ANALYSIS OF SOME METAL COMPLEXES BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

by

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ABSTRACT

ANALYSIS OF SOME METAL COMPLEXES BY
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High-performance liquid chromatography (HPLC) has been shown to be a rapid and effective method for the separation and quantitative determination of metal complexes. In recent years a great deal of work has been devoted to this area of analysis. The behavior of several metal complexes has been determined with various chromatographic systems. This particular study is primarily concerned with the chromatographic behavior of metal cupferrates although some work was done with metal hydroxyquinoline chelates. The metals investigated as cupferrate complexes include Al(III), Be(II), Co(III), Cu(II), Fe(III), Hg(II), La(II), Ni(II), and Pb(II). The behavior of these metal chelates was determined with normal and reversed-phase chromatographic systems. Three types of HPLC columns were utilized in the study: silica gel, a C_{18} bonded phase packing, and a CN bonded phase packing. Several mobile phases were also employed including pure and combined solvents. The best results were obtained on the CN bonded column with a hexane and chloroform solvent. Separation of the Al(III) and Be(II) cupferrates was possible within eleven minutes. The aluminum cupferrates results were reproducible and appear to be quantitative.

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LIST OF ABBREVIATIONS AND SYMBOLS

ABBREVIATION OR SYMBOL	DEFINITION
HPLC	High-performance liquid chromatography
LC	Liquid chromatography
LSC	Liquid-solid chromatography (adsorption chromatography)
LLC	Liquid-liquid chromatography (partition chromatography
Ox	Abbreviation for 8-hydroxyquinoline (oxine)
CupF	Abbreviation for Cupferron
μ	Abbreviation for micro
Conc.	Abbreviation for concentration
w.r.t.	Abbreviation for"with respect to"
β	Beta
t_r	Retention time of a given band measured from point of injection to center of peak (minutes)
t_o	The t_r value for mobile phase molecules injected as sample also t_r for unretained sample components (minutes)
k'	Partition constant or solute capacity factor; equal to total amount of solute in stationary phase divided by total amount of solute in mobile phase within column at equilibrium.
p	Partition coefficient, ratio of the solute distribution in the two phases during a liquid-liquid extraction.
N	Theoretical plate number, used as an indication of column efficiency.

ABBREVIATION OR SYMBOL	DEFINITION
UV	Ultraviolet
cm	Centimeter, 1×10^{-2} meter
mm	Millimeter, 1×10^{-3} meter
µm	Micron, 1×10^{-6} meter
nm	Nanometer, 1×10^{-9} meter
mL	Milliter, 1×10^{-3} liter
µL	Microliter, 1×10^{-6} liter
g	Grams

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CHAPTER I

INTRODUCTION

Modern liquid chromatography is an analytical procedure which possesses the capability for the high resolution separation of a wide range of sample types within times of a few minutes to perhaps an hour.[1-6] Since its relatively recent introduction in 1969, high-performance liquid chromatography has developed into a rapidly expanding field.[1,2] Several factors contributed to its popularity. The system combined versatility with convenience and permitted non-volatile and thermally unstable samples to be analyzed with the same precision and reliability demonstrated by gas chromatography.[2-4] However, the majority of chromatographic studies are concerned with the separation of organic and biochemical compounds and relatively few reports have been published dealing with the analysis of inorganic materials.

Purpose of Study

The purpose of this study was to develop a high-performance liquid chromatography system capable of metal analysis. This system would provide a rapid, reproducible and (possibly) quantitative separation of the various metals. The procedure investigated involves reacting the metals with an organic reagent to produce metal complexes which could then be chromatographically resolved. Cupferron (Ammonium salt of N-Nitosophenylhydroxylamine) and 8-hydroxyquinoline were the complexing agents investigated. However, due to difficulties encountered with the chromatographic behavior of 8-hydroxyquinolates,

the major portion of this presentation is concerned with the HPLC study of metal cupferrates.

Chromatography Methods

In the chromatographic process four separate mechanisms exist which describe the retention of sample molecules.[1,4] These mechanisms: partition, adsorption, ion exchange, and exclusion, give rise to the four chromatography methods: liquid-liquid, liquid-solid, ion exchange, and exclusion (gel). Liquid-liquid chromatography was the method utilized for the majority of work in this study, although the liquid-solid technique was briefly used in the analysis of cupferrates.

LLC

Liquid-liquid chromatography was developed in 1941 by Martin and Synge to separate acetylated amino acids.[1,3] This basic technique has been adapted to modern liquid chromatography. The procedure is essentially the same, however, improvements in packing and apparatus have permitted a major increase in speed, resolution and reproducibility.[5,6]

The partition chromatography system consists of a finely divided solid, called the support, on which a solvent (the stationary phase) is coated or chemically bonded such that it will not migrate.[2] A second liquid phase (the mobile phase) immiscible with the first flows over the support-partitioner combination allowing the two phases to come in contact over a large interface.[1,2] The sample then participates in a partition between the stationary phase where it is held in a fixed position and the mobile phase where migration occurs.

The solutes are presumed not adsorbed by the support.

The relative extent of partitioning into one phase or the other depends upon the molecular forces existing in the two phases.[2,4] For simplicity, the majority of these forces can be described in terms of polarity. The polarity consideration led to the development of two liquid-liquid chromatographic procedures, normal and reversed phase, which are based on the polarity of solvents and stationary phases.

Normal partition chromatography is comprised of polar stationary phases and non-polar solvents.[6] This system is best suited to the analysis of polar samples. Reversed-phase LLC, however, is the exact opposite, utilizing non-polar stationary materials with polar solvents. Reversed-phase allows the analysis of non-polar samples.[6] The characteristics of normal and reversed phase are illustrated by Table 1.[14]

TABLE 1

Normal vs. Reversed Phase

	Normal Phase	Reversed Phase
Packing Polarity	High	Low
Solvent Polarity	Low to Medium	Medium to High
Sample Elution Order	Least Polar First	Most Polar First
Effect of Increasing Solvent Polarity	Reduces Elution Time	Increases Elution Time

The large variety of partitioning phases which are available provide LLC with great versatility. Unique chemical interactions can allow separation which are difficult by other methods.[5] Liquid-liquid

chromatography generally separates on the basis of the type and sometimes the number of substituent groups and by differences in molecular weight.[1,2] Therefore, this technique allows the resolution of homologs and mixtures of compounds with different functional groups.[6] There is no universal partition system for all solutes, however, there is almost an infinite capability for separation by selecting an appropriate pair of partitioning liquids.[2]

LSC

The oldest method of chromatography is liquid-solid or adsorption, developed by Tsvet in 1903.[1] This technique was adapted to modern LC in the late 1960's. The samples usually analyzed by adsorption chromatography are organic-soluble, of intermediate molecular weight, and non-ionic.[4-6] The stationary phase or packing in liquid-solid chromatography is polar, while the mobile phase can be of various polarities. Solutes containing polar groups are strongly attracted to the adsorbent, while non-polar hydrocarbon groups are weakly attracted. The intermolecular forces which are normally responsible for adsorption are dispersion (London) forces, dipole (orentation, induction) forces, hydrogen bonds and weak covalent bonding.[2] Liquid-solid chromatography is not noted for its ability to separate homologs or other mixtures.[6]

Chromatographic Stationary Phases

Silica is the most common support in partition chromatography and adsorbent in liquid-solid chromatography.[2,4,6] Silica is available as either irregular porous particles, as porous spheres or as solid

spheres coated with thin layers (~1 μm) of porous material (porous-layer beads).[7-10]

Silica particles are composed of a three dimensional array of SiO_4 tetrahedra.[11] At the surface of the particle where the lattice is abruptly terminated, the surface silicon atoms can exist in three forms diagrammed in Figure 1.[2] Silica characteristics depend on the hydroxyl groups attached to the surface.

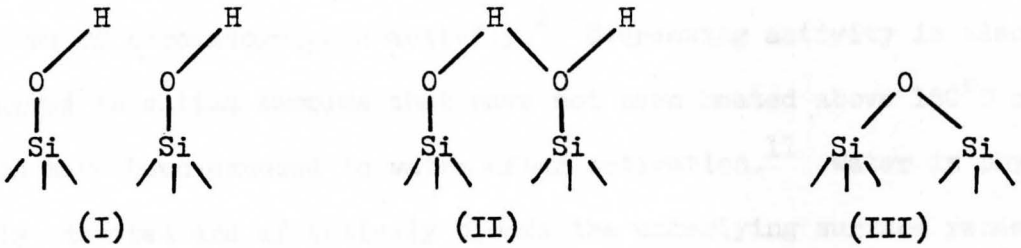


Figure 1. Structures possible for surface silicon atoms in silica particles.

The hydroxyls may exist in the free state (I) where no hydrogen bonding occurs with adjacent surface groups. This is the most active silica form. The hydroxyls may also exist as hydrogen bonded groups or clusters (II). Surface area and relative concentrations of different hydroxyl groups (I or II) vary among different silica, leading to corresponding differences in chromatographic properties.[2,11] The best porous silica for adsorption chromatography have surface areas of about 400 m^2/g and surface hydroxyls largely of type (I).[2] Heating silica above 200°C leads to loss of water (Figure 2) and conversion of surface hydroxyls into siloxane groups (III).[11]

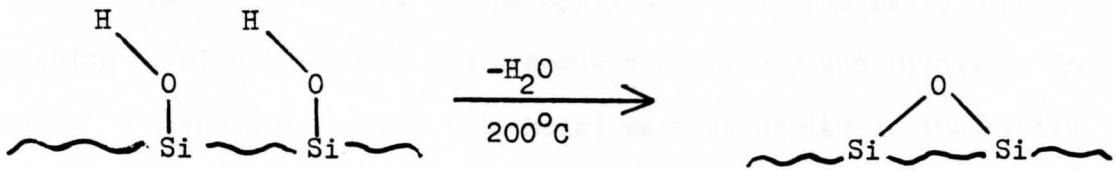


Figure 2. Dehydration of silica surface.

Siloxane groups are non-polar and do not contribute to the preferential retention of polar compounds on silica, therefore leading to loss of chromatographic activity.[2] Decreasing activity is also observed in silica samples that have not been heated above 150°C or which have been exposed to water after activation.[11] Water is physically adsorbed and effectively blocks the underlying surface rendering the silica packing less active or deactivated.[11]

In partition chromatography the silica particles are surrounded by an organic liquid. This organic material acts as the functional or active group of the column. The encasement of silica particles is accomplished by coating or chemical bonding.

Coating silica is usually performed by a technique called "in situ coating" where the stationary material is added to the mobile phase and pumped through a chromatography column prepacked with the silica support.[2] The stationary phase is adsorbed by the support and distributed throughout the column. The disadvantages to this type of mechanically held liquid phase are non-homogenous coating and stripping by the moving liquid. Therefore, the mobile phase must be saturated with the immiscible stationary phase, severely limiting the use of solvents.[1,2]

The first example of the application of chemically bonded packing materials to HPLC was described by Halasz and Sebestian in 1969.[11] Organic materials (alcohols) were esterfied to the silica surface by reacting silica chloride with an appropriate alcohol.[11] The bonded groups in such supports are oriented on the surface like the bristles of a brush, this type of packing has become known as "Halasz brushes". However, the alcohol groups can be easily hydrolyzed which is a major disadvantage.[11] The use of Halasz brushes and coated packing materials has largely been superseded by the more robust permanently bonded packing.[7-12]

The development of packing materials which have organic groups permanently bonded to the surface of the support is perhaps the most important breakthrough in partition chromatography.[11] This type of stationary phase cannot be extracted by the moving solvent and in most cases is thermally and hydrolytically stable.[7-12] More important, the use of an immiscible liquid phase is unnecessary. The mobile phase may be as closely similar or widely dissimilar to the stationary phase in terms of polarity, a condition which cannot be met with coated packing. This allows greater versatility in the separation of solute components.

There are several routes whereby organic materials may be bonded to the silica surface. It is possible to form a silicate ester ($\geq$Si-OR) by the reaction of surface silanol groups with alcohols.[11]

$$\geq\text{Si-OH} + \text{ROH} \longrightarrow \geq\text{Si-OR} \qquad (1)$$

This type of bond is hydrolytically unstable and reactions of this type are mainly used to prepare polar packings for normal partition

chromatography. The formation of an organic coating on the silica of the type $\geq Si-O-SiR_3$ can occur by reacting the surface silanol groups with an organochlorosilane.[11]

$$\geq Si-OH + R_3Si-Cl \longrightarrow \geq Si-O-SiR_3 \qquad (2)$$

The surface silanol groups can also be converted to chlorides using thionyl chloride followed by treatment with an organolithium (or Grignard) reagent to yield a product with the organic group directly bonded to the surface silicon atoms.[11]

$$\geq Si-OH + SOCl_2 \longrightarrow \geq Si-Cl$$
$$\geq Si-Cl + RLi \longrightarrow \geq Si-R \qquad (3)$$

The most popular partition phase of this type is the C_{18} surface bonded packing for reversed-phase.[10] The packing materials produced by the reactions illustrated in equations 2 and 3 are very stable and used mainly for reversed-phase partition chromatography.[7-12] The packings are resistant to aqueous solvents in the pH range of 3-10 and chemical modification by the majority of organic solvents.[11]

Mobile Phases and Solvent Effects

There are several basic solvent characteristics which dictate the use of a particular moving phase to achieve solute separation.[13-18] The major considerations are solvent strength, selectivity, compatibility, and viscosity.[1] Solvent strength refers to the way a solvent affects the migration rate of the sample. Solvent strength characterizes solvents on the basis of polarity and dielectric constant.[19] In liquid-liquid chromatography this term is not well defined. The strength of a given solvent depends entirely on the chromatography

system. Weak solvents produce large k' values (partition constant) whereas strong solvents give lower k' values.[1,2]

Solvent selectivity generally is the measure of the ability of a particular solvent to retain a solute. It is commonly associated with the presence or absence of hydrogen bonding between the sample and solvent.[1]

Compatibility is the most important consideration when evaluating a solvent. The mobile phase must be compatible with the entire HPLC system. Pump seals, valve packing and injector septums must be free from chemical attack. Decomposition of the sample and modification of the stationary phase must also be avoided.[2] When a UV detector is used the solvent should possess a relatively low UV cut-off.

Viscosity is also an important solvent property since eluents with lower viscosity minimize separation time and permit a rapid mass transfer.[2] Some of the minor considerations in solvent selection are toxicity, purity, and stability.[18]

Elution

There are two forms of elution utilized in liquid chromatography, isocratic and gradient.[1,2,6] Elution with a constant flow rate, constant k' of the sample and identical mobile phase composition throughout is called normal or isocratic elution.[4] This method has the advantages of simplicity, convenience, compatibility with all detectors and higher resolution.[5,6] However, this technique is limited to samples with k' values between 0.5 and 15.[1]

Gradient elution is the continuous or stepwise change in the mobile phase composition during a separation. In general, the solvent

strength is increased as the separation proceeds so that the k' value for each component is in the optimum range at 2-5 during most of the migration through the column.[2,5,6] Gradient elution has many advantages, it improves the detection of peaks with long retentions, covers a broad range of k' values, and can optimize resolution and separation time.[1] However, the complexity of equipment is increased and column regeneration is necessary after each sample elution. Isocratic elution was employed throughout this study of metal complexes.

CHAPTER II

RELATED STUDIES

The analysis of metal complexes by high-performance liquid chromatography has received much attention since Huber, Kraak, and Veening first documented the separation of metal β-diketonates.[22] Since this report in 1972, the behavior of several different metal complexes has been determined with various chromatography systems. A relatively complete listing of complexing agents and metals studied is presented in Table 2. The following is a brief summary of the conditions of analysis for these metal compleses.

Dialkyldithiocarbamates

The diethyldithiocarbamates were studied by Heizmann and Ballschmiter on a silica column (LiChrosorb SI 60, 30 μm particle diameter) with benzene as the mobile phase.[21,23] Detection of the complexes was by UV at 330 nm. Separation was possible for pairs of the metal chelates by using columns of various lengths and different solvent flow rates. The Cu(II) and Hg(II), Cu(II) and Ni(II), Cu(II) and Co(II), and Ni(II) and Co(II) chelates were resolved.[23] The Zn(II), Cd(II), and Pb(II) chelates were also determined; however, considerable peak tailing prevented separation.

