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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY: APPLICATIONS TO ORGANOMETALLIC AND METAL COORDINATION COMPOUNDS

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(First received September 3rd, 1981; revised manuscript received October 9th, 1981)

CONTENTS

1. INTRODUCTION

High-performance liquid chromatography (HPLC) has been applied extensively to the separation of numerous organic and biochemically active compounds during the last 10 years. The technique has also been used for the separation of metal complexes and organometallic systems, although this application has not been as thoroughly studied. It is encouraging to note, however, that the amount of interest in utilizing HPLC for inorganic systems appears to be increasing during recent years. Other chromatographic methods such as gas chromatography (GC), thin-layer chromatography (TLC) and "classical" (gravity feed) column chromatography (CCC) have also been used for the separation and determination of inorganic compounds. GC, however, is severely limited for separating metal–organic compounds because the method usually requires column operating temperatures at which many of these species either lack volatility or undergo decomposition. TLC and CCC suffer from difficulties such as long analysis times and lack of quantitative capability. In HPLC, the columns are usually constructed of stainless steel; the compounds of interest are thus isolated from the atmosphere and light and they can be separated with degassed, inert mobile phases at ambient temperatures. The use of HPLC for determining inorganic metal complexes which are often unstable, therefore, offers obvious advantages over previously employed TLC, CCC and GC techniques. It should be noted, however, that stainless steel can sometimes reduce metal complexes, and one must be alert to this possibility.

This paper will update our earlier review[1] on the same subject; some of the work which was covered in that paper will also be included here. The general subject of HPLC in inorganic analysis has also been reviewed recently by Schwedt[2]. We will review the application of HPLC for two classes of metal–organic systems: (1) organometallic compounds and (2) metal coordination complexes. Any complex containing a metal–carbon bond will be considered under the organometallic classification. Separations of organometallic complexes from coordination compounds and other substances will also be considered here. Within each of these two categories the subject will be subdivided into two classifications: (a) separations involving a single metal, (b) multi-element separations employing one or more ligands. The individual discussions have been organized according to ligand type.

This review has been limited to those applications where metals are complexed *prior* to HPLC elution; work involving post-column derivatization of metal ions with a ligand is not included. Also, ion-exchange separations of metal ions and metal–organic compounds primarily of biological interest are not covered, though some of the latter are included for other reasons. This review includes applications which utilize most of the known HPLC modes of operation, *i.e.* liquid–liquid partition, liquid–solid adsorption, reversed-phase, size exclusion and ion-exchange chromatographic techniques. We have attempted to cover the literature through 1980 along with later articles which happen to be known to us.

2. ORGANOMETALLIC COMPOUNDS

2.1. Single-metal complexes

2.1.1. Arene metal carbonyls

The first use of HPLC for the separation of organometallic species was reported in 1969[3]. A column containing Carbowax 400/Porasil C was used to separate four arene tricarbonylchromium complexes with isooctane as the mobile phase. The order of elution of these compounds (Fig. 1) is the inverse of that found with GC[4] (see also ref. 43). The same experimental conditions were later used to separate the two geometric isomers of 2,3-dimethylnaphthalenetricarbonylchromium[5]. The two geometric isomers of tricarbonyl[4b,5,6,7,8,8a,η-(9,10-dihydro-1,4-dimethoxy-9,10-o-benzenoanthracene)]chromium have also been separated, both analytically and on a small preparative scale, on Carbowax 400/Corasil I as well as on uncoated Corasil I[6].
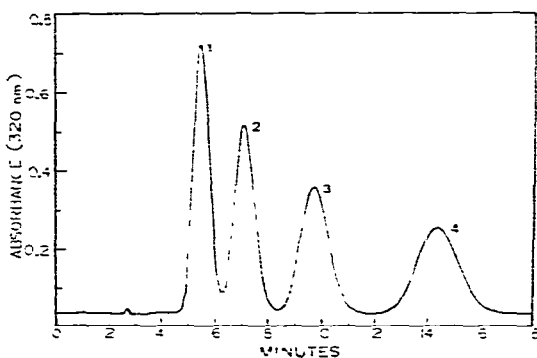
Fig. 1. HPLC separation of tricarbonylchromium complexes of (1) mesitylene, (2) m-xylene, (3) toluene and (4) benzene. Reprinted with permission from ref. 3 (Chemical Society, London).

Eberhardt et al.[7] have reported an extensive investigation of the HPLC behavior of 23 derivatives of benzenetricarbonylchromium on a Carbowax 400/Corasil column. Three tricarbonylchromium complexes of 9-phenylanthracene (I, II and III) have been separated on a preparative scale on silica with methylene chloride as eluent[8].

(I)   (II)   (III)
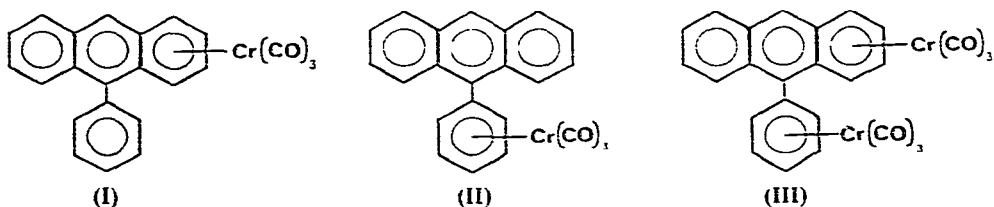
2.1.2. Cyclopentadienyl metal carbonyls

HPLC studies of tricarbonylcyclopentadienylmanganese complexes were first reported in 1973[7]. More recently, Vollhardt et al.[9] have separated a mixture of six

Fig. 2. Chromatogram of a mixture of IV–IX. Reprinted with permission[9].

dinuclear molybdenum complexes (IV–IX), using an Ultrasphere-ODS reversed-phase column with acetonitrile–water (5:1) (see Fig. 2).

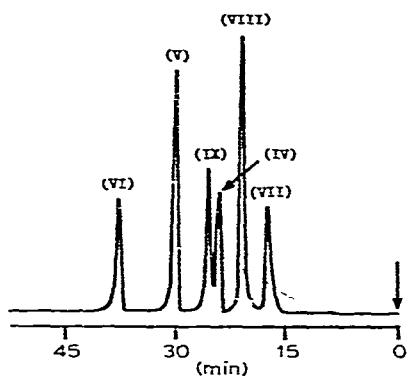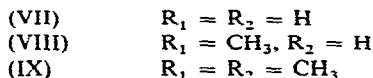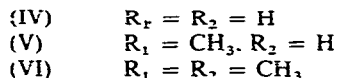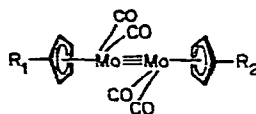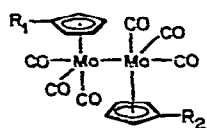| (IV) | $R_1 = R_2 = H$ | (VII) | $R_1 = R_2 = H$ |
| (V) | $R_1 = CH_3, R_2 = H$ | (VIII) | $R_1 = CH_3, R_2 = H$ |
| (VI) | $R_1 = R_2 = CH_3$ | (IX) | $R_1 = R_2 = CH_3$ |

Within each series of single or triply bonded molybdenum complexes, introduction of methyl groups on the cyclopentadienyl rings increases the hydrophobicity and thus also increases the retention time.

2.1.3. Dicyclopentadienyl complexes

A systematic study of the HPLC behavior of 26 dicyclopentadienyl complexes of iron was reported in 1973[7]. Surprisingly, little HPLC work with this type of complex has appeared recently. One important recent advance in HPLC techniques has been the development of element specific detectors. In particular, the inductively coupled plasma (ICP) detector enables one to detect and quantitatively to determine complexes of a single element in the presence of complexes of other elements, even though these different complexes may not be separable under the chromatographic conditions used. Gast et al.[10] demonstrated the usefulness of this method of detection in the separation of ferrocene, acetylferrocene and diacetylferrocene on a silica gel column with toluene as the mobile phase. Hausler and Taylor[11] have used size exclusion HPLC (μStyragel 100 Å) to separate ferrocene and 1,1'-diacetylferrocene from iron and copper coordination compounds with toluene as eluent and with ICP detection. They have also separated ferrocene, acetylferrocene, 1,1'-diacetylferrocene and bis(tetrapyrazolylborate)iron(II) with pyridine elution[12]. McKone[13] has developed an undergraduate laboratory experiment involving the separation of ferrocene, acetylferrocene and 1,1'-diacetylferrocene on a C_{18} reversed-phase column with a methanol–water (3:1) mobile phase.

2.1.4. Cyclopentadienyl–cycloolefin complexes

Sternberg and Vollhardt[14] have described the reactions of linear diynenes with dicarbonylcyclopentadienylcobalt to form cyclopentadienyl–cycloolefin cobalt complexes (eqn. 1).

$$(CH_2)_4 \underset{C\equiv C-H}{\overset{C\equiv C-(CH_2)_n-CH=CH_2}{\diagdown}} + \; CpCo(CO)_2 \xrightarrow[\Delta]{C_8H_{18}} \text{[structure]} \quad exo/endo \; (1)$$

$$(n=3,4)$$
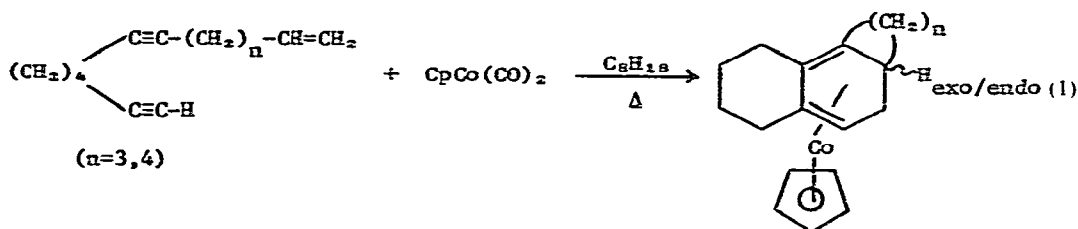
The exo- and endo-isomers were formed in approximately equal amounts in 65–75 % yields and were separated on a preparative scale on a C_{18} reversed-phase column with dioxane–acetonitrile (5:95) eluent. For the $n=4$ case, a third isomer, presumably arising from hydrogen shifts, was also found. Trimethylsilyl derivatives of the above were also studied (see below).

