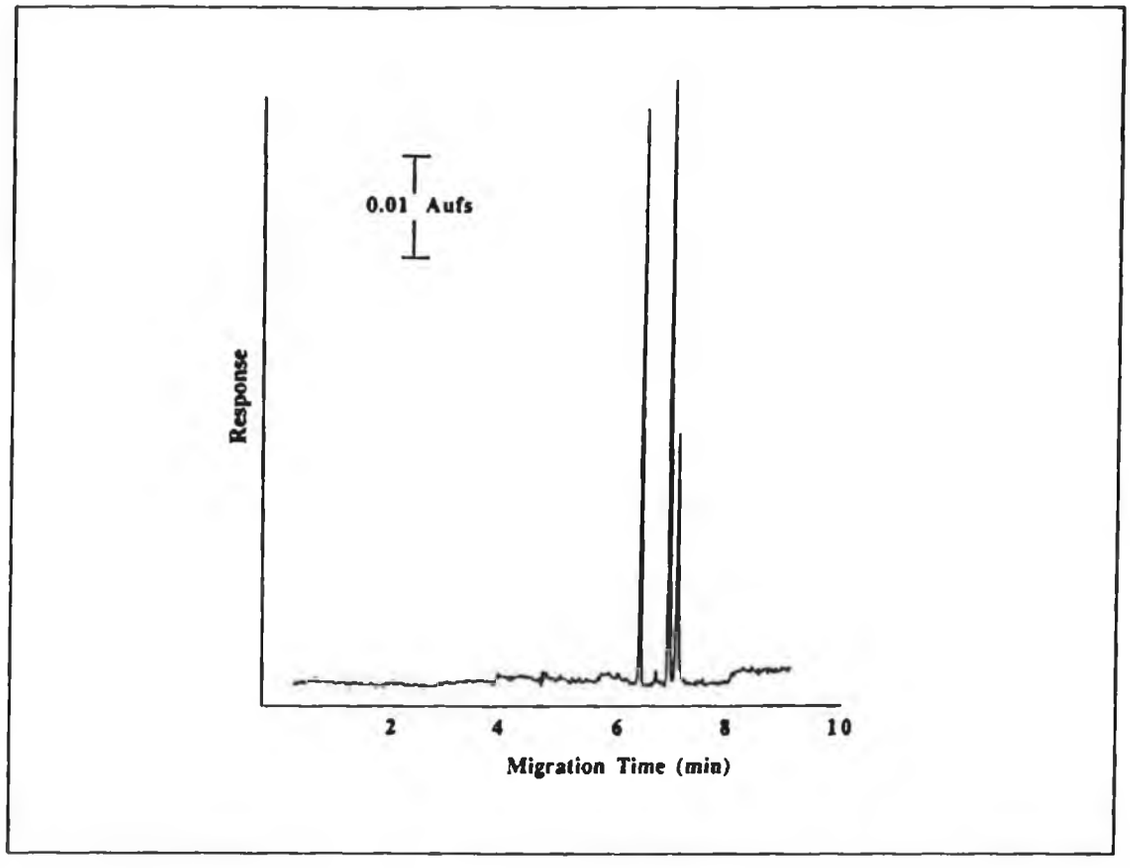

Figure 5.4

Electropherogram of metal ion separation in a commercial vitamin supplement. Precomplexation with PAR.[PAR] $1 \cdot 10^{-3}$ M. Electrophoretic buffer, 10 mM TAPS with $1 \cdot 10^{4}$ M PAR, pH 8.4; peak identification: (2) free PAR, (4) Fe^{+2}, (5) Zn^{+2}.

5.3.5 On-column complexation with peak stacking

To obtain adequate limits of detection for water analysis (pg/cm^3), most LC-based methods for the determination of metal ions involve a trace enrichment step. The most common procedures use bonded-phase or ion-exchange resins [39,40] to concentrate the sample off line. In CE, peak stacking has been shown to be an effective trace enrichment method. Sample stacking occurs as a result of the movement of sample ions across the stationary boundary, which separates the region of the injected sample solution from the rest of the capillary containing the support buffer [31,32]. Because of the matrix differences between those two regions, the ions experience a lower electric field in the support buffer region than in the sample region, thus the velocity of the ions decreases as they cross the stationary boundary. The slower moving ions will "stack up" into a smaller volume, thereby increasing the concentration in the sample zone [31].

To obtain the best sensitivity with the PAR reagent, a three-step stacking procedure was developed, which involved on-column complexation of the metal and PAR (Fig. 5.5). In order to maximize the degree of complexation, it was discovered that an initial plug of higher concentration PAR ($1 \cdot 10^{-3}$ *M*) was necessary, followed by a plug of metal ions in water. The reason for this is that the positively charged metal ions have a positive electrophoretic mobility, and therefore move more rapidly toward the cathode than does PAR (which has a negative electrophoretic mobility), thus mixing with the slower migrating negatively charged ligand. Without this initial plug of PAR, the buffer containing $1 \cdot 10^{-4}$ *M* ligand carries the metal ions, but the kinetics are not fast enough for complete complexation to occur. Stacking of metal ions may also be due to the presence of the PAR zone [45].

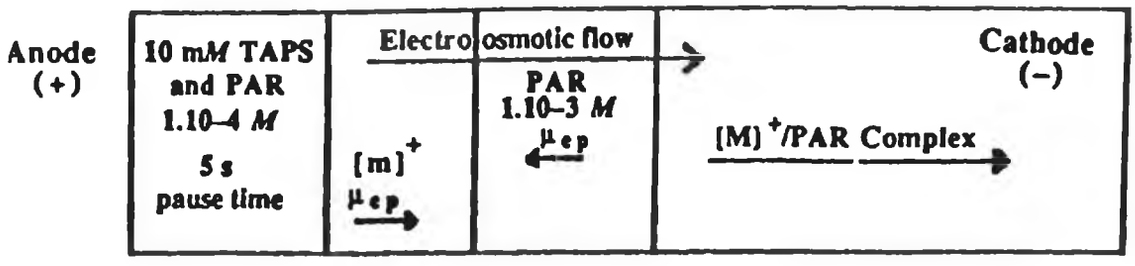

Figure 5.5

Schematic diagram of stacking with on-column complexation

The effect is clearly illustrated in Fig. 5.6a. In this case, no plug of PAR was introduced prior to the metal ion and the peak heights are substantially reduced. Even more importantly, under these conditions the metal ions which form relatively weak chelates, *e.g.*, Cu^{+2} and Zn^{+2} [16], do not have sufficient time to complex, and this results in broad peaks. Fig. 5.6b shows the CE separation of the metal chelates under the stacking conditions, where a 10–s electrokinetic injection of 1m*M* PAR is introduced prior to the metal ion. The advantage of introducing a more concentrated plug of PAR is demonstrated by this electropherogram. On-column complexation, with the metal ions injected in buffer identical to the electrophoretic buffer, achieved LODs similar to those obtained with precolumn complexation.

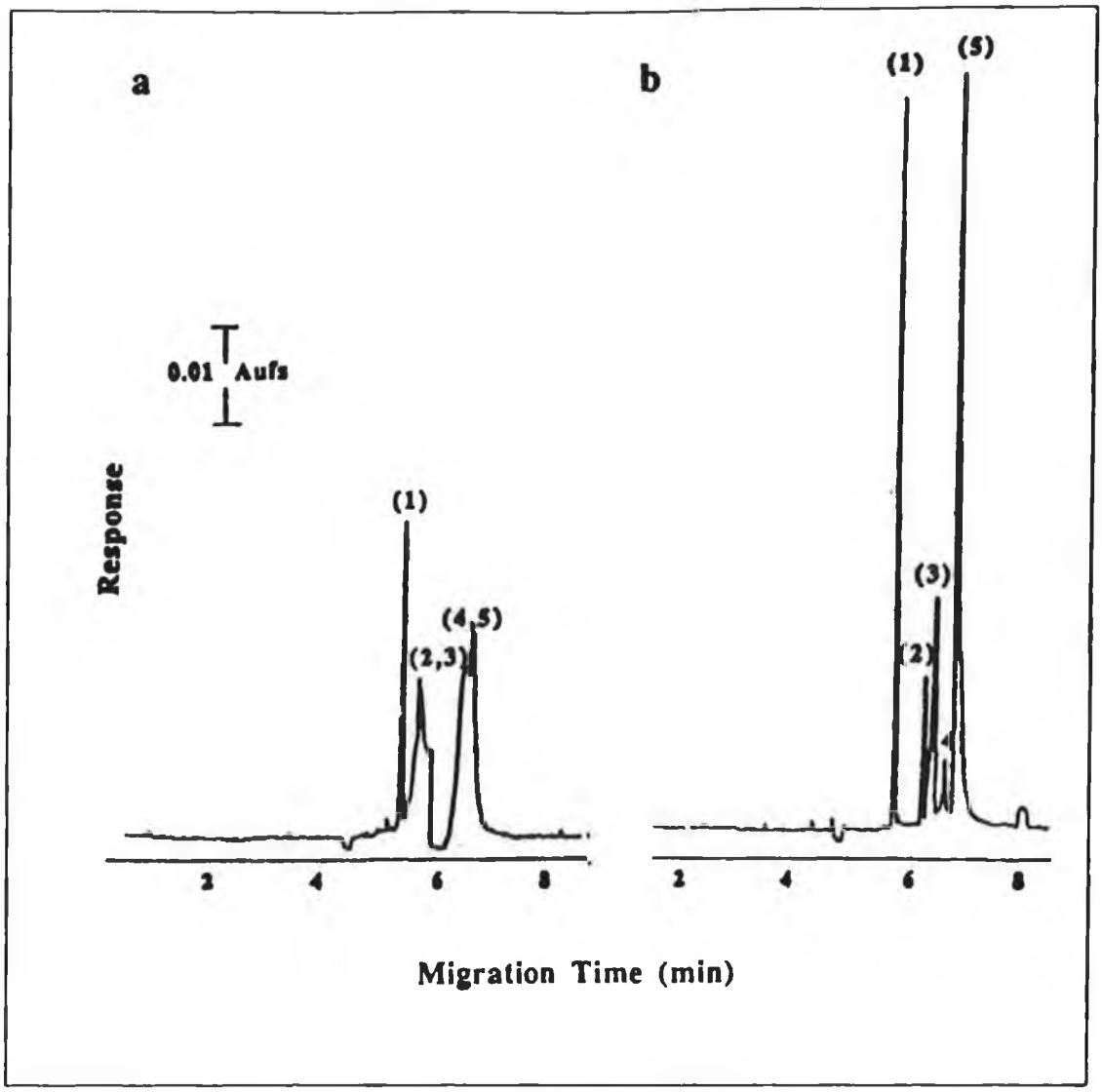

Figure 5.6

(a) CE separation under stacking conditions in the absence of a PAR plug.
(b) CE separation of metal/PAR chelates under stacking conditions and on-column complexation.
Peak identification: (1) Co^{+2}, (2) free PAR, (3) Cu^{+2}, (4) Fe^{+2}, (5) Zn^{+2}; stacking voltage, 10 kV; injection times, [PAR] $1 \cdot 10^{-3}$ M, 10 s; [M]+ $8 \cdot 10^{-7}$ M, 10 s;[PAR] $1 \cdot 10^{-4}$ M.

The effect of buffer concentration on sample stacking was investigated and it was observed that as the buffer concentration in the sample decreased, the peak heights increased. In this study, the optimum stacking effect was observed with pure water.

In order to optimize the on-column complexation procedure, the effect of injection time and voltage on peak height and column efficiency was investigated. Injection times between 5 and 35 s were investigated at 5–s intervals. As can be seen in Fig. 5.7, a maximum was reached at 15 s. Above this period, excess metal ion is introduced, resulting in a reduction in peak height. The injection voltage also had an influence on the efficiency of the separation. Obviously, the higher the voltage the greater the quantity of metal introduced, and, therefore, the larger the peak height. However, resolution became a factor at higher injection voltages as the peak width increased substantially with an increase in voltage. At voltages above 10 kV the efficiency was dramatically reduced (see Fig. 5.8). At 5 kV a sufficient quantity of ions was not introduced onto the capillary. Therefore, based on these experiments, a 10 kV electrokinetic injection for 15 s was determined to best provide both satisfactory baseline resolution and peak height response.

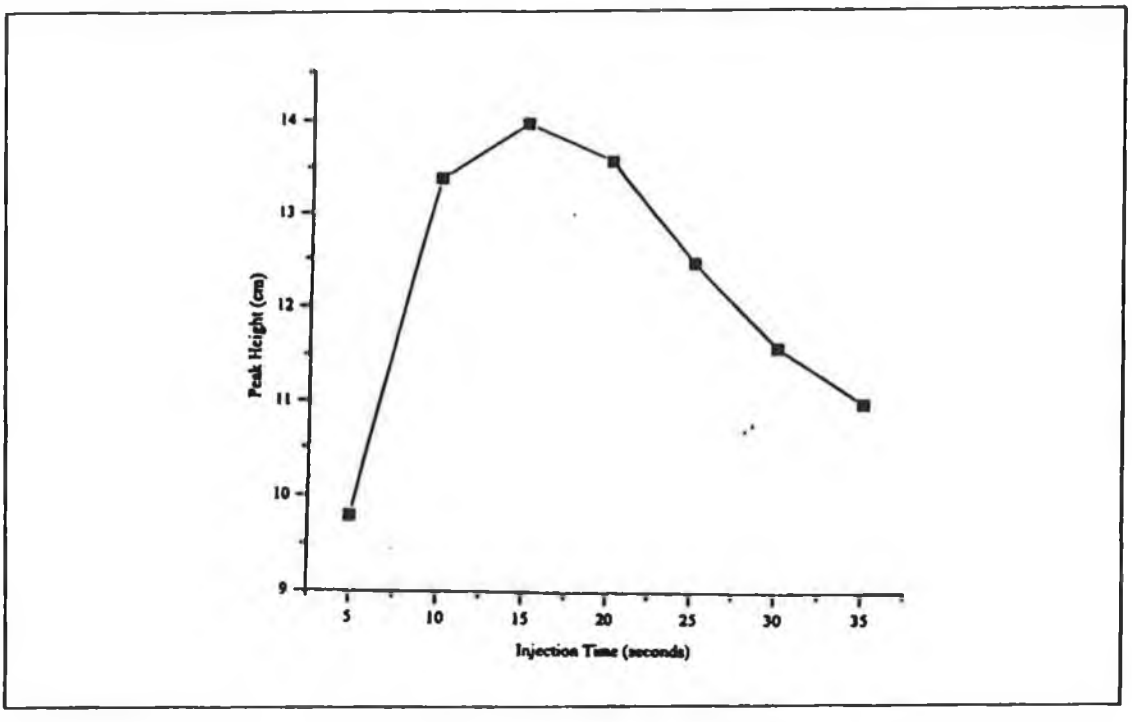

Figure5.7

Optimization of sample injection time during peak stacking. [M]+ $1 \cdot 10^{-6}$ M; stacking sequence, first plug [PAR] $1 \cdot 10^{-3}$ M, 10 s [M]+; pause time, 5 s. Electrophoretic buffer 10 mM TAPS containing $1 \cdot 10^{-4}$ M PAR. Stacking voltage, 10 kV.

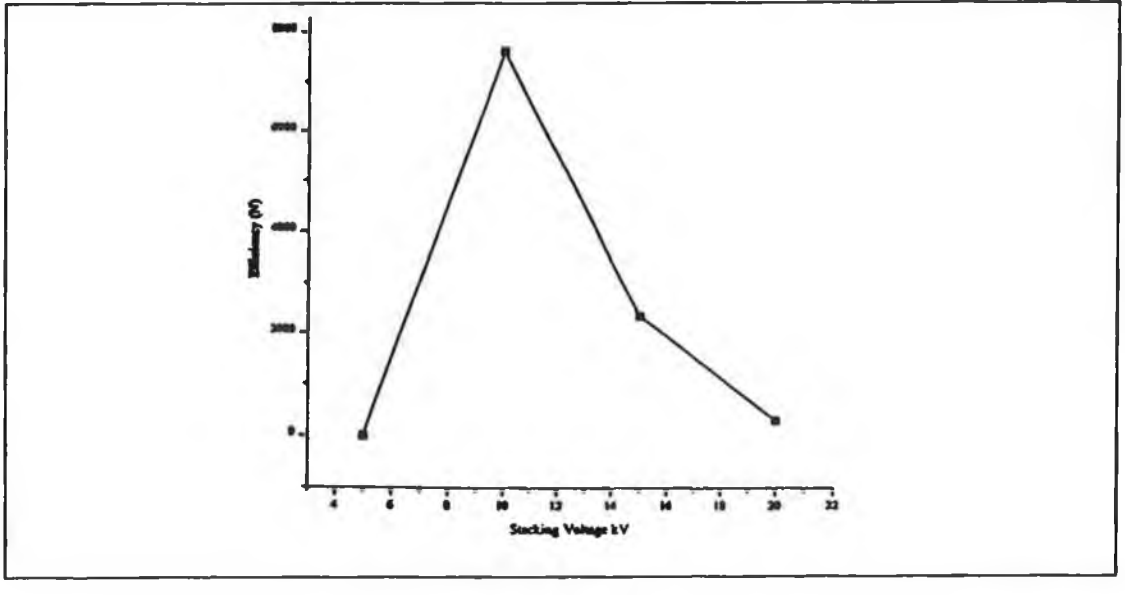

Figure 5.8

Effect of injection voltage on separation efficiency under stacking conditions. N, number of theoretical plates ($5.54\ (tm/W1/2)^2$)

The third step in the stacking program involved a pause time, whereby the run buffer is applied at 10 kV for a period of 5 sec, after which the voltage was ramped to 30 kV to allow separation to continue. If this step is omitted, complexation is incomplete. On the other hand, too large a pause interval results in a dramatic reduction in both resolution and efficiency. Fig. 5.9 shows the effect of pause time on the resolution of Fe^{+2} and Zn^{+2}. A pause time of 5 s was determined to be optimal.

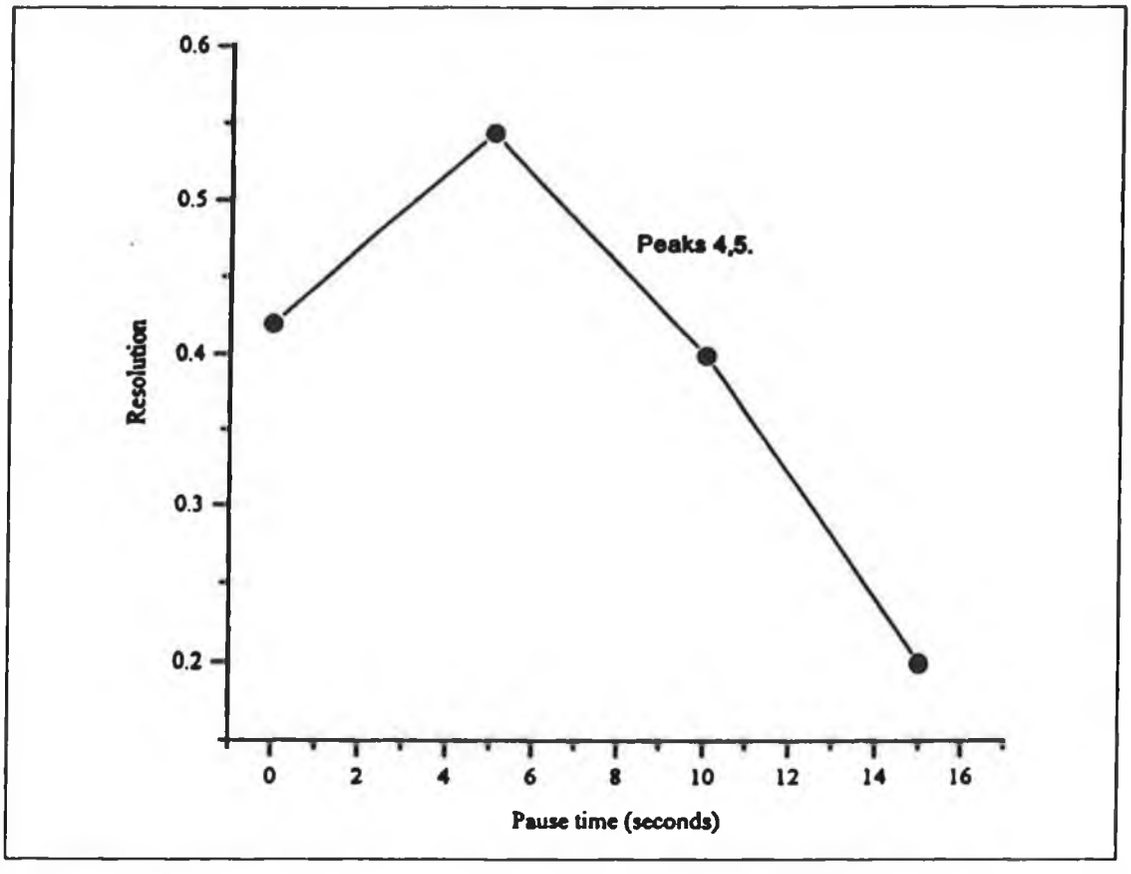

Figure 5.9

The effect of pause time on resolution under peak stacking conditions. 5s. Electrophoretic buffer, 10 mM TAPS @ 1·10 ^{4} M PAR, pH 8.4.

5.3.6 Limits of Detection

The combination of peak stacking and on-column complexation resulted in a 100-fold reduction in LOD for Co^{+2}, Fe^{+2}, and Zn^{+2} and a 10-fold reduction for Cu^{+2}. Detection limits were $1 \cdot 10^{-8}$ for Co^{+2}, Fe^{+2} and Zn^{+2} and $4 \cdot 10^{-7}$ for Cu^{+2} (S/N = 2). These correspond to mass detection limits of 0.2 fmol for Co^{+2}, Fe^{+2} and Zn^{+2} and 7.0 fmol for Cu^{+2}. Reproducibility of peak heights ranged from 0.8 to 1.9% R.S.D. for the metal ions studied. The linear dynamic range for these metals was greater than 3 orders of magnitude with an average correlation coefficient of 0.9987 (Table 5.2). The reproducibility is much better than that reported by Iki et al. for the determination of metals at the $2 \cdot 10^{-7}$ *M* level. The average R.S.D. reported was 12%. Even though there was a substantial loss of efficiency when the stacking method was employed, as can be seen in Fig. 5.6b, the peaks are still adequately resolved for quantitation.

