THE DETERMINATION OF TRANSITION METAL IONS IN ANAEROBIC ADHESIVES BY REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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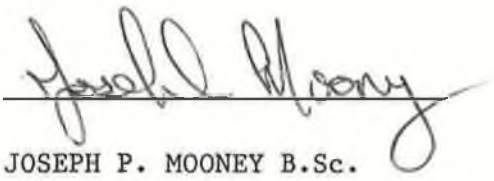
DECLARATION

I hereby declare that the contents of this Thesis are based on my own work.



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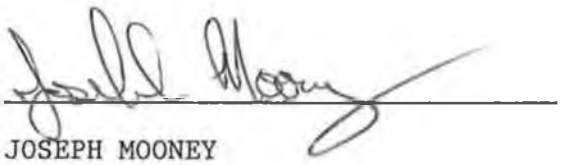
Signed: 

JOSEPH MOONEY

DEDICATION

TO MY FAMILY

AND

MY FIANCEE

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ABSTRACT

By using reverse phase high performance liquid chromatography (HPLC) and chelating agents such as dithiocarbamates or 8-hydroxyquinoline it was possible to separate a range of metal ions which are of importance in the polymerisation chemistry of anaeorbic adhesives.

These metal-chelate complexes can be formed 'in-situ' or externally. 'In-situ' complexation involves incorporating the chelating agent into the mobile phase and external formation involves mixing the appropriate metal ions with the chelating agent prior to HPLC separation.

Using sodium diethyldithiocarbamate as the chelating agent, it was possible to selectively separate metal ions such as Ni(II), Co(III) and Fe(III), using a C_{18} reverse phase system with ultra-violet detection at 254 nm. The main disadvantage of sodium diethyldithiocarbamate was that it does not have the selectivity to fully resolve Cu(II) and Fe(III). These are two most important metal ions in anaerobic adhesive chemistry, and it was therefore decided to investigate an alternative chelating agent.

This alternative ligand was 8-hydroxyquinoline, and using this it was possible to resolve Cu(II) and Fe(III) as their oxinate complexes using reverse phase high performance liquid chromatography with a mobile phase of acetonitrile: 0.02 M sodium acetate buffer (pH 6.0), (50:50, v/v) which was 5×10^{-3} M in 8-hydroxyquinoline and 0.1 M in KNO_3 with ultra-violet detection at 400nm.

An extraction system was then developed which extracted the metal ions, namely Cu(II) and Fe(III), from anaerobic adhesive formulations

and anaerobic adhesive raw materials. This extraction system showed that the raw materials used in the formulation contained levels of Cu(II) and Fe(III) which if not complexed by EDTA would cause premature polymerisation of adhesive. There was also Fe(III) present in the water which was throughout the analysis, so background corrections were needed to considered at every step.

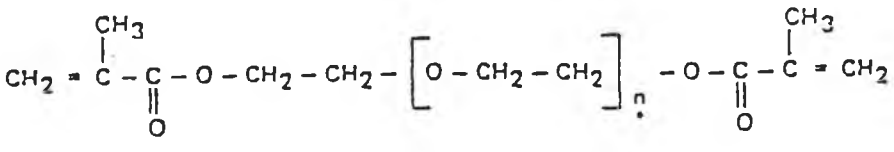
The extraction method was applied to anaeorbic adhesive products 290 and 270. Detection limits for Cu(II) and Fe(III) in PEGMA-based (polyethyleneglycoldimethacrylate) anaerobic adhesive formulations were 250 µg/ml and 600 µg/ml respectively and in TRI-EGMA (triethyl-eneglycoldimethacrylate) were 100 µg/ml and 250 µg/ml.

INTRODUCTION

A. USES AND CHEMISTRY OF ANAEROBIC ADHESIVES

Anaerobic adhesives are a range of products containing polymeric compounds developed for use in mechanical engineering applications. A wide range of polymers exist with varying strengths and viscosities depending upon the substituents. Anaerobic adhesive polymerise by radical polymerisation of the vinyl group of the corresponding acrylic ester (I). This free radical polymerisation can be initiated by peroxides, persulphates, or azo compounds. The reaction of the peroxide to form the free radical is catalysed by the presence of metal ions. This polymerisation reaction is beneficial when the metal ions are located at the surface of the mechanical parts to be bonded together, but is a distinct disadvantage if the metal ions are present in the adhesive formulation at the production stage.

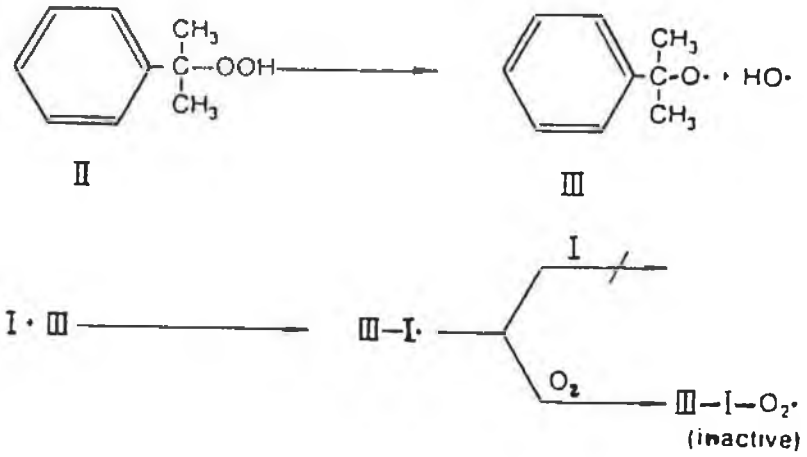
One of the early breakthroughs in anaerobic adhesive chemistry was made by Krieble in the early 1950's. He solved the problem of obtaining a stable anaerobic system by eliminating the monomer oxygenation step and using cumene hydroperoxide(II), a source of very stable free radicals, as a polymerisation initiator. This research overcame the problem associated with the early anaerobic adhesives that required continuous refrigeration or bubbling with air to inhibit polymerisation. Due to its high order of stability, cumene hydroperoxide underwent homolytic decomposition to a free radical species (III) at an exceedingly slow rate at room temperature.



* NOTE n = 3 average.

I

The free radicals produced reacted with the monomer (I) to initiate polymerisation, but polymerisation did not proceed in the presence of small amounts of oxygen which would react preferentially with the monomer radical to form an inactive species.



When the mixture of I and II was confined so as to exclude further contact with atmospheric oxygen, the oxygen dissolved in the system was consumed by the III-I· free radicals generated from II, and polymerisation eventually occurred. However, because the rate of spontaneous generation of the free radicals was extremely slow, the time necessary for the onset of polymerisation was exceedingly long.

The key to a practical anaerobic system is the fact that hydroperoxide undergoes a very facile oxidation-reduction reaction with transition metal ions, such as Cu(II) and Fe(III), leading to a rapid heterolytic decomposition of free radicals. The initiation reaction for this process can typically occur by the following general mechanism:

$$R\text{-}O\text{-}O\text{-}H + M^{n+} + H^{+} \rightarrow R\text{-}O^{\cdot} + M^{(n+1)+} + {}_2HO$$

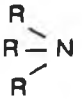
$$R\text{-}O\text{-}O\text{-}H + M^{(n+1)+} \rightarrow R\text{-}O\text{-}O^{\cdot} + M^{n+} + H^{+}$$

$$2R\text{-}O\text{-}O^{\cdot} \rightarrow 2R\text{-}O^{\cdot} + O_2$$

$$R\text{-}O^{\cdot} + H_2C{=}CH_2 \rightarrow R\text{-}O\text{-}CH_2\text{-}CH_2^{\cdot}$$

As can be seen from this general mechanism, the reaction of the peroxide to form the free radical is catalysed by the presence of metal ions, notably Cu(II) and Fe(III). When the anaerobic adhesive is placed on a transition metal surface under conditions which precludes further contact with oxygen, there is a rapid generation of free radicals. The dissolved oxygen is consumed and polymerisation proceeds at a commercially acceptable rate.

Formulation of the basic monomer-hydroperoxide system was followed by further work that identified a class of organic compounds know as trialkylamines (IV), as accelerators, for the polymerisation. It was also found that the presence of oxygen alone was not always adequate to ensure that the product would not polymerise during storage. The addition of small amounts of another class of organic compounds, the quinones (V), produced major improvements in the shelf stability of the product.



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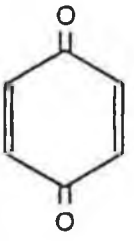
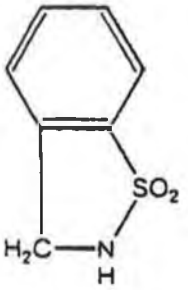


V

The "first generation" of anaerobic products cured fairly rapidly on "active" surfaces, such as iron and copper, where a surplus of transition metal ions were available on the surface. However, on "inactive" surfaces such as zinc plate or cadmium plate, where the surface concentration of metal ions is very low, curing was often slow or non-existent.

Importance was now focussed on decreasing the curing time of the system and decreasing the dependence of the composition of the surfaces to be bonded. Research efforts were now being directed towards developing new accelerators which would render the hydroperoxide much more sensitive to heterolytic decomposition by exceedingly low levels of transition metal ions. The result of this research was a co-accelerator system based on saccharin (VI) and dialkylarylamines (VII) which was capable of producing major increases in cure speed without a consequent negative effect on shelf stability.



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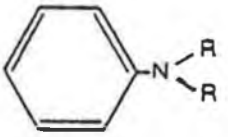


VII

Despite the considerable advances in anaerobic technology from 1950-1968, there were still significant limitations on formulation capabilities. The majority of attention had been devoted to improving the cure speed characteristics of the products. Formulation parameters were still basically limited to the thickener-plasticizer combinations of the 1950's. This was primarily due to the fact that the oxygen-quinone stabilised anaerobic systems were prone to destabilisation on addition of the variety of chemicals needed to produce other useful properties.

The destabilisation of anaerobic adhesives by a variety of chemical species was traced to the fact that these chemicals were

introducing small amounts of metal ions into the formulation. The incorporation of chelating agents to complex and deactivate these "tramp" metal ions produced major improvements in the ability of anaerobic adhesives to tolerate low levels of contamination.

In anaerobic adhesives, a range of free radical initiators can be used. Table 1 provides examples of some free radical initiators.

TABLE 1 FREE RADICAL INITIATORS

COMPOUND	RADICAL INITIATION STEP
HYDROGEN PEROXIDE	$HO-OH \rightarrow HO^{\cdot} + HO^{\cdot}$
ORGANIC PEROXIDES	$RO-OR' \rightarrow RO^{\cdot} + R'O^{\cdot}$
HYDROPEROXIDES	$RO-OH \rightarrow RO^{\cdot} + HO^{\cdot}$
PERACIDS	$RCOOOH \rightarrow R^{\cdot} + HO^{\cdot} + CO_2$
PERESTERS	$RCOOOR' \rightarrow R^{\cdot} + R'O^{\cdot} + CO_2$
DIACYL PEROXIDES	$(RCOOO)_2 \rightarrow R' + RO^{\cdot} + CO_2$
PERSULPHATES	$S_2O_8^{=} \rightarrow SO_4^{-\cdot} + SO_4^{-\cdot}$
AZO COMPOUNDS	$RN=NR' \rightarrow R^{\cdot} + R'^{\cdot} + N_2$

The use of redox free radical generation has many advantages. Redox catalysis systems use both a primary initiator precursor and a chemical reducing agent. A one electron transfer from the reducing

agent to the precursor occurs, giving rise to a free radical or free radical ion which may react as such or decompose further to another free radical and a stable ion or molecule. For example:

$$H_2O_2 + Fe(II) \rightarrow HO^{\cdot} + OH^{-} + Fe(III)$$

The above reaction illustrates the effects that can be caused by metal ions present in anaerobic adhesive formulations. The chelating agents added to the adhesive renders these metal ions inactive, but free metal ions, if present, can cause premature polymerisation of the adhesive.

Polymerisation in anaerobic adhesives occurs via the reactive vinyl group. Acrylate esters can polymerise and undergo typical addition reactions across the double bond, like other vinyl monomers. The ester group undergoes typical ester interchange reactions. As these vinyl groups are highly reactive, inhibitors are necessary to prevent them reacting spontaneously. The chemistry of anaerobic adhesives is very detailed and much research has been done on this class of compounds. Just as the chemistry of this system is diverse, so also are the uses to which anaerobic adhesives are put.

Anaerobic adhesives play a special role in the mechanical engineering industry. Some of the uses are listed in Table 2

Since much research has been focussed on the use of chelating agents in the detection of transition metal ions, the use of such chelating agents was the approach adopted for quantitation of the transition metals Cu(II) and Fe(III) in the anaerobic formulations.

TABLE 2 USES FOR ANAEROBIC ADHESIVES

Threaded parts
Gaskets
Sealing porous castings and welds
Mounting electrical rotors
Carburettor sealings
Mounting bearings
Permanent locking of threaded fasteners
Sealing threaded pipe fittings
Sealing plane surfaces

The two chelating agents investigated were sodium diethyldithiocarbamate (NaDEDTC) and 8-hydroxyquinoline (oxine). Of the two types of chelating agents, the use of dithiocarbamate has received much more widespread use. By forming a metal-chelate complex, it is possible to separate a number of transition metal ions by high performance liquid chromatography (HPLC).

As stated earlier, the reaction of the peroxide is catalysed by the presence of transition metals, predominately Fe(III) and Cu(II). Both of these transition metal ions can be introduced into the anaerobic adhesive during production. The sources of transition metal ion contamination are (i) pipes, tubing and containers used in production and (ii) raw materials. If and when these transition metal ions find a way into the anaerobic adhesive, they can cause premature

polymerisation of the product. This can occur in the production of the anaerobic adhesive or on the shelf. Obviously the loss of product at either of these two steps will cost time and money and is commercially damaging to the company.

The various pipes and containers used in the formulation step are specifically designed so as to introduce a minimal level of contamination. The main materials used are plastics and stainless steel. The plastics used are no real cause of any contamination problems, but stainless steel does contain Ni(II). The Ni(II) could possibly leach out, but this is not a significant problem.

The main source of transition metal ion contamination is from the metal ions present in the raw materials. Even though ethylenediamine tetraacetic acid (EDTA) is added to the formulation to 'mop-up' free transition metal ions, and when this is the case premature polymerisation can not occur.

A list of the various constituents used in a typical anaerobic adhesive formulation is shown in Table 3. Each of the constituents imparts a very specific property to the anaerobic adhesive, and much attention has been directed towards formulating an adhesive of a very high quality; hence the inclusion of such a wide range of compounds.

TABLE 3 CONSTITUENTS OF A TYPICAL ANAEROBIC ADHESIVE

FUNCTION	CONSTITUENT	PERCENTAGE
Monomer	Triethyleneglycoldimethacrylate	50-90%
Stabiliser	Solution of Quinone in a Dimethacrylate Ester	0.5%
Thickener	Bisphenol A Polyester	10-25%
Plasticiser	Tetraethylene Glycol-Di-(2-Ethylhexanoate)	20-30%
Polymerisation Initiator	Cumene Hydroperoxide	1-3%
Co-Accelerator	Saccharin	1-2%
Accelerator	Acetyl Phenyl Hydrazine or N,N-Dimethyl-p-Toluidine	0.5-1%
Thixotropes	Fumed Silica, Polyethylene	1-4%
Chelating Agents	Ethylenediaminetetra-acetic Acid, Tetrasodium Salt	400-800 ppm

To date, the adopted method of analysis for trace metals has been atomic absorption spectrometry (AAS) but this has a number of disadvantages when compared to HPLC. The use of AAS is metal specific, but only one metal can be analysed at a time, whereas HPLC can determine a range of transition metal ions in a mixture. By developing an HPLC method for routine analysis of the raw materials and the finished product, it was hoped that the method would be cost effective from the viewpoints of both money and time.

B. CHEMISTRY OF DITHIOCARBAMATES AND 8-HYDROXYQUINOLINE

B(i). Dithiocarbamate Chemistry

The use of dithiocarbamates (DTCs) in analytical chemistry stems from the fact that they are used as chelating agents for transition metal ions.

In the DTC ion the substituent/substituent sulphur bound to the nitrogen does to some degree influence the metal-sulphur bonding and also determine some analytical properties of the complex. The solubility of metal-DTC complexes in organic solvents increases as the size of the alkyl groups attached to the nitrogen increase. By chelating transition metal ions to a DTC ligand, the resulting complex is neutral or non-polar. Due to their relative non-polar nature, such complexes can be extracted using non-polar solvents, and separation can be achieved using normal or reverse phase HPLC. The structure of some commonly used DTC ligands is shown in Table 4.

TABLE 4 DITHIOCARBAMATE LIGANDS

NAME	ABBREVIATION	STRUCTURE
Diethyldithiocarbamate	DEDTC	R_1=R2=Ethyl
Benzylmethyldithiocarbamate	BMDTC	R_1 = Methyl R_2 = Benzyl
Diethoxyethyldithiocarbamate	DEODTC	R_1=R_2=Ethoxyethyl
Dihexyldithiocarbamate	DHDTC	R_1=R_2=Hexyl
Bis(n-butyl-2-napthyl) Methyldithiocarbamate	BNMDTC	R_1=n-Butyl R_2=2-Naphylmethyl
i-Pyrolidinedithiocarbamate (Tetramethylene dithiocarbamate)	TMDTC	II

B(ii). 8-Hydroxyquinoline Chemistry

Approximately two dozen cations form sparingly soluble complexes with 8-hydroxyquinoline (oxine). One advantage of oxine over DTC's is the fact that metal-oxine complexes are more stable that metal-DTC complexes. Most of the metal-oxinate complexes are soluble in chloroform or other non-polar solvents.

The reaction which occurs when an aqueous solution of a divalent metal ion, M^{2+}, is extracted with an organic solvent containing oxine (HQ) can be formulated as:

$$2(HQ)_{org} + (M^{2+})_{aq} = (MQ_2)_{org} + (H^{+})_{aq}$$

The equilibrium is clearly pH dependent, and by controlling the pH one can selectively separate cations with different formation constants.

C. CHROMATOGRAPHY OF METAL IONS

Metal-chelate complexes have been separated by various chromatographic methods, eg. thin layer chromatography (TLC), gas chromatography (GC), and high performance liquid chromatography (HPLC). An extensive range of ligands have been used to achieve chelation; eg. DTCs, acetylacetonates, β-diketonates, and dithizone 8-Hydroxquinoline.

C(i). GAS CHROMATOGRAPHY

Drasch et al. (1) reported the separation and determination of seven metallic species in biological material by GC. Following a liquid-liquid extraction, the metal ions were chelated with sodium diethyldithiocarbamate (NaDEDTC) and separation was achieved on an OV-101 column.

C(ii). HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Much of the early research on the use of DTC's as chelating agents for transition metal ions prior to reverse phase (RP) HPLC was conducted by Schwedt. Schwedt (2) separated Cu(II), Ni(II), and Pb(II) as their DEDTC complexes by RP-HPLC on a Nucleosil 10 C_{18} column with ultra-violet (UV) detection at 254 nm. Schwedt (3) subsequently separated Se(IV), Cr(III), Ni(II), Co(III), Pb(II), Cu(II) and Hg(III) by HPLC again as their DEDTC complexes. With the exception of Pb(II) and Cu(II), all metal complexes were separated on a LiChrosorb RP-8 column with a mobile phase of methanol: water (70:30, v/v). The Pb(II) and Cu(II) complexes co-eluted under these conditions.

Schwedt (4) then investigated the use of an alternative DTC ligand, i.e. sodium tetramethylenedithiocarbamate (NaTMDTC). Using this ligand, Schwedt (4) separated Cd(II), Pb(II), Ni(II), Co(III), Zn(II), Cu(II) and Hg(II) on a LiChrosorb RP-8 (10 μm) with a mobile phase consisting of methanol:water (70:30, v/v) and UV detection at 254 nm.

Another DTC complex was then used by Schwedt (5) for the determination of Cr(III) and Cr(VI) in water. This ligand was ammonium

pyrrolidinedithiocarbamate (APDTC); separation was possible using methanol: water (70:30, v/v) as mobile phase on a LiChrosorb RP-8 (5 μm) anayltical column.

It is also possible to separate metal-DTC complexes by normal phase HPLC (NP-HPLC) as was demonstrated by Uden et al.(6). Separation of Cu(II), Ni(II), and Co(III) as their DEDTC complexes was possible by NP-HPLC with acetonitrile: diethylether: petroleum hydrocarbon (5:15:80. v/v) as the mobile phase. The column was a Sperisorb Silica (8 μm) with a dual detection system consisting of (i) UV; and (ii) DC argon plasma emission spectroscopic detection. To stop on-column degradation, the column was pre-treated with pyridine. To verify that the peaks observed corresponded to the specific metal-DTC complex and not to degradation products, the DC argon plasma detector acted as a metal specific detector at the respective wavelength of each metal.

In a number of publications by Liska et al. (7-10), Zn(II), Cu(II), Mn(II), Ni(II), Pb(II), Cr(III), Co(III), Cd(II), and Fe(III) were separated by NP-HPLC as their DEDTC complexes on a LiChrosorb Si-60 column. The mobile phase was chloroform: cyclohexane (10:90, v/v) with UV detection at 254 nm. Liska et al. (7-10) studied the use of a range of other DTC complexes for transition metal ion separation which inclued bisdipropyl-DTC and bisdibutyl-DTC, and the use of mixed ligand complexes such as dipropyl-DTC/dibutyl-DTC, diethyl-DTC/dibutyl-DTC, and diethyl-DTC/dipropyl-DTC.

Heizmann and Ballschmiter (11) used NP-HPLC to separate Pb(II), Zn(II), Cd(II), Hg(II), Cu(II), Ni(II), and Co(II) as their DTC complexes at the nanogram level. Heizmann and Ballschmiter also investigated the use of three DTC complexes, i.e., (i) NaDEDTC, (ii)

benzylmethyldithiocarbamate (BMDTC), and (iii) diethoxyethy dithiocarbamate (DEOEDTC). Separation of Cu(II), Hg(II), Ni(II), and Co(III) as their DEDTC complexes was achieved at a low flow rate with a mobile phase consisting of benzene on a glass LiChrosorb Si-60 column. Using BMDTC as the chelating agent, it was possible to separate Co(III), Cd(II), and Ni(II) with benzene: cyclohexane (75:25, v/v) as the mobile phase. Using the DEOEDTC chelating agent and a mobile phase of carbon tetrachloride: acetonitrile (96:4), Heizmann and Ballschmiter separated Cu(II), Ni(II), Co(III), and As(III). The metal-DTC complexes were detected by UV detection at 254 nm.

Moriyasu and Hashimoto (12) also used a NP-HPLC technique to separate Hg(II), Cd(II), Pb(II), CR(III), Bi(III), and Cu(II) and their DEDTC complexes in the microgram to nanogram range. Separation was possible using a deactivated silica gel column, LiChrosorb Si-100, with a mobile phase of hexane (H_2O saturated):ethylacetate (98:2, v/v). Moriyasu et al. (12) investigated the possibility of selective wavelength detection for the various metal-DTC complexes.

In an article published by O'Laughlin and O'Brien (13), the separation of Ni(II), Co(III), Cu(II), Zn(II), Cd(II), Hg(II) and Pb(II) was reported on two different silica columns i.e., (i) Corasil; and (ii) μPorasil with toluene (100%) as mobile phase and UV detection at 254 nm. Better resolution of the peaks resulted from the use of the μPorasil column.

The use of DTCs in speciation studies has been investigated. Tande et al. (14) separated Cr(III) and Cr(VI) as their DEDTC complexes by RP-HPLC with UV detection at 254 nm. The analytical column was a LiChrosorb RP-8 (10 μm) with methanol: water (65:35, v/v)

as the mobile phase. The pH optimum for the complexation of the majority of metal ions and NaDEDTC is in the range pH 4.0-4.5 but in this range Cr(III) does not complex. For complexation of Cr(III) to occur, the pH needs to raised to pH 5.8.

Smith et al. (15-19) were the first to develop a direct injection technique, with on-column derivatisation of the metal ions to form DTC complexes 'in-situ'. The mobile phase was a ternary mixture of methanol: water: chloroform (70:30:10, v/v) which was 0.05% (w/v) in NaDEDTC. It was possible to separate Hg(II), Hg(I), Pb(II), and Cd(II) on an ODS-Hypersil column with UV detection at 350 nm. Poor reproductibility was exhibited with respect to peak shape and height for same of the metal-DTC complexes. Smith et al. (15-19) found that the addition of a small percentage of a chlorinated solvent to the mobile phase tended to stop peak tailing. Smith et al. (15-19) also separated Cd(II), Pb(II), Co(III), and Cu(II) as their DTC complexes under the conditions outlined above. Smith et al. also used the converse of this procedure by employing transition metal ions as a means of determining the amount of DTC present in solution. In effect they had the transition metals in this manner, as 'ion-pair' reagents. By adopting this approach, it was possible to detect methyldithiocarbamate (MDTC), ethyldithiocarbamate (EDTC), and dimethyldithiocarbamate (DMDTC). The DTC complexes of Fe(III), Mn(II), Ag(I), Cr(III), and U(IV), under the chromatographic conditions outlined, were found either to decompose or to give several peaks.

Bond and Wallace (20) reported a RP-HPLC method for the determination of Cu(II) with electrochemical detection. The Cu-DTC complex can be formed 'in-situ' by including the NaDEDTC in the mobile

phase or it can be formed externally. The mobile phase was acetonitrile: sodium acetate buffer (0.02 M)(70:30, v/v) with 0.2 M $NaNO_3$ as supporting electrolyte. The DC current was measured amperometrically at a potential of -0.6V versus Ag/AgCl.

In another publication by Bond and Wallace (21) the simultaneous determination of Cu(II), Ni(II), Co(III), Cr(III), and Cr(V) by RP-HPLC with electrochemical detection was demonstrated. To achieve separation of all six metal ions, the metal-DTC complexes were formed externally since Cr(VI), and Cr(III) are slow to chelate with NaDEDTC, and formation can take up to three hours. Formation of the Co(III)-DTC complex 'in-situ' was also unsatisfactory. Bond and Wallace (21) investigated the use of two DTC ligands, i.e. (i) NaDEDTC and (ii) APDTC. Using a mobile phase of acetonitrile: acetate buffer (0.02 M) (70:30, v/v), the retention times of the metal-APDTC complexes were shorter than those of the metal-DEDTC complexes. The metal-APDTC complexes were found to be more stable and more polar than the corresponding metal-DEDTC complexes. Bond and Wallace (22) demonstrated that the DTC complexes of both Ni(II) and Cu(II) could be formed 'in-situ', and hence proceeded to automate the procedure. The chromatographic eluent was acetonitrile: acetate buffer (0.02 M, pH 6.0), which was 0.005-0.01 M in $NaNO_3$ as supporting electrolye and contained between 10^{-3}M and 10^{-4}M NaDEDTC. Separation was achieved on a μBondapak C_{18} with both electrochemical and UV detection at 254 nm. It was found that resolution, with respect to Ni(II) and Cu(II), could be improved by replacing acetonitrile with methanol. However, the overall efficiency of the system was decreased by switching to methanol.

Bond and Wallace (23) then went on to investigate the preparation of the metal-DTC complexes. The methods available for metal-DTC formation were investigated for both APDTC and NaDEDTC. The three methods of metal-DTC complex formation available are:

(i) direct formation in a solvent suitable for subsequent chromatographic separation;

(ii) liquid-liquid extraction and removal of co-extracted ligand, to minimize possible interferences observed with some detection methods; and

(iii) pre-column formation; in which the metal-DTC complex is deposited onto a Sep-Pak (C_{18}) cartridge and then eluted in a solvent suitable for chromatography.