O'Laughlin and O'Brien also utilized silica (Corasil, 37-50 μm particle diameter) as the stationary phase to resolve the diethyldithiocarbamates.[24] Toluene was used as the eluent and the UV detection

wavelength was 270 nm. Separations were demonstrated for Ni(II) and Co(III), and Hg(II) and Pb(II). The Cu(II), Cd(II) and Zn(II) complexes were also determined, but similar retention times on the HPLC system chosen prevented simultaneous separation of all the metal chelates.[24]

A deactivated silica gel column (LiChrosorb SI 100, 10 μm particle diameter) was employed by Moriyasu and Hashimoto to resolve the metal diethyldithiocarbamates.[25] The detection was by UV at 254 nm. With a solvent system composed of water-saturated n-hexane and ethylacetate it was possible to separate Hg(II), Cu(II), Cd(II), Pb(II), Ni(II), and Bi(III) (listed in order of elution).[25] Complete peak resolution was not obtained with the system above so a second system was developed with improved results. Using the same column with a mobile phase consisting of water-saturated cyclohexane and ethylacetate an improved separation of Hg(II), Cu(II), Pb(II), Cr(III), Ni(II), Bi(III) and Co(III) was demonstrated.[25] Some other metal chelates were also tested on both systems: Fe(III), Sb(III), Zn(II), Ag(I), and Sn(III). These were not eluted from the column by the relatively nonpolar solvent systems. Moriyasu and Hashimoto also found that the retention times of the peaks increased slightly with decreasing amounts of sample.[25] This may cause irreproducible partition of the chelates on the silica column.

A reversed-phase chromatographic system was developed by Schwedt in the analysis of diethyldithiocarbamates.[26] A bonded column (LiChrosorb RP 8, 10 μm particle diameter) was used with a methanol and water mobile phase. The resolution of Se(II), Ni(II), Co(II), Cu(II), and Hg(II) chelates was possible. The Pb(II) and Cr(III)

complexes were also tested; however, Pb(II) was inseparable from Cu(II) and Cr(III) developed as two peaks, one of which interferes with Se(II).[26] The detection wavelength in the UV was 254 nm.

The benzylmethyldithiocarbamates were determined by Heizmann et. al. on a silica gel column (LiChrosorb SI 60, 30 μm particle diameter).[23] Resolution was demonstrated for the Cu(II), Ni(II), Cd(II), and Co(III) complexes using a benzene and cyclohexane mobile phase. The Zn(II) chelate was also tested, but because of peak tailing and a retention time similar to Cu(II), separation was not feasible. The UV detection wavelength for this study was 360 nm.

Heizmann et. al. resolved the diethoxyethyldithiocarbamates with a silica gel column (LiChrosorb SI 60, 40 μm particle diameter).[23] Carbon tetrachloride and acetonitrile composed the binary mobile phase. The detection wavelength in the UV was 360 nm. Separation of Cu(II), Co(III), and Ni(II) was attempted, but Cu(II) displays a large band which interferes with the other metals.[23] Several other metal diethoxyethyldithiocarbamates were also tested including Sb(III), Bi(III), Pb(II), Zn(II), and Cd(II). These complexes could not be separated due to excessive retention and large tailing effects.[23]

Diketobis(4-substituted)thiosemicarbazones

The chelates of glyoxalbis(2,2,3,3-tetramethylbutyl)thiosemicarbazone were resolved on an alumina column (Alox T, 30 μm particle diameter) using a benzene mobile phase.[23] The UV detection wavelength was 360 nm. The resolution of Hg(II), Cu(II), and Ni(II) was demonstrated. The Pb(II) chelate decomposed via a catalytic reaction on the stationary phase. Some of the other metals tested include Cd(II),

Zn(II), and Co(III). These complexes were strongly retained on the alumina column and failed to elute.

Heizmann and Ballschmiter investigated the diacetylbis(cyclohexyl)thiosemicarbazones using two different HPLC systems.[23] It was possible to resolve only two of the metals tested. The detection wavelength was 360 nm. In the first system Hg(II) and Cu(II) chelates were separated on a silica column (LiChrosorb SI 60, 40 μm particle diameter) with benzene and tetrahydrofuran as the eluent. The second system allowed a much improved separation of Hg(II) and Cu(II). In this system an alumina column (Alox T, 30 μm particle diameter) was used with a benzene mobile phase. Some other metal diacetylbis(cyclohexyl)thiosemicarbazones were also tested including Zn(II), Ni(II), Co(III), and Cd(II). These chelates all demonstrated a strong affinity for both stationary phases and could not be eluted from either system.

Diketobisthiobenzhydrazone

A gradient elution chromatography system was necessary to separate the metal chelates of 1,2-diacetylbisthiobenzhydrazone.[23] The Hg(II), Ni(II), Cu(II), and Pb(II) complexes were resolved on a silica column (LiChrosorb SI 60, 30 μm particle diameter). The UV detection wavelength for this study was 360 nm. A linear gradient program was used, the initial mobile phase consisting of n-heptane and benzene was slowly changed to 100% benzene.[23] This type of elution mode was required to achieve baseline separation of Hg(II), Ni(II), and Cu(II) and to allow elution of Pb(II) which is strongly retained.

Diphenylthiocarbazane

The behavior of metal dithizonates was investigated on a silica column (LiChrosorb SI 60, 30 μm particle diameter) by Lohmuller, Heizmann and Ballschmiter.[27] The solvent used was benzene and detection was in the visible range at 525 nm. It was possible to separate groups of the metal complexes by utilizing columns of various lengths and different solvent flow rates. The separations demonstrated include Hg(II), Ni(II), Co(II), and Pb(II); Hg(II), Cu(II), and Co(II); and Hg(II), Zn(II), and Pb(II). The Cd(II) complex was also determined, but has an affinity for the stationary phase and cound only be eluted with a polar solvent.[27] The simultaneous separation of all the metal chelates listed was impractical due to intense peak tailing by some of the metals.

The dithizone chelates were also studied by O'Laughlin et. al. on silica (Porasil, 10 μm particle diameter).[24] Toluene was the mobile phase and the UV detection wavelength was 270 nm. The separation of Hg(II) and Co(III) was demonstrated. The other metal dithizonates studied include Ni(II), Cu(II), Zn(II), and Pb(II). Separation of these complexes was not possible with the described HPLC system.[24]

β-Diketonates

Tollinche and Risby studied the HPLC of several β-diketonates on a silica column (HIEFF MicroPart, 5 μm particle diameter).[28] Their study included 2,4-pentanedione, 2,2,7,7-tetramethyl-3,5-heptanedione, 1,1,1-trifluoro-2,4-pentanedione, and 1,1,1,2,2,3,3-heptafluoro-4,6-octanedione.

The analysis of 2,4-pentanedione was carried out using an ethylene chloride and methanol mobile phase. It was possible to separate Be(II), Ru(III), Rh(III), Cr(III), Al(III), and Co(III).[28] A complete resolution of these metal chelates was not attained and partial peak overlap did occur. The UV detection wavelength was 280 nm.

Saitoh and Suzuki also investigated the chelates of 2,4-pentanedione.[29] A silica column (Merkogel OR-2000, 40 μm particle diameter) was employed with tetrahydrofuran as the eluent.[29] The resolution of Al(III) and Be(II) complexes was demonstrated at a detection wavelength of 310 nm. The Co(III), Fe(III), Cr(III), Cu(II), and Ni(II) complexes were also tested; however, extreme peak tailing and similar retention volumes did not allow further separations.

Huber et. al. studied the 2,4-pentanedione chelates using a column packed with diatomaceous earth (particle size 10-20 μm).[22] A ternary solvent system was utilized, composed of water, 2,2,4-trimethylpentane and ethanol. The UV detection wavelength was 310 nm. The 2,4-pentanedionate separations were presented in two groups: Be(II), Cu(II), Al(III), Cr(III), Ru(III), and Co(III); and Be(II), Fe(III), Cr(III) and Co(III). The Fe(III) complex could not be resolved from Cu(II) and Al(III) because of similar retention volumes and band tailing.

The 1,1,1-trifluoro-2,4-pentanedione complexes were studied on a silica column (HIEFF MicroPart, 5 μm particle diameter) with an eluent of n-heptane and methylene chloride.[28] It was possible to separate Cr(III) and Co(III), and Rh(III) and Co(III) complexes. The Al(III) chelate was also tested, but could not be resolved from the other metal complexes. The UV detection wavelength for this

study was 310 nm.

The 1,1,1-trifluoro-2,4-pentanedione complexes were investigated by Huber et. al. using a diatomaceous earth column (particle size 10-20 μm).[22] They were able to resolve the chelates of Be(II), Cu(II), Al(III), Cr(III), Ru(III), and Co(III) with a ternary mobile phase composed of water, 2,2,4-trimethylpentane and ethanol. The detection wavelength was 310 nm.

Uden, Bigley and Walters were able to resolve the Co(III) and Cr(III) chelates of 1,1,1-trifluoro-2,4-pentanedione.[30] Silica (Partisil, 10 μm particle diameter) was the stationary phase used with a detection wavelength of 254 nm. A methylene chloride and hexane eluent was used to achieve complete separation of the Co(III) and Cr(III) complexes.

The Co(III) and Cr(III) chelates of 2,2-dimethyl-3,5-hexanedione were also investigated by Uden et. al.[30] A silica gel column (Partisil, 10 μm particle diameter) was used with an acetonitrile and methylene chloride solvent. The UV detection wavelength was 254 nm. The Co(III) and Cr(III) complexes were resolved.

The 2,2,7,7-tetramethyl-3,5-heptanedione chelates were studied on a silica column (Partisil, 10 μm particle diameter) using a benzene mobile phase.[28] The detection wavelength was 310 nm and complete separation of Cr(III), Co(III), Fe(III), Ni(II), and Cu(II) complexes was demonstrated.

The metal complexes of 1,1,1,2,2,3,3-heptafluoro-4,6-octanedione were investigated by Tollinche et. al.[28] A silica stationary phase (Partisil, 10 μm particle diameter) was used with a methylene chloride and n-heptane eluent. Complete separation of

Co(III) and Cr(III) was demonstrated. Some additional metal chelates tested include Fe(III), Ni(II), Cu(II), and Rh(III). These failed to elute from the silica column.

Uden et. al. resolved the Co(III) and Cr(III) chelates of benzoylacetylacetone.[30] A silica stationary phase (Partisil, 10 μm particle diameter) was employed with a methylene chloride and acetonitrile solvent. The detection wavelength was 310 nm.

β-Ketoamines

The HPLC behavior of metal β-ketoamines has been extensively studied; however, it has been possible to resolve only a few metals. The study of N,N'-ethylenebis(acetylacetoneimine) was undertaken by Gaetani et. al.[31] A silica column (MicroPak CH, octadecylsilane on silica gel 10 μm particle diameter) was used with a mobile phase of methanol and phosphate buffer.[31] The separation of Co(III), Ni(II), and Cu(II) complexes was demonstrated. The Pd(II) complex was also determined, but could not be separated from the other complexes. The UV detection wavelength was 254 nm.

Uden, Parees and Walters used a reversed-phase chromatography system to separate the metal chelates of N,N'-ethylenebis(acetylacetoneimine).[32] The UV detection wavelength was 254 nm. A bonded silica column (Microbondapak C 18, 10 μm particle diameter) was used with a methanol, water and acetonitrile mobile phase. The Ni(II), Pb(II), and Cu(II) chelates were resolved.

The Ni(II) and Cu(II) chelates of N,N'-ethylenebis(acetylacetoneimine) were completely separated by Uden and Walters.[33] A silica column (Partisil, 10 μm particle diameter) was used with a

methylene chloride and acetonitrile solvent. The detection wavelength used in the study was 254 nm.

Gaetani et. al. characterized the N,N'-trimethylenebis(acetylacetoneimine) chelates using a silica column (MicroPak CH, octadecylsilane on silica gel, 10 μm particle diameter).[31] A 254 nm detection wavelength was employed. The Co(III), Ni(II), and Cu(II) chelates were separated using a methanol and phosphate buffer eluent.

Uden et. al. analyzed the majority of metal β-ketoamines using reversed-phase partition chromatography.[32] The study included N,N'-propylenebis(acetylacetoneimine), N,N'-butylenebis(acetylacetoneimine), N,N'-ethylenebis(trifluoroacetylacetoneimine), N,N'-propylenebis(trifluoroacetylacetoneimine), and N,N'-butylenebis(trifluoroacetylacetoneimine). A bonded silica column (Microbondapak C 18, 10 μm particle diameter) was used with an acetonitrile and water mobile phase. For each of the chelating agents, separation of the Ni(II), Pd(II), and Cu(II) complexes was demonstrated. The detection wavelength for the entire study was 254 nm.

The N,N'-ethylenebis(benzoylacetoneimine) chelates were investigated on a silica column (MicroPak CH, octadecylsilane on silica gel, 10 μm particle diameter) with a mobile phase of methanol and phosphate buffer.[31] A UV detection wavelength of 254 nm was used. The Cu(II) and Ni(II) complexes were resolved.

Tollinche et. al. studied the N,N'-ethylenebis(1,1,1-trifluoro-2,4-pentanedione) and N,N'-ethylenebis(1,1,1-trifluoro-2,4-pentanedioneimine) chelating agents.[28] The same HPLC was utilized in the analysis of both reagents, consisting of a silica column (Partisil, 10 μm particle diameter) and a mobile phase of methylene chloride and

acetonitrile. Similar chromatographic behavior was reported for metal complexes of both reagents. The Cu(II) and Ni(II) chelates were completely separated. The UV detection wavelength for the study was 254 nm.

Salicylaldimine

The N,N'-ethylenebis(salicylaldimine) chelates were investigated by Uden et. al. using reversed-phase partition chromatography.[32] The detection wavelength was 254 nm. The bonded silica column (Microbondapak C 18, 10 μm particle diameter) was used with an acetonitrile and water eluent. It was possible to resolve the Cu(II), Ni(II), and Pd(II) complexes.

The N,N'-ethylenebis(salicylaldimine) chelates were also studied on a normal partition chromatography system.[33] A silica stationary phase (Partisil, 10 μm particle diameter) was used with a methylene chloride and acetonitrile mobile phase. The Ni(II) and Cu(II) chelates were resolved. The detection wavelength for the study was 254 nm.

TABLE 2

The Metals and Their Respective Complexing Agents
Analyzed by High-Performance Liquid Chromatography

Complexing Agents	Metals
Dialkyldithiocarbamates	
diethyldithiocarbamate	Bi(III), Cd(II), Co(II), Co(III), Cr(III), Cu(II), Hg(II), Pb(II), Se(II), Zn(II)
benzylmethyldithiocarbamate	Cd(II), Co(III), Cu(II), Ni(II), Zn(II)
diethoxyethyldithiocarbamate	As(III), Co(III), Cu(II), Hg(II), Ni(II)
Diketobis(4-substituted)thiosemicarbazones	
glyoxalbis(2,2,3,3-tetramethylbutyl)thiosemicarbazone	Cu(II), Hg(II), Ni(II)
diacetylbis(cyclohexyl)thiosemicarbazone	Cu(II), Hg(II)

TABLE 2 cont.

Complexing Agents	Metals
Diketobisthiobenzhydrazone	
1,2-diacetylbisthiobenzhydrazone	Cu(II), Hg(II), Ni(II), Pb(II)
Diphenylthiocarbazane	
diphenylthiocarbazane (Dithiozone)	Cd(II), Co(II), Co(III), Cu(II), Hg(II), Ni(II), Pb(II), Zn(II)
β-Diketonates	
2,4-pentanedione	Al(III), Be(II), Co(III), Cr(III), Cu(II), Fe(III), Rh(III), Ru(III)
1,1,1-trifluoro-2,4-pentanedione	Al(III), Be(II), Co(III), Cr(III), Cu(II), Ru(III)
2,2-dimethyl-3,5-hexanedione	Co(III), Cr(III)
2,2,7,7-tetramethyl-3,5-heptanedione	Co(III), Cr(III), Cu(II), Fe(III), Ni(II)

TABLE 2 cont.

Complexing Agents	Metals
1,1,1,2,2,3,3-heptafluoro-4,6-octanedione	Co(III), Cr(III)
benzoylacetylacetone	Co(III), Cr(III)
β-Ketoamines	
N,N'-ethylenebis(acetylacetoneimine)	Co(III), Cu(II), Ni(II), Pd(II)
N,N'-trimethylenebislacetylacetoneimine)	Co(III), Cu(II), Ni(II)
N,N'-propylenebis(acetylacetoneimine)	Co(III), Cu(II), Pd(II)
N,N'-butylenebis(acetylacetoneimine)	Co(III), Cu(II), Pd(II)
N,N'-ethylenebis(trifluoroacetylacetoneimine)	Co(III), Cu(II), Pd(II)
N,N'-propylenebis(trifluoroacetylacetoneimine)	Co(III), Cu(II), Pd(II)
N,N'-butylenebis(trifluoroacetylacetoneimine)	Co(III), Cu(II), Pd(II)
N,N'-ethylenebis(benzoylacetoneimine)	Cu(II), Ni(II)
N,N'-propylenebis(1,1,1-trifluoro-2,4-pentanedione)	Cu(II), Ni(II)

TABLE 2 cont.

