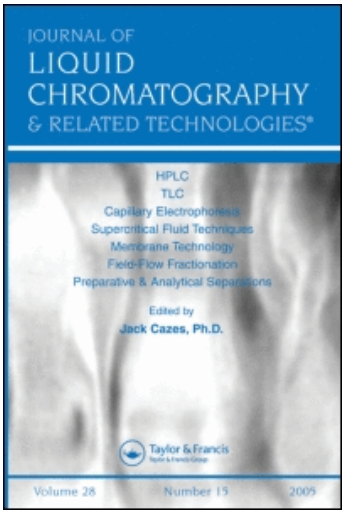

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High Performance Liquid Chromatography of Metal Chelates

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HIGH PERFORMANCE LIQUID CHROMATOGRAPHY OF METAL CHELATES

by

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1. INTRODUCTION

Liquid chromatography, especially ion-exchange chromatography, has been used very successfully for the separation of ionic metal chelates for many years. More recently, ion chromatography, an ion exchange method proposed by Small et al. (1) in 1975 for the determination of ionic species at low concentrations has undergone a period of rapid growth. Fritz (2) has recently published a book on ion chromatography which reviews work done in this field. Classical chromatographic methods for the separation of inorganic species and metal chelates has been reviewed by Michal (3) and, the somewhat more recently developed, reversed-phase extraction chromatographic methods by Cerrai and Ghersini (4) and Braun and Ghersini (5).

The first reported use of modern liquid chromatographic methods or high performance liquid chromatography, HPLC, of metal chelates is generally credited to Huber et al. (6). Rapid improvements in HPLC methodology over the past decade have significantly enhanced the potential usefulness of HPLC for the separation and determination of

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metal chelates and organometallic species. This is being recognized by a rapidly increasing number of scientists in many research areas including; analytical chemists interested in trace metal analysis; inorganic and coordination chemists interested in the separation of metal chelates and organometallic species; environmental chemists interested in speciation studies; biochemists investigating biologically active metal-containing species; and scientists in nuclear medicine trying to prepare organ selective imaging reagents, to name only a few.

Applications of HPLC to organometallic and metal coordination compounds have been reviewed by Willeford and Veening (7,8) and Schwedt (9). A book on liquid chromatography applied to environmental analaysis edited by Lawrence (10) is in press. Wong (11) has reviewed applications of HPLC to radiopharmaceuticals.

The present review stresses applications of HPLC to the separation and determination of metal chelates. Organometallic compounds are not included except where the work pertains to the separation or detection of metal chelates. An example might be the study of a metal specific detector which could be used for both types of compounds. Only selected papers on ion-exchange methods are included which, for example, might discuss a metal selective or specific detection system or involve chelating reagents in the separation process. No attempt was made to cover the field of ion chromatography.

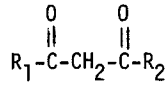
The author has attempted to include all the work reported on applications of HPLC to the separation of metal chelates through 1982 and more recent papers which came to his attention. Because work in this area is published in so many diverse journals, and the emphasis in many papers is not on HPLC, some very relevant work may have been overlooked. To partially compensate for these possible omissions, a

number of papers are cited which bear only indirectly on the HPLC of metal chelates. These include, for example, selected papers where metal chelates are used as modifiers or ion-pairing reagents for the separation of organic solutes. This particular area is growing rapidly and full coverage is outside the scope of the review. It is hoped only that the papers cited will introduce readers not familiar with this area to its potential usefulness.

2. METAL CHELATE SYSTEMS

2.1 β-Diketones

General Structure



Name	Abbreviation	Structure	
		R_1	R_2
1. Acetylacetone	H(acac)	methyl	methyl
2. Trifluoroacetylacetone	H(tfa)	trifluoromethyl	methyl
3. Dipivaloylmethane	H(dpm)	tert-butyl	tert-butyl
4. 1,1,1,2,2,3,3-Heptafluoro-4,6-octanedione	H(fod)	n-heptafluoropropyl	ethyl
5. 1,1,1,2,2,3,3-Heptafluoro-7,7-dimethyl-4,6-octanedione	H(hpm)	n-heptafluoro	tert-butyl

7.	Pivaloylacetone	H(pacs)	tert-butyl	methyl
8.	Thenoyltrifluoroacetone	H(tta)	2-thienyl	trifluoromethyl
9.	Furoyltrifluoroacetone	H(fta)	2-furyl	trifluoromethyl
10.	Benzoylacetone	H(bza)	phenyl	methyl
11.	Benzoyltrifluoroacetone	H(bzta)	phenyl	trifluoromethyl

Huber et al. (6) are generally credited as the first investigators to report on the separation of metal chelates by HPLC. They showed that the metal chelates of Be(II), Cu(II), Al(III), Cr(III), Ru(III) and Co(III) with H(acac) were eluted in the order given in under 25 minutes as a series of symmetrical and well resolved peaks. The elution of the chelates was monitored photometrically at 310 nm using a homemade flow-through photometric cell with a pathlength of 10 mm and a volume of 7.5 µl. A thick-walled glass column (2.7 mm i.d. and 10- or 25- cm in length) packed with diatomaceous earth with particle sizes in the ranges 5-10 µm and 10-20 µm was used. The stationary phase, consisting of the water-rich phase of a ternary two-phase system of water-2,2,4-trimethylpentane-ethanol, was supported on the diatomaceous earth. The mobile phase was the organic-rich phase which was pumped through the column at a constant flow rate of 1.8 mm sec^{-1} with a pressure drop of 17 bars (246 psi). A pre-column was employed to keep the mobile phase saturated with respect to the stationary phase and samples were injected onto the column through a rubber septum.

Although the apparatus used by these authors was somewhat primative compared to modern instrumentation and the non-bonded stationary phases posed some problems, they did report 2,200 theoretical plates for the Co(III) complex and a calculated peak capacity of their column of 14 peaks in 20 minutes. This is quite similar to what has been reported to date for metal coordination compounds by other investigators using more

sophisticated apparatus and modern columns. Several prescient comments were made by these authors. They attributed asymmetric peaks (tailing) for the Ni(II), Al(III) and Fe(III) complexes to hydrolysis reactions and found more symmetric peaks were obtained by including small amounts of the ligand in the mobile phase. Up to three peaks were observed for the Al(III) complex which had been allowed to stand for two weeks before it was injected into the chromatograph. These were attributed to hydrolysis products of the complex and it was suggested mixed hydroxy-acetylacetonato complexes could be separated by this technique. They also reported that the selectivity factor is determined by the metal ion and is nearly independent of the ligand for $Co(acac)_3$ and $Cr(acac)_3$, and $Co(tfa)_3$ and $Cr(tfa)_3$.

Tollinche and Risby (12) later reported on the separation of metal chelates by HPLC with acetylacetone; trifluoroacetyacetone; dipivaloylmethane, H(dpm); and 1,1,1,2,2,3,3-heptafluoro-4,6-octanedione, H(fod). The H(dpm) ligand was incorrectly named as a 2,2,7,7-complex in this paper and it might be noted that other authors have used H(fod) for the compound with R_1 as in Table 1 but R_2 = tert-butyl. The use of alumina, silica gel, polyurethane, and bonded phase column packings was studied and silica gel columns were found to give the best separations. The elution order was $Be(acac)_2$, $Ru(acac)_3$, $Cr(acac)_3$, $Al(acac)_3$ and $Co(acac)_3$ on a 10-μm Partisil column with a 95% CH_2Cl_2 and 5% CH_3CN mobile phase. The separation of cis(or fac) and trans(or mer) geometrical isomers of the unsymmetrical chelates of $Cr(tfa)_3$, $Co(tfa)_3$, $Ru(tfa)_3$, $Rh(tfa)_3$, $Co(fod)_3$ and $Cr(fod)_3$ on 5-μm and 10-μm Partisil and HIEF MicroPart silica columns were reported with various mobile phases including; n-heptane-isopropanol, n-hexane-benzene, toluene,

n-heptane-dichloromethane, n-hexane, and n-pentane. The trans (or mer) isomer always eluted first as expected due to less interaction with the polar silica stationary phase. Some separation of metal-acac complexes was reported on bonded phase columns (C-8 and C-18) but no retention of the metal-dpm or metal-hpm complexes for any mobile phase studied.

O'Brien (13) studied the separation of Ni(II), Fe(II), Cu(II), Mn(II) and Co(II) complexes with H(hfa), H(tfa) and H(hpm) as well as adducts of these species with di-n-butylsulfoxide (DBSO) on columns packed with alumina, Silica Gel H, Partisil A, Kel-F impregnated with DBSO, Corasil, and Corasil-C18 with various mobile phases including toluene, chloroform, ethyl acetate, and methanol-water. He obtained sharp peaks for the Ni(II), Mn(II) and Co(II) complexes with H(hfa) with the Corasil-C18-ethyl acetate system but no separation. A good separation of the $Ni(hfa)_2$ and $Mn(hfa)_2$ complexes was obtained on a Porasil A column with ethyl acetate as the mobile phase. Separation of the $Cu(hfa)_2$-2DBSO, $Ni(hfa)_2 \cdot 2DBSO$ and $Mn(hfa)_2 \cdot 2DBSO$ complexes was observed on the Corasil-C18 column with toluene as the eluent but the column was very inefficient and the peaks were very broad. Well shaped peaks but little separation was found for the Fe(II), Co(II), Ni(II), Mn(II) and Cu(II) complexes with H(hfa) on a 35-50 μm Porasil A column with ethyl acetate as the mobile phase. Linear calibration plots were obtained over the range 0.5-600 ng of metal injected with photometric detection at 300 nm when the chelates were injected one at a time.

Uden et al. (14) reported the mer and fac complexes of Co(III) and Cr(III) with various unsymmetrical β-diketones including H(tfa), H(bza), H(pac) could be separated by HPLC. The mer complexes of $Co(bza)_3$ and of $Cr(pac)_3$ eluted from a 10-μm Partisil column with

dichloromethane-acetonitrile as the mobile phase before the fac complexes. These authors used atomic emission spectroscopy with a DC plasma source to selectively detect metal containing species eluted from the column as well as photometric detection at 254 nm. The mer and fac species were collected and further characterized by mass spectrometry. The separation of geometrical isomers of the mixed ligand species of $Cr(tfa)(hfa)_2$ and $Co(tfa)(hfa)_2$ was also reported.

Schwedt (15) reported a detection limit of 150 pg of berylium based on the elution of $Be(acac)_2$ from a 7-μm silica column with photometric detection at 254 mm. Willett and Knight (16) determined chromium in orchard leaves by HPLC based on the elution and photometric detection of $Cr(acac)_3$ and claimed a detection limit of 1 ng. These authors reported that $Cr(acac)_3$ was irreversibly bound to active silanol groups on a μ-Porasil column but that reproducible results were obtained on a μ-Bondapak C18 column with 66% water- 36% acetonitrile as the mobile phase. Numerous other well defined peaks in the chromatograms obtained were attributed to other metal acetylacetonates but these were not identified. The identify of the chromium complex was confirmed by mass spectrometry.

Gurira and Carr (17) recently reported on the liquid chromatographic separation of a number of kinetically inert metal acetylacetonates and benzoylacetonates on bonded-phase columns (25-cmx4.6mm i.d., 5-μm Ultrasphere-ODS and a 15-cmx4-6 mm i.d., 5μm-Supelco-C18) with acetonitrile-water and methanol-water as mobile phases. The separation of seven complexes in less than 12 minutes is shown in Figure 1. The resolution was found to increase as the acetonitrile to water ratio decreased and the iridium and ruthenium acac complexes, which overlapped under the conditions shown in Figure 1,

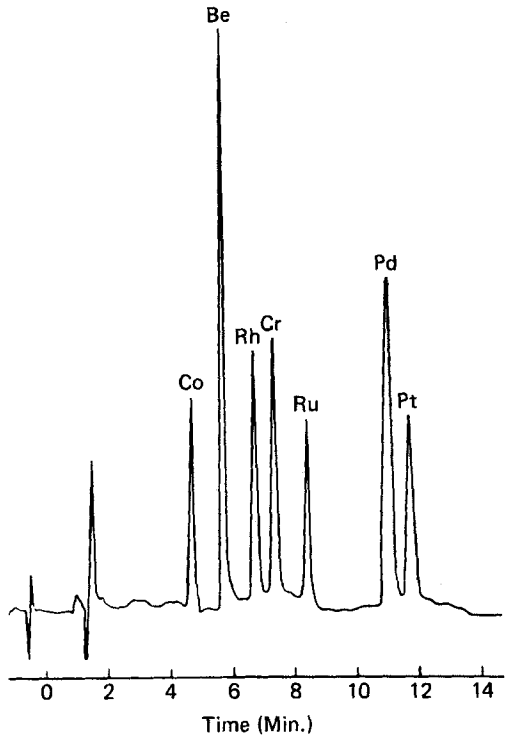


Fig. 1. HPLC separation of ACAC metal complexes on an Ultrasphere C_{18} column, 25-cm x 4.6-mm i.d., 5-µm particle; solvent, 40% acetonitrile/water; flow rate, 2 ml/min; detection at 254nm; 0.04 AUFS; sample, 20 µl of 1 x 10^{-5} in chelates. Reprinted by permission from Reference 17 (Preston Public.).

could be partially resolved with a 30-70 acetonitrile-water mobile phase. Mixtures of $Cr(acac)_3$ and $Cr(bza)_3$ gave two well defined peaks with the acac complex eluting first. Linear calibration curves were obtained over two orders of magnitude for the $Cr(acac)_3$ and $Co(acac)_3$ complexes with the curves passing through the origin indicating no decomposition or irreversible sorption at low

concentrations. Detection limits of less than 1 ng for both complexes were reported.

Yammamota et al. (18) reported on the gel chromatography of $Cr(acac)_3$ and $Co(acac)_3$ on styrene-divinylbenzene copolymer columns (TSK G-1000H, pore size 10^1A). Calibration curves of molar volume versus K_D were prepared based on the elution of Polystyrene 4000 (K_d taken as = 0 for this species) n-docosane, n-hexadecane, n-decane and n-pentane (K_d = 1). The authors noted that Irving (19) found partial molar volumes of $Cr(acac)_3$ of; 266.7 ml in benzene; 269.3 ml in tolune; 256.8 ml in carbon tetrachloride; and 269.5 ml in chlorofrom which were not too different than the molar volume of 273.3 for $Cr(acac)_3$ in the solid state. The elution of $Cr(acac)_3$ and $Co(acac)_3$ from the above column, however, gave effective molar volumes far different in many cases than those reported by Irving. Values obtained for the $Cr(acac)_3$ and $Co(acac)_3$ molar volumes, respectively, were, 250 and 240 ml in benzene; 160 and 140 ml in toluene; 120 and 110 ml in carbon tetrachloride; 350 and 340 ml in chloroform; and 160 and 140 ml in p-xylene.

The variation in K_d (or effective molar volume) of these chelates on gel chromatography with these different solvents was attributed to sorption effects on the column. An attempt was made to rationalize the variation in K_d with solubility parameters of the solvents, the complexes, and the gel and to rationalize the effective molar volumes found with solvation of the complexes in some solvents. The lower plate counts obtained for the complexes (N=230 to 3,000) than with n-alkanes (N$\sim$7,000) together with the molar volume data do indicate adsorption effects are important with the non-polar solvents and are probably the reason for the larger differences in K_d values observed for the complexes with p-xylene.

