ANALYSIS OF SOME TRANSITION METALS

BY HIGH PRESSURE LIQUID CHROMATOGRAPHY

by

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Submitted in Partial Fulfillment of the Requirements

for the Degree of

Master of Science

in the

Chemistry

Program

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YOUNGSTOWN STATE UNIVERSITY

August, 1980

ABSTRACT

ANALYSIS OF SOME TRANSITION METALS
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The feasibility of the use of ion exchange HPLC for the separation of metal ion mixtures is investigated using two approaches. The first approach involves the separation of metal ions by the cation exchange separation of chloro-complexes. Distribution coefficients for cadmium(II), copper(II), cobalt(II), nickel(II), and zinc(II) were determined in methanol or methanol-water solutions of lithium chloride. The separation of these metals using a Corasil strong cation exchange column is tried.

The other approach to the ion exchange HPLC separation of metal ion mixtures uses tartaric acid complexes. The anion exchange separation of cadmium(II), cobalt(II), copper(II), manganese(II), nickel(II), and zinc(II) using aqueous tartaric acid solutions is demonstrated. The quantitation of manganese(II) is also demonstrated. A Corasil strong anion exchange column and an Aminex A-27 column are used.

ACKNOWLEDGEMENTS

I would like to extend my thanks to Dr. Francis Smith for his guidance throughout this research. I would also like to thank Dr. Daryl Mincey and Dr. Steven Schildcrout for their time spent in the review of this manuscript.

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LIST OF SYMBOLS

SYMBOL	DEFINITION	UNITS OR REFERENCE
mL	Milliliter	1×10^{-3} liter
mg	Milligram	1×10^{-3} gram
µg	Microgram	1×10^{-6} gram
nm	Nanometer	1×10^{-9} meter
mmole	Millimole	1×10^{-3} mole
mol/L	Molarity	moles per liter
L	Liter	--
g	Gram	--
min	Minute	--
^{o}C	Degrees Celsius	--
HPLC	High Pressure Liquid Chromatography	--
UV	Ultraviolet	--
RI	Refractive Index	--
±	Plus or Minus	--
%	Percent	--

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CHAPTER I

INTRODUCTION

Ion Exchange

Ion exchange chromatography involves the substitution of one ionic species for another on a stationary phase. The stationary phase is a rigid matrix which carries functional groups having either positive or negative charges. If there is a positive charge, the exchange site (designated R^+) is associated with an anion X^-. If a solution containing anions Y^- is passed over the stationary phase, Y^- will exchange for X^- and will be retained on the stationary phase. See equation (1).

$$R^+X^- \quad + \quad Y^- \quad \Longleftrightarrow \quad R^+Y^- \quad + \quad X^- \qquad (1)$$
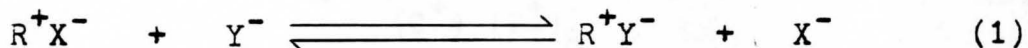
This process is called anion exchange.

The process of cation exchange is analogous. The exchange site is now negatively charged (R^-) and is associated with a cation B^+. If a solution of cations C^+ is passed over the stationary phase, exchange as shown in equation (2) occurs.

$$R^-B^+ \quad + \quad C^+ \quad \Longleftrightarrow \quad R^-C^+ \quad + \quad B^+ \qquad (2)$$
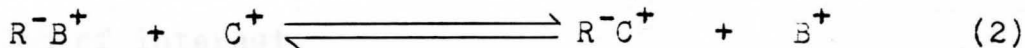
The separation of ions utilizing either cation or anion exchange chromatography is based on the relative affinity of the ions for the exchange site. That is, ions with a strong affinity will be retained on the column longer than

ions with a weaker affinity. In order to devise separation schemata for ions in a mixture it is necessary to have a measure of the affinity of each ion for the exchange site. This information is obtained by determination of the distribution coefficient, D, for each ion in the mixture. This coefficient is the ratio of the equilibrium concentration of a given species in the ion exchange medium to that in the solution.

The two types of distribution coefficients determined are based either on weight distribution (D_w) or volume distribution (D_v). D_w may be derived from the mass action expression for the ion exchange equilibrium. For example, if there is an equilibrium present such as that shown in equation (2), the mass action expression is,

$$K = \frac{(C^+)_R (B^+)}{(C^+)(B^+)_R} \tag{3}$$

The subscript R refers to the concentration of B^+ or C^+ bound to the stationary phase (usually a plastic resin). K is considered constant for: (1) a given ion exchange resin or other stationary phase, (2) a given loading, and (3) a given ionic strength. Loading refers to the actual number of exchange sites on the stationary phase occupied by the ion of interest.

In equation (3), the ratio of $(C^+)_R/(C^+)$ is equal to a distribution constant, D_B+, as shown in equation (4).

$$D_B+ = \frac{(C^+)_R}{(C^+)} \tag{4}$$

If the loading with respect to C^+ is low and if (C^+) is much less than (B^+), then $(B^+)_R$ and (B^+) may be considered constant to a very good approximation. If this is the case, and the concentration of C^+ is kept small, D_{B^+} becomes constant and is equal to D_w. D_w may thus be defined as shown in equation (5).

$$D_w = \frac{\text{amount of } C^+ \text{ per gram of dry resin}}{\text{amount of } C^+ \text{ per mL of solution}} \quad (5)$$

"Dry resin" refers to resin (or other stationary phase) which has been heated to 110° C to get rid of all water.

The volume distribution coefficient, D_v, is related simply to D_w by the expression,

$$D_v = D_w \rho \quad (6)$$

The symbol ρ is defined as kilograms of dry resin per liter of resin bed.

The utility of D_w is that elution orders of particular ions may be predicted on the basis of the relative magnitudes of the coefficients. That is, the higher the weight distribution coefficient, the longer a particular ion will be retained on an exchanger. The utility of D_v is that it may be related to the volume of eluent needed to elute a particular ion from an ion exchange column. Generally, it seems that D_w is determined more often than is D_v. The most probable reason for this is that one usually has some idea of the elution volume if the elution order is known.

Complexation Equilibria

It is well known that in many instances, many metal ions, in the presence of any one of a number of anions, will tend to form complexes with that anion. In the case of a divalent metal ion (M^{2+}) and chloride or tartrate ion (X^-), the following typical equilibria will be achieved (equations (7) through (10)).

$$M^{2+} + X^- \rightleftharpoons MX^+ \tag{7}$$

$$MX^+ + X^- \rightleftharpoons MX_2 \tag{8}$$

$$MX_2 + X^- \rightleftharpoons MX_3^- \tag{9}$$

$$MX_3^- + X^- \rightleftharpoons MX_4^{2-} \tag{10}$$
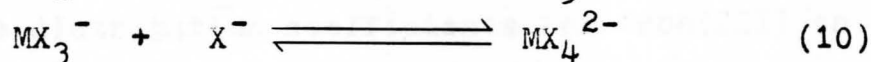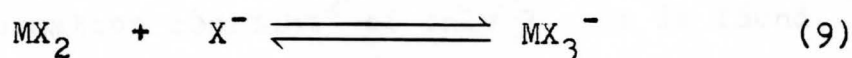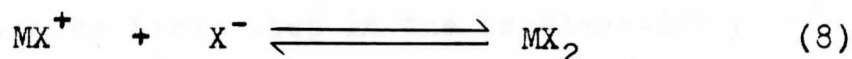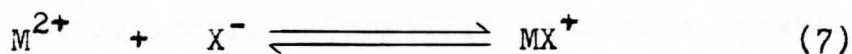
With many of the transition metals, the stability constants for the species formed in the above equilibria are such that the anionic species predominate, unless the concentration of X^- is low.

Chlorocomplexes

Chlorocomplexes will form in hydrochloric acid or any of the alkali-metal chlorides (i.e. KCl, NaCl, LiCl, or CsCl). In such a solution, the typical distribution coefficient will first rise with increasing acid or salt concentration, pass through a maximum, and then decrease. At low chloride concentration (less than 0.5 mol/L) the distribution coefficients will rise with the square of the chloride concentration. According to Schindewolfe[1], this is a result of the equilibrium shown in equation (11).

$$M^{2+} + 2Cl^- + 2RCl \rightleftharpoons R_2MCl_4 \tag{11}$$
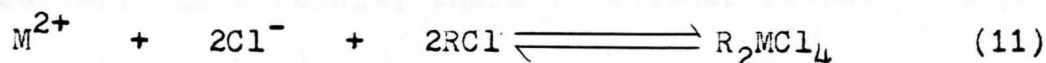
At high chloride concentrations, the distribution coefficients fall as a result of the displacement of the metal-chloride complex from the ion exchange functional group by chloride ions. This is illustrated in equation (12).

$$R_2MCl_4 \quad + \quad 2Cl^- \rightleftharpoons 2RCl \quad + \quad MCl_4^{2-} \qquad (12)$$
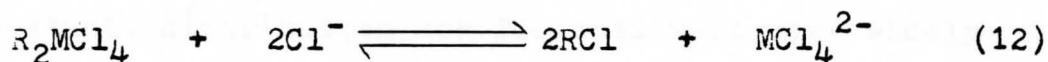
In solution, the stability of the chlorocomplex is not necessarily the same as the stability of the complex in the ion exchange medium itself. Iron(III) is a good example of this behavior. The first step in the complexation reaction has a formation constant[2] of only 3. It is found, however, that the distribution coefficients for iron(III) on a quaternary ammonium ion exchange resin are in excess of 10,000. Another example is cobalt(II). The second step in the complexation reaction has a formation constant[2] of only 0.2 yet distribution coefficients are in excess of 100. This means that although formation constants are small, large amounts of anionic complex are being formed. According to Rieman and Walton[2], this is due to the close association of the complex ions in the resin with the chloride ions fixed on the resin. As a result, ion pairs form causing the formation of the complexes $FeCl_4^-$ and $CoCl_4^{2-}$. In fact, ion exchange does not occur.

Tartaric Acid Complexes

Tartaric Acid complex formation is essentially the same as chloride complex formation. Tartaric acid is a weak acid, however. As a result, there is another equilibrium to

consider, i.e. the dissociation of the acid. The distribution coefficients are not only dependent on the tartaric acid concentration but also depend on the pH.

It is found that, if tartaric acid concentration is kept constant, distribution coefficients will vary widely with pH variations. The reason for this is that the concentration of the free tartrate anion is not the same as the concentration of the acid. At low pH, the acid is largely undissociated and very little free anion is present. As the pH is increased, more and more free anion is released until at high pH most of the acid is in the free anion form.

Distribution coefficients of the tartaric acid complexes follow much the same pattern as chlorocomplex distribution coefficients. They are low at low pH or low tartaric acid concentration, increasing with increasing pH or tartaric acid concentration, passing through a maximum and finally decreasing. The reasons for this behavior are the same as those given for the behavior of chlorocomplexes.

Stationary Phases in HPLC Ion Exchange

The ion exchange stationary phases used in HPLC are generally made of a silica backbone with attached ionic functional groups. Functional groups are generally of the form, $-SO_3H$ for strong cation exchangers and $\overset{+}{N}R_3$ for strong anion exchangers.

The conventional type of plastic resin, which finds some use in HPLC, is of two general types, microreticular (gel) and macroreticular (macroporous). With the gel type,

pore size depends on the degree of resin swelling as governed
by the amount of cross-linking present. Pores in the macro-
reticular type resins are permanent. These resins are
usually made of polystyrene with the degree of cross-linking
controlled by the percentage of divinylbenzene present.

Materials developed exclusively for use in HPLC are
the porous layer bead packings. Older porous layer exchangers
are generally a solid polymer or glass bead core surrounded
by a skin of ion exchange material. This skin of material
is comparable to a conventional ion exchanger. Newer porous
layer materials are porous layer silica beads treated with
organosilanes containing the appropriate ionic groups. These
organosilanes become chemically attached to the porous sur-
face of the silica bead rather than being attached as a skin
on the surface.

The porous layer materials are probably better for
HPLC use than are conventional type materials. The major
advantage over conventional materials is the greater speed
of mass transfer obtained with porous layer materials.
Since the ion exchange material is present as only a thin
layer on the bead surface, ions do not have to diffuse very
far into the material for exchange to occur. Another advan-
tage of porous layer materials is the fact that swelling
does not occur and they are stable if eluent composition is
changed, both of which can be problems with conventional
materials.

The major disadvantage of porous layer materials is
their relatively small capacities. While conventional

materials have capacities of, typically, from 3 to 5 milli-
equivalents per gram of dry material, porous layer materials
have capacities of only, typically, 5 to 15 microequivalents
per gram of dry material. This means that porous layer
packings give low column capacities which make trace analyses
difficult.

All of the materials mentioned above are usable with
HPLC systems. The ultimate choice of material rests with
the demands of the user.

CHAPTER II

HISTORICAL

Chloride Complexes

The use of anionic chloride complexes of metal ions
as a means of separation is really quite old. Around 1953,
two groups, Kraus and Moore[3] in the United States and
Jentsch[4] in Germany, performed experiments with the anion
exchange chromatography of metal ions in hydrochloric acid
using plastic resins of the quaternary ammonium type. They
found that, in several instances, the change of distribution
coefficient with changing concentration of hydrochloric acid
was over several powers of ten. These large differences in
distribution coefficients allowed good separations.

Separation was achieved by placing a solution of the
metal ions, in 12 mol/L hydrochloric acid, on top of the resin
bed (in an ion exchange column) and eluting the metal ions
with stages of decreasing hydrochloric acid concentration.
The effect is that those metal ions which form anionic com-
plexes will be retained on the column. As the hydrochloric
acid concentration is lowered, the distribution coefficients
of some of the metal ions begin to decrease. Those which
decrease first elute from the column first. The last metal
ion to elute from the column is the one with the highest
distribution coefficient.

In later work, Kraus and his coworkers[5,6] measured distribution coefficients for a large number of metal ions in hydrochloric acid concentrations up to 12 mol/L using radioactive tracers. For convenience, since a large number of elements were determined, they presented these as a series of semi-log graphs grouped into a periodic table, each element with its own graph.

For the separation of metal chlorocomplexes, the emphasis then seemed to shift from anion exchange to cation exchange. Also, about the same time, it was discovered that the addition of organic solvents to the chlorocomplex systems would cause enhanced complexation. This led to higher distribution coefficients on anion exchangers and lower distribution coefficients on cation exchangers[7].

Cation exchange chromatography involves the adsorption of metal ions as uncomplexed cations onto the resin. Chloride solutions are then passed through in stages of increasing chloride concentration. Those metal ions which form the most stable anionic complexes will elute first while those forming the least stable complexes will elute last[7].

Kember, Macdonald, and Wells[7] were probably the first to recognize the utility of and actually use organic solvents for the cation exchange separation of metal chlorocomplexes. They were able to completely separate copper(II) and nickel(II) using 4% (v/v) hydrochloric acid and 10% (v/v) water in acetone.

Fritz and Rettig[8] determined the distribution coefficients of 14 metals in acetone-water-hydrochloric acid media.

Acetone concentrations up to 89% and hydrochloric acid concentrations up to 1.0 mol/L were used. They demonstrated the separation of several synthetic mixtures of metal ions using their data. Strelow, Victor, van Zyl, and Eloff[9] determined the distribution coefficients of 54 metals in acetone-water-hydrochloric acid media. Acetone concentrations to 90% and hydrochloric acid concentrations of 0.1 to 3.0 mol/L were used. They also demonstrated the separation of several synthetic metal ion mixtures. Korkisch and Ahluwalia[10] determined the distribution coefficients of 20 metals in hydrochloric acid and the organic solvents methanol, ethanol, n-propanol, iso-propanol, methyl glycol, acetone, tetrahydrofuran, and acetic acid.

Other examples of cation exchange separations include the separation of calcium from magnesium in hydrochloric acid-ethanol[11], the separation of the rare earth elements and scandium from a large number of heavy metals[12], and the separation of barium and strontium in hydrochloric acid and the organic solvents acetone, methanol, ethanol, and dioxane[13].

Anion Exchange

Anion exchange of chlorocomplexes seems to have found more use in the investigation of the theoretical aspects of the ion exchange reaction than in actual separations. Marcus and coworkers have investigated the behavior of a number of metal ion systems using anion exchange. They determined[14] the distribution coefficients of zinc(II), cadmium(II), and mercury(II) in anhydrous ethanol solutions of hydrochloric

acid and lithium chloride. They used the data to investigate the resin invasion by electrolytes in these systems. They also determined[15] the distribution coefficients of rhenium(VII), copper(II), cadmium(II), and iron(III) in anhydrous methanol and anhydrous ethanol solutions of hydrochloric acid and tetramethylammonium chloride. These data were used to deduce the species formed both in the resin and in solution.

Marple[16] has investigated the mechanism of adsorption of cadmium(II) from chloride media using anion exchange. Horne[17] investigated the adsorption of zinc(II) from solutions of lithium chloride, calcium chloride, magnesium chloride, sodium chloride, hydrochloric acid, potassium chloride, cesium chloride, and ammonium chloride.

