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Briefs

Determination of Sub-Nanogram Quantities of Ruthenium by Flameless Atomic Absorption Spectrometry. CRAIG M. YOUNG AND JON M. BALDWIN, *Allied Chemical Corporation, Idaho Chemical Programs Operations Office, 550 Second Street, Idaho Falls, Idaho 83401.*

The determination has been applied to sub-nanogram quantities of ruthenium in a variety of matrices encountered in the solidification of nuclear waste. Detection limits ranged to below 10^{-10} g, depending on the sample matrix.

Microchem. J. **23**, 265–284 (1978).

Analytical Reactions of Substituted Cyanoferrates. 2. Pentacyanoamminoferrate (II) in Catalytic Spectrophotometric Determination of Sub-Parts per Million Amounts of Ag^{+}, Au^{3+}, and Hg^{2+} in Solution. M. K. GADIA AND M. C. MEHRA, *Environmental Contaminants Research Group, Département de chimie, Université de Moncton, Moncton, New Brunswick, Canada E1A 3E9.*

The ions catalyze ligand exchange reaction between pentacyanomminoferrate (II) ion and ferrozine to produce a strongly colored chelate absorbing at 562 nm. A mechanism is proposed and the effects of the experimental parameters have been determined.

Microchem. J. **23**, 278–284

The Linear Correlation of the $(\Delta R'_m)_t$ Function Determined for the Ortho Derivatives of Phenol, Aniline, Benzaldehyde, and Nitrobenzene. LEONARD OGIERMAN AND JÓZEF SLIWIOK, *Institute of Chemistry, Silesian University, 40-006 Katowice, Poland.*

Selected chromatographic data from a group of ortho derivatives of phenol, aniline, benzaldehyde, and nitrobenzene are helpful in attempting to predict R_f values of substances with known structures or in elucidating such structures with compounds having known R_f values.

Microchem. J. **23**, 285–290 (1978).

Spectrophotometric Determination of Cerium(IV), Arsenic(III), and Nitrite with Promazine Hydrochloride. H. SANKE GOWDA AND K. N. THIMMAIAH, *Department of Postgraduate Studies and Research in Chemistry, University of Mysore, Manasa Gangotri, Mysore-570006, India.*

Promazine hydrochloride reacts with cerium(IV) in acid solution to form a red compound which exhibits maximum absorbance at 505 nm. Arsenic(III) and nitrite are determined indirectly.

Microchem. J. **23**, 291–296 (1977).

Benzothiazole-2-aldehyde-2-quinolylhydrazone as a Reagent for the Extractive Spectrophotometric Determination of Copper(II). MAKOTO OTOMO AND HIDEMASA NODA, *Department of Synthetic Chemistry, Nagoya Institute of Technology, Showa-ku, Nagoya 466, Japan.*

Copper(II) was reacted with benzothiazole-2-aldehyde-2-quinolylhydrazone (BTAQH) at pH 8.3–12.6 to form a water-insoluble 1:2 complex, which was extracted with various organic solvents.

Microchem. J. **23**, 297–304 (1978).

Metallic Excess Determination in Nonstoichiometric Oxides and Chalcogenides. T. BESAGNI, F. LICCI, AND L. ZANOTTI, *Laboratorio Maspec del C. N. R., Via Spezia, 73, 43100 Parma, Italy.*

A polarographic procedure is described for determining the excess of a metallic element in nonstoichiometric oxides and chalcogenides. The method is based on the possibility of changing the reduction potential of an element in the presence of a strong complexing agent (EDTA).

Microchem. J. **23**, 305–311 (1978).

The Use of Redox Reactions in the Analysis of Dyes and Dye Industry Intermediates. III. An Indirect Determination of Brilliant Green with Ceric Sulfate. J. BAREK, A. BERKA, AND K. JAKUBEC, *Department of Analytical Chemistry, Charles University, 128 40 Prague 2, Czechoslovakia.*

The stoichiometry of the reaction of brilliant green with ceric sulfate was studied. An indirect determination of brilliant green was developed based on this oxidation.

Microchem. J. **23**, 312–320 (1978).

A New Reagent for the Spectrophotometric Microdetermination of Cadmium. H. ALEXAKI-TZIVANIDOU, G. KOUNENIS, AND B. ELEZOGLOU, *Laboratory of Physiology, Veterinary Faculty, University of Thessaloniki, Thessaloniki, Greece.*

2,2′-Dipyridyl-2-pyridylhydrazone (DPPH) is used in aqueous solution for the determination of cadmium. The yellow 1:2 metal-to-ligand complex formed has a molecular extinction coefficient of 5.5×10^4 liters $mole^{-1}$ cm^{-1} at the absorption maximum of 444 nm.

Microchem. J. **23**, 329–335 (1978).

2,4-Dioxo-4-(4-hydroxy-6-methyl-2-pyrone-3-yl)butyric Acid Ethyl Ester: Reagent for Identifying and Estimating Metal Ions in Ring Oven Analysis. ANTUN GERTNER AND DUBRAVKA PAVIŠIĆ, *Institute of Chemistry, Faculty of Pharmacy and Biochemistry, University of Zagreb, A. Kovačića 1, 4100 Zagreb, Yugoslavia.*

The ligand gave variously colored or fluorescent rings when reacted with several kinds of ions on a ring oven. With some ions, the reactions were sensitive enough to enable application of ring colorimetry or fluorometry for quantitation.

Microchem. J. **23**, 336–340 (1978).

The Use of Redox Reactions in the Analysis of Dyes and Dye Industry Intermediates. IV. The Oxidation of Benzidine, *o*-Tolidine, and *o*-Dianisidine with Chloramine T and *N*-Bromosuccinimide. J. BAREK, A. BERKA, AND K. JAKUBEC, *Department of Analytical Chemistry, Charles University, 128 40 Prague 2, Czechoslovakia.*

An extraction–photometric determination of the diamines was developed based on the reaction with an excess of the reagents and the measurement of the absorbance of the reaction product

Microchem. J. **23**, 341–352 (1978).

Analytical Applications of Picolinealdehyde Salicyloylhydrazone. I. Spectrophotometric Determination of Nickel and Zinc. M. GALLEGO,* M. GARCIA-VARGAS,* F PINO,* AND M. VALCARCEL,† **Department of Analytical Chemistry, Faculty of Sciences, University of Sevilla, Sevilla, Spain, and †Department of Analytical Chemistry, Faculty of Sciences, University of Cordoba, Cordoba, Spain.*

A study of the physical properties of the hydrazone was undertaken with the view that it could be used as an analytical reagent. With both nickel and zinc, yellow complexes are formed. In both cases, the method can determine parts per million amounts.

Microchem. J. **23**, 353–359 (1978)

Spectrophotometric Determination of Arsenic with Diethyldithiocarbamic Acid Silver Salt and Borohydride as Reducing Agent. MAISABEL BASADRE PAMPÍN, A. ALVAREZ-DEVESA, F. BERMEJO-MARTÍNEZ, AND MAHERMINIA BOLLAÍN-RODRÍGUEZ, *Department of Analytical Chemistry, Faculty of Chemistry, University of Santiago de Compostela, Santiago de Compostela, Spain.*

The method is a modification of a previously published method.

Microchem. J. **23**, 360–365 (1978).

Analytical Study of the Phenyl-2-pyridyl Ketone Azine–Palladium System. Photometric Determination of Palladium. M. GARCIA-VARGAS AND M. VALCARCEL, *Department of Analytical Chemistry, Faculty of Sciences, University of Sevilla, Sevilla, and University of Cordoba, Cordoba, Spain.*

A yellow 1:1 complex is formed in aqueous ethanolic solution which is used for the determination.

Microchem. J. **23**, 366–373 (1978).

Consecutive Determination of Rutin and Quercetin by Spectrophotometric Measurements. MIZUHO SAKAMOTO AND KIYOKO TAKAMURA, *Tokyo College of Pharmacy, 1432-1, Horinouchi Hachioji, Tokyo 192-03, Japan.*

Quercetin forms a more stable metal complex without interference from rutin and is determined as a tin complex. After quercetin is removed by using its oxidation with copper(II), rutin is determined as a rutin–copper(II) complex. The method is applicable to concentrations of the order of 10^{-5} *M*.

Microchem. J. **23**, 374–383 (1978).

Identification of the Order of Aromatic Alcohols and Their Derivatives by Means of Thin-Layer Chromatography. JÓZEF ŚLIWIOK AND LEONARD OGIERMAN, *Institute of Chemistry, Silesian University, 40-006 Katowice, Poland.*

Separation conditions were established for aromatic primary and secondary alcohols and their acetates.

Microchem. J. **23**, 384 – 389 (1978).

Preliminary Evaluation of Biacetyl Bis(2-Pyridyl)Hydrazone as an Analytical Reagent. A. G. ASUERO, *Department of Analytical Chemistry, Faculty of Sciences and Pharmacy, The University of Seville, Seville-4, Spain.*

The compound acts as a general chromogenic reagent, and the fundamental solution chemistry of the complexes formed with metal ions was studied.

Microchem. J. **23**, 390–399 (1978)

A Study of the Stability of Pyrimidine Series Cytostatics, Ftorafur and 5-Fluorouracil. The Effect of Oxidation on the Stability of Ftorafur and 5-Fluorouracil. HANA TOMÁNKOVÁ* AND JAROSLAV ZÝKA,† **The State Institute for Control of Drugs, Prague, and* †*Department of Analytical Chemistry, Charles University, 128-40 Albertov 2030, Prague, Czechoslovakia.*

The stability of solutions of the compounds was studied during oxidation with alkaline peroxide, acidic peroxide, and weakly acidic peroxide by means of thin-layer chromatography. The decrease in content was monitored spectrophotometrically in the ultraviolet region. The greatest decrease occurred in alkaline solutions, where the pyrimidine ring opened, yielding urea.

Microchem. J. **23**, 400–406 (1978)

A Device to Aid Installing and Removing Glass Gas Chromatographic Columns. H. M. STAHR* AND A. WUNDERLICH,† **Veterinary Diagnostic Laboratory* and †*Instrument Shop, Iowa State University, Ames, Iowa 50011.*

This device allows columns to be removed and replaced in a matter of seconds without stress.

Microchem. J. **23**, 407–408 (1978)

MICROCHEMICAL JOURNAL 23, 265–277 (1978)

Determination of Sub-Nanogram Quantities of Ruthenium by Flameless Atomic Absorption Spectrometry

CRAIG M. YOUNG[1] AND JON M. BALDWIN[2]

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INTRODUCTION

Atomic absorption in a combustion flame absorption cell has been used by many workers as a convenient means of determining ruthenium in ores and metallurgical concentrates (*7, 11, 12*) as well as in other media (*1, 5, 6, 9, 10*). The detection limit for ruthenium by flame atomic absorption is generally reported to be about 3 μg/g.

Recently, concern over the behavior of fission-product ruthenium in the waste from nuclear fuel reprocessing has spurred the investigation of more sensitive atomic absorption measurements of that element. As part of a study (*2*) of methods for suppressing Ru volatilization during fluidized-bed calcination of simulated waste from light-water reactor (LWR) fuel reprocessing, it was necessary to devise methods for measuring as little as 10^{-10} g of Ru. Radiotracer methods were ruled out by the conditions of the experiment. Sample matrices ranged from solid particulate materials to aqueous condensates and a variety of scrubber solutions. Ruthenium concentrations ranged from about 100 μg/g to 100 ng/g. Samples containing greater than 10 μg of Ru/g were conveniently analyzed with conventional atomic absorption techniques, using a fuel-rich air/acetylene flame and a 4-wt% addition of uranium as described by Montford and Cribbs (*9*). Samples containing 10 μg of Ru/g or less required a more sensitive method. In this paper we describe the development of flameless atomic absorption methods having the requisite detection limit, and suitable for the sample types mentioned above.

Flameless atomic absorption was first used for the determination of Ru by Guerin (*4*) who found a detection limit of 200 pg in aqueous solution. A similar detection limit has been reported by Everett (*3*). More recently, Megarrity and Siebert (*8*) have applied flameless atomic absorption to the

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determination of Ru in biological material after fusing the sample with KOH/KNO_3. Although they did not specifically state a detection limit, it appears that they also could detect about 200 pg of Ru. All of these workers conducted limited studies of interferences, not directly applicable to the present situation.

MATERIALS AND METHODS

A. Bench-scale fluidized bed calciner. The samples analyzed in this study were generated in the 3-in.-diameter calciner system shown in Fig. 1 (*2*). Samples were taken from various points in the system and included solids from the bed, cyclones, and filters, aqueous condensate, and solutions from the two scrubbers. The volatility studies were conducted primarily with a simulated LWR waste of the composition given in Table 1. It was intended to simulate a 1:1 blend of high-level (HLLW) and intermediate-level liquid waste (ILLW) such as would be encountered in the calcination of 1-year cooled commercial LWR waste. Chemical simulants were substituted, on a 1:1 weight basis, for some of the waste elements, as listed in Table 1. Some cations at less than 4 mol% of the total were not included in the synthetic feed. A limited number of experiments were also run with a simulated mixed Zr/Al waste feed with the composition shown in Table 2.

The simulated LWR waste, with a Ru concentration of 2 mg/ml, produced some scrubber solutions in the range 10 to 100 μg Ru/ml, but under

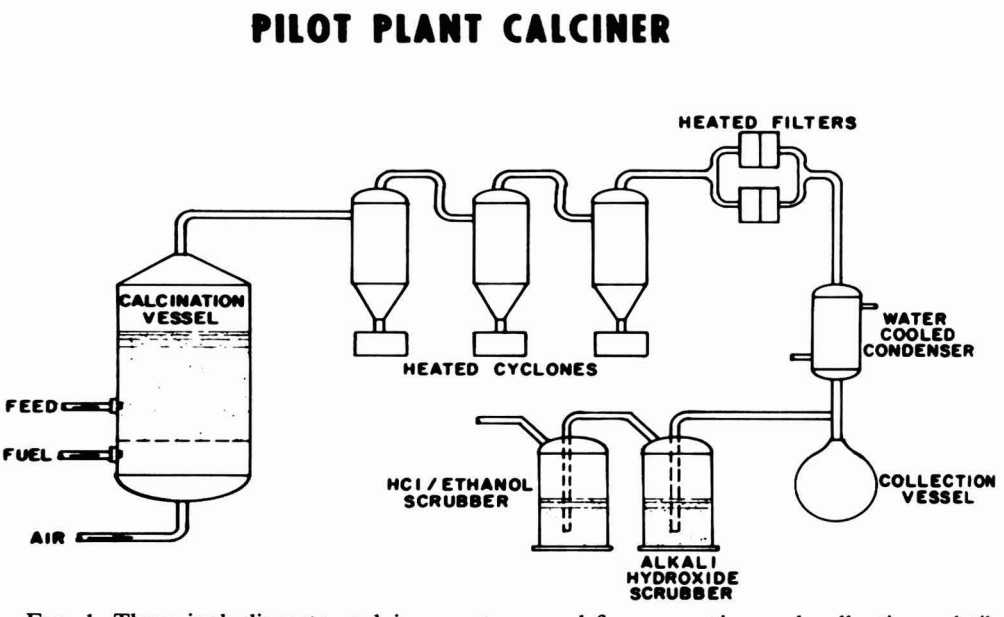

FIG. 1. Three-inch-diameter calciner system used for generating and collecting volatile ruthenium species.

TABLE 1

COMPOSITION OF SIMULATED COMMERCIAL WASTE[a]

LWR waste element	Concentration (M)	Reagent used in simulated feed[b]
Se	2.08×10^{-4}	—
Br	1.99×10^{-4}	—
Rb	4.72×10^{-3}	KNO_3
Zr	1.08×10^{-2}	$Sr(NO_3)_2$
Y	5.49×10^{-3}	R.E.N.
Zr	4.67×10^{-2}	$ZrO(NO_3)_2 \cdot 2H_2O$
Mo	4.28×10^{-2}	—
Tc	9.75×10^{-3}	—
Ru	2.68×10^{-2}	$Ru(NO_3)_3$
Rh	5.76×10^{-3}	$Co(NO_3)_2 \cdot 6H_20$
Pd	1.69×10^{-2}	$Ni(NO_3)_2 \cdot 6H_20$
Ag	9.9×10^{-4}	—
Cd	1.43×10^{-3}	$Cd(NO_3)_2 \cdot 4H_2O$
In	1.2×10^{-5}	—
Sn	2.46×10^{-4}	—
Sb	1.03×10^{-4}	—
Te	4.85×10^{-3}	TeO_2
Cs	2.26×10^{-2}	KNO_3
Ba	1.49×10^{-2}	$Ba(NO_3)_2$
La	1.12×10^{-2}	R.E.N.
Ce	2.12×10^{-2}	R.E.N.
Pr	1.07×10^{-2}	R.E.N.
Nd	3.39×10^{-2}	R.E.N.
Pm	2.84×10^{-4}	R.E.N.
Sm	7.93×10^{-3}	R.E.N.
Eu	1.33×10^{-3}	R.E.N.
Gd	9.17×10^{-4}	Gd_2O_3
Tb	1.33×10^{-5}	R.E.N.
U	6.17×10^{-2}	$UO_2(NO_3)_2 \cdot 6H_2O$
Np	2.39×10^{-3}	R.E.N.
Pu	5.25×10^{-4}	R.E.N.
Am	2.55×10^{-3}	R.E.N.
Cm	1.16×10^{-4}	R.E.N.
Na	0.26	$NaNO_3$
Fe	4.29×10^{-2}	$Fe(NO_3)_3 \cdot 9H_2O$
Cr	4.52×10^{-3}	CrO_3
Ni	1.60×10^{-3}	$Ni(NO_3)_2 \cdot H_2O$
Gd Soluble poison)	6.72×10^{-2}	Gd_2O_3
PO_4	0.105	H_3PO_4
Mn	2.99×10^{-3}	—
K	1.80×10^{-3}	KNO_3
SO_4	6.24×10^{-3}	$Fe_2(SO_4)_3 \cdot XH_2O$
Cl	5.29×10^{-3}	KCl
F	4×10^{-4}	—
Hg	7.61×10^{-3}	$Hg(NO_3)_2 \cdot H_2O$
I	2.31×10^{-3}	NaI
$\sum$ cations	0.759	

[a] A 1:1 blend of 150 gal/MTU HLLW and 75 gal/MTU ILLW; MTU = metric ton of uranium.

[b] R.E.N. = Rare earth nitate (atom percent): Ce, 57; Gd, 1.7; La, 16; Pr, 4.7; Sm, 2.6; Y, 2.9.

TABLE 2
COMPOSITION OF SIMULATED Zr/Al WASTE FEED

Zr/Al waste element	Concentration (M)
Al(III)	0.6
ZR(IV)	0.1
B(III)	0.1
Ca(II)	1.4
Ru(III)	2.5×10^{-4}
F^-	1.0

optimum conditions the condensate and scrubber solutions contained less than 1 μg of Ru/ml. The simulated Zr/Al waste, with a Ru concentration of 25 μg/ml, always produced condensate and scrubber solutions with less than 1 μg of Ru/ml. The various solid samples contained as little as 100 ng of Ru/g. With the sample preparation necessary for these solids the resulting final concentration in solution was as little as 20 ng/ml.

B. Measurement apparatus. Atomic absorption measurements were made with Varian atomic absorption spectrometers, Models AA-5 and AA-6, with a Varian Model 63 carbon rod atomizer as the absorption cell. The source of atomic resonance radiation was a commercial ruthenium hollow cathode lamp with neon filler gas, operated at 10 mA. The 349.9-nm ruthenium resonance line was observed with a spectral band pass of 0.17 nm. Background correction was accomplished with a Varian BC-6 simultaneous background corrector and a hydrogen continuum lamp. Transient absorption signals were recorded on a Varian Model A25 strip-chart recorder with 0.5-sec full-scale pen response.

Temperatures below 500°C were measured with an iron–constantan thermocouple; those above 500°C were measured with an E^2 Thermodot infrared thermometer, modified to have a 30-msec rise time. The temperature actually measured was that of the atomizer outer surface and did not correspond exactly to the temperature of the surface on which the sample was deposited. The sample surface temperatures were 10–20% higher than the temperatures measured in this work (J. M. Baldwin and D. A. Pavlica, unpublished observations).

C. Reagents. A stock solution was prepared by dissolving $RuCl_3 \cdot xH_2O$ in 0.1 N HCl to give about 2 mg of Ru/ml, and was standardized gravimetrically by hydrogen reduction to the metal. Working standards were prepared by diluting the stock solution with 0.2 N HCl. All other reagents were prepared from analytical reagent grade starting materials, with the exception of ammonium pyrrolidinedithiocarbamate, which was of unspecified purity. All aqueous solutions were prepared with water from a Millipore Milli-Q water system.

RESULTS AND DISCUSSION

A. Atomizer Temperatures

All samples dealt with in this study were in aqueous solution, or were converted to a solution in water or amyl acetate before analysis. Consequently, drying at 100°C for 60 sec was suitable in all cases. The ash temperature was 500°C maximum, for 20 sec for all matrices except the NaOH scrubber solution, which required a maximum temperature of 800°C, also for 20 sec. Ruthenium absorption was not detected at temperatures below 2500°C. Best sensitivity was obtained with an atomization temperature of 2800°C, maximum. At this temperature 3 sec were required to completely atomize the Ru. This heating regime was suitable for all samples but those in hydroxide matrix, where the ramp mode of heating was required to separate matrix absorption from Ru atomic absorption. The problems associated with the hydroxide matrix will be discussed in a following section.

B. Sheath Gas Composition

Initial tests with N_2/H_2 mixtures indicated that, although maximim sensitivity was obtained only upon inclusion of H_2 in the sheath gas mixture, the peak Ru absorbance is not a strong function of the N_2/H_2 ratio. Flows of 4.0 liters/min of N_2 and 0.5 liter/min H_2 were chosen for maximum sensitivity. Experience with this mixture subsequently revealed rapid deterioration of the graphite atomizer parts at high atomization temperatures. Destruction of the pyrolytic graphite coating was particularly rapid when the ramp heating mode was used, due to the relatively long time spent at high temperature.

Substitution of argon/10% methane for the H_2 resulted in about 40% decrease in sensitivity, but a considerable prolongation of graphite part lifetime due to continuous renewal of the surface by methane pyrolysis. The effects of the two gas mixtures on the graphite surface are illustrated in Fig. 2. Figure 2A shows a micrograph of contact electrodes that were, from left to right, unused, used for 100 atomizations in $N_2/Ar/10\%$ CH_4, and used for 50 atomization cycles with N_2/H_2. While the electrode exposed to the N_2/H_2 atmosphere shows appreciable deterioration, there has been little or no noticeable change in the electrode exposed to methane. Figures 2B–D show micrographs of the interior of atomizer tubes that are, respectively, (B) unused, (C) used for 20 atomization cycles with N_2/H_2, and (D) used for 30 atomization cycles with $N_2/Ar/CH_4$. The improvement in surface condition with methane addition is obvious. The $N_2/Ar/CH_2$ mixture was used throughout the remainder to this work.

C. Matrix Effects

1. Aqueous condensate samples. No molecular absorption or scattering

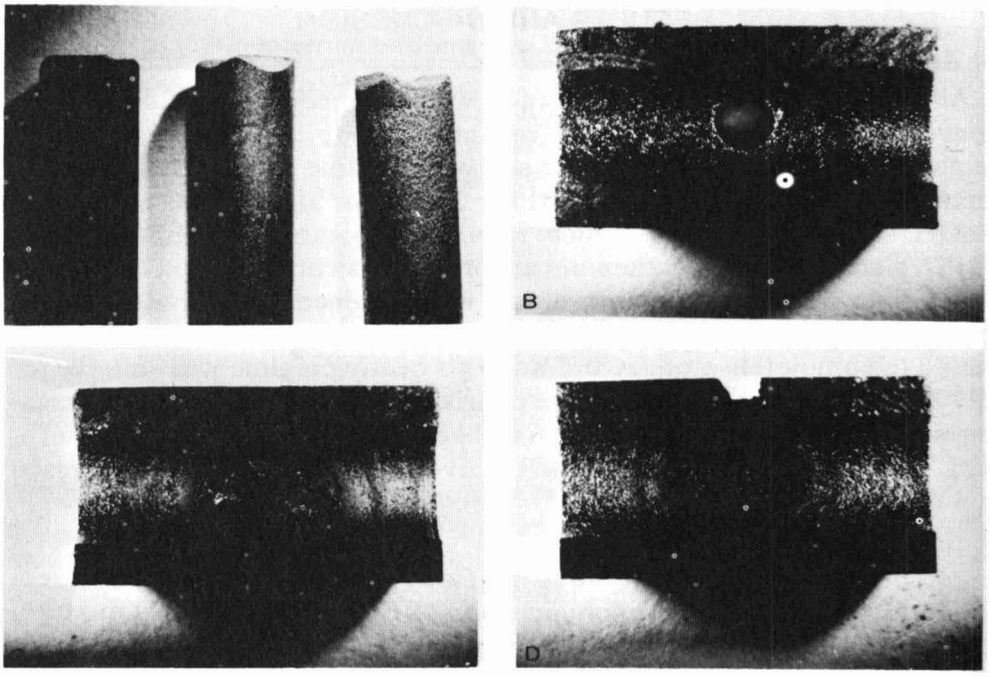

FIG. 2. Micrographs of graphite atomizer parts: (A) contact electrodes (left to right): unused; N_2/Ar/10% CH_4, 100 cycles; N_2/H_2, 50 cycles; (B) unused atomizer tube; (C) atomizer tube, N_2/H_2, 20 cycles; (D) atomizer tube, N_2/Ar/10% CH_4, 30 cycles.

was observed with this matrix. The only elements, other than Ru, expected to volatilize to a significant extent were Hg, I, and F. These elements were tested at up to 5000:1 weight ratio and produced no significant change in sensitivity, either alone or in combination. Ru in this matrix was readily measured by direct comparison with standards in 0.1 *N* HCl.

2. HCl/ethanol scrubber solutions. The initial composition of this scrubber solution was a mixture of equal volumes of 95% ethanol and 6 *N* HCl. Direct atomization of this matrix gave no evidence of molecular absorption or of scattering. The presence of the matrix does, however, reduce the recovery of Ru when compared to standards in 0.1 *N* HCl. Typical data are given in Table 3. The low recovery is apparently due to greater permeation of the ethanol solution into the graphite surface and can be eliminated by evaporating the sample to near dryness and redissolving it in 0.1 *N* HCl. There is also some indication that the peak heights may be more reproducible with direct application of the ethanolic solution. This is probably also attributable to the better wetting characteristics of the ethanol solution.

3. Alkali hydroxide scrubber solutions. Potassium or sodium hydroxide,

TABLE 3
Ru RECOVERY FROM HCl/ETHANOL

Concentration (μg/ml)	Percentage recovery[a]	
	Direct application	Evaporation
0.10	95.4 ± 13.4[b]	—
0.25	83.3 ± 7.6	90.7 ± 13.1
0.50	84.6 ± 3.6	98.9 ± 6.3
1.00	82.1 ± 3.6	99.6 ± 4.3

[a] Referred to standards in 0.1 *N* HCl.
[b] Mean ±1 SD.

initially 3 *M* in aqueous solution, was used as a first scrubber solution. When atomization from this matrix was attempted in the step heating mode, a maximum ash temperature of 1500°C for 25 sec was needed to completely remove background absorption by the alkali metal salt. Background removal with the simultaneous background corrector was not possible due to the large amount of matrix present. The severity of the background interference is indicated by the absorbance vs time trace of Fig. 3A, obtained with the step atomization mode. This operating mode was unsatisfactory due to the need for ashing the sample at 1500°C for 25 sec. Even with the $N_2/Ar/CH_4$ sheath gas mixture, deterioration of the

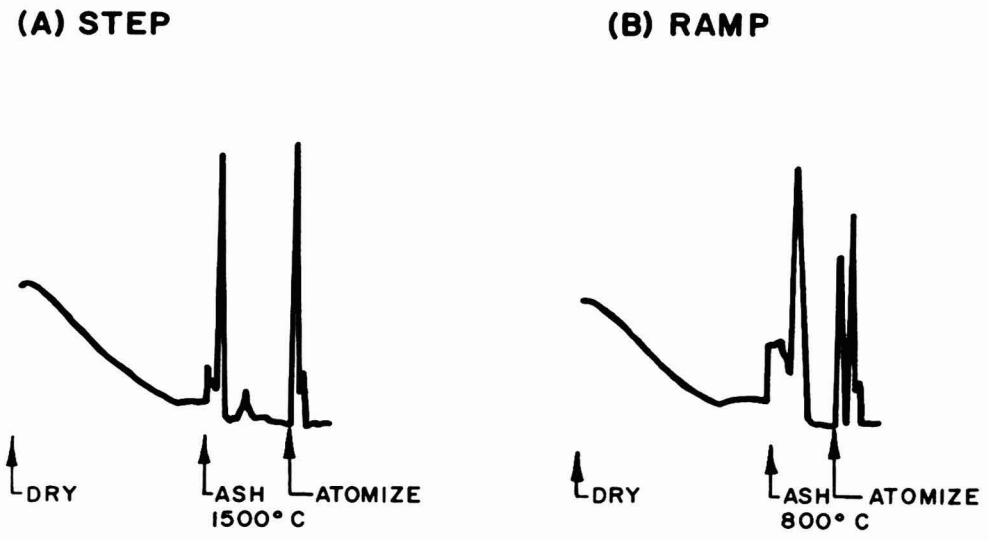

FIG. 3. Background absorption in atomization of the alkali hydroxide matrix: (A) step temperature program, (B) ramp temperature program.

graphite atomizer parts was too rapid. This difficulty was eliminated by atomizing Ru from these matrices in the ramp heating mode. The absorbance vs time trace with this mode is shown in Fig. 3B. This, combined with an ashing temperature of 800°C gave complete resolution of Ru atomic absorption from the background and acceptable atomizer life.

Recovery of Ru from the alkali matrix, measured against standards in 0.1 *N* HCl, is shown in Table 4 and is essentially complete, indicating that matrix matching of the standards is not necessary.

4. Solid samples. Solid samples were taken from various points in the calciner system but in all cases represented essentially the same chemical composition, the principal difference being one of particle size. The Ru in aqueous waste solutions is present as nitroso ruthenium nitrate complexes which, upon calcination, are converted to ruthenium oxides. The matrix is essentially a mixture of oxides of the waste constituents. A basic oxidizing flux was necessary to break down the calcine particles and convert the ruthenium to soluble form. The fluxing agents tested were potassium hydroxide, sodium hydroxide, and sodium peroxide. The hydroxides required addition of potassium nitrate or sodium peroxide to act as oxidants. All combinations of fluxes and oxidants provided adequate attack of the sample matrix at 675°C. At 675°C, the hydroxides tend to creep out of the crucible and descend along the sides. For this reason, sodium peroxide alone was chosen to fuse the samples. A 1-g sample was mixed with 5 g of sodium peroxide and heated at 675°C for 10 min. The ruthenium was leached from the solidified fusion mixture with water. Many of the matrix constituents, including iron, nickel, and zirconium, are precipitated from the basic leachate, providing a preliminary separation.

To separate the Ru from the remaining matrix constituents, solvent extraction was employed. Three complexing agents, ammonium pyrollidinedithiocarbamate (APDC), sodium diethyldithiocarbamate, and diethylammonium diethyldithiocarbamate were tested in combination

TABLE 4
Ru Recovery from Alkali Hydroxide Scrubber Solutions

Concentration (μg/ml)	Percentage recovery[a]	
	3 *M* NaOH[b]	3 *M* KOH[b]
0.10	90.3 ± 33.3[c]	87.1 ± 30.9
0.25	88.7 ± 12.0	100 ± 11.8
0.50	101.2 ± 3.2	98.8 ± 10.0
1.00	98.3 ± 12.0	105.3 ± 13.7

[a] Referred to standards in 0.1 *N* HCl.
[b] Initial scrubber solution composition.
[c] Mean ±1 SD.

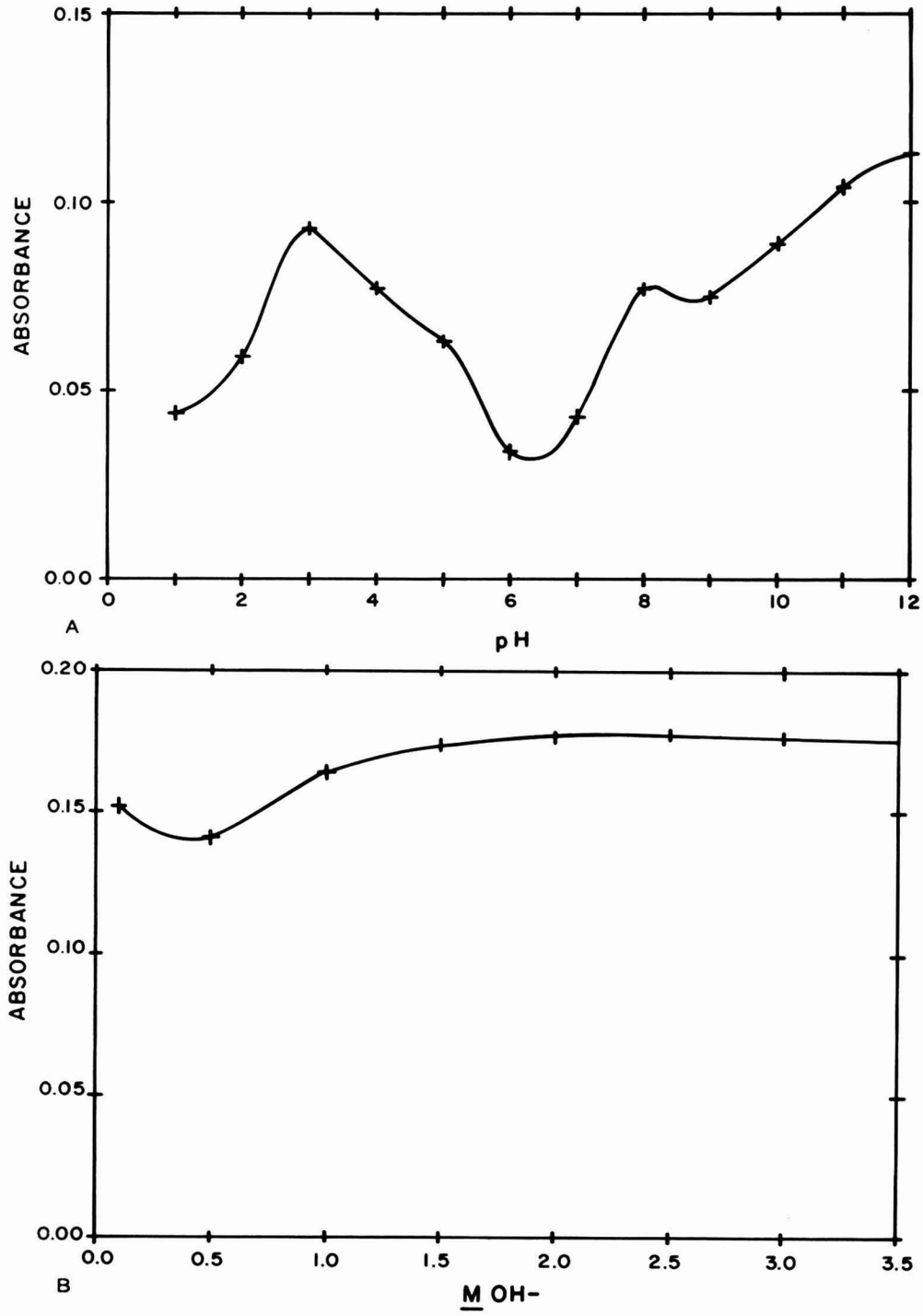

FIG. 4. The pH dependence of ruthenium extraction from (A) pH < 12 and (B) extremely basic solution.

with the solvents methyl isobutyl ketone, butanone, butanol, ethyl acetate, and amyl acetate. APDC in amyl acetate was found to give good recovery of Ru, is the least soluble in the aqueous phase, and could be pipetted into the atomizer with little difficulty. The APDC/amyl acetate system was used in all further studies. The recovery of Ru in this system is a complicated function of pH, as shown in Fig. 4A. However, in strongly basic solution, the recovery of RuO_4^{-2} is high and is essentially constant with $[OH^-] > 1.5$ (Fig. 4B).

To ensure the recovery of Ru from basic solution, it was necessary to prevent formation of Ru(III) and Ru(IV) hydroxides. This was conventiently done by adding I_2 to the basic solution, causing the *in situ* generation of hypoiodite. The hypoiodite oxidizes any ruthenium present to Ru(VI), with no danger of producing volatile RuO_4.

Spectrophotometry was used to investigate the oxidation state of ruthenium in the various steps in the procedure. A 0.1-g sample of RuO_2 was fused as described above. The melt was leached with water, and the spectrum was recorded on a Cary Model 16 recording spectrophotometer. This spectrum is shown in Fig. 5, curve A. The spectrum corresponds to that reported for RuO_4^{-2} (*13*). Concentrated hydrochloric acid was then

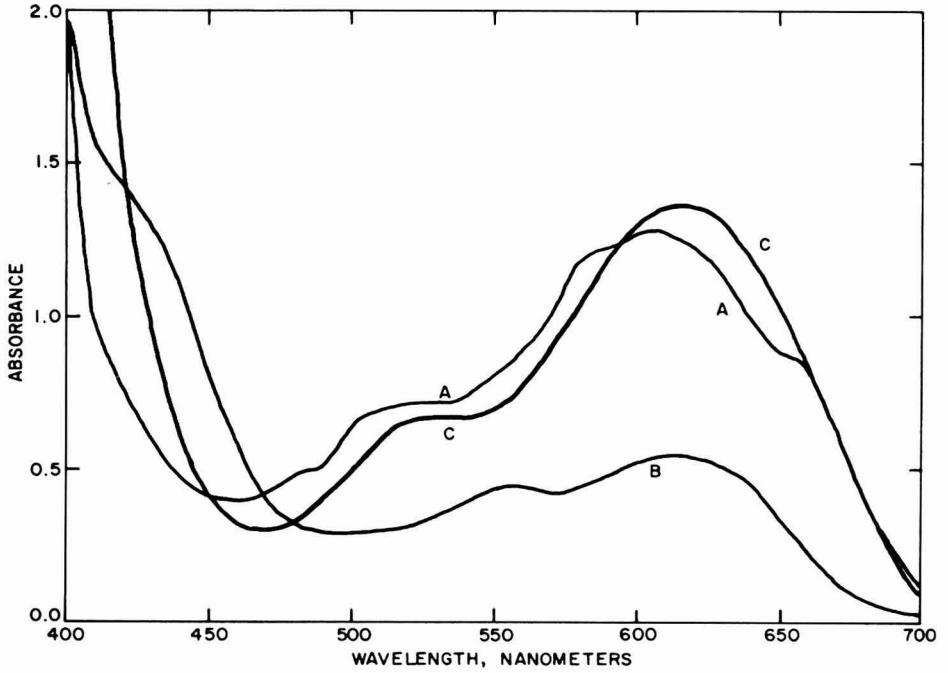

FIG. 5. Visible absorption spectra: (A) melt leachate; (B) reduced with HCl; (C) after I_2 addition.

added, and the spectrum was recorded (Fig. 5, curve B). This spectrum corresponds to that reported (*14*) for a mixture of Ru(IV) oxychloride complexes. The solution was then made 2 *M* in sodium hydroxide, and several iodine crystals were added. The mixture was stirred until the iodine dissolved. The spectrum is shown in Fig. 5, curve C. This spectrum matched that taken earlier of the melt leachate and RuO_4^{-2} (*13*). The solution was then extracted with APDC and amyl acetate. The spectrum is shown in Fig. 6, curve A. A sample of Ru(IV) chloride was also extracted with APDC and amyl acetate. This spectrum is shown in Fig. 6, curve B. Both spectra appeared the same indicating that an additional reduction of the ruthenium takes place upon the addition of APDC to the solution of RuO_4^{-2}.

The completeness of the separation of ruthenium from the matrix was investigated. Samples of the Zr/Al and LWR calcines were fused with sodium peroxide and leached with water. The leachate was acidified and spiked with ruthenium. The acidified leachates were examined by dc arc emission spectrometry and found to contain major amounts of aluminum and sodium and minor amounts of chromium, molybdenum, ruthenium, and zirconium (the latter only from Zr/Al calcine). After extraction, the

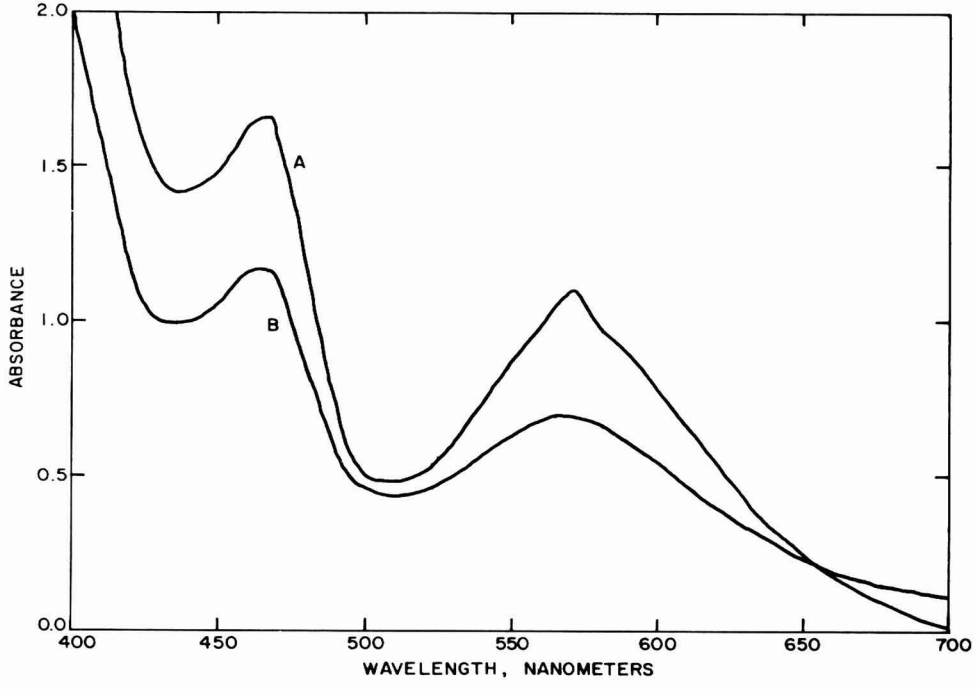

FIG. 6. Visible absorption spectra: (A) extract of APDC complex of sample; (B) extract of Ru(IV) and APDC.

TABLE 5
RESULTS FOR DETERMINATION OF RUTHENIUM IN SOLIDS

Sample	Makeup (wt% of Ru)	Found (wt% of Ru)	SD
Zr/Al-1	0.0086	0.0075	0.0008
Zr/Al-2	0.055	0.058	0.004
Zr/Al-3	0.27	0.25	0.02
LWR	0.76	0.77	0.02

organic phase was evaporated to near dryness and wet-ashed with nitric acid. The resulting solution was examined by emission spectrometry and found to contain major amounts of ruthenium and traces of aluminum and sodium. The recovery of ruthenium was 85.6 ± 0.7%.

The final extraction procedure consisted of making the leachate of the fusion mixture 2 *M* in OH^-, adding iodine crystals, adding 1 ml of a 1% solution of APDC in water, and extracting the ruthenium into 10 ml of amyl acetate. Time allowed for complexation had no significant effect on the recovery as long as it was in excess of 5 min. Extraction time was also not a significant parameter, as long as the solutions were shaken for at least 15 sec. The volume ratio did not affect the recovery significantly as long as it was 5:1 (aqueous/organic) or less.