	Precomplexed[1]		Stacking	
Metal	**LOD (*M*)**	**%RSD**	**LOD (*M*)**	**%RSD**
Co^{+2}	$3 \cdot 10^{-6}$	0.9	$1 \cdot 10^{-8}$	1.0
Cu^{+2}	$1 \cdot 10^{-6}$	3.1	$4 \cdot 10^{-7}$	0.8
Fe^{+2}	$1 \cdot 10^{-6}$	2.5	$1 \cdot 10^{-8}$	1.9
Zn^{+2}	$1 \cdot 10^{-6}$	1.0	$1 \cdot 10^{-8}$	1.0
Linear range	$1 \cdot 10^{-6}$ to $1 \cdot 10^{-4}$ *M*		$1 \cdot 10^{-8}$ to $1 \cdot 10^{-5}$ *M*	
Average r	0.9996		0.9987	

[1]Without stacking

Table 5.2

The Effect of Peak Stacking on Limit of detection (S/N =2)

5.3.7 Water samples

The use of this method for the analysis of a pond water sample was investigated. No Fe^{+2} or Zn^{+2} was found to be present in the original sample, so it was spiked with $1 \cdot 10^{-7}$ *M* metal ion. Fig. 5.10a shows the electropherogram obtained using precolumn complexation. Neither ion was detectable. Fig. 5.10b shows the same sample using on-column complexation and peak stacking. In this case Fe^{+2} and Zn^{+2} were both detected. There was some reduction in the sensitivity of the method when using the real sample ie., pond water. This is believed to be due to the addition of acid to remove the organic matter bound to metal ions at such low concentrations. The addition of acid makes the ionic strength greater than that of the nanopure water used in the development of the stacking method.

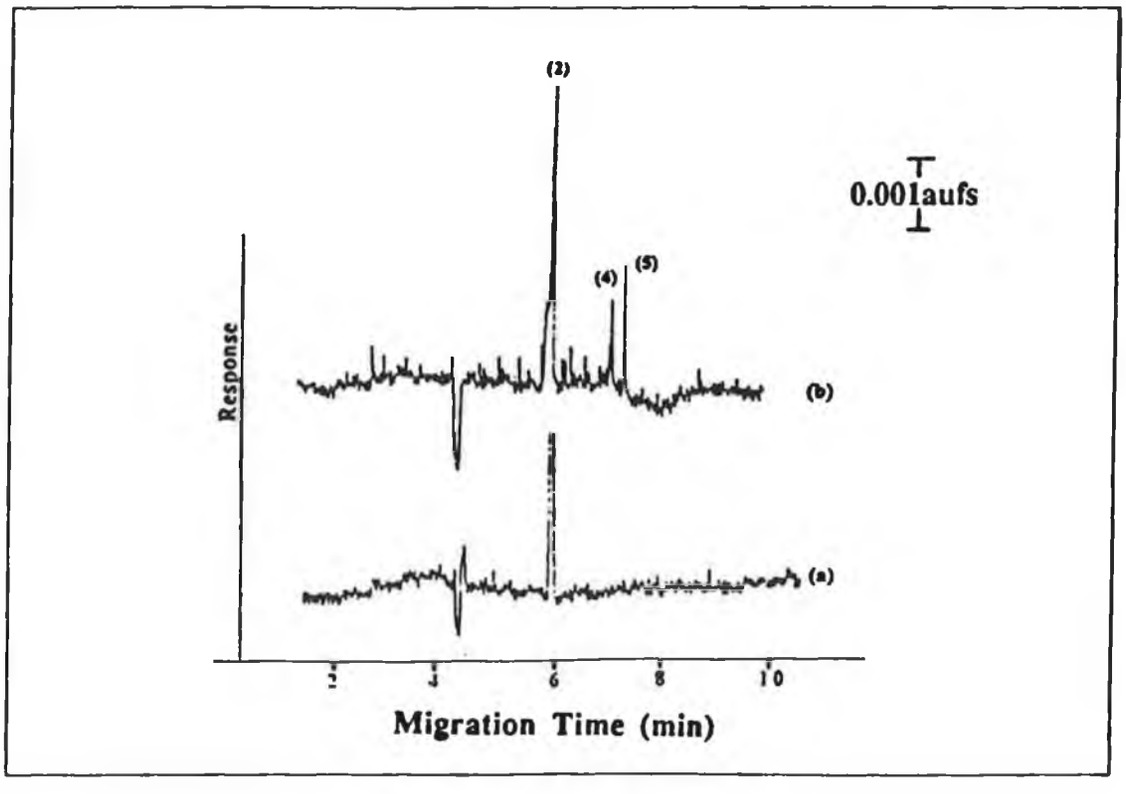

Figure 5.10

Water sample spiked with $1 \cdot 10^{-7}$ M (peak 4) Fe^{+2} and (peak 5) Zn^{+2} and analyzed by (a) precolumn complexation and (b) on-column complexation and peak stacking. Conditions are the same as in Fig. 5.6 [(peak 2) = free ligand].

5.4 CONCLUSIONS

The CE technique described, provides a rapid, simple and sensitive technique for determination of trace metal ions in aqueous samples. High separation efficiencies, small sample volume and simple equipment requirements have made CZE of great interest, particularly in the area of biological analyses. It is generally agreed that improvements in sample handling and introduction, and reduction in limits of detection in conventional absorbance detection can benefit its present practice [22]. Limits of detection could be improved if CZE was amenable to on-line preconcentration as in liquid chromatography.

Using sample stacking and on-column complexation, it was possible to achieve a 10-fold reduction in the LOD for Zn^{+2} and 100-fold reductions for Cu^{+2} and Co^{+2}, compared to precolumn complexation. The detecion limits are an order of magnitude lower than those reported using the same chelating reagent [16] and better than those reported using indirect detection [21] or electrochemistry [24]. In addition, the chelator PAR exhibits greater selectivity than that which can be obtained with indirect detection. The applicability of this technique to real samples was confirmed through the analysis of vitamin supplements and a pond water sample.

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Chapter 6.0

Determination of Pb(II) in aqueous samples by capillary electrophoresis using on-column complexation with PAR and preconcentration by peak stacking.

6.1 INTRODUCTION

6.1.1 Lead in the environment

6.1.1.1 *Sources of Lead in the Environment*

Lead is a common air pollutant in the urban environment and its toxicity together with its cumulative effect is well known. It is therefore one of the most important subjects in environmental studies. In the early years of the industrial revolution, much lead was added to the environment due to inefficient smelting processes and the poor design of early furnaces. Today, however, although lead continues to be used in a wide range of products, the greatest threat from lead in the environment is due to pollution from manmade organolead compounds, especially the use of tetraalkyl lead in petrol. Such compounds have been added to petrol since 1923 to improve the octane ratings for fuels used in high compression internal combustion engines.

Lead may exist as dissloved and suspended species in water. Lead in aqueous environments is most likely to be found in the +2 oxidation state. Only small quantities are likely to reach water via air deposition, drainage water or runoff from agricultural land. The most important source of lead is industrial effluents. Most of the lead in the environment is associated with anthropogenic inputs such as leaded gasolines, mining and smelting, combustion of coal, lead based paints, lead pipes and solder. In areas where limestone and galena (the major lead mineral, PbS) ores are found, natural waters may contain lead in solution up to 0.8 mg dm^{-3}. Surface waters otherwise contain significant amounts of lead only when subject to contamination by industrial wastes [1].

6.1.1.2 *Lead toxicity*

Lead poisoning usually results from cumulative toxic effects after continuous consumption over a long period of time, rather than occasional small doses. The adverse and toxic effects of lead on humans, particularly children, are well recognised. Lead reportedly interferes with a number of body functions, notably the nervous system, the hematopoietic system and the kidney. The prevention of the accumulation of lead, and its accurate control in the atmosphere, water, soils, food products etc., has received considerable attention. The determination of trace quantities of lead is of fundamental importance in this control strategy [2].

6.1.2 Analysis of Lead

Various analytical techniques such as atomic absorption [1], electrochemistry [3], inductively coupled plasma [4], and UV-visible spectrophotometry [5] have been utilized for lead analysis. However the majority of methods require sophisticated instrumentation.

6.1.2.1 *Spectrophotometric determination of lead*

A number of reagents are available for the spectrophotometric determination of lead, notably, diethyldithiocarbamate [7], 4-(2-pyridylazo) resorcinol [8] and diphenylcarbazone [9]. Spectrophotometric determination of Pb(II) using 2-(2-thiazolyazo)-*p*-cresol (TAC) reagent was reported by Ferreira et al. [5]. This ligand reacts with many ions with the formation of a coloured complex. The chelates have low solubility in water and thus this reagent is unsuitable for use in aqueous media. Ferreira and co-workers [5] described the use of this ligand in the determination of Pb(II) in the presence of a surfactant agent, TERGITOL NPX.

Determination of lead was demonstrated using direct injection and on-column derivitization using a dithiocarbamate ligand with HPLC [7]. This determination involved injection of the metal ions into a mobile phase containing sodium dithiocarbamate. Poor reproducibility was reported for low concentrations of lead, notably for 0.1ppm lead concentraion a coefficient of variation of 14.4% was recorded [7]. Detection was carried out at 350 nm as this wavelength was found to reduce interference from excess of reagent in the eluent. A 4-(pyridylazo) resorcinol-based continuous detection system for Pb(II) determination was employed by Jezorek and Freiser [8]. This reagent was favourable as it forms water-soluble complexes with a large number of metal ions, and these complexes exhibit high sensitivity for photometric detection.

The use of the ligand murexide has also been described for lead determination by complexation with the formation of a coloured complex [10]. This reagent has been used as a complexing agent for a large number of metal ions, and is known as a convenient ligand for the quantitative determination of metal ion concentrations in aqueous solutions with visible spectrophotometric detection [11].

Detection of 1 ng $cm^{-3}$of lead was described by Afkhami and co-workers [12] using a catalytic spectrophotometric determination by reduction of resazurin by sodium sulfide. The technique described, was based on the catalytic effect of Pb(II) on the reduction reaction of resazurin by sodium sulfide. This chemical reaction is followed spectrophotometrically by the measurement of reduction in absorbance of resazurin at 605nm. The calibration range of lead was found to be dependant on sulfide concentration.

6.1.2.2 *Atomic absorption spectrometry*

Determination of lead in various matrices has been demonstrated using HPLC with atomic absorption spectrometry by many groups. Matrices such as soil [13], mineral waters [14], seawater [15] and biological matrices [1] have all been described in the literature.

Determination of lead in urine has been reported as being a useful method of assessing environmental exposure to lead concentrations [1]. However matrices such as urine and blood contribute problems to the analyst. Subramanian et al. [1] described the utilization of graphite furnace AAS (GFAAS) with matrix modification for the determination of lead in human urine. An ammonium nitrate-nitric acid-graphite furnace AAS procedure was described, with detection limits in the ng cm^{-3} range reported. The study reported good recovery of Pb(II) from spiked human urine samples [1].

A more recent study has been reported involving the determination of lead in blood using flow injection flame AAS [17]. The study by Nygren et al. described a FAAS system without a preconcentration step for lead determination. The samples were treated with acid and subsequently introduced into a nebuliser interface prior to FAAS determination. A limit of detection of 0.02μg cm^{-3} was reported.

Hunt and Winnard [18] described a number of methods of matrix modification, prior to determination of lead by graphite furnace AAS. Detection limits in the low μg dm^{-3} range were reported. This method was utilized for the analysis of lead, among other toxic metal ions, in highly mineralized waters. The concept of stabilized temperature platform furnace (STPF) was employed with GFAAS. This procedure enabled interference-free graphite furnace analysis of complex matrices such as sea waters, and drinking

waters which contain a large number of interfering ions notably, calcium, sodium, magnesium, chloride, sulphate and bicarbonate. The limit of determination of this method was 1 μgdm^{-3} for Pb(II).

Determination of lead collected on air filters and *Sphagnum* moss using hydride generation AAS was recently described [19]. Detection limits in the range 0.3 ng cm^{-3} were reported, using optimum conditions of 0.75% HNO_3, 6% $(NH_4)_2S_2O_8$ and 2% sodium citrate for the sample solution at a flow rate of 6cm^3 min^{-1} and 7% $NaBH_4$ for the reducing solution (flow rate: 3cm^3 min^{-1}). Interferences using this technique were not found to be a problem.

6.1.2.3 *Electrochemical methods*

6.1.2.3.1 *Potentiometric methods*

The determination of Pb(II) has been described using a Pb(II) electrode with poly (hydroxamic acid) (PHXA) as the active material, and silicone rubber as the supporting material [20]. A detection limit of $4 \cdot 10^{-6} M$ Pb was reported. Silicone rubber was used as a supporting matrix due to the insolubility of PHXA in many organic solvents, thus making it impossible to prepare a thin membrane by solvent casting.

The use of Pb(II) ion-selective electrodes based on crown ethers was described by Sheen et al [21]. Detection limits of $1 \cdot 10^{-6} M$ Pb were reported, with a linear range in the region $1 \cdot 10^{-1}$-$1 \cdot 10^{-5}$ *M*. The crown ethers are encorporated here as neutral carriers in the Pb(II) poly (vinyl-chloride) membrane. Interferences from other cations were not found to be a problem.

6.1.2.3.2 *Voltametric methods*

Differential-pulse anodic stripping voltammetry (DPASV) has been utilized recently in the determination of trace quantities of lead in soils [3]. Open beaker, teflon bomb and microwave heating digestions were used for matrix modification, and complete dissolution (> 98%) of the samples, in acidic medium (HF,HNO_3) occurred for the open beaker and microwave methods. The high acidity however created a problem for DPASV in that it reduced the potential window available for analysis. Attempts were made to

counteract this hurdle in the form of (a) partial neutralization with NaOH, (b) dilution with water and (c) dilution with a constant weak acid electrolyte. Of the three attempts, the latter appeared to be the most preferable solution, as dilution with a constant weak acid electrolyte ($CH_3CO_2H + KNO_3$) made the procedure less dependent on the amount of acid remaining from the dissolution step.

6.1.2.4 *Determination of Pb(II) using 4-(2-pyridylazo) resorcinol (PAR)*

The use of the ligand PAR in the determination of lead has been well documented [8,22-24]. This ligand as reported in section 5.1.9.1, has been widely used for metal ion determinations, due to its metal complexing ability and water solubility [25]. Analysis of lead using this reagent has been documented using HPLC with cation-exchange [22-24] and reverse-phase columns [8].

Kawazu and Fritz [22] reported the determination of lead by the addition of the PAR reagent following elution of the metal ions from a cation-exchange column. Forced flow cation-exchange was utilized encorporating the macroreticular ion exchange resins, Amberlite-200 and Amberlyst-15. A volume of 80 cm^3 of eluent was passed through the column for equilibration, with subsequent separation of the metal ions. The colour forming reagent, PAR was added following separation via a mixing chamber, and detection was carried out spectrophotometrically at 525nm. A limit of detection of $2.3 \cdot 10^{-7} M$ was obtained.

Fritz and co-workers [23] reported a similar application using macroreticular resins for metal ion separations including lead. Forced-flow chromatography involving the application of pressurised eluent to the resin bed was employed, using strongly acidic eluents of hydrochloric acid in acetone -water. On-stream addition of the colour forming reagent (PAR) provided continuous determination and quantitation of the eluted metals. Detection limits in the 10^{-7} M region were reported.

Jezorek and Freiser [8] demonstrated the use of a 4-(2-pyridylazo) resorcinol based detection systems for trace metal ions, notably Pb(II). The study involved the use of a PAR-ZnEDTA detection system. Lead showed poor reaction kinetics when using the indicator PAR, but an improved

response was noted when PAR-ZnEDTA was utilised. An optimum response using this ligand occurred when a pH of 9 was used.

6.1.3 Trace enrichment of lead

Many methods describing trace analysis of lead involve a preconcentration step [13,15,26-31], with both on-line [13,15,28,30] and off-line [26,29,31] preconcentration techniques reported.

6.1.3.1 *Off-line preconcentration of lead*

Ueda at al. [29] demonstrated the preconcentration of lead using polystyrene resins with azobenzylphosphonic acid functionalities. Trace quantities of Pb(II) were quantitatively retained on the resin columns at neutral pH and eluted using 2m*M* HCl and 2*M* HNO_3. The technique was applied to trace lead determination in sea water and river water, prior to analysis by AAS and UV-visible spectrophotometry.

Off-line enrichment of lead from tap water using evaporation, extraction and extraction after evaporation was reported [31]. The study assessed advantages of evaporation and extraction techniques and detection was effected using FAAS giving a limit of detection of 10ppb. It was found that " extraction after evaporation" was the most suitable method of analysis as the extraction method alone resulted in a light scattering effect below 250 nm due to high total dissolved solids, therefore initial evaporation reduced this effect.

More recently Vernon and Wani [26] reported a comparative study involving three experimental methods for trace lead preconcentration off-line. The chosen preconcentration techniques included evaporation, solvent extraction and cation exchange. The evaporation technique involved acidification of samples, using nitric acid, followed by evaporation to 5-10cm^3 using a hot plate. Subsequent dilutions were carried out, until a final 20-fold concentration factor was achieved. The method of detection was atomic absorption spectrometry. Solvent extraction involved the use of a number of chelating agents, notably 8-hydroxyquinoline and ammonium pyrolidine dithiocarbamate (APDC). 500 cm^3 samples were taken, pH adjusted and

extracted into chloroform twice. A 20-fold concentration factor was again obtained. The final method using ion-exchange involved the encorporation of a Dowex 50 strong cation exchange resin. A 500 cm^3 sample was passed through the column and sorbed metals were eluted using nitric acid. Again a 20-fold concentration factor was achieved. The three techniques studied provided equal concentration factors, and all were found to be satisfactory methods for preconcentrating trace metals from surface waters. The ion-exchange method, however, did show a fall in efficiency at very high dilutions.

6.1.3.2 *On-line preconcentration methods*

On-line sample preconcentration methods offer a number of advantages over off-line methods. The notable advantage being minimal sample handling. This is important to ensure accurate determinations of metal ions without contamination problems.

Cassidy and Elchuk [28] reported the encorporation of a short bonded phase ion-exchanger for enrichment of the metal ions of interest, (Mn(II), Ni(II), Co(II), Cu(II), Zn(II) and Pb(II)), with subsequent on-line separation on a styrene-divinyl benzene resin column. Once the desired enrichment was achieved, the concentrated analytes were diverted onto the separation column, in the backflush mode. A detection limit of 1.7 pg cm^{-3} was reported for Pb(II) using an enrichment volume of 2 litres. Detection was carried out using post column reaction with PAR and spectrophotometric detection.

More recently, Zhang et al. [30] described the on-line preconcentration and determination of lead in potable water by flow injection AAS. For enrichment, a micro-column of activated alumina was used. The analyte ions were deposited on the alumina during the sampling stage, and elution was effected using nitric acid. A detection limit of 0.36 μg dm^{-3} was obtained using a 25 cm^3 sample volume at a sampling rate of 5 cm^3 min^{-1} for 5 min.

Fang et al. [15] demonstrated the detection of 0.3 μg dm^{-3} Pb(II) using on-line sorbent extraction and flow injection AAS. The preconcentration step involved complexation of the ions with dithiocarbamate ligands and subsequent adsorption of the complexes on silica adsorbent, with octadecyl functional groups. A 20 second loading time, at a flow rate of 3.3 cm^3 min^{-1} was used. Ethanol and methanol were used to elute the adsorbed analytes. The

ligand solution was introduced at a concentration of 0.5 mg cm^{-3} at 0.4 cm^3 min^{-1} and it was mixed with the acidified sample prior to concentration.

A similar procedure was carried out [13] using a C18 sorbent material for concentration, with dithicarbamate as the complexing agent and methanol as an eluent. Using a 20 second sample loading time at 8.7 cm^3 min^{-1}, a detection limit of 10 μg dm^{-3} was obtained. The procedure was applied to the analysis of environmental samples such as coal fly ash, loamey soil, lake, river and estuarine sediment. Determination was carried out using flame AAS.

Determination of trace quantities of lead by on-line preconcentration, flow injection AAS was described by Purohit et al. [27]. This technique involved the use of resins, synthesised from quinolin-8-ol and resorcinol with crosslinking agents such as formaldehyde or benzaldehyde, for Pb(II) preconcentration. A continuous flow manifold using resin micro-columns was used for trace lead determinations. Use of such chelating resins has been widely reported [32] for trace metal preconcentration. In this investigation [27] preconcentration of lead on microcolumns containing chelating resins was achieved by passing 5 cm^3 of $1 \cdot 10^{-7}$ *M* lead solution through at a flow rate of 2 cm^3 min^{-1}. Nitric acid was utilised to elute the chelated lead analytes, and quantitative uptake of lead by the resins was reported using these columns.

6.1.4 Lead determination using capillary electrophoresis (CE)

Only one publication to date has reported the determination of lead using CE [33]. Detection was carried out using mass spectrometry and indirect UV spectrophotometry. Twelve cations were separated and quantified including lead. The CE buffer contained 30 m*M* creatinine and 8m*M* HIBA, pH 4.8. Pressure injection was used with a separation voltage of 30 kV.

Additional studies involving CE analysis of inorganic ions include that carried out by Lu and Cassidy [34] in their use of mercury microelectrodes for metal ion determination. More recent studies involved complexation of metal ions using ligands, 8-hydroxyquinoline [35] and PAR [36] with subsequent separation of the metal chelates.

In this investigation a similar capillary electrophoresis approach to that described in chapter 5, incorporating complexation of lead with the PAR ligand was attempted. On-column complexation was carried out successfully

using an organised sequence of steps, with additional ligand included in the electrophoretic buffer solution to maintain complex stability. In addition to on-column complexation, by injection of the metal ion in nanopure water, a 10 fold reduction in limit of detection was achieved, due to a sample stacking effect which occurs on-line. In order to demonstrate the applicability of this technique to real samples, a pond water sample was spiked with $1 \cdot 10^{-6} M$ Pb(II) and injected electrokinetically allowing complexation with the PAR reagent to occur on-line. In addition on-line detection was effected by UV-visible spectrophotometry at 498nm.

6.1.5 Aims and Objectives

The aim of this study was to develop a method of Pb(II) determination utilizing capillary electrophoresis with visible spectrophotometric detection following complexation with a suitable ligand.

