Chromatographic and Electroanalytical Studies of Metal Ions in Industrial and Environmental Matrices

by

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of

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Declaration

I hereby declare that the contents of this thesis, except where otherwise stated, are based entirely on my own work, which was carried out in the School of Chemical Sciences, National Institute for Higher Education, Dublin and in the University of Wollongong, Wollongong, Australia.

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Malcolm R. Smyth

Malcolm R. Smyth

(Supervisor)

To my mother and my deceased father

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Abstract

Chromatographic and Electroanalytical Studies of Metal Ions in Industrial and Environmental Matrices

Mary Meaney

This thesis describes novel analytical methods for the determination of trace metal ions in a variety of industrial and environmental samples.

A method is described for the simultaneous determination of Cu(II) and Fe(III) in anaerobic adhesive formulations by high performance liquid chromatography with spectrophotometric detection. Limits of detection of 100 ppb and 250 ppb for Cu(II) and Fe(III) respectively were achieved. A second approach based on direct application of the adhesive formulation to the surface of a glassy carbon electrode was also developed.

The chromatographic method was further developed for the simultaneous determination of Fe(III) and Al(III) in soil and clay samples and information on the speciation of those metals in these matrices obtained by comparison with atomic absorption spectroscopy.

Detection of metal chelates and inorganic ions was then investigated using polymer modified electrodes in flowing streams. The ruthenium polymer used i.e. $[Ru(bpy)_2(PVP)_5Cl]Cl$ was shown to enhance sensitivity over bare glassy carbon electrodes by about 3-5 orders of magnitude. These polymer modified electrodes were then stabilised using UV irradiation and coating with other polymeric materials for use in flow injection analysis.

Chapter 1

The Determination of Metal Complexes using High Performance Liquid Chromatography

1.1. Introduction

The technique of high performance liquid chromatography (HPLC), although less than twenty years old, has already made a significant contribution to pharmaceutical, biochemical, clinical and environmental analysis. The invention of chromatography in its modern form is generally ascribed to Tswett [1], who in 1903 first showed that plant pigments could be separated by elution through a column packed with calcium carbonate. Tswett recognised that separation arose because of the different affinities of substances for the adsorbent with which the column was packed.

Traditionally, HPLC has been applied to the separation of organic and biochemically active compounds, but during the last ten to fifteen years, increasing attention has been paid to the use of HPLC for separation, identification and quantitative determination of inorganic compounds. With its many advantages of low detection limits, wide linear dynamic ranges, the possibility of quantitative, qualitative and simultaneous analysis, separation of isomeric compounds and the determination of non-volatile and thermally unstable compounds, HPLC would appear to be a powerful instrumental method for inorganic analysis.

The area of inorganic analysis on which much of this thesis was based was the analysis of metals as inorganic metal complexes. Owing to the toxicity of many trace metals, there is a need for a simple multi-element method of analysis, especially for trace metals in industrial and environmental studies.

The most commonly used techniques for the determination of trace metals are atomic absorption spectrometry (AAS)[2] and various colorimetric methods [3]. Other methods of metal analysis include anodic stripping voltammetry [4], and in more recent years inductively coupled plasma mass spectrometry [5]. However, most of these methods are either selective and thus time consuming, or involve very expensive instrumentation. Chromatographic methods, such as gas liquid chromatography, thin layer chromatography and classical (gravity feed) column chromatography have been used for the analysis of metal complexes, but these methods are severly limited and are not suitable for routine analysis.

The determination of metal ions as inorganic metal complexes using HPLC has received a lot of attention in recent years. Using this technique, the metal ions of interest are complexed with an organic ligand and subsequently the metal-ligand complexes are passed down a chromatographic column on which they are separated. "In-situ" complex formation has also been investigated, whereby on-column complexation is carried out by injection of the metal ions of interest into a mobile phase containing the ligand. A wide variety of organic ligands have been studied. The first separation of metal complexes was reported in 1967 by Huber et al. [6], when six metal-acetylacetonates were determined in 25 min. Since then a large number of ligands have been found suitable for this type of analysis; these include the dithiocarbamates, β-dithizonates, β-diketonates, hydrazones and semicarbazones, 1,10-phenanthrolines and ethylenediamines, porphyrins, 8-hydroxyquinolates and α-ketoamines. Many new ligands have

also been developed and have proved very successful. The application of each of these groups of ligands to the analysis of metal complexes will be discussed in detail.

1.2. Dithiocarbamates

Dithiocarbamates (dtc) have been the most commonly used ligands in the analysis of metal ions using HPLC. The popularity of this group of ligands may be explained by their ability to form strong metal complexes quickly with a wide variety of metal ions. The resulting complexes exhibit high absorbtivities resulting in low limits of detection when analysis is carried out using spectrophotometric detection, and also offer the added advantage that they may be determined electrochemically.

Numerous papers have been published on the determination of dithiocarbamate-metal complexes using both normal and reverse phase HPLC. One of the earlier reports was published by Liska et al. [7]. Diethyldithiocarbamate complexes of Zn(II), Cu(II), Mn(II), Ni(II), Pb(II), Cr(II), Co(II), Cd(II) and Fe(III) were separated by reverse phase HPLC using UV detection at 254 nm. This ligand has proved to be very popular and many reports of its use for the determination of metal ions using HPLC have subsequently been published.

Using this ligand Tande et al. [8] separated Cr(III) from Cr(IV) using reverse phase chromatography with an RP-8 column and a mobile phase of methanol-water (65:35). This report highlighted another advantage that this technique offers, in that metal species of different oxidation states may be analysed simultaneously. The optimised technique was applied to the analysis of both Cr(III) and Cr(VI) in water samples.

The simultaneous determination of both Cr(III) and Cr(VI) was also investigated by Eijarvi et al.[9]. Sodium

diethyldithiocarbamate was used as the ligand with a C-18 Hypersil column and a mobile phase of 75% aqueous methanol. Rectilinear graphs up to 5 ug ml^{-1} were achieved for both chromium species. In a previous article the same authors had reported on the simultaneous determination of Hg(II) and methyl mercury(I) [10]. Both species were determined at picogram levels using diethyldithiocarbamate as the complexing ligand. LiChrosorb RP-8 and RP-18, Hypersil RP-8 and RP-18 columns were all investigated using mobile phases of methanol-water, acetonitrile-water, tetrahydrofuran-water and methanol acetonitrile-water. The optimum system was found to be methanol-water at a ratio of 80:20, which resulted in detection limits of 30 pg Hg(II) and 80 pg MeHg(I) when using an injection volume of 5 ul with UV detection at 254 nm.

Muller and Lovett [11] used reverse phase HPLC with UV detection to determine trace levels of Pt(II), Pd(II), Rh(III), Co(III) and Ru(III) in aqueous solution following complexation with diethyldithiocarbamate. The metal complexes were extracted in acetonitrile from aqueous solution by the addition of a saturated salt solution. Analyses of real samples (platinum powder) were highly reproducible and detection limits of < 3 ng ml^{-1} of original solution were achieved.

The determination of trace levels of Cd(II), Pb(II) and Hg(II) as dithyldithiocarbamate complexes using reverse phase HPLC was reported by Drasch [12]. Detection limits of < 1 ng ml^{-1} were achieved using "in-situ" complexation. The use of other dithiocarbamates were also investigated, but provided no advantage, except for bis(trifluoroethyl)dithiocarbamate, which allowed complete separation of the Cd(II) complex from an

interfering oxidation product. However, this ligand is expensive and is not readily available.

Electrochemical detection was investigated as a mode of detection for the analysis of metal diethyldithiocarbamate complexes by Bond and Wallace [13]. Reverse phase HPLC was used with mobile phases of acetonitrile-water containing 0.02 M acetate buffer and 0.2 M $NaNO_3$ as supporting electrolyte. It was reported that the $Cu(dtc)_2$ complex undergoes reversible one-electron oxidation and reduction steps at platimum, gold and glassy carbon electrodes enabling copper ions to be determined at levels down to 1 ng. In this study, both external and "in-situ" complex formation were investigated, resulting in the successful determination of Cu(II) ions using "in-situ" complex formation.

In a subsequent paper by Bond and Wallace [14], the simultaneous determination of Cu(II), Ni(II), Co(II), Cr(III) and Cr(VI) was reported using electrochemical detection. For simultaneous determination of all five metals it was necessary to form the complexes prior to injection, but in the case of Cu(II) and Ni(II) separation could be achieved by including the dithiocarbamate ligand (10^{-3}) in the mobile phase. A C-18 μ-Bondapak column with acetonitrile-water as the mobile phase was used as in the previous study. In this case a similar separation could be achieved using both diethyldithiocarbamate and pyrrolidinedithiocarbamate.

In a further publication the system described above was automated for the determination of Ni(II) and Cu(II) [15]. The system reported consisted of a microprocessor-based instrumental method involving "in-situ" formation of the

complexes followed by separation on HPLC using a μ-Bondapak column with acetonitrile-water mixtures as mobile phases. Both electrochemical and spectrophotometric methods of detection were compared with the results being in good agreement, but the electrochemical detector was generally more sensitive, and by application of a suitable wave form, more selective. However, less maintenance was associated with the spectrophotometric detector and day to day reproducibility was higher with this form of detection. The results obtained, using this system, for drinking water, industrial effluent, urn water and copper refinery water compared favourable with AAS results on the same samples.

This automated system was extended further by Bond and Wallace [16] to determine Pb(II), Hg(II), Co(II), Ni(II) and Cu(II) simultaneously. The automated microprocessor could be operated continuously for several days using electrochemical and/or spectrophotometric detection. An attempt to remove excess ligand at the detection stage was also reported in this paper. An ion-exchange based suppressor column was used, but this had two major problems associated with it: (a) this column has deleterious effects on the determination of Pb(II) and Cd(II) ; and (b) the period of time over which the analysis could be left unattended was decreased. The system compared favourably (when run for a 72 hr period) with a system with manual injection and atomic absorption spectrometry in an off-line mode.

During this time, other workers were also investigating the separations which could be achieved using this technique. The determination of Cu(II), Co(II), Ni(II) and

Fe(III) by incorporating 0.05 % w/w sodium diethyl-dithiocarbamate in the eluent was reported by Smith et al. [17]. Reversed phase chromatography, with an Hypersil ODS column and 80:20 methanol:water containing the dithiocarbamate salt as eluent was shown to be suitable for the required separation. The complexes so formed were detected using a variable wavelength detector set at 350 nm and detection limits in the range of 1 - 10 ppm were reported.

In 1984 R.M. Smith [18] reported on the separation and detection of Cu(II), Co(II), Cd(II) and Pb(II) metal ions. Again "in-situ" complex formation using sodium diethyl-dithiocarbamate dissolved in the mobile phase proved to be the most successful method of separation. In this study in order to achieve reproducible results for the determination of Pb(II) ions, chloroform had to be added to the mobile phase to prevent precipitation of the complex.

Smith et al. [19] published a further paper on the separation of metal ions using HPLC in 1985. In this study the on-column derivatisation technique was used for the determination of Pb(II), Hg(II) and Cd(II). The determination of these metal ions had posed problems in preceding research in which sodium diethyldithiocarbamate was incorporated in the mobile phase. It had been suggested that the poor shapes of the peaks were due to exchange reactions of the chelates with the stainless steel columns [20], but in the report by Smith et al. [19], it was shown that the problem occurred at the derivatisation step and was not due to interaction with the column. The problem seemed to have arisen due to the partial precipitation of the complex. This problem was overcome by

addition of chloroform to the mobile phase as was suggested by Drasch et al. [21]. Using mobile phases of methanol-water-chloroform (70:20:10) containing sodium diethyldithiocarbamate, linear calibration graphs from 0.1 to 10 ppm for Pb(II) and 0.5 to 10 ppm for Hg(II) were achieved with spectrophotometric detection at 350 nm. Because the UV-visible spectrum of the Cd(II) complex was very similar to that of the ligand, this system was unsuitable for the determination of the Cd(II) ion.

In a report published by Bond and Wallace [22] ternary complex formation was used to separate Cu(II) and Hg(II) dithiocarbamate complexes, the separation of which had previously been reported as being extremely difficult. The equilibrium established during ternary complex formation may be described as follows:

$$M + nL_1 \rightleftharpoons M(L_1)_n$$

$$M + nL_2 \rightleftharpoons M(L_2)_n$$

$$M(L_1)_n + M(L_2)_n \rightleftharpoons nML_1L_2$$

The two ligands used in this study were pyrrolidinedithiocarbamate (pdtc) and morpholine dithiocarbamate (mdtc). Using electrochemical detection, a C-18 μ-Bondapak column and acetonitrile-water mobile phases containing $7x10^{-5}$ M mdtc and 10^{-5} M pdtc, it was possible to separate the Cu(II) and Hg(II) complexes. However, limitations of the method were discovered when the separation of Ni(II) and Co(II) was investigated. The resulting peaks were broad and were of little analytical use. It was shown that slow kinetics involved in the ternary complex formation were

responsible for this problem.

Although "in-situ" complex formation is becoming a popular method for the determination of trace metal ions using HPLC, it is not possible to use this method for the majority of metal ions. Bond and Wallace [23] carried out research into different methods of complex formation. Three different methods were investigated using pyrrolidinedithiocarbamate and diethyldithiocarbamate as the complexing ligands. The methods investigated were as follows (a) direct formation in a solvent suitable for subsequent chromatographic separation; (b) liquid-liquid extraction and removal of co-extracted ligand to mimimise possible interferences observed with some detection methods; and (c) precolumn formation in which the complex was deposited onto a Sep-pak cartridge and then eluted in a solvent suitable for chromatography. These methods were successfully applied to determinations of Cu(II), Ni(II), Co(II), Pb(II), Hg(II), Cd(II), Se(IV), Cr(III) and Cr(VI). "External" formation in the chromatographic solvent was found to be the simplest procedure, but it did not prove useful for all eight metal ions investigated. Liquid-liquid extraction offered the advantage of simplifying sample clean-up and /or preconcentration. Pre-column preparation offered similar advantages but has the added ability to concentrate samples even further than the liquid-liquid extraction approach, therefore this method is generally preferred.

In 1987, a method was presented that involved the simultaneous formation of metal dithiocarbamates, on-line preconcentration, and subsequently, separation of the heavy-metal ions Cd(II), Pb(II), Hg(II), Cu(II), Co(II), Ni(II)

and Bi(II) by reverse phase liquid chromatography [24]. The diethyldithiocarbamate ion pair was loaded onto a precolumn packed with C-18 bonded silica. The injected metal ions reacted instantaneously with the ligand to form stable complexes, which could be efficiently preconcentrated before separation on a C-18 column, with a gradient mobile phase of acetonitrile/water containing cetyltrimethylammonium bromide buffered to pH 6.8.

Other advances that have been made in this area, include the introduction of new ligands. In 1982, Shih and Carr [25] introduced bis(n-butyl-2-naphthylmethyldithiocarbamate). This ligand rapidly forms stable, kinetically inert and easily detectable complexes with a wide variety of metal ions. It has been found that the naphthyl group imparts high absorptivities to resulting complexes, and the combination of the bulky naphthyl- and butyl- groups tend to stabilise the metal complexes formed with this reagent. Ni(II), Fe(II), Cu(II), Hg(II) and Co(II) complexes of this ligand have been separated using three different columns: (a) a Waters Radial Pak-A C18 column; (b) a Supelcosil LC-18; column and (c) a Waters μ-Bondapak C18 column, using varying ratios of methanol-water as the mobile phase. Detection limits of 1-2 ng were achieved using a variable wavelength detector at 221 nm.

In 1986 the determination of Ni(II), Cd(II), Co(II), Cu(II) and Hg(II) in water using a new water soluble dithiocarbamate, bis(ethoxyethyl)dithiocarbamate, was reported [26]. In this study the sample was buffered to pH 10 and reacted with a 0.02 % solution of the ligand. The complexes formed were collected by sorption onto a silica pre-column from which unconsumed reagent was washed with water. The complexes

were then separated by HPLC on a C-18 column with a mobile phase of methanol-water-isopropyl ether-acetonitrile and UV detection at 254 nm.

A second report on the use of water soluble dithiocarbamates was made by Schwedt and Schneider [27]. The ligand investigated was N-methyl-N-(2-sulphoethyl) dithiocarbamate, and the separation of its complexes of Ni(II), Co(II) and Cu(II) were reported. The complexes were preformed in the presence of 0.1 M acetate buffer and were separated by ion-pair HPLC on a Nucleosil C-18 column with methanol-water-acetate buffer (6:3:1) as the mobile phase and detection at 400 nm. Detection limits of 0.8, 1.2 and 1.5 ng were achieved for Co(II), Cu(II) and Ni(II) respectively.

The determination of Co(II), Cu(II), Hg(II) and Ni(II) as bis(2-hydroxyethyl)dithiocarbamate complexes by HPLC was reported by King and Fritz [28]. Again the metal complexes are water soluble eliminating the need to extract them into an organic solvent prior to analysis. Reverse phase HPLC with UV detection at 405 nm was used throughout. This ligand was also used by Ge and Wallace [29] to separate and detect Cd(II), Co(II), Pb(II), NI(II) and Cu(II) using "in-situ" ligand exchange chromatography. The separation was achieved using a μ-Bondapak C-18 column with a mobile phase of 40% methanol containing 0.1 mM Zn bis(2-hydroxyethyl)dithiocarbamate as ligand exchange reagent and spectrophotometric detection at 300 nm. Detection limits ranged from 7.7 to 53 ppb, but could be improved for individual species by tuning the wavelength for maximum absorption.

Bis(dibenzyldithiocarbamate) complexes of Cu(II),

Hg(II), Ni(II) and Cd(II) were analysed using HPLC by Haj-Hussein [30]). A LiChrosorb Si 60 column was used with a mobile phase of cyclohexane-benzene (2:1) and UV detection at 315 nm.

Micro-HPLC, which involves the use of smaller injector valves, columns and detectors, therefore leading to lower consumption of eluent, has been applied to the determination of trace metal ions as metal complexes. Wenclawiak et al. [31] reported on the determination of Cd(II), Ni(II), Zn(II), Cu(II), Co(III) and Hg(II) as diethyldithiocarbamate complexes at the 40 - 500 picogram level. Consumption of the mobile phase was 10 - 20 times less than conventional HPLC.

In recent years, trace metal ions in environmental and industrial samples have been determined using the technique of HPLC determination of metal complexes. Bond and Wallace [32] reported on the simultaneous determination of trace metals in a zinc sulphate plant electrolyte. In this matrix, difficulties were encountered with many analytical techniques when trying to determine low concentrations of metal ions. Using solvent extraction followed by HPLC with either electrochemical or spectrophotometric detection, Cd(II), Co(II), Cu(II), Pb(II), Hg(II) and Ni(II) pyrrolidinedithiocarbamate complexes could be determined simultaneously. Interference due to Zn(II) complexes and excess ligand were removed by passing the sample through an anion exchange column. For this particular sample, "in-situ" complex formation proved unsuitable.

E. Inatimi [33] carried out multi-element analysis in some environmental samples, namely trade effluents, river

water and standard kale and marine samples. Normal phase HPLC was used with a silica gel column and a mobile phase of benzene. Dithiocarbamate complexes of Hg(II), Cu(II), Pb(II), Ni(II) and Mn(II) were analysed, following extraction at pH 8.5. The results obtained compared favourable with those obtained using AAS.

The determination of Ni(II), Pb(II), Zn(II) and Cu(II) in citrus leaves and rice flour by HPLC was reported by Ichinoki and Yamazaki [34]. They used hexamethylene-dithiocarbamate to form metal chelates which were separated on a C-18 column using a mobile phase of methanol-water-chloroform which contained the dithiocarbamate salt at a level of 0.01 M. UV detection at 260 nm was used, and the results agreed very well with the standard values.

The determination of Cu(II), Ni(II) and Pb(II) in urine was described by Bond et al. [35]. Direct injections of freshly acidified and filtered samples were made onto a C-18 μ-Bondapak column, which was protected by a guard column. The metals were complexed with pyrrolidinedithiocarbamate included in the mobile phase and were detected electrochemically or spectrophotometrically. Spectrophotometric detection was insufficiently sensitive for direct determination of Cu(II) and Ni(II) at normal to occupationally exposed levels, but these metal ions could be determined using electrochemical methods. The determination of Pb(II) was restricted to determinations in subjects over-exposed to Pb(II), unless additional clean-up was applied.

1.3. β-Diketonates

The first HPLC separation of metal complexes was carried out using acetylacetonates as ligands by Huber et al. [6]. They used a ternary two-phase system consisting of 2,2,4-trimethylpentane, water and ethanol and reported on the separation of six metal ions in 25 minutes. The metals separated were Be(II), Cu(II), Al(III), Cr(III), Ru(III) and Co(III) with UV detection at 310 nm.

During the 1970's, the separation of β-diketonate complexes of Co(III) and Cr(III) using size exclusion chromatography was reported by Yamamato et al. [36]. Saitoh and Suzuki [37] also reported the chromatographic behaviour of acetylacetonate complexes of Co(III), Fe(III), Cr(III), Al(III), Cu(II), Ni(II) and Be(II) during this time.

A study of several metal β-diketonate complexes on alumina, silica gel, bonded phases and open-pore polyurethane HPLC columns was carried out by Tollinche and Risby [38]. The metals studied included Cu(II), Co(III), Cr(III), Al(III), Be(II), Ru(III), Rh(III) and Ni(II). It was found that the best separations were obtained using normal phase silica gel columns with non-polar eluents such as 1,2-dichloroethane-methanol mixtures.

The feasibility of using gel permeation chromatography for the determination of Be(II)-β-diketonate complexes was demonstrated by Noda et al. [39]. Reverse phase HPLC of β-diketone metal chelates was investigated by Gurira et al. [40]. They studied the chromatographic behaviour of acetylacetonates of Mn(II), Be(II), Co(III), Rh(III), Ru(III),

Pd(II) and Pt(II) and the benzoylacetone complexes of Cr(III). The complexes were separated using either MeOH/water or MeCN/water mixtures as the mobile phases. All of the complexes gave highly symmetrical peaks which indicated that no adsorption or decomposition had taken place on the analytical column. The molar absorptivities of the complexes in the UV region were in the order of 10^4 resulting in detection limits in the nanogram range with UV detection at 280 nm.

Reverse phase HPLC was used to determine Cr(III) acetylacetonate in nitric acid digested samples of NBS standard reference material and orchard leaves [41]. The samples were digested in nitric acid, following which the samples were heated with acetylacetone (necessary for quantitative chelation). The complexes were extracted with chloroform, which was evaporated and the residue dissolved in ethanol before analysis. Separation was carried out on a μ-Bondapak column using 36% acetonitrile/water as the mobile phase. The experimental values obtained compared very well with the standard values for the reference materials.

The separation of Cr(III)- and Co(III)-β-diketonates by reverse phase chromatography was investigated by Wenclawiak et al. [42]. The geometrical isomers of 2,2,7-trimethyl-octane-3,5-dione chelates of Cr(III) and Co(III) were separated by reverse phase HPLC on a silica column surface bonded with n-octyl or n-octadecyl groups, with aqueous acetonitrile as the mobile phase and detection at 275 nm. The compounds could also be separated by normal phase HPLC, thin layer chromatography (TLC) or high performance TLC (HPTLC) with hexane/dichloromethane as mobile phase.

Henderson et al. [43] reported on the separation of tris-(1-phenylbutane-1,3-dionato) complexes of Cr(III), Co(III), Al(III), Ga(III) and Fe(III) at low temperatures on a Spherisorb S5-ODS-2 column. Temperatures ranging from ambient to -30° C were studied. Aqueous 90% methanol and aqueous 75% acetone provided the best separations over the widest temperature range, although the former was limited by its viscosity and the latter by high UV detection cut-off.

Very recently, two reports on the use of the ligand 1,3-dimethyl-4-acetyl-2-pyrazolin-5-one (DMAP) have been published. Morales and Bartholdi [44] separated U(VI), Fe(III), Th(IV), Cu(II), Zr(IV) and Np(IV) complexes containing the above ligand using a C-18 column and an acetonitile/water eluent. A high percentage of organic modifier was required because the complexes were found to be very hydrophobic and insoluble in water. They found that addition of a small amount of 1-phenyl-3-methyl-4-benzoyl-2-pyrazolin-5-one (PMBP) to the eluent improved the peak shape of the metal complexes.

A second report on the use of this ligand was published by Palmieri and Fritz [45]. The separations of the metal complexes of Fe(III), U(VI), Ga(III) and Cu(II) on both a C-18 silica column and a polystyrene-divinylbenzene column were investigated, with better separation being achieved on the polymeric column. Separations of the metal-DMAP complexes using different organic modifiers were compared. Good separation was acheived using mobile phases of acetonitrile:water (35:65) containing 0.50 mM DMAP and 0.02 M acetic acid. Separation was also achieved using tetrahydrofuran, but in this case the inversion of retention was apparent.

1.4. 8-Hydroxyquinolates

The first reported HPLC separation of metal 8-hydroxyquinolate (oxine) complexes was in 1979 by Berthod et al. [46]. 8-hydroxyquinoline is an ideal ligand to use for the separation of multi-element mixtures by HPLC because of its ability to complex with many metal ions to produce neutral chelates. Furthermore, since several of these chelates possess natural fluorescence, it is likely that the sensitivity could by improved using this mode of detection. In their report Berthod et al. discussed the separation of Cu(II), Co(II), Ni(II), Hg(II) and Fe(II) using reverse phase HPLC. Oxine (5×10^{-3} M) was dissolved in the mobile phase (methanol/water 60/40) making it possible to directly inject metallic species without precolumn derivatisation. A LiChrosorb RP-8 column was used throughout the study with UV detection at 385nm.

The 8-hydroxyquinolate chelates of Al(III) and Co(III) were separated by Hambali and Haddad [47]. They used a silica column and 5% methanol/chloroform as the mobile phase with UV detection at 254 nm. A rapid separation was obtained for the two compounds in less than 5 minutes, and the detection limits were in the nanogram range for both metals.

The separation of 8-hydroxyquinolate complexes of V(V), Mo(VI), W(VI), Co(III) and Cr(III) using reverse phase HPLC was reported in 1981 by Wenclawiak [48]. A stationary phase of Si 60 with mobile phases of either tetrahydrofuran/chloroform (6:4) or dioxan/chloroform (6:4) resulted in separation of the preformed metal complexes in less

than 8 minutes.

Hoffmann and Schwedt [49] compared pre-column and on-column derivatisation for the separation of oxinate complexes of Cr(VI), Co(II), Mn(II), Zn(II), Cu(II), Al(III) and Mn(III). Using on-column derivatisation, the separation of all the above metal complexes could be achieved on an RP-8 column (mobile phase: methanol/water-borate buffer pH 9.0 and 10^{-3} M oxine 60/40). Pre-column derivatisation suffered from problems associated with changes in the oxidation states of some metals during the derivatisation step. For example, it was found that the derivatisation of Mn(II) with oxine was not complete because the oxidation state changed to Mn(III) during the extraction procedure. Hence, pre-column derivatisation was the preferred technique.

The HPLC determination of Cu(II), Fe(III), Mn(III) and Al(III) as oxinate complexes in biological and other samples was reported by Bond and Nagaosa [50]. Reverse phase chromatography using an Altex Ultrasphere ODS column and a mobile phase of acetonitrile/acetate buffer (0.02 M pH 6.0) containing 5mM oxine and 0.4 M potassium nitrate was used. Spectrophotometric and electrochemical detection was investigated and detection limits in the low nanogram region were achieved. The technique was applied to the determination of Cu(II), Fe(III) and Al(III) in bovine liver and oyster tissue. The determination of Mn(III) could be achieved using a mobile phase containing 1 mM-Tris buffer (pH 8.8) after injection of externally prepared complexes.

The separation of Mo(VI), Al(III), Co(III) and Cu(II) oxine complexes was reported using a mobile phase

containing EDTA [51]. The complexes were separated by HPLC on a Rad-Pak C-18 column with a mobile phase of methanol-water (7:3) containing 50 M EDTA. Detection limits of 4.2, 8.4, 10.2 and 63 ng were achieved for Cu(II), Al(III), Co(III) and Mo(VI) respectively.

Micro-column chromatography was used for the determination of Cu(II), Al(III) and Ga(III) oxinates [52]. The metals were complexed with oxine in the presence of acetate buffer (pH 5.0) and the excess reagent was removed by washing with sodium hydroxide. The preformed chelates were separated on a Silasorb-600 column with chloroform/propan-2-ol as the mobile phase. Detection limits of 5 ng for Cu(II), 0.5 ng for Al(III) and 2 ng for Ga(III) were reported using spectrophotometric detection at 254 nm.

HPLC analysis of Cr(VI), V(V) and Mo(VI) using pre- in combination with on-column derivatization by oxine, 2,2'-bipyridyl and hydrogen peroxide was reported by Wu and Schwedt [53]. A Nucleosil C-8 column was used to separate anionic forms of the metals with a mobile phase of 40% acetonitrile containing 8 mM oxine, 1.6 mM 2,2'-bipyridyl and 0.01 M citrate buffer (pH 6.5). Solutes were pre-complexed in 2 mM oxine and 3.6 mM 2,2'-bipyridyl containing hydrogen peroxide in 0.01 M citrate at pH 4.2 before HPLC injection. Cr(VI) was eluted as CrO_4^{2-}, V(V) as a peroxo-complex with both oxine and bipyridyl and Mo(VI) as a peroxo-complex with oxine only. Detection was at 370 nm and detection limits of 0.7 ppm for Cr(VI), 1.0 ppm for Mo(VI) and 0.4 ppm for V(V) were attained.

The use of 8-hydroxyquinoline as a reagent in the determination of Zn(II), Co(II), Cu(II), Al(III), Ga(III),

In(III), Cr(III) and Fe(III) by HPLC was evaluated by Baiocchi et al. [54]. A LiChrospher C-18 column was used in conjunction with mixtures of water and methanol, acetonitrile, tetrahydrofuran, dioxan and dimethyl sulphoxide, buffered from pH 4.5 to 7.0 with 0.1 M acetate buffer, as mobile phases. Variation of the pH from 4.5 to 7.0 did not give any significant difference in the chromatographic results and pH 4.5 was found to be the most suitable with regard to the stability of the chelates. Aqueous acetonitrile, tetrahydrofuran and dioxan all proved to be suitable mobile phases and the detection limits reported were between 2 and 15 ppb.

Trace levels of Al(III) were determined by ion chromatography with fluorescent detection, following post-column derivatisation with 8-hydroxyquinoline [55]. 2 mM 8-hydroxyquinoline-5-sulphonate was used to complex the Al(III) after eluting from a cation exchange guard column (Dionex CG2). Fluorescent detection at 512 nm (excitation at 360 nm) was used to achieve a detection limit of 1 μg/ml of Al(III). Other commonly occurring metals did not interfere and the method was successfully applied to the determination of Al(III) in a reference alloy and in tap water.

1.5. 1,10-Phenanthrolines

In 1980 O'Laughlin and Hanson [56] used paired ion HPLC to separate 1,10-phenanthroline (phen) complexes of Fe(II), Ni(II) and Ru(II). They found that a good separation of Ni(II) and Ru(II) from Fe(II) could be achieved using a μ-Bondapak C-18 column with a mobile phase of methanol/0.5 % acetic acid (20/80) with 0.15 M methanesulfonate as the counter ion. The elution of the ruthenium complex was confirmed using fluorescence detection and that of the nickel complex with atomic absorption spectrometry. As the Ni(II) and Ru(II) complexes had identical retention volumes, the three metal complexes could not be determined simultaneously. In a later report by O'Laughlin and Hanson [57] separation of Fe(II), Ru(II) and Ni(II) as the corresponding 1,10-phenanthroline complexes was achieved. A polystyrene-divinylbenzene polymer-based bead column and a μ-Partisil cation exchange column were used in conjunction with a mobile phase of 4:1 acetonitrile:water. The technique was then extended to the separation of the labile 1,10-phenanthroline chelates of Zn(II), Cd(II), Co(II) and Cu(II) using lithium perchlorate as the ion pairing agent and also using a mobile phase containing 10^{-4} M ligand. UV detection at 265 nm was used throughout.

Rigas and Pietrzyk [58] reported on the use of Fe(II)-1,10-phenanthroline salts as mobile phase additives for the HPLC separation of inorganic analyte anions on a reverse stationary phase. Indirect detection was used to detect analyte anions by monitoring the effluent at 510 nm where the Fe(II)-1,10-phenanthroline complex absorbs. Several separations

of multicomponent mixtures illustrated excellent resolution and effeciency. For example, the separation of F^-, Cl^- and Br^- was achieved using a PRP-1 column and a mobile phase of aqueous 1.0×10^{-4} M $Fe(phen)_3SO_4$ and 1.0×10^{-4} M succinate at pH 6.1. Linear detector response with analyte anion (Cl^-,Br^-) concentration was found from 10 ng to over 10,000 ng of injected sample.

On-column adduct formation has been used to enhance the stability of metal xanthate complexes by including 1,10-phenanthroline in the chromatographic eluent [59]. The separation of Fe(II), Hg(II), Ni(II) and Cu(II) was achieved using a C-18 LiChrosorb column with a mobile phase of acetonitrile/water (0.05 M tetraethylammonium perchlorate (TEAP)) (70:30) containing 3×10^{-5} M xanthate and 6×10^{-5} M 1,10-phenanthroline. The determination of Co(II) could also be achieved by excluding the perchlorate from the mobile phase. Detection limits of 0.50 ppm were achieved for each metal using UV detection at 290 nm.

Cation exchange chromatography was employed by Yoneda et al. [60], who separated tri- and divalent 1,10 phenanthroline and ethylenediamine (en) chelates. $[Co(phen)_3]^{3+}$, $[Fe(phen)_3]^{2+}$, $[Co(en)_3]^{3+}$ and $[Ni(en)_3]^{2+}$ were separated on a SP-Sephadex cation exchange column using various concentrations of potassium bromide and potassium sulphate as eluents.

1.6. Ethylenediamines

Beckett et al. [61] reported on the separation of EDTA chelates of Pb(II), Cd(II) and Zn(II). Potentially fluorescent metal chelates using 4-aminophenylethylenediamine-tetra-acetic acid as the ligand were synthesised and the resulting chelates separated using HPLC with a mobile phase of 0.20 M sodium acetate buffer and a Partasil column. The fluorescence was developed by post column derivatisation with fluorescamine.

The separation of Bi(III), Cu(II) and Fe(III) as cyclohexane-1,2-diaminetetra-acetic acid chelates by reverse phase paired ion chromatography was reported by Inoue et al. [62]. Two techniques wee employed: (i) the preformed chelates in 0.1 M acetate buffer (pH 5) were separated on an ECR-ODS column with 0.01 M tetrabutylammonium bromide (TBAB) in aqueous 45% methanol as the mobile phase; and (ii) the metal ions in acetate buffer containing 5mM sodium potassium tartrate were separated by direct injection onto the ECR-ODS column with 0.01 M TBAB and 1 mM ligand in aqueous 40% methanol as the mobile phase. Detection was at 254 nm and both methods resulted in detection limits of 37 and 24 ng for Bi(II) and Cu(II) respectively, in 25 μl of chelated injected solution.

The separation of Fe(III), Cr(III), Ni(II), Cu(II), Mn(II) and Pb(II) by reverse phase HPLC using EDTA as the complexing agent in the mobile phase was carried out by Marina et al. [63]. A Spherisorb ODS-2 column was used with a mobile phase of 10% aqueous methanol containing 0.01 M EDTA and 0.2 M tetrabutylammonium bromide adjusted to pH 3.25 with phosphoric

acid. Detection limits for Fe(III), Mn(II) and Pb(II) were 100 pg, 30 ng and 2 ng respectively using UV detection at 254 nm.