Alkali-Metal Chlorides

In 1955, Kraus and coworkers[18] discovered that a number of metal ions were adsorbed more strongly onto anion exchangers from lithium chloride solutions than from hydrochloric acid solutions of the same concentration. For example, manganese was found to have a distribution coefficient of 550 in 12 mol/L lithium chloride and a distribution coefficient of only 4 in 12 mol/L hydrochloric acid. Similar effects were observed for the elements scandium(III), iron(III), cobalt(II), zinc(II), gallium(III), and gold(III). It was stated that, in general, the distribution coefficients were more than a factor of 100 greater in 12 mol/L lithium chloride than in 12 mol/L hydrochloric acid.

Tsintsevich, Gorokhova, and Tavroskaya[19] investigated
the cation exchange behavior of gallium(III) and indium(III)
in 1 to 5 mol/L lithium chloride, sodium chloride, potassium
chloride, and ammonium chloride aqueous solutions. They
found that for both metals, the distribution coefficients
decrease regularly with increasing concentration of salt.
The extent of the decrease depended on the salt used.

Bagbanly et al[20] separated tellurium, gallium, and
indium from the heavy metals lead, gold, iron, antimony,
silver, cobalt, and cadmium using anion exchange chromatography
and solutions of lithium chloride. Bagbanly, Guseinov, and
Allakhverdieva[21] used lithium chloride acidified with
hydrochloric acid for the anion exchange separation of
gallium, aluminum, iron, vanadium, and titanium. Tikhomirova[22]
has also separated the elements americium and europium from
one another using anion exchange and solutions of lithium
chloride.

Lee, Lee, and Kang[23] determined the cation exchange
distribution coefficients for cadmium, magnesium, zinc,
copper, and nickel and the anion exchange distribution
coefficients for cadmium and zinc using potassium chloride
solutions. The determinations were done in methanol-water
solutions up to 40% methanol and up to 1.0 mol/L potassium
chloride. The separation of a number of synthetic mixtures
of these metal ions was achieved using cation exchange
chromatography. Since acid was not used in any system,
the pH was such that hydrolysis of iron and nickel occured.

Salicylic acid or sulfosalicylic acid were added to the eluting media to prevent iron hydrolysis. Glycine was added to prevent nickel hydrolysis.

The number of published uses of chloride in the conventional ion exchange separation of metals is too great to list them all. The above is meant to give an insight into the evolution of the use of chlorocomplexes in ion exchange. It is believed, however, that the references given for the use of alkali-metal chlorides in non-acid media comprise a complete list of that work to date.

Tartaric Acid Complexes

The beginning of the use of tartaric acid as a complexing agent for ion exchange chromatography is less clear cut than for the use of chlorocomplexes. It appears, however, that Glass[24], in 1955, was the first to do so. He succeeded in separating americium and curium using 0.1 mol/L tartaric acid at pH = 4.0. Gallium and aluminum were studied in 1958 and determined to be inseparable using tartaric acid as the eluent[25]. Distribution coefficients for the tartaric acid complexes of zirconium and hafnium have been determined[26] but no separations have been reported.

One of the first groups to more extensively study the use of tartaric acid was that of Morie, Sweet, and Pitstick[27]. They determined anion exchange distribution coefficients for manganese(II), cobalt(II), zinc(II), copper(II), and iron(III) in tartaric acid concentrations from 2.12×10^{-3} mol/L to 8.50×10^{-2} mol/L at pH values from 2 to 13. A separation

of all five metals from one another was demonstrated using
2.12 X 10^{-3} mol/L tartaric acid at pH = 4.5. In a later
work, using the same conditions, Morie and Sweet[28] extended
the distribution coefficients to the tartaric acid complexes
of chromium, molybdenum, nickel, silver, cadmium, and mercury.
They also demonstrated a number of possible separations of
these metals along with those determined earlier. Other
work by Morie and Sweet[29] demonstrated the separation of
iron(III) and iron(II) using 8.50 X 10^{-2} mol/L tartaric acid
at pH = 2.0.

In 1973, Quershi, Varshney, and Kaushik[30] performed
distribution coefficient determinations using cation exchange
on the tartaric acid complexes of titanium(IV), vanadium(V),
iron(III), niobium(V), and UO_2(II) in formic, oxalic,
tartaric, and citric acid media. Several separations were
demonstrated.

Other cation exchange work is that done by Dadone,
Baffi, and Frache[31]. They determined distribution coeffi-
cients for the tartaric acid complexes of manganese,
cadmium, cobalt, nickel, copper, aluminum, and iron. These
were determined in 0.1 to 1.0 mol/L tartaric acid. A
mixture of all these metal ions, except aluminum, was
separated.

High Pressure Liquid Chromatography

There has been relatively little work done with the
ion exchange high pressure liquid chromatography of metal
ions. All of the work done using chlorocomplexes as the

basis of separation has been done using specially adapted equipment rather than equipment which is comercially available. In 1971, Seymour, Sickafoose, and Fritz[32] applied forced flow anion exchange chromatography to the quantitative determination of iron(III). The column used for the analysis was a stainless steel tube packed with a macroreticular anion exchange resin. For the analysis, the iron(III), in 6 mol/L hydrochloric acid, was adsorbed onto the resin. It was subsequently eluted with 2 mol/L hydrochloric acid. Quantitation was done photometrically with the use of a flow cell. A linear calibration curve of peak height versus micrograms of iron(III) was obtained. Analysis of NBS standards were used to verify the procedure.

Seymour and Fritz[33], using the same system described above, studied the separation of ten metals in mixed hydrochloric-perchloric acid media. The metals mercury, copper, lead, and tin were quantitated.

Kawazu and Fritz[34] reported the separation of seven metals using forced flow cation exchange chromatography. The eluents used were either 2-propanol and water or acetone and water. The eluted peaks were detected spectrophotometric- ally after the addition of the color-forming reagent 4-(2-pyridylazo) resorcinol (P.A.R.). Quantitations were not performed. Fritz and Story[35] demonstrated a number of separations using forced flow cation exchange with hydro- chloric acid in acetonitrile as the eluent. NBS standards were analyzed to verify the procedure. In a similar work, Kawazu[36] demonstrated the forced flow cation exchange

separation of copper, cobalt, manganese, vanadium, and nickel. Eluting agents used were hydrochloric acid in the organic solvents tetrahydrofuran, 2-propanol-tetrahydrofuran, dimethylsulfoxide, dimethylformamide, or acetone-dimethyl-formamide.

The use of tartaric acid as an eluent for high pressure liquid chromatography has also been demonstrated. Takata and Muto[37] separated the metals mercury, copper, zinc, nickel, lead, cadmium, and cobalt using cation exchange and 0.15 mol/L sodium tartrate-0.09 mol/L sodium chloride at pH = 3.0 as the eluent. Detection was by constant potential coulometry. Takata and Fujita[38] used a flow coulometric detector in the cation exchange separation of copper, zinc, nickel, cobalt, and cadmium in 0.2 mol/L sodium tartrate at pH = 3.8. Girard[39] used ion chromatography for the separation of copper, zinc, nickel, cobalt, and cadmium using 0.18 mol/L sodium tartrate, 0.04 mol/L tartaric acid, and 0.04 mol/L sodium chloride. Detection was by means of flow coulometry.

High pressure liquid chrmatography has not found much use in the ion exchange analysis of metals for essentially two reasons. They are: (1) many of the eluents are too corrosive to many commercially available instruments or are not adaptable to columns presently used for ion exchange in HPLC systems, and (2) conventional methods of detection, such as ultraviolet or refractive index, are not usable due to interference from the eluting media.

It is believed that the references given for the use of tartaric acid and HPLC are a complete compilation to date.

CHAPTER III

STATEMENT OF PROBLEM

The use of high pressure liquid chromatography as an analytical tool has increased dramatically since its inception in the late 1960's and early 1970's. Its use, in the vast majority of the cases, however, has been in the analysis of substances organic or biochemical in nature. Very little has been published about the use of ion exchange HPLC for the separation and/or quantitation of metals.

Many of the ion exchange methods published over the last thirty-five years have involved the use of strong acid eluents. The use of these strong acids with a conventional HPLC system, using bonded phase pellicular or micro-particulate columns, is not possible. Such use could possibly lead to irreversible pump damage and, very quickly, to column degeneration and destruction. It is thus desirable to find an ion exchange system using no acid or only weak acid for adaptation to comercially available HPLC systems.

The use of lithium chloride in methanol or methanol-water to cause chlorocomplex formation fits the necessary role well. Chlorocomplexes will form readily with lithium chloride and the solvent system is not detrimental to the HPLC system. As an aid in the application of such a system, distribution coefficients of the metals investigated were

required for the lithium chloride-methanol systems. Deter-
mination of these was necessary as these data are not
available in the literature.

The use of tartaric acid complexes in HPLC ion
exchange is also desirable as this system also meets the
necessary criteria for adaptation. The pH at which the
system may be used is variable and may thus be adjusted so
that damage to the instrument or column does not occur.
Distribution coefficients published by Morie et al[27,28] were
used as an aid to the investigation.

CHAPTER IV

MATERIALS AND APPARATUS

Materials

All chemicals used in this research were of analytical reagent quality.

Apparatus

Distribution Coefficients

All samples were shaken overnight with a Dubnoff Shaking Incubator (Precision Scientific, division of GCA Corp. Chicago, IL). Colorimetric analyses were performed using a Model 26 ultraviolet-visible spectrophotometer (Beckman Scientific Instruments, Irvine, CA 92713).

High Pressure Liquid Chromatography

Sample analyses were performed with a Model 204 Liquid Chromatograph with a Model M-6000A solvent delivery system and a Model U6K Universal Injector with a 2.0 mL injection loop (Waters Associates Inc., Milford, MA 01757). The three types of detectors used were a Model 440 fixed wavelength (254 nm) ultraviolet absorbance detector (Waters Associates Inc.), a Model 155 variable wavelength, ultraviolet-visible absorbance detector (Beckman Scientific Inst.), and a Model 401 refractive index detector (Waters Associates Inc.).

Peak recording was done with a Model DSRG 2 dual
channel recorder with a Model S-72170-60 electronic
integrator (Sargent-Welch Scientific Co., Skokie, IL 60076).

Spectra were taken using a Model 26 UV-visible
spectrophotometer with a Model 24-25ACC recorder (Beckman
Scientific Inst.).

Columns used in this work were Corasil strong anion
and strong cation exchange columns (Waters Associates Inc.)
and a 7.5 cm, 4mm I.D., stainless steel column packed with
Aminex A-27 strong anion exchange resin (BioRad Laboratories,
Richmond, CA 94804).

CHAPTER V

EXPERIMENTAL

Distribution Coefficients of Chlorocomplexes

Reagents

Reagent solutions used in the determination of
distribution coefficients of chlorocomplexes, and their
preparation, are as follows:

lithium chloride, 0.5 mol/L in 50% methanol-water	Dissolve 21.20 g of LiCl in 800 mL of 50% methanol-water and dilute to 1 L with same.
lithium chloride, 1.0 mol/L in 50% methanol-water	Dissolve 42.39 g of LiCl in 800 mL of 50% methanol-water and dilute to 1 L with same.
lithium chloride, 0.5 mol/L in anhydrous methanol	Dissolve 21.20 g of LiCl in 800 mL of anhydrous methanol and dilute to 1 L with same.
lithium chloride, 1.0 mol/L in anhydrous methanol	Dissolve 42.39 g of LiCl in 800 mL of anhydrous methanol and dilute to 1 L with same.
copper chloride, 0.1 mol/L	Dissolve 17.05 g $CuCl_2 \cdot 2H_2O$ in deionized water and dilute to 1 L.
cobalt chloride, 0.1 mol/L	Dissolve 23.79 g $CoCl_2 \cdot 6H_2O$ in deionized water and dilute to 1 L.
nickel chloride, 0.1 mol/L	Dissolve 23.80 g $NiCl_2 \cdot 6H_2O$ in deionized water and dilute to 1 L.

cadmium nitrate, 0.1 mol/L   Dissolve 30.90 g $Cd(NO_3)_2 \cdot 4H_2O$
                                    in deionized water and
                                    dilute to 1 L.

zinc nitrate, 0.1 mol/L   Dissolve 29.75 g $Zn(NO_3)_2 \cdot 6H_2O$
                                    in deionized water and
                                    dilute to 1 L.

Procedure

The procedure used for the determination of distri-
bution coefficients is similar to that used by Lee, Lee, and
Kang[23]. 1.00 mL of 0.1 mol/L metal ion solution was placed
into a 100 mL volumetric flask. The flask was diluted to
volume with the lithium chloride solution being investigated.
It was assumed that the 1.00 mL of water added with the metal
ion is negligible. After bringing to volume, the flask was
stoppered and the contents mixed well. Approximately 1.00 g
of ion exchange resin was weighed into a 250 mL erlenmeyer
flask and the weight recorded for use in calculations. The
contents of the 100 mL volumetric flask were emptied into
this erlenmeyer flask which was then tightly stoppered. The
resin-metal ion solution was then shaken overnight with an
automatic shaker (Precision Scientific) to allow equilibrium
to be reached. The next day, the resin was allowed to
settle and the supernatant solution was decanted into
another erlenmeyer flask. An aliquot of the decantate was
taken for metal analysis to determine the amount of metal
ion taken up by the resin. From these data, distribution
coefficients were calculated.

Metal Ion Analysis

The procedures used for the analysis of the metals were adapted from a number of sources. The analysis of cobalt makes use of the deep blue color of the cobalt-thiocyanate complex in acetone solutions[40]. Copper and zinc were analyzed using the color-forming reagent Zincon (2-carboxy-2'-hydroxy-5'-sulfoformazyl benzene)[41]. Nickel and cadmium were analyzed with the color-forming reagent P.A.R. (4-(2-pyridylazo) resorcinol)[42]. The complete procedures for the analyses are given in Appendix A.

Calculations

The distribution coefficients were calculated from equation (13) below.

$$D_w = \frac{\text{mmole of metal per gram of dry resin}}{\text{mmole of metal per mL of solution}} \quad (13)$$

The denominator, mmole of metal per mL of solution, was obtained directly from the results of the metal analyses. The numerator, mmole of metal per gram of dry resin, was obtained by difference. Since the initial amount of metal ion added was known, it was assumed that the difference between the amount of metal present in solution before equilibration with the resin and the amount present after equilibration represents that portion taken up by the resin.

Since undried resin was added to the flask, it was necessary to find the weight of dry resin added for use in the above calculation. For this, knowledge of the percentage of water present in the resin was required. Each week, an

accurately weighed amount of the undried resin was placed, overnight, in an oven at 120° C. The next day, the resin was cooled in a desicator and reweighed. The weight loss was due to the water in the resin. The percentage water was calculated from these data.

HPLC of Chlorocomplexes

Reagents

Reagents used in the HPLC separation of chloro-complexes are given below.

lithium chloride, 0.5 mol/L in anhydrous methanol
: Dissolve 21.20 g of LiCl in 800 mL of anhydrous methanol and dilute to 1 L with same.

lithium chloride, 0.5 mol/L in 50% methanol-water
: Dissolve 21.20 g of LiCl in 800 mL of 50% methanol-water and dilute to 1 L with same.

copper chloride, 0.1 mol/L in mobile phase
: Dissolve 1.71 g $CuCl_2 \cdot 2H_2O$ in mobile phase and dilute to 100 mL with same.

cobalt chloride, 0.1 mol/L in mobile phase
: Dissolve 2.38 g $CoCl_2 \cdot 6H_2O$ in mobile phase and dilute to 100 mL with same.

nickel chloride, 0.1 mol/L in mobile phase
: Dissolve 2.38 g $NiCl_2 \cdot 6H_2O$ in mobile phase and dilute to 100 mL with same.

zinc nitrate, 0.1 mol/L in mobile phase
: Dissolve 2.98 g $Zn(NO_3)_2 \cdot 6H_2O$ in mobile phase and dilute to 100 mL with same.

Samples

All samples used for HPLC analysis are the chloride
or nitrate salts of the metals investigated, dissolved in
the mobile phase being investigated. In all cases, the
concentrations of the samples are 0.1 mol/L.

HPLC Analysis

A bonded phase, pellicular, cation exchange column
(Waters Associates Inc.) was used for this part of the
investigation. Flow rates of 0.6 to 1.0 mL/min were tried.
Detection was by means of UV absorbance at 254 nm or by
refractive index, with the mobile phase serving as the
reference. Injection volumes were 1 to 5 μL of the 0.1
mol/L metal ion solutions.

Modifications

The mobile phases tested were: (1) 0.5 mol/L
lithium chloride in absolute methanol, and (2) 0.5 mol/L
lithium chloride in 50% methanol-water. The procedures for
making these solutions are outlined in the section on
reagents. After the solutions were made they were filtered
through a microporous filter (Millipore Corp., Bedford, Mass.
01730) to prevent contamination of the column by particulate
matter.