Solid standards with composition similar to the samples were not available. Known samples were synthesized by grinding weighed amounts of RuO_2 with various ruthenium-free calcine materials. The ruthenium content was determined by comparison with standards in 0.1 *N* HCl, using the fusion–extraction procedure. A comparison of known and measured ruthenium concentrations is given in Table 5.

SUMMARY

Flameless atomic absorption spectrometry has been applied to the determination of subnanogram quantities of ruthenium in a variety of matrices encountered in the solidification of nuclear waste. Detection limits ranged to below 10^{-10} g, depending on the sample matrix. Most matrix effects could be eliminated by proper selection of atomizer temperature program, allowing the use of a single set of standards in 0.1 *N* HCl. The one exception was the calcined solid matrix, where a fusion and extraction were used to dissolve the ruthenium and separate it from matrix constituents.

ACKNOWLEDGMENTS

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MICROCHEMICAL JOURNAL 23, 278–284 (1978)

Analytical Reactions of Substituted Cyanoferrates

2. Pentacyanoamminoferrate (II) in Catalytic Spectrophotometric Determination of Sub-Parts per Million Amounts of Ag^{+}, Au^{3+}, and Hg^{2+} in Solution[1]

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INTRODUCTION

Our increased concern toward the presence and distribution of heavy metals in the water bodies, air, and the soils has necessitated the development of precise, rapid, and sensitive analytical procedures. Among the various sophisticated procedures employed in environmental analytical work, the spectrophotometric and the electrometric technics perhaps still remain popular because of their simplicity and easy adaptiability (*15*).

The combined use of catalytic and spectrophotometric procedures has in fact extended the analytical capabilities into the sub-parts per million range. Fiegl and Caldas (*6*), Asperger *et al.* (*1–3*), and Pinter and Dresner (*14*) have analyzed trace amounts of Ag^{+}, Hg^{2+}, and Pd^{2+} by following the metal-catalyzed ligand displacement reactions between hexacyanoferrate (II) and 2-2′-bipyridyl and orthophenanthroline. Since then several others have explored similar reactions with other organic ligands either by "fixed time" or "initial reaction rate" methods and have found it feasible to quantitize sub-parts per million concentrations of Ag^{+}, Au^{3+} and Hg^{2+} in samples of interest (*4,5,10–13*).

The significance of these metal-catalyzed reactions of analytical importance has been recognized only recently though the underlying principle was known long ago. Yatsimirskii (*19*) has compiled a monogram reviewing such metal-catalyzed ligand exchange reactions.

In our continued investigations on the analytical reactions of substituted cyanoferrates (*8*), it has been found that the pentacyanoamminoferrate ion (PCAF) undergoes an exchange reaction with an organic ligand ferrozine which is catalyzed by Ag^{+}, Au^{3+}, and Hg^{2+} ions. The reaction is rapid and quantitative at sub-parts per million levels of these ions under controlled experimental conditions. In addition it has been

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noticed that even organomercurials can catalyze this reaction. Based on these observations an analytical procedure has been developed for the trace analyses of the three cations. The use of substituted cyanoferrates in catalytic spectrophotometric determinations does not appear to have occurred as yet. Harrop and Herington (*9*) have suggested some qualitative reactions of PCAF in conjunction with rubeanic acid.

MATERIALS AND METHODS

Reagents. (1) Stock solutions of the cations Hg^{2+}, Au^{3+}, and Ag^+ were prepared at a concentration of 1000 μg/ml by accurately weighing their certified analytical grade nitrates or chlorides in double-distilled deionized water. These were appropriately diluted when required. The Hg^{2+} solution was maintained at 0.01 *M* acidity with H_2SO_4.

(2) The reagent sodium pentacyanoamminoferrate (PCAF) used in this study was procured from Fisher Scientific Co. A 250-ml stock (0.01 *M*) of the reagent was prepared by weighing 0.7433 g of the compound in double-distilled deionized water. The reagent tends to become turbid after a few days of storage, hence it was prepared fresh for each new set of experiments, immediately before use.

(3) The reagent ferrozine [3-(2-pyridyl)-5,6-bis(4-phenylsulfonic acid)-1,2,4-triazine], as a sodium salt and procured from Aldrich chemicals, was used as such. A stock solution (0.01 *M*) was prepared by dissolving 5.685 g in 1 liter of double-distilled deionized water.

(4) The ammonium acetate/acetic acid buffer employed in this study was prepared by dissolving 62.5 g of CH_3COONH_4 in 175 ml of CH_3COOH and adding double-distilled deionized water to 250 ml.

The experimental studies were carried out at a constant temperature of 25 $\pm$ 0.5°C by using the Margini whirl constant-temperature water bath of the Blue M Electric Co.

The absorption data were recorded on the Spectronic-70 spectrophotometer from Bausch and Lomb, using optically matched 1-cm rectangular quartz calls.

Procedure. Place 5 ml of the ferrozine solution in several 50-ml volumetric flasks. Add to each flask varying amounts of the appropriately diluted cation solution to give the desired concentration (0–3 ppm) in 50 ml of solution. Dilute to approximately 44 ml with double-distilled deionized water. Add 1 ml of the buffer solution and suspend all the flasks into constant-temperature water for 5 min. Similarly run a blank with acetate buffer but free of any cation.

Also place a 50-ml volume of the pentacyanoamminoferrate stock (0.01 *M*) in the constant-temperature water bath for 5 min.

Transfer 5 ml of the pentacyanoamminoferrate solution after 5 min of equilibration in a water bath to each of the cation–ferrozine and blank

solutions. Mix well and allow the reaction to proceed at 25 ± 0.5°C for 10 min. Run a new blank for each set of experiments. Measure the absorbance of each solution against the blank after exactly 10 min at 562 nm. The mixing is so spaced that enough time is permitted for the operator to make the absorbance measurement at each concentration.

RESULTS AND DISCUSSION

The analytical reaction responds linearly for Hg^{2+} (0.01–0.4 ppm), Ag^{+} (0.02–0.5 ppm), and Au^{3+} (0.1–3.0 ppm) as seen from the experimental data in Fig. 1. The relative standard deviations (five determinations) at the lowest concentrations of quantization of cations, Hg^{2+} (0.01 ppm), Ag^{+} (0.02 ppm), and Au^{3+} (0.1 ppm), do not exceed 2.7, 4.7, and 4.3%, respectively, thereby offering good precision for analyses.

The data suggest that the order of sensitivity is $Hg^{2+} > Ag^{+} > Au^{3+}$. This may be rationalized on the basis of the cyanide complexes of these metals which also follow the same order. Thus, the stronger tendency of the Hg^{2+} ion for CN^{-} accelerates the release of CN^{-} from PCAF. This in turn partly or fully liberates Fe^{2+} from the cyanide complex to from the Fe^{2+}–ferrozine chelate imparting magenta color absorbing at 562 nm (*16*). The reaction sequence could be envisaged as similar to hexacyanoferrate systems (*2,6*).

$$[Fe(CN)_5(NH_3)]^{3-} + H_2O \rightleftharpoons [Fe(CN)_5(H_2O)]^{3-} + NH_3 \quad (1)$$

$$[Fe(CN)_5(H_2O)]^{3-} + H_2O + Hg^{2+} \rightleftharpoons HgCN^{+} + [Fe(CN)_4(H_2O)_2]^{2-} \quad (2)$$

$$[Fe(CN)_4(H_2O)_2]^{2-} + \text{ferrozine} \rightleftharpoons [Fe(CN)_4(\text{ferrozine})]^{2-} + 2H_2O \quad (3)$$

$$H^{+} + HgCN^{+} \rightleftharpoons Hg^{2+} + HCN. \quad (4)$$

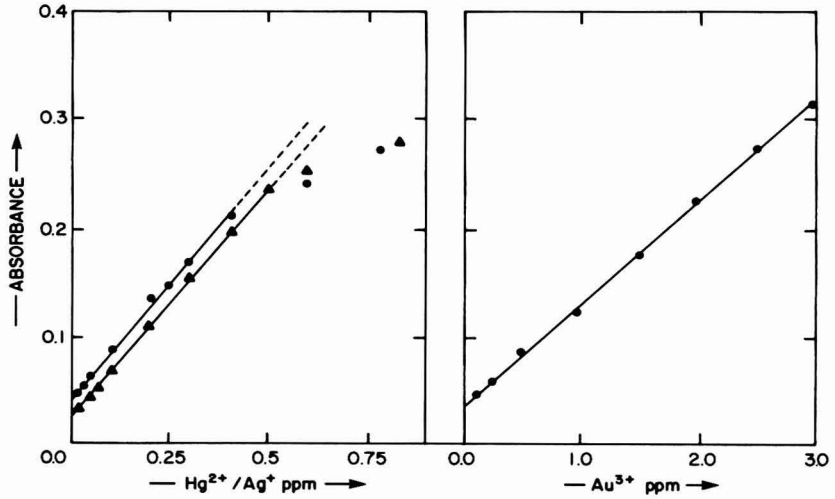

FIG. 1. Spectrophotometric response of Hg^{2+}, Ag^{+}, and Au^{3+} in the catalyzed displacement reactions.

Toma *et al.* (*18*) have also noticed the formation of hydroxocyanide as seen in Reaction (1) above as an intermediate in the substitution reactions of PCAF with some organo compounds. Reactions (2) and (3) depict chelate formation through the Hg^{2+}-catalyzed reaction as seen in Eq. (4). It is conceivable that a mixed cyanoferrozine species is formed since a similar $[Fe(CN)_4$ (orthophenanthroline)$]^{2-}$ chelate is known to exist in an aqueous solution (*17*). If the entire CN^- is released all at once, the stable $Hg(CN)_4^{2-}$ complex would inhibit the Hg^{2+}catalytic activity as postulated in Reaction (4) above. On the other hand, ferrozine being a bidenate ligand, it requires at least two coordination positions around Fe^{2+}. This is probably accomplished in Reactions (2) and (3) as cited above.

The metal ion catalytic effect is supported by the fact that in its absence direct reaction between PCAF and ferrozine is negligibly small, while even a 10-ppb concentration of Hg^{2+} is sufficient to force the indicator Reaction (3) to proceed toward the right rapidly. The experimental data in all three cases show that absorbance increases for the first 10 min and then levels off. The "fixed time" period of 10 min in this case is thus advantageous and appears justified. The slowing down of the reaction can be attributed to cyanide complex formation. With the progress of Reaction (4), enough CN^- molecules are accumulated to permit $Hg(CN)_4^{2-}$ formation and, similarly, the silver and the gold complexes. This in turn blocks the catalytic effect by reducing the free metal ion concentration in the medium.

The reagent concentrations and the pH have a pronounced effect on the reaction rate. The optimum concentrations for PCAF (10^{-4} *M*) and ferrozine (10^{-3} *M*) have been experimentally established with Au^{3+} as reference. Similarly pH must remain controlled in a narrow pH 4–6 range, as seen in Table 1. The behavior of mercury and silver ions is similar to that of the gold ion. At increased pH the hydrolytic precipitation or increased metal–cyano complex stability inhibits catalytic displacement reaction. At lower pH (<2) the reagent PCAF itself is somewhat unstable. Therefore all determinations were made at pH 4.8 controlled with $HOAC/NH_4OAC$ buffer.

The use of substituted cyanoferrate appears attractive in one sense that no initiation or quenching of the reaction at elevated temperatures is required as was found necessary in the hexacyanoferrate systems (*11,13*).

There are however some experimental interferences due to diverse ions similar to those seen in the hexacyanoferrate systems (*4*). Some data for Hg^{2+} are shown in Table 2. The interference due to Fe^{3+} is unexpected. It appears that in the presence of PCAF there is reduction to the Fe^{2+} state which forms directly the usual tris chelate with ferrozine. This fact has been made use of in the trace analysis of iron in environmental samples (*7*). The negative interferences due to Zn^{2+}, Cd^{2+}, and Cu^{2+} perhaps stem from their reactions with ferrozine, which inhibit full color development in the sys-

TABLE 1
THE PCAF, FERROZINE, AND pH EFFECTS ON THE REACTION RATE MEASURED AT 562 nm WITH 2 ppm OF Au^+ AT A "FIXED TIME" PERIOD OF 10 MIN

	Reagent PCAF ($M \times 10^5$)	Net absorbance	Reagent ferrozine ($M \times 10^4$)	Absorbance	pH	Net absorbance[a]
1.	2.0	0.047	1.0	0.170	2.0	0.100
2.	4.0	0.092	2.0	0.175	3.4	0.149
3.	6.0	0.135	4.0	0.185	4.0	0.162
4.	8.0	0.175	6.0	0.225	4.5	0.223
5.	10.0	0.225	8.0	0.220	4.8	0.225
6.	12.0	0.225	10.0	0.223	5.9	0.224
7.	16.0	0.225	12.0	0.220	7.0	0.085
8.	18.0	0.235	14.0	0.220	8.0	0.065
9.	20.0	0.230	—	—	9.0	0.020

[a] Absorption data at 10^{-4} *M*, PCAF, 10^{-3} *M*, ferrozine.

tem. The anions $S_2O_3^{2-}$ and S^{2-} are known for their direct complexation reactions with heavy metal ions.

In addition to trace inorganic mercury analysis, the system remains responsive to $(CH_3)Hg^+$ and $(C_2H_5)Hg^+$ ions as well. The comparative absorbance data are summarized in Table 3. This aspect could remain helpful in the total mercury analysis as is often required in aqueous environmental samples.

TABLE 2
EFFECT OF DIVERSE IONS ON Hg^{2+} CATALYSED DISPLACEMENT REACTIONS BETWEEN PCAF (10^{-4} *M*) AND FERROZINE (10^{-3} *M*) AT pH 4.8 AND 25 ± 0.5°C[a]

Diverse ion	Tolerance limit (ppm)	Diverse ion	Tolerance limit (ppm)
Ca^{2+}	10.0	Cl^-	20.0
Mg^{2+}	10.0	SO_4^{2-}	50.0
Ni^{2+}	10.0	CH_3COO^-	N.I.[b]
Al^{3+}	5.0	NO_3^-	N.I.[b]
Mn^{2+}	5.0	Na^+	N.I.[b]
Co^{2+}	2.0	NH_4^+	N.I.[b]
VO^{2+}	1.0	$S_2O_3^{2-}$	<0.4
Cu^{2+}	<0.4	S^{2-}	<0.4
Zn^{2+}	<0.4		
Cd^{2+}	<0.4		
Fe^{3+}	<0.4		
Au^{3+}	<0.4		
Ag^+	<0.4		

[a] Hg^{2+} concentration: 0.4 ppm.
[b] No influence.

TABLE 3
COMPARATIVE DATA ON ORGANOMERCURIALS AND IONIC MERCURY IN THE PCAF (10^{-4} *M*)–FERROZINE (10^{-3} *M*) SYSTEM AT pH 4.8 AND 25 ± 0.5°C

Concentration (ppm)	Net absorbance[a]		
	Hg^{2+}	$(CH_3)\ Hg^+$	$(C_2H_5)\ Hg^+$
0.05	0.062	0.072	0.073
0.10	0.087	0.100	0.110
0.20	0.140	0.141	0.162
0.30	0.186	0.185	0.201
0.40	0.210	0.220	0.235
0.60	0.255	0.252	0.270

[a] An average of at least five determinations.

In conclusion trace analysis of Ag^+, Au^{3+}, and Hg^{2+} by the use of PCAF and ferrozine systems is feasible. Unfortunately there are some interferences, however, analytical schemes can be devised to render this system useful in environmental analytical work.

SUMMARY

The sub-parts per million amounts of Ag^+, Au^{3+}, and Hg^{2+} ions in solution have been determined by a catalytic spectrophotometric reaction. These ions catalyze the ligand exchange reaction between pentacyanoamminoferrate (II) ion and ferrozine [3-(2-pyridyl)-5,6-bis(4-phenylsulfonic acid)-1,2,4-triazine] to produce a strongly colored chelate absorbing at 562 mm. The relative standard deviations for Hg^{2+} (10 ppb), Ag^+ (20 ppb), and Au^{3+} (100 ppb) do not exceed 5%. The reaction is rapid in slightly acidic medium at room temperature. A mechanism is proposed and the effects of the experimental parameters have been determined.

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The Linear Correlation of the $(\Delta R'_m)_i$ Function Determined for the Ortho Derivatives of Phenol, Aniline, Benzaldehyde, and Nitrobenzene

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INTRODUCTION

Separation of organic substances by means of adsorption thin-layer chromatography depends to large extent on their chemical structure and properties. Snyder (*1–3*) succeeded in proving that the energy of interactions between the molecule of the adsorbed compound and the adsorbent surface can be described as a sum of interactions between each atom or functional group from the molecule and the adsorbent surface:

$$S^0 = \sum^{i} Q_i^? .$$

Snyder's theory also succeeded in relating the adsorption equilibrium constant K° of a given substance to its specific surface and the adsorbent activity $\propto$ (*4, 5*), and for consequent modification of two substances P and P_i the following equation was derived:

$$(R'_m)_{P-i} - (R'_m)_P = \propto (Q_i^? - a_i\epsilon^0) = (\Delta R'_m)_i .$$

The $Q_i^?$ values can be directly determined on the basis of the experimental $\Delta R'_m$ data. The energy values of the single functional groups influence the total adsorption energy values S^0 for the molecule of a given substance. Relating the S^0 and $Q_i^?$ parameters to the chromatographically measurable R_m or ΔR_m values provides us with valuable information on the separation power of the chromatographic systems applied.

In our work the selected ortho isomers derived from phenol, aniline, benzaldehyde, and nitrobenzene were separated by means of adsorption thin-layer chromatography, using for each group of substances two developing systems. The results helped to determine the $(\Delta R'_m)_i$ values, which are a rough measure of the adsorption energy of functional groups present in the *ortho* position, compared with the reference compounds, such as phenol, aniline, benzaldehyde, and nitrobenzene. The linear correlation of the $(\Delta R'_m)_i$ function was established for two developing systems.

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TABLE 1
SEPARATION OF THE ORTHO-ISOMERIC DERIVATIVES OF PHENOL ($\times 10^2$)

Substance analyzed	Mobile phase (1)			Mobile phase (2)		
	R_f	R_m	$(\Delta R'_m)_i$	R_f	R_m	$(\Delta R'_m)_i$
Phenol	15.6	73.3	—	37.0	23.1	—
2-Aminophenol	1.8	173.7	100.4	7.0	121.3	98.2
Resorcinol	4.2	135.8	72.5	10.1	94.9	71.8
2-Metoxyphenol	21.8	55.5	−17.8	40.1	17.4	−5.7
2-Methylphenol	25.9	45.6	−27.7	44.1	9.4	−13.7
2-Chlorophenol	32.9	31.0	−42.3	49.3	1.2	−21.9
2-Acetylphenol	44.0	10.5	−62.8	61.2	−19.8	−42.9
Salicylaldehyde	47.1	5.0	−68.3	64.3	−25.6	−48.7
2-Nitrophenol	59.7	−17.0	−90.3	72.3	−41.7	−64.8

TABLE 2
SEPARATION OF THE ORTHO-ISOMERIC DERIVATIVES OF ANILINE ($\times 10^2$)

Substance analyzed	Mobile phase (3)			Mobile phase (4)		
	R_f	R_m	$(\Delta R'_m)_i$	R_f	R_m	$(\Delta R'_m)_i$
Aniline	45.9	7.2	—	56.1	−10.7	—
o-Diaminobenzene	15.4	74.0	66.8	26.3	44.7	55.4
2-Toluidine	18.5	64.4	57.2	30.1	36.6	47.3
2-Hydroxyaniline	21.2	57.0	49.8	36.6	23.9	34.6
2-Nitroaniline	46.9	5.2	−2.0	65.8	−28.4	−17.7
2-Metoxyaniline	51.8	−3.1	−10.3	68.4	−33.5	−22.8
2-Acetylaniline	54.5	−7.8	−15.0	72.8	−42.8	−32.1
2-Aminobenzaldehyde	58.1	−14.1	−21.3	74.6	−46.8	−36.1
2-Chloroaniline	62.8	−22.7	−29.9	78.1	−55.2	−44.5

TABLE 3
SEPARATION OF THE ORTHO-ISOMERIC DERIVATIVES OF BENZALDEHYDE ($\times 10^2$)

Substance analyzed	Mobile phase (5)			Mobile phase (6)		
	R_f	R_m	$(\Delta R'_m)_i$	R_f	R_m	$(\Delta R'_m)_i$
Benzaldehyde	53.9	−7.0	—	60.0	−17.6	—
2-Aminobenzaldehyde	29.8	37.2	44.2	36.9	23.3	39.9
2-Nitrobenzaldehyde	33.4	30.0	37.0	42.8	12.6	30.2
Salicylaldehyde	45.4	8.0	15.0	62.8	−22.7	−5.1
2-Tolualdehyde	51.2	−2.0	5.0	67.8	−32.3	−14.7
2-Metoxybenzaldehyde	53.1	−5.4	1.6	71.6	−40.2	−22.6
2-Chlorobenzaldehyde	59.8	−17.2	−10.2	75.0	−47.7	−37.1

TABLE 4
SEPARATION OF THE ORTHO-ISOMERIC DERIVATIVES OF NITROBENZENE ($\times 10^2$)

Substance analyzed	Mobile phase (7)			Mobile phase (8)		
	R_f	R_m	$(\Delta R'_m)_i$	R_F	R_m	$(\Delta R'_m)_i$
Nitrobenzene	58.4	−14.7	—	66.8	−30.4	—
o-Dinitrobenzene	16.6	70.1	84.8	19.7	61.0	91.4
2-Nitroaniline	21.6	56.0	70.7	26.0	45.4	75.8
2-Nitroacetophenone	26.1	45.2	59.9	30.9	34.9	65.3
2-Nitrobenzaldehyde	28.3	40.4	55.1	33.8	29.2	59.6
2-Nitrophenol	53.3	−5.7	9.0	59.8	−17.2	13.2
2-Nitrotoluene	66.9	−30.8	−16.1	74.5	−46.5	−16.1
2-Nitroanisole	71.4	−39.7	−25.0	78.2	−55.5	−25.1

EXPERIMENTAL

The ortho isomers of the substances examined were separated on ready-made glass plates (E. Merck, West Germany), covered with silica gel 60-F_{254} with a layer thickness of 0.25 mm, and activated at 110°C for 30 min. For each group of isomers (with respect to the character of the main substituent) the two three-component systems were developing in UV light, with a wavelength of 254 nm applied.

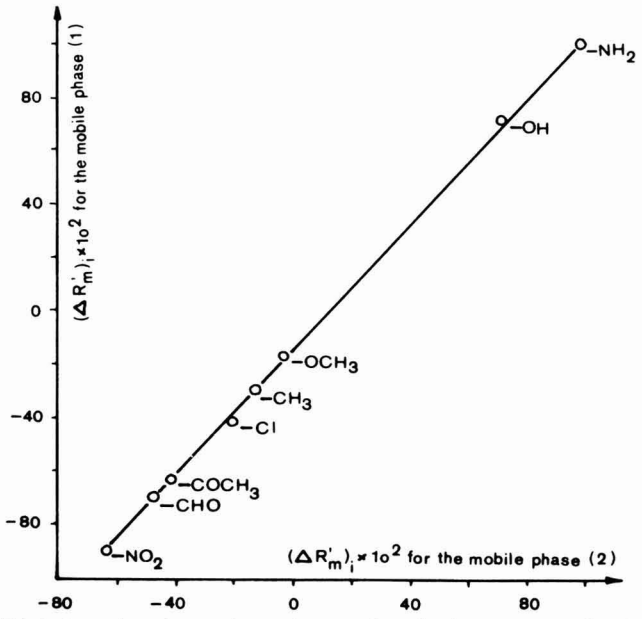

FIG. 1. The $(\Delta R'_m)_i$ function dependence in two developing systems for the ortho-isomeric derivatives of phenol.

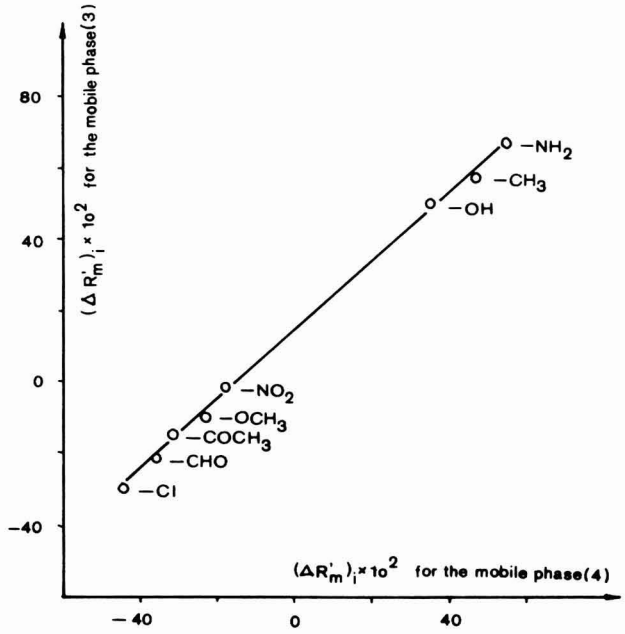

FIG. 2. The $(\Delta R'_m)_i$ function dependence in two developing systems for the ortho-isomeric derivatives of aniline.

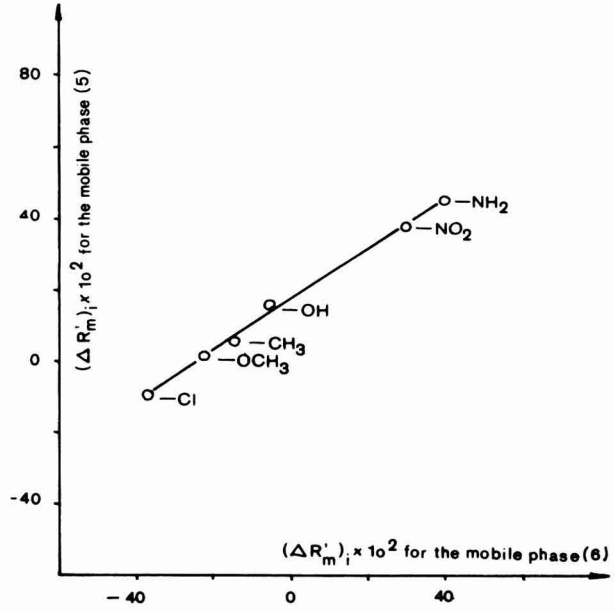

FIG. 3. The $(\Delta R'_m)_i$ function dependence in two developing systems for the ortho-isomeric derivatives of benzaldehyde.

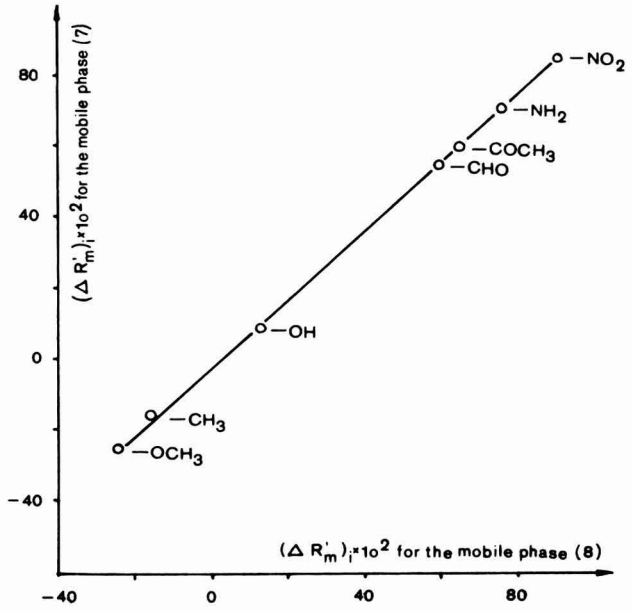

FIG. 4. The $(\Delta R'_m)_i$ function dependence in two developing systems for the ortho-isomeric derivatives of nitrobenzene.

	Ortho isomers of phenol		
1	*n*-Hexane–benzene–chloroform	v/v/v	1:1:1
2	Cyclohexane–dichloromethane–ethyl ether	v/v/v	4:1:1
	Ortho isomers of aniline		
3	Cyclohexane–benzene–ethyl acetate	v/v/v	2:1:1
4	Carbon tetrachloride–ethyl ether–acetone	v/v/v	6:3:1
	Ortho isomers of benzaldehyde		
5	*n*-Hexane–benzene–ethyl ether	v/v/v	3:1:1
6	Cyclohexane–dichloromethane–ethyl acetate	v/v/v	8:1:1
	Ortho isomers of nitrobenzene		
7	*n*-Hexane–benzene–ethyl acetate	v/v/v	7:2:1
8	Cyclohexane–chloroform–acetone	v/v/v	8:1:1

RESULTS

The results of the separation of the substances analyzed are shown in Tables 1–4, in the form of the R_f, R_m, and $(\Delta R'_m)_i$ coefficient values. By means of the Snyder equation the $(\Delta R'_m)_i$ values were calculated for the developing systems applied for different functional groups, present in the ortho position in phenol, aniline, benzaldehyde, and nitrobenzene. The results are also shown in Figs. 1–4 as a mutual dependence of $(\Delta R'_m)_i$

values for two developing systems and they demonstrate linear correlation, independent of the structure and properties of the substituents in the ortho position.

DISCUSSION

The investigations show that the adsorption energy Q_i^0 of a given substance differs considerably from the adsorption energy of its derivatives, which include an additional functional group in the ortho position. It results in significant differences in the R_f values for the basic compound and its ortho derivatives.

The interaction between the mobile phase and the molecules of the substances investigated is constant, similar to the "ortho" effect, which is related to the presence of intramolecular hydrogen bonds. It should be an explanation of the constant $(\Delta R'_m)_i$ values for the mobile phases investigated. The presentation of the experimental results in the form of mutual dependence between two $(\Delta R'_m)_i$ values from two different mobile phases results in the obtaining of a linear correlation for all the functional groups examined in the ortho position toward the $-OH$, $-NH_2$, $-CHO$, and $-NO_2$ groups in the benzene ring. This concept of additivity of chromatographic data could perhaps be applied in predicting the R_f values of substances with known structures and also in performing the opposite task, i.e. elucidating molecular structure from chromatographic data. In addition, the linear correlation of the $(\Delta R'_m)_i$ values, which is shown in Figs. 1–4, enables the group identification with different substituents in a constant position in a benzene ring.

SUMMARY

The additivity of selected chromatographic data with a group of ortho derivatives of phenol, aniline, benzaldehyde, and nitrobenzene has been proved with the help of the theoretical approach developed by Snyder (1–5), and this concept is assumed to be useful in the attempt to predict the R_f values of substances with known structures or in elucidating such structures with compounds having known R_f values.

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Spectrophotometric Determination of Cerium(IV), Arsenic(III), and Nitrite with Promazine Hydrochloride

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INTRODUCTION

Promazine hydrochloride, PMH, 10-(3-dimethylamino propyl) phenothiazine hydrochloride *(5)*, was proposed for the spectrophotometric determination of palladium. In the present investigation the authors have studied the reaction of PMH with cerium(IV) and proposed PMH as a sensitive reagent for the spectrophotometric determination of cerium(IV), arsenic (III), and nitrite.

EXPERIMENTAL

Reagents

Cerium(IV) sulfate solution. A stock solution of cerium(IV) sulfate was prepared in 0.5 *M* sulfuric acid and standardized with arsenic(III) solution.

Arsenic(III) solution. A 0.01 *N* sodium arsenite solution was prepared from Analar arsenic trioxide.

Sodium nitrite solution. Approximately 0.02 *N* sodium nitrite solution was prepared in doubly distilled water and standardized by the cerium(IV) sulfate method *(9)*.

PMH solution. A 0.2% solution of PMH was prepared in doubly distilled water and stored in an amber bottle in a refrigerator.

Diverse ions. Solutions of diverse ions of suitable concentrations were prepared using analytical grade reagents.

Apparatus. Beckman spectrophotometer Model DB with matched 1-cm silica cells was used for absorbance measurements.

Procedure for the Determination of Cerium(IV)

An aliquot of the stock solution containing 12.5–525 μg of cerium(IV), 5 ml of 5 *M* sulfuric or phosphoric acid, and 2.5 ml of 0.2% PMH solution were taken in a 25-ml volumetric flask, and the solution was diluted to the mark with doubly distilled water. The flask was shaken and the absorbance was measured at 505 nm against a corresponding reagent blank prepared in the same manner. The amount of cerium was then deduced from the calibration curve.

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Procedure for the Determination of Arsenic(III)

Different aliquots, 0.5, 1.0, 1.5, 2.0, . . . , 6.0 ml of arsenic(III) solution (3.75–65 μg) were taken in 12 25-ml volumetric flasks. A 13th 25-ml flask was used for a simultaneous blank determination. Five milliliters of 2 *M* sulfuric acid, 1.0 ml of osmium(VIII) (1.2 μg), and 2 ml of 0.001 *M* cerium(IV) sulfate were then added successively to each flask. After shaking the solution, 5 ml of 5 *M* phosphoric acid and 2.5 ml of 0.2% PMH solution were added and the volume was made up to the mark with doubly distilled water. All of the 13 solutions were shaken thoroughly and their absorbances were measured at 505 nm against the reagent blank containing no cerium(IV) and arsenic(III). The amount of arsenic(III) in a test solution was then deduced from the calibration curve constructed by plotting the concentration of arsenic(III) versus the difference in the absorbance readings between the blank (in the 13th flask) and the sample.

Procedure for the Determination of Nitrite

Five milliliters of 1 *M* sulfuric acid and 2 ml of 0.001 *M* cerium(IV) sulfate were taken in each of 12 25-ml volumetric flasks. Five milliliters of sodium nitrite solution containing 1–42.0 μg of nitrite was transferred into each flask and the flasks were shaken well. After 5 min, 5 ml of 5 *M* phosphoric acid and 2.5 ml of 0.2% PMH solution were added and the volume was made up to the mark with doubly distilled water. A blank containing all the reagents except nitrite was prepared in the 13th 25-ml flask. The absorbances of 13 solutions were measured at 505 nm against the reagent blank containing no cerium(IV) and nitrite. The amount of nitrite in a test solution was deduced from the calibration curve of the concentration of nitrite versus the difference in the absorbance readings between the blank (in the 13th flask) and the sample.

RESULTS AND DISCUSSION

Determination of cerium(IV). PMH is readily oxidized to the red-colored species by means of cerium(IV) at room temperature in the presence of sulfuric or phosphoric acid. The sensitivity and stability of the red species, believed to be a free radical *(3)*, depend on the nature and concentration of the acid medium. The sensitivity in three acid mediums is in the order of $H_3PO_4 > H_2SO_4 > HAc$. The stability of the red intermediate in 1 *M* H_3PO_4, H_2SO_4, and HAc is 50, 35, and 10 min, respectively. The absorbance readings are unstable in hydrochloric acid medium. Nitric acid medium cannot be used as it oxidizes PMH to a red intermediate. Maximum absorbance is achieved in 0.5–2 *M* sulfuric acid or in 0.5–2.5 *M* phosphoric acid solution. The maximum color intensity is not observed below 0.5 *M* sulfuric or phosphoric acid. The reagent is slowly oxidized at acidities higher than 2 *M* sulfuric acid or 2.5 *M* phosphoric

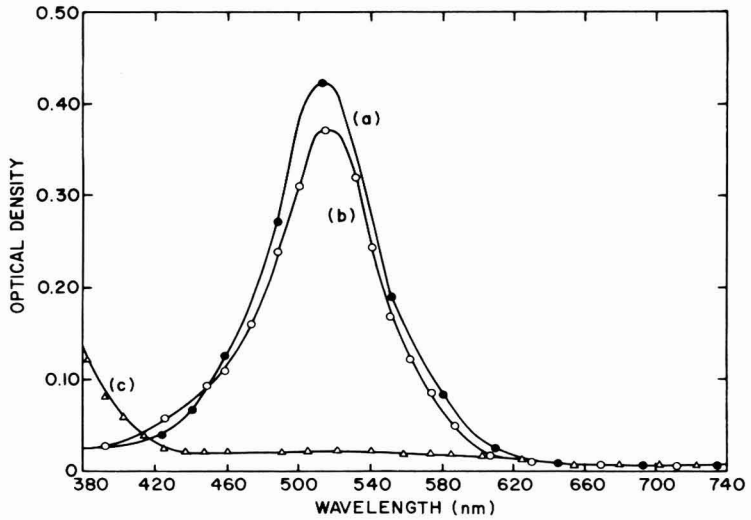

FIG. 1. Absorption spectra of (a) the red intermediate of PMH in 1 *M* phosphoric acid, (b) the red intermediate of PMH in 1 *M* sulfuric acid, (c) PMH in 1 *M* phosphoric or sulfuric acid. [PMH] = 6.25×10^{-4} *M*

acid. One molar solution of the phosphoric acid medium has been selected because of higher sensitivity and less interference of foreign ions.

Absorption spectra. The absorption spectra of the red intermediate in 1 *M* phosphoric acid [Fig. 1(a)], 1 *M* sulfuric acid [Fig. 1(b)], and PMH [Fig. 1(c)] are shown in Fig. 1. The maximum absorbance of the red-colored species is found at 505 nm. The reagent under similar conditions does not absorb around this wavelength, thus promoting excellent analytical conditions. The order of addition of reagents is not critical. The absorbance is not affected by temperature in the range 10–60°C. Above 60°C the absorbance gradually decreases with the rise of temperature.

Effect of reagent concentration. The effect of reagent concentration is examined by measuring the absorbance at 505 nm of solutions containing 8 μg/ml of cerium(IV) and a varying amount of PMH. An 11-fold molar excess of the reagent is necessary for the full development of the color intensity. The optimal amount of the reagent is 2.5 ml of 0.2% reagent solution in a final volume of 25 ml.

Calibration, range, and sensitivity. Beer's law is valid over the concentration ranges 0.5–15 ppm in sulfuric acid and 0.5–21 ppm in phosphoric acid. The optimum concentration ranges for the effective spectrophotometric determination evaluated by Ringbom's method are 1.2–14.6 ppm in sulfuric acid and 1.2–20.5 ppm in phosphoric acid. The molar absorptivity for the red-colored species is 6.429×10^3 liter mole^{-1} cm^{-1} in sulfuric acid and 7.098×10^3 liter mole^{-1} cm^{-1} in phosphoric acid at 505 nm. For log $I_0/I = 0.001$, the sensitivity of the reaction as calculated

from Beer's law data is 0.022 $\mu g/cm^2$ in sulfuric acid and 0.019 $\mu g/cm^2$ in phosphoric acid. Errors are in general about $\pm 2\%$.

The sensitivity of the proposed method is more than that of *o*-aminophenol *(8)*, sulfanilic acid *(6)*, and salicylhydroxamic acid *(4)*, which have been proposed as sensitive spectrophotometric reagents for cerium. The sensitivity of the present method is less than that of *o*-tolidine *(2)*, phenylanthranilic acid *(1)*, and xylenol orange *(7)*, which are used for the determination of cerium.

Effect of diverse ions. In order to assess the possible analytical applications of the reaction the effects of some ions which often accompany cerium are studied. For these studies, different amounts of the ionic species are added to 200 μg of cerium(IV) in 1 *M* phosphoric acid in 25-ml volumetric flasks and the color is developed as outlined in the procedure. The following amounts (μg/ml) of foreign ions are found to give less than 2% error in the determination of 8 μg/ml of cerium(IV): La(III) 4000, Pr(III) 4000, Nd(III) 4000, Gd(III) 4000, Dy(III) 4000, Ho(III) 4000, Er(III) 4000, Yb(III) 4000, Y(III) 4000, Tb(III) 4000, As(V) 4000, Os(VIII) 6, Ru(III) 5, Pd(II) 0.8, Pt(IV) 15, Ir(III) 18, Rh(III) 20, Au(III) 0.20, Cu(II) 525, Ni(II) 667, Co(II) 261, Fe(III) 300, Ag(I) 5, Mg(II) 2000, Zn(II) 1822, Th(IV) 232, Mo(VI) 250, Zr(IV) 500, U(VI) 1600, chloride 8400, bromide 3600, iodide 0.5, fluoride 4400, sulfate 15200, phosphate 8000, nitrate 8800, acetate 7200, citrate 6400, tartrate 7920, thiosulfate 0.8, and EDTA 0.9. The ions of dichromate, vanadate, iodate, permanganate interfere at all concentrations.

It can be seen that many diverse cations, especially lanthanides, do not interfere in the determination of cerium(IV). The major advantage of this method is that PMH can be used as a selective reagent for the determination of cerium(IV) in the presence of large quantities of other lanthanides in readily attainable oxidation states without the use of masking agents.

Determination of cerium in misch metal. The composition of the commercial misch metal is 50% Ce, 25% La, 15% Nd, 5% Fe, and 5% mixtures of Pr, Eu, Gd, and Er. Analyzed samples of misch metal were not available. Therefore synthetic mixtures corresponding to misch metal were prepared and the cerium content was determined following the standard procedure. The results are given in Table 1.

Determination of arsenic(III) and nitrite. Arsenic(III) and nitrite are indirectly determined spectrophotometrically. Arsenic(III) is quantitatively oxidized to arsenic(V) instantaneously by a known excess of cerium(IV) sulfate (20–100%) in 0.25 *M* sulfuric acid medium containing 1.2 μg of osmium(VIII) catalyst which does not interfere under the experimental conditions. Nitrite is oxidized to nitrate in 0.5 *M* sulfuric acid in 5 min by a known excess of cerium(IV) sulfate (20–100%). The unreacted cerium(IV) is determined colorimetrically by the proposed method. The reduction in the absorbance of the red color produced by the fixed amount

TABLE 1
DETERMINATION OF CERIUM IN SYNTHETIC MIXTURES CORRESPONDING TO MISCH METAL

Ce present (ppm)	La (ppm)	Nd (ppm)	Fe (ppm)	Pr (ppm)	Eu (ppm)	Gd (ppm)	Er (ppm)	Ce found (ppm)
2.00	1.00	0.60	0.20	0.050	0.050	0.050	0.050	1.99
4.00	2.00	1.20	0.40	0.100	0.100	0.100	0.100	4.00
5.00	2.50	1.50	0.50	0.125	0.125	0.125	0.125	5.10
7.00	3.50	2.10	0.70	0.175	0.175	0.175	0.175	6.99
8.00	4.00	2.40	0.80	0.200	0.200	0.200	0.200	7.99

of cerium(IV) sulfate, in the absence of other reducing substances, is directly proportional to the amount of arsenic(III) or nitrite present. Cerium(III), arsenic(V), and nitrate formed in the reaction are colorless and do not interfere. 0.15–2.6 μg/ml of arsenic(III) and 0.04–1.68 μg/ml of nitrite can be determined. This method can be used for the spectrophotometric determination of microquantities of other substances which are quantitatively oxidized by cerium(IV) in sulfuric acid to colorless, noninterfering products.

SUMMARY

Promazine hydrochloride is proposed as a new reagent for the spectrophotometric determination of cerium(IV), arsenic(III), and nitrite. The reagent forms a red-colored radical with cerium(IV) instantaneously in 0.5–2 *M* sulfuric acid or 0.5–2.5 M phosphoric acid solution. The red radical exhibits maximum absorbance at 505 nm. An 11-fold molar excess of the reagent is necessary for the full development of the color intensity. Beer's law is valid over the concentration range 0.5–15 ppm in sulfuric acid and 0.5–21 ppm in phosphoric acid. The sensitivities of the reaction in sulfuric and phosphoric acid media are 0.022 and 0.019 μg/cm^2, respectively. The effects of acidity, time, order of addition of reagents, temperature, reagent concentration, and diverse ions are reported. The proposed method offers the advantages of good sensitivity, simplicity, rapidity, selectivity, and a wider range of determination without the need for extraction. Arsenic(III) and nitrite are indirectly determined.