Some transition metal ions and bivalent metal ions were separated as EDTA complexes using anion exchange HPLC with UV detection [64]. Cr(III), Fe(III), Ca(II), Cd(II),Ni(II), Zn(II), Mn(II), Co(II), Pb(II) and Cu(II) complexes of EDTA were separated by HPLC. External complexation was carried out using 0.01 M EDTA (pH 5.7). Anion exchange chromatography with a Vydac column and a mobile phase of 1.0 M EDTA at pH 3.27 proved to be the optimum conditions for separation. UV detection at 220 nm resulted in detection limits in low ppm range.

1.7. Hydrazones and Semicarbazones

In 1973 Heizmann and Ballschmiter [65] reported the first succesful HPLC separation of Hg(II), Cu(II), Pb(II) and Zn(II) chelates of bisacetylbisthiobenzylhydrazone. They used normal phase chromatography with a Merkosorb SI 60 column and benzene as the mobile phase. Gradient elution, employing a second pump to add a polar component to the mobile phase, was used for the analysis of chelates with extremely different retention times e.g. of Hg(II) and Cd(II). Limits of detection in the nanogram range were achieved for Hg(II) and Cu(II) using UV detection.

In a second paper Heizmann and Ballschmiter [66] used 1,2-diketobisthiobenzhydrazones, dialkyldithiocarbamates and 1,2 diketobisthiocarbazones as ligands. Hg(II),Ni(II), Cu(II), Zn(II) and Pb(II) complexes were separated using a Lichrosorb SI 60 column and a mobile phase of benzene. Using this system the corresponding Zn (II) chelate had a long retention time and therefore was determined using gradient elution with n-heptane/benzene mixtures. Such a system allowed the Hg(II), Ni(II), Cu(II) and Pb(II) chelates to be separated in twelve minutes.

HPLC of chelates of 1,2-diketobisthiosemicarbazone were also studied. However, the widespread analytical use of this ligand in trace metal analysis is prevented by the low solubility of most of its metal chelates in organic solvents. The reports that have been published include the paper by Heizmann and Ballschmiter [66] in which Hg(II), Cu(II) and Ni(II) chelates of glyoxylbis(2,2,3,3-tetramethylbutyl)

thiosemicarbazones were separated. Normal phase HPLC with Alox T as the stationary phase and benzene as the mobile phase was used in this separation.

Metal biacetylbis-(4-phenylthiosemicarbazone) complexes of Zn(II), Cd(II), Pb(II), Ni(II),Cu(II) and Hg(II) were separated using reverse phase HPLC [67]. The metal complexes were separated on a C-18 column with 0.01 M sodium acetate in aqueous 67.5 % acetone (pH 8.8) as the mobile phase. UV detection at 425 nm was used throughout the study.

1.9. Porphyrins

Richter and Rienits [68] have published a report on the HPLC separation of Zn(II) and Mn(II) chelates of dimethyl derivatives of protoporphyrin IX. The complexes were separated on a Lichrosorb column using a mobile phase of acetone/hexane (15:85). The separated chelates were detected using UV detection at 405 nm. The technique was then successfully applied to the determination of these metals in cucumber etiochloroplasts and wheat etioplasts.

HPLC of metal tetraphenylporphyrin chelates of Mg(II), V(IV), Mn(III), Fe(III), Ni(II), Cu(II), Zn(II), Pd(II) and Cd(II) were studied by Saitoh et al. [69]. The chelates of Mg(II), V(IV), Ni(II), Cu(II), Zn(II) and Pd(II) were separated in 8 minutes using a Lichrosorb RP-18 column with actone/acetonitrile (40:60) as the mobile phase. Detection of the metal chelates was achieved using UV detection at 420 nm.

A second paper by Saitoh and Suzuki [70] reported on the separation of Ni(II), Cu(II) and Zn(II) as the corresponding tetraphenylporphyrin complex. However, in this study the bivalent metal ions were first extracted into carbon tetrachloride as their diethyldithiocarbamate complexes. After phase separation and removal of the solvent, the complexes were treated with 5,10,15,20-tetraphenylporphine in alcohol at 140^{o} for 60 minutes. Quantitative conversion into the porphyrin chelate was achieved for 0.25 to 4.5 μg of each metal. These chelates were then separated on a LiChrosorb C-18 column with acetonitrile/acetone (3:2) as the mobile phase and detected using UV detection at 412 nm. The method was

sucessfully applied to the determination of Cu(II) and Zn(II) in NBS bovine liver.

Specific retention behaviour of metal tetraphenylporphyrins in non-aqueous reverse phase HPLC was studied by Suzuki et al.[71]. The Co(III), Ni(II), Fe(III) and V(IV) complexes of tetraphenylporphine (TPP) were separated with short retention times from a Lichrosorb C-18 column with ethanol as the mobile phase. The Mn(III) and Co(III) complexes of TPP were retained. Although the retentions of other metal-TPP complexes were unaffected, those of the Mn(III) and Co(III) complexes, decreased dramatically with increasing concentration of ammonium chloride added to the mobile phase.

1.10. β-Dithizonates

In 1978 O'Laughlin and O'Brien [72] published a paper on the use of dithizone for the analysis of metal chelates using HPLC. These workers investigated the separation of Ni(II), Co(II), Cu(II), Zn(II), Hg(II) and Pb(II) on μ-Corasil and μ-Porasil columns using mobile phases ranging in polarity from heptane to iso-octane. The best separation was achieved on a μ-Porasil column with toluene as eluent. Detection limits for the metals studied ranged from 10 to 100 ng using UV detection at 275 nm.

The first successful HPLC separation of divalent metal dithizonates using reverse phase chromatography was reported by Henderson et al. [73]. This was accomplished using acidic polar modifiers such as acetic acid in the mobile phase in conjunction with the use of glass-lined stainless steel columns. An HPLC separation of Co(II), Cu(II), Pb(II), Zn(II) and Cd(II) was reported for those metal ions as their corresponding dithizonate complexes using UV detection at 254 nm.

HPLC of dithizonate metal complexes has been applied to the analysis of trace metals in some environmental samples, including trade effluents and aquatic biota, by Inatimi [33]. Normal phase HPLC was studied using silica gel columns with benzene as the mobile phase. A Hypersil column was used to separate dithizonate complexes of Hg(II), Cu(II), Ni(II), Co(II) and Pb(II). Trade effluents from manufacturing companies were then analysed for the presence of trace metals and the results were compared with AAS. The correlation of results

between both methods was very good, with the HPLC method taking up considerably less time. Water samples from the Thames, standard kale and biota from the aquatic environment were also analysed.

Hobbs et al. [74] reported on the use of dithizonate as a post-column reactor coupled to an ion-exchange HPLC system for the determination of metal ions in aqueous media. The dithizonate concentration was adjusted so that it would give a baseline absorbance of between 0.1 and 1.0 at 590 nm. Any metal ions which form a complex would then produce a negative absorbance peak. Good linear calibrations up to 100 ppm were obtained for Bi(II), Cd(II), Co(II), In(II), Ni(II), Pb(II) and Zn(II) using this system. An Aminex A9 column with a mobile phase of 0.2 M tartaric acid at pH 4.9 was used to separate the metal ions before complexation.

1.11. Other Ligands used in the Chromatography of Metal Chelates

Other ligands that are used in the chromatography of metal chelates include the resorcinol ligands. This group of ligands has become popular in recent years. On-column chelation with 4-(2-pyridylazo)resorcinol was reported by DiNunzio et al. [75]. The separation of Fe(II), Ni(II), Co(II) and Zn(II) as their respective resorcinol complexes was described. An Alltech C-18 or a Chromega C-2 column was used with mobile phases of methanol/0.01 M acetate buffer (pH 5.0) (1:1) or methanol/0.01 M sulphuric acid buffer (pH 2.54) (13:12:) respectively. Both mobile phases were 0.5 M in 4-(2-pyridylazo)resorcinol. UV detection at 546 nm for the C-2 column and at 525 nm for the C-18 was employed. Detection limits of 20, 1, 2, and 8 ppm were achieved for Zn(II), Fe(II), Ni(II) and Co(II) respectively.

The determination of Mo(VI), Cr(VI) and V(V) by ion-pair HPLC based on pre-column chelation with 4-(2-puridylazo)resorcinol was reported by Zhang et al. [76]. Preformed chelates were analysed on a Zorbax CN column with 1 mM tetrabutylammonium iodide/10 mM potassium hydrogen phosphate and 10 mM sodium hydrogen phosphate in aqueous 30% methanol as the mobile phase. Using UV detection at 540 nm, limits of detection of 0.82, 1.7 and 0.71 ppm were achieved for Mo(VI), Cr(VI) and V(V) respectively.

The determination of 4-(2-pyridylazo)resorcinol metal chelates by HPLC with electrochemical detection was studied by Ji et al. [77]. The separation of Co(II), Ni(II) and Fe(II) as complexes of the above ligand was achieved on a

YWG-CH ODS column with methanol/0.1 M phosphate buffer (pH 7.0) (1:1) as the mobile phase. Detection was carried out electrochemically at +1.2V vs SCE.

Another group of ligands that has been studied has been the phenolic ligands. Shijo and Sakai [78] reported on the separation of Cu(II), Co(II), Ni(II). Cr(III) and V(V) as 3-[-4-(5-bromo-2-pyridylazo)-3-hydroxy-N-propylanilino]propane-sulphonic (BPHPP) acid chelates. A Lichrosorb C-18 column with a mobile phase of aqueous 65% methanol containing 0.1 mM BPHPP, 0.1 M lithium chloride and 0.01 M acetate buffer (pH 4.0) was used in association with UV detection at 575 nm. The detection limits were 1 to 5 ppb.

The use of a similar ligand i.e. 2-(5-bromo-2-pyridylazo)-5-diethylaminophenol for the simultaneous determination of Mo(VI) and Cr(VI) by HPLC was reported by Lin and Zhang [79]. The precomplexed metal chelates were separated on a Shim-pack PNH_2 column with methanol-THF-water (2:3:15) containing 0.01 M lithium sulphate and 5mM tris buffer (pH7.7) as the mobile phase. UV detection at 600 nm resulted in detection limits of 0.2 and 120 ng for Cr(VI) and Mo(VI) respectively. The method was applied to the determination of these two metals in alloy steel and waste water.

The same ligand was studied by Chen et al. [80] for the separation of Pt(II),Pd(II) and Ir(II) chelates. Separation was achieved within ten minutes using ethylacetate/hexane or propan-2-ol/hexane as mobile phase and silica gel as the stationary phase. The Pt(II) and Pd(II) content of some samples were determined.

The determination of metal ions by HPLC separation of their hydroxamic acid chelates was reported by Palmieri and Fritz [81]. The metal ions in aqueous solution were mixed with N-methylfurohydroxamic acid (NMFH) and the complexes were separated on a PLRP S or a Zorbax C-8 column with aqueous acetonitrile containing 1 mM NMFH and hydrogen perchlorate as the mobile phase and UV detection at 304 nm. The composition of the mobile phase was varied according to the ion being determined; for the determination of Zr(IV) in antiperspirant, acetonitrile/water (1:3) was used with 0.01 M hydrogen perchlorate. Zr(IV), Hf(IV), Fe(III), Al(III), Nb(V) and Sb(III) were determined and detection limits between 0.5 and 5.8 uM were achieved.

As can be seen from this report many different ligands have been used in the determination of metal ions as metal complexes using HPLC. The most popular ligands used in these studies would appear to be the dithiocarbamates. However, many other ligands are now gaining in popularity. One such ligand is 8-hydroxyquinoline which was the main ligand used throughout these studies.

1.12. References

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Chapter 2

Theory

2.1. High Performance Liquid Chromatography

The technique of high performance liquid chromatography (HPLC), although only less than twenty years old, has made a significant contribution to pharmaceutical, biochemical, clinical and environmental analysis. The basis of high performance liquid chromatography was described in the late 1960's when the first HPLC chromatographs were introduced [1,2,3]. Since then HPLC has become a very successful analytical method as it offers the capability of separating complex mixtures of components in relatively fast time. In the following sections the essential features governing separation by this method are described.

2.1.1. Chromatographic Separation

Chromatography is essentially a physical method of separation in which the components to be separated are distributed between two phases; one of these is a stationary phase and the other is a mobile phase which percolates through or over the stationary phase. The chromatographic process is a result of repeated sorption/desorption steps which occur during the movement of the sample components along the stationary phase, and the separation is effected due to differences in the distribution coeffecients of the individual sample components within the system. In HPLC, the mobile phase is a liquid of low viscosity that flows through a column containing the stationary phase under a pressure gradient applied between the column ends.

In HPLC, the migration rate of an individual sample component is determined by the equilibrium distribution of the component between the stationary and flowing phases. Compounds that are distributed mainly in the mobile phase move rapidly through the column whereas those that are distributed mainly in the stationary phase move slowly. These differences in migration rates among the components of a sample mixture can lead to their separation as shown in Figure 2.1.

2.1.2. Retention and Equilibrium

The fundamental retention parameter in HPLC is the retention volume ,V_r, defined as the volume of the mobile phase that must flow through the column for elution of a given component. The retention time ,t_r, required for the elution of a solute from the column can be obtained from the equation:

$$t_r = V_r/F \qquad (2.1)$$

where F is the flow rate (ml min^{-1}) of the mobile phase [4]. The corresponding elution volume V_m for a non-retained component is given as:

$$V_m = t_o F \qquad (2.2)$$

where t_o is the time required for a non-retained component to pass through the column.

The migration of a solute down the column is dependent on the time spent in the mobile phase. Thus a useful

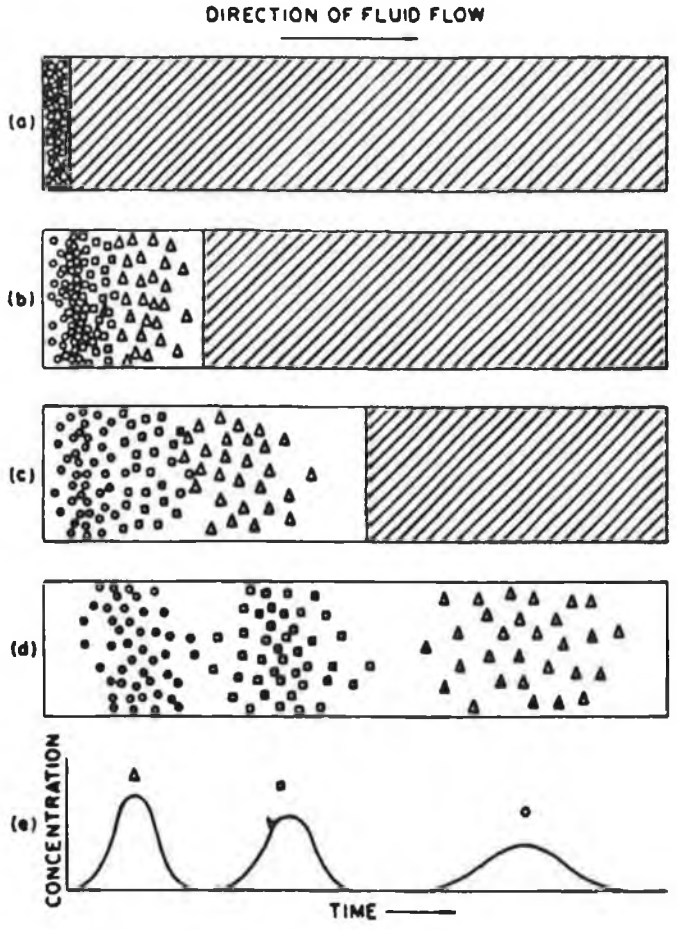

Figure 2.1. Schematic illustration of separation by the differential migration mode [4].

measure of retention is the retention ratio, R, which is the fraction of solute in the mobile phase, or the probability that a solute molecule will be found in the mobile phase at any given instant. R can be defined as:

$$R = n_m/(n_m + n_s) \qquad (2.3)$$

where n_m and n_s are the total number of moles of solute in the mobile and stationary phases, respectively. Since the capacity factor k' can be defined as:

$$k' = n_s/n_m \qquad (2.4)$$

$$R = 1/(1 + k') \qquad (2.5)$$

From these equations, it can be seen that the larger the value of R, the more rapidly the solute moves through the column. The average migration velocity, v_s, of the solute is therefore given by:

$$v_s = vR \qquad (2.6)$$

where v is the mobile phase velocity. Therefore, when R = 0 the solute does not migrate at all and when R = 1 it moves with the same velocity as the mobile phase. The migration velocity may also be defined as:

$$v_s = L/t_r \qquad (2.7)$$

where L is the column length, and v may be defined as:

$$v = L/t_o \qquad (2.8)$$

Inserting these relationships into eqn. 2.6 gives

$$t_r = t_o/R \qquad (2.9)$$

since $t_r = V_r/F$ and $t_o = V_m/F$, it can be shown that

$$V_r = V_m/R \qquad (2.10)$$

Substituting eqn. 2.5 into eqn. 2.10 yields a fundamental relationship between retention volume and k' or K:

$$V_r = V_m(1 + k') \qquad (2.11)$$

or

$$V_r = V_m + V_sK \qquad (2.12)$$

where K is the distribution constant and V_s is the volume of the stationary phase. The column dead volume, V_m, has no effect on differential migration of separation, since it is the same for all sample components. It is the value of V_sK that differs for different compounds and hence determines differential band migration.

2.1.3. Efficiency of Separation

The efficiency of column separation may be considered in terms of the height equivalent to a theoretical

plate (HETP) approach [5]. A theoretical plate is a purely imaginary concept, yet it is a convenient parameter for evaluating efficiency. The height of each plate is defined as:

$$H = L/N \qquad (2.13)$$

where N is the number of theoretical plates. For high efficiency a large number of plates and small values of H are required. The number of plates in a column length L has been shown from statistical considerations to be

$$N = 16(t_r/W)^2 \qquad (2.14)$$

where W is the extrapolated peak width measured at the baseline. In chromatography, it is desirable for W and H to be small as possible so that the peaks will be narrow and sharp. The physical processes that govern band broadening can adversely affect the width of a peak. In these situations peak widths are increased because these processes cause spreading of molecules in the analytical column.

2.1.4. Band Broadening

The broadening of solute bands as they travel through the chromatographic column is important, because it impedes separation and results in increased dilution of the solute by the mobile phase [4]. This broadening is due to differences in the rates of migration of the individual molecules. There are three main contributions to the variation

in migration rate for different solute molecules. First, the resistance to mass transfer between phases prevents instantaneous equilibration; this is usually the main cause of band broadening. Secondly, there is non-uniformity of flow rate within the column. Thirdly longitudinal diffusion leads to band broadening, with or without mobile phase flow.

2.1.4.1. Longitudinal Diffusion

Longitudinal diffusion results from molecular diffusion of the solute randomly in all directions. This causes a spreading of the analyte molecules along the column. Its contribution δ_L^2 to the total band broadening can be calculated directly from the Einstein equation:

$$\delta_L^2 = 2D_m t \qquad (2.15)$$

where D_m is the solute diffusion coefficient in the mobile phase and t is the time the average solute molecules spend in the mobile phase. On average, the solute molecules spend the time $t = L/v$ in the mobile phase, so that the variance in the mobile phase is given by

$$\delta_L^2 = 2D_m L/v \qquad (2.16)$$

The plate height contribution of longitudinal diffusion H_L is then obtained as

$$H_L = \delta_L^2/L = 2\gamma D_m/v \qquad (2.17)$$

where γ is an obstruction factor which recognises that longitudinal diffusion is hindered by the packing material, and is only important in HPLC at low flow rates when using small particle columns.

2.1.4.2. Mass Transfer into the Stationary Phase

Mass transfer in either the stationary or mobile phase is not instantaneous and, consequently, complete equilibrium is not reached under normal separation conditions. The result is that the solute concentration profile in the stationary phase is always displaced slightly behind the equilibrium position. The stationary phase contribution to mass transfer is given by:

$$H_s = qk'd^2v/D_s(1 + k')^2 \qquad (2.18)$$

where q is a configuration or shape factor, d is the measure of the distance travelled by a molecule in an "average step" and D_s is the diffusion coeffecient in the stationary phase.

2.1.4.3. Mobile Phase Mass Transfer

Solute molecules in the mobile phase are displaced not only by diffusion but also by flow. In a single flowstream, the flow rate is not uniform. Mobile phase in close proximity to the stationary phase moves very slowly, or not at all, whereas mobile phase in the centre of the flowstream moves quickly. This results in solute molecules near the stationary

phase moving shorter distances than those molecules at the centre of the flowstream. Furthermore, small- and large- scale flow inequalities are always present as a result of the irregular packing structure of the stationary phase. The flow pattern in packed stationary phases is very complicated but it is generally assumed that H_M, the mobile phase contribution to band broadening, can be related to the plate height contributions from flow profile (H_F) and diffusion effects (H_D). Therefore in liquid chromatography, the following relationship may be applicable because of coupling of flow and diffusion effects:

$$H_M = 1/(1/(H_F + 1/H_D)) \qquad (2.19)$$

In general, H_M increases with particle diameter and flow velocity and decreases with solute diffusivity.

2.1.4.4. Complete Plate Height Equation

The magnitude and relative importance of these various plate height contributions can vary greatly from one chromatographic technique to another, and even within a particular technique when stationary phase and operating conditions are changed. Although the preceding list of contributions to H for liquid chromatography is not comprehensive, it contains the major band-broadening factors, and the overall plate height can be expressed as their sum:

$$H = H_L + H_S + H_M \qquad (2.20)$$

Figure 2.2 illustrates the dependence of H on flow velocity as the sum of the individual terms given in equation 2.20. This dependence of H on flow rate is most commonly expressed as the Van Deemter equation.

$$HETP = A + B/v + (C_s + C_m)v \qquad (2.21)$$

Where A represents the contribution from eddy diffusion (non-uniformity of flow), B the contribution from longitudinal diffusion and C the contributions from mass transfer in the mobile and stationary phases, to the total column plate height. By differentiating equation 2.21 with respect to the mobile phase velocity and setting the result to zero, the optimum values of mobile phase velocity (u_{opt}) and plate height ($HETP_{opt}$) can be obtained.

$$v_{opt} = \sqrt{B/(C_M + C_S)} \qquad (2.22)$$

$$(HETP)_{min} = A + 2\sqrt{B(C_M + C_S)} \qquad (2.23)$$

In practice a flow velocity larger than v_{opt} is usually used to provide faster separation. Hence, band broadening is controlled by the mass transfer terms. Provided that the ascending portion of the van Deemter curve is fairly flat at higher velocities than v_{opt}, then the loss in efficiency will be small and well worth the gain in analysis time.

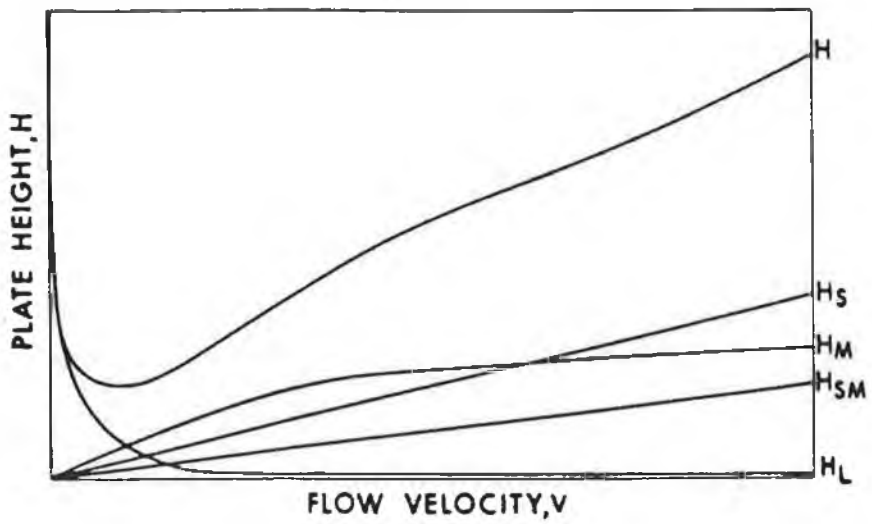

Figure 2.2. Relationship between band broadening and flow rate (van Deemter equation).

2.1.5. Resolution

The main objective in liquid chromatography is to obtain adequate separation between the components of a sample mixture. The degree of separation or resolution of two adjacent bands is commonly defined as the distance between band centers divided by the average band width [4]. It is defined as follows:

$$R_s = 2(t_2 - t_1)/W_1 + W_2 \qquad (2.24)$$

where t_1 and t_2 are the retention times of component 1 and 2 and W_1 and W_2 are their respective baseline widths. It is possible to define R_s in terms of k' and N as follows:

$$R_s = (\sqrt{N}/4)(\alpha - 1/\ )(k'_2/k'_2 + 1) \qquad (2.25)$$

where α is the separation factor between adjacent peaks and is equal to the ratio of their k' values. Using this equation, resolution may be controlled by varying α,N or k'.

The fundamental parameters α,N and k' can be adjusted more or less independently. N is determined mainly by the dynamics or rates of the various processes that take place during separation, and α and k'_2 are thermodynamic or equilibrium properties of the chromatographic system. It is possible to vary N by changing the length of the column, altering the flow rate of the mobile phase, using stationary phase materials of different particle size, and so on. Changes in α and k'_2 are achieved by selecting different stationary

and mobile phases, or by varying temperature and pressure.

It is often convenient to combine the plate number and capacity factor terms into a single parameter, the effective plate number N_{eff}:

$$N_{eff} = N(k'/(1 + k'))^2 \qquad (2.26)$$

For a constant value of α , R_s is proportional to N_{eff}. Hence N_{eff} is a more useful parameter than N for comparing the resolving power of different chromatographic columns, especially when the same stationary and mobile phases are involved.

2.2. Voltammetric Methods

2.2.1. Introduction

Voltammetry involves the measurement of current as a function of the potential applied to a working electrode with regard to a reference electrode in a 3-electrode cell. A plot of current versus applied potential is known as a voltammogram, and is used for the determination of the analyte species. The electrode at which the species reacts is known as the working electrode, and can be either the cathode or the anode. The working electrodes can be solid, stationary or dropping mercury electrodes. The first reproducible voltammetric apparatus was devised by Jaroslav Heyrovsky [6] in 1922. He used a dropping mercury cathode as his working electrode and called the technique "polarography" and since then the term polarography is restricted to current-voltage measurements obtained at a dropping mercury electrode (DME).

In subsequent years, voltammetric and polarographic methods were widely developed, until 1950 when the methods appeared to be mature and completely developed. However, the decade from 1955 to 1965 saw several major modifications. Pulse methods [7,8] were developed, which significantly increased the sensitivity and therefore the applications of the techniques. During the 1970's a revival of interest in voltammetry occurred, particularly in the area of environmental analysis. During the late 1970's voltammetric methods became increasingly used as detection methods in high performance liquid chromatography (HPLC).

In the following sections the theory associated with cyclic voltammetry and differential pulse voltammetry will be discussed in detail. However, before discussing the theory of these methods some general features of electrolysis will be introduced.

2.2.2. Basic Features of Electrochemical Processes

2.2.2.1. Modes of Mass Transport of Electroactive Species to an Electrode Surface

During electrolysis, three modes of mass transfer [9] are generally important:

(i) migration;
(ii) convection; and
(iv) diffusion.

Elimination of migrational modes of mass transfer of the electroactive species in solution is accomplished by addition of an excess of supporting electrolyte. The ions of the supporting electrolyte carry practically the total charge within the analyte solution, as these ions are in a large excess compared to the electroactive species. Therefore, migration currents of the electroactive species are negligible. This is shown more clearly when we examine the migration current in terms of transport number. The transport number of a univalent cation in the absence of a supporting electrolyte is

given as

$$t = c_+\lambda_+/c_+\lambda_+ + c_-\lambda_- \qquad (2.27)$$

where c_+ and c_- are the concentrations of electroactive cations and anions, and λ_+ and λ_- are their respective ionic mobilities. If we consider the addition of a 100 fold excess of univalent supporting electrolyte the migration current component of the electroactive cation is decreased to

$$t = \frac{c_+\lambda_-}{c_+\lambda_+ + c_-\lambda_- + 100c_+'\lambda_+' + 100c_-'\lambda_-'} \qquad (2.28)$$

where c_+' and c_-' are the concentrations of the cationic and anionic species of the supporting electrolyte, and λ_+' and λ_-' are their respective ionic mobilities. Therefore, the addition of a supporting electrolyte whose ions can neither be oxidised or reduced, causes the transference number of the electroactive species to decrease.

Convective mass transfer occurs under the influence of stirring or temperature gradients in solution. Although this mode of mass transfer of electroactive species to the electrode surface is utilised in stripping voltammerry, it is undesirable in voltammetric studies as it causes non-linear responses. Elimination of convective mass transfer is achieved by maintaining the solution in a quiescent state.

2.2.2.2. Overall Features of the Electrode Process

Electrochemical reactions that involve the transfer of charge at the solution/electrode interface are classed as heterogeneous processes. The rate of the electrochemical reaction is determined by a series of steps involving transport of electroactive species to the electrode and the transfer of charge at the interface [10]. Consider a simple electrochemical reaction of the type:

$$O + ne \rightleftharpoons R$$

Conversion of O to R involves the following steps:

(i) diffusion of O from bulk solution to the electrode surface;

(ii) transfer of electrons at the electrode surface to form R ; and

(iii) diffusion of R from the electrode surface into the bulk solution.

In addition to these steps, the overall electrode reaction can often involve homogeneous chemical reactions which either precede or follow the heterogeneous charge transfer step. Furthermore, other heterogeneous processes such as (a) adsorption or desorption of reactants and products, or (b) surface-mediated recombination of atoms or radicals, may be coupled with the electrode reaction [11].

The kinetics of the electrochemical reaction are

strongly affected by chemical reactions occurring in solution and by adsorption phenomena. Therefore, the overall rate of the electrode reaction is determined by all of the individual rates together.

2.2.2.3. Faradaic and Capacitance Currents

The total observed current in an electrochemical process arises from two clearly different processes at the electrode surface. The first process gives rise to the faradaic current which results from electron transfer across the electrode-solution interface. Faradaic currents result when either oxidation or reduction of electroactive species occurs. The magnitude of the faradaic current is determined by the mass transfer process, the method being used and whether the electrolysis is restricted by diffusion, electron transfer, chemical kinetics or adsorption [9]. In addition, the faradaic current depends on the surface area of the electrode and the applied potential.

The second process gives rise to the capacitance or charging current and results because the structure of the electrode-solution interface can change with changing potential or surface area (if a dropping mercury electrode is used). The interface between an electrolyte solution and an electrode is known as the electrical double layer. Figure 2.3. illustrates a widely accepted model of the electrode-solution interface. The electrode is covered by a sheath of oriented solvent molecules (water molecules as illustrated). Adsorbed anions or molecules, A, contact the electrode directly and are not fully

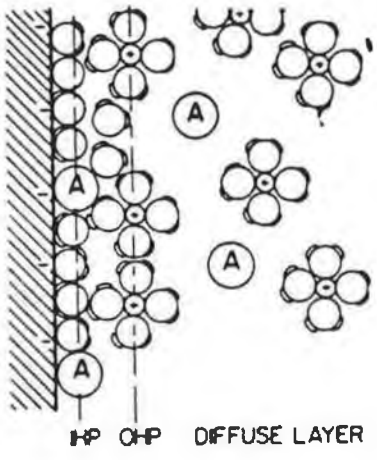

Figure 2.3. The proposed model for the electrode-solution interface.

solvated. The next layer of molecules carry their primary (hydration) shell and are separated from the electrode by the monolayer of water molecules adsorbed on the electrode surface. Beyond this layer lies a region referred to as the diffuse layer. In the diffuse layer, the random thermal motion tends to distribute the ions evenly throughout the solution, whereas the electrostatic forces tend to attract or dispel ions from the surface depending on their charge. These two tendencies counterbalance each other resulting in a non-uniform distribution of ions near the surface. Beyond the diffuse layer, the potential diminishes exponentially with distance from the surface and the strength becomes zero very close (usually less than 10^{-6} m) to the electrode [12].

In voltammetric studies, only faradaic currents are of interest and many methods are now available which discriminate against the capacitance current.

2.3. Theory of the Techniques Used in these Studies

2.3.1. Cyclic Voltammetry

The technique of cyclic voltammetry was developed in 1938 by Matheson and Nichols [13], and since its introduction it has become perhaps the most effective and versatile electroanalytical technique for the mechanistic study of redox systems [14-18]. Its effectiveness results from its capability for rapidly observing the redox behaviour of electroactive species over a wide potential range. Once a redox couple has been located, it can be characterised from the potentials of peaks on the cyclic voltammogram and from changes caused by variation of the scan rate.

Cyclic voltammetry (CV) consists of cycling the potential of a working electrode in an unstirred solution and measuring the resulting current. The potential of the working electrode is controlled versus a reference electrode such as a saturated calomel electrode (SCE) or a silver/silver chloride (Ag/AgCl) electrode.

The repetitive triangular potential excitation signal for CV causes the potential of the working electrode to sweep back and forth between two designated potentials. The potential excitation that is applied across the electrode-solution interface in order to obtain a cyclic voltammogram is illustrated in Figure 2.4. Single or multiple scans may be used. Often there is little change between the first and successive scans; however the changes that do result are important as they can reveal information about reaction

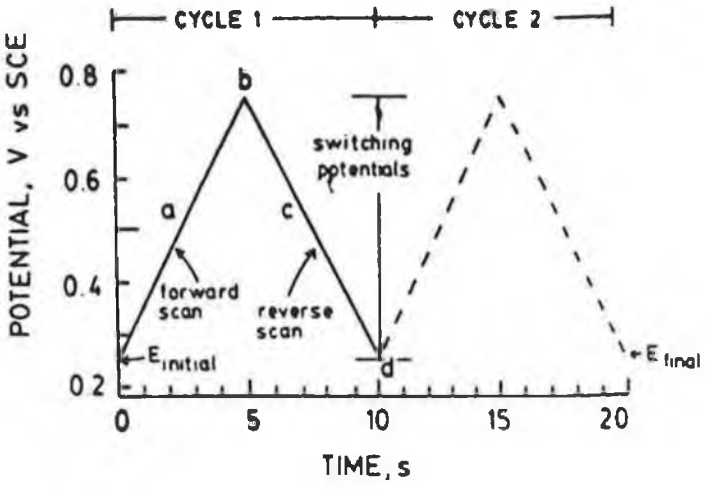

Figure 2.4. Typical potential-time excitation signal for cyclic voltammetry.

mechanisms [19].

To obtain a cyclic voltammogram, the current at the working electrode in an unstirred solution is measured during the potential scan. In Figure 2.5. a typical cyclic voltammogram of Fe(II) obtained at a carbon paste electrode using 1.0 M sulphuric acid as the supporting electrolyte is shown. In the forward scan, Fe(III) is electrochemically generated as indicated by the anodic current. In the reverse scan this Fe(III) is reduced back to Fe(II) as indicated by the cathodic current. Therefore, CV is capable of rapidly generating a new species during the forward scan and then probing its fate on the reverse scan. This is a very important aspect of the technique.

The important parameters of a cyclic voltammogram are listed below:

(i) the cathodic (E_{pc}) and anodic (E_{pa}) peak potentials; and

(ii) the cathodic (i_{pc}) and anodic (i_{pa}) peak currents.

