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主論文内容の要旨

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論文題名	Fundamental Study on Extraction-Atomic Absorptiometric Determination of Trace Metals in Sea Water. (溶媒抽出-原子吸光光度法による海水中の微量金属の定量に関する基礎的研究)
カドミウム、亜鉛、銅等の元素は天然状態においても主に河川水によって多量に海洋に運ばれている。そして海洋中において堆積し、収支は平衡が保たれて海水中の重金属濃度は一定に維持されていると考えられている。このような天然系に対して人為的に重金属が、いわゆる汚染によってもたらされた時、海洋における重金属濃度はどのようになるかは人類の将来にとって大きな問題である。そのような議論のもとになるものは海洋における重金属濃度の測定であるが、正しい値が得られているとは言い難い。 近年における機器分析の進歩は著しく、その所産の一つである原子吸光光度法は重金属の分析においても確かにすぐれた方法であるが、これを海水に直接適用するには、多量に共存する塩類の妨害と、ある重金属については測定感度が不足で	

あるという問題点を含んでいる。それ故、化学的手法により目的金属の分離と濃縮のために前段処理を行わなければならない。分離と濃縮の手法の中で、金属と有機試薬との錯形成を利用する溶媒抽出法は簡便、迅速でしかも特別な装置を必要とせず、また濃縮の程度を比較的自由に換えられることなどの利点を有している。さらに通常、金属と有機試薬との錯体は有色であるので、そのまま比色定量法としても応用出来る場合が多く、極めて重要な分離分析法である。

以上の観点から、著者は、新しい溶媒抽出法の開発に関する基礎的研究、ならびに開発した方法と原子吸光光度法との併用による海水中の亜鉛、カドミウム、ニッケル、マンガン、銅の測定法の確立を研究の目的とした。

PART I. ゼフィラミン存在下における金属の抽出の基礎的研究

金属イオンと有機試薬との錯体を有機溶媒に抽出する場合、その効率や生成する錯体のタイプと使用する溶媒の性質に依存している。すなわち、生成する錯体が陽もしくは陰電荷をもつ場合は、反対の電荷をもつ物質を共存させ、イオン対を形成させることにより、ほとんどすべての溶媒に抽出が可能である。一方、電

荷をもたない錯体は、すべての溶媒に抽出されるもの（生成した錯体が配位水分をもたず、配位飽和錯体と呼ばれるもの）と極性溶媒だけに抽出され、非極性溶媒には抽出されないもの（残余配位水をもち、配位不飽和錯体と呼ばれるもの）とがある。したがって感度、選択性、錯形成能力等の点ですぐれた有機試薬であつても、その金属錯体が配位不飽和錯体である時には、抽出性を失ないあつたとその応用範囲は狭くなる。

そこで著者は、溶媒抽出法の応用範囲を完全なものとするために、配位不飽和錯体の非極性溶媒への抽出法の研究をPART I.で行なつた。

これまで配位不飽和錯体の非極性溶媒への抽出は、残余配位水を中心有機塩基で置換してその抽出を可能にする方法が唯一であつた。著者は、このタイプの錯体がオ4級アンモニウム塩（陽イオン界面活性剤の一種）であるゼフィラミン（*zeph*）の存在下で非極性溶媒へ抽出可能となる新しい知見を得、以下に示す数種の錯体について抽出挙動を考察し、新しい溶媒抽出定量法を開発した。

GHA（グリオキザールビス（2-ヒドロキシアニル））はカルシウムを比色定量するための最もすぐれた有機試薬であるが、

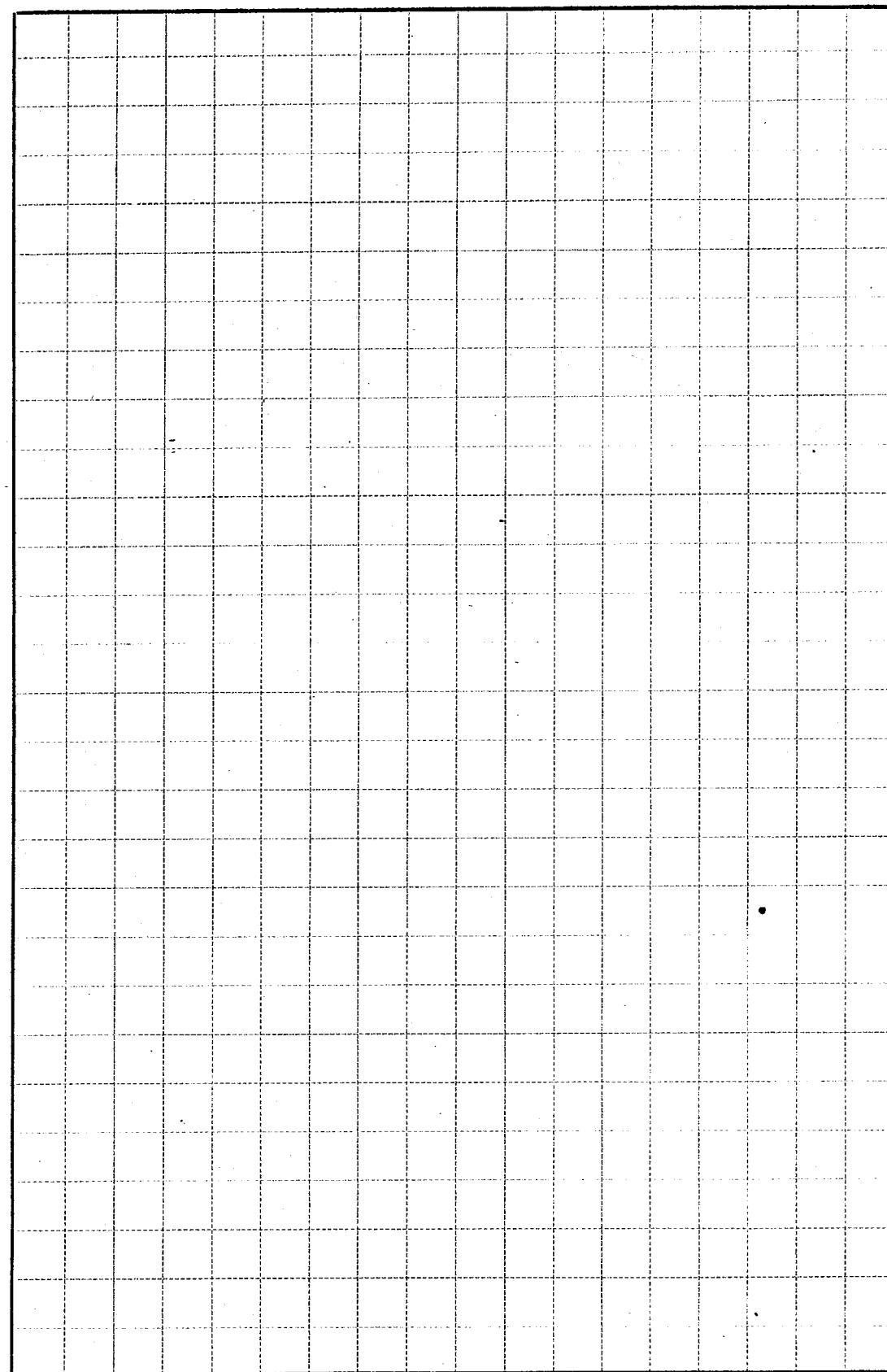
カルシウムとの錯体が非常に不安定であることが欠点であつた。しかし、ゼフィラミン存在下での抽出では長時間安定であることがわかつた。同時に通常のCa:GHA = 1:1錯体からCa:GHA = 1:2錯体に変化し、1.5倍の比色感度の増大を見た。SAPH（o-（サリチリデンアミ）フェノール）のニッケルならびに亜鉛錯体もまた、GHAのカルシウム錯体同様安定性欠点を有していたが、ゼフィラミン存在下での抽出により安定な錯体を得た。応用範囲の広いTTA（テノイルトリフルオロアセトン）のニッケル、コバルト錯体について、通常の、金属:TTA = 1:2錯体が金属:TTA:オ4級アンモニウム塩 = 1:3:1となつて抽出可能になることを見つけた。典型的な配位不飽和錯体であるマグネシウムのオキシソルミン錯体、Mg:オキシソルミン = 1:2錯体からゼフィラミン存在下でMg:オキシソルミン: *zeph* = 1:3:1の抽出可能な錯体となり、同時に1.5倍の比色感度の増大を得た。

以上に示したように、ゼフィラミンの使用により、これまですぐれていとされながらも、応用範囲に制限のあつた試薬を用いて、多くの金属が抽出可能となることを見つけた。この新しい現象を利用した溶媒抽出法による海水中の重金属の分

離濃縮が可能になることを提案した。
PART II. 海水中の微量金属の定量
8-ヒドロキシキノリン(オキシシン)
は、重金属との錯形成能力ならびに取り扱いの簡便さなどの点において最もすぐれた有機試薬である。しかしながら、鉄、アルミニウム、ニッケルの分離、濃縮を除いては、海水中の重金属の抽出法には応用されていない。その理由はオキシシンは亜鉛、カドミウムなどの2価金属と1:2(金属:オキシシン)の錯体を生成するが、それらは非極性溶媒には抽出不可能な配位不飽和錯体であるためであった。そこで著者は、PART I で示したゼフィラミンの存在下で、配位不飽和錯体が非極性溶媒に抽出可能になるという現象をふまえて、ゼフィラミン存在下でのオキシシンによる1,2-ジクロロエタンへの亜鉛、カドミウム、ニッケル、マンガン、銅の抽出条件を検討し、海水中のこれらの重金属の原子吸光度法との併用による定量法を確立した。
亜鉛、カドミウムは通常の、金属:オキシシン=1:2錯体が金属:オキシシン:30ppm=1:3:1錯体となり抽出可能となった。ニッケル、マンガン錯体はゼフィラミンが存在しない時でも抽出可能であるが、存在することにより、1:2錯体が

1:3:1錯体となり抽出がより有利になった。銅錯体は他の4元素と異なりゼフィラミンの有無にかかわらず抽出は完全であった。基礎的諸条件の検討の後、次のような定量操作を提示した。
採水後、酸性にした海水500mlを水酸化ナトリウム溶液でpHを約8とし、分液漏斗に取る。5×10⁻²Mゼフィラミン溶液を20ml、1×10⁻²Mオキシシン-1,2-ジクロロエタン溶液を20ml加え、20分間振とうする。有機相を分取し、再び2-N硝酸10mlと振とうして金属イオンを逐次抽出する。その後、原子吸光度法により、その濃度を測定する。
この方法を海水試料に応用し、感度、精度ならびに簡便さなどの点において満足すべき結果を得た。
ここで著者が提示した、オキシシン-ゼフィラミン系による抽出法は、現在用いられているジチゾン、APDCもしくはDDTCとの錯体をメチルイソブチルケトンまたはイソアミルアルコールに抽出する方法に比べ、抽出性が良いこと、抽出される錯体が非常に安定なこと、使用する溶媒の水との相互溶解度が小さいことなどの多くの利点を有している。また、この方法は操作が簡便で迅速性に富んでいて、このことから、保存中にみかけの定量値

が変化する可能性のある海水試料を採水
時に船上で処理することが出来るなど、
従来法に比べすぐれた濃縮、分離定量法
である。



Fundamental Study on Extraction-Atomic Absorptiometric
Determination of Trace Metals in Sea Water

by

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PART I. PRELIMINARY STUDIES FOR THE EXTRACTION OF METALS IN THE PRESENCE OF ZEPHIRAMINE

CHAPTER 1. INTRODUCTION

Most metal ions play an important role in the hydrosphere, biosphere and atmosphere. In recent years, with the rapid growth of human and industrial activities, pollution by heavy metals is becoming a major concern. Often, these pollutants are finally brought into the sea. Presently, the concentrations of these metals occur only as trace amounts in the oceans, but the concentration of these metals by living organisms is of critical importance; we must pay strict attention to the concentration of these substances by food chains.

For discussions about pollution, it is necessary to know baseline concentrations of those constituents accurately. However, there are a number of difficulties in the analysis of these trace amounts in sea water, because of their low concentration in the environment. Accurate, sensitive, simple and rapid methods for the determination of trace chemical constituents are required.

Generally speaking, chemical analyses involve two steps: the first is the separation of the desired constituent from the bulk sample and the second is the measurement of the amount of constituent present. Although practical techniques for these measurements have rapidly advanced with improvements of analytical instruments in recent years, basic techniques for their separation have changed little during this time.

Among the latter the most important techniques are as follows:

1. Distillation or evaporation
2. Precipitation
3. Chromatographic separation
4. Solvent extraction

In this study, solvent extraction has been adopted and investigated for the separation and concentration of some trace metal ions which form chelates with suitable organic reagents.

Although solvent extraction has long been familiar to the chemist, only in recent years has it begun to achieve recognition as one of the most powerful separation and concentration techniques. The extraction method has the advantages in simplicity, rapidity and ease.

Prior to the determination of an element by absorptiometry or by some other analytical methods, the element must be separated quantitatively and sufficiently concentrated so that the element can be determined accurately. Such conditions are achieved by the selection of a suitable organic reagent, organic solvent, pH-value of the aqueous phase and so on.

Solvent extraction plays an important role in the determination of trace elements, and today the metal chelate with a suitable organic reagent is widely used for extraction. According to the type of chelate formed, metal chelates are classified as follows:

1. Charged (valence-unsaturated) metal chelates
 - 1-a. Cationic chelate

- 1-b. Anionic chelate
- 2. Uncharged (valence-saturated) metal chelates
 - 2-a. Coordination-unsaturated chelate
 - 2-b. Coordination-saturated chelate

Typical examples are shown in Fig.I-1.

To extract a metal chelate into an organic solvent, the formation of an uncharged species is a probable prerequisite, because charged chelates are poorly extractable. But in the case of extraction of charged chelates, extractable chelates may be formed by the neutralization of charge attending the association of these ions purely on the basis of their electrostatic attraction. For example, tin(IV) oxinate (a positively charged chelate) or iron(III)-8-hydroxyquinoline-5-sulfonic acid chelate (a negatively charged chelate) is extracted by ion-association or coupling with an ion having opposite charge, such as chloride ion¹ or a cationic surface-active agent². The extractability of an uncharged chelate is greatly reduced by water molecules coordinated to the central atom. Namely, an uncharged metal chelate having no water molecule is extractable into organic solvents, but an uncharged metal chelate having a water molecule or water molecules is poorly extractable into organic solvents.

If the coordination number of a metal is twice as large as the charge of the metal cation the resulting chelates have no hydrate water, i.e., water coordinated to the metal. Such chelates are named "coordination-saturated". Therefore, chelates, such as cobalt(III) oxinate or nickel dimethyl-

glyoximate, may be easily extracted into all organic solvents. If the coordination number is more than two times of the charge, the resulting neutral chelate has "free" coordination sites which become occupied by water molecules. Such hydrated chelate compounds are often arbitrarily named "coordination-unsaturated"; these coordination-unsaturated chelates are poorly extractable into inactive organic solvents such as benzene, 1,2-dichloroethane and chloroform, because of the water molecules coordinated to the free coordination positions of the central atom. For example, typical and useful organic reagents such as 8-hydroxyquinoline(oxine) and its derivatives, and some β -diketones, form valence-saturated but coordination-unsaturated chelates with many divalent metals, and these chelates are poorly extractable into inactive solvents³. In order to make the extraction of coordination-unsaturated chelates possible, synergic extraction using neutral organic substances such as pyridine³⁻⁵ and its derivatives⁶, and tri-butyl phosphate⁷, has been studied.

In spite of the fact that there are a large number of coordination-unsaturated chelates, little is known about their extraction into inactive organic solvents. If coordination-unsaturated chelate can be quantitatively extracted into an organic solvent, the sphere of application of these solvents for extraction techniques will be widely extended. In this section, the author shows that some coordination-unsaturated chelates, which are poorly extractable and therefore have not been used for separation and concentration of metals, can be

quantitatively extracted into inactive solvents in the presence of a quaternary ammonium base; e.g., the chelates of calcium-GHA, nickel-and zinc-SAPH, nickel-and cobalt-TTA and magnesium-oxine become extractable in the presence of zephiramine which is a quaternary ammonium base. The possibility of using this phenomenon for the determination and concentration of metal ions is also discussed.

Organic reagents used

Chelating reagents:

Glyoxal bis-(2-hydroxyanil)	:GHA
o-(Salicylideneamino)phenol	:SAPH
Thenoyltrifluoroacetone	:TTA
8-Hydroxyquinoline	:Oxine

Quaternary ammonium bases:

Tetradecyldimethylbenzyl ammonium chloride	:Zephiramine(zeph.)
Cetyltrimethyl ammonium bromide	:CTMAB

The structure are shown in Fig.I-2.

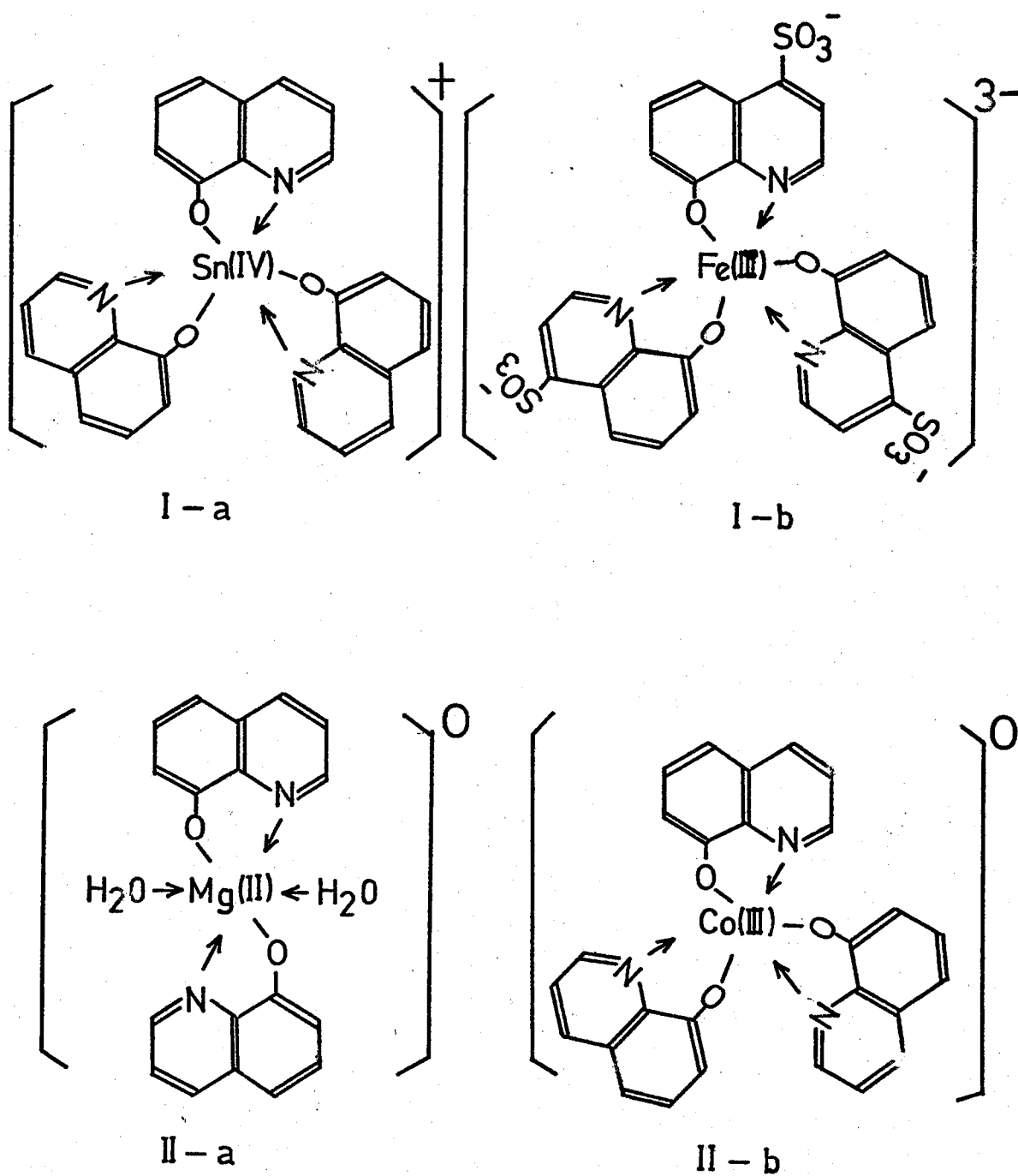
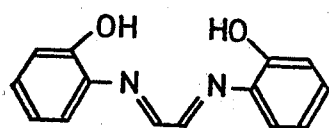
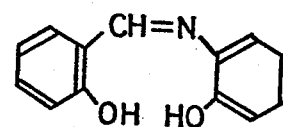


Fig. I-1. Structure of various chelates.

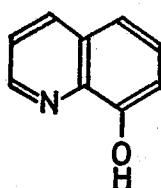
I-a, cationic chelate; I-b, anionic chelate;
 II-a, neutral and coordination unsaturated chelate;
 II-b, neutral and coordination saturated chelate.



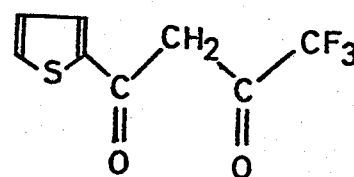
GHA



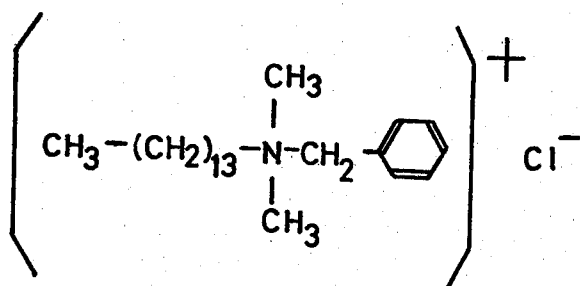
SAPH



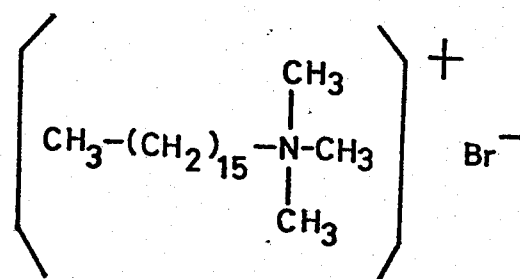
Oxine



TTA



Zephiramine



CTMAB

Fig. I-2. Structure of the reagent.

CHAPTER 2. EXPERIMENTAL

2-1. Reagents

Metal solutions

Calcium, 5×10^{-3} M. Dissolve 0.500 g of calcium carbonate in 10 ml of conc. HCl and dilute to 1 l with water.

Nickel, 1×10^{-2} M. Dissolve 2.81 g of nickel sulfate in 1 l of water.

Zinc, 1×10^{-2} M. Dissolve 2.87 g of zinc sulfate in 1 l of water.

Cobalt, 1×10^{-2} M. Dissolve 2.38 g of cobalt chloride in 1 l of water.

Magnesium, 1×10^{-2} M. Dissolve 2.46 g of magnesium sulfate in 1 l of water.

Standardize the metal solutions against a standard solution of EDTA.

Chelating reagents

GHA, 1.5×10^{-2} M. Dissolve 0.360 g of glyoxal bis-(2-hydroxyanil) in 100 ml of ethanol.

SAPH, 5×10^{-3} M. Dissolve 0.268 g of o-(salicylidenamino) phenol in 250 ml of ethanol.

TTA, 5×10^{-3} M. Dissolve 0.555 g of thenoyltrifluoroacetone in 500 ml of 1,2-dichloroethane.

Oxine, 1×10^{-2} M. Dissolve 0.726 g of 8-hydroxyquinoline in 500 ml of an organic solvent.

Quaternary ammonium bases

Zephiramine, 5×10^{-3} M. Dissolve 0.920 g of tetradecyl-

dimethylbenzyl ammonium chloride in 500 ml of water.

CTMAB, 5×10^{-3} M. Dissolve 0.911 g of cetyltrimethyl ammonium bromide in 500 ml of water.

Buffer solution

The pH of given solution was adjusted with acetate buffer (pH 4 - 6), phosphate buffer (pH 6 - 8) or borate buffer (> pH 8).

All the chemicals used were of analytical-reagent grade.

2-2. General procedure

A metal solution, a buffer solution and a quaternary ammonium base solution were placed in a separatory funnel. The chelating reagent solution and an organic solvent were added, and the funnel was shaken for a definite time. After the organic phase was dried with anhydrous sodium sulfate, the absorbance was measured against water as a reference and the blank correction was made.

The extraction efficiency was obtained by determining the metal concentration in the aqueous phase by atomic absorption spectrophotometry and by measuring the absorbance of the formed chelate in the organic phase.

CHAPTER 3. RESULTS AND DISCUSSION

3-1. GHA chelate

In this section, the sensitive extraction-spectrophotometric method for the determination of calcium is reported.

Calcium is an abundant element and plays an important role in environment, especially in biosphere. Calcium, for example, is a major constituent of the shell of molluscs and some types of marine plankton.

In natural samples, calcium is usually determined by flame photometry. This is a relatively insensitive method, and in practice, errors of the order of 0.5 ppm must be accepted. A search of the literature reveals a few available colorimetric methods: Sandell⁸ has quoted five in detail. Two of these are indirect, i.e., one involves determination of excess of oxalate by a ceric salt, and the other involves determination of phosphate by the molybdenum-blue method after removal of magnesium, a multiple-stage micro technique being used. Chloranilate and murexide are sensitive, and the presence of up to 10 ppm of magnesium is permissible when these reagents are used. But murexide is extremely unstable. o-Cresolphthalein complexone has been used for determining calcium after removal of magnesium, but the calibration graph is non-linear.

In 1957, Bayer⁹ synthesised a Schiff's base, glyoxal bis(2-hydroxyanil) (GHA). Shortly afterwards, Goldstein and Stark-Mayer¹⁰ found that the compound was a sensitive reagent for the detection of calcium, with which it forms a red-colored

chelate and it was subsequently used by Goldstein¹¹ as an indicator in the quantitative determination of calcium by ethylenediamine tetra-acetic acid (EDTA). Because of superiority in selectivity of the reaction for calcium, many papers on spectrophotometric determination of calcium with GHA have been reported. GHA has been, for example, used to determine calcium in natural samples, such as rain and sea water¹², soil extract, serum and plasma¹³⁻¹⁵. On the other hand, other workers^{16,17} have studied the reaction conditions of GHA, using pure calcium salts. The calcium-GHA chelate is extracted from an alkaline solution with chloroform, and the chloroform phase is clarified by centrifugation. Umland and Meckenstock¹⁶ found that the chloroform phase can be clarified and the color stabilized with the addition of a small amount of methanol, but they also found that the chelate was incompletely extracted with added methanol, recovery being only 15 per cent. In the absence of alcohol, as in Williams and Wilson's method¹⁷, the complex is stable for only 15 minutes after extraction. Lindstrom and Milligan¹⁸ pointed that the reagent and its chelate are unstable in the presence of water. It is concluded that GHA is superior to the other reagents in colorimetric determination of calcium, but a serious disadvantage is that the red color rather rapidly fades.

To overcome the instability, the color formation in a mixture of water and alcohol^{13,19,20} or extraction of the chelate into a mixture of alcohol and chloroform²¹ was tried,

all attempts, however, were not always satisfactory.

The author has found that a good color-stability is achieved by extraction of the chelate into 1,2-dichloroethane with the addition of zephiramine, a quaternary ammonium base.

3-1-1. Recommended procedure

A solution of calcium ($0 - 1.25 \mu$ mole), 2 ml of borate buffer (pH 12.8), 2 ml of ethanol and 1 ml of the GHA solution are placed in a separatory funnel, and dilute to 20 ml with water. After 1 - 2 min, 1 ml of the zephiramine solution and 20 ml of 1,2-dichloroethane are added and the funnel is shaken for 1 min. After the organic phase has been dried with anhydrous sodium sulfate, the absorbance is measured at 530 nm using water as a reference.

3-1-2. Absorption spectra

The absorption spectrum of calcium-GHA chelate extracted into 1,2-dichloroethane is shown in Fig.I-3. The extracted chelate is red colored and has an absorption maximum at 530 nm, where the reagent blank has no absorption when compared with water.

3-1-3. Conditions for extraction

Effect of pH. The aqueous solution was adjusted to various pH values with the borate buffer solution and the extraction was carried out as above. Constant absorbance readings were obtained in the pH range of 12.7 - 13.0 (Fig.I-4), and the

experiments were carried out at pH 12.8.

Color-stability of the extracted chelate. No change in the absorbance of the organic phase at 530 nm occurred for at least 6 hours (Fig.I-5). This proposed method appears to have solved the serious disadvantage of the color-instability of the Ca-GHA complex.

Effect of shaking time. Constant readings were obtained in the range of 4 - 7 min. of the shaking time (250 rpm).

Effect of time on color development. We must wait 1 - 2 minutes after adding the GHA because of its slow reaction rate with calcium; at the same time we must not wait too long because of GHA's instability in water. Thus, no later than two minutes after GHA addition, the organic solvent must be added, and the solution shaken for at least 4 minutes. If these instructions are followed, a color-stable solution results which can be read at any time within one hour.

Effect of GHA and ethyl alcohol concentrations. Since GHA is insoluble in water, GHA solution was prepared with ethyl alcohol. Effects of the concentrations of ethyl alcohol and GHA were studied. Constant readings were obtained in the ranges of 4×10^{-4} - 8×10^{-4} M of GHA (Fig.I-6) and 1.3 - 3.0 M of ethyl alcohol in the aqueous phase.

Effect of zephiramine concentration. The constant and maximum absorbance of the extract was obtained in the range of $(1 - 4) \times 10^{-4}$ M of zephiramine (Fig. I-7).

Extractability and molar absorptivity. About 20 ml of aqueous solution containing 50.0 μ g of calcium and the reagents

of appropriate concentrations was shaken with 20 ml of 1,2-dichloroethane; the extraction was repeated with another 20 ml of 1,2-dichloroethane after the separation of the first extract. The second extract showed no absorbance of Ca-GHA, and it was found that 100 % of calcium was extracted in a single extraction. The molar absorptivity of the extracted chelate was 1.3×10^4 which is higher than the value obtained by the conventional method, $(8 - 9) \times 10^3$.^{12,16-18}

Calibration curve. Varying amounts of calcium were extracted by the proposed method. Beer's law holds up to 50 μg of calcium, and Sandell's sensitivity for an absorbance value of 0.001 was 0.0031 $\mu\text{g cm}^{-2}$ (Fig.I-8).

3-1-4. Composition of the extracted chelate

GHA is a quadridentate ligand and its chelate with calcium is a 1 : 1 chelate which is unstable and not extractable into inactive solvent. In the presence of zephiramine, however, calcium-GHA chelate is extracted into 1,2-dichloroethane, and the color of the extract is very stable. So, the composition of the extracted chelate was investigated.

The calcium to GHA molar ratio in the extract was determined by the continuous variations method* (Fig.I-9). It was determined that composition of extracted chelate is $\text{Ca}(\text{GHA})_2$.

In order to examine the stoichiometry between the metal

* See appendix

and the zephiramine, two experiments were made by varying concentrations either of calcium or zephiramine. Fig.I-7 shows that the extraction was complete when the zephiramine concentration was more than 1×10^{-4} M regardless of concentrations of calcium, and a constant absorbance was obtained even when the zephiramine to calcium molar ratio was 0.25 - 20. Moreover, at a constant concentration of zephiramine, the relationship between calcium concentration and the absorbance was linear, regardless of the molar ratios of zephiramine to calcium, e.g., 1, 0.5 and 0.3, as shown in Fig.I-10. These experiments indicate that the presence of a certain amount of zephiramine is necessary for the formation of Ca : GHA = 1 : 2 chelate, but that there is no stoichiometric relationship between the amounts of zephiramine and calcium necessary for the formation of the extractable chelate. Probably the extracted chelate has no zephiramine in its composition.

For these reasons, the composition of the chelate extracted in the presence of zephiramine is assumed to be Ca : GHA = 1 : 2, having no zephiramine in its composition.

Regarding the calcium-GHA chelate, it may be concluded that the water molecules involved in the ordinary nonextractable 1 : 1 chelate are replaced by a GHA molecule (H_2A) by the action of zephiramine. The color-stability was achieved by expelling the water molecules.