Noda et al., (20) used gel chromatography on a polystyrene-divinylbenzene polymer (Shodex 801 gel, 10-15 μm) and an ethyl acetate mobile phase to isolate the mixed ligand complex Be(acac)(tta) from $Be(acac)_2$ and $Be(tta)_2$. Three well resolved peaks were obtained using UV detection at 308 nm and $Be(tta)_2$, Be(tta)(acac) and $Be(acac)_2$ eluted in the order given. The mixed ligand complex was eluted without dissociation and was characterized by its UV and H-NMR spectra.

Saitoh and Suzuki (with others) (21-24) had previously published a series of papers on the gel chromatography of β-diketones and their metal complexes. They found that $Cr(acac)_3$ and H(acac) were eluted from a Merckogel OR-2000 column with chloroform as the mobile phase as two well separated peaks (21). Retention behavior was reported to be due entirely to the sieve effect and K_d values were independent of concentration and column temperature with no evidence of adsorption on the stationary phase. In a later paper (22) these authors extended this study to other metal complexes: $Co(acac)_3$, $Fe(acac)_3$, $Cr(acac)_3$, $Al(acac)_3$, $Cu(acac)_2$, $Ni(acac)_2$ and $Be(acac)_2$. A general correlation of increasing retention with decreasing molar volume was found for H(acac) and the metal complexes on a Merckogel OR-2000 column with tetrahydrofuran as the mobile phase but these species fell on a different curve than the n-alkanes. Skewed peaks were observed for Cu(II) and Ni(II) complexes indicating on-column decomposition or adsorption. In a later paper (23), other mobile phases were studied. Effective molar volumes greater than reported by other methods were observed for the trivalent metal complexes in chlorofrom except for $Fe(acac)_3$. Other solvents studied included 1,4-dioxane, benzene, toluene, tetrahydrofuran, ethylacetate, acetone, ethylmethyketone,

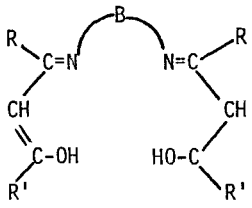
butylacetate, and methanol. Effective molar volumes for the trivalent chelates and $Be(acac)_2$ generally decreased in the order given for these solvents with K_d values greater than one for the last four solvents. Appreciable interaction of the metal chelates with the stationary phase is indicated which appeared to be quite dependent on the solvent. The same authors (with Shibukawa) (24) studied the gel chromatography of seven different β-ketones including; H(acac), H(tta), H(tfa), H(fta), H(bza), H(bzta), and H(dpm), and the Cr(III) chelates of all these species on two polyvinylacetate gels (Merckogel OR-PVA 500 and Merckogel OR-PVA 2000) and two polystrene gels (Bio-Beads S-X8 and Poragel 60A) with p-dioxane as the mobile phase. K_{av} values based on the relationship,

$$K_{av} = (V_e - V_o)/V_x$$

were found for all species studied. V_e is the elution volume of the solute, $V_x = V_t - V_o$ is the volume of the swollen gel phase, V_t the total column volume and V_o the retention volume of polystyrene (200,000 mol. wt.). The K_{av} values were found to depend strongly on the gels and could not be correlated with the molecular weights of the compounds. The dependance of the β-diketones and the metal chelates on the gel was different than for the n-alkanes as found in an earlier paper (22).

2.2 β-Ketoamines

General Structures



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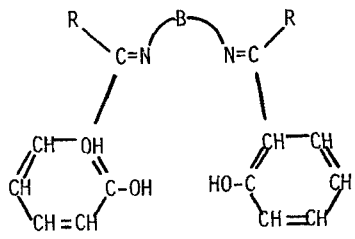


II

Name	Abbreviation	Structure
1. bis(acetylacetone)ethylene-diimine	H_2(bae) (Ref 25,26; H_2enAA_2) (Ref 27; H_2enAA)	I. R=R'=CH_3, B=ethylene
2. bis(salicylaldehyde)ethylene-diimine	H_2(salen) (Ref 25,26; H_2enSal_2)	II. R=H
3. bis(acetylacetone)propylene-diimine	H_2(bap) (Ref 26; H_2pmAA_2) (Ref 27; H_2(tm)AA)	I. R=R'=CH_3, B=propylene
4. bis(acetylacetone)butylene-diimine	H_2(bab) (Ref 26; H_2bmAA_2)	I. R=R'=CH_3 B=butylene
5. bis(phenylacetylacetone)-ethylenediimine	H_2(bpae) (Ref 27; H_2(en)BA)	I. R=CH_3 R'=phenyl B=ethylene
6. bis(trifluoroacetylacetone)-propylenediimine	H_2(btfap) (Ref 12; H_2(pntfa)	I. R=CH_3,R'=CF_3 B=propylene
7. bis(trifluoroacetylacetone)-ethylenediimine	H_2(btfae) (Ref 12,26; H_2(entfa))	I. R=CH_3,R'=CF_3 B=ethylene
8. bis(trifluoroacetylacetone)-butylenediimine	H_2(btfab)	I. R=CH_3-R'=CF_3- B=butylene

The ligands called β-ketoamines (7,12,25,26) are Schiff bases formed by the condensation of ethylenediamine, propylenediamine or butylenediamine with acetylacetone, trifluoroacetylacetone, phenylacetylacetone or salicyaldehyde. The separation of metal chelates with these ligands by HPLC was studied by several groups.

Uden and Walters (25) studied the separation of Ni(bae), Cu(bae), Ni(salen) and Cu(salen) on 10-μm Partisil columns using 80%

methylenedichloride-20% acetonitrile as the mobile phase. The Ni(bae) and Cu(bae) were eluted in the order given and well resolved. The Ni(salen) and Cu(salen) were also eluted in the same order with greater retention and resolution. The nature of the species eluted was confirmed by mass spectroscopy, ultraviolet spectroscopy and the Cu(salen) species by electron spin resonance spectroscopy. Peak areas were proportional to amount of chelate injected over the range 25 ng to 240 μg of chelate based on photometric detection at 254 nm. The peaks were rather broad and the Cu(salen) peak tailed. Plate counts with N ranging from 309-493 were reported.

The above authors (with Parees) (26) reported on the chromatography of Cu(II), Ni(II) and Pd(II) complexes of H_2(bae), H_2(salen), H_2(bap), H_2(bab) and H_2(btfae) using a 10-μm Bondapak-C18 column with methanol-water-acetonitrile mobile phases. The Ni(II) complexes with H_2(bae), H_2(bap) and H_2(bab) were well resolved using a 55% methanol-45% water mobile phase and eluted in the order given showing greater retention as methylene groups were added to the bridging group B in structure I. The same order was observed for the Cu(II) chelates with the latter complexes all having longer elution times than their Ni(II) analogs. The Pd(II) complex with H_2(bae) generally eluted between the Ni(II) and Cu(II) complexes but its elution behavior relative to these complexes varied with mobile phase composition. With 50% methanol-50% water Pd(bae) eluted with the Ni(bae) complex while with 20% acetonitrile-80% water it eluted after the Cu(bae) species. The elution order of the Cu(btfae), Cu(btfap) and Cu(btfab) complexes increased in the order given with a 50% acetonitrile-50% water mobile phase and the Ni(II) complexes with the same ligands followed a similar pattern. The Ni(II) species all eluted after the Cu(II) analogs which

is the reverse of what was observed for the H_2(bae), H_2(bap) and H_2(bab) complexes. The Cu(salen), Ni(salen) and Pd(salen) complexes eluted in the order given with a 20% acetonitrile-80% water mobile phase. A successively higher water content was required to resolve the metal complexes of H_2(bae), H_2(btfae), and H_2(salen). It was concluded that the H_2(bae) complexes showed an anomalous elution behavior in the reversed phase mode in that the elution order was the same (Ni(bae) before Cu(bae)) as on a silica column. The elution order of the H_2(btfae) and H_2(salen) metal complexes were in reverse order from those of the H_2(bae) metal complexes on the C18 column.

Gaetani et al. (27) reported on the chromatography of Co(II), Ni(II), Cu(II) and Pb(II) complexes with H_2(bae), H_2(bap) and H_2(bpae) on both C18 (10 μm-Micropak CH) and a 3- aminopropyltriethoxysilane bonded-phase column (10 μm-NH_2). The elution order of H_2(bae), Co(bae), Ni(bae) and Cu(bae) was reported for the C18 column with a 65% methanol-35% water mobile phase buffered at pH 7.8 with a phosphate buffer. The Pd(bae) peak overlapped the Ni(bae) peak. The elution of Ni(bae) before Cu(bae) was the same (anomalous?) order observed by Uden et al. (26) on a C18 column. The Cu(II) complexes with H_2(bae), H_2(bap) and H_2(bpae) eluted in the order given with a 50% methanol-50% water mobile phase buffered at pH = 7.8. The retention volume for the H_2(bpae) complex with Cu (II) was considerably greater (26.5 ml) compared to the other ligands due to the effect of the phenyl ring. The Cu(bae) and Ni(bae) peaks overlapped on the C18 column and could not be resolved but were well separated on the -NH_2 column and eluted in the order Cu(bae) before Ni(bae) with a 40% methanol-60% water mobile phase buffered at pH 7.8. The areas of the Ni(bae) and Cu(bae) complexes (injected separately) increased in a linear manner

with concentration and detection limits of 0.2 and 0.5 ng of metal injected for Ni and Cu, respectively, were reported. Plate counts reported varied from 346 to 940 and the peaks appeared symmetric.

Tollinche and Risby (12) also studied the separation of Ni(II) and Cu(II) complexes with H_2(btfae) and H_2(btfap) on a silica column (5 μm-HIEFF Micropart) using mobile phases of methylenedichloride with 20% n-heptane, with 10% n-heptane and with 1% acetonitrile. In all cases the elution order was Ni(btfae) before Cu(btfae) and Ni(btfap) before Cu(btfap).

Walters (28) reported on the use of multiple linear regression analysis to predict retention behavior as a function of solvent compositions for Cu(II), Ni(II) and Pd(II) complexes with H_2(bae) and H_2(bap) on μ-Bondapak C18, Partisil-ODS, and Alltech RP-8 columns (all 10-μm particle size). The retention data were fit to the equation,

$$t = a + bx + cy,$$

for ternary mobile phases consisting of methanol-water-acetonitrile. The retention time for the peak is given by t; a, b, and c are the experimentally determined multiple linear regression coefficients, x is the percentage methanol and y is the percentage acetonitrile. An abnormally high coefficient, b, for the Pd(bae) complex on the μ-BondapakC18 column accounts for the observed behavior of Pd(bae) in binary systems where it elutes with Ni(bae) with a water-methanol mobile phase and with Cu(bae) with a water-acetonitrile mobile phase. Use of the above equation permits calculation of the ternary solvent composition 21% methanol-21% acetonitrile-58% water for the elution of Pd(bae) halfway between the Ni(bae) and Cu(bae) complexes.

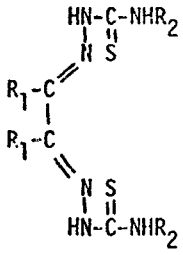
Calculations show that Ni(bae) and Cu(bae) can reverse their order of elution with certain compositions of the mobile phase consisting of

methanol-water-acetonitrile but there is no possibility of reversal on the RP-8 column in the system methanol-water-tetrahydrofuran. This approach was extended to the quarternary system methanol-water-acetonitrile and tetrahydrofuran. Modifier strength decreases in the order tetrahydrofuran, acetonitrile to methanol.

Calligares et al. (29) have reviewed the structural aspects of metal complexes with H_2(bae) and H_2(salen). Both tetradendate complexes tend to have the four donor atoms coplanar with only small deviations toward tetrahedral geometry. Axial coordination positions in the solid complexes for the Cu(bae) and Cu(salen) complexes are filled with water or another Lewis base. The (salen) ligand also gives dimeric species which have not been observed with (bae).

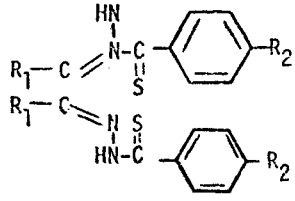
2.3 Thiosemicarbazones, Thiobenzhydrazones, Hydrazones and Dithizone

General Structures



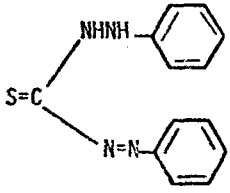
1,2-diketobisthiosemicarbazones

I

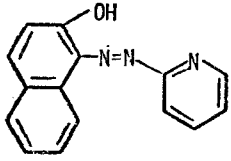


1,2-diketobisthiobenzhydrazones

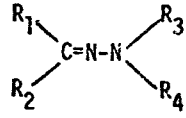
II



III
dithizone



IV
pan



V
substituted hydrazone

Name	Abbreviation	Structure
diacetylbis(thiobenzhydrazone)	H_2(tbh)	(II) R_1 = methyl, R_2 = H
glyoxalbis(2,2,3,3-tetramethyl butyl)-*thiosemicarbazone*	H_2(gbbtc)	(I) R_1 = H, R_2 = 2,2,3,3-tetra methylbuty.
diacetylbis(cyclohexyl) thiosemicarbazone	H_2(dahtc)	(I) R_1 = methyl, R_2 = cyclohexyl
dithizone	H(dz)	(III)
1-(2-pyridylazo)-2-napthol	pan	(IV)
pyridene-2-aldehyde-2-quinolylhydrazone	pac	(V) R_1=2-pyridyl, R_2=R_3=H, R_4=2-quinolyl
substituted hydrazone	L^1	R_1=R_2=methyl, R_3=methyl, R_4=phenyl
substituted hydrazone	L^2	R_1=R_2=ethyl, R_3=methyl, R_4=phenyl
substituted hydrazone	L^3	R_1=methyl R_2=isopropyl, R_3=methyl R_4=phenyl
substituted hydrazone	L^4	R_1=R_2=R_3=R_4=methyl
substituted hydrazone	L^5	R_1=R_2=R_3=methyl, R_4=phenyl
substituted hydrazone	L^6	R_1=H, R_2=R_3=methyl R_4=phenyl

Heizman and Ballschmiter (30) separated Hg(tbh) and Cu(tbh) on a 20-µm Merckosorb SI 60 column with a resolution, Rs, of 1.5 using benzene as the mobile phase and could determine $2x10^{-9}$g of Hg and $0.5x10^{-9}$ g of Cu with photometric detection. With gradient elution the H_2(tbh) chelates of Cu(II), Hg(II), Pb(II) and Zn(II) could be eluted in a reasonable time. In a later paper (31), the chelates of Hg(II), Ni(II), Cu(II), and Pb(II) were found to elute from a 30- µm LiChrosorb SI 60 column in the order given with various

n-heptane-benzene mobile phases. The Zn(II) complex could not be eluted under isocratic conditions. Resolution of various solute pairs varied from 0.5 to 3.2 with different n-heptane-benzene ratios with increasing resolution and longer retention volumes as the benzene concentration decreased. Gradient elution using n-heptane-chloroform as the mobile phase permited the elution of the all five chelates and well resolved peaks were shown for the Hg(II), Ni(II), Cu(II) and Pb(II) chelates. In the same paper (31), the above authors reported on the use of glyoxalbis(2,2,3,3-tetramethylbutyl)thiosemicarbazone), H_2(gbbtc), and diacetylbis(cyclohexylthiosemicarbazone), H_2(dahtc), as ligands. The Hg(II), Cu(II) and Ni(II) chelates with H_2(gbbtc) were eluted in the order given from an alumina (30 μm Alox T) column with benzene as the mobile phase. The Pb(II) chelate decomposed on the stationary phase and the Cd(II), Zn(II), and Co(III) chelates were strongly adsorbed. The Hg(II) and Cu(II) chelates with H_2(dahtc) were separated on both a Li Chrosorb SI 60 and an AloxT column using benzene-3% tetrahydrofuran as the mobile phase. The Pb(II) chelate with H_2(dahtc) also decomposed on the stationary phase and the Zn(II), Ni(II), Co(III), and Cd(II) chelates were very strongly adsorbed.

