IS-T--1466
DE90 014427

New Chelating Reagents for the Separation of Metal Complexes by High Performance Liquid Chromatrography

by

Mark Vernon Main

PhD Thesis

Date Transmitted: July 1990

Ames Laboratory is operated by Iowa State University
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of Energy under Contract no. W-7405-ENG-82.

New chelating reagents for the separation of metal complexes by high performance liquid chromatography

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New chelating reagents for the complexation of metal ions in aqueous samples, have been developed. The reagents synthesized and tested were 2,6-diacetylpyridine bis(N-methylenepyridiniohydrazone) dichloride (abbreviated H_2dapmp), 2,6-diacetylpyridine bis(N-methylene-N,N,N-trimethylammonio-hydrazone) dichloride (abbreviated H_2dapmta), and 2-acetylpyridine-4-ethyl-3-thiosemicarbazone (abbreviated Hapet). All three chelating reagents proved to be useful for the separation and determination of metal ions by reverse-phase high performance liquid chromatography. Both H_2dapmp and H_2dapmta formed kinetically stable metal complexes that did not dissociate on the chromatographic column.

H_2dapmp and H_2dapmta were prepared and characterized. Both complexing reagents contained quaternary ammonium groups which greatly enhanced the water solubility of the reagents and their metal-ion complexes. When the solid H_2dapmp was dissolved in water, an equilibrium was attained in which three geometric forms co-exist. A rate constant was determined for the equilibrium of the E,E isomer, into the E,Z and Z,Z geometrical isomers. Several metal ions, including Ti(IV), Fe(III), V(V), and U(VI), were determined quantitatively and selectively by complexation with one of the bis hydrazones and chromatographic separation of the metal ion complexes at pH 3.3.

At a pH of 7 it was possible to extend the technique and separate and determine 8 metal complexes, in a single run, under gradient conditions on a polystyrene-divinylbenzene polymer based column. Possible separation of rare earth complexes and ion-pairing behavior were also investigated. Linear calibration curve ranges and detection limits are presented for the metal complexes of Sn(II), Ti(IV), Mn(II), Co(II), Cd(II), Sb(III), Cu(II), Ni(II), Pr(III) and U(VI) under neutral conditions. Interferences due to the presence of other metal ions in solution were also investigated at both pH values.

Hapet was prepared and extensively characterized. Hapet complexes of In(III), Ga(III), Fe(III), Co(II), Zn(II), Cd(II), Bi(III), and Pd(II) were separated chromatographically using reverse-phase HPLC. Many of these metal ions were also tested for linear calibration ranges, and the effect of foreign ions in the sample solution. The effects of varying chromatographic conditions on metal complex retention are also described.

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Mark Vernon Main

A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of the
Requirements for the Degree of
DOCTOR OF PHILOSOPHY

Department : Chemistry
Major : Analytical Chemistry

Approved:

Fritz
In Charge of Major Work

For the Major Department

For the Graduate College

Iowa State University
Ames, Iowa

1990

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GENERAL INTRODUCTION

Presently, aqueous metal ions are determined by a variety of different analytical techniques (1,2) including electrochemical techniques, such as, polarography, amperometry, and coulometry; and chromatographic techniques, such as, ion chromatography. Mass measurements of metal ions continue to be popular although modern techniques (inductively coupled plasma-mass spectrometry (ICP-MS)) have generally replaced the more traditional methods (gravimetric analysis). Atomic spectrometry has been applied to metal determination through a variety of means including atomic absorption, emission, and fluorescence using a number of different sources (flames, graphite furnaces, arc/spark, inductively coupled plasma (ICP), microwave induced plasma (MIP), etc.). Spectrophotometric determination of metals ions through the use of color forming chelating reagents is also popular with new complexing reagents being introduced continuously.

Of these techniques, inductively coupled plasma-atomic emission spectroscopy (ICP-AES) and ICP-MS have gained the most in popularity in recent years. This is due to the many advantages of these techniques, such as extremely low limits of detection, large linear dynamic ranges, and the ability of the plasma to atomize and ionize metal ions in virtually any sample matrix. Although the ICP is an excellent source for atomizing and ionizing elements, AES and MS do have certain limitations in terms of spectral and mass interferences, respectively. For example, in the determination of arsenic, ICP-AES suffers from spectral interference due to the presence of Fe in the sample matrix, whereas ICP-MS suffers from a mass interference due to the presence of chloride in the sample (3). Also, both techniques give no information about the oxidation state of the elements in the original sample solution. Although both ICP-AES and ICP-MS do have some detection limitations, the most prohibitive aspects of the two techniques for a small analytical laboratory is their capital cost.

The present work will look at an alternative way of determining aqueous metal ions. This work will look at developing new chelating reagents for the determination of metal ions spectrophotometrically as their metal complexes after a liquid chromatographic separation of the various metal complexes of interest. This method is a powerful technique for determining metal ions due to the coupling of two very complementary techniques. Spectrophotometric detection is very a sensitive method for determining metal ions through the use of color forming reagents. This method does suffer from matrix effects since more than one metal ion normally will complex with the color forming reagent. Chromatography is a natural complement to spectrophotometric detection, because the various metal complexes can now be separated chromatographically and determined individually as they elute from the chromatographic column.

The determination of metal ions by the chromatographic separation of their metal complexes is a useful technique. Not all chelating reagents are suitable for this technique. Some of the requirements a chelating reagent must meet to be successful are discussed in the following paragraphs. Many compounds can behave as coordination compounds, but very few of these compounds can meet all of the following requirements.

The first desirable feature is for the chelating reagent to complex with a variety of metal ions, thereby making it possible to do a multi-element analysis. Obviously, the most ideal situation is to have a complexing reagent that forms coordination compounds with all of the metal ions of interest in the sample solution. It is also preferred that the complexing reagent forms these metal complexes selectively, thereby removing possible interferences, such as competition for a limited amount of chelating regent, chromatographic coelution, etc. In some instances selectivity can be acquired by varying the pH of the sample or by the introduction of a competing complexing reagent.

The coordination number of the metal ions should also be satisfied by the chelating reagent, and form only one type of metal complex. If the metal ion is not satisfied by the complexing reagent, many different types of metal complexes are potentially possible, depending on the type of matrix present. While it is preferable that only one type of metal complex is formed with each metal ion, the formation of more than one type of complex is acceptable provided the ratios between the various species remains constant at different metal concentrations.

In designing a metal complex it must be remembered that if the ligand becomes too voluminous, the different metal complexes will be very difficult to separate chromatographically. Larger ligands are more tolerable if the electron density in the the metal complex is shifted away from the metal atom towards the ligand (4).

Preferably, the metal complexes should also be thermodynamic and kinetically stable. This requirement is usually achieved by designing chelating reagents that behave as multidentates, and form 5- and 6-membered chelation rings with the metal ions. The low concentrations of solutes used in modern chromatography make it somewhat difficult to find complexes that will not fall apart to some extent during the chromatographic separation. Metal parts in the chromatographic system and ion-exchange properties of silica-based column packings contribute to the instability of some metal complexes. Addition of complexing reagent to the eluent (as well as to the sample) enhances the stability of the complexes and permits re-formation of the complexes if dissociation takes place (5-7). However, adding complexing reagent to the eluent is unnecessary when the metal complexes are kinetically inert. Although the dissociation might be favored thermodynamically, the kinetics of the dissociation are slow and the metal complexes traverse the chromatographic system intact.

The solubility of both the chelating reagent and the metal complexes should also be considered when separating metal complexes by HPLC. The most common method is to use a hydrophobic chelating reagent and extract the metal ions out of the aqueous phase and into an organic phase as hydrophobic metal complexes. A portion of the organic phase is then injected onto a chromatographic column, and the metal complexes are generally separated by normal-phase liquid chromatography. Although this method does allow for some enrichment of the sample and the removal of some of the matrix, it is a labor-intensive process.

An alternative approach to the extraction method is the direct injection method. In the direct injection mode greater hydrophilicity is intentionally designed into the synthesis of the chelating reagent (6-8). The goal is to add chelating reagent directly to an aqueous system without precipitation, form the metal complexes as water soluble species, and then inject a portion of the aqueous solution onto the chromatographic column and separate the metal complexes by reverse phase liquid chromatography. The direct injection method has several advantages over the extraction method in terms of sample preparation. The advantages gained by employing a direct injection technique include the elimination of a labor-intensive step (since an additional analytical step is eliminated), a reduction in analysis time, a reduction in solvent cost, and elimination of the problems of handling and disposing of potentially toxic and carcinogenic organic solvents.

The metal complexes should also lend themselves to easily detection. Typical detection methods include UV-vis absorption, fluorescence, and electrochemical detection. Since most metal chelates will absorb in the UV-vis spectral region, this is by for the most common detection method. Obviously, the detection limits will be lower if the complexes are strong UV-vis absorbers. This conditions can usually be improved by an extended conjugation system, since extinction coefficients generally increase as conjugation is increased (9). Since the metal complexes normally have some rigidity, fluorescence detection is also feasible in many instances, thereby lowering the detection limits further (10). Electrochemical detection is also popular provided that the metal complexes are responsive to oxidation-reduction reactions (11-15).

Due to the requirements listed above, there are many complexing reagents cited in the literature that prove unsuccessful for the chromatographic separation of metal complexes. Some chelating reagents do meet the requirements listed above and are useful for determining metal complexes chromatographically. Many review articles have been written on the subject (4,16-20). It should be noted that although many different complexing reagents have proved successful, many contain very similiar functionality. Some of the most common types of chelating functionality employed are dithiocarbamates, β-

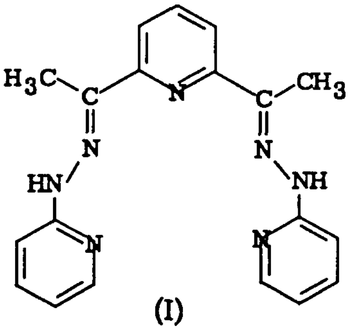
diketones/thiodiketones, oxinates/thiooxinates, and cyclic chelating agents, such as, crown ethers and porphrins. For example, in a recent review article dealing specifically with analytical applications of this technique, of the 37 references cited, 28 of the studies had employed some type of derivative of dithiocarbamate (20).

SECTION I. CHROMATOGRAPHIC DETERMINATION OF METAL IONS AFTER COMPLEXATION WITH BIS(QUATERNARY AMMONIUM HYDRAZONES) OF 2,6-DIACETYLPYRIDINE UNDER ACIDIC CONDITIONS

INTRODUCTION

Bis(hydrazones) of 2,6-diacetylpyridine constitute a class of chelating reagents that have received very little attention from analytical chemists. Much more work has been performed by inorganic chemists looking at the metal complexes in the solid state by X-ray diffraction or other spectroscopic techniques.

Curry et al. synthesized the first derivative of 2,6-diacetylpyridine that behaved as a quinquedentate ligand. In their work (1) 2,6-diacetylpyridine bis(2'-pyridylhydrazone) (H_2dapp) was shown to act as a quinquedentate ligand in the complexation of cobalt (II) ions where chelation occurred through the pyridyl nitrogens and the two imino nitrogens, as shown in (I). Webster and Palenik (2,3) investigated H_2dapp as a complexing agent for Zn(II) and Co(II) ions. Paolucci and Marangoni (4,5) investigated H_2dapp as a chelating reagent for the complexation of uranium(VI). The results of these studies supported Curry's early work (1), and indicated H_2dapp was acting as a quinquedentate ligand.



(I)

Several research groups in Europe have also studied the metal complexes of 2,6-diacetylpyridine bis(aroylhydrazone) in the solid state. These complexing reagents also have the ability to act as a quinquedentate ligand with chelation occurring through the pyridyl nitrogen, the two imino nitrogens, and the two carbonyl oxygens, producing four, 5-membered chelation rings, as shown in Figure 1. Many different derivatives have been prepared by reacting various hydrazides with 2,6-diacetylpyridine, as shown in Figure 2. The most frequent derivative investigated has been 2,6-diacetylpyridine bis(picolinoylhydrazone) (H_2dappc) and its metal complexes of copper(II) (6), manganese(II) (7), cobalt(II) (7,8), nickel(II) (7), and zinc(II) (7). Other metal complexes investigated include tin(IV) metal complexes of 2,6-diacetylpyridine bis(salicyloylhydrazone) (H_2daps) (9), and 2,6-diacetylpyridine bis(2-aminobenzoylhydrazone) (H_2dapa) (10); the uranium(VI) metal complex of 2,6-diacetylpyridine bis(4-methoxybenzoylhydrazone) (H_2dapmb) (11); the cadmium(II) metal complexes of H_2daps (12); and the manganese(II) metal complex of 2,6-diacetylpyridine bis(benzoylhydrazone) (H_2dapb) (13). However, cobalt(II), nickel(II), copper(II), and zinc(II) metal complexes have been the most popular chelates investigated and all four metals have been characterized as the H_2daps (12), the H_2dapb (13), the H_2dapa (14), and the 2,6-diacetylpyridine bis(2-thenoylhydrazone) (H_2dapt) (15) metal complex. As can be seen, much work has been done with this type of bis(hydrazone), although

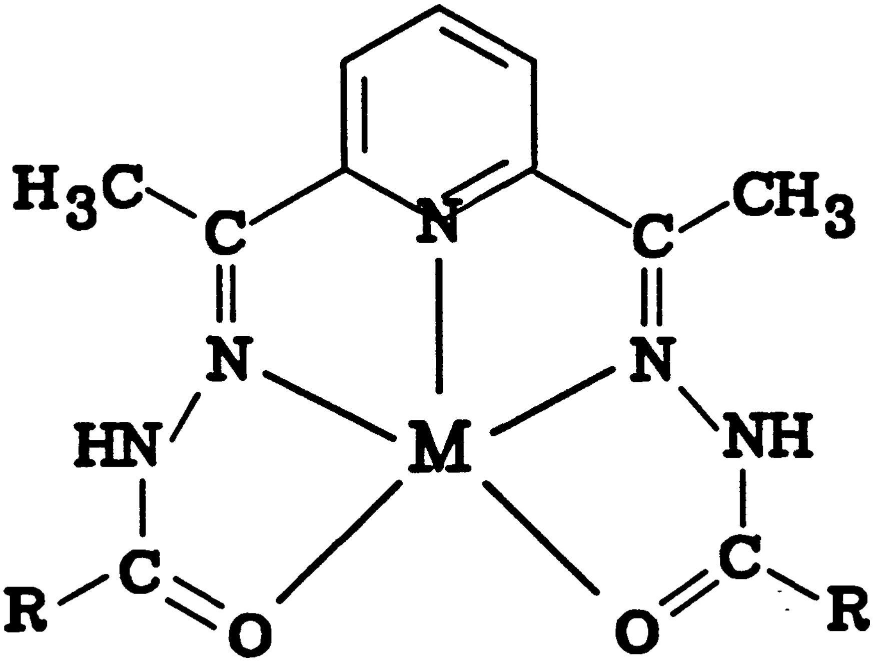
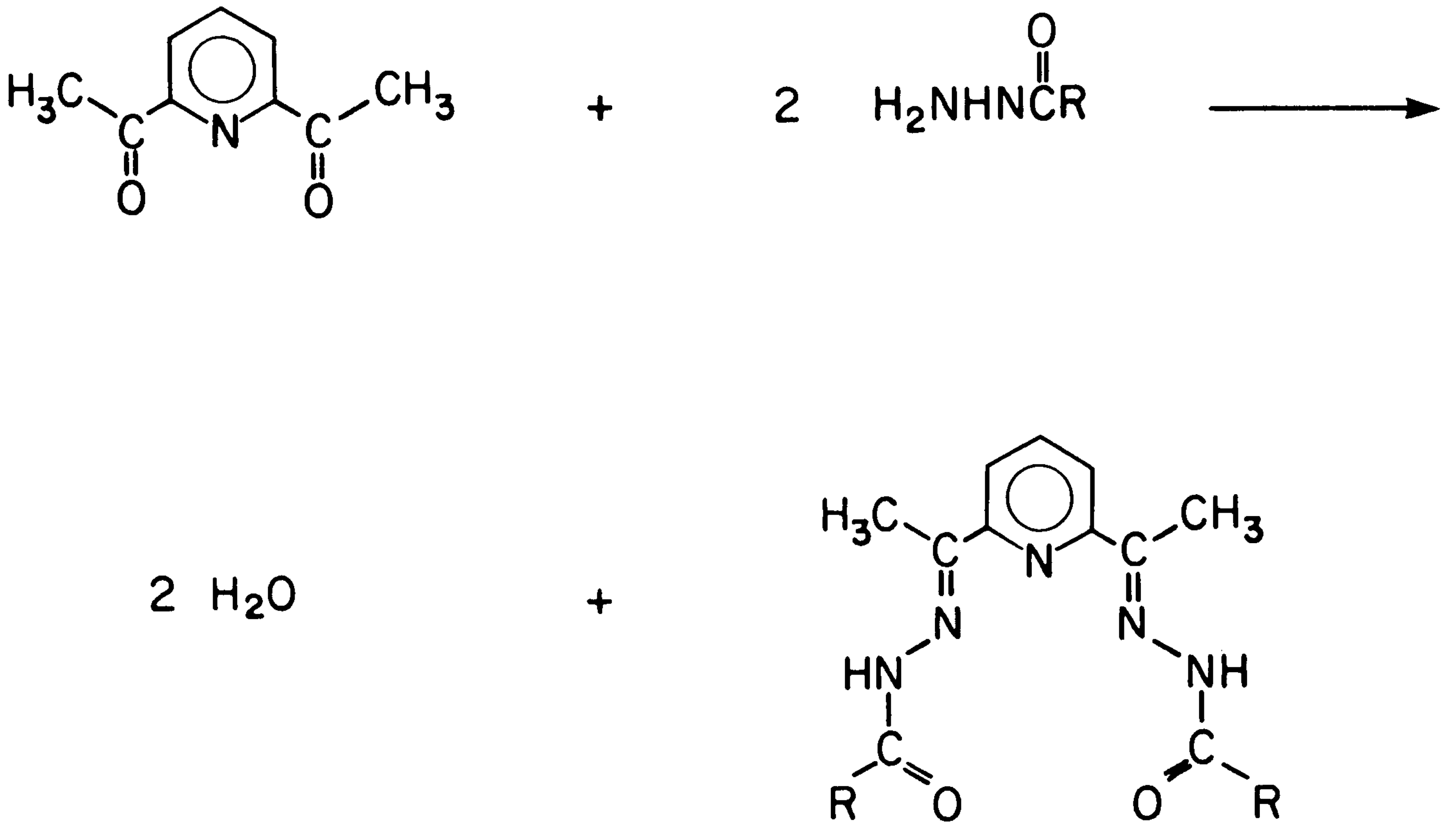


Figure 1: 2,6-diacetylpyridine bis(hydrazone) behaving as a quinquedentate ligand in the complexation of a metal ion (M).

Figure 2: Synthesis route of bis(hydrazones) of 2,6-diacetylpyridine.


H3C
CH3
N
O
+
2
H2NHNCR
2 H2O
HN
NH
R

the focus has been on the synthesis and spectroscopic characterization of the metal complexes in the solid state.

Analytical chemists have also recently become interested in adapting 2,6-diacetylpyridine bis(hydrazones) to analytical applications. Casoli et al. published a short paper on the use of H_2dapb for the chromatographic separation of uranium (VI) and copper (II) (16), and for the determination of uranium (VI) by spectrophotometry and by differential pulse polarography (17). Garcia-Vargas et al. used the same reagent to determine antimony in non-ferrous alloys (18). Bonilla-Alvarez et al. published a paper on the concentration and determination of uranium(VI) using 2,6-diacetylpyridine bis(furoylhydrazone) (H_2dapf) and 2,6-diacetylpyridine bis(pyridylhydrazone) (H_2dapp), two slightly more water soluble bis(hydrazones) (19).

In the present work two new derivatives of 2,6-diacetylpyridine bis(hydrazone) have been prepared and characterized. Both complexing reagents contained quaternary ammonium groups which greatly enhanced the water solubility of the reagents and their metal-ion complexes. These reagents are evaluated for separating high valency metal ions chromatographically as their metal ion complexes. The reagents were useful for determining and quantifying metal ions concentrations in aqueous samples selectively by controlling the pH.

EXPERIMENTAL

Synthesis and Characterization of H_2dapmp

To a 500 ml. round- bottom flask were added 0.012 mole of Girard's Reagent P (Aldrich) and 0.006 mole of 2,6-diacetylpyridine (Aldrich) in approximately 250 ml. of absolute ethanol. The mixture was refluxed for 3 hours and the resulting white precipitate was collected. The product (2,6-diacetylpyridine bis(N-methylenepyridiniohydrazone) dichloride (abbreviated H_2dapmp)) was then recrystallized from a 85%/15% ethanol-water solvent. Products were allowed to precipitate overnight before collection. The product (2,6-diacetylpyridine bis(N-methylene-N,N,N-trimethylammoniohydrazone) dichloride (abbreviated H_2dapmta)) was synthesized from Girard's Reagent T (Aldrich) and 2,6-diacetylpyridine in a similar manner.

The following procedures were used to characterize the H_2dapmp. The infrared spectrum was taken on a IBM IR/98 (KBr pellet). The NMR spectrum was obtained on a Nicholet NT-300 using D_2O (99.8%) as the solvent and chemical shift standard. The mass spectrum of the sample was taken on a Kratos MS-50 employing a Fast Atom Bombardment (FAB) procedure. The matrix was a thioglycerol/water mixture and the background was subtracted out electronically.

LC Studies

The chromatographic system consisted of a LKB 2150 HPLC Pump, LKB 2152 HPLC Controller, LKB 2040-204 Low Pressure Mixer, LKB 11300 Ultrograd Mixer Driver (mixing chamber volume of 0.5 ml.), Rheodyne 7000 injector (10 μl injection loop), and a Kratos Spectroflow 783 UV-vis detector. The column was a PLRP-S 100 Å 5 μm polystyrene-divinylbenzene (15 cm x 4.6 mm) column from Polymer Laboratories.

Eluents were prepared from Fisher HPLC grade acetonitrile and water purified with a Barnstead Nanopure II system on a percent volume basis. No corrections were made for non-additive volumes. Acids, bases and ion-pairing salts were all reagent grade or better. All eluents were prepared by diluting 0.5 M formate (pH=3.3) and 1.0 M $NaClO_4$ to yield 25 mM formate and 50 mM $NaClO_4$. Possible changes in apparent pH were ignored. The eluent components were mixed and filtered with 0.2 μm Nylon 66 filters (Rainin) before use in the chromatographic system. The flow rate was 1 ml/min. The detection wavelength was 340 nm.

Preparation of Solutions

Metal ion stock solutions were prepared either from metal ion salts or from the metal. Uranium (VI), thorium (IV), and iron (III) stock solutions were all made from the nitrate salts. Vanadium (IV) and (V) stock solutions were made from vanadyl sulfate and vanadium pentoxide in H_2SO_4, respectively. Titanium (IV) solutions were prepared by dissolving titanium metal in a HNO_3/H_2SO_4 mixture. Stock solutions for the interferent metals were all made from the chloride or nitrate metal salts.

The UV-vis spectra of H_2dapmp and titanium complex were taken at a pH of 3.3 in a 20 mM formate buffer. Both spectra were blanked with this formate buffer and contained 40 μM H_2dapmp. Enough Ti(IV) was added to H_2dapmp to yield 10 μM titanium complex.

