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Microchemical Journal

devoted to the application of microtechniques in all branches of science

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Microchemical Journal, Volume 26, Number 4, December 1981

Briefs

Cellulose Sorbents in the Investigations of Self-Association of Higher Fatty Acids. TERESA KOWALSKA, *Institute of Chemistry, Silesian University, 40-006 Katowice, Poland.*

Cellulose sorbents were used for the qualitative and semi-quantitative evaluation of intermolecular interactions with higher fatty acids.

Microchem. J. **26**, 447–452 (1981).

Flotation–Spectrophotometric Determination of Osmium (Ruthenium) with Thiocyanate and Capri Blue. Z. MARCZENKO AND J. UŚCIŃSKA, *Department of Analytical Chemistry, Technical University, ul. Noakowskiego 3, 00-664 Warsaw, Poland.*

The method is based on the ion associate formed by the anionic thiocyanate osmium complex with oxazine basic dye, Capri blue.

Microchem. J. **26**, 453–462 (1981).

Girard-P Derivative of 2-Pyridinecarboxaldehyde as Analytical Reagent. Spectrophotometric Determination of Copper. M. CALLEJÓN MOCHÓN AND J. L. GOMEZ ARIZA, *Department of Analytical Chemistry, Faculty of Chemistry and Pharmacy, University of Seville, Seville, Spain.*

The synthesis, characteristics, properties, and reactions of the reagents with metallic ions were studied. The reagents complexed with copper(II), nickel(II), cobalt(II), mercury(I), mercury(II), zinc(II), cadmium(II), iron(II), and iron(III) with absorption maxima located in the ultraviolet zone.

Microchem. J. **26**, 463–471 (1981).

Sensitive and Selective Ion Exchange Test for Microgram Detection and Spectrophotometric Determination of Picric Acid. SAIDUL ZAFAR QURESHI, IZZATULLAH, AND REETA BANSAL, *Chemistry Department, Aligarh Muslim University, Aligarh-202001, India.*

The microgram detection and determination of picric acid with sodium hypochlorite solution is based on its oxidation in alkaline solution and chlorination with excess of chlorine present in hypochlorite solution. A characteristic orange color is obtained.

Microchem. J. **26**, 472–476 (1981).

BRIEFS

Photometric Determination of Iron(III). WADALA A. BASHIR, *Department of Chemistry, College of Science, University of Mosul, Mosul, Iraq.*

The color reaction of iron(III) with tiron (1,2-dihydroxybenzene–3,5-disulfonic acid) was studied and proposed as a method for the determination of traces of that metal.

Microchem. J. **26,** 477–480 (1981).

Applications Involving Oxidation with Potassium Bromate. II, Potentiometric Titration of Chromium Alone or in Mixtures. I. A. ISMAIL, H. KHALIFA, AND M. ZAKY, *Faculty of Science, Zagazig University, Zagazig, Egypt.*

A method is given for the determination of chromium based on oxidation of the Cr(III) with a known excess of bromate to Cr(VI).

Microchem. J. **26,** 481–486 (1981).

Study of Various Substrates for Peroxidase-Coupled Peroxide Oxidations. J. D. ARTISS,[*,†] R. J. THIBERT,[†,‡] J. M. MCINTOSH,[†] AND B. ZAK,[†,‡] *Department of [*]Medical Biochemistry, St. Joseph Hospital, London, Ontario N6A 4V2, Canada, Department of [†]Chemistry, University of Windsor, Windsor, Ontario N9B 3P4, Canada, and Department of [‡]Pathology, Wayne State University School of Medicine and Detroit Receiving Hospital–University Health Center, Detroit, Michigan 48201.*

It appears from this study that the sodium sulfonates of 2,4-dichloro- and 2,4-dibromophenol when employed in the presence of 4-aminoantipyrene produce useful and sensitive colorimetric cosubstrate systems.

Microchem. J. **26,** 487–505 (1981).

Electrochemical Study of the Use of Tin(II) Complexed by Sorbitol in Volumetric Analysis. J. ZIMA, M. ROUŠAL, AND J. DOLEŽAL, *Department of Analytical Chemistry, Faculty of Natural Sciences, Charles University, Albertov 2030, 128 40 Prague 2, Czechoslovakia.*

A study was made of the possibility of using the reagent. Potentiometry, bipotentiometry, and biamperometry were used for determination of the equivalent point.

Microchem. J. **26,** 506–513 (1981).

Determination of Tungsten by Calcium Atomization Inhibition Titration. M. TADDIA, *"G. Ciamician" Chemical Institute of the University, I-40126, Bologna, Italy.*

A method for the determination of tungsten utilizing inhibition effect on calcium emission in hydrogen-air flame is described.

Microchem. J. **26,** 514–518 (1981).

BRIEFS

A Spectrophotometric Study of the Color Reaction of Zinc with 2,2′-Dipyridyl-2-pyridylhydrazone (DPPH), G. S. VASILIKIOTIS* AND H. ALEXAKI-TZIVANIDOU,† *Laboratory of *Analytical Chemistry, School of Physics and Mathematics and †Laboratory of Physiology, Veterinary Faculty, University of Thessaloniki, Thessaloniki, Greece.*

The optimal formation conditions, the composition, and the overall apparent instability constant of the yellow water-soluble zinc–DPPH complex were studied. A simple, rapid and very sensitive method for the determination of zinc was proposed.

Microchem. J. **26,** 519–526 (1981).

Schiff Bases Derived from Biacetyl as Analytical Reagents: A Review. A. G. ASUERO, *Applied Chemical Analysis Department, Faculty of Pharmacy, The University of Seville, Seville-4, Spain.*

A review of the literature of the subject. One hundred and twenty nine references are cited.

Microchem. J. **26,** 527–556 (1981).

Analytical Possibilities of Pyridine-2-acetaldehyde Benzoylhydrazone as a Chromogenic Reagent. M. GARCIA-VARGAS, J. M. BAUTISTA, AND P. DE TORO, *Faculty of Chemistry, University of Sevilla, Sevilla, Spain.*

A study of the physical properties and fundamental solution chemistry of the complexes formed by the reagent with 14 metal ions was carried out. A critical comparison of other reagents was made.

Microchem. J. **26,** 557–568 (1981).

Analytical Applications of Crown Ethers. I. Oxidation and Determination of Benzaldehyde. I. N. PAPADOYANNIS, TH. A. KOYIMITZSIS, AND G. S. VASILIKIOTIS, *Laboratory of Analytical Chemistry, University of Thessaloniki, Thessaloniki, Greece.*

The use of dicyclohexyl-18-crown-6 (DCC) as charge transfer reagent for the oxidation of benzaldehyde in organic solvents by aqueous solution of potassium permanganate is presented.

Microchem. J. **26,** 569–573 (1981).

Derivatives of Rhodanine as Spectrophotometric Analytical Reagents. I. Condensation at C-5 with Aromatic Pyridine and Nonpyridine Aldehydes. G. GALÁN ALFONSO AND J. L. GÓMEZ ARIZA, *Department of Analytical Chemistry, Faculty of Chemistry and Pharmacy, University of Seville, Seville, Spain.*

The synthesis, characteristics, and properties as well as reactions with metallic ions of five compounds are reported.

Microchem. J. **26,** 574–585 (1981).

Photometric Determination of Nitrite. S. FLAMERZ AND WADALA A. BASHIR, Departments of Chemistry, College of Education and College of Science, University of Mosul, Mosul, Iraq.

The ion reacts with *p*-aminobenzoic acid to form a diazonium ion, which is then coupled with α-naphthol to form a pinkish red azo dye.

Microchem. J. **26,** 586–589 (1981).

Investigation of the Association of the 1-Dodecene–Lauryl Alcohol Bicomponent System. TERESA KOWALSKA AND BEATA WALCZAK, *Institute of Chemistry, Silesian University, Katowice, Poland.*

The bicomponent system was investigated as an exemplary one, able to form the hydrogen-bonded mixed multimers, involving the olefinic double bond π electrons.

Microchem. J. **26,** 590–596 (1981).

MICROCHEMICAL JOURNAL 26, 447–452 (1981)

Cellulose Sorbents in the Investigation of Self-Association of Higher Fatty Acids

TERESA KOWALSKA

Institute of Chemistry, Silesian University, Katowice, Poland

Received December 3, 1980

INTRODUCTION

In our previous paper (*1*) the physicochemical sense of a semiquantitative comparison of higher fatty alcohols was discussed with respect to their ability to self-associate, taking advantage of the cellulose sorbents. It was pointed out that the low-activity cellulose sorbents proved to be suitable for such a purpose due to their rather weak interactions with the chromatographed alcohols, insignificantly perturbing the association states of the analyzed substances.

The aim of this study was to perform similar investigations and continue the discussion dealing with higher fatty acids.

EXPERIMENTAL

We investigated the following higher fatty acids: lauric (C_{12}), myristic (C_{14}), and palmitic (C_{16}). The sample purities in each case were higher than 99.5%. Carbon tetrachloride solutions of each acid were prepared in the following concentrations: 0.01, 0.025, 0.04, 0.05, and 0.10 mole/dm^3. Chromatographic paper Whatman 2 (Whatman, England) was applied as a sorbent. The introductory step depended upon drying it for 0.5 hr at 110°C; then 20-μl aliquots of each prepared sample were applied. Chromatograms were developed in decalin 16 cm high, then dried at room temperature for 24 hr and finally visualized with the 10% soln. of $K_2Cr_2O_7$ in 50% H_2SO_4. The visualized chromatographic spots were immediately copied and their areas planimetrically determined. The determination error never surpassed ±5%. The obtained results are given in Table 1 and Fig. 1.

On the basis of these results the ratios of the chromatographic spot areas for two different acids were calculated, taking into consideration all the investigated compounds and the applied sample concentrations. The obtained number values are shown in Table 2.

In Table 3 we present ratios of the chromatographic spot areas calculated against the spot area obtained with the sample having the highest concentration.

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TABLE 1
THE CHROMATOGRAPHIC SPOT AREAS ($\bar{S}$)[a] OF LAURIC, MYRISTIC, AND PALMITIC ACID ON THE CHROMATOGRAPHIC PAPER WHATMAN 2 (MOBILE PHASE: DECALIN) FOR VARIOUS CONCENTRATIONS OF THE APPLIED SOLUTIONS[b]

Substance	Concentration (moles/dm^3) 0.01	0.025	0.04	0.05	0.10
Lauric acid	74	135	200	215	280
Myristic acid	100	185	235	270	380
Palmitic acid	160	325	445	505	715

[a] Mean values from ten individual measurements (mm^2).
[b] Constant aliquots of the CCl_4 solutions: 20 μl.

DISCUSSION

It was already stated in our previous paper (1) that the behavior of higher fatty acids on a chromatographic paper differed significantly from that in a solution based upon a low-polar solvent (e.g., CCl_4). From ir spectroscopic investigations it becomes evident that in spite of the low acid concentration (0.05 mole/dm^3 in CCl_4) all the investigated compounds still appear in the form of cyclic dimers. On the other hand investigation of the same substances on a chromatographic paper allows the following conclusion: Due to interactions with the cellulose sorbent one observes opening of cyclic dimers, which are transformed into linear dimers and ultimately into the higher linear multimers.

The results presented in Tables 1–3 and Fig. 1 additionally confirm the above-formulated observations and conclusions. These results indicate that with acids one does not observe the straight-line dependence between

TABLE 2
RATIOS OF THE CHROMATOGRAPHIC SPOT AREAS ($\bar{S}'/\bar{S}''$) WITH THE INVESTIGATED HIGHER FATTY ACIDS

Discussed system of two acids	Concentration (moles/dm^3) 0.01	0.025	0.04	0.05	0.10
Myristic/lauric	1.35	1.37	1.18	1.17	1.29
Palmitic/myristic	1.60	1.76	1.89	1.87	1.88
Palmitic/lauric	2.16	2.41	2.22	2.35	2.55

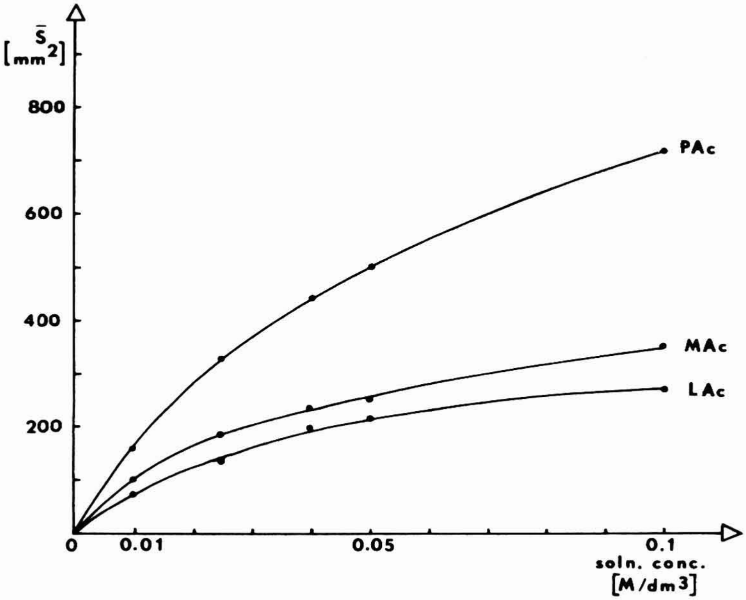


FIG. 1. The chromatographic spot areas ($\bar{S}$) of lauric, myristic, and palmitic acid vs concentrations of the applied sample solutions (sorbent: Whatman 2; mobile phase: decalin; sample aliquots: 20 μl).

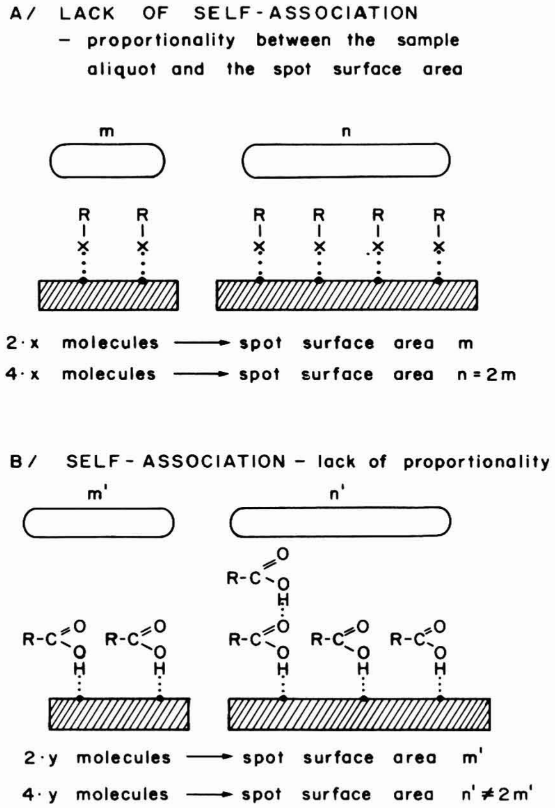
the same aliquot and the obtained spot surface area. A similar observation was valid for the higher fatty alcohols (*1*, *2*). The curves plotted in Fig. 1 indicate that the increasing number of acidic molecules applied upon a sorbent layer induces shift of the association equilibrium toward the higher linear multimers. Thus the obtained chromatographic spot areas

TABLE 3
RATIOS OF CHROMATOGRAPHIC SPOT AREAS ($\bar{S}'/\bar{S}''$)[a] CALCULATED AGAINST THE SPOT AREA OBTAINED WITH THE SAMPLE HAVING THE HIGHEST CONCENTRATION

	Concentration (moles/dm³)				
Substance	0.01	0.025	0.04	0.05	0.10
Lauric acid	3.78	2.07	1.40	1.30	1.00
Myristic acid	3.80	2.05	1.62	1.41	1.00
Palmitic acid	4.47	2.20	1.61	1.42	1.00

[a] $\bar{S}'$ = spot area of 0.1 mole/dm³ sample; $\bar{S}''$ = spot area of samples with given concentrations.

are lower than one would expect. The mechanism of this phenomenon can be explained with the following scheme:



Besides, as the data in Table 2 show, the similar number values (considering the relatively high error of chromatographic measurements) of ratios of the chromatographic spot areas with two different acids also suggest dependence of these areas on intermolecular interactions. As was introduced in our previous paper (*1*), the ratio of the chromatographic spot areas with two compounds able to self-associate is

$$\frac{\bar{S}'}{\bar{S}''} = \frac{A' + B'/2 + C'/3 + \dots}{A'' + B''/2 + C''/3 + \dots},$$

where $\bar{S}'$ and $\bar{S}''$ are the chromatographic spot areas of these compounds; $A', B', C', \dots$ and $A'', B'', C'', \dots$ are numbers of molecules of both substances appearing as monomers, dimers, trimers, etc., respectively.

We proved that under particular conditions the dissociation degrees of the consecutive multimers with two different substances are constant, depending exclusively upon temperature (*1*). Thus the conclusion can be drawn that when our self-association mechanism of obtaining chromatographic spots upon a paper is assumed, one can await stability of the $\bar{S}'/\bar{S}''$ ratio values independently from the applied sample aliquots. Certain deviations with acids (Table 2) can most probably be explained by their still slightly pronounced ability to form cyclic dimers.

The number data given in Table 3 prove that the association equilibria shift with the increasing sample aliquots toward the higher multimers. According to what was suggested previously (*1*), it may be possible to calculate the n''/n' values, which are ratios of the mean statistical lengths of open multimers, from the chromatographic data, depending upon concentrations of the applied solutions. The multimer length is understood as a number of monomer units, building the given multimer chain. The discussed ratio can be calculated from the dependence (*1*)

$$\frac{\bar{S}'}{\bar{S}''} \cong A \cdot \frac{n''}{n'}, \qquad A = \frac{c'}{c''},$$

where c' and c'' are the molar concentrations of the considered samples. The obtained number values are presented in Table 4.

The number data from Table 4 give evidence of the fact that with increasing aliquots of the applied samples the corresponding n'' values which reflect the mean statistical lengths of the open acidic multimer on the cellulose sorbent, also increase.

TABLE 4

THE A AND n''/n' NUMBER VALUES[a] OF THE CCl_4 SOLUTIONS WITH THE DISCUSSED HIGHER FATTY ACIDS

	Concentration (moles/dm^3)									
	0.01		0.025		0.04		0.05		0.10	
Substance	A	n''/n'	A	n''/n'	A	n''/n'	A	n''/n'	A	n''/n'
Lauric acid	10	0.38	4	0.52	2.5	0.56	2	0.65	1	1.00
Myristic acid	10	0.38	4	0.51	2.5	0.65	2	0.70	1	1.00
Palmitic acid	10	0.45	4	0.55	2.5	0.64	2	0.71	1	1.00

Note. Determinations performed by means of paper chromatography (sorbent: Whatman 2; mobile phase: decalin).

[a] n' corresponds with the 0.1 mole/dm^3 solution, n'' with the solutions of given concentration.

Thus the applicability of cellulose sorbents in both qualitative and semiquantitative self-association investigations is once again (*1*, *2*) demonstrated.

SUMMARY

The possibility was established of using cellulose sorbents for qualitative and semiquantitative evaluation of intermolecular interactions with higher fatty acids.

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MICROCHEMICAL JOURNAL 26, 453–462 (1981)

Flotation–Spectrophotometric Determination of Osmium (Ruthenium) with Thiocyanate and Capri Blue

Z. MARCZENKO AND J. UŚCIŃSKA

Department of Analytical Chemistry, Technical University,
ul. Noakowskiego 3, 00-664 Warsaw, Poland

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INTRODUCTION

Trace analysis lacks in sensitive spectrophotometric methods for the determination of osmium and ruthenium (*1, 4*). Many of the recently published sensitive spectrophotometric methods for determination of elements take advantage of the ion associates involving basic dyes. These associates can be extracted or floated. The latter case leads to extremely sensitive methods. Ion associates of anionic complexes of elements to be determined precipitate with basic dye at the phase boundary or on the walls of the separatory funnel when the aqueous phase is shaken with some organic solvents. The ion associate, separated from both liquid phases, is dissolved in a polar solvent and the absorbance of the solution obtained is measured (*3, 5*).

Recently, flotation–spectrophotometric method for the determination of rhodium with malachite green (*10*), platinum with crystal violet (*11*), palladium with methylene blue (*8*) or rhodamine 6G (*9*), and osmium with methylene blue (*12*), have been developed in our laboratory. In this paper a method is reported for the determination of osmium (ruthenium) based on its anionic thiocyanate complex associated with oxazine dye–Capri blue (CB). So far this dye has been used in the extraction–spectrophotometric determination of boron (*14*), gallium (*13*), and tantalum (*2*). Thiocyanate complexes of osmium and ruthenium were used previously for their extractive separation (*6*).

EXPERIMENTAL

Reagents and Apparatus

Osmium standard solution, 1 mg Os/ml. Weigh a glass ampoule containing approximately 0.5 g OsO_4, score it with a file, and then crush it carefully in a beaker containing about 100 ml of water. Collect the glass from the ampoule, wash carefully, dry, and weigh. Calculate the amount

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of osmium tetroxide used for preparation of the solution from the mass difference. Dilute the solution with water to obtain a concentration exactly 1 mg Os/ml. Working solutions are prepared by suitable dilution of the stock solution with water.

Ruthenium standard solution, 1 mg Ru/ml. Take 100 mg of ruthenium powder and fuse it with 2 g of sodium peroxide in a silver crucible. Dissolve the melt in water and acidify the solution with hydrochloric acid (1+3). Filter off the AgCl precipitate. Dilute the solution to 100 ml with hydrochloric acid to obtain 1 *M* HCl solution.

Capri blue (CB), 1 × 10^{-3} M solution (ca. 0.035%). A preparation of Capri blue (supplied by Gurr, England) was purified by crystallization from methanol. In order to check the effectiveness of the purification procedure, the following experiment was carried out: The commercial preparation was dissolved in water and Capri blue perchlorate was precipitated. After careful washing with dilute perchloric acid the precipitate was dried at 100°C. A known amount of the compound was then dissolved in methanol. The molar absorptivity of the solution was similar to that obtained for the solution of a preparation purified by crystallization from methanol or ethanol.

Ammonium thiocyanate, 5% solution.

A Spekol spectrophotometer and Specord UV–VIS spectrophotometer, 1-cm cells.

A pH meter Elpo N-512.

Procedure

Add 5 ml of ammonium thiocyanate solution to the sample solution containing not more than 16 μg of osmium (or 10 μg of ruthenium) and adjust the pH of the solution to 2–3 by means of diluted sulfuric acid and ammonia. Fill up the solution with water to 15 ml and heat on a boiling water bath for 15 min. Allow the solution to cool to room temperature and transfer it to a 100-ml separatory funnel. Add 1 ml of the Capri blue solution to 5 ml of diisopropyl ether and shake for 30 sec. Remove the aqueous phase and wash the ether phase by shaking with three portions of water (shaking time about 15 sec). Carefully discard the diisopropyl ether and dissolve the separated ion associate in methanol. Transfer the solution to a 25-ml volumetric flask and dilute to the mark with methanol. Measure the absorbance of the solution at 630 nm against a reagent blank prepared in the same way.

RESULTS AND DISCUSSION

Preliminary Studies

Studies were carried out in order to find a basic dye able to form an extractable or floatable ion associate with thiocyanate–osmium complex at suitable acidity of the aqueous phase. The following basic dyes were examined: crystal violet, malachite green, brilliant green, methyl green, Victoria blue B and Victoria blue 4R (triarylmethane dyes), rhodamine B and rhodamine 6G (xanthene dyes), Nile blue A, Meldola blue and Capri blue (oxazine dyes), methylene blue (thiazine dye) and safranine T (phenazine dye). The experiments were carried out in an acidity range from pH ca. 2 to 3 *M* sulfuric acid, at a dye concentration exceeding 20–30 times the concentration of osmium. The amount of osmium was 12 μg. The aqueous solutions were shaken with benzene, toluene, carbon tetrachloride, chloroform, diisopropyl ether, methylisobutyl ketone, and isoamyl alcohol.

Ion associates formed by thiocyanate ion with basic dyes are extracted with carbon tetrachloride, chloroform, methylisobutyl ketone, and isoamyl alcohol.

In the case of benzene, toluene, and diisopropyl ether, flotation of associates of thiocyanate–osmium complex takes place. At the same time ion associates of thiocyanate ion with basic dyes precipitate. In some cases the ion associate of thiocyanate–osmium complex with the dye can easily be separated from the associate of thiocyanate complex with this dye by washing with water; blank value is then insignificant.

The systems methylene blue–toluene and Capri blue–diisopropyl ether permit very sensitive flotation–spectrophotometric methods for the determination of osmium. In the case of rhodamine B and diisopropyl ether the method is highly sensitive but not precise. The method based on methylene blue has been reported previously (*12*). The present paper deals with the determination of osmium (ruthenium) with Capri blue.

Conditions of the Formation and Flotation of the Ion Associate in the System Os–SCN–CB

Thiocyanate–osmium complex forms with Capri blue ion associate which precipitates at the phase boundary during shaking with diisopropyl ether. The ion associate, separated in this way, is dissolved in methanol and the absorbance of the solution is measured. It has been found that the absorbance depends on the concentration of thiocyanate ions and acidity as well as on the time and temperature of heating of the osmium solution with thiocyanate. The anionic osmium complex that leads to maximum and constant final values is obtained when the solution (after adding thiocyanate) is heated for at least 10 min at thiocyanate concentration higher than 0.15 *M* and pH 0.5–3.5 (Fig. 1). For complete separation of osmium in the form of sparingly soluble ion associate, at least a 15-fold molar excess of Capri blue is necessary. Further increase in the dye concentration has no effect on the final results. However, the blank value increases (Fig. 2). Benzene, toluene, and diisopropyl ether were used as

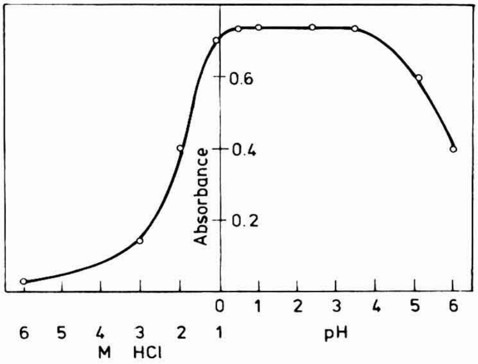


FIG. 1. Effect of the acidity on the formation and flotation of the ion associate Os–SCN–CB (absorbance of the methanol solution of the separated and washed ion associate).

floating solvents. Application of benzene and toluene leads to similar final results. In the case of diisopropyl ether absorbances are higher.

The change of the phase ratio by a factor of two has no effect on flotation separation of the ion associate. In order to achieve complete separation of osmium from the aqueous phase it is necessary to shake the phases for 30 sec.

The associate of thiocyanate ion with Capri blue separates together with the associate of thiocyanate–osmium complex. The former associate decomposes during washing with water and the dye passes to the aqueous phase. When shaking the precipitate and the isopropyl ether with three portions of water the excess of the dye is nearly completely removed; the absorbance of the blank does not exceed 0.07.

For dissolution of the separated and washed ion associate, ethanol,

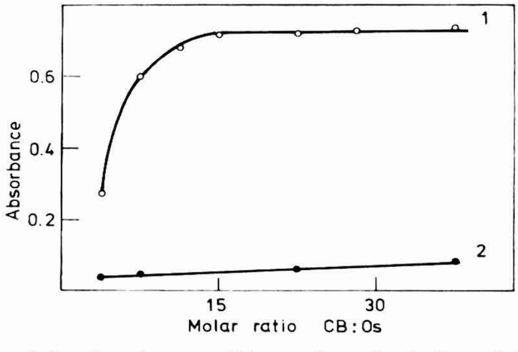


FIG. 2. Dependence of the absorbance of the methanol solution of the ion associate on the excess of Capri blue (flotation with diisopropyl ether). (1) Absorbance of the solutions measured against the blank. (2) Absorbance of the blank measured against water.

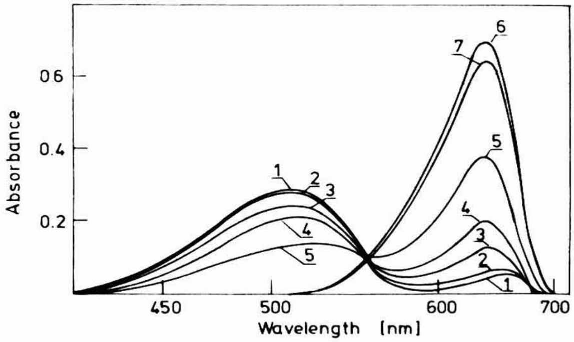


FIG. 3. Absorption spectra of Capri blue from the ion associate dissolved in dimethylformamide–water mixtures of volume ratio DMF:H_2O: (1) anhydrous DMF, (2) 100:1, (3) 50:1, (4) 10:1, (5) 5:1, (6) 1:1, (7) 1:4.

methanol, acetone, and dimethylformamide (DMF) were used. The precipitate is insoluble in ethanol, and partly soluble in acetone. After a short shaking it dissolves completely in methanol. It is readily soluble in dimethylformamide. However solutions in dimethylformamide have various colors, from pink through violet to blue. It has been found that the color of the solution depends on the amount of water in dimethylformamide. A blue solution of maximum absorbance is obtained for DMF–water mixtures containing 30–70% water (Fig. 3). It should be noticed that in the dimethylformamide–water mixtures dissolution of the separated ion associate proceeds more slowly than in anhydrous dimethylformamide. As can be seen in Fig. 4 the solutions of Capri blue in various solvents exhibit differences in the position of λ_{max}. The molar absorptivities of the solutions differ as well.

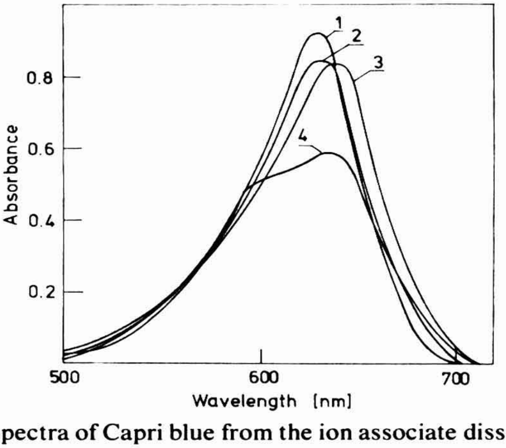


FIG. 4. Absorption spectra of Capri blue from the ion associate dissolved in: (1) methanol, (2) acetone; (3) DMF–water mixture (1 + 1), (4) spectrum of Capri blue in water (concentration of CB is the same in all cases).

Spectrophotometric Determination of Osmium (Ruthenium)

The above discussed experiments permitted preparation of a stand curve for the determination of osmium using thiocyanate and Capri b Beer's law is obeyed up to a concentration of 0.6 μg Os/ml. Molar sorptivity (ε) is 2.7 × 10^5 liters mol^{-1} cm^{-1} (specific absorptivity ε/(at.mass × 1000) = 1.42) at 630 nm.

Precision and accuracy of the method were verified by analyzing tions containing known amounts of osmium (Table 1).

The developed method is not selective. Under the described con other platinum metals form ion associates with thiocyanate and Ca as well. Gold, silver, copper, mercury, and tungsten belong to int metals, too; in conditions described under Procedure, they form able ion associates. Hence it is of great advantage to combine an separation method with the proposed, very sensitive method fo nation of osmium. The procedure becomes highly selective if th nation of osmium is carried out after its separation by distilla os-mium tetroxide using hydrogen peroxide or nitric acid as oxidi its When stronger oxidizing agents are used (e.g., perchloric ac ganate) ruthenium quantitatively distills as RuO_4 together with

It follows from the experiments carried out that ruther compound with thiocyanate and Capri blue similar to that o can be determined by the flotation–spectrophotometric conditions similar to those used for osmium. The result associate evaluation of the precision of the ruthenium determinatio aqueous proposed system are shown in Table 1.

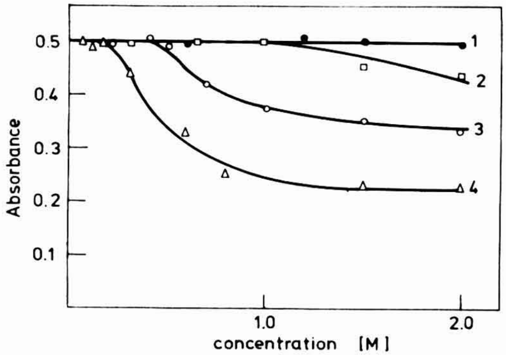
TABLE 1

RESULTS OF THE DETERMINATION OF OSMIUM AND RUTHENIUM W H THIO YANATE AND CAPRI BLUE

Metal				
Added (μg)	Found (μg)	Standard deviation (μg)	Relative standard deviation (%)	fidence limits
		Osmium		
4.00	3.97	0.19	4.7	3.97 ± 0.19
8.00	7.98	0.15	1.9	7.98 ± 0.16
12.00	11.98	0.12	1.0	11.98 ± 0.13
		Ruthenium		
2.00	1.96	0.13	6.5	1.96 ± 0.11
4.00	3.94	0.17	4.2	3.94 ± 0.15
8.00	8.04	0.17	2.1	8.04 ± 0.15

Note. *n* = 7.

[a] Probability level 0.95.



[illegible] Effect of various ions on the formation and flotation of the ion associate [illegible] CB (diisopropyl ether): (1) chloride, (2) nitrate, (3) perchlorate, (4) sulfate.

[illegible] does not interfere with the determination of osmium by the [illegible] ethod. Nitrate and perchlorate interfere above concentrations of [illegible] M, respectively. Sulfate interferes readily at concentrations [illegible] 0.2 M (Fig. 5).

C[illegible] were made to apply the elaborated method to the determination the[illegible]m and ruthenium in solutions containing both elements. The blu[illegible] converted to thiocyanate complexes. The thiocyanate–os- ture [illegible] was then extracted with diethyl ether containing peroxides dimeth[illegible] remained in the aqueous phase. Each of the metals was sociate pr[illegible]d by means of the Capri blue method after previous evap- can be se[illegible]yl ether and adjustment of pH and thiocyanate concentration to su[illegible] values. The results are shown in Table 2.

Composition of the Ion Associates

The molar ratio of osmium to Capri blue in the separated ion associate amounts to 1:5 as determined by the isomolar series method (Fig. 6). This result was confirmed by absorbance measurements of methanolic solu-

TABLE 2
RESULTS OF THE DETERMINATION OF OSMIUM AND RUTHENIUM AFTER EXTRACTIVE SEPARATION

Added		Found			
Osmium	Ruthenium	Osmium		Ruthenium	
20.0	2.0	18.2	18.0	2.0	2.2
5.0	10.0	5.3	5.4	8.8	9.2
10.0	5.0	8.6	9.3	5.2	5.0
10.0	10.0	9.2	10.1	8.8	9.3

Note. Data in micrograms.

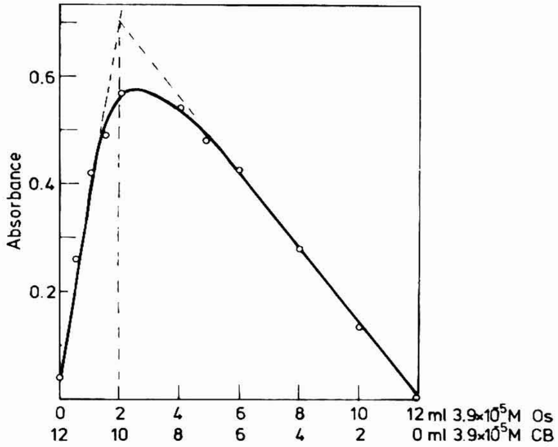


FIG. 6. Determination of the ratio of osmium to Capri blue in the ion associate by the isomolar series method (flotation with diisopropyl ether).

tions of the ion associate with a known amount of osmium and methanolic solutions of the dye, taken at a fivefold molar excess with respect to osmium in the first solutions. The absorbances obtained in both cases were practically identical.

In the case of flotation with toluene the obtained lower absorbances are due to the flotation of an ion associate of a different molar ratio of osmium to Capri blue. This ratio is 1:3 (Fig. 7).

The phenomenon of flotation of various ion associates when using various solvents was also observed in the case of ruthenium. As with osmium, flotation with toluene leads to the ion associate of a molar ratio of ruthenium to Capri blue equal to 1:3; diisopropyl ether floats the compound having a molar ratio of Ru:CB = 1:5.

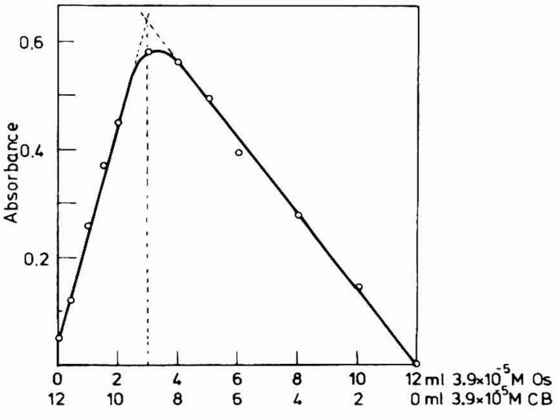


FIG. 7. Determination of the ratio of osmium to Capri blue in the ion associate by the isomolar series method (flotation with toluene).

The molar ratio of osmium to thiocyanate ions in the associate separated by means of diisopropyl ether was evaluated on the basis of the determination of the sulfur content in this compound. Removal of the free thiocyanate from the precipitate by washing was difficult and was achieved by shaking the precipitate with water and diisopropyl ether many times. The precipitate of the ion associate, after thorough washing, was oxidized by means of a mixture of concentrated hydrochloric acid and concentrated nitric acid (1 + 1). Thiocyanate sulfur was thereby oxidized to sulfate which was determined spectrophotometrically by the methylene blue method (*4*). The ratio of SCN^- to Os, based on seven determinations, amounts to 7.95 (SD = 0.40).

The ion associate that makes a basis for the proposed spectrophotometric method for the determination of osmium can be described by the formula: $[CB_3^+Os(SCN)_6^{3-}]\cdot 2[CB^+\cdot SCN^-]$. It can be supposed that the ion associate floated with toluene has the composition $[CB_3^+Os(SCN)_6^{3-}]$.

SUMMARY

A sensitive flotation–spectrophotometric method for the determination of osmium, based on the ion associate formed by the anionic thiocyanate osmium complex with oxazine basic dye, Capri blue (CB), has been developed. The ion associate is separated by shaking the aqueous solution (pH 2–3) with diisopropyl ether, washing the precipitate with water, and dissolving it in methanol. Molar absorptivity in this method amounts to 2.7×10^5 liters $mol^{-1}\ cm^{-1}$ at 630 nm. The molar ratio Os:SCN:CB in the separated associate is 1:8:5. Under the conditions of the determination of osmium, ruthenium can be determined as well. Metals that form anionic thiocyanate complexes, including other platinum metals, interfere. The method becomes highly selective for osmium and ruthenium after their separation by distillation as tetroxides. Osmium and ruthenium were determined with Capri blue after their extractive separation as thiocyanate complexes.

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Girard-P Derivative of 2-Pyridinecarboxaldehyde as Analytical Reagent. Spectrophotometric Determination of Copper

M. CALLEJÓN MOCHÓN AND J. L. GOMEZ ARIZA[1]

Department of Analytical Chemistry, Faculty of Chemistry and Pharmacy, University of Seville, Seville, Spain

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INTRODUCTION

Since 1936, when A. Girard and G. Sandulesco (*3*) synthesized three acethydrazides of quaternary ammonium salts, so called Girard's reagents T, P, and D, numerous applications of these reagents have been reported, especially for the recovery of substances which contain carbonyl groupings by the formation of the corresponding hydrazones. There are two reviews relating to this topic (*15, 16*), and Girard's reagents are already general ones for the isolation of steroids, hormones, and other substances of biological significance. Girard-T hydrazones undergo polarographic reduction (*4*) and have been used for polarographic identification of ketosteroids in samples of urine or blood, and subsequently have been proposed for the polarographic detection of aliphatic ketones, providing better results than analogous semicarbazonic derivatives (*2*). However, their use as spectrophotometric reagents is scant and almost reduced to Girard-T derivatives of 2-hydroxy-1-naphthaldehyde (*11, 12*) and resacetophenone (*13*); there are also some studies of Girard-T and -D complexes with copper(II), cobalt(II), nickel(II), and manganese(II) (*8, 9*), as well as of Girard-T hydrazones of isatin and methylisatin with metal ions (*14*), and Girard-T and -D hydrazones of aliphatic and aromatic aldehydes (*5*) in the same way. But Girard-P reagent or its derivatives have not been studied analytically, especially pyridine derivatives, which can be related to analogous ones of semicarbazide previously reported (*7*); therefore the study of pyridine derivatives of Girard-P reagent from an analytical approach is interesting and the present paper is the first of a series that will be devoted to this subject.

Pyridinium acethydrazone of picolinaldehyde chloride (PAHP), **I**, undergoes sensitive reactions with metal ions of the transition series, but

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[1] To whom all correspondence should be addressed.

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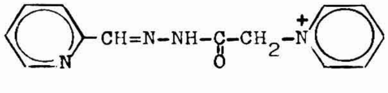
absorption maxima are placed in the ultraviolet region; ascorbic acid shifts the absorption band of the spectrum of the copper complex to the visible region and so it is possible to determine copper under high selectivity conditions.



EXPERIMENTAL

Apparatus

Unicam SP800, Unicam SP600, and Beckman DU spectrophotometers equipped with 1.0-cm glass or quartz cells, a Perkin-Elmer 621 infrared spectrophotometer, and a Phillips PW9408 digital pH meter with glass-calomel electrodes were used.

Reagents

Salts and solvents of analytical reagent grade purity or better were used throughout. All metal ion solutions were standardized.

Buffer solution at pH 6.4 contains 50.0 ml of 0.1 *M* KH_2PO_4 and 11.5 ml of 0.1 *M* NaOH, diluted to 100 ml; at pH 3.5 it contains 1.0 g of kalium hydrogenphtalate and 8.0 ml of 0.1 *M* HCl, diluted to 100 ml.