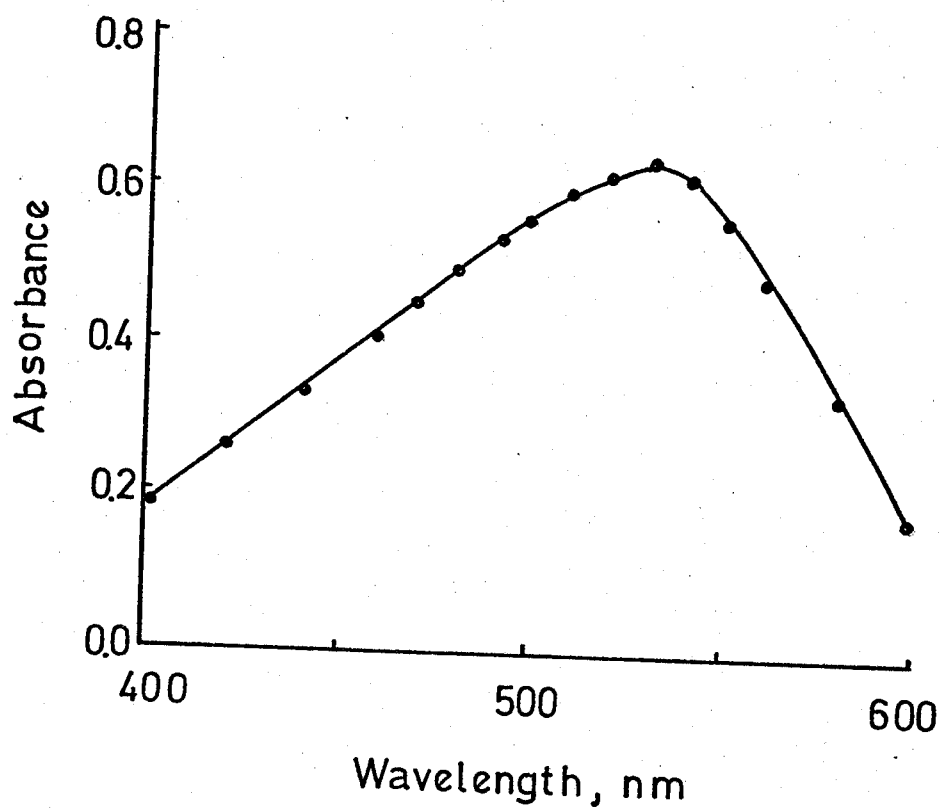


Fig. I-3. Absorption spectrum of Ca-GHA chelate in the organic phase.

$[\text{Ca}]_{\text{aq}} = 4 \times 10^{-5} \text{ M}$, $[\text{GHA}]_{\text{aq}} = 6 \times 10^{-4} \text{ M}$,
 $[\text{zeph}]_{\text{aq}} = 2 \times 10^{-4} \text{ M}$, and $\text{pH} = 12.8$.
 $V_{\text{aq}} = 25 \text{ ml}$ and $V_{\text{org}} = 20 \text{ ml}$. Reference: Water.

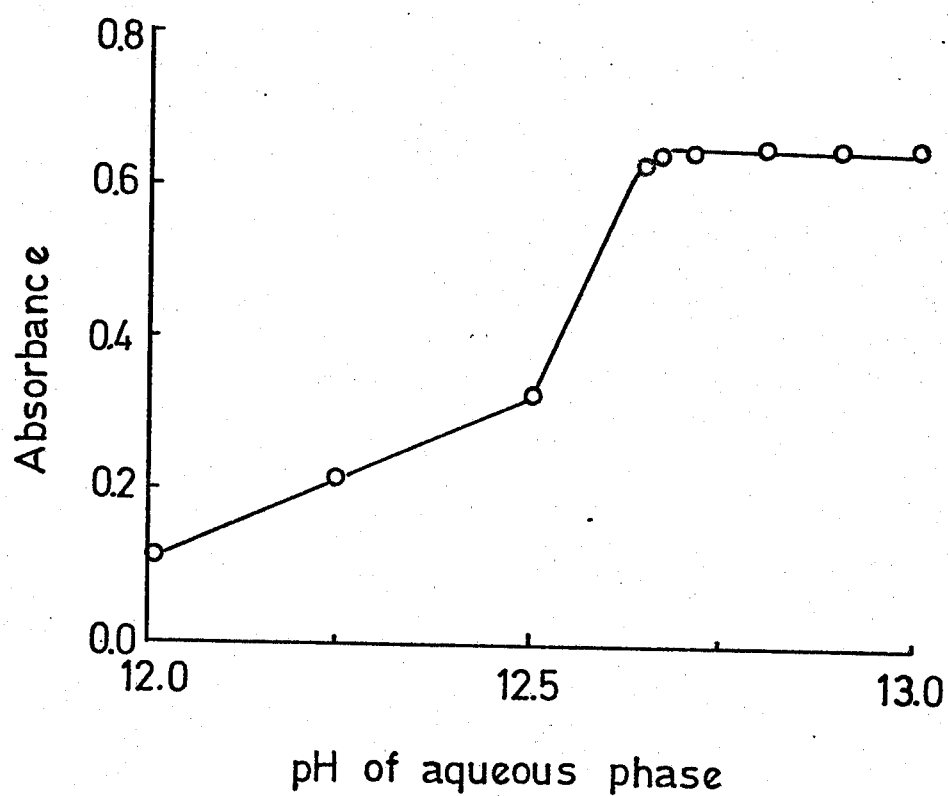


Fig. I-4. Effect of pH on the absorbance of Ca-GHA chelate.

Conditions are the same with those in Fig. I-3.

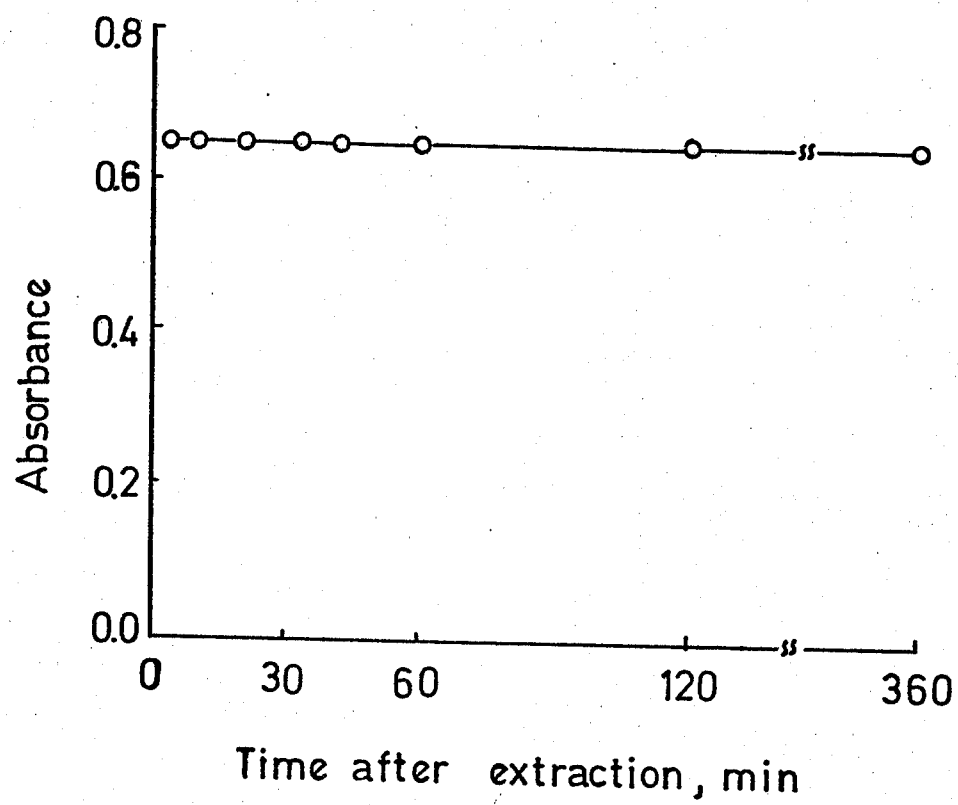


Fig. I-5. Stability of Ca-GHA chelate.

Conditions are the same with those in Fig. I-3.

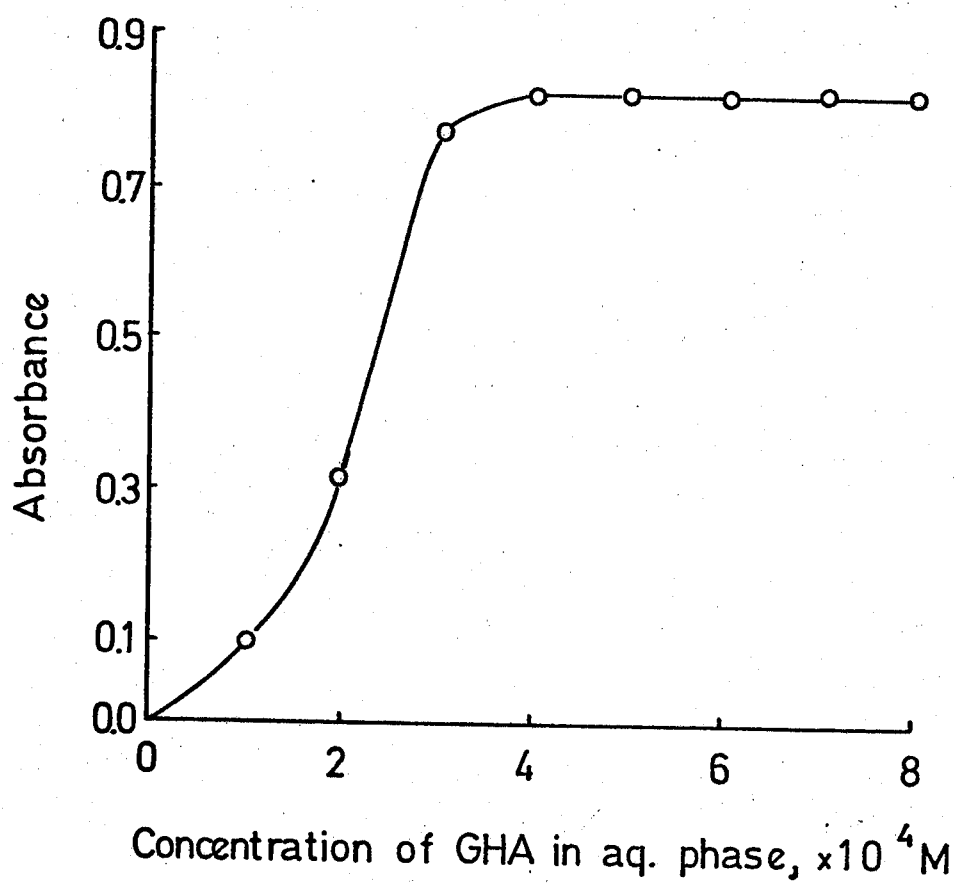


Fig. I-6. Effect of concentration of GHA on extraction of Ca-GHA chelate.

$[\text{Ca}]_{\text{aq}} = 5 \times 10^{-5} \text{ M.}$

The others are the same with those in Fig. I-3.

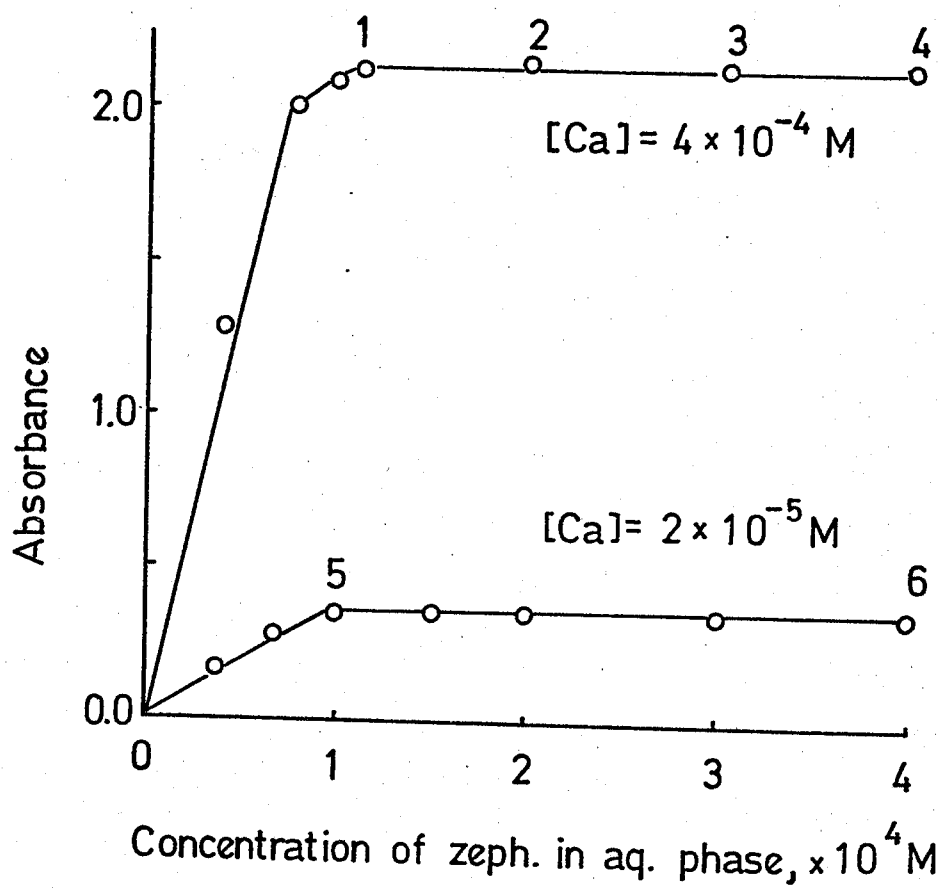


Fig. I-7. Effect of concentration of zephiramine on extraction of Ca-GHA chelate.

The number on the lines shows the $[zeph]/[Ca]$ ratio; (1) 0.25, (2) 0.5, (3) 1.0, (4) 1.5, (5) 5.0 and (6) 20. $[GHA]_{aq} = 7.5 \times 10^{-3}$ M, and $V_{org} = 50$ ml.

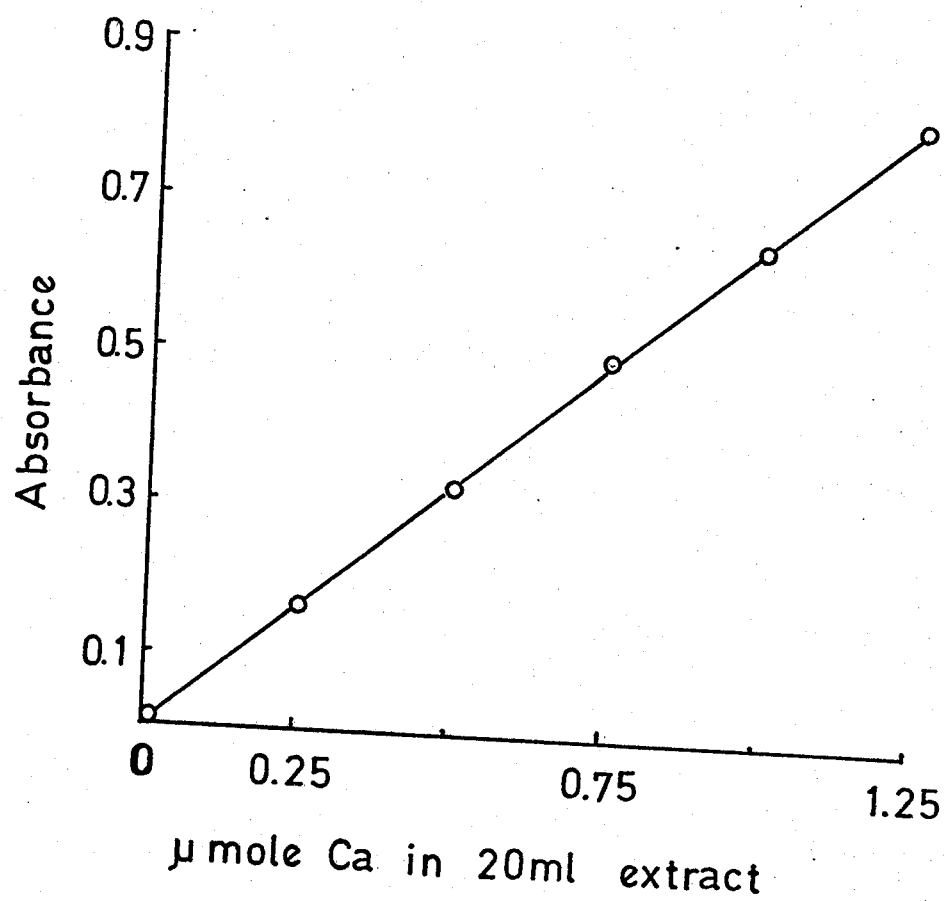


Fig. I-8. Calibration curve for calcium.
Conditions are the same with those in
Fig. I-3.

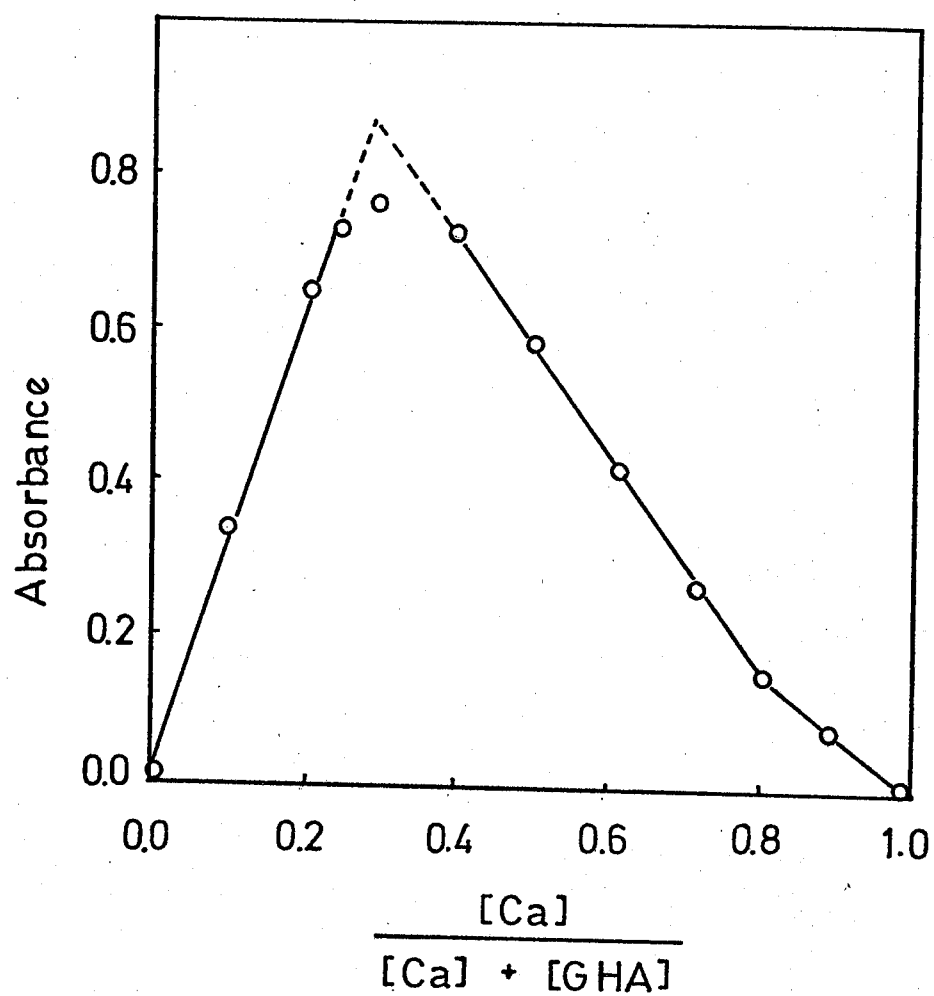


Fig. I-9. Continuous variations method for Ca-GHA chelate.

$$[\text{Ca}]_{\text{aq}} + [\text{GHA}]_{\text{aq}} = 2 \times 10^{-4} \text{ M.}$$

The others are the same with those in Fig. I-3.

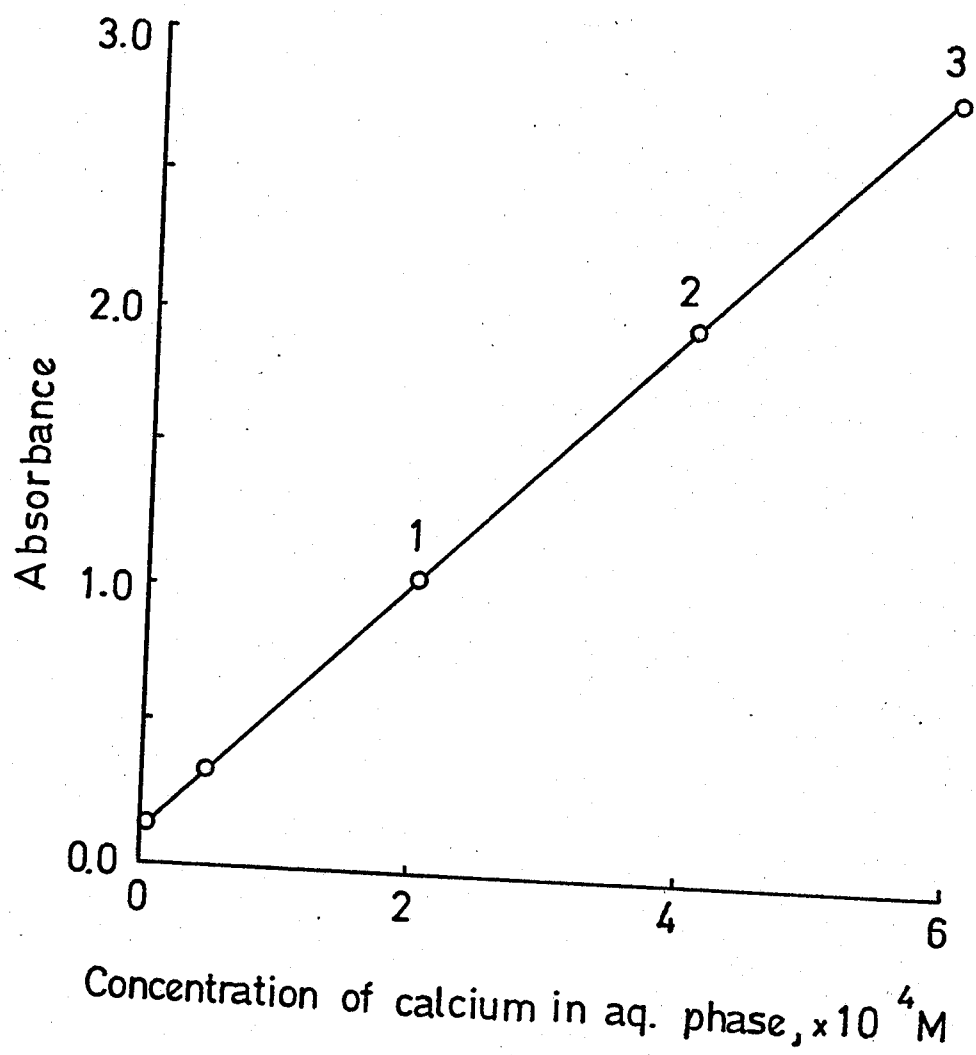


Fig. I-10. Absorbance of Ca-GHA chelate at a constant concentration of zephiramine.

The number on the line shows the $[\text{zeph}]/[\text{Ca}]$ ratio; (1) 1.0, (2) 0.5, and (3) 0.3.
 $[\text{GHA}]_{\text{aq}} = 5 \times 10^{-3}$ M, $[\text{zeph}]_{\text{aq}} = 2 \times 10^{-4}$ M,
 and $V_{\text{org}} = 50$ ml.

3-2. SAPH chelate

o-(Salicylidene-amino)phenol(SAPH), a tridentate ligand, has two hydroxy groups as "teeth" and a similar structure to GHA. SAPH (expressed as H_2A) forms a nonextractable $M(A)$ chelate with divalent cations such as cobalt, manganese, nickel and zinc^{22,23}. SAPH and its chelates are so unstable²² that the reagent is hardly applicable to the colorimetric determination of these metals.

The addition of zephiramine, however, changes the nonextractable nickel-or zinc-SAPH chelate to an extractable one, and increases the color-stability of the chelate as well.

3-2-1. Absorption spectra

Nickel

Fig.I-11 shows the absorption spectrum of nickel-SAPH chelate against the reagent blank extracted into 1,2-dichloroethane in the presence of zephiramine. The extracted chelate is yellow-colored and has an absorption maximum at 440 nm. No shift in the maximum absorption wavelength was observed when the pH value of the aqueous phase was varied from 9.0 to 12.5. In these experiments, the absorptions were measured at 440 nm using the reagent blank as a reference.

Zinc

The ordinarily nonextractable zinc-SAPH chelate also changed in the presence of zephiramine to an extractable one, showing an absorption maximum at 425 nm (Fig.I-12).

3-2-2. Conditions for extraction

Nickel

The effect of pH on the absorbance was examined and constant absorbance readings were obtained in the pH range of 10 - 11.5. pH 11.0 was chosen for subsequent experiments. The absorbance decrease slightly for a few minutes, but after this time no change in the absorbance at 440 nm was observed for at least 1-hour. Shaking times of 1 to 5 min. gave the identical results.

Because the SAPH solution was made using ethyl alcohol, the effects of varying concentrations of ethyl alcohol and SAPH were investigated. Constant readings were obtained in the range of $(1 - 2.5) \times 10^{-4}$ M of SAPH (Fig.I-13) and 0.43 - 0.86 M of ethyl alcohol in the aqueous phase.

The effect of the amount of zephiramine on the absorbance is shown in Fig.I-14. No change in the absorbance was seen in the range of $(2 - 6) \times 10^{-4}$ M of zephiramine.

Beer's law held for 0 - 25 μ moles of nickel, and the molar absorption coefficient at 440 nm was 2.4×10^4 (Fig.I-15).

Zinc

A constant absorbance was obtained in the pH range of 9.5 - 11.3, with concentrations of SAPH more than 4 times (Fig.I-16) and of zephiramine more than 6 times that of zinc (Fig.I-17). The absorbance decreased slightly for a few minutes immediately after shaking, and thereafter the color was stable for at least 1 hour.

A linear relationship was obtained up to at least 50 μ moles

of zinc between the concentration of zinc and the absorbance (Fig.I-18). The molar absorption coefficient at 425 nm is 8.4×10^3 .

3-2-3. Composition of the extracted chelates

Nickel

The chelate of nickel with SAPH is nonextractable and very unstable in the absence of zephiramine. In the presence of zephiramine, however, the nonextractable chelate changes to an extractable chelate, and its composition was investigated by the continuous variations method. As shown in Figs.I-19 and 20, the nickel-SAPH ratio in the extract was found to be 1 : 2, and the nickel-zephiramine ratio was also 1 : 2. Thus, the chelate extracted in the presence of zephiramine must have composition ratios of 1 : 2 : 2 (Ni : SAPH : zeph).

Zinc

The composition of zinc-SAPH chelate extracted into 1,2-dichloroethane was also investigated by the continuous variations method (Figs.I-21 and 22), and it was found that the chelate extracted in the presence of zephiramine has compositional ratio of 1 : 2 : 2 (Zn : SAPH : zeph).

Although nickel and zinc chelates usually give a 1 : 1 chelate, they change to 1 : 2 chelates in the presence of zephiramine. Thus, it is assumed that the 1 : 2 chelate has two negative charges as a result of higher chelation, and then the charged chelate couples with two molecules of zephiramine, the product being extracted into organic phase

as $\text{Ni(A)}_2(\text{zeph})_2$ or $\text{Zn(A)}_2(\text{zeph})_2$. Since this chelate contains no water molecules, its color is stable.

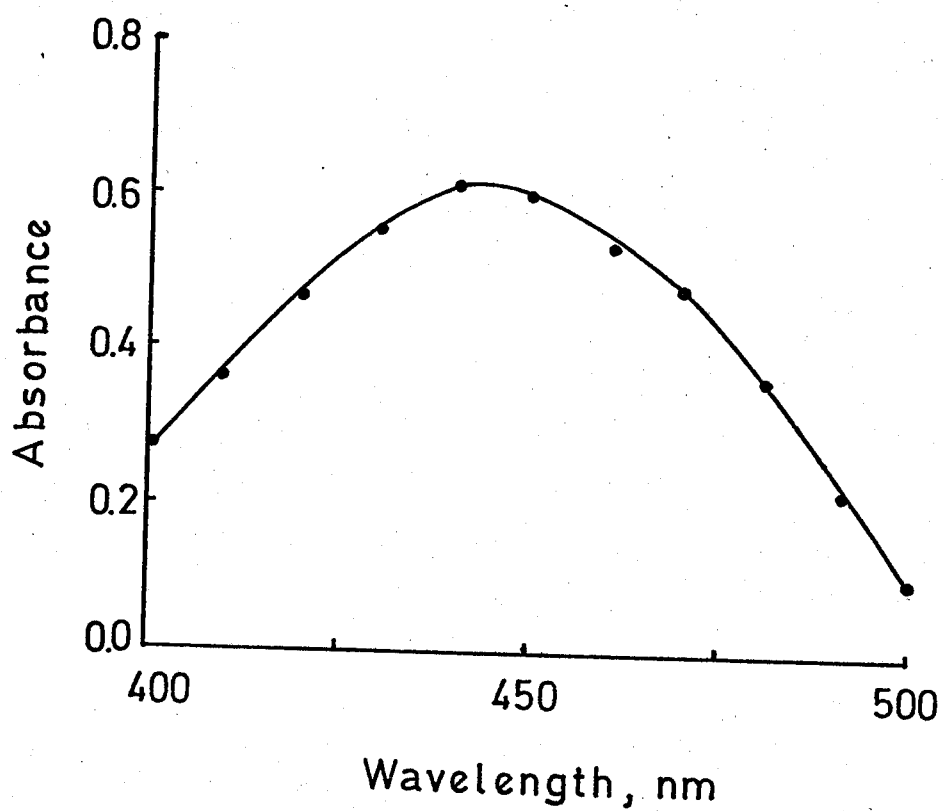


Fig. I-11. Absorption spectrum of Ni-SAPH chelate in the presence of zephiramine.

$[\text{Ni}]_{\text{aq}} = 2.5 \times 10^{-5} \text{ M}$, $[\text{SAPH}]_{\text{aq}} = 1.25 \times 10^{-4} \text{ M}$, $[\text{zeph}]_{\text{aq}} = 2.5 \times 10^{-4} \text{ M}$, and $\text{pH} = 11.0$.

$V_{\text{org}} = V_{\text{aq}} = 20 \text{ ml}$.

Reference: Reagent blank.

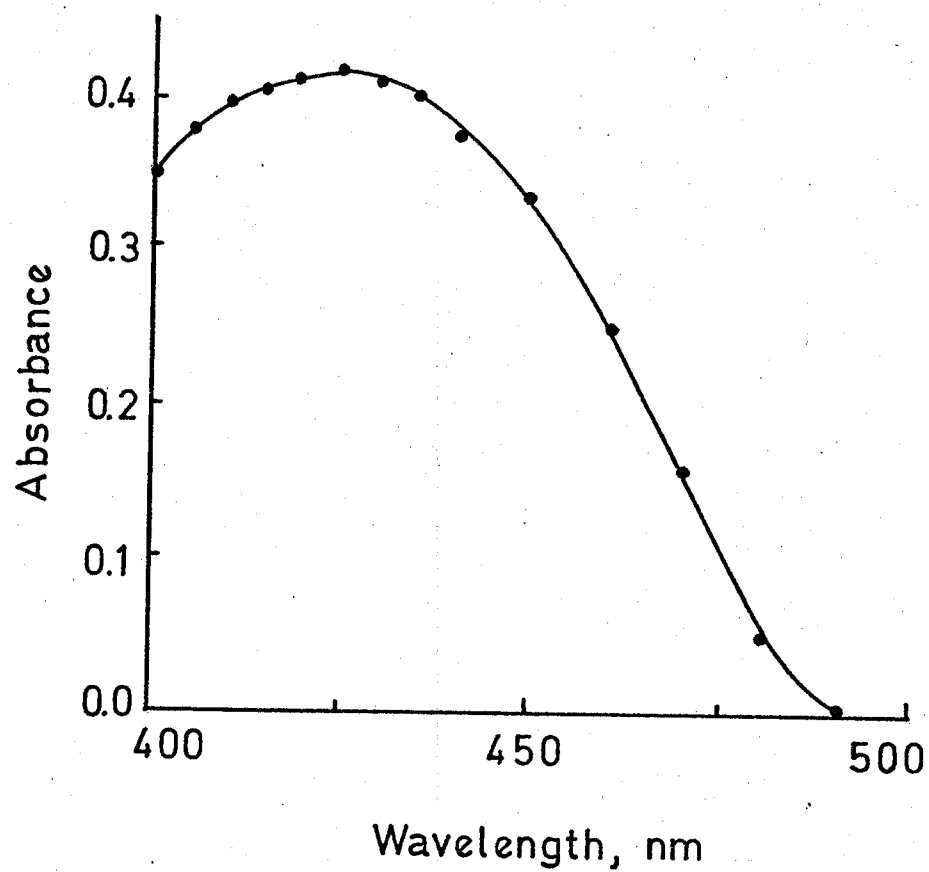


Fig. I-12. Absorption spectrum of Zn-SAPH chelate in the presence of zephiramine.

$[\text{Zn}]_{\text{aq}} = 5 \times 10^{-5} \text{ M}$, $[\text{SAPH}]_{\text{aq}} = 2.5 \times 10^{-4} \text{ M}$, $[\text{zeph}]_{\text{aq}} = 2.5 \times 10^{-4} \text{ M}$, and $\text{pH} = 11.0$.

$V_{\text{org}} = V_{\text{aq}} = 20 \text{ ml}$.

Reference: Reagent blank.