Gasparrini et al. (32) reported the separation of trans-[PdL_2Cl_2] complexes with various substituted hydrazones, L^1 through L^6, using a bonded phase 10-μm LiChrosorb DIOL column and 88% n-hexane-12% dichloromethane as the mobile phase. These complexes decomposed on unmodified silica columns. Chelates with all six ligands, L^1 through L^6, were separated in a single run and mixed chelates such as $Pd(L^2)(L^5)Cl_2$ could be separated as well. Both isocratic and gradient elution modes were studied. The capacity factor, k, was found to decrease with the chain length of R_1 or R_2 and also depended on the substituent, R_3, on the sp^3-nitrogen.

Schwedt and Budde (33) reported the Cu(II), Ni(II) and Co(III) complexes with pan could be extracted into chloroform, dissolved in acetonitrile after evaporating the chloroform and separated on a RP-2 column (LiChrosorb RP-2) using acetonitrile-water-citrate buffer (80:18:2) at pH=5 as the mobile phase. A Cu(II), Fe(III), Co(III) mixture could be treated in the same way and separated. Apparently Ni(II) and Fe(III) co-eluted. The elution of the chelates was monitored at 565 nm.

Lohmuller et al. (34) reported the elution of the Hg(II), Ni(II), Co(II) and Pb(II) chelates with dithizone on a LiChrosorb SI 60 column using benzene as the mobile phase. The Cu(II), Ni(II) and Zn(II) chelates were not resolved under these conditions but could be eluted individually. The lead peak tailed significantly and the cadmium chelate was strongly absorbed and could only be eluted by using a more polar eluent and still tailed badly. The elution of the peaks was monitored at 525 nm. O'Laughlin and O'Brien (35) obtained similar results on a Corasil and a μ-Porasil column using toluene as the mobile phase. The cobalt complex, assumed to be a Co(III) complex eluted after the lead complex and was fairly symmetrical. The lead complex tailed and apparently decomposed based on the slope of plots of peak area versus amount injected. Poor calibration plots were also obtained for Cu(II) and Hg(II). The elution of the peaks was monitored at 270 nm.

Henderson et al. (36) obtained similar results on the separation of the metal dithizonates to those reported above using aliphatic solvents with polar modifiers as the mobile phase. Results obtained using acetic acid as a modifier were claimed to be comparable to those obtained using toluene or benzene but except for a symmetrical elution peak for the

Pb(II) complex from a Spherisorb GP silica column using 10% hexane-0.2% acetic acid-0.1% butylamine in methylene dichloride as the mobile phase, the results shown do not support this conclusion. Although the authors apparently successfully eluted the $Ni(dz)_2$, $Zn(dz)_2$, $Cd(dz)_2$ and $Cu(dz)_2$ complexes, these peaks were not well separated and it is stated no metal separations were obtained except for that of $Co(dz)_2$ from all the above complexes. These authors also reported the $Hg(dz)_2$ complex decomposed and a peak near the column volume was due in part to a decomposition product of the $Hg(dz)_2$ complex. This is somewhat unexpected in view of the stability of the Hg(II) reported by other

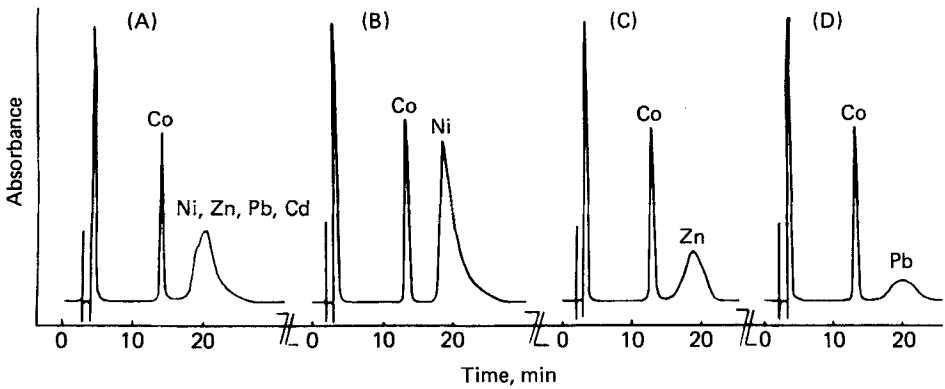


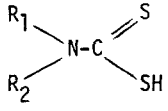
Fig. 2. Chromatograms of the mixture of some metal-dithizonates. (A) 5.2 ng $Co(dz)_3$, 2.50 ng $Ni(dz)_2$, 18.8 ng $Zn(dz)_2$, 52.6 ng $Pb(dz)_2$ and 5.2 ng $Cd(dz)_2$; (B) 5.2 ng $Co(dz)_3$ and 62.0 ng $Ni(dz)_2$; (C) 5.2 ng $Co(dz)_3$ and 47.0 ng $An(dz)_2$; (D) 5.2 ng $Co(dz)_3$ and 131.0 ng $Pb(dz)_2$. Mobile phase: 85%(V/v) methanol-water; Flow rate: 1.0 ml/min; Detection: 550 nm. Column: Shimadzu Zorbax ODS (4.6 mm x 250 mm). Reprinted by permission from Ref. 39 (Japan Soc. for Analytical Chemistry).

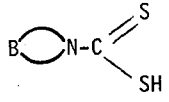
authors (37,38) and the symmetrical peaks observed for the $Hg(dz)_2$ complex by Lohmuller et al. (34) and O'Laughlin and O'Brien (35).

Ohashi et al. (39) reported that Co(II) could be determined by RP-HPLC in the 5.2 to 30 ng range. The Co(II) together with Ni(II), Zn(II), Pd(II) and Cd(II) were extracted into a chloroform solution of dithizone at pH 8.5 in the presence of disodium tartrate. Co(II) was assumed to form the $Co(dz)_3$ complex on extraction while the other metals formed the complexes $Pd(dz)_2$, $Zn(dz)_2$, $Ni(dz)_2$ and $Cd(dz)_2$. The $Co(dz)_3$ complex eluted from a Zorbax ODS column with 85% methanol-15% water as a sharp peak and was well separated from the peaks due to the other complexes which all eluted considerably later and gave broad peaks which tailed to some extent. A typical separation is shown in Figure 2. The significant difference in the elution behavior of the cobalt dithizone complex from those of the other divalent metals shown in Fig. 2 is the best chromatographic evidence for formulating the complex as $Co(dz)_3$.

2.4 Disubstituted Dithiocarbamates

General Structure





I
Dialkyldithiocarbamic acid

II
1-Pyrrolidine carbodithioic acid
($B=(CH_2)_4$)

Name	Abbreviation	Structure
Di-n-ethyldithiocarbamic acid	H(DDTC)	(I) $R_1=R_2$=ethyl

Benzylmethyl-dithiocarbamic acid	H(BMDTC)	(I)	R_1=methyl R_2=benzyl
Diethoxyethyl-dithiocarbamaic acid	H(DEDTC)	(I)	$R_1 = R_2$ = ethoxyethyl
Dihexyldithio-carbamic acid	H(DHDTC)	(I)	R_1=R_2=hexyl
bis(n-butyl-2-naphthyl-methyldithiocarbamate	H(BNMDTC)	(I)	R_1=n-butyl R_2=2-napthylmethyl
1-Pyrrolidinecarbo-dithioic acid (tetramethylenedithio-carbamic acid)	H(TMDTC)		(II)

Schwedt (40) reported the elution of Se(IV), Pb(II), Ni(II), and Cu(II) diethyldithiocarbamates in the order shown from 10-µm Nucleosil 10-C18 column with a 65% acetonitrile-35% water mobile phase. The elution of the peaks was monitored at 254 nm and picogram amounts of the metals detected. In a later paper Schwedt (41) showed that Se(IV), Cr(III), Ni(II), Co(III), Pb(II) (or Cu(II)), and Hg(II) diethyldithiocarbamates were well separated on a LiChrosorb RP-8 column with a 70% methanol-30% water mobile phase. The Pb(II) and Cu(II) chelates apparently co-eluted under these conditions. In a subsequent paper (42) the elution order of Pb(II), Ni(II), Co(III), Cu(II) and Hg(II) for the diethyldithiocarbamate metal complexes was reported on a 10-µm LiChrosorb RP-18 column with 65% acetonitrile -35% water as the mobile phase. The tetramethylenedithiocarbamate complexes were also studied and the observed elution order on a 10 µm-Chromosorb RP8 column was Cd(II), Pb(II), Ni(II), Co(III), Zn(II), Cu(II) and Hg(II).

Heizmann and Ballschmiter (31) reported on the normal phase chromatographic separation of metal substituted dithiocarbamates. The Cu(II), Hg(II), Ni(II) and Co(III) diethyldithiocarbamates were reported to elute in the order given from a 30-µm LiChrosorb SI 60

column with benzene as the mobile phase. The elution of the complexes was monitored at 330 nm and glass columns were employed to eliminate any contact between the chelates and metal parts. A flow-program was used to elute the Zn(II), Ni(II) and Co(III) benzylmethyldithiocarbomates (in that order) from a 40-μm LiChosorb SI 60 column with 75% benzene-25% cyclohexane as the mobile phase. The flow rate was increased from 8 ml/hr to 20 ml/hr after the elution of Ni(II). The same flow-program and column were used for the separation of Cu(II), Ni(II), Co(III) and As(III) diethoxyethyldithiocarbamates using 4% acetonitrile in carbon tetrachloride as the mobile phase. The As(III) peak was eluted last in just under 40 minutes and was very symmetrical. Elution of the peaks was monitored at 360 nm. No data on the plate counts were given but a value for N of about 500 based on the As(III) peak in Figure 11 in Reference 31 can be estimated. The observed retention times for Co(III), Cd(II), Ni(II), Cu(II) and Zn(II) decreased in the same order as R_F values for the benzylmethyldithiocarbamates increased on a thin layer plate (DC-Alufolie SiO_2) with 75% benzene-25% cyclohexane as the mobile phase.

Uden and Bigley (43) reported the separation of Cu(II), Ni(II) and Co(III) diethyldithiocarbamates by normal phase chromatography on a 4-mm x 25-cm stainless steel column packed with 8-μm Spherisorb SGP. The best separation was observed using 5% acetonitrile-15% diethylether-80% Skelly B as the mobile phase. The $Cu(DDTC)_2$, $Ni(DDTC)_2$ and $Co(DDTC)_3$ complexes eluted in the order given and a plate count of 1550 was reported for the $Co(DDTC)_3$ peak. A d.c. argon plasma emission spectrometer system in series with the photometric detector was used as a metal specific detector to confirm the peaks eluted were due to the metal complexes and not degradation products. Quantitative

studies based on the response of the photometric detector at 254 nm showed a linear reponse for $Co(DDTC)_3$ and $Ni(DDTC)_3$ from 5-500 ng of metal and for $Cu(DDTC)_2$ from 10-500 ng of the metal. On-column degradation was avoided by pretreatment of the column with pyridine. It was stated that solvent extraction of the metal complexes into chlorofrom would serve to separate the complexes from the reagent and preclude possible reactions of the chelating agent with the column but it was not clear whether this was done. It appears that the chelates studied were prepared and characterized as the pure solids and solutions of these were injected.

Liska et al. (44) noted mixed ligand complexes formed when mixtures such as $Ni(DDTC)_2$ and $Ni(DHDTC)_2$ were separated on a 10-μm Li-Chrosorb SI 60 column (stainless steel) with various combinations of chloroform, carbon tetrachloride, dichloromethane, cyclohexane, n-hexane and n-heptane as the mobile phases and UV detection at 325 nm. A third peak, apparently due to Ni(DDTC)(DHDTC), was observed that eluted between the peaks for the symmetrical chelates. Similar results were observed for other pairs of dialkyldithiocarbamates including diethyl with-dipropyl,-dibutyl,-dipentyl, and-diheptyl as well as other possible pair combinations. Capacity ratios, k, were given for the Ni(II) chelates of all the ligands mentioned above and for the mixed chelate species, L_1-Ni-L_2, as a function of the eluotropic strength, $\varepsilon°$, of the mobile phase. Liska et al. (45) unsuccessfully attempted to isolate the mixed ligand complex of Ni(DDTC)(DHDTC) by classical column preparative chromatography on glass columns packed with silica or alumina. Three colored zones were observed but they were not well separated and the middle zone, corresponding to the mixed ligand chelate, disappeared very quickly. It did prove possible to separate

and indentify all three species by two-dimensional thin layer chromatography using Silufol silica gel TLC plates. The molecular weight of both the symmetrical and mixed ligand Ni(II) chelates were determined in chloroform and the species were found to be monomeric. In a later paper in this series, Lehotay et al. (46) studied the elution behavior of various dialkyldithiocarbamate metal complexes and obtained well defined peaks for both the symmetrical and mixed ligand complexes. This work was done using a commercial HPLC unit with photometric detection at 254 and 280 nm. A metal column packed with 10-μm LiChromsorb SI 60 was used and various combinations of organic solvents used as the mobile phases. Mixtures of chloroform and dichloromethane with a less polar solvent (n-pentane, carbon tetrachloride or cyclohexane) gave the best results. Peaks resulting in exchange reactions such as

$$Ni(DDTC)_2 + Ni(DHDTC)_2 \rightarrow 2\ Ni(DDTC)(DHDTC)$$

were observed for all metal chelate pairs but no exchange reaction was observed for complexes of different metals with different ligands that led to mixed ligand complexes. The only reaction observed, for example, in the latter case was complete exchange,

$$Ni(DDTC)_2 + Cu(DHDTC)_2 \rightarrow Ni(DHDTC)_2 + Cu(DDTC)_2.$$

Liska et al. (47) showed that the diethyldithiocarbamate complexes of Zn(II), Cu(II), Mn(II), Ni(II), Pb(II), Cr(III), Co(II), Cd (II) and Fe(II) eluted in the order given from a stainless-steel column packed

with 10 μm-LiChrosorb SI 60 with 10% chloroform in cyclohexane as the mobile phase. The elution was monitored at 254 nm and well defined peaks attributed to each of the above species were generated in a single run on injection of 5 μl of a synthetic mixture of all the above complexes in chloroform (5.6×10^{-7} M for each compound).

O'Laughlin and O'Brien(35) reported an elution order of Cu(II), Ni(II), Hg(II), Zn(II), Cd(II), Co(III) and Pb(II) for the normal phase chromatographic separation of the diethyldithiocarbamate complexes on a 37-50-μm Corasil column with toluene as the mobile phase. The $Ni(DDTC)_2$-$Co(DDTC)_3$ and $Hg(DDTC)_2$-$Pb(DDTC)_2$ couples were separated but peaks due to Cu(II), Ni(II), Hg(II) and Zn(II) were not resolved on this rather inefficient column. The $Pb(DDTC)_2$ peak tailed badly and a large peak near the column void volume was observed. Elution of the peaks was monitored at 270 nm. A good separation of a mixture of $Co(DDTC)_3$ and $Co(dz)_3$ was observed on a μ-Porasil A column with no evidence of any mixed chelate with this system. The dithizone complex was assumed to be $Co(dz)_3$. The composition of cobalt dithizonate is still an unresolved question but recent evidence tends to support the $Co(dz)_3$ formula (39,48) although in the absence of oxygen and with a large excess of reagent as in extraction studies $Co(dz)_2$ may be present. Budesinsky and Sagat (37) reported a β_n value for $Co(dz)_2$.