Complexing Agents	Metals
N,N'-ethylenebis(1,1,1-trifluoro-2,4-pentanedioneimine)	Cu(II), Ni(II)
Salicylaldimine	
N,N'-ethylenebis(salicylaldimine)	Cu(II), Ni(II), Pd(II)

CHAPTER III

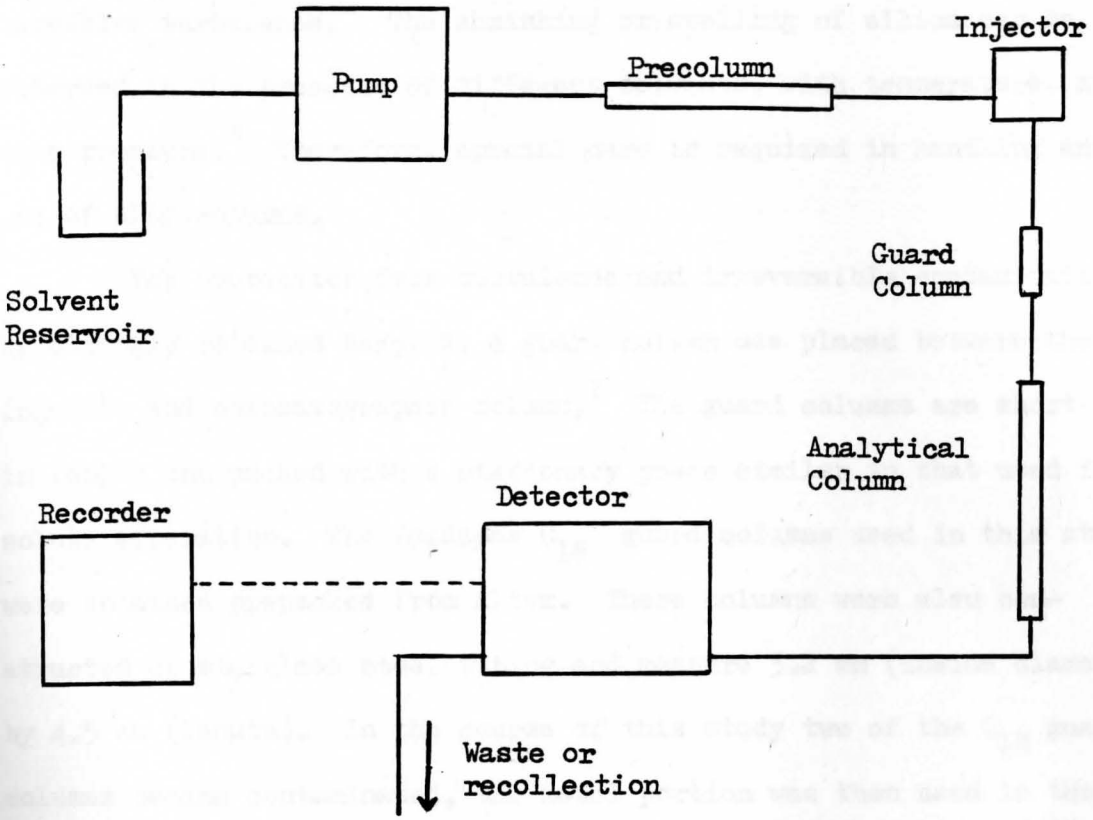
MATERIALS AND APPARATUS

Reagents

The materials used in this study were of reagent grade or better. Deionized water was used for all aqueous solutions. The solvents used for HPLC work are listed with some of their chromatographic characteristics in Table 4. The composition of mobile phases was based on these characteristics. Solvents were filtered to remove any particulate matter using Millipore (ultraporous) filters before use in the chromatography system.

Equipment

The high-performance liquid chromatograph used for this study was of multicomponent design. The individual components were obtained from various instrument manufacturing companies. A simplified schematic diagram is depicted in Figure 3. The analytical columns and their properties are described separately in Table 5. These columns were purchased prepacked in order to insure maximum efficiency and reproducibility. The columns are constructed of stainless steel tubing of various lengths with diameters of 2-5 mm. The stationary phase is mechanically packed dry or as a slurry. Maximum efficiency is obtained by aligning the highly porous silica particles in the column in something less than maximum density.[8,12] This delicate particle alignment can be disrupted by any physical or thermal shock.[8] Shifting of



Pump: High pressure capacity chromatography pump (Model 6000 A)
Waters Associates

Injector: Interrupted flow high pressure injector
Waters Associates

Detector: Variable wavelength spectrophotometric detector (Model 155)
Beckman (Altex)

Recorder: Duel pen potentiometric strip chart recorder with electronic integrator (Model DSRG-2)
Sargent-Welch

(Columns listed in Table 5)

Figure 3. Simplified schematic representation of high-performance liquid chromatograph, (and components used).

the particle bed can occur at high solvent flow rates or by generating excessive turbulence.[8] The shrinking or swelling of silica can be observed in the presence of different solvents, with temperature, and with pressure.[8] Therefore, special care is required in handling and use of HPLC columns.

For protection from turbulence and irreversible contamination by strongly retained samples, a guard column was placed between the injector and chromatographic column.[1] The guard columns are short in length and packed with a stationary phase similar to that used for solute separation. The Bondapak C_{18} guard columns used in this study were obtained prepacked from Altex. These columns were also constructed of stainless steel tubing and measure 3.2 mm (inside diameter) by 4.5 cm (length). In the course of this study two of the C_{18} guard columns became contaminated, the metal portion was then used in the preparation of guards for the other analytical columns. The C_{18} packing was removed and the metal tubing purged with acetone to remove any particulate matter. One of these columns was dry packed with Corasil using the tap-fill method to act as a guard for the μ-Porasil column. The other column was dry packed with Bondapak Phenyl to act as a guard for the μ-Bondapak CN column. The Phenyl stationary phase is similar to the CN packing in chromatographic behavior. Both the Corasil and Phenyl packings were obtained from Waters Associates.

A pre-column was also used in conjuction with the guard and analytical columns. This column measuring 4.6 mm x 25 cm was dry packed with silica (37-53 μm particle size) using the tap-fill method.[34] The pre-column is placed between the pump and injector so its contribution does not interfere with sample separation. This column

was provided so that potentially destructive solvents are tamed before they flow into the analytical column, this effect was described by Whatman Inc.[34]

> It has been found that mobile phases - aqueous and organic/aqueous - that contain buffer salts or bases will dissolve silica and the silica backbones of bonded phase microparticle media. The dissolving rate is, in fact, quite rapid - dissolving up to 220 µg/mL in a mobile phase of CH_3CN/H_2O (40:60), pH 10.7, NH_4OH at 1mL/min at 65°C.[34]

The analytical columns were operated in different modes during the analysis of cupferron chelates. The µ-Bondapak CN column is of intermediate polarity and efficient in both normal and reversed-phase partition chromatography systems by changing the composition of the mobile phase. The µ-Partisil column was also operated in different modes. By using non-polar organic solvents, the column was used for an adsorption chromatography system. When the non-polar solvents were saturated with water the µ-Partisil column was deactivated and reacted as a highly polar column for partition chromatography. Methanol was also used to deactivate the µ-Partisil column by bonding to the silanol groups forming a surface area composed of $\geq Si-O-CH_3$.

Column Efficiency

Column efficiency was also an important equipment operating parameter considered in this study. Efficiency of the analytical column is imperative for accurate results. Efficiency is based on the concept of theoretical plates, N, existing in the analytical column.[8] This value is used to relate the separation capacity of a particular column. The calculation of N (equation 4) is demonstrated in figure 4.

$$N = 16(V_r/W)^2 \qquad (4)$$

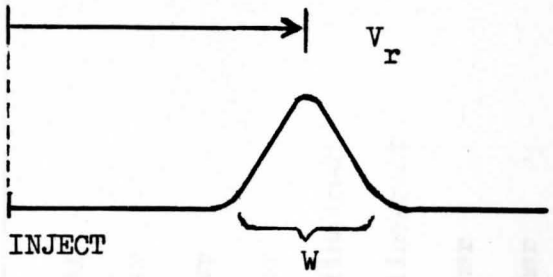


Figure 4. Calculation of N from retained peak, V_r = volume of solvent needed to elute peak center and W = width at base of peak, also measured in volume.

The plate count was determined before and after use for each of the columns used in this study. For all cases this value remained constant. It should be noted that this value is slightly higher than the manufacturers theoretical value because guard columns were in place for this measurement.

The column efficiency can also be monitored by the partition constant, k', (equation 5). To calculate this value it is necessary to measure the elution times of an unretained peak and a retained peak.

$$k' = (t_r - t_o)/ t_o \qquad (5)$$

TABLE 3

Reagents Used

Material	Formula	Grade of Reagent	Manufacturer
Aluminum Chloride	$AlCl_3 \cdot 6H_2O$	Reagent Grade	Baker
Beryllium Sulfate	$BeSO_4 \cdot 4H_2O$	Reagent Grade	Baker
Cobalt Chloride	$CoCl_3 \cdot 6H_2O$	Reagent Grade	Baker
Copper Chloride	$CuCl_2 \cdot 2H_2O$	Reagent Grade	Baker
Iron Chloride	$FeCl_3 \cdot 6H_2O$	Analytical	Mallinckrodt
Mercury Chloride	$HgCl_2$	Analytical	Mallinckrodt
Lanthanum Nitrate	$La(NO_3)_3 \cdot 6H_2O$	Reagent Grade	Fisher
Nickel Chloride	$NiCl_2 \cdot 6H_2O$	Reagent Grade	Fisher
Lead Chloride	$PbCl_2$	Reagent Grade	Fisher
Sodium Sulfate (anhydrous)	Na_2SO_4	Reagent Grade	Baker
8-Hydroxyquinoline	C_9H_7NO	Reagent Grade	Baker

TABLE 3 cont.

Material	Formula	Grade of Reagent	Manufacturer
Cupferron	$C_6H_9N_3O_2$	Analytical	Fisher
Acetic Acid	CH_3COOH	Analytical	Mallinckrodt
Ammonium Hydroxide	NH_4OH	Analytical	Mallinckrodt
Methanol	CH_3OH	HPLC	Fisher
Ethanol	C_2H_5OH	Reagent Grade	Rossville
Acetonitrile	CH_3CN	HPLC	Burdick and Jackson
Chloroform	$CHCl_3$	Reagent Grade	Baker
Tetrahydrofuran	C_4H_8O	Reagent Grade	Baker
Hexane	C_6H_{14}	HPLC	Fisher
Methylene Chloride	CH_2Cl_2	Reagent Grade	Fisher
Nitrobenzene	$C_6H_5NO_2$	Reagent Grade	Baker
Acenaphthene	$C_{12}H_{10}$	Reagent Grade	Baker
m-Dinitrobenzene	$C_6H_4N_2O_4$	Reagent Grade	Fisher

TABLE 4

HPLC Solvents

Solvent	Dielectric Constant	Viscosity	Maximum UV Cut-off	Solvent Strength
Hexane	1.89	0.33	195	0.00
Chloroform	4.81	0.58	245	0.40
Acetonitrile	37.5	0.44	189	0.65
Ethanol	24.3	1.20	210	0.88
Methanol	32.6	0.60	205	0.95
Water	80.4	1.00	-	large

The solvents in this table are listed in order of increasing polarity with Hexane the least polar and water with the highest polarity.

Dielectric constant value was based on a temperature of 20°C (obtained from the Handbook of Chemistry and Physics 55th edition).

UV cut-off was obtained from reagent bottles.

Solvent strength based on results for alumina column.[2]

TABLE 5

HPLC Columns

Column	Functional Group	Surface Nature	Particle Size (Avg.)	Solute Retention	Manufacturer
μ-PORASIL (analytical)	Si-OH	polar	10 μm	adsorption	Waters Assoc.
μ-BONDAPAK CN (analytical)	Si-CN	intermediate polarity	10 μm	partition (reversed-phase or normal)	Waters Assoc.
μ-BONDAPAK C_{18} (analytical)	Si-$C_{18}H_{37}$	non-polar	5 μm	partition (reversed-phase)	Beckman (Altex)
BONDAPAK C_{18} (guard)	Si-$C_{18}H_{37}$	non-polar	10 μm	partition (reversed-phase)	Beckman (Altex)
BONDAPAK Phenyl (guard)	Si(Ph)(Ph)	intermediate polarity	10 μm	partition (reversed-phase or normal)	column: Altex packing: Waters
CORASIL (guard)	Si-OH	polar	10 μm	adsorption	column: Altex packing: Waters
Pre-Column	Si-OH	polar	37-53 μm	———	Whatman Inc.

TABLE 6

Analytical Column Efficiency

Analytical Column	Column Dimensions	Solvent	Flow Rate (mL/min)	Sample	Plates Per Column (Literature)	Plates Per Column (Experimental)
μ-PORASIL	3.9 mm x 30 cm	Hexane	7.5	Nitrobenzene	3000	4200 +
μ-BONDAPAK CN (Reversed Phase)	3.9 mm x 30 cm	Acetonitrile and water (40:60)	2.5	Acenaphthene	2500	3100 +
μ-BONDAPAK CN (Normal Phase)	3.9 mm x 30 cm	Hexane	3.0	m-Dinitrobenzene	3000	3500 +
μ-BONDAPAK C_{18}	4.6 mm x 15 cm	Methanol and water (70:30)	1.0	Acetophenone Nitrobenzene Benzene, Toluene	2300 *	3600 * +

* The plate count for the u-BONDAPAK C_{18} column was calculated from the Benzene peak. The sample solution was supplied by Altex.

+ In all cases guard columns were in place for theoretical plate determination.

k' values for the columns were not calculated.

Column Dimensions: inside diameter x length

CHAPTER IV

EXPERIMENTAL

Preparation and Extraction of 8-Hydroxyquinoline Chelates

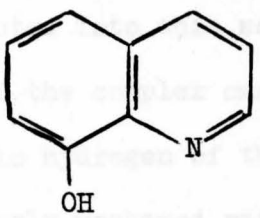


Figure 5. Structure of 8-Hydroxyquinoline (oxine).

8-Hydroxyquinoline is a white crystalline compound with a molecular weight of 145.15 and a melting point of 75-76°C.[35] It is often referred to by its trivial name "oxine" and can be recrystallized from a mixture of water and alcohol in almost colorless needles.[36] The reagent is sparingly soluble in cold water (3.6×10^{-3} M at 20-25°C), but has a high solubility in mineral acids and in dilute alkali to form yellow solutions.[37,38] This solubility increase in acidic solutions is caused by the formation of hydroxyquinolium ions H_2Ox^+, whereas, in alkaline solutions oxinate ions, Ox^- are formed.[37]

Oxine is also freely soluble in alcohol, chloroform, benzene and other organic solvents.[39] In this study oxine solutions were prepared by dissolving 1.00 gram of the solid in 100.mL of chloroform, (6.89×10^{-2} M). This solution was stored in a brown glass bottle, since the reagent is somewhat sensitive to light. For extractions,

the partition coefficient of the neutral compound between chloroform and aqueous phase at pH 6-9 is 460 at 25°C.[39] Because of its amphoteric nature, the partition of oxine is diminished under pH 6 and over pH 9.[40]

Hydroxyquinoline is one of the most versatile of organic reagents and is known to react with at least 50 metals.[41] Most metaloxinates are extremely soluble in chloroform (insoluble in aqueous) and can be completely extracted into this solvent forming yellow solutions. The formation of the complex can be described as follows: The metal replaces the acidic hydrogen of the oxine hydroxyl group.[3] At the same time the previously unshared pair of electrons on the nitrogen is donated to the metal, thereby forming a five-membered ring.[3,37]

Aluminum(III) and copper(II) were the metals used to form complexes with oxine. Divalent and trivalent metals form different complexes, illustrated by the reactions in Figures 6 and 7. The neutral chelate compounds of the type described are essentially organic in nature.[37] The metal ion becomes on of the members of an organic ring structure and its usual properties and reactions are no longer applicable.[38]

In this study, the Al(III) and Cu(II) solutions were prepared by dissolving the metal chloride salt in an acetic acid buffer (1.0 M). The buffer was produced by diluting concentrated acetic acid with deionized water and titrating with ammonium hydroxide to a pH of 7 (measured using Sargent-Welch pH meter). This pH was chosen since it provides the optimum condition for the complete extraction of Al(III) and Cu(II) as oxinates.[39] The concentrations were calculated such that 5.mL

