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*Microchem. J.* **25,** 1−7 (1980)

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The spectrophotometric determination of vanadium(V) as a mixed thiocyanate-3-hydroxy-2-methyl-1-phenyl-4-pyridone complex and as a mixed thiocyanate-3-hydroxy-2-methyl-1-(4-tolyl)-4-pyridone complex is described.

*Microchem. J.* **25,** 8−13 (1980)

**Analytical Applications of Biacetyl Bis(4-Phenyl-3-Thiosemicarbazone) and Bipyridylglyoxal Bis(4-Phenyl-3-thiosemicarbazone).** A. G. ASUERO AND M. GONZALEZ-BALAIRON, *Department of Applied Chemical Analysis, Faculty of Pharmacy, The University of Seville, Seville-4, Spain.*

This paper is the continuation of the study and applications of the two compounds. The determinations of cadmium and bismuth are described and the formations of cobalt−nickel and copper−nickel complexes have been reported.

*Microchem. J.* **25,** 14−45 (1980)

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*Microchem. J.* **25,** 46−47 (1980)

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*Microchem. J.* **25**, 48−54 (1980).

**Polarography of o- and p-Nitrophenyl Acetic Acids.** P. S. RAGHAVAN, M. S. SETHI, B. S. GARG, AND MAHENDRA K. GADIA,* *Department of Chemistry, A.R.S.D. College, New Delhi—110021, India, and *Atlantic Analytical Services Limited, P.O. Box 489, Springdale, Newfoundland, AOJ 1TO, Canada.*

Polarography was carried out in aqueous buffers of constant ionic strength. The nitro group underwent a diffusion-controlled reduction over the whole pH range.

*Microchem. J.* **25**, 55−60 (1980).

**Determination of Phosphatidylcholine in Amniotic Fluid.** HUGH Y. YEE, TINA M. L. YEE,* AND BOBETTE JACKSON, *Departments of Pathology and *Obstetrics/ Gynecology, 4707 St. Antoine, Detroit, Michigan 48201.*

The method requires 1 ml or less of sample for fluids having a total phospholipid concentration greater than 25 mg/liter. Following extraction with chloroform-methanol, the solvent is passed through a column of hydroxylapatite that removes all acidic phospholipids and allows the passage of phosphatidylcholine and sphingomyelin. Hydrolysis with periodate-sulfuric acid selectively releases inorganic phosphate from the phosphatidylcholine.

*Microchem. J.* **25**, 61−71 (1980).

**A New Method of Determining Chlorine and Bromine in Compounds of the Platinum Group Metals by the Oxygen Flask.** P. BORDA, *Department of Chemistry, The University of British Columbia, Vancouver, British Columbia, V6T 1W5, Canada.*

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*Microchem. J.* **25**, 72−74 (1980).

**Solvent Extraction and Spectrophotometric Determination of Palladium(II) with Some Nitrogen-Containing Heterocyclic Hydrazones in the Presence of Chloride Ions.** MAKOTO OTOMO AND ICHIRO NAKAYAMA, *Department of Synthetic Chemistry, Nagoya Institute of Technology, Showa-ku, Nagoya 466, Japan.*

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*Microchem. J.* **25**, 75–81 (1980).

**The Recovery of Prostaglandins and Other Related Metabolites of Arachidonic Acid from Human Blood Plasma.** K. C. SRIVASTAVA, K. P. TIWARI, AND R. K. BANSAL, *Department of Community Health and Environmental Medicine, Odense University, J. B. Winsløwsvej 19, DK 5000 Odense C, Denmark.*

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*Microchem. J.* **25**, 82–85 (1980).

**Simple and Specific Microdetermination of Organoiodine via Oxygen Flask Combustion.** F. W. CHENG, *ICI Americas Inc., Wilmington, Delaware 19897.*

Oxygen flask combustion is followed by an argentimetric titration with an iodide ion-selective electrode as indicator. Some advantages of this procedure over mercurimetric titration are detailed.

*Microchem. J.* **25**, 86–92 (1980).

**Spectrophotometric Estimation of Microgram Amounts of Sodium *N*-Chloro-*p*-toluenesulfonamide (Chloramine-T) and Sodium *N*-Chlorobenzenesulfonamide (Chloramine-B).** NETKAL M. MADE GOWDA, V. M. SADAGOPA RAMANUJAM, AND NORMAN M. TRIEFF, *Division of Environmental Toxicology, Department of Preventive Medicine and Community Health, University of Texas Medical Branch, Galveston, Texas 77550.*

An ultraviolet spectrophotometric method for the assay of microgram amounts (1–80 $\mu$g/ml) of the substances in aqueous solution is described.

*Microchem. J.* **25**, 93–97 (1980).

**Allethrin Labeled with Tritium.** W. B. BURTON, *Metabolism and Residue Analysis Department, Biological Sciences Research Center, Shell Development Company, A Division of Shell Oil Company, P.O. Box 4248, Modesto, California 95352.*

Radioactive allethrin labeled with tritium was prepared by the catalytic exchange process producing a material that was approximately 50% pure. Twenty milligrams was purified on silica gel layers yielding 14 mg of [$^3$H]allethrin, specific activity 61 mCi/mmol. Its purity, chemical and radiochemical as determined by proven methods, was $97 \pm 1\%$ m/m.

*Microchem. J.* **25**, 98–101 (1980).


**A New Method for the Determination of Magnesium and Its Application to Natural Waters.** M. TERNERO AND F. PINO, *Department of Analytical Chemistry, Faculty of Sciences, University of Sevilla, Sevilla, and* D. PÉREZ-BENDITO AND M. VALCÁRCEL, *Department of Analytical Chemistry, Faculty of Sciences, University of Córdoba, Córdoba, Spain.*

A kinetic method is described for the determination of trace amounts of magnesium in the presence of calcium. The procedure is based on the inhibition of magnesium-catalyzed aerial oxidation of the 1,4-dihydroxyphthalimide dithiosemicarbazone reaction by traces of magnesium.

*Microchem. J.* **25**, 102–110 (1980).


**A Contribution to the Use of Tetravalent Manganese in Solutions of Sulfuric Acid as An Oxidimetric Reagent.** A. BERKA, J. BAREK, AND M. CAFOURKOVÁ, *Department of Analytical Chemistry, Charles University, Albertov 2030, 128 40 Prague 2, Czechoslovakia.*

The preparation of solutions of manganese(IV) sulfate in 9 *M* sulfuric acid was studied for the 0.005–0.1 *M* concentration range of manganese(IV) ions and the stability of these solutions was studied. It was verified that manganese(IV) sulfate can be used for determining low concentrations of organic substances by direct or indirect titrations.

*Microchem. J.* **25**, 111–116 (1980).


**Spectrophotometric Method for the Determination of Microgram Amounts of Titanium Using an Extraction Technique with Diphenylglyoxal bis(2-Hydroxybenzoylhydrazone).** M. SILVA AND M. VALCÁRCEL, *Department of Analytical Chemistry, Faculty of Sciences, University of Cordoba, Cordoba, Spain.*

The reagent forms an orange-yellow complex with titanium(IV) in weakly acid solution. This complex can be extracted into benzyl alcohol in 0.1 *N* sulfuric acid. The extracted complex exhibits an absorption maximum at 500 nm with a molar absorptivity of $1.5 \times 10^4$ $M^{-1}$ cm$^{-1}$. A stoichiometric ratio of 1:3 is indicated.

*Microchem. J.* **25**, 117–123 (1980).

**The Detection of Potassium Sulfate in Excess of Potassium Pyrosulfate.** B. J. MEEHAN, S. A. TARIQ, AND R. J. MAGEE, *Department of Inorganic and Analytical Chemistry, La Trobe University, Bundoora, Victoria 3083, Australia.*

The detection of $K_2SO_4$ in excess of $K_2S_2O_7$ is successfully carried out when the concentration of $K_2SO_4$ is greater than 1% by weight in solid solutions through the characteristic sulfate band at 981 $cm^{-1}$ in the Raman spectrum of solid samples.

*Microchem. J.* **25,** 124–128 (1980).

**The Polarography of Oximes. I: 9,10-Phenanthraquinone Monoxime.** M. S. SETHI, P. S. RAGHAVAN, B. S. GARG, AND R. P. SINGH, *Department of Chemistry, University of Delhi, Delhi - 110007, India, and* MAHENDRA K. GADIA, *Atlantic Analytical Services Ltd., P. O. Box 489, Springdale, Newfoundland A0J 1T0, Canada.*

Polarography of the oxime was done in buffers of constant ionic strength. The oxime group underwent diffusion-controlled reduction over the whole pH range (3.50–13.40).

*Microchem. J.* **25,** 129–135 (1980).

# Investigations of the Thermal Decomposition of Polyvinyl Chloride by Means of Gas Chromatography

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## INTRODUCTION

The course of thermal decomposition of polyvinyl chloride has already been widely investigated. The patterns of decomposition products are fairly differentiated and depend on both the reaction conditions and on the nature of accompanying substances (*1 −3, 6, 7, 9−13*). The obtained reaction products, hydrochloride being the main one, strongly influence the kinetics and mechanism of the discussed process (*3 −5, 8, 10*). These facts explain the necessity of further investigations aiming at a better understanding of the decomposition process of polyvinyl chloride.

This paper discusses thermal decomposition of polyvinyl chloride with particular attention on chromatographic identification of the obtained reaction products and the role of oxygen in the process.

## EXPERIMENTAL

*Characteristics of the Applied Polyvinyl Chloride (PVC)*

Polymeric, emulsive PVC (in form of a powder): E 68 (in the range of the K number from 68 to 72); flow weight, 0.56 g/cm³; volatile part, 0.3% (mainly moisture); thermal stability at 180°C, 32 min; PVC stabilized with the 0.3% addition of $Na_2CO_3$.

*Conditions of Thermoxidative Separation and of GC Analysis*

Thermal decomposition of the polymeric PVC E 68 was performed inside the silite oven in a stream of air, its flow rate 1 cm sec⁻¹, the reaction time 15 min, at 300, 400, 500, and 600°C.

The sample of 1000 ± 5 mg in a quartz vessel was introduced into a quartz pipe placed in an oven at a demanded temperature. The temperature regulation of the oven allowed a measurement correctness in the

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TABLE 1
THE PRODUCTS OF THERMOXIDATIVE DECOMPOSITION DERIVED FROM THE
POLYMERIC PVC E 68 AT 300−600°C

| Number | The determined decomposition product | The determined amount of decomposition product [mg g$^{-1}$] The temperature of thermoxidative decomp. [°C] | | | |
|---|---|---|---|---|---|
| | | 300 ± 10 | 400 ± 10 | 500 ± 10 | 600 ± 10 |
| 1 | HCl | 551.00 | 562.30 | 563.10 | 562.60 |
| 2 | $CO_2$ | 20.79 | 115.57 | 234.30 | 253.63 |
| 3 | CO | 6.69 | 33.93 | 53.18 | 49.42 |
| 4 | $CH_4$ | 0.31 | 4.03 | 7.19 | 8.20 |
| 5 | $C_2H_6$ | 0.16 | 2.55 | 3.47 | 3.74 |
| 6 | $C_2H_4$ | 0.92 | 2.21 | 3.75 | 4.68 |
| 7 | $C_3H_8$ | 0.12 | 0.89 | 1.28 | 1.47 |
| 8 | $C_3H_6$ | 0.16 | 1.07 | 1.99 | 2.94 |
| 9 | $\Sigma C_4$ | 0.39 | 1.91 | 2.45 | 2.83 |
| 10 | $\Sigma C_5$ | 0.81 | 2.95 | 3.89 | 4.53 |
| 11 | $\Sigma C_6$ | 0.22 | 0.54 | 0.59 | 0.57 |
| 12 | $C_6H_6$ | 42.24 | 53.91 | 60.12 | 62.75 |
| 13 | $C_6H_5CH_3$ | 0.73 | 1.63 | 2.32 | 3.29 |
| 14 | $C_6H_4(CH_3)_2$ | 0.58 | 0.83 | 1.17 | 1.35 |
| 15 | $H_2$ | 0.02 | 0.07 | 0.35 | 0.85 |
| 16 | Carbonized residue | 336.84 | 162.10 | 29.10 | 12.10 |
| 17 | $\Sigma C_m H_n$ | 46.64 | 72.52 | 88.22 | 96.35 |

range ±10°C. Additionally the temperature of the sample was measured with a Ni−CrNi thermocouple fastened in the quartz pipe next to the vessel with the investigated sample.

The volatile products of decomposition were filtered through glass wool to separate fumes and tar products, and then they were bubbled through distilled water to absorb hydrochloride.

Gases and vapors were sucked into a 5-liter bottle by means of aspiration. Hydrochloride absorbed in water was determined titrometrically, using the mercurometric method of measurement. The carbonized residue was determined from the mass difference before and after completed thermoxidative decomposition. Water being one of the decomposition products was not determined. The stable gases and hydrocarbon vapors were analyzed by means of GC, using the N 503 type of gas chromatograph produced by Mera Elmat (Poland). For separation and determination of the stable gases the PCD unit was applied. To identify $CO_2$ a steel column was used, length 2 m, inner diameter 4 mm, filled with the 100/200 mesh silica gel; the working temperature of a column was 90°C and the carrier gas (He) flow rate was 40 cm$^3$ min$^{-1}$. With CO, similar measuring conditions were applied. The only difference depended upon the material
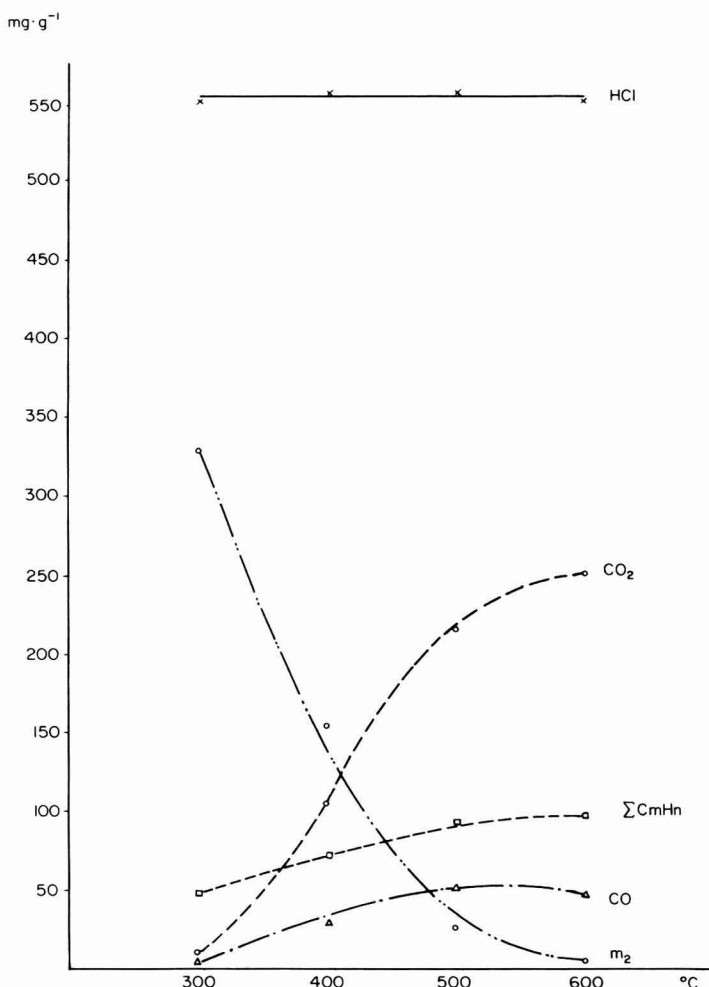
FIG. 1. The amount of the thermoxidative decomposition products derived from PVC vs the reaction temperature.

used for column filling. In this case the molecular sieves A 5 (80 mesh) were used instead of silica gel. For separation and determination of the aliphatic and aromatic hydrocarbons, a PID unit was applied.

The $C_1-C_3$ hydrocarbons were determined on a column filled with $Al_2O_3$ (60/80 mesh); the column length was 2 m and its inner diameter was 4 mm, the working temperature was 80°C, the carrier gas (Ar) flow rate 40 cm$^3$ min$^{-1}$, the air flow rate 500 cm$^3$ min$^{-1}$, and the hydrogen flow rate was 50 cm$^3$ min$^{-1}$.

The aliphatic hydrocarbons ($C_4-C_6$) and the aromatic hydrocarbons were determined using a 2-m-long column (inner diameter 4 mm), the col-
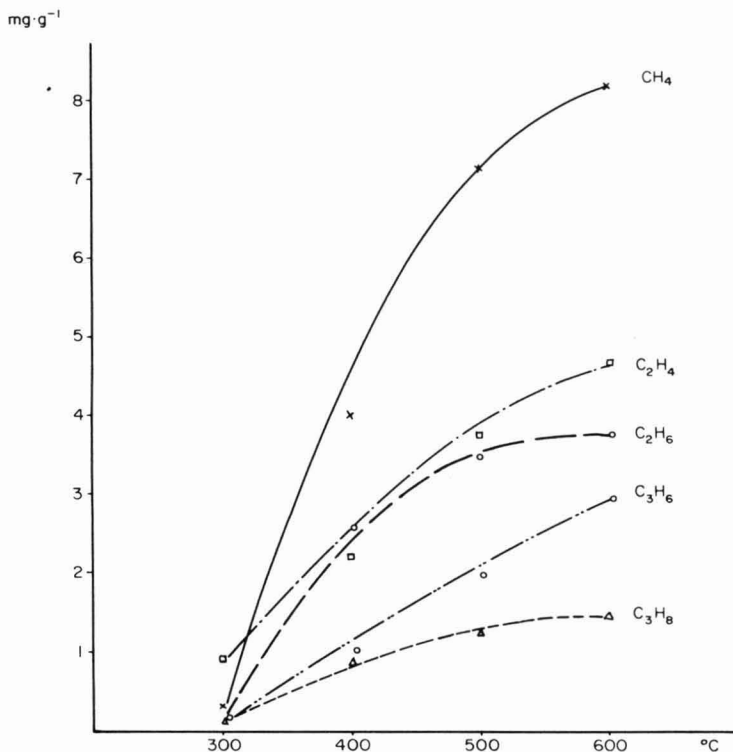
FIG. 2. The amount of aliphatic products of the thermoxidative decomposition derived from PVC vs the reaction temperature.

umn filling being 20% GE-SE 30 on Chromosorb PDMSC (60/80 mesh). The working temperature was 80°C and the gas flow rates were the same as when determining the $C_1-C_3$ hydrocarbons.

## DISCUSSION

Our investigations reveal that the basic reaction in the course of oxopyrolysis of PVC is elimination of hydrochloride, which is almost fully completed at 300°C and does not undergo any further, serious changes at the higher, examined temperatures. Due to elimination of HCl the polymer units containing $\pi$ bonds arise in macromolecules, which at 300°C form the basic product as a carbonized residue. At this temperature the products of oxidation and degradation of the chain give only an insignificant fraction of the total product yield (Table 1, Figs. 1−3). The increase of temperature obviously accelerates these concurrently running reactions. This can be deduced from our results. Thus with the increase of temperature the amount of carbonized residue rapidly decreases with the simultaneous increase of the $CO_2$ yield, which can be regarded as the second main product of oxopyrolysis.
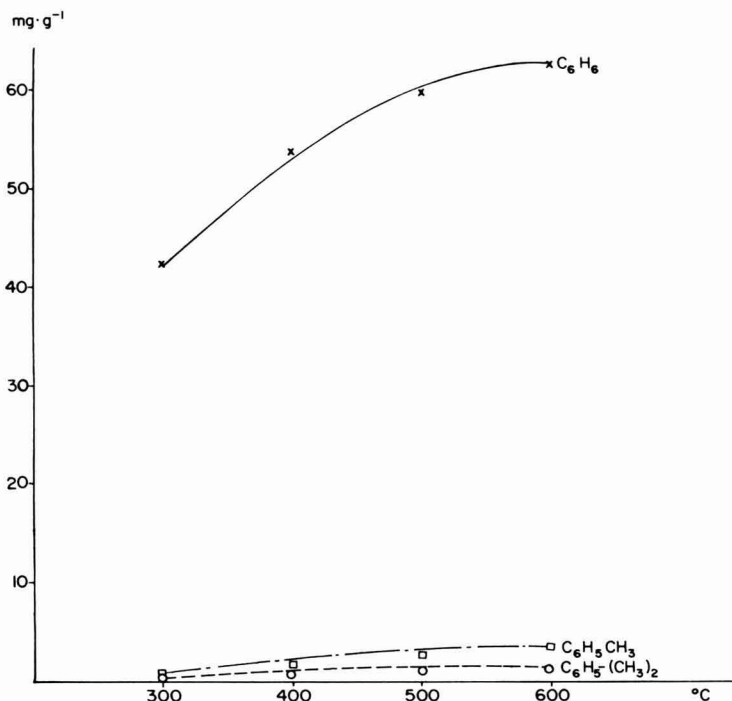
FIG. 3. The amount of benzene and its derivatives coming from the thermoxidative decomposition of PVC vs the reaction temperature.

Another process which seems to accompany oxidation depends on fragmentation of the carbon chain and on aromatization and isomerization of the products. Thus the main hydrocarbon products appear to be benzene, toluene, xylenes, and some other aromatic hydrocarbons, as well as methane and its homologs. It should be added that in each case the increase in temperature promotes the more rapid accumulation of the compounds with double bonds than of the saturated ones.

According to the experimental data the attack of a molecular oxygen on a fragment of a polyvinyl chloride molecule:
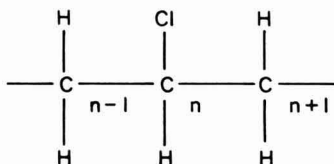


leads toward obtaining the following reaction products: $CO_2$, $CO$, $HCl$, and a number of hydrocarbons. Calculations based upon the extended CNDO, INDO, and MINDO methods reveal that net charge on a chlorine

atom approximately equals $-0.2$, while with the $C_n$ atom its net charge value equals $+0.2$, due to the $-I$ inductive effect of the chlorine substituent.

If the electron configuration of an oxygen molecule is taken into consideration (with this molecule stretched along the $x$ axis):

$$\{K,K\ (\sigma 2s)^2\ (\sigma^*2s)^2\ (\sigma 2p_x)^2\ (\pi 2p_y)^2\ (\pi 2p_z)^2\ (\pi^*2p_y)^1\ (\pi^*2p_z)^1\}$$
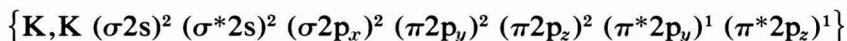
then one of the electron pairs $(\pi 2p_y)^2$, $(\pi 2p_z)^2$ could be accepted by the positively charged $C_n$ atom (in the case of a suitable steric position of an oxygen molecule):
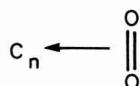


The nucleophilic attack of an oxygen molecule is simultaneously supported with an electromeric effect at a $C_n$ carbon atom. In addition the inductive effect of oxygen $(-I)$ tends to complete the following levels: $(\pi^*2p_z)^1$ or $(\pi^*2p_y)^1$ (with the $XZ$ plane stretched at the sheet of paper). Electrons are withdrawn from the occupied frontal orbital of a hydrocarbon chain and the following bonds are weakened: $(\sigma sp_C - sp_C)^2$, $(\sigma sp_C - s_H)^2$, $(\sigma p_{xCl} - sp_C)^2$, and therefore the oxygen molecule tends toward the following change of configuration:

$$\{K,K\ (\sigma 2s)^2\ (\sigma^*2s)^2\ (\sigma 2p_x)^2\ (\pi 2p_z)^2\ (\pi^*2p_y)^2\ (\pi^*2p_z)^2\},$$
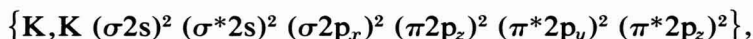
which promotes dissociation of an $O-O$ bond, formation of a $C-O$ bond, and dissociation of the following bonds: $H-C_n$, $C_{n-1}-C_n$, $C_{n+1}-C_n$, and $C_n-Cl$.

This brings about a qualitative explanation of the observed, experimental facts of obtaining hydrochlorine, carbon oxide, carbon dioxide, and hydrocarbons in the course of the oxidative pyrolysis, which are most probably formed through recombination of various free radicals:
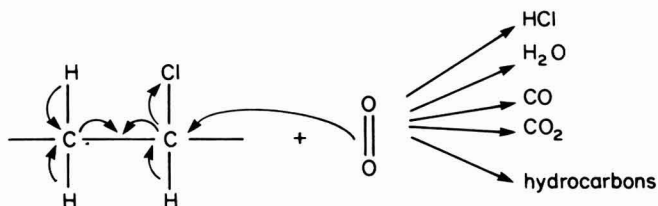


Thus a certain linkage was established between the experimental results and the theoretical considerations.

## SUMMARY

Gas chromatographic analysis was performed on the products of thermal decomposition of polyvinyl chloride with particular attention on the identification of the obtained reaction products and the role of oxygen in the process.

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# Spectrophotometric Determination of Vanadium(V) as a Mixed Thiocyanate-4-Pyridone Complex

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## INTRODUCTION

In our previous papers it has been reported that 3-hydroxy-2-methyl-1-phenyl-4-pyridone (HX) (7) and 3-hydroxy-2-methyl-1-(4-tolyl)-4-pyridone (HY) (5) are very suitable reagents for the extraction and spectrophotometric determination of vanadium(V) from solutions of hydrochloric, sulfuric, and perchloric acids.

We now report the application of HX and HY for the extraction and spectrophotometric determination of vanadium(V) as a mixed thiocyanate–HX (HY) complex. HX has been used as a very suitable reagent for the spectrophotometric determination of titanium and tungsten as a mixed thiocyanate-3-hydroxy-2-methyl-1-phenyl-4-pyridone complex (3, 6).

## MATERIALS AND METHODS

*Reagents.* A standard solution of vanadium(V) (0.01 $M$) was prepared by dissolving 1.17 g ammonium vanadate in water and making up to a volume of 1 liter. The solution was standardized gravimetrically with cupferron (2). HX and HY were synthesized as described earlier (1, 4). The solutions of these reagents in chloroform served as the organic phase. All the chemicals were analytically pure.

*Apparatus.* A Perkin-Elmer Coleman 124 spectrophotometer with 1-cm cells was used to record spectra and for all spectrophotometric measurements. A pH meter, Radiometer model TTT 1, was used for pH measurements.

*Procedure.* An aqueous solution (about 5 ml) containing 10–100 $\mu$g of vanadium(V) was adjusted to a pH of about 1.6 (1.5) with $H_2SO_4$ and 1 ml of $8 \times 10^{-2}$ $M$ KSCN (1 ml of $9 \times 10^{-2}$ $M$ KSCN) and up to 10 ml water was placed in an Erlenmeyer flask. After the addition of 10 ml of $2.5 \times 10^{-3}$ $M$ HX (HY) in chloroform the solution was shaken for 15 min with a mechanical shaker. The equilibrium was achieved in 2–3 min. After the

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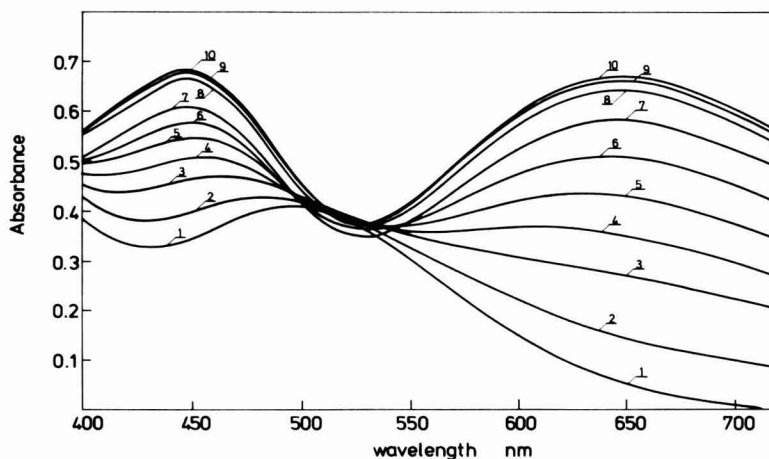
FIG. 1. Dependence of the absorption spectrum of vanadium−thiocyanate−HX complex on thiocyanate concentration. $1 \times 10^{-4} M$ V(V), $2.5 \times 10^{-3} M$ HX, pH = 1.6 (1) without thiocyanate. Thiocyanate concentration: (2) $1 \times 10^{-4} M$; (3) $3 \times 10^{-4} M$; (4) $5 \times 10^{-4} M$; (5) $1 \times 10^{-3} M$; (6) $2 \times 10^{-3} M$; (7) $3 \times 10^{-3} M$; (8) $5 \times 10^{-3} M$; (9) $1 \times 10^{-2} M$; (10) $2 \times 10^{-2} M$.

phases were separated the absorbance of the organic phase was measured at 650 and 450 nm against a reagent blank.

## RESULTS AND DISCUSSION

### Optimal Conditions for Extraction

The vanadium(V) complex formed with thiocyanate in a solution of sulfuric acid can be extracted with 3-hydroxy-2-methyl-1-phenyl-4-pyridone (HX) or 3-hydroxy-2-methyl-1-(4-tolyl)-4-pyridone (HY) in chloroform as green mixed complexes which have a maximum absorbance at 450 and 650 nm. The influence on the extraction of vanadium of the concentration of potassium thiocyanate and sulfuric acid in the aqueous phase and of the HX or HY concentration in the organic phase were studied and the results are shown in Figs. 1−4. For the quantitative extraction the minimal concentration of potassium thiocyanate must be $6 \times 10^{-3} M$, of HX or HY $4 \times 10^{-4} M$, and the pH of the aqueous phase must be in the range $1.3 - 1.9$. Under these conditions it is possible to determine $1 - 10$ ppm of vanadium. Beer's law is obeyed in this range. The molar absorptivity at 450 and 650 nm amounts to $6.8 \times 10^3$ liters $mol^{-1} cm^{-1}$. The Sandell photometric sensitivity is $0.0075$ $\mu g$ V $cm^{-2}$. The reproducibility of the measurements, expressed as standard deviation, is $0.2-2\%$, depending on the vanadium concentration. The color of the vanadium−thiocyanate−HX (HY) complex in the organic phase is stable for at least 24 hr.

### Influence of Diverse Ions

The influence of various cations and anions on the determination of
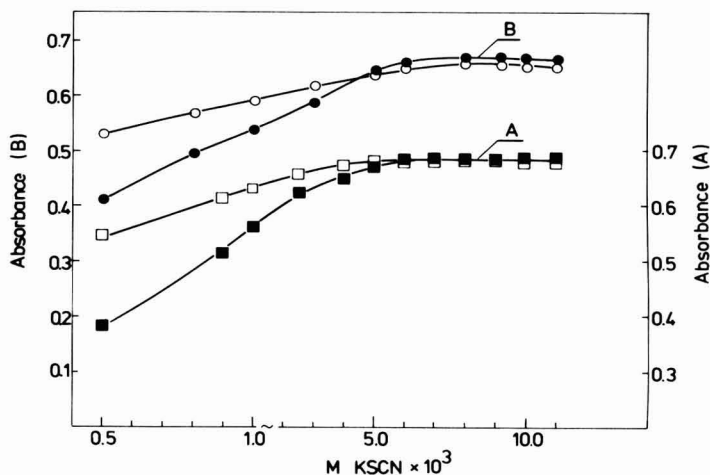
FIG. 2. Dependence of absorbance of vanadium–thiocyanate–HX (HY) complex on KSCN concentration. $1 \times 10^{-4} M$ V(V), $2.5 \times 10^{-3} M$ R, R = HX (■,□), pH = 1.6, (■) 650 nm, (□) 450 nm. R = HY (●,○), pH = 1.5, (●) 650 nm, (○) 450 nm.

vanadium was studied. It was found that chloride, sulfate, nitrate, phosphate, acetate, and bromide do not interfere in an amount 5000-fold higher than that of vanadium. Potassium, sodium, strontium, barium, calcium, manganese(II), chromium(III), and nickel are tolerated in a 1000-fold amount. Tartrate, perchlorate, aluminium, zinc, cadmium, magnesium, and cobalt are tolerated in a 500-fold amount, and oxalate, copper, and
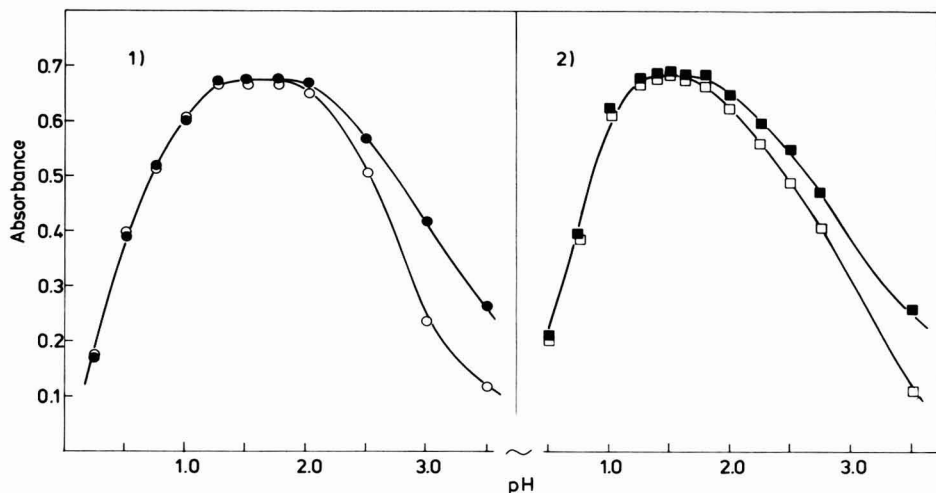


FIG. 3. Effect of pH in the aqueous phase on the absorbance of vanadium–thiocyanate–HX (HY) complex in chloroform. $1 \times 10^{-4} M$ V(V); (1) $2.5 \times 10^{-3} M$ HX, $8 \times 10^{-3} M$ KSCN, (●) 650 nm, (○) 450 nm; (2) $2.5 \times 10^{-3} M$ HY, $9 \times 10^{-3} M$ KSCN, (■) 650 nm, (□) 450 nm.
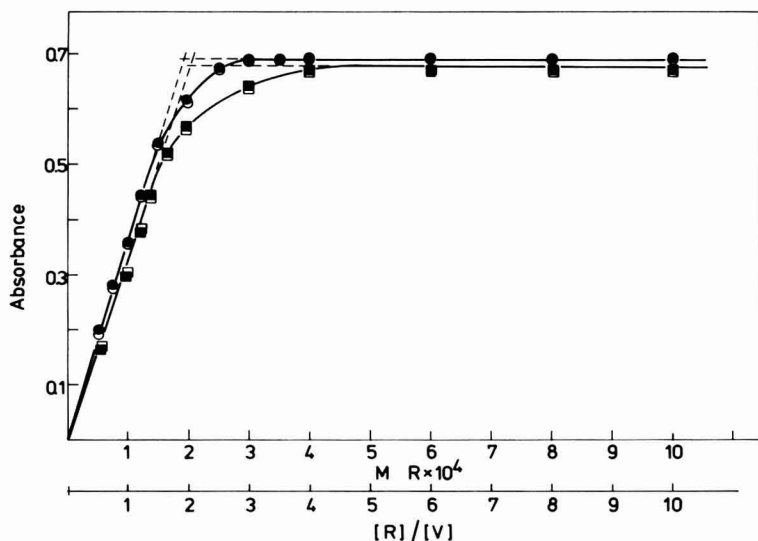
FIG. 4. Determination of V−HX (HY) ratio by mole-ratio method. R = HX (●,○); concn. V(V) = 1 × 10⁻⁴ $M$, concn. KSCN = 8 × 10⁻³ $M$, pH = 1.6, (●) 650 nm, (○) 450 nm. R = HY (■,□); concn. V(V) = 1 × 10⁻⁴ $M$, concn KSCN = 9 × 10⁻³ $M$, pH = 1.5, (■) 650 nm, (□) 450 nm.

plumbum in a 100-fold amount. Fluoride, citrate, uranyl, thorium, and gallium may not exceed a 10-fold amount. Large amounts of uranyl, thorium, and gallium decrease the absorbance because they react with HX and HY giving colorless complexes and thus consume HX (HY) for their own extraction and decrease the concentration of HX (HY) and the extraction of vanadium. Iron(III), molybdenum(VI), titanium(IV), palladium(II), tungsten(VI), and zirconium(IV) should be removed before vanadium determination.

*Composition of the Complexes*

The ratio of vanadium to thiocyanate was determined by Job's method of continuous variation. The concentration of HX (HY) was kept constant and in a large excess. The results obtained (Fig. 5a) indicate that the molar ratio of V:SCN is 1:1 in extracted V−SCN−HX and V−SCN−HY complexes. The experiments show that only absorbance at 650 nm can be used for the determination of the molar ratio of vanadium to thiocyanate. In the range of excess of vanadium to thiocyanate vanadium is not completely extracted as a mixed thiocyanate complex because of the increased absorbance at 450 nm which shows a partial formation of V−HX (HY) complexes (5, 7). The ratio of vanadium to HX (HY) was determined by Job's method and the mole-ratio method. In these experiments the concentration of thiocyanate was kept constant and in a large excess. The
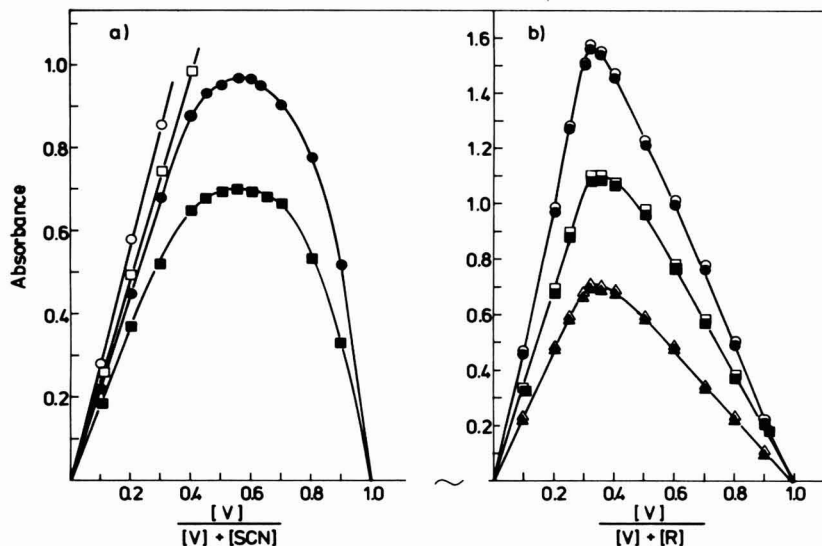
FIG. 5. Determination of the composition of the complexes by Job's method. (a) Concn. HX = $1 \times 10^{-2} M$ ($\square,\blacksquare$), pH = 1.6, [V] + [SCN] = $4 \times 10^{-4} M$, 450 nm ($\square$), 650 nm ($\blacksquare$); concn. HY = $1 \times 10^{-2} M$ ($\bigcirc, \bullet$), pH = 1.5, [V] + [SCN] = $6 \times 10^{-4} M$, 450 nm ($\bigcirc$), 650 nm ($\bullet$). (b) R = HX ($\triangle,\blacktriangle,\bigcirc,\bullet$), concn. KSCN = $8 \times 10^{-3} M$, pH = 1.6, [V] + [R] = $8 \times 10^{-4}$ $M$, 650 nm ($\bigcirc$), 450 nm ($\bullet$); [V] + [R] = $4 \times 10^{-4} M$, 650 nm ($\triangle$), 450 nm ($\blacktriangle$). R = HY ($\square,\blacksquare$), concn. KSCN = $9 \times 10^{-3} M$, pH = 1.5, [V] + [R] = $6 \times 10^{-4} M$, 650 nm ($\square$), 450 nm ($\blacksquare$).

results obtained (Figs. 4 and 5b) indicate that the molar ratio of vanadium to HX (HY) is 1:2. The molar ratio of vanadium:thiocyanate:HX (HY) in the extracted mixed complex is 1:1:2.