Mangia et al (49) reported the separation of Cu(II), Ni(II), Mn(II) and Co(II) as the diethyldithiocarbamate complexes on a 10-μm silica column with 20% methylene chloride-80% hexane as the mobile phase. The response of the photometric detector at 254 nm was determined for different amounts of Co(II). The cobalt complex was prepared by extracting a solution containing 0.05-5 ppm of Co(II) and NaDDTC with

carbon tetrachloride. An aliquot of the organic phase was injected and a linear calibration curve over the region 1-100 mg of metal injected was obtained. It seems likely the complex was $Co(DDTC)_3$ and not $Co(DDTC)_2$ as reported by the authors. In a subsequent paper (50) with Gaetani and Laureri this complex is formulated as $Co(DDTC)_3$. In this paper, an excellent separation of Cu(II), Ni(II), Mn(III) and Co(III) complexes with diethyldithiocarbamate was reported on a 10-μm MicroPak-CN column with 85% hexane-15% dichloromethane as the mobile phase. The peaks were monitored at 254 nm and a plate count for the Co(III) peak of 1040 was reported. Although the authors prepared the $Mn(DDTC)_3$ and $Co(DDTC)_3$ complexes by extraction of the divalent cations into carbon tetrachloride, they observed a change in the shape of the chromatographic peaks with time. They attributed this to oxidation to the Mn(III) and Co(III) complexes and confirmed the fact that these two metals eluted as $Mn(DDTC)_3$ and $Co(DDTC)_3$ by mass spectroscopy. It is not clear how mass spectrometry can be used to confirm the oxidation state and more likely it only indicated the approximate stoichiometry. In this same paper the separation of $As(DDTC)_3$, $Sb(DDTC)_3$ and $Bi(DDTC)_3$ was reported on a 10-μm MicroPak CN column with 90% hexane-8% dichloromethane-2% acetonitrile as the mobile phase. An interesting separation of $Ni(DDTC)_2$ and $Pd(DDTC)_2$, which are isostructural in the solid phase, was achieved using toluene as the mobile phase in which a large separation factor, $[Pd(DDTC)_2]/[Ni(DDTC)_2] = 9$, was observed with detection at 320 nm. A separation factor for the $Zn(DDTC)_2/Cd(DDTC)_2$ pair, also isostructural in the solid state, was 2.1 with 80% toluene-20% dichloromethane as the mobile phase. Linear calibration plots were obtained over the range 1.0-25 ng of metal injected with detection

limits for Cu, Ni, Pd and Co between 0.1 and 0.3 ng of metal. The quantitative studies were all done on solutions of the metals in carbon tetrachloride obtained by solvent extraction of the complexes from an aqueous solution of the metal containing a 10-100-fold excess of Na(DDTC).

Moriyasu and Hashimoto (51) used adsorption chromatography on deactivated silica columns (10-μm LiChrosorb SI 100 or SI 60) to separate and determine Hg(II), Cu(II), Cd(II), Pb(II), Cr(VI), Ni(II), Bi(II), and Co(III) as the diethyldithiocarbamate complexes. Cr(VI) is reduced to Cr(III) by the reagent and the peak observed was likely due to the Cr(III) complex. These chelates eluted in the order shown with 98% hexane (H_2O saturated) -2% ethylacetate as the mobile phase. Linear calibration plots were reported for the Hg(II), Cu(II) and Ni(II) complexes but not for the Pb(II), Bi(III) or Cd(II) complexes. In a subsequent paper, the same authors (52), noted that mixed ligand complexes similar to those reported by Liska et al. (44) formed when two different disubstituted dithiocarbamate complexes of Ni(II) or Cu(II) were mixed. The mixed ligand species was separated on a deactivated silica column (Shodex Silipak) with a 83.3% hexane-16.7% ethylacetate mobile phase which was saturated with water. Rate constants for the reactions,

$$NiA_2 + NiB_2 \rightleftharpoons 2NiAB$$

and

$$CuA_2 + CuB_2 \rightleftharpoons 2CuAB,$$

were reported for ligands where A=diethyldithiocarbamate and the structure of B was I with $R_1 = R_2 = CH_3$, I with $R_1 = R_2 =$ $CH_3CH_2CH_2$-; I with $R_1 = R_2$ = benzyl; II with B = $(CH_2)_4$; II with B = $(CH_2)_5$; and II with B =

$-CH_2CH_2OCH_2CH_2^-$. Rate constants for Ni(II) were in the range 10^1-$10^2 M^{-1}s^{-1}$ and for the more labile Cu(II) complexes the constants were on the order of $10^3 M^{-1}s^{-1}$.

Moriyasu et al. (53) also reported that the nickel chelate of dithiocarbamate derived from (+)-or(-)-ephedrine gave a single peak while that from (±)-ephedrine gave two peaks and that optically active and racemic ephedrines were determined.

Schwedt (54) determined Cr(III) and Cr(VI) in the presence of each other by separation of the different reaction products with ammonium pyrrolidinedithiocarbamate (II with B = $(CH_2)_4$). Cr(VI) reacted at room temperature and Cr(III) only at 60°C. The reaction products were separated on 5-µm LiChrosorb RP-18 (RP-8) columns with 80% (70%) acetonitrile -20%(30%) water as the mobile phases. The reaction products were detected at 254 nm and both Cr(III) and Cr(VI) were determined in waste water. Tande et al. (55) reported Cr(III) formed the $Cr(DDTC)_3$ chelate at pH 5.8 with NaDDTC in aqueous solution but not at pH 4. Cr(VI), added as $K_2Cr_2O_7$, reacted with NaDDTC at both pH4 and pH 5.6 to give three peaks attributed to an unidentified disulfide, a species formulated as $Cr(S_2CN(C_2H_5)_2)_2(OS_2CN(C_2H_5)_2)$ and $Cr(S_2CN(C_2H_5)_2)_3$. The ratio of the last two peaks was found to be constant at different Cr(VI) concentrations and by preparing standard curves for both peaks 2 and 3, originating from Cr(VI) and reacted at pH 5.6, and a third calibration curve for $Cr(S_2CN(C_2H_5)_2)_3$ when Cr(III) was reacted with the ligand at pH 5.6, it was possible to determine Cr(III) and Cr(VI) simultaneously in an unknown water sample.

Häring et al. (56) determined Co, Cu and Ni in the 0.2-10 µg/L range by preconcentration of the diethyldithiocarbamate complexes on a

LC 5-Phenyl reversed-phase precolumn from water and the elution of these chelates from a LC 5-phenyl reversed phase analytical column with 75% methanol-25% water. They found it necessary to use glass or Teflon to construct the analytical system to avoid ligand exchange, memory and contamination phenomena.

Edward-Inatimi and Dalziel (57) reported that copper, nickel, mercury, lead, cobalt, manganese, and bismuth diethyldithiocarbamates (neither the oxidation state of the metal or stoichiometry of the complexes are given in this paper) could be extracted from a pH 8.5 aqueous buffer into 5 ml of an organic solvent. A 5-µl portion of the organic extract was injected into the HPLC and the metal chelates separated on a 5-µm Hypersil column using benzene as the mobile phase. The elution of the peaks, in the order given above, was monitored at 280 nm. Linear calibration plots based on peak height were reported for all six metals and limits of detection based on metal concentration in original aqueous sample from 50-500 ppb was reported. The peak for lead was poorly defined and the calibration plot for lead had the smallest slope.

Bond et al. (58) showed that the Cu(II) diethyldithiocarbamate or the Cu(II) 1-pyrrolidenecarbodithioate chelates could be detected electrochemically in the 10^{-6} to 10^{-7} M concentration range. The $Cu(DDTC)_2$ was found to undergo one-electron reduction and oxidation steps at a platinum, gold, or glassy carbon electrode. Copper could be detected down to levels of 1 ng with no interference from a 10-fold excess of 20 selected ions. Peaks were also observed using electrochemical detection for the chelates of Cd(II), Pb(II), Co(III) and Fe(III) and it was assumed this method would be suited for multi-element analysis. Excellent agreement was reported for copper in

tap water when determined by HPLC with electrochemical detection and when determined by atomic absorption methods. In one variation of the above procedure, the reagent was included in the mobile phase and aqueous solutions of Cu(II) were injected with on-column formation of the complex.

Smith et al. (59) have also reported that Cu(II), Ni(II), Co(II), Pb(II) and Fe(III) could be determined by reversed-phase liquid chromatography on Hypersil-ODS by direct injection of aqueous solutions of these ions. The mobile phase included Na(DDTC) and the complexes were formed directly (on-column), separated and the peaks detected at 350 nm. A mixture containing 100 ppm Cu (II), 5 ppm Co(II) 110 ppm Pb(II) and 100 ppm Cd(II) gave four well defined peaks eluting in the order Cd(II), Pb(II), Co(III), Cu(II) using 75% methanol-35% water containing 0.05% Na(DDTC) as the mobile phase. The Co(II) eluted as the Co(III) complex according to these authors. It was found that Hg(I) and Hg(II) mixture gave two peaks and Zn(II) gave one peak but in these cases the retention times were not reproducible. Smith et al. (60,61) also have reported on the use of transition metal cations as "ion-pair" reagents in the HPLC separation of dithiocarbamates. This appears to involve the formation of metal chelates and is not ion-pair chromatography in the sense that this term is now used.

Sheh and Carr (62) have proposed a new ligand, bis(n-butyl-2-napthylmethyldithiocarbamate)zinc II, ($Zn(BNMDTC)_2$) for multi-element trace metal analysis. These authors note that it was reported that metal-DDTC complexes dissociate at low concentrations (51), have maximum absorption bands at very different wavelengths precluding optimization of response without multi-channel spectrophotometric capabilities, and in some cases tend to react on the

column with metal components. The napthyl group in the proposed reagent absorbs strongly at 221 nm and the bulky side groups are claimed to stabilize metal complexes with this ligand. They use two pre-columns, the first as silica column to saturate the mobile phase with silica and a second, a silica column derivatized with diaminosilane, to remove trace metals from the mobile phase. The analytical column used in this work was a 10-μm Waters RCM-100 C18 which was used to minimize generation of interferences from metal frits in conventional columns. The metal complexes were prepared by reacting metal salts with an excess of $Zn(BNMDTC)_2$ dissolved in the mobile phase buffered at pH 8.25 with Tris. The pH was adjusted to 8.25 prior to the addition of methanol with phosphoric acid. A typical chromatogram is shown in Fig. 3. The best results were obtained using methanol-water mobile phases although other mobile phases were studied. Retention values, k, on other columns varied dramatically even though the same mobile phase was employed (although the elution order remained the same). Columns studied included a 5-μm Supelcosil LC-8 and a 10 μm-Waters μ-Bondapak C18 in addition to the RCM-100 column. Linear calibration plots with a zero intercept were obtained for Hg(II), Cu(II) and Fe(III) using the RCM-100 column.

Hutchins et al. (63) reported that Co(III), Cr(III), Cu(II), Hg(II), Ni(II), Pb(II), Se(IV), and Te(IV) diethyldithiocarbamates could be separated on a Waters Radial Pak C18 column using a ternary mobile phase consisting of 40% methanol-35% acetonitrile-25% water provided the column was conditioned by prior injection of a concentrated mixture of the complexes or with 0.005 M EDTA. The Pb(II), Cd(II) and Fe(III) complexes gave poor peak shapes attributed to substitution reactions with nickel from metal components in the chromatographic system. A

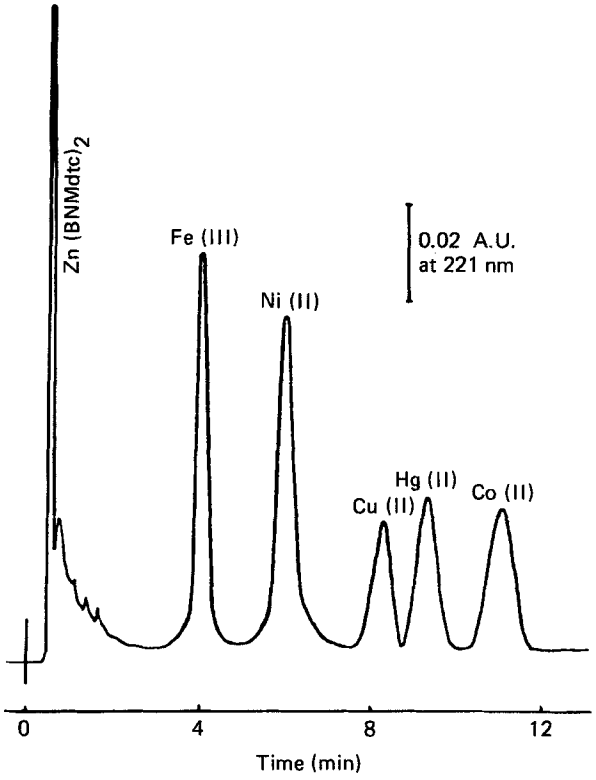


Fig. 3. Chromatogram of the metal $(BNMDTC)_n$ complexes. Sample was 20 µl of a synthetic mixture which was 1 x 10^{-4}M in each complex. Flow rate 2 ml min^{-1}; pressure drop less than 1500 psi. Waters Radial Pak C_{18} column. Mobile phase 95% methanol-5% water and 1.0mM in Tris. pH=8.25. Reprinted by permission from Ref. 62 (Elsevier).

typical separation is shown in Figure 4. The first peak eluted has been attributed by other investigations as a disulfide and was identified by Hutchins et al (63) as bis(diethylthiocarbonyl)disulfide (disulfiram) produced by the oxidation of diethyldithiocarbamic acid. The lack of any peak for Zn(II) in Figure 4 was attributed to the fact this species

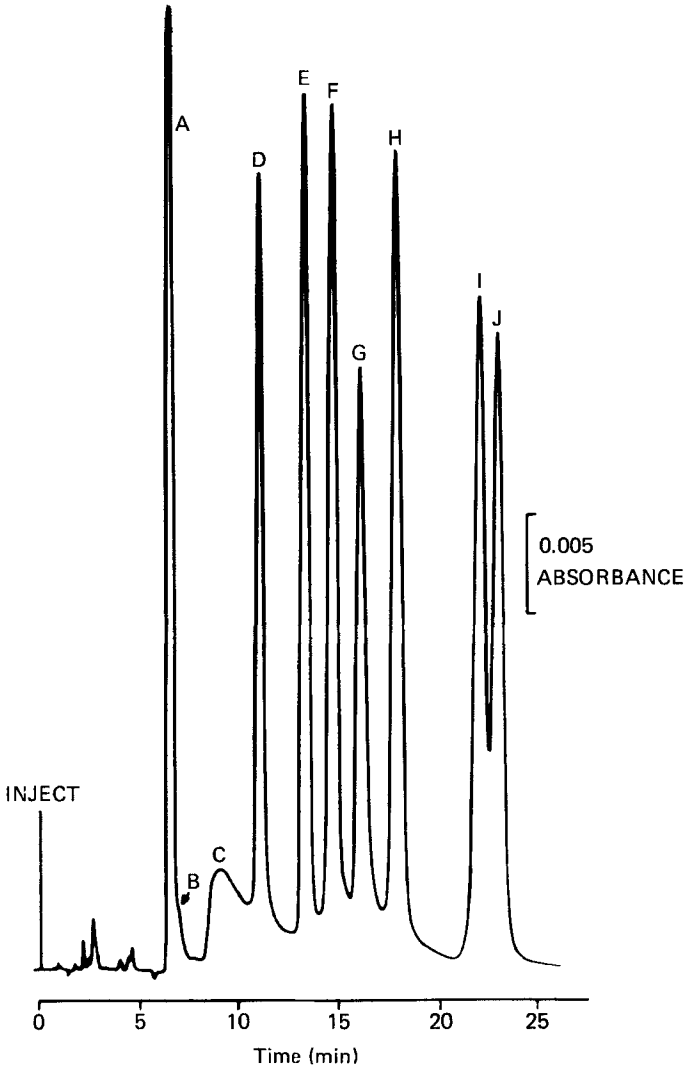


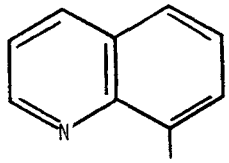
Fig. 4. Separation of a mixture of diethyldithiocarbamate complexes on a Waters Radial Pack C_{18} column. Conditions: mobile phase methanol-acetonitrile-water (40:35:25) flow rate 2.0 ml/min, detector wavelength 254 nm; detector sensitivity 0.05 a.u.f.s; injection volume 10 µl. A = disulfiram; B = Cd(II), 0.12 µg; C = Pb(II), 0.11 µg; D = Ni(II), 0.14 µg; E = Co(III), 0.07 µg; F = Cr(III), 0.11 µg; G = Se(IV), 0.32 µg; H = Cu(II), 0.18 µg; I = Hg(II), 0.51 µg; J = Te(IV), 0.17 µg. Reprinted by permission from Ref. 63 (Elsevier).

is unstable and reacted with the stainless steel components in the system (presumably the stainless steel spreader plates on the column or in the U6K injector). Applications of the above method to the determination of metals and organic brightening agents in electroplating solutions are discussed.