2.1.5. Metal cluster complexes

Several transition metal cluster complexes, including $H_4Ru_4(CO)_{12}$ and $Ru_3(CO)_{12}$, have been separated on a C_{18} reversed-phase column with methanol–water (3:1) as the mobile phase[15]. The reactions of a number of tetranuclear clusters, including the hydride clusters $H_4Ru_4(CO)_{12}$ and $H_2Ru_4(CO)_{13}$, with carbon monoxide have been studied by Fox et al.[16]; the course of the reactions was monitored by infrared spectroscopy and analytical HPLC and the products of the reactions were separated on a μPorasil column. $H_2Ru_4(CO)_{13}$ reacts with carbon monoxide in hexane at 25°C to produce $Ru_3(CO)_{12}$, $Ru(CO)_5$ and H_2. $H_4Ru_4(CO)_{12}$ first reacts with carbon monoxide to produce $H_2Ru_4(CO)_{13}$ and H_2; the $H_2Ru_4(CO)_{13}$ produced then reacts further as described above. The reaction of $H_2Ru_4(CO)_{13}$ is essentially first order in the cluster and first order in carbon monoxide ($\Delta H^{0*} = 12.5$ kcal/mol; $\Delta S^{0*} = -36.6$ cal/mol·°K).

2.1.6. Metal carbonyl complexes containing olefin-type ligands

The first application of reversed-phase HPLC to organometallic compounds is apparently that of Graf and Lillya[17] who separated cis- and trans-tricarbonyl-(3,5-hexadienone)iron; separations of similar cis–trans isomeric pairs for five other substituted hexadienones and one substituted pentadienone are also reported. The column contained ODS Permaphase, and 20% aqueous methanol was used as the mobile phase. This reversed-phase mode was chosen so that the synthesized tricarbonylirondienone complexes could be eluted in narrow peaks unobscured by large amounts of less polar starting materials which are strongly retained on the column. The same authors[18] also used HPLC to study the stereospecific synthesis of dienone iron tricarbonyl complexes by Friedel–Crafts acylation. Pryde[19] has reported the use of HPLC to study the thermal rearrangements of four isomeric tricarbonyl(phenylcycloheptatriene)iron complexes. A 5-μm Hypersil silica column was packed under acetone and conditioned with diethyl ether followed by n-hexane.

Baseline separations of isomers, which differ from each other only in the position of a hydrogen atom in the 7-membered ring, were achieved in less than 5 min. Peak identifications were confirmed by mass-spectral analysis.

Reversed-phase HPLC has been used to monitor the reactions of $Ru_3(CO)_{12}$ with 1,4-diazabutadienes (DAB) and of $Ru_2(CO)_6(DAB)$ with DAB[20]. The first of these reactions proceeds according to eqn. 2 and leads to a product of the structure X.

$$2\ Ru_3(CO)_{12} + 3\ DAB \rightarrow 3\ Ru_2(CO)_6(DAB) + 6\ CO \qquad (2)$$
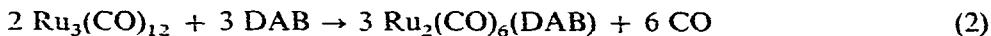
For R = isopropyl, the reaction was found to be second order [first order in $Ru_3(CO)_{12}$ and in DAB]. For R = tert.-butyl (t-Bu) the reaction again seems to be second order. The rate of reaction of tert.-butyl-DAB is slower than that of isopropyl (iso-Pr)DAB by a factor of 40. The reaction of $Ru_2(CO)_6(DAB)$ with DAB involves a complicated series of intermediates including those shown (XI–XIII). Notice that formation of these intermediates involves the making and breaking of carbon–carbon bonds. These and other intermediates were identified chromatographically. There is evidence for exchange of coordinated and free DAB when $Ru_2(CO)_6$(iso-PrDAB) reacts with tert.-BuDAB. The HPLC characteristics of $M_2(CO)_6(DAB)$ (M = Ru, Fe) on silica. CN-bonded, diol-bonded and ODS-bonded stationary phases have also been studied[21].

(X)

(XI)   (XII)   (XIII)
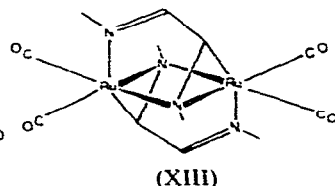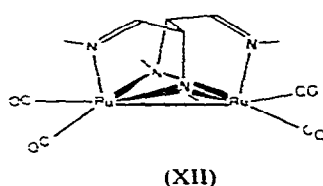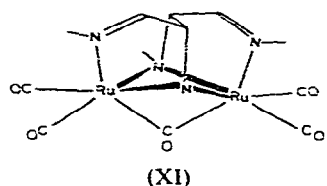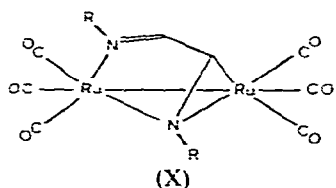
2.1.7. Metal carbonyl coordination complexes

The $M_2(CO)_6(DAB)$ complexes discussed above, including the intermediates and final products of the reactions, also involve metal–nitrogen coordination and thus could be considered under this heading. The intermediates XI and XII are bis-(alkylimino-alkylaminoethane) complexes (abbreviated as IAE). The capacity factors for silica, CN-bonded, diol-bonded and ODS-bonded stationary phases with a variety of eluents for $Ru_2(CO)_5$(t-BuIAE), $Ru_2(CO)_5$(cyclohexyl-IAE), $Ru_2(CO)_4$(cyclohexyl IAE) and $Mo_2(CO)_6$(t-BuIAE) have been determined[21]. ICP detection has been used to follow the separation of a mixture of five molybdenum complexes, including tetracarbonylbipyridylmolybdenum, using gradient elution on a C_8 reversed-phase column[10]. Reversed-phase HPLC on a C_8 bonded-silica support has been used to study the reaction of $Fe_2(CO)_9$ with di-tert.-butylsulfurdiimine
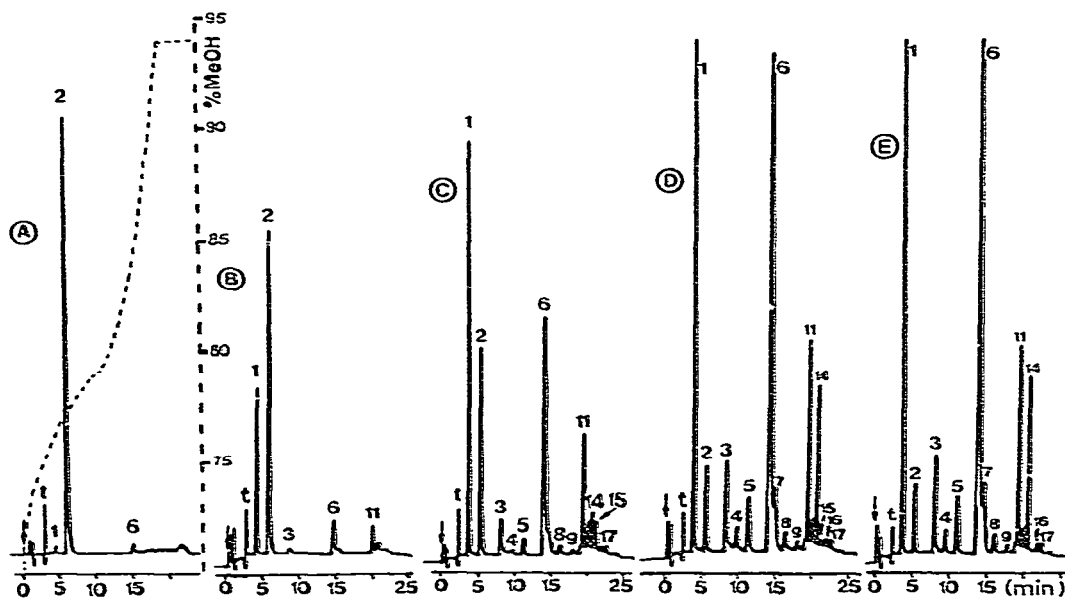
Fig. 3. Example of monitoring the reaction of $Fe_2(CO)_9$ with DBSD using gradient elution. Chromatograms of samples taken from the mixture at various times: A. after 5 min; B. after 1 h; C. after 2 h; D. after $3\frac{1}{2}$ h; E. after $4\frac{1}{2}$ h. Stationary phase: Zorbax C-8. Mobile phase: gradient from 70 to 94% (v·v) methanol–water, gradient shape indicated in A. t = Toluene. Reprinted with permission[22].

(DBSD)[22]. Gradient elution with 70–94% methanol in water resulted in the separation of seventeen products within 30 minutes (see Fig. 3). Structures of some of the products identified are given below (XIV–XIX). ICP detection has also been employed with this system[10].
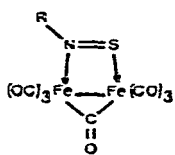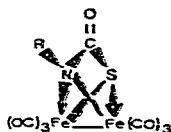
(XIV) (XV) (XVI)
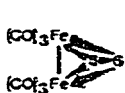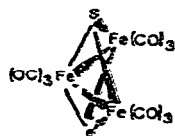
(XVII) (XVIII) (XIX)

Creber and Wan[23] have recently used electron spin resonance as a detector in an HPLC system to demonstrate the formation of the 3,5-di-*tert*.-butyl-*o*-quinonetetracarbonylrhenium complex (XX). The stationary phase was Partisil-10

(XX)
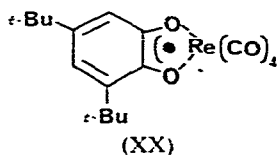
and the eluent was benzene. This first successful use of this detection mode holds promise for future separations and studies of other organométallic radicals.

2.1.8. Metallocarboranes

The original use of HPLC for the separation of several pairs of cyclopentadienylcobaltacarboranes by Evans and Hawthorne[24] has been extended by Plzák et al.[25]. Capacity factors on silica-gel columns with four different eluents were measured for eleven cyclopentadienylcobalt complexes of boranes (mostly carboranes), including two bis-cyclopentadienyl complexes. One such complex of iron was also included. Though no actual separations of these complexes were reported, separations were shown to be feasible in many cases. Capacity factors for ten bis-carborane cobalt complexes with bridging groups between the carborane ligands are also reported, and a separation of the methoxy-bridged complex from the ethoxy-bridged complex is demonstrated.