Chromatographic samples were prepared by taking an aliquot of stock metal solution and adding enough reagent to make the final sample 0.4-4 mM H_2dapmp. All solutions were buffered at pH = 3.3 (20 mM formate). All adjustments in pH were done with NaOH. Samples also contained $NaClO_4$ (20 mM). All samples were allowed to stand 30 minutes before injection to ensure complete formation of the complexes. The samples for the interference studies were prepared as described except that the H_2dapmp was held constant at 2mM, and no $NaClO_4$ was added. Metal complexes were formed by adding excess reagent to a somewhat acidic sample and then adjusting the pH to 3.3. Sample and interferent metals were both present before the addition of reagent. All interferent samples were allowed one hour to equilibrate before injection.

RESULTS AND DISCUSSION

Selection and Synthesis of Chelating Reagents

Bis(aroylhydrazones) of 2,6-diacetylpyridine are easily prepared and form very stable complexes with a number of metal cations. However, these reagents and their metal ion complexes are very insoluble in water and require a high proportion of an organic solvent to keep in solution. Girard's Reagent P and Reagent T both have a quaternary ammonium group in order to form water-soluble hydrazones after reaction with an aldehyde or ketone. Both the P reagent and T reagent reacted readily with 2,6-diacetylpyridine to form a product with good water solubility that formed strong complexes with a number of metal ions. The structural formula of these reagents is shown in Figure 3.

Most of the chromatographic studies were performed with H_2dapmp although the H_2dapmta appears to function in a similar manner. The physical characteristics for H_2dapmp are given in Table 1.

Conditions for Detection

The metal complexes of H_2dapmp and the free reagent both absorb strongly in the UV spectral region. Spectra for H_2dapmp and titanium (IV) complex are shown in Figure 4. The other metal complexes studied had a yellow or yellow green color and gave spectra similar to that for the titanium complex, except for the iron (III) complex which was brown in color. Detection at 340 nm

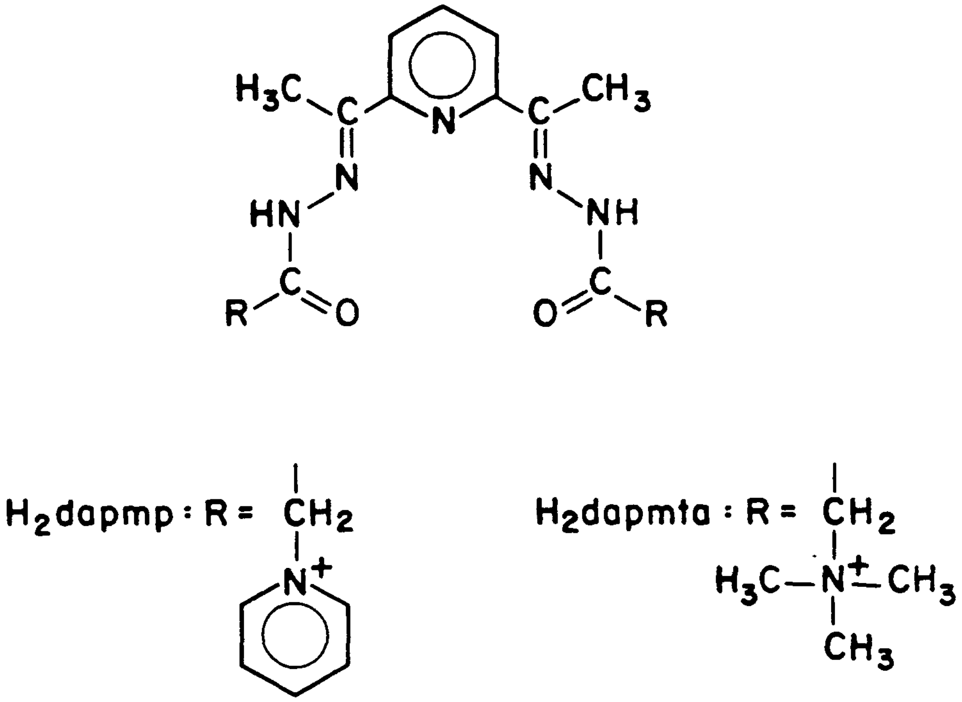
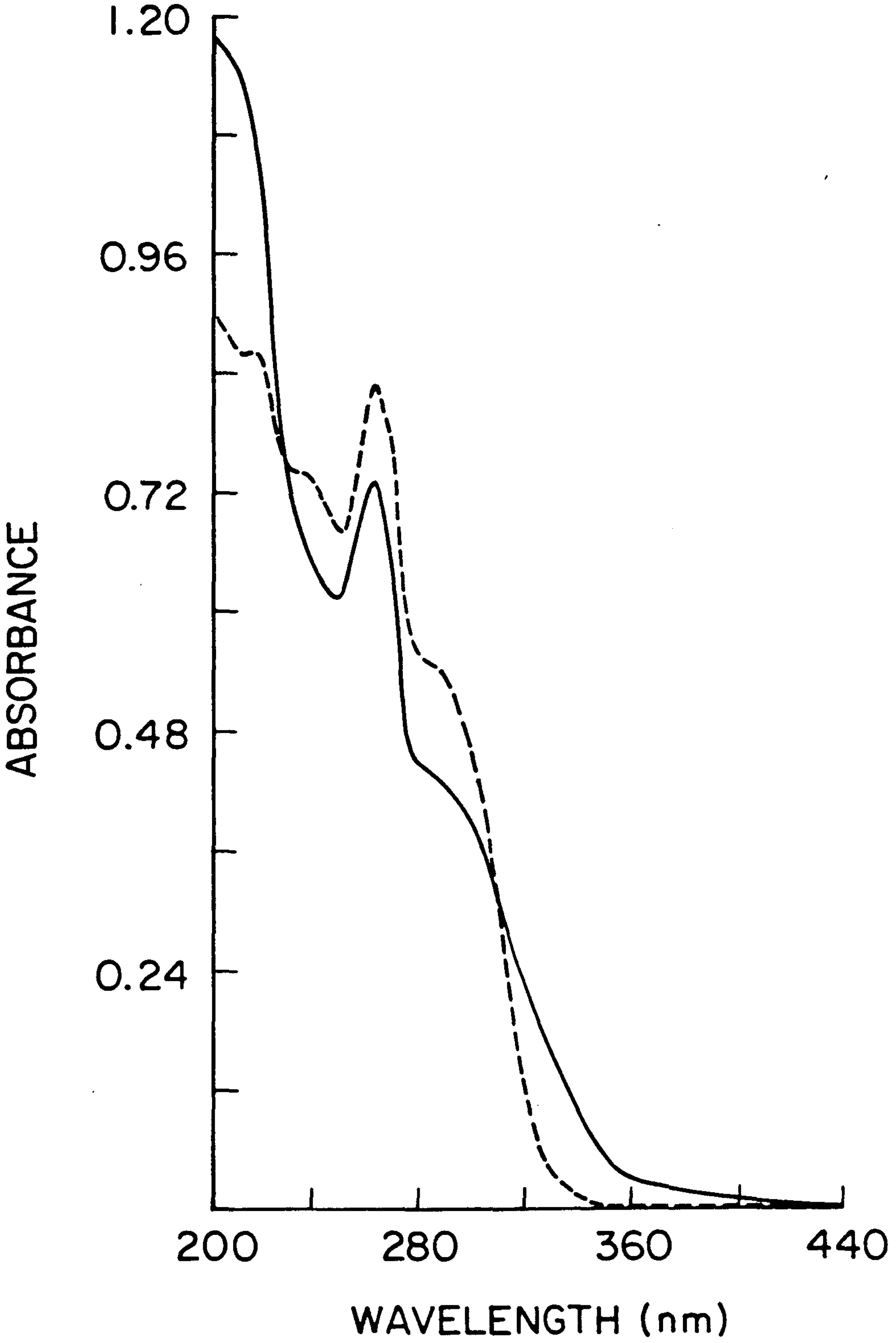


Figure 3: Structure of H_2dapmp and H_2dapmta.

Table I. Characterization of H_2dapmp

Melting range (°C)	IR maxima (cm^{-1})	Major mass spectral lines m/z (%)	Proton shift of NMR (integration)
>300	3441	466.2 (22.0)	8.80-8.59 (6.57)
	3132	430.2 (100)	8.59-8.44 (3.00)
	3053	352.2 (17.2)	8.15-7.67 (11.9)
	2976	337.1 (28.8)	6.03-5.84 (2.45)
	1690	280.1 (14.5)	5.64-5.53 (1.60)
	1636	254.1 (11.7)	5.53-5.24 (1)
	1570	215.6 (14.4)	2.63-2.55 (1.56)
	1489	181.0 (14.8)	2.42-2.36 (2.75)
	1273		2.30-2.22 (3.78)
	685		

Figure 4: UV-vis absorbance spectra of H_2dapmp (---) and titanium complex (——).


1.20
0.96
0.72
0.48
0.24
ABSORBANCE
200
280
360
440
WAVELENGTH (nm)

provided good sensitivity while minimizing the absorbance of the reagent. The molar absorptivities of the metal complexes studied were in the 3,000 to 8,000 $(M \cdot cm)^{-1}$ range at the detection wavelength.

Chromatographic Conditions

It was decided to make the reagent more selective by working at an acidic pH. Earlier work (19) with 2,6 diacetylpyridine bis(furoylhydrazone) showed that maximum formation of iron (III), titanium (IV) and uranium (VI) complexes occurred at or below pH 3.0. Therefore, a pH of 3.3 was selected for the present work. Formate buffers were incorporated into the eluent and samples to maintain the pH at 3.3.

All chromatographic separations were carried out on a column packed with styrene-divinylbenzene polymeric beads. Previous work (20) had shown that the use of such columns avoids the partial decomposition of metal-organic complexes that often results when silica-based column packings are used.

All separations of the metal complexes employed mixtures of water and acetonitrile in the eluent. Other authors have shown that acetonitrile is a better wetting agent than methanol for polymeric resins and yields better separations (20).

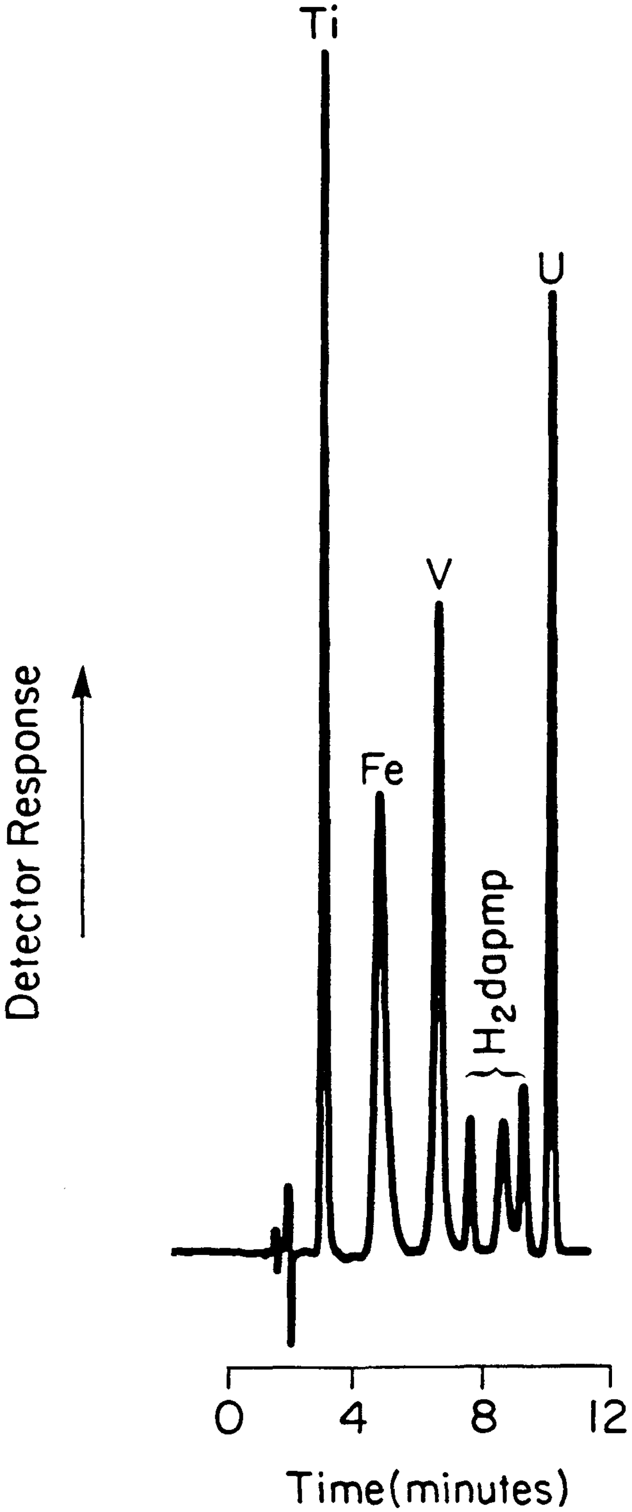
The two quaternary ammonium groups impart a 2+ charge to the complexing reagent, which will attract anions to form an ion pair. The chromatographic retention times of the reagent as well as the metal complexes are likely to be affected by the particular anion that is present. To avoid possible difficulty, sodium perchlorate is added to the eluent. Perchlorate was chosen because it is known as a good inorganic anion for ion pairing (20).

Several metal ion complexes of H_2dapmp can be separated by isocratic elution using acetonitrile-water eluents containing 18% to 28% acetonitrile. However, better separations were obtained using gradients in which the percentage of acetonitrile in the eluent was increased. Figure 5 shows a separation of titanium (IV), iron (III), vanadium (V) and, uranium (VI) complexes under gradient conditions. The concentrations of the metals in order of elution were $5x10^{-5}$ M, $1x10^{-4}$ M, $1x10^{-4}$ M, and $5x10^{-5}$ M. The four metal-complex peaks were separated nicely with little tailing. The complexes were sufficiently stable that it was not necessary to add reagent to the eluent.

Characteristics of the Reagents

The chromatogram in Figure 5 shows three peaks from the excess H_2dapmp reagent. At first, it was thought that the extra peaks were impurities in the reagent. Several attempts were made to purify the reagent further, but three reagent peaks remained in the chromatograms. It was finally ascertained that the multiple reagent peaks were due to the E,E-H_2dapmp, the E,Z-H_2dapmp, and the Z,Z-H_2dapmp geometrical isomers, as shown in Figure 6. Other

Figure 5: Separation of metal complexes of H_2dapmp on a PLRP-S column. Eluent conditions: gradient elution; solvent A, acetonitrile/water (10:90); solvent B, acetonitrile/water (40:60); linear gradient 70% A, 30% B at 0.0 min. to 100% B at 10 min.


Ti
Fe
V
H_2dapmp
U
Detector Response
0
4
8
12
Time(minutes)

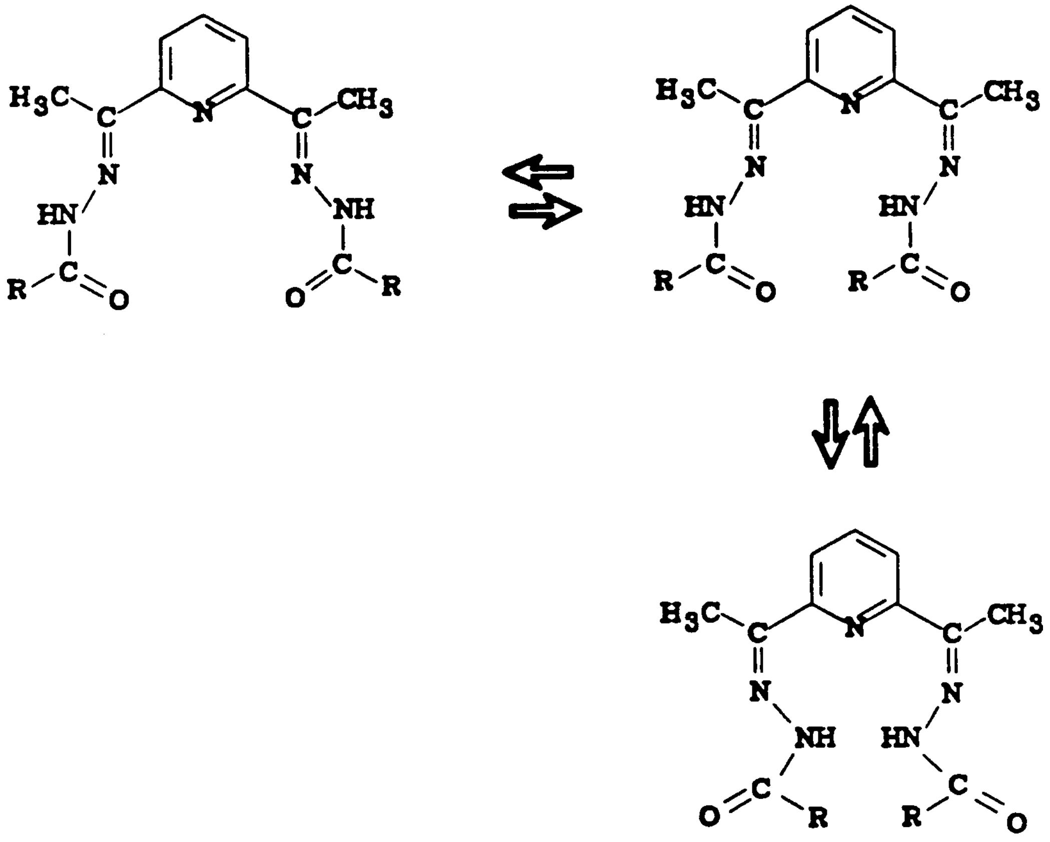


Figure 6: Three geometrical isomers of bis (hydrazones) of 2,6-diacetylpyridine.

researchers have also reported E/Z geometrical isomers in similar hydrazone reagents (16,21).

The earliest-eluting reagent peak in Figure 5 appears to be the chelating geometric isomer because it decreased significantly if an additional amount of metal ion was added to the sample solution. However, when an excess of metal ion (titanium (IV)) was added to the sample and the solution was chromatographed several days later, all three reagent peaks were absent. This indicates that an equilibrium exists between the three geometrical isomers. This equilibrium has been studied by other researchers on N-acyl- and N-aroylhydrazones in organic solvents (21).

In the synthesis and purification it appears that the hydrazone reagents crystallize as the E,E isomer. When solid H_2dapmp was brought into solution as quickly as possible and a portion of the solution injected onto the LC column, the first reagent peak was quite large and the second and third peaks were almost missing. Table II shows that subsequent injections of the reagent solution after various time periods resulted in the first peak decreasing in peak height, whereas the second and third peaks were increasing in peak height. The E,E isomer was apparently being converted into the E,Z and Z,Z forms, so that an equilibrium was reached in which all three geometric isomers are in solution. Figure 7 shows that the relative peak height of the E,E-H_2dapmp decreased linearly with time immediately after dissolution. The proportionality constant

Table II. Equilibration of E,E-H_2dapmp into E,Z-H_2dapmp and Z,Z-H_2dapmp isomers after dissolution

	Relative peak height		
Time (min)	E,E Isomer	E,Z Isomer	Z,Z Isomer
16.8	59.0	1.5	0.7
23.3	55.9	2.2	1.6
29.4	53.1	3.1	2.7
35.5	50.3	3.4	3.3
43.8	47.2	4.1	4.1
55.2	42.6	5.2	5.3
63.2	40.2	5.9	6.2
78.6	35.9	6.8	7.1

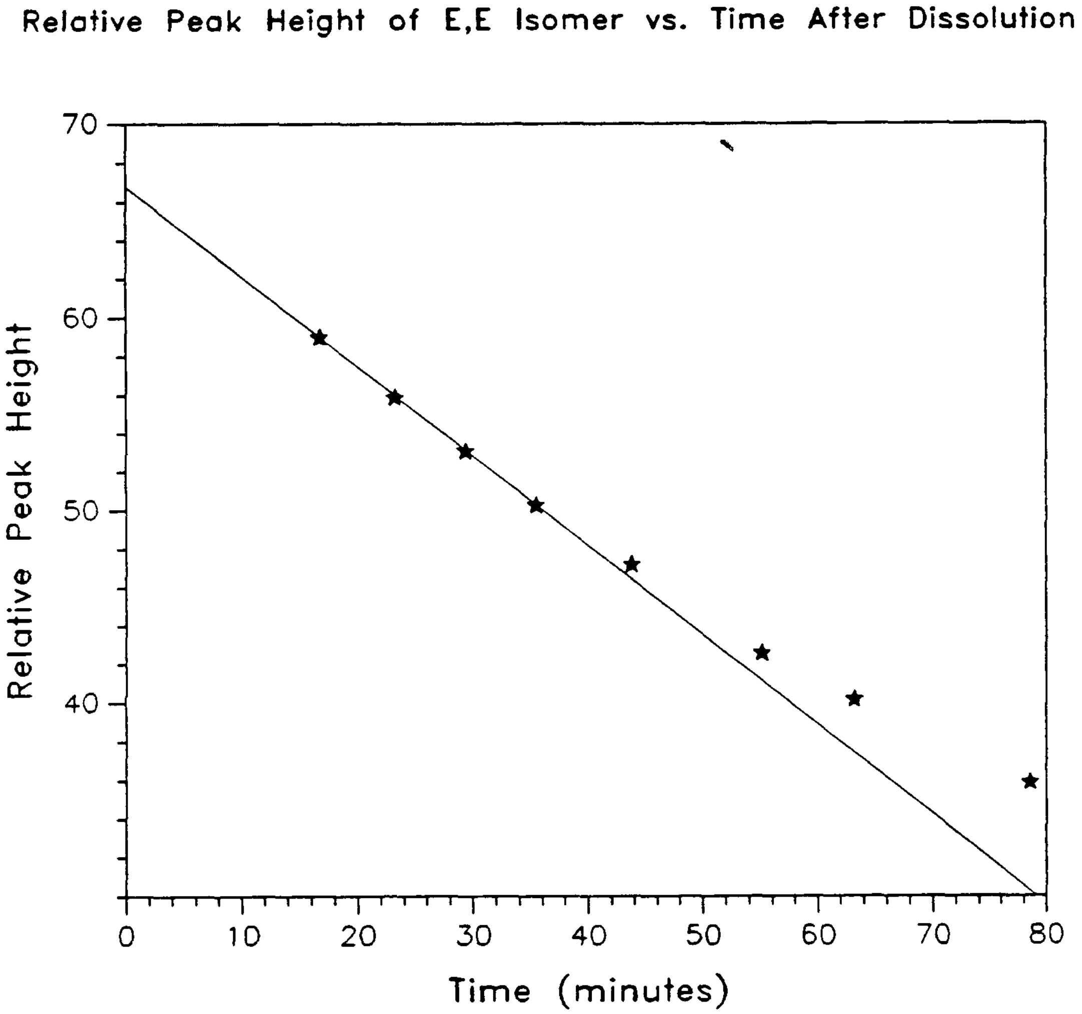


Figure 7: Plot of peak height vs. time for the equilibration of E,E-H_2dapmp into the E,Z- and E,E-H_2dapmp geometrical isomers after dissolution.

between the relative peak height and the concentration of H_2dapmp was obtained by extrapolating back to time zero in Figure 7, and using the known concentration of H_2dapmp initially present. Assuming that the relative peak height was directly proportional to the concentration of E,E-H_2dapmp present, it was possible to calculate the concentration of H_2dapmp present at the various times as shown in Table III. From the integrated rate expression:

$$\ln[\text{E,E-H}_2\text{dapmp}] = -kt + \ln[\text{E,E-H}_2\text{dapmp}]$$

a first order rate constant was calculated by plotting ln[E,E-H_2dapmp] verses time as shown in Figure 8. Using all eight data points in Figure 8, a first order rate constant of 8×10^{-3} min^{-1} was calculated for the conversion of E,E-H_2dapmp into the other isomers at pH = 3.3. This rate constant is only an approximation, however, since no strict control of temperature was undertaken.

