ION-PAIR CHROMATOGRAPHY
OF SOME TRANSITION METAL CHELONATES

by

Somsakdi Sagwansin

Submitted in Partial Fulfillment of the Requirements
for the Degree of
Master of Science
in the
Chemistry
Program

Francis W. Smith — February 1st, 1983
Adviser **Date**

Sally M. Hotchkiss — February 9, 1983
Dean of the Graduate School **Date**

YOUNGSTOWN STATE UNIVERSITY
March, 1983

ABSTRACT

ION-PAIR CHROMATOGRAPHY
OF SOME TRANSITION METAL CHELONATES

Somsakdi Sagwansin
Master of Science
Youngstown State University, 1983

In the widespread use of high performance liquid chromatography (HPLC) there has been only limited application of the method for the separation of metal ions. This report describes the first successful separation of iron(III), copper (11), and lead(II) by this method utilizing reverse-phase ion-pair chromatography. Chelonates of iron(III), copper(II), and lead(II) with trans-1,2-diaminocyclohexane-N,N,N^{1},N'-tetraacetic acid (DCyTA) were separated on a C-18 Ultrasphere-Ion Pair stainless steel column using 20% methanol as the mobile phase containing 0.01 M tetrabutylammonium ions as the pairing ions. The eluted peaks were detected at 254 nm and their identity was also confirmed in the visible region. This successful separation adds to the couple of papers already published showing the potential of reverse-phase ion-pair chromatography for metal separations by HPLC.

TABLE OF CONTENTS

ACKNOWLEDGEMENTS

I would like to thank Dr. Francis Smith for his advice and support. I would like to extend my thanks to Dr. Daryl Mincey and Dr. Friedrich Koknat for the review of this thesis.

TABLE OF CONTENTS (CONT.)

LIST OF SYMBOLS

SYMBOL	DEFINITION	UNITS OR REFERENCE
C_a	Total concentration of all the species of DCyTA	See Eq. (17)
C_M	Total concentration of all the metal species	moles/liter
D	Distribution coefficient	See Eq. (3)
DCyTA	Trans-1,2-diaminocyclohexane-N,N,N',N'-tetraacetic acid	See Appendix B
HPLC	High performance liquid chromatography	none
IPC	Ion-pair chromatography	none
k'	Solute capacity factor	See Eq. (4)
K	Equilibrium constant	See Eq. (2)
K_{abs}	Absolute stability constant	See Eq. (11)-(12)
K_{eff}	Effective stability constant	See Eq. (21)-(22)
LC	Liquid chromatography	none
M	Molarity or molar	moles/liter
nm	Nanometer	1×10^{-9} meter
UV	Ultraviolet	none
$\alpha_{M^{m+}}$	Fraction of the metal total concentration in the form M^{m+}	none
$\alpha_{Y^{4-}}$	Fraction of DCyTA total concentration in the form Y^{4-}	See Eq. (18)-(19)
μL	Microliter	1×10^{-6} liter
μm	Micrometer or micron	1×10^{-6} meter

LIST OF FIGURES

FIGURE PAGE

CHAPTER I

INTRODUCTION

Ion-Pair HPLC

High performance liquid chromatography (HPLC) is one of the fastest growing analytical techniques. However it has been mainly applied to organic and biochemical samples with only a few applications to the separation of metals or their complexes. The usefulness of HPLC for ion-exchange separation is quite limited, partly because ion-exchange packings suffer from a number of disadvantages when used in liquid chromatography columns. The columns are often irreproducible from lot to lot and tend to be unstable. Column efficiency is less than with other LC packings. Further there is not much choice among ion-exchange packings so that the range of selectivity available is quite limited.

An attractive alternative to ion-exchange is the technique of ion-pair chromatography (IPC) which can overcome the difficulties mentioned above. IPC as applied to HPLC is a relatively new technique which was first introduced in the mid-1970s. There are two modes used in IPC, normal-phase mode and reverse-phase mode. Reverse-phase IPC was used in this investigation so only this mode will be described. In reverse phase chromatography, a nonpolar stationary phase and a polar mobile phase are used. Nonpolar and nonionic species will be retained in the stationary phase and polar, ionic compounds

will be eluted. Separation of ionic compounds can be accomplished by making them to act as nonionic, nonpolar species. This is achieved by the addition of counter-ions in the mobile phase and the separation is then determined by degrees of retention in the stationary phase. If an anion designated Y^- is to be separated then a counter-ion of opposite charge must be added in the mobile phase. Suppose the counter-ion to be added is tetrabutylammonium ion, Bu_4N^+. By adjusting the pH with an appropriate buffer so that the sample is in Y^- form, the equilibrium shown in equation (1) will exist.

$$Y^- + Bu_4N^+ \rightleftharpoons Y^-Bu_4N^+ \qquad (1)$$

The ion-pair formed is then retained by the nonpolar stationary phase. The equilibrium constant of equation (1) is defined in equation (2) as follows.

$$K = \frac{(Y^-Bu_4N^+)}{(Y^-)\ (Bu_4N^+)} \qquad (2)$$

K is constant for a particular system at: (1) a given pH and ionic strength, (2) a given mobile phase composition, and (3) a given temperature and concentration.

The distribution of charged components may be defined in terms of a distribution coefficient, D. This is shown in equation (3).

$$D = \frac{\text{concentration of components in stationary phase}}{\text{concentration of components in mobile phase}}$$

$$= \frac{(Y^-Bu_4N^+)}{(Y^-)} \qquad (3)$$

The distribution of ionic components between the stationary phase and the mobile phase may be defined more precisely in terms of the amount of the components instead of the concentration. In this case, the term capacity factor, k', is defined in equation (4).

$$k' = \frac{\text{total amount of components in stationary phase}}{\text{total amount of components in mobile phase}} = \frac{DV_s}{V_m} \qquad (4)$$

Here V_s is the volume of stationary phase and V_m refers to the volume of mobile phase. The larger k' is, the better a given column will retain the species of interest.

From equation (2) and (3) the equilibrium constant, K, may be related to the distribution coefficient, D, as follows,

$$K = \frac{D}{(Bu_4N^+)} \qquad (5)$$

and from equations (2), (3), and (4), the capacity factor k' is related to the equilibrium constant K as follows:

$$k' = K(Bu_4N^+)(V_s/V_m) \qquad (6)$$

It can be seen from equations (5) and (6) that the concentration of the counter-ion, Bu_4N^+, may be used as a variable to control the extent of retention in a column. Since the equilibrium constant, K, varies with changes in pH and mobile phase etc., these parameters may be used in controlling the retention of the components.

In deriving the equations above it has been assumed that the sample ion and the counter-ion were paired, and that

the resulting ion-pair had neutral, nonpolar characteristics and so was retained on a reverse-phase column. Since a counter-ion such as the tetrabutylammonium ion contains an organic portion, it can be viewed in terms of the partition of the organic part, first into the stationary phase, followed by ion exchange at the surface. However, in a normal case with small counter-ions the organic portion of the counter-ion is not adsorbed[1-3] and the equations derived can be used as a qualitative guide.

Metal Complexes Equilibria

Metal ions in the presence of anions or neutral molecules may form complexes. For Fe^{3+} in the presence of chloride ions, the resulting equilibria can be written as follows:

$$Fe^{3+} + Cl^- \rightleftharpoons FeCl^{2+} \quad (7)$$

$$FeCl^{2+} + Cl^- \rightleftharpoons FeCl_2^+ \quad (8)$$

$$FeCl_2^+ + Cl^- \rightleftharpoons FeCl_3 \quad (9)$$

$$FeCl_3 + Cl^- \rightleftharpoons FeCl_4^- \quad (10)$$

It should be noted that several complex species are formed simultaneously. This could present some problems in HPLC separation (see Conclusions). The problems associated with the formation of more than one complex species may be best solved by the use of a class of compounds called chelons.

Chelon Complexes

Chelons belong to a special class of compounds that react with metal ions in a 1:1 ratio. The complexes formed with chelons are very stable and soluble in aqueous solvents. A well-known chelon reagent is ethylenediaminetetraacetic acid (EDTA). A metal complex formed by a chelon is a metal chelonate. Chelons have been used and applied successfully in the analyses of metals including HPLC.

DCyTA Complexes Equilibria

Trans-1,2-diminocyclohexane-N,N,N^1,N^1-tetraacetic acid, abbreviated as DCyTA, is a chelon reagent. Complexation equilibria involved with this compound are similar to those with EDTA. In general it is convenient to abbreviate the acid form of DCyTA as H_4Y. Since it reacts with metal ions in a 1:1 ratio and under normal circumstances, the complexes formed with iron(III), lead(II), copper(II) can be written as FeY^-, PbY^{2-}, and CuY^{2-}, respectively. It should be noted that DCyTA loses hydrogen ions in this process and the equilibrium constants of these reactions thus are pH dependent.

The values of absolute stability constants, K_{abs}, as tabulated in the literature are calculated from the following equations:

$$M^{m+} + Y^{4-} \rightleftharpoons MY^{-(4-m)} \qquad (11)$$

$$K_{abs} = \frac{(MY^{-(4-m)})}{(M^{m+})(Y^{4-})} \qquad (12)$$

The reaction in equation (11) is an idealized situation in which there are no other equilibria involved. In reality the reaction is complicated by other equilibria in particular the effect of pH and it is necessary to calculate the extent of this effect.

H_4Y in a solution dissociates to form the following species:

$$H_4Y \rightleftharpoons H_3Y^- + H^+ \qquad K_1 \qquad (13)$$

$$H_3Y^- \rightleftharpoons H_2Y^{2-} + H^+ \qquad K_2 \qquad (14)$$

$$H_2Y^{2-} \rightleftharpoons HY^{3-} + H^+ \qquad K_3 \qquad (15)$$

$$HY^{3-} \rightleftharpoons Y^{4-} + H^+ \qquad K_4 \qquad (16)$$

The total or analytical concentration, C_a, of all the species is written as follows:

$$C_a = (H_4Y) + (H_3Y^-) + (H_2Y^{2-}) + (HY^{3-}) + (Y^{4-}) \qquad (17)$$

If $\alpha_{Y^{4-}}$ is defined as the fraction of the total concentration in the form of Y^{4-} then we can write

$$\alpha_{Y^{4-}} = \frac{(Y^{4-})}{C_a} \qquad (18)$$

From equations (13)-(18), it is possible to derive $\alpha_{Y^{4-}}$ in terms of the dissociation constants and concentration of H^+ as follows.

$$\alpha_{Y^{4-}} = \frac{K_1K_2K_3K_4}{K_1K_2K_3(H^+) + K_1K_2(H^+)^2 + K_1(H^+)^3 + (H^+)^4} \qquad (19)$$

Obviously the values of $\alpha_{Y^{4-}}$ are dependent on pH and can be readily calculated at any pH by substituting into equation (19). As pH goes up the value of $\alpha_{Y^{4-}}$ increases.

From equations (12) and (18), we may arrive at equations (20) and (21) as follows:

$$K_{abs} = \frac{(MY^{-(4-m)})}{(M^{m+})\alpha_{Y^{4-}} C_a} \tag{20}$$

and

$$K_{abs}\alpha_{Y^{4-}} = \frac{(MY^{-(4-m)})}{(M^{m+})C_a} = K_{eff} \tag{21}$$

The effective stability constant, K_{eff}, is variable with $\alpha_{Y^{4-}}$ and thus variable with pH. K_{eff} is an actual measure for the formation of a complex for it takes other equilibria in the system into account. K_{eff} is thus more helpful than K_{abs} in determining the stability of a complex. K_{abs} is a constant and will be equal to K_{eff} if $d_{Y^{4-}}$ is unity, which is the case where pH becomes very high.

K_{eff} derived in equation (21) takes only the effect of pH into account. In the presence of other complexing agents, such as ammonia for example, their reactions with metal ions will affect the formation of the desired complex. If both the pH and complex effects are to be taken into account, equation (21) may be modified, using a similar derivation, as follows,

$$K_{abs}\alpha_{Y^{4-}}\alpha_{M^{m+}} = \frac{(MY^{-(4-m)})}{C_M C_a} = K_{eff} \tag{22}$$

where the new term C_M refers to the total concentration of all the metal species and $\alpha_{M^{m+}}$ is the fraction of the metal concentration in the form M^{m+}. $\alpha_{M^{m+}}$ can be calculated from stability constants of an interfering complex and equilibrium concentration of this complexing substance. Derivation of $\alpha_{M^{m+}}$ is similar to that of $\alpha_{Y^{4-}}$ and will not be shown here. From

the calculated values of $\alpha_{Y^{4-}}$ and $\alpha_{M^{m+}}$, K_{eff} can easily be obtained through equation (22).