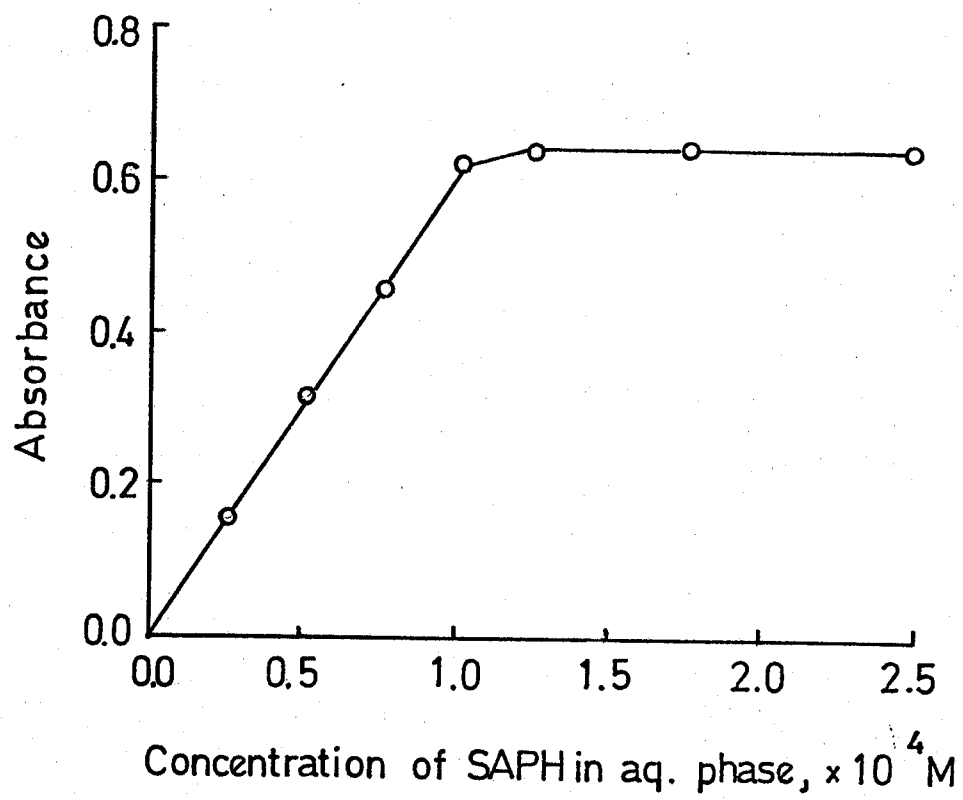


Fig. I-13. Effect of concentration of SAPH on extraction of Ni-SAPH chelate.

Conditions are the same with those in Fig. I-11.

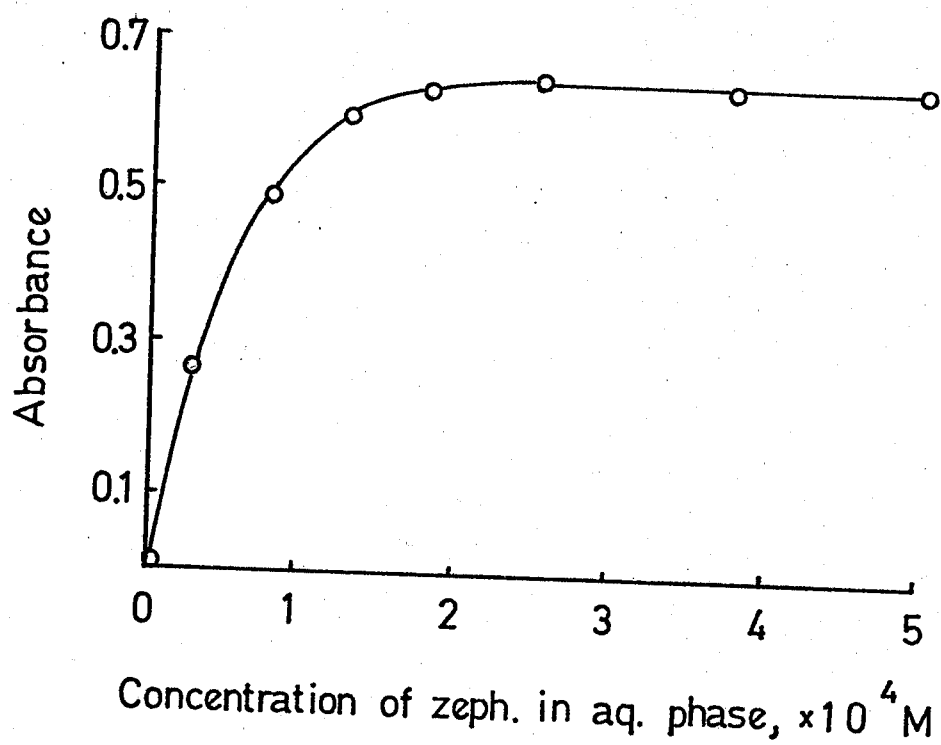


Fig. I-14. Effect of concentration of zephiramine on extraction of Ni-SAPH chelate.

Conditions are the same with those in Fig. I-11.

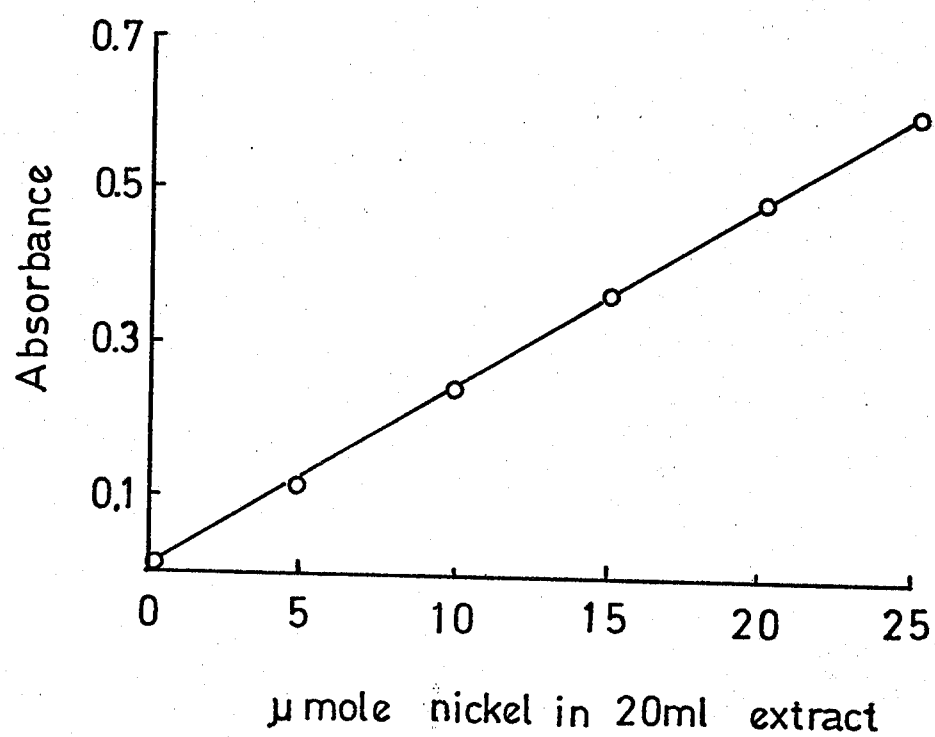


Fig. I-15. Calibration curve for nickel.

Conditions are the same with those in Fig. I-11.

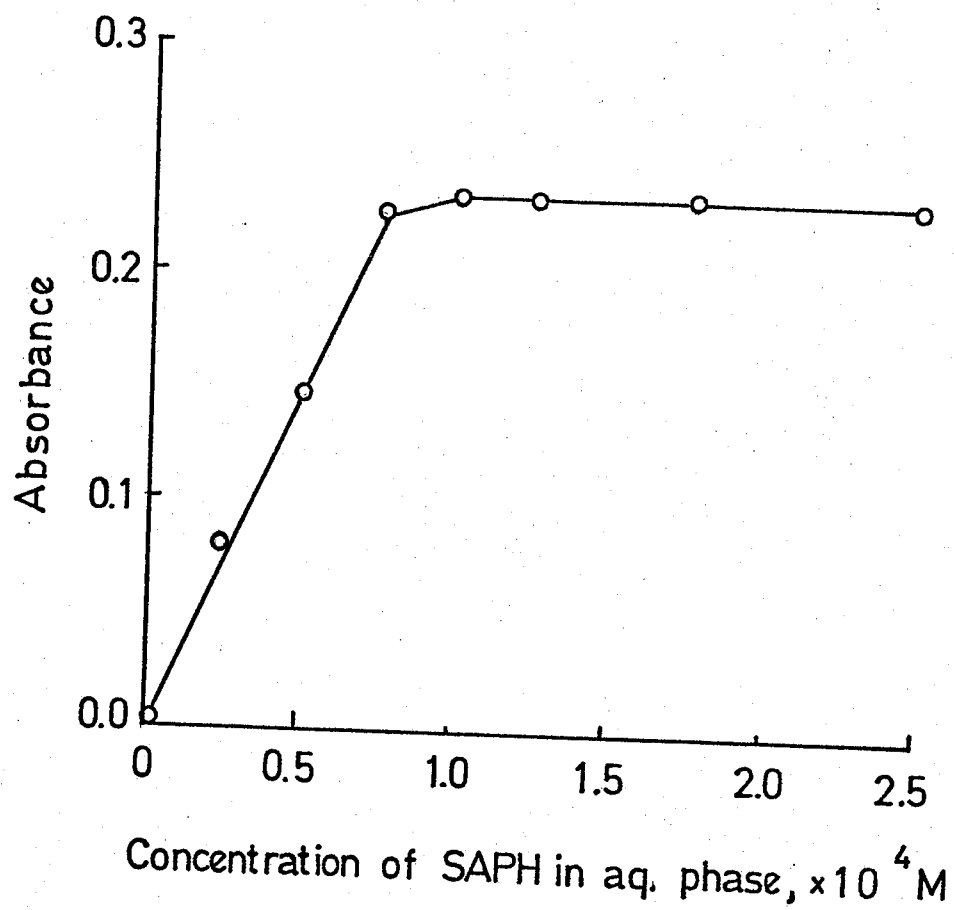


Fig. I-16. Effect of concentration of SAPH on extraction of Zn-SAPH chelate.

$[\text{Zn}]_{\text{aq}} = 2.5 \times 10^{-5} \text{ M.}$

The others are the same with those in Fig. I-12.

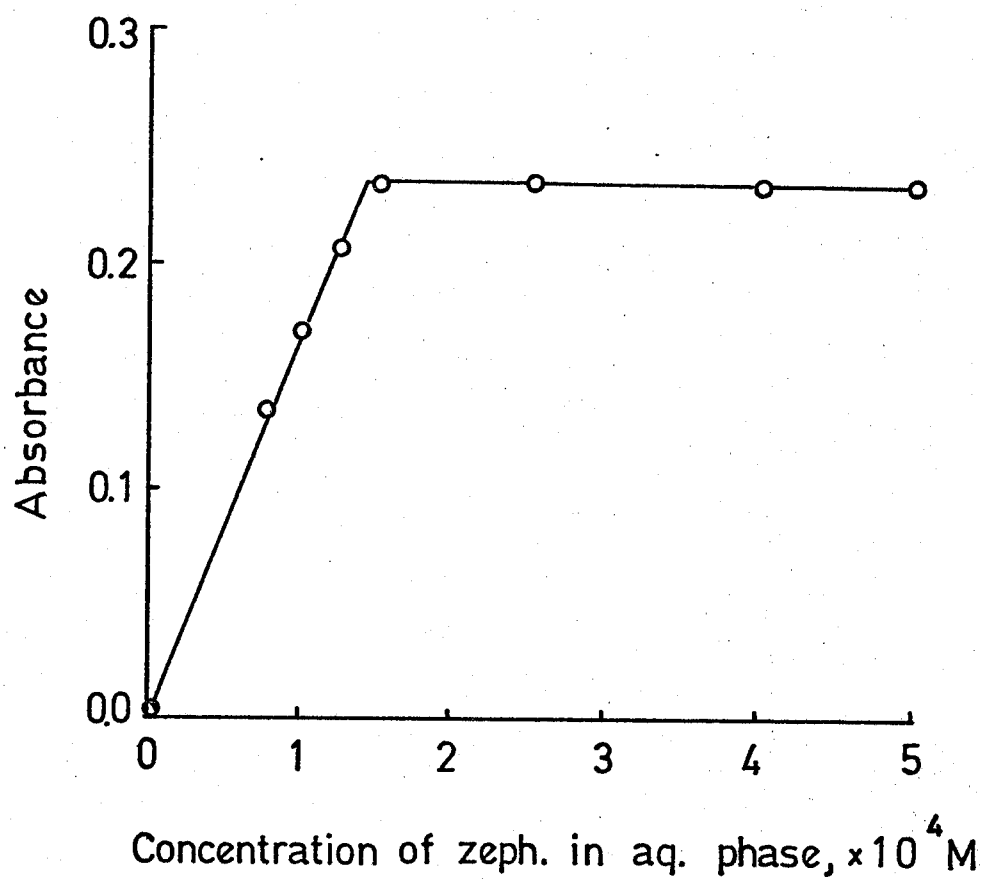


Fig. I-17. Effect of concentration of zephiramine on extraction of Zn-SAPH chelate.

$[\text{Zn}]_{\text{aq}} = 2.5 \times 10^{-5} \text{ M.}$

The others are the same with those in Fig. I-12.

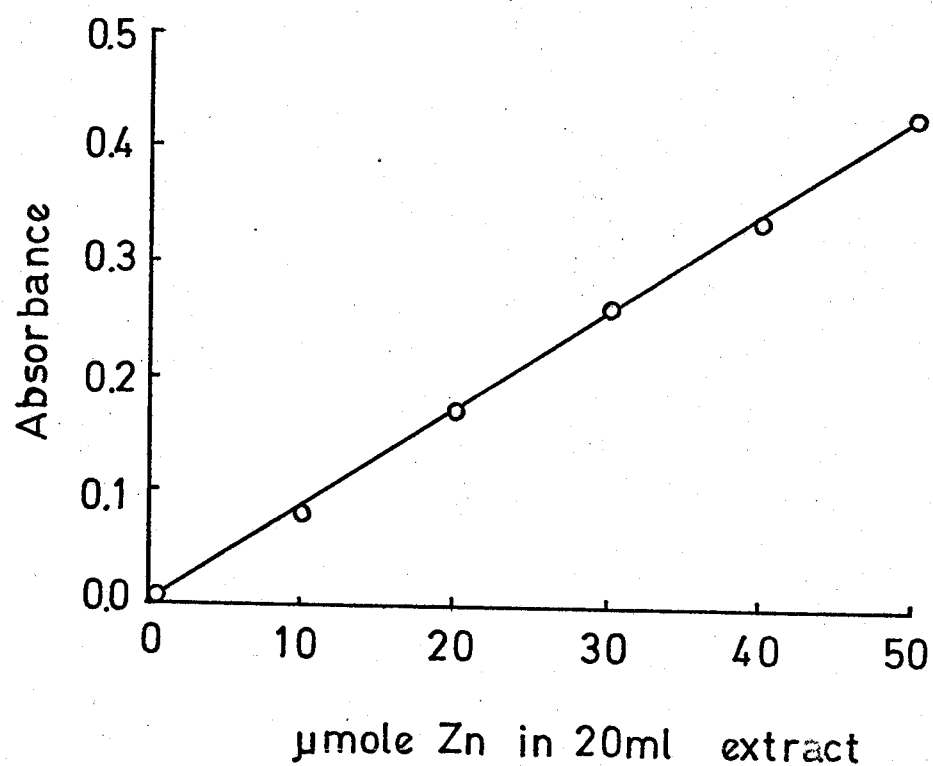


Fig. I-18. Calibration curve for zinc.

Conditions are the same with those in Fig. I-12.

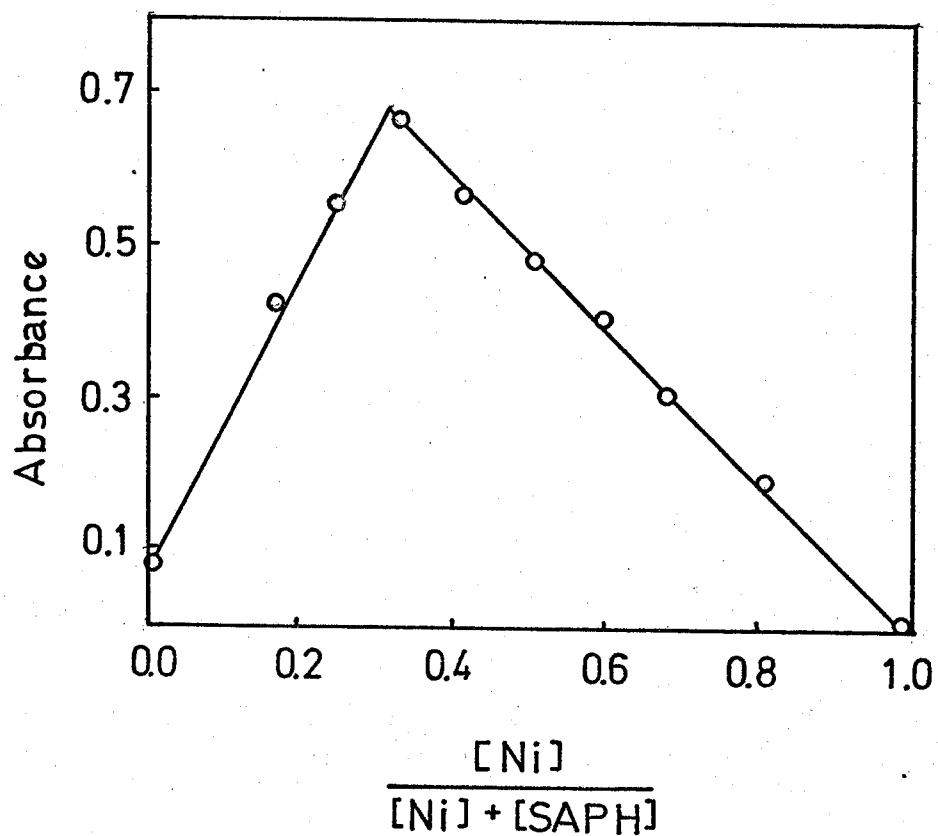


Fig. I-19. Nickel to SAPH ratio in the presence of zephiramine.

$$[Ni]_{aq} + [SAPH]_{aq} = 1.35 \times 10^{-4} \text{ M},$$

$$[zeph]_{aq} = 9.0 \times 10^{-5} \text{ M}.$$

The others are the same with those in Fig. I-11.

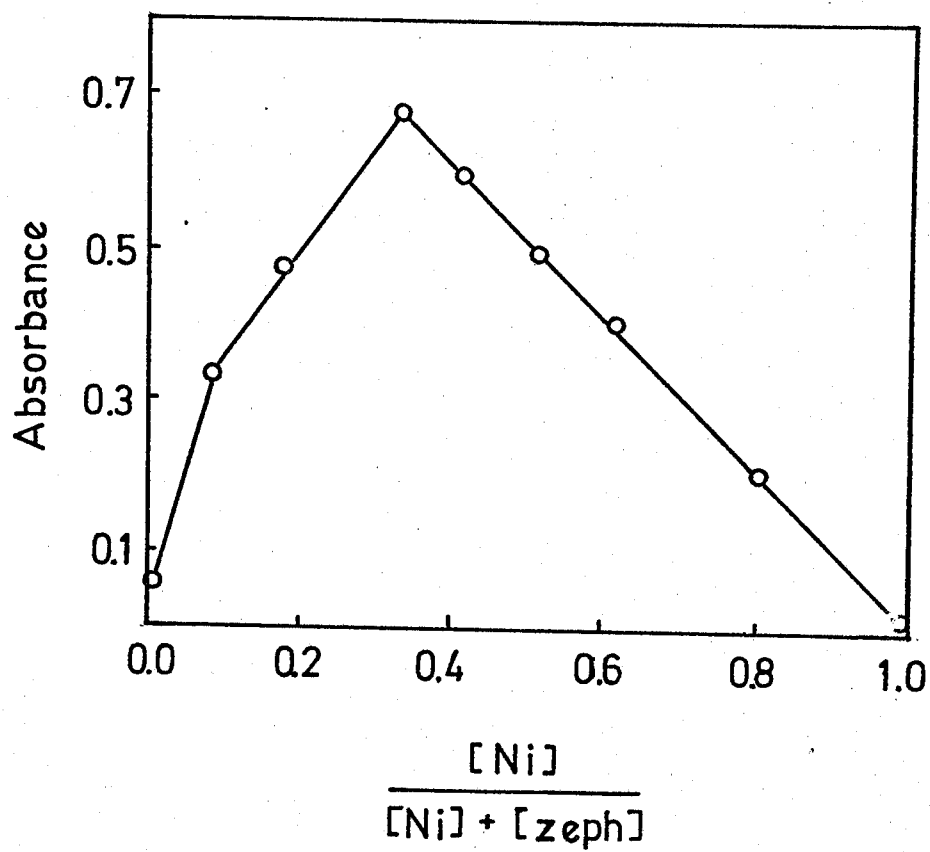


Fig. I-20. Nickel to zephiramine ratio in the Ni-SAPH chelate.

$$[Ni]_{aq} + [zeph]_{aq} = 1.35 \times 10^{-4} \text{ M},$$

$$[SAPH]_{aq} = 9.0 \times 10^{-4} \text{ M}.$$

The others are the same with those in Fig. I-11.

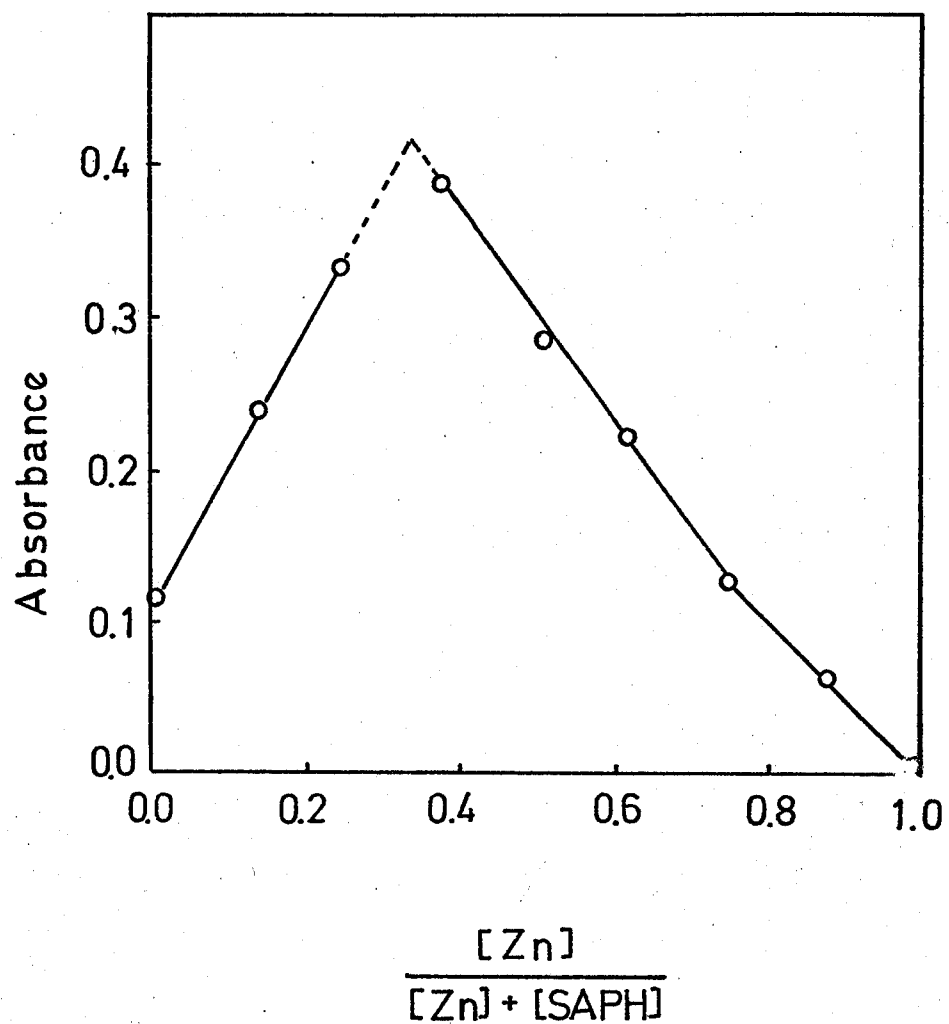


Fig. I-21. Zinc to SAPH ratio in the presence of zephiramine.

$[Zn]_{aq} + [SAPH]_{aq} = 1.0 \times 10^{-4} \text{ M.}$

The others are the same with those in Fig. I-12.

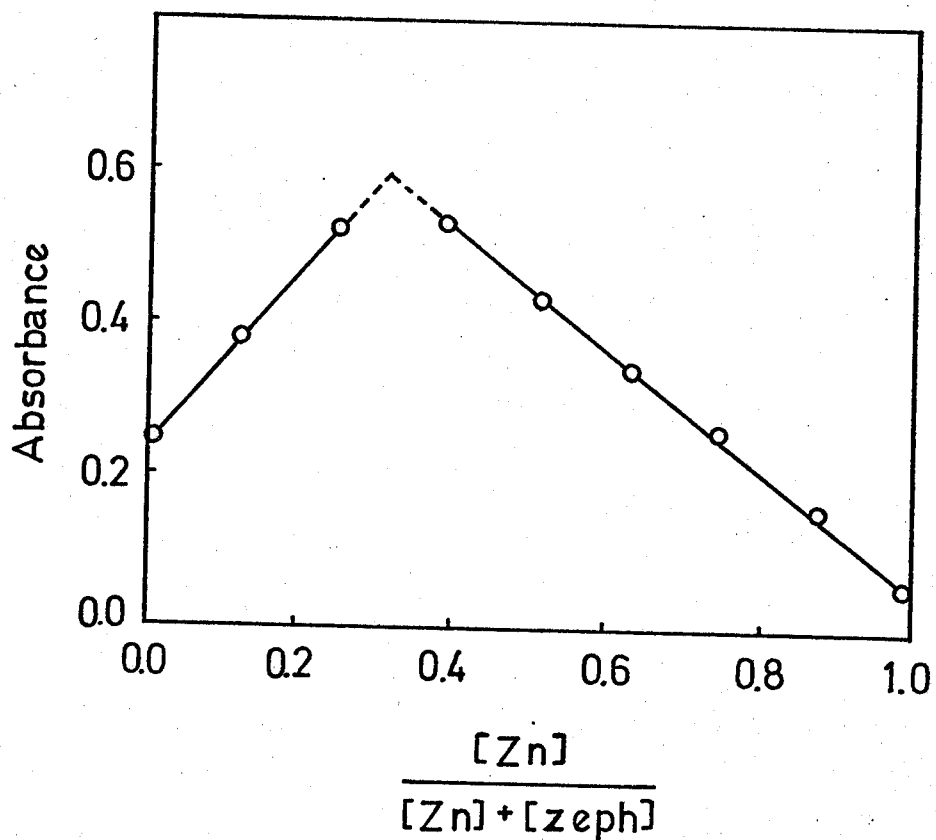


Fig. I-22. Zinc to zephiramine ratio in the Zn-SAPH chelate.

$$[Zn]_{aq} + [zeph]_{aq} = 1.0 \times 10^{-4} \text{ M},$$

$$[SAPH]_{aq} = 4.0 \times 10^{-4} \text{ M}.$$

The others are the same with those in Fig. I-12.

3-3. TTA chelate

Thenolytrifluoroacetone (TTA) is a bidentate ligand and forms valence-saturated (for the central atom) but coordination-unsaturated chelates, $M(TTA)_2 \cdot 2H_2O$, with nickel and cobalt(II). The chelates can be extracted into some oxygen-containing solvents such as alcohols and ketones,²⁴⁻²⁶ or into inactive solvents such as benzene and 1,2-dichloroethane by means of synergism.^{4,6} Such chelates, however, are rarely extracted directly into inactive solvents because of water molecule interference.

Nickel-and cobalt-TTA chelates are also extracted into 1,2-dichloroethane, an inactive organic solvent, in the presence of zephiramine or cetyltrimethyl ammonium bromide (CTMAB).

3-3-1. Absorption spectra

The absorption spectra of Ni-and Co-TTA chelates formed in the presence of the quaternary ammonium base were measured using water as a reference (Fig.I-23). As seen in Fig.I-23, the effects of zephiramine and CTMAB on the extraction are the same for nickel and cobalt. Although the spectra have no peaks, the wavelength of 420 nm was used hereafter where the reagent blank showed no absorption.

3-3-2. Conditions for extraction

The effect of pH on the extractions of nickel-and cobalt-TTA chelates in the presence of a quaternary ammonium base

was examined by changing the pH of the solutions from 4 to 9. Since constant and maximum absorbances at 420 nm were obtained in the pH range from 5 to 9 for nickel and cobalt, pH 7.0 was chosen for further experiments. Shaking times from 2 to 10 min. had the same effect on the extraction efficiency; the extracted chelates were stable for at least 1 hr. The molar concentration of TTA more than 10 times to nickel and 6 times to cobalt gave a constant absorbance, respectively.

The presence of quaternary ammonium bases produced irregular patterns of absorbance (Figs. I-24 and 25) caused by turbidity in the 1,2-dichloroethane phase; clear solutions with constant absorbances were observed for molar concentration ratios of quaternary ammonium base more than one, for nickel and cobalt.

3-3-3. Composition of the extracted chelate

The metal-TTA molar ratios of the chelates extracted in the presence of the quaternary ammonium bases were determined by the continuous variations method (Figs. I-26 and 27) and the equilibrium-shift method. Both methods show that the nickel- and cobalt-chelates have the composition of metal : TTA = 1 : 3. The ordinary $M(TTA)_2 \cdot 2H_2O$ chelates change to the coordination-saturated chelate, $M(TTA)_3$, two molecules of water having been expelled by the action of quaternary ammonium bases. It is reasonable to assume that the $M(TTA)_3$ chelates have a minus one charge resulting from higher chelation; as a result the chelates are extracted into the inactive solvent

after coupling with a positively charged quaternary ammonium base. Figs. I-24 and 25 show that the 1 : 1 molar ratio of quaternary ammonium bases to the metal is sufficient for the complete extraction. Thus, it is suggested that extracted chelates have the compositions of $\text{Ni}(\text{TTA})_3(\text{zephiramine})$ and $\text{Co}(\text{TTA})_3(\text{CTMAB})$, respectively.

Quaternary ammonium base has two effects on the extraction: one is to form a highly coordinated chelate, and the other is to couple with the formed highly coordinated chelate.

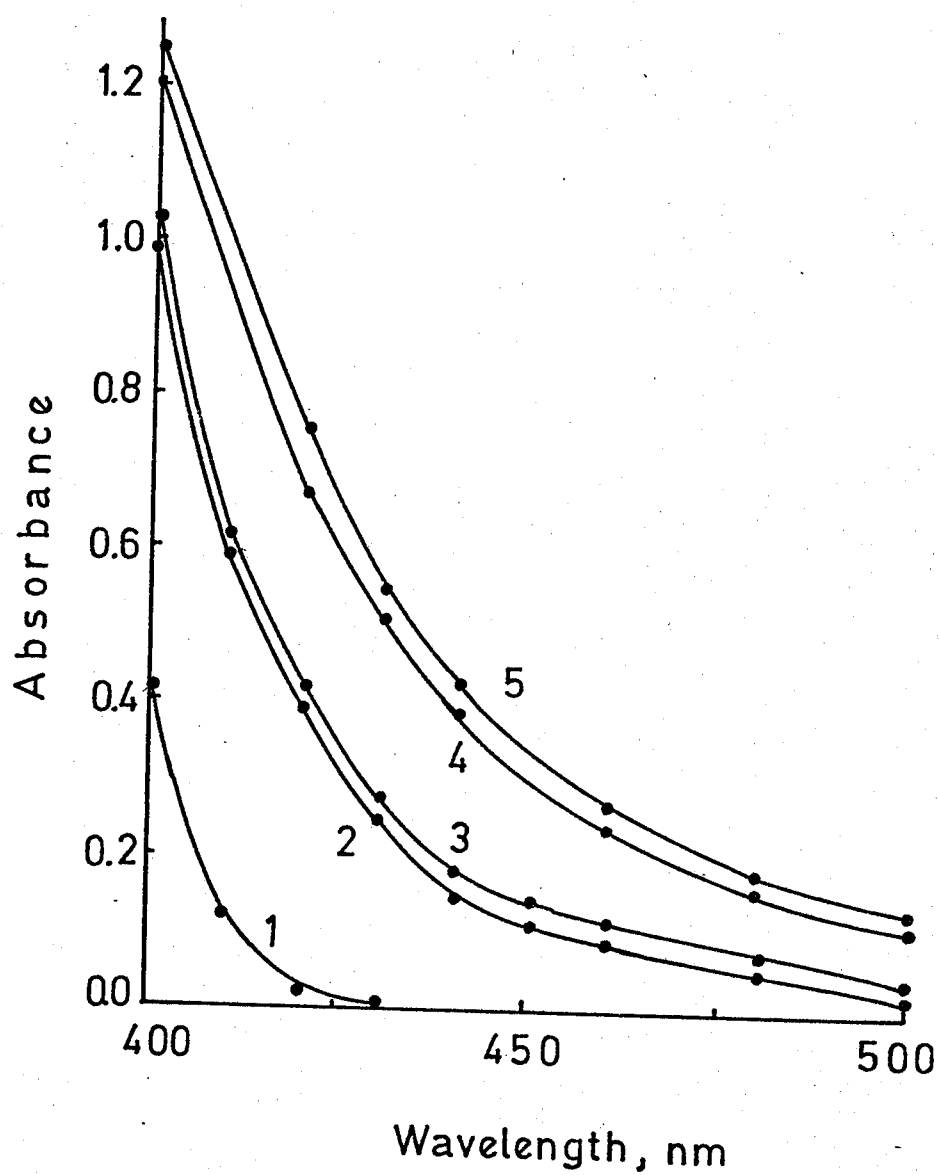


Fig. I-23. Absorption spectra of TTA chelates.