Synthesis of the Reagent

Girard-P reagent (9.4 g) and picolinaldehyde were dissolved in 100 ml of hot ethanol. Glacial acetic acid (5–10 ml) was added to the mixture and then it was refluxed for 2 hr and allowed to cool to room temperature. The pale-yellow crystalline product was filtered off and recrystallized from ethanol. The synthesis yield was about 70% (m.p. 242–244°C; found C 56.5%, H 4.4%, N 20.5%; calculated for $C_{13}H_{13}N_4OCl$, C 56.2%, H 4.6%, N 20.1%).

Properties of the Reagent

PAHP, as other derivatives of Girard-P reagent, exhibited a higher solubility in water (360 g/liter) and other hydroxylic solvents: methanol (143 g/liter) and ethanol (8 g/liter), and their aqueous mixtures. Contrary to this, few polar solvents (chloroform, benzene, nitrobenzene) showed solubilities below 1 g/liter.

The infrared spectrum of the reagent (KBr discs) was recorded. The spectrum exhibited numerous bands caused by the aromatic portion of the molecule and also those typical of Girard's reagents: amide I (1690 cm^{-1}), amide II (1570 cm^{-1}), $\nu^{>C=N}$ (1640 cm^{-1}), ν^{-NH-} (3050 cm^{-1}).

The ultraviolet spectrum of an aqueous solution of the reagent, in neutral medium, showed an absorbance maximum at 289 nm (ϵ = 18,600 liters mol^{-1} cm^{-1}), which was in agreement with the literature (*8*). The spectra of ethanol and methanol solutions were identical to the aqueous ones, but apolar solvents (chloroform, benzene, etc.) underwent a light bathochromic shift.

Stability of the Reagent

The stability of an aqueous solution (in neutral medium) of PAHP was investigated at 25°C for 24 hr. It was verified that this stability was quite satisfactory for this time period. Acid (pH 2.1) and alkaline (pH 9.9) media, specially acid media, decrease the absorbance of the solutions remarkably in a 4-hr period. Also, the absorbance is affected by oxidizing agents but not by reducing ones.

Reaction with Metal Ions

The samples were prepared in a 25-ml volumetric flask, with 2 to 5 ppm of metallic ion, 5 ml of 0.5% PAHP aqueous solution, 5 ml of buffer solution, and 5 ml of 0.5 *M* KCl to fix the ionic strength at 0.1, and then diluted to the mark with distilled water. The spectrum from 250 to 750 nm was recorded against a reagent blank. Table 1 shows the results obtained to pH 6.4. Generally, the solutions of the chelates are colorless and the absorption spectra show a band between 315 and 375 nm. There are two exceptions: the chelates of copper(II) and iron(II), whose spectra exhibit another maximum at 700 and 600 nm respectively, although their sensibility is low. The absorption maxima of the spectrum of Cu(II)–PAHP solutions, in the presence of ascorbic acid, rise to 460 and 350 nm. This reaction is particularly interesting, because of its sensibility is good and the

TABLE 1

PHOTOMETRIC CHARACTERISTICS OF THE COMPLEXES IN SOLUTION

Cation	λ_{max}(nm)	ϵ_{max}(liters mol^{-1} cm^{-1})	pH
Cu(II)	335	30,480	6.4
	460[a]	4,200	3.5
Ni(II)	340	26,500	6.4
Zn(II)	331	29,100	6.4
Co(II)	326	22,700	6.4
Cd(II)	328–332	16,800	6.4
Pd(II)	323	26,100	6.4
Hg(II)	325–330	10,500	6.4
Hg(I)	323	48,000	6.4
Fe(III)	322	13,400	6.4
Fe(II)	322	10,100	6.4

[a] In the presence of ascorbic acid.

absorption maximum ($\lambda_{max} = 460$ nm) is shifted bathochromically in relation to the maxima of the other complexes.

Procedures

Recommended procedure for the determination of copper. Place the copper solution (about 250 μg) in a 25-ml volumetric flask. Add 10 ml of 1.0% reagent solution in water, 5 ml of 1.0% ascorbic acid solution in water, and 5 ml of buffer solution (pH 3.5), and dilute to the mark with distilled water. Allow this to stand for 15 min and measure the absorbance at 460 nm against a reagent blank.

Determination of copper in "white metal E-8". Weigh accurately a suitable amount (about 0.15 g) of the white metal, and place it in a flask fitted with a reflux condenser. Add 10 ml of 1:3 concentrated nitric acid–concentrated hydrochloric acid, reflux for 2 hr, and then concentrate almost to dryness. Transfer the residue completely in a 100-ml volumetric flask and dilute with distilled water to the mark. Transfer between 3.0 and 3.5 ml of the solution into a 25-ml calibrated flask and determine the copper as described under Recommended Procedure.

RESULTS

Spectral Characteristics

Typical spectral curves corresponding to the appearance of individual hydrogen complexes of the ligand are represented in Fig. 1. Similar curves for the copper complex in the presence and absence of ascorbic acid show absorption maxima at 460 and 335 nm, respectively.

Influence of Acidity and Adherence to Beer's Law

The absorbance of the Cu(II)–PAHP complex is constant in the pH

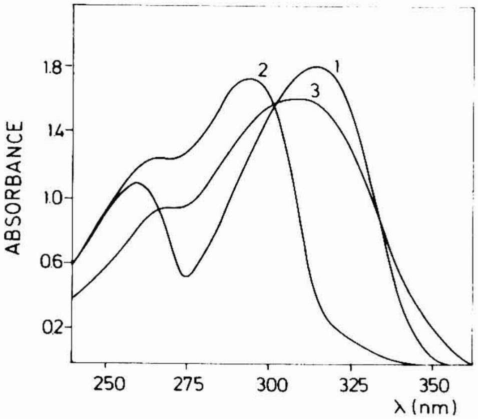


FIG. 1. Absorbance spectra of PAHP at different pH: (1) pH 1.7, (2) pH 6.9, (3) pH 10.7.

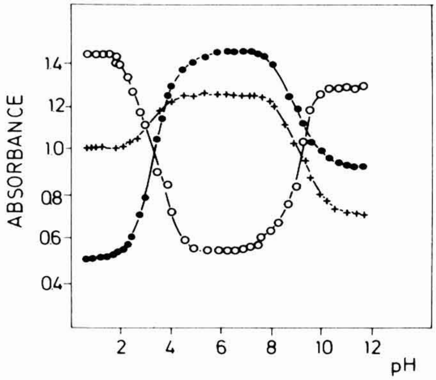


FIG. 2. Effect of the pH on the absorbance of PAHP. ●, 285 nm; ○, 310 nm; ×, 260 nm. $C_L = 8.0 \times 10^{-5}$ M.

interval 5.8–7.2 (for λ = 335 nm). A pH of 6.4 was selected for determination (using KH_2PO_4/NaOH buffer). In the presence of ascorbic acid the optimum pH interval is located between 3.1 and 4.3 (λ = 460 nm) and the determinations were made with pH 3.4 phthalate/HCl buffer.

The adherence to Beer's law was verified between 0.5 and 2 ppm in the absence of ascorbic acid and between 2 and 10 ppm in the presence of this compound.

Effect of Time on the Complexes

The stability of the aqueous solutions of the complexes prepared in the optimum pH interval was verified for 24 hr. Cu(II)–PAHP complex rapidly reaches a constant value of absorbance. In the presence of ascorbic acid the absorbance increases initially for 15 min but remains constant after this time interval.

Effect of Foreign Ions on the Determination of Copper

The effect of the most important cations and anions was investigated. The limiting value of the concentration of a foreign ion was taken as that value which caused an error of less than ± 5% in the determination of 1 ppm of copper, or 10 ppm if ascorbic acid is present. The results are summarized in Table 2. It is remarkable that ascorbic acid eliminates important interferences: Hg(I) and (II), Fe(III), Cd(II), Zn(II), and Co(II), particularly. Therefore the determination of copper should be preferably performed in this medium.

Precision Datum

This was obtained by multiple analyses of series of solutions containing 10 ppm of copper (method with ascorbic acid). The precision of the absor-

TABLE 2

DETERMINATION OF COPPER IN THE PRESENCE OF FOREIGN IONS

Foreign ion	Tolerance limit/ppm[a] a	Tolerance limit/ppm[a] b	Foreign ion	Tolerance limit/ppm[a] a	Tolerance limit/ppm[a] b
Pb(II)	50	100	Cd(II)	1	100
Ag(I)	100	100	Zn(II)	2	100
Hg(I) and (II)	1	100	Co(II)	1	100
Au(III)	2	100	Ni	1	100
Pd(II)	1	—	Mn(II)	100	100
Bi(III)	4	100	Sn(II)	30	100
Fe(III)	1	100	Sb(III)	—	100
Al	100	100	Th	100	100
Cr(III)	1	100	Ce(IV)	100	100
Pt(IV)	10	50	La	100	100
W(VI)	100	50	Alkalines	100	100
Mo(VI)	100	20	Alkaline-earths	100	100
V(V)	2	100			
I^-	100	2	U(VI)	100	100
SCN^-	100	2	PO_4^{3-}	100	100
$S_2O_3^{2-}$	100	100	F^-	100	100
Citrate	100	100	Tartrate	100	100

[a] a, without ascorbic acid; b, with ascorbic acid.

bance measurements for copper is 1.1% (relative standard deviation), corresponding to 0.11 ppm of copper.

Sensitivity of Determination

The sensitivity is expressed as effective molar absorptivity for copper corresponding to the linear part of the dependence between absorbance and the total concentration of metal. The value of ϵ_{460} is 4200 (liters mol^{-1} cm^{-1}).

Determination of Copper in the "White Metal E-8"

The proposed method has been satisfactorily applied to the determination of copper in the "white metal E-8" (Cu, 4.57%; Pb, 3.13%; Sb, 9.5%; Zn, 0.04%; Cd, 0.14%; Sn, 82.62%). Six determinations were carried out and the relative standard deviation was 1.8%.

Composition and Stability of Complexes

The stability constants of hydrogen complexes of PAHP were determined by the usual spectrophotometric methods (*10*, *16*). Because of donor-electron properties of pyridinic nitrogen atoms, the basic character of the compound is explained; likewise, the scheme proposed for the

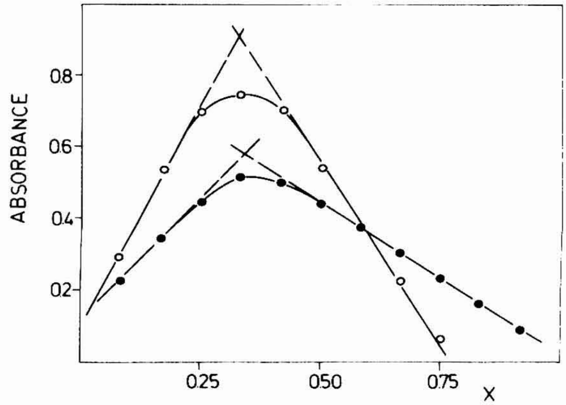


FIG. 3. Continuous variation in equimolar solutions. $X = C_M/(C_M + C_L)$, ●, without ascorbic acid, λ = 350 nm, $C_M + C_L = 9.12 \times 10^{-6}\ M$, pH 6.4; ○, with ascorbic acid, λ = 460 nm, $C_M + C_L = 1.73 \times 10^{-3}\ M$, pH 3.7.

dissociation of Girard's reagents and their hydrazones (*8*) supports the acid dissociation of the molecule of PAHP. The absorbance–pH plot was constructed for wavelengths 285 and 310 nm (Fig. 2). At extreme pH values (alkaline or acid medium) the sole presence of L^- or fully protonated complex H_2L^+ may be assumed, and was confirmed in Fig. 2 by the constant value of the absorbance. But also, the appreciable separation between the two pK values undergoes a third zone of constant absorbance (pH 6 to 7.5); the partial protonated complex HL exists in this pH zone as a sole species. If A_0, A_1, and A_2 are the constant absorbances corresponding to pH 11, 6–7.5, and 1.5, and the species of L^-, HL, and H_2L^+, respectively, the molar absorptivities ϵ_0, ϵ_1, and ϵ_2 are: $\epsilon_0 = A_0/C_L$, $\epsilon_1 = A_1/C_L$, $\epsilon_2 = A_2/C_L$, where C_L is the analytical concentration of the reagent. The value found of pK_1, PK_2, ϵ_0, ϵ_1, and ϵ_2 are given in Table 3.

The ratio of metal to ligand in the Cu(II)–PAHP complex was determined by the method of continuous variations in equimolar solutions.

TABLE 3

STABILITY CONSTANTS, MOLAR ABSORPTIVITIES, AND ISOSBESTIC POINTS OF HYDROGEN COMPLEXES

Complex	L^-		HL		H_2L^+
log K_n[a]			9.2		3.3
λ_{max}(nm)	308		296		313
$\epsilon_{\lambda_{max}} \cdot 10^{-4}$	3.2		3.4		3.6
$\lambda_{isos.}$(nm)		302		302	

[a] $K_n = [H_nL]\,[H_{n-1}L]^{-1}\,[H]^{-1}$.

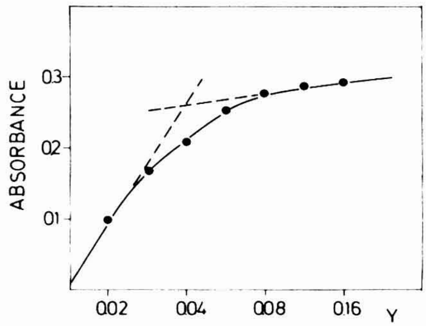


FIG. 4. Change in absorption characteristics of binary complex of PAHP with copper ion with variable amounts of ascorbic acid, molar ratio plot. $Y = C_M/C_{\text{ascorbic}}$, $C_M + C_L = 1.73 \times 10^{-3}$ M, $\lambda = 460$ nm, pH 3.7.

The curve is given in Fig. 3 and reveals that a 1:2 (Cu(II)–PAHP) complex is formed at 6.4 pH value, $\lambda = 350$ nm.

The nature of the Cu(II)–PAHP–ascorbic acid complex was considered, and three points can be made in relation to this topic: (i) solutions of the complex precipitate (white precipitate) in the presence of iodide ion, which is probably due to the formation of CuI, and suggests that ascorbic acid reduces to the copper(II) ion at the lower valence state; (ii) other reducing agents such as sodium sulfite, hydroxylammonium chloride, and hydrazinium sulfate, which also reduce Cu(II) to Cu(I), do not exert an influence analogous to that of ascorbic acid on the spectral characteristics of the Cu(II)–PAHP complex. Thus, ascorbic acid not only operates as a reducing agent but also participates in the complexation reaction; (iii) the ratio metal–ligand of the copper–PAHP complex in the presence of an excess of ascorbic acid (continuous variations method) is the same without this agent (Fig. 3); an attempt was made to determine the number of ascorbic acid molecules present in one molecule of complex; therefore the absorbance was measured at 460 nm for mixtures containing Cu(II) and PAHP in the proportions 1:2 and different amounts of ascorbic acid (Fig. 4). It was found that a 24-fold molar excess of ascorbic acid is required for maximum color formation.

The effective stability constant of the complexes was determined (*1*) and the values obtained are: $K_{12} = 1.4 \times 10^{13}$ (pH 6.4) and $K_{12(\text{ascorbic})} = 9.0 \times 10^{7}$ (pH 3.5).

SUMMARY

The synthesis, characteristics, properties, and reactions with metallic ions of pycolinaldehyde derivatives of Girard-P reagent have been studied. The reagents form complexes with Cu(II), Ni(II), Co(II), Hg(II) and (I), Zn(II), Cd(II), and Fe(III), and (II), which exhibit a good sensibility but whose spectra have the absorption maxima located in the ultraviolet

zone. The spectrum of copper complex in the presence of ascorbic acid shows another absorption band at the visible region and this makes the spectrophotometric determination of copper very selective. The influence of the different experimental parameters on the formation of copper complex without ascorbic acid and with this compound was studied, and the optimum conditions for the determination of copper were established. The precision of the procedure, expressed in terms of relative standard deviation, was 1.1%. The method has been tested on "white metal E-8" samples.

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MICROCHEMICAL JOURNAL 26, 472–476 (1981)

Sensitive and Selective Ion Exchange Test for Microgram Detection and Spectrophotometric Determination of Picric Acid

SAIDUL ZAFAR QURESHI,* IZZATULLAH, AND
REETA BANSAL

Chemistry Department, Aligarh Muslim University, Aligarh-202001, India

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INTRODUCTION

A spectrophotometric method for the determination of small amounts of picric acid using methyl blue has been reported (*3*). Propericiazine (II) has been used as a reagent for gravimetric and colorimetric determination of picric acid on a microgram scale (*11*). A number of general tests for the determination of nitrophenols have been described (*4, 6*) where these methods were found inefficient for the determination of picric acid in presence of other nitro compounds. Another spectrophotometric method for the determination of picric acid, which was described by Rawat *et al.* (*10*), uses zinc and ammonium chloride as reducing agents to produce a blue color. A review of the literature reveals that a yellow product known as bromopicrin is obtained by the reaction of hypobromite and picric acid (*1*). The kinetics and mechanism of this reaction was studied by Hunter and co-workers (*5*). As far as we are aware, no studies on the reaction between picric acid and sodium hypochlorite have been made. Therefore, this reaction which produces an orange color can be employed as a specific test for the detection of picric acid by means of the resin spot technique (*7, 8, 9*). The use of this reaction has also been applied for spectrophotometric determination.

EXPERIMENTAL

Apparatus. Absorbance measurements were made with a Bausch & Lomb Spectronic-20 spectrophotometer.

Reagent. A 30% sodium hydroxide solution was prepared in demineralized water and chlorine gas was continuously bubbled through this solution for an hour.

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* Present address: Chemistry Department, Faculty of Science, University of Sulaimania, Sulaimania, Iraq.

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Solution. Picric acid (AnalaR) solution 0.1 *M* was prepared in 98% methanol as stock solution. More dilute solutions were prepared from the stock solution.

Resin. Amberlite IR-400 (20–50 mesh) was used after the usual regeneration and conversion into OH^- form.

Procedure for the detection of picric acid. Place about 4–6 ion-exchange resin beads in OH^- form on a white spot plate and dry them by blotting with filter paper. Add a drop of test solution, followed by a drop of reagent solution. If the resin turns orange this indicates a positive test.

Procedure for the determination of picric acid. In a 10-ml standard volumetric flask add 0.5 ml sodium hypochlorite to an aliquot volume of picric acid solution containing 30 μg–0.25 mg. Make the solution up to the mark with methanol. Then measure the absorbance of the orange color solution at 480 nm against a blank solution at room temperature (25 ± 1°C).

RESULTS

A number of nitrophenols were tested and only picric acid gave the characteristic orange color, both in solution and in the resin phase. The detection limits were found to be 1.15 and 0.70 μg in solution phase and in the resin phase, respectively.

The ion exchange test was performed in the presence of a large amount of foreign substances; no interference was given by hydrocarbons and their derivatives, alcohols, ethers, carboxylic acids, carbohydrates, phenols, heterocyclic bases, aldehydes, ketones, amides, nitriles, and amines.

Determination of Picric Acid

Absorption spectrum. The absorption spectrum of a solution containing 140 μg of picric acid treated as described under procedure was taken against deionized water. The maximum absorption occurred at 480 nm.

Optimum condition. The optimum condition for the formation of the orange color was determined and maintained throughout the studies.

There was no effect of time on the stability of orange color for 1 hr. However, a slight decrease in absorbance was recorded afterward. Therefore, it was recommended that the absorbance should be measured within this period.

The effect of reagent concentration was studied by adding different volumes of sodium hypochlorite solution to a constant amount (140 μg) of picric acid. It was found that a maximum absorbance of the orange color occurred with 0.5 ml of the reagent, beyond which absorbance was constant (Fig. 1).

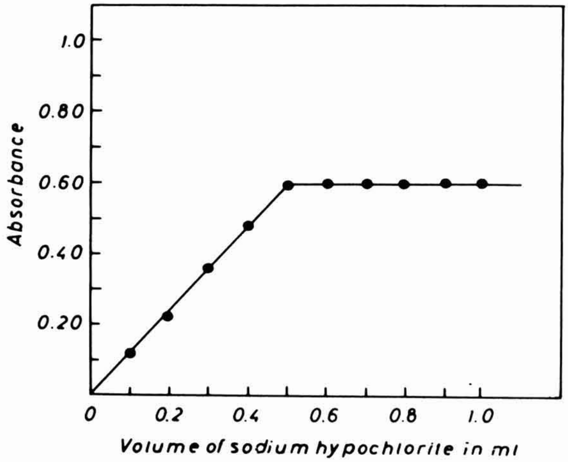


FIG. 1. Effect of sodium hypochlorite concentration.

Calibration graph. The sodium hypochlorite–picric acid system conforms to Beer's law over the range between 30 and 250 μg. Results are shown in Fig. 2.

Study of precision. The reproducibility of results was checked by ten replicate determinations, each containing 100 μg of picric acid. The accuracy of method was also checked by five unknown samples and the error was found to be ±2.5% with a standard deviation of 1.85 μg.

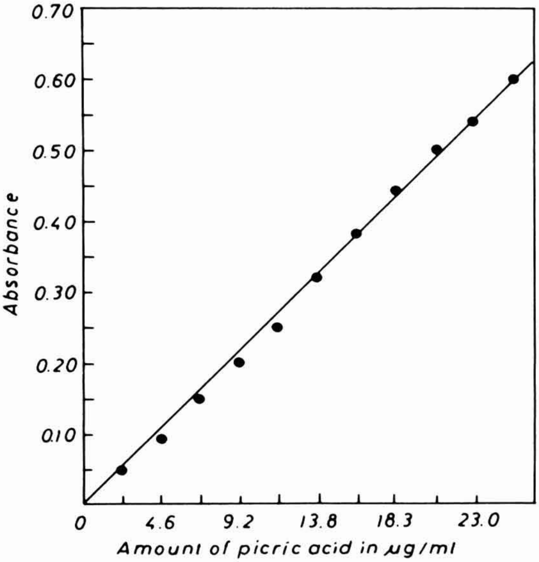


FIG. 2. Calibration curve for picric acid.

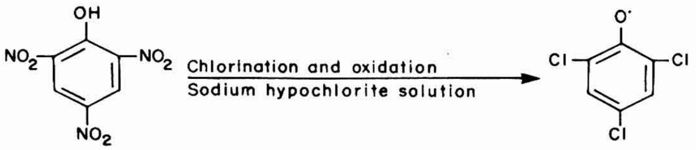
Study of charge on the complex. The charge on the complex was determined by adding two types of resin: (a) Amberlite IR-400, an anion-exchange resin and (b) Amberlite IR-120, a cation-exchange resin. The anion-exchange resin turned orange, as they are exchanged by the negatively charged complex.

Effect of foreign substances in picric acid determination. In order to study the applicability of the method in the presence of other organic compounds, the determination of picric acid was made in the presence of these compounds (amount given in parentheses): Sulfanilic acid (8.57 mg), sulfamic acid (6.50 mg), thiourea (3.80 mg), *p*-nitroaniline (6.90 mg), acetanilide (6.75 mg), benzylmethyl ketone (5.36), cyclopentanone (3.36 mg), histidine (7.75 mg), phenylalanine (8.46 mg), ethylenediamine (3.00 mg), aniline (9.32 mg), diphenylamine (5.08 mg), methylamine (4.65 mg), benzonitrile (3.10 mg), acetaldehyde (6.75 mg), phloroglucinol (4.55 mg), thymol (4.50 mg), 4-acetaminophenol (4.55 mg), chlorobenzene (5.63 mg), toluene (4.60 mg), pyrogallol (2.52 mg), resorcinol (4.40 mg), hydroquinone (5.50 mg), β-naphthol (2.88 mg).

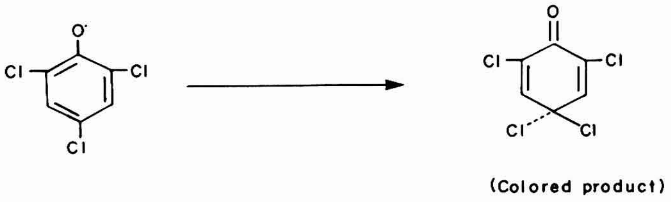
Other organic compounds do not interfere with the determination of picric acid.

DISCUSSION

The phenolic group of picric acid is easily attacked by oxidizing agents, resulting in the formation of phenoxy radicals. Usually such radicals are very unstable and reactive and therefore undergo rapid transformation into other products. The reaction is based on the double action of sodium hypochlorite: (i) oxidation in alkaline solution and (ii) chlorination, which is responsible for the colored product. Alkaline sodium hypochlorite solution contains an excess amount of chlorine which can convert nitrophenol with or without solvent into the 2,4,6-trichloro-derivatives (*2*), which undergo oxidation and chlorination as follows:



The final colored product may be due to the absorption of one more chlorine atom to form trichlorophenol chloride.



A look at the result shows that color produced by picric acid, both in solution and in the resin phase, can be used as a specific test for picric acid. The sensitivity of the picric acid can be enhanced by performing the test on the resin phase.

SUMMARY

The microgram detection and determination of picric acid with sodium hypochlorite solution is based on its oxidation in alkaline solution and chlorination with excess of chlorine present in hypochlorite solution. A characteristic orange color is obtained, which can be used as a specific test. The sensitivity of the picric acid was enhanced by performing the resin spot test. This color reaction was applied for the determination of picric acid spectrophotometrically.

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Photometric Determination of Iron (III)

WADALA A. BASHIR

Department of Chemistry, College of Science, University of Mosul, Mosul, Iraq

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INTRODUCTION

Iron (III) can photometrically be determined with thiocyanate (*3*), salicylic acid (*4*), or with 8-hydroxyquinoline (*2*). However, the use of these reagents requires a rigorous control of the experimental conditions to ensure quantitative determination.

Tiron (1,2-dihydroxybenzene–3,5-disulfonic acid) is a capital reagent for iron (III) and its color reaction needs a thorough study to consider the following procedure as a standard method for the determination of the titled ion. Therefore, in this study every attempt was made to represent the method as an outstanding analytical one.

MATERIALS AND METHODS

Reagents

All reagents used were of analytical grade.

Standard iron (III) solution (100 μg/ml). Ferric sulfate (0.0358 g) was dissolved in distilled water. Enough concentrated sulfuric acid was added to give a colorless solution, and the volume was diluted to 100 ml with distilled water.

Tiron solution (1%). The disodium salt (1.0 g) of the compound was dissolved in distilled water and the volume was completed to 100 ml with distilled water.

Buffer (pH 10) solution. Ammonium chloride (6.4 g) was dissolved in distilled water; 57 ml of concentrated ammonia solution was added and the volume was made up to 100 ml with distilled water (*1*).

Interfering ions solution. A 1000 μg/ml solution of each ion was prepared.

Apparatus

All absorbance measurements were made with 1-cm glass cells in a Bausch & Lomb Spectronic 20 spectrophotometer.

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Procedure

To a series of 25-ml volumetric flasks, an aliquot of the sample solution containing 10–250 μg iron (III) was transferred; 2 ml of 1% tiron solution and 4 ml of pH 10 buffer solution were added and the volume was made up to the mark with distilled water. The absorbances were measured, against a reagent blank prepared in the same manner but containing no iron (III), at 470 nm using 1-cm glass cells. The color develops immediately and is stable for a long time (>7 days). A straight-line standard curve passing through the origin was obtained, indicating that Beer's law was followed over the concentration range 10–250 μg of iron (III) in a final volume of 25 ml, i.e., 0.4–10 ppm. The Sandell sensitivity and apparent molar absorptivity, calculated from Beer's law data, were 0.0098 μg/cm^2 and 5.7×10^3 liter mol^{-1} cm^{-1}, respectively.

RESULTS AND DISCUSSION

For the preliminary experiments, 200 μg of iron (III) was taken and the final volume was made up to 25 ml. The absorption spectrum of the complex shows a single broad band with maximum absorbance at 470 nm, where the blank does not absorb at all. All measurements were therefore made at this wavelength.

It is known that chelate formation, and thus in most cases color development, is pH dependent and therefore the effect of pH on color formation and intensity was first established. A series of (pH 9–12) buffer solutions were tested. Buffers of pH 9 and 12 developed a pale yellow color, for both sample and blank, slowly, and those of pH 9.5, 10.5, and 11 inhibited color development while pH 10 gave an intense red color immediately and a colorless blank. Since this later buffer showed the effective result, its volume effect on the absorbance was then examined. A volume of 1–10 ml of the effective buffer was tested and the experiments showed that 4 ml exerted the most pronounced effect; larger volumes showed a slight decrease in the absorbance, whereas volumes smaller than 4 ml could not develop the red color. Other compositions ($NaHCO_3$ + Na_2CO_3 and H_3BO_3 + NaOH) for pH 10 buffer solution were tried but no fruitful results were obtained.

Next, the concentration effect of tiron for maximal absorbance was studied. Various volumes (1–10 ml) of 1% tiron were used; all volumes gave constant absorbance and 2 ml of the prescribed concentration was selected for the procedure. The order of addition of reagents, which is important in most cases, was then examined for optimal absorption and the best order was determined to be the sample solution, tiron reagent, and then the buffer solution.

Under the above optimized conditions, a check for the wavelength of

TABLE 1

ACCURACY AND PRECISION OF THE METHOD[a]

Amount of iron (III) (μg)	Accuracy (E_r%)[b]	Precision (RSD%)[c]
10	−3.0	1.4
100	+2.0	1.1
250	−0.8	0.45

[a] Five determinations for each.

[b] Relative error.

[c] Relative standard deviation.

maximum absorption and the stability period of the colored complex was made. The results showed that the colored complex still retains its maximum absorption at 470 nm and develops color immediately with a long-time stability period (>7 days).

After construction of the standard curve, the accuracy and precision of the described method were checked. The results are shown in Table 1.

The interfering effect of some ions was studied and their tolerable amounts in the presence of 8 ppm iron (III) were: Ca^{+2} (1500 ppm), Al^{+3} (1000 ppm), Mg^{+2} (800 ppm), As^{+3} (700 ppm), Hg^{+2} (600 ppm), Se^{+4} (460 ppm), Ce^{+4} (200 ppm), NO_2^- (1000 ppm), F^- (500 ppm), and $H_2PO_4^-$ (400 ppm).

Finally, studies concerning the chelate composition and its thermodynamic stability constant under the conditions of determination are in progress.

CONCLUSION

The new optimized conditions for the photometric determination of iron (III) with tiron make the method fairly simple and rapid. Furthermore, the method can be used to determine iron (III) in the presence of considerable amounts of fluoride and phosphate ions, which are known as strong complexing agents for iron (III). The rapidity and simplicity of the method make it advantageous for use in automated analysis. Also, the reagents used are cheap and easily available.

SUMMARY

A sensitive colored reaction of tiron with iron (III) is described. It is based on a complex formation between tiron and iron (III) in basic medium. The method is suitable to determine 0.4–10 ppm of iron (III) with a relative standard deviation of 0.45–1.4% depending on the concentration level, molar absorptivity of 5.7×10^3 liter mol^{-1} cm^{-1}, and Sandell sensitivity index of 0.0098 $\mu g/cm^2$.

Because of being simple and rapid, this method can certainly be used in routine analysis.

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Applications Involving Oxidation with Potassium Bromate

II. Potentiometric Titration of Chromium Alone or in Mixtures

I. A. ISMAIL, H. KHALIFA, AND M. ZAKY

Faculty of Science, Zagazig University, Zagazig, Egypt

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INTRODUCTION

Various metal ions can be oxidized by bromate; the subsequent sulfite reduction of the excess of bromate yields bromide. This bromide can be titrated potentiometrically with mercury(I); the approach has been applied to the determination of manganese (*2*). The bromate oxidation of chromium(III) to dichromate, on the basis of electrode potentials, proceeds to completion; it was found, however, that a catalyst such as cobalt(II) must be present. Sulfite reduces chromium(VI) to chromium(III) and unreacted bromate to bromide. Potentiometric titration of the bromide with mercury(I) using a silver amalgam indicator electrode allows the determination of chromium alone or certain binary or ternary metal mixtures. Iron(III) is reduced by the sulfite to iron(II) and does not interfere. By means of the oxidation of iodide, some metal ions can be determined by potentiometric titration of unreacted iodide with mercury(II).

EXPERIMENTAL

Solutions

All solutions were prepared from high purity chemicals and deionized water and were standardized by recommended methods.

Potassium bromate, 0.05 *M*, prepared by dissolving and diluting the calculated amount of the salt with water and standardizing by either a potentiometric back-titration under slightly acidic conditions of an excess of potassium iodide with mercury (II) (*1*) or by direct potentiometric titration with mercury(I) of bromide produced by sulfite reduction under slightly acidic conditions and boiling 5 min.

Mercury(I) nitrate, 0.05 *M*, prepared by reducing the calculated amount of mercury(II) nitrate, dissolved in the least amount of water, with mercury metal, separating the excess mercury by filtration, and diluting to

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volume with water. The solution was standardized against iron(III) solution in the presence of ammonium thiocyanate (*3*).

Other solutions, prepared to 0.05 *M* and standardized by recommended methods include CyDTA, potassium chromium(III) sulfate, potassium dichromate, manganese(II) sulfate, nickel sulfate, copper(II) sulfate, aluminum, iron, and potassium iodide. A 0.1 *M* cobalt sulfate solution was used as the catalyst, 10% sulfuric acid for acidification, and 20% sodium sulfite solution as the source of sulfite.

Apparatus

The titration assembly consisted of a 150-ml beaker fitted with a silver amalgam indicator electrode and a saturated calomel electrode (with salt bridge), a 5-ml buret, a magnetic stirrer, and a potentiometer (E512 Metrohm Herisau). The silver electrode was fashioned from spectrographically pure silver rod (2-mm diameter; 30-mm length) fitted to glass tube by polyethylene and paraffin wax.

Procedures

In the following procedures, the silver amalgam electrode is used as the indicator electrode when either bromide is titrated with mercury(I) or iodide or CyDTA with mercury(II).

Procedure A, determination of chromium alone. Transfer to a 150-ml beaker a solution containing 0.9 to 6.1 mg of chromium(III), add a known, excess volume of the 0.05 *M* bromate solution (1–3.8 ml) and 2 ml of 0.1 *M* cobalt sulfate. Boil 15 min to oxidize chromium completely and expel liberated bromine. Add 2 ml of the sodium sulfite solution and a few drops of sulfuric acid to reduce chromium(VI) to chromium(III) and unreacted bromate to bromide, boil to expel sulfur dioxide, cool, and titrate bromide with mercury(I). Express the result as chromium(III).

Procedure B, analysis of binary mixture of chromium and iron. For a mixture of chromium(III) and iron(III), dilute the sample (1–2.5 mg of each metal) to 20 ml with water, add a known, excess volume of 0.05 *M* bromate (3 ml) and 2 ml of 0.1 *M* cobalt sulfate. Continue as in Procedure A. The result corresponds to chromium(III).

Acidify slightly an identical diluted sample with a few drops of sulfuric acid, add a known, excess volume of 0.05 *M* potassium iodide (4 ml), and boil 10 min to expel liberated iodine, maintaining the volume by addition of portions of water. Cool in ice water (5°C) and titrate the unreacted iodide with mercury(II). Express the result as iron.

Procedure C, analysis of binary mixture of chromium and manganese. Dilute the sample (1–2.5 mg of each metal) to 20 ml with water, add a known, excess amount of 0.05 *M* bromate (3 ml) and 2 ml of 0.1 *M* cobalt sulfate, and boil thereby forming chromium(VI) and manganese(IV).

Continue as in Procedure A. The result corresponds to the sum of chromium(III) and manganese(II).

Make an identical, diluted sample alkaline with sodium peroxide and boil 20 min. Chromium(III) is thereby oxidized to chromium(VI) and manganese(II) to hydrated manganese(IV) oxide. Cool, acidify slightly with sulfuric acid, add 2 ml of 1:3 hydrochloric acid, and boil 10 min to yield manganese(II) and to expel liberated chlorine. Cool, add a known, excess volume of 0.05 *M* potassium iodide (5 ml) to reduce chromium(VI) to chromium(III), and titrate the excess of iodide with mercury(II). Express the result as chromium.

Manganese is calculated by difference.

Procedure D, analysis of chromium with nickel, copper, or aluminum. Dilute the sample (1–2.5 mg of chromium with 1–2.5 mg of one of the other metals) to 20 ml with water, acidify with a few drops of sulfuric acid, and add a known, excess volume of 0.05 *M* bromate (3 ml) and 2 ml of 0.1 *M* cobalt sulfate. Continue as in Procedure A. The result corresponds to chromium(III).

Add to an identical, diluted sample a known, excess volume of 0.05 *M* CyDTA, boil 15 min, cool, buffer with *hexamine*, and titrate unreacted CyDTA with mercury(II) at either pH 7.0 (nickel or copper) or pH 6.5 (aluminum). The result corresponds to the sum of chromium and the second metal.

The second metal is obtained by difference.

Procedure E, analysis of chromium(III) and chromium(VI) present together. Dilute the sample (1–2.5 mg each of the two oxidation states) to 20 ml with water, add 2 ml of 0.1 *M* cobalt sulfate, and a known, excess volume of 0.05 *M* bromate (3 ml), and continue as in Procedure A. The result corresponds to chromium(III).

Acidify an identical, diluted sample with a few drops of sulfuric acid; add a known, excess volume of 0.05 *M* potassium iodide (3 ml); allow to stand 2 min for complete reduction of chromium(VI) to chromium(III), and titrate the unreacted iodide with mercury(II). The result corresponds to chromium(VI).

Procedure F, analysis of ternary mixtures containing chromium(III). For a mixture with iron and either nickel or aluminum, dilute the sample (1–2.5 mg of each of the three metals) to 20 ml with water; add 2 ml of 0.1 *M* cobalt sulfate and a known, excess volume of 0.05 *M* bromate (3 ml), and continue as in Procedure A to obtain a value for chromium(III). Acidify an identical, diluted sample with a few drops of sulfuric acid, add a known, excess volume of 0.05 *M* potassium iodide (3 ml), and continue as in the second paragraph of Procedure B to obtain a value for iron. Add to a third identical, diluted sample, a known, excess volume of 0.05 M CyDTA and continue as in the second paragraph of Procedure D. The

TABLE 1
DETERMINATION OF CHROMIUM(III)

Chromium (mg)		Percentage error	End point break (mV/0.1 ml)
Taken	Found		
0.95	0.95	0.00	174
1.42	1.42	0.00	171
1.90	1.91	+0.53	170
2.37	2.39	+0.84	166
2.84	2.85	+0.35	157
3.32	3.31	−0.30	169
3.79	3.80	+0.26	156
4.27	4.27	0.00	176
4.74	4.75	+0.21	161
5.22	5.21	−0.19	165
5.69	5.69	0.00	182
6.16	6.17	+0.16	171

result corresponds to the sum of chromium(III), iron(III), and the third metal (aluminum or nickel), which is then found by difference.

For a mixture of chromium(III), chromium(VI), and iron(III), dilute the sample (1−2.5 mg of each of the metals) to 20 ml with water and proceed as in Procedure A to obtain a result for chromium(III). Acidify a similar, diluted sample, with a few drops of sulfuric acid; add a known, excess volume of 0.05 *M* potassium iodide, and continue as in the second paragraph of Procedure E to establish a value for chromium(VI). Acidify a third similar, diluted sample with a few drops of sulfuric acid; add a

TABLE 2
ANALYSIS OF BINARY MIXTURES

Chromium (mg)		Second metal (mg)	
Taken	Found	Taken	Found
2.37	2.37	1.20 Fe	1.22
1.19	1.18	2.40 Fe	2.39
2.32	2.32	1.32 Mn	1.33
1.16	1.16	2.64 Mn	2.65
2.32	2.32	1.39 Ni	1.39
1.16	1.16	2.79 Ni	2.78
2.32	2.32	0.63 Al	0.63
1.16	1.16	1.26 Al	1.26
2.32	2.32	1.58 Cu	1.58
1.16	1.16	3.16 Cu	3.16
2.32	2.31	1.26 Cr(VI)	1.27
1.16	1.17	2.53 Cr(VI)	2.52

TABLE 3
ANALYSIS OF TERNARY MIXTURES

Chromium (mg)		Second metal (mg)		Third metal (mg)	
Taken	Found	Taken	Found	Taken	Found
2.32	2.32	1.20 Fe	1.19	1.26 Al	1.27
1.16	1.16	2.40 Fe	2.40	0.63 Al	0.63
2.32	2.32	1.32 Mn	1.32	2.79 Ni	2.78
1.16	1.16	2.64 Mn	2.63	1.39 Ni	1.40
2.32	2.32	1.20 Fe	1.19	2.79 Ni	2.79
1.16	1.16	2.40 Fe	2.41	1.39 Ni	1.38
2.32	2.31	1.20 Fe	1.21	1.26 Cr(VI)	1.26
1.16	1.16	2.40 Fe	2.41	2.53 Cr(VI)	2.52

known, excess volume of 0.05 *M* potassium iodide (4 ml), and proceed as in the second paragraph of Procedure B to obtain a value for the sum of chromium(VI) and iron(III). Iron is found by difference.

RESULTS AND DISCUSSION

The results of determining 1 to 6 mg of chromium alone or in certain binary and ternary mixtures are summarized in Tables 1, 2, and 3. The values, which are the average of duplicates, are in good agreement with the amounts taken. The end point potential breaks are sharp and large for the titration of bromide with mercury(I), or CyDTA or iodide with mercury(II) (171, 172, and 307 mV per 0.1 ml of 0.05 *M* titrant, respectively). Under weakly acidic conditions, the bromate oxidation of chromium(III) goes to completion only when a metal ion such as cobalt(II), nickel(II), or manganese(II) is present. Cobalt is more effective than nickel in promoting this oxidation and equally effective as manganese, which, however, tends to be oxidized to manganese(IV) by bromate. The procedures presented should be applicable to the rapid and reliable determination of chromium(III) alone or with a variety of metals in diverse practical samples.