It is known that at pH values lower than 3, the cationic forms $VO_2^+$ and (to a smaller degree) $VO^{3+}$ prevail in the solution of vanadium(V). Since we isolated and identified in the solid state similar mixed chloro complexes $VO_2Cl(HX)_2$ and $VO_2Cl(HY)_2$ (5, 7), it is most probable that a $VO_2(NCS)(HX)_2$ or $VO_2(NCS)(HY)_2$ complex is formed and transferred into the organic phase.

## SUMMARY

The spectrophotometric determination of vanadium(V) as a mixed thiocyanate-3-hydroxy-2-methyl-1-phenyl-4-pyridone (HX) complex and as a mixed thiocyanate-3-hydroxy-2-methyl-1-(4-tolyl)-4-pyridone (HY) complex is described. The extracted complexes in chloroform have a maximum absorbance at 450 and 650 nm. The optimal conditions for the extraction and spectrophotometric determination of vanadium(V) are determined. The solutions of the V−SCN−HX and V−SCN−HY complexes in chloroform obey Beer's law in the range 1−10 ppm of vanadium, and are stable for at least 24 hr. The molar absorptivity of the method is $6.8 \times 10^3$ liters mol$^{-1}$ cm$^{-1}$. The molar ratio V:SCN:HX (HY) of the extracted complex is 1:1:2.

## ACKNOWLEDGMENT

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# Analytical Applications of Biacetyl Bis(4-phenyl-3-thiosemicarbazone) and Bipyridylglyoxal Bis(4-phenyl-3-thiosemicarbazone)[1]

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## INTRODUCTION

*Brief Review of Thiosemicarbazone Compounds*

Thiosemicarbozones are azomethines characterized by the presence of the grouping $>C=N-NH-C(=S)-NH-$ (Scheme 1). Pioneering works
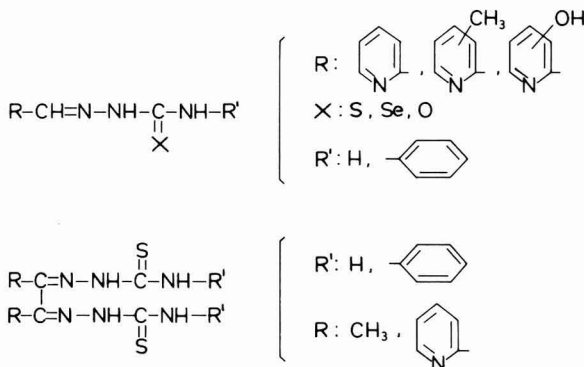


dealing with analytical applications of thiosemicarbazones were done by Scott *et al.* (*123*), Nillson (*104*), Hangas *et al.* (*57*), Hovorka and Holzbecher (*62–65*), Sircar *et al.* (*96, 126*) and Bhargava *et al.* (*21*) over 30 years ago. Since the reactions given by various thiosemicarbazone derivatives with some metal ions were very sensitive, the mentioned workers suggested their use as reagents in inorganic analysis. Thiosemicarbazones easily form highly colored and usually stable chelates with transition metal ions, which permit colorimetric determinations in the 1–20 ppm range. The proposed methods of analysis have recently been reviewed by Singh *et al.* (*124*) and by Cano *et al.* (*32*). The structural aspects of thiosemicarbazones have also been reviewed (*28*).

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Thiosemicarbazide (*4, 79, 119*) and phenylthiosemicarbazide (*4, 100*) itself have been used in inorganic analysis for the determination of trace amounts of cations, and in organic analysis for characterization and determination (*77, 87, 102, 120, 136, 137*) of aldehydes and ketones. The colorimetric determination of aromatic aldehydes can be carried out by means of their thiosemicarbazones (*17*). Mercurimetric (*78*) and potentiometric methods (*29, 30*) have been developed for the determination of thiosemicarbazones.

Thiosemicarbazones have been studied less than oximes (*37*) but more than phenylhydrazones (*3*), semicarbazones (*124*), pyridylhydrazones (*5*), or organoselenium compounds (*14, 18, 19, 34, 67, 68, 94, 108−110, 112*). There is no doubt that the thiosemicarbazones most studied have been picolinealdehyde thiosemicarbazone (PAT) (*1, 31, 34, 97, 124*) and biacetylmonoxime thiosemicarbazone (*62, 63, 66, 124, 147−151*). Two excellent papers dealing with the PAT−metal ions interactions have been published by Legget and McBride (*82, 83*). Analytical applications of thiosemicarbazones derived from cyclohexanone (*45*), 2-hydroxy-1-naphthaldehyde (*70*), the derivatives of certain furan compounds (*71*), from 2,2-dihydroxybenzophenone (*85*), and other hydroxylated derivatives (*139*), from 5-substituted furfurals and furylpentadienals (*93*), from cyclohexane-1,3-dione and acetylacetone (*115−117*), from β-resorcylidene (*128*) and β-resorcylaldehyde (*129*), and from salicylaldehyde (*152−154*) have also been reported. Some thiosemicarbazones have been tested in the spectrophotometric determination of rhenium (*24*). Mass spectra of chelate compounds of cobalt(II) with thiosemicarbazones (*72*), as well as their electron impact study (*73*), have been reported. The color reaction of thiosemicarbazones with sodium nitroprusside (*74*) is known. In general, phenylthiosemicarbazones (*15, 33, 43, 60, 88−90, 103*) have been less studied than thiosemicarbazones.

A search of chemical abstracts in recent years shows a widespread use of thiosemicarbazones as compounds with biological significance (*42, 95*). For example, we can mention the thiosemicarbazones derived from α-(N)-heterocyclic aldehydes (*122*), from isatinthiosemicarbazone (*41, 84*), from 1-formylisoquinoline thiosemicarbazone (*98*), from formylpyridine thiosemicarbazone (*105*), from methylchalcone (*111*), from aliphatic carbonyl compounds (*35*) and pirrolidine (*125*), as well as the bisthiosemicarbazones derived from kethoxal (*23*) and 3-ethoxy-2-oxobutyraldehyde (*36*). Likewise numerous papers have appeared dealing with coordination compounds with thiosemicarbazones as ligands. Thiosemicarbazones have also been very employed in organic synthesis (i.e., *13, 61, 138, 144*).

The presence of a donor group in α with respect to the thiosemicarbazide chain makes possible the formation of two five-membered chelate

rings, whereas the introduction of a new thiosemicarbazide chain ensures four coordination sites, the formation of three five-membered chelate rings, being, thus, possible. In both cases the chromogenic powder of reagents as well as the stability of chelates formed with metal ions increase. Likewise, the introduction of a benzene ring at the end of the thiosemicarbazide chain increases the sensitivity of the thiazone grouping.

In general, the duplication of the "analytical functional grouping" in a molecule improves its analytical properties (101). Dithiosemicarbazones were first studied by Bähr (11) who to the simplest member of the group gave the trivial name of "thiazone." In 1962, Gingras et al. (46) reported a study dealing with dithiosemicarbazones and their copper complexes. Glyoxal dithiosemicarbazone have been proposed for the spectrophotometric determination of Ag(I) and Hg(II) (25), and Pd(II) (92, 134). A wide study on dithiosemicarbazones derived from glyoxal, biacetyl, and benzil has been carried out by Gonzalez-Duarte (51, 52). The polarographic of copper, cobalt, iron, and nickel complexes with thiosemicarbazones have been studied by Toropova et al. (140 – 145). The electrochemical reduction of thiosemicarbazones in aqueous-alcohol media (75, 76) has been the subject of several investigations. The polarographic behavior of thiosemicarbazide and its derivatives (87) has also been studied.

Diphenylthiosemicarbazones were introduced in analysis by Niederschulte and Ballschmiter (103) and Ballschmiter (15) in 1972 – 1973, who suggested their use in extractive spectrophotometric procedures. By using this type of compounds, the determination of trace amounts in the 1 ppm range is possible. Recently Heizmann and Ballschmiter (60) have reported high-performance liquid chromatographic separation of several metal chelates at nanogram levels by adsorption chromatography on silica gel with 1,2-diketobisthiosemicarbazones and 1,2-diketobisthiobenzhydrazones being used as chelate-forming ligands.

Biacetyl bis(4-phenyl-3-thiosemicarbazone) (BBPT) (6) has been employed in the spectrophotometric determination of copper (7), zinc (8), mercury (9), and palladium (48). The behavior of bipyridylglyoxal bis(4-phenyl-3-thiosemicarbazone) (BGPT) as reagent (50) and its application to the spectrophotometric determination of zinc, cadmium, and copper (49) have also been reported. This paper is a continuation of the study and applications of both reagents, carrying out a number of spectrophotometric determinations. Some new analytical possibilities have been suggested. The complexing properties of BBPT and BGPT are superior to those shown by biacetyl monothiosemicarbazone (121) and picolinealdehyde 4-phenyl-3-thiosemicarbazone (47), respectively.

Bisthiosemicarbazones are structurally related to bis(thiobenzoyl)hy-

drazones (*12, 58−60*) and to Schiff bases obtained from the condensation
of α-diketones with 2-aminothiophenol (*69, 92, 135*) (Scheme 2).
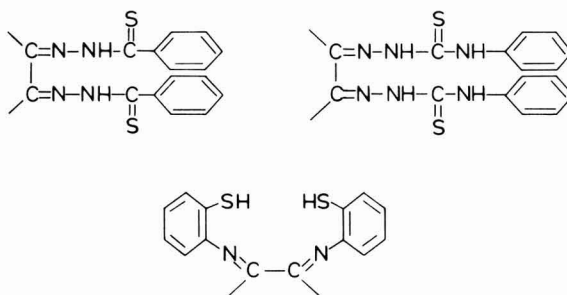


## MATERIALS AND METHODS

### Synthesis of the Reagents

Phenylthiosemicarbazide (20 mmol) was dissolved in 200 ml of
ethanol−water (3 + 2 v/v) and the solution was warmed to 70−80°C under
magnetic stirring. Four drops of 1 *N* hydrochloric acid were added to the
mixture. Then, the α-dicarbonilic compound (10 mmol) dissolved in 20 ml
of ethanol was added drop by drop from a separating funnel. The com-
pound precipitated in warmth. The solution was allowed to cool and the
product separated by filtration and washed with a few milliliters of hot
ethanol. The compounds were used without further purification. The an-
alytical results, melting points and yield of reaction are found in Table 1.

### Apparatus

Measurements of absorbances at fixed wavelengths, for analysis, were
made with a Unicam SP 600 (BGPT) and a Coleman 55 (digital) (BBPT)
spectrophotometers in 1-cm cells. Spectral curves were recorded with a
Unicam SP 8000 instrument. The infrared spectra of the solid reagents
were obtained with a Perkin Elmer 621 grating spectrophotometer. The
potassium bromide was spectroscally pure. The pH of the aqueous−
ethanol or aqueous−dimethylformamide solution was measured with a
glass-calomel electrode combination, and all analytical weighing were
done on a Sartorius type balance.

### Reagents

Analytical reagent grade materials were used whenever available and
distilled water was used throughout.

Biacetyl bis(4-phenyl-3-thiosemicarbazone) (BBPT) reagent stock so-
lutions at 0.1% (w/v) in dimethylformamide and at 0.025% (w/v) in toluene
or chloroform containing a 5% (v/v) solution of dimethylformamide were
used. The later solution is stable for several months when stored in the

TABLE 1
DIPHENYLTHIOSEMICARBAZONE COMPOUNDS SYNTHESIZED[a]

| Compound | Formula | Analysis (%) | | mp | IR spectra | |
|---|---|---|---|---|---|---|
| | | Found | Calc. | | | |
| Glyoxal bis(4-phenyl-3-thiosemicarbazone) (GBPT) | $C_{16}H_{16}N_6S_2$ | C 53.70 | 53.93 | 223–225 | 3250 m | NH stretch (1,2-CBPT) |
| | | H 4.44 | 4.49 | | 3270 and 3120 m | NH stretch (GBPT) |
| | | N 23.47 | 23.59 | | 3180 m | NH stretch (BBPT) |
| | | S 18.27 | 17.97 | | | |
| Biacetyl bis(4-phenyl-3-thiosemicarbazone) (BBPT) | $C_{18}H_{20}N_6S_2$ | C 55.98 | 56.25 | 218–220 s | 2930 and 2860 w | asym. and sym. $CH_2$ stretch |
| | | H 4.94 | 5.20 | | 1600 to 1430 s | (multiplet) aromatic C–H stretch; C=N; C=C stretch |
| | | N 21.60 | 21.87 | | | |
| | | S 16.84 | 16.67 | | 900 to 400 s | (multiplet) C–H in plane deformations |
| Cyclohexane-1,2-dione bis(4-phenyl-3-thiose micarbazone) (1,2-CBPT) | $C_{20}H_{22}N_6S_2$ | C 55.78 | 58.53 | 165–167 | (755 and 695) s | 1,2-CBPT monosubstituted |
| | | H 5.30 | 5.36 | | (745 and 685) s | GBPT phenyl |
| | | N 19.79 | 20.48 | | (750 and 690) s | BBPT |
| | | S 15.46 | 15.60 | | 400 to 200 s | C–H out-of-plane def. |
| | | | | | 1300 m | C–N stretch; in BBPT masked by $CH_3$ sym. bending (1340 s) |
| | | | | | 1250 and 1190 m | 1,2-CBPT |
| | | | | | 1250 and 1190 m | GBPT |
| | | | | | 1250 and 1175 m | BBPT   C=S stretch |

[a] Yield > 85%.

dark at low temperature and was prepared by dissolving 75 mg of BBPT in 5 ml of dimethylformamide and diluting to 250 ml with the organic solvent.

Bipyridylglyoxal bis(4-phenyl-3-thiosemicarbazone) reagent stock solution at 0.1% (w/v) in ethanol or at 0.2% (w/v) in dimethylformamide.

Standardized solutions of metals (20): Hg(II), 4.694 g/liter; Pb(II), 2.018 g/liter; Bi(III), 2.544 g/liter; Pd(II), 1.116 g/liter; and Mn(II), 1.000 g/liter.

Buffers pH 4.2, 4.5, 4.7, and 5.0 (acetic acid/sodium acetate), pH 8.2 (boric acid/sodium hydroxide), and pH 9.5 and 9.8 (ammonia/ammonium chloride), prepared by conventional methods.

For interference tests, the various ions were added in the form of their readily available soluble salts.

*General Procedures*

*Study of the reagents.* Solutions ($0.9-1.6 \times 10^{-5}$ $M$) were prepared at different pH values and their spectra were recorded over the range 450–200 nm and the effect of time was studied at each pH value. The effect that various oxidizing or reducing agents exerted over the absorption spectra of the ligand was also studied. Samples were prepared in the usual way, with the exception that 5 ml at 0.1 and at 1% solution of oxidizing or reducing agent was added. Likewise 1 ml of the reagent dissolved in dimethylformamide (BBPT, $4.0 \times 10^{-4}$ $M$; BGPT, $2.2 \times 10^{-4}$ $M$) was added in 25-ml volumetric flasks and the solutions were diluted with the various solvents.

*Aqueous–organic solutions.* The solution containing the metal ion was pipetted into a 25-ml measuring flask, the solution of the diphenyl-thiosemicarbazone compound, dimethylformamide (BBPT) or ethanol (BGPT), potassium nitrate solution to adjust ionic strength[2,3,4,5] (otherwise stated), the solution to the pH adjustment (hydrochloric acid, sodium hydroxide, or a buffer solution), and distilled water were then added in that order by pipet. After mixing, the solution was left to stand for about 5 min.[6] The absorbance was measured in a quart cell (BBPT) or glass cell (BGPT) against a reagent blank (or distilled water) as reference. The final solution contained 60% (v/v) of organic solvent in both cases.

*Extraction.* The solution containing the metal ion and the pH adjustment solution were pipetted into a 25-ml measuring flask and distilled water was added to the mark. Ten milliliters of this solution was trans-

[2] When use buffer ionic strength was not added (otherwise stated).

[3] In the case of the Ni-BGPT system the complex formation is slower in the absence of KCl or $KNO_3$, but the stability of the system was the same.

[4] In the case of the Co-BGPT system ionic strength was not added. The absorbance measurements changed in the presence of KCl or $KNO_3$. In acid medium and in the presence of the mentioned salts precipitation occurred.

[5] The absorbance is maximum for the Fe-BGPT system at an ionic strength of $\mu = 0.15$ with $I^-$, $BrO_3^-$, $SCN^-$, or $NO_3^-$. At an ionic strength greater than 0.15 precipitation occurred.

[6] For cobalt in acidic medium 30 min.

ferred by pipet to a separating funnel. To this 10 ml of BBPT reagent at 0.025% in toluene or chloroform containing a 5% (v/v) were added, shaking vigorously for 30 sec. After the phases had separated, the absorbance of the organic phase (dried upon anhydrous sodium sulfate) was measured in a glass cell of 10 mm pathlength against a reagent blank (or solvent) as reference.

## RESULTS AND DISCUSSION

*Synthesis*

Since the reagents had been obtained in only 20–30% yield (7, 50), an attempt was made to improve their preparation. The method of synthesis used is similar to the one used by Barret and Bays (158), and led to good results in the obtainment of GBPT, BBPT, and 1,2-CBPT. Phenylthiosemicarbazide is always in excess in the medium with respect to the α-dicarbonilic compound. Not having to reflux the solution precludes phenylthiosemicarbazide decomposition; thus excellent yields in the synthesis are obtained. The formation of thiosemicarbazones is acid catalyzed (56). The method failed when it was applied to benzil and α-furil, probably because a mixture of products was obtained as indicated by Gingras *et al.* (46). With bipyridylglyoxal no precipitation occurred.

*IR Spectra*

The localization of the $C=S$ band of thiosemicarbazones in the infrared spectra (22, 155–157) has been studied. The absorption bands associated with the imine groups are also well known (39, 40). The principal bands obtained with the dithiosemicarbazones and their assignments are listed in Table 1.

*UV Spectra*

In spite of the fact that UV absorption spectroscopy is an old tool for chemists, there is not much information available (114, 132) about electronic absorption spectra in comparison with other kinds of spectrometries. However, studies have been carried out on electronic absorption spectra of the $>C=C-C=N-$ grouping (38), on semicarbazones (26, 80, 107, 113), and phenylsemicarbazones (53), thiosemicarbazones (113), and phenylthiosemicarbazones (54), benzaldehyde thiosemicarbazones, and their palladium complexes (10), and on 2,4-dinitrophenylhydrazones (80, 106).

The formula of both reagents indicates the possibility of thiol-thione tautomerism in either or both of the $-NH-C(=S)-$ structures, as was proved with the dependence of the spectra with pH (7, 50) and by rmn spectrometry (50). This tautomerism is also observed in a solvent such as dimethylformamide. Slight shifts were observed in the UV spectra of BBPT when the temperature decreased. This change was reversible.

TABLE 2

ULTRAVIOLET SPECTRA OF THE REAGENTS IN COMMON SOLVENTS

| Solvent | Dielectric constant (55) | BBPT $\lambda$ (nm) | BBPT $\epsilon$ (liters mol$^{-1}$ cm$^{-1}$) | BGPT $\lambda$ (nm) | BGPT $\epsilon$ (liters mol$^{-1}$ cm$^{-1}$) |
|---|---|---|---|---|---|
| Water | 80 | 343 | 42400 | 318 | 63300 |
| Dimethylformamide | 37 | 348–354 | 34400 | 328 | 62200 |
| | | 420–430 | 5750 | | |
| Ethanol | 24 | 347–352 | 41800 | 331 | 61100 |
| Acetone | 21 | 345–350 | 41700 | | |
| Butil alcohol | 17 | 350–355 | 41000 | | |
| Ethyl acetate | 6 | 348–352 | 39000 | | |
| Chloroform | 5 | 350–360 | 37200 | 333 | 38900 |
| Toluene | 2.4–2.2 | 348–354 | 38200 | | |
| Benzene | 2.4–2.2 | 356–364 | 34400 | | |
| Dioxane | 2.4–2.2 | 360–365 | 34400 | 330 | 57800 |
| Carbon tetrachloride | 2.4–2.2 | 360–364 | 36200 | 335 | 52200 |

TABLE 3
CHARACTERISTICS OF SOME BBPT AND BGPT METAL-ION COMPLEXES

| Complex | $\lambda_{max}(nm)$ | Optimum pH range | Compliance with Beer's law | $\epsilon$ (liters mol⁻¹ cm⁻¹) at $\lambda$(nm) | Optimal working range (ppm) | Stability hours (pH) | Relative error ($P = 0.05$) | Optimum amount of reagent/ppm (ml at 0.1%) |
|---|---|---|---|---|---|---|---|---|
| Bi-BBPT | 395–405 | 5.9–7.2 (6.7) | 1–9 | 25000 (405) 22100 (420) | 1.5–4.5 | 24 (6.7) | 0.87 (405) 0.75 (420) | 1 |
| Cd-BBPT | 430 | 6.2–10.7 (6.7) | 0.5–4 | 22400 (430) 21500 (440) | 1.5–4 | 24 (6.7) | 0.23 (430) | 0.5 |
| Bi-BGPT | 390 | 5–9 (5.5) | 1–11 | 18300 (390) 15400 (410) 14000 (420) | 2–9 | 0.5–2 (5.3) 0.5–3 (9.7) | | |
| Mn-BGPT | 390 | 9.5–10.5 (9.5) | 0.2–1.2 | 42000 (390) 33300 (410) 22200 (420) | 0.4–1.1 | 24 (9.8) | | |
| Hg-BGPT | 370 | 4.5–10 (5.5) | 1.5–7.5 | 25300 (370) 20600 (390) 9900 (410) | 2.2–5.6 | 0.5 (5.3) 24 (9.7) | | |
| Pb-BGPT | 390 | 8.5–10.5 (9.8) | 1–7 | 28600 (390) 23000 (410) 17400 (420) | 1.1–4.0 | 0.5–2 (9.8) | | |
| Pd-BGPT | 380 | 5–9 (5.3) | 1–11 | 20400 (380) 18200 (400) 14000 (420) | 1.1–4.0 | 4 (5.3) 3 (9.7) | | |
| Co-BGPT | 390 | 4.5–9 (5.3) | 0.2–1.6 | 32800 (390) 27800 (410) 23300 (420) | 0.4–1.4 | 24 (5.3) 24 (9.7) | 0.39 | 0.4 |
| | 420 | 1.4–0.1 | 0.5–3 | 17900 (430) 11700 (450) | 0.7–2.3 | 0.5–4 (1.4) | 0.47 | 1.1 |
| Ni-BGPT | 370 | 6–9.8 (6.3) | 0.2–1.4 | 37700 (370) 35800 (390) 29000 (410) | 0.3–1.4 | 24 (6.3) 24 (9.7) | 0.37 | 0.55 |
| Fe-BGPT | 640 | 5–11 (5.5) | 1–7 | 7900 (640) 4900 (660) | 1.6–4.5 | 0.3–2 (6.3) | 0.17 | 0.75 |

The characteristics of BBPT and BGPT in different media are shown in Table 3. Changing from water to other less polar solvents produced a batochromic and hipochromic shift in the principal UV absorption band. This band is due to an n → π transition (127).

## Study of the Hydrolysis of Reagents

The instability of sulfur compounds as reagents is generally well recognized. The changes in the spectra with time were dramatic (Fig. 1A) in the case of BBPT and in dilute solution, when the ratio dimethylformamide–water was 1:3. When the ratio was 3:2 the absorbance decreased only about 2.3 and 3.9% at pH 6.0 and 10.0, respectively. The decomposition had little effect at longer wavelengths in most BBPT-concentrated solutions (Fig. 1B).

BGPT, in common with thiosemicarbazones derived from aldehydes and ketones pyridiniques (47, 48, 91), does not suffer hydrolysis.

## Influence of Reducing and Oxidizing Agents

The presence of two $>C=S$ groups in the molecule suggests that the instability might result from the oxidation of one or both of these groups. However, the addition of small amounts of either ascorbic acid or hydroxylamine hydrochloride had little effect upon reagent stability. The oxidation of the $>C=Se$ groups proceeds to $>C^+-Se-Se-C^+<$ for selenosemicarbazide oxidation (27, 112). An attempt was made to follow oxidation spectrophotometrically as a function of pH and time, but the interpretation of data was very difficult. The $>C=S$ instead led to $>C^+-S-S-C^+<$ which shows a marked tendency to be disproportionate. However, the oxidation of cyclohexane-1,3-dione bisthiosemicarbazone proceeds either inter- or intramolecularly (116), yielding well-defined products (Fig. 2).
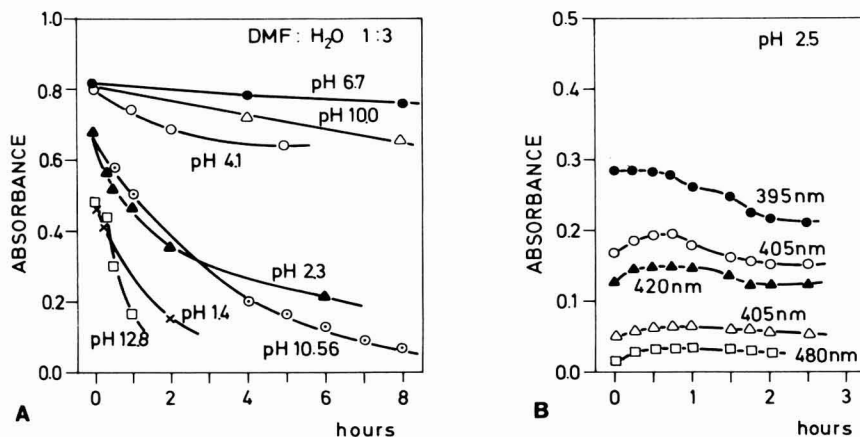


FIG. 1. Stability of BBPT reagent against pH. $C_{BBPT}$: (A) $1-2 \times 10^{-5} M$; (B) $5.8 \times 10^{-4} M$.
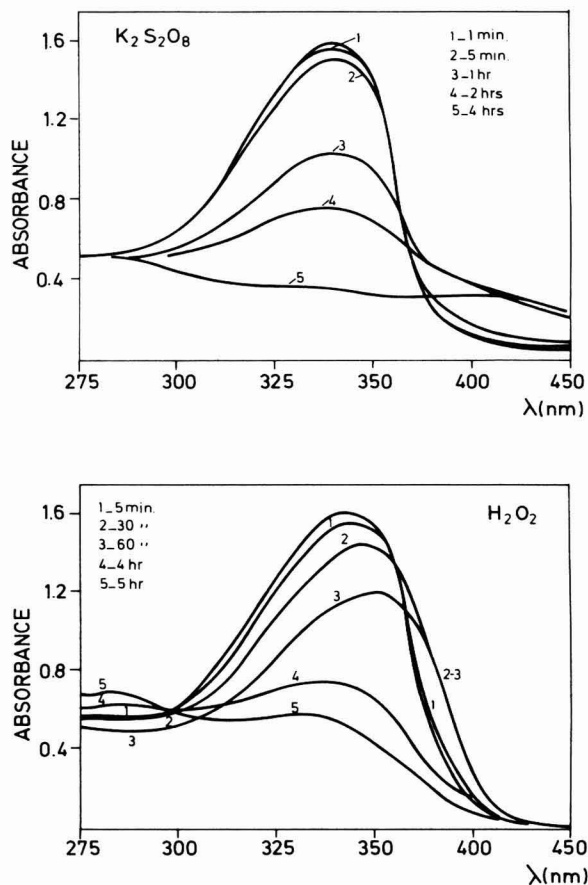
FIG. 2. Effect of oxidizing and reducing agents on the absorption spectra of BBPT.

## Chelate Formation

The chelate compounds are easily prepared by adding a few drops of solutions of BBPT and BGPT to a solution of heavy metals. Due to the presence of the double thiazone grouping, two complexes can be formed in solution with some cations, depending on whether the reagents or the metal ion was in excess.

Bismuth with BBPT at acidic pH forms a yellow complex which turns orange when the pH increases. The yellow complex is positively charged since it precipitates with phthalate and has a ratio Bi:BBPT of 1:1. The orange complex has a ratio Bi:BBPT of 1:2 and it is neutral as proven by solvent extraction tests and by the fact that it was not retained on either a cationic or an anionic ion-exchange resin. When a great excess of BBPT is employed, it forms a new complex having an absorption maximum at 405–420 nm and a high molar absorptivity, about $2.5 \times 10^4$ liters mol$^{-1}$ cm$^{-1}$ (see Figs. 3 and 4).
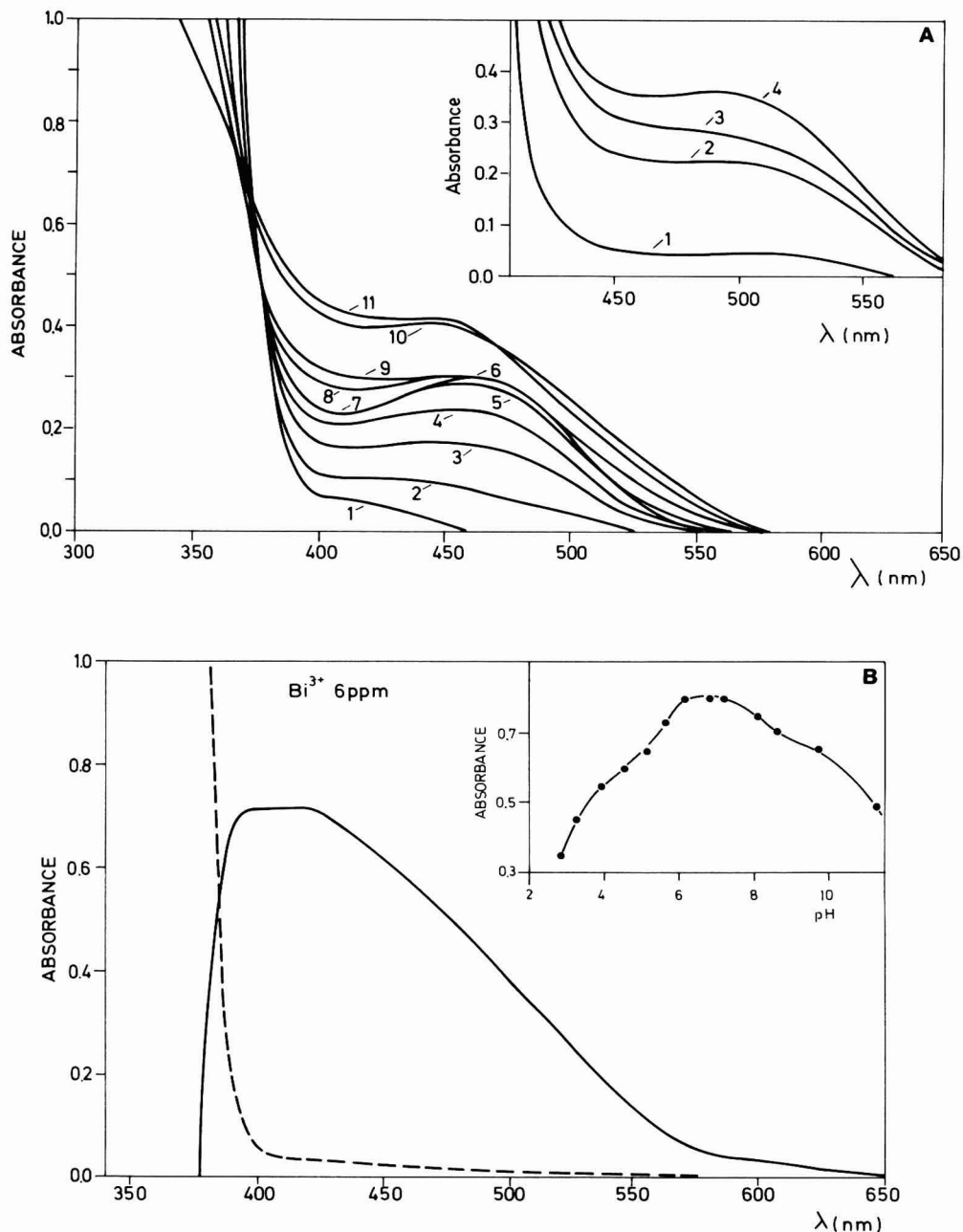
FIG. 3. (A) Absorbance-pH curves for the Bi-BBPT system. pH: 1, 2.00; 2, 2.70; 3, 2.87; 4, 2.97; 5, 3.42; 6, 3.57; 7, 4.76; 8, 5.55; 9, 5.90; 10, 7.95; 11, 8.70 and 12.0; inset: absorbance pH-curves for the Bi extracted with BBPT dissolved in $CHCl_3$. pH: 1, 2.65; 2, 11.45; 3, 9.20; 4, 3.67. $C_{Bi}$ = 6 ppm; Bi:BBPT, 1:2. (B) Absorbance-pH curve and absorption spectrum for the Bi-BBPT complex formed in excess of reagent. $C_{Bi}$ = 6 ppm; $C_{BBPT}$ = $5.8 \times 10^{-4}$ $M$.
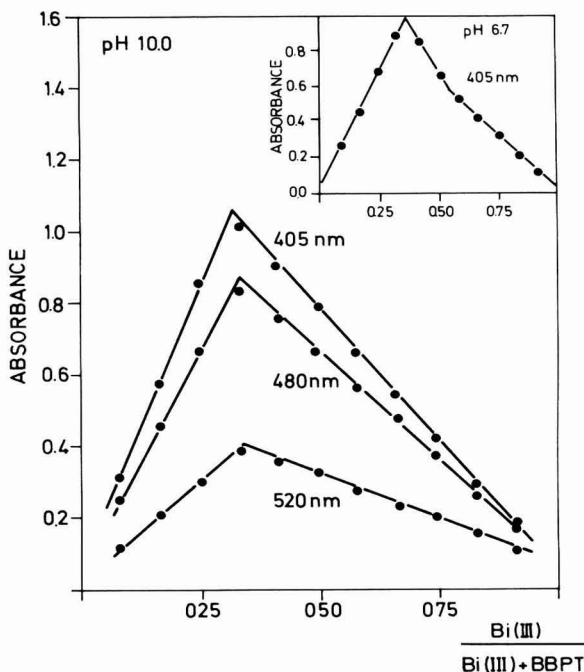
FIG. 4. Determination of composition of the complex by Job's method at pH 6.7 (acetic acid/sodium acetate buffer) and at pH 10 (ammonia/ammonium chloride buffer). $C_{Bi} + C_{BBPT}$ $= 1.37 \times 10^{-4}$ $M$.

Cadmium forms a 1:2 complex at pH 6.7, whereas at pH 10 it forms a 1:1 complex (Fig. 5). The photometric characteristics of the Cd-BBPT and Bi-BBPT systems are depicted in Table 4. The results of interference tests are included in Table 5.

Preliminary tests showed that the BBPT−metal ion complexes were extracted from aqueous-dimethylformamide solutions into numerous organic solvents. However, when the reagent was dissolved in the organic solvent, the complexes were not extracted. If the solvent contains some dimethylformamide, i.e., 5% v/v, the selectivity of the reaction increases, since only copper, mercury, palladium, cadmium, and bismuth were extracted (Fig. 6). The absorption spectra of such chelates underwent a decrease in molar absorptivity with a slight batochromic shift in absorption spectra with respect to the aqueous-dimethylformamide medium.

Because of the overlap of the absorption bands within the range 350−500 nm of the complexes formed by BBPT with zinc and cadmium, and the same pH of their formation, the possibility of determining cadmium or zinc in mixtures is excluded when working in a medium containing 60% v/v dimethylformamide. Consequently, the extraction appears to be advantageous for the spectrophotometric determination of mixtures of
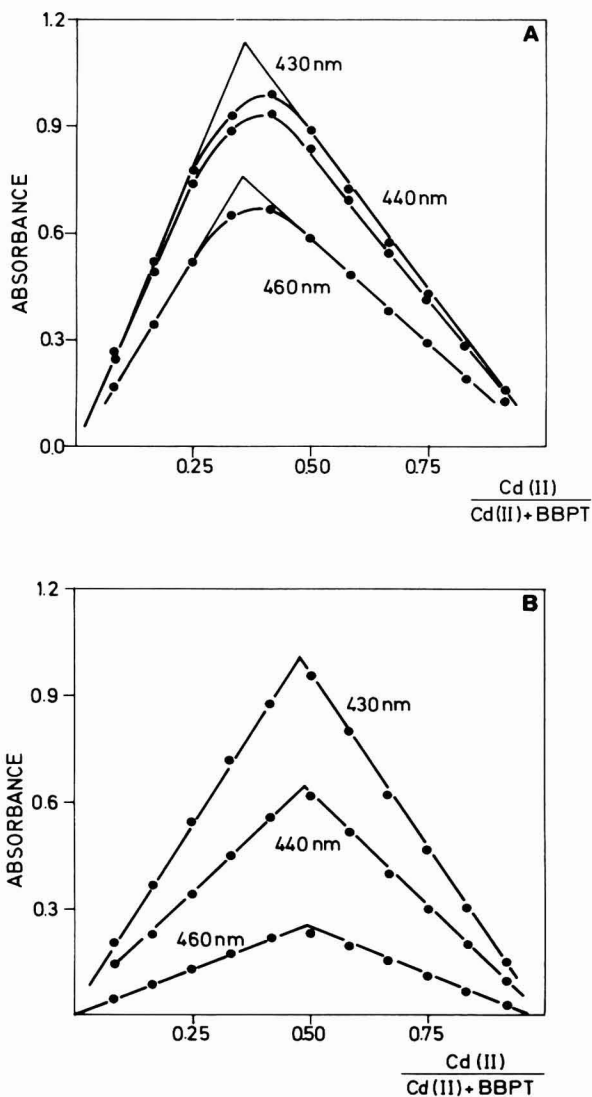
FIG. 5. Determination of the composition of the cadmium-BBPT complex by Job's method. (A) at pH 6.7; (B) at pH 10. $C_{Cd} + C_{BBPT} = 0.92 \times 10^{-4}$ $M$.

cadmium and bismuth, which show different absorption spectra in the presence of zinc and lead.

