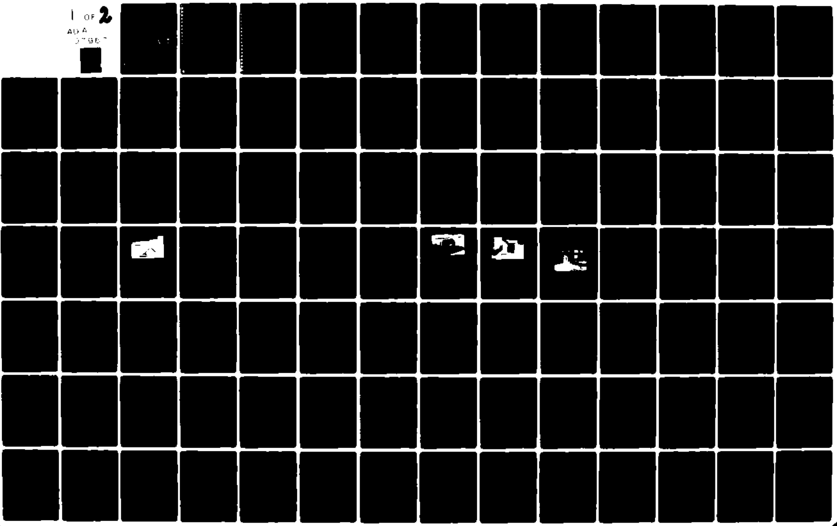
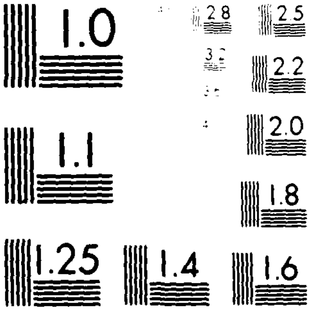
AD-A107 967 AIR FORCE INST OF TECH WRIGHT-PATTERSON AFB OH F/G 7/4
SEPARATION OF METAL IONS (FE(+3), CU(+2), NI(+2), ZN(+2)) BY US--ETC(U)
1979 K T DENBLEYKER
UNCLASSIFIED AFIT-CI-79-310T-S NL

1 OF 2
AD A
107967



796



MICROCOPY RESOLUTION TEST CHART
[illegible]

AD A107967

UNCLASS

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

## REPORT DOCUMENTATION PAGE

READ INSTRUCTIONS BEFORE COMPLETING FORM

1. REPORT NUMBER
79-310T-S

2. GOVT ACCESSION NO.
AD-A107 967

3. RECIPIENT'S CATALOG NUMBER

4. TITLE (and Subtitle)
Separation of Metal Ions ($Fe^{+3}$, $CU^{+2}$, $Ni^{+2}$, $Zn^{+2}$) by Use of Bonded Phases in Thin-Layer Chromatography and High-Performance Liquid Chromatography

5. TYPE OF REPORT & PERIOD COVERED
THESIS/~~DISSERTATION~~

6. PERFORMING ORG. REPORT NUMBER

7. AUTHOR(s)
Capt Kenneth T. DenBleyker

①

8. CONTRACT OR GRANT NUMBER(s)

9. PERFORMING ORGANIZATION NAME AND ADDRESS
AFIT STUDENT AT: Ohio State University

10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS

11. CONTROLLING OFFICE NAME AND ADDRESS
AFIT/NR
WPAFB OH 45433

12. REPORT DATE
1979

13. NUMBER OF PAGES
74

14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)

LEVEL ✓

15. SECURITY CLASS. (of this report)
UNCLASS

15a. DECLASSIFICATION DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)
APPROVED FOR PUBLIC RELEASE; DISTRIBUTION UNLIMITED

DTIC
SELECTE
DEC 2 1981
S D
H

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

23 NOV 1981

Fredric C. Lynch

18. SUPPLEMENTARY NOTES
APPROVED FOR PUBLIC RELEASE: IAW AFR 190-17

**FREDRIC C. LYNCH, Major, USAF**
**Director of Public Affairs**
Air Force Institute of Technology (ATC)
Wright-Patterson AFB, OH 45433

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)
ATTACHED

**81 11 30 024**

DD FORM 1 JAN 73 1473 EDITION OF 1 NOV 65 IS OBSOLETE

UNCLASS

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

DTIC FILE COPY

79-123T

Kenneth T. DenBleyker B. Sc. ChE
CAPT.; USAF
1979
94 pages
M. Sc. (Analytical Chemistry)
The Ohio State University

# SEPARATION OF METAL IONS ($Fe^{+3}$, $Cu^{+2}$, $Ni^{+2}$, $Zn^{+2}$) BY USE OF BONDED PHASES IN THIN-LAYER CHROMATOGRAPHY AND HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

## ABSTRACT

The purpose of this research was to obtain quick, chromatographic separations of $Fe^{+3}$, $Cu^{+2}$, $Ni^{+2}$ and $Zn^{+2}$ metal ions using thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC) on bonded stationary phases and to compare the TLC separations with TLC separations obtained on plain silica gel. Eight bonded-phase substrates were synthesized, immobilizing amino, dithiocarbamate and β-diketone groups on a silica surface. Separations were obtained on these bonded phases and on two types of silica using up to thirty mobile phases. An immobilized trifluoro β-diketone substrate used with a 10% trifluoroacetylacetone in acetone mobile phase gave a well resolved separation of the four metals in TLC. An HPLC column packed with this substrate, and using the same

mobile phase as in TLC, showed well resolved separation of $Cu^{+2}$, $Zn^{+2}$ and $Ni^{+2}$. $Fe^{+3}$ was not injected onto the column because of its extremely long retention time. Detection of the metals was accomplished by spraying with diphenylcarbazide for TLC and by visible and atomic absorption spectrometry for HPLC.

~~79-123T~~
79-310T-S

Kenneth T. DenBleyker B. Sc. ChE
CAPT.; USAF
1979
94 pages
M. Sc. (Analytical Chemistry)
The Ohio State University

# SEPARATION OF METAL IONS ($Fe^{+3}$, $Cu^{+2}$, $Ni^{+2}$, $Zn^{+2}$) BY USE OF BONDED PHASES IN THIN-LAYER CHROMATOGRAPHY AND HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

## ABSTRACT

The purpose of this research was to obtain quick, chromatographic separations of $Fe^{+3}$, $Cu^{+2}$, $Ni^{+2}$ and $Zn^{+2}$ metal ions using thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC) on bonded stationary phases and to compare the TLC separations with TLC separations obtained on plain silica gel. Eight bonded-phase substrates were synthesized, immobilizing amino, dithiocarbamate and $\beta$-diketone groups on a silica surface. Separations were obtained on these bonded phases and on two types of silica using up to thirty mobile phases. An immobilized trifluoro $\beta$-diketone substrate used with a 10% trifluoroacetylacetone in acetone mobile phase gave a well resolved separation of the four metals in TLC. An HPLC column packed with this substrate, and using the same

**81 11 30 024**

mobile phase as in TLC, showed well resolved separation of $Cu^{+2}$, $Zn^{+2}$ and $Ni^{+2}$. $Fe^{+3}$ was not injected onto the column because of its extremely long retention time. Detection of the metals was accomplished by spraying with diphenylcarbazide for TLC and by visible and atomic absorption spectrometry for HPLC.

# SEPARATION OF METAL IONS ($Fe^{+3}$, $Cu^{+2}$, $Ni^{+2}$, $Zn^{+2}$) BY USE OF BONDED PHASES IN THIN-LAYER CHROMATOGRAPHY AND HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

A Thesis

Presented in Partial Fulfillment of the Requirements
for the Degree Master of Science

by

Kenneth T. DenBleyker, B.Sc. ChE

The Ohio State University
1979

Approved by

Adviser

Department of Chemistry

# PREFACE

The metal ions $Fe^{+3}$, $Cu^{+2}$, $Ni^{+2}$ and $Zn^{+2}$ have been difficult to separate in a quick, one-step, chromatographic process. Some of these metals, and in a few cases, all of them have been reported as separable on silica gel, alumina, paper, or on these substrates which were impregnated with various reagents. Separation of all four of these metal ions using bonded stationary phases, however, has never been reported.

Bonded stationary phases have the advantage of longer life over plain silica gel coated with a liquid stationary phase, especially when used within a pH range of 2 to 8 or with organic mobile phases.

This study was undertaken to obtain quick, isocratic, chromatographic separations of these four metal ions using bonded stationary phases and to compare these separations with those using plain silica gel.

# ACKNOWLEDGEMENTS

I would like to thank my adviser, Dr. Thomas R. Sweet for his advice, cooperation and patience throughout the course of this research.

I would also like to thank the United States Air Force, Air Force Institute of Technology for this assignment to Ohio State University in order to obtain this degree.

Above all, I am extremely grateful to my fiancé, Cathy, without whose love and patience throughout this period, I would never have succeeded in this endeavor.

# VITA

| | |
|---|---|
| December 23, 1950 | Born - Paterson, New Jersey |
| 1973 . . . . . . . | B.S. ChE, New Jersey Institute of Technology, Newark, New Jersey |
| 1973-1977 . . . . | Lieutenant, USAF; Scientific and Technical Exploitation Program Manager; Foreign Technology Division, Wright-Patterson AFB, Ohio |
| 1977-1979 . . . . | Captain, USAF; Student; Ohio State University, Columbus, Ohio |

# TABLE OF CONTENTS

## LIST OF FIGURES

## LIST OF FIGURES (cont.)

# LIST OF TABLES

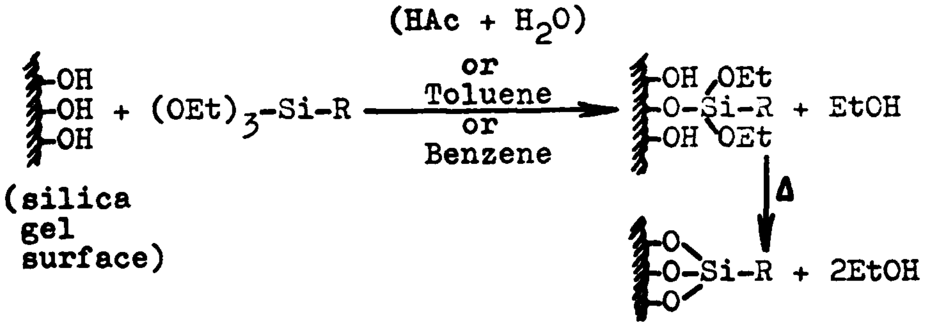
## LIST OF TABLES (cont.)

# INTRODUCTION

The purpose of this research was to obtain simple, chromatographic separations of $Fe^{+3}$, $Cu^{+2}$, $Ni^{+2}$ and $Zn^{+2}$ ions using bonded stationary phases and to compare these separations with those obtained using plain silica gel.

Thin-layer chromatography (TLC) was performed on these four metal ions. Retention factor ($R_f$) values were obtained on eight bonded-phase substrates and on two silica gel substrates using up to thirty different mobile phases. High-performance liquid chromatography (HPLC) was also performed on these metal ions on one bonded phase using a single solvent.

Synthesis of bonded-phase substrates is, generally, fairly simple. Dried silica gel is reacted with an organic triethoxysilane. The silanol groups on the surface of silica react with the ethoxy groups of the silane as shown in Equation 1.



(1)

This step is done in benzene or toluene media, or in aqueous, dilute acetic acid. Curing by heating is done to ensure complete bond formation between the silica gel matrix and the silane as shown in Equation 1.[1] The final product can be used as packing for a chromatography column and as a substrate (stationary phase) for thin-layer chromatography. The silylation step can also be accomplished using a trichlorosilane in place of a triethoxysilane with similar results.

Optimum loading of silane onto the silica seems to occur when a mixture of 10 ml of a 10% silylating solution and 5 grams of silica gel is used.[2]

The R group in the immobilized chain in Equation 1 can be reacted with various organic reagents to add other functional groups to the chain.

The chromatographic process which takes place on silica gel is primarily physical adsorption. The metal ions are adsorbed onto and released from the silica surface at various rates. These rates are contingent upon ionic charge, pH, solvent polarity, type of solvent, metal complex formation constants and reaction reversibility (if a complexing agent is present), and temperature among other factors.

In bonded-phase chromatography, the process is predominately partition chromatography. This is analogous to a solvent extraction process, so the $R_f$ values in TLC or

---

[1] Leyden, D.E., Luttrell, G.H.; Anal. Chem., 47, 1612 (1975).

[2] Ibid

the retention times in HPLC of the metal ions are dependent upon their distribution ratios between the stationary phase and the mobile phase. The distribution ratio is dependent upon pH, metal complex formation constants, reversibility and solubility of the metal ion and/or complex in the mobile phase among other factors.

The silica surface of the bonded-phase substrates used in this research is only about 10-11% silylated. Since this leaves many surface silanol groups available, both partition and adsorption chromatography occurs.

Understandably, the actual process or processes occuring can be quite complex. No attempt will be made to explain them in detail. However, this research has uncovered several interesting separations of the four metal ions, $Fe^{+3}$, $Cu^{+2}$, $Ni^{+2}$ and $Zn^{+2}$. Even in cases where no separations were obtained, some substrates showed great promise for the extraction of trace metals in routine purification of samples or as packing material for a "guard" column in HPLC to keep trace metals in a sample from being retained in an analytical column.

## LITERATURE REVIEW

Chromatography of metal ions was investigated by G.M. Schwab and his co-workers as early as 1937. A column packed with alumina was used, but, due to the basic nature of the alumina, ions such as $Fe^{+3}$, $Cu^{+2}$ and $Cr^{+3}$ formed precipitates which then acted as adsorbents.[1]

Since then, papers have been published on separations of metal ions and metal complexes on substrates of silica, alumina, paper and other materials. Reverse-phase chromatography using substrates impregnated with various reagents has also been reported. Other authors reported extraction and separations of metal ions with ion-exchange resins. However, very few investigators reported metal separation on bonded phases and only two, current articles report the separation of up to three transition metals on reverse-phase bonded silica. A summary of some of these papers is given below.

Frache and Dadone performed TLC of 60 metal ions on silica gel without binder using nitric acid/acetone solvent systems. $R_f$ values reported showed $Fe^{+3}$, $Ni^{+2}$ and $Zn^{+2}$ or $Fe^{+3}$, $Ni^{+2}$ and $Cu^{+2}$ would be separable using a 10% 12N nitric acid/90% acetone solvent system.[2]

They also performed TLC of 61 metal ions on silica

---

[1] Smith, O.C.; Inorganic Chromatography; D. Van Nostrand Co., Inc., New York, 1953, pp 21.

[2] Frache, R., Dadone, A.; Chromatographia, 5, 449 (1972).

gel without binder, using sulfuric acid/acetone solvent systems. $R_f$ values were reported which indicate that $Fe^{+3}$, $Ni^{+2}$ and $Cu^{+2}$ are separable with pure acetone. $Cu^{+2}$ and $Zn^{+2}$ did not appear separable using various concentrations of sulfuric acid in acetone.[3]

Frache, et al. reported the separation of Ni from Fe, Cu and Zn by TLC on silica gel without binder. The solvent systems used were various concentrations of phosphoric acid in acetone.[4]

Varshney attempted several TLC separations involving 46 metal ions on silica gel without binder and a methanol/ 50% nitric acid (9:1) solvent system. A $Fe^{+3}$, $Ni^{+2}$ mixture could be separated from a mixture of $Cu^{+2}$ and $Zn^{+2}$, but no further separation was reported.[5]

Qureshi and Thakur studied the chromatographic behavior of 47 metal ions on Silica Gel G using a mobile phase of 1M aqueous sodium chloride solution with 30% acetone. $R_f$ values reported were: $Fe^{+3}$ (0.00-0.88), $Cu^{+2}$ (0.00-0.21), $Ni^{+2}$ (0.90), $Zn^{+2}$ (0.00-0.48). The only reported separation of these four metals was that Ni was separable from Cu and Zn.[6]

---

[3] Frache, R., Dadone, A.; Chromatographia, 6, 433 (1973).

[4] Frache, R., Dadone, A., Baffi, F.; Chromatographia, 9, 83 (1976).

[5] Varshney, R.G.; Curr. Sci., 45, 54 (1976).

[6] Qureshi, M., Thakur, J.S.; Sep. Sci., 11, 467 (1976).

The oxinates of Cu, Co, and Ni were separated on a silica column by Hilliard and Freiser by using a 1% ethanol in chloroform mobile phase. Retention factors were reported for Cu, Co, Ni, Fe and Al.[7]

Rao and Shekar showed a TLC separation of the xanthates of $Cu^{+2}$, $Ni^{+2}$ and $Zn^{+2}$ on Silica Gel G using a toluene/benzene (10:1) solvent system.[8]

Oksala and Krause reported TLC separation of acetylacetonates of $Fe^{+3}$, $Cu^{+2}$, $Ni^{+2}$, $Co^{+3}$ and $Cr^{+3}$ on Silica Gel GF with a diethyl ether mobile phase. $R_f$ values reported were: $Cr^{+3}$ (0.93), $Co^{+3}$ (0.63), $Cu^{+2}$ (0.30), $Fe^{+3}$ (0.00-0.25), $Ni^{+2}$ (0.00). From these values, it appears that $Cu^{+2}$ and $Ni^{+2}$ are easily separable. However, the $Cu^{+2}$ and the $Fe^{+3}$ $R_f$ values and the elongated spot for $Fe^{+3}$ indicate doubtful separability of $Fe^{+3}$ from $Cu^{+2}$ or $Ni^{+2}$.[9]

Uden and Bigley separated diethyldithiocarbamates of $Cu^{+2}$, $Ni^{+2}$ and $Co^{+3}$ on a column using 8 $\mu$m silica and a mobile phase of acetonitrile/diethyl ether/Skelly B (petroleum hydrocarbon) (5:15:80). Detection was by UV and D.C. argon plasma emission spectroscopy.[10]

---

[7] Hilliard, L.B., Freiser, H.; Anal. Chem., 24, 752 (1952).

[8] Rao, A.L.J., Shekar, C.; Fresenius' Z. Anal. Chem., 277, 126 (1976).

[9] Oksala, R.H. Jr., Krause, R.A.; Anal. Chim. Acta, 85, 351 (1976).

[10] Uden, P.C., Bigley, I.E.; Anal. Chim. Acta, 94, 29 (1977).

Lohmueller, *et al.* attempted TLC separations of several metal dithizonates, among which were $Zn^{+2}$, $Cu^{+2}$ and $Ni^{+2}$, on 30-40 μm silica and on alumina. Eluents used were benzene, carbon tetrachloride, tetrahydrofuran, acetonitrile, toluene and chloroform. The metals were eluted in the order of $Cu > Zn > Ni$, but the separation showed overlap of the sample spots. The authors speculated that separation on a column would be very difficult.[11]

Singh, *et al.* attempted TLC separation of morpholine-4-carbodithioate (MCDT) complexes of 10 metal ions on Silica Gel G using various mobile phases. Using *n*-propanol as a mobile phase, $R_f$ values were: Ni (0.10), Cu (0.86). Using chloroform, $R_f$ values were: Fe (0.12), Ni (0.40), Cu (0.60).[12] Apparently, these three metals can be separated.

Uden and Walters succeeded in HPLC separation of $Cu^{+2}$ and $Ni^{+2}$ Schiff base chelates on 10 μm silica. The *N*,*N*'-ethylene*bis*(acetylacetoneimine) complexes of Cu and Ni were separated as were the *N*,*N*'-ethylene*bis*(salicyldimine) complexes of Cu and Ni using a methylene chloride/acetonitrile (4:1) solvent system.[13]

Gagliardi and Deutschmann reported TLC separation of *S*-methyl-*N*-2(-pyridyl)methylenedithiocarbazate (PMTC)

---

[11] Lohmueller, M., Herzmann, P., Ballschmiter, K.; J. Chromatogr., 137, 165 (1977).

[12] Singh, N., Kumar, P., Kansal, B.D.; Chromatographia, 11, 408 (1978).

[13] Uden, P.C., Walters, F.H.; Anal. Chim. Acta, 79, 175 (1975).

complexes of Fe, Cu, Zn, Co and Pd. An alumina substrate and a chloroform mobile phase were used.[14]

Baffi, <u>et</u> <u>al</u>. report the separation of Zn from Ni, Cu and Fe by TLC on cellulose using various concentrations of a tartaric acid, ammonium hydroxide and ethanol mixture as a solvent system.[15]

Soljic and Grba obtained successful separation of Ni, Mn, Co, Cu, Fe and Zn using TLC on cellulose. $R_f$ values obtained, using a solvent system of acetone/hydrochloric acid/water (86:8:7), were: $Ni^{+2}$ (0.09), $Mn^{+2}$ (0.20), $Co^{+2}$ (0.34), $Cu^{+2}$ (0.60), $Fe^{+3}$ (0.83), $Zn^{+2}$ (0.94). This separation was also attempted using silica gel but was unsuccessful.[16]

Kuroda, <u>et</u> <u>al</u>. performed TLC of 41 metal ions on triethylaminoethyl-cellulose using various hydrochloric acid/methanol (1:1) solvent systems. Using 1.0$\underline{M}$ hydrochloric acid, $R_f$ values were: $Fe^{+3}$ (0.91), $Ni^{+2}$ (0.94), $Cu^{+2}$ (0.87), $Zn^{+2}$ (0.37). For 6.0$\underline{M}$ hydrochloric acid, $R_f$ values were: $Fe^{+3}$ (0.79), $Ni^{+2}$ (0.84), $Cu^{+2}$ (0.65), $Zn^{+2}$ (0.42). For 11.2$\underline{M}$ hydrochloric acid, $R_f$ values were: $Fe^{+3}$ (0.51), $Ni^{+2}$ (0.68), $Cu^{+2}$ (0.53), $Zn^{+2}$ (0.48).[17] The best separa-

---

[14] Gagliardi, E., Deutschmann, G.; Mikrochim. Acta, <u>2</u>, 23 (1976).

[15] Baffi, F., Dadone, A., Frache, R.; Chromatographia, <u>9</u>, 280 (1976).

[16] Soljic, Z., Grba, V.; Z. Anal. Chem., <u>278</u>, 363 (1976).