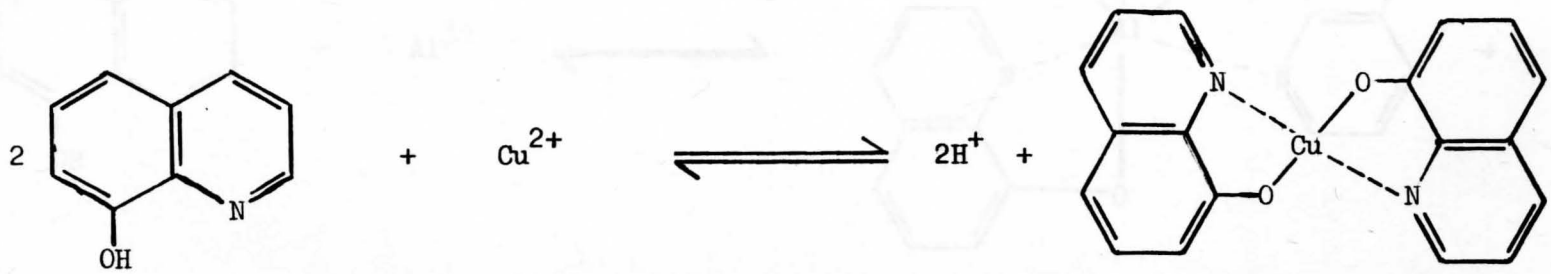


Figure 6. Complexation reaction for 8-Hydroxyquinoline with a divalent metal ion. (Cu (II) used as example)

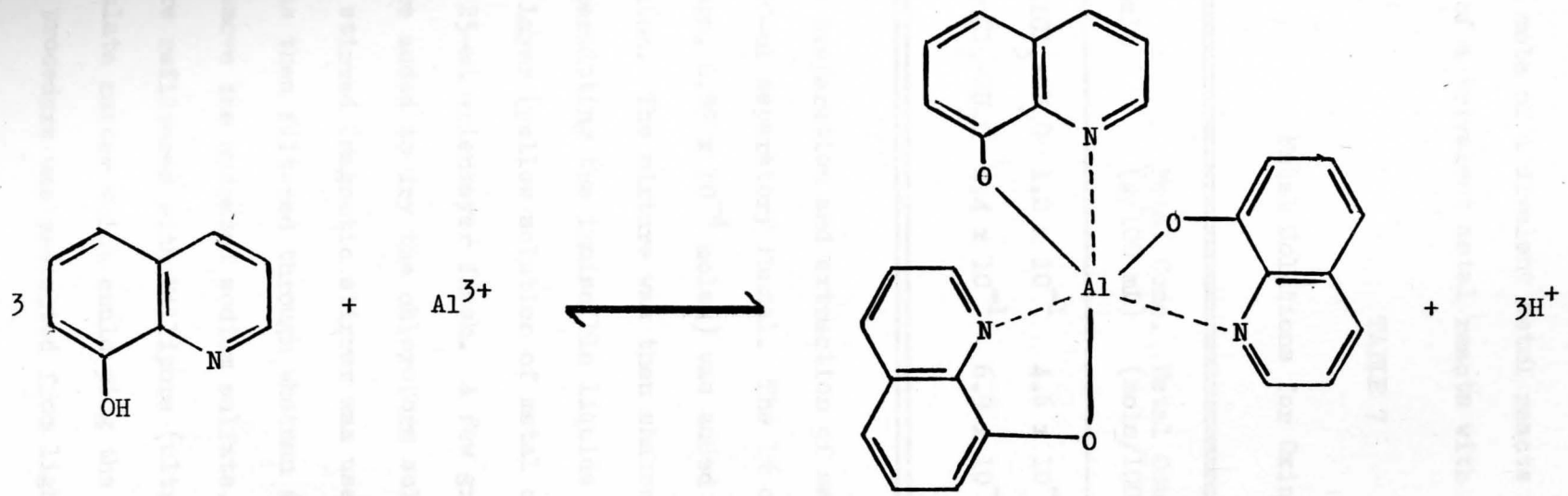


Figure 7. Complexation reaction for 8-Hydroxyquinoline with a trivalent metal ion. (Al(III) used as example)

of the metal solution would completely react with 10 mL of oxine (assuming 1 mole of a divalent metal reacts with 2 moles of oxine, and 1 mole of a trivalent metal reacts with 3 moles of oxine).

TABLE 7

Metal Solutions for Oxine Study

Metal Ion	Salt	Metal Conc. (g/100 mL)	Metal Conc. (mole/100 mL)	% Extraction at pH 7 (Theoretical optimum)
Al(III)	$AlCl_3 \cdot 6H_2O$	1.2×10^{-1}	4.6×10^{-3}	100
Cu(II)	$CuCl_2 \cdot 2H_2O$	4.4×10^{-1}	6.9×10^{-3}	100

The preparation and extraction of metal oxinates was carried out in a 250-mL separatory funnel. The 1% oxine solution (10. mL oxine in chloroform, 6.89×10^{-4} moles) was added to 5. mL of the aqueous metal solution. The mixture was then shaken for 2 minutes and allowed to stand, permitting the immiscible liquids to separate. The lower chloroform layer (yellow solution of metal oxinate) was then drawn off into a 25-mL erlenmeyer flask. A few grams of anhydrous sodium sulfate were added to dry the chloroform solution. The flask was corked and stirred (magnetic stirrer was used) for 3-4 minutes. The solution was then filtered through Whatman #2 (qualitative) filter paper to remove the hydrated sodium sulfate. Before HPLC analysis the samples were refiltered with Millipore (ultraporous) filters to remove any particulate matter which could plug the analytical column. The entire procedure was protected from light as much as possible.

The metal-oxinate samples and oxine reagent were stored, in tightly capped glass bottles, in the dark at room temperature. These solutions were stable for up to 10 days before becoming cloudy (indication of complex decomposition).

TABLE 8

Metal Oxinates

Metal Complex	MW	Complex Conc. in Extract (g/mL)	Metal Conc. in Extract (g/mL)	Color
Al $(C_9H_6NO)_3$	459.43	1.1×10^{-2}	6.2×10^{-4}	yellow
Cu $(C_9H_6NO)_2$	351.85	1.2×10^{-2}	2.2×10^{-3}	yellow

In Table 8 the concentrations were calculated assuming the metal was entirely reacted and completely extracted. Thus, concentrations are only approximate.

Preparation and Extraction of Cupferron Chelates

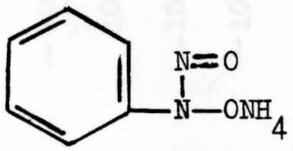


Figure 8. Structure of Cupferron (Ammonium salt of N-Nitrosophenylhydroxylamine)

Cupferron is a white or buff-colored crystalline powder with a molecular weight of 155.16 and a melting point of 163-164°C.[37,38] The reagent is soluble in water and alcohol, but is generally used in aqueous solutions.[42,43] In this study, the 1% cupferron solutions were prepared by dissolving 1.00 gram of the solid in 100 mL of acetate buffer, pH 7, (6.44×10^{-2} M). In aqueous solutions cupferron is a weak acid (pK = 4.16 at 25°C).[38] The partition coefficient of the undissociated acid between chloroform and aqueous phase is high (log p = 2.18 at 25°C).[42]

The reagent was first introduced by Baudisch in 1909, as a specific precipitant for copper (II) and iron (III).[37,38] Since then it has been found that almost all the heavy metals are precipitated as insoluble cupferrates, in weakly acidic solutions.[42,43] The complexation reaction can be described as follows: The metal replaces the acidic hydrogen of the free acid nitrosophenylhydroxylamine. At the same time, a pair of electrons from the nitroso (-N = O) group are donated to the metal, thereby forming a five membered ring.[42]

Several metals were used to investigate the chromatographic behavior of metal-cupferrates (Table 9). Divalent and trivalent metals form different complexes, aluminum (III) and beryllium (II)

TABLE 9

Metal Solutions for Cupferron Study

Metal Ion	Salt	Metal Ion Conc. (g/100 mL)	Metal Ion Conc. (moles/100 mL)	(Optimum) pH for Extraction	% Extracted at pH optimum (Theoretical)
Al (III)	$AlCl_3 \cdot 6H_2O$	1.2×10^{-1}	4.3×10^{-3}	7.0	100
Be (II)	$BeSO_4 \cdot 4H_2O$	5.8×10^{-2}	6.4×10^{-3}	7.0	~100
Co (III)	$CoCl_3 \cdot 6H_2O$	3.8×10^{-1}	6.4×10^{-3}	7.0	100
Cu (II)	$CuCl_2 \cdot 2H_2O$	4.1×10^{-1}	6.4×10^{-3}	4.0	~100
Fe (III)	$FeCl_3 \cdot 6H_2O$	2.4×10^{-1}	4.3×10^{-3}	4.0	~100
Hg (II)	$HgCl_2$	1.3	6.4×10^{-3}	4.0	98
La (III)	$La(NO_3)_3 \cdot 6H_2O$	6.0×10^{-1}	4.3×10^{-3}	7.0	90
Ni (II)	$NiCl_2 \cdot 6H_2O$	3.8×10^{-1}	6.4×10^{-3}	7.0	50
Pb (II)	$PbCl_2$	1.3	6.4×10^{-3}	4.0	~100

were used as examples in Figures 10 and 11. These neutral chelate compounds are essentially organic in nature. The metal ion becomes one of the members of an organic ring structure and its usual properties and reactions are no longer applicable.[38] These metal complexes are soluble in ethyl acetate, diethyl ether, benzene, isoamyl alcohol and chloroform. For this study, in all cases, chloroform was used for extraction.

The solutions of metal cupferrates and the pure reagent are somewhat unstable. Exposure to heat or strong acid leads to the formation of nitrosophenylhydroxylamine (free acid, reverse of complexation reaction) (Figure 9). The free acid is very unstable and decomposed on standing into nitrosobenzene and other products. Decomposition can be detected by the cloudy appearance of the chloroform solutions.

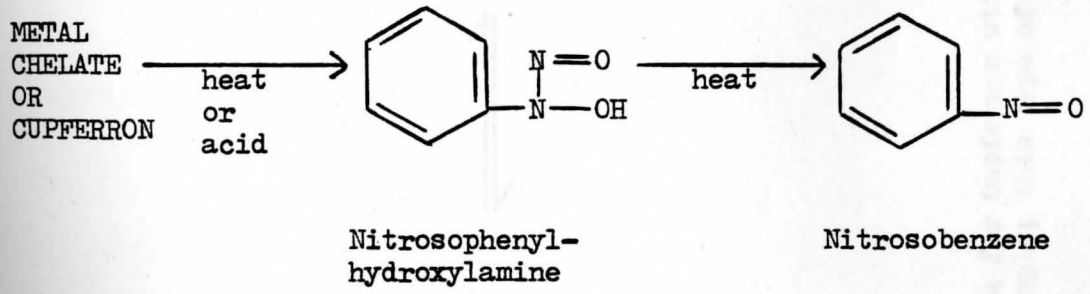


Figure 9. Decomposition of cupferron and its metal chelates.

For the investigation of cupferrates the metal solutions were prepared by dissolving a soluble metal salt in acetate buffer. The metal salt, ion concentration, and pH of the acetate buffer are given in Table 9. The concentrations were calculated such that

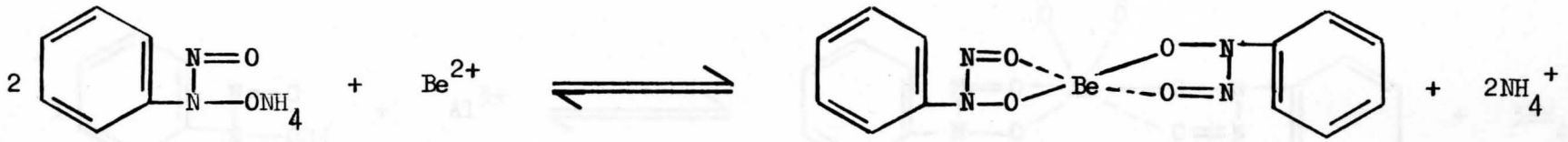


Figure 10. Complexation reaction for Cupferron with a divalent metal ion. (Be (II) used as example; this type of complex is also formed for Cu (II), Hg (II), Ni (II), and Pb (II).)

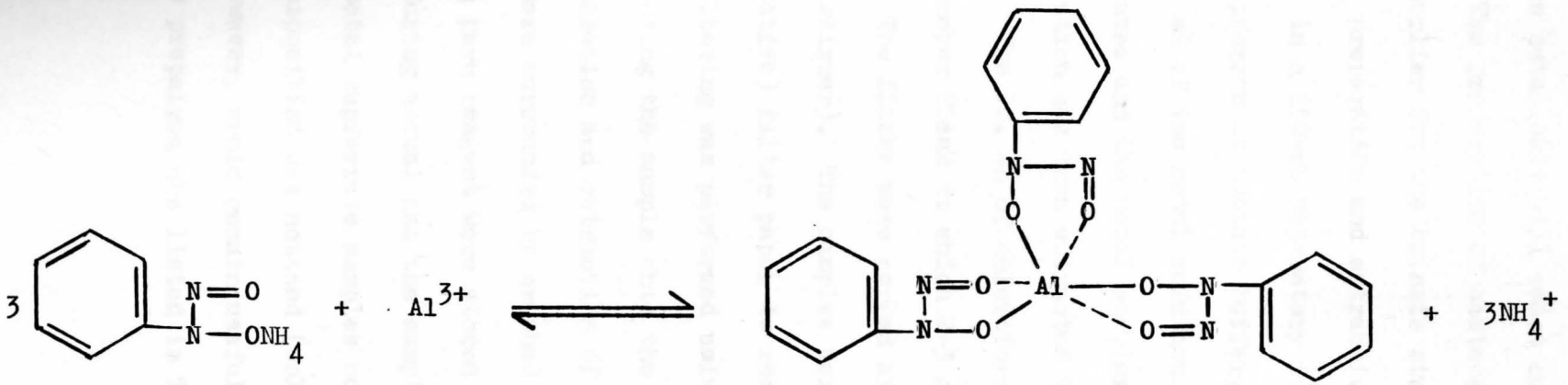


Figure 11. Complexation reaction for Cupferron with a trivalent metal ion. (Al (III) used as example; this type of complex is also formed for Co (III), Fe (III), and La (III).)

5. mL of the metal salt will react completely with 10. mL of cupferron solution. The preparation of acetate buffer is identical to that described earlier for the oxinate study.

The preparation and extraction of metal cupferrates was carried out in a 250-mL separatory funnel. The 1% cupferron solution (10. mL, cupferron in acetate buffer, pH 7, 6.44×10^{-4} moles) was added to 5. mL of the metal solution. The mixture was then shaken for 2-3 minutes and the metal complex allowed to precipitate. The aqueous solution was then extracted twice with 10. mL portions of chloroform. The 20. mL of chloroform solution was collected in a 25-mL erlenmeyer flask to which 2-3 grams of anhydrous sodium sulfate was added. The flasks were corked and stirred for 3 minutes (using a magnetic stirrer). The samples were then filtered using Whatman #2 (quantitative) filter paper to remove the hydrated sodium sulfate. A second filtering was performed using Millipore (ultraporous) filters before injecting the sample onto the analytical column. At all stages of the preparation and extraction of the metal cupferrates the solutions were surrounded by crushed ice (whenever possible). The samples and pure reagent were stored in tightly capped bottles in the freezer. During actual use the sample bottles were kept in crushed ice. The metal cupferrate samples could be stored for up to 2 days before decomposition was noticed (solutions became cloudy). The pure reagent; however, would remain useful for up to 5 days. The metal cupferrates prepaired are listed in Table 10.

TABLE 10

Metal Cupferrates

Metal Complex	MW	Complex Conc.* in Extract (g/mL)	Metal Conc.* in Extract (g/mL)	Color
$Al(C_6H_5N_2O_2)_3$	438.46	4.9×10^{-3}	3.0×10^{-4}	clear
$Be(C_6H_5N_2O_2)_2$	283.33	4.7×10^{-3}	1.5×10^{-4}	clear
$Co(C_6H_5N_2O_2)_3$	470.38	7.6×10^{-3}	9.5×10^{-4}	pink
$Cu(C_6H_5N_2O_2)_2$	337.87	5.3×10^{-3}	1.0×10^{-3}	blue
$Fe(C_6H_5N_2O_2)_3$	467.33	5.0×10^{-3}	6.0×10^{-4}	brownish red
$Hg(C_6H_5N_2O_2)_2$	474.91	7.6×10^{-3}	3.2×10^{-3}	clear
$La(C_6H_5N_2O_2)_3$	550.39	5.2×10^{-3}	1.3×10^{-3}	clear
$Ni(C_6H_5N_2O_2)_2$	333.03	2.7×10^{-3}	4.8×10^{-4}	green
$Pb(C_6H_5N_2O_2)_2$	481.51	7.7×10^{-3}	3.3×10^{-3}	clear

* Concentrations calculated are only approximate, based on theoretical % extraction at optimum pH.[42,43] It was also assumed complexation reaction and experimental extraction were complete.

Detection of Metal Complexes

A variable-wavelength UV detector was used in conjunction with the liquid chromatograph for sample detection. In order to ascertain the optimum detection wavelength of the metal oxinates and metal cupferrates, the UV spectra were obtained.