The absorption spectra of cobalt, nickel, and iron-BGPT complexes are shown in Fig. 7, which also includes the cobalt and nickel-BGT complexes for the sake of comparison. The cobalt complex in acid medium suffers a batochromic shift from 390 to 420 nm. As the stoichiometry in acid medium (Fig. 8) is the same as that obtained at pH 5.3, it seems that

TABLE 4
INTERFERENCE TESTS WITH BBPT

| Tolerance (ppm) | Ion added | |
|---|---|---|
| | Effect of foreign ions on the determination of 2 ppm of Cd with BBPT | |
| 12 | Mn(II) | |
| 25 | Zr(IV) | |
| 50 | As(III), Al(III) | |
| 100 | Alkaline, alkaline earths, La(III), $UO_2$(II), Ce(IV), Th(IV), Cr(III), Mo(VI), W(VI), Tl(I), Se(IV), $PO_4^{3-}$, $P_2O_7^{4-}$. | |
| 180 | $F^-$ | |
| 250 | $Br^-$, $I^-$, $SCN^-$, $S_2O_3^{2-}$ | |
| 1000 | tartrate, $B_4O_7^{2-}$ | |
| 20 | Ag(I), Pb(II), Hg(II), Au(III) | $S_2O_3^{2-}$ as masking; 250 ppm |
| 20 | Sb(III), Sn(II) | Tartrate as masking; 1000 ppm |
| 50 | Mn(II) | Tartrate as masking; 1000 ppm |
| 10 | Fe(III) | $F^-$ as masking; 180 ppm |
| | Effect of foreign ions on the determination of 3 ppm of Bi with BBPT | |
| 12 | Mn(II) | |
| 25 | Al(III), Be(II) | |
| 50 | Th(IV), As(III) | |
| 90 | Alkaline, alkaline earths, La(III), Ce(IV), $UO_2$(II), Tl(I), W(VI), Mo(VI), Se(IV), $PO_4^{3-}$, $SCN^-$, citrate | |
| 1000 | Tartrate, citrate, $B_4O_7^{2-}$ | |
| >3000 | $CN^-$ | |

the change observed in absorption spectra with the decrease of pH is due to protonation.

The relative facility with which the cobalt(II) ion is oxidized when it is bonded to ligand that contains nitrogen atoms as donor groups is well known. Thus, an attempt was made to evaluate the oxidation state of cobalt in the complex. Oxidizing and reducing agents at pH 5.3 and 1.4 did not exert an effect over the absorption spectra of the cobalt complex, if the reagent (oxidizing or reducing agent) was added before or after cobalt complexation. After running a stream of gaseous nitrogen through the solution of reagent and cobalt, they were mixed and the spectrum was recorded. At pH 5.3 the spectrum obtained in inert atmosphere (Fig. 9A) was superior in absorbance to that obtained in the presence of air. Although this is not conclusive it seems that the cobalt is at least partially oxidized in the complex. At pH 1.4, the chelate was not formed in the absence of air, although it was later formed when the sample was left to stand in air, thus indicating an oxidation number of three.

Iron(II) in acid medium with BGPT dissolved in ethanol gives a red complex which turns promptly to yellow, whereas at basic pH it gives a green complex. The rate of change of green to yellow decreases as the pH

TABLE 5
IRON-BGPT SYSTEM

| Cation | pH | Amount of cation (ppm) | λ(nm) | Color | Comments |
|---|---|---|---|---|---|
| Fe(II) | 2.5 | 2 | 415 | Yellow | Unstable. The absorbance increases for 2 hr |
| | 6.3 | 10 | 380 600–650[a] | Green | The green color changes to yellow for 3 hr; then precipitation occurs |
| | 9.7 | 10 | 380 640 550–580[a] | Green | First the absorbance decreases; then precipitation occurs |
| Fe(II) plus ascorbic acid | 2.4 | 10 | 420 600 | Dirty green | It does not change to yellow. The absorbance decreases and disappears with time |
| | 6.3 | 5 | 380 640 550–580 | Green | It is stable between 20 min and 2 hr |
| | 9.7 | 5 | 380 640 550–580 | Green | It shows the same behavior as shown without ascorbic acid |
| Fe(III) | 2.5 | 2 | 415 | Yellow | The absorbance increases for 3 hr; then it is stable and shows the same spectra as obtained with iron(II) |
| | 6.3 | 1 | 385 | Yellow | It is formed immediately |
| | 9.7 | | | Green | It shows the same spectrum as obtained with Fe(II) plus ascorbic acid and with Fe(II) |

[a] Shoulder.
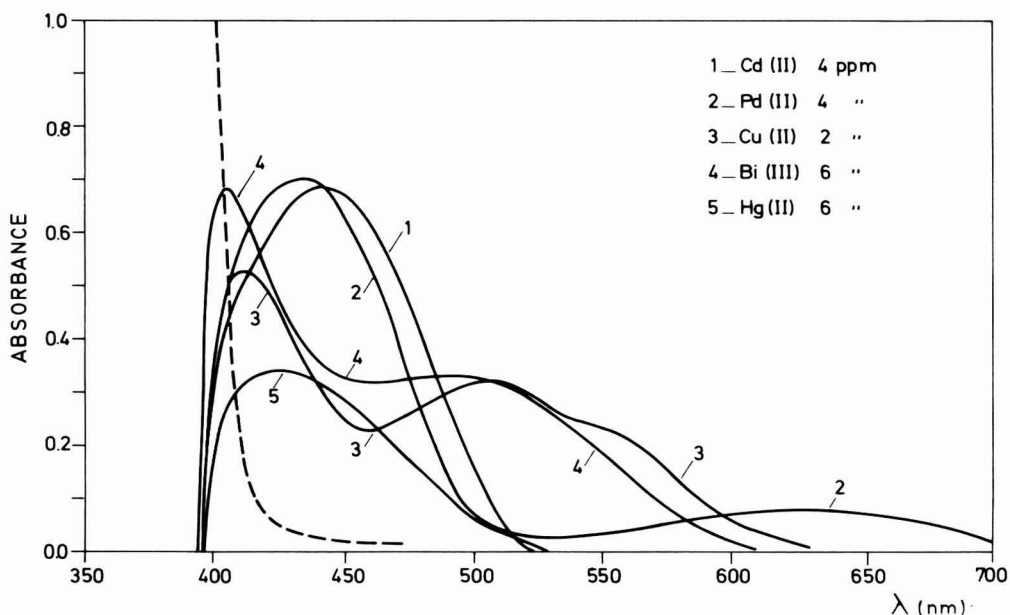
FIG. 6. Absorption spectra of the metal ion – BBPT complexes extracted into chloroform containing 5% of dimethylformamide at pH 4.7 (acetic acid/sodium acetate buffer).

increases. If dimethylformamide is used instead of ethanol, at acid pH it produces a dirty green coloration which also changes immediately to yellow. Iron(III) in acid medium with BGPT gives the yellow color, whereas at basic pH it gives the green one. The characteristics of the iron-BGPT system are included in Table 5. In Table 4 the photometric characteristics of BGPT-metal ions complexes are also summarized. The ion-exchange tests of cobalt, nickel, and iron-BGPT complexes are depicted in Table 6. The results of tests for tolerance of cobalt, nickel, and iron are given in Table 7.

Since the nickel-BGPT complex was not formed in acid medium, an attempt was made to determine cobalt in the presence of nickel. Nevertheless, nickel interfered in the determination of cobalt at pH 1.4 and this interference was not eliminated by adding an excess of reagent. The spectra of samples which contained 2 ppm of cobalt and various amounts of nickel (Table 8, Fig. 10) were recorded in order to confirm this fact. As we have previously indicated, the nickel alone plus BGPT was not absorbed under the same circumstances. The characteristic band of the Co-BGPT chelate ($\lambda_{max}$ = 420 nm) suffered a progressive bato- and hipochromic shift as the amount of nickel increased. The samples prepared were stable for about 3 hr.

An analogous behavior was observed in the study of the Cu-BGPT-Ni
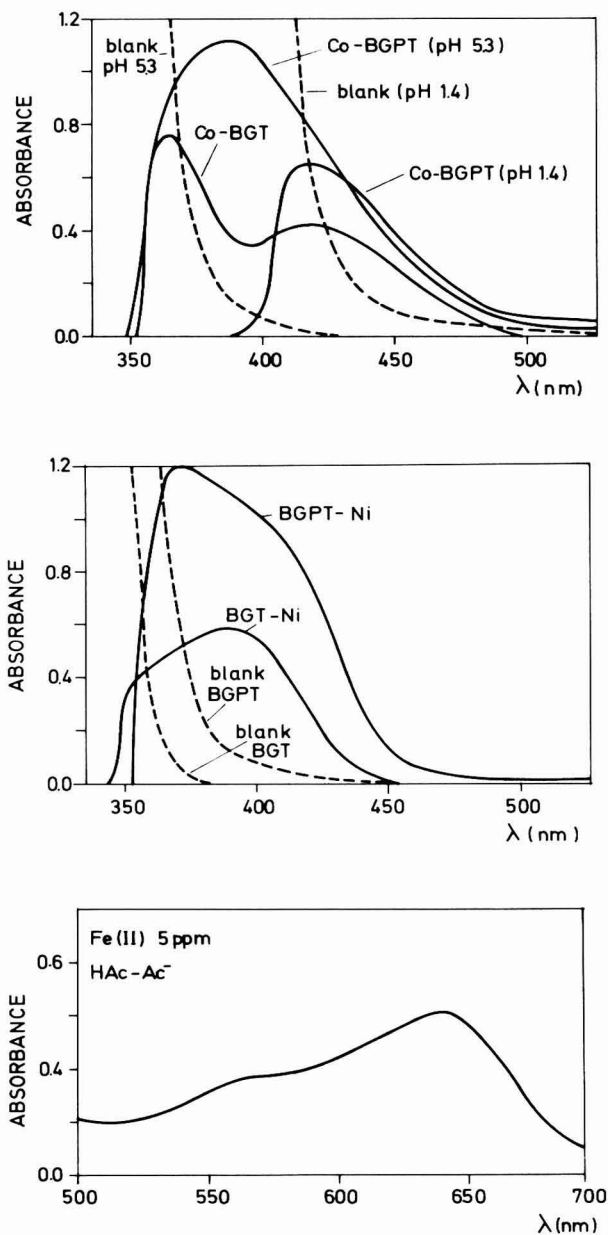
FIG. 7. Absorption spectra of the reaction products of BGPT with cobalt, nickel, and iron. The spectra of the corresponding dithiosemicarbazone (BGT) are included for the sake of comparison.
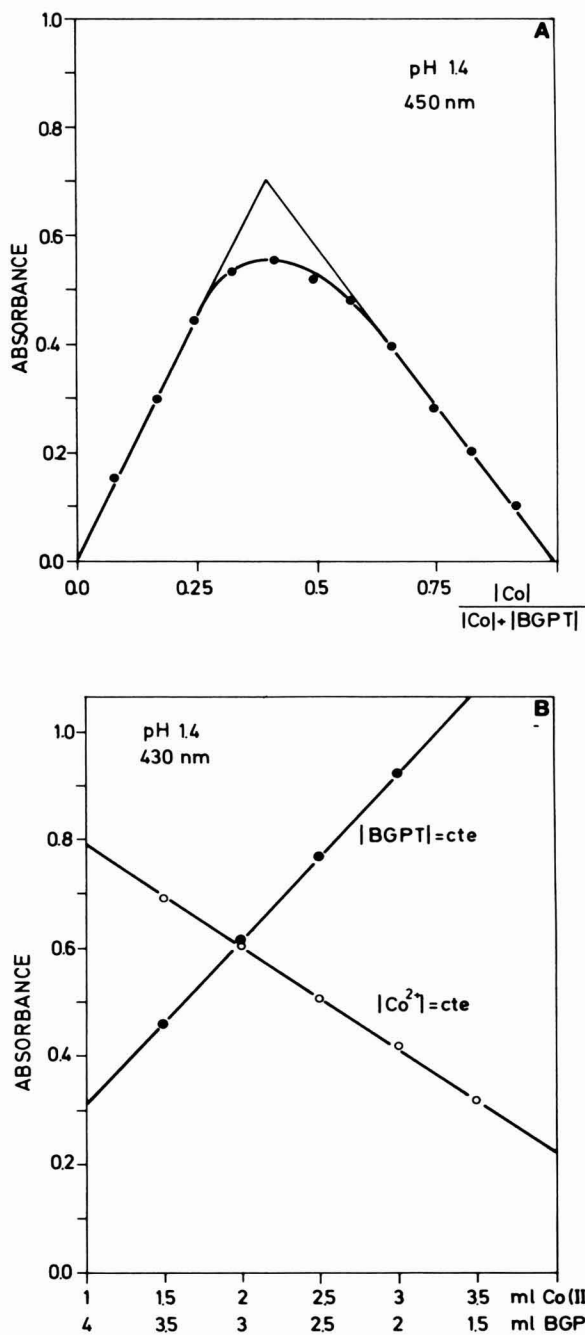
FIG. 8. Composition of the cobalt-BGPT complex at pH 1.4. (A) Composition determined by Job's method. (B) Composition determined by the slope ratio method.
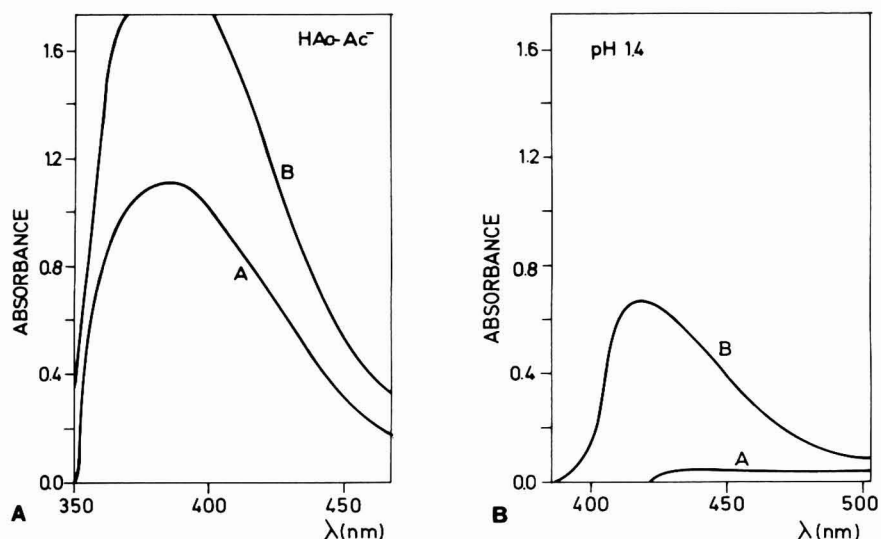
FIG. 9. Spectrum of Co-BGPT measured immediately after running a strem of gaseous nitrogen through the solution (A); and spectrum measured in the presence of air (B).

system. At first, nickel should not interfere either in the determination of copper at pH 2.5. Nevertheless, the characteristic band of the Cu-BGPT complex suffered a batochromic shift (Table 9, Fig. 11) from 420 to 429 nm. The ratio Cu:Ni of 1:30 was critical, because greater amounts of copper did not exert more modification on the absorption spectra. The stability of samples was about 4 hr.

A possible explanation of this strange behavior can be the formation of heteropolinuclear Co-BGPT-Ni and Ni-BGPT-Cu complexes. So far as we know, this is the first time that this interesting phenomenon has been reported by the use of a thiosemicarbazone reagent. A comprehensive

TABLE 6
ION-EXCHANGE TESTS (DOWEX 50-X8 RESIN, SODIUM FORM)[a,b,c]

| Complex/medium | Acid (hydrochloric acid) | Weak acid | | Basic (ammonia buffer) |
| | | without buffer | Acetic acid/ sodium acetate buffer | |
| --- | --- | --- | --- | --- |
| Co-BGPT | + | + | − | − |
| Ni-BGPT | | + | − | − |
| Fe-BGPT | + | + | − | − |

[a] In the presence of potassium chloride or potassium nitrate the complexes were not retained in acid and basic medium.
[b] The complexes were not retained in the anionic resin.
[c] +, Retained in the resin; −, not retained.

TABLE 7
INTERFERENCE TESTS WITH BGPT

| Tolerance (ppm) | Ion added | |
|---|---|---|
| | Effect of foreign ions on the determination of 1 ppm of Co at pH 5.3 | |
| 2 | Sb(III), Ce(IV), Pb(II) | |
| 5 | Au(III) | |
| 20 | Sn(II), Mn(II), $C_2O_4^{2-}$ | |
| 100 | Tl(I), As(III), Se(IV), Al(III), Uo$_2$(II), Zr(IV), Be(II), Th(IV), La(III), alkaline, alkaline earths | |
| 1000 | $PO_4^{3-}$, $S_2O_3^{2-}$, tartrate, $F^-$, $SCN^-$ | |
| 2 | Bi(III) | Tartrate as masking; 1000 ppm |
| 5 | W(VI) | Tartrate as masking; 1000 ppm |
| 10 | Hg(II) | $S_2O_3^{2-}$ as masking; 1000 ppm |
| 25 | Pb(II) | $S_2O_3^{2-}$ as masking; 1000 ppm |
| 100 | Mo(VI) | $S_2O_3^{2-}$ as masking; 1000 ppm |
| | Effect of foreign ions on the determination of 1.5 ppm of Co at pH 1.4 | |
| 1 | Bi(III), Ni(II) | |
| 2 | Au(III) | |
| 5 | Ag(I), Pb(II), W(VI), Hg(II) | |
| 10 | Ce(IV) | |
| 25 | Sb(III) | |
| 50 | Zr(IV) | |
| 100 | Mo(VI), Sn(II), Se(IV), Al(III), UO$_2$(II), As(III), Tl(I), Be(II), Th(IV), La(III), Mn(II), alkaline, alkaline earths | |
| 200 | Cd(II) | |
| 1000 | Zn(II) | |
| | Effect of foreign ions on the determination of 0.8 ppm of Ni(II) at pH 6.3 | |
| 1 | Pb(II), Cr(VI), Sb(III) | |
| 5 | As(III), Au(III), Sn(II) | |
| 25 | Ce(IV), Mn(II) | |
| 100 | Tl(I), Mo(VI), Se(IV), Al(III), UO$_2$(II), Zr(IV), Th(IV), La(III), Be(II), alkaline and alkaline earths | |
| 150 | $C_2O_4^{2-}$ | |
| 1000 | Tartrate, $PO_4^{3-}$, $S_2O_3^{2-}$, citrate, $F^-$, $B_4O_7^{2-}$, $SCN^-$, $ClO_4^-$ | |
| 15 | Ag(I) | $S_2O_3^{2-}$ as masking; 1000 ppm |
| 50 | Hg(II) | $S_2O_3^{2-}$ as masking; 1000 ppm |
| 80 | Au(III) | $S_2O_3^{2-}$ as masking; 1000 ppm |
| 5 | Fe(III) | $F^-$ as masking; 1000 ppm |
| 80 | Sb(III) | Tartrate as masking; 1000 ppm |
| | Effect of foreign ions on the determination of 4 ppm of Fe(II) at pH 5.5 | |
| 1 | Ni(II), Pd(II) | |
| 5 | W(VI) | |
| 10 | Co(II), Cu(II), Au(III), Bi(III), Sb(III), Ce(IV) | |
| 20 | $F^-$ | |
| 25 | V(V), Cd(II), Pb(II), La(III) | |
| 60 | Zn(II) | |
| 100 | Ag(I), Tl(I), Hg(II), As(III), Mo(VI), Pt(IV), Al(III), Cr(VI), Zr(IV), Th(IV), alkaline and alkaline earths | |
| 800 | $PO_4^{3-}$ | |
| 1000 | citrate, tartrate, $SCN^-$, $ClO_4^-$ | |

TABLE 8
COBALT – BGPT – NICKEL SYSTEM (AT pH 1.4)

| Co/Ni (weight) | $\lambda_{max}$(nm) | Absorbance |
|---|---|---|
| 1:0 | 420 | 0.700 |
| 1:0.1 | 420 | 0.700 |
| 1:0.12 | 420 | 0.700 |
| 1:0.17 | 420 | 0.700 |
| 1:0.25 | 420 | 0.690 |
| 1:0.50 | 421 | 0.675 |
| 1:1 | 423 | 0.625 |
| 1:2 | 426 | 0.600 |
| 1:4 | 429 | 0.600 |
| 1:6 | 431 | 0.600 |
| 1:8 | 432 | 0.580 |
| 1:10 | 432 | 0.570 |
| 1:20 | 434 | 0.555 |
| 1:30 | 434 | 0.550 |
| 1:40 | 435 | 0.520 |
| 1:50 | 436 | 0.500 |
| 1:100 | 438 | 0.380 |
| 1:200 | 441 | 0.235 |
| 1:500 | 446 | 0.130 |
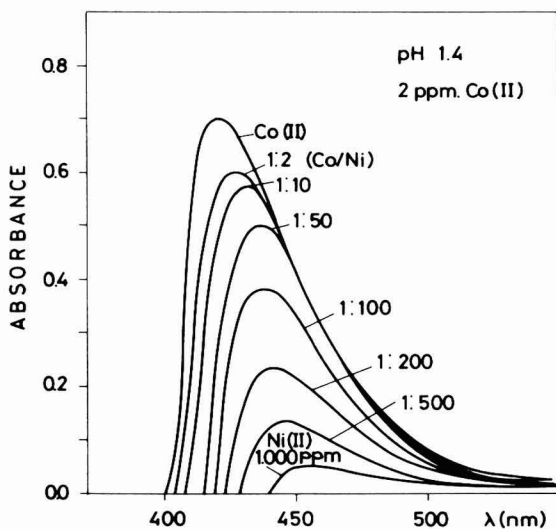


FIG. 10. Spectra of the Co-BGPT-Ni system.

TABLE 9
COPPER–BGPT–NICKEL SYSTEM (AT pH 2.5)

| Cu/Ni (weight) | $\lambda_{max}$(nm) | Absorbance |
|---|---|---|
| 1:0 | 420 | 0.720 |
| 1:0.1 | 420 | 0.700 |
| 1:0.12 | 420 | 0.685 |
| 1:0.17 | 420 | 0.675 |
| 1:0.25 | 422 | 0.640 |
| 1:0.5 | 423 | 0.620 |
| 1:1 | 424 | 0.600 |
| 1:2 | 425 | 0.545 |
| 1:4 | 426 | 0.510 |
| 1:6 | 426 | 0.500 |
| 1:8 | 426 | 0.495 |
| 1:10 | 427 | 0.490 |
| 1:20 | 428 | 0.480 |
| 1:30 | 429 | 0.460 |
| 1:40 | 429 | 0.455 |
| 1:50 | 429 | 0.445 |
| 1:100 | 429 | 0.445 |
| 1:200 | 429 | 0.440 |
| 1:300 | 429 | 0.440 |
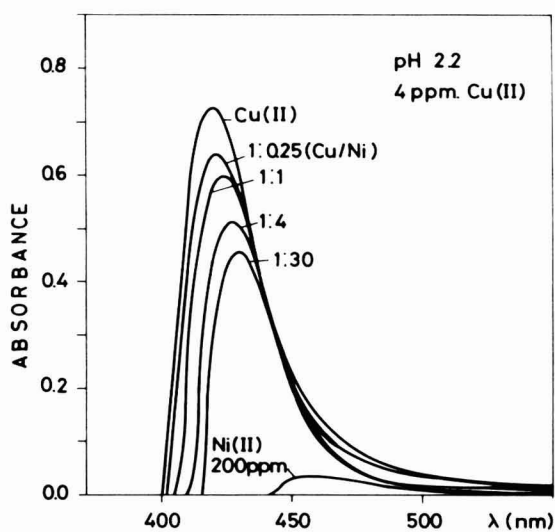


FIG. 11. Spectra of the Cu-BGPT-Ni system.

study of this phenomenon is not being undertaken because the authors are not able to find a technique of study. Our laboratory is mainly concerned with the establishment of empirically spectrophotometric methods of analysis.

## CONCLUSION

Diphenylthiosemicarbazones suffer from three serious drawbacks in their use as analytical reagents. The first is their sparse solubility in water, ethanol (other than BGPT), and methanol which necessitates the use of a considerable amount of water-miscible organic solvent to prevent precipitation of the reagent on the reaction mixture. Dimethylformamide was found to be most suitable for this purpose. The second is the well-recognized instability of sulfur compounds which implies the storage of reagents in the dark at low temperature and the use of freshly prepared reagent solutions. The third, even more serious, is the lack of selectivity in its behavior with regard to metal ions. In spite of this, the color reaction given by these reagents with various cations has attractive sensitivities and it would be interesting to establish selective methods of analysis using these reagents. Thiosemicarbazones have been reported numerous times in the literature, but little analytical significance has yet been established with these interesting compounds in connection with their usefulness in the spectrophotometric determination of cadmium or bismuth, and no analytical significance has been established regarding their ability to form heteropolinuclear complexes.

The presence of the nitrogen piridinique confers more solubility and stability to thiosemicarbazones, but shifts the maximum absorption of complexes to shorter wavelengths. Due to the high molar absorptivity of BGPT−metal ion complexes and to the fact that it chelates numerous metal ions, it would be interesting as a preconcentration reagent, i.e., to concentrate trace metal ions from sea water. It is also necessary to say that great interest has developed in the chemistry of thiosemicarbazones due, on one hand, to its pharmacological properties and, on the other hand, to the increasing interest in the coordination chemistry of ligand with SN atoms as donor groups.

## SUMMARY

This paper is a continuation of the study and applications of biacetyl bis(4-phenyl-3-thiosemicarbazone) (BBPT) and bipyridylglyoxal bis(4-phenyl-3-thiosemicarbazone) (BGPT). A number of spectrophotometric determinations have been carried out. BBPT is suggested as a reagent for the extractive spectrophotometric determination of cadmium and bismuth. The formation of heteropolinuclear cobalt-BGPT-nickel and copper-BGPT-nickel complexes at acid pH have been reported.

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# Indirect Spectrophotometric Determination of Borate Using Nitron as Reagent

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## INTRODUCTION

In previous papers, nitron has been proposed as a reagent for the indirect spectrophotometric determination of nitrate (1) and perchlorate (2). In the present article a modified procedure is described for borate. The latter ion is converted into tetrafluoborate which is precipitated with a large excess of nitron as nitron tetrafluoborate. After separating off the precipitate, the excess nitron is determined in the supernatant liquid by a spectrophotometric method. This method is based on the formation of the blue ion-association complex nitron cobaltothiocyanate, which can be extracted with a mixed organic solvent.

## EXPERIMENTAL

*Nitron reagent (0.1 M).* Dissolve 3.2 g of nitron in 20 ml of warm 5% acetic acid, filter into a dark plastic bottle, add 2.5 g of AR 46% hydrofluoric acid, and dilute to 100 ml with distilled water.

*Calibration curve.* Place 2-ml portions of standard borate solution containing from 0.12 to 1.1 mg of borate in 10-ml polythene beakers or centrifuge tubes. Add to each beaker or tube 2 ml of the nitron reagent measured with a pipet. Allow to stand in an ice bath for 1 hr. Filter through a sintered glass funnel or centrifuge off the precipitate. Pipet 1 ml of the supernatant liquid into a separatory funnel containing 30 ml of water. Add two drops of 1 $M$ sulfuric acid, 10 ml of 0.05 $M$ cobaltous sulfate, and 5 ml of 4 $M$ potassium thiocyanate solution, and mix. Extract the blue complex with four 10-ml portions of 30% (v/v) cyclohexanone in carbon tetrachloride. Combine the extracts and dilute to 50 ml with the solvent. Measure the absorbance at 625 nm in a 1-cm cell against the solvent.

Run a blank by treating 0.5 ml of the same nitron reagent with cobaltous sulfate and potassium thiocyanate, extracting the complex as described above and measuring the absorbance against the solvent.

Subtract the absorbance values of the borate standards from that of the blank. Draw the relation between the obtained values ($A$ difference) and the borate ion concentration. A straight line passing by the origin will be obtained.

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*Determination of borate.* Treat 2 ml of the test solution with an equal volume of the reagent and continue as previously mentioned. Carry out the blank using the same reagent solution. Find the difference in absorbance and read off the borate ion concentration from the calibration graph.

## RESULTS AND DISCUSSION

The validity of the method was examined by analyzing standard samples of AR boric acid; the method proved to be reproducible at milligram levels with a coefficient of variation of about $\pm 2\%$. Since analysis of almost all boron-containing compounds requires a preliminary treatment which ultimately results in an aqueous boric acid sample, this procedure may be regarded as a method for determination of boron. Sulfate, phosphate, and weak acids and bases do not interfere. Chloride does not interfere up to a concentration of 0.1 $M$. Bromide, iodide, chromate, chlorate and nitrite interfere even at concentrations as low as 0.01 $M$. These intereferences can be easily eliminated (2). Bromide and iodide are decomposed with chlorine water, nitrite with hydrazine sulfate or urea, chromate with hydrazine sulfate or sulfurous acid, and chlorate with formaldehyde or sulfurous acid; the procedures are outlined elsewhere (1, 2). Nitrate, perchlorate, tungstate, and molybdate, however, should be absent.

For a detailed discussion, see Ref. (2).

## SUMMARY

An indirect method is outlined for the spectrophotometric determination of small amounts of borate. Borate is quantitatively precipitated as nitron tetrafluoborate in the presence of excess nitron. After separating off the precipitate, the excess of reagent is determined in the supernatant liquid as nitron cobaltothiocyanate.

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# Solvent Extraction and Photometric Determination of a Microgram of Cerium with N-p-Tolyl-p-chlorobenzohydroxamic Acid

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## INTRODUCTION

Hydroxamic acids are widely used as analytical reagents for the solvent extraction and spectrophotometric determination of various metal ions (*1–5, 8, 10, 14, 17, 22*). Several other organic reagents are used for the extraction and spectrophotometric determination of cerium (*7, 11, 15, 20, 21*). Thenoyl trifluoroacetone (TTA) was used by Khopkar and Dey for the determination of cerium (*9*), and Smith and Moore (*16*) have modified the procedure and extracted the cerium at pH 5.4. This method is not selective since many common metals also form complexes with TTA at this pH. Onishi and Toita (*13*) have reinvestigated the method and determined cerium, first extracted with cupferron and then with TTA. However, none of these reagents are suitable for the rapid determination of cerium and the interference study was not extensive. With this object a rapid method for the simultaneous solvent extraction and spectrophotometric determination of cerium has been described. The cerium forms an orange-red-colored complex with N-p-tolyl-p-chlorobenzohydroxamic acid which is extracted with chloroform at pH 8.5 to 9.0. The extracted complex has a maximum absorbance at 460 nm. A clean-cut separation from many commonly occurring metal ions is easily accomplished by this method. Beer's law is obeyed within the range of 0.5 to 28 ppm of cerium. The molar absorptivity is $4.5 \times 10^3$ liters $mol^{-1}$ $cm^{-1}$. The method is sensitive, precise, and equally applicable for the extraction of a microgram quantity of cerium. The present method surpasses the reagent N-phenylbenzohydroxamic acid (*12*).

## EXPERIMENTAL

*Reagents and equipments.* All chemicals used are of analytical purity. The reagent N-p-tolyl-p-chlorobenzohydroxamic acid was prepared as previously described (*6*) and recrystallized from benzene and petroleum ether. Purity was checked by mp (166°C, reported (*18*) 166°C), elemental analysis, TLC, IR, and UV spectra.

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A stock solution of cerium was prepared by dissolving the requisite amount of ceric sulfate. The final concentration (5.6 ppm) was determined volumetrically (19).

A 0.1% (w/v) reagent solution was prepared in chloroform. The absorption spectrum of cerium complex was measured on a VSU2-P spectrophotometer. The pH measurements were made with a Systronics digital pH meter equipped with glass-calomel electrodes.

## PROCEDURE

Transfer 1 ml of cerium solution ($1 \times 10^{-4}$ M) into a 100-ml separatory funnel. Add 10 ml of the reagent solution of chloroform and adjust the pH to 8.5–9.0 with 0.05 M ammonium hydroxide, shake the contents for 5–10 min, and allow the phase to separate. Transfer the chloroform layer to an Erlenmeyer flask containing anhydrous sodium sulfate. Swirl the flask to remove the droplets of water and wash sodium sulfate with 3(×2)-ml of chloroform. Finally dilute the extract to 25 ml with chloroform and measure the absorbance at 460 nm against reagent blank.

## RESULTS AND DISCUSSION

*Absorption Spectra*

The absorption spectrum of the orange-red cerium complex has a maximum absorbance at 460 nm (Fig. 1). The reagent blank showed no absorbance at this wavelength. All absorbance measurements were done at 460 nm. The system obeys Beer's law over the range of 0.5 to 28 ppm of
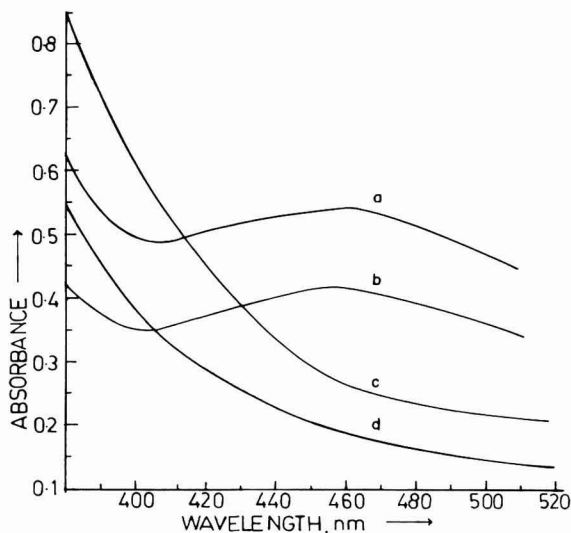


FIG. 1. Absorption spectra of (a) cerium(IV)−N-p-tolyl-p-chloroBHA extracted from chloroform, (b) carbon tetrachloride, (c) N-butylalcohol, and (d) amylalcohol.
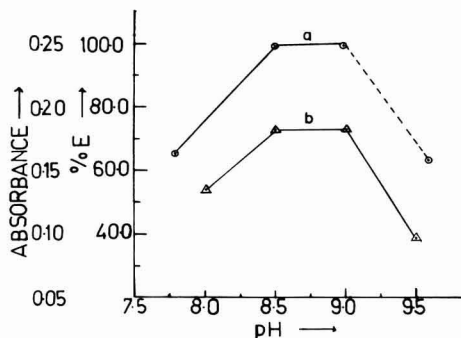
FIG. 2. Effect of pH on the extraction of cerium complex. (a) Percentage extraction vs pH and (b) OD vs pH.

cerium at 460 nm and the molar absorptivity of the extracted species was $4.5 \times 10^3$ liters mol$^{-1}$ cm$^{-1}$.

*Effect of pH*

The effect of pH on the extraction of cerium(IV) is shown in Fig. 2 and Table 1. The maximum color intensity was obtained at pH 8.5−9.0.

*Effect of Solvent*

The cerium−*N*-*p*-tolyl-*p*-chlorobenzohydroxamate complex was extracted from various solvents viz. carbon tetrachloride, chloroform, amyl alcohol, and *n*-butyl alcohol. It was observed that chloroform is the most suitable solvent for the extraction of cerium. The data are given in Table 2 (Fig. 2).

*Effect of Temperature*

The absorbance in chloroform extract of the cerium complex is constant over the range 15−35°C.

TABLE 1
EFFECT OF pH ON THE EXTRACTION OF
CERIUM(IV)−*N*-*p*-TOLYL-*p*-CHLOROBENZOHYDROXAMATE COMPLEX[a]

| Set numbers | pH | Percentage $E$[b] | Distribution ratio |
|:-----------:|:----:|:----------------:|:------------------:|
| 1 | 8.0 | 75 | 4.0 |
| 2 | 8.5 | 100 | —[c] |
| 3 | 9.0 | 100 | —[c] |
| 4 | 9.5 | 52.8 | 2.2 |

[a] Cerium, 5.6 ppm; maximum absorbance, 460 nm.

[b] Percentage $E = 100 \, D/(D + (Ce_{aq}/Ce_{org}))$, assuming that the volume of both phases was $V_{org} = V_{aq}$ and the distribution coefficient $D$, was determined from $D =$ concentration of Ce in organic phase/total Ce taken − Ce extracted inorganic phase.

[c] Too high to measure.

TABLE 2
EFFECTS OF SOLVENTS ON THE EXTRACTION OF
CERIUM(IV)-*N*-*p*-TOLYL-*p*-CHLOROBENZOHYDROXAMATE COMPLEX[a]

| Solvent | Color | Wavelength of maximum absorbance (nm) | $\epsilon$ ($\times 10^3$) |
|---------|-------|------------------------|---------------------|
| Chloroform | Orange-red | 460 | 4.5 |
| Carbon tetrachloride | Orange-red | 460 | 3.4 |
| N-Butyl alcohol | Orange-red | 460–465 | 2.2 |
| Amyl alcohol | Orange-red | 460 | 1.6 |

[a] Cerium, 5.6 ppm.

## Stability, Shaking Time, and Recovery

The complex is stable under optimum conditions. The absorbance remains unchanged for 10 days. Extraction of cerium is very rapid. Shaking for 2 min was enough to attain equilibrium. It was confirmed that cerium is extracted into organic phase quantitatively. Cerium (5.6 ppm) was extracted according to the procedure and the aqueous phase was reexamined by repeating the extraction with another 10 ml of reagent solution and measuring the absorbance of the organic phase. There was no difference between the absorbance of the second organic phase and reagent blank.

## Effect of Reagent Concentration

The solvent extraction behavior of cerium with *N*-*p*-tolyl-*p*-chlorobenzohydroxamate complex was studied by taking various concentrations of reagent. The results indicate that the lower concentration of reagent

TABLE 3
EFFECT OF ANIONS ON THE EXTRACTION OF CERIUM(IV)[a]

| Anion | Added as (mg) | Absorbance |
|-------|---------------|------------|
| $NO_2^-$ | $NaNO_2$ (60) | 0.90 |
| $F^-$ | NaF (60) | 0.90 |
| $Cl^-$ | NaCl (60) | 0.90 |
| $Br^-$ | NaBr (60) | 0.91 |
| $I^-$ | NaI (60) | 0.90 |
| $CN^-$ | KCN (50) | 0.90 |
| $CH_3COO^-$ | $CH_3COOH$ (60) | 0.90 |
| $Cit^{3-}$ | Citric acid (40) | 0.89 |
| $Tart^{3-}$ | Tartric acid (40) | 0.89 |
| $SO_3^{2-}$ | $Na_2SO_3$ (40) | 0.90 |
| $SO_4^{2-}$ | $Na_2SO_4$ (40) | 0.91 |
| $PO_4^{3-}$ | $Na_3PO_4$ (80) | 0.90 |
| $NO_3^-$ | $NaNO_3$ | 0.90 |

[a] Cerium = 30 $\mu$g/5 ml; $\times$ pH = 8.5.

gives incomplete extraction. In the present method 10 ml of 0.1% reagent is adequate for quantitative extraction. Excess reagent has no effect on the absorbance.

## Effect of Diverse Ions

The effect of diverse ions on the extraction of cerium was studied by the recommended procedure. The experiments were made on the aqueous solution containing a fixed amounts of cerium in the presence of various amounts of diverse ions given in Tables 3 and 4. Moderate amount of many ions can be tolerated.