$[\text{metal}]_{\text{aq}} = 1.25 \times 10^{-4} \text{ M}$, $[\text{TTA}]_{\text{org}} = 5 \times 10^{-3} \text{ M}$, $[\text{Q.A.B.}]_{\text{aq}} = 5 \times 10^{-4} \text{ M}$, and $\text{pH} = 7.0$.

(1) Reagent blank, (2) Ni with CTMAB,
(3) Ni with zeph, (4) Co with CTMAB, and
(5) Co with zeph.

$V_{\text{org}} = 10 \text{ ml}$ and $V_{\text{aq}} = 20 \text{ ml}$.

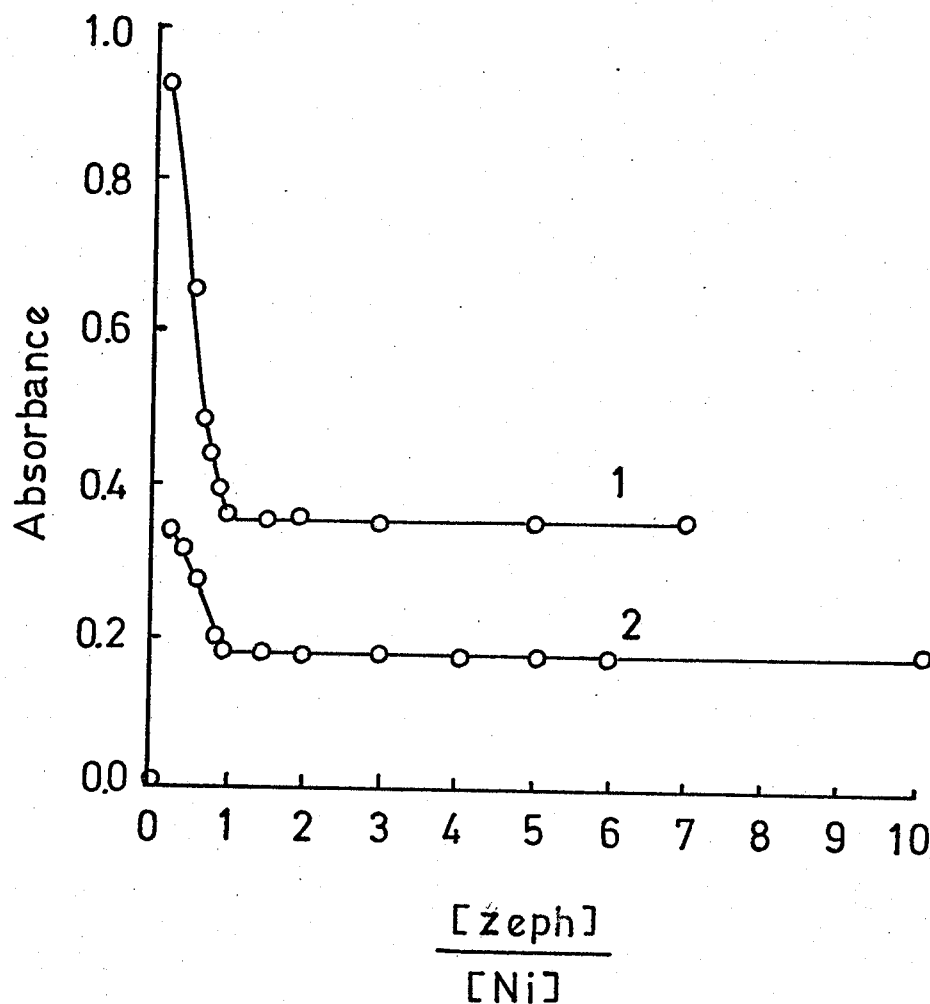


Fig. I-24. Effect of zephiramine on Ni-TTA chelate.

$[\text{TTA}]_{\text{org}} = 5 \times 10^{-3} \text{ M}$, and $\text{pH} = 7.0$.

(1) $[\text{Ni}]_{\text{aq}} = 2.5 \times 10^{-4} \text{ M}$, and (2) $[\text{Ni}]_{\text{aq}} = 1.25 \times 10^{-4} \text{ M}$.

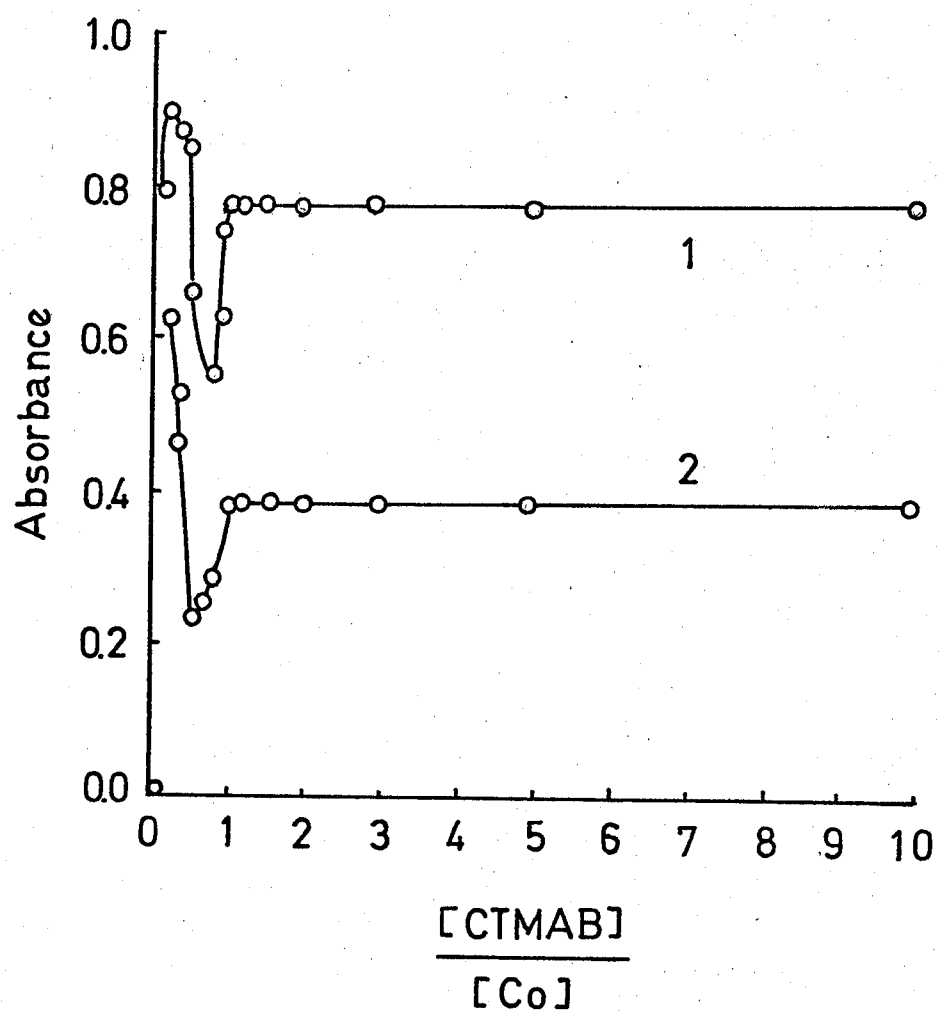


Fig. I-25. Effect of CTMAB on Co-TTA chelate.

$[\text{TTA}]_{\text{org}} = 5 \times 10^{-3} \text{ M}$, and $\text{pH} = 7.0$.

(1) $[\text{Co}]_{\text{aq}} = 2.5 \times 10^{-4} \text{ M}$, and (2) $[\text{Co}]_{\text{aq}} = 1.25 \times 10^{-4} \text{ M}$.

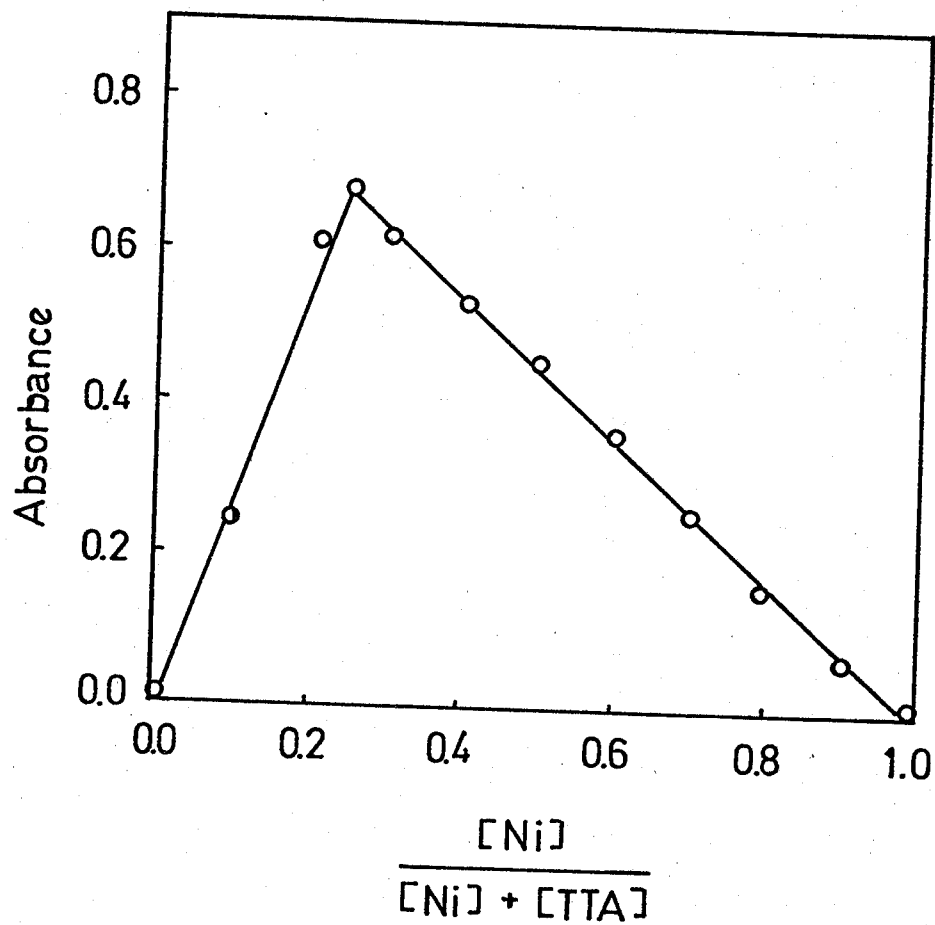


Fig. I-26. Continuous variations method for Ni-TTA chelate in the presence of zephiramine.

$$[\text{Ni}]_{\text{aq}} + [\text{TTA}]_{\text{org}} = 2.5 \times 10^{-3} \text{ M},$$

$$[\text{zeph}]_{\text{aq}} = 7.5 \times 10^{-4} \text{ M}, \text{ and } \text{pH} = 7.0.$$

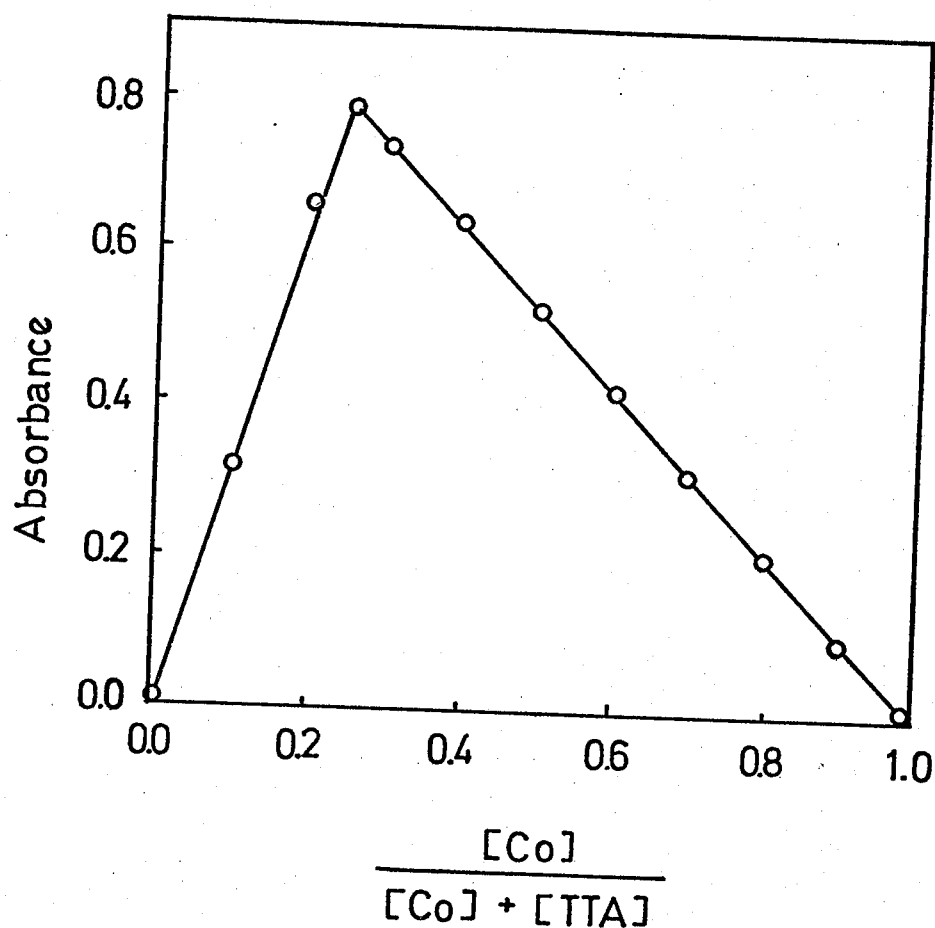


Fig. I-27. Continuous variations method for Co-TTA chelate in the presence of CTMAB.

$$[Co]_{aq} + [TTA]_{org} = 2.5 \times 10^{-3} \text{ M},$$

$$[CTMAB]_{aq} = 7.5 \times 10^{-4} \text{ M}, \text{ and } pH = 7.0.$$

3-4. Oxine chelate

8-Hydroxyquinoline(oxine) forms chelates with many divalent metals. Among these, magnesium oxinate is poorly extractable into inactive solvents such as benzene, 1,2-dichloroethane and nitrobenzene; this results from the formation of magnesium oxinate dihydrate, $\text{Mg}(\text{OX})_2 \cdot 2\text{H}_2\text{O}$, which has strongly coordinated water molecules. If the coordinated water molecules in the chelate are replaced by other neutral organic molecules, i.e. if the coordination is saturated, the chelate can be readily extracted into inactive solvents. For this purpose, synergic extractions in the presence of *n*-butylamine,²⁷ butylcellosolve^{28,29} and pyridine⁵ have been suggested.

In this section, it is shown that the magnesium-oxine chelate can also be quantitatively extracted into inactive solvents in the presence of zephiramine.

3-4-1. Absorption spectra

The absorption spectra of Mg-oxine chelate and the reagent blank extracted in the presence of zephiramine are shown in Fig.I-28. The magnesium chelate has an absorption maximum at 390 nm in the organic phase. All absorbance measurements were therefore carried out at 390 nm. The molar absorption coefficient at this wavelength is 6.5×10^3 .

3-4-2. Conditions for extraction

Effect of pH. The solvent extraction behavior of the

Mg-oxine system in the presence of zephiramine was studied over the pH range 8 to 10. Fig.I-29 shows the percentage extraction of Mg-oxine chelate into 1,2-dichloroethane as function of pH. Distribution ratio of magnesium in the extraction with various solvents are plotted as a function of pH in Fig.I-30.

As shown in Figs.I-29 and 30, the extraction begins to occur around pH 8.5 and becomes 100 % efficient at pH 9.5.

Effect of shaking time. Shaking time was varied from 1 min. to 30 min; a 5 minute shaking time was found to be optimum. The complex in the organic phase was optically stable for at least 1 hour.

Effect of oxine concentration. The concentration of oxine in the organic phase was varied from zero to 1.25×10^{-3} M, other factors being kept constant. Complete extraction was obtained using a concentrations higher than 0.75×10^{-3} M (Fig.I-31).

Effect of zephiramine concentration. In studies of the effect of zephiramine concentration, it was found that magnesium was completely extracted in the range of $(1.5 - 2.5) \times 10^{-3}$ M of zephiramine in the aqueous phase (Fig.I-32).

3-4-3. Composition of the extracted chelate

Composition of the extracted magnesium-oxine chelate in the presence of zephiramine was examined by the equilibrium-shift* and continuous variations method.

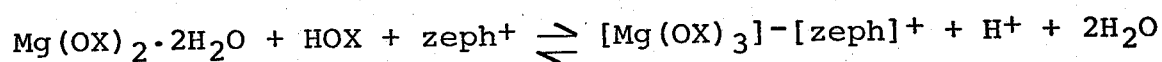
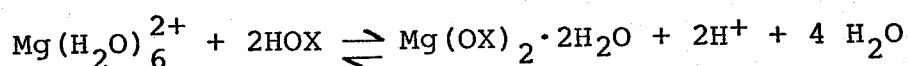
* See appendix.

The dependence of $\log D$, D being the distribution ratio, on pH and $\log[\text{oxine}]$ are shown in Figs. I-30 and 33. All these plots gave linear lines having a slope of three, indicating that the extracted magnesium chelate has a composition of $[\text{Mg}(\text{OX})_3]^-$. The results shown in Fig. I-34 reveal that the extracted chelate has a composition of $\text{Mg}(\text{OX})_3(\text{zeph})$.

3-4-4. Effect of organic solvent

By using nitrobenzene, 1,2-dichloroethane, chloroform and carbon tetrachloride, a chelate of the same composition was extracted, but the nature of organic solvent affected the extractability of the chelate.

From the above results, the following system is suggested for the extraction.



Then,

$$K_{\text{ex}} = \frac{[\text{Mg}(\text{OX})_3(\text{zeph})]_{\text{org}} [\text{H}^+]^3}{[\text{Mg}^{2+}] [\text{HOX}]_{\text{org}}^3 [\text{zeph}^+]}$$

is given.

For the extraction of ion-associated chelates, it has been generally said that the higher the dielectric constant of the organic solvent used, the greater the extractability. For the extractable magnesium-oxine chelate, the extraction constants (K_{ex}) and the dielectric constants of the organic solvents are listed in Table I-1 and their relationship is shown in Fig. I-35.

The relationship showed a good accordance with the general rule for extraction by ion-association. The best solvent was nitrobenzene and the second was 1,2-dichloroethane. As nitrobenzene was not good for having a bad smell, practically 1,2-dichloroethane was preferred.

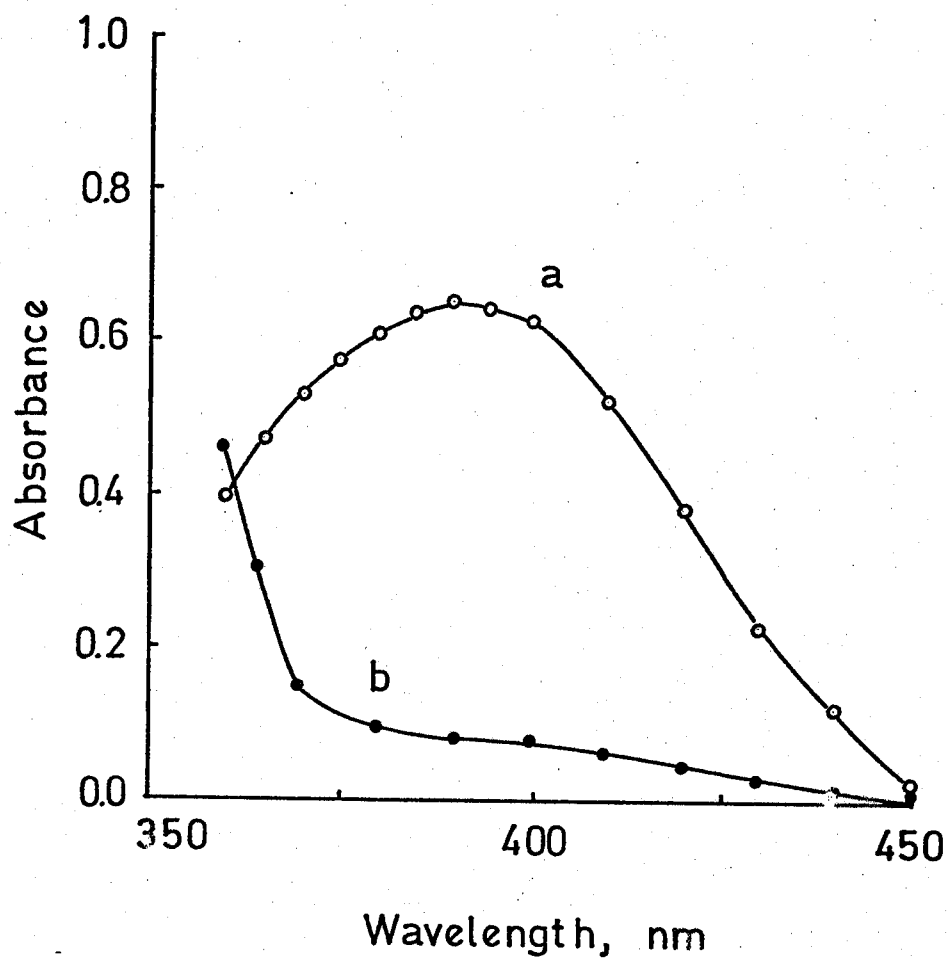


Fig. I-28. Absorption spectra of Mg-oxine chelate and reagent blank in the presence of zephiramine.

$[Mg]_{aq} = 5 \times 10^{-5} \text{ M}$, $[HOX]_{org} = 5 \times 10^{-3} \text{ M}$,
 $[zeph]_{aq} = 1 \times 10^{-4} \text{ M}$, and $pH = 11.0$.

(a) Mg-oxine-zephiramine chelate vs. reagent blank, (b) reagent blank vs. water.

$V_{aq} = 20 \text{ ml}$ and $V_{org} = 10 \text{ ml}$.

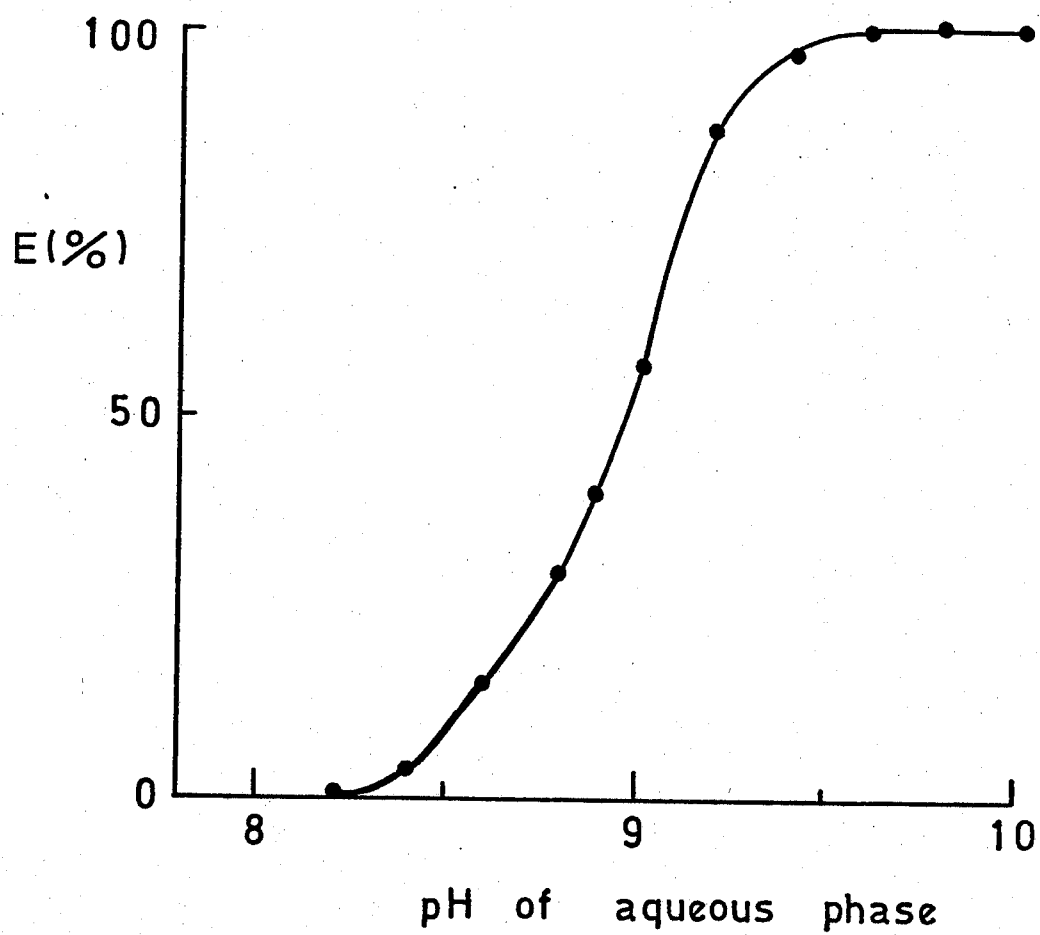


Fig. I-29. Effect of pH on extraction of magnesium oxinate.

The others are the same with those in Fig. I-28.

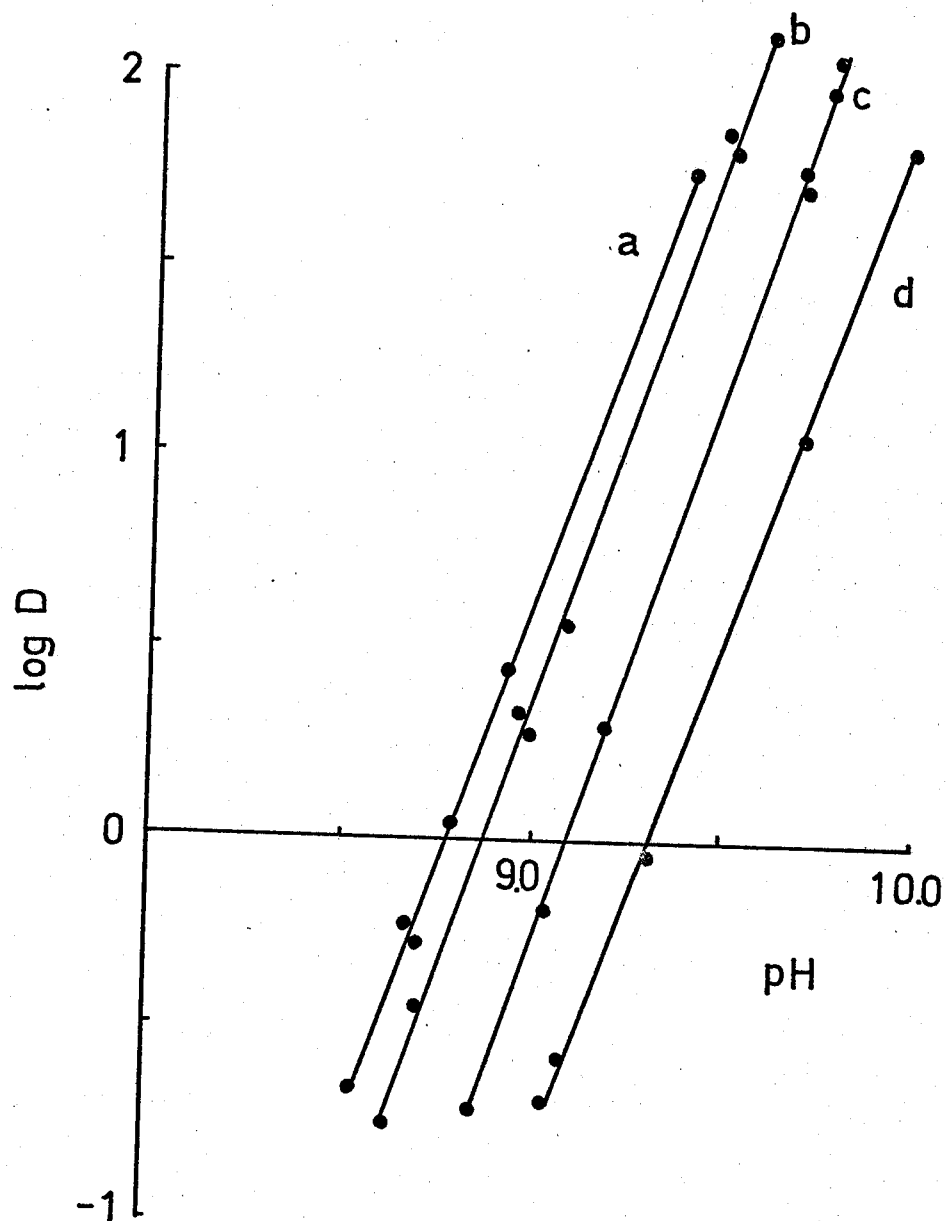


Fig. I-30. Dependence of $\log D$ on pH at extraction of Mg oxinate with various solvents in the presence of zephiramine.

$[Mg]_{aq} = 1 \times 10^{-4} \text{ M}$, $[HOX]_{org} = 1 \times 10^{-2} \text{ M}$,
and $[zeph]_{aq} = 1 \times 10^{-3} \text{ M}$.

(a) nitrobenzene, (b) 1,2-dichloroethane,
(c) chloroform, and (d) carbon tetrachloride.

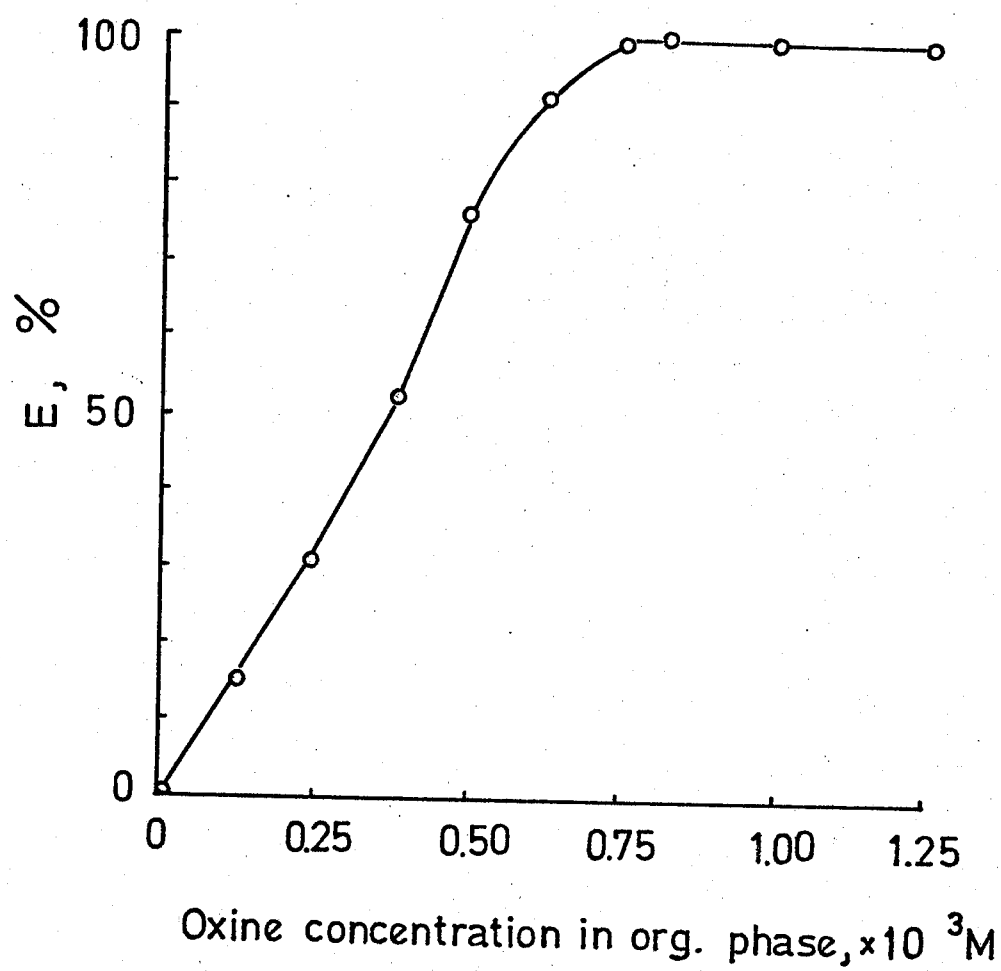


Fig. I-31. Effect of concentration of oxine on extraction of Mg-oxine chelate in the presence of zephiramine.

$[Mg]_{aq} = 2.5 \times 10^{-5}$ M.

The others are the same with those in Fig. I-28.