[17] Kuroda, R., Takahashi, T., Oguma, K.; Bull. Chem. Soc. Jpn., <u>49</u>, 815 (1976).

tion appears to be with 6.0<u>M</u> hydrochloric acid.

Kuroda, et al. also performed TLC of 47 metal ions on diethylaminoethyl-cellulose using various concentrations of aqueous sodium thiosulfate as the mobile phase. Although they reported some separations of some metal ions, the extremely close or identical $R_f$ values for $Fe^{+3}$, $Cu^{+2}$, $Ni^{+2}$ and $Zn^{+2}$ showed that a mixture of these four metals was not separable under these conditions.[18]

Shimizu, et al. investigated TLC of 57 metal ions on diethylaminoethyl-cellulose using sulfuric acid/ammonium sulfate solvent systems of various concentrations. Separations of $Fe^{+3}$, $Cu^{+2}$, $Ni^{+2}$ and $Zn^{+2}$ did not look promising due to high $R_f$ values (0.88-1.00) of all these metals with various concentrations of the solvents.[19]

Shimizu, et al. also attempted TLC separations for 58 metal ions on carboxymethyl-cellulose with sulfuric acid/ammonium sulfate solvent systems. These results, also, did not look promising for the separation of Fe, Cu, Ni and Zn.[20]

Shimizu and Kobayashi reported TLC $R_f$ values for 48 metal ions on cellulose phosphate using various concentrations of phosphoric acid as a mobile phase. The $R_f$ values

---

[18] Kuroda, R., Matusue, H., Oguma, K.; Radioanal. Chem., 36, 119 (1977).

[19] Shimizu, T., Katsuno, M., Iizuka, N.; J. Chromatogr., 106, 230 (1975).

[20] Shimizu, T., Kogure, Y., Arai, H., Suda, T.; Chromatographia, 9, 85 (1976).

reported indicate possible separations of $Fe^{+3}$ from $Cu^{+2}$, $Ni^{+2}$ and $Zn^{+2}$ at concentrations of 1-2<u>M</u> phosphoric acid.[21] No other separations of these four metals looked feasible.

TLC separation of Fe, Cu, Ni and Zn appears possible on a cellulose phosphate substrate using a 0.025<u>M</u> sulfuric acid/1.0<u>M</u> ammonium sulfate solvent system.[22]

Qureshi, *et al*. performed TLC of 40 metal ions using a stannic antimonate substrate. Various aqueous and mixed-solvent systems were used employing dimethyl sulfoxide, hydrochloric acid and nitric acid. No separations for $Fe^{+3}$, $Ni^{+2}$, $Cu^{+2}$ and $Zn^{+2}$ were reported.[23]

Qureshi, *et al*. also used stannic arsenate substrate for TLC studies of 42 metal ions. Solvent systems employed were, nitric acid, hydrochloric acid, aqueous sodium nitrate, arsenic acid and tartaric acid. They reported separation of $Fe^{+2}$ and $Ni^{+2}$ using 1.0<u>M</u> hydrochloric acid as a solvent, and $Ni^{+2}$ and $Cu^{+2}$ using 1.0<u>M</u> nitric acid.[24]

Dingman, *et al*. report a column separation of $Cu^{+2}$, $Zn^{+2}$, $Ni^{+2}$ and $Co^{+2}$ on polyamine-polyurea resins using

---

[21] Shimizu, T., Kobayashi, H.; Chromatographia, <u>10</u>, 611 (1977).

[22] Shimizu, T., Nakawaza, K., Kikuchi, T.; Chromatographia, <u>9</u>, 574 (1976).

[23] Qureshi, M., Varshney, R.G., Rajput, R.P.S.; Sep. Sci., <u>11</u>, 553 (1976).

[24] Qureshi, M., Varshney, R.G., Fatima, N.; Sep. Sci., <u>12</u>, 321 (1977).

hydrochloric acid as an eluent.[25]

Huber, et al. attempted column separations of acetylacetonates and trifluoroacetylacetonates of Be, Al, Cr, Fe, Co, Ni, Cu, Zn, Zr and Ru. The solid support consisted of diatomaceous earth (Kieselguhr, Merck), while 2,2,4-trimethylpentane, ethanol and water was employed as the mobile phase. The promising separations reported were Fe, Be, Cr and Co and Cu, Be, Ru and Co.[26]

Probably the first successful attempt to separate metal ions using reverse-phase chromatography was made by Erlenmeyer and Dahn. Diatomaceous earth was mixed with 8-hydroxyquinoline and used as the adsorbent in a column. Water was the eluent. Among other elements present, four transition metals were reported separable. Their elution order was $Cu^{+2} > Ni^{+2} > Zn^{+2} > Fe^{+3}$. No $R_f$ values were reported.[27]

Muchova and Jokl reported TLC $R_f$ values for 24 metal ions on silica impregnated with tributyl phosphate. Solvents used were lactic acid, tartaric acid, citric acid, iminodiacetic acid, nitrilotriacetic acid, glycine and EDTA. No significant separations of Fe, Cu, Ni and Zn

---

[25] Dingman, J. Jr., Siggia, S., Barton, C., Hiscock, K.B.; Anal. Chem., 44, 1351 (1972).

[26] Huber, J.F.K., Kraak, J.C., Veening, H.; Anal. Chem., 44, 1554 (1972).

[27] Smith, O.C.; Inorganic Chromatography; D. Van Nostrand Co., Inc., New York, 1953, pp 41.

were reported.[28]

$R_f$ values for TLC of 15 metal ions on EDTA-impregnated silica were reported by Srivastava and Dua. Two solvent systems were used. $R_f$ values using ethanol/sodium hydroxide (80:40) were: Cu (0.61), Fe (0.64), Ni (0.57). $R_f$ values using methanol/sodium hydroxide (60:40) were: Cu (0.70), Fe (0.73), Ni (0.67). Separation was not complete due to such close $R_f$ values.[29]

Srivastava, et al. used diethylenetriamine impregnated silica and ethanol/acetone/acetic acid solvent systems to perform TLC separations of 18 metals. Using ethanol/acetone/acetic acid (70:50:20), $R_f$ values reported were: $Zn^{+2}$ (0.38), $Ni^{+2}$ (0.42), $Fe^{+2}$ (0.71), $Cu^{+2}$ (0.22). Using the same solvents in a mixture of (40:20:50), $R_f$ values were: $Zn^{+2}$ (0.39), $Ni^{+2}$ (0.48), $Fe^{+2}$ (0.84), $Cu^{+2}$ (0.28).[30] It appears that either of these solvent systems would effectively separate $Zn^{+2}$, $Ni^{+2}$, $Fe^{+2}$ and $Cu^{+2}$ ions.

Honjo, et al. used cellulose impregnated with monothiothenoyltrifluoroacetone (STTA) and a pH 5 solvent system of hydrochloric acid, pyridine and sodium acetate in an attempt to separate metals by TLC. Among the other sep-

---

[28] Muchova, A., Jokl, V.; Chem. Zvesti, 30, 629 (1976).

[29] Srivastava, S.P., Dua, V.K.; Fresenius' Z. Anal. Chem., 286, 255 (1977).

[30] Srivastava, S.P., Dua, V.K., Gupta, K.; Chromatographia, 11, 539 (1978).

arations reported, $Cu^{+2}$, $Fe^{+2}$ and $Ni^{+2}$ were separated.[31]

Testa used paper impregnated with tri-n-octylamine and developed with various concentrations of aqueous hydrochloric acid to separate $Fe^{+3}$ from $Ni^{+2}$ and $Cu^{+2}$, and to separate $Zn^{+2}$ from $Ni^{+2}$.[32]

Capitan, et al. reported $R_f$ values for 30 metal ions on paper impregnated with 5,5'-methylenedisalicylic acid using solvent systems of butanol/50% nitric acid (7:3), dioxane/50% nitric acid (1:1), acetone/10% nitric acid (7:3) and pyridine/acetic acid (1:1). $R_f$ values using the dioxane/nitric acid solvent system were; $Fe^{+3}$ (1.00), $Cu^{+2}$ (0.74), $Zn^{+2}$ (0.05). For the acetone/nitric acid system, $R_f$ values reported were: $Fe^{+3}$ (0.47), $Cu^{+2}$ (1.00), $Zn^{+2}$ (0.65).[33]

Kojdl used chelating paper incorporating $\alpha(\beta)$-alanine-N,N'-diacetic acid groups in attempts to separate 10 metal ions. Various strengths of hydrochloric acid were used as eluents. Ni and Zn separate at pH=2.5. Fe and Cu separate at pH=0.25 and 0.5. Ni can be separated from Cu and Fe at pH=1.5.[34]

Separations of $Mn^{+2}$, $Fe^{+3}$, $Co^{+2}$, $Ni^{+2}$, $Cu^{+2}$ and $Zn^{+2}$

---

[31] Honjo, T., Fujioka, Y., Itoh, H., Kiba, T.; Anal. Chem., 49, 2241 (1977).

[32] Testa, C.; J. Chromatogr., 5, 236 (1961).

[33] Capitan, F., Salinas, R., Martinez, J.L.; Ars. Pharm., 16, 561 (1975).

[34] Kojdl, I.; Chromatographia, 9, 401 (1976).

were attempted on papers impregnated with paraffin, with zirconium phosphate and with zirconium tungstate using various mixtures of hydrochloric acid, n-butanol, isopropanol, acetone and diphenyl sulfoxide as the mobile phases. The best separation of $Fe^{+3}$, $Ni^{+2}$, $Cu^{+2}$ and $Zn^{+2}$ occurred on zirconium phosphate treated paper using the solvent system of 9.0M hydrochloric acid/n-butanol/acetone (1:1:3).[35]

Singh and Tandon obtained $R_f$ values of 28 metal ions using hydrous zirconium oxide paper and various aqueous mobile phases. $R_f$ values reported show the possibility of separating Ni, Cu and Zn or Ni, Cu and Fe using a 0.01M hydrochloric acid mobile phase. Ni, Cu, Fe and Zn all appear to be separable using a 0.5M ammonium chloride mobile phase or using a mixture of 0.01 moles of hydrochloric acid and 0.5 moles of ammonium chloride in 1 liter of water.[36]

Qureshi and Sharma report several separations of 48 metal ions on paper impregnated with stannic arsenate. A solvent system of 1M dimethyl sulfoxide/nitric acid (1:9) was used to separate $Fe^{+3}$ and $Ni^{+2}$. $R_f$ values reported were: $Fe^{+3}$ (0.00-0.34), $Zn^{+2}$ (0.66-0.90).[37]

---

[35] Durairaj, K., Arulraj, S.J., Patel, C.C.; Indian J. Chem. Sect. A, 15A, 476 (1977).

[36] Singh, N.J., Tandon, S.N.; Chromatographia, 10, 309 (1977).

[37] Qureshi, M., Sharma, S.D.; Chromatographia, 11, 153 (1978).

Gaetani, et al. were successful in using bonded-phase HPLC to separate metal chelates of some $\beta$-ketoamines. Two columns, one using Micropak CH ($C_{18}$-bonded silica, 10 $\mu$m diameter), and one using silica bonded to $\gamma$-aminopropyltriethoxysilane were used. A mobile phase consisted of methanol and 0.006M phosphate or borate buffer. Separation and detection of $Cu^{+2}$ and $Ni^{+2}$ were reported on both columns.[38]

Uden, et al. used bonded-phase HPLC to separate N,N'-ethylenebis(salicyldimine) complexes of Cu, Ni and Pd on 10 $\mu$m, $C_{18}$-bonded silica. A mobile phase of acetonitrile/water (20:80) was used.[39]

Most chromatographic work in the separation of metal ions has been done using TLC or paper chromatography. Little has been reported using HPLC and even less has been reported using bonded-phase substrates for either TLC or HPLC separation of metal ions.

---

[38] Gaetani, E., Laureri, C.F., Mangia, A., Parolari, G.; Anal. Chem., 48, 1725 (1976).

[39] Uden, P.C., Parees, D.M., Walters, F.H.; Anal. Lett. 8, 795 (1975).

# THEORY

The efficiency of a chromatographic separation depends basically upon the differences of the rates at which the solutes are adsorbed onto the stationary phase in adsorption chromatography, or the differences in the partition coefficients of the solutes between the stationary phase and the mobile phase in partition chromatography.

Using mathematical models, it is possible to simulate the behavior of chromatographic systems.

The distribution coefficient, or the partition coefficient, $K_d$, defined in Equation 2, is the ratio of the concentration of solute in the stationary phase, $C_s$, to the concentration of solute in the mobile phase, $C_m$.

$$K_d = \frac{C_s}{C_m} \tag{2}$$

An additional parameter useful in describing the performance of a partition chromatography system is the capacity factor, k'.

$$k' = K_d\left[\frac{V_s}{V_0}\right] \tag{3}$$

$V_s$ is the volume of the stationary phase and $V_0$ is the void volume of the stationary bed (the total internal column volume less the total volume of the packing).

If a sample mixture is to be separated, its components must have different k' values. For a pair of components, this may be expressed in terms of the ratio of the distribution coefficients, $\alpha$, the separation factor.

$$\alpha = \frac{K_{d_2}}{K_{d_1}} = \frac{k'_2}{k'_1} \qquad (4)$$

In thin-layer chromatography (TLC), solute movement can be quantified by comparing the distance moved by the solute with that moved by the solvent. This parameter is the retention factor, $R_f$, and is expressed in Equation 5.

$$R_f = \frac{\text{distance moved by solute}}{\text{distance moved by solvent front}} \qquad (5)$$

$R_f$ is related to k' in column chromatography by Equation 6.

$$R_f = \frac{1}{1 + k'} \quad \text{or} \quad k' = \frac{1}{R_f} - 1 \qquad (6)$$

Height equivalent to a theoretical plate (HETP or simply H) is a measure of efficiency of a chromatographic system.

Local plate height, $H_l$, is defined as the proportionality constant between the differential increase in the variance, $\sigma^2$, of a Gaussian profile spot and the differential length of migration.[1]

$$d(\sigma^2) = H_l dx \qquad (7)$$

The average plate height is derived from measurements

---

[1] Guiochon, G., Siouffi, A.; J. Chromatogr. Sci., 16, 470 (1978).

on developed plates:

$$\overline{H} = \frac{\int_0^x H_1 dx}{\int_0^x dx} = \frac{\int_0^x d(\sigma^2)}{x} = \frac{\sigma^2}{R_f(L - z_o)} \qquad (8)$$

where L is the final distance of the solvent front above the level of the solvent reservoir and $z_o$ is the distance of the original sample spot above the solvent reservoir (see Figure 1).[2]

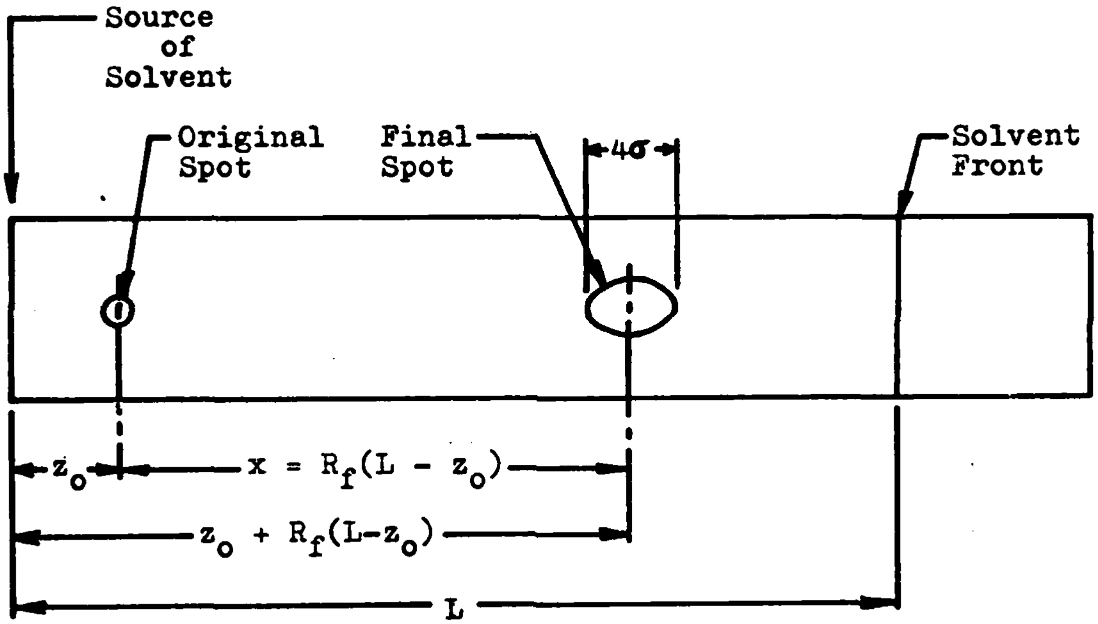


Figure 1. Symbols and Coordinates Used in TLC Theory.

In Figure 1, $4\sigma$ is the effective zone length of the final sample spot. It contains approximately 95.5% of the total sample, assuming a Gaussian distribution of the

---

[2] Guiochon, G., Siouffi, A.; J. Chromatogr. Sci., 16, 470 (1978).

molecules in the spot.

The parameters given here are approximate due to the much more complex process of TLC than of column chromatography. In TLC, one side of the plate is exposed to a vapor phase which may not be totally saturated with solvent vapor, thus creating a mobile phase flux perpendicular to the plate. The substrate not yet wetted by the mobile phase is in contact with the solvent vapor which it progressively adsorbs. This reduces its porosity and increases the apparent velocity of the mobile phase. The mobile phase does not penetrate all the channels of the substrate simultaneously. The solvent is attracted inside the small channels where capillary forces are large. This frontal solvent draws a column of solvent behind it which moves through larger channels where flow resistance is weaker. From the solvent front to the back, increasingly larger channels are filled, resulting in a gradient in the mobile phase layer thickness.[3]

Because of these processes, mobile phase velocity is a complex function of space and time and TLC theory is not treated in a rigorous manner. The conventional parameters ($\alpha$, k' and $R_f$) are used since they are complex averages of local values.[4]

---

[3] Guiochon, G., Siouffi, A.; J. Chromatogr. Sci., 16, 470 (1978).

[4] Ibid

In column chromatography, resolution is a good measure of the system's efficiency. Resolution, $R_s$, is defined as the peak separation divided by the mean peak width for two solutes. The complete equation for resolution can be written as;

$$R_s = -\frac{1}{2}\left[\frac{1-\alpha}{1+\alpha}\right]\left[\frac{\overline{k'}}{1+\overline{k'}}\right]N^{1/2} \qquad (9)$$

where N is the number of theoretical plates and;

$$\overline{k'} = \frac{k'_1 + k'_2}{2} \qquad (10)$$

For baseline separation or "just resolved" solutes, $R_s = 1.5$. If the desired degree of separation or $R_s$ value is known, the number of plates the column possesses can be determined using Equation 9.[5]

The efficiency of a column in terms of N can also be determined from the peak of a single solute using either Equation 11 or Equation 12.

$$N = 16\left[\frac{V'_R}{W}\right]^2 \qquad (11)$$

$$N = 5.54\left[\frac{V'_R}{W_{1/2}}\right]^2 \qquad (12)$$

Figure 2 shows the parameters used in these equations. $V'_R$ is the adjusted solute retention volume, the retention volume ($V_R$) minus $V_0$. W is the peak width, and

---

[5] Knox, J.H.; J. Chromatogr. Sci., 15, 352 (1977).

$W_{1/2}$ is the peak half-width (the width of the peak at one-half of the peak height).

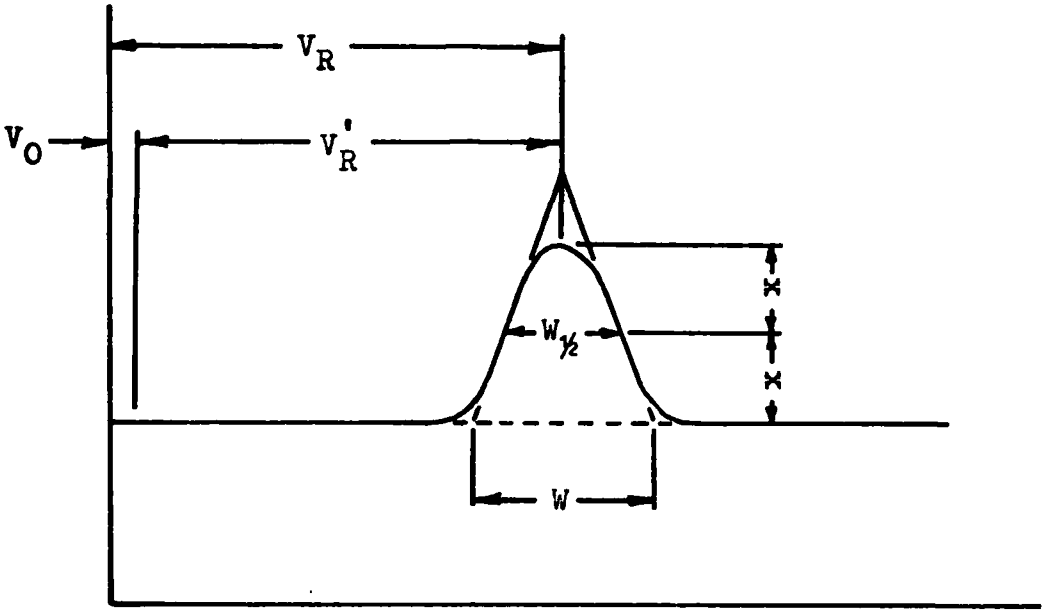


Figure 2. Chromatogram Showing Parameters Needed to Calculate N.

The number of theoretical plates also depends upon the the type of solute, flow rate of the mobile phase, temperature, method of sample introduction and column length. Hence, changing these parameters might be attempted in order to increase N and increase the efficiency of a column.

## EXPERIMENTAL

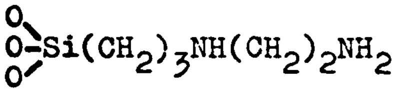
### Substrates

Approximately 300 thin-layer chromatograms were made during the course of this research using two types of silica gel substrates and eight bonded-phase substrates.