Another problem associated with complex formation is the precipitation resulting from hydrolysis of metal ions. The precipitation reaction may compete strongly with the chelon-metal ion reaction and K_{eff} may be greatly lowered to the point where the formation of the complex is not possible. The column used in this study can only be used in the 2-8 pH range within which the hydrolysis effect can be ignored.

Stationary Phase

Columns for modern IPC are the same as columns used in bonded-phase chromatography (BPC). For reverse-phase IPC **packings,** a nonpolar group usually an alkane is chemically bonded to the silica support. An example of this process is shown in equation (23) as follows:

$$\text{Si-OH} + \text{ROSiR}_3 \longrightarrow \text{Si-O-SiR}_3 \qquad (23)$$

Sample retention is directly proportional to the number of hydrocarbon groups attached to the silica support. The most widely used alkyl group is C_{18}, octadecylsilane (ODS). Either porous or pellicular type of packing material is commercially available. Some columns are specifically designed for IPC separations such as the column used in this investigation.

CHAPTER II

SURVEY OF DEVELOPMENTS

Historical

Historically, ion-pair chromatography may be traced back into the early twentieth century when strychnine in hydrochloric acid solution was observed to be extracted by chloroform. This discovery led to a new analytical technique called ion-pair extraction, which was used extensively in pharmaceutical analyses. Perhaps the turning point of ion-pair partitioning to its full usefulness in chromatography was due to Schill[4,5] around 1960. Extensive studies by Schill and coworkers gave rise to several applications[6-8] in ion-pair partition systems. Their work contributed greatly to modern ion-pair HPLC. Before the development of HPLC, Levine[9,10] had developed gravity-feed systems to use with the ion-pair method by adding the counter-ion to the mobile phase. High pressure systems studied by Haney resulted in a reverse-phase ion-pair HPLC[11].

HPLC of Metal Complexes

In 1961, Maeck, Booman, Kussy, and Rein[12] did experiments on ion-pair extraction of metal ions into methyl isobutyl ketone from five complexing media, HCl, NaOH, HF, H_2SO_4, and HNO_3, at different concentration. Fifty-seven metal ions were studied with three ion-pairing reagents,

tetrapropylamine, tetrabutylamine, and tetrahexylamine. Interesting results were obtained. They postulated that an ion-pair was formed from the close association of one quaternary amine ion and one singly charged metal complex. The ion-pair formed then was believed to be as written, $(R_4N)^+(M^{m+}A_{m+1})^-$.

Very little work has been done with HPLC of metal complexes particularly by the ion-pair method. In 1980, O'Laughlin and Hanson[13] from the University of Missouri successfully separated 1,10-phenanthroline complexes of iron (11), ruthenium(II), and nickel(II) using reverse-phase, ion-pair HPLC. $Ru(phen)_3^{2+}$ or $Ni(phen)_3^{2+}$ were separated from $Fe(phen)_3^{2+}$ on the 10-μm particle size μ-Bondapak-CN column. Methanesulfonic acid, added to the aqueous mobile phase which contained acetic acid, was used as the ion-pairing reagent. A wavelength in the UV region at 265 nm was used to detect the elution of the three complex species. $Fe(phen)_3^{2+}$ and $Ru(phen)_3^{2+}$ could also be detected in the visible region at 512 nm and 448 nm respectively. However, they did not observe the elution peaks of the labile complexes $Zn(phen)_3^{2+}$, $Cd(phen)_3^{2+}$, and $Co(phen)_3^{2+}$.

In 1981, Hirowatari, Koumura, Kumamoto, Hattori, and Aoshima[14] from Japan utilized EDTA to separate iron(III), copper(II), and bismuth(III) by reverse-phase ion-pair HPLC. In their experiments the three metal chelonates were separated on an ODS-Hypersil column and measured at 254 nm. The tetrabutylammonium counter-ion was added as 0.01 M Bu_4NBr in a 1:9 MeCN-H_2O mobile phase with 0.01 M $NH_4H_2PO_4$ as a buffer.

Beckett and Nelson[15] from the University of Wyoming illustrated the HPLC separation of zinc(II), cadmium(II), and lead(II) by complexing them with the synthesized 4-aminophenylethylenediamine tetraacetic acid, 4-NH_2Ph(EDTA). A Whatman Partisil-SAX anion-exchange column was used followed by post-column derivatization with fluorescamine for fluorescence detection. The aqueous mobile phase was buffered with sodium acetate to pH 6.5.

In the same year Fraley, Yates, Mahanan, Stalling, and Petty[16] from the University of Missouri performed an anion-exchange separation of nitrilotriacetic acid(NTA) and EDTA complexes of calcium(II), copper(II), magnesium(II), and zinc(II). The aqueous mobile phase was buffered with 0.05 M $(NH_4)_2SO_4$ to pH 6.0. Inductively-coupled plasma-atomic emission spectrometry (ICP-AES) was used to detect the metal complexes separated by HPLC.

O'Laughlin[17] , again in 1982, continued his work to separate 1,10-phenanthroline metal complexes of iron(II), ruthenium(II), and nickel(II) by the ion-pair method. This time all the three complexes above were separated from each other on μ-Partisil-SCX(cation exchange) and the Hamilton-PRP-1(polystyrene-divinylbenzene bead) columns. $HClO_4$, added to the acetonitrile-water mobile phase, was used as the pairing reagent. The separation of the labile complexes $Zn(phen)_3^{2+}$, $Cd(phen)_3^{2+}$, $Co(phen)_3^{2+}$, and $Cu(phen)_3^{2+}$ was also achieved by this technique with $LiClO_4$ as the pairing reagent and with a mobile phase 10^{-4} M in 1,10-phenanthroline. The concentra-

tion of 10^{-3} M in the ligand was too high for UV detection at 265 nm. In all cases, the retention was proved to be due to the perchlorate ion and not the hydronium ion or lithium ion. The retention volumes decreased with an increase in the perchlorate ion concentration. The effects of various parameters on retention and resolution were reported. The separation by this technique was not achieved on μ-Partisil-SAX(anion-exchange), μ-Bondapak-C18, μ-Bondapak-CN, or Zorbax-CN columns.

The references cited for the separation of metals by reverse-phase, ion-pair HPLC are believed to be a complete survey of all work reported up to the present.

CHAPTER III

STATEMENT OF THE PROBLEM

High performance liquid chromatography is a relatively new technique which has had a great impact in analytical chemistry. However it has been used almost exclusively in the analyses of biochemical and organic compounds. The use of HPLC for the separation of inorganic species has been quite restricted. This is undoubtedly due in part to the fact that strongly acidic solutions cannot be used with commercial HPLC equipment. Several factors should be considered in analyses of metals by HPLC: (1) detection of metal ions after separation, (2) identification of each metal peak, (3) interference from undesired species, and (4) quantitative analysis.

Metal ions may be separated either directly or after complexation. Separating metal ions directly is of limited usefulness due to detection difficulties. Most metal ions cannot be detected by conventional HPLC detectors. The use of complexing substances to form metal complexes eliminates the detection problem since metal complexes are easily detected by conventional UV and/or VIS spectrophotometry.

One major drawback in HPLC of metals by the reverse-phase ion-pair method associates with the limited range of pH to be used in the system. Reverse-phase columns can only be used in the pH range 2-8 and the use of strong acids may

damage the pump and other components in the HPLC system. Thus it is necessary to find a system in the operating pH range for HPLC to be performed.

DCyTA is a suitable complexing agent for reverse-phase ion-pair HPLC for the following reasons: (1) DCyTA contains an organic portion (see Appendix B) which absorbs in the UV region. (2) Complexation with metal ions can be achieved in the normal operating range of the pH and so damage to the instruments or column will not occur. (3) The chelon itself is not harmful to the HPLC system. (4) The absolute stability constants for most metal ion-DCyTA complexes are very large (of the order of 10^{20}), thus the complexes are very stable (see Appendix A for K_{eff}). (5) DCyTA complexes metal ions in a 1:1 ratio, so it is very convenient in preparing sample solutions.

The purpose of this study was to investigate the behavior of various metal chelonates of DCyTA using reverse-phase ion-pair HPLC with a view to achieving metal separations.

CHAPTER IV

CHEMICALS AND INSTRUMENTS

Chemicals

The reagent grade chemicals were used in this research unless otherwise specified. The followings are the list of reagents used:

$Cd(C_2H_3O_2)_2 \cdot 2H_2O$	'Baker Analyzed' Reagent
$Co(C_2H_3O_2)_2 \cdot 4H_2O$	Fisher Scientific Company
$Cu(C_2H_3O_2)_2 \cdot H_2O$	Fisher Scientific Company
$Fe(NO_3)_3 \cdot 9H_2O$	'Baker Analyzed' Reagent
$Pb(C_2H_3O_2)_2 \cdot 3H_2O$	Mallinckrodt
$Zn(C_2H_3O_2)_2 \cdot 2H_2O$	Fisher Scientific Company
DCyTA monohydrate, 98%	Aldrich Chemical Company
Bu_4NOH, 1 M solution in methanol	Eastman Kodak Company
Methanol	Fisher Scientific Company
NaOH	Mallinckrodt
Acetic acid	'Baker Analyzed' Reagent
Water	Distilled and deionized

Instruments

Spectra

The spectra were taken on a Model 26 ultraviolet-visible spectrophotometer and recorded by a Model 24-25ACC recorder (Beckman Scientific Instruments, Irvine, CA 92713).

HPLC System

Separation of metal chelonates was performed using a Model 330 Isocratic Liquid Chromatograph, a Model 110 A solvent delivery system, and a Model Altex 210 Injector with a 20 μL injection loop (Beckman Scientific Inst.). A precolumn packed with μ-Bondapak-C_{18}/Corasil (waters Associates Inc., Milford, MA 01757) was used between the injector and the column with the purpose to protect the column from particulate matter since the precolumn could always be repacked. The Altex Ultrasphere-Ion Pair stainless steel column (4.6 mm I.D. and 15 cm in length), packed with 5-μm particle size μ-Bondapak-C_{18} Was obtained from Beckman Scientific Instruments, Inc.

Sample elution was monitored with a Hitachi Model 100-10 variable wavelength, ultraviolet-visible spectrophotometer. Elution peaks were recorded with a Model DSRG-2 dual channel recorder (Sargent-Welch Scientific Co., Skokie, IL 60076).

A Sargent Model IP pH meter was used for all pH measurements. A Hamilton Microliter Syringe (25μL) was used to inject samples into the injection loop. This was then used to inject onto the column.

CHAPTER V

EXPERIMENTAL

Procedure

Mobile Phase

From reviewing all the related work previously done by other workers and taking pH and complex effects into account, it was decided to prepare the mobile phase in the following manner: 10 mL of 1 M tetrabutylammonium hydroxide titrant (1 M in methanol) was pipetted into a big graduate cylinder; methanol was added until the 200 mL mark was reached. 2 mL of glacial acetic acid was then pipetted into the graduate cylinder. The solution was transferred to a 1000 mL volumetric flask and diluted to the mark with water. The pH was adjusted to 7.2 using 5 M stock solution of NaOH in 20% methanol. In this manner the mobile phase consisted of 0.01 M Bu_4N^+ and 0.03 M acetic acid (as a buffer) in 20% methanol solvent. After the solution had been made, it was filtered through a 0.45 μm filter (Millipore Corp., Bedford, MA 01730) to prevent contamination of the column from particulate matter.

Samples

All samples used for HPLC analyses were the acetate or nitrate salts of the metal ions investigated. All metal chelonates were prepared by dissolving stoichiometric amounts of solid DCyTA and metal salts in the mobile phase. In all cases, equimolar amounts of DCyTA and metal ion were used. The final concentration of each metal chelonate was 0.001 M. More dilute solutions were obtained by diluting the prepared solutions proportionally with the mobile phase. Thus for example, the 0.001 M iron(III) chelonate was prepared as follows: 0.0404 g $Fe(NO_3)_3 \cdot 9H_2O$ and 0.0364 g DCyTA were dissolved in mobile phase and diluted to 100 mL with same. The other metal chelonate solutions were prepared in the same way.

Spectra

In order to determine the best wavelength for detection of the metal chelonates, the absorbance of the metal chelonates was measured against the mobile phase at various wavelengths on the Beckman Model 26 UV-visible spectrophotometer. The recorded spectra are shown in the next chapter.

High Performance Liquid Chromatography

Metal chelonates were injected onto the column by way of a 20 μL fixed-volume injection loop and eluted at a flow rate of 1.0 mL/min. Sample elution was monitored by means of

UV absorbance at 254 nm. The wavelengths in the visible region at 375 nm and 720 nm were used to confirm the elution of iron(III) ions and copper(II) ions, respectively.