## Analytical Data

To test the reliability of the method, standard cerium samples were analyzed for cerium. The results presented in Table 5 show that cerium can be determined precisely and accurately.

TABLE 4
EFFECT OF CATIONS ON THE EXTRACTION OF THE CERIUM(IV)[a]

| Cation | Added as (mg) | Absorbance |
|--------|---------------|------------|
| $Ag^+$ | $AgNO_3$ (80) | 0.90 |
| $Be^{2+}$ | $BeSO_4$ (80) | 0.90 |
| $Mg^{2+}$ | $Mg(NO_3)_2$ (100) | 0.90 |
| $Ca^{2+}$ | $Ca(NO_3)_2$ (80) | 0.90 |
| $Sr^{2+}$ | $SrCl_2$ (80) | 0.90 |
| $Ba^{2+}$ | $BaCl_2$ (80) | 0.91 |
| $Cu^{2+}$ | $CuSO_4$ (60) | 0.90 |
| $Cd^{2+}$ | $CdSO_4$ (80) | 0.89 |
| $Zn^{2+}$ | $ZnCl_2$ (80) | 0.90 |
| $Hg^{2+}$ | $Hg(NO_3)_2$ (80) | 0.91 |
| $Pb^{2+}$ | $Pb(C_2H_3O_2)_2 \cdot 3H_2O$ (100) | 0.90 |
| $Mn^{2+}$ | $Mn(C_2H_3O_2)_2 \cdot 4H_2O$ (100) | 0.92 |
| $CO^{2+}$ | $CO(C_2H_3O_2)_2 \cdot 4H_2O$ (100) | 0.91 |
| $Ni^{2+}$ | $NiCl_2$ (80) | 0.90 |
| $UO_2^{2+}$ | $UO_2(NO_3)_2 \cdot 6H_2O$ (60) | 0.90 |
| $Ga^{3+}$ | $GaI_3$ (60) | 0.89 |
| $La^{3+}$ | $La(NO_3)_3 \cdot 6H_2O$ (80) | 0.89 |
| $Pr^{3+}$ | $Pr(NO_3) \cdot H_2O$ (60) | 0.90 |
| $Nd^{3+}$ | $Nd(NO_3)_3 \cdot H_2O$ (60) | 0.90 |
| $As^{3+}$ | $AsF_3$ (60) | 0.90 |
| $Fe^{3+}$ | $FeCl_3$ (100) | 0.90 |
| $Sb^{3+}$ | $SbCl_3$ (100) | 0.91 |
| $In^{3+}$ | $InBr_3$ (80) | 0.91 |
| $Cr^{3+}$ | $Cr_2O_3$ (80) | 0.92 |
| $Ti^{4+}$ | $TiCl_4$ (30) | 0.90 |
| $Zr^{4+}$ | $Zr(NO_3)_4 \cdot 5H_2O$ (30) | 0.89 |
| $Th^{4+}$ | $Th(NO_3)_4$ (80) | 0.90 |

[a] Cerium(IV) = 30 $\mu g$/25 ml; pH = 8.5.

TABLE 5
ANALYTICAL DATA ON THE EXTRACTION OF CERIUM

| Cerium ($\mu g$/25 ml of chloroform) | Cerium found ($\mu g$) | Error | Standard deviation[a] ($\sigma$) |
|---|---|---|---|
| 3.00 | 2.99 | −0.01 | ±0.01 |
| 6.00 | 6.02 | +0.02 | ±0.02 |
| 9.00 | 8.99 | −0.01 | ±0.01 |
| 12.00 | 11.98 | −0.02 | ±0.02 |
| 18.00 | 18.01 | +0.01 | ±0.01 |
| 24.00 | 24.00 | 0.00 | ±0.01 |
| 30.00 | 30.01 | +0.01 | ±0.01 |
| 36.00 | 36.01 | +0.01 | ±0.01 |

[a] Average of eight determinations.

## SUMMARY

A spectrophotometric method for determination of a microgram quantity of cerium with N-p-tolyl-p-chlorobenzohydroxamic acid is described. The orange-red-colored complex is extracted from chloroform at pH 9 which absorbs between 460 and 465 nm. Beer's law is obeyed at this wavelength. A clean-cut separation from many commonly occurring metal ions is easily accomplished. The system obeys Beer's law within the range of 0.5−28 ppm of cerium(IV). The molar absorptivity of cerium-N-p-tolyl-p-chlorobenzohydroxamic acid complex is $4.5 \times 10^3$ liters mol$^{-1}$ cm$^{-1}$.

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# Polarography of *o*- and *p*-Nitrophenyl Acetic Acids

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## INTRODUCTION

Polarographic studies on aromatic nitro compounds, in general, show that they give two steps: The first corresponding to reduction to the hydroxylamine and the second, which occurs generally at more negative potentials in acidic medium, to the reduction of the hydroxylamine to the amine. However, *o*- and *p*-nitrophenols and *o*- and *p*-nitroanilines (5) show a single-step 6*e* reduction in alkaline media. 5-Nitrobarbituric acid, 5-nitrouracil, and 5-nitroorotic acid (3) yield 6*e* waves over a wide pH range. Similar observations have been reported for reductions of 2-nitroresorcinol (6), 5-nitroace-naphthene (1), and 7-nitrooxine-5-sulfonic acid (2) in a limited pH range. The reduction of these compounds should obviously be different, due to the formation of such phenylhydroxylamines as can be converted into the rapidly reducible quinonoid forms to give the respective amine as the final product. However, no data are available in literature on the reduction of nitro groups which have $-CH_2COOH$ in *o*- or *p*-positions. The present investigation, therefore, deals with the polarographic reduction of *o*- and *p*-nitrophenyl acetic acids with a view to finding the effect of $-CH_2COOH$ on the reduction of the nitro group.

## MATERIALS AND METHODS

A manual dc polarographic setup was used and the dme had the following characteristics: $m = 2.84$ mg/sec and $t = 3.05$ sec in 0.1 $M$ KCl (open circuit) at $h = 40.0$ cm (uncorrected for back pressure). Temperature was maintained at $30 \pm 0.5°$C. All pH measurements were made with a Philips pH meter, *o*- and *p*-nitrophenyl acetic acids (Aldrich & Co.) were recrystallized from alcohol before use. AnalaR BDH chemicals were used to prepare HCl/KCl, $Na_2HPO_4$/citric acid, boric acid/NaOH,

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and $Na_2HPO_4$/NaOH buffer systems of constant ionic strength 1.0 $M$ (by KCl). Triton X-100 was used as maximum suppressor. Nitrogen used to deoxygenate the solutions was previously passed through vanadous solutions (9) and water. The mercury used for the dme was first purified chemically and subsequently distilled under reduced pressure.

## RESULTS AND DISCUSSION

In order to identify the wave due to the nitro group, preliminary studies were made with phenyl acetic acid and the nitrophenyl acetic acids under identical conditions. The nitro group gave a well-defined wave in solutions of pH 4.0, 5.0, 6.0, 10.0, 11.0, and 12.0. At all other pH a maximum was present which was suppressed by 0.001% Triton X-100. To correlate the results the same concentration of Triton X-100 was used at all pH values.

The values of $i_d$ and $E_{1/2}$ for the nitro group reduction are shown in Table 1. It can be seen that the wave heights are almost identical for both isomers at all pH values. This indicates that the number of electrons involved in the reduction process is the same (provided $D$ is the same), though in strongly acidic and strongly alkaline media the predominant species are the unionized and the ionized forms, respectively. This also indicates that electroactive nature of the nitro group in both ionized and unionized forms is maintained. Apparently the $-COOH$ in the unionized and ionized forms does not appreciably affect the nitro group reduction. This could be expected considering the fact that the $-COOH$ is linked to the ring via a $-CH_2$ group. The shift in $E_{1/2}$ with pH (up to pH 10) is as expected for nitro group reduction. The reason for constancy of $E_{1/2}$ at high pH values, however, is not clear. The change in slope of the $E_{1/2}$ vs pH plot and the first intersection of linear sections at nearly pH 4.0 indicates that the rate of reduction is not entirely unaffected by the $-COO^-$ group. The pH at the intersection for the $o$- and $p$-isomer roughly corresponds to their respective $pK_a$ values (8) of 4.00 and 3.85.

In order to analyze the nature of the reduction of the nitro group the tests of $i_d$/C, $i_d$/h, and $i_d$/$h^{1/2}$ were made at all pH values. Further, the cathodic shifts in $E_{1/2}$ with concentration and the slopes of $E_{1/2}$ vs log $i/(i_d-i)$ were also studied. All these tests showed that the nitro group underwent diffusion-controlled irreversible reduction in both the isomers. The $E_{1/2}$ for the $o$-isomer is more cathodic than that of the $p$-isomer at all pH values (Fig. 1).

The diffusion coefficient of both isomers were individually determined by using a McBain-Dawson cell and applying the King-Cathcart equation (4). This value of $D$ found in the solution identical to those used for polarography was incorporated in the Ilkovic equation to get the number of electrons involved in the nitro group reduction. At pH = 5.0 this

## TABLE 1

KINETIC PARAMETERS $\alpha n_a$, $-\log k°_{f,h}$ AND $E_{1/2}$ FOR NITRO GROUP REDUCTION OF o- AND p-NITROPHENYL ACETIC ACIDS AT VARIOUS pH VALUES[a]

| pH | o-nitrophenyl acetic acid | | | | | p-nitrophenyl acetic acid | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | $i_d$ | $-E_{1/2}$ (SCE × 10^6) | $D$ (cm² sec^{-1}) | $\alpha n_a$ | $-\log k°_{f,h}$ | $i_d$ | $-E_{1/2}$ (SCE × 10^6) | $D$ (cm² sec^{-1}) | $\alpha n_a$ | $-\log k°_{f,h}$ |
| 2.0 | 8.40 | 0.300 | 3.48 | 0.216 | 3.33 | 8.40 | 0.275 | 3.60 | 0.302 | 3.09 |
| 3.0 | 8.30 | 0.335 | 3.61 | 0.210 | 3.74 | 8.35 | 0.315 | 3.48 | 0.286 | 3.44 |
| 4.0 | 8.40 | 0.380 | 3.58 | 0.194 | 4.13 | 8.35 | 0.355 | 3.62 | 0.270 | 3.96 |
| 5.0 | 8.40 | 0.455 | 3.70 | 0.182 | 5.19 | 8.40 | 0.430 | 3.47 | 0.252 | 4.91 |
| 6.0 | 8.30 | 0.540 | 3.45 | 0.170 | 5.86 | 8.35 | 0.505 | 3.58 | 0.237 | 5.62 |
| 7.0 | 8.35 | 0.620 | 3.65 | 0.158 | 6.21 | 8.30 | 0.580 | 3.71 | 0.216 | 5.96 |
| 8.0 | 8.45 | 0.700 | 3.42 | 0.140 | 6.72 | 8.35 | 0.660 | 3.48 | 0.194 | 6.04 |
| 9.0 | 8.40 | 0.785 | 3.56 | 0.131 | 7.32 | 8.40 | 0.740 | 3.74 | 0.178 | 6.64 |
| 10.0 | 8.45 | 0.835 | 3.60 | 0.120 | 8.22 | 8.35 | 0.780 | 3.56 | 0.152 | 7.01 |
| 11.0 | 8.35 | 0.835 | 3.43 | 0.108 | 9.11 | 8.45 | 0.788 | 3.67 | 0.132 | 7.98 |
| 12.0 | 8.45 | 0.835 | 3.70 | 0.104 | 9.87 | 8.40 | 0.780 | 3.78 | 0.130 | 8.66 |

[a] Concentration, 0.6 m$M$; temperature, 30°C; $\mu = 1.0$ $M$; 0.001% Triton X-100.
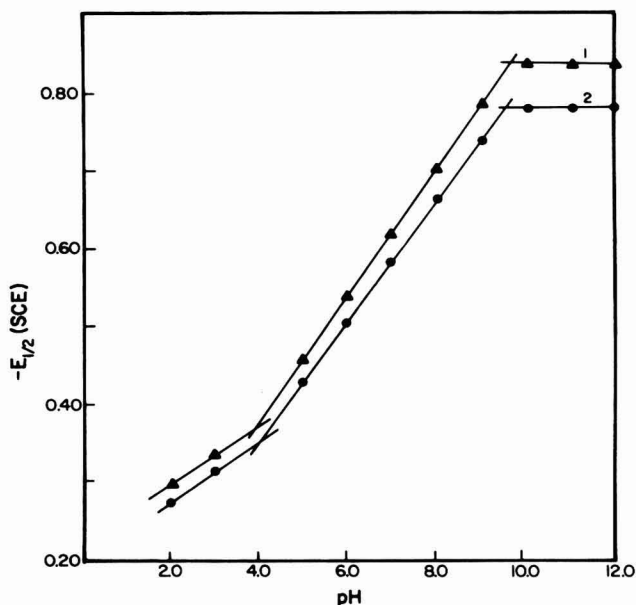
FIG. 1. Variation of $E_{1/2}$ with pH ($\mu = 1.0\ M$, 30°C). Curve 1: $o$-Nitrophenyl acetic acid (0.6 m$M$). Curve 2: $p$-Nitrophenyl acetic acid (0.6 m$M$).

yielded a value of 5.91 and 6.12 for $o$- and $p$-nitrophenyl acetic acid, respectively. It could reasonably be inferred that in both isomers the nitro group reduction involved six electrons per molecule. Since the wave heights were pH independent and the values of $D$ were found to be nearly the same at all pH values (Table 1), it could be concluded that both isomers were reduced to the respective amines.

Koutecky's method (7) was used to calculate the values of kinetic parameters ($\alpha n_a$ and $-\log k°_{f,h}$) listed in Table 1. It could be seen in Fig. 2 that $-\log k$ vs $E$ plots are linear, thus showing that there is only one slow process that determines the rate of the reduction of the nitro group. Values of $\alpha n_a$ showed a decrease and $-\log k°_{f,h}$ showed an increase with increasing pH values (Table 1). This points to the extent of irreversibility getting enhanced with increasing pH, presumably owing to the lesser availability of hydrogen ions. The $-CH_2COO^-$ ($H^+$) group containing a $-CH_2$ with electron-withdrawing centers on either side probably permits the formation of quinonoid structures, in both isomers, which are readily reducible. The 6e reduction of $o$- and $p$-nitrophenol and $o$- and $p$-nitroanilines also involve the rapidly reducible quinonoid forms (5, 10). The probable mechanism for the reduction of the nitro group in $o$-nitrophenyl acetic acid (likewise in $p$-isomer) may be shown as follows:
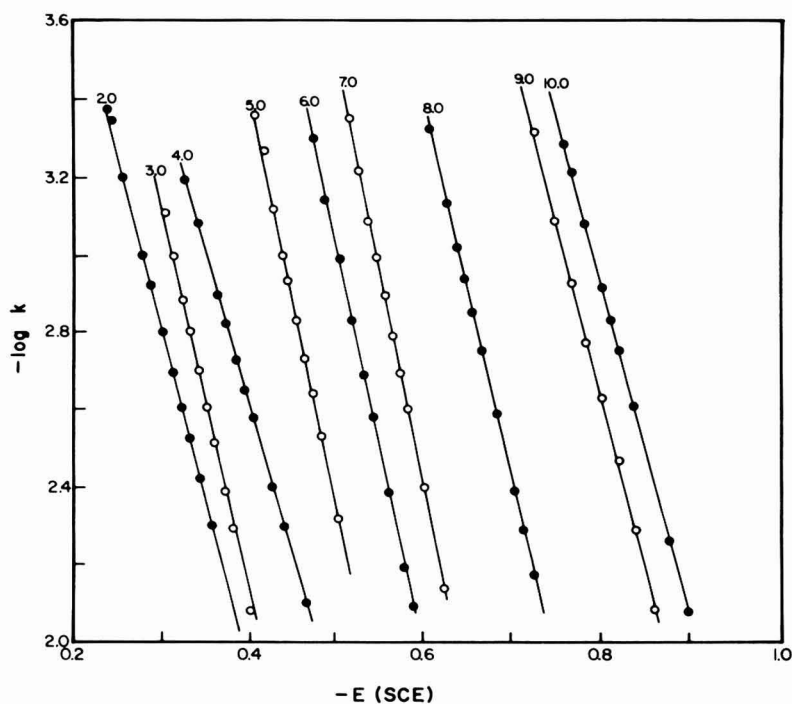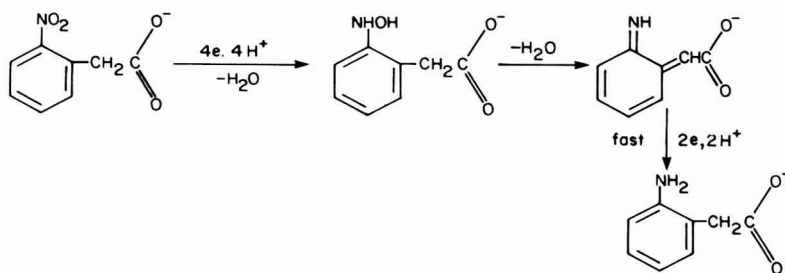
FIG. 2. −Log $k$ vs −$E$ (SCE) plots of 0.6 m$M$ $o$-nitrophenyl acetic acid at various pH values. ($\mu$ = 1.0 $M$, 30°C).

## SUMMARY

Polarography of $o$- and $p$-nitrophenyl acetic acids has been carried out in aqueous buffers (pH 2.0 to 12.0) of constant ionic strength (1.0 $M$) at 30 ± 0.5°C. The nitro group underwent a diffusion-controlled reduction (6$e$) over the whole pH range. The number of electrons involved in the reduction was found by incorporating the values of diffusion coefficients, obtained by using a McBain-Dawson cell, into the Ilkovic equation. Koutecky's method has been used to compute the kinetic parameters ($\alpha n_a$ and −log $k°_{f,h}$) for the reduction of the nitro group and a reduction mechanism is proposed.

## ACKNOWLEDGMENTS

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# Determination of Phosphatidylcholine in Amniotic Fluid

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## INTRODUCTION

In 1969, Nelson (*13*) suggested that the amniotic fluid phospholipid content and, in particular, the phosphatidylcholine (lecithin) concentration was associated with fetal lung maturity. Studies have shown that phosphatidylcholine (PC) is the principal phospholipid found in amniotic fluid accounting for 60–90% of the total with sphingomyelin (S), phosphatidylethanolamine (PE), phosphatidylinositol (PI), phosphatidylserine (PS), phosphatidylglycerol (PG), and lysophosphatidylcholine comprising almost all of the remaining amount. It is generally agreed that the measurement of PC would serve as a reliable indicator of fetal lung maturity. Several investigations have revealed that high concentrations were generally found with normal neonatal respiration whereas lower ones indicated a high risk of respiratory distress. It was also demonstrated that a PC concentration of about 35 mg/liter appeared to be critical (*2, 3, 5, 14, 15*).

The quantitative measurement of PC in amniotic fluid was described by Bhagwanani *et al.* (*3*). The phospholipids were extracted with 2:1 chloroform–methanol, separated by thin-layer chromatography, and following removal from the silica gel and digestion with perchloric acid, were quantitated by phosphate measurement. Recoveries of 86.7 to 96% were found. Since neither centrifuged fluid nor acetone precipitation was used in this study, quantitative results were obtained in contrast to those reported by Nelson that were about 20–30% lower. Large losses have been subsequently found for the acetone precipitation step (*16*), as well as losses due to centrifugation (*4, 21*).

Direct measurement of PC in amniotic fluid may be accomplished by an enzymic method using an initial hydrolysis with phospholipase D with the liberated choline determined by using choline oxidase, phenol, peroxidase, and 4-aminoantipyrine (*1*). High performance liquid chromatography has also been employed to quantitate the PC concentration (*11, 17*).

We have developed a method for measuring the phosphatidylcholine concentration of amniotic fluid that is relatively simple, rapid, and utilizes

readily available chemicals and materials. Following extraction of the phospholipids from the amniotic fluid with chloroform−methanol, the solvent is passed through a column containing an absorbent, calcium hydroxy phosphate (hydroxylapatite), which retains the acidic phospholipids and allows the passage of the phosphatidylcholine and sphingomyelin. The column is then washed with methanol to complete removal of the PC and S. The combined solvents are evaporated, and the residue is reacted with 1 ml of an alcoholic solution of periodic acid and sulfuric acid for 45 min at 100°C. After cooling and dilution, a 0.5-ml aliquot is removed for phosphate color development and measurement.

## MATERIALS AND METHODS

*Reagents*

*Adsorbent*. Wash 20 g of the adsorbent, calcium hydroxy phosphate (Bio-Gel HTP; Bio-Rad Laboratories, Richmond, Calif. 94804), with 150 ml of methanol three times, then two times with 150 ml of acetone, and with 100 ml of diethyl ether two times. Decant the solvent and fines off each washing. Spread out and let air dry until the odor of ether vapors is no longer detectable. Heat the adsorbent in a 100°C oven for 18 hr. Remove and let cool in a dessicator.

*Molybdate solution*. Dissolve 3 g of ammonium molybdate tetrahydrate into water. Add 6.6 ml of sulfuric acid and mix. Let cool and dilute to 100 ml.

*Reducing solution*. Dissolve 0.3 g hydrazine sulfate and 0.03 g stannous chloride dihydrate into water. Add 2.8 ml of sulfuric acid and mix. Dilute to 100 ml. Store in the refrigerator. Prepare fresh every 2 weeks.

*Phosphorus standard, 1 mg/liter P*. Dissolve 438 mg of potassium dihydrogen phosphate per 100 ml of water. Dilute 10 ml of this solution to 100 ml. In a volumetric flask containing water, add 6.6 ml of sulfuric acid and 1 ml of the 1:10 diluted solution, mix, and dilute to 100 ml.

*Sulfuric acid, 5 mol/liter*. Carefully add 28 ml of sulfuric acid to water and mix. When cool, dilute to 100 ml.

*Periodic acid, 0.1 mol/liter*. Dissolve 0.6 g $H_5IO_6$ per 25 ml. Discard any unused portion after 2 weeks and prepare fresh.

*Hydrolysis mixture*. Mix 3 ml of the 5 mol/liter sulfuric acid, 1 ml of the 0.1 mol/liter periodic acid, and 6 ml of ethanol. Prepare fresh and discard any unused portion.

*Methanol, J. T. Baker Cat. No. 6-P707 (do not substitute)*. If the specified methanol is not available, place 3.5 g silver nitrate and 7.5 g potassium hydroxide in a 2-liter round bottom flask. Add 20 ml of water to dissolve the salts. Add 1800 ml of methanol with swirling. Let stand 12 hr. Distill and discard the first 100 ml. Redistill and store in an amber glass bottle.

*Phospholipids*. Phosphatidylcholine, sphingomyelin, phosphatidyl-

serine, phosphatidylethanolamine, and phosphatidylinositol were obtained from the Sigma Chemical Company, St. Louis, Missouri 63128. Dissolve 10 mg of each of the phospholipids into 100 ml of chloroform.

*Diethylaminoethyl cellulose (DEAE), Whatman DE 22.* Wash with 2:1 chloroform:methanol. Let air dry.

*Ethanol.* Purify as described for the methanol.

*Water.* Use distilled water with a low organic residue content.

Glass columns, 180 × 5.8 mm, 4-ml capacity with polyethylene filter disk (Cat. No. QS-6 E) were obtained from Isolab, Inc., Akron, Ohio 44321. The chloroform was reagent grade.

## Method

*Glassware.* Rinse the test tubes which will be used to collect the eluate from the column chromatography with 2 ml of methanol by vortexing. Repeat twice. Rinse the tube with distilled water and dry in the oven. Do not cover these tubes with any type of plastic wrap or film.

*Extraction of the amniotic fluid.* Transfer 1 ml of a well-mixed, *uncentrifuged* amniotic fluid specimen to a 15-ml glass-stoppered centrifuge tube. If the total phospholipid phosphorus (TPP) concentration is less than 1.4 mg/liter P, use 2 ml of fluid, or if it is greater than 7 mg/liter use 0.5 ml. The TPP is assayed as described previously *(23)*. Add 4 ml of 2:1 chloroform–methanol, cap, and vortex the tube for 1 min. Centrifuge at 2500 rpm to separate the layers. Aspirate off the top layer. Carefully decant the clear solvent to a clean test tube (the protein layer at the interface will adhere to the side of the tube if the decantation is carefully done).

*Storage.* If the analysis cannot be done immediately, do not transfer the solvent extract to a clean tube, but store the glass-stoppered centrifuge tube containing it in the refrigerator.

*Column chromatography.* Pack a column by transferring enough DEAE to the glass column to give a height of one-eighth inch after packing the adsorbent by tapping the column. Transfer 0.10 g of HTP adsorbent to the column in small portions with gentle tapping of the column to pack the material down. Place a small plug of glass wool at the top of the packing. Wash the column with 4 ml of chloroform. Using a Pasteur pipet, transfer the solvent extract of the fluid to the HTP column. Let the solvent drain through, and collect the eluate in a *methanol cleaned* test tube. Elute the column with 4 ml of purified methanol and collect it in the same tube with the chloroform–methanol. Mix the two solvent layers by gently tapping the tube. Evaporate to dryness in a 55°C water bath with the aid of an air stream.

*Hydrolysis.* Add 1 ml of hydrolysis mixture to the dried residue and to a methanol cleaned tube for a blank. Mix and place all tubes in a 100°C heating block for 45 min. At the end of the incubation, remove all tubes,

let cool, and without delay add 2 ml of water to all tubes and mix.

*Color development.* Add 0.5 ml of the 1 mg/liter P standard to a clean tube. Transfer 0.5 ml of the hydrolyzed sample and 0.5 ml of the hydrolysis blank to labeled tubes. Add 0.5 ml water for a reagent blank. Add 2 ml of the molybdate reagent to all tubes and mix. Add 1 ml of the reducing solution to all tubes and mix. Let stand 5 min. Measure the absorbances of all tubes against the reagent blank at 660 nm.

*Calculations.* Subtract the hydrolysis blank value from absorbances of tubes that were carried through hydrolysis. Multiply by a dilution factor of 2.4 and a conversion factor of 25 to obtain mg/liter of phosphatidyl-choline.

## RESULTS AND DISCUSSION

The quantitative measurement of amniotic fluid phosphatidylcholine concentration necessitates that the analytical steps used do not result in any losses of phospholipid material. The analysis of amniotic fluid phospholipids may consist of the following steps: (a) sample preparation, (b) extraction, (c) acetone precipitation, (d) chromatographic separation, and (e) spectrophotometric measurement.

### Sample Preparation

*Effect of centrifugation.* Because amniotic fluid samples usually contain cells, cellular debris, vernix caseosa, etc., many investigators advocate centrifugation of the fluid for removal of this material prior to analysis. A detailed study by Wagstaff *et al.* (*21*) revealed losses of 71.1% of the acetone precipitable lecithin and 64.6% of the sphingomyelin from fluids obtained during the 40th week of gestation by centrifuging at 750$g$ for 10 min. With conditions of 1100$g$ for 10 min, both of these phospholipids were lost to the extent of 30% each, and with fluids obtained at 36 weeks gestation, losses of 60% were found.

Cherayil *et al.* (*4*) found that a force of 1400$g$ was required to insure a thin-layer chromatographic migration pattern with adequate separation of the lecithin/sphingomyelin (L/S) spots for densitometry. Using a group of 13 fluids, clinically valid information could be obtained with use of a $g$-force as low as 180$g$ for 5 min, while at 27,138$g$ for 15 min, 65.5% false negatives were found for fluids from 32−42 weeks gestation and 47.4% at weeks 36−42.

The conclusion reached was that a standardized $g$-force, time, and temperature must be specified and followed for the preparation of the fluid, especially if the well-accepted L/S ratio of 2 is to be used as a criterion of fetal maturity.

We confirmed that there is a loss due to centrifugation by dividing six samples into two aliquots with one being extracted without centrifugation and the other centrifuged at 200$g$ for 10 min at 4°C before extraction. The TPP was measured for both aliquots with a method described (*23*). The

TABLE 1
EFFECT OF CENTRIFUGATION (200g FOR 10 MIN AT 4°C) ON THE
TOTAL PHOSPHOLIPID CONCENTRATION

| Sample No. | Concentration (mg/liter P) | | Recovery (%) |
|---|---|---|---|
| | Before centrifugation | After centrifugation | |
| 1 | 19.1 | 17.0 | 89.0 |
| 2 | 2.6 | 2.0 | 76.9 |
| 3 | 2.7 | 1.8 | 66.7 |
| 4 | 3.9 | 2.9 | 74.4 |
| 5 | 10.2 | 8.0 | 78.4 |
| 6 | 6.8 | 5.6 | 82.4 |

data obtained is given in Table 1. Significant losses of 11−33% were found with an average loss of about 22%.

Haahti *et al.* (*10*) have demonstrated that the composition of vernix consist mainly of nonpolar lipids with only trace amounts of phospholipids, so that a possible explanantion according to Wagstaff (*21*) is that the losses may be due to the adsorption of the phospholipids on the surfaces of the cellular debris or particulate matter. On the basis of all of this evidence, we believe that for a quantitative recovery of the phospholipids, centrifugation of the fluid should be avoided.

*Storage.* However, it has been found necessary to centrifuge amniotic fluids to remove the extraneous material, so that the fluid contents do not deteriorate upon storage. Indications are that hydrolytic enzymes are responsible. To obviate this problem, Bhagwanani *et al.* (*3*) recommended that the fluid be extracted immediately upon receipt with chloroform−methanol, and the solvent extract be stored at 4°C until assayed. Our experience has confirmed that samples treated and stored in this manner have excellent stability.

Since it may not be permitted and/or convenient to transport a chloroform−methanol solvent extract of the fluid to another laboratory for analysis, we recommend that the solvent be evaporated to dryness and the dried residue be shipped. Upon receipt of the residue, it should be processed for analysis or redissolved in chloroform−methanol for storage under refrigeration.

*Extraction.* The extraction of the phospholipids from amniotic fluid is carried out by using a 2:1 chloroform−methanol solvent mixture as described by Folch *et al.* (*6*) with a solvent to sample ratio of 4:1. This extraction procedure has been demonstrated to be quantitative.

*Acetone precipitation.* Phosphatidylcholine found in amniotic fluid may be classified as surface and nonsurface active. The surface active PC consist predominantly of the dipalmitoyl ester, and it is relatively acetone

insoluble. Gluck *et al.* (*7*), in their method for obtaining the L/S ratio using thin-layer chromatography, utilize a cold acetone precipitation step to separate the surface from the nonsurface active PC. Subsequently, other investigators (*12, 16*) have found variable recoveries of 50–90% for acetone precipitation, so that the use of this step will not yield quantitative recoveries of the total PC. Whether or not it is necessary to separate these two classes of PC for the assessment of fetal lung maturity has not been resolved definitively. We have chosen to omit the acetone precipitation step in order to obtain quantitative recoveries of all of the phosphatidylcholine.

*Separation and Measurement*

*Phosphate release.* The proposed method for measuring phosphatidylcholine described in this communication utilizes the release of inorganic phosphate by hydrolysis with periodate and sulfuric acid (*18*) followed by formation and reduction of the phosphomolybdate complex for quantitation (*22*). This hydrolysis reaction was demonstrated to liberate phosphate from glycerophosphatides, but not from sphingomyelin (*18*). Data in Table 2 indicate that at a concentration of up to 100 mg/liter of sphingomyelin, only 6% of it will react while the PC is recovered quantitatively. Since this hydrolysis is not specific for PC, it is necessary to remove any interfering phospholipid or to isolate the PC.

*Column chromatography.* Because it was possible to measure PC selectively in the presence of sphingomyelin, we sought a means of separating the PC from the other phospholipids found in amniotic fluid. Some of the other phospholipids which may be present are: phosphatidylinositol, phosphatidylserine, phosphatidylethanolamine. Thin-layer chromatography with silica gel has been used extensively for the separation of phospholipids, but it is relatively time consuming and difficult to perform on a routine basis. For quantitation, the area where the phospholipid has migrated must be removed and the phospholipid eluted for further reaction and measurement.

Gosselin and Foidart (*9*) described the removal of the acidic phos-

TABLE 2
REACTION OF PHOSPHATIDYLCHOLINE AND SPHINGOMYELIN WITH
PERIODATE-SULFURIC REAGENT

|  | Concentration (mg/liter) | | Recovery (%) |
|---|---|---|---|
|  | Taken | Found |  |
| Sphingomyelin | 50 | 3.0 | 6.0 |
|  | 100 | 5.5 | 5.5 |
| Phosphatidylcholine | 50 | 51.3 | 102.6 |
|  | 100 | 97.5 | 97.5 |

pholipids from PC and S in a chloroform–methanol solution by a batch-wise treatment with dry diethylaminoethyl cellulose (DEAE). This adsorbent has been used to fractionate the phospholipids by column chromatography (8, 19).

A similar fractionation using calcium hydroxy phosphate (hydroxylapatite) as an adsorbent was described by Slomiany and Horowitz (20). We have investigated the use of the hydroxylapatite to remove the acidic phospholipids from PC. Preliminary investigations indicated that 0.10 g of the adsorbent would be sufficient to adsorb completely the amounts of phospholipids found in 5 ml of amniotic fluid. This adsorbent could not be used in a batchwise procedure such as the DEAE, since it contains phosphate which could interfere with subsequent measurements dependent on phosphate quantitation. Instead, we use a microcolumn with which both the PC and S could be eluted with a 7:3 solution of chloroform–methanol with retention of all of the other phospholipids. Since this solvent composition was quite similar to the 2:1 one commonly used for extraction, we decided to use the extract without further processing and pass it directly through the column. Approximately 80% of the PC and S were passed through, and elution with a small volume of methanol completed the collection. To confirm that the column did allow all of the PC and S through with retention of the other phospholipids, solutions of each of the pure materials at a concentration of 50 mg/liter were processed. Data in Table 3 show that 5–7% of the PI, PE, and PS were not retained. Fortunately, the highest concentration that the combined acidic phospholipids would reach in amniotic fluid is about 50 mg/liter (20% of the total phospholipid concentration), so that the interference would probably be about 2 mg/liter.

*Linearity and specificity.* The linearity of the phosphate release and quantitation after column chromatography was assessed by taking PC standards of 12.5–150 mg/liter concentration through the entire procedure. These standards were compared to those of the same concentration without chromatographic treatment. The data are given in Table 4. Excellent linearity was found, so that a wide range of concentrations is

TABLE 3
RETENTION OF PHOSPHOLIPIDS BY HYDROXYLAPATITE COLUMN

| Phospholipid | Concentration (mg/liter) | | Recovery (%) |
|---|---|---|---|
| | Taken | Found | |
| Phosphatidylcholine | 50 | 50.2 | 100.4 |
| Sphingomyelin | 50 | 53.8 | 107.6 |
| Phosphatidylinositol | 50 | 2.3 | 4.6 |
| Phosphatidylethanolamine | 50 | 2.3 | 4.6 |
| Phosphatidylserine | 50 | 3.5 | 7.0 |

TABLE 4
PHOSPHATIDYLCHOLINE STANDARDS CARRIED THROUGH PROCEDURE

| | Concentration (mg/liter) | |
| --- | --- | --- |
| | Found | |
| Taken | Without chromatography | With chromatography |
| 12.5 | 14.8 (118.4)[a] | 14.1 (112.8) |
| 25.0 | 24.6 (98.4) | 25.9 (103.6) |
| 50.0 | 50.9 (101.8) | 48.6 (97.2) |
| 100.0 | 96.0 (96.0) | 94.7 (94.7) |
| 150.0 | 144.9 (96.6) | 137.0 (91.3) |

[a] Percentage given in parentheses.

available for use without dilution of the sample. The chief limitation is column overload that restricts the use of samples containing more than 175 mg/liter of phospholipid. To circumvent this, a smaller sample volume of 0.5 ml is used in these cases.

To determine the specificity of the phosphate releasing reaction, solutions containing ratios of 1:1, 2:1, and 3:1 lecithin (PC)/sphingomyelin were assayed by the proposed method. These data are shown in Table 5. Quantitative recoveries of PC in the presence of S were made, so that the procedure is specific provided that any interfering phospholipids have been removed (18).

*Precision.* Day-to-day precision was checked over a period of 2 weeks. At a PC concentration of 175 mg/liter, the SD was 16 mg/liter, and at 50 mg/liter, the SD was 4 mg/liter. Coefficients of variation were 9.4 and 8.0%, respectively.

*Recovery study.* Recoveries of PC were carried out in two ways. The first was to add PC in increasing amounts to a five-component mixture containing all of the major amniotic fluid phospholipids. The percentage

TABLE 5
PHOSPHATIDYLCHOLINE-SPHINGOMYELIN MIXTURES TAKEN THROUGH PROCEDURE

| | Concentration (mg/liter P) | | | |
| --- | --- | --- | --- | --- |
| | | PC found | | |
| PC/S ratio | PC + S taken | Calc | Without chromatography | With chromatography |
| 1:1 | 4.29 | 2.14 | 2.28 (106.5)[a] | 2.16 (100.9) |
| 2:1 | 4.48 | 2.99 | 2.95 (98.7) | 3.12 (104.3) |
| 3:1 | 4.25 | 3.19 | 3.24 (101.6) | 3.60 (112.9) |

[a] Percentage given in parentheses.

TABLE 6
ANALYSIS OF A FIVE-COMPONENT PHOSPHOLIPID MIXTURE

| | Mixture | | | | |
|---|---|---|---|---|---|
| | A | B | C | D | E |
| Phospholipid | Percentage in mixture | | | | |
| Phosphatidylcholine | 60 | 65 | 75 | 85 | 90 |
| Sphingomyelin | 15 | 10 | 8 | 5 | 5 |
| Phosphatidylinositol | 15 | 20 | 10 | 5 | 3 |
| Phosphatidylethanolamine | 5 | 3 | 4 | 3 | 1 |
| Phosphatidylserine | 5 | 2 | 3 | 2 | 1 |
| Analysis of: | Concentration (mg/liter P) | | | | |
| Total phospholipid | 4.78 | 3.90 | 4.63 | 4.38 | 4.50 |
| PC taken | 2.87 | 2.54 | 3.47 | 3.72 | 4.05 |
| PC found | 3.00 | 2.76 | 3.48 | 4.08 | 4.20 |
| Recovery (%) | 104.5 | 108.7 | 100.3 | 109.7 | 103.7 |

of PC was varied from 60 to 90% which is comparable to that found in amniotic fluid. Data in Table 6 show recoveries of 100.3–109.7%. In the second recovery study, PC was added in concentrations of 11–240 mg/liter to aliquots of an amniotic fluid pool. Recoveries ranged from 104.3 to 113.7%, and these data are shown in Table 7. The recovery data indicates that the proposed method is suitable for the quantitative determination of phosphatidylcholine.