2.1.9. Alkyl and aryl complexes of main group metals

The importance of alkyl and aryl complexes of heavy metals, particularly mercury and lead, in environmental chemistry has brought about investigations of the use of HPLC for their separation and detection. Early work by Fanasaka et al.[26], Brinckman et al.[27] and Botre et al.[28] has been discussed in our earlier review[1]. More recently, electrochemical detection has been used to monitor the separation of Hg^{2-}, $MeHg^+$, $EtHg^+$ and $PhHg^-$ on an ODS column with 40% aqueous methanol containing 0.06 mol/l ammonium acetate and $5 \cdot 10^{-4}$ M 2-mercaptoethanol at pH 5.5 as mobile phase[29]. The separation of $MePh_3Sb^-$, Et_3Pb^+, Me_3Pb^+ and $MeHg^+$ on LiChrosorb NH_2 with the same eluent minus the 2-mercaptoethanol is also reported. MacCrehan and Durst[30] have used differential-pulse electrochemical detection with an ODS column and the same eluent to separate $MeHg^+$, $EtHg^+$ and $PhHg^+$. Good separations were obtained even in the presence of interfering reducible cations such as Cd^{2+}, Pb^{2+} and Cu^{2+}. The method has been used for quantitative determination of methyl mercury in tuna fish and shark meat. MacCrehan[31] has recently discussed the advantages of differential-pulse detection (DPD) of organometallic cations; Me_3Sn^+, Et_3Sn^+ and n-Bu_3Sn^+ are simultaneously detected after separation on a cation-exchange column. Alkyltin cations can be more reliably measured with DPD than with amperometric detection.

Gast and Kraak[32] have reported the separation of organomercury compounds on a silica-gel column with 10% butanol in n-hexane saturated (ca. 0.01%) with tetramethylammonium chloride as eluent. Clean separations of mixtures of diphenylmercury, propylmercury bromide, ethylmercury chloride, methylmercury chloride and phenylmercury chloride were achieved with an elution time of about 20 min. Reversed-phase chromatography on a C_8 column with 40% acetonitrile in 0.1 M aqueous sodium bromide (pH 3.5) was also shown effectively to separate a mixture of

mercury(II) chloride, methylmercury chloride, ethylmercury chloride, propylmercury bromide and 3-chlorophenylmercury acetate. Both UV detection at 205 nm and post-column derivatization (dithizone) were used. For alkylmercury compounds, the lowest limit of detection (80 ppb, 10[9]) was obtained with dithizone reaction detection while for the phenylmercury compounds, UV detection was most sensitive (60 ppb). ICP has been used for the detection of alkylmercury compounds and of lead compounds in gasoline[10]. This detector shows enhanced sensitivity for alkylmercury compounds compared to UV detection; ICP detection shows clearly the presence of four lead compounds in the gasoline sample while UV detection reveals only a very broad band of unseparated components.

Burns et al.[33] have compared gas–liquid chromatography (GLC) and HPLC for the separation of tin tetraalkyls and alkyltin halides. For ethyltin compounds, they found that both GLC and HPLC are satisfactory for identification and quantitative applications. The detection limits for GLC are smaller than those of HPLC (differential refractometric) by factors of 4–5. However, for methyltin compounds, a redistribution reaction (eqn. 3) occurs on the GLC column so that it was not possible to examine all four methyltin compounds simultaneously. Reversed-phase HPLC with acetone–n-pentane (60:40) elution produced a satisfactory separation (see Fig. 4). Vickrey et al.[34] have described an off-line HPLC–graphite-furnace atomic-absorption sampling procedure which offers certain advantages; this is illustrated by the reversed-phase C_{18} chromatography of tetraphenyllead. Parks et al.[35] have described the use of graphite-furnace atomic-absorption detection with size-exclusion HPLC and reversed-phase HPLC for characterizing controlled-release biocidal organometal macromolecules such as organotin silicates and poly(tri-n-butyltin methacrylate–methyl methacrylate). Among the compounds studied by Hausler and Taylor[11] using size-exclusion HPLC with ICP detection are Ph_3Si–$C\equiv C$–$SiPh_3$, Ph_3Si–CH $=CHCH_3$, Ph_4Pb and $(CH_3CH=CH)_4Pb$.
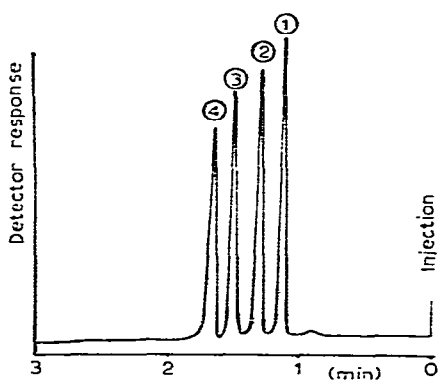
$$(CH_3)_4Sn + CH_3SnCl_3 \rightarrow (CH_3)_3SnCl + (CH_3)_2SnCl_2 \qquad (3)$$
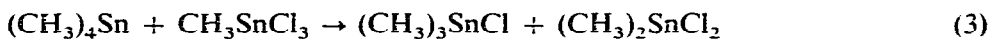
Fig. 4. Chromatogram of admixture of (1) (CH$_3$)$_4$Sn, (2) (CH$_3$)$_3$SnCl, (3) (CH$_3$)$_2$SnCl$_2$ and (4) CH$_3$SnCl$_3$. Reprinted with permission[33].

2.2. *Complexes containing two or more different metals*

2.2.1. *Cyclopentadienyl–cycloolefin complexes*

The work of Sternberg and Vollhardt[14] on the reactions of diynenes with dicarbonylcyclopentadienylcobalt has already been discussed in section 2.1.4. Similar reactions with trimethylsilyl-substituted diynenes have also been carried out leading to trimethylsilyl-substituted cycloolefin complexes. When *exo*- and *endo*-isomers were formed (eqn. 1), these were found also to be separable on a reversed-phase C_{18} column with dioxane–acetonitrile (5:95) as eluent. Gesing and Vollhardt[36] have studied the reaction of 1,6-bis(trimethylsilyl)-1,5-hexadiyne with dicarbonylcyclopentadienylcobalt. Eighteen characterizable complexes were isolated from these reactions, including cyclobutadienecobalt–, cyclopentadienonecobalt– and cobalt cluster–η^5-C_5H_5 complexes. Nine of these complexes, all trimethylsilyl-substituted cyclobutadienes, eluted as a single band from alumina with diethyl ether, but were separable on an ODS reversed-phase HPLC column. The structure of one of these complexes is shown (XXI); this is typical of the compounds synthesized and separated. An extensive discussion of the use of reversed-phase HPLC as an analytical and preparative tool for the characterization and isolation of organometallic compounds, especially CpCo complexes of cyclobutadienes and cyclohexadienes, has recently appeared[9].

(XXI)
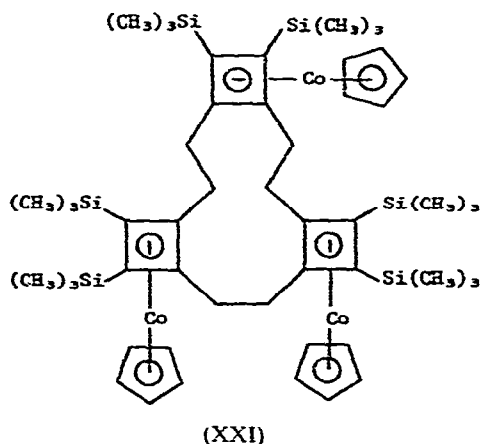
2.2.2. *Metal cluster complexes*

$H_2FeRu_3(CO)_{13}$ has been separated from $H_4Ru_4(CO)_{12}$ and $Ru_3(CO)_{12}$ on a C_{18} reversed-phase column using methanol–water (3:1) as eluent[15]. UV irradiation of benzene solutions of $HFeCo(CO)_{10}(PPh_3)_2$ gives at least three products which are separated by elution with methanol–water (4:1)[15,37]. Reactions of the mixed-metal tetranuclear clusters $H_2FeRu_3(CO)_{13}$, $H_2FeRu_2Os(CO)_{13}$, $H_2FeRuOs_2(CO)_{13}$, $H_4FeRu_3(CO)_{12}$, $HCoRu_3(CO)_{13}$, $H_2FeRu_3(CO)_{12}L$ (L=PMe_2Ph, PPh_3), [$HFeRu_3(CO)_{13}$]$^-$ and [$CoRu_3(CO)_{13}$]$^-$ with CO under relatively mild conditions have been monitored by infrared spectroscopy and by analytical liquid chromatography and the products separated by HPLC[16]. These reactions result in fragmentation

to give trimeric and monomeric products which are greatly dependent on metal composition and/or structure. The tetrahydride cluster $H_4FeRu_3(CO)_{12}$ first reacts with carbon monoxide to produce H_2 and $H_2FeRu_3(CO)_{13}$, the latter then fragmenting to form $Ru_3(CO)_{12}$, $Fe(CO)_5$ and H_2. The last reaction proceeds largely by a second-order, [CO]-dependent path for which $\Delta H^{0*} = 20.0 \pm 2.0$ kcal/mol and $\Delta S^{0*} = -25.4 \pm 5.8$ cal/mol·°K) [cf. values for $H_2Ru_4(CO)_{13}$ given in section 2.1.5.].

2.2.3. Metal carbonyl complexes containing olefin-type ligands

The work of Gast and Kraak[21] on diazabutadiene complexes of the general formula $M_2(CO)_6(DAB)$ discussed previously (section 2.1.6.) includes a study of two mixed metal species, $CoMn(CO)_6(iso-PrDAB)$ and $CoMn(CO)_6(t-BuDAB)$. Capacity factors for these complexes on silica. CN-bonded, diol-bonded and ODS-bonded stationary phases with a variety of eluents were measured.

2.2.4. Alkyl and aryl complexes of main group metals

Among the compounds studied by Hausler and Taylor[11] were three mixed metal species, $Ph_3SnC \equiv CSi(Ph)_2C \equiv CSnPh_3$, $Ph_3SnC \equiv CSiPh_3$ and $Ph_3PbC \equiv CGePh_3$. After size-exclusion HPLC, a refractive-index detector showed separation of the first of these from the other two. Detection of Sn species by ICP shows separation of the first two compounds while Pb monitoring shows a single peak for the last compound.