SUMMARY

A rapid and reliable determination of chromium was developed based on bromate oxidation of chromium(III) to chromium(VI). The reaction is complete under weakly acidic conditions and with cobalt(II) present as a catalyst. Unreacted bromate and chromium(VI) are then reduced with sulfite to bromide and chromium(III). The bromide is titrated potentiometrically with mercury(I) using a silver amalgam indicator electrode. Iron(III) if present is reduced by sulfite to iron(II) and does not interfere. Some binary and ternary metal mixtures containing chromium can be resolved by the determination of chromium, alone or with another metal, by the above procedure coupled with procedures for further sample portions involving the potentiometric titration of unreacted CyDTA or iodide, or both, with mercury(II).

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Study of Various Substrates for Peroxidase-Coupled Peroxide Oxidations[1]

J. D. Artiss,*·† R. J. Thibert,†·‡ J. M. McIntosh,† and B. Zak†·‡

*Department of Medical Biochemistry, St. Joseph Hospital, London, Ontario N6A 4V2, Canada, †Department of Chemistry, University of Windsor, Windsor, Ontario N9B 3P4, Canada, and ‡Department of Pathology, Wayne State University School of Medicine and Detroit Receiving Hospital–University Health Center, Detroit, Michigan 48201

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INTRODUCTION

Many enzymes exhibit a high degree of specificity toward a particular substrate and in some instances even isomers of the same substrate (*11*). However, the horseradish peroxidase–hydrogen peroxide complex, as used in coupled enzyme assays, does not exhibit such distinctive restriction toward chromogenic substrates. This lack of specificity can be advantageous in that it allows a wide range of substrates to be used (*2, 7–9, 12–18, 20, 22, 35*) in various coupled reactions. However, at the same time, the oxidative nonspecificity can also be detrimental in that it permits interfering substances (*3, 19, 24, 25, 30*), which may or may not be of common occurrence, to compete in the enzymatic reaction.

Various compounds interfere with peroxidase (POD)-coupled reactions by different modes. Some may either compete as a proton donor with the intended substrate, or they may keep the intended substrate in a reduced state, subsequently causing a negative interference due to the decreased amounts of intended chromogen generated (*3, 19*). This negative characteristic is especially pronounced in those situations where the interfering substance in either reduced or oxidized forms does not contribute to the final reaction color. Tolbutamide has been reported (*30*) to produce a positive interference by interacting directly with the chromogen. Hemoglobin (*24, 25*) adds to the final color by contributing some overlap of its characteristic spectrum to the total color where a particular concern is the region near 410 nm owing to the strong Soret band. Bilirubin (*24, 25*) is particularly interesting because it not only competes as a proton donor but also contributes some spectral overlap and possibly the spectra of its

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oxidation products to the final color. Therefore, due primarily to chance (*24*, *25*), measurements made in the region of 500 nm involving the Trinder reaction using 4-aminoantipyrene (4-AAP) and phenol (Ph) are seemingly less affected by bilirubin contamination, because the inherent color of the bilirubin and perhaps its oxidation products partially compensate for the decreased concentration of the intended chromogen.

Sharp (*29*) and Sharp *et al.* (*30*) have postulated that in order to minimize the interference of compounds which behave as oxygen acceptors, chromogenic substrates should themselves be very reactive. In other words, the initial velocity and affinity of the intended substrates should be much higher than those of any competing species. They suggest that this would necessitate a comparison of the presently available oxygen acceptor chromogens.

Apart from a high initial velocity, a good chromogenic substrate should be quite sensitive and stable. In order to take advantage of the compensatory effects of bilirubin as well as to avoid the Soret band of hemoglobin, the wavelength of measurement would ideally be in the region of 500 nm (*24*). Solubility is also a consideration as both the substrates and chromogen should be readily soluble in aqueous solutions. In order to alleviate possible instrumental problems, a reaction resulting in a small blank would be both preferable and advantageous.

This study was, therefore, undertaken with the intent of determining the general structural configuration necessary to meet the above requirements for a cosubstrate which would substitute for phenol (*35*) in the peroxidase-catalyzed, oxidative coupling reaction with 4-aminoantipyrene. In addition, a previously proposed substitute for 4-AAP, 3-methyl-2-benzothiazolinone hydrazone (MBTH) was investigated (*4*, *9*, *10*).

MATERIALS AND METHODS

Chemicals

Peroxidase (from horseradish) [hydrogen-peroxide oxidoreductase, EC 1.11.1.7] was purchased from Boehringer-Mannheim Canada, Dorval, Quebec. One unit, as defined by BMC, is that amount of enzyme which catalyzes the oxidation of 1 μmol of guaiacol by H_2O_2 per minute at 25°C at pH 7.0.

The following compounds were purchased from Fisher Scientific, Don Mills, Ontario: sulfuric acid, 33% fuming sulfuric acid; *p*-bromophenol; sodium 4-hydroxynaphthalenesulfonate, sodium *p*-phenolsulfonate, and phenol.

The following compounds were purchased from Aldrich, Milwaukee, Wisconsin: chlorosulfonic acid, 2,3-dichlorophenol, 3,5-dichlorophenol, 3,4-dichlorophenol, 2,5-dichlorophenol, 2,4-dichlorophenol, 2,6-dichlorophenol, 4,5-dihydroxynaphthalene-2,7-disulfonic acid, disodium salt dihydrate, *m*-chlorophenol, *o*-chlorophenol, N,N-diethylaniline, 4-aminoantipyrene, and dimethylaminoantipyrene.

4-Chlorophenol and 2,2′-methylenebis(*p*-chlorophenol) were purchased from ICN Pharmaceuticals, Montreal, Quebec.

The following compounds were purchased from Pfaltz and Bauer, Stamford, Connecticut: 3,5-dichloro-4-hydroxybenzoic acid, 3,5-dibromo-2-hydroxybenzoic acid, 2,4-dibromophenol, and 1-naphthylamine-7-sulfonic acid.

Homovanilic acid and *p*-hydroxyphenylacetic acid were purchased from Sigmá Chemical Co., Saint Louis, Missouri.

Apparatus

The spectrophotometer used throughout the study was an ACTA MVI, equipped with automatic cell changer and circulating water bath, Beckman Instruments, Analytical Instruments, Toronto, Ontario.

Reagents

Stock hydrogen peroxide. This solution was prepared by diluting 6 ml of 30% H_2O_2 to 1 liter. It was then stored in a brown bottle at 0–4°C. Prior to use this reagent was standardized (the concentration of the particular reagent used was 6.68×10^{-2} mol/liter).

Hydrogen peroxide standards. These standards were prepared by diluting the stock hydrogen peroxide solution to 100, 200, and 300 μmol/liter in H_2O as required.

Tris-HCl buffer: This buffer was prepared to be 50 mmol/liter, pH 7.8, containing 0.2% (w/v) Triton X-100.

Peroxidase solution. This solution was prepared to contain 0.1 *U*/ml peroxidase in Tris-HCl buffer.

Dimethylaminoantipyrene, 4-AAP, and MBTH solutions were prepared to be 6 mmol/liter in Tris-HCl buffer. All other substrates were prepared to be 22.5 mmol/liter in Tris-HCl buffer.

The stock hydrogen peroxide was standardized in a manner proposed by Kolthoff and Sandell (*21*). That is, 25 ml of stock H_2O_2 solution was mixed with 10 ml of 1:5 H_2SO_4 and titrated with potassium permanganate. The permanganate was prepared and standardized exactly according to Kolthoff and Sandell (*21*).

Sodium 2-hydroxy-3,5-dichlorobenzenesulfonate (HDCBS) was prepared as described previously (*1*). Tables 1 (^{13}C) and 2 (^{1}H) contain the nuclear magnetic resonance (NMR) data.

Where the sulfonation of the phenols could lead to more than one isomer, the product structures were assigned on the basis of their ^{13}C and

TABLE 1

CARBON-13 NUCLEAR MAGNETIC RESONANCE SPECTRA[a]

Compound	C_1	C_2	C_3	C_4	C_5	C_6
Sodium 4-hydroxy-3-chlorobenzenesulfonate	154.5	120.6	128.0	135.8	126.2	117.0
Sodium 2-hydroxy-4-chlorobenzenesulfonate	154.3	117.7	138.3	120.6	129.3	127.0
Sodium 2-hydroxy-5-chlorobenzenesulfonate	152.7	129.7	127.7	124.5	133.5	119.5
Sodium 4-hydroxy-2,3-dichlorobenzenesulfonate	163.3	124.7	129.8	135.8	126.8	111.5
Sodium 2-hydroxy-4,5-dichlorobenzenesulfonate	165.6	123.0	135.2	112.8	128.9	130.4
Sodium 4-hydroxy-3, 5-dichlorobenzenesulfonate	161.9	[124.0][b]	[125.9][b]	126.6	[125.9][b]	[124.0][b]
Sodium 2-hydroxy-3, 5-dichlorobenzenesulfonate	148.3	132.9	130.5	126.3	124.7	123.2

[a] All spectra were run in D_2O and are reported in parts per million relative to CH_3OH set at δc = 49.3 relative to tetramethylsilane internal standard. All spectra were run on a Bruker CXP-100 operating in the Fourier transform mode.

[b] Numbers contained within brackets may be interchanged.

^1H NMR spectra. ^{13}C spectra were calculated using standard tables of functional group shift increments, comparisons with the unsulfonated phenols and trends appearing within the series of products. As expected, significant deviations from calculated spectra appeared when *ortho* substituents were present.

Sodium 4-hydroxy-3-chlorobenzenesulfonate was prepared by a modification of the synthesis of HDCBS. Eight milliliters of *o*-chlorophenol were added to a 25-ml round-bottom flask equipped with a reflux condenser, thermometer, heating mantel, and magnetic stirrer. The phenol was brought to about 80°C at which time 9 ml of 33% oleum were added slowly so that the temperature did not rise above 150°C. As the reaction mixture cooled slowly to about 100°C the material inside the flask solidified. The solid material was transferred with a small amount of water into a beaker containing 20 g of NaCl, stirred, and then allowed to sit for 30 min at 23°C. This material was then filtered, washed with 1 *N* HCl and recrystallized twice from H_2O. The yield based on *o*-chlorophenol was 70%. The material appeared as off-white microneedles from H_2O. Tables 1 (^{13}C) and 2 (^1H) contain the NMR data.

Sodium 2-hydroxy-4-chlorobenzenesulfonate was prepared in a manner similar to the above, with the following modification. Four milliliters of *m*-chlorophenol and 4 ml of 33% oleum were used as starting materials. The reaction mixture was stirred at 80–90°C for about 12 hr, the temperature maintained upon cooling after the initial rise to 140–150°C. By this time the reaction mixture had solidified. The crude sodium salt was obtained upon mixing with 10 g NaCl. Upon recrystallization, the material appeared as white flakes from H_2O in a 31% yield based on *m*-chlorophenol. Tables 1 (^{13}C) and 2 (^{1}H) contain the NMR data.

Sodium 2-hydroxy-5-chlorobenzenesulfonate was prepared with some difficulty in the purification procedures in a manner similar to the above. Four milliliters of 33% fuming sulfuric acid were added slowly to 9.9 g of *p*-chlorophenol, which had been melted at 44°C. The reaction mixture was stirred at 80°C for 9 hr at which time it was poured onto 15 g of NaCl. Upon filtration under reduced pressure the crude product was washed twice with ether, boiled with charcoal in 150 ml of H_2O, filtered through Hyflo Super Cel and extracted twice with benzene before it was recrystallized once from H_2O. The yield, based on *p*-chlorophenol, was 20%. Tables 1 (^{13}C) and 2 (^{1}H) contain the NMR data.

Sodium 4-hydroxy-2, 3-dichlorobenzenesulfonate was prepared in a manner similar to the above. To 5 g of melted 2,3-dichlorophenol, 4 ml of 33% oleum was added slowly, with mixing. This mixture was stirred for 8 hr at 95°C. At this time the reaction mixture was transferred with a minimal amount of H_2O to a beaker containing 25 g of NaCl and allowed to sit for 30–60 min. The crude product was filtered under reduced pressure and then dissolved in 200 ml of H_2O. This solution was neutralized with NaOH and then boiled with charcoal and filtered through Hyflo Super Cel. Upon cooling, the solution was extracted with 2 × 200 ml of ether, boiled to reduce the volume and allowed ty crystallize. The product was redissolved in a small amount of water and was isolated by precipitation with acetone. The yield, based on 2, 3-dichlorophenol, was 10%. Tables 1 (^{13}C) and 2 (^{1}H) contain the NMR data.

Sodium 2-hydroxy-4, 5-dichlorobenzenesulfonate was prepared in exactly the same way as sodium 4-hydroxy-2, 3-dichlorobenzenesulfonate except that the starting material was 3, 4-dichlorophenol and the reaction was carried out for 10 hr. The yield was 47% based on 3,4-dichlorophenol. Tables 1 (^{13}C) and 2 (^{1}H) contain the NMR data.

Sodium 4-hydroxy-3, 5-dichlorobenzenesulfonate was prepared exactly as the above except that the reaction mixture solidified upon cooling to 90°C, after the initial rise in temperature, upon addition of the oleum. Benzene was used in place of ether for the extraction process. The yield based on 2,6-dichlorophenol was 35%. Tables 1 (^{13}C) and 2 (^{1}H) contain the NMR data.

Sodium 2-hydroxy-5-bromobenzenesulfonate was prepared in exactly the same manner as sodium 4-hydroxy-3, 5-dichlorobenzenesulfonate except that 5.19 g *p*-bromophenol was used as starting material and no organic solvent extraction was necessary. The yield was 22% based on *p*-bromophenol. Table 2 contains the proton NMR data.

Sodium 2-hydroxy-3, 5-dibromobenzenesulfonate was prepared in a manner similar to the chloro analog. To 7.7 g of melted 2,4-dibromophenol, 8 ml of H_2SO_4 were added slowly. This mixture was stirred for 40 hr at 100 ± 5°C, and then poured onto 8 g of NaCl. After cooling to 4°C for 1 hr the crude mixture was filtered under reduced pressure. This material was redissolved in H_2O and boiled with charcoal for 5 min. Upon filtering through Hyflo Super Cel the solution was boiled to reduce its volume. The product was recrystallized twice giving a yield of 51% based on 2,4-dibromophenol. Proton NMR data are given in Table 2.

TABLE 2

PROTON NUCLEAR MAGNETIC RESONANCE SPECTRA[a]

Compound	Nuclear magnetic resonance spectral data
Sodium 2-hydroxy-3,5-dichlorobenzenesulfonate	7.57 (d, J = 2.5, 1H) 7.83 (d, J = 2.5, 1H)
Sodium 4-hydroxy-3-chlorobenzenesulfonate	7.90 (d, J = 2.5, 1H) 7.68 (dd, J = 2.5, 8.6, 1H) 7.50 (d, J = 8.6, 1H)
Sodium 2-hydroxy-4-chlorobenzenesulfonate	7.70 (d, J = 8.6, 1H), 7.03 (m, 2H)
Sodium 2-hydroxy-5-chlorobenzenesulfonate	7.65 (d, J = 2.5, 1H) 7.33 (dd, J = 2.5, 8.6, 1H), 6.88 (d, J = 8.6, 1H)
Sodium 4-hydroxy-2, 3-dichlorobenzenesulfonate	7.60 (d, J = 8.6, 1H), 6.55 (d, J = 8.6, 1H)
Sodium 2-hydroxy-4,5-dichlorobenzenesulfonate	7.73 (s, 1H), 6.84 (s, 1H)
Sodium 4-hydroxy-3, 5-dichlorobenzenesulfonate	7.75 (s, 2H)
Sodium 2-hydroxy-5-bromobenzenesulfonate	7.90 (d, J = 2.5, 1H), 7.60 (dd, J = 2.5, 8.6, 1H), 6.95 (d, J = 8.6, 1H)
Sodium 4-hydroxy-3, 5-dibromobenzenesulfonate	7.80 (s, 2H)
Sodium 2,2′-methylene-bis(2-hydroxy-5-chloro benzenesulfonate)	7.45 (d, J = 3, 2H) 7.23 (d, J = 3, 2H), 3.61 (s, 2H)

[a] Tabulation follows the order chemical shift (δ) in parts per million from trimethylsilylpropane sulfonate, multiplicity, coupling constant, and number of protons. Spectra were run in D_2O, on a JEOLCO 60 HL spectrometer, J values given in Hz.

Shultz (*31*) has reported a method for the monosulfonation of biphenyls. His procedures were modified slightly due to differences in the solubilities of the starting materials in an attempt to monosulfonate 2,2′-methylenebis(*p*-chlorophenol). Proton NMR data (Table 2) suggest that the product was the disulfonate, sodium 3,3′-methylenebis(2-hydroxy-5-chlorobenzenesulfonate). This material was prepared by dissolving 5.3 g of 2,2′-methylenebis(*p*-chlorophenol) in 325 ml $CHCl_3$. Chlorosulfonic acid, 5.2 ml in 10 ml of $CHCl_3$, was added slowly and the solution was stirred for 14 hr. The precipitate was removed by filtration under reduced pressure and then washed with 100 ml of $CHCl_3$. The combined $CHCl_3$ fractions were mixed with an additional 2.6 ml $ClSO_3H$ in 5 mL $CHCl_3$ for another 3 hr at which time the suspension was filtered. The combined solid fractions were dissolved in 200 ml of boiling water and neutralized with NaOH. Undissolved material was removed by filtering through Hyflo Super Cel. The volume was reduced by boiling. After two recrystallizations the yield was 43%. Table 2 contains the ^1H NMR data.

Procedure

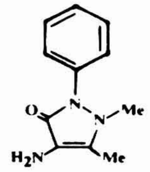
Four hundred microliters of each of a pair of substrates were mixed with 100 μl of each of the H_2O_2 standards plus a blank which contained H_2O in place of H_2O_2. The reactions were initiated by the addition of 100 μl of peroxidase. Prior to mixing, all reagents were stored on ice. The contents of each tube were transferred to cuvettes at 37°C, and placed into the spectrophotometer with 75 sec. The change in absorbance at the λ_{max}, for each pair, was monitored for at least 30 min. At this time the spectrum of the blank reaction was scanned against water and then the spectrum of each standard against the blank. In a few cases, when the color produced faded quickly, the tests were repeated and the spectra of these were scanned at a time corresponding to maximum color development.

RESULTS AND DISCUSSION

This study deals with the comparison of several cosubstrates involved in peroxidase-coupled reaction with 4-AAP and MBTH. With the exception of HDCBS none of the sulfonation reactions to prepare the final compounds were optimized as a large amount of material was not necessary for the present study. Elemental analysis was carried out only on HDCBS (*1*) as it proved to be of greatest interest. The purity of the other compounds was considered to be good enough for the study on the basis of the ^{13}C and ^1H NMR spectra and the solubility of the compounds.

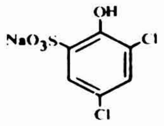
Since a number of the spectra obtained in this study are similar, only representative spectra will be given. In those instances where spectra were obtained but are not shown, it will be noted as to which spectrum it is similar.

I. 4-Aminoantipyrene

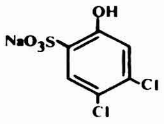


II. Sodium 2-hydroxy-3,5-dibromobenzenesulfonate

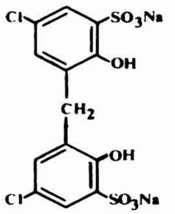
III. Sodium 2-hydroxy-3,5-dichlorobenzenesulfonate



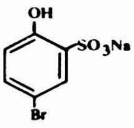
IV. Sodium 2-hydroxy-4,5-dichlorobenzenesulfonate



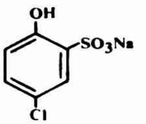
V. Sodium 3,3'-methylenebis[2-hydroxy-5-chlorobenzenesulfonate]



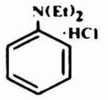
VI. Sodium 2-hydroxy-5-bromobenzenesulfonate



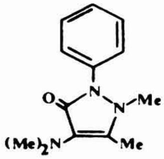
VII. Sodium 2-hydroxy-5-chlorobenzenesulfonate



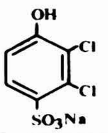
VIII. N,N-Diethylaniline hydrochloride



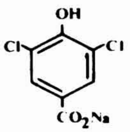
IX. Dimethylaminoantipyrene



X. Sodium 4-hydroxy-2,3-dichlorobenzenesulfonate



XI. Sodium 3,5-dichloro-4-hydroxybenzoate



XII. Sodium 2-hydroxy-4-chlorobenzenesulfonate

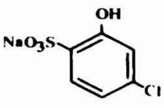
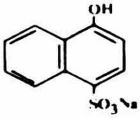


FIG. 1. Structures of the cosubstrates studied. All structures are numbered to correspond to the numbers in the text. Me = CH_3, Et = CH_2CH_3.

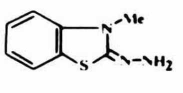
Figure 1 shows the structures of those compounds which were studied. Each compound is numbered and is referred to by that number where it is convenient.

Figure 2 shows the spectra obtained from the coupling of phenol to

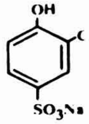
XIII. Sodium 1-napthol-4-sulfonate



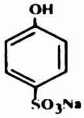
XIV. 3-Methyl-2-benzothiazolinone hydrazone



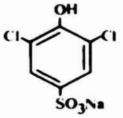
XV. Sodium 4-hydroxy-3-chlorobenzenesulfonate



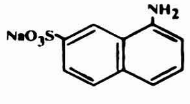
XVI. Sodium 4-hydroxybenzenesulfonate



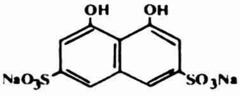
XVII. Sodium 4-hydroxy-3,5-dichlorobenzenesulfonate



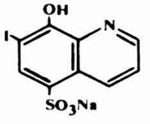
XVIII. Sodium 1-napthylamine-7-sulfonate



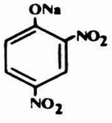
XIX. Disodium 4,5-dihydroxynapthalene-2,7-disulfonate



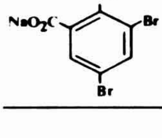
XX. 8-Hydroxy-7-iodo-5-quinolinesulfonic acid



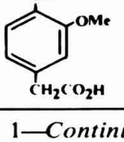
XXI. Sodium 2,4-dinitrophenoxide



XXII. Sodium 2-hydroxy-3,5-dibromobenzoate



XXIII. Homovanillic acid



XXIV. p-hydroxyphenylacetic acid

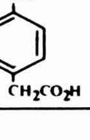


FIGURE 1—*Continued.*

4-AAP. The λ_{max} is broad and centered around 505 nm. As this is probably the most popular cosubstrate combination (*23, 24–27, 35*) for peroxidase-coupled reactions, it will be used as a basis with which to compare all of the substrates in this study. Since in several of the reactions the structure of the generated chromogen is unknown it is not possi-

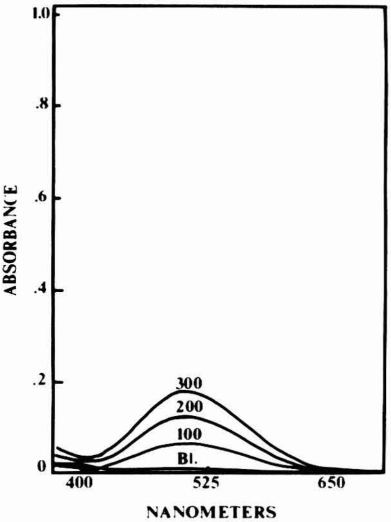


FIG. 2. Spectra resulting from the POD-catalyzed coupling of phenol to 4-AAP, at three concentrations (100, 200, 300 μmol/l) of H_2O_2 as well as the blank (Bl.) reaction. The blank was scanned against H_2O while the standards were scanned against the blank.

ble to discuss comparative molar absorptives. For this reason all comparisons of sensitivity will be made in terms of the following equation:

$$\frac{A\ \lambda_{\max} \text{ of test substrate}}{A_{505} \text{ for (4-AAP + Ph)}} = \text{Factor.}$$

Because it was necessary, for reasons of solubility, to prepare the sodium sulfonate of many of the compounds studied, it was considered important to compare the reaction of sodium 4-hydroxybenzenesulfonate (XVI) with 4-AAP to the parent phenol. Spectral data suggest that the only difference in the spectra of the two reactions is a slight hypsochromic shift in the $\lambda_{\max}$ of the sulfonated phenol reaction to 500 nm. Table 3 indicates that there are no differences in the sensitivities, stabilities, or times required for maximum color development of the two reactions, while the initial rate is slightly slower for the sulfonated compound.

The purported reaction mechanism for the nonenzymatic coupling reaction (*5*, *6*, *32*, *33*) predicts that in order for it to proceed, any group *para* to the hydroxyl group must be displaced. It would appear, based on sensitivity and rates, that a *para* chloro group (VII) of the monochlorinated compounds is more readily removed than that of a *para* sulfonic acid (XV). Where a hydrogen is *para* to the hydroxyl (XII), a rate and sensitivity difference between the latter two is demonstrated. This is consistent with the observations of Faust and Mikulewicz (*6*) and might be

TABLE 3

COMPARATIVE DATA OF ALL SUBSTRATES STUDIED

Cosubstrates	λ_{max} (nm)	Factor[a]	Time required for maximum color development (min)	Initial rate[b] (ΔA/min)	Decrease at 30 min (%)	Spectra similar to figure
I and II	510	4.1	11.0	0.134	2.5	4
I and III	510	4.0	10.0	0.146	2.0	4
I and IV	502	3.1	20.0	0.053	1.6	2
I and V	515–520	2.6	13.0	0.061	0.0 at 60 min	6
I and VI	505	2.6	17.0	0.063	0.4	2
I and VII	500–505	1.1	15.0	0.066	1.4	2
I and VIII	555	2.5	6.5	0.070	17.3	9
IX and III	510	2.4	20.0	0.034	0.0 at 60 min	4
I and X	517	2.3	10.0	0.072	14.4	6
I and XI	505–510	2.1	9.0	0.074	17.2	6
I and XII	500	1.7	11.0	0.058	2.3	1
I and XIII	485–490	1.2	10.0	0.047	6.1[c] increase	Not shown
XIV and VIII[d]	590	1.2	15.0	0.200	1.9	7
XIV and III[d]	540	1.1	15.0	0.084	1.0	10
I and XV	505	1.1	7.5	0.520	12.7	1
I and phenol	505	1.0	13.0	0.043	0.0	1
I and XVI	500	1.0	13.0	0.031	0.0	2
I and XVII	505–10	1.0	15.0	0.023	0.5	1
I and XVIII	485–90	0.8	>30.0[e]	0.029	0.0	Not shown
I and XIX	600–610	0.6	7.0	0.025	12.8	Not shown

Note. Refer to Fig. 1.

[a] Factor is defined in Equation 1.

[b] Initial rate of the 300 μM standard.

[c] Absorbance appeared to plateau, but then continued to rise.

[d] ΔA of blk and stds paralleled at 15 min; however, A continued to increase with respect to H_2O.

[e] Absorbance at 30 min, although absorbance continued to increase.

explained on the basis of: (a) steric effects and/or (b) H and SO_3 are poor leaving groups compared to Cl.

These observations are somewhat substantiated by the dichloro-substituted hydroxybenzenesulfonates (Table 3). With the exception of the brominated analog, sodium 2-hydroxy-3, 5-dichlorobenzenesulfonate (III) shown in Fig. 3 was the most sensitive cosubstrate studied. Note that the sodium sulfonate of this compound is *ortho* to the hydroxyl as is one chloro, while the second chloro is *para*. The second compound of the sequence also has the *ortho* sulfonate and *para* chloro (IV); however, the

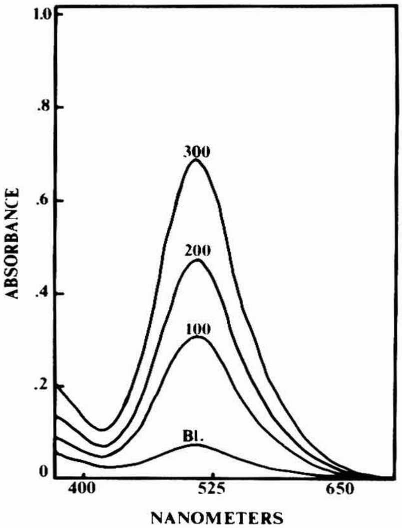


FIG. 3. Spectra resulting from the POD-catalyzed coupling of sodium 2-hydroxy-3, 5-dichlorobenzenesulfonate (III) with 4-AAP at three concentrations (100,200,300 μmol/l) of H_2O_2 as well as a blank (Bl.) reaction. The blank was scanned against H_2O while the standards were scanned against the blank.

second chloro is now *meta* to the hydroxyl. Possibly because Cl is a better leaving group than is H, or perhaps due to the rearrangement of the chloro group, the sensitivity of the dichlorinated compound is about 1.8 times that of the monochloro (*meta* to hydroxyl) compound (XII). However, because the initial rates are nearly equal, the leaving group argument seems compromised while credence is lent to the possibility of rearrangement. The results of the remaining two compounds (X, XVII) of this series are consistent with the above data in that they are sulfonated *para* to the hydroxyl and show a decreased sensitivity. However, the initial rate of sodium 4-hydroxy-2,3,-dichlorobenzenesulfonate (X) appears to be inconsistent in that it is relatively high. This might be explained on the basis of steric hindrance or the rearrangement of the displaced *para* substituent. That is to say, the initial velocity appears to be slower in those instances where rearrangement would result in a chloro group adjacent to the sulfonate.

It is noteworthy to mention that what would superficially appear to be a trivial exchange of carboxyl for sulfonate totally contradicts the trends set forth above. The sulfonation of 2, 4-dibromophenol, in the 6 position, yields one of the most sensitive cosubstrates studied. However, the carboxyl (XXII) analog gives no more than a slight reaction (Table 3). Conversely, sodium 4-hydroxy-3, 5-dichlorobenzoate (XI) yields twice the color, when coupled to 4-AAP, than does the sulfonated analog (XVII), although the color is considerably less stable. Svobodova and co-workers (*33, 34*) have reported that *o*-hydroxybenzoic acid does not react with 4-AAP in the nonenzymatic oxidative coupling reaction while *p*-hydroxybenzoic acid does (*33*). The enzymatic and nonenzymatic reactions appear to be consistent with each other but the individual compounds tend to show some ambiguity with respect to the manner in which they react.

Table 3 tabulates the data for the coupling of HDCBS to dimethylaminoantipyrene (IX). This reaction yields approximately 60% of the color of the 4-AAP–HDCBS reaction. Table 3 indicates that the initial rate of this reaction is slower than that of the 4-AAP–HDCBS reaction. This has been explained (*32*) as being due to the fact that the 4-AAP is the reactive species in both instances. In other words, demethylation of the dimethylaminoantipyrene is the rate-limiting step.

The sodium sulfonate *para* to the hydroxy of 1-naphthol (XIII) appears to have an increased initial rate and sensitivity compared to the 4-hydroxybenzenesulfonate. The reactions of sodium 1-napthylamine-7-sulfonate (XVIII) and 4, 5-dihydroxynaphthalene-2, 7-disulfonic acid disodium salt (XIX) with 4-AAP produce less color than the 4-AAP–phenol coupling. It may be impractical to compare the disubstituted case of 4, 5-dihydroxynaphthalene-2, 7-disulfonate with the previous monohydroxy-monosulfonated compounds as this is the only instance in which the hydroxy and sulfonic acid groups are *meta* to each other. This particular arrangement would appear, however, to be detrimental to the reaction.

The reaction of the disodium salt of 3, 3′-methylenebis(2-hydroxy-5-chlorobenzenesulfonic acid) (V) with 4-AAP yields a spectrum (Fig. 4). This reaction appears to be extremely stable with no noticeable change in the absorbance at 515 nm, 45 min after full color development. The alkyl substitution *ortho* to the hydroxyl of the phenol does not appear to have altered the reaction apart from an added degree of stability to the generated chromogen.

The use of MBTH and N, N-dimethylaniline as cosubstrates in peroxidase-coupled reactions for the determinations of glucose and uric acid have been proposed (*4, 9, 10*). In a preliminary study no difference was found between the reactions of the dimethyl and diethyl analogs. Further studies were subsequently performed only with N, N-diethylaniline. Under the described reaction conditions there is a large blank reaction (Fig. 5). Figure 6 demonstrates that there is a continuous increase in the blank reaction for at least 30 min from the initiation of the reaction. Considering the apparent continual increase in absorbance, the high blank, and the fact that there is very little sensitivity gained over the phenol–4-AAP reaction, the merits of this cosubstrate system are not obvious.

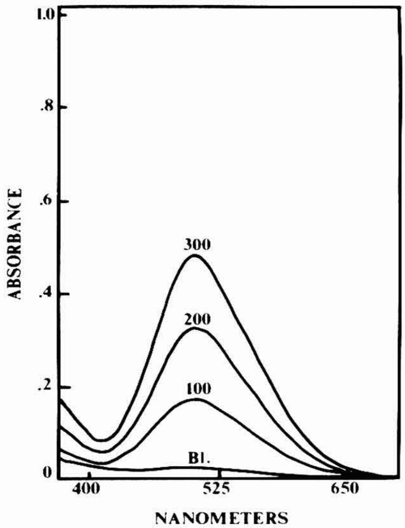


FIG. 4. Spectra resulting from the POD-catalyzed coupling of sodium 3,3′-methylenebis(2-hydroxy-5-chlorobenzenesulfonate) (V) with 4-AAP, at three concentrations (100, 200, 300 μmol/l) of H_2O_2 as well as the blank (Bl.) reaction. The blank was scanned against H_2O while the standards were scanned against the blank.

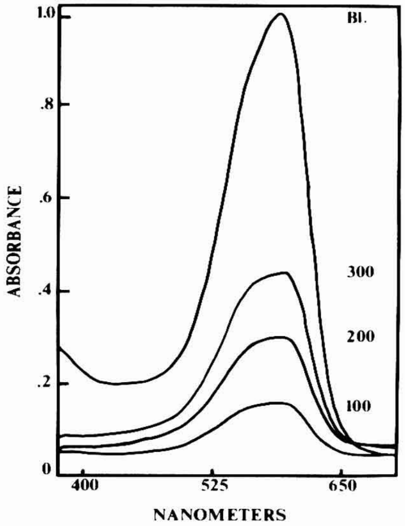


FIG. 5. Spectra resulting from the POD-catalyzed coupling of MBTH (XIV) and N,N-diethylaniline · HCl (VIII), at three concentrations (100, 200, 300 μmol/l) of H_2O_2 as well as the blank (Bl.) reaction. The blank was scanned against H_2O while the standards were scanned against the blank.

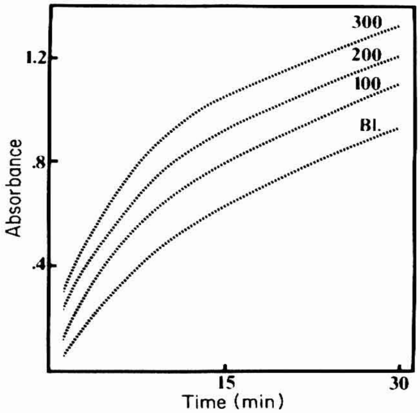


FIG. 6. Rate of color development, at 590 nm, of the POD-catalyzed coupling of MBTH (XIV) and N,N-diethylaniline·HCl (VIII) at three concentrations (100, 200, 300 μmol/l) of H_2O_2 as well as the blank (Bl.) reaction. The instrument was blanked with water.

Kabasakalian and co-workers (*18*) and Rautela and Liedtke (*28*) have proposed the use of N, N-diethylaniline·HCl, and 4-AAP as cosubstrates for peroxidase-coupled glucose (*18*) and cholesterol (*28*) procedures. Figure 7, the spectra of the chromogen of this coupling, shows the λ_{max} to be at about 555 nm with an increased sensitivity of about 2.5 times over that

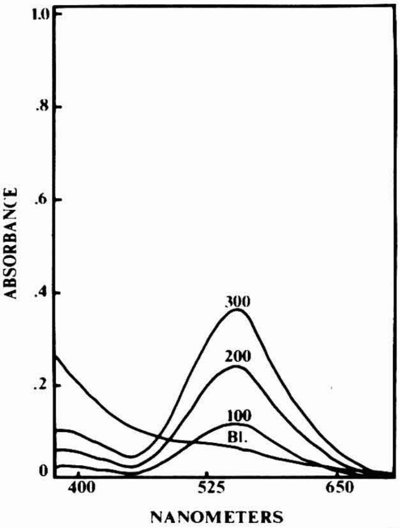


FIG. 7. Spectra resulting from the POD-catalyzed coupling of N,N-diethylaniline (VIII) and 4-AAP, at three concentrations (100, 200, 300 μmol/l) of H_2O_2 as well as the blank (Bl.) reaction. The blank was scanned against H_2O while the standards were scanned against the blank.

of the phenol–4-AAP reaction (Table 3). However, the generated chromogen is apparently relatively unstable. Twenty-five minutes after maximum color development the absorbance at 555 nm had decreased by about 17%.

As a matter of interest, the use of MBTH and HDCBS as cosubstrates was studied. The results were quite similar to those obtained from the N,N-diethylaniline–MBTH reaction. As may be seen in Fig. 8, there is a very large blank reaction and as in the latter the blank reaction appears to be continuous (Fig. 9). The sensitivity of this cosubstrate system appears to be similar to the phenol–4-AAP combination.

Homovanillic acid (XXIII) and *p*-hydroxyphenylacetic acid (XXIV) are used separately as substrates for fluorometric determinations of peroxidase (*11, 13, 15*). Under the described conditions, they do not produce chromogens in the visible region with 4-AAP. This is consistent with nonenzymatic studies (*6, 32*).

8-Hydroxy-7-iodo-5-quinolinesulfonic acid (XX) was considered to be a possible cosubstrate with 4-AAP. There did appear to be a reaction taking place; however, the former imparted a very intense yellow color to the solution which made absorbance measurements difficult.

Sodium 2, 4-dinitrophenoxide (XXI) did not couple with 4-AAP. This observation is consistent with the nonenzymatic studies of Faust and Mikulewicz (*6*).

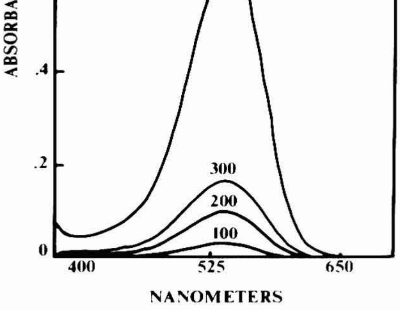


FIG. 8. Spectra resulting from the POD-catalyzed coupling of MBTH and HDCBS at three concentrations (100, 200, 300 μmol/l) of H_2O_2 as well as the blank (Bl.) reaction. The blank was scanned against H_2O while the standards were scanned against the blank.

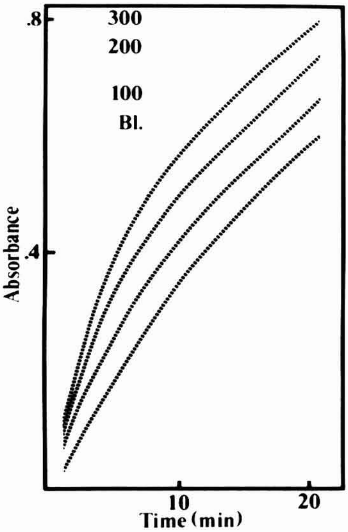


FIG. 9. Rate of color development, at 540 nm, of the POD-catalyzed coupling of MBTH and HDCBS, at three concentrations (100, 200, 300 μmol/l) of H_2O_2 as well as the blank (Bl.) reaction. The instrument was blanked with water.

SUMMARY

It would appear from this study that the sodium sulfonates of 2, 4-dichloro- and 2, 4-dibromophenol when employed in the presence of 4-AAP produce useful and sensitive, colorimetric, cosubstrate systems. What may prove to be of equal importance is the relatively high initial rate of the coupling reactions, as it has been suggested (*29*, *30*) that this might help alleviate interference by competitive proton donors.

As long as the general structure of the phenols is maintained, increased aromaticity tends to add stability to the chromogen. This is, of course, an asset when performing equilibrium analysis.

The use of MBTH, in the presence of either aromatic amines or alcohols, appears to have little obvious merit. Apart from the continual increase in absorbance of the blank, that is, a spontaneous coupling of the cosubstrates, as well as the comparatively high blank absorbance, this material does not seem to be as sensitive as some of the other reactions studied.

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Electrochemical Study of the Use of Tin(II) Complexed by Sorbitol in Volumetric Analysis

J. ZIMA, M. ROUŠAL, AND J. DOLEŽAL

Department of Analytical Chemistry, Faculty of Natural Sciences, Charles University, Albertov 2030, 128 40 Prague 2, Czechoslovakia

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In previous works, the use of standard solutions of tin(II) chloride complexed with glycerol in volumetric analysis for the reductometric determination of inorganic and organic substances was proposed (*1, 2*) and evaluated (*3*). The high viscosity of the solutions must be decreased by the addition of ethanol. The titrations were mostly carried out in a saturated sodium carbonate solution. The Sn(II) and Sn(IV) salts are complexed with some polyols, e.g., sorbitol, in a manner similar to glycerol. To increase the ligand concentration to the required level, the standard solutions also contained sodium carbonate, the solution in which most of the determinations are carried out.

This work is concerned with a study of the standardization of standard tin(II) chloride solutions in sorbitol (Sn(II)–sorb.), and of their stability in an inert atmosphere and in the air. The reversibility of the redox system was studied polarographically and voltammetrically and the reaction of Sn(II)–sorb. with dichromate was followed spectrophotometrically. The possibility of determining some inorganic and organic compounds is also mentioned.

EXPERIMENTAL

Apparatus. The titrations were carried out in a vessel adapted for work in an inert atmosphere using an SCE and a Pt indicator electrode (A = 0.35 cm^2) or two identical Pt electrodes (A = 0.35 cm^2). Potentiometric (EP) measurements were carried out using a Radiometer PHM-64 pH meter, bipotentiometric and biamperometric measurements were carried out using a titration adapter from the J. Heyrovský Institute of Physical Chemistry and Electrochemistry (the biamperometric signal was converted to mV on the pH meter scale). Polarographic measurements were carried out using an LP 7 instrument with an EZ 7 recorder (Laboratorní Přístroje, Prague), voltammetric measurements using a PO 4 polarograph (Radiometer, Denmark), and spectrophotometric measurements using a Unicam SP-800 instrument (Unicam, England). The area of the RDPtE

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was 0.053 cm^2. An ABU 12 burette (25 ml) (Radiometer, Denmark) was employed.