Bannister et al. (64) reported that Na(DDTC) reacted with cis-dichlorodiamminoplatinum(II) in urine to form $Pt(DDTC)_2$. The latter chelate was extracted into chloroform and determined by HPLC. The elution of the $Pt(DDTC)_2$ from a μ-Bondapak CN (C-18 or alkylphenyl columns were unsatisfactory) with 82% heptane-18% isopropanol was monitored at 254 nm. Platinum in urine at the 25 ng/ml level could be determined with a relative precision of 2.5% and accuracy of 4%.

2.5 8-Hydroxyquinoline

General Structure



I

Name	Abbreviation	Structure
8-hydroxy-quinoline(oxine)	H(Ox)	I

Bethod et al. (65) used reversed-phase chromatography on a RP-8 column to separate the Cu(II), Co(II), Ni(II), Hg(II) and Fe(II) chelates of 8-hydroxyquinoline. Solutions of Cu(II), Co(II), Ni(II) and Hg(II) were injected into a column already holding 8-hydroxyquinoline.

The chelates were then separated using a 55% methanol-45% water mobile phase which was also $5x10^{-3}$M in oxine. Electrochemical, UV absorption and atomic absorption methods were used to detect the peaks eluted. Hambali and Haddad (66) reported the separation of Al(III) and Co(III) chelates with 8-hydroxyquinoline on a 10-μm LiChrosorb SI 60 column using a 5% methanol-95% chloroform mobile phase. The elution of the peaks was monitored at 254 nm and linear calibration curves of peak area versus amount of metal ion injected over the ranges 0-0.5 μg for Co(III) and 0-0.20 μg for Al(III) were obtained. Detection limits of 0.9 ng and 17ng for Co(III) and Al(III) were reported. These authors were unable to separate Co(II) by TLC as the oxinate due to oxidation to Co(III) and the formation of two spots on the TLC plate and Co(II) was not studied in the column work. Wenclawiak (67) reported the separation of the V, Mo, W, Co and Cr chelates with 8-hydroxyquinoline on a SI 60 silica column with a 60% tetrahydrofuran-40% chloroform mobile phase. The oxine complexes were formed prior to injection on the column. The peaks eluted in the order given and detection limits based on absorption at 254 nm of <1 ng for V, Mo, and Cr, 0.5 ng for Co, and 1.5 ng for W were reported. Hoffman and Schwedt (68) compared the pre-column derivatization method proposed by Wenclawiak (67) and the on-column injection method of Bethod (65) for the possible separation of Mn(II) and Mn(III) in natural waters. They found that Co(II), Mo(VI), Mn(II) and Mn(III) could not be separated by the pre-column derivatization method and the separation of chelates by this method was not reproducible. The on-column derivatization method, however, was successful and peaks (with retention time in minutes) for Cr(VI), (2.45), Co(II) (4.01 and 4.09), Mn(II) (4.60), Zn(II) (4.88), Cu(II) (12.29), Al(III) (19.26), and Mn(III) (25.77) were obtained on a RP-8

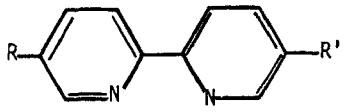
column with a 60% methanol-40% water mobile phase which was 10^{-3}M in oxine. The best results were obtained on a 5-μm RP-18 column according to the authors but no data was given for this column. It was claimed that Mn(II) and Mn(III) could be determined in river water and fertile soils. The authors note that many metals change their oxidation state by reaction with air during the derivatization step and that two peaks were observed for Co(II) if injected immediately after derivatization (with the pre-column derivatization method) but only one peak if allowed to stand) for several hours prior to injection. They made the rather strange observation that "If Al(III) is present with Mn(III), several peaks with t_R values differing from those of the pure oxinates will result". Al(III) thus appears to interfer with the Mn(III) determination which makes it difficult to see how the method could be used to determine Mn II/Mn(III) ratios in "fertile soils".

Watanabe et al. (69) reported trace elements in sea water could be preconcentrated by formation of the 8-hydroxyquinoline complexes and adsorption of these metal complexes on a C18 column (Bondapak Porasil B). Quantitative recovery of copper and manganese spikes (10 and 9 μg/L, respectively) was reported on elution of the complexes from the column with methanol.

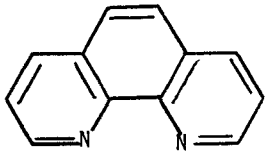
It is somewhat surprising that relatively few papers have been published on the separation of metal oxinates in view of the large number of metal ions known to form stable chelates with this ligand and the fluorescent nature of many of these chelates. It is well known that divalent metals tend to form complexes in which additional molecules of neutral oxine apparently displace coordinated water. This might explain the greater reproducibility of the direct injection method reported by Hoffman and Schwedt (68). It appears additional research is needed in this area.

2.6 Bipyridine and 1,10-Phenanthroline

General Structure



I. 2,2'-Bipyridine



II. 1,10-Phenanthroline

Name	Abbreviation	Structure
2,2'-bipyridine	bpy	I. R=R'=H
2,2'-bipyridine dicarboxylate	bpy-1	I. R=R'= $-COO^-$
2,2'-bipyridine monocarboxylate-monoester	bpy-2	I. R= $-COO^-$ R'= $-COOC_2H_5$
"	bpy-3	I. R = $-COO^-$ R'= $-COOC_{18}H_{37}$
2,2'-bipyridine diester	bpy-4	I. R=R'= $-COOC_2H_5$
"	bpy-5	I. R=R'= $-COOC_{18}H_{37}$
"	bpy-6	I. R= $-COOC_{18}H_{37}$ R'= $-COOC_{16}H_{33}$
"	bpy-7	I. R= $-COOC_{18}H_{37}$ R'= $-COOC_{20}H_{47}$
1,10-phenanthroline	phen	II.

Valenty and Behnken (70) were the first to report on the use of reversed-phase paired-ion HPLC to separate ionic metal chelates. They studied the separation and quantitation of $Ru(bpy)_3^{+2}$ derivatives with the general structure, $(bpy)_2Ru(L)$, where the ligand L was bpy-1 through bpy-7. The charge on the metal chelate thus varied from a net zero for $Ru(bpy)_2$(bpy-1), the dicarboxylate species, to plus one for

the $Ru(bpy)_2(bpy\text{-}2)$ and $Ru(bpy)_2(bpy\text{-}3)$ the mono-ester species, to plus two for the diester species $Ru(bpy)_2(bpy\text{-}4)^{+2}$, $Ru(bpy)_2(bpy\text{-}5)^{+2}$, $Ru(bpy)_2(bpy\text{-}6)^{+2}$ and $Ru(bpy)_2(bpy\text{-}7)^{+2}$. The observed elution order for the Ru(II) tris chelates was $Ru(bpy)_2(bpy\text{-}1)$, $Ru(bpy)(bpy\text{-}3)^{+1}$, $Ru(bpy)_2(bpy\text{-}6)$, $Ru(bpy)_2(bpy\text{-}5)$, and $Ru(byp)_2(byp\text{-}7)$ with retention times of 1.1, 2.4, 6.8, 7.3 and 8.0 min., respectively, on a μ-Bondapak-C18 column using a 20-minute linear solvent gradient from 50% THF-50% H_2O to 100% THF. The flow rate was 2.0 ml/min and elution of the species was monitored photometrically at 254 nn and 280 nm. The mobile phase was kept 0.015M in methanesulfonic acid and 0.5% in acetic acid. The species $Ru(bpy)_2(bpy\text{-}1)$, $Ru(bpy)_2(bpy\text{-}2)$, and $Ru(bpy)_2(bpy\text{-}4)$ eluted in the order given on the same column and under the same conditions as above with a 10-min linear solvent gradient from 10% THF-90% H_2O to 40% THF-60% H_2O (both solvents 0.005M in n-heptanesulfonic acid and at pH=3.5). Observed retention times were 4.9, 7.7 and 10.4 minutes respectively. The peaks were sufficiently sharp that the diester species $Ru(bpy)_2(bpy\text{-}5)^{+2}$, $Ru(bpy)_2(bpy\text{-}6)^{+2}$ and $Ru(bpy)(Bpy\text{-}7)^{+2}$ which differ by only two methylene units in the hydrocarbon tails on the ester could be separated. The neutral dicarboxylate species, $Ru(bpy)_2(bpy\text{-}1)$, eluted first followed by the monoester species with a charge of plus one. Because a different gradient program was used as well as a different counter ion (n-heptane sulfonate for the bpy-1, bpy-2 and bpy-4 species), it is not possible to compare retention times for the monoester species with $R=COOC_2H_5$ (bpy-4) and $R = -COOC_{18}H_{37}$ (bpy-5) or the retention of the dicarboxylate species (bpy-1) with the two counter ions. Although the retention time was shorter for the

dioctadecyl ester than the diethyl ester it should be noted that the 7.7 min retention time for the former species was obtained for a gradient program going up to 100% THF and the 10.4 min retention time for the diethyl ester involved a gradient program going only to a maximum concentration of 40% THF in water after ten minutes. Under isocratic condtions it would be expected that the diethylester would elute first. These authors reported a detection limit for the species, $Ru(bpy)_2(bpy\text{-}5)$, of $1.x10^{-12}$ mol at a S/N ratio of 2 at 280 nm and $e^{280} = 5x10^4$ L/mole cm. The detector response was linear over the range $5x10^{-12}$ to $2x10^{-8}$ mol/L of the above compounds with precision of ±2% at the $2.5x10^{-9}$ molar level. The authors concluded that monitoring hydrolysis reactions of the above diester compounds by HPLC was superior to photometric absorption or fluorimetric methods.

O'Laughlin and Hanson (71) reported that the kinetically inert tris(1,10-phenanthroline)iron(II) and ruthenium(II) chelates could be separated by paired-ion chromatography on a μ-Bondapak CN column (10-μm particle size) using methanol-water or acetonitrile-water mobile phases which were also 0.015 M in methanesulfonic acid and 0.5% in acetic acid. The retention volumes for both chelates (and the resolution) decreased with increasing concentration of the organic component and a 20% methanol-80% water composition permitted the separation of the two species in under ten minutes with baseline resolution.

The effect of pH and pairing ion concentration on the resolution was studied. The former appeared to have relatively little effect over the range pH 2.9 to 6.0 on the separation although the retention volumes for both species first decreased to minimum values around pH 4.5 and then increased. Capacity factor values, k, for $Fe(phen)_3^{+2}$ decreased from 2.40 at pH 2.9 to 1.93 at pH 4.5 and then increased to

2.81 at pH=6.0. Both capacity factors and the resolution increased as the concentration of the pairing ion was increased. With n-heptanesulfonate as the pairing ion and using a 40% methanol-60% water mobile phase (also 0.06M in acetic acid), k values for Fe(II) and Ru(II) increased from 1.46 and 1.72, respectively, at 10^{-4}M heptanesulfonate to 2.46 and 2.92 at 10^{-2} M heptanesulfonate. The plate count (based on the iron(II) peak) also increased from 1350 to 1860 and the resolution, R_s, from 0.84 to 1.02.

The elution of the $Fe(phen)_3^{+2}$ and $Ru(phen)_3^{+2}$ species was monitored using a variable wavelength photometric detector at 448 nm and 512 nm in the visible (corresponding to the wavelength for maximum absorbance in the visible for the $Ru(phen)_3^{+2}$ and $Fe(phen)_3^{+2}$ species) and at 265 nm where both species adsorbed strongly. The elution of the $Ru(phen)_3^{+2}$ species was also monitored fluorimetrically at 565 nm. It was found that the kinetically inert $Ni(phen)_3^{+2}$ species eluted with the same retention volume as the $Ru(phen)_3^{+2}$ species and, in the absence of the latter, the elution of the $Fe(phen)_3^{+2}$ and $Ni(phen)_3^{+2}$ species could be monitored at 265 nm. Plots of peak area versus nanograms of metal injected were linear for both complexes. No separation of the above complexes was observed on a μ-Bondapak C18 column with methanesulfonate as the pairing ion at methanol concentrations greater than about 60%. Some separation was evident at 50% methanol-50% water with retention volumes for the $Fe(phen)_3^{+2}$, $Ni(phen)_3^{+2}$ and $Ru(phen)_3^{+2}$ of 9.01, 9.25 and 10.48 ml, respectively. All the peaks tailed badly and could not be resolved. O'Laughlin (72) later reported a better separation of the $Fe(phen)_3^{+2}$ and $Ni(phen)_3^{+2}$ on a μ-Bondapack C18 column was possible using a 30% acetonitrile-70% water mobile phase containing 2.0g

$LiClO_4$/L. Some tailing was still evident although baseline separation of the peaks was possible with retention volumes of 21.2 and 25.0 ml, respectively, for the Fe(II) and Ni(II) species. No peaks were observed on either column for the kinetically labile Co(II), Zn(II) or Cd(II) complexes although the possibility that these complexes could be separated if the ligand was added to the mobile phase was suggested.

O'Laughlin (73) in a subsequent paper showed that well resolved peaks for the labile Zn(II) and Cd(II) complexes with 1,10-phenanthroline were obtained on a μ-Partisil SCX column using an 80% acetonitrile -20% water mobile phase which was 0.048M in $LiClO_4$ and 10^{-4}M in 1,10-phenanthroline. The elution of the peaks was monitored at 265 nm. Separate peaks for the inert $Ni(phen)_3^{+2}$, $Ru(phen)_3^{+2}$ and $Fe(phen)_3^{+2}$ were obtained on the μ-Partisil SCX column using a 80% acetonitrile -20% water mobile phase which was 0.06M in $HClO_4$. The elution order did not change for the $Fe(phen)_3^{+2}$ and $Ni(phen)_3^{+2}$ species as the acetonitrile concentration was decreased to 50% but the retention volumes and the resolution of the two peaks increased in a uniform manner. The retention volumes for these two complexes varied inversely with the square of the perchlorate ion concentration but did not appear to be a function of the hydrogen ion concentration. It was shown (72) that the retention volumes for the kinetically inert complexes were independant of the 1,10-phenanthroline concentration in the mobile phase but those for the Co(II), Zn(II) and Cd(II) complexes varied with the 1,10-phenanthroline concentration. A typical separation of several metal complexes at one ligand concentration is shown in Figure 5. When the 1,10-phenanthroline concentration was 10^{-4}M the Cd(II) species eluted last.