A Beckman 26 recording spectrophotometer was used to obtain the UV spectra of the metal complexes studied. These spectra are all contained in the Appendix section of this presentation. Chloroform was used as the solvent for the sample and as the reference in the instrument. From the UV bands it was determined that the optimum detection wavelength was 254 nm for metal oxinates and 280 nm for metal cupferrates.

The UV spectra for the iron cupferrate chelate were also obtained in methanol, acetonitrile, and hexane. This was performed to determine if a shift in the UV band was brought about by changes in the solvent. It was found that the spectrum of the iron complex was identical in all four solvents. Therefore, it was assumed that similar behavior was demonstrated by the other cupferron chelates.

CHAPTER V

RESULTS AND DISCUSSION

Throughout this study the experimental conditions for chromatography analysis were standardized as much as possible to allow easy comparison of results. This includes the solvent flow rate (1 mL/min), detector sensitivity (0.2 absorbance units, on Altex UV detector), sample injection volume (1 μL, see Tables 8 and 10 for approximate solute concentrations in g/mL), recorder chart speed (1 cm/min) and column temperature (ambient). The detection wavelength was also held constant for the study of 8-hydroxyquinolates (254 nm) and cupferrates (280 nm).

The column effectiveness was also maintained throughout the study, this was verified by periodic evaluations of N (see Table 6). For solvent changeovers, the columns were conditioned with the new mobile phase before injecting samples. This conditioning involved pumping eluent through the column for 40 to 50 minutes.

Chromatographic Behavior of Metal Oxinates

The metal oxinates were studied with reversed-phase chromatography using a μ-Bondapak C_{18} column (see Table 11). The pure reagent was also tested to determine the interference produced by excess oxine in solutions of the metal chelates. The mobile phases listed in the table of results were not the only solvent systems tested, but are representative of the other systems (this is also

TABLE 11

HPLC Results for Metal Oxinates:
u-Bondapak C_{18} Column (reversed-phase)

	Mobile Phase	Sample	t_r (min)	Observations
1.	Methanol/H_2O (70:30)	C_9H_7NO	3.8	Oxine and Al-complex eluted as broad tailing peaks at same t_r.
		$Al(C_9H_6NO)_3$	3.8	
		$Cu(C_9H_6NO)_2$	*	
2.	Methanol (anhydrous)	C_9H_7NO	4.2	With anhydrous methanol, peaks narrowed, tailing decreased and t_r was slightly increased.
		$Al(C_9H_6NO)_3$	4.2	
		$Cu(C_9H_6NO)_2$	*	
3.	Ethanol (100%)	C_9H_7NO	4.2	Using an ethanol mobile phase produced the same results obtained for methanol.
		$Al(C_9H_6NO)_3$	4.2	
		$Cu(C_9H_6NO)_2$	*	
4.	Acetonitrile	C_9H_7NO	4.0	The t_r values decreased slightly w.r.t. Ethanol and Methanol. The Al-Ox peak was sharp and the oxine peak broad with tailing.
		$Al(C_9H_6NO)_3$	4.0	
		$Cu(C_9H_6NO)_2$	*	

TABLE 11 cont.

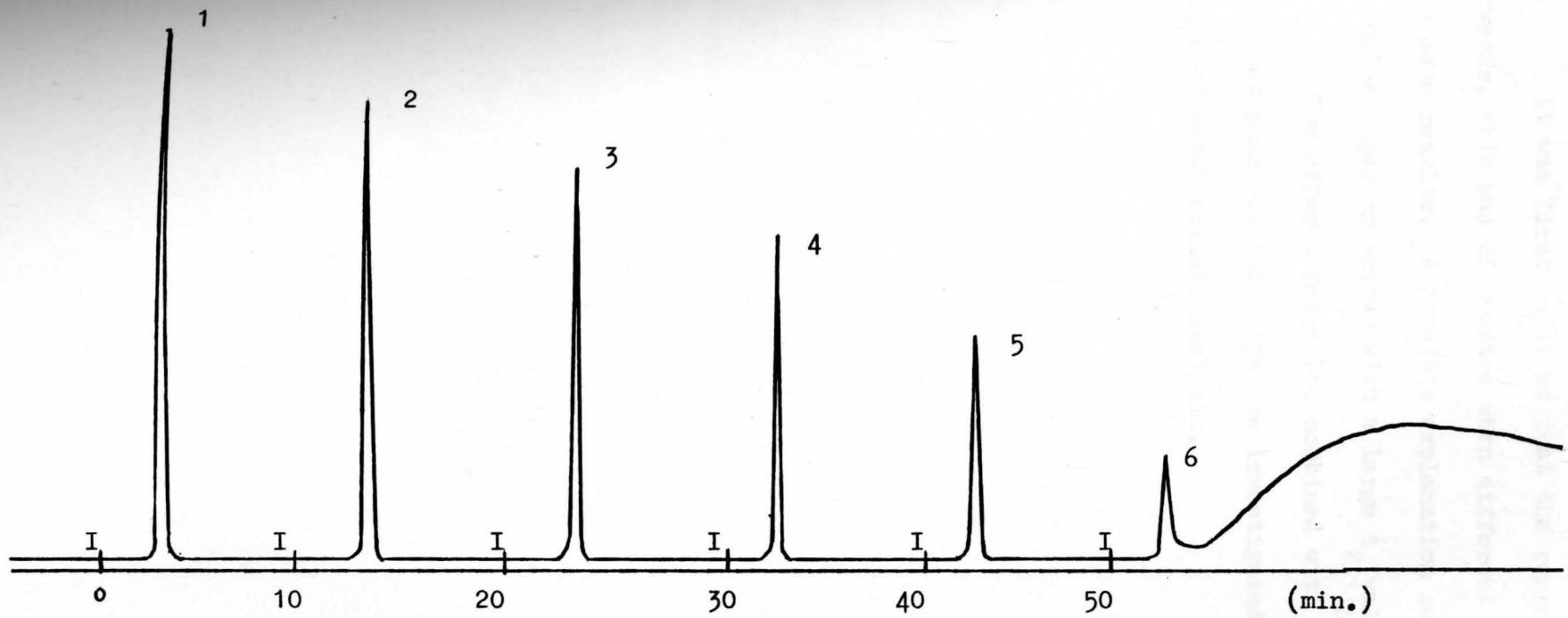
	Mobile Phase	Sample	t_r (min)	Observations
5.	Chloroform	C_9H_7NO	4.2	The Cu-complex was eluted in a broad, tailing peak.
		$Al(C_9H_6NO)_3$	4.2	The Al-Ox peak was sharp. The reagent peak was
		$Cu(C_9H_6NO)_2$	4.2	broad with some tailing.
6.	Hexane	C_9H_7NO	5.2	The reagent peak was very broad with considerable
		$Al(C_9H_6NO)_3$	*	tailing, 30-40 minutes were required to elute peak.
		$Cu(C_9H_6NO)_2$	*	

* Sample not eluted.

t_r Retention time (measured from injection point to center of peak, in minutes).

true for the metal cupferrates). The first mobile phase, methanol/water was tested with various amounts of water (1-30%, 30% highest water content possible since the samples are in chloroform). Acetate buffer (1 M, pH 6 and pH 7) was also tried in place of water. However, t_r remained as reported for mobile phase 1, the only change observed was a reduction of band tailing (also band width) with decreasing amounts of water. When the water content was reduced to approximately 5%, the samples behaved as in anhydrous methanol (mobile phase 2). This type of result was also obtained for combinations of chloroform and hexane (samples behaved as in chloroform until 40% hexane was reached, from 40%-100% hexane samples behaved as reported for mobile phase 6). I can offer no explanation for this rapid change of solute behavior.

With mobile phases 1-6 an elution effect was observed which can only be described as "solute hang-back" (see Figure 12). What seems to be occurring is a strong retention of a portion of the introduced sample then a delayed elution. Chromatography literature did not contain any reference to this type of elution problem. This effect was first noticed with methanol and water and later reproduced in all the mobile phases. After equilibration with the mobile phase, successive injections (1 μL) of the 1% oxine solution were made allowing an average of 10-12 minutes between samples. With each input of sample the peak area noticeably decreased (peak area for the 5th injection was approximately 25% of the 1st) until the 6th injection, when a second peak was obtained. This peak was extremely broad and 80-90 minutes were required for complete elution.



I= point of sample injection

Column: μ-Bondapak C_{18}

Solvent: Methanol / H_2O (70:30)

Flow rate: 1 mL/min

Chart speed: 1cm/min

Detect. Sen: 0.2

Detect. wavelength: 254 nm

Sample size: 1 μL

Sample: 1% Oxine in $CHCl_3$

(t_r for sample 3.8 min.)

Figure 12. Illustration of Solute Hang Back Effect.

It was first believed that the reagent was contaminated, however, this was discounted when different samples of oxine produced the same results. A possible explanation could be the formation of an oxine dimer or trimer with a large t_r value.

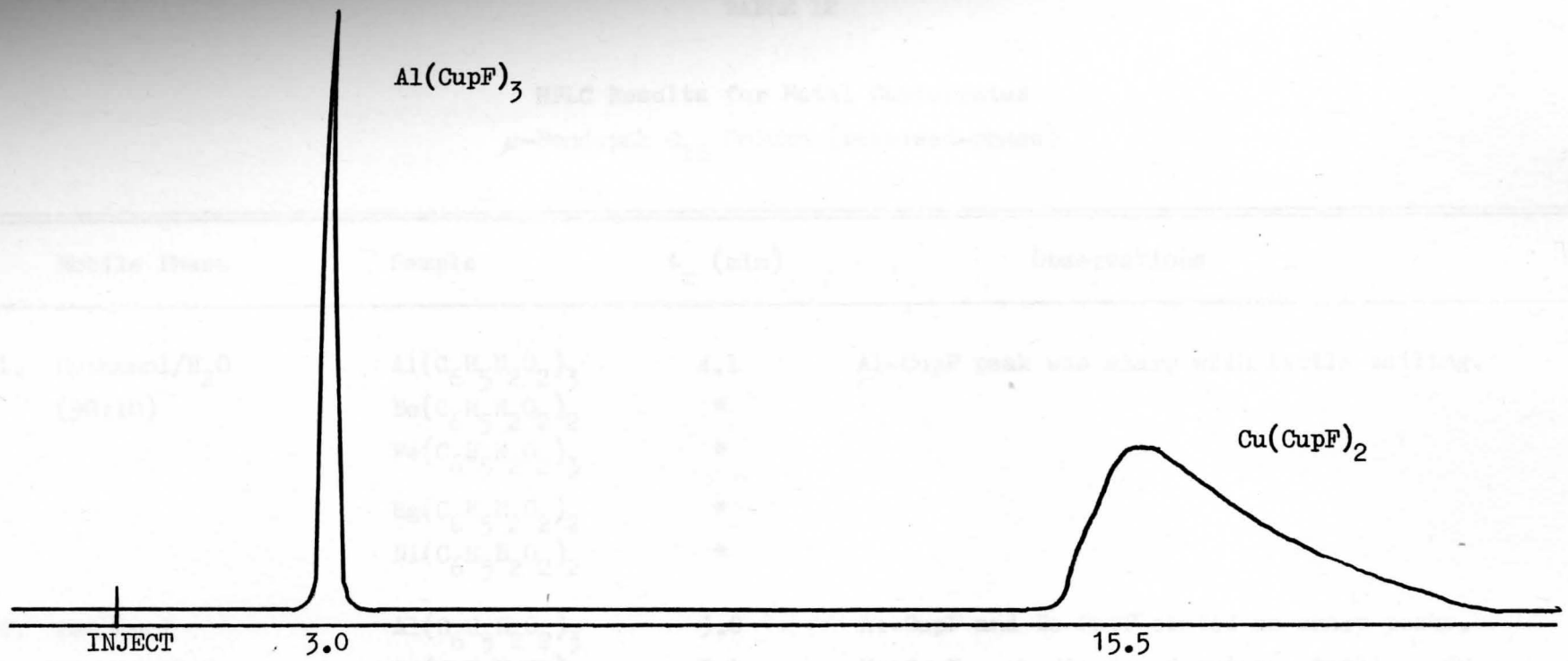
The effects described combined with the fact that separations were not possible (with systems investigated) led to the discontinuation of metal oxinate analysis.

Chromatographic Behavior of Metal Cupferrates

The metal cupferrates were investigated with partition (normal and reversed phase) and adsorption chromatography. Three columns were used with a variety of mobile phases to determine the chromatographic behavior of several metal cupferrates (all metal CupF were not tested with each system). An extraction of pure cupferron was also tested to determine if any interference would develop from excess reagent. However, since CupF prefers aqueous to organic and has a low molar absorbtivity at 280 nm, a peak was not observed for this sample.

With the C_{18} column, the same rapid change in sample behavior described in the previous section for pure oxine was observed for metal cupferrates. This occurred with the methanol/water system (changing slowly to anhydrous methanol) and the acetonitrile/water changeover to acetonitrile. However, it was possible to achieve a separation of Al-CupF and Cu-CupF, which were extracted together (see Figure 13). This system is of little value as an analytical method because of interference by other metal chelates (see Table 12) and band tailing which would not allow quantitation.

The μ-Porasil column was used in the activated and deactivated form to study the metal cupferrates. With both silica forms, the samples were strongly adsorbed. Al-CupF was an exception and could be eluted from the activated silica column with a chloroform mobile phase (in the absence of any interference). This type of system could possibly be used for the analysis of Al (III) in the presence of metals. The only drawback is the removal of the strongly adsorbed samples. This would require an involved column regeneration procedure.



Column: μ-Bondapak C_{18}

Solvent: Methanol

Flow rate: 1 mL/min.

Chart speed: 1 cm/min.

Detect. Sens. 0.2

Detect. Wavelength: 280 nm

Concentration of Al(III) solution (g/mL)

6.0×10^{-5}

Concentration of Cu(II) solution (g/mL)

7.1×10^{-4}

Figure 13. Chromatogram for the separation of aluminum and copper.

TABLE 12

HPLC Results for Metal Cupferrates
μ-Bondapak C_{18} Column (reversed-phase)

	Mobile Phase	Sample	t_r (min)	Observations
1.	Methanol/H_2O (90:10)	$Al(C_6H_5N_2O_2)_3$	4.1	Al-CupF peak was sharp with little tailing.
		$Be(C_6H_5N_2O_2)_2$	*	
		$Fe(C_6H_5N_2O_2)_3$	*	
		$Hg(C_6H_5N_2O_2)_2$	*	
		$Ni(C_6H_5N_2O_2)_2$	*	
2.	Methanol (anhydrous)	$Al(C_6H_5N_2O_2)_3$	3.0	Al-CupF and Be-CupF eluted as sharp peaks.
		$Be(C_6H_5N_2O_2)_2$	3.1	Hg-CupF peak displayed extreme tailing, 20
		$Cu(C_6H_5N_2O_2)_2$	15.5	minutes were required for complete elution.
		$Fe(C_6H_5N_2O_2)_3$	*	Cu-CupF developed a broad, slightly tailing
		$Hg(C_6H_5N_2O_2)_2$	3.0	peak. Al (III) and Cu (II) also extracted
		$Pb(C_6H_5N_2O_2)_2$	*	together and separated.(see Figure 13).

TABLE 12 cont.

	Mobile Phase	Sample	t_r (min)	Observations
3.	Ethanol (100%)	$Al(C_6H_5N_2O_2)_3$	3.0	Al-CupF, sharp peak.
		$Be(C_6H_5N_2O_2)_2$	*	
		$Cu(C_6H_5N_2O_2)_2$	*	
		$Hg(C_6H_5N_2O_2)_2$	*	
4.	Acetonitrile/H_2O (90:10)	$Al(C_6H_5N_2O_2)_3$	4.1	Al-CupF, sharp peak with some tailing.
		$Be(C_6H_5N_2O_2)_2$	*	
		$Cu(C_6H_5N_2O_2)_2$	*	
		$Ni(C_6H_5N_2O_2)_2$	*	
5.	Acetonitrile	$Al(C_6H_5N_2O_2)_3$	3.0	Sharp peaks were obtained for Al-CupF and
		$Be(C_6H_5N_2O_2)_2$	2.9	Be-CupF. Fe-CupF was eluted as a broad tailing
		$Cu(C_6H_5N_2O_2)_2$	*	peak.
		$Fe(C_6H_5N_2O_2)_3$	3.0	
		$Ni(C_6H_5N_2O_2)_2$	*	

TABLE 12 cont.