Before its initial use, the cation exchange column
was flushed for one hour with the lithium chloride solution
to be used. This allowed the column to be converted to

the lithium form. After each day's use, the column was
flushed with about 15 mL of methanol (15 minutes at 1.0
mL/min) to retard the growth of mold and bacteria and to
prevent rapid column degeneration. On each day after the
initial conditioning, a 15 minute flush with the mobile
phase, before use, was required to recondition the column.

HPLC of Tartaric Acid Complexes

Reagents

Reagents used in the HPLC analysis of tartaric acid
complexes are given below.

cadmium nitrate, 0.1 mol/L in
mobile phase

Dissolve 3.09 g $Cd(NO_3)_2 \cdot 4H_2O$
in mobile phase and dilute
to 100 mL with same.

cobalt chloride, 0.1 mol/L in
mobile phase

Dissolve 2.38 g $CoCl_2 \cdot 6H_2O$
in mobile phase and dilute
to 100 mL with same.

copper chloride, 0.1 mol/L in
mobile phase

Dissolve 1.71 g $CuCl_2 \cdot 2H_2O$
in mobile phase and dilute
to 100 mL with same.

manganese chloride, 0.1 mol/L in
mobile phase

Dissolve 1.98 g $MnCl_2 \cdot 4H_2O$
in mobile phase and dilute
to 100 mL with same.

nickel chloride, 0.1 mol/L in
mobile phase

Dissolve 2.38 g $NiCl_2 \cdot 6H_2O$
in mobile phase and dilute
to 100 mL with same.

zinc nitrate, 0.1 mol/L in
mobile phase

Dissolve 2.98 g $Zn(NO_3)_2 \cdot 6H_2O$
in mobile phase and dilute
to 100 mL with same.

cadmium nitrate, 1×10^{-3} mol/L
in mobile phase

Dilute 1.00 mL of 0.1 mol/L
$Cd(NO_3)_2 \cdot 4H_2O$ to 100 mL
with mobile phase.

cobalt chloride, 1 X 10^{-3} mol/L in mobile phase	Dilute 1.00 mL of 0.1 mol/L $CoCl_2 \cdot 6H_2O$ to 100 mL with mobile phase.
copper chloride, 1 X 10^{-3} mol/L in mobile phase	Dilute 1.00 mL of 0.1 mol/L $CuCl_2 \cdot 2H_2O$ to 100 mL with mobile phase.
manganese chloride, 1 X 10^{-3} mol/L in mobile phase	Dilute 1.00 mL of 0.1 mol/L $MnCl_2 \cdot 4H_2O$ to 100 mL with mobile phase.
nickel chloride, 1 X 10^{-3} mol/L in mobile phase	Dilute 1.00 mL of 0.1 mol/L $NiCl_2 \cdot 6H_2O$ to 100 mL with mobile phase.
zinc nitrate, 1 X 10^{-3} mol/L in mobile phase	Dilute 1.00 mL of 0.1 mol/L $Zn(NO_3)_2 \cdot 6H_2O$ to 100 mL with mobile phase.
manganese standard, 0.1000 mol/L stock solution	Dissolve 0.9896 g $MnCl_2 \cdot 4H_2O$ in mobile phase and dilute to 50 mL with same.
sodium hydroxide, 3.0 mol/L	Dissolve 12.0 g NaOH in deionized water and dilute to 100 mL. Make fresh before each use.
tartaric acid, 2.12 X 10^{-3} mol/L pH = 4.5	Dissolve 0.318 g d-tartaric acid in 995 mL deionized water. Adjust to pH = 4.5 with 3.0 mol/L NaOH and dilute to 1 L.
tartaric acid, 2.12 X 10^{-2} mol/L pH = 3.0	Dissolve 3.18 g d-tartaric acid in 995 mL deionized water. Adjust to pH = 3.0 with 3.0 mol/L NaOH and dilute to 1 L.
tartaric acid, 4.25 X 10^{-2} mol/L	Dissolve 6.38 g d-tartaric acid in 990 mL deionized water. Adjust to pH = 3.0 with 3.0 mol/L NaOH and dilute to 1 L.

Samples

The samples prepared for HPLC analysis were the chloride or nitrate salts of the metals investigated. They were prepared by dissolving the salt in the tartaric acid solution to be used as the mobile phase. A number of sample concentrations were tried. Initially, 5 to 10 μL of 0.1 mol/L solutions of the salts were injected. As work progressed, however, it was discovered that injection volumes up to 500 μL could be used with no ill effects on column performance. As a result, sample concentrations were reduced to cover the range of 1×10^{-4} to 1×10^{-3} mol/L. Standard curves were determined for manganese(II).

HPLC Analysis

Two types of anion exchange packings were used for the analyses. Initially, work was done with a Corasil anion exchange column (Waters Associates Inc.). Corasil is a bonded phase, pellicular, anion exchange packing. This column degenerated, however, to the point of uselessness. At this point work was shifted to a column packed with Aminex A-27 (BioRad Laboratories, Richmond, CA 94804), an 8% cross-linked divinylbenzene-polystyrene resin of 13.5±1.5 micron diameter. Unlike the pellicular column, which was received ready to use, the Aminex A-27 was received in the dry, unpacked form. The resin was slurry packed into a stainless steel column (4 mm I.D.) according to a procedure given by Scott and Lee[43].

With the Corasil column, flow rates of 0.7 to 3.0 mL/min were tried. Detection was by means of a UV detector (Waters Associates Inc.) at 254 nm. Injection volumes up to 10 μL were tried.

With the Aminex A-27 column a flow rate of 1.2 mL/min was used throughout. Detection was by means of a UV-visible variable wavelength spectrophotometer (Beckman Inst.) or by refractive index (Waters Associates Inc.).

Modifications

The mobile phase used with the Corasil column was 2.12×10^{-3} mol/L tartaric acid at pH = 4.5. The mobile phases used with the Aminex A-27 column were: (1) 2.12×10^{-3} mol/L tartaric acid at pH = 4.5, (2) 2.12×10^{-2} mol/L tartaric acid at pH = 3.0, (3) 4.25×10^{-2} mol/L tartaric acid at pH = 3.0. The details for mixing these solutions are given in the section on reagents. Mold growth was a problem with the tartaric acid solutions. To minimize any problems that may have been caused by this, the solutions were usually filtered before each use with a microporous filter (Millipore Corp.). Also, fresh tartaric acid solutions were prepared every three days.

Before initial use, the Corasil column was flushed with 60 mL (1 hour at 1.0 mL/min) of the tartaric acid mobile phase to convert the column to the tartrate form. At the end of each day, the column was flushed with 15 mL of deionized water and 15 mL of absolute methanol to prevent column deterioration and mold growth. Each day, after

the initial conditioning, the column was flushed with 15
mL of water and 15 mL of the tartaric acid mobile phase
before running. This ridded the column of the absolute
methanol.

Since the Aminex A-27 column was slurry packed using
the tartaric acid mobile phase, conditioning to the tartrate
form was not required. For overnight or weekend storage, the
column was left in the tartaric acid mobile phase. For
longer storage, mold growth on the column became a problem.
To prevent this, a solution of 1 X 10^{-3} mol/L manganese, in
the mobile phase, was run onto the column. The manganese
inhibited the growth of mold. After this treatment, a one
hour flush with the tartaric acid mobile phase was required
to recondition the column. Occasionally, after weekend
storage, a small amount of mold growth occured, as evidenced
by an increased pressure drop across the column. Flushing
the column for one-half hour corrected this and ridded the
column entirely of mold. In cases where excessive mold
growth occured, the column was unpacked and the resin
reconditioned.

CHAPTER VI

RESULTS

Distribution Coefficients of Chlorocomplexes

The results of the distribution coefficient measurements for chlorocomplexes of the metals copper(II), cobalt(II), cadmium(II), nickel(II), and zinc(II) are given in Tables 1 through 4. Results are not given for cadmium(II) in anhydrous methanol as these measurements were not performed.

TABLE 1

ANION EXCHANGE DISTRIBUTION COEFFICIENTS
IN ANHYDROUS METHANOL

	Lithium Chloride (mol/L)		
Metal	0	0.5	1.0
Copper(II)	280	6900	1300
Cadmium(II)	---	---	---
Cobalt(II)	10	>25000	>25000
Nickel(II)	<0.10	<0.10	<0.10
Zinc(II)	1400	1100	350

TABLE 2

ANION EXCHANGE DISTRIBUTION COEFFICIENTS
50% METHANOL-WATER

Metal	Lithium Chloride (mol/L)		
	0	0.5	1.0
Copper(II)	70	220	260
Cadmium(II)	150	>25000	>25000
Cobalt(II)	2.0	4.0	3.0
Nickel(II)	<0.10	<0.10	<0.10
Zinc(II)	110	290	150

TABLE 3

CATION EXCHANGE DISTRIBUTION COEFFICIENTS
IN ANHYDROUS METHANOL

Metal	Lithium Chloride (mol/L)		
	0	0.5	1.0
Copper(II)	>25000	<0.10	<0.10
Cadmium(II)	---	---	---
Cobalt(II)	>25000	17	0.10
Nickel(II)	51	0.10	<0.10
Zinc(II)	1400	16	16

TABLE 4

CATION EXCHANGE DISTRIBUTION COEFFICIENTS
IN 50% METHANOL-WATER

	Lithium Chloride (mol/L)		
Metal	0	0.5	1.0
Copper(II)	7000	7.0	2.0
Cadmium(II)	>25000	<0.10	<0.10
Cobalt(II)	>25000	72	21
Nickel(II)	>25000	1.0	0.30
Zinc(II)	5700	<0.10	<0.10

HPLC of Chlorocomplexes

Cation exchange of the metals copper(II), cobalt(II), nickel(II), and zinc(II) was studied with 0.5 mol/L lithium chloride in anhydrous methanol as the eluting medium. Copper(II) was studied with elution by 0.5 mol/L lithium chloride in 50% methanol-water as well. The cation exchange distribution coefficients tabulated in the previous section were used as a guide to the relative retention times and the order of elution of each metal.

In all cases, except copper(II) in 0.5 mol/L lithium chloride-anhydrous methanol and zinc, which is undetected at 254 nm, multiple peaks were observed with UV detection at 254 nm. Figures 1 through 3 show the peaks obtained for copper(II), cobalt(II), and nickel(II), respectively, with elution by 0.5 mol/L lithium chloride in anhydrous methanol.
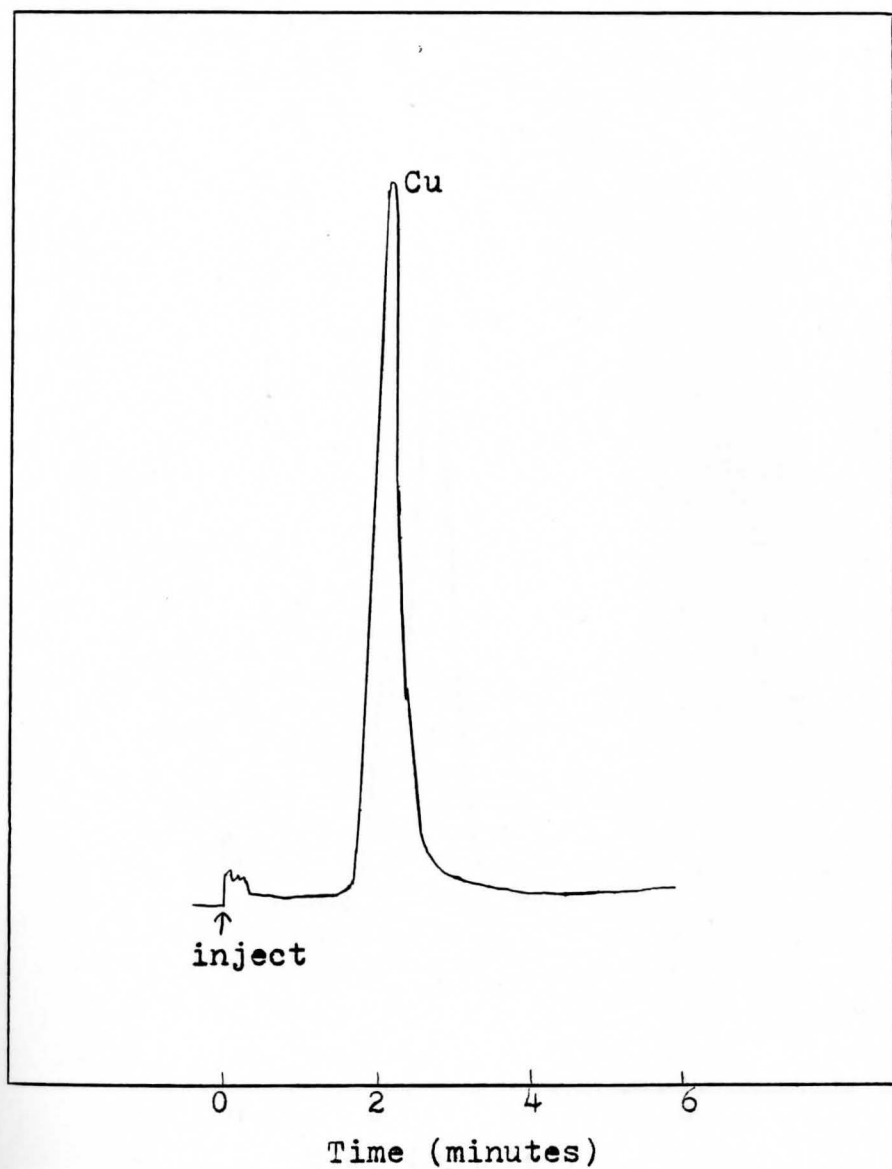
Cu

inject

Time (minutes)

Fig. 1. Chromatogram of copper(II), 0.1 mol/L.
Conditions: column, Corasil cation exchange;
eluent, 0.5 mol/L LiCl in anhydrous methanol; flow rate,
1.0 mL/min; injection volume, 1.5 µL; detection, UV
absorbance at 254 nm.

Co

↑
inject

0   2   4   6

Time (minutes)

Fig. 2.   Chromatogram of cobalt(II), 0.1 mol/L.
Conditions: column, Corasil cation exchange;
eluent, 0.5 mol/L LiCl in anhydrous methanol; flow rate,
1.0 mL/min; injection volume, 2.0 μL; detection, UV
absorbance at 254 nm.