O'Laughlin also reported (72) that the neutral bis-(cyano)bis-(1,10-phenanthroline)iron(II) complex,

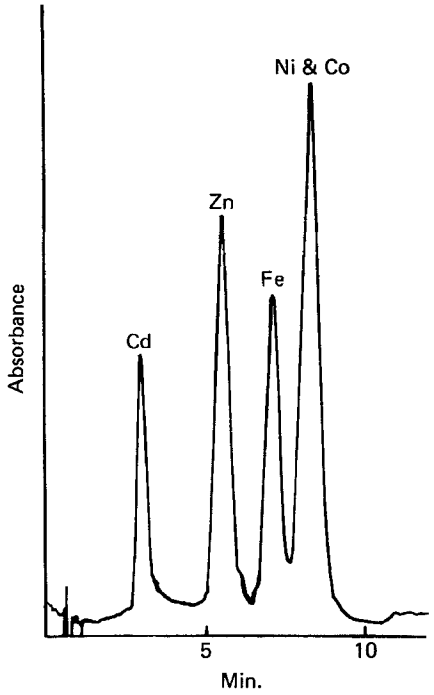


Fig. 5. Separation of $M(phen)_3^{+2}$ complexes on a PRP-1 column. Mobile phase 35% CH_3CN-65% H_2O with 4g $LiClO_4$ per L and 3.5 x 10^{-5}M in phen. From Ref. 72.

$Fe(CN)_2(phen)_2$, could be separated from the $Fe(phen)_3^{+2}$ species on both a μ-Bondapak C18 and a PRP-1 (polystyrene-divinylbenzene) column. Rentention of the $Fe(CN)_2(phen)_2$ species on the μ-Bondapak C18 column decreased as the acetonitrile content of an acetonitrile-water mobile phase decreased from 90% to 70% and then increased slightly. If the mobile phase contained 2.0g of $LiClO_4$/L the $Fe(phen)_3^{+2}$ species eluted before the $Fe(CN)_2(phen)_2^{+2}$ species when the acetonitrile concentration was over 70% and after the

$Fe(CN)_2(phen)_2$ species at lower acetonitrile concentrations. This was suggested as a method for the determination of the cyanide ion and linear calibration plots of peak area of the $Fe(CN)_2(phen)_2$ peak versus amount injected were obtained when the peak was monitored at either 540 nm or at 242 nm.

It is interesting to note that the elution order of the inert chelates was $Fe(phen)_3$, $Ru(phen)_3^{+2}$ and $Ni(phen)_3^{+2}$ on the μ-Bondapak CN, μ-Bondapak C18 and PRP-1 columns but in the inverse order on the μ-Partisil SCX column regardless of the organic to water ratio in the mobile phase with either ClO_4^- or CH_3SO_3- as of the pairing ion. Although the concentration of the pairing ion and ratio of organic solvent to water in the mobile phase had the largest effect on resolution and retention volumes it appears the cation exchange sites on the μ-Partisil SCX, do have an effect on elution order and selectivity.

Yoneda (74) has noted an inversion in the elution order for the $Co(phen)_3^{+3}$ and $Fe(phen)_3^{+2}$ species on an SP-Sephadex cation-exchange column as the KBr concentration in the mobile phase was increased. At low KBr concentrations the $Co(phen)_3^{+3}$ was more strongly retained than the $Fe(phen)_3^{+2}$ but at higher KBr concentrations the order was inverted with the inversion occuring at 0.3 mol dm^{-3}. No inversion was noted for the corresponding ethylenediamine complexes $Co(en)_3^{+3}$ and $Fe(en)_3^{+2}$ even up to 1 mol dm^{-3} KBr. On the contrary, when K_2SO_4 was used in the mobile phase the $Co(en)_3^{+3}$ - $Fe(en)_3^{+2}$ did show an inversion in elution at 0.18 mol dm^{-3} but not up to 0.2 mol dm^{-3} for the phen complex pair. This was interpreted in terms of ion association of the hydrophobic $Fe(phen)_3^{+2}$ and $Co(phen)_3^{+2}$ with the large and relatively poorly hydrated Br^- ion. On the other hand there is little

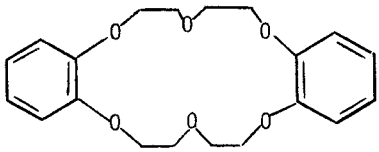
tendency toward association of the hydrophillic SO_4^{-2} ion and the phen species. The opposite is expected for the en species. This suggests that not only the nature but the concentration of the pairing ion might be critical with regard to selectivity in ion-pair chromatography of charged metal chelates.

Lundgren and Schilt (75) studied the adsorption of metal ions on Amberlite XAD-2 styrene-divinylbenzene resins coated with the ferroin type ligand 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triamine (PDT) as a function of pH and anion. They attributed the adsorption of this ligand and metal complexes to π-electron overlap between the styrene moieties on the resin and the adsorbate molecules. The order of increasing distribution coefficients for divalent metals perchlorates on XAD-2 resin columns coated with PDT was substantially different than the elution order observed by O'Laughlin (73) for the divalent metal phenanthroline complexes on the PRP-1 column.

Mangia and Lugari (76) studied the separation of the tris(2,2'-bipyridine) nickel(II) and-iron(II) complexes by ion-pair chromatography. Three well separated peaks, for the 2,2'-bipyridene ligand, the Ni(II) chelate and the Fe(II) chelate were found to elute in that order from a 10-μm -Bondapak-CN column with a 60% methanol-40% water mobile phase which was 0.02 M in KNCS. The retention volume for the Ni(II) complex was found to decrease in an exponential manner with increasing concentration of the pairing ion, NCS^-. The data were fitted to a linear equation, $1/V_R = 1.241+0.511 \log [NCS^-]$, with a coefficient of correlation of 0.9948 and a standard deviation of the slope of 2.1%. It was noted this elution order and pairing ion dependence was similar to that found by O'Laughlin (73) for the 1,10-phenanthroline complexes on a Partisil SCX column. It should be

pointed out, however, that O'Laughlin and Hanson (71) observed the opposite elution order ($Fe(phen)_3^{+2}$ before the $Ni(phen)_3^{+2}$ complex) on a μ-Bondapak CN column and an increase in V_R with pairing ion (heptanesulfonate) concentration.

2.7 Crown Ethers



Dibenzo-18-crown-6

The crown ethers, such as dibenzo-18-crown-6, are capable of forming cationic complexes with many metals. Kolthoff (77) has reviewed the the use of crown ethers, aza-crown compounds, and related compounds such as the cryptands. The use of crown ethers as anchor groups on ion-exchange resins is also reviewed as well as the use of crown ethers in extraction chromatography.

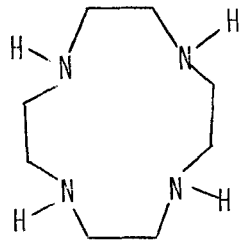
Mangia et al. (78) studied the HPLC separation of dibenzo-18-crown-6, DBC, complexes of Cd(II), Pb(II), Ag(I) and Hg(II) halides on a 10-μm Micropak CH column (octadecyl) with a methanol-aqueous phosphate or borate buffer. Only one peak due to the free ligand was observed for the first three complexes at any pH of the mobile phase. Peaks attributed to $HgCl_2$-DBC, $HgBr_2$-DBC and HgClBr-DBC were observed for the Hg(II) halides in addition to a peak for the free ligand. The mixed halide peak was obtained as well as the $HgCl_2$-DBC and $HgBr_2$-DBC peaks when an alkaline solution containing Br^- and Cl^- was treated with Hg(II) acetate and extracted with a

solution of DBC in methylene dichloride. The plate count reported for the $HgBr_2$-DBC peak of only 290 was very low and three peaks attributed to $HgCl_2$-DBC, HgClBr-DBC and $HgBr_2$-DBC were only partially resolved.

Kimura and Shono (79) have recently reviewed work on the application of crown ethers in liquid chromatography. The separation of LiCl, NaCl, CsCl, RbCl and KCl in that order on a crown-ether modified silica, the synthesis of which was previously reported by Kimura et al. (80), is reported. On a different crown-ether modified silica the KCl eluted between RbCl and CsCl and the alkaline earth chlorides $MgCl_2$, $CaCl_2$, $SrCl_2$ and $BaCl_2$ eluted in the order shown. Peak widths tended to be very broad and the peaks tailed significantly. Resolution of the peaks in the alkaline earth series was poor.

It would appear that the crown ethers should have great potential in HPLC, especially using ion pair HPLC. Although some interesting separations such as that of the alkali metals using extraction chromatography with DBC have been reported (81), it seems that the usefulness of these interesting ligands in HPLC has yet to be fully exploited.

2.8 Macrocyclic Amines



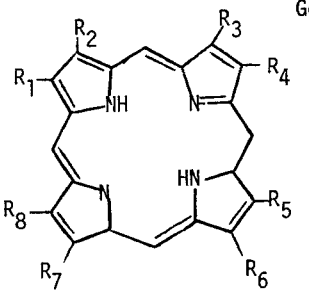
Cyclam

Macrocyclic amines such as the compound above given the trivial name cyclam by Troutner et al. (82), are formal analogs of the crown ethers with the imino group replacing the oxygen atoms. Troutner et al. (82) reported cyclam was an efficient complexing agent for Tc-99m and has potential use as a radiopharmaceutical. Hoffman et al. (83) have studied the separation of the Tc-cyclam complex by HPLC.

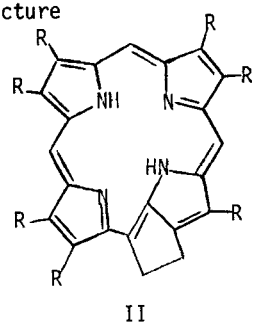
Royer et al. (84) studied the separation of optical isomers of Co(III) derivatives of macrocyclic amines similar to cyclam but with six nitrogen atoms in the ring which they call 21N6. The $Co(21N6)^{+3}$ species was resolved by eluting from a SP-Sephadex C-25 column (180x2 cm) in the sodium form. The separation of geometric isomers of Co(III) complexes with unsymmetric ligands similar to 21NG was also reported.

2.9 Porphyrins

General Structure



I



II

Metalloporphyrins occur commonly in nature. Richter and Rienits (85) reported on the separation of zinc protoporphyrin (IX) and magnesium protoporphyrin (IX) as the neutral dimethylesters (protoporphyrin has the general structure of I with $R_1=R_3=R_5=R_8=CH_3$, $R_2=R_4=CHOHCH_3$ and $R_6=R_7=CH_3CH_2COOH$). These authors used a 10-μm LiChrosorb silica column with 15% acetone-85% hexane as the mobile phase. The formation of the magnesium chelate is regarded as an

essential step in the biosynthesis of chlorophyll and these authors note this is one of the least well understood steps due partially to inadequate methods for routine assay of the magnesium chelate. HPLC seems to offer a viable solution.

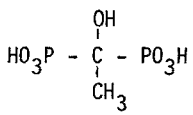
Hajibrahim et al. (86) showed that the Ni(II) complex of etioporphyrin (structure I with $R_1=R_3=R_5=R_7=CH_3$ and $R_2=R_4=R_6=R_8=CH_2CH_3$) and octaethylprophyrin (structure II with $R=C_2H_3$) could be separated from the more polar vanadyl desoxophyhloerythroetioporhyrin on a 10-μm Sphersorb silica column using a linear solvent gradient program from 2% to 50% chloroform in hexane in 20 min. with photometric detection at 400 nm. They also reported nickel and vanadyl petroporphyrins extracted from Boscan crude oil were separated using a concave solvent gradient from 10% toluene-90% hexane to 50% toluene-50% chloroform over 25 min with the nickel fraction detected at 395 nm and the vanadyl complexes at 572 nm. Spencer et al. (87) used the basic scheme suggested by Hajibrahim et al. (86) to separate vanadium containing compounds into two groups, porphyrin and non-porphyrin. The latter was partially separted using gradient elution programs and was a highly complex mixture with many vanadium containing complexes. These authors used flame emission and furnace atomic absorption to follow the elution of the vanadium complexes. On-line flame emission could not be successfully used to monitor the peaks from the HPLC according to these authors due to the lack of sensitivity, solvent effects (especially with a gradient program) and poor compatability of the nebulizer uptake ratio and column flow rate. The furnace AA methods involved discrete sampling and it was thought some vanadium species could be lost in the dry ashing step. These authors (87) claim a simpler and faster procedure was developed for the isolation of the vanadium porphyrin fraction nearly

free of vanadium non-porphyrins and nickel porphrins than procedures used previously (86).

Although metalloporphyrins are generally considered to be "non-chromotographable" by gas chromatorgraphy, Marriott (88) has recently successfully separated a number of metalloporphyrins on a capillary column with Kovats retention index values over 5,000. There was no evidence of metal exchange among the different porphyrins. Considering the difficulty with the GLC separation of these chelates, the stability of the porphyrin chelates with many metals, and the ease of detection with photometric detector, it is surprising to this reviwer that more work has not been reported on the HPLC of different metal porphyrin complexes. Hui et al. (89) have studied the TLC behavior of the tetraphenylporphyrin chelates of manganese, iron, cobalt, nickel, copper, zinc, rhodium, cadmium, mercury and lead on silica gel and alumina. A number of good separations can be deduced from the published R_F data and sharp separations by HPLC on silica columns should be possible. The intense colors of these chelates should permit selective detection in the visible region of the spectrum.

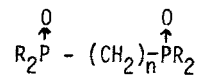
2.10 Organophosphorus Reagents

General Formulas



I

II



III

Name	Abreviation	Structure
Hydroxyethylidene-diphosphonate	HEDP	I
Tri-n-butylphosphate	TBP	II R=-OC_4H_9
Methylenebis(di-n-hexyl)phosphine oxide	MHDPO	III n=1 R=C_6H_{13}

Pinkerton et al. (90) reported on the separation of Tc-99m complexes with HEDP. These complexes are used as imaging agents in nuclear medicine. The chemistry of formulation of the Tc-99m complex involves the reduction of a mixture of $^{99m}TcO_4^-$ and the daughter product $^{99}TcO_4^-$ with a reducing agent ($NaBH_4$) and a stabilizer (ascorbic acid). The resulting mixture is somewhat complex and contains several complexes containing Tc-99 or Tc-99m. These authors were able to separate a number of these species on a 13-μm Aminex A-27 anion-exchange column. An aqueous sodium acetate solution at pH=8.4 was used as the mobile phase. Detection of the species eluted was based on photometric detection at 250 nm and 405 nm and radiometric detection. The efficiency of the column as determined by the number of plates generated was found to increase sharply around 50°C from around N=400 at temperatures below 40° to over 4,000 at 60°C based on the peak width of the most prominent peak. Wong (11) has reviewed application of HPLC for the separation of Tc-99m complexes with pryophosphates, methylene diphosphonates and other ligands.