6.2 EXPERIMENTAL

6.2.1 Apparatus

Separations were performed on 0.1*M* NaOH-treated fused silica capillaries, 50 μm i.d. x 300μm o.d., obtained from Polymicro Technologies, (Phoenix, AZ). The high voltage power supply was a Glassman MJ30P400 power supply (Glassman, High Voltage, Whitehouse Station, NJ), the input from which was placed in a plexiglass box with an interlock in the access door for safety. UV-visible on-column detection with a C4 capillary electrophoresis absorbance detector (ISCO, Lincoln, NE) was carried out at 498 nm. The total length of the capillary was 85 cm and the length to the detector was 75cm.

6.2.2 Reagents

The stock solutions were prepared by dissolving the appropriate amount of lead nitrate in nanopure water. An electrophoretic buffer of 10 m*M* TAPS (N-tris [hydroxymethyl] methyl-3-aminopropane sulphonic acid) (Sigma, St. Louis, MO) was used throughout the investigation. The pH was adjusted to the required value using 0.1*M* NaOH. The working PAR solution was prepared by dissolving the reagent in the electrophoretic buffer at pH 9.0.

6.2.3 Procedure

6.2.3.1 *Precolumn complexation*

Lead/PAR chelates were formed by adding an appropriate quantity of metal ion stock solution to 10m*M* TAPS buffer, adjusted to pH 8.4 containing $1 \cdot 10^{-3} M$ PAR. The Metal complex formed immediately and was injected electrokinetically within 30 minutes of preparation. A lower concentration of PAR ($1 \cdot 10^{-4} M$) was added to the electrophoretic buffer to prevent dissociation of the unstable lead chelates [35]. Injection of the complexes was carried out at 10 kV for 10 seconds, and separation was effected using 30 kV resulting in a current of 5 μA.

6.2.3.2 *On-column complexation and sample stacking*

A three-step sequence was followed to enable optimum complexation on-capillary. For sample stacking, samples were injected in nanopure water, employing the three step sequence outlined below to effect on-column complexation, and sample enrichment.

Step	Operation
1	1mM solution of PAR, pH 8.4 was injected at 15 kV onto the capillary for a period of 10 seconds.
2	The sample solution containing the metal ion was then injected in nanopure wateronto the capillary for a period of 20 seconds at 15 kV.
3	The anodic end of the capillary was again placed in the run buffer (containing 0.1mM PAR) and 15 kV was applied for 5 seconds (pause time).

The final step enabled adequate complexation of the ligand and the metal ion. The voltage was then ramped to 30 kV to enable separation to occur.

6.2.3.3 *Sample preparation*

The water samples were obtained from a small pond at the University of Kansas in Lawrence, KS. Samples were collected in polyethylene bottles. The samples were spiked with $1 \cdot 10^{-6} M$ Pb(II) and were acidified using nitric acid to release any metal bound to organic matter present in the samples.

6.3 RESULTS AND DISCUSSION

6.3.1 Pb(II)/PAR complexation

Preliminary investigations involved selection of a suitable concentration of ligand, and selection of an optimum pH for complex formation. It was found (Figure 6.1) that a 10 fold molar excess of ligand was necessary to enable optimum complex formation. The plot of absorbance versus ligand concentration, for precomplexed solutions demonstrates that for a 0.1m*M* Pb(II) standard a PAR concentration of 1m*M* or greater was necessary.

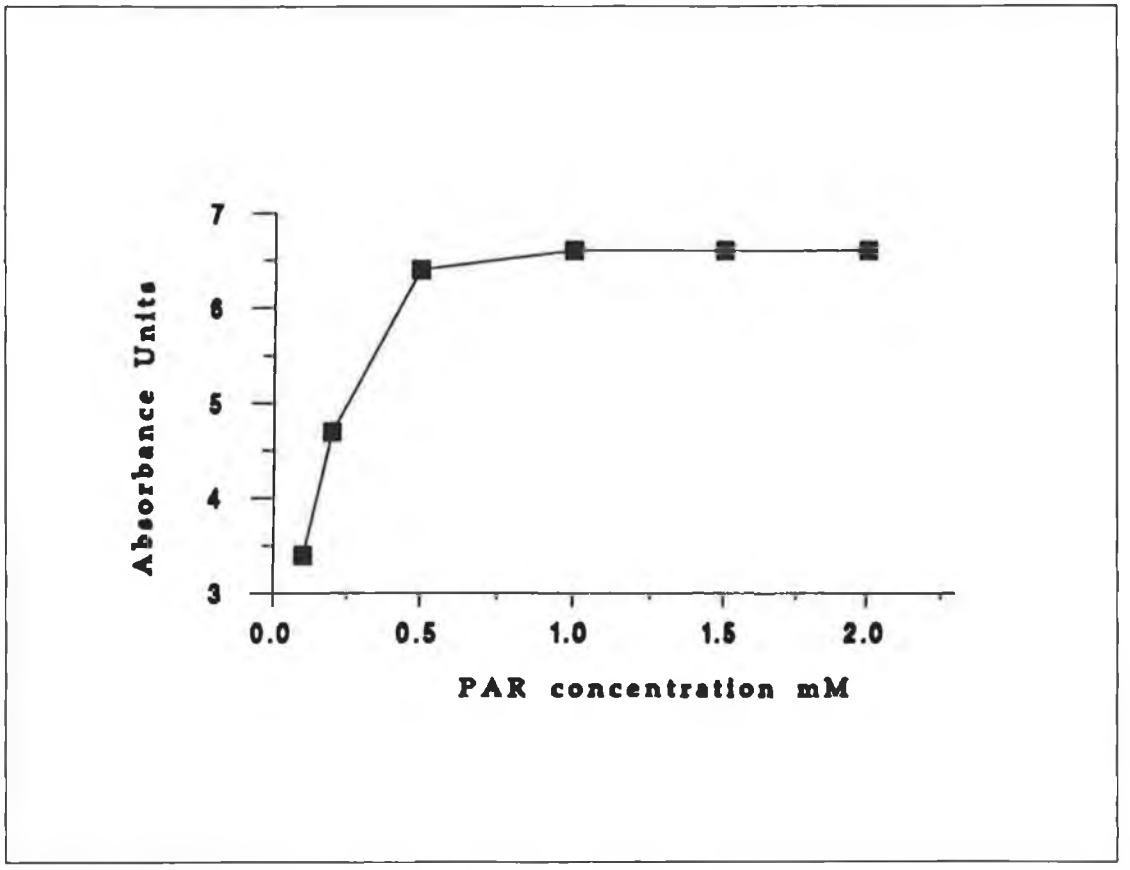

Figure 6.1

Effect of Ligand (PAR) concentration on complexation. [Pb]=0.1mM. pH 8.4, detection @ 498nm

Figure 6.2 shows the effect of pH on the lead/PAR complex stability over time. At low pH values , for example at pH 6.0, PAR chelates are unstable, show low absorptivity and after a 20 minute period disintegrate. At alkaline pH, it was found that a much more stable chelate is formed, a factor also noted by Jezorek et al. [8]. At pH 8 the chelate remains stable for a period of up to 10 minutes, following which it degrades, as is evident from its absorbance value. However at pH 9 it was found that the chelate remains stable for up to 40 minutes. Thus from these observations a time limit of 30 minutes at pH 9 was selected within which injections were to be carried out, in order to ensure maximum sensitivity of the PAR chelate.

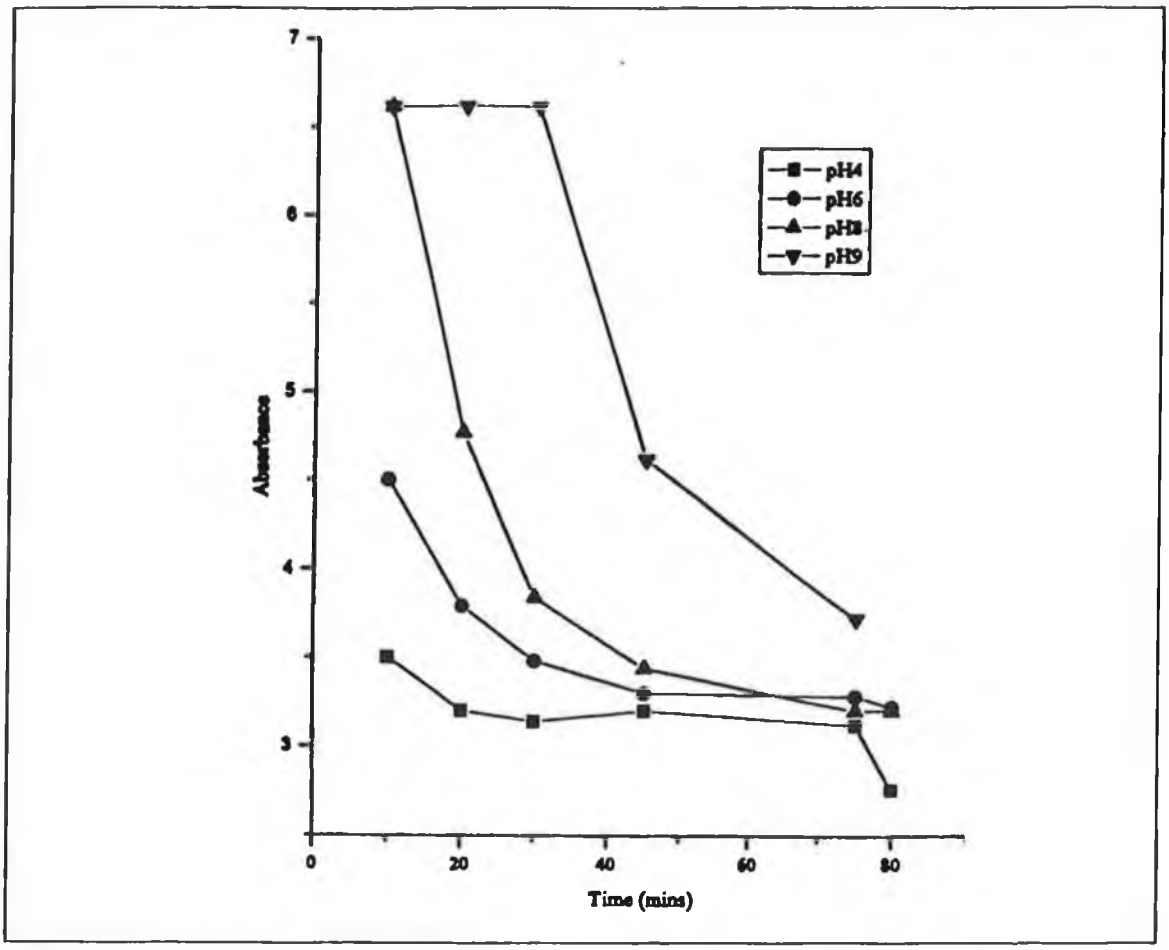

Figure 6.2

Effect of pH variation on Pb(II)/PAR complex stability.
[M^+]=0.1mM, [PAR]=1mM.

6.3.2 Electrophoretic buffer composition

Previous investigations (section 5.3.2) found zwitterionic buffer TAPS to provide very satisfactory separations. Because higher voltages can be used, this results in more efficient separations due to lower currents (5μA). Due to the instability of the Pb/PAR chelate it was necessary to include a small concentration of the ligand ($1 \cdot 10^{-5} M$) in the electrophoretic buffer in order to enhance complex stability, and thus obtain efficient separations. Recent investigations by Iki [36] and Swaile [35] reported similar advantages resulting from the inclusion of ligand in the running buffer. Figure 6.3 illustrates clearly the effect of varying PAR concentration in the electrophoretic buffer. At very low concentrations, ie., $1 \cdot 10^{-5} M$ PAR, efficiency is poor, with a typical theoretical plate number of 554 for an 85cm column. When the ligand concentration was increased a substantial improvement in efficiency was observed. The optimum concentration of ligand which achieved the best efficiency ie. 10,275 plates/85cm, was found to be $5 \cdot 10^{-4} M$ PAR. Above this concentration the background absorbance from the ligand itself posed a problem, due to the slight absorbance due to PAR at the wavelength of detection (498nm).

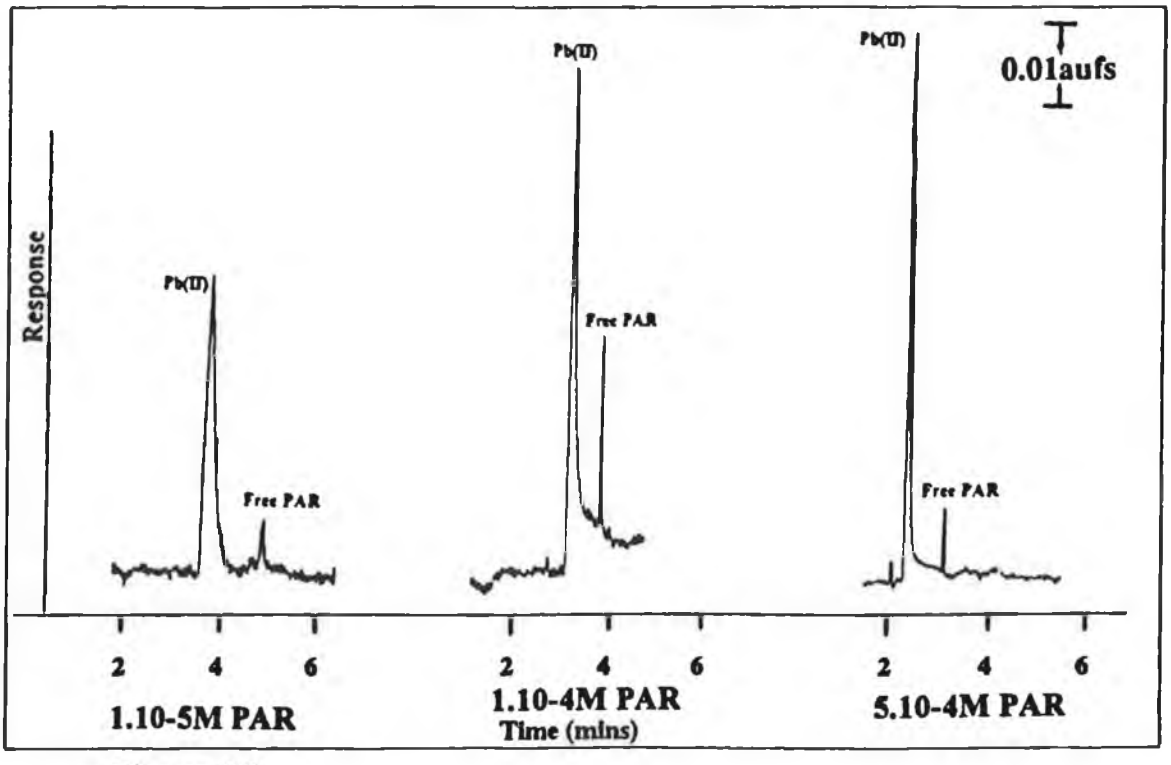

Figure 6.3

Effect of [PAR] in the electrophoretic run buffer on separation efficiency.([Pb]=$3 \cdot 10^{-3} M$)

6.3.3 Interferences

In addition to the effect of PAR concentration on separation efficiency, its importance in resolving Pb(II) from other interfering metal cations was also demonstrated (Figure 6.4). The only metal cation found to interfere with Pb(II) was Co(II). It was found that the greatest factor in resolving these two species sufficiently was the ligand concentration in the running buffer. A concentration of $5 \cdot 10^{-4}$ *M* PAR was found to provide optimum resolution.

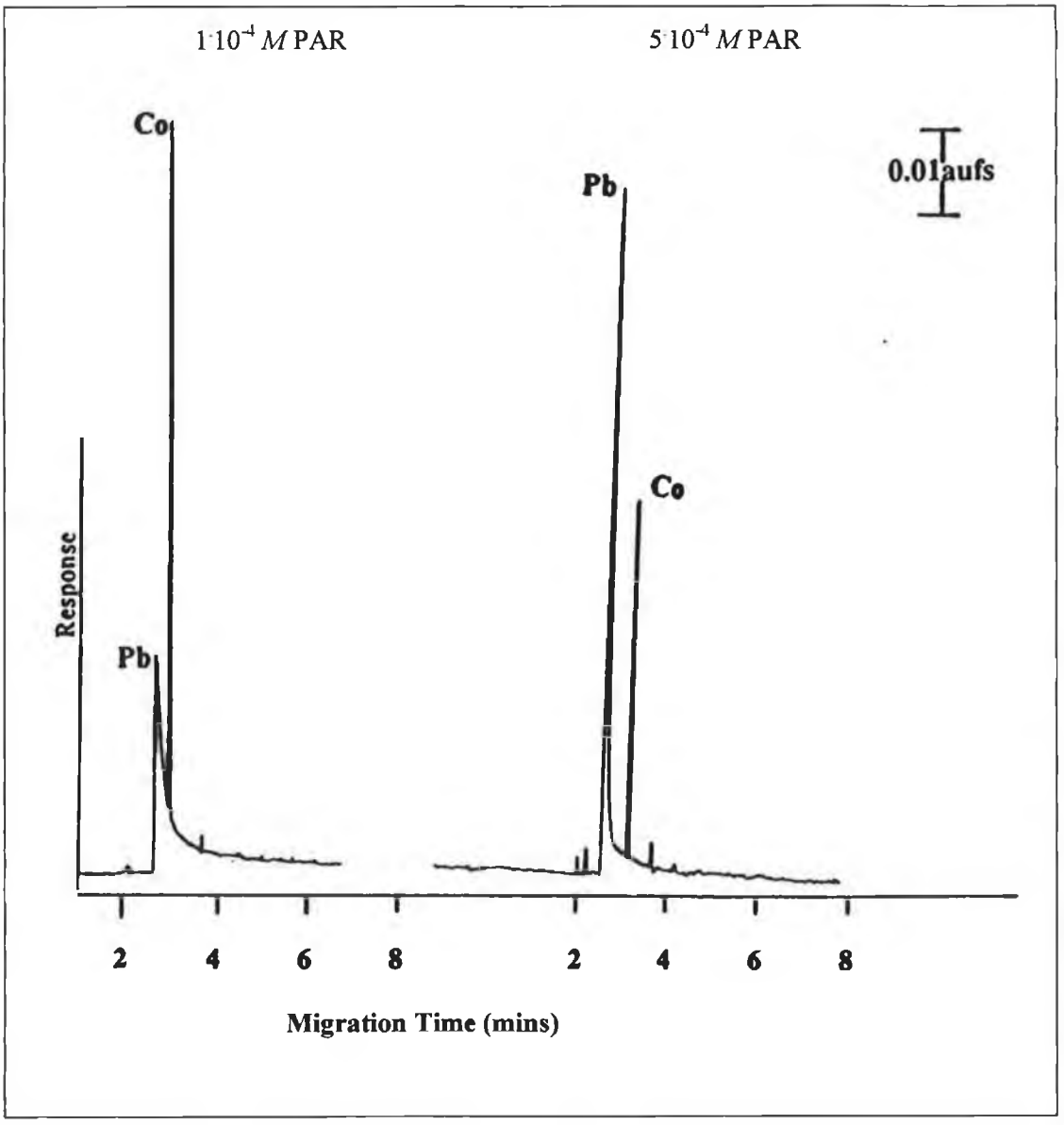

Figure 6.4

Effect of [PAR] in electrophoretic buffer on resolution of Pb^{+2} from Co^{+2}

6.3.4 Metal ion separations

Figure 6.5 illustrates the separation of Pb(II) and free PAR using the precomplexation technique, with electrokinetic injection of the complex. The optimum conditions selected for separation include 10kV injection for 10 seconds with a separation voltage of 30 kV. The electrolyte employed consisted of 10 m*M* TAPS containing $5 \cdot 10^{-4}$ *M* PAR to effect efficient separation and to maintain stability of the chelate on-capillary.

Use of this precomplexation technique achieved a limit of detection of $1 \cdot 10^{-5} M$, which in terms of mass detectability is $2 \cdot 10^{-13}$ *M* Pb(II). A linear dynamic range over two orders of magnitude (10^{-3}-$10^{-5} M$) was evident and reproducibility for six replicate sample injections was ~4 % CV. The poor limits of detection obtained here are insufficient for environmental applications, where concentrations are very often much lower. A contributing factor to the poor limit of detection in this procedure is the poor quality of the detector, as noted previously in section 5.3.3.. The inclusion of $5 \cdot 10^{-4} M$ PAR to the electrophoretic buffer may also be a contributary factor, as the background absorbance is higher than in its absence. There have been a number of reports in the literature concerning methods of improving sensitivity for CE. Among these reports, the use of a Z-cell has been employed in order to lengthen the effective pathlength [37]. Solid phases have also been encorporated into the capillary to enable on-line enrichment [38]

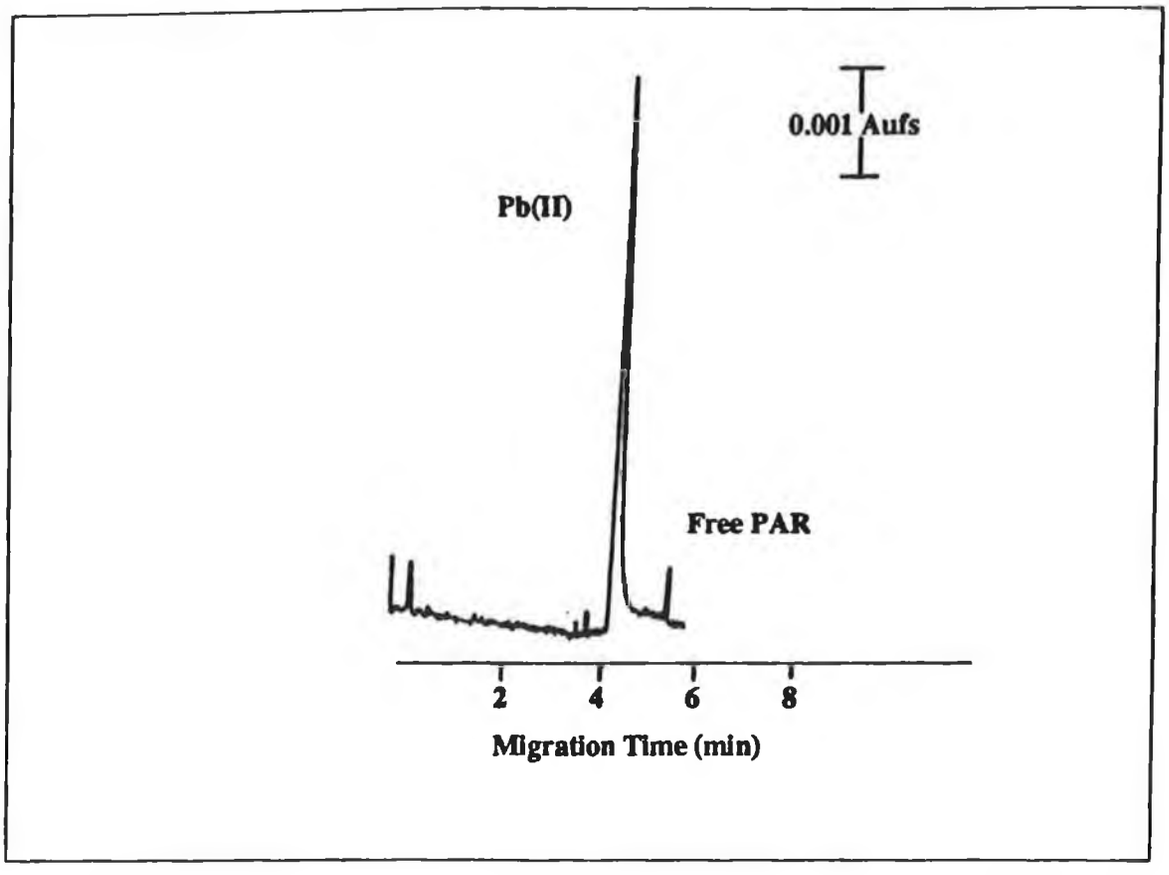

Figure 6.5
Precomplexation of Pb ($3 \cdot 10^{-3}$M) with PAR ($1 \cdot 10^{-3}$M) Electrokinetic injection; 10 kV, 10 s. Detection; 498nm. Separation voltage; 30 kV, Electrolyte; 10 mM TAPS & $5 \cdot 10^{-4}$M PAR

6.3.5 On-column complexation and trace enrichment

On-column complexation has been widely employed for metal ion analysis using HPLC [39]. The on-column formation of complexes allows the direct introduction of metal ions into the chromatographic system, which eliminates tedious sample preparation steps. On-column complexation requires good kinetics of complexation and no ligand interference in the detection of the specific metal ions [39].

