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Spectrophotometric Study of Copper(II)–2,2′-dipyridyl-2-pyridylhydrazone Complex Formation. H. ALEXAKI-TZIVANIDOU AND G. S. VASILIKIOTIS, *Laboratory of Animal Physiology and Laboratory of Analytical Chemistry, University of Thessaloniki, Thessaloniki, Greece.*

Two copper complexes were formed, one at pH 6.5–8.5 and the other at pH 12.0–12.5. A sensitive procedure for the determination of copper at less than a 1-ppm level is proposed.

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Three complexes are formed between vanadium and xylenol orange. In slightly alkaline solution, the formation of the complexes is instantaneous. Two of these form complexes with hydrogen peroxide.

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Steroids. XXIV. Study of the Formation of 3,20-Dihydroxyiminoprednisolone. M. MARIÁN AND B. MATKOVICS, *Biological Isotope Laboratory, "A.J." University, Szeged, Hungary.*

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Microchem. J. **26**, 17–21 (1981).

Microdetermination of Carbon and Hydrogen in Organofluorine Compounds. A. B. FARAG, *Chemistry Department, Faculty of Science, Mansoura University, Mansoura, Egypt A.R.E.* AND M. E. ATTIA AND H. N. A. HASSAN, *National Rearch Centre, Dokki, Cairo, Egypt A.R.E.*

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Spectrophotometric Determination of Phosphorus in Steel Using Phosphoantimonyl Molybdate Complex. P. K. GUPTA AND R. RAMACHANDRAN, *Analytical Chemistry Section, National Physical Laboratory, New Delhi-110012, India.*

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Microchem. J. **26**, 32–39 (1981).

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Microchem. J. **26**, 40–44 (1981).

Spectrophotometric Determination of Aluminum and Gallium with Pyrogallol Red and Cetyltrimethylammonium Ions. CZESŁAW WYGANOWSKI, *Institute of General Chemistry, Technical University, 90-924 Łédź, Poland.*

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Microchem. J. **26**, 45–50 (1981).

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The compounds are extracted into chloroform–isoamyl alcohol and separated by thin-layer chromatography. The plates are then sprayed with 2,4,7-trinitro-9-fluorenone in acetone and the colored spots scanned.

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The reaction between the reagent and the metal ions was studied as well as the influence of foreign ions.

Microchem. J. **26**, 55–60 (1981).

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A comparison was performed of selected glycols in respect to their ability to form intramolecular hydrogen bonds.

Microchem. J. **26**, 68–74 (1981).

Use of Phenyl Acetic Acid in Determination of Iron. B. B. SINHA, *Analytical Division, Central Fuel Research Institute, P.O. F.R.I, District Dhanbad, Bihar, India,* AND J. ADAM, *Analytical Laboratory, Institute of Geological Science, Charles University, Prague 2, Albertov 6, Czechoslovakia.*

An effective comlexometric method of determination of iron by separating iron from other metals as a salt of phenylacetic acid is described.

Microchem. J. **26**, 75–79 (1981).

Ion Flotation of Zinc Using Ethylhexadecyldimethylammonium Bromide. CURTIS W. MCDONALD AND OLUBUNMI A. OGUNKEYE, *Department of Chemistry, Texas Southern University, Houston, Texas 77004.*

Zinc ions react with the surfactant to form a surface active sublate which can be removed from aqueous chloride solutions by ion flotation.

Microchem. J. **26**, 80–85 (1981).

Identification of Glucan Synthetase Glycosidically Bound to Amylotectin from Cotton Leaf. CHONG W. CHANG, *USDA, Science and Education Administration-Agricultural Research, Western Cotton Research Laboratory, 4135 E. Broarway Road, Phoenix, Arizona 85040.*

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Microchem. J. **26**, 86–94 (1981).

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N,N'-Dichloro-*p*-nitrobenzenesulfonamide (Dichloramine-N) as a New Oxidimetric Reagent. Synthesis and Analytical Application. NETKAL M. MADE GOWDA,[1] V. M. SADAGOPA RAMANUJAM,[1] NORMAN M. TRIEFF,[1] G. JOHN STANTON,[2] AND DANDINASIVARA S. MAHADEVAPPA.[3] [1]*Division of Environmental Toxicology, Department of Preventive Medicine and Community Health and* [2]*Department of Microbiology, University of Texas Medical Branch, Galveston, Texas 77550 and* [3]*Department of Chemistry, University of Houston, Houston, Texas 77004.*

Analytical applications of the compound as a redox titrant in the determination of diverse reductants such as ascorbic acid, methionine, sulfite, arsenite, glutathione, and indigocarmine are described.

Microchem. J. **26**, 95–104 (1981).

Thermal Analysis of Neuroleptic Drugs and Vitamins. MAREK WESOŁOWSKI, *Institute of Chemistry and Analytics, Medical Academy, 80-416 Gdansk-6, Al. K, Marksa 107, Poland.*

The possibility has been demonstrated to employ thermal techniques for the identification of particular dosage forms and the qualitative and quantitative monitoring composition of neuroleptic and vitamin drugs.

Microchem. J. **26**, 105–119 (1981).

Alternating Current Polarography of Metal Carbonyl Complexes in Nonaqueous Solvents. PAUL M. WIESSMAN, DONALD B. NUZZIO, AND JOSIAH S. WINTERMUTE, JR., *Chemistry Department, Fairleigh Dickinson University, Madison, New Jersey 07940.*

Using tetrabutylammonium perchlorate as an electrolyte in nonaqueous solvents, such as methanol or *N,N*-dimethylformamide, and using a mercury pool auxiliary electrode, reproducible alternating current polarograms were taken of transition metal complexes and organometallic compounds.

Microchem. J. **26**, 120–126 (1981).

Thin-Layer Chromatographic Separation of Serotonin from Epinephrine and Norepinephrine. S. EBRAHIMIAN AND J. PAUL, *Chemistry Department, University of Bridgeport, Bridgeport, Connecticut 06602.*

The method involves the use of ethylenediaminetetraacetic acid-impregnated silica gel G plates and *n*-butanol–ethanol–acetic acid–water (8:2:1:3) as developer.

Microchem. J. **26**, 127–131 (1981).

Some Analytical Applications of Aromatic Sulfonyl Haloamines: Estimation of Indigocarmine by Chloramine-B, Bromamine-T, and Dibromamine-T. DANDINASIVARA S.

MAHADEVAPPA,* K. S. RANGAPPA,* B. T. GOWDA,* AND NETKAL M. MADE GOWDA,†
**Department of Post-graduate Studies and Research in Chemistry, University of Mysore, Manasagangotri, Mysore 570006, India, and †Division of Environmental Toxicology, Department of Preventive Medicine and Community Health, University of Texas Medical Branch, Galveston, Texas 77550.*

The oxidation product was estimated by spectrophotometric measurements at 410 nm. The sulfonamides formed by reduction of the haloamines were detected by TLC and paper chromatography.

Microchem. J. **26**, 132–140 (1981).

MICROCHEMICAL JOURNAL 26, 1–9 (1981)

Spectrophotometric Study of the Copper(II)–2,2′-Dipyridyl-2-pyridylhydrazone Complex Formation

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INTRODUCTION

In a continuation of our studies on the analytical applications of 2,2′-dipyridyl-2-pyridylhydrazone (DPPH) (*1, 2, 11*) we examined the reaction of this reagent with copper(II) ions. It was concluded that two copper complexes are formed with DPPH, one at pH below 8.5 and the second at pH above 11.2. These complexes are stable and have high molecular extinction coefficients. These considerations prompted an investigation for a possible copper spectrophotometric microdetermination. In this paper the results of the above investigation are reported.

Actually in the absence of interfering cations there is a possibility of a sensitive determination of copper at less than a 1-ppm level. The interferences are due to a number of cations which also form colored complexes with DPPH. The development of a general procedure for a spectrophotometric copper determination is also reported.

EXPERIMENTAL

Apparatus

A Unicam SP 800 recording spectrophotometer and a Zeiss PMQ-II spectrophotometer (both equipped with 10-mm matched quartz cells) were used for absorption spectra studies and quantitative measurements, respectively.

pH measurements were performed with an L.Pusle ACL-112 pH meter, equipped with a combination electrode.

Reagents and Solutions

Analytical grade reagents from BDH, (Poole, Dorset, England) were used throughout this work and their solutions were prepared from twice-distilled water.

2,2′-Dipyridyl-2-pyridylhydrazone (DPPH). An ethanolic solution of

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equimolar quantities (about 0.4 *M*) of 2-pyridylhydrazone and di-(2-pyridyl) ketone (Aldrich Chemical Co., Milwaukee, Wisc.) was refluxed for 2 hr and an equal volume of water was added to facilitate the crystallization of the crude product. This product was recrystallized from an ethanol–water mixture. The purity of the final product was checked by mp (138°C) and by TLC on silica gel G (Merck, Darmstadt, West Germany).

A 10^{-2} *M* ethanolic solution of DPPH was prepared and this reagent was stored in a dark bottle. The used ethanol was distilled from an all-Pyrex glass apparatus.

Standard copper(II) solution, $10^{-4}M$. The standard copper(II) solution was prepared from a stock copper ion solution $10^{-2}M$ of $CuSO_4 \cdot 5H_2O$ and it was standardized iodometrically.

Buffer solutions. The following buffer solutions were used: Perchloric acid for pH below 3.5, acetic acid–sodium acetate for pH 3.5–6.0, boric acid–borax for pH 6.5–9.0, borax–sodium hydroxide for pH 9.5–11.0, and sodium hydroxide for pH above 11.0.

Recommended Procedure

Take an aliquot of the solution to be analyzed, which should contain no more than 35 μg of copper. Dilute the aliquot to about 22 ml with water, add 1.3 ml of the 10^{-2} *M* DPPH solution, and mix it thoroughly. Adjust the pH value to 12.2 ± 0.2 and dilute it to 25.0 ml. Read the absorbance of the formed complex at 448 nm against a reagent blank. The amount of the copper present is calculated from a previously prepared calibration curve.

RESULTS AND DISCUSSION

The effects of the following parameters on the formation reaction of the copper(II)–DPPH complex, were evaluated at a constant ionic strength of 0.1, regulated by a 1 *M* sodium perchlorate solution.

Acidity and DPPH Concentration

The pH study was carried out at the pH range from 1 to 13. It indicates that a strong pH dependence of the Cu(II)–DPPH reaction exists. In Fig. 1 some characteristic spectra of solutions containing copper(II) ions and excess of DPPH at different pH values (all versus a reagent blank) are given. It is observed that at pH values below 8.5 the formed complex shows a maximum absorption at 478 nm. As the pH value increases above 9, a gradual hypsochromic shift is observed and at pH about 11.2 the maximum absorption is at 448 nm. No shift of this maximum is observed at higher pH values. The formation of two complexes at least is obvious, one predominating at pH values below 8.5 and the second at pH values above 11.2.

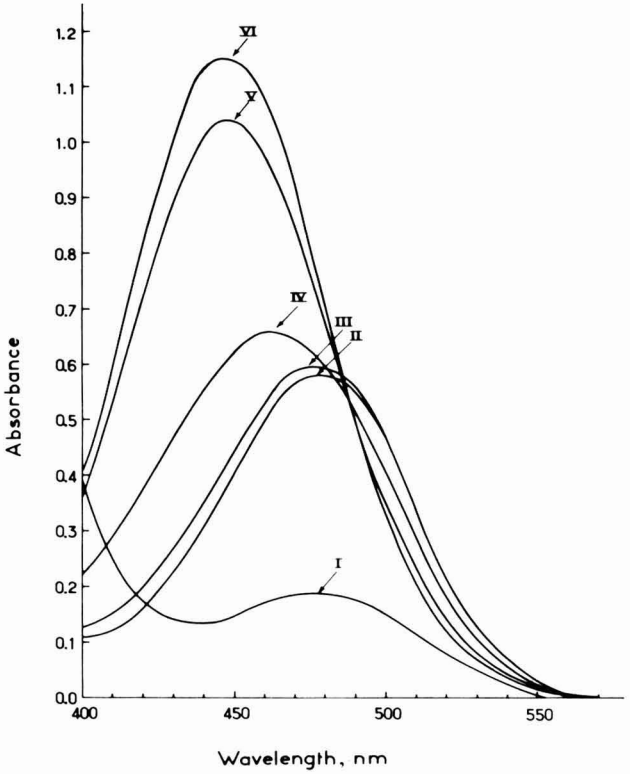
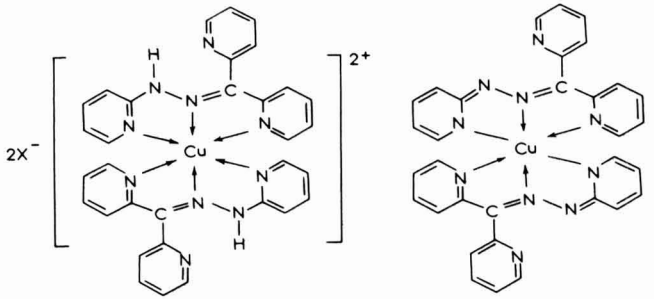


FIG. 1. Absorption spectra of Cu(II)–DPPH complexes at different pH values (DPPH in excess). In all cases copper conc. 3.0×10^{-5} *M*, DPPH conc. 5.0×10^{-4} *M*, $\mu = 0.1$, at (I) pH 4.95, (II) pH 8.06, (III) pH 8.90, (IV) pH 9.97, (V) pH 11.45, and (VI) pH 12.90.

It is known (*4*, *8*) that copper(II) ions may appear to be four- or six-coordinated. Considering that in the presence of an excess of DPPH a 1:2 copper(II)–DPPH complex is mainly formed and that in this case the copper is six-coordinated, this shift of maximum may be attributed to the gradual deprotonation of the two imino groups of the DPPH molecules at pH values above 9. This deprotonation most probably is completed at pH 11.2 and this was concluded from the fact that there is no shift of the maximum absorbance at measured pH values from 11.2 to 13.0. At pH from 11.9 to 12.6 the extinction coefficient has an actual high value ($\sim 3.8 \times 10^4\ M^{-1} \cdot cm^{-1}$) and is stable. This pH value was selected as the optimum pH for the Cu(II) determination. At λ_{max} 478 nm there is also a region of pH values from 6.5 to 8.5 with a constant molecular extinction coefficient but it has a lower value ($\sim 1.9 \times 10^4\ M^{-1} \cdot cm^{-1}$). This study was done with 3.0×10^{-5} *M* copper(II) concentration in the presence of 5.0×10^{-4} *M* DPPH and $\mu = 0.1$.

According to previous investigations (*4*, *7*, *10*, *12*), the 1:2 protonated



I II

Cu(II)–DPPH complex most probably has the structure **I**, where X^- is a monovalent ion, while the 1:2 deprotonated complex has the structure **II**. In the case of **II** a hypsochromic shift was noted although an extented conjugated system of double bonds seems to be formed.

In Fig. 2, some characteristic spectra of solutions containing DPPH and an excess of copper(II) ions are given at different pH values. The mea-

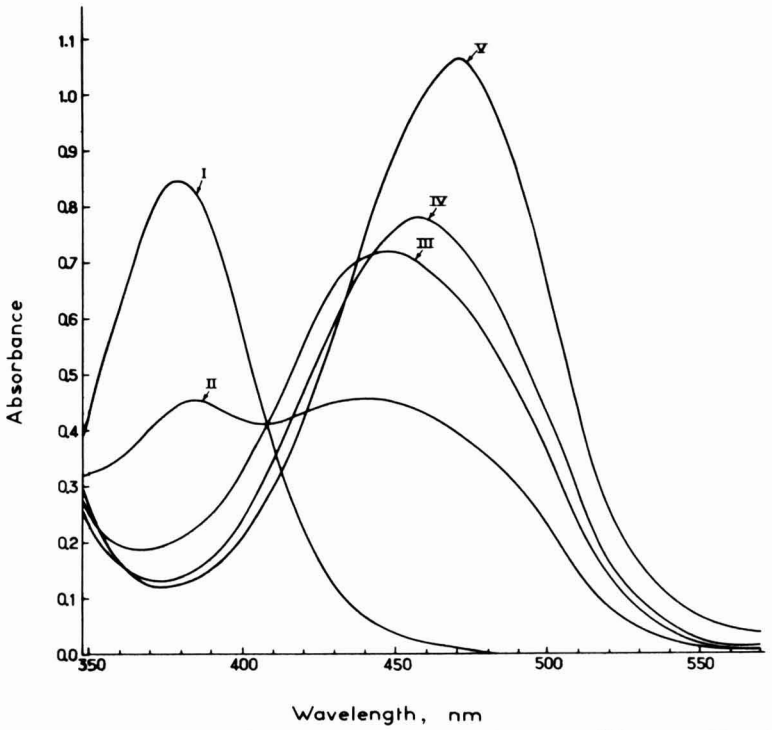


FIG. 2. Absorption spectra of Cu(II)–DPPH complexes at different pH values (Cu in excess). In all cases copper conc. $5.0 \times 10^{-4}M$, DPPH conc. $5.0 \times 10^{-5}M$, $\mu = 0.1$, at (I) pH 2.05, (II) pH 3.94, (III) pH 5.00, (IV) pH 7.03, and (V) pH 11.37.

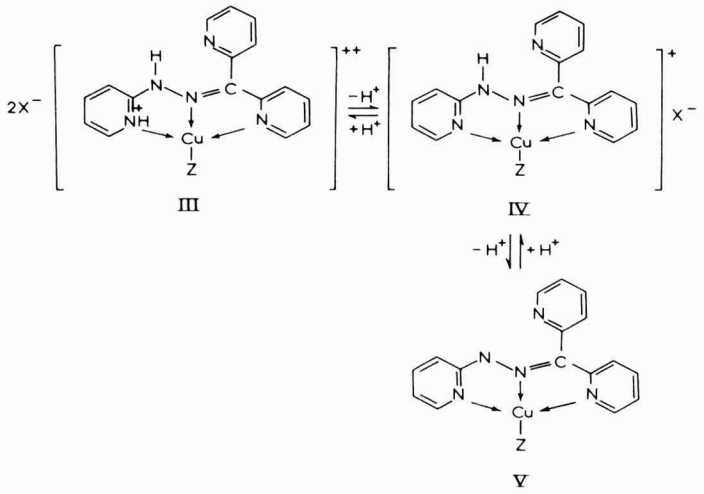
surements at pH > 7 were done immediately after complex formation to avoid any copper hydroxide precipitation upon standing. Here we can see the formation of three complexes. The first complex, with λ_{max} at 380 nm, predominates at pH < 3.5. The second complex, with λ_{max} at 448 nm, predominates at pH ~ 4.5 to 6.5 and the formation of a third one is obvious for pH > 6.5 with a bathochromic shift of λ_{max} to 470 nm. As both the mono- and the bis-copper complexes have been reported (*4, 5*), it is most probable that in the presence of an excess of Cu(II) ions the 1:1 Cu(II)–DPPH complex is formed. In this case copper must be four-coordinated and the continuous bathochromic shift of λ_{max} as the pH values become higher may be related to the deprotonation of the copper–DPPH system. This hypothesis is in accordance with the results of other papers (*3–5, 12*) dealing with copper complexes with similar ligands.

The possible structures of the above Cu(II)–DPPH complexes at the three different protonation stages are suggested to be as **III**, **IV**, and **V**, where X and Z are monovalent anions.

Both the 1:1 and the 1:2 complexes are immediately formed over the whole pH range. The 1:2 complex at the pH ranges 6.5–8.5 and 11.9–12.6 remained stable for at least 3 hr in the presence of diffuse daylight.