These parameters are labelled in Figure 2.5. One method for measuring i_p involves extrapolation of a baseline current. The establishment of a correct baseline is essential for the accurate measurement of peak currents. This is not always easy, particularly for more complicated systems. The second sweep generally causes the main problem since the baseline is not the same as the residual current obtained by an identical experiment in supporting electrolyte. Difficulty in obtaining

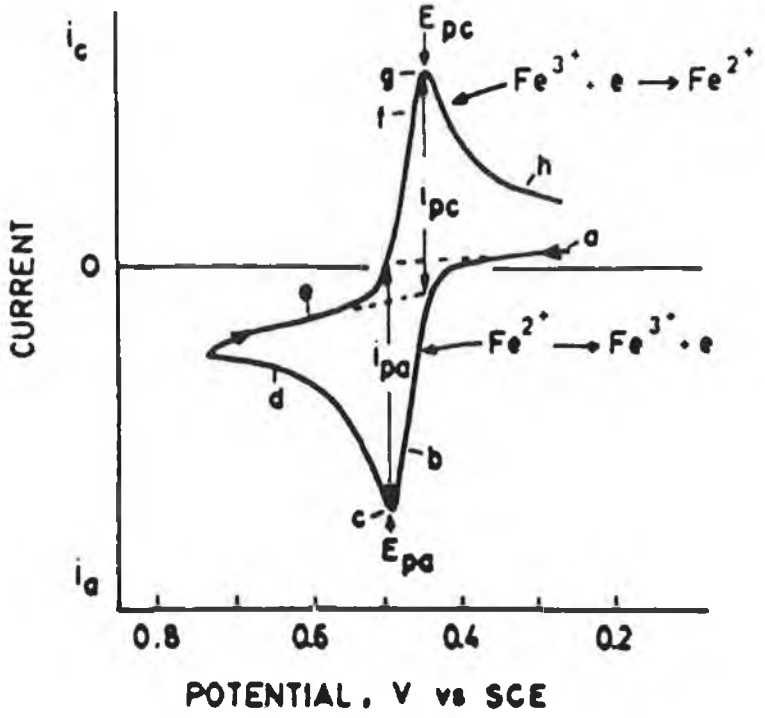

Figure 2.5. A typical cyclic voltammogram of Fe(II) in 1 M sulphuric acid.

accurate peak currents has been suggested to be perhaps one of the biggest problems in CV [20].

Having established a correct baseline, the peak current measured depends on two steps:

(a) movement of the electroactive material to the working electrode; and

(b) the rate of the electron transfer reaction.

The rate of electron transfer for a reduction process is a function of potential and is theoretically described as:

$$k_f = k^o \exp\ [-(\alpha nF/RT)(E-E^{o'})] \qquad (2.29)$$

where k^o is the standard heterogeneous electron-transfer rate constant, α is the transfer coefficient, $E^{o'}$ is the formal reduction potential, n is the number of electrons transferred in the electrode process, F is the Faraday constant and R is the universal gas constant. The electron tranfer rate constant for the reverse process, k_b, is similarly controlled by the applied potential and is denoted by:

$$k_b = k^o \exp[((1-\alpha)nF/RT)(E-E^{o'})] \qquad (2.30)$$

When the electron transfer process is reversible, the difference between anodic and cathodic peak potentials is 59/n mV. This relationship may be used to evaluate n. Under

reversible conditions the electron transfer reaction at the electrode surface is fast enough to maintain the concentrations of the oxidised and reduced forms in equilibrium with each other. The equilibrium ratio for a given potential at the electrode surface is determined ty the Nernst Equation:

$$E = E^{o'} + (RT/nF)(\ln([R]/[O]_{x=o})) \qquad (2.31)$$

where O and R are the oxidised and reduced forms respectively and x is the distance from the electrode surface. It should be noted that the 59/n mV separation of peak potentials is independent of scan rate for a reversible couple, but is slightly dependent on switching potential and cycle number [21].

Electrochemical irreversibility is caused by slow electron exchange of the redox species with the working electrode. It is characterised by a separation of peak potentials that is greater than 59/n mV and is dependent on scan rate. At high scan rates, the electron transfer reaction may not be fast enough to maintain equilibrium conditions as the potential changes.

In cyclic voltammetry the peak current, i_p, is given by the following equation [19]:

$$i_p = k\, n^{3/2}\, A\, D^{1/2}\, C^o\, v^{1/2} \qquad (2.32)$$

where k is the Randles-Sevcik constant, A is the area of the electrode, D is the diffusion coefficient, v is the scan rate and C^o is the concentration of the species in bulk solution.

The dependence of i_{pc} and i_{pa} on $v^{1/2}$ is a further characteristic identifying a reversible system.

CV has become increasingly popular in all fields of chemistry as a means of studying redox states [22]. The technique enables a wide potential range to be rapidly scanned for reducible or oxidisable species. This capability, together with the ability to use a variable time scale make this one of the most versatile electroanalytical techniques available today. However, it must be said that its advantages lie mainly in qualitative rather than quantitative analysis. Quantitative measurements are best obtained using other techniques e.g., step, pulse or hydrodynamic techniques.

Perhaps the most useful aspect of CV is its application to the qualitative diagnosis of electrode reactions which are coupled to homogeneous chemical reactions [15-18].

2.3.2. Differential Pulse Voltammetry

The technique of differential pulse polarography was introduced in the early 1960's by Barker [7] in an effort to overcome some of the problems associated with direct current (DC) polarography. This new technique overcame certain deficiencies of DC techniques by:

(i) recording data only at the end of a drop life;

(ii) maximising flux by using a potential pulse;

(iii) discriminating against charging current by subtraction of background currents using a timed sample-and-hold sequence.

The use of this technique resulted in a lowering of detection limits and also in the presentation of data in a more acceptable form. Later, the same approach was found to be useful for solid and mercury film electrodes and from this, differential pulse voltammetry (DPV) became one of the most popular electroanalytical techniques in the 1970's.

In DPV, a normal DC voltage ramp is applied to the electrode. At a fixed time before application of the pulse, the current flowing, i_{ft1}, is sampled. A small-amplitude pulse, ($E \leq 100$ mV), is then superimposed on the voltage ramp as shown in Fig. 2.6. The current flowing at the end of the pulse i_{ft2} is again sampled at a fixed time after application of the pulse. The difference between the two currents, i, is the parameter measured. The theory of DPV has been discussed by Osteryoung and Hasebe [23]. In these calculations it was assumed that the electrode reaction was fast and that there were no other chemical kinetic complications. For a reversible electrode process the differential pulse current was shown to be given by the expression

$$i(E) = \epsilon(\delta^2 - 1)\, i_d/(\delta^2 + \epsilon)(1 + \epsilon) \qquad (2.33)$$

where

$$\delta = \exp\ (-nF/RT\ E/2) \qquad (2.34)$$

and

$$\epsilon = \exp\ ((nF/RT)(E - E_{1/2})) \qquad (2.35)$$

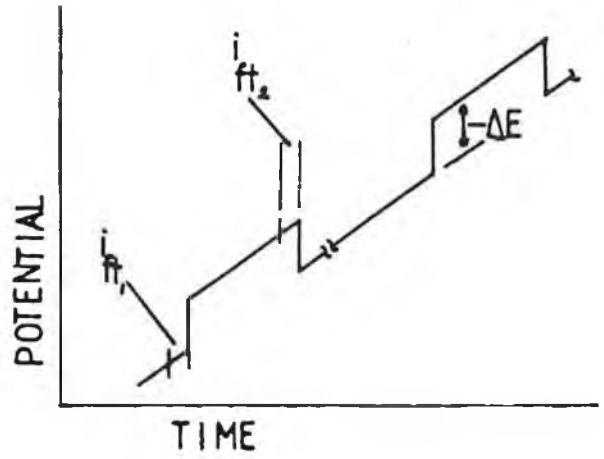

Figure 2.6. Waveform used in DP voltammetry.

The maximum value of $\Delta i(E)$, where $\Delta i(E) = i_p$, is found by differentiating eqn. 2.33 and is given by

$$i_p = i_d \, (\delta - 1)/(\delta + 1) \qquad (2.36)$$

It may also be noted that eqn 2.33 actually gives the derivative current, whereas the DPV experiment employs a differential current measurement. Agreement of theory with experiment arises from the fact that the DC contribution is small. These relationships have been experimentally verified by Christie and Osteryoung [24]. Saito and Himeno [25], have re-examined in detail these relationships using the reduction of Cd(II), Co(II), Tl(I) and Zn(II).

In normal pulse voltammetry the current may be calculated by the Cottrell Equation:

$$i_d = nFAD^{1/2}C^o/(\pi t_m)^{1/2} \qquad (2.37)$$

where n, F, A, D and C^o have their usual significance and t_m is the time in seconds, measured from pulse application, at which the current is measured. If the peak difference current in DPV pulse is denoted by i_{DPV} and the diffusion current in normal pulse voltammetry (NPV) is denoted by i_{NPV}, equation 2.33 can be rewritten as:

$$i_{DPV} = i_{NPV}(\delta - 1)/(\delta + 1) \qquad (2.38)$$

The ratio $(\delta - 1)/(\delta + 1)$ depends only on the number of electrons transferred and on the pulse amplitude, ΔE, and is

always less than one. According to Oateryoung and Hasbe [23] it can be shown that in the absence of complicating kinetic factors, equation 2.38 always applies, regardless of electrode geometry. Therefore, the procedure for calculating the DPV current is to calculate the NPV current and apply equation 3.38.

2.3.3. Electrochemical Detection in Liquid Chromatography and Flow Injection Analysis

2.3.3.1. Introduction

Liquid chromatography with electrochemicl detection (LCEC) is coming into widespread use for the trace determination of easily oxidisable and reducible analyte species. Very low detection limits have been achieved, in the order of 0.1 pmoles. The modern interest in electrochemical detectors for liquid chromatography was stimulated by the recognition that this technique was ideal for the study of aromatic metabolism in the mammalian central nervous system. Since the first commercial detectors became available in 1974, a wide range of applications have been explored [26].

Liquid chromatography (LC) and hydrodynamic electrochemistry are very compatible techniques and when combined result in important advantages for the determination of many analyte species. The three major advantages offered by this combination are:

(i) high selectivity;

(ii) low detection limits and;

(iii) low cost.

The use of modern LC techniques requires selective detection with a rapid response time, wide dynamic range, and low active dead volume. Because electrochemistry is a surface technique, small volume cells can easily be constructed that fit these criteria.

2.3.3.2. Fundamental Principles

As with cyclic voltammetry, hydrodynamic voltammetry involves the measurement of the current produced under the influence of an applied potential. However, in this case the working electrode is held at a fixed operating potential and the analyte flows past the electrode surface. In the first application of voltammetric methods to flowing systems, it was observed that the measured voltammetric signal depends on the hydrodynamic parameters of the measuring system. This can be readily explained as the voltammetric signal is a result of a mass transfer process.

2.3.3.3. Mass Transfer Processes Under Hydrodynamic Conditions

The first and so far most commonly used theory concerning the kinetics of heterogeneous chemical reactions taking place in stirred solution has been developed by Nernst

[27]. According to this theory, there is a thin layer of static liquid immediately adjacent to the surface of the electrode through which diffusion of the reacting species takes place.

Beyond this layer, known as the diffusion layer with thickness δ , the analyte is transported by convection. Inside the diffusion layer, the solution is assumed to be unstirred, and the concentration distribution within the layer is linear. On the basis of these assumptions the flux of component taking part in the heterogeneous chemical reaction can be given by the following equation:

$$j = D(C-C^{o})A/\delta \qquad (2.39)$$

where j is the mass flux, D is the diffusion coefficient, C is the concentration in bulk solution, C^{o} is the concentraton on the surface of the electrode and A is the surface area of the electrode.

However, the experimental observations do not support Nernst's assumptions, namely the liquid is not stationary in the vicinity of the electrode surface and the concentration destribution is not linear. In spite of these limitations, the Nernst relationship is still being used. From this equation it can be seen that every effect that decreases δ, such as an increase in flow rate, increases the mass flow. However, even though this description is qualitatively correct, the relationship is not suitable for the quantitative description of the effect of these parameters.

The exact treatment of the mass transport involving convection and diffusion was given by Levich [28]. The

fundamental statement of Levich's theory is that "the transport of a solute in a liquid is governed by two quite different mechanisms. First, there is molecular diffusion as a result of concentration differences; second, solute particles are entrained by the moving liquid and are transported with it. The combination of these two processes is called convective diffusion of solute in a liquid." Either of the two strongly differing processes, convection and diffusion, can be predominant in one or another point of the liquid. However, in the vicinity of the solid surface both processes play significant roles.

In addition to convective diffusion, the migration of ions due to the effect of electrical attraction plays a role in an electrode process. Furthermore, the rate of a homogeneous chemical reaction may depend upon a heterogeneous chemical reaction in which it is involved. The overall concentration distribution relating to a heterogeneous chemical reaction can be given as follows:

$$dC_i = (D_i \nabla C_i) - V\nabla C_i + z_i F\nabla(u_i C_i \nabla\Phi) + R_i \qquad (2.40$$

where D_i is the diffusion coefficient of species i, C_i is the concentration of species i, V is the flow velocity vector, z_i is the number of charges transported by i, u_i is the ionic mobility, Φ is the strength of the electric field, and R_i is the rate of the homogeneous chamical reaction.

In the above equation, the first term relates to the concentration gradient of species i, and the second relates to the macroscopic flow velocity of the fluid and the

concentration of i. The third term relates to migration and the fourth one is the rate of the homogeneous chemical reaction. This equation can only be solved in a few cases, usually in the cases of systems with very simple geometry.

Levich has solved the convective diffusion limiting current equation with respect to some of these simple geometries namely, rotating disk electrodes, tubular electrodes, flat surfaced electrodes and conical microelectrodes. In the studies undertaken in this research project the electrodes of interest under hydrodynamic conditions were the flat surfaced electrodes.

2.3.3.4. Flat Surfaced Electrodes

Flat surfaced electrodes have been used extensively in practice. They are commonly used in association with thin layer cells as detection systems for chromatographic eluents. The convective diffusion to a flat plate was first examined by Levich [28]. In his deduction, Levich assumed the following: the flow with a velocity V is laminar in the vicinity of the plate and the length h and width b of the plate is much greater than the thickness of the hydrodynamic boundary layer. Using these assumptions, the following equation was obtained for the limiting current:

$$i_L = 0.63nFD^{2/3}\nu^{-1/6}bh^{1/2}V^{1/2}c \qquad (2.41)$$

Trumpler and Zeller [29] carried out experiments which verified this equation, while Wrangler and Nilsson [30] proved it to be

applicable for both laminar and turbulent conditions.

2.3.4. Modified Electrodes

2.3.4.1. Introduction

A modified electrode is created by deliberately coating a clean electrode surface with a thin film of a chosen material, with the objective of altering its properties [32]. Work in this area began in 1975 when Murray's group covalently attached silane derivatives to a variety of materials [33]. Since then numberous methods for electrode modification have been reported [34-35]. Chemically modified electrodes (CME'S) have been employed for many different analytical applications. Their mode of operation can be broadly classified into four types [34]:

(i) ionic interaction (positive and negative);
(ii) complexation;
(iii) size exclusion; and
(iv) electrocatalysis.

The first two types may achieve extra sensitivity and selectivity by electrostatic attraction or complexation of the analyte onto the electrode surface. The third type may introduce specificity, as interferences may be discriminated against on the basis of size. The catalytic processes achieve extra sensitivity and selectivity by enhancing the electron transfer process for a particular analyte. It is this type of

electrode modification procedure that was investigated in these laboratories; therefore, this procedure will be discussed further.

2.3.4.2. Electrocatalysis

Electrocatalysis, in polymer modified electrodes, is normally achieved through the introduction of an electroactive polymer onto the surface of an electrode. These polymer modified electrodes may be formed either by adsorbing the appropriate polymer onto the electrode surface or by electrochemically generating the polymer layer at the electrode surface.

To be analytically useful, the CME must be capable of producing an electrical signal which is related to the concentratiion of the analyte. The basis of signal generation has been discussed by Wallace [36], in which he points out that the signal may be due to a change in resistance of the CME, a change in the potential difference across the CME, or to gereration of a current arising from oxidation or reduction of the analyte (voltammetric/amperometric methods). Voltammetric methods will be discussed further as these were the techniques used in this study.

The generation of a voltammetric signal at a CME is similar to that at a conventional electrode in that the analyte must be transported to the electron transfer boundary before the redox process occurs. However, the electron transfer boundary is not as clearly defined in CME's as in conventional electrodes. When a catalytic CME is used then a process similar

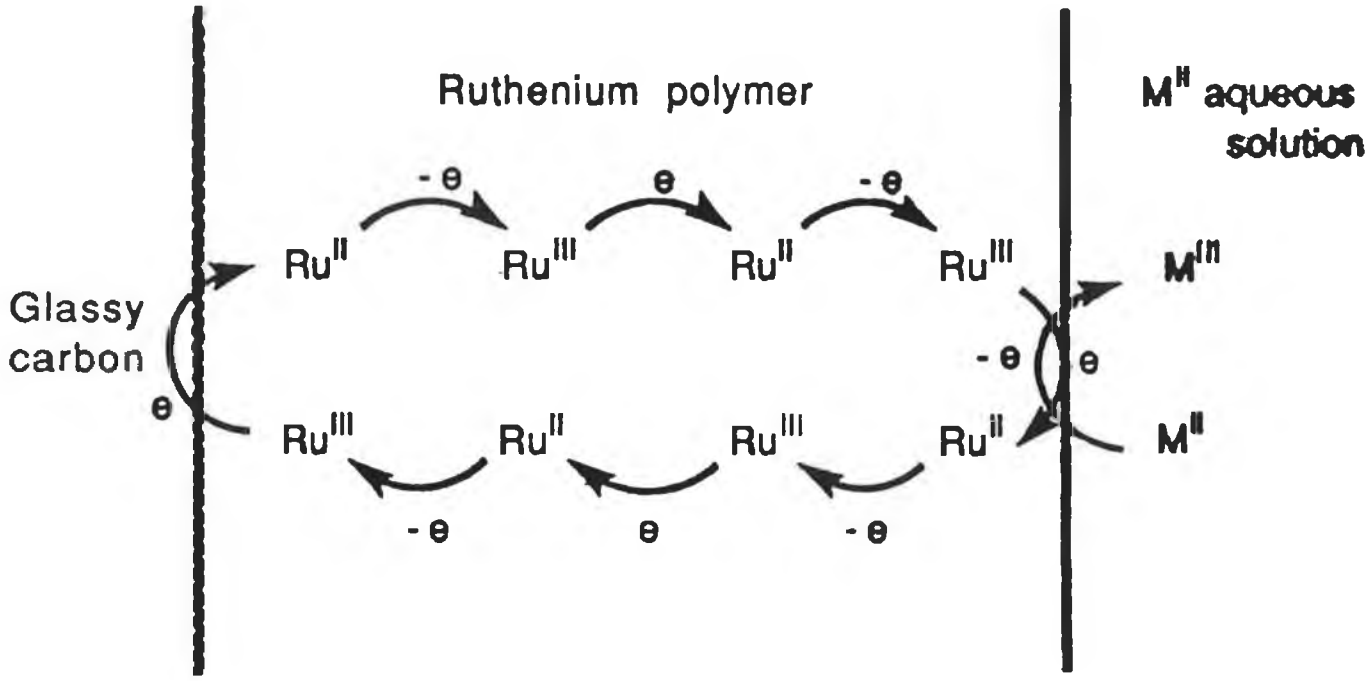

Figure 2.7. Proposed electron transfer mechanism for a ruthenium redox polymer.

to that depicted in Figure 2.7 may be observed. The analyte migrates to the electrode surface, or as in this case the modifier-solution boundary, where it may then be oxidised or reduced by the electrocatalyst according to:

$$Ru(II) \rightarrow Ru(III) + e$$

$$Ru(III) + Ru(II) \rightarrow Ru(II) + Ru(III)$$

$$Ru(III) + Fe(II) \rightarrow Fe(III) + Ru(II)$$

The overall electron transfer process is much more complicated than that at a clean metal surface. Following electron exchange at the surface, the charge must be transported in some way from the substrate surface to the modified boundary. This may occcur via a charge hopping process (self-exchange reactions) or some limited diffusion procedure. Whether electron hopping or molecular diffusion takes place will depend on which of these processes is faster. In some cases the analytes may diffuse short distances to allow electron hopping to occur. This theory is supported by work carried out by Kuo and Murray [37] who measured d-d distances of 2.5 - 3.4 nm for the ferricyanide ion. For electron self-exchange to occur, distances of 0.92 nm are required. However, it has also been pointed out that the mechanism of transport will vary with analyte concentration and also with the morphology of the polymer [38]. Responses obtained at mediator electrodes can be further complicated if some of the analyte diffuses through to the bare electrode surface.

A theoretical treatment of charge hopping has been undertaken by A.R. Hillman [32]. In this study he derives an equation relating the observed current, i_F, to the sum of the fluxes of direct (j_Y) and mediated (j_B) charge transfer and the concentration gradient at the electrode/interface. The equation is given as follows:

$$(i_F/nFA) = j_o = j_B + j_Y = -D_E\ db/dx + D_Y\ dy/dx = k_{ME}y \quad (3.42)$$

where D_E is the diffusion coeffecient of the electron, D_Y is the diffusion coefficient of the analyte and k_{ME} is the effective rate constant.

This equation was derived for a rotating disk electrode, as this is the principal technique used for quantitative data acquisition at modified electrode surfaces. This is the case as the controlled steady state solution mass transport properties at rotating disk electrodes give straight forward access to the concentration of analyte at the polymer/solution interface from its bulk solution value [32]. Consequently, most theoretical treatments are analysed in this form.

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Chapter 3

The Analysis of Trace Metal Ions in Anaerobic Adhesives

3.1. Introduction

3.1.1. Loctite - A Company Profile

Loctite is a worldwide organisation, manufacturing adhesives and sealants for many industries and trades. The Loctite corporation was founded in the U.S.A. in 1953 by Vernon K. Krieble, Professor of Chemistry at Trinity College in Hartford, Connecticut. The venture was based on a patent describing the first anaerobic adhesive, a liquid that could be used to bond metal parts in the absence of oxygen, that cures at room temperature [1]. The company expanded rapidly and today has centres at fifteen locations worldwide. The company's headquarters are still based in Connecticut, where the main manufacturing base for the United States is situated. The main manufacturing and Research and Development centres for Europe are located in Dublin. This plant supplies 90% of Loctite products for the European market at present.

Loctite (Irl) Ltd. was established in the early 1970's with a manufacturing plant in Ballyfermot. The Research and Development facility was set up in Tallaght in 1976. The success of the company necessitated the opening of another large production centre in Tallaght in 1981. At present, in Dublin, the company employs 300 people in its various facilities. Sixty of these work in the Research and Development section, which accounts for nearly 50 % of the total people, 130, employed in Loctite Research and Development centres worldwide.

3.1.2. Loctite Products

A list of the categories of products manufactured by Loctite worldwide are shown in Table 3.1. Anaerobic adhesives and sealants and cyanoacrylate adhesives are the major products manufactured in Dublin. The present study was based on analysis of some of the anaerobic adhesives and sealants.

3.1.2.1. Anaerobic Adhesives and Sealants

Anaerobic adhesives and sealants were introduced specifically for use in mechanical engineering applications. They are formulated to give a wide range of products with various strengths and viscosities. These products are employed in a wide variety of bonding, locking, sealing and retaining applications. They are designed to remain in the inert liquid form in air, but once confined between closely-fitting, active metal surfaces (e.g. nut and bolt), they polymerise, in the absence of air, into tough heat and solvent-resistant solid materials, which bond the parts together.

The major component in any of these formulations is the monomer used. In these studies, we concentrated on products based on methacrylate monomers; namely, polyethylene glycol dimethacrylate (PEGMA) and triethylene glycol dimethacrylate (TRIEGMA). These formulations are produced and marketed by Loctite Irl. The chemical formula for these monomers are shown in Fig. 3.1.

These monomers both contain reactive vinyl side-chains which can polymerise in the presence of

Table 3.1. Loctite Products

Anaerobic Adhesives and Sealants (methacrylates)

Methacrylate Primers

Superglues (cyanoacrylates)

UV Curing Adhesives

Epoxy Based Adhesives

Silicone Based Adhesives

Rust Primers

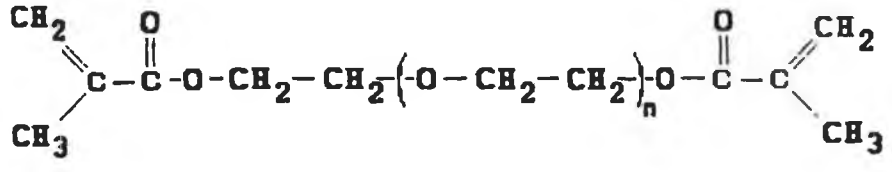


Figure 3.1. The chemical formula of methacrylate monomers, where n = 3 for TRIEGMA and n = 4 or more for PEGMA.

persulphate, peroxide or other redox catalyst systems to form polymeric materials which are resistant to heat, water, acids or alkalis and sunlight.

There are two basic types of polymerisation; addition polymerisation and condensation polymerisation. Vinyl polymers, such as the methacrylate monomers, polymerise by addition processes. In addition, initiation is achieved by addition of a free radical to the terminal double-bond of the monomer molecule (Fig. 3.2.). In the case of anaerobic adhesives, if anaerobic conditions are maintained, the methacrylate free radical thus formed will add on to the terminal double-bond of another monomer molecule. Chain propagation proceeds as the growing methacrylate free radical sucessively attacks more terminal double-bonds, extending the length of the polymer chain with each addition. Termination of the chain occurs when two free radicals combine or when no more unreacted terminal double-bonds are available.

The generation of free radicals for polymerisation may take place by two major routes: (i) thermal cleavage and (ii) redox reaction. The radicals necessary for polymerisation of the methacrylate monomers in anaerobic adhesives and sealants are produced through a redox mechanism. Transition metal ions such as copper(II) and iron(III) can initiate such redox polymerisation reactions according to the following reaction mechanism.

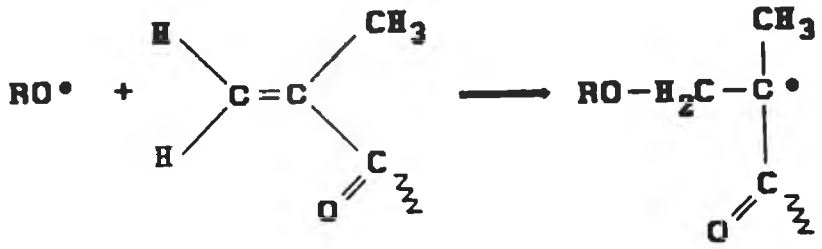
$$ROOH + M^{n+} + H^{+} \longrightarrow RO^{\cdot} + M^{(n+1)+} + H_2O$$

$$ROOH + M^{(n+1)+} \longrightarrow ROO^{\cdot} + M^{n+} + H^{+}$$

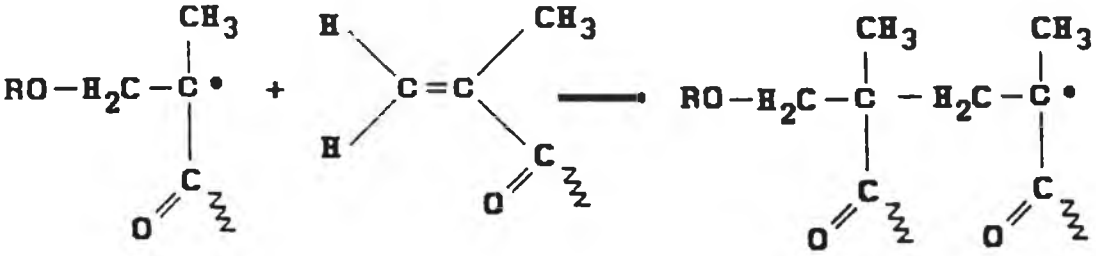
$$2\ ROO^{\cdot} \longrightarrow 2\ RO^{\cdot} + O_2$$

$$RO^{\cdot} + H_2C{=}CH_2 \longrightarrow ROCH_2CH_2^{\cdot}$$

1. Initiation



2. Propagation



3. Termination

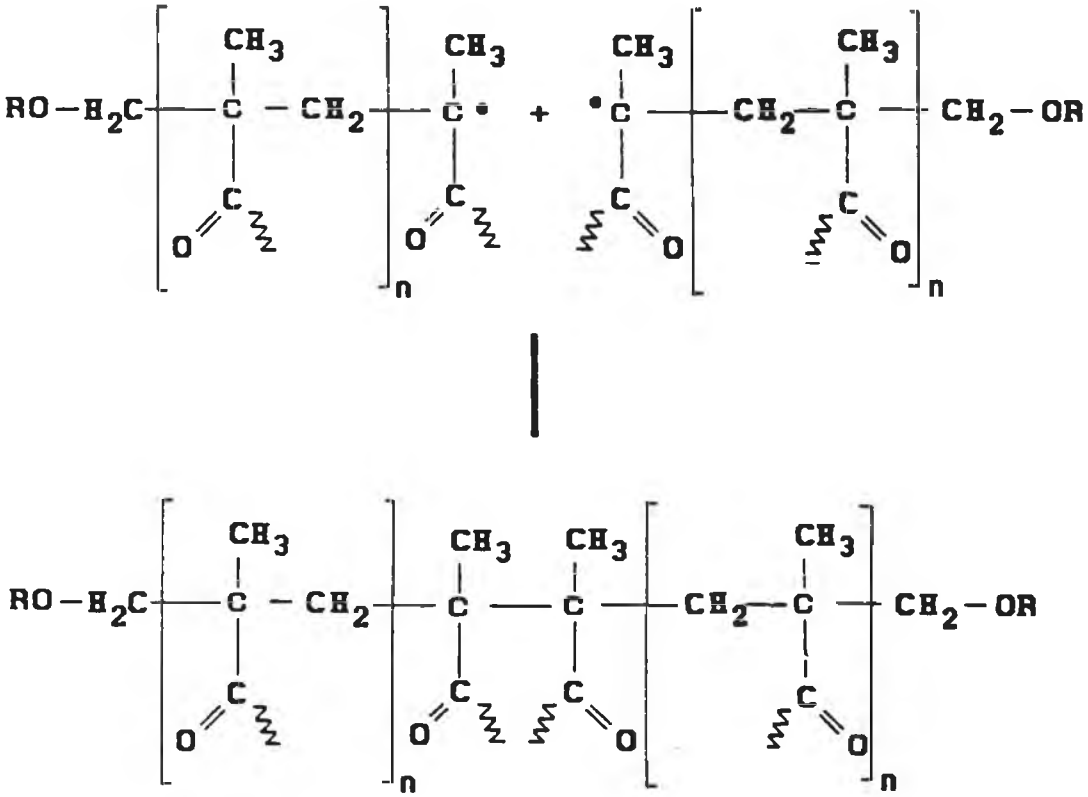


Figure 3.2. Polymerisation of methacrylate monomer in the presence of a free radical.

This can be advantageous, in that low temperature polymerisation favours linear polymers which result in better adhesive properties. In addition, better control of polymerisation rates and more efficient catalytic activity result when redox systems are used. In practice, polymerisation of adhesive applied to a metal surface is readily initiated, but can be a problem if the metal ion is present in the formulation to start with. Hence, the analysis of such formulations for the presence of transition metal ions is important for Quality Control purposes. To overcome some of the problems associated with these free metal ions, chelating agents are included to "mop-up" contaminating metal ions which would encourage polymerisation and so shorten shelf life.

Apart from the monomers and catalysts used, typical formulations also contain accelerators (such as amines, strong acids and reducing agents), stabilisers (such as phenolics and chelating agents), thickeners, plasticisers and fillers, all of which are possible sources of trace metal contamination.

3.1.2.2. Cyanoacrylate Adhesives (Superglues)

Cyanoacrylate adhesives offer advantages in speed, strength and simplicity. They bond in seconds at room temperature, and will join metals, plastics, rubbers, ceramics, wood, leather, paper and cork in any combination. These adhesives also have a high resistance to humidity and solvents.

In this case , the monomer involved is the cyanoacrylate molecule. An acidic stabiliser is used in minute amounts to keep the adhesive in the liquid form in the bottle

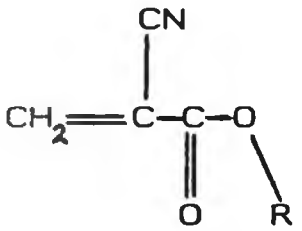
(Fig. 3.3.). When the liquid adhesive is applied to the surfaces to be bonded, partially ionised molecules of water vapour, which are normally found on surfaces exposed to the atmosphere, react with the stabiliser molecules, and thus neutralise each other (Fig. 3.4). The lack of stabiliser and the catalytic action of the remaining water vapour induces polymerisation. The hydroxyl groups of the ionised water act as nucleophiles, and polymerisation proceeds via nucleophilic attack of the cyanoacrylate monomer molecules. The adhesive molecules begin to link up from the surface to form a polymer. As the chains from both surfaces grow and interweave, they produce a bond with high structural strength.

3.1.2.3. Other Adhesives

Other Loctite products include ultraviolet-curing adhesives, epoxy resins, acrylics and silicone adhesives. Recently, there has been a great deal of activity in the development of adhesives for the electronics industry. The company also manufactures lubricating and cleaning materials. These products are not manufactured on a large scale in Ireland and as their chemistry was not significant in relation to this project, they will not be discussed further.

3.1.3. Sales and Markets

Loctite products are sold worldwide to industrial, domestic and automotive maintenance markets. Loctite (Irl) Ltd.



Where R = H or alkyl

Figure 3.3. The chemical structure of a typical cyanoacrylate monomer.

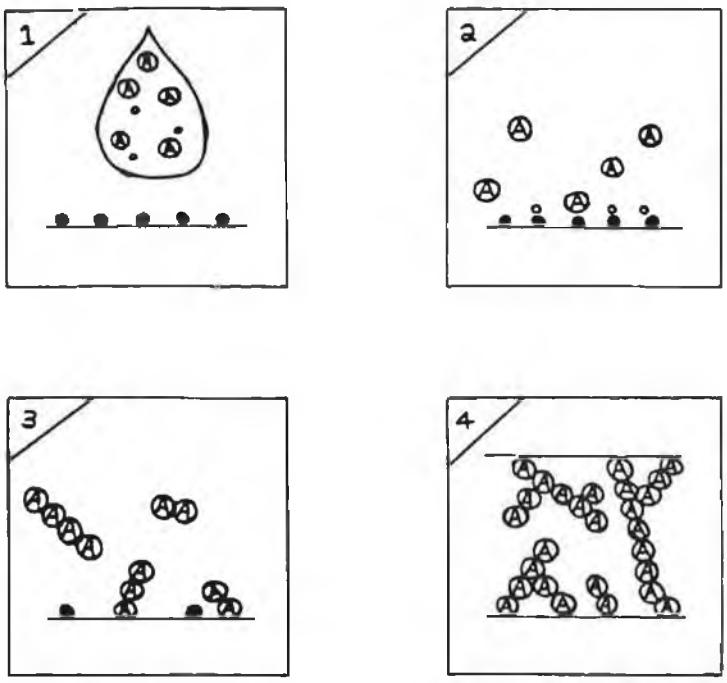


Figure 3.4. The curing mechanism for cyanoacrylate ashesives, where o are the acidic stabiliser molecules, ● are partially ionised water vapour molecules and Ⓐ are the cyanoacrylate molecules.

supplies 90% of the European Market, parts of Asia and are currently exporting to Japan. The European headquarters are based in Paris. All sales staff are time-served engineers, trained by Loctite in the U.K. Distributers are also trained and attend technical seminars arranged by the company.

3.1.4. Determination of Trace Metal Ions

3.1.4.1. Atomic Absorption Spectrometry (AAS)

In the Loctite laboratories AAS is routinely used especially for the analysis of Cu(II) and Fe(III). These transition metals act as activators and are desirable up to a certain level in various raw materials, intermediates and products. However, at higher levels these metals cause premature curing, and therefore shorten the shelf life of the products.

AAS is used for the analysis of Cu(II) and Fe(III) following either extraction or ashing of the adhesive sample. The extraction procedure involves the use of dilute hydrochloric acid and the extraction is carried out from a solution of the material in chloroform. Alternatively, the sample is ashed in a muffle furnace followed by boiling with dilute HCl to produce a concentrated solution of the metals.