O'Laughlin and Jensen (91) reported on the use of MHDPO and the related MEHDPO (structure III - R=ethylhexyl and n=1) as stationary phases for the separation of the lanthanide elements by extraction chromatography using 9M nitric acid as the stationary phase. Although this work and similar studies by Siekierski and Fidelis (92) with TBP as

the stationary phase was done using classical column techniques, the very sharp separations obtained and low plate heights suggest some of these systems might be of interest to people using modern HPLC equipment. Extensive reviews on extraction chromatography of inorganic species have been published by Cerrai and Ghersini (4) and Braun and Ghersini (5). Bushee et al. (93) noted the lack of any research using modern HPLC equipment and extraction reagents previously found useful in extraction chromatography. The separation of Cd(II), Zn(II) and Hg(II) was reported by these authors on a C18 column (several different types were studied) using an aqueous mobile phase which was 2M in LiCl and saturated with TBP. The elution of the metals was monitored using a refractive index detector and by atomic emission spectroscopy with an ICP inductively coupled plasma source. The results obtained were encouraging and suggest that with modifications in the equipment (glass lined columns, Teflon valves) the method has considerable potential.

3. DETECTION SYSTEMS

Most metal chelates have strong molecular absorption bands in the ultraviolet and many have useful absorption bands in the visible region of the spectrum. Consequently, simple ultraviolet detectors or variable wavelength photometric detectors were employed in most of the published work on the HPLC of metal chelates. In some cases, the less sensitive refractive index detector or the more sensitive and selective fluorimetric detectors were employed. All three are commonly used with HPLC equipment and in terms of cost, sensitivity, and convenience the photometric (and fluorimetric where it can be used) detectors are hard to match. Except for the fluorimetric detector and, to a lesser degree, the variable wavelength photometric detector they lack specificity.

Detectors which respond to specific metals or metal chelates are very useful to development of HPLC methods for metal chelate separations and are of great interest in many potential applications such as speciation studies. Some of these more specific detection systems which have been used in the HPLC of metal containing species are covered in more detail in the following sections.

3.1 Atomic absorption, AA, and Flame Emission.

Manahan and Jones (94) passed the effuent from a Chelex ion-exchange resin in the Cu(II) form directly to a Perkin-Elmer Model 403 AA unit. Samples containing EDTA (ethylenediaminetetraacetic acid) or NTA (nitrilotriacetic acid) resulted in copper being stripped from the column and the elution of the Cu(II) chelate was monitored using flame atomization by the atomic absorption due to copper. A detection limit of 5×10^{-7} millimoles of EDTA or NTA (and presumably for Cu(II), was reported. O'Laughlin and Hansen (71) used the same technique to confirm the elution of $Ni(phen)_3^{+2}$ (Section 2.6) but noted the poor sensitivity of AA as compared to photometric detection. Jones and Manahan (95) calculated minimum detectable amounts of copper which can be detected by flame AA when eluted as the Cu-NTA and Cu-EDTA complexes from a Chelex ion exchange column as 3.13 and 6.72 ng but were only able to detect 10.7 and 23.6 ng of Cu as these complexes experimentaly.

Freed (96) directly coupled a HPLC unit to a Beckman Total Consumption burner and used flame emission to monitor the elution of the alkaline earths and the lanthanide elements from a Zipax-SCX cation exchange (pellicular resin) column. With 0.01M nitric acid as the mobile phase and the detection system used in the non-selective mode (no slits), he was able to monitor the elution of Ca, Sr, and Ba. Up to five lanthanides could be eluted as separate peaks from the same column

with 0.2M citric acid as the mobile phase and the elution of the peaks monitored by flame emission in the non-selective mode. Plots of concentration versus response were claimed to be linear in the 2-10 ppm range.

Although flame AA or emission might be useful to confirm the elution of a particular metal, the inherent noise from the flame, background absorption or emission (which might be particularly troublesome with organic solvents or gradient elution methods) and the relatively poor sensitivity of both flame AA and emission methods in this mode of operation limits the usefulness of these methods of detection at trace concentration levels and more attention has been given to furnace AA and flame emission with plasma excitation sources.

Brinkman et al. (97) coupled a commercial graphite furnace AA unit with an HPLC unit for the specific detection of organometallic compounds at the nanogram level. The graphite furnace AA detector (GFAA) was operated either in a rapid sampling mode for achieving complete resolution or in a batch survey mode for maximum sensitivity. This device used an autosampler which was rotated and caught specific sized samples of the column effuent in sampling cups. An aliquot of each cup was automatically pipetted into the graphite furnace and the sample dryed, ashed and atomized in a manner consistent with the particular sample. A "reconstituted" chromatographic peak could be constructed from the individual detector pulses. Applications of the GFAA-HPLC system to a number organometallic compounds including alkyl arsenic, lead, mercury and tin compounds were reported. Parks et al. (98) used a GFAA-HPLC system similar to that just described for the analysis of biocidal organotin moieties and organotin silicates by size exclusion HPLC and reversed phase HPLC.

Vickrey et al. (99) noted a problem with the "pulsed mode" sampling in the work described above (97,98). Sharp peaks could be missed because the sampling rate depends on the rate of furnace analysis and subsequent cool-down time. They developed a method which stores an eluate sample which contains a peak (determined by UV detector response) in a capillary tube. The contents of the tube are later incrementally analyzed off-line by the furnace AA. The entire operation was controlled by a Motorola 6800 based Heathkit microprocessor. The detection limits for $Pb(Rh)_4$ was about 20 pg for solutions of $Pb(Ph)_4$ directly analyzed by GFAA and 480 pg of $Pb(Ph)_4$ for samples subjected to liquid chromatographic separation (LCGFAA) analysis using this equipment. They note that the off-line storage of peaks allows a much higher number of AA data points to be collected per peak. It appears that if the solute did not absorb in the UV its retention time would have to be known.

Van Loon (100) reviewed atomic spectroscopy as applied to the detection of metal containing species separated by chromatography. In an earlier paper Van Loon et al. (101) proposed the use of atomic fluorescence as a metal specific detection system in metal speciation-chromatography studies.

3.2 DC Plasma and Inductively Coupled Plasma Atomic Emission

Uden et al. (102) described a DC argon plasma detector and interface system to a liquid chromatograph. A detection limit of 3.5×10^{-10} g Cu/s^{-1} was obtained for this detector for the elution of $Cu(en)(acac)_2$ by monitoring the 324.7 nm copper emission line. Calibration plots were linear over the range 30-4,000 ng Cu. The separation of a number of metal (Cu(II), Hg(II), Cr(III)) chelates and

mixed chelates (of en, acac, β-ketoamine, hfa, tfa) by reversed phase HPLC on a C18 column and the detection of these species by atomic emission is reported. The authors note that solvent systems common to reverse-phase and ion-exchange systems cause few problems but that hydrocarbon and halocarbon solvents common to normal phase separations present difficulties. It is stated these can be minimized with a new nebulizer system.

Gast et al. (103) reported on the use of an inductively coupled plasma atomic emission spectrometer, ICP-AE, as a detector in HPLC. The high temperature of the plasma which excites useful emission lines for essentially all the metallic elements and elimination or depression of problems caused by compound formation in flames are claimed to make the ICP very attractive for the continuous monitoring of a large number of elements at very low levels. These authors investigated the usefulness of ICP-AE detection for a number of iron and molybdenum carbonyl complexes, ferrocene, organoarsenic compounds, organolead and organotin compounds. Detection limits were found to be in the nanogram per mL range and a linear response range of four orders of magnitude was reported. Solvents containing up to 100% acetonitrile, ethanol and higher alcohols caused no serious problems. Only xylene and toluene could be used for normal phase LC. Other solvents put out the plasma. Fraley et al. (104) reported on minimum detectable concentrations, MDL, that could be detected by ICP-AE based on the injection of aqueous solutions of twenty-five different metal solutions (one at a time) through a short dummy column. They claimed passage through the dummy column gave idealized Gaussian peaks due to diffusion processes on the column but apparently did not consider peak broadening effects on this very "inefficient column". MDL values from 1.3 (for Mn) to 1800 μg/L

(for Mg) were reported. These were far different than the MDL values found for continuous aspiration of 1 and 15 μg/L, respectively, for Mn and Mg. Bushee et al. (93) reported similar differences in relative response and found that when using ICP peak heights alone, the ratio of direct ICP to ICP/HPLC response was about 18.0.

Hausler and Taylor (105) noted that a number of investigators demonstrated the use of ICP-AE as a sequential multielement detector using a single channel and usually an aqueous solvent. They discussed the use of ICP-AE as a simultaneous multielement detector in gel permeation chromatography. The separation and detection of organically bound metal compounds using toluene as the mobile phase is reported. Multiple metal peaks (for each individual metal) were observed in some cases in the chromatography of a Conoco C-21 standard which contained 21 elements at specified concentration levels. The Conoco C-21 standard does not necessarily have all the metals present in only one form and the multiple peaks for some metals was expected. In a subsequent paper (106), these authors used pyridine as the mobile phase for the SEC separation of the Conaco-21 standard and a simpler mixture of four metal containing compounds including ferrocene, 1,1'-diacetylferrocene, acetylferrocene and bis(tetrapyrazolylborate)iron (II). These four compounds were clearly separated and gave four peaks on elution with pyridine but a much different chromatogram was obtained on elution with toluene. This, plus other evidence, was interpreted in terms of solute interaction with pyridine. Several peaks were observed for the elution of $Cu(acac)_2$ with pyridine on the 100A μ-Sytragel column but the number of peaks and retention times were not reproducible on successive injections. Coal liquefaction process solvents were also examined by this technique for organically bound trace metals. Several metals show

complex chromatograms indicating the metal is present in more than one form. Although interesting data were obtained using the ICP-AE detection system , the multiple peaks observed for some metals in complex samples are difficult to interpret and the multiple peaks found for just one metal compound, $Cu(acac)_2$, with pyridine suggest additional studies on simple systems are needed.

Jinno and Tsuchida (107) have recently described an interface system for coupling a micro high performance liquid chromatograph (0.5 mm i.d. x12cm Teflon column packed with 5μm ODS material, Jasco SC-01 column) to an ICP-AE unit. A flow rate of only 25 μℓ/min was used and peak heights and widths with UV detection and ICP-AE detection were very similar for $Cu(acac)_2$ and $Cu(DDC)_2$ samples. No significant differences were observed with methanol or methanol-water mobile phases and it was conjectured that gradient elution could be used. A mixture of Cu(II), Zn(II), Fe(III) and Co(III) diethyldithiocarbamates was injected and the peaks were resolved when monitored at specific emission wavelengths for each metal but it was not possible to resolve the four peaks with UV detection because of the small differences in the retention times. The authors are clearly using the term "resolution" in two different ways which is unfortunate and could lead to confusion.

3.3 Electrochemical Detection

MacCrehan and Durst (108) used differential pulse voltammetry to detect organomercury species eluted from a 5-μm Spheriosorb ODS column. The organomercury cations were reacted with 2-mercaptoethanol and the neutral complexes eluted with a 40% methanol-60% aqueous phase buffered at pH 5.5 and 0.06 M in ammonium acetate. The advantages of the differential pulse method and the amalgamated gold working electrode are discussed. MacCrehan (109) extended this method to the determination of

organotin compounds and reported that triphenyltin gave a linear response with concentration in the range 10^{-4} to 10^{-6} mol/L. MacCrehan and Durst (110) described a dual electrode system employing two sequential generator/detector electrodes in which analytes are first electrolyzed and then detected electrochemically at the second electrode. They note their system has particular advantages for the detection of species such as the organotin cations and other complexes with relatively high oxidation or low reduction potentials. Lyle and Saleh (111) describe the amperometric detection of Cu^{+2} and Zn^{+2} eluted from an Ionex-SA (15-17 μm) column using a specially designed dropping mercury electrode.

Bond and Wallace (58) discussed the detection of metal diethyldithiocarbomates electrochemically (see Section 2.4). In a later paper, Bond and Wallace (112) described an automated method for the determination of nickel and copper at trace levels in a wide variety of matrices based on the HPLC separation of the diethyldithiocarbamate or pyrrolidine dithiocarbamate chelates of Ni(II) and Cu(II) with both electrochemical and photometric detection of the metal chelates. The entire operation from sample injection to the waveform applied to the electrochemical detector and data reduction is under microprocessor control. The metal complexes are formed in situ and the ligand was made a component of the mobile phase (70% acetonitrile-30% aqueous acetate buffer at pH6 and 10^{-4}M in ligand). Various electrochemical waveforms were studied at a glassy carbon electrode. Detection was based on the oxidation (not reduction) of the complexes which eliminated the serious problem of oxygen interference in the reductive mode. Electrochemical detection limits of 0.1 and 0.2 ng of nickel and copper, respectively, were reported with DC detection at +0.70 volts vs Ag/AgCl as compared to

photometric detection limits of 0.2 ng Ni/L and 1.0 ng Cu. The authors report that this automated system has been applied in industrial situations for 18 months and that the extreme versatility of the system has enabled a system of continuous monitoring to be developed. It appears to the present author that the excellent work described by Bond and Wallace shows the great potential in the area of trace metal analysis for methods based on the HPLC separation of metal chelates.

Bethod et al. (65) discussed the electrochemical detection of metal oxinates (See Section 2.5).

Lewis et al. (113) used electrochemical detection for the determination of the pertechnetate ion after separation from potential interferences by HPLC on a NH_2-bonded phase column. The determination of total TcO_4^- ($^{99m}TcO_4^-$ + $^{99}TcO_4^-$) is an important problem in radiopharmacy. These authors studied the reduction of TcO_4^- at both solid (glassy carbon) and static mercury drop electrodes with various applied waveforms (sampled DC, normal pulse, and differential pulse). The determination of TcO_4^- down to the 10^{-8}M range in $^{99}Mo/^{99m}Tc$ generator effluents was reported.

3.4 Post-Column Reactors

Hirose et al. (114) described a post-column reaction technique for the detection of the lanthanides eluted from an ion exchange column. The lanthanides were reacted with xylenol Orange and the metal complexes detected photometrically at 630 nm. Elchuck and Cassidy (115) describe a post-column reaction system for the determination of metal cations following the separation of these cations on a cation exchange column. The eluate is mixed with a chromogenic reagent, sodium 4-(2-pyridylazo)-resorcinol, and the metal complexes monitored at 530 to 540 nm. These authors applied this technique to the determination of lanthanides (115)

and transition metals (116-118). With an on-column trace enrichment procedure they were able to determine a number of metals in the pg/mL range. Beckett and Nelson (119) separated potentially fluorescent metal chelates of 4-aminophenylethylenediaminetetraacetic acid on a 10-μm Partisil-SAX column and detected the chelates on elution by reaction with fluorescamine and fluorimetric detection. The fluorescamine derivatives of these chelates exhibited a linear fluorescence detector response over the range $5x10^{-7}$ to $5x10^{-11}$ g of metal ion. No change in relative sensitivity was observed with different metal ions.

4. HPLC SEPARATION OF OPTICAL AND GEOMETRIC ISOMERS

Strazza and Polcaro (120) recognized the advantages of a fast separation method and used HPLC to separate the cis- and trans-isomers of labile Co(III) species. Cis-$[Co(en)_2(OCOC_6H_5)_2]$ NO_3 was mixed with NH_4Cl and the formation of the trans-$[Co(en)_2(OCOC_6H_5)_2]^+$ and an intermediate species thought to be trans-$[Co(en)_2(OCOC_6H_5)Cl]^+$ was evidenced by the appearance of three peaks in a chromatogram of the mixture after it was heated for 10 min. at 130°C. These authors used a 10-μm Micropak-SI column and the mobile phase was a mixture of 95% ethanol, 2-propanol, 25% aqueous ammonium acetate, and 1N acetic acid in methanol in the ratio 140:60:2.5:0.15. Isocratic elution, not gradient as suggested in a recent review (7), was used and the benzoato complexes were detected at 580 nm. The cis- and trans-$[Co(en)_2CO_3]^+$ species as well as intermediate species were also separated on the same column with a mobile phase as above but in the ratios, 60:140:2.5:0.15, with detection at 340 nm. This work appears to be either an example of ion exchange chromatography on a silica column or of ion-pair chromatography with the acetate ion as the pairing ion.