2.2.5. Organopolymetallic compounds

The recently discovered ability of zerovalent triphenylphosphine complexes of platinum and palladium to insert into Hg–C and M–M' bonds (M = Hg, Cd, Zn; M' = Ge, Sn) has resulted in the synthesis of a number of organometallic compounds containing polymetallic chains. Germylpalladium and germylplatinum complexes were also synthesized by oxidative insertion of palladium and platinum triphenyl-phosphines into Ge–H bonds. Bochkarev et al.[38] have used silica columns with UV detection (254 nm) and n-hexane–diethyl ether mixtures as eluents to separate penta-fluorophenyl complexes containing Ge–Hg–Pt–Ge, Sn–Hg–Pt–Ge and Ge–Cd–Pt–Ge groups. These complexes cannot be separated by GLC because of low volatility and limited thermal stability. HPLC makes it possible to monitor the reactions involved and to obtain information on relative reaction rates, mechanisms and stabilities of reaction products.

3. METAL COORDINATION COMPLEXES

3.1. Separations of single-element multi-ligand coordination complexes

A number of reports have appeared in which coordination complexes of the same metal with a variety of ligands are separated by HPLC. Many of these studies have led to interesting observations concerning stereochemical, kinetic, thermodynamic and structural properties of metal coordination compounds.

3.1.1. β-Diketonates

The elution behaviour of various β-diketone ligands and β-diketonates of

Cr(III) on size-exclusion columns has been studied by Saitoh *et al.*[39] and by Suzuki *et al.*[40]. In the initial paper, acetylacetone and its Cr(III) chelate were selected as model compounds for studying their gel chromatographic behavior on Merckogel OR-2000[39]. Two poly(vinyl acetate) gels and two polystyrene gels were investigated in the later work[40]. The ligands included acetylacetone, trifluoroacetylacetone, benzoylacetone, furoyltrifluoroacetone, benzoyltrifluoroacetone, theonyltrifluoroacetone and dibenzoylmethane, as well as their corresponding Cr(III) chelates. It was found that the elution order of neither the β-diketone ligands nor their Cr(III) chelates on any gel followed the expected order of their molecular weights; the distribution coefficients of the ligands and chelates were found to depend strongly on the gel and could not be correlated with the molecular weights of the compounds.

A recent communication by Noda *et al.*[41] demonstrated the feasibility of gel permeation chromatography for the separation of mixed-ligand complexes of several beryllium(II) β-diketonates. The chelates studied included the bis(acetylacetonato), the bis(theonyltrifluoroacetonato) and the mixed-ligand complexes.

Uden *et al.*[42] employed normal-phase HPLC on silica for the separation of pairs of geometrical isomers of octahedral cobalt(III) and chromium(III) chelates of trifluoroacetylacetone [H(TFA)], benzoylacetone [H(BAA)] and 2,2-dimethylhexane-3,5-dione [H(PAM)]. Both isocratic and gradient elution modes were used with UV detection at 254 nm. It was found that adsorption HPLC with a mobile phase of medium polarity (6% acetonitrile in methylene chloride) permits ready resolution of isomer pairs of non-volatile complexes such as Co(BAA)$_3$ and Co(PAM)$_3$. In all cases, it was found that the *mer* isomer elutes before the *fac* isomer, and for a given ligand, chromium complex isomers elute before the analogous cobalt species. The authors also found that it was possible to achieve good resolution of mixed ligand complexes of chromium with hexafluoroacetylacetone [H(HFA)] and HTFA using either isocratic or gradient elution.

Acetylacetonato-cobalt(II) and -cobalt(III) complexes as well as those of copper(II) have recently been separated using size-exclusion HPLC and detected with an ICP atomic-emission spectrometer by Hausler and Taylor[12].

3.1.2. β-Ketoamines

Uden *et al.*[43] applied reversed-phase HPLC successfully to the separation of neutral tetradentate chelates of copper(II), nickel(II) and palladium(II) with a range of fluorinated and non-fluorinated β-ketoamine and salicylaldimine ligands. A bonded octadecyl substrate was used as the reversed-phase functionality, and elution was carried out isocratically using methanol–water–acetonitrile. UV detection at 254 nm was employed. The separation of the N,N'-ethylenebis(acetylacetoneimine) [H$_2$(enAA$_2$)], N,N'-propylenebis(acetylacetoneimine) [H$_2$(pnAA$_2$)] and N,N'-butylenebis(acetylacetoneimine) [H$_2$(bnAA$_2$)] chelates of nickel(II) showed that substitution of methyl groups into the ethylene bridge leads to greater partition on the C$_{18}$ substrate and longer retention times. It is interesting to note that the order of elution is the reverse of that noted in gas chromatography for the same compounds (see also ref. 3). Other interesting variations of retention and resolution as a function of ligand structure and the metal used are reported in this paper.

3.1.3. Hydrazones

Gasparrini et al.[44] have separated several palladium(II) complexes of substituted hydrazones on a LiChrosorb DIOL column with n-hexane–dichloromethane mixtures. Complexes which were separated included those of the type trans-[PdL₂Cl₂] where L is the substituted hydrazone ligand. Ligands which were studied included the N-methyl-N-phenylhydrazones of methyl isopropyl ketone, of diethyl ketone, of methyl-n-propyl ketone, of acetone and of acetaldehyde as well as the N,N-dimethylhydrazone of acetone. Mixed ligand complexes of the type trans-[PdLL′Cl₂] were also studied. They found that unmodified silica columns were not effective, as the complexes were generally unstable and strongly adsorbed. The LiChrosorb DIOL column was found to be very effective and permitted the use of non-polar mobile phases in which the complexes were readily soluble. A number of six-component separations were reported using either isocratic or gradient elution operating conditions. It was also found that the capacity factor (k') decreases as the chain-length of the ketone increases, a result which is consistent with normal-phase HPLC separations. The high sensitivity of the technique permits trace amounts of these complexes to be determined. The method can also be employed to monitor the progress of the synthesis of these complexes and can provide information about their purity and rate of ligand exchange.

3.1.4. Dithiocarbamates

A distinct advantage of the use of the dialkyldithiocarbamate (DTC) anion over many other ligands is its insolubility in organic solvents and its ability to form complexes with many metal ions. Extraction into organic solvents transfers only the neutral chelate, leaving behind excess ligand. Hence, DTC complexes lend themselves uniquely to HPLC studies.

Liška et al.[45] have studied the separation and identification of a series of nickel(II) bisdialkyldithiocarbamate complexes by HPLC. Silica gel was used as the stationary phase and chloroform–cyclohexane mixtures as the mobile phase with UV detection at 325 nm. The capacity factors (k') of a series of DTC complexes from the dimethyl- to dioctyl- were measured as a function of mobile phase composition. It was found that there was a substantial influence of the N-alkyl substituent and the mobile phase composition on the capacity factors; the limit of detection was in the range of 10^{-9}–10^{-10} M Ni(II). The separation and quantification of Ni(II)–DTC complexes was found to be severely complicated by the formation of new complexes via ligand exchange reactions. These side reactions were investigated further in a later paper by Liška et al.[46]. In this study it was observed that a third compound was formed for every pair of Ni(II) complexes with different N-alkyl substituents. For example, a mixture of nickel(II) bisdiethyl- and bisdihexyl-DTC separated by either HPLC or TLC produced a mixture of three substances of which two were identical with the original symmetrical complexes. It was demonstrated that mixed-ligand complexes were formed by ligand-exchange reactions in solution. The results were verified by TLC analysis. Some of these compounds could be isolated and were characterized by molecular-weight determinations and elemental analysis. The HPLC separation of multi-ligand DTC complexes of Cu(II), Co(II), Zn(II) and Pb(II) was reported in a subsequent paper by Lehotay et al.[47]. It was demonstrated again that symmetrical Zn(II) bisdialkyl–DTC complexes as well as symmetrical Pb(II) bisdial-

kyl–DTC complexes used to prepare the sample mixture produced mixed ligand complexes as described by the equilibrium shown in eqn. 4.

$$MA_2 + MB_2 \rightleftharpoons 2\ MAB \tag{4}$$

This reaction was found not to occur between bisdialkyl–DTC complexes of Cu(II) or Co(II). A typical chromatogram showing the separation of Zn(II) bisdialkyl–DTC complexes is shown in Fig. 5. The authors have concluded from this work that HPLC is an effective method to separate mixtures of metal bisdialkyl–DTC complexes, although the determination of these compounds in the environment or in fungicides is hindered by the ease with which ligand exchange between them occurs.
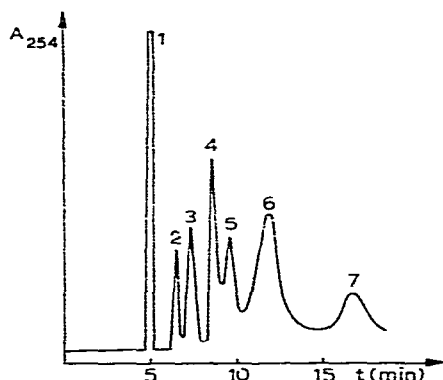
Fig. 5. Separation of Zn(II) bisdialkyl–DTC complexes. Mobile phase: chloroform cyclohexane (10:90). Column: 25 × 0.46 cm. LiChrosorb SI 60, particle size 10 μm. Peaks: 1 = inert substance; 2 = Zn(II) bisdibutyl–DTC; 3 = mixed-ligand complex, Zn(II) dipropyl–DTC dibutyl–DTC; 4 = Zn(II) bisdipropyl–DTC; 5 = Zn(II) diethyl–DTC dibutyl–DTC; 6 = Zn(II) diethyl–DTC dipropyl–DTC; 7 = Zn(II) bisdiethyl–DTC. Reprinted with permission[47].