The two types of silica used were Silica Gel G: (Type 60) for TLC (E. Merck, Darmstadt, Germany) and Silica Gel 7 (J.T. Baker: "Baker TLC Reagent"). Silica Gel 7 is pure silica of particle sizes approximately 40 μm. Silica Gel G is 87% 63-200 μm silica and 13% calcium sulfate binder.

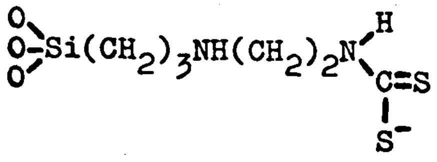
All eight bonded-phase substrates were made using Silica Gel 7 and performing silylation reactions and various organic syntheses which will be discussed later in detail. Below are the eight bonded-phase substrates and their chemical structures.

1. Z-6020 Amine

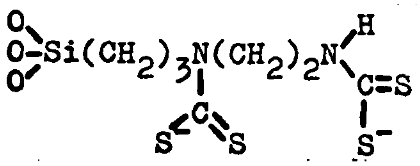


$$\begin{matrix}\mathrm{O} & \\ \mathrm{O{-}Si(CH_2)_3NH(CH_2)_2NH_2} & \\ \mathrm{O} & \end{matrix}$$

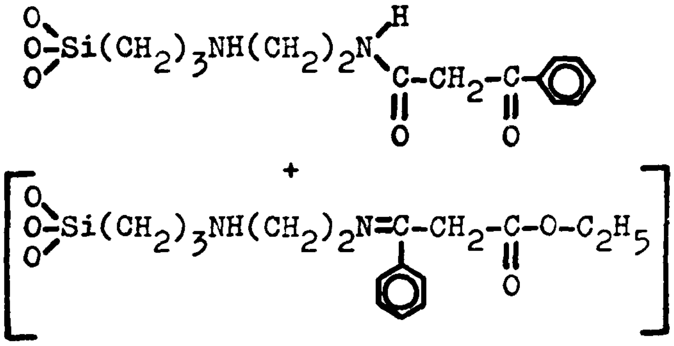
2. Z-6020 Dithiocarbamate



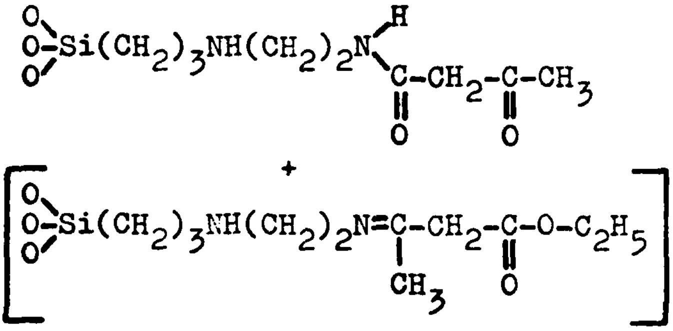
3. Z-6020 <u>bis</u>-Dithiocarbamate



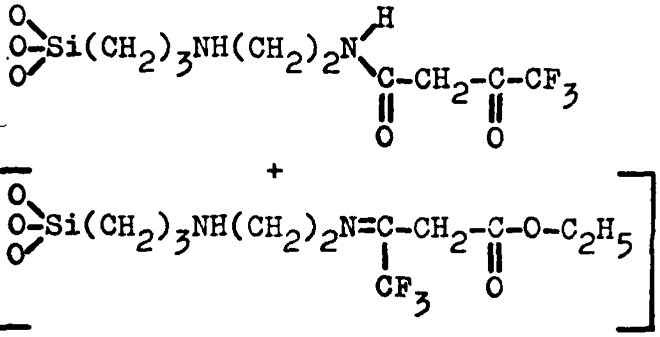
4. Z-6020 β-Diketone-1



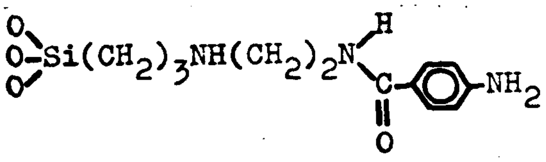
5. Z-6020 β-Diketone-2



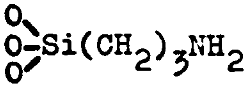
6. Z-6020 β-Diketone-3



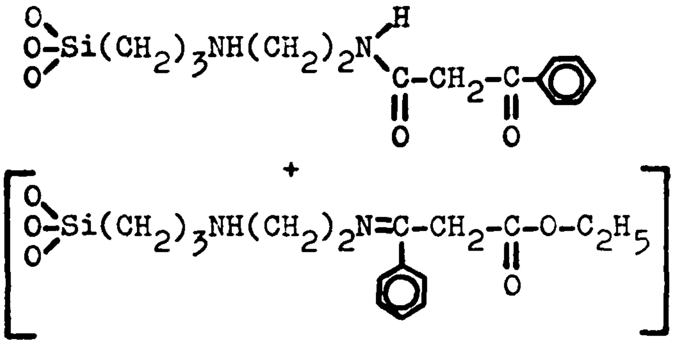
7. Z-6020 Arylamine



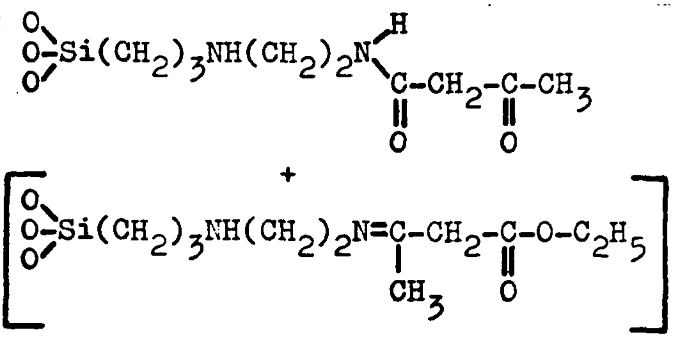
8. γ-Amine



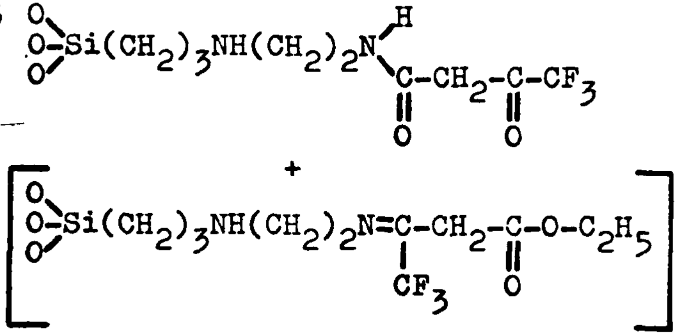
4. Z-6020 β-Diketone-1



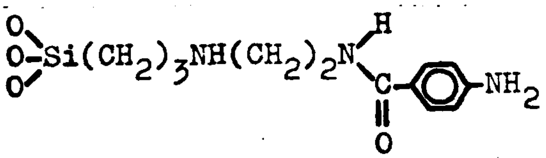
5. Z-6020 β-Diketone-2



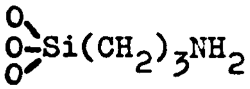
6. Z-6020 β-Diketone-3



7. Z-6020 Arylamine



8. γ-Amine



For the three Z-6020 $\beta$-Diketones, a mixture of two structures was produced.[1] The first one (not in parentheses) is the structure of interest, and is the one that is more effective in the partitioning of the metals between the stationary phase and the mobile phase during chromatographic runs.

Synthesis of Substrates

1. Z-6020 Amine

To synthesize Z-6020 Amine, 180 grams of Silica Gel 7 was vacuum dried at 120° C, 28 in Hg, for 24 hours. The dried silica was then mixed with 972 ml of toluene and 108 ml of 3-(2-aminoethylamino)propyltrimethoxysilane (Dow Corning Z-6020 Silane) in a 2000 ml round-bottom flask. The mixture was refluxed for approximately 6 hours and then filtered and washed with toluene, isopropanol, acetone, methanol and acetone again. It was then vacuum dried at 100° C for about 1 hour and stored in a container at room temperature. C, H, N analyses confirmed the amine structure and showed 10-11% loading on the silica surface.

2. Z-6020 Dithiocarbamate

Z-6020 Amine (60 grams) was added to 200 ml of benzene, 40 ml of isopropanol, 40 ml of carbon disulfide, and 10 ml of a 10% tetramethylammonium hydroxide solution in methanol, and stirred for 20 minutes. The mixture was

---

[1] Chow, F.K., Grushka, E., Anal. Chem., 50, 1346 (1978).

filtered, washed with isopropanol, air dried at room temperature and stored in a refrigerator.[2]

3. Z-6020 bis-Dithiocarbamate

Z-6020 Amine (60 grams) was added to 300 ml of distilled water, 75 ml of 0.25N sodium hydroxide, 75 ml of isopropanol and 60 ml of carbon disulfide and stirred for 20 minutes. The mixture was filtered, washed with isopropanol, air dried at room temperature and stored in a refrigerator.[3]

4. Z-6020 β-Diketone-1

Z-6020 Amine (60 grams) was added to 648 ml of toluene and 72 ml of ethyl benzoylacetate (Aldrich Chemical Co. or Eastman Kodak Co.) in a 2000 ml round-bottom flask. The mixture was refluxed for approximately 6 hours, filtered, washed with toluene, isopropanol and acetone, air dried at room temperature and stored in a dessicator over calcium chloride.

The resultant powder was a mixture of two products as shown previously on page 23. However, the first product, the β-diketone, was the one of interest.[4]

5. Z-6020 β-Diketone-2

Z-6020 Amine (60 grams) was added to 648 ml of tolu-

---

[2] Leyden, D.E., Luttrell, G.H.; Anal. Chem., 47, 1612 (1975).

[3] Ibid.

[4] Chow, F.K., Grushka, E.; Anal. Chem., 50, 1346 (1978).

ene and 72 ml of ethyl acetoacetate (Aldrich Chemical Co.) in a 2000 ml round-bottom flask. The mixture was refluxed for approximately 6 hours, filtered, washed with toluene, isopropanol and acetone, allowed to air dry at room temperature, and stored in a dessicator over calcium chloride.

Two products, again, were present as shown on page 23, but, again, the first product, the β-diketone, was the one of interest.

6. Z-6020 β-Diketone-3

Z-6020 Amine (21 grams) was added to 225 ml of toluene and 25 ml of ethyl 4,4,4 trifluoroacetoacetate (Aldrich Chemical Co.) in a 500 ml round-bottom flask and refluxed for 6 hours. The mixture was filtered, washed with toluene, isopropanol and acetone, allowed to air dry at room temperature, and stored in a dessicator over calcium chloride.

The two products shown on page 23 were present. The first product, the β-diketone, was the one of interest.

7. Z-6020 Arylamine

Z-6020 Amine (120 grams) was mixed with 26.64 grams of p-nitrobenzoyl chloride dissolved in 1140 ml of chloroform and 60 ml of triethylamine. The mixture was stirred for 2 hours, filtered, washed with chloroform and air dried at room temperature. This product was added to 25 grams of sodium dithionite in 1000 ml of distilled water, boiled and stirred for 30 minutes. The mixture was filtered, washed with distilled water and then acetone, air dried at room temperature, and stored in a dessicator over calcium chloride.[5]

8. $\gamma$-Amine

Silica Gel 7 (175 grams) was vacuum dried at 120° C, 28 in Hg, for 24 hours. The dried silica was mixed with 945 ml of toluene and 105 ml of $\gamma$-aminopropyltriethoxysilane (Pierce Chemical Co.) in a 2000 ml round-bottom flask. The mixture was refluxed for 6 hours, filtered, washed with toluene, isopropanol, acetone, methanol and acetone again, air dried at room temperature and stored in a dessicator over calcium chloride.[6] (Note: A sample of $\gamma$-Amine which was vacuum dried at 100° C turned brownish-yellow, possibly due to oxidation to a nitro compound.)

## Manufacture of TLC Plates

The substrate to be used was coated as a 0.25 mm thick layer on 20 cm x 20 cm glass plates. The plates were thoroughly washed with water and detergent and dried before coating. Five of these plates were placed in a plastic jig as shown in Figure 3 and wiped clean with acetone. A slurry of the substrate to be used was made in accordance with the quantities shown in Table I.

---

[5] Weetall, H.H.; Biochim. Biophys. Acta, 212, 1 (1970).

[6] Chow, F.K., Grushka, E.; Anal. Chem., 50, 1346 (1978).

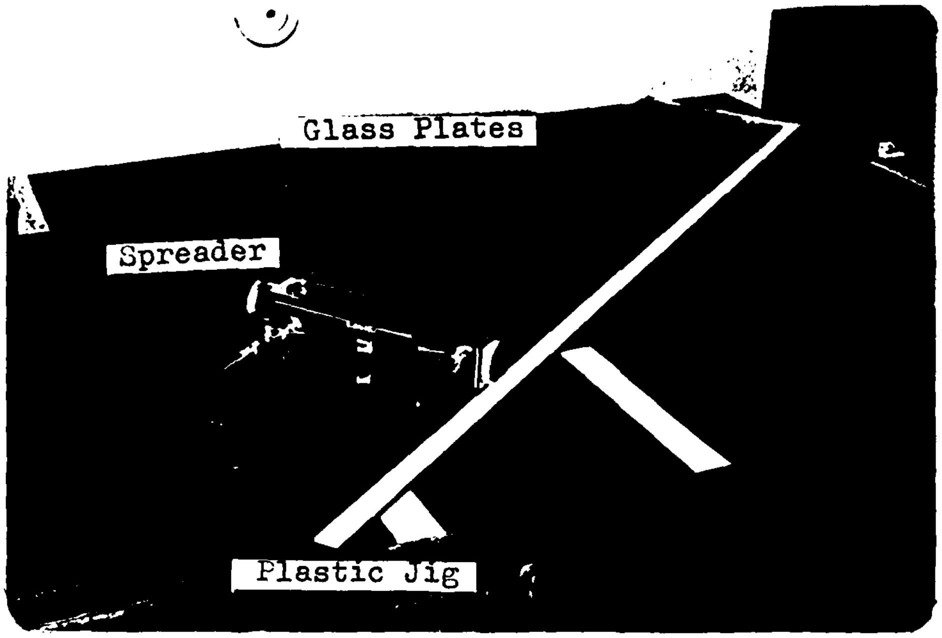


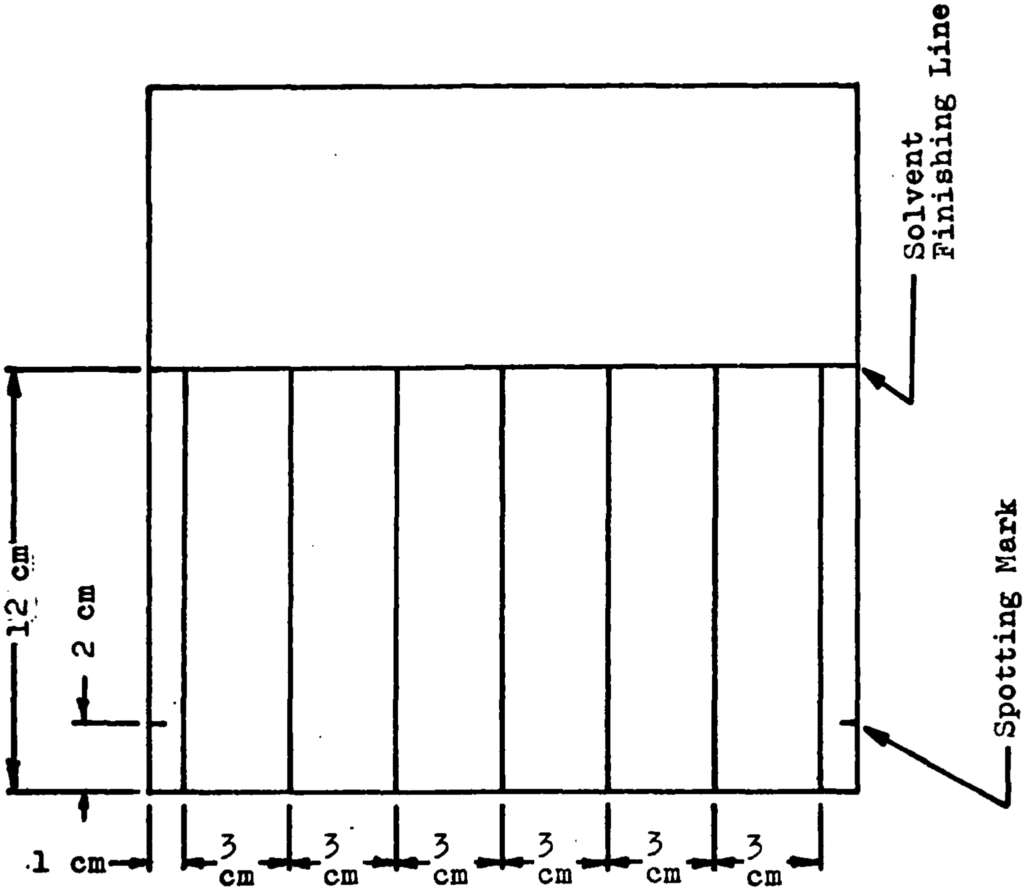
Figure 3. Preparation of TLC Plates.

The slurry was placed in a Desaga-Brinkman variable-thickness spreader set at 0.25 mm layer thickness. The spreader was then drawn smoothly over each plate and the plates were allowed to air dry (see Figure 3). The middle three plates were inspected for uniformity of layer thickness and set aside for "scoring." Plates number one and five were used to catch excess slurry. The substrate was scraped from them, dried and stored for reuse.

After the coated plates had dried, they were "scored." A thin spatula blade was used to scrape away thin lines of substrate from the plates in a pattern shown in Figure 4.

TABLE I. Preparation of Substrate Slurries for TLC Plates (Enough Material to Cover 3 - 20 x 20 cm Glass Plates at a Substrate Thickness of 0.25 mm).

| SUBSTRATE | GRAMS OF DRY SUBSTRATE | ml OF LIQUID |
|---|---|---|
| Silica Gel G | 30 | 60 ml $H_2O$ |
| Silica Gel 7 | 20 | 62-67 ml Isopropanol |
| Z-6020 Amine | 20 | 42 ml $H_2O$ |
| Z-6020 Dithiocarbamate | 25 | 37 ml Isopropanol |
| Z-6020 *bis*-Dithiocarbamate | 30 | 57 ml Isopropanol |
| Z-6020 β-Diketone-1 | 20 | 42 ml Isopropanol |
| Z-6020 β-Diketone-2 | 20 | 38 ml Isopropanol |
| Z-6020 β-Diketone-3 | 20 | 39 ml Isopropanol |
| Z-6020 Arylamine | 20 | 35 ml [50% Isopropanol<br>50% $H_2O$] |
| γ-Amine | 20 | 45-47 ml Isopropanol |



20 cm x 20 cm Square Glass Plate (Coated)

Figure 4. "Scoring" Pattern of a TLC Plate

In this manner, six different samples could be spotted on one plate (one sample in each 3-cm wide strip) and developed simultaneously with minimal interference between samples (i.e., latitudinal spreading).

The marks 2 cm from the lower edge of the plate are the spotting marks and serve as the location for a line on which the samples are spotted and the "zero" point for $R_f$ measurements.

The line 12 cm from the lower edge of the plate prevents the developing solvent from travelling any farther than 12 cm up the plate. This line, being 10 cm from the spotting marks, allows sufficient development time and simplifies calculation of $R_f$ values.

Samples of 1% aqueous solutions of ferric, cupric, nickel and zinc perchlorates were spotted onto the finished TLC plates. The perchlorates were reagent grade and obtained from G. Frederick Smith Chemical Co.

Generally, 1.0-1.5 $\mu$l of aqueous solution (10-15 $\mu$g of perchlorate) per spot were applied to the plates by a Hamilton 10 $\mu$l syringe along the spotting line (see Figure 5).

The exceptions to this were the plates coated with substrates of either Z-6020 $\beta$-Diketone-1 or Z-6020 $\beta$-Diketone-3. Both of these substrates are water repellent and could not be spotted with purely aqueous solutions. A solution of 60% aqueous metal perchlorate and 40% isopropanol could be spotted easily onto these plates. Approximately 2.0 $\mu$l of this solution (12 $\mu$g of sample) per spot were applied to the plate.

## Development and Detection

The spotted TLC plates were developed in a glass tank shown in Figure 6 using ascending solvent development. The internal volume of the tank is approximately 5 liters. In order to assist in saturation of the atmosphere inside the tank, the walls were lined with Whatman No. 1 filter paper which was saturated with the developing solvent.

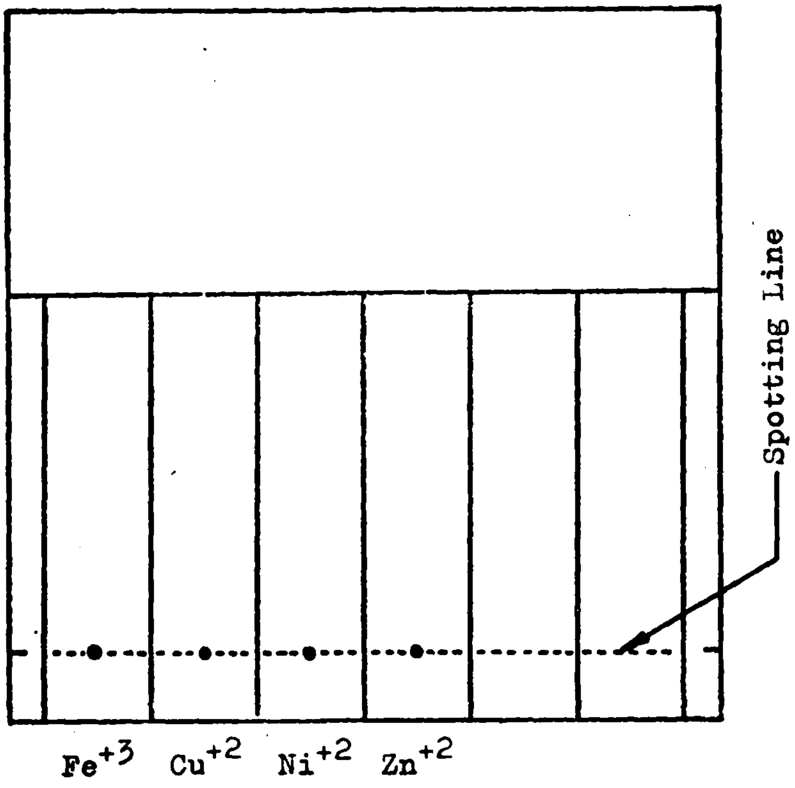


Figure 5. TLC Plate Showing Metal Perchlorate Samples.