Care and Maintenance

Solution Stability

All solutions were filtered through a microporous filter (0.45 μm) before use. This removed all particulate matter and most micro-organisms and served to protect the column. Solutions which became turbid or showed the presence of particulate matter were discarded.

The System

The prepared mobile phase was not allowed to remain static in the system. At the end of each experiment, before shutting down the instruments, about 25 mL of 50% methanol was pumped through the solvent delivery system and the column.

The Column

Great care was taken in handling the column to avoid mechanical shocks which might have serious effect to the column packing. When not in use, the column was stored with the bellows attached in its original box.

CHAPTER VI

RESULTS

Spectra

All the spectra taken used the mobile phase as the reference. Samples were dissolved in the mobile phase and spectra were recorded against a blank of pure mobile phase in the reference cell. Spectra of various metal chelonates are shown in Figures 1 through 8. In each case the mobile phase referred to was a mixture of methanol/H_2O-acetic acid-NaOH-Bu_4NOH described in Chapter V.

The spectrum of DCyTA alone is shown in Figure 9 and the spectrum of uncomplexed lead(II) ions is shown in Figure 10.

Comparison of the spectra in Figure 1 to 8 gives us the idea of what common wavelength should be used to detect these metal chelonates in HPLC. In this case the detection wavelength at 254 nm was chosen for detection of the following three complexes of lead(II), copper(II), and iron(III). These three metal chelonates were chosen for HPLC separation in this project because they absorb very strongly in the UV region as compared to the other three metal chelonates.

Figure 9 shows that at wavelengths lower than 254 nm the complexing agent absorbs relatively strongly and can interfere with the detection of metal chelonates.

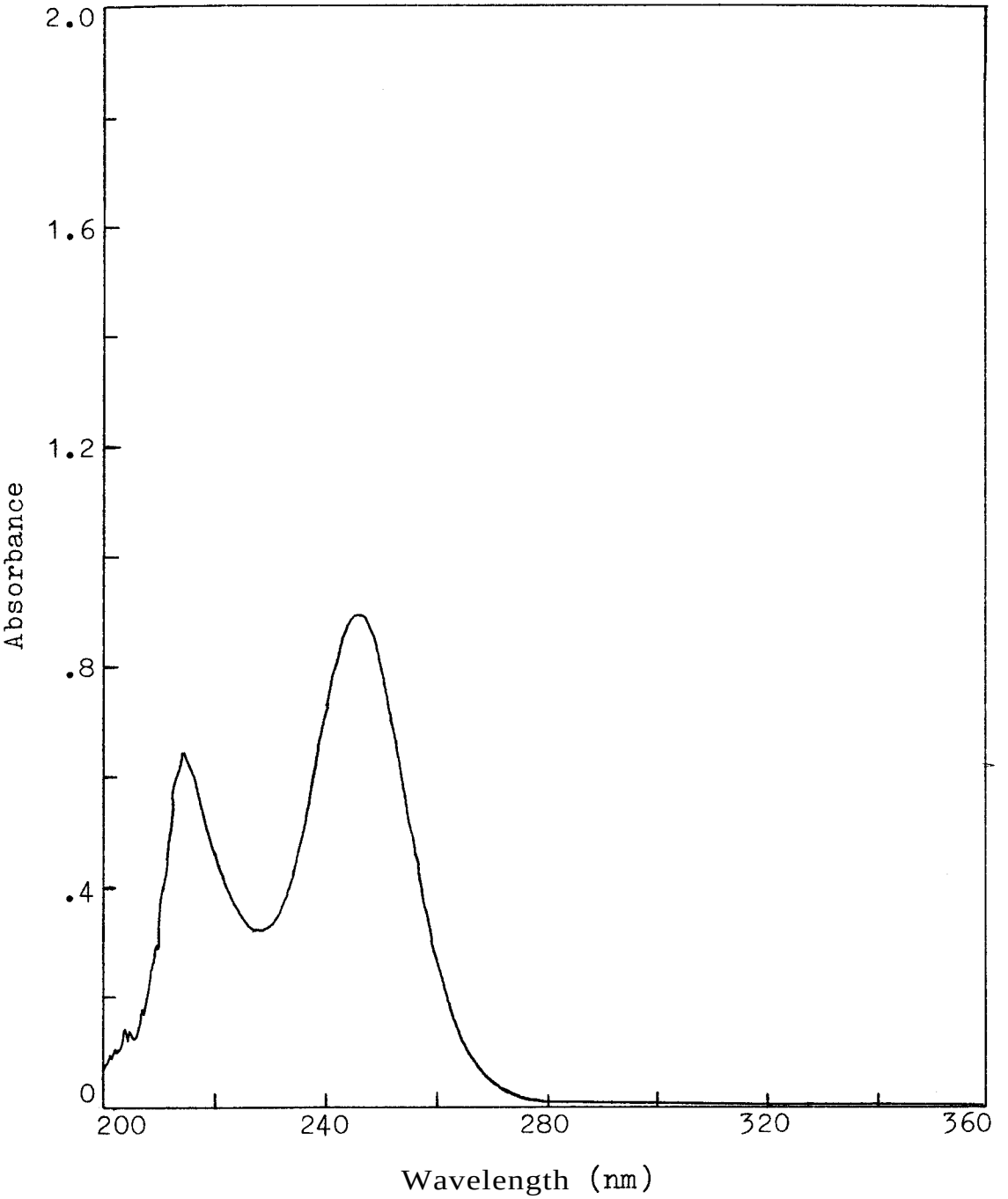


Fig. 1. Spectrum of 0.0001 M lead(II) chelonate in UV region.

Sample: 0.0001 M $Pb(C_2H_3O_2)_2 \cdot 3H_2O$ and 0.0001 M DCyTA in mobile phase (20% methanol, 0.01 M Bu_4NOH, 0.03 M acetic acid, adjusted to pH 7.2 with NaOH).

Reference: mobile phase.

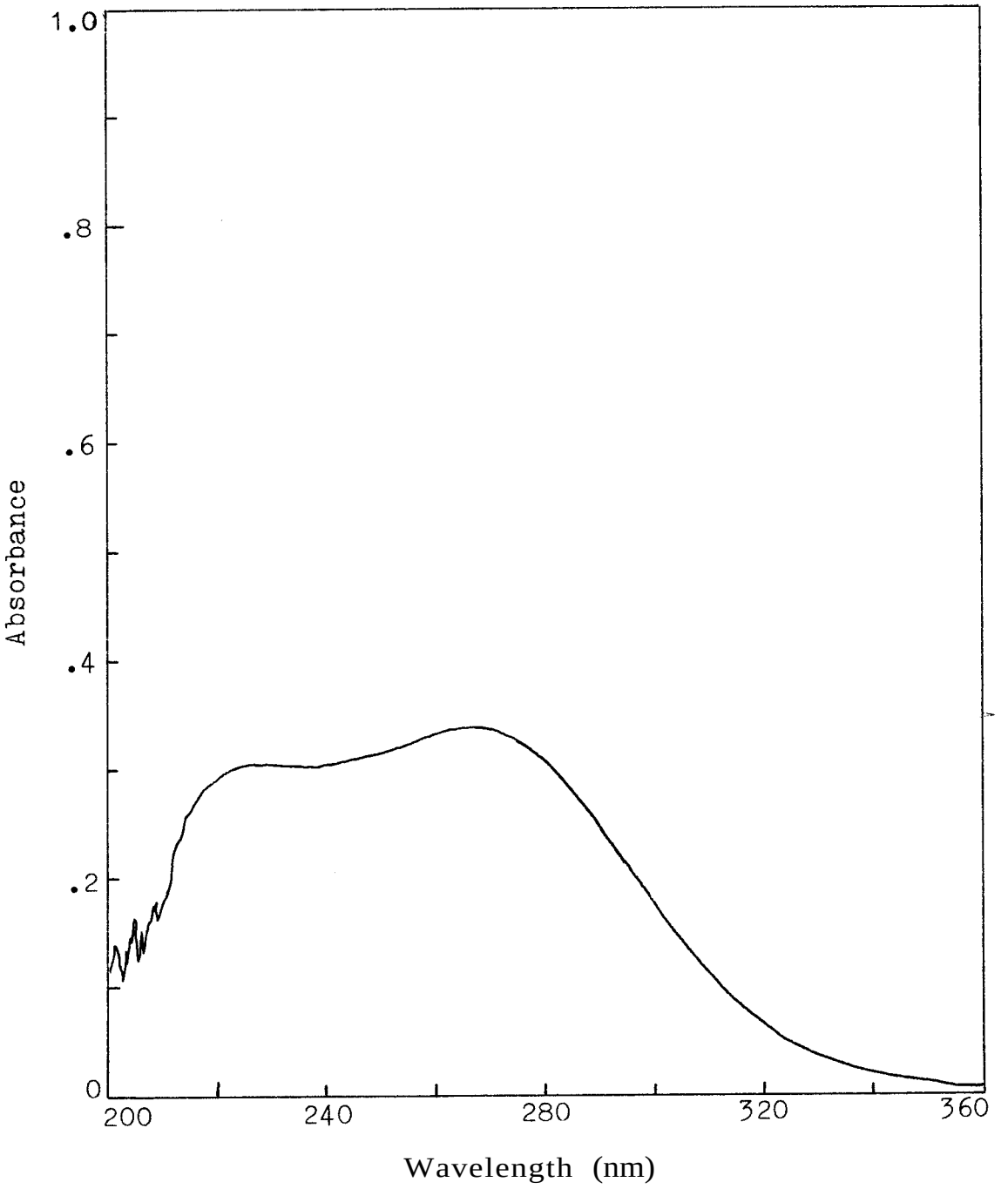


Fig. 2. Spectrum of 0.0001 M copper(II) chelonate in UV region.
Sample: 0.0001 M $Cu(C_2H_3O_2)_2 \cdot H_2O$ and 0.0001 M DCyTA in mobile phase (see Figure 1).
Reference: mobile phase.

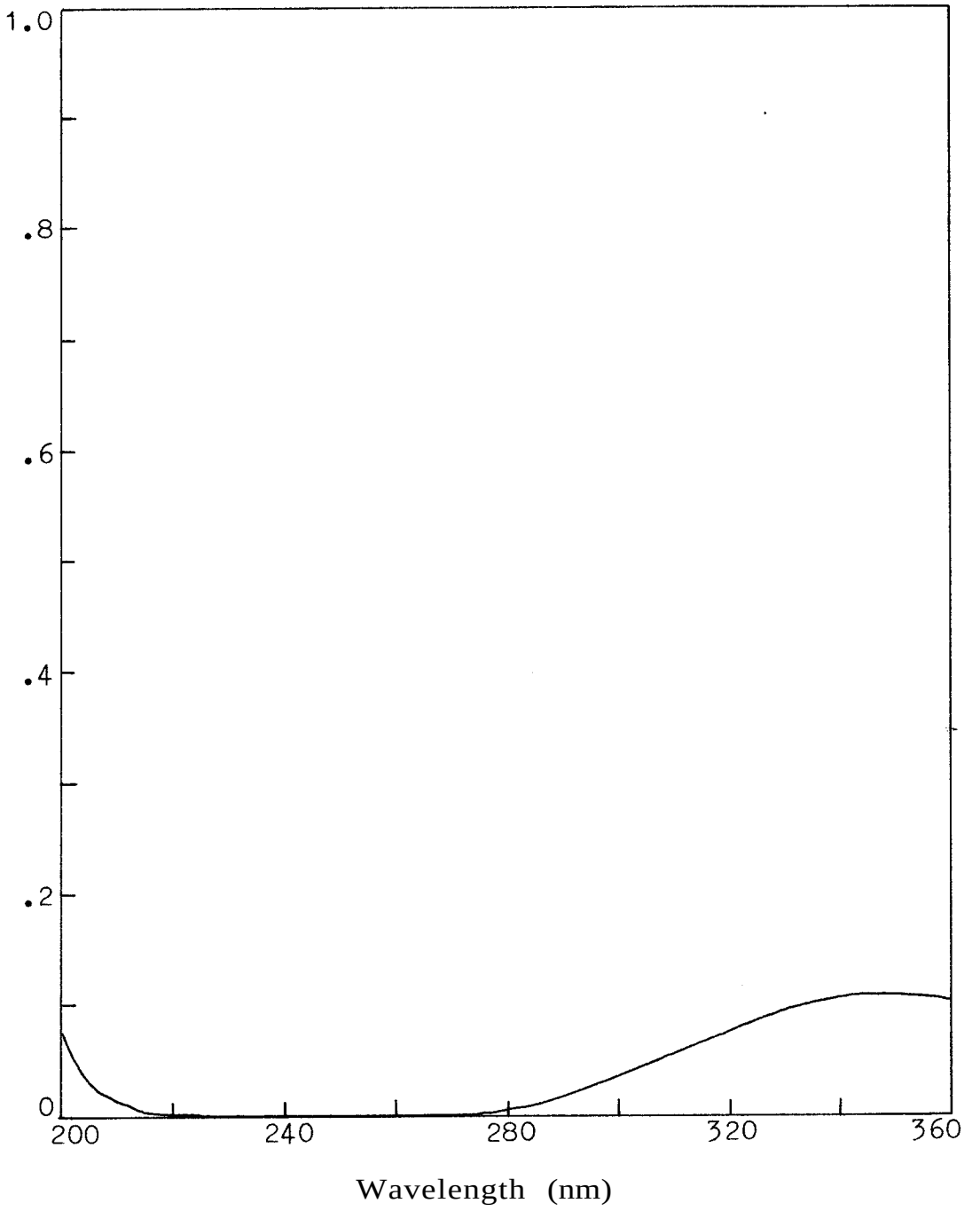


Fig. 3. Spectrum of 0.001 M copper(II) chelonate in visible region.
Sample: 0.001 M $Cu(C_2H_3O_2)_2 \cdot H_2O$ and 0.001 M DCyTA in mobile phase (see Figure 1).
Reference: mobile phase.