The existence of three geometric forms of the reagent causes no real problem in the separation of metal ion complexes because none of the metal complexes studied had a retention that coincided with one of the reagent isomers. However, the existence of three isomers means that a larger excess of the reagent will be needed to completely complex the metal ions in the sample. Possibly a smaller excess of the reagent could still be used if a sufficient time is

Table III. Calculated concentrations of E,E-H_2dapmp as a function of time after dissolution

Time (min)	Concentration of E,E-H_2dapmp, M	ln[E,E-H_2dapmp]
0.0[a]	$1.20X10^{-3}$	-6.725
16.8	$1.06X10^{-3}$	-6.855
23.3	$1.00X10^{-3}$	-6.908
29.4	$9.54X10^{-4}$	-6.955
35.5	$9.04X10^{-4}$	-7.009
43.8	$8.48X10^{-4}$	-7.073
55.2	$7.65X10^{-4}$	-7.176
63.2	$7.22X10^{-4}$	-7.234
78.6	$6.45X10^{-4}$	-7.346

[a] This concentration was known by dissolving a known amount of H_2dapmp volumetrically.

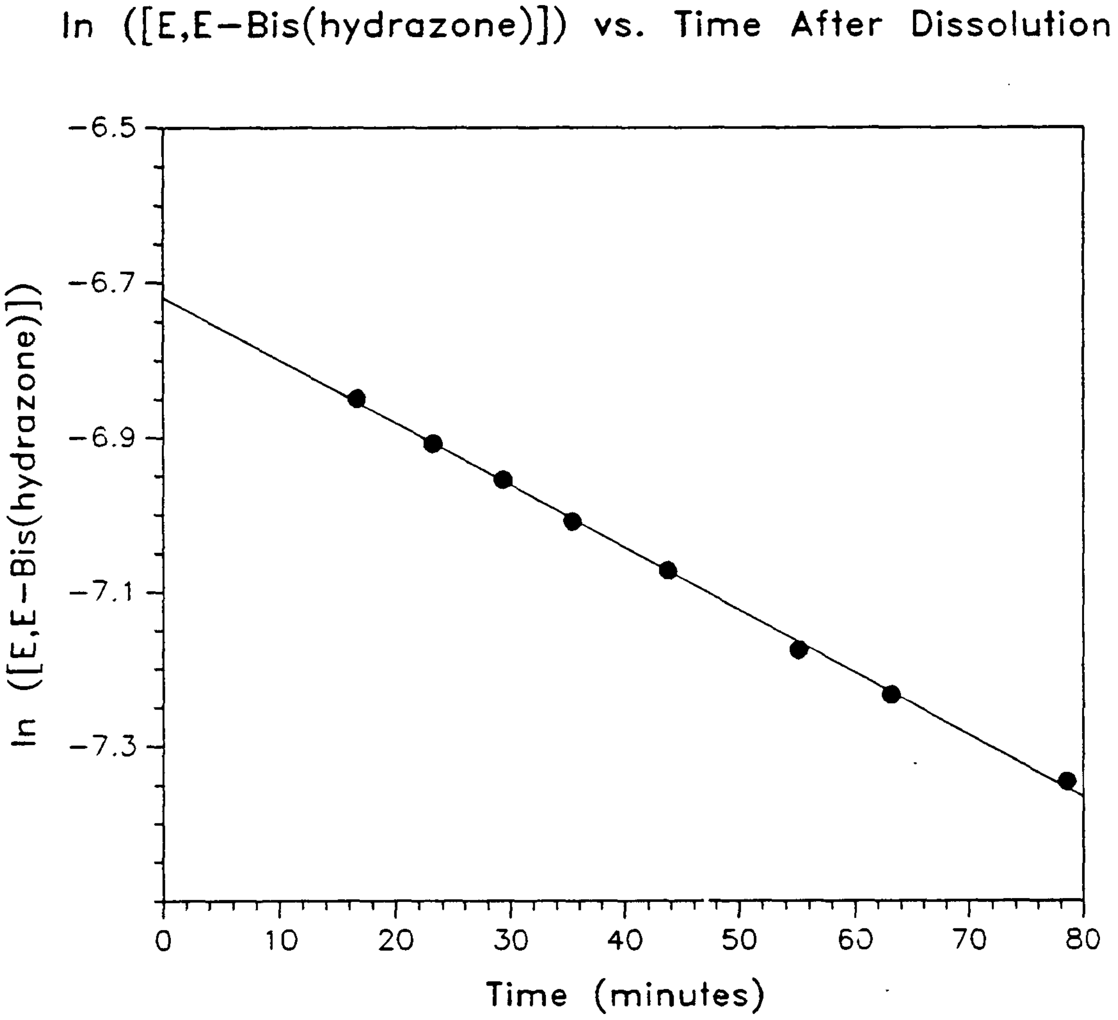


Figure 8: Plot of ln ([E,E-H_2dapmp]) vs. time after dissolution of E,E-H_2dapmp.

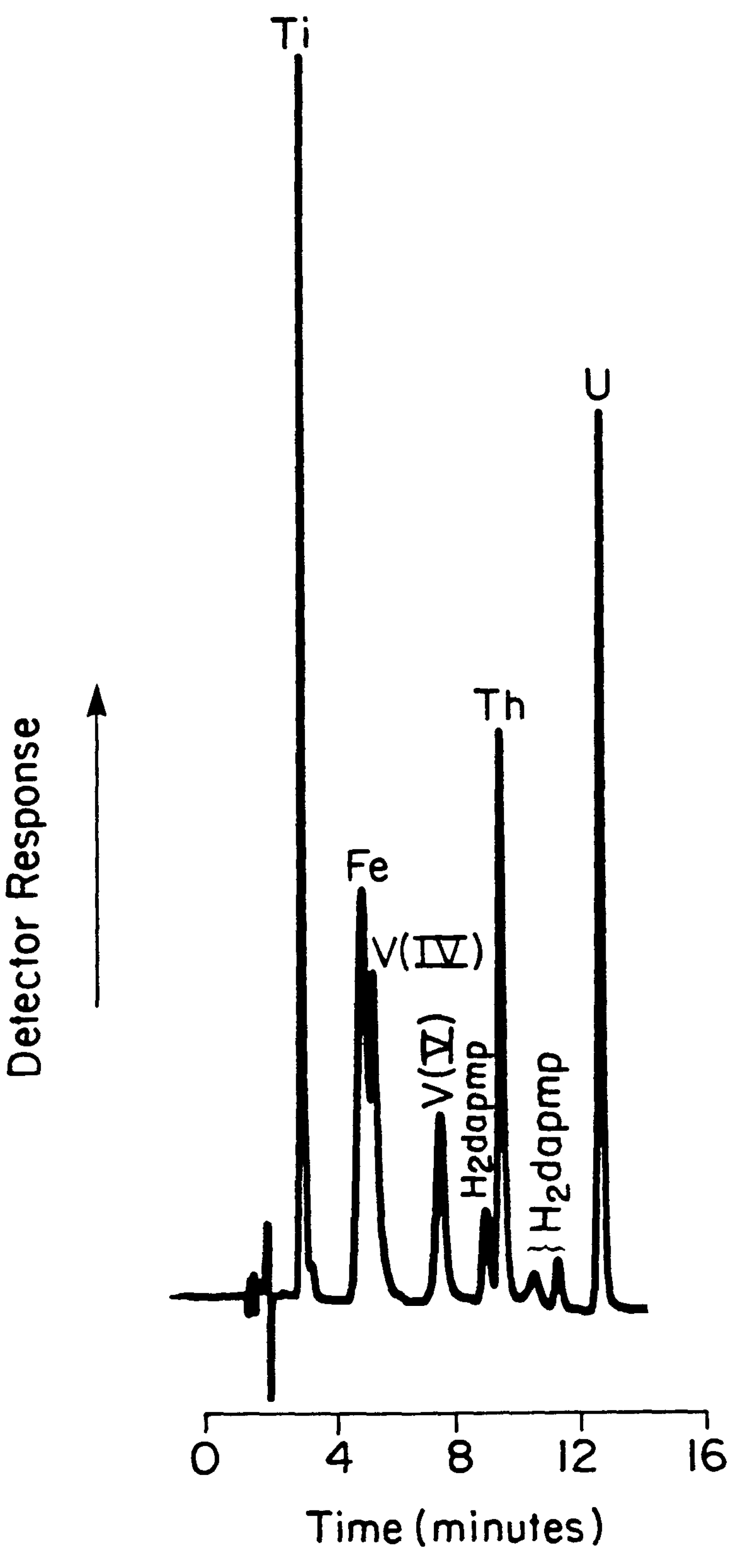
provided for equilibration. Presumably only the E,E isomer reacts with a metal ion.

Chromatographic Separation of Metal-hydrazone Complexes

At an approximately neutral pH a fairly large number of metal ions were found to form colored complexes with H_2dapmp and H_2dapmta. However, the hydrazone reagents become more selective as the sample was made more acidic. At pH 3.3, chromatographic peaks for the following metal ion complexes were obtained: titanium (IV), iron (III), vanadium (IV) and (V), thorium (IV) and uranium (VI). Figure 9 shows that most of these complexes can be completely resolved using gradient elution.

In Figure 9 it will be noted that peaks for both vanadium (IV) and (V) complexes with H_2dapmp were obtained. This indicates that speciation of different oxidation states of vanadium should be possible. However, complexation and chromatographing a sample containing only vanadium (IV) gave peaks for both vanadium (IV) and (V) complexes. This happened even when a freshly prepared solution of vanadium (IV) was used and the complexation was carried out under an inert gas. Apparently partial oxidation occurred during complexation. If the sample was prepared in 10 mM ascorbic acid, only the peak from the vanadium (IV) complex was observed in the chromatogram. If vanadium (V) was also present in the sample containing the ascorbic acid, it was reduced to vanadium (IV). Vanadium (V) can be

Figure 9: Separation of metal complexes of H_2dapmp on a PLRP-S column. Eluent conditions: gradient elution; solvent A and B same as Figure 3; linear gradient 80% A, 20% B at 0.0 min. to 100% B at 16 min.

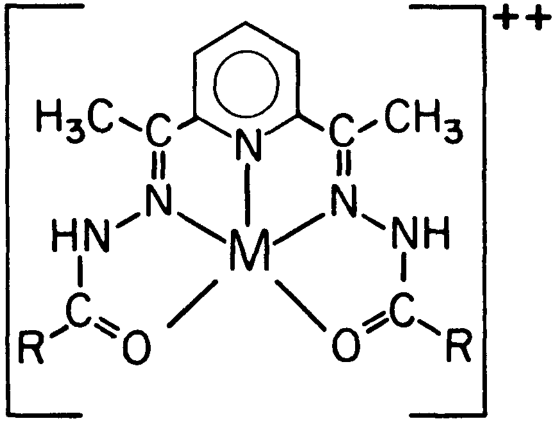

Ti
U
Th
Fe
V(IV)
V(V)
H_2dapmp
H_2dapmp
Detector Response
0
4
8
12
16
Time (minutes)

complexed with H_2dapmp and chromatographed to give only the vanadium (V) peak.

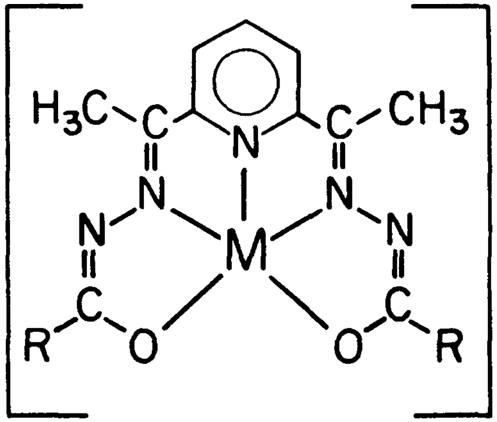
Characteristics of the Metal Complexes

In Figure 9 it will be noted that only the complexes of the very heavy metals thorium (IV) and uranium (VI) elute after the first reagent peak. Although it may not be meaningful to speculate on the reason for this retention behavior, there are some conclusions that can be drawn. The most obvious conclusion is that thorium and uranium are forming metal complexes that are more hydrophobic than the free reagent, whereas the other metals are forming metal complexes that are more hydrophilic than the free reagent. One possible reason for this difference in polarity could be due to the type of metal complex formed during chelation. It is known that crystallized metal-hydrazone complexes can be prepared that are neutral or positively charged (11). These two types of metal complexes are illustrated in Figure 10, where M represents a metal ion with a 2+ charge. In the charged complex, no protons are removed during chelation, resulting in a 2+ charge in the coordination center after chelation. However, in the neutral complex, the 2+ charge initially present on the metal ion is neutralized during chelation by the removal of the two enol protons. This ability to form neutral or positively charged complexes could explain the retention behavior of the metal complexes. For example, in the case of thorium(IV) and uranium(VI) the type of metal complex formed could be of the

Figure 10: Structure of metal complexes of M-H_2dapmp (charged coordination center) and M-dapmp (neutral coordination center), where M represents a divalent metal ion.



Charged



Neutral

neutral type, whereas the other metal ion complexes could be of the charged type as shown in Figure 10. The exact nature of the metal complexes formed has not been established.

Comparison of H_2dapmp and H_2dapmta

Although most of the work was done with H_2dapmp, the other reagent (H_2dapmta) gave very similar results. Table IV shows that H_2dapmta gives very similar retention times of the metal complexes when compared with H_2dapmp. The order of elution was identical for both reagents, the only difference being that H_2dapmta was more hydrophilic than H_2dapmp, as were its metal complexes.

Calibration and Quantitation

Calibration curves were prepared for titanium (IV), uranium (VI) and iron (III) under isocratic elution conditions and for vanadium (V) under gradient conditions in order to test whether quantitative determinations are feasible. The elution conditions, working ranges, and limits of detection are given in Table V. All metal complexes eluted between 3 and 8 min under the conditons used.

Good linear calibration curves were obtained over a concentration range of at least two orders of magnitude for the first three metal ions and over almost three orders of magnitude for vanadium (V). Possibly longer working ranges could be obtained by using a larger amount of reagent during the complexation step. These results indicate that good quantitative results should be obtained

Table IV. Comparison of retention times for H_2dapmp and H_2dapmta metal complexes

Metal ion	Retention times of metal complexes[a] H_2dapmp (min)	 H_2dapmta (min)
Ti(IV)	3.4	2.8
Fe(III)	5.5	4.6
V(IV)	5.8	4.9
V(V)	8.2	7.1
Th(IV)	10.0	
U(VI)	13.3	12.0

[a] Eluent conditions: same as Figure 9.

Table V. Eluent conditions for calibration of various metals by HPLC using H_2dapmp as the complexing agent[a]

metal ion	elution conditions	linear calibration curve range, M	detection limit, M
Ti(IV)	18% CH_3CN 82% H_2O	$9X10^{-7}$ to $1X10^{-4}$	$8X10^{-8}$
U(VI)	28% CH_3CN 72% H_2O	$1X10^{-6}$ to $3X10^{-4}$	$2X10^{-7}$
Fe(III)	25% CH_3CN 75% H_2O	$4X10^{-6}$ to $7X10^{-4}$	$4X10^{-7}$
V(V)	19% CH_3CN[b] 81% H_2O to 40% CH_3CN 60% H_2O	$2X10^{-6}$ to $1X10^{-3}$	$3X10^{-7}$

[a] For all eluents: Flow rate = 1.0 ml/min; UV detection, λ = 340 nm, injection volume = 10 μl, 25 mM formate (pH = 3.3), 50 mM $NaClO_4$.

[b] Gradient elution: 10 min linear gradient.

with this chromatographic method. Of course, quantitative results in any gradient method is only as accurate as the reproducibility of the solvent gradient itself.

Interferences

The effect of possible interfering ions was studied by adding varying amounts of selected foreign ions to samples of metal ions to be determined. A foreign ion was considered to interfere when the peak height of a metal complexes was changed by more than ± 4%. Results are given in Table VI. For convenience, the concentrations of foreign ions were varied by decades. The + or - in parentheses indicates the direction of the error. However the actual interference begins somewhere between the concentration listed and a 10-fold higher concentration. Positive errors were always due to the interferent ion forming a weak metal complex with H_2dapmp and undergoing dissolution on the chromatographic column. This dissociation produced a broad chromatographic peak that had the effect of shifting the baseline of the chromatogram, thereby increasing the peak heights of all of the analytes that coeluted. Negative errors were probably due to competition between the analyte metal ions and the interferents ions for a limited amount of H_2dapmp that had been added to the sample.

Table VI. Concentration (M) of interferent ions tolerated in determination of sample ions[a]

Interferent Ion	Sample Ions			
	Ti(IV)	Fe(III)	V(V)	U(VI)
Na^+	0.1	0.1	0.1	0.1
Ca^{2+}	0.1	0.1	0.1	0.1
Co^{2+}	0.001	0.0001(+)	0.0001(+)	0.001
Mn^{2+}	0.001(-)	0.01	0.01	0.001(-)
Zn^{2+}	0.001(-)	0.01	0.01	0.001(-)
Cu^{2+}	0.001(-)	0.001(-)	0.0001(+)	0.0001(-)
Al^{3+}	0.01	0.01	0.01	0.01
Hg^{2+}	0.001	0.001	0.001	0.001
ZrO^{2+}	0.0001(-)	0.001	0.001	0.001
Ho^{3+}	0.01	0.01	0.001(+)	0.01

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[a] Eluent conditions: same as Figure 5. The concentration of Ti(IV), Fe(III), V(V), and U(VI) were 2 x 10^{-5} M, 1 x 10^{-4} M, 1x10^{-4} M and 3 x 10^{-5} M, respectively.

CONCLUSIONS

Bis(quaternary ammonium hydrazones) of 2,6-diacetylpyridine proved to be very useful chelating reagents in the quantitative determination of aqueous metal ions. The reagents were selective for high valence metal ions under acidic conditons, and divalent transition metal ions could be tolerated in most situations. Quantitation of the metal complexes of Ti(IV), Fe(III), V(V), and U(VI) was possible, due to a linear relationship between relative peak height and concentration of metal ion in solution. The metal complexes were also kinetically stable and the addition of reagent to the eluent was not necessary, as is typically the case when separating metal complexes chromatographically. The solubility of the free ligand and the metal complexes was not a problem in aqueous samples because both the free ligands and the metal complexes all have permanent positive charges, due to the presence of the quaternary ammonium groups. Some speciation was also possible as shown by the chromatographic separation of metal complexes of vanadium (IV) and (V). Gradient elution was the preferred method for a the multi-element analysis, due to the differences in retention of the various metal complexes.

There were also many interesting properties displayed by the free reagents. For example, an equilibration of the free reagent between three different geometrical species in aqueous solution was apparent due to the elution of the free reagent as three chromatographic peaks. It was also shown that in the synthesis of the free reagent, H_2dapmp crystallized out of solution exclusively as the E,E-H_2dapmp geometrical isomer.

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SECTION II. CHROMATOGRAPHIC DETERMINATION OF METAL CHELATES OF 2,6-DIACETYLPYRIDINE BIS(N-METHYLENEPYRIDINIOHYDRAZONE) UNDER NEUTRAL CONDITIONS

INTRODUCTION

This section of this thesis will look at the possibility of extending the work performed in the area of separating metal ions as complexes through the use of derivatives of 2,6-diacetylpyridine bis(hydrazone). Earlier work, both in Section I, and by other authors (1,2), has shown that many metal ions can complex with bis(hydrazone) derivatives under various pH conditions. Pelizzi et al. has shown that manganese(II), cobalt(II), nickel(II), zinc(II), and copper(II) all form metal complexes with a variety of different bis(hydrazones) under neutral pH conditions (3-7). H_2dapmp displayed a great deal of selectively under acidic conditions and only complexed high valency metal ions, as was shown in Section I. However, under neutral pH conditions H_2dapmp complexes with many additional metal ions, and many of these metal ions can be separated chromatographically.

This section will also investigate separating rare earth metal ions as the H_2dapmp metal complexes. Traditionally, the separation of rare earth metal ions has been considered a very difficult separation. Knight et al. have obtained very nice separations of the lanthanides by ion chromatography followed by post-column chelation using PAR or Arsenazo III (8). Other authors have also separated rare earth metal ions chromatographically, as their metal complexes, with some success (9,10).

The effect of varying the concentration of ion-pairing reagent in the eluent, on the retention times of the metal complexes will also be investigated. Both the free ligand and the metal complexes do have permanent positive charges, due to the presence of the quaternary ammonium groups. Therefore, the retention times of the metal complexes should vary with the concentration of the ion-pairing reagent in the eluent.

EXPERIMENTAL

Preparation of Stock Solutions

Solutions of H_2dapmp were prepared by dissolving the H_2dapmp in water. Metal ion stock solutions were prepared either from metal ion salts or from the metal. Uranium (VI) and copper (II) stock solutions were made from the nitrate salts. Manganese (II) solutions were made from the sulfate salt. Nickel (II), cadmium (II), and cobalt (II) stock solutions were made from the chloride salts. Tin (II) and antimony (III) stock solutions were made from stannous chloride in H_2SO_4 and antimony trioxide in HCl, respectively. Titanium (IV) solutions were prepared by dissolving titanium metal in a HNO_3/H_2SO_4 mixture. Stock solutions for the interferent metals were all made from the chloride or nitrate metal salts.

LC Studies

The chromatographic system consisted of a LKB 2150 HPLC Pump, LKB 2152 HPLC Controller, LKB 2040-204 Low Pressure Mixer, LKB 11300 Ultrograd Mixer Driver (mixing chamber volume of 0.5 ml.), Rheodyne 7000 injector (10 μl injection loop), and a Kratos Spectroflow 783 UV-vis detector. The column was a PLRP-S 100 Å 5 μm polystyrene-divinylbenzene (15 cm x 4.6 mm) column from Polymer Laboratories.

Eluents were prepared from Fisher HPLC grade acetonitrile and water purified with a Barnstead Nanopure II system on a percent volume basis. No corrections were made for non-additive volumes. Acids, bases and ion-pairing salts were all reagent grade or better. Eluents were prepared by diluting 1.0 M ammonium acetate (pH = 7.0) and 1.0 M $NaClO_4$ to yield 20 mM ammonium acetate and 50 mM $NaClO_4$. Possible changes in apparent pH were ignored. Eluents used in interference studies were also 4 mM tartrate. Eluents used in ion-pairing studies were 4mM ammonium acetate (pH=7.0), with varying amounts of $NaClO_4$ present. Ion-pairing studies were done under isocratic conditions. The eluent components were mixed and filtered with 0.2 μm Nylon 66 filters (Rainin) before use in the chromatographic system. The flow rate was 1 ml/min. The detection wavelength was 340 nm.

Preparation of Sample Solutions

Chromatographic samples were prepared by taking an aliquot of stock metal solution and adding enough reagent to make the final sample 1-5 mM H_2dapmp. All solutions were buffered at pH = 7.0 with ammonium acetate. All adjustments in pH were done with NaOH. All samples were allowed to stand at pH = 7.0 for 10 minutes before injection. Samples containing titanium (IV) and uranium (VI) were allowed to remain at an acidic pH (2-3.3) for 30 min before raising the pH to 7. Solutions used in ion-pairing studies were 4 mM ammonium acetate and the $NaClO_4$ concentrations were varied in an attempt to match the $NaClO_4$ concentrations of the eluent. The upper concentration of

$NaClO_4$ was limited to about 70 mM $NaClO_4$, due to precipitation. The samples for the interference studies were prepared as described except that the H_2dapmp was held constant at 4 mM, and tartrate (16 mM) was also present.