	Mobile Phase	Sample	t_r (min)	Observations
6.	Methanol/Chloroform (80:20)	$Al(C_6H_5N_2O_2)_3$	4.1	Tailing band was obtained for Al-CupF.
		$Be(C_6H_5N_2O_2)_2$	*	
		$Cu(C_6H_5N_2O_2)_2$	*	
		$Fe(C_6H_5N_2O_2)_3$	*	
		$Hg(C_6H_5N_2O_2)_2$	*	
7.	Chloroform/Acetonitrile (80:20)	$Al(C_6H_5N_2O_2)_3$	2.6	Sharp peaks were obtained for Co-CupF, Fe-CupF, Be-CupF and Al-CupF. The Cu-CupF band displayed extreme tailing and 30-40 minutes were required to completely elute the peak.
		$Be(C_6H_5N_2O_2)_2$	2.5	
		$Cu(C_6H_5N_2O_2)_2$	8.1	
		$Co(C_6H_5N_2O_2)_3$	2.6	
		$Fe(C_6H_5N_2O_2)_3$	2.6	
		$Ni(C_6H_5N_2O_2)_2$	*	
8.	Chloroform	$Al(C_6H_5N_2O_2)_3$	4.1	Al-CupF and Cu-CupF both produced peaks which involved considerable tailing.
		$Be(C_6H_5N_2O_2)_2$	*	
		$Cu(C_6H_5N_2O_2)_2$	18.0	
		$Fe(C_6H_5N_2O_2)_3$	*	

TABLE 12 cont.

	Mobile Phase	Sample	t_r (min)	Observations
9.	Hexane	$Al(C_6H_5N_2O_2)_3$	*	Elution of the metal-cupferrates was not possible with this solvent.
		$Be(C_6H_5N_2O_2)_2$	*	
		$Cu(C_6H_5N_2O_2)_2$	*	
		$Fe(C_6H_5N_2O_2)_3$	*	
		$Ni(C_6H_5N_2O_2)_2$	*	

* Sample not eluted.

t_r Retention time (measured from injection point to center of peak, in minutes)

TABLE 13

HPLC Results for Metal Cupferrates

μ-Porasil Column (Adsorption Chromatography)

	Mobile Phase	Sample	t_r (min)	Observations
1.	Acetonitrile	$Al(C_6H_5N_2O_2)_3$	4.8	Al-CupF and Cu-CupF are inseparable because of
		$Be(C_6H_5N_2O_2)_2$	*	the broad, tailing peak obtained for Cu-CupF.
		$Cu(C_6H_5N_2O_2)_2$	5.5	(activated silica)
		$Fe(C_6H_5N_2O_2)_3$	*	
		$Hg(C_6H_5N_2O_2)_2$	*	
		$Pb(C_6H_5N_2O_2)_2$	*	
2.	Chloroform	$Al(C_6H_5N_2O_2)_3$	4.6	Sharp peak obtained for Al-CupF.
		$Cu(C_6H_5N_2O_2)_2$	*	(activated silica)
		$Fe(C_6H_5N_2O_2)_3$	*	
		$Ni(C_6H_5N_2O_2)_2$	*	

TABLE 13 cont.

	Mobile Phase	Sample	t_r (min)	Observations
3.	Hexane	$Al(C_6H_5N_2O_2)_3$	*	(activated silica)
		$Cu(C_6H_5N_2O_2)_2$	*	
		$Fe(C_6H_5N_2O_2)_3$	*	
		$Ni(C_6H_5N_2O_2)_2$	*	
4.	Methanol (anhydrous)	$Al(C_6H_5N_2O_2)_3$	*	(deactivated silica)
		$Be(C_6H_5N_2O_2)_2$	*	
		$Cu(C_6H_5N_2O_2)_2$	*	
		$Hg(C_6H_5N_2O_2)_2$	*	
		$Ni(C_6H_5N_2O_2)_2$	*	
		$Pb(C_6H_5N_2O_2)_2$	*	
5.	Chloroform/Methanol (95:5)	$Al(C_6H_5N_2O_2)_3$	4.4	Broad, tailing band observed for Al-CupF.
		$Be(C_6H_5N_2O_2)_2$	*	(deactivated silica)
		$Fe(C_6H_5N_2O_2)_3$	*	
		$Ni(C_6H_5N_2O_2)_2$	*	

TABLE 13 cont.

	Mobile Phase	Sample	t_r (min)	Observations
6.	Chloroform (water saturated)	$Al(C_6H_5N_2O_2)_3$	*	(deactivated silica)
		$Be(C_6H_5N_2O_2)_2$	*	
		$Fe(C_6H_5N_2O_2)_3$	*	
		$Ni(C_6H_5N_2O_2)_2$	*	
7.	Hexane (water saturated)	$Al(C_6H_5N_2O_2)_3$	*	(deactivated silica)
		$Be(C_6H_5N_2O_2)_2$	*	
		$Fe(C_6H_5N_2O_2)_3$	*	
		$Ni(C_6H_5N_2O_2)_2$	*	

* Sample not eluted.

t_r Retention time (measured from injection point to center of peak, in minutes).

The best results in this study were obtained with the μ-Bondapak CN column. This column with intermediate packing polarity was operated in normal and reversed phase. Converting to normal phase involves activating the silica packing by passing methylene chloride and tetrahydrofuran through the column for several minutes. The column is reconverted to reversed phase by using methanol or water in the mobile phase.

Using the CN column with a chloroform mobile phase it was possible to elute all of the metal cupferrates (see Table 14). However, all of the solutes displayed a similar t_r value. In order to achieve separation, several combinations of methanol/chloroform and acetonitrile/chloroform were tested. It was found that as the percentage of methanol or acetonitrile was increased the metal cupferrate peak area decreased in size, while t_r remained constant. It was also determined that the complexes were not held back on the column, since an immediate change to the chloroform mobile phase failed to elute sample bands. The UV spectra of the metal cupferrates (using Fe-CupF) were obtained in methanol, acetonitrile and hexane for comparison to chloroform (see Appendix). This was performed to determine if a UV shift occurs in the UV spectrum of metal CupF with a change of solvent. This proved not to be the case. The only other feasible possibility is the decomposition of the metal chelates by some reaction with the column and solvent.

The best separation obtained on the CN column was that of aluminum and beryllium (see Figure 14) with a mobile phase of hexane/chloroform (80:20). This optimum ratio was determined after testing several combinations of hexane and chloroform. The elution behavior

TABLE 14

HPLC Results for Metal Cupferrates

μ-Bondapak CN Column (normal and reversed phase)

	Mobile Phase	Sample	t_r (min)	Observations
1.	Methanol (anhydrous)	$Al(C_6H_5N_2O_2)_3$	4.2	Sharp band for Al-CupF.
		$Cu(C_6H_5N_2O_2)_2$	*	
		$Fe(C_6H_5N_2O_2)_3$	*	
		$Ni(C_6H_5N_2O_2)_2$	*	
2.	Acetonitrile	$Al(C_6H_5N_2O_2)_3$	4.5	Sharp bands for Al-CupF, Be-CupF and Fe-CupF
		$Be(C_6H_5N_2O_2)_2$	4.5	with some tailing.
		$Cu(C_6H_5N_2O_2)_2$	*	
		$Fe(C_6H_5N_2O_2)_3$	4.8	
		$Ni(C_6H_5N_2O_2)_2$	*	
3.	Chloroform/Methanol (90:10)	$Al(C_6H_5N_2O_2)_3$	4.2	Sharp bands for Al-CupF and Be-CupF.
		$Be(C_6H_5N_2O_2)_2$	4.2	
		$Fe(C_6H_5N_2O_2)_3$	*	

TABLE 14 cont.

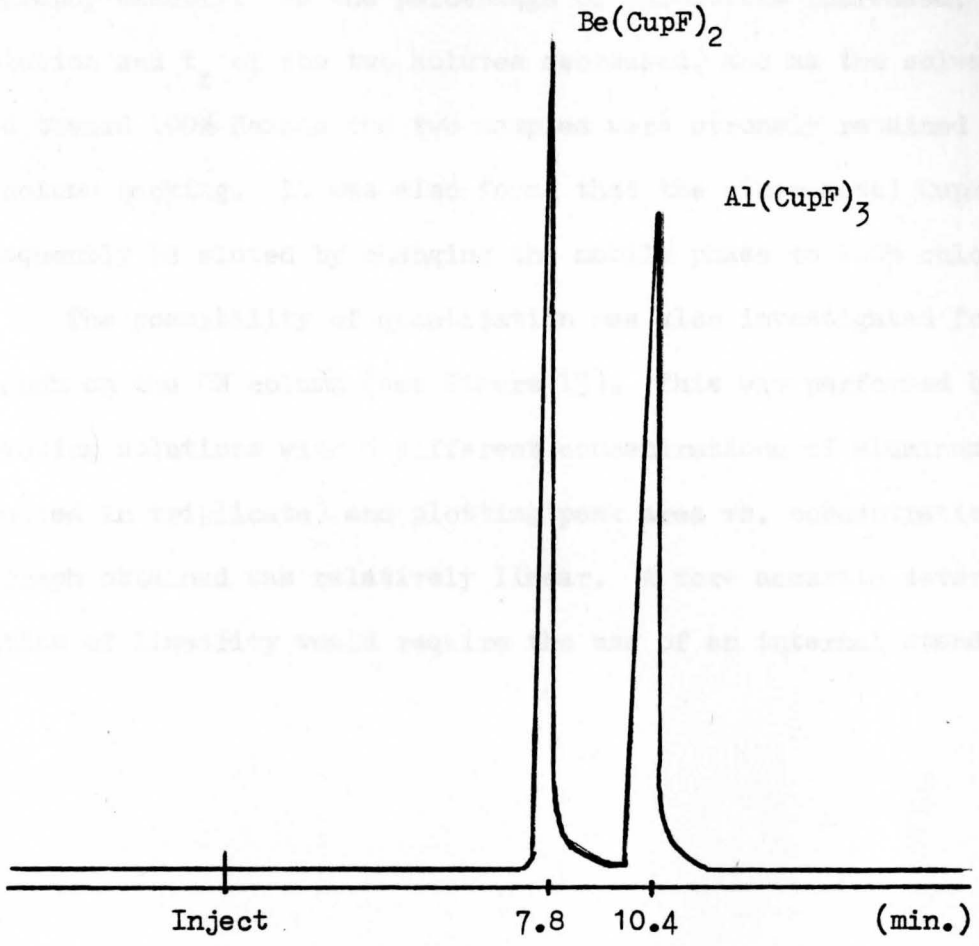
	Mobile Phase	Sample	t_r (min)	Observations
4.	Chloroform	$Al(C_6H_5N_2O_2)_3$	4.2	Sharp bands were obtained for Al-CupF, Be-CupF,
		$Be(C_6H_5N_2O_2)_2$	4.4	Co-CupF, Fe-CupF and Ni-CupF. Broad tailing
		$Co(C_6H_5N_2O_2)_3$	4.4	bands were recorded for Cu-CupF, Hg-CupF,
		$Cu(C_6H_5N_2O_2)_2$	4.4	La-CupF and Pb-CupF.
		$Fe(C_6H_5N_2O_2)_3$	4.4	
		$Hg(C_6H_5N_2O_2)_2$	4.4	
		$La(C_6H_5N_2O_2)_3$	4.4	
		$Ni(C_6H_5N_2O_2)_2$	4.4	
		$Pb(C_6H_5N_2O_2)_2$	4.4	
5.	Hexane	$Al(C_6H_5N_2O_2)_3$	*	(No Sample Elution)
		$Be(C_6H_5N_2O_2)_2$	*	

TABLE 14 cont.

	Mobile Phase	Sample	t_r (min)	Observations
6.	Hexane/Chloroform (80:20)	$Al(C_6H_5N_2O_2)_3$	10.3	The best separation possible between Al-CupF and Be-CupF, with no interference (See Figure 14)
		$Be(C_6H_5N_2O_2)_2$	4.8	
		$Co(C_6H_5N_2O_2)_3$	*	
		$Cu(C_6H_5N_2O_2)_2$	*	
		$Fe(C_6H_5N_2O_2)_3$	*	
		$Hg(C_6H_5N_2O_2)_2$	*	
		$La(C_6H_5N_2O_2)_3$	*	
		$Ni(C_6H_5N_2O_2)_2$	*	
		$Pb(C_6H_5N_2O_2)_2$	*	

* Sample not eluted.

t_r Retention time (measured from injection point to center of peak, in minutes).



Column: μ-Bondapak CN
Solvent: Hexane / Chloroform (80:20)
Flow rate: 1 mL/min
Chart speed: 1 cm/min
Detect. Sens: 0.2
Detect. wavelength: 280 nm
Sample size: 1 μL

Sample

$Al(CupF)_3$ + $Be(CupF)_2$

Al conc. (g/mL) 6.0×10^{-5}

Be conc. (g/mL) 9.5×10^{-5}

Figure 14. Chromatogram for the separation of aluminum and beryllium.

of Be-CupF and Al-CupF obeyed the principles of reversed-phase chromatography exactly. As the percentage of chloroform increased, resolution and t_r of the two solutes decreased, and as the solvent moved toward 100% Hexane the two samples were strongly retained by the column packing. It was also found that the other metal CupF could subsequently be eluted by changing the mobile phase to 100% chloroform.

The possibility of quantitation was also investigated for aluminum on the CN column (see Figure 15). This was performed by extracting solutions with 5 different concentrations of aluminum (repeated in triplicate) and plotting peak area vs. concentration. The graph obtained was relatively linear. A more accurate determination of linearity would require the use of an internal standard.

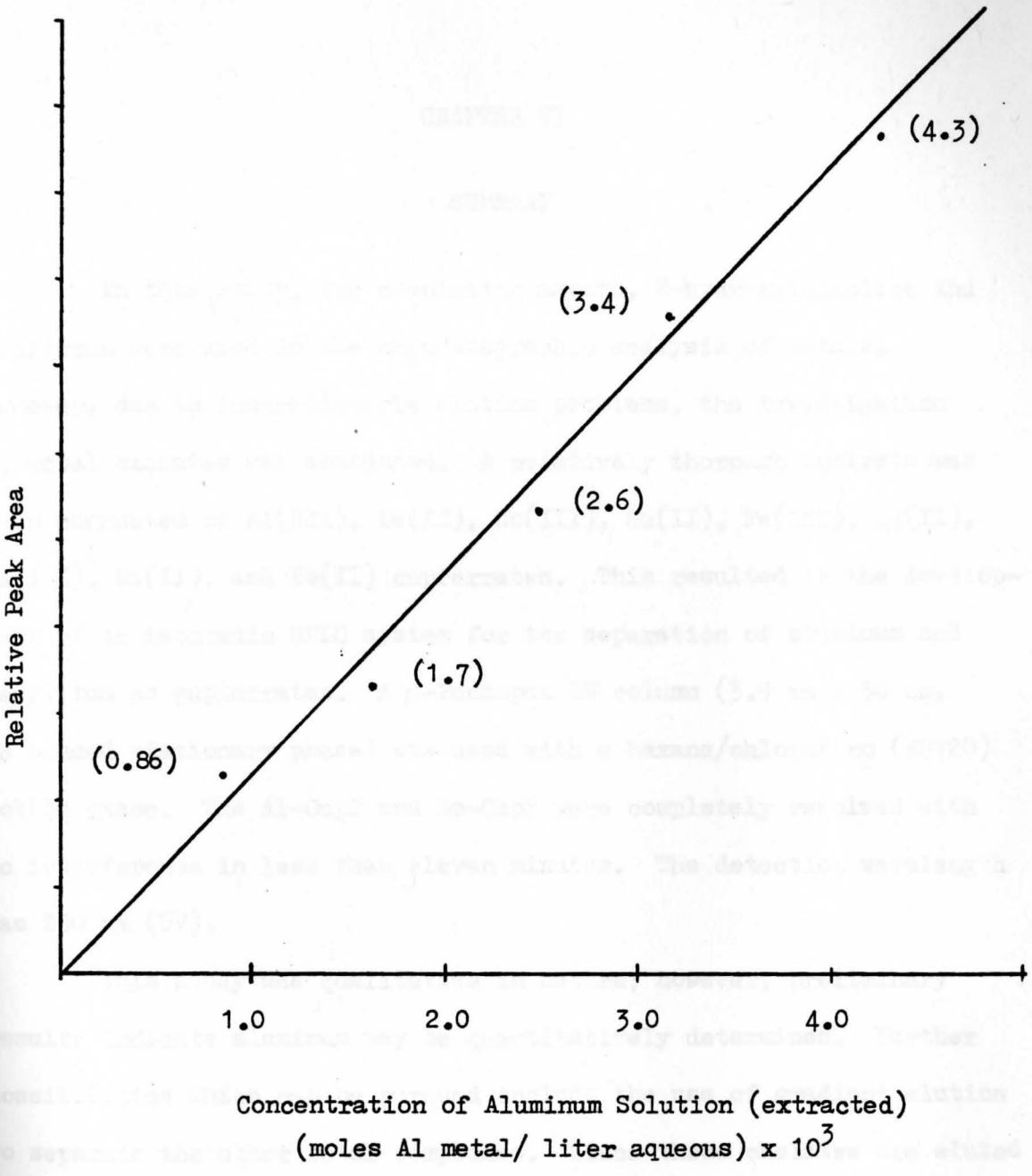


Figure 15. Demonstration of linearity for aluminum extractions

CHAPTER VI

SUMMARY

In this study, two complexing agents, 8-hydroxyquinoline and cupferron were used in the chromatographic analysis of metals. However, due to insurmountable elution problems, the investigation of metal oxinates was abandoned. A relatively thorough analysis was then conducted of Al(III), Be(II), Co(III), Cu(II), Fe(III), Hg(II), La(III), Ni(II), and Pb(II) cupferrates. This resulted in the development of an isocratic HPLC system for the separation of aluminum and beryllium as cupferrates. A μ-Bondapak CN column (3.9 mm x 30 cm, CN bonded stationary phase) was used with a hexane/chloroform (80:20) mobile phase. The Al-CupF and Be-CupF were completely resolved with no interference in less than eleven minutes. The detection wavelength was 280 nm (UV).