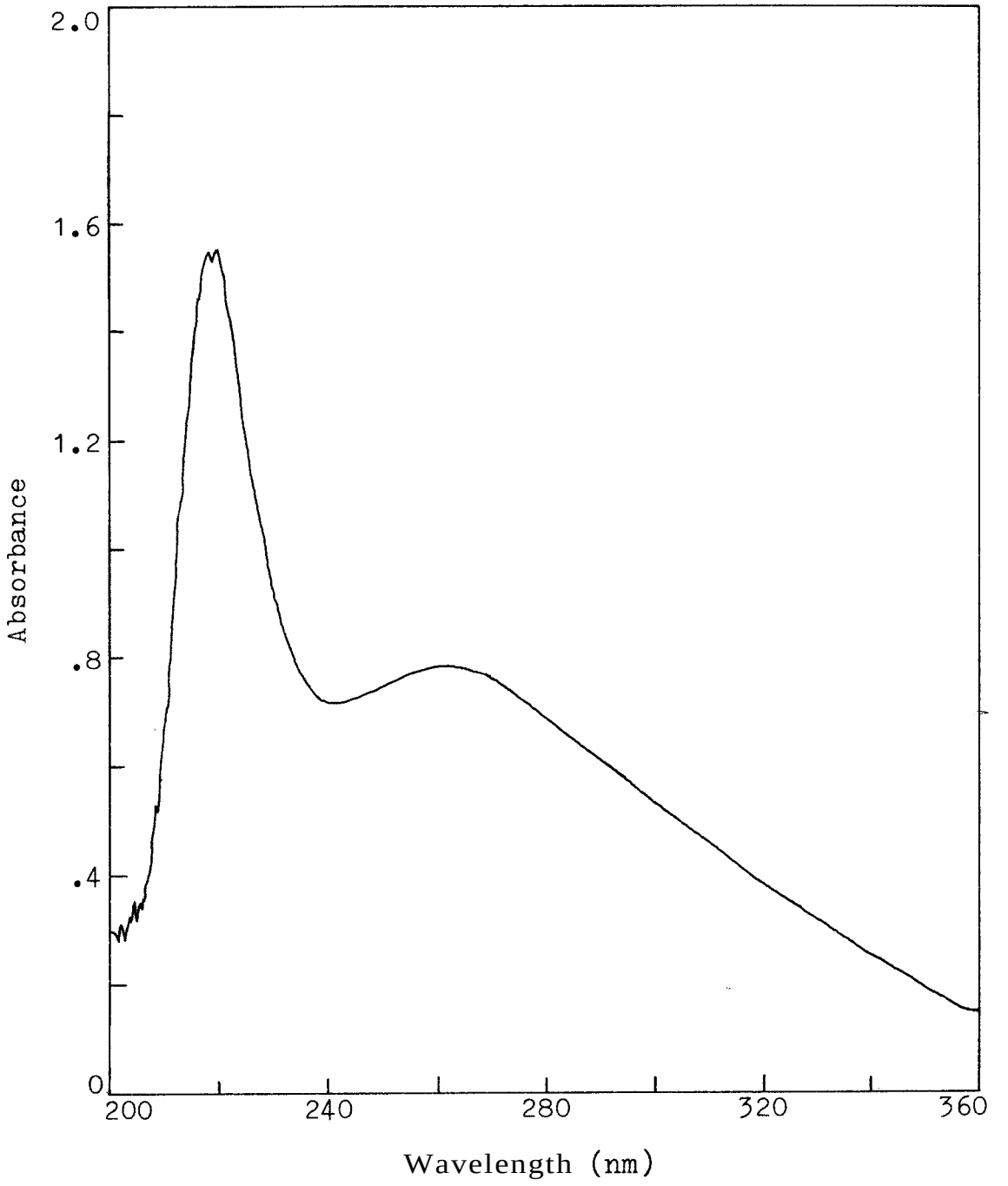


Fig. 4. Spectrum of 0.0001 M iron(III) chelonate in UV region.
Sample: 0.0001 M $Fe(NO_3)_3 \cdot 9H_2O$ and 0.0001 M DCyTA in mobile phase (see Figure 1).
Reference: mobile phase.

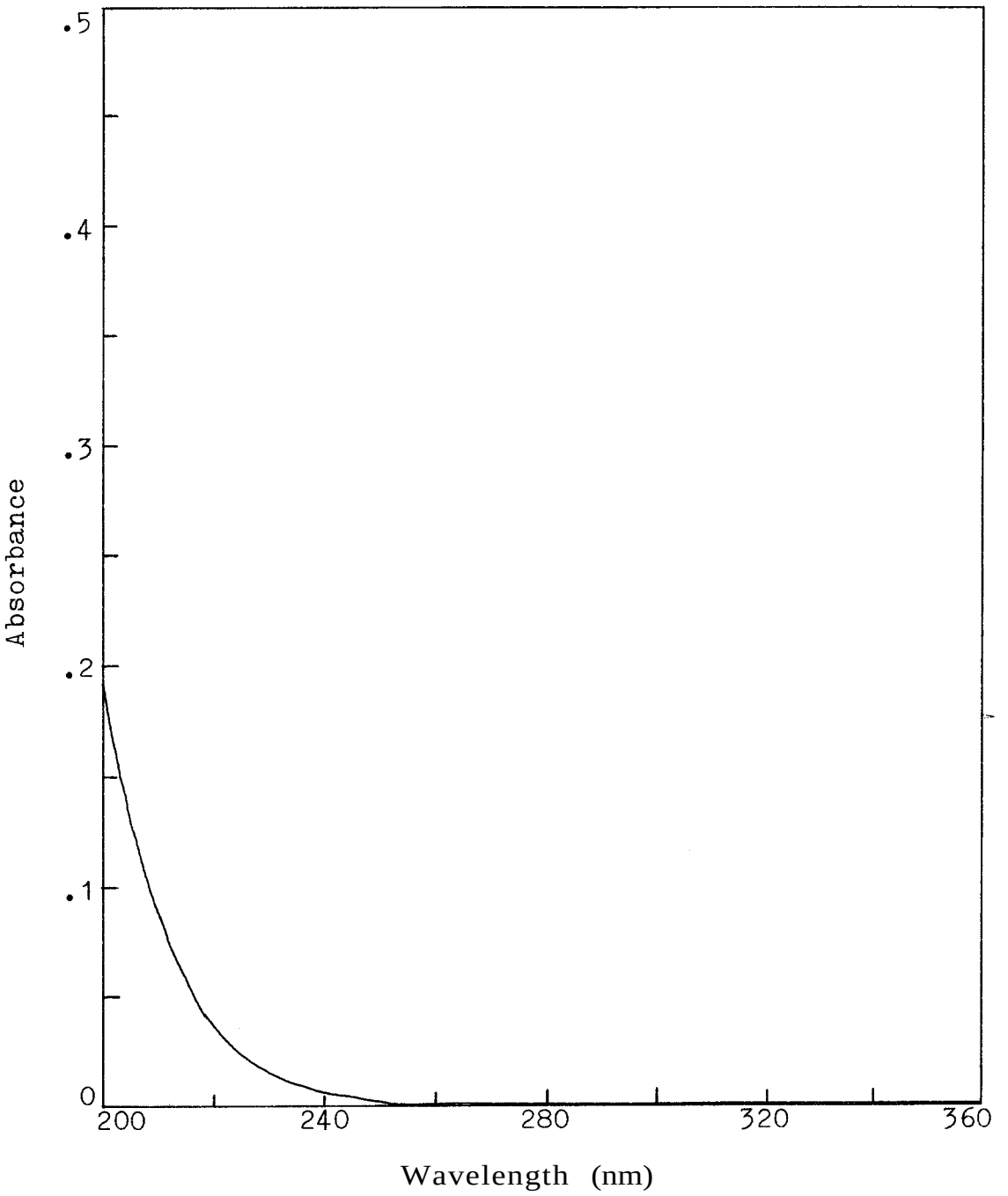


Fig. 5. Spectrum of 0.0001 M iron(III) chelonate in visible region.
Sample: 0.0001 M $Fe(NO_3)_3 \cdot 9H_2O$ and 0.0001 M DCyTA in mobile phase (see Figure 1).
Reference: mobile phase.

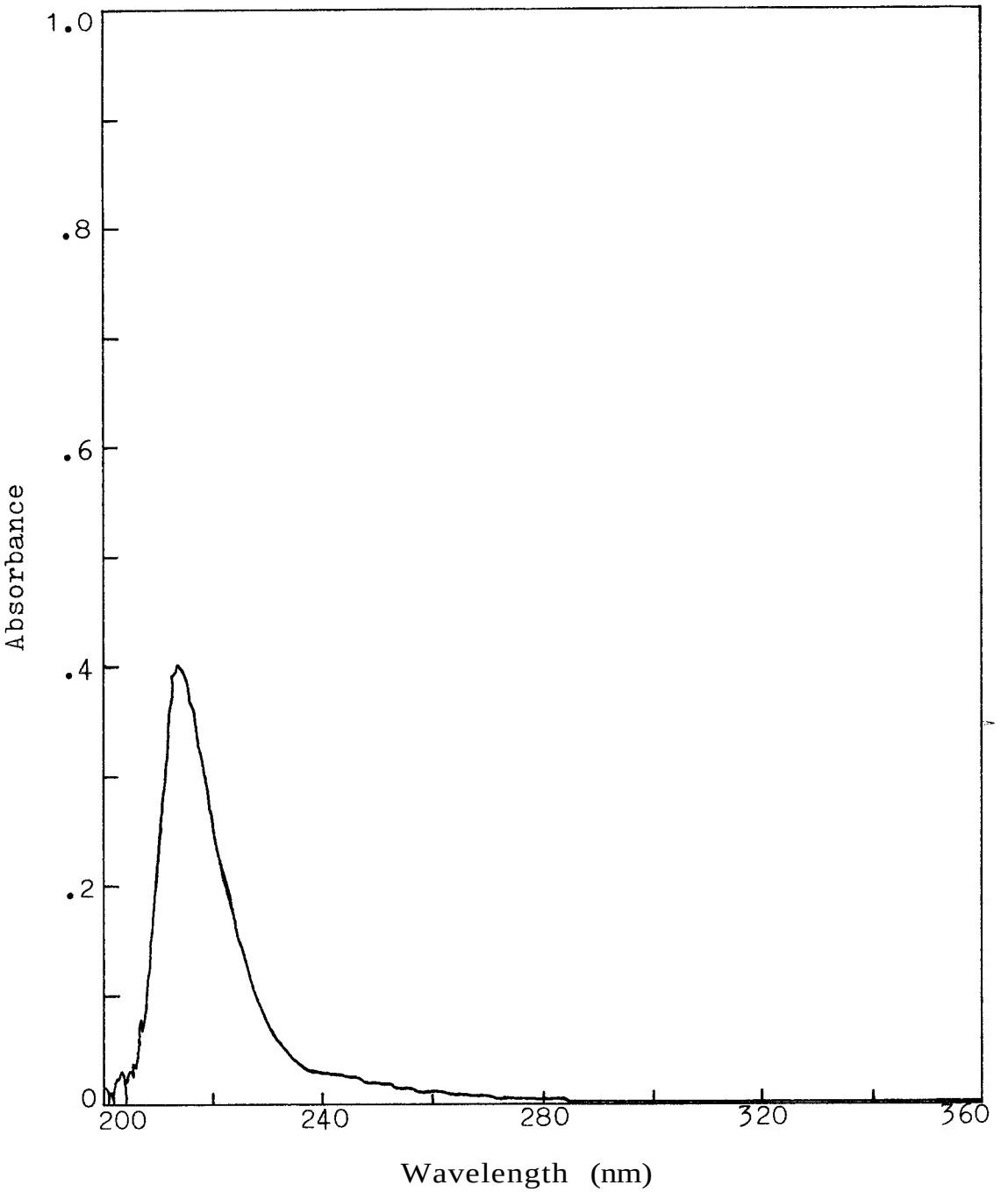


Fig. 6. Spectrum of 0.001 M zinc(II) chelonate in UV region.