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Benzothiazole-2-aldehyde-2-quinolylhydrazone as a Reagent for the Extractive Spectrophotometric Determination of Copper (II)

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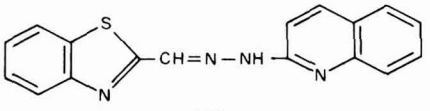
INTRODUCTION

Various tridentate hydrazone ligands containing a modified ferroin linkage (−N=C−C=N−NH−C=N−) have been suggested as highly sensitive colorimetric or fluorimetric reagents for divalent transition metal ions (*3, 7, 8, 13, 14*). These ligands are unique in having dissociable imino hydrogen on a nitrogen that is not directly involved in complexation. The extractability of the bis complexes into water-immiscible solvents as well as their high molar absorptivity makes this group of chelating agents very valuable for applied work.

During our investigation of the effect of modification of the ligand structure on the spectral properties of the metal complexes formed, it was found that benzothiazole-2-aldehyde-2-quinolylhydrazone(I, BTAQH) undergoes a particularly sensitive reaction with copper(II) ions. This ligand was first employed in the spectrophtometric determination of palladium(II) after extraction of the complex into benzene (*11*). In this paper are described an extractive spectrophotometric method for determining less than 0.75 ppm of copper with BTAQH and the nature of the complex formed.



(I)

EXPERIMENTAL

Reagents

BTAQH. The ligand was prepared by interaction of benzothiazole-2-aldehyde and 2-hydrazinoquinoline in stoichiometric amounts and was recrystallized to constant melting point (281–283°C) from ethanol (*11*).

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The acid dissociation constants of BTAQH were measured spectrophotometrically in 20% (v/v) aqueous dioxane in the presence of 0.1 *M* sodium perchlorate and were found to be pK_1 (azomethine-hydrogen) -0.22, pk_2 (benzothiazole-hydrogen) 3.2_1, pK_3 (quinoline-hydrogen) 5.2_1, and pK_4 (imino-hydrogen) >13.1 at 25°C. A solution of 3.3×10^{-4} *M* BTAQH in benzene was freshly prepared daily.

Copper(II) solution. Copper(II) standards were obtained by dilution of 0.01 *M* copper(II) perchlorate stock solution prepared from pure (99.99%) copper powder.

Buffer solution. An approximately 0.1 *M* sodium borate solution was adjusted to the desired pH with 2 *M* hydrochloric acid or 2 *M* sodium hydroxide.

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

Apparatus

All absorbance measurements were made with a Nippon Bunko UVIDEC-1 digital double-beam spectrophotometer using matched 1.00-cm quartz cells. For pH measurements a Toa Dempa HM-6A pH meter was used with a combination electrode.

RESULTS AND DISCUSSION

Characteristics of the Copper(II) Complex

Copper(II) ion forms a water-insoluble complex with BTAQH. The colored complex occurs over the pH range 5.7–13.8. The complex had been found to be extractable from aqueous solution into organic solvents such as benzene, toluene, chloroform, carbon tetrachloride, *o*-dichlorobenzene, lower aliphatic alcohols, and ketones such as methyl isobutyl ketone. The species of interest has a wavelength of maximum absorption at 523 nm in benzene (Fig. 1). At this wavelength there is no interference from excess ligand. Copper(II) can be completely extracted from aqueous solution (pH 9.0) with one 10-ml portion of BTAQH in benzene over the concentration range studied.

Effects of pH and Excess Reagent

A pH study was carried out over the pH range 6.1–13.7. The data are plotted in Fig. 2, indicating that maximum absorbance is obtained between pH 8.3 and 12.6. In more acidic or more alkaline solutions, the absorbance decreases because of incomplete complex formation and of hydrolysis of the complex, respectively.

A mole ratio study made at pH 9.0 demonstrates that, in order to get maximal and reproducible absorbance, at least a 16-fold excess of BTAQH in the organic phase is necessary. An excess of the reagent up to 80-fold does not interfere with the formation and extraction of the complex.

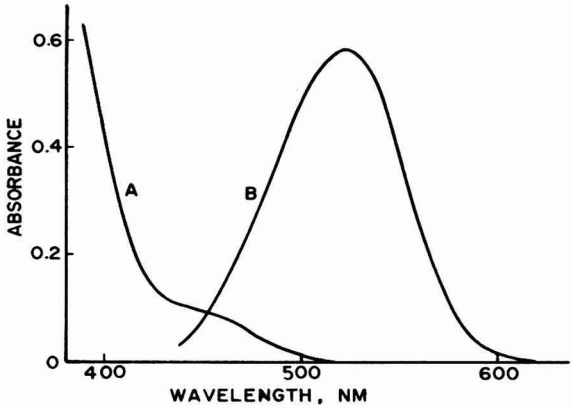


FIG. 1. Absorption spectra obtained under the experimental conditions given in the proposed procedure. Curve A is the spectrum of 3.3×10^{-4} *M* BTAQH in benzene. Curve B is the spectrum of the copper(II)–BTAQH complex obtained by the reaction of copper ions and BTAQH at concentrations of 3.7×10^{-6} and 3.3×10^{-4} *M*, respectively.

Extraction Rate

The rate of extraction of copper(II) is influenced not only by the excess of ligand but also by the organic solvents used for the extraction and by the auxiliary complexing agents present. The higher the concentration of the ligand and the greater the polarity of the organic solvents, the smaller the time necessary for complete extraction of copper(II), because the ligand and resulting chelate are only slightly water soluble at the pH required for reaction. The rate is improved by the addition of pyridine or aliphatic primary amines; addition of 0.01–0.06 *M* pyridine is satisfactory for quantitative extraction within 10–15 min.

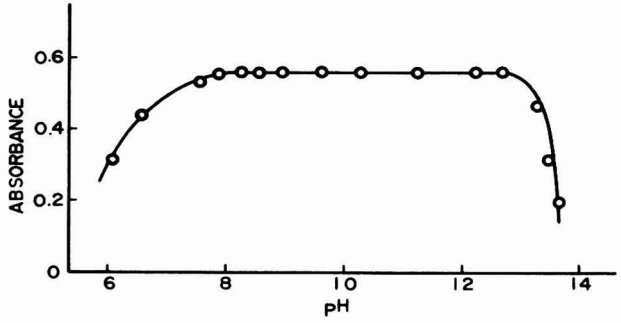


FIG. 2. The pH dependence of the copper(II)–BTAQH complex formation. Initial copper concentration in the aqueous phase and BTAQH concentration in benzene are 3.7×10^{-6} and 3.3×10^{-4} *M*, respectively.

Conformance to Beer's Law and Sensitivity

The analytical species of interest obeys Beer's law over the range studied of 0.15×10^{-5} to 1.2×10^{-5} *M* copper(II) in the organic phase. The sensitivity of the reaction as defined by Sandell (*15*) is 6.7×10^{-4} μg/cm^2. The best value for the molar absorptivity at 523 nm, as determined by least squares, was $7.50 \times 10^4 M^{-1}$ cm^{-1}. The color intensity of the complex was constant over the measured 1-hr period.

In Table 1 are summarized the spectral properties of the copper(II) complexes with several tridentate hydrazones described to date: The present ligand, BTAQH, is found to be the most sensitive reagent of those tested for copper(II). It is of interest to note that quinoline-2-aldehyde-2-benzothiazolylhydrazone (QABTH), which differs from BTAQH only in the interchange of substitution groups, forms a copper(II) complex with its maximum absorption lying at a shorter wavelength and a considerably lower molar absorptivity than those of the corresponding BTAQH complex. The same relation is found between the copper(II) complexes of benzothiazole-2-aldehyde-2-pyridylhydrazone and pyridine-2-aldehyde-2-benzothiazolylhydrazone.

TABLE 1
SPECTRAL PROPERTIES OF COPPER(II) COMPLEXES OF TRIDENTATE HETEROCYCLIC HYDRAZONES

Hydrazone (R_1–CH=N–NH–R_2)	Solvent	λ_{max} (nm)	ϵ_{max} ($\times 10^{-4}$)	Reference
$R_1 = R_2$ = 2-pyridyl	Chloroform	480	6.28	(*13*)
R_1 = 2-pyridyl, R_2 = 2-quinolyl	80% aq. ethanol	474	4.58	(*3*)
R_1 = 2-pyridyl, R_2 = 2-benzothiazolyl	80% aq. ethanol	436	3.46	(*3*)
	Benzene	470	5.00	(*12*)
R_1 = 2-quinolyl, R_2 = 2-pyridyl	Benzene	524	5.5	(*18*)
	Chloroform	512	5.8	(*18*)
$R_1 = R_2$ = 2-quinolyl	Benzene	540	5.8	(*16*)
	Nitrobenzene	536	4.73	(*6*)
R_1 = 2-quinolyl, R_2 = 2-benzothiazolyl	Chloroform	500	2.7	(*12*)
R_1 = 6-phenanthridyl, R_2 = 2-pyridyl	Chloroform	522	7.1	(*18*)
R_1 = 6-phenanthridyl, R_2 = 2-quinolyl	Benzene	543	6.4	(*18*)
	Chloroform	536	6.6	(*18*)
R_1 = 2-benzothiazolyl, R_2 = 2-pyridyl	Benzene	501	6.50	(*12*)
R_1 = 2-benzothiazolyl, R_2 = 2-quinolyl	Benzene	523	7.50	Present work
$R_1 = R_2$ = 2-benzothiazolyl	60% aq. dioxane	464	3.25	(*17*)
R_1 = 2-α-naphthothiazolyl, R_2 = 2-benzothiazolyl	60% aq. dioxane	476	4.13	(*17*)

Effect of Diverse Ions

The possible interference of various ions was examined by introducing them into a solution containing 3.8 μg of copper(II). The tolerance limit of an ion was fixed as the maximum amount causing an error no greater than 2% in the absorbance of the extract solution. The results are summarized in Table 2. Cationic interference includes cadmium, iron(II, III), uranium(VI), and, to a lesser extent, nickel and zinc. Iron and uranium give negative errors, whereas cadmium, nickel, and zinc give positive errors by forming colored complexes. Common anions do not interfere with the formation or extraction of the copper(II) complex. However, the following anions, because of the strong copper(II) complex, have been shown to interfere: EDTA, pyrophosphate, and citrate.

Recommended Procedure

Dissolve the sample to be analyzed by appropriate means. Separate copper from the interfering ions by common ion-exchange procedures (*2, 9, 10*). Aliquots of the sample should be taken to contain 0.09 to 0.75 ppm copper(II) ion. Add 2.5 ml of 1% aqueous pyridine solution and 10 ml of 0.1 *M* sodium borate–hydrochloric acid buffer (pH 9.0) to the copper sample in a 50-ml separatory funnel. Dilute to approximately 20 ml with deionized water and equilibrate with exactly 10 ml of 3.3×10^{-4} *M* BTAQH in benzene for 10–15 min. Transfer an aliquot of the benzene extract to a 1.00-cm cell and measure the absorbance of the solution at 523 nm against the reagent blank. Determine the concentration of copper(II) in the sample from a previously prepared calibration curve.

TABLE 2
EFFECT OF FOREIGN IONS ON DETERMINATION OF 3.8 μg OF COPPER(II)

Tolerance limit ([Ion]/[Cu(II)])	Ion
≥12,000	F^-, Cl^-, Br^-, NO_3^-, ClO_4^-, CH_3COO^-, SO_3^{2-}, SO_4^{2-}, CO_3^{2-}, PO_4^{3-},
≤3,000	I^-
≤1,200	$C_2O_4^{2-}$
≥120	SCN^-, $S_2O_3^{2-}$, tartrate, VO_3^- MoO_4^{2-}, WO_4^{2-}, Mg^{2+}, Ca^{2+}, Be^{2+}, Mn^{2+}, Pb^{2+}, Pd^{2+}, Hg^{2+}[a], Ag^+, Au^{3+}
0–20	EDTA, $P_2O_7^{4-}$, citrate, UO_2^{2+}, Fe^{2+}, Fe^{3+}, Ni^{2+}, Zn^{2+}, Cd^{2+}

[a] Potassium iodide was added.

Composition and Structure of the Complex

Owing to the relative slow complexation rate and, consequently, to the influence of the mass action law, the common Job or mole ratio plots of the equilibria in two-phase systems (*5*) did not furnish any reasonable results. The extraction method (*1*, *4*), where metal distribution is followed in the presence of a reasonable excess of ligand, was found to be satisfactory. It is known that, when the various solution and extraction equilibria are taken into account, an expression for the metal distribution ratio can be derived which, after transformation, leads to the equation:

$$\log D_{Cu} = \log K_{ex} - \log \alpha_{Cu} + n \log[HL]_{org} - n \log[H^+],$$

where D_{Cu} denotes the distribution ratio of copper, K_{ex} is the extraction constant defined by $[CuL_n]_{org}\,[H^+]^n/[Cu^{2+}]\,[HL]^n_{org}$, n is the number of hydrazone molecules bound per metal atom, and α_{Cu} is the side-reaction coefficient for copper(II). If a reasonable excess of chelating agent is used, its concentration in the organic phase, $[HL]_{org}$, can be set equal to its overall analytical concentration, C_{HL}. At constant pH, a logarithmic plot of the metal distribution against the analytical ligand concentration should be a straight line with slope equal to n. Experimental data showed n equal to 2 and a 1:2 Cu:HL ratio (Fig. 3). This conclusion was supported by a logarithmic plot of the metal distribution against pH, where the ligand concentration in benzene had been held constant. The value of K_{ex} was then estimated from these data to be $3.3_2 \times 10^{-9}$.

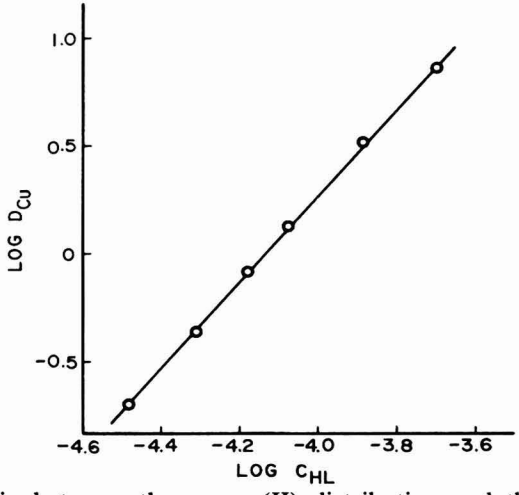
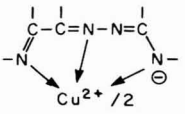
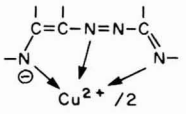


FIG. 3. Relationship between the copper(II) distribution and the analytical BTAQH concentration. Initial copper concentration in the aqueous phase is 3.7×10^{-6} *M*.

For the copper(II) chelate of BTAQH, two resonance forms can be written when deprotonation takes place at the imino group. It would



(A) -C=N- form



(B) -N=N- form

appear that structure (A) is favored energetically, because of two effects opposite to each other, the electron surplus associated with the sulfur of benzothiazole and the electron deficiency associated with the nitrogen of quinoline; in other words, the -C=N- structure in the heterocyclic ring on the aldehyde moiety is more preferable than that on the hydrazine moiety. A similar consideration of the copper(II) complex of QABTH, on the other hand, would suggest that the reverse is true and the -N=N- structure is more favorable. The spectral properties of the copper(II) complexes with the other hydrazones, containing the benzothiazolyl grouping, listed in Table 1 can be interpreted by the preference of the -C=N- or -N=N- structural form. Zatka *et al.* (*18*) have concluded that extension of the π system in the aldehyde group substituent would be of greater importance for the spectral properties of the chelates than if the same change would occur in the neighborhood of the hydrazine grouping. Our observations, however, suggest that introduction of an electron-donative heterocyclic substituent, such as benzothiazolyl or probably the benzimidazolyl group, into the aldehyde moiety of the free ligand would serve to improve the wavelength of maximum absorption accompanied by a corresponding increase in the molar absorptivity of the metal complexes formed.

SUMMARY

Benzothiazole-2-aldehyde-2-quinolylhydrazone (BTAQH) was used for the spectrophotometric determination of trace amounts of copper(II) after the extraction process. Copper(II) reacts with BTAQH at pH 8.3–12.6 to form a water-insoluble 1:2 complex, which can be extracted with many kinds of organic solvent. The extracted species with benzene has an absorption maximum at 523 nm and obeyed Beer's law over the range 0.09 to 0.75 ppm of copper. The molar absorptivity is $7.50 \times 10^4\ M^{-1}\ cm^{-1}$ at 523 nm. The spectral properties of the copper(II) complexes with some tridentate hydrazones containing benzothiazole ring as a functional group were also discussed.

ACKNOWLEDGMENT

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Metallic Excess Determination in Nonstoichiometric Oxides and Chalcogenides

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INTRODUCTION

The determination of very small amounts of an element in the matrix of that same element is a very interesting analytical chemistry problem. Material technology poses such a problem. For instance, in certain circumstances some semiconductor materials must be prepared as completely stoichiometrically as possible, because their electrical properties are strongly influenced by a component excess in the lattice. Several works have dealt with stoichiometry determination in semiconductor compounds such as lead telluride (*2*), lead and tin telluride (*3, 5–7*), or copper and indium telluride (*10*) with an accuracy no better than 0.1%. However, none of these procedures has been able to determine a small excess of a component with respect to an exact stoichiometry. Electrochemical (*4*) and gas-chromatographic (*1, 8, 9*) methods, which determine small amounts of zinc or cadmium in their oxides or sulfides, have been published for only a few II-VI compounds.

We propose a very simple method, developed in a standard analytical chemical laboratory, which does not require sophisticated equipment and which can be applied to a vast range of materials. The method consists of the sample dissolution and the stripping voltametric determination of microamounts (1 to 50 μg) of free cation in the presence of a 10^3- to 10^5-times larger quantity of the same cation masked by a suitable complexing agent. Ethylenediaminetetraacetic acid (EDTA), because of the very stable complexes it forms, markedly changes the reduction potentials of numerous elements, and for this reason was used as a complexing agent. The polarographic procedure was found to be the most suitable for determining uncomplexed amounts of an element in the presence of the same element opportunely masked. Neither the atomic absorption nor the mass spectrometry technique accomplishes this. This is true also for other typical analytical methods (i.e., X-ray fluorescence and electron microprobe) which detect specific elements, no matter which chemical bond is present. The solvent extraction method using a chelating agent is not suitable because chemical equilibria are disturbed during the extraction proce-

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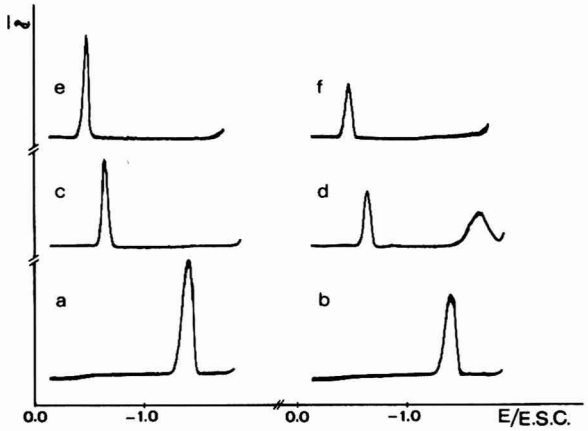

FIG. 1. Polarograms with superimposed sinewave tension. (a) Zn^{2+} (1.2×10^{-4} *M*) in 1 *M* NH_4OH. (b) Zn^{2+} (0.8×10^{-4} *M*) + Zn–EDTA (0.4×10^{-4} *M*) in 1 *M* NH_4OH. (c) Cd^{2+} (0.3×10^{-4} *M*) in 1 *M* KCl. (d) Cd^{2+} (0.2×10^{-4} *M*) + Cd–EDTA (10^{-5} *M*) in 1 *M* KCl. (e) Pb^{2+} (0.4×10^{-4} *M*) in CH_3COOH/CH_3COONa buffer. (f) Pb^{2+} (0.2×10^{-4} *M*) + Pb–EDTA (0.2×10^{-4} *M*) in CH_3COOH/CH_3COONa buffer.

dure. The proposed method can be applied to the determination of metallic excess in zinc, cadmium, and lead oxide and chalcogenide samples.

OUTLINE OF THE METHOD

A 0.1- to 1-g precisely weighed sample was dissolved in an acidic medium (see below) and combined with standardized EDTA, which was enough to complex a quantity of metal exactly according to the correct stoichiometry. The solution obtained was neutralized and then diluted to the proper volume with water (usually up to 1 to 10 mg of sample/ml of water). Suitable fractions of this solution were analyzed by means of the stripping voltametric technique with a hanging-drop mercury electrode. Depending on the cation examined, appropriate supporting electrolytes and preelectrolysis potentials were used. According to the quantity of added EDTA, a cation excess, when present, was revealed up to 10^{-3} to 10^{-2}% in weight. Quantitative determinations were obtained by calibration curves performed under identical analytical conditions.

EXPERIMENTAL METHOD

Apparatus. A Tacussel electrochemical apparatus was used for all the measurements. The stripping voltametric analyses were performed with a PRGS-type polarograph connected to a Metrohm hanging-drop mercury electrode. Direct polarographic measurements were obtained by using the PRGS polarograph connected with a ADAPAL unit, which supplies a superimposed ac signal. Potentiometric titrations were carried out by a TITRIMAT titrator associated with an automatic reagent-supply system

TABLE 1
PEAK POTENTIALS VS SCE IN DIFFERENT ELECTROLYTES

Peak	Electrolytes		
	KCl (1 *M*) (volts)	CH_3COOH (0.1 *M*) + CH_3COONa (0.2 *M*) (volts)	NH_4OH (1 *M*) (volts)
Zn	−1.0		−1.38
Zn−EDTA	−1.70		<−1.9
Cd	−0.63		−0.79
Cd−EDTA	−1.63		<−1.9
Pb	−0.43	−0.43	
Pb−EDTA	−1.66	<−1.6	

and a synchronous recorder. A mercury cup and a saturated calomel were used as a working electrode and a reference electrode, respectively. All the analyses were performed in a 50-ml polarographic cell. An M5 Mettler microbalance was used for sample weighing.

Reagents. A 0.1 *M* EDTA solution was prepared from purified salt (*12*) and potentiometrically standardized versus standard zinc solution. Standard zinc, cadmium, and lead solutions were prepared by dissolving 99.999% pure metals in diluted hydrochloric or nitric acid. Supporting electrolyte solutions were 0.2 *M* sodium acetate + 0.1 *M* acetic acid, 1 *M* ammonia, and 1 *M* kalium chloride. Sodium hypophosphite was used as 10% (w/v) solution. All the reagents, inorganic acids, and distilled water were tested by polarographic analysis, before use, and were found to be free of detectable traces of examined metals.

Sample preparation. Oxides. They were easily dissolved in a few mil-

TABLE 2
ANALYTICAL CONDITIONS IN THE ANODIC-STRIPPING PROCEDURE

Metal	Supporting electrolyte	Preelectrolysis potential vs SCE (volts)	Peak potential vs SCE (volts)	Instrumental sensitivity ($\mu A/\mu g$)[a]
Zn	NH_4OH (1 *M*)	−1.5	−1.25	0.017[b] 0.031[c]
Cd	NH_4OH (1 *M*)	−1.0	−0.75	0.017[b] 0.025[c]
Pb	CH_3COOH (0.1 *M* + CH_3COONa (0.2 *M*)	−0.7	−0.41	0.005[b] 0.008[c]

[a] Solution volume: 50 ml.
[b] Preelectrolysis time: 5 min.
[c] Preelectrolysis time: 10 min.

liliters of diluted hydrochloric or nitric acid and treated as previously described.

Sulphides. A powdered sample of 0.1 to 1 g was treated with 10 to 20 ml of 6 *M* hydrochloric acid and moderately warmed, and 1 to 2 ml of concentrated nitric acid was added in order to completely oxidize sulfide ions which might interfere in the following analytical steps. The resulting solution was gently warmed in order to evaporate most of the acids. The general procedure was then followed.

Selenides and tellurides. Ten to twenty milliliters of 1:1 hydrochloric:nitric acid mixture was used to dissolve 0.1 to 1 g of sample. The solution was gently warmed in order to evaporate residual nitric acid. Hydrochloric acid was added to obtain a 1 *M* solution for tellurides and a 6 *M* solution for selenides. Five to ten milliliters of hypophosphite solution was carefully added and the solution was allowed to stand for about 30 min at 70 to 80°C. After cooling the solution was neutralized and EDTA was added. Finally, the precipitate was centrifuged and the resulting solution was diluted to proper volume.

Polarographic measurements. Metals dissolved in different electrolytes were polarographically examined both with and without EDTA present. Typical polarograms are shown in Fig. 1. They were recorded by a polarographic procedure with superimposed sinewave tension and synchronous detection. Standard conditions for the polarograms were: drop time, 2 sec; potential sweep speed, 150 mV/min; deaeration time 10 min; temperature, 20°C. No maximum suppressor was used. The reduction potentials vs SCE in different electrolytes are summarized in Table 1. Cation analyses by the stripping voltametric procedure were performed under the following standard conditions: solution volume, 50 ml; drop volume, 0.150 mm^3; preelectrolysis time, 5 min; potential sweep speed, 150 mV/min; deaeration time, 10 min; temperature, 20°C. Polarogram results are summarized in Table 2.

RESULTS AND DISCUSSION

The proposed procedure was checked by known solutions. Measurements performed by the polarographic technique with a superimposed ac signal showed that typical cation peaks were decreased by the EDTA addition. At the same time, in a kalium chloride medium, a second peak appeared at plus negative potentials and its height was increased with EDTA concentration. For ammonia and acetate–acetic acid media, the second peak did not appear before electrolyte discharge: In both cases only metal peak decreases were observed when EDTA was added. Systematic runs to verify the functioning of the two polarographic waves versus added EDTA were not performed. It can only be confirmed that the polarographic wave of the free cation disappeared when an equimolar

TABLE 3
SYNTHETIC SAMPLE ANALYSIS

Matrix	Cation excess (μg)/sample (mg)		Standard deviation
	Added	Found[a]	(μg)
CdS	0.1	0.09	0.01
	2	2.1	0.1
	10	9.5	0.4
PbTe	0.5	0.52	0.05
	5	5	0.3
	10	9.9	0.3
ZnSe	0.5	0.5	0.01
	5	5.1	0.4
	8	7.8	0.5

[a] Any value was the average of three determinations.

quantity of metal and of EDTA was present in the solution. In the stripping voltametric analyses the preelectrolysis potentials were fixed on the basis of previous experiments. They were kept low enough to avoid metal-complex reduction even if their concentration was 10^3 to 10^4 times larger than uncomplexed cations. Solutions as concentrated as 10^{-3} M (for zinc–EDTA), 3×10^{-3} M (for cadmium–EDTA) and 2×10^{-3} M (for lead–EDTA) did not show any interference when examined under the conditions described. In these media calibration curves were carried out for each cation: Proportional results were obtained in the ranges: (0.3×10^{-6}) to 10^{-5} M (for zinc), $(0.2-5)\times 10^{-6}$ M (for cadmium) and $(0.5-5) \times 10^{-6}$ M (for lead). Synthetic samples containing a known metal excess were analyzed following the above procedure and the results are summarized in Table 3. In Table 4 the results of some II-VI and IV-VI compounds examined are reported. When compared with direct po-

TABLE 4
ANALYSIS OF COMPOUNDS II-VI AND IV-VI

Sample nominal composition	Found composition
$Cd_{1+x}S$[a]	$Cd_{1.048}S$
$Cd_{1-x}S$[a]	$Cd_{0.983}S$
PbO[b]	$Pb_{1.107}O$
ZnTe[b]	$Zn_{0.987}Te$

[a] Doped polycrystalline samples prepared in our Laboratory.
[b] Commercial polycrystalline samples.

larography, the stripping voltametric technique was preferred for its higher sensitivity; furthermore the results obtained were more accurate and reproducible. By using our proposed sample dissolution, sulfur does not interfere in the subsequent procedure. To the contrary, the presence of either selenium or tellurium in solution hinders the polarographic analysis even if a masking agent (such as citrate ions) is present. An elemental reduction was used to separate both elements. Hypophosphite was a suitable reducing agent in hydrochloric acid medium (*11*) and it does not interfere with the polarographic procedure. The addition of EDTA and buffer solution directly onto the precipitate minimizes the eventual losses of metallic substances adsorbed on the precipitate.

CONCLUSIONS

We have described a simple and versatile polarographic method for the determination of cation excess in II-VI and IV-VI oxides and chalcogenides. When the cation is defective within the sample, it is nevertheless possible to determine the defective quantity by adding suitable amounts of the element examined to the solution until a specific peak is obtained. The method can be used in the evaluation of the exact stoichiometry of both commercial polycrystalline powders and laboratory-produced single crystals. Furthermore it can also be applied for determining concentration gradients resulting from thermal diffusion processes, for example, after CdS has been thermally treated with cadmium for obtaining heavily doped CdS.

SUMMARY

A very simple polarographic procedure is described for determining the excess of a metallic element in nonstoichiometric oxides and chalcogenides. The method was based on the possibility of changing the reduction potential of an element in the presence of a strong complexing agent (EDTA). After sample dissolution, metals were complexed with an EDTA amount according to an exact stoichiometry; the solution was analyzed by a stripping voltametric procedure with a suitable preelectrolysis potential and the uncomplexed excess of the element was measured. Solutions as dilute as 10^{-6} *M* in free cation were examined with an accuracy better than 10% in the presence of a 10^3- to 10^4-times larger concentration of masked cations. With a simple modification the method can also be applied to stoichiometric analysis of cation-defective samples.

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MICROCHEMICAL JOURNAL 23, 312–319 (1978)

The Use of Redox Reactions in the Analysis of Dyes and Dye Industry Intermediates

III. An Indirect Determination of Brilliant Green with Ceric Sulfate[1]

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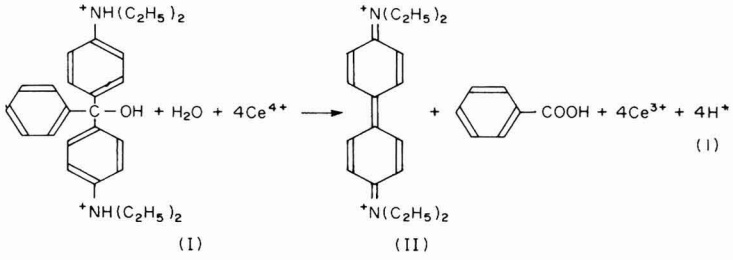
INTRODUCTION

Brilliant green (I) belongs to the group of triphenylmethane dyes which earlier were determined chiefly by reductometric titrations (*4,5*), whereas oxidimetric titrations have been studied only recently (*2,3,7*). In the framework of the systematic study of redox reactions of dyes and dye industry intermediates, the present paper deals with the oxidation of brilliant green by ceric sulfate.

It has been found that this reaction can be described by Eq. (1) and can be utilized analytically by determining spectrophotometrically or ascorbinometrically the reaction product, diphenoquinone-(4,4′)bis(diethylimine) (II).



EXPERIMENTAL

Reagents. The following reagents were used: ceric sulfate, 0.01 and 0.001 *M* solutions in 0.25 *M* sulfuric acid; ferrous sulphate, 0.01 *M* solution in 0.25 *M* sulfuric acid, whose titer was determined daily using a

[1] Part II. An Indirect Determination of Crystal Violet with Ceric Sulphate. *Coll. Czech. Chem. Commun.*, in press.

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bichromate standard solution; ascorbic acid, 0.01 *N* solution (equiv. = mole/2) in redistilled water, whose titer was determined daily using a bichromate standard solution (*1*); hydrazinium sulfate, 0.01 *M* solution in redistilled water; brilliant green, 0.025 *M* solution of the pure substance (A Company for Marketing Tar Dyes, Prague) in distilled water, whose titer was determined titanometrically (*4*). More dilute solutions were prepared by accurate dilution of this stock solution.

N,N,N',N'-tetraethylbenzidine, 0.005 *M* solution in 0.02 *M* hydrochloric acid, was prepared by dissolving the accurately weighed amount of the pure substance (The Research Institute for Organic Syntheses, Pardubice-Rybitví) in hydrochloric acid and diluting to 1 liter with distilled water. A 0.00025 *M* solution was prepared by accurately diluting this stock solution with redistilled water.

Thin-layer chromatographic experiments were carried out on commercial plates, "Silufol UV 254" (Kavalier, Votice).

Apparatus. Potentiometric titrations were performed with an Acidimeter EK millivoltmeter (Druopta, Prague), with a bright platinum indicator and a saturated calomel reference electrodes. A 10-ml buret with 0.02-ml divisions was used, and its orifice was adjusted so that one drop corresponded to 0.02 ml. For titrations under an inert atmosphere, a specially modified 25-ml buret was used, with 0.1-ml divisions. The solutions were stirred by an electromagnetic stirrer (Laboratorní přístroje, Prague).

Spectrophotometric measurements were carried out on a Specord UV VIS instrument (C.Zeiss, Jena) with 0.5- and 1.0-cm quartz cuvettes. A Spekol instrument with a Ti titration adaptor (C.Zeiss, Jena) and a 30-ml cuvette were employed for spectrophotometric titrations with an Agla microburet (Burroughs Wellcome & Co., London) with a total volume of 0.5 ml and with 0.0002-ml divisions. The infrared spectra were measured on an UR 20 instrument (C.Zeiss, Jena) in a 0.1-mm cuvette.

PROCEDURES AND RESULTS

Study of the Reaction Stoichiometry

As commercial brilliant green preparations contain oxidizable impurities, it was impossible to determine the reaction stoichiometry from the measured number of electrons exchanged. Therefore, the validity of Eq. (1) was verified by detecting and identifying the oxidation products and determining the diphenoquinone-(4,4')-bis(diethylimine) formed.

Spectrophotometric Verification of the Formation of Diphenoquinone-(4,4')-bis(diethylimine)

To 10 ml of 0.01 *M* ceric sulfate in 0.25 *M* sulfuric acid, 5 ml of 0.0025 *M* brilliant green solution were added, the excess of the reagent was removed after 2 min by potentiometric titration with 0.01 *M* ferrous sulfate,

and the solution was diluted to 500 ml with distilled water. The absorption spectrum of this solution contained a sharp absorption peak at 469 nm, whose position and intensity were identical with those of the absorption maximum of the product of the oxidation of *N,N,N',N'*-tetraethylbenzidine measured analogously.

Verification of the Formation of Diphenoquinone-(4,4')-bis(diethylimine) by Thin-Layer Chromatography and Infrared Spectroscopy

The well-known fact that this substance is reduced to *N,N,N',N'*-tetraethylbenzidine by strong reductants (e.g., ascorbic acid) was utilized. In the first case, the following procedure was employed: To 10 ml of 0.01 *M* ceric sulfate in 0.25 *M* sulfuric acid, 5 ml of 0.0025 *M* brilliant green were added, and after 2 min 15 ml of a 0.01 *N* solution of ascorbic acid were added. The pH was then adjusted to 7 with 0.1 *M* sodium hydroxide (universal indicator paper), and the solution was extracted with two 10-ml portions of benzene. The combined extracts were evaporated to dryness *in vacuo*, the residue was dissolved in 0.5 ml of acetone, and an approximately 0.5-μl aliquot was placed at the start of chromatogram, together with a standard solution prepared by dissolving 10 mg of *N,N,N',N'*-tetraethylbenzidine in 10 ml of acetone. The chromatographic procedure was performed in an ascending arrangement in a closed glass bottle, whose atmosphere was saturated by the vapors of the elution system from a strip of filter paper. A 4:1 mixture of benzene and ethanol was used as the elution system, and the spots were detected by spraying with 0.0025 *N* ceric sulfate. It was found that pure *N,N,N',N'*-tetraethylbenzidine and the substance obtained by ascorbinometric reduction of the products of oxidation of brilliant green with ceric sulfate yield spots with identical positions.

In the second case, 100 ml of a 0.0025 *M* brilliant green solution were added to 200 ml of 0.01 *M* ceric sulfate; 0.5 g of zinc powder was added after 30 min. The solution was stirred during the reduction, and the zinc was filtered off after solution discoloration. The pH was then adjusted to 8 with 0.1 *M* sodium hydroxide (universal indicator paper), and the solution was extracted with four 50-ml portions of benzene. The combined extracts were evaporated to dryness *in vacuo*, the residue was recrystallized from ethanol, and the ir spectrum of its saturated solution in tetrachloromethane was measured from 800 to 1700 cm^{-1}. It was found that the spectrum obtained is identical with that of *N,N,N',N'*-tetraethylbenzidine.

Verification of the Formation of Benzoic Acid Using ir Spectroscopy

The benzoic acid formed was isolated from the reaction mixture by extraction with ether, the ether was evaporated, and the residue was identified using the ir spectrum as follows: To 100 ml of a 0.0025 *M*

brilliant green solution, 200 ml of 0.01 *M* ceric sulfate were added, and the solution was allowed to stand 30 min. Then 0.5 g of zinc powder was added, and the solution was stirred until it became colorless. The zinc was filtered off through an S3 frit, the pH was adjusted to 8 with 1 *M* sodium hydroxide (universal indicator paper), the precipitate formed was filtered off using an S4 frit, and the pH of the filtrate was adjusted to 7 with 1 *M* sulfuric acid. The solution was then extracted by four 25-ml portions of ether, and the combined extracts were evaporated to dryness *in vacuo*. The residue was dissolved in tetrachloromethane, and its ir spectrum was obtained from 800 to 1700 cm^{-1}; it was identical with the spectrum of pure benzoic acid.

Spectrophotometric Determination of the Diphenoquinone-(4,4')-bis(diethylimine) Formed

The time required for completion of the reaction and the stability of the coloration formed in time had to be found first. To 10 ml of 0.001 *M* ceric sulfate in 0.25 *M* sulfuric acid, 5.00 ml of 0.00025 *M* dye solution were added, the mixture was diluted to 50 ml with water, and the absorbance of this solution was measured at certain time intervals at 469 nm in a 0.5-cm cuvette. The results are given in Table 1, from which it follows that the absorbance must be measured 2 min after the solution preparation.

The amount of diphenoquinone-(4,4')-bis(diethylimine) formed by the oxidation of brilliant green was determined as follows: In a 100-ml volumetric flask, 5.00 ml of a solution containing 3.54×10^{-5} to 28.40×10^{-5} mol of brilliant green, 10 ml of 0.001 *M* ceric sulfate in 0.25 *M* sulfuric acid were measured, and the mixture was diluted with distilled water to the mark. The absorbance of the solution was measured after 2 min in 0.5-cm cuvettes at 469 nm. The blank determination was carried out simultaneously, with the diphenoquinone-(4,4')-bis(diethylimine) formed reduced by adding 20 ml of 0.001 *N* ascorbic acid, subtracting the blank value from the determined value. The amount of the diphenoquinone-(4,4')-bis(diethylimine) formed was then found from a calibration curve obtained with pure *N,N,N',N'*-tetraethylbenzidine oxidized by ceric ions; to 10 ml of 0.001 *M* ceric sulfate, 1–5 ml of 0.00025 *M* tetraethylbenzidine were added, the solution was diluted to 100 ml, and its absorbance was measured after 2 mins in a 0.5-cm cuvette at 469 nm. The results obtained

TABLE 1
THE TIME DEPENDENCE OF THE ABSORBANCE OF THE SOLUTION FORMED BY THE OXIDATION OF BRILLIANT GREEN WITH CERIUM SULFATE[a]

t (min)	2	5	10	20	30
A	1.18	1.17	1.16	1.15	1.14

[a] Brilliant green concentration, 1.42×10^{-5} *M*; wavelength, 469 nm.

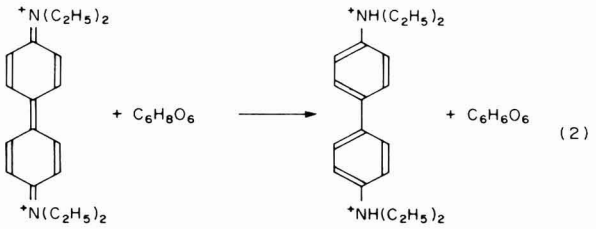
are given in Table 2, from which it follows that 1 mol of diphenoquinone-(4,4′)-bis(diethylimine) is formed from 1 mol of brilliant green.

Analytical Use of the Reaction

The presence of oxidizable impurities in brilliant green samples prevents determination based on back-titration of the unreacted oxidant. However, the results above have shown that the reaction can be utilized analytically, assuming that the amount of brilliant green can be determined from the amount of the diphenoquinone-(4,4′)-bis(diethylimine) produced by the oxidation, which can be determined ascorbinometrically or spectrophotometrically.

An Indirect Determination of Brilliant Green Based on Ascorbino-metric Titration of the Diphenoquinone-(4,4′)-bis-diethylimine Formed

The diphenoquinone-(4,4′)-bis(diethylimine) produced by the oxidation of brilliant green can be quantitatively reduced to *N,N,N′,N′*-tetraethylbenzidine by ascorbic acid, according to Eq. (2). As the initial quinonediimine is intensely yellow, and the *N,N,N′,N′*-tetraethylbenzidine is colorless, brilliant green can be indirectly determined in this way. Visual titration is impossible in practical samples, because of the presence of colored impurities, but spectrophotometric titration yielded good results.



(2)

In establishing the optimum conditions for this determination, the time required for completion of the reaction was determined first, and then it was verified that the unreacted ceric sulfate can be quantitatively removed by adding excess hydrazinium sulfate which does not reduce the quinonediimine formed. The recommended procedure is as follows:

To 10.00 ml of 0.01 or 0.001 *M* ceric sulfate in 0.25 *M* sulfuric acid are added 5.00 ml of a solution containing 0.5 to 5 or 0.05 to 0.5 mg of brilliant green. Titration with 0.1 or 0.01 *N* ascorbic acid is started after 2 min. The standard solution is added from an Agla microburet, and the absorbance is measured at 469 nm. The end point is found graphically from the titration curve. An amount of 1 ml of 0.1 or 0.01 *N* ascorbic acid corresponds to 21.052 or 2.105 mg of the chloride of brilliant green, respectively.

TABLE 2
THE DETERMINATION OF THE STOICHIOMETRY OF THE BRILLIANT GREEN OXIDATION WITH CERIC SULFATE ON THE BASIS OF THE SPECTROPHOTOMETRIC DETERMINATION OF THE DIPHENOQUINONE-(4,4′)-BIS(DIETHYLIMINE) PRODUCED

Taken (mol of brilliant green)	Found (mol of diphenoquinone-(4,4′)-bis(diethylimine))
3.54×10^{-5}	3.24×10^{-5}
10.64×10^{-5}	10.96×10^{-5}
17.74×10^{-5}	18.09×10^{-5}
28.40×10^{-5}	29.80×10^{-5}

The accuracy and reproducibility of this determination can be seen in Table 3. The titration curves are depicted in Fig. 1.

An Indirect Determination of Brilliant Green Based on the Spectrophotometric Determination of the Diphenoquinone-(4,4′)-bis(diethylimine) Produced

The exceptionally high molar absorption coefficient of the quinonediimine produced makes possible a sensitive spectrophotometric determination of brilliant green. As the reaction mixture after the oxidation of practical samples of brilliant green with ceric sulfate contained impurities absorbing at 469 nm, i.e., at the wavelength of the absorption maximum of the diphenoquinone-(4,4′)-bis(diethylimine) to be determined, the following procedure had to be employed:

To 5.00 ml of a solution containing 0.7 – 6.0 mg of brilliant green, 10 ml of 0.01 *M* ceric sulfate in 0.25 *M* sulfuric acid are added, the mixture is diluted with distilled water to 1 liter after 2 min, and its absorbance is measured at 469 nm in a 0.5-cm cuvette. The blank determination is carried out simultaneously; the diphenoquinone-(4,4′)-bis(diethylimine) produced is reduced after 2 min by adding 20 ml of 0.01 *N* ascorbic acid. The blank solution absorbance at 469 nm is subtracted from the value

TABLE 3
THE ACCURACY AND REPRODUCIBILITY OF THE INDIRECT ASCORBINOMETRIC DETERMINATION OF BRILLIANT GREEN

Taken (mg)	Found (mg)[a]	Standard deviation (mg)
3.734	3.743	0.013
0.0809	0.0829	1.3×10^{-5}

[a] The values found are averages of 10 determinations, from which the standard deviation was also calculated.