Fig. 3. Chromatogram of nickel(II), 0.1 mol/L. Conditions: column, Corasil cation exchagne; eluent, 0.5 mol/L LiCl in anhydrous methanol; flow rate, 1.0 mL/min; injection volume, 2.0 μL; detection, UV absorbance at 254 nm.
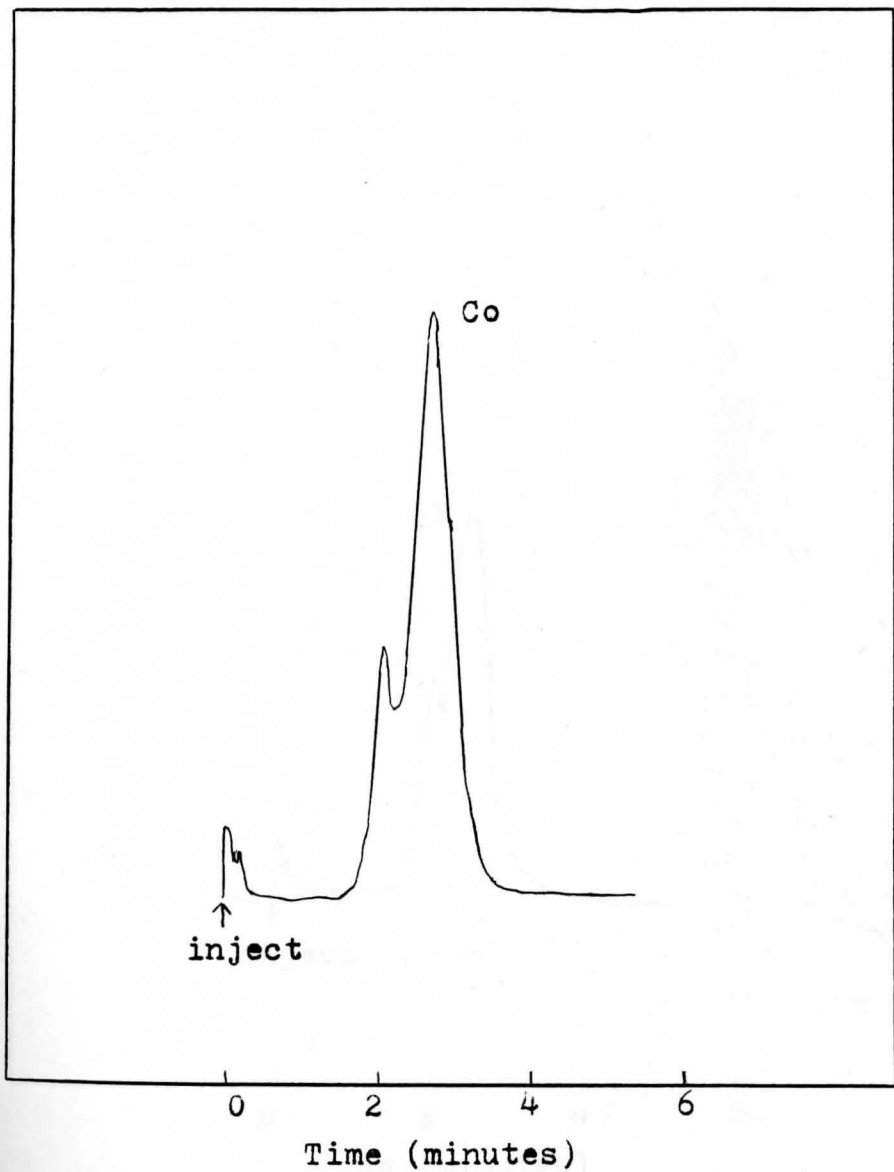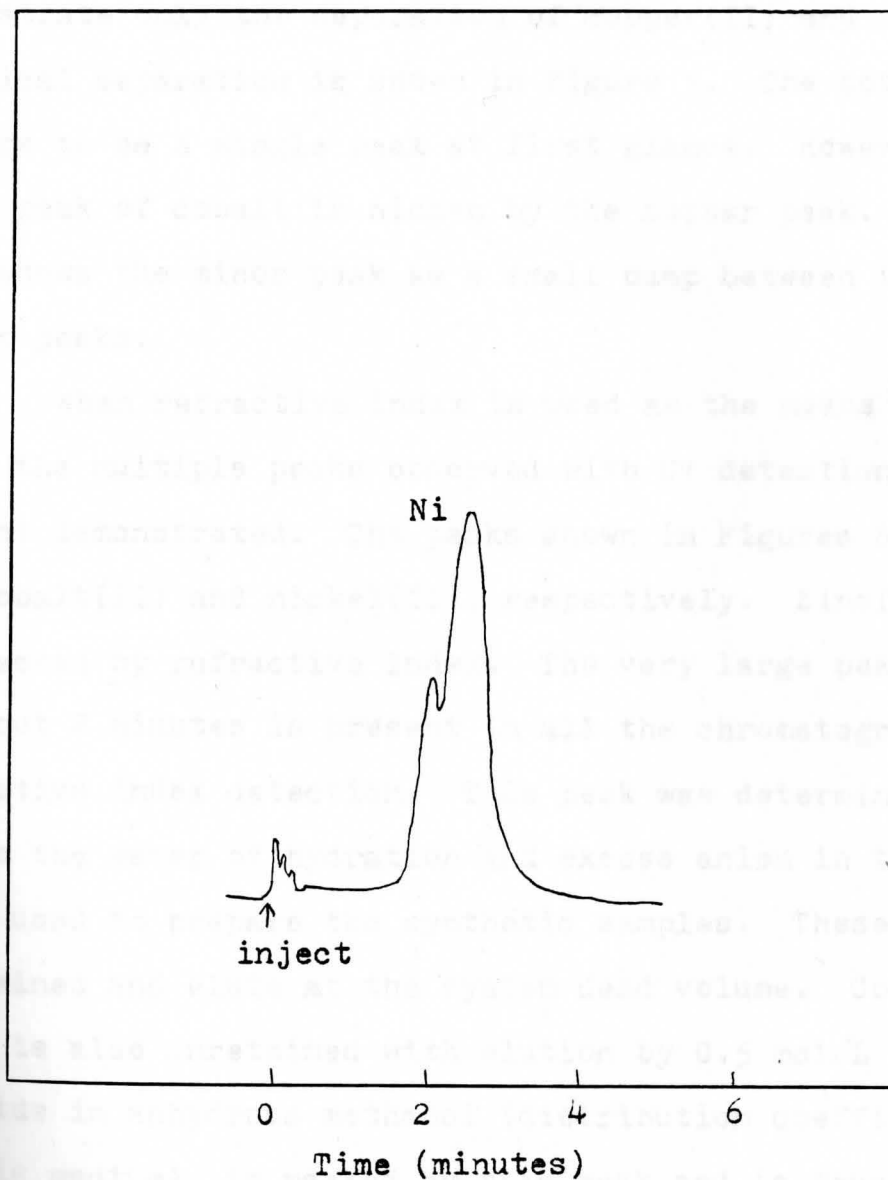
In each case, the flow rate was 1.0 mL/min. Figure 4 shows
the multiple peaks observed for copper(II) with elution by
0.5 mol/L lithium chloride in 50% methanol-water at three
flow rates.

Due to the relative closeness of the retention times,
and the fact that zinc(II) is undetected, it was possible to
demonstrate only the separation of copper(II) and cobalt(II).
A typical separation is shown in Figure 5. The cobalt(II)
appears to be a single peak at first glance. However, the
minor peak of cobalt is hidden by the copper peak. A closer
look shows the minor peak as a small bump between the two
larger peaks.

When refractive index is used as the means of detec-
tion, the multiple peaks observed with UV detection at 254 nm
are not demonstrated. The peaks shown in Figures 6 and 7 are
for cobalt(II) and nickel(II), respectively. Zinc(II) is
undetected by refractive index. The very large peak appearing
at about 2 minutes is present in all the chromatograms with
refractive index detection. This peak was determined to be
due to the water of hydration and excess anion in the metal
salts used to prepare the synthetic samples. These are
unretained and elute at the system dead volume. Copper(II),
which is also unretained with elution by 0.5 mol/L lithium
chloride in anhydrous methanol (distribution coefficient = 0
in this medium), is masked by this peak and is thus undetect-
able using refractive index. Copper(II) elution with 0.5 mol/L
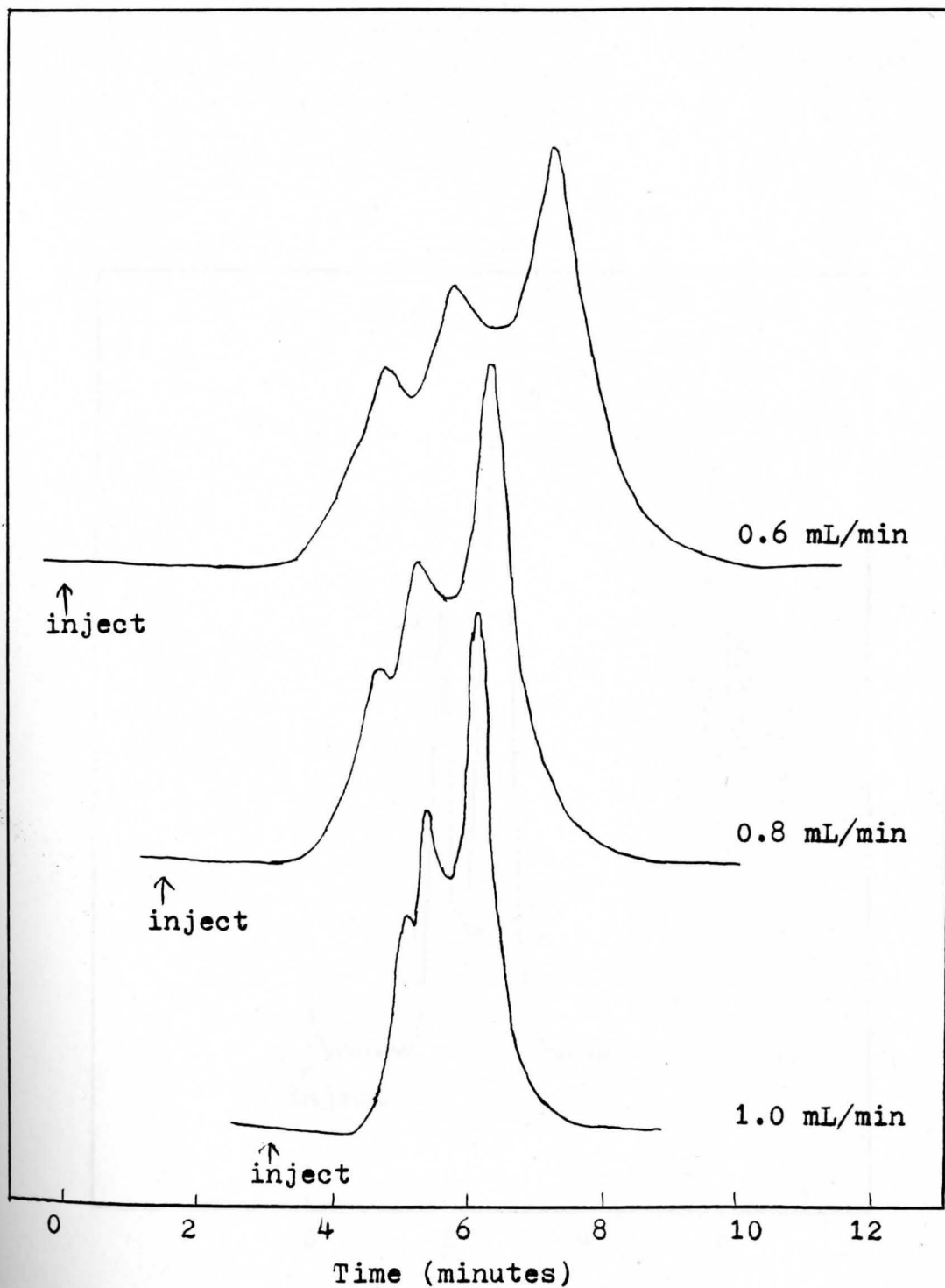lithium chloride in 50% methanol-water was not tried with

Fig. 4. Chromatograms of copper(II), 0.1 mol/L.
Conditions: column, Corasil cation exchange;
eluent, 0.5 mol/L LiCl in 50% methanol-water; flow rates,
0.6, 0.8, and 1.0 mL/min; injection volume, 1.0 µL in each
case; detection, UV absorbance at 254 nm.
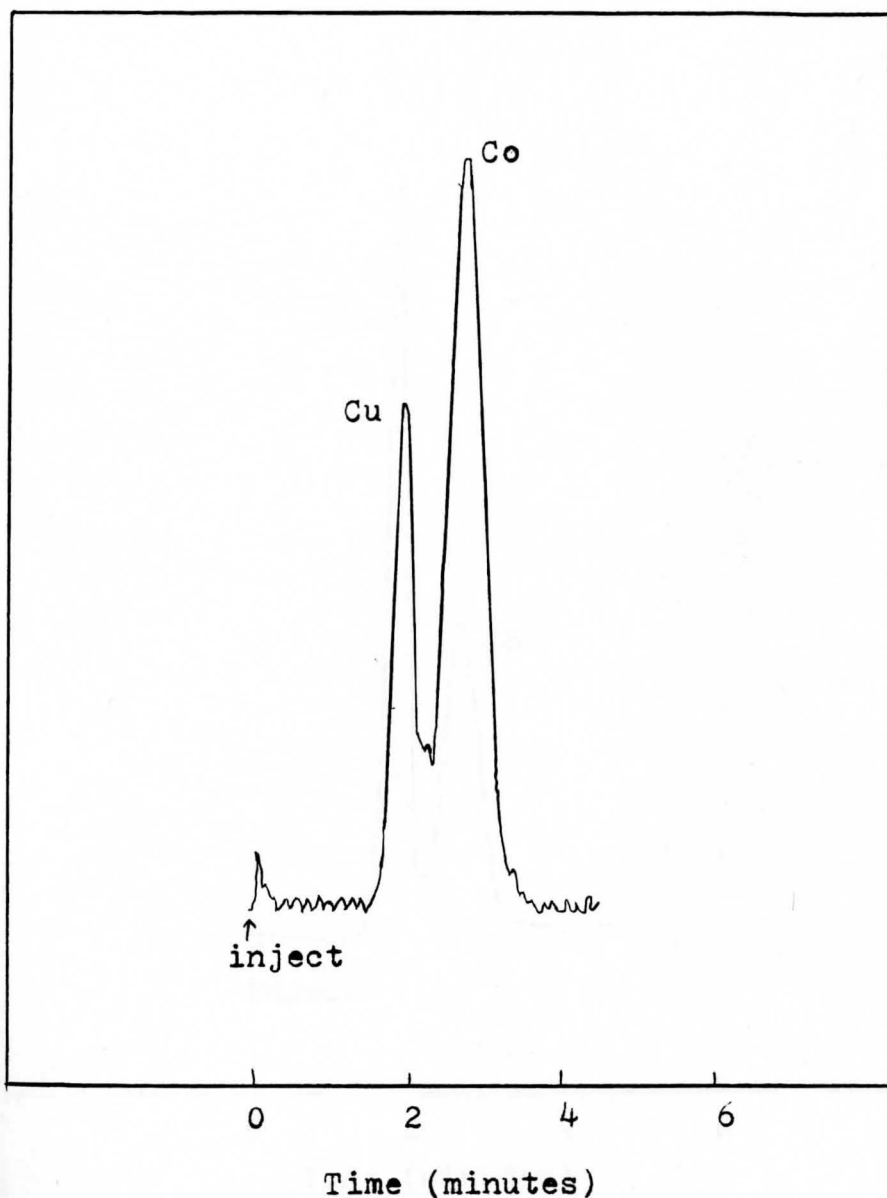
Time (minutes)

Fig. 5. Chromatogram of the separation of copper(II) (0.05 mol/L) and cobalt(II) (0.1 mol/L).
Conditions: column, Corasil cation exchange; eluent, 0.5 mol/L LiCl in anhydrous methanol; flow rate, 1.0 mL/min; injection volume, 1.0 μL; detection, UV absorbance at 254 nm.