From precomplexation studies it was found that Pb/PAR chelates are somewhat unstable on-column, but this factor was overcome by the inclusion of ligand in the electrophoretic run buffer. On-column complexation in this determination achieved two advantages, notably, (a) the elimination of a sample

preparation step, and (b) the substantial sensitivity enhancement which is achieved using on-column complexation as opposed to precomplexation. By encorporating a three step sequence for on-column complexation, optimum complexation and " peak stacking" is achieved.

Figure 6.6 illustrates an electropherogram of Pb(II) determination by CE under stacking conditions with on-column complexation. In order to achieve optimum sensitivity enhancement, the stacking steps were optimized as in section 5.3.6. On-column complexation did not occur completely unless the three steps described were carried out. An initial plug of ligand was required prior to sample introduction. The effect of this first step is clearly illustrated in Figure 6.7. It was found that in the absence of this ligand plug, complexation was not complete, but by electrokinetic injection of PAR for 10 seconds (10kV), a substantial improvement was seen to occur.

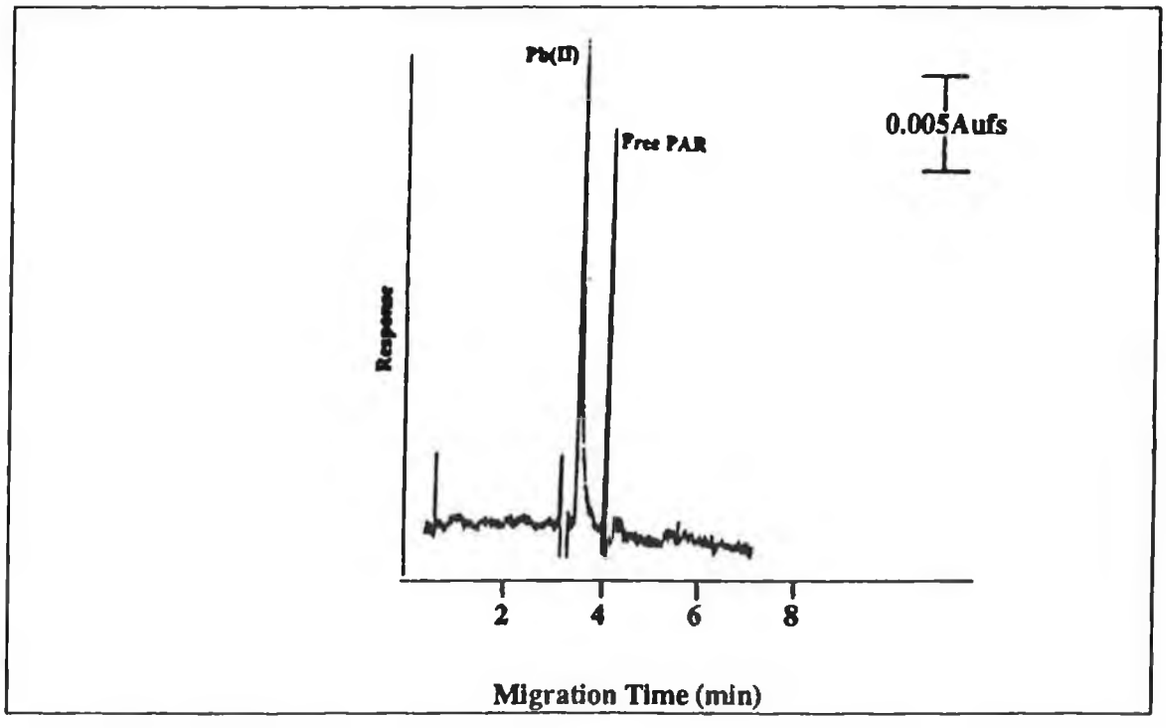

Figure 6.6

Determination of Pb(II) by CE under stacking conditions with on-column complexation.

Stacking sequence; 1st plug $1 \cdot 10^{-3}$M PAR 10s. [metal ion] $6 \cdot 10^{-7}$M; Pause time $5 \cdot 10^{-4}$M PAR(in electrolyte) 5 s. Stacking voltage 15 kV, separation voltage 30 kV; 498nm.

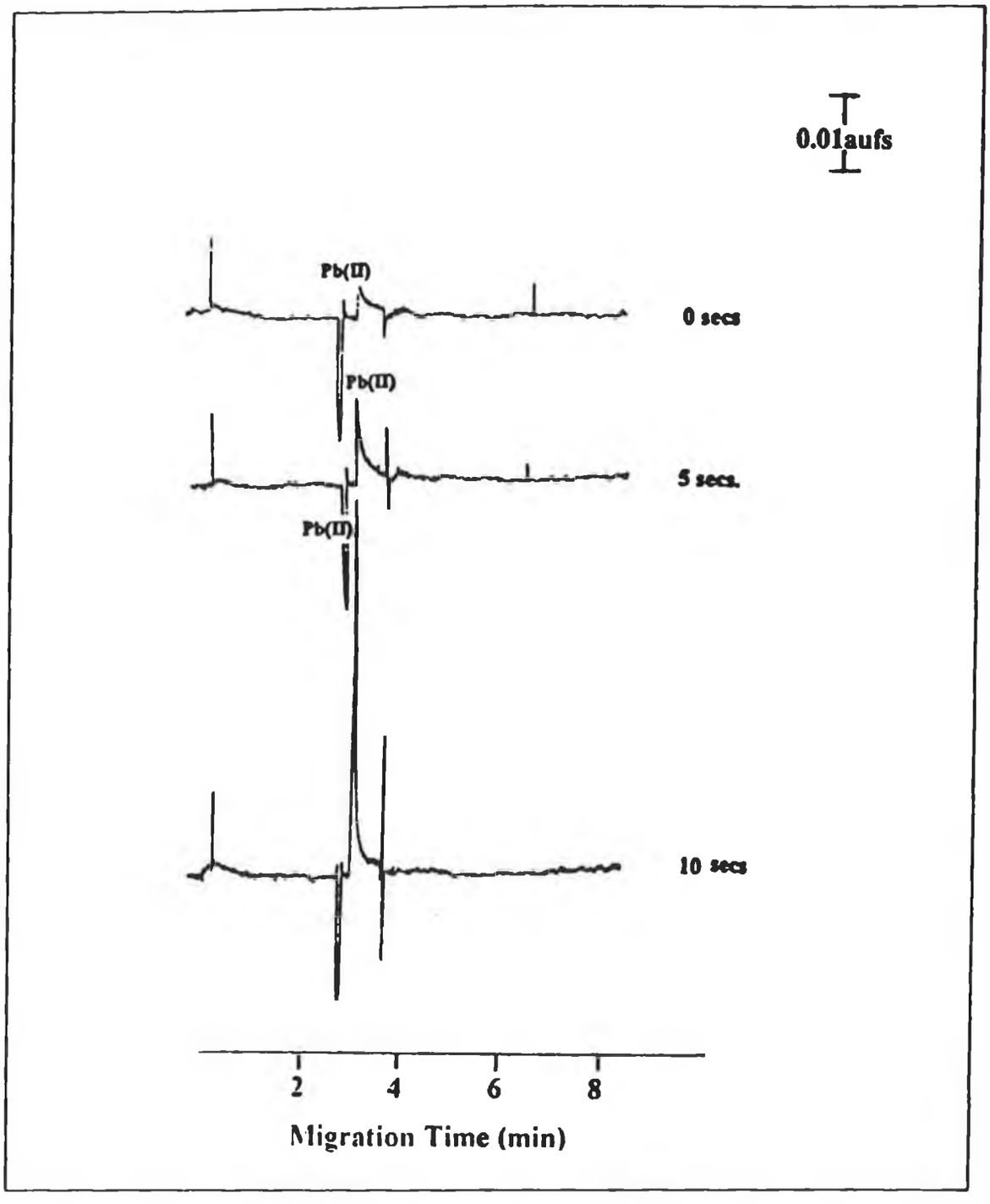

Figure 6.7

Effect of injection time of the PAR plug on Pb(II) peak height using on-column complexation.

Careful selection of the sample injection time was also essential to effect optimum on-column complexation. An injection time of 20 seconds (figure 6.8), provided an optimum peak height response, beyond which peaks broadened due to overloading. For CE injection it is necessary to maintain short injection times as well as low injection voltages to obtain efficient separation [40]. In this determination of lead, although an injection time of 20 seconds provided the optimum response, a combination of 15 kV with a 15 sec. injection time provided the most satisfactory electropherogram. Figure 6.9 shows that voltage has a continually increasing effect on peak stacking, however voltages above 15 kV were unsuitable due to overloading of the capillary. At high voltages a high concentration of ions move into the running buffer [41]. However using the three step sequence, overloading of the column causes severe band broadening. This effect appeared to be due to the more rapidly moving cations mixing with the slow moving ligand and in the absence of sufficient ligand to coincide with the large influx of cations the complexes appear as a broad band. Thus selection of correct injection voltage and time is vital for on-line complexation.

The final step in the stacking sequence involved a "pause time". This pause time can be defined for this procedure, as the time allowed for complete complexation to occur. During this time a low voltage is applied to enhance mixing of the PAR and metal ion, prior to application of the separation voltage. Figure 6.10 shows that in the absence of a pause time all the metal ions have not been complexed. By applying a low voltage for a short time period (5 seconds), mixing of the ligand and metal ion is enhanced, due to more rapid migration of the positively charged lead ions towards the negative electrode. In doing so the ions encounter the plug of ligand and complexation occurs. Following the pause time, the voltage is ramped to 30 kV to effect normal separation.

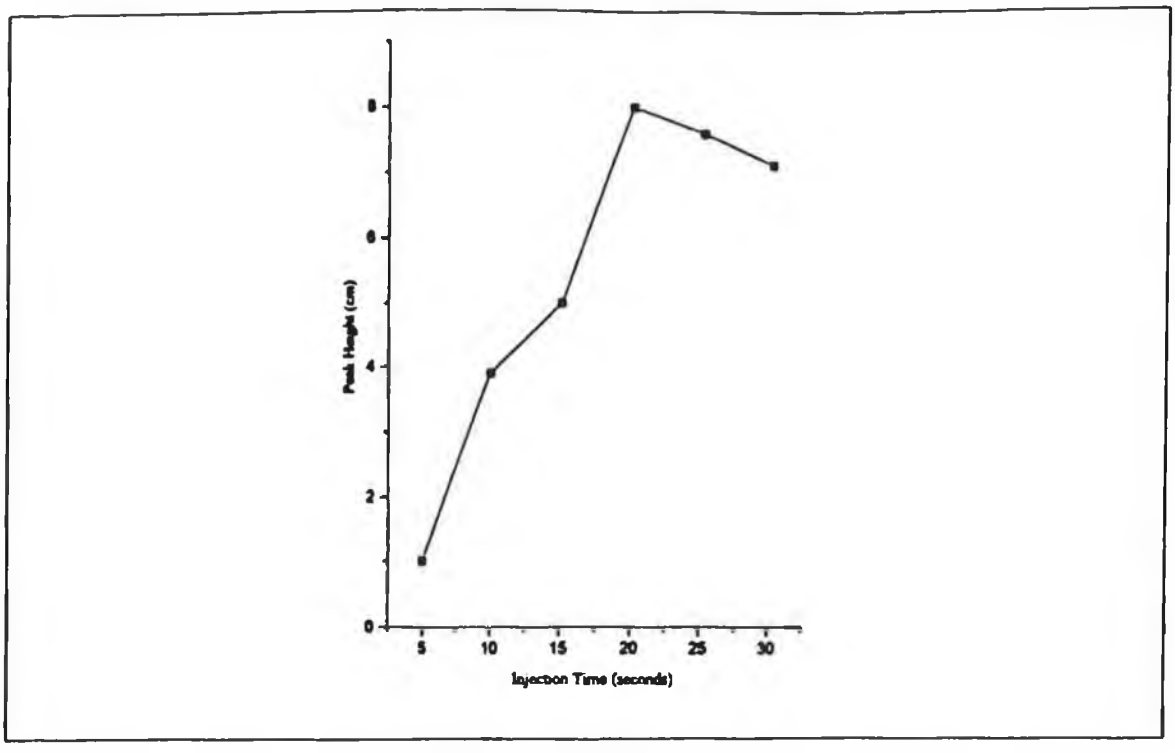

Figure 6.8

Effect of sample injection time on peak height. [Pb] = $5 \cdot 10^{-6}$ M. Stacking sequence; 1st plug, 10 s.; Metal ion; Pause time = 5 s.

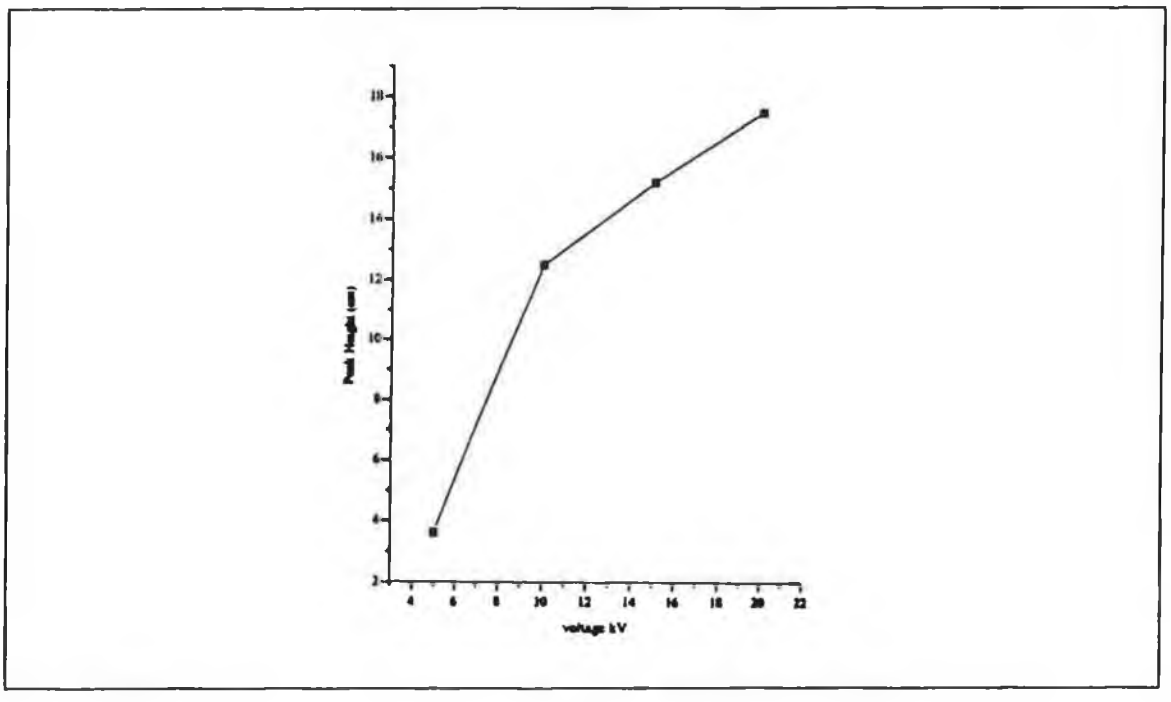

Figure 6.9

Effect of voltage on peak stacking. Stacking sequence: 1st plug 10s.; Metal ion 15 s.; Pause time 5s. [Pb] = $5 \cdot 10^{-6}$M

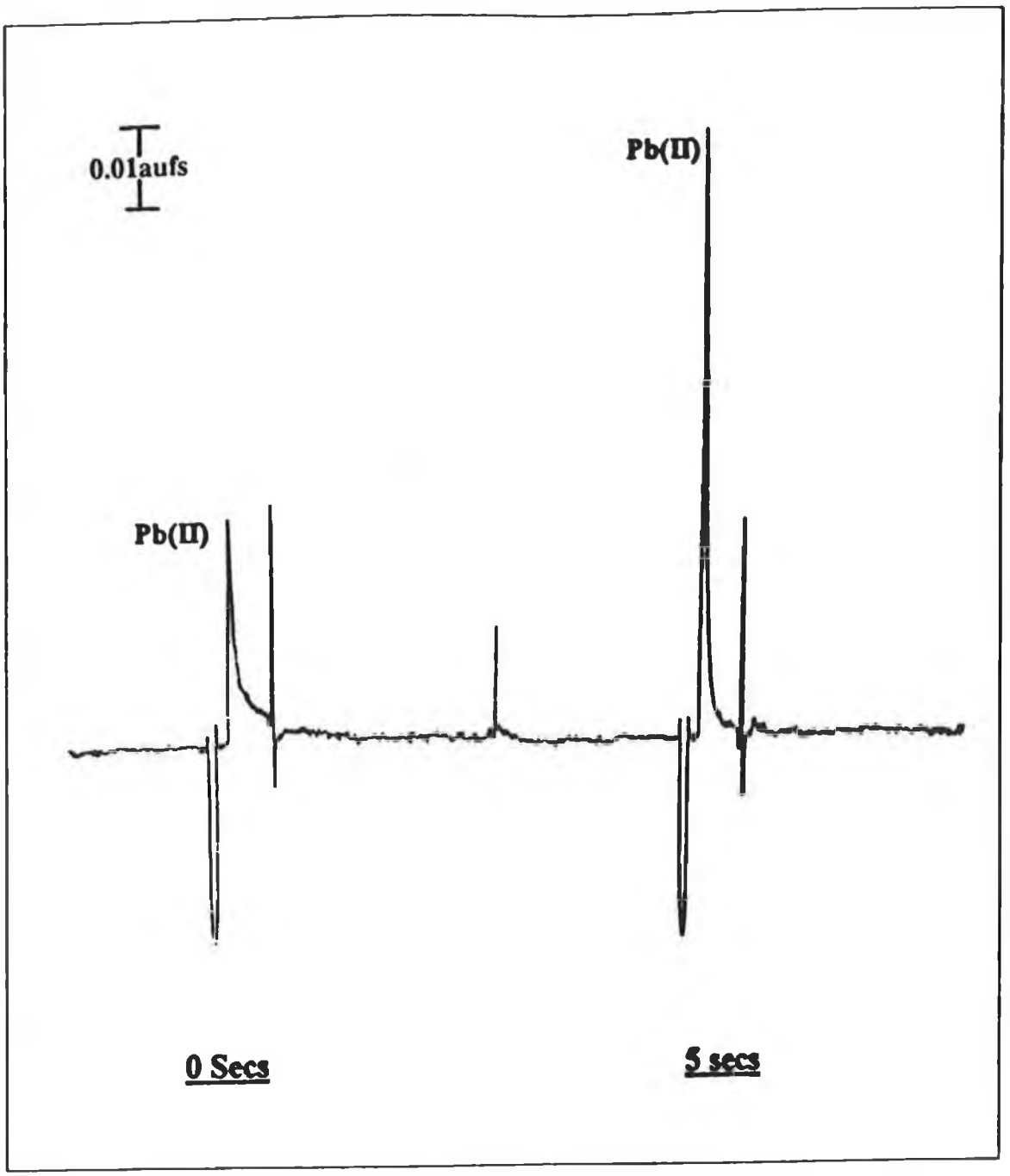

Figure 6.10
Effect of "Pause Time" length on peak height.

6.3.6 Peak stacking

By injecting the sample ions in a buffer solution equivalent in ionic strength to that of the run buffer a small degree of peak stacking was seen to occur. This effect may have been due to the presence of the PAR plug in the on-column complexation sequence [42]. Further stacking however is enhanced by injection of the sample in nanopure water. Injection of the ions in a solution which has a much lower ionic strength than the run buffer, results in the development of a field accross the sample ions on application of the voltage [41]. This causes the ions to migrate more rapidly until they reach the buffer boundary at which time the ions slow down, and subsequently stack up into a smaller volume. In the case of the three step sequence employed in this study, two boundaries confront the analyte ions, notably, the run buffer and the PAR plug. The latter boundary is thought to also enhance the stacking effect somewhat [42]. From the investigation it was found that the greatest stacking effect was noted when nanopure water was used. This is illustrated by a drop in peak height when stacking a Pb(II) concentration of $5.10^{-6}M$. Upon addition of even small quantities of buffer (1m*M*) to the sample the stacking effect deteriorates to zero (Figure 6.11).