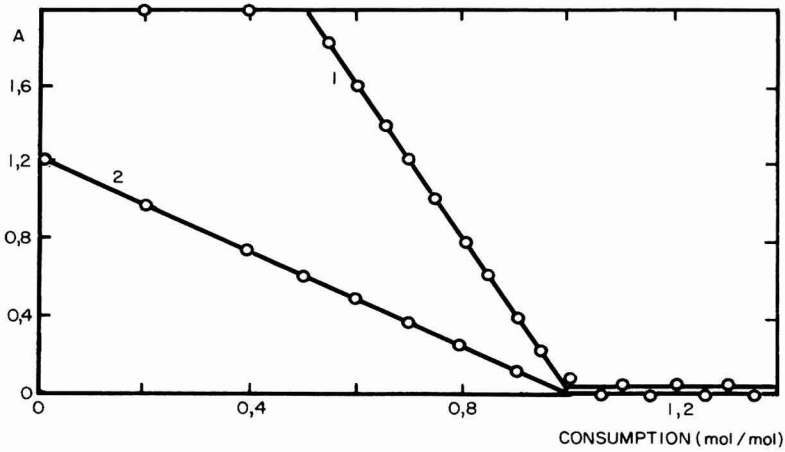


FIG. 1. Spectrophotometric titration of diphenoquinone-(4,4′)-bis(diethylimine) with ascorbic acid. Curve 1, 8.87×10^{-6} mol titrated with 0.1 *N* ascorbic acid; curve 2, 1.92×10^{-7} mol titrated with 0.01 *N* ascorbic acid.

obtained in the determination, and the dye content is determined from a calibration curve obtained with a standard solution of brilliant green of pure *N,N,N′,N′*-tetraethylbenzidine. The accuracy and reproducibility of this determination follows from Table 4.

DISCUSSION

The study of the stoichiometry of the brilliant green oxidation with cerium sulfate has shown that the reaction obeys Eq. (1). The presence of oxidizable impurities in practical samples of brilliant green prevents the determination based on direct titration with ceric sulfate or on back-titration of its unreacted excess. On the other hand, new methods employing the ascorbinometric or spectrophotometric determination of the diphenoquinone-(4,4′)-bis(diethylimine) produced yield very good results.

TABLE 4

THE ACCURACY AND REPRODUCIBILITY OF THE DETERMINATION OF BRILLIANT GREEN BASED ON THE MEASUREMENT OF THE ABSORBANCE OF THE DIPHENOQUINONE-(4,4′)-BIS(DIETHYLIMINE) FORMED

Taken (mg)	Found (mg)[a]	Standard deviation (mg)
0.747	0.726	0.04
2.240	2.158	0.08
3.734	3.842	0.10
5.227	5.452	0.11

[a] The values found are averages of seven determinations, from which the standard deviation was also calculated.

In a practical sample, the brilliant green contents obtained titanometrically and by the new method with the ascorbinometric and spectrophotometric measurement amounted to 70.94, 71.10, and 72.35%, respectively. These results are very good, considering the high contents of impurities in brilliant green samples and the fact that the new methods determine amounts of the dye that are 10-fold lower than in the titanometric determination.

The newly developed methods can be recommended for practical determinations of brilliant green chiefly because of their rapidity, relative simplicity, and the fact that the complex apparatus required for titanometric titrations under an inert atmosphere is not required.

SUMMARY

The stoichiometry of the reaction of brilliant green with ceric sulfate was studied. An indirect determination of brilliant green based on its oxidation with excess ceric sulfate and the ascorbinometric or spectrophotometric determination of the diphenoquinone-(4,4')-bis(diethylimine) produced has been developed.

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Investigation of the Association of Higher Fatty Alcohols by Means of Paper Chromatography

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INTRODUCTION

Intermolecular interactions, particularly those through hydrogen bonds, seriously affect the results of chromatographic investigations. The so-called "ortho" effect has been known for a long time, and more recent investigations attempt, among other things, to describe the influence of mobile phase components on the R_f values of substances under examination (*1*). Graham and Daly's results (*1*) were a sort of chromatographic confirmation of the "hydrogen bond index" values determined for a large group of phenols by Sears and Kitchen (*2*), who used IR spectroscopy as a measuring technique.

The purpose of our work is to introduce chromatographic paper as a low-activity layer to test the influence of a mobile phase on the establishment of association equilibria.

EXPERIMENTAL

Whatman 2 chromatographic paper was used as an immobile phase, and the mobile phases applied are described in Table 1. Carbon tetrachloride solutions (0.125 *M*/liter) of myristyl alcohol and cetyl alcohol were used. For each experiment 5-μl amounts of a given sample were applied at starting points, developing chromatograms 16 cm high. Each chromatogram was dried with an electric fan and visualized with a 5% solution of $K_2Cr_2O_7$ in 50% H_2SO_4. Chromatographic spot areas were determined immediately after visualization and were then planimetrically evaluated. The results are shown in Figs. 1 and 2 and Tables 2 and 3. In addition, IR spectroscopic measurements were performed for the following samples:

1. a 0.125 *M*/liter decaline solution of cetyl alcohol;
2. a decaline solution of the cetyl alcohol–methyl alcohol bicomponent system (0.125 *M*/liter of cetyl alcohol, 0.125 *M*/liter of methyl alcohol);
3. a decaline solution of the cetyl alcohol–propyl alcohol bicomponent system (0.125 *M*/liter of cetyl alcohol, 0.125 *M*/liter of propyl alcohol);

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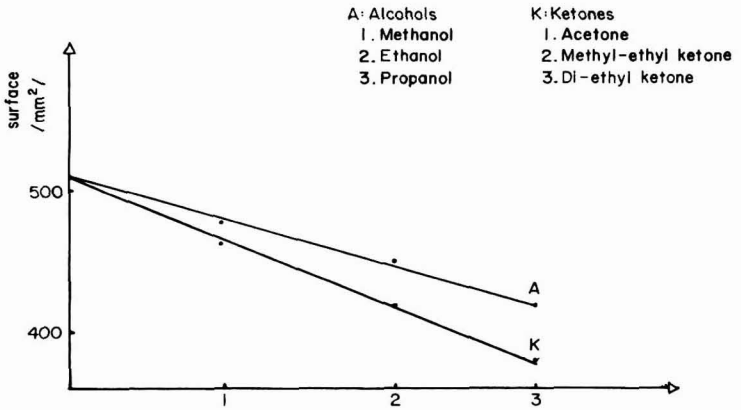


FIG. 1. Changes of the chromatographic spot areas of myristyl alcohol depending on the alcohol (A) and ketone (K) in the bicomponent developing systems.

4. a decaline solution of the cetyl alcohol–dimethyl ketone bicomponent system (0.125 *M*/liter of cetyl alcohol, 0.2 *M*/liter of dimethyl ketone);

5. a decaline solution of the cetyl alcohol–diethyl ketone bicomponent system (0.125 *M*/liter of cetyl alcohol, 0.2 *M*/liter of diethyl ketone).

The IR spectra were run by means of a UR-20-type spectrophotometer (Carl Zeiss, Jena, East Germany) in the range 3800 to 3050 cm^{-1}. NaCl cells 1 mm thick and an LiF prism were applied, under the following conditions: slot 4, a measuring speed of 64 cm^{-1}/min, and a

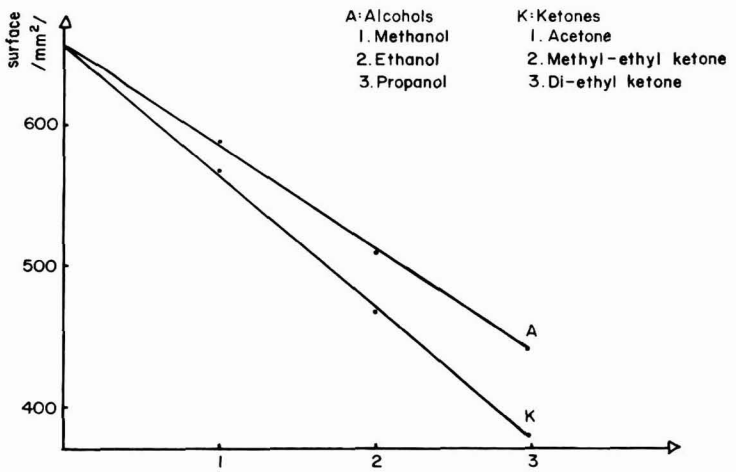


FIG. 2. Changes of the chromatographic spot areas of cetyl alcohol depending on the alcohol (A) and ketone (K) in the bicomponent developing systems.

TABLE 1
THE MOBILE PHASES

Mobile phase	Description	Volume ratio of components	Dielectric constant
Decaline	a	—	2.20
Decaline–methanol	b	100:0.5	2.76
Decaline–ethanol	c	100:0.5	2.49
Decaline–propanol	d	100:0.5	2.38
Decaline–dimethyl ketone	e	100:0.5	2.40
Decaline–methyl ethyl ketone	f	100:0.5	2.34
Decaline–diethyl ketone	g	100:0.5	2.31

TABLE 2
THE CHROMATOGRAPHIC SPOT AREAS OF MYRISTYL ALCOHOL AND CETYL ALCOHOL AT THE CHROMATOGRAMS DEVELOPED IN DECALINE AND IN DECALINE–ALIPHATIC ALCOHOL BICOMPONENT SYSTEMS

Substance analyzed	Chromatographic spot area (mm^2) in the mobile phase[a]			
	a	b	c	d
Myristyl alcohol	511	475	455	419
Cetyl alcohol	658	591	510	440

[a] Description of the mobile phases according to Table 1; the analytical results are mean values taken from 10 measurements.

TABLE 3
THE CHROMATOGRAPHIC SPOT AREAS OF MYRISTYL ALCOHOL AND CETYL ALCOHOL AT THE CHROMATOGRAMS DEVELOPED IN DECALINE AND IN DECALINE–KETONE BICOMPONENT SYSTEMS

Substance analyzed	Chromatographic spot area (mm^2) in the mobile phase[a]			
	a	e	f	g
Myristyl alcohol	511	460	420	382
Cetyl alcohol	658	571	467	375

[a] Description of the mobile phases according to Table 1; the analytical results are mean values taken from 10 measurements.

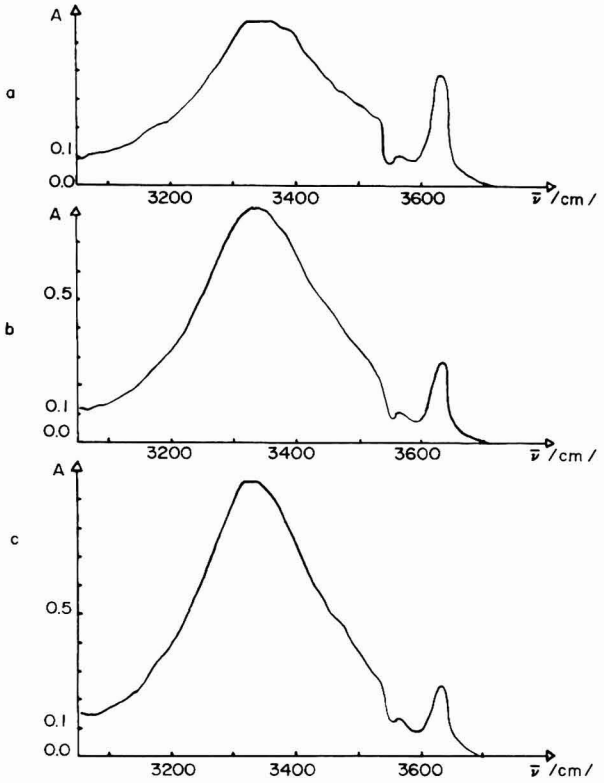


FIG. 3. The IR spectra in the range 3800 to 3050 cm^{-1} of: (a) cetyl alcohol; (b) the cetyl alcohol–propyl alcohol system; (c) the cetyl alcohol–methyl alcohol system. All samples were dissolved in decaline; in each case the cetyl alcohol concentration was 0.125 *M*/liter, and the methyl and propyl alcohol concentrations were also 0.125 *M*/liter.

registration width of 100 cm^{-1}/20 mm. All the spectra were obtained as a function $\%T = f(\bar{\nu})$ and they were recalculated at 10-cm^{-1} intervals as $A = f(\bar{\nu})$, using instead of the log I_0/I values the log $T_{b(\bar{\nu})}/T_{(\bar{\nu})}$ values ($T_{b(\bar{\nu})}$ is the percentage of the background transmission and $\bar{\nu}$ is the frequency value; $T_{(\bar{\nu})}$ is the percentage of the sample transmission). The spectra obtained are shown in Figs. 3 and 4. For each spectrum a graphic separation of the stretching vibration band of the free hydroxyl groups was performed and its integral intensity, defined as

$$i = \int_{\bar{\nu}_1}^{\bar{\nu}_2} \log \frac{T_{b(\bar{\nu})}}{T_{(\bar{\nu})}} d\bar{\nu},$$

was planimetrically evaluated. The results are given in Table 4.

DISCUSSION AND CONCLUSIONS

As seen from the results given in Tables 2 and 3 and Figs. 1 and 2, the chromatographic spot areas of myristyl alcohol are smaller than those

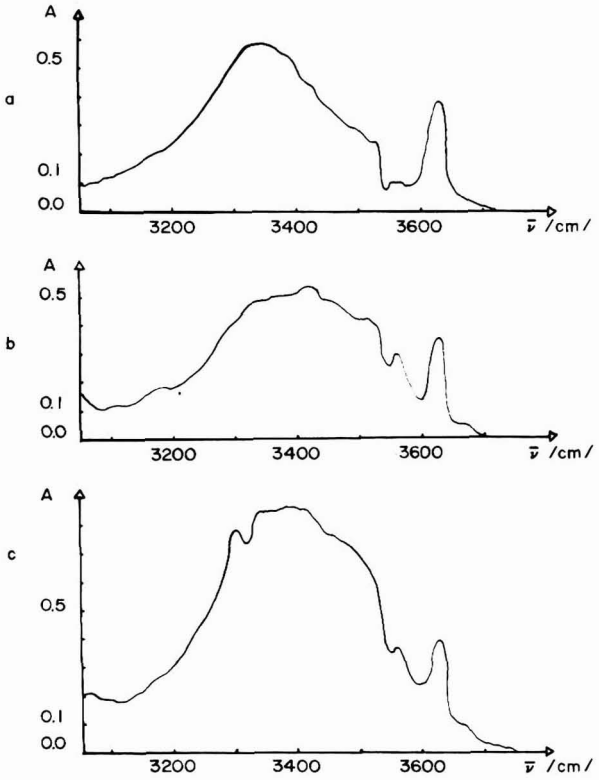


FIG. 4. The IR spectra in the range 3800 to 3050 cm^{-1} of: (a) cetyl alcohol; (b) the cetyl alcohol–diethyl ketone system; (c) the cetyl alcohol–dimethyl ketone system. All samples were dissolved in decaline; in each case the cetyl alcohol concentration was 0.125 M/liter, and the dimethyl and diethyl ketone concentrations were 0.2 *M*/liter.

of cetyl alcohol. In addition, as shown in Figs. 1 and 2, the higher the carbon chain length of alcohol or ketone which modifies decaline, the lower the chromatographic spot areas with both alcohols examined. There is still another phenomenon to be discussed: The relative decrease

TABLE 4

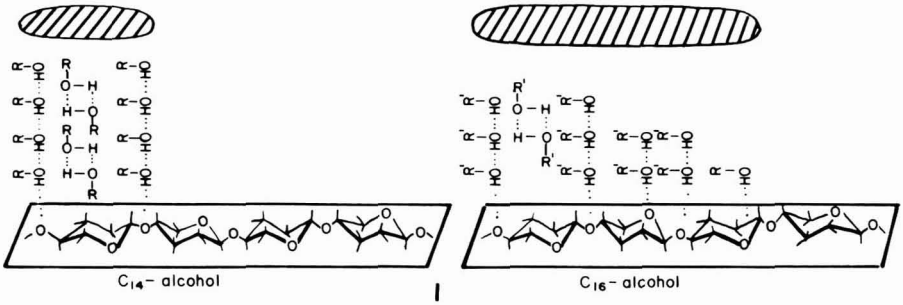
THE INTEGRAL INTENSITY VALUES OF THE FREE HYDROXYL STRETCHING VIBRATION BANDS IN THE IR SPECTRA OF THE SAMPLES EXAMINED

Sample	i (cm^{-1})
Cetyl alcohol	11.00
Cetyl alcohol–propyl alcohol	8.75
Cetyl alcohol–methyl alcohol	7.25
Cetyl alcohol–diethyl ketone	8.30
Cetyl alcohol–dimethyl ketone	7.60

of the chromatographic spot areas is more rapid with modification of the basic component of the mobile phase with ketone than with modification with alcohol.

Let us begin by explaining the fact that the chromatographic spot areas are smaller with myristyl alcohol than with cetyl alcohol. According to our previous observation (T. Kowalska, unpublished results) concerning higher fatty alcohols with from 12 to 20 carbon atoms in a molecule, the larger the number of carbon atoms in the molecule of a given alcohol, the higher the concentration of free, nonbonded hydroxyl groups. Thus there is good reason to assume that with increasing carbon chain length of higher fatty alcohols at least one of the following effects should play an important role: (a) increasing concentration of completely free, nonbonded molecules in a given alcohol; (b) shift toward linear multimers with relatively lower numbers of n-mers; and/or (c) decreasing ability to form cyclic multimers.

Taking into account this complex situation, the difference between the chromatographic spot areas of myristyl alcohol and cetyl alcohol is schematically explained by the following drawing.[1]



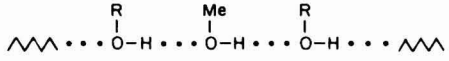
I

To determine why, with an increase in the carbon chain length of an alcohol or a ketone which modifies decaline as a basic component of the mobile phase, decreases in the chromatographic spot areas were observed with both myristyl alcohol and cetyl alcohol, additional measurements were performed by means of IR spectroscopy. As the data given in Figs. 3 and 4 and Table 4 show, modifications of decaline with methyl and propyl alcohols and with dimethyl and diethyl ketones contribute toward decreases in the integral intensity values of the free hydroxyl stretching vibration bands. Thus it can be deduced that the polar components of the mobile phases, of both alcohols and ketones contribute toward increases in the association degrees of the chromatographed substances. As stated

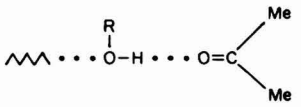
[1] Schemes I to III explain the difference between the chromatographic spot areas of myristyl alcohol and cetyl alcohol through the difference between their abilities to self-associate.

earlier, the chromatographic spot areas depend strongly upon the concentrations of the free hydroxyl groups (Scheme I). The chromatographic spot areas are smaller in the mobile phases modified with polar components than in a mobile phase of pure decaline. This effect is stronger with increasing carbon chain length of an alcohol or a ketone which modifies decaline. The following statement appears to explain this phenomenon. The longer the carbon chain of an alcohol or a ketone, the less effective their interactions with the chromatographed substances. The higher integral intensity values of the free hydroxyl bands with the cetyl alcohol–propyl alcohol and cetyl alcohol–diethyl ketone systems (8.75 and 8.30 cm^{-1}, respectively), compared with those for the cetyl alcohol–methyl alcohol and cetyl alcohol–dimethyl ketone systems (7.25 and 7.60 cm^{-1}, respectively) serve as a proof of the statement. If we now reverse our manner of reasoning, we can state that the shorter the carbon chain of an alcohol or a ketone of a mobile phase, the greater the possibility of forming mixed multimers, i.e., multimers which include both the molecules of the chromatographed substance and those of the mobile phase component. To support this assumption we compared the 3560- to 3450-cm^{-1} ranges of the spectra of Figs. 4a, b, and c. With the spectrum of cetyl alcohol dissolved in decaline (Fig. 4a), one observes no absorption arm in this region, as it takes place with the samples of the cetyl alcohol–ketone systems (Figs. 4b and c). The presence of an intense absorption arm in the range 3560 to 3450 cm^{-1} with the samples of the a/m bicomponent systems gives evidence of a shift of the association equilibria toward multimers with lower n-mer numbers (probably with modest contributions of dimers). The mixed multimers most likely decompose when the chromatogram is dried and in this way additional free hydroxyls are liberated, which may interact directly with the chromatographic layer. The lower the aliphatic chain of an alcohol or a ketone, the more convenient the conditions for developing the situation described above; i.e., a larger number of free hydroxyls will arise when the chromatogram is dried and a larger area of the layer will be occupied by the chromatographed substance.

In conclusion, we should stress the difference in the nature of interactions between chromatographed substances (myristyl and cetyl alcohols), on the one hand, and alcohols or ketones as components of the mobile phase, on the other. Methyl and propyl alcohols can build their molecules into the multimer chain, owing to their OH functional groups, which include both "basic" oxygen and "acidic" hydrogen atoms. It can be shown in the following way:

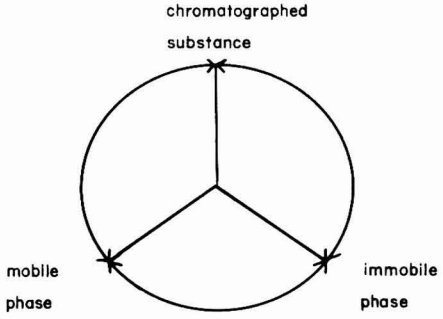


The ketone molecules possess the "basic" oxygen atom only, which limits their role to that of terminal molecules, able to end the multimer chain:



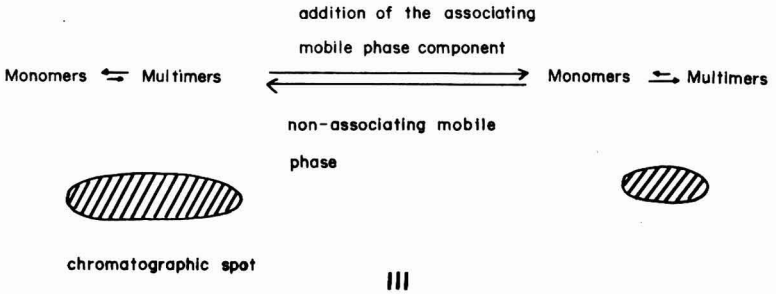
Thus with equal quantities of chromatographed substances and with alcohols and ketones of similar concentrations as components of the mobile phase, ketone–chromatographed substance interactions are much less likely to occur than alcohol–chromatographed substance interactions. The impossibility of building the ketone molecule into the multimer chain of the substance analyzed is probably the main reason why the decrease of the chromatographic spot areas is greater with ketones than with alcohols as the mobile phase components.

The given state of thermodynamic equilibria in a chromatographic system composed of a chromatographed substance, a mobile phase, and an immobile phase can generally be described by the following scheme:



II

Increasing the polarity of the mobile phase through the addition of a component able to form hydrogen bonds affects this thermodynamic equilibrium system; it contributes toward an increase in the dissociation degree of the chromatographed substance, and, eventually, to a decrease in its chromatographic spot area.



III

Scheme III attempts to describe the dependence by means of a model.

SUMMARY

Paper chromatography investigations were performed with the aim of tracing the adsorption mechanism of the selected higher fatty alcohols on a paper layer and determining the role of polar solvents in establishing thermodynamic equilibria.

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MICROCHEMICAL JOURNAL 23, 329–335 (1978)

A New Reagent for the Spectrophotometric Microdetermination of Cadmium

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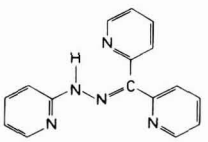
INTRODUCTION

The complexes of different metal ions [Zn(II), Cu(II), Pd(II), V(V), Co(II), Fe(II), Fe(III)] with the reagent 2,2′-dipyridyl-2-pyridylhydrazone (DPPH) (I) have been described in a number of recent papers (*1–4, 9*). In the cases examined DPPH acts as a di- or tridentate chelating agent and forms mono-, bis-, or tricomplexes, according to the preferred metal coordination number. In alkaline solutions deprotonation of the chelate compounds at the imino group takes place, giving in most cases intensively colored neutral complexes.



(I)

As part of a study concerning the analytical possibilities of DPPH, the results on the cadmium(II)–DPPH complex are described in this paper and a rapid, reproducible, and senstive method for the spectrophotometric determination of cadmium with DPPH is proposed.

EXPERIMENTAL

Apparatus

Absorbance measurements were made with a Gilford 2400-2 spectrophotometer and a Unicam SP 800 recording spectrophotometer, both equipped with 1-cm matched quartz cells.

Reagents

Reagents of A. R. quality were used wherever possible throughout this work. The ethanol was distilled and the water was redistilled from an all-Pyrex glass apparatus.

Standard cadmium(II) solution, 10^{-4} M. This solution was freshly prepared from a stock 1000-ppm Cd(II) ("Titrisol" Merck) solution.

Sodium perchlorate solution, 1M.

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Buffer solutions. The adjustment of pH was achieved with boric acid–borax mixtures (for pH 6.5 to 9.0), borax–sodium hydroxide mixtures (for pH 9.5 to 11.0), and sodium hydroxide (for pH above 11).

2,2'-Dipyridyl-2-pyridylhydrazone (DPPH) ethanolic solution, 10^{-2} M. The reagent DPPH was synthesized by refluxing an ethanolic solution of equimolar quantities of di-2-pyridylketone and 2-pyridylhydrazone as previously described (*3*). A 10^{-2} *M* working solution was prepared by dissolving 0.27531 g of DPPH in 100 ml of ethanol.

Procedure

Dilute a sample of test solution, containing 5 to 50 μg of cadmium, to about 20 ml. Add 1.25 ml of the DPPH solution and mix. Adjust the pH to 12.30 ± 0.20 with sodium hydroxide solution. Dilute to 25 ml and read the absorbance at 444 nm versus a reagent blank. The concentration of cadmium is determined by a previously prepared calibration graph.

RESULTS AND DISCUSSION

The study of the cadmium(II)–DPPH complex formation was carried out at an ionic strength of 0.1, regulated by the sodium perchlorate solution.

Absorptimetric characteristics. Cadmium(II) forms a yellow complex with DPPH, showing a maximum absorbance at 444 nm. The spectrum of this complex in the visible is shown in Fig. 1 (curve A). This spectrum was obtained as soon as possible after the reaction, at pH 12.29, of excess

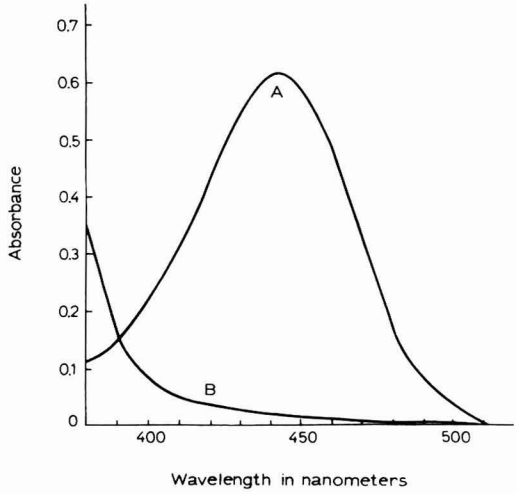

FIG. 1. Absorption spectra. Curve A is the spectrum of the cadmium(II)–DPPH complex, at pH = 12.29 and μ = 0.1, obtained on the reaction of cadmium(II) ions and DPPH, at concentrations 2.4×10^{-4} and 2.4×10^{-5} *M*, respectively. Curve B is the spectrum of a 2.4×10^{-5} *M* DPPH solution, obtained under the same conditions.

cadmium(II) (2.4×10^{-4} *M*) with DPPH (2.4×10^{-5} *M*), before a cadmium precipitate was observed. In Fig. 1 the spectrum of DPPH (2.4×10^{-5} M) is also given, under the same experimental conditions (curve B).

Comparison of the two spectra shows that at the wavelength of maximum absorbance for the complex, i.e., 444 nm, the absorbance of the reagent is slight. Consequently, this wavelength is suitable for use as the analytical wavelength.

Effect of acidity. A pH study was carried out in order to determine the optimum pH conditions for the cadmium(II)–DPPH complex formation. As shown in Fig. 2, the maximum color development of the complex solution takes place at high pH values, i.e., between 12 and 13. In this pH range one may consider the absorbance of the solution to be independent of pH.

Stability of the color of the complex. When DPPH solution is added to a cadmium(II) solution, under the experimental conditions given in the proposed procedure, the color formation is instantaneous and the absorbance of the complex solution remains stable for at least 2 hours, despite the presence or absence of diffuse daylight.

Molar composition of the complex. Job's method of continuous variations and the molar ratio method (*7*) have been used to evaluate the stoichiometric ratio of metal to ligand in the complex. The results are shown in Figs. 3 and 4, respectively. The 1:2 metal-to-ligand molar ratio, under the experimental conditions given, was ascertained by both methods.

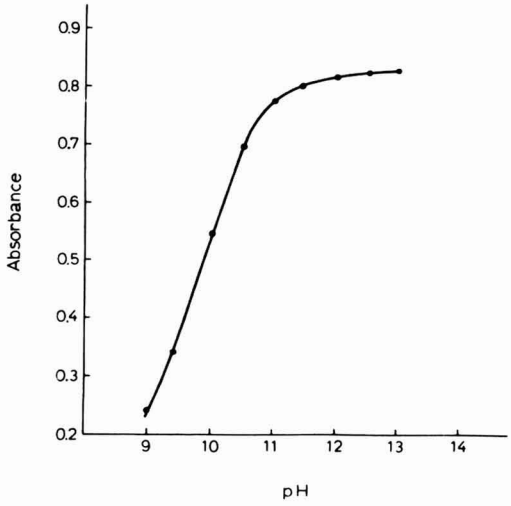

FIG. 2. The effect of acidity on the cadmium(II)–DPPH complex formation. Concentration of reactants: for cadmium(II), 1.5×10^{-5} *M*; for DPPH, 5×10^{-4} *M*. $\mu = 0.1$, $\lambda = 444$ nm.

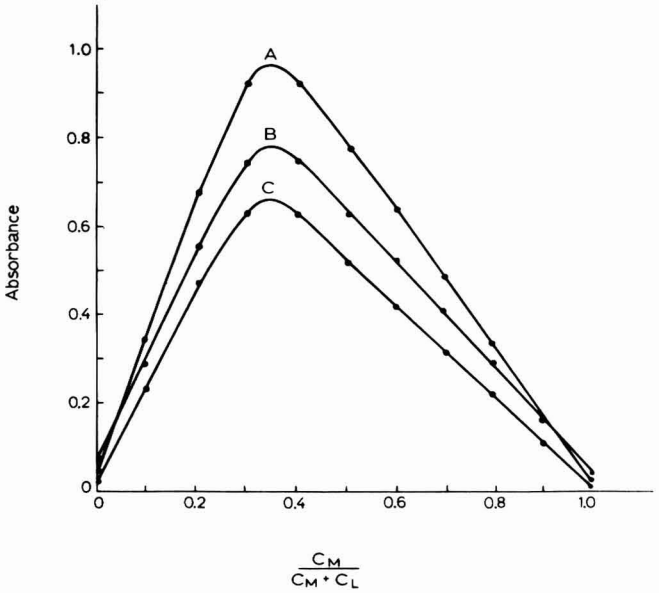

FIG. 3. Composition of the cadmium(II)–DPPH complex by Job's method. Total molarity: $C_M + C_L = 6 \times 10^{-5}$ *M*. $\mu = 0.1$, pH = 12.3 ± 0.2, λ = 444 (A), 425 (B), 465 (C) nm.

The absorbance curves of the solutions used for drawing the graphs in Figs. 2, 3, and 4 show no shift of the wavelength of maximum absorbance, lying at 444 nm. From this it can be deduced that one complex is mainly formed in the pH range investigated, whatever the metal-to-ligand molar

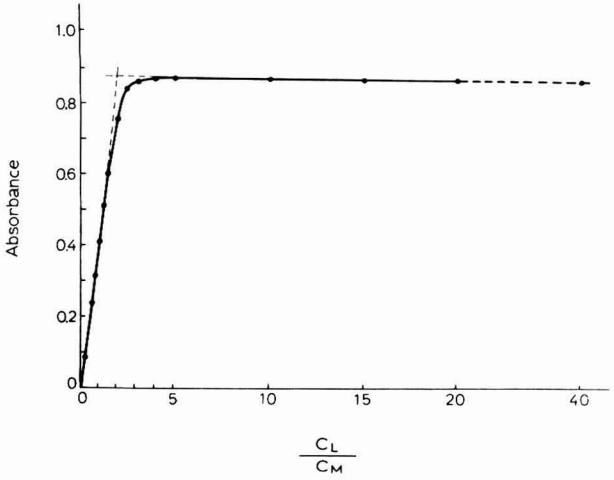

FIG. 4. Composition of the cadmium(II)–DPPH complex by the molar ratio method. Concentration of cadmium(II) (fixed component) = 1.6×10^{-5} *M*. $\mu = 0.1$, pH = 12.3 ± 0.2, λ = 444 nm.

concentration in the solution. This is partly shown in Fig. 4, by drawing the Job curve at three different wavelengths.

Effect of reagent concentration. The molar ratio plot shown in Fig. 3 indicates that a four-fold excess of reagent is sufficient for complete color development.

The overall apparent instability constant of the complex. Two spectrophotometric methods, the method of Harvey and Manning (*6*) and the method of Turner and Anderson (*5, 8*), have been used to determine by approximation the overall apparent instability constant, K, of the cadmium(II)–DPPH complex. The determination was made at pH 12.3 ± 0.1 and at an ionic strength of 0.1. Seven determinations of K were made, in total by the two methods, and the results agree closely with each other. K was found to be of the order of 10^{-12}.

Beer's law and optimal concentration range. A straight line was obtained over the concentration range 2×10^{-6} to 1.8×10^{-5} M (or 0.2 to 2 ppm) cadmium(II), when absorbance was plotted against concentration under the experimental conditions given in the recommended procedure.

The optimal range of the cadmium(II) concentration for accurate absorption measurements, as deduced from Ringbom's plot, was about 0.4 to 1.5 ppm cadmium(II).

Sensitivity. The molar extinction coefficient of the complex, under the experimental conditions given in the proposed procedure, was calculated from Beer's law plot and found to be 5.5×10^4 liters mole^{-1} cm^{-1}. Using Sandell's defintion of sensitivity, the sensitivity of the color reaction was 0.002 μg of cadmium(II)/cm^2 for an absorbance of 0.001 at 444 nm.

Precision. The precision of the method was evaluated at different cadmium(II) concentrations. Three sets, with six solutions each, were prepared containing 4×10^{-6}, 1×10^{-5}, and 1.4×10^{-5} M cadmium(II), respectively, and the absorbance was measured under optimal conditions. The mean absorbance value, the standard deviation (in absorbance units), and the coefficient of variation for the three sets respectively were as follows: 0.219 ± 0.002, 1.1%; 0.541 ± 0.006, 1.2%; 0.768 ± 0.010, 1.3%.

Effect of diverse ions. In order to study the effect of diverse ions on the determination of cadmium, a fixed amount of cadmium—enough to give a final cadmium concentration of 1×10^{-5} M—was taken with varying amounts of foreign ions, up to a 4000:1 mole ratio of ion:cadmium where necessary, and the recommended procedure was applied. An error of ±2% in the absorbance reading was considered tolerable.

Tolerances for various foreign ions are shown in Table 1.

Iron, if present up to a five-fold molar excess compared with cadmium, can be eliminated by fluoride at a 100:1 fluoride-to-iron molar concentration.

On the other hand Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Hg^{2+}, Pb^{2+}, and EDTA should not be present.

TABLE 1
EFFECT OF DIVERSE IONS ON THE DETERMINATION OF CADMIUM AT A CONCENTRATION OF 1×10^{-5} *M*

Iron	Ion:cadmium molar ratio
Li^+, Na^+, NH_4^+, As^{3+}, molybate, tungstate, bicarbonate, perchlorate, acetate, oxalate, citrate, tartrate	4000
K^+, Cl^-, I^-, NO_3^-, SCN^-, ascorbate	2000
Phosphate	1000
F^-, Br^-	500
Ca^{2+}, Ba^{2+}	100
CN^-	80
Cr^{3+}	20
Bi^{3+}, vanadate	10
Ag^+	3
Sb^{3+}	2
Mg^{2+}	1

SUMMARY

2,2′-Dipyridyl-2-pyridylhydrazone (DPPH) allows a simple, rapid, and sensitive spectrophotometric microdetermination of cadmium in aqueous solution. The yellow 1:2 metal-to-ligand complex formed has a molecular extinction coefficient of 5.5×10^4 liters mole^{-1} cm^{-1} at the absorption maximum of 444 nm. The determination of cadmium is carried out at pH 12.3 ± 0.2. Beer's law is obeyed over the concentration range of 0.2 to 2 ppm and the Sandell sensitivity of the color reaction is 0.002 μg of cadmium/cm^2 for an absorbance of 0.001.

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MICROCHEMICAL JOURNAL 23, 336–340 (1978)

2,4-Dioxo-4-(4-hydroxy-6-methyl-2-pyrone-3-yl)butyric Acid Ethyl Ester: Reagent for Identifying and Estimating Metal Ions in Ring Oven Analysis

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INTRODUCTION

The title compound (Ligand) (*1*, *3*) was synthesized by the sodium ethoxide-catalyzed condensation of dehydracetic acid and diethyl oxalate (M. Laćan *et al.*, unpublished). The product forms colored metal chelates with Cu(II), Co(II), and Ni(II) ions (*3*), and fluorescent (blue in ethanolic solution) complexes with ions of Al(III) and Be(II) (*1*). We have examined the usefulness of Ligand as a qualitative and quantitative broad-spectrum reagent for metal ions in ring oven (*6*, *7*) analysis. In this study we have tried to use Ligand as ligand for 18 kinds of ions, and to make the reaction selective by masking ions previously with common chelating agents [D. Pavišić *et al.*, in preparation; see also ref. (*2*, *4*, *5*)].

MATERIALS AND METHODS

Reagents. A stock solution of Ligand [recrystallized before use (*7*)] was prepared by dissolving 0.3 g in 100 ml of glacial acetic acid. Working solutions were made up freshly by mixing equal volumes of stock solution, 96% ethanol, and redistilled water.

Masking agents were aqueous solutions of EDTA (0.05 *M*), NTA (0.1 *M*), and CDTA (0.05 *M*).

Standard solutions containing the metal ions were prepared by dissolving appropriate salts in redistilled water and adjusting to a final concentration of 1 μg of ion/μl of solution. The following salts were used: $AlCl_3 \cdot 6H_2O$, $BeSO_4 \cdot 4H_2O$, $Bi(NO_3)_3 \cdot 5H_2O$, $CaCl_2 \cdot 6H_2O$, $CdCl_2 \cdot H_2O$, $Co(NO_3)_2 \cdot 6H_2O$, $CuSO_4 \cdot 7H_2O$, $FeCl_3 \cdot 6H_2O$, $HgCl_2$, K_2SO_4, $MnCl_2 \cdot 4H_2O$, $NiSO_4 \cdot 6H_2O$, $Pb(NO_3)_2$, $SbCl_3$, $SnCl_2 \cdot 2H_2O$, $Sr(NO_3)_2$, and $ZnSO_4 \cdot 7H_2O$.

All chemicals were reagent grade.

Materials and equipment. A modified ring oven (*2*) was used with filter paper No. 589² from Schleicher and Schuell (Dassel, Federal Republic of Germany) as the capillary support. Fluorescence of complexes deposited

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inside the ring zone was observed under 366-nm radiation filtered from a Camag Universal-UV-Lampe (Muttenz, Switzerland).

Procedures

Identification reactions. A known volume of standard solution was applied to the middle point of the support placed on the ring oven heated to 105°C. The metal ion was thereupon eluted into the ring zone by washing six times with a freshly prepared working solution of Ligand. The color of the ring zone was recorded under visible and uv illumination.

Setup of standard scales for semiquantitative estimations by ring colorimetry or fluorometry (*4*). Increasing volumes of standard solutions were used to prepare, in the way just described, a series of 10 standard rings. The smallest mass of ion deposited in a ring zone giving discernible coloration was used to characterize the sensitivity of reaction with Ligand.

Masking. Standard solution is applied and subsequently treated with the required volume of complexing agent solution. This treatment is followed by reaction with Ligand.

Estimation of Al(III), Cr(III), and Fe(III) in mixtures. Two runs are required. In the first run applied sample is immediately eluted with solution of Ligand, and the brown, nonfluorescent ring is compared to the Fe(III) standard series. In the second run, applied sample is first treated with EDTA solution which masks the iron and chromium ions. Elution with Ligand now produces a grayish-violet ring with the color due to a Cr(III)–EDTA complex, and it is compared to a corresponding series of Cr(III) standards. Under uv illumination the ring turns blue and can be compared to the Al(III) standards. Results from all comparisons allow the calculation of the amounts of Al(III), Cr(III), and Fe(III) in the sample.

RESULTS AND DISCUSSION

Of the 18 ions examined, 15 produced colored or fluorescent rings on treatment with Ligand. Only seven kinds of ion, however, gave colors or fluorescences sufficiently intense for quantitative work. The colors obtained with the 15 reacting ions are recorded in Table 1. Detection limits reflecting reaction sensitivities are entered for the seven ions that reacted with enough intensity.

Table 2 shows a summary of ring colors produced by Ligand after pretreatment with various masking agents. These colors resulted from the combination of contributing shades from free Ligand and from the particular complexes. These data show that any complexing agents used were only able to mask the ions of Cu(II), Co(II), Cr(III), Fe(III), Ni(II), and Pb(II), and were unable to mask the ions of Al(III), and Be(II).

TABLE 1

SUMMARY OF COLORS OR FLUORESCENCE OF PRODUCTS OBTAINED BY REACTING VARIOUS IONS WITH 2,4-DIOXO-4-(4-HYDROXY-6-METHYL-2-PYRONE-3-YL) BUTYRIC ACID ETHYL ESTHER ON A CAPILLARY SUPPORT[a]

Ion-generating element	Color of product[b]	Detection limit (μg)
Be(II)	bl. (fluorescence)	0.1
Al(III)	bl. (fluorescence)	0.2
Co(II)	ygr.	2.0
Ni(II)	ygr.	1.0
Cu(II)	gr.	0.2
Fe(III)	br.	0.2
Cr(III)	olive gr.	2.0
Hg(II)	pale y. (fluorescence)	
Cd(II)	pale y. (fluorescence)	
Bi(III)	light br.	
Pb(II)	gr.	
Sn(II)	ygr.	
Sr(II)	ygr.	
Ca(II)	ygr.	
K(I)	ygr.	
Zn(II)	—	
Mn(II)	—	
Sb(III)	—	

[a] Ring oven temperature, 105°C.
[b] bl. = blue; br. = brown; gr. = green; y. = yellow; ygr. = yellowish green.

TABLE 2

COLORS OR FLUORESCENCE OF PRODUCTS OBTAINED IN THE SAME MANNER AS THOSE SHOWN IN TABLE 1, BUT AFTER PRETREATMENT WITH CHELATING AGENTS

Ion-generating element	Chelating agent		
	NTA	EDTA	CDTA
Be(II)	bl. (fluorescence)[a]	bl. (fluorescence)	bl. (fluorescence)
Al(III)	bl. (fluorscence)	bl. (fluorescence)	bl. (fluorescence)
Cu(II)	ygr.	ygr.	ygr.
Fe(III)	bry.	bry.	bry.
Ni(II)	y.-olive gr.	olive gr.	light olive gr.
Co(II)	brp.	brp.	ybr.
Cr(III)	gy.	gyv.	v.
Pb(II)	y.	y.	y.