Composition of the Complex and Its Stability

The composition of the complex was studied by the mole ratio method at two pH values: (a) At pH 7.9 ± 0.1: Absorption spectra at this pH value showed, as expected, that λ_{max} varies with the metal-to-ligand ratio. In the presence of an excess of metal, λ_{max} is at 462 nm, and in the presence of an excess of DPPH, λ_{max} is at 478 nm. Plots in Fig. 3 have been made at the



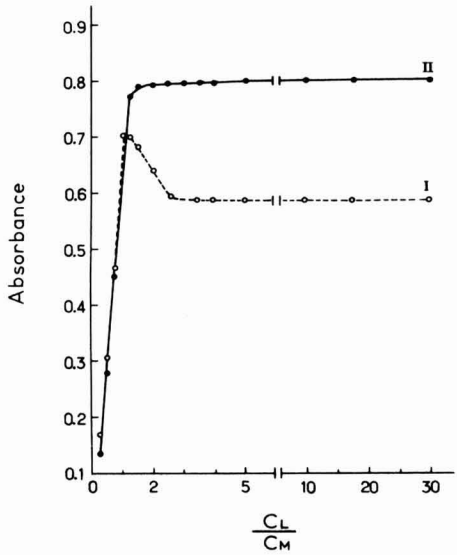


FIG. 3. Mole ratio method for the determination of the copper–DPPH complex composition. Copper conc. $4.0 \times 10^{-5}M$, pH 7.9 ± 0.1, and $\mu = 0.1$. Curve I at λ_{max} 462 nm and curve II at λ_{max} 478 nm.

above wavelengths and in the first case the formation of the 1:1 complex is confirmed while in the second case the formation of the 1:2 complex is confirmed. From these data it is deduced that at a mole ratio of 1:1.25 metal:DPPH, the bis complex predominates and that a three-fold excess of DPPH is required to obtain a reproducible color formation with the highest optical density. (b) At pH 12.30 ± 0.05: Similar results were obtained for the metal-to-DPPH mole ratio variation. In Fig. 4, the mole ratio plots are given at λ_{max} 470 nm for the 1:1 complex (excess of metal) and at λ_{max} 448 nm for the 1:2 complex (excess of DPPH). As at this high pH value, it is almost impossible to accept any protonated form of the Cu(II)–DPPH complex the noticed shift of λ_{max} may be attributed to the initial formation of the 1:1 deprotonated (neutral) complex and then by increasing the DPPH-to-metal ratio, to the formation of the 1:2 twice-deprotonated complex.

The apparent stability constant of the 1:2 Cu(II)–DPPH complex was determined according to Harvey and Manning (*6*) for the two pH values 7.5 ± 0.1 and 12.2 ± 0.1 at an ionic strength of 0.1 and 22 ± 0.5°C. The mean value was found to be of the order of 1×10^{14} and 1×10^{10}, respectively.

Absorption Spectra

The absorption spectra of DPPH and its bis complex with Cu(II) at pH 7.55 (λ_{max} 478 nm) and 12.25 (λ_{max} 448 nm), measured against a reagent

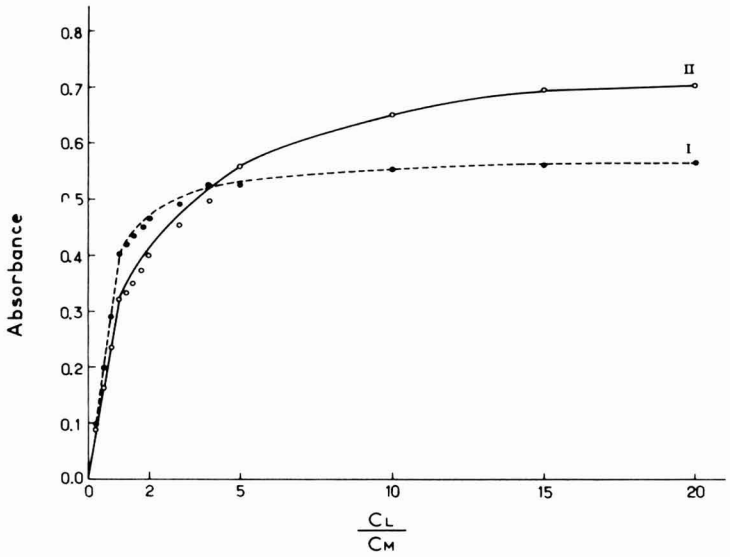


FIG. 4. Mole ratio method for the determination of the copper–DPPH complex composition. Copper conc. $2.0 \times 10^{-5}M$, pH 12.30 ± 0.05, and $\mu = 0.1$. Curve I at λ_{max} 470 nm and curve II at λ_{max} 448 nm.

blank, are presented in Fig. 5. At both pH values, DPPH does not exhibit significant absorbance and, therefore, an excess of reagent is not critical.

Beer's Law and Sensitivity of the Reagent

Calibration results at pH 6.5–8.5 as well as at pH 11.9–12.6 showed that the Beer's law was obeyed from 0.5 to 5.0×10^{-5} M (or 0.3 to 3.2 ppm) and from 0.25 to 2.5×10^{-5} M (or 0.15 to 1.6 ppm) in copper(II) ion, respectively.

The optimum range of concentration, evaluated by Ringbom's method (*9*) is from 1.0 to $4.0 \times 10^{-5}M$ (or 0.6 to 2.5 ppm) for the first case and 0.5 to $2.0 \times 10^{-5}M$ (or 0.3 to 1.3 ppm) for the second case.

The molecular extinction coefficient of this complex is about 1.9×10^4 $M^{-1} \cdot cm^{-1}$ (at 478 nm and pH region 6.5–8.5) and 3.8×10^4 $M^{-1} \cdot cm^{-1}$ (at 448 nm and pH region 11.9–12.6). The sensitivity, as defined by Sandell, is 0.00334 and 0.00167 $\mu g \cdot cm^{-2}$ of copper(II), respectively.

Interferences

The reagent forms colored complexes with a number of cations like Zn^{2+}, Cd^{2+}, Hg^{2+}, Pb^{2+}, Mn^{2+}, Ni^{2+}, and Co^{2+} which interfere. Iron(III) interferes also but it can be masked with fluorides. Common anions such as fluoride, chloride, bromide, nitrate, sulfate, perchlorate, acetate, trichloroacetate, and borate do not interfere up to a ratio of 1:500 Cu:ion.

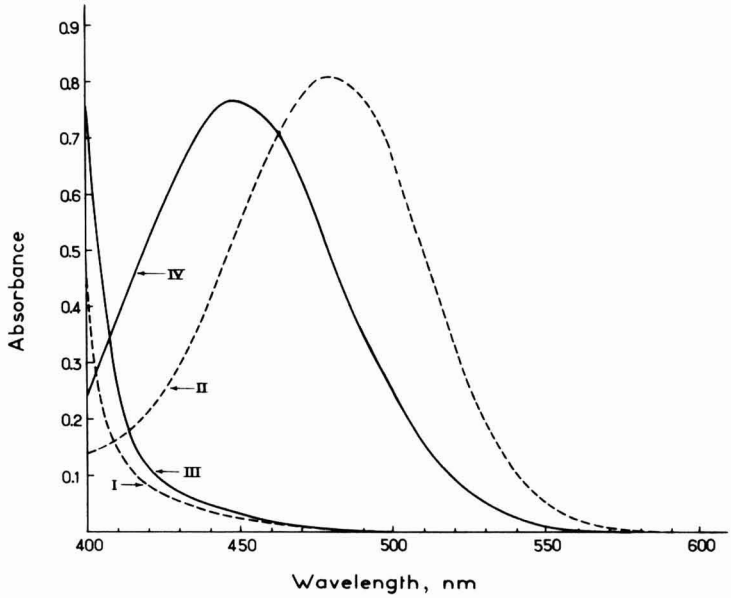


FIG. 5. Absorption spectra (a) At pH 7.55:(I) DPPH conc. $5.0 \times 10^{-4}M$, (II) Cu–DPPH complex from DPPH conc. $5.0 \times 10^{-4}M$ and Cu(II) conc. $4.0 \times 10^{-5}M$. (b) At pH 12.25: (III) DPPH conc. $5.0 \times 10^{-4}M$, (IV) Cu–DPPH complex from DPPH conc. $5.0 \times 10^{-4}M$ and Cu(II) conc. $2.0 \times 10^{-5}M$. In all cases $\mu = 0.1$.

The criterion for an interference was taken as a more than ±2% difference in the measured value of absorbance, compared to that found with copper alone.

CONCLUSIONS

According to this work, copper(II) can be determined with the reagent 2,2′-dipyridyl-2-pyridylhydrazone (DPPH) at pH 6.5–8.5 and 11.9–12.6. In the second case the pH adjustment is more critical but the sensitivity of the reaction is higher.

SUMMARY

The reaction of copper(II) ions with 2,2′-dipyridyl-2-pyridylhydrazone (DPPH) has been studied. Two copper complexes were found to be formed. One at pH 6.5 to 8.5 and the other at pH 11.9 to 12.6 with molecular extinction coefficients of $1.9 \times 10^4\ M^{-1} \cdot cm^{-1}$ at the absorption maximum of 478 nm and $3.8 \times 10^4\ M^{-1} \cdot cm^{-1}$ at 448 nm, respectively. A sensitive spectrophotometric procedure for the determination of copper at less than a 1-ppm level is proposed.

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MICROCHEMICAL JOURNAL 26, 10–16 (1981)

Analytical Applications of Mixed-Ligand Complexes

III. Vanadium(V)–Xylenol Orange Reagent Mixture for the Spectrophotometric Determination of Traces of Hydrogen Peroxide

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INTRODUCTION

We earlier described a sensitive method for the determination of hydrogen peroxide in the presence of the peroxy acids of sulfur with the aid of titanium(IV)–xylenol orange–hydrogen peroxide mixed-ligand complex (*6*). Xylenol orange (denoted as XO) was found to be suitably resistant toward the attack of strong oxidizing agents. This method, however, can not be applied to systems containing phosphate ions. For the determination of hydrogen peroxide in human fluids, Matsubara and Takamura (*3*) recently proposed the use of the vanadium(V)–XO–H_2O_2 system. It was pointed out that sulfate, phosphate, copper(II), iron(III), and aluminum ions interfere strongly with the method, but that the interference can be avoided simply by diluting the samples to be analyzed. The method has the advantage that urea, amino acids, and other carboxylic acids (e.g., acetic, citric, glycolic, lactic, tartaric, pyruvic acids, etc.) do not impede the estimation of hydrogen peroxide. The method proved useful in our laboratory too, but some of the experiences and results obtained made it necessary to extend our knowledge of this complex system.

EXPERIMENTAL

Chemically pure substances were used throughout, without further purification. XO was analyzed by the method of Murakami *et al.* (*2*). If not stated otherwise, XO was added to the slightly alkaline vanadium(V) solution, the pH was then adjusted with acetate buffer (the final concentration of buffer was always 0.02 *M*) and the hydrogen peroxide was added last. Spectra were recorded with a Unicam SP 800 spectrophotometer, and a Radiometer PHM 64 Research pH meter was used for pH measurements.

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RESULTS

The spectra in Fig. 1 show that at least two vanadium(V)–XO complexes occur. At lower concentrations of XO a 2:1 vanadium:XO complex is formed, which is transformed into a less colored 1:1 complex when the concentration of ligand is increased. With the appearance of the 1:1 complex, an isosbestic point can be observed at 546 nm. It also seems probable that a third complex, with 1:2 composition, is formed in the presence of higher XO concentrations (Fig. 2). It should be emphasized that the formation of these complexes is slow when XO is added to a slightly acidic (pH 4) vanadate solution; the absorbance maxima can be reached only after 24 hr. Complex formation is considerably faster when XO is added to slightly alkaline vanadate solution and the pH is adjusted to 4 afterward. It is therefore advisable to use alkaline vanadate solution, or even better to prepare the reagent mixture as in the procedure of Matsubara and Takamura.

If hydrogen peroxide is added in increasing quantity to the 2:1 vanadium(V)–XO complex, a new complex is formed. The 576-nm band decreases, the broader 450-nm band increases, and an isosbestic point occurs at 512 nm (see Fig. 3). Similar behavior can be observed when hydrogen peroxide is added to the 1:1 vanadium (V)–XO complex, although the change in absorbance is different. Figure 4 gives some hints concerning the compositions of the ternary complexes. When hydrogen peroxide is added to the 2:1 or 1:1 complexes, curves having limiting values are obtained, and the intersection points of the corresponding tangents reveal that one molecule of hydrogen peroxide is combined with

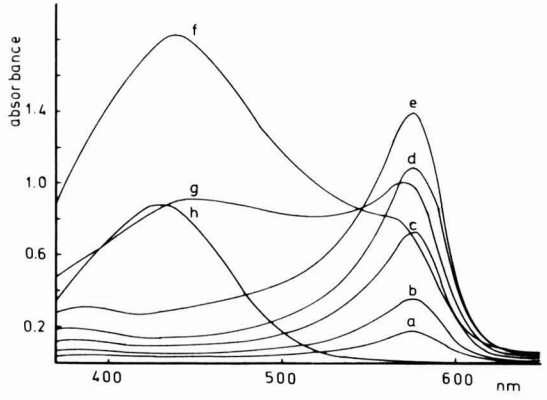

FIG. 1. Absorption spectra of vanadium (V)–XO (a–g) and XO (h) at pH 4 (0.02 *M* acetate buffer). V(V): (a–g) 5.0×10^{-5} *M*; (h) 0.0. XO (a) 3.05×10^{-6} *M*; (b) 6.1×10^{-6} *M*; (c) 1.22×10^{-5} *M*; (d) 1.83×10^{-5} *M*; (e) 3.05×10^{-5} *M*; (f) 9.7×10^{-5} *M*; (g) 5.49×10^{-5} *M*; (h) 3.66×10^{-5} *M*.

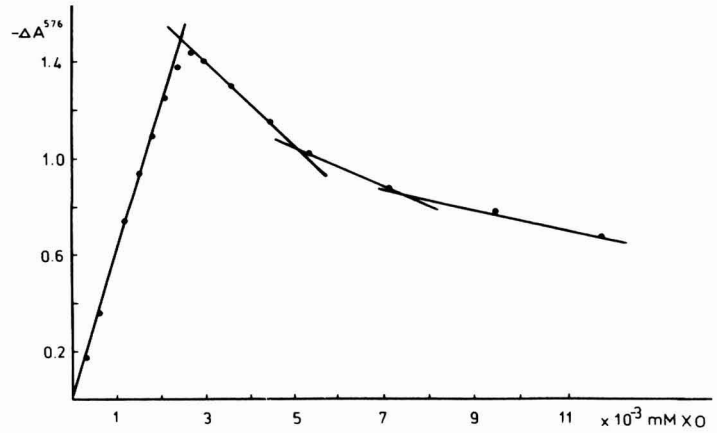

FIG. 2. Influence of XO concentration on the absorbance of the V(V)–XO complex at 576 nm. V(V): 5.0×10^{-3} m*M*, pH 4.

each vanadium atom in both cases. This was confirmed by the continuous variation method.

Our measurements corroborated the finding of Matsubara and Takamura that the absorbance at 560 nm in the V(V)–XO–H_2O_2 system reverts to its original value (i.e., the absorbance in the absence of hydrogen peroxide) on the further addition of XO (Fig. 5). On the basis of this observation, Matsubara and Takamura (*3*) put forward the idea that the decrease in the absorbance of the V(V)–XO complex on the action of hydrogen peroxide is caused by a redox reaction: "Then it becomes feasi-

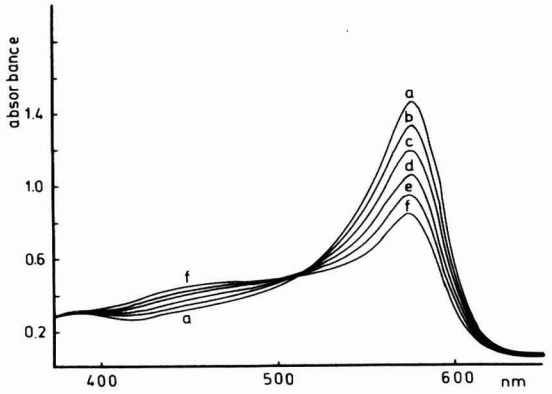

FIG. 3. Influence of hydrogen peroxide on the absorption spectra of the V(V)–XO complexes. V(V): 5×10^{-3} m*M*; XO: 3.05×10^{-3} m*M*. H_2O_2: (a) 0.0; (b) 9.98×10^{-4} m*M*; (c) 1.99×10^{-3} m*M*; (d) 2.99×10^{-3} m*M*; (e) 3.99×10^{-3} m*M*; (f) 4.99×10^{-3} m*M*. Spectra taken 5 min after addition of hydrogen peroxide.

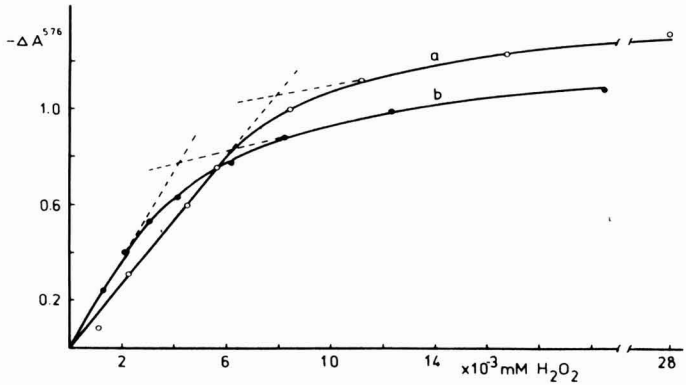

FIG. 4. Decreases in the absorbances at 576 nm of the V(V)–XO complexes of different compositions on the addition of hydrogen peroxide, Curve (a): V(V) 5.2×10^{-3} m*M*; XO 2.65×10^{-3} m*M*; pH 4. Curve (b): V(V) 5.2×10^{-3} m*M*; XO 5.7×10^{-3} m*M*; pH 4.

ble to think that oxidation of XO bonded to vanadium(V) as a ligand takes place by the adjacent hydrogen peroxide, probably with the catalytic action of vanadium(V), as a result of which the absorption peak at 582 nm disappeared completely at when $[XO] \leq [H_2O_2]$. The broad band around 460 nm may be attributed to some oxidation products of XO.'' To check this assumption, we measured the absorbance of the iron(III) formed when vanadium(V), vanadium(V)–XO, and vanadium(V)–XO–H_2O_2 were added to an acidic iron (II) sulfate solution. The data in Table 1 show clearly that the total oxidizing capacity of the three-component system does not display any change in time; the differences between the found and calculated values are within the error limits of ordinary spectrophotometric measurements.

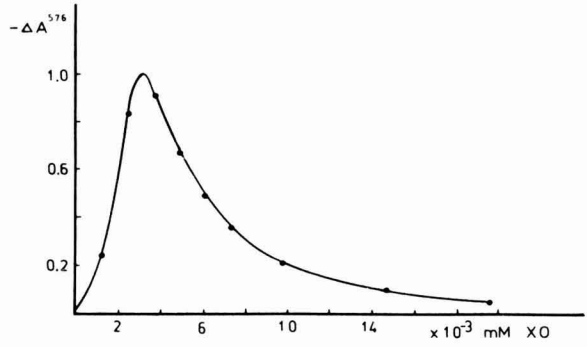

FIG. 5. Effect of XO concentration on the absorbance decrease caused by hydrogen peroxide at 576 nm. V(V): 4.0×10^{-3} m*M*; H_2O_2: 7.7×10^{-3} m*M*; pH 4.