TABLE 7
RECOVERY OF ADDED PHOSPHATIDYLCHOLINE[a]

| Concentration (mg/liter) | | | |
|---|---|---|---|
| Added | Found | Recovered | Recovery (%) |
| 0 | 36.0 | | |
| 11.5 | 48.0 | 12.0 | 104.3 |
| 28.8 | 66.8 | 30.8 | 107.0 |
| 57.5 | 98.0 | 62.0 | 107.8 |
| 115.0 | 160.0 | 124.0 | 107.8 |
| 240.0 | 308.8 | 272.8 | 113.7 |

[a] Varying amounts of a PC standard (100 mg/liter) were added to an amniotic fluid pool extract containing 36 mg/liter phosphatidylcholine.

## SUMMARY

A relatively rapid method for measuring phosphatidylcholine (lecithin) quantitatively in amniotic fluid has been described that requires 1 ml or less of sample for fluids having a total phospholipid concentration greater than 25 mg/liter. Following extraction with chloroform–methanol, the solvent is passed through a calcium hydroxyphosphate column which

removes the acidic phospholipids and allows passage of the phosphatidylcholine and sphingomyelin. Hydrolysis with periodate-sulfuric acid selectively releases inorganic phosphate from the phosphatidylcholine that is measured by reduction of the formed phosphomolybdate complex to the usual blue color. Various mixtures of phospholipids were carried through the entire procedure with excellent recoveries. Phosphatidylcholine added to an amniotic fluid pool was also quantitatively recovered, so that the method appeared completely suitable for routine clinical laboratory use.

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# A New Method of Determining Chlorine and Bromine in Compounds of the Platinum Group Metals by the Oxygen Flask

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## INTRODUCTION

Since the midfifties, Schönigers (4) oxygen flask method has become one of the most frequently used techniques for the determination of halogens in organic compounds (3). In spite of its widespread application, problems arise when Cl, Br, and I are determined by this technique in organometallic derivatives of the platinum group metals. The difficulties in this type of analysis are usually attributed to the formation of sparingly soluble metal–halogen complexes which subsequently interfere with the titration of Cl, Br, and I ions. For the purpose of circumventing such interferences, a number of other decomposition methods in addition to the oxygen flask technique have been described. These include fusion of the sample with $Na_2O_2$ (5) followed by the masking of interfering metal elements with EDTA, or the reduction of the platinum metal ions by magnesium (1) to the elemental form. However, such decomposition methods for the routine analysis of Cl and Br in organometallics are rather cumbersome and tedious.

We now report that halides in the presence of the group 8 metals can indeed be determined simply by the oxygen flask technique, if the initial sample is mixed and ignited with ammonium fluoride.

## MATERIALS AND METHODS

The materials used were $NH_4F$; 0.5 $N$ NaOH; 2.0 $N$ $HNO_3$; hydrogen peroxide (30% solution); and 0.02 $N$ $AgNO_3$. All reagents used in this study were of BDH Analar grade. The final argentometric titrations of chloride and bromide ions were followed potentiometrically (2) with an Orion pH meter (Model 801) employing a silver indicator in combination with double-junction reference electrodes.

*Procedure.* The analytical samples containing 0.30 to 2.0 mg of Cl or Br were weighed onto a piece of filter paper and mixed with approximately 4 mg of ammonium fluoride. The mineralisation of the samples by ignition was then carried out in a 500-ml oxygen flask containing 10 ml of 0.5 $N$ NaOH and a few drops of hydrogen peroxide as an absorbent solution.

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TABLE 1
ANALYSIS OF Cl AND Br IN THE PRESENCE OF PLATINUM GROUP METALS

| Compound | Percentage | | Difference |
|----------|------|-------|------------|
|          | Calcd | Found | |
| $K_2PtCl_4$ | 34.16 | 33.80 | −0.36 |
|  |  | 34.24 | +0.08 |
|  |  | 34.19 | +0.03 |
| $C_{12}H_{36}N_6P_6PtCl_2$ | 9.97 | 9.78 | −0.19 |
| $PdCl_2$ | 40.00 | 40.29 | +0.29 |
|  |  | 40.22 | +0.22 |
| $C_{12}H_{36}N_6P_6PdCl_2$ | 11.30 | 11.19 | −0.11 |
| $C_{54}H_{45}P_3RuBr_2$ | 15.28 | 15.46 | +0.22 |
| $C_6H_{15}S_3RhCl_3$ | 22.16 | 21.96 | −0.20 |
| $C_{20}H_{20}N_4ClRhCl_2$ | 20.23 | 20.23 | ±0.00 |
| $C_{36}H_{30}P_2Rh(CO)Cl$ | 5.13 | 5.38 | +0.25 |
| $(NH_4)_2IrCl_6$ | 48.24 | 48.50 | +0.26 |
|  |  | 47.85 | −0.39 |
|  |  | 48.00 | −0.24 |

After the combustion was complete, the flask was shaken thoroughly and was left to stand for 0.5 hr. The stopper and gauze then were rinsed down with 5 ml of distilled water, and the contents of the flask were boiled for 3 min. After the flask had cooled to room temperature, the absorbent solution was acidified with 2 $N$ nitric acid and washed into a 250-ml beaker. The final titrimetric determination of the halogens was carried out potentiometrically with 0.02 $N$ silver nitrate in aqueous acetone solution.

## RESULTS AND DISCUSSION

The determination of chlorine and bromine in test samples by this method gave the results summarized in Table 1. The data indicate that this simple technique can be utilized for the analysis of halogens not only in organometallics, but also in inorganic complexes of the platinum group metals. In the final titrimetric determination of chlorine and bromine no interference is experienced from metallic elements, thereby suggesting that during the decomposition process kinetically inert metal species are formed.

## SUMMARY

A new manner of determining Cl and Br in organometallic derivatives of the platinum group metals by the oxygen flask method is described. No interference by the metallic elements is found if the sample is mixed before ignition with ammonium fluoride.

## ACKNOWLEDGMENTS

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# Solvent Extraction and Spectrophotometric Determination of Palladium(II) with Some Nitrogen-Containing Heterocyclic Hydrazones in the Presence of Chloride Ions

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## INTRODUCTION

Terdentate heterocyclic hydrazone ligands containing a modified ferroin linkage ($-N=C-C=N-NH-C=N-$) have been widely used for creation of organic reagents applied in photometric inorganic analysis, because they can be comparatively readily synthesized in the laboratory and give highly sensitive chromogenic reactions with divalent transition metal ions. The secondary amine proton in this grouping has been shown to be quite labile when the ligand undergoes coordination with metal ion. The extraction behavior of the palladium(II) complexes with such hydrazones has been interpreted in terms of the formation of an extractable uncharged ternary complex between the monopositive palladium(II) complex with the ligand and a suitable monodentate anion (*1, 2, 4, 5*).

In the present investigation we have synthesized three heterocyclic hydrazone compounds (I), all containing a phthalazine nucleus in the
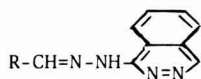
R-CH=N-NH- (I)

R: 2-pyridyl (PAPhH), 2-quinolyl (QAPhH), 2-benzothiazolyl (BAPhH)

hydrazine group substituent, and applied these compounds to the extractive spectrophotometric determination of palladium(II) in the presence of chloride ions. Of the reagents examined, BAPhH with a $\pi$-electron donative heterocyclic substituent, 2-benzothiazolyl, in the aldehyde moiety of the ligand merits considerable attention as a sensitive spectrophotometric agent for palladium(II).

## EXPERIMENTAL

*Reagents*

*PAPhH, QAPhH, and BAPhH.* The ligands were prepared by interaction of the particular aldehyde and 1-hydrazinophthalazine hydrochloride

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in stoichiometric amounts and were recrystallized to a constant melting point from 5% aqueous hydrochloric acid solution (PAPhH, QAPhH) or ethanol containing small amounts of pyridine (BAPhH). The former two were obtained as the monohydrochloride. The purity of these compounds was checked by elemental analysis. Aqueous solutions of PAPhH and QAPhH and a chloroform solution of BAPhH were freshly prepared daily.

*Standard palladium(II) solution.* Palladium(II) standards were obtained by dilution of 0.01 $M$ palladium(II) sulfate stock solution, which was 1.5 $N$ in sulfuric acid and had been standardized compleximetrically (2).

All other reagents were of analytical grade and were used as received.

## Apparatus

All absorbance measurements were made in a Hitachi Model 124 spectrophotometer using matched 1.00-cm quartz cells. For pH measurements a Toa Dempa HM-5A pH meter was used.

## Recommended Procedures

*Determination with PAPhH or QAPhH.* Transfer the sample solution containing less than 65 $\mu$g of palladium(II) to a 50-ml separatory funnel along with 1 ml of 6 $N$ sulfuric acid and 4 ml of $1 \times 10^{-3}$ $M$ PAPhH (or QAPhH) hydrochloride. Dilute to 10 ml with demineralized water and equilibrate with exactly 10 ml of chloroform for 5–10 min. Transfer an aliquot of the chloroform extract to a 1.00-cm cell and measure the absorbance of the solution at 537 nm (for PAPhH complex) or 580 nm (for QAPhH complex) against the reagent blank.

*Determination with BAPhH.* To the sample solution containing up to 60 $\mu$g of palladium(II) in a 50-ml separatory funnel add 3 ml of 6 $N$ sulfuric acid and 0.5 ml of 1 $M$ sodium chloride. Adjust the volume to 10 ml with water. Add exactly 10 ml of $2.5 \times 10^{-4}$ $M$ BAPhH solution in chloroform and extract the complex for 10–15 min. Measure the absorbance of the chloroform extract at 587 nm.

## RESULTS

### Characteristics of the Complexes

The palladium(II) complexes formed with the reagents in the presence of chloride ions are sparingly soluble in water, but readily soluble in various organic solvents such as partially halogenated hydrocarbons, acetate esters, ketones, and aliphatic alcohols. Chloroform and 1,2-dichloroethane proved to give the highest absorbance at the wavelengths of maximum absorption, the former being chosen as the solvent.

The visible absorption spectra of the three palladium(II) complexes extracted into chloroform are illustrated in Fig. 1. The palladium(II) complexes of QAPhH and BAPhH give two absorption maxima at 578 and 600, and 587 and 623 nm, respectively, whereas the PAPhH complex shows a maximum absorption at 537 nm and a big shoulder at about 565
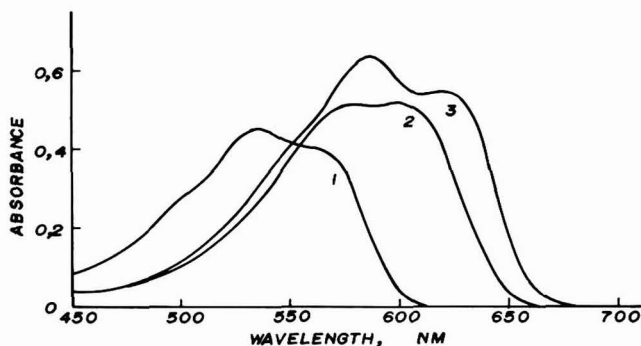
FIG. 1. Absorption spectra of the palladium(II) complexes in chloroform. Concentration of palladium(II) is $3.75 \times 10^{-5}$ M (39.8 $\mu$g). (1) Pd(II)–PAPhH; (2) Pd(II)–QAPhH; (3) Pd(II)–BAPhH.

nm. In each case, the reagent blank solution was known to exhibit practically no absorbance in the visible region studied, because the reagent forms almost unextractable cationic species with hydrogen ions; for example, BAPhH in 20% (v/v) aqueous ethanol forms monoprotonated and diprotonated cationic species around pH 3.5 and below pH 1.5, respectively (Fig. 2).

*Effects of Experimental Conditions*

The absorbance of the organic phase was measured as functions of the initial concentrations of sulfuric acid, sodium chloride, and the reagent. The effect of the shaking period for the extraction was also investigated.
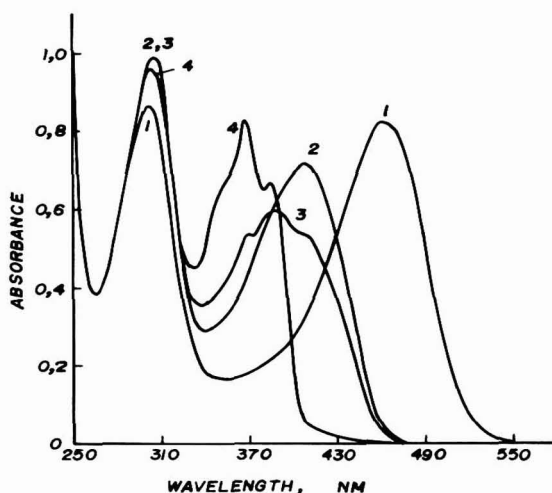


FIG. 2. Absorption spectra of BAPhH in 20% (v/v) aqueous ethanol at $\mu = 0.1$. Concentration of BAPhH is $1.31 \times 10^{-5}$ M. (1) In 0.72 M KOH; (2) at pH 5.09; (3) at pH 3.46; (4) at pH 1.25.

The rate of extraction of palladium(II) into the chloroform solution of BAPhH depends on the sulfuric acid concentration of the aqueous phase; the higher the acid concentration, the shorter the time necessary for complete extraction of palladium(II). A shaking period of about 7 min is required under the optimal condition of the sulfuric acid concentration. The optimal conditions for the determination of palladium(II) are summarized in Table 1. All the palladium(II) complexes extracted under the optimal conditions showed constant color for at least 1 hr.

*Conformance to Beer's Law*

All the analytical species of interest obey Beer's law over the concentration range studied of up to about $6 \times 10^{-5}$ $M$ palladium(II) in the organic phase (Fig. 3). The molar absorptivities of the complexes were calculated from the data used to plot the calibration curves to be $1.24 \times 10^4$, $1.37 \times 10^4$, and $1.68 \times 10^4$ $M^{-1}$ cm$^{-1}$ for the PAPhH, QAPhH, and BAPhH systems, respectively. The reaction with BAPhH is sensitive enough to make this reagent attractive for analytical application.

*Stoichiometry of the Complexes*

Job's method of continuous variations and the molar ratio method were used to evaluate the stoichiometric ratios of metal to ligand in the complexes. The 1:1 molar ratio, under the experimental conditions given, was ascertained by both methods. As mentioned above, the presence of chloride ions served an important role for the formation of the extractable palladium(II) complexes. The fourth coordination site of palladium(II) would therefore be occupied by a chloride ion to form a 1:1:1 uncharged complex, as described earlier (2).

TABLE 1

OPTIMAL CONDITIONS FOR THE DETERMINATION OF PALLADIUM(II)

| Reagent | $H_2SO_4$ concentration (*M*) | NaCl concentration (*M*) | Reagent concentration (*M*) | Shaking period (min) |
|---------|-------------------------------|--------------------------|-----------------------------|----------------------|
| PAPhH | 0.05–1.25 | $3 \times 10^{-4}$–0.2[b] | $4 \times 10^{-4}$ | 5–10 |
| QAPhH | 0.35–0.85 or 3.05–4.85[a] | $3 \times 10^{-4}$–$2 \times 10^{-3b}$ | $4 \times 10^{-4}$ | 5–10 |
| BAPhH | 0.65–4.25 | $2 \times 10^{-3}$–0.25[b] | $2.5 \times 10^{-4c}$ | 10–15 |

[a] Between 1 and 2.5 $M$, orange yellow precipitates were formed on the boundary of the two phases.

[b] A similar result was also obtained with potassium bromide in place of sodium chloride.

[c] The concentration in chloroform.
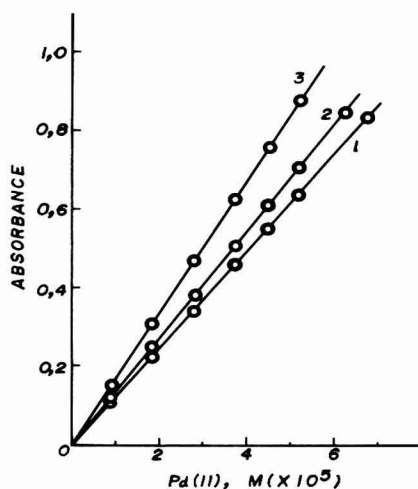
FIG. 3. Calibration curves for palladium(II) determination. (1) Pd(II)−PAPhH system; (2) Pd(II)−QAPhH system; (3) Pd(II)−BAPhH system.

## Effect of Diverse Ions

In order to study the effect of diverse ions on the determination of palladium(II) with BAPhH, which is the most promising reagent of the three for palladium(II), a fixed amount of palladium(II), 39.8 $\mu$g, was taken with varying amounts of foreign ions and the recommended procedure was applied. An error of $\pm 2\%$ in the absorbance reading was considered tolerable. Tolerances for various foreign ions are shown in Table 2.

TABLE 2
EFFECT OF FOREIGN IONS ON DETERMINATION OF 39.8 $\mu$g OF PALLADIUM(II)

| Tolerance limit ([Ion]/[Pd(II)]) | Ion |
| --- | --- |
| $\geq 10,000$ | $NO_3^-$, $C_2O_4^{2-}$, $PO_4^{3-}$, tartrate, citrate |
| $\geq 100$ | $Al^{3+}$, $Ba^{2+}$,[a] $Be^{2+}$, $Bi^{3+}$, $Ca^{2+}$, $Cd^{2+}$, $Co^{2+}$, $Cu^{2+}$, $Fe^{2+}$, $Fe^{3+}$, $Mn^{2+}$, $Ni^{2+}$, $Os^{8+}$, $Pb^{2+}$, $Sc^{3+}$, $Sr^{2+}$,[a] $Th^{4+}$, $Tl^+$, $U^{6+}$, $V^{5+}$, $Zn^{2+}$ |
| $\leq 100$ | $As^{5+}$, $Cr^{3+}$, $Hg^{2+}$, $Mo^{6+}$,[b] $Pt^{4+}$, $Ru^{3+}$ |
| $\leq 10$ | $Rh^{3+}$, $W^{6+}$[b] |
| $\leq 3$ | $Au^{3+}$, $Ir^{3+}$ |
| $0-1$ | $I^-$, $SCN^-$, EDTA |

[a] Nitric acid was used instead of sulfuric acid.

[b] 2 ml of 1 $M$ tartaric acid was added.

Platinum(IV) and ruthenium(III) at about 100-fold ratio to palladium(II) do not interfere. Gold(III) and iridium(III) up to a foreign ion/ palladium(II) ratio of 2 or 3 interfere slightly. However, iodide, thiocyanate, and EDTA interfere seriously by forming stable complexes with palladium(II).

## DISCUSSION

It is interesting to compare with each other the spectral properties of the palladium(II) complexes extracted into the organic solvent. The highest molar absorptivity as well as the greatest bathochromic shift in the maximum absorption, observed in the BAPhH complex, implies that the $-C=N-$ structure in the heterocyclic ring on the aldehyde moiety would be of great importance for the spectral properties of the complex; in the light of the bond order, the relative preference of the $-C=N-$ structural form would increase in the sequence benzothiazolyl > quinolyl > pyridyl. Moreover, in the BAPhH complex, the above structural form would be favorably stabilized by two effects opposite to each other, the electron surplus associated with the sulfur of benzothiazole and the electron deficiency associated with the nitrogen of phthalazine. This situation for interpreting the spectral properties of the metal−hydrazone complexes is applicable to the palladium(II) (2), copper(II) (3), and cadmium(II) (M. Otomo, from unpublished data) complexes of terdentate heterocyclic hydrazones $R_1-CH=N-NH-R_2$, where $R_1$ = 2-pyridyl, 2-quinolyl, 6-phenanthridyl, or 2-benzothiazolyl, and $R_2$ = 2-pyridyl or 2-quinolyl.

## SUMMARY

Three terdentate hydrazones, all containing the 1-phthalazino grouping in the hydrazine moiety but differing in the heterocyclic substituent in the aldehyde moiety, have been used as analytical reagents for palladium(II), the optimal conditions for the extractive spectrophotometric determination of palladium(II) in the presence of chloride ions being deduced. These compounds are highly selective and sensitive reagents for palladium(II), since they are not extracted into chloroform from sulfuric acid solutions and do not react with other platinum group metals. The desirable spectral properties of the palladium(II) complex of benzothiazole-2-aldehyde-1-phthalazinohydrazone (BAPhH) have also been discussed with respect to preference of the $-C=N-$ structural form in the heterocyclic ring on the aldehyde moiety of the ligand.

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# The Recovery of Prostaglandins and Other Related Metabolites of Arachidonic Acid from Human Blood Plasma

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## INTRODUCTION

Prostaglandins and other related metabolites of arachidonic acid are biosynthesized in most biological systems in infinitely small amounts. A large number of publications (*1*) have appeared that include quantitative data on these substances. On account of their generally low levels in most tissues coupled with the presence of several different compounds of similar chemical nature, the quantitative data are inconsistent. This is thought to be due to binding of these substances to proteins and probably to some other macromolecules. The present report is an attempt to show that these substances can be extracted almost quantitatively from blood plasma into organic solvents of common use.

## MATERIALS AND METHODS

[1-$^{14}$C]Arachidonic acid (specific activity: 60.2 mCi/mmol) was purchased from The Radiochemical Centre, Amersham. Standard prostaglandins $E_2$, $D_2$, $F_{2\alpha}$, and 6-keto-$PGF_{1\alpha}$ were obtained as a generous gift from The Upjohn Company, Kalamazoo, Michigan, and thromboxane $B_2$ ($TxB_2$) from ONO Pharmaceutical Company Ltd., Higashiku, Osaka, Japan.

Labeled thromboxane $B_2$ was prepared by incubating [1-$^{14}$C]arachidonic acid with human platelets (*2*). Other labeled prostaglandins and related metabolites were prepared by incubating labeled arachidonic acid with rat lung and some other tissue homogenates (*3*). The metabolites were extracted into an organic solvent and subjected to a three-system TLC on silica gel G plates. The developed plates were kept in an iodine chamber whereby different metabolite zones were shown and identified with the help of unlabeled standard samples of prostaglandins which were added before extracting the incubation mixture with an organic solvent.

Stock solutions contained about 80 pmol (10,000 dpm) of labeled metabolites $PGE_2$, $PGD_2$, $PGF_{2\alpha}$, $TxB_2$, and 6-keto-$PGF_{1\alpha}$, and 20 μg of unlabeled standard samples of each per 150 μl methanol. The stock solu-

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tion of the HHT and HETE mixture contained 400 pmol (50,000 dpm) of labeled material only in 150 $\mu$l methanol.

*Preparation of platelet-rich-plasma (PRP).* Venous blood was withdrawn by venipuncture from a healthy person and mixed with 0.1 vol of 3.8% trisodium citrate solution. The mixture was centrifuged in an Adams Dynac centrifuge (Catalog No. 0102) at 700 rpm for 15 min and the supernatant (PRP) was collected in a siliconized flask. The PRP was stored at 4°C until use.

*Extraction of prostaglandins and related metabolites from PRP.* Four organic solvents, viz., ethyl acetate, ether, chloroform, and methylene chloride, were separately used for the extraction of the prostaglandins and related metabolites from plasma. Each metabolite was separately added to PRP and was then recovered by extraction into the above four solvents separately. As an example, the procedure for the recovery of $PGE_2$ from PRP by extraction with the above solvents is described here. Twelve glass-stoppered test tubes were arranged into four groups, each comprising three test tubes, and 1 ml of PRP was taken in each test tube. $PGE_2$ (150 $\mu$l) from the stock solution was added to PRP in each test tube, and the contents were mixed well on a Fisons Whirlimixer and allowed to stand for 20 min. The solution in each tube was then acidified (about 150 $\mu$l of 1 $N$ HCl) to pH 3.0. After allowing the solution to stand for 5 min, 1 ml ethanol was added to it, and the solution was mixed on a Whirlimixer. Among the four groups of test tubes, each group was marked for extraction with one particular solvent. The solvent (2 ml) was now added to the contents of the three test tubes of the respective group, and the contents were mixed on a Whirlimixer and allowed to stand for about 15 min. The contents were then centrifuged and the separated organic phase from each test tube was collected separately in test tubes with the help of disposable glass pipets. The residual matter in the test tubes was further extracted twice using 2 ml of the respective solvent each time. Throughout the extraction, the tubes containing the PRP−metabolite mixture were kept in ice-cold water. All the extracts (12 in all, 3 of each solvent) were separately evaporated to dryness at 40−50°C in a stream of nitrogen (which also removed most of the HCl). The residues thus obtained were separately dissolved in 1100 $\mu$l methanol and their radioactivity was measured by placing the methanolic solution in 10 ml toluene cocktail (4 g PPO and 100 mg POPOP per liter) in an LKB 81000 liquid scintillation counter. Labeled $PGE_2$ (150 $\mu$l) from the stock solution was also taken out for counting of radioactivity at the same time the material was added to PRP. From the counts obtained for 12 residues and 150 $\mu$l $PGE_2$ from the stock solution, the percentage recovery of labeled $PGE_2$ from each PRP sample was calculated. The values reported in Table 1 are means of three trials with each solvent (corresponding to three test tubes of each group). A similar procedure was followed with the remaining three solvents.

TABLE 1

EXTRACTION OF PROSTAGLANDINS AND OTHER RELATED ARACHIDONIC ACID METABOLITES FROM HUMAN BLOOD PLASMA

| Compound | Solvent[a] | Percentage recovery | | | | |
|---|---|---|---|---|---|---|
| | | 1st Extr. | 2nd Extr. | 3rd Extr. | Extr. 1 + 2 | Extr. 1 + 2 + 3 |
| $PGD_2$ | 1 | 68.46 ± 0.07 | 10.98 ± 0.58 | 1.44 ± 0.34 | 79.45 ± 0.51 | 80.88 ± 0.60 |
| | 2 | 65.26 ± 1.26 | 8.96 ± 0.08 | 1.39 ± 0.35 | 74.22 ± 1.29 | 75.25 ± 1.25 |
| | 3 | 55.86 ± 3.58 | 7.35 ± 2.58 | 5.46 ± 2.00 | 63.21 ± 1.64 | 68.67 ± 0.95 |
| | 4 | 65.74 ± 2.01 | 11.59 ± 1.20 | 3.42 ± 0.44 | 77.34 ± 1.58 | 80.77 ± 1.33 |
| $PGE_2$ | 1 | 78.08 ± 2.75 | 10.96 ± 2.42 | 0.40 ± 0.04 | 89.03 ± 0.49 | 89.43 ± 0.51 |
| | 2 | 70.00 ± 1.68 | 11.60 ± 1.56 | 1.48 ± 0.43 | 81.60 ± 1.09 | 83.08 ± 0.78 |
| | 3 | 68.74 ± 1.21 | 8.45 ± 1.00 | 1.18 ± 0.11 | 77.05 ± 0.47 | 78.37 ± 0.54 |
| | 4 | 67.79 ± 1.67 | 8.76 ± 0.96 | 1.17 ± 0.16 | 76.55 ± 0.74 | 77.73 ± 0.88 |
| $PGF_{2\alpha}$ | 1 | 73.78 ± 1.95 | 11.32 ± 0.79 | 1.10 ± 0.15 | 85.20 ± 1.85 | 86.30 ± 1.74 |
| | 2 | 65.14 ± 2.45 | 12.42 ± 1.23 | 4.09 ± 1.02 | 77.57 ± 1.76 | 81.65 ± 0.78 |
| | 3 | 67.88 ± 2.89 | 8.34 ± 0.56 | 1.74 ± 0.38 | 76.22 ± 2.36 | 77.87 ± 2.02 |
| | 4 | 62.79 ± 2.41 | 10.68 ± 1.41 | 3.13 ± 0.16 | 73.48 ± 1.47 | 76.61 ± 1.42 |
| 6-Keto-$PGF_{1\alpha}$ | 1 | 79.25 ± 1.07 | 8.70 ± 0.74 | 1.30 ± 0.31 | 87.94 ± 1.43 | 89.30 ± 1.18 |
| | 2 | 67.25 ± 1.45 | 14.20 ± 0.11 | 2.90 ± 0.44 | 81.44 ± 1.47 | 84.24 ± 0.98 |
| | 3 | 74.82 ± 2.69 | 12.67 ± 1.87 | 3.96 ± 0.46 | 87.49 ± 1.75 | 91.45 ± 1.32 |
| | 4 | 64.76 ± 1.95 | 14.20 ± 1.72 | 3.36 ± 0.44 | 78.96 ± 0.69 | 82.32 ± 0.99 |
| $TxB_2$ | 1 | 74.19 ± 2.80 | 9.76 ± 0.62 | 1.12 ± 0.28 | 83.95 ± 2.40 | 85.07 ± 2.17 |
| | 2 | 68.17 ± 1.75 | 11.74 ± 0.76 | 2.33 ± 0.44 | 79.91 ± 1.19 | 82.24 ± 1.48 |
| | 3 | 76.33 ± 0.47 | 9.16 ± 0.34 | 2.60 ± 0.34 | 85.49 ± 0.33 | 88.09 ± 0.58 |
| | 4 | 69.94 ± 0.88 | 7.19 ± 0.23 | 2.74 ± 0.31 | 77.14 ± 0.73 | 79.88 ± 0.78 |
| HHT and HETE mixture | 1 | 69.70 ± 1.53 | 9.63 ± 0.93 | 1.49 ± 0.53 | 79.34 ± 2.20 | 80.85 ± 1.75 |
| | 2 | 69.99 ± 0.96 | 7.57 ± 0.72 | 0.62 ± 0.36 | 77.57 ± 0.86 | 78.19 ± 0.50 |
| | 3 | 67.48 ± 1.07 | 8.18 ± 0.63 | 1.45 ± 0.02 | 75.66 ± 0.54 | 77.11 ± 0.52 |
| | 4 | 62.12 ± 2.81 | 9.10 ± 1.32 | 2.05 ± 0.34 | 71.22 ± 1.62 | 73.28 ± 1.27 |

[a] Solvents: (1) ethyl acetate; (2) ether; (3) chloroform; (4) methylene chloride.

It may be added that the residues obtained after evaporation of extracts may be used for obtaining quantitatively prostaglandins and related metabolites free from water-soluble impurities by extracting the metabolites from the residues into ethyl acetate.

## RESULTS AND DISCUSSION

It is evident from Table 1 that with almost all the experimental solvents two extractions of PRP containing the metabolites give sufficiently high recovery yields of metabolites and thus the third extraction can be safely omitted as loss of material would be almost insignificant (cf. Table 1).

Ethyl acetate and diethyl ether appear to be the most effective solvents for nearly all the metabolites and the recovery yields obtained from two extractions range from 75 to 90% (cf. Table 1). The use of these two solvents for routine work has an added advantage in that during extraction they constitute the upper phase which can be separated out more easily.

Although chloroform and methylene chloride give recovery yields comparable to those obtained on extraction with ethyl acetate and diethyl ether, they are somewhat unsuitable for routine work as they constitute the lower phase, the separation of which is considerably tedious, particularly when small volumes of plasma and solvents are handled.

Some previous workers (1) have reported poor recovery of the highly polar metabolite, viz., 6-keto-PGF$_{1\alpha}$, from chloroform extract. In contrast to this, the present extraction procedure shows that this metabolite can be recovered to the extent of 80−90% even in less polar solvents—chloroform, methylene chloride, or ether. The higher recovery yields of the metabolite in the present work can probably be attributed to the presence of ethanol (in the extraction mixture) which augments the extraction of this metabolite into organic solvents all of which are miscible with ethanol.

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# Simple and Specific Microdetermination of Organoiodine via Oxygen Flask Combustion

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## INTRODUCTION

The microdetermination of organochlorine and organobromine by oxygen flask combustion and mercurimetric titration (*1*) has been found to be reliable by several collaborative studies (*9, 10*). However, this procedure cannot be applied to organoiodine determination. Lower results are frequently observed. The source of errors can be traced from two noticeable difficulties. In the first place, the absorbing liquid, the alkaline hydrogen peroxide, may not be an effective agent for converting iodine quantitatively to iodide under the flask combustion condition. Second, the mercurimetric titration may not be the optimum choice for the final titration of iodide because of the unfavorable conversion factor involved.

In 1972, Lalancette *et al.* (*6*) succeeded in preventing iodine loss in combustion by using a more effective absorbing agent, namely, hydrazine, to replace hydrogen peroxide. However, they retained the mercurimetric titration which resulted in unsatisfactory performance as reported in an AOAC collaborative study (*7*). They had to omit several data points in order to obtain an acceptable standard deviation of the means.

We have also experienced difficulty in using the AOAC method for iodine determination while examining several quaternary ammonium iodides. Four approaches were taken in determining the iodine contents. Because of limited supply of material, only 1–3 mg of each sample was used for each determination. The methods used were:

1. direct mercurimetric titration for iodide, which produced the theoretical value of iodine percentage;

2. mercurimetric titration after flask combustion with alkaline hydrogen peroxide as an absorbing agent, omitting the boiling off step as previously suggested (*2*), which showed 93–96% recovery;

3. AOAC procedure (*7*) using 3 mg each sample, much smaller than the amount required by that procedure, which showed that iodine was almost completely lost;

4. argentimetric titration with an iodide ion-selective electrode as indicator after oxygen flask combustion using hydrazine as the absorbing

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agent. It is this procedure which is the subject of this communication and which produced a 100% recovery.

The results are tabulated as follows:

| Sample | Iodine(%) | | | |
| | Direct titration method | Alkaline $H_2O_2$ method | AOAC method | Electrode indicator titration method |
| --- | --- | --- | --- | --- |
| A | 26.50 | 25.45 | None found | 26.50 |
| B | 27.56 | 25.80 | None found | 27.80 |

It is not surprising that the AOAC method was not applicable to smaller samples. Generally there are two drawbacks with the AOAC procedure (7). First, it requires a large sample size (i.e., containing 6−9 mg iodine). For a compound containing 30% iodine, a large sample size of about 20 mg is required in order to supply the minimum amount of iodine (i.e., 6 mg). Second, as mentioned earlier, the unfavorable mercurimetric titration requires a specified type of microburette to avoid possible titration error (6). Using a more dilute titrant [say 0.001 $M$ Hg(NO$_3$)$_2$ solution] will not improve the situation because simultaneously a large blank is produced.

After an extensive comparative study we conclude that the argentimetric titration with an iodide ion-selective electrode as the indicator is the optimum choice when preceded by oxygen flask combustion with hydrazine as the absorbing agent for microdetermination of organoiodine. The author has found one report in literature on this subject with a similar approach differing only in performance in titration (11).

## MATERIALS AND METHODS

*Reagents*

a. 0.001 $N$ silver nitrate standard solution
b. Saturated solution of hydrazine sulfate in water
c. 0.05% bromophenol blue in alcohol
d. 0.5 $N$ potassium hydroxide solution
e. 0.05 and 0.5 $N$ nitric acid solution
f. 20% sodium nitrate solution
g. Potassium iodide reference solution—containing about 0.1 mg iodide/ml

*Equipment*

a. Electrometer, read to 0.1 mv (e.g., Corning Model 101)
b. Iodide ion-selective electrode (e.g., Orion 94-53)

c. SCE reference electrode, bridged with sodium nitrate solution
d. 10-ml burette, readable accurately in each 0.5-ml delivery
e. Gran's Plot 10% correction paper (e.g., Orion 90-00-90)
f. Magnetic stirrer

*Procedure*

Weigh sample (containing 0.2−0.5 mg iodine) and conduct the oxygen flask combustion with 10 ml water, 4 drops saturated hydrazine sulfate solution, and 1 ml 0.5 N potassium hydroxide solution as absorbing liquid.

After the combustion is complete, shake and let the flask stand for 2−5 min for complete absorption. Transfer all of the contents into a 150-ml-tall form beaker with about 60 ml of water, and add 5 ml of 20% sodium nitrate solution. Adjust the pH to a value of 3.5 with nitric acid in the presence of bromphenol blue indicator. Add enough water to a final volume of 100 ml. Insert the SCE and iodide ion-selective electrode into the beaker and connect them to an electrometer. Fill a 10-ml burette with 0.001 N silver nitrate standard solution and proceed to titrate, placing the sample solution on the insulated magnetic stirrer, and continue stirring.

Read from the electrometer the electrode potential (in mV) during constant stirring to 0.1 mV accurately.

*Option of Preliminary Estimation*

The initial millivolt reading (i.e., before adding silver nitrate titrant) may be used to estimate the iodide content from a calibration curve previously prepared with potassium iodide reference solution. However, this preliminary estimation may not be always reliable.

*Titration*

Read accurately the initial millivolts and successively read after each addition of exactly 0.5 ml silver nitrate titrant. Plot the millivolt titration point vs milliliters of titrant on Gran's plot 10% correction paper. Only three or four titration points are necessary to define a straight line which can be extrapolated to the endpoint of the titration, which is the intercept of the line of the horizontal axis (i.e., the ml titrant axis). The iodine content of the sample can then be calculated from the determined endpoint.

Figure 1 illustrates an iodide titration on Gran's plot. Point A is the iodide endpoint of the titration. Point B is the endpoint of total titration of iodide and another halide, if present.

## RESULTS AND DISCUSSION

Experimental results are given in Table 1 which demonstrates the suitability of the procedure for microdetermination of iodine content in organic compounds: *o*-iodobenzoic acid is the British Drug Houses Ltd. (BDH) microstandard, the three samples A, B, and C are preparatory

GRAN'S PLOT WITH 10% CORRECTION
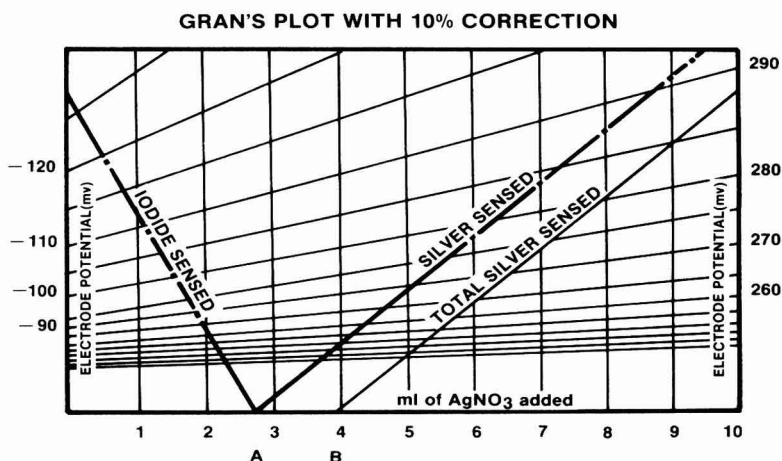


FIG. 1. Argentimetric titration for iodide with an iodide ion-selective electrode as indicator. Point A is the iodide endpoint. Point B is the total titration endpoint of mixed halides.

synthetic drug candidates which have been subjected to purity tests including a complete elemental analysis, while the last three iodo compounds are Eastman reagent grade chemicals within 2°C variation of their melting point. The latter compounds may be considered 99+% pure. Since all samples are preliminarily subjected to the same oxygen flask combustion according to Lalancette *et al.* (6), no further comment about it will be necessary. Several advantages of the argentimetric titration with an ion-electrode indicator over the mercurimetric titration will be discussed.

*Simplicity*

Unlike mercurimetric titration, the unused hydrazine left in the combustion product will not interfere with the iodide ion-selective electrode as an indicator in the argentimetric titration. This makes it simple for the analyst to titrate immediately after combustion, thus eliminating the elimination of hydrazine. Table 2 shows the corresponding electrode potentials in titration of a pair of potassium iodide solutions, one with and another without 4 drops of hydrazine sulfate solution. No interference is observed.