[a] brp. = brownish pink; bry. = brownish yellow; gy. = gray; gyv. = grayish violet; v. = violet; ybr. = yellowish brown.

TABLE 3

RESULTS OF Al(III) ESTIMATION IN MIXTURES CONTAINING Cr(III) AND Fe(III) BY RING COLORIMETRY OR FLUOROMETRY ON A RING OVEN, USING 2,4-DIOXO-4-(4-HYDROXY-6-METHYL-2-PYRON-3-YL) BUTYRIC ACID ETHYL ESTER AS THE REAGENT

Al(III) (μg)		Absolute error	Relative error	Components (μg)	
Present	Estimated[a]	(μg)	(%)	Cr(III)	Fe(III)
1.1	1.0	−0.1	−9.1	1.0	2.0
5.2	5.0	−0.2	−3.8	5.0	5.0
9.5	10.0	+0.5	+5.2	10.0	10.0
0.6	0.5	−0.1	−13.4	10.0	15.0
0.2	0.2	0.0	0.0	20.0	20.0

[a] Means of 5−10 estimations.

Basing on results described in preceeding paragraphs a procedure was worked out for estimation of Al(III), Cr(III), and Fe(III) in mixtures without separating the components. First, a sample was analyzed on a ring oven using Ligand as the reagent. The resulting brown ring allowed estimation of Fe(III) without interference from the other ions, by ring colorimetry. The fluorescence of Al(III), however, was quenched by Cr(III) and Fe(III). Another run was made in which applied sample was pretreated with EDTA, then reacted with Ligand. In this run the complexing agent has masked Fe(III) and Cr(III) against reacting with Ligand, but Cr(III)−EDTA passed together with Al(III)−Ligand into the ring zone, imparting its color to the ring under visible illumination. Thus Cr(III) could be estimated by ring colorimetry in visible light, and Al(III) by ring fluorometry under a uv lamp. Results of Al(III) estimations in various synthetic samples are presented in Table 3. These results show that Al(III) can be estimated in an amount equivalent to its detection limit (0.2 μg), in mixtures containing as much as 100 times this amount of both Cr(III) and Fe(III).

SUMMARY

Ligand gave variously colored or fluorescent rings when reacted with several kinds of ions on a ring oven (Schleicher and Schuell filter paper 589[2]). With ions of Al(III), Be(II), Co(II), Cr(III), Cu(II), Fe(III), and Ni(II) the reactions were sensitive enough to enable application of ring colorimetry or fluorometry (excitation at 366 nm) for quantitation (detection limits, 0.1−2.0 μg of metal). A procedure for estimation of Al(III), Cr(III), and Fe(III) in mixtures was based on reaction with Ligand in combination with EDTA pretreatment, but omitting ion separation. Al(III) could be estimated reliably at its detection-limit-level, in the presence of up to 100 times as much of both Cr(III) and Fe(III).

ACKNOWLEDGMENTS

The authors wish to thank Prof. Dr. Z. Štefanac and V. Drevenkar, mr. chem., of the Institute for Medical Research, Yugoslav Academy of Sciences and Arts, for the preparation of the title compound.

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The Use of Redox Reactions in the Analysis of Dyes and Dye Industry Intermediates

IV. Oxidation of Benzidine, *o*-Tolidine, and *o*-Dianisidine with Chloramine T and *N*-Bromosuccinimide[1]

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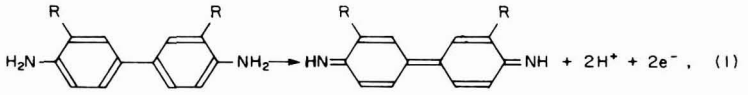
INTRODUCTION

Study of the oxidation of conjugated aromatic diamines, including benzidine, *o*-tolidine, and *o*-dianisidine, is interesting for two reasons. First, they are important intermediates in the manufacture of dyes. Consequently, suitable methods for their determination are needed; oxidimetric methods (*1*, *6*) exhibit certain advantages over the diazotization methods (*3*) that have been used most frequently. Second, it has been found (*2*, *7*) that the oxidation mechanism of these substances is most probably connected with their carcinogenic effects.

During oxidation of these substances, the nitrogen oxidation state may change or the aromatic rings may be destroyed to a lesser or greater extent, resulting in varying consumptions of oxidant. From the analytical point of view, the two-electron oxidation of the substances to the corresponding quinonediimines according to Eq. (1) seems to be most promising:



where R is H in benzidine, CH_3 in *o*-tolidine, and OCH_3 in *o*-dianisidine. The selectivity of the determination based on this oxidation reaction can be substantially improved by determining the amount of the oxidation product formed instead of measuring the amount of the reagent consumed. Therefore, the oxidation of benzidine, *o*-tolidine, and *o*-dianisidine with chloramine T and *N*-bromosuccinimide was studied, as it could be expected that the diphenoquinonediimine produced in the first reaction step would undergo the substitution of the imine residue by a halogen,

[1] Part III. An Indirect Determination of Brilliant Green with Ceric Sulfate, *Microchem. J.* 23, 312–319 (1978).

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with formation of *N,N'*-dihalogenodiphenoquinonediimine extractable into chloroform. Considering the structure of this product it could further be expected that it would have an exceptionally high molar absorption coefficient permitting a sensitive spectrophotometric determination.

To verify the above assumptions, the oxidation of benzidine, *o*-tolidine, and *o*-dianisidine with chloramine T and *N*-bromosuccinimide was studied in the present paper. The oxidation products were identified first, then the reaction mechanism was proposed on the basis of spectrophotometric and potentiometric monitoring of the reaction course, and finally the possibilities of analytical use of the reactions were considered.

EXPERIMENTAL

Reagents. Chloramine T, a 0.01 *M* solution, was prepared by dissolving the accurately weighed substance in distilled water and diluting to 1 liter. The titer was determined using arsenic(III) oxide (*8*). *N*-Bromosuccinimide, a 0.01 *M* solution, was prepared by dissolving the accurately weighed substance in distilled water and diluting to 1 liter. The titer was determined using arsenic(III) oxide (*4*). Benzidine, *o*-tolidine, and *o*-dianisidine, 0.005 and 0.0005 *M* solutions in 0.02 *M* hydrochloric acid, were prepared by dissolving the accurately weighed substances in 100 ml of 0.2 *M* hydrochloric acid and diluting to 1 liter with distilled water.

All chemicals used were of p.a. purity.

Apparatus. Potentiometric titrations were carried out in an automatic titrator consisting of a PHM 64 pH meter, TTT 60 titrator, ABU 12 buret, and REC 61 recorder (Radiometer, Copenhagen), with a bright platinum indicator and a saturated calomel reference electrodes. Spectrophotometric measurements were performed using a Specord UV VIS instrument (C. Zeiss, Jena) in 0.5 and 1.0-cm quartz cuvettes. The infrared spectra were obtained on an UR 20 instrument (C. Zeiss, Jena) in a 0.1-mm cuvette. The mass spectrum was obtained on a JEOL MS D 100 instrument.

PROCEDURES AND RESULTS

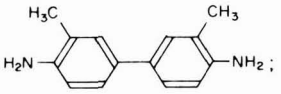
Identification of the Reaction Products

The oxidation product of *o*-tolidine in reaction with chloramine T was isolated in solid state, and its infrared and mass spectra were obtained as follows: To a solution of 2 g of *o*-tolidine in 200 ml of 0.02 *M* hydrochloric acid were added 10 g of chloramine T dissolved in 200 ml of distilled water. The mixture was mixed thoroughly and set aside for 15 min at laboratory temperature. The clear solution above the precipitate was decanted, and the precipitate was washed with 200 ml of distilled water and after 15 minutes filtered off through an S4 frit, washed again with distilled water and a small amount of ethanol, and dissolved in 100 ml of

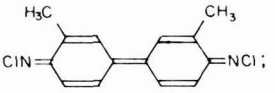
warm (50°C) chloroform. The undissolved residue was filtered off through cotton wool. The chloroform solution was cooled to 0°C and after 2 hr the crystals formed were filtered off through an S4 frit and dried in a desiccator containing silica. Thin-layer chromatography indicated that the substance is chemically homogeneous.

The isolated substance was heated to 150°C when it decomposed, and the mass spectrum of the vapor was recorded immediately. Ions with relative molecular masses of 212, 278, and 280 were present in the highest contents. On the basis of the elemental analysis of the corresponding lines, these relative molecular masses were assigned to the following compounds:

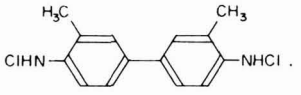
212: the initial *o*-tolidine,



278: *N*,*N*′-dichloro-3,3′-dimethyl-diphenoquinonediimine,



and 280: *N*,*N*′-dichloro-*o*-tolidine,



As the infrared spectrum of the isolated oxidation product does not contain an absorption maximum in the region 3100 $-3600\ cm^{-1}$, where the N−H bond vibration would be reflected, it can be assumed that the product is *N*,*N*′-dichloro-3,3-dimethyl-diphenoquinonediimine, whereas the initial *o*-tolidine and its *N*,*N*′-dichloroderivative are formed only during its thermal decomposition.

On the basis of the similarity of the visible absorption spectra and on the basis of potentiometric monitoring of the reaction of benzidine, *o*-tolidine, and *o*-dianisidine with chloramine T and *N*-bromosuccinimide (see below), it can be assumed that analogous products are also formed in the reactions of the other diamines with the two oxidants.

Potentiometric Monitoring of the Reaction

Direct potentiometric titrations of the diamines with both chloramine T and *N*-bromosuccinimide were carried out. To 5.00 ml of the diamine solution were added 20 ml of distilled water, and the solution was titrated with a 0.01 *M* titrant, recording the titration curve. The titration curves

with chloramine T could not be evaluated because of slow potential stabilization, but the curves with *N*-bromosuccinimide (Fig. 1) indicated that the reactions involve two steps. The number of moles of *N*-bromosuccinimide per mole of diamine was determined from the titrant consumptions corresponding to the individual potential breaks. The data are given in Table 1, from which it follows that the first step corresponds to the oxidation of aromatic diamines to the diimines according to Eq. (3) (a consumption of 1 mol of the oxidant) and the second step corresponds to substitution by bromine according to Eq. (5) (a total consumption of 3 mol of the oxidant/mol of the diamine). The second potential break was not obtained with *o*-dianisidine, apparently because of instability of the *N,N'*-dibromo-3,3'-dimethoxydiphenoquinonediimine formed (see the results of the spectrophotometric following of the reaction course).

Spectrophotometric Monitoring of the Reaction

The visible absorption spectra of the diamine solutions were measured after adding an accurately known excess of an oxidant. To 5.00 ml of a 0.0005 *M* diamine solution, 0.2, 0.5, 1.0, 2.0, and 5.0 ml of 0.01 *M* chloramine T or 0.1, 0.2, 0.5, 1.0, and 2.0 ml of 0.01 *M* *N*-bromosuccinimide were successively added, the solution was diluted to 50 ml with distilled water after 2 min, and the absorption spectrum was measured from 330 to 800 nm in a 0.5-cm cuvette.

It was found (see Table 2) that maxima identical with those of the corresponding diphenoquinonediimines (*2*) appear in the measured spectra at an oxidized substance-to-oxidant molar ratio of 0.8 to 2. These maxima disappear only at higher oxidant excesses, and new maxima appear, corresponding to *N,N'*-dibromo or *N,N'*-dichloro-dipheno-

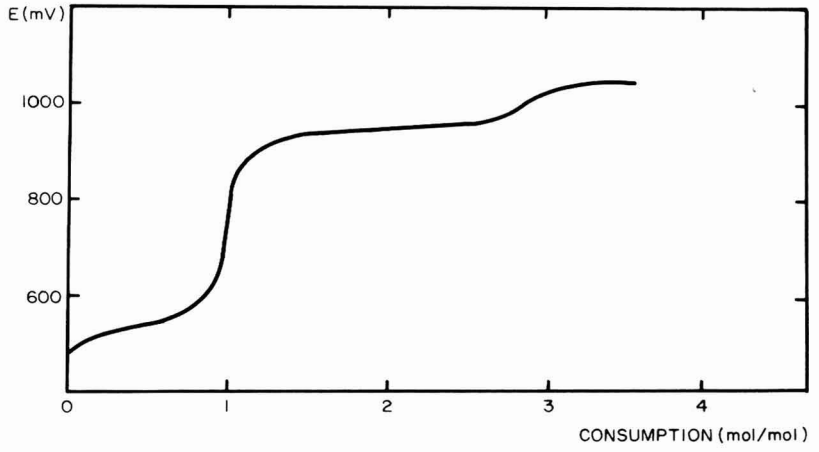


FIG. 1. Potentiometric titration curve of *o*-tolidine titrated with *N*-bromosuccinimide.

TABLE 1
CONSUMPTION OF N-BROMOSUCCINIMIDE IN MOLES PER MOLE OF AN AROMATIC DIAMINE IN POTENTIOMETRIC TITRATION

Diamine	Consumption (mol/mol)	
	First break	Second break
Benzidine	1.18	3.06
o-Tolidine	1.56	3.06
o-Dianisidine	1.16	—

quinonediimines that are readily extracted into chloroform. The only exception is the oxidation of *o*-dianisidine with *N*-bromosuccinimide, when the *N,N'*-dibromo-3,3'-methoxydiphenoquinonediimine produced is rapidly decomposed with formation of a colored substance not extractable into chloroform. Changes in the absorption spectrum of *o*-tolidine at various molar excesses of *N*-bromosuccinimide are given in Fig. 2 for the sake of illustration.

Spectrophotometric Determination of Benzidine, o-Tolidine, and o-Dianisidine with Chloramine T

The possibility of determining the above substances by reaction with chloramine T and measurement of the absorbance of the reaction product extracted into chloroform was verified. First, the optimum time of the reagent action and the optimum amount of the reagent were determined,

TABLE 2
THE DEPENDENCE OF THE POSITION OF THE ABSORPTION MAXIMA OF THE DIAMINE SOLUTIONS ON THE MOLAR EXCESS OF CHLORAMINE T (A) AND N-BROMOSUCCINIMIDE (B)

Diamine		Absorption maxima (nm)				
		0.8[a]	2.0[a]	4.0[a]	8.0[a]	20[a]
Benzidine	A	425[b]	425	425	428	363
	B	425	438	—[c]	—[c]	—[c]
o-Tolidine	A	437[b]	437	437	439	410
	B	437	442	395	405	—[c]
o-Dianisidine	A	441[b]	441	441	450	435
	B	441	441	520	—[c]	—[c]

[a] Reagent molar excess.

[b] The absorption maxima positions in the first column are the values for diphenoquinonediimine (benzidine), 3,3'-dimethyldiphenoquinonediimine (*o*-tolidine), and 3,3'-dimethoxydiphenoquinonediimine (*o*-dianisidine).

[c] A precipitate is formed.

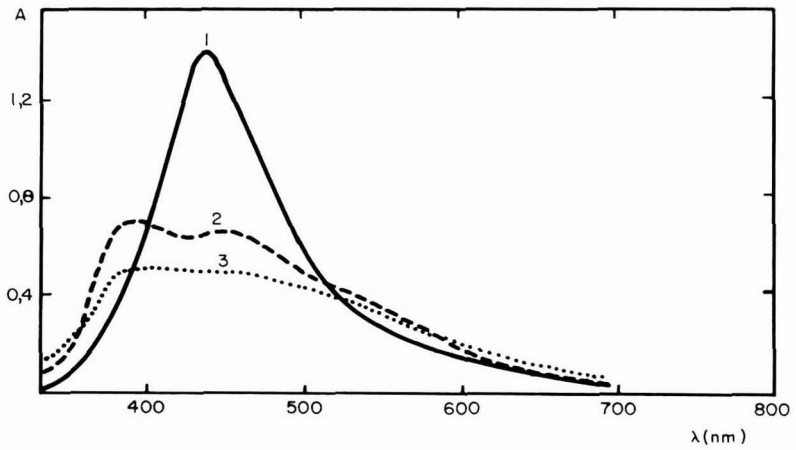


FIG. 2. Absorption spectrum of *o*-tolidine after oxidation with a twofold (1), fourfold (2), and eightfold (3) molar excess of *N*-bromosuccinimide.

and then the validity of the Lambert–Beer law was verified over the concentration range used.

The optimum time of the reagent action was found as follows: To 1.00 ml of a 0.0005 *M* solution of an aromatic diamine, 2 ml of 0.01 *M* chloramine T were added, and the solution was allowed to stand at laboratory temperature. After time *t*, 0.5 g of sodium chloride was added, and the solution was extracted with four 10-ml portions of chloroform. The combined extracts were diluted to 50 ml with chloroform, and the absorption spectrum was measured immediately from 330 to 800 nm in a 1-cm cuvette.

The spectra obtained for $t = 5$ min are given in Fig. 3, and the depen-

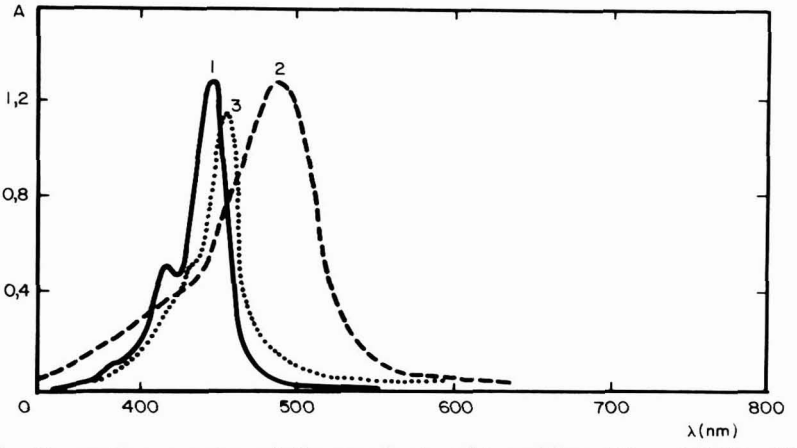


FIG. 3. Absorption spectra of the products of benzidine (1), *o*-tolidine (2), and *o*-dianisidine (3) oxidation with chloramine T, after their extraction into chloroform.

dence of the absorbance on the time of chloramine T action is given in Table 3. It follows from the table that the optimum time for treatment with excess chloramine T is 5 min for benzidine and *o*-tolidine and 2 min for *o*-dianisidine. It further follows that the oxidation product of *o*-tolidine is most stable in an aqueous medium, whereas that of *o*-dianisidine is least stable and decomposes very rapidly.

The following procedure was employed in determining the optimum molar excess of chloramine T: To 1.00 ml of 0.0005 *M* benzidine or *o*-tolidine, or 2.00 ml of 0.0005 *M* *o*-dianisidine, 0.5, 1.0, 2.0, 3.0, 5.0, and 10.0 ml of 0.01 *M* chloramine T were added, and after 5 min (benzidine and *o*-tolidine) or 2 min (*o*-dianisidine) 0.5 g of sodium chloride was added. The solution was then extracted with four 10-ml portions of chloroform. The combined extracts were diluted with chloroform to 50 ml, and the absorbances were measured in 1-cm cuvettes at the wavelengths corresponding to the maxima of the *N,N'*-dichlorodiphenoquinonediimines.

The results obtained are given in Table 4, from which it follows that a 20 to 40-fold excess of chloramine T is optimal. At higher reagent excesses the reaction product extraction into chloroform becomes less efficient, which results in obtaining lower absorbance values.

Calibration Curve Construction

Volumes of 0.20 to 1.00 ml of 0.0005 *M* benzidine and *o*-tolidine, or 0.40 to 2.00 ml of 0.005 *M* o-dianisidine were gradually measured, adding to them 2.0 or 3.0 ml of 0.01 *M* chloramine T. After 5 or 2 min, 0.5 g of sodium chloride was added, and the solution was extracted with four 10-ml portions of chloroform. The combined extracts were diluted to 50 ml with chloroform, and their absorbances were measured in a 1-cm cuvette at 444 (benzidine), 458 (*o*-tolidine), or 488 nm (*o*-dianisidine). The dependences obtained are given in Figs. 4 and 5, from which it follows that the Lambert–Beer law is obeyed for concentrations from 1×10^{-5} to 5×10^{-4} (benzidine and *o*-tolidine) and 2×10^{-4} to 1×10^{-3} *M* (*o*-dianisidine).

TABLE 3

THE DEPENDENCE OF THE ABSORBANCE OF THE APPROPRIATE *N,N'*-DICHLORODIPHENOQUINONEDIIMINES ON THE TIME OF TREATMENT WITH EXCESS CHLORAMINE T

Test substance	Wavelength (nm)	Absorbance				
		0[a]	2[a]	5[a]	10[a]	20[a]
Benzidine	444	1.06	1.20	1.28	1.16	1.15
o-Tolidine	458	1.12	1.13	1.14	1.10	1.09
o-Dianisidine	488	0.62	0.63	0.62	0.55	0.43

[a] *t* in minutes.

TABLE 4

THE DEPENDENCE OF THE ABSORBANCE OF THE APPROPRIATE *N,N'*-DICHLORODIPHENOQUINONEDIIMINES ON THE MOLAR EXCESS OF CHLORAMINE T

Molar excess of chloramine T	Absorbance		
	Benzidine (444 nm)	*o*-Tolidine (458 nm)	*o*-Dianisidine (488 nm)
10	0.54	0.62	0.43
20	1.30	1.15	1.20
40	1.30	1.15	1.26
60	1.28	1.12	1.23
100	0.97	1.11	0.96
200	0.82	0.96	0.80

Recommended Procedure for the Determination

To 1.00 ml of a sample solution containing 0.02–0.1 mg of benzidine or *o*-tolidine, or 0.05–0.25 mg of *o*-dianisidine, 2.00 ml of 0.01 *M* chloramine T are added (3.00 ml with *o*-dianisidine). After 5 min (2 min with *o*-dianisidine), 0.5 g of solid sodium chloride is added, and the solution is extracted with four 10-ml portions of chloroform. The combined extracts are diluted with chloroform to 50 ml, and their absorbances are measured immediately in 1-cm cuvettes at 444 (benzidine), 458 (*o*-tolidine), or 488 nm (*o*-dianisidine). The content of the diamine is found from the calibration curve obtained with the pure substance by the above procedure.

It was found that this procedure permits the determination of 0.02–0.1 mg of benzidine and *o*-tolidine with a precision better than ±4 or 3%,

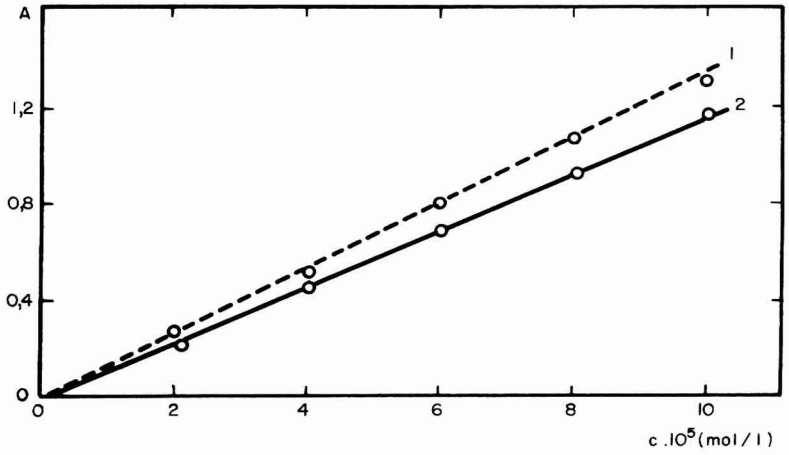


FIG. 4. Calibration curve for the spectrophotometric determination of benzidine (1) and *o*-tolidine (2), based on the measurement of the absorbance of the reaction product with chloramine T extracted into chloroform.

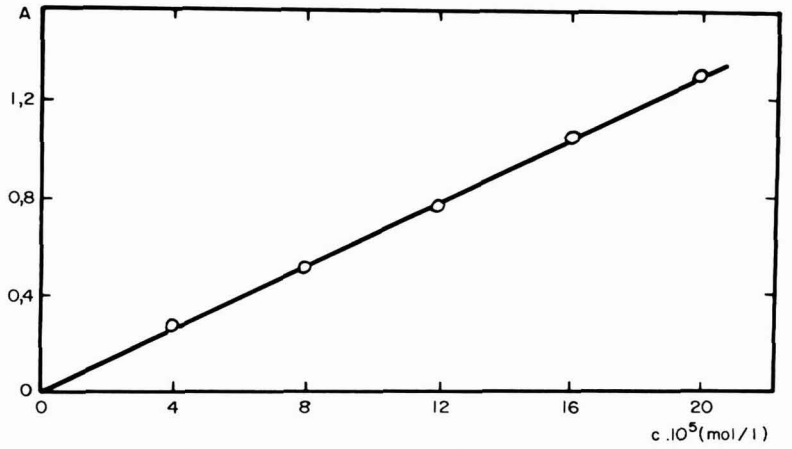


FIG. 5. Calibration curve for the spectrophotometric determination of *o*-dianisidine, based on the measurement of the absorbance of the reaction product with chloramine T extracted into chloroform.

respectively, and of 0.05–0.25 mg of *o*-dianisidine with a precision better than ±2%.

Spectrophotometric Determination of Benzidine and o-Tolidine with N-Bromosuccinimide

A procedure analogous to that employed with chloramine T has shown (5) that the optimum conditions involve a 2-min treatment with a 100-fold molar excess of *N*-bromosuccinimide.

Calibration Curve Construction

Volumes of 0.02 to 1.00 ml of 0.0005 *M* benzidine or *o*-tolidine were measured, 5.0 ml of 0.01 *M* *N*-bromosuccinimide were added, and the solution was extracted with four 10-ml portions of chloroform after 2 min. The combined extracts were diluted with chloroform to 50 ml, and the absorbance was measured at 455 nm for benzidine and 465 nm for *o*-tolidine in 1-cm cuvettes. The dependences obtained are depicted in Fig. 6, from which it follows that the Lambert–Beer law is obeyed in a concentration range of 1×10^{-4} to 5×10^{-4} *M*.

Recommended Procedure for the Determination

To 1.00 ml of a solution containing 0.02–0.1 mg of benzidine or *o*-tolidine, 5.0 ml of 0.01 *M* *N*-bromosuccinimide are added, and the solution is extracted with four 10-ml portions of chloroform after 2 min. The combined extracts are diluted to 50 ml with chloroform, and the absorbance is measured immediately in a 1-cm cuvette at 455 nm for benzidine and 465 nm for *o*-tolidine. The diamine content is found from the calibration curve constructed using the pure substance by the above procedure.

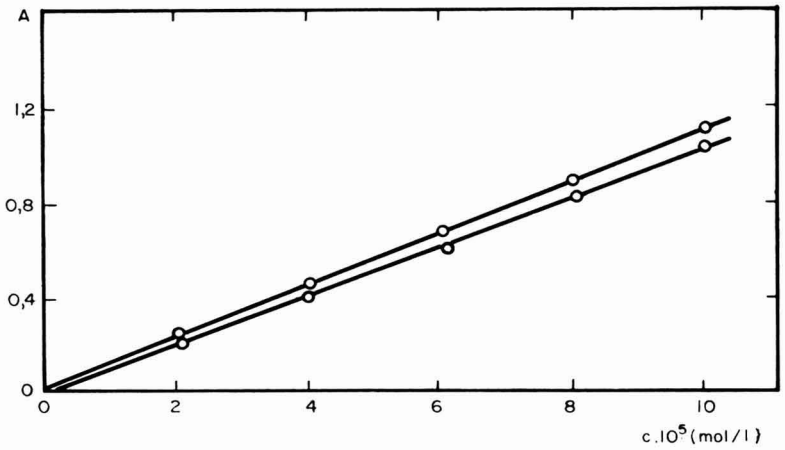
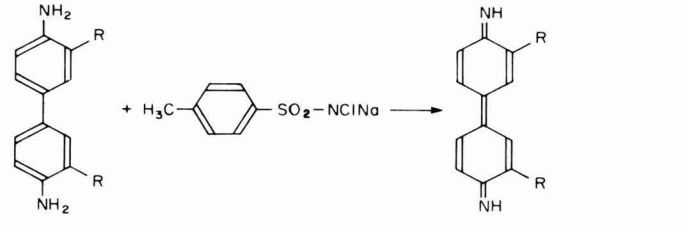


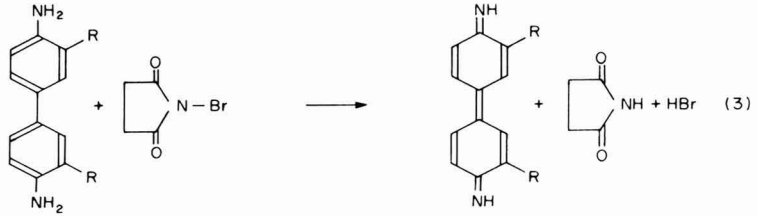
FIG. 6. Calibration curve for the spectrophotometric determination of benzidine (1) and *o*-tolidine (2), based on the measurement of the absorbance of the reaction product with *N*-bromosuccinimide extracted into chloroform.

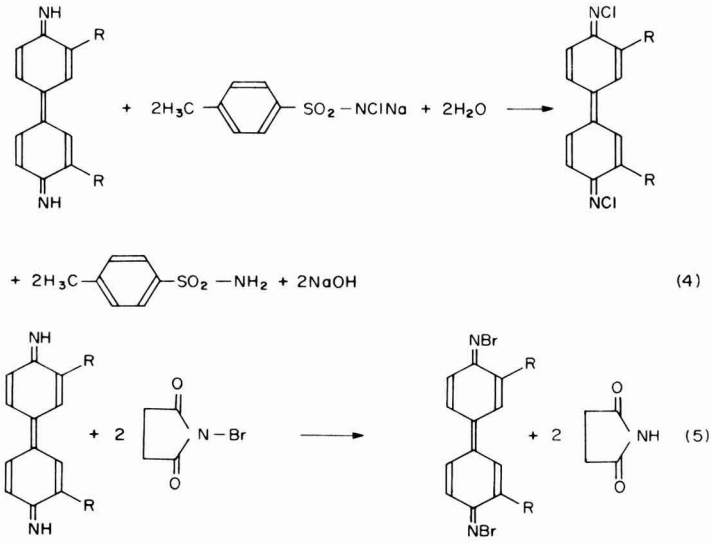
The procedure permits the determination of 0.02–0.1 mg of benzidine and *o*-tolidine with a precision better than ±4 and 2%, respectively.

DISCUSSION

It follows from the data presented in the Experimental section that the reaction of benzidine, *o*-tolidine, and *o*-dianisidine with chloramine T and *N*-bromosuccinimide consists of two steps. In the first step all three diamines are oxidized by both reagents to the corresponding quinone-diimines, according to Eq. (2) or (3). In the second step, the imine hydrogen is substituted by a halogen according to Eq. (4) or (5).







It has further been verified that the *N,N'*-dihalogenoderivatives of the corresponding quinonediimines are readily extracted into chloroform. This possibility for separation of the reaction products, together with their high molar absorption coefficients, permit the spectrophotometric determination of the aromatic diamines in the presence of other oxidizable substances.

The optimum conditions have been found for the determination of benzidine, *o*-tolidine, and *o*-dianisidine with chloramine T and of benzidine and *o*-tolidine with *N*-bromosuccinimide. The products of the oxidation of *o*-dianisidine with *N*-bromosuccinimide are unsuitable for spectrophotometric determination. An advantage of the proposed methods is their applicability to practical samples containing oxidizable admixtures.

SUMMARY

The oxidation of benzidine, *o*-tolidine, and *o*-dianisidine with chloramine T and *N*-bromosuccinimide was studied, the stoichiometry was established, and a mechanism was proposed. An extraction–photometric determination of these diamines was developed, based on the reaction with excess chloramine T or *N*-bromosuccinimide and the measurement of the absorbance of the *N,N'*-dihalogeno-diphenoquinonediimines formed, after their extraction into chloroform.

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MICROCHEMICAL JOURNAL 23, 353–359 (1978)

Analytical Applications of Picolinealdehyde Salicyloylhydrazone

Spectrophotometric Determination of Nickel and Zinc.

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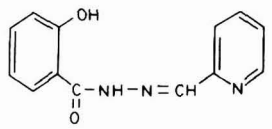
INTRODUCTION

Aroylhydrazones have been used as photometric (*2, 7, 8, 9*), fluorimetric (*5,6*) reagents, qualitative reagents for mercury (10), and metallochromic indicators (3).

The salicyloylhydrazone of picolinealdehyde has been used by Domiano and co-workers (*1*) to study the structure in solid state of the complex that it forms with nickel. In this paper, the characteristics and analytical properties of salicyloylhydrazone of picolinealdehyde (SHPA) and the spectrophotometric determination of small amounts of nickel and zinc are described.



SHPA

MATERIALS AND METHODS

Equipment

Unicam SP 8000, Unicam SP 600 s-2, and Beckman DU spectrophotometers equipped with 1.0-cm glass or quartz cells were used. A Unicam SP 1000 ir spectrophotometer was also used, as well as a digital pH meter (Philips PW 9408) with glass–calomel electrodes.

Chemicals and Solutions

Synthesis of the reagent. A 0.65-ml volume of picolinealdehyde was added to 1 g of salicyloylhydrazide dissolved in 20 ml of hot absolute ethanol. The yellow crystals were filtered off and recrystallized twice

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from ethanol. The crystals obtained (yield 60%) melted at 220°C. *Anal.* Calcd for $C_{13}H_{11}N_3O_2$: C, 64.7%, H, 4.6%, N, 17.4%. Found: C, 64.9%; H, 4.7%; N, 17.4%.

Reagents. The 0.075% and 0.1% solutions of salicyloylhydrazone of picolinealdehyde in ethanol were used. Solutions of nickel(II) were standardized with dimethylglyoxime, and solutions of zinc(II), with EDTA. Acetic acid–sodium acetate buffer solution of pH 4.8 was prepared. All solvents and reagents were of analytical grade.

Procedure

Determination of nickel. To the solution containing 6.3 to 31.3 μg of nickel, add 10 ml of 0.1% reagent solution, 4.5 ml of acetate buffer, and 3 ml of 0.5 *M* KCl solution, and dilute the mixture to the mark with distilled water in a 25-ml standard flask. Measure the absorbance of this solution at 375 and 385 nm against a reagent blank prepared in a similar way without nickel.

Determination of zinc. To the solution containing 6.3 to 23.8 μg of zinc, add 10 ml of the 0.075% reagent solution, 1 ml of 0.5 *M* KCl solution, and 5 ml of acetate buffer solution, and dilute to the mark with distilled water in a 25-ml standard flask. Measure the absorbance of this solution at 365 and 375 nm against a reagent blank prepared in a similar manner without zinc.

RESULTS AND DISCUSSION

Analytical Properties of the Reagent

The infrared spectrum of SHPA in KBr disks was obtained, and the bands were assigned to the stretching vibrations of the -NH- bond (3270 cm^{-1}), the = CH bond (3030 cm^{-1}), and the $\geq$CO bond (1655 cm^{-1}).

An aqueous reagent solution of 5×10^{-5} *M* concentration shows maximum absorption at 300 nm with a molar absorptivity of 2.8×10^4 $M^{-1}cm^{-1}$.

SHPA has solubilities in water, methanol, ethanol, chloroform, nitrobenzene, benzene, and amyl alcohol of 0.2, 1.1, 1.0, 0.04, 0.7, 0.5 and 0.9 g/liter, respectively.

The Phillips and Merrit method (*4*) was used for the determination of the ionization constants. The average p*K* values found were 3.5 ± 0.1 and 9.2 ± 0.1 (Fig. 1). The first p*K* may be that of the protonated pyridine nitrogen atom, and the second, that of the hydroxyl group.

Oxidizing substances in moderate concentration do not alter the absorption spectra of SHPA for at least 2 hr, but reducing substances do.

The reaction of the reagent with 35 cations at different pH values was investigated; it reacts with nickel(II), zinc(II), copper(II), cobalt(II), iron(II and III), palladium(II), bismuth(III), titanium(IV), and va-

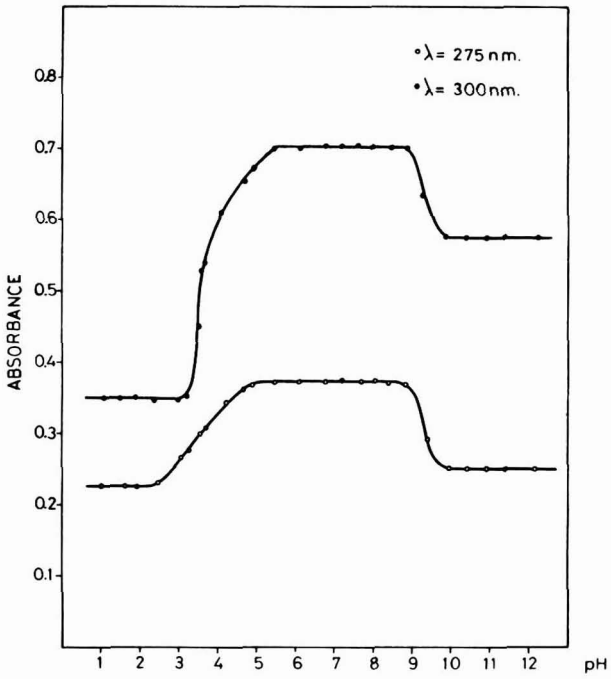


FIG. 1. Photometric determination of the ionization constants of SHPA.

nadium(V). From this study it may be concluded that the complexes of nickel and zinc are those that present the greatest absorptivity.

Formation and Study of Nickel and Zinc Complexes

When dilute nickel(II) or zinc(II) solutions and a 0.1% solution of SHPA are mixed a yellow complex is obtained. The absorption spectra of both complexes remain stable for at least 24 hr and are shown in Fig. 2.

Influence of pH. The effect of pH on the color development was studied by preparing series of solutions with 0.75 ppm of nickel or 1.0 ppm of zinc varying from pH 1 to 11. The optimum pH range for formation of the nickel complex is 5.0 to 6.3, and of that for the zinc complex, 5.5 to 6.2 (Fig. 3).

Stoichiometry of the complexes. The results obtained by Job's method in acetate buffer for solutions of nickel (2.66×10^{-4} *M*) or zinc (1.33×10^{-4} *M*) and SHPA are shown in Fig. 4. The metal:ligand ratio for both complexes is 1:2. The same results are obtained by the molar ratio method.

Charge of the complexes. This study was carried out with the ion-exchange resins Dowex 50-X8 (cation exchanger) and Dowex 1-X8 (anion exchanger), and neither complex was retained in either of the resins.

Nature of the complexes. From the results obtained it may be supposed that the nickel and zinc complexes have an analogous structure in aque-

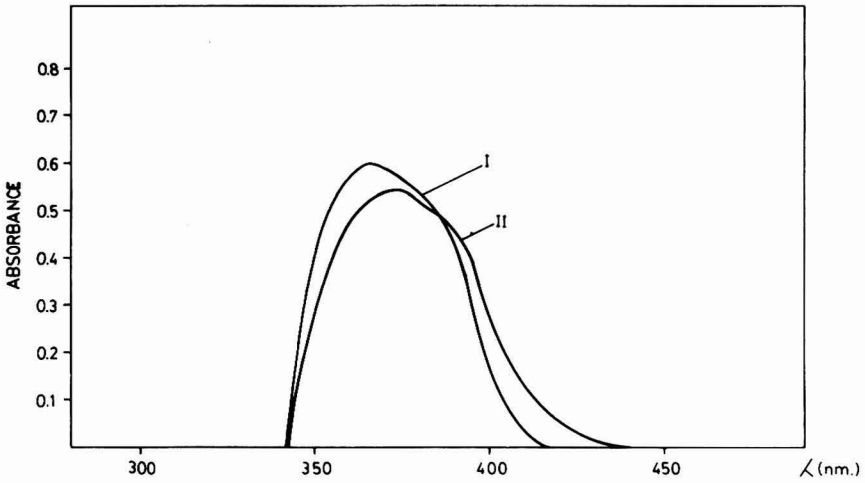
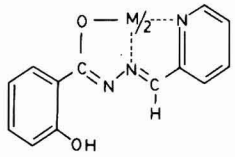


FIG. 2. Absorption spectra of solutions of nickel (0.75 ppm) and zinc (0.75 ppm) complexes. (I) Zn–SHPA; (II) Ni–SHPA.

ous solution. Following the conclusions of Domiano and co-workers (*1*) the reagent acts as a terdentate ligand which forms an octahedral complex of the M $(SHPA)_2$ type.



Spectrophotometric Determination of Nickel

The optimum conditions for the formation of the nickel complex have been indicated previously. The calibration graph proved to be linear over the range 0.25–1.0 ppm of nickel. The molar absorptivity for the complex, calculated statistically from Beer's law, is $3.9\times10^4\ M^{-1}\ cm^{-1}$. The optimal concentration range evaluated by Ringbon's method is 0.55–0.85 ppm of nickel. The relative error ($P = 0.05$) of the method is ±0.3% at 375 nm.

Numerous cations and anions were examined by applying the method to 0.75-ppm solutions of nickel. The following ions did not interfere at 100 ppm: tungsten(VI), calcium, barium, strontium, alkaline metal ions, thyocyanate, phosphate, fluoride, tartrate, and citrate. Arsenic(III), oxalate, and thiosulfate do not interfere at the 50-ppm level. Molybdenum(VI), tin(II), lanthanum, aluminium, and borate do not interfere at the 10-ppm level. Antimony(III) and manganese(II) at 10 ppm interfere.

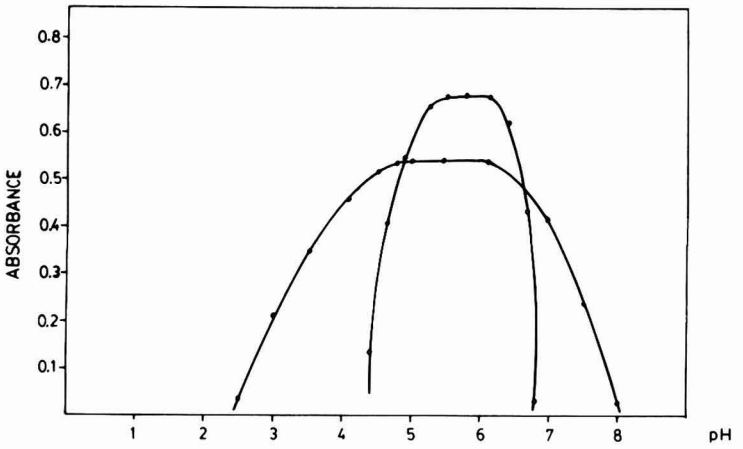


FIG. 3. Absorbance versus pH graphs of nickel (1.0 ppm) and zinc (0.75 ppm) complexes: (●) nickel complex; (o) zinc complex.

Uranium(VI), cerium, berylium, and silver do not interfere at the 2-ppm level. The most serious interferences are from vanadium(V), titanium, iron(II and III), bismuth, copper, palladium, cadmium, zinc, cobalt, lead, mercury(II), and EDTA.