Approximately 100-150 ml of solvent were added to the tank. This gave a solvent depth of about 5 mm. The tank was covered with a glass lid and the tank atmosphere was allowed to equilibrate for about 20 minutes. The TLC plate was inserted into the tank and allowed to develop. All plate developments were carried out at temperatures between 21° and 24° C. Development times ranged from 10 to 90 minutes depending upon the solvent and substrate used.

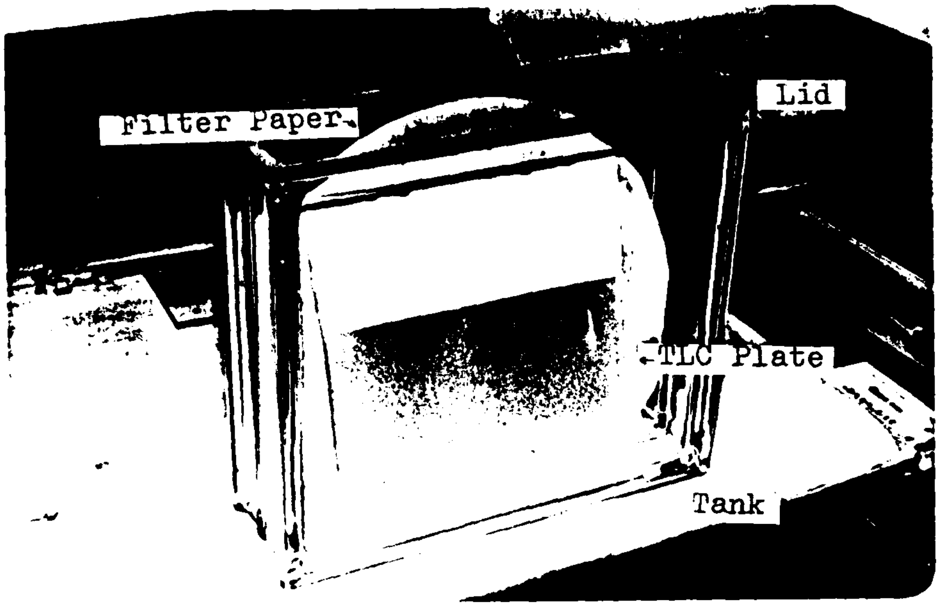


Figure 6. Development of a TLC Plate.

After the developing solvent front reached the finishing line, the plate was removed from the tank and allowed to air dry in a vertical position.

Sample spots were detected by spraying the developed plate with 1% diphenylcarbazide (DPC) in ethanol (see Figure 7). The colors of the metal-DPC complexes are: Fe (orange-brown), Cu (violet-brown), Ni (purple-pink), Zn (pink). In some cases, the plate was also sprayed with 1% aqueous potassium ferrocyanide. This colored the Fe spot blue and facilitated detection when the Fe-DPC spot was too faint.

After detection, each plate was numbered and photographed, giving a permanent record of the chromatograms.

The distance of each sample spot from the spotting marks was measured and $R_f$ values were calculated.

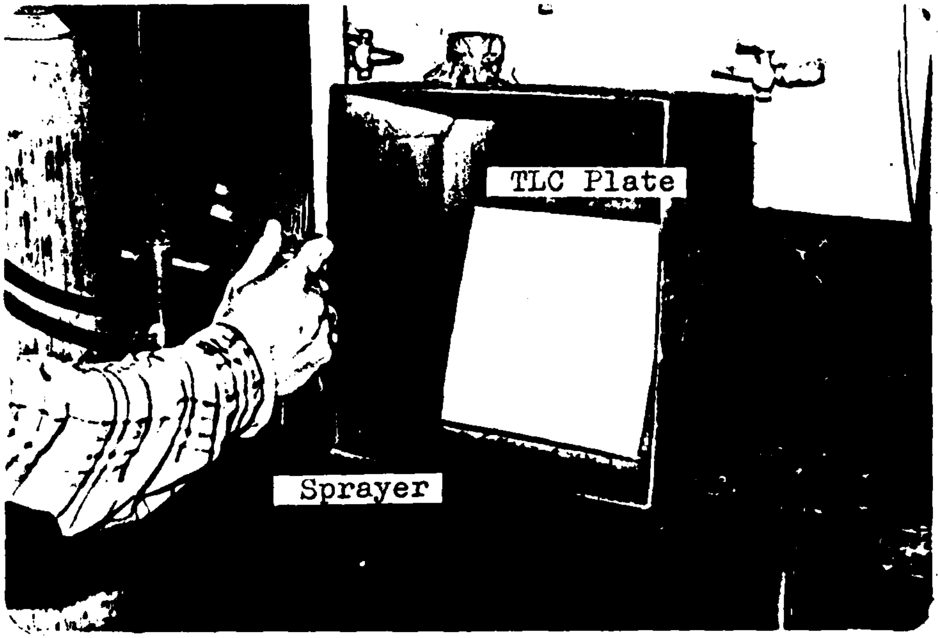


Figure 7. Detection of Sample Spots By Spraying With A Complexing Reagent.

HPLC Separations

Three HPLC columns were made using 2 mm I.D. Type 316 stainless steel tubing and Waters Associates minimum dead-volume fittings. Column lengths were 60, 30 and 15 cm. Z-6020 $\beta$-Diketone-3 was packed into each column using the modified tap-fill dry packing method reported by Kirkland. Plate heights on the order of 0.2 to 0.3 mm for a 1 cm/sec mobile phase velocity were obtained using this technique for Zipax® ($<37\ \mu$m) packing material.[7]

---

[7] Kirkland, J.J.; J. Chromatogr. Sci., 10, 129 (1972).

The inlet of each column, in turn, was connected to a Waters Associates HPLC pump and loop injector system. The column was fed into a Markson 0.25 ml capacity flow-cell which was housed in a Beckman DB UV/Visible spectrophotometer. The Beckman DB was hooked-up to a chart recorder and a 10X scale expander. The system set-up is shown in Figure 8.

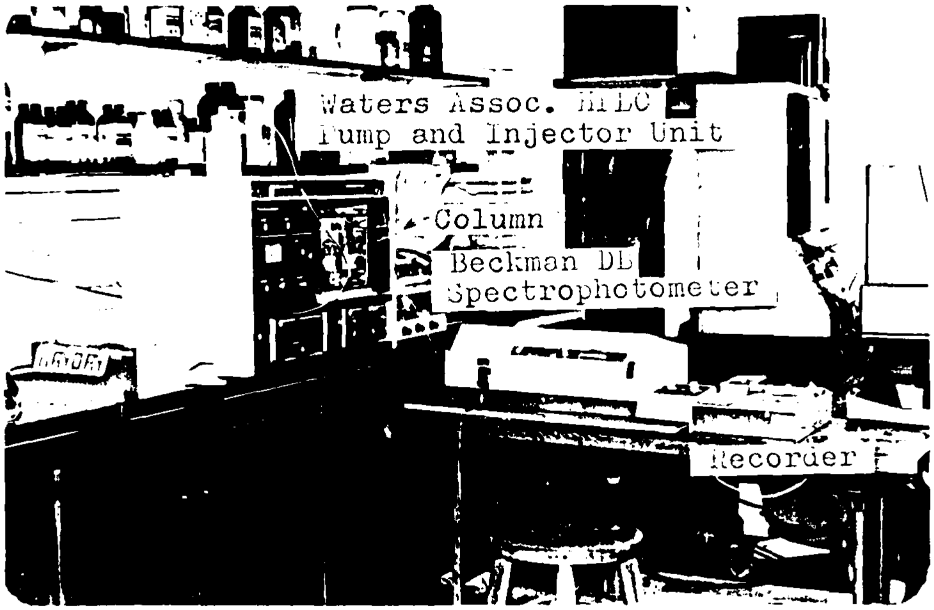


Figure 8. HPLC Equipment Set-Up.

Sample solutions of the perchlorates of Cu, Zn and Ni were prepared for use in the HPLC unit. Solution A was 0.05 grams of cupric perchlorate in 5 ml of 10% trifluoroacetylacetone (TFAA) in acetone (10,000 ppm Cu). Solution B was 0.05 grams of zinc perchlorate in 5 ml of 10% trifluoroacetylacetone (TFAA) in acetone (10,000 ppm Zn). Solution C was 0.05 grams of nickel perchlorate in 5 ml of

10% trifluoroacetylacetone (TFAA) in acetone (10,000 ppm Ni).

Chromatograms were obtained for Cu, Zn and Ni at various flow rates of the 10% TFAA in acetone mobile phase in the following ways.

1). With the spectrophotometer wavelength dial set at 368 nm, 10 $\mu$l of Solution A was injected into the HPLC unit. From absorbance curves previously run between 320 and 800 nm, 368 nm was indicated as the wavelength of maximum absorbance of the Cu-TFAA solution.

2). Several 10 $\mu$l samples of Solution B were injected into the HPLC unit. The spectrophotometer wavelength dial was set at a different value between 340 and 410 nm for each run.

3). Two, 10 $\mu$l samples of Solution C were injected into the HPLC unit. The detection wavelengths were 368 and 373 nm.

In each instance, the HPLC runs were carried out until complete peaks were observed or, if no peak was observed, until a large amount of time had elapsed (i.e., $>$ 25 min).

After considering flow rates, pump back-pressure and sample retention times, it was decided to employ the 30 cm long column for the remainder of the HPLC research.

Chromatograms were obtained for four, 2 $\mu$l samples of Solution A (Cu) injected onto the 30 cm column at mobile phase flow rates of 0.5, 1.0, 1.5 and 2.0 ml/min. From the parameters obtained from the Cu peaks, HETP was calculated at each of the four flow rates and plotted against flow rate in order to obtain the optimum flow rate.

A sample mixture of 8 $\mu$l of Solution C (Ni) and 2 $\mu$l of Solution A (Cu) was injected onto the column in order to obtain the Cu and the Ni retention times. The flow rate of the mobile phase (10% TFAA in acetone) was 1.0 ml/min.

Solutions A, B, and C were all diluted ten-fold with 10% TFAA in acetone to form Solution D (1,000 ppm Cu), Solution E (1,000 ppm Zn) and Solution F (1,000 ppm Ni), respectively. One ml each of Solutions D, E and F were mixed together to form Solution G.

The tubing at the column outlet was disconnected and 10 $\mu$l of Solution G was injected onto the column with the 10% TFAA in acetone mobile phase flow rate set at 1.0 ml/min.

Samples of eluent from the column outlet were collected continuously in 15-second intervals for a 7-minute period. Each of these 28 samples was diluted up to 20 ml with acetone and analyzed for Cu, Zn and Ni using a Perkin-Elmer Model 303 Atomic Absorption Spectrophotometer. The percent absorption readings obtained were converted into absorbance units and plotted against the eluent volume for each metal. Finally, a chromatogram showing the retention times of all three metals was constructed.

## RESULTS AND DISCUSSION

### % Loading and Structure Conformation

Samples of the substrates of Z-6020 Amine and γ-Amine were sent to M-H-W Laboratories; Phoenix, Arizona for C, H, N analyses.

The Z-6020 Amine samples showed silica surface loadings of 10.00% to 10.83%. Of this 10-11% loading, the theoretical percentages of C, H and N in the chain are 59.4%, 12.9% and 27.7%, respectively. The largest deviation from theoretical percentages was in a sample containing 66.86% C, 11.27% H and 21.87% N. The relative deviations were 12.56% for C, 12.64% for H and 21.05% for N.

The γ-Amine samples showed silica surface loadings of 6.00% to 7.70%. Theoretical percentages of C, H and N in the chain are 62.1%, 13.8% and 24.1%, respectively. The greatest deviation from this was a sample containing 72.08% C, 13.00% H and 23.96% N. The relative deviations were 16.07% for C, 6.15% for H and 0.58% for N.

Both the Z-6020 Amine and the γ-Amine structures were more or less confirmed by the percentages of C, H and N in the immobilized chain. The disparity in the N percentage in the Z-6020 Amine may be due to matrix effects on the inner nitrogen in the immobilized chain.

### Thin-Layer Chromatography (TLC)

TLC plates using 2 types of silica gel substrates and 8 bonded-phase substrates were developed with various mobile phases. Tables II and III and Tables V through XII show $R_f$ values for $Fe^{+3}$, $Cu^{+2}$, $Ni^{+2}$ and $Zn^{+2}$ ions on the ten different substrates.

Two or more TLC runs were made for most of the combinations of stationary phases and mobile phases. $R_f$ values listed are the mean values of several identical runs. $R_f$ values were reproducible to within 20% for all runs and to within 4% for 99% of all the runs.

One of four symbols may appear in the $R_f$ tables, above or in place of the $R_f$ value. These symbols are explained below.

| | |
|---|---|
| - | Sample spot was too faint to be detected. |
| * | Tailing or excessive spot elongation existed. |
| + | Fronting existed (the bulk of the spot was at a lower portion of the plate than the rest of the spot). |
| ? | Sample spot was very faint (possibility of error in measurement and $R_f$ value). |

Some of the mobile phases used on the Silica Gel 7 substrate were pH buffers. The amounts of the constituents of these buffers are shown in Table IV. Figure 9 shows graphs of the $R_f$ values of the four metal ions for various values of pH.

## TABLE II. $R_f$ Values for Metal Ions On Silica Gel G Stationary Phase With Various Mobile Phases.

| MOBILE PHASE | METAL ION | | | |
|---|---|---|---|---|
| | $Fe^{+3}$ | $Cu^{+2}$ | $Ni^{+2}$ | $Zn^{+2}$ |
| $H_2O$ | 0.00 | *.08 | *.69 | *.07 |
| Acetone | 0.00 | .01 | .02 | .01 |
| Ethanol | 0.00 | .01 | .03 | .01 |
| 12<u>M</u> HCl | - | - | - | - |
| 6<u>M</u> $H_2SO_4$ | - | - | - | - |
| 10% 12<u>M</u> HCl<br>90% Acetone | - | *.64 | .44 | - |
| 10% 0.5<u>M</u> HCl<br>90% Acetone | - | .08 | .15 | .27 |
| 10% 0.1<u>M</u> HCl<br>90% Acetone | 0.00 | .01 | *.05 | *.08 |
| 90% 0.1<u>M</u> HCl<br>10% Acetone | - | .34 | *.74 | .40 |
| 90% 0.1<u>M</u> $H_2SO_4$<br>10% Acetone | - | .80 | .94 | - |
| 90% 0.1<u>M</u> $HNO_3$<br>10% Acetone | - | .65 | *.91 | - |
| 50% $H_2O$<br>50% Acetone | 0.00 | - | - | - |
| 1<u>M</u> Ethylenediamine<br>in $H_2O$ | 0.00 | - | *.76 | - |
| 0.1<u>M</u> Ethylenediamine<br>in $H_2O$ | .01 | *.28 | *.94 | *.28 |
| 10% Acetylacetone<br>in Acetone | .87 | .83 | *.45 | *.55 |
| 10% Trifluoroacetylacetone<br>in Acetone | .86 | .82 | .79 | .80 |
| 10% Hexafluoroacetylacetone<br>in Acetone | .45 | .42 | *.36 | *.38 |

TABLE II. $R_f$ Values for Metal Ions On Silica Gel G Stationary Phase With Various Mobile Phases (cont.).

| MOBILE PHASE | METAL ION | | | |
|---|---|---|---|---|
| | $Fe^{+3}$ | $Cu^{+2}$ | $Ni^{+2}$ | $Zn^{+2}$ |
| 2% 1-Benzoylacetone in Acetone | .79 | .77 | * .70 | * .14 |
| 2% Dibenzoylmethane in Acetone | .53 | .51 | * .45 | * .30 |
| 2% Benzoyltrifluoroacetone in Acetone | .14 | * .13 | * .16 | .15 |
| 2% Salicylaldehyde in Ethanol | .07 | * .59 | * .67 | .03 |
| 2% Salicylaldoxime in Chloroform | .02 | * .32 | * .41 | 0.00 |
| 1% 8-Hydroxyquinoline in Chloroform | 0.00 | .02 | 0.00 | 0.00 |
| 0.2% Dithiooxamide in Ethanol | 0.00 | 0.00 | 0.00 | 0.00 |
| 1% Dimethylglyoxime in Ethanol | 0.00 | * .29 | .03 | .01 |
| 2% Sodium Diethyldithiocarbamate in Acetone | * .76 | .78 | * .74 | * .78 |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |

TABLE III. $R_f$ Values for Metal Ions On Silica Gel 7 Stationary Phase With Various Mobile Phases.

| MOBILE PHASE | METAL ION | | | |
|---|---|---|---|---|
| | $Fe^{+3}$ | $Cu^{+2}$ | $Ni^{+2}$ | $Zn^{+2}$ |
| $H_2O$ | 0.00 | * .07 | * .19 | * .10 |
| HCl (pH=1.00) | .94 | .99 | .99 | - |
| HCl (pH=2.00) | .10 | .53 | * .61 | .51 |
| HCl (pH=3.00) | .01 | * .12 | * .19 | * .18 |
| 90% 0.1$\underline{M}$ $H_2SO_4$ 10% Acetone | - | .66 | .73 | - |
| 1$\underline{M}$ Ethylenediamine in $H_2O$ | 0.00 | * .10 | * .09 | * .10 |
| 0.1$\underline{M}$ Ethylenediamine in $H_2O$ | .01 | .21 | * .43 | * .25 |
| 1$\underline{M}$ Ethylenediamine in Acetone | 0.00 | 0.00 | 0.00 | 0.00 |
| 0.1$\underline{M}$ Ethylenediamine in Acetone | 0.00 | 0.00 | .02 | 0.00 |
| 10% Acetylacetone in Acetone | .72 | .71 | * .55 | * .63 |
| 10% Trifluoroacetylacetone in Acetone | .70 | .68 | .68 | .69 |
| 2% 1-Benzoylacetone in Acetone | .69 | .69 | * .65 | * .45 |
| 2% Dibenzoylmethane in Acetone | .81 | .83 | * .79 | * .59 |
| 2% Benzoyltrifluoroacetone in Acetone | .80 | .80 | .82 | .79 |
| 2% Salicylaldehyde in Ethanol | * .24 | .78 | .83 | * .24 |
| 2% Salicylaldoxime in Chloroform | + .11 | .59 | .57 | .01 |
| 1% 8-Hydroxyquinoline in Chloroform | .02 | .07 | .02 | .02 |

TABLE III. $R_f$ Values for Metal Ions On Silica Gel 7 Stationary Phase With Various Mobile Phases (cont.).

| MOBILE PHASE | METAL ION | | | |
|---|---|---|---|---|
| | $Fe^{+3}$ | $Cu^{+2}$ | $Ni^{+2}$ | $Zn^{+2}$ |
| 0.2% Dithiooxamide in Ethanol | +<br>.05 | +<br>.08 | +<br>.12 | +<br>.10 |
| 1% Dimethylglyoxime in Ethanol | .02 | .65 | *<br>.07 | *<br>.10 |
| 2% Sodium Diethyldithiocarbamate in $H_2O$ | +<br>.01 | +<br>.01 | +<br>.01 | +<br>.01 |
| 2% Sodium Diethyldithiocarbamate in Acetone | *<br>.44 | *<br>.40 | *<br>.43 | *<br>.45 |
| pH 1.00 Buffer | .70 | .87 | .97 | .86 |
| pH 1.50 Buffer | *<br>.21 | .97 | .99 | .95 |
| pH 2.00 Buffer | .04 | .69 | *<br>.94 | *<br>.69 |
| pH 2.50 Buffer | *<br>.15 | .91 | .94 | .92 |
| pH 3.00 Buffer | *<br>.14 | .89 | .92 | .85 |
| pH 4.00 Buffer | *<br>.13 | .93 | .93 | .87 |
| pH 5.00 Buffer | .04 | .93 | .96 | .83 |
| pH 6.00 Buffer | .01 | *<br>.11 | .90 | *<br>.11 |
| pH 7.00 Buffer | .01 | *<br>.09 | .91 | *<br>.09 |
| | | | | |
| | | | | |
| | | | | |
| | | | | |