TABLE 1
ABSORBANCE OF IRON(III) FORMED

Waiting time (min)	Solutions measured				Calculated difference	
	1	2	3	4	(2 + 3)	4 − (2 + 3)
30	0.106	0.302	0.107	0.405	0.409	−0.004
60	0.204	0.298	0.106	0.406	0.404	+0.002
180	0.106	0.300	0.108	0.407	0.408	−0.001

Note. Composition of solutions: 1, 11.93×10^{-3} m*M* $NaVO_3$, pH 4; 2, 11.93×10^{-3} m*M* $NaVO_3$ + 6.94×10^{-3} m*M* XO, pH 4; 3, 5.97×10^{-3} m*M* H_2O_2, pH 4; 4, 11.93×10^{-3} m*M* $NaVO_3$ + 6.94×10^{-3} m*M* H_2O_2, pH 4. Analysis: After the given waiting period, 10 ml 0.05 *M* $FeSO_4$ and 5.3 ml 20% H_2SO_4 were added to each solution to adjust the sulfuric acid concentration to 0.4 *M*, and the volume was made up to 50 ml. After the solution had stood for 1 hr in the dark, the absorbance of the iron (III) was measured at 304 nm in a 0.20-cm cell against a blank solution containing acetate buffer, sulfuric acid, and $FeSO_4$.

DISCUSSION

In acetate buffer solution of pH 4 at lower concentrations of XO, vanadium(V) forms a 2:1 complex with an absorbance maximum at 576 nm (Fig. 1). When the concentration of XO is increased, the 2:1 complex is transformed into a 1:1 complex. Transformation is accompanied by a decrease in the absorbance at 576 nm, and an isosbestic point can be observed at 546 nm. The difference spectrum has a maximum at 520 nm. Our finding is in contrast with that of Budevsky and Přibil (*1*), who speak of 1:1 and 1:2 complex formation. This contradiction may arise from the fact that the XO preparation they used was not pure enough. Nevertheless, the formation of a third complex, with 1:2 composition, has also been observed (see Fig. 2).

On the addition of hydrogen peroxide, interactions take place with both 2:1 and 1:1 complexes, resulting in a decrease in the absorbances of the binary complexes, at 576 nm and an increase at 440 nm. These measurements suggest that each vanadium atom binds one hydrogen peroxide molecule, i.e., 2:1:2 and 1:1:1 mixed-ligand complexes are formed.

The fact that the absorbance at 576 nm increases when excess XO is added to the mixed-ligand complexes can easily be understood when the fairly mobile equilibria in the systems are considered. It should be noted, however, that the formation of the ternary complex is not an instantaneous process; it is necessary to wait a few minutes for the setting-in of the equilibrium.

The data in Table 1 support our hypothesis that the decrease in the absorbance at 576 nm when hydrogen peroxide is added to the binary complex does not arise from a redox reaction between hydrogen peroxide and XO under the catalyzing action of vanadium(V), as was assumed by

Matsubara and Takamura (*3*): in 3 hr there was no observable change in the total oxidizing capacity of the system. The ternary complex formation can further be supported by the isosbestic point at 510 nm (Fig. 3), although this wavelength depends slightly on the composition of the binary complex and the hydrogen peroxide concentration: higher concentrations of the latter may result in the partial displacement of XO by hydrogen peroxide. Similar behavior was found by Shtokala and Kulik (*5*) in the zirconium–XO–H_2O_2 system.

As regards the role of hydrogen peroxide in the formation of mixed-ligand complexes with the transition metals, a generalization can not be given. For example, in the case of titanium(IV)–XO the addition of hydrogen peroxide results in an increase in the absorbance at 520 nm, whereas a decrease occurs at 576 nm, together with an increase at 460 nm, in the present system. In the case of vanadium(V)–PAR–H_2O_2 (*4*) there is an increase in the absorbance at 540 nm in an 0.1 *M* acid solution, while a decrease is observed at the same wavelength when the pH is adjusted to 4–5.

It was mentioned above that the rate of formation of the binary complex V(V)–XO depends strongly on the starting pH: in the alkaline region the reaction is almost instantaneous, while at pH 4 or lower about 1 day is necessary for equilibrium to be reached. This behavior can be explained by the formation of vanadium(V) aggregates in slightly acidic medium.

The rates of formation of the ternary complexes are also higher at higher pH's, but it is more advisable to add the sample containing hydrogen peroxide to the slightly acidic solution to avoid the spontaneous decomposition of hydrogen peroxide. At pH 4, however, it is necessary to wait for at least 20 min for the equilibrium absorbance to be attained. It should be added that the specific decrease in the absorbance, i.e., $\Delta A/[H_2O_2] = \Delta E$, depends on the concentration ratio [V(V)]/[XO] because the composition of the binary complex species is determined by the latter. This explains the observed fact that ΔE changes when the experimental conditions, e.g., the ratio [V(V)]/[XO] or the pH, are varied. To avoid appreciable error in the analysis of hydrogen peroxide, it is advisable to use the simultaneously determined ΔE value or a calibration curve, especially if the binary complex reagent mixture is made from a new XO preparation.

SUMMARY

Vanadium(V) forms 2:1, 1:1, and 1:2 binary complexes with xylenol orange (XO). In slightly alkaline solution the formation of the complexes is almost instantaneous, whereas it takes a day for the equilibria to be reached at pH 4. On the action of hydrogen peroxide, both the 2:1 and the 1:1 complexes are transformed into mixed-ligand complexes, with 2:1:2 and 1:1:1 compositions, respectively. No intramolecular redox reaction takes place between XO and hydrogen peroxide. Peroxo complex formation can be used for estimation of hydrogen

peroxide if the decrease in absorbance of the 576-nm band of the binary complexes is measured. The molar absorptivity decrease depends on the ratio [V(V)]/[XO] and the pH.

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Steroids

XXIV. Study of the Formation of 3,20-Dihydroxyiminoprednisolone

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INTRODUCTION

The oxime-formation reaction of ketosteroids with hydroxylamine is a well-known and frequently employed process. In this paper we deal with the formation of the 3,20-dioxime of prednisolone (11β, 17α,21-trihydroxypregna-1,4-diene-3,20-dione). A number of publications have already reported on the formation of the 3,20-dioximes of pregn-4-ene-3,20-diones (*1*, *14*). These compounds are of pharmacological interest. For example, they exert a depressant effect on the central nervous system, as is the case with 3,20-dioximes of progesterone derivatives or aldosterone-3,20-dioxime, utilized in immunology (*13*). The steroid oximes are the starting materials in further syntheses (e.g., the Beckmann rearrangement); these reactions depend on the steric state of the oxime groups (*6*).

EXPERIMENTAL

Prednisolone-3,20-dioxime was prepared by two procedures.

Reaction A. Prednisolone (1 g) was dissolved in pyridine (20 ml) and hydroxylamine hydrochloride (4 g) was then added. The mixture were kept for 3 hr (reaction A1), or for 6 hr in a boiling-water bath, followed by standing at room temperature for 18 hr (reaction A2). Later on, an identical volume of ice-cold water was added to the solution, and the precipitate was extracted with diethyl ether. The ether was evaporated and the residue was dissolved in ethanol, from which the product was precipitated by water. The earlier steps the dissolution in ethanol and precipitation with water were repeated two or three times. The last crystallization was performed from ethanol–water too.

Yield: 0.89 g (85%). Analysis: $C_{21}H_{30}N_2O_5 \cdot H_2O$. Found: C, 61.39%; H, 8.09%; N, 6.93%. Calcd: C, 61.76%; H, 7.84%; N, 6.85%.

Reaction B. Prednisolone (1 g) was dissolved in ethanol (36 ml), and hydroxylamine hydrochloride (4 g) and soldium acetate (3.2 g) were added. After refluxing for 3 hr, the mixture was filtered and evaporated to

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low volume. The precipitate formed on the addition of ice water (5 ml) was dissolved in ethanol, and again precipitated with water as above.

Yield: 0.95 g (91%). Analysis: $C_{21}H_{30}N_2O_5 \cdot H_2O$. Found: C, 61.33%; H, 8.17%; N, 6.77%. Calcd: C, 61.76%; H, 7.84%; N, 6.85%.

The results of the other examinations on the products are listed in Tables 1–3.

Thin-layer chromatography of the starting material and oximes were performed on Kieselgel G (250 nm) plates. The running mixtures: (A) chloroform–ethanol, 90:10; (B) benzene–dioxane–diethyl ether, 100:65:65. In these systems the products of reactions A and B separated into four and two components, respectively.

A CAMAG (Mutenz, FRG) lamp (uv light at 254 nm) and color-forming reagents were used to detect the spots (Tables 1 and 2). The oxime spots gave an intense color at room temperature with a 2% aqueous solution of $CuCl_2$. The products of the oxime reaction did not give any color with either 2,4-dinitrophenylhydrazine (which indicates the keto group) or tetrazolium blue (indicator of the 21-OH, 20-CO group). For quantitative separation a thick layer (0.5 mm) of adsorbent and the earlier-described running mixtures were applied. After separation the individual bands were eluted with diethyl ether or ethanol. The proportions of the components were determined by weight measurement.

The ir spectra of the compounds were recorded in KBr by a Unicam SP 200 spectrophotometer. The ir spectra of the products of reactions A and B and of the separated components were almost identical. A broad, split OH band (3150–3550 cm^{-1}) was to be found in the spectrum of each product; this is indicative of strongly associated OH. The band at 1655 cm^{-1} can be ascribed to the C=N bond. The bands at 800–1460 cm^{-1} are attributed to deformational β and γ OH vibrations and NO vibrations.

TABLE 1
CHROMATOGRAPHIC PROPERTIES OF THE PRODUCTS

Compound	R_f values		Color formed		
	Solvent A	Solvent B	Reagent A	Reagent B	Reagent C
Compound 1	0.19	0.51	Green	None	None
Compound 2	0.30	0.64	Green	None	None
Compound 3	0.41	0.72	Brownish-green	None	None
Compound 4	0.50	0.80	Brownish-green	None	None

Note. Solvent A: chloroform:ethanol (90:10), solvent B: benzene:dioxane:diethylether (100:65:65), reagent A: 2% $CuCl_2$ in water, reagent B: 0.5% tetrazolium blue in 2.5 *N* NaOH (Ref. (5)), reagent C: 0.3% 2,4-dinitrophenylhydrazine in methanol 0.3% HCl (Ref. (5)).

TABLE 2
SOME SPECTRAL AND OTHER CHARACTERISTICS OF THE COMPOUNDS

Compound	uv λ_{max} (nm) in ethanol	Ext. coefficient ϵ	Color formation with $CuCl_2$ λ_{max} (nm)	mp (°C)	Relative amount in reaction		
					A1	A2	B
Product A	256	12.500 and 1.500	343	174			
Product B	256	13.000 and 1.500	350	167–169			
Compound 1	264	—	350	142–144	46	53	55
Compound 2	245	—	350	150–152	28	19	45
Compound 3	250	—	334	172	14	18	—
Compound 4	240	—	334	171	12	10	—

Palm and Werbin (*8, 9*) dealt with the analysis of these bands as regards the oximes of aromatic compounds, and in certain cases demonstrated characteristic differences between the isomeric oximes.

The uv spectra were recorded with a Specord UV VIS spectrophotometer.

For characterization of the compounds, the colors of their copper complexes were also examined. The material was dissolved in ethanol (0.2 mg/ml), and 0.1 ml 2% $CuCl_2$ solution in water was added at room temperature for each 2 ml of solution. The products of the oxime-formation reaction gave green colors. The intensities and spectra of these colors were established. The absorption maxima of compounds 1 and 2 differ from those of compounds 3 and 4.

In the solid phase each of the products is stable at room temperature. The products of reactions A and B and the individual separated components were dissolved in pyridine or in ether, or in pyridine–ether (1:1), respectively, and kept at room temperature for several weeks or refluxed for 24 hr. Under such conditions it was not possible to detect decomposition or the transformation of compounds 1 and 2 into compounds 3 and 4. In the above solutions, chromatographically separated compounds 1 and 2 give a mixture of 1 and 2, while separated compounds 3 and 4 give a mixture of 3 and 4. In a water-saturated pyridine–ether (1:1) solution at room temperature, in 4 days the mixture of 3 and 4 was converted quantitatively to a mixture of 1 and 2.

DISCUSSION

Analysis of the above data shows that oxime formation in pyridine medium gives rise to prednisolone-3,20-dioxime that can be resolved into four components. In the reaction in ethanolic medium, only two of these are formed.

TABLE 3
INFRARED ABSORPTION BANDS OF THE COMPOUNDS 1–4

Frequencies in cm^{-1} in the interval 800–1700 cm^{-1}					
		Separated compounds			
Product	Product	1	2	3	4
795 m	795 m	795 m	800 m	793 w	795 w
875 s	875 s	877 s	865 s	878 m	873 m
890 m	890 s	890 m	893 s		
925 s	922 s	925 s	922 s	928 s	923 s
965 s	964 s	963 s	965 s		
				975 s	973 s
990 m	990 m	990 m	990 m	990 s	990 s
1035 s	1038 s	1038 s	1039 s	1030 m	1030 m
				1042 m	1042 m
1075 w	1070 w	1075 w	1078 w	1078 m	1078 m
1115 s	1115 s	1115 s	1116 s	1120 s	1120 s
1158 w	1160 w	1160 w	1160 w	1158 w	1160 w
1173 w	1170 w	1170 w	1170 w		
1245 w	1142 w	1145 w	1145 w		
1275 w	1270 w				
		1290 w	1293 m	1290 s	1285 s
1350 m	1345 m	1345 w	1348 m	1350 w	1350 w
1370 m	1370 m	1375 w	1375 m	1379 m	1378 m
1390 m	1390 m	1390 w	1390 w	1390 w	1390 w
1445 s	1440 s	1445 s	1453 s		
				1463 s	1463 s
1655 s	1655 s	1655 s	1655 s	1655 s	1655 s

Note. s, Strong; m, medium; w, weak.

Theoretically, the appearance of the *syn* and *anti* geometrical isomers is to be expected in the formation of asymmetric ketoximes. Confirmation of the presence of the two isomers and their distinction have been carried out for some steroid ketoximes with various conformations; for example, the *syn* and *anti* isomers of the 3-ketoxime have been separated in the cases of 11α-hydroxyprogesterone (*14*) and cholest-4-ene-3-one (*10*).

On the formation of the steroid-20-ketoximes, the occurrence of the isomer better stabilized by H-bond formation is to be expected (*11*). In general, only one of the isomers can be detected in the case of the 20-ketoximes (*3, 11*). However, the *syn* and *anti* isomers of pregnenolone-20-oxime are known, and the isomerization of these on the action of uv light has been described (*12*). *Syn* and *anti* isomers of 3,20-diaminopregn-5-ene in the 3 and 20 positions are also known (*7*).

The oxime isomers are known to differ in a number of their properties, e.g., they have different melting points (*10*), and different chromato-

graphic natures (*3, 4*). Aromatic oximes have been reported to give different colours with $CuCl_2$ on a thin layer in the cases of the *syn* and *anti* isomers (*4*). The steroid oxime isomers can undergo isomerization in organic solvents or on the action of uv light (*2, 10, 12, 14*). The phenomena observed in our experiments are in accordance with these data. The infrared spectra of the compounds point to the same structure.

It may be concluded from our examinations that compounds 1, 2, 3, and 4 are isomers, and that the proportions of the isomers formed in pyridine or in ethanolic medium are different.

Further investigations are necessary to establish the configurations of the individual compounds.

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Microdetermination of Carbon and Hydrogen in Organofluorine Compounds

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INTRODUCTION

Fluorinated organic compounds owing to their thermal stability need special methods for the determination of their carbon and hydrogen. The fluorine decomposition products either poison the tube fillings, or they are not completely eliminated from the combustion products causing erroneous carbon figures (*8*). The simultaneous presence of fluorine combustion products together with the volatile silicon tetrafluoride, produced from reaction with the silica combustion tube, make the quantitative elimination of fluorine interference more difficult.

Owing to the reasons mentioned above and because of the importance of organic fluorine compounds, intensive investigations (*15*) have been carried out in order to find a suitable method for the determination of carbon and hydrogen in these compounds. Other studies concerning this problem have also been reported (*6, 8, 11, 16*). Various internal (*1, 5, 13, 14, 15, 17*) and external (*9*) absorbents have been suggested for the elimination of fluorine decomposition products, but the application of internal absorbents was more popular.

Recently, however, Bishara *et al.* (*3*) described a method for the microdetermination of carbon and hydrogen in fluorinated compounds in which external absorbents were recommended. In that method, the combustion products, before entering the carbon dioxide absorption tube, were conducted through a silica gel–hydrochloric acid mixture and the displaced chlorine is trapped either in acidified silver nitrate solution or in silica gel transfused with silver nitrate. Another Anhydrone tube was usually inserted to ensure that the gas stream passing to the soda asbestos tube is completely dry.

Although good results for carbon and hydrogen have been reported (*3*) for a relatively wide range of organofluorine compounds, the application

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of five absorption tubes for the elimination of fluorine interference is considered to be a disadvantage.

In the present work a standard absorption tube packed with silica gel previously treated with acidified thorium nitrate solution has been tested for the efficient elimination of fluorine decomposition products.

Four different combustion methods have been used in this investigation: (1) Cobalto–cobaltic oxide method (*7*); (2) rapid straight empty tube method (*12*); (3) rapid empty tube method of Belcher–Ingram (*2*); and (4) rapid flash combustion method (*10*).

EXPERIMENTAL

Reagents and Materials

All reagents were M.A.R. grade. Anhydrone and soda asbestos 14–22 mesh granules and quartz wool were used.

Cobalto–cobaltic oxide granules used as a combustion filling was prepared as previously described (*7*).

Preparation of the Silica Gel–Thorium Nitrate Mixture

The colorless coarse grains of silica gel are crushed and sieved to about 1.25 mm mesh size. The grains are washed with dilute hydrochloric acid (1:3 v/v) three times, and then with bidistilled water until the washings are chloride free.

Silica gel is dried first at 120°C for 2 hr and then overnight at 200°C. Acidified thorium nitrate solution was prepared by adding few drops of concn nitric acid to 10 ml of saturated thorium nitrate solution.

A few drops of the acidified thorium nitrate solution are added to the silica gel in a stoppered flask which is shaken vigorously. This process is repeated several times until saturation, i.e., just before the silica gel stuck to the glass.

Two-thirds of a standard Pregl absorption tube is filled with the treated silica gel and after inserting a short quartz wool plug the last part of the absorption tube is filled with Anhydrone to absorb any moisture diffused from the silica gel layer by the fast flow of oxygen.

The silica gel absorption tube was connected between the Anhydrone and soda asbestos tubes and kept at room temperature (ca. 25°C).

Procedure

The procedures used for cobalto–cobaltic oxide (*7*) rapid straight empty tube (*12*), rapid empty tube of Belcher–Ingram (*2*), and flash (*10*) combustion methods are as described elsewhere.

RESULTS AND DISCUSSION

The study of the combustion products of an organic compound is very important not only in the determination of a number of its constituent

elements but also in eliminating the interferences due to the presence of some of these elements.

Fluorine differs from other halogens in that its oxidation products can react with a silica combustion tube. Belcher and Goulden (*1*) suggested that during the preliminary combustion, hydrogen fluoride is produced, which attacks the combustion tube in the vicinity of the sample boat yielding silicon tetrafluoride. Some of this product, on passing through the hot zone of the tube, decomposed, giving rise again to hydrogen fluoride, and leaving a deposit of silica in the tube. It is possible that free fluorine is produced during combustion, especially in the case of fluorocarbons. The free element may also react with the silica combustion tube producing silicon tetrafluoride according to the reaction:

$$2F_2 + SiO_2 \rightarrow SiF_4 + O_2 \quad (1)$$

As a general approximation the combustion products may contain hydrogen fluoride, free fluorine, and silicon tetrafluoride. Evidently, all these products are passed uneffected in the Anhydrone tube but react in different ways with the alkaline reagent in the carbon dioxide absorption tube causing erroneous carbon figures.