Spectrophotometric Determination of Zinc

The optimum conditions for the formation of the zinc complex have

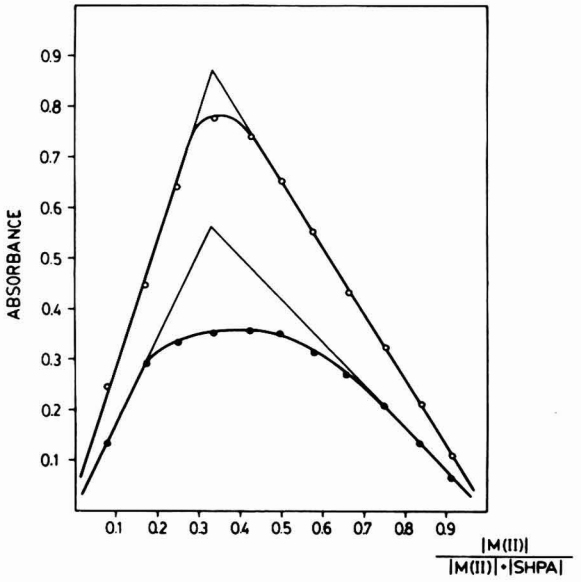


FIG. 4. Stoichiometry of nickel and zinc complexes by the continuous variations method: (o) nickel complex at 375 nm; (●) zinc complex at 365 nm.

TABLE 1
DETERMINATION OF NICKEL AND ZINC IN STANDARD SAMPLES

Sample	Metal present (%)	Metal found (%)
Zinc concentrate[a] S 31.2, Fe 10.0 Pb 0.91, Cu 0.10 As 0.13, Mn 1.12 Cd 0.22	51.4 (zinc)	51.4 ± 0.2 (zinc)
Steel[b] C 0.093, Si 0.67 S 0.023, P 0.018 Mn 0.94, Cr 18.4 Ti 0.46, Pb 0.0015 Ta 0.0017, Co 0.034	9.47 (nickel)	9.49 ± 0.01 (nickel)

[a] Bureau of Analysed Samples, Ltd. (41dG).
[b] British Chemical Standards (335).

been indicated above. Beer's law is obeyed between 0.25 and 0.95 ppm of zinc and the molar absorptivities at 365 and 375 nm are 4.8×10^4 and $4.4 \times 10^4\ M^{-1}\ cm^{-1}$, respectively. Ringbom's graph shows that 0.45 to 0.75 ppm of zinc is the minimum range of error. The relative error ($P = 0.05$) of the method is ±0.1% at 365 nm.

The interferences on 0.71 ppm of zinc have been investigated: 100 ppm of tungsten(VI), molybdenum(VI), calcium, barium, strontium, magnesium, alkaline metal ions, tartrate, citrate, thiosulfate, and phosphate do not interfere. Tin(II), fluoride, and thyocyanate do not interfere at the 50-ppm level, and antimony(III), lanthanum, aluminium, arsenic(III), manganese(II), oxalate, and borate do not interfere at the 25 ppm level. At the level of 1 or 2 ppm, uranium(VI), vanadium(V), titanium, iron, bismuth, copper(II), palladium, cadmium, nickel, cobalt, lead, mercury(II), silver, and EDTA interfere.

Standard Samples

Table 1 summarizes the results obtained for eight determinations of nickel and zinc in two standard samples. Iron has been separated by extraction with ether in the steel analysis.

SUMMARY

With a view to the use of picolinealdehyde salicyloylhydrazone as analytical reagent, a study of the physical properties and chemical reactions of this substance has been carried out. It reacts with nickel (λ_{max}= 375 nm, $\epsilon = 3.9\times10^4\ M^{-1}cm^{-1}$) or zinc ($\lambda_{max}$= 365 nm, $\epsilon = 4.8\times10^4\ M^{-1}cm^{-1}$) to produce a yellow 1:2 complex in both cases. Spectrophotometric determinations of trace amounts of nickel and zinc have been established.

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Spectrophotometric Determination of Arsenic with Diethyldithiocarbamic Acid Silver Salt and Borohydride as Reducing Agent

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Numerous methods for the spectrophotometric determination of arsenic use a large quantity of reagents. This is a great disadvantage because some of the reagents are difficult to preserve. These methods involve the reduction of arsenic to arsine by expelled hydrogen.

Vasak and Sedivec (*8*) obtained arsine by bubbling a pyridine solution of diethyldithiocarbamic acid silver salt into the test solution to form a red complex. This method (as opposed to that of Gutzeit) has many advantages (*4*), but it has the disadvantage of the unpleasant smell of pyridine.

The method of Bode *et al.* (*1*), in which pyridine is replaced by a brucine-chloroform solution, was used in this work.

A tin(II) chloride solution was used for reducing arsenic, but it is very unstable with time (*7*), and it must be frequently prepared. Granulated zinc in acid solution was used as well.

Recently, borohydride was used as a reducer in the determination of arsenic and other elements by atomic absorption spectrophotometry (*6, 2*), so borohydride was used as the reducing agent in the arsenic determination procedure we propose, taking the place of tin(II) chloride and granulated zinc.

EXPERIMENTAL

Reagents. A standard solution of arsenic(III) is prepared from 1,2303 g of arsenic trioxide dissolved in 40 ml of distilled water, containing 12 g of sodium hydroxide, and 10 ml of phosphoric acid are added. Diluting with distilled water up to 1000 ml provides a solution that contains 1 g of arsenic(III) per liter.

The following reagents are also used:

An aqueous solution of copper(II) sulfate containing 2 g of crystallized copper sulfate, $CuSO_4 \cdot 5H_2O$, per 100 ml;

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An aqueous solution of potasium iodide, KI, containing 2 g/100 ml;

An aqueous solution of 10% lead acetate starting from 12 g of $Pb(CH_3COO)\cdot 3H_2O$/100 ml;

A silver diethyldithiocarbamate solution, AgDDC, containing 2.5 g of AgDDC reagent and 0.215 g of brucine/100 ml of chloroform;

Granulated metallic zinc free from arsenic;

An aqueous solution of 15% sodium borohydride, containing 5 g of borohydride/100 ml of aqueous solution of sodium borohydride;

Hydrochloric acid, diluted (1:1);

Concentrated nitric acid ($d=1,40$), 65%;

Concentrated sulphuric acid ($d=1,84$), 95–97%.

Apparatus. The apparatus used in this study included an apparatus for arsenic determination with diethyldithyocarbamate silver salt using metal-

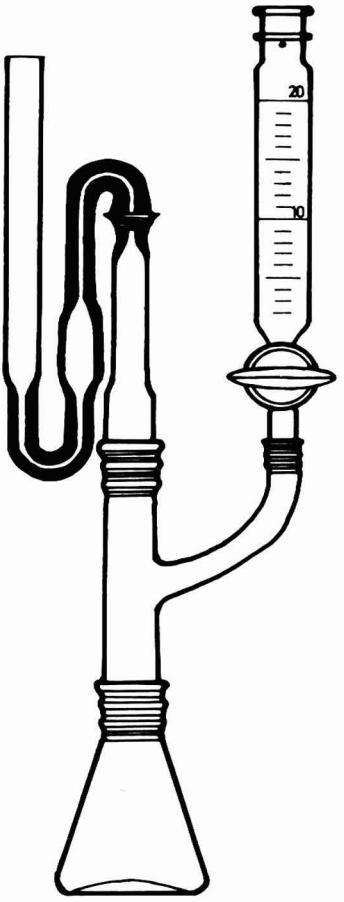

FIG. 1. Modified apparatus for arsenic determination with diethyldithiocarbamate silver salt using borohydride as reducer.

lic zinc as reducer and a modified apparatus for use with borohydride as the reducer (Fig. 1). The spectrophotometer used was a Varian Techtron Model 635 with double beam equipped with cells of 1-cm thickness with a Bausch-Lomb Omniscribe Model E 5131 SF graphic recorder.

Procedure. The sample is placed in the Erlenmeyer flask of the apparatus, and 0.5 g of sodium borohydride is added and diluted to a volume about 50 ml. With the apparatus tightly sealed, 5 ml of dilute chlorhydric acid contained in the separation funnel is slowly added dropwise. Testing shows that the free arsenamine is totally gathered by the silver diethyldithyocarbamate solution in 30 min. This reduction time is half that necessary for reduction with zinc.

Absorbance measurements are obtained in the spectrophotometer at 508 nm, using a 1-cm-thick cell and a silver diethyldithiocarbamate in brucine–chloroform solution as reference.

RESULTS

Absorption Spectrum and Beer's Law

With the above-mentioned method the absorption spectrum of the arse-

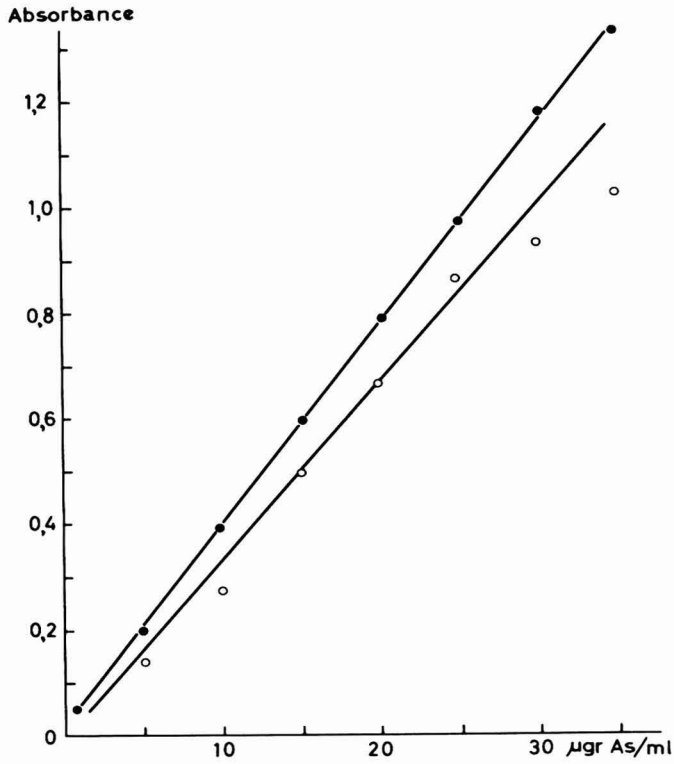

FIG. 2. Beer's law. Reduction method: ○ —— ○, with Zn; ● —— ●, with $NaBH_4$.

nic–diethyldithiocarbamate complex was determined and showed a maximum absorption at 508 nm and a molar extintion coefficient of 13,200 liters/mol cm. The proposed method is more sensitive than that of Vasak and Sedivec.

Beer's law was tested over the concentration interval from 1 to 35 μg of arsenic/ml, with which the work interval (in relation to the Vasak and Sedivec method) was extended (Fig. 2).

Following the procedures of Ringbom (*5*) and Cannon and Butterwort (*3*), we showed that the optimum interval of application is between 3 and 35 μg of arsenic/ml (Fig. 3).

Reproducibility and Precision

Two sets of samples, one with concentrations of 10 μg of arsenic/ml and one with concentrations of 20 μg of arsenic/ml, were prepared. Statistical study of these concentrations gave standard deviations of 0.029 and 0.040 μg/ml and mean relative errors of 5 and 3.5% respectively.

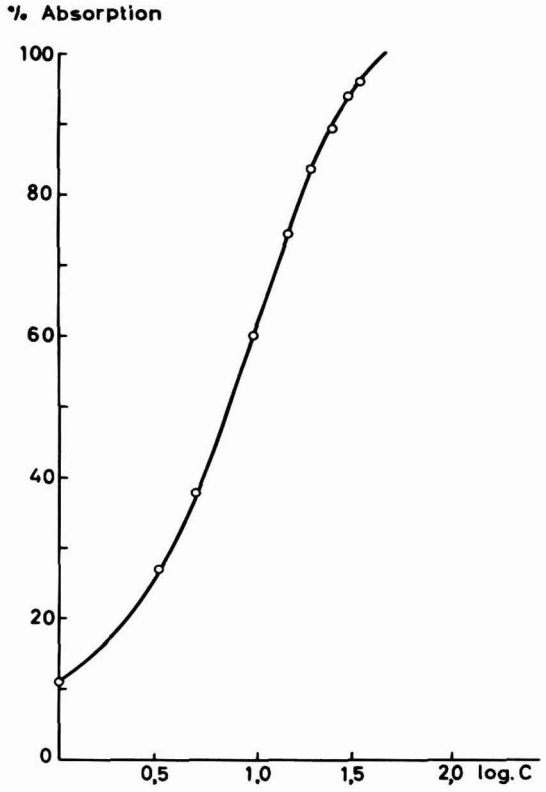

FIG. 3. Ringbom's line. Optimum interval of application of Beer's law.

TABLE 1
RESULTS OF ZINC AND BOROHYDRIDE REDUCTION METHODS

Soil number	Arsenic found (μg/g)		Difference (A-B)
	Method A[a]	Method B[b]	
1	29.7	29.0	1.1
2	25.2	26.0	0.8
3	21.1	23.0	1.9
4	30.7	31.4	0.7
5	42.4	46.6	4.2
6	39.9	43.5	3.6
7	43.9	46.4	2.5
8	43.9	45.0	1.1

[a] Zinc reduction method.
[b] Borohydride reduction method.

Economic Study of the Method

An economic comparative study between the proposed method and the Vasak and Sedivec method was made. Considering that the number of reagents in our method is less than that in the Vasak and Sedivec method and referring to the price lists from the Merck and Fluka firms from which the reagents were obtained, we determined that the saving, using the proposed method, amounts to 34%.

Application to Arsenic Determination in Soils

The results obtained with the zinc and borohydride reduction methods are presented in Table 1.

From these results and a former study on the method's reproducibility, it can be seen that the proposed method gives very acceptable results for the spectrophotometric determination of arsenic, within the usual limits of variability for these determinations.

SUMMARY

A modification of the Vasak and Sedivec method for the spectrophotometric determination of arsenic using borohydride as reducer was proposed. The absorption spectrum, fulfilment of Beer's Law, interval of application, and reproducibility of the method were all examined. Statistical and economic studies of the method were carried out. It is proposed that this method be applied in the determination of arsenic in soils.

ACKNOWLEDGMENT

The authors are grateful to Dr. M. Lopez Lamas for his kind contribution to modified apparatus design.

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Analytical Study of the Phenyl-2-pyridyl Ketone Azine–Palladium System. Photometric Determination of Palladium.

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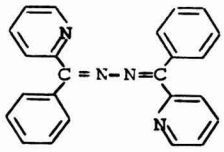
INTRODUCTION

Phenyl-2-pyridyl ketone azine (PPKA) has been described and used



PPKA

previously as a photometric reagent (*5*) and metallochromic indicator (*6*) for estimation of traces of iron. In this paper the analytical behaviour of the Pd(II)–PPKA system is examined and a sensitive and selective method for determination of traces of palladium is proposed.

Other azine reagents have been proposed by us as analytical photometric reagents for palladium, 6-methylpicolinealdehyde (*2*) and di-2-pyridylketone (*3*) azines, but the results of this paper are more interesting.

MATERIALS AND METHODS

Equipment

Absorption measurements were made with Unicam SP 800 and Perkin-Elmer Coleman 55 (digital) spectrophotometers, equipped with 1-cm glass or quartz cells. A digital pH meter, Philips PW 9408, with glass-calomel electrodes was also used.

Chemicals and Solutions

Phenyl-2-pyridyl ketone azine reagent solutions, 0.05 and 0.1% in ethanol, were used. Standard palladium solutions were made by dissolving the appropriate amount of $PdCl_2$ in dilute hydrochloric acid. They were standardized gravimetrically with dimethylglyoxime and were used to

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prepare standard solutions containing 114.5 and 111.6 ppm of Pd(II). Buffer solution, pH 4.75, was prepared by dissolving 56 g of sodium acetate in water, adding 25 ml of glacial acetic acid, and diluting to one liter.

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

Procedure

Determination of palladium. To a solution containing 22.3–223 μg of palladium, add 10 ml of 0.05% PPKA solution and adjust to pH 1.5–3.0 with 2 *N* sulfuric acid. Heat the sample in a water bath at 50–70°C for 30 min, transfer to a 25-ml standard flask, and dilute to volume with water. Measure the absorbance at 425 and 450 nm against a reagent blank prepared in a similar way without palladium.

Determination of palladium in catalysts. Weigh accurately a 0.75-g amount of palladium in calcium carbonate into a 100-ml beaker, and add 10 ml of water and 10 ml of aqua regia, avoiding spurting. Evaporate almost to dryness and dilute to 100 ml with water in a calibrated flask.

Treat the sample of palladium in active carbon (ca. 0.3 g) in a similar manner, but add 35 ml of 60% perchloric acid and 10 ml of concentrated nitric acid.

Aliquots are taken from these solutions, the palladium yellow complex is developed, and the analysis is completed as described above.

Determination of palladium in chalcopyrite. Weigh accurately a 47-g amount of mineral and place in a 1-liter Erlenmeyer flask; add 50 ml of water and 200 ml of concentrated hydrochloric acid. Boil for 3 hr. Add 250 ml of aqua regia and then concentrate to about 100 ml. Filter and dilute the solution to about 500 ml. Add 20 ml of tellurium(IV) solution (1 mg/ml) and 100 ml of 20% tin(II) chloride dihydrate solution (*4*, *7*). Filter off and add 30 ml of concentrated nitric acid over the precipitate and 15 ml of concentrated hydrochloric acid. Evaporate the solution to dryness and add 3 g of ammonium iodide. Heat the mixture and repeat the treatment with ammonium iodide two or three times. Then add 5 ml of aqua regia and 5 ml of 60% perchloric acid and reduce the volume to about 2 ml. Dilute to 25 ml with water in a standard flask. Analyze suitable aliquots of this solution for palladium as described above.

RESULTS AND DISCUSSION

Formation and Study of Palladium Complexes

When dilute solutions of palladium(II) and PPKA are mixed a yellow complex is obtained, which, after remaining unchanged for about 1 day, gradually changes into a red–violet complex.

Yellow complex. At pH 2.2 a stable color is attained in 2 hr at room temperature. However, when the solution is heated at 60°C for 30 min it

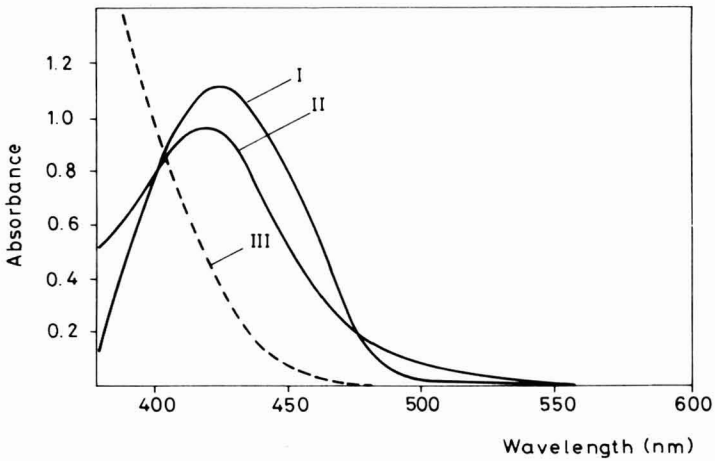

FIG. 1. Absorption spectra of the yellow Pd(II)–PPKA complex. Concentration of palladium is 9.2 ppm. (I) At pH 2.2; (II) at pH 5.30 (HAc/Ac^-); (III) reagent blank of I.

also gives a stable solution, their absorption spectrum (λ_{max} = 425 nm) coincides with the previous one (Fig. 1).

Several solutions containing 4.6 ppm of palladium prepared by the method explained previously were heated at different temperatures (50, 60, 70, and 80° C) for various times (5, 15, 30, and 60 min). The absorbance at 425 and 450 nm was measured at different times after heating.

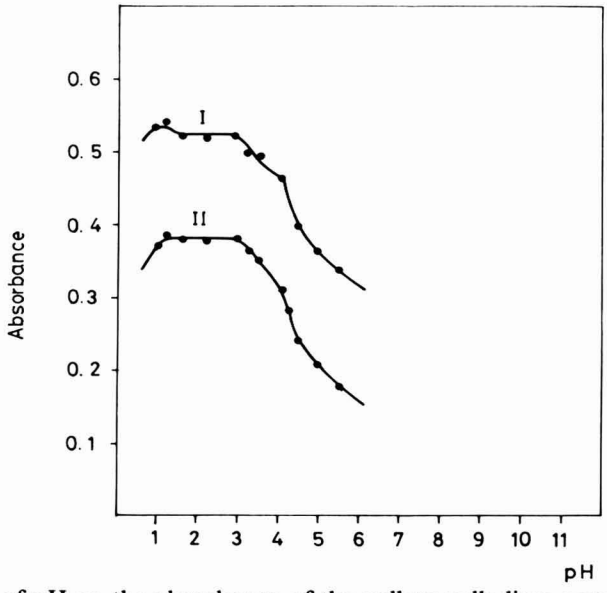

FIG. 2. Effect of pH on the absorbance of the yellow palladium complex. (I) At 425 nm; (II) at 450 nm.

The data obtained indicate that the absorbance remained constant (for 10 hr at least) after the solutions have been heated at 50–70°C for 30 min.

The spectrum of the yellow complex apparently exhibits a hypsochromic shift from 425 to 420 nm at the higher pH 3; this behavior is caused by a shift in the spectrum of the reagent as the pH is raised (Fig. 1).

The effect of pH on the color development is studied by preparing series of solutions with 4.6 ppm of palladium. No change in absorbance is observed over the range 1.5–3.0 (Fig. 2).

The results obtained by Job's method at pH 2.2 for solutions of palladium and PPKA heated at 60°C for 30 min are shown in Fig. 3. The metal:ligand ratio of the palladium yellow complex is 1:1. The same result is obtained by the slope ratio method.

Red–violet complex. The yellow complex changes slowly at any pH with the time (over 30 days) to give a red–violet solution which has an absorption maximum at 530–540 nm (Fig. 4).

The results obtained by Job's method in acetate buffer for solutions at room temperature are shown in Fig. 5. The metal:ligand ratio is 1:1 for the 2-hr-old complex, but becomes 1:3 after 1 or 2 months. The curves show that a simple complex is formed initially, but, on aging, a variety of complexes exist, ranging from 1:1 to 1:3 in composition. Similar results are obtained for solutions heated at 60°C for 4 hr.

Temperature, time of heating, oxidizing and reducing agents, chloride and acetate ions, and ultraviolet light or sunlight do not affect the slow formation of the red–violet complex.

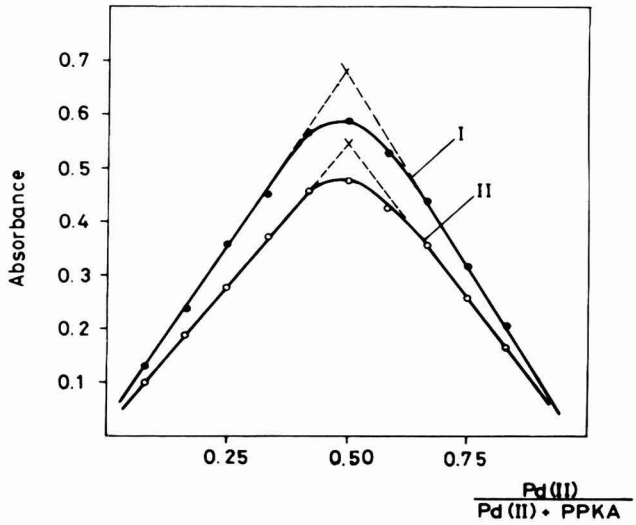

FIG. 3. Composition of yellow complex by continuous variations method. (I) At 425 nm; (II) at 450 nm.

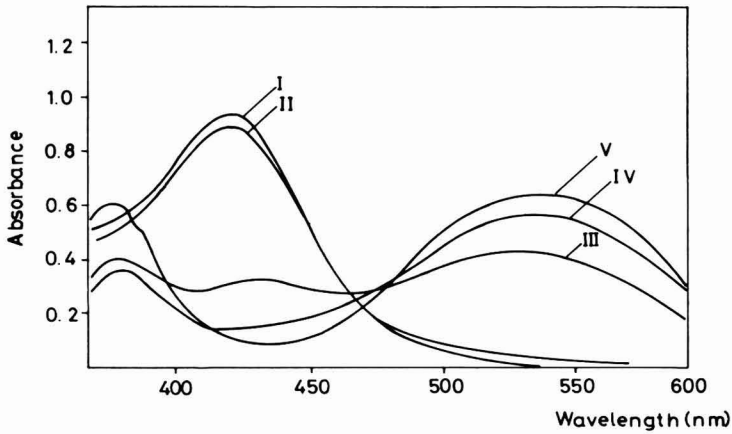

FIG. 4. Variation of the absorption spectra of the Pd(II)–PPKA solutions heated at 60°C for the first 30 min at pH 5.30 (HAc/Ac^-). (I) Initial; (II) after 1 day; (III) after 4 days; (IV) after 10 days; (V) after 30 days.

Although these azine reagents can be transformed easily in solution into hydrazones and carbonyl compounds or into carbonyl compounds and hydrazine by hydrolysis of the $\gtrless C = N$ groups (*2*) catalyzed in acidic medium, the reaction between Pd(II) and the hydrazine or the ketone does not produce the red–violet color under different experimental conditions

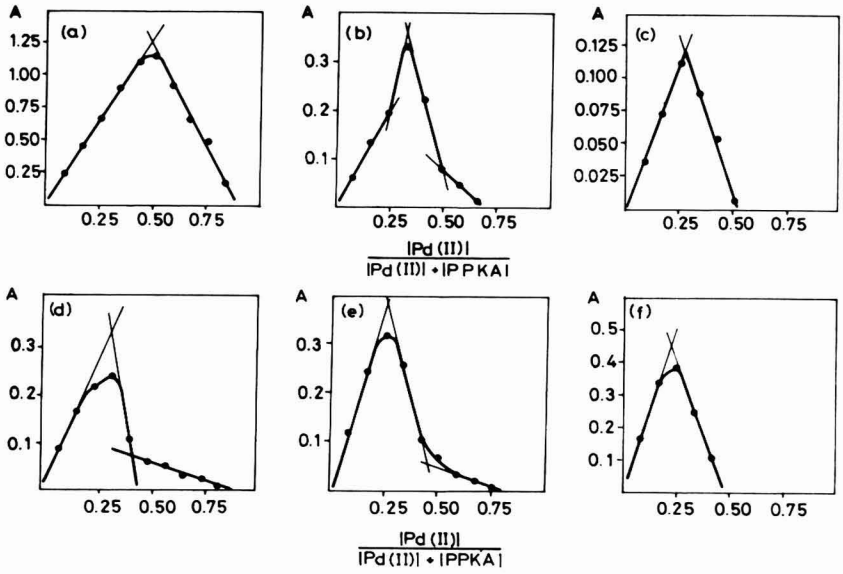

FIG. 5. Change in the composition of the Pd(II)–PPKA complexes with time at pH 5.30 (HAc/Ac^-). At 420 nm: (a) 2 hr; (b) 10 days; and (c) 30 days. At 530 nm: (d) 4 days; (e) 10 days; and (f) 30 days.

(pH, temperature, ionic strength, etc.). On the other hand, the presence of hydrazine in the Pd(II)–PPKA system [PPKA is transformed into hydrazone in the presence of excess hydrazine in solution, by means of the exchange of $\geq C = N$ groups (*8*, *9*)] prevents the evolution from the yellow to the red–violet complex. It can be supposed that the hydrazone is not involved in the formation of the red–violet complex.

From these results we can assume that the azine group is also the ligand in the red–violet complex, but the number of coordination positions is lower than in the yellow complex.

The red–violet complex is not suitable for practical use. Only the yellow complex is adequate for the determination of traces of palladium.

Analytical Applications of the Palladium Yellow Complex

Palladium complex. The calibration graph is linear over the range 0.9–8.9 ppm of palladium at 425 and 450 nm. The molar absorptivities for the complex, calculated statistically from Beer's law, are 10.4×10^3 and 10.2×10^3 $M^{-1}cm^{-1}$ at 425 and 450 nm, respectively. The optimal palladium concentration ranges, evaluated by Ringbom's method, are 1.6–8.9 (425 nm) and 2.2–5.6 ppm (450 nm). The relative error ($P=0.05$) of the method is ±0.6% at 425 nm and ±0.9% at 450 nm.

The influence of numerous cations and anions was examined by applying the method to 4.5-ppm solutions of palladium. The results obtained for noble metal ions are shown in Table 1. Rhodium(III) and platinum(IV) up to a foreign-ion/palladium ratio of 2 or 3 do not interfere. Rh(III) at about a fivefold ratio to palladium interferes slightly. Osmium(VIII) and ruthenium(IV) do not interfere when present at the same concentration as palladium.

Other foreign ions can be tolerated at the levels given in Table 2. Copper(II), nickel, bismuth, mercury(II), and thiosulfate produce serious interference when present at the same concentration as palladium.

TABLE 1
INTERFERENCE OF THE NOBLE METALS IN THE DETERMINATION OF 4.5 ppm OF Pd AT pH 2.2

Rh taken	Pd found (ppm)		Ru taken	Pd found (ppm)		Os taken	Pd found (ppm)		Pt taken	Pd found (ppm)	
(ppm)	425 nm	450 nm	(ppm)	425 nm	450 nm	(ppm)	425 nm	450 nm	(ppm)	425 nm	450 nm
50	5.2	5.1	10	—	6.3	50	6.2	7.9	50	5.5	5.1
25	4.7	4.7	5	4.6	5.5	25	5.2	6.3	25	4.8	5.0
10	4.7	4.6	2	4.5	5.0	10	4.9	5.3	10	4.5	4.5
5	4.6	4.5	1	4.5	4.5	5	4.6	4.5	5	4.5	4.5
2	4.6	4.5	0.5	4.5	4.5	2	4.5	4.5	2	4.5	4.5

TABLE 2
INFLUENCE OF FOREIGN IONS IN THE DETERMINATION OF 4.5 ppm OF Pd

Ions that interfere at level of				Ions that do not interfere up to 100 ppm	
4.5 ppm	10 ppm	15 ppm	50 ppm		
Cu^{2+}	Cr^{6+}	Mn^{2+}	Be^{2+}	As^{3+}	La^{3+}
Ni^{2+}	Au^{3+}	Zn^{2+}		Al^{3+}	Mg^{2+}
Bi^{3+}	Co^{2+}	UO_2^{2+}		Sb^{3+}	Sn^{2+}
Hg^{2+}	Ag^{+}			Zr^{4+}	F^{-}
$S_2O_3^{2-}$				Th^{4+}	$C_2O_4^{2-}$
				Fe^{3+}	EDTA
				Ca^{2+}[a]	PO_4^{3-}
				Sr^{2+}[a]	Tartrate
				Ba^{2+}[a]	Citrate

[a] Adjust pH with hydrochloric acid.

Analysis of industrial catalysts and minerals. The techniques described above have been applied to the determination of trace amounts of palladium in catalysts such as Pd in calcium carbonate and Pd on active carbon and minerals, e.g., chalcopyrite. The results obtained for five determinations of palladium in each of the catalysts are compared with those obtained by gravimetry with nioxime (1,2-cyclehexanedione dioxime) (*1*, *10*) and are shown in Table 3.

TABLE 3
DETERMINATION OF PALLADIUM IN CATALYSTS AND MINERALS

Sample	Nominal content in Pd (%)	Pd found (%)	
		Gravimetric method	PPKA method
$CaCO_3$–Pd[a] catalyst[a]	5	4.85	5.09 ± 0.03
Active carbon–Pd catalyst[b]	10	9.76	9.80 ± 0.08
Active carbon–Pd catalyst[c]	10	9.67	9.9 ± 0.14
Chalcopyrite[d]	—	—	1 ppm[e]

[a] Nominal 5% Pd (Merck). Sample of 0.7867 g/100 ml taken.
[b] Nominal 10% Pd (Merck). Sample of 0.3242 g/100 ml taken.
[c] Nominal 10% Pd (Merck). Sample of 0.3338 g/100 ml taken.
[d] Sample originating from Sudbury [contains an average 7.6 ppm of metals belonging to the platinum group (*1*)].
[e] Average value for palladium. Results obtained from three determinations.

SUMMARY

Phenyl-2-pyridyl ketone azine reacts with palladium(II) to produce a yellow 1:1 complex (λ_{max}= 425 nm, ϵ = 10.4 × 10^3 M^{-1} cm^{-1} in aqueous ethanolic solution) and a red–violet 3:1 complex (λ_{max}= 530–540 and 380–390 nm). The yellow complex in aqueous ethanolic solution has been used for the spectrophotometric determination of trace amounts of palladium. The method has been applied to the determination of palladium in some catalysts and one mineral.

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Consecutive Determination of Rutin and Quercetin by Spectrophotometric Measurements

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INTRODUCTION

Among the naturally occurring flavonoids, rutin and quercetin have become interesting in recent years because of their antioxidizing action in biological systems and their utility as a capillary stabilizer in the clinical field. A number of reports have so far dealt with the spectrophotometric and the fluorometric determinations of rutin and quercetin by using metal complex formation with them (*5*). Much attention has been paid to the analysis of such components in drug mixtures (*1, 2, 4, 7*). However, to determine the components consecutively, some separation procedures are usually needed prior to the spectrometric measurements. A convenient method for the consecutive determination without any pretreatment for separation is desirable.

Rutin and quercetin are characterized by their behavior in some reactions. For example, quercetin has a tendency to form stable metal complexes compared to rutin because of the structural difference with respect to the available chelating sites in these molecules (*6*). On the contrary, quercetin is particularly apt to be oxidized by oxygen rather than rutin (*9*), especially in the presence of some metal ions.

Based on such the differences in their properties, the present work was initiated in an attempt to provide desirable methods for the consecutive determination of rutin and quercetin. In addition to conventional spectrophotometry, the application of a two-wavelength spectrophotometry to a smaller amount of rutin and quercetin.

EXPERIMENTAL

Visible absorption spectra were recorded on a Hitachi two-wavelength double-beam spectrophotometer, Model 356.

Rutin and quercetin were obtained commercially. All other chemicals were reagent grade and used without further purification. Stock solutions of metal ions were prepared as follows: copper(II) solution (0.10 *M*), by dissolving $CuSO_4 \cdot 5H_2O$ in redistilled water; zirconium(IV) solution (0.01 *M*); by dissolving $ZrO(HO_3)_2 \cdot 2H_2O$ in methanol; and tin(II) solution (0.10

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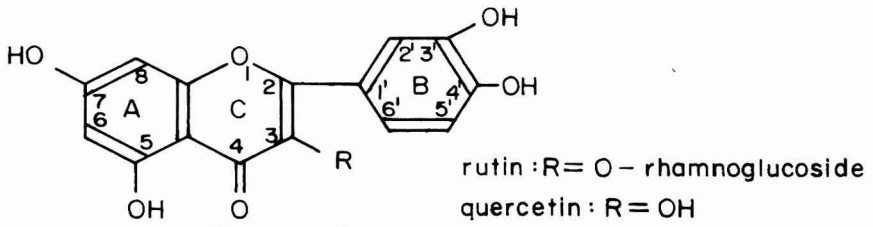
M); by dissolving $SnCl_2 \cdot 2H_2O$ in methanol. The stock solutions of copper(II) and zirconium(IV) were standardized by titration with EDTA, and the tin(II) solution was standadized by titration with I_2. Methanol solutions of 1.0×10^{-3} *M* of rutin and 1.0×10^{-3} *M* quercetin were made, each of which was used as a stock solution. These stock solutions were diluted with methanol to make solutions of the desired concentrations before each use.

Test solutions were prepared by mixing the metal solutions with the solution of rutin or quercetin at different ratios, followed by adding methanol to a constant volume, so that the amount of water did not exceed 0.4%.

RESULTS AND DISCUSSION

Determination of a Single Component in the Mixtures of Rutin and Quercetin

Determination of quercetin using the complex formation with tin(II). A chelating tendency of flavonoids usually depends on the situation of substituents in their molecules. In quercetin, 3-hydroxyl and 4-keto groups are favorably located to form the chelate ring with a metal ion, while 5-hydroxyl and 4-keto groups are available in rutin, in which the chelation at the 3-position is sterically hindered by *O*-rhamnoglucoside (Scheme 1). The chelation at the former groups is found to be more stable than that at the latter for several metal ions (*6, 11*). Further chelation often takes place at the 3′- and 4′-positions, where two hydroxyl groups are located adjacently in both rutin and quercetin, resulting in the formation of 1:2 (flavonoid:metal) type complexes.



SCHEME 1

The absorption spectra of 3.0×10^{-5} *M* rutin and quercetin obtained in methanol solutions in the presence of 6.0×10^{-5} *M* tin(II) are shown in Fig. 1. In the quercetin–tin(II) system, the absorbance of quercetin at 370 nm decreases markedly, and that at a new absorption peak which appears at 455nm increases by the addition of tin(II). The molar ratio and the continuous variation plots indicated that the new peak was attributed to 1:2 complex between quercetin and tin(II). In the rutin–tin(II) system, a new peak is faintly recognized at around 430 nm and the decrease in the absorbance of rutin at 360 nm is rather slight compared to the quercetin–

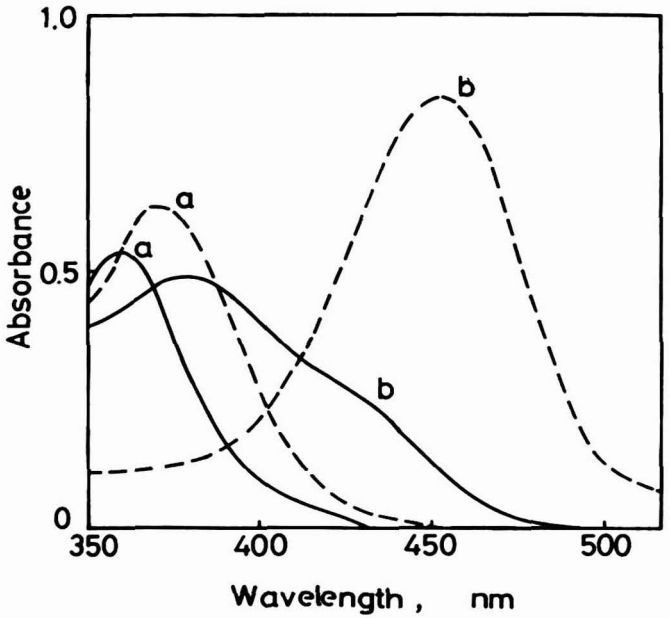


FIG. 1. Absorption spectra of 3.0×10^{-5} *M* rutin (—) and quercetin (---) obtained in a methanol solution in the absence (a) and in the presence (b) of 6.0×10^{-5} *M* Sn(II).

tin(II) system. With the increasing concentration of tin(II), the new peak became apparent at 431 nm, and it was confirmed as corresponding to the formation of 1:2 rutin–tin(II) complex.

The difference in the stability between rutin- and quercetin–tin(II) complexes is observed in Fig. 1, so that quercetin may be easily determined without an interference from rutin under the appropriate conditions. The conditions were then actually examined by measuring the absorbance at 470 nm at 15 min after adding 1.0×10^{-4} *M* tin(II) to the sample solutions containing quercetin and rutin in different concentration ratios. In the quercetin–tin(II) system, the absorbance at 470 nm remains unchanged within 30 min, and a linear relationship was found to hold between the absorbance and the concentration of quercetin, ranging from 1.0×10^{-6} to 2.0×10^{-5} *M*. The results are shown in Table 1. This method is found to be applicable to the determination of quercetin in the concentration range of 3.0×10^{-6} to 2.0×10^{-5} *M* with a relative error of within ±4%, unless the concentration of rutin exceeds half the amount of quercetin.

Determination of rutin with removal of quercetin through a copper(II)-catalyzed oxidation. The absorption spectra of 5.0×10^{-5} *M* rutin and quercetin obtained in methanol solutions in the presence of 5.0×10^{-3} *M* copper(II) are shown in Fig. 2A. In the rutin–copper(II) system, the new absorption peak corresponding to the 1:1 complex between rutin and

TABLE 1
DETERMINATION OF QUERCETIN IN THE PRESENCE OF RUTIN

Concentration, $\times 10^{-5}$ M			
Added		Found	Error
Quercetin	Rutin	Quercetin	(%)
0.100	0.050	0.109	+9.0
0.300	0.100	0.312	+4.0
0.600	0.300	0.618	+3.0
1.00	0.500	1.04	+4.0
2.00	1.00	2.03	+1.3

copper(II) appears at longer wavelength than the peak of rutin (*11*), and the spectra are virtually unchanged for 2 hr (curve a).

In the quercetin–copper(II) system, the absorption peak of quercetin disappears, and the broad band corresponding to both the 1:1 and 1:2 quercetin–copper(II) complexes appears anew over the wavelength region from 410 to 520 nm (*11*) (curve b), but the absorbance decreases significantly with time (curve c). In general, flavonoids having a hydroxyl group at the 3-position such as quercetin are known to be easily oxidized by air, especially under a catalysis of copper(II) (*6*). Contrary to this, rutin is stable to oxidation even in the presence of copper(II) (*9*), and the absorbance of the rutin–copper(II) complex is unchanged for several hours. From the expectation that the catalytic oxidation of quercetin may be enhanced with increasing pH of the solution, an experiment similar to the above was examined for the methanol solution containing 0.1 M sodium acetate. The absorption spectra of 1.0×10^{-5} M rutin and quercetin in the presence of 1.0×10^{-4} M copper(II) obtained in such a medium are shown in Fig. 2B, in which the spectra were recorded at 30 min after adding copper(II) to the solution. The absorption band of the quercetin–copper(II) complexes disappeared within 30 min (curve b′), and the absorption peak of the rutin–copper(II) complex is observed distinctly (curve a′, $\lambda_{max} = 435$ nm). Thus the use of this medium is favorable for the present purpose.

Based on these results, the determination of rutin in the presence of quercetin was examined. The spectra of the rutin– and quercetin–copper(II) systems were measured in methanol solution containing 0.1 M sodium acetate. A linear relationship was found to hold between the absorbance at 440 nm and the concentration of rutin, indicating that the oxidation products of quercetin barely affect the absorbance at 440 nm. The spectra remained unchanged within 60 min, but some fluorescent precipitates were gradually produced when the concentrations of rutin and copper(II) exceeded 2.0×10^{-5} and 4.0×10^{-4} M, respectively.

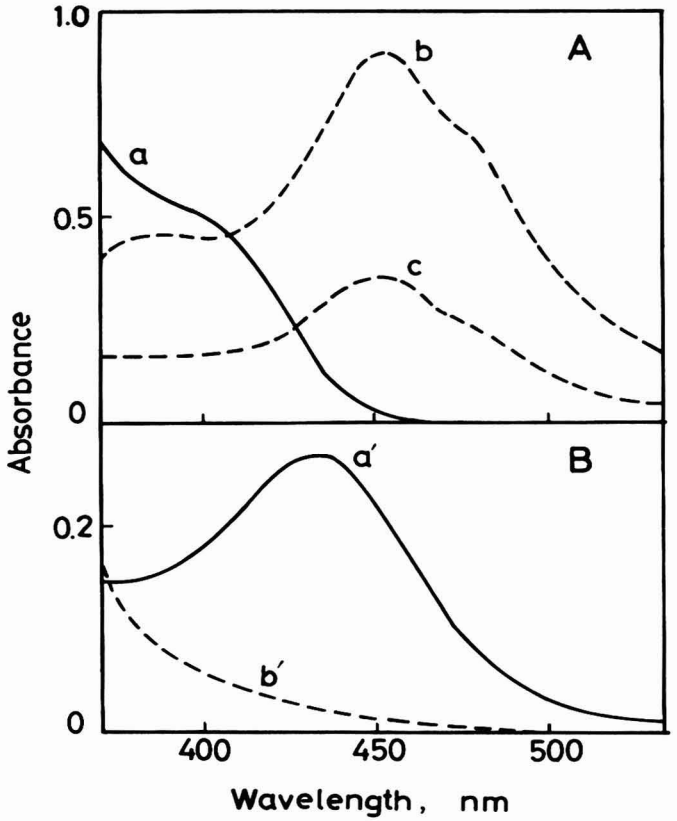


FIG. 2. Absorption spectra of rutin (—) and quercetin (---) in the presence of Cu(II) in methanol solution (A) and in a methanol solution containing 0.1 *M* CH_3COONa (B). Concentrations of rutin and quercetin: (A) 5.0×10^{-5} *M and (B)* 1.0×10^{-5} *M*. Concentrations of Cu(II): (A) 5.0×10^{-3} *M* and (B) 1.0×10^{-4} *M*. Spectra were recorded at the beginning (a and b) and after 30 min (a′ and b′) and 2 hr (c) or reaction.