Although direct formation in the chromatographic solvent was the simplest, it is not suitable for multi-element determination. Liquid-liquid extraction is recommended for the determination of range of metal ions and has the advantage of simplifying sample clean-up and/or preconcetration. Pre-column formation offers similar advantages to liquid-liquid extraction, but has the added ability to concentrate samples even further.

Bond and Wallace, (24) then applied their findings to the determination of Cd(II), Co(II), Pb(II), Hg(II), and Ni(II) in zinc sulphate plant electrolyte, based on separation as their APDTC complexes by RP-HPLC. The metal ions were extracted using a 1 M sodium acetate buffer, pH 4.0-4.2 which was 1% (w/v) in APDTC. At acidic pH values, APDTC complexes were more stable than their NaDEDTC

complexes. Dual detection was used with UV at 254 nm and DC amperometry at an applied potential of +1.20 volts versus Ag/AgCl at glassy carbon.

The use of solvent extraction followed by NP-HPLC of metal-DTC complexes was investigated by Inatimi (25). The mobile phase was benzene with UV detection at 280 nm. Samples of environmental importance, namely trade effluent and fish samples, were assayed for the following metal ions Cu(II), Ni(II), Hg(II), Pb(IV), Co(III), Mn(II), and Bi(III), as their DTC complexes. The use of HPLC in this study compared favourably to other analystical methods, such as activation analysis, atomic absorption spectrometry, volumetric analysis and polarographic analysis.

Hutchins et al. (26) investigated the use of a C_{18} "radial pak" compression module column for the RP-HPLC separation of Cu(II), Hg(II), Ni(II), Pb(II), Se(IV), and Te(IV) as their DEDTC complexes. A mobile phase consisting of methanol:acetonitrile: water (40:35:25, v/v) was used. It was found that the "radial pak" C_{18} column required conditioning prior to use. This was achieved either through an injection of a concentrated solution of metal-DTC onto the column, or by flushing the column with 0.005 M EDTA followed by addition of 1.25 x 10^{-5}M EDTA to the mobile phase. The DTC complexes of Pb(II), Cd(II), and Fe(III) gave rise to poor peak shape, and Hutchins et al. postulated this was due to substitution reactions with nickel present in the stainless steel; hence addition of EDTA should neutralize this reaction.

The micro RP-HPLC method was developed by Wenclawiak et al. (27) for the determination of Cu(II), Ni(II), and Zn(II) as their DEDTC

complexes in drinking water. Separation was achieved using a micro-analysis Polygosil column with a ternary mobile phase of methanol: water: chloroform (70:20:10, v/v) with a flow rate in the range 3-7 µl/min. The absorbance maximum was monitored for each metal-DTC complex with limits of detection of 40-500pg on-column.

The use of DTCs in conjunction with RP-HPLC has been used as a technique to selectively detect both organic and inorganic mercury compounds, as was demonstrated by Lajunen et al. (28). A mobile phase composition of methanol: water (85:15, v/v) was used with a 5 m particle size RP-18 Hypersil column. The limits of detection were 30 and 80 pciogram for Hg(I) and $CH_3Hg(I)$ respectively. This shows the selectivity of using DTCs in speciation studies of inorganic and organic mercury compounds.

Separation of eight metal-DTC complexes was reported by Ichinoki et al. (29) using hexamethyleneammonium hexamethylene-dithiocarbamate (HMA-HMDTC). With a mobile phase of methanol: water: chloroform: 0.01M HMA-HMDTC (76:16.5:6:1.5), it was possible to separate Cd(II), Ni(II), Pb(II), Zn(II), Cu(II), Hg(II), Co(III) and Bi(III). It was found that the Mn(II) and Fe(II) complexes dissociated on-column.

Another DTC chelating agent was used by Shih et al. (30) to separate Fe(III), Ni(II), Cu(II), Hg(II), and Co(III) as their (bis)N-butyl-2-naphthylemthyldithiocarbamate (BNMDTC) complexes. The metal-BNMDTC complexes were found to be thermodynamically stable and kinetically inert at the 1×10^{-8}M level. The column used was a µ-Bondapak C_{18} column with methanol: water (80:20, v/v) as the mobile phase and UV detection at 221 nm.

Jinno et al. (31) interfaced an inductively coupled plasma atomic

emission spectrometer (ICP-AES) to an HPLC system as a metal selective detector. By using the ICP-AES as an alternative to the more conventional UV detector, it was possible to selectively detect Cu(II), Zn(II), Fe(III), and Co(III) as their DEDTC complexes.

A micellar mobile phase was used by Kirkbright et al. (32) to separate metal-DEDTC complexes on a μBondapak CN column. The surfactant used was cetyltrimethylammonium bromide (CTAB), above its critical micelle concentration.

Other metal ions which have been separated as their DTC complexes include Pt(II), Pd(II), and Rh(II). Mueller et al. (33) separated Pt(II), Pd(II), and Rh(II) as DEDTC complexes on a C_{18} Econosphere column with acetonitrile: acetate buffer (0.1 M) pH 6.0 as eluent. It was demonstrated that none of the following metal-DTC complexes interferred with Pd(II) or Pt(II), ie., Cr(III), Co(III), Fe(III), Mn(II), Hg(II), Pb(II), Cu(II) and Cd(II).

Lohmuller et al. (34) have demonstrated that diphenyl thiocarbazone (dithizone) can be used as a chelating agent for the detection of a number of metal ions. By applying a NP-HPLC method, it was possible to detect Co(III), Hg(II), Ni(II), Zn(III), Cu(II), and Pb(II) as their dithizone complexes with UV detection at 525 nm, using a LiChrosorb Si-60 column and benzene as the mobile phase. A number of the metal-dithizone complexes did show excessive tailing.

A selection of publications exist on the use of dithizone as a chelating agent in both RP-HPLC and NP-HPLC (35-37).

The use of 8-hydroxyquinoline (oxine) as a chelating agent in metal ion analysis by HPLC was first investigated by Berthod et al. (38). The separation of four metal-oxine complexes was achieved on a

LiChrosorb RP-8 column with a methanol: water (55:45, v/v) mobile phase at pH 8.5. The mobile phase contained 5 x 10^{-3}M oxine with UV detection at 385 nm. The addition of oxine to the mobile phase facilitates 'in-situ' formation of the complexes.

A NP-HPLC method, utilising oxine, was developed by Hambali et al. (39). The initial chromatographic data was extrapolated from thin layer chromatography of the metal-oxinate complexes. The mobile phase was chloroform: methanol (99:1, v/v) using a silica column. Using these conditions it was possible to separate Al(III) from Fe(III) as oxinate complexes.

The range of metal ions one could determine as oxinate complexes was extended to five by Wenclawiak (40). They again used a NP-HPLC method to separate V(IV), Mo(VI), W(VI), Co(III), and Cr(III) on a Si-60 silica column with tetrahydrofuran: chloroform (60:40, v/v) as mobile phase and UV detection at 254 nm. Elution of all five complexes was achieved in less than eight minutes.

Hoffman et al. (41) made a direct comparison between pre-column and on-column formation of Mn(II)-oxinate and Mn(III)-oxinate. For pre-column formation, a NP-HPLC method was used with tetrahydrofuran: chloroform (60:40, v/v) as the mobile phase using a Si-60 column. A RP-HPLC approach was used for on-column formation of the metal-oxinate complexes. It was possible to separate Cr(VI), Co(II), Mn(II), Zn(II), Cu(II), Al(III), and Mn(III) on a RP-8 column with methanol: water: borate buffer (pH 9.0) (60:20:20, v/v). It was not possible to separate Co(II), Mo(VI), Mn(II), and Mn(III) by pre-column formation due to lack of selectivity.

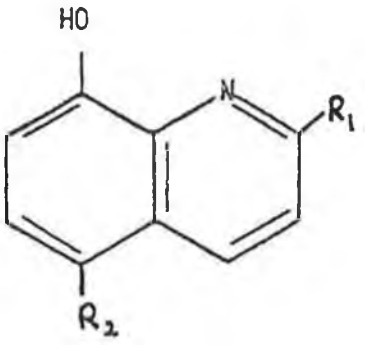
Jones et al. (42) used a derivative of oxine to separate Al(III),

Ga(II), In(IV), Mg(II), Cd(II), and Sn(II) as their 8-hydroxyquinoline -5-sulphonate complexes. Detection was achieved using fluorescence spectrometry, since a 10-fold increase in sensitivity over absorption systems can be achieved. The limit of detection for Ga-oxinate-5-sulphate was 1 µl/l for a 100 µl for a 100 µl injection. The structure of some commonly used hydroxyquinoline ligands (X) are listed in Table 5.

Bond and Nagaosa (43) separated Fe(III), Cu(II), Al(III), and Mn(II) as oxinate complexes by RP-HPLC. An 'in-situ' formation method was developed for the determination of Fe(III), Cu(II), and Al(III) as oxinate complexes. Separation was achieved on a C_{18} column with acetonitrile:0.02 M acetate buffer (pH 6.0) (50:50, v/v) which was 5 x 10^{-3} oxine and 0.04 M in KNO_3 as eluent with electrochemical and UV detection at 400 nm. The presence of KNO_3 is essential for fast and quantitative formation of the Fe-oxine complex. Detection of Mn(III)-oxinate was not feasible by 'in-situ' formation of the complex, so external formation was necessary. It was possible to detect Fe(III) in drinking water by performing a pre-concentration step using silica Sep-Pak cartridges. By using the Sep-Pak cartridge, it was possible to concentrate the Fe(III)-oxinate complex fifty-fold.

Huber et al. (44) are generally credited as the first investigators to report on the separation of metal chelates by HPLC. Included in the study were the acetylacetonates and trifluoroacetyl-acetonates of Be(II), Al(III), Cr(III), Fe(III), Co(III), Cu(II), Ni(II), and Zn(II). The mobile phase was a ternary liquid-liquid system composed of water, 2,2,4 trimethylpentane and ethanol. The chelating agents used by Huber et al. (44) were β-diketones, of which

GENERAL STRUCTURE



8-Hydroxyquinoline

(X)

TABLE 5 HYDROXYQUINOLINE LIGANDS

NAME	ABBREVIATION	STRUCTURE
8-Hydroxyquinoline	OX	$R_1 = R_2 = H$
2-Methyl-8-Hydroxyquinoline	MOX	$R_1 = CH_3$
8-Hydroxyquinoline-5-Sulphonate	OXS	$R_1 = H$ $R_2 = SO_3H$

same ten to twelve have been used as chelating agents for the separation of metal ions (25,26,45,46,47,48,49).

Another important class of chelating agents used in the HPLC of metal ions is the β-ketoamines. There are some eight chelating agents in this class which have been used (45,47,48,52).

Yet another group of chelating agents used for the separation of metal ions by HPLC are (i) crown ether complexes; (ii) macrocyclic amines; (iii) porphyrins; and (iv) organophosphorous compounds (57-66). Other chelating agents of importance are (i) bipyridine and (ii) 1,10-phenanthroline (50, 53-56).

D. OTHER ANALYTICAL METHODS FOR DETERMINATION OF METAL IONS

D(i). Electroanalytical Techniques

There has been widespread use of electroanalytical techniques for the determination of metal ions and metal complexes. These techniques include (i) polarography; (ii) potentiometric titration; (iii) ion selective electrodes; (iv) coulometry; (v) chronopotentiometry; (vi) amperometric titration; (vii) anodic stripping voltammetry; (viii) cathodic stripping voltammetry; and (ix) potentiometric stripping analysis.

The polarographic behaviour of Cu(II), Pb(II), Zn(II), Cd(II), Ni(II) and Bi(III) as their DEDTC complexes in benzene: methanol (50:50, v/v) with $LiClO_4$ or LiCl as supporting electrolyte was examined by Cordova et al. (67). A selective extraction system based

on benzene allows the polarographic determination of the above mentioned metals without mutual interference.

Gevorgyan et al. (68) titrated biamperometrically Hg(II) and Bi(III) with 0.003 M Pb(II)-DEDTC in benzene using two rotating platinum electrodes.

D(ii). Radiochemical Techniques

The use of dithiocarbamates continues to grow in what might be termed radiochemical applications. An interesting application is the use of the chelating agent to pre-concentrate trace amounts of metals prior to their determination by activation analysis.

Lo et al. (69) used Pb-DEDTC as a reagent for enriching Hg(II) and Cu(II) in seawater prior to irradiation in neutron activation analysis. The metals were selectively concentrated from a large volume of water to a small volume of chloroform solution. The same authors (70) used Pb-DEDTC as a pre-concentration agent for Hg(II) in urine prior to its determination by neutron activation analysis.

D(iii). Thermoanalytical Techniques

For convenience and simplicity the thermochemistry of metal dithiocarbamates can be divided into two sub-sections concerned with (a) volatility, and (b) thermal decomposition.

D(iii). (a) Volatility

Hanso et al. (72) have reported on the sublimation behaviour of a wide variety of metal chelates, including metal-DTC complexes. It was concluded that a variety of metals may be separated and purified via DTC complexation.

Lorionou et al. (73) have studied the thermolysis of Ni(II), Zn(II), Cd(II), In(IV), Tl(I), Cu(II), Sb(IV), As(III), and Bi(III) as their DTC complexes by termogravimetry (TG), differential thermal analysis (DTA), and differential scanning calorimetry (DSC) in air and in an inert atmosphere. The DSC data was used to derive the melting point and the heat of fusion of these complexes.

D(iii). (b) Thermal Decomposition

Afanasova et al. (74) have studied the thermal stability of a wide variety of metal-DTC complexes and reported the following order of stability:

Ni(II) > Co(III) > Zn(II) > Cd(II) > Pb(II) > Sb(IV) > Bi(III).

The initial thermal decomposition temperatures of the metal-DTC complexes were within the range 150-255°C. Thermal analysis does not, in most cases, give information as to the metal bound to the DTC, but in some situations it is possible to speculate.

D(iv). Spectroscopic Techniques

The use of the following spectroscopic techniques will be discussed in the next sections:

(a) Ultra Violet-Visible Spectrometry (UV-Vis)

(b) Atomic Absorption Spectrometry (AAS)

(c) X-Ray Fluorescence Spectrometry (XRF)

D(iv). (a) Ultra Violet-Visible Spectrometry

Kleweska et al. (75) described an analytical procedure for the determination of trace Cu(II) in biological material. Autopsy specimens were neutralized with concentrated H_2SO_4:HNO_3 and extracted with a carbon tetrachloride solution of zinc dibenzyl-DTC. The absorbance of the organic phase was determined at 440 nm.

One major problem with the determination of metal-DTC complexes by UV-Vis spectrometry is due to the similarity in spectra of all metal-DTC complexes. The use of UV-Vis spectrometry does not allow selective determination of the metal ion bound to the chelating agent.

D(iv). (b) Atomic Absorption Spectrometry

Yanagisana et al. (76) reported an AAS method for the determination of both Cr(III) and Cr(VI). Both are extracted into isobutylmethylketone followed by complexation with NaDEDTC for Cr(VI)

and oxine for Cr(III). Both Cr(VI) and Cr(III) can be determined by AAS at 357.9 nm.

Lo et al. (77) used a solvent extraction method to extract trace metal from seawater using NaDEDTC followed by a back-extraction. The metal-DTC complexes were determined by AAS.

D(iv). (c) X-Ray Fluorescence Spectrometry

Using X-Ray fluorescence spectrometry (XRF), a multi-element approach was developed by Linder et al. (78). Trace metal ions present in pharmaceutical products were treated with H_2SO_4/H_2O_2. The metal ions were co-precipitated from a buffer solution with the dibenzylamine salt of dibenzylDTC. This method allows for the simultaneous determination of up to thirteen metal-DTC complexes.

THEORY

A. COMPLEXATION REACTIONS IN ANALYTICAL CHEMISTRY

The formation of metal complexes can serve as the basis of a number of accurate and convenient methods to determine a metal ion. Many cations will form complexes in solution with a variety of substances that have a pair of free electrons (e.g. on N, O, S atoms in a molecule), as is the situation with the DTC ligands and oxine which have donor S, O, and N atoms present in their molecular structure. The number of molecules of the ligand which are involved in the complexation will depend on the co-ordination number of the metal and on the number of complexing group on the ligand molecule.

Solvent extraction has one of its most important applications in the separation of metal cations. This separation can be accomplished in several ways. The uncharged inorganic ligands tend to dissolve in the organic layer, while the metal ion remains in the polar aqueous layer. Metal ions do not tend to dissolve appreciably in the organic layer. For these ions to become "soluble" in the organic phase, the charge must be neutralised. There are two principal ways to do this.(79)

A(i). Ion-Association Complexes

In one approach, the metal ion associates with an organic ion of large molecular weight, typically the solvent. This organic ion displaces the co-ordinated water which is associated with the metal ion. The metal ions become solvated by the solvent to make it "solventlike" and is then extracted into this organic phase. For

example, it is well known that Fe(III) can be quantitatively extracted from hydrochloric acid into diethyl ether. The mechanism is not completely understood, but evidence exists that the chloro complex of Fe(III) is co-ordinated with the oxygen atom of the solvent (the solvent displaces the co-ordinated water), and this ion associates with a solvent molecule that is co-ordinated with a proton:

$$\{ (C_2H_5)_2O \ : \ H^+, \ FeCl_4[(C_2H_5)_2O]_2^- \ \}$$

The most widely used method of extracting metal ions is formation of a chelate molecule with an organic chelating agent.

A(ii). Metal Chelates

In order to achieve a distribution of a metal between an aqueous and an organic phase, the metal ion must first be converted to a neutral species. Such a neutral molecule can be obtained by the formation of a chelate in which the ligands neutralise the charge on the metal ion and displace the water of hydration. Most chelating agents are weak acids that ionise in water; the ionisable proton is displaced by the metal ion when the chelate is formed and the charge of the organic compound neutralises the charge of the metal ion.

The usual practice is to add the chelating agent to the organic phase. The extraction process can be thought to consist of four equilibrium steps, each with an equilibrium constant. The equilibrium distribution of the neutral metal chelate between the aqueous and the organic phase is described by the partition coefficient (K). In

addition to the distribution equilibrium of the neutral chelate, a number of other competing equilibria are involved. For this reason, all complexes which are formed in aqueous solution have to be considered, if an overall distribution coefficient (K_m) of the metal is to obtained. K_m is derived from a number of equations which describe the single equilibria involved. The distribution equilibria are characterised by their respective partition coefficients; the dissociation equilibria by their formation constants. Figure 1 represents a schematic situation for a simple case of an equilibrium in which it is assumed that no chemical reactions occur in the non-polar (organic) phase, and intermediate and anionic complexes are not formed in the polar aqueous phase.

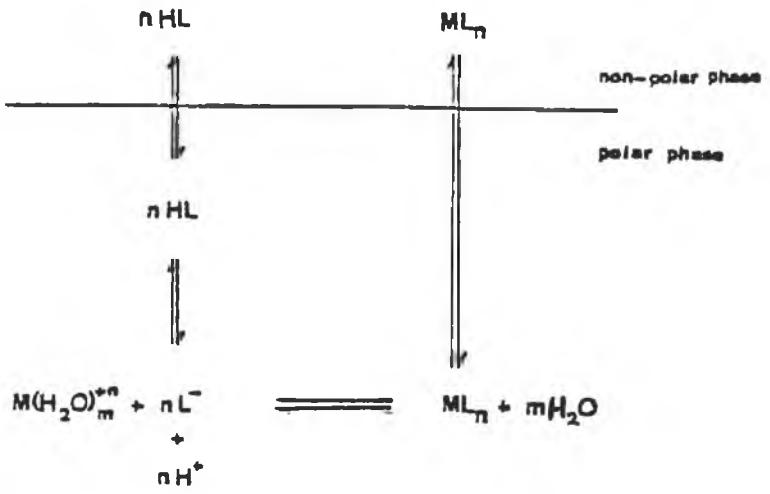


FIGURE 1. SCHEMATIC REPRESENTATION OF EQUILIBRIA INVOLVED IN EXTRACTION OF METAL CHELATES

The metal is present as the hydrated ion and in the neutral chelate in the polar phase, and in the neutral chelate in the non-polar phase (79). Therefore, the total distribution coefficient of the metal is

given by:

$$K_m = \frac{[M(H_2O)_m^{+n}]_p + [ML_n]_p}{[ML_n]_{np}} \quad \ldots\ldots 1$$

where $[M(H_2O)_m^{+n}]$ = the conc. of the species $[M(H_2O)_m]$ in the polar phase.

$[ML_n]_{np}$ = the concentration of the species $[ML_n]_{np}$ in the polar phase.

M = metal
L = ligand
n = ionic charge
m = the coordination number of the metal

The protonated ligand and the metal chelate will undergo distribution between the two phases. The partition coefficients for each of these equilibria are:

$$K_{HL} = \frac{[HL]_p}{[HL]_{np}} \text{ and } K_{ML_n} = \frac{[ML_n]_p}{[ML_n]_{np}} \quad \ldots\ldots 2$$

In addition, the protonated form of the ligand and the metal chelate also undergo dissociation in the polar phase. The formation constants for these equilibria are defined as follows:

$$K_{HL}^{*} = \frac{[HL]_p}{[H^+]_p[L^-]_p} \text{ and } K_{ML_n}^{*} = \frac{[ML_n]_p}{[M(H_2O)_m^{-n}]_p[L^-]_p^n} \quad \ldots\ldots 3$$

Equations 1, 2, and 3 can be combined to yield an expression which describes the dependence of the total distribution coefficient (K_M) on the hydrogen ion concentration in the polar phase and the ligand concentration in the non-polar phase.

$$K_M = K_{ML_n}\left\{1 + \frac{1}{K_{ML_n}^*}\left(\frac{K_{HL}^*}{K_{HL}}\right)^n\left(\frac{[H^+]_p}{[HL]_{ap}}\right)^n\right\} \qquad \ldots\ldots 4$$

For a more general case, the stepwise formation of the metal chelate complex and the participation of hydroxyl and other anions have to be considered as well. The following general equation can be written for the total distribution coefficient of the metal between the two phases;

$$K_M = \frac{\Sigma[\{M(H_2O)_{m-2j-k-l}L_j(OH)_kA_l\}^{+n-j-k-l}]_p}{[ML_n]_{ap}} \qquad \ldots\ldots 5$$

where j = the number of chelating ligands

k = the number of hydroxyl anions

l = the number of anions of type A

From equation 5, it is evident that the total distribution coefficient of the metal ion is not constant. For the simple case, however, described by equation 4, K_M becomes almost constant if the term which contains the equilibrium constants and the concentrations of the hydrogen ion and the chelating agent becomes small compared to one.(80) The order of stability of complexes of a limited number of divalent metals has been shown, other things being equal, to be fairly independent of the nature of the chelating agents, with the following stability sequence:

Pd > Cu > Ni > Pb > Co > Zn > Cd > Fe > Mn > Mg

It is important that the pH of the solution be carefully controlled since chelate stability generally decreases as the reagent acidity increases. The effect of pH on extraction efficiency (%E) for a number of metal dithizone complexes is demonstrated in Figure 2. By increasing pH the extraction efficiency (%E) increases for the various metal dithizone complexes.

A more recent development in analytical separations has been the introduction of solid-phase extraction cartridge. The range of solid-phase extraction cartridges available is very extensive.

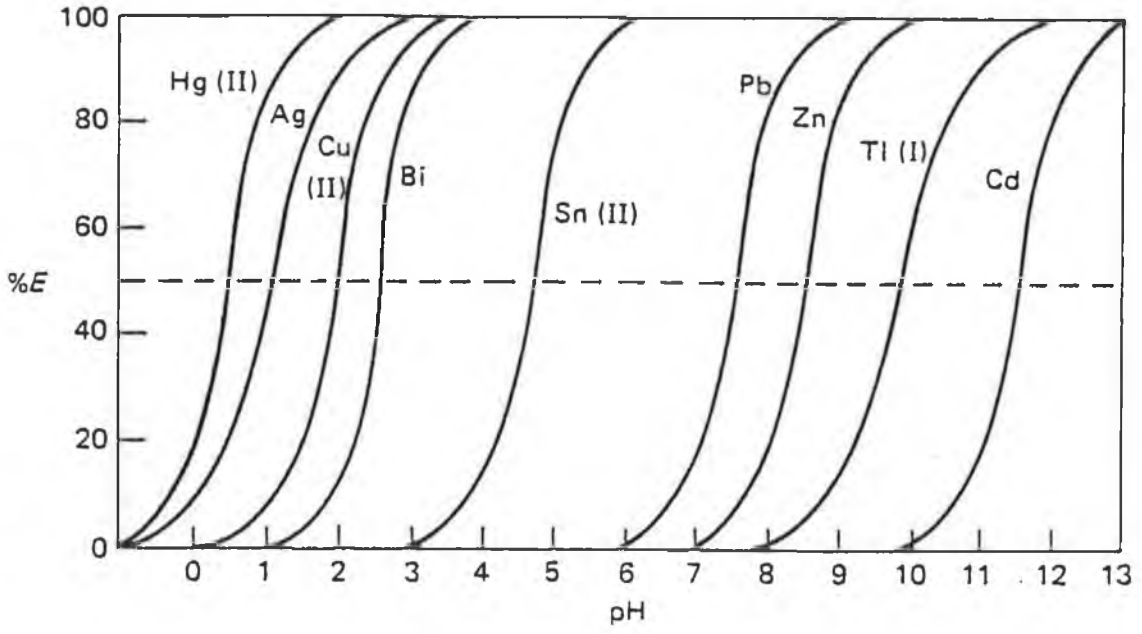


FIGURE 2 QUALITATIVE EXTRACTION CURVES FOR METAL DITHIZONATES IN CARBON TETRACHLORIDE

Depending on the cartridge used, be it a C_{18} or Si cartridge, they are used as a solid phase extraction 'support'. For example, by forming a metal-oxine complex in dichloromethane and then passing this

solution through a silica cartridge, the metal-oxine complexes are retained by the silica cartridge. The metal-oxine complex can be subsequently eluted with methanol. This shows how the polarity of a metal-oxine complex can be utilised to selectively extract it from various solutions. Solid-phase extraction cartridges can be compared to packing material used in HPLC columns. A list of solid phase supports is listed in Figure 3 both polar and non-polar solid phase support materials are available (81).