*Sensitivity*

The iodide ion-selective electrode is a very sensitive indicator for argentimetric titration. The limit of detection for silver ion is about $10^{-8}$ *M*. Therefore a precise titration can be achieved using very dilute titrant (say 0.001 *M*) and/or a small sample (say $1-3$ mg). Table 3 includes some analyses with sample weights under 1 mg.

TABLE 1

DETERMINATION OF IODINE IN ORGANIC COMPOUNDS

| No. | Sample | Percentage iodine | | Remark |
| --- | --- | --- | --- | --- |
| | | Theor. | Found | |
| 1 | o-Iodobenzoic acid<br>IC$_7$H$_5$O | 51.17 | 51.07 (see Table 3)<br>avg. of 10 runs | Mico standard |
| 2 | Sample A<br>IC$_{14}$H$_{33}$N$_2$O$_4$ | 31.75 | 31.53; 31.55<br>31.49; 31.50 | |
| 3 | Sample B<br>IC$_{23}$H$_{28}$NO | 26.50 | 26.80; 26.80<br>(27.09); 26.79 | |
| 4 | Sample C<br>IC$_{22}$H$_{28}$NO | 27.56 | 27.89; 27.85<br>27.90; 27.70 | |
| 5 | p-Iodobenzoyl chloride<br>IC$_7$H$_4$OCL | 47.66 | 47.94<br>47.99 | Calcd Cl% 13.49<br>Found 13.37 |
| 6 | p-Iodobenzene sulfonyl chloride<br>IC$_6$H$_4$SO$_2$Cl | 41.70 | 41.76<br>41.89 | Calcd Cl% 11.66<br>Found 11.39 |
| 7 | 2-(p-Iodophenyl)-3-(p-nitrophenyl-<br>5-phenyl-2H-tetrazolium chloride*<br>IC$_{19}$H$_{13}$N$_5$O$_2$Cl | 25.12 | 25.11<br>24.93 | Calcd Cl% 7.02<br>Found 6.70 |
| 8 | 2-iodoacetamide<br>IC$_2$H$_4$ON | 68.65 | 68.62<br>68.44 | |

* Recrystallized from ether−MeOH mixture.

TABLE 2
TITRATION POINTS OF POTASSIUM IODIDE SOLUTION CONTAINING 0.2940 mg IODIDE[a]

| AgNO$_3$ added (ml) | mv Reading (mV) | |
| --- | --- | --- |
| | KI solution without hydrazine | KI solution with hydrazine |
| 0.00 | −115.2 | −115.5 |
| 0.50 | −107.9 | −107.9 |
| 1.00 | − 97.0 | − 97.0 |
| 1.50 | − 76.5 | − 76.5 |
| Titration endpoint | 1.92 ml AgNO$_3$ | 1.92 ml AgNO$_3$ |

[a] Testing solution: 2 ml KI reference solution, 5 ml 20% NaNO$_3$ solution, 1 ml 0.5 N KOH solution and with or without 4 drops of saturated hydrazine sulfate solution. The mixture is diluted with water to a final volume of 100 ml and adjusted to a pH value of 3.5 with bromophenol blue indicator and 0.5 N nitric acid.

## Specificity

The iodide ion-selective electrode is a specific indicator in presence of other halides. Therefore the argentimetric titration can determine iodide in mixed halide solution. The other halide can, sometimes, be simultaneously estimated. Samples 5 and 6 in Table 1 demonstrate this case. Figure 1 illustrates the titration endpoints. Point A is the iodide titration endpoint, while point B is the endpoint of titration for total halides in the mixture. Mercurimetric titration lacks such selectivity.

TABLE 3
PRECISION STUDY: ANALYSIS OF o-IODOBENZOIC ACID[a]

| Sample weight (mg) | Iodine found (%) | Deviation from mean | |
| --- | --- | --- | --- |
| | | $d$ | $d^2 \times 10^{-4}$ |
| 1.060 | 51.17 | 0.10 | 100 |
| 1.065 | 51.25 | 0.18 | 324 |
| 1.205 | 50.93 | 0.14 | 136 |
| 0.455 | 50.95 | 0.12 | 144 |
| 0.820 | 51.01 | 0.06 | 36 |
| 0.980 | 51.19 | 0.12 | 144 |
| 1.200 | 51.25 | 0.18 | 324 |
| 1.135 | 50.95 | 0.12 | 144 |
| 1.140 | 50.93 | 0.14 | 136 |
| 1.050 | 51.10 | 0.03 | 9 |
| Mean: | 51.07 | 0.12 | $1497 \times 10^{-4}$ |

[a] Theoretical percentage iodine = 51.17%. Average percentage standard deviation = $[\sum d^2/(n - 1)]^{1/2}$.

Precision and accuracy are established by the analyses of the BDH standard, o-iodobenzoic acid. Table 3 presents the data which show the average recovery over 99.8%. The standard deviation is 0.13. These results are comparable with any other reliable micromethods.

The current trend of using microprocessors to improve the titration should be noted. The present procedure is readily adapted to automation, such as an ion-selective analysis system, set up in some laboratories (4, 5), and a minicomputer in connection with Gran's plot in some ICI laboratories (3).

## SUMMARY

A rapid, simple, and reliable procedure for specific microdetermination of organoiodine is described. This procedure consists of oxygen flask combustion followed by an argentimetric titration with an iodide ion-selective electrode as indicator. Some advantages of this procedure over mercurimetric titration currently used in an AOAC method are detailed.

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# Spectrophotometric Estimation of Microgram Amounts of Sodium N-Chloro-p-toluenesulfonamide (Chloramine-T) and Sodium N-Chlorobenzenesulfonamide (Chloramine-B)

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## INTRODUCTION

Both chloramine-T (CAT: sodium N-chloro-p-toluenesulfonamide) and chloramine-B (CAB: sodium N-chlorobenzenesulfonamide) have multifarious reactions with a wide range of functional groups. In the recent literature, important and novel molecular transformations including vicinal hydroxyamination of olefins and allylic amination techniques have emerged (8). CAT has been used as a disinfectant, antiseptic, and scrubbing agent (19, 20). Both CAT and CAB have wide applications in analytical and synthetic chemistry (8, 13) due to their potentiality as oxidizing and chlorinating agents (1–3, 9–13, 15–19, 23). The available titrimetric methods for the assay of CAT and CAB are limited to the determination of milligram amounts of the compounds. The recent spectrophotometric assay of CAT developed in our laboratory (18), although useful for higher concentrations, introduces significant error if the amount is less than 200 μg/ml. The present investigation deals with an accurate ultraviolet spectrophotometric method for the assay of CAT and CAB in the range 1 to 80 μg/ml. The linearity of absorbance with concentration of CAT or CAB in aqueous solution at $\lambda_{max} = 205$ nm is the basis for the present method developed. To our knowledge, this is the first report of a spectrophotometric estimation of CAB, and an ultraviolet spectrophotometric estimation of CAT. Advantages of the present method include high sensitivity (microgram amounts), rapidity, and reproducibility.

## MATERIALS AND METHODS

*Chloramine-T.* The chloramine-T (Eastman Kodak) was freed from possible dichlorocontaminant by washing several times with carbon tetrachloride and dried in a vacuum desiccator over $CaCl_2$ (12). The purity of

the sample, determined iodometrically (7), was 99% based on active chlorine titrated. One hundred milligrams of this purified sample were dissolved in 100 ml of distilled water and used as the stock solution for the assay.

*Chloramine-B.* The chloramine-B was prepared by bubbling pure chlorine gas through a solution of benzenesulfonamide in 4 $M$ NaOH over a period of 1 hr at 70°C (15). CAB obtained was filtered, washed with small quantities of water, and dried. The dried sample was washed with carbon tetrachloride to remove the dichlorocontaminant and unreacted benzenesulfonamide. The purity (>99%) of the sample was checked iodometrically. The stock solution was prepared by dissolving 100 mg of this sample in 100 ml of distilled water.

Spectrophotometry was carried out with a Gilford Model 250 dual-source spectrophotometer fitted with digital readout. The uv spectra of CAT and CAB were obtained using the automatic scanning unit of the instrument.

*Procedure.* Different solutions containing varying amounts of CAT or CAB were prepared by appropriate dilution (1–80 $\mu$g/ml) of the stock solutions. The absorbance of each solution was read at 205 nm ($\lambda_{max}$ for CAT or CAB) (Fig. 1 under Results and Discussion) using the uv-visible spectrophotometer. The amount corresponding to the known absorbance was calculated using the standard plot (see Fig. 2 under Results and Discussion).

For the estimation of a mixture of CAT and CAB, appropriate amounts of the two were mixed and the absorbance was read. By using the standard plots of CAT and CAB, the total amount of the two present in a mixture was computed.

## RESULTS AND DISCUSSION

The ultraviolet spectra of CAT and CAB are presented in Fig. 1. The absorption maximum for both CAT and CAB appears at 205 nm. The fact that CAT and CAB have the same $\lambda_{max}$ suggests that this absorption maximum at 205 nm is characteristic of the $-SO_2NCl^-$ group. The absorbance measurements of CAT and CAB were carried out at 205 nm at room temperature. The standard plots of absorbance versus microgram amounts of chloramines (CAT or CAB) were linear in the range 1 to 80 $\mu$g/ml obeying Beer's Law (Fig. 2). The large value of the molar extinction coefficients (CAT: $\xi_{max} = 9714.0$; CAB: $\xi_{max} = 9525.7$, both calculated from slopes obtained by the least mean square method) is indicative of the high sensitivity of the present method.

Since the slopes of these two plots have almost the same value, the total amounts of CAT and CAB in a mixture can be determined using any one of the plots. For an approximately 50% mixture of CAT and CAB, the

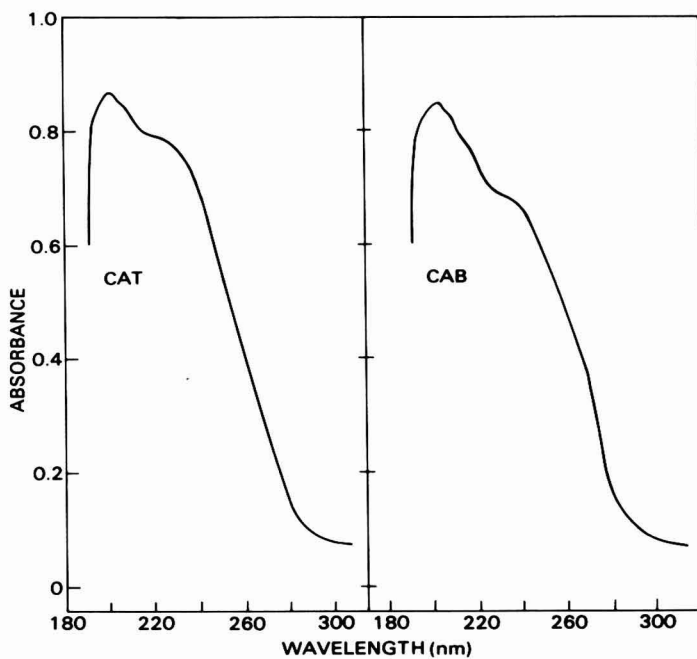FIG. 1.  Ultraviolet spectra of chloramine-T and chloramine-B (both 20 $\mu$g/ml) in aqueous solutions  at  room  temperature.
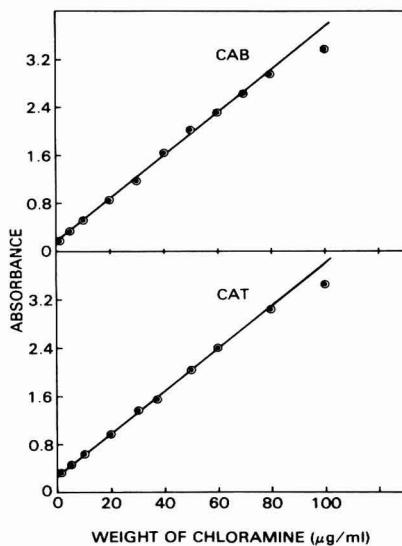


FIG. 2.  Standard plots (absorbance vs $\mu$g/ml of CAT or CAB) for the ultraviolet spectrophotometric assay of chloramine-T and chloramine-B at room temperature. CAT: $\lambda_{max}$ = 205 nm, slope = 0.034486, $r$ = 0.9996; CAB: $\lambda_{max}$ = 205 nm, slope = 0.035586, $r$ = 0.9990.

TABLE 1
ESTIMATION OF CHLORAMINE-T AND CHLORAMINE-B IN A MIXTURE

Mixture of CAT and CAB

| Amount Taken | | Amount Found[a] | | | | | |
|---|---|---|---|---|---|---|---|
| | | I | | II | | III | |
| $\mu$g/ml | $\mu$mol/ml | $\mu$g/ml | $\mu$mol/ml | $\mu$g/ml | $\mu$mol/ml | $\mu$g/ml | $\mu$mol/ml |
| 19.503 | 0.0710 | 19.250 | 0.0701 | 19.23 | 0.0700 | 19.240 | 0.0700 |
| 29.255 | 0.1065 | 29.723 | 0.1082 | 28.81 | 0.1049 | 29.267 | 0.1066 |
| 39.006 | 0.1420 | 39.468 | 0.1437 | 38.56 | 0.1404 | 39.014 | 0.1420 |
| 48.758 | 0.1775 | 49.457 | 0.1800 | 48.10 | 0.1751 | 48.779 | 0.1776 |
| 58.509 | 0.2130 | 59.202 | 0.2155 | 58.20 | 0.2119 | 58.701 | 0.2137 |

[a] I, from CAT plot; II, from CAB plot; III, average of I and II.

error will be considerably minimized if the average value obtained from the two plots is taken (Table 1). The ultraviolet spectrophotometric determination of the total amounts of CAT and CAB in a mixture has the added advantage over the usual titrimetric procedure in that the former can be applied to microgram levels, while titrimetry is limited to milligram levels.

## SUMMARY

A novel ultraviolet spectrophotometric method for the assay of microgram amounts (1–80 $\mu$g/ml) of CAT and CAB in aqueous solution has been developed. The method is highly sensitive, simple, and rapid within the limits of the experimental conditions described. The present method is also applicable for the determination of these two compounds present in a mixture since they have identical $\lambda_{max}$ and nearly the same $\xi_{max}$. The ultraviolet spectra of the compounds have been presented and the values of $\lambda_{max}$ and $\xi_{max}$ are reported.

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# Allethrin Labeled with Tritium

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## INTRODUCTION

Allethrin was needed in radioactive form as a model compound in certain studies in which we were interested. Several workers have reported the specific labeling of allethrin using isotopes of carbon and hydrogen (*1–3*). These preparations involved the synthesis of labeled chrysanthemum acid, or the cyclopentenone moiety, followed by esterification to yield allethrin. A more direct method involving less time was desired, thus, various tritiation techniques on the intact molecule were considered. Further, $^3H$ labeling has an additional advantage over $^{14}C$, as often products of high specific activity can be made at a reasonably low cost. Some of the methods use an exchange reaction of protons in the molecule with $^3H$ in the tritiated donors, which are available commercially at high activities. For our use such a label would be satisfactory, as it is known that the exchange of tritium from esters in biological systems is slow enough not to limit their use for metabolism studies. From the techniques available, a catalytic exchange method was chosen.

## METHODS

*Preparation*

Unlabeled allethrin was obtained from K & K Chemicals, Inc., North Hollywood, California. It was analyzed by TLC and found to contain several impurities and was purified yielding a sample comparable to reference allethrin by TLC and ir analysis. The purified allethrin was forwarded to Nuclear Dynamics Inc., El Monte, California, for tritiation. The technique used to incorporate the radioactivity was termed the Moravek tritiation process. This particular exchange allows for shorter exposure times and milder conditions than other similar methods. Although the details are proprietary, it is a catalytic exchange from a hydroxylic solvent allowing for random tritium labeling. The noble metals are commonly used as catalysts. Exchange by this method is performed by stirring the materials together at room temperature for a day or more, and, as expected, the success of the procedure depends on the stability of the compound to the reaction conditions. The sample of allethrin was exposed to 50 Ci of tritium donor for 48 hr. After the exchange all labile

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tritium was removed by repeated lyophylization. The material upon re-
turn from Nuclear Dynamics had a total activity of about 42 mCi and
weighed approximately 0.2 g. At least four breakdown materials had
formed during the tritiation process, and the [³H]allethrin content as de-
termined by TLC was only slightly over 50%. It was purified by
preparative-layer chromatography (PLC).

*Purification*

As was discovered by two-directional chromatography of light-exposed
samples, [³H]allethrin decomposed in the light. Subsequently all chro-
matography was carried out in darkened chambers in subdued light.

The radioactive allethrin was purified by PLC using Merck-Darmstadt
silica gel F254 precoated 2-mm plates. To ascertain if the procedure was
satisfactory, 270 mg of unlabeled allethrin was applied to a silica gel plate
and developed with hexane-diethylether (1:2). The band containing the
product was located by ultraviolet detection, eluted from the silica gel,
and isolated for purity tests. The recovery of high-purity allethrin was
94%. As a result of this, a sample of 4–5 mCi, 20 mg, of [³H]allethrin (ca.
54% pure) was streaked as a narrow band on silica gel layers 20 mm from
the bottom edge and developed with the same solvent mixture as before.
The plate after sample application was air dried and placed in the de-
veloping chamber; the atmosphere in the developing tank was saturated
with respect to the developing solvent mixture. The chromatographic
plate was removed when the solvent had reached the premarked solvent
front line. The migration solvent was removed by evaporation. After
being dried the layers were suitably identified with radioactive marking
ink and placed in direct contact with a sheet of Kodak "no screen" X-ray
film for 18 hr. After exposure from the radioactive components on the
plate, the film was developed, fixed, and washed according to the man-
ufacturer's directions. The section containing allethrin was collected and
extracted with diethyl ether and methylene chloride. The combined sol-
vents were removed on a rotary flask evaporator. The weight of [³H]alle-
thrin was 14 mg containing about 2.5 mCi of activity. Its purity was deter-
mined by TLC and ir analysis, and its specific activity by standard
radiometric procedures. Additional product was purified in a similar man-
ner with like results. The volatility of the [³H]allethrin in the system used
was greater than expected, therefore, as a safe handling procedure the
operations were performed in a fume hood.

*Characterization*

Infrared analyses were performed to determine the chemical purity as
well as for identification purposes. Standard spectra of pure (*cis* and *trans*
mixed isomers) allethrin were obtained in carbon disulfide solution (ca.
1%) in a 1.0-mm sodium chloride cell using a Beckman IR-12 spec-

trophotometer with normal instrument settings. The spectrum of the tritiated allethrin was obtained in the same manner and the absorbence determined by a baseline procedure. The purity was greater than 96% m/m with no significant difference of absorptivities between the solution of the standard and the radioactive allethrin. The isomer ratio was 1 *cis* to 4 *trans*.

The presence of [³H]allethrin, radioactive impurities, and their relative amounts were determined by thin-layer chromatography (TLC), including cochromatography with authentic samples. Merck-Darmstadt silica gel F254 precoated 0.25-mm plates were used. The materials were spotted and developed as previously described. The solvent systems used are listed in Table 1.

After the developed plates were dried some were sprayed with Omni-spray (intensifier for autoradiography) manufactured by the New England Nuclear Corporation, Boston, Massachusetts. The plates were again dried and exposed to X-ray film at dry ice temperature. After several days, depending on the activity present, the film was developed by the standard procedure. The radiochemical purity as determined by dilution techniques appeared to be over 98%. The purity by zone counting of the chromatogram sections using a liquid scintillation spectrophotometer was found to be greater than 97%. The [³H]allethrin and the unlabeled reference standard had the same $R_f$ value when chromatographed together or separately in five different solvent systems.

The specific activity of the [³H]allethrin was determined by weighing a known amount and diluting with cyclohexane and counting an aliquot of the solution with a Packard Tri-Carb liquid scintillation spectrophotometer Model 3003. The instrument efficiency was measured by counting a [³H]toluene standard. The quench for the sample and solvent system was determined and the appropriate correction made to the calculations. The specific activity was found to be 61 mCi/mmol or 201 $\mu$Ci/mg.

## RESULTS

The preparation of allethrin labeled with tritium has been successfully completed. The material obtained by a catalytic exchange method

TABLE 1
CHROMATOGRAPHY OF ALLETHRIN ON SILICA GEL LAYERS

| Solvent systems | Ratio | $R_f$ value |
|---|---|---|
| Diethyl ether-hexane | 2:1 | 0.84 |
| Benzene-carbon tetrachloride | 4:1 | 0.78 |
| Hexane-ethyl acetate | 3:1 | 0.55 |
| Hexane-diethyl ether | 2:1 | 0.48 |
| Benzene-ethyl acetate | 17:3 | 0.43 |

(Moravek process) was about 50% pure with four radiolabeled breakdown products. The sample was purified on silica gel layers in approximately 20-mg portions, yielding 14 mg, 2.5 mCi, of [³H]allethrin. The labeled allethrin decomposed on silica gel in the light so the chromatography was performed in the dark. Analysis showed a chemical and radiochemical purity of 97 ± 1% m/m. The specific activity was determined to be 61 mCi/mmol, and its isomer ratio 1:4, E to Z.

## SUMMARY

Radioactive allethrin labeled with tritium has been synthesized because it was needed as a model compound in studies of interest. It was prepared by a catalytic exchange process producing a material that was approximately 50% pure containing several breakdown products. Portions of about 20 mg each were purified on silica gel layers yielding 14 mg of [³H]allethrin, specific activity 61 mCi/mmol. Its purity, chemical and radiochemical, as determined by proven methods was 97 ± 1% m/m.

## ACKNOWLEDGMENT

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# A New Kinetic Method for the Determination of Magnesium and Its Application to Natural Waters

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## INTRODUCTION

Determination of traces of magnesium is not usually performed by kinetic methods, there being various analytical techniques employed for this purpose: flame photometry, adsorption spectrophotometry in the presence of several dyes, polarography, atomic absorption spectrophotometry, as well as titrimetric methods for solutions of most concentrates.

Some kinetic procedures have been described. Besides the differential kinetic method of Pausch and Margerum (*11*), other methods proposed are based on the catalytic effect of magnesium in descarboxylation reactions (*4*) or on the measurement of the rate of crystallization in a precipitation reaction (*2*). Moreover, in recent years many kinetic methods of analysis based on catalytic rates modified by inhibition or activation have been developed for determining several species in solution (*5, 6*). Both aspects—inhibition and activation—have been applied to enzymatic as well as nonenzymatic catalysis. Thus, the activating effect of Mg(II) in some enzyme-catalyzed reactions (*1, 3, 7–10*) has been described—and applied in some cases to its determination.

The autoxidation of 1,4-dihydroxyphthalimide dithiosemicarbazone (OH-PDT) catalyzed by manganese(II) has been previously described and applied to kinetic determination of manganese(*12*) and as an indicator reaction of endpoint catalytic titration (*13, 14*). The magnesium ion produces an inhibitory effect on this catalyzed reaction, probably due to its interaction with the reagent. In this paper, we established that magnesium can be determined by a kinetic method which allows determination of as small a concentration as $3.29 \cdot 10^{-5}\ M$, even in the presence of calcium. The kinetic determination of magnesium based on an inhibitory effect on a nonenzymatic reaction is proposed here for first time.

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## MATERIALS AND METHODS

*Equipment*

All absorbance measurements were made with a Pye-Unicam SP 8000 spectrophotometer with a 1.0-cm glass cell and equipped with a thermostat for kinetic measurements.

*Chemicals and Solutions*

1,4-Dihydroxyphthalimide dithiosemicarbazone (OH-PDT) reagent solution (0.05%) in dimethylformamide(DMF) synthesized from 1,4-dihydroxyphthalimide dioxime and thiosemicarbazide (*12*), were used. Standard manganese solutions were prepared from manganese sulfate (ignited at 500°C), dissolved in hot dilute sulfuric acid, and standardized by titration with EDTA; the stock solutions (1000 g liter$^{-1}$) were diluted just before use. Standard magnesium solutions were prepared from magnesium carbonate and standardized by titration with EDTA; the stock solutions (0.600 g liter$^{-1}$) were diluted just before use.

All solvents and reagents were of analytical reagent grade.

*Procedure*

*Determination of magnesium.* The optimum operating conditions were chosen so that the rate of the catalyzed reaction was very large compared with that of the inhibited reaction. To a solution containing up to 13 $\mu$g of Mg(II) in a 10-ml volumetric flask, add 0.35 $\mu$g of Mn(II), 5 ml of 0.858 $M$ ammonia solution, and 1 ml of 0.05% reagent solution in DMF and dilute to 10 ml with bidistilled water (inhibited reaction). Prepare a similar solution containing no Mg(II) (catalyzed reaction). Transfer a portion of the reaction mixtures to a thermostated 1.0-cm cell at 33 $\pm$ 0.1°C and record the rate of change of absorbance at 594 nm with time of the sample containing the catalyzed reaction against that of the sample solution containing the inhibited reaction ($\Delta A$ per minute)$_{sample}$. Record also the rate of change of absorbance at 594 nm for the catalyzed reaction against a blank containing no Mn(II) ($\Delta A$ per minute)$_{blank}$. Begin the measurements 8 min after preparation of the samples. The percentage of inhibition is calculated

$$\% \text{ Inhibition} = \frac{(\Delta A/\text{min})_{sample}}{(\Delta A/\text{min})_{blank}} \times 100 \ .$$

The concentration of magnesium is determined from the calibration plot of percentage inhibition vs concentration of magnesium.

*Determination of magnesium in natural waters.* In a 10-ml volumetric flask pipet an aliquot of the sample (without previous dilution) containing between 8 and 13 $\mu$g of magnesium and follow the technique described above. The content of magnesium is determined from the calibration graph.

## RESULTS AND DISCUSSION

The aerial oxidation of the reagent (OH-PDT) in alkaline medium is very slow. In the presence of traces of Mn(II), the oxidation rate is increased and new absorption bands at 550 and 594 nm appear rapidly (red-violet color). The kinetics of this catalyzed reaction has been studied and has been applied to the determination of Mn(II) in the range $10-90$ ng ml$^{-1}$ (12). The mechanism has been also discussed and probably involves the oxidation of manganese(II) to manganese(III) and possibly manganese(IV), by atmospheric oxygen in alkaline medium. The Mn(III) serves as a mediator in the electron transfer: Mn(III) + OH-PDT$_{red}$ = Mn(II) + OH-PDT$_{ox}$. The role of magnesium(II) in the inhibited reaction has not been elucidated completely, but probably consists of interaction with the substrate, thereby stopping the cycle and causing partial inhibition of the catalytic effect of Mn(II).

### Inhibitory Effect of Magnesium(II)

Under the operating conditions and in absence of manganese(II), the magnesium ion interacts with the reagent, appearing as a new absorption band at 420 nm whose formation is very slow. In the presence of Mn(II)
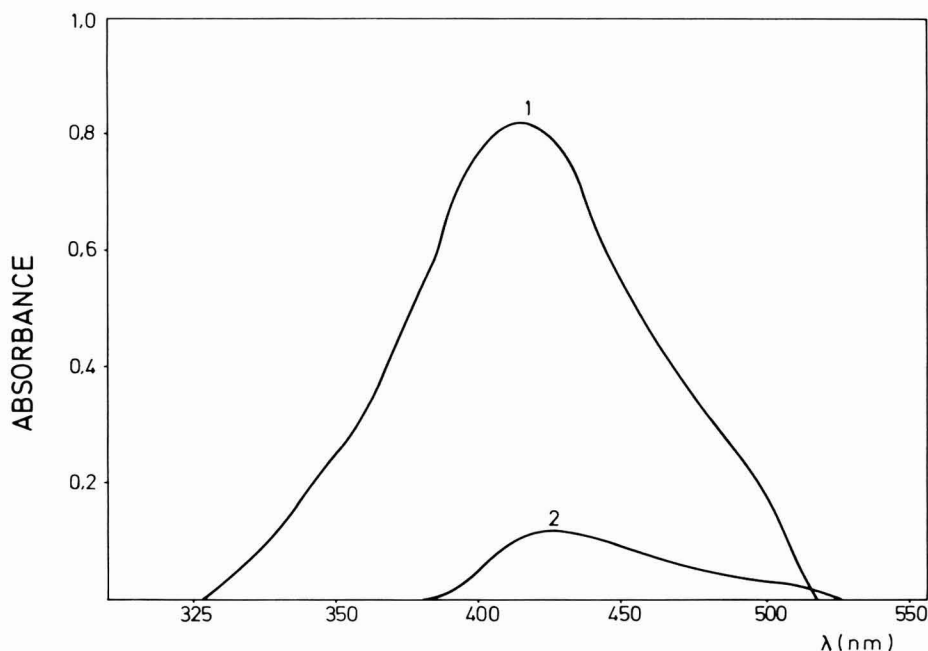


FIG. 1. Absorption spectra of the product of the reaction of OH-PDT with magnesium(II). Curve 1: in the presence of manganese; curve 2: without manganese; both against a similar sample containing no magnesium. Conditions: $6.17 \cdot 10^{-5}$ $M$ Mg(II), 35 ng Mn ml$^{-1}$, 0.429 $M$ ammonia, and $1.6.10^{-4}$ $M$ OH-PDT. The curves were recorded after 15 min.
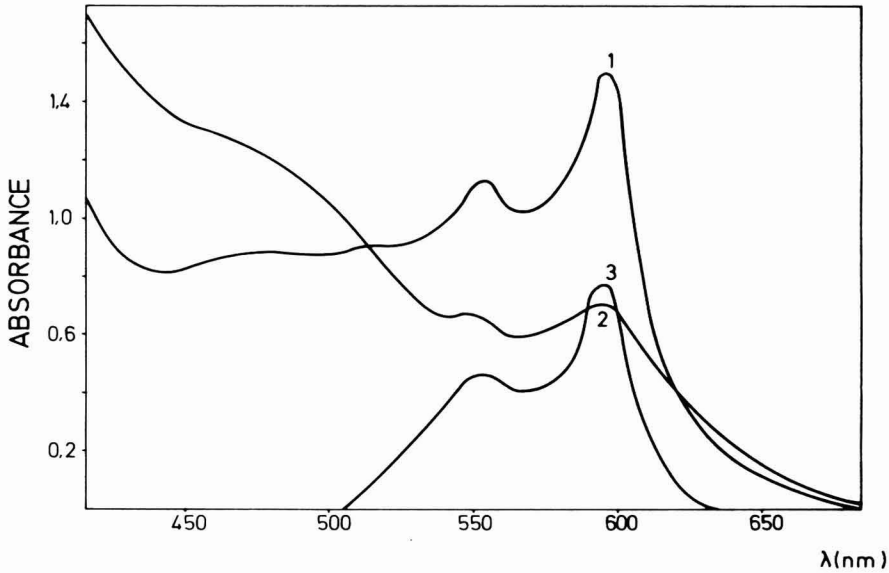
FIG. 2. Effect of magnesium(II) on the absorption spectra of the autoxidation product of OH-PDT. Curve 1: in the presence of Mn(II), without Mg(II); curve 2: with Mn(II) and Mg(II) (both 1 and 2 against a distilled water reference; curve 3: differential spectra. Conditions: $6.17 \cdot 10^{-5}$ $M$ Mg(II), 35 ng Mn ml$^{-1}$, 0.429 $M$ ammonia, and $1.6 \cdot 10^{-4}$ $M$ OH-PDT. The curves were recorded after 15 min.
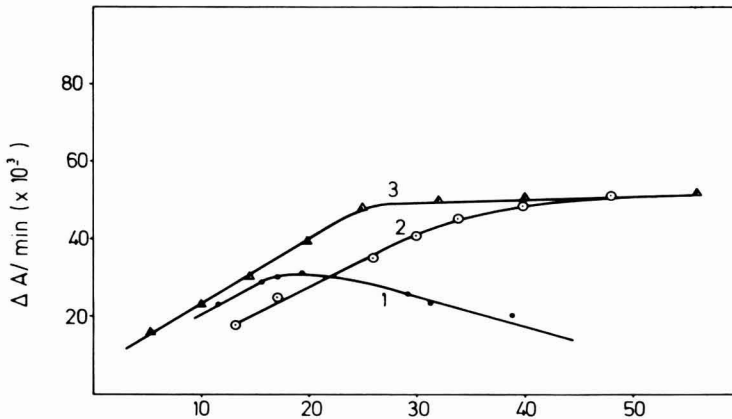


FIG. 3. Effect of OH-PDT concentration, $\times$ $10^{-5}$ $M$ (curve 1); ammonia concentration, $\times$ $10^{-2}$ $M$ (curve 2); and manganese concentration, ng ml$^{-1}$ (curve 3) on the reaction rate. Conditions for curve 1: $6.17 \cdot 10^{-5}$ $M$ Mg(II), 50 ng Mn ml$^{-1}$, and 0.214 $M$ ammonia; for curve 2: $6.17 \cdot 10^{-5}$ $M$ Mg(II), 50 ng Mn ml$^{-1}$, and $1.6 \cdot 10^{-4}$ $M$ Oh-PDT; for curve 3: $6.17 \cdot 10^{-5}$ $M$ Mg(II), 0.429 $M$ ammonia, and $1.6 \cdot 10^{-4}$ $M$ OH-PDT.

the rate of formation of this band is increased (Fig. 1), decreasing the absorptivity of the band at 594 nm. Therefore, the magnesium acts as an inhibitory agent in the manganese(II)-catalyzed aerial oxidation of OH-PDT (Fig. 2). The rate of change of absorbance at 594 nm for the sample containing the catalyzed reaction against a similar sample containing magnesium is proportional to the magnesium concentration.

*Effect of Reaction Variables*

During the course of this study, the temperature was maintained at 33°C, a value previously established for the catalyzed reaction.

The effect of reagent, ammonia, and manganese concentrations on the reaction rate is shown in Fig. 3. The effect of reagent concentration was studied in the range $1.1-3.9\cdot10^{-4}$ $M$. The absorbance–time curves of the samples prepared according to the experimental section show that the absorbances increase linearly during the first 15 min. The slopes of these curves during this time ($\Delta A$/min) depend linearly on reagent concentration
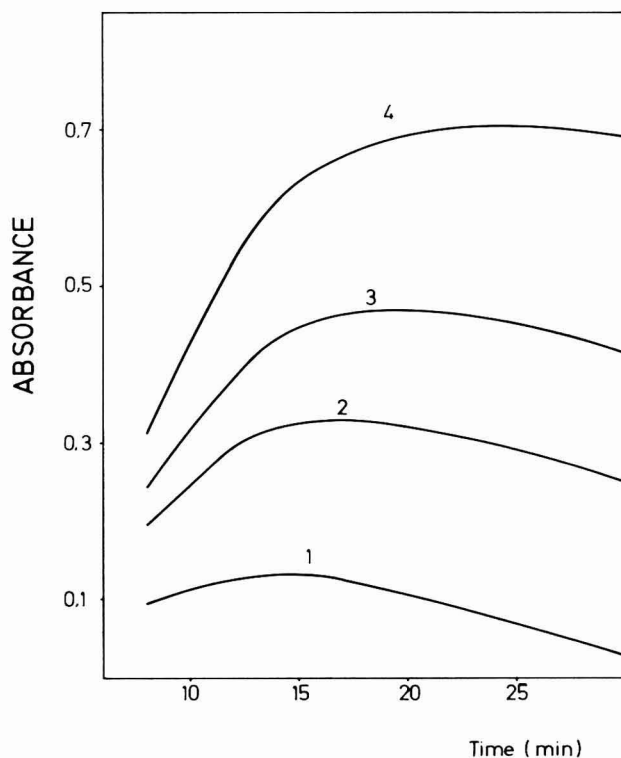


FIG. 4. Effect of magnesium concentration. Curves 1–4 correspond to 3.29, 4.11, 4.94, and $5.76\cdot10^{-5}$ $M$ Mg(II), respectively. Conditions: 35 ng Mn ml$^{-1}$, 0.429 $M$ ammonia, and $1.6\cdot10^{-4}$ $M$ OH-PDT.

up to $1.6-2.1 \cdot 10^{-4}$ $M$ ($1-1.3$ ml of a 0.05% solution), but the slope of the relationship then changes. A $1.6 \cdot 10^{-4}$ $M$ concentration of reagent was selected as the optimum value of maximum inhibition. The reaction rate also depends linearly on the ammonia concentration up to 0.350 $M$. A concentration of 0.429 $M$ was selected. The reaction is first order with respect to manganese(II) concentration in the range $2-25$ ppb. A concentration of 35 ng Mn ml$^{-1}$ was selected.

## Calibration Graph

The absorbance–time curves for solutions containing the catalyzed reaction were recorded against a similar solution containing different amounts of Mg(II) (Fig. 4). The $\Delta A$/min values were obtained from the straight portion of the curves (initial rates). The percentage inhibition was calculated as indicated in the experimental section and plotted against magnesium concentration. The calibration graph was linear in the concentration range $3.29-5.35 \cdot 10^{-5}$ M (Fig. 5). The relative standard deviation for $4.52 \cdot 10^{-5}$ $M$ was 1.2% ($n = 11$).

## Determination of Magnesium in Presence of Calcium and Transition Metals: Application to Natural Waters

The effect of calcium is shown in Table 1. As can be seen, the presence of calcium in an amount double that of the magnesium ion does not interfere with the determination of magnesium.

In order to assess possible analytical applications of the kinetic method, the influence of some transition metals was examined. The results are shown in Table 2. Small amounts of heavy metal ions which normally are
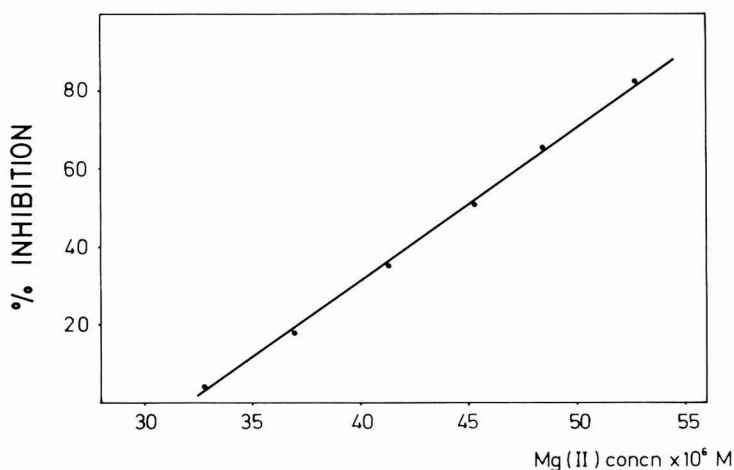
FIG. 5. Calibration graph for magnesium(II). Conditions: 35 ng Mn ml$^{-1}$, 0.429 $M$ ammonia, and $1.6 \cdot 10^{-4}$ $M$ OH-PDT.