Then the determination of rutin was tried for the mixed solution of rutin and quercetin by measuring the absorbance at 440 nm at 30 min after adding 4.0×10^{-4} *M* copper(II). The results given in Table 2 indicate that this method is suitable for the determination of rutin ranging 2.0×10^{-6} to 2.0×10^{-5} *M* with a relative error of within ±4%, unless the concentration of quercetin exceeds half the amount of rutin.

Consecutive Determination of Rutin and Quercetin by Two-Wavelength Spectrophotometry

Two-wavelength spectrophotometry has recently been developed for the consecutive determination of two components in a given solution with a high sensitivity (*10*). When the absorption maxima of two components (A and B) are not so far apart each other, the resulting spectrum exhibits ill-defined two peaks, as illustrated in Fig, 3 (curve C). By irradiation with

TABLE 2
DETERMINATION OF RUTIN IN THE PRESENCE OF QUERCETIN

Concentration $\times 10^{-5}$ M			
Added		Found	Error
Rutin	Quercetin	Rutin	(%)
0.100	0.050	0.110	+10
0.200	0.100	0.218	+4.0
0.600	0.300	0.612	+2.0
1.00	0.500	0.992	−0.8
2.00	1.00	1.93	−3.5

light rays of two different wavelengths to the solution at the same time, only the absorbance of one component can be obtained with eliminating that of the other. Supposing that B is the desired component to determine, let us select two wavelengths, λ_1 and λ_2, as indicated in Fig. 3, i.e., the difference in the absorbance at λ_1 and λ_2 [denoted ΔA $(\lambda_2 - \lambda_1]$ on peak A is equal to zero, while ΔA $(\lambda_2 - \lambda_1)$ on peak B gives a maximum value. Then the absorbance reading in a two-wavelength spectrophotometer just corresponds to the value of ΔA $(\lambda_2 - \lambda_1)$ on peak B, which is dependent on the concentration of B but independent of that of A.

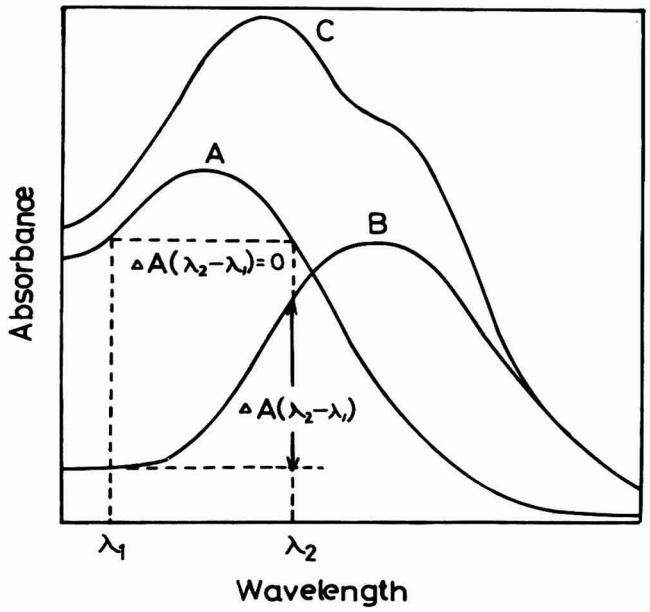


FIG. 3. Principle of consecutive determination of two components by two-wavelength spectrophotometry.

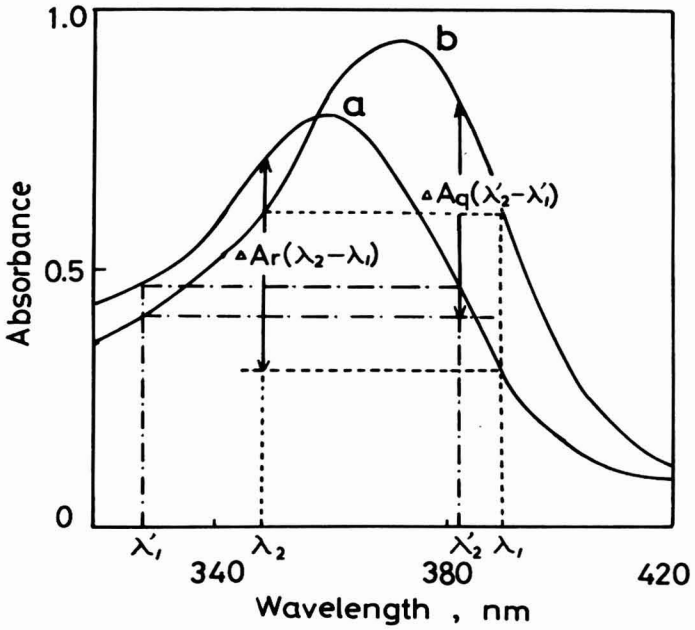


FIG. 4. Absorption spectra of 3.0×10^{-5} *M* rutin (a) and quercetin (b) obtained in a methanol solution. λ_1 (350 nm) and λ_2 (391 nm) were set for rutin, and λ_1' (328 nm) and λ_2' (385 nm) were set for quercetin.

The method was applied to the mixed samples of rutin and quercetin. Rutin and quercetin exhibit the absorption maxima in methanol solution at 360 and 370 nm (see Fig. 4), respectively, and both spectra obey Beer's law. As depicted in Fig. 4, 391 and 350 nm were chosen as λ_1 and λ_2, respectively, for the determination of rutin, and the values of ΔAr ($\lambda_2 - \lambda_1$) on peak a were read in the spectrophotometer. Similarly, 328 (λ_1') and 385 (λ_2') nm were selected for quercetin. The results are tabulated in

TABLE 3
CONSECUTIVE DETERMINATION OF RUTIN AND QUERCETIN

Concentration, $\times 10^{-5}$ *M*					
Added		Found		Error (%)	
Rutin	Quercetin	Rutin	Quercetin	Rutin	Quercetin
5.00	0	4.87	—	−0.6	—
4.00	1.00	3.85	0.970	−1.3	−3.0
3.00	2.00	2.90	2.00	−3.3	0
2.00	3.00	1.92	2.95	−4.0	−1.7
1.00	4.00	0.980	3.93	−2.0	−1.8
0	5.00	—	5.00	—	0

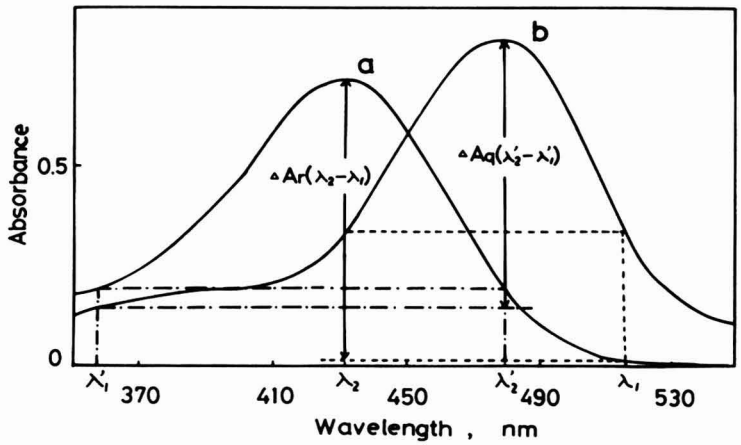


FIG. 5. Absorption spectra of 3.0×10^{-5} *M* rutin (a) and quercetin (b) obtained in a methanol solution in the presence of 1.0×10^{-3} *M* Zr(IV). λ_1 (521 nm) and λ_2 (432 nm) were set for rutin, and λ_1' (353 nm) and λ_2' (480 nm) were set for quercetin.

Table 3. The lower limit of detection for both rutin and quercetin is 1.0×10^{-5} *M*. Rutin as well as quercetin can be determined within a relative error of within ±4%, even one of them is present four times in excess of the other.

To apply this method to these components in much smaller amounts, an enlargement of the wavelength difference between their absorption maxima was attempted in the following experiment using zirconium(IV). The absorption spectra of 3.0×10^{-5} *M* rutin and quercetin in the presence of 1.0×10^{-3} *M* zirconium(IV) are given in Fig. 5. The absorption peaks appearing at 432 and 480 nm correspond to 1:2 complexes of rutin–zir-

TABLE 4
CONSECUTIVE DETERMINATION OF RUTIN AND QUERCETIN

Concentration, $\times 10^{-5}$ *M*					
Added		Found		Error (%)	
Rutin	Quercetin	Rutin	Quercetin	Rutin	Quercetin
3.00	0.500	2.90	0.480	−3.3	−4.0
2.00	1.00	2.00	0.960	0	−4.0
1.00	2.00	0.980	1.93	−2.0	−3.5
0.500	3.00	0.520	2.85	+4.0	−1.7
0.300	0.040	0.310	0.037	+1.7	−7.5
0.200	0.100	—	0.096	—	−4.0
0.100	0.200	—	0.201	—	+0.5
0.040	0.300	—	0.310	—	+3.0

conium(IV) and quercetin–zirconium(IV), respectively (*8*). Both peaks follow Beer's law in the concentration range of 1.0×10^{-6} to 3.8×10^{-5} *M* and remain unchanged for about 24 hr. The results are listed in Table 4. In the range of 5.0×10^{-6} to 3.0×10^{-5} *M*, both rutin and quercetin can be determined with a relative error within ±4%, even one of them is present six times in excess of the other. The lower limit of the detection reached is on the order of 10^{-6} *M*.

This method may be used to advantage in the consecutive determination of rutin and quercetin in the concentration range of 10^{-5} to 10^{-6} *M* without any separation procedure prior to the absorption measurements.

SUMMARY

The consecutive determination of rutin and quercetin without any pretreatment for separation was examined in methanol solutions by a conventional and a two-wavelength spectrophotometry. Based the tendency of quercetin to form more stable metal complexes compared to rutin, quercetin can be determined through the tin(II) complex formation without interference from rutin. The method was applied to the determination of quercetin in the concentration range of 3.0×10^{-6} to 2.0×10^{-5} *M*.

Quercetin is apt to be oxidized by oxygen rather than rutin, especially in the presence of copper(II), whereas rutin is not decomposed under such a condition. After removal of quercetin through copper(II)-catalyzed oxidation, rutin ranging in concentration from 2.0×10^{-6} to 2.0×10^{-5} *M* was determined by the absorbance measurement of rutin–copper(II) complex in slightly alkaline methanol media.

Both rutin and quercetin were determined directly by two-wavelength spectrophotometry, without adding any complex forming metals; the lower limit of detection was about 1.0×10^{-5} *M*. The method was extended to the determination of a smaller amounts of rutin and quercetin using the absorption peaks of their zirconium(IV) complexes, and the determination of both components in the range of 5.0×10^{-6} to 3.0×10^{-5} *M* was made with a relative error of within ±4%.

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Identification of the Order of Aromatic Alcohols and Their Derivatives by Means of Thin-Layer Chromatography

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In a number of papers dealing with thin-layer chromatography a linear correlation has been established between the R_m coefficient values and the number of methylene groups in a molecule of investigated compounds (*1–6*). This fact enabled identification of individual components of the selected homologous groups (*1, 3, 5*).

Our paper takes advantage of the a/m dependence and concerns determination of the order of aromatic alcohols as well as of their esters on a thin layer.

EXPERIMENTAL

Separation of the selected aromatic alcohols and their acetates by means of adsorption thin-layer chromatography was performed using the ready-made glass plates (E. Merck, West Germany), covered with a 0.25 mm-thick 60-F_{254} silica gel and activated for 30 min at 110°C.

The following mobile phases were applied: alcohols: (A) cyclohexane–dichloroethane–ethyl acetate (v/v/v, 4:1:1); (B) Carbon tetrachloride–ethyl ether–ethyl acetate (v/v/v, 8:2:1), esters: (C) cyclohexane–benzene–chloroform (v/v/v, 8:1:1); (D) *n*-hexane–carbon tetrachloride–ethyl ether (v/v/v, 8:1:1). Chromatograms were visualized under uv light, wave length of 254 nm, or with a 5% solution of phosphoromolybdic acid.

RESULTS

The results of separation of aromatic alcohols as well as of their acetates are given in form of the R_f and R_m coefficients in Tables 1 and 2.

DISCUSSION

The R_m values of the examined homologous groups of aromatic alcohols and their acetates show a linear correlation based on the number of car-

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TABLE 1
THE R_f AND R_m COEFFICIENT VALUES OF THE AROMATIC ALCOHOLS IN THE ADSORPTION THIN-LAYER CHROMATOGRAPHY

Separated substances	Mobile phase (A)		Mobile phase (B)	
	R_f	R_m ($\times 10^2$)	R_f	R_m ($\times 10^2$)
Primary alcohols				
Benzyl alcohol	35.4	26.1	44.1	10.5
2-Phenylethanol	30.3	36.2	38.7	20.0
3-Phenyl-1-propanol	26.4	44.5	34.1	28.6
4-Phenyl-1-butanol	22.0	55.0	29.9	37.0
Secondary alcohols				
1-Phenyl-1-ethanol	44.3	10.0	53.9	− 6.8
1-Phenyl-1-propanol	52.4	− 4.3	60.5	−18.5
1-Phenyl-1-butanol	59.2	−16.2	68.0	−32.7
1-Phenyl-1-pentanol	65.8	−28.4	73.7	−44.7
Tertiary alcohols				
2-Phenyl-2-propanol	72.6	−42.3	79.1	−57.8

bon atoms in a molecule. This dependence is given in Figs. 1 and 2 and remains unchanged for all the applied mobile phases.

Simultaneously it seems significant that the discussed dependence R_m

TABLE 2
THE R_f AND R_m COEFFICIENT VALUES OF THE ACETATES OF AROMATIC ALCOHOLS IN THE ADSORPTION THIN-LAYER CHROMATOGRAPHY

Separated substances	Mobile phase (C)		Mobile phase (D)	
	R_f	R_m ($\times 10^2$)	R_f	R_m ($\times 10^2$)
Acetates of primary alcohols				
Benzyl alcohol	36.2	24.6	49.6	0.7
2-Phenylethanol	31.6	33.5	44.2	10.1
3-Phenyl-1-propanol	28.0	41.0	38.7	20.0
4-Phenyl-1-butanol	23.4	51.5	32.8	31.2
Acetates of secondary alcohols				
1-Phenyl-1-ethanol	43.1	12.0	60.6	−18.7
1-Phenyl-1-propanol	48.8	2.0	66.5	−29.8
1-Phenyl-1-butanol	53.6	− 6.3	71.2	−39.3
1-Phenyl-1-pentanol	57.8	−13.6	75.4	−48.7
Acetates of tertiary alcohols				
2-Phenyl-2-propanol	65.9	−28.6	82.7	−67.9

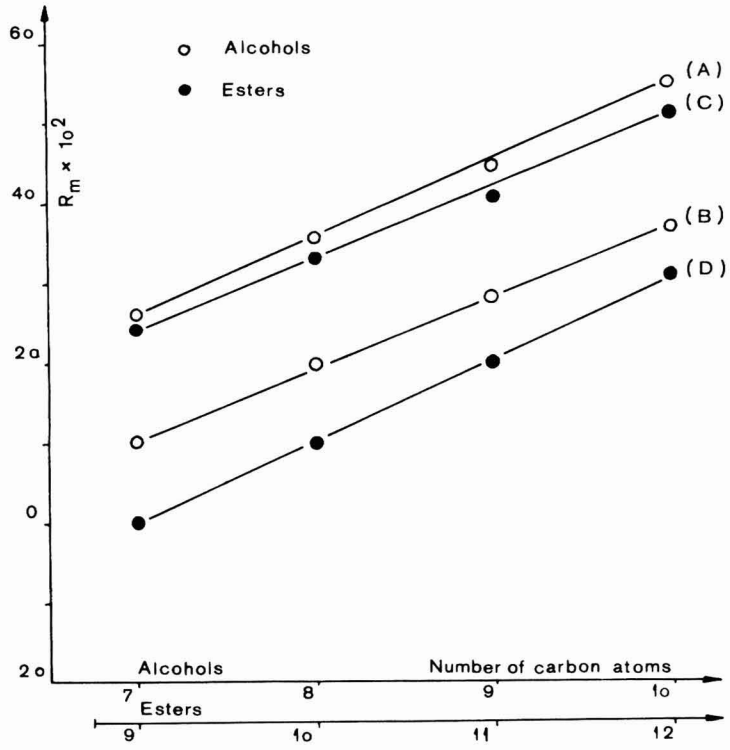

FIG. 1. The R_m coefficient values vs the number of carbon atoms for primary alcohols and their acetates.

$= f(c)$ for two mobile phases (A) and (B) in the case of alcohols and (C) and (D) in the case of esters demonstrates the parallel course.

Presentation of the results in form of the R_m coefficient dependences for both mobile phases also shows a linear correlation, no matter what the order of the examined homologous orders of alcohols and their acetates are. The above-mentioned dependence is given in Figs. 3 and 4.

With the separation of primary alcohols and their esters, changes in adsorption of the individual homologs are caused by increases of the carbon side chain length, and they induce lowering of the R_f values. For the homologous order of secondary alcohols and their esters, one observes the reverse effect. In this case separation of a single species is influenced by steric effects to a greater extent. Thus the hydroxyl and ester functional groups are less adsorbed on a layer, which is reflected in the increased R_f values. In the discussed homologous order the R_f coefficient value increases with the growing length of the carbon side chain.

The highest R_f coefficient values are characteristic of the tertiary alcohols and their esters, where the functional group is sterically hindered to the highest extent.

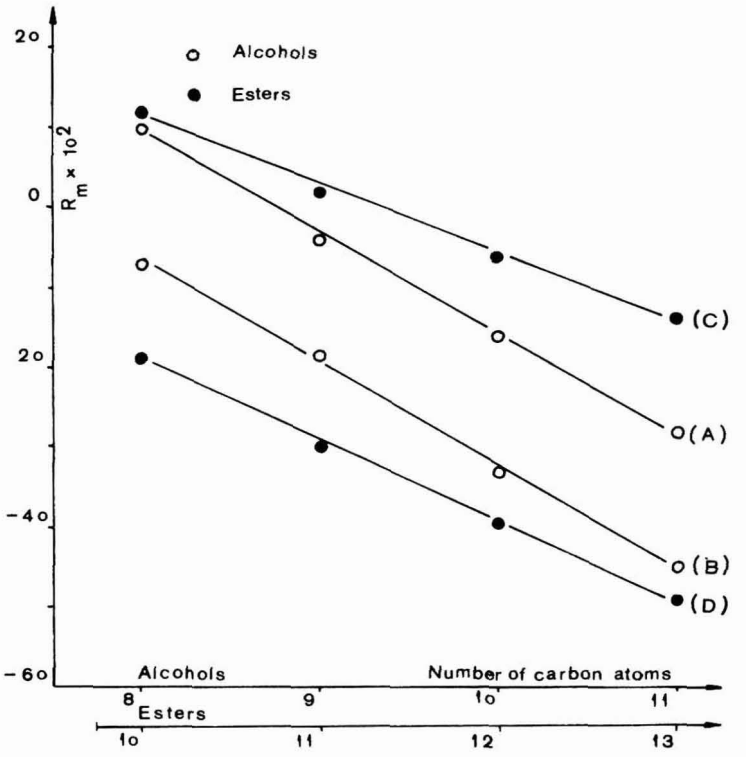

FIG. 2. The R_m coefficient values vs. the number of carbon atoms for secondary alcohols and their acetates.

The results of chromatographic separation of the investigated homologous orders shown in the form of the mutual dependences of R_m coefficents demonstrate a linear course with all groups of alcohols and the corresponding esters. The a/m fact presented in Figs. 3 and 4 is evidence of the regularity with which substances belonging to the same group of chemical compounds, but differing with respect to functional group order, can be characterized by the constant value of the R_m change in the same two mobile phases. A constant parameter will also be given by the ratio of the R_m value differences for two homologs, determined in two mobile phases. The discussed dependence is given by the following equation:

$$\frac{R_{m\alpha}^{(B)} - R_{m\beta}^{(B)}}{R_{m\alpha}^{(A)} - R_{m\beta}^{(A)}} = tg\alpha = \text{const.}$$

In this equation use was made of determinations presented in Fig. 3, in which: $R_{m\alpha}^{(A)}$, $R_{m\beta}^{(A)}$, $R_{m\alpha}^{(B)}$, and $R_{m\beta}^{(B)}$ are the R_m values for the α and β substances determined in the (A) and (B) mobile phases.

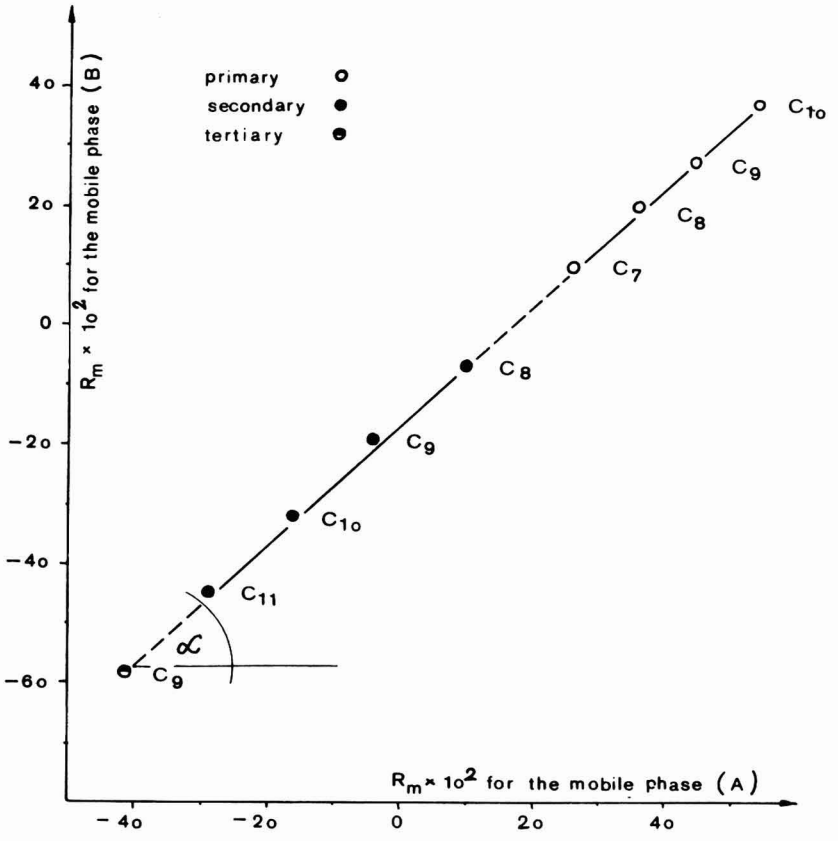

FIG. 3. The R_m coefficient values for aromatic alcohols in two mobile phases.

On the basis of the conducted investigations the possibility of determining functional group order of aromatic alcohols and their esters was established, employing the chromatographic results. Using data arranged in form of the $R_m = f(c)$ function, obtained for two different mobile phases, as well as data obtained on the basis of the linear correlation between the R_m coefficients in the (A) and (C) mobile phases vs the similar coefficients in the (B) and (D) mobile phases, a new method has been established of distinguishing the functional group order of the discussed homologs.

SUMMARY

Separation conditions for homologous orders of primary and secondary aromatic alcohols as well as their acetates were established applying adsorption thin-layer chromatography. A linear correlation was found for the R_m coefficient values depending upon the number of carbon atoms in the analyzed homolog. In addition it was established that the R_m coefficient values of aromatic alcohols in two mobile phases demonstrate a linear dependence, regardless of the order of the examined homologous groups.

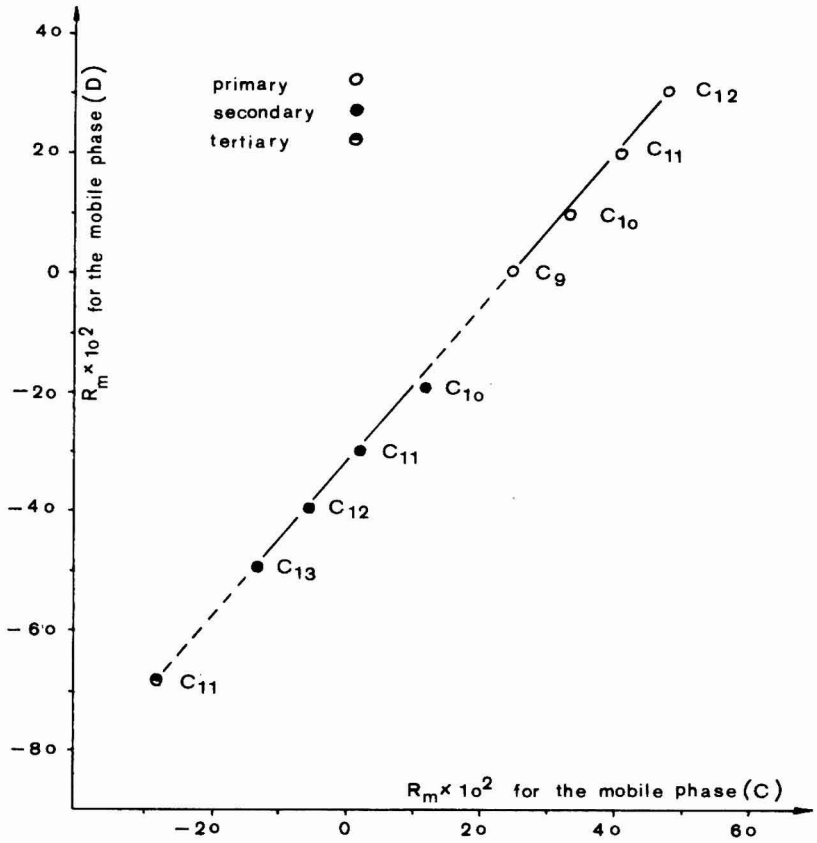

FIG. 4. The R_m coefficient values for acetates of aromatic alcohols in two mobile phases.

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Preliminary Evaluation of Biacetyl Bis(2-Pyridyl)Hydrazone as an Analytical Reagent

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INTRODUCTION

As early as 1915, 2-hydrazinopyridine was prepared by allowing hydrazine hydrate and 2-chloropyridine to react together (*5*). It forms a complex salt with iron which is structurally similar to those formed by dipyridyl and phenanthroline, but in contrast to the complex salts with dipyridyl and phenanthroline, this is very unstable and is immediately oxidized by air to the corresponding ferric salt (*20*).

2-Quinolylhydrazine was prepared first by Perkinson and Robinson in 1913 (*15*). It shows no analogy to the cuproine compounds since in a neutral or faintly alkaline medium this compound forms colored complexes with Cu(II) and Co(II); however, with Cu(I) no coloration appears (*12*).

Considerable attention has been directed in recent years to the use of pyridyl and quinolylhydrazones as suitable reagents in spectrophotometry and fluorimetry. Since Lions' *et al.* annotation in 1962 that the pyridylhydrazone of picolinaldehyde (PAPHY) acts as a tridentate chelate compound forming chelates with many heavy metals (*6*), and the consequent apparition of a survey dealing with its analytical possibilities by Cameron et al. (*4*), this reagent and its analogs have found extensive use in analytical chemistry. It is impossible to give here a complete survey of all the publications which have dealt with these compounds; this has been done recently by Katyal and Dutt (*13*).

Nevertheless, there are a few papers which were not included in the above-mentioned paper, and other papers have since been published. Heterocyclic hydrazones of *o*-hydroxyaldehydes are fluorimetric for a number of ions, specially Zn(II), Al(III), Ga(III), In(III), and Sc(III) (*18*). A spectrophotometric determination of cobalt can be performed with the use of 2-benzoylpyridine-2-pyridylhydrazone (*7*), benzil mono-(2-pyridyl)hydrazone (*16*), and 2,2′-dipyridyl-2-pyridylhydrazone (*19*). These reagents have been claimed by the various authors to offer advantages over the nitroso reagents in such determinations. 2,2′-Dipyridyl-2-pyridylhydrazone also has been used for determining iron in serum (*1*).

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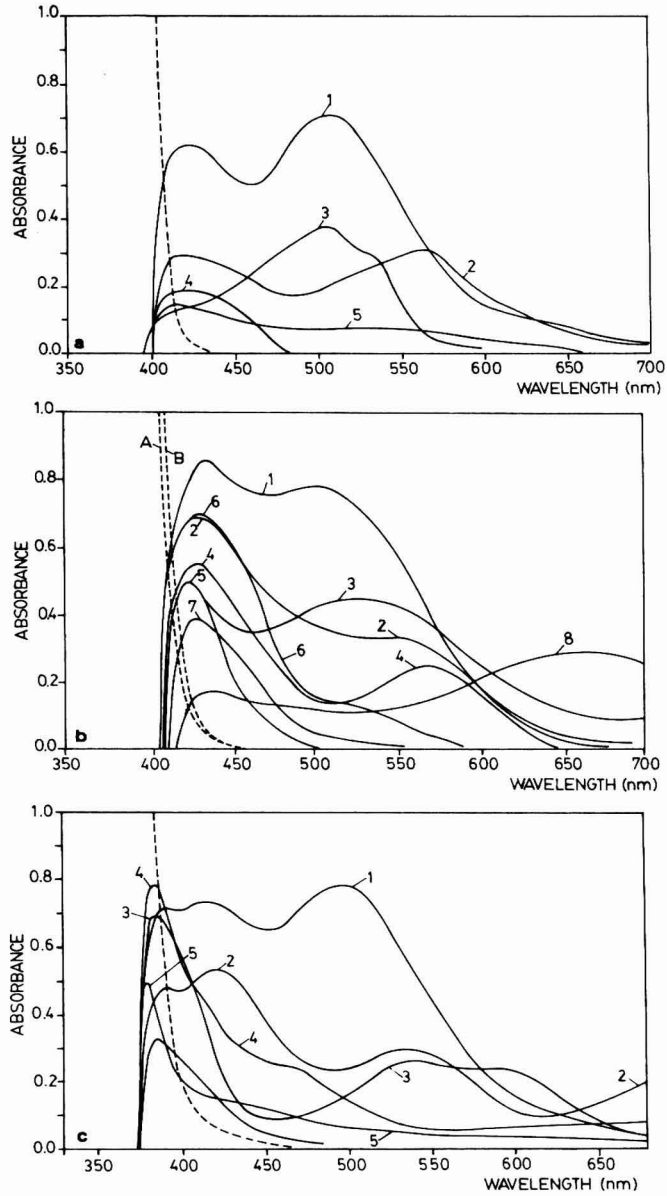


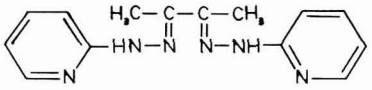
FIG. 1. Absorption spectra of the reaction products of BBPH with metal ions at different pH values. (a) At pH 1.70 (hydrochloric acid): (1) Co(II), 4 ppm; (2) Pd(II), 12 ppm; (3) Rh(III), 12 ppm[A]; (4) Cu(II), 6 ppm[B]; (5) Pt(IV), 12 ppm. (b) At pH 4.70 (acetate buffer): (1) Co(II), 4 ppm; (2) Fe(II), 4 ppm; (3) Rh(III), 12 ppm[C]; (4) Fe(II), 4 ppm; (5) Ni(II), 4 ppm; (6) Cu(II), 8 ppm; (7) Cu(II) 8 ppm, plus ascorbic acid[D]; (8) Pd(II), 12 ppm; A, reagent blank; B, reagent blank of the Rh(III). (c) At pH 9.68 (ammonia buffer)[E]: (1) Co(II), 4 ppm; (2) Pd(II), 12 ppm; (3) Cu(II), 4 ppm[F]; (4) Ni(II), 4 ppm; (5) Fe(III), 4 ppm; (6) Cu(II) 4 ppm, plus ascorbic acid. Two milliliters of BBPH at 0.2% in ethanol in the three cases. [A] The development of the colors given by BBPH with the noble metal ions requires a previous heating of

A sulfonated form of 2-benzoylpyridine-2-pyridylhydrazone has been synthesized to circunvent the solubility problems of the later in determinations of the formation constants and acid dissociation constants of the metal complexes (*8*). The preconcentration of trace metal ions by combined complexation–anion exchange with the use of this reagent also has been reported (*9*).

The reaction between cobalt(II), PAPHY, and the dyestuff eosin at pH 5.6 has been used to form a ternary complex which is then extracted into a chloroform–acetone mixture, allowing a spectrophotometric and fluorimetric determination of cobalt (*11*),

The formation of osazones is a general property of all α-oxiketones and is also presented by compounds such as benzoine, acetoine, and related compounds. In spite of the fact that the osazones derived from such hydrazones and 1,2-diketones can act as tetradentate ligands forming much more stable complexes, they have not been studied much as reagents. Schilt *et al.* have reported on new mono and bis(hydrazones) of benzil and pyridyl (*17*), although no practical application of these compounds has been made yet. 2,2′-Pyridyl bis(2-pyridyl)hydrazone seems to behave as a molecular duplicate of PAPHY.

However, this paper deals with the synthesis and fundamental solution chemistry of the complexes formed by biacetyl bis(2-pyridyl)hydrazone (BBPH) with the metal ions. This compound is closely related to some of those previously mentioned.



BBPH

MATERIALS AND METHODS

Synthesis of the Reagent

The reagent was synthesized according Lions's *et al.* procedure (*14*). Ice-cold diacetyl (0.8 ml, Merck) and 2-pyridylhydrazine (2.1 g, Aldrich) were dissolved in 100 ml of ethanol–water, 1:1, and allowed to stand overnight in the refrigerator. The yellow compound obtained had a melting point of 212°C. Anal. Calc for $C_{12}H_{16}N_6$: C, 62.68; H, 5.97; N 31.34. Found: C, 62.1; H, 6.1; N, 30.6. This product was used after one recrystallization in ethanol.

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the samples in a water vath; [B] The same spectra is obtained with Cu(II) plus ascorbic acid; [C] This sample containes 5 ml of dimethylformamide to prevent its precipitation; [D] The pH value of this sample is lower than 4.7, due to the solid ascorbic acid added; [E] The samples prepared at this pH value contain 10 ml of dimethylformamide; [F] The absorbance decreases with time.

Apparatus

Spectral curves and other measurements at varying wavelengths were made on a Pye Unicam SP 8000 recording spectrophotometer. Analytical measurements at fixed wavelength were made on a Coleman 55 (digital) spectrophotometer. Stoppered silica cells on 1-cm optical path were used for all measurements. The pH values were measured by employing a pH meter with a combined electrode (glass and calomel), calibrated with aqueous standard buffer solutions. No correction was made for differences in ionic strength between standard and other solutions. As the pH values were measured in aqueous–ethanolic or aqueous–dimethylformamide solutions, the combined electrode did not display the same function as in water. The pH values measured in mixed media with ethanol or dimethylformamide were not corrected. All observations were made at room temperature (in the range 18 to 24°C).

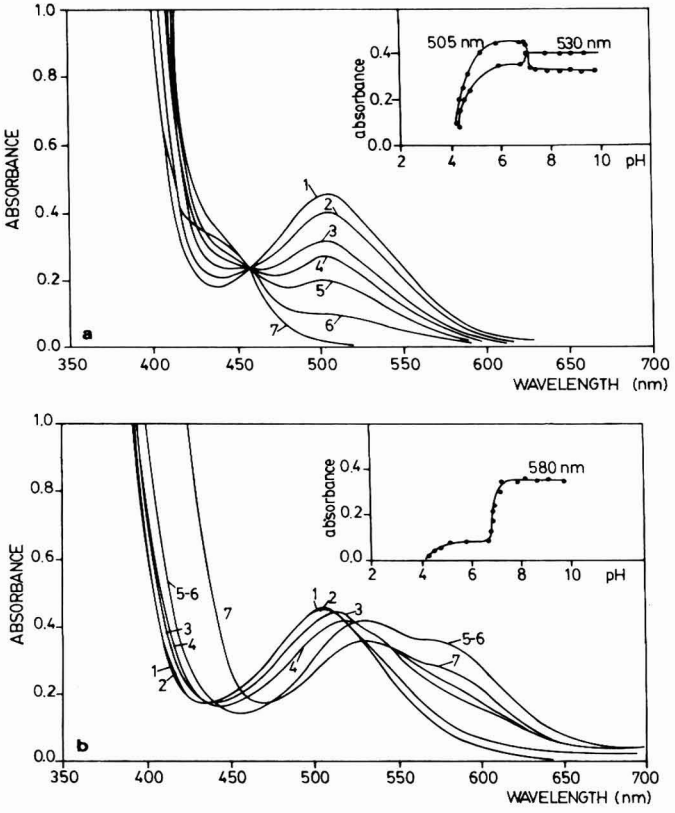


FIG. 2. Variation of the absorption spectrum for the copper(II)–BBPH system. (a) pH: (1) 5.90; (2) 5.20; (3) 4.80; (4) 4.57; (5) 4.41; (6) 4.31; (7) 2.40. (b) pH: (1) 6.80; (2) 6.97; (3) 7.03; (4) 7.14; (5) 8.82; (6) 9.27; (7) 12.8. C_{Cu} = 4 ppm; 2.5 ml of BBPH at 0.1% in dimethylformamide; reference, water.

Reagents

Analytical-reagent-grade materials were used whenever available, and distilled water was used throughout. Ethanol of synthesis was purified by column distillation, and the fraction distilled at 77–78°C was used. Biacetyl bis(2-pyridyl)hydrazone stock solutions at 0.2 and 0.1% in ethanol or dimethylformamide were employed. The concentrations of the standard solutions of metals (2–5 g/liter) were determined by weighing the oxides [Fe(III), Fe(II)] or the dimethylglyoximates (Ni, Pd), or by chelatometry (*3*) with PAN, murexide, or xylenol orange as metallochromic indicators (Cu, Co, Ni, Zn, Cd, Bi, Pb). The other elements were from standard solutions prepared in a previous investigation in this laboratory. Working solutions of appropriate concentration were pre-

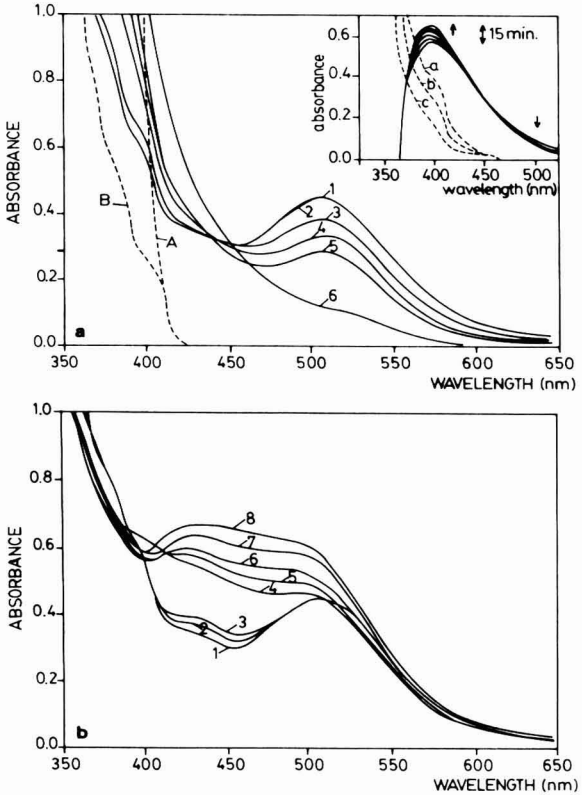


FIG. 3. Variation of the absorption spectrum for the cobalt(II)–BBPH system (a) In a strongly acidic medium: (1) HCl, 1 *M*; (2) HCl, 2 *M*; (3) HCl, 3 *M*; (4) HCl, 4 *M*; (5) HCl, 6 *M*; (6) HCl, 12 *M*; reference, water; A, reagent blank (HCl, 2 *M*, and HCl, 6 *M*); B, reagent blank (HCl, 12 *M*). On top right: absorption spectra of cobalt(II)–BBPH system in perchloric acid medium of 60%; a, blank measured immediately; b, after 15 min; c, after 1 and 15 days. (b) pH: (1) HCl, 1 *M*; (2) 1.22; (3) 1.65; (4) 4.9; (5) 5.62; (6) 5.90; (7) 6.21; (8) 6.48; reference, water C_{Co} = 4 ppm; 1 ml of BBPH at 0.1% in dimethylformamide.

pared as needed from the stock solutions. Buffer solutions were prepared by conventional methods.

Procedures

Aqueous solution. The solution containing the metal ion was pipetted into a 25-ml measuring flask. The solution of the pyridylhydrazone compound, 2.5 ml of potassium nitrate solution, 1 *M* (to maintain the ionic strength of the final solution at 0.10 ± 0.01), the solution for adjustmen of pH, and distilled water were then added in that order by pipet. After mixing, the solutions were allowed to stand for about 5 min, and the absorbance was measured against a blank or distilled water as reference.

Qualitative extraction tests. Aliquots of 5 ml of the former solutions were pipetted into glass-stoppered tubes. Five milliliters of chloroform were added, and the mixture was shaken vigorously for 30 sec, the colors of the aqueous and organic layers being noted. Alternatively a test was made by adding solid sodium perchlorate (about 0.1 g) to the aqueous solution before adding the organic solvent.

RESULTS AND DISCUSSION

For each complex, series of aqueous solutions containing the same concentration of complex and different pH values were prepared, and the visible absorption spectra of the solutions were recorded. Figures 1, 2, and 3 depict the most important complexes at pH 1.7 (hydrochloric acid), 4.70 (acetate buffer), and 9.68 (ammonia buffer). Likewise, the Tables 1, 2, and 3 depict the color of these complexes at these pH values as well as the qualitative extraction tests of the complexes formed with the use of chloroform as organic solvent.

TABLE 1
COLORS OF THE COMPLEXES IN AQUEOUS SOLUTION AT pH 1.70 AND EXTRACTED INTO CHLOROFORM

Metal ion	Color of the complex	Color of the extracted complex	
		Aqueous layer	Organic layer
Co(II)	Red	Red	—[a]
Cu(II)	Yellow	Yellow	—
Pd(II)	Greyish	—	Purple
Rh(III)[b]	Pink	Pink	—

[a] In the presence of sodium perchlorate cobalt is distributed between the aqueous (red–orange) and organic (red–purple) layers.

[b] The reactivities of Ir and Ru complexes have not been studied due to their lack of availability.

TABLE 2
COLORS OF THE COMPLEXES IN AQUEOUS SOLUTION AT pH 4.70 AND EXTRACTED INTO CHLOROFORM

Metal ion	Color of the complex	Color of the complex extracted	
		Aqueous layer	Organic layer[a]
Fe(II)	Orange	Orange	—
Co(II)	Red	Orange	Red
Ni(II)	Yellow	Yellow	—
Cu(II)	Yellow	Yellow	—
Pd(II)	Green	—	Green
Pt(IV)	Yellow–Olive	—	Yellow
Rh(III)	Pink	Orange	Pink[b]

[a] By adding sodium perchlorate cobalt is extracted totally, iron is distributed between the organic (orange) and the aqueous (pink) layers, and nickel is also distributed between both phases.

[b] Without acetate buffer the organic layer is colorless.