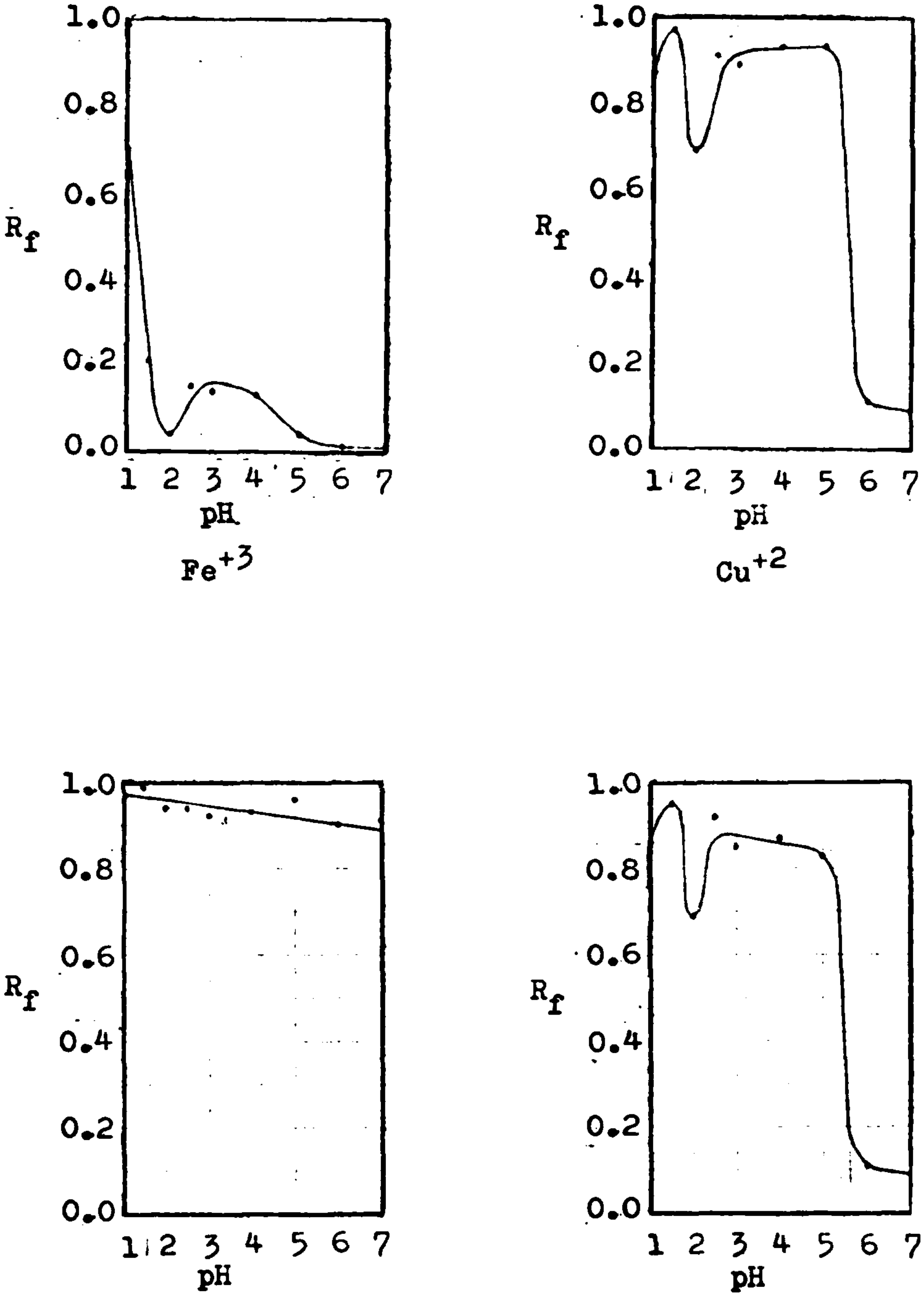
TABLE IV. pH Buffer Solutions Used With Silica Gel 7 TLC Plates.[1]

| pH | SOLUTION |
|---|---|
| 1.00 | 250 ml 0.2M KCl + 670 ml 0.2M HCl |
| 1.50 | 250 ml 0.2M KCl + 207 ml 0.2M HCl |
| 2.00 | 250 ml 0.2M KCl + 65 ml 0.2M HCl |
| 2.50 | 500 ml 0.1M KHPhthalate + 388 ml 0.1M HCl |
| 3.00 | 500 ml 0.1M KHPhthalate + 223 ml 0.1M HCl |
| 4.00 | 500 ml 0.1M KHPhthalate + 1.0 ml 0.1M HCl |
| 5.00 | 500 ml 0.1M KHPhthalate + 226 ml 0.1M NaOH |
| 6.00 | 500 ml 0.1M $KH_2PO_4$ + 56 ml 0.1M NaOH |
| 7.00 | 500 ml 0.1M $KH_2PO_4$ + 291 ml 0.1M NaOH |

$H_2O$ added to bring the final volume of each solution up to 1000 ml.

---

[1] Weast, R.C.; CRC Handbook of Chemistry and Physics, 54th Edition; CRC Press, Cleveland, 1973, pp D-113.



$Fe^{+3}$

$Cu^{+2}$

$Ni^{+2}$

$Zn^{+2}$

Figure 9. Graphs of $R_f$ vs. pH for Metal Ions On Silica Gel 7 Stationary Phase.

TABLE V. $R_f$ Values for Metal Ions On Z-6020 Amine Stationary Phase With Various Mobile Phases.

| MOBILE PHASE | METAL ION | | | |
|---|---|---|---|---|
| | $Fe^{+3}$ | $Cu^{+2}$ | $Ni^{+2}$ | $Zn^{+2}$ |
| $H_2O$ | 0.00 | 0.00 | 0.00 | 0.00 |
| Benzene | 0.00 | 0.00 | 0.00 | 0.00 |
| HCl (pH=1.00) | .02 | .01 | .05 | .01 |
| 1M Ethylenediamine in $H_2O$ | 0.00 | .98 | .98 | .02 |
| 0.1M Ethylenediamine in $H_2O$ | 0.00 | .11 | .26 | 0.00 |
| 1M Ethylenediamine in Acetone | 0.00 | .04 | .04 | 0.00 |
| 0.1M Ethylenediamine in Acetone | 0.00 | .02 | .01 | 0.00 |
| 10% Acetylacetone in Acetone | 0.00 | *<br>.09 | 0.00 | 0.00 |
| 10% Trifluoroacetylacetone in Acetone | 0.00 | 0.00 | .02 | .06 |
| 2% 1-Benzoylacetone in Acetone | 0.00 | .02 | 0.00 | .01 |
| 2% Dibenzoylmethane in Acetone | 0.00 | 0.00 | 0.00 | 0.00 |
| 2% Benzoyltrifluoroacetone in Acetone | 0.00 | .06 | 0.00 | .01 |
| 2% Salicylaldehyde in Ethanol | 0.00 | 0.00 | 0.00 | 0.00 |
| 2% Salicylaldoxime in Chloroform | 0.00 | .15 | 0.00 | .01 |
| 1% 8-Hydroxyquinoline in Ethanol | 0.00 | - | 0.00 | 0.00 |
| 2% 8-Hydroxyquinoline in Benzene | 0.00 | .12 | 0.00 | 0.00 |
| 1% Dimethylglyoxime in Ethanol | 0.00 | 0.00 | 0.00 | 0.00 |

TABLE V. $R_f$ Values for Metal Ions On Z-6020 Amine Stationary Phase With Various Mobile Phases (cont.).

| MOBILE PHASE | METAL ION | | | |
|---|---|---|---|---|
| | $Fe^{+3}$ | $Cu^{+2}$ | $Ni^{+2}$ | $Zn^{+2}$ |
| 2% Sodium Diethyldithiocarbamate in Acetone | 0.00 | .27 | .25 | .21 |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |

TABLE VI. $R_f$ Values for Metal Ions On Z-6020 Dithiocarbamate Stationary Phase With Various Mobile Phases.

| MOBILE PHASE | METAL ION | | | |
|---|---|---|---|---|
| | Fe$^{+3}$ | Cu$^{+2}$ | Ni$^{+2}$ | Zn$^{+2}$ |
| 1<u>M</u> Ethylenediamine in $H_2O$ | 0.00 | 0.00 | 0.00 | - |
| 1<u>M</u> Ethylenediamine in Acetone | 0.00 | .23 | - | - |
| 10% Acetylacetone in Acetone | 0.00 | 0.00 | 0.00 | 0.00 |
| 2% Salicylaldehyde in Ethanol | 0.00 | 0.00 | 0.00 | 0.00 |
| 2% Salicylaldoxime in Chloroform | 0.00 | 0.00 | 0.00 | 0.00 |
| 1% 8-Hydroxyquinoline in Chloroform | 0.00 | 0.00 | 0.00 | 0.00 |
| 1% Dimethylglyoxime in Ethanol | 0.00 | 0.00 | 0.00 | 0.00 |
| 2% Sodium Diethyldithiocarbamate in $H_2O$ | 0.00 | 0.00 | 0.00 | 0.00 |
| 2% Sodium Diethyldithiocarbamate in Acetone | .30 | .31 | .31 | .30 |
| 2% Sodium Diethyldithiocarbamate in Ethanol | .49 | .49 | .48 | - |
| 2% Sodium Diethyldithiocarbamate in Isopropanol | .35 | .35 | .34 | - |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |

TABLE VII. $R_f$ Values for Metal Ions On Z-6020 bis-Dithiocarbamate Stationary Phase With Various Mobile Phases.

| MOBILE PHASE | METAL ION | | | |
|---|---|---|---|---|
| | $Fe^{+3}$ | $Cu^{+2}$ | $Ni^{+2}$ | $Zn^{+2}$ |
| 2% Sodium Diethyldithiocarbamate in $H_2O$ | 0.00 | 0.00 | 0.00 | 0.00 |
| 2% Sodium Diethyldithiocarbamate in Acetone | .25 | .27 | .27 | .12 |
| 2% Sodium Diethyldithiocarbamate in (50% Acetone + 50% $H_2O$) | 0.00 | 0.00 | .01 | .02 |
| 2% Sodium Diethyldithiocarbamate in (50% Acetone + 50% Benzene) | .18 | .14 | .15 | .08 |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |

TABLE VIII. R$_f$ Values for Metal Ions On Z-6020 $\beta$-Diketone-1 Stationary Phase With Various Mobile Phases.

| MOBILE PHASE | METAL ION | | | |
|---|---|---|---|---|
| | Fe$^{+3}$ | Cu$^{+2}$ | Ni$^{+2}$ | Zn$^{+2}$ |
| 1$\underline{M}$ Ethylenediamine in Acetone | 0.00 | - | - | - |
| 10% Acetylacetone in Acetone | 0.00 | * .68 | .02 | * .05 |
| 10% Trifluoroacetylacetone in Acetone | 0.00 | .75 | .01 | .03 |
| 10% Hexafluoroacetylacetone in Acetone | .03 | .05 | .01 | .01 |
| 2% 1-Benzoylacetone in Acetone | 0.00 | * .19 | 0.00 | .01 |
| 2% Dibenzoylmethane in Acetone | 0.00 | * .18 | 0.00 | 0.00 |
| 2% Benzoyltrifluoroacetone in Acetone | 0.00 | .29 | 0.00 | .01 |
| 2% Salicylaldoxime in Chloroform | 0.00 | * .45 | .34 | .04 |
| 2% 8-Hydroxyquinoline in Chloroform | 0.00 | .75 | - | - |
| 0.2% Dithiooxamide in Ethanol | 0.00 | 0.00 | 0.00 | 0.00 |
| 1% Dimethylglyoxime in Ethanol | 0.00 | * .13 | 0.00 | 0.00 |
| 2% Sodium Diethyldithiocarbamate in Acetone | 0.00 | .48 | .48 | - |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |

TABLE IX. $R_f$ Values for Metal Ions On Z-6020 $\beta$-Diketone-2 Stationary Phase With Various Mobile Phases.

| MOBILE PHASE | METAL ION | | | |
|---|---|---|---|---|
| | $Fe^{+3}$ | $Cu^{+2}$ | $Ni^{+2}$ | $Zn^{+2}$ |
| 1$\underline{M}$ Ethylenediamine in $H_2O$ | 0.00 | ?<br>.96 | .97 | .05 |
| 0.1$\underline{M}$ Ethylenediamine in $H_2O$ | 0.00 | ?<br>.99 | ?<br>.99 | .04 |
| 10% Acetylacetone in Acetone | 0.00 | .70 | .03 | .03 |
| 10% Trifluoroacetylacetone in Acetone | 0.00 | .70 | .01 | .02 |
| 2% 1-Benzoylacetone in Acetone | 0.00 | .60 | .03 | .02 |
| 2% Dibenzoylmethane in Acetone | 0.00 | .53 | .01 | .01 |
| 2% Benzoyltrifluoroacetone in Acetone | 0.00 | .42 | 0.00 | .02 |
| 2% Salicylaldehyde in Ethanol | 0.00 | *<br>.02 | *<br>.08 | 0.00 |
| 2% Salicylaldoxime in Chloroform | .04 | *<br>.45 | *<br>.45 | .02 |
| 1% 8-Hydroxyquinoline in Chloroform | +<br>0.00 | .66 | .07 | *<br>.10 |
| 0.2% Dithiooxamide in Ethanol | 0.00 | 0.00 | 0.00 | 0.00 |
| 1% Dimethylglyoxime in Ethanol | 0.00 | .38 | .01 | 0.00 |
| 2% Sodium Diethyldithiocarbamate in $H_2O$ | 0.00 | 0.00 | .02 | .01 |
| 2% Sodium Diethyldithiocarbamate in Acetone | +<br>0.00 | .40 | .38 | .22 |
| | | | | |
| | | | | |
| | | | | |

TABLE X. $R_f$ Values for Metal Ions On Z-6020 $\beta$-Diketone-3 Stationary Phase With Various Mobile Phases.

| MOBILE PHASE | METAL ION | | | |
|---|---|---|---|---|
| | $Fe^{+3}$ | $Cu^{+2}$ | $Ni^{+2}$ | $Zn^{+2}$ |
| 1M Ethylenediamine in Acetone | 0.00 | .02 | 0.00 | .01 |
| 10% Acetylacetone in Acetone | 0.00 | .16 | .03 | .03 |
| 10% Trifluoroacetylacetone in Acetone | 0.00 | .81 | .12 | .41 |
| 2% 1-Benzoylacetone in Acetone | 0.00 | .03 | .01 | .01 |
| 2% Benzoyltrifluoroacetone in Acetone | 0.00 | .69 | .09 | .24 |
| 2% Salicylaldoxime in Chloroform | 0.00 | .61 | .60 | .01 |
| 1% 8-Hydroxyquinoline in Chloroform | 0.00 | .66 | .06 | .06 |
| 1% Dimethylglyoxime in Ethanol | 0.00 | .01 | 0.00 | 0.00 |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |

TABLE XI. $R_f$ Values for Metal Ions On Z-6020 Arylamine Stationary Phase With Various Mobile Phases.

| MOBILE PHASE | METAL ION | | | |
|---|---|---|---|---|
| | $Fe^{+3}$ | $Cu^{+2}$ | $Ni^{+2}$ | $Zn^{+2}$ |
| 1$\underline{M}$ Ethylenediamine in $H_2O$ | 0.00 | .92 | .96 | .01 |
| 0.1$\underline{M}$ Ethylenediamine in $H_2O$ | 0.00 | .43 | *.49 | .01 |
| 1$\underline{M}$ Ethylenediamine in Acetone | 0.00 | .01 | - | - |
| 0.1$\underline{M}$ Ethylenediamine in Acetone | 0.00 | 0.00 | 0.00 | 0.00 |
| 10% Acetylacetone in Acetone | +0.00 | .08 | 0.00 | .01 |
| 10% Trifluoroacetylacetone in Acetone | +0.00 | .05 | .01 | +.06 |
| 2% 1-Benzoylacetone in Acetone | 0.00 | .02 | 0.00 | 0.00 |
| 2% Dibenzoylmethane in Acetone | +0.00 | .01 | 0.00 | 0.00 |
| 2% Benzoyltrifluoroacetone in Acetone | +0.00 | .29 | 0.00 | .03 |
| 2% Salicylaldoxime in Chloroform | .02 | .16 | .05 | 0.00 |
| 2% Salicylaldoxime in Ethanol | .01 | *.04 | .02 | 0.00 |
| 2% Salicylaldoxime in Acetone | .01 | *.08 | *.04 | 0.00 |
| 1% 8-Hydroxyquinoline in Chloroform | *.12 | .28 | 0.00 | .02 |
| 0.2% Dithiooxamide in Ethanol | 0.00 | 0.00 | 0.00 | 0.00 |
| 1% Dimethylglyoxime in Ethanol | 0.00 | .01 | 0.00 | 0.00 |
| 2% Sodium Diethyldithiocarbamate in $H_2O$ | 0.00 | 0.00 | 0.00 | 0.00 |
| 2% Sodium Diethyldithiocarbamate in Acetone | .23 | .33 | .30 | .22 |

TABLE XII. $R_f$ Values for Metal Ions On $\gamma$-Amine Stationary Phase With Various Mobile Phases.

| MOBILE PHASE | METAL ION | | | |
|---|---|---|---|---|
| | $Fe^{+3}$ | $Cu^{+2}$ | $Ni^{+2}$ | $Zn^{+2}$ |
| $H_2O$ | 0.00 | .01 | .03 | 0.00 |
| 1M Ethylenediamine in $H_2O$ | 0.00 | .97 | .98 | .03 |
| 0.1M Ethylenediamine in $H_2O$ | 0.00 | ?<br>.92 | .93 | .03 |
| 10% Acetylacetone in Acetone | +<br>0.00 | *<br>.53 | 0.00 | .02 |
| 10% Trifluoroacetylacetone in Acetone | 0.00 | .80 | .01 | .04 |
| 2% 1-Benzoylacetone in Acetone | 0.00 | .59 | 0.00 | .01 |
| 2% Dibenzoylmethane in Acetone | 0.00 | .54 | 0.00 | .01 |
| 2% Benzoyltrifluoroacetone in Acetone | 0.00 | .08 | .01 | .02 |
| 2% Salicylaldehyde in Ethanol | 0.00 | 0.00 | 0.00 | 0.00 |
| 2% Salicylaldoxime in Chloroform | 0.00 | .17 | .16 | 0.00 |
| 1% 8-Hydroxyquinoline in Chloroform | 0.00 | .36 | 0.00 | 0.00 |
| 0.2% Dithiooxamide in Ethanol | 0.00 | 0.00 | 0.00 | 0.00 |
| 1% Dimethylglyoxime in Ethanol | 0.00 | .15 | 0.00 | 0.00 |
| 2% Sodium Diethyldithiocarbamate in $H_2O$ | 0.00 | 0.00 | 0.00 | 0.00 |
| 2% Sodium Diethyldithiocarbamate in Acetone | 0.00 | *<br>.59 | *<br>.56 | .26 |
| | | | | |
| | | | | |

All $R_f$ values were calculated using Equation 5 on page 17. The distance moved by the solute was measured from what appeared to be the most concentrated point of the sample spot.

Comparison of Silica G and Silica Gel 7 substrates shows that the metal ions generally have higher $R_f$ values on type G than on type 7. This is probably because the Silica Gel G consists of larger particles. There is less surface area for the solutes to be adsorbed, so they move through the stationary bed faster. Also, the G type contains 13% calcium sulfate as a binder which is fairly inert as an adsorbent and further decreases silica surface area available.

It was also interesting to note that sample spots on the G type were somewhat more compact than on type 7. This was also possibly due to the smaller amount of adsorption on the G type particles, so there was a lesser chance for solute spreading.

$Ni^{+2}$ is separable from $Fe^{+3}$, $Cu^{+2}$ and $Zn^{+2}$ on either type of silica using water as the mobile phase. Also, a mixture of $Cu^{+2}$ and $Zn^{+2}$ was easily separable from $Fe^{+3}$.

Better separations were obtained with ethylenediamine (en)/water mobile phases than with ethylenediamine (en)/acetone on Silica Gel 7. $R_f$ values were zero or close to zero for the latter system. This suggests that the metal-en complexes are more soluble in water than in acetone and/or that the formation constants of the metal-en complexes are higher in water than in acetone. The $R_f$ values were higher and the metal separation was better for 0.1M en in water than for 1M en in water. The pH of the 1M en is high and perhaps aided in the formation of metal hydroxides which precipitated and were highly retained by the silica.

It appears that all four metals might be separable with a Silica Gel 7 substrate and a 0.1M en in water solvent system if the tailing of the $Zn^{+2}$ spot was decreased. An efficient HPLC silica column might accomplish this.

Three of the four metals can be effectively separated from each other on a Silica Gel 7 substrate with the following solvent systems: water, 0.1M en in water, pH = 2.00 hydrochloric acid, 10% acetylacetone in acetone, 2% salicylaldehyde in ethanol, 2% salicylaldoxime in chloroform, 1% dimethylglyoxime in ethanol, pH 2.00 buffer.

A study was also done on the effect of pH on the $R_f$ values of the metals ions on Silica Gel 7. The pH buffers in Table IV were used and the results are shown in Table III and Figure 9. $Fe^{+3}$, $Cu^{+2}$ and $Zn^{+2}$ exhibit odd behavior in the pH range of 1 to 2.5. The graphs of Figure 9 did not give any promising outlook for the separation of $Cu^{+2}$ and $Zn^{+2}$, so most of the bonded-phase TLC was done using chelating reagents in organic solvents. The separations would then depend upon competition of the immobilized group with the chelating group in the mobile phase for the metal ions, rather than a pH dependent solvent extraction type of process.

On the Z-6020 Amine substrate, a pH = 1.00 hydrochloric acid mobile phase hardly even separates two metals from each other. $R_f$ values were 0.05 or lower for all metals.

The most feasible separation on this substrate occured using a 0.1$\underline{M}$ en in water mobile phase. $Cu^{+2}$, $Ni^{+2}$ and $Zn^{+2}$ or $Cu^{+2}$, $Ni^{+2}$ and $Fe^{+3}$ were separable. Competition between the immobilized diamine and the en in the mobile phase for the metal ions occured here.

As on the silica gels, $R_f$ values of the metals were higher in the aqueous en solutions than in the acetone/en mobile phases.

The $\beta$-diketones used in the mobile phases generally gave very small $R_f$ values for the metals ($<0.10$), and did not give good separation of any metals on the Z-6020 Amine substrate. The diamine group immobilized on the silica appears to form a stable chelate, so the metals are not easily released to the $\beta$-diketone in the mobile phase. Other evidence for a strong diamine complex is the difficulty of detection of the sample spots after spraying with diphenylcarbazide (DPC). This occured on the Z-6020 Amine plates and on other plates when an en mobile phase was used. The metals tended to stay in the diamine chelate rather than to complex with diphenylcarbazide and form the more highly colored metal-DPC complexes.

The Z-6020 Dithiocarbamate and the Z-6020 <u>bis</u>-Dithiocarbamate show no real promise for the separation of $Fe^{+3}$, $Cu^{+2}$, $Ni^{+2}$ and $Zn^{+2}$. The stationary phase totally retained the four metals for almost every mobile phase used. The most movement the metals exhibited on these plates was when mobile phases of sodium diethyldithiocarbamate (NaDDC) in organic solvents were used. Even then, $R_f$ values were almost identical for all the metals and separations were not possible.