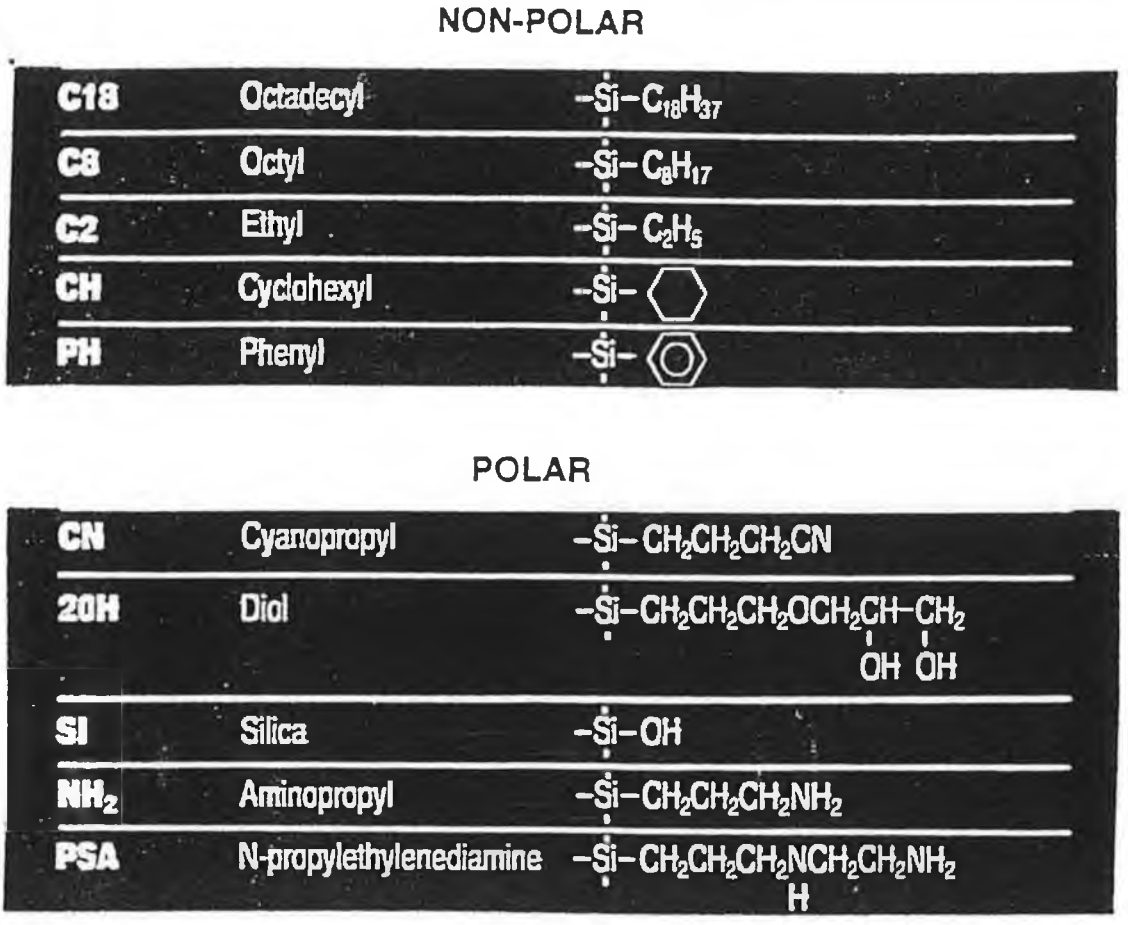


FIGURE 3 BONDED PHASE AVAILABLE FOR SOLID PHASE EXTRACTION CARTRIDGES

B. HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

B(i). Theory of Separation

In order to achieve separation in chromatography, one must first have retention (82). The fundamental relationship between the retention volume (V_r), the quantity of stationary phase (V_s) and the partition coefficient (K) is expressed in the following equation:

$$V_r = V_m + KV_s \qquad \ldots\ldots 6$$

where V_m is the volume of mobile phase within the column. The retention volume can be determined directly from the chromatogram as $V_R = Ft_r$, where F = the flow rate of solvent and t_r = the retention time of the component. A more practical expression of solute retention is given by k', the capacity factor:

$$k' = \frac{n_s}{n_m} \qquad \ldots\ldots 7$$

where n_s is the total number of moles of solute component in the stationary phase and n_m is the total number of moles of solute component in the mobile phase. It can subsequently be show that

$$V_r = V_m(1 + k') \qquad \ldots\ldots 8$$

and that:

$$k' = \frac{(t_r - t_m)}{t_m} \qquad \ldots\ldots 9$$

thus the capacity factor, k', is a measure of sample retention by the column and can be determined from the chromatogram directly.

A measure of the separation efficiency of a column is given by the number of theoretical plates (N) the column is equivalent to, which is defined in terms of the retention time (t_r) and the base width of the peak (t_w):

$$N = 16\left(\frac{t_r}{t_w}\right)^2 \qquad \ldots\ldots 10$$

The efficiency of any column is best measured by the height equivalent to a theoretical plate (H) which is expressed as:

$$H = L/N \qquad \ldots\ldots 11$$

where L is the length of the column.

The resolution R_s of two components is defined as being equal to the peak separation divided by the mean base width of the peaks:

$$R_s = \frac{2(tr_A - tr_B)}{(tw_B + tw_A)} \qquad \ldots\ldots 12$$

A more practical expression which uses the selectivity factor $\alpha = k_2'/k_1'$ is given by:

$$R_s = 1/4(N)^{\frac{1}{2}} \left(\frac{(\alpha - 1)}{\alpha}\right)\left[\frac{k'_2}{[k'_2 + 1]}\right] \qquad \ldots\ldots 13$$

The kinetic factors giving rise to band broadening in HPLC must also be considered, and are similar to those considered in other modes of chromatography, though their relative contributions may be different. Each of the rate factors contributing to the band broadening is considered, and when combined give a general expression for the overall plate height, commonly referred to as the Van Deemter equation.

Broadening of a chromatographic zone as it passes through the column can arise from the variable channels which the solute molecules may follow through the packing. This effect is known variously as the multi-path, the eddy-diffusion or non-uniform flow term (H_f). The contribution of this effect to the overall plate height (H) is proportional to the particle size of the packing (dp) and is expressed as:

$$H_f \approx (dp) \qquad \ldots\ldots 14$$

where λ is a measure of how uniformly the column is packed.

Whenever a concentration gradient exists in the column then spreading will occur due to random processes. This interdiffusion of the two molecular species, which occurs in all directions independent

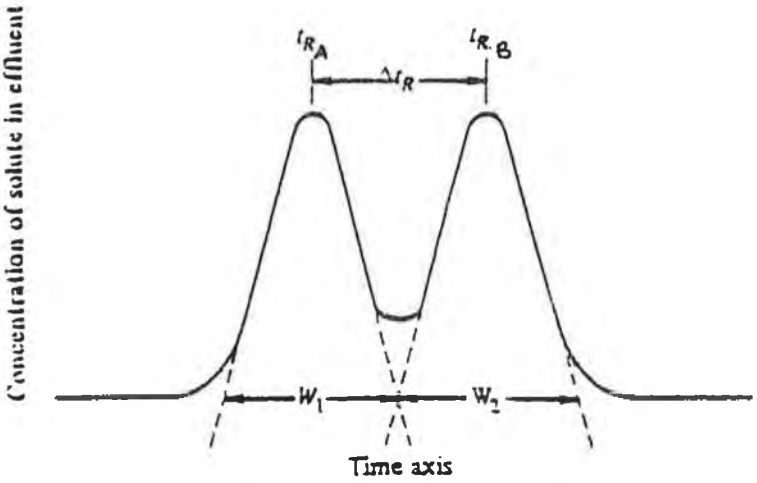


FIGURE 4 DEFINITION OF RESOLUTION

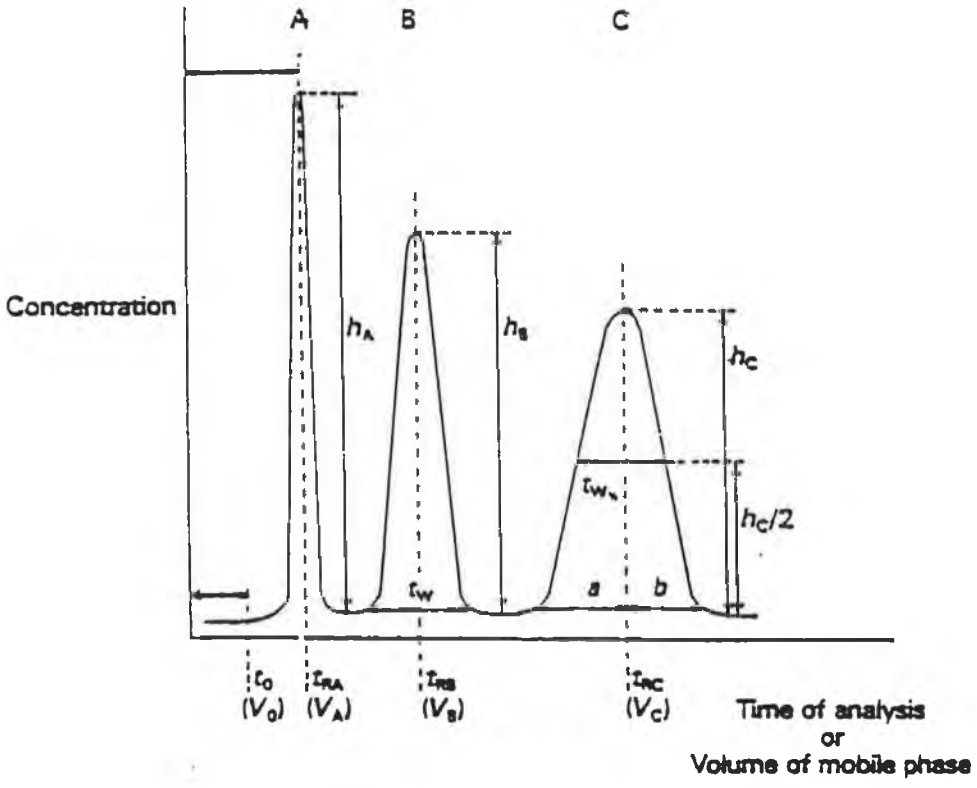


FIGURE 5 SAMPLE CHROMATOGRAM SHOWING CHROMATOGRAPHIC PARAMETERS OF ANALYSIS

of whether solvent is flowing or not, is dependent upon two factors: first, the diffusion coefficient (D_m) for interdiffusion of solute and solvent molecules, and second, the time over which this diffusion occured. The longitudinal diffusion contribution to the plate height of the column is expressed as:

$$H_{dm} \simeq \frac{D_m}{u} \qquad \ldots\ldots 15$$

Where u is the mobile phase flow rate. There is also an analagous contribution to the overall plate height due to diffusive spreading in the stationary phase. Using similar reasoning as above, this is expressed as:

$$H_{ds} \simeq \frac{D_s}{u} \qquad \ldots\ldots 16$$

where D_s is the interdiffusion coefficient.

The assumption in the plate theory that the transfer of solute molecules between the mobile phase and stationary phases is instantaneous is invalid due to the finite rates of mass transfer within the mobile and stationary phase. This contribution to the effective plate height may itself by regarded as arising from two separate effects. These two effects are collectively known as mobile-phase mass transfer contribution which is expressed as:

$$H_{em} \simeq \frac{(dp)^2 u}{D_m} \qquad \ldots\ldots 17$$

Analagous arguments can be developed to take account of stationary phase mass transfer effects which can similarly be expressed as:

$$H_{es} \simeq \frac{d^2u}{D_s} \qquad \ldots\ldots 18$$

Where d is the thickness of the conceptual stationary phase and D_s is the diffusion coefficient of solute molecules within it.

Finally, the presence of substantial amounts of immobile solvent, which is either trapped in the interstices between the packing material or in the deep pores within the particles, is a further cause of band broadening. This is termed stagnant mobile phase mass transfer, expressed as:

$$H_{em'} \simeq \frac{(dp)^2u}{D_m} \qquad \ldots\ldots 19$$

It has been shown that the overall plate height arising from these kinetic factors can be expressed mathematically as:

$$H = \frac{1}{(1/H_f) + (1/H_{em})} + (Hdm + Hds) + Hem' + Hes \qquad \ldots 20$$

where:

$$H_f = C_f dp$$

$$H_{em} = \frac{C_{em}(dp)^2 u}{D_m}$$

$$H_{dm} = \frac{C_{dm} D_m}{u}$$

$$H_{ds} = \frac{C_{ds} D_s}{u}$$

$$H_{em'} = \frac{C_{em'} (dp)^2 u}{D_m}$$

$$H_{es} = \frac{C_{es}(d)^2 u}{D_s}$$

and C_f, C_{em}, C_{dm}, C_{ds}, C_{es} and $C_{em'}$, are plate height coefficients (82). This equation is commonly presented in its simplified form:

$$H = Au_{0.33} + \frac{B}{u} + Cu + Du \qquad \text{..... 21}$$

Where A, B, C and D are constants which have been derived from consideration of the kinetics of eddy diffusion, longitudinal diffusion and mass transfer respectively.

This equation is called the Van Deemter equation (79). Other factors which can effect resolution and bank broadening are tubing

volume used in the instrumentation, column connections containing voids and the level of temperature control

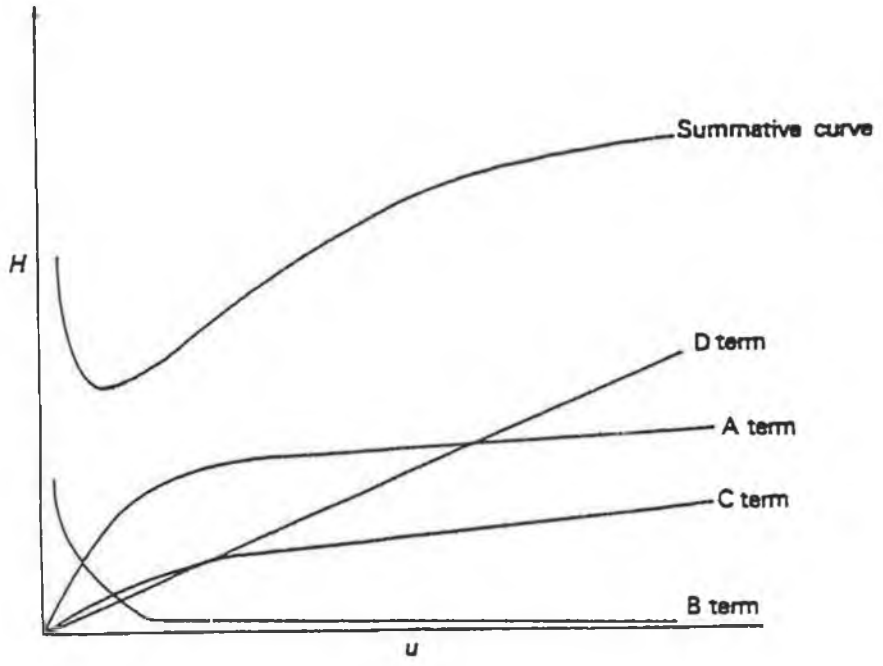


FIGURE 6 PLOT OF PLATE HEIGHT (H) VERSUS LINEAR VELOCITY (u) SHOWING CONTRIBUTIONS FROM THE FOUR TERMS IN EQUATION

B(ii). HPLC Instrumentation

In HPLC, filtered eluant is drawn from the solvent reservoirs, the eluant composition being determined by the proportion of each solvent delivered to the column via a high pressure pump and a solvent mixing system. The mixture is applied to the top of the column and the components of the mixture are then carried down through the column by the eluant at a rate which is inversely proportional to their attraction for the packing material. The passage of the solutes

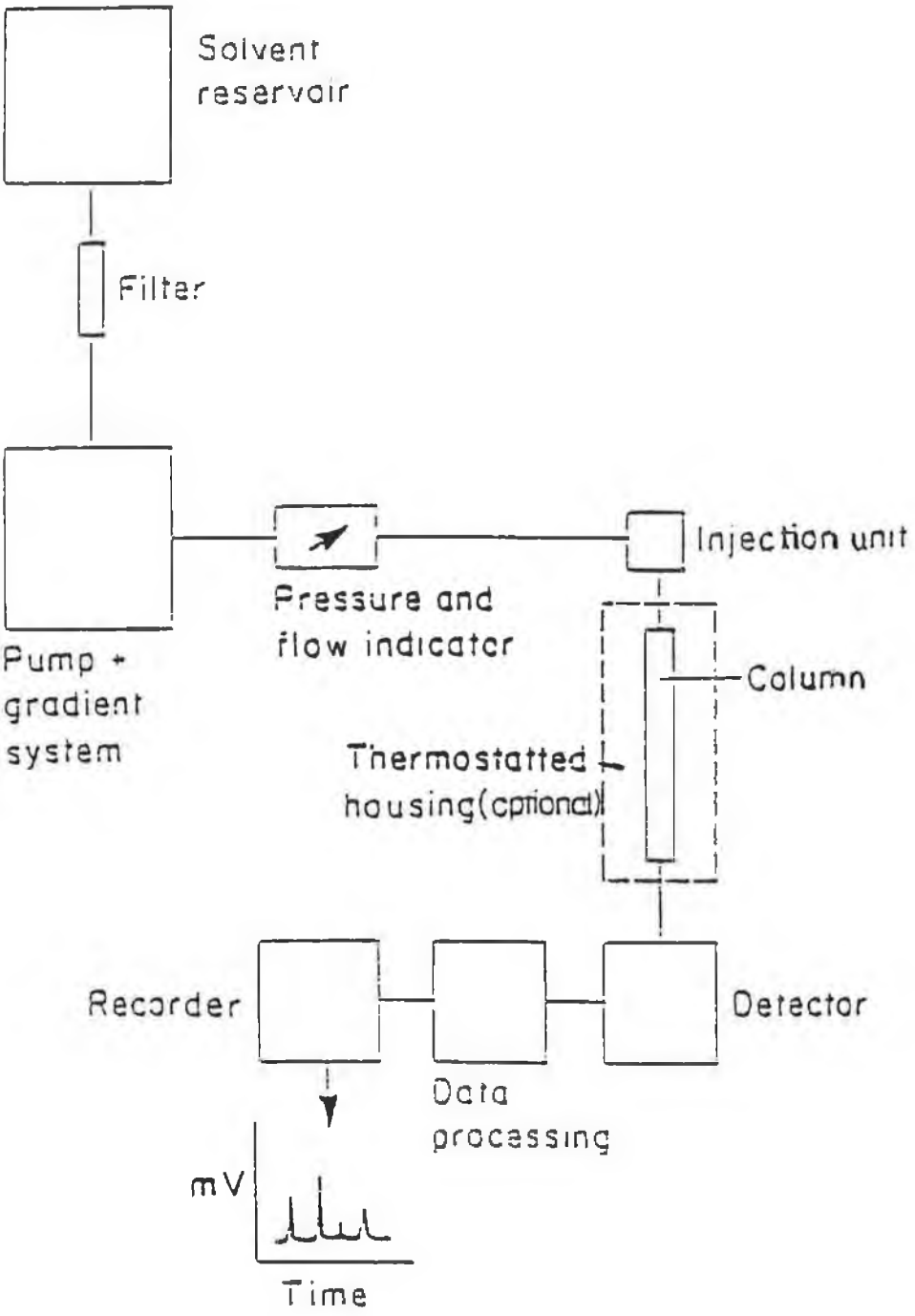


FIGURE 7 BLOCK DIAGRAM OF A HIGH PERFORMANCE LIQUID CHROMATOGRAPH

from the column is monitored by the detector and the response displayed on either a chart recorder or on an integrator. Separation of a multi-component mixture can be achieved by using an isocratic system or a gradient type system (83). A typical HPLC system is illustrated in Figure 7.

B(iii). Synthesis of Bonded Phase Materials

The majority of packings for modern HPLC are based on microporous particles of varying size, shape and porosity (82). The surface of these particles can be modified subsequently by either physical or chemical means to afford access to any of the classical modes of chromatography. The most common material used is silica, as it can withstand relatively high pressures and is available with large surface area (200 - 300 $m^2 g^{-1}$).

The severe restrictions on classical liquid-liquid partition chromatography with regard to solvent stripping of the stationary phase from the analytical column and the incompatability with gradient elution techniques, led to the development of a range of chemically-bonded stationary phases.

The principal limitation is in the range of pH of the eluent used, as cleavage or hydrolysis of the stationary phase can occur; similar restrictions apply to certain oxidizing solutions. Almost all commercially available bonded materials use silica gel as the support.

The most widely used bonded-phase materials are those derived from the reaction of the surface silanol groups with organochloro-

silanes, which leads to linkage of the stationary phase to the support via a siloxane (Si-O-Si) bond: for octadecylsilane (ODS, $C_{18}H_{37}Si$) - bonded phases, the reagent used is octadecyltrichlorosilane. A typically commerical material will have 50-70% of the available sites derivatised. The advantage of such supports is the stability of the siloxane linkages to column inlet pressures and hydrolysis, thus allowing use of solvent at 6000 psi in the pH range 2.0 - 8.5.

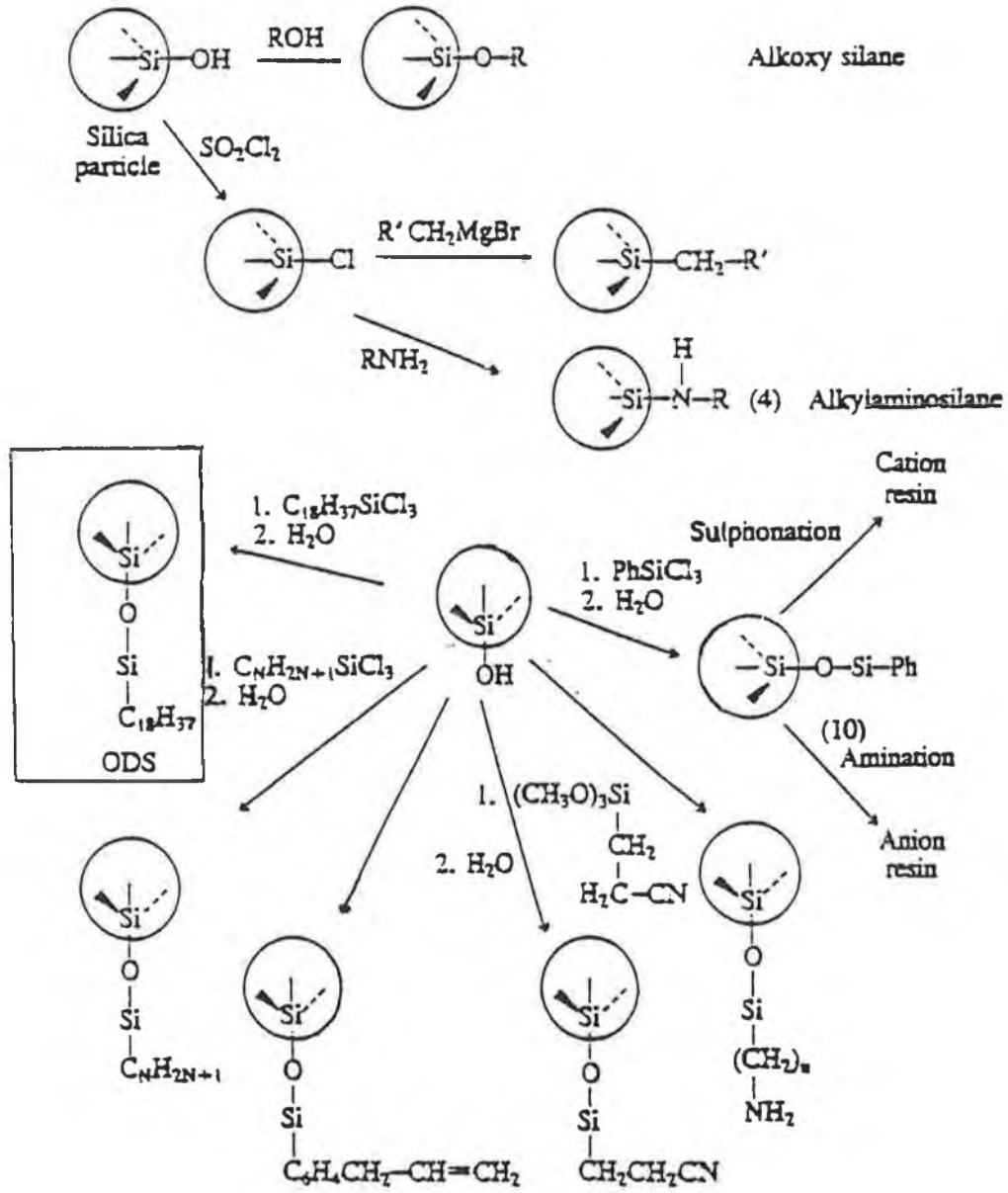


FIGURE 8 REACTION SCHEMES FOR PREPARATION OF CHEMICALLY-BONDED STATIONARY PHASES

Residual silanol present in the silica particle can cause tailing and these groups can be blocked or 'capped' using trimethylsilyl chloride. This makes the particles completely non-polar. But in some instances, the existence of these residual silanol groups is required and imparts a secondary affect to the separation of components in a mixture.

Using bonded phase chemistry it is possible to manufacture a range of packing materials with various functional groups and polarities (Figure 8).

EXPERIMENTAL

A. APPARATUS

Ultraviolet spectra were recorded using a Shimadzu Model UV-240 recording Spectrophotometer with matching 1 cm quartz cells. Readings of pH were made on a Philips Model PN9410 digital pH meter with an Orion Model 91-05 saturated calomel electrode (S.C.E.) containing a temperature compensating device. The pH meter was first calibrated with standard buffer solutions at pH 4.0, pH 7.0 and pH 10.0.

Gas chromatograms were obtained on a Hewlett-Packard Model 5890 gaschromatograph in conjunction with a flame ionisation detector. The area/height under the peaks was measured with a Shimadzu Model C-R3A integrator. The column used was a BP-1 column (0.53 m i.d., 25 metre), supplied by SGE (Australia).

The injection method was splitless injection with 'cold trapping' of the sample. The temperature program used was as follows:

Initial T	80°C for 1min
Rate	25 °C/min
Final T	280°C for 1min
Injector T	300°C
Detector T	300°C

The carrier gas was helium with a column head pressure of 15 psi. Liquid chromatograms were obtained with an Applied Chromatography Systems (ACS) Model 352 ternary gradient pump connected to a Rheodyne Model 7125 injection valve. The ACS Model 352 HPLC uses high pressure mixing and was programmed via an RS232 connection to a Commodore Microprocessor. The eluant was monitored using a Shimadzu Model SPD-6A variable wavelength detector. Peak analysis was performed by a

Shimadzu Model C-R3A recording integrator.

The chromatographic system was allowed to equilibrate for one to one and a half hours before injection of any samples. Stainless steel columns and radial PAK columns were used throughout the analysis. The columns used were a μBondapak C_{18}(10μ), Resolve C_{18}(5μ), a Nova-Pak C_{18}(4μ), all manufactured by Waters. The Rad-PAK C_{18} column was used in conjunction with a radial-PAK compression module supplied by Waters Associates. A LiChrosorb C_{18}(10μ) and a Nucleosil - 10 C_{18} column were supplied by HPLC Technology. A Supelco LC-18-DB (5μm), (fully end-capped) column was supplied by Supelchem.

B. REAGENTS

The metal ion standards were prepared from their appropriate salts. A stock solution of each metal ion (100μg/ml) was prepared in water and was henceforth stored in the fridge at 4°C. The deionised water used throughout the study was first distilled before being passed through a Waters Milli-Q water purification system to remove metals, organic and particulate matter.

Acetate buffers were prepared using Analar grade sodium acetate and Analar grade acetic acid. Analar grade sodium nitrate and potassium nitrate were supplied by BDH. The sodium nitrate was prepared as a 5% (w/v) stock solution dissolved in deionised water.

All glassware was soaked overnight in 10% nitric acid to remove any contaminants present. It was then rinsed with deionised water and air dryed.

The chelating agents used were APDTC, NaDEDTC and oxine of analar grade supplied by BDH. the stock solutions of chelating agents were prepared and stored in the fridge. The stock solution of NaDEDTC was 1% (w/v) dissolved in deionised water. The stock solution of oxine was 0.5% (w/v) buffered to pH 4.0 with 1 M acetate buffer.

The Sep-Pak cartridges, (both C_{18} and silica) were manufactured by Waters. Prior to depositing the metal-chelate complexes on the Sep-Pak cartridges they must first be solvated. To solvate the C_{18} cartridges, they were washed with 5 ml of methanol followed by a complete flushing with 10-20 ml of water. Solvation activates the surface groups on the silica particles, be they C_{18} or silica, so that the metal-chelate complexes can be retained. The silica Sep-Pak cartridges were washed with 5-10 ml of methanol prior to use. After this initial solvation step the cartridges should not be allowed to dry out completely.

The anaerobic adhesive raw materials used in the formulation of a typical product were supplied by Loctite (Tallaght, Ireland). Two finished products, called Product 270 and Product 290, were also supplied by Loctite (Tallaght, Ireland).