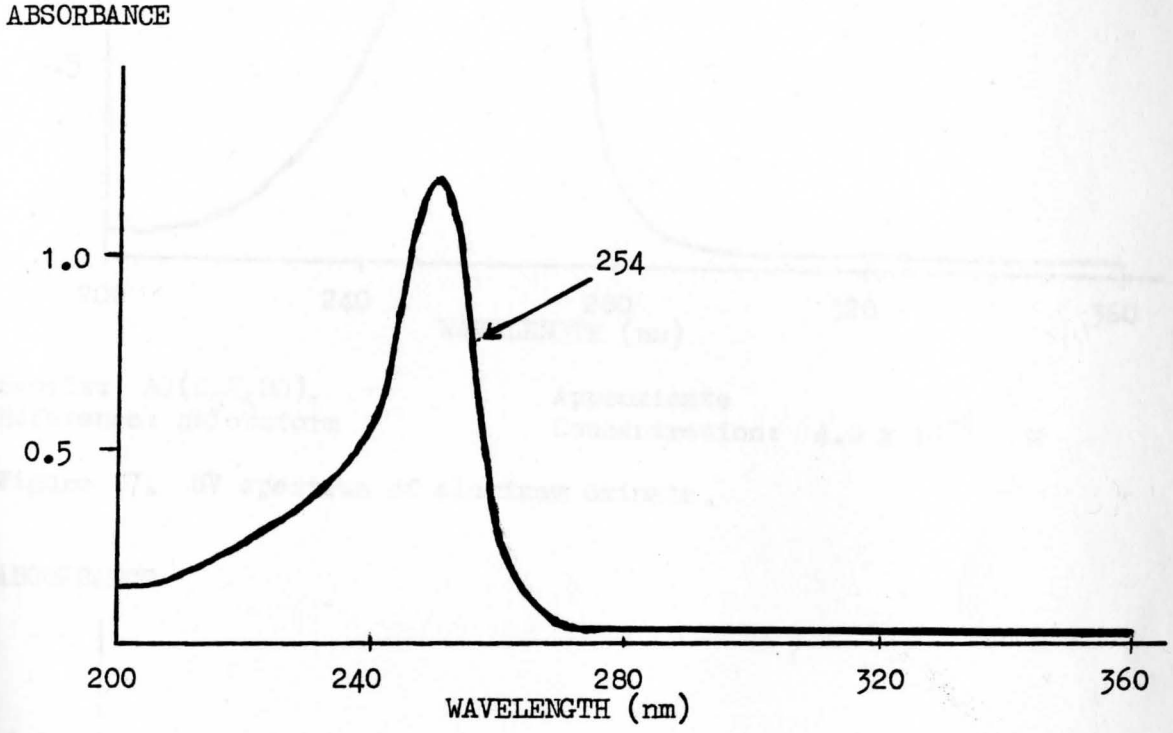
This study was qualitative in nature; however, preliminary results indicate aluminum may be quantitatively determined. Further possibilities which can be pursued include the use of gradient elution to separate the other metal complexes. Since these chelates are eluted from the CN column with chloroform, a gradient program which allows a gradual solvent change from hexane/chloroform (80:20) to 100% chloroform, should allow a separation for most of the metal cupferrates.

APPENDIX

UV Spectra for the Metal Complexes

The UV spectra for the metal complexes and pure reagents were obtained using the Beckman 26 double beam, recording spectrophotometer. The samples were extractions in chloroform (for concentrations, see Tables 8 and 10) which were diluted 1:50 with chloroform (for analysis of solvent behavior other organic solvents were used for the dilution and reference). Chloroform was also used in the reference cell of the instrument, when appropriate. The instrumental conditions for this determination are as follows:

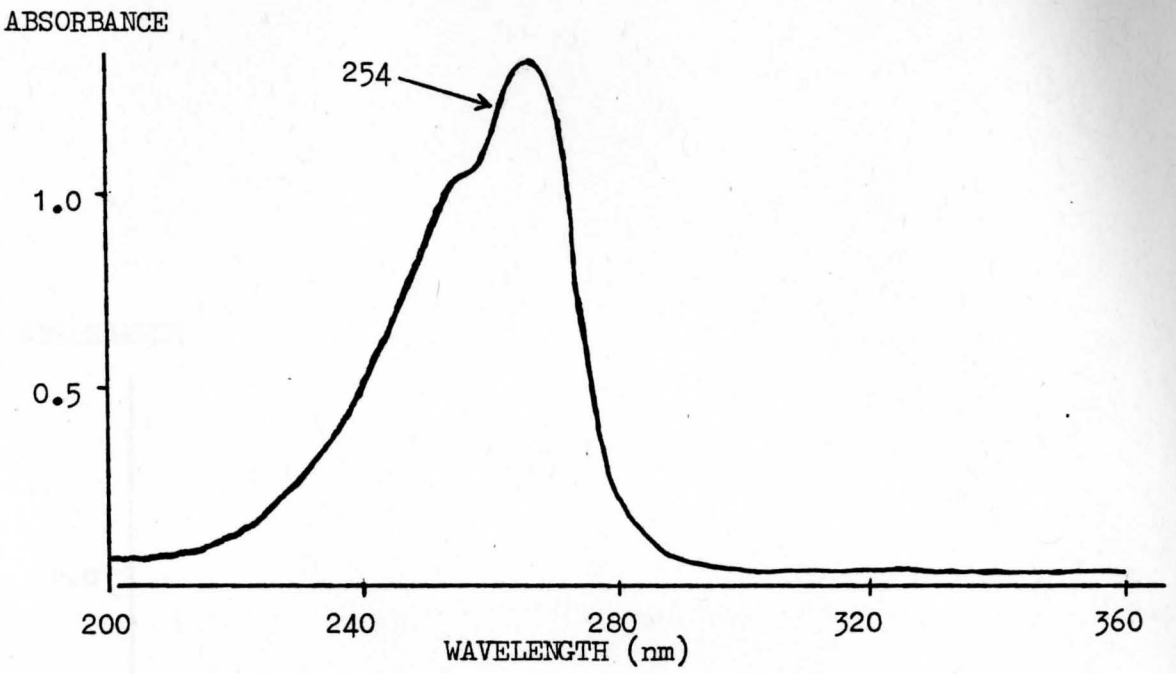
Period	200-360 nm (UV)
Scan Speed	20 nm/min.
Scale	0-2 absorbance units



Sample: 8-Hydroxyquinoline
Reference: Chloroform

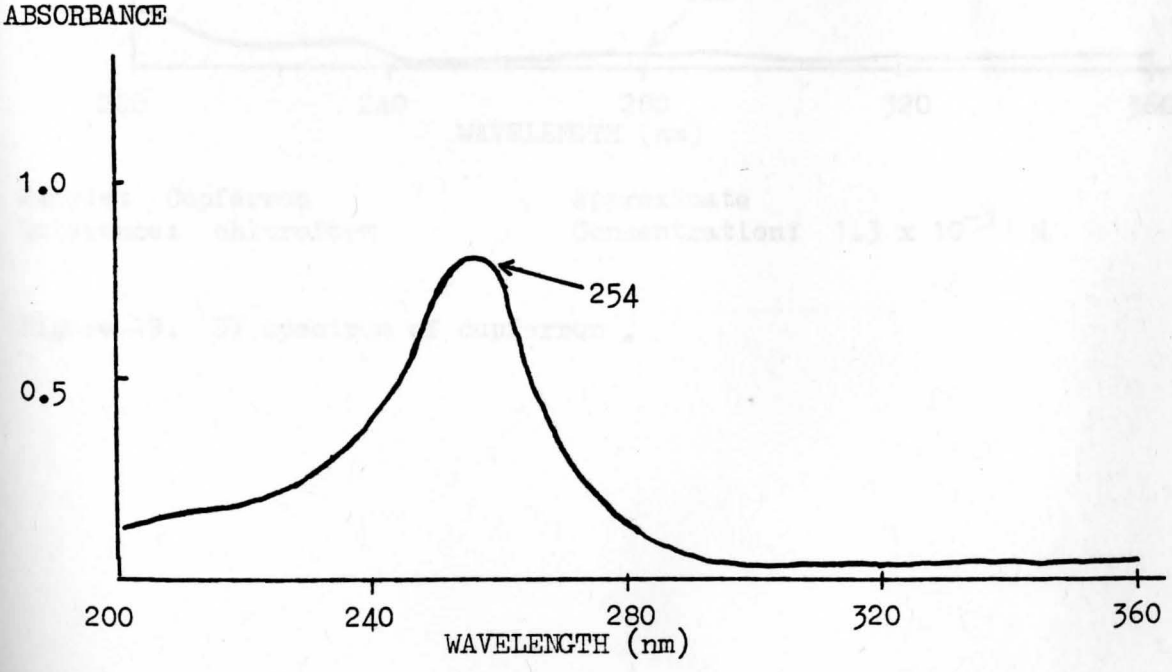
Approximate
Concentration: 1.4×10^{-3} M

Figure 16: UV spectrum of 8-hydroxyquinoline.



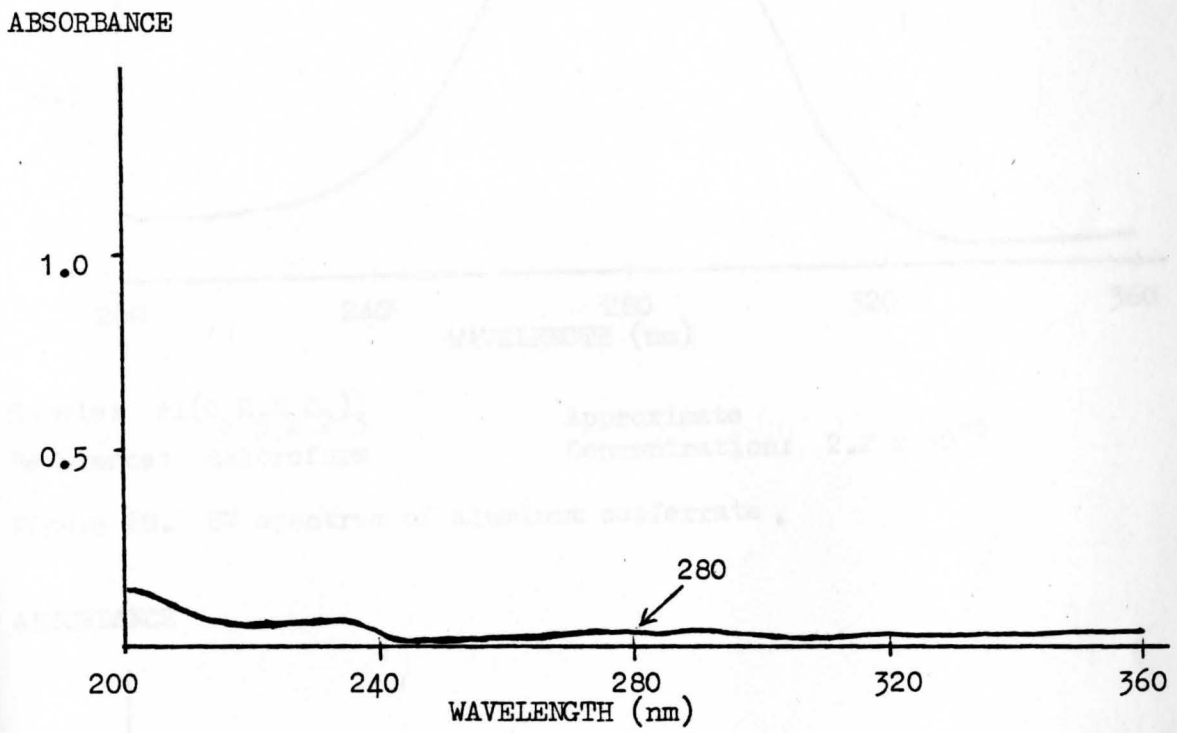
Sample: $Al(C_9H_6NO)_3$ Approximate
Reference: chloroform Concentration: 4.8×10^{-4} M

Figure 17. UV spectrum of aluminum oxinate.



Sample: $Cu(C_9H_6NO)_2$ Approximate
Reference: chloroform Concentration: 6.8×10^{-4} M

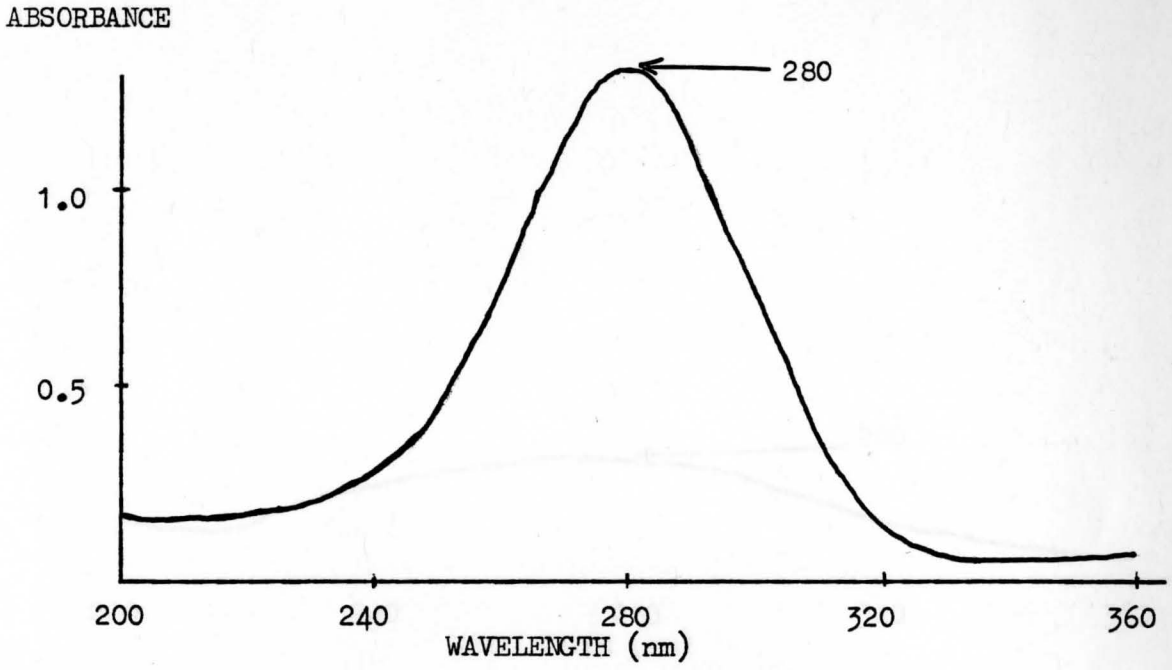
Figure 18. UV spectrum of copper oxinate.