Reagents. $SnCl_2 \cdot 2H_2O$, $K_2Cr_2O_7$, H_2O_2, I_2, KI, chloramine T, $Hg(NO_3)_2$, HCl, $Na_2S_2O_3$, $NaAsO_3$, NaCl, EDTA, TAR, Na_2CO_3, K_2CO_3, $KHCO_3$, $NaHCO_3$, NH_4OH, *N*-bromosuccinimide, pure ethanol, sorbitol for medical use, redistilled monoethanolamine, all p.a. substances from Lachema, Brno, Czechoslovakia, $K_3Fe(CN)_6$, p.a. from Merck, GFR, and $AgNO_3$, p.a. from Evans Medical Supplies Ltd., England, were used.

Organic peroxides were obtained from the factory in Velvěty, Czechoslovakia: cyclohexanone peroxide and dibenzoyl peroxide as the solid substances, methylethylketone peroxide and methylcyclohexanone peroxide in solution in methylethyl ketone and methylcyclohexane, respectively. The *m*-chloroperbenzoic acid used was the product of Fluka, England and was of p.a. purity.

RESULTS

Preparation and Titer Determination of 0.1 N Sn(II)–Sorbitol

A 0.1 *N* $SnCl_2$ solution in 3 *M* sorbitol in saturated Na_2CO_3 (Sn(II)–sorb.) was prepared. The solutions employed were freed of oxygen before use. The tin content in the $SnCl_2 \cdot 2H_2O$ employed was determined in 1 *M* HCl medium using potassium dichromate (*4*).

The following procedure was followed in all titrations: 5.00 ml of standard 0.1 *N* $K_2Cr_2O_7$ solution was pipetted into 45 ml of the appropriate electrolyte, the solution was freed of oxygen by a stream of inert gas, and was then titrated with the Sn(II)–sorb. solution with stirring and maintenance of an inert atmosphere over the solution. The Sn(II)–sorb. titer was determined in the following media: sat. Na_2CO_3, sat. $NaHCO_3$, sat. Na_2CO_3 + conc. NH_4OH, and 2 *M* carbonate buffers. The results are listed in Table 1. The titer in an electrolyte containing sat. Na_2CO_3 was the same as that in 1 *M* HCl.

It follows from the dependence on the ionic strength (Na_2CO_3) that the carbonate concentration can be decreased to 1.5 *M*. At lower concentrations, the steepness of the titration curves is decreased. The results obtained in determination of the Sn(II)–sorb. titer using a standard 0.1 *N* $K_3Fe(CN)_6$ solution were practically the same as those found using potassium dichromate (optimal electrolyte—sat. Na_2CO_3). Potential equilibration during the titration is much more rapid than with $K_2Cr_2O_7$.

The determination of $K_2Cr_2O_7$ at elevated temperatures up to +75°C is accompanied by variously large negative errors, resulting from reduction of Cr(VI) by sorbitol. The determination of Sn(II)–sorb. with standard 0.1 *N* $K_2Cr_2O_7$ and 0.1 *N* $K_3Fe(CN)_6$ solutions in media of sat. $NaHCO_3$, sat. Na_2CO_3, and sat. Na_2CO_3 + conc. NH_4OH (24:1) was studied in

TABLE 1

DETERMINATION OF THE TITER OF 0.1 *N* Sn(II)–SORB, USING POTASSIUM DICHROMATE

Electrolyte	pH	Taken (mg Cr(VI))	Found (mg Cr(VI))	Titer (0.1 *N*) Sn(II)–sorb.	Error (%)	E^{f}_{0} (SCE) (mV)
HCl		8.664	8.681	0.0998	+0.20	
$NaHCO_3$	8.3	8.664	8.491	0.1020	−2.00	−590
Na_2CO_3	11.5	8.664	8.681	0.0998	+0.20	−750
Na_2CO_3 + NH_4OH	12.5	8.664	8.820	0.0982	+1.80	−790
Carbonate buffer	9.3	8.664	8.543	0.1014	−1.40	
Carbonate buffer	10.0	8.664	8.595	0.1008	−0.80	
Carbonate buffer	10.6	8.664	8.647	0.1002	−0.20	

connection with possible indirect determinations. The best results were obtained in sat. Na_2CO_3 medium where the error was not below −1%.

Study of the Stability of Standard 0.1 N Sn(II) Solutions in Various Media

The stability of the prepared solutions, 0.1 N Sn(II) in 3 *M* sorb. in sat. Na_2CO_3, 0.1 *N* Sn(II) in 0.5 *M* sorbitol in water, and 0.1 *N* Sn(II) in monoethanolamine, was studied by determination of standard 0.1 *N* $K_2Cr_2O_7$ in sat. Na_2CO_3 in an inert atmosphere (Fig. 1). The standard solution was stored in an inert atmosphere. The Sn(II)–3 *M* sorb. solution is most stable (the factor does not change for ca. 12 days); the factor of the Sn(II)–0.5 *M* sorb. solution in water changes immediately after preparation. The instability of the third solution, 0.1 *N* Sn(II)–monoethanol-

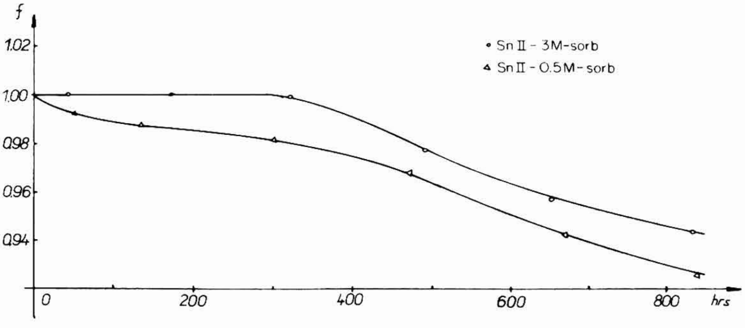

FIG. 1. The stability of standard Sn(II)–3 *M* sorb. solutions in sat. Na_2CO_3 and Sn(II)–0.5 *M* sorb. in water, depending on time.

amine renders it practically unusable. $SnCl_2 \cdot 2H_2O$ is practically insoluble in triethanolamine medium. The factor of a Sn(II)−3 M sorb. solution left in the air decreases quite rapidly.

Polarographic and Voltammetric Study of the Sn(IV)−sorb./Sn(II)−sorb. System

This study was carried out in sat. Na_2CO_3 medium. Polarographic study revealed an anodic wave at $E_{1/2} = -0.83$ V, corresponding to the oxidation Sn(II)−sorb.→Sn(IV)−sorb. and a cathodic wave at $E_{1/2} = -0.94$ V (SCE) corresponding to Sn(II)−sorb.→Sn(O). In the voltammetric study, the presence of sorbitol so affected the electrode reaction that practically no polarization curve was obtained for the studied system. The polarization voltage and current suitable for use of BiP and BiA indication of the equivalence point had to be found experimentally.

Spectrophotometric Study of the Reduction of Cr(VI) with Sn(II)−sorb.

A transient brown color (2 min), apparently corresponding to an undefined complex of Cr(IV) or Cr(V) with sorbitol, is always formed during the reduction of Cr(VI) with Sn(II)−sorb. After addition of the reducing agent Sn(II)−sorb. to the solution, the potential decreases by ca. 150 mV to positive values, i.e., an oxidant stronger than $K_2Cr_2O_7$ in the given medium is temporarily formed. Attempts to retain the unstable intermediate in solution for the time necessary for carrying out a reproducible recording were unsuccessful, even at excess complexing agent or at decreased temperatures down to 0°C. It was only possible to determine that the unknown complex absorbs in a broad region between ca. 680 and 450 nm, with a poorly defined maximum around 500 nm. Similar complexes of Cr(V) with this type of ligand are described in the literature (*5*−*10*).

Determination of Some Inorganic and Organic Redox Systems

Unless otherwise mentioned, all determinations were carried out in the following manner: x ml of a solution of the substance to be determined was pipetted into 45 ml of sat. Na_2CO_3. Then oxygen was removed from the solution by a stream of inert gas with constant mixing. During this time, the potential of the indication electrode stabilized and then titration with Sn(II)−sorb. was carried out in an inert nitrogen atmosphere.

Table 2 gives the results of the determination of the substances: the substance, the amount in milligrams in 50 ml of solution, the percentage error, the standard deviation, the variation coefficient, and the slope (ΔmV/Δml) for 0.02 ml of reagent at the equivalence point for EP and BiP indication of the equivalence point. The results of the determination of inorganic peroxides and chloroperbenzoic acid are given in Table 3; the

TABLE 2

DETERMINATION OF SOME SUBSTANCES WITH THE Sn(II)–SORB. STANDARD SOLUTION

Substance	Amount determined (mg)	Error (%)	Standard deviation	Coefficient of variation (%)	Slope (ΔmV/Δml)	
					EP	BiP
$K_2Cr_2O_7$	0.866–26.00	+0.20 to 1.04	0.010–0.136	0.36–1.11	5800	18000
$K_3Fe(CN)_6$	16.48–329.26	+0.20 to 0.67	0.25–0.57	0.16–1.93	17500	16000
H_2O_2	0.775–15.508	−0.89 to +1.54	0.018–0.032	0.21–2.71	3200	10000
Chloramine T	5.44–108.78	+0.74 to 1.60	0.15–0.30	0.27–2.80	3700	5000
I_2	6.58–131.60	+0.12 to 3.04	0.12–0.39	0.26–2.08	15700	14000
Ag^+	5.39–107.87	+0.20 to 0.93	0.09–0.33	0.30–2.02	11000	4500
Hg^{2+}	5.52–110.32	+0.82 to 1.36	0.20–0.72	0.65–3.58	6000	8500
N-bromosuccineimide	7.39–36.95	−0.92 to +1.17	0.15–0.21	0.42–2.47	8000	18500
TAR	5.39–16.16	+1.95 to 2.21	0.07–0.14	0.83–1.18	2200	—
CHP[a]	2.952–5.904	+0.44 to 0.51	0.018–0.035	0.60–0.62		
DBP	1.275–5.100	+0.27 to 0.39	0.019–0.035	0.68–1.48		
MEKP	2.104–5.252	−1.86 to −1.95	0.027–0.037	0.71–1.29		
MCHP	1.974–4.935	+0.83 to 1.42	0.024–0.034	0.68–1.18		

[a] As mg of act. O_2.

TABLE 3

DETERMINATION OF MgO_2, $NaBO_3 \cdot 4H_2O$, AND *m*-CHLOROPERBENZOIC ACID WITH A STANDARD Sn(II)–SORB. SOLUTION

MgO_2					
Taken (mg)	5.36	5.41	5.36	4.02	4.03
Found (mg)	5.40	5.43	5.39	4.04	4.01
Error (%)	+0.74	+0.37	+0.56	+0.50	−0.50
$NaBO_3 \cdot 4H_2O$					
Taken (mg)	27.65	38.72	27.68	13.85	14.00
Found (mg)	27.23	38.49	27.75	13.90	13.90
Error (%)	−1.52	−0.59	+0.25	+0.36	−0.73
m-Chloroperbenzoic acid					
Taken (mg)	8.372	8.431	8.263	8.348	8.465
Found (mg)	8.281	8.314	8.066	8.231	8.348
Error (%)	−1.09	−1.38	−2.38	−1.40	−1.38

results of the determination of $K_2Cr_2O_7$ with EP, BiP, and BiA indication of the equivalence point are given in Fig. 2.

Determination of Organic Peroxides

The following substances were successfully determined using Sn(II)–sorb.: cyclohexanone peroxide (CHP), dibenzoyl peroxide (DBP), methylethylketone peroxide (MEKP), methylcyclohexanone peroxide (MCHP), and *m*-chloroperbenzoic acid. Only the latter could be determined directly using a sample dissolved in a minimal amount of ethanol diluted with oxygen-free sat. Na_2CO_3 to 50 ml. Then titration was carried out with EP, BiP (I = 2–3 μA), and BiA (U = 800 mV) indication of equivalence. Solutions containing 0.1 or 0.05 N of the solid CHP and DBP, respectively, in isopropanol (IPA) were prepared and were determined indirectly by titration of the unconsumed amount of Sn(II)–sorb. with dichromate. IPA solutions were prepared from the MEKP and MCHP solutions and were determined indirectly. The EP, BiP (I = 3 μA), and BiA (U = 1000 mV) methods were used for determination of the equivalence point with all four organic peroxides. All the results are in very good agreement with those obtained by the iodometric methods (*11*), used for standardization.

DISCUSSION

The use of the sorbitol complex of divalent tin in reductometry was studied in connection with electrochemical study of the use of complexing agents in inorganic analysis. It follows from the formal redox potential values obtained from the titration curves for the determination of chromium that this is a medium strong reducing agent. These results are in

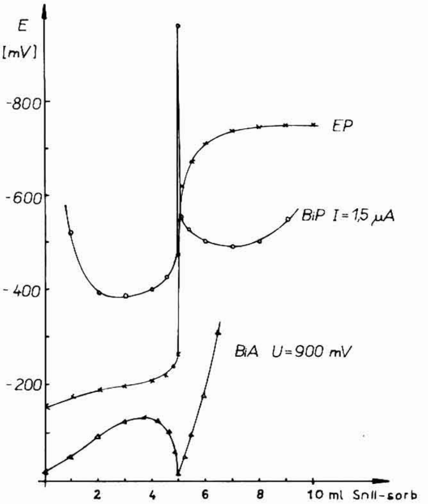

FIG. 2. The determination of potassium dichromate with Sn(II)–sorb. using Ep, BiP (I = 1.5 μA), and BiA (U = 900 mV) equivalence point indication.

agreement with a study of the stability of 0.1 N Sn(II)–sorb. solutions, which are much more stable than Sn(II) solutions in hydrochloric acid in the air (see Fig. 1). The standard solutions were standardized using dichromate potassium and hexacyanoferrate. It follows from the dependences on the ionic strength and pH that an aqueous solution of sodium carbonate with a concentration $\geqslant 1.5\ M$ is most suitable. In this medium, even the determination of Sn(II)–sorb. with 0.1 N $K_2Cr_2O_7$ and 0.1 N $K_3Fe(CN)_6$ (for back-titration) can be carried out. It follows from polarographic study of the Sn(IV)–sorb./Sn(II)–sorb. system that the reaction is irreversible. The effect of sorbitol on the electrode reactions of the Sn(IV)/Sn(II) system at a PtRDE prevented voltametric study.

The nature of the brown color, corresponding to an intermediary complex of Cr with sorbitol and formed during reduction of dichromate with Sn(II)–sorb., could not be clarified by spectrophotometric study. It follows from Table 2 that this newly proposed reagent can be used with sufficient precision for the potentiometric, bipotentiometric, or biamperometric determination of a number of inorganic substances. The high value of the slopes of the bipotentiometric titration curves permits determinations at a microlevel.

In addition to the substances mentioned above, the following elements were studied: (a) Ce(IV), Mn(VII), Br(V), Fe(III), V(V), U(VI), Ir(IV); and (b) Pt(IV), Pd(II), Bi(III), Cu(II), Se(IV), Se(VI), Te(VI). Ions in group (a) are not reduced quantitatively even at elevated temperatures or in excess reagent. The ions given in (b) are reduced to the metal, either by sorbitol alone or Pt(IV), Pd(II), or else the equivalence point in both direct and indirect titrations does not correspond to any marked potential change. Among organic substances, some organic peroxides, *N*-bromosuccinimide and thiazolylazoresorcinol, have been determined successfully. Further possible uses of the standard Sn(II)–sorb. reagent will be studied.

SUMMARY

The possibility of using the sorbitol complex of divalent tin in volumetric analysis was studied. Potassium dichromate and potassium hexacyanoferrate were used for standardization. The dependences on the pH and ionic strength indicate that the most suitable titration medium is sat. Na_2CO_3. The titrant is stable for 14 days in an inert atmosphere. It was found polarographically and voltammetrically that the Sn(IV)–sorb./Sn(II) sorb, system is irreversible. Standard solutions of Sn(II)–sorb. were used to determine $K_2Cr_2O_7$, $K_3Fe(CN)_6$, H_2O_2, and inorganic and organic peroxides, I_2, Hg(II), Ag(I), chloramine T, chlorinated lime, *n*-bromosuccinimide, and TAR. Potentiometry, bipotentiometry, and biamperometry were used for determination of the equivalence point.

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Determination of Tungsten by Calcium Atomization Inhibition Titration

M. TADDIA

"G. Ciamician" Chemical Institute of the University, I-40126 Bologna, Italy

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INTRODUCTION

Flame emission inhibition titration is an established analytical technique. Indirect methods for anions and cations based on their inhibitor effect on atomic emission of calcium have been reported (*1*, *4*, *5*). When the analyte exhibits only a moderate depressant effect on the calcium signal, the atomization inhibition titration (AIT) is advantageously integrated with a subsequent release step employing lanthanum or similar agent (*1*, *5*). Since many atomic absorption spectrophotometers have flame emission capability, it has been found convenient to develop new AIT methods for elements that would require a nitrous oxide–acetylene flame for their AAS determination. The present communication describes the application of the AIT-release technique for the determination of tungsten.

EXPERIMENTAL

Reagents. A tungsten stock solution (1000 ppm) was prepared from sodium tungstate dihydrate. Titrant solution of calcium (20 ppm) was prepared by dilution of 1000 ppm Ca solution which was made by dissolving calcium chloride dihydrate in water. The nitrate salt was used for the preparation of titrant solution of lanthanum (1000 ppm). All reagents used were of analytical grade and all solutions were prepared with deionized–distilled water.

Apparatus. Flame emission measurements were made with a Perkin-Elmer Mod. 372A atomic absorption spectrophotometer with flame emission capability. A hydrogen–air flame with a nitrous oxide burner head was used. The gas flow was adjusted to obtain maximum depressive effect with minimum noise. The instrument was used at the 2 nm nominal bandpass. The emission intensity was measured at the 422.7 nm calcium atomic emission line. Before processing each series of samples, the gain control was adjusted while aspirating a standard to always provide the same reading. Constant rate infusion pumps and 20-ml syringes were used

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for delivery of the calcium and lanthanum titrants. Titration curves were recorded on a Leeds-Northrup Mod. 681A potentiometric recorder.

Procedure. The sample solution (100 ml) 0.1 *M* in hydrochloric acid was placed in a 150-ml beaker on a magnetic stirrer. Injection and aspiration tubes were positioned and stirring was started. Soon after aspiration has begun, calcium solution flow and recording were initiated simultaneously. Titrant addition was stopped when the appropriate calcium emission signal was reached. Lanthanum was then added until a new constant emission reading was obtained. The signal enhancement due to the lanthanum releasing effect was determined. A calibration graph was constructed by analyzing a series of standards. A blank measure was done under the same conditions. Titrant flow rate was 3.0 ml min^{-1} for calcium and 4.0 ml min^{-1} for lanthanum. The aspiration rate was 4.4 ml min^{-1}.

RESULTS AND DISCUSSION

Tungstate is known to depress the calcium atomic absorption signal both in oxy-hydrogen (*2*) and air–acetylene (*3*) flame. Recently we have shown (*5*) that tungstate interferes in the vanadium determination by AIT using calcium as monitor metal. These observations have led to the suggestion that the tungsten determination by AIT could be feasible. A preliminary investigation of the tungsten interference on the calcium atomic emission in hydrogen–air flame was still necessary because no quantitative data were available. A measurement of the calcium signal as a function of the interferent concentration was done. The interference curve is shown in Fig. 1. With rising interferent concentration the depression of the signal approaches saturation. For a molar ratio of calcium to tungsten equal to 1.5, tungsten depresses the calcium emission to approximately 58% of the emission of calcium alone. This behavior suggests that a so called "volatilization interference" occurs, that is tungstate forms an involatile compound with the analyte. Calcium depression is proportional to tungsten concentration up to 1 ppm W so the determination by a release

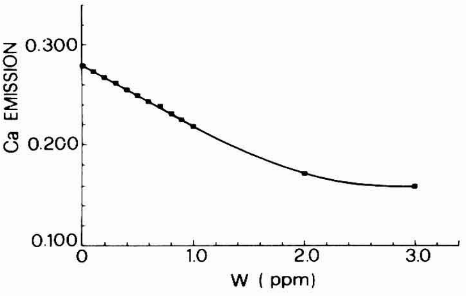

FIG. 1. Effect of tungsten on the emission of calcium in the hydrogen–air flame (1 ppm Ca, 0.1 *M* HCl).

effect was possible. Because of the dependence of the calcium signal from the acid concentration, all experiments were performed in 0.1 *M* hydrochloric acid. Inhibition titrations were carried out on solutions containing 0.2−1.0 ppm W. Calcium addition was discontinued when a predetermined emission intensity (I_0) was reached. Lanthanum was added to release calcium inhibited by tungsten to obtain a constant sharp enhancement in the signal (ΔI). Values of I_0 between 0.100 and 0.110 were found suitable to give reproducible ΔI. A linear increase in the magnitude of ΔI by increasing the tungsten concentration from 0.2 to 0.8 ppm was observed (Fig. 2). Detection and determination limits were calculated for a 95% confidence level and a 5% relative standard deviation. The detection limit was 0.013 ppm and the determination limit was 0.12 ppm. Nine replicate determinations on 0.20 ppm W provided a relative standard deviation of 7.4%. Accuracy was tested by analyzing synthetic samples containing known amounts of tungsten. Results are shown in Table 1. The recovery is always better than 95%. Although AIT offers a better detection limit compared to AAS (1 ppm), specificity is certainly poorer. The effect of 13 foreign ions on the tungsten determination was investigated. Table 2 summarizes the effect of these interfering ions. As expected, all the chemical species that form refractory compounds with calcium are strongly interferents (higher ΔI) because lanthanum releases them from the refractories. Data obtained for phosphate, silicate, sulfate, aluminum, vanadium, and titanium may be correlated with their ability to form involatile compounds with calcium. Cations such as nickel, cobalt, and iron interfere contrariwise. This was ascribed to a concomitant enhancing and releasing effect on calcium of these cations. The well-known releasing effect of iron did not need further investigations. The enhancing effect for nickel and cobalt was tested apart. A signal enhancement of approximately 10% was found by measuring the emission of 1 ppm calcium in the

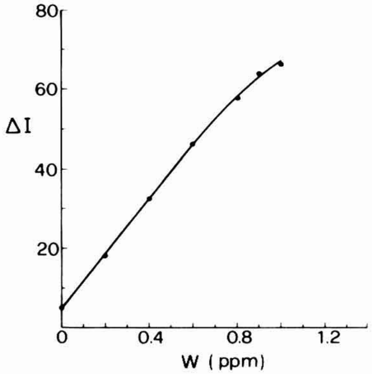

FIG. 2. Calibration graph for the determination of tungsten. For conditions see text.

TABLE 1
ACCURACY AND PRECISION OF SYNTHETIC SAMPLES

W added (ppm)	Number of determinations	W found, mean value (ppm)	Error (%)	s (ppm)
0.20	9	0.19	−5.0	0.014
0.40	4	0.39	−2.5	0.017
0.60	4	0.61	+1.7	0.012
0.80	3	0.78	−2.5	

presence of 10 ppm nickel or cobalt. The releasing effect on calcium inhibited by tungsten was tested for 1 ppm calcium in the presence of 0.4, 1.0, and 2 ppm tungsten. By adding 10–50 ppm nickel or cobalt the calcium emission signal was released.

In conclusion AIT of tungsten can be suggested in analytical practice if precaution is taken to remove the interferences above by preliminary separation steps based on ion-exchange and solvent extraction.

TABLE 2
EFFECT OF VARIOUS IONS ON TUNGSTEN DETERMINATION AT A CONCENTRATION OF 0.4 ppm[a]

Ion	Source	Concentration[b] (ppm)	ΔI
$H_2PO_4^-$	KH_2PO_4	0.10	75
		0.50	246
SiO_3^{2-}	Na_2SiO_3	0.10	61
		0.50	117
SO_4^{2-}	Na_2SO_4	0.20	51
		0.50	108
Al^{3+}	$Al(ClO_4)_3$	1.00	79
		10.0	174
Ti^{4+}	$TiCl_4$	0.20	92
		0.50	144
VO^{2+}	V_2O_5	0.50	67
		1.00	77
Cr^{3+}	$CrCl_3$	10.0	29
$Mo_7O_{24}^{6-}$	$(NH_4)_6Mo_7O_{24}$	10.0	57
Mn^{2+}	$MnCl_2$	10.0	36
Ni^{2+}	$NiCl_2$	10.0	0
Co^{2+}	$CoCl_2$	10.0	8
Cu^{2+}	$Cu(NO_3)_2$	10.0	34
Fe^{3+}	$FeCl_3$	10.0	24

[a] With no interference present, $\Delta I = 32$.

[b] As element.

SUMMARY

A method for the determination of tungsten utilizing inhibition effect on calcium emission in hydrogen–air flame is described. The technique is based on atomization inhibition titration (AIT) and subsequent release by means of lanthanum. The signal enhancement following the lanthanum addition is proportional to the tungsten concentration up to 0.8 ppm W. A detection limit of 0.013 ppm and a determination limit of 0.12 ppm were found. The effect of 13 foreign ions on the tungsten determination was investigated.

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A Spectrophotometric Study of the Color Reaction of Zinc with 2,2′-Dipyridyl-2-pyridylhydrazone (DPPH)

G. S. VASILIKIOTIS*,[1] AND H. ALEXAKI-TZIVANIDOU†

*Laboratory of Analytical Chemistry, School of Physics and Mathematics, and †Laboratory of Physiology, Veterinary Faculty, University of Thessaloniki, Thessaloniki, Greece

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INTRODUCTION

The "ferroin" type compound 2,2′-dipyridyl-2-pyridylhydrazone (DPPH) previously synthesized (*1, 12*) has been proven to give intensely colored complexes with several metal ions (*1, 2, 4, 5, 12*). By these reactions sensitive methods of determination for a number of cations can be developed.

In this communication the analytical properties of the zinc–DPPH complex are studied and the use of DPPH for the colorimetric as well as the complexometric determination of zinc is investigated.

MATERIALS AND METHODS

Apparatus

Unicam SP 800 and a Gilford 2400-2 spectrophotometers, equipped with matched 1-cm quartz cells, were used as well as an L. Pusle pH meter with a combined electrode.

Reagents

2,2′-Dipyridyl-2-pyridylhydrazone (DPPH) ethanolic solution, 10^{-2} M. The synthesis of the reagent has previously been described (*1, 2, 12*). Dissolve 0.2753 g of DPPH in 100 ml of ethanol. The solution is stable for several months if kept in an amber glass bottle.

Stock zinc solution, 10^{-2} M. This solution was prepared by dissolving zinc powder (Merck, p.a. reagent) in a minimal quantity of diluted hydrochloric acid and diluting to the volume with redistilled water. More diluted solutions, when necessary, were obtained by accurate dilution of the stock one. The solutions were standardized by EDTA titration.

Double-distilled water, from an all-Pyrex-glass apparatus, was used throughout this work.

All the other reagents used were of analytical grade (E. Merck or BDH).

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[1] Correspondence and reprints: Professor G. S. Vasilikiotis, Laboratory of Analytical Chemistry, University of Thessaloniki, Thessaloniki, Greece.

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Procedure

Take an aliquot of the sample, containing 12.5 to 50 μg of zinc(II), in a 50-ml volumetric flask and dilute it with redistilled water to 40 ml. Add 2 ml of the ethanolic solution of DPPH; bring the pH between 11.1 and 11.6 with sodium hydroxide or perchloric acid and dilute to the volume. Read the absorbance at 442 nm. Calculate the zinc content using a standard or a calibration graph.

RESULTS AND DISCUSSION

The study of the reaction of zinc with DPPH was made at an ionic strength of $\mu = 0.1$, regulated with sodium perchlorate. Perchloric acid and sodium hydroxide solutions were used for the pH regulation.

Absorption spectra. DPPH forms a yellow complex with zinc(II) in alkaline solutions. In Fig. 1 the uv/vis spectra of DPPH (A) and the zinc–DPPH complex (B) are given. It is considered that in B all the quantity of DPPH is in the zinc–DPPH complex form, as a five-fold metal excess was used. It is evident that at 442 nm, the wavelength of maximum absorption of the complex, the absorption of the ligand is negligible. This wavelength is consequently suitable as an analytical one. Changes in the metal-to-ligand molar ratio (from 10:1 to 1:10) do not alter the maximum

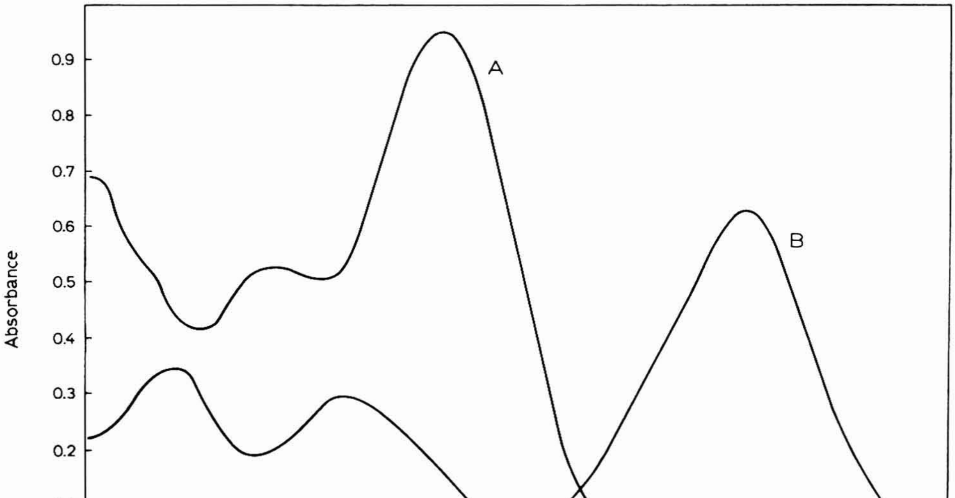


FIG. 1. Absorption spectra at pH 11.2. (A) DPPH, $C_{DPPH} = 4.8 \times 10^{-5}\ M$. (B) Zinc–DPPH complex, $C_{Zn} = 1.2 \times 10^{-4}\ M$, $C_{DPPH} = 2.4 \times 10^{-5}\ M$.

wavelength. From this fact it may be considered that under the proposed procedure principally one complex is formed.

Influence of the pH. The effect of the pH on the complex formation is shown in Fig. 2. Complete color development is observed above pH 10.9 and absorbance is constant for a range of at least 1.5 pH units. There is no shift of the maximum wavelength through the whole pH range studied and it may be, hence, considered that principally a single complex is formed through the pH range of about 8 to 12.5.

Speed of formation and stability of the complex. In the optimal pH range the formation of the zinc–DPPH complex is almost instantaneous and the absorbance of the complex solution remains unchanged for at least 20 hr, even in the presence of diffuse daylight.

Effect of the solvent. As an ethanolic solution of DPPH was used, the effect of the solvent was examined on the formation of the complex in water–ethanolic media. Tests performed at pH values between 11 and 12 have shown that changes in the ethanol content of the solvent between 1.5 and 50% have no effect on the molecular extinction coefficient of the complex at 442 nm. On the other hand, increasing the ethanol content to above 10% diminishes the stability of the color with time (e.g., for 12% ethanol a 3% decrease is observed in 4 hr).

Molar composition of the complex. The Job's plot at three wavelengths (λ) and three different total concentrations (Fig. 3), as well as the molar

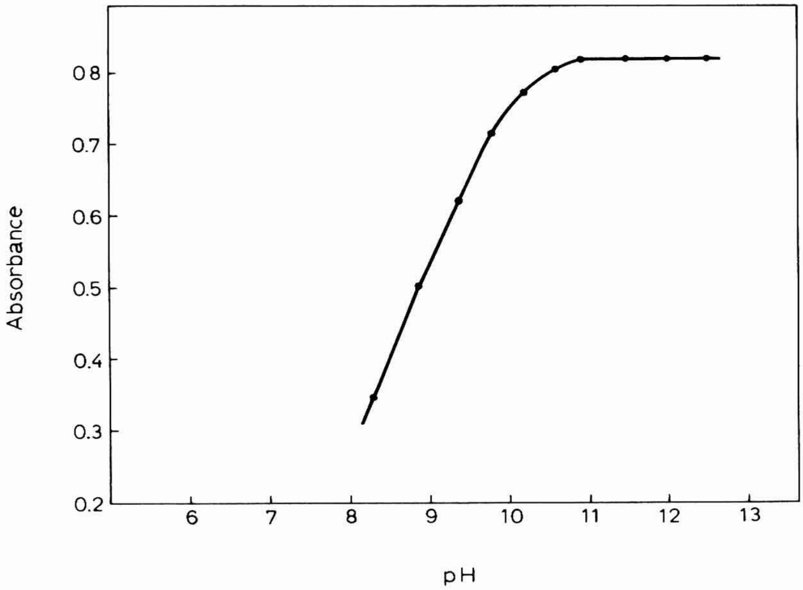


FIG. 2. Effect of pH on the complex formation. $C_{Zn} = 1.5 \times 10^{-5}\ M$. $C_{DPPH} = 2 \times 10^{-4}\ M$. Absorbance curve taken at 442 nm.

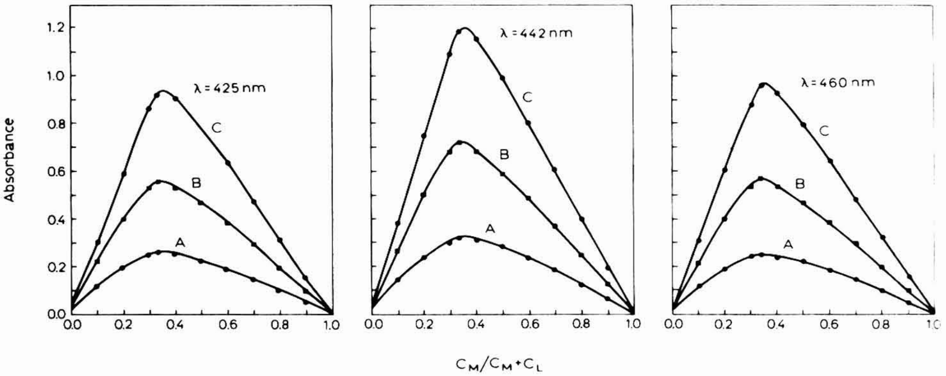
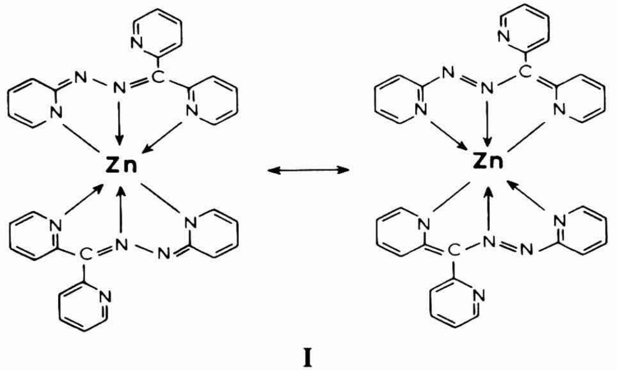


FIG. 3. The Job's plot at three wavelengths and three total concentrations, taken at pH 11.2 ± 0.1. Zinc (II) and DPPH total concentration, (A) 2.4×10^{-5} M, (B) 4.8×10^{-5} M, (C) 7.2×10^{-5} M.

ratio plot (Fig. 4) show that the main complex formed is a 2:1 DPPH-to-zinc species. As previously mentioned, no shift of the maximum wavelength was observed by changing the metal-to-ligand mole ratio, by both methods, demonstrating the formation of a single main complex. Following the conclusions of Lions and Martin (9) and those of Geldard and Lions (7), the reagent may act as a tridentate ligand forming a complex with zinc of the L_2Zn general type. In alkaline solution the ligand is deprotonated in the imino group and the complex possibly has the structure **I**.



I

Isolation and structure of the complex. The solid zinc–DPPH complex was prepared by dissolving 0.55 g of DPPH in 50 ml of hot ethanol and separately 0.13 g of zinc powder in a minimal quantity of hot diluted hydrochloric acid. The two hot solutions were mixed by stirring and then made alkaline with 4 M sodium hydroxide solution. After diluting this mixture with water and cooling, an orange precipitate was formed which was filtered, twice recrystallized from an ethanol–water (3:1) mixture and

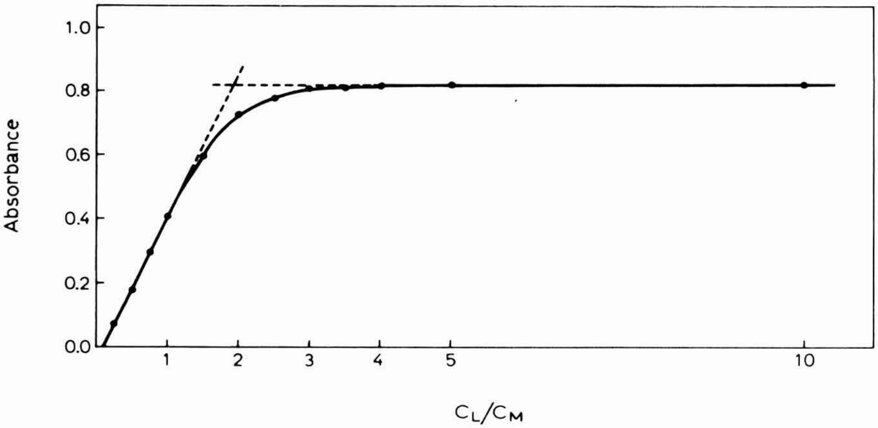


FIG. 4. The molar ratio plot taken at 442 nm and pH 11.2 ± 0.1. Zinc (II) concentration 1.6 × 10^{-5} *M*.

dried in a vacuum desiccator over calcium chloride. About 0.45 g of the complex was obtained. Its melting point was 151–154°C. Calculated for $C_{32}H_{24}N_{10}Zn$ (L_2Zn): C = 62.60%, H = 3.94%, N = 22.81%. Found: C = 62.03%, H = 4.09%, N = 23.03%. In Fig. 5 the mass spectrum of the isolated solid is given. The molecular ion was found to be $M = m/e = 611 \pm 1$. This value justifies a molecular weight of 313.9 for $C_{32}H_{24}N_{10}Zn$.

Both the elemental analysis and the mass spectrum indicate that the structure of the isolated zinc–DPPH complex may be that shown in **I**.

Effect of the reagent concentration. As shown in Fig. 4, under the proposed conditions a fourfold excess of ligand is sufficient for full color development. Apparently, a larger excess is necessary for possible analytical purposes.

Determination of the overall apparent instability constant of the complex. This determination was made at pH 11.1, ionic strength 0.1, and temperature 25 ± 0.5°C by (*a*) the Harvey and Manning (*8*) method and (*b*) the Turner and Anderson (*6, 11*) method. By both methods the value

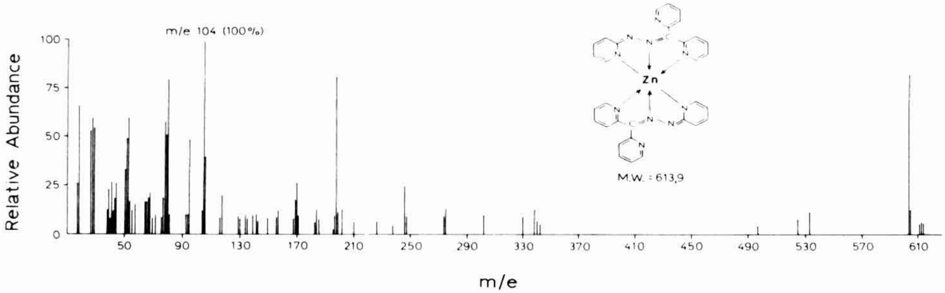


FIG. 5. The mass spectrum of the isolated zinc–DPPH complex.

obtained was of the order of 10^{-12} (about 2.5×10^{-12} by the first and about 2.8×10^{-12} by the second method).

Conformity to Beer's law and sensitivity of the reaction. The Beer's law plot is a straight line passing through the origin for a zinc (II) concentration of 2×10^{-6} to 2×10^{-5} *M* (or about 0.13 to 1.30 ppm), while the Ringboom's graph shows an optimal concentration range from 4×10^{-6} to 1.4×10^{-5} *M* (or about 0.25 to 0.92 ppm).

The molecular extinction coefficient of the zinc–DPPH complex at 442 nm was found to be 5.25×10^4 $M^{-1}\cdot\text{cm}^{-1}$. The Sandell's sensitivity was calculated to be 0.00123 μg Zn/cm^2 for an absorbance of 0.001 at 442 nm. This is a very good sensitivity compared to that of other zinc reagents.

Precision. The precision of the method was evaluated by analyzing solutions of known zinc concentration. Three sets of solutions of 10 samples each were prepared. The results are shown in Table 1.

Interference of foreign ions. The following quantities of foreign ions do not interfere with the determination of 1 ppm zinc, an error of less than ±3% being acceptable: 1000 ppm of Br^-, I^-, SO_4^{2-}, NO_3^-, $(COO^-)_2$, CH_3COO^-, tartrate, Ba^{2+}, Sb^{2+}, Bi^{2+}; 500 ppm of Cl^-, PO_4^{3-}, SCN^-, Sr^{2+}; 300 ppm of F^-, Na^+, K^+, Ca^{2+}, citrate; 200 ppm of Sn^{2+}, WO_4^{2-}; 100 ppm of As^{3+}; 50 ppm of Al^{3+}, Zr^{2+}; 25 ppm of MoO_4^{2-}; 15 ppm of Th^{4+}, UO_2^{2+}; 8 ppm of Mn^{2+}; 1 ppm of Be^{2+}, Cr^{3+}, VO_3^-, Co^{2+}, Pb^{2+}; 0.5 ppm of Ni^{2+}, Hg^{2+}. Iron, copper, and cadmium interfere strongly. Iron and copper can be eliminated by cyanate masking (*3*).