Sample: 0.001 M $Zn(C_2H_3O_2)_2 \cdot 2H_2O$ and 0.001 M DCyTA in mobile phase (see Figure 1).

Reference: mobile phase.

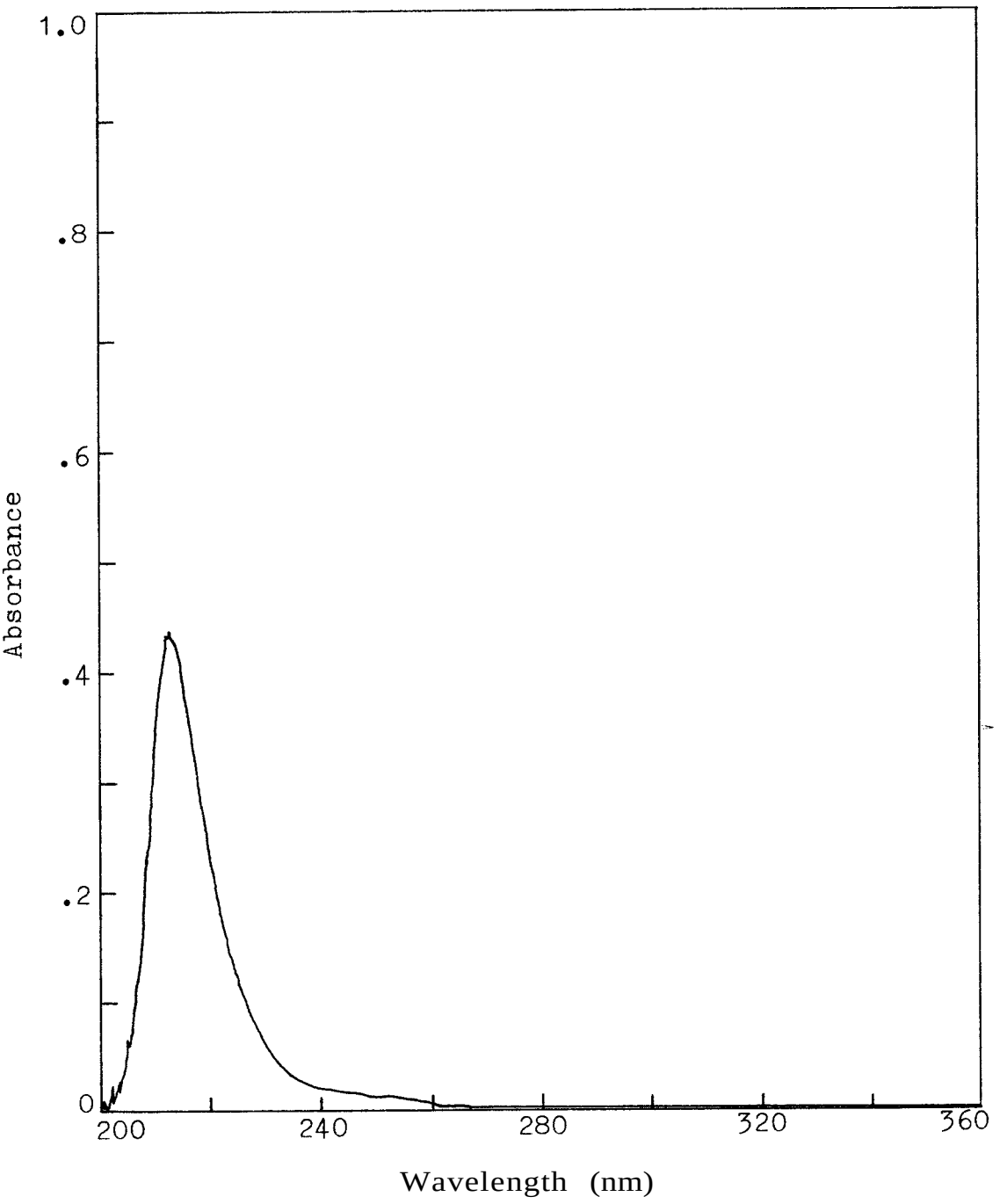


Fig. 7. Spectrum of 0.001 M cadmium(II) chelonate in UV region.
Sample: 0.001 M $Cd(C_2H_3O_2)_2 \cdot 2H_2O$ and 0.001 M DCyTA in mobile phase (see Figure 1).
Reference: mobile phase.

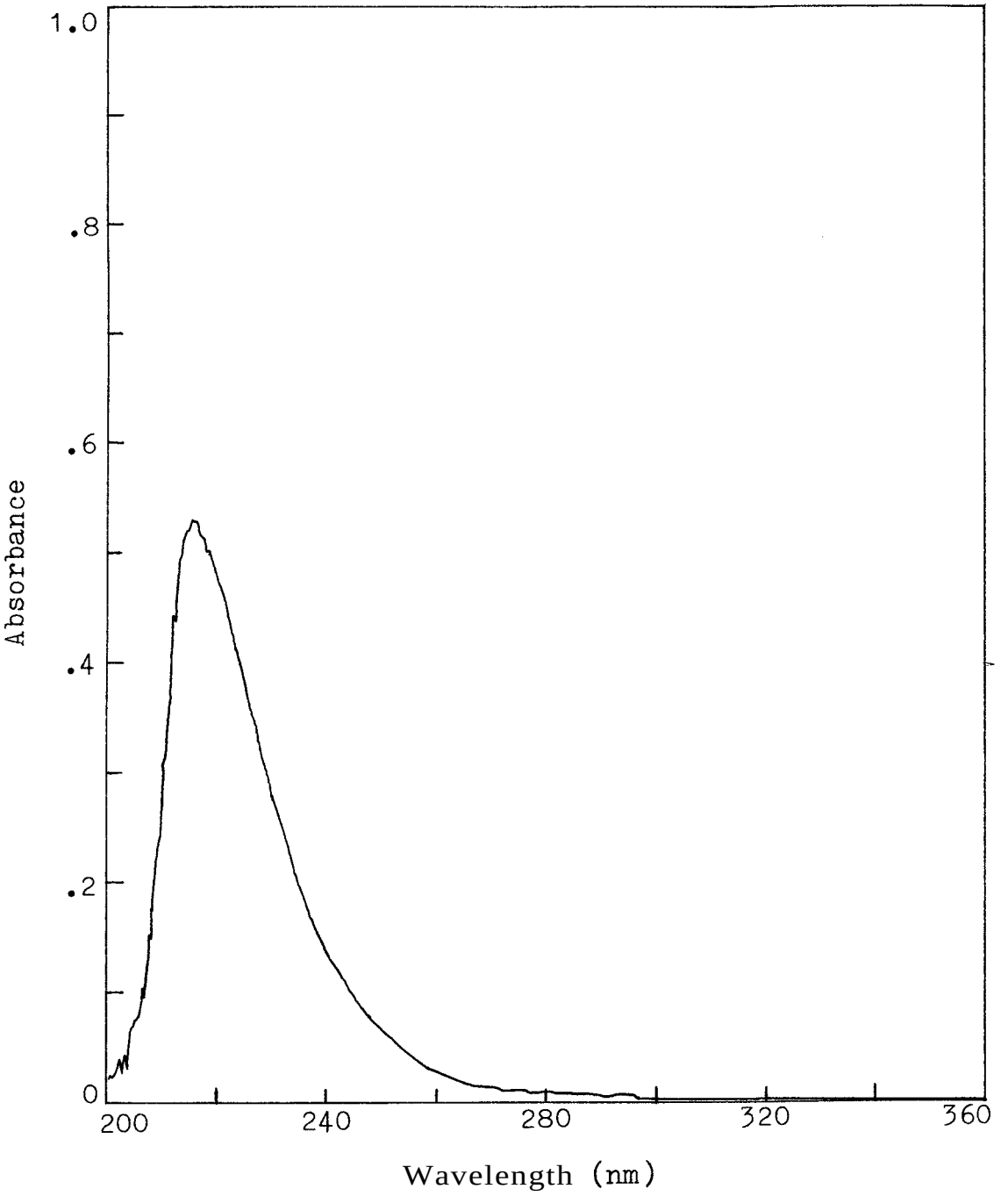


Fig. 9. Spectrum of 0.001 M DCyTA in UV region.
Sample: 0.001 M DCyTA in mobile phase (see Figure 1).
Reference: mobile phase.

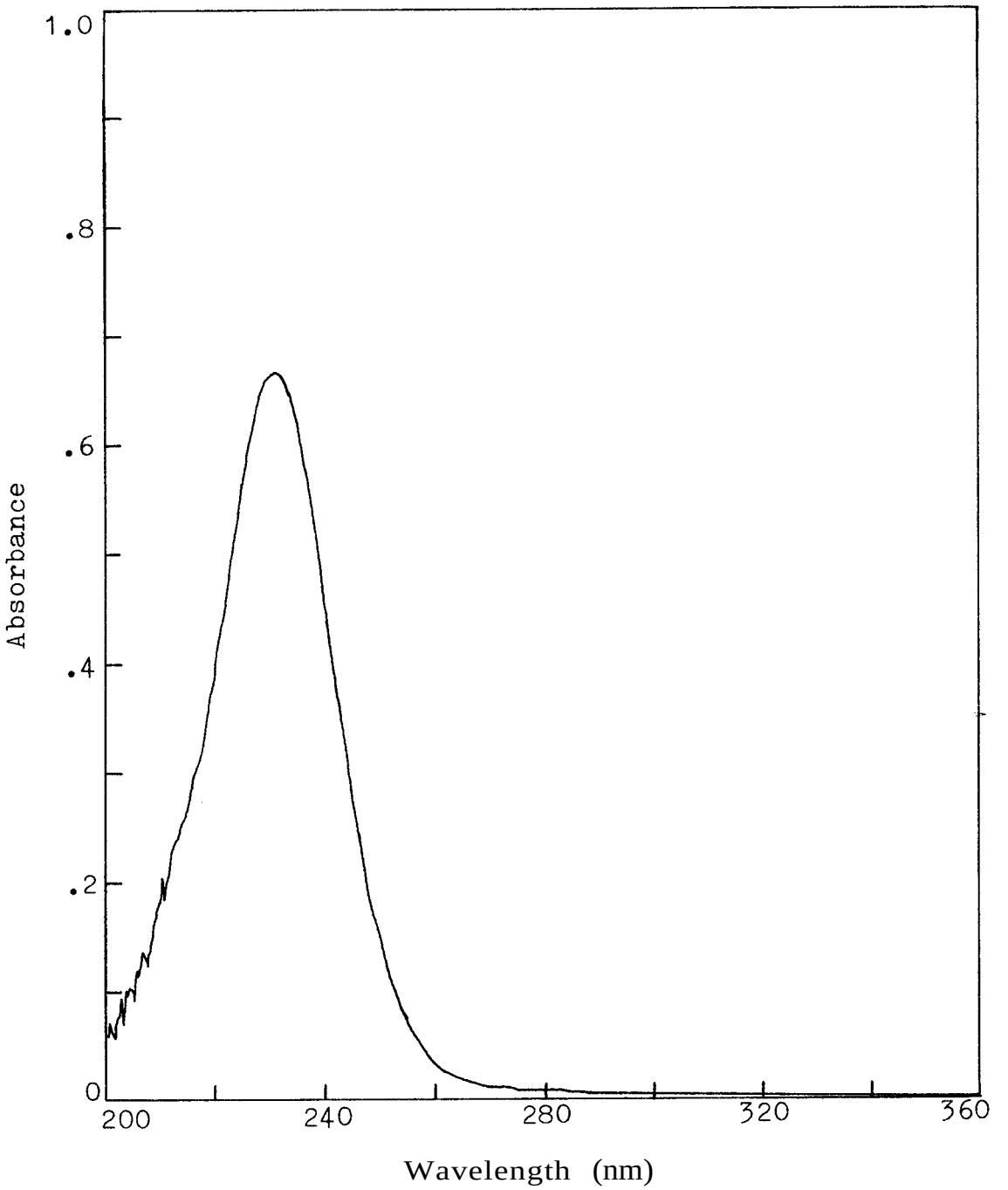


Fig. 10. Spectrum of 0.0001 M lead(II) ions in UV region.

Sample: 0.0001 M $Pb(C_2H_3O_2)_2 \cdot 3H_2O$ in mobile phase (see Figure 1).