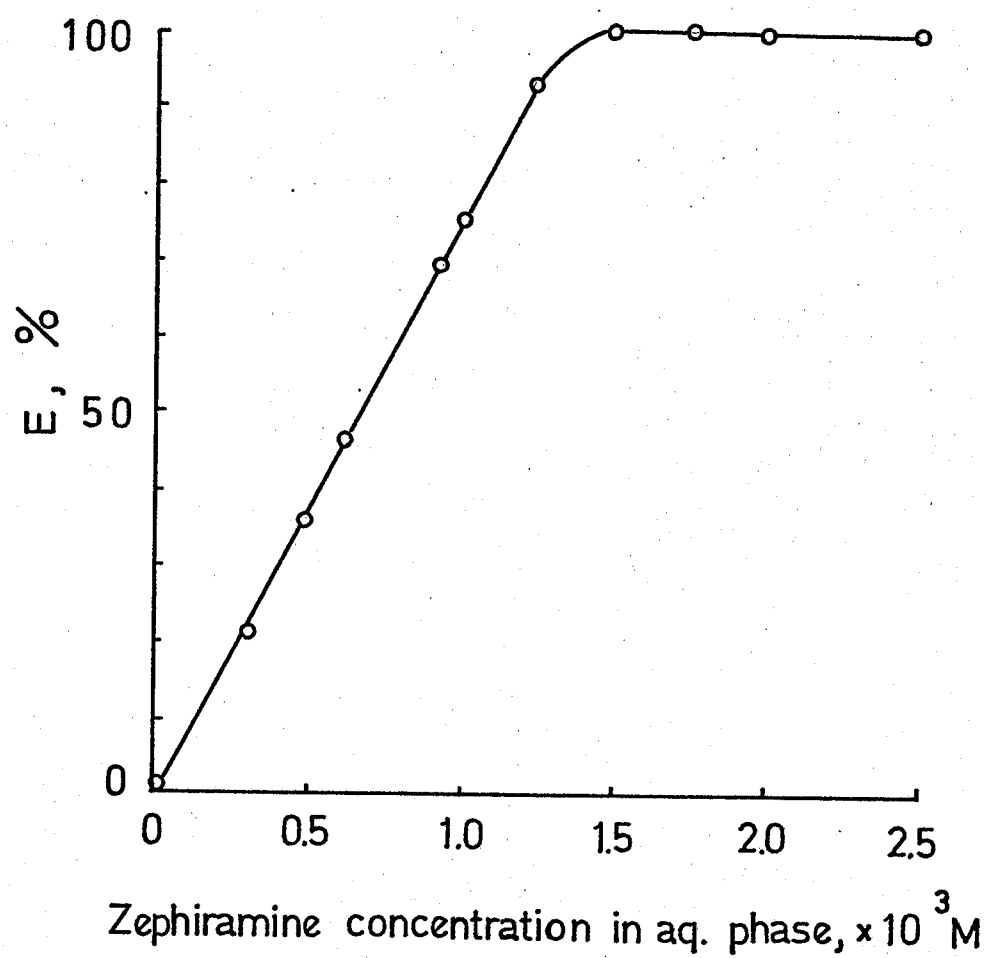


Fig. I-32. Effect of concentration of zephiramine on extraction of Mg-oxine chelate.

$[Mg]_{aq} = 2.5 \times 10^{-5}$ M.

The others are the same with those in Fig. I-28.

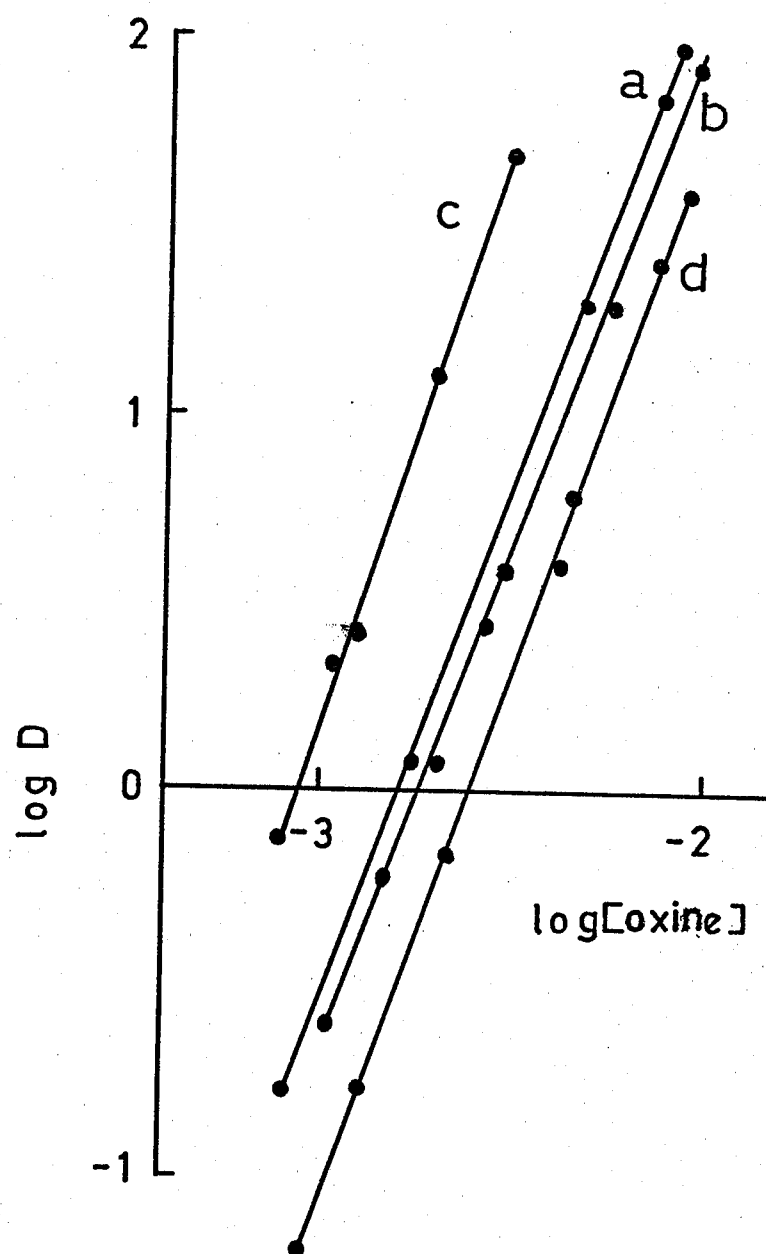


Fig. I-33. Dependence of $\log D$ on $\log [\text{oxine}]$ in the presence of zephiramine.

(a) nitrobenzene at $\text{pH} = 9.50$, (b) 1,2-dichloroethane at $\text{pH} = 9.50$, (c) chloroform at $\text{pH} = 10.05$ and (d) carbon tetrachloride at $\text{pH} = 9.80$.

The others are the same with those in Fig. I-28.

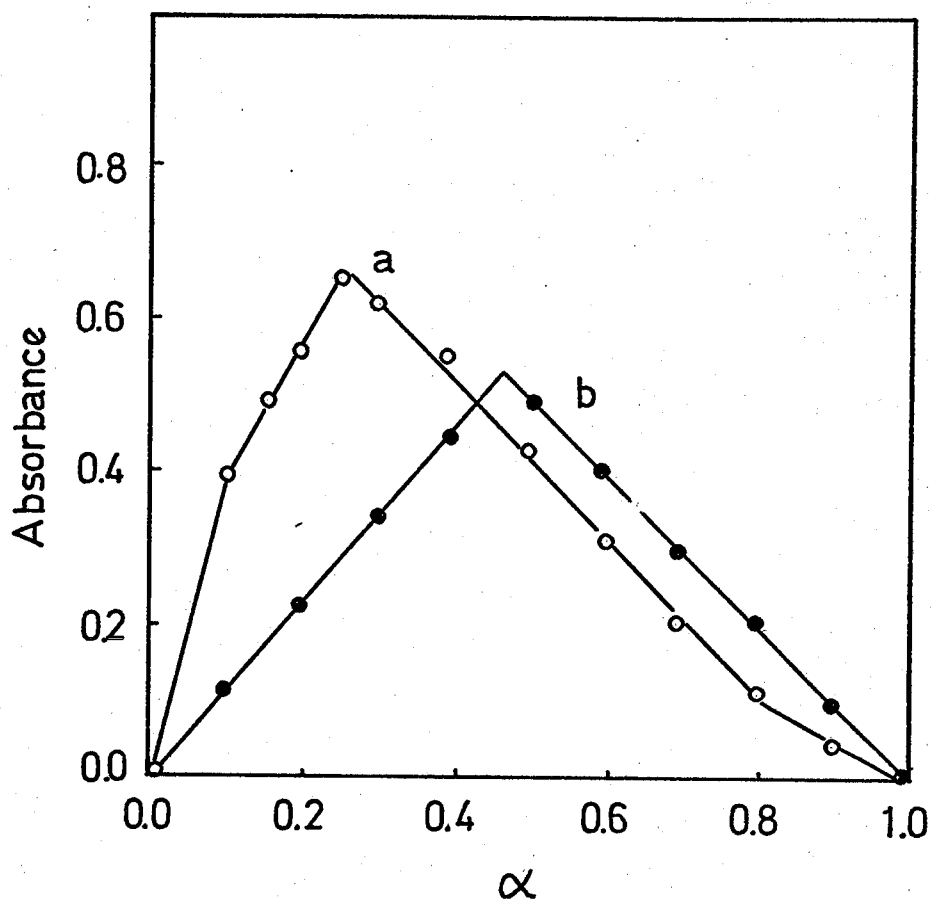


Fig. I-34. Continuous variations method for magnesium-oxine and magnesium-zephiramine relationships.

(a) $\alpha = [\text{Mg}]/[\text{Mg}] + [\text{HOX}]$; $[\text{Mg}] + [\text{HOX}] = 5 \times 10^{-4} \text{ M}$, and $[\text{zeph}] = 5 \times 10^{-4} \text{ M}$,

(b) $\alpha = [\text{Mg}]/[\text{Mg}] + [\text{zeph}]$; $[\text{Mg}] + [\text{zeph}] = 1 \times 10^{-4} \text{ M}$, and $[\text{HOX}] = 5 \times 10^{-3} \text{ M}$.

pH = 9.5.

Table I-1

Extraction constants and dielectric constants of the organic solvents used.

	<chem>CCl4</chem>	<chem>CHCl3</chem>	<chem>C2H4Cl2</chem>	<chem>C6H5NO2</chem>
Dielectric constants (α)	2.2	4.8	10.4	34.8
- log K_{ex}	18.5	18.0	17.7	17.3

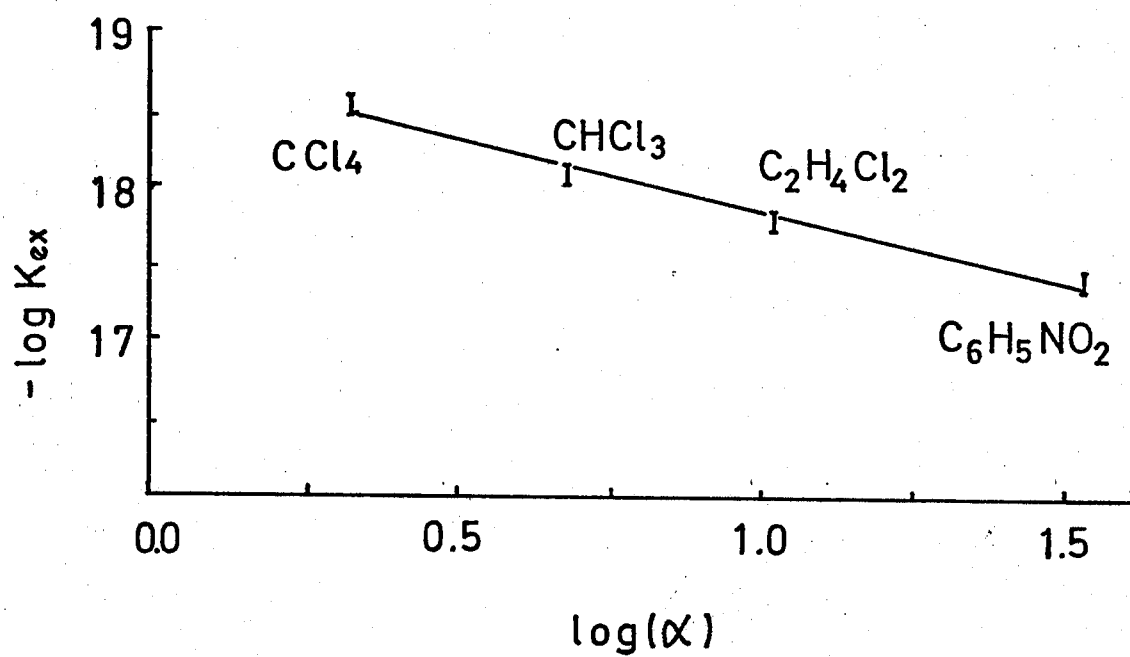


Fig. I-35. Relationship between K_{ex} and dielectric constant (α).

PART II . DETERMINATION OF TRACE METALS IN SEAWATER

CHAPTER 1. INTRODUCTION

In recent years, the determination of trace elements in sea water has recieved an increasing amount of attention and the use of modern instrumental methods has been successful in this field.

Riley³⁰ has summerized the available data for the method of determination of heavy metals in seawater and has concluded that much of the early classical work is unreliable because of faulty analytical techniques and contamination. At present, atomic absorption spectrophotometry and neutron activation analysis offer the best promise for the determination of trace elements in the sea.

Table II -1 indicates the mean abundances of some heavy metals in sea water,^{31,32} but it must be emphasized that the analytical data for each element varies quite widely. Concentrations of heavy metals in seawater are usually one or more orders of magnitude less than the detection limits of commonly-used methods such as spectrophotometry, direct atomic absorption spectrophotometry and so on. The most common concentration techniques that have been employed are co-precipitation, solvent extraction and the use of ion-exchange columns. Of these techniques, as mentioned in PART I, solvent extraction of the trace elements for atomic absorption analysis is superior for simplicity and speed.

Although many of these elements exist in seawater at

concentrations below their detection limits using atomic absorption, they can often readily be concentrated by extraction into a small amount of suitable solvent which results in an increase in sensitivity. Clearly, a combination of solvent extraction and atomic absorption spectrophotometry presents a useful method for the determination of trace elements in saline waters. Alkali and alkaline earth metals, which are the main part of the constituents, and interfere seriously with atomic absorption spectrophotometry, are easily eliminated by solvent extraction. So nowadays, solvent extraction is widely used before the atomic absorptiometric determination of metals.

It is well known that ammonium pyrrolidine dithiocarbamate (APDC) and sodium diethyldithiocarbamate (DDTC) are useful chelating agents for a number of transition metals and permit a wide pH range for extraction. Sprague and Slavin³⁶ have shown that the extraction of APDC and methyl isobutyl ketone (MIBK) makes it possible to separate trace elements from 25 % potassium chloride solutions and have suggested its use for sea water. Afterwards, many investigations have used the systems of APDC-MIBK,^{37,38} APDC-ethyl acetate³⁹ and DDTC-MIBK⁴⁰ to separate a number of heavy metals from sea water. However, these methods have many problems. For instance, extractabilities of heavy metals with these systems are not always complete, and such organic solvents have appreciable solubility (~10 %) in water and are not suitable for routine analyses because of their rather high costs.

On the other hand, oxine is a more typical and powerful chelating agent as compared with APDC and DDTC.⁴⁰⁻⁴³ In previous papers, precipitation reaction of oxine with aluminum,^{44,45} iron,⁴⁶ vanadium⁴⁷ and cadmium⁴⁸ has been used for the determination of these metals in sea water. As seen in the summary by Stary,³ many papers on chelation and extraction of metal oxinates have been reported, but there is no information on the simultaneous extraction of heavy metals in sea water. Some metal oxinates, such as zinc and cadmium oxinates, are not ordinarily extracted into suitable organic solvents.

In PART I, it was shown that some non-extractable chelates change to extractable chelates in the presence of a quaternary ammonium base. This finding can be applied to the extraction of trace metals from sea water, and followed by atomic absorption spectrophotometry. In fact, the extraction system of oxine-1,2-dichloroethane in the presence of zephiramine makes it possible to extract several heavy metals simultaneously; e.g. nickel, manganese, cadmium, zinc and copper in sea water. Besides the simultaneous extraction of several metals, this method has the advantages of oxine being an inexpensive and stable chelating agent and the solubility of 1,2-dichloroethane being low (~ 0.1 %) in water.

Table II-1

Abundance of selected trace metals in the oceans (ppb).

Trace metal	Chester and Stoner ^a		Goldberg ^b
	Range	Average	Average
Zn	0.6 - 12.6	1.4	3
Cd	0.04 - 0.3	0.07	0.02
Ni	0.5 - 5.2	1.2	2
Mn	0.12 - 1.2	0.22	0.3
Cu	0.3 - 3.8	0.8	2

a Ref.31

b Ref.32

CHAPTER 2. EXPERIMENTAL

2-1. Reagents

Oxine solution, 1×10^{-2} M. Dissolve 1.45 g of the reagent in 1 l of 1,2-dichloroethane.

Metal solutions, 1×10^{-2} M. Each solution of cadmium, zinc, nickel, manganese and copper was prepared by dissolving cadmium nitrate or the other metal sulfates, and standardized with EDTA. The solutions were diluted for use.

Zephiramine solution, 5×10^{-2} M. Dissolve 18.4 g of tetradecyldimethylbenzylammonium chloride in 1 l of water.

Buffer solution. The pH of a solution was adjusted with acetate buffer (pH < 6.2), borate buffer (pH 6.2 – 12.0) and sodium hydroxide solution (pH > 12.0).

All the chemicals used were of analytical-reagent grade.

2-2. General procedure

A metal solution, a buffer solution and the zephiramine solution were placed in a separatory funnel and diluted to 20 ml with water. The oxine-1,2-dichloroethane (20 ml) was added and the funnel was shaken for a definite time. After the organic phase was dried by filtration through a paper filter, the absorbance was measured against the reagent-blank. Distribution ratios were obtained by determining the metal concentration left in the aqueous phase by atomic absorption spectrophotometry.

CHAPTER 3. RESULTS AND DISCUSSION

3-1. Chelation and extraction of heavy metals with 8-hydroxyquinoline

8-Hydroxyquinoline(oxine) is nearly insoluble in water and ether, but quite soluble in alcohol and 1,2-dichloroethane. Oxine has a hydrogen atom which is replaceable by a metal ion, and has a heterocyclic nitrogen which forms a five-membered chelate ring with the metal ion. Oxine is known to react with at least 43 metals which are generally the same metals that precipitate with ammonia.⁴⁹ The divalent and trivalent metal oxinates have the general formulas of $M(\text{oxine})_2$ and $M(\text{oxine})_3$ respectively. Among these, some divalent metal oxinates are of neutral charge but coordination-unsaturated chelates which are poorly extractable into inactive solvents. For example, zinc, cadmium and magnesium form $M(\text{oxine})_2 \cdot 2\text{H}_2\text{O}$ which are not extractable into inactive solvents. For this reason, oxine can not be used directly for the simultaneous extraction and concentration of these metals from sea water. In this section, the fact that nonextractable metal oxinates change to extractable chelates by the addition of zephiramine and the conditions for application to simultaneous extraction of these metals from sea water are investigated.

3-1-1. Zinc

Absorption spectra

The absorption spectrum of zinc-oxine chelate extracted

in the presence of zephiramine is shown in Fig.II-1. The bright yellow zinc oxinate solution has a strong absorption at 400 nm and the reagent also shows considerable absorption at this wavelength (Fig.II-1). The absorbances were measured against the reagent-blank as a reference. The molar absorption coefficient of zinc-oxine chelate at 400 nm is 7.0×10^3 .

Effect of pH

The aqueous solution was adjusted to various pH values with acetate and borate buffer solutions, and the extraction was carried out by the above procedure (2-2). The extraction of zinc-oxine chelate begins at about pH 6.7, increases sharply and attains 100 % above pH 8.0 (Fig.II-2).

Effect of shaking time

With other variables constant, the extraction period was varied from 1 to 30 min. The optimum shaking time was found to be 5 min. The colour of the extract is quite stable for at least 5 hours.

Effect of oxine concentration

Varying amounts of oxine were added to 1,2-dichloroethane and the each solution was shaken with zinc solution of pH 8.5. A constant absorbance of the extract is obtained from 0.8×10^{-3} to 1×10^{-3} M of oxine, and the 100%-extraction is achieved (Fig.II-3).

Effect of zephiramine concentration

It was found that the extraction begins at about 1×10^{-5} M and becomes 100 % above 5×10^{-4} M of zephiramine in aqueous solution (Fig.II-4).

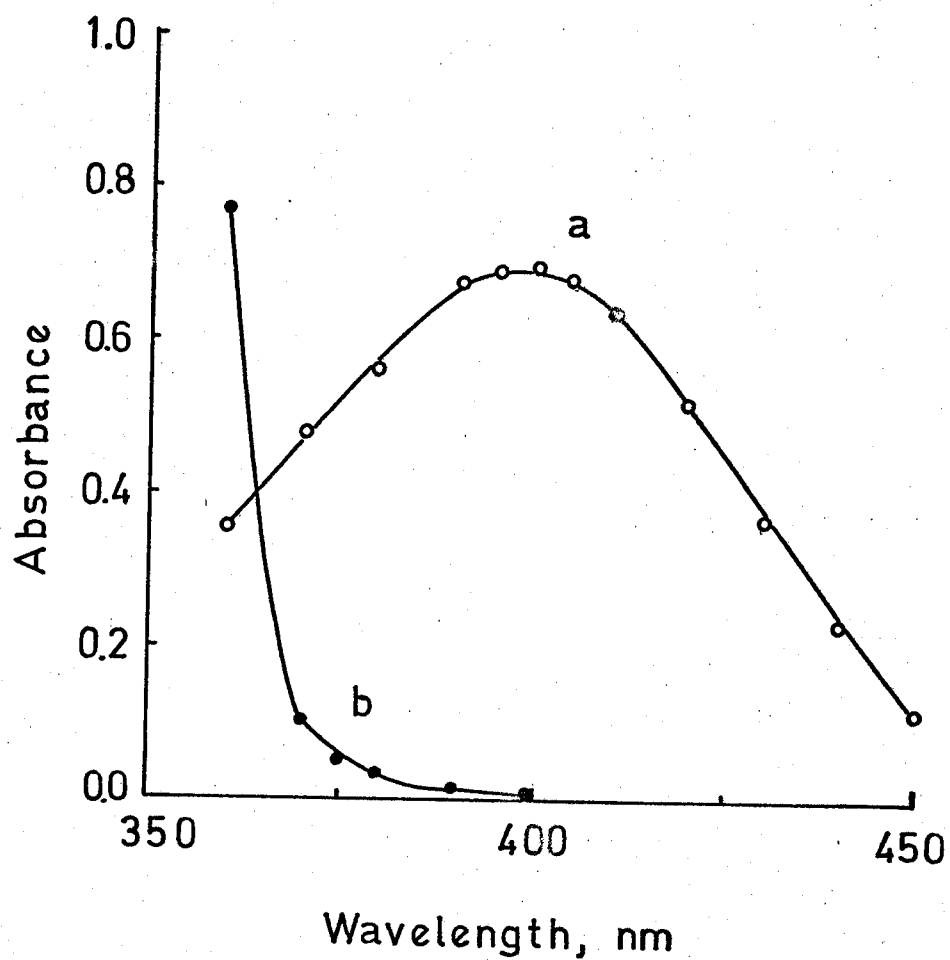


Fig. II-1. Absorption spectra of Zn-oxine chelate and reagent blank in the presence of zephiramine.

$[Zn]_{aq} = 1 \times 10^{-4} \text{ M}$, $[HOX]_{org} = 1 \times 10^{-2} \text{ M}$,
 $[zeph]_{aq} = 1 \times 10^{-3} \text{ M}$, and $pH = 9.0$

(a) Zn-oxine-zephiramine chelate vs. reagent blank, (b) reagent blank vs. water.

$V_{org} = V_{aq} = 20 \text{ ml.}$

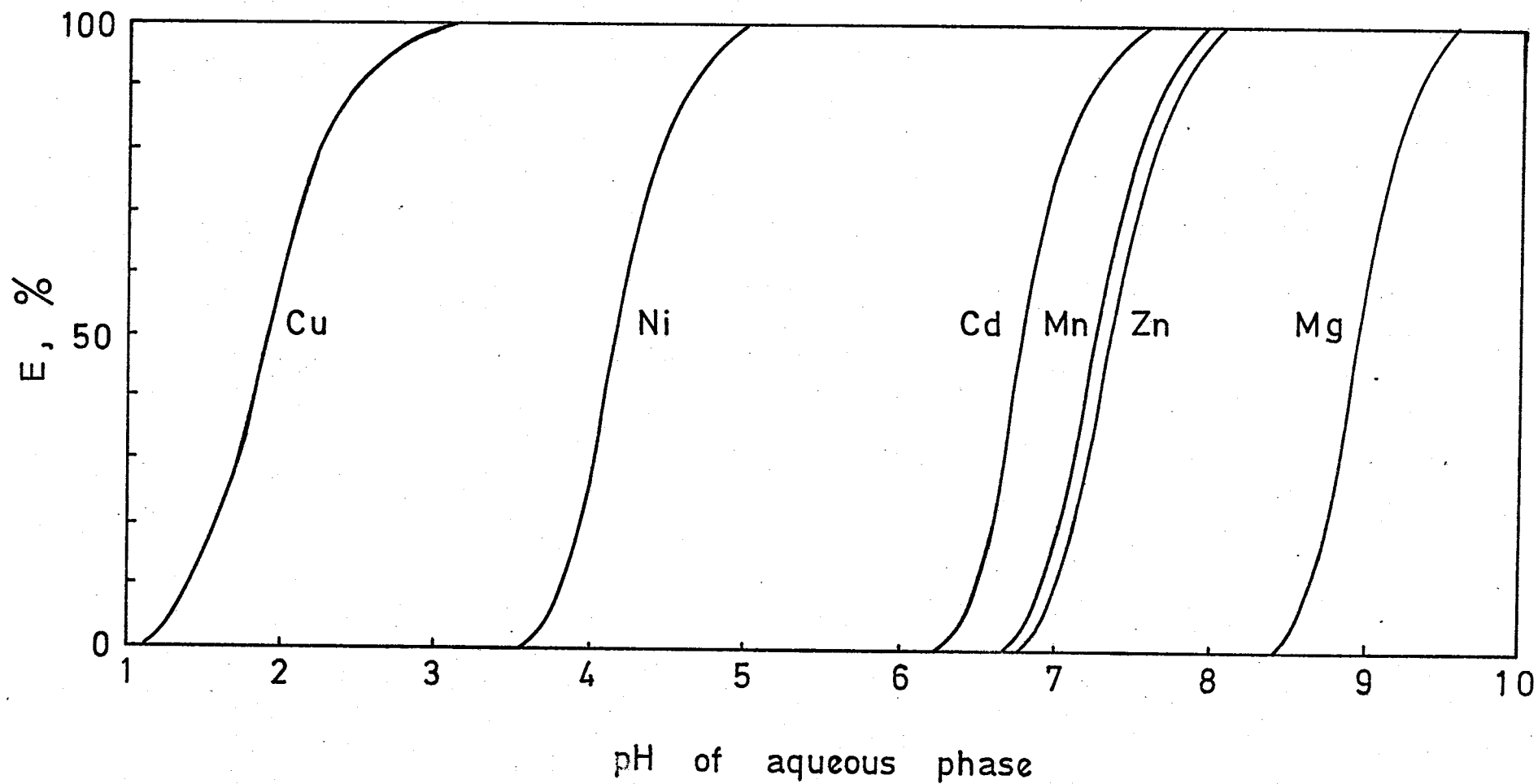


Fig. II-2. Effect of pH on extraction of metal oxinates.

$[\text{metal}]_{\text{aq}} = 1 \times 10^{-4} \text{ M}$, $[\text{HOX}]_{\text{org}} = 1 \times 10^{-2} \text{ M}$, $[\text{zeph}]_{\text{aq}} = 1 \times 10^{-3} \text{ M}$,
 $V_{\text{org}} = V_{\text{aq}} = 20 \text{ ml}$.

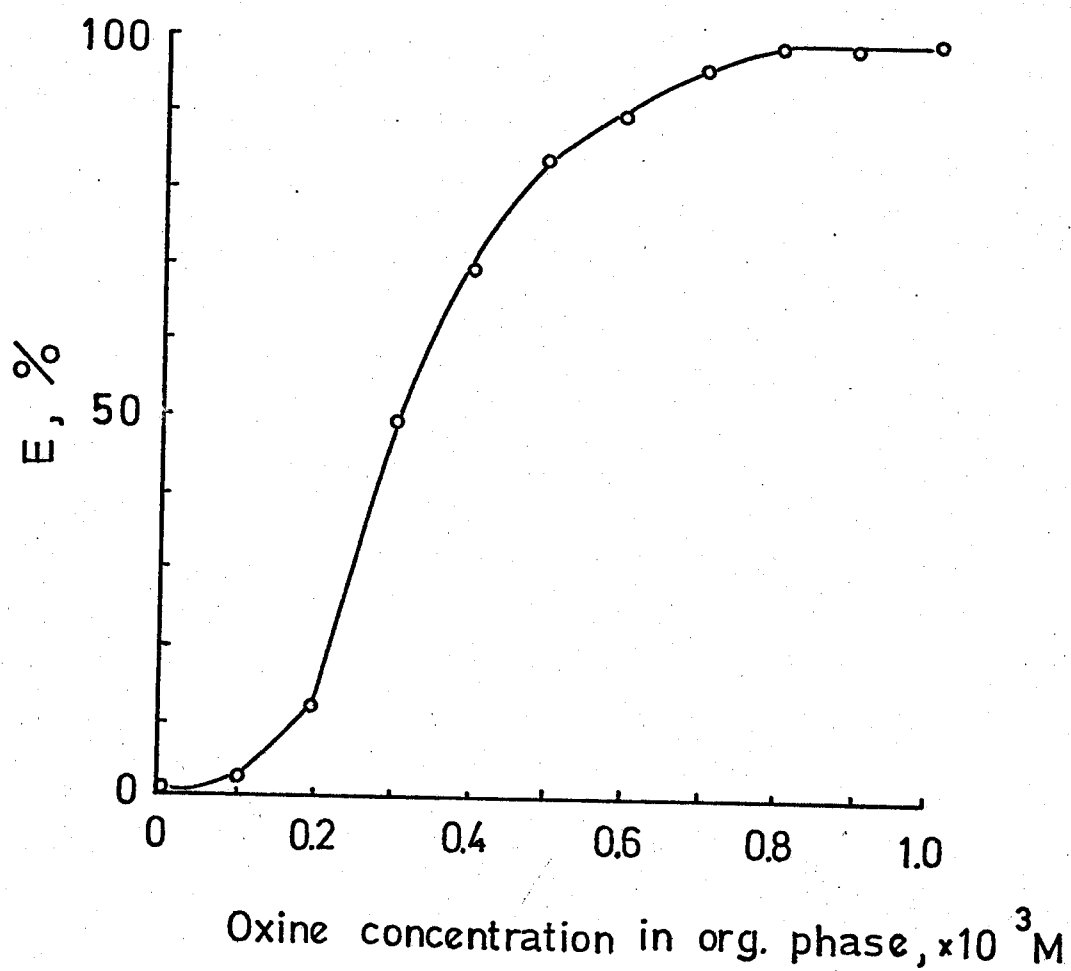


Fig. II-3. Effect of concentration of oxine on extraction of Zn-oxine chelate in the presence of zephiramine.

Conditions are the same with those in Fig. II-1.

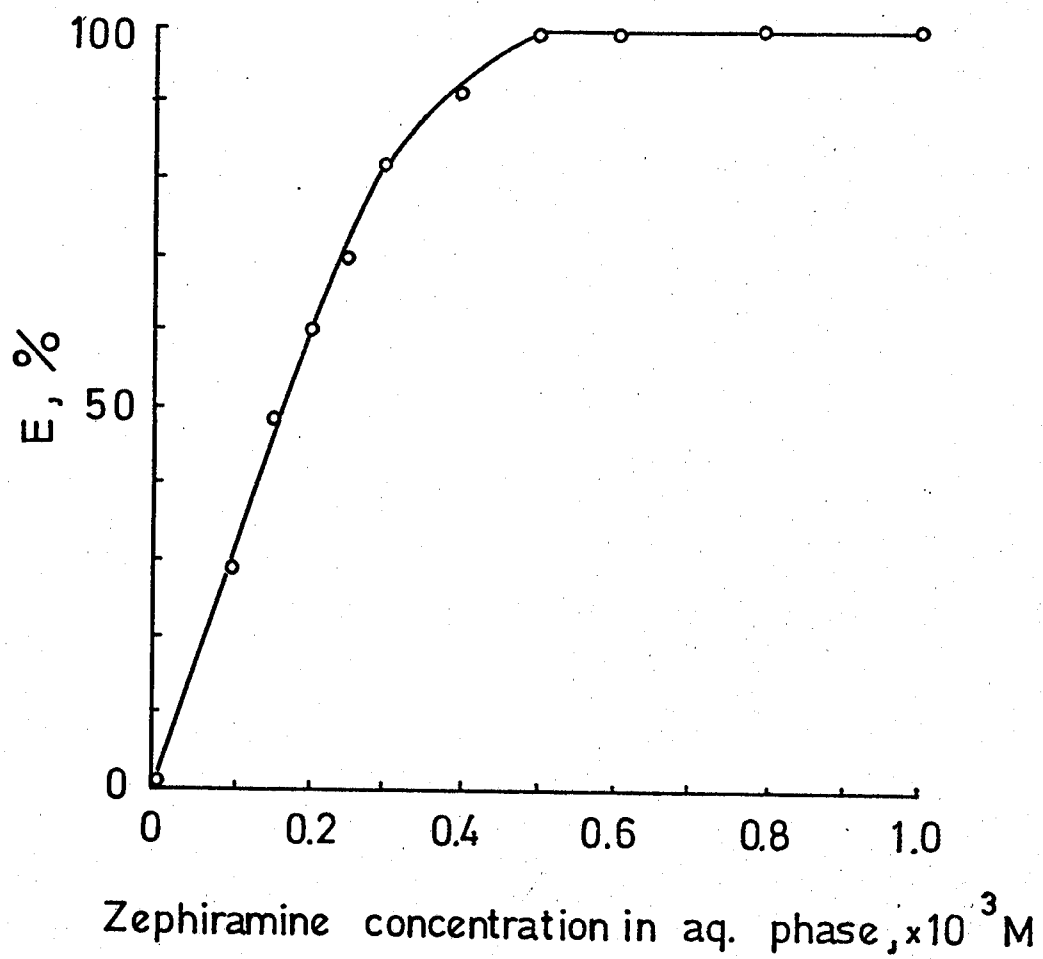


Fig. II-4. Effect of concentration of zephiramine on extraction of Zn-oxine chelate.