Complexometric titration of zinc. The reaction of zinc (II) with DPPH at pH 10.9–12.4 is stoichiometric, rapid, and very sensitive. Moreover, the obtained value for the instability constant of the complex, $K_{\mathrm{Zn(DPPH)_2}}$, at the above mentioned pH range allows the following relation to be valid (*10, 13*):

$$5 < pK_{\mathrm{Zn(DPPH)_2}} < pK_{\mathrm{ZnEDTA}} - 4.$$

Hence, DPPH is suitable as a metallochromic indicator for the complexometric determination of zinc at this pH interval, in the absence of interfering ions. This is shown in Fig. 6. Experimental data have shown

TABLE 1

PRECISION

Zn (ppm)		Percentage relative error	Standard deviation	Coefficient of variation
Taken	Found			
0.195	0.193	−1.02	0.0025	1.28
0.654	0.658	+0.61	0.007	1.07
1.046	1.054	+0.76	0.012	1.15

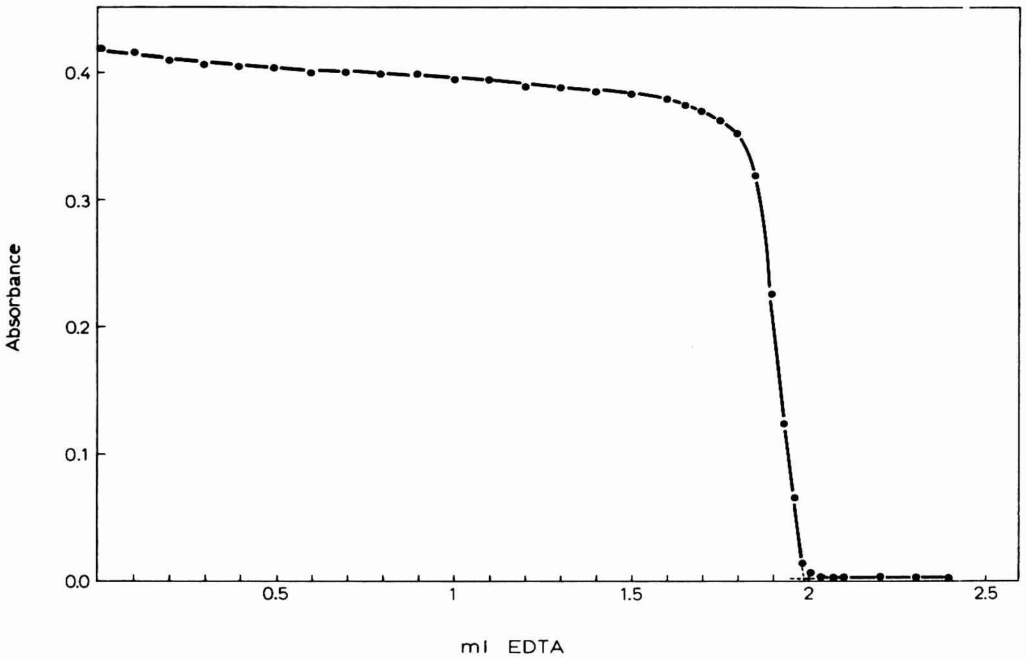


FIG. 6. Complexometric titration of 159.8 μg Zn (II) at pH 11.1, with an EDTA solution of 10^{-5} *M*, in the presence of DPPH at a concentration of about 10^{-5} *M*.

that the optimal concentration for zinc is 5×10^{-6} to 1×10^{-4} *M*, for EDTA 1×10^{-3} *M*, and that for DPPH is of the order of 1×10^{-5} *M*.

The precision of the complexometric determination was found to be the same as that of the spectrophotometric one.

SUMMARY

A spectrophotometric study of the zinc complex with 2,2′-dipyridyl-2-pyridylhydrazone (DPPH) is presented. The optimal formation conditions, the composition, and the overall apparent instability constant of the yellow, water-soluble zinc–DPPH complex are studied. A simple, rapid, and very sensitive spectrophotometric method for the determination of zinc with DPPH is proposed. At pH 10.9 to 12.4 the molecular extinction coefficient of the complex is 5.25×10^4 $M^{-1}\cdot$cm^{-1} at 442 nm. DPPH can be also used as a methallochromic indicator for the complexometric titration of zinc.

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Schiff Bases Derived from Biacetyl as Analytical Reagents: A Review

A. G. ASUERO

Applied Chemical Analysis Department, Faculty of Pharmacy,
The University of Seville, Seville-4, Spain

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INTRODUCTION

Since the appearance in 1905 of a short paper published by Tschugaev (*115*), dealing with dimethylglyoxime, numerous papers have been published on the analytical applications of the various dioximes, and several reviews have appeared in the literature. The two last surveys were made by Egneus (*32*), who summarized the analytical procedures in condensed form and Singh *et al.* (*104*). Nevertheless, the other Schiff bases derived from biacetyl have never been reviewed in spite of the fact that among these compounds some with very interesting properties and applications can be found. The aim of this paper is to review the analytical literature which deals with investigations carried out with the use of the mentioned compounds.

BIACETYL BIS-SIMMETRICAL DERIVATIVES

The Iron–Methine Chromophore

On mixing alcoholic or aqueous solution of glyoxal with ferrous salts and excess methylamine, a strong blue color develops, which changes slowly to a deep purple red. Biacetyl, methylglyoxal, phenylglyoxal, and cyclohexane-1,2-dione condense as glyoxal does with methylamine and ferrous salts (*62*), but no colored complexes are obtained from benzil under similar conditions. The blue color is destroyed by acidification whereas the red coloration remains stable. Krumholtz (*62*) assumed that the red coloration was due to the ferrous complex of the corresponding mono-*N*-methyl-imine in accordance with Feigl (*35*) and Kuester (*128*). The substitution of the *N*-methyl by *N*-phenyl group changes the behavior of the α-diimine against ferrous salts. The intensive color is related to the presence of the chromogenic group $>C(=N-)-C(=N-)-$, and it seems to be of secondary importance as to whether or not the $>C=N-$ groups belong to a heterocyclic ring. The visible spectra of 1,10-phenanthroline, 2,2′-bipyridine and biacetyl bismethylimine–iron(II) complexes exhibit

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single maxima of high molar absorptivity (8,700 to 11,500 nm) and have a shoulder on the violet side of the point of maximum absorption. The range of maxima is from 510 to 568 nm. The iron(II) complex of biacetyl bis(hydrazone) does not conform to this class, having a single symmetrical peak of a somewhat lower molar absorptivity at 444 nm (*20*).

In Situ Reactions: The Iron(II)–Biacetyl–Hydrazine Reaction

A great number of reagents are known for the colorimetry of iron(II). Most of them possess the chain –N–C–C–N– and the preparation of some of them dates from 1898 (*92*). Among the compounds which possess the mentioned grouping, the ferrous complex of biacetyl bis(hydrazone) is found, to which Busch and Bailar (*20*) attributed the formula $Fe(C_4H_{10}N_4)_3FeCl_4$. Fifteen years ago, in 1965, an investigation was begun (*23*) whose main purpose was to obtain that complex *in situ* from the metallic ion and the organic ingredients, in solution. The idea was to prepare colored systems useful for photometric measurements by using the starting materials of an organic reagent instead of the reagent itself. This seemed more suitable than to add a new reagent to the numerous already existing ones for the colorimetry of iron.

The direct approach in synthesizing a Schiff base from the condensation of an *o*-hydroxy, *o*-amino, or *o*-mercaptoamine with a carbonyl compound often results in the undesirable side reaction involving ring closure with the formation of a heterocyclic compound. Nevertheless, in the presence of certain metal ions this rearranges to give the Schiff base chelate quantitatively (Schiff, 1879) (*58*) (Fig. 1). This fact, the preparation of formaldoxime (*128*) for the colorimetry of manganese and the *in situ* synthesis of some organic reagents used for the precipitation and gravimetric determination of metal ions (*42, 43*), can be cited as

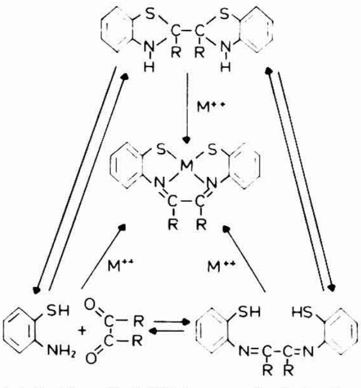

FIG. 1. The manner in which the Schiff base chelate is formed from the heterocyclic reagent (2,2′-substituted 2,2′-benzothiazoline).

antecedents of this technique. Although in the later case the ressemblance is not complete since in gravimetry the reaction product between the metal ion and the organic reagent is separated from the solution forming a new phase, whereas in the case of the formation of a soluble compound, this remains in equilibrium in the homogeneous phase. Also it may occur, and inconveniently so, that the components of the reagents react with the metal ions separately.

As more recent examples of this *in situ* technique, the following can be cited: the reactions of hydrolytic degradation and exchange of $>C=N-$ groups that are of great importance for the formation of certain iron (*111*) and copper (*117*) complexes; the hydrolysis of *S*-2-(3-mercaptoquinoxalinyl)thiouronium chloride (*29*) which occurs rapidly in ammonia buffer at pH 10, to give a red coloration with nickel(II); and the extraction–spectrophotometric study made of complexes of cobalt and nickel with *in situ* generated nitroso derivatives (*84*) of resorcinol and salicyl acid.

There are indubitable advantages to working in this manner. The fact of not having to isolate, nor purify the reagent, made its preparation more rapid and economical; and, in some cases, the formation of the organic metallic complex displaces the equilibrium and favors the formation of some compounds in the synthesis of which difficulties appear. Sometimes different products are obtained instead of the expected products, due to the possibility of collateral reactions. In any case this fact can be verified later by synthesizing the reagent and making a comparison of the spectra obtained in the two cases. If the formation of these new complexes is accompanied by colors whose reproducibilities are well within a determinated condition, new analytical applications may be reported. The applications of this technique appear to be numerous. In the author's opinion, the possibilities which the *in situ* technique offers have still not been exploited properly by the analyst.

By mixing Fe(II), biacetyl, and hydrazine (*23*) in a given condition of temperature and pH a red-orange color is developed which is very stable and has an absorption maximum at 490 nm. Among the variables which exercise a decisive influence in the reaction, one is the pH; it is not produced either in alkaline or acid medium. If the pH rises to about 9 with sodium bicarbonate and if, once the solution is heated, the solution is acidified to pH 4–7, then an increment in the molar absorptivity is produced. This coloration has been applied to the colorimetric estimation of iron (*46, 61*). The ferrous complex of biacetyl bis(hydrazone) has a different color from the one obtained *in situ* with iron(II), biacetyl, and hydrazine, and it absorbs at 442 nm. Biacetylhydrazone azine is not as good a reagent as biacetyl bis(hydrazone) for the colorimetry of iron (see Table 1). Thus, it is possible that the iron(II)–biacetyl–hydrazine complex was

TABLE 1

SCHIFF BASES DERIVED FROM BIACETYL AND RELATED COMPOUNDS AS ANALYTICAL REAGENTS FOR CATIONS

Reagent	Cation	max (nm)	liters mol^{-1} cm^{-1}	Medium, comments	Reference
Biacetyl bis(*N*-methylimine)	Fe(II)	568	10,700		(*62*)
Biacetyl + hydrazine	Fe(II)	490	9,800	pH 5.5–6	(*23*)
		490	11,500	First, pH 9.4, then pH 4–7	(*46*)
			13,800	Extr. into nitrobenzene	
Biacetyl bis(hydrazone)	Fe(II)	442	6,900	NaCH COO; 20% ethanol	(*23*)
Biacetyl monohydrazone azine	Cu(I)	350	7,230	Extr. into benzyl alcohol	(*114*)
Biacetylhydrazone azine	Fe(II)	460	5,585	$NaCH_3COO$, 10% ethanol	
Biacetyl oxime azine	Ni(II)			Complex prepared and studied	(*110*)
Biacetyl monothiosemicarbazone	Cu(II)	410	6,300	pH 5 (acetate buffer)	(*90*)
	Ni(II)	400	12,400	pH 8 (phosphate buffer)	
2,2′-Dimethyl-2,2′-bis-benzothiazoline	Zn(II)	350	3.88	Pyridine as solvent	(*58*)
		510	3.33[a]	Pyridine as solvent	
	Cd(II)	335	3.69[a]	Pyridine as solvent	
		463	3.31[a]	Pyridine as solvent	
Biacetyl bis(thiosemicarbazone)	Co(II)	510	1,710	pH 10 (ammonia buffer)	(Ref. *32* in *7*)
		450	4,710	pH 3 (phthalate/HCl buffer)	
Biacetyl bis(salicylhydrazone)	Zn(II) Cd(II)			Gravimetrically; Mg, Mn, Co, Cu interferes	(*96*)
Biacetyl bis(5-bromosalicylhydrazone)	Zn(II) Cd(II)			Gravimetrically	(*96*)
Biacetylmonoxime 2,4-dinitrophenylhydrazone	Co(II)			Qualitative detection	(*2*)
Biacetylmonoxime *p*-nitrophenylhydrazone	Co(II)	520		Excess of reagent separated with ethyl ether	(*2*)
Biacetylmonoxime *p*-nitrophenylhydrazone + ethylenediamine	Co(II)	520			(*83*)

Biacetylmonoxime thiosemicarbazone	Fe(II), Co(II), Ni(II), Cu(II), Bi(III)				*(13, 103)*
	Mn(II)			Microchemical test	*(54)*
	Mn(III)	540		pH 9.8–11.7	*(55)*
	Mn(III)			Polarography	*(119, 121)*
	Mn(II)	346			*(78)*
	Fe(II)	507	6,250	0.01 *N* $HClO_4$	*(112)*
	O_2	540		pH 10 (ammonia buffer, 10–100 ppm O_2)	*(120)*
Biacetylmonoxime phenylthiosemicarbazone	Mn(III)	550	3,600	pH 9–12; extr. in $CHCl_3$	*(22)*
	Cu(II)	395	10,300	pH 4.8; extr. in amyl alc.	*(21)*
	Bi(III)	375	17,000	pH 3.2–5.0; extr. in amyl alc.	*(21)*
Biacetylmonoxime 2-benzothiazolylhydrazone	Pd(II)			Qualitative detection	*(40)*
	Pd(II)	560	5,110	Extr. from pH 1.8 into $CHCl_3$	*(1)*
Biacetylmonoxime-ethylenediimine	Ni(II), Pd(II)			Gravimetrically	*(72)*
Biacetylmonoxime-*o*-phenylenediimine	Ni(II), Pd(II)			Gravimetrically	*(72)*
Biacetyl bis(4-phenyl-3-thiosemicarbazone)				Separation of several metals by pH-dependent extraction and by thin-layer chromatography	*(11)*
				Extractive spectrophotometric determinations	
				High-performance liquid chromatography separation of several metals at ng levels	*(52)*
	Cu(II)	435 530	12,700 8,200	pH 1.8–11.9; 60% dimethylformamide	*(5)*
	Zn(II)	440	21,500	pH 5.5–9.9; 60% dimethylformamide	*(6)*
	Hg(II)	420	14,500	pH 4.2–9.7; 60% dimethylformamide	*(8)*
Biacetyl bis(4-hydroxybenzoylhydrazone)	Several	440	21,000–6,500	20 μM of cation added to 1 mM ligand in 10 mM sodium hydroxide	*(66)*
Biacetyl bis(benzoylhydrazone)				Chelates with tin(IV), lead(II), and lead(IV) prepared and spectral data obtained	*(25)*
Biacetylmonosemioxamazone				Reactionability with metal ions	*(56)*
Biacetylmonosalicyloylhydrazone				Reactionability with metal ions	
Biacetyl oxime hydrazone				Reactionability with metal ions	
Biacetyl oxime salicylalhydrazone				Reactionability with metal ions	
Biacetyl oxime salicyloylhydrazone				Reactionability with metal ions	

TABLE 1—*Continued*

Reagent	Cation	max (nm)	liters mol^{-1} cm^{-1}	Medium, comments	Reference
Biacetyl bis(thiobenzhydrazone)	Pb(II)	400–	3,000–		*(50)*
	In(III)	500	14,000		
	Sb(III)				
	Bi(III)				
				Separation of chelates of Co(II), Hg(II), Pb(II), and Zn(II) on glass columns packed with silica gel, Merckosorb S560, Perisorb A, or alumina	*(51)*
Biacetyl mono(2-pyridyl)hydrazone	Co(II)	505	23,500	2 *M* HCl pH 6; 4–25% ethanol	*(9)*
Biacetyl bis(thiobenzohydrazone)	Cu(II)	530		Determination of Cu in Al–Cd alloys	*(10)*
Biacetyl bis(2-pyridyl)hydrazone	Co(II)			Qualitative studies	*(3)*
	Pd(II)				
	Cu(II)				
Biacetylmonoxime-2-pyridylhydrazone				Reactionability with metal ions	(Ref. *4* in *4*)
Pyruvylidine-2-hydrazinobenzothiazole	Cd(II)			Ident. limit 0.5 μg	*(69)*
	Cd(II)	460	4,600	pH 11–12; extr. into benzene	*(89)*
Glyoxal bis(*N*-methyl-imine)	Fe(II)	554	8,800		*(62)*
Glyoxal dithiosemicarbazone	Ag(I)	335	43,000	pH 1–7; EDTA as masking agent	*(103)*
	Hg(II)	335	43,000	pH 1–7; EDTA as masking agent	*(103)*
	Pd(II)	385	13,000	pH 1–9.5; EDTA as masking agent	*(4)*
		600	2,800		
Glyoxal bis(4-hydroxybenzoylhydrazone)	Ca(II)	460	15,000	CN^- as masking agent	*(66)*
	Cd(II)	460	25,000	CN^- as masking agent	
Glyoxal bis(2-mercaptoanil)				Reactionability with metal ions	*(128)*
	Hg(II)			Qualitative, 0.5 μg filter paper, 5 μg test tube	*(113)*
	Hg(II)			0.3–0.96 μg; ring-oven technique	*(73)*

2,2'-Bisbenzothiazoline	Zn(II)	380	4.12[a]	Pyridine as solvent	*(58)*
		590	3.56[a]	Pyridine as solvent	
	Cd(II)	376	4.10[a]	Pyridine as solvent	
		575	3.64[a]	Pyridine as solvent	
Benzyl bis(2-pyridyl)hydrazone	Co(II)	531	4,600	pH 7 (ammonium acetate buffer)	*(95)*
	Fe(II)	635	5,800	pH 7 (ammonium acetate buffer)	
Benzyl bis(2-hydroxybenzoylhydrazone)	Ca(II)	432	17,600	pH 12; 60% dimethylformamide	*(101)*
	Ti(IV)	460	16,600	pH 3.5–6.5; 60% dimethylformamide	*(102)*
		500	15,500	Extr. into benzyl alcohol (pH 1.0–2.0)	
	Sn(II)	450	22,500	pH 3.5–6.5; 60% dimethylformamide	*(100)*
		455	35,400	Extr. in isobutyl methyl ketone from pH 2.45 to 3.20	
		475	28,800		
	In(III)	440	46,300	pH 6.2; 60% dimethylformamide	*(99)*
2,2'-Pyridyl dihydrazone	Fe(II)	488	8,240	pH 4.5–7.8	*(45)*
		486	9,170	Extr. into nitrobenzene	
2,2'-Pyridyl bis(2-pyridyl)hydrazone	Co(II)	480	25,400	pH 7 (ammonium acetate buffer)	*(95)*
	Fe(II)	595	8,300	pH 7 (ammonium acetate buffer)	
		573	10,400	pH 7 (ammonium acetate buffer)	
2,2'-Pyridyl dipyrazinyl hydrazone	Co(II)	508	29,700	pH 7 (ammonium acetate buffer)	*(94)*
	Fe(II)	601	10,300	pH 4	
		450	19,000	pH 4	
2,2'-Pyridyl bis(2-hydroxybenzoylhydrazone)	Ca(II)	435	10,300	pH 8.5–10.5; 60% dimethylformamide	*(101)*
	In(III)	440	46,300	pH 6.2; 60% dimethylformamide	*(99)*
Bis(6-methyl-2-pyridyl)glyoxal dihydrazone	Cu(II)	440	8,700	Extr. into nitrobenzene	*(116)*
2,2'-Pyridylmonoxime	Co(II)	482	27,400	pH 7 (ammonium acetate buffer)	*(93)*
2-Pyridylhydrazone	Fe(II)	529	9,030	pH 4	
		454	8,020	pH 4	
Benzyl mono(2-pyridyl)hydrazone	Co(II)	535	27,000	60% ethanol–water; pH 8 HCl 2 *M*	*(85)*
Benzyl mono(2-quinolyl)hydrazone	Cu(II)	520	40,000	pH 6; extracted in benzene	*(14)*
2,2'-Pyridyl 2-pyridylhydrazone	Co(II)	489	28,000	pH 7 (ammonium acetate buffer)	*(95)*
	Fe(II)	621	5,800	pH 7 (ammonium acetate buffer)	

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[a] Equals log ε.

formed by a ligand of greater molecular mass than biacetyl bis(hydrazone). Zimmerman and Lochte (*127*) have found that when heated at reflux in a sodium carbonate medium biacetyl and hydrazine, it forms poliazines. As a confirmation of this hypothesis, the compound possesses a lower number of N−H bonds than biacetyl bis(hydrazonate)iron(II) and a higher molecular mass (*44*), about 1400.

A review dealing with chelate complexes of α-diimines and related bidentate ligands with iron(II) and their applications as spectrophotometric reagents (*49*) has been recently published by Hashmi.

Biacetylhydrazone Azine

The studies with biacetylhydrazone azine (*109*) revealed that this ligand forms compounds with iron(II) and nickel(II) of the general type $M_2L_3I_4$. The compounds seem to contain bridged tetrafunctional coordination similar to pyridinaldazine complexes, but unlike pyrinaldazine, however, this appears to be the only stable mode of chelation for this ligand. All attempts to prepare compounds of the type ML_2X_2 have been unsuccessful, and the absence of any time dependence in the absorption spectrum of FL_3I_4 suggests the presence of only one complex species.

Color Reactions of Carbazides with Biacetyl and Biacetyldioxime

When semicarbazide hydrochloride and biacetyldioxime are heated with hydrochloric acid, a red color develops (*106*) which is fairly stable for several days. A similar color is produced by heating semicarbazide hydrochloride with biacetyl. Adding ammonia caused a bluish violet color to develop. Experiments were performed with many organic compounds to discover to what extent these effects are characteristic of semicarbazides.

Bisthiosemicarbazones

The interest offered by the study of chelate complexes containing SN donor atoms is widely shown in the chemical literature. Thiosemicarbazones have been recently reviewed by Singh *et al.* (*103*) and Asuero *et al.* (*7*).

Bis(thiobenzhydrazones)

Except for the dithiosemicarbazones, there are only a few other examples of coordination to copper where the donor atoms are sulfur and nitrogen. One of these examples is biacetyl bis(thiobenzhydrazone) (*10*). This reagent can be used for the determination of copper in the range 6−488 μg. The sample is dissolved in 50% H_2SO_4; the reagent (dissolved in 50% H_2SO_4) is added, and the complex is extracted with $CHCl_3$ after waiting a few minutes. The complex is violet in color and possesses absorption maximum at 530 and 375 nm. The following are the only elements that interfere: Sb, As, and Au; these can be removed by distillation from HBr solution. The reagent is particularly useful in the determination of copper in the range 0.01–0.1% in Al–Cd alloys.

The application of 1,2-diketo bis-thiobenzhydrazones (*50*) as ligands for the extraction and spectrophotometric determination of metal ions has been studied. The 1,2-bis(thiobenzhydrazones) form highly colored and easily extractable chelates with Pb(II), In(III), Sb(III), Bi(III), and elements of the 1st, 2nd and 8th subgroups. Some absorption spectra were given, and the separation of the complexes by thin-layer chromatography was considered. The application of biacetyl bis(thiobenzhydrazone) and 2,3-pentanedione bis(*p*-methoxi)thiobenzhydrazone (*51*) for the determination of Cd(II), Co(III), Hg(II), Pb(II), and Zn(II) has been studied in detail. The chelates of Co(II), Hg(II), Pb(II), and Zn(II) with biacetyl bis(thiobenzhydrazone) (*51*) were used as test materials in a study of separation on glass columns (2 and 6 mm) packed with silica gel, Mercko Sorb 5560; Perisorb A or alumina-Benzene was used as eluent (at 3 to 60 ml per hr) and detection was by spectrophotometry with the use of a 10-mm flowthrough cell (capacity 8 μl) with 50 μl of sample solution; 2 ng of Hg and 0.5 ng of Cu, present as their chelates, can be separated and detected. 1,2-Diketo bis(thiobenzhydrazones) have also been used as chelate forming ligands in high-performance liquid chromatographic separations (*52*) of several metal ions chelates at nanogram levels by adsorption chromatography on silica gel.

Glyoxal Bix(2-hydroxyanil) Derivatives

In 1957, Bayer reported that glyoxal bis(2-hydroxyanil) (*12*) forms colored inner complex salts with copper, cobalt, nickel, cadmium, manganese, and uranyl ions. Glyoxal reacts with *o*-aminophenol to form a Schiff base which gives a red, water-soluble complex with calcium. This reaction was used a year later by Feigl and Goldstein for the detection of both glyoxal and *o*-aminophenol (*36*) with detection limits of 1 and 25 μg, respectively. The same year Goldstein and Stark-Mayer were able to make the reagent specific for calcium (*45*), also describing the preparation of the reagent itself, although an improved procedure (*31*) was reported later by Drewy. Glyoxal bis(2-hydroxyanil) was also proposed as an indicator for the complexometric determination of calcium (*39*). The bright red color formed in alcoholic solution and at high pH constitutes the basis for direct methods for calcium. It is impossible to give here a complete survey of all the publications which deal with glyoxal bis(2-hydroxyanil) derivatives. A study of the colorimetric determination of calcium using reagents of the glyoxal bis(2-hydroxyanil class was carried out by Michigan and Lindstrom (*74*) in 1972. The use of the mentioned type of reagents in the spectrophotometric determination of indium, scandium, thallium, and uranium, was reported by Leather (*65*) in 1973. Recently glyoxal bis(2-hydroxyanil) has been proposed as an indicator for the complexometric determination of cobalt(II), nickel(II), manganese(II), and silver(I) (*82*).

Biacetyl unlike glyoxal reacts with *o*-aminophenol to give a heterocyclic compound (*58*) because a ring closure occurs in the reaction.

Schiff bases and their uses have been reviewed (*30*) by Dey.

Glyoxal Bis(2-mercaptoanil)

Another kind of ligand which provides an additional example of coordination where the donor atoms are sulfur and nitrogen may be biacetyl bis(2-mercaptoanil), but the condensation of glyoxal, biacetyl, or benzyl with *o*-aminobenzenethiol does not give the expected Schiff base (*58*). However, the product obtained in the condensation of glyoxal with 2-aminobenzenethiol (*128*) responds to mercury salts alone and has been used in a specific test (*113*) for mercury. When a solution of glyoxal bis(2-thioanil) in chloroform was added to an aqueous solution of a mercury salt, followed by sodium hydroxide, a red product was obtained which was extractable into chloroform. The limits of identification were 0.5 and 5 μg for filter paper and test tube, respectively. It was possible to identify 30 μg of Hg(I) or 25 μg of Hg(II) in the presence of 4500 μg of Cu, Ni, Co, or Cl^-, 2500 μg of I^-, 900 μg of Pd(II), or 500 μg of Ag. In like manner an extremely selective determination of mercury (0.3–0.96 μg) can be carried out by the ring-oven technique (*73*) by using glyoxal bis(2-thioanil). Sulfide interferes; Cu and Ag (20 μg) interfere. The method was highly effective in determining mercury in alloys and inorganic samples. Undoubtedly, the heterocyclic compound in the presence of mercury rearranges to give the Schiff base chelate, which implies a greater selectivity in reaction with metal ions.

Biacetyl Bis(benzoylhydrazones)

The quelating behavior of biacetyl bisbenzoylhydrazone (*25*) with tin(IV), lead(II), and lead(IV) has been reported. The spectral data indicate that the ligand is a tetradentate in all of the complexes except SnL_2; in this complex the ligand is bidentate, and a pseudotetraedral structure exists. Instead, biacetyl bis(salicyloylhydrazone) and biacetyl bis(5-bromo-salicyloylhydrazone) (*96*) have been used in the gravimetric determination of zinc and cadmium. Zinc or cadmium can be precipitated from hot solutions containing ammonia (>0.05 *M* Zn or Cd) with a threefold excess of the reagent. The precipitate, consisting of the ammonium salt of the metal reagent complex, is separated by filtration and dried to constant weight. Five or six single determinations can be carried out in 3 hr. For Zn (≃ 7 to 98 mg) and for Cd (≃ 12 to 116 mg) the errors range from +0.3 to −0.4% and from +0.25 to −0.3%, respectively, for the unsubstituted reagent, and from +0.2 to −5.9% and from −0.2 to −0.9%, respectively, for the bromo derivative. The bromo derivative can be used for precipitating weights of Zn up to 40 mg per sample only. Interference is caused by Mg, Mn, Co, and Cu. Ni interferes if present in amounts larger than those of Zn or Cd.

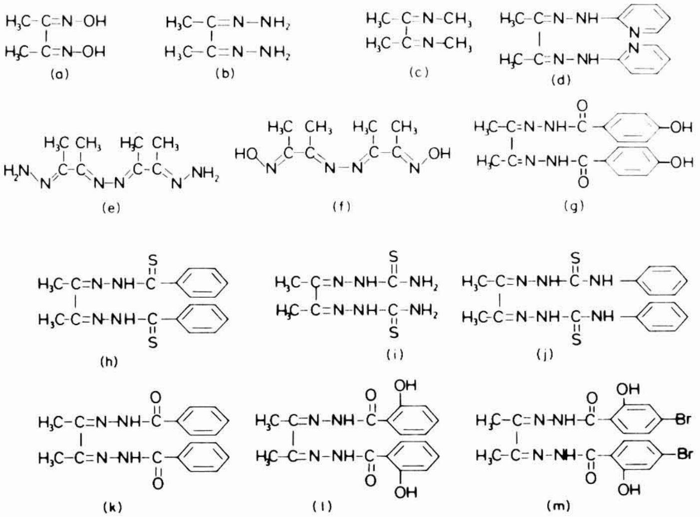
Aroylhydrazines react with carbohydrates in alkaline solution (*67, 68*) to form a colored product which forms complexes with various cations having distinct absorption and fluorescence spectra. Really glyoxal and methylglyoxal aroylhydrazone are the reaction products. The reactions between cations and the bis-aroylhydrazones of α-diketones were observed, because calcium in biological materials increased the sensitivity of method of glucose determination which depends upon the formation of these hydrazones (*67, 68*). However, the derivative of glyoxal is superior to the corresponding biacetyl derivative. It has been proposed for the determination of calcium and cadmium, in the presence of CN^- or citrate, respectively, when other cations are present. Beer's law was obeyed in the range 0 to 50 m*M* in the final solution for both cations: for the Ca chelate $\epsilon = 1.5 \times 10^4$ and for the Cd chelate $\epsilon = 2.8 \times 10^4$ liters mol^{-1} cm^{-1} at 460 nm (*67*). Various bis(aroylhydrazones) were compared, mainly those derived from benzoyl-, 4-chlorobenzoyl-, 4-aminobenzoyl-, 4-hydroxybenzoyl-, 3-nicotinyl-, and 4-nicotinylhydrazide. All aroylhydrazones were found to give a visible color with calcium and cadmium.

Bis(pyridylhydrazones)

Although the synthesis of 2-pyridylhydrazine was first reported by Fargher and Furness as early as 1915 (*34*), the analytical applications of complex compounds derived from ligands of the Schiff base type possessing the 2-pyridylhydrazine residue were first reported in 1963 (*61*). For many years pyridine itself has been known as a reagent. Replacement on the benzene ring by the pyridine ring in a molecule provides an additional position of coordination also increasing its solubility in water, ethanol, and other organic solvents. Thus, pyridylhydrazones are found much more applications than phenylhydrazones (*2*) as reagents. The main pyridylhydrazones studied have been derived from aldehydes and ketones pyridiniques and the general line followed has been to increase the π-system of the molecule in order to obtain a greater sensitivity in reaction with metal ions.

Biacetyl bis(2-pyridyl)hydrazone was synthesized in 1958 by Lions and Martin (*70*) who isolated the copper(II) and cobalt(III) complexes of this compound. Some planar quadridentate chelate compounds have also been prepared from biacetyl bis(2-pyridyl)hydrazone and transition metal ions favoring square planar four-coordination (*26*). The preliminary evaluation of biacetyl bis(2-pyridyl)hydrazone (*3*) has been reported. It appears to be a promising reagent for the colorimetric estimation of cobalt and palladium. The study of equilibrium forms, dissociation constants, behavior at various pH, and effect of various solvents on pyridylhydrazones derived from biacetyl has also been reported (*88*). The analytical properties of glyoxal bis(2-pyridyl)hydrazone (*4*) have also been reported. In spite of this apparent similarity, there is a great difference of both glyoxal and biacetyl bis(2pyridyl)hydrazone. 2-Pyridylhydrazine appears to be an excellent reagent for the spectrophotometric determination of glyoxal (*4*).

For the structure of bis-simetrical derivatives see Scheme I.



SCHEME I. (a) Dimethylglyoxime; (b) biacetyl bis(hydrazone); (c)biacetyl bis(*N*-methylimine); (d) biacetyl bis(2-pyridyl)hydrazone; (e) biacetyl hydrazone azine; (f) biacetyl oxime azine; (g) biacetyl bis(4-hydroxybenzoylhydrazone); (h) biacetyl bis(thiobenzohydrazone); (i) biacetyl bis(thiosemicarbazone); (j) biacetyl bis(4-phenyl-3-thiosemicarbazone); (k) biacetyl bis(benzoylhydrazone); (l) biacetyl bis(2-hydroxybenzoylhydrazone) (salicyloylhydrazone); (m) biacetyl bis(5-bromosalicyloylhydrazone).

BIACETYLMONOXIME AND THEIR DERIVATIVES

Biacetylmonoxime

The group $-CO-C(=N-OH)-$ has a great analytical importance and it confers upon the molecule the property of inner-complex formation (*128*). One of the most interesting reactions of monoximes of α-diketones is the so-called "iron-blue reaction," which was first observed by Whiteley (*127*). The relationship between structure and the iron-blue reaction with ferrous iron has been investigated by Feigl *et al.* (*35*). Factors such as enolizability, steric arrangement, and the possibility of the two carbon atoms are part of a ring structure have importance.

Biacetylmonoxime has been proposed for the spectrophotometric determination of cobalt as cobalt(III), of rhenium and molybdenum and palladium (*104*). The other monoximes structurally related to biacetylmonoxime have also found numerous analytical applications as reagents for metal ions (*17, 59, 60, 97, 104, 105, 125*). Biacetylmonoxime has been recently proposed for homogeneous precipitation (*48*) of nickel.

Determination of urea with biacetylmonoxime in blood (*24*) agreed with those results found by the NaOBr method, while they showed variable results in urine. Urea is condensed with biacetylmonoxime to give a yellow complex, and the hydroxylamine liberated is taken up by the addition of phenazone, the reaction being catalyzed by iron(III). This reaction has been used for determining urea in blood (*16*). As low concentrations of urea are not in relationship with the color produced, the sample and standards are diluted with urea solution. The absorbance is measured at 460 nm.

Urea in microliter samples of serum and urine after deproteinization with trichloroacetic acid has been spectrophotometrically determined after the reaction with biacetylmonoxime and *N*-phenanthranilic acid. The range of the method (*81*) is 0.125 to 1 mg of urea per ml of final solution. The recovery of urea was 97 to 102% from serum and 96 to 103% from urine. The oxidizing and reducing agents present in serum or urine interfere. Biacetylmonoxime has also been used in the automatic determination of urea (*123*) in a protein medium without dialysis. Precisely, the first AutoAnalyzer (*28, 75*) was built to perform blood urea–nitrogen analysis because this is one of the most requested analyses in the clinical laboratory. The sensitivity of the biacetylmonoxime reaction is greatly improved by adding phenazone. In order to avoid the need for dialysis, the sample was diluted about 700-fold in two steps. The reaction mixture is warmed at 95°C and the absorbance measured at 460 nm. It is possible to analyze 60 samples per hour.

The conditions for the formation of fluorescent derivatives of citrulline, allantoin, allantoic acid, carbanoyl aspartic acid, and urea are given by condensation with biacetylmonoxime (*37*) in media $H_2SO_4-H_3AsO_4$ and H_3PO_4. The addition of an adjuvant such as Fe did not alter the specificity of the reactions, but addition of phenazone or thiosemicarbazide did.

The urea–iron(III)–biacetylmonoxime–thiosemicarbazide color constitutes the basis of another microchemical method (*18*) adaptable to AutoAnalyzers using continuous flow or transfer systems, permitting a rapid and simple determination of urea clearance. The automatic determination of urea in biological fluids without dialysis can also be carried out by means of biacetylmonoxime–thiosemicarbazide (*19*). In the presence of Fe^{3+} in an acidic medium thiosemicarbazide and urea reacts with biacetylmonoxime to give a pink condensation product. The color intensity is determined at 520 nm. The apparatus is simple and permits the simultaneous estimation of glucose with *o*-toluidine. Results give a linear response from 0 to 2.5 g/liter, good reproducibility, negligible contamination, and absence of interference with other substances normally present in biological fluids.

The combination diacetylmonoxime–thiosemicarbazide has also been used as a spray reagent for the determination of urea (*80*) in urine. An alcoholic solution of the mixture was specific for urea and its aryl derivatives, and could be used for paper and thin-layer chromatographic determinations.

Biacetylmonoxime phenylhydrazones

Biacetylmonoxime 2,4-dinitrophenylhydrazone has been used in the qualitative detection of cobalt and biacetylmonoxime *p*-nitrophenylhydrazone in the spectrophotometric determination of cobalt (*2*). A new method for the spectrophotometric determination of cobalt based on the reaction of cobalt with biacetylmonoxime *p*-nitrophenylhydrazone in the presence of ethylenediamine has been developed. The color development is complete after 3 hr at 90°C and the excess of reagent is extracted with ether as in the original procedure. Beer's law is obeyed over 0.05 to 0.6 ppm Co. The method has been applied routinely to plant samples containing from 0.02 to 1.5 ppm of cobalt (*83*).

Biacetylmonoxime Salicylal- and Salicyloylhydrazone

The apparent stability constants (log K) of Ni(II) chelate of biacetylmonoxime salicyalhydrazone (11.4 ± 0.18) and of Ni(II) (11.2 ± 0.11), Ca(II) (4.24 ± 0.04), Sr(II) (2.91 ± 0.04), and Ba(II) (4.26 ± 0.02) chelates of biacetylmonoxime salicyloylhydrazone were determined potentiometrically in 1:1 H_2O–MeOH mixtures. The effect of the central atom on the stability of chelates formed was examined (*53*, *54*, *126*).

Biacetylmonoxime Hydrazone

Biacetylmonoxime hydrazone (*57*) reacts analogously to biacetylmonoxime or other biacetylmonoxime derivatives used for proof of the structure of α-dioxime salts.

Biacetylmonoxime-2-benzothiazolylhydrazone

This colorless compound reacts sensitively with palladium(II) (*40*) over a wide range of pH. The complex compound formed can be extracted into chloroform (limit of dilution and identification 1 in 2 × 10^5 and 0.25 μg, respectively). The method is selective and it is possible to detect palladium in alloys by treating a small amount of the sample with one to three drops of aqua regia and then with a few drops of the reagent. The red complex extracted in chloroform (Pd:reagent, 1:2 at pH 1.8) is very appropriate for the spectrophotometric determination of palladium (λ_{max} = 560 nm, ϵ = 5110 liters mol^{-1} cm^{-1}). Beer's law is obeyed up to 15 ppm of palladium. One of the principal advantages of the method is the great tolerance for foreign ions of the platinum group. In the determination of 1 ppm of palladium, 41 metals including Ir, Pt(II), Rh, and Ru were tolerated at concentrations of 1000 ppm, Ce(III) and Sn(II) at 100 ppm, and V(V) at 10 ppm (*1*).

Bisbiacetylmonoxime-ethylenedi-imine and Bisbiacetylmonoxie-o-phenylenedi-imine

These diimine compounds containing two salt-forming and two coordinating nitrogen atoms give rise to tetradentate ligands which form precipitates with nickel and palladium (72). The precipitates can be used for the gravimetric determination of these metal ions.

Biacetyl Oxime Azine

The nickel complex of biacetyl oxime azine (*110*) was prepared and the IR, magnetic, and pH titration data were investigated in order to propose an appropriate structure for the complex. The reagent was prepared from biacetylmonoxime and N_2H_4 in refluxing ethanol.

Biacetylmonoxime Thio- and Phenylthiosemicarbazone

The microchemical confirmation of manganese(II) in solution (*54*) can be carried out either on a spot plate or paper impregnated with an alcoholic solution of biacetylmonoxime thiosemicarbazone (BMT). BMT has also been applied to the microanalysis of Mn(II) at pH 6.2 measuring the absorbance at 346 nm after 40 min standing of the samples. Limit of identification is 0.1 μg Mn/ml. (*78*).

Biacetylmonoxime and thiosemicarbazide react *in situ* (50°C) with nickel ion in an ammonia medium to give a red-orange coloration, very stable, which has λ_{max} at 350–360 nm (*13*). When BMT is employed for the colored reaction the absorbance is higher than that obtained with the mixture of biacetylmonoxime and thiosemicarbazide. As the ratio of Ni to

BMT was 1:2 it is tentatively postulated that the compound formed *in situ* has the formula $Ni(BMT)NiX_2$. In the case of cobalt the synthesis *in situ* of BMT is not convenient as each of these substances can react with cobalt separately.