Both of these procedures result in the determination of total metal content, with the ashing procedure resulting in more accurate values. However, the total metal content is not always a good indicator of stability, as only the free metal ions will initiate the redox process and cause premature

curing. AAS also has the disadvantage that each metal must be determined individually which is a time consuming process. Therefore, there is a need for a multi-element approach which can determine "free" metal concentration. This chapter discusses the development of two approaches which were examined in this regard: (i) the determination of trace metals as metal complexes using high performance liquid chromatography (HPLC); and (ii) the direct voltammetric determination of trace metals in a typical anaerobic adhesive coated on a glassy carbon electrode.

3.1.4.2. HPLC of Metal Complexes

The determination of trace metals using HPLC-based technology, where a chelating agent has been added to the mobile phase, has been a topic of much interest in recent years. In particular, the dithiocarbamates have received much attention in this regard. Uden and Bigley [2] reported a method for the separation of Cu(II), Ni(II) and Co(III) as their diethyldithiocarbamate complexes using sodium diethyldithiocarbamate (NaDEDTC) as the ligand in a normal-phase HPLC system. Schwedt [3] described a reversed-phase system for the determination of Cu(II), Ni(II) and Pb(II) using the same ligand. Bond and Wallace [4] further developed this approach by forming metal-dithiocarbamate complexes "in situ". This was achieved by incorporating NaDEDTC into the mobile phase, and permitted the direct injection of the metal ions onto the column. This approach proved successful for some metal ions, e.g., Cu(II), but in the case of Cr(III)

and Cr(IV), complexation was found to take up to 2-3 h, rendering this procedure inapplicable for these ions. Bond and Wallace [5] therefore developed a procedure which involved the formation of the DEDTC complexes of the metal ions externally before injection onto the reversed-phase column which contained NaDEDTC in the mobile phase. In this way, these authors were able to determine Cu(II), Ni(II), Co(III), Cr(III) and Cr(IV) simultaneously.

For anaerobic adhesives, the main metallic contaminants to be determined are Cu(II) and Fe(III) as it is known that these ions are mostly responsible for the premature curing of the adhesives in their containers. Until now, there have been no reports on the simultaneous determination of these metal ions as dithiocarbamate complexes by HPLC. Compared with this group of ligands, there has been relatively little work reported on the use of 8-hydroxyquinoline (oxine) as a possible alternative. A paper by Bond and Nagaosa [6] highlighted the possible use of this reagent for the separation of Cu(II) and Fe(III) using HPLC. A recent paper has also demonstrated the possibility of using this ligand as a pre-column chelating agent for HPLC [7]. In this paper the separation of eight metal-oxine chelates, including Cu(II) and Fe(III), was reported. It was decided, therefore, to investigate oxine more fully in the context of the determination of these metal ions in some typical anaerobic adhesives.

3.1.4.3. Analysis using a direct voltammetric approach

The voltammetric method investigated in the course

of this work involved the electrochemical analysis of the adhesive following application of the adhesive formulation directly onto the surface of a glassy carbon electrode. This approach, utilising in effect a modified electrode, should minimise changes in metal speciation during sample pretreatment and should provide a simple analytical method amenable to automation.

Procedures suitable for the incorporation of chemical or biochemical substances or ion-exchange sites onto electrode surfaces have been developed in various laboratories [8-12]. Such modified surfaces can then be used to perform a variety of functions. They have been employed to preconcentrate analytes prior to voltammetric analysis and hence improve sensitivity. For example, dimethylglyoxime has been used to preconcentrate Ni(II) [9] and EDTA to preconcentrate Ag(I) [10]. Alternatively electrocatalysts have been attached to electrode surfaces, and by speeding up what would otherwise be a sluggish electron transfer process, increased sensitivity has been attained [11]. Another approach has been to improve performance by covering conventional electrode surfaces with a protective membrane. By excluding species on the basis of size or charge, then increased selectivity has been obtained [12].

To date, no literature pertaining to the approach adopted in this study, i.e. the application of the analyte directly onto the electrode surface, have been found. This approach offers a very simple method of analysis that has the advantages that: (i) very little sample is required; and (ii) since this is a direct approach, little or no chemical changes in the species to be analysed should occur.

3.2. HPLC Analysis of Cu(II) and Fe(III) in Typical Anaerobic Adhesive Formulations

3.2.1. Experimental

3.2.1.1. Reagents and Standard Solutions

All chemicals used were of analytical reagent grade. All raw materials used in the anaerobic adhesive formulations and the product formulations were supplied by Loctite (Tallaght, Ireland). All aqueous solutions were prepared in distilled water which was further purified by passing it through a Milli-Q water purification system. All organic solvents were of HPLC grade. Sample preparation cartridges (Sep-Pak) were obtained from Waters Associates. The C_{18} column used was obtained from Supelchem, and was a 25 cm x 4.6 mm i.d. stainless-steel column containing LC-18-DB (5 μm particle size) packing material. A guard column of C_{18} packing material was used to protect the analytical column.

3.2.1.2. Instrumentation

The HPLC system used consisted of an Applied Chromatography Systems (ACS) Model 352 ternary gradient pump connected to a Rheodyne 7125 injection valve and a Shimadzu SPD-6A variable-wavelength spectrophotometric detector. Ultraviolet and visible spectra were obtained using a Shimadzu Model UV-240 scanning spectrophotometer.

3.2.1.3. Methods

3.2.1.3.1. Optimisation of complex formation

Each test solution was prepared by taking 100 µl of a 1 x 10^{-2} M solution of the metal ion and making up to 10 cm^3 with the solution under investigation. A delay time of 20 s was employed prior to recording the absorbance of each solution at 400 nm and at 30 s intervals. The effect of ligand concentration, pH, solvent composition and KNO_3 concentration on the rate of complex formation was investigated.

3.2.1.3.2. Extraction Procedure

The anaerobic adhesive samples analysed were based on the monomers polyethylene glycol dimethacrylate (PEGMA) and triethylene glycol dimethacrylate (TRIEGMA). The anaerobic adhesive formulations were made up by sequential addition of the individual constituents, at the appropriate concentrations. A 10 cm^3 aliquot of the sample was extracted with 25 cm^3 of 0.10 M HCl. This solution was then analysed by injecting it directly onto the HPLC column.

3.2.2. Results and Discussion

3.2.2.1. Optimisation of the Complexation Reaction

Before investigating the HPLC separation of the Fe(III)- and Cu(II)-oxine complexes, the important factors

affecting the complexation reactions were investigated using UV spectrophotometry.

3.2.2.1.1. Effect of Ligand Concentration

The ligand concentration was varied between 1 x 10^{-4} and 5 x 10^{-3} M for the analysis of Fe(III) and Cu(II) oxine complexes. The absorbance was measured at 30 s intervals and the results for Fe(III) are shown in Fig. 3.5. The maximum absorbance of the Fe(III) oxine complex (at 400 nm) occurred when the concentration of ligand was 5 x 10^{-3} M (at higher ligand concentrations no further increase in absorbance was noted). Under these conditions the Cu(II) complex precipitated out of solution and therefore could not be analysed.

3.2.2.1.2. Effect of pH

Using a ligand concentration of 5 x 10^{-3} M, and varying the pH of the aqueous phase between 2.0 and 10.0 resulted in low absorbance values for the Fe(III)-oxine complex at pH 2.0, and in unsteady absorbances with respect to time in solutions of pH greater than 8.0 (Fig. 3.6.). A pH of 6.0 was found to be optimum, taking into consideration the absolute value of the absorbance obtained and the time taken to record a steady value. Under these conditions, the Cu(II)-oxine complex again precipitated out of solution.

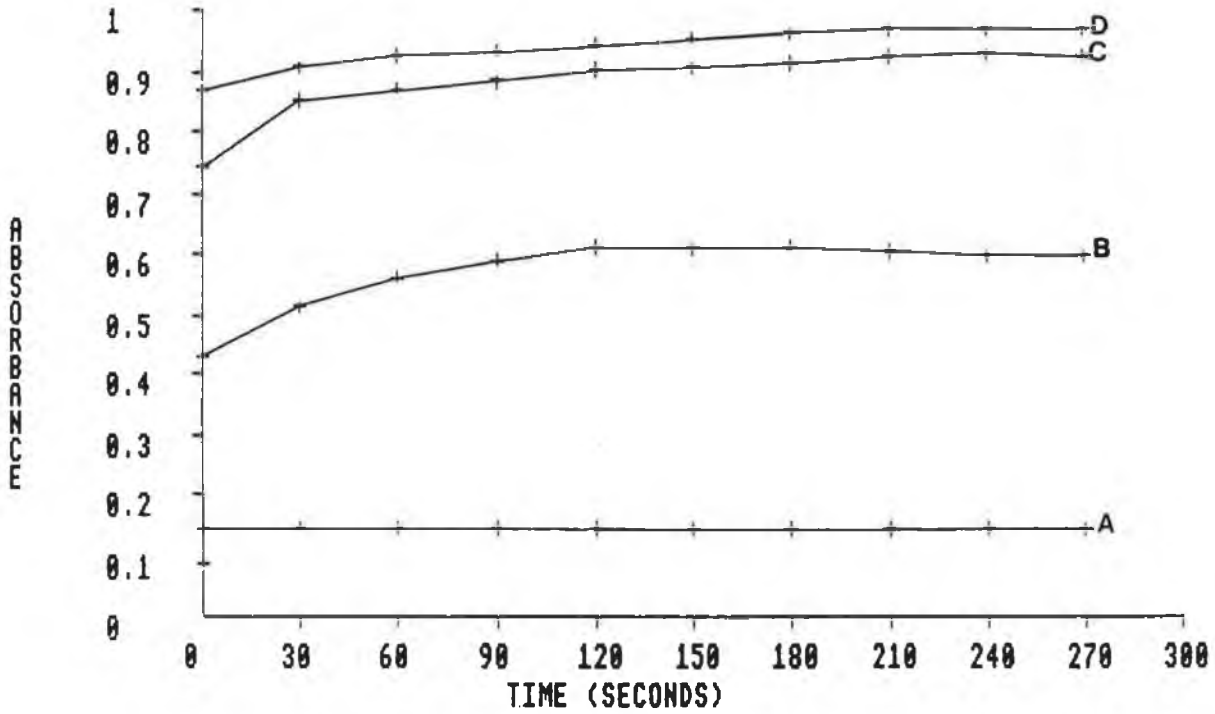


Figure 3.5. The effect of ligand concentration on absorbance at 400 nm for a 1 x 10^{-4} M Fe(II) solution with:

A = 5 x 10^{-4} M;

B = 1 x 10^{-3} M;

C = 2.5 x 10^{-3} M; and

D = 5 x 10^{-3} M ligand.

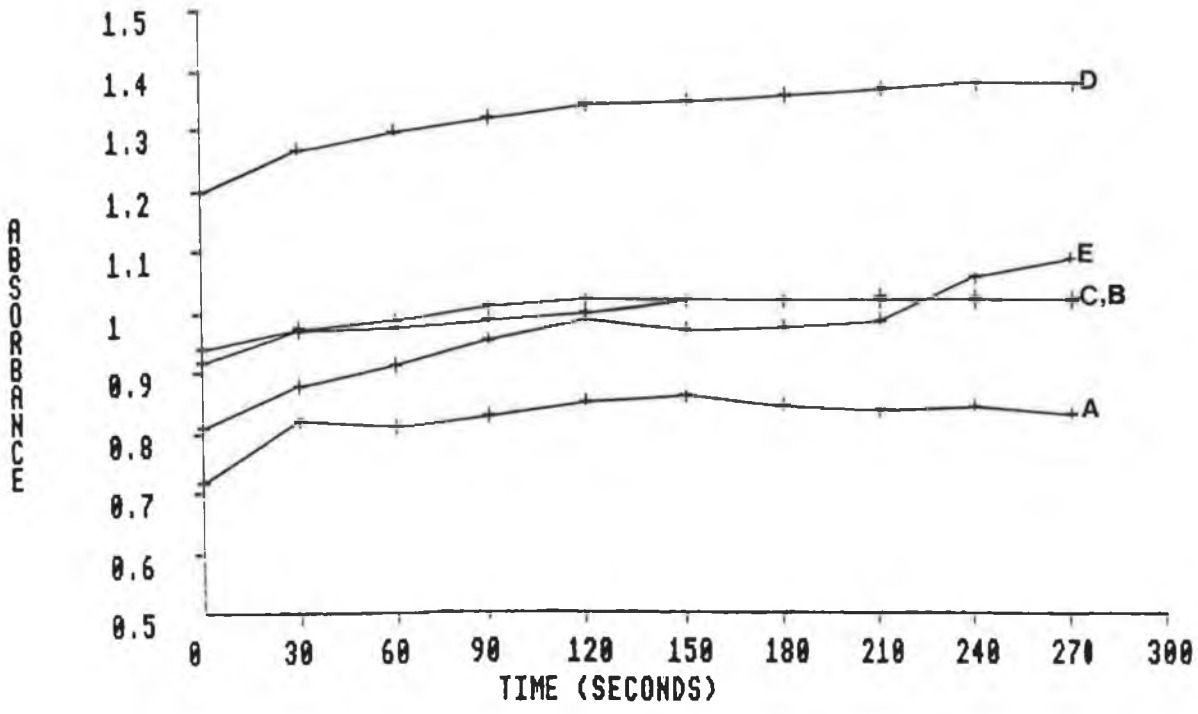


Figure 3.6. The effect of pH on absorbance at 400 nm with a 1 x 10^{-4} M Fe(III) solution and a ligand concentration of 5 x 10^{-3} M at:

A = pH 2.0;

B = pH 4.0;

C = pH 6.0;

D = pH 8.0; and

E = pH 10.0.

3.2.2.1.3. Effect of Solvent Composition

Solvent compositions of 80:20, 60:40, 50:50 and 40:60 0.02 M acetate buffer (pH 6.0):acetonitrile were investigated using the optimum ligand concentration of 5 x 10^{-3} M and the optimum pH of 6.0 (Fig. 3.7.). The Cu(II)-oxine complex was found to be unstable at the 80:20 and the 60:40 solvent ratios. Both the Cu(II)- and the Fe(III)-oxine complexes gave rise to the highest absorbances using the 50:50 solvent mixture, suggesting that such solvent mixtures should be used for the HPLC analysis.

3.2.2.1.4. Effect of Potassium Nitrate Concentration

As Bond and Nagaosa [6] had reported better stability of the Fe(III)-oxine complex in the presence of KNO_3, it was decided to investigate this factor by varying the concentration of this salt from 0.1 to 0.8 M. This study was carried out using a ligand concentration of 5 x 10^{-3} M at pH 6.0 in a solvent system of 50:50 0.02 M acetate buffer: acetonitrile. With Fe(III), the complex was found to give the steadiest reading of absorbance using a KNO_3 concentration of 0.1 M. On increasing the KNO_3 concentration, slightly higher readings of absorbance were obtained, but these tended to fluctuate. With Cu(II), the complex again gave the highest absorbance reading for a KNO_3 concentration of 0.1 M, but the absorbances decreased on increasing the KNO_3 concentration further (Fig. 3.8.). A KNO_3 concentration of 0.1 M was therefore employed in further studies.

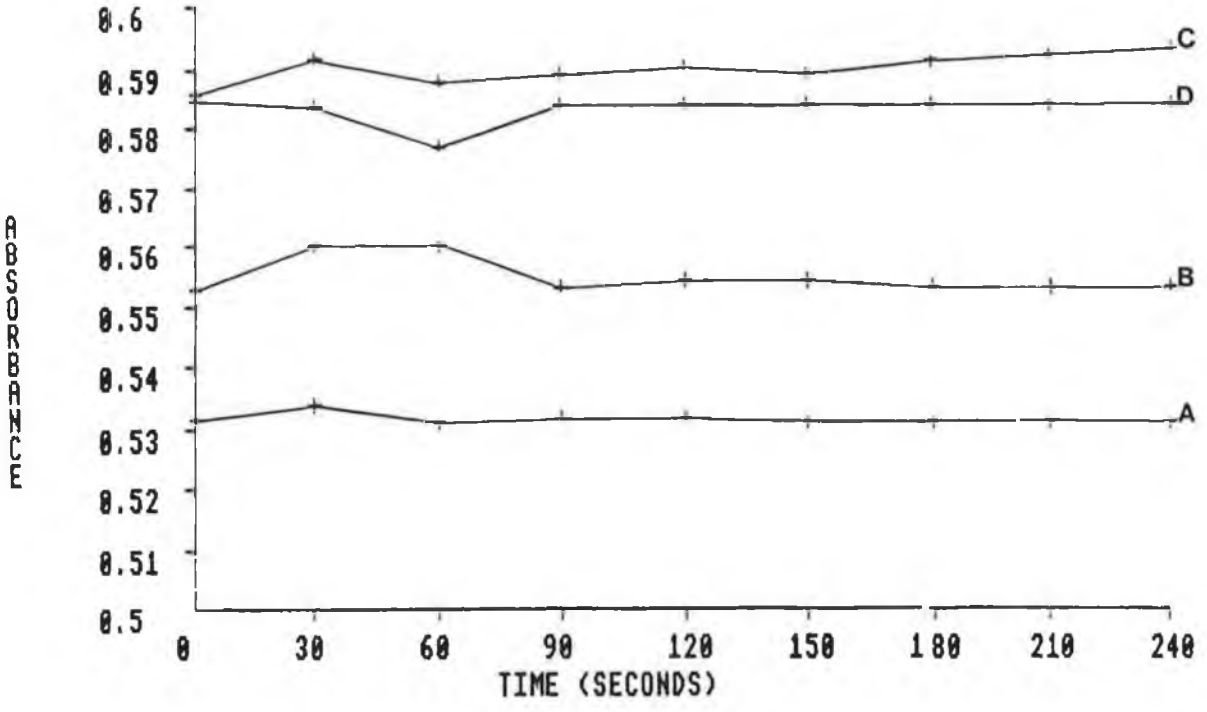


Figure 3.7. The effect of solvent composition on absorbance at 400 nm for a 1 x 10^{-4} M Fe(III) solution with a ligand concentration of 5 x 10^{-3} M at pH 6.0 in:

A = 80:20;

B = 60:40;

C = 50:50; and

D = 40:60 0.02 M acetate buffer: acetonitrile.

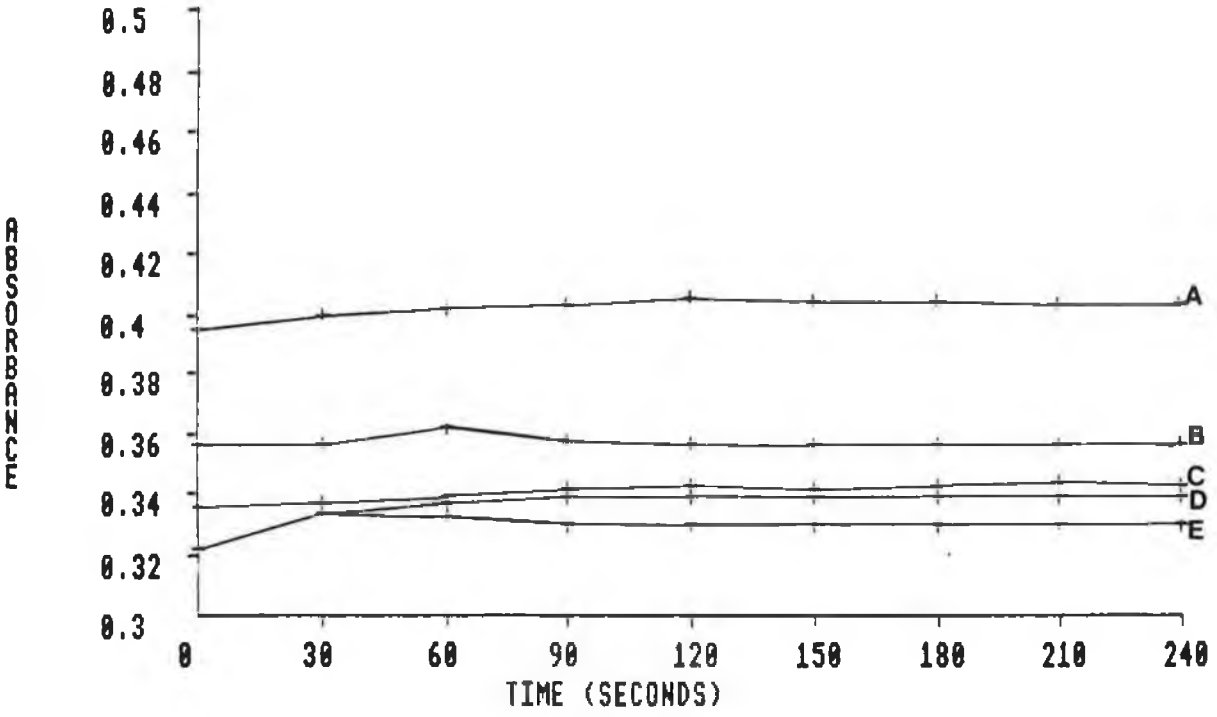


Figure 3.8. The effect of KNO_3 concentration on absorbance at 400 nm for a 1 x 10^{-4} M Cu(II) solution with a ligand concentration of 5 x 10^{-3} M at pH 6.0 in 50:50 0.02 M acetate buffer: acetonitrile at:

A = 0.1 M;

B = 0.2 M;

C = 0.4 M;

D = 0.6 M; and

E = 0.8 M KNO_3 concentration.

3.2.2.2. Optimisation of the HPLC Separation Process

Based on the spectrophotometric studies, the mobile phases investigated for the separation of the metal complexes contained acetonitrile (made 1 x 10^{-2} M in oxine) and 0.02 M acetate buffer (pH 6.0, made 0.2 M in KNO_3) in various ratios. The separation of six metal ions, namely Cu(II), Fe(III), Zn(II), Ni(II), Co(III) and Cr(III), was investigated using this system, and the results for four metals are given in Table 3.2. With Ni(II), unsymmetrical peaks were obtained for all solvent ratios investigated, whereas for Co(III), peaks were found to be irreproducible. At 70:30 and 65:35 solvent ratios, it was not possible to resolve the Cr(III)-oxine complex from the solvent front. At 60:40 and 55:45 solvent ratios, it was not possible to resolve the oxine complexes of Zn(II) and Cr(III). At a solvent ratio of 50:50, a good resolution was obtained for four of the metal oxinate complexes (Fig. 3.9. & 3.10.). Increasing further the ratio of the aqueous phase provided no better separation, and mainly had the effect of increasing the retention times of the four peaks. The 50:50 solvent ratio was therefore employed in all further investigations. These studies were carried out using a detection wavelength of 400 nm. When the detection wavelength was lowered into the ultraviolet region, much greater background signals were obtained, presumably due to absorption of the ligand itself. When higher wavelengths were investigated a loss in sensitivity for all of the metal complexes was noted. The UV absorption profiles of the separated metal oxinate complexes can be seen in Fig. 3.10. A detection wavelength of

Table 3.2. Effect of mobile phase composition on retention time(mins).

Metal	Mobile phase Composition (CH_3CN:Buffer)						
	70:30	65:36	60:40	55:45	50:50	45:55	40:60
Cr(III)	-	-	3.3	3.5	3.4	3.5	3.6
Zn(II)	3.5	3.7	3.8	4.1	4.6	5.3	7.1
Cu(II)	4.1	4.5	4.7	5.2	6.0	7.1	9.2
Fe(III)	4.7	5.3	5.9	7.2	9.05	12.1	18.9

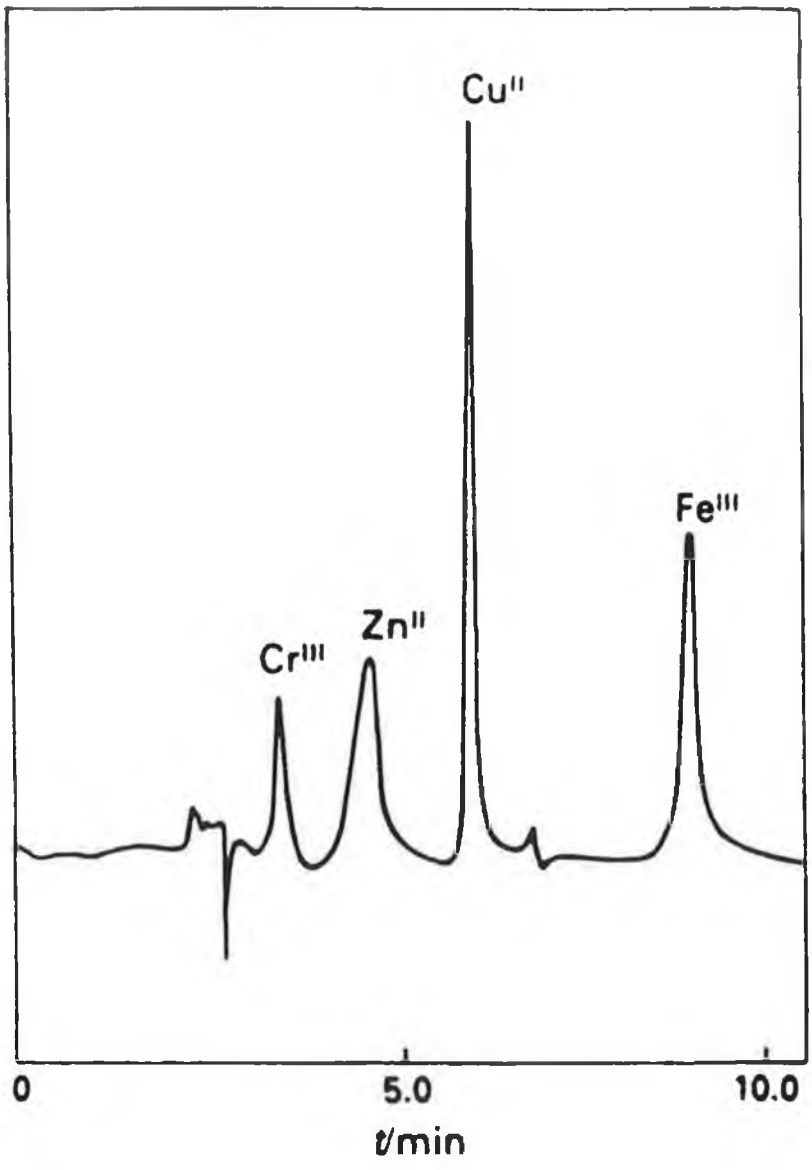


Figure 3.9. HPLC separation of Cu(II), Fe(III), Zn(II) and Cr(III) as their oxinate complexes using 50:50 acetonitrile (made 1 x 10^{-2} M in oxine) : 0.02 M acetate buffer (pH 6.0, made 0.2 M in KNO_3) at 400 nm.

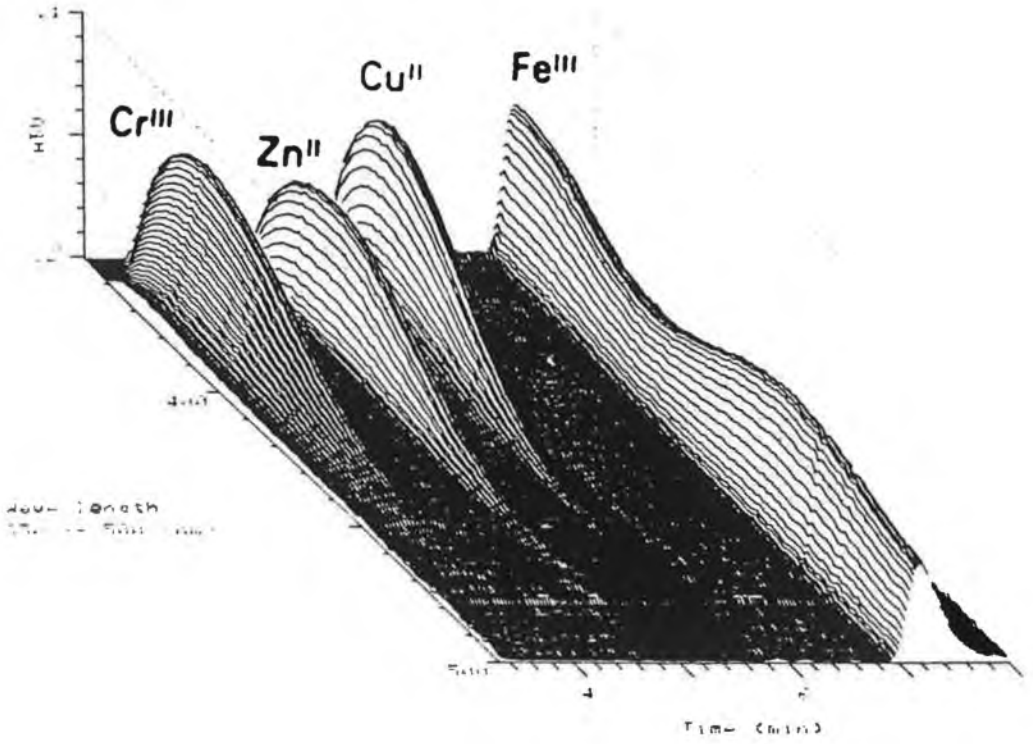


Figure 3.10. The UV absorption profiles of the Cu(II), Fe(III), Zn(II) and Cr(III) oxinate complexes following separation by HPLC using the conditions described in Fig. 3.9.

400 nm was therefore used throughout this project. Based on the optimum conditions, limits of detection of 0.5, 0.8, 1.5 and 5.0 p.p.m. were obtained for the direct injection of standard solutions of Cu(II), Fe(III), Zn(II) and Cr(III), respectively, on to the column.

As discussed by Bond and Nagaosa [6], it is possible to lower the limits of detection by first complexing the metal ions with oxine before injection onto the column. This approach was investigated by taking 50 cm^3 of a 1.0 ppm solution of the metal ion of interest, adding 10.0 cm^3 of 0.5 % solution of oxine in 1.0 M acetate buffer of pH 4.0 and extracting twice with 10.0 cm^3 portions of dichloromethane. The combined extract was then passed through a silica Sep-Pak column and the complex eluted with 2.5 cm^3 of methanol before injection onto the HPLC column. In this way, a 20-fold concentration of the sample was achieved, and the limits of detection of Cu(II) and Fe(III) were decreased to 0.1 and 0.5 ppm, respectively. The main reason why these limits could not be further decreased lies in the problem associated with the background level of these ions in the water that was used in this study. Attempts to purify the water further by passing it through another ion-exchange column have so far proved unsucessful. In addition, the use of commercial HPLC grade water has also been investigated in this regard, but this showed no lower levels of contaminant metal ions than that encountered with the Milli-Q-grade water.

3.2.2.3. Development of an Extraction Procedure

A typical anaerobic adhesive formulation contains 50-80 % of the appropriate monomer, plus minor constituents such as stabilisers, thickeners and plasticisers (Table 3.3.). In the development of an appropriate extraction scheme, the nature of the matrix is a major consideration. Initial studies were, therefore, directed to the efficient extraction of the monomers used, i.e., PEGMA and TRIEGMA. The extractants investigated were 0.1 M HCl, 0.02 M acetate buffer pH 6.0 and 0.02 M phosphate buffer pH 6.0. It was hoped that extraction could be carried out using acetate buffer at pH 6.0, as this extractant could be analysed directly by HPLC. However, this extraction procedure resulted in low extraction efficiencies, especially for the extraction of Fe(III). These losses were thought to be due to the precipitation of a Fe-acetate complex during the extraction procedure. A phosphate buffer of pH 6.0 was then investigated, but again very low extraction efficiencies resulted. As 0.1 M HCl is routinely used by Loctite for the extraction of metals prior to analysis using AAS, this extractant was investigated. Extraction efficiencies of 102 % and 103 % were achieved for Cu(II) and Fe(III), respectively, and analysis of the extract could be carried out directly using HPLC with "in-situ" complex formation.

This extraction technique, coupled with HPLC using "in-situ" complex formation was used for the analysis of the monomer after sequential addition of the individual components listed in Table 3.3, at the concentration level at which they

Table 3.3. Constituents of a typical anaerobic adhesive formulation.

Polyethyleneglycol dimethacrylate (monomer)
Saccharin
Maleic acid
N,N-dimethyl-p-toluidine
1-acetyl-2-phenylhydrazine
Cumene hydroperoxide
Polyethylene glycol di(2-ethylhexanoate)
1,4-naphthoquinone
Na_4EDTA

would normally be present in the final product. A final ormulation was also investigated using this method. These investigations showed that no interferences were co-extracted. As was anticipated, no metal contaminants were detected in the particular samples analysed but when spiked with metal ions the technique showed that it had the capability of detecting Cu(II) and Fe(III) if present above the 0.5 ppm level.

This project was investigated further in association with J.P. Mooney [13], with a view to lowering the detection limits. The extraction scheme described previously, in which the adhesive formulations were extracted with 0.1 M HCl was modified in the following manner. An 0.1 M HCl solution was mixed with a 1 x 10^{-2} M solution of oxine and then extracted with dichloromethane. The organic phase containing the metal-oxinate complexes was passed through a Sep-Pak cartridge, where the complexes were retained. The complexes were later eluted with methanol prior to analysis. Using this extraction scheme, the oxinate complexes were found not to be fully retained on the Sep-Pak cartridges, and on investigation using gas liquid chromatography (GLC) it was found that some of the dimethacrylate species in the adhesives were being co-extracted [13]. These compounds are relatively polar and have high water solubilities, and hence are extracted to a certain extent with 0.1 M HCl. These low relative molecular mass compounds aid the dissolution of metal ion-oxine complexes which are formed later in the scheme and cause their non-retention on the silica Sep-Pak column. This problem was overcome by first dissolving PEGMA in dichloromethane (20 : 80) before extraction with 0.1M HCl. For TRIEGMA-based adhesives, formulations were dissolved

in dichloromethane on a 50 + 50 basis.

Using this extraction scheme extraction efficiencies for Cu(II) and Fe(III) at the 1 ppm level from PEGMA were found to be 85 $\pm$ 3 and 100 $\pm$ 5 % and from TRIEGMA 78 $\pm$ 3 and 100 $\pm$ 5 %, respectively. The limits of detection for Cu(II) and Fe(III) in PEGMA were 250 ppb and 600 ppb and in TRIEGMA 100 and 250 ppb, respectively. This extraction technique was also investigated for the analysis of the individual components as they were added sequentially to the PEGMA and TRIEGMA monomers. On addition of the individual components to PEGMA a slight increase in extraction efficiency was noted, this was presumably due to the presence of metals in the components. However, for TRIEGMA, the extraction efficiency for both metals decreased by about 25-30 % on addition of some of the components listed in Table 3.3. The reason for this decreased extraction efficiency has not yet been fully resolved, but is probably due to some form of complexation or physical change in the sample formulations containing this monomer and the metal ions of interest.

Two final formulations were also investigated using this method. Both formulations investigated contain EDTA at the 300-400 ppm level in order to complex any "free" metal ions which are present. Although EDTA is present at this concentration level in total, it is only present in the above formulation in the "soluble" form to the extent of approximately 20 ppm. A series of experiments were therefore carried out in which the products were spiked with Cu(II) and Fe(III) at levels between 1.0 and 50.0 ppm. Levels of these ions were only detected by the HPLC method when levels greater

than about 20.0-25.0 ppm Cu(II) or Fe(III) were spiked to the formulations. This indicates that the method developed is specific for covalent metal ions which are soluble in 0.1 M HCl and which form a stronger complex with oxine than any other complexing agent in the system.

3.3. Direct Voltammetric Determination of Trace Metal Ions and Other Constitutents in Typical Anaerobic Adhesive Formulations

3.3.1. Experimental

3.3.1.1. Reagents and Standard Solutions

The adhesive sample and the individual components which make up a typical adhesive formulation (Table 3.3) were supplied by Loctite (Irl) Ltd. The Cu(II) and Fe(III) standard solutions were prepared from their corresponding nitrate salts (Ajax Chemicals) in distilled water which had been passed through a Milli-Q water purification system. Sodium dodecyl sulphate (SDS) (Sigma) supporting electrolyte solutions were also prepared in this water. All reagents used were of analytical grade.