Conditions are the same with those in Fig. II-1.

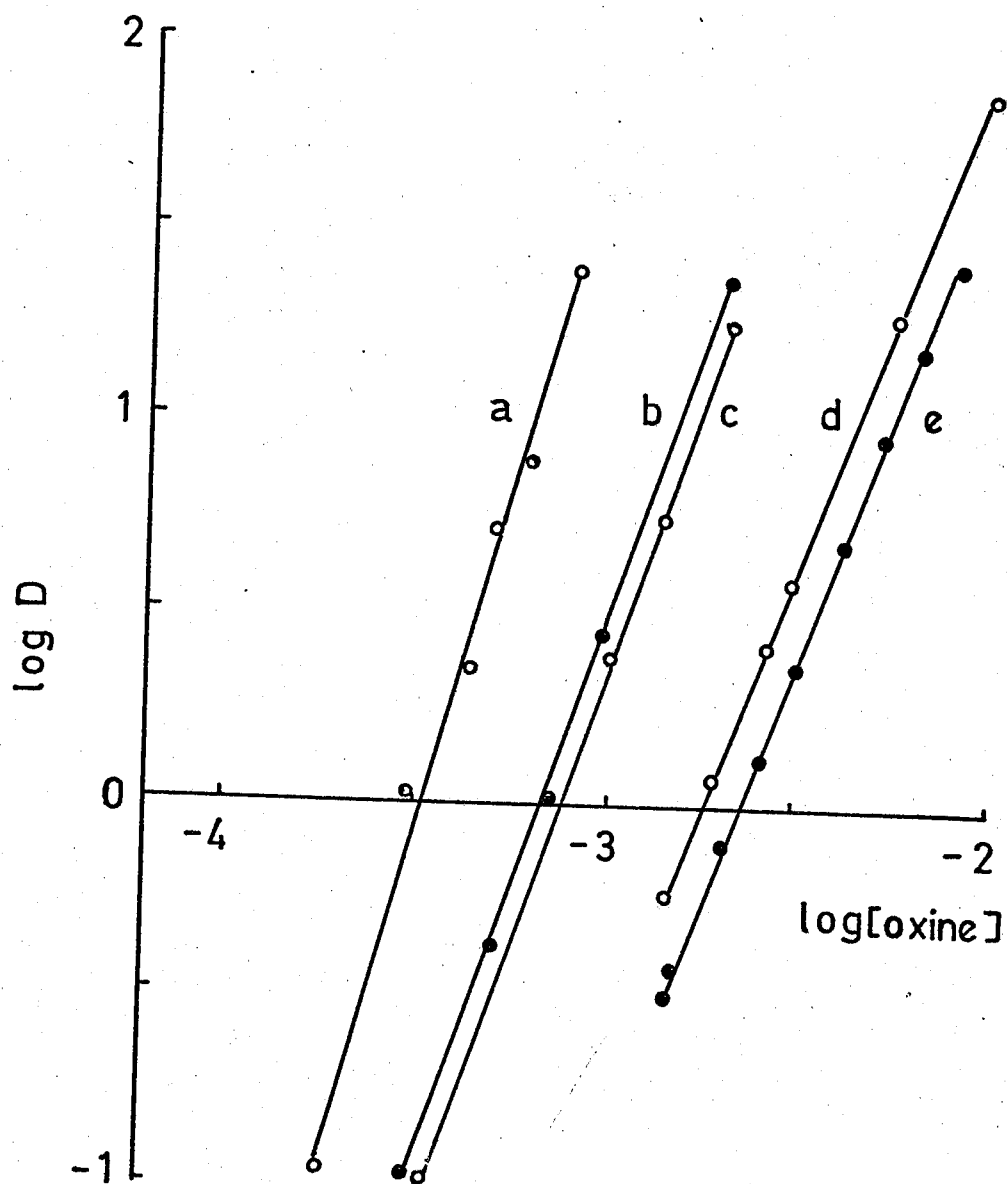


Fig. II-5. Dependence of $\log D$ on $\log [\text{oxine}]$ in the presence of zephiramine.

$[\text{zeph}]_{\text{aq}} = 1 \times 10^{-3} \text{ M};$

(a) Zn at pH = 8.5, (b) Mn at pH = 8.5,

(c) Cd at pH = 8.0, (d) Mg at pH = 9.5,

(e) Ni at pH = 4.95.

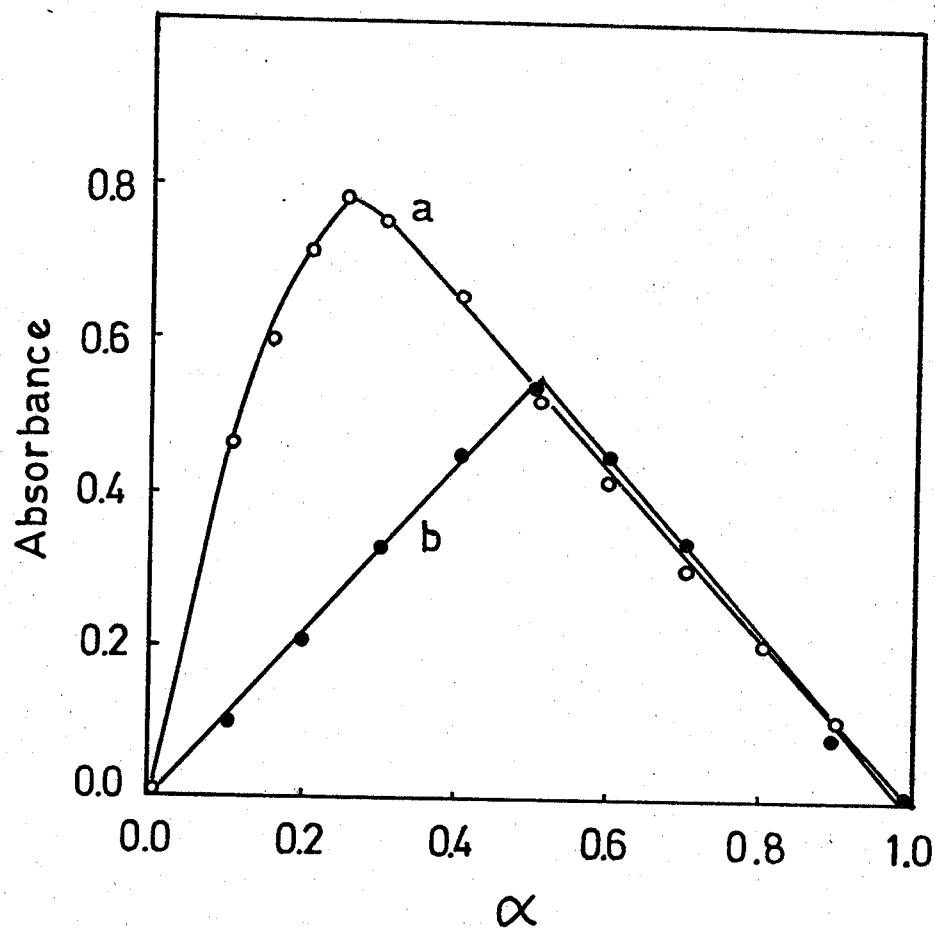


Fig. II-6. Continuous variations method for zinc-oxine and zinc-zephiramine.

(a) $\alpha = [\text{Zn}]/[\text{Zn}] + [\text{HOX}]$; $[\text{Zn}] + [\text{HOX}] = 5 \times 10^{-4} \text{ M}$, and $[\text{zeph}] = 1 \times 10^{-3} \text{ M}$;

(b) $\alpha = [\text{Zn}]/[\text{Zn}] + [\text{zeph}]$; $[\text{Zn}] + [\text{zeph}] = 2 \times 10^{-4} \text{ M}$, and $[\text{HOX}] = 5 \times 10^{-3} \text{ M}$.

pH = 10.9.

Composition of the extracted chelate

Dependence of $\log D$ on $\log [\text{oxine}]$ and the results of continuous variations method are shown in Figs. II-5 and II-6 which show that the composition of the chelate is $[\text{Zn}(\text{OX})_3(\text{zeph})]$.

3-1-2. Cadmium

Cadmium forms an extractable chelate, $\text{Cd}(\text{OX})_2 \cdot 2(\text{HOX})$, but this chelate is decomposed on shaking for extraction.^{1,3} Therefore, cadmium can not be extracted with oxine. But we can quantitatively extract cadmium with oxine in the presence of zephiramine.

Absorption spectra

The absorption spectra of Cd-oxine chelate and reagent blank in the presence of zephiramine are shown in Fig. II-7. The extracted chelate has an absorption maximum at 400 nm, and its molar absorption coefficient is 6.5×10^3 .

Effect of pH

The effect of pH on the extractions in the presence of zephiramine was examined by changing the pH of the solution from 6 to 10. Fig. II-2 shows the plot of percentage extraction of Cd-oxine chelate as function of pH. The extraction reaches 100 % at pH 7.9.

Effect of shaking time

Five-minute shaking was found to be the desired time, and the chelate is stable for at least 6 hours.

Effect of oxine concentration

Varying amounts of oxine were added to the organic phase,

other factors being kept constant, and the 100%-extraction was attained with a concentration higher than 3×10^{-3} M (Fig.II-8).

Effect of zephiramine concentration

Fig.II-9 shows that cadmium is quantitatively extracted in the range of 6×10^{-4} – 1×10^{-3} M of zephiramine in the aqueous phase.

Composition of the extracted chelate

Results of the equilibrium shift method and the continuous variations method are shown in Fig.II-5 and II-10. Both methods showed that the cadmium chelate has the composition of metal : oxine : zeph = 1 : 3 : 1.

3-1-3. Nickel

Since nickel oxinate has no coordinated water molecules, $\text{Ni}(\text{OX})_2$ can be extracted into organic solvents in the absence of zephiramine. In the absence of zephiramine, however, a higher concentration of oxine is needed to extract nickel quantitatively as compared with the extraction in the presence of zephiramine. Moreover, the extraction equilibrium of nickel is attained after several hours in the absence of zephiramine.¹ It is obvious that the method in the presence of zephiramine is superior to that in the absence of zephiramine for a routine analysis. The conditions for the extraction of nickel were examined in the presence of zephiramine.

Absorption spectra

Absorption spectra of the nickel chelates are shown in Fig.II-11. In the presence of zephiramine, the absorbance

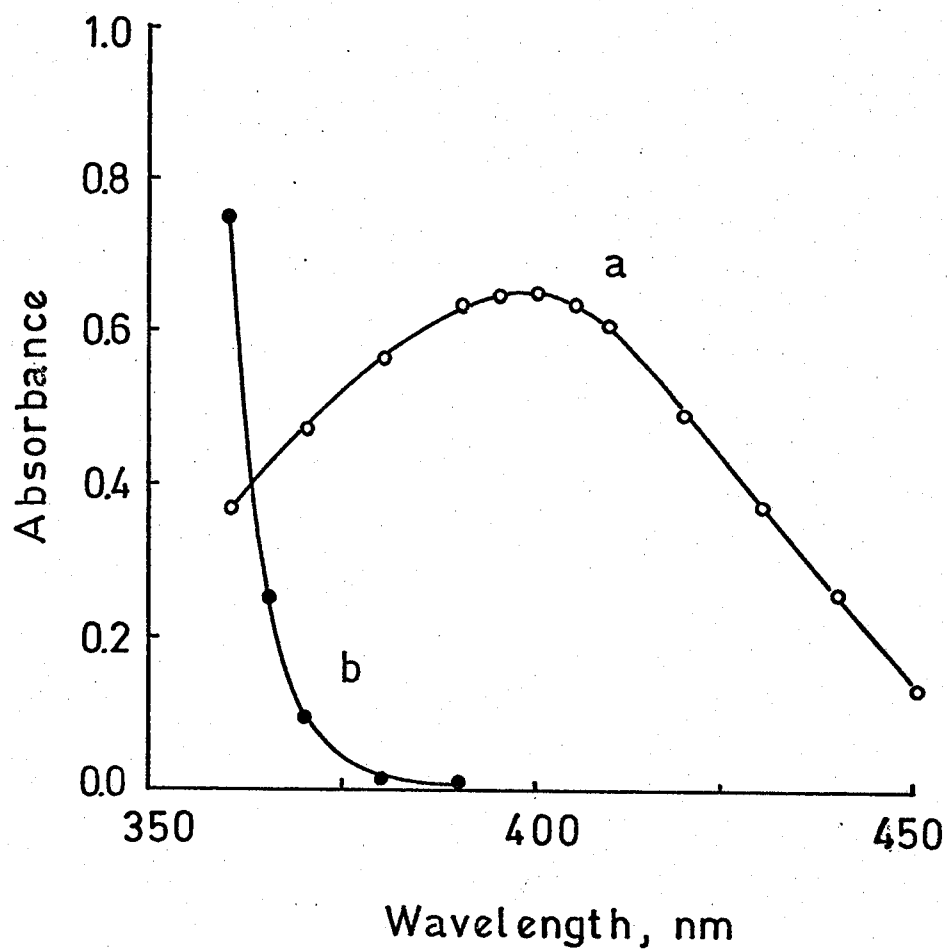


Fig. II-7. Absorption spectra of Cd-oxine chelate and reagent blank in the presence of zephiramine.

$[\text{Cd}]_{\text{aq}} = 1 \times 10^{-4} \text{ M}$, $[\text{HOX}]_{\text{org}} = 1 \times 10^{-2} \text{ M}$,
 $[\text{zeph}]_{\text{aq}} = 1 \times 10^{-3} \text{ M}$, and $\text{pH} = 8.5$.

(a) Cd-oxine-zephiramine chelate vs. reagent blank, (b) reagent blank vs. water.

$V_{\text{org}} = V_{\text{aq}} = 20 \text{ ml}$.

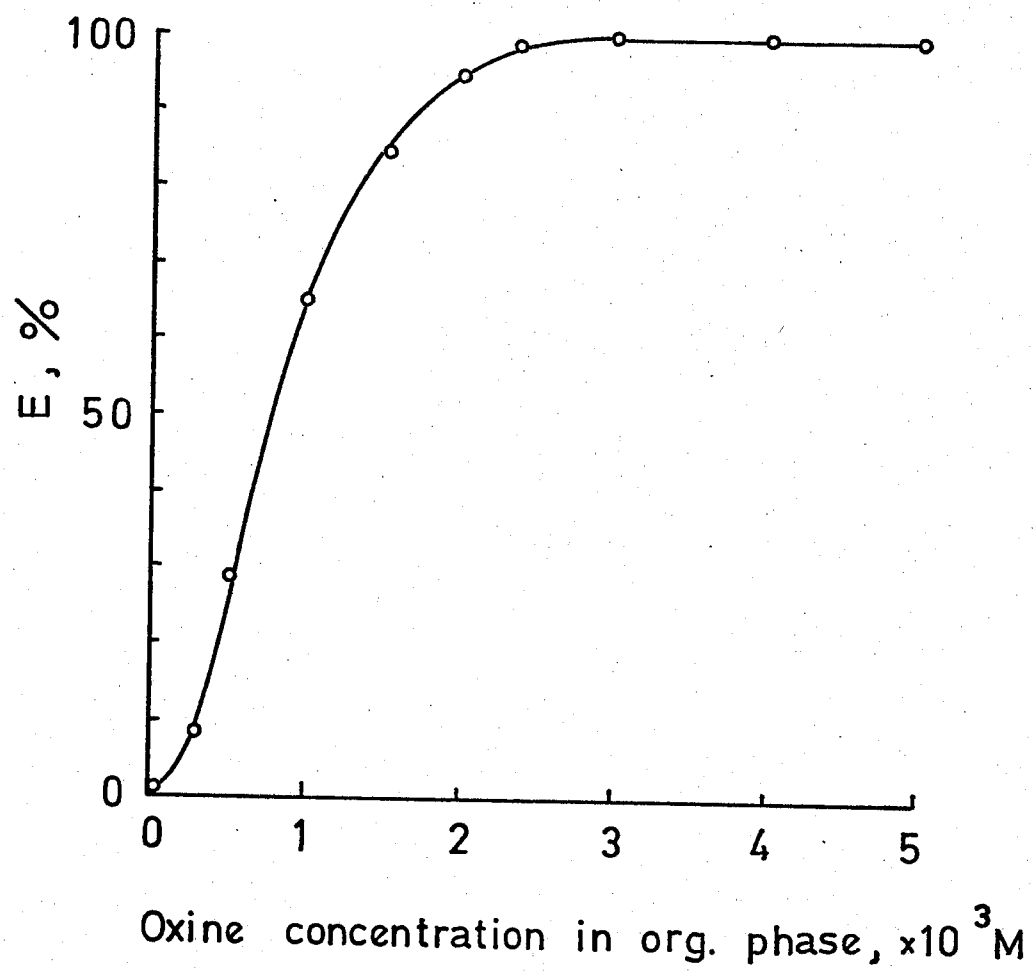


Fig. II-8. Effect of concentration of oxine on extraction of Cd-oxine chelate in the presence of zephiramine.

Conditions are the same with those in Fig. II-7.

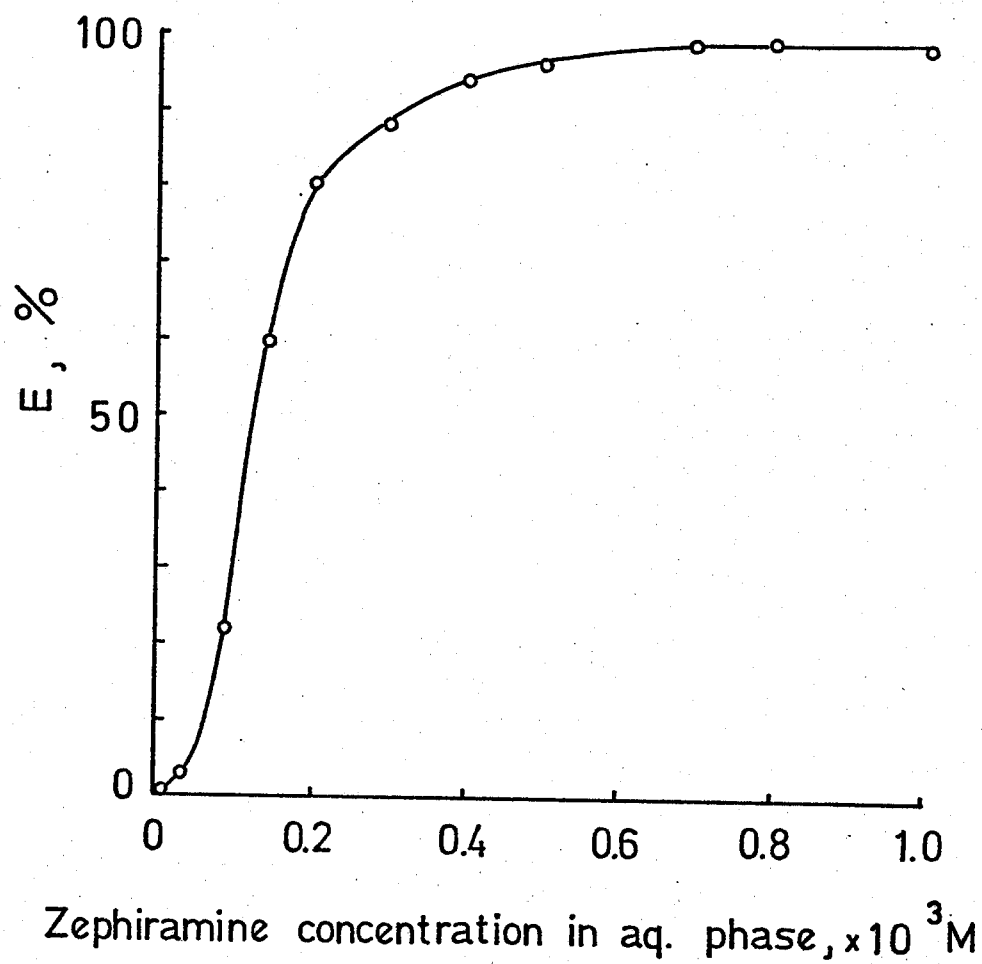


Fig. II-9. Effect of concentration of zephiramine on extraction of Cd-oxine chelate.

Conditions are the same with those in Fig. II-7.

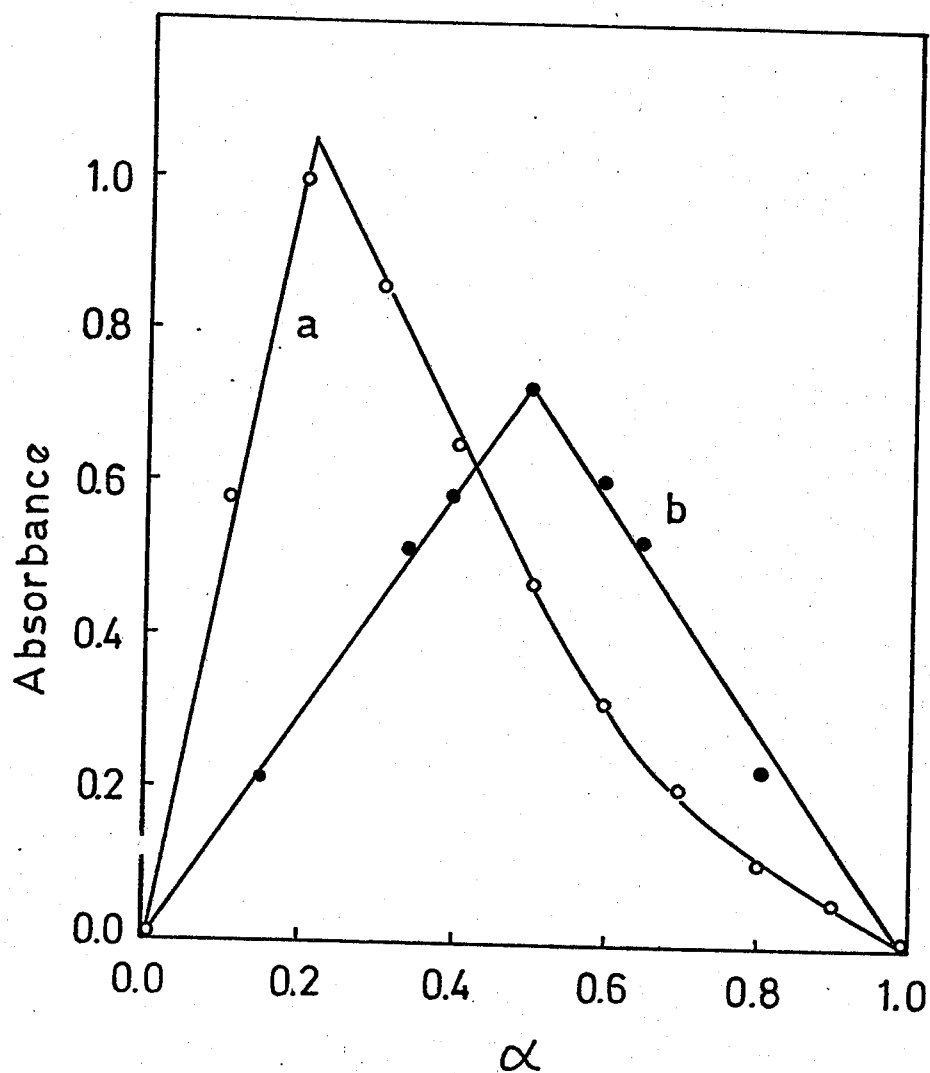


Fig. II-10. Continuous variations method for cadmium-oxine and cadmium-zephiramine.

(a) $\alpha = [\text{Cd}]/[\text{Cd}] + [\text{HOX}]$; $[\text{Cd}] + [\text{HOX}] = 1 \times 10^{-3} \text{ M}$, and $[\text{zeph}] = 5 \times 10^{-3} \text{ M}$,

(b) $\alpha = [\text{Cd}]/[\text{Cd}] + [\text{zeph}]$; $[\text{Cd}] + [\text{zeph}] = 5 \times 10^{-4} \text{ M}$, and $[\text{HOX}] = 1 \times 10^{-2} \text{ M}$.

pH = 8.0

increases and the wavelength of absorption maximum shifts from 370 nm to 400 nm. The molar absorption coefficient of the nickel chelate is 4.0×10^3 at 370 nm in the absence of zephiramine and 7.3×10^3 at 400 nm in the presence of zephiramine.

Effect of pH

As shown in Fig.II-2, the extraction begins at about pH 3.5 with a sharp increase and becomes 100 % above pH 4.9 in the presence of zephiramine.

Effect of shaking time

In the absence of zephiramine, the extraction is time-consuming, but the optimum shaking time is shortened to 10 minutes in the presence of zephiramine. The color of the extract is stable for at least 6 hours.

Effect of oxine concentration

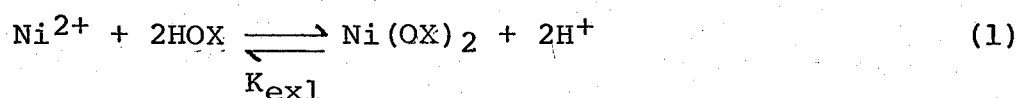
In the presence of zephiramine, the effect of the concentration of oxine was examined (Fig.II-12). The 100%-extraction is obtained with a concentration higher than 7×10^{-3} M of oxine.

Effect of zephiramine concentration and composition of the extracted chelate

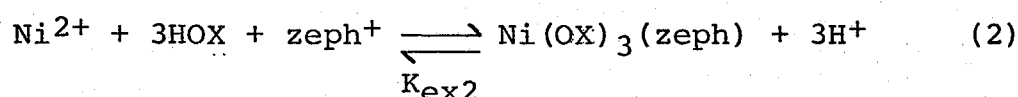
In the case of nickel, nickel oxinate is extracted in the absence of zephiramine, but the extractability of the nickel oxinate is enhanced with addition of zephiramine. High extractability is due to the fact that the ordinary nickel oxinate, $\text{Ni}(\text{OX})_2$, changes to another nickel oxinate having an absorption maximum at 400 nm in the presence of zephiramine (Fig.II-11). From Figs.II-5 and II-13, it is concluded that

the extracted nickel chelate has a composition of Ni : oxine = 1 : 3. The extraction system of nickel chelate is assumed as follows:

In the absence of zephiramine;



In the presence of zephiramine;



The chelates of the two types are completely extracted into organic phase at the pH higher than 5.5. So, we obtain

$$\frac{K_{\text{ex2}}}{K_{\text{ex1}}} = \frac{[\text{Ni}(\text{OX})_3(\text{zeph})]_{\text{org}}[\text{H}^+]}{[\text{Ni}(\text{OX})_2]_{\text{org}}[\text{HOX}]_{\text{org}}[\text{zeph}^+]} \quad (3)$$

In the presence of 1×10^{-3} M zephiramine, nickel is completely extracted as a chelate of $\text{Ni}(\text{OX})_3(\text{zeph})$ and its molar absorption coefficient is 7.30×10^3 at 400 nm. On the other hand, in the absence of zephiramine, that of $\text{Ni}(\text{OX})_2$ is 2.35×10^3 at 400 nm. When the concentration of zephiramine is lower than 1×10^{-3} M at pH 5.5, the absorbance of organic phase at 400 nm is given by the following equation,

$$\begin{aligned} \text{Abs}_{400\text{nm}} &= \epsilon_1[\text{Ni}(\text{OX})_2] + \epsilon_2[\text{Ni}(\text{OX})_3(\text{zeph})] \\ &= \{2.35 \times 10^3 \times X + 7.30 \times 10^3 \times (1 - X)\} \times C_{\text{Ni}} \\ \text{or } \text{Abs}_{400\text{nm}} &= (7.30 - 4.95X) \times 10^3 \times C_{\text{Ni}} \end{aligned} \quad (4)$$

Where, ϵ_1 and ϵ_2 are the molar absorption coefficients of $\text{Ni}(\text{OX})_2$ and $\text{Ni}(\text{OX})_3(\text{zeph})$, respectively, X is the portion of $\text{Ni}(\text{OX})_2$ of the extracted chelates and C_{Ni} is the total

concentration of nickel.

The equation (3) is rewritten by the equation (5).

$$\frac{K_{ex2}}{K_{ex1}} = \frac{[1 - X][H^+]}{[X][HOX]_{org}[zeph]} \quad (5)$$

At various concentrations of zephiramine, absorbances at 400 nm were measured (Fig.II-14). From equation (4), the value of X is calculated at a certain concentration of zephiramine, and the dependency of $\log ([1 - X]/[X])$ on $\log [zeph]$ is shown in Fig.II-15. A linear curve having inclination of one (Fig.II-15) indicates that the extracted nickel chelate has a composition of $Ni(OX)_3(zeph)$ in the presence of zephiramine.

3-1-4. Manganese

Absorption spectra

In the absence of zephiramine, manganese(II) is completely extracted into organic solvents of 0.10 M oxine. As shown in Fig.II-16, the absorbance of the extracted chelate is enhanced and the wavelength of absorption maximum shifts from 390 nm to 400 nm in the presence of zephiramine. The molar absorption coefficients of the extracted chelates are 4.8×10^3 at 390 nm in the absence of zephiramine and 7.6×10^3 at 400 nm in the presence of zephiramine.

Effect of pH

In the absence of zephiramine, manganese is extracted in the pH range 9.0 - 11.0¹. In the presence of zephiramine, the extraction of Mn-oxine chelate begins at pH 6.7 and reaches 100 % at pH 7.5.

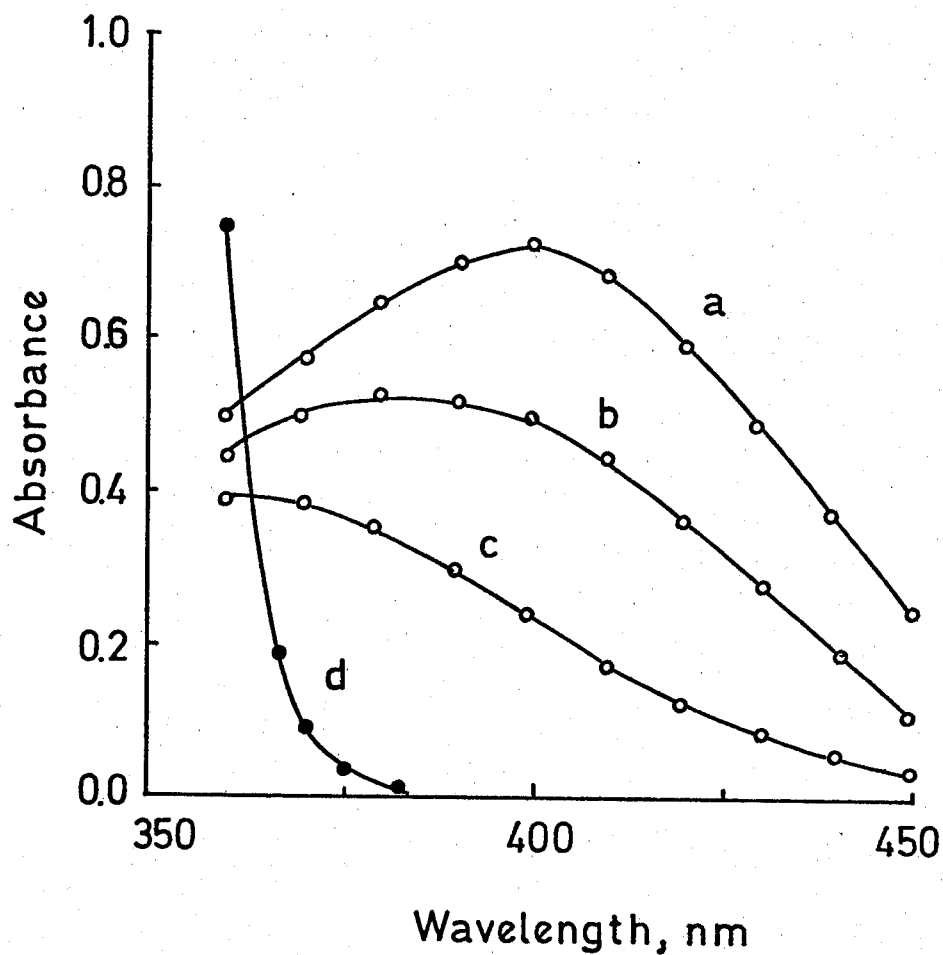


Fig. II-11. Absorption spectra of Ni-oxine chelate and reagent blank.