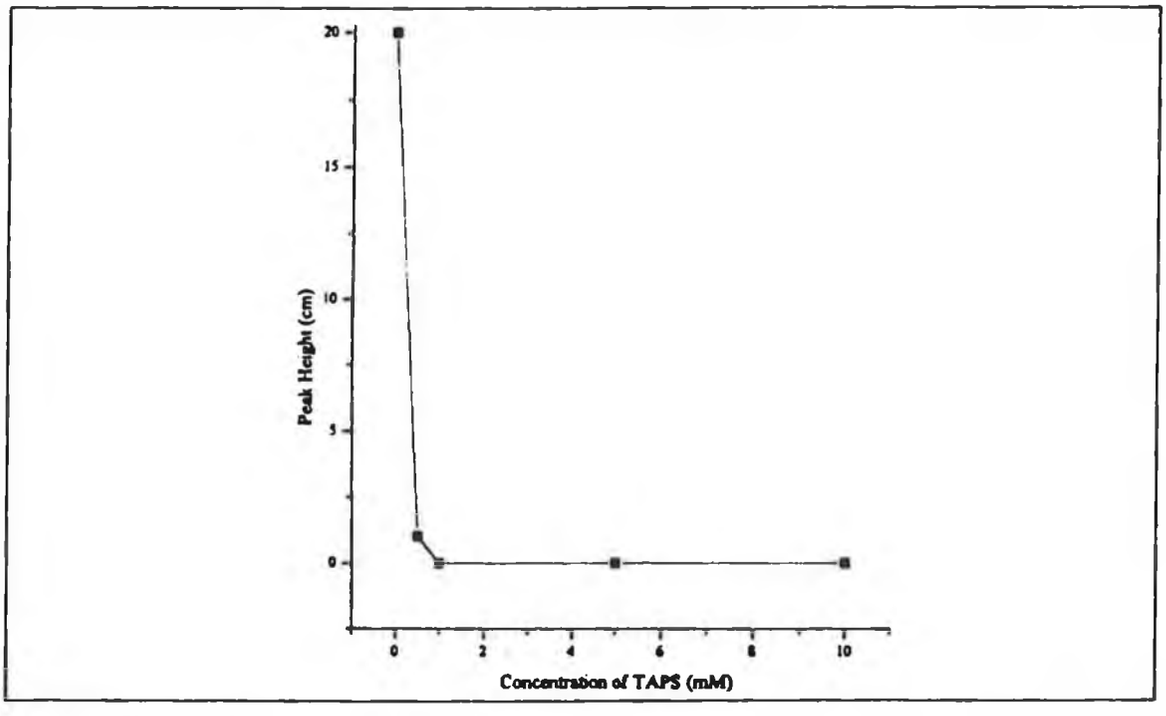

Figure 6.11
Effect of solution ionic strength on peak stacking.
[Pb+2] = 5·10⁻⁶M

6.3.7 Limits of detection

Table 6.1 sumarises precolumn and on-column complexation data. From the investigation it was found that by employing on-column complexation with peak stacking, a 100 fold improvement in LOD was achieved. This corresponds to a mass detection limit of $8.2 \cdot 10^{-15}$ m, (ie., 8 fmol) for a 82 nl injection volume of Pb(II). A linear dynamic range for the calibration was obtained over two orders of magnitude, and reproducibility for six replicate injections of $1 \cdot 10^{-7} M$ was 3% CV. Use of the sample stacking procedure for Pb(II) did not demonstrate a loss in efficiency, but rather an improvement over the precomplexation approach.

	Precolumn complexation (off-line)	**Oncolumn complexation stacking**
LOD	$1 \cdot 10^{-5} M$	$1 \cdot 10^{-7} M$
Mass LOD	$2.4 \cdot 10^{-13}$m	$8.2 \cdot 10^{-15}$m
Volume injected	24nl	82nl
% RSD	3.9 %	3.0 %
N	10175	19944
Linear range	$1 \cdot 10^{-5}$-$1 \cdot 10^{-3}$M	$1 \cdot 10^{-7}$-$1 \cdot 10^{-5}$M

Table 6.1

Summary of precolumn and on-column complexation data for Pb(II) with PAR.

6.3.8 Sample analysis

With the aim of illustrating the applicabiltiy of this technique for Pb(II) analysis in aqueous systems, a pond water sample was spiked with a fixed concentration ($1 \cdot 10^{-6}$ *M*) of metal standard solution (Figure 6.12). The sensitivity of the method decreased somewhat when applied to the real sample as was seen previously in section 5.3.8. It is believed that this reduction in sensitivity is due to the addition of acid initially to release the organic matter present.

The acid has the effect of contributing more ions to the sample solution, and thus it has a higher ionic strength than nanopure water. This factor was confirmed by comparing samples containing (a) nanopure water plus Pb(II) ions and (b) nanopure water with Pb(II) and HNO_3. The latter showed slightly worse sensitivity than the former, though not significantly worse. This result suggests that this method is unsuitable for samples with a high ionic strength matrix [40].

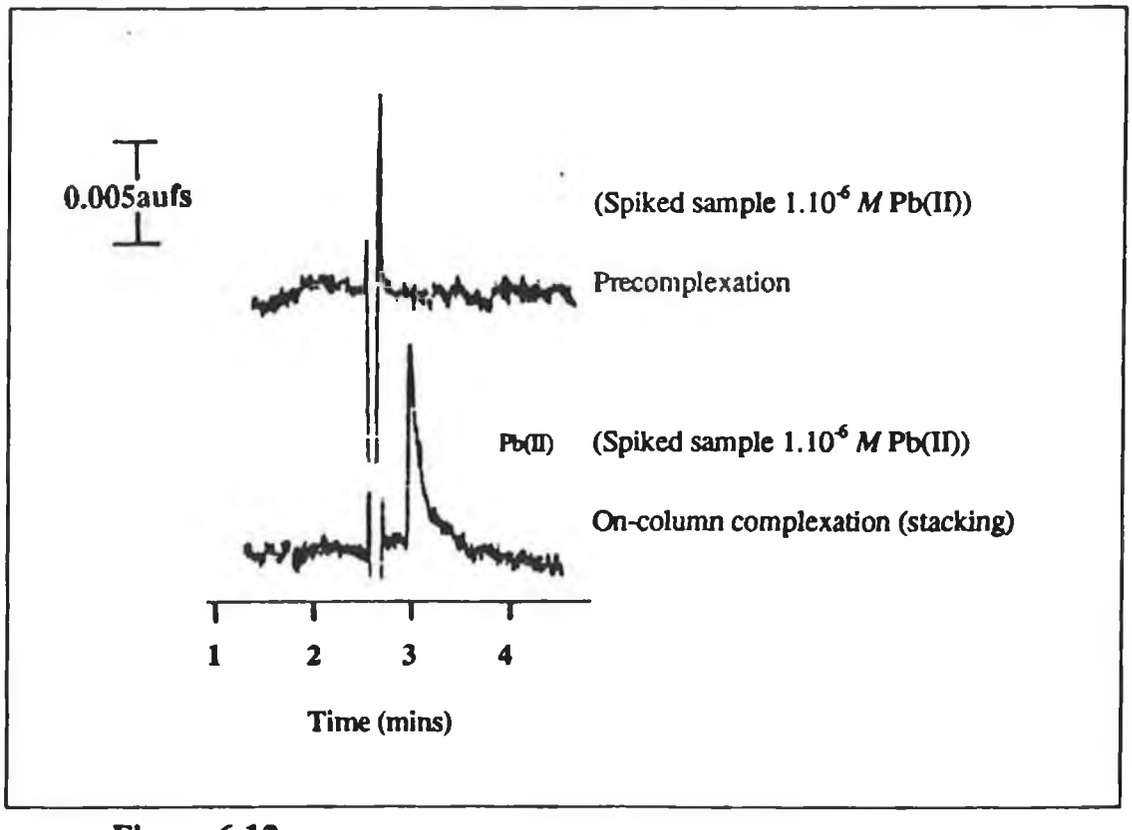

Figure 6.12
Analysis of Pb(II) in pond water under optimised peak stacking conditions.

6.4 CONCLUSIONS

The procedure described in this section is similar to that described in section 5.0. However the CE technique was optimised for Pb(II) because of the need for sensitive detection methods for this metal, due to the concern about its toxicity in the environment. By employing on-column sample stacking, sample pretreatment was minimized, and detection limits were enhanced by a factor of 100. Due to the lability of the lead/PAR chelate, careful selection of ligand concentration in both sample and buffer was required. The limits of detection obtained from this study are comparable to flow injection systems using large sample preconcentration volumes [27,30]. Such off-line preconcentration procedures are often slow, prone to contamination and probably subject to incomplete recoveries. However this system offers the advantage of on-line sample concentration which has the additional advantage of selectivity and applicability to on-line sample detection using visible spectrophotometry, which reduces the degree of sample handling. The latter is an important factor in method development.

Stacking can only be effective on samples of low ionic strength. The problem of using sample stacking on real samples lies in the effect of the ionic strength associated with the sample itself. Because peak stacking results from differences in sample and run buffer ionic strengths.

Capillary electrophoresis offers a simple, rapid, reproducible and sensitive technique for the analysis of lead in aqueous samples. It provides many advantages over previous methods, particularly the ease of application and selectivity when using the ligand. With developments in the area of detection pathlength, the limit of detection could be enhanced further, making this technique even more attractive to the analyst.

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Chapter 7

Comparison of ion-exchange post-column derivitization chromatography with capillary electrophoresis for speciation of Cr(III) and Cr(VI).

7.1 INTRODUCTION

7.1.1 Chromium speciation

Chromium exists in the environment predominantly in two oxidation states, Cr(III) and Cr(VI). Speciation of trace amounts of Cr(III) and Cr(VI) ions is very important in environmental, clinical and biological research because of the unique biochemical roles of chromium [1]. There is little conclusive evidence of the toxic effects of Cr(III). In most cases it is considered to be an essential element [1]. However, Cr(VI) has been reported as a carcinogen [2]. In order to achieve better understanding of chromium toxicity it is necessary to differentiate Cr(III) from Cr(VI) in environmental and biological samples. Traditionally, total chromium content has been determined using atomic absorption spectrometry (AAS) [3,4]. Some speciation work has been carried out using ion-exchange chromatography [5-10], solvent extraction [11] and electrochemical methods with two stage determination [12,13]. Adjusting the charges of both chromium species to the same charge status is sometimes necessary for them to be retained simultaneously on a chromatographic column [6,7,15,16].

The basic requirement for trace metal speciation is the ability to quantitatively determine each of the forms of the metal present independently and without interference from other forms. One way of doing this is by physically separating the species present, followed by their determination using an appropriate physicochemical measurement procedure [8,9,17]. A second way of speciating metals is by employing a physicochemical analysis procedure that converts all forms of the metal to a specific species, which is then measured [15,16].

Clearly the conversion of metal species from one form to another can have serious drawbacks, including incomplete conversion, introduction of contaminants, interference from other metals present, and generally a complex sample pretreatment procedure [15,16]. Thus, the preferred approach is one which physically separates the individual species present followed by direct quantitation.

With the development of HPLC and other chromatographic methods, a variety of separation modes, via chelate formation, have been employed to

separate various metal ions followed by colorimetric, spectroscopic or electrochemical detection of the separated species [8,11-13,15,19,20]. For metals such as chromium however which can exist as anionic (Cr(VI)) or cationic (Cr(III)), speciation presents a more challenging problem. Several approaches have been tried.

The application of HPLC to the analysis of metal ions has increased rapidly in the last decade [21]. It is a convenient instrumental technique for separating and determining metal ions. Among the techniques reported is the application of HPLC to the speciation of chromium. Usually, speciation of Cr(III) and Cr(VI) is achieved by one species being eluted with the solvent front and the other being eluted later [9]. Therefore, hyphenated techniques such as, HPLC-AAS, HPLC-ICP AES, HPLC-DCP etc were needed for speciation studies [21].

7.1.2 Chromium speciation using Ion-chromatography

7.1.2.1 *Ion-exchange techniques*

Chromium is particularly well suited to ion-exchange techniques, since chromate and chromic ions have opposite charges. This factor was exploited by Dionex [8] recently who developed a method of chromium speciation using an ion-exchange column which consisted of both anion and cation exchange sites. The procedure involved precomplexation of Cr(III) ions with the ligand, 2,6-pyridine dicarboxylic acid (PDCA), followed by separation of the complex anion and chromate anion, with subsequent post column derivitization of Cr(VI) and UV-visible detection at 520nm. By using a 250µl loop size detection limits of the order, 0.3ppb and 30ppb were achieved for hexa-and trivalent chromium respectively [8].

The use of a Dionex cation-exchange guard column for Cr(VI) and Cr(III) separation was reported by Gammelgaard et al. [7]. However in order to detect Cr(VI), this species was reduced to Cr(III) using potassium sulfite. This subsequently enabled chemiluminescence detection by the luminol-hydrogen peroxide system. Detection limits for both species were in the ppb range.

Williams et al.[6] used a similar reduction system for Cr(VI) to Cr(III) prior to detection by post-column reaction with luminol chemiluminescence.

Very high sensitivity was again obtained, with the limit of detection in the 0.1-0.3 μgdm^{-3} range.

The use of direct current plasma atomic emission spectrometry (DCP-AES), as an element selective method of detection for ion-chromatographic determination of Cr(III) and Cr(VI) species was described in a recent report [9]. This procedure involved the use of either anion or cation exchange columns for species separation and conversion of either species was unnecessary. A cation exchange column enabled separation of both species whereby the chromate anion eluted with the solvent front and the Cr(III) cation eluted later. An eluent containing HCl, which acted as a counter ion for Cr(III) elution and also for column regeneration was used. A similar approach was followed using the anion-exchange column, in which case the Cr(III) cation was expected to elute with the solvent front, but was actually retained on the column. However upon acidification of the sample Cr(III) was separated from the chromate anion. Results using either the anion or cation exchange columns were found to be identical, and detection limits in the low ppb range were reported using on-column preconcentration [9].

Saleh et al.[22] described a method encorporating ion-chromatography with conductivity detection for chromium speciation in aquatic samples. The method for Cr(III) determination involved the use of a low capacity cation exchange column and an eluent containing tartaric acid, ethylene diamine and acetonitrile at pH 2.9. A low capacity anion-exchange column was also used for Cr(III) determination as a complex anion with EDTA, and this column was also used for Cr(VI) determination without complexation. Detection limits using this technique were poor, approximately 0.5 $mgdm^{-3}$ for both species.

A rapid ion-exchange technique for separation and preconcentration of Cr(III) and Cr(VI) was reported recently by Johnson [10]. The procedure involves the use of anion and cation-exchange columns constructed from plastic syringes packed with the appropriate material. The aqueous samples were passed through the column before being eluted with a nitric acid solution. The eluate was then analysed for Cr(III) and Cr(VI) using graphite furnace AAS off-line. Detection limits using this technique are ~0.02 $\mu g\ dm^{-3}$ using a 200 cm^3 sample volume.

7.1.2.2 *Ion-pair chromatography*

Several authors reported the use of ion-pair chromatography for chromium speciation [17,23]. When quaternary ammonium salts are used as ion-pairing reagents, anionic forms of Cr(VI) are retained on the column. Use of sulphonates as ion-pairing reagents enables the retention of Cr(III) on the column. By using ion-pair chromatography, retention behaviour of the analytes can be controlled.

A high performance ion-pair chromatographic method for Cr(III) and Cr(VI) separation, using a phosphate buffer containing tetrabutylammonium bromide as the eluent was described by Trojanowicz et al. [17]. Detection was carried out on-line by encorporating post-column reaction detection using diphenylcarbazide (DPC). This reagent has been widely used in the past as a selective spectrophotometric reagent for Cr(VI) determination [24]. The detection procedure involved oxidation of Cr(III) to Cr(VI) using cerium (IV)sulphate , and simultaneous analysis of both species achieved detection limits of 7μgdm^{-3} (Cr(III)) and 13 μgdm^{-3} (Cr(VI)).

In a similar report using reversed-phase ion-pair chromatography for chromium speciation, Cr(III) was chelated to EDTA prior to analysis. A C8 column was utilised to separate the Cr(III) chelate from the Cr(VI) anion using an eluent containing acetonitrile and tetrabutyl ammonium ion. UV-spectrophotmetric detection at 242nm was employed, with detection limits in the region 0.4 ng and 1.6 ng (20 µl injection) for Cr(III) and Cr(VI) respectively [23].

7.1.3 Chromium speciation using chelation chromatoghraphy

In recent years there have been several reports on the use of chelating ligands for chromatographic separation of trace elements. In the separation and determination of chelated metal ions liquid chromatography has frequently been used. The most frequently used chelating agents are dithiocarbamates [12,18,20] and 8-hydroxyquinoline [19]. Simultaneous determination of Cr(III) and Cr(VI) using chelation chromatography has not been widely documented.

The use of sodium diethyldithiocarbamate for chelation with both Cr(III) and Cr(VI) was described by Tande et al. [18]. This ligand was found

to react with both species at room temperature to form neutral chelates, which were subsequently separated using a reversed-phase analytical column and detected using UV photometric detection at 254nm.

Use of a dithiocarbamate ligand was also demonstrated by Bond and Wallace [12] for chromium speciation. Electrochemical detection with platinum, gold and glassy carbon electrodes was employed, with the latter producing the best response. Detection limits in the nanogram per volume region were reported.

Eijarvi et al. [20] developed a method of simultaneous Cr(VI) and Cr(III) determination, by precomplexing with diethyldithiocarbamate or ammonium pyrollidine dithiocarbamate ligands. Solvent extraction was employed to extract the metal chelates with subsequent separation of the chelates on reversed-phase columns using a variety of eluent combinations. Complex formation of Cr(III) was found to be vey slow due to the hydrolysis of Cr(III) ions. Heating of the reaction mixture at 60 degrees for 20 minutes was found to produce a stable complex of Cr(III)APDC. Average detection limits of 40-95 pg, using a 5 µl injection volume were achieved and reproducibility was in the region of 1 and 5% RSD. Linear calibration curves over a wide calibration range, up to the 5ppm level, with a linear regression coefficient of 0.999 for complexes of both Cr(III) and Cr(VI) were reported. The pH was found to be a critical factor for extraction of Cr(DTC) chelates. pH values in the range 5.8-6.0 was selected, and below this range only Cr(III) was extracted, ie., at pH 4.5.

Isshiki et al.[15] reported the determination of Cr(VI) and Cr(III) in sea water by complexation with quinolin-8-ol. The Cr(VI) species was reduced to Cr(III) using hydroxylamine and subsequently collected on a macroporous resin. Detection was carried out using graphite furnace AAS off-line. Complex formation between the ligand and Cr(III) ions was achieved by heating the sample in a microwave oven for a short period. The pH was found to affect extraction of Cr(III) from the seawater samples, in that recovery of Cr(III) increased with increasing pH, with a maximum recovery occurring at pH 8.0. Recoveries were not reproducible when using the resin method at room temperature. It was found that by heating the Cr(III) ligand mixture in a microwave oven, followed by cooling, Cr(III) was quantitatively collected by the column extraction method, with good reproducibility. In the investigation the effects of coexisting organic materials in seawaters were assessed. From

the study it was found that organic materials were thought not to affect Cr(III) recovery.

7.1.4 Atomic absorption spectrometry (AAS)

Atomic absorption methods have been the most widely employed methods in chromium determinations. Following a number of preparative steps eg. solvent extraction [11], ion-exchange [5-10] and most frequently conversion from one oxidation state to another [15], atomic absorption has been the preferred detection method.

In the past this technique has been used in the determination of total chromium by Willie et al.[25] in which case no attempt at chromium speciation was made. Later determinations of chromium species using AAS involved the separation of Cr(VI) and Cr(III) species by coprecipitation with Fe(III) oxide, and separate determination of total dissolved chromium, by conversion to Cr(VI) [3], followed by extraction with ammonium pyrrolidinedithiocarbamate (APDC) into methyl isobutylketone (MIBK).

A method of simultaneous determination of Cr(VI) and Cr(III) by flame atomic absorption spectrometry involving a chelating ion-exchange flow injection system was developed by Milosacljevic et al.[5]. This procedure involved an approach encorporating a chelating ion-exchange column containing 8-quinolinol for the separation and preconcentration of tri and hexavalent chromium with on-line flow injection to the detector. The technique operated the principle of on-line retention of the Cr(III) cationic species, but Cr(VI) passes directly to the AAS detector. A valve system was operated such that following detection of Cr(VI), a nitric acid eluent passed through the chelating exchange column in order to elute the retained Cr(III) analyte and thus it is directed to the detector. Detection limits of 85 ng cm^{-3} and 16 ng cm^{-3} for Cr(VI) and Cr(III) respectively were obtained using a $1cm^3$ sample loop.

A solvent extraction method encorporating ammonium pyrrolidine-carbodithioate-methyl isobutyl ketone (APCD-MIBK) was utilized for chromium complexation prior to detection using graphite furnace atomic absorption spectrometry [11]. Procedures were developed for the selective determination of Cr(VI) and the simultaneous determination of [Cr(III) +

Cr(VI)] without the need to convert Cr(III) to Cr(VI). The value of Cr(III) was obtained by difference.

Sperling et al. [4] developed a chromium speciation technique which involved total chromium determination by oxidation of Cr(III) to Cr(VI) using potassium peroxydisulfate, in which case Cr(III) was calculated by difference. Determination of Cr(VI) was carried out by flow injection on-line sorbent extraction preconcentration coupled with AAS. Sodium diethyl dithiocarbamate was used as the complexing agent and a C18 bonded silica reversed-phase sorbent as the column material. Detection limits were of the order of 16-18 ng dm^{-3} using 40 µl samples.

Recent investigations of chromium speciation using high performance flow flame atomic absorption spectrometry were described by Posta et al. [26]. Detection limits of 0.03 mg dm^{-3} Cr(III) and 0.02 Cr(VI) $mgdm^{-3}$ without preconcentration were reported, however, when a preconcentration step was encorporated using a $5cm^3$ loop, a detection limit of 0.5 μgdm^{-3} was obtained for Cr(VI). Separation of Cr(VI) and Cr(III) was effected by ion-pair chromatography on a C18 column using tetrabutyl ammonium acetate as the ion-pairing reagent. Following separation, on-line detection was carried out by high pressure nebulization into the flame AAS. This speciation technique was applied to Cr(VI) and Cr(III) speciation in samples such as drinking water, waste water and soil.

One of the sources of occupational exposure to chromium is that of the welding process. A speciation procedure for the determination of chromium in welding fumes was reported by Naranjit and co-workers [27]. The technique involved separation of Cr(III) and Cr(VI) in aqueous extracts of welding fumes by using anion- and cation-exchange resins, with detection using atomic absorption spectrometry.

7.1.5 Spectrophotometric Methods for Chromium Determination

The use of diphenylcarbazide as a complexation reagent has been widely documented for the determination of Cr(VI) due to its specificity for this species. A number of applications for Cr(III) and Cr(VI) speciation have involved the oxidation of the trivalent species to Cr(VI) with subsequent reaction using the DPC reagent for spectrophotometric detection [17,24].