RESULTS AND DISCUSSION

Chromatographic Conditions

Excess H_2dapmp reagent was added to the metal ion samples, the sample was then buffered to pH 7.0 using ammonium acetate, and the samples were allowed to stand 10 min before injection. Samples containing metal ions that hydrolyze readily (such as titanium (IV) and uranium (VI)) were allowed to stand 30 min at an acidic pH before raising the pH to 7.

At pH 7 H_2dapmp and its metal ion complexes absorb strongly in the UV spectral region. Above 340 nm the free reagent absorbs only slightly while the metal ion complexes continue to absorb. The molar absorptivities of metal complexes at 340 nm range from 3,000 to 12,000 $(M \cdot cm)^{-1}$.

The chromatographic column was packed with a polymeric resin to avoid the possibility of metal complex decomposition by interaction with ion exchange groups in silica-base resins. Eluents contained varying proportions of acetonitrile and water, an ammonium acetate buffer to maintain a suitable pH, and sodium perchlorate to supply an appropriate anion for ion pairing with the positively charged quaternary ammonium groups in the reagent.

Chromatographic Separations

Preliminary qualitative tests showed that H_2dapmp forms yellow complexes with many metal ions at pH 7. Some of the metal ion complexes of H_2dapmp are sufficiently stable to give good chromatographic peaks, while others are apparently somewhat unstable and give no chromatographic peaks at all. Figure 1 shows a nice separation of the complexes of Mn(II), Co(II), Sb(III), Cu(II), and Ni(II). This separation was obtained using a gradient in which the percentage of acetonitrile in the eluent was increased. The complexes were sufficiently inert that the addition of H_2dapmp to the eluent was not necessary. Figure 1 also shows three peaks for the excess H_2dapmp added to the sample. These 3 peaks were due to the E,E; E,Z; and Z,Z geometrical isomers of the reagent. Similiar hydrazone E/Z isomers have been reported by other authors (11,12), as was discussed in Section I.

At pH 7.0 chromatographic peaks were obtained for the following metal ion complexes: tin (II), titanium (IV), manganese (II), cobalt (II), cadmium (II), antimony (III), copper (II), nickel (II), and uranium (VI). Figure 2 shows that most of these complexes can be completely resolved using gradient conditions. Cd(II) is not shown in Figure 2; it coeluted with Co(II). All metal ions were present at approximately $1X10^{-4}$ M.

Although Figure 2 shows a sharp peak for Sn(II), there are precautions that should be noted. All Sn(II) stock solutions had to be prepared daily because older solutions produced more than one chromatographic peak. Sn(IV) also produced a chromatographic peak with the same retention time as the Sn(II) peak, although, at a greatly reduced peak height. The intensity of the color was

Figure 1: Separation of metal complexes of H_2dapmp on a PLRP-S column. Eluent conditions: gradient elution; solvent A, acetonitrile/water (10:90); solvent B, acetonitrile/water (40:60); linear gradient 86% A, 14% B at 0.0 min to 40% A, 60% B at 13 min.

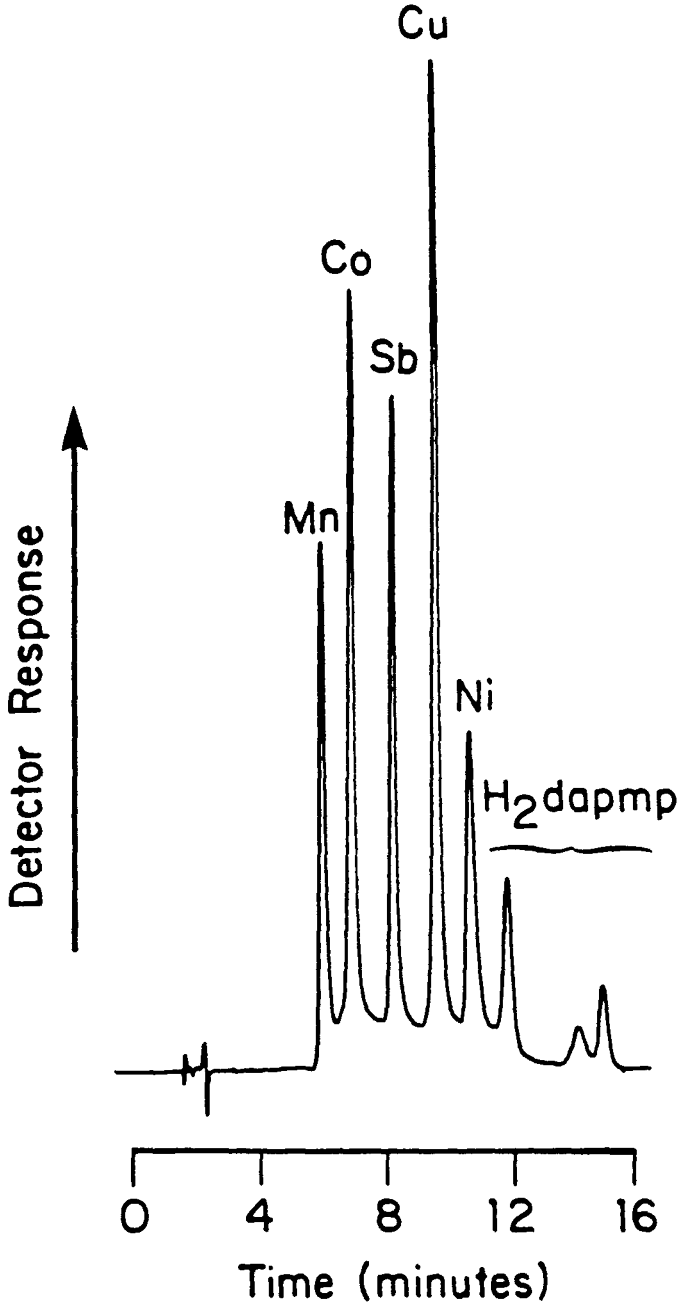
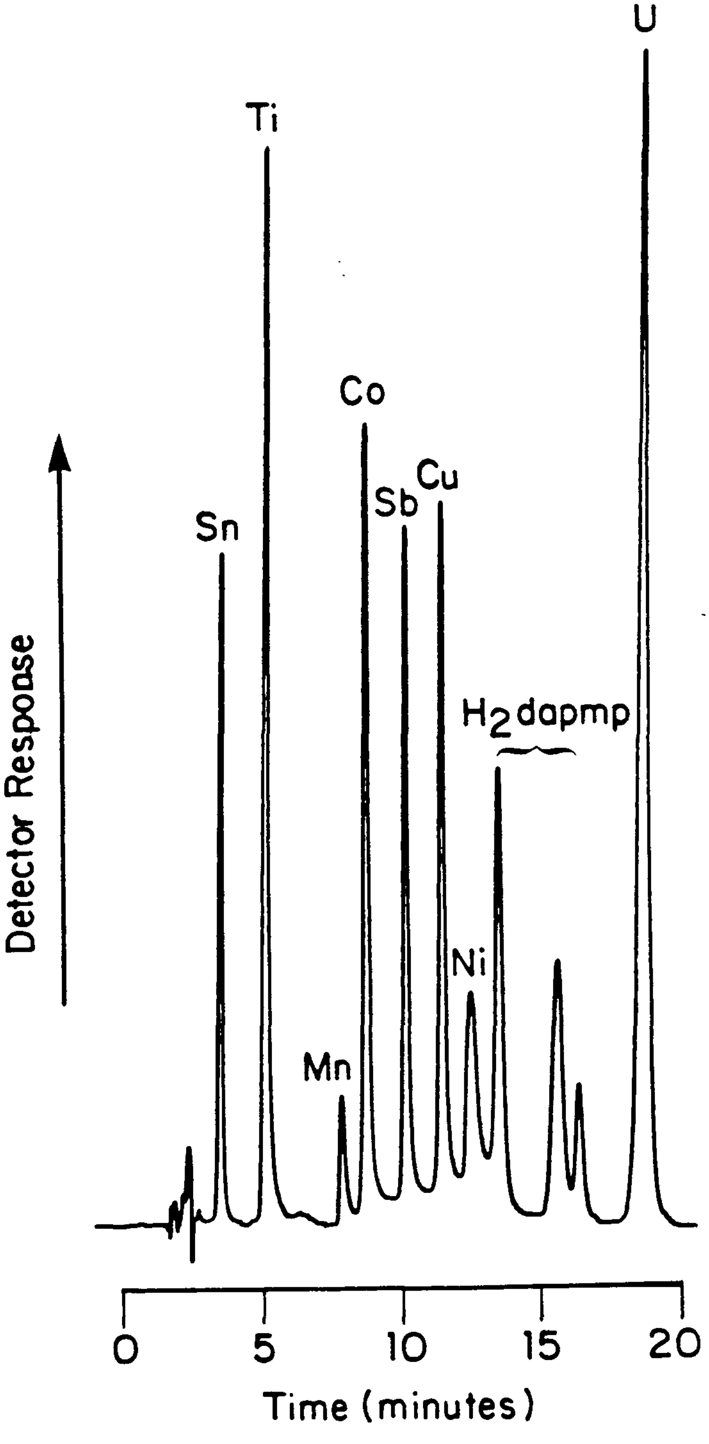

Cu
Co
Sb
Mn
Ni
H_2dapmp
Detector Response
0
4
8
12
16
Time (minutes)

Figure 2: Separation of metal complexes of H_2dapmp on a PLRP-S column. Eluent conditions: gradient elution; solvent A and B same as Figure 1; linear gradient 95% A, 5% B at 0.0 min. to 30% A, 70% B at 18 min.


U
Ti
Co
Sb
Cu
Sn
Detector Response
H_2dapmp
Ni
Mn
0
5
10
15
20
Time (minutes)

also much weaker. This would indicate that Sn(II) might form such a strong complex that Sn(IV) was being reduced to Sn(II) to some degree. It was assumed that if reduction of Sn(IV) was taking place, then the presence of ascorbic acid might encourage this reduction. The addition of ascorbic acid did produce both Sn(II) and Sn(IV) chromatographic peaks, but abnormally high system peaks were also observed. It appears that ascorbic acid forms other complexes that elute unretained in the system peak. This is supported by the fact that the height of the Sn(II) peak was reduced by the presence of ascorbic acid and the height of the Sn(IV) was only increased modestly. It is unclear how easy it will be to handle Sn(II) in real samples.

Nickel (II) was another metal ion with undesirable characteristics. At concentrations below $1X10^{-4}$ M Ni(II) only one chromatographic peak was observed, but at higher concentration of nickel an additional broad peak was observed which eluted under the Sb(III) and Cu(II) peaks. Nickel(II) sample solutions were heated in an attempt to correct this problem, but this resulted in numerous smaller peaks along the baseline.

Of the eight metals shown in Figure 2, titanium(IV) and uranium(VI), also worked nicely under acidic conditions, as shown earlier in Section I. Iron(III) and vanadium(V) which had been previously chromatographed successfully at a pH of 3.3, proved to be unworkable at a pH of 7.0 due to the poor chromatographic performance of the metal complexes. Although titanium(IV) and uranium(VI) both worked nicely at pH 7, it should be noted that these two metals formed complexes very slowly under neutral pH conditions as can be seen in Figure 3. Therefore, when these two metal ions are present in the sample solution, the pH was lowered prior to injection of the sample to allow for complete formation of the Ti(IV) and U(VI) complexes.

Separation of Rare Earth Complexes

At an approximately neutral pH many of the rare earth ions were found qualitatively to form complexes with H_2dapmp. A chromatographic separation of five of the six lowest rare earths was obtained, as shown in Figure 4. Although separation of the individual rare earth complexes was good, the rising chromatographic baseline was unacceptable. This rising baseline was probably caused by the instability of the the rare earth complexes during the chromatographic separation. The baseline was only increased between the chromatographic peak and the first reagent peak (the complexing form of the reagent). This would be expected because after dissociation the retention of the free ligand would now be greater. Therefore, metal complexes that dissociated almost immediately after injection probably contributed to the rising baseline closest to the excess complexing reagent peak, since much of the journey through the column would be as the free ligand. Whereas metal complexes that

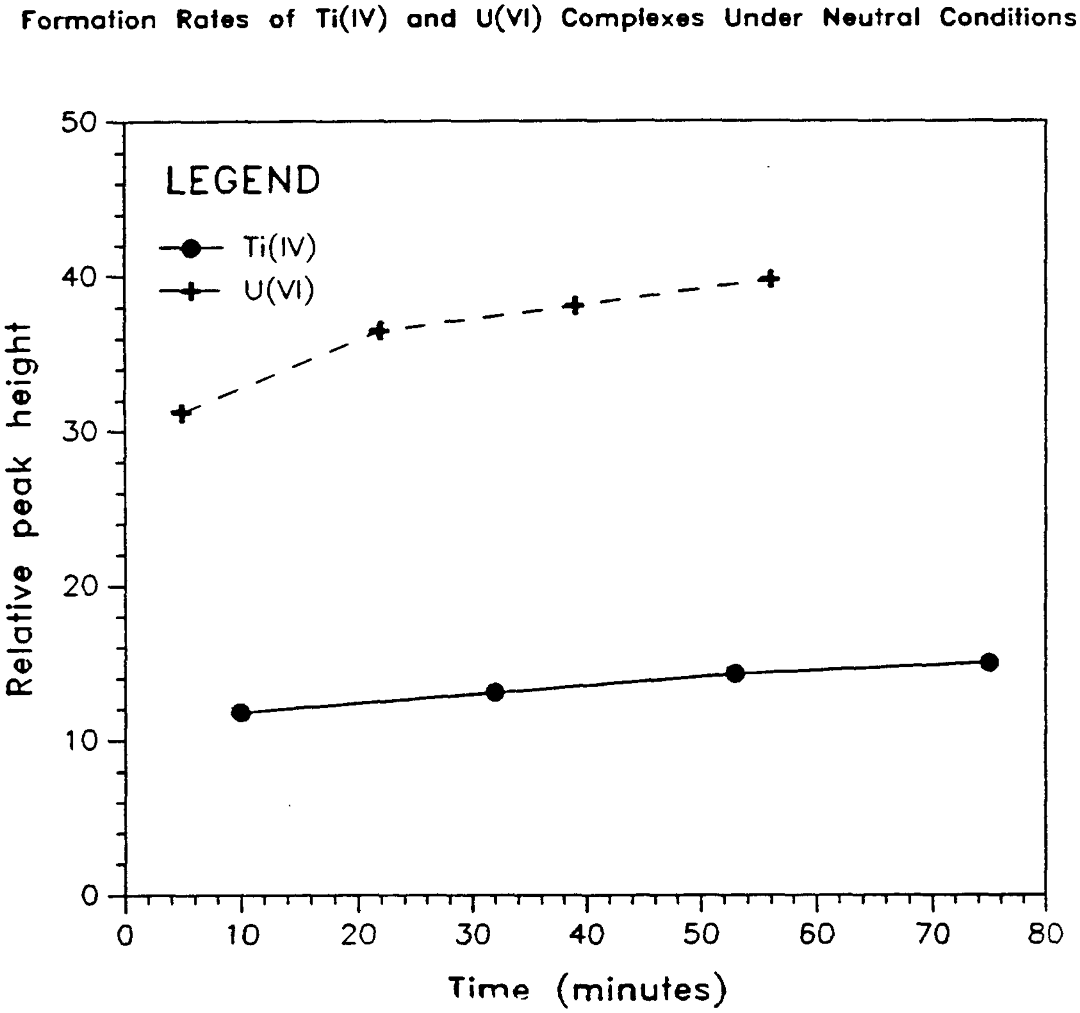
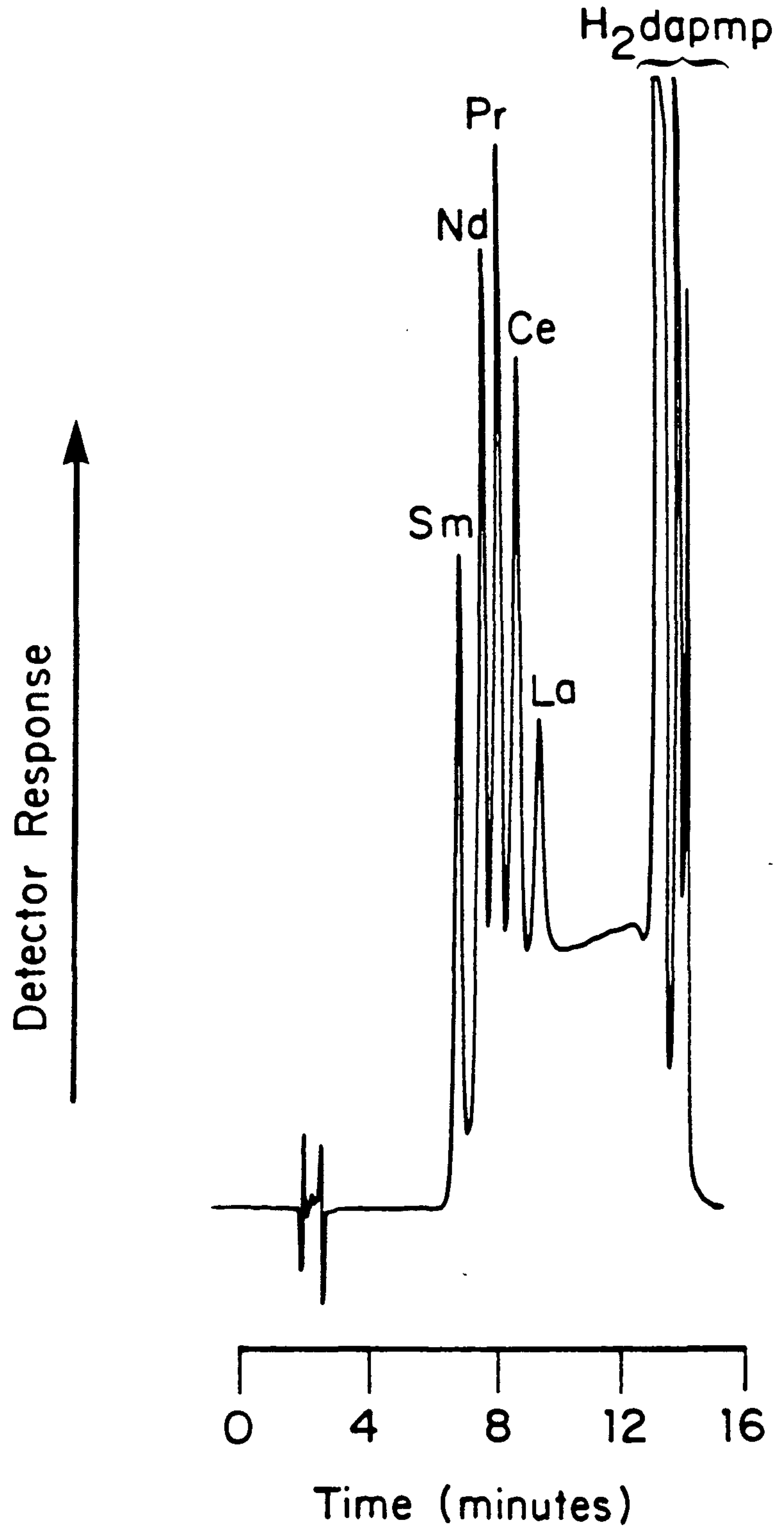


Figure 3: Formation rates of titanium and uranium complexes at pH=7.

Figure 4: Separation of rare earth complexes of H_2dapmp on a PLRP-S column. Eluent conditions: gradient elution; solvent A and B same as Figure 1; linear gradient 82% A, 18% B at 0.0 min, to 70% A, 30% B at 10 min. At 10 min. 100% B for 4 min.



H$_2$dapmp
Pr
Nd
Ce
Sm
La
Detector Response
0
4
8
12
16
Time (minutes)

dissociated immediately before eluting from the column would contribute to the rising baseline directly after the chromatographic peak of the analyte.

Several attempts were made to eliminate this rising baseline in the separation of the rare-earth metal complexes. Changing the observation wavelength provided no improvement in the baseline, because at a longer observation wavelength the molar absorptivity of both the rare-earth metal complexes and the free ligand decrease in a linear fashion. Attempts were also made to remove the baseline problem by adding reagent to the eluent. However, a stable baseline could not be obtained when H_2dapmp was present in the eluent, probably due to the equilibration of the geometrical isomers. Lowering the temperature of the eluent and column did have a significant effect on the rising baseline. Cooling the system reduced the dissociation of the metal complexes considerably but did not eliminate the rising baseline entirely.

It is worth noting that spectrophotometric studies indicated all rare earths did complex with H_2dapmp, but rare earths larger than europium(III) typically showed no chromatographic peak. Quantitation was possible even with the rising baseline. Figure 5 shows a linear relationship between log(relative peak height) and log(praseodymium concentration).

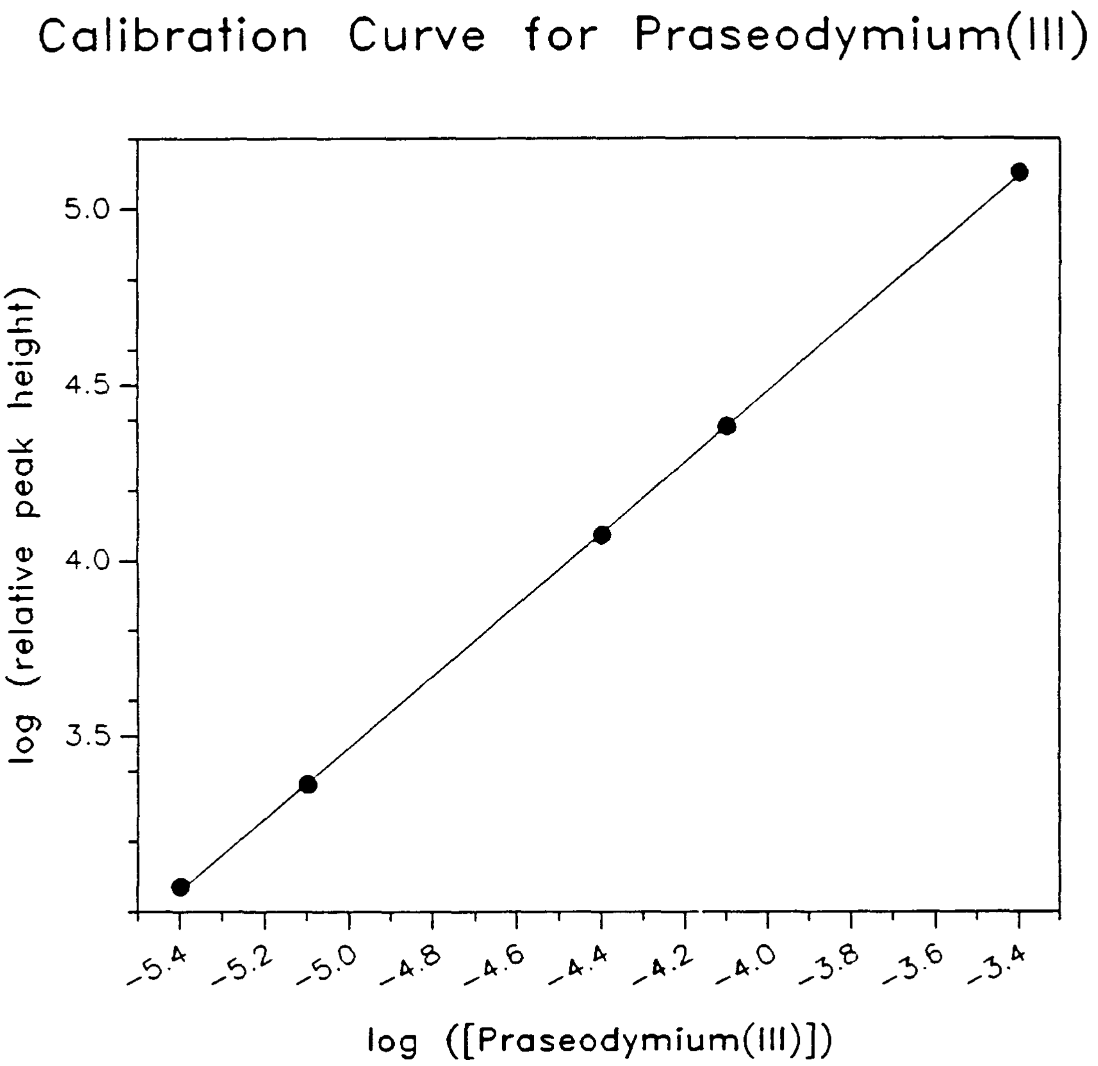


Figure 5: Calibration curve for Praseodymium (III), under isocratic elution conditions, 17% CH_3CN / 83% H_2O, 20 mM ammonium acetate (pH =7).