Moriyasu and Hashimoto[48] have carried out additional studies on the lability of ternary Ni(II) chelates of DTC by HPLC. They describe an HPLC method to determine the kinetic rate constants for the formation and disproportionation of these complexes. They verified that thoroughly deactivated silica columns produced satisfactory chromatograms for Hg(II), Cu(II), Ni(II) and Co(III) without dissociation of the chelates during chromatography. Contrary to the findings of Lehotay et al.[47], Moriyasu and Hashimoto[48] found that the Cu(II) chelates are more labile than those of Ni(II) and that during chromatography, disproportionation did in fact occur. Moriyasu and Hashimoto[48] also state that Lehotay et al.[47] claim non-lability for Co(III) complexes. This is incorrect; Lehotay et al. claimed non-lability for Co(II) complexes. Moriyasu and Hashimoto[49] have also done a more extensive kinetic and equilibrium HPLC study of dialkyl–DTC chelates of Ni(II) and Cu(II). The equilibrium of ternary complex formation from two symmetrical complexes is the same as that shown in eqn. 4. They found that the HPLC separation process was sufficiently rapid, and that kinetically unstable ternary complexes can be eluted without disproportionation during chromatography. It was found possible to halt the progress of fast exchange reactions at the moment of injection. The chromatograms, therefore,

indicated the concentration of the species before chromatography, facilitating the determination of equilibrium constants for the Ni(II) and Cu(II) chelates. The equilibrium equation for the process shown in eqn. 4 is given in eqn. 5.
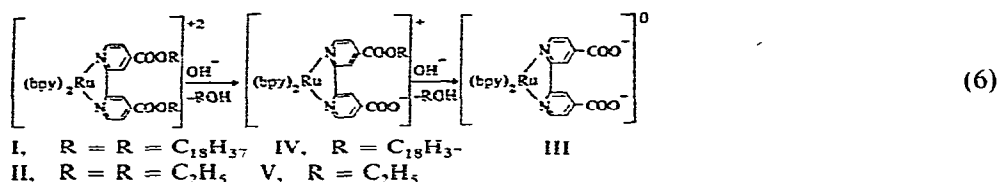
$$K = \frac{[MAB]^2}{[MA_2][MB_2]}$$

(5)

Experimentally determined K values were always found to be 4.0. The authors suggest that disproportionation takes place by collision of two ternary metal chelates, the rate constants being on the order of 10^1-10^2 l/mol·sec for Ni(II) chelates and 10^3 l/mol·sec for Cu(II) chelates.

The interesting possibility of simultaneously determining Cr(III) and Cr(VI) in waste water by separating the complexes of these chromium species with ammonium pyrrolidinedithiocarbamate using reversed-phase HPLC has been examined by Schwedt[50]. Reaction conditions and products were examined and the procedure was found to be applicable to the simultaneous determination of both chromium species. Tande et al.[51] have used DTC for the simultaneous determination of Cr(III) and Cr(VI) in water by means of reversed-phase HPLC with methanol–water (65:35) as the mobile phase and UV detection at 254 nm. They found that at room temperature and pH 5.8, DTC reacts with both Cr(III) and Cr(VI). Detailed examination of this reaction by HPLC revealed that three products were formed, an unidentified disulfide and two chromium–DTC chelates of different composition.

3.1.5. Bipyridines

Paired-ion HPLC is rapidly becoming very useful and popular for the separation of ionic compounds on reversed-phase columns. This can be accomplished by the addition of a counter-ion (usually an ionic alkyl compound) to the mobile phase causing the ionic species of interest to form ion pairs. The ion pair behaves as though it were a non-ionic, neutral species, thus favoring sorption on hydrophobic stationary phases. The mechanism and application of reversed-phase paired-ion HPLC has been described extensively[52,53]. It can be seen, therefore, that this chromatographic technique is especially useful for reversed-phase HPLC studies of cationic and anionic complexes, an area which has received very little attention thus far.

Valenty and Behnken[54] have reported the first use of reversed-phase paired-ion HPLC to separate ionic metal complexes. They investigated the elution of the diester, monoester-monocarboxylate and dicarboxylate derivatives of tris(2,2'-bipyridyl)ruthenium(II). These compounds are of interest because they can be employed as photopromoted electron transfer reagents and they can be distinguished on the basis of water solubility and molecular charge as shown in the hydrolytic reactions of I and II (eqn. 6).

$$\left[(bpy)_2 Ru \begin{array}{c} N \\ N \end{array} \begin{array}{c} COOR \\ COOR \end{array} \right]^{+2} \xrightarrow[-ROH]{OH^-} \left[(bpy)_2 Ru \begin{array}{c} N \\ N \end{array} \begin{array}{c} COOR \\ COO^- \end{array} \right]^{+} \xrightarrow[-ROH]{OH^-} \left[(bpy)_2 Ru \begin{array}{c} N \\ N \end{array} \begin{array}{c} COO^- \\ COO^- \end{array} \right]^{0}$$

(6)

I, $R = R = C_{18}H_{37}$ IV. $R = C_{18}H_3$-   III
II. $R = R = C_2H_5$ V, $R = C_2H_5$

Linear gradient elution with aqueous tetrahydrofuran (THF) containing ion-pairing reagents was used on a reversed-phase C_{18} column. Eluted components were monitored with UV detection at 254 and 280 nm. The ion-pairing reagents which were added to the mobile phase were 0.015 M methanesulfonic acid (for the separation of I, III and IV) and 0.005 M heptanesulfonic acid (for the separation of II, III and V). The chromatogram in Fig. 6 shows a separation of II, III and V accomplished in 11 min with a linear gradient programmed from 10 to 40% THF in water. The order of elution is exactly what one would predict; compound III is the most polar, although it has a net charge of zero; the two carboxylate groups cannot form an ion pair with the counter ion since they are anionic. Compound II is the least polar, even though it has a net cationic charge of $+2$, and can thus form a relatively non-polar ion pair with the sulfonate counter ion. This paper is a particularly interesting example which illustrates the versatility of paired-ion reversed-phase HPLC for the separation of ionic metal complexes.
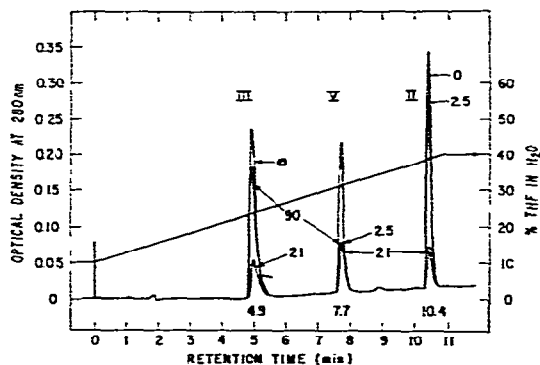

Fig. 6. Reversed-phase paired-ion HPLC separation of tris(2,2'-bipyridyl)ruthenium(II) derivatives. Chromatograms of the hydrolysis reaction of II in aqueous $1.0 \cdot 10^{-2}$ M pH 10 borate buffer. Traces shown at reaction times of 0, 2.5, 21, 90 and ∞ min. The diagonal line across the figure shows the composition of mobile phase with 0.005 M n-heptanesulfonic acid buffered to pH ca. 3.5. Reprinted with permission from ref. 54 (American Chemical Society).

3.1.6. EDTA, ethylenediamines and amino acids

Jones and Manahan[55] have demonstrated that copper(II) chelates of EDTA, NTA (nitrilotriacetic acid), EGTA [ethylenebis(oxyethylenenitrilo)tetraacetic acid] and CDTA [(1,2-cyclohexylenedinitrilo)tetraacetic acid] can be separated by HPLC using a 5-cm weak anion-exchange resin column and an aqueous 0.05 M ammonium sulfate mobile phase. The eluted copper chelates were detected by atomic absorption. The chelates were observed to elute from the column as a function of charge and size in the order: $Cu_2(EGTA)$, $Cu(NTA)^-$, $Cu(EDTA)^{2-}$, $Cu(CDTA)^{2-}$. This analysis was developed principally to determine chelating agents as pollutant components of natural and waste waters. The chelated copper(II) ion, added to the ligand mixture, served as a convenient "indicator metal" to provide sensitive and selective detection of the ligands by atomic absorption. This work illustrates the utility of atomic absorption as a species-specific detector. Yoshikawa et al.[56] have also reported an ion-exchange HPLC separation of cationic and anionic mixed ligand complexes of

Co(III) with NH_3, NO_2^-, en, dien, trien, carbonate, oxalate, EDTA and imino-diacetic acid. Separations were achieved on strongly acidic and strongly basic resins using aqueous sodium chloride, and UV detection at 254 nm. The method was applied to kinetic studies and isomer separations.

It has been shown recently by Warner and Legg[57] that the geometric diastereoisomeric forms of en–amino acid–Co(III) complexes can be separated by analytical and preparative HPLC on Whatman LP-1 (10–20 μm) silica particles with an isopropanol–triethylammonium bicarbonate (70:30) buffer system (pH 9). The absorbance at 510 nm was monitored. The complexes studied included $[Co(en)_2tyr]^{2-}$, $[Co(en)_2asp]^{2+}$ and $[Co(EDDA)(en)]^-$, where tyr = tyrosine, asp = asparagine and EDDA = ethylenediaminediacetic acid. Even though complete resolution was not achieved for the diastereoisomeric $[Co(en)_2aa]^{2+}$ complexes (aa = amino acid), the compounds were rapidly separated from several other mixture components and mirror-image circular dichroism spectra were generated. Typical analytical and preparative chromatograms for $[Co(en)_2(tyr)]^{2+}$ are shown in Fig. 7. The *sym-cis* and *unsym-cis* isomers of $[Co(EDDA)(en)]^-$ were also rapidly and completely resolved by this method. The results were consistent with those previously obtained by TLC.

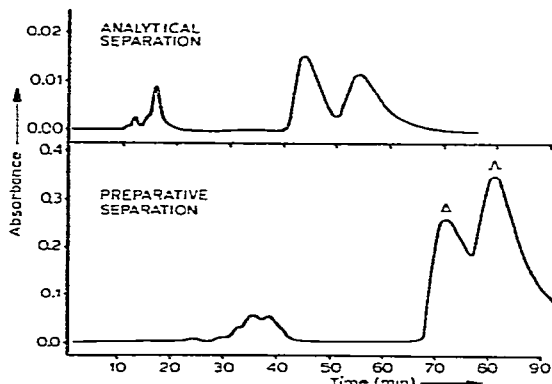Fig. 7. Analytical and preparative separations of $[Co(en)_2(tyr)]^{2+}$ diastereomers. Analytical separation conditions: flow-rate, 6 ml/min; approximately 2 mg of mixture were applied in a 20-μl sample volume; elution monitored at 510 nm. Preparative separation conditions: flow-rate, 3 ml/min, approximately 100 mg of mixture were applied in a 500-μl sample volume with a 20-min flow program; elution monitored at 510 nm. Reprinted with permission from ref. 57 (American Chemical Society).