The on-line oxidation of Cr(III) by Ce(IV), to allow determination of total chromium, from which Cr(III) was obtained by subtraction has been reported [16]. Cr(VI) could be determined in the presence of Cr(III) as the latter species does not interfere in the Cr(VI)-DPC reaction. A flow injection analysis system enabled rapid sequential determination of these species, with detection limits in the region of 18 ng cm^{-3} for Cr(VI) and 55 ng cm^{-3} for Cr(III).

A type of multi component spectrophotometric method of chromium speciation has been developed by Fang et al.[28]. Sequential analysis of chromium(VI) and Cr(III) involved the determination of the total amount of chromium upon reaction with 2-(5-bromo-2-pyridylazo)-5-diethylamino phenol after the determination of Cr(VI) with diphenylcarbazide. This technique was applied to determination of chromium species in aquatic samples.

A spectrophotometric determination of Cr(III) and Cr(VI) by use of a Cr(VI), Cr(III), chrome azurols-cetylpyridinium bromide-hydroxylamine hydrochloride-zinc (II) system was reported recently [29]. This study illustrated the effect of zinc addition to the reaction whereby the absorbance wavelength maximum was shifted, thus achieving greater sensitivity than in its absence. However this method only determines total chromium under the conditions used, as the Cr(VI) is reduced to Cr(III) by the hydroxylamine in the solution.

7.1.6 Additional detection methods in chromium speciation studies

Sequential determination of Cr(VI) and Cr(III) was described by Cox and co-workers [14] using flow injection analysis-inductively coupled plasma, atomic emission spectrometry (FIA-ICP AES). A micro-column of activated alumina was used in the FIA manifold to separate and preconcentrate Cr(VI) from Cr(III) in synthetic aqueous solutions prior to ICP detection at 267.72 nm. Using a 2 ml sample volume detection limits of 1.4 and 0.2 μg dm^{-3} were achieved for Cr(III) and Cr(VI) respectively.

Neutron activation analysis was demonstrated as a detection method in the determination of chromium species by Lan et al.[31]. A two step method was employed based on determination of each species separately due to

different coprecipitation yields with $Pb(PDC)_2$ at different pH values. Firstly Cr(VI) was coprecipitated at pH 4.0 and then Cr(III) was precipitated at pH 9.0. Total chromium was determined by reduction of Cr(VI) followed by coprecipitation at pH 9.0.

X-ray fluorescence spectrometry has been utilized by a number of groups for Cr(VI) and Cr(III) detection. Leyden et al.[32] reported separation of chromium species on a chelating ion-exchange resin with subsequent x-ray flourescence detection. The quantity of Cr(VI) was determined by subtraction of the Cr(III) value from the total chromium. Determination of Cr(III) was carried out using a Chelex-100 ion-exchange resin, which selectively retained the cationic Cr(III) but anionic Cr(VI) was unretained. Sodium bisulphite was utilized for the reduction process. Ahern and coworkers [33] also utilized X-ray fluorescence spectrometry to the determination of chromium species in seawater. Dissolved Cr(III) and (VI) were coprecipitated separately from sea water, and chromium in the precipitates and particulate matter was then determined by thin film X-ray fluorescence. Arber et al. [34] also applied this detection mode to Cr(III) and Cr(VI) speciation in occupational hygiene samples.

7.1.7 The Capillary Electrophoresis Challenge

A wide variety of analytical methods have been employed in the area of chromium speciation. Many methods have involved conversion of one species to another with subsequent off-line element specific (eg. AAS) detection [3,4], a step which is generally undesirable in trace analysis. Further investigations have involved the use of both cation and anion exchange columns for separation of the species again followed by element specific detection, without the need for conversion of the species [5]. Chromium speciation by chelation chromatography has also seen widespread use, with dithiocarbamate ligands being the most commonly employed ligands [12,18,20], however complex formation of the Cr(III) has been shown to be slow [20], often requiring a heating step.

The most frequently used method of analysis for the determination of Cr(III) and Cr(VI) is ion chromatography. The vast majority of ion-chromatographic applications involve simultaneous determination of more than one analyte [35,36]. Increasingly it is claimed however that ion

chromatography has finally met its match in capillary zone electrophoresis [37]. From the literature it has been shown that separation efficiency achieved by CE far exceeds that of ion-chromatography [38]. The attractive feature of CE is the possibility of separation of cations neutrals and anions in a single run, thus eliminating the need for conversion of species.

However, detection limits are less attractive than those obtained with ion-chromatography and CE does not lend itself well to the variety of preconcentration techniques available to IC. These hurdles may not be impossible to overcome. A greater challenge for many real samples concerns analytical reproducibility in routine use. Regardless of the fact that separation selectivities in electrophoresis and ion exchange are quite different, IC would always remain in some form. IC is hardly threatened at the moment, however, continued developments in CE for ion analysis is confirming its applicability [38,39], and CE offers a number of attractions when compared with IC.

7.1.8 Aims and objectives

The aim of this investigation is to compare the performance of an ion-chromatographic method of chromium speciation with that of a capillary electrophoretic procedure.

The objectives of the project were as follows:

*optimisation and adaptation of an ion-chromatographic technique already developed by Dionex;

*development of a capillary electrophoretic technique for chromium speciation;

*comparison of LOD, reproducibility, efficiency and general performance of ion chromatofraphy and capillary electrophoresis.

7.2 EXPERIMENTAL

7.2.1 Ion-chromatography

7.2.1.1 *Apparatus*

The chromatographic equipment consisted of a Dionex 4500 ion chromatograph, equipped with an eluent resevoir and eluent degas module. A microinjection valve was used, which consisted of a 50µl fixed loop (Rheodyne) 4000psi injection valve. Loading of the sample was effected via a removable needle, guide fitted into the valve body, which is air actuated via valve 5 on the pump module.

The ion-chromatographic system consisted of a guard column (HPIC-CG5) [8] and separator (HPIC-CS5) column followed by a post-column derivitization system. The latter consisted of a waters 501 HPLC pump connected to the column via a mixing tee, and subsequently the eluents were mixed further in a small column (containing glass beads) mounted on-line (Figure 7.1). Detection was carried out using a Schimadzu SPD-6A UV-visible variable wavelength detector. Cr(III) precomplexation was stabilized rapidly with the aid of a microwave.

7.2.1.2 *Reagents*

All of the chemicals used were of analytical reagent grade. Liquid chromatography quality water was obtained by purifying water in a milli-Q filtration system (Millipore, Bedford, MA, USA.). 1,5-diphenylcarbazide (DPC), 2,6-pyridine dicarboxylic acid (PDCA), Na_2HPO_4, NaI, $CH_3CO_2NH_4$ and LiOH (Aldrich Chemicals) reagents were used without purification.

Spectrophotometric grade (96%) sulphuric acid was used in the preparation of the post column reagent. The tri- and hexavalent chromium standards were prepared from the solid salts, $Cr(NO_3)_3 \cdot 9H_2O$ and K_2CrO_4 respectively.

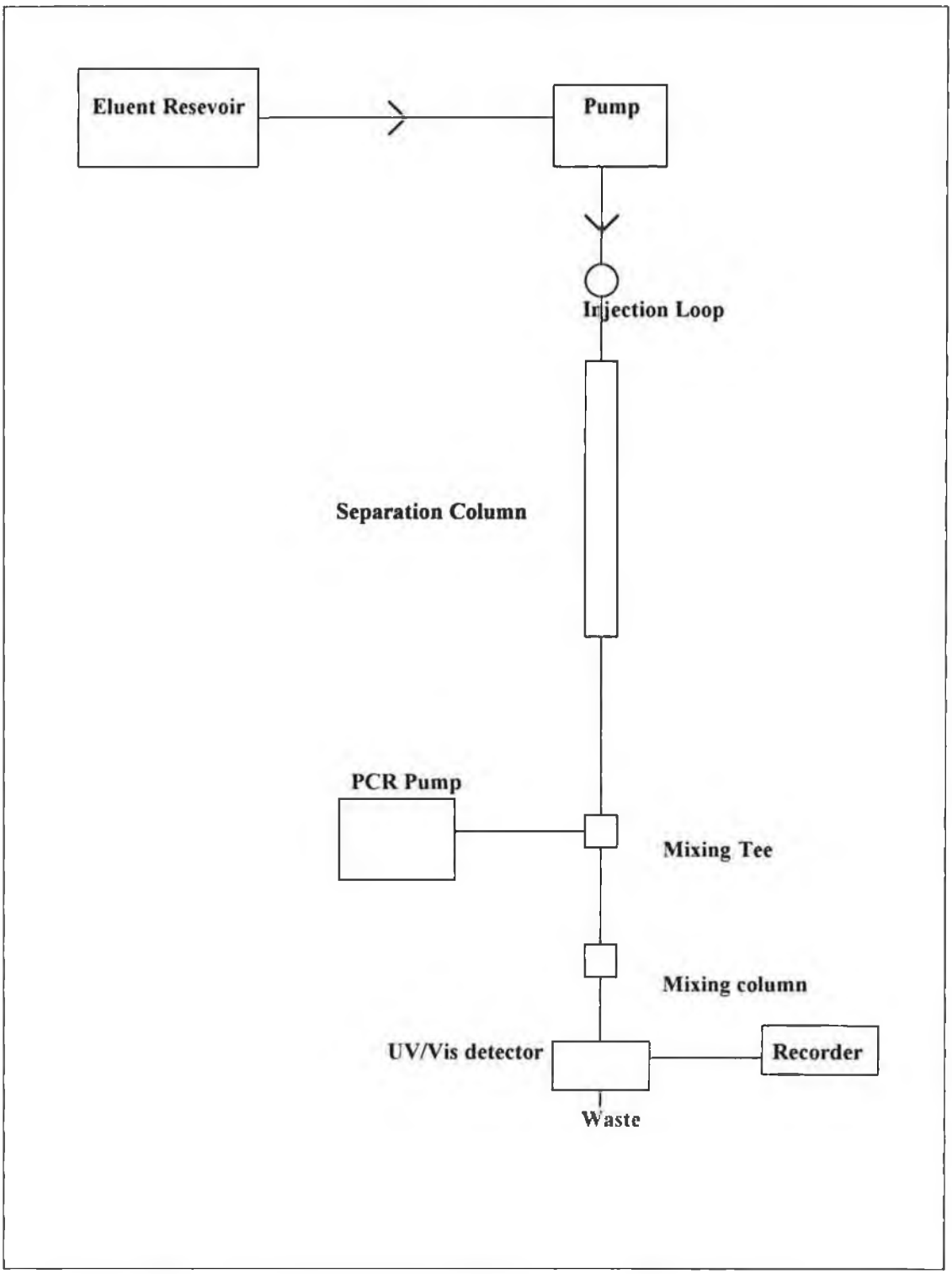


Figure 7.1.

Schematic diagram of the ion-chromatographic system for chromium speciation.

7.2.1.3 *Procedure*

7.2.1.3.1 *Standard preparation*

Cr(VI) standards were prepared by dissolving an appropriate quantity of K_2CrO_4 in milli-Q water. Cr(III) was separated as the $Cr(PDCA)^-_2$complex, and thus required complexation prior to injection. Because of the slow kinetics of complex formation for Cr(III), a precolumn derivatization with PDCA was used to form the Cr(III)-PDCA complex. A microwave was used to rapidly heat the solutions thus aiding rapid complexation. Cr(III) complexes were prepared by addition of the PDCA mobile phase solution, adjusted to pH 6.8 using 0.1*M* NaOH, (containing 5m*M* PDCA) to appropriate volumes of the Cr(III) stock solution.

7.2.1.3.2 *Post-column reagent (PCR) preparation*

The post column reagent (DPC) was stored in the dark as it was found to degrade when exposed to light. Preparation of the PCR involved initial dissolution of the DPC reagent in methanol with subsequent slow addition of the acid (H_2SO_4). Early addition of the acid causes degradation of the DPC reagent.

7.2.1.3.3 *Chromatographic procedure*

The ion-chromatographic procedure involved injection of the Cr(III)-PDCA complex, and direct injection of Cr(VI) as the CrO_4^{2-} anion, using a 50µl loop size, with the ions mixed in a mixing tee with the PCR. Cr(VI) rapidly forms a coloured complex and the trivalent form does not complex with DPC. Both solutions were detected spectrophotometrically at 540nm.

7.2.1.3.4 *Simultaneous determination of Cr(VI) and Cr(III)*

A mixed sample standard containing tri-and hexavalent chromium was prepared by the addition of the PDCA eluent to a solution containing appropriate volumes of each species. The solution was then heated for 60 seconds using a microwave, to allow complete complexation of Cr(III) and PDCA. Cr(VI) does not complex with this reagent. The mixture was then injected and separated, following which the eluent was mixed with 1,5-diphenylcarbazide reagent post-column, which formed a coloured complex with the hexavalent species. The wavelength maximum for both species is in

close proximity (Cr(VI) @540nm and Cr(III) @ 541nm) and therefore a wavelength average of 540nm was chosen for simultaneous speciation.

7.2.2 Capillary Electrophoresis (CE).

7.2.2.1 *Apparatus.*

The CE system used here is identical to that described previously in section 5.2. and shown in Figure 5.1. The columns used were 75 μm i.d.fused silica capillary columns (Polymicro Technologies) 95 cm in total length, and 85cm to the detector.

7.2.2.2 *Reagents*

Electrophoretic buffers of phosphate, borate and acetate (Sigma, St. Louis, MO) were used throughout the study, with pH adjustment using sodium hydroxide and acetic acid.

7.2.2.3 *CE procedure*

7.2.2.3.1 *Standard preparation*

Preparation of mixed Cr(III) and Cr(VI) standards involved addition of the appropriate quantity of both stocks to a solution containing 5m*M* PDCA. This solution was heated using a microwave to aid Cr(III) complexation. The solution was then allowed to cool prior to addition of 0.2m*M* DPC reagent. Cr(VI) DPC complexation occurred immediately. Optimum concentrations of ligands were found from previous HPLC determinations.

7.2.2.3.2. *Simultaneous chromium determinations using CE.*

Following Cr(VI) complex formation the solutions were injected immediately, using electrokinetic injection at 5kV for 10 seconds. The electrophoretic buffer solution of 20mM acetate pH 4.0 was applied at 20 kV to effect separation. Detection was carried out on-line at 540nm.

7.3. RESULTS AND DISCUSSION

7.3.1 Ion-chromatography

7.3.1.1 *Mobile phase conditions*

The HPIC-CS5 separator column is a general purpose transition metal column. The column has both anion and cation exchange capacity. These columns are designed for rapid and sensitive determination of transition metals in a wide variety of matrices [8]. Transition and heavy metals have a high affinity for the cation exchange resin of the separator column, but many have very similar affinities for the ion exchange resin, making separation difficult. Eluents which contain metal complexing acids, such as PDCA, can be used to enhance separation [40]. Inclusion of these acids in the eluents results in the formation of anionic metal complexes, which can compete with the organic acid anion for the exchange site of the resin.

Separation of the metals depends on the relative affinity of their respective anionic complexes for the resin [40]. Separation of Cr(VI) and Cr(III) was accomplished using an eluent containing 2m*M* PDCA which was found to give optimum separation. The effect of this reagent on Cr(VI), is shown in Figure 7.2. As the concentration of PDCA increases in the eluent, above 2m*M* the peak height decreases slightly but the effect levels off at ~ 4mM PDCA. Below 2m*M* peaks are very small. The effect of this ligand on Cr(III) is such that optimum peak height is observed using 3m*M* PDCA, however, peak shape is most satisfactory using a 2m*M* concentration. Thus taking both species into account a 2m*M* PDCA eluent was selected.

Figure 7.3 illustrates the effect of PDCA concentration in the chromium standard solutions on the chromatography. From the plot of PDCA concentration versus peak height for Cr(III) it is evident that 5mM ligand is necessary to obtain an optimum response. Above this concentration no change is observed, but below 5mM the ligand becomes limiting, resulting in a reduced response. This factor however depends on the working range of standards under observation. In this case 5mM ligand was used in all standard solutions in the range 10ppb-1ppm (~10^{-6} *M*-10^{-4} *M*). In the lower region ie. ppb, such a concentration of ligand is unnecessary. However it was found that at least a 10 fold excess of ligand is required to give optimum complexation

and response. This limitation was previously reported by Ryan et al.[41] for metal ion determination using 8-hydroxyquinoline. Above 10^{-4} *M* Cr(III) a higher concentration of ligand is required.

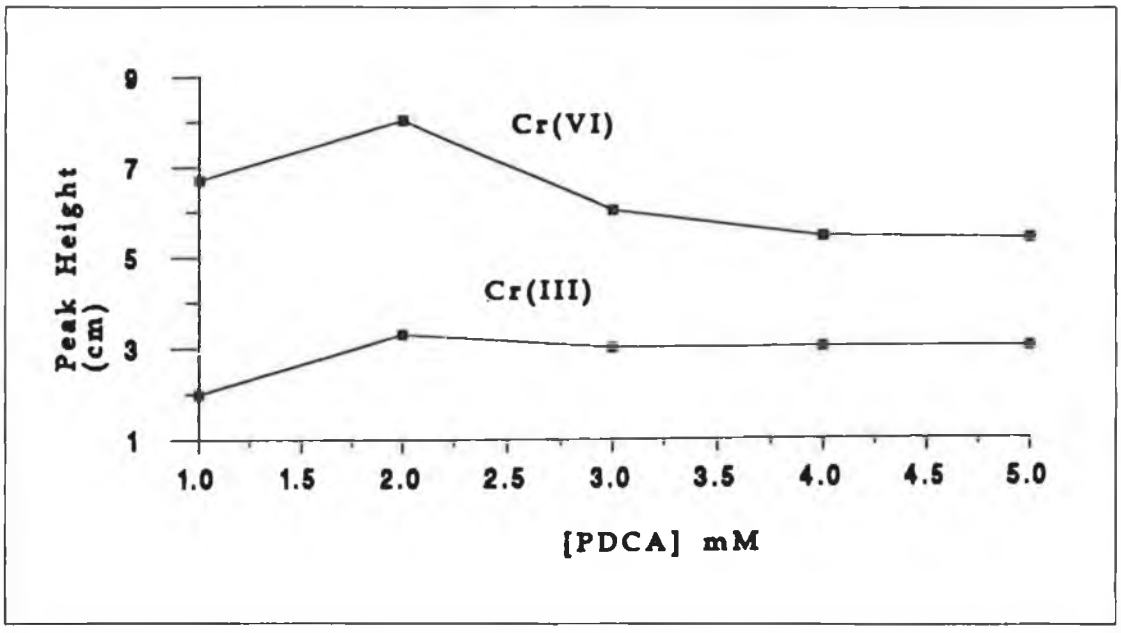


Figure 7.2

Effect of eluent PDCA concentration.
2mM Na_2HPO_4, 10mM NaI, 50mM $CH_3CO_2NH_4$ and 2.8 mM LiOH. [Cr(VI)]=0.01ppm & [Cr(III)]=0.01ppm

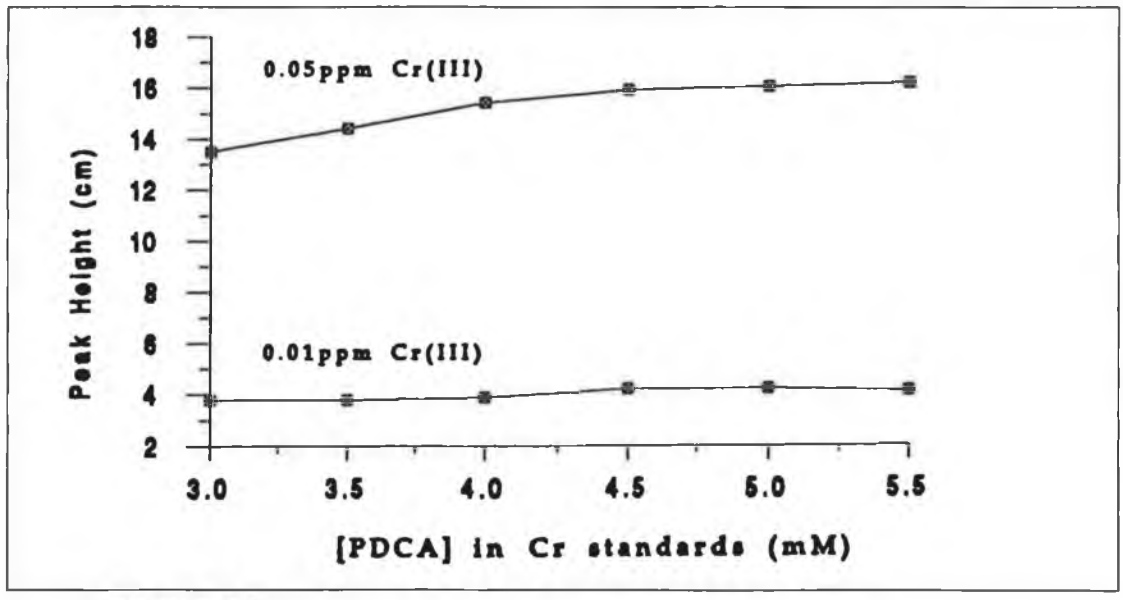


Figure 7.3

Effect of [PDCA] in standards on Cr(III). [Cr(III)]=0.01ppm.

7.3.1.2 *Mobile phase counterions*

The eluent used in the ion-chromatographic separation was adapted from that used by Dionex in a similar separation [8]. However, due to the poor separation of both species, individual components in the mobile phase were varied and the effect noted. Variation of LiOH concentration, $CH_3CO_2NH_4$, and Na_2HPO_4 showed little effect on separation, ie., the resolution of the two species did not change. The sodium iodide component was found to have the greatest effect on Cr(III) peak shape. At 10m*M* NaI, peak width and retention times of Cr(III) was large, resulting in very poor resolution of the peaks. By increasing the concentration of NaI, the resolution improved, but beyond 20m*M* no further improvement was noted, thus 20m*M* was selected for the eluent (Figure 7.4).