$[\text{Ni}]_{\text{aq}} = 1 \times 10^{-4} \text{ M}$, $[\text{HOX}]_{\text{org}} = 1 \times 10^{-2} \text{ M}$, and $\text{pH} = 4.95$.

(a) Ni-oxine chelate vs. reagent blank in the presence of $1 \times 10^{-3} \text{ M}$ zephiramine, (b) Ni-oxine chelate in the presence of $4 \times 10^{-4} \text{ M}$ zephiramine, (c) Ni-oxine chelate in the absence of zephiramine, (d) reagent blank vs. water.

$V_{\text{org}} = V_{\text{aq}} = 20 \text{ ml}$.

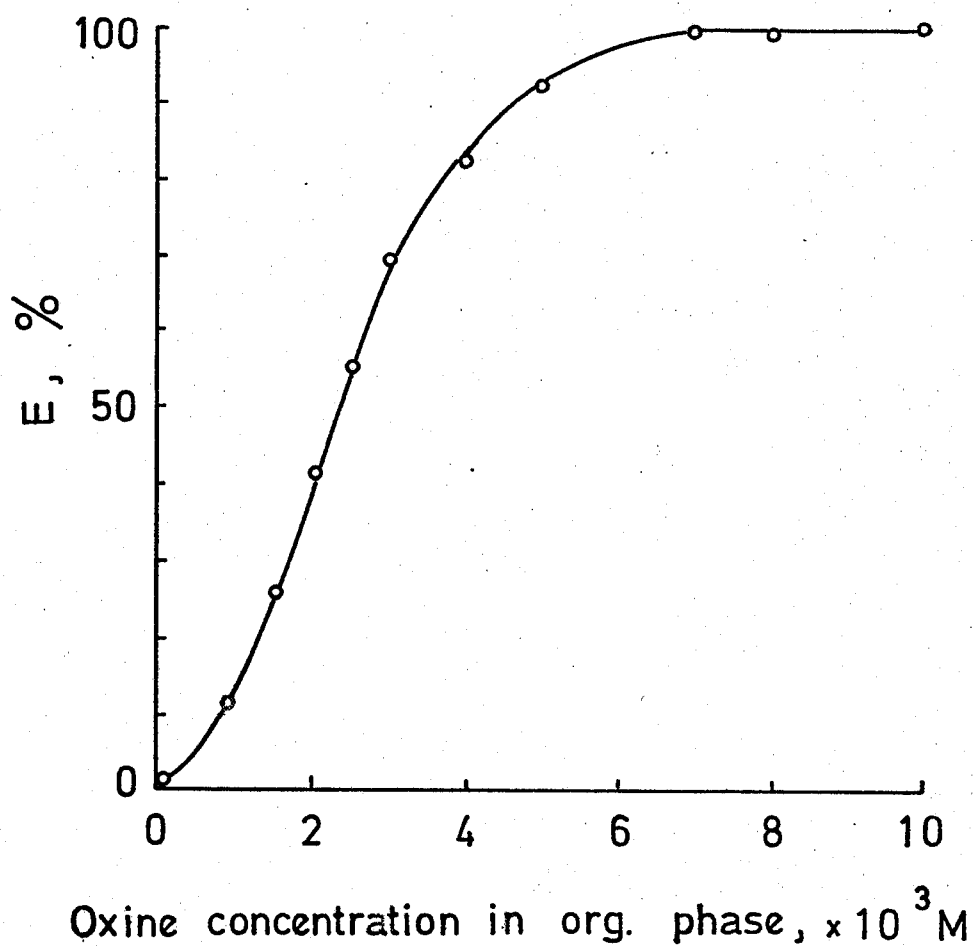


Fig. II-12. Effect of concentration of oxine on extraction of Ni-oxine chelate in the presence of zephiramine.

$[\text{zeph}]_{\text{aq}} = 1 \times 10^{-3} \text{ M.}$

The others are the same with those in Fig. II-11.

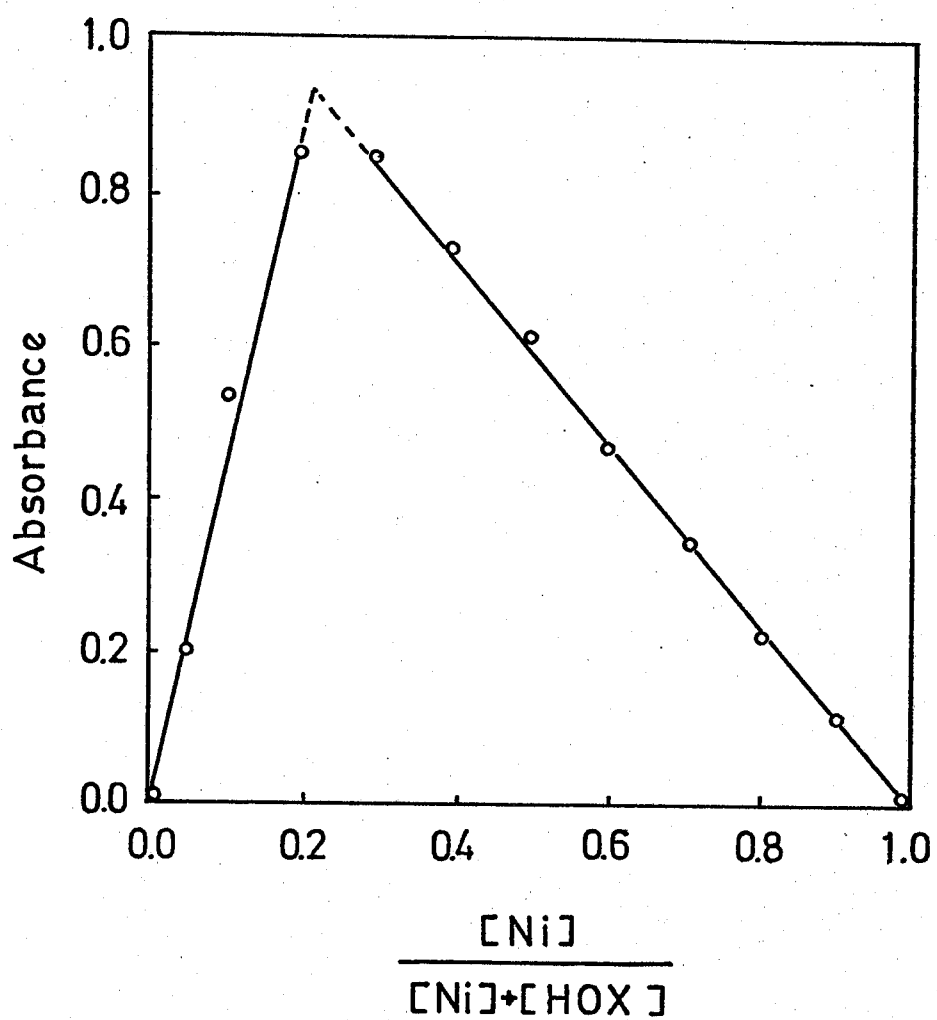


Fig. II-13. Continuous variations method for Ni-oxine chelate.

$[Ni] + [HOX] = 1 \times 10^{-3} \text{ M}$, $[zeph] = 5 \times 10^{-3} \text{ M}$,
and $\text{pH} = 5.8$.

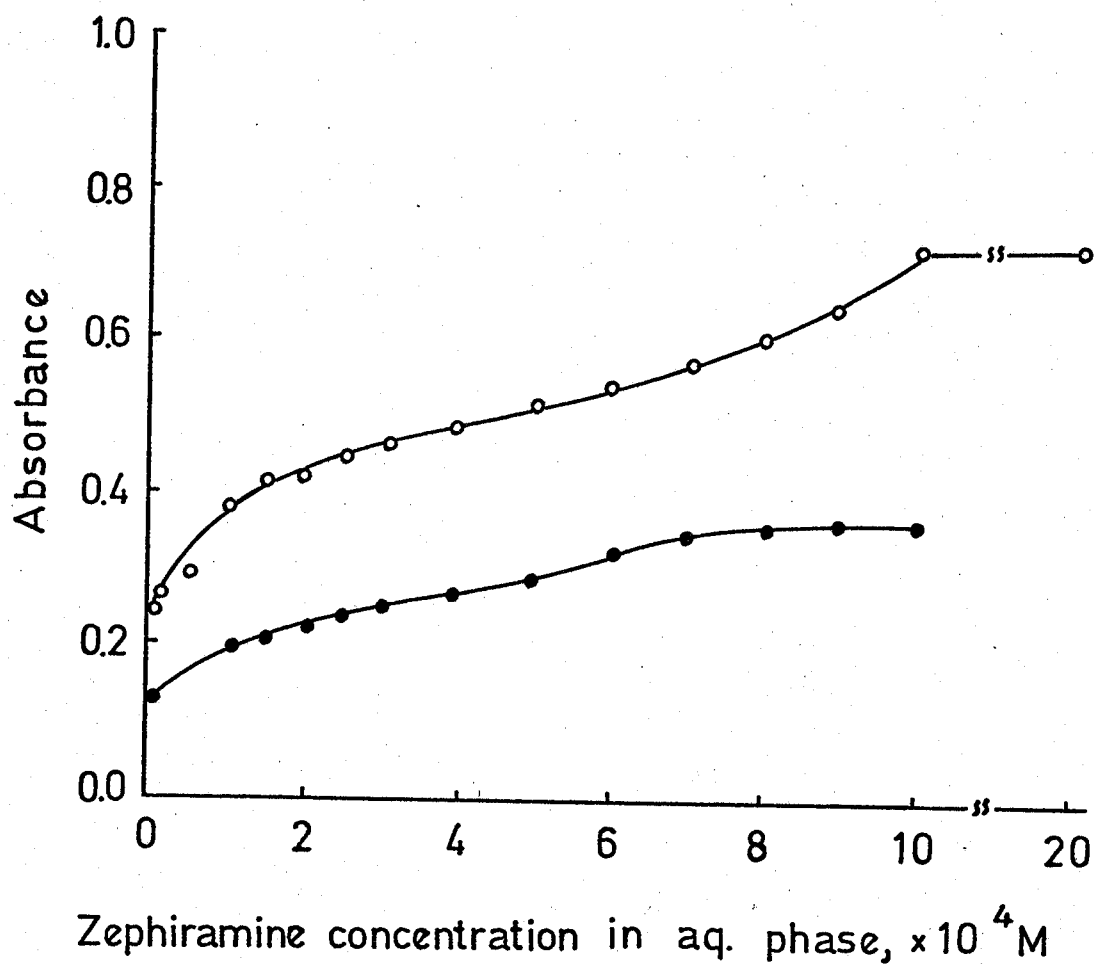


Fig. II-14. Effect of concentration of zephiramine on the absorbance of Ni-oxine chelate.

$[\text{HOX}]_{\text{org}} = 1 \times 10^{-2}$ M, and pH = 5.5.

O $[\text{Ni}]_{\text{aq}} = 1 \times 10^{-4}$ M, and ● $[\text{Ni}]_{\text{aq}} = 5 \times 10^{-5}$ M.

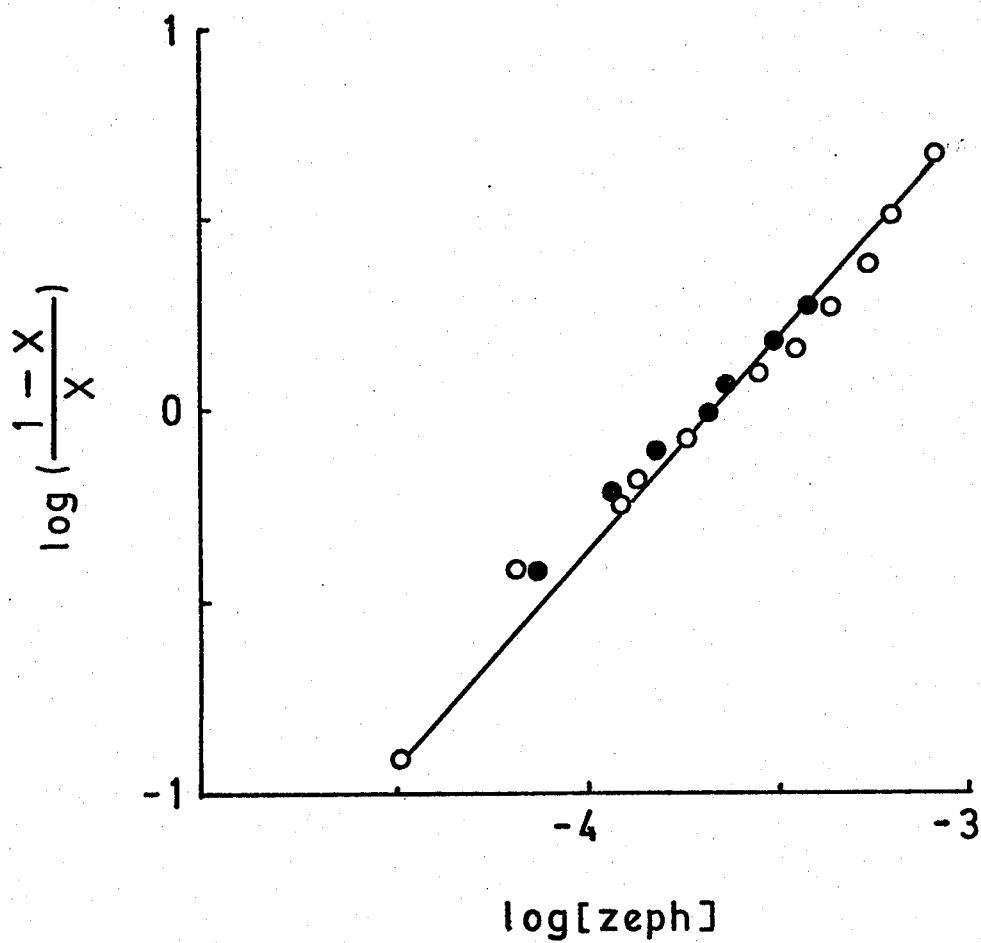


Fig. II-15. Zephiramine dependence on formation of Ni-oxine-zephiramine chelate from Ni-oxine chelate.

Conditions are the same with those in Fig. II-14.

Effect of shaking time

The optimum period for shaking was found to be 5 minutes, and the color of the extract is stable for at least 6 hours.

Effect of oxine concentration

In the absence of zephiramine, a higher concentration of oxine such as 0.1 M is necessary to extract manganese, but in the presence of zephiramine manganese is quantitatively extracted with a lower concentration of oxine (Fig.II-17).

Effect of zephiramine concentration and composition of the extracted chelate

The same effect of zephiramine on manganese extraction is observed as in the extraction of nickel (Fig.II-16). Effect of the concentration of zephiramine is shown in Figs.II-18 and II-19, and it is easily concluded that manganese is extracted as a chelate of $\text{Mn}(\text{OX})_3(\text{zeph})$ in the presence of zephiramine. The continuous variations method gives the same result (Fig.II-20).

3-1-5. Copper

The coordination number of copper is ordinarily four, and its chelate with oxine has a composition of $\text{Cu}(\text{OX})_2$. This chelate is a coordination-saturated chelate which is easily extracted in the absence of zephiramine. Moreover, the composition of the extracted Cu-chelate does not change with the addition of zephiramine. The Cu-oxine chelate has an absorption maximum at 410 nm, and its absorption coefficient is 5.6×10^3 in either the absence or the presence of zephiramine (Fig.II-21). As shown in Fig.II-2, copper is easily extracted at a low pH region.

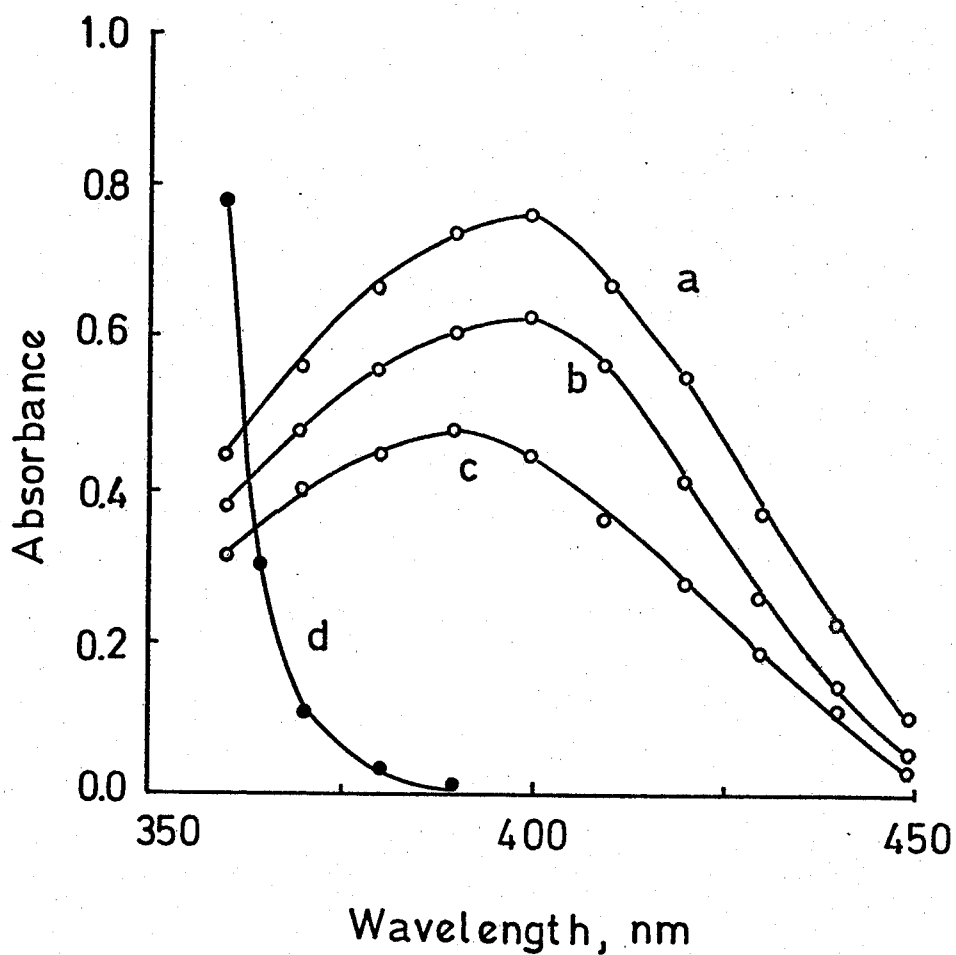


Fig. II-16. Absorption spectra of Mn-oxine chelate and reagent blank.

$[\text{Mn}]_{\text{aq}} = 1 \times 10^{-4} \text{ M}$, $[\text{HOX}]_{\text{org}} = 1 \times 10^{-2} \text{ M}$, and $\text{pH} = 9.2$.

(a) Mn-oxine chelate vs. reagent blank in the presence of $1 \times 10^{-3} \text{ M}$ zephiramine, (b) Mn-oxine chelate in the presence of $4 \times 10^{-4} \text{ M}$ zephiramine, (c) Mn-oxine chelate in the absence of zephiramine, (d) reagent blank vs. water.

$V_{\text{org}} = V_{\text{aq}} = 20 \text{ ml}$.

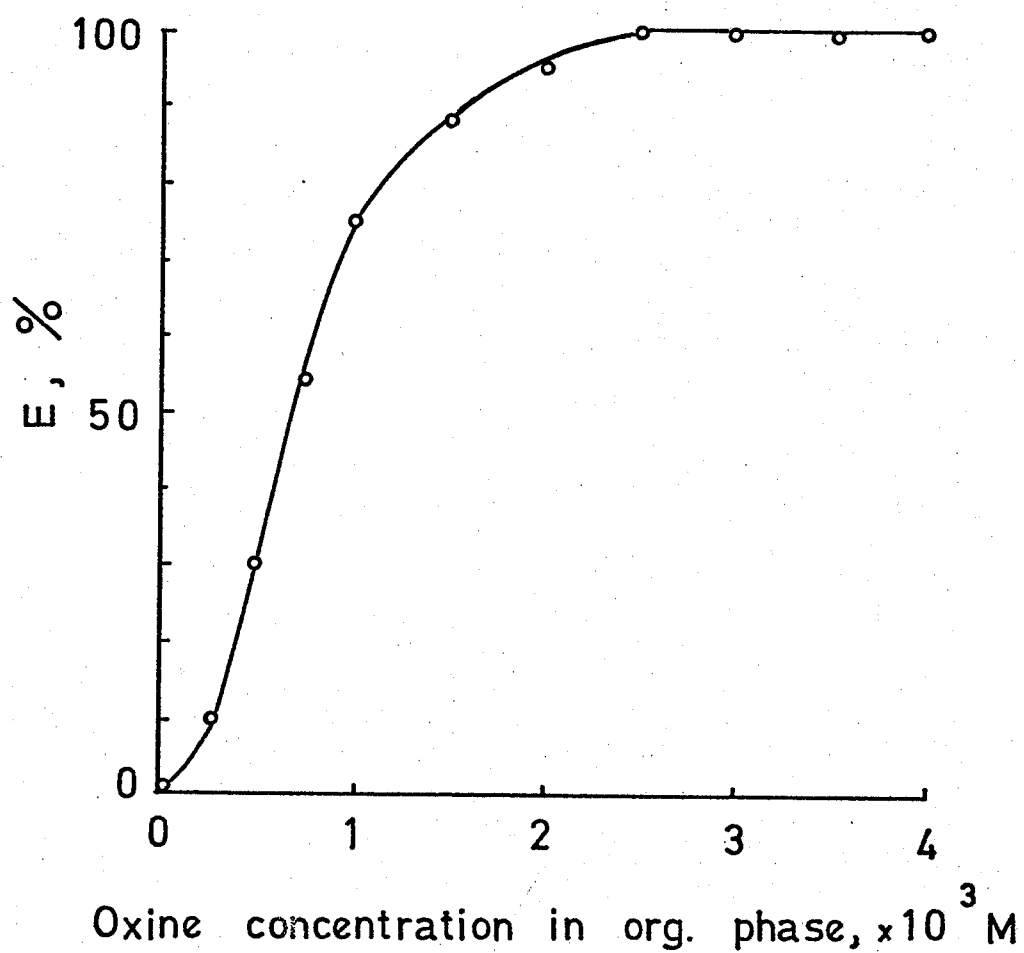


Fig. II-17. Effect of concentration of oxine on extraction of Mn-oxine chelate in the presence of zephiramine.

[zeph] = 1×10^{-3} M.

The others are the same with those in Fig. II-16.

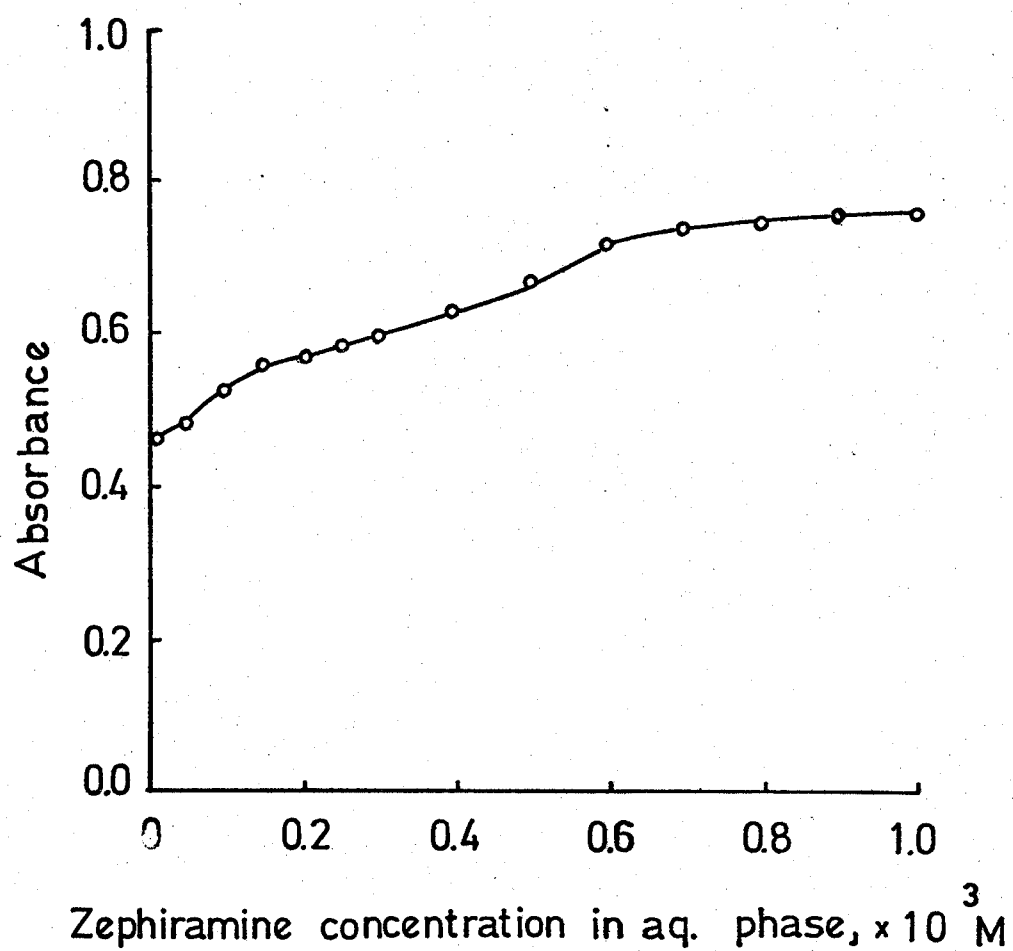


Fig. II-18. Effect of concentration of zephiramine on the absorbance of Mn-oxine chelate.

Conditions are the same with those in Fig. II-16.

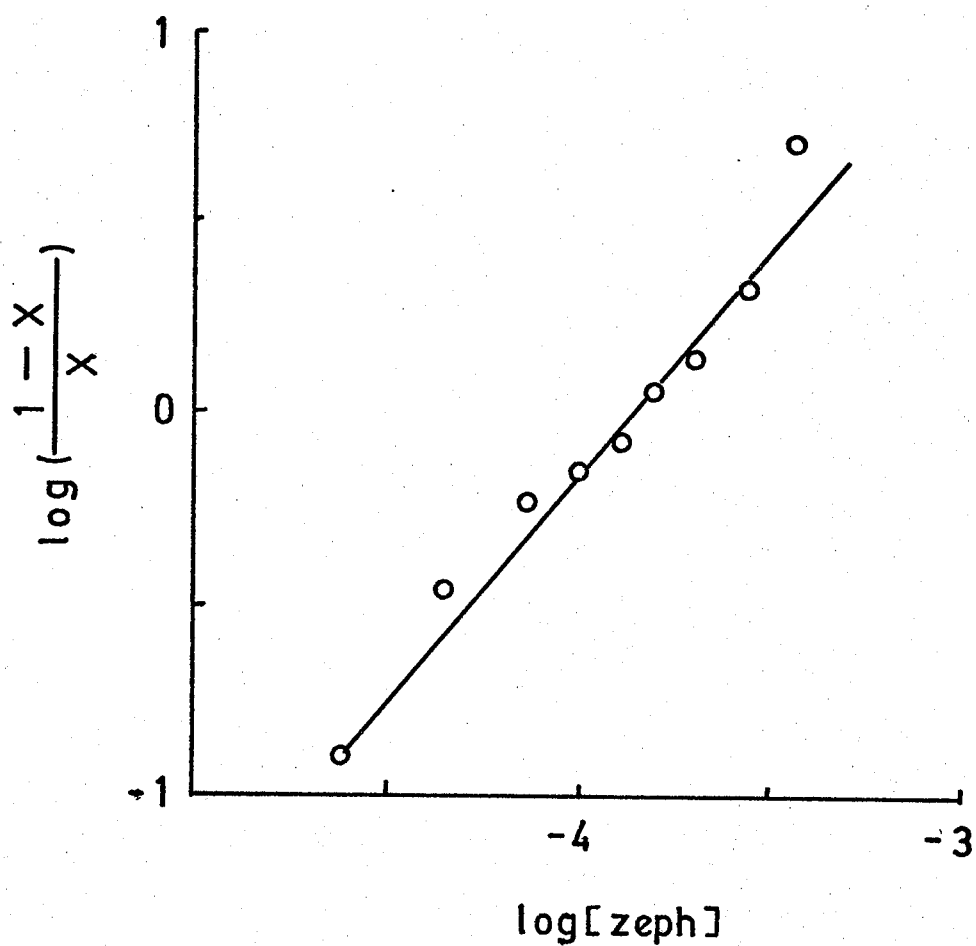


Fig. II-19. Zephiramine dependence on formation of Mn-oxine-zephiramine chelate from Mn-oxine chelate.

Conditions are the same with those in Fig. II-16.

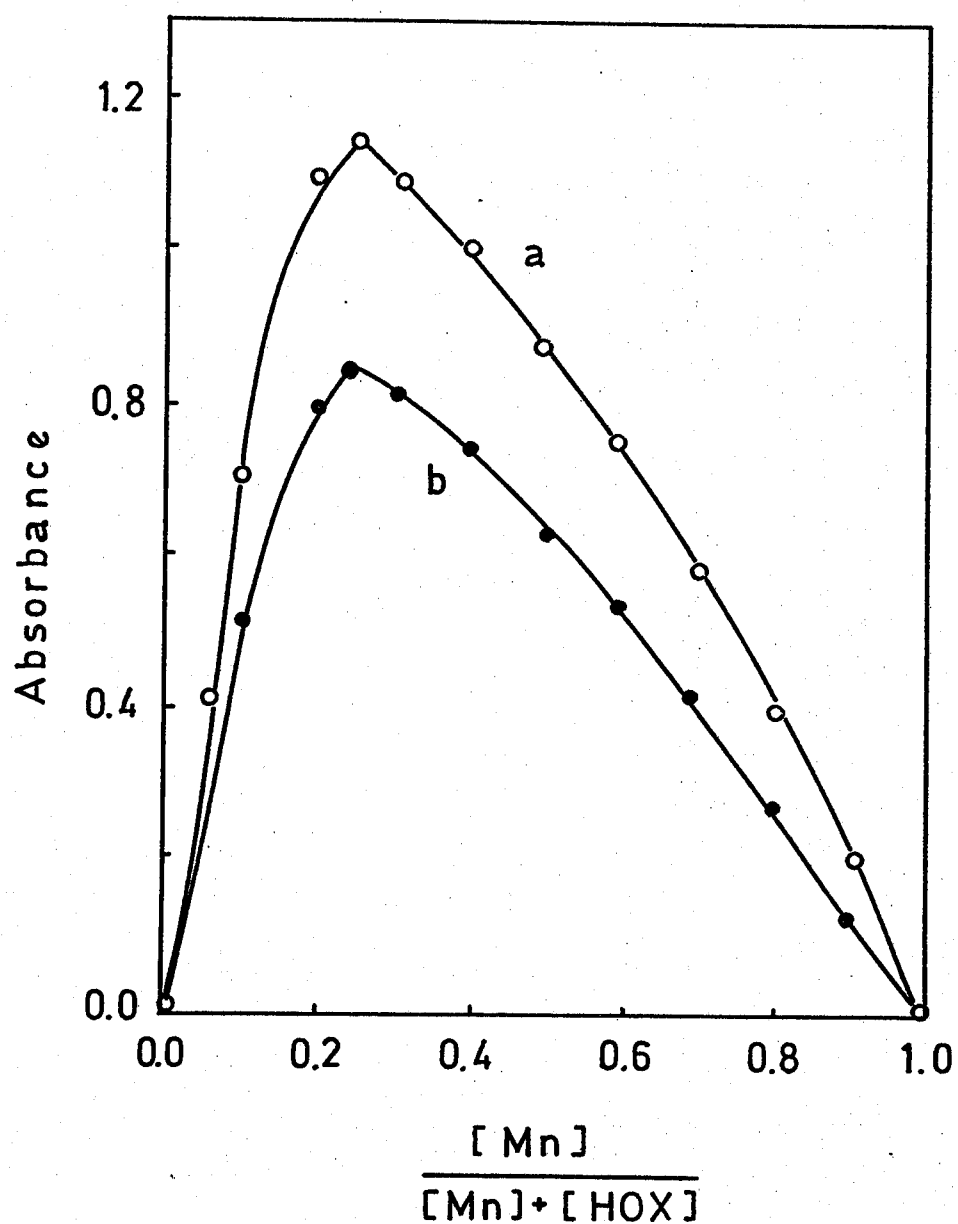


Fig. II-20. Continuous variations method for Mn-oxine chelate.

$[Mn] + [HOX] = 1 \times 10^{-3} \text{ M}$, $[zeph] = 5 \times 10^{-3} \text{ M}$,
and $\text{pH} = 8.5$.

(a) Absorbance at 400 nm, and (b) absorbance
at 420 nm.