C. TECHNIQUES

C(i). Ultraviolet Spectral Investigations

The optimisation of the parameters for complex formation between oxine and the two metal ions, Fe(III) and Cu(II), was

preformed by taking 40 µl of the appropriate metal ion standard (1 x 10^{-2}M) and making up to 4 ml with the solution under investigation. This gave a final metal concentration of 1 x 10^{-4}M in metal ion. The UV absorbance was measured at 30 second intervals at 400 nm. At the beginning of each UV investigation, the lamp(s) of the spectrometer were allowed to equilibrate for 15 to 30 min before analysis.

The parameters which affected complexation and subsequent HPLC separation were ligand concentration in the mobile phase, pH of solution for complexation, solvent composition and effects of KNO_3 concentration on complexation. The Chromatographic conditions used by Bond and Nagaosa (43) were optimised for the present study.

The effect of varying the ligand concentration over the range 1 x 10^{-4}M to 5 x 10^{-3}M on the complexation of the metal ions was monitered by UV spectrometry at 400 nm. The values of absorbance (A) were plotted versus ligand concentration, and the optimum ligand concentration determined.

Using the optimum ligand concentration, the pH of the complexation solution was adjusted in the pH range 2.0-10.0 using dilute HCl or dilute NaOH. The absorbance was measured at 30 second intervals after a 20 second equilibration time. Absorbance (A) was then plotted versus pH.

Bond and Nagaosa (43) used a mobile phase of acetonitrile: 0.02M acetate buffer (pH 6.0) (50:50, v/v). Using UV spectral analysis at 400 nm and varying the percentage acetonitrile in the mobile phase, the optimum solvent ratio was established. It was also necessary to repeat this step under chromatographic conditions. Again a plot of absorbace (A) at 400 nm vs solvent composition was drawn for both

Cu(II)- and Fe(III)-oxinate complexes, yielding optimum solvent ratios.

It had been stated (43) that improved stability of the Fe(III)-oxinate complex was posible by the addition of KNO_3 to the mobile phase. Using the optimum conditions obtained above and adjusting the KNO_3 concentration between 0.1M and 0.8M, the UV absorbance was measured at 400 nm. The results obtained were used to plot a graph of absorbance (A) vs time, and from this graph the optimum KNO_3 concentration was obtained.

D. EXTRACTION PROCEDURES

D(i). Dithiocarbamate Extraction Procedure

Metal ion solutions were buffered to pH 4.0 with 1M sodium acetate buffer prior to the addition of 1-2 ml of a 5% (w/v) sodium nitrate solution. The $NaNO_3$ helps break down coloid which form in the presence of excess ligand. Next, 1 ml of a 1% (w/v) sodium diethyl-dithiocarbamate solution was added (or an alternative DTC ligand). This solution was then mechanically shaken for 15 min to allow for complete complexation to occur. This aqueous mixture was then passed through a C_{18} Sep-Pak cartridge. The cartridge was then dried and the metal-DTC complexes eluted in 5 ml of a non-polar solvent, such as dichloromethane or hexane, prior to chromatographic investigation.

It is also possible to form the metal-DTC complexes in-situ by incorporating the appropriate DTC ligand into the mobile phase. The

metal ions can then be directly injected into the chromatographic solvent containing the DTC ligand. One disadvantage of this system is the existence of a relatively high background level, due to ligand, at 254 nm (the wavelength used for detection of metal-DTC complexes). Some metal ions, such as Cr(III) and Cr(VI), take 2-3 hours to form complexes, and due to this long formation time a direct injection technique will not work.

D(ii). 8-Hydroxyquinoline Extraction Procedure

Extractions were performed by dissolving 2 ml of a PEGMA based formulation in 8 ml of dichloromethane (or 5.0 ml of TRI-EGMA-based formulation in 5.0 ml of dichloromethane) and extracting the metal ions with 10 ml of a 0.1 M HCl. After centrifugation, the HCl extract was passed through a C_{18} Sep-Pak cartridge and the eluate mixed with 10 ml of 1 x 10^{-2}M oxine. This solution was then extracted twice with 10 ml portions of dichloromethane. The dichloromethane fractions were combined and passed through a silica Sep-Pak cartridge. The cartridge was then air dried for 1-2 min. The metal-oxinate complexes were then eluted with 2.5 ml of methanol, before being injected into the chromatographic system.

E. GAS CHROMATOGRAPHIC INVESTIGATIONS

Using the Hewlett-Packard Model 5890 gas chromatograph it was possible to determine which of the monomer constituents were

extracting into the 0.1M HCl solution used in the extraction procedure for oxine, outlined earlier.

The dichloromethane solution, which contains the metal-oxinate complexes, was passed through a silica Sep-Pak cartridge. The dichloromethane eluant was collected and evaporated to dryness at 60°C on a heating block. The residue was reconstituted in 100 µl of hexane and 1 µl injected for analysis.

F. LIQUID CHROMATOGRAPHIC INVESTIGATIONS

F(i). Dithiocarbamates

In ascertaining the chromatographic behaviour of various metal-DTC complexes, the samples were prepared as outlined previously. The metal-DTC complexes, dissolved in dichloromethane, were then injected into the chromatographic system and detected using a UV detector at 254 nm.

The solvents used throughout the chromatographic investigations, using DTC ligands, were acetonitrile: water or acetonitrile: sodium acetate buffer (pH 5.5) mixtures. Prior to using the solvents they were filtered through a 0.45 µm Sartorius disposable filter and degassed using helium for approximately 30 min. The chromatographic system was allowed to equilibrate for one to one and a half hours before the injection of any samples. To form DTC complexes of Cr(III) and Cr(VI), it was necessary to shake for 2-3 hours as prior to injection.

F(ii) 8-Hydroxyquinoline

In ascertaining the chromatographic behaviour of the metal-oxine complex, two methods for sample preparation were used. By incorporating oxine into the mobile phase it was possible to directly inject the metal ions of interest into the chromatographic system and monitor the eluant at 400 nm. The second approach was to use the extraction scheme outlined previously on page 56. This extraction scheme was developed so that the metal-oxine complexes were formed externally and thus functions as a sample pre-concentration step.

The solvents used throughout the chromatographic investigations, employing oxine as the ligand, were acetonitrile: 0.02M acetate buffer (pH 6.0) (50:50 v/v). The mobile phase also contained oxine and KNO_3, so it was necessary to filter the mobile phase through a 0.45 μm Sartorius disposable filter and then degas for 30-45 min under helium. It was also necessary to flush the chromatographic system for one hour, with the mobile phase, prior to the injection of any samples. It was not possible to recycle the mobile phase, so fresh mobile phase was prepared daily. Due to the presence of KNO_3 and oxine in the mobile phase, the chromatographic system was flushed out each day with methanol for one hour. This was to prevent damage to the pump head, lines and the analytical column.

Based on the studies reported under UV spectral investigations, it was required to investigate what effects these parameters caused under chromatographic conditions. The effects on retention due to changes in mobile phase composition was investigated over the range, acetonitrile: acetate buffer, 70:30 (v/v) to 40:60 (v/v). The

concentration of KNO_3 in the mobile phase was adjusted to ascertain if it caused any significant effects on the separation or complexation chemistry. It was increased step-wise from 0.1 M to 0.4 M KNO_3. As an alternative wavelength, the use of 350 nm was examined.

G METHOD FOR ANAEROBIC ADHESIVE EXTRACTION

2 ml of the anaerobic adhesive formulation was taken and dissolved in 8 ml of dichloromethane. This solution was placed in a separating funnel and 10 ml of 0.1 M HCl added. The mixture was shaken for 10-15 min followed by centifugation at 3,000 rpm for 5 min.

The HCl layer was removed and passed through a C_{18} Sep-Pak cartridge. The eluate was then mixed with 10 ml of 1 x 10^{-2}M oxine (pH 4.0, 1M acetate buffer) for 10 min to allow complexation to occur. This solution was then extracted twice with 10 ml of dichloromethane and the two fractions combined.

The dichloromethane extract was then passed through a silica Sep-Pak cartridge, which retained the metal-oxine complexes. A slow stream of nitrogen or air was passed through the cartridge to dry it and remove traces of dichloromethane. The metal-oxine complexes were then eluted in 2.5 ml of methanol. For analysis, 20 µl or 50 µl of the methanol solution was then injected into the chromatographic system.

RESULTS AND DISCUSSION

A. DETERMINATION OF METAL IONS USING DITHIOCARBAMATES AS CHELATING AGENT

Using RP-HPLC, Bond and Wallace (22) showed that it was possible to separate a number of metal ions. The initial stage was to from the appropriate metal-DTC complex using NaDEDTC as the chelating agent. The formation of these metal-DTC complexes has been outlined in the Experimental section.

Once formed, samples were injected into the chromatographic system. Initial investigations of metal-DTC complexes were made on a Rad-Pak C_{18} column in conjunction with a radial compression module. The mobile phase used was acetonitrile: acetate buffer (pH5.5) (70:30, v/v) with UV detection at 254 nm.

Using the Rad-Pak C_{18} column, it was possible to elute Ni(II), Co(III), Cu(II) and Fe(III) as their appropriate DEDTC complexes. However, excessive tailing of the peaks was noted. The Rad-Pak C_{18} column is not end-capped, and this tailing could be as a result of uncapped silanol groups present on the column packing material. It has been stated in a number of articles (13) that certain metal-DEDTC complexes are unstable and readily degrade on-column. This point is emphasised if one examines the chromatogram of $Fe(DEDTC)_3$ (Fig. 1). A peak with a retention time of 5 min is due to degradation of $Fe(DEDTC)_3$ on column. This degradation peak was also present in the chromatographic separation of $Co(DEDTC)_3$, but was not as pronounced as with $Fe(DEDTC)_3$.

Employing the Rad-Pak C_{18}, column it would appear from the retention times (Table 6) and poor peak shape that resolution of

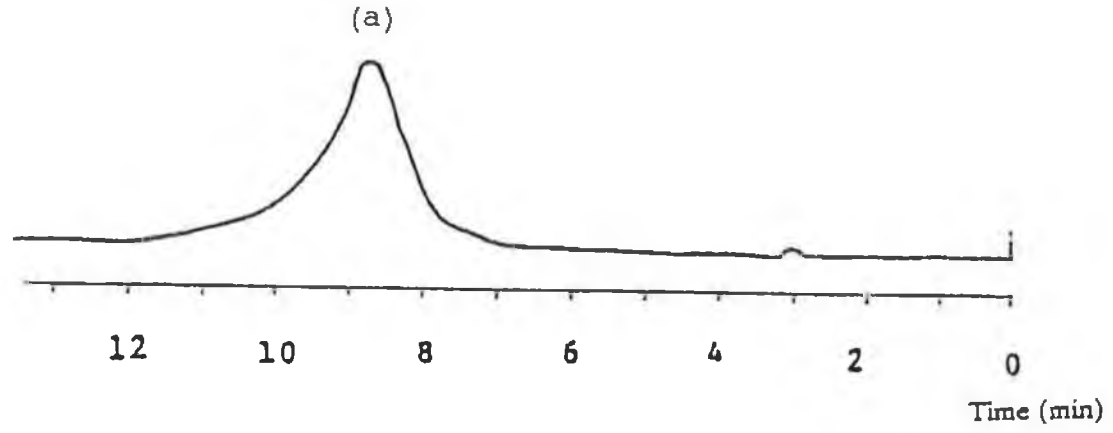


FIGURE 9(a) Separation of (a) $Cu(DEDTC)_2$ on a Rad-Pak C_{18} cartridge. Mobile phase acetonitrile:0.02 M acetate buffer (pH 5.5) (70:30, v/v) with a flow rate of 1.0 ml/min and UV detection at 254 nm.

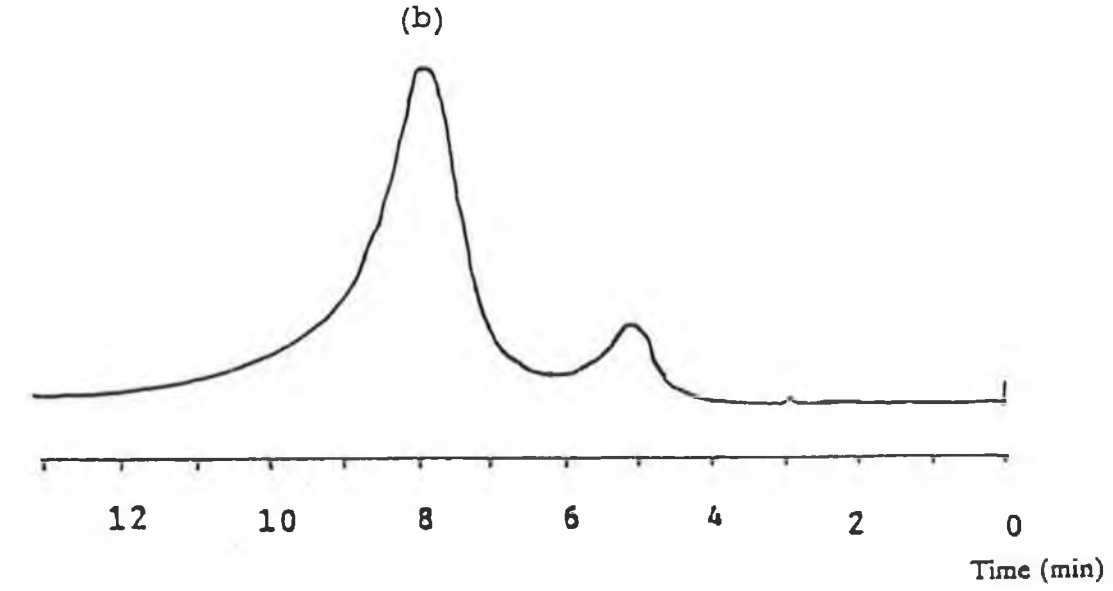


FIGURE 9(b) Separation of (b) $Fe(DEDTC)_3$ on a Rad-Pak C_{18} cartridge. Mobile phase acetonitrile:0.02 M acetate buffer (pH 5.5) (70:30, v/v) with a flow rate of 1.0 ml/min and UV detection at 254 nm.

Cu(II) and Fe(III) as their DEDTC complexes would not be possible. The solvent composition of acetonitrile: acetate buffer was adjusted, but did not exhibit the selectivity to resolve the two metal-chelates (See Table 6 for results).

It was now decided to try a μBondapak C_{18} column (10 μm). The column packing here is a C_{18} 'end-capped' material. This should stop or decrease the peak tailing. The mobile phase was adjusted with the acetate buffer being replaced by water. The new mobile phase was acetonitrile: water (70:30, v/v) as a starting point. Using the μBondapak column there was a marked improvement in peak shape with only a minor degree of tailing. (Figure 10(a) and 10(b)), with $Fe(DEDTC)_3$ giving rise to the second peak at 6.3 min (Fig. 10 (a)).

The degradation peak was not identified but it was postulated that it was thiuram disulphide, which has been shown to be a degradation product of DTC ligands. Again, as was the situation with the Rad-Pak C_{18} column, the retention times of $Cu(DEDTC)_2$ and $Fe(DEDTC)_3$ were similar (Table 6). Due to the instability of the $Fe(DEDTC)_3$ complex it was not possible to use the μBondapak column with any level of success. Under the chromatographic conditions outlined, it was possible to resolve to some degree, $Ni(DEDTC)_2$ from the other complexes.

As stated in the Experimental section, the metal-DTC complexes were eluted from the C_{18} Sep-Pak with dichloromethane. It is this solvent containing the metal-DTC complexes which was injected into the chromatographic system. The presence of dichloromethane can cause immiscibility problems that subsequently leads to poor peak shape, excessive tailing and even break-down of the metal-DEDTC complexes, as

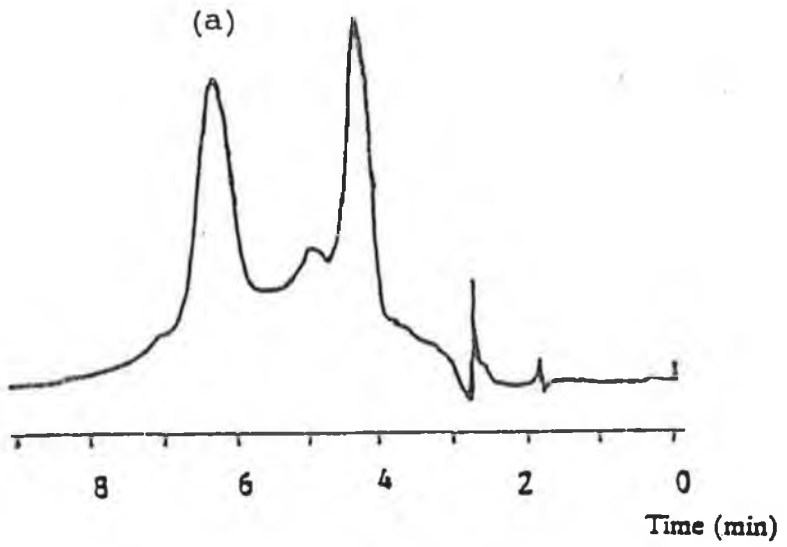


FIGURE 10(a); Chromatogram of (a) $Fe(DEDTC)_3$ on a μ-Bondapak C_{18} column.

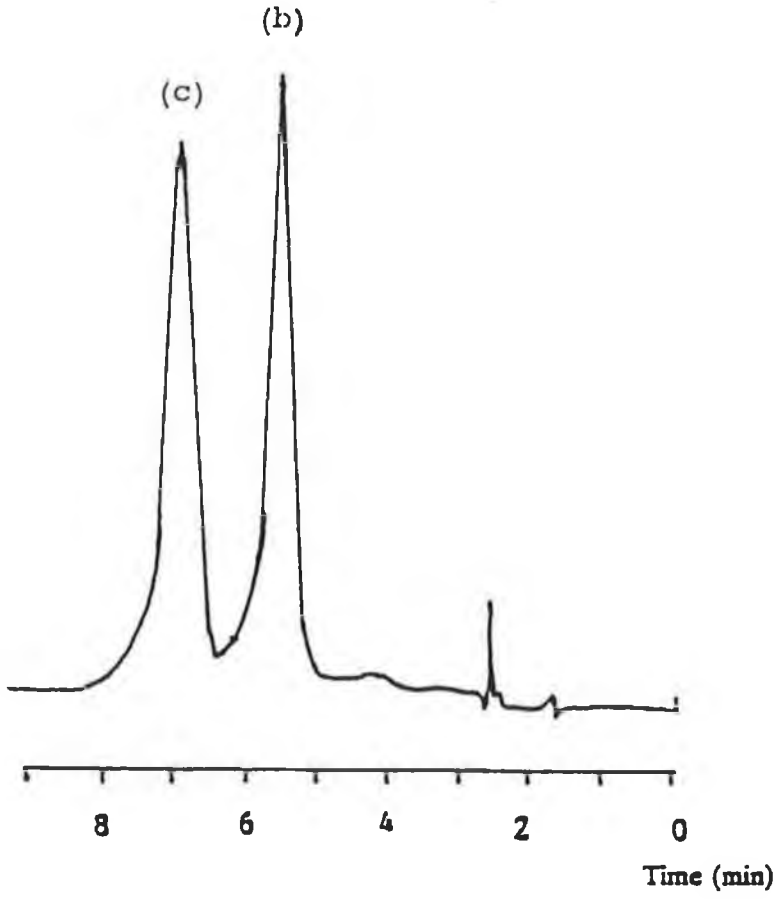


FIGURE 10(b); Separation of (b) $Ni(DEDTC)_2$ and (c) $Cu(DEDTC)_2$ on a μ-Bondapak C_{18} column. Mobile phase in both cases was acetonitrile:water (70:30, v/v) with a flow rate of 1.5 ml/min and UV detection at 254 nm.

has occurred in the study (Table 6).

To try and resolve $Cu(DEDTC)_2$ and $Fe(DEDTC)_3$ the μBondapak column was replaced with a Nucleosil 10 C_{18} column. The mobile phase was acetonitrile: water (70:30, v/v) with UV detection at 254 nm. The retention times on the Nucleosil 10 column, under the same conditions as were used for the other C_{18} column, were much longer. The retention times for $Fe(DEDTC)_3$ and $Cu(DEDTC)_2$ were approximately doubled. There is no clear explanation why this occurred. The only significant difference between the columns is in the manufacture of the bonded phases.

A sample chromatogram showing retention of Fe(III), Cu(II) and Ni(II) on the Nucleosil 10 column can be seen in Figure 11(a) and 11(b).

The percentage carbon loading on the silica particles is different and this results in increased retention of the metal-DEDTC complexes on the Nucleosil 10 column. The increased retention of the complexes on the Nucleosil 10 column implies that the column is more non-polar than the μBondapak column used previously. This point emphasises the differences which exist between C_{18} columns from different manufacturers. Relatively low concentrations of metal-DTC complexes had to be injected onto the Nucleosil 10 column, because peak splitting (due to column over-loading) was evident at high concentrations.

The retention time of $Zn(DEDTC)_2$ was 13.3 min and $Fe(DEDTC)_3$ at 12.8 min. So, if all three metal ions, Zn(II), Cu(II) and Fe(III) were present in a mixture, it would not be possible to resolve and quantitate the three metal ions. Since base-line resolution using

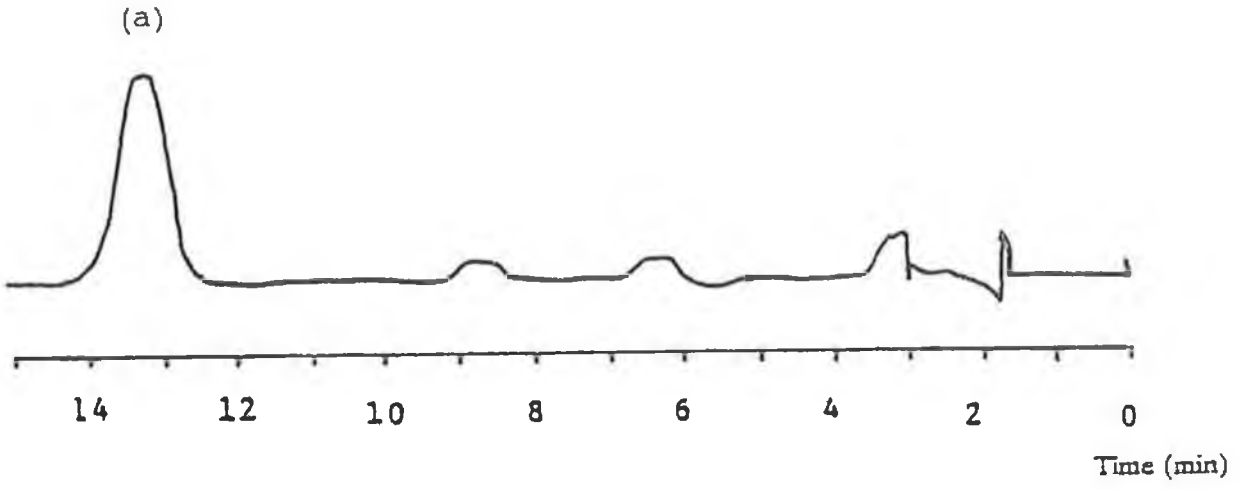


FIGURE 11(a); Chromatogram of (a) $Cu(DEDTC)_2$ on a Nucleosil 10 C_{18} column.

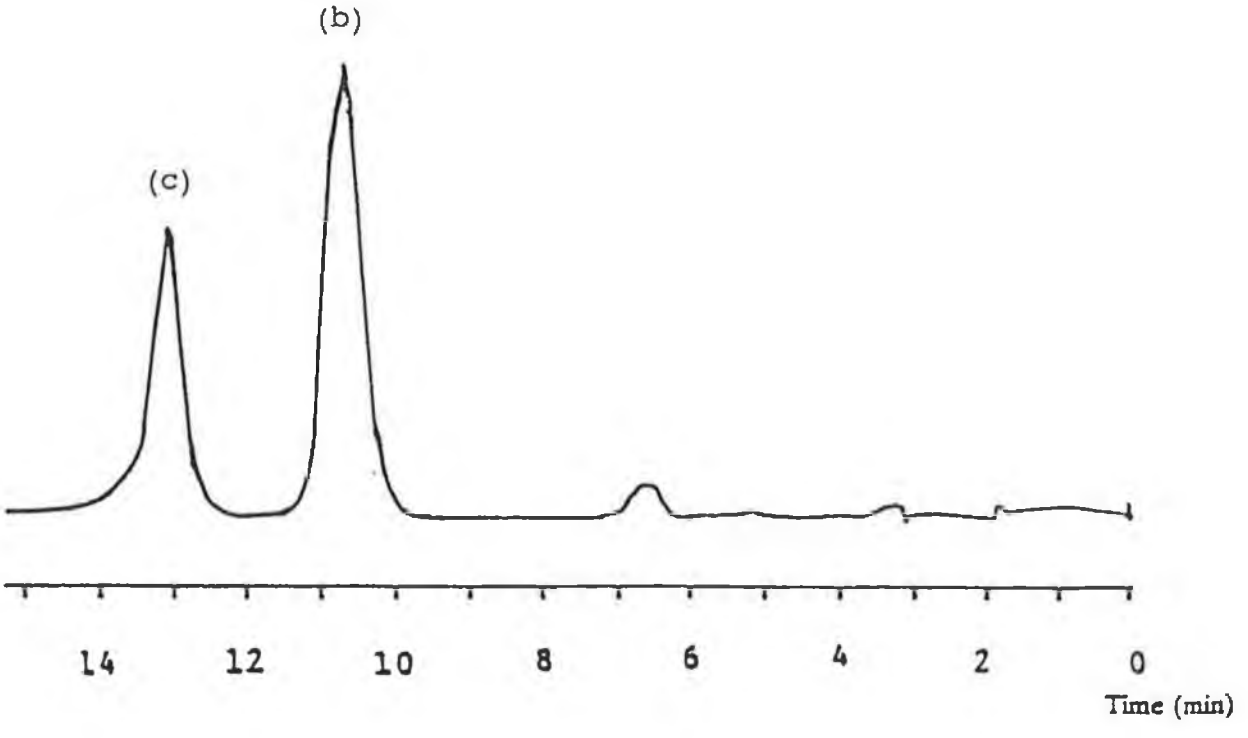


FIGURE 11(b); Separation of (b) $Co(DEDTC)_3$ and (c) $Fe(DEDTC)_3$ on a Nucleosil 10 C_{18} column. Chromatographic parameters as in FIGURE 10(b).

these C_{18} columns was not possible to date, alternative C_{18} columns with smaller particle size packing materials were investigated. The first of these high resolution columns was the Novapak C_{18} (4 μm) column, manufactured by Waters Associates (15 cm column).

The Novapak column posed a number of problems. The small particle size resulted in excessive build up in column back-pressure. Column back-pressures in excess of 4,000 psi were common with a mobile phase composition of acetonitrile: water (70:30, v/v) with typical flow rates of 1.0 ml/min - 1.5 ml/min.

Another high resolution column was tried, namely the Resolve C_{18} (5 μm) column. This column did not separate the DTC complexes of Cu(II) and Fe(III) and the DTC complexes of Cu(II), Fe(III), Ni(II), and Co(III) had very short retention times (less than 5 min for each). The mobile phase was acetonitrile: water (70:30, v/v), with typical flow rates of 1.0 ml/min - 1.5 ml/min with UV detection at 254 nm. The residual silanol groups on the column packing material were believed to impart a secondary effect and shortening the retention times of the complexes on the column.

Since it appeared that retention of the metal-DEDTC complexes increased as a more non-polar column was used, the analytical column was replaced by a Supelco LC-18-DB column which is fully end-capped. This end-capping results in a slightly more non-polar column than a non- endcapped C_{18} column.