In the present work an absorption tube packed with silica gel–thorium nitrate and connected between water and carbon dioxide absorption tubes is suggested for the elimination of interferences of the different fluorine oxidation products.

As mentioned previously, hydrogen fluoride, silicon tetrafluoride, and free fluorine are the expected decomposition products of fluorinated compounds. Hydrogen fluoride can react with thorium nitrate mounted on the silica gel according to the reaction:

$$4\ HF + Th(NO_3)_4 \rightarrow ThF_4 + 4\ HNO_3 \quad (2)$$

Thorium tetrafluoride can be precipitated all over the silica gel in the absorption tube.

Free fluorine, if present, can react with the silica gel–thorium nitrate mixture according to the reaction (*4*)

$$7\ F_2 + 6\ H_2O \rightarrow 12\ HF + O_2 + O_3 + F_2O \quad (3)$$

Silicon tetrafluoride is expected to react with thorium nitrate loaded on silica gel according to the reaction

$$SiF_4 + Th(NO_3)_4 + 4\ H_2O \rightarrow H_4SiO_4 + ThF_4 + 4\ HNO_3 \quad (4)$$

That is, thorium tetrafluoride and orthosilicic acid are precipitated over the silica gel in the absorption tube.

The proposed silica gel–thorium nitrate tube was extensively examined for the elimination of fluorine interference in carbon and hydrogen determination for a wide range of fluorinated and perfluoro compounds using the different empty (*2, 10, 12*) tubes and packed (*7*) tube methods. Tables 1 to 4 show the results obtained by the cobalto–cobaltic oxides (*7*), rapid

TABLE 1

MICRODETERMINATION OF CARBON AND HYDROGEN IN FLUORINATED ORGANIC COMPOUNDS BY THE COBALTO–COBALTIC OXIDE METHOD

No.	Compound	Carbon and hydrogen percentage	Average[a] carbon and hydrogen percentage ($\dot{x}$, %)	Relative accuracy of the mean percentage	Standard deviation (S)	Confidence limits $\dot{x} \pm ts/n^{1/2}$[b] $t = 0.95$
1	4-Fluorobenzoic acid	60.01	59.94	−0.07	0.208	59.94 ± 0.24
		3.59	3.55	−0.04	0.094	3.55 ± 0.11
2	Perfluoronaphthalene	44.14	43.97	−0.17	0.017	43.97 ± 0.02
3	Trifluoroacetanilide	50.80	50.80	—	0.134	50.80 ± 0.16
		3.20	3.47	+0.27	0.148	3.47 ± 0.17
4	2,3-Dichloro-6-fluoro-quinoxaline	44.27	44.40	+0.13	0.125	44.40 ± 0.14
		1.39	1.39	—	0.168	1.39 ± 0.19
5	2,3-Dimethoxy-6-fluoro-quinoxaline	57.69	57.74	+0.05	0.104	57.74 ± 0.12
		4.36	4.53	−0.17	0.124	4.53 ± 0.14
6	2,4-Dioxo-7-fluoro-(1H,3H) quinazoline	53.34	53.48	−0.14	0.095	53.48 ± 0.11
		2.79	2.93	−0.14	0.030	2.93 ± 0.03

[a] Average of four determinations.

[b] n is the number of determinations.

TABLE 2

MICRODETERMINATION OF CARBON AND HYDROGEN IN FLUORINATED ORGANIC COMPOUNDS BY THE RAPID STRAIGHT EMPTY TUBE METHOD

No.	Compound	Carbon and hydrogen percentage	Average[a] carbon and hydrogen percentage ($\dot{x}$, %)	Relative accuracy of mean percentage	Standard deviation (S)	Confidence limits $\dot{x} \pm ts/n^{1/2}$[b] $t = 0.95$
1	4-Fluorobenzoic acid	60.01	60.06	±0.05	0.169	60.06 ± 0.20
		3.59	3.53	−0.06	0.098	3.53 ± 0.12
2	Perfluoronaphthalene	44.14	44.14	—	0.177	44.14 ± 0.15
		—	—	—	—	—
3	Trifluoroacetanilide	50.80	50.80	—	0.156	50.80 ± 0.18
		3.20	3.41	+0.21	0.155	3.41 ± 0.18
4	2,3-Dichloro-6-fluoro-quinoxaline	44.27	44.27	—	0.361	44.27 ± 0.41
		1.39	1.39	—	0.255	1.39 ± 0.29
5	2,3-Dimethoxy-6-fluoro-quinoxaline	57.69	57.63	−0.06	0.229	57.63 ± 0.27
		4.36	4.38	+0.02	0.126	4.38 ± 0.15
6	2,4-Dioxo-7-fluoro-(1H,3H)quinazoline	53.34	53.26	−0.08	0.155	53.26 ± 0.18
		2.79	2.89	+0.10	0.060	2.89 ± 0.07

[a] Average of four determinations.

[b] n is the number of determinations.

TABLE 3

MICRODETERMINATION OF CARBON AND HYDROGEN IN FLUORINATED ORGANIC COMPOUNDS BY THE RAPID EMPTY TUBE OF BELCHER–INGRAM

No.	Compound	Carbon and hydrogen	Average[a] carbon and hydrogen percentage ($\dot{x}$, %)	Relative accuracy of the mean	Standard deviation (S)	Confidence limits $\dot{x} \pm ts/n^{1/2}$[b] $t = 0.95$
1	4-Fluorobenzoic acid	60.01	60.16	−0.15	0.147	60.16 ± 0.17
		3.59	3.77	−0.18	0.067	3.77 ± 0.08
2	Perfluoronaphthalene	44.14	44.22	−0.08	0.164	44.22 ± 0.19
		—	—	—	—	—
3	Trifluoroacetanilide	50.80	50.84	−0.04	0.136	50.84 ± 0.16
		3.20	3.47	−0.27	0.103	3.47 ± 0.12
4	2,3-Dichloro-6-fluoro-quinoxaline	44.27	44.38	−0.11	0.145	44.38 ± 0.17
		1.39	1.50	−0.11	0.292	1.50 ± 0.34
5	2,3-Dimethoxy-6-fluoro-quinoxaline	57.69	57.69	—	0.152	57.69 ± 0.17
		4.36	4.53	−0.17	0.204	4.53 ± 0.23
6	2,4-Dioxo-7-fluoro-(1H,3H)quinazoline	53.34	53.55	−0.21	0.095	53.55 ± 0.11
		2.79	2.88	−0.09	0.054	2.88 ± 0.06

[a] Average of four determinations.

[b] n is the number of determinations.

TABLE 4

MICRODETERMINATION OF CARBON AND HYDROGEN IN FLUORINATED ORGANIC COMPOUNDS BY THE FLASH COMBUSTION METHOD

No.	Compound	Carbon and hydrogen percentage	Average[a] carbon and hydrogen percentage ($\dot{x}$, %)	Relative accuracy of the mean	Standard deviation (S)	Confidence limits $\dot{x} \pm ts/n^{1/2}$[b] $t = 0.95$
1	4-Fluorobenzoic acid	60.01	59.95	−0.06	0.271	59.95 ± 0.20
		3.59	3.45	−0.14	0.143	3.45 ± 0.10
2	Perfluoronaphthalene	44.14	43.34	−0.80	0.501	43.34 ± 0.13
		—	—	—	—	—
3	1,3-Dimethyl-2,4-dioxo-6-fluoroquinazoline	57.70	57.81	+0.11	0.101	57.81 ± 0.12
		4.33	4.36	+0.30	0.146	4.36 ± 0.17
4	1,3-Dimethyl-2,4-dioxo-7-fluoroquinazoline	57.70	57.92	+0.22	0.242	57.92 ± 0.28
		4.33	4.46	+0.13	0.111	4.46 ± 0.13
5	1,3-Dimethyl-2-oxo-6-fluoro-4-thionoquinazoline	53.56	53.61	+0.05	0.099	53.61 ± 0.12
		4.05	4.24	+0.19	0.066	4.24 ± 0.08
6	1,3-Dimethyl-2-oxo-7-fluoro-4-thionoquinazoline	53.56	53.58	+0.02	0.110	53.58 ± 0.13
		4.05	4.29	+0.24	0.073	4.29 ± 0.09
7	2,4-Dioxo-7-fluoro-(1H,3H)-quinazoline	53.34	53.46	+0.12	0.175	53.46 ± 0.21
		2.79	2.78	−0.01	0.206	2.78 ± 0.24
8	2,4-Dithiono-7-fluoro-(1H,3H)quinazoline	45.27	45.47	+0.20	0.059	45.47 ± 0.07
		2.37	2.33	−0.04	0.179	2.33 ± 0.21
9	1,4-Dimethyl-2,5-dioxo-6-fluoroquinoxaline	57.69	57.73	+0.04	0.177	57.73 ± 0.21
		4.36	4.26	−0.10	0.060	4.26 ± 0.07

10	1,4-Dimethyl-2,3-dithiono-6-fluoro-quinoxaline	49.89 3.77	50.21 3.77	+0.32 0.00	0.022 0.131	50.21 ± 0.03 3.77 ± 0.15
11	2,3-Dichloro-5-fluoro-quinoxaline	44.27 1.39	44.15 1.51	−0.12 +0.12	0.157 0.094	44.15 ± 0.18 1.51 ± 0.11
12	2,3-Dichloro-6-fluoroquin-oxaline	44.27 1.39	44.33 1.36	+0.06 −0.03	0.121 0.174	44.33 ± 0.14 1.36 ± 0.20
13	2,3-Dimethoxy-6-fluoro-quinoxaline	57.69 4.36	57.79 4.32	+0.10 −0.04	0.385 0.067	57.79 ± 0.45 4.32 ± 0.08
14	2,3-Diethoxy-6-fluoroquinox-aline	61.01 5.55	61.24 5.44	+0.23 −0.11	0.130 0.083	61.24 ± 0.15 5.44 ± 0.10
15	2,-Trifluoromethyl-1,5,8-trimethyl-(1H)imidazo-(4,5-g)-6,7-dioxo(5H,8H)-quinoxaline	50.00 3.55	50.10 3.59	+0.10 +0.04	0.197 0.103	50.10 ± 0.23 3.59 ± 0.12
16	2-Trifluoromethyl(1H)imidazo-(4,5-g)6,7-dioxo(5H,8H)-quinoxaline	42.64 4.35	42.53 4.23	−0.11 −0.12	0.139 0.215	42.53 ± 0.16 4.23 ± 0.25
17	2-Trifluoromethyl-1,6,7-trichloroimidazo(4,5-g)-quinoxaline	35.17 0.59	35.09 0.78	−0.08 +0.19	0.216 0.067	35.09 ± 0.25 0.78 ± 0.08

[a] Average of four determinations.

[b] *n* is the number of determinations.

straight empty tube (*12*), rapid empty tube of Belcher–Ingram (*2*), and flash combustion methods (*10*).

As is evident from these results, quantitative carbon and hydrogen recoveries are obtained by the four different combustion procedures for the organofluorine compounds analyzed. However, low carbon recoveries were always obtained in the analysis of perfluoronaphthalene by the flash combustion method (cf. Table 4). This is presumably due to incomplete decomposition of this termally stable fluorocarbon by this combustion technique. Other compounds gave more or less good results by the flash combustion method (cf. Table 4).

As is clear from the results presented in Tables 1–4, some compounds contained nitrogen and/or sulfur beside C, H, O, and F. No interference was observed from these additional elements, suggesting that all of the other cumbustion products were retained by the additional reagents.

It is worth mentioning that one tube filled with a 2.5-g silica gel–thorium nitrate mixture had a useful life of about 40–60 analyses depending on the fluorine content.

Trials for the simultaneous gravimetric measurement of fluorine (assuming that all fluorine in the organic compound is completely converted to silicon tetrafluoride) with carbon and hydrogen in organofluorine compounds were unsuccessful. Although correct carbon and hydrogen values were obtained, the fluorine figures were always low by about 1–2%. The slightly low fluorine figures are most reasonably attributed to the presence of small amounts of other fluorine decomposition products. That is the trace presence of hydrogen fluoride and perhaps free fluorine, which is completely absorbed by the silica gel absorption tube, required a higher conversion factor for calculation. Actually, these results offered true evidence for the presence of silicon tetrafluoride as a major product in the decomposition of fluorinated organic compounds by any of the four combustion methods described.

In conclusion, the external absorption tube packed with silica gel–thorium nitrate mixture and kept at room temperature is recommended for the elimination of fluorine interference in carbon and hydrogen determination of fluorinated organic compounds. A further advantage of the proposed reagent is that it proved quite suitable for use with any of the four methods for analyses of aliphatic or aromatic compounds whether partially or highly fluorinated.

SUMMARY

A simple method is described for the elimination of the interferences of fluorine in carbon and hydrogen determination of fluorinated organic compounds. Samples are burnt in a rapid flow of oxygen using the cobalto–cobaltic oxide, rapid straight empty tube, rapid empty tube of Belcher–Ingram, and flash combustion methods.

The combustion products are passed through Anhydrone, silica gel–thorium nitrate, and

soda asbestos absorption tubes, respectively. Carbon and hydrogen are determined gravimetrically. Acceptable results are generally obtained for a wide range of partially and highly fluorinated organic compounds.

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Spectrophotometric Determination of Phosphorus in Steel Using Phosphoantimonyl Molybdate Complex

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INTRODUCTION

Small quantities of phosphorus are usually determined spectrophotometrically by the "heteropoly blue" method both in the reduced and unreduced forms. The method has not been applied to the determination of phosphorus in steel because of the interference of ferric and nitrate ions and suitable modifications in the method have to be made to overcome such interferences. Hague *et al.* (*2*) have eliminated this interference by reducing ferric ions to ferrous with sodium sulfite and removing nitrate ions by fuming with perchloric acid. Similarly in another method, use of fluoride ions has been recommended to overcome the interference due to ferric ions (*3*). Hydrazine sulfate has been used as the reducing agent for the formation of the heteropoly blue complex. A number of other reducing agents have also been used for the formation of the complex. Of these stannous chloride (*7*), 1-amino 2-naphthol 4-sulfonic acid (*1*), and ascorbic acid (*4*) have been commonly applied. Murphey and Riley (*6*) have introduced antimony and ascorbic acid as a mixed reducing agent whereby the reduction is carried out at room temperature. The formation of the heteropoly blue complex takes place rapidly thus eliminating the problems associated with the long period of color development which takes place in the absence of antimony. The complex has been found to contain phosphorus and antimony in 1:2 ratio. The incorporation of antimony in the complex favors selective reduction of phosphoantimonyl molybdate without any effect on excess molybdate present in the system. The reproducibility of the determination is quite good and the method has been suggested in the standard specification for determination of phosphorus in water and waste water (*5*, *8*).

This work was undertaken to apply the use of the mixed reducing agent in the determination of phosphorus in steel in view of its promising nature. The method is comparatively fast and quite reproducible which is an essential requirement in quality control laboratories of steel industry.

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EXPERIMENTAL

Apparatus and Reagents

Absorbance measurements are made in 1.0-cm matched cells with a Hilger uv spectrophotometer, Model H.700. A reagent blank is used in the reference cell. However, in most of the cases the reagent blank does not differ in absorbance from pure solvent. pH measurements are carried out on Cambridge Model 105 pH meter from J. N. Marshall Company. Higher acidity of the solution is controlled with 1:3 perchloric acid. The antimony content of the complex is determined on atomic absorption spectrophotometer Pye Unicam Model SP 1900. The following reagents and solutions are used:

Ammonium molybdate. Dissolve 40 g of A.R. ammonium molybdate in water and dilute to 1 liter. Store the solution in Pyrex glass bottle.

Ascorbic acid (0.1 *M*). Dissolve 1.32 g of ascorbic acid in 75 ml of water. The solution has to be prepared fresh daily.

Potassium antimonyl tartarate. Dissolve 0.2743 g of potassium antimonyl tartarate in distilled water and dilute to 100 ml.

Acids. Analar grade sulfuric, nitric, perchloric, and hydrofluoric acid were used, and acidity adjustments are made with 5 *N* sulfuric acid.

Standard phosphorus solution. Potassium dihydrogen phosphate

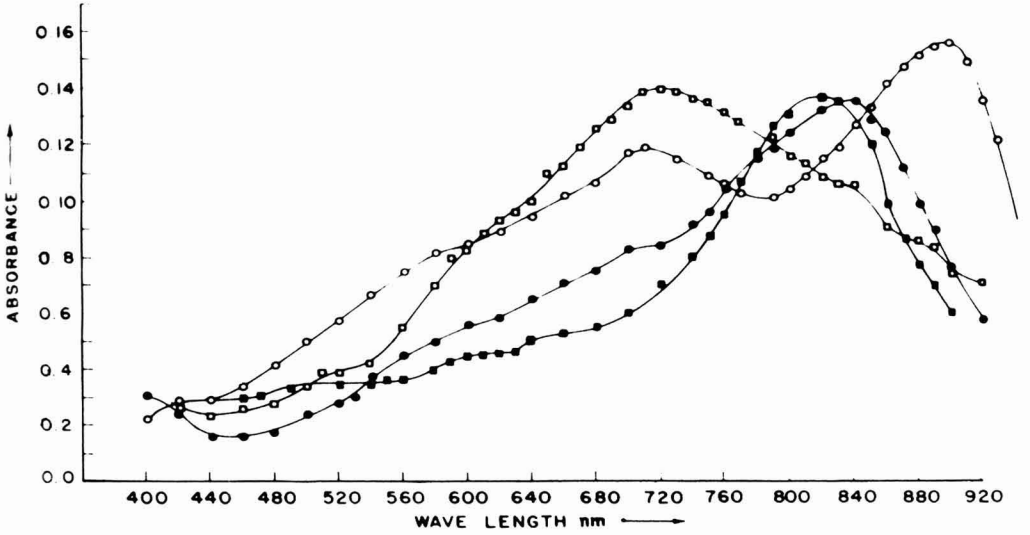

FIG. 1. Absorption spectra of heteromolybdenum blue with different reducing agents in water (phosphorus concentration 0.2 ppm). Reducing agents: (○) ascorbic acid + antimony; (●) ascorbic acid; (■) hydrazine sulfate; (□) stannous chloride.

equivalent to 25 mg/liter of phosphorus is prepared in water and subsequent dilutions are made from this solution.

Sodium sulfite solution. A 10% solution in water is used after filtration.

Mixed reagent. Mix thoroughly 125 ml of 5 *N* sulfuric acid and 37.5 ml of ammonium molybdate solution. To this solution add 75 ml of ascorbic acid and 12.5 ml of potassium antimonyl tartarate solution. Mix thoroughly. This reagent should be prepared fresh before use.

Absorption spectra of phosphomolybdate using different reducing agents viz. (a) hydrazine sulfate, (b) stannous chloride, (c) ascorbic acid, and (d) antimony and ascorbic acid mixed reagent are drawn in water (Fig. 1) and steel (Fig. 2). A comparison of these spectra indicates that the maxima in the case of ascorbic acid and antimony occur at 882 nm. In other cases the maxima appear at lower wavelengths. This indicates that antimony is a component of phosphomolybdate complex, which has been confirmed by elemental analysis. In the case of steel, the stannous chloride method could not be applied due to the instability of color on account of the narrow range of acidity and reagent concentration.

In order to determine the atomic ratio of antimony to phosphorus in the complex, the complex formed from 10 μg of phosphate is quantitatively extracted with methyl isobutyl ketone. The antimony content in the extract is determined by atomic absorption spectrophotometry. The ratio of antimony to phosphorus was found to be 1:2.