3.3.1.2. Instrumentation

A Princeton Applied Research Corporation (PARC) Model 174 Polarographic Analyser was operated in conjunction with a three electrode cell system which employed a platinum auxilary electrode and a Ag/AgCl reference electrode. Square wave voltammograms were generated using a Bioanalytical Systems (BAS) Model 100A Electrochemical Analyser. All voltammograms were recorded on an Omniscribe X-Y recorder.

3.3.1.3. Methods

A 3.0 mm diameter glassy carbon electrode (Metrohm) was prepared by diamond polishing down to 3 μm, followed by a buff with aluminium oxide. The electrode was then held at +1.80 V for 30 s in 0.1 M SDS supporting electrolyte. A 10 μl sample of the adhesive solution (prepared by dissolving 0.05 g of the adhesive in 0.5 ml acetone) was then applied to the surface of the electrode using a pipette and allowed to dry for 3 min. The electrode was then placed in the cell, and the electrode scanned from -0.80 -> +1.80 V in 0.1 M SDS using differential pulse voltammetry (DPV). The electrode was subsequently cleaned between runs by wiping with a tissue soaked in acetone, and the surface repolished with aluminium oxide.

3.3.2. Results and discussion

3.3.2.1. Choice of Solvent

Direct application of the adhesive to the electrode surface was not successful, owing to the fact that the thick coating gave rise to a lack of conductivity, resulting in insensitive voltammetry. Consequently, a variety of solvents were investigated for dissolution of the adhesive. Due to the complex organic nature of the adhesive formulation under study, the formulation is not soluble in aqueous systems, and only sparingly soluble in many organic solvents. The formulation was found to be soluble in glacial acetic acid, acetone and dichloromethane, but only partially soluble in acetonitrile or

methanol. However, mixed solvents such as acetone/water and dichloromethane/acetonitrile were also found to dissolve the adhesive. Since acetone gave rise to the fastest drying time, and produced the most uniform coating, this was the solvent of choice.

In order to achieve the best possible sensitivity, it was required to find the lowest dilution of adhesive in acetone which could be used. This was found to be 0.05 g adhesive in 0.5 cm^3 acetone. At lower volumes of acetone, the adhesive layer became too thick and non-conductive, resulting in a loss of response.

The volume of adhesive/acetone solution applied to the surface was varied and the optimum value found to be 10 ul. At higher applied volumes, the coating again became too thick and non-conductive for analysis. Dip-coating was also investigated as a means of applying the adhesive/acetone solution onto the surface of the electrode, but this proved irreproducible.

Efficient removal of the adhesive from the surface was then achieved by wiping the electrode surface with a tissue soaked in acetone prior to polishing with a slurry of aluminium oxide.

3.3.2.2. Choice of Supporting Electrolyte

It was decided to carry out the analysis using an aqueous supporting electrolyte, since the adhesive coating is insoluble in such media, and the voltammetric behaviour of metal ions in aqueous solutions is well understood. Sodium nitrate,

sodium chloride, tetraethylammonium perchlorate and tetrabutylammonium perchlorate were all investigated in this regard, but did not prove suitable because they gave rise to ill-defined peaks and irreproducible behaviour, presumably due to a lack of surface "wetting". This behaviour was improved upon, however, by employing the surfactant sodium dodecyl sulphate, where the resultant increase in surface contact between the adhesive coating and this supporting electrolyte gave rise to well defined voltammetric behaviour, which could be used for analytical purposes.

3.3.2.3. Electrode Pretreatment

In the initial stages of this project, major problems were encountered with irreproducibility of response. These were attributed to differences in the surface of the glassy carbon electrode between runs. It is well known that electrochemical pretreatment of glassy carbon can be used to control these problems [14-17], and in this work it was found that holding the potential of the working electrode at a high anodic potential (+1.80 V) prior to coating the surface with an adhesive/acetone solution (to which both copper(II) and iron(III) had been spiked at the 10.0 and 3.0 ppm levels), resulted in an increased sensitivity for all the peaks shown in the differential pulse voltammogram (Figure 3.11.). At less positive potentials, the currents obtained following pretreatment were diminished. The optimum time for this pretreatment step was found to be 30 s, after which the currents obtained did not increase any further. The relative standard deviation in the measurement of peak

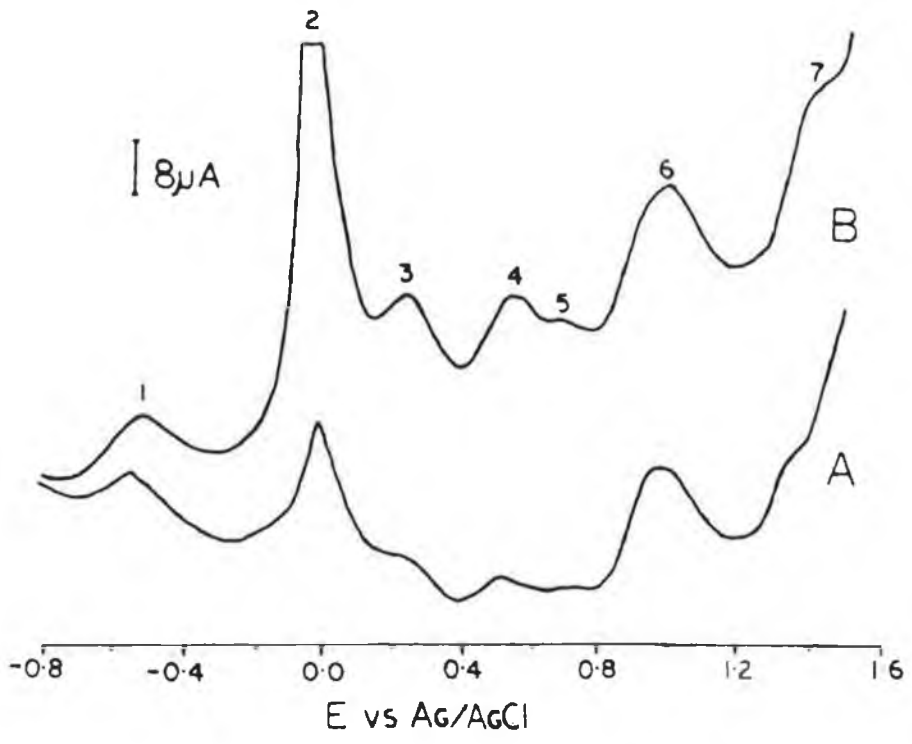


Figure 3.11. The effect of pretreatment on the differential pulse voltammetric behaviour of an ashesive modified electrode.

A. Non-electrochemically pretreated electrode

B. Electrochemically treated electrode

Conditions: scan rate 10 mV s^{-1}; duration between pulses 0.5 s; pulse amplitude 25 mV; pulse width 60 ms.

electrodecurrent was of the order of 3-4% for five electrodes prepared successively in one day, and of the order of 4-6% when peak currents were compared over a one month period.

3.3.2.4. Analysis of the coated adhesive

The analysis of the adhesive was carried out using DPV from -0.8 -> +1.4 V in a 0.1 M SDS supporting electrolyte using a scan rate of 10 mV s^{-1}. Similar experiments were carried out using square wave voltammetry (SWV), but neither the Barker nor Osteryoung variants of SWV gave rise to any further peaks. The Osteryoung variant showed increased resolution, but this was at the expense of loss in sensitivity for some of the peaks. It was decided, therefore, to use DPV for the rest of the study.

In an attempt to identify the peaks shown in Figure 3.11., the glassy carbon electrode was coated sequentially with the monomer, i.e. polyethyleneglycol dimethacrylate, dissolved in acetone, followed by solutions of the monomer which were spiked with the individual components listed in Table 3.3 at the concentration levels normally found in the adhesive formulation. The effects of these additions are shown in Figure 3.11. In this way, peaks 1 and 5 were found to be due to the monomer, peak 4 due to cumene hydroperoxide, and peak 3 due to 1-acetyl-2-phenylhydrazine. The other compounds listed in Table 3.3 did not give rise to electrochemical activity under the conditions used in this experiment and at the concentration levels studied. The only peaks that are therefore not due to constituents of the adhesive are peaks 2,6 and 7. It was decided

to investigate, therefore, whether or not these peaks were due to metal ion contamination.

When copper(II) ions were spiked into the adhesive sample, an increase was seen in peak 2, which increases linearly with concentration between 0.25 - 10.0 ppm (Figure 3.12.). To check if this peak was due to copper(II) ions in the adhesive coating, DPV was carried out using a bare glassy carbon electrode in a 0.1 M SDS supporting electrolyte containing copper(II) ions. This resulted in a peak at + 0.11 V (Figure 3.13a). When the same electrode was removed from this solution washed and coated with adhesive, the peak at + 0.11 V decreased in magnitude, and a new peak appeared at - 0.05 V (Figure 3.13b.), which corresponds to peak 2 seen in Figure 3.11. The height of this peak increased with time, as copper(II) ions presumably diffused into the adhesive coating from the external solution where they were complexed by the EDTA present. When the electrode was again removed from solution, washed and coated with a sample of adhesive spiked with copper(II) ions, a single peak at - 0.05 V was exhibited using DPV (Figure 3.13c.).

These results indicate that peak 2 in Figure 3.11. is in fact due to the $CuEDTA^{2-}$ complex, and this was confirmed by spiking a sample of the adhesive with some of this complex formed externally. When copper(I) chloride was spiked into the adhesive, no additional peaks or increases in existing peaks were found. This indicates that the electrochemical reaction involves oxidation of copper contained within the chelate from the zero to the +2 state. This peak can therefore be used to determine the amount of copper(II) ions complexed within the adhesive product, and the DPV method may also be able to

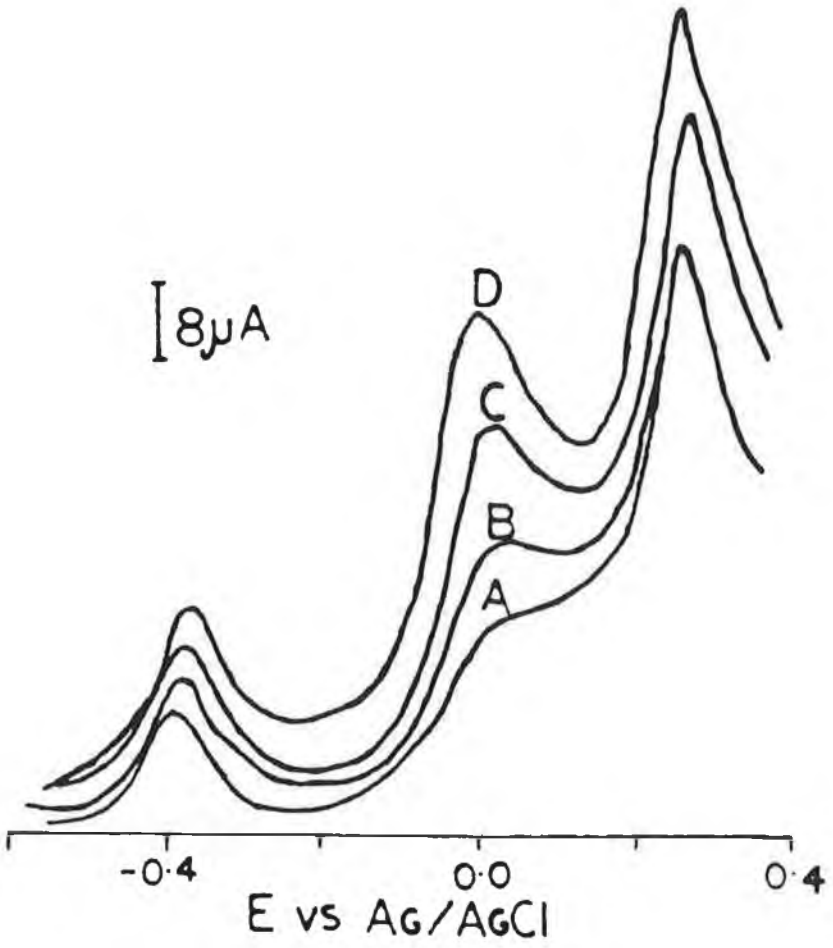


Figure 3.12. Effect of increasing Cu(II) concentration on the height of peak 2 obtained for an electrochemically pretreated adhesive modified electrode.

A. unspiked adhesive

B. A + 0.25 ppm Cu(II)

C. A + 0.50 ppm Cu(II)

D. A + 0.75 ppm Cu(II)

Conditions: as in Figure 3.11.

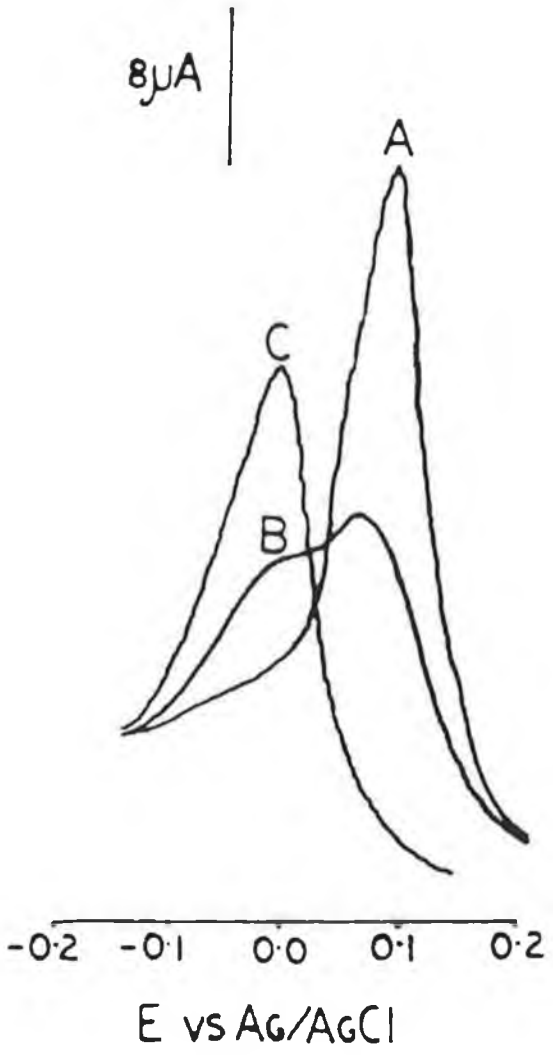


Figure 3.13. Differential pulse voltammograms of Cu(II.)

A. Analysis of 5.0 ppm Cu(II) at a bare glassy carbon electrode in 0.1 M SDS.

B. Analysis of 5.0 ppm Cu(II) at an ashesive modified electrode in 0.1 M SDS.

C. Analysis of adhesive coating containing Cu(II) spiked into the ashesive at the 5.0 ppm level, and run in 0.1 M SDS.

Conditions as in Figure 3.11.

determine "free" from "bound" copper once the complexing capacity of the formulation has been exceeded.

When the adhesive was spiked with iron(III) before coating on the electrode, a new peak appeared (peak 7) which increased in size with 2.0-5.0 ppm additions, after which no further increases were noticed (Fig. 3.11). Addition of $FeEDTA^-$ did not result in the appearance of this peak, and the response for iron(III) at a bare glassy carbon electrode was at + 0.40 V. Addition of iron(II) to the adhesive before coating also produced no new peaks. It would appear, therefore, that iron(III) is complexed to some other constituent in the adhesive formulation, and that it exists primarily in the complexed form.

The reason why one sees a peak for the oxidation of the reduced forms of the monomer, cumene hydroperoxide and 1-acetyl-2-phenylhydrazine, considering that these organics normally undergo irreversible reduction in solution, is not well understood, but could be associated with the fact that these compounds are held within the coating and thus do not diffuse away from the glassy carbon electrode surface.

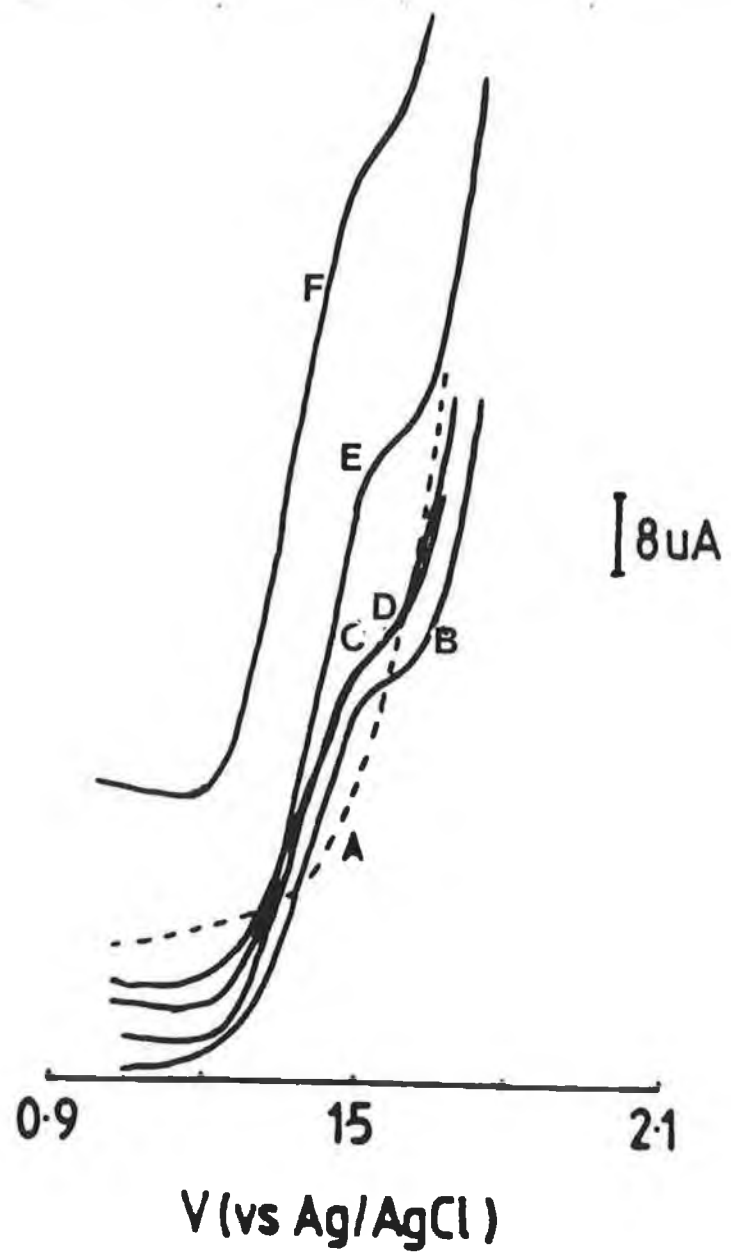


Figure 3.14. Effect of increasing Fe(III) concentration on the height of peak 7 obtained for an electrochemically pretreated adhesive modified electrode.

A. unspiked adhesive

B. A + 1.0 ppm Fe(III)

C. A + 2.0 ppm Fe(III)

D. A + 3.0 ppm Fe(III)

E. A + 4.0 ppm Fe(III)

F. A + 5.0 ppm Fe(III)

Conditions as in Figure 3.11.

3.4. Conclusions

Both of the projects undertaken have shown the possibility of determining metal ions, especially Cu(II) and Fe(III), in anaerobic adhesives. In this respect the possible application of HPLC as a technique for the separation and simultaneous determination of trace metals has been demonstrated. Under optimum conditions Cu(II), Fe(III), Zn(II) and Cr(III) could be analysed simultaneously as their oxinate complexes and the application of this technique for the determination of trace amounts of transition metal ions in anaerobic adhesive formulations has proved successful. The HPLC technique offers the advantage over AAS in that only the "free" metal ions are detected. This is the fraction of metal ions that are of interest in adhesive formulation chemistry as only "free" metal ions will cause premature curing of the formulations. It also offers the possibility of multi-element analysis, thus saving analysis time.

The second study carried out has demonstrated the possibility of using a direct voltammetric approach utilising an adhesive modified electrode to detect some of the major constituents in a typical adhesive formulation. The possibility of determining Cu(II) and Fe(III) within the anaerobic formulation has also been demonstrated. Using this technique both the free and the complexed Cu(II) species could be analysed simultaneously. The analysis of Co(II), Ni(II) and Cr(III) was also investigated but no increases in any of the background responses or the presence of additional peaks were noted within

the potential range -0.8 -> +1.4 V. However, it may be possible that analysis of these metals and many more could be carried out using different conditions of analysis. It may also be possible in the future to adapt such a method for Quality Control purposes, and work is underway to develop adhesive coated microelectrodes in this regard.

3.5. References

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Chapter 4

The Analysis of Trace Metal Ions in Soils and Clays

4.1. Introduction

Soils around the world have developed from the weathering of a wide range of materials, ranging greatly in age from coastal soils whose age can be measured in tens or hundreds of years to ancient rocks millions of years old. The large variation in climate across the continents has resulted in many different soil types. The climate can affect soil weathering either directly due to extreme hot or cold temperatures, or indirectly by promoting or inhibiting plant growth. The weathering processes which result in the formation of soils from the parent materials, include physical effects such as shattering by frost action and chemical change by the dissolving action of water. Vegetation affects the rate of weathering in several ways. Roots can enlarge crevices in rock fragments and split them as they expand. They can also affect the porosity of the soil, and consequently the volume of water flowing through it which speeds up the rate of chemical weathering. As well as removing the more soluble materials, chemical weathering results in the formation of clay from the degradation products of other primary minerals.

Although there are many different soil types, all contain similar constituents although these may be present in different proportions. These are mineral particles of a range of sizes (sand, silt and clay), organic matter, water-containing plant nutrients and air spaces or pores. The properties of these constituents vary not only from soil to soil but also within the same soil. For example, the topsoil usually contains

much more organic matter than in the underlying subsoil. The subsoil frequently contains much more clay than the topsoil but in some soils may contain less [1].

The main chemical constituents of the mineral particles in most soils are silica, aluminium oxide and iron oxide which together account for around 90% of the chemical species present [2]. There are also smaller concentrations of a range of other elements, several of which are plant nutrients. The most important of these are collated in Table 4.1. One of the goals of soil science is an understanding of the mechanisms of the uptake of these nutrients by plants growing in the soil. To achieve this goal it is essential that the concentration and form of a trace element or major nutrient in the soil solution, and the factors that control these, are known and understood. In an effort to understand these processes it is necesssary to examine the structure of the soil itself.

4.1.1. Soil Structure

The size of soil particles varies 10,000 fold from the smallest particles of clay (which cannot even be seen with the best optical microscope to fine gravel) [2]. Soil particles have been graded into 8 different sizes as shown in Table 4.2. There is a significant variation in surface area among these particles, with clay particles having by far the largest surface area. Therefore, studies of the solid phases in soils should focus mainly on those solids that are common in clay fractions.

Soil clays consist mainly of polymers, i.e. compounds or mixtures of compounds formed from the bonding together of

Table 4.1. Elements present in soils

Essential	Functional	Potentially Significant
Na	Cr	Rb
K	Ni	Cs
Mg	Si	Be
Ca	Se	Sr
Mo	Br	Ba
Mn	I	Tl
Fe		W
Co		Ag
Cu		Au
Zn		Cd
B		Hg
C		Al
N		Ge
P		Sn
O		Pb
S		As
Cl		F

Table 4.2. Size and surface area of soil particles

	Size (microns)	Surface area of same volume of diff. size factors
Gravel	2,000 - 20,000	0.1 - 1
Coarse sand	500 - 2,000	1 - 4
Medium sand	200 - 500	4 - 10
Fine sand	100 - 200	10 - 20
Very fine sand	50 - 100	20 - 40
Coarse silt	20 - 50	40 - 100
Silt	2 - 20	100 - 1000
Clay	<2	>1000

repeating molecular units. In clays, the solid phase is said to be "crystalline" if the repeating structure persists throughout a molecular region whose diameter is at least 3 μm. If the structure is regular over distances less than 3 μm, the solid phase is said to be "amorphous" [2]. However, this distinction is only used as a general guide, because among the crystalline solids in soil clays there is wide variation in molecular order, where disorder is introduced by isomorphic substitutions of cations and anions and by irregular stacking of crystalline layers.

With respect to inorganic polymers of soil clays, the most important structural units found are the silica tetrahedron i.e. SiO_4^{4-} and the octahedral complex, i.e. MX_6^{m-6b}, which consists of a metal cation M^{m+} and six anions, X^{b-}. Both of these units can polymerise to form sheet structures as shown in Figure 4.1. Crystalline solids that contain only silica tetrahedral sheets do not exist, but carbonate, oxide, oxyhydroxide and hydroxide solids that have metal cations are widespread (Table 4.3) [3].

Goethite is the most thermodynamically stable of the iron oxides and therefore is the one most commonly found in soils. Gibbsite is the most important of the aluminium minerals listed, whereas birnessite is the most commonly found manganese-containing mineral [3]. The molecular structures of goethite and gibbsite are shown in Figure 4.2.

The organic solids in soils are termed humic substances. They are very complex in nature and there are many uncertainties regarding the structural chemistry of these solids. This renders it nearly impossible to describe a developed

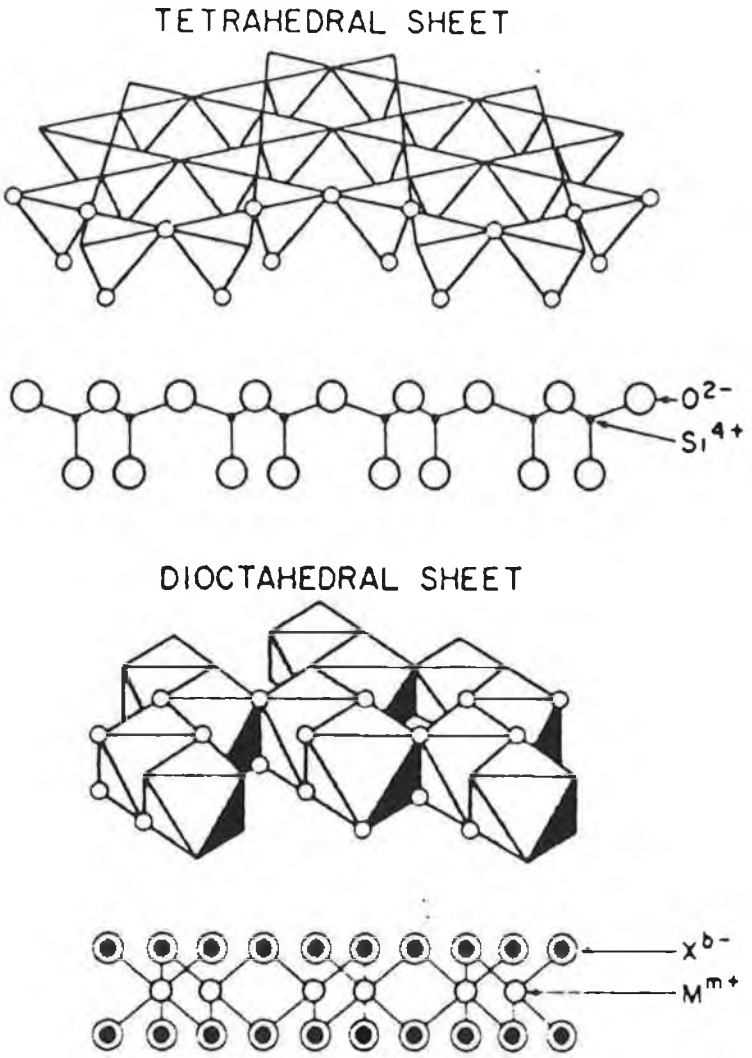

Figure 4.1. Sheet polymeric structures of SiO_4^{4-} and $MX_6^{(m-6b)}$. The open circles at the polyhedral vertices in each perspective view are shown directly below in a projection along the crystallographic a axis.

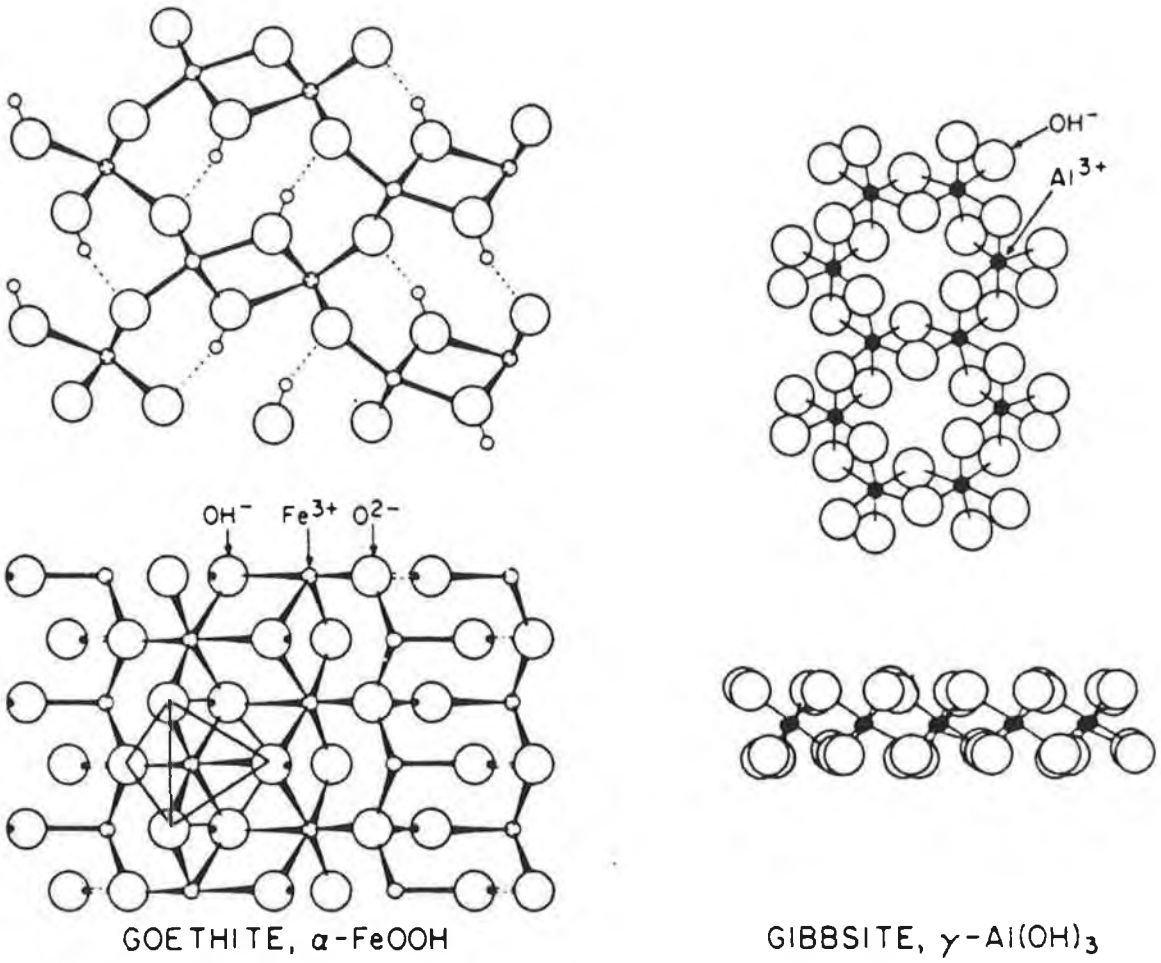

Figure 4.2. The molecular structure of goethite and gibbsite along the crystallographic c axis (upper) and a (lower). Hydrogen bonds in goethite are indicated by dashed lines, and an $Fe(OH)_3$ octahedron is outlined in the a axis projection.

Table 4.3. Metal oxides, oxyhydroxides and hydroxides commonly found in clay fractions of soils (1).

Name	Chemical formula	Name	Chemical formula
Anatase	TiO_2	Hamatite	αFe_2O_3
Birnessite	$Na_{0.7}Ca_{0.3}Mn_7O_{14}$ $2.8H_2O$	Menite	$FeTi_1O_3$
Boehmite	γ-AlOOH	Lepidocrocite	γ-FeOOH
Ferrihydrite	Fe_2O_3 2FeOOH $2.6H_2O$	Lithiophorite	(Al,Li) $MnO_2(OH)_2$
Gibbsite	γ-$Al(OH)_3$	Maghemite	γ-Fe_2O_3
Goethite	α-FeOOH	Magnetite	$FeFe_2O_4$

γ-denotes cubic close packing of anions and α denotes hexagonal close-packing.

molecular structure. However, it is known that they are synthesised from phenolic compounds which result from the decomposition of proteins and carbohydrates. These precursors are shown in Figure 4.3. From this figure it is evident that many chemical and microbological processes mediate the polymerisation reactions leading to the formation of organic solids. Hence, the amount and nature of the humic substances varies greatly from soil to soil [3].

At this stage in time, the metallic species present in soil have been characterised to a certain degree. However, the exact requirements of plants are not fully understood. As mentioned earlier in this report it is necessary for the metal species to be soluble in the liquid phase of soil before these species become available to plants. Even though the solubility of many of these metallic species are known, the exact percentage of metal available to plants has not been fully quantified. Therefore in recent years much work has been carried towards the development of specific extraction techniques.

4.1.2. Sample Pretreatment Procedures

There are many different reasons for requiring analysis of different metal fractions in soil. In recent years the large increase in intensive farming around the world has resulted in the depletion of many essential elements required for growth of plants. To counteract this, minerals have been added to the soil, but if the correct balance is to be maintained the exact percentage of the elements of interest in

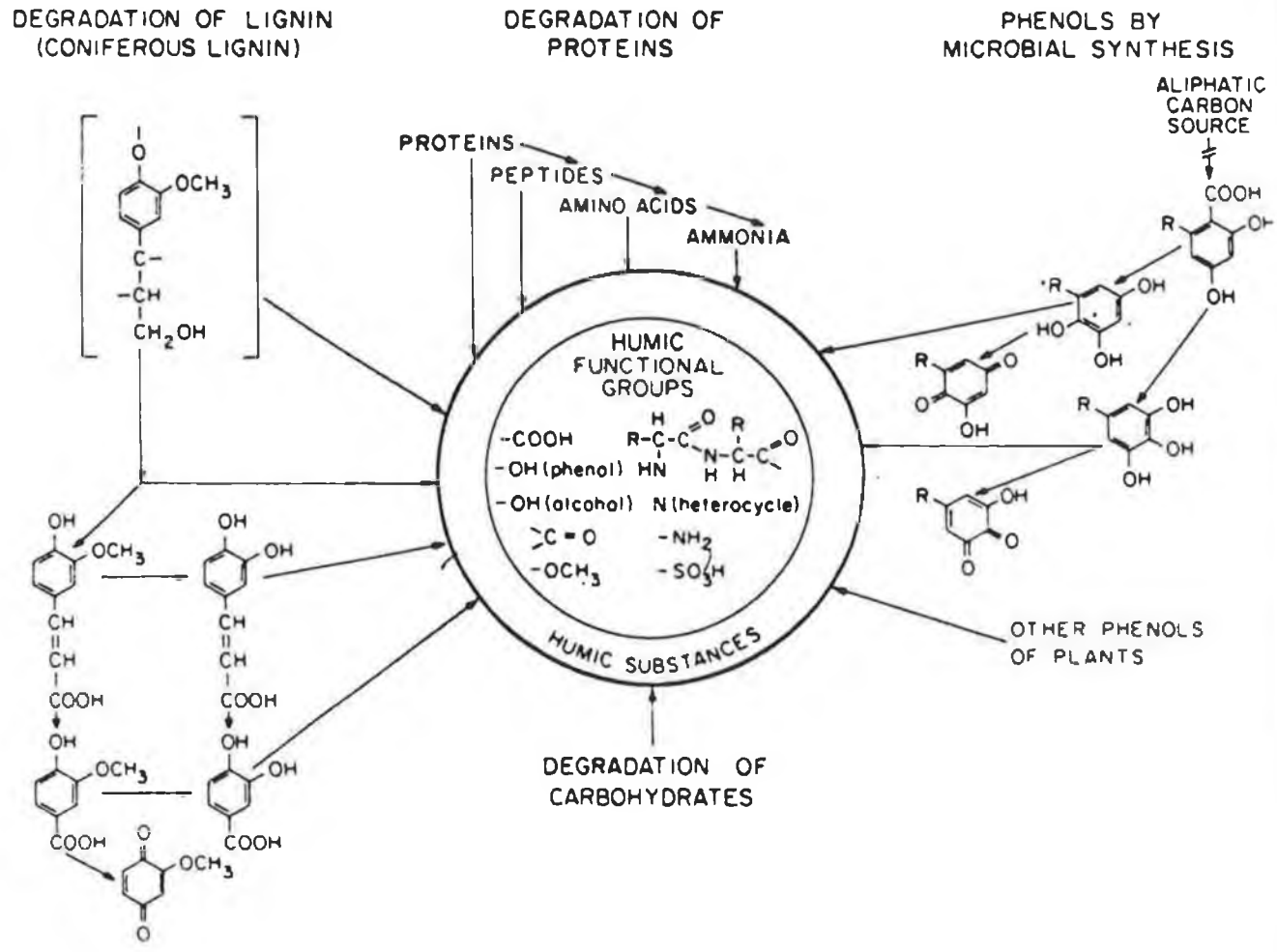

Figure 4.3. The structural precursors of humic substances in soils(1).

soil must be known. However, this is a very difficult task, as the total metal in soil does not represent that percentage which is available to crops. To tackle this problem, selective extraction procedures have been developed, some of which give an indication of the concentration of metal "available" to crops. These extraction techniques act as guidelines, because, owing to the complexity of soil, it would be impossible for any one technique to extract one phase completely without some contamination within the system. Also, as the components of the "available" metals are not fully understood it is not possible to say that any one extraction technique simulates that of plant assimilation.