TABLE 1
EFFECT OF CALCIUM ION[a]

| Ca(II) taken ($\times 10^5$ $M$) | Percentage inhibition | Mg(II) found ($\times 10^5$ $M$) |
|---|---|---|
| 0.00 | 50.0 | 4.53 |
| 0.62 | 47.9 | 4.48 |
| 1.24 | 54.1 | 4.62 |
| 2.49 | 48.9 | 4.50 |
| 4.99 | 49.1 | 4.51 |
| 7.48 | 45.8 | 4.42 |
| 9.98 | 43.7 | 4.38 |

[a] Conditions: $4.53 \cdot 10^{-5}$ $M$ magnesium(II), 35 ng Mn ml$^{-1}$, 0.429 $M$ ammonia, $1.6 \cdot 10^{-4}$ $M$ OH-PDT.

in natural waters, do not interfere in the determination of magnesium.

The results for the determination of magnesium in natural waters were checked by atomic absorption spectrometry and are shown in Table 3. The titrimetric results obtained by using the conventional metalchromic indicator were in agreement with both results in Table 3 but the volume of the sample should be larger than 100 ml if the sample has not been previously concentrated. In using the atomic absorption spectrometry method sample dilution is necessary. However, with the method proposed here volumes of 0.5−3 ml are sufficient.

## SUMMARY

A kinetic method is described for the determination of trace amounts of magnesium in the presence of calcium. The procedure is based on the inhibition of the manganese(II)-catalyzed aerial oxidation of 1,4-dihydroxyphthalimide dithiosemicarbazone reaction by

TABLE 2
EFFECT OF TRANSITION METALS[a]

| Transition metal | Concentration ($M$) | Percentage inhibition | Mg(II) found ($\times 10^5$ $M$) |
|---|---|---|---|
| Fe(II) | $3.6.10^{-5}$ | 54.1 | 4.62 |
| Fe(III) | $3.6.10^{-5}$ | 47.8 | 4.48 |
| Co(II) | $3.4.10^{-5}$ | 50.0 | 4.53 |
| Ni(II) | $3.4.10^{-5}$ | 50.0 | 4.53 |
| Cu(II) | $3.1.10^{-5}$ | 52.0 | 4.56 |
| Zn(II) | $3.0.10^{-5}$ | 54.1 | 4.62 |
| Cd(II) | $1.7.10^{-5}$ | 52.0 | 4.56 |
| Hg(II) | $9.9.10^{-6}$ | 45.8 | 4.44 |
| Sn(II) | $2.1.10^{-6}$ | 50.0 | 4.52 |
| Pb(II) | $1.2.10^{-6}$ | 54.1 | 4.62 |

[a] Conditions: $4.53.10^{-5}$ $M$ Mg(II), 35 ng Mn ml$^{-1}$, 0.429 $M$ ammonia, $1.6.10^{-4}$ $M$ OH-PDT.

TABLE 3
DETERMINATION OF MAGNESIUM IN NATURAL WATERS

| Natural water sample | Ca(II) present[a] (M) | Mg(II) found (M)[b] | |
|---|---|---|---|
| | | Kinetic method | Atomic absorption method |
| Commercial | $3.45 \cdot 10^{-4}$ | $1.65 \cdot 10^{-3}$ | $1.74 \cdot 10^{-3}$ |
| Commercial | $5.46 \cdot 10^{-4}$ | $1.57 \cdot 10^{-4}$ | $1.81 \cdot 10^{-4}$ |
| Untreated | $6.13 \cdot 10^{-4}$ | $2.16 \cdot 10^{-4}$ | $2.40 \cdot 10^{-4}$ |
| Treated | $4.95 \cdot 10^{-4}$ | $1.93 \cdot 10^{-4}$ | $2.17 \cdot 10^{-4}$ |

[a] EDTA titration less the magnesium.
[b] Average of three separate determinations.

traces of magnesium(II). The reaction is followed spectrophotometrically by measuring the rate of change in absorbance at 594 nm. The calibration graph (percentage inhibition vs magnesium concentration) is linear in the range $3.29 - 5.35 \cdot 10^{-5}$ M with an accuracy and precision of 1.2%. The method has been applied to the determination of magnesium in natural waters at low concentrations.

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# A Contribution to the Use of Tetravalent Manganese in Solutions of Sulfuric Acid as an Oxidimetric Reagent

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## INTRODUCTION

Among the compounds of tetravalent manganese, primarily manganese dioxide is used for oxidimetric determinations; the action of this substance, especially on organic compounds, has been discussed in reviews (*4, 8*). The reactivity of manganese dioxide depends, among other factors, on the method of preparation and the active surface area. Thus it would be interesting to determine the usefulness of manganese(IV) sulfate as an oxidimetric reagent. The preparation of this substance has been described and its analytical applications have been the subject of a preliminary communication (*5*).

In this work, 0.005–0.1 *M* solutions of manganese(IV) sulfate were prepared and their stability was studied, along with the determination of their titer using primary standards and the possibility of analytical applications in direct and indirect determinations.

## EXPERIMENTAL

*Reagents*

Solutions (0.005, 0.01, 0.05, and 0.1 *M*) of manganese(IV) sulfate were prepared (*5*) by dissolving 0.8, 1.58, 7.9, or 15.8 g of potassium permanganate with intense stirring for 20 hr in 9 *M* (or 8 *M*) sulfuric acid and diluting the solution with 9 *M* (8 *M*) sulfuric acid to 1000 ml. The conversion of heptavalent manganese to tetravalent was confirmed spectrophotometrically (*5*). The titer was determined daily by potentiometric titration with ferrous sulfate of appropriate molarity (*5*).

Solutions (0.005, 0.01, 0.05, and 0.1 *M*) of ferrous sulfate were prepared by dissolving 2.8, 5.6, 28.0, or 56.0 g of ferrous sulfate heptahydrate in 50 ml of 4 *M* sulfuric acid and diluting with water to 1000 ml. The titer was determined daily using dichromate (*7*).

A 0.005 *M* solution of oxalic acid, a 0.005 *M* solution of potassium iodide, and a 0.005 *M* solution of sodium hexacyanoferrate were prepared by dissolving the accurately weighed amounts of the solid substances in

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distilled water. More dilute solutions were prepared by accurate dilution of these solutions. Solutions (0.005 $M$) of arsenite and hydrazinium sulfate were prepared in the usual way.

Solutions (0.01 $M$) of hydroquinone, $p$-aminophenol, and metol were prepared by dissolving the accurately weighed substances in distilled water. (More dilute solutions were prepared by accurate dilution of these solutions.) The titer was checked cerimetrically (6) or by oxidation with compounds of trivalent manganese (1, 2).

## Apparatus

The potentiometric titrations were carried out using an Acidimetr AK millivoltmeter (Druopta, Prague) with a platinum foil indicator electrode and a saturated calomel reference electrode.

The constant temperature of the reaction mixture was maintained using a U10 Ultra-Thermostat (Mechanik Prüfgeräte Medingen, German Democratic Republic).

## Procedure

*Study of the stability of manganese(IV) sulfate solutions.* The titer of the solutions of manganese(IV) sulfate was determined 24 hr after preparation and followed for 21 days. Each individual value is the average of five measurements which did not differ by more than 1.2% rel. The stability of the solutions with various molarities of manganese(IV) ions was studied with reference to dependence on the concentration of sulfuric acid, on the temperature, and on the effect of light by potentiometric titration with a ferrous sulfate solution (5) of suitable molarity.

*Determination of the titer of manganese(IV) sulfate solutions with ferrous sulfate.* A volume of 10.00 ml of a solution of manganese(IV) sulfate in 9 ml of sulfuric acid was titrated potentiometrically with a standard solution of ferrous sulfate of suitable molarity. The titer of the ferrous sulfate was found daily using potassium bichromate (7).

*Determination of the titer of manganese(IV) sulfate solutions using potassium iodide or sodium hexacyanoferrate.* A volume of 30.00 ml of 0.002 $M$ potassium iodide or sodium hexacyanoferrate was titrated potentiometrically with a 0.005 $M$ manganese(IV) solution in 9 $M$ sulfuric acid.

*The titration determination of hydroquinone, p-aminophenol, and metol.* Into a beaker containing 50 ml of distilled water was measured 10.00 ml of a solution containing $1-6$ mg hydroquinone, $1.5-7$ mg $p$-aminophenol, or $2-9$ mg metol and the solution was titrated potentiometrically with a 0.005 $M$ solution of manganese(IV) sulfate.

*Indirect determination of oxalic acid.* A volume of 10.00 ml of a solution containing $3-6$ mg oxalic acid was mixed with about a twofold excess of manganese(IV) sulfate, resulting in complete oxidation of oxalic acid to carbon dioxide and water. The unreacted manganese(IV) sulfate was de-

termined by potentiometric titration with a 0.01 $M$ solution of ferrous sulfate after 2−240 min.

## RESULTS AND DISCUSSION

### Determination of the Titer of Tetravalent Manganese Ions in 9 M Sulfuric Acid with Ferrous Sulfate

Compounds of divalent iron have been titrated by solutions of tetravalent manganese using ferroin as indicator (5). With reverse potentiometric titration with 0.1−0.05 $M$ solutions of manganese(IV) sulfate the potential of the indicator platinum electrode stabilizes instantaneously; the potential of the inflection point lies in the region around 900 mV (vs SCE), the potential break at the equivalence point is 400 mV for 0.03 ml of 0.01 $M$ ferrous sulfate.

### Study of the Stability of Tetravalent Manganese Ions

The measurements (3) confirmed that, in 9 $M$ sulfuric acid, solutions of manganese(IV) sulfate are relatively stable at laboratory temperature, but that the concentration of 9 $M$ sulfuric acid must be maintained, not only for rapid preparation (5), but also for greater stability (3). This is especially important with solutions of lower molarity. For example, the titer of a 0.005 $M$ solution of the reagent in 9 $M$ sulfuric acid decreased by 2.6% rel. in 21 days, while in 8 $M$ sulfuric acid it decreased by 37% rel. after the same time. Reagent solutions of higher molarity are more stable; the molarity of a 0.05 $M$ reagent solution in 9 $M$ sulfuric acid decreased by 1.5% rel. after 21 days, in 8 $M$ sulfuric acid, by 3.5% rel.

In 9 $M$ sulfuric acid the solutions are relatively stable up to 40°C; the titer decreased by 1.5% rel. after 4 hr at this temperature, which can be compensated for by carrying out a blank determination.

Analytical use of this reagent above 40°C cannot be recommended. The change in molarity caused by daylight is negligible.

### Determination of the Titer of Manganese(IV) Sulfate solutions with iodide ions

Potentiometric titration of potassium iodide, useful for determining the titer of 0.005 $M$ solutions, was carried out with good results in media in which the concentration of sulfuric acid around the equivalence point was 1.5−3 $M$ (Table 1). In media containing more concentrated sulfuric acid, equivalence is attained at a reagent consumption lower than the theoretical value, apparently as a result of oxidation of a small amount of iodide to iodine in the medium. In media with 1.5−3 $M$ sulfuric acid the potential break around the equivalence point was about 100 mV per 0.03 ml of 0.005 $M$ reagent solution; the potential of the inflection point was around 500 mV (vs SCE). The potential stabilizes in the vicinity of the equivalence point after 2−4 min.

TABLE 1
DIRECT POTENTIOMETRIC TITRATION OF 0.005 $M$ KI WITH A
0.005 $M$ SOLUTION OF Mn(SO$_4$)$_2$

| KI added (mg) | Reagent consumption (ml) | | SD (ml) | H$_2$SO$_4$ Concn. ($M$) |
|---|---|---|---|---|
| | Theor. | Actual | | |
| 8.30 | 5.00 | 5.01 | 0.020 | 1.5 |
| 16.60 | 10.00 | 9.98 | 0.023 | |
| 8.30 | 5.00 | 5.02 | 0.020 | 3 |
| 16.60 | 10.00 | 10.01 | 0.012 | |

[a] Each value is the average of seven measurements, from which the standard deviation was calculated.

## Determination of the Titer of Manganese(IV) Sulfate Using Hexacyanoferrate Ions

Potentiometric titration of sodium hexacyanoferrate is also useful for determining the titer of 0.005 $M$ reagent solutions, with about 1.5 $M$ sulfuric acid concentrations around the equivalence point (Table 2). The potential break around the equivalence point is about 200 mV per 0.03 ml reagent; the potential of the inflection point is about 700 mV (vs SCE). The potential stabilization around the equivalence point takes about 5 min.

Satisfactory results were not obtained in the study of the oxidation of arsenite and hydrazinium sulfate, which were also considered as possible primary standards.

## Study of the Analytical Application of Manganese(IV) Sulfate

Hydroquinone, $p$-aminophenol, and metol were used as model substances in the direct potentiometric titration. The results are summarized in Table 3. Because of a deeper nonstoichiometric oxidation of these substances at higher concentrations of sulfuric acid, the determination was carried out in media with acidity below 1.5 $M$ sulfuric acid. Under

TABLE 2
DIRECT POTENTIOMETRIC TITRATION OF 0.005 $M$ Na$_4$(Fe(CN)$_6$) WITH A 0.005 $M$ SOLUTION
OF Mn(SO$_4$)$_2$ IN 1.5 $M$ H$_2$SO$_4$ MEDIUM

| Na$_4$(Fe(CN)$_6$) added (mg) | Reagent consumption (ml) | | SD (ml) |
|---|---|---|---|
| | Theor. | Actual[a] | |
| 24.20 | 5.00 | 5.00 | 0.021 |
| 48.40 | 10.00 | 10.01 | 0.021 |

[a] Each value is the average of seven measurements, from which the standard deviation was calculated.

| Added (mg) | Found (mg) | SD (mg) |
|---|---|---|
| *p*-Aminophenol | | |
| 1.456 | 1.438 | 0.025 |
| 3.640 | 3.657 | 0.024 |
| 7.280 | 7.315 | 0.023 |
| Metol | | |
| 1.732 | 1.706 | 0.025 |
| 4.330 | 4.330 | 0.034 |
| 8.660 | 8.700 | 0.028 |
| Hydroquinone | | |
| 1.101 | 1.090 | 0.017 |
| 2.750 | 2.774 | 0.016 |
| 5.500 | 5.550 | 0.019 |

these conditions a two-electron exchange occurs; the potential value of the indicator electrode remains stable for 10 min after attaining equivalence. The potential around the equivalence point stabilizes during 2 min.; its change at equivalence is about 150 mV per 0.03 ml of 0.005 $M$ manganese(IV) sulfate solution. The potential of the inflection point for all three substances is about 600 mV (vs SCE). A volume of 1.00 ml of 0.005 $M$ manganese(IV) sulfate solution corresponds to 0.505 mg hydroquinone, 0.725 mg *p*-aminophenol, or 0.865 mg metol.

The possibility of determining low concentrations of substances by the action of excess reagent and back-titration was demonstrated by quantitative determination of oxalic acid in 9 and 1.5 $M$ sulfuric acid at laboratory temperature. Theoretical results were obtained in all cases, corresponding to quantitative oxidation to carbon dioxide and water.

It follows from the results that the presence of manganese(IV) sulfate in sulfuric acid medium enables determination of low concentrations of organic substances. Solutions with lower reagent molarity can be prepared in relatively stable form, but their stability depends on the presence of sulfuric acid, the high concentration of which need not always satisfy the conditions for the stoichiometric course of the studied redox reactions. Thus the samples of *p*-aminophenol, hydroquinone, and metol were adjusted by addition of distilled water before the determination.

## SUMMARY

The preparation of solutions of manganese(IV) sulfate in 9 $M$ sulfuric acid as well as the stability of these solutions was studied for $0.005-0.1$ $M$ concentration of manganese(IV) ions. Potentiometric titration of solutions of primary standard substances, potassium iodide

and sodium hexacyanoferrate, was recommended for determining the titer of 0.005 $M$ reagent solutions. It was verified that manganese(IV) sulfate can be used for determining low concentrations of organic substances by direct titration determination of hydroquinone, $p$-aminophenol, and metol and by indirect determination of oxalic acid.

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# Spectrophotometric Method for the Determination of Microgram Amounts of Titanium Using an Extraction Technique with Diphenylglyoxal bis(2-Hydroxybenzoylhydrazone)

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## INTRODUCTION

Bis(aroylhydrazones) have been little used as analytical reagents. Lever (4) has reported photometric and fluorimetric methods for the determination of calcium(II), cadmium(II), lanthanum(III), and bismuth(III) with bis(4-hydroxybenzoylhydrazone) of glyoxal, methylglyoxal, and dimethylglyoxal. Monoaroylhydrazones have been recently investigated by us as analytical reagents for the determination of nickel(II) and zinc(II) (1), iron(II) (3), and vanadium(V) (2).

In the present work the bis(2-hydroxybenzoylhydrazone) of diphenylglyoxal (BSHB) has been evaluated as a reagent for the spectrophotometric determination of titanium(IV). This reagent has been employed by us for the colorimetric determination of calcium(II) (7), indium(III) (5), and tin(II) (6).

## MATERIALS AND METHODS

### Apparatus

A Perkin–Elmer 402 spectrophotometer was used for recording spectra in the ultraviolet and visible regions of the spectrum and an SP6-500 (digital) spectrophotometer was used for measurements at fixed wavelengths. A Radiometer PHM 62 pH meter, with glass–calomel electrodes, was used for pH measurements.

### Reagents

All solutions were prepared with analytical reagent grade chemicals using distilled water.

*Diphenylglyoxal bis(2-hydroxybenzoylhydrazone) reagent solutions.* A 0.1% (m/v) solution was prepared in dimethylformamide (DMF). A 0.1% (m/v) reagent solution in benzyl alcohol was prepared by dissolving 0.1 g

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of reagent in 3 ml of dimethylformamide and diluting to 100 ml with benzyl alcohol. These solutions were stable for at least 1 week (7).

*Standard titanium(IV) solution: 1.233 g liter⁻¹.* The appropriate amounts of $TiO_2$ and potassium hydrogen sulfate were added in a platinum crucible and the mixture was fused. After total fusion the residue was extracted with sulfuric acid (1 + 4) and was diluted to 1 liter with distilled water. This solution was standardized gravimetrically with Cupferron.

## Procedure

*Direct photometry.* The sample solution must contain between 12.5 and 62.5 µg of titanium in a 25-ml volumetric flask. Add 5.0 ml of the BSHB solution in DMF and 10 ml of dimethylformamide. Adjust the pH to about 3.5 to 4.5 with diluted sulfuric acid and dilute with distilled water to the mark. Measure the absorbance of the colored complex at 460 nm against distilled water.

*With extraction.* Take an aliquot of titanium(IV) solution (5−25 µg) in a 100-ml separatory funnel and then add 5.0 ml of 0.1 N sulfuric acid, 2 g of sodium perchlorate, and 10 ml of reagent solution in benzyl alcohol. Shake the contents for 1.5 min and allow the phases to separate. Tap off the organic phase and dry over anhydrous sulfate. Measure the absorbance of this solution at 500 nm against distilled water. Prepare a calibration plot with known amounts of titanium(IV).

## Other Experimental Procedures

Details for suitable experimental procedures for the analysis of several samples follow. The iron present has been eliminated from a determine volume of the dissolved sample in hydrochloric acid (1 + 1) medium by extraction with ethylic ether.

*Special steel.* Add a quantity of steel (2.0096 g), 32 ml of water, and 8 ml of concentrated sulfuric acid in a glass container. Warm the mixture gently until complete disolution. Cool, add 6 ml of concentrated nitric acid, and continue heating until the nitrous vapors disappear. Cool and dilute to 100 ml in a volumetric flask with distilled water.

*Nickel alloy.* Add an exactly weighed amount of alloy (1.9682 g) and 25 ml of regia aqua in a glass container. Warm gently to complete disolution. Cool and dilute to 100 ml in a volumetric flask with distilled water.

*Bauxite.* Dry the sample at 100 °C and weigh an amount (0.7511 g) into a 250-ml Kjeldahl flask. Add 5 ml of concentrated hydrochloric acid, 5 ml of concentrated nitric acid, and 5 ml of concentrated sulfuric acid; mix, and heat on a water bath for 60−90 min. Continue the heating for 20 min after the appearance of sulfuric acid fumes. Cool and add 50 ml of distilled water and 2.5 ml of concentrated hydrochloric acid to the residue, and heat the mixture on a water bath for 30 min. Filter the hot mixture into a 250-ml volumetric flask. Fuse the residue with about 0.5 g of potassium

pyrosulfate, dissolve the smelt in a small volume of water and hydrochloric acid, and add this to the filtrate in the 250-ml volumetric flask. Cool and dilute the filtrate exactly to 250 ml with distilled water.

*Portland cement.* Into a glass container add a known amount of cement (2.3202 g), 50 ml of water, and 50 ml of concentrated hydrochloric acid. Place the glass on a sand bath until complete disolution and evaporate to dryness at 100−105°C. Cool and add 25 ml of concentrated hydrochloric acid and the same volume of distilled water. Digest 10 min, dilute with 50 ml of hot water, and filter quickly. Wash the precipitate of silica with hydrochloric acid (1 + 100). Concentrate on a sand bath to approximately 50 ml, cool, and dilute to 100 ml with distilled water in a volumetric flask.

## RESULTS AND DISCUSSION

### Study of the Titanium(IV)−BSHB Complex in Solution

*Absorption Spectrum Stability*

Diphenylglyoxal bis(2-hydrobenzoylhydrazone) in weak acid medium reacts with titanium(IV) to produce an orange-yellow complex. The absorption spectrum of the Ti(IV)−BSHB complex appears in Fig. 1. This chelate presents two absorption maximums, one at 460 nm and other at 375 nm. At 460 nm the orange-yellow complex shows several advantages in contrast with the other wavelength: major molar absorptivity and possibility to realize the photometric measurements against distilled water.

The presence of at least 60% (v/v) dimethylformamide is necessary in
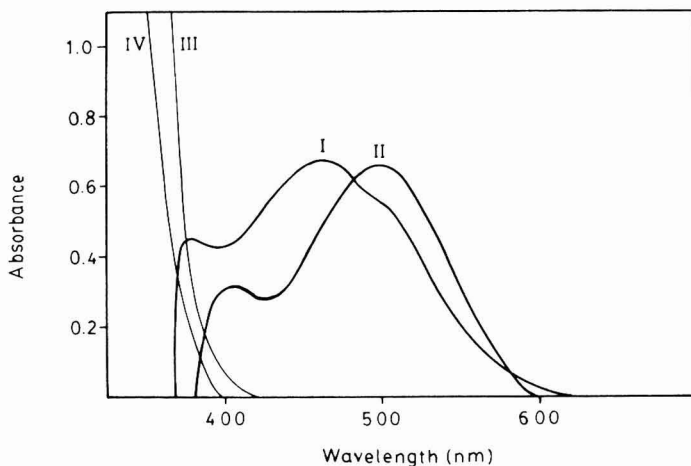


FIG. 1. Absorption spectra of Ti(IV)−BSHB complex. Concentration of Ti(IV), 4.17 × $10^{-5} M$; concentration of reagent, 4.18 × $10^{-4} M$ in aqueous dimethylformamide medium and 2 × $10^{-3} M$ in benzyl alcohol. (I) Orange-yellow complex in aqueous dimethylformamide medium at pH 4.0; (II) amber complex extracted into benzyl alcohol at pH 1.0; (III and IV) reagent blanks against water.

the reaction medium to prevent the precipitation of the excess of reagent and the titanium complex. The complex is formed quickly and its absorbance at 460 nm remains stable for at least 6 hr.

## Effect of pH

The influence of pH on the reaction was studied in the range 1.5–10. The optimum pH range for formation of the titanium complex is 3.5–6.5 (Fig. 2).

## Nature of the Complex

The stoichiometry of the chelate was determined by Job's method and the molar ratio method at both wavelengths where this complex presents the two absorption maximums. In both cases the stoichiometric ratio 1:3 for metal: ligand is found (Fig. 3).

The electrical charge of the complex in solution was studied by means of ion-exchange resins. At pH 4.0 the complex was retained by a cation-exchange resin (Dowex 50-X8, sodium form) and was completely separated from an anion-exchange resin, (Dowex 1-X8, chloride form); therefore, under these experimental conditions, the complex offered a global positive charge.

## Extraction of the Colored Complex

In order to try to eliminate some of the disadvantages of the photometric technique in aqueous dimethylformamide medium (elevated percentage of dimethylformamide and large number of interferences) the complex Ti(IV)–BSHB was extracted.
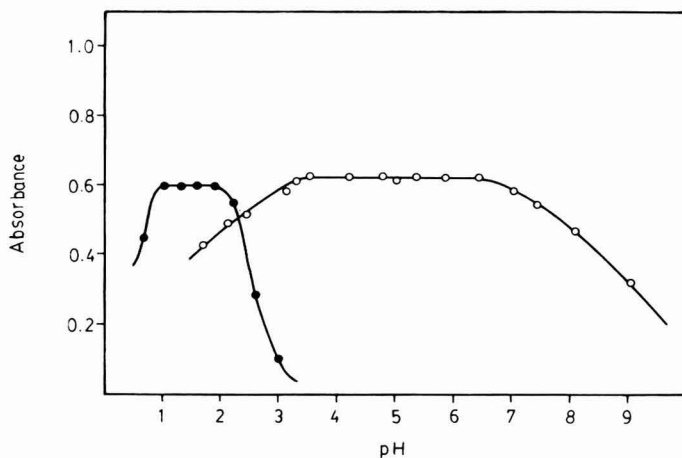


FIG. 2. Influence of pH on the formation of Ti(IV)–BSHB complex. Concentration of Ti(IV), $4.17 \times 10^{-5}$ M. (○) In aqueous dimethylformamide solution ($\lambda = 460$ nm), and (●) extracted into benzyl alcohol ($\lambda = 500$ nm).
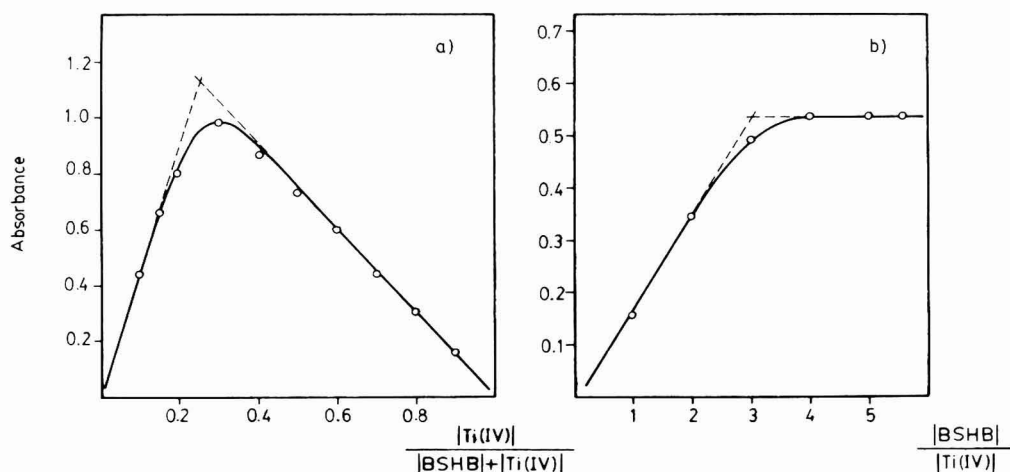
FIG. 3. Composition of Ti(IV)−BSHB chelate in solution at pH 4.0 Concentration of titanium, $1.2 \times 10^{-3} \, M$ ($\lambda = 460$ nm). (a) Continuous variations method; and (b) molar ratio method.

Only when oxygenated organic solvents such as methylisobutyl ketone, benzyl alcohol, butylic alcohol, cyclohexanol, and so on, were used was the extraction of the complex quantitative. The presence of sodium perchlorate as salting-out agent was necessary for complete development of color in the organic phase according to the global positive charge of the complex. Benzyl alcohol gave better sensitivity and in this solvent a bathochromic shift of the absorption maximum was produced ($\lambda_{max} = 500$ nm) with respect to the homogeneous medium ($\lambda_{max} = 460$ nm).

This complex was completely extracted between pH 1.0 and 2.0 (Fig. 2) and the optimum pH value was adjusted with 0.1 $N$ sulfuric acid solution. The absorbance of the complex solutions remains constant for at least 24 hr under these experimental conditions.

## Photometric Determination of Titanium with BSHB

### Direct Spectrophotometry

Based on the experimental procedure described above, the calibration graph was found to obey Beer's law in the range 0.5−2.5 ppm of titanium(IV). The molar absorptivity of the reaction was calculated to be $(1.66 \pm 0.02) \times 10^4 \, M^{-1} \, cm^{-1}$ at 460 nm. To determine the precision of the proposed method the same amount of titanium (1.5 ppm) was determined in 11 samples. The relative error ($P = 0.05$) of the method is ±0.53%.

### Extraction Spectrophotometry

*Effect of reagent concentration, salting-out agent, and shaking time.* A 0.002 $M$ reagent solution was adequate for complete extraction of titanium from pH 1.0 solutions. The absorbance of the complex was also studied as

a function of the amount of salting-out agent and the shaking time. Experimental data gave the following results: at least 2.0 g of sodium perchlorate and 1.5 min of equilibration are necessary for the maximum development of the extraction.

*Characteristics of the method.* Under the optimal experimental conditions for the formation of the titanium(IV) complex in benzyl alcohol, Beer's law is obeyed between 0.5 and 2.5 $\mu$g ml$^{-1}$ of titanium and the molar absorptivity at 500 nm is $(1.55 \pm 0.02) \times 10^4 M^{-1}$ cm$^{-1}$. The sensitivity of the method, according to Sandell, is 0.0031 $\mu$g cm$^{-2}$. The optimum concentration range, evaluated by Ringbom's method, is 1.0–2.25 ppm of titanium. The measurement of 11 individual samples each containing 2.0 ppm of titanium, gave a relative error ($P = 0.05$) of $\pm0.64\%$.

*Interferences.* The selectivity of the proposed method was investigated by determination of 20 $\mu$g/10 ml of titanium in the presence of a series of other ions. The results are shown in Table 1. Some masking agents (thioglycollic acid, ascorbic acid, thiosulfate, tartrate) were used to eliminate the more serious interferences. Two parts per million of titanium can be determined in the presence of large concentrations of silver(I), mercury(II), chrome(III), cobalt(II), nickel(II), zinc(II), aluminum(III), calcium(II), copper(II), cadmium(II), bismuth(III), sulfate, chloride, nitrate, and so on.

TABLE 1
INTERFERENCE OF FOREIGN IONS IN THE DETERMINATION OF 2.0 ppm OF TITANIUM

| Tolerance limit ($\mu$g/10 ml) | Ion added |
|---|---|
| 10,000 | Ag(I),[a] Hg(II),[a] Tl(I),[a] Al(III), Cr(III), Co(II), Ni(II), Zn(II), Ca(II), Mg(II), Sr(II), Ba(II), Rb(I), NH$_4$(I), Na(I), K(I), ClO$_4^-$, SO$_4^{2-}$, NO$_3^-$, Cl$^-$, CO$_3^{2-}$ |
| 5,000 | Pb(II),[a] Th(IV), Be(II), AsO$_2^-$ |
| 2,500 | Bi(III),[a] Cu(II),[a] Cd(II),[a] Li(I), La(III), B$_4$O$_7^{2-}$, S$_2$O$_3^{2-}$, SCN$^-$, S$_2$O$_8^{2-}$, I$^-$ |
| 1,000 | Tartrate, citrate, IO$_3^-$, BrO$_3^-$ |
| 500 | Sb(III), V(V),[b] Sn(II),[a] PO$_4^{3-}$ |
| 250 | Pd(II),[c] Mn(II), Se(IV), Fe(III),[b] oxalate |
| 50 | Mo(VI),[b] W(VI), In(III)[a] |
| 20 | Zr(IV)[d] |

[a] Thioglycollic acid: 2000 ppm.
[b] Ascorbic acid: 1500 ppm.
[c] Thiosulfate: 250 ppm.
[d] Tartrate: 100 ppm.

TABLE 2

DETERMINATION OF TITANIUM IN ALLOYS AND MINERAL SAMPLES

| | Titanium (%) | |
|---|---|---|
| Sample | Reported value | Found[a] |
| Special steel (BCS, No. 335) | 0.46 | 0.45 ± 0.01 |
| Nimonic 90 alloy (BCS, No. 310/1) | 2.43 | 2.40 ± 0.01 |
| Bauxite (BAS, No. 87) | 2.25[b] | 2.26 ± 0.01[b] |
| Portland cement (BCS, No. 372) | 0.33[b] | 0.34 ± 0.01[b] |

[a] Average of five separate determinations.
[b] Percentage as $TiO_2$.

*Determination of titanium in standard samples.* This method has been satisfactorily applied to determination of titanium in some standard samples: special steel, nickel alloy, bauxite, and Portland cement. In Table 2 are shown the results obtained for each analyzed sample.

## SUMMARY

Diphenylglyoxal bis(2-hydroxybenzoylhydrazone) forms an orange-yellow complex with titanium(IV) in weakly acid solution. This complex can be extracted into benzyl alcohol in 0.1 $N$ sulfuric acid medium. In this solvent the extracted complex exhibits an absorption maximum at 500 nm with a molar absorptivity of $1.5 \times 10^4 \, M^{-1} \, cm^{-1}$. Job's method and the molar ratio method indicate a 1:3 stoichiometric ratio. The interferences have been investigated and the reagent has been used successfully for the determination of titanium in minerals and alloys.

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# The Detection of Potassium Sulfate in Excess of Potassium Pyrosulfate

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## INTRODUCTION

In aqueous solutions, the detection of sulfate in the presence of pyrosulfate is not possible because of the rapid hydrolysis of pyrosulfate (2) according to the equation

$$S_2O_7^{2-} + H_2O \rightarrow 2SO_4^{2-} + 2H^+(aq).$$
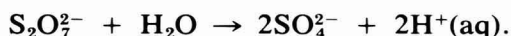
It is, therefore, necessary to carry out the qualitative analysis of sulfate in the presence of pyrosulfate on solid samples.

X-Ray diffraction patterns have been used for the identification of pure compounds as well as a mixture of compounds (1). Consequently, the X-ray diffraction method was investigated for the detection of $K_2SO_4$ in excess of $K_2S_2O_7$. As sulfate and pyrosulfate exhibit several infrared and Raman active vibrations (5), these vibrational spectroscopic methods were also investigated for the purpose of detection of $K_2SO_4$ in $K_2S_2O_7$.

## EXPERIMENTAL METHODS

*Chemicals.* Anhydrous $K_2SO_4$ (BDH Analar, 99.5%) was dried in an oven at 110°C. $K_2S_2O_7$ (E. Merck analytical reagent grade) and KBr (E. Merck spectroscopic grade) were dried as previously described (3, 4).

*Procedure.* Four standard solutions with concentrations of $1.035 \times 10^{-2}$, $5.130 \times 10^{-2}$, $18.310 \times 10^{-2}$, and $34.800 \times 10^{-2}\,m$ in $K_2SO_4$ in $K_2S_2O_7$ were prepared by fusing their weighed amounts in a tube furnace at 450°C. The resultant clear melts were then frozen and ground to a fine powder under a dry nitrogen atmosphere.

The X-ray diffraction measurements were carried out on a Siemens counter-tube goniometer using nickel-filtered $CuK\alpha$ radiation.

The infrared spectra of the pressed disks containing $1-1.5$ mg of the solid solutions and 200 mg of KBr were measured on a Perkin–Elmer 225 grating infrared spectrophotometer.

For the raman spectroscopy, the samples were packed under anhydrous conditions into melting point tubes to a height of 1 cm, sealed with

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plasticine, and mounted in the Spex Ramalog laser raman spec-trophotometer, and the spectra were measured at 800 mW from 200 to 13 $cm^{-1}$.

## RESULTS AND DISCUSSION

The $d$-values and the relative intensities of $K_2SO_4$, $K_2S_2O_7$, and the standard solutions of $K_2SO_4$ in $K_2S_2O_7$ are shown in Table 1. The $d$-values of most of the lines in the $K_2SO_4$ diffraction pattern were found to be the same as, or close to, those in the diffraction pattern of $K_2S_2O_7$, and hence could not be used for the identification of $K_2SO_4$ in solid solutions. The $d$-value of the $K_2SO_4$ line at 2.90 Å (it is different from any $K_2S_2O_7$ line) and its high intensity suggested that it might be a value diagnostic line, especially if present in the diffraction pattern of the solid solutions. How-

TABLE 1

X-Ray Diffraction Patterns of $K_2SO_4$ (1), $K_2S_2O_7$ (2), and the Solutions of $K_2SO_4$ in $K_2S_2O_7$ (3–6)

| | | $d$-Value in Å ($I$) | | | |
|---|---|---|---|---|---|
| (1) | (2) | (3) | (4) | (5) | (6) |
| — | 6.30(7) | 6.30(7) | 6.30(7) | 6.30(7) | 6.30(7) |
| — | 4.84(11) | 4.79(16) | 4.79(16) | 4.79(16) | 4.79(16) |
| — | 4.48(5) | 4.41(16) | 4.41(6) | 4.41(6) | 4.41(6) |
| — | 4.23(5) | 4.19(12) | 4.23(5) | 4.23(12) | 4.23(12) |
| 4.11(19) | 4.11(8) | 4.11(8) | 4.11(7) | 4.11(6) | 4.11(6) |
| — | 3.83(100) | 3.78(65) | 3.83(77) | 3.78(67) | 3.83(14) |
| — | 3.48(33) | 3.56(30) | 3.56(30) | 3.56(35) | 3.59(52) |
| 3.37(4) | 3.37(58) | 3.40(20) | 3.40(36) | 3.37(29) | 3.45(11) |
| — | 3.23(80) | 3.24(100) | 3.23(100) | 3.24(100) | 3.24(100) |
| 3.16(3) | 3.12(11) | 3.13(10) | 3.01(49) | 3.01(56) | 3.19(94) |
| 3.02(4) | 3.01(62) | 2.98(45) | 3.01(55) | 3.02(51) | 3.05(94) |
| 3.00(4) | 2.83(9) | 2.85(6) | 2.87(13) | 2.88(9) | 2.88(36) |
| 2.90(100) | 2.73(12) | 2.74(8) | 2.74(12) | 2.71(11) | 2.74(10) |
| 2.66(2) | 2.66(8) | 2.63(6) | 2.63(6) | 2.63(6) | 2.60(13) |
| 2.50(4) | 2.56(9) | — | — | — | — |
| 2.49(4) | 2.45(5) | 2.45(6) | 2.45(6) | 2.46(5) | 2.48(8) |
| — | 2.40(8) | 2.40(6) | 2.40(5) | 2.40(5) | 2.40(3) |
| — | 2.35(19) | 2.35(24) | 2.37(20) | 2.35(29) | 2.34(19) |
| — | 2.32(5) | — | — | — | — |
| — | 2.26(19) | 2.25(6) | 2.29(16) | 2.25(21) | 2.25(13) |
| 2.22(6) | 2.18(3) | 2.20(6) | 2.19(10) | 2.18(11) | 2.20(9) |
| 2.07(4) | 2.10(9) | 2.10(10) | 2.10(10) | 2.10(11) | 2.09(9) |
| 2.06(7) | 2.01(12) | 2.00(6) | 2.01(12) | 2.00(17) | 2.00(10) |
| — | 1.92(14) | 1.93(10) | 1.93(14) | 1.93(12) | 1.93(28) |
| 1.88(4) | 1.89(5) | 1.89(8) | 1.89(8) | 1.90(30) | 1.90(30) |
| — | 1.85(6) | 1.85(5) | 1.85(5) | 1.85(5) | 1.85(6) |
| 1.7(2) | 1.76(8) | — | — | — | — |

ever, this line was absent in the diffraction pattern of even the most concentrated solid solution used, i.e., $34.8 \times 10^{-2}$ $m$ in $K_2SO_4$ and could not, therefore, be used for the detection of $K_2SO_4$ by the X-ray diffraction technique.