Sample: Cupferron
Reference: chloroform

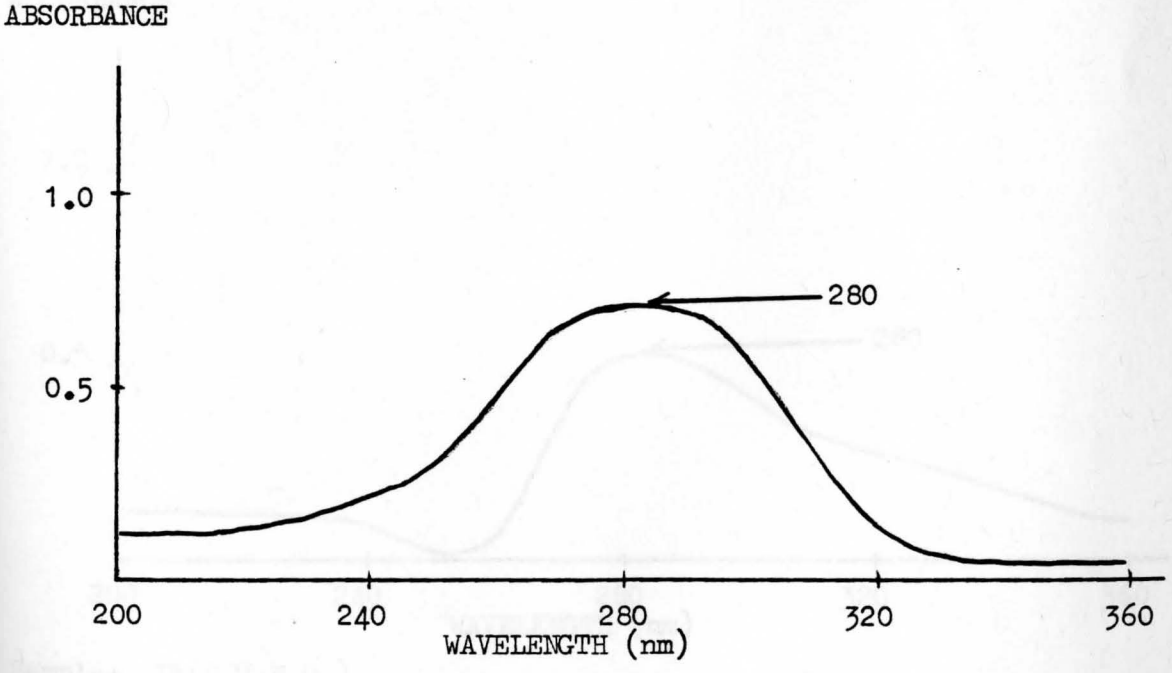
Approximate
Concentration: 1.3×10^{-3} M

Figure 19. UV spectrum of cupferron .



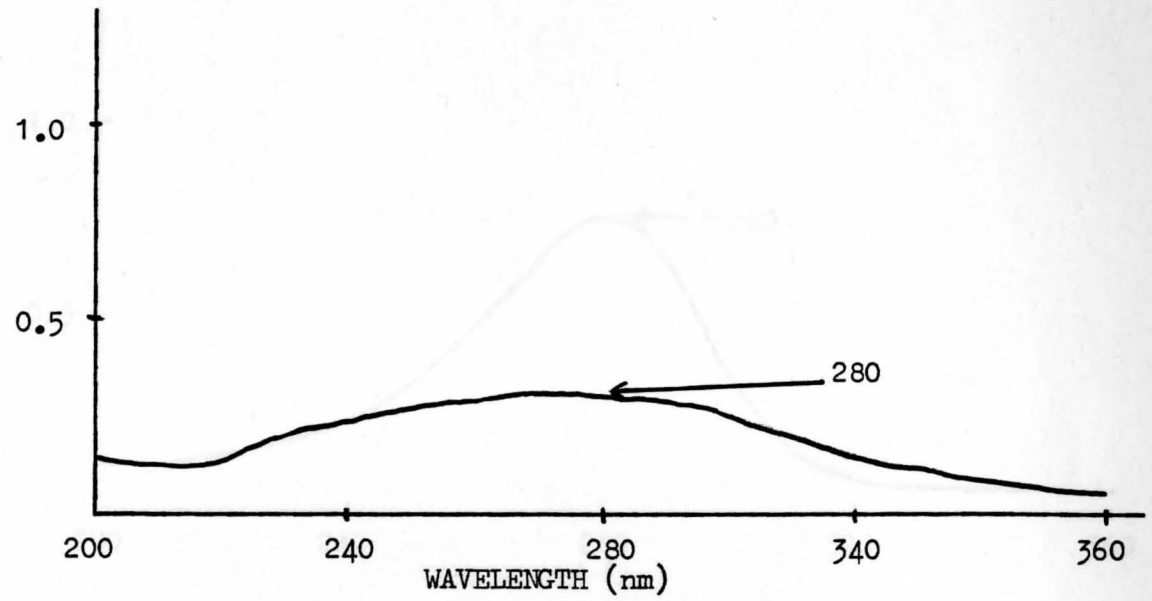
Sample: $Al(C_6H_5N_2O_2)_3$ Approximate
Reference: chloroform Concentration: 2.2×10^{-4}

Figure 20. UV spectrum of aluminum cupferrate.



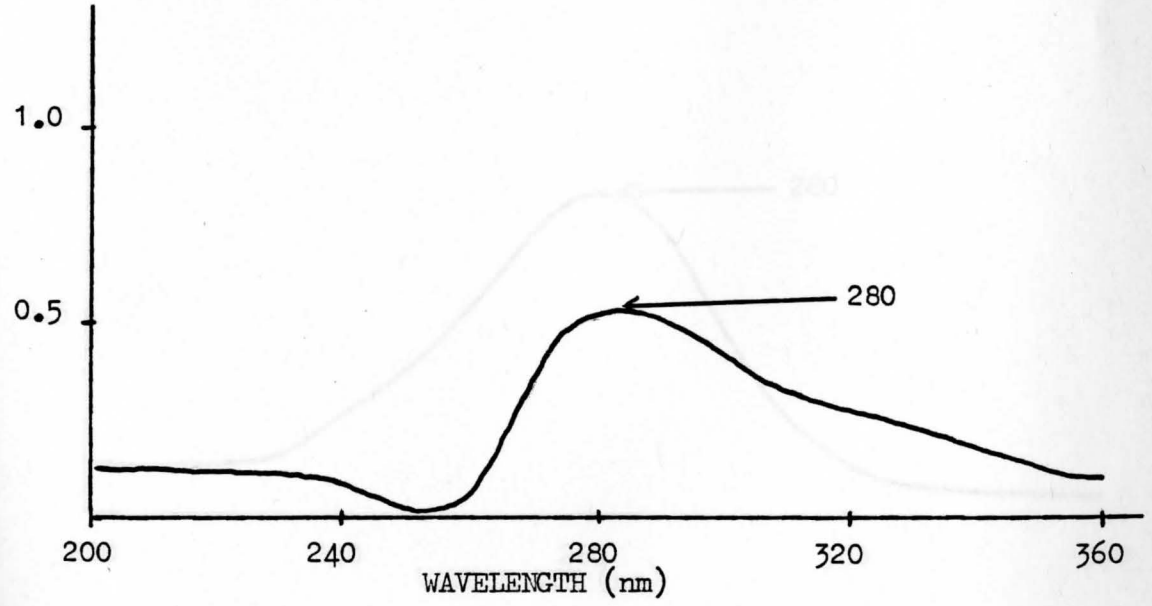
Sample: $Be(C_6H_5N_2O_2)_2$ Approximate
Reference: chloroform Concentration: 3.3×10^{-4}

Figure 21. UV spectrum of beryllium cupferrate.



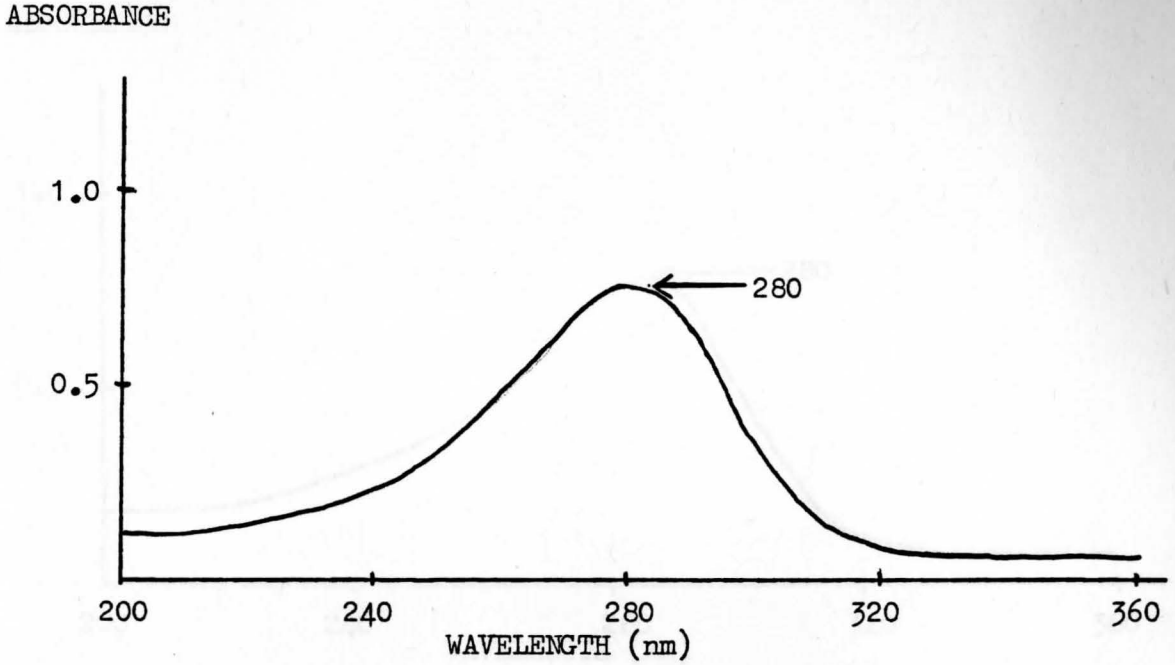
Sample: $Cu(C_6H_5N_2O_2)_2$ Approximate
Reference: chloroform Concentration: 3.1×10^{-4}

Figure 22. UV spectrum of copper cupferrate.



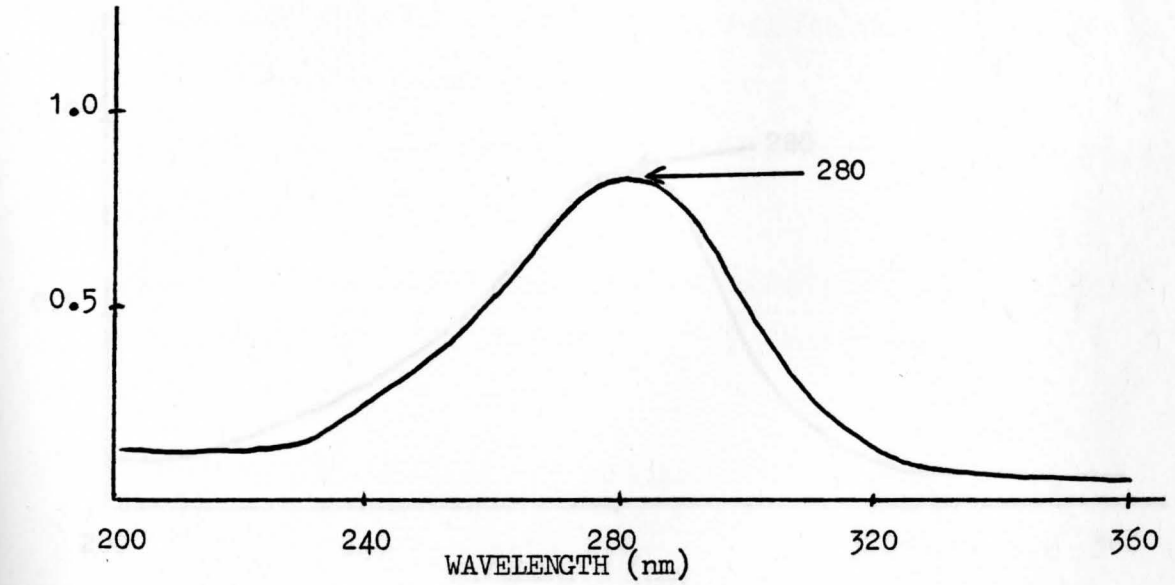
Sample: $Pb(C_6H_5N_2O_2)_2$ Approximate
Reference: chloroform Concentration: 3.1×10^{-4}

Figure 23. UV spectrum of lead cupferrate.



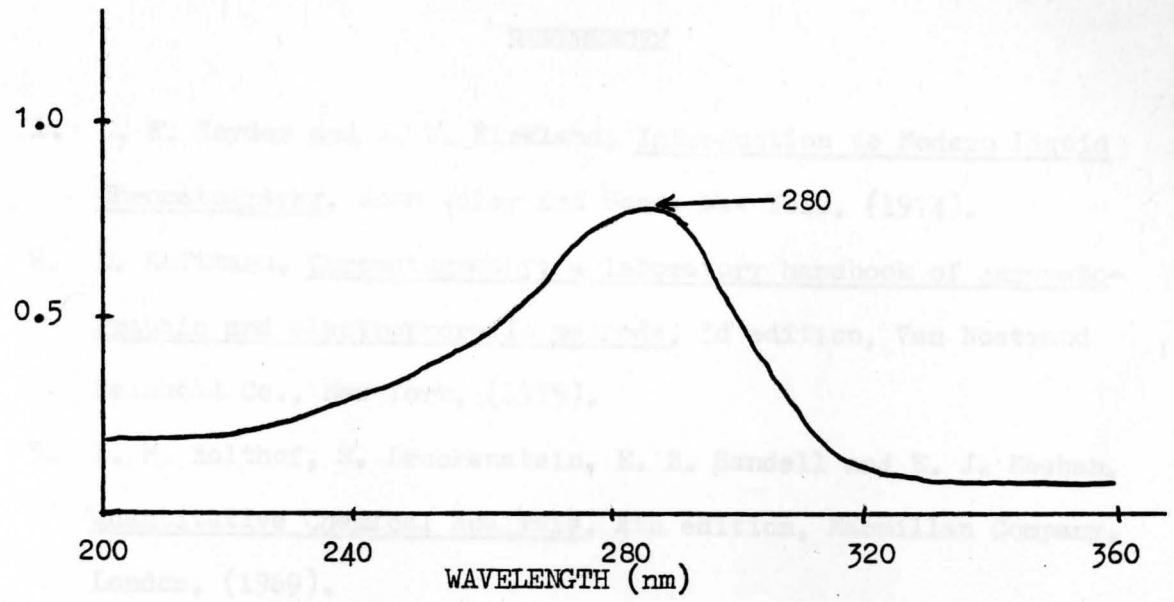
Sample: $Fe(C_6H_5N_2O_2)_3$ Approximate
Reference: chloroform Concentration: 2.1×10^{-4}

Figure 24. UV spectrum of iron cupferrate (in chloroform).



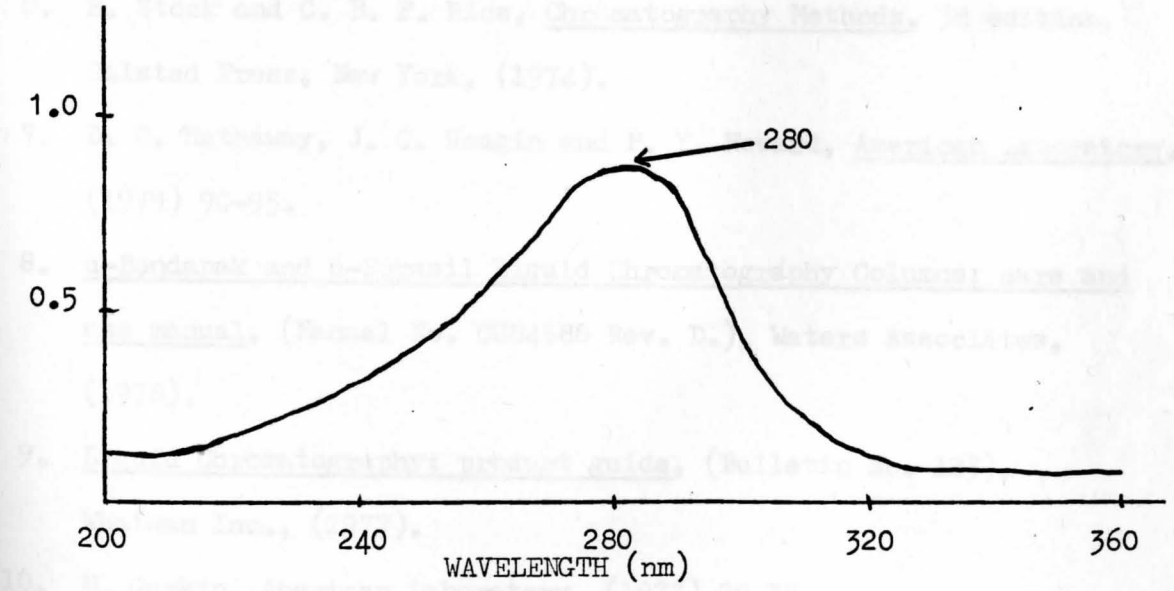
Sample: $Fe(C_6H_5N_2O_2)_3$ Approximate
Reference: methanol Concentration: 2.1×10^{-4}

Figure 25. UV spectrum of iron cupferrate (in methanol).



Sample: $Fe(C_6H_5N_2O_2)_3$ Approximate
Reference: n-hexane Concentration: 2.1×10^{-4}

Figure 26. UV spectrum of iron cupferrate (in hexane).



Sample: $Fe(C_6H_5N_2O_2)_3$ Approximate
Reference: acetonitrile Concentration: 2.1×10^{-4}

Figure 27. UV spectrum of iron cupferrate (in acetonitrile).

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