Fig. 6. Refractive index detection of cobalt(II),
0.1 mol/L.
        Conditions: column, Corasil cation exchange;
eluent, 0.5 mol/L LiCl in anhydrous methanol, flow rate,
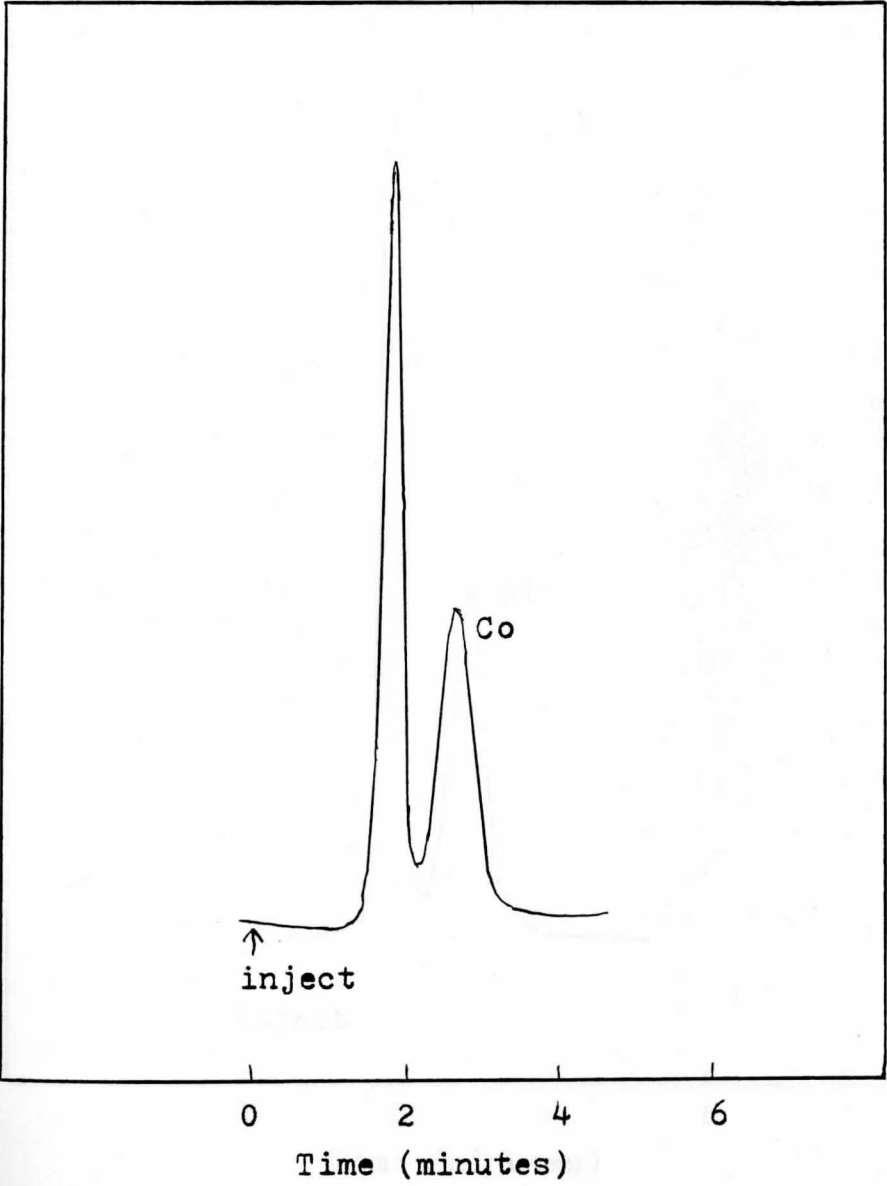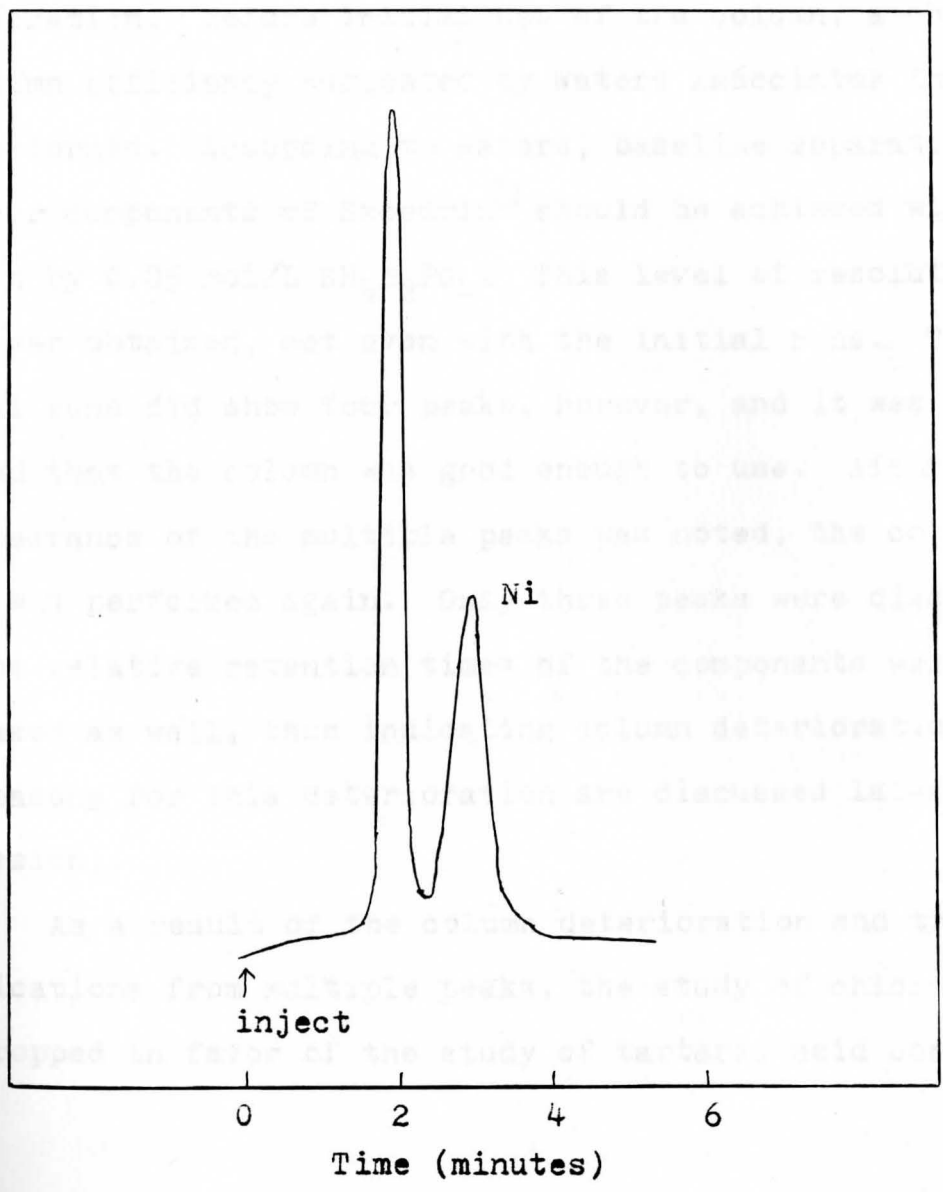1.0 mL/min; injection volume, 5.0 μL.

Fig. 7. Refractive index detection of nickel(II), 0.1 mol/L.
Conditions: column, Corasil cation exchange; eluent, 0.5 mol/L LiCl in anhydrous methanol; flow rate, 1.0 mL/min; injection volume, 5.0 µL.

refractive index detection. Also, there were no demonstrable separations with any eluting medium and detection by refractive index.

It was found that, as time passed, the multiple peaks observed for the metals with UV detection disappeared gradually. The reason for this was found to by column deterioration. Before initial use of the column, a check on column efficiency suggested by Waters Associates Inc. was performed. According to Waters, baseline separation of the four components of Excedrin® should be achieved with elution by 0.05 mol/L $NH_4H_2PO_4$. This level of resolution was never obtained, not even with the initial runs. The initial runs did show four peaks, however, and it was decided that the column was good enough to use. After the disappearance of the multiple peaks was noted, the column check was performed again. Only three peaks were discernible and the relative retention times of the components were decreased as well, thus indicating column deterioration. The reasons for this deterioration are discussed later (see Discussion).

As a result of the column deterioration and the complications from multiple peaks, the study of chlorocomplexes was dropped in favor of the study of tartaric acid complexes.

HPLC of Tartaric Acid Complexes

Anion Exchange Separations on Corasil

Anion exchange of the metals copper(II), cobalt(II), manganese(II), and zinc(II) was studied using 2.12×10^{-3} mol/L tartaric acid at pH = 4.5 as the eluting medium. Anion exchange distribution coefficients determined by Morie et al[27] were used as a guide for the separations. A list of these is given in Table 5 below. Also given are the retention times of each metal at a flow rate of 1.0 mL/min.

TABLE 5

ANION EXCHANGE DISTRIBUTION COEFFICIENTS AND
RETENTION TIMES IN 2.12×10^{-3} MOL/L TARTARIC ACID

Metal	Distribution[a] Coefficient	Retention[b] Time
Cobalt(II)	3	2.0 min
Copper(II)	30	3.0 min
Manganese(II)	1	1.7 min
Zinc(II)	7	2.3 min

[a]The distribution coefficients are from Morie et al[27].
[b]The retention times are given for a flow rate of 1.0 mL/min on a Corasil strong anion exchange column.

The only mode of detection used was UV absorbance at 254 nm. All metals absorbed adquately at this wavelength for detection. Generally, the amount of metal ion injected was between 0.5×10^{-6} and 1.0×10^{-6} mole in a 5 to 10 μL volume.

Copper(II) was an exception to this, absorbing very strongly at 254 nm so that only 1.0×10^{-8} mole of metal ion needed to be injected.

A few separations were demonstrated, but deteriorating column conditions precluded the demonstration of others. The separation of manganese(II), zinc(II), and copper(II) is shown in Figure 8. The flow rate was 1.0 mL/min. The large peak seen between 5 and 6 minutes is due to nitrate from the metal salts used as samples. Potassium nitrate, injected at the same concentration, gave a similar peak. Also, if only chloride salts of the metals were used, the peak disappeared. The separation of manganese(II) and copper(II) in Figure 9 shows the copper(II) peak more clearly. The flow rate was 1.0 mL/min here also.

Cobalt(II), although giving a very nice peak and a retention time between that for manganese(II) and zinc(II), could not be separated from these two metals. The retention times are so close that when a separation was tried, one large peak at an intermediate retention time rather than three separate peaks was obtained. It should be possible to separate copper(II) and cobalt(II) nicely but this separation could not be demonstrated due to rapid column degeneration.

Column degeneration became a noticeable problem after about three weeks of use. After four weeks, the column had deteriorated so badly that it was useless. When first received, a check was performed to determine column efficiency. A mixture of aspirin and thymine eluted with 0.75 mol/L $NH_4H_2PO_4$ at pH = 4.8 was used to check the plate
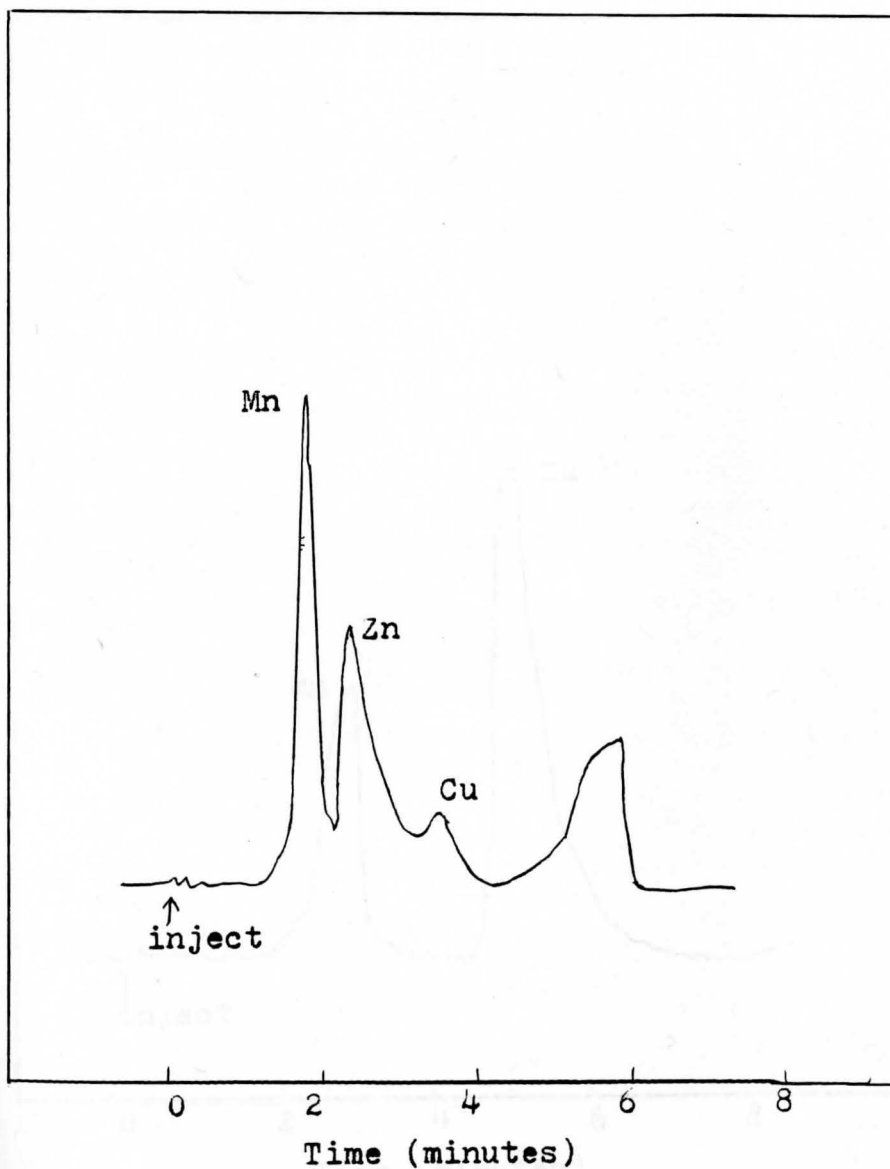
Fig. 8. Chromatogram of the separation of manganese(II)
(0.1 mol/L), zinc(II) (0.1 mol/L), and copper(II) (0.05 mol/L).
Conditions: column, Corasil anion exchange; eluent,
2.12×10^{-3} mol/L tartaric acid, pH = 4.5; flow rate,
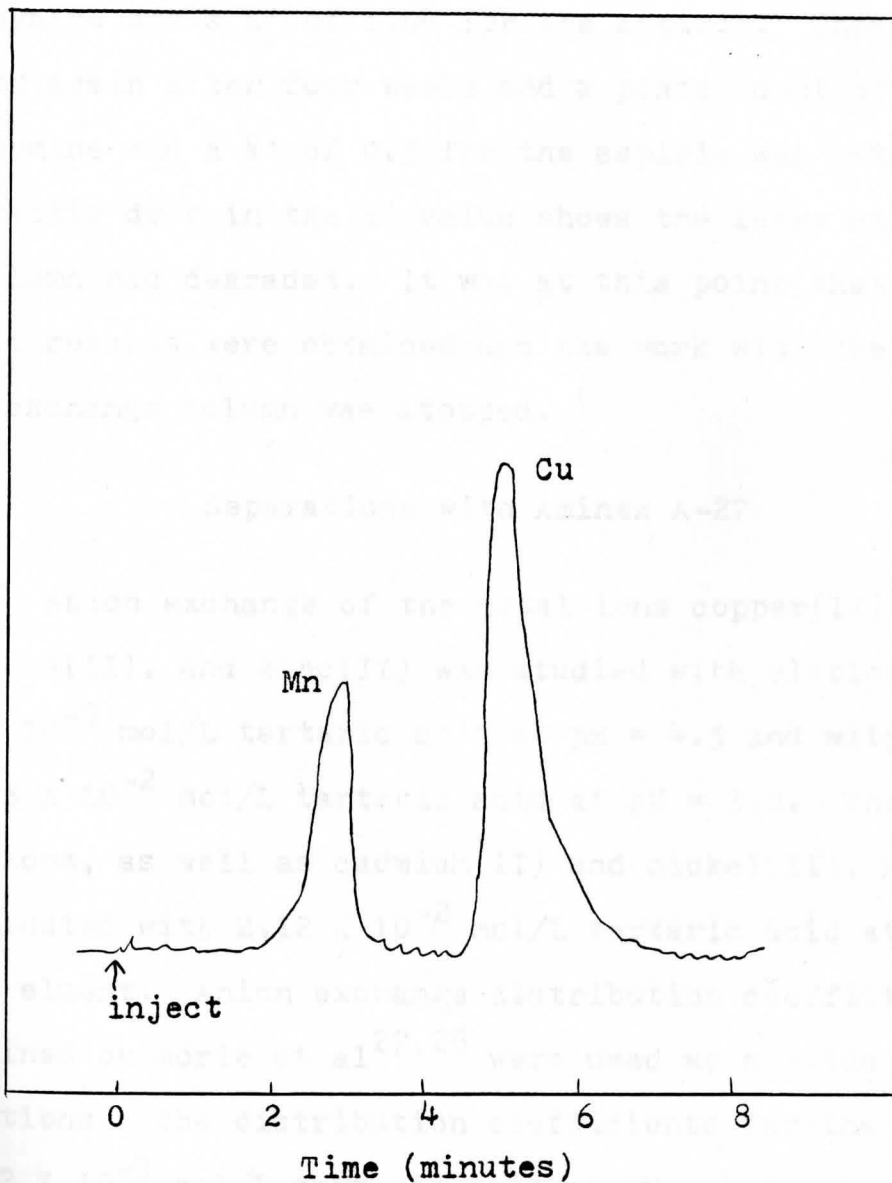1.0 mL/min; injection volume, 10 μL; detection, UV absorbance
at 254 nm.

Fig. 9. Chromatogram of the separation of manganese(II) (0.1 mol/L) and copper(II) (0.05 mol/L). Conditions: column, Corasil anion exchange; eluent, 2.12×10^{-3} mol/L tartaric acid, pH = 4.5; flow rate, 0.7 mL/min; injection volume, 5.0 μL; detection, UV absorbance at 254 nm.

count and the retentive capacity of the column (k'). According to Waters Associates Inc., using thymine as a basis for the plate count, a value of greater than 300 plates should be obtained. Using aspirin to check the retentive capacity, a k' value of at least 0.6 to 0.9 should be obtained. The initial runs with the column gave a plate count of 290 for the thymine and a k' of 1.04 for the aspirin. These were checked again after four weeks and a plate count of 260 for the thymine and a k' of 0.5 for the aspirin was obtained. The drastic drop in the k' value shows the large extent that the column had degraded. It was at this point that very erratic results were obtained and the work with the Corasil anion exchange column was stopped.

Separations with Aminex A-27

Anion exchange of the metal ions copper(II), cobalt(II), manganese(II), and zinc(II) was studied with elution by 2.12×10^{-3} mol/L tartaric acid at pH = 4.5 and with elution by 4.25×10^{-2} mol/L tartaric acid at pH = 3.0. These metal ions, as well as cadmium(II) and nickel(II), were also studied with 2.12×10^{-2} mol/L tartaric acid at pH = 3.0 as the eluent. Anion exchange distribution coefficients determined by Morie et al[27,28] were used as a guide for the separations. The distribution coefficients for the metals in 2.12×10^{-3} mol/L tartaric acid at pH = 4.5 are given in the previous section in Table 5. The retention times given there are for the Corasil column only, however. The distribution coefficients for the metals in the other tartaric

acid solutions are given below in Tables 6 and 7. It should be noted that these values were read from small semi-log graphs and are only approximate.

TABLE 6

ANION EXCHANGE DISTRIBUTION COEFFICIENTS AND
RETENTION TIMES IN 2.12 X 10^{-2} MOL/L TARTARIC ACID

Metal	Distribution[a] Coefficient	Retention[b] Time
Cadmium(II)	3	7.0 min
Cobalt(II)	2	8.2 min
Copper(II)	15	18.6 min
Manganese(II)	1	2.2 min
Nickel(II)	15	16.8 min
Zinc(II)	8	17.0 min

[a]The distribution coefficients are from Morie et al[27,28].
[b]The retention times are for a flow rate of 1.2 mL/min on a 7.5 cm Aminex A-27 column.