On the Supelco LC-18-DB column, the metal-DEDTC complexes had extremely long retention times. The mobile phase was acetonitrile: water (70:30, v/v); other mobile phase compositions were investigated, but the above ratio was found to be optimum for this column. The

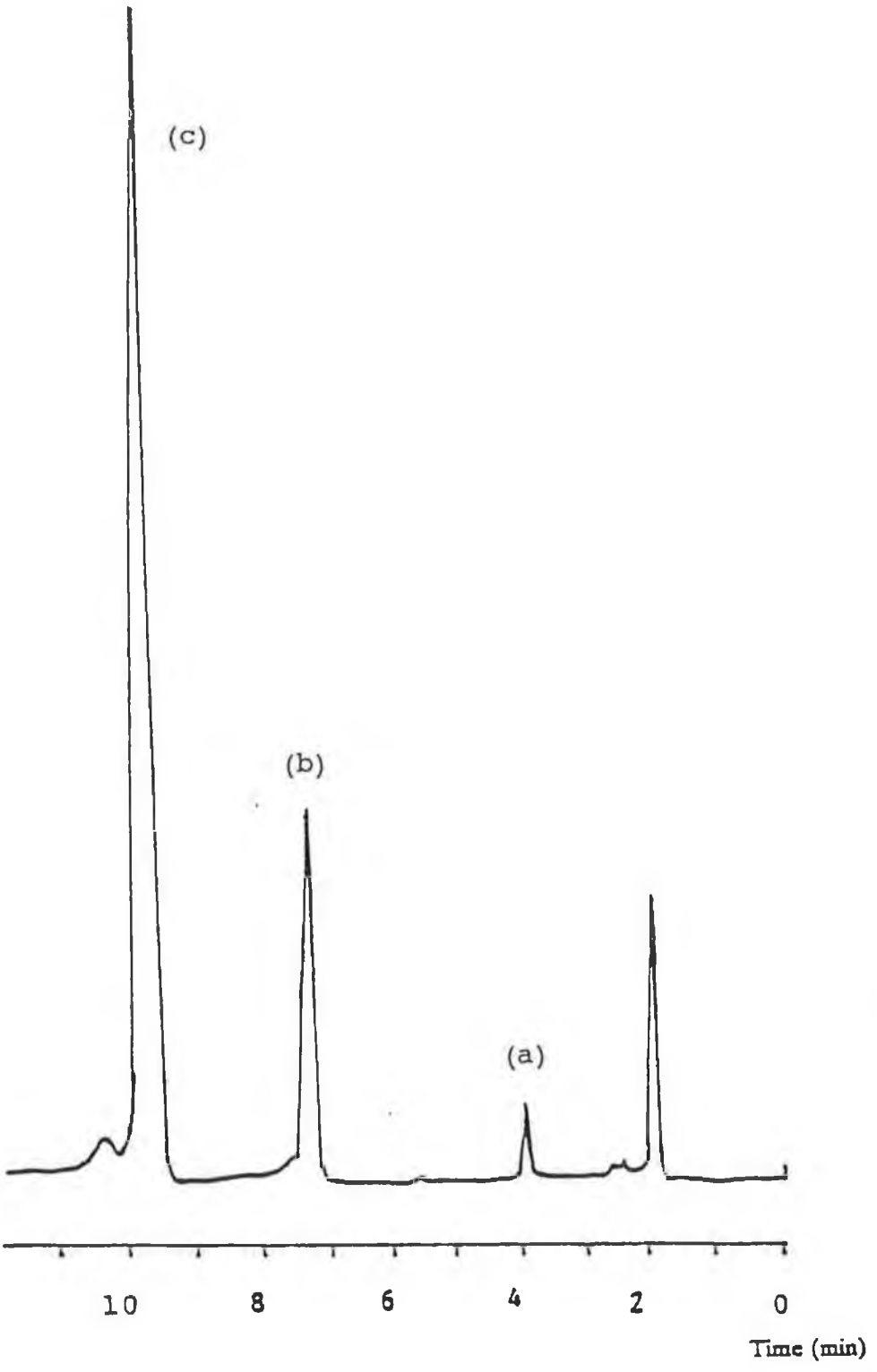


FIGURE 12; Chromatogram of NaDEDTC showing a number of degradation peaks on a Supelco LC-18-DB column. Mobile phase acetonitrile: water (70:30, v/v) with a flow rate of 1.0 ml/min and UV detection at 254 nm.

$Ni(DEDTC)_2$ complex had the shortest retention time of 14.6 min at a flow rate of 1.0 ml/min (See Table 6). Even though the complexes had long retention times, good peak shape was obtained using this column. This agrees with the point made earlier that the non end-capped silanol groups caused band broadening.

The DEDTC complexes of Fe(III) and Zn(II) showed a number of degradation peaks in their chromatograms. The $Fe(DEDTC)_3$ complexes degraded to the point that the main peak present in the chromatogram was due to the ligand itself i.e. NaDEDTC. This was confirmed by making an injection of NaDEDTC and comparing peaks in the two chromatograms (Fig. 12). The chromatogram of $Zn(DEDTC)_2$ was similar to that of $Fe(DEDTC)_3$ in that a number of degradation peaks were present. The main peak due to $Zn(DEDTC)_2$ was split and had a retention time from 21.5 min - 22.5 min.

The retention times of Cu(II) and Fe(III) on this column were to close for baseline resolution. The $Zn(DEDTC)_2$ peak co-eluted with both $Fe(DEDTC)_2$ and $Cu(DEDTC)_2$. Retention times were too long to make the method possible from a time point of view. Figure 13 shows the separation of Ni(II), Co(III) and Cu(II) on the Supelco column as their DEDTC complexes.

At this point in time the research was diverted to the possibility of using ammonium pyrrolidonedithiocarbamate (APDTC) as the chelating agent. It has been stated (21) that the metal ion complexes of APDTC are more stable than their corresponding metal-DEDTC complexes.

The column used was a Supelco LC-18-DB (5 μm) with a mobile phase of acetonitrile: water (70:30 v/v) and a flow rate of 1.0 ml/min. The

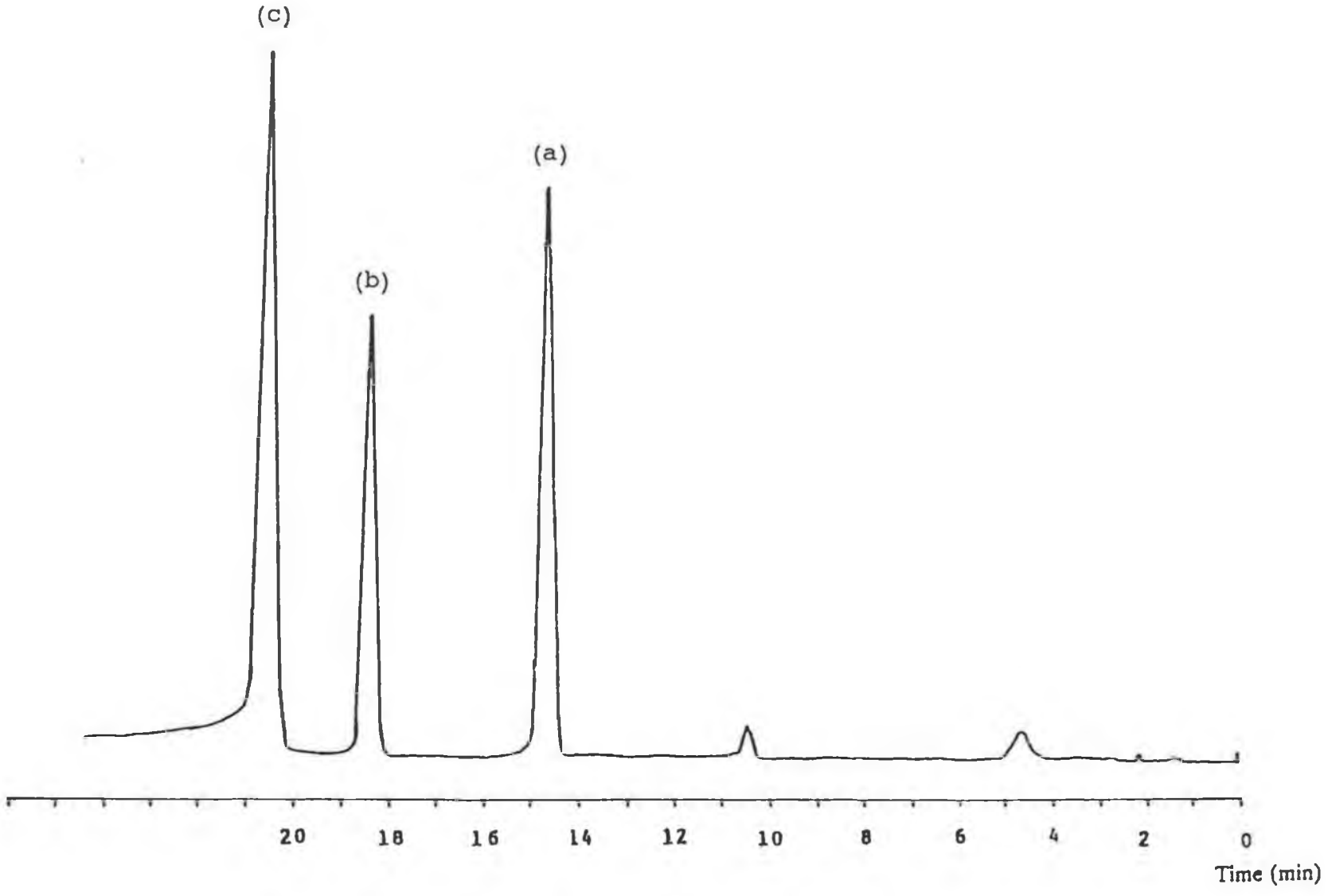


FIGURE 13; Separation of (a) $Ni(DEDTC)_2$, (b) $Co(DEDTC)_3$ and (c) $Cu(DEDTC)_2$ on a Supelco LC-18-DB column. Mobile phase acetonitrile:water (70:30, v/v) with a flow rate of 1.0 ml/min and UV detection at 254 nm.

metal-APDTC complexes were formed externally following the same procedure as was used for the metal-DEDTC complexes. Using APDTC as chelating agent, the Cu(II) and Fe(III) complexes had retention times of 13.7 min and 14.0 min respectively. As was the situation with DEDTC as complexing agent, baseline resolution was not achieved. The $Fe(APDTC)_3$ complex did show some tailing. Also present in the chromatograph was a large peak at 7 min due to degradation of $Fe(APDTC)_3$, showing instability of the complex (Fig. 14).

To achieve base-line resolution, the mobile phase composition of acetonitrile: water was adjusted. The ratio's investigated were adjusted between acetonitrile: water (70:30, v/v) to acetonitrile: water (30:70, v/v). The net results of these adjustments was an increase in retention times when the acetonitrile concentrations was increased. Alterations to the mobile phase composition did not have the desired effect of resolving the complexes of Cu(III) or Fe(III) as either their DEDTC or APDTC complexes.

Since it was not possible to separate the metal-DTC complexes under the conditions outlined, it was necessary to investigate the use of an alternative chelating agent.

Bond and Nagaosa (43) have used 8-hydroxyquinoline (oxine) to separate Cu(II) and Fe(III) in less than 10 min by RP-HPLC. The mobile phase used was acetonitrile: 0.02 M acetate buffer (pH 6.0) which was 5×10^{-3}M in oxine and 0.4M KNO_3. With this in mind, the present research was devoted to the use of oxine as chelating agent to separate Cu(II) and Fe(III).

COLUMN	CHROMATOGRAPHIC CONDITIONS		RETENTION TIMES OF COMPLEXES (MIN)					
			Ni(III)	Co(III)	Cu(II)	Fe(III)	Zn(II)	Na(I)
RAD-PAK C_{18}	Mobile phase:	acetonitrile: acetate buffer (70:30,v/v)	6.6	7.5	8.8			
	Flow Rate:	1.0 ml/min				(5.0)		
	Detection:	UV @ 254 nm				7.9		
	Ligand:	NaDEDTC						
µBondapak	Mobile Phase:	acetonitrile: water (70:30, v/v)				(4.4)		
	Flow Rate:	1.5 ml/min	5.6	6.0	6.9	6.2		
	Detection:	UV @ 254 nm						
	Ligand:	NaDEDTC						
Nucleocil-10	Mobile Phase:	acetonitrile: water (70:30, v/v)						
	Flow Rate:	1.5 ml/min	9.1	10.6	13.3	12.8	12.9	
	Detection:	UV @ 254 nm						
	Ligand:	NaDEDTC						
Supelco LC-18-DB	Mobile Phase:	acetonitrile: water (70:30, v/v)		(10.0)		(10.0)	(10.7)	(3.5)
	Flow Rate:	1.0 ml/min	14.6	18.3	20.5	(17.0)	(14.4)	(7.5)
	Detection:	UV @ 254 nm				20.7	17.6	10.0
	Ligand:	NaDEDTC						
Supelco LC-18-DB	Mobile Phase:	acetonitrile: water (70:30, v/v)			(7.0)			
	Flow Rate:	1.0 ml/min			13.7	(7.0)		
	Detection:	UV @ 254 nm				(7.6)		
	Ligand:	APDTC				14.0		

( ) Degradation Peaks

B. DETERMINATION OF METAL IONS USING 8-HYDROXYQUINOLINE AS CHELATING AGENT

B(i). Ultra-violet Spectral Investigations

The chromatographic parameters outlined by Bond and Nagaosa (43) to separate Cu(II) and Fe(III) were first optimised using UV spectroscopy. The parameters to be optimised were:

(i) concentration of chelating agent in the mobile phase;

(ii) pH of the mobile phase;

(iii) solvent composition (acetonitrile: acetate buffer);

(iv) KNO_3 concentration in the mobile phase.

To determine the optimum concentration of chelating agent which should be added to the mobile phase, the concentration used by Bond and Nagaosa was taken as the starting point and levels were adjusted around this value. The concentration range investigated was from 1×10^{-4} to 5×10^{-3} oxine (above a concentration of 5×10^{-3}M, oxine was found not to be soluble). These concentrations closely simulate the chromatographic conditions used to separate Cu(II) and Fe(III) as their oxinate complexes.

It was also noted that under the conditions investigated the Cu(II)-oxinate complex precipitated out of solution. From the results obtained it was possible to draw a graph of absorbance (A) at 400 nm vs time (sec). From this graph it was possible to obtain the optimum concentration of chelating agent which should be added to the mobile phase.

The results are shown in Figure 15. From this graph the optimum chelating agent was determined as being 5×10^{-3}M. This concentration would now be used in all further investigations.

Using the optimum concentration of chelating agent, the pH of the solution was adjusted over the pH range 2.0 - 10.0 to determine the optimum pH for formation of the metal-oxine complex. A graph of absorbance (A) vs time (sec) was constructed and from this it was possible to obtain the pH value which gave rise to the most stable complexes. The results are shown in Figure 16. The Cu(II)-oxine complex again precipitated out of solution. It can be seen from Figure 16 that at pH values above pH 8.0 the absorbance did not show a stable reading with respect to time. It can be seen that the optimum pH is pH 4.0 or 6.0. It was decided to use a pH of 6.0 for complex formation since less adjustments to the mobile phase were required at pH 6.0.

The effect of solvent composition on complex formation was also investigated. The solvent composition could cause degradation of the complexes, as was seen when using DEDTC and a high percentage of water in the mobile phase. Solvent compositions of acetonitrile: 0.02M acetate buffer, pH 6.0 (80:20, v/v) to acetonitrile: 0.02M acetate buffer, pH 6.0 (40:60, v/v) were investigated and the results of absorbance (A) vs time (in sec) were plotted. From Figure 17 it was then possible to select what solvent composition was optimum for complexation formation.

At a solvent composition of acetonitrile: 0.02M acetate buffer, pH 6.0 (80:20, v/v), the Cu(II) complex precipitated out of solution. The Cu(II) complex also exhibited some instability at a 60:40, (v/v)

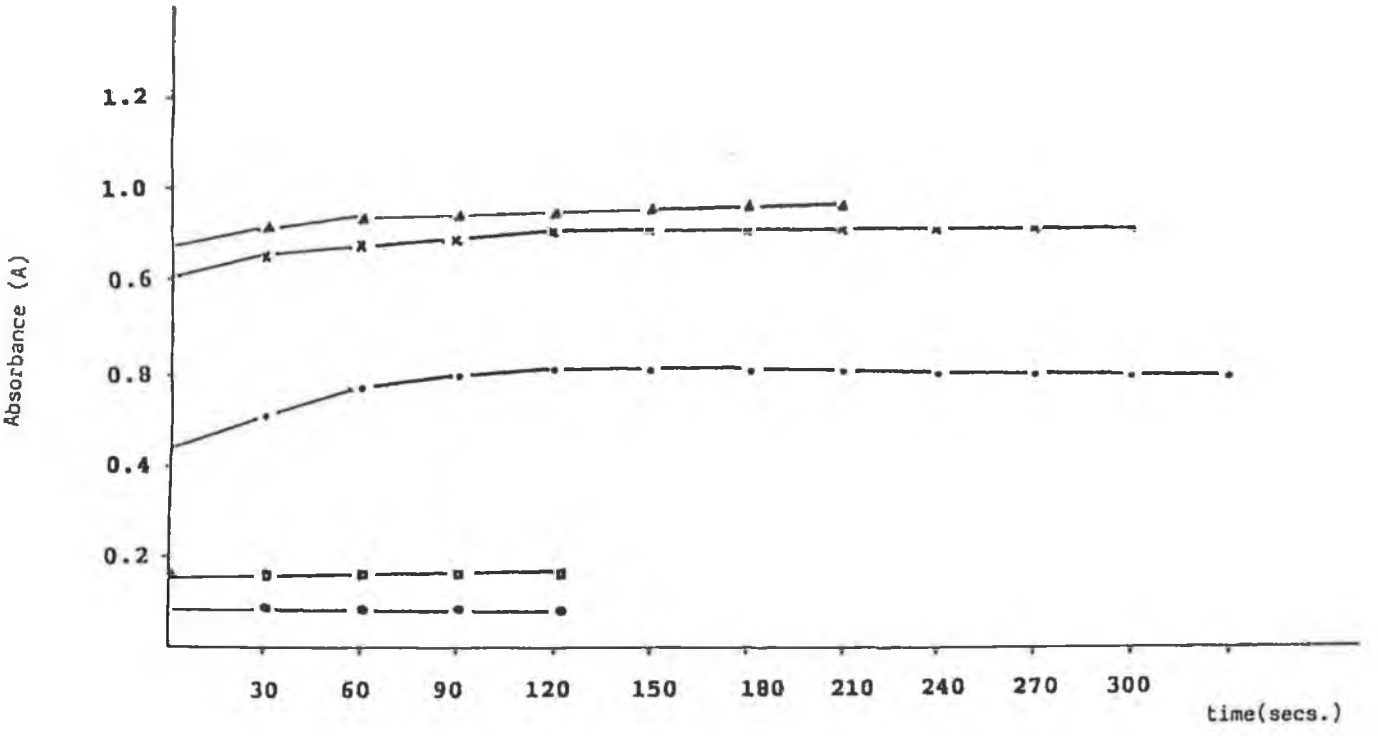


FIGURE 15 Effect of varying ligand concentration on absorbance (A) of metal-oxine complex. [Fe(III)] = 1×10^{-4} M

△ = 5×10^{-3} M □ = 1×10^{-4} M

× = 2.5×10^{-3} M ○ = 5×10^{-4} M

• = 1×10^{-3} M

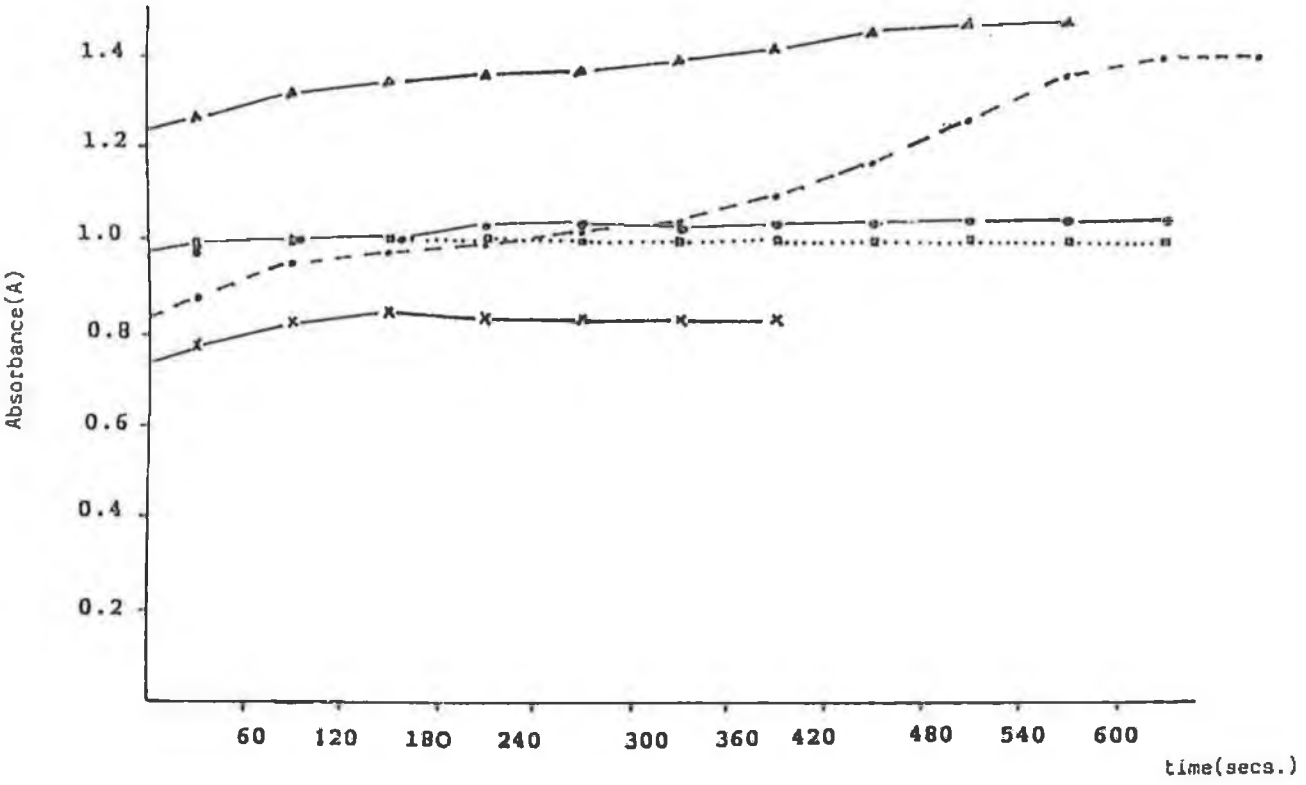


FIGURE 16 Effect of pH on complex formation.
[Fe(III)] = 1 x 10^{-4} M

△ = pH 8.0 • = pH 10.0
□ = pH 6.0 × = pH 2.0
○ = pH 4.0

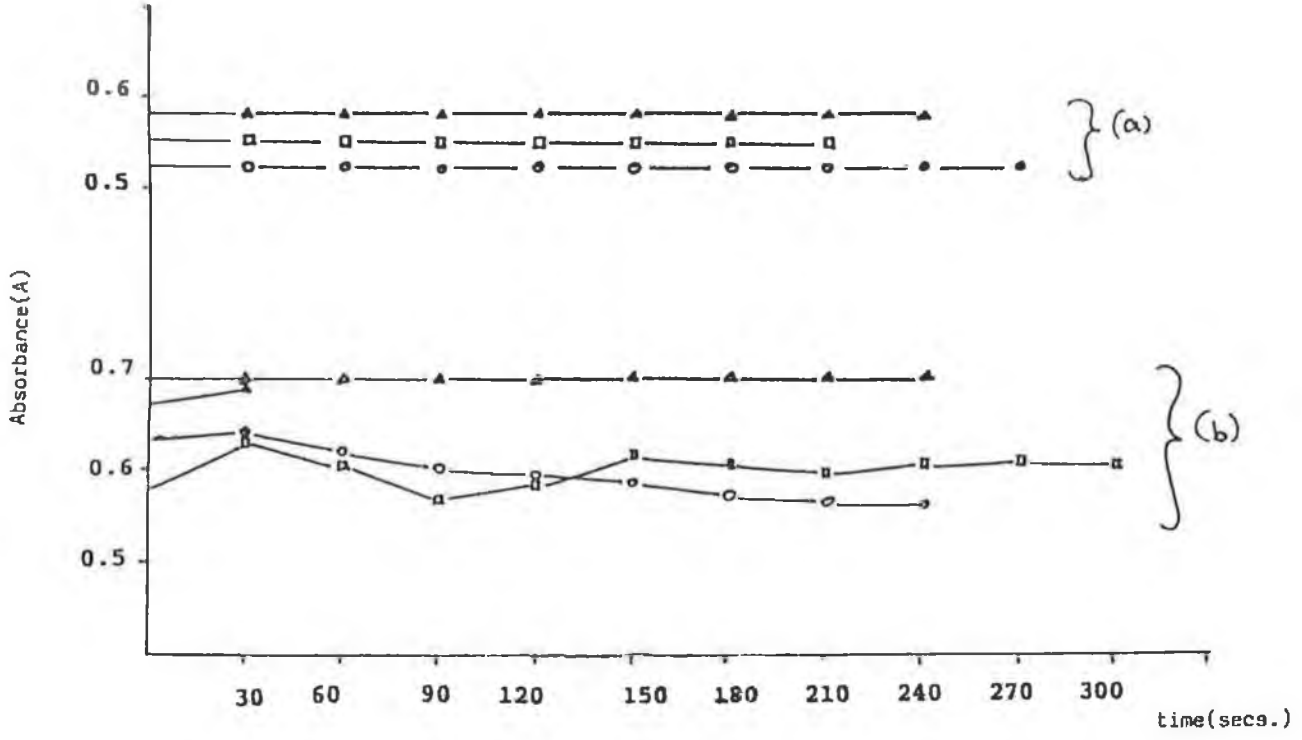


FIGURE 17 Effect of varying solvent composition* on complex formation. (a) [Fe(III)] = 1 x 10^{-4} M (b) [Cu(II)] = 1 x 10^{-4} M

△ = 60:40, 50:50 (v/v)

□ = 40:60 (v/v)

○ = 20:80 (v/v)

* acetonitrile:0.02 M acetate buffer.