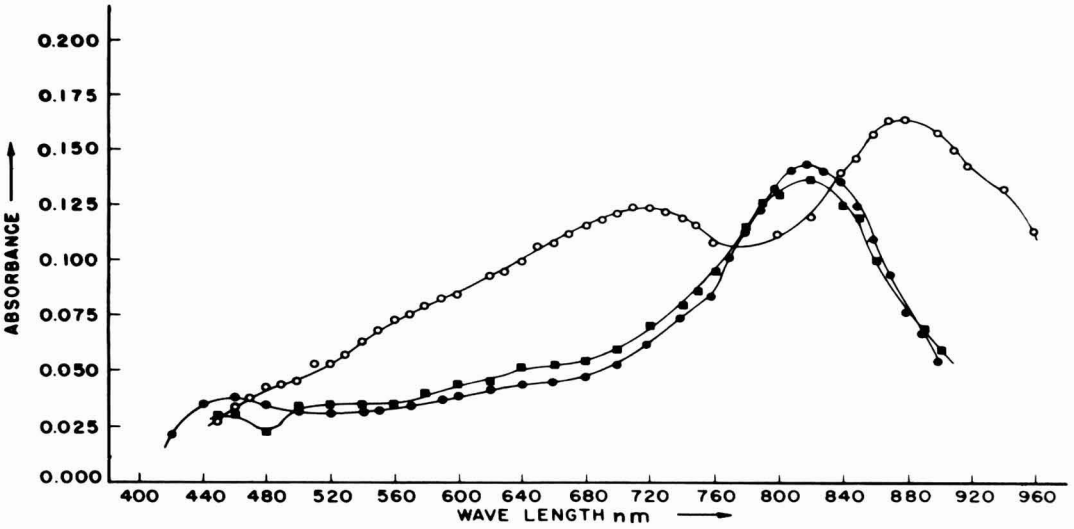

FIG. 2. Absorption spectra of heteromolybdenum blue with different reducing agents in steel (phosphorus concentration 0.2 ppm (10 μg/50 ml)). (○) Ascorbic acid + antimony; (●) ascorbic acid; (■) hydrazine sulfate.

STANDARD PROCEDURE

Carbon Steel

Take 20–50 mg of sample in a 50-ml Erlenmeyer flask and add 5 ml of dilute nitric acid (1:1). Heat until the sample is in solution, add a few drops of hydrofluoric acid in the case of high-silica steel. Heat to just dryness. Two to three milliliters of perchloric acid (60%) is then added and the solution evaporated to fuming stage. The solution should be heated gently on a hot plate until perchloric acid starts refluxing on the walls of the flask and thick white fumes start coming out. This indicates the complete removal of nitric acid and ensures the conversion of all the phosphorus to orthophosphates. In case more than 0.05% arsenic is present 5 ml of hydrobromic acid is added and the solution is brought to fuming stage. Thus all the arsenic is volatilized as arsenic pentabromide and an excess of hydrobromic acid is also removed. To the solution add 10 ml of water and 6–10 ml of sodium sulfite solution and heat to boiling for 1 to 2 min. Complete removal of sodium sulfite is indicated by the absence of brown color in the solution. If the solution is turbid it is filtered. The filtrate is diluted to 40 ml with water and 8 ml of mixed reagent is added to it. The volume is made up to 50 ml and absorption spectra are measured at 880 nm after 10 min. Phosphorus content is calculated from the standard calibration curve drawn for standard steels.

It has been found that for the complete formation of "phosphomolybdenum blue" different conditions should be carefully controlled viz. (a) acidity, (b) molybdate concentration, (c) time necessary for completion of reaction, (d) hydrogen ion concentration, (e) presence of interfering ions etc.

Acidity

Variation of acidity from 0.1 to 2 *N* with respect to perchloric acid has shown that the complex is stable between acidity .2 *N* to 2 *N*.

Acidity	0.2 *N*	0.5 *N*	1 *N*	1.2 *N*	2 *N*
Absorbance for 0.2 ppm	0.164	0.164	0.164	0.162	0.160

However, acidity corresponding to 1 *N* is chosen as this would eliminate the interference of silicon and ferric ions if present in the solution.

Molybdate Concentration

Two to three milliliters of a 4% solution of ammonium molybdate is found to be suitable excess for a final volume of 50 ml.

Color Development

Ten minutes time is allowed for the formation of the complex. No heating is necessary. No change in the intensity is found for at least 24 hr.

Absorption Curves

The absorption spectra for various concentrations of phosphorus in steel are shown in Fig. 3. There is an absorption maximum at 880 nm with an inflection point at 710 nm.

Calibration Curve

A calibration curve is drawn using B.C. steels containing different concentrations of phosphorus ranging from 0.01 to 0.08% and following the same procedure as indicated above. It is clear from Fig. 4 that Beer's Law is obeyed up to a phosphate concentration of 1 μg/ml.

Effect of Other Ions

Copper, nickel, chromium, and vanadium do not interfere with the development of phosphomolybdenum blue color but give colored ions which may interfere resulting in higher absorbance unless due compensation is made. It has been found that as much as 35% nickel, 15% copper, 5% vanadium, and 6% chromium may be present without causing any appreciable error in determination of phosphorus. Niobium and tungsten are removed after precipitating them as acids. No phosphorus is lost if carbides are decomposed with prolonged heating with nitric/perchloric acid. The amount of iron in the final solution should not exceed 30 mg/50 ml. In case of higher concentration of iron, the solution becomes turbid after 30 min.

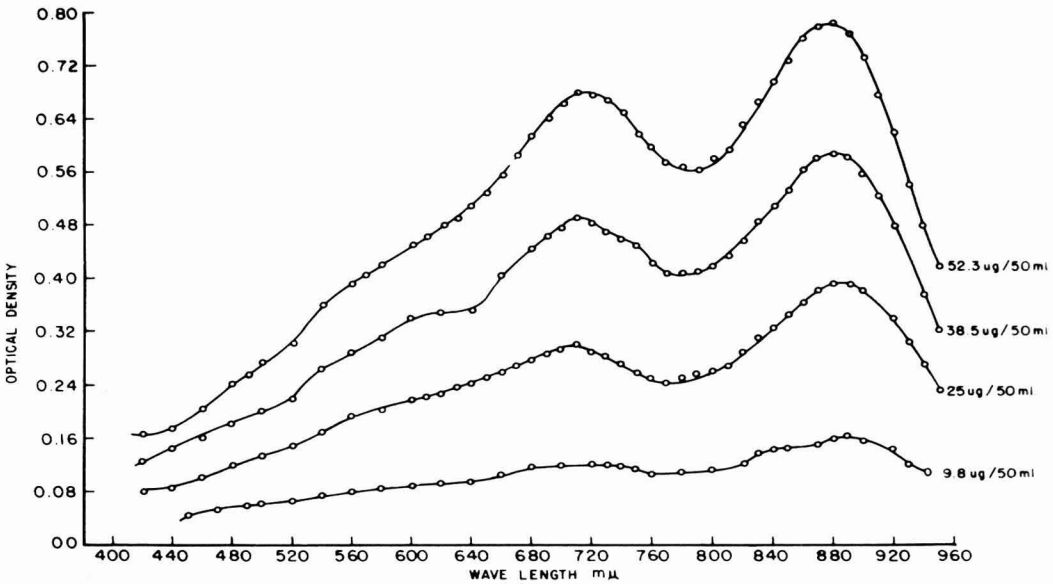

FIG. 3. Absorption spectra for various concentrations of phosphorus in steel using ascorbic acid and antimony as reducing agents.

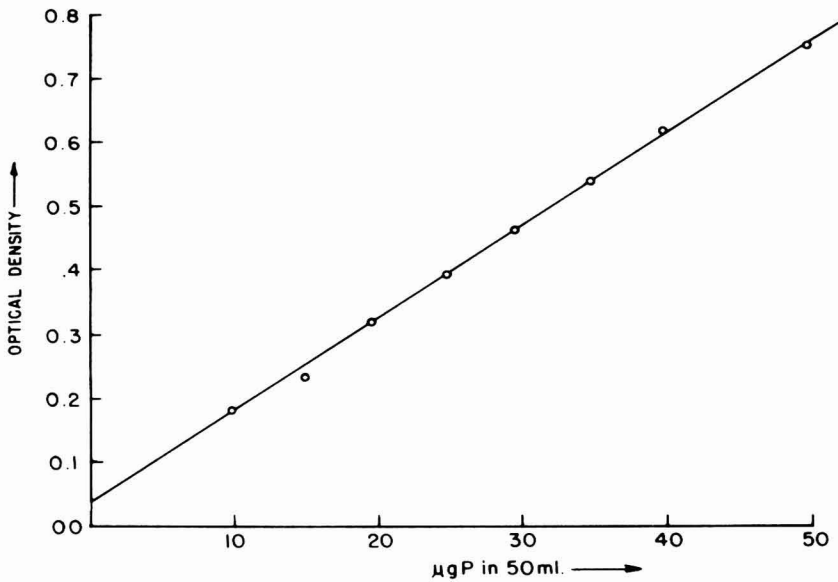

FIG. 4. Calibration curve for phosphorus in steel.

Determination of Phosphorus in Alloy Steel (Table 1)

For steels containing less than 2% chromium, 35% nickel, 5% vanadium, proceed as in the case of carbon steel. For high-chromium steel add 3 ml diluted 1:1 hydrochloric acid. If the chromium content is more than 2%, a correction for the absorption of the blank solution may be necessary. The blank solution is prepared through all the steps in the determination of steel except that 5 ml of 5 *N* sulfuric acid is substituted for ammonium molybdate solution.

Determination of Phorphorus in Cast Iron

Take 20–50 mg of the sample in a 100-ml Erlenmeyer flask and dissolve the sample in 10–15 ml (1:1) nitric acid (sp gr 1.2) by heating gently. Add a few drops of hydrofluoric acid to drive off silica in the case of high-silica samples. Take the sample solution to almost dryness and heat with perchloric acid (1–3 ml) and digest gently until thick white fumes of perchloric acid start coming. Continue heating for 1–2 min. If the arsenic content of the sample is more than 0.05%, hydrobromic acid treatment must be done before reduction with sodium sulfite. Dilute the solution and filter off graphitic carbon and proceed as in the case of carbon steel.

DISCUSSION

The incorporation of antimony in ascorbic acid facilitates the reduction of heteropoly blue complex. The antimony atom is found to be a con-

TABLE 1

VALUES OF PHOSPHORUS FOR DIFFERENT SAMPLES OBTAINED BY THE PRESENT METHOD IS MENTIONED BELOW

	Sample	Type of steel	Certified value for phosphorus	Phosphorus found (%)	Deviation
1	BCS No. 232	Carbon	0.076	0.074	−0.002
2	BCS No. 240	Carbon	0.028	0.027	−0.001
3	BCS No. 163	Carbon	0.049	0.048	−0.001
4	BCS No. 235	Carbon	0.021	0.020	−0.001
5	BCS No. 224	Carbon	0.012	0.012	Nil
6	BCN No. 225	Carbon	0.021	0.022	+0.001
			Percentage of phosphorus by volumetric method		
Cast iron					
7			0.024	0.025	+0.001
8			0.053	0.051	−0.002
9			0.019	0.020	+0.001
10			0.025	0.025	Nil
11			0.059	0.057	−0.002
12			0.071	0.071	Nil

stituent of the phosphomolybdate complex and is present in 1:2 molar ratio. The selective reduction of phosphomolybdate complex is carried out at room temperature without affecting the ammonium molybdate which is present in excess in the solution thus eliminating the uncertainties of the effect of diverse ions associated with heating of the solution which is necessary in the case of ascorbic acid and other reducing agents. It was, however, found that iron in the ferric form interferes in the formation of phosphoantimonyl molybdate complex. The iron present in the solution has to be reduced to ferrous form by using strong sodium bisulfite solution. The ferrous ions could be tolerated up to 30 mg in 50 ml of solution. In case of greater amounts, the solutions become turbid within 20 min. This does not offer a serious disadvantage to the determination of phosphorus in steel as the high sensitivity of the method permits considerable dilution of the sample. The sensitivity of the method in terms of Sandell's sensitivity was calculated to be 0.0012 and the molar extinction coefficient was found to be 25,670. This implies that the recommended procedure enables us to determine as low as 0.0002% phosphorus in steel.

SUMMARY

An improved method for the spectrophotometric determination of phosphorus in steel has been described. Reduction of the phosphomolybdate complex with ascorbic acid and antimony has been found to be advantageous over other methods. No heating is necessary for the formation of the complex and it is stable for more than 24 hr. The "molybdenum blue" formed shows maximum absorption at 882 nm and the molar extinction coefficient is 25,670. The method has been found useful for the determination of microquantities of phosphorus in steel.

ACKNOWLEDGMENTS

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MICROCHEMICAL JOURNAL 26, 40–44 (1981)

Microdetermination of Thiocyanate and Selenocyanate by Densitometry of Thin-Layer Chromatograms

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INTRODUCTION

Separation and determination of thiocyanates and selenocyanates became increasingly interesting in recent years because of the environmental pollution problems.

A number of workers (*1, 2*) have studied the separation of cyanide, thiocyanate, and selenocyanate by paper and thin-layer chromatography but no accurate determination of these anions, after their separation, has been reported.

Because of the simplicity and the relative accuracy of the densitometric methods, for the quantitative evaluation of compounds separated by TLC, we decided to study this problem. The present work reports the results of in situ measurements by light absorption and reflection of thiocyanates and selenocyanates, separated on a TLC in the presence of cyanates and cyanides.

EXPERIMENTAL

Reagents

The potassium salts of thiocyanate, selenocyanate, cyanate, and cyanide were from E. Merck, Darmstadt. Standard solutions of these salts were prepared by dissolving the appropriate quantity in distilled water. The standardization of the above solutions was performed as follows: The thiocyanates were titrated with a silver nitrate solution according to the Volhard method, the selenocyanates were titrated also by a silver nitrate solution conductometrically and the cyanides were titrated according to the Liebig–Deniges method.

Apparatus

The measurements of light absorption and reflection were performed with a Universal Densitometer, Vitatron type TLD 100, automatic

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"flying-spot" scanner. Peak areas, automatically integrated by the recorder, were expressed in integrator units.

Thin-Layer Chromatography

TLC plates (0.25 mm thick) were prepared as follows: 25 g of silica gel G were mixed by constant stirring with 60 ml of distilled water. The slurry obtained was spread over glass plates, 20 × 20 cm, in the usual way, allowed to dry for 12 hr. The plates were stored over silica gel without activating.

On the starting point of the plates, 1–10 μl of each solution was spotted gradually by means of a Hamilton microsyringe. A known volume (i.e., 3.00 μl) of a solution which contained the mixture of the tested anions was also spotted.

The plates were developed in an equilibrated chamber, filled with a solvent system of 1-butanol:acetone:water (45:45:10), until the solvent front ascended about 7 cm from the spotting line. (The composition of the solvent system was found experimentally.) After the visualization of the developed spots by spraying with a solution of 5×10^{-2} M ammonium ferric sulfate in water, the plates were redeveloped in the same chamber, until the solvent front ascended about 14 cm from the spotting line.

Alternatively we can spread the half of the area of a plate (upper part) so when the front of the solvent will reach the spread area selenocyanates will be retained as insoluble complex, while the soluble complex of iron–thiocyanates will proceed.

Quantitative Evaluation

The plates were scanned and light absorption and reflection was measured for the colored compounds thiocyanate and selenocyanate. The

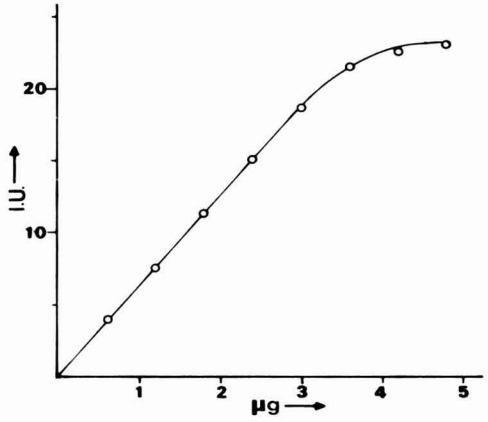

FIG. 1. Reflectance calibration curve for thiocyanate.

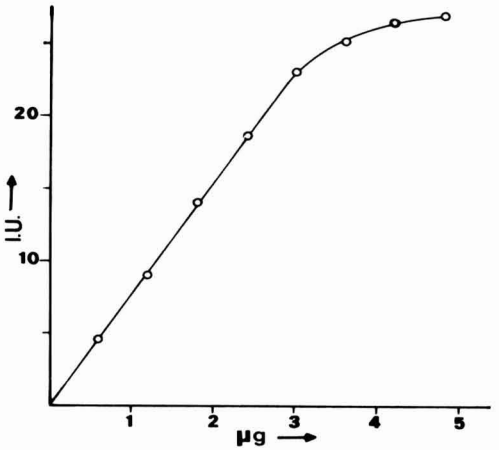

FIG. 2. Transmittance calibration curve for thiocyanate.

integrator units for each compound were refered to the corresponding calibration curves, constructed for each compound in the same way.

RESULTS AND DISCUSSION

For the TLC separation of the three anions a systematic study with several solvent systems was performed first. A number of them, including several suitable solvents for paper chromatography were generally unsatisfactory for TLC separation (*3*). The most suitable solvent system proved to be 1-butanol:acetone:water (45:45:10). This system enables satisfactory separation of the thiocyanate and selenocyanate in mixture with cyanide after spraying the plate with a solution of ammonium ferric sulfate. The chromatographic spots are compact and suitable for densitometric registration.

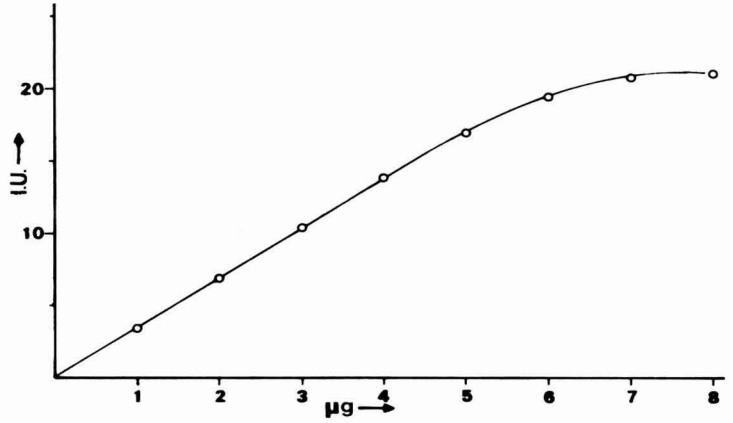

FIG. 3. Reflectance calibration curve for selenocyanate.

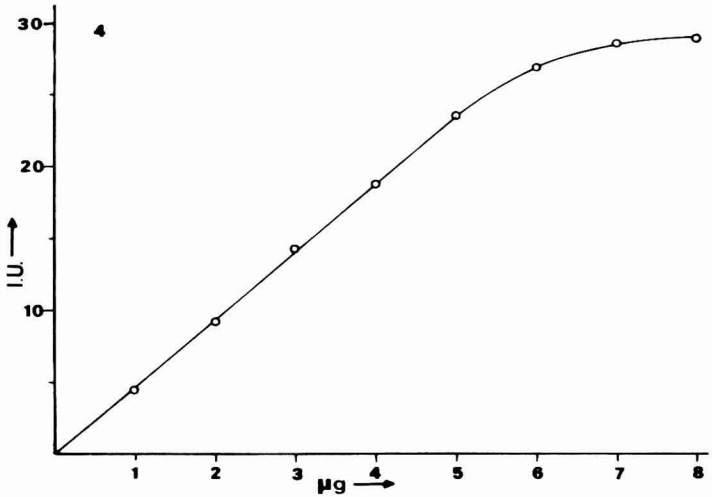

FIG. 4. Transmittance calibration curve for selenocyanate.