The modes of detection used were UV absorbance and refractive index. At the beginning of the investigation, a UV detector with a fixed wavelength of 254 nm (Waters Associates Inc.) was used. It was discovered, however, that the metals could not be detected with this instrument, possibly due to an instrument malfunction. Refractive index was tried as a means of detection and proved to be quite adequate. Later in the investigation, a variable wavelength, UV-visible detector

(Beckman Scientific Inst.) was used. UV and visible spectra of the metals were taken to determine the optimum wavelength for detection. It was found that 230 nm was best suited for the detection of all metal ions investigated. It was found, however, that tartaric acid also absorbs strongly in this region of the spectrum. The result of this absorption by tartaric acid is an erratic baseline with UV detection at 230 nm. It was decided that refractive index is a better means of detection and it was, therefore, used throughout this part of the investigation.

TABLE 7

ANION EXCHANGE DISTRIBUTION COEFFICIENTS AND
RETENTION TIMES IN 4.25 X 10^{-2} MOL/L TARTARIC ACID

Metal	Distribution[a] Coefficient	Retention[b] Time
Cobalt(II)	3	7.0 min
Copper(II)	10	11.7 min
Manganese(II)	1	1.9 min
Zinc(II)	8	10.8 min

[a]The distribution coefficients are from Morie et al[27].
[b]The retention times are for a flow rate of 1.2 mL/min on a 7.5 cm Aminex A-27 column.

With 2.12 X 10^{-3} mol/L tartaric acid at pH = 4.5 as an eluent, it was found that poor peak shapes were obtained for metal ions retained on the column for more than a few minutes. The peak shapes for manganese(II) and cobalt(II),

which eluted rather quickly at 1.7 and 3.3 minutes, respectively (1.2 mL/min flow rate), were fine but zinc(II) and copper(II) tailed severely giving peaks that were very spread out. As a result, this concentration of tartaric acid was not studied further.

A number of separations are possible with either 2.12 X 10^{-2} mol/L tartaric acid at pH = 3.0 or 4.25 X 10^{-2} mol/L tartaric acid at pH = 3.0 as the eluent. Since the distribution coefficients for the metals in these tartaric acid concentrations are similar, it was found that the same separations were possible for each eluent, the only difference being the retention times.

Using 4.25 X 10^{-2} mol/L tartaric acid at pH = 3.0 as the eluent, the separation of manganese(II), cobalt(II), and copper(II) is demonstrated in Figure 10. Note the small size of the copper peak. For an unknown reason, the refractive index detector was very insensitive to the copper(II)-tartrate complex. The separation of manganese(II), cobalt(II), and zinc(II), using the same conditions, is shown in Figure 11. In both cases, the flow rate was 1.2 mL/min.

With 2.12 X 10^{-2} mol/L tartaric acid at pH = 3.0 as the eluent at a flow rate of 1.2 mL/min, the following separations were demonstrated: cobalt(II) and nickel(II), Figure 12; manganese(II), cadmium(II), and zinc(II), Figure 13; manganese(II), cadmium(II), and nickel(II), Figure 14; and manganese(II), cobalt(II), and copper(II), Figure 15.

It will be noted that, in Figure 15, the peak for copper(II), which should appear at about 18 minutes, is not
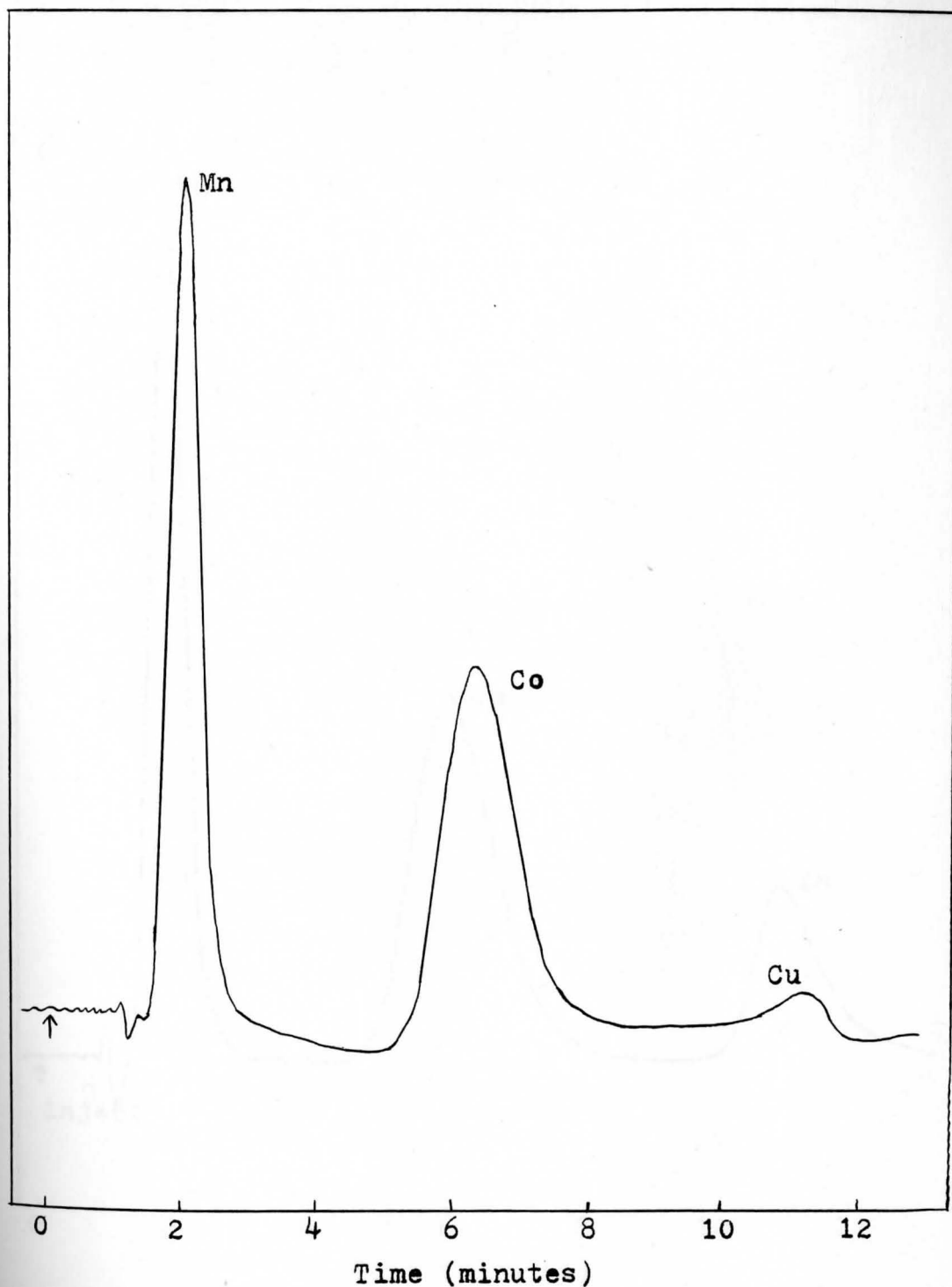
Fig. 10. Chromatogram of a mixture containing
0.1 mol/L each of manganese(II), cobalt(II), and copper(II).
Conditions: column, Aminex A-27; eluent,
4.25×10^{-2} mol/L tartaric acid, pH = 3.0; flow rate,
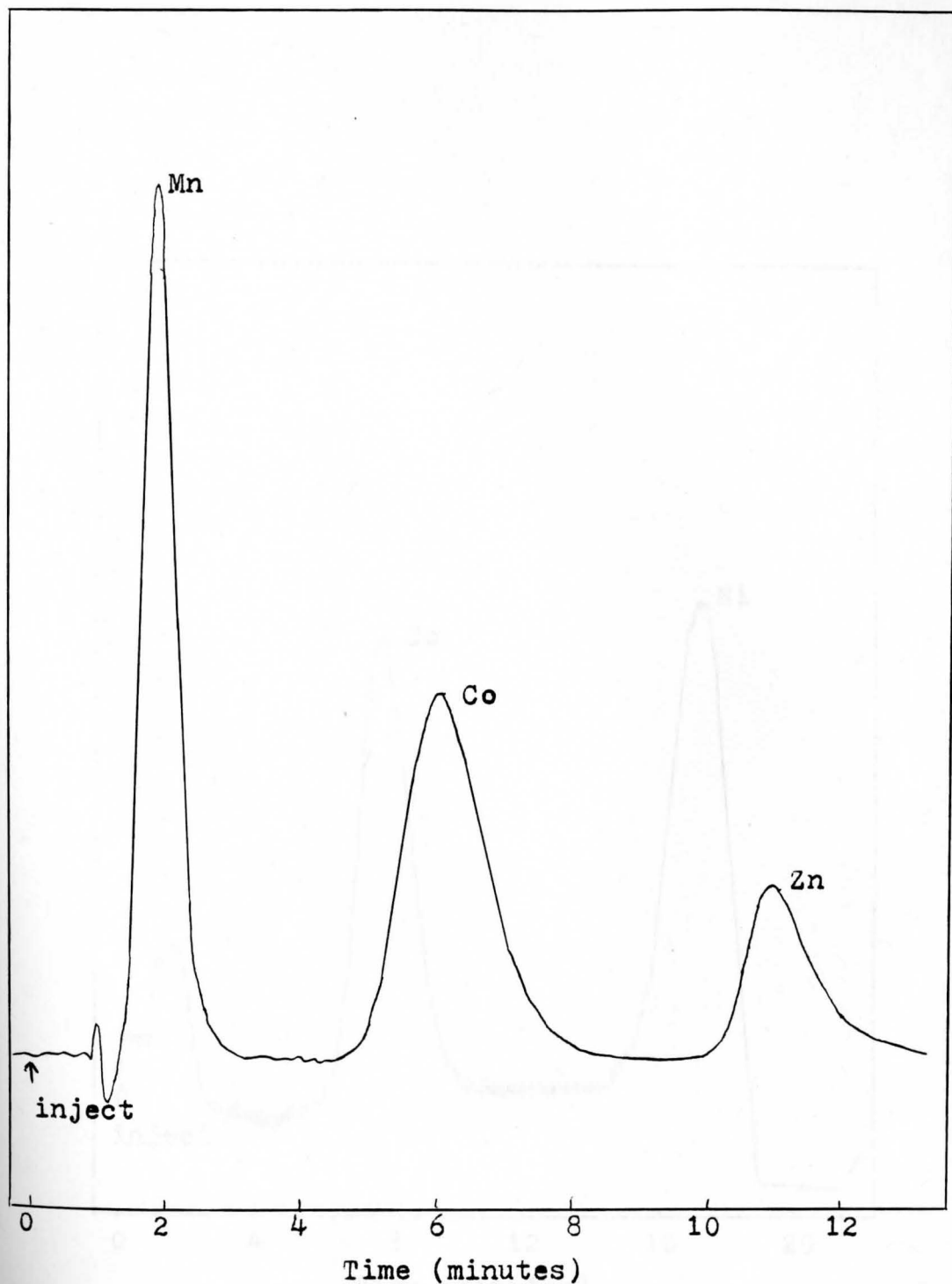1.2 mL/min; injection volume, 6.0 µL; detection, refractive
index.

Fig. 11. Chromatogram of a mixture containing 0.1 mol/L each of manganese(II), cobalt(II), and zinc(II). Conditions: column, Aminex A-27; eluent, 4.25×10^{-2} mol/L tartaric acid, pH = 3.0; flow rate, 1.2 mL/min; injection volume, 6.0 µL; detection, refractive index.
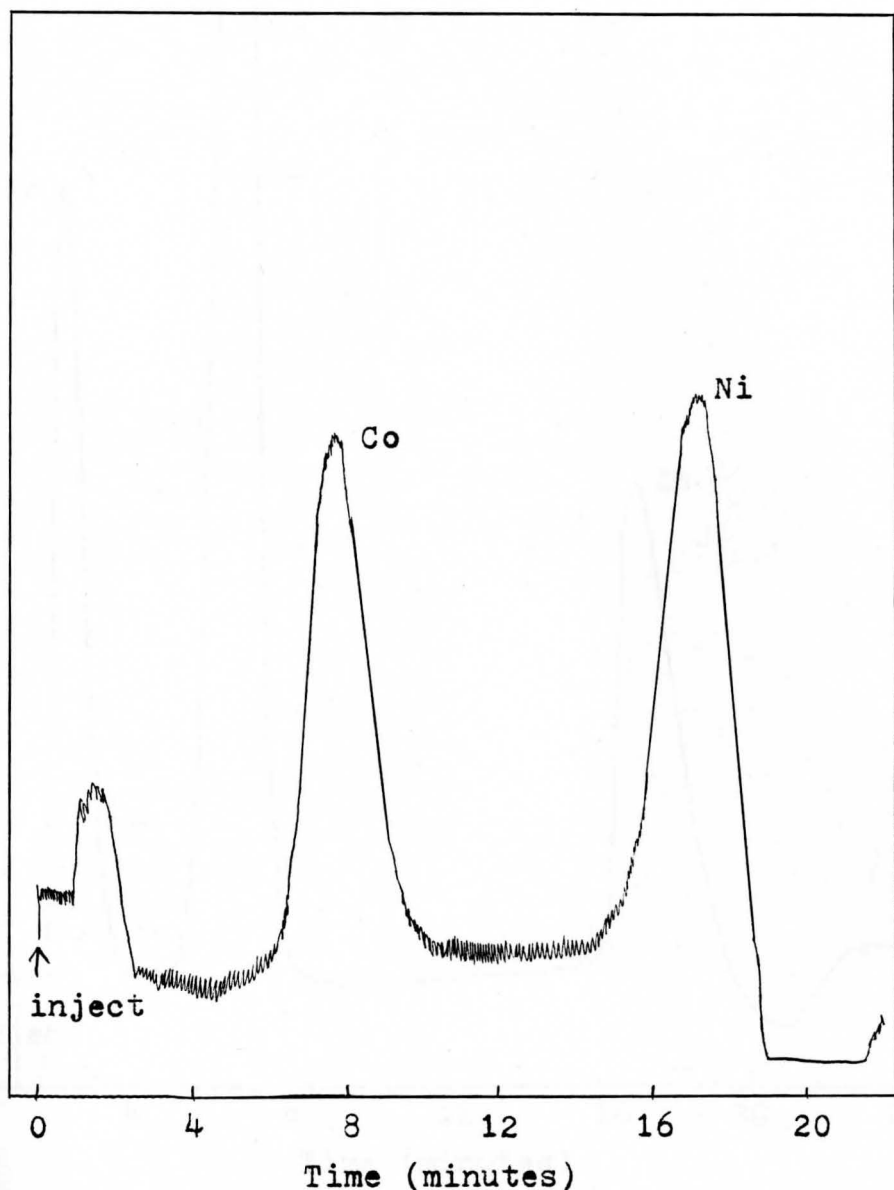
Fig. 12. Chromatogram of a mixture containing
0.001 mol/L each of cobalt(II) and nickel(II).
Conditions: column, Aminex A-27; eluent,
2.12×10^{-2} mol/L tartaric acid, pH = 3.0; flow rate,
1.2 mL/min; injection volume, 250 µL; detection,
refractive index.