Extraction techniques can be divided into two main groups:

(i) dissolution techniques and
(ii) digestion techniques

Dissolution techniques do not break down the structure of the soil and tend to represent "available" metal fraction, compared with digestion techniques which are destructive and represent total metal fractions. The most important of these extraction procedures will be discussed with respect to the determination of iron and aluminium.

4.1.2.1. Dissolution Extraction Techniques

There are four main extractions associated with dissolution techniques: the oxalate extraction; the

dithionite-citrate-bicarbonate extraction; the pyrophosphate extraction; and the ethylenediaminetetra-acetic acid (EDTA) extraction. Due to the complexity of soil, dissolution techniques alone cannot identify individual metal compounds, but can be used to divide these compounds into various fractions or phases. A dissolution technique is considered selective, if its dissolution profile reaches a plateau after some time.

Ideally the chosen dissolution technique should dissolve the compound in question quantitatively leaving the other constituents unchanged. For soils and clays no such technique exists because soils normally contain several compounds with similar dissolution properties. Adding to their complexity, the dissolution properties of soil constituents are affected by their crystallinity i.e. big "perfect" crystals are more difficult to dissolve than small "imperfect" crystals. Furthermore, partial substitution of one element by another may increase the stability of the mineral against dissolution, e.g. the dissolution rate of synthetic goethite in HCl was found to decrease markedly when some of the iron atoms were substituted by aluminium [4].

4.1.2.1.1. Oxalate Extraction

The oxalate extraction was originally proposed by Tam [5] for the determination of the components in what he called "the inorganic gel complex", i.e. an undefined mixture of aluminium, iron and silicon oxides in soils. This technique was later modified by Schwertmann [6], to extract the so called "active" fraction of iron oxides in soils. In this

modification, light should be excluded during extraction, because oxalate-extractable iron was found to be very light sensitive. The technique consists of either a 2 hour (according to Schwertmann [7]) or a 4 hour (according to McKeague and Day [8]) extraction with 0.2 M ammonium oxalate at pH 3 in the dark, and is now by far the most popular technique for estimating amorphous, non-crystalline or poorly ordered oxides in soils and clays.

Oxalate "dissolves" metals due to its complexation properties. This dissolution results in clear extracts, and at pH 3 the oxalate system is well buffered, and minor variations in pH have little influence on the oxalate extractable metal. However, if the pH is increased above 4, the buffer capacity is increased and therefore the oxalate-extractable metal is increased [8]. After extraction, the extracts should not be left for too long before analysis, because light induces decomposition of oxalate which may result in precipitation of the metal, or, if stored, the extracts should be protected against light.

The major problem associated with the oxalate extraction is that a dissolution plateau may take weeks to achieve. There is an initial high dissolution rate which is severely reduced after 2 - 4 hours, but it continues to increase until all iron oxides are dissolved. This strong decrease in the dissolution rate after 2 - 4 hours indicates preferential dissolution of a certain metal fraction. Based on investigations of some iron oxide enriched materials from soils, this fraction was shown to consist mainly of ferrihydrite, together with minor amounts of lepidocrocite and goethite.

Preferential dissolution of ferrihydrite, as well as some attack on more well crystallised iron oxides, especially lepidocrocite and magnetite , by the oxalate extraction has also been demonstrated by numerous investigators of synthetic and geological materials [9 - 16]. Because it is a strong ligand, oxalate also extracts metals from organic complexes.

Although the oxalate technique in itself cannot be used for phase identification in soils, it could be a very useful aid for fractionating compounds prior to the real phase identification by X-ray diffraction and Mossbauer spectroscopy. As with the other dissolution techniques, this technique may help to quantify that fraction of metals available to plants.

4.1.2.1.2. Dithionite-Citrate-Bicarbonate (DCB) Ectraction

This extraction was proposed by Mehra and Jackson [17] in 1960 for estimating "free" iron oxides in soil and clay. This technique is based on the fact that iron (II) compounds are more soluble than iron (III) compounds, although even the most soluble iron oxides e.g. goethite may be dissolved provided the redox potential of the extractant is low enough. Using the thermodynamic data collected by Sadiq and Linsay [18], it may be calculated that dithionite, a very powerful reductant at a concentration of 0.05 M can reduce all iron (III) oxides to iron(II) at pH's below 9 -> 10. The lower pH limit is also important, as iron sulphide and/or sulphur may precipitate if the pH drops below 6 -> 6.5. This gives an optimum pH range of 7 -> 8. To achieve this the solution must be buffered. Citrate was therefore added as a ligand to the extraction method as

dithionite in solution is rapidly oxidised by air to sulphate and thereby loses its reducing properties. The presence of the ligand prevents reprecipitation of the dissolved iron. Using this extraction method a sample of soil and clay is extracted for 15 minutes at 370° K at a pH of 7.3 with a citrate-bicarbonate solution, to which is added sodium dithionite. The reproducibility and robustness of this extraction are good. This extraction is also used for the extraction of aluminium compounds.

The compounds which are intended to be "dissolved" using this procedure are primarly oxides of different crystallinity althrough DCB extracts will also include the comparatively small fraction of water-soluble, exchangeable and organic-bound metals. However, the ability of this extraction to dissolve oxides is affected by the size of the oxide crystals, and several investigators [8, 14, 15] have shown that big crystals, especially of magnetite but also of goethite and of haematite, are only partial dissolved by DCB extraction even if they have been crushed prior to extraction. There is also some evidence that iron may be extracted from iron-silicates using this technique [8, 14, 17]. Despite these uncertanties, DCB extractions are commonly used for the extraction of iron and aluminium oxides in soils and clays.

4.1.2.1.3. EDTA Extract

Extraction with weakly alkaline solutions of EDTA was introduced by Borggard [13, 19, 20]. This extraction was proposed for selective extraction of amorphous iron oxides in

soils and clays. Like oxalate, EDTA forms strong complexes with iron and aluminium as well as other di- and trivalent metals [21] and the extracts can be easily clarified by low-speed centrifugation or filtration.

The EDTA extraction is considered to be selective as well as robust. However, for individual extraction series, at fixed EDTA concentrations, pH's and soil: solution ratios, dissolved metals increase slightly in concentration even after 3 months. Experiments on synthetic and natural iron oxides and silicates show that EDTA only dissolved amorphous iron oxides leaving the well crystallised iron oxides and silicates behind [13].

Although EDTA extractions may give selective dissolution of certain metal fractions, the nature of these fractions is still rather obscure. EDTA extracts include exchangeable metal as well as most of the metal in organic complexes [22,23]. However, for many soils investigated by Borggard [24,25], the major part of the EDTA extractable metal seemed to consist of ill-defined amorphous or poorly crystalline oxides. Close correlation between EDTA-extractable metal and oxalate-extractable metal for most soils investigated by Borggaard [25] may indicate that EDTA dissolves oxides similar to those dissolved by oxalate. On the other hand, some substantial differences were noted between the two techniques for a few soils, suggesting that some difference may exist between the two methods.

Future work could confirm the selectivity of the EDTA technique, but in general the EDTA technique is far too slow to be used in routine soil analysis. Unfortunately, attempts to

speed up the exraction by increasing extraction temperature or by lowering the pH to below 7.5 were unsuccessful [19, 20]. Therefore, the EDTA technique may only serve special purposes, such as being a reference technique against which other less time consuming techniques can be evaluated.

4.1.2.1.4. Pyrophosphate Extraction

Potassium or sodium pyrophosphate solutions have been used for many years for estimating iron and aluminium present in soils as part of organic complexes [26]. However,there is a major problem associated with this technique in that the extracts contain suspended materials which cause poor reproducibility. This poor reproducibility was demonstrated by the great effect of centrifugation efficiency on pyrophosphate-extractable iron by McKeague and Schuppli, 1982 [27]. The effect of pH and the use of either the sodium or potassium salt, was investigated by Loveland and Digby ,[28], and again problems with reproducibility were encountered. Much work [22,28,29], has been done to improve reproducibility, especially by trying to clarify the extracts prior to the measurement of the metal concentration, but a reproducible method is still unavailable.

Pyrophosphate does not dissolve iron by forming iron-pyrophosphate complexes or, if so, only to a limited extent. From the only stability constant for an iron pyrophosphate complex found in the literature, i.e. log K = 22.2 for di (monohydrogen-pyrophosphate) iron (III), it may be calculated that such a complex is highly unstable compared to

amorphous iron oxides at pH 10 [21]. This unstability of iron pyrophosphate complexes compared to iron and aluminium oxides has also been shown by McKeague et al. [30]. According to Jeanroy and Guillet [22], the dissolution occurs via a solubilisation mechanism. This dissolution is thought to occur by adsorption of pyrophosphate onto soil particles resulting in increased negative charges thereby resulting in increased solubility of these particles in water.

The reproducibility problems and the uncertainties about the dissolution mechanism and the suspended materials leave the nature of pyrophosphate-extractable metal unresolved. In the absence of better techniques, pyrophosphate may still be used provided the extraction and clarification procedure are precisely described.

4.1.2.1.5. Other Dissolution Techniques

The four main extraction techniques used for trace metal analysis in soils have been discussed above, but as the demand for more specific extraction techniques increase, the number of extractions investigated also expands. This expansion has also been due to the requirement of simpler and faster techniques.

One such extraction is the use of hydroxylamine for the extraction of iron and aluminium from soils. In 1983, Chao and Zhou [31] found that acid hydroxylamine dissolved virtually no magnetite when extracting amorphous iron oxides from soils and sediments. They recommended hydroxylamine as the best extractant for amorphous iron oxides, and suggested that it

should be tested as an extractant for soil samples. One obvious reservation regarding its use in soil samples is the strong acidity of the solution (pH 1.0), which could cause significant dissolution of other crystalline species. An investigation of the use of hydroxylamine for the extraction of iron and aluminium from soils was carried out by Ross et al.[32] in 1985. The results of this investigation were similar to what would have been expected from an oxalate extraction. A further investigation was carried out by McKeague [30] who concluded that hydroxylamine could be used instead of oxalate as an extractant for soils resulting in easier analysis, when using atomic absorption spectrometry (AAS). This recommendation was made since the hydroxylamine extracts did not clog up the AAS burner as much as the oxalate samples did.

The determination of aluminium has also been achieved using 8-hydroxyquinoline, followed by extraction of the Al-hydroxyquinolate complex with an organic solvent, usually chloroform, and measurement of either the absorbance or fluorescence of the complex. The spectrophotometric methods are similar in sensitivity to the more commonly used aluminium methods [33], but the fluorometric methods are up to 100 times more sensitive [34]. Both methods have been sucessively applied to the analysis of Al in soil extracts [33,35,36 and 37].

In 1978, Bloom et al. [38] used butyl acetate as the solvent (instead of chloroform) for the determination of aluminium in soil extracts. In this method, a 15-minute reaction time was used to allow reduction and complexation of interfering iron(III). Some erroneous results were obtained due to the presence of contaminating metals, which will also react with

8-hydroxyquinoline resulting in falsely high absorbance values.

May et al.[39] modified this 8-hydroxyquinoline method to measure low concentrations of monomeric aluminium in neutral waters. To eliminate interferences from cations, pretreatment steps were carried out. These pretreatments may have caused changes in the aluminium species present. In 1983, James et al. [40] reported a further modification of the 8-hydroxquinoline method to determine labile and total aluminium in soil extracts. An extraction time of 15 seconds was used to measure labile aluminium and a second wavelength was used to account for interferences from other metals. Non labile aluminium was calculated as the difference between labile and total aluminium. Good reproducibility was reported and the technique appeared to minimise changes in aluminium speciation during the extraction.

The use of 8-hydroxyquinoline as an extractant for other metal ions has not been reported. This is probably due to the inability of the detection systems used, i.e. absorption and fluorescent techniques, to differentiate between the different metal complexes. However, these problems may be overcome using high performance liquid chromatography (HPLC), whereby separation takes place prior to detection.

Another extraction that is gaining in popularity is the acetate extraction. This extraction technique has been shown to extract a manganese fraction similar to the fraction taken up by plants [41]. The analysis was carried out using inductively coupled plasma emission spectrometry (ICPES). Ammonium acetate extractions have also been carried out for determination of bismuth, cadmium and lead [42]. The results were compared to

HNO_3 and aqua regia digestion techniques, and as anticipated the acetate extract contained a lower metal ion concentration, as the soil structure was not destroyed.

Many other organic ligands have also been used as extractants for the analysis of metals. These include tetrazolium salts [43], thioanilide [44], thiocyanate [45], ammonium-pyrollidine-1-carbodithioate-isobutyl methyl ketone [46], and phosphonic acid chelating agents [47]. Some of these ligands may become popular in the future for selective metal extractions, but at present the main extractions carried out are those based on the oxalate and DCB extraction procedures described above.

4.1.2.2. Digestion Techniques

These techniques consist mainly of acid digestions. They are very seldom used, unless total metal content is required, as they are techniques in which the soil structure is totally destroyed. This leads to disruption of the chemical equilibria and hence changes in the composition of the metal species present.

An example of such a technique is that used by An Foras Taluntais [48]. In this technique, the soil is ignited at 500^{o}C for 16 hours. Following this it is digested using 66% HCl for 24 hours. The digests may then be analysed using AAS, spectrophotometry or as in the work reported in this chapter, HPLC.

Other digestion techniques that have been used include the use of aqua regia. McGrath et al. [49] used a

simplified aqua regia method to extract iron, zinc, gold, nickel, cadmium, lead, chromium and manganese from soils and sewage sludges. The extracts were analysed using AAS and good percentage recoveries were reported.

In soils which are uncontaminated, it is very likely that a large percentage of the metals present will be in the soil matrix, which is only completely dissolved when sodium carbonate fusion or hydrogen fluoride dissolution are used. Other acid digestion techniques, including the use of aqua regia, are likely therefore to give smaller recoveries for uncontaminated soils. Although block digestion with aqua regia does not give absolute total metal concentration, the method has given results sufficiently close to the accepted values for reference materials and therefore can be used in routine monitoring of the metal contents of agricultural soils and sludges.

Merry et al.[50] used aqua regia for digestion of soil after addition of hydroxyl-ammonium chloride for the determination of antinomy and arsenic. This digestion appeared to extract the total metal fraction, but no comparisons with stronger extraction procedures were investigated. The determination of total selenium in soil and plant materials was reported following a digestion procedure with phosphoric acid, nitric acid and hydrogen peroxide [51]. The extracted selenium was analysed fluorimetrically and the results were similar to those obtained from a HNO_3-$HClO_4$ digestion method..

Multi-element analysis of standard reference materials with total reflection X-ray fluorescence was carried out by Gerwinski et al. [52]. Analysis of the digests obtained

following digestion with HNO_3 as well as sequential digestion with HNO_3, HF and H_3BO_3 showed recoveries of 94-104%, and the results obtained agreed well with the published values for the reference materials.

4.1.3. Instrumental Methods of Analysis for Determination of Trace metals in soils.

The analysis of metals in soil extracts has been carried out using a large number of methods. The main techniques in use include atomic spectroscopy (AAS, AES, ICP, and GF AAS), X-ray fluorescence, neutron activation analysis, electroanalytical and chromatographic techniques.

4.1.3.1. Atomic Spectrometry.

From the literature it would appear that AAS and ICP methods are the most commonly used for the determination of metals in soil extracts. Since these methods determine total metal concentration, speciation within the soils is carried out using selective extractions such as those discussed in the previous section.

The determination of cadmium, copper, manganese, nickel, lead, and zinc by AAS after extraction with EDTA has been reported [53]. Detection limits of 0.05 - 0.25 ppm were achieved. AAS was also used, following a similar extraction, for the determination of cadium, cobalt, nickel and lead by Iu et al.[54]. The detection limits for the determination of these metals was further improved using graphite furnace AAS following

an EDTA extraction procedure [55].

Petrov et al. (56) determined cadmium, cobalt, copper, manganese, nickel, lead and zinc in soil extracted with acetate using AAS. A limit of detection of 1 -> 2 ppm was obtained using preconcentration techniques, but low recoveries and irreproducible results were obtained when measuring manganese and zinc after pre-concentration.

Determination of trace metals in raw sewage was reported by Das et al. [57]. The author used AAS following extraction with HNO_3 and HCl. Nitric acid was also used as an extractant by Schmidt et al. for the determination of cadmium, copper, iron, lead, and zinc [58].

Wolf et al. [59] determined trace metals in soils and used their results as an indication of environmental pollution. Moss and soil samples were dissolved by pressure ashing with HNO_3 at 140^{o}C, digestion with aqua regia or total ashing with HF. Seventeen metals including iron, aluminium, copper and nickel were then determined by AAS, ICP-AES or NAA. Portnaya et al.[60] carried out determinations of trace heavy metals in soils following ashing. Traces of cadmium, lead, tin, copper, cobalt, molybdenum and vanadium were determined using AAS following co-precipitation with chrompyrazole II. Detection limits ranged from 0.5 - 1 ppm.

Burridge and Hewitt [61] also used co-precipitation to determine metals using atomic spectroscopy. They determined aluminium in soil extracts by carbon arc emission spectrometry after co-precipitation with iron using 8-hydroxyquinoline. This method was shown to be effective for determining aluminium in soil extracts containing from 0.02 to 20 g ml^{-1} of aluminium.

Aluminium, calcium and magnesium were determined in KCl extracts by Bruce et al.,[62]. The concentration of aluminium was estimated using four different methods of analysis (i) titrimetry, (ii) spectrophotometry, (iii) AAS and (iv) ICP-ES. All methods effectively measured total aluminium. Method (iii) and (iv) were also used to determine calcium and magnesium in the extracts. Method (iv) gave higher results for aluminium, possibly owing to the presence of colloidal matter. However, this method also allowed determination of all three elements in the same aliquot.

In recent times, much work has been carried out on development and improvement of methods for the determination of single elements and groups of elements. This is especially so for metals such as lead, molybdenum and cadmium which are of great importance in pollution. Baucell et al. [63] developed methods for the direct determination of cadmium and molybdenum in soil extracts by graphite furnace atomic-absorption spectrometry (GFAAS) and ICP spectrometry.

Further improvements in the determination of heavy metals include the direct determination of these metals in solid samples. In 1982, Stuper et al. [64] described a method for the direct, routine AAS determination of copper, iron, manganese and magnesium in soil suspensions. The method was also found to be suitable for the determination of lithium, calcium, strontium, barium, aluminium, chromium and titanium.

Kenneth et al. [65] developed a method for the determination of lead in soil by GFAAS with direct introduction of slurries. The sensitivity of this technique was 0.6 $\mu g\ g^{-1}$ soil in a typical slurry. Cadmium was also analysed by this

technique by Hinds et al. [66]. Analyte recoveries were quantitative provided that the soil was finely ground prior to analysis. The importance of soil particle size was studied and it was shown that insufficient grinding led to poor recovery from larger particles. A further improvement in the sensitivity and accuracy of this technique was reported by De Kersabiec et al. [67]. They reported that these improvements could be achieved for the analysis of cadmium, lead, mercury, antimony and arsenic, by diluting the solid soil sample with graphite powder.

Trace metals in solid samples have also been determined using ICP by Lorber et al. [68]. Detection limits in the range 0.02-5 ppm were achieved for a uranium matrix. In total, 22 elements were determined, including chromium, zinc, lead, aluminium and iron.

The determination of metals in solid samples may become important for total metal determination, as it does not involve any extraction procedures. However, there is still a need for less time-consuming multi-element methods of analysis of soil extracts.

4.1.3.2. X-Ray Fluorescence

Another method of analysis that has been used for the analysis of soil extracts is X-ray fluorescence (XRF) spectroscopy. Zsolnay et al. [69] used X-ray fluorescence with gallium as an internal standard for the determiation of vanadium, chromium, nickel, copper, zinc and arsenic. They could determine these elements at the 50 ppm level within a precision

and accuracy of ± 20% or less. To determine "mobile" forms of nickel, copper, zinc and cadmium in soil. Bryking et al. [70] used XRF spectroscopy following extraction with an acetate buffer solution at pH 4.8.

Davydov et al.[71] reported the possible use of the instrumental photon activation technique in the analysis of soil. The applicability of photon activation analysis to the determination of elements in soil was examined by mathematical modelling. This technique was shown to be suitable for the determination of nineteen metals in the samples.

The simultaneous determination of 24 elements in geochemical samples (which were analysed as powdered pellets), was reported by Xu et al.[72]. This technique offers rapid multi-element analysis but little information relating to the forms of metal analysed is obtained unless the technique is carried out in conjunction with a selective extraction.

4.1.3.3. Electroanalytical Techniques.

Electroanalytical techniques have been used by Wu Chung et al. [73] in the determination of micro amounts of lead and cadmium. Adsorption polarography was used in this study, which resulted in a method capable of the simultaneous analysis of iodides of both metals. Copper and lead were determined in EDTA extracts of soils using differential pulse anoidic stripping voltammetry (DPASV) by Edmonds et al. [74]. Simultaneous determination of toxic trace metals (cadmium, copper, lead and zinc) by DPASV was reported by Reddy et al. [75] following extraction with aqua regia. A comparison of

microprocessor-controlled voltammetry with flame AAS was carried out following HCl and HNO_3 acid digestion steps [76]. A hanging mercury drop electrode was used and the results agreed very favourable with flame AAS for the determination of zinc, cadmium, lead and copper.

O'O'-dialkyl-thio-carbamayl phosphorodithioate complexes of copper, nickel, zinc, cobalt and lead were determined in soil by Ulakhovich et al. [77] using a polarographic method. Microgram quantities of copper could be separated from other metals by extraction from aqueous 6 M HCl in $CHCl_3$. However, the determination of metals as complexes is more important when determining metals using HPLC.

4.1.3.4. Chromatographic Techniques.

The first separation of metal co-ordinated complexes using HPLC was reported in 1972 by Huber et al. [78]. It was shown in this study that six metals could be separated in 25 minutes using acetylacetonates as ligands. Complexes of beryllium, copper, aluminium, chromium, ruthenium and cobalt were separated with UV detection at 310nm.

Since then many other ligands such as ketoamines, hydrazones and semicarbazones, dithiocarbamates, dithizonates, 8-hydroxyquinolates, 1, 10 phenanthrolines, ethylenediamines and porphyrins have been used for the separation of metals, as metal complexes using HPLC (see chapter 1). However, this mode of detection has not yet been routinely applied to the analyses of soil extracts.

Drasch et al. [79] carried out quantitative

determination of heavy metals in soils by HPLC after chelate extraction with dialkyl-dithiocarbamates or dithionite. Metal complexes of cadmium, lead, magnesium, copper, nickel and cobalt were investigated using either a C_8 or a C_{18} column with methanol-water-chloroform mixtures as the mobile phase.

The determination of selenium in soils using HPLC with fluorescence detection, following extraction with HF, HNO_3 and $HClO_4$, was described by La Manna et al.[80]. Selenium was determined as a napthalene-2,3-diamine complex using a mobile phase of ethyl acetate : cyclohexane (1:19). Fluorescence detection at 525 nm following excitation at 380 nm was used.

HPLC has also been investigated for the determination of molybdenum in soils and plants [81]. HF was used as an extractant and α-benzoin oxime complexes of molybdenum could be separated from the tungsten complexes. UV detection was used at 315 nm.

Even though the use of HPLC for the analysis of soil samples is only at an early stage of investigation, it would appear that this technique offers a relatively simple multi-element approach. As many metal complexes can be separated using HPLC, it may be possible to develop a system whereby the metals may be extracted as complexes and these metal-complexes then analysed directly using HPLC. This approach would offer the advantages of multi-element analysis with very little alteration of the metal speciation present within the soil sample. This type of analysis may also make it possible to determine those metal species which are available to plants.

In this project the analysis of trace metals in soil

and clay was investigated using HPLC. This technique was investigated as, already mentioned, it offers the possibility of simultaneous multi-element analysis. Several different extraction techniques were carried out and the extracts were analysed using both HPLC and AAS. This comparison between the two analytical methods was carried out in order to evaluate the newly developed HPLC approach against a standard well documented technique.

The possibility of using oxine as an extractant for "available" metal was also investigated. This approach enabled direct injection of the extracted metal complexes onto the HPLC column. Such an approach should minimise errors as the extract can be analysed directly without the need for any digestion steps.

4.2. Experimental

4.2.1. Reagents and Standard Solutions

All chemicals used were of analytical grade. Soil samples were taken from a fixed locality on the NIHE campus, sieved to obtain a particle size of 2 mm or less, and dried in an oven at 110 ^{o} C. The clay sample used was an untreated Wyoming montmorillonite described by Breen et al. [82]. All aqueous solutions were prepared in distilled water, further purified by passage through a Milli-Q water purification system. All organic solvents used were of HPLC-grade. Silica sample preparation cartridges (Sep-Pak) were obtained from Waters. The C_{18} column used in this study was obtained from Supelchem, and was a 25 cm x 4.6 mm steel column containing LC-18-DB (5 μm particle size) packing material. A Guard-PAK (Waters) guard column containing 10-μm μBondapak C_{18} packing material (end capped) was used to protect the analytical column.

4.2.2. Apparatus

The HPLC system used in this study consisted of an Applied Chromatography Systems (ACS) Model 352 ternary gradient pump connected to a Rheodyne 7125 injection valve and a Shimadzu Model SPD-6A variable-wavelength spectrophotometric detector. AAS was carried out using an Instrumentation Laboratory (IL) Model 357 AA/AE spectrophotometer.

4.2.3. Methods

4.2.3.1. Digestion/extraction procedures

(a) Hydrofluoric acid digestion. Dried soil or clay (0.1 g) was shaken with 5.0 ml 40% hydrofluoric acid in a PTFE vessel for 24 hr, and the extract diluted to 1 part in 300 parts water, prior to analysis.

(b) Hydrochloric acid digestion. Dried soil or clay (0.1 g) was shaken with 100.0 ml 36% hydrochloric acid for 24 hr. Analysis was carried out on 1:100 and 1:25 dilutions in water for soil and clay samples, respectively.

(c) Dithionite-citrate-bicarbonate-extraction. This was carried out according to the method of Mehra and Jackson [17].

(d) Oxalate extraction. This was carried out according to the method of McKeague and Day [8].

(e) Oxine extraction for Fe(III). Dried soil or clay (0.5 g) was extracted for 4 h with 40.0 ml of (0.5%) oxine dissolved in 0.02 M acetate buffer, pH 4.0. A 2.0-ml aliquot of each extract was then passed through a silica Sep-Pak. The metal ion-oxine complex was then eluted with 4 ml methanol before analysis.

(f) Oxine extraction for Al(III). This was carried out according to the method of James et al. [40], with the following modifications: (i) the oxine concentration was reduced to 0.5 %, (ii) the reaction was stopped by centrifugation instead of extraction with butyl acetate, which interfered with subsequent HPLC analysis.

4.2.3.2. HPLC Analysis

The conditions used for the HPLC analysis of Fe(III) and Al(III) were the same as those reported in chapter 3. Separation was achieved with a mobile phase of acetonitrile (containing $1x10^{-2}$ M oxine)-0.02 M acetate buffer pH 6.0 (containing 0.2 M potassium nitrate) (50:50) at 1 ml/min. Standard solutions of metal ions or extracts from soil or clay samples were injected directly onto the column through the injection port without any external formation of the complex.

4.2.3.3. Atomic Absorption Spectrometry

The conditions used for AAS measurements for Fe(III) were: light source, hollow cathode; lamp current, 8 mA; wavelength, 248.3 nm; slit width, 80um; burner head, single slot; band pass, 0.3 nm; flame description, air-acetylene, oxidising, fuel lean, blue. For Al(III) they were: light source, hollow cathode; lamp current, 8 mA; wavelength, 309.3 nm; slit width, 320 um; burner head, nitrous oxide; band-pass, 1.0 nm; flame description, nitrous oxide-acetylene, fuel rich, red.

4.2.3.4. X-ray Diffraction

Oriented samples for X-ray diffraction (XRD) analyses were prepared by evaporating an aqueous slurry of soil or clay onto a microscope slide (15 x 10 mm). The slide was then placed in the goniometer of a Philips Model PW1050 diffractometer

operating at 40 kV and 20 mA using CuK radiation (λ = 1.5418 A). The XRD profiles were recorded at $2^{\circ}(2\theta)$ min^{-1} from 0-60 (2θ). In the case of the hydrofluoric acid digestion, the extract contained almost no solid matter, and a small portion of the extract was poured onto the glass slide, where some crystals formed.

4.3. Results and Discussion

4.3.1. X-ray Diffraction (XRD) Studies

The XRD profile of the untreated soil sample is shown in Fig. 4.4a, and illustrates that the major, indexable, crystalline component of this soil sample is α-quartz (Q). In contrast, the XRD profile of the untreated clay (C) sample (Fig. 4.4b) indicates the presence of several impurities including mica (M), kaolin (K), quartz (Q) and Feldspar (F), but exhibits no peaks commensurate with crystalline Fe- or Al-containing species such as goethite, lepidocrocite or gibbsite, respectively.

The effect of the increasing severity of the three extraction/digestion procedures reported in this study on the XRD traces obtained for the clay sample is illustrated in Fig. 4.3c-e. The DCB extract was not washed prior to collecting the XRD data, and this accounts for the three characteristic sodium chloride (N) peaks shown in Fig. 4.4c. The XRD trace obtained for the hydrochloric acid digest is shown in Fig. 4.4d, and shows that treatment with 36% hydrochloric acid causes no noticeable degradation of the structural clay lattice, and reflects the greater resistance of aluminous silicates to acid attack compared to the magnesium rich analogues. The reduced intensity of the Q and F peaks in the DCB extract most probably reflects the physical loss of sample rather than preferential extraction, whilst the Feldspar peak for the hydrochloric acid digested clay (Fig.4.3d) lies below the 005 reflection marked C at around $28^{\circ}(2\theta)$. The XRD trace

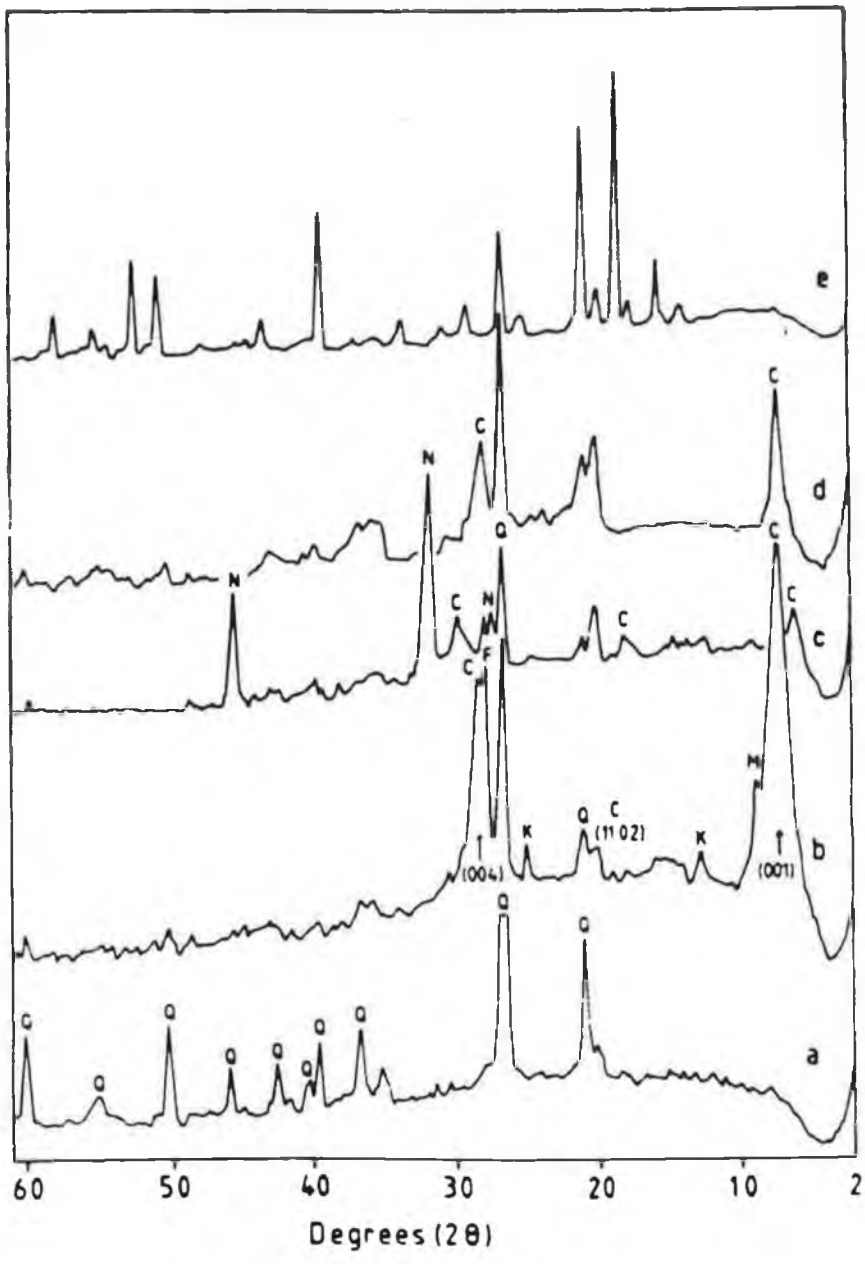

Figure 4.4. XRD profiles for (a) untreated soil, (b) untreated clay, (c) DCB-extracted clay, (d) hydrochloric acid digested clay, and (e) hydrofluoric acid digested clay.

obtained for the hydrofluoric acid digest of the clay (Fig. 4.4e) illustrates emphatically that treatment with hydrofluoric acid has a devastating effect on the clay, leaving few identifiable reflections. In a similar manner, only the hydrofluoric acid digestion procedure had any marked effect on the diffraction profile of the soil sample.