The infrared spectra of $K_2S_2O_7$ and $K_2SO_4$ and that of a typical solution of $K_2SO_4$ in $K_2S_2O_7$ are shown in Fig. 1. The absorption bands of $K_2SO_4$ were observed to correspond to regions of strong absorption of $K_2S_2O_7$. The spectra of $K_2S_2O_7$ and those of the solutions of $K_2SO_4$ in $K_2S_2O_7$ were thus found to be more or less similar. The infrared spectroscopy could, therefore, not be used for the detection of $K_2SO_4$ in $K_2S_2O_7$.

The raman spectra of $K_2S_2O_7$, $K_2SO_4$, and two solutions of $K_2SO_4$ in $K_2S_2O_7$ in the interested region are shown in Fig. 2. A strong and narrow band at 981 cm$^{-1}$ in the $K_2SO_4$ spectrum was absent in the $K_2S_2O_7$ spectrum and was found to be present in the spectra of the solutions of $K_2SO_4$
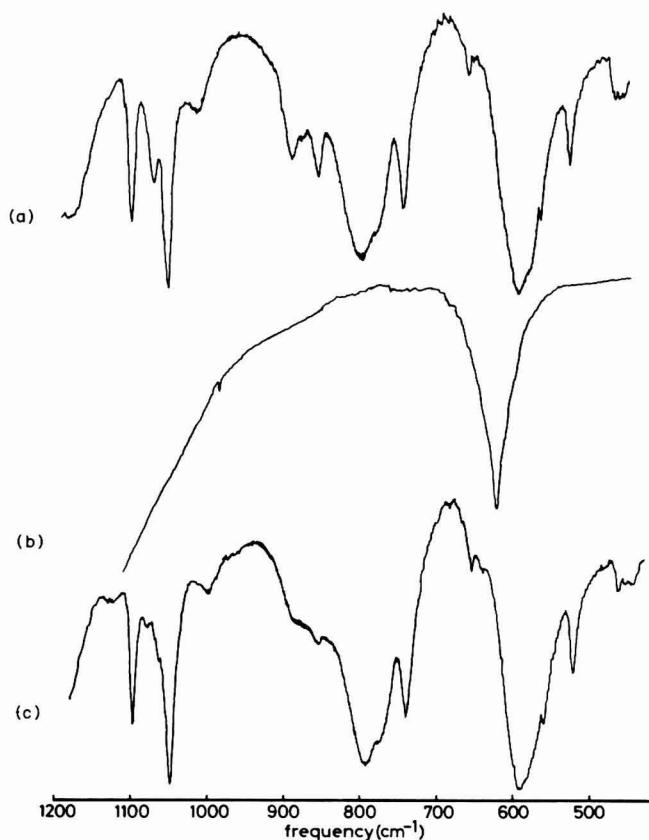


FIG. 1. Infrared spectra of (a) $K_2S_2O_7$, (b) $K_2SO_4$, and (c) a typical solution of $K_2SO_4$ in $K_2S_2O_7$ ($5.13 \times 10^{-2}$ $m$).
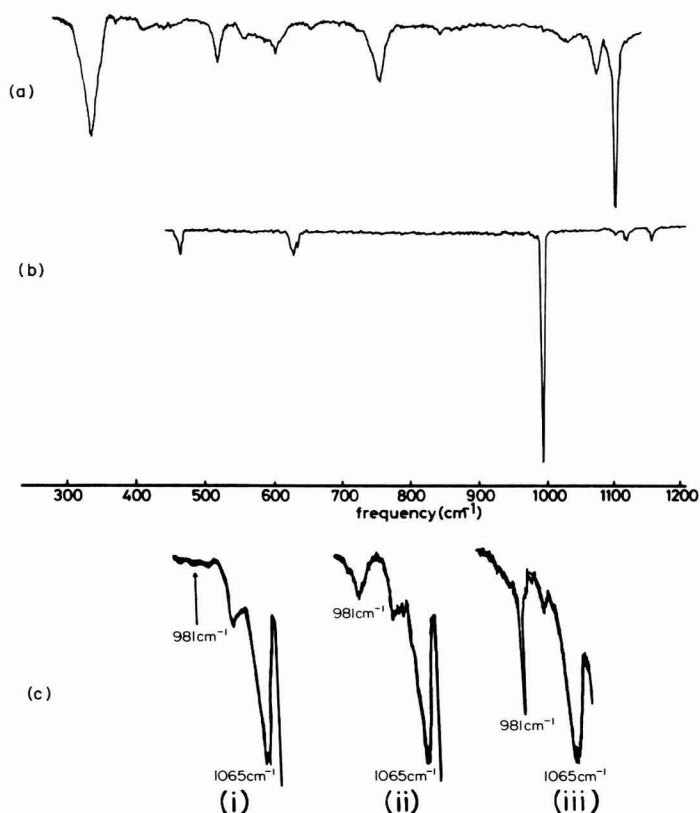
FIG. 2. Raman spectra of (a) $K_2S_2O_7$, (b) $K_2SO_4$, (c-i) $K_2S_2O_7$, (c-ii) $5.13 \times 10^{-2} m$ solution of $K_2SO_4$ in $K_2S_2O_7$, and (c-iii) $34.8 \times 10^{-2} m$ solution of $K_2SO_4$ in $K_2S_2O_7$.

in $K_2S_2O_7$. The band at 981 cm$^{-1}$ was characteristic of $K_2SO_4$ and was found to be free from the interference of the large excess of $K_2S_2O_7$. This could be used for the detection of $K_2SO_4$ in $K_2S_2O_7$ by raman spectroscopy. When the concentration of $K_2SO_4$ in solid solutions of $K_2S_2O_7$ is $5.13 \times 10^{-2} m$ or more (i.e., 1% $K_2SO_4$ by weight), raman spectroscopy was found to be a satisfactory method for the detection of $K_2SO_4$ in excess of $K_2S_2O_7$.

## SUMMARY

X-Ray diffraction, infrared, and raman spectroscopic methods were investigated for the detection of $K_2SO_4$ in excess of $K_2S_2O_7$ in solid solutions.

The X-ray diffraction lines of $K_2SO_4$ were found to be overlapped by the diffraction pattern of $K_2S_2O_7$ and infrared studies indicated that $K_2SO_4$ absorption bands corresponded to regions of strong absorption in $K_2S_2O_7$. The detection of sulfate could not be carried out by the X-ray diffraction and infrared methods. However, the raman method indicated that a strong and narrow $K_2SO_4$ band at 981 cm$^{-1}$ could unambiguously be used for the detection of

sulfate in solid solutions of $K_2SO_4$ in $K_2S_2O_7$, as pyrosulfate showed no absorption around this band.

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# The Polarography of Oximes

## I. 9,10-Phenanthraquinone Monoxime

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## INTRODUCTION

The polarographic behaviour of oximes have been studied by several authors (2, 5–8, 12, 16, 17, 20, 21). In general, the reduction products of an oxime group at the dme were the corresponding amines. This process was shown to involve four electrons per molecule. It has been shown (4, 5) with $\alpha$-ketoximes that, unlike simple oximes, these compounds are reduced to the corresponding amines even in strongly basic media. The present investigation deals with the polarographic behavior of 9,10-phenanthraquinone monoxime, which is an $\alpha$-ketoxime on which no polarographic work is reported so far. This compound has been shown to be a good complexing and precipitating agent (22) and hence was chosen for the present study.

## MATERIALS AND METHODS

9,10-Phenanthroquinone monoxime was prepared, from its parent quinone, as described by Goldschmidt (10). Stock solutions of the parent quinone and its oxime were prepared in purified ethanol. All other chemicals were of analytical reagent grade. HCl–KCl, $Na_2HPO_4$–citric acid, and boric acid–NaOH buffers were used. All experiments were carried out in 40% (v/v) alcoholic media. The ionic strength was adjusted to 0.5 $M$ with KCl. pH measurements were made with a Philips pH meter (Model PR 9405M). Nitrogen gas used for deoxygenation of the solutions was purified as described by Meites (18, p. 89) and was passed through a 40% (v/v) alcohol–water mixture prior to its entry in the polarographic cell. No maximum suppressor was needed. The temperatures were maintained with an accuracy of ±0.5°C. All potentials are referred to that of saturated calomel electrode (SCE). A manual dc polarographic setup was used. The

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capillary characteristics were: $m = 2.35$ mg/sec and $t = 3.00$ sec/drop in 0.5 $M$ KCl (open circuit) at $h_{corr.} = 48.4$ cm. The mercury used for the dme was first purified chemically and subsequently distilled under reduced pressure.

Millicoulometric analyses were carried out at pH 3.50, 7.50, 10.45, and 13.40. Controlled potential electrolyses were also performed at the above mentioned pH's. The diffusion coefficients were determined by using a McBain–Dawson diaphragm cell and applying the King–Cathcart equation (14). Ultraviolet spectra were taken with a Beckmann DU 2 spectrophotometer.

## RESULTS AND DISCUSSION

In order to identify the wave(s) due to the reduction of the oxime group and due to lack of data on 9,10-phenanthraquinone in the same buffer system and ionic strength preliminary studies on 9,10-phenanthraquinone and 9,10-phenanthraquinone monoxime were carried out under identical conditions at 35°C. It has already been established (1), in a study in the pH range 4.13 to 7.20, that 9,10-phenanthraquinone undergoes a reversible 2$e$ reduction. We extended the work up to pH 13.40. A well-defined wave was obtained for 9,10-phenanthraquinone only up to pH 11.0 beyond which ill-defined waves were obtained. In the pH range 3.50 to 13.40, 9,10-phenanthraquinone monoxime exhibited a well-defined wave whose height was almost constant. Below pH 3.50 ill-defined waves were observed which were not reproducible presumably due to their decomposition under these conditions (22). Further, the wave heights observed with 9,10-phenanthraquinone monoxime were almost twice the wave height obtained with 9,10-phenanthraquinone under similar conditions. This indicated that the number of electrons involved in the reduction of 9,10-phenanthraquinone monoxime was four. This was confirmed by other methods described later. The wave heights of 9,10-phenanthraquinone monoxime at pH 11.0 were compared with those of 9,10-phenanthraquinone at pH 11.0 since beyond this pH irregular waves were observed with 9,10-phenanthraquinone. A number of workers (2, 5, 6, 8, 12, 17, 20) have reported a 4$e$ reduction for oximes culminating in the formation of the corresponding amines. In most cases it has been observed that the wave heights of oximes decreased in strongly basic media which has been attributed to the lesser tendency of the anion to be reduced at the electrode (3, 20) or to the slow rate of recombination of the oxime anion (at pH $>$ p$K_a$) with H$^+$ prior to its reduction (23). In our case, however, the wave heights remained unaltered with the change in pH. This is explained later.

Having established the identity of the wave due to the oxime group, a

detailed investigation was carried out to examine the nature of the wave. The tests of $i_d/C$, $i_d/h^{\frac{1}{2}}$, negative shifts of $E_{\frac{1}{2}}$ with concentration, slopes of $\log i/(i_d-i)$ vs $E$ plots, and the temperature coefficients of $E_{\frac{1}{2}}$ and $i_d$ were carried out at pH 3.50, 7.50, 10.45, and 13.40. All these tests showed that the oxime group underwent diffusion-controlled irreversible reduction.

The diffusion coefficient $D$ of the oxime was determined under conditions similar to polarographic test solutions with regard to composition and pH. The values of $D$ obtained were 4.21, 4.29, 4.10, and 4.30 $\times$ $10^{-6}$ cm²/sec at pH 3.50, 7.50, 10.45, and 13.40, respectively. These values of $D$ were determined by using a McBain−Dawson cell and applying the King−Cathcart equation (14). It can be seen that $D$ does not change much with pH and a mean value of $D$ was taken for further calculations. Since the wave is diffusion controlled, the value of $D$ can be incorporated into the Ilkovic equation to obtain the value of the number of electrons involved in the reduction process, $n$. A value of $n = 4.09 \simeq 4.0$ was obtained at pH 3.50 and the $n$ values approximated to 4 at other pH's as well. This implies that the reduction of the oxime group goes up to the amine stage.

Millicoulometric studies at the pH's mentioned above also yielded a value of $n$ close to 4. Controlled-potential electrolysis (cpe), using a mercury pool cathode, was also carried out at the four pH's mentioned earlier. The resulting solution showed a positive test for amine. A hydrochloride from the solution had mp 110°C which is in accord with that of the hydrochloride of 9,10-aminophenanthrol (13). The reduction was also carried out with $H_2S$ in hot solution and the uv spectrum of the resulting solution was superimposable on that of the solution resulting from controlled-potential electrolysis. It is known that with $H_2S$ the oxime is reduced to 9,10-aminophenanthrol (19).

The wave heights of 9,10-phenanthraquinone monoxime were also compared with those of $\alpha$-nitroso $\beta$-naphthol (I), and 7-nitrooxine-5-sulfonic acid (II) which, under similar conditions, show a 4e (9) and a 6e wave (11), respectively. The values of $D$ for I and II were also determined under similar conditions. Solutions (0.6 m$M$) of 9,10-phenanthraquinone monoxime, I, and II when studied separately gave $i_d = 6.30$, 6.10, and 9.00 $\mu$A, respectively, in 40% ethanolic solutions of pH 5.00. The value of $n$ for 9,10-phenanthraquinone monoxime came out to be respectively 4.13 and 4.20 when compared with I and II.

The $E_{\frac{1}{2}}$ values showed a cathodic shift with pH. This showed that $H^+$ are involved in the potential-determining step. The p$K_a$ of the oxime has been found to be 8.8 in water and 10.1 in 75% dioxane (22). It can be justifiably inferred that the oxime anion is the predominant species in the bulk of the solution at pH > 11.0. The existence of a wave at pH > 11.0, whose height remains unaltered, indicates that either the oxime anion is

being reduced directly or the recombination of the anion with $H^+$, at the electrode surface, is fast. Since $E_{\frac{1}{2}}$ values showed a cathodic shift even beyond pH 11.0, it can be concluded that the anion is not reduced. The $E_{\frac{1}{2}}$ of the reduction of the anion is expected to be pH independent (23) at pH $> pK_a$, i.e., the slope of the $E_{\frac{1}{2}}$–pH plot is expected to be zero in this region. The slope is, however, observed to be 70 mV/pH with 9,10-phenanthraquinone monoxime in this region. Oximes are known to exhibit two waves at pH $\tilde{\ } pK_a$, corresponding to separate reduction of the oxime and its monoanion to an $\alpha$-aminoketone (20). Where there is no separation in current–voltage curves, logarithmic analysis can reveal the existence of two waves (23). In the present study only one wave was observed and the log plots were linear. This is an additional evidence for the nonreducibility of the anion and it could be concluded that the neutral oxime molecule is reduced since the height of the wave is maintained in the alkaline region too. Hence it is reasonable to infer that the anion recombines with $H^+$, at the electrode surface, prior to being reduced and that the rate of recombination is fast as compared to the rate of the reduction of the oxime at the dme, i.e., at the electrode surface there is a rapid establishment of equilibrium between the anion and $H^+$ on the one hand and the neutral oxime on the other.

The $E_{\frac{1}{2}}$–pH plot showed two segments and the pH at the intersection was about 9.9 which is near the expected $pK_a$ of the oxime on the basis of $pK_a$'s reported (22) in aqueous and 75% dioxane media. The plots of $E$ vs $[\log i/(i_a-i) - 0.546 \log t]$ were linear at all pH values. $\alpha n_a$ values at various pH values were calculated from the slopes of these plots using the relevant equation (18, pp. 242–248). These values of $\alpha n_a$ (Table 1) were nearly 0.54 in the pH range 3.50–8.50, and 0.72 in the pH range 9.45–13.40. These values of $\alpha n_a$ were introduced in the following equation to obtain the value of $p$, the number of $H^+$ involved in the rate-determining step (18, pp. 242–248):

$$dE_{\frac{1}{2}}/d(\text{pH}) = 0.061 \, p/\alpha n_a.$$

The value of $p$ obtained in the pH region 3.50–8.50 was 0.35. Whereas a value of $p = 0$ or $p = 1$ could be meaningfully interpreted, the value $p = 0.35$ is intriguing. A probable mechanism is, however, given in which the slow uptake of the first electron leads to the formation of the oxime radical. The subsequent reduction to the hydroxylamine stage, loss of a molecule of $H_2O$ to the imine stage, and the ultimate step to form the amine are proposed to be fast steps. The last two steps have been shown to be fast in a number of mechanisms with other oximes (17, 20). In the pH range 9.45–13.40 the value of $p$ emerged to be 0.83 or within the precision of the measurements, $p = 1$. Consequently, the course of the reduction in

this pH region through the rate-determining step can be shown accordingly. The values of kinetic parameters $\alpha n_a$ and $-\log k_{f,h}^0$ have been calculated by Koutecky's procedure (15) and are given in Table 1. The values of $\alpha n_a$ thus calculated were close to those calculated from the log plots. The value of $-\log k_{f,h}^0$ increased successively with pH showing thereby that the reduction became more and more irreversible as pH is increased.

On the basis of the foregoing discussion the following mechanisms are proposed:
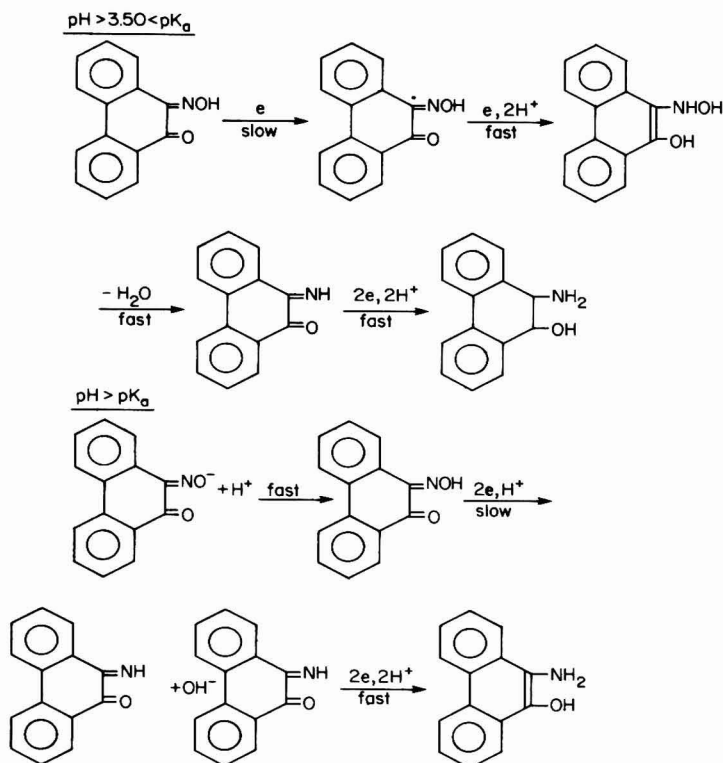
## SUMMARY

Polarography of 9,10-phenanthraquinone monoxime has been carried out in buffers (pH 3.50 to 13.40) of constant ionic strength 0.5 $M$ in 40% alcoholic solutions at 35 ± 0.5°C. The oxime group underwent diffusion-controlled reduction (4$e$) over the whole pH range studied. The number of electrons involved in the reduction was found coulometrically as well as by incorporating the value of the diffusion coefficient, obtained by using a McBain–Dawson cell, into the Ilkovic equation. Controlled potential electrolyses and uv spectroscopic methods were used to identify the products. Koutecky's method was used to compute the kinetic parameters ($\alpha n_a$ and $-\log k_{f,h}^0$) for the reduction of the oxime group and reduction mechanisms are proposed.

TABLE 1

POLAROGRAPHIC CHARACTERISTICS OF 9,10-PHENANTHRAQUINONA MONOXIME IN
BUFFERS, CONTAINING 40% ETHANOL, AT 35° C.

| pH | $-E_{1/2}{}^b$ (vs SCE) | $i_d$ ($\mu$A) | Slope of log plot$^c$ (mV) | $\alpha n_a$ From slope of log plot | Koutecky's method | $-\log k_{f,h}^0{}^d$ (N.H.E.) |
|---|---|---|---|---|---|---|
| 3.50 | 0.129 | 6.40 | 110 | 0.54 | 0.53 | 1.90 |
| 4.55 | 0.174 | 6.30 | 109 | 0.55 | 0.55 | 2.31 |
| 5.60 | 0.221 | 6.35 | 110 | 0.54 | 0.53 | 2.70 |
| 6.40 | 0.248 | 6.30 | 113 | 0.53 | 0.55 | 3.04 |
| 7.50 | 0.320 | 6.35 | 110 | 0.54 | 0.58 | 3.41 |
| 8.50 | 0.350 | 6.40 | 105 | 0.57 | 0.61 | 3.74 |
| 9.45 | 0.399 | 6.35 | 80 | 0.75 | 0.69 | 4.68 |
| 10.45 | 0.468 | 6.30 | 80 | 0.75 | 0.76 | 5.67 |
| 11.10 | 0.483 | 6.40 | 83 | 0.72 | 0.72 | 6.08 |
| 12.50 | 0.553 | 6.40 | 83 | 0.72 | 0.71 | 6.48 |
| 13.40 | 0.648 | 6.40 | 80 | 0.75 | 0.76 | 7.85 |

[a] Concentration of oxime = 0.6 m$M$; ionic strength = 0.5 $M$.
[b] From the intercept of the log plot.
[c] $E$ vs [$\log i/(i_d-i) - 0.546 \log t$].
[d] Koutecky's method.

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# BOOK REVIEWS

**Microorganisms and Minerals.** Edited by EUGENE D. WEINBERG. Marcel Dekker, New York, 1977. xiv + 492 pp., $44.50.

Microbiology may be the ''wave of the future.'' Microbes have answers to many of our problems: energy, waste disposal, production of hormones, clean-up after oil spills, single-cell food protein—the list is almost endless. Therefore ''Microorganisms and Minerals'' is an important book. Although its value to the microchemist is limited, it does have possibilities. The extreme sensitivity of many organisms to infinitesimal concentrations of certain cations suggests their use for the infrequent assay that does not justify the purchase of expensive equipment. The development of such procedures could prove rewarding.

<div align="right">

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**Membrane Proteins and Their Interactions with Lipids.** Edited by RODERICK A. CAPALDI. Marcel Dekker, New York, 1977. ix + 260 pp. $34.75

The tremendous increase in information about the cell membrane has completely altered the concept of this structure from a simple barrier to the location of vital cell processes. A full understanding of the structure and function of the cell membrane has become critical for comprehending cell activities and the resultant effects on the entire multicellular organism. For the microchemist, knowledge of the complex biomolecular structure and interactions for the performance of specific tasks may be essential for certain types of analyses. In this monograph, the contributions of nine investigators are combined to present information on one aspect of the cell membrane: the interactions between lipids and proteins of the membrane.

The book consists of six review articles, followed by an index to all authors cited in the six articles. A subject index is not included. The first article, by the editor R. Capaldi, discusses structural properties of membrane proteins. The article defines the difference between intrinsic and extrinsic membrane proteins and methods for isolation of these proteins for study. The second article, by J. Segrest and R. Jackson, is concerned with molecular properties of membrane proteins. Special attention is given to the specific points of protein–lipid interaction, the lipid-associating domains of the membrane protein. The third article, by G. Lenaz, reviews information on the lipid portion of the cell membrane and how lipid properties affect the lipid–protein interaction. An important part of this discussion is the essential role of the lipid portion in the physiological functioning of the cell membrane. The fourth article, by R. Baskin, discusses the $Ca^{2+}-Mg^{2+}$ ATPase protein found as part of the sarcoplasmic reticulum. The article presents fascinating information about this enzyme, an intrinsic membrane protein, and its essential role in the uptake of $Ca^{2+}$ following muscle contraction. The fifth article, by R. Barnett, reviews information on another cell membrane protein, the $Na^+$, $K^+$ ATPase. Information derived from NMR and ESR techniques has made possible a detailed study of this enzyme and its interactions with membrane lipids. The

<div align="center">136</div>

last article, by K. Simons, H. Garoff, and A. Helenius, reviews information on the glycoproteins making up part of the membrane surrounding the Semiliki Forest virus.

This monograph is Volume 1 of a series entitled "Membrane Proteins," edited by R. Capaldi. It is a well-presented, comprehensive review of the information which will be essential reading for all those interested in membrane structure and function.

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**Potentiometric Water Analysis.** By D. MIDGELY AND K. TORRANCE. John Wiley & Sons, Inc., New York, 1978. 409 pp., $42.00.

This book is certain to be the definitive text for potentiometric theory in general and estimation of the ionic content of water in practice. In two parts, the first deals with the mathematical aspects of potentiometry and goes on to cover the choice, use, and maintenance of electrodes and auxiliary equipment used in laboratory, field, and continuous stream monitoring. Particularly welcome is the completeness of the listing of possible errors in sampling and analytical procedure and various methods of detection and avoidance of these errors.

The last chapter of Part I forms a bridge between theory and laboratory practice and could equally well be the first chapter of Part II.

This second part is actually a detailed laboratory manual for the determination of some 33 ions or ionic groupings found in various waters. For ions not in this list, references abound to published papers of other researchers; in connection with this, it should be noted that the bibliography is excellent and conveniently arranged.

A welcome sequel to Part II would be a similar collection of laboratory methods for use in ecological investigations of air and similar fields and also of trace elements in soil.

Last but not least, it is very up-to-date in its references to other researchers.

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**Table of Molecular Weights.** Edited by M. WINDHOLZ, S. BUDAVARI, M. N. FERTIG, AND G. ALBERS-SCHÖNBERG. Merck & Co., Rahway, New Jersey, 1978. vi + 257 pp. (Soft cover)

This is a companion volume to the 9th edition of the *Merck Index,* providing access to the more than 9000 compounds listed in it, through their high resolution molecular weights. The compounds appear in order of their increasing molecular weights which are reported to six decimal places. The data appear in columnar form, giving the molecular weight, empirical formula, compound name, and monograph number from the *Merck Index.* There are also tables showing: (1) the atomic masses of the most abundant isotopes, and (2) atomic masses and abundances of naturally occurring isotopes.

This computer-selected list provides a link between high-resolution molecular weights of compounds and the detailed descriptions in the *Merck Index,* 9th edition. Mass spectroscopists will find this companion to the *Merck Index* of tremendous utility.

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**The Analysis of Organic Materials. An International Series of Monographs.** Edited by R. BELCHER AND D. M. W. ANDERSON. **The Determination of Sulphur-containing Groups. Volume 2. Analytical Methods for Thiol Groups.** By M. R. F. ASHWORTH. Academic Press, London and New York, 1976. xi + 288 pp., £9.80.

The analytical methods described are for compounds in which the thiol group is attached to a carbon atom joined to hydrogen or another carbon. Such topics are covered as various means of oxidation, mercaptide formation, acetylation, addition to double bonds, reactions with disulfides, reduction, reaction with bases, acids, peroxides, as well as polarographic and chromatographic methods. Those working or intending to work in this field will find the book of considerable value.

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**Molecular and Crystal Structure Models.** By ANNE WALTON. Halsted Press, John Wiley & Sons, New York, 1978. 201 pp., $22.50.

The need for this "consumer guide" to molecular and crystal structure models is made apparent by the wide variety of uses of models described in the book, and by the abundance of commercial and homemade types among which to choose. The author's confession that self-indulgence was among her motives for writing the book helps to explain the level of enthusiasm and the sparkle that are evident in what might have been a dry catalog of specifications. Dr. Walton has produced a handy reference volume that will be valuable to researchers, to teachers, and, especially, to students.

The introductory chapter emphasizes the importance of model building, lists the chemical requirements that models ought to fulfill, classifies the types on the market, recommends the color codes that should be observed, and refers to previous surveys of the subject. The following four chapters describe in some detail the structural features, costs, advantages, and shortcomings of the various space-filling molecular and crystal structure models, ball-and-spoke models, and skeletal models. Chapter 6 covers the special models needed to represent macromolecular structures, such as those of protein and nucleic acid molecules, and the polyhedral models that are so useful in depicting mineral and inorganic structures.

Chapter 7 leaves the structures of molecules and crystals to describe the models used to display the shapes of atomic and molecular orbitals. Some of these indicate merely the angular dependences of atomic wave functions, some show the electron density distributions about atoms, and some illustrate such bonding characteristics as hybridization, sigma and pi bonds, and delocalized bonding. Next is a chapter on dynamic models, which include those with the facility for demonstrating inversions and pseudorotations, magnetic models that mimic electrostatic interactions, and bubble rafts that can simulate defects in two-dimensional structures. Chapter 9 considers the devices (ball punches, jigs, templates, etc.) and techniques that are essential in the construction of accurate homemade models. The final chapter discusses the two-dimensional representations of structures through pictures, projections, and computer images; stereoscopic photographs are mentioned, but not the stereoscopic drawings of crystal and molecular structures generated by Carroll Johnson's ubiquitous computer program. An appendix lists 71 manufacturers or suppliers of models; of these, 25 are American and 33 British.

The descriptions in the book are complemented by 58 photographs of models. Further arguments for the importance of models (or at least of pictures) are provided unintentionally

by those cases where words alone are used to portray complex structures; the writing is clear, compact, and literate, but both teacher and students are handicapped by the absence of visual aids.

A primary lesson of the book is that the model of a molecular or crystal structure that is "best" depends upon the features that need to be shown. No one type can display all of the complications of molecular architecture, space filling, atomic size ambiguity, bonding, steric hindrance, or dynamic behavior that exist in real matter; models are necessarily idealizations of a few properties. Dr. Walton has sorted out these details of purpose, function, and availability, and she has given us a good practical guide to the science and art of model building.

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**Ultratrace Metal Analysis in Biological Sciences and Environment.** Edited by TERENCE H. RISBY. American Chemical Society, Washington, D.C., 1979. vii + 263 pp., $36.50.

Metals can be either beneficial or harmful to humans and biota depending on their concentration and the chemical form. Further, all forms of ecological systems are affected to varying extents by different kinds of metals. Although metals are nondestructible and nonbiodegradable, they may transfer from one biological system to another, from the biological system to the environment, and vice versa. With current interest in the study of environmentally related biological and biomedical problems, knowledge and information on the kinds and quantities or concentration levels of some trace metals present in biological systems and the environment are essential to the screening of various toxic effects and the effective control of the redispersion or redistribution of such metallic pollutants from one system to another. This volume brings together many research scientists and environmental chemists to discuss the sensitive analytical methodology and rapid and accurate instrumental techniques to determine the ultratrace levels of metals in biological and environmental systems. It collects and compiles some 14 papers presented at a Symposium which was sponsored by the Division of Analytical Chemistry at the 174th Meeting of the American Chemical Society, Chicago, Illinois, August 29–30, 1977. It belongs to Advances in Chemistry Series 172. An index is provided.

The first paper contributed by W. Mertz discusses the recent progress of nutritional trace element research and its implications for trace element analysis and future direction of development. The second paper by F. N. Abercrombie *et al.* demonstrates the advantages and limitations of the simultaneous multielement analysis of biologically related samples using Induction-Coupled Argon Plasma (ICAP) optical emission spectroscopy. The sample categories covered include grains, feeds, fish, bovine liver, orchard leaves, and human kidney stones. The elements that have been analyzed from these materials are. Cu, Ni, V, Cr, P, Co, Pb, K, Zn, Mn, Fe, Sr, Na, Al, Ca, Mg, Si, B, and Be, etc.

In Chapter 3, the health implications of trace metals in the environment are reviewed by K. Bridbord and H. P. Stein. Potential adverse health effects of occupational exposures to trace metals are discussed: cancer—As, Be, Cr, Ni, and perhaps Cd; chronic lung disease—Be and Cd; neurologic and reproductive disorders—Pb and Hg; and kidney disorders—Pb and Cd. The following paper by G. E. Bentley *et al.* describes a procedure for the determination of molybdenum (Mo) in serum, red blood cells, and urine using atomic

absorption spectrophotometry (AAS) and electrothermal atomization. The method can be used to distinguish between industrially exposed and unexposed individuals.

The analysis of size-fractionated and time-dependent particulate matter for metals by AAS or by neutron activation analysis (NAA) is reported in Chapter 5 by G. J. Rosenberger *et al.* Twelve elements were detected and analyzed by NAA and eight elements were analyzed and four were detected by AAS. In Chapter 6, the collection and analysis of airborne metallic elements Hg, Pb, and Mn, are summarized by R. J. Thompson. The methods used in this study include AAS, NAA, SSMS, OES (optical emission spectrometry), X-ray fluorescence (XRF), and X-ray diffraction (XRD). Elemental compositional levels and ranges of metals are considered. The concentrational differences between sites can vary by $10^5$.

A preliminary report on some specific nickel compound-induced transformations in tissue culture is presented by M. Costa in the 7th paper. The basic idea of this study is that the carcinogenetic process in tissue culture represents a complex array of changes of which transformation is an integral part. Of particular concern here is $Ni_3S_2$. The 8th paper also focuses more on the development and application of specific analytical procedure, based on ICAP–AES, for the rapid, simultaneous determination of a number of trace elements in human urine. A detailed experimental procedure is presented by W. J. Hass, Jr. *et al.*

In Chapter 9, D. S. Auld reports the investigation of the role of zinc in biochemical processes and states the importance of zinc to both nucleic acid and protein metabolism. It shows that zinc functions in the catalytic step of peptide hydrolysis and in the binding step of ester hydrolysis. Chapter 10 by C. P. Weisel *et al.* describes a modified standard addition method for determining Cd, Pb, Cu, and Fe in sea water-derived samples by the heated graphite furnace flameless AAS.

Chapter 11 by R. E. Lee, Jr. and F. V. Duffield considers the sources of environmentally important metals in the atmosphere and their biologically toxic effects. Seventeen environmentally important metals have been identified: As, Be, Cd, Cr, Cu, Fe, Hg, Mg, Mn, Ni, Pb, Ti, Sb, Se, Sn, V, and Zn. The major sources of these metals with emphasis on fine particulate emissions are reviewed. In the next paper, J. J. Dulka *et al.* reports the uptake of Al, Cd, Cr, Co, Cu, Fe, Pb, Mg, Mn, Mo, Ni, Ag, Sn, and Zn by *Bacillus subtilis* strain 168. The data were obtained during different phases and analyzed by AAS. It was mentioned that these metals seem to be required for growth although the roles these metals played were not postulated. However, the clinical, biochemical, and nutritional effects of zinc in humans were shown in the 13th paper by A. S. Prasad. Zinc is essential for DNA synthesis. The activities of many zinc-dependent enzymes have been shown to be affected adversely in zinc-deficient tissues.

In the concluding paper of the volume, A. A. Yunice summarizes the results of the investigation of serum copper in relation to age. Serum Cu and ceruloplasmin concentrations were determined in 180 male and 44 female subjects ranging in age between 20 and 89 years. In male population, there was significant increase with age with $P < 0.25$. In the female population, correlation of serum Cu with age was not significant. In neither the males nor the females were there any altered serum ceruloplasmin levels with age. In addition, the role of Cu in the biological system and the environment has been reviewed in relation to other ultratrace and bulk metals, with particular reference to the aging processes. A particular emphasis was placed on hormonal imbalance and free-radical theory. This book is highly recommended to analytical chemists, biochemists, environmental chemists, and engineers as an excellent reference book for treating the effects of trace metals on biochemical- and biomedical-related environmental problems.

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**Kirk–Othmer Encyclopedia of Chemical Technology, Vols. 2, 3, 4, and 5,** 3rd ed. Editorial Board, HERMAN F. MARK, DONALD F. OTHMER, CHARLES G. OVERBERGER, AND GLENN T. SEABORG; Executive Editor, MARTIN GRAYSON; Associate Editor, DAVID ECKROTH. Wiley-Interscience, New York. Vol. 2, 1978, xxv + 1036 pp.; Vol. 3, 1978, xxv + 958 pp.; Vol. 4, 1978, xxv + 930 pp.; Vol. 5, 1979, xxv + 880 pp.; Index to Vols. 1–4, 1979, 213 pp. $120.00 per volume; subscription price, $95.00 per volume.

These volumes are the continuation of material started with Volume 1, reviewed earlier in this journal (*Microchem. J.* **24**, 389 (1979)).

Volume 2 has 66 contributors and covers substances beginning with the letter A—alkoxides, metal, to antibiotics. Alkyd resins, alkylation, amides, amines, analgesics, anthraquinone derivatives, etc., are included. Under each section is an extensive bibliography.

Volume 3 has 66 contributors and covers substances beginning with the letter A—antibiotics (continued from Volume 2)—to some subjects beginning with the letter B—bagasse to bleaching agents. Included are such subjects as antifreezes, antimony compounds, antioxidants, antistatic agents, appetite-suppressing agents, asbestos, arsenic, bakery processes, barium compounds, batteries, bearing materials, benzyl alcohol, beryllium, biomedical automated instrumentation, etc.

Volume 4 has 77 contributors, completes the subjects beginning with the letter B, and covers some starting with the letter C. Blood, coagulants, and anticoagulants are the first subjects covered. Then follow such subjects as blood fractionation, boron compounds, bromine compounds, butadiene, butylenes, cadmium compounds, calcium compounds, calorimetry, carbohydrates, carbon compounds, etc., ending with cardiovascular agents.

Volume 5 has 86 contributors and continues the subjects beginning with the letter C, starting with castor oil and ending with chlorosulfuric acid. Included are catalysis, celullose, cement, ceramics, cerium, cesium, chelating agents, chemicals in war, chemiluminescence, several categories under chemotherapy, chlorine oxygen acids, chlorophenols, etc. It is assumed that additional volumes will be coming to cover the rest of the alphabet.

In general, this is an enormous undertaking and is being accomplished extremely well. There are many tables and figures, and each section has its own bibliography. Numerous sections, or groups of sections on closely related subjects, are very extensive, covering as many as 100 pages or more. Examples are the sections on boron compounds and on carbon (not carbon compounds), which have 138 and 153 pages, respectively.

The Index to Volumes 1 to 4, consisting of 213 pages, is very valuable for finding individual compounds and subjects.

The entire series contains a wealth of information, and its placement in all libraries is highly recommended.

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# Erratum

Volume 24, No. 3 (1979), in the article, "The American Microchemical Society: An Informal History," by David B. Sabine and Herbert K. Alber, pp. 265-274: Page 273, the portion of Table 2 between the years of 1968 and 1973 should read:

| | |
|---|---|
| K. Fleischer | 1968−9 |
| D. Green | 1969−70 |
| J. Kobliska | 1970−71 |
| A. Prezioso | 1971−2 |
| T. Kielty | 1972−3 |

The following name should be added to the end of the table:

| | |
|---|---|
| E. Ross | 1979−80 |