Fig. 13. Chromatogram of a mixture containing
0.001 mol/L each of manganese(II), cadmium(II), and zinc(II).
Conditions: column, Aminex A-27; eluent,
2.12×10^{-2} mol/L tartaric acid, pH = 3.0; flow rate,
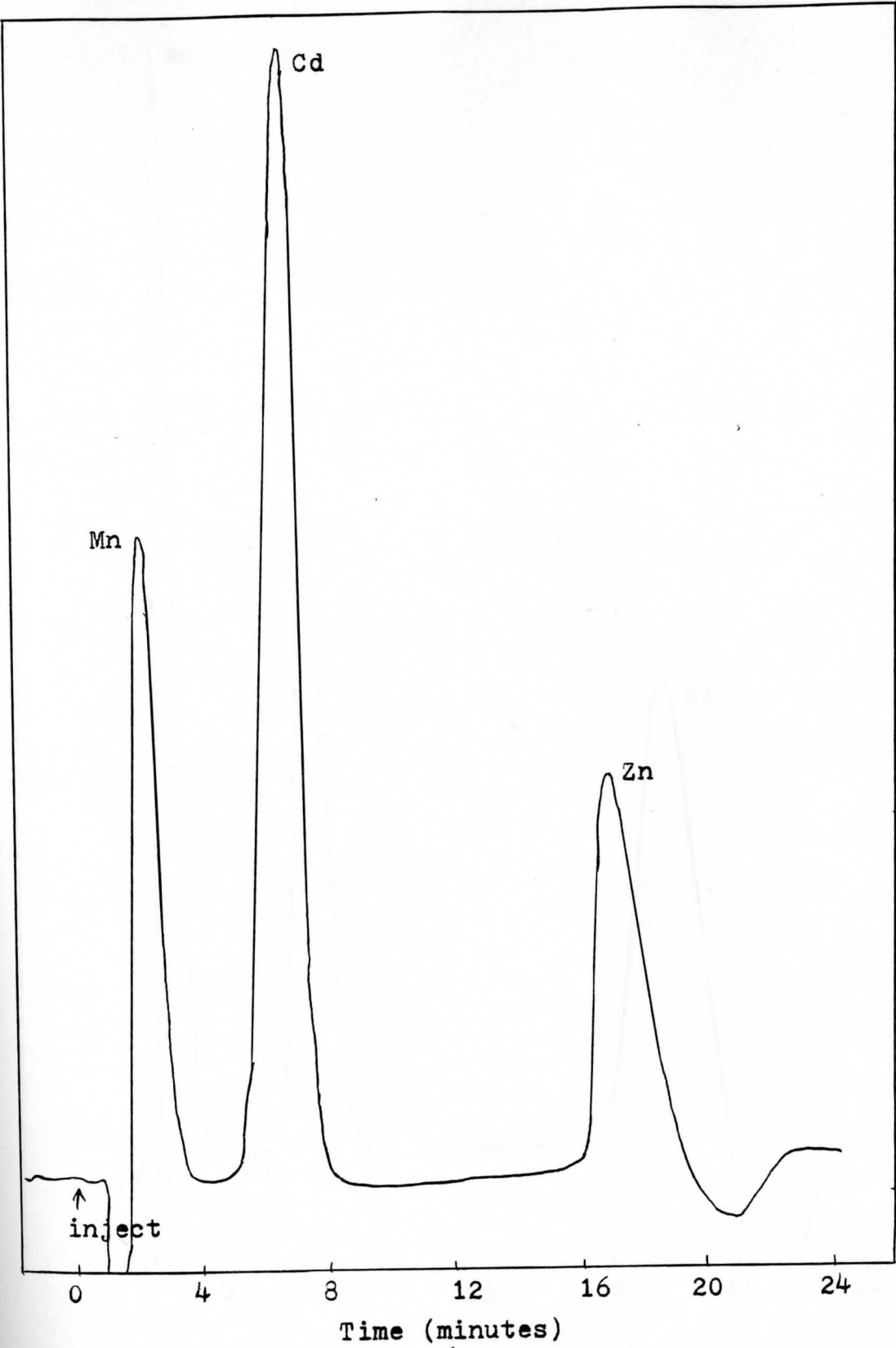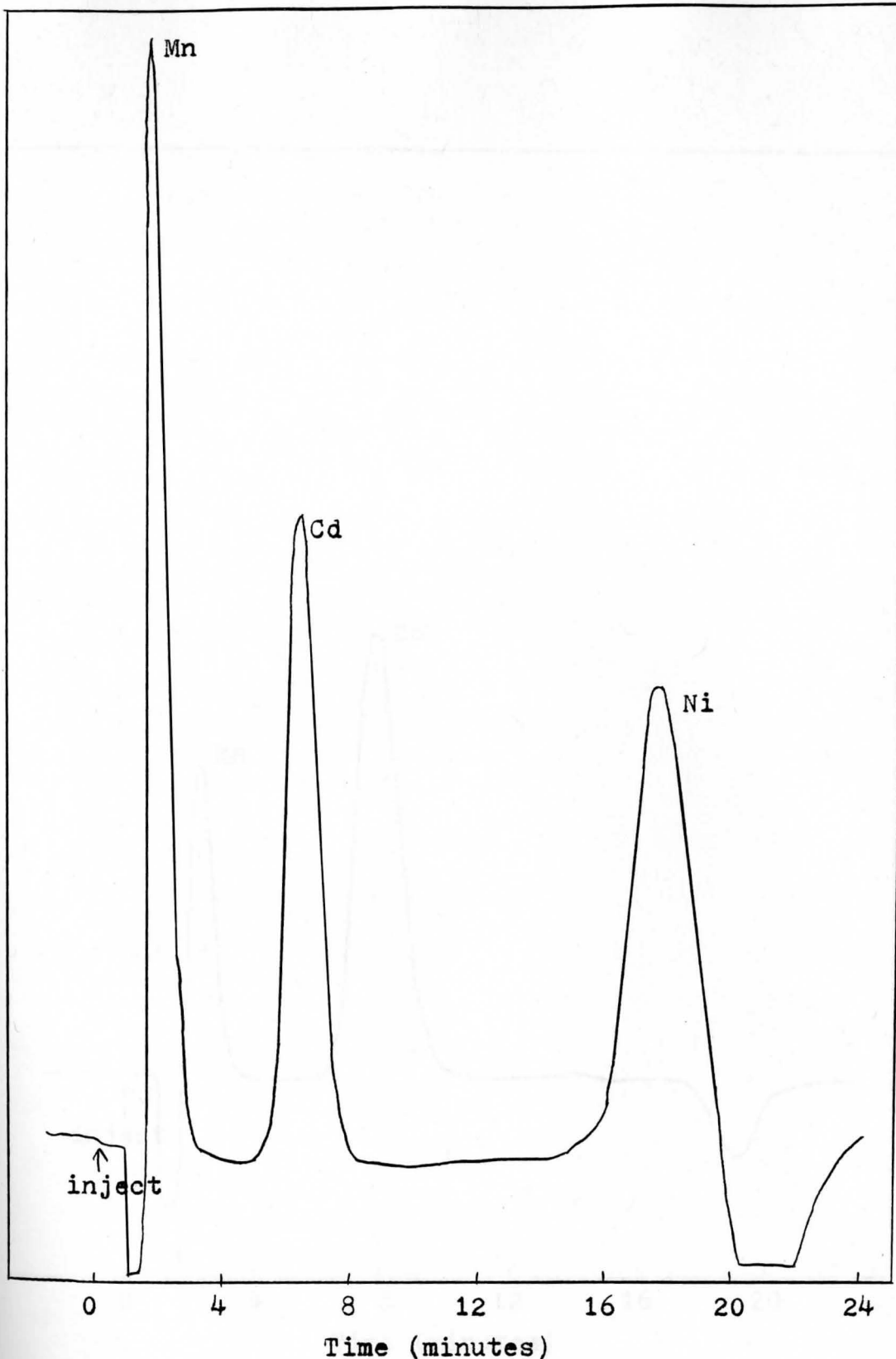1.2 mL/min; injection volume, 500 µL; detection, refractive
index.

Fig. 14. Chromatogram of a mixture containing
0.001 mol/L each of manganese(II), cadmium(II), and nickel(II).
Conditions: column, Aminex A-27; eluent
2.12×10^{-2} mol/L tartaric acid, pH = 3.0; flow rate,
1.2 mL/min; injection volume, 250 µL; detection, refractive
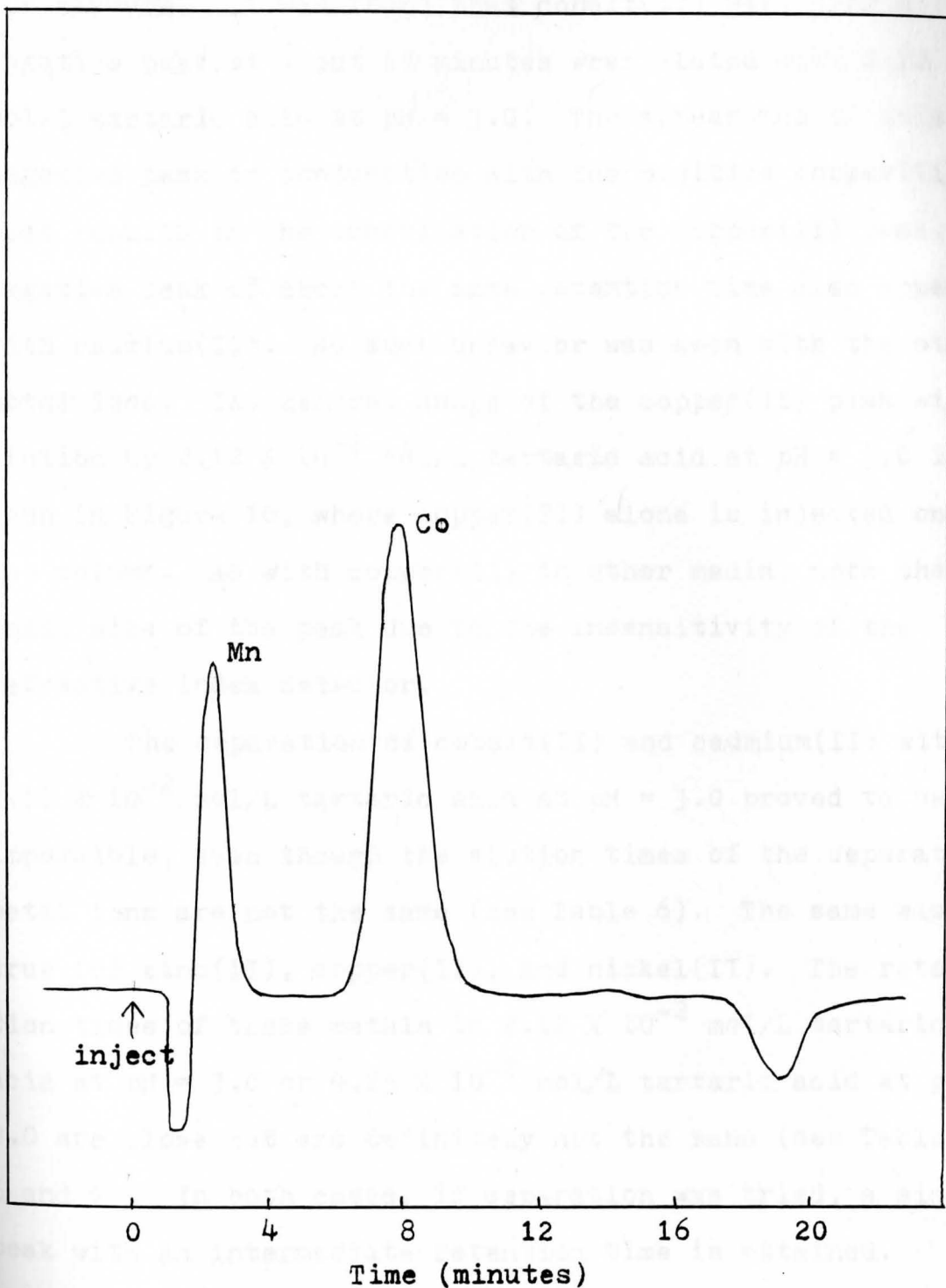index.

Fig. 15. Chromatogram of a mixture containing
0.001 mol/L each of manganese(II), cobalt(II), and copper(II).
Conditions: column, Aminex A-27; eluent,
2.12 X 10^{-2} mol/L tartaric acid, pH = 3.0; flow rate, 1.2 mL/
min; injection volume, 250 µL; detection, refractive index.

present. This is due to the cobalt(II) present in the metal ion mixture. It was found that cobalt(II) will give a negative peak at about 18 minutes when eluted with 2.12×10^{-2} mol/L tartaric acid at pH = 3.0. The appearance of this negative peak in conjunction with the positive copper(II) peak results in the cancellation of the copper(II) peak. A negative peak of about the same retention time also appeared with cadmium(II). No such behavior was seen with the other metal ions. The general shape of the copper(II) peak with elution by 2.12×10^{-2} mol/L tartaric acid at pH = 3.0 is seen in Figure 16, where copper(II) alone is injected onto the column. As with copper(II) in other media, note the small size of the peak due to the insensitivity of the refractive index detector.

The separation of cobalt(II) and cadmium(II) with 2.12×10^{-2} mol/L tartaric acid at pH = 3.0 proved to be impossible, even though the elution times of the separate metal ions are not the same (see Table 6). The same was true for zinc(II), copper(II), and nickel(II). The retention times of these metals in 2.12×10^{-2} mol/L tartaric acid at pH = 3.0 or 4.25×10^{-2} mol/L tartaric acid at pH = 3.0 are close but are definitely not the same (see Tables 6 and 7). In both cases, if separation was tried, a single peak with an intermediate retention time is obtained. Apparently, the resolution of the Aminex A-27 column is not fine enough to allow the separation of metals with retention times closer than a few minutes. Perhaps if a longer column were used, the problem could be solved.

Cu

inject

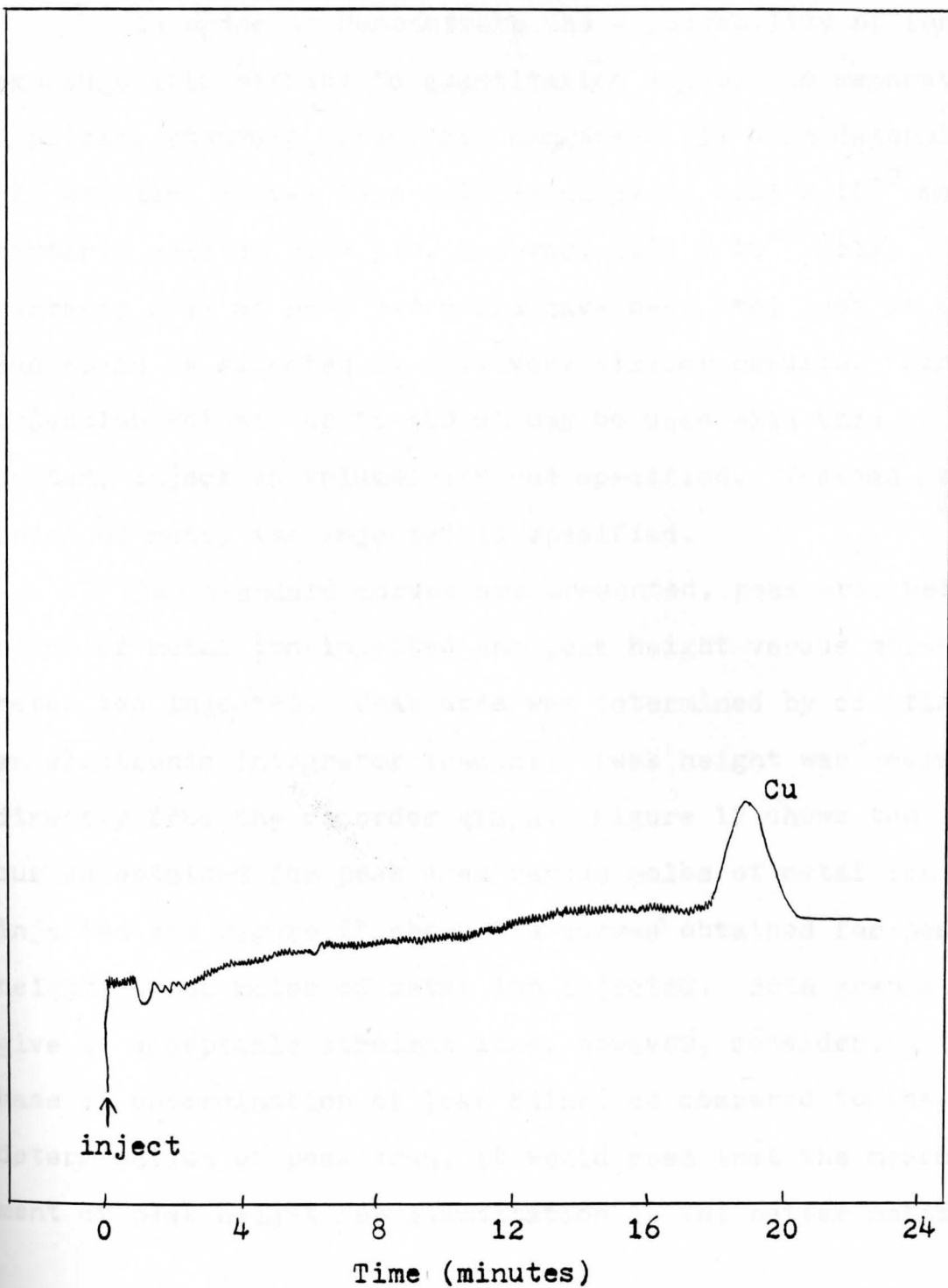0   4   8   12   16   20   24

Time (minutes)

Fig. 16. Chromatogram of copper(II), 0.001 mol/L.
Conditions: column, Aminex A-27; eluent,
2.12×10^{-2} mol/L tartaric acid, pH = 3.0; flow rate,
1.2 mL/min; injection volume, 250 µL; detection, refractive
index.

Quantitation

In order to demonstrate the applicability of ion exchange HPLC methods to quantitation as well as separation, duplicate standard curves for manganese(II) were determined. The standard curves were determined using 4.25×10^{-2} mol/L tartaric acid at pH = 3.0, however, 2.12×10^{-2} mol/L tartaric acid at pH = 3.0 could have been used just as well and would be expected to give very similar results. Since injection volumes up to 500 uL may be used with this system, injection volumes are not specified. Instead, the moles of metal ion injected is specified.