The colored spots of selenocyanate and thiocyanate were scanned for light absorption and reflection. The amount of each compound in the mixture was calculated from calibration curves constructed in the following way to increase the precision. Various amounts of the compounds studied, were spotted on a plate. The plate was then developed in the same way as the unknown sample, the spots were scanned and the integrator units were plotted against known amounts of the compound.

The sensitivity of the reflectance measurements was found to be lower than those of transmittance (*4*). The detection limits of these compounds, corresponding to a signal-to-noise ratio of 2:1 are in the range of 0.2 μg for thiocyanate and 0.01 μg for selenocyanate.

Various attempts to obtain densitometric registration of the colorless, nonfluorescent cyanide spots were unsuccessful. A blue color, obtained by spraying these spots with two solutions of ferrous and ferric ions were

TABLE 1
DENSITOMETRIC MICRODETERMINATION OF THIOCYANATE AND SELENOCYANATE IN PRESENCE OF CYANIDE AFTER SEPARATION BY TLC

Compound	R_f (×100)	Added (μg)	Found (μg)[a]	
			R	T
Cyanide	0	2.50	—	—
Thiocyanate	98	1.80	1.77 ± 0.04	1.79 ± 0.02
Selenocyanate	50	3.00	3.02 ± 0.02	2.98 ± 0.01

[a] Average value of 12 determinations. R, Reflectance; T, transmittance.

not suitable for quantitative evaluation. The presence of cyanates do not interfere in this separation and determination because they do not appear after visualization of the plates. Most probably they are hydrolized during the development process.

The results from 12 independent determinations performed on separate thin-layer plates with an artificial mixture of the three compounds are shown in Table 1. The precision of the densitometric methods is satisfactory, as it is shown in Table 1. In Figs. 1 and 3 the reflectance calibration curves for thiocyanate and selenocyanate are shown. In Figs. 2 and 4 the transmittance calibration curves for thiocyanate and selenocyanate are also shown.

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MICROCHEMICAL JOURNAL 26, 45–50 (1981)

Spectrophotometric Determination of Aluminium and Gallium with Pyrogallol Red and Cetyltrimethylammonium Ions

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INTRODUCTION

The sensitivity of color reactions between metal ions and some metallochromic indicators, used as reagents, has recently been greatly increased by the sensitizing action of some cationic surfactans, e.g., cetyltrimethylammonium ion (CTA) cetylpyridinium ion (CP), cetyldimethylbenzylammonium ion (CDBA), or tetradecyldimethylbenzylammonium ion (zephiramine). In the presence of mentioned quaternary bases a considerable bathochromic shifts are observed as well as significant increase of absorbance being the basis of much more sensitive spectrophotometric methods than those in the absence of such bases. Consequently, some very sensitive spectrophotometric methods for determining traces of metals have been developed (*1*).

The reagents which are commonly applied in conjunction with cationic surfactants are triphenylmethane dyestuffs. Beside triphenylmethane dyes in spectrophotometric determination of traces of metals other chelating dyestuffs (xanthene dyes) were used (*5, 6, 10*). Sensitivity of the latter methods is lower than that of triphenylmethane dyes. Pyrogallol red has been used for spectrophotometric determination of aluminium (*8*) and gallium (*7*); the aluminium complex has been measured at 525 nm, whereas the wavelength of maximal absorption for the gallium complexes is at 530 nm. Beer's law is obeyed up to 0.5 μg of Al/ml and 4 μg of Ga/ml.

This paper describes a sensitive method for the determination of aluminium and gallium on the basis of the ternary color systems formed with pyrogallol red in the presence of cetyltrimethylammonium ions.

EXPERIMENTAL

Reagents and Apparatus

Aluminium stock solution: 1 mg Al/ml. Dissolve 8.5900 g of aluminium sodium sulfate in water and 2 ml of concentrated sulfuric acid, and dilute with water to 500 ml. Dilute as required with 0.02 *M* sulfuric acid.

Gallium stock solution: 1 mg Ga/ml. Dissolve 0.1000 g of the metal in 10 ml of 7 *M* hydrochloric acid and dilute the solution with 1 *M* hydrochloric

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acid to 100 ml. Dilute further with 1 *M* hydrochloric acid as required.

Pyrogallol red, sodium salt (PR): 4.8 × 10⁻⁴ M. Dissolve 50 mg of the reagent in 125 ml of ethanol and dilute with water to 250 ml.

Cetyltrimethylammonium bromide (CTA): 2.6 × 10⁻³ M. Dissolve 0.2500 g of the reagent in water and dilute with water to 250 ml.

Buffer solution (pH 6.9). An aqueous 10% hexamine solution, adjust the pH to 6.9 with dilute hydrochloric acid.

Apparatus. Spectrophotometer Varian Techtron Model 635, 10-mm cells, N-512 pH meter (Polish).

Procedure

Dilute a sample of test solution, containing 2.5 to 10 μg of aluminium or gallium to about 10 ml in a beaker. Add 2 ml of PR and 3 ml of CTA, adjust the pH to approx. 6.9 with ammonia. Transfer this solution to a 25-ml standard flask, add 5 ml of buffer solution, fill with water to the mark, and mix. Measure the absorbance at 615 nm for gallium or after 15 min for aluminium at 610 nm against a reagent blank.

RESULTS AND DISCUSSION

Absorption Spectra

To study the spectral properties of the colored complexes, the absorption spectra of the PR–CTA systems with aluminium and gallium as well as these ones without CTA were recorded (Fig. 1). Aluminium and gallium form an orange–red complex with PR in the acid range. Maximal

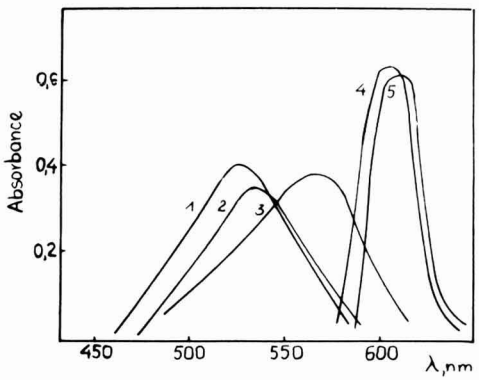

FIG. 1. Absorption spectra of: pH 5.0 (1) Al–PR complex—$C_{Al} = 1.5 \times 10^{-5}$ *M*, $C_{PR} = 4 \times 10^{-5}$ *M*; (2) Ga–PR complex—$C_{Ga} = 3 \times 10^{-5}$ *M*, $C_{PR} = 4 \times 10^{-5}$ *M* measured against a blank; pH 6.9: (3) pyrogallol red—$C_{PR} = 2 \times 10^{-5}$ *M* measured against water; (4) Al–PR–CTA complex—$C_{Al} = 1.5 \times 10^{-5}$ *M*, $C_{PR} = 4 \times 10^{-5}$ *M*, $C_{CTA} = 3 \times 10^{-4}$ *M*; (5) Ga–PR–CTA complex—$C_{Ga} = 5.7 \times 10^{-6}$ *M*, $C_{PR} = 4 \times 10^{-5}$ *M*, $C_{CTA} = 3 \times 10^{-4}$ *M* measured against a blank.

absorbance for the aluminium complex occurred at pH 4.8–5.2, and that for the gallium complex at 4.2–5.5. In the presence of CTA a bathochromic shift of the absorption maximum from 525 to 610 nm for Al, and 530 to 615 nm for Ga takes place.

Effect of pH

The absorbance and optimum wavelength of the ternary complex (aluminium and gallium) changes with pH (4–8). This is illustrated by the absorption spectra of the Al–PR–CTA complex shown in Fig. 2. Further measurements were carried out at 610 nm for aluminium and at 615 nm for gallium. At these wavelengths more detailed studies of the dependence of the absorption of the ternary complex on the pH were carried out. The obtained results are shown in Fig. 3, in which it is shown that maximum intensity can be obtained in the pH range 6.8 to 7.2 for the aluminium complex and for the gallium complex. In order to maintain a constant pH value of the color solutions hexamine buffer was used. Hexamine does not affect the absorbance of the ternary complex.

Effect of PR and CTA Concentrations

The dependence of the absorbance of the ternary complex on the PR concentration was examined of CTA equal to 3×10^{-4} *M*. The concentration of the reagent (PR) varied in the range 1 to 8×10^{-5} *M*. The maximum absorbance was observed when the molar excess of PR over aluminium was somewhat higher than twofold and for gallium above sevenfold molar amounts of reagent.

The effect of changes in the concentration of CTA on the absorbance of

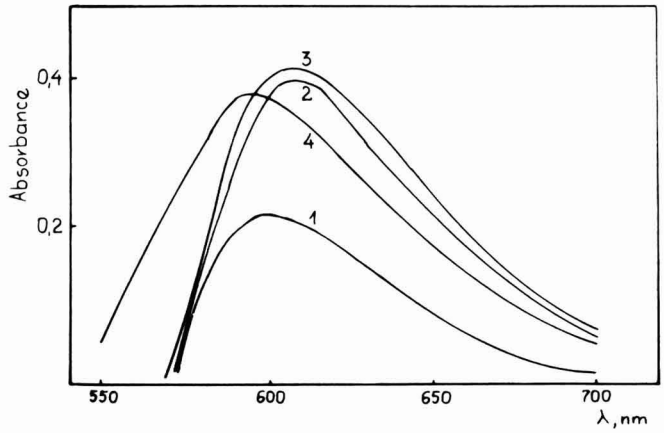

FIG. 2. Absorption spectra of aluminium complex with PR and CTA in dependence on pH, $C_{Al} = 0.2$ μg/ml, $C_{PR} = 4 \times 10^{-5}$ *M*, $C_{CTA} = 3 \times 10^{-4}$ *M*; (1) pH 4, (2) pH 5, (3) pH 7, (4) pH 7.8.

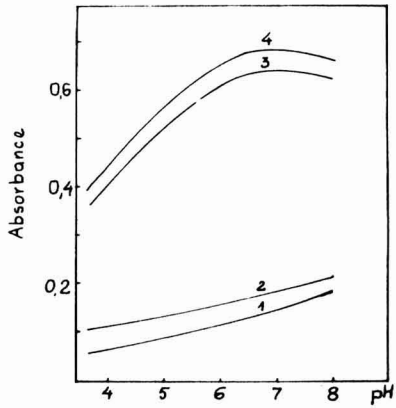

FIG. 3. Effect of pH at 610 and 615 nm, $C_{Al} = C_{Ga} = 0.4$ μg/ml, $C_{PR} = 4 \times 10^{-5}$ *M*, $C_{CTA} = 3 \times 10^{-4}$ *M* of: (1) PR + CTA at 615 nm, (2) PR + CTA at 610 nm measured against water: (3) Ga–PR–CTA at 615 nm, (4) Al–PR–CTA at 610 nm measured against blank.

the ternary complex was determined by measuring the absorbance. The molar ratio of metal ions to PR was constant and equal to 1:2 for aluminium and 1:7 for gallium, while the molar excess of CTA ions was variable. At low CTA concentration (twofold ratio to metal) of the ternary complexes (λ_{max}, 600 nm) is formed. As the molar excess of CTA increases to 15 to 50-fold a shift of the absorption maximum to 610 nm for aluminium and 615 nm for gallium is observed, while the absorbance is maximum and constant. Further increase in the molar excess of CTA to 60- to 100-fold results in a shift of the absorption maximum toward shorter wavelengths (Al λ_{max}, 595 nm; Ga λ_{max}, 607 nm) while the absorbance decrease. On the basis of the obtained results it can be supposed that various complexes are formed in the ternary system as the CTA concentration increases. These complexes show different absorption spectra and obviously different numbers of CTA molecules bounded. The absorption spectra of the Al–PR–CTA complex for various CTA concentrations are shown in Fig. 4. A 20-fold molar excess of CTA with respect to aluminium and 50-fold to gallium ensure the formation of the ternary complex with a maximum absorbance at λ_{max}, 610 nm for Al and at λ_{max}, 615 nm for Ga.

Beer's Law

Calibration graphs were prepared by the procedure. Beer's law was obeyed over the range 0.1–0.4 μg/ml of aluminium or gallium. A molar absorptivity of 4.8×10^4 liter mol^{-1} cm^{-1} was obtained for the aluminium complex, and 1.0×10^5 liter mol^{-1} cm^{-1} for the gallium complex.

Effect of Foreign Ions

The influence of foreign ions on the determination of aluminium or gallium was ascertained by the standard procedure. The most commonly

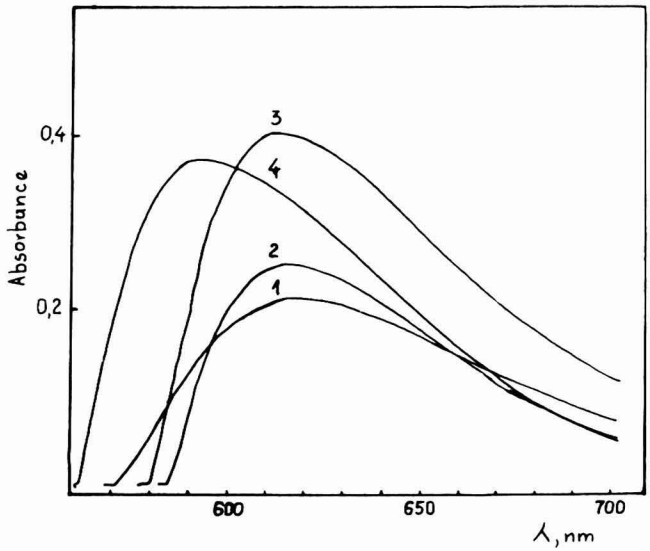

FIG. 4. Absorption spectra of the Al–PR–CTA complex in dependence on CTA concentration $C_{Al} = 7.4 \times 10^{-6}$ *M*, $C_{PR} = 4 \times 10^{-5}$ *M*; (1) 1.5×10^{-5} *M*, (2) 8×10^{-5} *M*, (3) 3×10^{-5} *M*, (4) 5.2×10^{-4} *M*.

encountered ions were added individually to a solution containing 10 μg of aluminium or gallium. These metal ions can be determined in the presence: aluminium: 500-fold weight excess, Mg, Tl, B; 100-fold, Mn, Zn; 50-fold, Te, Se, Pb; 20-fold, Pd, Pt, Cd; 10-fold, Bi, Ni, Re; 5-fold, Co, Hg, Ag, Cr; gallium: 500-fold weight excess, Mn, Cd, Mg, B, Tl, As; 100-fold, Se, Te; 70-fold, Co, Ni, Pb; 10-fold, Bi, Hg, Ag, Cr. Numerous metals interfere, e.g., Cu, Fe(III), Fe(II), Sb, V, Mo, W, Sn. EDTA also interferes. The presence of tartrates, citrates and acetates depress the absorbance. These interfering ions can be removed by preliminary extraction of these metals with cupferron (aluminium in the aqueous phase) (*2*, *4*, *9*); however, gallium can be separated by extraction of gallium from 7 *M* hydrochloric acid (containing ascorbic acid) with di-isopropyl ether (*2*, *3*).

Separation of Aluminium

The sample solution containing 10 μg of aluminium is adjusted with the concentration hydrochloric acid to 1–2 M. Add 1 ml of 5% cupferron solution and after 10 min shake with two portions of chloroform. The aqueous phase containing of aluminium adjust to pH 5–6 with ammonia and analyze for aluminium by procedure.

Separation of Gallium

To 5 ml of 7 *M* hydrochloric acid, containing 10 μg of gallium add 100 mg of ascorbic acid. After 15 min shake with two portions of di-iospropyl

ether. Wash the ether phase with 7 *M* hydrochloric acid and evaporate with 5 ml of 0.01 *M* sulfuric acid to approx. 2 ml. Dilute with 3 ml of water. The determination of gallium should be carried out according to the recommended procedure.

Complex Formation

Job's method of continuous variations have been used to establish the molar ratio of the aluminium complex and gallium complex in the presence of CTA. A Job plot shows the molar ratio of metal ions to PR is 1:2.

SUMMARY

Spectrophotometric methods are described for the determination of microgram amounts of aluminium and gallium based on the formation of a ternary complex between the metal, pyrogallol red, and cetyltrimethylammonium bromide. The complexes have absorbance maxima at 610 and 615 nm, respectively, with molar absorptivities of 4.8×10^4 (Al) and 1.0×10^5 liter mol^{-1} cm^{-1} (Ga). Numerous metals interfere. Gallium can be separated by extraction of gallium from 7 *M* hydrochloric acid with di-isopropyl ether. However, aluminium can be separated by extraction of interfering ions with cupferron.

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Determination of Aminophenol Isomers in Water Samples by Extraction and Thin-Layer Chromatography Densitometry

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INTRODUCTION

Aminophenol isomers are valuable organic intermediates in the preparation of dyes and photographic chemicals. These compounds are also the main metabolites of aniline both *in vivo* and *in vitro*. Various methods have been proposed for the determination of aminophenols (*1*, *2*, *5*). However, these methods are difficult for determining aminophenol isomers in a sample simultaneously. Only high-speed liquid chromatography (*3*, *4*) has been used for simultaneous quantitation of aminophenols in mixture.

In the present paper a method for simultaneous determination of aminophenol isomers in water samples is proposed. The aminophenols are first extracted into an organic solvent and then are separated on a TLC plate. The amount of each aminophenol is determined by direct densitometric measurement of the light absorption or reflection of the visualized TLC spots.

MATERIALS AND METHODS

Reagents

Standard solutions of o-, m-, and p-aminophenol. These solutions were prepared by dissolving the appropriate amounts (Merck, purified by sublimation before use) in distilled water.

Chromogenic reagent. 2,4,7-Trinitro-9-fluorenone (TNF), 0.5% (w/v) in acetone.

TLC plates. These plates (0.25-mm-thick layer) were prepared as follows: 20 g of silica gel G (Merck) were mixed by constant stirring with 40 ml of distilled water. The slurry obtained was spread over glass plates, 20 × 20 cm, in the usual way, allowed to dry for 30 min, and activated at 110°C for 1 hr; the plates were stored over silica gel.

All other reagents and solvents were analytical-grade reagents.

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Apparatus

Thin-layer chromatographic equipment. A developing tank (27 × 27 × 7.5 cm) with glass top.

Densitometer. The measurements of light absorption and reflection were performed with a Universal Densitometer, Vitatron type TLD 100 automatic "flying-spot" scanner. Peak areas, automatically integrated by recorder, were expressed in integrator units.

Procedure

Two hundred milliliters of water sample is transferred to a separatory funnel and 6 g of sodium chloride is added (in the case of seawater there is no need for NaCl). The pH is adjusted to 7–8 and 12 ml of chloroform–isoamylalcohol (1:2) is added. After 2 min of shaking, the two phases are separated. The volume of organic phase withdrawn after separation is 10 ml.

Ten microliters of the organic phase are spotted on a TLC plate. The plate is developed in the equilibrated tank, filled with a solvent system of toluene–ethanol–1-butanol (4:1:1) until the solvent front ascended about 15 cm from the start line. After development the chromogenic reagent is spread on the plate and the spots of each aminophenol are visualized. Then the plate is scanned in the densitometer using appropriate filters and light absorption or reflection for the colored spots are measured. The integrator units for each aminophenol were referred to the corresponding calibration curve.

Calibration curves for each aminophenol were constructed by plotting numbers of integrator units against known amounts of compound spotted on the TLC plate.

RESULTS AND DISCUSSION

A systematic study of the extraction and TLC separation of the three aminophenols was performed.

Various conditions (such as solvent systems, pH of the aqueous phase, salt effect) have been tested for the quantitative extraction of the aminophenol. Chloroform–isoamylalcohol mixture (1:2) showed good extraction efficiency. The pH of the aqueous phase must be in the range 7–8, where aminophenol species are in neutral form. The presence of sodium chloride in the aqueous phase increases the extraction of the aminophenols. In the presence of more than 3 g of NaCl per 100 ml the aminophenols are extracted quantitatively.