Paired-ion reversed-phase HPLC was recently employed by Buckingham *et al.* for the rapid separation of similar cobalt(III) bis(ethylenediamine) amino acid complexes[58]. The complexes studied were of the diastereomeric type $\varDelta[Co(en)_2aa]X_2$ where aa represents an amino acid (gly, pro, val, leu or phe). It was possible to separate these five complexes on a reversed-phase packing in less than 15 min with *p*-toluenesulfonate or hexanesulfonate as the pairing-ion. The complexes were detected at 480 nm. It was shown that these complexes elute in the order of the relative hydrophobicities of the parent amino acid. At high sample loadings, concentration-dependent peak-splitting effects were noted.

A reversed-phase paired-ion separation of L-methionine dipeptide complexes of palladium(II) was recently reported by Lam-Thanh *et al.*[59]. They used quaternary ammonium salts as the pairing ions with UV detection at 380 nm.

3.1.7. Carbonates, benzoates and camphorates

Strazza and Polcaro have used HPLC for rapid separations of relatively labile carbonato- and benzoatocobalt(III) complexes formed in solid-state reactions[60]. Included in the study were the *cis* and *trans* forms of $[Co(en)_2Cl_2]^+$ and $[Co(en)_2(OCOC_6H_5)_2]^-$. HPLC was performed on a silica column (10 μm particles) using a gradient which was programmed from an eluent which was a mixture of 95 % ethanol, 2-propanol and 25 % aqueous ammonium nitrate to an eluent consisting of 1 M acetic acid in methanol. The column effluent was monitored at 340 and 580 nm respectively for each of the two separations.

An interesting HPLC separation of the four diastereomers of tris[(+)-3-acetylcamphorato]chromium(III) on Corasil II with 15 % THF in n-hexane has been reported by Minor and Everett[61]. Retention times were 12 min for Δ-*trans*, 14 min for Λ-*trans*, 20 min for Λ-*cis*, and *ca.* 50 min for Δ-*cis*.

3.1.8. Diphosphonates

Diphosphonate complexes of technetium-99m are widely used as skeletal imaging agents in nuclear medicine. A typical skeletal imaging agent is prepared on the day of use by mixing $^{99m}TcO_4^-$ and its daughter $^{99}TcO_4^-$ with a reducing agent $(Sn^{2+}, NaBH_4$ or $NH_2OH)$, a diphosphonate ligand [hydroxyethylidene diphosphonate, $CH_3C(PO_3H_2)_2(OH)$, HEDP] and an antioxidant stabilizer (ascorbic acid). The resultant radio-pharmaceutical solution contains Tc–diphosphonate complexes, reductant–diphosphonate complexes, excess diphosphonate and stabilizer. Pinkerton *et al.*[62] have developed the first ion-exchange HPLC procedure to separate and characterize the component complexes in radio-pharmaceutical Tc–HEDP solutions. They used a 25-cm column containing Aminex A-27 anion-exchange resin and aqueous sodium acetate (pH 8.4) as the eluent. Three modes of detection were used: (1) single wavelength absorbance at 405 nm, (2) UV–visible rapid scanning (250–660 nm) and (3) radiometric scintillation. The results indicated that the radio-pharmaceutical solutions consist of mixtures of numerous Tc-containing species of unknown oxidation state, configuration and coordination environment. A typical chromatogram, shown in Fig. 8, illustrates that there are at least seven components containing ^{99m}Tc activity.

Wong[63] has reviewed the application of HPLC for the analysis of mixtures containing ^{99m}Tc labeled radiopharmaceuticals. The review includes work which has been reported on ^{99m}Tc adducts with pyrophosphate, methylene diphosphonate, ethylene diphosphonate, diethylenetriamine pentaacetic acid and hum an serum albumin.

3.1.9. Crown ethers

Reversed-phase HPLC was employed by Mangia *et al.*[64] to study the chromatographic behavior of Hg(II) halide complexes with the macrocyclic polyether, dibenzo-18-crown-6(2,3,11,12-dibenzo-1,4,7,10,13,16-hexaoxacyclooctadeca-2,11-diene) (DBC). This compound acts as a hexadentate ligand with Hg(II) halides. Micropak CH (octadecylsilane on silica) columns were used with mobile phases consisting of methanol–phosphate or borate buffer mixtures (pH 7–10) to separate the complexes which were detected by monitoring the UV absorbance at 254 nm. It was shown that several of the mercury halides and mixed halides could be separated very rapidly as their crown ether complexes by this method.
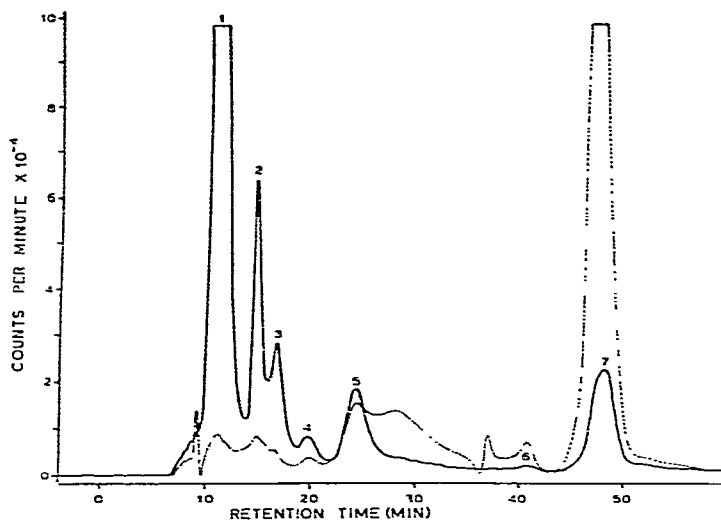
Fig. 8. HPLC of 3.3 mM Tc(NaBH$_4$)–HEDP spiked with ^{99m}Tc and prepared in air (flow-rate, 0.156 ml min; temperature, ambient). Solid line, γ detection at 140 keV (50-keV window); broken line, visible detection at 405 nm (0.1 a.u.f.s.). Reprinted with permission from ref. 62 (American Chemical Society).

3.1.10. Other ligands

Hausler and Taylor have shown[12] that complexes such as bis(tetrapyrazolylborate)iron(II) and bis(o-aminobenzaldehyde)ethylenediimine copper(II) can be separated from a series of metal–organic complexes by size-exclusion HPLC. The main objective of this work was to demonstrate the application of HPLC to the determination of organically bound metals in coal-derived materials. Metal-specific detection was employed.

The capacity factors of various bis(tetraazadiene)nickel complexes were measured by Gast and Kraak[21] on silica, CN-bonded and ODS-bonded stationary phases with THF–n-heptane mixtures. In the reversed-phase mode, the order of elution was exactly reversed from that observed in the normal-phase mode.

3.2. Multi-element separations of coordination complexes

3.2.1. β-Diketonates

The first HPLC separation and determination of metal coordination complexes was reported in 1972 by Huber et al.[65]. They showed that liquid–liquid partition HPLC with a ternary two-phase system consisting of water–2,2,4-trimethylpentane–ethanol could be used for the isocratic separation of several metal acetylacetonates. The water-rich (polar) phase served as the stationary liquid and was supported on silica particles (5–10 and 10–20 μm). The hydrocarbon-rich (non-polar) phase served as the eluent. The column effluent was monitored at 310 nm. It was shown that six metal acetylacetonates, Be(II), Cu(II), Al(III), Cr(III), Ru(III) and Co(III), could be separated and determined in 25 min. Three of the complexes, Ni(II), Al(III) and Fe(III), produced asymmetric peaks due to hydrolytic reactions of the complexes with the water-rich stationary phase. The Al(III) acetylacetonate was

found to undergo hydrolysis in the stationary phase producing several hydroxoacetylacetonatoaluminum(III) species which could be separated on the column. This initial paper on HPLC of metal coordination complexes demonstrated the utility of the technique for separating such compounds and for studying their dissociation equilibria.

Size-exclusion HPLC has found very limited application for the study of metal complexes; however, several reports dealing with this mode of separating metal acetylacetonates are of interest and are included in this review. Yamamoto et al.[66] studied the elution of metal acetylacetonates by use of size-exclusion chromatography. They used a 10 Å pore size styrene–divinylbenzene copolymer gel with a variety of organic solvents (carbon tetrachloride, chloroform, benzene, toluene, and p-xylene) as the isocratic mobile phase. Differential refractometry was employed for detection. They found (surprisingly) that, by a suitable choice of solvent, polystyrene gel columns can be used for the separation of chelates with very similar sizes such as cobalt(III) and chromium(III) acetylacetonates. They speculated that in certain solvents such as chloroform (known to form solvated complexes with metal acetylacetonates), it is the solvated species which are the effective entities being separated according to molecular size of the polystyrene gel. The chromatographic behavior of the Co(III), Fe(III), Cr(III), Al(III), Cu(II), Ni(II) and Be(II) acetylacetonates on size-exclusion Merckogel OR-2000 columns was studied by Saitoh and Suzuki[67]. They used THF as the mobile phase and refractometric detection. They reported the elution characteristics and distribution coefficients for the chelates comparing the data with several alkanes. In a later paper by Suzuki and Saitoh[68], the elution characteristics of Al(III), Cr(III), Fe(III), Co(III) and Be(II) acetylacetonates on the same gel with a variety of organic solvents (chloroform, benzene, toluene, 1,4-dioxane, ethyl acetate, butyl acetate, acetone, ethyl methyl ketone and methanol) were reported.