Ion-pairing Studies

The positively-charged quaternary ammonium groups in the H_2dapmp attract counter anions. With chloride as the counter ion, no chromatographic separations of metal complexes could be obtained. Good separations were only possible when a good ion-pairing anion such as perchlorate was present as the counter ion. The dependence on retention times of several metal complexes on the perchlorate concentration in the eluent was therefore studied. Figure 6 shows a linear dependence of the log of adjusted retention time on the log of perchlorate concentration, for the Cu(II), Sb(III), and Co(II) metal complexes.

If the distribution mechanism was just a simple extension of extraction principles, then the data should fit the linear equation

$$\log(t_r) = m \log[ClO_4^-] + b$$

where m equals the positive charge of the analyte. However, the slopes of the plots shown in Figure 6 were 0.48 for Co(II), 0.45 for Sb(III), 0.44 for Cu(II). Clearly other retention mechanism besides simple ion-pairing must be involved, due to the very low values of m obtained for the complexes. To ensure that coordinated anions were not responsible for such low positive charges the experiment was also preformed on the free reagent. Figure 7 shows a linear dependence of the log of adjusted retention time on the log of perchlorate concentration for the Cu(II) and Pr(III) metal complexes and also for the E,E-

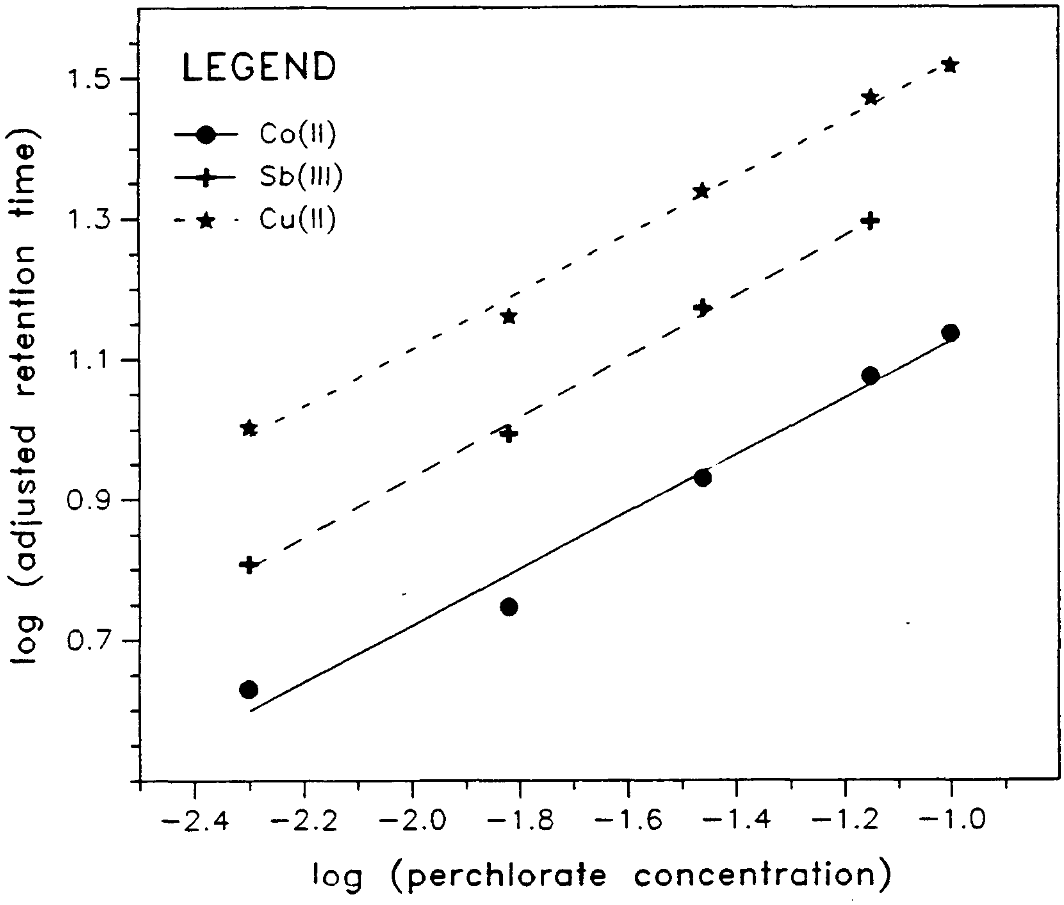


Figure 6: Dependence of log (adjusted retention time) for Cu(II), Sb(III), and Co(II) metal complexes of H_2dapmp on the log of $NaClO_4$ concentration in the eluent. Eluent conditions: 15% CH_3CN / 85% H_2O, 4mM ammonium acetate.

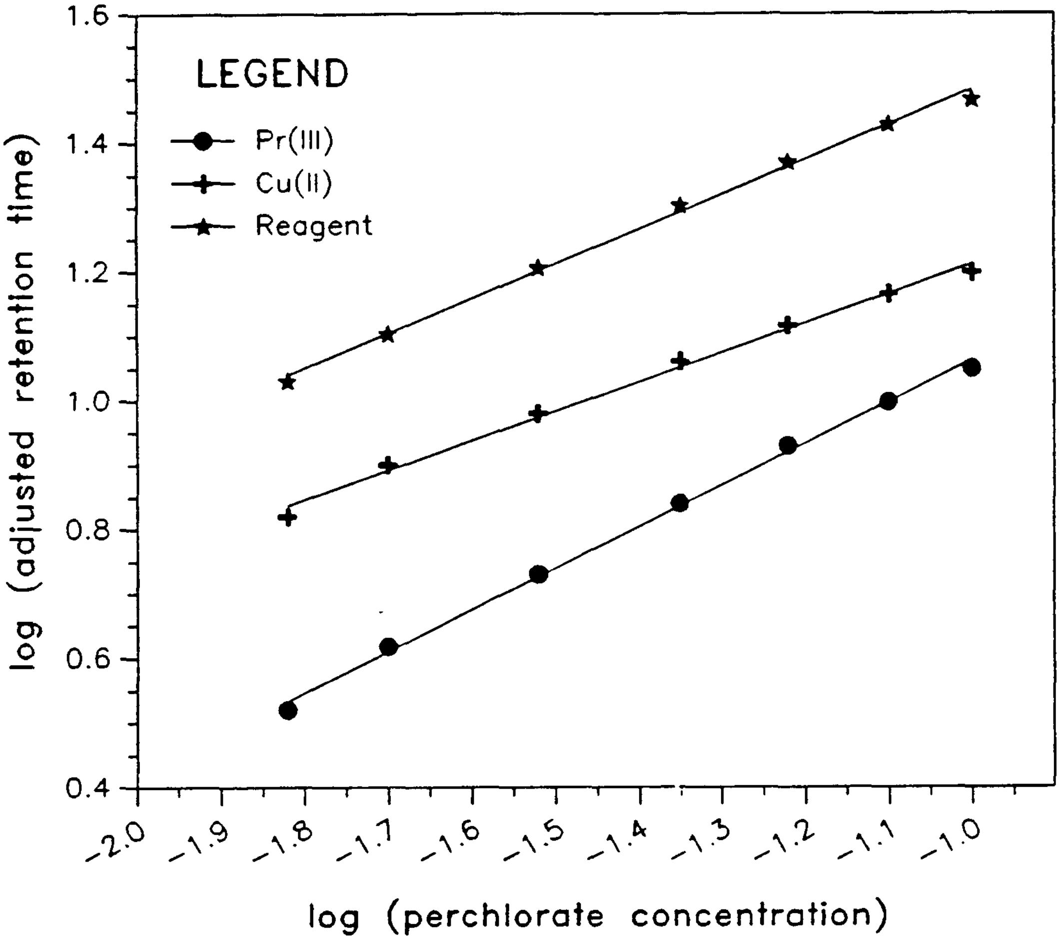


Figure 7: Dependence of log (adjusted retention time) for Pr(III), Cu(II) metal complexes of H_2dapmp, and E,E-H_2dapmp on the log of $NaClO_4$ concentration in the eluent. Eluent conditions: 18% CH_3CN / 82% H_2O, 4mM ammonium acetate.

H_2dapmp. The slopes of these three plots were 0.47, 0.66, and 0.56, respectively. These results also indicate that other retention mechanisms are occurring besides simple ion-pairing. Although in some instances this type of experiment works quite nicely for obtaining the charge of the metal complex (13), other authors have shown this not to be true in all instances (14,15).

Calibration and Quantitation

Calibration curves were prepared for each metal ion to test whether quantitative determinations are feasible. All metals were tested under gradient conditions with the exception of Sn(II). The elution conditions, linear working ranges, and detection limits are given in Table 1. All metals gave good linear calibration curve over a concentration range of at least two orders of magnitude with the exception of Ni(II), Mn(II), and Cd(II). The working range of Ni(II) at low concentrations was limited due to frontal tailing of the first reagent peak or the employment of a stainless steel system. It should be noted that although titanium(IV) and uranium(VI) formed metal complexes slowly under neutral conditions, both gave very nice linear working ranges, as shown in Figure 8.

Interferences

The effect of possible interfering ions was studied by adding varying amounts of selected foreign ions to samples of metal ions to be determined. A foreign ion was considered to interfere when the peak height of a metal complex

Table I. Eluent conditions for calibration of various metals by HPLC using H_2dapmp as the complexing agent[a]

metal ion	elution conditions	linear calibration curve range, M	detection limit, M
Sn(II)	11% CH_3CN 89% H_2O	$3X10^{-6}$ to $3X10^{-4}$	$3X10^{-7}$
Mn(II)	Gradient[b]	$3X10^{-5}$ to $1X10^{-3}$	$2X10^{-6}$
Co(II)	Gradient[b]	$7X10^{-7}$ to $1X10^{-3}$	$3X10^{-7}$
Cd(II)	Gradient[b]	$3X10^{-5}$ to $7X10^{-4}$	$1X10^{-6}$
Sb(III)	Gradient[b]	$4X10^{-6}$ to $8X10^{-4}$	$6X10^{-7}$
Cu(II)	Gradient[b]	$3X10^{-6}$ to $6X10^{-4}$	$5X10^{-7}$
Ni(II)	Gradient[b]	$3X10^{-5}$ to $3X10^{-4}$	$5X10^{-6}$
U(VI)	Gradient[c]	$2X10^{-6}$ to $4X10^{-4}$	$4X10^{-7}$
Ti(IV)	Gradient[c]	$2X10^{-6}$ to $8X10^{-4}$	$2X10^{-7}$

---

[a] For all eluents: Flow rate = 1 ml/min; UV detection, λ = 340 nm, injection volume = 10μl, 20 mM ammonium acetate (pH = 7.0), 50 mM $NaClO_4$.

[b] Gradient conditions same as in Figure 1.

[c] Gradient conditions same as in Figure 2.

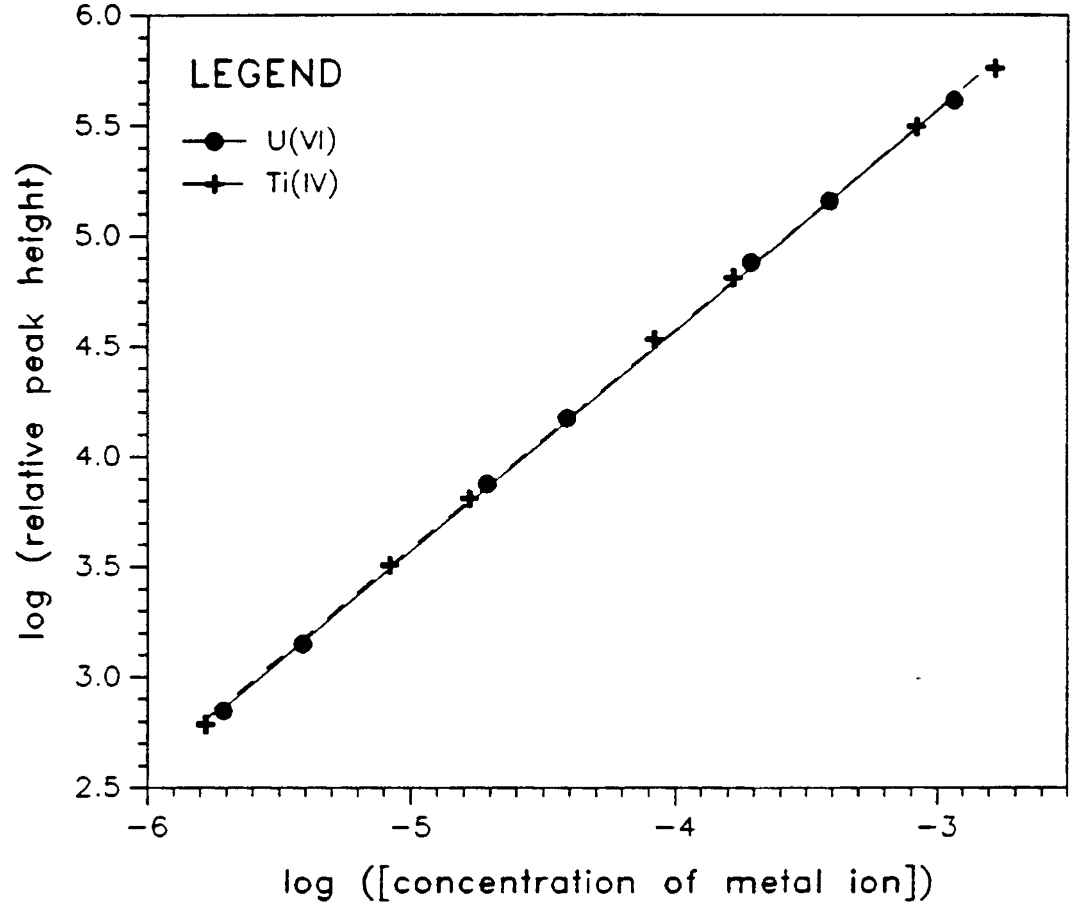


Figure 8: Calibration curve for titanium (IV) and uranium (VI) metal complexes of H_2dapmp, under neutral pH conditions.

was changed by more than ± 5%. For convenience, the concentrations of foreign ions were varied by decades. The results are given in Table II. The + or - signs in parentheses indicate the direction of error if the concentration of the foreign ion is increased one order of magnitude. All positive deviations were due to the co-elution of broad tailing peaks caused by complexation with foreign ions. Negative errors were unexplainable since there appeared to be excess reagent in all instances.

Tartrate was added to the sample and eluent throughout the interference study in an attempt to keep interferent ions from precipitating in the sample or on the column. The presence of tartrate appeared to have no effect on the metal complexes, although it did suppress the Sb(III) response slightly. It should be noted that although antimony works at pH=7, it is probably more desirable to work with it at a more acidic pH.

Table II. Concentration (M) of interferent ions tolerated in determination of sample ions

	Sample Ions[a]						
Interferent Ion	Sn(II)	Ti(IV)	Mn(II)	Co(II)	Sb(III)	Cu(II)	U(VI)
Na^+	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Mg^{2+}	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Al^{3+}	0.001(-)	0.01	0.01	0.01	0.01	0.01	0.01
Zn^{2+}	0.001	0.0001(+)	0.0001(+)	0.0001(+)	0.0001(+)	0.0001(+)	0.001
Hg^{2+}	0.001	0.001	0.001	0.001	0.001	0.0001(-)	0.001
Fe^{3+}	0.001	0.0001(-)	0.0001(+)	0.0001(+)	0.0001(+)	0.0001(+)	0.001
ZrO^{2+}	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Pb^{2+}	0.001	0.001	0.0001(-)	0.001	0.0001(-)	0.001	0.001
Bi^{3+}	0.001	0.001	0.00001(+)	0.0001(+)	0.0001(+)	0.001	0.001
Sm^{3+}	0.001	0.001	0.001	0.00001(+)	0.0001(+)	0.0001(+)	0.001

[a] Eluent conditions: same as Figure 4. The concentration of the sample metals (in the same order as above, starting with Sn(II)) were 4 x 10^{-5}M, 5 x 10^{-5}M, 2 x 10^{-4}M, 7 x 10^{-5}M, 8 x 10^{-5}M, 6 x 10^{-5}M, and 4 x 10^{-5}. See text for explanation of values cited.

CONCLUSIONS

2,6-diacetylpyridine bis(N-methylenepyridiniohydrazone) was shown to be an even more powerful chelating reagent for the chromatographic separation of metal complexes under neutral pH conditions. At a pH of 7 it was possible to separate and determine 8 metal complexes in a single run under gradient conditions on a polystyrene-divinylbenzene polymer based column. Not only could these metal complexes be separated, but all gave nice linear calibration curve, including the following metal chelates: Sn(II), Ti(IV), Mn(II), Co(II), Cd(II), Sb(III), Cu(II), Ni(II), and U(VI). Separation of some rare earth complexes also proved successful under neutral conditions, but these metal complexes did appear to be kinetically unstable. Ion-pairing behavior was also apparent in the retention of the metal complexes as demonstrated by a direct correlation between retention times of the metal complexes and concentration of ClO_4^- in the eluent. Interferences due to the presence of other metal ions in solution were found to be minimal.

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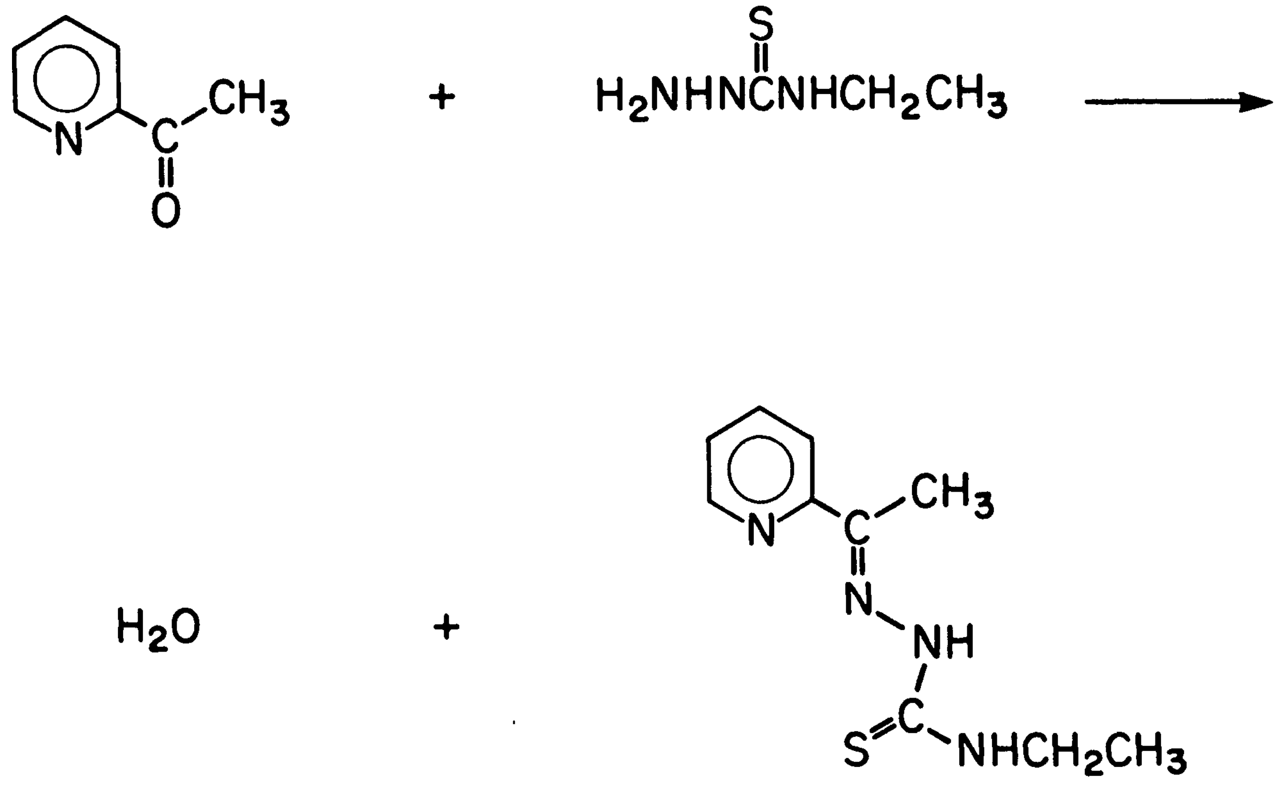
SECTION III. CHROMATOGRAPHIC DETERMINATION OF METAL COMPLEXES OF 2-ACETYLPYRIDINE-4-ETHYL-3-THIOSEMICARBAZONE

INTRODUCTION

Thiosemicarbazones constitute a class of compounds with many useful chemical properties. The synthesis of thiosemicarbazones is generally conducted by condensing a thiosemicarbazide with an aldehyde or ketone. The resulting thiosemicarbazone can act as a chelating agent for metal ions by bonding through the sulfur atom and imino nitrogen. In the present work a new thiosemicarbazone was prepared by condensing 2-acetylpyridine with 4-ethyl-3-thiosemicarbazide as shown in Figure 1.

Thiosemicarbazones have found numerous medical and analytical applications. Thiosemicarbazones derived from 2-acetylpyridine have alone found many potential applications in the literature, including, antimalarial, antitumor, and antibacterial activities(1-3). Metal complexes of thiosemicarbazones have also proven useful for their desirable medicinal properties (3-8). In 1978, Singh et al. wrote an excellent comprehensive review article on the analytical applications of thiosemicarbazone and semicarbazones (9). The article contains 28 different derivatives of thiosemicarbazones and 86 references, although many applications were as indicators in the titration of metal ions against EDTA. Other analytical applications include spectrophotometry, fluorimetry, and gravimetric reagents.

Figure 1: Synthesis route of 2-acetylpyridine-4-ethyl-3-thiosemicarbazone.



Since Singh's review many new derivatives of thiosemicarbazones have been prepared and evaluated by researchers in Spain. Most of this work, however, has been focused on evaluating new symmetrical and asymmetrical derivatives of thiocarbohydrazide as spectrophotometric analytical reagents (10-21).

Inorganic chemist in India have also recently studied thiosemicarbazones as coordinating reagents for metal ions, and review articles have also been written on this subject (22,23). Jain et al. have recently investigated metal complexes of 2-acetylpyridine-4-phenyl-3-thiosemicarbazone (abbrev. Happt). All of this work (24-27) has been in the area of magnetic EPR and spectral studies of solid metal coordination compounds of Happt.

In the present work 2-acetylpyridine-4-ethyl-3-thiosemicarbazone (abbreviated Hapet) has proved useful for the determination of metal ions through the chromatographic separation of their metal complexes. The 2-acetylpyridine derivative is fairly soluble in methanol/water solution, thereby making it possible to simply add buffered reagent to the sample and inject a portion of the sample into the liquid chromatograph. Although, many metals can be separated chromatographically employing Hapet, the Ga(III) and In(III) metal complexes seemed to behave quite nicely.