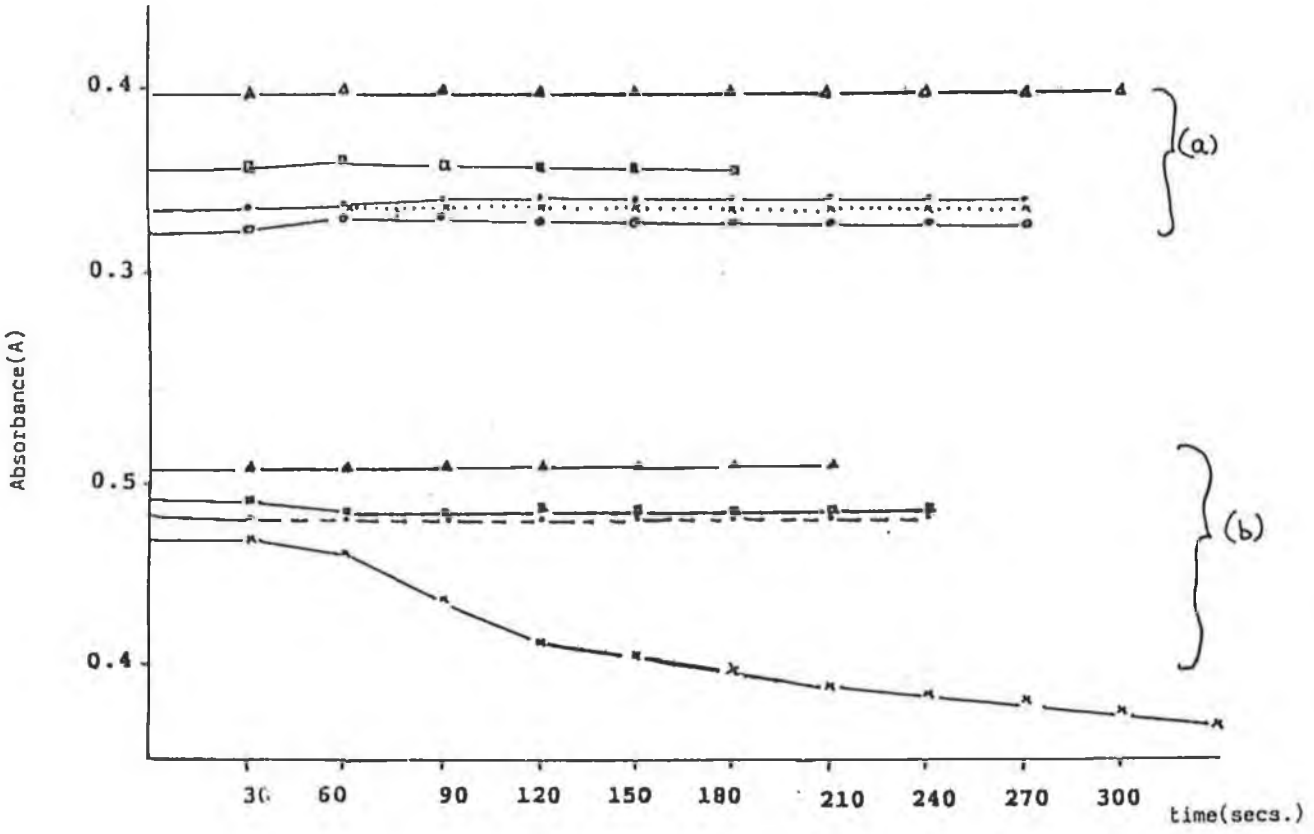


FIGURE 18 Effect of varying KNO_3 concentration on complex formation. (a) [Fe(III)] = 1 x 10^{-4} M (b) [Cu(II)] = 1 x 10^{-4} M

(a)		(b)	
△	= 0.6 M	△	= 0.1 M
□	= 0.2 M	□	= 0.4 M
•	= 0.8 M	•	= 0.2 M
×	= 0.1 M	×	= 0.6 M
○	= 0.4 M		

composition. The solvent composition selected was 50:50, (v/v), which was the mobile phase composition used by Bond and Nagaosa (43).

Finally, the effect of varying concentrations of KNO_3 on complex formation was investigated. Using the optimised conditions developed to this point, the effect of KNO_3 up to a concentration of 0.8 M KNO_3 was investigated. The results are displayed graphically in Figure 18. A plot of absorbance (A) vs time (sec) yielded the optimum concentration required for complex formation. At a concentration of 0.6 M KNO_3 the Cu(II)-oxinate complex readily degraded. In the case of Fe(III)-oxinate complex, increasing the concentration of KNO_3 from 0.1 M to 0.8 M had no dramatic improvement on complex stability or absorbance. As a precautionary measure 0.1 M KNO_3 was added to all mobile phases used.

From the UV spectral investigations the optimum conditions for complex formation were found to be:

(i) 5×10^{-3} oxine in mobile phase;

(ii) pH 6.0, mobile phase;

(iii) acetonitrile: 0.02 M acetate buffer, (50:50, v/v);

(iv) 0.1 M KNO_3.

B(ii). Chromatographic Investigations

The next step was to investigate how the chromatographic peaks for Fe(III)- and Cu(II)-oxinate complexes would be affected by mobile phase composition and KNO_3 concentration. Firstly Fe(III), Cr(III), Cu(II), Ni(II), Co(III) and Zn(II) were injected separately

into the chromatographic system with a mobile phase of acetonitrile: 0.02 M acetate buffer (pH 6.0) which was 5 x 10^{-3}M in oxine and 0.1 M in KNO_3. The metal-oxine complex as were formed 'in-situ' (on a Supelco LC-18-DB 25cm column) and detected by a UV detector set at 400 nm. Under these conditions it was possible to separate a multi-component mixture of Cr(III), Zn(II), Cu(II) and Fe(III) in less than 10 min (Figure 19). On injecting Ni(II) and Co(III) into the chromatographic system very unsymmetric, irreproducible peaks were obtained. A base-line resolution of 3 min was achieved for Fe(III)-oxine and Cu(II)-oxine using oxine as chelating agent.

The effects of solvent composition on retention of the metal-oxinate complexes was then ascertained. The mobile phase composition was adjusted from acetonitrile: acetate buffer, pH 6.0 (70:30, v/v) to acetonitrile: acetate buffer, pH 6.0 (40:60, v/v). The most marked effect of this was on the retention of the Fe(III)-oxine complex. As the concentration of this polar fraction (acetate buffer) was increased, the retention time of the Fe(III)-oxine complex increased. The retention time increased from 9 min to 19 min on increasing the percentage acetate buffer by 10%. On decreasing the concentration of the acetate buffer, all retention times were very similar and resolution was not possible.

At mobile phase compositions with a percentage acetonitrile above 55%, it was not possible to detect the Zn(II)-oxine complex, and at a percentage acetonitrile of less than 45%, the Cu(II)-oxine complex gave rise to a very broad peaks. The optimum mobile phase composition was chosen to be acetonitrile: 0.02 M acetate buffer (50:50, v/v) and was used in all subsequent separations.

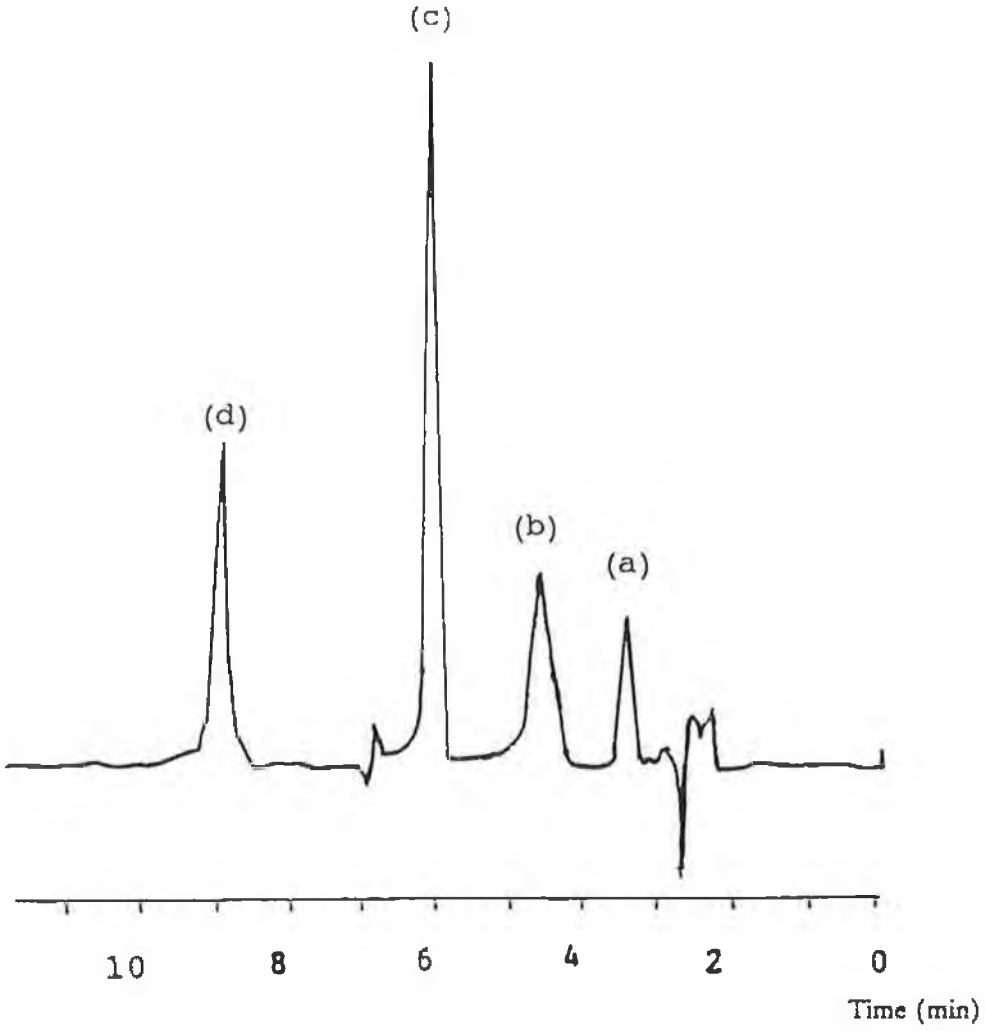


FIGURE 19; Separation of (a) Cr(III), (b) Zn(II), (c) Cu(II), and Fe(III) following `in-situ' formation of their oxinate complexes on a Supelco LC-18-DB column. Mobile phase acetonitrile:0.02 M acetate buffer (pH 6.0) which was 5×10^{-3} M in oxine and 0.1 M in KNO_3 with UV detection at 400 nm.

The wavelength used to detect the metal-oxine complex was 400 nm. At 350 nm it is possible to detect the metal-oxine complexes of Zn(II), Cu(II) and Fe(III), but due to an interfering peak it was not possible to detect Cr(III)-oxine. In all future investigations the UV detector was set at 400 nm. At wavelengths less than 350nm the oxine present in the mobile phase causes a very high background level and thus increases the level of detection.

The effects on KNO_3 on the separation was assessed by adjusting its concentration in the mobile phase from 0 to 0.4 M. The net result was no increase in peak height on adding KNO_3 to the mobile phase. As stated previously, a level of 0.1 M KNO_3 was added to the mobile phase as a precautionary measure.

Using the optimum conditions determined to date, calibration curves were constructed for Cu(II) and Fe(III). Both of these calibration curves were linear over the concentration range 1 - 100 µg/ml investigated. For a 20 µl direct injection of each metal ion, the limits of detection for Cr(III), Zn(II), Cu(II) and Fe(III) (as aqueous injections) were 5.0 µg/ml, 1.5 µg/ml, 0.5 µg/ml and 0.8 µg/ml respectively.

The metal standard (50 ml) was mixed with a 0.5% oxine solution and then extracted into dichloromethane. This solution was then passed through a silica Sep-Pak cartridge which retains the relatively polar metal-oxinate complexes. These metal-oxine complexes were then eluted form the cartridge with 2.5 ml of methanol. Using this pre-concentration step, a 20 fold increase in peak height was possible. This preconcentration can be seen in Figure 20(a) and 20(b). In Figure 20(a), the peak at 9 min represents an aqueous injection of

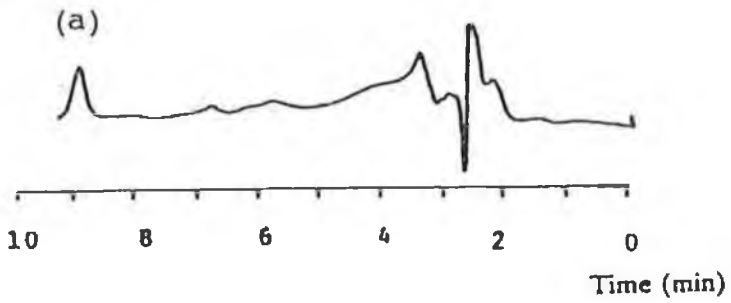


FIGURE 20(a); Direct injection of a 1 µg/ml Fe(III) solution. (a) Fe(III)-oxine complex. Mobile phase acetonitrile:0.02 M acetate buffer (pH 6.0) which was 5×10^{-3} M in oxine and 0.1 M in KNO_3 with UV detection at 400 nm.

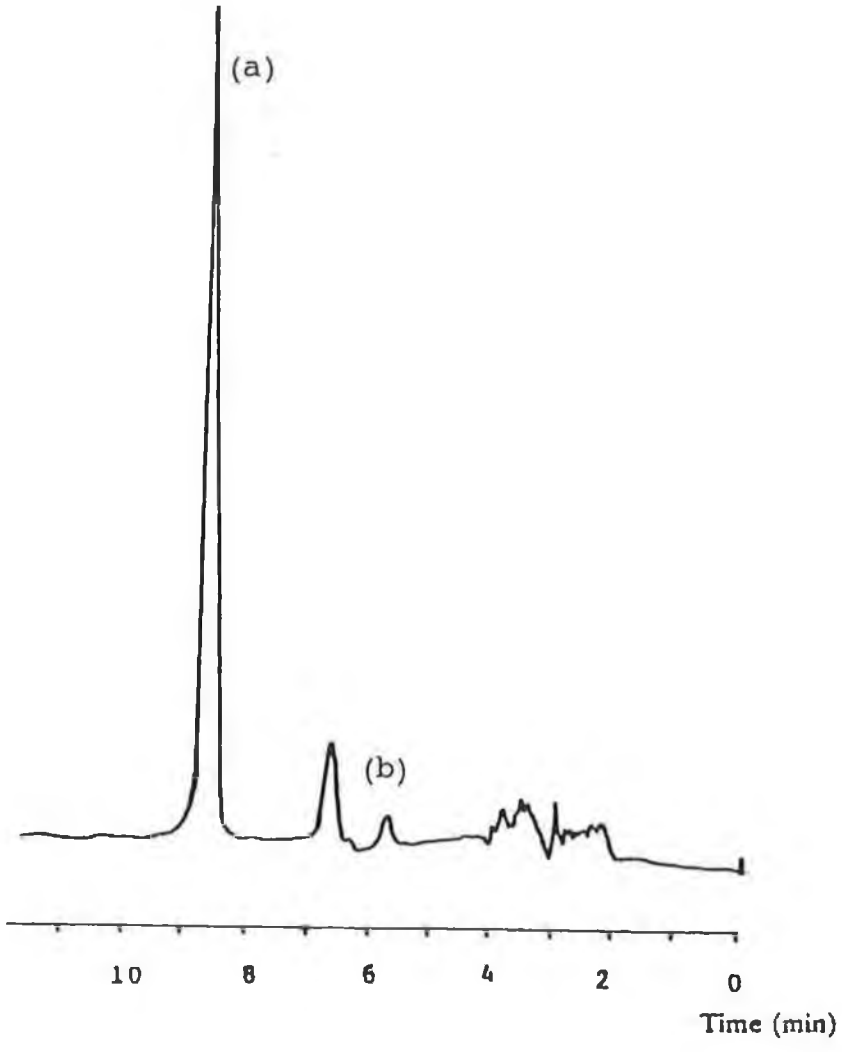


FIGURE 20(b) Chromatogram of a 1 µg/ml Fe(III) solution preconcentrated on a silica Sep-Pak cartridge. (b) Cu(II)-oxine (a) Fe(III)-oxine. Conditions as in FIGURE 21.

1 μg/ml Fe(III) solution. This can then be compared to Figure 20(b) which represents the preconcentration sample.

The use of this preconcentration step has a distinct advantage in that it improves the limit of detection for both Fe(III)-oxine and Cu(II)-oxine complexes. One distinct disadvantage of the preconcentration step is however, that it increases the background level of Fe(III) which is present in the deionised water. There also exists a trace amount of Cu(II) in deionised water. The concentration of Fe(III) present in the millipore water was approximately 0.6 μg/ml.

The problem associated with this background contamination due to Fe(III) is that it will cause quantitation errors when assaying the anaerobic adhesive raw materials for Fe(III) and Cu(II). To try and remove the Fe(III) present, the deionised water was mixed with a solution of oxine to try and remove the Fe(III) contamination and the deionised water was passed through an Amberlite ion exchange column. Neither of the above methods reduced significantly the level of Fe(III) contamination in the water.

A comparison was then made of the background concentration of Fe(III) in tap water, deionised water and HPLC grade water. The concentration of Fe(III) in each was almost the same which suggests that the purification methods used for both deionised and HPLC-grade water are not very efficient. The concentration of Cu(II) in the tap water was 20 times higher than both deionised and HPLC-grade water. The Cu(II) in tap water comes from the copper piping. The level of Cu(II) and Fe(III) contamination can be seen in Figure 21.

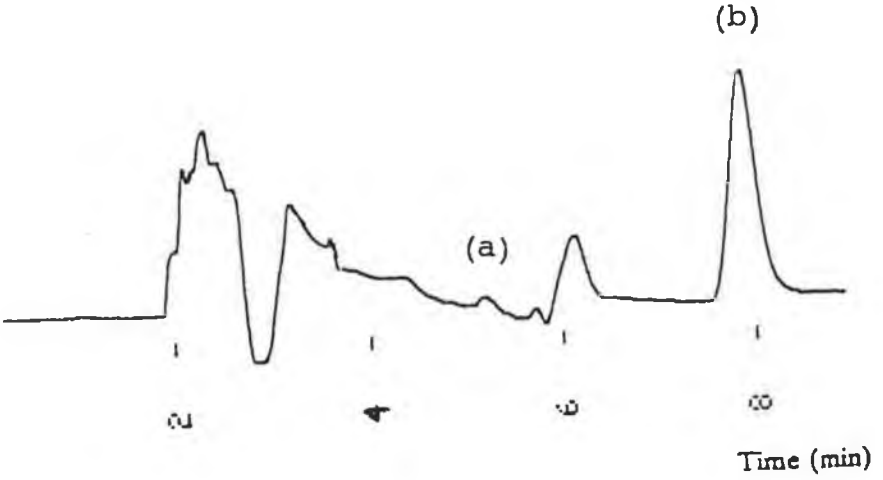


FIGURE 21; Chromatogram showing the background concentration of Cu(II) and Fe(III) present in deionised water. (a) Cu(II)-oxine (b) Fe(III)-oxine. Mobile phase acetonitrile:0.02 M acetate buffer (pH 6.0) which was 5 x 10^{-3} M in oxine and 0.1 M KNO_3 with a flow rate of 1.0 ml/min and UV detection at 400 nm.

B(iii). Determination of Cu(II) and Fe(III) in Anaerobic Raw Materials

In analysing the anaerobic raw materials for Cu(II) and Fe(III), the various components were added step by step in the correct proportions, and the mixture at each step was assayed for the concentration of metal ions, particularly Fe(III) and Cu(II), present.

The anaerobic raw materials were assayed initially to assess the concentration of both Cu(II) and Fe(III) present in the raw material. Then the raw materials were 'spiked' with a known concentration of Cu(II) and Fe(III) and this 'spiked' sample was extracted to determine the percentage recovery of both metal ions and determine the concentration of Fe(III) and Cu(II) in the raw materials as they were added together. To determine the average percentage recovery upon adding the various components to the raw material, an aqueous standard of the same concentration as the level 'spiked' into the raw material components was extracted.

The fully developed extraction scheme is outlined under Experimental. Initially, aqueous samples which were extracted gave clean chromatograms, and using this extraction it was possible to determine Zn(II), Cu(II) and Fe(III) as their oxinate complexes, but it was not possible to detect Cr(III) due to a large interfering peak. This can be seen in Figure 22.

Since a typical anaerobic adhesive formulation contains 50-90% of the appropriate monomer, initial studies were therefore directed to the efficient extraction of Fe(III) and Cu(II) from the monomers. The two basic monomers predominantly used are TRI-EGMA and PEGMA.

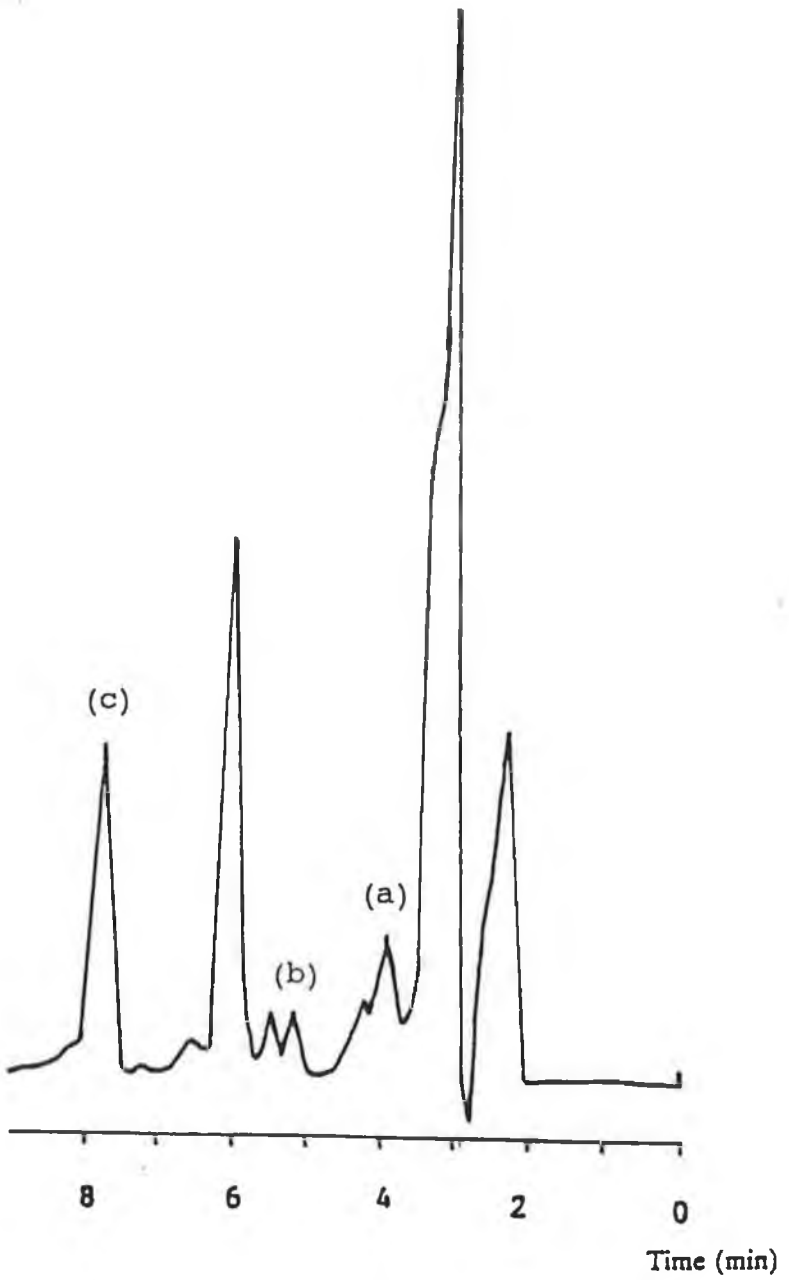


FIGURE 22 Separation of 0.5 µg/ml (a) Zn(II), (b) Cu(II), and (c) Fe(III) as their oxinate complexes on a Supelco LC-18-DB column. Conditions as FIGURE 21.

The extraction step involved mixing the monomer with 10 ml of 0.1 M HCl, extracting the metal ions into dichloromethane after forming neutral complexes with oxine, and then depositing the metal-oxine complexes on a silica Sep-Pak cartridge. However, in the case of PEGMA, this resulted in non-retainment of the metal-oxine complexes on the Sep-Pak cartridge.

To determine what was causing the non-retainment of the Cu(II)-oxine complex, the dichloromethane was evaporated down and the residue was reconstituted in 100 μl of hexane. This hexane fraction was analysed by gas chromatography. The results of this investigation are shown in Figure 23(a). This shows that some of the PEGMA monomer components have extracted into the 0.1M HCL and then into the dichloromethane. The monomer components extracted into the HCL due to the presence of polar groups, typically alcohol functional groups on the monomer.

With these monomer components extracted into the HCl then they dissolve in the dichloromethane along with the metal-oxine complex. The metal-oxine complexes are not therefore retained on the silica Sep-Pak but prefer to stay dissolved in the more non-polar dichloro-methane-monomer phase.

To overcome this non-retainment problem, the monomer, PEGMA, was dissolved in dichloromethane in a ratio of 1:4, (v/v) monomer: dichloromethane. This should result in less dissolution of the monomer components in the HCl phase. First dissolving the monomer in dichloromethane and then adding the HCl creates two distinct polarity phases, and the net result is that less of the monomer components were extracted into the 0.1M HCl. This can be seen in Figure 23(b).

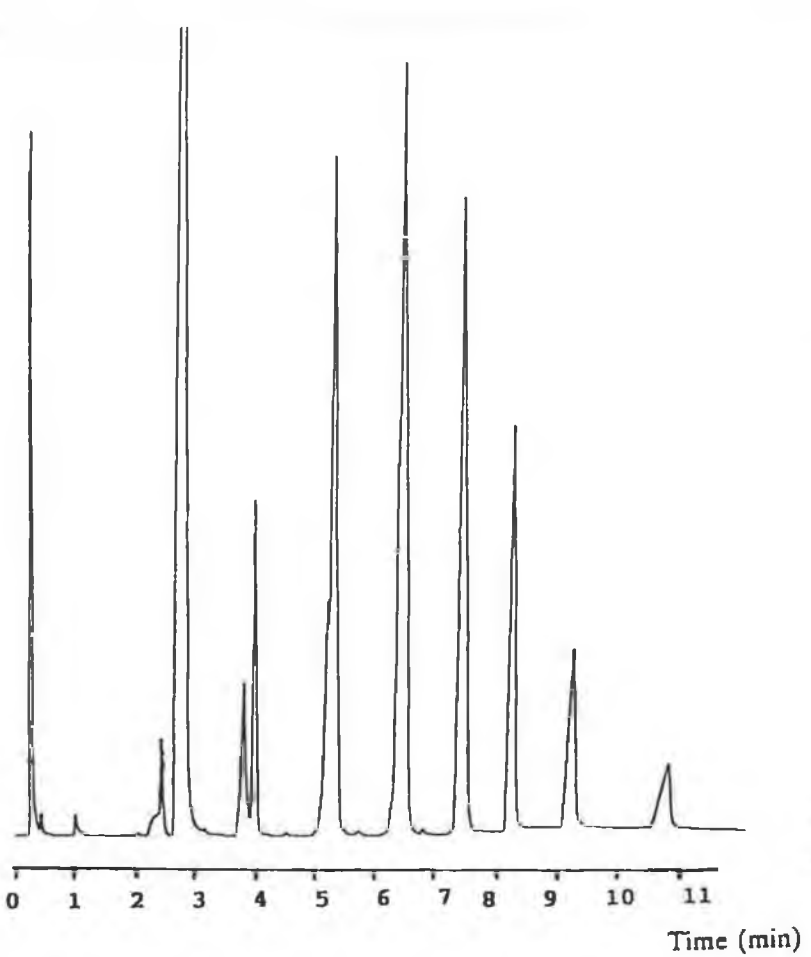


FIGURE 23(a); GC of PEGMA extract.