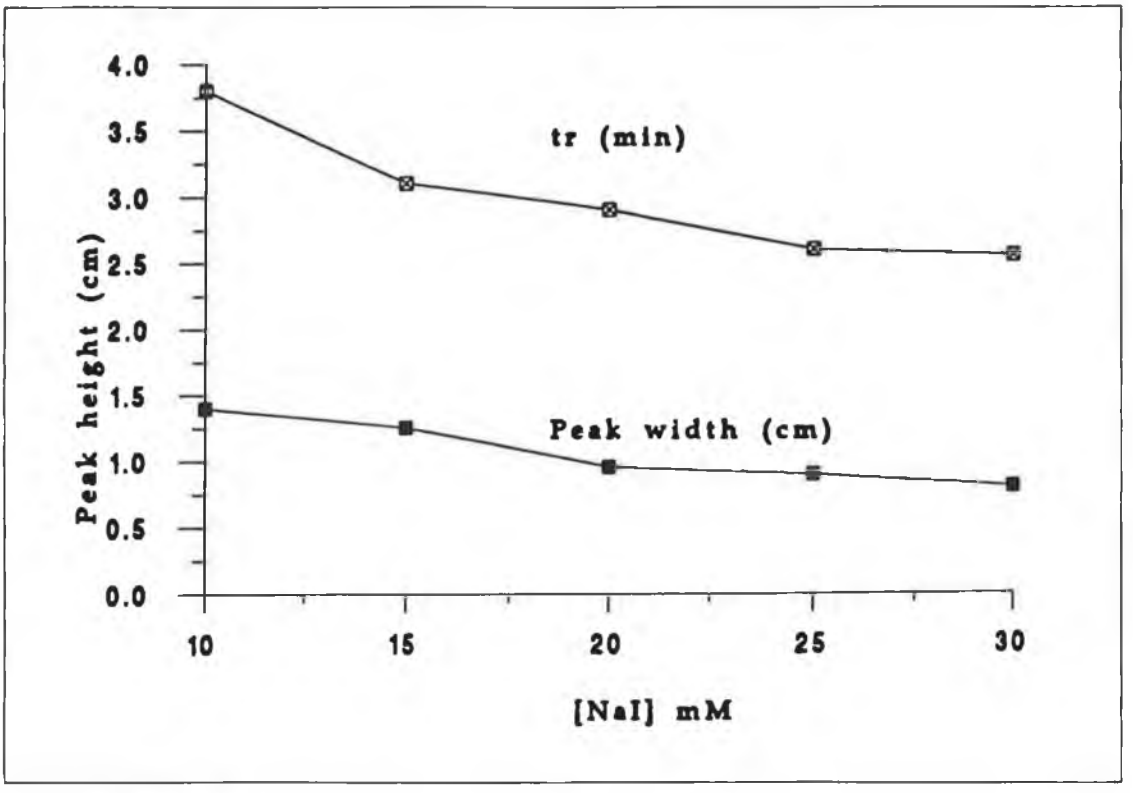


Figure 7.4

Effect of eluent NaI concentration on Cr(III) peak width and retention times. Eluent conditions. 5mM PDCA, 50 mM $CH_3CO_2NH_4$,2mM Na_2HPO_4 and 2.8 mM LiOH. [Cr]=0.01 ppm.

Investigation of the optimum eluent conditions showed that the concentrations of components in the eluent suggested by Dionex were optimum also for our system. However a slight variation in the concentration of PDCA in the standards was employed to optimise complex formation, in addition to microwave heating which enabled shorter analysis times. The optimum conditions selected for ion chromatographic separation of Cr(VI) and Cr(III) are shown in Table 7.1 and Figure 7.5 shows a typical chromatogram of Cr(VI) and Cr(III) separated under these conditions. It was found that reduction in the efficiency of the method resulted from a degraded post column reagent solution. It was essential that the DPC reagent be prepared correctly and stored free of light, in order to obtain maximum detection sensitivity.

Guard Column	**HPIC-CG3(Dionex)**
Separator Column	**HPIC-CS5(Dionex)**
Mobile phase [PDCA]	**2 m*M***
Eluent flow rate	**1 cm^3 min^{-1}**
Eluent pH	**pH 6.8**
[PCR]	**0.2 m*M***
PCR flow rate	**1 cm^3 min^{-1}**

Table 7.1

Chromatographic conditions

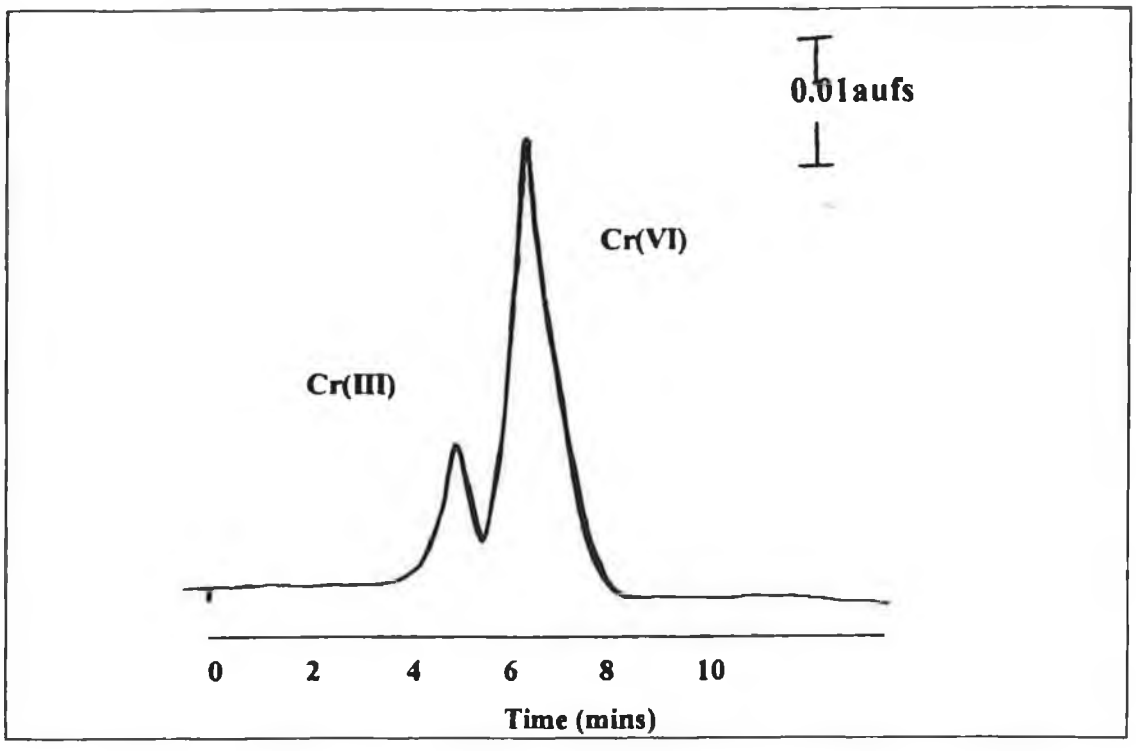


Figure 7.5

Chromatogram of Cr speciation using ion-exchange chromatography.

Eluent conditions, 5mM PDCA, 20 mM NaI, 2.8 mM LiOH, 2mM Na_2HPO_4 and 50 mM $CH_3CO_2NH_4$. [Cr(VI)] = 0.1ppm, [Cr(III)] = 0.5ppm

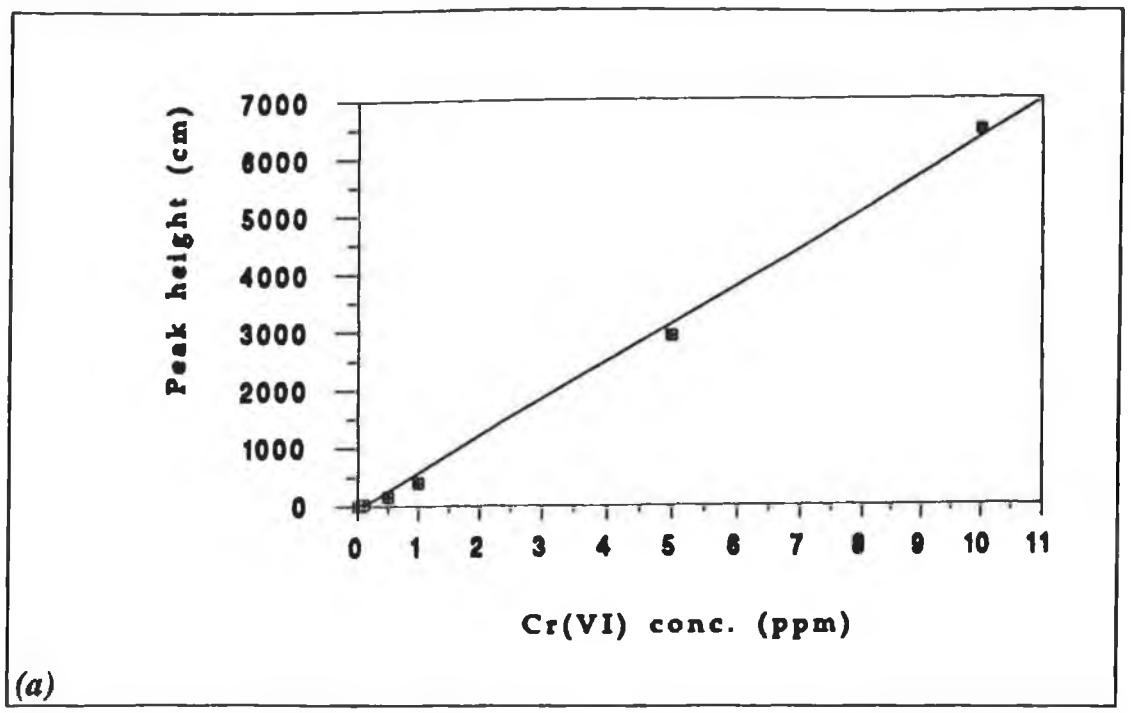
7.3.1.3 *Limits of Detection for Cr(VI) and Cr(III) Using Ion-Chromatography.*

The criteria for limit of detection used in these studies was a signal (in peak height) to background noise ratio of 3:1. Table 7.2 sumarises the data obtained. Calibration plots for both species were linear over 2 orders of magnitude, with regression values of 0.9980 and 0.9970 for Cr(III) and Cr(VI) respectively (figure 7.6 a & b). Using a 50µl loop size limits of detection of 0.5ppb (Cr(VI)) and 10ppb (Cr(III)) were obtained. In the study reported by Dionex [8], a loop size of 250µm was utilised, and detection limits of 0.3ppb and 30ppb, for hexa- and trivalent chromium respectively, were noted. Thus by incorporating such a loop size increase in this procedure a further improvement in LOD could be achieved. Six repeated injections of chromium standards demonstrated reproducibility values of 1.5% CV for Cr(VI) and 3.3% CV for Cr(III).

	Cr(VI)	Cr(III)
LOD	0.5ppb	10ppb
Linear Dynamic Range	0.5ppb-10ppm	10ppb-10ppm
Reproducibility (%CV)	1.5	3.3
Regression	0.99702	0.99805
n	6	6

Table 7.2

Limits of detection and reproducibility for the ion-chromatographic separation of Cr(III) and Cr(VI)



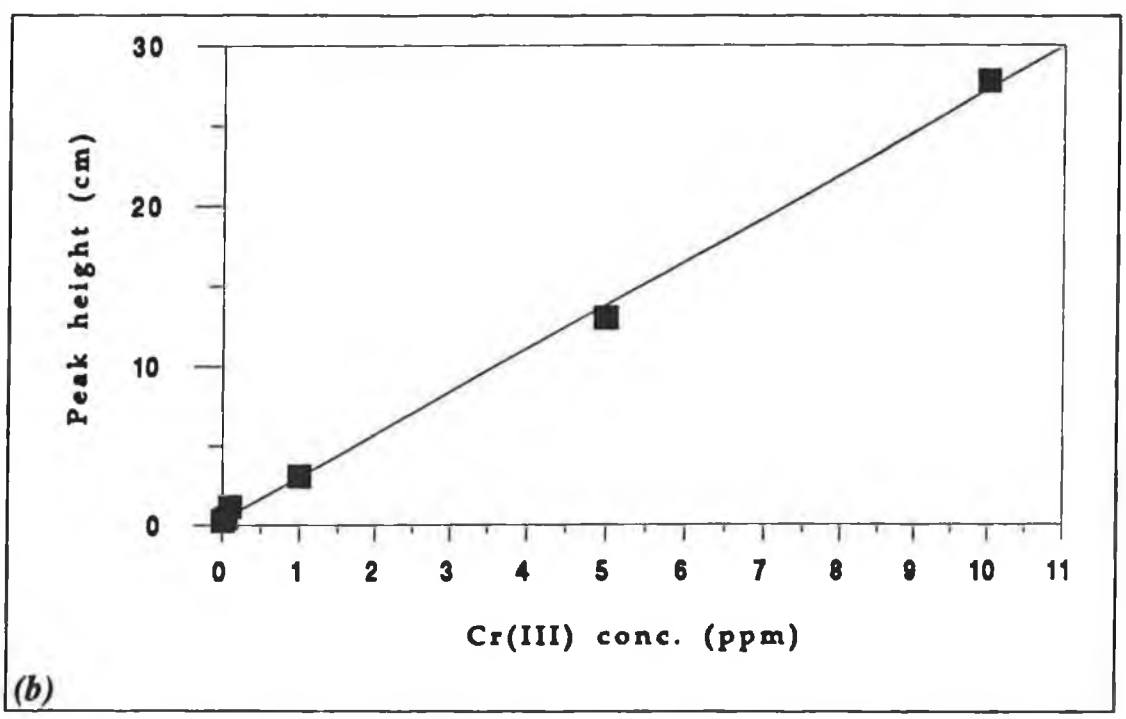


Figure 7.6

Calibration curves for chromium determinations. (a) Cr(VI) and (b) Cr(III). Conditions as in Table 7.1

7.3.2 Separation of Cr(VI) and Cr(III) using capillary electrophoresis

7.3.2.1 *Complex formation and stability*

Initially the study involved development of a procedure of complexing both species simultaneously with the appropriate ligands prior to their subsequent injection onto the capillary. As the CE system was not equipped for post column derivitization, it was necessary to precomplex the chromate anion also prior to analysis by CE.

By initial heating of the solution containing Cr(VI), Cr(III) and PDCA it was possible to effect Cr(III) complexation, and subsequently, following adequate cooling, the diphenylcarbazide reagent was added to allow complexation of the chromate species. Heating of the solution in a microwave enables rapid complexation of Cr(III), and does not have an adverse effect on Cr(VI). However it was found that the solution must be completely cooled, otherwise Cr(VI) complexation will not occur due to degradation of the DPC reagent. Figure 7.7 demonstrates the stability of Cr(VI) diphenylcarbazide complex over time. Although no significant decrease in response occurs, optimum absorbance was observed at 35 minutes following addition of the reagent to Cr(VI), and a small drop off in absorbance was observed thereafter.

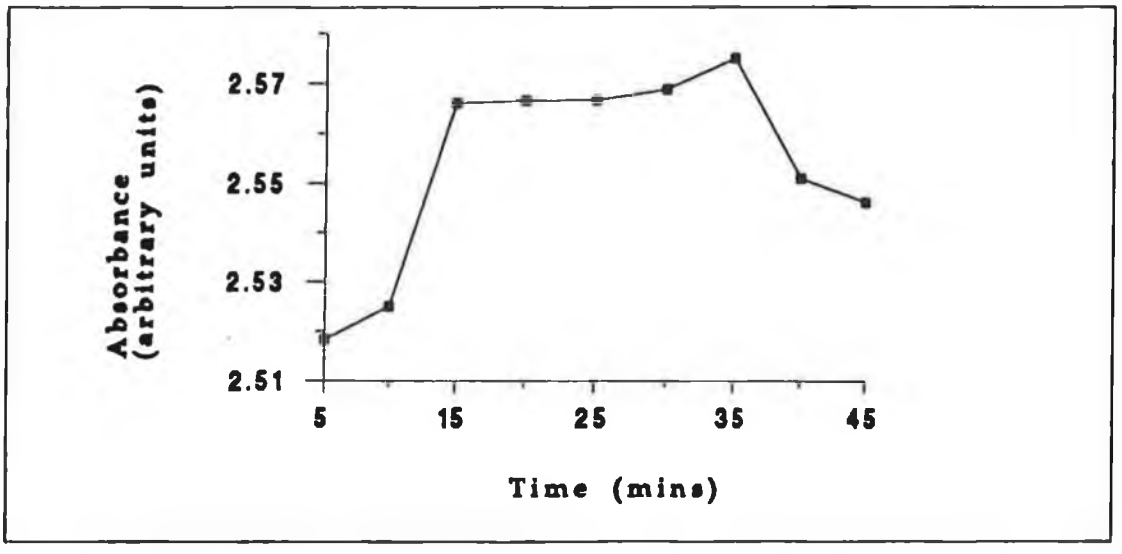


Figure 7.7
Stability of Cr(VI) DPC complex. [Cr(VI)] = 0.01mM, [DPC] = 0.2mM

7.3.2.2 *Electrophoretic buffer composition*

A number of buffers were investigated to assess their effect on chromium separation, notably borate pH 9.0, phosphate pH 6.8 and acetate pH 4.0. All buffers achieved adequate separation of both species, however a more satisfactory peak symmetry was obtained using acetate pH 4.0. At alkaline pH, the efficiency of Cr(VI) was poor, in addition two peaks for chromium DPC occurred. On lowering the pH the migration order of these peaks began to shift. The peak relating to the chromate species varied linearly with concentration, and the other peak maintained the same height over the entire concentration range. Figures 7.8 a-d illustrate the effect of different buffers on Cr(VI) analysis. The poor efficiency noted with alkaline pH values may be attributed to the considerable current value associated with the high conductivity of phosphate and borate buffers. These high currents (~ 40-60μA) have the effect of causing joule heating on the capillary [42], which in turn may be causing breakdown of the Cr-DPC complex on-capillary, resulting in the two peaks. When using low pH buffer, ie., acetate, currents are almost halved, but are still significant (~27μA).

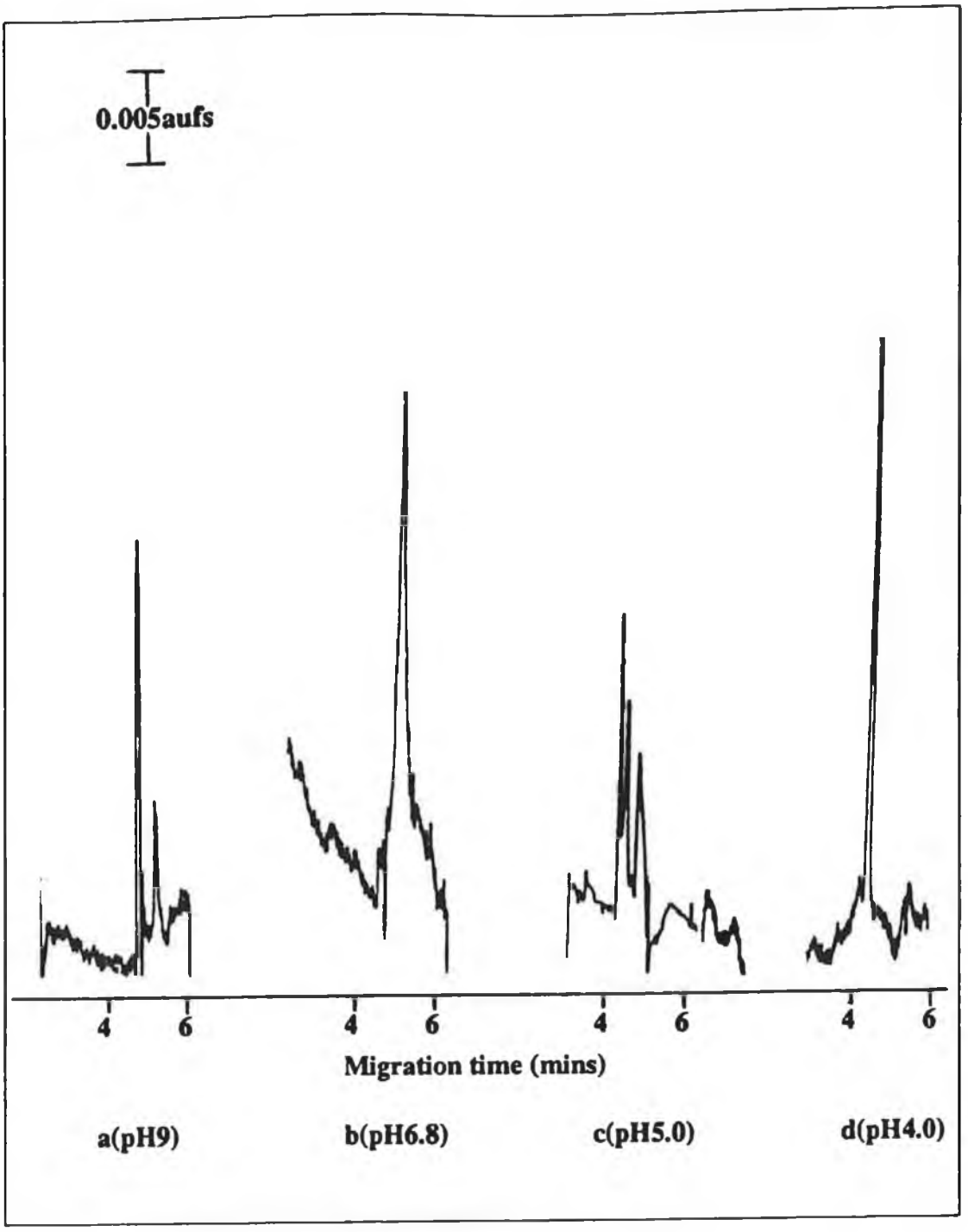


Figure 7.8

Effect of Buffer type and pH on CE analysis of Cr(VI)-DPC. [Cr(VI)]=0.05 mM; [DPC]=0.2 mM

7.3.2.3 *Chromium speciation using CE*

7.3.2.3.1 *On-capillary complexation*

Attempts were made to effect on-capillary complexation of Cr(VI) as it rapidly forms complexes with diphenylcarbazide reagent [24]. The procedure used involved electrokinetic injection of ligand for 10 seconds at 5 kV, followed by injection of metal ion under similar injection conditions. No migration of the ligand occurs until a high voltage is applied. Figure 7.9 illustrates clearly that on-line complexation is unsuccessful. The disadvantage of such a technique is the inability to load a large concentration of chromate anions due to their negative charge, and their subsequent affinity for the positive electrode. Even if a higher injection voltage is used, injection of these negative ions is still unsuccessful, thus the sensitivity of this method is unsatisfactory (ie.,~ 10^{-4} *M* LOD Cr(VI)).