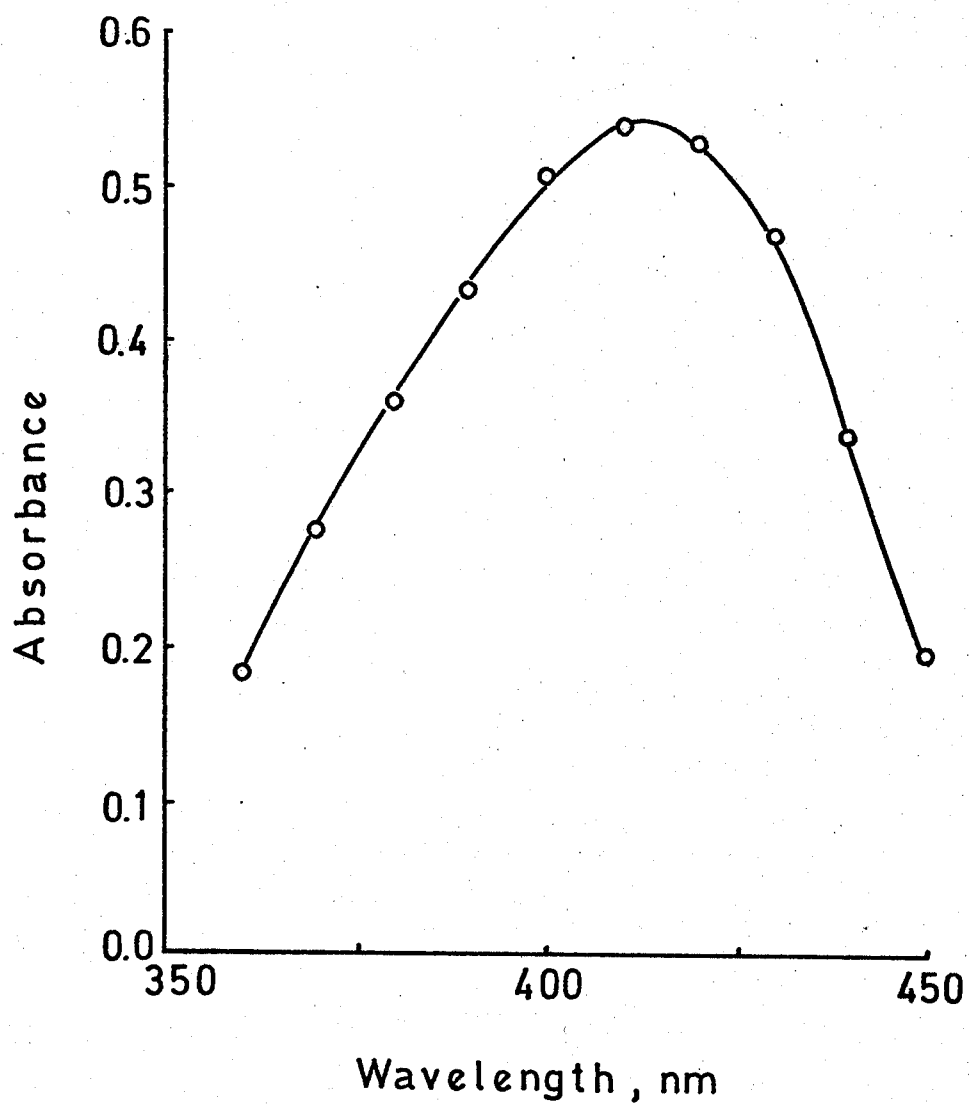


Fig. II-21. Absorption spectrum of Cu-oxine chelate in the absence of zephiramine.

$[\text{Cu}]_{\text{aq}} = 1 \times 10^{-4} \text{ M}$, $[\text{HOX}]_{\text{org}} = 1 \times 10^{-2} \text{ M}$,
and $\text{pH} = 4.7$.

$V_{\text{org}} = V_{\text{aq}} = 20 \text{ ml}$.

3-2. Determination by atomic absorption spectrophotometry

As stated in Chap.3-1, we can simultaneously extract five divalent heavy metals with oxine at a pH higher than 8.0 in the presence of zephiramine. These same metals have been extracted and concentrated from sea water by the above proposed method, and have been measured by atomic absorption spectrophotometry after back-extraction into an aqueous solution.

3-2-1. Distribution coefficients of the metal oxinate between sea water and 1,2-dichloroethane

The sensitivity of the whole method depends upon the ratio of seawater sample to the amounts of extractive solvent and back-extractive aqueous solution, and the success depends also upon separability of the desired elements from the bulk constituents. The method is dependent on the use of large seawater/solvent ratios in order to achieve a concentration effect as well as separation from the bulk constituents. When a large seawater/solvent ratio is used, the extraction-percentages of the metals will decrease, since the relationship among the distribution coefficient (D), the extraction-percentage (E), and the volumes of organic phase (V_{org}) and aqueous phase (V_{aq}) is given by the expression:

$$E = \frac{100 D}{D + V_{aq}/V_{org}}$$

This relationship is graphically illustrated in Fig.II-22. As shown in Fig.II-2, the distribution ratios of five metals

are approximately 1000 above pH 8.0. For subsequent experiments, a ratio of 25 is used.

3-2-2. Zephiramine concentration

Zephiramine is a positively charged large ion and has a tendency to associate with a negatively charged ion, for example, sulfate in seawater. Therefore, a higher concentration of zephiramine is required for the extraction of metals in seawater than in distilled water (Fig.II-23). So, 20 ml of 5×10^{-2} M zephiramine solution must be added to 500-ml seawater sample for complete extraction.

3-2-3. Back-extraction

In atomic absorption measurements, some organic solvents are not suitable to be burned in the flame because of the formation of interfering products. To avoid such an interference and increase the extraction efficiency, a back-extraction technique with an aqueous solution was tried.

When the pH of a solution is lower than 1, the metal ions investigated do not form any oxinate (Fig.II-2). If 20 ml of dichloroethane phase containing metal oxinate is shaken with 5 ml of 2 N nitric acid solution ($\text{pH} < 0$), the metals are completely back-extracted into the nitric acid phase. Finally, the metal ions in the nitric acid solution are determined by atomic absorptiometry.

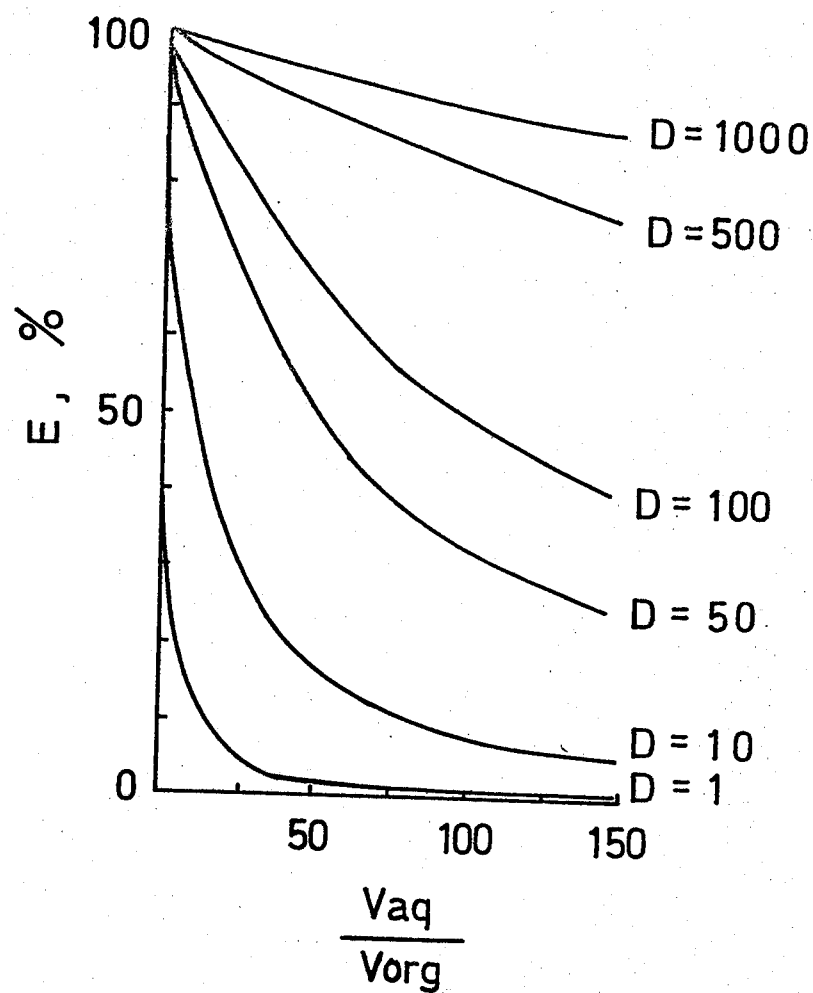


Fig. II-22. Percentage of extraction as a function of phase ratio for each distribution coefficient.

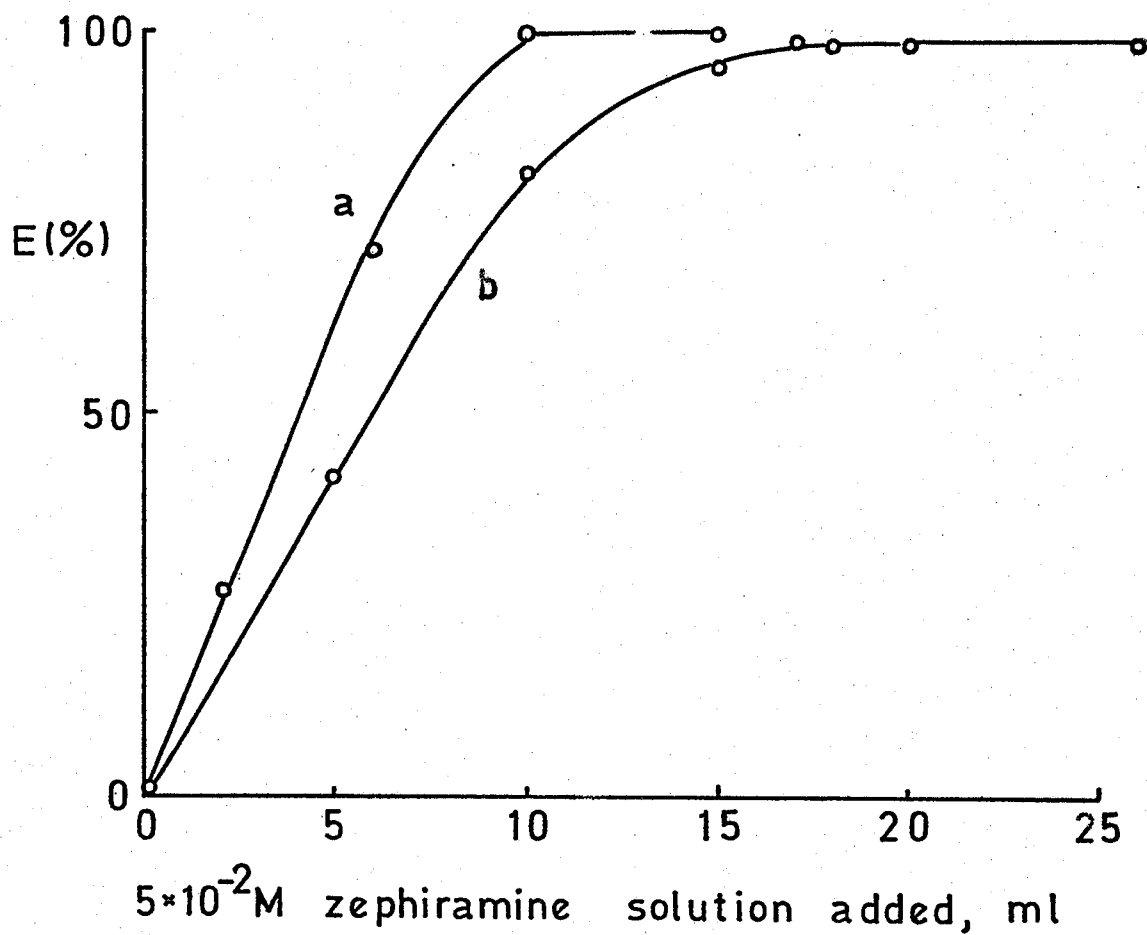


Fig. II-23. Effect of concentration of zephiramine on extraction of metals from artificial seawater.

$[\text{Zn}] = [\text{Cd}] = 10 \text{ ppb}$, $[\text{HOX}]_{\text{org}} = 1 \times 10^{-2} \text{ M}$, and $\text{pH} = 8.1$.

$V_{\text{aq}} = 500 \text{ ml}$ and $V_{\text{org}} = 20 \text{ ml}$.

(a) extraction from distilled water, and

(b) extraction from artificial seawater.

3-2-4. Recommended procedure

A seawater sample is acidified with the addition of hydrochloric acid (the final pH is about 1) and stored in a polyethylene bottle.⁵⁰⁻⁵² The seawater sample (1 l) is divided into two portions. Aliquots of 500 ml are placed in 1-l separatory funnels and the pH is adjusted to about 8.2 with the addition of sodium hydroxide and borate buffer solution. Twenty ml of 5×10^{-2} M zephiramine solution is then added to each funnel followed by 20 ml of oxine-1,2-dichloroethane solution (1×10^{-2} M). The funnel is shaken for 10 min. by a mechanical shaker. After phase separation, each organic phase is combined and transferred to a 100-ml separatory funnel. After the addition of 5 ml of the 2 N nitric acid solution, the funnel is shaken for 3 min. After the aqueous phase is separated, concentration of each metal back-extracted into the aqueous phase is determined by atomic absorption spectrophotometry.

The conditions of the atomic absorption spectrophotometry are summarized in Table II-2.

3-2-5. Calibration curve

Before use of an artificial seawater, the metals which are contained from the reagents used in preparation of the artificial seawater⁵³ are taken out by the same oxine-extraction method as applied to seawater samples. An aliquot of 500 ml of the artificial seawater is placed in a 1-l separatory funnel, and increments of the five metals are added. The metals

Table II-2

Conditions of atomic absorption spectrophotometry.

Parameter	Zn	Cd	Ni	Mn	Cu
Line used, Å	2139	2289	2320	2795	3247
Lamp current, mA	15	10	15	15	10
Absorption sense	150	200	145	240	220
Detection limit, ppb	0.18	0.2	1.0	0.15	0.3

Atomic absorption spectrophotometer: Hitachi 207 Atomic Absorption Spectrophotometer

Flame: Air (1.8 kg/cm², 13 l/min)-acetylene(0.4 kg/cm², 3 l/min)

are determined by the procedure mentioned above. The calibration curves are shown in Fig. II-24.

3-2-6. Precision of the method

The precision of the method was tested by replicate analyses of known concentrations of the metals added to seawater. After a seawater sample was analysed by the proposed method, known concentrations of five metals were added and recoveries of the metals were determined. The results are summarized in Table II-3, and good accuracy and reproducibilities were achieved for all metals except manganese.

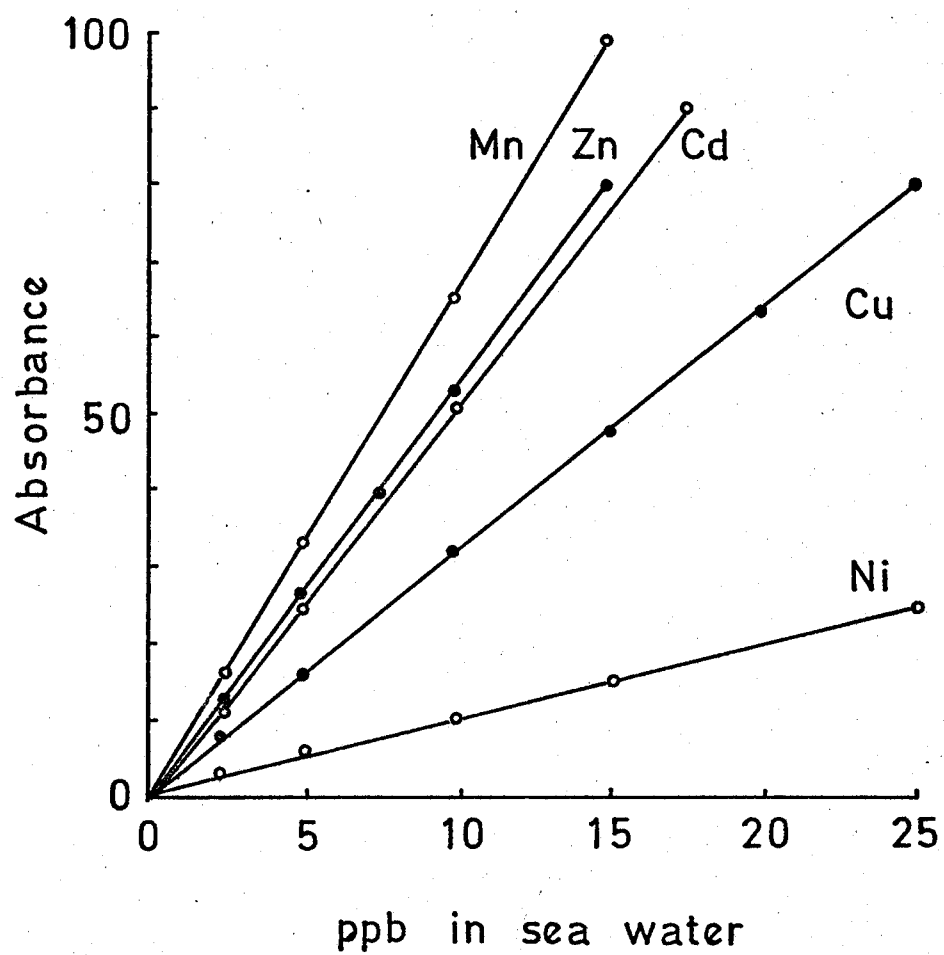


Fig. II-24. Calibration curves for the metals.

Table II-3

Recovery of metals added to seawater.^a

	Zn	Cd	Ni	Mn	Cu
Original concentration, $\mu\text{g/l}$	5.0 ± 0.2	<0.2	<1.0	0.3 ± 0.1	0.5 ± 0.1
Metal added, $\mu\text{g/l}$	4.9	2.5	5.8	5.5	4.7
Recovery, % ^b	98 ± 3	98 ± 1	98 ± 2	78 ± 1	99 ± 1

a The sample is the surface water of Mutsu Bay

b Average of 6 determinations

3-3. Application to sea water

The proposed technique was applied to the determination of five elements in seawater. The seawater samples were taken from the surface waters of Tachimachi in Hakodate, Hakodate Bay, Mutsu Bay and Eastern China Sea, and the sample waters were acidified and stored in polyethylene bottles. In the laboratory, the samples were analyzed against standards prepared in the similar matrix, and the results are shown in Table II-4. For the metal contents of the surface waters which were analysed, no pronounced differences could be detected in concentration when compared with the data for world-wide surface waters by R. Chester and J. H. Stoner.³¹

Table II-4

Concentration of trace metals in surface seawater from the various regions (ppb).

		Zn			Cd			Ni			Mn			Cu		
		1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Hakodate Bay (Sep., 1974)	A	6.2	6.2	6.0	<0.2	<0.2	0.2	1.5	2.0	1.0	0.5	0.4	0.5	0.6	0.3	0.6
	B	5.8	5.8	6.0	0.2	0.2	0.2	1.5	2.0	2.0	0.2	0.15	0.15	0.5	0.3	0.3
	C	6.0	6.0	6.0	<0.2	<0.2	<0.2	1.0	1.0	1.0	0.4	0.2	0.2	0.5	0.3	0.3
Tachimachi (Sep., 1974)	A	4.0	4.2	4.8				1.0	2.0	1.0	0.2	0.15	0.2	0.5	0.6	0.5
	B	3.8	3.6	3.6		<0.2		1.0	1.0	1.0	0.2	<0.15	<0.15	0.8	0.8	0.8
	C	3.4	3.4	3.4				1.0	1.0	1.0	0.2	0.2	0.2	0.5	0.5	0.5
Mutsu Bay (June, 1972)	A	9.5	6.3	6.3				1.0	<1.0	1.0	1.2	0.6	0.6	0.5	0.6	0.6
	B	5.1	4.8	4.8		<0.2		<1.0	<1.0	<1.0	0.3	0.3	0.2	0.5	0.5	0.5
	C	7.5	7.3	7.5				1.0	1.0	1.0	1.0	0.9	0.9	0.8	0.6	0.6
Eastern China Sea (Feb., 1975)	A	4.0	4.0	4.0				<1.0	<1.0	<1.0	0.4	0.3	0.5	1.2	1.0	1.0
	B	5.5	5.3	5.3		<0.2		1.5	1.0	1.0	1.9	1.5	1.5	6.8	5.6	6.3
	C	3.8	3.6	3.0				1.0	<1.0	<1.0	0.2	<0.15	<0.15	0.8	0.6	0.8

SUMMARY

PART I.

A nonextractable metal chelate, which is a valence-saturated but coordination-unsaturated chelate and has not been used for determination, separation and concentration of that metal, changes to an extractable chelate with the addition of a quaternary ammonium base. This finding suggests the possibility of extraction of many trace metals from natural waters with good results for separation and concentration of metals, from the bulk composition of the waters.

GHA chelate

1. The nonextractable Ca-GHA chelate can be changed to an extractable one with the addition of zephiramine, and the color of the extracted chelate is stable for at least 6 hours.
2. The ordinary nonextractable chelate has a composition of $\text{Ca}(\text{GHA})$, while the extracted chelate has a composition of $\text{Ca}(\text{GHA})_2$.
3. Molar absorption coefficient is 1.3×10^4 at 530 nm, and the proposed method is more sensitive than the previous method, in which the molar absorption coefficient is 8×10^3 .
4. Sensitivity by Sandell's expression is $0.003 \mu\text{g cm}^{-2}$, and it would be possible to determine a trace of calcium in various natural samples.

SAPH chelates

1. Nickel or zinc forms a 1 : 1 unstable and nonextractable chelate with SAPH, but this chelate changes to the stable and

extractable chelate having a composition of $M(SAPH)_2(zeph)_2$ in the presence of zephiramine.

2. The extracted chelates of nickel and zinc have absorption maxima at 440 nm and 425 nm, and molar absorption coefficients are 2.4×10^4 and 8.4×10^3 , respectively.

TTA chelates

1. The nonextractable chelate of nickel and cobalt, $M(TTA)_2$, also changes to extractable chelate having the composition of $M(TTA)_3(Q.A.B.)$ in the presence of a quaternary ammonium base (Q.A.B.).

Oxine chelate

1. The nonextractable magnesium chelate, $Mg(oxine)_2$, changes to the extractable chelate, $Mg(oxine)_3(zeph)$, in the presence of zephiramine.

2. The extracted chelate has an absorption maximum at 390 nm, and the molar absorption coefficient is 6.5×10^3 .

PART II.

A method for simultaneous extraction of five heavy metals was achieved by using chelation with oxine in the presence of zephiramine. After extraction and concentration by the proposed method, the five heavy metals in seawater were determined by atomic absorption spectrophotometry.

1. The five heavy metals in seawater, zinc, cadmium, nickel, manganese and copper, are simultaneously extracted in the presence of zephiramine at the pH higher than 8.0.

2. The extracted chelate of Zn, Cd, Ni or Mn has a composition of $M(oxine)_3(zeph)$.

3. In the proposed extraction-atomic absorption spectrophotometric method, the detection limits are as follows:

Zn = 0.18 ppb, Cd = 0.2 ppb, Ni = 1 ppb, Mn = 0.15 ppb, and Cu = 0.3 ppb.

4. The proposed method has the advantages in its speed, relatively good precision and high sensitivity.

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APPENDIX I. Apparatus used

Spectrophotometer: Hitachi Perkin-Elmer 139 UV-VIS Spectrophotometer and Hitachi Model 101 Spectrophotometer.

Atomic Absorption Spectrophotometer: Hitachi 207 Atomic Absorption Spectrophotometer.

pH Meter: Hitachi-Horiba F-5_{ss} pH Meter.

Mechanical Shaker: Iwaki Model KM Shaker.

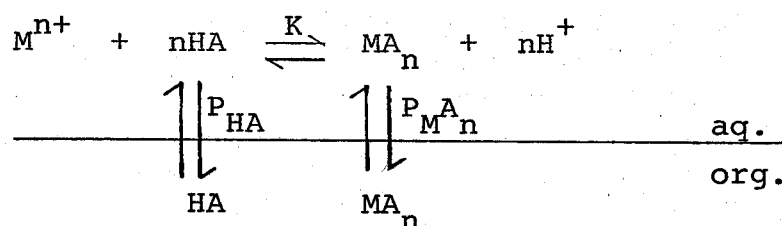
APPENDIX II. Method for determination of composition of an extracted metal chelate

In order to consider the chemical mechanism of chelate formation, it is necessary to determine the composition of the chelate formed. In this study, the composition of a metal chelate is mainly determined by continuous variations method and equilibrium shift method.

II-1. Continuous variations method

In this method, the concentrations of the two reaction components are varied, while the sum of the concentration is kept constant. The molar ratio of concentrations of the added components shown at the maximum absorbance is equal to the compositional ratio of the complex.

We consider the following extraction scheme:



The formation constant, K , and the partition coefficients, P_{HA} and P_{MA_n} , are given by the expressions:

$$K = \frac{[MA_n][H^+]^n}{[M^{n+}][HA]^n} \quad (1)$$

$$P_{HA} = \frac{[HA]_{org}}{[HA]} \quad (2)$$

$$P_{MA_n} = \frac{[MA_n]_{org}}{[MA_n]} \quad (3)$$

At the system containing x C moles of the metal and $(1 - x)$ C moles of the reagent, let us denote the concentration of the complex in aqueous phase $[MA_n]$ by αC , then the concentration in the organic phase $[MA_n]_{org}$ is expressed as $\alpha C P_{MA}$. And, the equation (1) are written as follows:

$$K = \frac{\alpha C (1 + P_{HA})^n [H^+]^n}{[xC - \alpha C (1 + P_{MA})][(1 - x)C - n\alpha C (1 + P_{MA})]^n} \quad (4)$$

Equation (4) is differentiated with respect to x at constant pH:

$$\begin{aligned} \frac{\partial K}{\partial x} = 0 = \frac{\partial \alpha}{\partial x} \left[\frac{1}{\alpha} + \frac{1 + P_{MA}}{x - \alpha(1 + P_{HA})} + \frac{n^2(1 + P_{MA})}{1 - x - n\alpha(1 + P_{MA})} \right] \\ - \frac{1}{x - \alpha(1 + P_{MA})} + \frac{n}{1 - x - n\alpha(1 + P_{MA})} \end{aligned} \quad (5)$$

At the extremum, $\partial \alpha / \partial x = 0$. Then, from equation (5) we obtain

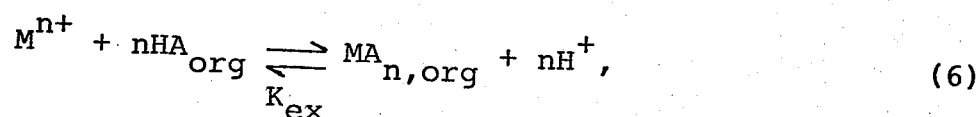
$$n = \frac{1 - x}{x}.$$

Thus, the n in the chelate, MA_n , is given by the ratio of the

molar fractions of the components at the extremum. Fig.App.-1 shows the typical curve for MA_n . This method can be also used to determine the composition of mixed chelate compounds as $M(A)_n(B)_m$.

II-2. Equilibrium shift method

This is a highly useful method for determining the composition of an extracted chelate compound, based on the concept of the extraction constant. If the extraction process is described by the equation



the extraction constant is

$$K_{ex} = \frac{[MA_n]_{org} [H^+]^n}{[M^{n+}] [HA]_{org}^n} \quad (7)$$

Equation (7) is more conveniently written in logarithmic form,

$$\log K_{ex} = \log \frac{[MA_n]_{org}}{[M^{n+}]} - npH - n\log[HA]_{org} \quad (8)$$

The ratio $[MA_n]_{org}/[M^{n+}]$ is experimentally determined, provided that the metal gives no compound other than MA_n with the reagent. From equation (8), we obtain

$$\log D = \log K_{ex} + npH + n\log[HA]_{org}$$

Thus if the reagent concentration is varied at a constant pH value, the plotting of $\log D$ as a function of $\log [HA]_{org}$ gives a straight line with the slope of n (Fig.App.-2). This method

can also be successfully applied to determine the composition of a complex of type MA_nB_m .

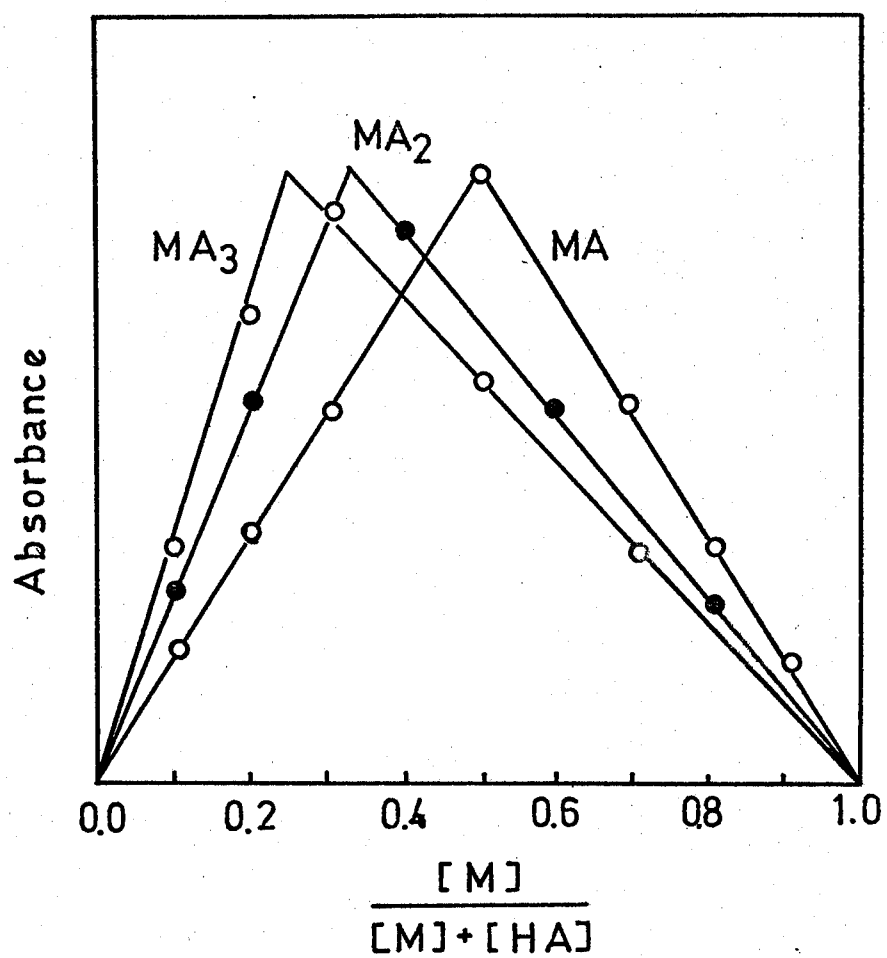


Fig. App.-1. Determination of composition of extracted chelate by continuous variations method.

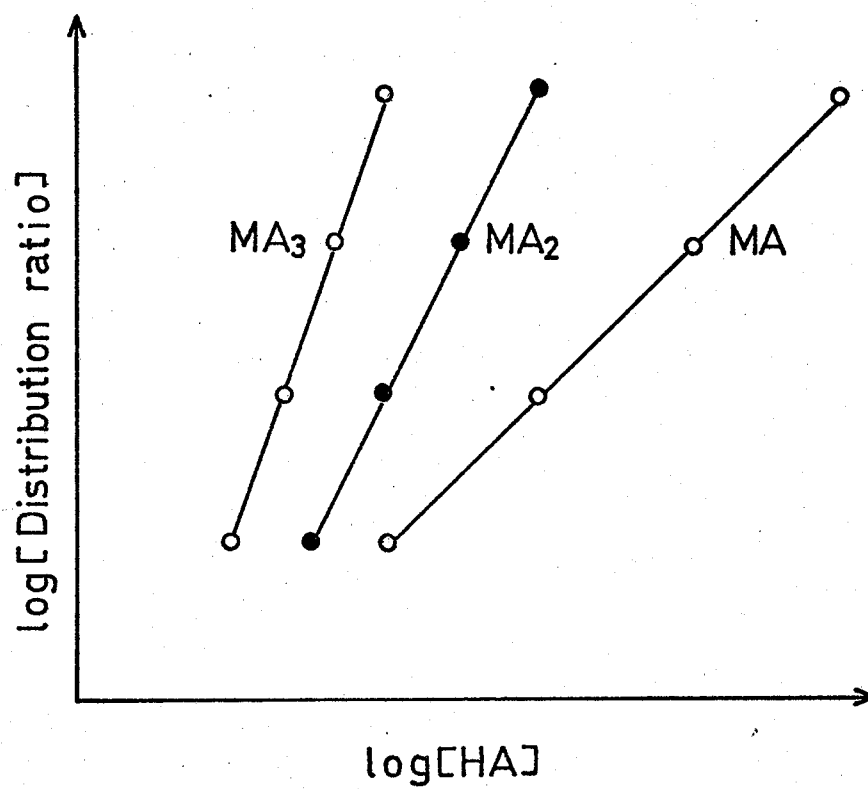


Fig. App.-2. Determination of composition of extracted chelate by equilibrium shift method.