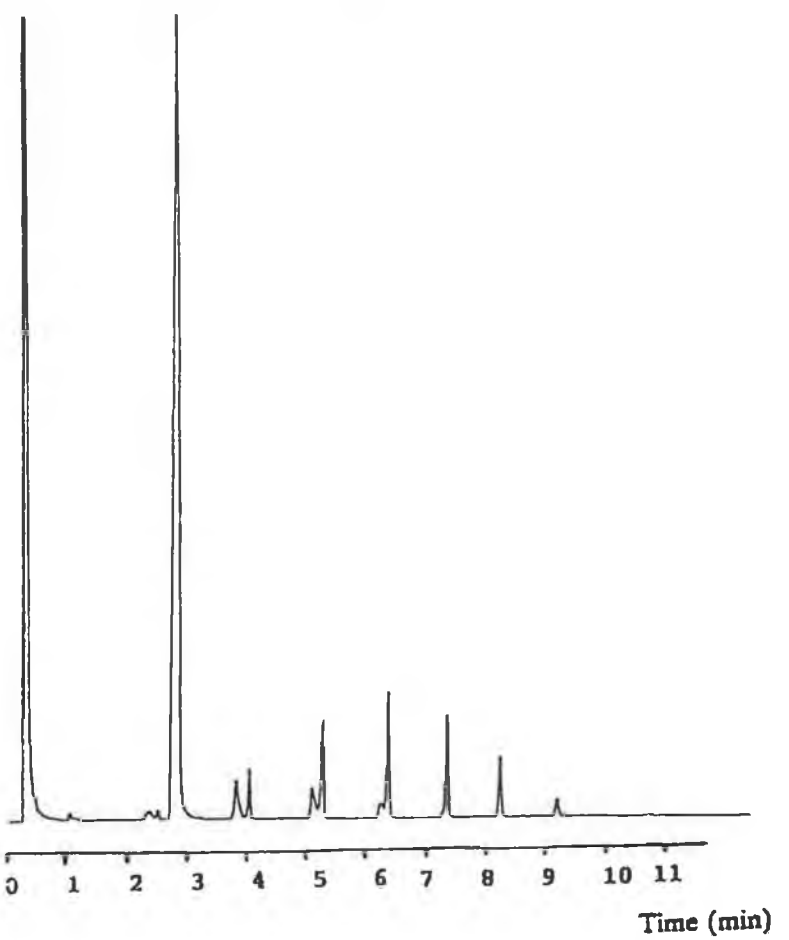


FIGURE 23(b); Chromatogram of PEGMA dissolved in dichloromethane prior to GC analysis.

In the case of TRI-EGMA it was necessary also to dissolve the monomer in dichloromethane in a ratio of 1:1, (v/v) monomer to dichloromethane. This co-extraction of monomer components was not as much of a problem with the TRI-EGMA, since it is predominantly one component. By dissolving both monomers in the dichloromethane prior to extraction an improvement was obtained in the percentage recovery of both Cu(II) and Fe(III) from the anaerobic products.

The extraction effeciencies for Cu(II) and Fe(III) were evaluated at the 1 μg/ml level and are listed in Table 7.

TABLE 7. EXTRACTION EFFICIENCIES FOR Cu(II) AND Fe(III) FROM PEGMA AND TRI-EGMA

MONOMER	METAL ION	EXTRACTION EFFICIENCY (%)
TRI-EGMA	Cu(II)	78 ± 3
TRI-EGMA	Fe(III)	100 ± 5
PEGMA	Cu(II)	85 ± 3
PEGMA	Fe(III)	100 ± 5

The recovery of Cu(II)-oxine from both of the monomers is less than the recovery of Fe(III)-oxine. The Fe(III)-oxine complex is more non-polar than the Cu(II)-oxine complex. This is shown by the retention times on a C_{18} column, and due to this factor the Cu(II)-oxine complex is not completely extracted into dichloromethane. This accounts for the slight loss in recovery of the Cu(II)-oxine complex.

At this point in the research it was necessary to determine the limit of detection of the procedure. For PEGMA, the limit of detection for Cu(II) and Fe(III) as extracts, were 250 μg/ml and 600 μg/ml and in TRI-EGMA were 100 μg/ml and 250 μg/ml respectively. When compared to the limits of detection for aqueous standards, the

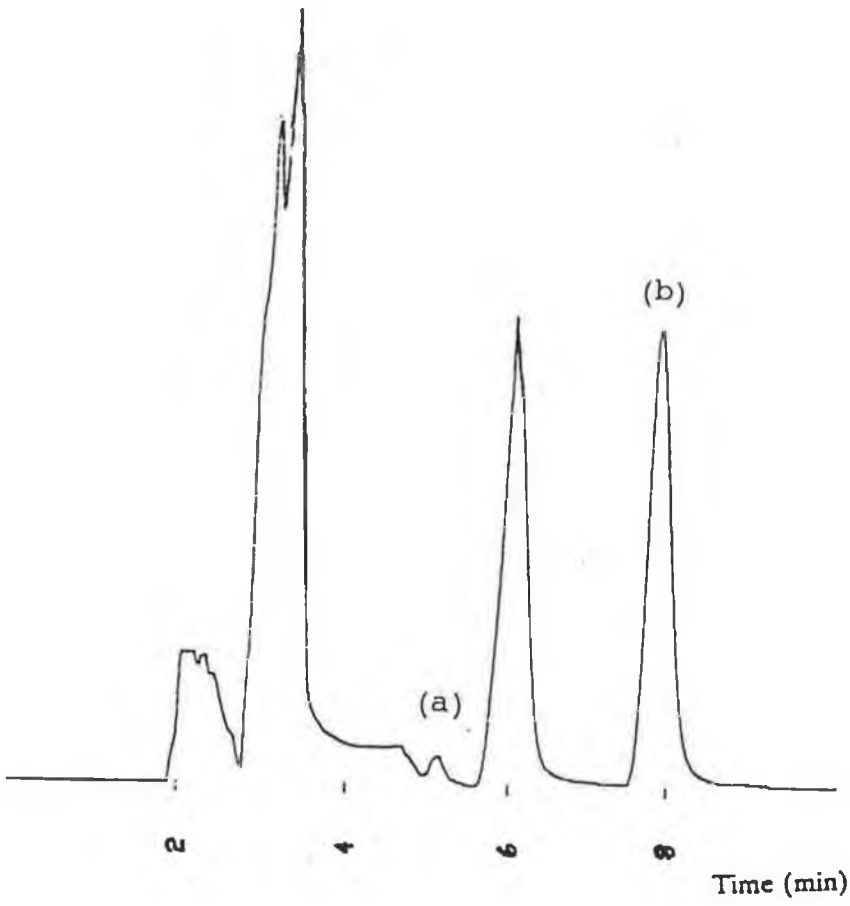


FIGURE 24; Chromatogram of the background level of (a) Cu(II)-oxine and (b) Fe(III)-oxine extracted from TRI-EGMA. Conditions as in FIGURE 21.

preconcentration step significantly improves the limit of detection.

A chromatogram of a typical extract of TRI-EGMA is shown in Figure 24. This extract is of the monomer with no Cu(II) or Fe(III) 'spiked' into it. A small peak at 5.0 min is due to Cu(II) present in the monomer extract. This small concentration is mainly due to the presence of Cu(II) in the monomer, but a small contribution comes from the deionised water, as was discussed earlier. The rather large peak at 8 min is due mainly to the Fe(III) contamination present in the deionised water used to prepare the 0.1 M HCl. As stated earlier, it was not possible to remove this background concentration of Fe(III) but it can be subtracted from all samples 'spiked' with Fe(III) from now on.

The monomers (PEGMA and TRI-EGMA) were both 'spiked' with 2.5 μg/ml of each metal ion and extracted. This extraction of Cu(II) and Fe(III) was used to determine extraction efficiencies for the monomer, and also to give the efficiencies as the various components were added to the monomer. A typical 'spiked' sample of monomer, containing 2.5 μg/ml Cu(II) and Fe(III) is shown in Figure 25. A peak at 6.0 min present in the chromatogram is due to oxine present in the uncomplexed form.

In order to investigate the influence of the other constituents of a typical anaerobic adhesive on the extraction of Cu(II) and Fe(III) from TRI-EGMA- and PEGMA-based formulations, test formulations were prepared based on Tri-EGMA or PEGMA as the monomer and adding the individual constituents in a step-wise procedure, at the concentration level typical of a final product, and then extracting the metal ions Cu(II) and Fe(III) at each stage.

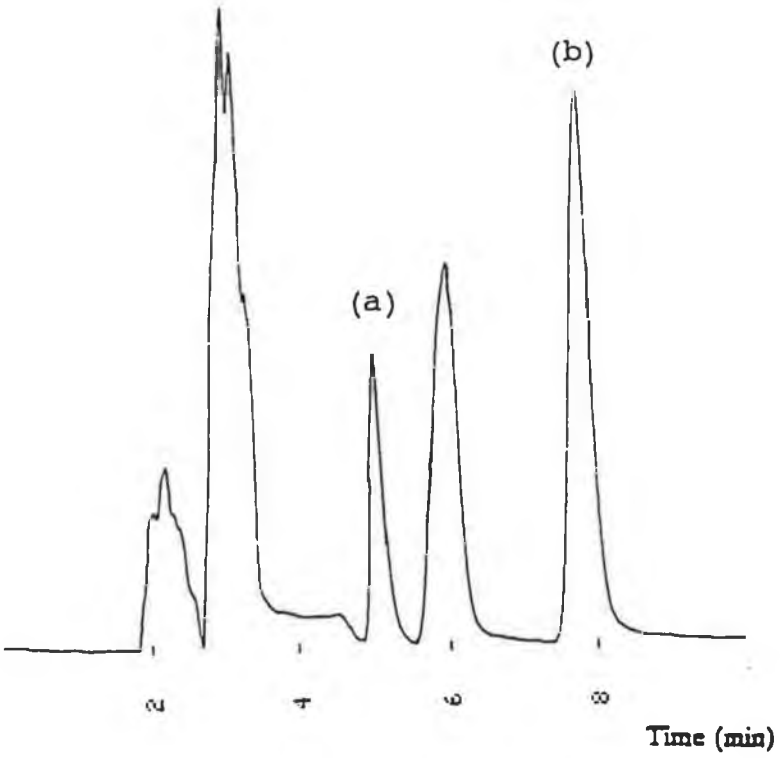


FIGURE 25; Chromatogram of TRI-EGMA which was `spiked' with 2.5 µg/ml of both Cu(II) and Fe(III). (a) Cu(II)-oxine (b) Fe(III)-oxine. Chromatographic parameters as in FIGURE 21.

By adding the various constituents to the monomer and extracting, it was possible to assess two important factors, these being;

(i) changes in percentage recovery as the constituents were added.

(ii) the introduction of Cu(II) and Fe(III) to the formulation by the raw materials.

The second point is of more importance since the introduction of these metal ions by the raw materials will cause premature polymerisation of the adhesive.

For Cu(II), the extraction efficiency from the 'spiked' TRI-EGMA was 78%. As the components were added to the monomer the extraction efficiency decreased by some 25-30%. This decrease was not as pronounced in PEGMA based formulations. This loss in extraction efficiency can be seen in Figure 26 where concentration (μg/ml) of metal vs added components is plotted. In adding the raw materials step-wise to TRI-EGMA, the Cu(II)-oxine complex is somehow complexed chemically or physically bound to the anaerobic adhesive formulation and hence not extracted. The background level of Cu(II) is approximately 0.4 μg/ml. The slight increase in extraction efficiency of Cu(II) from PEGMA was due to the addition of trace amounts of Cu(II) being introduced by the raw materials as they were added.

A comparison of the extraction of the final product (formulated in the laboratory) and the same product 'spiked' with Cu(II) and Fe(III) can be seen in Figure 27(a) and 27(b). This chromatogram shows a large increase in peak height for both Cu(II) and Fe(III) in the final product formulation.

This kind of increase in peak height due to Cu(II) and Fe(III)

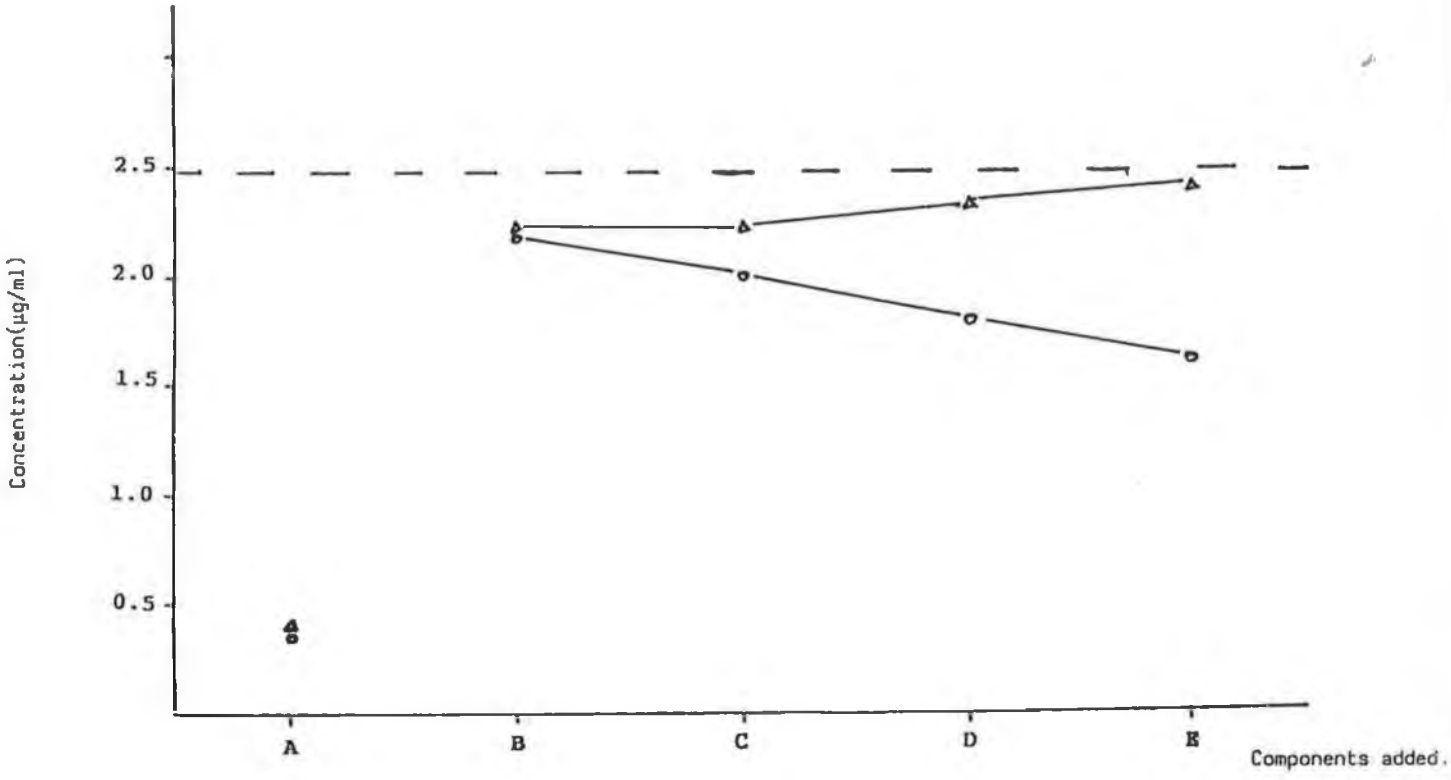


FIGURE 26; Plot of concentration (µg/ml) Cu(II) vs component added to monomer.

A = monomer, B = A + spike, C = B + cumene hydroperoxide
D = C + thickener, E = D + thixotrope

○ = TRI-EGMA

△ = PEGMA

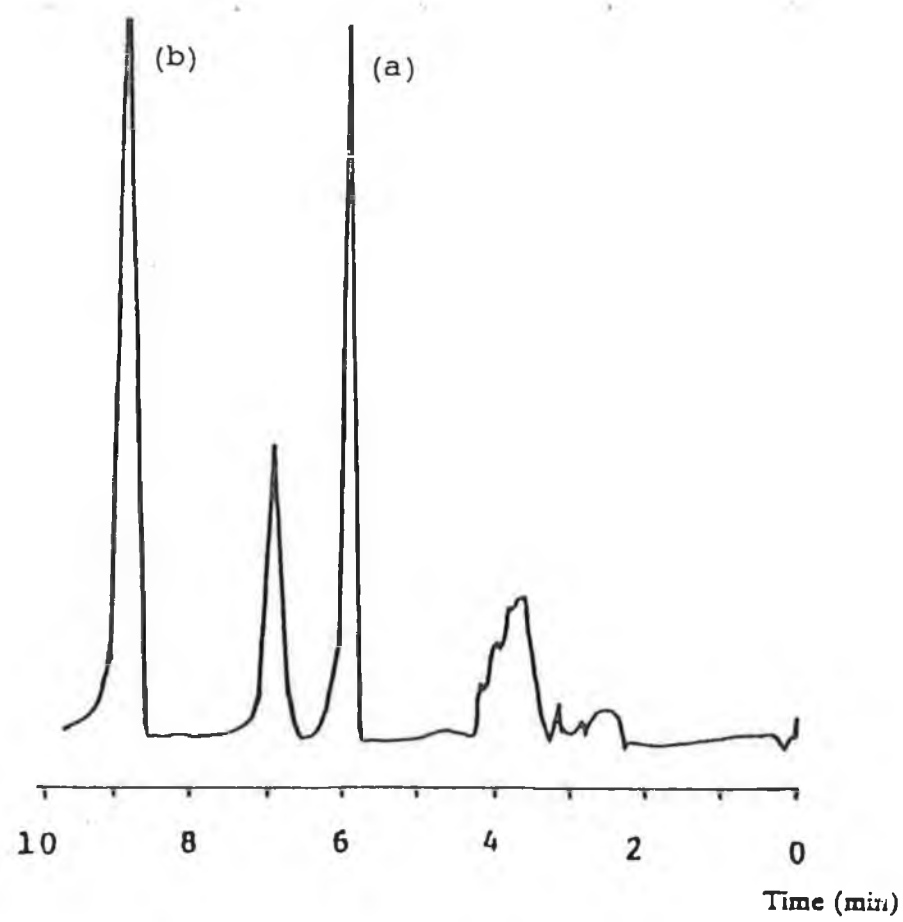


FIGURE 27(b); Chromatogram of final product formulated in the laboratory, 'spiked' with 2.5 µg/ml of (a) Cu(II)-oxine and (b) Fe(III)-oxine. Chromatographic conditions as in FIGURE 21.

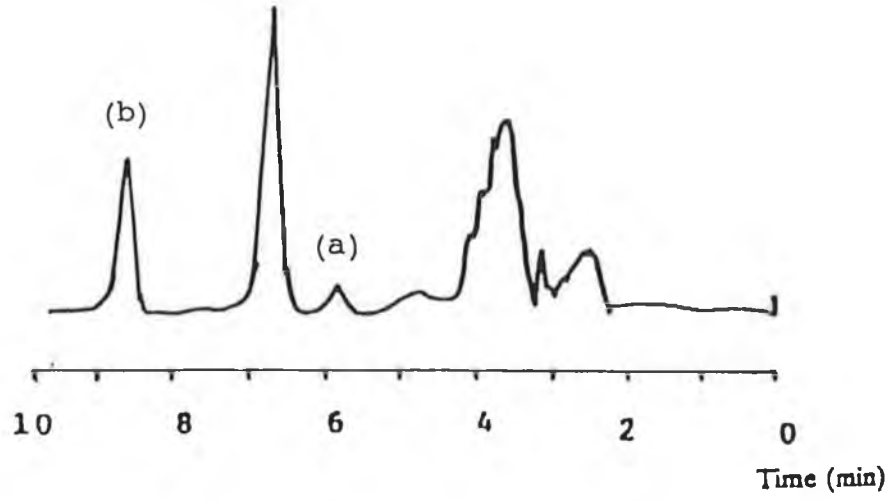


FIGURE 27(a); Chromatogram of final product formulated in the laboratory showing (a) Cu(II)-oxine and (b) Fe(III)-oxine. Mobile phase and chromatographic parameters as in FIGURE 21.

'spiked' into the formulation as the constituents were added was typical of the extraction method.

The situation for the extraction of Fe(III) from both PEGMA and TRI-EGMA was somewhat different. The extraction efficiency of Fe(III) from PEGMA and TRI-EGMA was 100% (±5%) in both cases. As the various constituents were added to TRI-EGMA the extraction efficiency decreased by 25-30%, again indicating that Fe(III) is being bound chemically or physically by the TRI-EGMA. This loss in recovery must be considered in the analysis of a product which is TRI-EGMA based.

The extraction of Fe(III) from PEGMA showed a similar trend to the extraction of Cu(II) from PEGMA (Figure 28). The recovery of Fe(III) from PEGMA was initially at the 100% (±5%) level, and as the constituents were added, the levels of Fe(III) present showed an increase. This increase in the level of Fe(III) comes from the raw materials which were added to PEGMA, step-by-step and then extracted. This again indicates how metal ions are introduced into the formulation of a typical anaerobic adhesive.

One of the raw materials listed in Table 3, page 10, was not yet added into the formulations. This constituent was the chelating agent ethylenediaminetetraacetic acid tetrasodium salt (NaEDTA). This chelating agent is added to a typical anaerobic adhesive to complex the metal ions, mainly Cu(II) and Fe(III), which, as we have seen, get introduced into the formulation at the production stage. The concentration of EDTA added to a typical anaerobic adhesive is in the range 400-800 µg/ml. The actual solubility of EDTA in an anerobic adhesive is only 20-25 µg/ml. This low level of solubility was to be expected, since EDTA is a relatively polar compound. Since EDTA forms

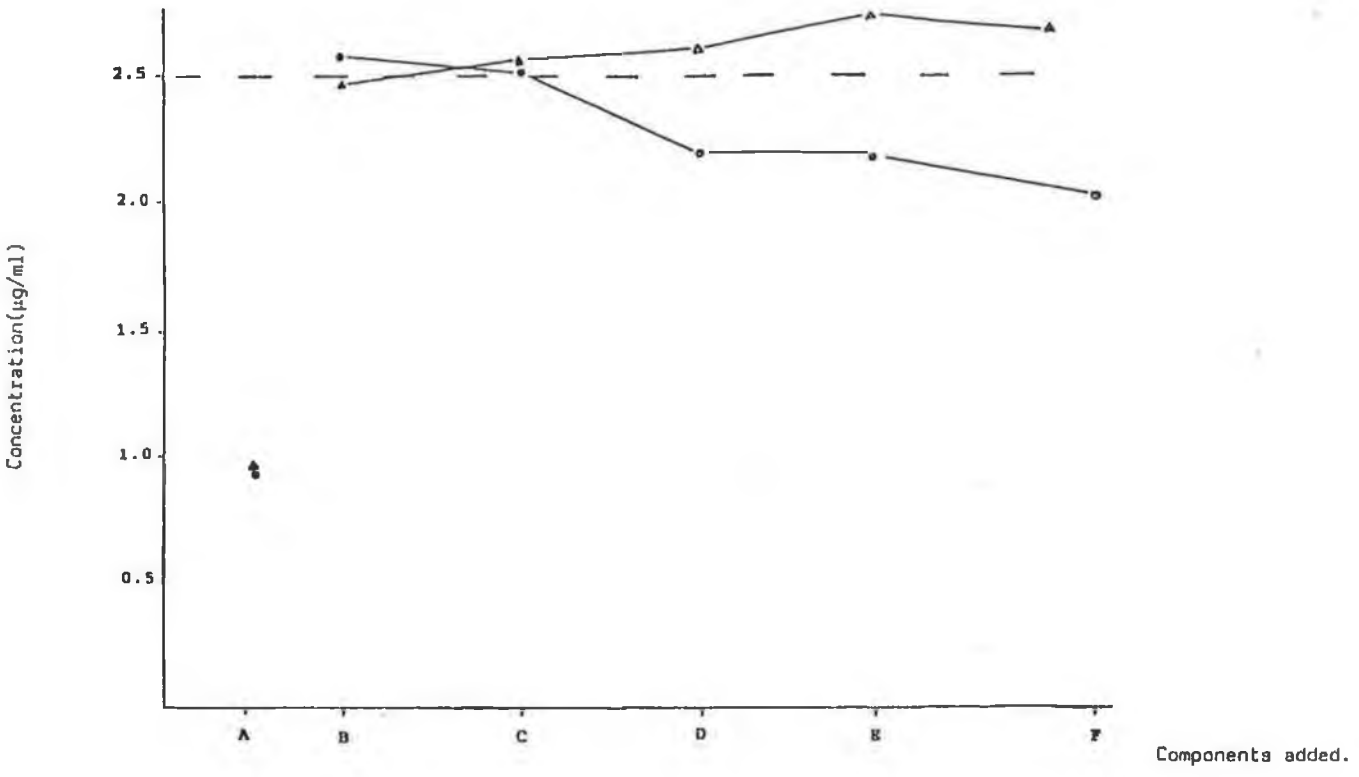


FIGURE 28; Plot of concentration (µg/ml) Fe(III) vs component added to monomer.

A = monomer, B = A + spike, C = B + cumene hydroperoxide
D = C + thickener, E = D + saccharin, F = E + thixotrope

○ = TRI-EGMA

△ = PEGMA

1:1 complexes with most metal ions, it can only tolerate a concentration of Cu(II) and/or Fe(III) in the adhesive at or below the solubility level of 20-25 µg/ml.

To evaluate the usefulness of EDTA in a typical formulation, a series of experiments were performed where EDTA was added at a concentration of 400 µg/ml and the Cu(II) was 'spiked' in at levels between 1.0 µg/ml and 50 µg/ml. It was not until the concentration of metal ions in the adhesive increased above 20-25 µg/ml that the metal ions were detected. At concentrations less than 20 µg/ml, the metal ions Cu(II) and Fe(III) were only present at their respective background concentrations. Figure 29 shows a typical final formulation containing EDTA which was 'spiked' with 10 µg/ml of both Cu(II) and Fe(III). Upon the addition of the metal ions and then extracting, no increase in concentration was recorded due to the presence of EDTA in the formulation. A rather large peak appeared, however, at 3.0 min. This peak had not appeared in any of the chromatograms to date, so it was determined that this peak was due to EDTA or some EDTA complex.

The addition of EDTA stabilises the anaerobic adhesive and stops premature polymerisation of the product. In the chromatograms, there were no peaks which interfered with the metal ions Cu(II) and Fe(III) as their oxinate complexes. This is one of the advantages of the long extraction step in that it also acts as a sample clean-up procedure as well as an extraction/pre-concentration step.

It was observed that some raw materials introduced rather a high concentration of Cu(II) and Fe(III) into the test anaerobic adhesive formulated in the laboratory. For example, two types of silica are used as fillers for anaerobic adhesives. They are (i) fumed silica

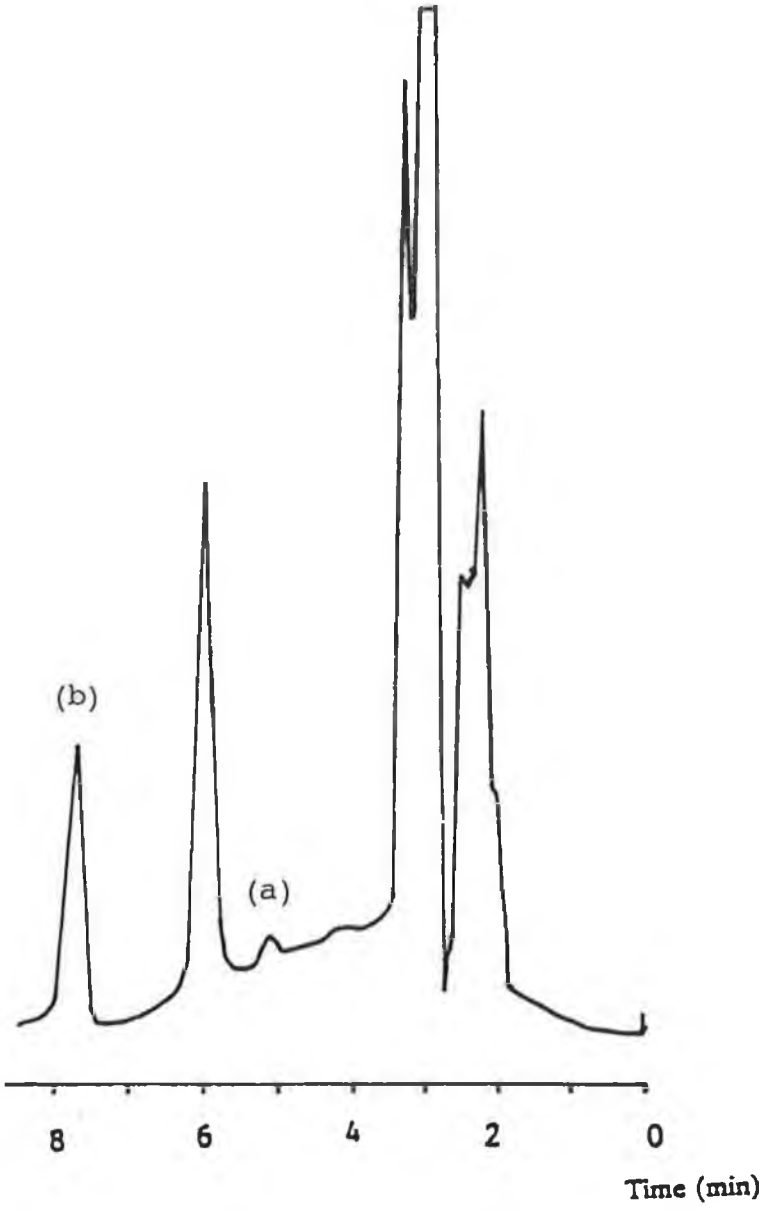


FIGURE 29; Chromatogram of final product containing 400 µg/ml of EDTA and spiked with 10 µg/ml of (a) Cu(II) and (b) Fe(III). Chromatographic parameters as in FIGURE 21.

and (ii) hydrophobic silica. The test anaerobic adhesive formulated was prepared with fumed silica and another with hydrophobic silica before they were extracted. Both formulations were 'spiked' with Cu(II) and Fe(III) at the same concentration (i.e. 2.5 μg/ml). The results can be seen in Figure 30(a) and 30(b). It was noted that the hydrophobic silica contained rather higher concentrations of Cu(II) and Fe(III); typically in the order of 0.5 μg/ml of each.