EXPERIMENTAL

Synthesis and Characterization of Hapet

To a 100 ml round-bottom flask were added 0.027 mol of 2-acetylpyridine (Aldrich) and 0.027 mol of 4-ethyl-3-semicarbazide (Aldrich) in approximately 45 ml of absolute ethanol. The mixture was refluxed for 3 h. after the reaction mixture had cooled 25 ml of water was added and approximately 45 ml of the ethanol/water solvent was removed under vacuum keeping the temperature below 35°C at all times. The product was then collected and washed with 10 ml of a 1:1 solution of methanol:water. At this point the product still had a very detectable odor, probably due to the presence of excess 2-acetylpyridine. The product was recrystallized in 60% methanol and 40% H_2O which yielded white needle-shaped crystals.

The following procedures were used to characterize the Hapet. The infrared spectrum was taken on a IBM IR/98 (KBr pellet). The NMR spectrum was obtained on a Nicholet NT-300 using $DCCl_3$ as the solvent and TMS as the chemical shift standard. The mass spectrum of the sample was taken on a Kratos MS-50. Elemental analysis of the crystals resulted in 53.96 %C, 6.42 %H, 25.16 %N, and 14.67 %S; calculated for $C_{10}H_{14}N_4S$; 54.03 %C, 6.35 %H, 25.20 %N, and 14.42 %S.

LC Studies

The chromatographic system consisted of a LKB 2156 solvent conditioner, LKB 2249 HPLC gradient pump (mixing chamber volume of 0.5 ml), Rheodyne 7125 injector (20 μl injection loop) and a Kratos Spectroflow 783 UV-vis detector. Both the pump and injector were titanium components. The column was a PLRP-S 100-Å 5 μm polystyrene-divinylbenzene (15 cm x 4.6 mm) column from Polymer Laboratories.

Eluents were prepared from Fisher HPLC grade acetonitrile and methanol mixed with water purified with a Barnstead Nanopure II system on a percent volume basis. No corrections were made for non-additive volumes. Acids, bases, and ion-pairing salts were all reagent grade or better. All eluents were prepared by diluting aqueous buffers. Possible changes in apparent pH were ignored, except in the study of the affect on retention times of the metal complexes with changing eluent pH. Most eluents contained 5×10^{-4} M Hapet, 25mM $NaClO_4$, and 21 mM ammonium acetate (pH=7), unless stated otherwise. Eluent components were mixed and filtered with 0.2 μm Nylon 66 (Rainin) filters before use. Eluents were prepared daily. The flow rate was 1.0 ml/min.

Eluents used in the ion-pairing studies, where the adjusted retention time was measured as a function of the perchlorate concentration, were all 50:50 acetonitrile/water and 12 mM ammonium acetate (pH=7).

Preparation of Solutions

Metal stock solutions were made from the metal ion salts. Anions present in these salts included chloride, sulfate, nitrate, and perchlorate. Gallium and palladium were standard metal solutions purchased from Aldrich.

The UV-vis spectra of Hapet and gallium complex were taken at a pH of 4.0 and 7.0 in a 20 mM formate and ammonium acetate buffer, respectively. All spectra were blanked with the corresponding buffer and contained 40 μM Hapet. Enough Ga(III) was added to Hapet to yield 10 μM gallium complex.

Chromatographic samples were prepared by taking an aliquot of stock metal solution and adding enough reagent to make the final sample 1-3 mM Hapet. Metal complexes were formed by adding excess reagent to a somewhat acidic sample and then adjusting the pH. All samples solutions were buffered at the pH of the eluent, and all adjustments in pH were done with NaOH. The samples for the interference studies were prepared as described except that the Hapet was held constant at 3mM. Sample and interferent metals were both present before the addition of reagent.

Hapet was moderately soluble in methanol at room temperature. It was possible to prepare stock solutions of the reagent in methanol at 5 mM and dilute to 1 mM, Hapet without any precipitation of the reagent. The physical characteristics for Hapet are given in Table I.

Table I. Characterization of Hapet

Melting range (°C)	IR maxima (cm^{-1})	Major mass spectral lines m/z (%)	Proton shift of NMR (integration)
127-8	696	222.1 (78)	8.72 (0.36)
	756	207.1 (8)	8.61-8.59 (0.36)
	906	144.1 (9)	7.97-7.94 (0.38)
	1028	134.1 (20)	7.75-7.69 (0.41)
	1068	121.1 (100)	7.32-7.27 (0.43)
	1155	106.1 (28)	3.82-3.77 (0.63)
	1182	79.0 (36)	2.41 (0.88)
	1329	78.0 (31)	2.05 (0.09)
	1373	60.0 (13)	1.36-1.31 (1)
	1452		
	1493		
	1583		
	1601		
	2850		
	2920		
	3028		
	3061		
	3082		

RESULTS AND DISCUSSION

Conditions for Detection

Hapet and its metal ion complexes absorb in the UV spectral region. The metal complexes also absorb in the visible region, whereas Hapet shows little visible color under the pH range used in this study (pH 3.3 to 7). Observation wavelengths were chosen to maximize the molar absorptivities of the metal complexes and minimize the molar absorptivity of the free reagent. All of the work performed at pH 7 had an observation wavelength of 380 nm, whereas a longer wavelength (405 nm) was employed for work performed under more acidic conditions. Spectra for the reagent and the Ga complex at pH 7 are shown in Figure 2. As the pH was lowered, the spectrum of the free reagent underwent a bathochromic shift, as shown in Figure 3. Most of the other metal complexes gave spectra similiar to the Ga(III) chelate. Extinction coefficients of the metal complexes varied with pH. Most were slightly lower when working under acidic conditions (pH 4.3) at 405nm, as shown in Table II. The exceptions were Cd(II) and Zn(II) with extinction coefficients reduced greatly under acidic conditions, being only 600 $(M\cdot cm)^{-1}$ and 400 $(M\cdot cm)^{-1}$, respectively. The absorbance, due to the presence of Hapet in the eluent, was subtracted out electronically.

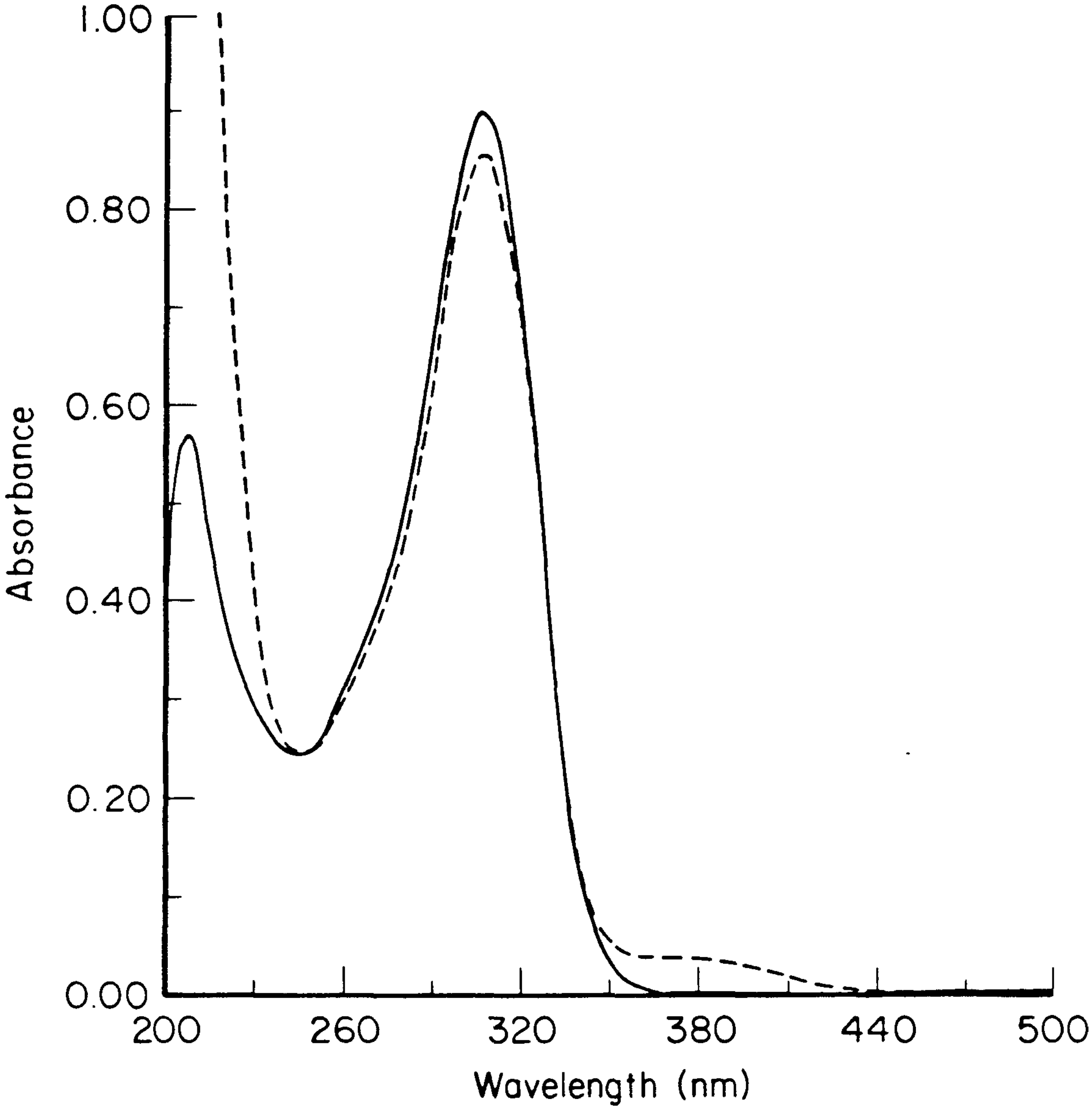


Figure 2: UV-vis absorbance spectra of Hapet (__) and gallium complex (---) at pH 7.

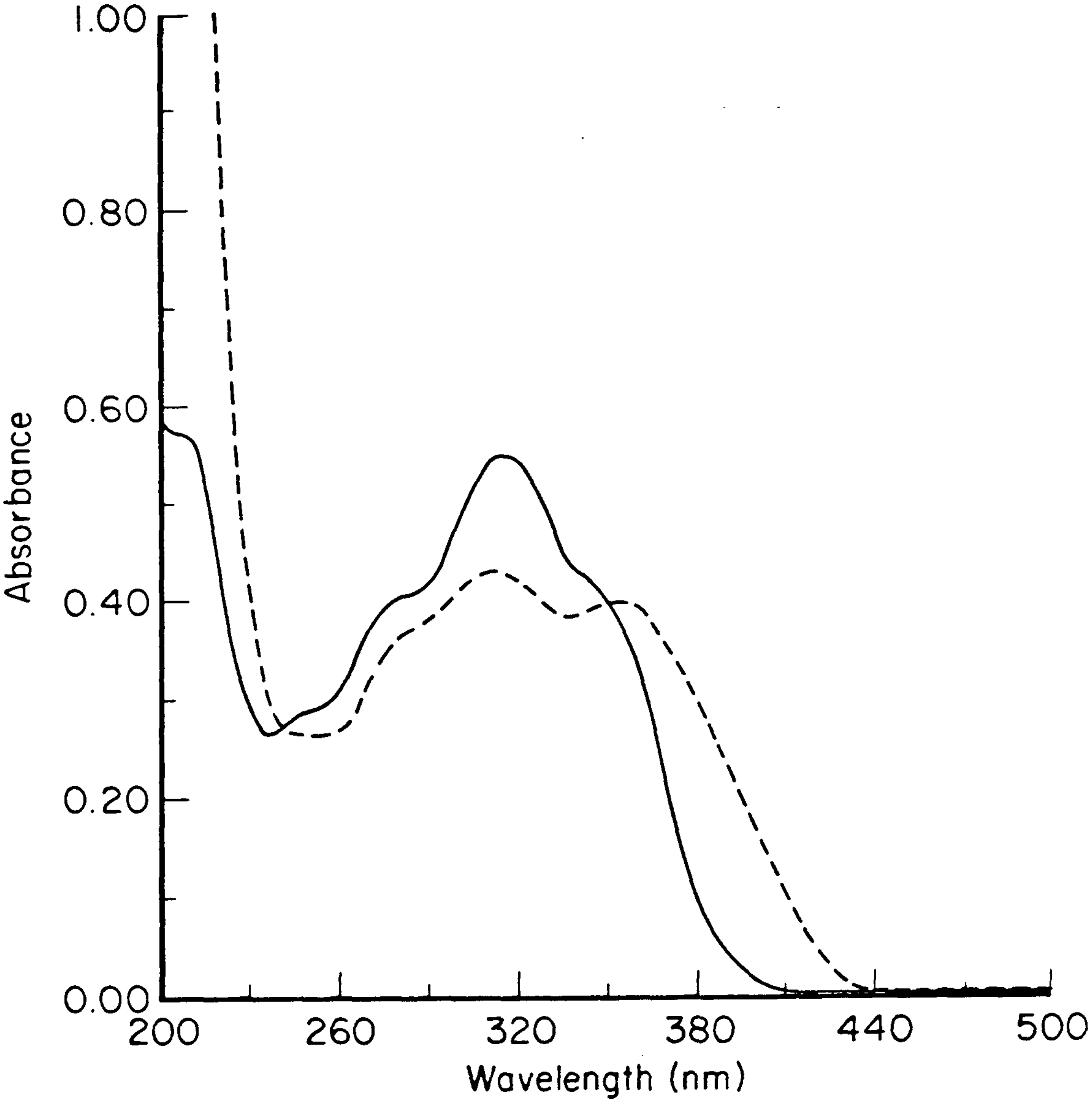


Figure 3: UV-vis absorbance spectra of Hapet (__) and gallium complex (---) at pH 4.

Table II. Extinction coefficients of metal complexes of Hapet

Metal	Extinction coefficients	
	λ=380nm	λ=405nm
Ni(II)	19,000	2,600
Co(II)	12,000	11,000
Fe(III)	11,000	8,800
V(V)	1,800	6,100
Bi(III)	6,300	3,600
Pd(II)	6,400	4,000
Ga(III)	10,000	11,000
In(III)	19,000	4,300
Zn(II)	12,000	650
Cd(II)	--------	420
Cu(II)	7,100	5,200
Pb(II)	1,500	260
Hg(II)	--------	1,800

[a] Extinction coefficients at 380 nm and 405 nm were determined at pH 7.0 and 4.3, respectively.

Eluent

Due to the instability of the metal complexes on the column, Hapet was added to the eluent. The concentration of Hapet in the eluent had little effect on the retention time of the metal complex, as shown in Table III. At low concentrations of Hapet, however, the chromatographic peaks were considerably broader. Figure 4 shows a separation of 5 metal complexes employing $1X10^{-4}$ M Hapet in the eluent. Under these conditons both the Zn(II) and Cd(II) metal ion-complexes produced broad chromatographic peaks, as can be seen from the chromatogram. If the concentration of the reagent was increased to $5X10^{-4}$ M Hapet, the peak shapes improved tremendously, as is shown in Figure 5. Further increases in the concentration of Hapet in the eluent had no further improvement in peak shape of the analytes. A concentration of $5X10^{-4}$ Hapet was use throughout most of this work.

The percentage of acetonitrile in the eluent had a significant effect on the retention time of the metal complexes. As the percentage of acetonitrile was increased, the retention time of the metal complexes were reduced as is shown in Figure 6. It should be noted that the slopes of the plots shown in Figure 6 are not parallel, indicating that the elution order can be affected by the percentage of acetonitrile in the eluent. For example, at a low percentage of acetonitrile the Ni(II) metal complex was the first peak to elude. At high percentages of acetonitrile both the Co(II) and Fe(III) metal complexes elute before the Ni(III)

Table III. Adjusted retention time of metal complexes as a function of varying amounts of Hapet in the eluent

Concentration of Hapet, M	Adjusted retention time of metal complexes (min)					
	Co	Fe	In	Zn	Cd	Ni
$1.0X10^{-4}$	2.40	2.81	3.76	4.70	9.16	2.78
$2.5X10^{-4}$	2.36	2.78	3.70	4.46	9.12	2.76
$5.0X10^{-4}$	2.27	2.65	3.52	4.24	8.78	2.68
$1.0X10^{-3}$	2.32	2.79	3.58	4.21	8.70	2.69
$2.5X10^{-3}$	2.38	2.78	3.68	4.26	8.90	2.78

Figure 4: Separation of metal complexes of Hapet on a PLRP-S column, using 1X10^{-4} M Hapet in the eluent. Eluent conditions: isocratic elution; 60% CH_3CN, 40% H_2O, 25 mM $NaClO_4$, 21 mM NH_4OAc, and 1X10^{-4} M Hapet.

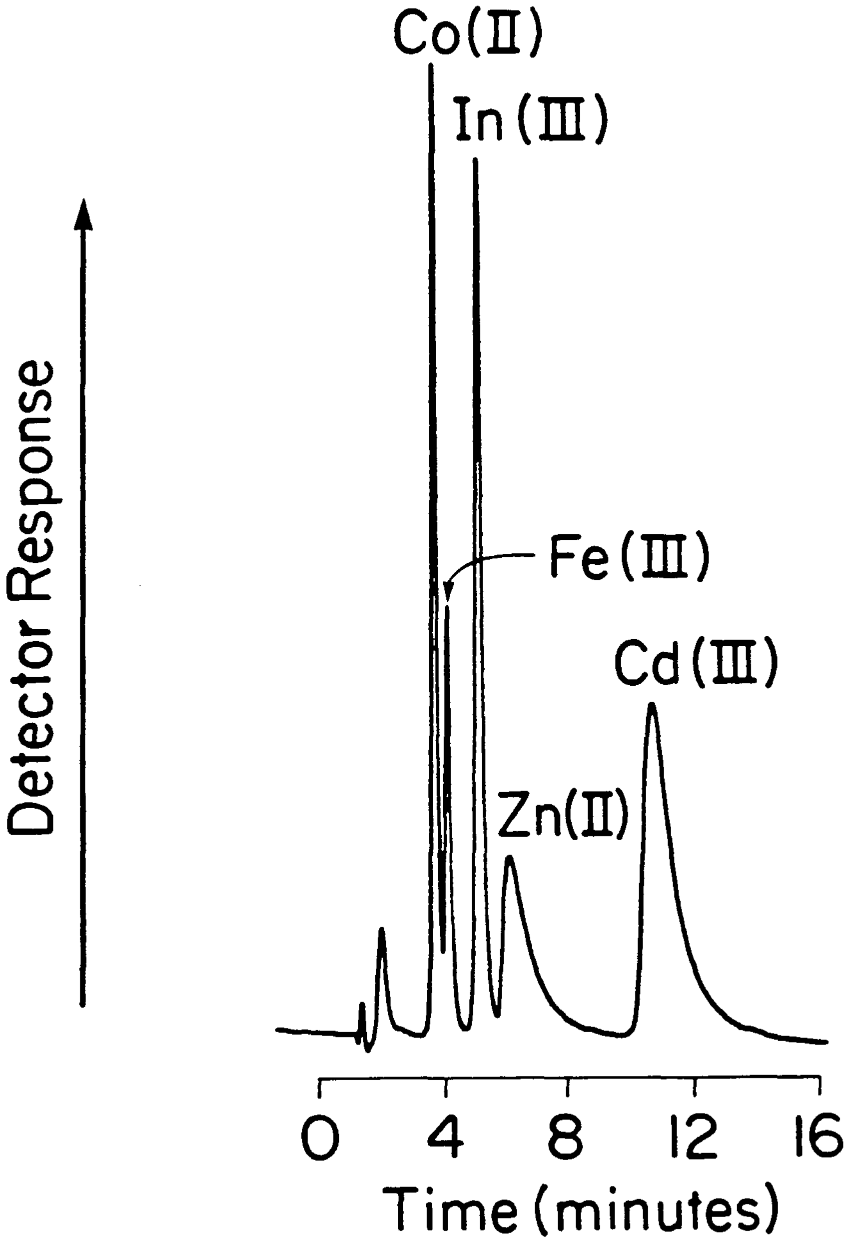
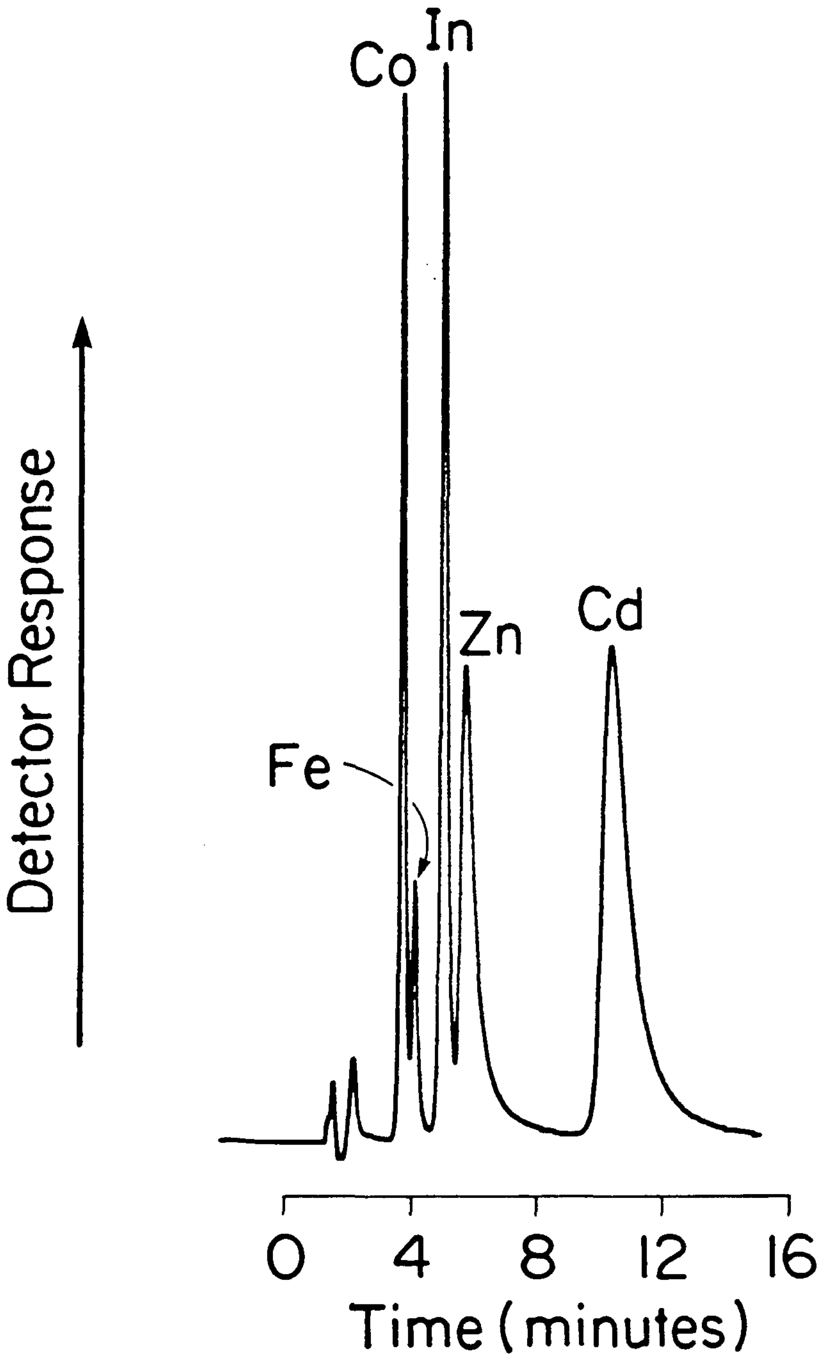

Co(II)
In(III)
Fe(III)
Cd(III)
Zn(II)
Detector Response
0
4
8
12
16
Time (minutes)

Figure 5: Separation of metal complexes of Hapet on a PLRP-S column, using $5X10^{-4}$ M Hapet in the eluent. Eluent conditions: isocratic elution; 60% CH_3CN, 40% H_2O, 25 mM $NaClO_4$, 21 mM NH_4OAc, and $5X10^{-4}$ M Hapet.