An extensive study of the elution behavior of a series of metal β-diketonates on alumina, silica gel, bonded-phases, and open-pore polyurethane HPLC columns has been carried out by Tollinche and Risby[69]. Metal β-diketonates which were studied included: (1) acetylacetonates (acac), Cu(acac)$_2$, Co(acac)$_2$, Cr(acac)$_3$, Al(acac)$_3$, Be(acac)$_2$, Ru(acac)$_3$, Rh(acac)$_3$, Co(acac)$_3$; (2) 2,2',7,7'-tetramethyl 3,5-heptanedionates (thd), Co(thd)$_3$, Cr)thd)$_3$, Fe(thd)$_3$, Cu(thd)$_2$, NNi(thd)$_2$; (3) 1,1,1-trifluoro-2,4-pentanedionates (tfa), Al(tfa)$_3$, Cr(tfa)$_3$, Co(tfa)$_3$, Ru(tfa)$_3$, Rh(tfa)$_3$; and (4) 1,1',1',2,2',3,3'-heptafluoro-4,6-octanedionates (fod), Co(fod)$_3$, Cr(fod)$_3$. It was found that the best separations were obtained on normal-phase silica gel columns with non-polar eluents and UV detection at 280 nm. A typical six-component separation of several metal acetylacetonates is shown in Fig. 9. The authors also studied a number of different mobile phases and found that several cis-'rans isomers of the metal β-diketonates could be separated in the normal-phase mode.

3.2.2. β-Ketoamines

Normal-phase HPLC (liquid–solid) was employed by Uden and Walters[70] for the isocratic separation of neutral copper and nickel chelates of N,N'-ethylenebis(acetylacetoneimine) [H$_2$(enAA$_2$)] and N,N'-ethylenebis(salicylaldimine) [H$_2$(enSal$_2$)] on 10-μm diameter silica with UV detection at 254 nm.

Reversed-phase and normal-phase HPLC separations of several β-ketoamine metal chelates were reported by Gaetani et al.[71]. Included in the study were several
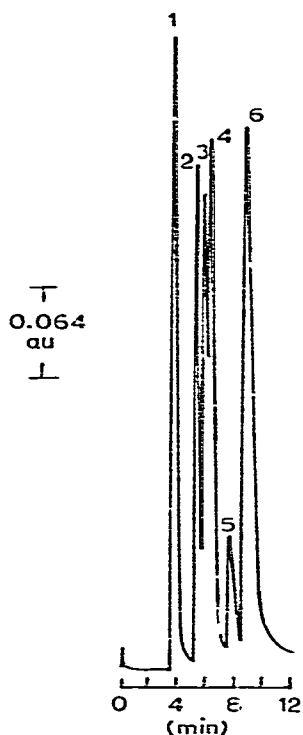
Fig. 9. HPLC separation of metal acetylacetonates on a 5-μm Partisil column (25 cm). Conditions: eluent, 1,2-dichloroethane–methanol (98.6:1.4); detector, UV at 280 nm. Components: (1) Be(acac)$_2$; (2) Ru(acac)$_3$; (3) Rh(acac)$_3$; (4) Cr(acac)$_3$; (5) Al(acac)$_3$; (6) Co(acac)$_3$. Reprinted with permission from ref. 69 (Preston Publications).

chelates of H$_2$(enAA$_2$), N,N'-trimethylenebis(acetylacetoneimine) [H$_2$(tmAA$_2$)] and N,N'-ethylenebis(benzoylacetoneimine) [H$_2$(enBA$_2$)] with Co(II), Ni(II), Cu(II) and Pd(II). Rapid separations of CoenAA$_2$, NienAA$_2$ and CuenAA$_2$ (< 5 min) were obtained on a reversed-phase C$_{18}$ column with methanol–phosphate buffer (pH 7.8). Also, Ni(en)BA$_2$ and Cu(en)BA$_2$ were successfully separated on a polar (normal-phase)–NH$_2$ bonded-phase column using a methanol–phosphate buffer. Detection limits were in the sub-nanogram range for nickel and copper.

3.2.3. Hydrazones and semicarbazones

In 1973 Heizmann and Ballschmiter[72] reported the first successful liquid–solid HPLC separations of mercury(II), copper(II), lead(II) and zinc(II) chelates of bis-acetylbisthiobenzoylhydrazone. They used Merckosorb SI 60, Perisorb A and alumina as the column packing and reported satisfactory correlation with TLC data and limits of detection in the nanogram range for Hg(II) and Cu(II). This paper represents one of the important early breakthroughs in the field of HPLC of metal chelate systems.

In a later paper, Heizmann and Ballschmiter[73] demonstrated the possibilities of separating the 1,2-diketobisthiosemicarbazone and 1,2-diketobisthiobenzhy-drazone chelates of Cd(II), Hg(II), Cu(II), Ni(II) and Co(III) isocratically on silica as

the adsorbent. Mobile phases which were studied consisted mostly of binary mixtures containing benzene, n-hexane, chloroform, acetonitrile, cyclohexane and n-heptane. Detection was performed by monitoring the UV absorbance at 360 nm. It was shown that the elution characteristics of the chelates were influenced by varying the substituents on the ligand molecules. The metal chelates could be detected at the nanogram level.

3.2.4. Dithiocarbamates

Uden and Bigley[74] employed the liquid–solid adsorption mode for the isocratic separation of Cu(II), Ni(II) and Co(III) diethyldithiocarbamates on 8-μm spherical silica particles with an eluent consisting of 5 % acetonitrile, 15 % diethyl ether and 80 % Skelly B (light petroleum hydrocarbon). Detection of the chelates was accomplished by means of UV absorption at 254 nm; this method was supplemented by using a metal-specific argon-plasma detector in order to demonstrate that the observed peaks corresponded to the metal chelates and not decomposition products. A detailed description of the construction and evaluation of this metal-specific HPLC detection system has been published by Uden et al.[75]. This paper was the first report in which a d.c. argon plasma emission source (DCP) was used for the detection of metallic species after HPLC separation. The authors pointed out that the advantages of plasma sources are that they generally exhibit superior sensitivities (sub-nanogram levels) and detection limits compared to flame techniques and that they are also suitable for multi-element determinations. Model compounds which were used to evaluate the DCP included β-diketonate, β-ketoamine and diethyldithiocarbamate complexes of Cu(II), Ni(II), Cr(III), Co(III) and Hg(II).

Heizmann and Ballschmiter, in their paper[73] dealing with the separation of semicarbazone and hydrazone metal complexes, also included a study of the separation of the dithiocarbamates of Cd(II), Hg(II), Cu(II), Ni(II) and Co(II) using normal-phase HPLC and UV detection at 360 nm.

In 1979, Liška et al.[76] described the normal-phase separation of mixtures of Zn(II)–, Cu(II)–, Mn(II)–, Ni(II)–, Pb(II)–, Co(II)–, Cd(II)– and Fe(II)–DTC complexes on LiChrosorb SI 60 (10 μm) with chloroform–hexane as the mobile phase and UV detection. They also studied the influence of the nature of the ligand N-substituents and mobile phase composition on the chromatographic properties of the Cu(II)–DTC complexes.

Schwedt[77] has reported a 12-min isocratic separation of Se–, Cu(II)–, Ni(II)– and Pb(II)–DTC complexes by reversed-phase HPLC using acetonitrile–water mixtures as the mobile phase and UV detection at 254 nm. He has also reported the separation of Se, Cr(III), Ni(II) and Co(II) chelates by reversed-phase HPLC[78]. Additionally, the possibilities of reversed-phase HPLC separation of the tetramethylenedithiocarbamates of Cd(II), Pb(II), Ni(II), Co(II), Zn(II), Cu(II) and Hg(II) on LiChrosorb RP-8 using methanol–water (70:30) and UV detection was studied[79]. This separation is shown in Fig. 10.

Edward-Inatimi and Dalziel[80] have reported a multi-element analysis scheme using solvent extraction and normal-phase HPLC on Hypersil with spectrograde benzene and UV detection at 280 nm. They separated mixtures of DTC complexes of Cu(II), Ni(II), Hg(II), Pb(II), Co(II), Mn(II) and Bi(III) and were able to determine these in the ppb range. They tested their procedure successfully using standard steels.
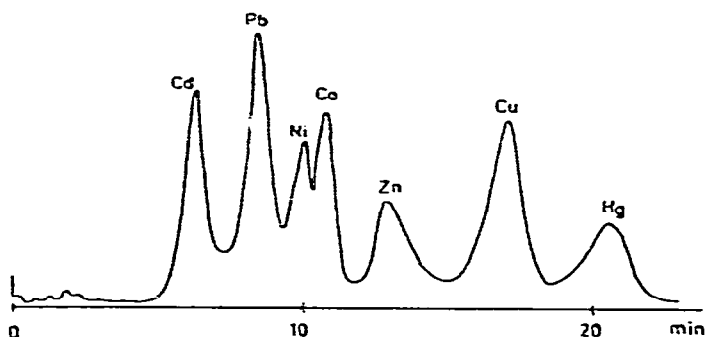
Fig. 10. Reversed-phase HPLC of metal tetramethylenedithiocarbamates. Column, 250 × 4.6 mm I.D., LiChrosorb RP-8 (10 μm); mobile phase, methanol–water (70:30); flow-rate, 1.2 ml min; detector, 254 nm. Reprinted with permission from ref. 79 (Vieweg).

Reversed-phase HPLC with electrochemical detection has been used by Bond and Wallace[81] for a specific and very sensitive determination of copper as its diethyl-dithiocarbamate or its pyrrolidinedithiocarbamate complex [Cu(DTC)$_2$]. Two procedures are reported. In one of these. the copper is complexed prior to injection; the second method involves adding a dithiocarbamate salt to the moving phase causing complex formation *in situ* when the metal ion solution is injected. Cyclic voltammograms showed that these Cu(DTC)$_2$ chelates undergo reversible one-electron reduction and oxidation steps at platinum, gold and glassy carbon electrodes in an acetonitrile–water medium. It was found that electrochemical detection is extremely sensitive with a limit of detection of 1 ng of copper. A large number of anions and cations (in 20-fold excess) were tested as potential interferents. Several of the metal ions such as Cd(II), Pb(II), Co(III) and Fe(III) were found to produce HPLC peaks, but did not interfere with the determination. Applications to a wide range of industrial effluents were successful. The detection limit of the HPLC method is comparable to that of atomic absorption. but the HPLC procedure would be the preferred technique when only small volumes of samples are available.