This introduction of Cu(II) and Fe(III) into the adhesive by the raw material will cause obvious deleterious effects. This indicates the importance in a method to quantify the concentration of metal ions in the raw materials and the finished products.

The next stage was to extract Cu(II) and Fe(III) from a anaerobic adhesive formulated in the plant. Two typical finished products were analysed; these were Product 270 and Product 290. Both of these formulations are PEGMA-based anaerobic adhesives and contained the chelating agent EDTA plus some dye stuffs. The difference between these two products is due to the fact that Product 270 contains a filler, making it more viscous than Product 290.

Since the two products contain EDTA, by 'spiking' them with Cu(II) or Fe(III) at concentrations less than 20-25 μg/ml it was not possible to detect any increase in the levels of these metal ions above the background concentrations. The fact that the metal ions 'spiked' into the products were not detected by extracting as their oxine complexes proves that the method developed is specific for uncomplexed metal ions in anaerobic adhesives. The metal ion complexes of EDTA are not broken down by the extraction scheme and release the bound metal ions into the anaerobic adhesive. This proves

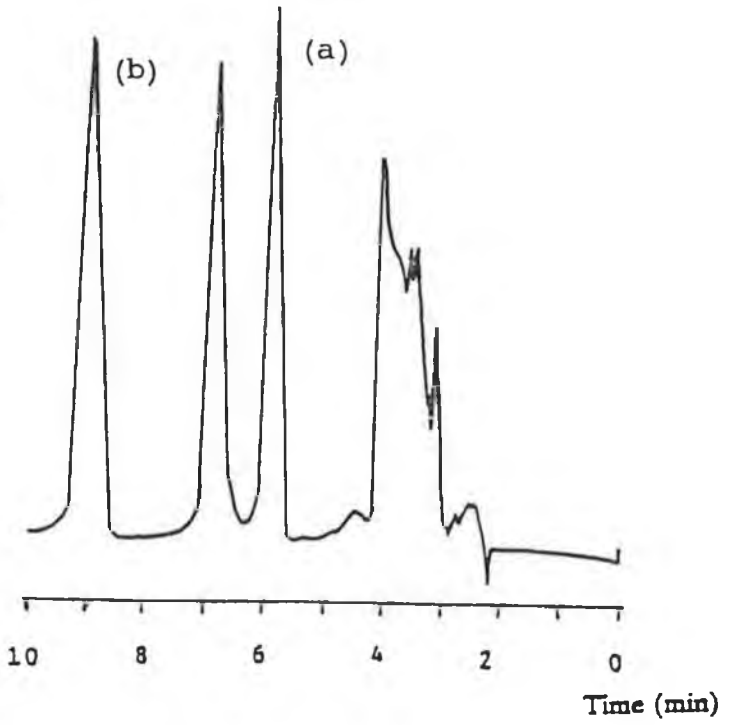


FIGURE 30(a); Chromatogram of final product (laboratory formulation) containing fumed silica and spiked with 2.5 µg/ml of (a) Cu(II) and (b) Fe(III). Chromatographic parameters as in FIGURE 21.

FIGURE 30(b); Chromatogram of final product (laboratory formulation) containing hydrophobic silica and spiked with 2.5 µg/ml of (a) Cu(II) and (b) Fe(III). Chromatographic parameters as in FIGURE 21.

that the EDTA added to the anaerobic adhesives is functioning efficiently, and also proves the selectivity of the method developed to determine 'free' metal ions in anaerobic adhesives and raw materials used in their formulation.

Typical chromatograms for Loctite Products 290 and 270 can be seen in Figures 31 and 32 respectively. These chromatograms of Product 270 and Product 290 were both spiked with 10 μg/ml of Cu(II) and 10 μg/ml of Fe(III). The concentration of the two metal ions in the 'spiked' products was slightly above the expected background concentration. This indicates that the adhesive is at a saturation level for metal ions. Most of the Cu(II) and Fe(III) ions which were 'spiked' into the adhesive were chelated by the soluble EDTA, but the system had become saturated and the 'free' metal ions were extracted and detected. It is at this point that the problems arise. These 'free' metal ions in the adhesive can trigger the reactions which lead to polymerisation of the anaerobic adhesive.

Finally, another important metal ion to anaerobic adhesive chemistry is Mn(II). The possibility of determining Mn(II)-oxine by RP-HPLC was investigated. The use of the acetonitrile: acetate buffer (pH 6.0) mobile phase proved unsuccessful, and no peak was detected for Mn(III)-oxine by in-situ formation. In a publication by Hoffman et al. (45), the Mn(II)-oxine complexes formed in a solution of oxine (0.1%, v/v) which was at pH 9.0 employing a borate buffer system. Using 40 mls. of 0.1 M borate buffer (pH 8.6) it was possible to form oxine complexes of a number of metal ions, namely Fe(III), Cu(II), Co(III) and Mn(II). The mobile phase was the same as was used to separate Cu(II) and Fe(III) in the anaerobic adhesive extraction

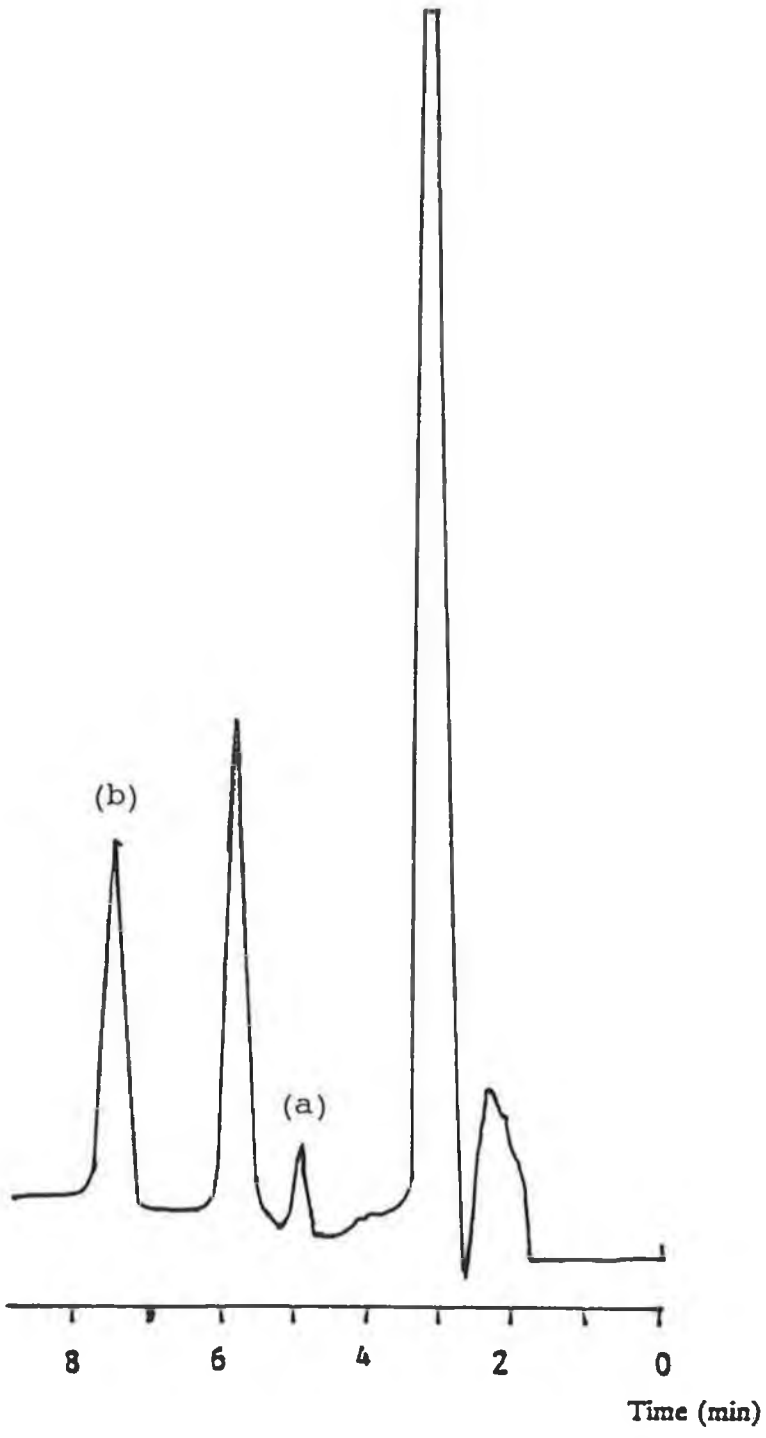


FIGURE 31; Chromatogram of Loctite product 290 with (a) Cu(II)-oxine and (b) Fe(II)-oxine present. Mobile phase, acetonitrile: 0.02 M acetate buffer (pH 6.0) which was 5 x 10^{-3} M in oxine and 0.1 M in KNO_3. Separation on a Supelco LC-18-DB column with UV detection at 400 nm.

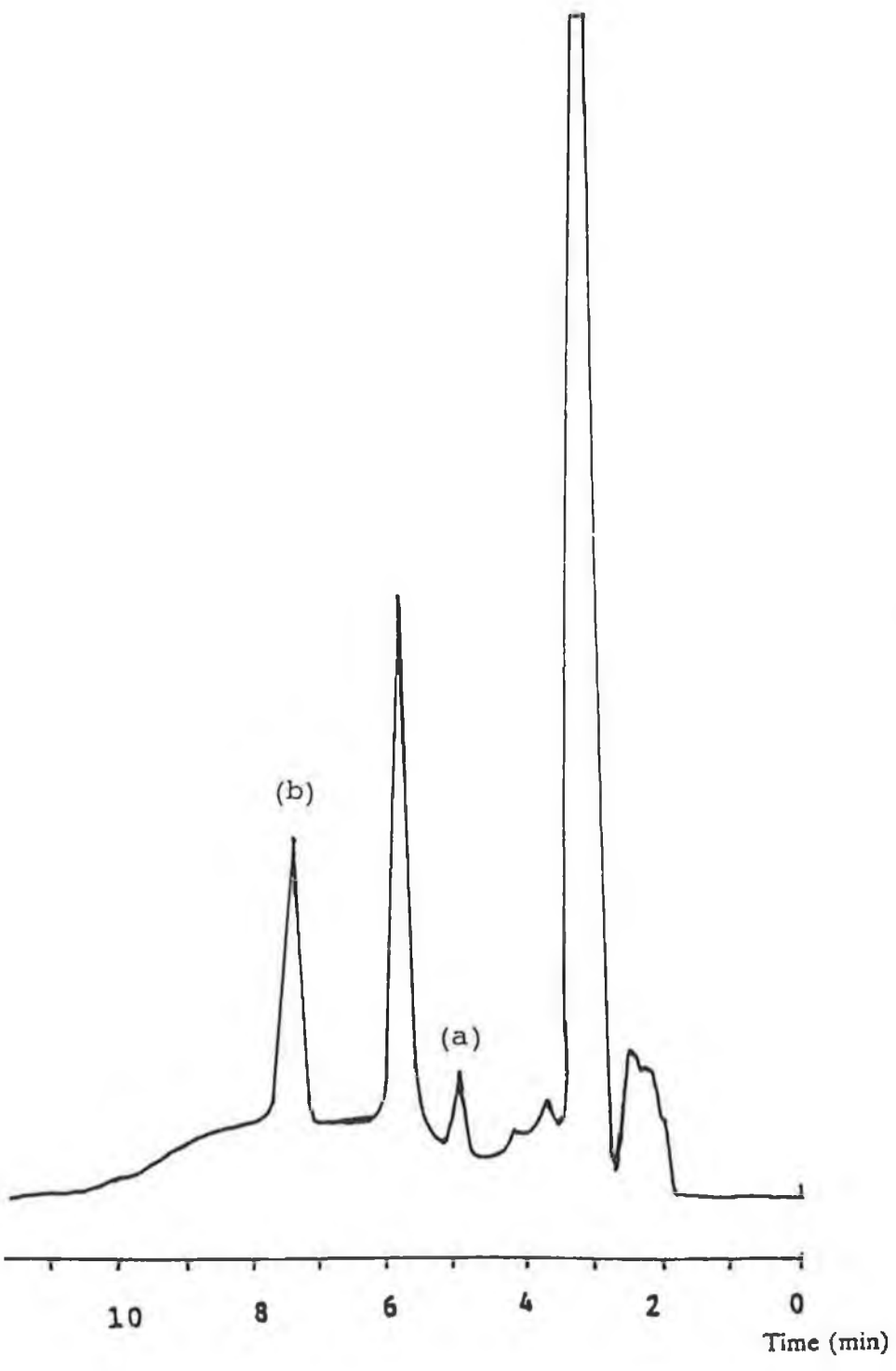


FIGURE 32 ; Chromatogram of Loctite product 270 showing the presence of (a) Cu(II)-oxine and (b) Fe(III)-oxine. Chromatographic parameters as in FIGURE 21.

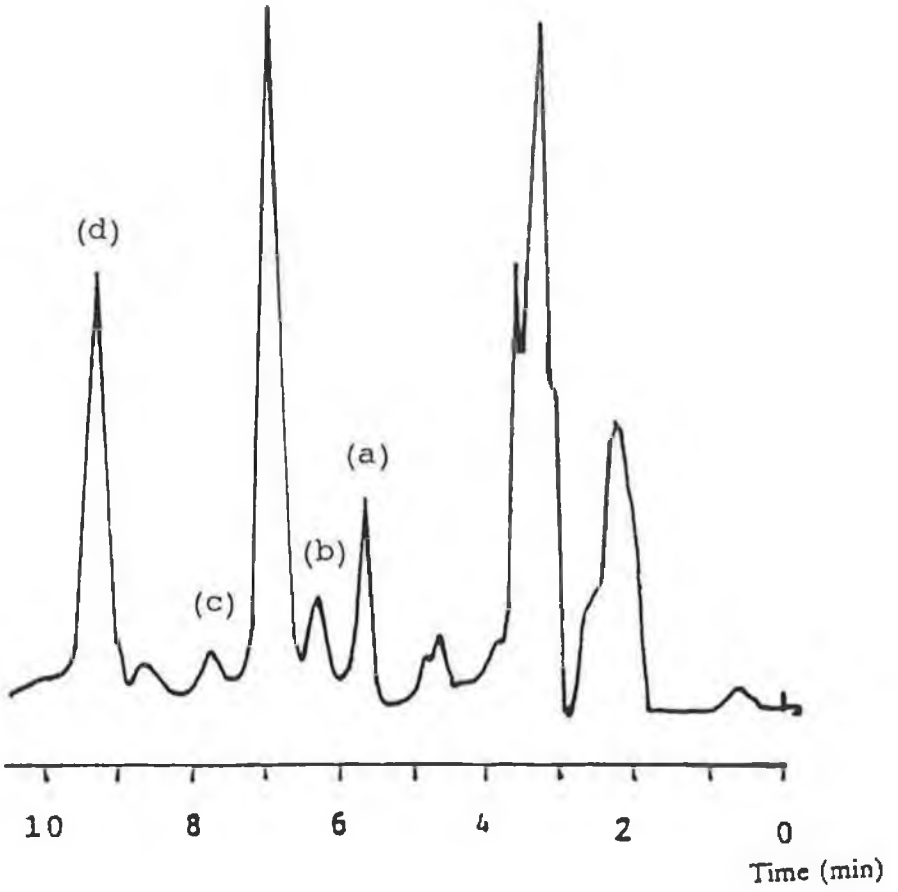


FIGURE 33; Separation of (a) Cu(II), (b) Co(III), (c) Fe(III), and (d) Mn(II) as their oxinate complexes. Complexes formed in borate buffer. Chromatographic conditions as in FIGURE 21.

samples.

The adjustments were made to the extracting solution, instead of using 0.1 M HCl a 0.1 M borate buffer (pH 8.6) was used which was 0.1% oxine. This borate system facilitated the formation of Mn(II) and Co(III) at higher pH values. Figure 33 shows the separation of (i) Cu(II)-oxine (ii) Co(III)-oxine (iii) Fe(III)-oxine and (iv) Mn(II)-oxine in one single injection by pre-forming the complexes.

Using the borate buffer system the oxinate complexes of Zn(II) and Ni(II) did not form. The column used was the Supelco LC-18-DB column with a mobile phase of acetonitrile: 0.02 M acetate buffer (pH 6.0) which was 5 $x10^{-3}$M in oxine and 0.1 M in KNO_3. This demonstrates the versatility of oxine as a chelating agent of metal ions followed by RP-HPLC.

CONCLUSION

It has been shown that by using RP-HPLC following the extraction/ preconcentration step outlined it was possible to determine the concentrations of Fe(III) and Cu(II) in anaerobic adhesives and the raw materials used in their formulation. Using a mobile phase of acetonitrile: 0.02M acetate buffer (pH 6.0) which was 5×10^{-3}M oxine and 0.1 M KNO_3 separation of Fe(III) and Cu(II), by 3 min, was achieved on a Supelco LC-18-DB with UV detection at 400 nm.

The method developed here is specific for 'free' metal ions present in the adhesive formulation. The EDTA present in the formulations complexes metal ions up to a concentration of 20-25 µg/ml. The EDTA metal ion complexes are more stable than the oxine complexes and so do not degrade and release the metal ions into the adhesive and cause polymerization.

The method developed is sensitive, due to the preconcentration step and selective in that it determines 'free' metal ions and not those complexed by EDTA in anaerobic adhesive formulations.

REFERENCES

1. G. Drasch, L. Von Meyer and G. Kavert, Z. Anal. Chem., 1982, 311, 695.

2. G. Schwedt, Chromatographia, 1978, 11, 145.

3. G. Schwedt, Z. Anal Chem., 1978, 288, 50.

4. G. Schwedt, Chromatographia, 1979, 12, 289.

5. G. Schwedt, Z. Anal. Chem., 1979, 295, 382.

6. P.C. Uden and I.E. Bigley, Anal. Chim. Acta., 1977, 94, 29.

7. O. Liska, J. Lethotay, G. Guiochon and H. Colin, J. Chromatogr., 1979, 172, 379.

8. J. Lehotay, O. Liska, E. Brandsteterova and G. Guiochon, J. Chromatogr., 1979, 172, 379.

9. O. Liska, J. Lehotay, E. Brandsteterova and G. Guiochon, J. Chromatogr., 1979, 171, 145.

10. O. Liska, G. Guiochon and H. Coltn, J. Chromatogr., 1979, 171, 145.

11. P. Heizmann and K. Ballschmiter, J. Chromatogr., 1977, 137, 153.

12. M. Moriyasu and Y. Hashimoto, Anal. Lett., 1978, A11, 593.

13. J. O'Laughlin and T. O'Brien, Anal. Lett., 1978, 11, 829.

14. T. Tande, J. Pettersen and T. Torgrimsen, Chromatographia, 1980, 13, 607.

15. R. Smith, A. Butt and A. Thakur, Analyst, 1985, 110, 35.

16. R. Smith, R. Morarji, W. Salt and R. Stretton, Analyst, 1980, 105, 184.

17. R. Smith, Anal. Proc., 1980, 21, 73.

18. R. Smith and E. Yankey, Analyst, 1982, 107, 744.

19. R. Smith, R. Morarji and W. Salt, 1981, 106, 129.

20. A. Bond and G. Wallace, Anal. Chem., 1981, 53, 1204.

21. A. Bond and G. Wallace, Anal. Chem., 1982, 54, 1706.

22. A. Bond and G. Wallace, Anal. Chem., 1983, 55, 718.

23. A. Bond and G. Wallace, Anal. Chim. Acta., 1984, 164, 223.

24. A. Bond and G. Wallace, J. of L. Chromatogr., 1983, 6(10), 1799.

25. E. Edward-Inatimi, J. Chromatogr., 1983, 256, 253.

26. S. Hutchins, P. Haddad and S. Dilli, J. Chromatogr., 1982, 252, 185.

27. B. Wenclawiak, P. Schultze and V. Vegts, Mikrochimica. Acta., 1984, 11, 251.

28. Lajuneu Lauri, E. Erkki and N. Pirjo, Finn. Chem. Lett., 1984, 146.

29. S. Ichonoki, and M. Yamazaki, Anal. Chem., 1985, 57, 2219.

30. Y. Shih and P. Carr, Anal. Gutm. Acta., 1982, 142, 55.

31. K. Jinno and H. Tsuchida, Anal. Lett., 1982, 15(A5), 427.

32. G. Kirkbright and F. Mullins, Analyst, 1984, 109, 493.

33. B. Mueller and R. Lovett, Anal. Chem., 57, 2693.

34. M. Lohmuller, P. Heizmann and K. Ball Schmiter, J. Chromatogr., 1977, 137, 165.

35. P. Hobbs, P. Jones and L. Ebdon, Anal. Proc., 1983, 20, 613.

36. P. Hendersen, R. Chaffee and F. Novak, J. Chromatogr. Sci., 1981, 19, 79.

37. K. Oshashi, S. Iwai and M. Horiguchi, Bunseki Kagaku, 1982, 31, E285.

38. A. Berthod, M. Kolosky and O. Vittori, Analusis, 1979, 7, 395.

39. C. Hambili and P. Haddad, Chromatographia, 1980, 13, 633.

40. B. Wenclawiak, Z. Anal. Chem., 1981, 308, 120.

41. B. Hoffman and G. Schwest, HRC and CC, 1982, 5, 439.

42. P. Jones, K. Barron and L. Ebdon, Anal. Proc., 1985, 22, 373.

43. A. Bond and Y. Nagaosa, Anal. Chim. Acta., 1985, 178, 197.

44. J. Huber, J. Kraak and Veening, Anal. Chem., 1972, 44, 1544.

45. C. Tollinche, and T. Risby, J. Chromatogr. Sci., 1978, 10, 448.

46. H. Noda, K. Saitoh and N. Sueuki, Chromatographia, 1981, 14, 189.

47. P. Uden, I. Bigley and F. Walters, Anal. Chim. Acta., 1978, 100, 555.

48. P. Uden, D. Parees and F. Walters, Anal. Lett., 1975, 8, 795.

49. K. Saitoh, M. Satoh and N. Suzuki, J. Chromatogr., 1974, 92, 291.

50. S. Valenty and P. Behnken, Anal. Chem., 1978, 50, 834.

52. E. Gaetani, C. Laureri, A. Mangia and G. Parolari, Anal. Chem., 1976, 48, 1725.

53. J. O'Laughlin and R. Hanson, Anal. Chem., 1980, 52, 2263.

54. J. O'Laughlin, Anal. Chem., 1982, 54, 178.

55. H. Yoneda, C. OH, and S. Yamazaki, Bull. Chem. Soc. Jap., 1980, 53, 2403.

56. A. Mangia and M. Lugari, J. Liq. Chromatogr., 1976, 13, 122.

57. A. Mangia, G. O Parolari, E. Gaetani and C. Laureri, Anal. Chim. Acta., 1977, 92, 111.

58. K. Kimura and T. Shono, J. Liq. Chromatogr., 1982, 5, 223.

59. D. Troutner, J. Simon, A. Ketring, W. Volkert and Holmes, J. Nucl. Med., 1980, 21, 443.

60. T. Hoffman, W. Volkert, D. Troutner and R. Holmes, J. of App. Rad. and Isotopes, in Press, 1983.

61. D. Royor, G. Grant, D. Van Derveer and M. Cartillo, Inorg. Chem., 1982, 21, 1902.

62. M. Richter and K. Rientits, FEBS Lett, 1980, 116, 211.

63. S. Hajibrahim, P. Tibbetts, C. Watts, J. Maxwell, G. Eglinton, H. Collin and G. Guiochon, Anal. Chem., 1978, 50, 549.

64. W. Spencer, J. Galobardes, M. Curtis and L. Rogers, Sep. Sci. and Tech., 1982, 17, 797.

65. T. Pinkerton, W. Heineman and E. Deutsch, Anal. Chem., 1980, 52, 1106.

66. J. O'Laughlin and D. Jensen, J. Chromatogr., 1969, 32, 567.

67. R. Cordona, A. Oliva, and R. Schrebler, Talanta, 1977, 24(4), 259.

68. A. Gevorgyan, E. Molodezhen, Sh. Tanpou, V. Kha Deev, and V. Kostyleu deposited DOC 1975, Viniti, 2034.

69. J. Lo, J. Wei and S. Yem, Anal. Chem., 1977, 49, 1146.

70. J. Lo, J. Wei and S. Yem, Anal. Chim. Acta., 1978, 50, 1545.

72. T. Honjo, H. Imura, S. Shima and T. Kiba, Anal. Chem., 1978, 50, 1545.

73. S. Larionov, Term. Anal., Tezisy. Dokl. Vses. Sovech., 1979, 1, 202.

74. G. Afanasova, I. Shkhiyants, N. Nechitailo and V. Sher., in Neftekhimiva, 1971, 11, 911.

75. A. Klewska and M. Strycharska, Chem. Anal. (Warsaw), 1967, 12(6), 121.

76. M. Yanagisawa, M. Suzuki, and T. Takevchi, Microchim. Acta., 1973, 3, 475.

77. J. Lo, J. Wei, and S. Yem, Anal. Chem., 1982, 54, 2536.

78. H. Linder and I. Bigley, Anal. Chem., 1977, 94, 29.

79. G. Christian, Analytical Chemistry.

80. J. Huber and J. Kraak, Anal. Chem., 1972, 44, 1554.

81. Analytichem International, Handbook on "Sorbent Extraction Technology".

82. A. Braithwaite and F. Smith, Chromatographic methods, Wiley Interscience, London, 1986.

83. Williard, Merritt, Dean and Settle, Instrumental methods of analysis, Wadsworth, Belmont, London, 6th Edition, 1987.

84. D. Skoog and D. West, Analytical Chemistry, Saunders, Philadelphia, 4th Edition, 1982..

85. J. Mooney, M. Meaney, M. R. Smyth, R. Leonard and G. Wallace, Analyst, 1987, 112, 1555.