4.3.2. Digestion/extraction methods

The results obtained using both HPLC and AAS for the percentages of Fe(III) and Al(III) in the soil and clay samples following the various digestion/extraction procedures detailed under Experimental are given in Table 4.4. A typical trace obtained for the separation of Fe(III) and Al(III) using HPLC is shown in Fig. 4.5. In this trace responses due to the simultaneous extraction of Fe(III) and Al(III) are evident. A detection wavelength of 400 nm was employed, which lies between the $_{max}$ values for the oxine complexes of Fe(III) and Al(III) at 450 and 375 nm, respectively. Limits of detection of the order of 1-2 ppm for Fe(III) and Al(III) were typically achieved using both methods.

4.3.2.1. Hydrofluoric Acid Digestion

The values obtained for the percentage Fe(III) in the soil and the clay represent the "total" metal content, because the hydrofluoric acid completely digests the samples, as shown from the XRD trace in Fig. 4.4e. The results obtained using both HPLC and AAS are in good agreement, and show that

Table 4.4. Concentrations of Fe(III) and Al(III) obtained using HPLC and AAS following digestion /extraction techniques.

Method / Extractant	Sample	HPLC % Fe(III)	HPLC % Al(III)	AAS % Fe(III)	AAS % Al(III)
HF	Soil	2.38±0.18	0.09±0.002	2.36±0.12	2.64±0.09
	Clay	1.60±0.05	0.25±0.005	1.64±0.12	7.51±0.44
HCl	Soil	1.69±0.04	0.20±0.01	1.71±0.02	0.42±0.01
	Clay	0.75±0.01	0.14±0.01	0.75±0.01	0.15±0.01
DCB	Soil	1.39±0.06	1.33±0.04	1.46±0.03	1.70±0.04
	Clay	0.18±0.01	$0.004\pm1\times10^{-4}$	0.16±0.01	$0.003\pm1\times10^{-4}$
Oxalate	Soil	0.08±0.01	0.04±0.005	0.08±0.005	0.07±0.005
	Clay	0.04±0.005	0.04±0.001	0.04±0.001	0.08±0.001
Oxine	Soil	0.05±0.005	nd	0.05±0.005	nd
	Clay	0.02±0.001	nd	0.02±0.001	nd

nd = not detected

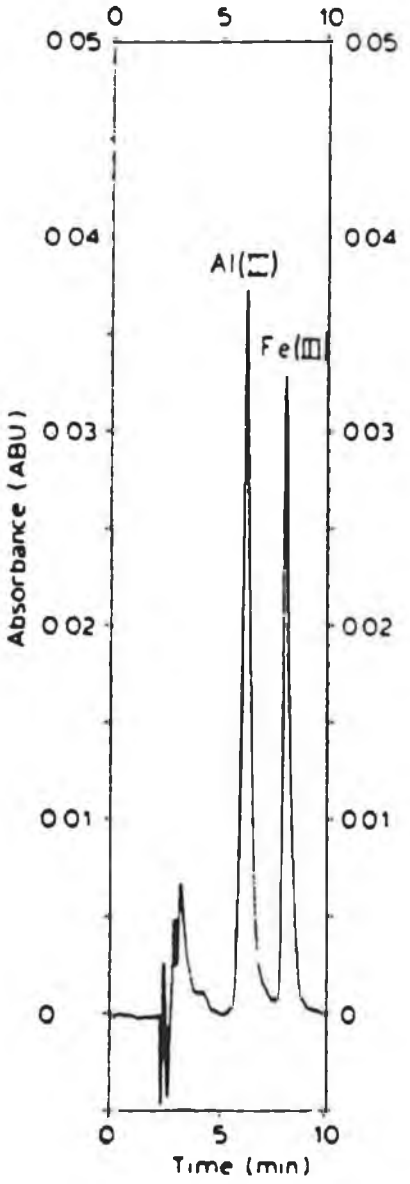

Figure 4.5. HPLC separation of a 10 ppm mixture of Fe(III) and Al(III) with spectrophotometric detection. (Conditions: flow rate 1 ml min^{-1}, detection wavelength 400 nm and mobile phase 50:50 acetonitrile (containing $1x10^{-2}$ M oxine) - 0.02 M acetate buffer pH 6.0 (containing 0.2 M potassium nitrate).

the Fe(III) content of the soil sample exceeds that of clay.

The values obtained for the percentage Al(III) in the soil and clay using HPLC were consistently lower than those obtained using AAS. This is probably due to the fact that Al(III) forms a much stronger complex with fluoride ions than with oxine, and hence prevents any "in situ" formation of the Al(III)-oxine complex on the column. With the high temperatures achieved by the rich nitrous oxide-acetylene flame, the Al(III)-fluoride complexes will be atomised, hence giving rise to a more accurate value for the Al(III) content in the soil or clay. Consequently, greater cognisance should be afforded the AAS results, particularly since the value of 7.51% Al(III) in the clay sample is close to that reported previously (82).

4.2.3.2. Hydrochloric Acid Digestion

The values obtained for the percentage Fe(III) in the soil and clay samples were shown to be in good agreement using both instrumental methods of analyses. In the case of Al(III), the results for the clay were in good agreement, but the percentage Al(III) in the soil was found to be nearly double using AAS when compared to HPLC. The reason for this discrepancy is not clear, but may be due to the nature of the "extra-framework" Al(III) in the respective matrices. From the results of the XRD studies, it is clear that the 36% hydrochloric acid digestion is not as effective as hydrofluoric acid at breaking down the structural units of the soil and clay matrices and thus this digestion procedure can only yield results that approximate to "total" metal ion content.

4.2.3.3. Dithionite-citrate-bicarbonate Extraction

This extraction technique, which is based on the strong reducing properties of dithionite, was developed to extract oxides of different crystallinity, including water-soluble, exchangeable and organic-bound metal [17]. The optimum pH for this extraction is 7-8, and this is maintained by the buffering capacity of the bicarbonate anion. Citrate is employed to complex the reduced metal ion.

The values obtained for the percentages of Fe(III) and Al(III) (Table 4.4) show that whilst the HPLC and AAS results for percentage Fe(III) are in good agreement, the HPLC results for percentage Al(III) in the soil sample are somewhat lower than those obtained by AAS. This probably reflects competition between citrate and oxine for Al(III), which is a problem in the HPLC assay, but not in AAS where the citrate complex would be broken down. Consequently, since the HPLC method relies on complexation of free metal ion with oxine, the difference between the two results may relate to the amount of AL(III)-citrate complex present following extraction. One further interpretation, which cannot be ruled out, is that the DCB method extracts organic-bound Al(III) which would be determined by AAS but not by HPLC. The higher values for the percentage Al(III) in the soil compared to those obtained following hydrochloric acid digestion may be explained by the possible presence of semi-crystalline iron oxides containing a substantial amount of Al(III). It is difficult to obtain evidence for the presence of these phases, because as Schulz and Schwertmann [83] have found in both naturally occurring and

synthetic goethites, the substitution of Fe(III) by Al(III) substantially reduces the intensity of peaks in the diffraction profile.

4.2.3.4. Oxalate Extraction

The oxalate extraction was introduced by McKeague and Day [8] to extract the "active" fraction of metal ions from soils. This includes amorphous, non-crystalline or poorly ordered metal oxides and also includes organic-bound metal. The technique is based on complexation of metal ions by oxalate at pH 2 or 3, but little difference was noticed in the metal ion concentrations obtained. The results obtained for the percentage Fe(III) and Al(III) in soil and clay samples following this extraction procedure at pH 3 are given in Table 4.4. Once again there is good agreement between the HPLC and AAS values for the percentage Fe(III), but not for the percentage Al(III). However, it is unlikely that the source of this discrepancy is due to the competitive chelation observed in the DCB method, because there is a digestion step using nitric acid-sulphuric acid prior to analysis, which should break down any Al(III)-oxalate complex and/or organic-bound Al(III). Unfortunately, this acid digestion step lowers the pH of the extract prior to the analysis stage, and studies with comparable standard solutions indicate that these low pH values suppress absorbance readings in AAS and affects the complexation of Al(III) with oxine, resulting in lower values than anticipated for the HPLC method. The reason that the values obtained for the percentage Al(III) using HPLC are

higher in the soil and lower in the clay following the oxalate extraction compared with the dithionite-citrate-bicarbonate extraction may be due to the fact that oxalate is a better extractant of amorphous Al(III) species [8] which are suggested to be more prevalent in the soil than the clay from the XRD studies.

4.2.3.5. Oxine Extraction

Methods employing oxine as an extractant for "available" Al(III) in soils have been reported in the literature [38,40], but this extractant has not been widely used for determinations of Fe(III) in soil and clay samples. We have therefore investigated the use of oxine for Fe(III) determinations in these matrices based on the method of James et al. [40] reported for Al(III).

The extraction method involving oxine was optimised with respect to time of extraction, pH and isolation of the complex using silica Sep-Pak cartridges. The extraction time varied between 2 and 72 h, but periods in excess of 4 h gave comparable results. Previous studies [84,85] have shown that the formation of the oxine-Fe(III) complex is optimal in the pH range 4-6, although analysis of the soil extracts indicated that slightly higher values were obtained at pH 4 than pH 6. Furthermore, the use of a silica Sep-Pak was found to be effective means for isolation of the complex formed, in addition to acting as a means of sample "clean-up", as observed previously. It was found that 4 ml of methanol was required to quantitatively remove 20 ug of oxine-Fe(III) complex from the

Sep-Pak.

The results in Table 4.4 show that there is good agreement between the HPLC and AAS results for the percentage Fe(III) in soil and clay samples using this extraction method. No detectable concentrations of Al(III) were found in any of the oxine extracts by either HPLC or AAS, although spiking a soil sample with 5ppm Al(III) resulted in HPLC and AAS values of 5.03 and 5.3 ppm Al(III),respectively. This suggests that oxine will only extract extremely labile Al(III) from these matrices.

4.3.4. Conclusions

A comparison of the results in Table 4.4 show a good agreement in the percentage Fe(III) values obtained from both soil and clay using both HPLC and AAS. The percentage Fe(III) extracted using the various methods decreased in the order HF > HCl > DCB > Oxalate > Oxine. This is to be expected considering the different mechanisms by which these extractions/digestions operate. It is interpreted from the results that the hydrofluoric acid extraction yields a value related to the "total" Fe(III) content. Because of the specific nature of the DCB and oxalate extraction procedures for crystalline and non-crystalline oxides, respectively, the different values for the percentage Fe(III) arising from these procedures can be explained. the difference between the DCB and oxalate extractions for Fe(III) can be attributed to the amount of crystalline iron oxides present in these matrices, even though they were too small in particle size to be observed using XRD

analyses. The difference between the oxalate results and those obtained using most probably due to "exchangeable" Fe(III) species.

The results obtained with the 36% hydrochloric acid digestion suggest a small ingress of acid into the octahedral layer, thus leaching out a small amount of Fe(III) associated with the lattice structure. In the case of Al(III), the percentage values obtained in both soil and clay were found to be in good agreement for the hydrochloric acid digestion and the DCB extraction for the clay using both HPLC and AAS. The results obtained using the other extraction procedures, however, were found to be much lower using HPLC compared to AAS, especially for the soil. This is mainly due to the competition between oxine and the various extractants employed for Al(III).

Although this investigation has necessarily limited itself to the analysis of a single soil and a single clay sample, it has, however, highlighted the possibility of employing HPLC (i) as a multi-element approach to the determination of metal ions in soils and clays, and (ii) to provide information on the speciation of metal ions, provided that experiments have been carried out taking into consideration the matrix involved as well as the sample preparation. If lower limits of detection were required than are possible using the approaches described in this paper, then these could be achieved for the HPLC methods by employing the technique of "external formation" of the oxine-Fe(III) or oxine-Al(III) complex prior to injection onto the column, and for the AAS method by employing a flameless approach to

atomisation. It would also be possible to use electrochemical detection for the HPLC analysis of the metal complexes. The use of electrochemical detection for such analysis is discussed in the next chapter.

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Chapter 5

The Utilisation of Polymer Modified Electrodes in the Determination of Trace Metal Ions in Flowing Streams

5.1. Introduction

As the popularity of high performance liquid chromatography (HPLC) and flow injection analysis (FIA) grows, the need for fast reliable detectors increases. In an ideal situation these detectors would have the following properties, (a) infinite stability, (b) excellent signal to noise ratios, (c) high sensitivity, (d) wide linear dynamic range and (e) the capability of detecting all solutes equally [1]. However, in practice no such detector is available at present. The most popular detectors that are in use today are electrochemical, spectrophotometric (both UV-vis and fluorescent) and refractive index detectors, each detector having specific advantages and disadvantages. Electrochemical detection (ED) in flow injection analysis and high performance liquid chromatography has become very popular in recent years [2]. This can mostly be attributed to the sensitivity and selectivity that may be achieved using this mode of detection.

Electrochemical detection may be subdivided into the following modes, conductrimetric, for the determination of ionic species and amperometric, coulometric and polarographic modes, for the determination of compounds which can be electrolytically oxidised or reduced at a working electrode [1]. These detectors offer selectivity, as not all compounds are electroactive. Further selectivity may be introduced by variation of the potential applied to the detector, to discriminate between different electroactive species. Other advantages associated with ED include reliability and simple low volume cell design (0.1->5.0 ul). However, ED requires

the use of conductive mobile phases, usually containing inorganic salts, resulting in conditions that are compatible with reversed-phase and ion-exchange chromatography but cause difficulties when used in conjunction with other chromatographic techniques.

Other forms of detection available for use in HPLC or FIA include UV-visible, fluorescence and refractive index detectors [1]. UV-vis detectors are the most widely used detectors in HPLC and FIA. They offer high sensitivities, detection limits in low to subnanogram ranges, and a linear dynamic range of 10^4 -> 10^5. However, their use is limited to determination of compounds which absorb UV-vis radiation and problems due to background absorption by the solvents and other matrices are commonly responsible for loss of sensitivity and selectivity.

Fluorescence detectors are also frequently used in flowing systems. As only a small number of all inorganic and organic compounds are naturally fluorescent high selectivity for fluorescent compounds is possible in conjunction with high sensitivity [1].

In contrast to fluorescent detection, which is only applicable to small numbers of compounds, refractive index detection is used as a universal detector [1]. This mode of detection relies on the inherent property of all molecules to bend light to varying extents at a surface solution interface. Unfortunately this mode of detection is insensitive and suffers from interference problems caused by any small changes in the background eluent.

These are the four main types of detection in routine use for detection in flowing solutions. In this study the use of electrochemical detection was investigated in both stationary and flowing solutions. Many different types of electrodes are available for use in electrochemical detection, some of which will now be discussed.

5.1.2. Working Electrodes

As in stationary voltammetry the main working electrodes used in flowing systems are either based on mercury or carbon. The mercury-based electrodes include the dropping mercury electrode (DME), the hanging mercury drop electrode (HMDE), the mercury film electrode (MFE) and the static mercury drop electrode (SMDE). The solid electrodes commonly employed are carbon paste, glassy carbon, gold, silver and platinum.

5.1.2.1. Mercury-based Electrodes

Mercury-based electrodes are mainly used for the analysis of compounds which undergo reductive processes, as the low oxidation potential of mercury prevents its use for oxidizable species. These electrodes have one major advantage in that surface contamination is rarely a problem as the electrode surface is continuously renewed.

The use of mercury electrodes as liquid chromatography detectors was introduced by Kemula [3], although these early studies still utilized relatively large-volume cells. For example, Bond et al.[4] reported on the use of a

large volume continuous flow cell for use in the zinc electrorefining process. Lower detection limits using small volume cells, incorporating the use of conventional DME's have been described by Koen et al.[5], Fleet and Little [6] and many others [7-9]. However, these electrodes suffer from problems which include high background currents, turbulence caused by liquid flow in the region of the drop and the need for complex cell design and therefore are not routinely used.

Stationary hanging mercury drop electrodes, as well as thin film mercury electrodes deposited on many different substrates e.g. platinum, glassy carbon, gold, nickel, are also in use [10-13]. These types of mercury electrodes exhibit properties that are very similar to those exhibited by solid electrodes.

5.1.2.2. Solid Electrodes

Solid electrodes have been used extensively as amperometric detectors for HPLC and FIA, mainly in the amperometric mode of measurement [14-18]. Many of these electrodes, in particular those based on carbon electrodes, approach ideal behaviour.

5.1.2.2.1. Carbon Paste Electrodes

One of the first electrode materials used for HPLC-ED was carbon paste, which was used to detect catecholamines based on their anodic oxidation [14]. Detection limits for carbon paste electrodes are among the lowest

reported for electrochemical detection. For instance, Caliguri and Mefford [15] have shown that detection limits of 100 fg can be achieved using carbon paste electrodes, in conjuction with microbore chromatography, for the determination of catecholamines. Such low detection limits are possible due to the low residual currents that are associated with these electrodes.

However, these electrodes have also several drawbacks associated with them. The main disadvantage is the variability in the preparation of carbon paste leading to poor reproducibility. A major limitation to their application is the fact that these electrodes can only be used for anodic oxidation, as carbon paste contains oxygen, which is reduced at negative potentials and results in a large residual current. Carbon paste electrodes also tend to be soluble in organic solvents, hence causing problems in HPLC, especially when the organic solvent concentration exceeds 20%.

5.1.2.2.2. Composite Carbon Electrodes

As carbon paste electrodes are unstable in organic solvents, several solid binders have been investigated so that carbon materials can be used as "composite" carbon electrodes. Polyvinyl chloride, chloroprene rubber and cerasin wax have all been used with graphite powders to form a more stable electrode material [16-18]. The use of polyethylene in conjunction with carbon black and graphite powder has been shown to give a composite carbon black electrode which exhibits excellent signal-to-noise ratios and stability at positive potentials.

Electrodes made from Kel-F and graphite have also been prepared, and although they have been found to have a lower absolute sensitivity to electroactive compounds than solid electrodes baseline noise is reduced leading to lower limits of detection [19].

5.1.2.2.3. Glassy Carbon Electrodes

Glassy carbon is a gas permeable substrate which is formed by heating phenol-formaldehyde resins in an inert atmosphere. It is the most popular material for amperometric detection following chromatographic separation, as these electrodes can be operated at both positive and negative potentials [20-24]. In aqueous solution, a dynamic range of approximately 2 V is achievable. The detector response has been shown to be relatively constant with time, in comparison with a solid electrode such as platinum which showed a decrease in response with repeated injections of potassium ferricyanide [25]. The use of glassy carbon has the added advantage that the electrode surface can be reactivated by electrochemical pretreatment without the need for cell disassembly [26-28]. Glassy carbon electrodes have also been used as substrates for electrode modification, whereby an increase in sensitivity and selectivity and a decrease in electrode fouling can be simultaneously achieved [29-33].

5.1.2.2.4. Metallic Electrodes

Metallic electrodes have not been used extensively in conjuction with flowing systems, as the detection limits are not as low as those obtained using glassy carbon. However, platinum electrodes have been used sucessively with capillary columns. For example detection limits of less than 1 pg have been obtained for hydroquinone [34].

Other metallic electrodes include copper and nickel, which have been used for the detection of amines and amino acids [35,36]. The use of cadmium electrodes as detectors in flowing systems has also been investigated, and these electrodes have been found to be useful for the determination of nitrate and nitrite [37].

5.1.3. Modified Electrodes

Electrode modification has become very poplular in recent years. Many different types of approaches to modification have been investigated these include

(a) Physical pretreatment

(b) Immobilised enzyme electrodes and

(c) Chemically modified electrodes

The aims of such modification procedures are to increase sensitivity and selectivity and also in the case of some modified electrodes, to reduce problems associated with electrode fouling.

5.1.3.1. Physical Pretreatment

Various approaches have been used to achieve modification of the surface of carbon electrodes used in flowing streams. One approach has been based primarily on physical pretreatment. Gregg [38] reported a method involving electrochemical pretreatment of the surface of a glassy carbon electrode at +1.32 V until a steady baseline was achieved, followed by disconnecting the reference electrode for two minutes. After re-equilibration, he reported that this pretreatment resulted in enhanced sensitivity for the HPLC determination of timolol and oxprenolol, but slightly decreased sensitivity for prenalterol. In a further publication, Gregg [39] also reported that preanodisation at 6.0 V for two minutes at a current $\leq$ 2mA was required for the determination of oxprenolol in blood by HPLC-ED. Iwamoto et al.[40] reported that the limit of detection for serum methionine by HPLC-ED could be lowered 360 times by holding the potential of the glassy carbon electrode at +1.90 V for two minutes before using this electrode at +1.70 V for detection purposes. In terms of stability of response, Wang and Peng [41] reported increased stability of glassy carbon electrodes for FIA determination of NADH and dopamine after polishing, rinsing, sonication and holding the potential constant at +1.50 V for thirty minutes. No significant decreases in response for these two compounds were noticed after 360 injections. Significant decreases were found, however, for both phenol and chlorophenol.

The effect of polishing a glassy carbon electrode and subsequent performance as an electrochemical detector for

HPLC has been studied by Hoogvliet et al.[42]. They reported that extensive polishing can render a glassy carbon electrode more adsorptive and therefore more susceptible to blocking of the response. This can be overcome by polishing with alumina particles of size <6 μm followed by 10-30 linear potential scans from 0.0-> +1.5 V. This was demonstrated to be important for the amperometric detection of epinephrine. Cathodic pretreatment has also been demonstrated to be important for the determination of amino acids (such as L-cystine and L-histidine) and proteins (such as albumins, globulins, ribonucleases and lysosomes) using Cu wire (1 mm diameter). Cathodic pretreatment resulted in the Cu electrode exhibiting rapid, reproducible and near Nernstian responses towards these biological compounds [43]. In addition, the application of a triangular wave from 0.0 -> +1.8 V at 1 V s^{-1} for 30 s has been shown to be important for on-column electrochemical detection of various catechols using graphite fibres located inside the chromatographic column [44].

5.1.3.2. Immobilised Enzyme Electrodes

A second approach to modification of electrode surfaces prior to their use in flowing streams has been to immobilise enzymes onto the surface. These techniques have long been used in the development of amperometric enzyme electrodes, but have only recently been applied to on-line analysis using (primarily) FIA. Some examples from the recent literature include the use of glucose oxidase immobilised onto either a Clark oxygen electrode [45] or nylon net [46] for the

determination of glucose down to sub mM levels, and chymotrypsin covalently immobilised onto the surface of a IrO_2-coated titanium electrode for detection of N-benzoyl-L-tyrosine ethyl ester[47].

5.1.3.3. Chemically Modified Electrodes

The design of chemically modified electrodes for electroanalysis has been the subject of much research in the last few years. In attempts to enhance the sensitivity of voltammetric methods, the use of CMEs has three major advantages, the first is that the coating employed can be used to provide a surface that is capable of efficient pre-concentration. The second advantage can be seen in terms of the facilitation of faster electron transfer reactions and the third in better surface protection, resulting in decreased memory effects. A variety of different approaches to electrode modification have been reported.

One of the first approaches was introduced by Brown et al.[48] whose method involved modification of an electrode surface through the irreversible adsorption of selected aromatic hydrocarbons. Using this approach, Landum et al.[49] described the electrochemical characteristics of methyl viologen on gold, whereas Davis and Murray [50] studied the behaviour of iron porphyrins on SnO_2.

A second approach has been to modify the electrode surface through the covalent attachment of electroactive groups via silanisation reactions on oxide surfaces. Hence, Abruna et al.[51] have described the use of trichlorosilane for this

purpose, whereas Wrighton and co-workers have used trichlorosilylferrocene to modify platinum [52], gold [53] and germanium [54] electrodes.

A third approach has been to investigate the use of polymer modified electrodes. These electrodes are usually based on a solid substrate which can be coated either with electrochemically-generated polymer layers, such as polypyrrole [55,56], or with chemically generated redox polymers, such as those containing ruthenium(II)-ruthenium(III) active sites [57-59].

Electrochemically generated polymers may be subdivided into two main groups; conducting and non-conducting polymers. Conducting polymers are characterised by their ability to electrochemically switch from an oxidised (conducting) form to a neutral (non-conducting) form. The pyrrole [60,61] and thiophene [62-64] containing polymers have been most extensively studied resulting in many potential applications including storage batteries [65], semiconductor devices [66], electrochemical sensors [67,31] and detectors [68].

Non-conducting polymers may also be synthesised electrochemically. Such polymers include poly-N-ethyltyramine [69] and poly-N-o-hydroxybenzyl aniline [70], whose applications and chemical properties are still under investigation.

Chemically-generated redox polymers have been found to enhance the electron transfer characteristics of some sluggish electrode processes. Redox polymers containing ruthenium sites have been studied extensively in recent years,

and it is well known that they can be used to improve the characteristics of less facile electron transfer reactions. The mediation by the Ru(II/III) couple has been shown to enhance the sensitivity of oxidation processes. For example the response obtained for the oxidation of Fe(II) has previously been studied [71,58]. This enhancement is thought to be due to the electrocatalytic properties of the ruthenium sites in the polymer, through which rapid charge transfer can occur, possibly via a charge hopping process. This process is depicted diagramatically in Figure 5.1.

These redox polymers (dissolved in an appropriate low-boiling organic solvent) are usually applied directly onto the electrode surface using a pipette and the solvent is allowed to evaporate. Other methods of introducing ruthenium onto an electrode surface have been reported based on electrostatic interactions between polyelectrolytes [72], incorporation of ruthenium oxide in polypyrrole [73], formation of a bilayer electrode such as Pt-ruthenium polymer-polypyrrole [74] and attachment of ruthenium(II) to amino-functional groups on graphite electrode surfaces [75].

The use of chemically modified electrodes in flowing streams has been investigated by several workers. Baldwin and co-workers [76,77] have reported on the use, for instance, of Cobalt-phthalocyanine modified electrodes for the determination of mercaptopurines in plasma and oxalic acid in blood and urine using HPLC-ED, whereas Marko-Varga et al. used Meldola Blue (C.I. Basic Blue 6) on carbon rods to mediate the response of adsorbed glucose dehydrogenase [78]. In these experiments glucose could be determined between 5 µM and 2 mM

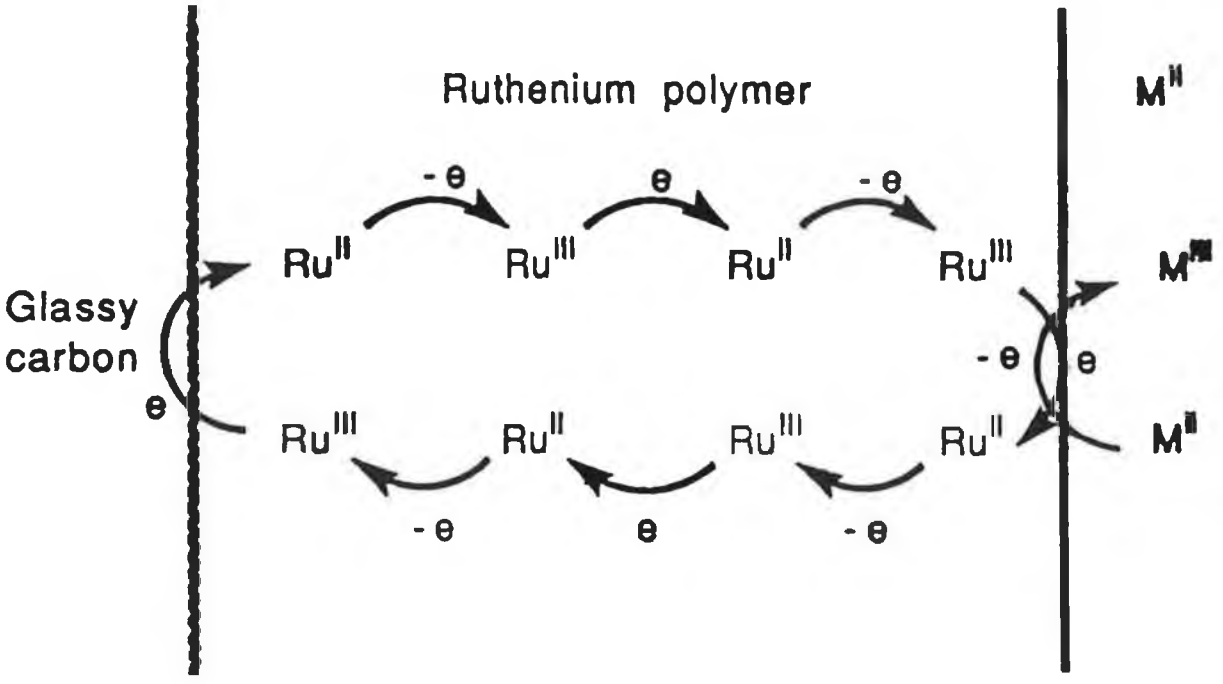


Figure 5.1. Proposed electron transfer mechanism for the ruthenium redox polymer.

concentration. In a recent publication, Kulesa et al.[79] have reported on the use of a mixed metal Ni-$Fe(CN)_6$ deposit on a vitreous carbon electrode for the HPLC determination of Fe(III). The benefit of this approach is that there was no need to remove O_2 from the eluant.

Another early attempt in this direction was made by Wang and Hutchins [80] who coated a vitreous carbon electode with cellulose acetate, then hydrolysed the film in OH^- to increase its porosity. By hydrolysing for different time periods, the authors could achieve films of different porosities. The coated electrodes showed high selectivity towards smaller analytes in both HPLC and FIA, and electrode poisoning due to protein adsorption was minimised. The method was applied to the determination of paracetamol in diluted urine. In a recent publication Wang et al. [81] have described the incorporation of a cobalt phthalocyanide catalyst into cellulose acetate. The mixed coating was shown to exhibit properties superior to those of the two components alone and improved stability and selectivity was demonstrated.

A reduction in surface poisoning by SCN^- in the FIA determination of NO_2^- at a vitreous carbon disc (7.5 mm^2) coated with poly-(4-vinyl-pyridine) incorporating $IrCl_6^{4-}$ as redox mediator was also reported by Cox and Kulkarni [82]. In this case, however, the response was found to be halved when compared with a non-modified electrode , and the response was non-linear. The modified electrode, however, showed decreased interference from Pb(II), Mn(II) and Fe(II). In further studies Cox et al.[83] have reported stable modified electrodes for the FIA determination or thiocyanate. Platinum

electrodes that were modified by adsorption of iodine and coated with cellulose acetate, and glassy carbon electrodes that were modified by anodisation in a $RuCl_3/K_4Ru(CN)_6$ mixture, were used for the determination of SCN^- in urine; interference by uric and ascorbic acids was eliminated by controlled potential electrolysis prior to the determination.

The use of polymer-modified electrodes for the FIA determination of electroinactive anions has been reported by Ikariyama and Heineman [84]. This method is based on repetitive doping-undoping of the polypyrrole coating due to influx/regress of anions such as PO_4^{3-} and CO_3^{2-}. Concentrations of these ions between 10 µM -> 1 mM could be determined. The coating was found to be stable for two weeks in an anaerobic atmosphere. A similar study using polyaniline modified electrodes for the determination of electroinactive anions, was carried out by Ye and Baldwin [85]. Using this polymer, NO_2^-, ClO_4^- and SO_4^- could be determined. A very interesting aspect of these electrodes was that the selectivity for various anions could be controlled by manipulation of the composition of the electrolyte solution.

Other recent publications include a paper by Thomsen et al. [86] in which the voltammetric determination of traces of nickel(II) involving a medium exchange flow system and a chemically modified carbon paste electrode containing dimethylglyoxime, were discussed. Under flowing conditions, the CME was highly stable and showed no signs of fatigue after more than 4 h of continuous operation. This system was used for the determination of nickel in NBS fly ash. Tay et al. [87] have reported on the determination of hydrogen peroxide in FIA using

a platinum-dispersed Nafion-modified glassy carbon electrode. This electrode showed increased stability and sensitivity for this determination compared to a conventional platinum electrode.

As can be seen from the literature, the use of modified electrodes has become increasingly popular. These electrodes have many advantages over conventional electrodes, they offer increased sensitivity and selectivity and in some cases a reduction in electrode poisoning is achieved. However, the use of modified electrodes in flowing systems has suffered from some problems relating to stability of the electrode coating. In this study a Ru-polymer modified electrode based on the polymer $[Ru(bpy)_2(PVP)_5Cl]Cl$ (where bpy = bipyridyl and PVP = polyvinyl pyridine) was investigated for use in flowing solutions. Some procedures for stabilisation of these electrodes were also investigated.

5.2. Experimental

5.2.1. Reagents and Standard Solutions

All chemicals were of analytical-reagent grade. Metal salts were obtained from Ajax Chemicals. Deionised water was produced by passing distilled water through a Millipore Milli-Q water purification system. The $[Ru(bpy)_2(PVP)_5Cl]Cl$ polymer was prepared following the method reported by Clear et al.(88). The bis-(2-hydroxyethyl) dithiocarbamate (HDTC) was synthesised as described previously (89). The metal dithiocarbamate complexes were prepared by mixing stoichiometrically, aqueous solutions of both ligand and the corresponding metal. HPLC grade methanol and acetonitrile were obtained from BDH.

5.2.2. Instrumentation

For studies in the stationary cell, a Princeton Applied Research Corporation (PARC) Model 174 polarographic analyzer was operated with a three electrode cell system (incorporating a platinum auxiliary electrode and a Ag/AgCl reference electrode) to obtain the electrochemical responses. Metrohm glassy carbon electrodes (3 mm diameter) were used as working electrode substrates.

The flowing system consisted of a Dionex thin layer cell and a Bio-Analytical Systems LC-3A amperometric detector or a Dionex pulsed amperometric detector. The working electrode was a 3 mm diameter glassy carbon electrode.

Galvanostatic generation of polymer coatings were achieved using a PARC Model 173 Potentiostat/Galvanostat. Chronopotentiograms were also recorded using the PARC Model 173. Potentiostatic generation of polymer coatings were achieved using a Bioanalytical Systems (BAS) Model CV27 cyclic voltammograph.

5.2.3. Electrode preparation and modification

Metrohm glassy carbon electrodes (3 mm diameter) were used throughout these studies. These electrodes were diamond-polished in stages down to 3 μm, followed by a buff with an aluminium oxide slurry. The electrodes were rinsed with distilled water and dried prior to modification. Ruthenium polymer modified electrodes were prepared by pipetting 5 ul of a $1x10^{-3}M$ solution of $[Ru(bpy)_2(PVP)_5Cl]Cl$ in methanol onto the surface of the electrode. The electrode was then covered with an aluminium coated beaker and the solvent allowed to evaporate in the dark, leaving a thin film of polymer chemisorbed to the surface. In the flowing system a Dionex thin layer cell was used. In this cell the working electrode was a 3 mm glassy carbon electrode which was coated using the same procedure as for the stationary cell.

5.2.4. Stabilisation procedures

The following procedures were employed to stabilise the $[Ru(bpy)_2(PVP)_5Cl]Cl$ polymer modified electrode.

(a) UV irradiation

Dry modified electrodes were irradiated by holding under a 200w mercury lamp (Ultraviolet Products) for a specified period of time.

(b) Coating with conductive polymers

(i) Polypyrrole: after leaving the modified electrode to dry for at least 24h, it was placed in an 0.5 M aqueous solution of pyrrole (Sigma) that was 1.0 M in NaCl. The polypyrrole layer was then generated galvanostatically at a current density of 0.5mA cm^{-2}. The thickness of the polypyrrole layer was controlled by varying the amount of charge passed and the coating time.

(ii) Poly(3-methylthiophene): poly(3-methylthiophene) coatings were generated galvanostatically by scanning from 0.0-> + 1.50 V (vs Ag/AgCl) at a scan rate of 50 mV s^{-1} in an 0.5 M solution of 3-methylthiophene in acetonitrile that was 0.25 M in $HClO_4$. The thickness of the coating was controlled by varying the number of scans.

(c) Coating with a non-conductive polymer: N-ethyltyramine was synthesised according to Wallace et al.(69). Poly(N-ethyltyramine) was generated potentiostatically by scanning from 0.0-> +1.50 V (vs Ag/AgCl) at a scan rate of 50 mV s^{-1} in an 0.1 M solution of N-ethyltyramine in methanol that was 0.5 M in NaOH.