Various solvent systems have been tested for the TLC separation of the extracted aminophenols. The most suitable solvent system proved to be toluene–ethanol–1-butanol (4:1:1), which gives good separation of the

TABLE 1
DENSITOMETRIC DETERMINATION OF AMINOPHENOL ISOMERS IN SPIKED SEAWATER SAMPLES AFTER EXTRACTION AND TLC SEPARATION

Spiked seawater (200 ml)	Compound	Added (μg)	Found[a] (μg)
A	*o*–	2.0	2.07 ± 0.03
	m–	3.0	3.04 ± 0.05
	p–	1.5	1.44 ± 0.06
B	*o*–	5.0	4.95 ± 0.06
	m–	2.0	2.06 ± 0.04
	p–	4.0	4.08 ± 0.03
C	*o*–	8.0	8.09 ± 0.02
	m–	10.0	10.04 ± 0.06
	p–	3.0	2.98 ± 0.04

[a] Five determinations.

aminophenols. The R_f values of the three isomers under procedure described above are: 0.59 for *p*-aminophenol, 0.72 for *m*-aminophenol, and 0.82 for *o*-aminophenol.

The spots of the aminophenols are colorless and nearly nonfluorescent. These spots are visualized by spraying the chromatographic plate with a solution of 0.5% (w/v) 2,4,7-trinitro-9-fluorenone in acetone. The obtained colored spots are compact and suitable for densitometric measurements. These spots were scanned for light absorption and reflection. In both cases there is a linear relationship between the number of integrator units and the amount of aminophenol on the TLC plate. The range of linearity was found to be 0–3 μg. The absolute detection limit corresponding to a signal equal to twice the background signal is 0.1 μg.

The interferences from various compounds such as aniline, pyridine, phenols, nitrophenols, etc., have been examined. Under the recommended procedure none of these compounds interfere.

The precision and accuracy of the entire procedure was evaluated with spiked seawater samples. Replicate analyses were carried out at various concentrations. The results of five independent determinations of the aminophenol isomers in three spiked seawater samples are summarized in Table 1. These results confirm the recovery of aminophenols and the precision of the developed method.

This method is suitable for the simultaneous determination of aminophenol isomers in various water samples. Further, it may be applied for the determination of aminophenols in biological materials after appropriate pretreatment, since aqueous samples can be analyzed.

SUMMARY

A TLC densitometric method for the determination of the aminophenol isomers in water samples has been developed. The aminophenols are first extracted into chloroform–isoamylalcohol (1:2) mixture. The extracted aminophenols are separated on a TLC plate and visualized by spaying solution of 2,4,7-trintro-9-fluorenone in acetone. The colored spots are scanned for light absorption or reflection and the amount of each aminophenol is evaluated.

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Application of 2,2′-Diquinoxalyl to the Spectrophotometric Determination of Gold(III) and Chromium(VI)

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INTRODUCTION

The reaction of gold(III) and chromium(VI) with the product of reduction of 2,2′-diquinoxalyl under the influence of strong reductive agents was examined as a continuation of works on the use of 2,2′-diquinoxalyl as a reagent for the chemical analysis.

2,2′-Diquinoxalyl (2,2′-DQx) reacting with tin(II) or titanium(III) in the presence of concentrated mineral acids and acetic acid is reduced and the colorless solution of reagent becomes blue from the reduced form of 2,2′-diquinoxalyl (2,2′-DQx_{red}).

The obtained colored product has one absorbtion maximum at $\lambda = 685$ nm at the visible light range and the determined molar absorbtion coefficient is $\epsilon = 3.3 \times 10^4$. The determined formal potential of the 2,2′-diquinoxalyl oxidized, 2,2′-diquinoxalyl reduced system in 6 mol/dm^3 hydrochloric acid is +0.41 (1). Detailed conditions for chemical and electrolytic reduction, reaction scheme, and application of this reaction for the determination of tin(II) and titanium(III) with spectrophotometric (*2–4*), potentiometric (*5*), and chromatographic (*6*) methods were elaborated earlier.

The oxidation reaction of 2,2′-DQx_{red} under the influence of hydrogen peroxide was applied for the kinetic determination of copper(II), iron(III), and platinum(IV) (*7*). These ions catalyze oxidation of 2,2′-DQx_{red} to 2,2′-diquinoxalyl. The present examination is connected with ions which potentials show possibility of direct reaction with 2,2′-DQx_{red}, where 2,2′-DQx_{red} is used as a reductive agent and the examined ion as an oxidizing agent.

Oxidation of 2,2′-DQx_{red} to 2,2′-diquinoxalyl causes decoloration of solutions, because 2,2′-diquinoxalyl has no maximum of absorbtion at the visible light range.

The spectrophotometric method of determination of chromium(VI) and gold(III) based on the measurement of absorbance of the solution before and after insertion of examined ions was elaborated.

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EXPERIMENTAL

Reagents and Equipment

2,2′-Diquinoxalyl (2,2′-DQx) was synthesized as previously described (*8, 9*). A standard solution of 2,2′-diquinoxalyl in concentration 3.4×10^{-3} mol/dm^3 was prepared by dissolving the 2,2′-diquinoxalyl in concentrated hydrochloric acid (1:1) and then diluting to obtain the required concentration.

A standard solution of tin(II) in concentration 0.5 mol/dm^3 was prepared from the weighted sample of diaqueous stannous chloride. The concentration of the obtained solution was determined bromometrically.

A standard solution of titanium(III) in concentration 0.1 mol/dm^3 was prepared from a weighted amount of titanium chloride by dissolving it in hydrochloric acid (1:1). Titanium(IV) was reduced to titanium(III) by passing the solution through the column with amalgamated cadmium. The titer of solutions of titanium(III) was determined potentiometrically with iron(III) or chromium(VI).

A standard solution of gold(III) containing 0.1 mg of gold in 1 ml was prepared from analar $HAuCl_4$. A standard solution of chromium(VI) containing 0.1 mg of chromium(VI) in 1 ml was prepared from analar $K_2Cr_2O_7$.

Hydrochloric acid and hydrogen peroxide were spectrally pure. Distilled water from glass apparatus was used for solutions. Oxygen was removed by blowing with carbon dioxide and the reduction was carried out in an airless atmosphere. All solutions of reagents were blown with carbon dioxide directly before the reaction. Spectrophotometer Spekol.

Method Used

The solution of 2,2′-diquinoxalyl in concentration 3.06×10^{-5} mol/dm^3 in 6 mol/dm^3 hydrochloric acid was inserted into measuring flasks of 25-cm^3 volume and swept with carbon dioxide for 3–4 min.

Solutions of tin(II) in molar ratio to 2,2′-diquinoxalyl 1:1 or solutions of titanium(III) in ratio 2 mol of titanium(III) to 1 mol of 2,2′-diquinoxalyl were placed into flasks. After 60 min the solutions containing examined ions were placed into flasks and then filled up to the measuring mark with hydrochloric acid.

The absorbance of the solution containing the reduced form of 2,2′-diquinoxalyl (2,2′-DQx_{red}) was measured at the light wavelength $\lambda = 685$ nm, and cuvettes with $d = 1$ cm were used.

Reaction of Gold(III) and Chromium(VI) with 2,2′-DQx_{red}

The blue product obtained after reduction of 2,2′-diquinoxalyl with tin(II) or titanium(III) shows absorbance $A = 0.98$ if the conditions given

earlier were fulfilled. The absorbance remains unchanged for 1 hr and then slow decoloration of the solution is observed as an effect of oxidation with oxygen diffusing from the air. After the maximum absorbance of colored solution of 2,2′-DQx_{red} in hydrochloric acid is measured 0.5–6.0 $\mu g/cm^3$ of gold(III) in 5–25 $\mu g/cm^3$ acetic acid was introduced.

The absorbance of the samples containing DQx_{red} with no gold(III) added and containing colored solutions of 2,2′-DQx_{red} with examined ions was measured. The dependence of ΔA (difference of absorbance of the blank sample and examined sample) upon the reaction time for introduced amounts of examined ions was measured. The time of measurement was 3–30 min.

The reaction of oxidation of chromium(VI) was carried out for concentrations from 0.1 to 1.0 $\mu g/cm^3$ in hydrochloric acid and acetic acid. The influence of gold(III) and chromium(VI) on the oxidation reaction in the presence of hydrogen peroxide in concentration 7.0×10^{-5} mol/dm^3 was examined.

Obtained average values ΔA in the reaction of 2,2′-DQx_{red} with gold(III) and chromium(VI) in hydrochloric acid and acetic acid were shown in Table 1. Quantitative curves for the determination of examined ions are in Fig. 1.

From the absorbance of the solution obtained in a period of 10 min, for the molar absorbtion coefficient for 2,2′-DQx_{red}, $\epsilon = 3.3 \times 10^4$, the amount of 2,2′-$DQx_{red}$ oxidized to 2,2′-DQx under the influence of introduced ions, was calculated (Table 2). It was established that the molar ratio of $K_2Cr_2O_7$ to 2,2′-DQx is 1:3 and that of $HAuCl_4$ to 2,2′-DQx is 1:1.

The Influence of Concentration of Hydrochloric Acid and Foreign Ions on the Reaction of 2,2′-DQx_{red} with Gold(III) and Chromium(VI)

The reaction was carried out in hydrochloric acid in concentration from 4 to 8 mol/dm^3. The concentration of 2,2′-diquinoxalyl was 3.06×10^{-5} mol/dm^3. Only slight differences in the reaction with the hydrochloric acid in concentration 6–8 mol/dm^3 were observed. For this reason the acid of concentration 6 mol/dm^3 was proposed for analytic purposes. For the acid in lower concentration the repeatability of results was worse. It was ascertained that NH_4(I), Na(I), Zn(II), Pb(II), Ni(II), Co(II), and Al(III) in 1000-fold excess do not interfere.

DISCUSSION AND CONCLUSIONS

It was proved that the reaction of 2,2′-DQx_{red} with gold(III) and chromium(VI) is, as was predicted, a redox reaction. The stoichiometry of the reaction was estimated. It is obvious from it that under the described conditions of the oxidation of 2,2′-DQx_{red} to 2,2′-diquinoxalyl 3

TABLE 1

AVERAGE VALUES OF Δ A IN THE REACTION OF OXIDATION OF 2,2′-DQx_{red} WITH THE USE OF CHROMIUM(VI) AND GOLD(III) IN HYDROCHLORIC ACID AND ACETIC ACID

	6 mol/dm³ HCl						6 mol/dm³ HCl + H_2O_2				
	Cr(VI) (μg/ml)						Cr(VI) (μg/ml)				
min	0.1	0.2	0.3	0.5	0.8	1.0	0.1	0.2	0.3	0.5	0.8
3	0.10	0.19	0.26	0.40	0.74	0.87	0.10	0.19	0.25	0.41	0.73
5	0.11	0.18	0.25	0.39	0.74	0.87	0.10	0.18	0.25	0.41	
7	0.10	0.18	0.24	0.38	0.74	0.86	0.10	0.19	0.24	0.40	
10	0.10	0.17	0.24	0.38	0.74	0.86	0.10	0.20	0.25	0.40	
15	0.11	0.18	0.24	0.37	0.74	0.86	0.10	0.20	0.23	0.38	
30	0.10	0.17	0.24	0.36	0.72	0.85	0.08	0.20			
	Au(III) (μg/ml)						Au(III) (μg/ml)				
3	0.05	0.13	0.28	0.40	0.62	0.95	0.08	0.12	0.26	0.34	0.45
5	0.06	0.12	0.28	0.42	0.64	0.95	0.08	0.15	0.27	0.35	0.45
7	0.04	0.13	0.28	0.41	0.64	0.95	0.09	0.16	0.28	0.36	0.45
10	0.05	0.12	0.27	0.42	0.65	0.95	0.08	0.17	0.30	0.36	0.42
15	0.05	0.12	0.28	0.43	0.65	0.95	0.09	0.18	0.31	0.38	0.41
20	0.05	0.12	0.28	0.43	0.65	0.95	0.10	0.20	0.34	0.36	
30	0.05	0.12	0.28	0.43	0.65	0.95	0.09	0.20	0.34	0.36	0.38
	CH_3COOH						CH_3COOH + H_2O_2				
	Cr(VI) (μg/ml)						Cr(VI) (μg/ml)				
min	0.1	0.2	0.3	0.5	0.8	1.0	0.1	0.2	0.3	0.5	0.8
3	0.08	0.16	0.26	0.40	0.76	0.98	0.10	0.19	0.27	0.44	0.70
5	0.08	0.16	0.25	0.40	0.76	0.98	0.11	0.18	0.25	0.43	
7				0.40		0.98	0.09	0.18	0.21	0.35	
10	0.08	0.17	0.26	0.40	0.76	0.98	0.08	0.13	0.18		
15	0.08	0.16	0.26	0.39	0.76	0.96	0.08	0.10			
30	0.08	0.16	0.27	0.40	0.77	0.96	0.07				
	Au(III) (μg/ml)						Au(III) (μg/ml)				
	4	6	8	12	20		4	6	8	12	20
3	0.08	0.23	0.36	0.50	0.83		0.04	0.06	0.10	0.13	0.18
5	0.10	0.23	0.35	0.52			0.04	0.06	0.08	0.13	0.20
7			0.37	0.56			0.05	0.07	0.10	0.13	0.18
10	0.15	0.24	0.41	0.58			0.06	0.06	0.10	0.13	0.19
15	0.14	0.25					0.05	0.08	0.11	0.12	
20							0.06	0.08	0.10	0.14	
30	0.16	0.32					0.06	0.08	0.10	0.12	

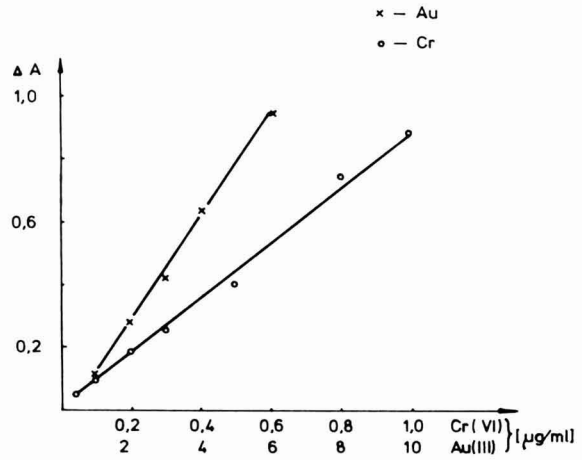

FIG. 1. Quantitative curves for determination of chromium(VI) and gold(III) in hydrochloric acid 6 mol/dm^3.

mol of the reagent reacts with 1 mol $K_2Cr_2O_7$ or 1 mol of the reagent reacts with 1 mol $HAuCl_4$, so that in the oxidation process of 1 mol 2,2′-DQx_{red} take part two electrons. The obtained results are in coincidence with the two-electron character of the reduction of 2,2′-DQx to DQx_{red} (*1*) and with the two-electron character of the oxidation of 2,2′-DQx_{red} with iron(III) (*7*).

It can be supposed, from the stoichiometry of the reaction, that gold(III) as a complex $AuCl_4^-$ oxidizing 2,2′-DQx_{red} is reduced to gold(I) in a form of a complex $AuCl_2^-$. Chromium(VI) is reduced to chromium(III). The reaction of oxidation was also carried out in the presence of hydrogen peroxide to act as a control if the redox reaction is not accompanied by catalytic reactions caused by ions of chromium(III) and gold(I).

There are no differences between oxidation caused by chromium(VI) in

TABLE 2
MOLAR RATIO OF REAGENTS IN THE REACTION OF OXIDATION OF 2,2′-DQx_{red} WITH THE USE OF GOLD(III) AND CHROMIUM(VI)

Concentration of $K_2Cr_2O_7$ ($\times 10^{-6}$ mol)	Concentration of 2,2′-DQx ($\times 10^{-6}$ mol)	Molar ratio $K_2Cr_2O_7$: 2,2′-DQx	Concentration of $HAuCl_4$ ($\times 10^{-6}$ mol)	Concentration of 2,2′-DQx ($\times 10^{-6}$ mol)	Molar ratio $HAuCl_4$: 2,2′-DQx
0.96	3.03	1:3.14	5.08	3.64	1.39:1
1.92	5.45	1:3	10.2	8.48	1.20:1
2.88	7.27	1:2.52	15.2	13.0	1.17:1
7.69	22.4	1:2.91	20.3	19.7	1.03:1
9.62	26.1	1:2.71	30.5	28.8	1.06:1

the presence or absence of hydrogen peroxide. There are some differences in the oxidizing reaction of gold(III) in the presence of hydrogen peroxide. It can be caused by the reaction of gold(III) with hydrogen peroxide, which can behave here as a reductive agent. The catalytic effect of ions of gold and chromium in lower oxidation state was not observed.

Parameters of the reaction and the influence of the concentration of hydrochloric acid and the foreign ions were examined. Quantitative curves for the determination of chromium(VI) in concentration 0.05–1.0 $\mu g/cm^3$ and gold(III) in concentration 0.5–6.0 $\mu g/cm^3$ were estimated.

The presented results show a possibility of using 2,2′-diquinoxalyl for quantitative determination of chromium and gold in concentrated acids. It is often important in technical analysis.

SUMMARY

The reaction of 2,2′-diquinoxalyl with tin(II) and titanium(III) was used for spectrophotometric determination of chromium(VI) and gold(III). The conditions of the reactions were determined. The curves for determination of chromium(VI) and gold(III) were found. The influence of foreign ions was examined.

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Semiautomatic Indirect Titration of Alkaline-Earth Ions with Catalytic Endpoint Indication

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INTRODUCTION

This paper forms part of an investigation about the application of manganese(II)-catalyzed autoxidation of the 1,4-dihydroxyphthalimide dithiosemicarbazone(OH-PDT) reaction (*6*) as an indicator system in catalytic titrations.

Aminopolycarboxylic acids (e.g., EDTA and EGTA) have an inhibitory effect on this catalytic reaction and this provides a direct method of determining these ligands and an indirect method of determining some metal ions (such as Mn(II) and Ni(II)) by a semiautomatic back-titration method which are described in an earlier paper (*8*). The titration is monitored spectrophotometrically at 600 nm where the oxidation product absorbs.

The present paper describes a semiautomatic method for detecting the endpoint in a titration of EDTA by Mn(II) (catalyst) and its application to a back-titration method for alkaline-earth ions and specially for (Ca + Mg) in water or milk by the indicator reaction indicated above.

The use of catalyzed reactions for the indication of endpoints in titrimetric analysis has been described in literature (*1*, *5*) but the determination of alkaline-earth ions has not deserved much attention. In other methods which have been described for the determination of these ions, the endpoint has been determined visually (*4*) or thermometrically (*5*) by using the manganese(II)-catalyzed hydrogen peroxide–resorcinol system.

The proposed method in this paper is the first application of semiautomatic catalytic titration for alkaline-earth determination with photometric monitoring. It shows advantages over the conventional one using metalchromic indicators, because of the lower range of concentration of metal ion which can be determined. It is a rapid, simple, accurate, and sensitive way of determining small amounts of alkaline-earth ions.

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MATERIAL AND METHODS

Reagents

1,4-Dihydroxyphthalimide dithiosemicarbazone solution. Prepare 0.05% (w/v) solution in dimethylformamide (DMF). The reagent was synthesized from 1,4-dihydroxyphthalimide dioxime and thiosemicarbazide (*6*).

Standard manganese(II) solution. Prepare from manganese sulfate (ignited at 500°C), dissolved in hot dilute sulfuric acid, and standardize by titration with EDTA. Dilute the stock (1000 $g \cdot liter^{-1}$) just before use.