Reference: mobile phase.

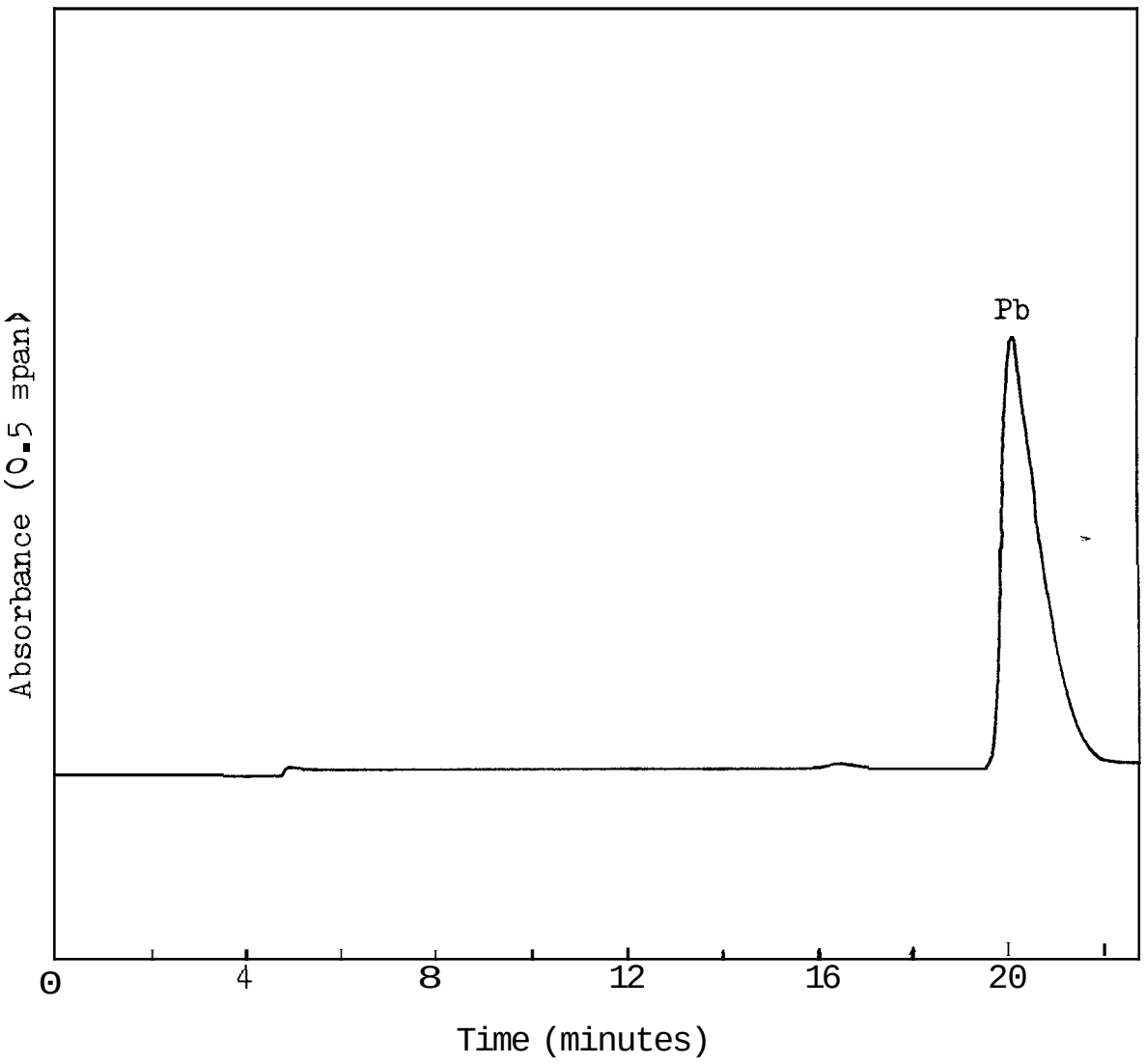
Figure 10 demonstrates that lead(II) ion is a species different from lead(II) chelonate and has a different spectrum. Note that lead(II) ion itself has a very strong absorbance in the UV region.

All the metal ions used were in the forms of acetate and nitrate salts. Since acetate and nitrate have very weak absorbances in UV region[18], the effects of these anions can be ignored.

HPLC of Metal Chelonates

Reverse-phase, ion-pair chromatography of lead(II), copper(II), and iron(III) chelonates was studied using the mobile phase described previously as the eluting medium. The detection was monitored by UV absorbance at 254 nm. All the three metal chelonates absorbed very strongly at this wavelength. The wavelengths in the visible region at 720 nm and 375 nm were used to confirm the elution peaks of copper(II) chelonate and iron(III) chelonate respectively. The metal chelonates injected were between 2×10^{-8} and 2×10^{-9} moles in a 20 μL volume. The flow rate of 1.0 mL/min was used in all cases.

Figures 11 to 15 show the peaks obtained for the complexes of lead(II), copper(II), and iron(III) detected in both UV and visible regions. Three different retention times of the three metal chelonate were obtained. Each metal gave a single and strong absorbance peak. Due to these excellent results, it was possible to demonstrate the separation of these three metal chelonates using the same condition.



Pig. 11. Chromatogram of 0.001 M lead(II) chelonate. Conditions: column, C-18 Ultrasphere-Ion Pair; eluent, mobile phase (see Figure 1); injection volume, 20 μL; flow rate, 1.0 mL/min; detection, UV absorbance at 254 nm; sensitivity, 0.5 span.

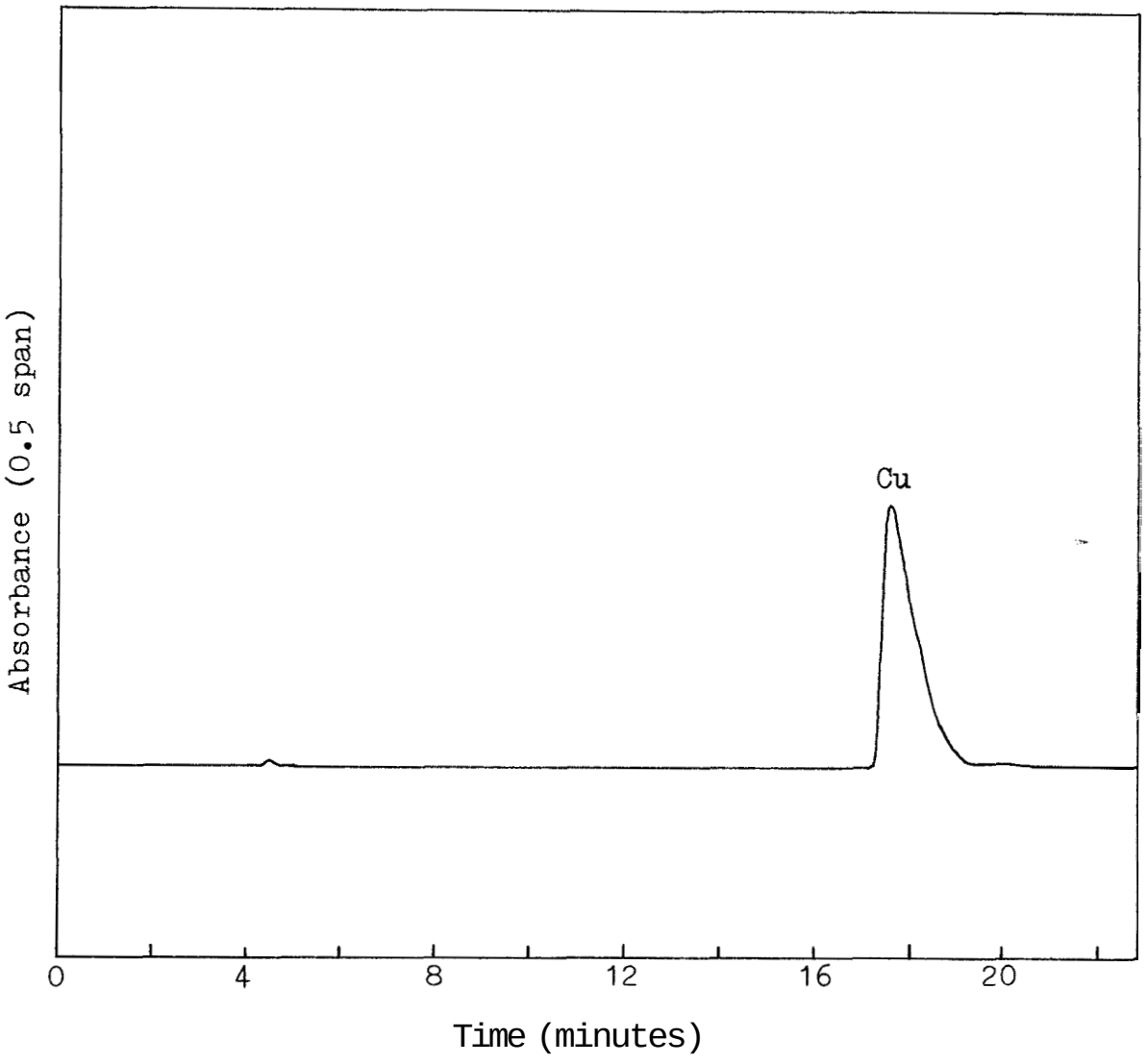


Fig. 12. Chromatogram of 0.001 M copper(II) chelonate. Conditions: column, C-18 Ultrasphere-Ion Pair; eluent, mobile phase (see Figure 1); injection volume, 20 μL; flow rate, 1.0 mL/min; detection, UV absorbance at 254 nm; sensitivity, 0.5 span.

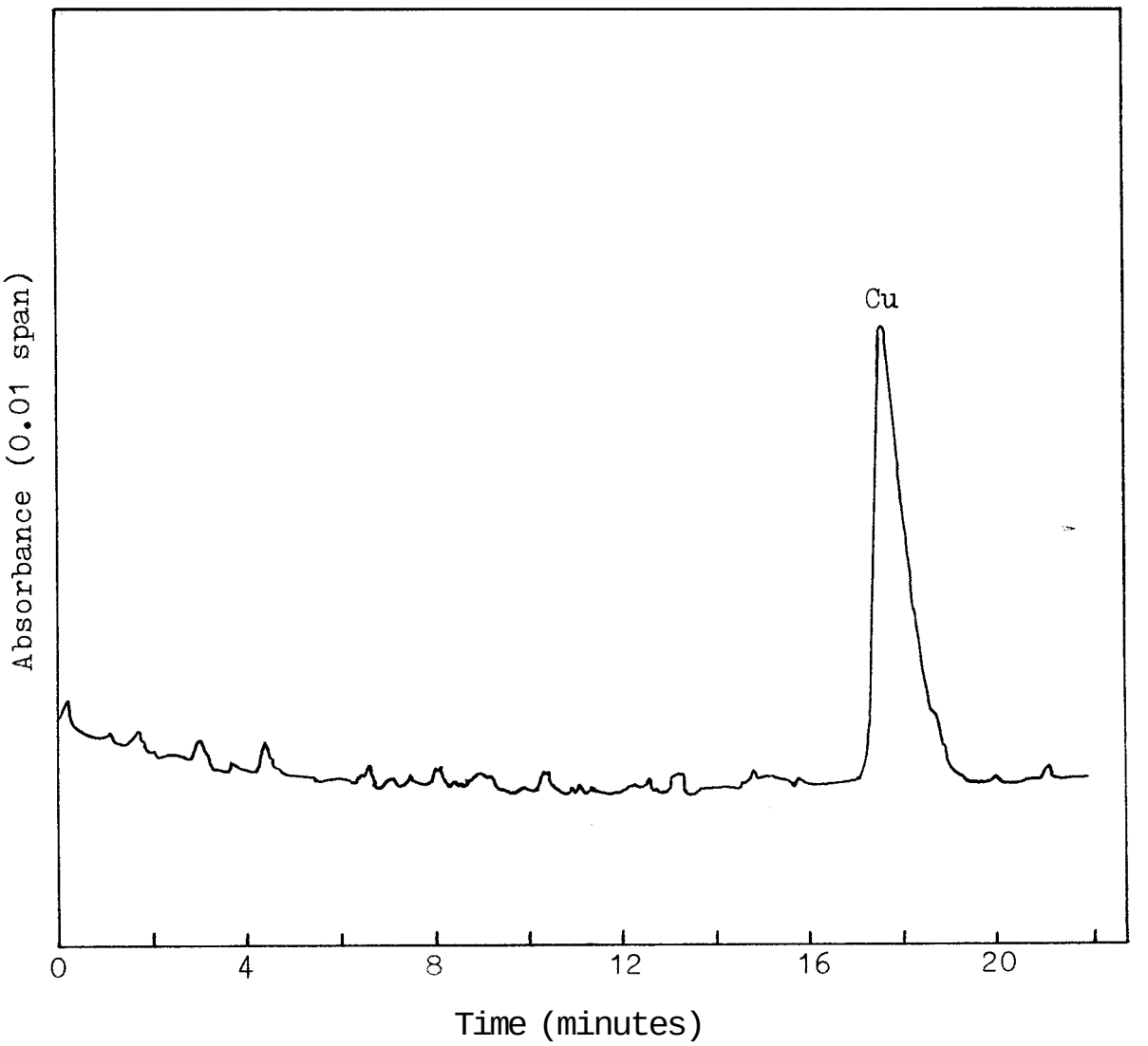


Fig. 13. Chromatogram of 0.001 M copper(II) chelonate. Conditions: column, C-18 Ultrasphere-Ion Pair; eluent, mobile phase (see Figure 1); injection volume, 20μL; flow rate, 1.0 mL/min; detection, visible absorbance at 720 nm; sensitivity, 0.01 span.