5.3. Results and Discussion

5.3.1. Electrochemical Studies in a Stationary System.

Initial voltammetric experiments were carried out in a conventional 3-electrode cell. After coating a glassy carbon electrode with the ruthenium-containing polymer ($[Ru(bpy)_2(PVP)_5Cl]Cl$), well defined oxidation and reduction responses due to the Ru(II)/Ru(III) couple were observed in 1 M $NaNO_3$ (Fig.5.2a). The peaks were symmetrical in shape, and as observed by previous workers [58], the addition of Fe(II) to the solution results in an electrocatalytic oxidation at the same potential observed for the Ru(II)/Ru(III) system (Fig.5.2b). At the same time, however, it is clear that the mediated reduction of Fe(III) is less efficient and that reduction of Fe(III) partly occurs at the bare glassy carbon electrode as can be seen from the peak around 0.0 V in Fig.5.2b.

In an attempt to optimise the electrochemical responses of the Ru(II)/Ru(III) couple, the following parameters were investigated:

(a) scan rate

(b) polymer thickness and

(c) supporting electrolyte.

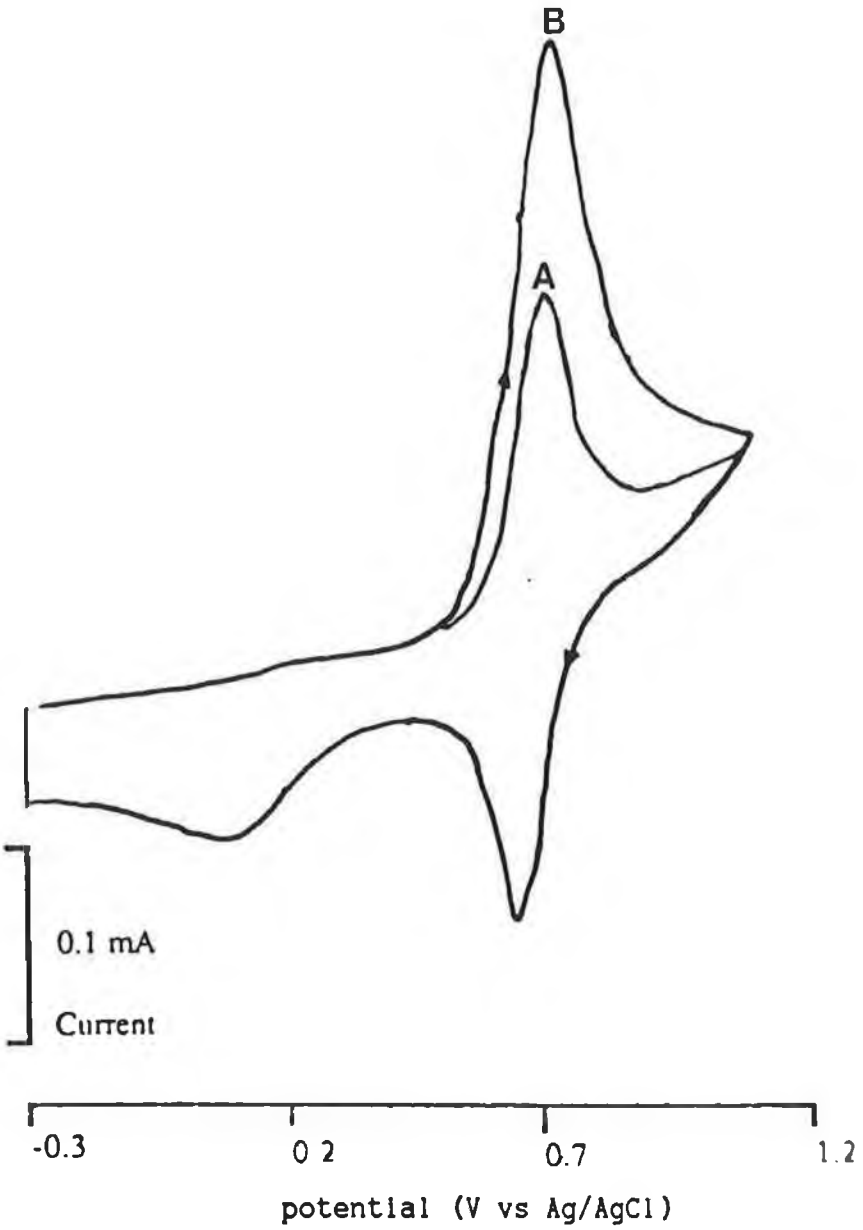


Figure 5.2. Cyclic voltammetry for (A) an electrode coated with ruthenium polymer and (B) the analysis of 5 x 10^{-3} M Fe(II) using this electrode. (Electrolyte: 1 M $NaNO_3$ Scan rate: 200 mV sec^{-1})

5.3.1.1. Effect of Scan Rate

In 1M $NaNO_3$, the currents for both the oxidation and reduction responses for the Ru(II)Ru/(III) couple varied linearly with scan rate up to 300 mV/sec (Fig.5.3).This indicates that although the response is due to a surface bound species, it becomes diffusion controlled at higher scan rates.

5.3.1.2. Effect of Polymer Coverage

The effect of polymer coverage on peak current for the analysis of Fe(II) ($2.5x10^{-4}$M) can be seen in Table 5.1. From this it can be seen that increasing the coverage from 2.5 -> $7.5x10^{-9}$ moles/cm^2 had little effect on peak heights.

5.3.1.3. Effect of Supporting Electrolyte

The effect of supporting electrolyte on the potential of the Ru(II)/Ru(III) redox couple obtained with the ruthenium polymer are shown in Table 5.2. Some marked effects were observed. Firstly, the Ru(II)/Ru(III) peak-to-peak separation varies between 4-15 mV; the more ideal response (less peak-peak separation) being observed in 0.1 M H_2SO_4 + 0.9M Na_2SO_4. The combination of low pH and high ionic strength presumably results in increased mobility and consequently rapid charge transfer within the polymer.

Using this supporting electrolyte, an i_{pa}/i_{pc} value of 0.92 was obtained, which is also indicative of fast reversible electron transfer, but suggests that the charge

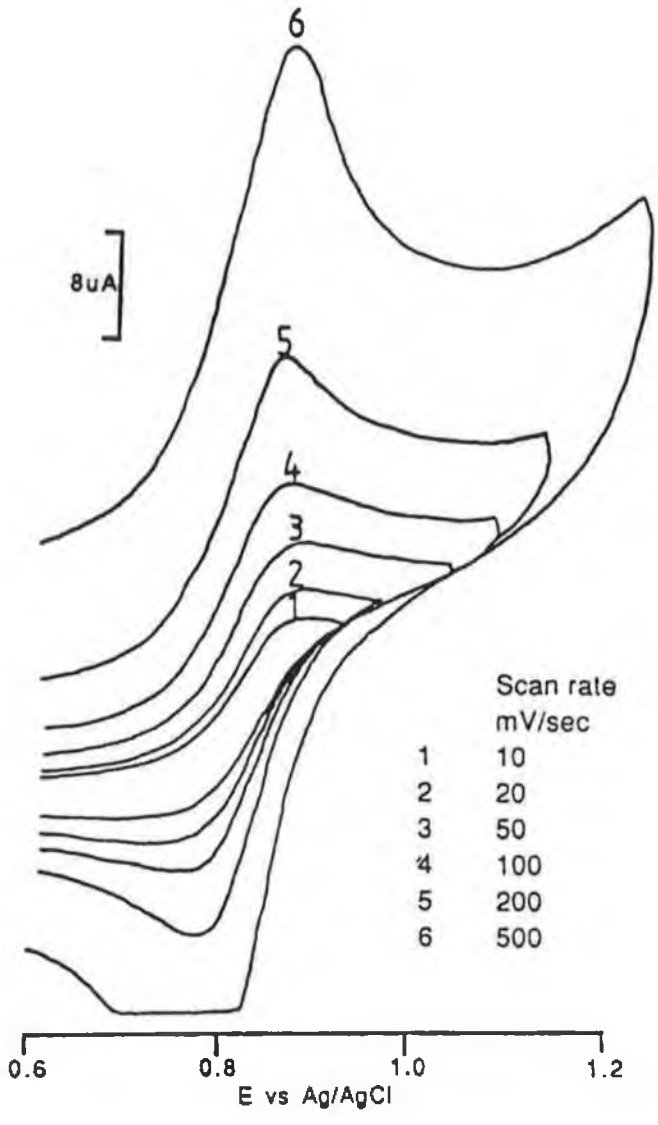


Figure 5.3. Variation of the response of the modified electrode with scan rate in 1 M $NaNO_3$.

Table 5.1. Variation of the Fe(II) response with polymer coating thickness.

Analyte	Polymer coating (moles / cm^2)	Peak height (mA)
Fe^{2+} (2.5×10^{-4} M)	2.5×10^{-8}	0.10
	5.0×10^{-8}	0.09
	7.5×10^{-8}	0.11

Scan rate: 100mV/sec

Electrolyte: 1.0 M $NaNO_3$

Table 5.2. Electrochemical responses at glassy carbon modified electrodes in various electrolytes.

Supporting electrolyte	1.0 M $NaNO_3$	1.0 M KCl	1.0 M H_2SO_4	0.1 M H_2SO_4 + 0.9M Na_2SO_4	
Polymer coating $\frac{moles}{cm^2} \times 10^{-8}$	7.5	10	12.5		7.5
Ru (E_{pa})	0.78	0.81	0.72		0.69
Ru ($E_{pa} - E_{pc}$)	0.14	0.11	0.08		0.04
Fe (E_{pa})	0.73	0.82	0.72	0.59	0.73
Fe ($E_{pa} - E_{pc}$)	0.10	0.15	0.07	-0.06	0.01
Ru (i_{pa} / i_{pc})	1.04	0.97	1.07		0.92
Fe (i_{pa} / i_{pc})	1.27	1.10	1.42	0.52	0.71

(Scan rate: 100 mV and V vs. AgCl)

transfer associated with the Ru(II) species is slightly faster than that associated with the more highly charged Ru(III) species. In this mixed electrolyte a double peak was observed on addition of Fe(II), with responses at +0.59 V and +0.73 V vs Ag/AgCl. This feature can probably be explained as a ligand exchange reaction which can be initiated thermally or photochemically. However, this double peak phenomenon results in difficulties when analysing the voltammograms for peak currents and therefore 1.0M $NaNO_3$ was chosen for further investigations.

5.3.1.4. Electrochemical Responses of Various Ions in Stationary Cells

Using 1.0M $NaNO_3$ as supporting electrolyte, a polymer coverage of $5x10^{-8}$ moles/cm^2 and a scan rate of 100 mV/sec, data was obtained for the addition of a variety of analytes as shown in Table 5.3.

On a bare glassy carbon electrode, the oxidation of nitrite (NO_2^-) gave a broad response at 0.95 V vs Ag/AgCl. The corresponding response on a polymer-modified electrode occurred at +0.80 V vs Ag/AgCl, indicating a reduction in the overpotential of NO_2^- oxidation. The sensitivity for determination of NO_2^- increased by a factor of three using the coated electrode (Fig. 5.4).

Although the other species investigated showed an anodic shift in oxidation potential of the Ru(II)/Ru(III) couple, responses were more sensitive and better defined. On bare glassy carbon, a response was observed at +0.34 V vs

Table 5.3. Electrochemical responses at modified and non-modified glassy carbon electrodes in 1 M $NaNO_3$.

Analyte	Ep (V)	Ep-$Ep_{1/2}$ (V)	Sensitivity (mA/mole)
		Coated Electrode	
NO_2^-	0.80	0.05	56
HDTC	0.75	0.04	20
$Ni(HDTC)_2$	0.79	0.07	20
$Cu(HDTC)_2$	0.80	0.06	12
Fe^{II}	0.79	0.09	46
		Uncoated Electrode	
NO_2^-	0.95	0.10	19
HDTC	0.34	0.05	3
$Ni(HDTC)_2$	0.64	0.07	12
$Cu(HDTC)_2$	0.50	0.08	16
Fe^{II}	0.62	0.07	30

(Scan rate: 100 mV and V vs Ag/AgCl)

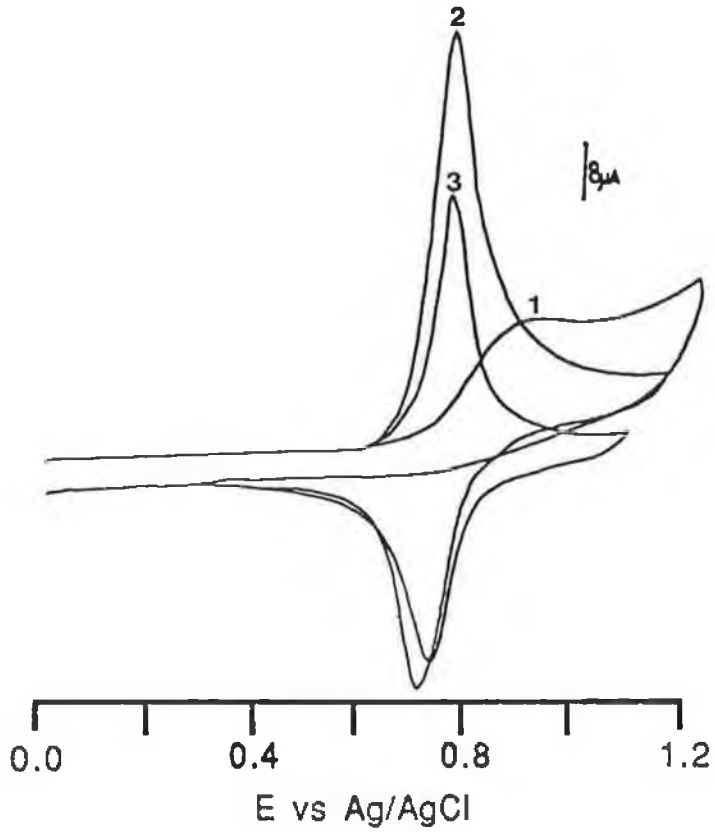


Figure 5.4. Cyclic voltammetry showing oxidation of 40 ppm NO_2^- in 1.0 M $NaNO_3$, at (1) an uncoated glassy carbon electrode, (2) a polymer coated electrode.

Curve 3: ruthenium polymer background response.

Scan rate: 100 mV sec^{-1}.

Ag/AgCl for $5x10^{-4}$ M HDTC in 1.0 M $NaNO_3$. Major problems with reproducibility were encountered, possibly due to adsorption of a thiuram disulphide, oxidation product on the electrode surface. To obtain reproducible results, the electrode had to be removed from solution, polished and washed between scans. Using a polymer-coated electrode, a response was observed at +0.75 V vs Ag/AgCl and no reproducibility problems were encountered. Furthermore, the modified eletrode enhanced the sensitivity by a factor of six.

As the objective of this study was to produce a detection system capable of operation in conjunction with HPLC or FIA for the determination of metal complexes, metal complexes of HDTC were synthesised. The first complex that was studied was the $Ni(HDTC)_2$ complex. On bare glassy carbon this complex gave an oxidation response at +0.64 V vs Ag/AgCl. Again this analyte showed adsorption problems, which required polishing of the electrode to achieve reproducibility between scans. However, use of the polymer-modified electrodes resulted in no adsorption being observed and an enhancement in sensitivity by a factor of four.

A $2.5x10^{-6}$ M solution of the $Cu(HDTC)_2$ complex was also investigated using modified and unmodified electrodes. In this case, the bare glassy carbon electrode showed a greater response than that obtained with a coated electrode. This is presumably due to the fact that the oxidation of the $Cu(HDTC)_2$ complex is relatively fast on bare glassy carbon.

5.3.2. Electrochemical Studies in a Flowing System

In order to circumvent problems associated with the high background signal,(i.e. the presence of the Ru(II)/Ru(III) at 0.75 V) the operation of the modified electrode in flowing solutions was investigated. The flowing system offers the advantage that at potentials $\geq$ 0.80 V all the Ru-sites are in the Ru(III) oxidation state, the only response observed being due to the analyte signal. Hence, lower detection limits, without the errors associated with background subtraction, can be achieved. The system was optimised with respect to.

(a)Polymer coverage;
(b)Supporting Electrolyte;
(c)Applied Potential; and
(d)Flow Rate.

5.3.2.1. Effect of Polymer Coverage

The polymer coverage was varied from 2 -> 8 x 10^{-8} moles/cm^2. Unlike the stationary system, where polymer coverage had little effect on the magnitude of the response, increased coverage resulted in a reduction in the response magnitude (Table 5.4). As will be discussed later this variation of response with coverage contributes to problems with reproducibility.

Table 5.4. Variation of the $Ni(HDTC)_2$ response with polymer coating thickness.

Analyte	Polymer Coating (moles / cm^2)	Peak height (nA)
$Ni(HDTC)_2$	2×10^{-8}	4280
(5×10^{-4} M)	4×10^{-8}	3200
	8×10^{-8}	2920

Applied potential : 1.2 V vs Ag/AgCl.

Eluent : 1 M Na NO_3

Flow rate : 1 ml/min

5.3.2.2. Effect of Supporting Electrolyte

As with the stationary cell, the choice of supporting electrolyte influences the analytical response for both modified and conventional electrodes. The electrolytes investigated were 0.1 M $NaNO_3$, 0.1 M Na_2SO_4 and 0.09 M Na_2SO_4 + 0.01 M H_2SO_4. For the determination of NO_2^- or $Ni(HDTC)_2$ using sodium nitrate or sodium sulphate as electrolyte, the analytical responses were very similar. In contrast to results obtained in the stationary cell the mixed supporting electrolyte of sodium sulphate and sulphuric acid which gave optimum responses in the stationary cell resulted in lower sensitivity in the flowing system. Obviously, under convection control, the effect of the supporting electrolyte is different than under diffusion control, as would be encountered in the stationary cell. The results for sodium sulphate and sodium nitrate were very similar and either could have been chosen for further studies. In these studies sodium nitrate was used throughout.

5.3.2.3. Effect of Applied Potential

In the flowing system, the potential applied for detection ensures that all of the ruthenium is present as Ru^{III}. As the analyte reaches the electrode surface, it can therefore be reduced as shown in eq.(5.1).

$$Ru^{III} + Fe^{II} \rightarrow Ru^{II} + Fe^{III} \qquad (5.1)$$

In this study the potential applied was varied from + 0.80 V to + 1.4 V vs Ag/AgCl in 0.20 V increments. Both $Ni(HDTC)_2$ and NO_2^- were injected into the system. Increasing the potential resulted in increased sensitivity (Table 5.5), but above + 1.2 V, the background noise also increased due to oxidation of the eluent. Hence experimental work in the flowing system was carried out at + 1.2 V vs Ag/AgCl.

5.3.2.4. Effect of Flow Rate

Using 0.1M $NaNO_3$ as supporting electrolyte the effect of flow rate on the magnitude of response was investigated. The results for the detection of NO_2^- can be seen in Table 5.6. At very low flow rates, i.e. 0.1 ml/min, a broad response was obtained. However, these conditions resulted in the largest peak area corresponding to the maximum electrolytic conversion of the analyte. Flow rates of 0.5 and 1.0 mL/min resulted in similar peak heights and peak areas and faster flow rates e.g. 5ml/min, produced a loss of sensitivity. The flow rate in further work was therefore set at 1.0 ml/min.

5.3.2.5. Electrochemical Responses of Various Ions in the Flowing Cell

Using the optimum conditions cited above the responses of various analytes were investigated. Some typical results are summarised in Table 5.7. From this table it can be seen that the use of modified electrodes results in a significant sensitivity enhancement for the species studied,

Table 5.5 Variation of the $Ni(HDTC)_2$ response with applied potential. (a)

Analyte	Applied potential (V)	peak height (nA)
$Ni(HDTC)_2$ ($5x10^{-4}$)	1.0	600
	1.2	972
	1.3	1092
	1.4	1260

(a) Applied potential: vs Ag/AgCl

Eluent: 1 M $NaNO_3$

Flow rate: 1 ml/min

Table 5.6. Variation of the NO_2^- response with flow rate in 0.1 M $NaNO_3$ at a coated glassy carbon electrode.

Flow rate (mL/min)	Peak height (nA)	Peak width (min)
0.1	440	0.90
0.5	624	0.25
1.0	672	0.20
5.0	568	0.10

Applied potential: 1.2 V vs. Ag/AgCl.
Concentration of NO_2^- : 1 ppm

Table 5.7. Electrochemical responses at modified and non-modified glassy carbon electrodes in flowing solutions. (a)

Analyte(b)	Peak height (nA) Uncoated electrode	Peak height (nA) Coated GC electrode (c)	Increase Factor
$Cd(HDTC)_2$	612	2220	3.6
$Co(HDTC)_2$	276	1512	5.7
$Cu(HDTC)_2$	252	840	3.3
$Ni(HDTC)_2$	432	1656	3.8
$Pb(HDTC)_2$	1040	4960	4.8
$Zn(HDTC)_2$	588	2280	3.9
HDTC	262	2920	11.2
NO_2^-	180	860	4.8

(a) Eluent: 0.1M $NaNO_3$ at 1.0 mL/min.

(b) Concentrations: Metal/HDTC complexes: 2.5×10^{-4} M, HDTC : 5×10^{-4} M, NO_2^- : 1 ppm.

(c) Polymer coating : $3 \times 10^{-}$ moles/cm^2

while noise levels remain essentially unchanged.

A typical flow injection analysis trace showing repetitive injections of NO_2^- is shown in Fig.5.5.
Other analytes showed similar short term reproducibility. The response for NO_2^- was found to be linear with concentration in the 0-2 ppm range with a limit of detection of 3 ppb.

In an attempt to further enhance the sensitivity, pulsed amperometric detection was applied. The conditions were optimised for NO_2^- and $Ni(HDTC)_2$ with respect to applied potential, pulse amplitude and pulse duration. The optimum conditions involved the application of a base potential of +0.7 V vs Ag/AgCl for 60 msec and an anodic pulse of 300 mV for 420 msec. This mode of detection enhanced the signal for both the modified and the conventional electrode by a factor of three.

As can be seen from the results (Table 5.7), these modified electrodes, when used in flowing systems, can provide increased sensitivity without any increase in background noise. However, problems with irreproducibility between different coated electrodes were encountered. The main factor thought to be responsible for this irreproducibility was the thickness of the polymer coating. As previously shown (Table 5.4) the magnitude of the analyte response varied with polymer thickness.

Furthermore, when the coated electrodes were examined under a microscope it was noted that the coating thickness decreased from the periphery to the centre. These characteristics render it extremely difficult to reproduce the coated electrode surface. Another problem associated with the flowing system was the slow removal of the polymer from the substrate. This resulted in changes in the thickness of the

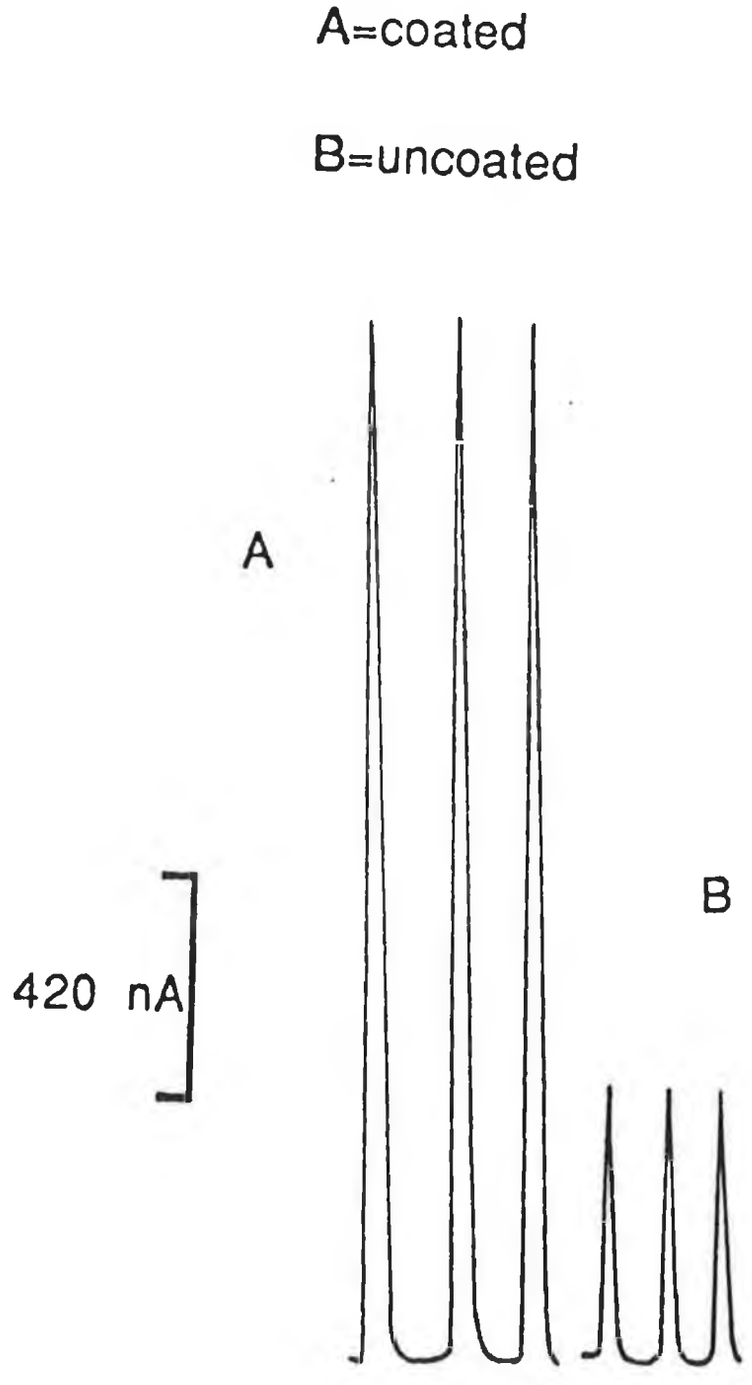


Figure 5.5. Comparison of coated and uncoated electrodes in a flowing system (0.1 M $NaNO_3$ at 1 ml min^{-1}) for analysis of $Ni(HDTC)_2$ (2.5×10^{-4} M).

polymer coating causing responses to decrease with time during some experiments. In an attempt to overcome some of these problems, methods of stabilisation of the ruthemium polymer for use in flowing solutions were investigated.

5.3.3. Stabilisation Procedures

5.3.3.1. Effect of UV Irradiation

It is well known that treatment of polymeric materials with UV irradiation can increase the degree of cross-linking in the polymer and lead to greater mechanical stability [90]. Such treatment may also activate groups on the electrode surface and induce bonding with the polymeric material. Furthermore, ligand exchange reactions in the electroactive ruthenium moieties could also lead to enhanced cross-linking, as earlier reported experiments have shown that the chloride ligand is photochemically labile [91].

On injection of test analytes such as NO_2^- and $Ni(HDTC)_2$, into flowing streams the peak currents increased linearly with increasing analyte concentration. A slight decrease in sensitivity was observed, however, when the signals obtained were compared to a non-irradiated coating. The effect of UV irradiation on the responses obtained for nitrite in flowing streams are shown in Table 5.8. As expected, an increase in irradiation time resulted in a decrease in sensitivity, presumably due to decreased penetration of analyte and/or counterion into the polymer layer, or by a change in the electrocatalytic properties of the polymer-bound ruthenium

Table 5.8. A comparison of sensitivities obtained for the deterimation of NO_2^- at bare, non-stabilised and stabilised ruthenium polymer modified electrodes in flowing solutions.

Stabilisation Procedure		Sensitivity (mA $mole^{-1}$) Bare	non-stabilised	stabilised	$t_{1/2}$ (h)
UV irradiation					
time (min)	0	88	364	-	8
	5			310	12
	10			320	24
	15			302	>48
	20			288	>48
	60			207	>48
coating with poly(3-methylthiophene)					
number of scans	0	92	368		8
	1			342	>48
	2			274	>48
coating with polypyrrole					
coating time (min)	0	87	285		8
	4			242	12
coating with poly(N-ethyltyramine)					
number of scans	0	83	219		8
	1			209	>48
	2			201	>48
	3			211	>48

moieties. A marked increase in stability is observed, however, and for short irradiation times, this loss in sensitivity can be minimised. A similar result was obtained for analysis of $Ni(HDTC)_2$, as shown in Table 5.9.

5.3.3.2. Coating with conductive polymers

Coating the ruthenium polymer modified electrode with either polypyrrole or poly(3-methylthiophene) caused the Ru(II)/Ru(III) redox couple to be obscured in cyclic voltammetry in a stationary cell, due to the high background responses associated with these polymers. Scanning to potentials anodic enough to observe oxidation of the Ru(II) sites resulted in oxidation of the polypyrrole coating, although the coating still adhered well to the surface of the ruthenium polymer. In flowing streams, the coating procedures again resulted in a decrease in sensitivity for both NO_2^- and $Ni(HDTC)_2$ compared to the conventional ruthenium polymer modified electrode (Tables 5.8 and 5.9). This can again be explained by a decreased penetration of analyte and/or counterion into the ruthenium polymer layer. This loss of sensitivity was also found to increase with increasing thickness of the conductive polymer coating. As with the UV irradiation treatment, however, stability was shown to be improved for both NO_2^- and $Ni(HDTC)_2$ using both polypyrrole and poly(3-methylthiophene) as protective coatings, except in the case of the polypyrrole coated electrode for the determination of $Ni(HDTC)_2$ (Tables 5.8 and 5.9). This increase in stability was particularly evident when using the

Table 5.9. A comparison of sensitivities obtained for the determination of $Ni(HDTC)_2$ at bare, non-stabilised and stabilised ruthenium polymer modified electrodes in flowing streams.

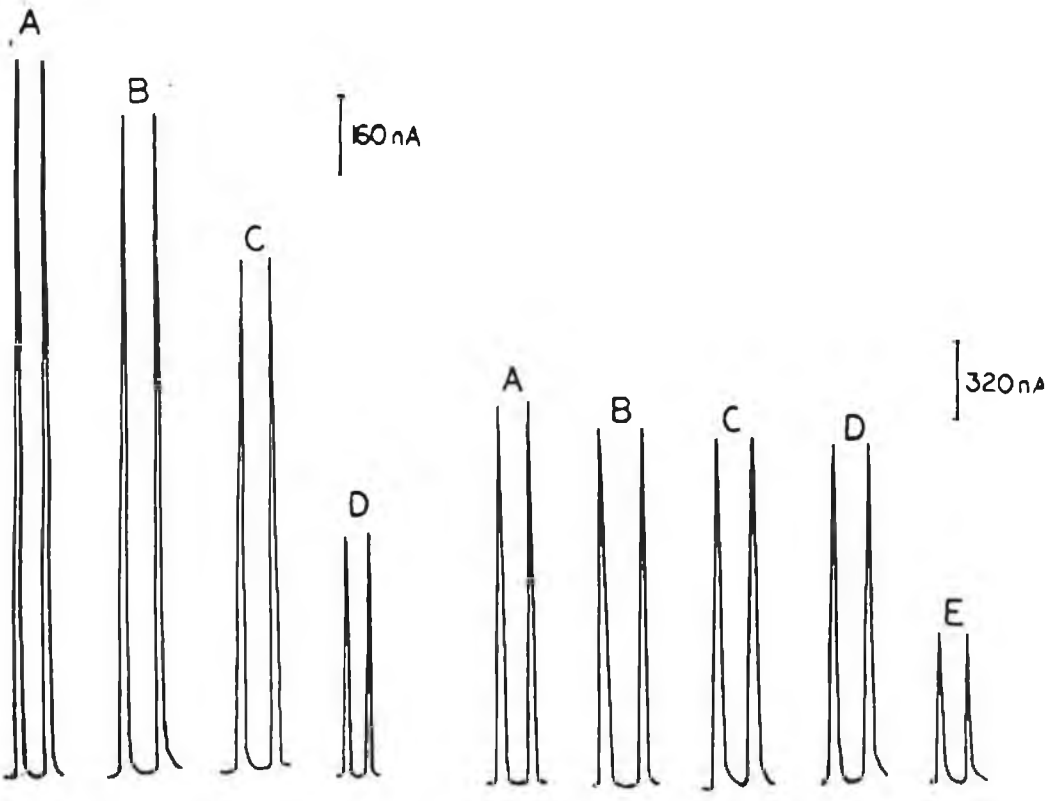
Stabilisation Procedure		Sensitivity (mA $mole^{-1}$) Bare	Non-stabilised	Stabilised	$t_{1/2}$ (h)
UV irradiation time (min)	0	111	307		8
	10			216	>48
Coating with poly(3-methylthiophene) number of scans	0	114	186		8
	1			173	>48
Coating with polypyrrole Coating time (min)	0	115	282		8
	4			232	4
Coating with poly(N-ethyltyramine) number of scans	0	117	217		8
	3			212	>48

poly(3-methylthiophene) coating, where a typical stabilised electrode had a half-life of >48 h compared to a half-life of 8 h for a typical non-stabilised electrode. This is illustrated in Figure 5.6 for typical traces obtained using these electrodes.

5.3.3.3. Coating with a non-conductive polymer

No response due to the Ru(II)/Ru(III) couple could be observed in cyclic voltammetry after coating the ruthenium polymer with poly(N-ethyltyramine). In the flowing system, however, only a slight loss in sensitivity was observed when the non-conductive polymer coated electrode was compared with a conventional ruthenium polymer modified electrode for both NO_2^- and $Ni(HDTC)_2$ (Tables 5.8 and 5.9). This was coupled, however, to a marked increase in stability, as can be seen from a comparison of the half-lives of the stabilised electrode for the determination of both analytes when compared to a non-stabilised electrode.

The reason for the lower loss in sensitivity of the non-conductive polymer coated electrode compared to the UV irradiated electrode or the two conductive polymer coated electrodes may be explained by the fact that poly(N-ethyltyramine) can preconcentrate analytes such as NO_2^- at its surface. This was demonstrated when a glassy carbon electrode was coated solely with poly(N-ethyltyramine), and the performance of this modified electrode compared to that of a bare electrode for both nitrite and $Ni(HDTC)_2$. In doing this it was found that the enhancement due to the



(a) A : t = 0 min;
B : t = 30 min;
C : t = 8 h;
D : response for nitrite at a bare electrode.

(b) A : non-stabilised electrode at t = 0 min;
B : electrode stabilised by coating with poly(3-methylthiophene) following one scan between 0.0->+1.50 V t = 0 min;
C : electrode as in B; t = 30 min;
D : electrode as in B; t = 16 h;
E : response for nitrite at bare electrode.

Figure 5.6. A comparison of the determination of NO_2^- (5.0 ppm) using (a) a non-stabilised ruthenium polymer modified electrode, and (b) a ruthenium polymer modified electrode stabilised by coating with poly(3-methylthiophene), in a flowing system (0.1 M $NaNO_3$ at 1 ml min^{-1}).

poly(N-ethyltyramine) coating was not as great as that obtained for a ruthenium modified electrode coated with this polymer. This would suggest that both layers contribute to the maintenance of the current response seen for this electrode.

5.4. Conclusions

The advantage of modified electrodes coated woth electrocatalytic polymers for detection in flowing solutions has been demonstrated. The ruthenium polymer used has been shown to enhance the response for various analytes. At the same time it circumvents problems associated with adsorption of oxidation products. Limitations associated with the instability of chemisorbed modifiers were observed and various ways of overcoming this problem were investigated.

These investigations have shown that a ruthenium polymer modified electrode can be stabilised for use in flowing solutions, using a variety of approaches. In all cases this increase in stability was at the expense of a slight loss in sensitivity. Of the approaches reported, treatment with UV irradiation was the simplest way to achieve better stability; if the modified electrode was to be used in an organic solution, coating with poly(3-methylthiophene) or poly(N-ethyltyramine) provides the best protection for the organic soluble ruthenium polymer. Polypyrrole can also be used for stabilisation, but the decrease in sensitivity was more marked for this coating than with the other protective polymer coatings.

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