Co
In
Zn
Cd
Fe
Detector Response
0
4
8
12
16
Time (minutes)

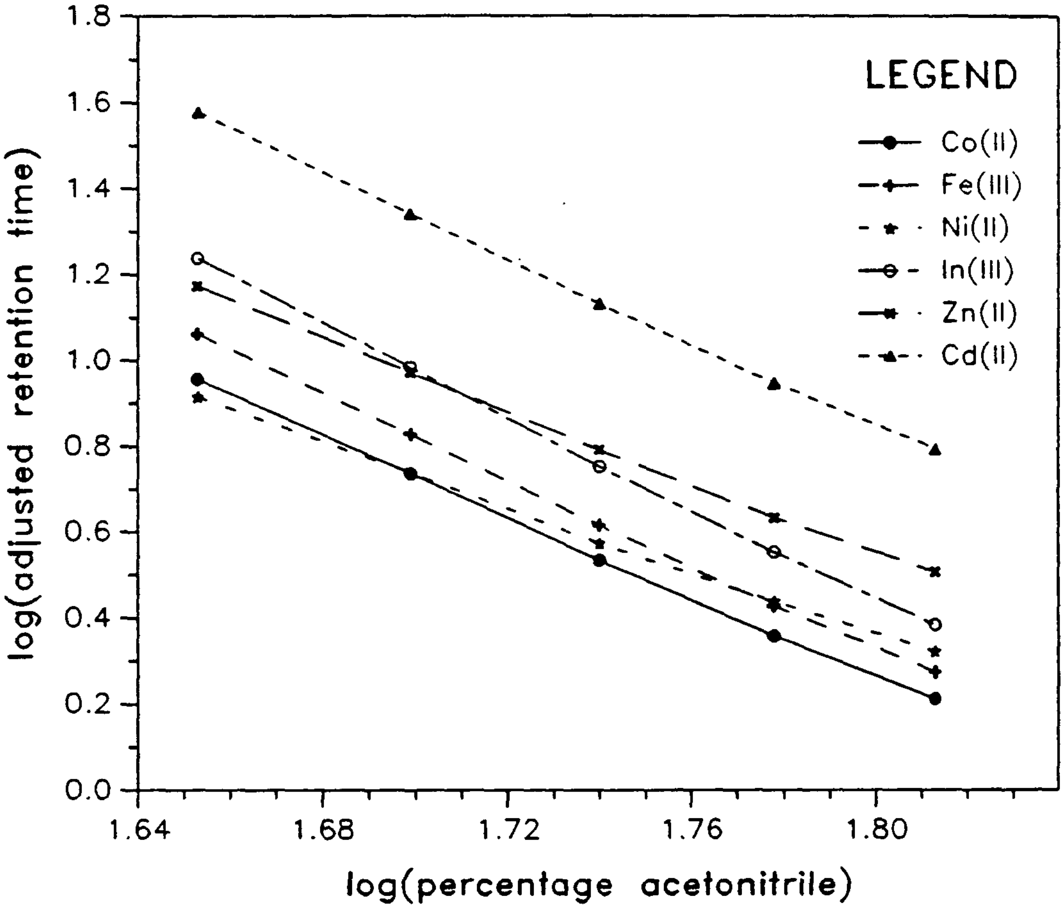


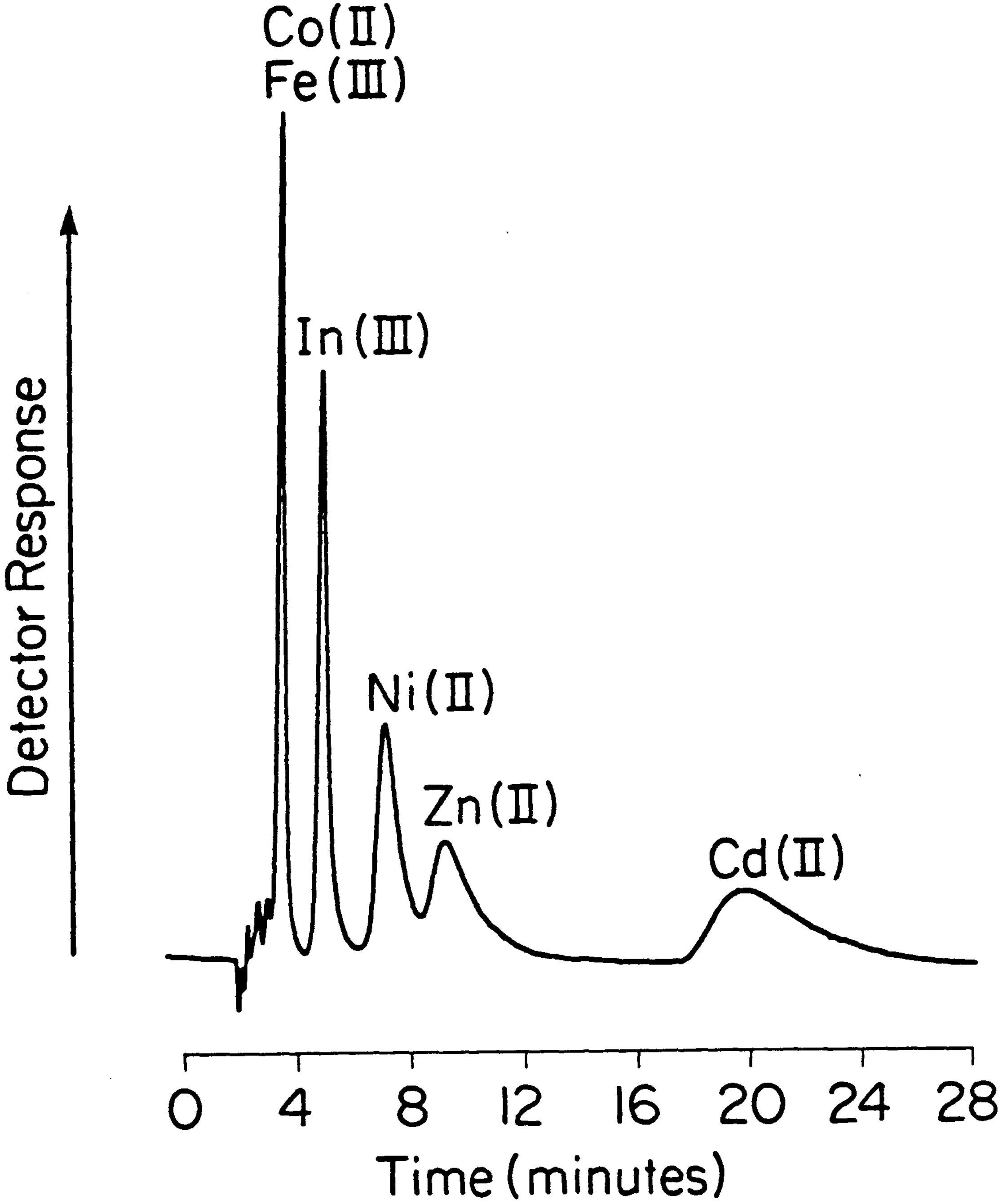
Figure 6: Dependence of log (adjusted retention time) for Ni(II), Co(II), Fe(III), In(III), Zn(II), and Cd(II) metal complexes of Hapet on the log of acetonitrile percentage in the eluent. Eluent conditions: 25 mM $NaClO_4$, 21 mM NH_4OAc (pH=7), $5X10^{-4}$ M Hapet, and varying amounts of CH_3CN/H_2O.

complex. The elution order of Zn(II) and In(III) could also be reversed by varying the percentage of acetonitrile in the eluent.

Methanol was also tested as a potential organic modifier in the chromatographic separation of Hapet metal complexes. The chromatographic peaks were considerable broader when using methanol in the eluent, as shown in Figure 7. Other authors have observed similar affects when switching from acetonitrile to methanol as an organic modifier (28). It should be noted that methanol is also a much weaker organic modifier than acetonitrile, requiring a much higher percentage to obtain a similiar chromatographic separation.

The concentration of the ion-pairing reagent (ClO_4^-) in the eluent had a significant effect on the retention time of many of the metal complexes. Figure 8 shows the effect that varying the perchlorate concentration in the eluent had on the retention time of the metal complexes. As the concentration of perchlorate in the eluent was increased, the retention time of the Co(II), Fe(III), and In(III) metal complexes increased. This would indicate that a positive charge resides on these three metal complexes. By comparison, as the perchlorate concentration was increased the retention time of the Cd(II) and Zn(II) metal complexes did not increase. These results indicate that Cd(II) and Zn(II) form neutral metal complexes with Hapet under pH neutral conditions, since their retention times should not increase if the metal complexes are neutral. At high concentrations,

Figure 7: Separation of metal complexes of Hapet on a PLRP-S column using methanol as an organic modifier. Eluent conditions: isocratic elution; 85% CH_3OH, 15% H_2O, 25 mM $NaClO_4$, 21 mM NH_4OAc, and $5X10^{-4}$ M Hapet. All metals present at approximately $1X10^{-4}$ M in sample solution.


Co(II)
Fe(III)
In(III)
Ni(II)
Zn(II)
Cd(II)
Detector Response
0
4
8
12
16
20
24
28
Time (minutes)

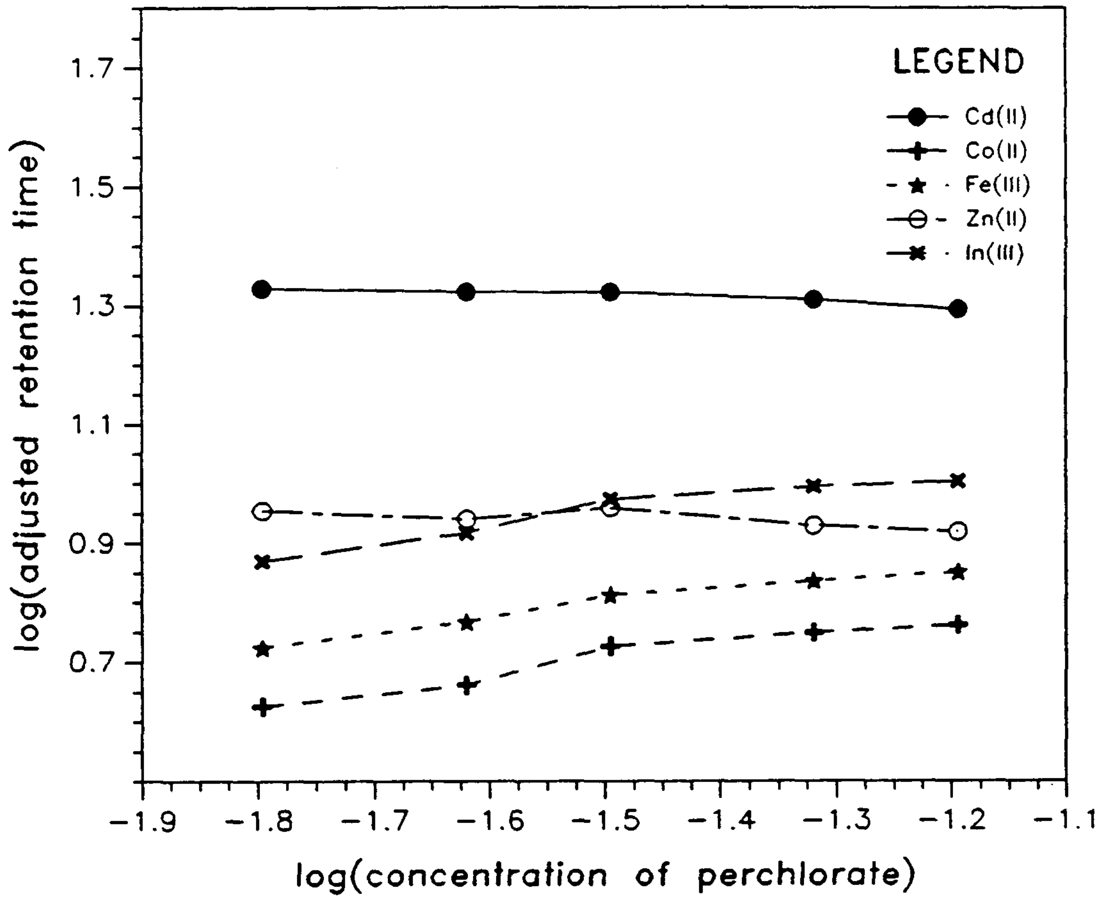


Figure 8: Dependence of log (adjusted retention time) for Co(II), Fe(III), In(III), Zn(II), and Cd(II) metal complexes of Hapet on the log of $NaClO_4$ concentration in the eluent. Eluent conditions: 50% CH_3CN / 50% H_2O, 12mM NH_4OAc (pH=7.0), $5X10^{-4}$ M Hapet, and varying amounts of $NaClO_4$.

Zn(II) and Cd(II) metal complexes were also much more insoluble than the other metal complexes at pH 7. This would also indicate neutral complexes. Figure 8 shows the elution order can also be altered by varying the concentration of $NaClO_4$ in the eluent. At low concentrations of $NaClO_4$ (33mM), Figure 9 shows that the In(III) metal complex eludes before the Zn(II) metal chelate. As the concentration is increased the two metal complexes now coelute, as illustrated in Figure 10. Further increases in the concentration of $NaClO_4$ result in the Zn(II) metal complex actually eluting before the In(III) metal complex (Figure 11). It should be noted that Figures 9 thru 11 do not correlate directly with Table III because different amounts of acetonitrile were used in the two studies.

Figure 12 shows the retention times of some metal complexes were unaffected by the eluent pH, such as Ga, Co, Fe, and In metal complexes. However, the retention time of the Zn, Ni, and Cd metal complexes varied greatly with pH. Formation of neutral complexes with Hapet by these metal ions would require the removal of acidic protons, so a dependence of retention time on pH is not surprising. Either the the thiol and thione form of the semicarbazone could be involved in chelation of metal ions, since the exact structures of the metal complexes in solution was not determined.

Figure 9: Separation of metal complexes of Hapet on a PLRP-S column, using 33 mM $NaClO_4$ as an ion-pairing reagent. Eluent conditions: isocratic elution; 60% CH_3CN, 40% H_2O, 33 mM $NaClO_4$, 14 mM NH_4OAc, and $5X10^{-4}$ M Hapet.

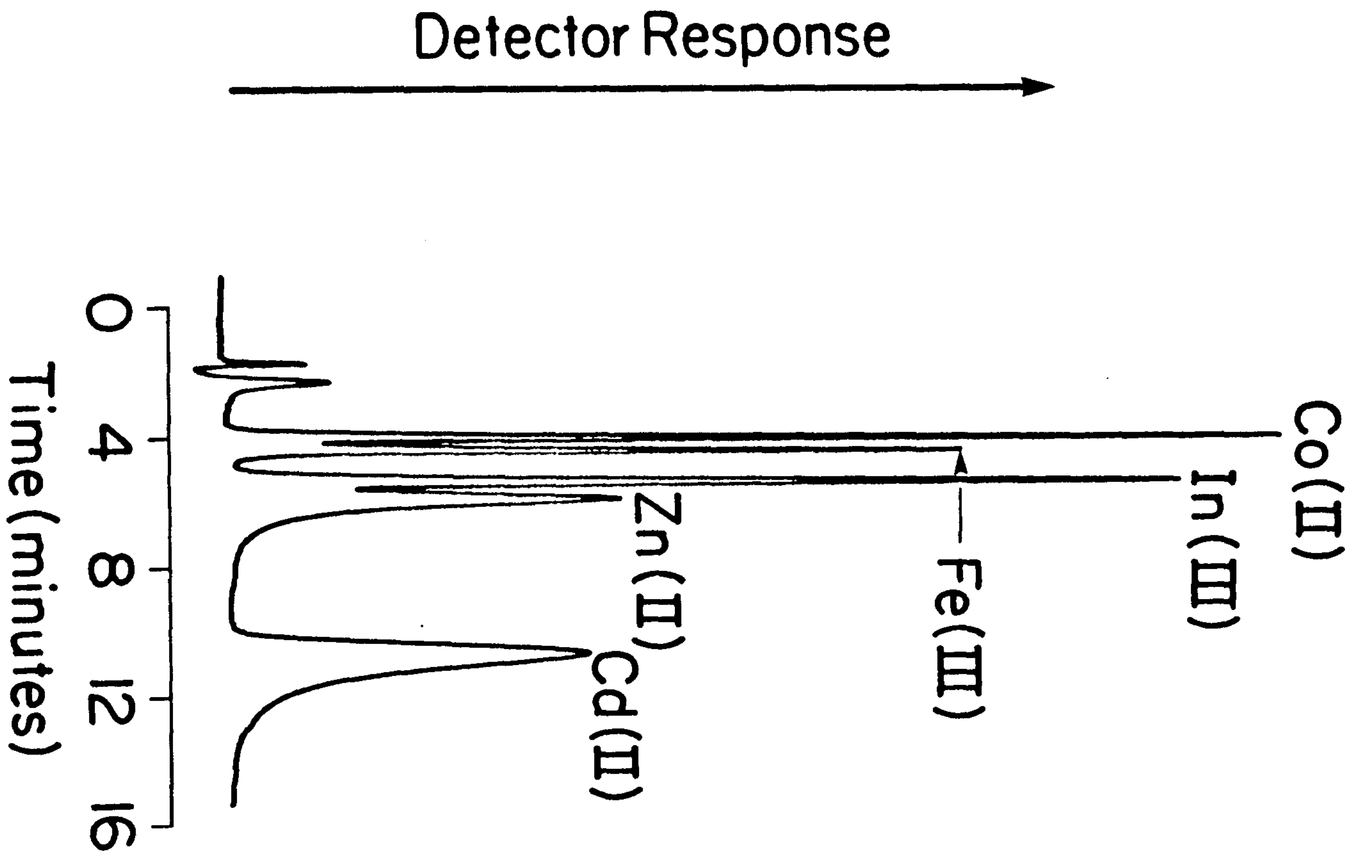

Detector Response
Co(II)
In(III)
Fe(III)
Zn(II)
Cd(II)
0
4
8
12
16
Time (minutes)

Figure 10: Separation of metal complexes of Hapet on a PLRP-S column, using 60 mM $NaClO_4$ as an ion-pairing reagent. Eluent conditions: isocratic elution; 60% CH_3CN, 40% H_2O, 60 mM $NaClO_4$, 14 mM NH_4OAc, and $5X10^{-4}$ M Hapet.

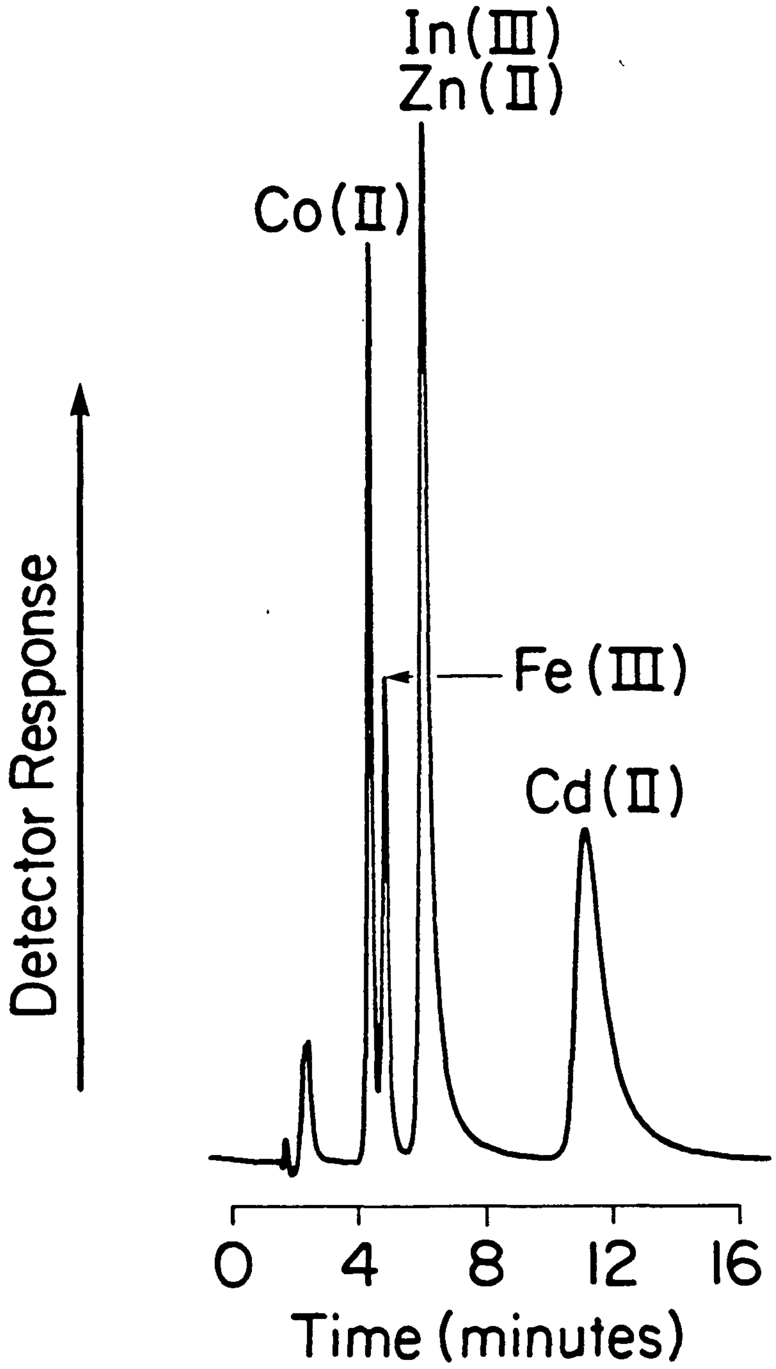
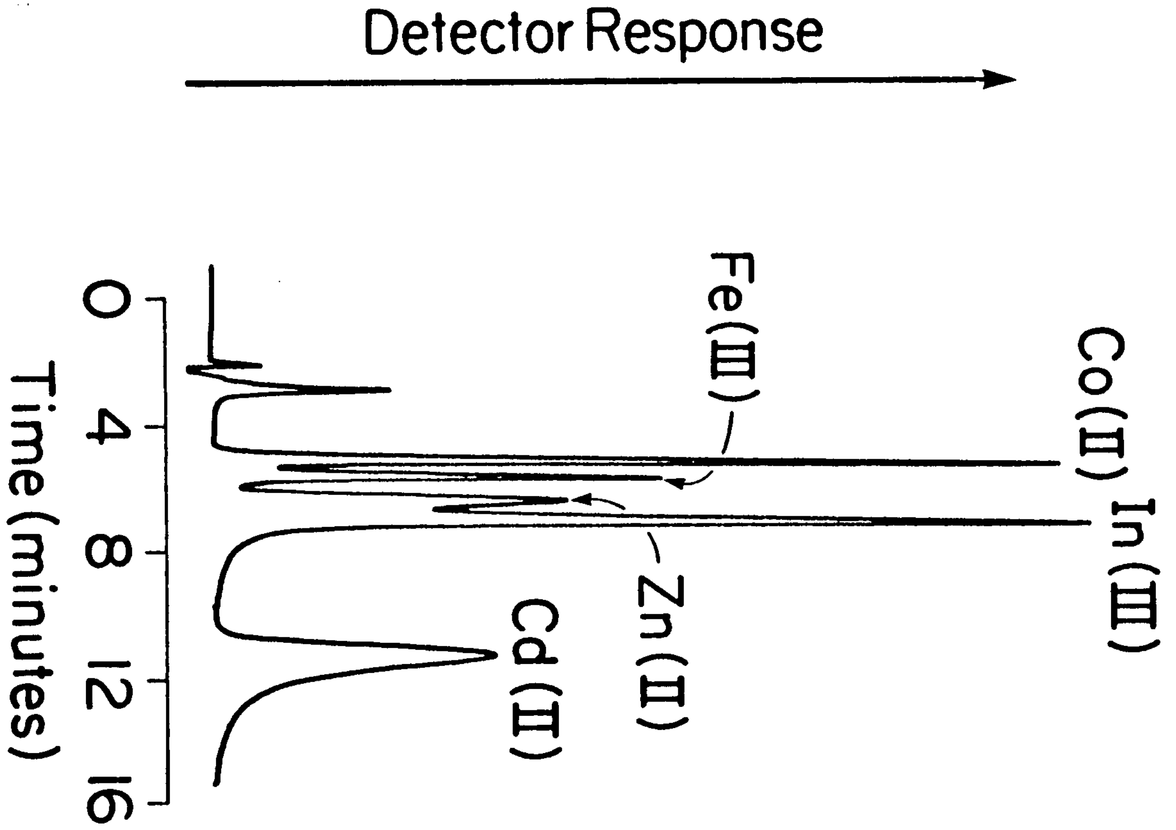

In(III)
Zn(II)
Co(II)
Fe(III)
Cd(II)
Detector Response
0
4
8
12
16
Time (minutes)

Figure 11: Separation of metal complexes of Hapet on a PLRP-S column, using 200 mM $NaClO_4$ as an ion-pairing reagent. Eluent conditions: isocratic elution; 60% CH_3CN, 40% H_2O, 200 mM $NaClO_4$, 14 mM NH_4OAc, and $5X10^{-4}$ M Hapet.