The use of dithiocarbamate stationary phases is also limited since acidic mobile phases attack the nitrogen and release carbon disulfide from the substrate.

Because of the stable chelates formed, the dithiocarbamate-bonded silica substrates probably would be very good for extracting trace metals in purifying chemical samples. Also, the material could be used as packing in a "guard" column to keep trace metals from accumulating in an analytical column.

The three Z-6020 β-Diketone substrates usually showed good separations or at least a promising outlook for separation of the four metals. The best results usually occured when a β-diketone mobile phase was used. This way, the immobilized β-diketone and the β-diketone in the mobile phase compete for the metal ions. The metals usually had fairly high $R_f$ values and the sample spots were usually compact. This could be due to the quick equilibration and reversibility of metal-$\beta$-diketonate formation.

A 2% salicylaldoxime in chloroform mobile phase on a Z-6020 $\beta$-Diketone-1 TLC plate offered a good outlook for separation of the four metals. The $Cu^{+2}$ spot tailed into the $Ni^{+2}$ spot, but an efficiently packed column might combat this problem. In any case, three of the four metals were easily separable from each other.

A 2% sodium diethyldithiocarbamate in acetone mobile phase showed the best separation on the Z-6020 $\beta$-Diketone-2 substrate. $Cu^{+2}$ and $Ni^{+2}$ spots had very close $R_f$ values. A longer development length for a TLC plate or a long length of packed column could conceivibly separate all of the four metals.

By far, the best separation obtained during the course of this research occurred on the Z-6020 $\beta$-Diketone-3 substrate using a 10% triflucroacetylacetone (TFAA) in acetone mobile phase. All spots except for $Zn^{+2}$ were very compact.

The development of this particular system was repeated many times, at times using two different batches of the substrate. Reproducibility was always within 4%. Figure 10 shows a typical Z-6020 $\beta$-Diketone-3 plate developed with 10% TFAA in acetone. Fe, Cu, Ni and Zn perchlorates were spotted separately in the first four strips of the plate. The last two strips were spotted with a mixture of equal amounts of Fe, Cu, Ni and Zn perchlorates. Spots were a total of 10-15 $\mu$g of sample.

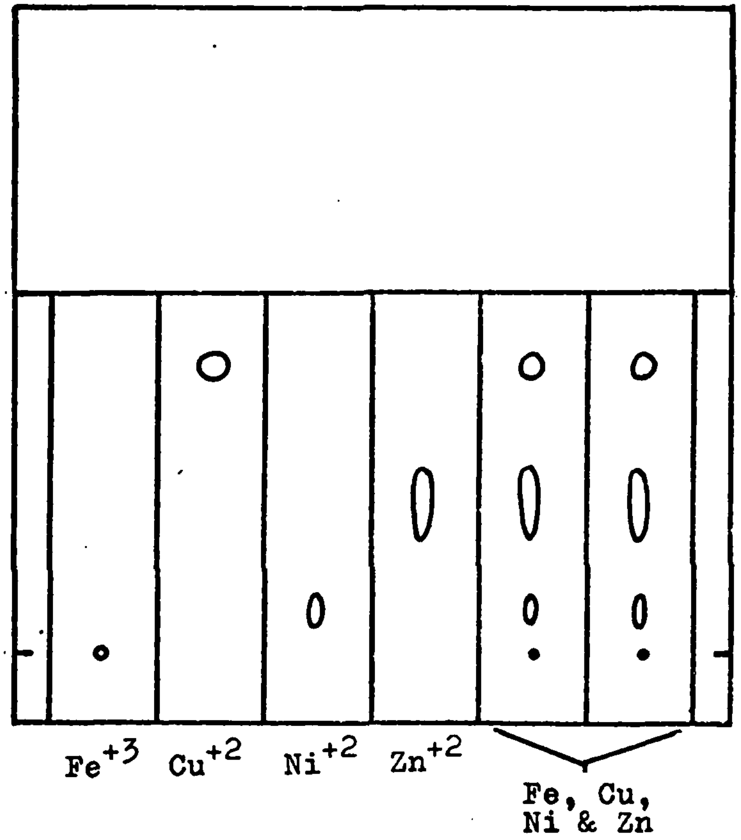


Figure 10. Developed TLC Plate of Z-6020 $\beta$-Diketone-3 Substrate and 10% Trifluoroacetylacetone in Acetone Mobile Phase.

Z-6020 $\beta$-Diketone-3 plates were also spotted with various amounts of total sample of Fe, Cu, Ni and Zn perchlorates. With samples ranging from 3.2 $\mu$g to 19.2 $\mu$g,

$R_f$ values showed reproducibility to within 4% even for varying sample loading. A typical plate is shown in Figure 11.

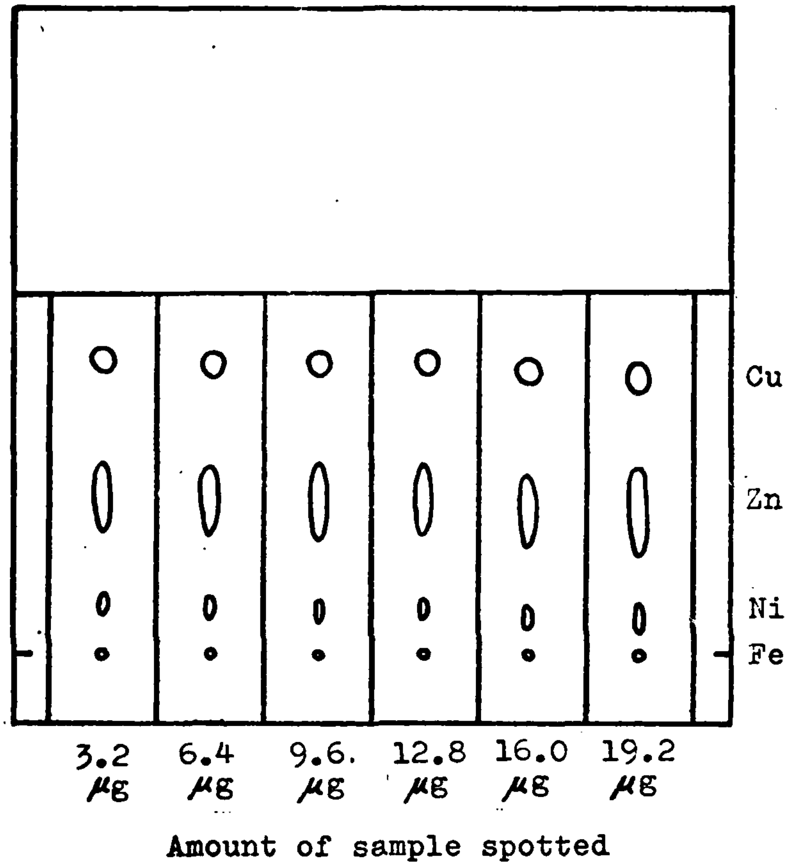


Figure 11. Developed TLC Plate of Z-6020 β-Diketone-3 Substrate and 10% Trifluoroacetylacetone in Acetone Mobile Phase (Various Sample Loadings).

$R_f$ values for the metal ions on the Z-6020 Arylamine and the γ-Amine substrates were substantially higher than $R_f$ values on the Z-6020 Amine substrate for the same solvents. The Z-6020 Amine forms stable chelates with the metals whereas the Z-6020 Arylamine has a primary amine and a secondary amine which are separated by eight atoms and tend to form weaker complexes, and the $\gamma$-Amine has only one amino group available to the metal ions, forming a weaker complex. Since these complexes are much less stable than the diamine chelate, the metals tend to be released more easily and travel farther along the stationary bed.

$R_f$ values for the metal ions on the $\gamma$-Amine were generally slightly higher than on the Z-6020 Arylamine for the same solvents. The presence of a secondary amine and a primary amine coupled with the slight resonance of the aryl ring in the Z-6020 Arylamine tends to retard the metals more than on the straight chain $\gamma$-Amine.

The best separation on the Z-6020 Arylamine occurred using a 2% salicylaldoxime in chloroform mobile phase. Figure 12 shows a typical TLC plate developed using this system. All $R_f$ values are rather small,but each metal spot is very compact. Longer TLC development length would give a better visualization of actual separations.

Separations on the $\gamma$-Amine substrate were not very good due to spot spreading and similar $R_f$ values for the metals. The best separations obtained on this substrate were with mobile phases of 10% TFAA in acetone and with 2% NaDDC in acetone. Still, only two metals were separable from each other and from a mixture of the other two metals.

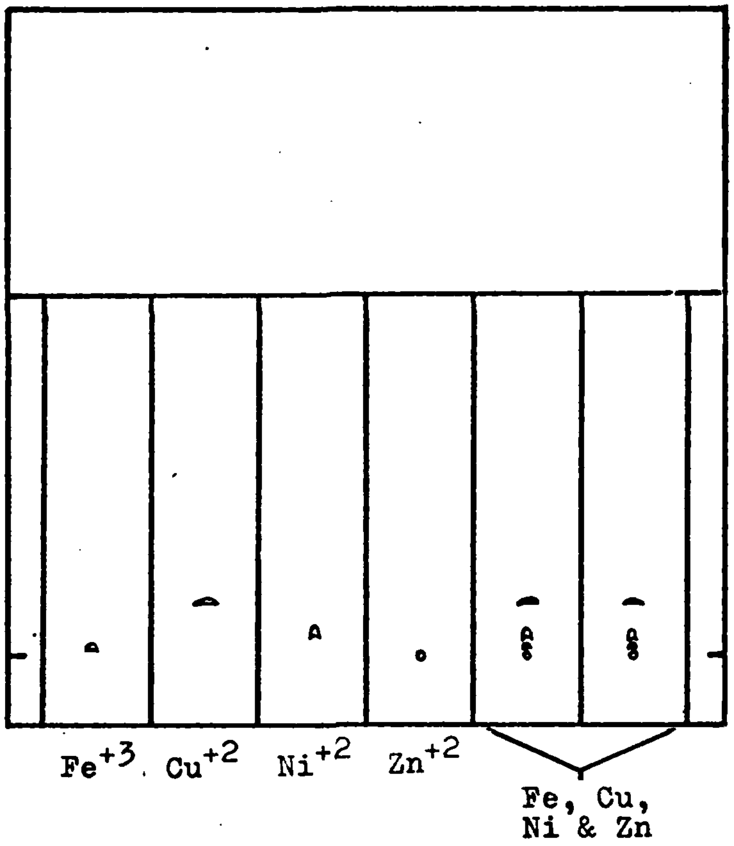


Figure 12. Developed TLC Plate of Z-6020 Arylamine Substrate and 2% Salicylaldoxime in Chloroform Mobile Phase.

$R_f$ values and final spot lengths obtained from developed TLC plates can be used to calculate the local plate height for a certain substrate-solute system. This is easily done with the help of Figure 1 and Equation 8.

Table XIII lists average plate height ($\overline{H}$) for the four substrate-solute systems on a Z-6020 $\beta$-Diketone-3 plate. Intermediate parameters are also listed. The number of plates (N) for a system is simply the length that the solvent front travels up the plate divided by the average plate height ($\overline{H}$). The length that the solvent front travelled was always 10 cm, or 100 mm so,

$$N = \frac{100}{\overline{H}} \tag{13}$$

The number of plates is also listed in Table XIII.

TABLE XIII. Table of Parameters Obtained From Z-6020 β-Diketone-3 TLC Plate Using 10% Trifluoroacetylacetone in Acetone Mobile Phase.

| Metal Ion | $4\sigma$ (mm) | $\sigma$ (mm) | $\sigma^2$ (mm$^2$) | $R_f$ | $\overline{H}$ (mm) | N |
|---|---|---|---|---|---|---|
| $Fe^{+3}$ | 3.0 | 0.75 | 0.5625 | 0.00 | ∞ | 0 |
| $Cu^{+2}$ | 6.0 | 1.50 | 2.25 | 0.81 | 0.0278 | 3600 |
| $Ni^{+2}$ | 8.0 | 2.00 | 4.00 | 0.12 | 0.3333 | 300 |
| $Zn^{+2}$ | 24.0 | 6.00 | 36.00 | 0.41 | 0.8780 | 114 |

Equations 4, 6, 9 and 10 were used to calculate the approximate number of plates needed to separate certain pairs of metal ions on Z-6020 β-Diketone-3 substrate with a 10% TFAA in acetone mobile phase. A value of 1.5 was used for $R_s$ in Equation 9 for the calculations of N. This would give baseline separation or a "just resolved" mixture. The three metal ion pairs to be separated, and their corresponding N values are listed in Table XIV. Intermediate values, $\alpha$ and $\overline{k'}$ are also listed.

TABLE XIV. Calculated Number of Theoretical Plates Needed to Separate Pairs of Metal Ions ($R_s$=1.5) On Z-6020 $\beta$-Diketone-3 Stationary Phase With 10% Trifluoroacetylacetone in Acetone Mobile Phase.

| Metal Ion Pair | $\alpha$ | $\overline{k'}$ | N |
|---|---|---|---|
| $Cu^{+2}$-$Zn^{+2}$ | 6.135 | 0.8368 | 83.72 |
| $Zn^{+2}$-$Ni^{+2}$ | 5.096 | 4.3860 | 30.06 |
| $Ni^{+2}$-$Fe^{+3}$ | $\infty$ | $\infty$ | 9.00 |

Keeping in mind that it is dependent upon the solute, N should be used only as a "ballpark" value to determine the feasibility of a separation.

For a "just resolved" separation of $Cu^{+2}$ and $Zn^{+2}$ on a $\beta$-Diketone-3 plate using a 10% TFAA in acetone mobile phase, N should be at least 84 plates. Looking back at Table XIII, N=3600 for Cu and N=114 for Zn. N for the Cu-Zn mixture is probably some complex function of the two separate N values for the metals. It presumably would be somewhere between the values of 114 and 3600. The excellent resolution of the Cu and Zn spots in Figures 10 and 11 do not discount this presumption. If 84 plates are theoretically needed to get a "just resolved" separation of $Cu^{+2}$ and $Zn^{+2}$, it appears from the figures that N is at least 114 in order to get the wide separation shown. A more rigorous treatment of this theory, if practical or even possible, is beyond the scope of this research.

## Reusability of Z-6020 Amine

To test the reusability of the Z-6020 Amine substrate under various pH conditions, samples of the substrate were stirred with various pH solutions of hydrochloric acid and solutions of ethylenediamine (en).

### pH=1.00

About 10 grams of Z-6020 Amine were stirred for 30 minutes with a hydrochloric acid/water mixture ($\approx$75ml) of pH=1.00. The powder was then filtered, washed with water and dried. C, H, N analysis showed only 7.3% loading.

TLC plates made from this substrate and developed with aqueous 0.1M ethylenediamine gave the $R_f$ values: $Fe^{+3}$ (0.01), $Cu^{+2}$ (0.61), $Ni^{+2}$ (0.79), $Zn^{+2}$ (0.01). Each value was greater than for the substrate that was not mixed with hydrochloric acid. It appears that at low pH, the Si-C bond is broken, releasing the previously immobilized $(CH_2)_3NH(CH_2)_2NH_2$ chain which tended to retard the movement of the metal ions through the stationary bed.

### pH=2.00

About 10 grams of Z-6020 Amine were stirred for 30 minutes with $\approx$75 ml of pH=2.00 hydrochloric acid/water. The powder was filtered, washed with water and dried. C, H, N analysis showed 10.4% loading.

TLC plates made with this substrate and developed with aqueous 0.1M ethylenediamine gave the $R_f$ values:

$Fe^{+3}$ (0.00), $Cu^{+2}$ (0.11), $Ni^{+2}$ (0.26), $Zn^{+2}$ (0.00). These values were identical to the values obtained for the untreated Z-6020 Amine substrate. The constancy of the % loading and the reproducibility of the $R_f$ values indicate that the Z-6020 Amine would be reusable at this pH.

pH=8.00

About 10 grams of Z-6020 Amine were stirred for 30 minutes with $\approx$75 ml of an ethylenediamine/water mixture of pH=8.00. The powder was filtered, washed with water and dried. C, H, N analysis showed 10.0% loading.

TLC plates made from this substrate and developed with aqueous 0.1M ethylenediamine gave the $R_f$ values: $Fe^{+3}$ (0.00), $Cu^{+2}$ (0.12), $Ni^{+2}$ (0.28), $Zn^{+2}$ (0.00). These values were almost identical to those for the untreated substrate. It appears that the Z-6020 Amine is also stable at this pH.

pH=11.35

About 10 grams of Z-6020 Amine were stirred for 30 minutes with $\approx$75 ml of aqueous 0.1M ethylenediamine (pH=11.35). The powder was filtered, washed with water and dried. C, H, N analysis showed only 8.7% loading.

TLC plates made from this substrate and developed with aqueous 0.1M ethylenediamine gave the $R_f$ values: $Fe^{+3}$ (0.01), $Cu^{+2}$ (0.58), $Ni^{+2}$ (0.74), $Zn^{+2}$ (0.01). These values were quite a bit different from values obtained from the untreated substrate. It appears that at high pH, the Si-O bonds are broken and the previously immobilized groups are released from the silica surface.

From the % loading and the reproducibility of the chromatograms, it appears that the Z-6020 Amine substrate can be used in a pH range of 2 - 8 and still be reliably reused. This is obviously important to know when operating HPLC columns packed with this substrate. The substrate can be used outside this pH range for a single TLC run, however, reproducibility cannot be expected in a column or a plate which is to be used again.

The treatment of the substrate with hydrochloric acid or with ethylenediamine caused a change in the total % loading of the silane on the silica, however, percentages of C, H and N were still in the same proportions to each other. It is reasonable to assume that the C-C, C-H, C-N and N-H bonds were left intact and only the Si-C or the Si-O bonds were attacked, as was postulated earlier.

It is also reasonable to assume that the other amine and $\beta$-diketone bonded-phase substrates are reusable in the pH range of 2 - 8. The dithiocarbamates, of course, give off carbon disulfide in contact with acidic solutions, so the reusability of these substrates is more severly pH limited.

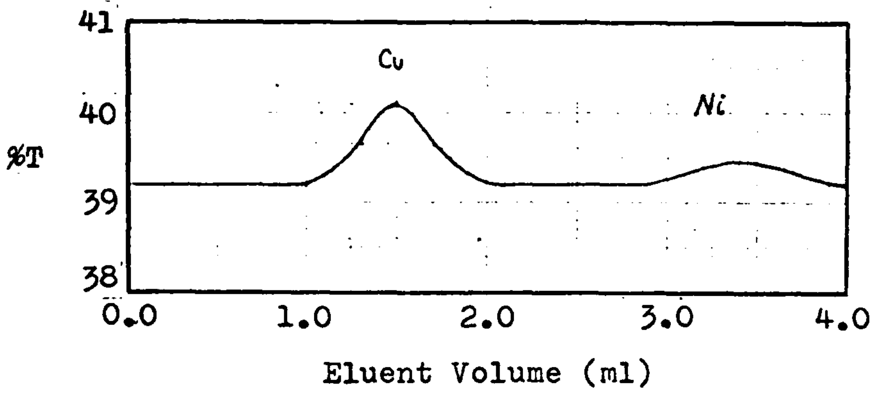
## High-Performance Liquid Chromatography (HPLC)

Only $Cu^{+2}$, $Ni^{+2}$ and $Zn^{+2}$ ions were injected onto the HPLC columns which were packed with Z-6020 $\beta$-Diketone-3.

$Fe^{+3}$ was not injected because of its 0.00 $R_f$ value observed on TLC plates. The $Fe^{+3}$ ion would probably be retained at the top of the column, or, if it was eluted, it would probably take an extraordinarily long time.

$Cu^{+2}$, $Ni^{+2}$ and $Zn^{+2}$ mixtures were injected onto the 30 cm long column but only $Cu^{+2}$ and $Ni^{+2}$ could be detected using visible spectrophotometry. Even then, because of the low extinction coefficients of the complexes at the detector wavelengths, high concentrations (10,000 ppm) of the metals were needed.

Figure 13 shows a typical chromatogram obtained from a mixture of 2 μl of (10,000 ppm) Cu and 8 μl of (10,000 ppm) Ni. The mobile phase was 10% TFAA in acetone.



30 cm long Z-6020 β-Diketone-3 column
2 μl of (10,000 ppm) Cu + 8 μl of (10,000 ppm) Ni
Flow rate = 1.0 ml/min
Detection wavelength = 373 nm

Figure 13. Typical HPLC Chromatogram for the Separation of Cu and Ni.

There is a good chance that the high amounts of sample overloaded the column. However, the Cu peak appeared to show a fairly Gaussian profile, so 2 $\mu$l samples of (10,000 ppm) Cu were used to determine the optimum flow rate of the mobile phase. Figure 14 shows the chromatograms for Cu at four different mobile phase flow rates. These figures, along with Figure 2 and Equation 12, were used to calculate the number of theoretical plates (N) in the column. $V_R'$ was approximated as $V_R$ since $V_0$ is small compared to $V_R$. The height equivalent to a theoretical plate (H) in cm, was calculated according to Equation 14 since the column length was 30 cm.

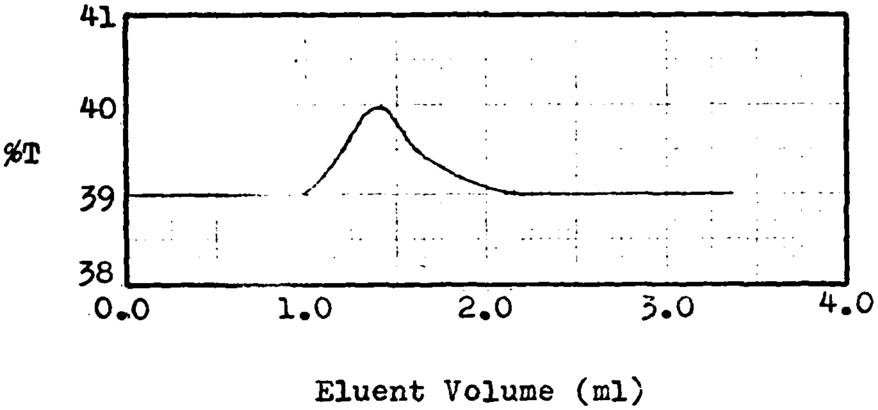
$$H = \frac{30}{N} \qquad (14)$$

These values are listed in Table XV.