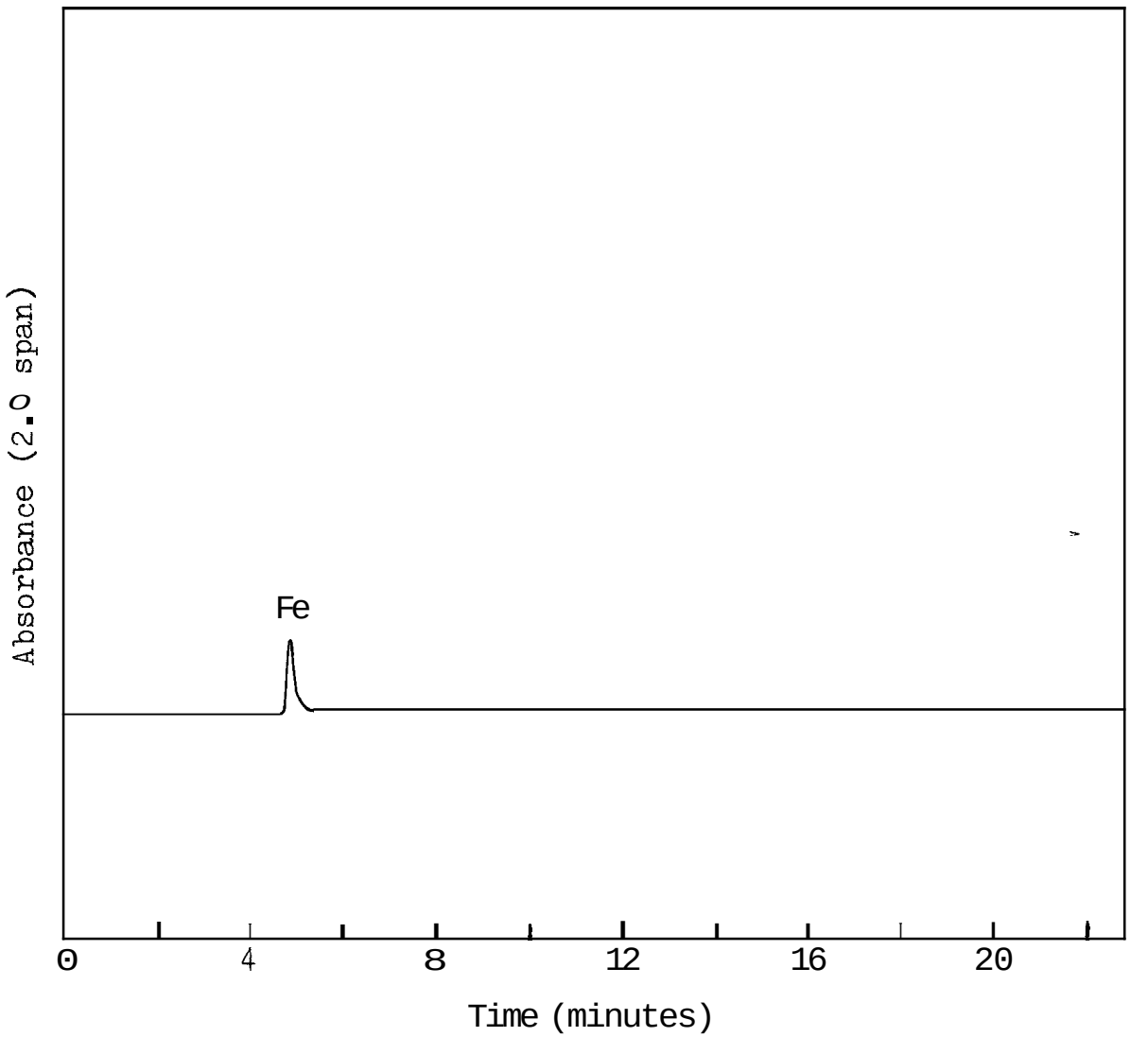


Fig. 14. Chromatogram of 0.0001 M iron(III) chelonate. Conditions: column, C-18 Ultrasphere-Ion Pair; eluent, mobile phase (see Figure 1); injection volume, 20 μL; flow rate, 1.0 mL/min; detection, UV absorbance at 254 nm; sensitivity, 2.0 span.

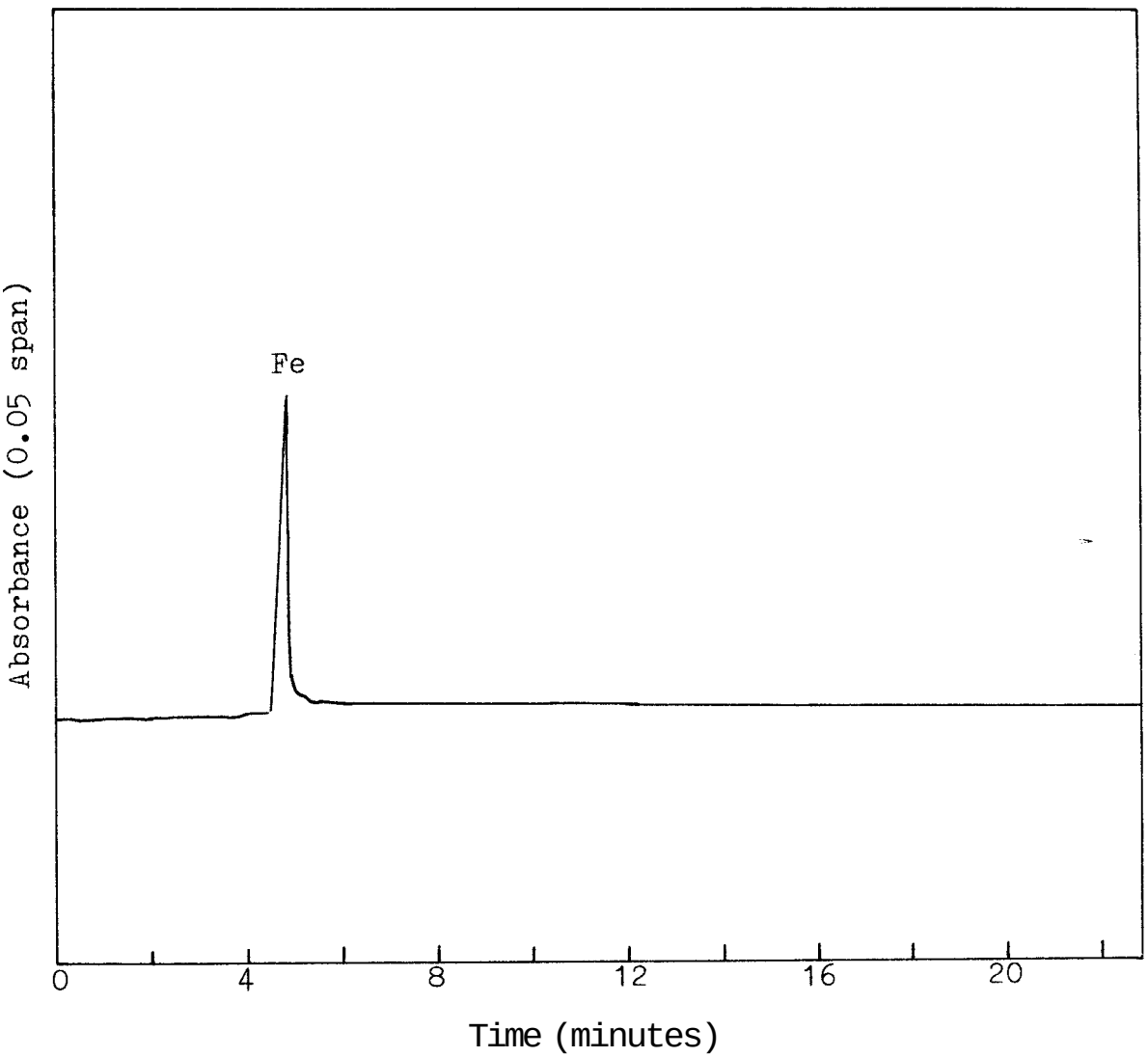


Fig. 15. Chromatogram of 0.0001 M iron(III) chelonate. Conditions: column, C-18 Ultrasphere-Ion Pair; eluent, mobile phase (see Figure 1); injection volume, 20 µL; flow rate, 1.0 mL/min; detection, visible absorbance at 375 nm; sensitivity, 0.05 span.