The determination of traces of oxygen (6 μg; 10–100 ppm O_2) in gases and solution (*120*) has been carried out. The method is based upon the pronounced color change occurring during the oxidation of the manganese(II) chelate to that of manganese(III). The oxidation is practically instantaneous at pH 10 in ammonia buffer solution (Mn:BMT, 1:20). The absorbance is measured at 540 nm. The manganese(III)–BMT complex has been utilized for the polarographic determination of manganese (concentration range $5 \times 10^{-5} - 3 \times 10^{-4}$, 8% aqueous–ethanolic medium, pH 10.5 (*119*, *121*). At a concentration of 1 μg/ml, the same amounts of cobalt, nickel, copper, iron, and an excess of tervalent chromium (10:1) do not interfere.

The acid–base and chelating properties of BMT have been studied. Two kinds of complexes MHL^+ and $M(H_2L)$ were found for Co(II) and Fe(III) and MHL^+ complexes were found for Cu(II), Ni(II), and Zn(II). The $Co(HL)_2$ complex is converted into $CoHL_2^-$ on deprotonation. The stability of complexes of the MHL^+ type varies (*118*) along the series Zn < Fe ≈ Ni < Co ≈ Cu.

Ferrous iron forms with BMT in an aqueous ethanolic medium and in absence of air, in strongly acidic medium a red complex (ϵ_{507} = 6250 liters mol^{-1} cm^{-1}) (*112*). In the presence of ascorbic acid the red complex is stable even in the presence of air. The advantage of the method is the low pH value of determination of iron; the disadvantage is the dependence of absorbance on the concentration of perchloric acid and the possibility of precipitation of the reagent at too low pH values (≥0.1 *M* HClO). The method is not interfered by an excess of the ions Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, Li^{2+}, Zn^{2+}, and Th^{4+} (500:1), Cd^{2+} (50:1), Sc^{3+} (5:1); the ions Bi^{3+}, Co^{2+}, Ni^{2+}, and particularly Cu^{2+}, Ag^+, and also Hg^{2+}, precipitating with the reagent interfere.

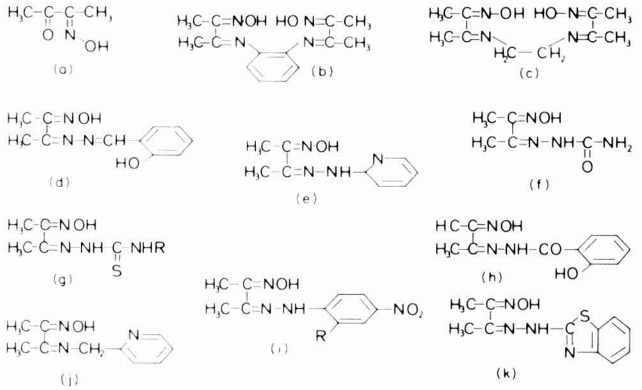
A colorimetric determination of manganese with the use of BMT has also been proposed by Holzbecher and Jezek (*55*).

Biacetylmonoxime 4-phenyl-3-thiosemicarbazone has been applied to the spectrophotometric determination of manganese (*22*), bismuth, and copper, and their mixtures (*21*). Sodium tartrate was used as masking agent for bismuth.

For the structures of various biacetylmonoxime derivatives see Scheme II.

BIACETYL-MONO DERIVATIVES

We have previously mentioned the analytical applications of biacetylmonoxime itself.



SCHEME II. (a) Biacetylmonozime; (b) biacetylmonoxime-*o*-phenylenedi-imine; (c) biacetylmonoxime-ethylenedi-imine; (d) biacetylmonoxime salicylalhydrazone; (e) biacetylmonoxime 2-pyridylhydrazone; (f) biacetylmonoxime semicarbazone; (g) biacetylmonoxime thiosemicarbazone (R = H) and phenylthiosemicarbazone (R = $-C_6H_5$); (h) biacetylmonoxime salicyloylhydrazone (2-hydroxybenzoylhydrazone); (i) biacetylmonoxime p-nitrophenylhydrazone (R = H) and 2,4-dinitrophenylhydrazone (R = $-NO_2$); (j) biacetylmonoxime-2-picolylamine-imine; (k) biacetylmonoxime 2-benzothiazolylhydrazone.

Biacetylmonosemioxamazone and Biacetylmonosalicyloylhydrazone (*56*)

Their reactionability with metal ions (*57*) has been described.

Biacetyl Monothiosemicarbazone

It has been used in the spectrophotometric determination (*90*) of copper and nickel.

Biacetylmonohydrazone Azine

The monohydrazone of biacetyl azine (*114*) has been employed in the spectrophotometric determination of copper as copper(I). The 1:1 complex (λ_{max} = 350 nm, ϵ = 7.239 × 10^3 liters mol^{-1} cm^{-1}) is extracted in benzyl alcohol. $S_2O_3^{2-}$, SCN$^-$, $C_2O_4^{2-}$, and citrate do not interfere up to a ratio of 90:1; Zn(II), Hg(II), V(V), Mn(II) at 40:1; Ag(I) and Co(II) at 30:1; Fe(III), Hg(II), Sb(III) at 20:1; Au(III), Pt(IV), Fe(II) at 15:1; and Pb(II) at 4:1.

Pyruvylidine-2-Hydrazinobenzothiazole

The yellow compound obtained by the condensation of 2-hydrazinobenzothiazole and pyruvic aldehyde forms with cadmium ions in an alkaline medium an orange precipitate which can be dissolved in benzene; colored compounds are also produced with Cu, Ni, Co, Pb, Au, Mn, Tl, Ce, Hg, and Fe, but these metals can be masked by suitable masking agents. Identification limits is 1:10^5. Five micrograms of cadmium can be detected in the presence of 0.5 mg Pb, 1 mg Co, 2 mg Ni, 5 mg Cu, and 0.5 mg of Fe, Au, Ce, Hg, Mn, or Tl. With the test proposed

1 μg of cadmium can be identified in a sample containing 99.8% of zinc (*69*).

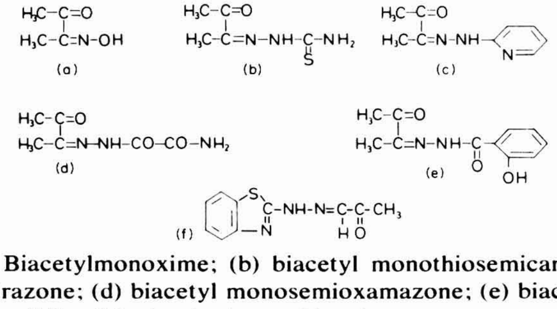
Cadmium is extracted from alkaline solutions (pH 11–12) of tartrate (to avoid precipitation of hydroxide of possible interferences) with the reagent dissolved in benzene. The yellow complex shows (*89*) maximum absorbance at 370 nm and a shoulder at 460 nm. At this latter wavelength the absorbance of reagent is negligible and it is selected for the analytical work. In the presence of Cu, Ni, Co, Pb, Mn, Tl, Hg(I), and Fe(II) a demasking procedure using cyanide is used. The tolerable amounts of diverse ions were (in ppm): 200 Ni, 100 Co, 50 Pb, 25 Fe(III), 15 Hg(II), 15 Cu(II), 15 Tl(I), and 5 Mn. As much as 1000 ppm or more of zinc could be tolerated without interference.

Biacetyl Mono(2-pyridyl)hydrazone

Biacetyl mono(2-pyridyl)hydrazone has been proposed by Asuero *et al.* (*9*) as a reagent for the spectrophotometric determination of cobalt. The 1:2 orange-red Co:BPH complex has λ_{max} at 505 nm with a molar absorptivity of 2.35×10^4 liters mol^{-1} cm^{-1}. The cobalt chelate is formed at acidities between 2 and 10^{-6} *M* in aqueous ethanolic solutions (4–50% of ethanol). The apparent pH value for the development of maximum absorbance is about 4.8. The optimum working range is 0.3–1.2 ppm of cobalt. The proposed method was applied to the determination of cobalt in nitrates of zinc, cadmium, mercury, lead, uranyl, and iron(III). For the structures of biacetyl monoderivatives see Scheme III.

BIACETYL

When a dilute solution of protein is mixed with a small amount of 10% KOH solution and then one drop of 1% diacetyl solution is added, it forms a pink color with green fluorescence. The reaction depends on the presence of the group NH:C(NH$_2$)NHR. Arginine, agmatine, creatine, dicyanamide, and guanidine acetic acid also give the pink color (*48*) but not



SCHEME III. (a) Biacetylmonoxime; (b) biacetyl monothiosemicarbazone; (c) biacetyl mono(2-pyridyl)hydrazone; (d) biacetyl monosemioxamazone; (e) biacetyl monosalicyloylhydrazone; (f) pyruvylidine-2-hydrazinobenzothiazole.

the fluorescence. The active material is not diacetyl itself, but an unstable aldol resulting from the action of alkali. Creatine which has a free NH_2 group gives the colored reaction, but not creatinine which has a lactam form (*36*), thus suggesting that the unstable aldol condenses with the NH_2 group of the respective guanidino compound. The presence of the NH_2 group is essential for the colored reaction. Methylguanidine, nitro, and aminoguanidine give no color under similar conditions. The diketone need to be diacetyl, but must have the two CO groups adjacent to one of them. $C_6H_5COCOCH_3$ is preferable to diacetyl because it is less volatile and gives a purer color. A colorimetric method for determination of creatine based on this reaction has been proposed (*64*) by Lang. The method is essentially the same for arginine in protein, except that the protein is first hydrolyzed and the hydrolysate decolorized with kaolin before comparison with the standard arginine solution similarly treated. the limit of error is less than 5%. A comment on the paper of Lang dealing with the mechanism of the biacetyl reaction has been reported (*76*) by Muller. The reactions previously studied by Harden and Norris (*48*) have been confirmed and their spectral absorption characteristics studied (*87*) by Roche.

For many years, the color reaction and fluorescence of protein and their derivatives in the presence of biacetyl has been known. Biacetyl on reaction with acid amides also leads to a characteristic fluorescence. This reaction and those on the peptide bond in protein were studied by fluorescence variation, absorption, and fluorescence spectra. Probably a new conjugate double-bond system was produced (*37*) by the reaction of $>C=0$ and $-NH$.

Very acid solutions of α-diketones, such as biacetyl and carbazole, or 1-phenyl-1,2-propanedione with traces of nitrate are reagents (*108*) for urea and guanidine.

Hydroxylammine can be determined by its reaction with diacetyl to give dimethylglyoxime (*86*) which then reacts with nickel(II).

RELATED REAGENTS

The Schiff bases derived from glyoxal, benzyl, and cyclohexane-1,2-dione can be considered as structurally related reagents. Some of them have been previously mentioned. Strictly speaking, those derived from dipyridylglyoxal and bis(6-methyl-2-pyridyl)glyoxal cannot be considered as related to biacetyl derivatives, because the presence of a donor group in the chain makes the molecule behave as a duplicate of the corresponding derivative of pyridine 2-carboxaldehyde and 6-methyl-2-pyridylcarboxaldehyde, respectively.

Benzil mono(2-pyridyl)hydrazone forms with cobalt a 1:2 (Co:L) red complex at 2 *M* HCl, pH 8, which has λ_{max} at 535 nm with $\epsilon = 2.72 \times 10^4$

liters mol^{-1} cm^{-1}. The apparent pH for the formation is approximately 7.5. Determination of cobalt in selected metal samples—high nickel steel, Co–Mo–W steel, and Cu–Ni–Zn alloy—was carried out, and the results obtained after ion exchange separation and without prior separation were consistent.

The 1:1 copper benzyl:mono(2-quinolyl)hydrazone complex (*14*) may be extracted into benzene at pH 6. The absorption maximum occurs at 520 nm and the apparent molar absorptivity is 40,000 liters mol^{-1} cm^{-1}. Cobalt(II) is coextracted since there is an enhancement of absorbance, and Fe(III), Cd(II), and Fe(II) form precipitates in the aqueous phase accompanied by a decrease in the absorbance of the complex in the organic layer.

Dipyridylglyoxal dihydrazone forms at pH 4.5–7.8 with iron(II) a red 3:2 (L:Fe) complex (λ_{max} = 488 nm, ϵ = 8240 liters mol^{-1} cm^{-1}, which can be extracted into nitrobenzene (λ_{max} = 486 nm, ϵ = 9170 liters mol^{-1} cm^{-1}. The *in situ* reaction synthesis mixing α-pyridyl, hydrazine, and ferrous ion under adequate pH and temperature conditions (*40*) was also studied. Bis(6-methyl-2-pyridyl)glyoxal dihydrazone forms with copper(I) ions a 1:1 orange complex having λ_{max} at 440 nm with ϵ = 8700 liters mol^{-1} cm^{-1}, which has been used for the selective determination of trace amounts of copper (*116*) in different materials.

Diphenyl and dipyridylglyoxal bis(benzoylhydrazone) have been studied (*85*) as analytical reagents. Diphenylglyoxal bis(2-hydroxybenzoylhydrazone) reacts with calcium(II) (*87*) at pH 12 to produce a 2:3 (Ca:L) yellow complex having λ_{max} at 432 nm and ϵ = 1.76×10^4 liters mol^{-1} cm^{-1}. When masking agents were added common cations do not interfere. The procedure which does not require a preliminary extraction step has been applied to the determination of calcium in water and food. Diphenyl bis- and dipyridylglyoxal bis(2-hydroxybenzoylhydrazone) have been used in the spectrophotometric determination of indium (*99*). The 1:4 (In:L) complexes having λ_{max} at 440 nm were formed at pH 6.2 in a solution containing a 60% of dimethylformamide. Beer's law was obeyed between 0.5 and 2 ppm of indium(III) and the molar absorptivities were about 4.63×10^4 liters mol^{-1} cm^{-1}. Interferences were numerous in both methods. Diphenylglyoxal bis(2-hydroxybenzoylhydrazone) has also been applied to the spectrophotometric determination of tin(II) (*100*) and titanium (*102*) using extraction techniques.

The chromogenic properties of some mono- and bishydrazones of benzyl and 2,2'-pyridyl (*95*) as well as the ones of 2-pyridyl and pyrazinylhydrazones of some pyridyl, pyrazinyl, and pyridazinyl Ketones of isatin and phenylglyoxal (*94*) have been evaluated by Schilt *et al.* Certain pyridinyl and diazinyl hydrazones containing one or more ferroin groups (*93*) have also been studied by Schilt *et al.*

Metal complexes of 2,2′-pyridyl bis(2-pyridyl)hydrazone have been prepared by Black *et al.* (*15*).

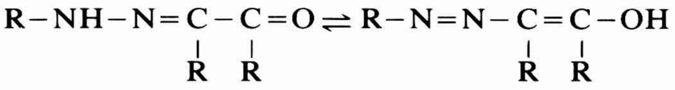
ACID–BASE PROPERTIES OF LIGANDS

The complexing properties of reagents depend upon the presence and arrangement of electron donor groups in the reagent molecule and upon its acid–base properties.

The nitrogen atom of the oxime group has weak basic properties, and a protonation according to the equation $>C{=}NOH + H^+ = \, >C{=}NHOH^+$ takes place in acid medium. Because biacetylmonoxime decomposes in acid solution a modified spectrophotometric technique was used (*33*) in order to obtain more accurate results for the dissociation constant of the conjugated acid of biacetylmonoxime.

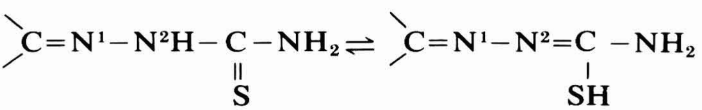
Salicylalhydrazone and salicyloylhydrazone of biacetylmonoxime possess a similar structure. However, due to the exchange of the $-N{=}CH-$ group in the first for the $-NH-CO-$ group in the second, one may assume different basicity (*54, 124*) to form coordination bonds.

From the study of biacetyl mono(2-pyridyl)hydrazone (*88*) it is deduced that the reagent is found preferably in the keto-form. In high alkalinity, instead, the deprotonation of the imino-nitrogen takes place (*61*) in common with numerous pyridylhydrazones. However, the intense color of benzyl mono(2-quinolyl)hydrazone (*14*) is most likely due to the keto-enol tautomerism in which the enol form of the ligand takes the structure of a highly conjugated azo dye:



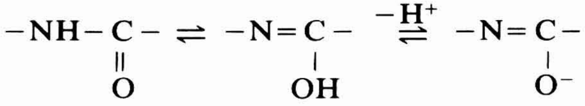
$$R-NH-N=\underset{R}{\underset{|}{C}}-\underset{R}{\underset{|}{C}}=O \rightleftharpoons R-N=N-\underset{R}{\underset{|}{C}}=\underset{R}{\underset{|}{C}}-OH$$

In the case of thiosemicarbazones, it has been confirmed that the N^2-hydrogen atom (*38*) is a requirement for the tautomeric equilibrium illustrated below, which is necessary for the formation of complexes:



$$>C=N^1-N^2H-\underset{S}{\underset{\|}{C}}-NH_2 \rightleftharpoons \, >C=N^1-N^2=\underset{SH}{\underset{|}{C}}-NH_2$$

Benzoylhydrazones suffer one ionization process in accord with the following (*98*) equilibrium:



$$-NH-\underset{O}{\underset{\|}{C}}- \rightleftharpoons -N=\underset{OH}{\underset{|}{C}}- \overset{-H^+}{\rightleftharpoons} -N=\underset{O^-}{\underset{|}{C}}-$$

The phenolic group of 2-hydroxybenzoylhydrazones (salicyloylhydrazones)

TABLE 2

pK_a OF BIACETYL DERIVATIVES (AND RELATED LIGANDS)

Ligand	pK_a	Method	Medium	Assignment	Reference
Biacetyl bis(2-pyridyl)hydrazone	5.9	Spect.	3% v/v ethanol	Pyridine N	*(88)*
	6.4 and 5	Spect.	20% v/v ethanol	Pyridine N	
Biacetyl bis(thiosemicarbazone)	10.1	Spect-pot.	20% v/v ethanol; 0.25 *M* KNO_3	Thiol groups	*(77)*
Biacetyl bis(4-phenyl-3-thiosemicarbazone)	10.95	Spect.	7 + 3 v/v water–dimethylform.	Thiol groups	*(5)*
Biacetyl mono(2-pyridyl)hydrazone	4.6	Spect.	3.2% v/v ethanol	Pyridine N	*(88)*
	4.8	Spect. titr.	20% ethanol	Pyridine N	
	14.5	Spect.	3.2% v/v ethanol	Imine N	
Pyridine	5.17	Spect.	1% ethanol, = 0.1		*(112)*
Biacetylmonoxime	1380	Spect.	30°C	K_A^{HMH+}	*(33)*
	1440		25°C		
	1490		20°C		
Biacetylmonoxime semicarbazone	10.33	Spect. titr.	20% ethanol; 0.25 *M* KNO_3	Oxime grouping	*(77)*
Biacetylmonoxime thiosemicarbazone	11.2	Spect. titr.	20% ethanol	Oxime group	*(77)*
	11.13 ± 0.09	Spect.	40% ethanol	Oxime group	*(118)*
	11.16 ± 0.09	Pot.	40% ethanol	Oxime group	*(118)*
	12.74 ± 0.08	Spect.	40% ethanol	Thiol group	
	12.64 ± 0.08	Pot.	40% ethanol	Thiol group	
Biacetylmonoxime phenylthiosemicarbazone	10.40	Spect.	20% DMF	Oxime grouping	*(22)*
	11.50			Thiol group	
Biacetylmonoxime salicylalhydrazone	3.58 ± 0.26	Spect.	Methanol–H_2O 1:1	Azomethine N	*(124)*
	10.10 ± 0.28	Spect.	Methanol–H_2O 1:1	Phenol. group	*(124)*
	11.49 ± 0.17	Spect.	Methanol–H_2O 1:1	Oxime group	*(124)*
Biacetylmonoxime salicyloylhydrazone	2.43 ± 0.07	Spect.	Methanol–H_2O 1:1	Azomethine N	*(124)*
	6.98 ± 0.16	Spect.	Methanol–H_2O 1:1	Phenol. group	*(124)*
	11.86 ± 0.42	Spect.	Methanol–H_2O 1:1	Oxime group	*(124)*

Biacetylmonoxime 2-pyridylhydrazone	5.9	Spect.	4% v/v ethanol	Pyridine N	(*88*)
	11.4	Spect.	4% v/v ethanol	Oxime group	
Dimethylglyoxime	10.54	Spect.	aq.		(*32*)
	12.03	Pot	50% dioxan–0.1 *M* $NaClO_4$		
α-Furilmonoxime	9.16 ± 0.04	Spect.			(*125*)
Glyoxal bis(2-pyridyl)hydrazone	5.2	Spect.	20% ethanol	Pyridine N	(*4*)
	4.1			Pyridine N	
Glyoxal dithiosemicarbazone	9.3 ± 0.3	Spect.	aq.	Thiol group	(Ref. *25* in *7*)
	10.2 ± 0.2			Thiol group	
Cyclohexane-1,2-dione bis(thiosemicarbazone)	10.1	Spect. titr.	20% v/v ethanol; 0.25 *M* KNO_3	Thiol groups	(*77*)
Bis(6-methyl-2-pyridyl) glyoxal dihydrazone	4.82	Spect.		Pyridine N	(*116*)
Benzil bis(benzoylhydrazone)	9.07 ± 0.05	Spect.		Keto-enol.	(*98*)
Benzil bis(2-hydroxybenzoylhydrazone)	8.20 ± 0.05	Spect.	Water–DMF (3 + 2 v/v)	Phenol. groups	(*101*)
2,2′-Pyridyl bis(benzoylhydrazone)	2.60 ± 0.05	Spect.		Pyridine N	(*98*)
	8.63 ± 0.05	Spect.		Keto-enol.	
2,2′-Pyridyl bis(2-hydroxybenzoylhydrazone)	3.27 ± 0.05	Spect.	Water–DMF (3 + 2 v/v)	Pyridine N	(*101*)
	7.85 ± 0.05			Phenol. group	
2,2′-Pyridyl bis(thiosemicarbazone)	3.8	Spect.	2% v/v ethanol	Pyridine N	(Ref. *32* in *7*)
	10.4	Spect.		Thiol group	
2,2′-Pyridyl bis(4-phenyl-3-thiosemicarbazone)	3.20	Spect.	2:3 ethanol–H_2O	Pyridine N	(Ref. *50* in *7*)
	3.27	Spect.		Pyridine N	
	11.68	Spect.		Thiol group	
	11.62	Spect.		Thiol group	

is first deprotonated. Then, at high pH values an additional equilibrium occurs, which is formulated as previously indicated for benzoylhydrazones (*124*). The azomethine nitrogen of both benzoyl- and salicyloylhydrazones can be protonated in acidic medium (*124*).

The various biacetyl derivatives and related compounds which have been used as analytical reagents for cations are summarized in Table 1. The ionization constants of various ligands are also included in Table 2.

SUMMARY

A review of the analytical uses of Schiff bases derived from biacetyl and related compounds is given. Biacetylderivatives have found use in qualitative and gravimetric analysis, and in spectrophotometric and chromatographic procedures. Biacetylmonoxime has been broadly used in the spectrophotometric determination of urea in biological fluids. Biacetyl itself has also found use as an analytical reagent.

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Analytical Possibilities of Pyridine-2-Acetaldehyde Benzoylhydrazone as a Chromogenic Reagent

M. GARCIA-VARGAS,[1] J. M. BAUTISTA, AND P. DE TORO

Faculty of Chemistry, University of Sevilla, Sevilla, Spain

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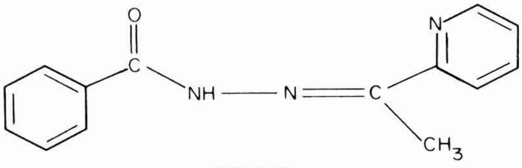
INTRODUCTION

Hydrazones have been widely used for the spectrophotometric determination of metal ions and several papers have dealt with the use of aroylhydrazones as analytical reagents. In previous studies on the analytical applications of benzoyl- and salicyloyl-hydrazones (*3–7, 10–13*), it was observed that these compounds form colored complexes with several cations. Most of these complexes are soluble in aqueous–ethanolic solutions and may be extracted in various organic solvents of low dielectric constant.

This paper is concerned with an investigation of the spectrophotometric properties in aqueous 3:2 (v/v) ethanol solutions and the solvent extraction behavior of pyridine-2-acetaldehyde benzoylhydrazone (PABH) complexes with Fe(II), Fe(III), Ni(II), Pd(II), V(V), Ti(IV), Hg(II), Mn(II), Zn(II), Bi(III), Co(II), Cu(II), Pb(II), and Ga(III).



(PABH)

MATERIALS AND METHODS

Apparatus

A Unicam SP800 spectrophotometer was used for recording spectra in the ultraviolet and visible regions of the spectrum and a Unicam SP6-400 spectrophotometer was used for measurements at fixed wavelengths. Quartz or silica (according to the wavelength required) 1-cm optical path cells were used. A Perkin–Elmer 257 infrared spectrophotometer was

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[1] Present address: Department of Analytical Chemistry, Faculty of Chemistry, University of Cadiz, Puerto Real (Cadiz), Spain.

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also used as well as a digital pH-meter (Crison digit 74) with glass–calomel electrodes.

Synthesis of PABH

Equimolecular amounts of pyridine-2-acetaldehyde and benzoylhydrazide were mixed in ethanol. Several drops of concentrated hydrochloric acid were added and the mixture was refluxed for 60 min. The white PABH compound was separated by filtration. The product obtained was recrystallized twice in ethanol (yield 36.7% and mp 154–156°C).

The analysis result was as follows. Calculated for PABH ($C_{14}H_{13}N_3O$), C 70.21%, H 5.43%, and N 17.55%; found, C 70.40%, H 5.40%, and N 17.74%.

Reagents

All reagents were of analytical-reagent grade and all solutions were prepared using distilled water. Pyridine-2-acetaldehyde benzoylhydrazone stock solution at 0.1% (m/v) in ethanol was employed. The concentrations of the standard solutions of metal ions (1–6 g/liter) were determined gravimetrically (*2*) by weighing the oxides (iron), the dimethylglyoximates (nickel, palladium), or the cupferronates (vanadium, titanium). The other standard solutions were prepared in a previous investigation in this laboratory. Working standard solutions were prepared from these solutions as necessary. Phtalate, chloroacetate, acetate, and ammonia buffers were prepared by conventional methods.

General procedures

Direct method. In a 10-ml standard flask containing metal ion solution, 4 ml of reagent solution, 2 ml of buffer solution, and 1 ml of 1 *M* potassium chloride solution were added and diluted to the mark with distilled water. The solutions were allowed to stand for about 30 min, and the absorbance was measured against a reagent blank.

Extraction method. Aliquots of 3 ml of the former solutions were pipetted into separating funnels. Three milliliters of chloroform were added, and the mixture was shaken for 30 sec. The color of the aqueous and organic layers was noted and the absorbance of the organic solutions was measured against a reagent blank. When the metal was distributed or was not extracted, the mixture was shaken for 30 sec plus. If extraction still remained incomplete a test was made by adding 0.01 g of sodium perchlorate to the aqueous phase before adding the chloroform.

RESULTS AND DISCUSSION

Properties of Pyridine-2-Acetaldehyde Benzoylhydrazone

Infrared spectrum. The infrared spectrum of PABH in potassium bromide disks was obtained. In agreement with Domiano *et al.* (*1*) the bands were assigned to the characteristic vibrations (cm^{-1}) of: the N−H stretching mode at 3182 m (this frequency is low to correspond to a free N−H absorption, therefore may be assigned to a bonded N−H absorption), the =C−H stretching mode at 3030−3010 w (corresponding to the vibrations of the phenyl and pyridine rings), the $-CH_3$ stretching mode at 2980 and 2875 w, the C=O stretching mode at 1655 vs (amide I band), the N−H deformation at 1540 s (amide II band, probably with contribution of the C=N stretching mode), and the C−N stretching mode at 1285 s (amide III band).

Solubility and ultraviolet spectrum in organic solvents. PABH has a solubility in water, methanol, ethanol, acetone, isoamyl alcohol, methyl isobutyl ketone, ethyl ether, carbon tetrachloride, benzene, and toluene of 0.9 (80.37, 20°C), 37.7 (33.62, 20°C), 17.6 (24.30, 25°C), 40.5 (20.7, 25°C), 5.6 (14.7, 25°C), 9.9 (13.11, 20°C), 1.5 (4.34, 20°C), 2.9 (2.24, 20°C), 6.0 (2.28, 20°C), and 3.8 (2.38, 25°C) g/liter, respectively. The numbers in parentheses are the corresponding dielectric constants and temperatures of the pure liquids. The solubility of the reagent in chloroform (4.81, 20°C) and N,N-dimethylformamide (37.6, 25°C) is higher than 50 g/liter. PABH is more soluble than other related reagents, such as pyridine-2-acetaldehyde (*7*) and pyridine-2-carbaldehyde (*3*) salicyloylhydrazones, PASH and SHPA, respectively. Although PASH and SHPA, with a hydrophilic OH group would be expected to be more soluble than PABH, this is not so, probably because of the hydrogen bonding. In alkaline medium the solubility of PASH and SHPA increases because the OH groups are deprotonated.

A reagent solution of PABH in water or chloroform of 8.35×10^{-5} *M* concentration shows an absorption maximum at 300 or 295 nm, with molar absorptivity of 2.32×10^4 or 1.89×10^4 liters $mol^{-1}cm^{-1}$, respectively.

Influence of pH on the aqueous reagent solutions. Ionization constants. The effect of change in pH on the spectra of aqueous solutions of the reagent (8.35×10^{-5} *M*) shows bathochromic shifts at pH less than 4 or greater than 10. PABH shows at pH 1−3 two absorption maxima at 265 and 320 nm (the latter of greater molar absorptivity) and an isosbestic point at 308 nm. At pH > 12 an absorption maximum at 285−315 nm and an isosbestic point at 315 nm is also showed by the reagent.

The Phillips and Merrit method (*9*) was used for the spectrophotometric determination of the ionization constants. Figure 1 depicts the plots of absorbance vs pH of PABH at four wavelengths. The average p*K* values, in aqueous medium at ionic strength 0.1, was found to be 3.6 ± 0.3 and 11.1 ± 0.1. The first p*K* may be caused by protonation of the pyridine nitrogen atom. SHPA and PASH have pK_1 values of 3.5 and 4.0, respectively. The second p*K* may be due to deprotonation of the hydrogen atom of the −CONH− group. The acid−base behavior can be explained by the

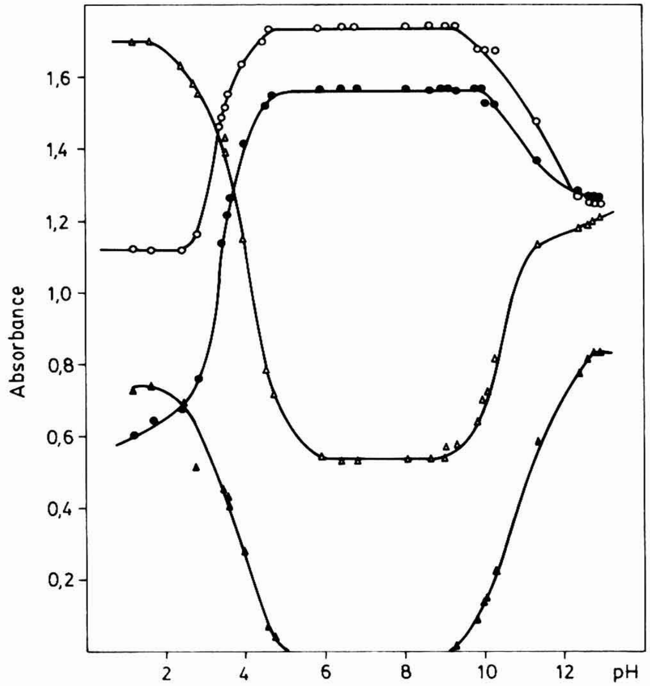
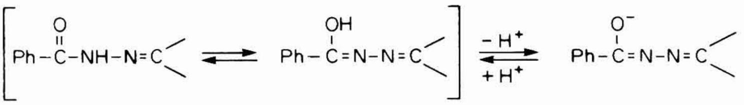


FIG. 1. Plots of absorbance vs pH of the reagent ($C_R = 1.34 \times 10^{-4}$ *M*): (○) at 300 nm, (△) at 325 nm, (●) at 285 nm, and (▲) at 340 nm. The absorbance of each solution was measured with time and then calculated by extrapolation the absorbance value at zero reaction time.

existence of tautomeric forms of the benzoylhydrazone molecule. The deprotonation is enhanced by coordination (*8*):



Stability of reagent solutions. Effect of redox substances. The reagent is stable in ethanol or chloroform for several days, but hydrolysis to pyridine-2-acetaldehyde and benzoylhydrazide occurs in dilute aqueous solutions (1.34×10^{-4} *M*). The rate of hydrolysis of the ligand increases as the pH value decreases, but in acetate or ammonia buffers decreases when the ethanol-to-water ratio is increased. PABH is somewhat more unstable compared with the other salicyloylhydrazones (SHPA or PASH). The reagent is added to the metal ion solution in excess to avoid this trouble.

Reducing (such as sodium sulfite, hydroxilamine, and ascorbic acid) or oxidizing (such as oxygenated water and potassium persulfate) agents in 0.4% (m/v) concentration affect the absorption spectra of the reagent in acetate buffer, but in chloroacetate buffer they do not. This is probably because the reagent is more rapidly hydrolyzed than oxidized. The absorption spectra of PABH in ammonia buffer is also affected by hydroxilamine, ascorbic acid, or oxygenated water, but sulfite or persulfate have no perceptible effect. On the other hand, reducing agents at lower concentration have no perceptible effect.

Reactivity of PABH with metal ions. The reactions of 31 metal ions with the reagent were tested in phtalate, acetate, and ammonia buffers, to determine possible applications of the reagent to the spectrophotometric analysis. PABH forms soluble colored chelates with Pb(II), Mn(II), Ga(III), Zn(II), Ti(IV), Hg(II), Ni(II), Bi(III), V(V), Fe(II), Pd(II), Cu(II), Co(II), and Fe(III), as shown qualitatively in Fig. 2.

Spectrophotometric Study of Metal Ion-PABH Systems

Formation of the complexes in aqueous ethanolic solution. The addition of a 0.1% (m/v) solution of reagent in ethanol to a solution of metal ions immediately produces a complex. Maximal absorption bands appear between 353 and 405 nm, except for iron(II) (λ_{max} = 648 nm). Absorption peaks and molar absorptivities of the complexes formed in three different buffers are shown in Table 1. The complex solutions show a difference in absorption with the pH. In the case of the Hg(II)–, Pb(II)–, Mn(II)–, Zn(II)–, and Co(II)–PABH systems, when pH increases the absorption maximum shows a bathochromic shift from 6 to 32 nm. Ga(III)–,

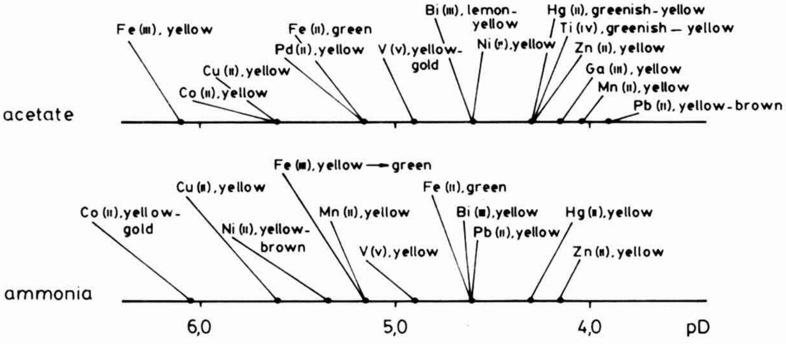


FIG. 2. pD values and colors of PABH reactions with metal ions in aqueous 40% v/v ethanol solution. pD = –log (minimum detectable mass of metal ion, g/volume, ml).

TABLE 1

ABSORPTION MAXIMA AND MOLAR ABSORPTIVITIES OF THE METAL CHELATES OF PABH IN AQUEOUS ETHANOLIC SOLUTION

	Phtalate buffer			Acetate buffer			Ammonia buffer		
Metal ion	Conc. (ppm)	λ_{max} (nm)	ϵ $(l \cdot mol^{-1} \cdot cm^{-1})$	Conc. (ppm)	λ_{max} (nm)	ϵ $(l \cdot mol^{-1} \cdot cm^{-1})$	Conc. (ppm)	λ_{max} (nm)	ϵ $(l \cdot mol^{-1} \cdot cm^{-1})$
Hg(II)	80	348	1.5×10^3	40	348	0.9×10^3	100	350–380	2.9×10^3
Pb(II)	60	360	1.1×10^3	60	368	4.4×10^3	10	366	1.8×10^4
Ga(III)	13.5	380–390	1.1×10^4	13.5	370–390	1.1×10^4	4.5	366	5.9×10^3
Bi(III)	10	370[a]	4.8×10^3	3	370	2.0×10^4	3	360	3.0×10^4
Fe(II)	20	375	7.4×10^3	0.5	354[b]	2.6×10^4	20	372	8.1×10^3
Fe(II)	—	—	—	20	648[c]	3.9×10^3	20	648[d]	2.7×10^3
Mn(II)	200	—	—	200	354	0.2×10^3	10	365–375	1.4×10^4
Zn(II)	10	359	3.1×10^3	50	375	3.4×10^3	10	375	1.6×10^4
V(V)	20	390[e]	7.1×10^3	20	375[f]	7.3×10^3	20	380	0.6×10^3
Cu(II)	4	378	3.1×10^4	4	365–375	2.1×10^4	4	366	3.8×10^4
Pd(II)	1	346	4.4×10^4	1	405	3.7×10^4	1	343	3.8×10^4
Co(II)	10	362	4.8×10^3	2	365–370	1.5×10^4	2	360–380	1.5×10^4
Ni(II)	10	370	8.4×10^3	4	376	2.5×10^4	4	365	2.3×10^4
Ti(IV)	200	—	—	40	359[g]	2.2×10^3	200	353	0.4×10^3

[a] Shoulder at 390–400 nm ($\epsilon = 0.3 \times 10^3$).

[b] Shoulder at 390–400 nm ($\epsilon = 1.1 \times 10^3$).

[c] Shoulder at 430–460 nm ($\epsilon = 5 \times 10^3$).

[d] Shoulder at 580–600 nm ($\epsilon = 1.6 \times 10^3$).

[e] Shoulder at 410–420 nm ($\epsilon = 6.6 \times 10^3$).

[f] Shoulder at 400–420 nm ($\epsilon = 6.5 \times 10^3$).

[g] $\lambda_{max} = 380$ nm ($\epsilon = 0.2 \times 10^3$).

Bi(III)–, V(V)–, and Cu(II)–PABH systems show a reverse behavior with a bathochromic shift from 10 to 24 nm. These facts can be explained by the existence of the cationic or anionic form of the reagent at low or high pH values, respectively, in which the color is enhanced by coordination (Fig. 3). The extension of the π-system of the molecule in alkaline media leads to an even more intensely colored reagent. In the case of the Fe(II)–PABH system, two different complexes, one yellow (λ_{max} = 354 or 372 nm) and the other green (λ_{max} = 648 nm), are formed according to pH. Ascorbic acid ensures that iron remains in the lower oxidation state. Hydroxilamine or hydrazine cannot be recommended because of the possible formation of pyridine-2-acetaldehyde oxime or hydrazone by exchange of C=N groups (*14*). Fe(III) and PABH quickly form a yellow complex in phtalate and acetate buffers that changes to a green complex in ammonia buffer. This fact may be explained by the reduction of iron(III) by the reagent.

The presence of at least 40% (v/v) ethanol is necessary in the reaction medium to prevent the precipitation of the excess of reagent and its complexes.

Influence of pH. The effect of pH on the color development was studied by preparing a series of solutions, with different concentrations according

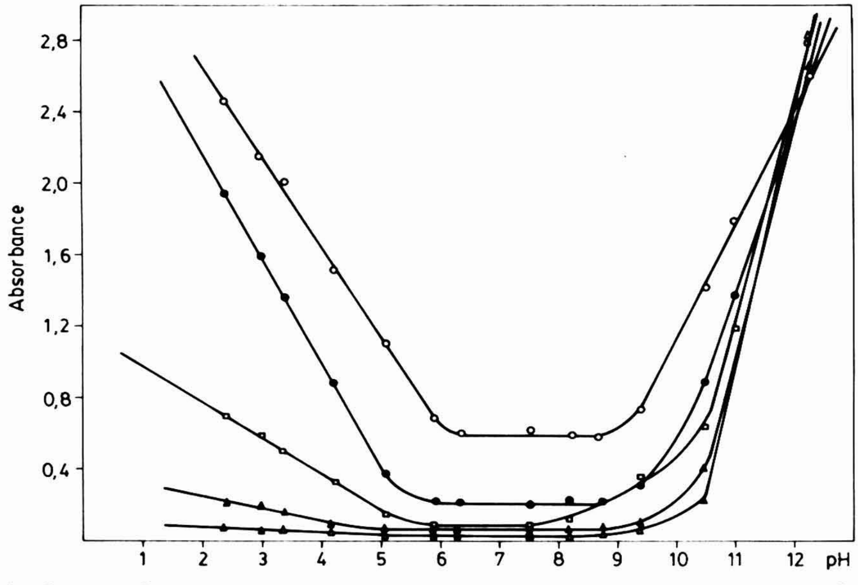


FIG. 3. Curves of absorbance vs pH of a reagent blank solution: (○) at 350 nm, (●) at 360 nm, (□) at 370 nm, (▲) at 380 nm, and (△) at 390 nm. The samples were prepared in 10-ml volumetric flasks, with 4 ml of 0.1% m/v aroylhydrazone solution in ethanol, 1 ml of 1 *M* KCl solution, different volumes of HCl or NaOH solutions and diluted to volume with distilled water. The absorbances were immediately measured against distilled water.

to the metal ion, and varying from pH 2 to 12. The optimum pH range for the formation of the metal chelates is shown in Table 2. Hg(II), Pb(II), Mn(II), Zn(II), Co(II), Ni(II), and Bi(III) ions have the optimal pH range in neutral or alkaline medium, while for Ga(III), V(V), and Cu(II) it is located in acid medium. As the other metal complexes are partially or totally destroyed by the addition of acid, this may permit the establishment of selective spectrophotometric methods for the determination of gallium, vanadium, and copper. Besides, the reagent itself absorbs little at the wavelength of maximum absorption of these complexes.