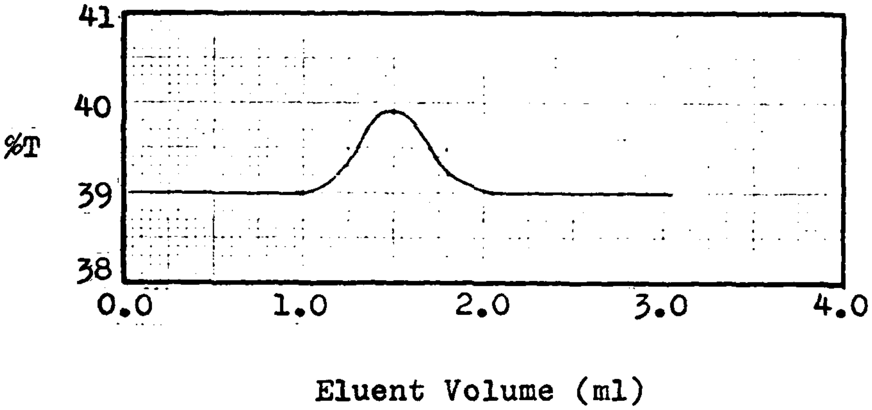
The values in Table XV were used to plot the H vs. mobile phase flow rate graph of Figure 15. The figure shows the optimum flow rate occurring at approximately 1.1 ml/min.

A sample of 333.3 ppm for each metal (1,000 ppm total), to be detected by atomic absorption spectrophotometry, was injected onto the column with the mobile phase flow rate set at 1.0 ml/min.

The diluted samples aspirated into the atomic absorption spectrophotometer ranged in maximum concentrations from $\approx$0.005 ppm to $\approx$0.015 ppm according to the metal.



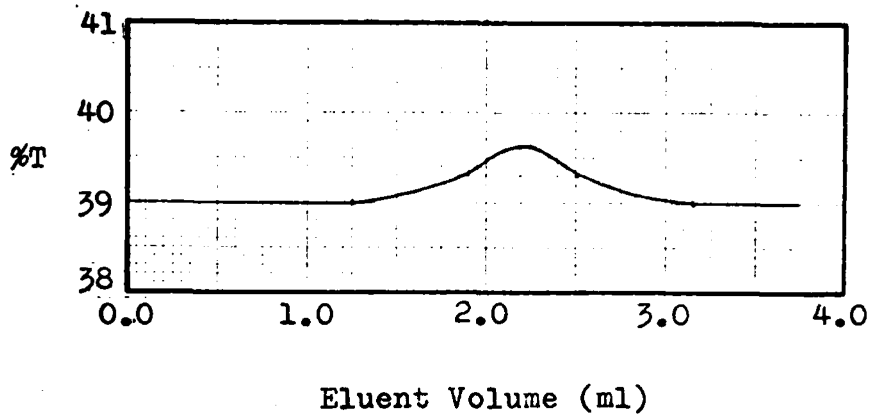
a) Flow Rate = 0.5 ml/min



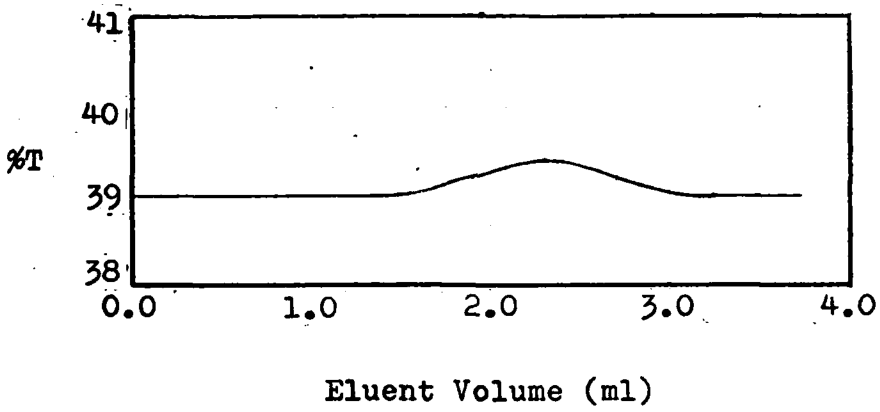
b) Flow Rate = 1.0 ml/min

30 cm long Z-6020 $\beta$-Diketone-3 column
2 $\mu$l of (10,000 ppm) Cu
Detection wavelength = 368 nm

Figure 14. HPLC Peak Shapes of Cu.



c) Flow Rate = 1.5 ml/min



d) Flow Rate = 2.0 ml/min

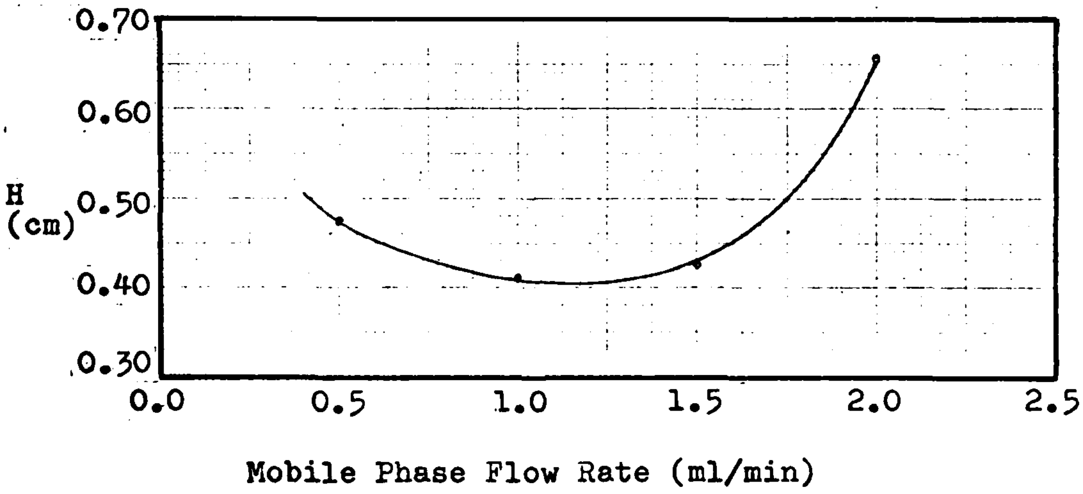
30 cm long Z-6020 β-Diketone-3 column
2 μl of (10,000 ppm) Cu
Detection wavelength = 368 nm

Figure 14. HPLC Peak Shapes of Cu (cont.).

TABLE XV. Table of Parameters Obtained From a 30 cm HPLC Column.

| Flow Rate (ml/min) | $V'_R$ (ml) | $W_{1/2}$ (ml) | N | H (cm) |
|---|---|---|---|---|
| 0.5 | 1.42 | .42 | 63.33 | .474 |
| 1.0 | 1.49 | .41 | 73.17 | .410 |
| 1.5 | 2.18 | .61 | 70.76 | .424 |
| 2.0 | 2.30 | .80 | 45.79 | .655 |

30 cm long Z-6020 β-Diketone-3 column
2 μl of (10,000 ppm) Cu
Detection wavelength = 368 nm



30 cm long Z-6020 β-Diketone-3 column
2 μl of (10,000 ppm) Cu
Detection wavelength = 368 nm

Figure 15. Graph of Height Equivalent to a Theoretical Plate (H) vs. Mobile Phase Flow Rate.

There was a higher maximum concentration of Cu in these samples than Zn since all of the Cu was eluted with a smaller volume of mobile phase than was all of the Zn. Zn had a higher maximum concentration than Ni for the same reason.

Tables XVI, XVII and XVIII list the samples of the three metals aspirated into the atomic absorption spectrophotometer and their corresponding % absorption values. The % absorption was converted to absorbance units and was also listed in the tables.

---

TABLE XVI. Atomic Absorption Readings for HPLC Samples of Cu.

| Sample Tube # | Time Frame Sampled (min) | % Absorption | Absorbance |
|---|---|---|---|
| 1 | 0.00-0.25 | 0.5 | 0.0022 |
| 2 | 0.25-0.50 | 0.5 | 0.0022 |
| 3 | 0.50-0.75 | 0.5 | 0.0022 |
| 4 | 0.75-1.00 | 0.7 | 0.0031 |
| 5 | 1.00-1.25 | 1.1 | 0.0048 |
| 6 | 1.25-1.50 | 0.8 | 0.0035 |
| 7 | 1.50-1.75 | 0.6 | 0.0026 |
| 8 | 1.75-2.00 | 0.5 | 0.0022 |
| 9 | 2.00-2.25 | 0.5 | 0.0022 |
| 10 | 2.25-2.50 | 0.5 | 0.0022 |
| 11 | 2.50-2.75 | 0.5 | 0.0022 |

TABLE XVII. Atomic Absorption Readings for HPLC Samples of Zn.

| Sample Tube # | Time Frame Sampled (min) | % Absorption | Absorbance |
|---|---|---|---|
| 1 | 0.00-0.25 | 4.0 | 0.0177 |
| 2 | 0.25-0.50 | 4.0 | 0.0177 |
| 3 | 0.50-0.75 | 3.7 | 0.0164 |
| 4 | 0.75-1.00 | 3.3 | 0.0146 |
| 5 | 1.00-1.25 | 4.0 | 0.0177 |
| 6 | 1.25-1.50 | 3.3 | 0.0146 |
| 7 | 1.50-1.75 | 3.7 | 0.0164 |
| 8 | 1.75-2.00 | 3.7 | 0.0164 |
| 9 | 2.00-2.25 | 4.7 | 0.0209 |
| 10 | 2.25-2.50 | 4.7 | 0.0209 |
| 11 | 2.50-2.75 | 7.3 | 0.0329 |
| 12 | 2.75-3.00 | 4.3 | 0.0191 |
| 13 | 3.00-3.25 | 4.3 | 0.0191 |
| 14 | 3.25-3.50 | 3.7 | 0.0164 |
| 15 | 3.50-3.75 | 3.3 | 0.0146 |
| 16 | 3.75-4.00 | 4.0 | 0.0177 |
| 17 | 4.00-4.25 | 3.7 | 0.0164 |

TABLE XVIII. Atomic Absorption Readings for HPLC Samples of Ni.

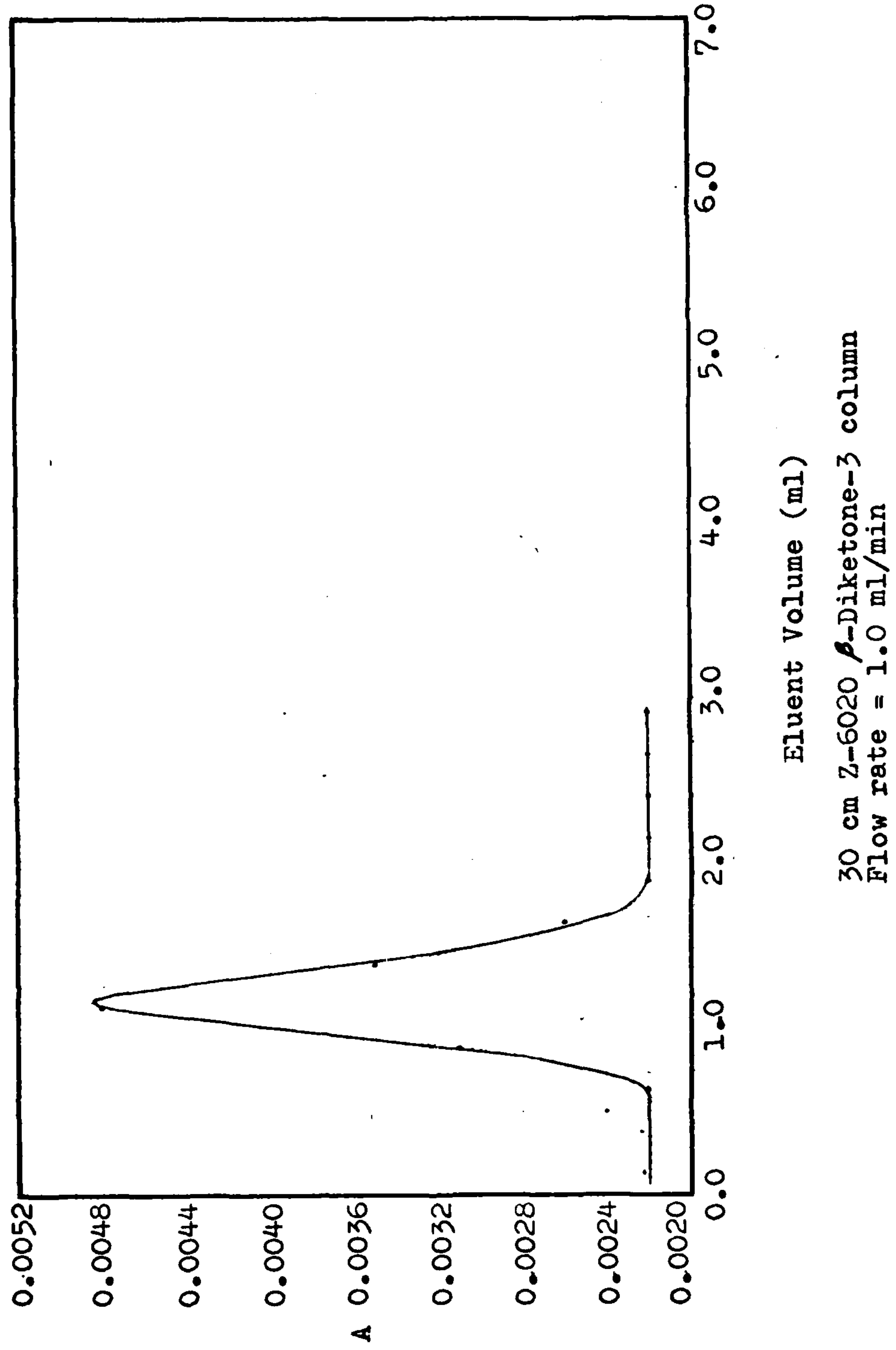
| Sample Tube # | Time Frame Sampled (min) | % Absorption | Absorbance |
|---|---|---|---|
| 12 | 2.75-3.00 | 0.0 | 0.0000 |
| 13 | 3.00-3.25 | 0.1 | 0.0004 |
| 14 | 3.25-3.50 | 0.1 | 0.0004 |
| 15 | 3.50-3.75 | 0.1 | 0.0004 |
| 16 | 3.75-4.00 | 0.2 | 0.0009 |
| 17 | 4.00-4.25 | 0.2 | 0.0009 |
| 18 | 4.25-4.50 | 0.6 | 0.0026 |
| 19 | 4.50-4.75 | 0.6 | 0.0026 |
| 20 | 4.75-5.00 | 1.0 | 0.0044 |
| 21 | 5.00-5.25 | 0.8 | 0.0035 |
| 22 | 5.25-5.50 | 0.6 | 0.0026 |
| 23 | 5.50-5.75 | 0.6 | 0.0026 |
| 24 | 5.75-6.00 | 0.2 | 0.0009 |
| 25 | 6.00-6.25 | 0.2 | 0.0009 |
| 26 | 6.25-6.50 | 0.2 | 0.0009 |
| 27 | 6.50-6.75 | 0.1 | 0.0004 |
| 28 | 6.75-7.00 | 0.0 | 0.0000 |

The absorbance values in Tables XVI, XVII and XVIII were plotted against eluent volume in Figures 16, 17 and 18. The flow rate of the mobile phase was 1.0 ml/min. Each eluent sample was taken for 0.25 minutes, so the absorbance for each sample is actually the sum of all the infinitessimal absorbances ($\Sigma\Delta A$) in a 0.25 ml wide segment of the abcissa of the chromatograms. As an approximation, the actual absorbance reading was called the mean over this segment and was plotted as the midpoint of the 0.25 ml segment.

Samples could be taken for smaller time intervals in an attempt to obtain greater accuracy, but this would decrease the amount of metal in each sample. Metals in samples taken at too small an interval may go undetected by atomic absorption.

Curves were drawn through the points in Figures 16, 17 and 18 as close to a Gaussian profile as the points would permit.

Chromatograms from Figures 16, 17 and 18 were combined by offsetting the baselines of the Cu and the Ni peaks and are shown in Figure 19 as a typical chromatogram of the Cu-Zn-Ni system. All absorbance values are to scale.



30 cm Z-6020 β-Diketone-3 column
Flow rate = 1.0 ml/min

Figure 16. HPLC Chromatogram of Cu Using Atomic Absorption Spectrophotometry for Detection.

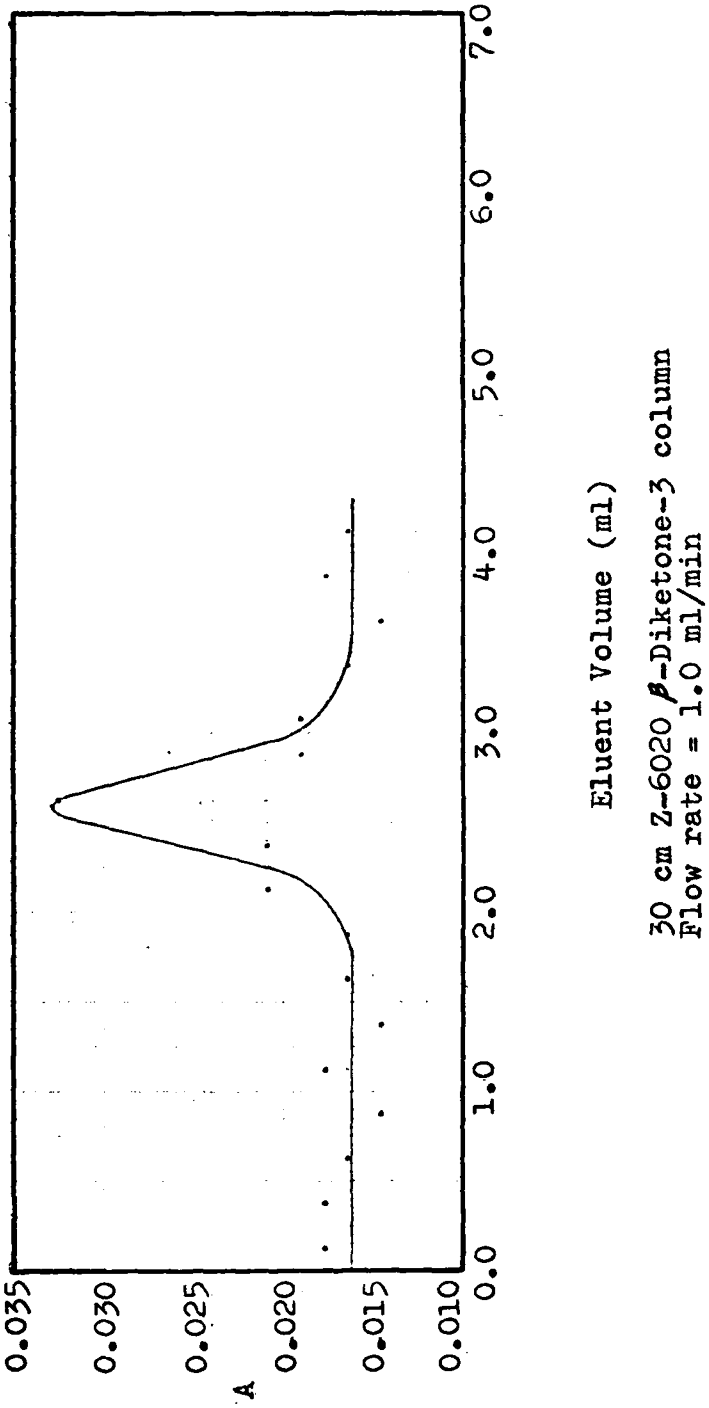
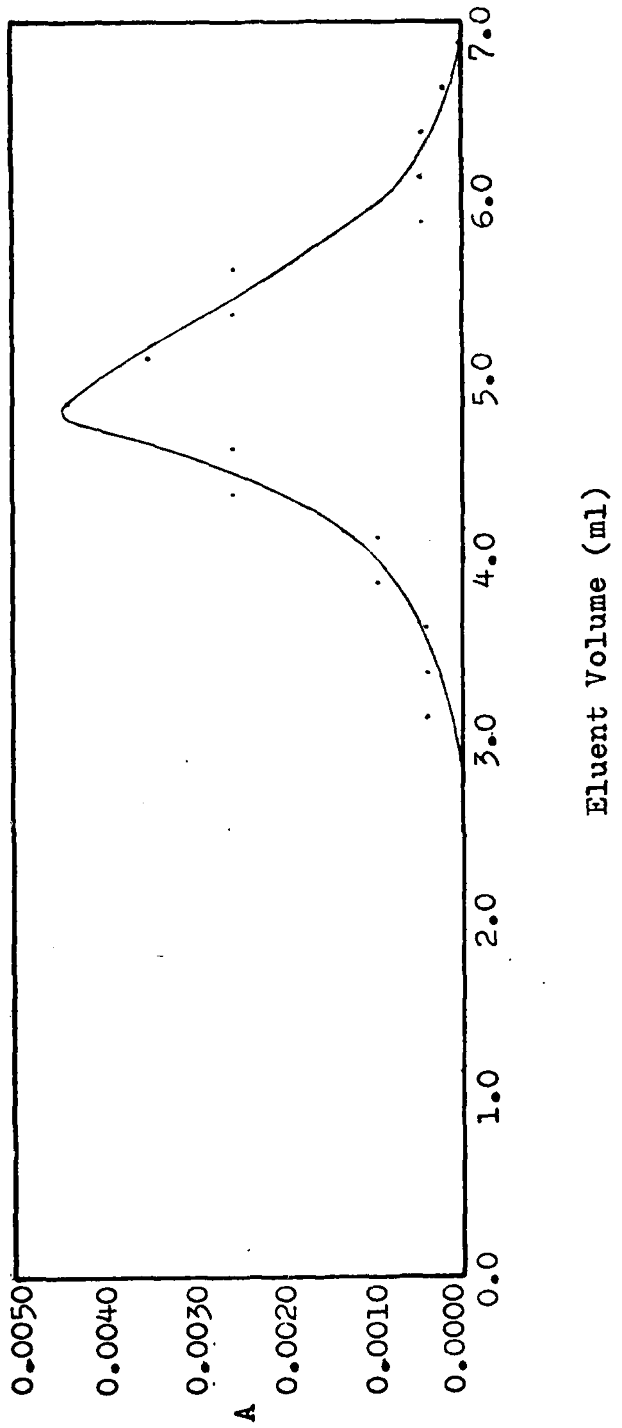


Figure 17. HPLC Chromatogram of Zn Using Atomic Absorption Spectrophotometry for Detection.