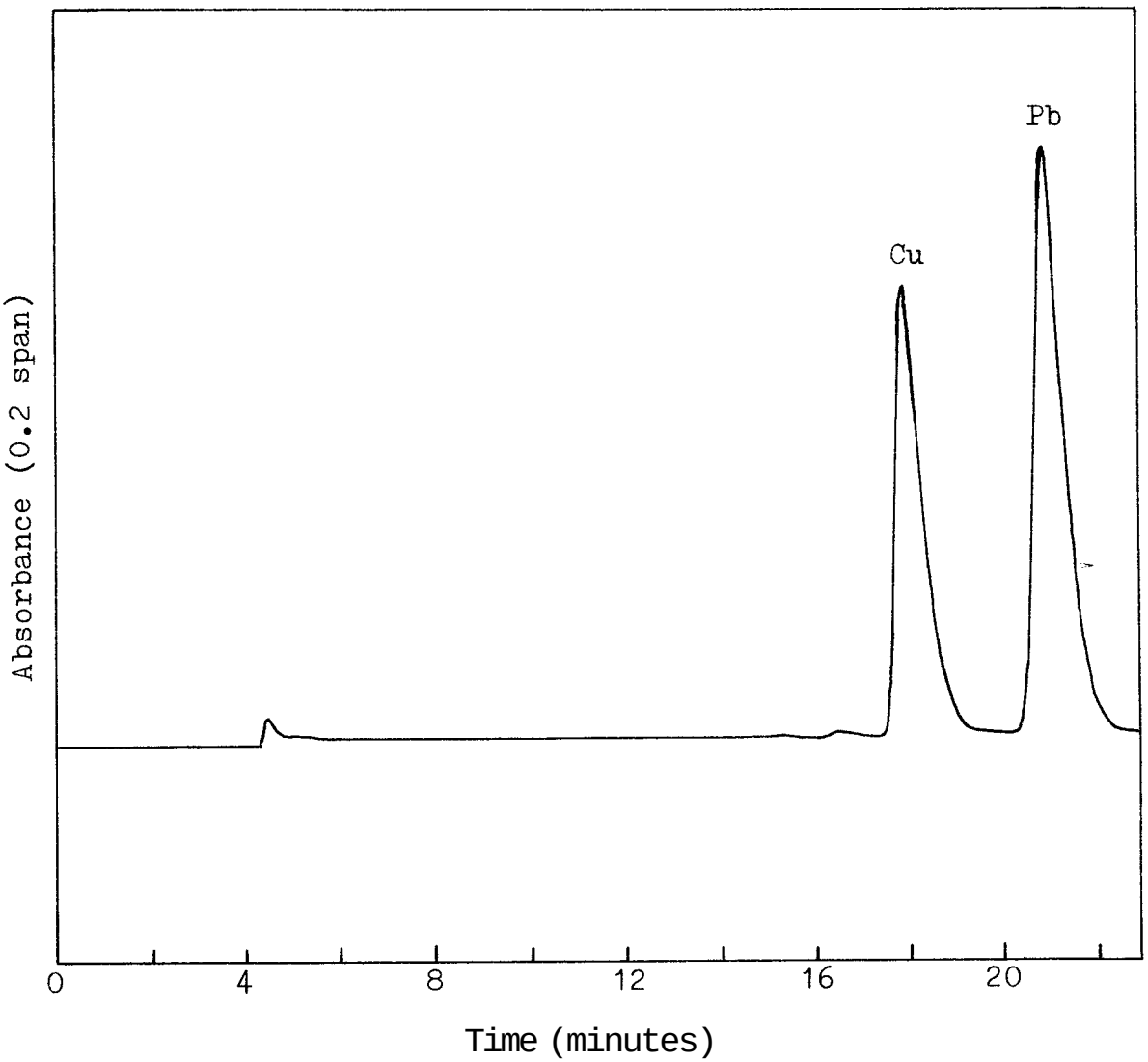
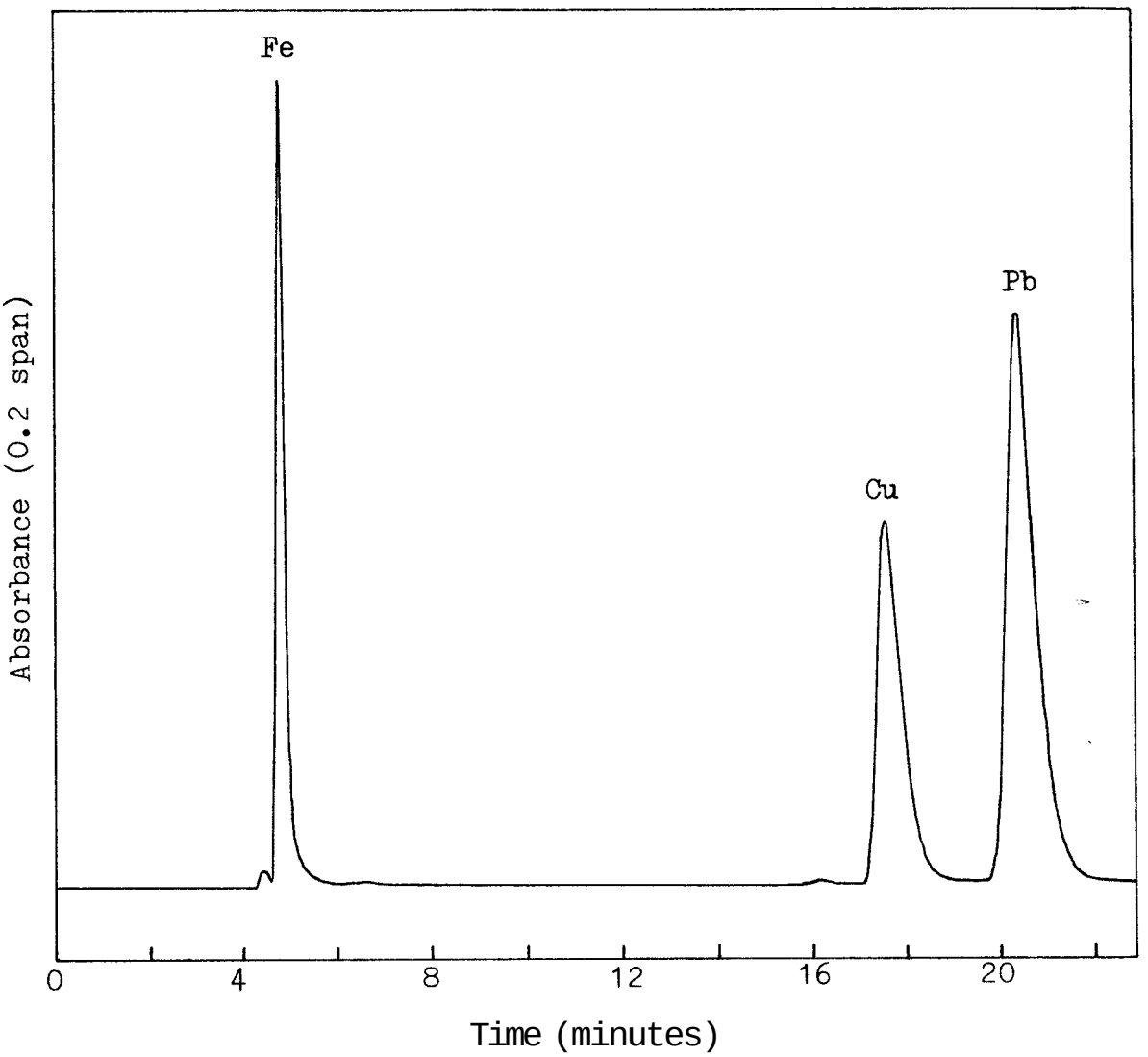


Fig. 16. Separation of a mixture of 0.0004 M lead(II) chelonate and 0.0005 M copper(II) chelonate.

Conditions: column, C-18 Ultrasphere-Ion Pair; eluent, mobile phase (see Figure 1); injection volume, 20μL; flow rate, 1.0 mL/min; detection, UV absorbance at 254 nm; sensitivity, 0.2 span.



Pig. 17. Separation of a mixture of 0.0004 M lead(II) chelonate, 0.0004 M copper(II) chelonate, and 0.0001 M iron (III) chelonate.

Conditions: column, C-18 Ultrasphere-Ion Pair; eluent, mobile phase (see Figure 1); injection volume, 20μL; flow rate, 1.0 mL/min; detection, UV absorbance at 254 nm; sensitivity, 0.2 span.

Figure 16 shows the separation of a mixture of lead(II) chelonate and copper(II) chelonate using the same conditions as in Figures 11 to 15. Two peaks with good resolution of the two metal chelonates were obtained as expected. Separation of a mixture containing lead(II) chelonate, copper(II) chelonate, and iron(III) chelonate is demonstrated in Figure 17.

Interferences due to contamination by the reagents can be avoided by a modification of the method described by Kunkel[19]. Metal ions impurities which are present in the mobile phase and in the complexing agent can be eliminated by adjusting the pH to 10.0 ± 0.2, digesting by heating to incipient boiling for one hour, filtering out the precipitated metal hydroxides with a 0.45-micron membrane filter. Thus the remaining solution should be free from metal ions impurities.

CHAPTER VII

CONCLUSIONS

The ultimate goal of this investigation is to develop practical analytical procedures for the analysis of metal ions. HPLC has been widely used for the analysis of organic and biochemical compounds but very few, if any, practical procedures have been published for the separation and determination of metals in mixtures. In general, gravity fed ion-exchange methods have been widely used for the separation of complex mixtures such as silicates and metal alloys. The procedures are generally rather slow because after separation the various fractions have to be analyzed separately for each component. Adaptation of the voluminous literature on ion-exchange separations to HPLC has been very slow, partly because of the incompatibility of liquid chromatography instrumentation with the strong acids often used in conjunction with ion-exchange resins. A second major problem has been the lack of suitable detections for metal ions. Recent work has employed coulometric and other electrochemical detections, and a few workers have successfully utilized atomic absorption to detect metals in the liquid chromatograph effluent.

The results reported in this investigation add to a couple of papers already published which show the potential of IPC for metal separations. Initially the behavior of some metal chlorocomplexes in reverse-phase IPC was investigated.

However multiple peaks were observed for Co and Ni, probably corresponding to the series of chlorocomplexes which can exist as discussed on page 4. Detections of chlorocomplexes, in both UV and visible regions, were experienced with difficulties. Therefore this approach was abandoned in favor of the chelon DCyTA. DCyTA forms strong complexes with many metals (see Appendix A) and the problem of detection is largely overcome by the fact that all the separated metals would have a large organic molecule attached in the chelonate, allowing for the possibility of a common detection system.

Further work should be aimed first at confirming quantitative results for the metals already considered and then establishing suitable conditions for the separation of other metals. The separation of the various metal chelonates could be optimized by varying four parameters, namely the nature and concentration of the counter-ion, the pH, the nature and concentration of the organic solvent in the aqueous mixture, and the temperature. All these variables may affect k' values and often have a large effect on solvent selectivity.

We can imagine a procedure in which a rock, mineral or metallic alloy would be brought into solution by a suitable procedure followed by removal of corrosive acids or other fluxes, etc. which may interfere in the chromatographic separation, perhaps by evaporating to dryness. After dissolution of the residue and addition of any necessary buffer to adjust the pH, the chelon solution would be added. The mixture could then be separated by IPC followed by automatic

quantitation of the eluted peaks. While such a procedure would not be adequate for major components, it could give good precision for minor and trace metals and of course would have the advantage of great speed.

APPENDIX A

TABLE 1

$\alpha_{Y^{4-}}$ AS A FUNCTION OF pH FOR DCyTA

pH	$\alpha_{Y^{4-}}$ [a]
2	1.4×10^{-16}
3	3.2×10^{-13}
4	1.2×10^{-10}
5	1.4×10^{-8}
6	8.8×10^{-7}
7	1.8×10^{-5}
8	2.0×10^{-4}

[a]The values of $\alpha_{Y^{4-}}$ are calculated from equation (19) using the dissociation constants from Meites.[20]

TABLE 2

K_{eff} AS A FUNCTION OF pH
OF SOME METAL CHELONATES

pH	K_{eff} of Cu(II) Chelonate[a]	K_{eff} of Pb(II) Chelonate
2	2.8×10^{5}	7.1×10^{3}
3	6.4×10^{8}	1.6×10^{7}
4	2.4×10^{11}	6.0×10^{9}
5	2.8×10^{13}	7.1×10^{11}
6	1.8×10^{15}	4.4×10^{13}
7	3.5×10^{16}	8.9×10^{14}
8	3.9×10^{17}	9.9×10^{15}

[a]Effective stability constants are calculated from equation (22) by assuming that the complex and hydrolysis effects in this pH range be negligible. K_{abs} used in calculation are from Meites.

APPENDIX B

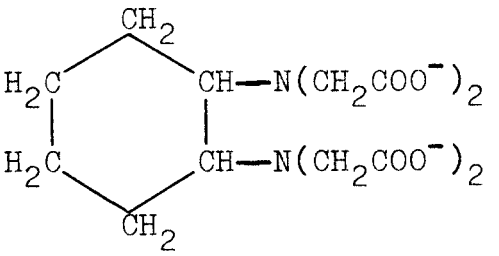


Fig. 18. Representation of a DCyTA molecule

REFERENCES

1. Horvath, C., et al. Anal. Chem. 1977, 49, 2295.
2. Scott, R. P. W. and Kucera, P. J. Chromatogr. 1977, 142, 213.
3. Deelder, R. S. and Linssen, H. J. M. J. Chromatogr. in press.
4. Schill, G,, Modin, R., and Persson, B. A. Acta Pharm. Suecica. 1965, 2, 119.
5. Schill, G. Acta Pharm. Suecica. 1965, 2, 13.
6. Eksborg, S. and Schill, G. Anal. Chem. 1973, 45, 2092.
7. Karger, B. L. and Persson, B. A. J. Chrom. Sci. 1974, 12, 521.
8. Borg, K. O., Gabrielsson, M., and Johnsson, T. E. Acta Pharm. Suecica. 1974, 11, 313.
9. Levine, J. J. Ass. Off. Anal. Chem. 1974, 57, 237.
10. Kaplan, G. B. and Levine, J. J. Ass. Off. Anal. Chem. 1974, 57, 735.
11. Wittmer, D. P., Nuessle, N. O., and Haney, W. G. Anal. Chem. 1975, 47, 1422.
12. Rein, James E., et al. Anal. Chem. 1961, 33, 1775.
13. O'Laughlin, Jerome W. and Hanson, Russell S. Anal. Chem. 1980, 52, 2263.
14. Aoshima, Shozo., et al. Bunseki Kagaku. 1981, 30, 534.
15. Beckett, James R. and Nelson, David A. Anal. Chem. 1981, 53, 909.
16. Petty, James., et al. Appl. Spectrosc. 1981, 35, 525.
17. O'Laughlin, Jerome W. Anal. Chem. 1982, 54, 178.
18. Silverstein, Robert M., et al. Spectrometric Identification of Organic Compounds, 3rd edition. New York: John Wiley and Sons, 1974.
19. Kunkel, R. and Manahan, S. E. Anal. Chem. 1973, 45, 1465.
20. Meites, Louis. Handbook of Analytical Chemistry, 1st ed. New York: McGraw-Hill, 1963.

UNCITED REFERENCES

Day, R. A. and Underwood, A. L. Quantitative Analysis, 3rd ed. Englewood Cliffs: Prentice-Hall, 1974.

Peters, Dennis G., et al. Chemical Separations and Measurements. Philadelphia: Saunders, 1974.

Schram, Steven B. The LDC Basic Book on Liquid Chromatography. St. Petersburg: Milton Roy, 1980.

Snyder, L. R. and Kirkland, J. J. Introduction to Modern Liquid Chromatography, 2nd edition. New York: John Wiley and Sons, 1979.

Tollinche, C. A. and Risby, T. H. J. Chrom. Sci. 1978, 16, 448.

Waters Associates. Paired-Ion Chromatography. An Alternative to Ion-Exchange.