3.2.5. Dithizonates
Dithizone (DZ) has been used extensively as an effective analytical solvent-extraction reagent in the past. Its advantages as a potential ligand for multi-element HPLC analysis include its ability to form neutral chelates with a large number of divalent metal ions as well as high chelate molar absorptivities (30,000–100.000 l/mol · cm) in the visible region of the spectrum (500–530 nm). This property permits the elution of DZ complexes from HPLC columns with UV-absorbing solvents.

Lohmüller et al.[82] reported the first successful separation of several groups of DZ chelates including those of Pb(II), Zn(II), Cd(II), Hg(II), Cu(II) and Co(II) using normal-phase HPLC on 30-μm silica particles with a series of organic moving phases (benzene, carbon tetrachloride, tetrahydrofuran, acetonitrile, toluene and chloroform). The absorbance at 525 nm was monitored. Several three- and four-component mixtures were successfully separated. A typical three-component separation of the mercury, nickel and cobalt chelates is shown in Fig. 11. An HPLC separation of several metal dithizonates, M(DZ)$_2$, was developed concurrently with the work of
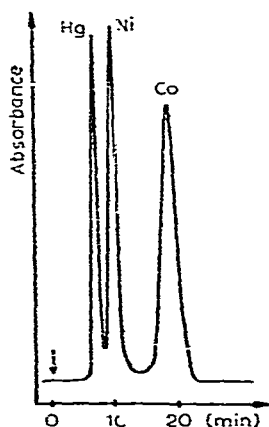
Fig. 11. HPLC separation of metal dithizonates. Packing, LiChrosorb SI 60 (30 μm); glass column, 300 × 2 mm; solvent, benzene; flow-rate, 10 ml/h. Reprinted with permission[82].

Lohmüller *et al.* and was reported in 1978 by O'Laughlin and O'Brien[83]. This study included an investigation of the behavior of Ni(II), Co(II), Cu(II), Zn(II), Hg(II) and Pb(II) dithizonates on 37–50-μm μ-Corasil and 10-μm μ-Porasil with a variety of mobile phases ranging in polarity from heptane to isopropanol. The best separations, however, were obtained on μ-Porasil with toluene as eluent and a variable-wavelength UV–visible spectrophotometer operated at 275 nm. Detection limits for the metals ranged from 10 to 100 ng.

Henderson *et al.*[84] have recently published a normal-phase HPLC separation of a number of divalent dithizonates using non-aromatic solvents and glass-lined columns with a variety of binary and ternary mobile phases containing acidic or basic modifiers such as acetic acid and various amines. Detection was accomplished by visible absorption at 475–525 nm. They also emphasize the advantages of DZ as a multi-element ligand for HPLC analysis, *i.e.* many divalent complexes have very high formation constants and very high molar absorptivities. HPLC separations of DZ complexes of Co(II), Cu(II), Pb(II), Zn(II) and Cd(II) are reported.

3.2.6. 8-Hydroxyquinolates

Several 8-hydroxyquinolates including those of Cu(II), Co(II), Ni(II), Hg(II) and Fe(II) were separated by Berthod *et al.*[85]. They used reversed-phase HPLC and three modes of detection: UV absorption, electrochemical and atomic absorption. This was the first reported HPLC separation of metal 8-hydroxyquinolates.

The 8-hydroxyquinoline chelates of Al(III) and Co(III) were recently separated by Hambali and Haddad[86]. They used a silica column and 5 % methanol–chloroform as the mobile phase with UV detection at 254 nm. A rapid separation was obtained for the two compounds in less than 5 min and detection limits were in the nanogram range for both metals.

It appears that 8-hydroxyquinoline would be an ideal ligand to use for the separations of multi-element mixtures by HPLC because of its ability to complex with many metal ions to produce neutral chelates. Furthermore, since several of these chelates possess native fluorescence, it is likely that the sensitivity could be improved considerably using this mode of detection.

3.2.7. 1,10-Phenanthrolines and ethylenediamines

Paired-ion HPLC was used successfully for the separation of the 1,10-phenanthroline (phen) chelates of Fe(II), Ni(II) and Ru(II) by O'Laughlin and Hanson[87]. They found that an excellent separation of $Ni(phen)_3^{2+}$ or $Ru(phen)_3^{2+}$ from $Fe(phen)_3^{2-}$ could be achieved on a μBondapak-CN column with methanol–(0.5%) aqueous acetic acid (20:80) containing 0.015 M methanesulfonate as the counter-ion. Detection was achieved by monitoring the UV absorption signal at 265 nm. Nickel was determined in the 0–30 ng range by monitoring the elution of $Ni(phen)_3^{2+}$ at 265 nm. The $Ru(phen)_3^{2+}$ and $Ni(phen)_3^{2+}$ species eluted with the same retention volume. Iron and nickel or iron and ruthenium can thus be determined simultaneously. The elution of $Ru(phen)_3^{2-}$ was confirmed with a fluorescence detector and that of $Ni(phen)_3^{2+}$ with atomic absorption. The effect of various ion-pairing reagents, pH and mobile phase composition on the retention of these complexes was studied. No elution peaks were observed for the labile phen complexes of Co(II), Zn(II) or Cd(II). In a later report by O'Laughlin[88], the three complexes $Ni(phen)_3^{2+}$, $Ru(phen)_3^{2+}$, and $Fe(phen)_3^{2+}$ were separated from one another as well as from the ligand and from a bis-$Ru(phen)_2X^{2+}$ chelate. This separation is shown in Fig. 12 and was accomplished by paired-ion HPLC on Partisil SCX with acetonitrile–water–perchloric acid mixtures as the mobile phase, and detection at 265 nm.

Cation-exchange chromatography was employed by Yoneda et al.[89] who separated two pairs of tri- and divalent ethylenediamine (en) and phen chelates:
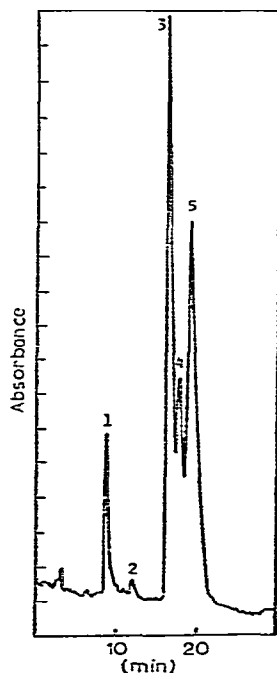

Fig. 12. HPLC separation of $Ni(phen)_3^{2+}$, $Ru(phen)_3^{2+}$ and $Fe(phen)_3^{2-}$. Column: μPartisil-SCX; mobile phase: acetonitrile–water (80:20)–0.06 M HClO$_4$; detector: UV at 265 nm. Components: (1) phen; (2) $Ru(phen)_2X^{2-}$; (3) $Ni(phen)_3^{2+}$; (4) $Ru(phen)_3^{2-}$; (5) $Fe(phen)_3^{2-}$. Reprinted with permission[88].

$[Co(en)_3]^{3-}$–$[Ni(en)_3]^{2-}$ and $[Co(phen)_3]^{3-}$–$[Fe(phen)_3]^{2-}$. The separation was achieved on an SP-Sephadex cation-exchange column using various concentrations of aqueous potassium bromide and potassium sulfate solution as eluents. The absorbance of the various complexes was monitored at appropriate visible wavelengths. The chromatographic behavior and order of elution of these complexes as a function of ionic strength was also studied. Yoneda[90] has discussed the stereochemical aspects of optical resolution of several octahedral ethylenediamine, diethylenetriamine, triethylenetetramine and amino acid metal chelates of Co(III) and Cr(III) on ion-exchange resins.

3.2.8. Porphyrins

A report published recently by Richter and Rienits[91] deals with the HPLC separation of zinc and magnesium chelates of protoporphyrin IX as the dimethyl ester derivatives. The complexes were eluted on 10 μm LiChrosorb with acetone–hexane (15:85) with detection at 405 nm. This determination was successfully applied to the examination of the protoporphyrin IX chelates arising from the incubation of cucumber etiochloroplast and wheat etioplast.

4. CONCLUSION

HPLC was first applied to metal–organic systems in 1969; approximately 50 papers on the subject appeared in the 9 years since then. This number has approximately doubled in the past 3 years, a fact which indicates that applications of HPLC in organometallic and coordination chemistry are being developed rapidly. This trend may be expected to continue.

HPLC has been shown to be an effective means for the separation and determination of many different types of coordination complexes and organometallic compounds. One area which holds a great deal of potential is the use of HPLC for multi-element determinations by employing a single ligand to complex mixtures of dissolved metal ions prior to HPLC separation. The advantage of such an approach is the possibility of achieving multi-element determinations in a single run. It should be pointed out, however, that chromatographic adjustment of selectivity for the elution of metal chelates is characterized by narrower limits in reversed- or normal-phase HPLC than would be possible in ion-exchange separations of the free metal ions or their halo-complexes. Also, the development of HPLC element specific detectors such as ICP and others based on atomic absorption and electrochemical properties has made available sensitive and selective methods for the detection and determination of trace amounts of metals.

Among the exciting developments in organometallic chemistry in recent years has been the use of HPLC to monitor the course of reactions, identify intermediates and determine reaction rates and the composition of equilibrium mixtures. The separation of isomeric complexes is often possible by HPLC when all other methods fail. The most significant advantages of HPLC for the study of inorganic systems are that the method is capable of excellent resolution and low level detection of highly non-volatile and thermally unstable systems. It is principally because of these reasons that we expect to see much greater use being made of this technique in inorganic chemistry as more chemists become aware of its possibilities.

5. SUMMARY

A review of the applications of high-performance liquid chromatography to organometallic compounds and metal coordination complexes since 1969 is presented. The review covers high-performance liquid chromatographic separations which have been reported for arene metal carbonyls, cyclopentadienyl metal carbonyls, dicyclopentadienyl complexes, metal cluster complexes, metallocarboranes, cyclopentadienyl–cycloolefin complexes, alkyl and aryl complexes and organopolymetallic compounds. The second part of the review summarizes separations of metal coordination compounds, including complexes of the following ligand types: β-diketonates, β-ketoamines, hydrazones, semicarbazones, dithiocarbamates, dithizonates, bipyridines, phenanthrolines, 8-hydroxyquinolates, EDTA, ethylenediamines, amino acids, carbonates, camphorates, diphosphonates, crown ethers and porphyrins.

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