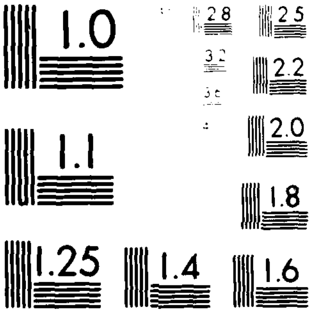
30 cm Z-6020 β-Diketone-3 column
Flow rate = 1.0 ml/min

Figure 18. HPLC Chromatogram of Ni Using Atomic Absorption Spectrophotometry for Detection.

AD-A107 967   AIR FORCE INST OF TECH WRIGHT-PATTERSON AFB OH   F/G 7/4
SEPARATION OF METAL IONS (FE(+3), CU(+2), NI(+2), ZN(+2)) BY US--ETC(U)
1979   K T DENBLEYKER
UNCLASSIFIED   AFIT-CI-79-310T-S   NL

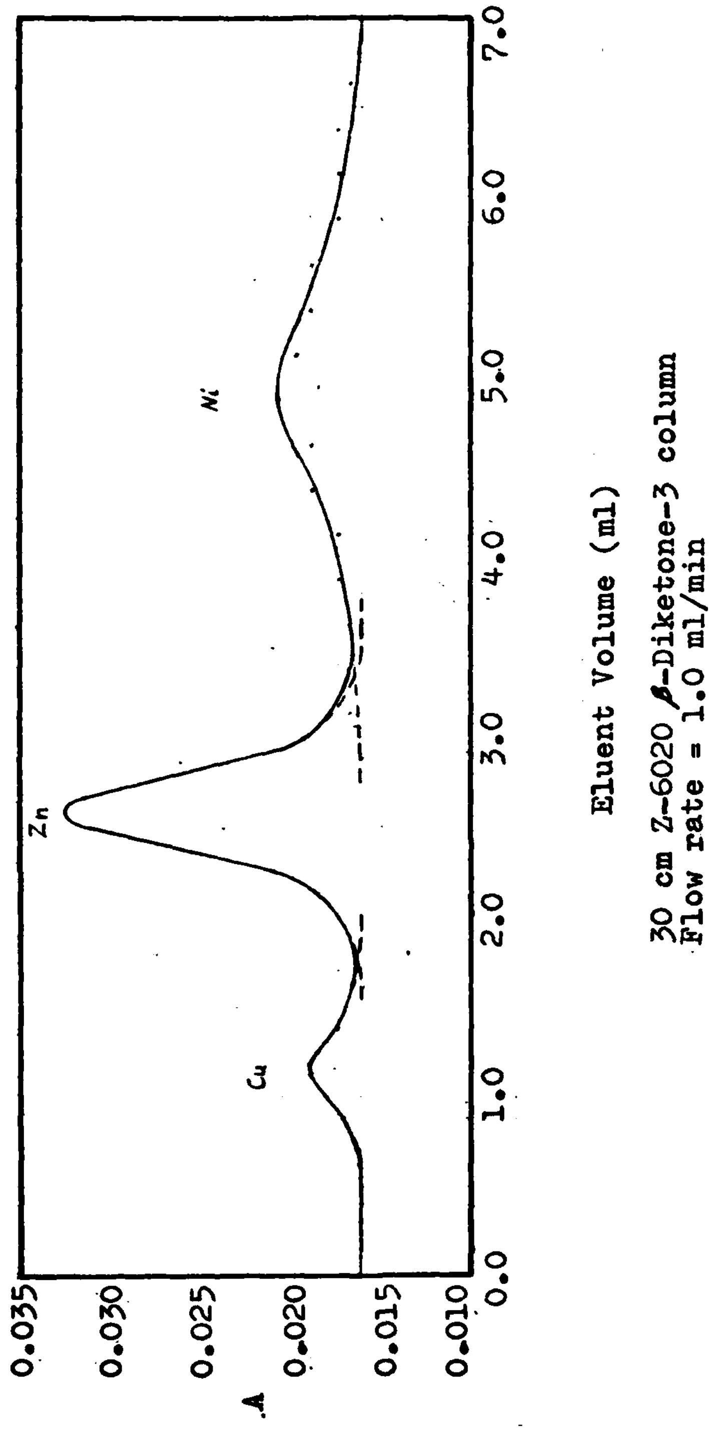
2 OF 2
AD A
[illegible]

END
DATE
FILMED
1 -82
DTIC



MICROCOPY RESOLUTION TEST CHART

NATIONAL BUREAU OF STANDARDS 1963-A



30 cm Z-6020 β-Diketone-3 column
Flow rate = 1.0 ml/min

Figure 19. HPLC Chromatogram for Cu, Zn and Ni Using Atomic Absorption Spectrophotometry for Detection.

The resolution between two chromatographic peaks is the ratio of the difference of their retention volumes to the average base peak width.

$$R_s = \frac{V_{R_2} - V_{R_1}}{\frac{1}{2}(W_1 + W_2)} \tag{15}$$

$V_R$ and W are values defined in Figure 2.

The resolution between adjoining metal ion peaks is listed in Table XIX. Intermediate values are also listed.

TABLE XIX. Resolution Between Metal Ion Peaks on an HPLC Chromatogram.

| Metal Ion Pair | $V_{R_1}$ (ml) | $V_{R_2}$ (ml) | $W_1$ (ml) | $W_2$ (ml) | $R_s$ |
|---|---|---|---|---|---|
| $Cu^{+2}$-$Zn^{+2}$ | 1.16 | 2.58 | 0.96 | 0.98 | 1.45 |
| $Zn^{+2}$-$Ni^{+2}$ | 2.58 | 4.91 | 0.98 | 3.27 | 1.10 |

30 cm Z-6020 β-Diketone-3 column
Flow rate = 1.0 ml/min

Calculations were made using measurements from Figures 16, 17 and 18. The base peak width for Ni was estimated as well as the peak shape would allow. Tangents were drawn almost as if the peak exhibited a Gaussian profile.

$Cu^{+2}$ and $Zn^{+2}$ are almost baseline resolved. For $Zn^{+2}$ and $Ni^{+2}$, $R_s$ = 1.10. For $R_s$ = 1.00, resolution is about 94% complete, which corresponds to a 3% overlap of peak areas. $R_s$ = 1.10 is certainly sufficient for most analytical purposes.

The number of theoretical plates for each solute was calculated using Equation 12. The results are listed in Table XX.

TABLE XX. Calculated Number of Theoretical Plates (N) for an HPLC Column.

| Metal Ion | $V'_R$ (ml) | $W_{1/2}$ (ml) | N |
|---|---|---|---|
| $Cu^{+2}$ | 1.16 | 0.43 | 40.32 |
| $Zn^{+2}$ | 2.58 | 0.50 | 147.50 |
| $Ni^{+2}$ | 4.91 | 1.14 | 102.77 |

30 cm Z-6020 β-Diketone-3 column
Flow rate = 1.0 ml/min

The N values for Zn and Ni are within order of magnitude agreement with the N values for TLC (see Table XIII). However, the HPLC value for N for Cu is almost one hundred times smaller than for the TLC value. Some factors which may contribute to this large difference are listed below.

1) There is a slight possibility that the HPLC column was overloaded with the amount of sample injected (10 μg). This would cause the bulk of the least easily retained metal, Cu, to be flushed through the column quickly. This decreases $V'_R$ and decreases N by a factor of $(V'_R)^2$.

2) Total TLC development took approximately 20 minutes. In HPLC, Cu was eluted in about 1.2 minutes. Cu had a period about seventeen times longer to equilibrate

between stationary and mobile phases in TLC than in HPLC. This could account for great differences between the N values in TLC and HPLC.

3) All parameters in Table XIII were obtained by visual measurement. Since Cu travelled the farthest of the metals, there was a chance that it spread the most. The visual detection limit might have been poor enough to give a false final spot length. It is possible, then, that the value measured as $4\sigma$ did not contain 95.5% of all the Cu. If this was true, the $4\sigma$ value should be larger than the value listed for Cu in Table XIII. Hence, $\overline{H}$ would increase and N would decrease, bringing it closer to the value calculated from the HPLC column.

Because of a non-rigorous treatment of the theory and the limits of visual measurements, the TLC in this research should not be compared too closely with the HPLC. The two different methods have two different applications. TLC should be used as a quick, first-look separative method for a solute-solvent-substrate system. Its results can be applied to HPLC and then the HPLC system can be optimized. Once this is done, the HPLC system can be used effectively as a very quick analytical method for samples which must be separated on a repetative basis.

# CONCLUSIONS

In general, $R_f$ values for metal ions were higher on Silica Gel G than on Silica Gel 7. An effective separation of three of the four metals, $Fe^{+3}$, $Cu^{+2}$, $Ni^{+2}$ and $Zn^{+2}$, can be accomplished with the following solvent systems on Silica Gel 7 substrate: water, 0.1M en in water, pH=2.00 hydrochloric acid, 10% acetylacetone in acetone, 2% salicylaldoxime in chloroform, 2% salicylaldehyde in ethanol, 1% dimethylglyoxime in ethanol and a pH 2.00 buffer. Separation of all four metals might be possible on an efficient silica column using an aqueous 0.1M en mobile phase.

The Z-6020 Amine substrate, using an aqueous 0.1M en mobile phase, separates three of the four metals from each other.

The Z-6020 Dithiocarbamate and the Z-6020 bis-Dithiocarbamate exhibited poor ability to separate the four metals.

The Z-6020 β-Diketone substrates usually showed good separations. By far, the best separation of all four metals obtained during this research occurred on the Z-6020 β-Diketone-3, an immobilized trifluoroacetylacetone substrate. The mobile phase was 10% trifluoroacetylacetone (TFAA) in acetone.

Another fairly good separation of all four metals was on Z-6020 Arylamine, an immobilized analine substrate, with a 2% salicylaldoxime in chloroform mobile phase.

Separations of Fe, Cu, Ni and Zn on the $\gamma$-Amine, an immobilized n-propyl amine, were not very good. At best, two metals were separable from each other and from a mixture of the other two metals.

On TLC plates that showed only moderate tailing, it is conceivable that better separations could be obtained on an efficiently packed HPLC column.

Using the Z-6020 $\beta$-Diketone-3 substrate and a 10% TFAA in acetone mobile phase, about 84 theoretical plates are needed to separate $Cu^{+2}$ and $Zn^{+2}$. To separate $Zn^{+2}$ and $Ni^{+2}$, approximately 30 plates are needed. Separation of $Ni^{+2}$ and $Fe^{+3}$ requires only 9 plates.

With the exception of the dithiocarbamates, the bonded-phase substrates appear to be reusable if used in a pH range of 2 - 8 or with organic mobile phases.

HPLC runs with $Cu^{+2}$ on a column packed with Z-6020 $\beta$-Diketone-3 showed an optimum mobile phase flow rate of about 1.1 ml/min. The mobile phase was 10% TFAA in acetone. For HPLC separation of $Cu^{+2}$, $Zn^{+2}$ and $Ni^{+2}$, the elution order of the three metals was $Cu > Zn > Ni$. The resolution between the $Cu^{+2}$ and $Zn^{+2}$ peaks is 1.45. The resolution between the $Zn^{+2}$ and $Ni^{+2}$ peaks is 1.10. The three metals are sufficiently separated for most analytical purposes.

TLC should be used primarily to obtain quick separation for a new solute-solvent-substrate system. Its results can be applied to HPLC. The HPLC system can then be optimized and used as a quick analytical method for samples which must be separated on a repetative basis.

# BIBLIOGRAPHY

## Books

1. Akhrem, A.A., Kuznetsova, A.I.; Thin Layer Chromatography; Israel Program for Scientific Translations, Jerusalem, 1965.

2. Bobbitt, J.M., Schwarting, A.E., Gritter, R.J.; Introduction to Chromatography; Reinhold Book Corp., New York, 1968.

3. Burger, K.; Organic Reagents in Metal Analysis; Pergamon Press Ltd., London, 1973.

4. De, A.K., Khopar, S.M., Chalmers, R.A.; Solvent Extraction of Metals; Van Nostrand Reinhold Co., London, 1970.

5. Dean, J.A.; Chemical Separation Methods; Van Nostrand Reinhold Co., London, 1969.

6. Flashka, H.A., Barnard, A.J. Jr.; Chelates in Analytical Chemistry, Volumes 1,2,3, and 4; Marcel Dekker, Inc., New York, 1967.

7. Grushka, E.; Bonded Stationary Phases in Chromatography; Ann Arbor Science Publishers, Inc., Ann Arbor, 1974.

8. Karger, B.L., Snyder, L.R., Horvath, C.; An Introduction to Separation Science; John Wiley and Sons, Inc., New York, 1973.

9. Kertes, A.S., Marcus, Y.; Solvent Extraction Research; John Wiley and Sons, Inc., New York, 1969.

10. Korkisch, J.; Modern Methods for the Separation of Rarer Metal Ions; Pergamon Press Ltd., London, 1969.

11. McKay, H.A.C., Healy, T.V., Jenkins, I.L., Naylor, A.; Solvent Extraction Chemistry of Metals; MacMillan and Co., Ltd., London, 1967.

12. Martell, A.E., Calvin, M.; Chemistry of Metal Chelate Compounds; Prentice Hall, Inc., New York, 1952.

13. Morrison, G.H., Freiser, H.; Solvent Extraction in Analytical Chemistry; John Wiley and Sons, Inc., New York, 1957.

14. Pierce, A.E.; Silylation of Organic Compounds; Pierce Chemical Co., Rockford, 1968.

15. Sekine, T., Hasegawa, Y.; Solvent Extraction Chemistry; Marcel Dekker, Inc., New York, 1977.

16. Smith, I., Feinberg, J.G.; Paper & Thin Layer Chromatography & Electrophoresis; Longman Group Ltd., London, 1972.

17. Smith, O.C.; Inorganic Chromatography; D. Van Nostrand Co., Inc., New York, 1953.

18. Stahl, E.; Thin Layer Chromatography, A Laboratory Handbook; Academic Press, Inc., New York, 1965.

19. Stary, J.; The Solvent Extaction of Metal Chelates; The MacMillan Co., New York, 1964.

20. Stock, R., Rice, C.B.F.; Chromatographic Methods; Chapman and Hall Ltd., London, 1974.

21. Weast, R.C.; CRC Handbook of Chemistry and Physics, 54th Edition; CRC Press, Cleveland, 1973.

22. Wolf, F.J.; Separation Methods in Organic Chemistry and Biochemistry; Academic Press, Inc., New York, 1969.

23. Zlatkis, A., Kaiser, R.E.; HPTLC (High Performance Thin Layer Chromatography); Elsevier Scientific Publishing Co., Amsterdam, 1977.

24. Zolotov, Y.A.; Extraction of Chelate Compounds; Humphrey Science Publishers, Ann Arbor, 1970.

## Journals

1. Baffi, F., Dadone, A., Frache, R.; Chromatographia, 9, 280 (1976).

2. Bauman, A.J., Weetall, H.H., Weliky, N.; Anal. Chem., 39, 932 (1967).

3. Berg, E.W., Acosta, J.J.C.; Anal. Chim. Acta, 40, 101 (1968).

4. Brinkman, U.A.Th., deVries, G., Kuroda, R.; J. Chromatogr., 85, 187 (1975).

5. Capitan, F., Salinas, R., Martinez, J.L.; Ars. Pharm., 16, 561 (1975).

6. Chow, F.K., Grushka, E.; Anal. Chem., 50, 1346 (1978).

7. Cox, G.B.; J. Chromatogr. Sci., 15, 385 (1977).

8. Dingman, J. Jr., Siggia, S., Barton, C., Hiscock, K.B. ; Anal. Chem., 44, 1351 (1972).

9. Durairaj, K., Arulraj, S.J., Patel, C.C.; Indian J. Chem. Sect. A, 15A, 476 (1977).

10. Frache, R., Dadone, A.; Chromatographia, 5, 449 (1972).

11. Frache, R., Dadone, A.; Chromatographia, 6, 433 (1973).

12. Frache, R., Dadone, A., Baffi, F.; Chromatographia, 9, 83 (1976).

13. Gaetani, E., Laureri, C., Manzia, A., Parolari, G.; Anal Chem., 48, 1725 (1976).

14. Gagliardi, E., Deutschmann, G.; Mikrochim. Acta, 2, 23 (1976).

15. Gilpin, R.K., Sisco, W.R.; J. Chromatogr., 124, 257 (1976).

16. Guiochon, G., Siouffi, A.; J. Chromatogr. Sci., 16, 470 (1978).

17. Hemetsberger, H., Maasfeld, W., Ricken, H.; Chromatographia, 9, 303 (1976).

18. Hilliard, L.B., Freiser, H.; Anal. Chem., 24, 752 (1952).

19. Honjo, T., Fujioka, Y., Itoh, H., Kiba, T.; Anal. Chem., 49, 2241 (1977).

20. Horvath, C., Melander, W.; J. Chromatogr. Sci., 15, 393 (1977).

21. Huber, J.F.K., Kraak, J.C., Veening, H.; Anal. Chem., 44, 1554 (1972).

22. Hunt, D.C., Wild, P.J., Crosby, N.T.; J. Chromatogr., 130, 320 (1977).

23. Karger, B.L., Giese, R.W.; Anal. Chem., 50, 1049A (1978).

24. Kirkland, J.J.; J. Chromatogr. Sci., 10, 129 (1972).

25. Knox, J.H.; J. Chromatogr. Sci., 15, 352 (1977).

26. Kojdl, I.; Chromatographia, 9, 401 (1976).

27. Kuroda, R., Matusue, H., Oguma, K.; Radioanal. Chem., 36, 119 (1977).

28. Kuroda, R., Takahashi, T., Oguma, K.; Bull. Chem. Soc. Jpn., 49, 815 (1976).

29. Leyden, D.E., Luttrell, G.H.; Anal. Chem., 47, 1612 (1975).

30. Lohmueller, M., Heizmann, P., Ballschmiter, K.; J. Chromatogr., 137, 165 (1977).

31. Majors, R.E.; Analusis, 3, 549 (1975).

32. Majors, R.E.; Anal. Chem., 44, 1722 (1972).

33. Majors, R.E.; Int. Lab., July-Aug 1972, 25.

34. Majors, R.E.; J. Chromatogr. Sci., 15, 334 (1977).

35. Moyers, E.M., Fritz, J.S.; Anal. Chem., 48, 1117 (1976).

36. Muchova, A., Jokl, V.; Chem Zvesti, 30, 629 (1976).

37. Oksala, R.H. Jr., Krause, R.A.; Anal. Chim. Acta, 85, 351 (1976).

38. Phillips, R.J., Fritz, J.S.; Anal. Chem., 50, 1504 (1978).

39. Qureshi, M., Sharma, S.D.; Chromatographia, 11, 153 (1978).

40. Qureshi, M., Thakur, J.S.; Sep. Sci., 11, 467 (1976).

41. Qureshi, M., Varshney, R.G., Fatima, N.; Sep. Sci., 12, 321 (1977).

42. Qureshi, M., Varshney, R.G., Rajput, R.P.S.; Sep. Sci., 11, 533 (1976).

43. Rao, A.L.J., Shekar, C.; Fresenius' Z. Anal. Chem., 277, 126 (1976).

44. Rehak, V., Smolkova, E.; Chromatographia, 9, 219 (1976).

45. Shimizu, T., Katsuno, M., Iizuka, N.; J. Chromatogr., 106, 230 (1975).

46. Shimizu, T., Kobayashi, H.; Chromatographia, 10, 611 (1977).

47. Shimizu, T., Kogure, Y., Arai, H., Suda, T.; Chromatographia, 9, 85 (1976).

48. Shimizu, T., Nakawaza, K., Kikuchi, T.; Chromatographia, 9, 574 (1976).

49. Singh, N., Kumar, R., Kansal, B.D.; Chromatographia, 11, 408 (1978).

50. Singh, N., Tandon, S.N.; Chromatographia, 10, 309 (1977).

51. Soljic, Z.; Chromatographia, 11, 430 (1978).

52. Soljic, Z., Grba, V.; Z. Anal. Chem., 278, 363 (1976).

53. Srivastava, S.P., Dua, V.K.; Fresenius' Z. Anal. Chem., 286, 255 (1977).

54. Srivastava, S.P., Dua, V.K., Gupta, K.; Chromatographia, 11, 539 (1978).

55. Sugawara, K.F., Weetall, H.H., Schucker, G.D.; Anal. Chem., 46, 489 (1974).

56. Tollinche, C.A., Risby, T.H.; J. Chromatogr. Sci., 16, 448 (1978).

57. Uden, P.C., Bigley, I.E.; Anal. Chim. Acta, 94, 29. (1977).

58. Uden, P.C., Bigley, I.E., Walters, F.H.; Anal. Chim. Acta, 100, 555 (1978).

59. Uden, P.C., Parees, D.M., Walters, F.H.; Anal. Lett., 8, 795 (1975).

60. Uden, P.C., Walters, F.H.; Anal. Chim. Acta, 79, 175 (1975).

61. Unger, K.K., Becker, N., Roumeliotis, P.; J. Chromatogr., 125, 115 (1976).

62. Varshney, R.G.; Curr. Sci., 45, 54 (1976).

63. Walton, H.F.; Anal. Chem., 50, 36R (1978).

64. Weetall, H.H.; Biochim. Biophys. Acta, 212, 1 (1970).

65. Weetall, H.H.; Res./Dev., 22, 18 (1971).