Two standard curves are presented, peak area versus moles of metal ion injected and peak height versus moles of metal ion injected. Peak area was determined by counting an electronic integrator tracing. Peak height was measured directly from the recorder graph. Figure 17 shows the curves obtained for peak area versus moles of metal ion injected and Figure 18 shows the curves obtained for peak height versus moles of metal ion injected. Both graphs give an acceptable straight line, however, considering the ease of determination of peak height as compared to the determination of peak area, it would seem that the measurement of peak height for quantitation is the better method.

Fig. 17. Quantitation of manganese(II); peak area versus moles of metal injected.
Conditions: column, Aminex A-27; eluent, 4.25×10^{-2} mol/L tartaric acid at pH = 3.0; flow rate, 1.2 mL/min; detection, refractive index.
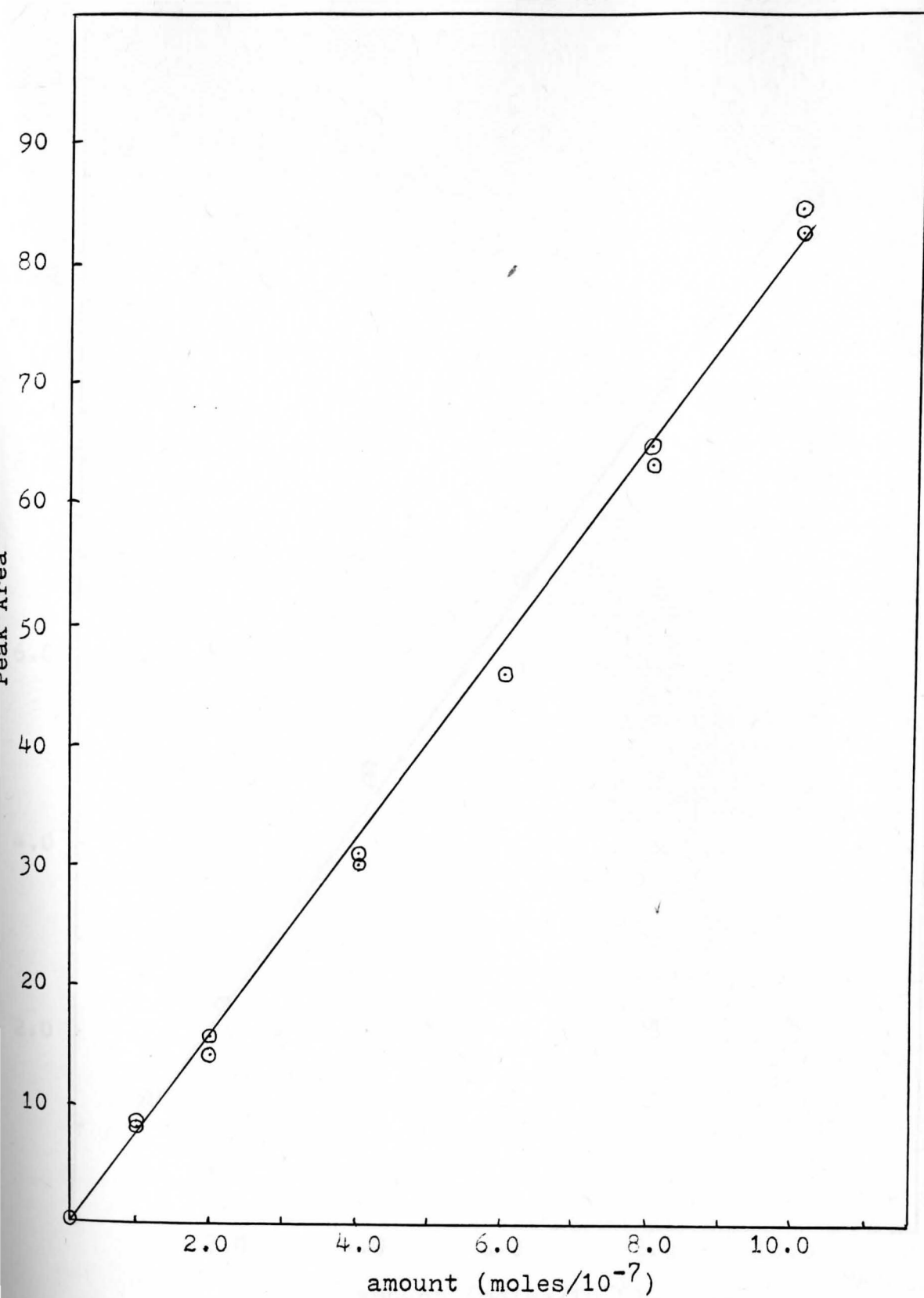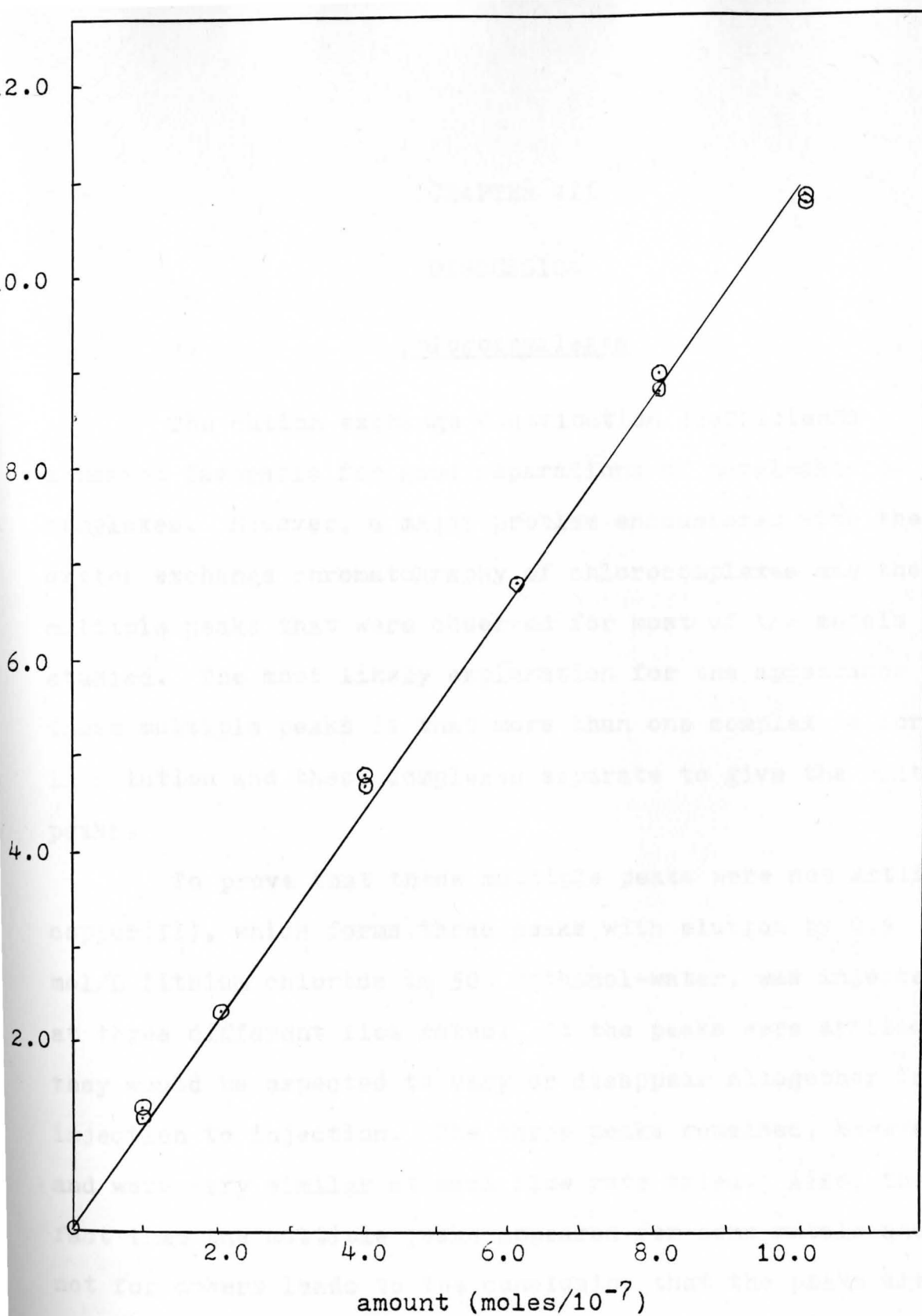
Fig. 18. Quantitation of manganese(II); peak height
versus moles of metal injected.
Conditions: column, Aminex A-27; eluent, 4.25×10^{-2}
mol/L tartaric acid at pH = 3.0; flow rate, 1.2 mL/min;
detection, refractive index.

CHAPTER VII

DISCUSSION

Chlorocomplexes

The cation exchange distribution coefficients
appeared favorable for good separations of metal-chloro-
complexes. However, a major problem encountered with the
cation exchange chromatography of chlorocomplexes was the
multiple peaks that were observed for most of the metals
studied. The most likely explanation for the appearance of
these multiple peaks is that more than one complex is formed
in solution and these complexes separate to give the multiple
peaks.

To prove that these multiple peaks were not artifacts,
copper(II), which forms three peaks with elution by 0.5
mol/L lithium chloride in 50% methanol-water, was injected
at three different flow rates. It the peaks were artifacts,
they would be expected to vary or disappear altogether from
injection to injection. The three peaks remained, however,
and were very similar at each flow rate tried. Also, the
fact that the multiple peaks appeared for some metals and
not for others leads to the conclusion that the peaks are
real and not artifacts.

The use of anion exchange chromatography may be more
favorable for the separation of chlorocomplexes. Looking at

the distribution coefficients for anion exchange, it will be seen that appreciable coefficients are obtained in the samples with no chloride present in the solution phase. This type of behavior was also noted by Katzin and Gebert[44]. Apparently, in media with a lowered dielectric constant (i.e. with respect to water), complexation is enhanced enough to cause the metal to form a complex with the chloride present on the anion exchange resin, which is in the chloride form.

It should thus be possible to separate metal ions using only a column in the chloride form and a methanol or methanol-water eluent. In order to insure that the column stays in the chloride form, a small concentration of chloride could be dissolved in the eluent. Unfortunately, time did not allow this to be tested. It may be that, through the use of such a system, the multiple peaks observed with cation exchange chromatography would not be present.

Tartaric Acid Complexes

From the results it can be seen that the use of tartaric acid as an eluting medium for HPLC ion exchange shows a great amount of promise. The six metal ions separated in this investigation are only a demonstration of the utility of this system. These six metals are only a few of those for which distribution data have been published. Any metal which forms a tartrate complex has the potential of being separated with this system.

The problems encountered with the tartaric acid system were few, mostly being concerned with the detection

of the complexes and with the growth of mold. Tartaric acid absorbs strongly in the ultraviolet region of the spectrum. The spectra of tartaric acid and the metal-tartrate complexes studied overlap to a great extent. For UV detection under these circumstances, the best situation would be to keep the tartaric acid concentration as low as possible to minimize the absorption by the tartaric acid. This is the one big advantage of the Corasil system. The concentration of tartaric acid used there is only 2.12 X 10^{-3} mol/L. The complexes were able to be detected even at 254 nm which is on a shoulder far from the absorbance peak of 230 nm for most of the complexes. With the Aminex system, the concentrations used were 10 to 20 times those used with the Corasil system. Under these circumstances, the absorbance of the tartaric acid was no longer negligible. This led to a very erratic baseline that changed with every small change in flow rate (due to pump irregularities) and responded drastically to pump noise. Nonetheless, the absorbance of the tartrate complexes was greater than that of the tartaric acid and the complexes were still detectable with UV. One conceivable way around this problem would be to use a double-beam detector so that the absorbance due to the tartaric acid could be cancelled.

One mode of detection, although not investigated, should not be overlooked as a possibility. That is, the use of visible absorbance for the detection of colored complexes such as copper(II), cobalt(II), and nickel(II). This would allow the detection, selectively, of these metals

in the presence of other metals which may elute at the same time or be impossible to detect in any other manner.

The problems encountered with mold growth are discussed in the results section where the care of the columns is outlined. It was very important that the tartaric acid solutions be filtered before each use. This insured that all mold growth was taken from the mobile phase before it entered the column.

Column Deterioration

With both the Corasil anion exchange and the Corasil cation exchange columns, deterioration after a short period of use was a real problem. According to the consultants at Waters Associates Inc., it appears that the problem is associated with the ionogenic groups bound to the silica beads in the column. They speculated that these groups are not really chemically bound to the silica beads. Instead, they are simply adsorbed to the surface of the beads. As such, they are subject to diffusion effects and, slowly but surely, will detach from the silica bead and wash out with the eluent eventually leaving the column stripped of ionogenic groups.

CHAPTER VIII

CONCLUSIONS

From this investigation, the following conclusions may be reached: (1) The separation of metal-chloro complexes by cation exchange HPLC is not feasible in the solvent systems studied. Other solvent systems should be studied, as well as the applicability of anion exchange for achieving useful separations. (2) The separation of metal-tartrate complexes by anion exchange HPLC using a conventional type ion exchange material, such as Aminex A-27, is possible and potentially useful. The work done in this investigation is believed to be the first sucessful application of ion exchange HPLC to metal ion analysis using a commercially available instrument with conventional type detection methods (i.e. UV detection or refractive index detection). Although more work needs to be done to apply the system to a wider range of metals, the six metals separated serve as a demonstration of the utility of the system. (3) The bonded phase silica ion exchange materials presently available for use with HPLC systems are inadequate to the tasks for which they are needed. The useful life of the materials is so short, and their relative cost so high, that use of these materials would not be recommended. Until ways of extending the useful life of the materials are demonstrated, their usefulness will be severely limited. Fortunately, conventional

materials, such as Aminex A-27, which are very stable, are readily available and are relatively easy to use.

APPENDIX A

METALS ANALYSIS

Reagents

Reagent solutions used in the analysis of cobalt(II), copper(II), cadmium(II), nickel(II), and zinc(II), and their preparation, are as follows:

ammonium thiocyanate, 50% (w/v)	Dissolve 50.0 g of NH_4SCN in deionized water and dilute to 100 mL with same.
Zincon, 0.13% (w/v/)	Dissolve 0.13 g Zincon in 2 mL of 1.0 mol/L NaOH and dilute to 100 mL with deionized water.
P.A.R., 0.10% (w/v) in anhydrous methanol	Dissolve 0.10 g P.A.R. in anhydrous methanol and dilute to 100 mL with same.
borate buffer, pH = 9.0	Stir 213 mL of 1.0 mol/L NaOH into 600 mL of deionized water. Add 37.3 g of KCl and 31.0 g of H_3BO_3, dissolve, dilute to 1 L with deionized water.
sodium hydroxide, 1.0 mol/L	Dissolve 20.0 g of NaOH in deionized water and dilute to 500 mL with same.

Cobalt(II) Procedure

The analysis of cobalt(II) makes use of the deep blue color of the cobalt-thiocyanate complex in acetone solutions. The procedure used is similar to that given by Sandell[40]. An accurately measured aliquot of the sample to by analyzed was

placed into a 100 mL volumetric flask. 10.0 mL of 50% (w/v) ammonium thiocyanate and 50 mL of acetone were added and the flask was brought to volume with deionized water. This was mixed thoroughly, allowed to stand for 5 minutes, and read on a spectrophotometer at 625 nm against a reagent blank. The blank and standards were treated in the same manner as the samples. In those aliquots with methanol present, an equivalent volume of methanol was added to the blank, standards, and other samples with no methanol present.

Copper(II) and Zinc(II) Procedure

Copper(II) and zinc(II) were analyzed using the color-forming reagent Zincon (2-carboxy-2'-hydroxy-5'-sulfo-formazylbenzene)[41]. An aliquot of the sample to be analyzed was placed into a 50 mL volumetric flask. 3.00 mL of Zincon and 10.0 mL of borate buffer, pH = 9.0, were added and the flask brought to volume with deionized water. The contents were mixed thoroughly and allowed to stand at least 5 minutes. Readings were taken at 620 nm for zinc(II) analysis and at 600 nm for copper(II) analysis against a reagent blank. The blank and standards were treated the same as the samples.

Cadmium(II) and Nickel(II) Procedure

Cadmium(II) and nickel(II) were analyzed using the color-forming reagent, P.A.R. (4-(2-pyridylazo) resorcinol)[42]. An aliquot of the sample to be analyzed was placed into a 50 mL volumetric flask. 1.00 mL of the alcoholic P.A.R. and 5.0 mL of borate buffer, pH = 9.0, were added and the flask

brought to volume with deionized water. The contents were
mixed thoroughly and allowed to stand for 5 minutes.
Readings were taken at 494 nm for both cadmium(II) and
nickel(II) against a reagent blank. The blank and standards
were treated in the same manner as the samples.

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