Stability of the complexes. The stability of the complexes has been tested by measuring the absorbances of the absorption maxima with time (Fig. 4). From this figure it may be concluded that most metal complexes are stable for at least several hours. The green Fe(II)–PABH complex is formed immediately in aqueous media, and remains stable for 10 min in the optimum pH range, then the absorbance at 648 nm decreases, because of the precipitation of the complex. It is inferred that in aqueous media the green complex is not of great analytical interest. It is to be noted that although the reagent is partially hydrolyzed in aqueous media, however, most of its metal complexes are stable.

Stoichiometry of the complexes. The stoichiometry of the metal chelates in the optimum pH range was evaluated by the continuous variation method (Table 3). The metal:ligand ratio for iron(II), zinc, cobalt, and nickel was found to be 1:3. The results obtained for iron(II) were also confirmed by extraction of the chelate from aqueous–ethanolic medium

TABLE 2

OPTIMUM pH RANGE FOR THE FORMATION OF METAL CHELATES OF PABH

Metal ion	Concentration (ppm)	Wavelength measured (nm)	pH range[a]
Hg(II)	100	360	9.50–10.25
Pb(II)	10	360	6.75–7.75 (9.0–11.0)
Ga(III)	4.5	370	3.0–3.25 (5.0–6.4)
Bi(III)	5	380	8.75–10.0 (5.2–6.3)
Fe(II)	10	648	5.9–10.5
Fe(II)	2	370	7.0–8.0
Mn(II)	5	370	8.0–9.25
Zn(II)	4	370	6.75–9.0
V(V)	1	380	3.0–3.6
Cu(II)	1	370	2.5–3.5 (6.8–7.5)
Co(II)	10	370	6.25–7.0 (8.5–9.0)
Ni(II)	2	376	5.25–8.30

[a] pH ranges in parentheses corresponding to other optimum pH ranges of lower absorbances.

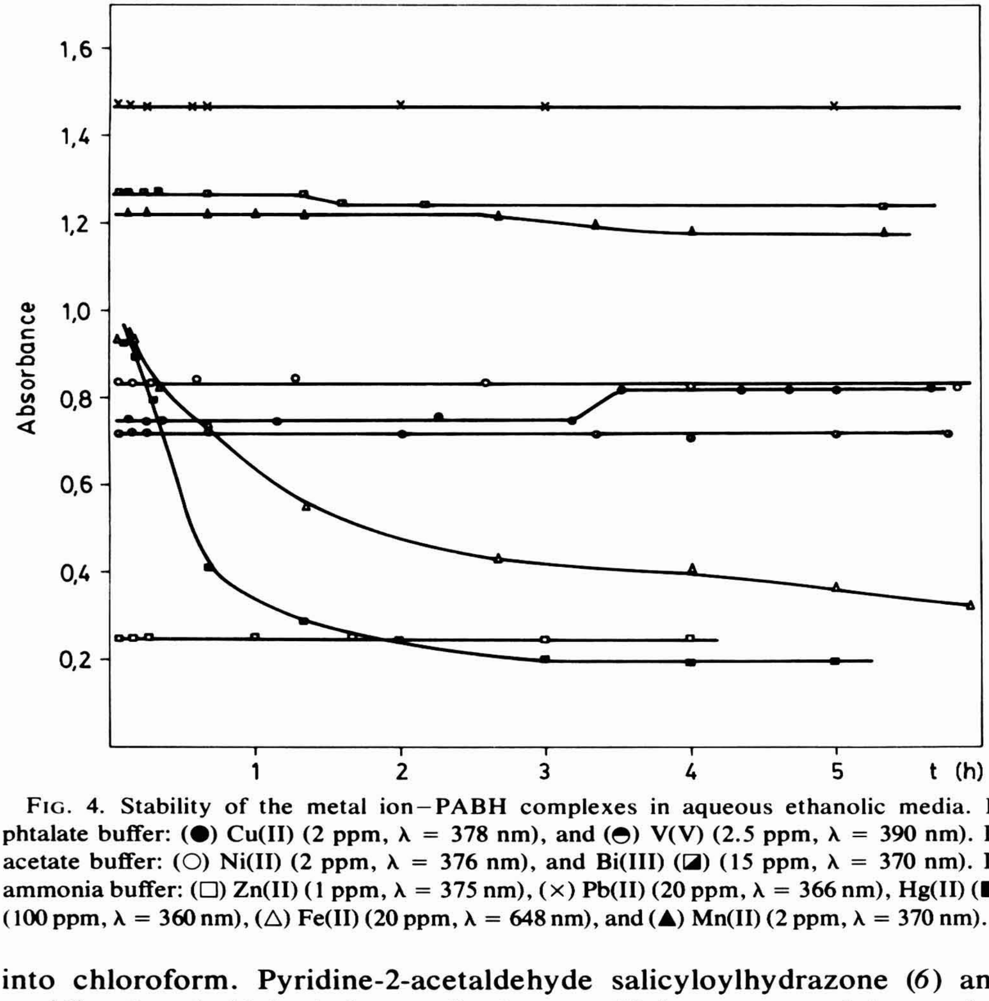


FIG. 4. Stability of the metal ion–PABH complexes in aqueous ethanolic media. In phtalate buffer: (●) Cu(II) (2 ppm, λ = 378 nm), and (◒) V(V) (2.5 ppm, λ = 390 nm). In acetate buffer: (○) Ni(II) (2 ppm, λ = 376 nm), and Bi(III) (◪) (15 ppm, λ = 370 nm). In ammonia buffer: (□) Zn(II) (1 ppm, λ = 375 nm), (×) Pb(II) (20 ppm, λ = 366 nm), Hg(II) (■) (100 ppm, λ = 360 nm), (△) Fe(II) (20 ppm, λ = 648 nm), and (▲) Mn(II) (2 ppm, λ = 370 nm).

into chloroform. Pyridine-2-acetaldehyde salicyloylhydrazone (*6*) and pyridine-2-carbaldehyde benzoylhydrazone (*5*) from a green 3:1 complex with iron(II). It is apparent that the three reagents act as a bidentate ligand. However, pyridine-2-carbaldehyde salicyloylhydrazone forms a green 2:1 complex with iron(II) (*5*). As all molecules contain the ferroin group, it is evident that the hydroxide group and the methyl group play an important part in the chelate character of the ligand. On the other hand, the latter reagent forms a yellow 2:1 complex with nickel or zinc (*3*). Nevertheless, all of these ligands react with vanadium(V) giving a yellow 1:1 complex (*7, 4*).

The apparent stability constants for the complexes were estimated from the data obtained by Job's method or by means of the equation

$$K_{\text{app}} = \frac{a - (\Delta A/\epsilon)}{n^n\,(\Delta A/\epsilon)^{n+1}},$$

TABLE 3

STOICHIOMETRY OF THE METAL–PABH CHELATES IN AQUEOUS ETHANOLIC MEDIA

Metal ion	Metal conc.[a] (M)	Wavelength (nm)	Buffer	Metal/ligand ratio
Hg(II)	4.98×10^{-3}	360	Ammonia	1:2
Pb(II)	1.93×10^{-3}	360	Ammonia	1:1
Fe(II)	1.43×10^{-2}	648	Ammonia	1:3[b]
Mn(II)	3.64×10^{-3}	370	Ammonia	1:2
Zn(II)	7.65×10^{-4}	370	Ammonia	1:3
V(V)	4.91×10^{-4}	380	Phtalate[c]	1:1
Pd(II)	3.74×10^{-3}	390	Ammonia	1:2[d]
Co(II)	3.39×10^{-3}	370	Ammonia	1:3
Ni(II)	1.70×10^{-3}	376	Acetate	1:3

[a] The ligand concentration is half the metal ion concentration in order to give samples and blanks a 40% v/v ethanol concentration.

[b] The stoichiometric ratio found in chloroform is the same.

[c] In acetate buffer the metal/ligand ratio found is to be 2:3.

[d] A metal/ligand ratio of 1:1 is also found.

where a = metal ion total concentration, ΔA = sample absorbance in ligand excess minus the sample absorbance at the stoichiometric ligand amount, ϵ = molar absorptivity at the measured wavelength, and n = number of ligands. K_{app} values of 10^4-10^5, 10^6-10^8, and $10^{16}-10^{17}$ were obtained for ML, ML_2, and ML_3 (where, M = metal and L = ligand) complexes, respectively. The metal–PABH systems are moderately dissociated in aqueous–ethanolic solution.

Extraction of the complexes. For each complex, a series of aqueous solutions containing different concentrations of metal ion at three pH values were prepared and extracted into chloroform, after 5 min reaction time. Table 4 shows the absorption maxima of the complexes in phtalate (pH = 2.7), acetate (pH = 4.9), and ammonia (pH = 10) buffers. The study of extraction of the metal chelates is interesting because: (1) The absorption maxima shift to a spectral region where the absorption of the reagent becomes lower, (2) molar absorptivities are higher, and (3) PABH is more stable in organic solvents. From the data of the above table, the establishment of a selective method for vanadium(V) appears possible with the extraction being performed from aqueous solutions of relatively low pH values. The study of extraction–spectrophotometric possibilities of copper, nickel, and cobalt also appears of interest.

CONCLUSION

PABH possesses the chromogenic grouping –N=C–C=N–NH–CO– and acts as a bidentate or tridentate ligand and five-membered rings are produced. As has been previously indicated, the –NHCO– group can be

TABLE 4
ABSORPTION MAXIMA OF THE METAL–PABH CHELATES IN CHLOROFORMIC SOLUTION

	Phtalate buffer			Acetate buffer			Ammonia buffer		
Metal ion	Conc. (ppm)	λ_{max}	Absorbance	Conc. (ppm)	λ_{max}	Absorbance	Conc. (ppm)	λ_{max}	Absorbance
Hg(II)	80	No extraction		40	380	0.44	100	370[a]	2.70
Pb(II)	60	390	0.240	60	385	1.36	10	380–385	1.40
Fe(II)	20	No extraction		20	660	0.45	20	640[b]	0.22
Mn(II)	200	No extraction		200	350[c]	0.70	10	370[d]	2.70
Zn(II)	10	383	1.15	50	370[c]	2.70	10	370[e]	2.72
V(V)	7.5	405	2.06	7.5	397[f]	2.28	7.5	395–400	0.23
Cu(II)	4	390	1.38	4	380	2.30	4	370–380	2.50
Pd(II)	1	400	0.26	1	390	0.28	1	390	0.23
Co(II)	10	388	0.67	2	360–370	1.28	2	370–380	0.96
Ni(II)	10	388	0.41	4	385	2.32	4	380–385	2.35

[a] Shoulder at 380–400 nm (with absorbance = 2.6).
[b] λ_{max} = 370 nm (with absorbance = 2.05) and shoulder at 540–560 nm.
[c] Shoulder at 370–380 nm (with absorbance = 0.57).
[d] Shoulder at 390–400 nm (with absorbance = 2.62).
[e] Shoulder at 390–400 nm (with absorbance = 2.60).
[f] Shoulder at 420 nm (with absorbance = 1.64).

deprotonated in alkaline solutions. The stability of PABH is lower than that of PASH, but the sensitivities of both for metal ions are similar. Nevertheless, the metal chelates formed by the latter reagent are more insoluble. There is no doubt that the hydroxide group in salicyloylhydrazide has a definitive effect on the complex. PABH was selected because the solubilities of both reagent and its metal complexes are superior to those of other salicyloylhydrazones.

The study of the chelating reactions used in flame and flameless atomic absorption and other details of these reactions as well as some analytical applications will be reported later.

SUMMARY

With a view to the use of pyridine-2-acetaldehyde benzoylhydrazone is an analytical reagent, a study of the physical properties and fundamental solution chemistry of the complexes formed by PABH with Fe(II), Fe(III), Ni(II), Pd(II), V(V), Ti(IV), Hg(II), Mn(II), Zn(II), Bi(III), Co(II), Cu(II), Pb(II), and Ga(III) metal ions has been carried out. A critical comparison of pyridine-2-carbaldehyde and pyridine-2-acetaldehyde salicyloylhydrazones and pyridine-2-acetaldehyde benzoylhydrazone as analytical reagents is given.

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MICROCHEMICAL JOURNAL 26, 569–573 (1981)

Analytical Applications of Crown Ethers

I. Oxidation and Determination of Benzaldehyde

I. N. PAPADOYANNIS,[1] TH. A. KOYIMTZIS, AND
G. S. VASILIKIOTIS

*Laboratory of Analytical Chemistry, University of Thessaloniki,
Thessaloniki, Greece*

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It is well known that crown ethers have remarkable complexing properties (*6*). These compounds form complexes with many cations especially those of alkali and alkaline earth cations, both in solution and in the crystalline state. The cations are located in the center of the etheric ring in the plane of the oxygen atoms. The cations are held there principally by electrostatic ion–dipole forces. These positive charged complexes form ion pairs with various anions (ion association complexes). Most of these ion pairs are soluble in organic solvents (*7*). Thus using such crown compounds it is possible to transfer cations and anions from aqueous solutions to organic solvents.

The complex properties of the crown ethers, the stability constants, and the structure of their complexes have been examined by various workers (*2, 3, 5*). Generally there is an increasing interest of examining the properties of these compounds but little work has been done on their analytical applications (*1, 4*). Having this in mind we started an investigation on the possible application of crown ethers in chemical analysis, such as the determination of water in organic solvents, the determination of nitrophenols, the oxidation and determination of aldehydes and alcohols, and the extraction of various ions from aqueous solution e.t.r.

In the present paper we report the first results of our investigation in this field. These results refer to the oxidation and determination of benzaldehyde in organic solvents, by dicyclohexyl-18-crown-6-potassium permanganate complex (DCC-K^+ MnO_4^-).

EXPERIMENTAL

Reagents. 2,5,8,15,18,21-Hexaoxatricyclo-|20,4,0,^{9,14}| hexacosan

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[1] Part of the work to be submitted as Ph.D. Thesis.

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(dicyclohexyl-18-crown-6) was from Fluka AG. A 10^{-2} M solution of this crown ether was prepared in benzene.

Standard solutions of benzaldehyde (E. Merck) were prepared by dissolving appropriate amounts in benzene.

A standard solution of permanganate (2.5×10^{-3} N) was prepared by dissolving appropriate amounts in 0.01 N sulfuric acid and standardized against Mohr's salt.

All other reagents and solvents were of analytical grade reagents.

Procedure. One to eight milliliters of organic solvent sample (containing up to 5 μmol of benzaldehyde) is transferred to a separatory funnel, followed by 1 ml of 10^{-2} M crown ether solution in the same solvent, and the volume is adjusted to 10 ml. Then 10 ml of permanganate solution (2.5×10^{-3} N) is added to the funnel followed by 0.2 ml of sodium molybdate solution, 5×10^{-3} M. The mixture is shaken for 5 min. The two phases are separated and the absorbance of the aqueous phase is measured at 525 mm against distilled water using a 10-mm cell. From a previous permanganate calibration curve the amount of unreacted permanganate is found.

RESULTS AND DISCUSSION

It is known that potassium permanganate is solubilized in benzene by complexing with crown ether. This complex has been used in organic chemistry as an efficient oxidizing agent (*3*). This type of reagent has been used in our work for quantitative aspects for the oxidation of benzaldehyde in organic solvents. For this purpose, originally potassium permanganate was solubilized in benzene using dicyclohexyl-18-crown-6 (DCC) as a complexing agent. Aliquots of this solution were mixed with various volumes of benzaldehyde solution in benzene. To acidify this mixture phenylacetic acid or trifluoroacetic acid was added in the presence of a catalyst (molybdate). The mixture was allowed to react for a certain period of time and the excess of permanganate was extracted with water and determined by titration or spectrophotometrically.

Results obtained by this procedure were not reproducible and no stoichiometry between benzeldehyde and permanganate consumed could be found. In most cases manganate dioxide was formed.

Then we tried to oxidize benzaldehyde with an acidified aqueous solution of permanganate using crown ethers as ion transfer reagents. For this purpose equal volumes of benzaldehyde solution in benzene and aqueous permanganate solution were shaken. Previously crown ether was added to the organic phase and catalyst was added to the aqueous phase. After finishing the shaking the two phases were separated and the unconsumed permanganate in the aqueous phase was determined by absorbance measurements. Blank experiments were used to determine the possible decomposition of permanganate during the procedure period.

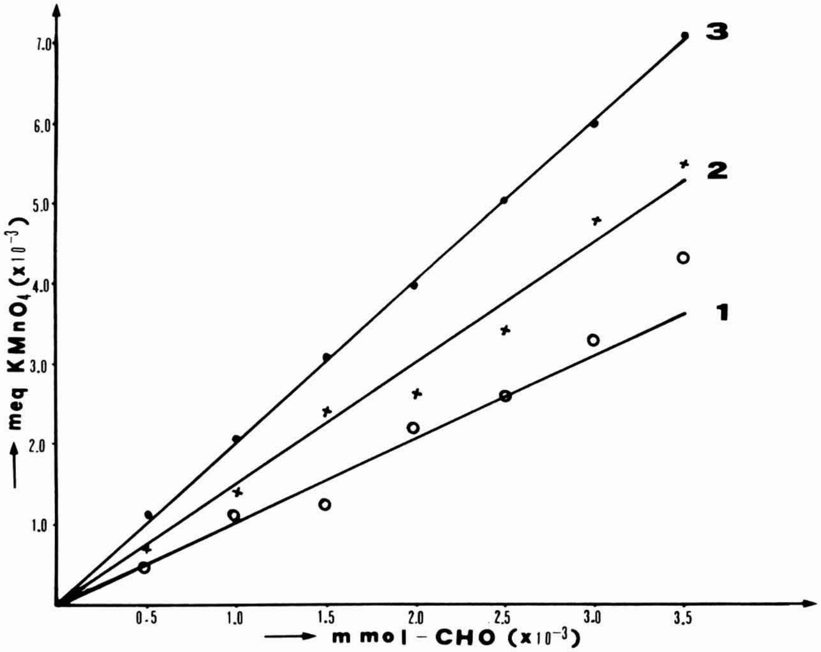

FIG. 1. Amounts of permanganate consumed for the oxidation of benzaldehyde in benzene: (1) in the absence of crown ether, (2) in the presence of crown ether but without catalyst, and (3) in the presence of crown ether and catalyst. Initial concentration of permanganate is 2.5×10^{-3} *N* and of DCC 1×10^{-2} *M*.

Results obtained by this procedure showed that the presence of crown ether and the catalyst in the reaction mixture plays an important role in the oxidation of benzaldehyde.

The amounts of permanganate consumed for the oxidation of various amounts of benzaldehyde (after shaking the two phases for 5 min) are given in Fig. 1.

In Fig. 1 curve 1 represents the results obtained in the absence of crown ether. These results show poor reproducibility, and no stoichiometric relation could be found. In all cases the amount of permanganate consumed for 1 mmol of benzaldehyde was less than 2 meq. The results were also unsatisfactory even when oxidation time was increased.

Curve 2 represents the results obtained in the presence of crown but without a catalyst. These results show only the extent of the oxidation of benzaldehyde comparing with the results of curve 1.

Curve 3 represents the results obtained in the presence of crown ether and a catalyst (molybdate ions). These results show good reproducibility and a stoichiometry was found corresponding to 2 meq. of permanganate per mmol of benzaldehyde in all cases. This stoichiometry suggests that

TABLE 1
RESULTS OF THE SPECTROPHOTOMETRIC DETERMINATION OF BENZALDEHYDE IN THE PRESENCE OF DICYCLOHEXYL-18-CROWN-6 AND CATALYST

Solvent (10 ml)	Added (μmol)	Found[a] (μmol)
Benzene	0.50	0.48 ± 0.07
	1.00	1.05 ± 0.05
	1.50	1.55 ± 0.08
	3.00	2.92 ± 0.09
Cyclohexane	0.50	0.47 ± 0.06
	1.00	0.94 ± 0.08
	1.50	1.52 ± 0.07
	3.00	3.05 ± 0.09

[a] Average value of five determinations.

benzaldehyde is quantitatively oxidized to benzoic acid. Thus curve 3 could be used as a calibration curve for quantitative determination of benzaldehyde in organic solvents. A systematic study was undertaken to find out the optimum condition for such a determination.

Various catalysts such as vanadate, molybdate, and cobalt ions were tested. Of these catalysts molybdate was superior to others for quantitative oxidation of benzaldehyde.

For a quantitative oxidation the concentration of crown ether in the organic phase must be more than twice the concentration of benzaldehyde.

Replicate analyses were performed at various concentrations of benzaldehyde in benzene and cyclohexene. The results of five determinations at each level are summarized in Table 1. These results show good precision of the developed procedure.

Further work on the oxidation of various aldehydes and alcohols and on other analytical applications of the crown ethers is in progress and results will be published in due time.

SUMMARY

The use of dicyclohexyl-18-crown-6 (DCC) as charge transfer reagent for the oxidation of benzaldehyde in organic solvents by aqueous solution of potassium permanganate is presented. The oxidation in the presence of a catalyst is found to be quantitative and suitable for the determination of benzaldehyde. Results for the determination of benzaldehyde in benzene and cyclohexane are given.

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Derivatives of Rhodanine as Spectrophotometric Analytical Reagents

I. Condensation at C-5 with Aromatic Pyridine and Nonpyridine Aldehydes

G. GALÁN ALFONSO AND J. L. GÓMEZ ARIZA[1]

Department of Analytical Chemistry, Faculty of Chemistry and Pharmacy, University of Seville, Seville, Spain

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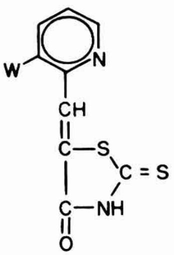
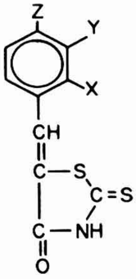
INTRODUCTION

Derivatives of rhodanine and 2-thiohydantoin were first studied by Feigl (*16, 17*) and later by Dubský (*13, 14*), who called attention to their use as analytical reagents, emphasizing the possibilities of *p*-dimethylaminobenzylidenerhodanine; other workers (*36, 37*) have considered these reagents in detail, but have always focused on the qualitative applications. The only one which has been taken into consideration as a spectrophotometric reagent is *p*-dimethylaminobenzylidenerhodanine (cited above), about which numerous papers have been published (*1, 2, 4–12, 18, 21, 22, 27, 29, 30–35, 41*). To supply the lack of information with a photometric focus, concerning the other derivatives, our Department has undertaken two series of studies devoted to exploring the spectrophotometric possibilities of the aromatic derivatives at C-5 of the azolidine ring. The study dealing with 2-thiohydantoin derivatives has already provided several papers (*3, 23, 24, 26*); this paper is the first concerning rhodanine derivatives. Besides, pyridine derivatives of rhodanine have been synthesized for the first time.





Derivatives pattern	W	X	Y	Z	Nominated
Nonpyridine	—	H	H	H	A
	—	OH	H	H	B
	—	H	H	OCH_3	C
Pyridine	H	—	—	—	D
	CH_3	—	—	—	E

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[1] The authors are indebted to Professor F. Pino Perez for his valuable advice and discussion.

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EXPERIMENTAL

Apparatus

The apparatus consisted of a Unicam SP 800, Unicam SP 600, and Beckman DU Spectrophotometry equipped with 1.0-cm glass or quartz cells, a Perkin-Elmer 621 infrared spectrophotometer, and a Phillips PW 9408 digital pH meter with glass-calomel electrodes.

Reagents

Salts and solvents of analytical reagents grade purity or better were used throughout. All metal ion solutions were standardized.

Buffer solution, pH 4.7. Dissolve 56.0 g of sodium acetate and 25.0 ml of glacial acetic acid in water and dilute to one liter.

Preparation of the reagents. The reagents were prepared by the method of Wheeler and Hoffman with some modifications because the reaction was carried out in an aqueous medium instead of a fused one as proposed by the authors. Rhodanine (0.01 mol) was dissolved in 20 ml of 0.4 *M* sodium hydroxide and a solution of the aldehyde (0.01 mol) in 15 ml of ethanol was added; 5 ml of glacial acetic acid was slowly added to the mixture and the product rapidly began to appear. The mixture was heated for 15 min at 60°C, then allowed to stand overnight in the refrigerator, and the crystalline product was filtered off under suction and recrystallized in the solvent adequate for each one. The colors, melting points, and yields of these compounds are summarized in Table 1. The elemental analyses agreed with the values calculated for the empirical formulas.

Procedures

Spectrophotometric determination of dissociation constants. One milliliter of a solution of the reagent studied (1.0×10^{-3} *M*) in ethanol was

TABLE 1
CHARACTERISTICS OF THE REAGENTS STUDIED

Compound	Abbreviation	Molecular formulas	m.p. (°C)	Color and appearance
5-(Phenyl)methylene-rhodanine	A	$C_{10}H_7NOS_2$	199–201	Yellow needles
5-(*O*-Hydroxy-phenyl)-methylenerhodanine	B	$C_{10}H_7NO_2S_2$	198–200 d.	Dark-yellow needles
5-(*p*-Methoxy-phenyl)-methylenerhodanine	C	$C_{11}H_9NO_2S_2$	252–256	Yellow needles
5-(2-Pyridyl)methylene-rhodanine	D	$C_9H_6N_2OS_2$	262–264 d.	Yellow needles
5-(6-Methyl-pyridyl)-methylenerhodanine	E	$C_{10}H_8N_2OS_2$	243–246 d.	Reddish microcrystals

placed in a 25-ml standard flask and 5 ml of 0.5 *M* potassium chloride was added to give an ionic strength of 0.1. The pH values were adjusted with sodium hydroxide solution and hydrocloric acid and the solution was diluted with water to the mark. The absorbance was measured at the adequate wavelengths for each reagent against water as blank (1-cm cells).

The dissociation constants were determined by the methods of Phillips and Merrit (*28*) and Hilldebrand and Reilley (*19*).

Reactions with metal ions. The reactions of numerous ions with the reagents were tested in acetic–acetate medium. The solutions were prepared in 25-ml standard flasks, with 4 ppm of metal ions, a fivefold molar ratio of reagent (dissolved in ethanol), 10 ml of ethanol that prevents the precipitation of the complexes, 5 ml of buffer solution, and dilution to volume with distilled water; the spectrum from 200 to 700 nm was measured against a reagent blank.

RESULTS AND DISCUSSION

The ultraviolet spectra of the reagents in water and ethanol were measured. The results are summarized in Table 2. The reagents show a band between 350 and 400 nm which exhibits two peaks in some reagents. Also in Table 2 we have included analogous data of 2-thiohydantoin derivatives for comparison. Both rhodanine and 2-thiohydantoin derivatives show

TABLE 2
ULTRAVIOLET CHARACTERISTICS OF RHODANINE AND 2-THIOHYDANTOIN DERIVATIVES[a]

	Rhodanine derivatives				2-Thiohydantoin derivatives			
	Water		Ethanol		Water		Ethanol	
Aldehyde	λ_{max}	ϵ_{max}	λ_{max}	ϵ_{max}	λ_{max}	ϵ_{max}	λ_{max}	ϵ_{max}
Benzal	378	26000	375sh	29000	360	32000	362	34000
	371	26250	370	32000	288	7700	294	7500
	275	5500	275	6500				
Salicyl	380	24250	384	26000	376	26000	383	29000
	277	6500	280	8000	307–287sh	7700	301–290sh	7700
Anisal	396	30250	390	33000	384	35000	384	38000
	287	6500	280	7500	292	9000	297	9500
Pycolin	380	31000	379	31500	384	20000	384	17200
	370	30500	368	31250	365	18500	369	19500
	283	8000	282	7500				
6-Methyl-pycolin	386	28500	383	30000	384	30700	384	17200
	372	24750	371	26500	368	30200	369	16500
	288	5000	285	5500	295	9000	305	4500

[a] ϵ_{max}, liters mol^{-1} cm^{-1}; λ_{max}, nm.

TABLE 3
INFRARED SPECTRA OF RHODANINE DERIVATIVES

Assignment	A	B	C	D	E
ν^{NH} and ν^{OH} (phenol)	3150	3100–3400	3150	3150	3200
$\nu^{C=O}$ (amide I band)	1705s	1710s	1690s	1725s	1725s
$\nu^{C=C}$	1675s	1620m	1650m	1615s	1635s
δ^{NH} (amide II band)	1595w	1580w	1570s	1560w	1580w
Aromatic ring (multiplet)	1600–1450	1600–1450	1600–1450	1600–1450	1600–1450
ν^{O-CH_3}			2850m		
$\nu^{C=S}$	1240s	1270m	1245s	1230s	1245s
Aromatic $\delta^{CH}_{\text{in plane}}$ (multiplet)	1225–950	1225–950	1225–950	1225–950	1225–950
Aromatic $\delta^{CH}_{\text{out-of-plane}}$ (multiplet)	900–690	900–690	900–690	900–690	900–690

similar spectra characteristics which is in accord with their structural resemblance.

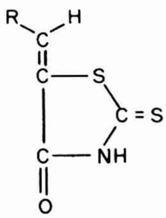
The infrared spectra of the derivatives allow the assignments for the most important bands, but their extensive overlapping makes detailed interpretation very difficult. The bands assigned to the characteristic vibrations are shown in Table 3.

Stability of the Reagents

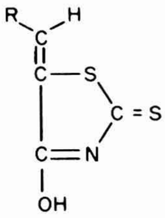
All the reagents are stable in ethanol and 40% (v/v) ethanol–water solutions (4.10^{-5} *M*) for a 100-hr time period. The stability in the ethanol–aqueous medium shows a pH dependence; so, they are stable for at least 100 hr in acid and neutral media (pH 1.5 to 7), but in alkaline medium (about pH 11) the absorbance of the maximum decreases by about 16% in 100 hr for B and E, 6% for C, 30% for D, and 66% for A. This behavior can be attributed to the hydrolitic breakdown of the bond $\mathrm{H}{-}\overset{(3)}{\underset{|}{\mathrm{N}}}{-}\overset{(4)}{\underset{|}{\mathrm{C}}}{=}\mathrm{O}$ analogous to hydantoin derivatives (*39*).

Acid–Base Behavior

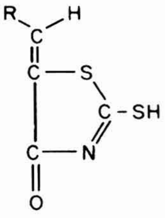
The acid–base behavior can be explained by the existence of tautomeric forms of the rhodanine molecule (*40*), but derivatives at C-5 of rhodanine exhibit only three tautomeric forms (four for rhodanine alone), since the keto-enol tautomerism between C-5 and C-4 atoms is eliminated by the double bond at C-5. Besides, **III** is probably the form mainly present in solution, because of the greater acidity of the thiomidol group,



I



II



III

formed between C−2 and N−3 atoms, in front of the imidol group formed between the N−3 and C−4; this is in accord with Erlenmeyer's (*15*) assertion that decreasing acidity of the groupings is −S−CS−NH > −O−CO−NH > −S−CO−NH and also with the stabilization of −CO− by conjugation effect. (4)

Typical spectral curves corresponding to the appearance of individual hydrogen complexes of the reagents are presented in Fig. 1. The equilibria are marked by shifts, on the absorption spectra and the isosbestic points

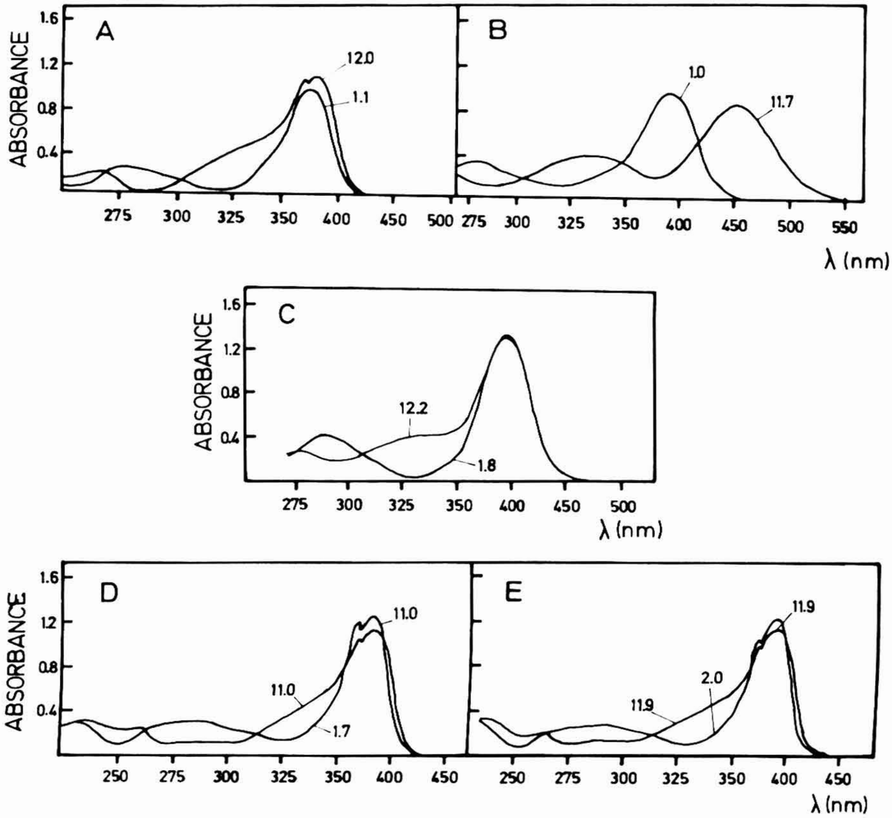
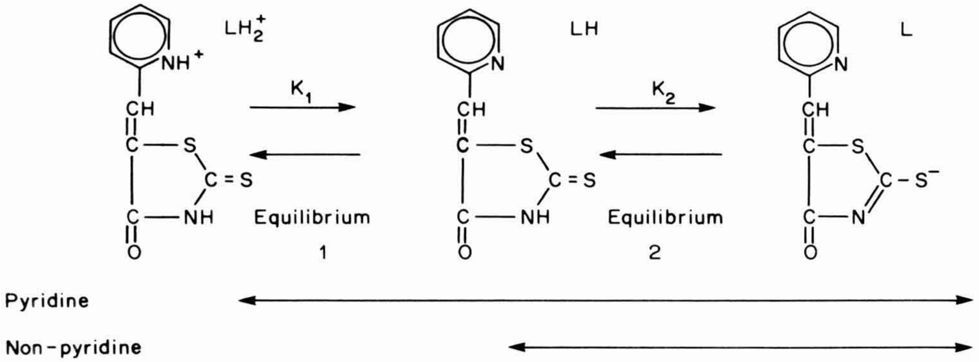


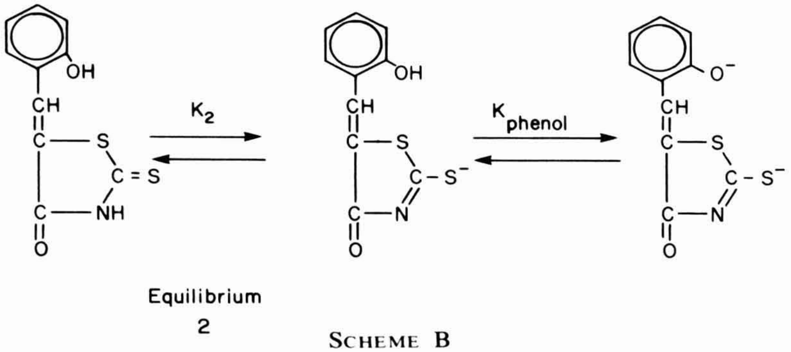
FIG. 1. Absorbance spectra of the reagents at different pH.

which consequently appear. Reagents behave according to two patterns (Scheme A) either for pyridine or nonpyridine derivatives.



SCHEME A

The overall equilibrium of the pyridine reagents involves two hydrogen ions and Scheme A encloses Equilibrium 1 plus Equilibrium 2. In nonpyridine derivatives Equilibrium 1 is not possible and only Equilibrium 2 occurs; there is, however, an exception for salicyladehyde derivative Scheme B, the −OH grouping of which also makes possible the occurrence of a second dissociation equilibrium in alkaline medium.



SCHEME B

The spectral characteristics of the reagents in solution at different pH values (Figs. 1 and 2 and Tables 4–6) support the hypothetical schemes assumed before. Spectra of pyridine derivatives show light changes in strong acid medium. The protonation of the pyridine nitrogen atom affects only slightly the resonance of the molecule; however, the influence of alkaline medium is greater and the broad band at 288 nm, corresponding to the rhodanine ring, decreases ahd shifts bathochromically to form a shoulder of the other band. This is probably due to the conjugation $\mathrm{O{=}\underset{(4)}{\overset{|}{C}}{-}\underset{(3)}{N}{=}\underset{(2)}{\overset{|}{C}}{-}S^-}$ caused by the increasing of the pH value. Nonpyridine

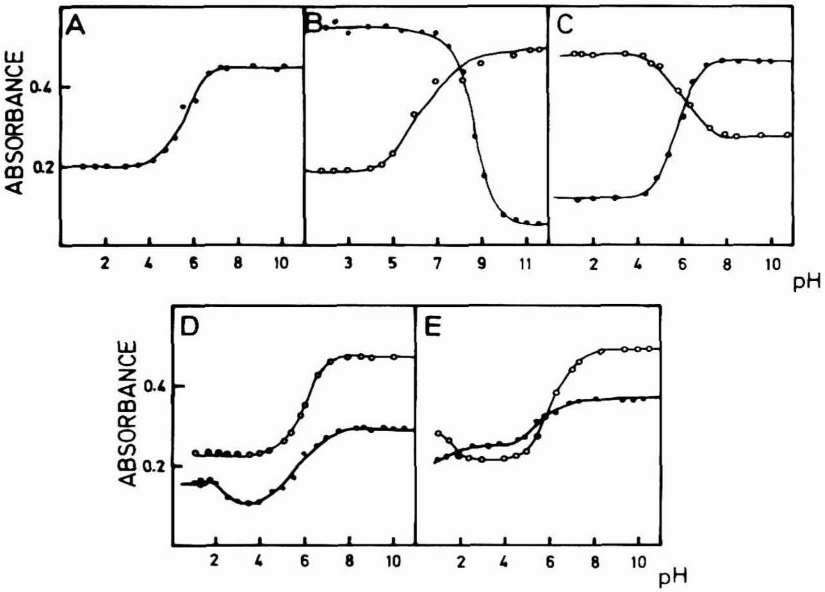


FIG. 2. Effect of the pH on the absorbance of the reagents. (A) ● 325 nm; (B) ● 390 nm, ○ 325 nm; (C) ● 332 nm, ○ 430 nm; (D) ● 410 nm, ○ 335 nm; (E) ● 410 nm, ○ 335 nm. $C_L = 4.0 \times 10^{-5}$ *M*.

reagents show spectral changes only in alkaline medium but which are analogous to the pyridine ones. The spectral characteristics of salicyl derivative are exceptional, because the band at 390 nm shifts bathochromically in more alkaline medium. This is caused by the deprotonation of phenol which affects remarkably the resonance of the molecule and changes the location of the band assigned to the benzene conjugated with the pentagonal ring.

The p*K* values obtained by spectrophotometric methods are listed in Table 4.

Reactionability of the Reagents

It is common to cite the silver affinity of rhodanine and related deriva-

TABLE 4

STEPWISE STABILITY CONSTANTS OF HYDROGEN COMPLEXES

	$\log K_n = \log [H_nL][H]^{-1}[H_{n-1}L]^{-1}$		
Reagent		*n* phenol = 1	*n* = 2
A		5.6	—
B	8.6	6.2	—
C		5.8	—
D		5.7	2.4
E		6.0	1.9

TABLE 5
ISOSBESTIC POINTS IN ABSORPTION SPECTRA OF REAGENTS

Reagent	Complexes	pH	λ_{isos} (nm)
A	L, HL	4.0–7.0	303
B	L, HL	4.0–9.0	305
	HL, H_2L	7.0–11.0	348,418
C	L, HL	4.0–8.0	307
D	L, HL	2.0–3.5	—
	HL, H_2L	4.0–7.0	313
E	L, HL	1.5–2.5	—
	HL, H_2L	4.0–8.0	313

tives, but there is no agreement about the grouping that holds this property. Feigl states the silver specificity of –NH– group (*16*, *17*), and Dubský asserts that grouping $S{=}\overset{|}{C}{-}NH{-}\overset{|}{C}{=}$ is responsible for the reactionability of these reagents; according to Kulberg *et al.* (*20*), a hydrogen atom of $\overset{(3)}{-NH-}$ may be substituted by silver undergoing $S{=}C{-}NAg{-}\overset{|}{C}{=}$, but another Voznesenskii *et al.* (*38*) assume that both S and N atoms contribute to the bonding as $\underbrace{S{=}\overset{|}{C}{-}N}Ag{-}C{=}$ or $\underbrace{Ag S{-}\overset{|}{C}{=}}N{-}\overset{|}{C}{=}$.