Detector Response
Fe(III)
Co(II)
In(III)
Zn(II)
Cd(II)
0
4
8
12
16
Time (minutes)

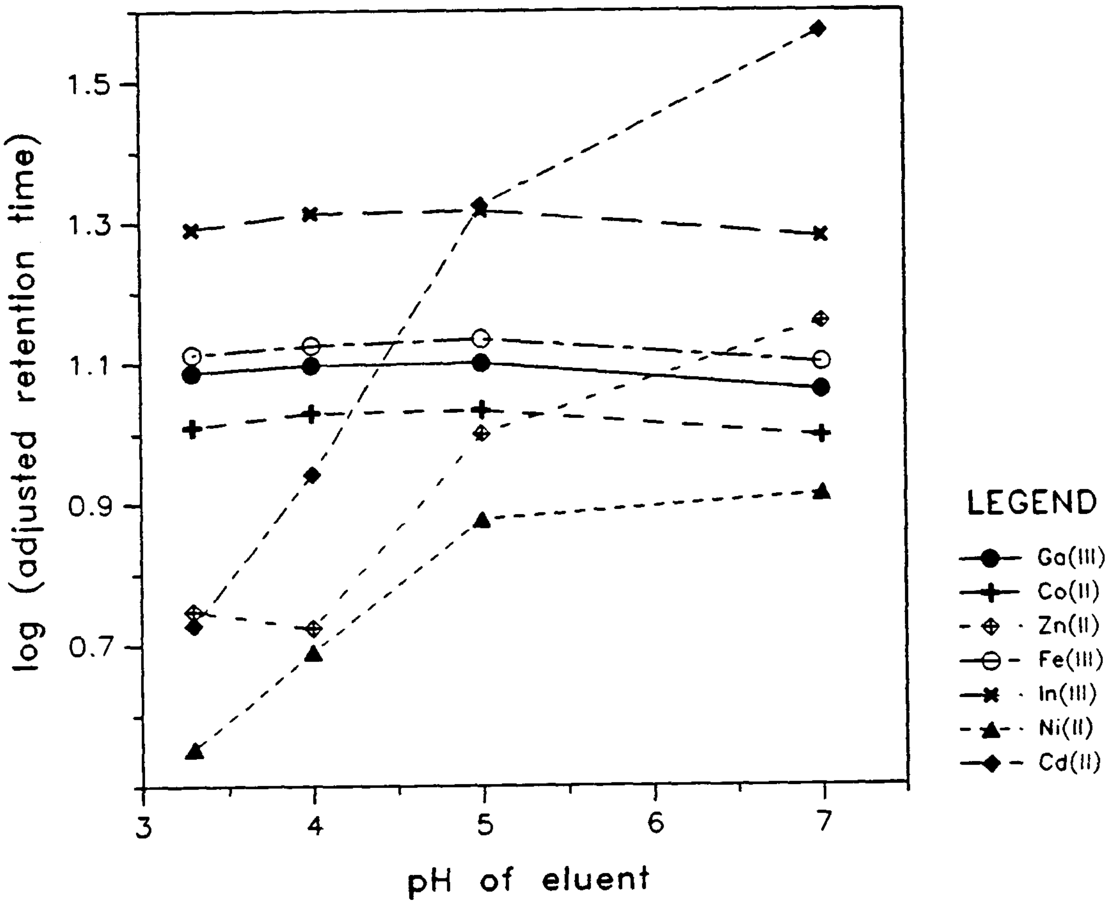


Figure 12: Dependence of log (adjusted retention time) for Ni(II), Co(II), Fe(III), Ga(III), In(III), Zn(II), and Cd(II) metal complexes of Hapet on the pH of the eluent. Eluent conditions: 55% CH_3CN/45% H_2O, 50 mM $NaClO_4$, $5X10^{-4}$ M Hapet, and various buffers.

Platinum Group Metal Complexes

Most of the platinum group metal ions appeared to form metal complexes with Hapet under acidic conditions. Of these metals, palladium behaved quite nicely. It gave only one chromatographic peak and its metal complex formed relatively quickly (few minutes). All of the other platinum group metals tested (Pt(IV), Ru(III), Au(III), and Ir(IV)) either formed metal complex with Hapet very slowly, or formed more than one type of metal complex. Most of the metal ions formed more than one type of metal complex and produce more than one chromatographic peak. It did appear, however, that by increasing the temperature of the sample solution, Pt(IV) formed predominantly one type of complex with Hapet.

Chromatographic Separation of Metal-Hapet Complexes

Many of the metal complexes could be separated successfully under both neutral (pH=7) and acidic (pH=4) conditions. Ni(II), Co(II), Fe(III), In(III), and Ga(III) metal complexes all produced nice chromatographic peaks under both pH conditions. Figure 13 shows chromatographic separations of Ni(II), Co(II), Fe(III), Ga(III), and In(III) metal complexes, under acidic conditions. Almost identical results were obtained under neutral conditions.

Although an excellent separation of complexed gallium(III) and indium(III) was obtained (Figure 14), the iron(III)-Hapet complex co-eluted with the gallium peak. It was hoped that reduction of iron from (III) to (II) and

Figure 13: Separations of metal complexes of Hapet on a PLRP-S column at a pH of 4. Eluent conditions: isocratic elution; 60% CH_3CN, 40% H_2O, 25 mM $NaClO_4$, 12 mM Acetate (pH=4), and $5X10^{-4}$ M Hapet. All metals present at approximately $1X10^{-4}$ M in sample solution, except gallium(III) which was present at $5X10^{-4}$ M.

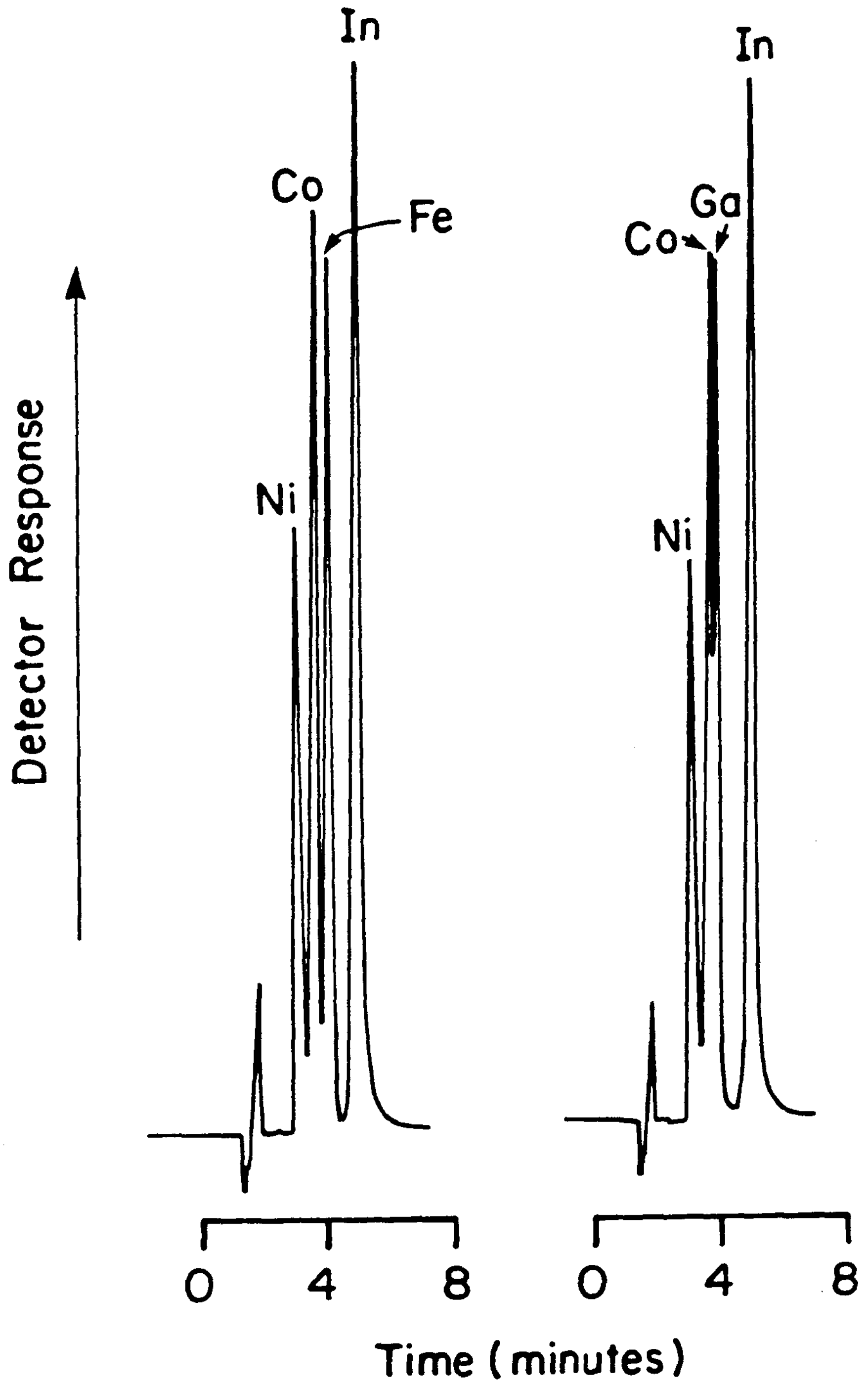
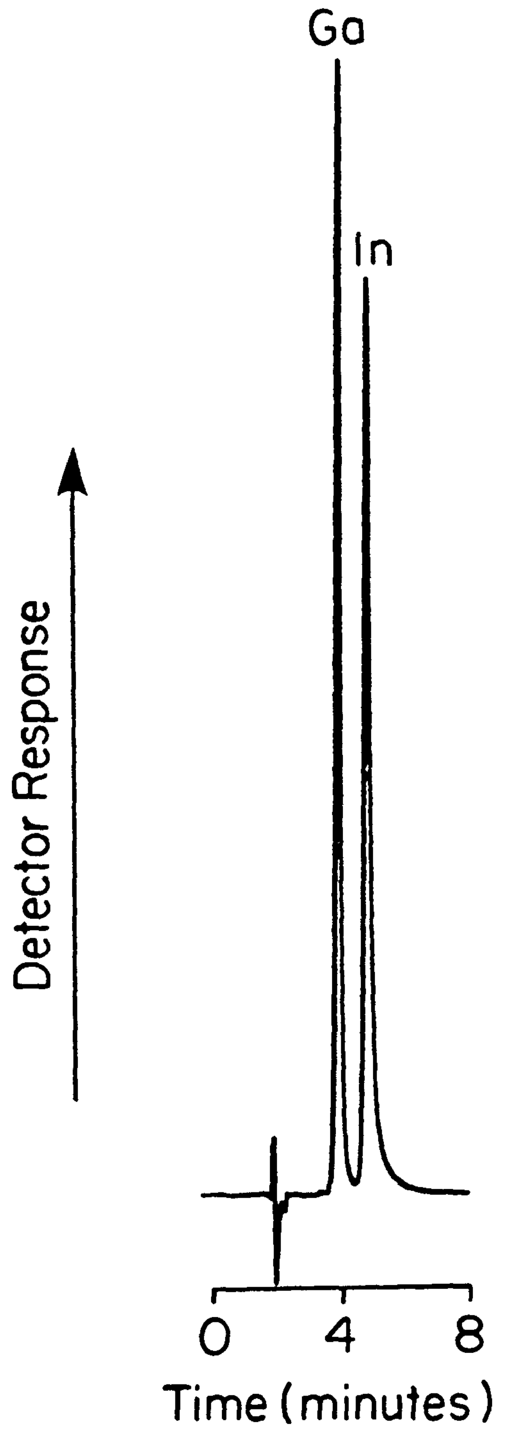

In
Co
Fe
Ni
In
Ga
Co
Ni
Detector Response
0
4
8
0
4
8
Time (minutes)

Figure 14: Separation of gallium(III) and indium(III) metal complexes of Hapet on a PLRP-S column at a pH of 4. Concentrations were $6X10^{-5}$ M and $1X10^{-4}$ M, respectively. Eluent conditions: isocratic elution; 60% CH_3CN, 40% H_2O, 25 mM $NaClO_4$, 12 mM Acetate (pH=4), and $5X10^{-4}$ M Hapet.


Ga
In
Detector Response
0
4
8
Time (minutes)

complexation with 1,10-phenanthroline would permit the chromatographic determination of gallium without interference from iron. This treatment did remove the iron chromatographic peak, but the gallium peak was also missing from the chromatogram. Attempts were also made to resolve the iron and gallium peaks by replacing some of the acetonitrile in the eluent with tetrahydrofuran. However, increasing proportions of THF in the eluent only decreased the already poor resolution of the two metal complexes.

Calibration and Quantitation

Calibration curves were prepared for In(III), Co(II), Zn(II), Ni(II), Ga(III), Fe(III), and Cd(II) under isocratic elution conditions. The elution conditions, linear calibration curve range, and limits of detection are shown in Table IV. All metal complexes eluted between 3 and 8 minutes under the conditions used.

Good linear calibration curves were obtained for all metal over at least 2 orders of magnitude, the only exception being the Zn(II) chelate. These results indicate that good quantitative results are possible using this method. The lower range of some metal complexes was limited by excess reagent peaks that coeluted with these metal complexes. The upper concentration range of Zn(II) and Cd(II) at a pH of 7 was limited by the solubility of the metal complexes. Although most of the calibration work was preformed under neutral pH conditions, it should be noted that In(III) also gave a nice linear calibration curve from $7X10^{-7}$ M to $1X10^{-3}$ M at a pH of 4.3. For analysis of Ni(II), Co(II),

Table IV. Eluent conditions for calibration of various metals by HPLC using Hapet as the complexing agent[a]

metal ion	elution conditions	linear calibration curve range, M	detection limit, M
In(III)	60% CH_3CN 40% H_2O	4×10^{-7} to 7×10^{-4}	9×10^{-8}
Co(II)	60% CH_3CN 40% H_2O	3×10^{-7} to 6×10^{-4}	1×10^{-7}
Zn(II)	60% CH_3CN 40% H_2O	3×10^{-6} to 2×10^{-4}	1×10^{-6}
Ni(II)	60% CH_3CN 40% H_2O	3×10^{-6} to 7×10^{-4}	4×10^{-7}
Ga(III)	60% CH_3CN 40% H_2O	6×10^{-7} to 3×10^{-4}	3×10^{-7}
Fe(III)	60% CH_3CN 40% H_2O	3×10^{-6} to 7×10^{-4}	6×10^{-7}
Cd(II)	70% CH_3CN 30% H_2O	7×10^{-8} to 3×10^{-5}	2×10^{-8}

[a] For all eluents: Flow rate = 1.0 ml/min; UV detection, λ = 380 nm, injection volume = 10μl, 25 mM $NaClO_4$, 21 mM NH_4OAc (pH = 7.0).

Ga(III), Fe(III), and In(III) a pH of 4 appears to work quite nicely for the determination. If Zn(II) or Cd(II) are also present in the sample, however, the pH should be raised to increase their retention times and remove them as potential interferents.

Interferences

The possibility of interfering ions was tested by adding various metal ions to sample solutions containing Ni(II), Co(II), Fe(III), Ga(III), and In(III). A foreign ion was considered to interfere if the peak height of the metal complex was changed by more than ± 5%. The results are shown in Table V. For convenience, the concentration of the foreign ions were varied by decades. All concentrations shown could be tolerated in the determination. The + or - in parentheses indicates the interferents effect on the analytes peak height, if the concentration of the foreign ion was increased one order of magnitude. It should be noted that the metal complexes of Zn(II) and Cd(II) interfere in the analyses of many of the metal complexes at a pH of 4, as shown in Table V. However, these two interferents can be removed by raising the pH of the eluent, and actually be chromatographed successfully under neutral conditions.

Table V. Concentration (M) of interferent ions tolerated in determination of sample ions

Interferent Ion	Sample Ions[a]				
	Ni(II)	Co(II)	Fe(III)	Ga(III)	In(III)
Na^+	0.1	0.1	0.1	0.1	0.1
Ca^{2+}	0.1	0.1	0.1	0.1	0.1
Al^{3+}	0.01	0.01	0.01	0.01	0.01
Mn^{2+}	0.001	0.001	0.001	0.001	0.001
Hg^{2+}	0.001(-)	0.001	0.001(-)	0.001	0.001
Pb^{2+}	0.001	0.001	0.001	0.001	0.001
Ce^{3+}	0.001	0.001	0.001	0.001	0.001
Bi^{3+}	0.001	0.001	0.001	0.001	0.001
Cu^{2+}	0.0001(+)	0.0001(+)	0.0001(+)	0.0001(+)	0.0001(+)
Zn^{2+}	0.00001(+)	0.0001(+)	0.0001(+)	0.0001(+)	0.001
Cd^{2+}	0.001	0.001	0.0001(+)	0.0001(+)	0.0001(+)

[a]Eluent conditions: same as Figure 14. The concentration of the sample metals were all 1 X 10^{-4} M, except for Ga(III) which was 5 X 10^{-5}M. See text for explanation of values cited.

CONCLUSIONS

2-Acetylpyridine-4-ethyl-3-thiosemicarbazone was shown to be a useful reagent for separating many metal ions chromatographically. The reagent complexed many metal ions under both acidic and neutral conditions, including Ni(II), Co(II), Fe(III), Ga(III), In(III), Zn(II), and Cd(II). Although many metal complexes could be separated chromatographically, these complexes were kinetically unstable and free ligand did have to be added to the eluent to keep the metal complexes from dissociating on the chromatographic column.

There were a variety of chromatographic components available to optimize the separations, such as the concentration of the ion-pairing reagent in the eluent, the pH of the eluent, and the percentage organic modifier in the eluent. All of the metal tested gave nice linear calibration curves over approximately two orders of magnitude and few interferences were noted.

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GENERAL CONCLUSIONS

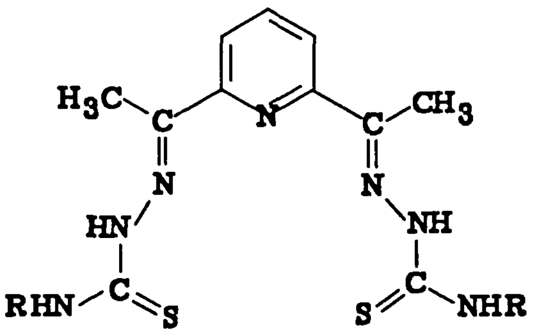
This dissertation has described the synthesis and analytical application of three new chelating reagents for the separation and determination of metal complexes by HPLC. All three reagents were applicable to a direct injection sample preparation, and produced nice chromatographic peaks for the metal complexes under reverse-phase chromatographic conditions. By using hydrophilic reagents metal ions could be determined as their metal complexes without an extraction step during the sample preparation. This provides a quick and easy method for metal ion determination and is superior to determining metal ions by an extraction method.

FUTURE WORK

The future holds many challenges for the analytical chemist. As the 90's progress environmental chemistry will become considerable more significant, from both a public opinion, and hence industrial view. The analytical demands put forward by this surge of environmental awareness will put great pressure on an already shrinking scientific labor force to develop methods that are quick, cheap, and applicable to the small analytical laboratory. The determination of metal ions by chromatographic separation of their metal complexes is one method that does meet these requirements. Its popularity has grown considerable throughout the 80's and will surely continue into the 90's.

The increased use of organic complexing reagents for the determination of metal ions provides for many opportunities to develop new and even more powerful chelating reagents. Although these complexes should be thermodynamically and kinetically stable, they must also form complexes relatively quickly. The reagents also should display a great deal of specificity in terms of which metal ions are complexed. Specificity allows for a large variety of different sample matrixes without any additional methods development. The reagent should also form metal complexes that are applicable to both the sample preparation and to the chromatographic separation. This requirement holds true whether working in the extraction or direct injection mode.

One area that deserves future investigation is in the synthesis of a bis(thiosemicarbazone) similiar to the bis(hydrazones) prepared in this work. All preliminary work conducted by the author has indicated that a bis(thiosemicarbazone), as shown below, would form very stable complexes with a variety of sulfur liking metal ions. It is also very likely that these metal complexes could be separated chromatographically with relative ease. The only obstacle to overcome would be the very limited solubility of the free reagent. All derivatives prepared by the present author, where R = -H, $-CH_2CH_3$, or $-C_5H_6$, have had extremely limited solubilities in both water and many organic solvents. One solution to this solubility problem would be to introduce a very polar functional group, such as, a quaternary ammonium group into the compound by varying the R group. This work would require the synthesis of a new thiosemicarbazide, since only a limited number are presently commercially available.



ACKNOWLEDGEMENTS

This work was performed at Ames Laboratory under contract no. W-7405-eng-82 with the U.S. Department of Energy. The United States government has assigned the DOE Report number IS-T 1466 to this thesis.

The author would also like to thank the following people for their contribution to this work.

First thanks go to Dr. James S. Fritz whose guidance and direction were very much appreciated. You alone are responsible for my ability to perform independent research, and this trait will surely serve me well in the future.

I would also like to thank all of the members of my committee, including Dr. Johnson, Dr. Houk, Dr. Barton, and Dr. Oulman. All contributed to my education in many significant ways, whether it be friend, teacher, role model, etc.

Dr. Cotton and Dr. Tabatabai participation as substitute committee members is also greatly appreciated.

I would also like to thank all group members both past and present for their opinions and comradery. However, two members do deserve special recognition: Jim Lockridge for his advice and enthusiasm of organic chemistry, and Roy Strasburg whose friendship was not only treasured, but many days essential. May life treat you well, Roy.

Special thanks are also extended to my family who made me what I am

today. Your significant contribution to me, and hence this work, can never be denied.

Lastly, I would like to thank my wife, Linda. Your love and unreserved confidence in me made all the difference in the world.