The speed and high efficiency of HPLC are attractive features for the separation of both labile geometric isomers and for the frequently much more difficult separation of optical isomers. The column chromatographic separation of optical isomers by derivatization (i.e. as diastereoisomers) has been used for some years. Direct chromatographic resolution of enantiomers is a newer approach which has been reviewed by Audebert (121). Yoneda (122) has reviewed the resolution of enantiomers of octahedral metal complexes and discuss the fundamental factors involved in the resolution of optically active species in terms of the "fit" between a pairing ion such as d-tartrate and the Ω-$[Co(en)_3]^{+3}$ or Δ-$[Co(en)_3]^{+3}$ form of the complex. Yukimoto and Yoneda (123) reported on the separation of enantiomers in the series fac-$[Co(\beta\text{-ala})_n(\alpha\text{-ala})_{3-n}]$ on an anion exchange column charged with chiral and achiral anions such as d-tartrate, antimony d-tartrate, chloride, or sulfate. Nakazawa et al. (124) extended the above studies to the separation of geometric isomers of monopositive cobalt(III) complexes such as $[Co(gly)_2en]^+$. Yamazaki and Yoneda (125) reported on the resolution of racemic cations on an anion exchange column and developed general equations for the retention volumes and selectivity ratios for two enantiomers with monopositive cations such $[Co(gly)_2en]^+$, $[Co(gly)_2(NH_3)_2]^+$ and $[Co(N_3)_2trien]^+$. If a dinegative chiral selector ion such as $[Sb_2\text{-d-tartrate})^{-2}$ is used in the mobile phase it is more strongly held on the anion exchange column than the ion-pair 1:1 complex with a net charge of minus one. Thus, only relatively low concentrations of the chiral selector ion are required in the mobile phase to elute the enantiomer pairs unlike the case when a cation exchange column is used. Calculated values for the optimum chiral selector ion concentration are compared with experimental

results and the latter are shown to agree well with the theory developed. Although the above work does not involve the use of HPLC, the demonstration that outer sphere ion pairing results in separation of enantiomers and the discussion in these papers on how the "fit" of ion pairs affects retention volumes should be of great interest to research workers investigating factors which relate to selectivity in ion-pair HPLC.

Buckingham et al. (126) described the separation of a series of amino acid Co(III) bis(ethylenediamine) complexes, $[Co(en)_2AA]X_2$ by ion pair HPLC on a 10-μm Bondapak C18 column. An excellent separation of the $\Delta[Co(en)AA]I_2$ complexes was achieved where AA was glycine, proline, valine, leucine, phenylalanine with retention times of 5.6,6.8,8.8,10.8 and 13.8 min., respectively. A linear gradient from 0 to 100% methanol in 15 minutes was employed with the mobile phase kept 5mM in p-toluene-sulfonate and at pH 3.5. The elution order was the same with 5mM hexanesulfonate as the pairing ion but the percentage methanol had to be increased ten-fold to achieve comparable k values. The similarily in elution order to that of the amino acids on silica was interpreted to mean there was a preferred orientation of the charged complex with regard to the hydrocarbonaceous stationary phase such that the amino acid side chain participates significantly in the retention mechanism. This feature can be exploited for the separation of the Δ-S and Λ-S or Δ-R and Λ-R diastereoisomeric mixtures. The nature of the counter ion I^-, Cl^-, or ClO_4^- did not affect the resolution. These authors reported an unusual phenomenon which they believed was clearly due to the association of two (or more) different cationic species. At constant pairing-ion concentration when the sample loading of two charged complexes such as Λ-$[Co(en)_2gly)^{+2}$ and

Λ-$[Co(en)_2Pro]^{+2}$ was increased each peak split into two peaks. This peak splitting was also seen at constant loading of sample when the pairing-ion concentration was decreased. It was not due to overloading as shown by varying the concentration of a single species. Achiral complexes, $[Co(NH_3)_5X]^{+2}$, showed similar peak splitting. These experiments show that there must be an interaction between two (or more) complex ions which infuences their respective distribution ratios according to the above authors. They pointed out this effect could easily be avoided for analytical purposes by proper choice of the counter ion concentration.

Minor and Everett (127) reported that the resolution of electrically neutral complexes such as $M(\beta\text{-dik})_3$ was generally difficult but by using chiral β-diketone ligands such as (+)-3-acetylcamphor, atc, four stereoisomers are possible; Δ-cis, Λ-cis, Δ-trans and Λ-trans. The four stereoisomers of $Cr(atc)_3$ were separated on a Corasil II silica column using a 15% THF -85% hexane mobile phase with a flow rate of 0.4 ml/min.

Warner and Legg (128) describe a "bare bones" liquid chromatographic system suitable for the preparative separation by synthetic inorganic chemists of geometrical isomers and diastereomers of metal complexes. A Merck 4x60 cm "Lo-Bar" column was slurry packed with Whatman LP-1 silica and 70/30 IS-TEA (isopropyl alcohol-2M triethylammonium carbonate buffer at pH 9). Analytical and preparative separations of $[Co(en)_2Tyr]^{+2}$ diastereomers were shown with the elution of the species monitored at 510 nm.

5. CHELATES AS MODIFIERS

A number of authors have used metal chelates as a component of the mobile or stationary phase to modify the elution behavior of organic

solutes. Chow and Gruska (129) chemically bonded dithiocarbamate and β-diketone groups by reacting carbon disulfide or ethylbenzoylacetone, respectively, with a previously bonded-amine Partisil-10. They loaded the bonded-phase material with Cu(II) and noted this stationary phase permitted selective separations of aromatic amines and other compounds not attainable without the Cu(II) bonded-phase. Karger et al. (130-132) explored the use of metal chelates in the mobile phase to enhance selectivity in HPLC. They observed that a metal chelate in the mobile phase was equivalent to a counter ion such as a quaternary ammonium ion in ion-pair chromatography. However with aromatic acids ($RCOO^-$ or RSO_3^-) the functional group selectivity and steric or isomeric selectively was much more pronounced with metal chelates such as C_{12}-dien-Zn(II) relative to the usual counter ions. They noted that the loading of the metal chelate from a mobile phase 10^{-3}M in C_{12}-dien-Zn(II) onto a Lichrospher-C8 column was slow and required roughly 50 column volumes of mobile phase to be passed through the column but that the column quickly reached equilibrium if the salt concentration (ammonium acetate) was changed. The selectivity and high performance of these systems was interpreted in terms of ligand exchange reactions in the outer coordination sphere of the chelate with chiral metal chelates in the mobile phase such as L-2-isopropyl-4-octyl-diethylenetriamine-Zn(II) (C_3*-C_8-dien-Zn(II)). These authors (132) showed it was possible to resolve the optical enantionmers of all the common amino acids except D,L-Dns-Proline (Dns, dansyl). The latter was resolved using L-proline-n-octylamide with a stoichiometric amount of Ni(II), instead of C_3*-C_8-dien-Zn(II).

Lochmuller and Hangac (133) described the use of the square planar and coordinately unsaturated complex, bis(2,2,6,6-tetramethylheptane-

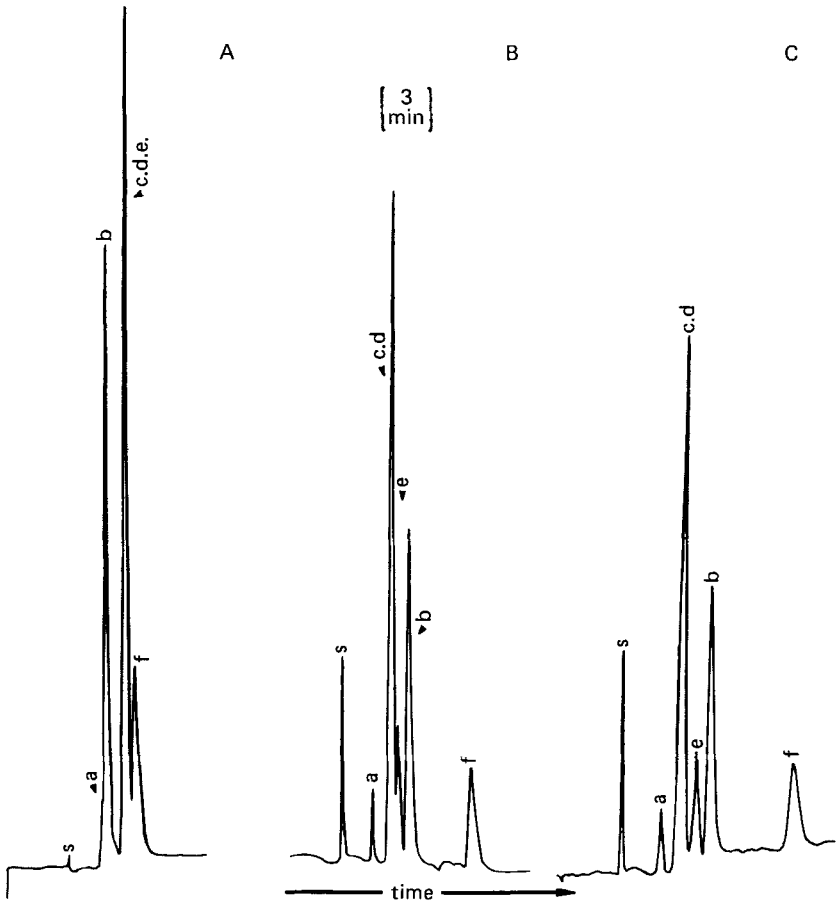


Fig. 6. Chromatograms of amine and pyridine derivatives with and without the Ni(DPM) mobile-phase additive: Column: Whatman ODS-3 (25cm x 4cm); chromatographic conditions: A. 60/40 methanol/water; flow rate: 1 ml/min; temperature: 30°C, B. 1.25 x 10^{-4}M Ni(DPM) - 60/40 methanol/water flow rate: 1 ml/min; temperature: 30°C; C. 4.0 x 10^{-4}M Ni(DPM) - 60/40 methanol/water; flow rate: 1 ml/min; temperature: 30°C Peak a: aniline; b: pyridine; c: o-toluidine; d: 2-picoline; e: p-toluidine; f: 4-picoline. Reprinted by permission from Reference 133 (Preston Public.).

3,5-dionato)nickel(II), ($Ni(dpm)_2$), as a mobile phase modifier. This neutral complex interacts with polar solute molecules and the interplay of factors such as the dipole moment, structure, and solvation effects influence the net interaction. The complex is transparent in the 210-290 nm range permitting UV detection of solutes. The separation of some amine and pyridine derivatives with and without the modifier is shown in Figure 6.

Davankov et al. (134) reported on the resolution of racemic amino acids using ligand exchange on a polystyrene resin containing residues of chiral heterocyclic α-amino acids and charged with Cu(II) ion. Sugden et al. (135) described the resolution of amino acids on a microparticulale silica column holding a chiral Cu(II)-proline complex. Gubitz et al. (136) also used a chiral Cu(II)-proline complex which was chemically bonded to the silica support via 3-glycidoxypropyltrimethoxysilane. Carunchio et al. (137) used a porous silica gel modified with N(aminoethylamino)propyl groups and Λ (+) $[Co(en)_2(NO_2)]Br$ to separate optical isomers by an outer-sphere complex mechanism. Corradine (138) used a cross-linked dextran gel with amino-Co(III) complex groups to separate some mono-and dinucleotides.

In 1975 Porath et al. (139) introduced metal chelate affinity chromatography for the fractionation of proteins. Hansson and Kagedal (140) coupled iminodiacetic acid to Sepharose activated with 1,4-bis-(2,3-epoxypropoxy)butane. This column was loaded with Zn(II) or Cu(II) and used to separate protein fractions.

The use of metal chelates in trace metal analysis and the use of metal ions and chelates as modifiers in the separation of organic solutes has recently been discussed by Raja (141).

6. CONCLUSIONS

It has been shown by many investigators that HPLC can be used for the separation and determination of metal chelates even at trace concentration levels. With the use of modern microprocessor controlled apparatus, routine monitoring of trace metals by HPLC has become possible. A very nice example of this possibility is the automated HPLC method for the determination of copper and nickel in industrial plant solutions developed by Bond and Wallace (112) and described in Section 3.3. This procedure involves a chemical step (formation of the nickel and copper dithiocarbamate complexes), a separation step (HPLC of chelates), and the detection step (either photometric or electrochemical) with most experimental variables controlled by the microprocessor-based system which also generates the proper electrochemical wave from (for the electrochemical detector), collects, stores, and analyses the data. Other applications of such systems, perhaps with different chelates, seem certain to be forthcoming.

The use of HPLC in speciation studies promises to be a rapidly developing area. The simultaneous determination of Cr(III) and Cr(VI) by HPLC described by Schwedt (54) and of Mn(II) and Mn(III) by Hoffman and Schwedt (68) suggest the usefulness of HPLC in this area. The use of HPLC to separate metal porphyrin species in crude oil described by Hajibrahim et al. (86) and Spencer et al. (87) is another area of great potential in the speciation area. The use of metal specific detector systems such as GFAA and ICP-AE systems coupled with HPLC separation methods will undoubtedly be more widely used in this area. Electrochemical detection systems would also appear to have a bright future in speciation studies.

It seems very likely that HPLC will be much more widely employed by coordination chemists in the near future. The rapid separation of mixed ligand species, geometric and optical isomers of coordination compounds, and the usefulness of HPLC in kinetic studies will certainly be exploited.

The use of metal chelates as modifiers in the mobile phase to increase selectivity in the separation of organic solutes is another area in which rapid growth is already in progress. The relatively few applications referenced in Section 5 should only be viewed as an introduction to this rapidly developing area.

Although much progress has been made in the last decade in the HPLC of metal chelates, some problems have surfaced repeatedly and should be kept in mind. Metal chelates can and do dissociate to some extent during the separation process and tailing peaks and unsatisfactory chromatographic separations will be the result. This may be a very minor or quite insignificant problem for kinetically inert chelates but a major problem for labile chelates even though the thermodymanic formation constants are large. A possible solution in the case of labile chelates is to include the ligand as a component of the mobile phase and to buffer the mobile phase at a pH when the conditional equilibrium constant is sufficiently large. This, of course, raises the possibility of interaction by the chelating reagent with metallic components of the HPLC system. All glass or other metal-free systems can be employed to avoid this problem but this increases the experimental difficulties and may put unacceptably low pressure limits on the system. Commercially available pumps have been used with many mobile phases including chelating reagents with little problem. There is some evidence that suggest the major source of metallic contamination

of the sample occurs is the sample preparation and injection process. It should also be kept in mind that reduction of metal complexes may occur by contact with metallic surfaces and oxidation of Co(II) and Mn(II) in the chelate formation step has frequently been observed.

Peak splitting has been reported by several investigators. Hausler and Taylor (105) reported multiple peaks for $Cu(acac)_2$ with pyridine as the mobile phase (sec. 3.2) and Buckinghan et al. (126) noted peak splitting for both chiral and achiral Co(III) complexes with amino acids (Sec. 4).

While some unresolved problems remain, this reviewer believes that HPLC methodology will be employed much more widely in the near future for the determination of trace metals, in speciation studies, in coordination chemistry, in radiochemistry, in the separation of geometric and optical isomers, and that metal chelates will increasingly be used as modifiers in difficult separation problems.

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