BBPH is an interesting chromogenic reagent, giving reactions with numerous cations. Its chelate-forming ability is retained on standing; this implies that the $>C=N-$ bonds do not break unlike other Schiff bases derived from biacetyl, such as bis(thiosemicarbazones) (*10*) and bis(phenylthiosemicarbazones) (*2*). It has been found that the presence of an ammonia buffer favors precipitation of the reagent. To prevent precipitation the samples prepared at pH 9.68 contained an excess of dimethylformamide (10 ml). Five milliliters were not enough to prevent precipitation.

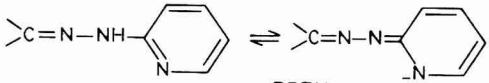
TABLE 3
COLORS OF THE COMPLEXES IN AQUEOUS SOLUTION AT pH 9.68 AND EXTRACTED INTO CHLOROFORM

Metal ion	Color of the complex	Color of the extracted complex	
		Aqueous layer	Organic layer
Co(II)	Red	—	Red
Ni(II)	Yellow	—	Yellow
Cu(II)	Blue–Purple	—	Green
Cu(I)	Yellow	Yellow	—[a]
Pd(II)[b]	Brown	Purple	Green

[a] By adding sodium perchlorate, the yellow copper(I) complex is extracted, and an orange precipitate appears in the liquid–liquid interphase.

[b] The other noble metals do not react.

In agreement with numerous bibliographies these azomethine compounds can be deprotonated in basic solutions as shown by the following (*13*):



BBPH

The deprotonation is enhanced by coordination.

The presence of pyridine nitrogen atoms confers to the molecule, apart from two possitions of coordination, a high solubility in ethanol, dimethylformamide, and organic solvents. Although sparingly soluble in water, it can dissolve in water–ethanol mixtures, in acetic acid–sodium acetate medium, or in dilute hydrochloric acid.

The reactions of BBPH with a number of metal ions were screened to determine possible applications of the reagent to spectrophotometric analysis. In the case of the copper–BBPH system the solutions show a marked difference in absorption with the pH. At low pH values it is yellow. When the pH increases the color changes first to orange and then to red with the appearance of an isosbestic point at 458 nm (Fig. 2a). At basic pH the color is purple and an isosbestic point appears at 520 nm, although it is not well-defined. These changes rule out the use of this metal complex as acid–base indicator. The intense color of the complex of copper with BBPH is lost upon the addition of EDTA, which indicates that BBPH might be useful as a metal indicator in the complexometry determination of copper.

When a volume of BBPH solution is added to a cobalt(II) solution, a red color is formed, which persists in a 1 to 2 *M* hydrochloric acid medium. Upon increasing the concentration of the hydrochloric acid, the color changes progressively from red to orange to yellow (isosbestic point at about 440 nm). These colorations are stable for several hours, but fade slowly with time. Nevertheless the yellow complex is stable in a medium containing 60% perchloric acid for several weeks (Fig. 3a). As the other metal complexes are destroyed by the addition of acid, there is no doubt that this will permit the establishment of a selective spectrophotometric method for the determination of cobalt.

The palladium(II) complex is deprotonated in acidic solutions. Thus, the establishment of a selective method for determining this cation also appears possible with the extraction being performed from aqueous solutions of relatively low pH values. From low pH values to alkaline ones the color is purple (blue-purple), blue (greenish-blue), green (green), and yellow (green), in the absence of buffer substances and with a ratio of Pd to BBPH of 1:1.33. The colors in parentheses are the corresponding colors of the complexes extracted into chloroform.

The iron(III)–BBPH complex at pH 4.7 changes on standing to the iron(II) complex. The reagent acts as its own reducing agent to provide iron(II) for its color reaction. In the formation of this latter complex the presence of acetate ion and a great excess of reagent seems to play a decisive role.

The cadmium complex in alkaline medium shows a great absorptivity (about 3.6×10^4 liter/mol^{-1} cm^{-1}), but its coloration is nonspecific.

CONCLUSION

This kind of hydrazone possesses the double chromogenic grouping >C=N-NH-C=N- and functions as a tetradentate chelate compound. The three chelate compound loops of the complex ions are five-membered. As has been previously indicated, the secondary amino grouping of the pyridylhydrazone residues can be deprotonated in basic solutions. Thus, two different kinds of colored complexes can be formed. Extension of the π-system of the molecule should lead to even more sensitive reactions. Thus, quinolylhydrazones are superior in sensitivity to pyridylhydrazones (*13*). Nevertheless the sensitivity of benzil bis(2-pyridyl)hydrazone for metals is less than BBPH. There is no doubt that the nature of R in R-C(=O)-C(=O)-R has a definitive effect on complex formations. Although the simplest member of this class of ligands is glyoxal bis(2-pyridyl)hydrazone, BBPH was selected due to the inductive effect ($+I$) of the methyl groups. Both the inductive ($-I$) and mesomeric effects of the aromatic ring alter the electron density of the nitrogen atoms resulting in the weakening of the bonds. The solubilities of BBPH and its metal complexes are superior to those of benzil bis(2-pyridyl)hydrazone.

The acid dissociation constants of the ligand and further details of the chelating reactions as well as some supporting analytical applications will be reported later.

SUMMARY

With the purpose of introducing biacetyl bis(2-pyridyl)hydrazone as an analytical reagent, the pyridylhydrazone literature has been reviewed. BBPH acts as a general chromogenic reagent. The fundamental solution chemistry of the complexes formed by BBPH with the metal ions has been studied. BBPH appears to be a promising reagent for the colorimetric estimation of cobalt and palladium. It may be advantageously compared with benzil bis(2-pyridyl)hydrazone which has the same basic chelate structure.

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A Study of the Stability of Pyrimidine Series Cytostatics, Ftorafur and 5-Fluorouracil

The Effect of Oxidation on the Stability of Ftorafur and 5-Fluorouracil

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INTRODUCTION

The present paper deals with oxidation changes of cytostatics Ftorafur and 5-fluorouracil and is connected to the study of the stability of these cytostatics in dependence on uv radiation, temperature, and the medium pH (*3*). Hydrogen peroxide was selected as the oxidant, as it can be assumed that oxidation of organic compounds with hydrogen peroxide proceeds analogously to the oxidation with atmospheric oxygen (*2, 4*).

The cytostatics were oxidized at laboratory temperature, in light and in dependence on the medium pH. The degree of oxidation and its rate were monitored using thin-layer chromatography; the decrease in the content of the cytostatically effective substances was followed spectrophotometrically.

REAGENTS AND APPARATUS

5-Fluorouracil from Roche, Basle, Switzerland.
Ftorafur from Medexport, USSR.
Urea, p.a., from Lachema, Czechoslovakia.
A 1% aqueous solution of Ftorafur, pH 5.02.
A 1% aqueous solution of 5-fluorouracil, pH 5.2.
A 0.1% aqueous solution of urea.
The organic solvents used were of p.a. purity from Lachema, Czechoslovakia.

Chromatographic Plates

Silufol UV 254, 150 × 150 mm from Kavalier, Czechoslovakia.
Lucefol Quick, 200 × 200 mm, from Kavalier, Czechoslovakia.
DC-Fertigplatten Kieselgel, 50 × 200 mm from Merck, Germany.

Chromatographic Systems

(S1) Ethyl acetate: acetone: water (35:20:5).

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(S2) *n*-Butanol: 0.1 *N* diethylamine (2:1).
(S3) Ethyl acetate: water:formic acid (50:15:10).

Ehrlich detection reagent: 1% f-Dimethylamino benzaldehyde in 90 ml of methanol and 10 ml of concentrated hydrochloric acid.

A 0.1% ninhydrine solution in methanol.

A 0.04% bromocresol green solution in 80% methanol (8 drops of a 30% aqueous sodium hydroxide are added per 100 ml of the reagent) (*1*). Acids form yellow spots on a green background.

A 0.04% bromocresol purple solution in 50% methanol, with the pH adjusted to 10 with sodium hydroxide. Acids form yellow spots on a blue background (*1*).

A Varian Techtron 635 spectrophotometer.

A PHM 4d pH meter, Radiometer, Denmark.

A Fluorotest Universal UV lamp, Hanau.

MD 30 and MD 100 microsamplers, Dipptra, Czechoslovakia.

EXPERIMENTAL

The 1% solutions of Ftorafur and 5-fluorouracil were oxidized with hydrogen peroxide at laboratory temperature, in the light and in alkaline (0.1 *N* NaOH), acidic (0.1 *N* H_2SO_4), and neutral (distilled water, the pH of 1% cytostatic solutions = 5) media. The solutions of the cytostatics were followed in certain intervals over a period of 21 days, using thin-layer chromatography on silica gel and cellulose. The decrease in the content of the cytostatically effective substances was monitored spectrophotometrically, either directly in the cytostatic solutions or after prior separation by TLC. Unoxidized solutions of Ftorafur and 5-fluorouracil were stored and studied under the same conditions.

(A) 1% Aqueous Solutions of the Cytostatics (pH 5)

(a) Chromatographic monitoring of the oxidation products of Ftorafur and 5-fluorouracil. The stability of the 1% aqueous solutions of the cytostatics was monitored at certain time intervals during oxidation with a 1% hydrogen peroxide solution, using TLC with reagent systems specified above. Amounts of 20 μl of 1% aqueous solutions of the cytostatics were applied to the chromatographic plates and the plates were developed in a common manner in saturated chambers. The substances were detected in uv light at 254 nm, using the detection reagents specified above.

(b) Spectrophotometric determination of the Ftorafur and 5-fluorouracil content. This determination was carried out in certain time intervals directly or after preliminary chromatographic separation, in 0.1 *N* hydrochloric acid. The cytostatic solutions were diluted to a concentration of about 8–10 μg/ml. The absorption maxima of 5-fluorouracil and Ftorafur are located at 267 and 272 nm, respectively.

The chromatographic separations were performed on Silufol UV 254 in the S1 system, using 1% solutions of the studied cytostatics and 1% standard solutions (20-μl volumes were applied to the plate). After detection in uv light at 254 nm, the spots of the cytostatics were eluted from the chromatogram with a 0.1 *N* solution of hydrochloric acid, to obtain a resultant cytostatic concentration of 8–10 μg/ml.

(c) *Monitoring of the decrease in pH of the cytostatic solutions.* These changes were monitored at certain time intervals electrometrically.

(B) 1% alkaline and acidic solutions of the cytostatics

(a) The chromatography of the oxidation products in 0.1 *N* NaOH and 0.1 *N* H_2SO_4 was carried out in the same way as in section A.

(b) The spectrophotometric determination of Ftorafur and 5-fluorouracil in the cytostatic solutions was performed in the same way as in section A.

RESULTS AND DISCUSSION

The stability of the Ftorafur and 5-fluorouracil cytostatics oxidized by 1% hydrogen peroxide for 21 days at laboratory temperature and in the light was studied in alkaline (0.1 *N* NaOH), acidic (0.1 *N* H_2SO_4), and neutral (pH 5) aqueous solutions, determining the content of the cytostatics by uv spectrophotometry. The greatest decrease in the cytostatic content occurred in the alkaline medium. Whereas the decrease in the unoxidized alkaline solutions of 5-fluorouracil was negligible after 21 days, that in the oxidized solutions amounted to almost 50% (Table 1, Fig. 1). The content of Ftorafur decreased by 35% in the oxidized alkaline solutions and by only 8% in the unoxidized solutions (Table 1, Fig. 1). Because tetrahydrofuran is dissociated from the N1 pyrimidine ring upon oxidation of Ftorafur (I), with formation of 5-fluorouracil (II) that absorbs light in the same wavelength region as Ftorafur (the respective absorption maxima at 267 and 272 nm in (0.1 *N* HCl), the Ftorafur content could be determined only after prior chromatographic separation. In the further stage of the oxidation, the pyrimidine ring was opened between N3 and C4 and between C6 and N1, with formation of urea (III).

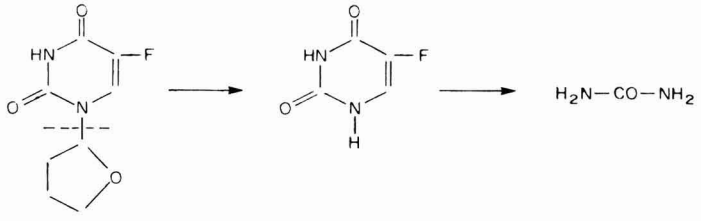

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TABLE 1

SPECTROPHOTOMETRIC DETERMINATION OF THE DECREASE IN THE 5-FLUOROURACIL (FU) AND FTORAFUR (FT) CONTENT IN SOLUTIONS OXIDIZED WITH HYDROGEN PEROXIDE

Time	Content (%)					
	FT in 0.1 *N* NaOH		FU in 0.1 *N* NaOH		FU at pH 5	
	Oxidized	Unoxidized	Oxidized	Unoxidized	Oxidized	Unoxidized
After preparation	100.00	100.00	100.00	100.00	100.00	100.00
1st day	75.00	98.00	72.20	99.70	98.02	100.22
4th day	70.00	94.00	65.15	99.08	96.00	100.22
7th day	68.00	94.00	59.30	98.77	93.20	99.10
10th day	67.05	92.50	56.00	98.20	92.16	99.70
15th day	65.00	92.00	52.85	97.90	92.00	99.40
21st day	65.00	92.00	51.80	97.40	90.12	99.31

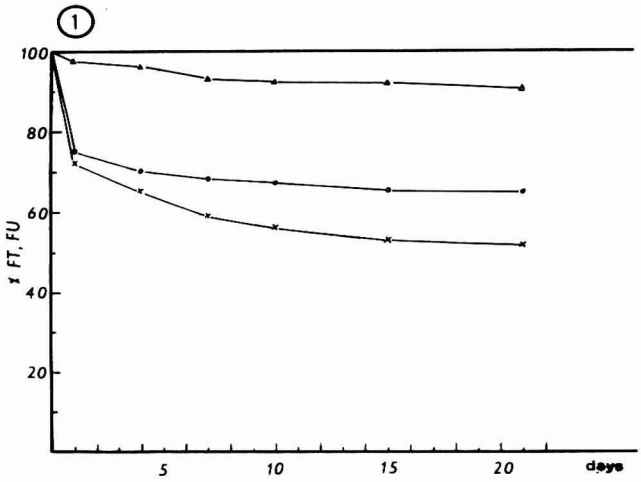

FIG. 1. The decrease in Ftorafur (FT) and 5-fluorouracil (FU) content in oxidized solutions of the cytostatics: (Δ) 1% aqueous solution of 5-fluorouracil (pH 4.8); (o) 1% solution of Ftorafur in 0.1 *N* NaOH; (x) 1% solution of 5-fluorouracil in 0.1 *N* NaOH.

This reaction was very rapid in the alkaline solution; urea was detected in the solutions as early as after several hours from the beginning of the oxidation, whereas in the unoxidized solutions only traces of urea appeared after 7 days (Figs. 2 and 3). In the oxidized Ftorafur alkaline solutions, another substance with R_f 0.43 was detected in addition to urea (Fig. 3). This substance can be detected with the Ehrlich reagent but has not been identified.

In the weakly acidic medium (pH 5), the decrease in the content of 5-fluorouracil upon oxidation was slow, about 10% after 21 days (Table 1, Fig. 1). Ftorafur dissociated tetrahydrofuran from N1 on the pyrimidine ring under these conditions, with formation of 5-fluorouracil (Fig. 3). Urea was not detected in these solutions. A pronounced decrease in the pH occurred during oxidation of the cytostatic weakly acidic solutions, from 4.85 to 3.04 with 5-fluorouracil and from 4.8 to 3.08 with Ftorafur, within 10 days. The pH of unoxidized cytostatic solutions remained unchanged (Table 2). Thin-layer chromatography detected two substances of acidic nature in the oxidized solutions of the cytostatics and these substances probably cause the decrease in the pH. The R_f values on Lucefol were 0.19 and 0.32 in the S2 system and 0.36 and 0.86 in the S3 system.

In the acidic medium (0.1 *N* H_2SO_4), Ftorafur dissociated tetrahydrofuran on N1 of the pyrimidine ring, with formation of 5-fluorouracil. In addition to 5-fluorouracil, two other substances detectable in uv light at 254 nm were produced (Fig. 3). In 5-fluorouracil solutions only a single substance detectable in uv light was formed and remained at the start; the

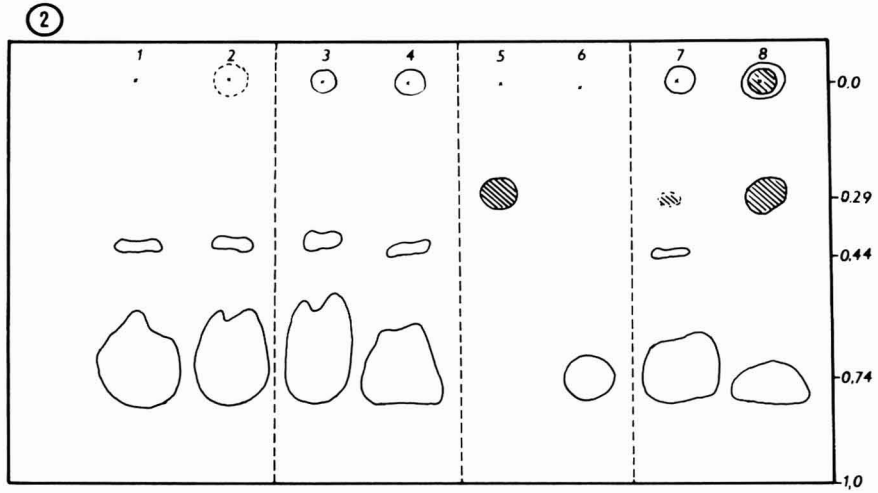

FIG. 2. Chromatographic study of 5-fluorouracil solutions oxidized with hydrogen peroxide, in dependence on the pH (after 7-day oxidation). Silufol UV 254, the S1 system; detection: UV 254 (open figures) and 1% *p*-DMAB (shaded figures). (1) 1% aqueous solution of FU, 20 μl; (2) 1% aqueous solution of FU oxidized by H_2O_2, 20 μl; (3) 1% solution of FU in 0.1 *N* H_2SO_4, 20 μl; (4) 1% solution of FU in 0.1 *N* H_2SO_4 oxidized by H_2O_2, 20 μl; (5) standard solution of urea, 5 μl; (6) standard solution of FU, 20 μl; (7) 1% solution of FU in 0.1 *N* NaOH, 20 μl; (8) 1% solution of FU in 0.1 *N* NaOH oxidized by H_2O_2, 20 μl.

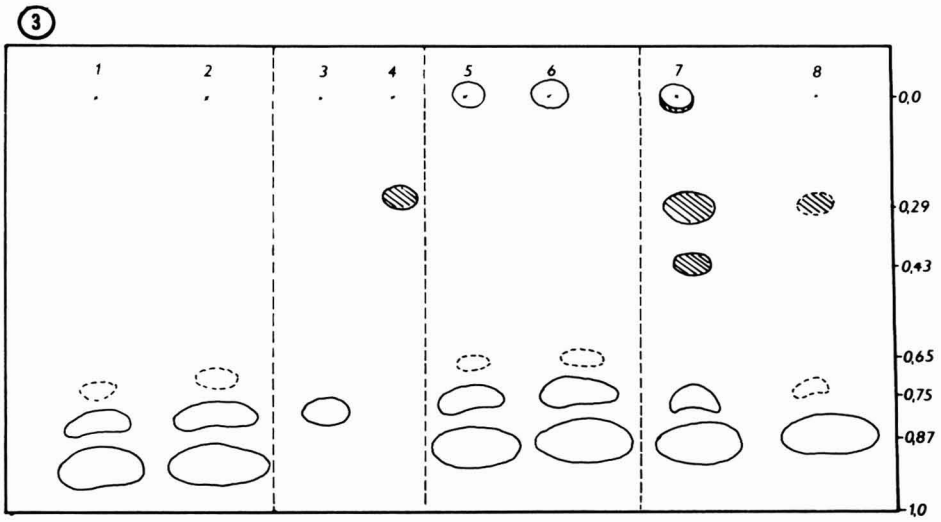

FIG. 3. Chromatographic study of Ftorafur solutions oxidized with hydrogen peroxide, in dependence on the pH (after 7-day oxidation). Silufol UV 254, the S1 system; detection: UV 254 (open figures) and 1% *p*-DMAB (shaded figures). (1) 1% aqueous solution of FT, 20 μl; (2) 1% aqueous solution of FT oxidized by H_2O_2, 20 μl; (3) standard solution of FT, 20 μl; (4) standard solution of urea, 5 μl; (5) 1% solution of FT in 0.1 *N* H_2SO_4, 20 μl; (6) 1% solution of FT in 0.1 *N* H_2SO_4 oxidized by H_2O_2, 20 μl; (7) 1% solution of FT in 0.1 *N* NaOH oxidized by H_2O_2, 20 μl; (8) 1% solution of FT in 0.1 *N* NaOH, 20 μl.

TABLE 2
THE DECREASE IN THE pH IN 1% AQUEOUS SOLUTIONS OF FTORAFUR (FT) AND 5-FLUOROURACIL (FU) OXIDIZED WITH HYDROGEN PEROXIDE

	pH			
	After preparation	After 2 hr	After 3 days	After 10 days
FU/H_2O	5.2	5.2	5.1	5.09
FU/H_2O_2	4.84	4.52	3.54	3.04
FT/H_2O	5.02	5.02	4.99	4.94
FT/H_2O_2	4.78	4.61	3.29	3.08

substance with R_f 0.44 was present in the initial 5-fluorouracil substance (Fig. 2).

CONCLUSION

The 1% aqueous solutions of cytostatics Ftorafur and 5-fluorouracil were oxidized by 1% hydrogen peroxide at laboratory temperature and in the light for 21 days. The oxidation was followed by TLC and spectrophotometrically in the uv region, after chromatographic separation. The greatest decrease in the Ftorafur and 5-fluorouracil contents occurred in alkaline media (0.1 *N* NaOH), namely, by 50% with 5-fluorouracil and by 35% with Ftorafur, over 21 days (Table 1, Fig. 1). The oxidation in the alkaline medium led to opening of the pyrimidine ring between N3 and C4 and between C6 and N1, with formation of urea (Figs. 2 and 3).

The pH of 1% aqueous solutions of the cytostatics considerably decreased during the oxidation, from 4.85 to 3.04 with 5-fluorouracil and from 4.8 to 3.08 with Ftorafur, within 10 days (Table 2). TLC detected two acidic substances in these solutions which probably cause the decrease in the pH.

SUMMARY

The stability of 1% solutions of cytostatics Ftorafur and 5-fluorouracil was studied during oxidation with hydrogen peroxide in alkaline (0.1 *N* NaOH), acidic (0.1 *N* H_2SO_4), and weakly acidic (pH 5) solutions by thin-layer chromatography. The decrease in the cytostatic content was monitored spectrophotometrically in the uv region. The greatest decrease in the cytostatic content occurred in alkaline solutions, where the pyrimidine ring opened between N3 and C4 and between C6 and N1, with formation of urea.

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A Device to Aid Installing and Removing Glass Gas Chromatographic Columns

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INTRODUCTION

Columns used for gas chromatography are most practically made of glass (*1*). This allows careful filling and reduces the reactivity of the column and sample. When glass columns are used in close proximity, difficulty arises in installing and removing columns from column ovens (Fig. 1). Our laboratory has four gas chromatographs with multiple columns and all present difficulty when columns are changed. Often expensive columns are broken in this process.

EXPERIMENTAL

Wing nuts were welded to regular Swagelok ¼- and ⅛-in. column fittings to make column nuts (Fig. 1). O rings made of silicone rubber were in-

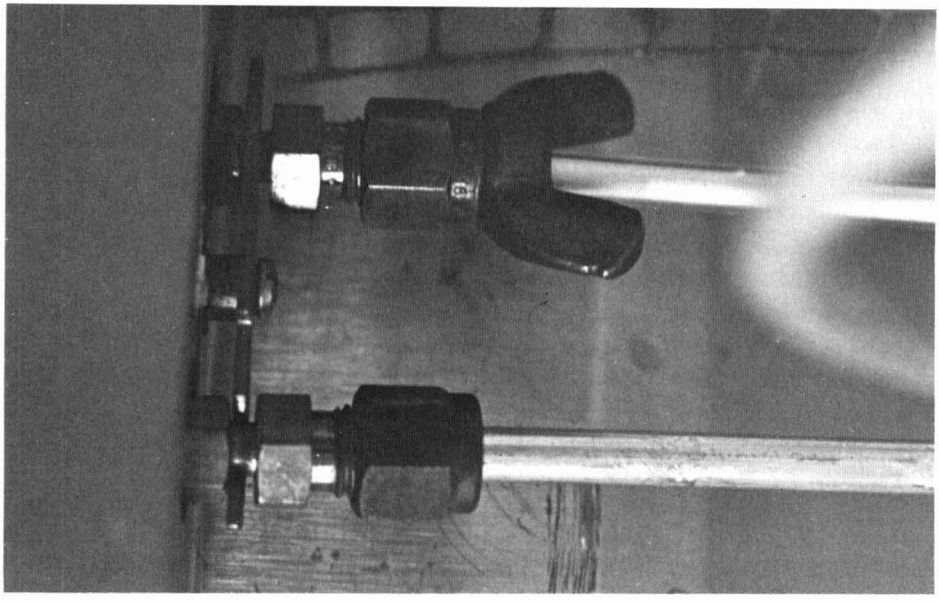

FIG. 1. Column oven with nuts in place.

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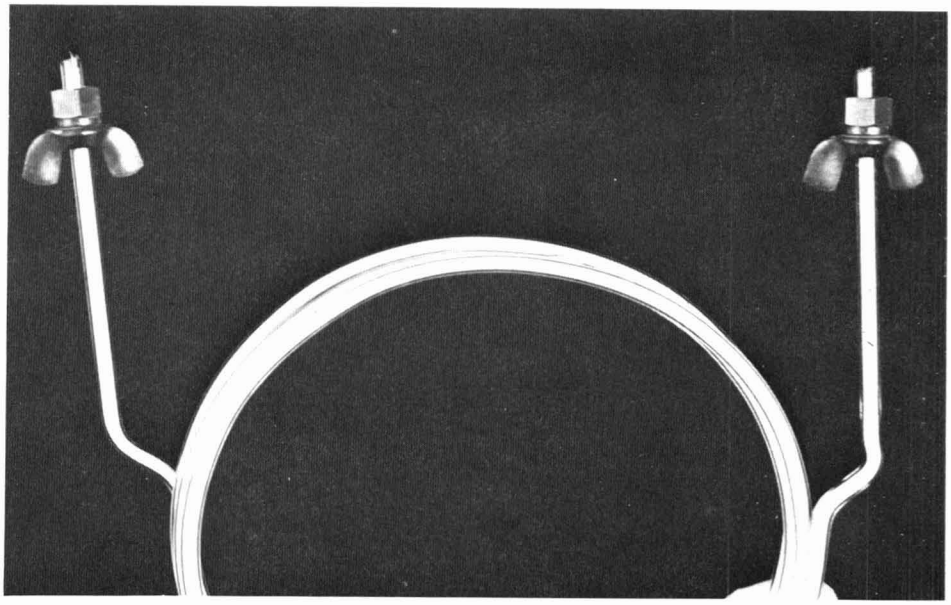

FIG. 2. Column nuts on columns.

stalled below the column nuts to prevent loss of column nuts from the column end position. Used ferrules or a tape wrap are used for this purpose too, depending on the application and temperature. Ordinary Swagelok column fittings may be used with metal columns. O rings or plastic ferrules of Teflon, Vespel, or other materials such as graphite or lead may be used without modification with these nuts.

RESULTS AND DISCUSSION

Columns may be removed easily without stress. The "built-in" wrench effect allows column change in seconds without breaking columns or even burning fingers. It greatly benefits the positioning of columns in close spaces like a four-column biomedical oven or a two-column rectangular oven, shown in Fig. 1. It even allows a better "feel" to prevent breaking by twisting glass too much during installation. Best of all, it allows high-temperature switching of columns in seconds, greatly enhancing speed and selectivity of analysis. We attempted to use every quick disconnect system we could conceive of before this system was devised by Mr. A. Wunderlich.

The Iowa State University Foundation will make the column nuts available to those interested in obtaining some.

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BOOK REVIEWS

Anodic Oxidation. By SIDNEY D. ROSS, MANUEL FINKELSTEIN, AND ERIC J. RUDD. Academic Press, New York, 1975. x + 339 pp. $37.00

Electrochemical methods—both as analytical tools and micropreparative techniques—are becoming more and more adopted in microchemistry. Both the sample size and the concentration range correspond to limits within which techniques are considered as microchemical. Even if the basic principle of electrochemical procedures are simple and seem deceivingly straightforward, the successful application of electrochemistry demands on the part of the experimenter both skill and knowledge of theory. For reduction processes useful information is available based on polarographic and related techniques; for oxidation processes such information is much more limited. Therefore, the appearance of this volume will be greeted by all interested in applications of electrooxidation (and even more so because the senior author has been for years one of the world's leading contributors to understanding of this area).

The book consists of two parts: In the first, the principles and methods used in electrochemical investigation are summarized in 78 pages. Such summary presents a difficult choice of problems and leads to evaluations based on somewhat limited evidence. Most of the material presented here can be found in more detail elsewhere, e.g., in *Experimental Electrochemistry for Chemists* by D. T. Sawyer and J. L. Roberts, Jr., in numerous specialized monographs, and in review articles. Perhaps it would have been better to restrict this discussion to principles and refer to other sources. In the evaluation of information which can be obtained from polarography the most important aspect, namely, distinguishing the sequence of chemical and electrochemical steps and, in particular, information on the nature of the electroactive species and chemical reactions preceding the electrode process proper, is not mentioned.

The real strength of this monograph is in the second part, giving a survey of anodic oxidations of hydrocarbons, Kolbe acid oxidation, oxidation of amines, amides, hydrazines, alcohols, phenols, and esters. Of the more commonly encountered types only aldehydes are missing. Each chapter is an authoritative review, where anybody interested in oxidation of a special type of compound can find essential and up-to-date information.

The volume is strongly recommended to all those who use electrooxidation in microchemical procedures.

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Advances in Physical Organic Chemistry, Volume 12. Edited by V. GOLD AND D. BETHELL. Academic Press, New York/London, 1976. viii + 318 pp. $27.25.

This volume of this excellent and informative series contains three reviews: "Structure and Mechanism in Organic Electrochemistry," by L. Eberson and K. Nyberg (Lund, Sweden) (pp. 1–129); "Acid–Base Properties of Electronically Excited States of Organic Molecules," by J. F. Ireland and P. A. H. Wyatt (St. Andrews, Scotland) (pp. 130–221); and "Application of Radiation Techniques to the Study of Organic Radicals," by P. Neta (Carnegie–Mellon University, Pittsburgh, Pennsylvania). Of these, in the opinion of the reviewer, the first is of particular interest to microchemists.

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Organic electrochemistry is dealt with by two types of chemists: physical or analytical chemists who became interested in applications of electrochemical methods to organic compounds and organic chemists who have been seduced by novelty and the possibilities of electrochemical methods. The authors are outstanding representatives of the rarer breed, belonging to the second category. The review demonstrates not only their familiarity with organic chemistry, but also a profound understanding of the essential aspects of electrochemistry. Individual topics dealt with are the classification of electroorganic processes, the mechanisms of such processes, and the techniques used in their elucidation. Particular attention is devoted to three timely problems—competition between reaction of radical ions and doubly charged ions, disproportionation and the so-called ECE mechanism, and the nature of coupling of primary products of the electrode process. The roles of adsorption and of the electrode material are discussed together with available knowledge of the electron transfer proper and the relationship between the structure of organic compounds and their reactivity in electrode processes.

In many instances, a fresh, novel approach is used. By using an almost telegraphic style, the authors have managed to include a wide scope of material. The presentation is thought-provoking and inspiring. Is there a better recommendation for a review article?

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Chemical Technicians' Ready Reference Handbook. By G. J. SHUGAR, R. A. SHUGAR, AND L. BAUMAN. McGraw–Hill, New York, 1973. xiv + 463 pp. $23.95.

Handbook for Chemical Technicians. By H. J. STRAUSS and edited by M. KAUFMAN. McGraw–Hill, New York, 1976. Various pagination [454 pp]. $19.50.

Many a supervisor of a chemical laboratory dreams of drafting a manual that he could use for in-house indoctrination of technicians, especially those having little or no post-high-school training. Few supervisors realize their dream.

In this age of paraprofessionalism, many instructional, and informational aids for the training of technicians are becoming available, including motion picture films, slide and filmstrip presentations, programmed workbooks, source works, and textbooks. A single publisher has issued the two works under review. Both can be recommended to libraries supporting either chemical education or research and application studies in the chemical sciences. Technicians may profit by having a bench copy available.

To a large extent these two works complement each other. The overlap in the material presented is not great. Shugar and co-workers focus on the chemical *laboratory* technician and include detailed general procedures for many common laboratory operations and techniques. In contrast, Strauss and Kaufman direct their work to the chemical technician, broadly conceived, including the pilot plant technician and even the skilled chemical plant operator. They have adopted a "summary" style for the text and have provided many tables of useful data.

Shugar and co-authors in their Preface claim to offer an "omnibook" so that the "technician has at his fingertips all of the information, procedures, techniques, and methods that he needs." A more modest representation would have been appropriate. To some extent these authors "favor" the technician being involved in chemical analysis. Consequently, the work may be of special value to quality control groups. The determination of elements in organic compounds by combustion and digestion methods is treated in some detail; this topic would only seem to warrant consideration with the few analytical technicians assigned to such tasks. No attention is given to the *general* tests that are run by the majority of quality control

technicians in the pharmaceutical and chemical industries, such as arsenic, heavy metals, color (APHA), residue after ignition, and mesh size.

Strauss and Kaufman in their efforts to provide a broad-based reference work include useful chapters on thermochemistry and kinetics, metals and alloys, fluid mechanics, engineering operations, and an excellent 61-page chapter on hazards and safety practices.

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Handbook for Chemical Technicians. By HOWARD J. STRAUSS. Edited by MILTON KAUFMAN. McGraw-Hill, New York, 1976. vii + 446 pp., $19.50.

This handbook is, for the first time, designed particularly to meet the essential demands of day-to-day problem solving in the chemistry laboratory. Primarily, it is intended to provide chemical technicians, plant operators, engineers, basic research workers, students, and others with a fundamental knowledge of chemistry, a thorough understanding of all aspects of chemical technology, and an easy-to-use guide indispensable for running a laboratory.

The book consists of 10 comprehensive, information-packed, and well-compiled chapters. The informative materials and data presented include: units and measurements; thermal, electrical, and mechanical units; review of some chemistry fundamentals; thermochemistry and kinetics; elementary organic chemistry; metals, alloys, and metal testing; fluid mechanics; engineering operations such as blending, filtration, and drying; safety practices, procedures, first aid, and waste disposal, and so forth. The presentation of each subject is such as not only to cover a great number of practical useful data but also to include a short exposition of the pertinent theory and examples illustrating the use of the tabular or graphic information. A total of some 138 tables, 144 figures, and 208 illustrated examples are well surveyed, compiled, demonstrated, and cited. An index appears at the end of the book.

Specifically, several good features of the book are worth emphasizing. For instance, special attention has been given to organizing and clarifying all data and descriptions of laboratory operations. All illustrated examples of calculations involved in measurement and analysis are worked out with step-by-step explanations. All solutions include detailed discussions of experimental procedures and equation solving. The descriptions of the subject matter are functionally detailed and practically emphasized. All data selected are the best or most common values available from the reliable reference sources or literature. These values cover inorganic, organic, analytical, and physical chemistry as well as basic applied physics and engineering sciences. In addition, of special value is the chapter on safety procedures which meet the current laboratory need for safe operations. Among these are demonstrations of the necessary precautions for the safe handling of materials and the effective wearing of specific types of protective clothing; the correct way of protecting workers in the laboratory from fire, explosive and radioactive materials, and electric and toxic hazards; and the proper procedures of disposing dangerous wastes, and so on.

In summary, this is a well-compiled and concise working reference book for all areas of scientific laboratories. All practical workers will find this all-under-one-roof approach to be an excellent aid for reviewing new methods of problem solving. The many well-drafted graphs and diagrams and the sufficient tables of reliable, easy-to-apply values will help technicians to choose the most efficient methods for their daily laboratory work. Furthermore, this handbook is suitable for students or professional beginners as a self-teaching text. As for its value in

both reference guideline and teaching qualities, laboratory workers, students, technicians, and researchers would not hesitate to have one for their own use.

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Relaxation Kinetics. C. F. BERNASCONI. Academic Press, New York, 1976. xii + 288 pp., $29.50.

One of the areas dealing with analysis of dilute solutions and sometimes in small volumes, which therefore may be considered to belong to microchemistry in a wider sense, is the study of fast reactions. Even though investigations in this field, involving fast flow of solutions and rapid mixing or electrochemical perturbations, as in polarography, were initiated in the twenties and thirties and developed in the forties, it was only after introduction in the fifties of techniques, in which chemical equilibrium is rapidly perturbed (usually by a change in a physical property—such as temperature, pressure, or electric field), that such methods were widely studied and adopted. Such investigations involved following the readjustment of the perturbed system to the equilibrium conditions existing at new imposed physical conditions. The time needed for adjustment to the new equilibrium conditions, called relaxation time, offers information about rates of chemical reactions involved in the reestablishment of the equilibrium.

Measurements of relaxation times have so far predominantly dealt with applications to problems of inorganic chemistry, mainly to the studies of rates of complex-formation and of proton-transfer reactions. More limited applications to the study of equilibria involving organic compounds have been interpreted as due to the absence of a proper text. This situation should be rectified by the reviewed volume, aimed at potential researchers in the field of fast kinetics and at graduate students, as an introduction. It accomplishes this aim in a very successful way.

The first eight chapters are devoted to development of the mathematical apparatus, particularly equations relating relaxation times to specific rate constants and concentration changes. The ninth chapter describes evaluation of relaxation times from experimental data. Chapters 11–16 deal with principles and experimental approaches used in the individual techniques, namely, temperature-, pressure-, electric field-, and concentration-jump, ultrasonic, and stationary field methods. Applications are discussed briefly, but literature is quoted extensively up to 1973 (with inclusion of some very recent references).

The mathematical treatment is easy to follow, descriptions are clearly written, and evaluations are critical and objective. It is a very suitable introduction for any microchemist who is interested (or made to become interested) in the study of fast reactions. Some problems are included, no answers are given, but references to original literature are provided. An author's index is missing (used frequently as subject index!), but the quality of the production is up to the usual Academic Press standards and the appearance is very pleasing.

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Stereoselective Reductions. Edited by MICHAEL P. DOYLE AND CHARLES T. WEST. Halstead Press (Wiley), New York, 1977. xviii + 419 pp. $30.00.

This is Volume 6 in a series of "Benchmark Papers in Organic Chemistry." The purpose of the series is to take some major topic, in this case, stereoselective reductions, and select

the key papers that will provide historical significance, scientific elegance, and contemporary impact.

The Editors have grouped these papers into four major sections: Stereoselective Reductions of the Carbon–Carbon Double Bond, 16 papers; Stereoselective Reductions of the Carbon–Carbon Triple Bond, 9 papers; Stereoselective Reductions of the Carbon–Oxygen Double Bond, 36 papers; and Enzyme Catalyzed Reductions, 3 papers. Each of the major sections is subdivided into subsections of one to six papers and each section and subsection is provided with editorial comments and an introduction which give perspective and significance.

Two of the papers are in German, the rest are in English. Since each of the papers is reproduced as it originally appeared, the general appearance of the volume is uneven; however, the reproduction is clear, and even the finest printing is easily legible.

The articles in this collection trace the development, preparation, and application of stereoselective reducing agents and record the advances in understanding the stereoselective reduction process.

In addition to an index of the original paper authors and a subject index there is also an index of all of the authors cited in each of the papers.

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New Concepts in Safety Evaluation. Edited by MYRON A. MEHLMAN, RAYMOND E. SHAPIRO, AND HERBERT BLUMENTHAL. Halstead Press (Wiley), New York, 1976. xix + 455 pp. $24.50.

This is Volume 1, Part 1, of a projected new series, Advances in Modern Toxicology. The goal is to provide any researcher involved in the study of toxicology, the newest and most important concepts essential in dealing with the flood of new compounds and industrial processes and with the ever increasing concern of the public for the effects of these chemicals and processes on the environment.

The 13 chapters involving 21 authors range from "how to" details, literature reviews, and presentation of data for evaluation to opinions. Each chapter is independent of the others and a listing will give the best overview of the contents and scope of this volume.

Part I, Interactions, contains two chapters, Role of Environmental Agents in Modifying Their Biological Activity and Modern Concepts in Nutritional Status and Foreign Compound Toxicity. Part II, Conceptual and Methodological Tools, contains five chapters: "Potential Contribution of Inbred Syrian Hamsters to Future Toxicology, Concepts in Health Evaluation of Commercial and Industrial Chemicals, Assessment of the Value of Systemic Toxicity Studies in Experimental Animals, The Current Methodology in Teratological Research, and Nonlethal Parameters as Indices of Acute Toxicity. Part III, Pharmacokinetics, A New Tool, is a single long chapter dealing with Pharmacokinetic Studies in Evaluation of the Toxicological and Environmental Hazard of Chemicals. Part IV, Metabolism and Biochemical Toxicity, describes Radioautographic Methods for Physiologic Disposition and Toxicological Studies, the Significance of Metabolite-Mediated Toxicities in the Safety Evaluation of Drugs, and the Distribution, Metabolism and Perinatal Toxicity of Pesticides with Reference to Food Safety Evaluation. Part V, Transplacental Toxicity, deals with Transplacental Toxicity of Diethylstilbestrol.

Each chapter has an extensive bibliography. There are many tables and charts, all presented in an easily legible form.

This volume was distributed to the participants in the Twentieth Anniversary Gordon Conference on Toxicology and Safety Evaluation.

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Contamination Control in Trace Element Analysis. By MORRIS ZIEF AND JAMES MITCHELL. John Wiley & Sons, New York, 1976. 262 pp., $22.50.

As methods for analysis of trace elements become more refined, the problem of contamination becomes more and more acute. It is of little value to develop highly sensitive methods if contamination destroys the validity of the results obtained. The authors, on the basis of their own practical success in controlling contamination, have prepared this highly practical manual describing how to control contamination in trace element analysis. The book is part of a series of monographs on analytical chemistry and its applications edited by P. J. Elving. The book includes seven chapters, three appendices, and a subject index. The first chapter is an introduction to the subject and problems of trace analysis. Chapter 2 is a review of basic aspects of quantitative ultratrace analysis. Chapters 3, 4, and 5 discuss the type of laboratory control needed for ultratrace analysis, the types of sample containers and apparatus to be used, and the degree of reagent purification required. Contamination control during routine analytical operations is described in detail in Chap. 6. The final chapter, Chap. 7, discusses selected methods for the determination of ultratrace elements in reagents and materials. Although this chapter has nothing to do with the book's objectives, it is still an interesting chapter. Appendix I is a brief review of abbreviations, symbols, and definitions. Appendix II details storage conditions necessary to keep the loss of trace elements to a minimum. Appendix III is a highly practical listing of suppliers of specialty products for the analytical laboratory. Each of the seven chapters includes numerous figures, tables, and photographs, plus a list of references to the pertinent literature. The authors have done a superb job of identifying the problem and of presenting practical methods for the control of contamination in trace metal analysis. All those involved in trace metal analysis will find this to be an interesting and valuable book.

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Erratum

Volume **22**, No. 4 (1977), in the article, "Kinetic Potentiometric Determination of Creatinine with a Picrate-Ion-Selective Electrode," by E. P. Diamandis, M. A. Koupparis, and T. P. Hadjiioannou, pp. 498–504: Pages 501 and 502: Figures 2 and 3 should be reversed (the legends are correct). Page 503: The first sentence of the last paragraph of the text should read: "In conclusion, the proposed kinetic method is faster than the end point spectrophotometric method."