The reactions of numerous metallic ions with the reagents were tested in acetic–acetate medium, and the results are summarized in Table 7; analogous results for 2-thiohydantoin derivatives (*23*, *26*) were included

TABLE 6
MAXIMUM MOLAR ABSORPTIVITIES OF INDIVIDUAL HYDROGEN COMPLEXES

	$\epsilon \times 10^{-4}$ (liters mol^{-1} cm^{-1}) for wavelength λ (nm)							
Reagent	L		HL			H_2L		
A	2.75 (380)	2.67 (371)	2.5 (378)	0.75 (280)		—	—	
B	2.1 (450)	1.0 (330)	2.5 (390)			2.4 (390)	0.85 (280)	
C	3.2 (396)		3.3 (398)	1.05 (288)		—	—	
D	2.8 (382)	2.6 (369)	3.1 (382)	3.0 (369)	0.8 (287)	3.1 (382)	3.0 (369)	0.8 (287)
E	2.8 (389)	2.5 (372)	3.1 (388)	2.6 (372)	0.7 (287)	3.1 (388)	2.6 (372)	0.7 (287)

TABLE 7

Photometric Characteristics of the Complexes in Solution[a]

Cation	A		B		C		D		E	
	λ_{max}	ϵ_{max}	λ_{max}	ϵ_{max}	λ_{max}	ϵ_{max}	λ_{max}	ϵ_{max}	λ_{max}	ϵ_{max}
Au(III)	422	8.7×10^3	446	9.43×10^3	435	6.4×10^3	413	1.08×10^4	420	1.12×10^4
Pd(II)	418	2.18×10^4	454	2.63×10^4	440	2.26×10^4	413	2.92×10^4		
Cu(II)	425	2.22×10^3	450	4.13×10^3	444	1.46×10^4	420	6.35×10^3	426	1.59×10^3
Cu(I)	428	1.4×10^4	457	1.65×10^4	446	1.47×10^4	422	1.14×10^4	462	1.03×10^4
Hg(II)	440	1.01×10^3	448	4.52×10^3	442	3.72×10^4	414	2.26×10^4	420	2.36×10^4

[a] λ_{max}, nm; ϵ_{max}, liters mol^{-1} cm^{-1}.

for comparison. These results surprise us, because reagents give good reactions, from an analytical point of view, only with Pd(II), Au(III), Hg(II), and Cu(I) and (II), but not with Ag(I). This fact is apparently not in keeping with all the hypotheses reviewed in the first paragraph. However, we must emphasize that the only derivative of rhodanine that has been extensively studied is *p*-dimethylaminobenzylidenerhodanine, and the others have been looked at only from a qualitative perspective; therefore, the *p*-dimethylaminebenzylidene grouping must itself contribute to the reaction with silver instead of exerting only a resonance effect. The other derivatives, although they also react with silver (*25*), are interesting only as qualitative reagents and do not show bands in the visible zone of the spectrum that may be useful for spectrophotometric determinations.

SUMMARY

The synthesis, characteristics, properties, and reactions with metallic ions of five aromatic (three nonpyridine and two pyridine) derivatives of rhodanine have been studied. Nonpyridine derivatives exhibit one p*K* value in aqueous solution and two pyridine reagents, and with Pd(II), Au(III), Hg(II), and Cu(I) and (II) form stable complexes which show an absorption band, at the visible zone of the spectrum, with molar absorptivities adequate for spectrophotometric measurements. Finally, suggestions have been made about the group responsibile for the reactionability.

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Photometric Determination of Nitrite

S. FLAMERZ AND WADALA A. BASHIR

Departments of Chemistry, College of Education and College of Science,
University of Mosul, Mosul, Iraq

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INTRODUCTION

Nitrite, when correlated with other nitrogen forms in water, can provide an index of organic pollution. Therefore, a sensitive, selective, and rapid method for nitrite determination seems very desirable. Consequently, a method based on the formation of an azo dye (anion) has been developed and is proposed for nitrite determination.

MATERIALS AND METHODS

Reagents

Unless otherwise stated, all chemicals used were of analytical grade.

Standard nitrite solution (100 μg/ml). Sodium nitrite (0.1499 g) was dissolved in distilled water; 1 ml of spectroscopic chloroform and a pellet of sodium hydroxide were added (*1*), and the solution was diluted with distilled water to 1 liter. Less concentrated solutions were prepared by dilution.

p-Aminobenzoic acid (PABA) solution (0.3%). *p*-Aminobenzoic acid (0.3 g) was dissolved in 116 m*M* hydrochloric acid and the volume was diluted to 100 ml with the same hydrochloric acid solution.

α-Naphthol solution (0.2%). The compound (0.2 g) was dissolved in 100 ml of N,N-dimethylformamide.

Sodium hydroxide solution (2%). NaOH (2.0 g) was dissolved in 100 ml of distilled water.

Interfering ions solution. A 1000 μg/ml solution of each tested ion was prepared.

Apparatus

All spectrophotometric measurements were made with 1-cm glass cells in a Shimadzu UV-210A double-beam spectrophotometer.

Procedure

To a series of 10-ml volumetric flasks, transfer not more than 7.0 ml of sample solution containing 1–13 μg of nitrite. Add 1 ml of 0.3% *p*-

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aminobenzoic acid solution, 1 ml of 0.2% α-naphthol solution, and 1 ml of 2% sodium hydroxide solution. Dilute to the mark with distilled water and shake thoroughly; the color develops immediately and is stable for a long time (>30 hr). Measure the absorbances against a reagent blank at 519 nm. The standard curve obtained is rectilinear, indicating that Beer's law is obeyed over the concentration range 1–13 μg of nitrite in a final volume of 10 ml, i.e., 0.1–1.3 ppm. The apparent molar absorptivity in the region of least photometric error was found to be 3.5×10^4 liter mol^{-1} cm^{-1} and the Sandell sensitivity index is 0.0013 μg cm^{-2}.

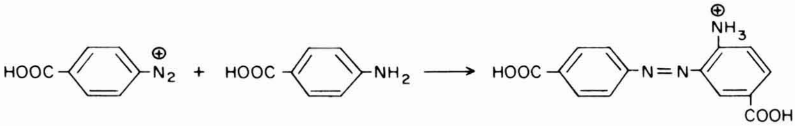
RESULTS AND DISCUSSION

For the following experiments, 10 μg of nitrite was taken and the final volume was made up to 10 ml. Under the conditions of the preliminary investigations and of the prescribed procedure, the colored azo dye showed maximum absorption at 519 nm. This wavelength was kept in all subsequent experiments.

Choice of Diazo Component

For the evaluation of a method for nitrite with α-naphthol in basic medium, many primary aromatic amines, including orthanilic acid, *o*-aminophenetole, *p*-aminophenetole, *o*-nitroaniline, and *p*-aminobenzoic acid were tested for optimal conditions. Of these amines, only the latter showed the desired results. Furthermore, *p*-aminobenzoic acid is easily soluble in mineral acids, giving a colorless solution; easily diazotized; and gives a water-soluble azo dye under the conditions of determination, thus eliminating the process of extraction, a time-consuming procedure. Therefore, it was chosen and further investigated.

Effect of p-aminobenzoic acid concentration and hydrochloric acid concentration. The effect of PABA concentration for maximal color intensity was studied and, using the prescribed procedure, one milliliter of (0.1–0.35%) PABA in 104 m*M* hydrochloric acid solution was added to a series of nitrite solutions. The results show that 0.2–0.3% gave optimum constant absorbance and 0.3% concentration was selected. The 0.35% concentration decreases the absorbance, which might be attributed to self-coupling of the acid as follows:



Then the hydrochloric acid concentration, which is essential for the diazotization reaction, was examined. PABA (0.3 g) was dissolved in 100 ml of 80–232 m*M* hydrochloric acid; 104–174 m*M* hydrochloric acid gave maximal color intensity and the 116 m*M* concentration was chosen. The optimal volume of this composite (PABA + HCl) reagent for maximum absorption was tested, and 1 ml of the composite reagent gave optimal absorbance.

Choice of Coupling Agent

For the determination of nitrite with PABA, many coupling agents for effective results were tried. Tiron (disodium salt), β-naphthol, and α-naphthol were tested. Only α-naphthol gave the promised results. β-Naphthol is toxic, and tiron, although water soluble, gave a very pale yellow color in the method. Therefore, α-naphthol was studied further.

Effect of α-naphthol concentration. α-Naphthol is water insoluble, therefore it was dissolved in organic solvents; the available solvents, ethanol and N,N-dimethylformamide, were used. N,N-dimethylformamide showed a 10% absorbance increase over ethanol; for this reason it was used. A 0.5–2.5 -ml quantity of 0.2% α-naphthol in DMF was tested for effective results, and 1 ml of α-naphthol was selected for the procedure.

Choice of Alkaline Solution

The alkaline solution is very necessary for developing the pinkish red color. Sodium acetate, sodium carbonate, and sodium hydroxide were tested. Sodium acetate develops a reddish orange color (λ_{max} 491 nm) with a lower molar absorptivity; sodium carbonate develops the pinkish red color but with a 14% decrease in absorbance when compared with sodium hydroxide at the same concentration. Consequently, sodium hydroxide was the choice and 1 ml of 2% solution gave the optimum results.

Accuracy, Precision, and Sensitivity of the Method

After construction of the standard curve, the accuracy and precision (five replicates) of the described method were checked. The results are shown in Table 1. The method seems to be very reliable. The sensitivity of the method (expressed as the amount of nitrite corresponding to an absorbance of 0.001) in a 1-cm cell at 519 nm was 0.0013 μg · cm^{-2}.

TABLE 1

ACCURACY AND PRECISION OF THE METHOD

Amount of nitrite (μg)	Accuracy (E_r%[a])	Precision (RSD%[b])
1	0.00	1.70
7	0.00	0.28
13	+0.08	0.30

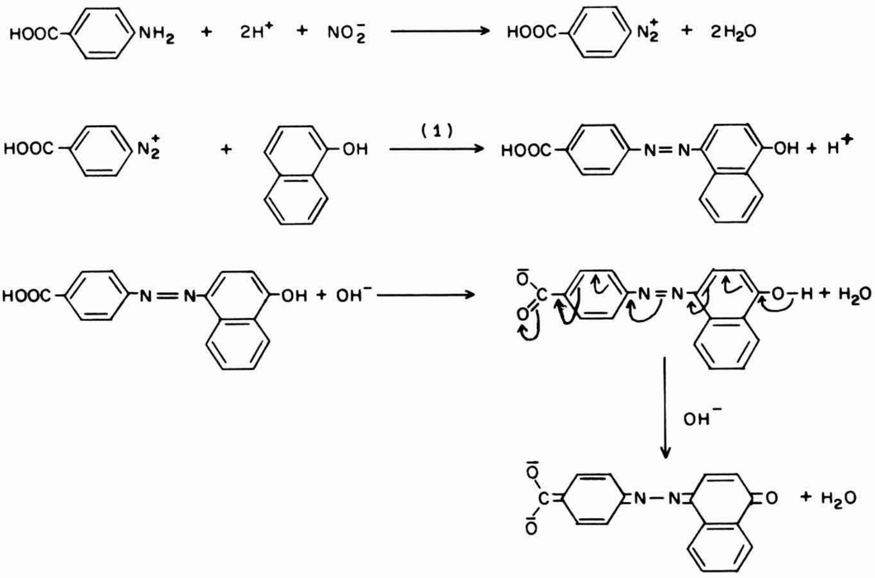
[a] Relative error.

[b] Relative standard deviation.

The interfering effect of some ions was studied and their tolerable amounts, in the presence of 1 ppm nitrite, were: Ammonia (120 ppm), bicarbonate (150 ppm), carbonate (60 ppm), chloride (300 ppm), cyanide (60 ppm), fluoride (130 ppm), iodide (220 ppm), nitrate (90 ppm), phosphate (140 ppm), sulfate (550 ppm), sulfide (8 ppm), calcium (45 ppm), cadmium (20 ppm), cobalt (II) (20 ppm), iron (III) (15 ppm), magnesium (27 ppm), mercury (II) (40 ppm), lead (II) (50 ppm), tin (II) (35 ppm), and phenol (150 ppm). These figures correspond to the amount of diverse ions causing an error of less than 2% in the determination.

Suggestion of Reaction Mechanism (2)

The proposed reaction sequence may be the following steps:



SUMMARY

A sensitive colored reaction for nitrite determination is developed. It involves the reaction of the titled ion with *p*-aminobenzoic acid to form a diazonium ion, which is then coupled with α-naphthol in alkaline medium to form a pinkish red soluble azo dye absorbing maximum at 519 nm. The described method is capable of determining 0.1 – 1.3 ppm of nitrite with a relative standard deviation of 0.3 – 1.7% depending on the concentration level, molar absorptivity of 3.5×10^4 liter $\mathrm{mol^{-1}\ cm^{-1}}$, and Sandell sensitivity index of 0.0013 $\mu\mathrm{g\ cm^{-2}}$. Diazotization and coupling are very fast and control of temperature is unnecessary. Moreover, the colored azo dye is stable and its intensity is in direct proportion with nitrite concentration over a wide range.

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MICROCHEMICAL JOURNAL 26, 590–596 (1981)

Investigation of the Association of the 1-Dodecene–Lauryl Alcohol Bicomponent System

TERESA KOWALSKA AND BEATA WALCZAK

Institute of Chemistry, Silesian University, Katowice, Poland

Received February 5, 1981

INTRODUCTION

Investigation of the associative interactions involving hydrogen bonds is still seriously restricted through limitations of the available analytical methods and techniques. Besides one deals with the relatively low interaction energies, which makes the considered hydrogen-bonded structures unstable. This is a further difficulty of the discussed investigations. Particularly difficult measuring conditions appear in such cases, in which the double-bond π-electrons are Lewis bases and not the electronegative oxygen, nitrogen, or sulfur atoms with their nonbonding electron pairs. For the above mentioned reasons literature concerning hydrogen bonds which involve π-electrons is but a small fraction of the otherwise abundant literature dealing with various theoretical and practical aspects of this problem.

The aim of this work is to present a new proposal of investigating the π-electron involving hydrogen bonds, taking advantage of the 1-dodecene–lauryl alcohol binary system. The novelty depends upon using chromatographic paper and pointing out the correlations between the results obtained with this type of sorbent and those gained from IR spectroscopy and the dielectric permeativity measurements.

EXPERIMENTAL

In the experiment we used the following analytical grade reagents: 1-dodecene (Fluka, Switzerland) and lauryl alcohol (Apolda, German Democratic Republic), as well as Whatman 1 and 4 chromatographic papers (Whatman, England). The reagents were additionally purified through distillation and crystallization to the purity level surpassing 99%.

Then for IR purposes the 1-dodecene–lauryl alcohol system samples were prepared in the form of the CCL_4 solutions, preserving the following molar ratios of both components: 0.8:0.2, 0.6:0.4, 0.4:0.6, 0.2:0.8, and 0:1.0. A concentration of lauryl alcohol was constant in each sample and equal 0.2 M/dm^3.

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For the dielectric permeativity measurements and the investigations involving chromatographic paper the binary systems were prepared, which preserved the same molar ratios as given above, but without any additional solvent.

The IR spectroscopic investigations were performed in the following way: Spectra were run by means of the UR-20 type IR spectrophotometer produced by Carl Zeiss, Jena (German Democratic Republic) in the range of 3800–3100 cm^{-1}. The working conditions were as follows: the LiF prism; the NaCl cells, their thickness 0.627 mm; the measuring temperature 30 ± 0.2°C; the slot program 4; the registration width 20 mm/100 cm^{-1}. The registered spectra reproduced the $\%T = f(\bar{\nu})$ dependence and thus one had to recalculate them in the 10 cm^{-1} intervals to obtain another dependence, remaining in accord with the Lambert–Beer law, where $A = \ln I_0/I = f(\bar{\nu})$. Instead of the I_0/I ratio values we introduced the T_b/T ones, where T_b is the transmittance of the background, and T is the transmittance of a sample for a given $\bar{\nu}$ wavenumber value. With the recalculated spectra the integral intensities of certain bands were measured, defined as:

$$\text{integral intensity} = \int_{\bar{\nu}_1}^{\bar{\nu}_2} \log \frac{T_{b(\bar{\nu})}}{T_{(\bar{\nu})}}\, d\bar{\nu}.$$

These bands corresponded with the valency vibrations of free and bonded hydroxyl groups, and they were first graphically separated and then planimetrically evaluated. The measurement error never surpassed $\pm 0.2\ \mathrm{cm}^{-1}$.

The dielectric permeativity measurements were performed in the following way: The 1-dodecene, lauryl alcohol, and the binary system samples were consecutively introduced to the measuring cell of a dielectrometer (type OH-302, produced by Radelkis, Hungary) and the necessary values were to be found at the calibrated apparatus scale. These measurements were performed at 20°C.

Investigations involving chromatographic paper were performed in the following way. The paper sheets were first dried at 110°C and then 5-μl aliquots of each sample were applied upon the sorbent layer. After an additional 10 min in the room conditions they were placed for 24 hr in a chamber saturated with iodine vapors. The visualized spot surface areas were planimetrically evaluated and the measurement error was ±2%.

DISCUSSION

First the changes of the integral intensity values were observed with the valency vibrations of free and bonded lauryl alcohol hydroxyl groups, which appeared when adding the increasing amounts of 1-dodecene. The obtained results are given in Table 1 and Fig. 1. As seen from the data given in Table 1 and Fig. 1, addition of the first portion of 1-dodecene caused an increase of the integral intensity value corresponding with the monomeric OH groups, which was most probably connected with splitting of multimers containing exclusively the lauryl alcohol molecules (i.e., the self-associates). Destruction of the purely alcoholic multimer structure seemed to introduce a certain disorder among the molecules, which was connected with accumulation of some quantities of free hydroxyl groups. Nevertheless the further addition of 1-dodecene was accompanied by the slow decrease of the discussed parameter number values. Simultaneously one observed an increase of the integral intensity value with the bonded hydroxyl band. It was most probably caused by the increasing participation of 1-dodecene in the mixed multimers, proportional to the increase of the olefin concentration in the system.

The role of the investigations taking advantage of the chromatographic paper was as follows. Application of equal aliquots of the binary system samples and evaluation of the obtained spot surface areas aimed to make a comparison between the IR spectroscopic results and these gained from employing the cellulose sorbent. From our previous work (*2–5*) it was already known that chromatographic paper was not active enough to significantly interfere with the natural associative equilibria of the investigated substances.

Taking advantage of the spot surface areas of pure 1-dodecene and lauryl alcohol the additive spot surface areas were calculated of the binary systems, defined as:

$$S_{\mathrm{add}} = S_{\mathrm{1\text{-}D}} \cdot x_{\mathrm{1\text{-}D}} + S_{\mathrm{LA}} \cdot x_{\mathrm{LA}},$$

where S_{add} is the additive spot surface area; $S_{\mathrm{1\text{-}D}}$ and S_{LA} are the spot

TABLE 1

THE INTEGRAL INTENSITY VALUES OF THE VALENCY VIBRATION BAND OF FREE (*b*) AND BONDED (*B*) LAURYL ALCOHOL HYDROXYL GROUPS FOR THE 1-DODECENE–LAURYL ALCOHOL BINARY SYSTEM HAVING THE CHANGING MOLAR FRACTION RATIOS[a]

The molar fraction ratio in the 1-D–LA system	*b* ($\mathrm{cm^{-1}}$)	*B* ($\mathrm{cm^{-1}}$)
0.0:1.0	18.0	78.1
0.2:0.8	18.5	78.5
0.4:0.6	18.2	76.6
0.6:0.4	17.8	81.9
0.8:0.2	17.5	85.6

[a] The lauryl alcohol concentration in each sample equals 0.2 M/dm^3 (the $\mathrm{CCl_4}$ solutions); the cell thickness d = 0.627 mm; the measuring temperature is 30°C.

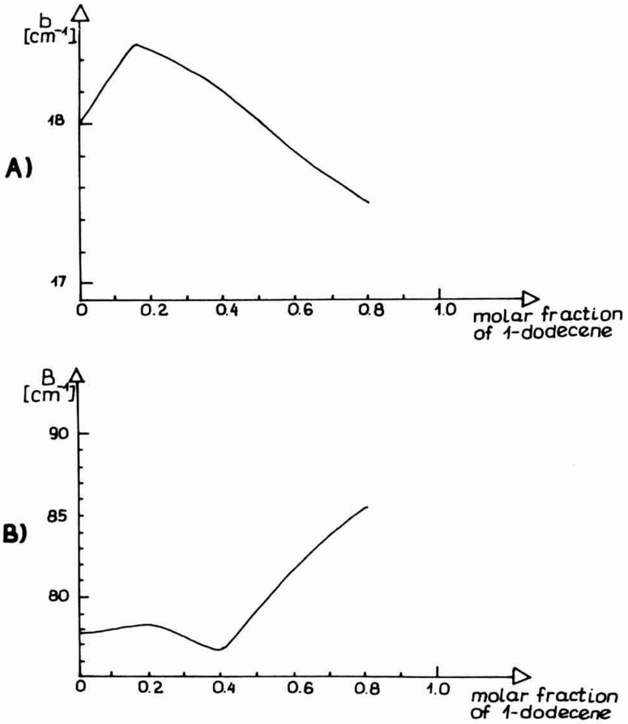


FIG. 1. The integral intensity values of the valency vibration band of free *b* (A) and bonded *B* (B) lauryl alcohol hydroxyl group vs the molar fraction of 1-dodecene in the binary system.

surface areas of pure 1-dodecene and lauryl alcohol; and $x_{\text{1-D}}$ and x_{LA} are the molar fractions of 1-dodecene and lauryl alcohol. The experimentally established values enabled determination of a deviation from the spot surface additivity, accepted as:

$$\Delta S = S_{\text{exp}} - S_{\text{add}}.$$

The obtained results are given in Figs. 2 and 3.

With both Whatman 1 and 4 papers we gained a similar picture of the spot area changes, highly dependent upon the molar ratio of the system components. Thus, introduction of the first portion of 1-dodecene to lauryl alcohol was reflected in the significant, positive deviation from additivity of the discussed areas. This effect is good proof, similar to that based upon the IR results, of the strong alteration of the characteristic lauryl alcohol association equilibria. Transfer from the uniform to mixed multimers is obviously connected with the important structural changes of these macroaggregates. Introduction of further quantities of 1-dodecene did not effect such drastic deviations, as was seen in the first step. With

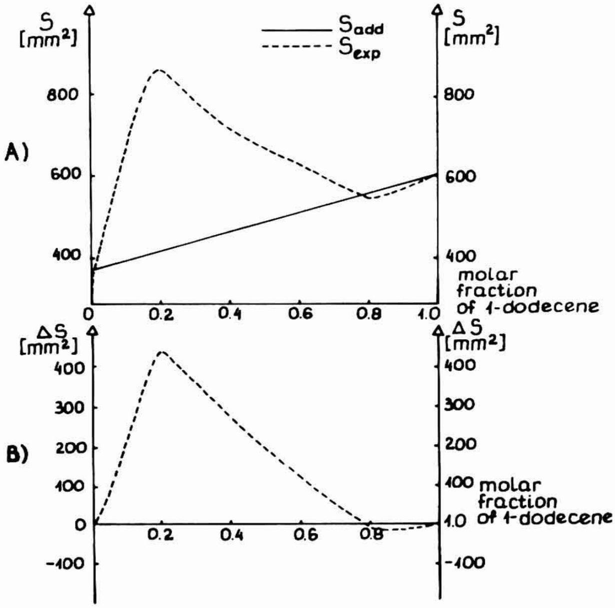


FIG. 2. The spot surface areas (A) and the deviations from additivity of these areas (B) for the 1-dodecene–lauryl alcohol binary system with the changing molar fraction ratios upon the Whatman 1 chromatographic paper.

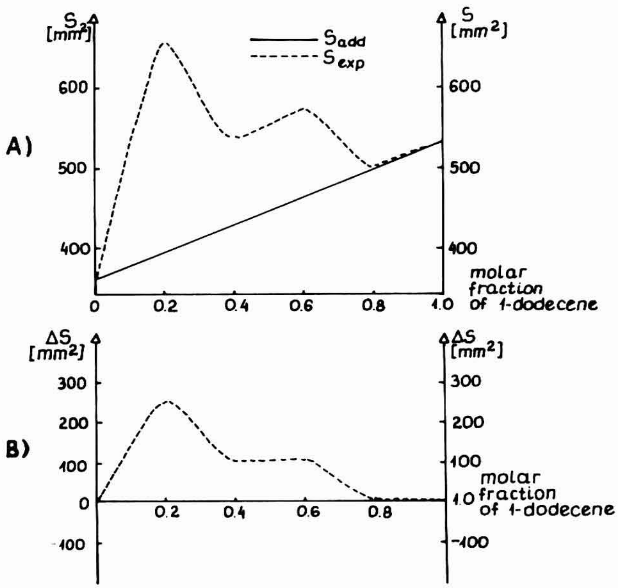


FIG. 3. The spot surface areas (A) and the deviations from additivity of these areas (B) for the 1-dodecene–lauryl alcohol binary system with the changing molar fraction ratios upon the Whatman 4 chromatographic paper.

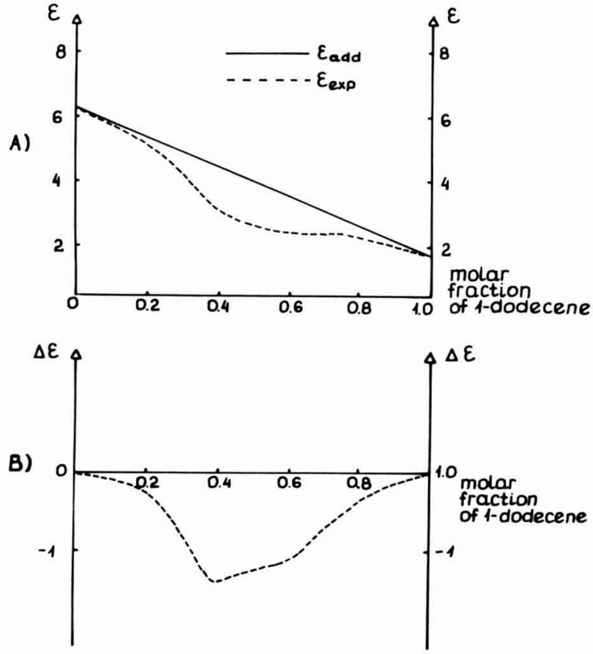
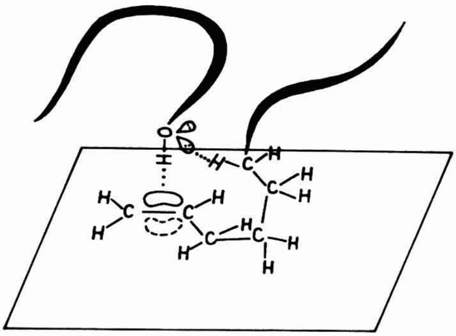


FIG. 4. The experimental and additive dielectric permeation values (A) and the deviations from additivity (B) for the 1-dodecene–lauryl alcohol binary system with the changing molar fraction ratios.

the molar ratio of 1-dodecene vs lauryl alcohol equal 0.8 to 0.2 the deviation from additivity practically disappeared.

Further information was gained from the dielectric permeativity measurements. The results are shown in Fig. 4. In this case we also took advantage of the experimental and the additive dielectric permeativity values, as well as the deviations from additivity (*1*).

The results from Fig. 4 also support our statement that introduction of 1-dodecene to lauryl alcohol is connected with a rearrangement of the multimer structures characteristic for the alcoholic self-associates. In addition, the dielectric permeativity results give evidence of the fact that the experimental dielectric permeativity values of the mixed multimers are lower than the additive ones. The dielectric measurements reflect the mean situation inside the entire system and thus it is difficult to determine upon this basis the structure of the mixed multimers. Nevertheless, the negative deviations from additivity suggest a certain tendency toward the better charge compensated multimers, i.e., toward the cyclic mixed multimers. With the 1-dodecene–lauryl alcohol mixed multimers the following model can be proposed:



Thus, it would be the model assuming two hydrogen bonds per one 1-dodecene and one lauryl alcohol molecule: the first one between the hydroxylic hydrogen bond and the olefinic π-electrons, and the second one between the nonbonding electron pair of the hydroxylic oxygen atom and the γ or δ hydrogen atom from the olefinic aliphatic chain.

SUMMARY

The 1-dodecene–lauryl alcohol bicomponent system was investigated as an exemplary one, able to form the hydrogen-bonded mixed multimers, involving the olefinic double bond π-electrons. We applied the following analytical techniques: IR spectroscopy, dielectric permeativity measurements, and examinations taking advantage of the cellulose sorbents.

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MICROCHEMICAL JOURNAL 26, 597–601 (1981)

BOOK REVIEWS

Quantitative Analysis of Organic Mixtures, Part One: General Principles. By T. S. MA AND ROBERT E. LANG. Wiley, New York, 1979. xvii + 366 pp., $23.50.

It is fitting and proper that textbooks for analytical chemistry should deal with ideal situations. However, in the everyday world of the working analyst such conditions are rare. The authors have addressed this non-ideal problem.

They have stated four objectives to be met by this monograph:

1. To guide the reader in selecting a suitable method to analyze a new mixture.
2. To suggest ways and means for checking a procedure when discrepancies arise.
3. To show how to improve existing methods and develop new ones.
4. To review the literature covering the analysis of organic mixtures.

To meet these objectives the authors have covered the following major subjects: Problems in the Analysis of Organic Mixtures, Preparation of the Sample and Selection of the Analytical Method, Determination of Several Components in a Mixture With and Without Separation and the Separation of Acidic, Basic, and Neutral Substances.

This is not a laboratory manual; there are no detailed specific methods. Instead, by means of numerous illustrative tables and over 3000 references, the authors guide, suggest, show how, and review. It is a guide book, a reference book, and should be available to anyone facing the problem of analyzing a mixture of organic compounds.

A complete author and subject index is included.

In the forthcoming Part Two the authors will examine specific mixtures encountered in pharmaceuticals, polymers, pollutants, agriculture, and natural products among others.

BILL ELPERN, *9 Surrey Way*
White Plains, N.Y. 10607

The Lewis Acid–Base Concepts. An Overview. By WILLIAM B. JENSEN. Wiley–Interscience, New York, 1980. xi + 364 pp., $32.50.

This monograph presents a comprehensive exposition of the Lewis acid–base concepts and clearly demonstrates their significance as integrating principles in current chemical problems. The author describes the development of the Lewis concept both from historic and fundamental bonding theory points of view. An informative treatment of the Pearson hard–soft acid–base theory is placed in context. Students and researchers will find the text a usefully broad and penetrating treatment of contemporary work in this central area. Reviews of the work of Gutmann, Drago, and Olah are positive features. One surprising omission to the discussion on the role of solvation on acid–base strength: ion molecule findings of proton affinities in the gas phase.

HENRY FREISER, *Department of Chemistry*
University of Arizona
Tucson, Arizona 85721

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Inorganic Reaction Chemistry. Systematic Chemical Separation. By D. T. Burns, A. Townshend, and A. G. Catchpole. Wiley, New York, 1980. 248 pp., \$57.50. Paperback, \$27.95.

The intention of the authors of this book was to refocus attention on an important but somewhat neglected field of chemical analysis: inorganic qualitative analysis. The advent of instrumentation has supplanted this type of analysis for the most part; however, there are numerous situations where these techniques are more suitable than the instrumental methods. This book is a volume in the "Ellis Horwood Series in Analytical Chemistry," edited by R. A. Chalmers and M. Mason.

The volume includes six chapters, a list of references after each chapter, a highly informative foreword, a list of reagents and how to prepare them, and a subject index. Chapter 1 is an interesting review of the history of qualitative analysis. Chapters 2 and 3 discuss the physicochemical basis for reactions in aqueous solutions and present a theoretical structure for systematic inorganic qualitative analysis. Chapters 4, 5, and 6 are devoted to a presentation of qualitative procedures, especially semi-micro procedures and the ring–oven technique.

The authors have clearly succeeded in their objectice and have produced a fine book. Those who have not considered qualitative analysis as an alternative should definitely examine this book as a source of useful information.

Donald F. Logsdon, Jr.
Chapman College, Sacramento, California 95655

Organic Chemistry. By T. W. Graham Solomons. Wiley, New York, 1980. xiii + 1110 pp., \$26.95.

This is definitely an elementary text in the sense that it is the chemistry major's first real study of organic chemistry. In this second edition, material considered extraneous has been omitted and many sections have been completely rewritten, especially that on nucleophilic substitution. There is a bibliography of 6 pages and an index of 38 pages.

Although this book, of itself, has little relevance for microchemists, it is one that should be in every chemist's personal library for reference or review.

David B. Sabine, *185 Old Broadway*
Hastings-on-Hudson, New York 10706

The Particle Atlas, 2nd ed. Volume V, **Light Microscopy Atlas and Techniques.** By Walter C. McCrone, John G. Delly, and Samuel J. Palenik. Volume VI, **Electron Optical Atlas and Techniques.** By Walter C. McCrone, John A. Brown, and Ian M. Stewart. Ann Arbor Science Publishers, Ann Arbor, Mich. Volume V, 1979, pp. 1139–1454, \$90.00. Volume VI, 1980, pp. 1455–1703, \$90.00.

These two volumes update the previous four volumes of the 2nd edition of the "Particle Atlas" and are a welcomed addition.

They are of the same excellent quality as that of the earlier work, both in content and printing, and extend the total number of particle types from the 609, which were covered in Volumes II and III, to 1022. Color photomicrographs for the additional 412 particulate compositions appear in Volume V; their scanning electron microscope pictures (as well as those of some additional materials) appear in Volume VI. In addition, energy dispersive X-ray patterns of most of these materials are included in Volume VI as well as the transmission electron microscope and selected area electron diffraction patterns for certain particles.

The photomicrographs for the 412 new substances (which extend the coverage of materials in the same categories as before, such as pollens, minerals, industrial chemicals, and drugs) are the primary subject matter presented in Volume V. New topics covered in Volume V include a short description on the laser Raman microprobe and the Hoffman and Gross technique for enhancing contrast by appropriate light modulation. Other updates are given on dispersion staining and current recommended refractive index liquids because the polychlorinated biphenyls have been replaced. A brief description is given on how to conduct qualitative microchemical reactions for particle identification. This includes a description of the equipment, procedures, and certain selected examples, such as the methods for the qualitative identification of calcium, zinc, carbohydrate material, reducing sugars, cellulose, and lignin, as well as other common particulate constituents.

A separate short chapter is included on the determination of the geographical origin of dust samples (including minerals; biological components such as pollens, leaf fragments, and hairs; and combustion products, as well as industrial products and fertilizers). Also short chapters are included on the microscopical examination of air pollutants, the identification of asbestos by polarized light microscopy, particle analysis in the crime laboratory, and the application of particle study in art and archeology conservation and authentication.

Volume VI details the advances made in particle identification by the electron optical methods since the appearance of Volume III, which was written in 1973. Short chapters are included on the advances in electron optical methods of particle analysis, the identification of submicrometer particles, and some techniques for handling particles in scanning electron microscopy. This chapter will be most appreciated by users of scanning electron microscopes, for it includes particle mounting, collection, and metal-coating techniques as well as single-particle preparation techniques. The major coverage is the description and electron micrographs of the particles. A separate chapter is given to the identification of asbestos by electron microscopy, and the use of automation in laboratory instrumentation with emphasis on its application to particle analysis; the electron microscope analyzers, the ion microprobe, and related particle identification equipment are included in the discussion.

Both Volumes V and VI include a survey of books and articles representative of the literature covered by each volume. As indicated before, both of these volumes are excellent and highly recommended. There is no other work available giving the coverage presented in the "Particle Atlas."

PETER F. LOTT, *Chemistry Department*
University of Missouri
Kansas City, Missouri 64110

Kirk-Othmer Encyclopedia of Chemical Technology. Vols. 8, 9, and 10., 3rd ed. Editorial Board, HERMAN F. MARK, DONALD F. OTHMER, CHARLES G. OVERBERGER, AND GLENN T. SEABORG; Executive Editor, MARTIN GRAYSON; Associate Editor, DAVID ECKROTH. Wiley–Interscience, New York. Volume 8, 1979, xxvi + 930 pp., $120.00, subscription price $95.00. Volume 9, 1980, xxiv + 902 pp., $145.00, subscription price $120.00. Volume 10, 1980, xxvi + 962 pp., $145.00, subscription price $120.00. Index to Vols. 5–8, 1980, 200 pp.

These volumes are the continuation of the material started with Volume 1 and it and subsequent volumes were reviewed in this *Journal* (*Microchem. J.* **24**, 389 (1979); **25**, 141 (1980); **26**, 146 (1981).

Volume 8 has 65 contributors and covers subjects beginning with diuretics (continued from the previous volume) and includes such items as driers, drying, dyes, eggs, elastomers, electrical conductors, electrochemical processing, electrodialysis, electrode plating, electrostatic sealing, embedding, and emulsions.

Volume 9 has 47 contributors and continues the subjects beginning with E and includes material on enamels, energy management, engineering data, enzymes, epinephrine and norepinephrine, epoxidation, epoxy resins, esterification, ethanol, ethers, ethylene, evaporation, exhaust control, expectorants, explosives, and extraction. It then starts to treat subjects beginning with the letter F and includes fans, fats, feedstocks, felts, fermentation, and ferrites.

Volume 10 has 63 contributors and continues with subjects beginning with the letter F. Included are chapters on ferroelectrics, fertilizers, fiber optics and fibers, fillers, films, filtration, fine chemicals, flame retardants, flavors, flocculation agents, flotation, fluidization, fluid mechanics, fluorine and its compounds, etc.

The index to Volumes 5 to 8 consists of 200 pages and includes the material under the headings from castor oil to emulsions.

Volumes 8 to 10, like the previous ones, contain a wealth of information written by experts in the various fields and no library can be complete without adding the series to its collection.

AL STEYERMARK, *Department of Chemistry*
Rutgers, The State University of New Jersey
Newark, New Jersey 07102

Corrosion of Nickel and Nickel–Base Alloys. By WAYNE Z. FRIEND. Wiley, New York, 1980. viii + 459 pp., $39.95.

This book, which is part of the "Corrosion Monograph Series" sponsored by the Electrochemical Society, summarizes in one volume information concerning the corrosion-resisting properties and limitations of alloys having primarily nickel as the major alloying constituent. Thus, with few exceptions, it treats the corrosion behavior of alloys containing generally 40% or more nickel. This includes pure nickel, nickel–copper alloys, nickel–chromium alloys, nickel–iron alloys, complex nickel super alloys, etc. (e.g., Hastelloy alloys, Inconel alloys, Incoloy alloys, etc., but not stainless steels). Information is provided on the attack of nickel alloys by both aqueous solutions (e.g., hot acids) and nonaqueous vapor (e.g., chlorine). The book brings together data which were primarily previously available in specific literature on corrosion resistance and the factors affecting it for nickel alloys. It is well indexed, has excellent literature citations, and provides the data to permit one to evaluate and select the appropriate nickel alloy for satisfactory service under severely corrosive conditions.

PETER F. LOTT, *Chemistry Department*
University of Missouri
Kansas City, Missouri, 64110

The Asbestos Particle Atlas. By WALTER C. MCCRONE. Ann Arbor Science Publishers, Ann Arbor, Mich., 1980. v + 122 pp., $85.00.

Asbestos is the name given to a group of fibrous hydrous mineral silicates, primarily chrysotile, amosite, and crocidolite. The health hazard of asbestos is well known, but not as well known are methods that can be used to identify asbestos and not always recognized is the fact that generally the method of choice is polarized light microscopy and dispersion staining. This book (which is to a large extent an offset reproduction of typewritten pages) provides the information for the recognition of asbestos in varied materials by the microscopic method. It is a "how to do it" book. It tells you what you need and how to use it (a polarizing microscope, a dispersion staining objective, and immersion liquids). It both provides the theory and shows the final practical result through color photomicrographs of the different stages in the identification procedure. Photomicrographs are given for the different forms of asbestos; other related nonasbestos materials such as mineral wool, glass wool, polyester fiber, and paper; and actual asbestos-containing insulation samples.

Very briefly the major sections of the book consist of an introduction to the mineralogy of asbestos, a section on optical crystallography which also includes refractive index measurements and dispersion staining, a text section on the identification of asbestos by polarized light microscopy, a discussion of the photomicrographic color plates, and the 35 color plates, which generally have 12 photomicrographs per plate. Also included is a short section on Köhler illumination, exposure control in photomicrography, information on the purchase of a suitable microscope, a bibliography, and an excellent index.

Although it would be difficult for a total novice to learn the whole technique of asbestos identification by reading the book, a person with some background in microscopy and optical crystallography would be successful without major difficulty. He would also know, although it is not explicitly stated in the book, that the absolutely necessary asbestos standards, dispersion staining objective, and appropriate immersion liquids can be purchased from McCrone Accessories and Components. Without question, the book will be a necessity for anyone who undertakes problems in asbestos identification.

PETER F. LOTT, *Chemistry Department,*
University of Missouri
Kansas City, Missouri 64110

MICROCHEMICAL JOURNAL 26, 602 (1981)

Announcement

Microchemical Workshop—1982

The Department of Continuing Education of the Pennsylvania State University will conduct, on July 6, 7, and 8, 1982, at the University Park Campus, a Microchemical Workshop on the general theme of "Titrimetry." The technical program will consist of a number of intensive lectures and lecture demonstrations presented by recognized scientists from the United States and from abroad.

By utilizing the housing, food service, and meeting and recreational facilities of the University, expenses will be minimal.

For more information concerning

the technical program, contact:
Howard J. Francis, Jr.
The Pennwalt Corporation
900 First Avenue
King of Prussia, Pennsylvania 19406
Telephone: (215) 265-3200

registration and housing, contact:
Ronald Avillion
The Pennsylvania State University
J. Orvis Keller Building
University Park, Pennsylvania 16802
Telephone: (814) 865-0313

MICROCHEMICAL JOURNAL 26, 603–604 (1981)

Author Index for Volume 26

Microchemical Journal

devoted to the
application of
microtechniques
in all branches
of science

Editor: Al Steyermark

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1981

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Number 4, December 1981

INFORMATION FOR AUTHORS

The MICROCHEMICAL JOURNAL will invite research papers on all phases of chemistry, including clinical and biochemical, which involve small-scale manipulation. This will include both organic and inorganic work on preparation, purification, separation, detection, determination, trace analysis, and all types of instrumentation.

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1. Ames, S. R., Swanson, W. J., and Harris, P. L. Biochemical studies on vitamin A. XIV. Biopotencies of geometric isomers of vitamin A acetate in the rat. *J. Amer. Chem. Soc.* **77**, 4134–4136 (1955).

2. Pease, D. C., "Histological Techniques for Electron Microscopy," 274 pp. Academic Press, New York, 1960.

3. Stern, K. C., Electrophoresis and ionophoresis. *In* "Physical Techniques in Biological Research" (G. Oster, ed.), Vol. 2, pp. 61–121. Academic Press, New York, 1956.

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