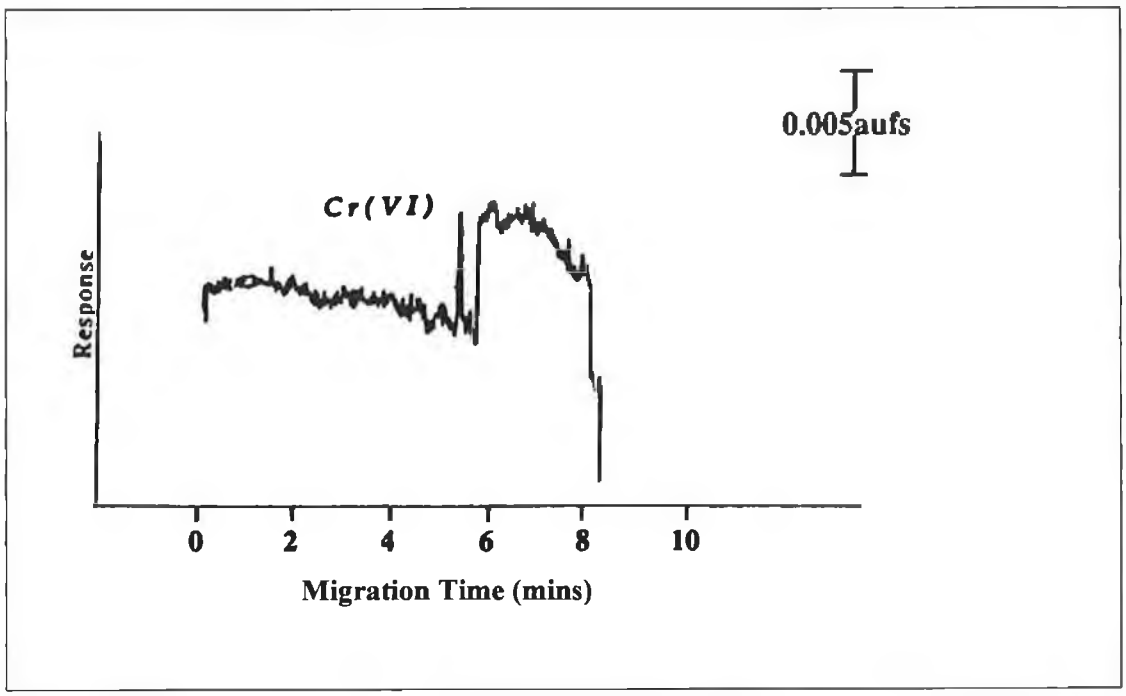


Figure 7.9
On-line complexation of Cr(VI) with 1,5-diphenylcarbazide. [DPC]=0.2mM [Cr(VI)]=0.1mM. Electrophoretic buffer; 20 mM acetate pH 4.0.

7.3.2.3.2 Off-line complexation

Complexation of both species was carried out off-line by initial heating of the solution containing both chromium species with 5mM PDCA, which resulted in a coloured complex with a wavelength maximum of 541nm corresponding to the $Cr(PDCA)_2^-$ complex. Following cooling of this solution, diphenylcarbazide reagent was added to effect Cr(VI) complexation. As neither ligand complexed with the other species in solution, there was no interfering effect from the ligands in solution. In addition as the ligands themselves do not absorb in the 540nm range, free uncomplexed ligand does not interfere with the separation. From the electropherogram shown in Figure 7.10. it can be seen that a peak migrates between Cr(VI) and Cr(III), however this does not interfere with the two species of interest. Complete resolution of the species is obtained. The optimum CE conditions for separation of Cr(III) and Cr(VI) are summarised in Table 7.3.

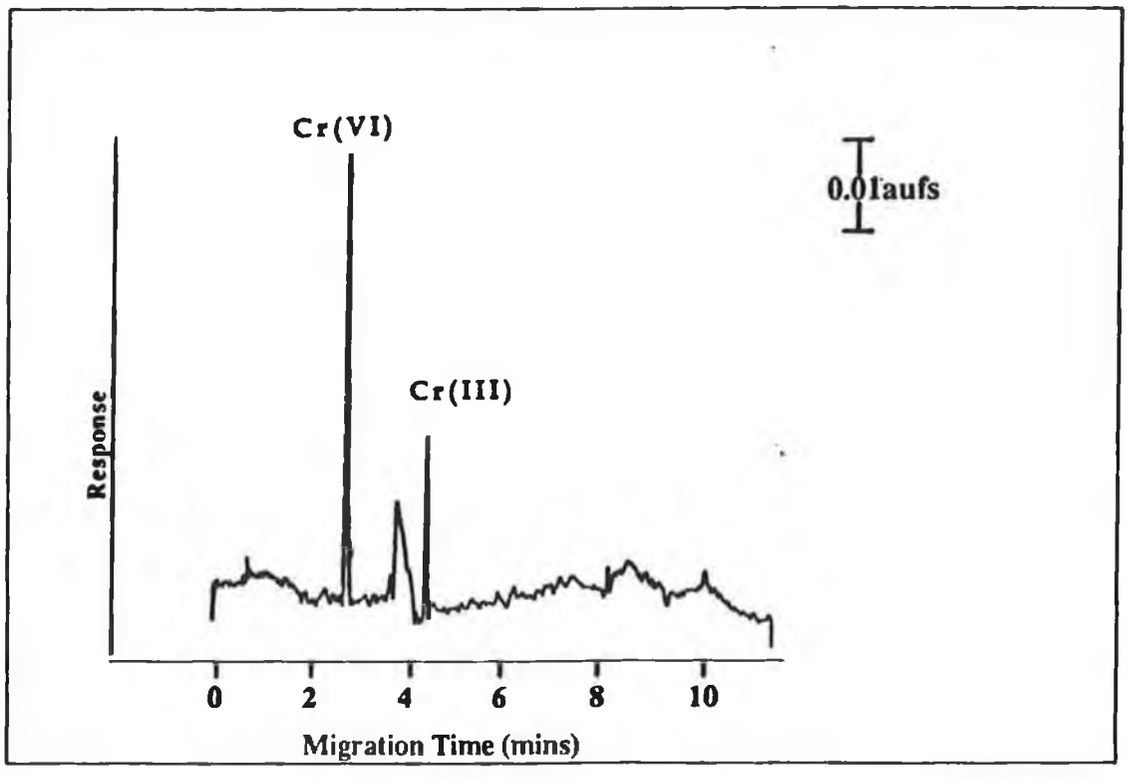


Figure 7.10
CE separation of Cr(VI) and Cr(III) using precomplexation. [PDCA]=5mM; [DPC]=0.2mM; Electrophoretic buffer = 20 mM actetate, pH 4.0;Injection voltage 10s @ 5kV; Separation voltage 20 kV.[Cr(VI)]=0.01mM,[Cr(III)]=0.1mM

Electrophoretic buffer	**20 m*M* Acetate**
Buffer pH	**4.0**
Injection voltage	**5 kV**
Injection time	**10 sec.**
Separation voltage	**20 kV**
Total capillary length	**95cm**
Effective capillary length.	**85cm**
Wavelength average.	**540nm.**

Table 7.3

Optimum separation conditions for capillary electrophoresis

7.3.2.4 *Effect of sample ionic strength*

Due to the nature of the CE system, the type of solution containing the analytes will determine the quantity of ions loaded onto the capillary. A method of signal enhancement is electroinjection of samples prepared in highly diluted buffer or water [43]. In conventional electroinjection, samples are prepared in a buffer solution that is the same concentration as that used in the separation, and the number of ions injected is rather limited. However if the sample is prepared in water (or diluted buffer) an enhanced electric field strength at the injection point exists when a high voltage is applied [43]. This can greatly enhance the number of ions injected onto the column.

In the case of chromium enrichment, it was found that almost a 10 fold improvement in limit of detection can be achieved by preparing the sample in water rather than buffer solution. Inclusion of even a small concentration of buffer (ie 10^{-4} *M*) caused a reduced effect on sensitivity enhancement. Figure 7.11 illustrates the effect of varying the ionic strength of the sample solution on LOD for Cr(VI) determination. From the plot it can be noted that, upon inclusion of buffer there is an obvious reduction in sensitivity enhancement. By plotting calibration curves for Cr(VI) analysis in buffer and water, it was found that the latter provided a more sensitive method as illustrated by the larger slope value for the sample in nanopure water (Figures 7.12 a & b).

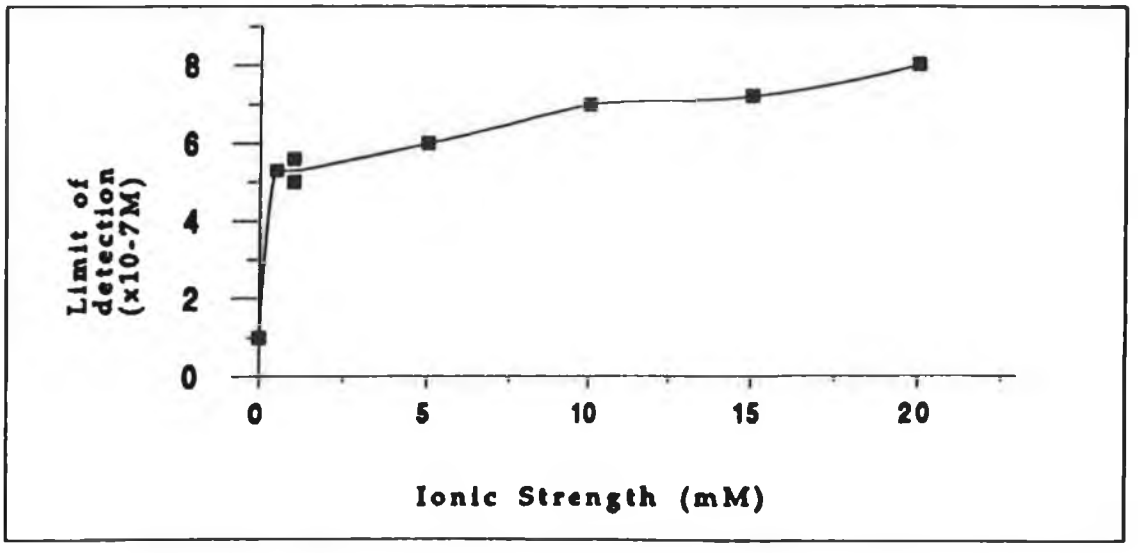
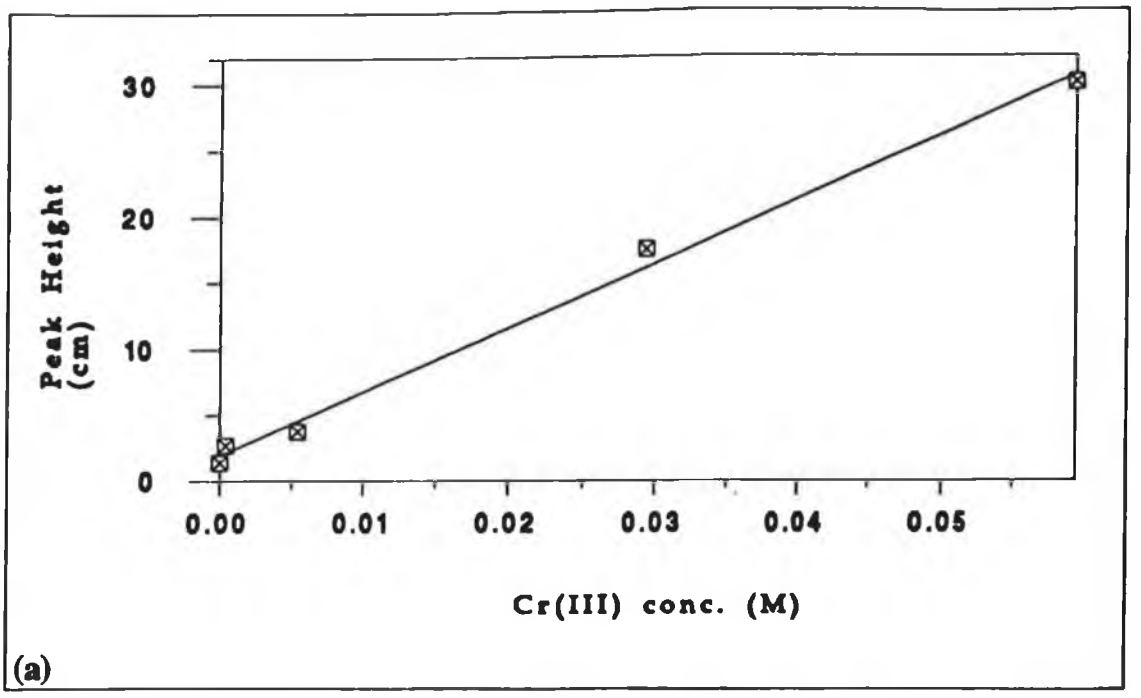


Figure 7.11
Effect of sample ionic strength on Cr(VI) limit of detection



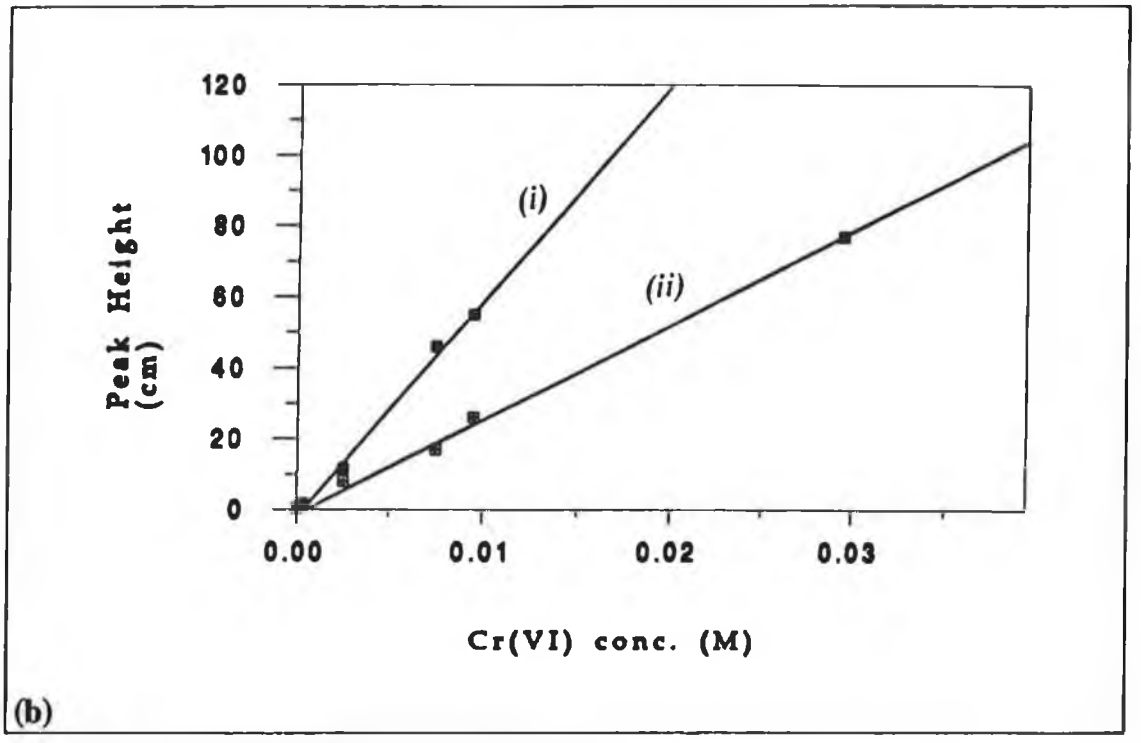


Figure 7.12 a & b
(a) Calibration curve for CE analysis of Cr(III) in H_20 and
(b) Calibration curves for CE analysis of Cr(VI) in (i) nanopure water and (ii) 20mM acetate buffer. CE conditions as for Figure 7.9.

7.3.2.5 *Limit of detection for Cr(VI) and Cr(III) determination using capillary electrophoresis*

The limit of detection obtained in these investigations corresponds to a signal to background noise ratio of 3:1. Table 7.4 summarises data obtained for Cr(VI) and Cr(III) speciation using capillary electrophoretic analysis. By injection of the analytes in nanopure water detection limits of 1.10^{-7} *M* for Cr(VI) and 1.10^{-6} *M* for Cr(III) were achieved. By carrying out six replicate electrokinetic injections of the mixture of species a coefficient of variation of 5.3% and 2.0% for hexa-and trivalent chromium respectively was noted. The calibration plot for Cr(VI) in water was linear over three orders of magnitude, with a regression value of 0.9980. Cr(III) has a linear dynamic range in the region 10^{-6} - 10^{-4} *M* with a linear regression of 0.9977.

	Cr(VI)	Cr(III)
LOD	$10^{-7}M$ (~5ppb)	$10^{-6}M$(~50ppb)
Linear range	10^{-7}-10^{-4} *M*	10^{-6}-$10^{-4}M$
Regression	0.9980	0.9977
*Reproducibility	5.3	2.0
n	6	6

n = number of sample replications.

*Reproducibility as % coefficient of variation

Table 7.4

Limits of detection and reproducibility for CE separation of Cr(VI) and Cr(III)

7.3.2.6 *Comparison of HPIC and CE for chromium speciation*

7.3.2.6.1 *Complementary use of HPLC and CE*

The aim of these investigations focussed on comparing the performance of two different analytical techniques in the speciation of chromium in aqueous solutions. Both procedures had distinct advantages, and particular differences in a number of areas were observed, notably, limit of detection, efficiency and resolution, analysis time and solvent volumes used. CE has advantages and disadvantages when compared with HPLC. The relative merits of each for a particular application will determine the technique chosen.

CE offers similar selectivity to that obtained using HPLC, and accordingly, combinations of the techniques can be adopted in method validation studies. Some examples of combined use of CE and HPLC in biological analyses are given in the literature [44,45]. Simplicity and ease of operation are key features of CE. The time taken to begin a CE analytical sequence, from receipt of samples, will be less than for an equivalent HPLC determination. There is no requirement to condition CE capillaries before each analytical run, unlike HPLC columns. Electrolytes are typically water based and can be stored for several days. Electrolyte requirements may be in the order of 20 cm^3 per day compared with HPLC, which often uses as much as a litre of mobile phase per day. These reductions also minimise disposal costs. Table 7.5 compares separation characteristics of both capillary electrophoresis and ion chromatography.

The major drawback with current CE technology is the limited preparative options offered. In general there is up to an order of magnitude difference when directly comparing the sensitivity of CE and HPLC at a common wavelength, due to the small path lengths charcteristic of CE capillary columns [37]. The quality of CE separations is greatly affected by the nature of the sample matrix. High sample salt contents or high percentage organic content in the sample can have major effects on the resolution achieved [46].

	HPLC		CE	
	Cr(VI)	Cr(III)	Cr(VI)	Cr(III)
Reproducibility**	1.5	3.3	5.3	2.0
LOD (ppb)	0.5	30	5	50
Efficiency *	234	272	125,000	90,000
Resolution @	0.57		7.15	
Run Time (min)	4.8		5.6	
Solvent Volumes (cm^3)	~1000		~20	

**Reproducibility as % CV (n=6)

* Efficiency in terms of theoretical plate number.

@ Resolution between neighbouring Cr peaks.

Table 7.5

Comparison of HPLC and CE for chromium speculation

7.3.2.6.2. *Precision*

The poor precision (5.3%) obtained for Cr(VI) analysis using CE is a characteristic feature of home-made CE equipment [37]. Using commercial instrumentation can achieve comparable precision to that obtained in HPLC (~2%). In this determination of Cr(VI) using HPIC a precision value of 1.5% was achieved, as expected. Cr(III) on the other hand had a lower coefficient of variation for CE (3.3%).

Precision in CE is related largely to sample concentration with improved precision noted for higher sample concentrations [37]. Sample injection into the capillary is carried out electrokinetically, ie., by immersing the capillary into the sample solution, and subsequently applying a voltage. Mechanical timing of this procedure introduces possible injection variability, especially with short injection times (ie., < 3 s).

7.3.2.6.3 *Sensitivity*

Due to the small path lengths characteristic of capillary columns, ie.75μm i.d., detection limits are normally an order of magnitude higher than for HPLC according to reports on pharmaceutical analysis using CE [37]. A number of approaches are available to enlarge this path length ie. Z-cell [47], which could achieve better sensitivity, comparable to that of HPLC. In this study a LOD of 0.5ppb Cr(VI) was achieved using ion-chromatography, but using CE the LOD was found to be 5ppb. The values of Cr(III) determination however, using both CE and IC are in close correlation, which may suggest that the path length is not the contributing factor in this case. The reason proposed for reduced sensitivity with Cr(VI) is the possible breakdown of the DPC complex on-capillary due to joule heating, or some other on-capillary reactions.

Even though the LOD using CE is higher than IC, the efficiency of separation and resolution of the two species is much greater for CE, as summarised in Table 7.5. A factor of 1000 improvement in efficiency is achieved using CE, in addition to a factor of 12 improvement in resolution. Both procedures obtain satisfactory limits of detection without the need for preconcentration methods which often add problems to the analytical procedure.

7.3.2.6.4 *Analysis time*

Analysis times using HPLC are decidedly longer than CE due to the preparation of mobile phases and column equilibration. Because of the simplicity of the electrophoretic buffer solution, containing only one component, notably, acetate, the preparation time is minimal. In addition, equilibration of the capillary only requires a short application of voltage upon the electrophoretic buffer prior to each run. Analytical run times following injection are similar for both techniques, and could be further shortened for the CE procedure by using a shorter capillary, due to the degree of resolution of the species.

An attractive feature of CE is the small solvent volumes used in the applications. This factor is important in industrial / environmental analyses, as it reduces the volumes of analytes (often toxic) for disposal, in addition to reducing the costs of disposal.

7.4 CONCLUSION

CE is viewed increasingly as a complimentary technique to HPLC [44,45]. Methods have been validated and data has been successfully submitted to regulatory authorities. The combined use of HPLC and CE for confirming analytical results is a powerful aspect of method validation. The features and disadvantages of CE, compared with HPLC, suggest that the choice between the techniques for a particular application is dependant on the nature of the sample and the requirements of the assay.

In this investigation CE was found to have the characteristics of a valid analytical technique for chromium speciation, which compared well in the area of detection limit and reproducibility, and achieved even better efficiency than ion-chromatography. The technique used offered a rapid method of analysis and the atraction of small solvent volumes is an appealing factor to the environmental analyst. Ion-chromatography has been used for many years in metal ion analysis, and CE does not as yet offer an alternative due to the slight differences in sensitivity. However it can be used as a parallel validation procedure, and further improvements, in the area of detection could encourage its independent use for metal ion analysis.

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