Standard calcium(II), magnesium(II), strontium(II), and barium (II) solution. Prepare from their carbonate salts and standardize by titration with EDTA (*7*), except calcium carbonate which is primary standard.

Standard EDTA solution. Prepare from dihydrate disodium salt, dissolved in distilled water and standardize against standard calcium carbonate solution using murexide as indicator.

All solvents and reagents were of analytical-reagent grade.

Apparatus

A Metrohm E 1009 photometric titrator with a titration vessel and stirrer was coupled with a Metrohm E 536 potentiometric recorder (potentiograph) and a Metrohm E 436 automatic constant-rate burette.

Procedure

Preparation of equipment. Fill the autoburette with a 9.1×10^{-4} *M* manganese(II) solution. The wavelength of the photometric titrator was fixed at 600 nm and the rate of titrant addition at 4 $ml \cdot min^{-1}$.

Semiautomatic catalytic titration of alkaline-earth ions. To a solution containing between 40 and 150 μg of calcium(II) or magnesium(II) or 20 and 250 μg of strontium(II) or 20 and 450 μg of barium(II), in a 100-ml titration vessel, a suitable volume of 1.01×10^{-3} *M* standard EDTA solution, 2 ml of 0.05% (w/v) OH-PDT solution in DMF, and 10 ml of 0.429 *M* ammonium hydroxide solution are added and diluted to around 50 ml with distilled water. The stirrer is switched on and when the relative absorbance attain the 0.20–0.25 value, the function switch is turned to "Record" and the absorbance is monitored on the potentiograph to obtain the titration curve. The endpoint is obtained graphically from the intersection of the straight segments of the titration curve occurring before and after the equivalence point. The content of alkaline-earth ions is then calculated by the proportional method (*2*) or by working curves obtained by plotting the end volumes against the amount of metal ion.

The volume of 1.01×10^{-3} *M* standard EDTA solution added depends

on the metal ion present to obtain the endpoint in the optimal range titrant volume (3–7 ml for 10 mm/ml scale register chart and 1–7 ml for 20 mm/ml). The recorder has to be calibrated in each titration using the burette reading, for best accuracy.

Determination of the total concentration in calcium and magnesium in natural waters. In a titration vessel pipet an aliquot of the sample (1–2 ml), a known suitable volume of 1.01×10^{-3} *M* standard EDTA solution, and continue as in the preceding titration. The sample is titrated with 9.1×10^{-4} *M* manganese(II) solution by the indicated procedure.

Determination of the total concentration in calcium and magnesium in milk. Twenty-five milliliters of the sample is treated with an equal volume of trichloroacetic acid. The filtered is diluted to 100 ml with distilled water, neutralizing previously the sample. In a titration vessel an aliquot of this prepared sample is pipetted and continued as in preceding titrations.

RESULTS AND DISCUSSION

From the point of view of equilibrium laws, the back-titration method can only be used when the conditional constants of complexes of the titrated metal are much larger that for the titrant metal ion (Mn(II)), so that during the back titration of excess inhibitor (EDTA), the substitution reaction between Mn(II) and the metal ion–EDTA complex can not take place. Although the stability constant of Mn–EDTA complex is larger than the stability constants of alkaline-earth–EDTA complexes, the back titration is possible and the excess of titrant does not intervene in the substitution reaction. This is probably due to the relatively slow kinetics of the displacement reaction and to the adjustment of the reported experimental conditions which slows the substitution reactions to a point that their effect is negligible.

The shape of the catalytic titration curve and the sharpness of the endpoint were affected by the concentration of Mn(II), OH-PDT, and the rate of titrant addition. However, all these factors can be kept constant and the results are reproducible under a given set of experimental conditions.

Recorded curves for the semiautomatic titration of calcium(II), magnesium(II), strontium(II), and barium(II) with manganese(II) are shown in Fig. 1. The endpoint was obtained by extrapolating the linear segments of the titration curve.

Working curves were obtained by plotting the endpoint volumes against the amount of metal ion. These plots were linear for each concentration of titrant Mn(II) and volume of EDTA solution added. Working curves were prepared for each metal ion and for each of the following ranges, titrating

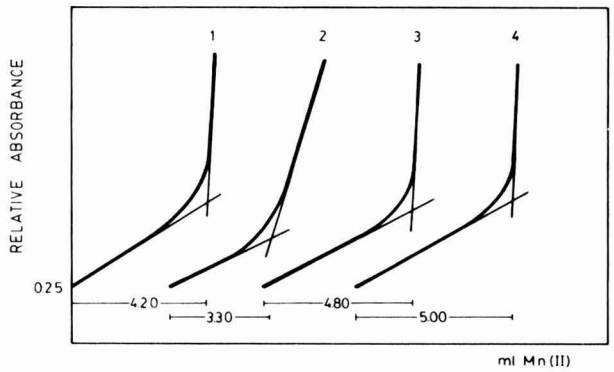

FIG. 1. Recorded curves for the semiautomatic indirect catalytic titration of alkaline-earth ions. 1, 50.0 μg of Ca(II); 2, 50.0 μg of Mg(II); 3, 64.7 μg of Sr(II); and 4, 60.0 μg of Ba(II). Conditions: 5 ml of 1.01×10^{-3} *M* EDTA solution, 10 ml of 0.429 *M* ammonium hydroxyde solution titrated with 9.1×10^{-5} *M* Mn(II) solution. Theoretical endpoints: 4.18, 3.29, 4.74, and 5.07 ml, respectively.

with 9.1×10^{-4} *M* manganese(II) solution and adding the indicated volume of EDTA solution:

Ca(II)	10–150 μg	5 ml of 1.01×10^{-3} *M* EDTA solution
Mg(II)	10–75 μg	5 ml of 1.01×10^{-3} *M* EDTA solution
	75–150 μg	8 ml of 1.01×10^{-3} *M* EDTA solution
Sr(II)	20–250 μg	5 ml of 1.01×10^{-3} *M* EDTA solution
Ba(II)	20–450 μg	5 ml of 1.01×10^{-3} *M* EDTA solution

Four standard metal ion solutions were sufficient for each working curve. For reproductibility it was essential to maintain all working conditions constant.

The metal ion can be also be found by the proportional method (*2*). The volume of EDTA found by substracting the volume of unconsumed (back-titrated) EDTA from the known volume of added EDTA to the sample was equivalent to the quantity of the metal ion in the sample.

Results for aqueous calcium(II) and magnesium(II) solution of known concentration are shown in Table 1. In the titration of magnesium(II) the autocontrol switch was not utilized in order to obtain better accuracy and precision than the proportional method. For the first approach the average error and relative standard deviation were 1.16 and 0.42%, respectively, for calcium and 2.76 and 0.74% in the range 10–75 μg and 1.10 and 0.16% in the range 75–150 μg, respectively, for the magnesium.

Results for the indirect titration of strontium(II) and barium(II) solutions of known concentration are shown in Table 2. In the titration of strontium, the working curve method lead to the best accuracy. The aver-

TABLE 1
SEMIAUTOMATIC INDIRECT CATALYTIC TITRATION OF Ca(II) AND Mg(II)
(9.1×10^{-4} *M* MANGANESE(II) SOLUTION TITRANT)

EDTA taken	Ca(II) taken (μg)	Ca(II) found (μg)		Percentage error and RSD (n = 11)			
		Method A	Method B	Method A		Method B	
5 ml $1.01 \times 10^{-3}M$	10.0	9.1	10.0	−9.0		0.0	
	30.0	28.4	29.5	−5.3		−1.6	
	50.0	49.2	49.5	−1.6		−1.0	
	70.0	69.0	68.8	−1.0		−1.4	
	100.0	100.3	99.0	0.3	(±0.44)	−1.0	(±0.42)
	150.0	148.8	147.0	−0.8		−2.0	
Average				3.00		1.16	
	Mg(II) taken (μg)	Mg(II) found(μg)		Percentage error and RSD (n = 11)			
		Method A	Method B	Method A		Method B	
5 ml $1.01 \times 10^{-3}M$	12.5	13.2	12.0	5.6		−4.2	
	25.0	26.5	26.0	6.0		4.0	
	37.5	38.6	39.0	2.9		4.0	
	50.0	49.7	50.0	−0.6	(±0.69)	0.0	(±0.74)
	62.5	63.0	63.5	0.8		1.6	
Average				3.18		2.76	
8 ml $1.01 \times 10^{-3}M$	87.5	88.0	88.0	0.6		0.6	
	100.0	103.5	103.0	3.5		3.0	
	112.5	114.5	113.5	1.7		0.8	
	125.0	125.6	124.5	0.5	(±0.22)	−0.4	(±0.16)
	137.5	141.1	139.0	2.6		1.1	
	150.0	153.2	151.0	2.1		0.7	
Average				1.83		1.10	

Note. Method A, corresponds to proportional method; Method B, corresponds to working curve method.

age error and relative standard deviation were 1.14 and 0.80%, respectively, for this method.

The method has been applied to the determination of total concentration in calcium and magnesium (total hardness) in natural waters and in sterilized milk. The excess of back-titrated EDTA solution, was calculated from proportional method and the total concentration of calcium and magnesium was easily calculated from these data. The excess of EDTA can be also be found by a working curves obtained by titrating standard EDTA solution with the same manganese titrant.

The sum of calcium and magnesium in natural waters was determined directly in a little aliquot (1−2 ml). The results are shown in Table 3 and are expressed in moles per litre (*M*). The total hardness, in parts per million of $CaCO_3$ was easily calculated from these data.

The analysis of sterilized milk required the treatment indicated in experimental.

TABLE 2
SEMIAUTOMATIC INDIRECT CATALYTIC TITRATION OF Sr(II) AND Ba(II)
(9.1×10^{-4} *M* MANGANESE(II) SOLUTION TITRANT)

EDTA taken	SR(II) taken (μg)	Sr(II) found (μg)		Percentage error and percentage RSD (n = 11)			
		Method A	Method B	Method A		Method B	
5 ml $1.01 \times 10^{-3}M$	21.6	23.8	22.0	10.2		1.8	
	64.7	66.8	67.0	3.2		3.5	
	107.8	106.7	108.0	−1.0	(±0.80)	0.2	(±0.80)
	129.4	129.8	130.0	0.3		0.4	
	172.5	172.9	174.0	0.2		0.8	
	215.6	211.2	215.0	−1.5		−0.3	
	258.7	252.7	256.0	−2.3		−1.0	
Average				2.67		1.14	
	Ba(II) taken (μg)	Ba(II) found (μg)		Percentage and percentage RSD (n = 11)			
		Method A	Method B	Method A		Method B	
5 ml $1.01 \times 10^{-3}M$	20.0	21.0	18.0	−5.0		−10.0	
	60.0	59.8	56.0	−0.3		−6.6	
	100.0	103.5	99.0	3.5		−0.1	
	160.0	162.3	157.0	1.4		−1.8	
	200.0	202.3	197.0	1.1	(±0.60)	−1.5	(±0.69)
	250.0	249.8	246.0	−0.1		−1.6	
	350.0	351.1	355.0	0.3		1.4	
	450.0	456.6	453.0	1.3		0.6	
Average				1.62		.95	

Method A: correspond to proportional method
Method B: correspond to working curve method

TABLE 3
DETERMINATION OF TOTAL CONCENTRATION IN Ca(II) AND Mg(II) IN SEVERAL SAMPLES

Sample	Total concentration found (M)[a]		
	Catalytic titration		Metalchromic indicator
	Method A	Method B	
Commercial natural waters	7.16×10^{-4}	7.17×10^{-4}	7.27×10^{-4}
	2.09×10^{-3}	2.08×10^{-3}	2.08×10^{-3}
Natural water			
Treated	7.04×10^{-4}	7.10×10^{-4}	7.12×10^{-4}
Not treated	8.18×10^{-4}	8.31×10^{-4}	8.53×10^{-4}
Sterilized milk	1.11×10^{-2}	1.10×10^{-2}	1.09×10^{-2}

[a] Average of two separate determinations.

The results are compared with those obtained using eriochrome black T as indicator in complexometric titration. The aliquot of the sample, in the case of natural waters, should be higher than 100 ml, if the sample has not been previously concentrated. However, with this proposed method volumes of 1–2 ml are sufficient.

SUMMARY

A semiautomatic spectrophotometric method of endpoint indication for indirect catalytic titrations of microamounts of alkaline-earth ions is described. The manganese(II)-catalyzed autoxidation of 1,4-dihydroxyphthalimide dithiosemicarbazone is used as indicator reaction. Calcium and magnesium (10–150 μg), strontium (20–250 μg), and barium (20–450 μg) can be determined by adding a known excess of EDTA to the sample solution and back-titrating the unconsumed inhibitor agent with standard manganese(II) solution. This catalytic method was applied to determine the total concentration in calcium and magnesium (total hardness) in natural waters and milk. The method is fast, accurate (1–3%), precise (0.2–0.8%, relative standard deviation), and specially useful for the determination of microamounts where metallochromic indicator are ineffective (for concentrations ranges below 10^{-4} *M*).

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MICROCHEMICAL JOURNAL 26, 68–74 (1981)

Investigation of Self-Association of the Selected Glycols on Cellulose Sorbents

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INTRODUCTION

Experimental difficulties accompanying investigations on self-association through hydrogen bonds promote efforts aiming toward the establishing of new analytical techniques, more successfully penetrating nuances of mentioned interactions. On the other hand some more detailed mechanisms of interactions at the phase borders, e.g., adsorption of the solution components on solid surfaces or the related effects in chromatography, require taking into assumption hydrogen bonds to better explain the very essence of those phenomena.

The aim of this paper was to introduce a new, microchemical approach taking advantage of chromatographic paper as a low-active sorbent and enabling qualitative comparison of the selected glycols with respect to their ability to self-associate. Investigations concerned the following compounds: 1,2-ethanediol, 1,3-propanediol, 1,4-butanediol, and 1,5-pentanediol. Here it should be stressed that in the abundant literature dealing with both theoretical and experimental aspects of hydrogen bond considerations having to do with substances including more than one functional group seem to be represented seldomly.

EXPERIMENTAL

All glycols used in the presented experiment, i.e., 1,2-ethanediol, 1,3-propanediol, 1,4-butanediol, and 1,5-pentanediol, were the reagents of analytical grade. First, to qualitatively compare these substances with respect to their ability to self-associate, the investigation was performed employing the ir absorption spectroscopy. The ir spectra of the 0.1 M CCl_4 solutions of 1,3-propanediol, 1,4-butanediol, and 1,5-pentanediol were measured in the range of 3800–3000 cm^{-1}, i.e., in the range of stretching vibrations of the free and bonded hydroxyl group (due to insolubility of 1,2-ethanediol in CCl_4 spectroscopic examination of this compound was omitted). Spectra were run using the UR-20 type ir spectrophotometer (Carl Zeiss, Jena, East Germany) and the following working conditions: NaCl cells, their thickness $d_1 = 3.00$ mm with the solutions of 1,3-propanediol and 1,5-pentanediol, and $d_2 = 1.01$ mm with the solution of 1,4-

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butanediol; measuring temperature, 30 ± 0.2°C; the LiF prism; the slot program 4; registration speed, 64 cm^{-1}/min; registration width 20 mm/100 cm^{-1}. The obtained spectra are given in Figs. 1–3.

Then graphical separation was performed of the valency absorption bands of the free and bonded hydroxyls with each examined glycol, and the areas under the both types of curves were integrated. The obtained number values were accepted as integral intensities under the given measuring conditions. They are presented in Table 1.

The proposed new technique employing chromatographic paper was as follows. We used the Whatman 1, 2, 3, and 4 chromatographic papers (Whatman, England), which were first dried at 110°C for 1 hr. Then we spotted the 5-μl amounts of the investigated glycols upon the applied papers and left the sheets for 10 min to let substances occupy characteristic surfaces upon the paper layer. Finally the glycol spots were visualized with the iodine vapors for 24 hr and their surfaces were planimetrically measured. The determination error never surpassed ±5% (Table 2).

DISCUSSION AND CONCLUSIONS

The spectroscopic results shown in Table 1 give evidence of differentiated ability of the discussed glycols to self-associate. If we accept the integral intensity b and B values as a relative measure of concentration of the free and bonded hydroxyls in each system (which remains in agreement with the foundations of ir absorption spectroscopy), and the b/B ratio values as a relative measure of tendency of those hydroxyls to appear in

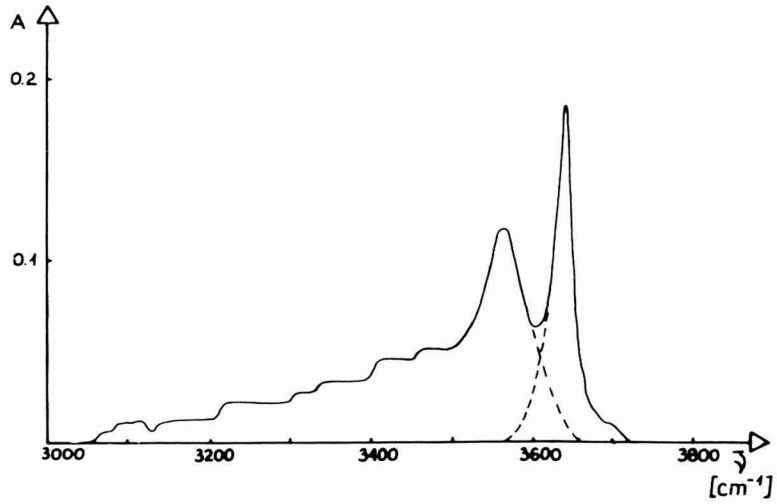

FIG. 1. The ir spectrum of the 0.1 M CCl_4 solution of 1,3-propanediol in the range of 3800–3000 cm^{-1}; measuring temperature, 30°C.

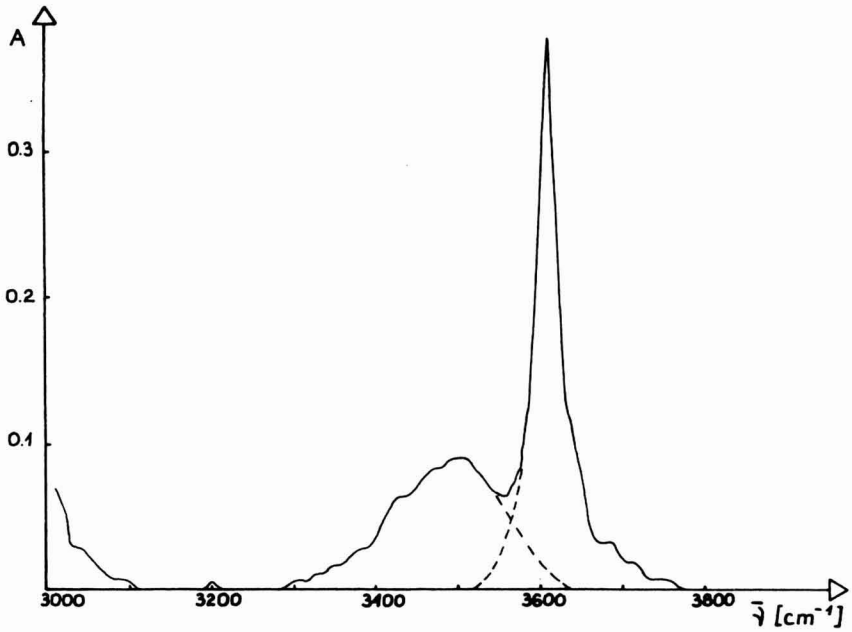

FIG. 2. The ir spectrum of the 0.1 *M* CCl_4 solution of 1,4-butanediol in the range of 3800–3000 cm^{-1}; measuring temperature, 30°C.

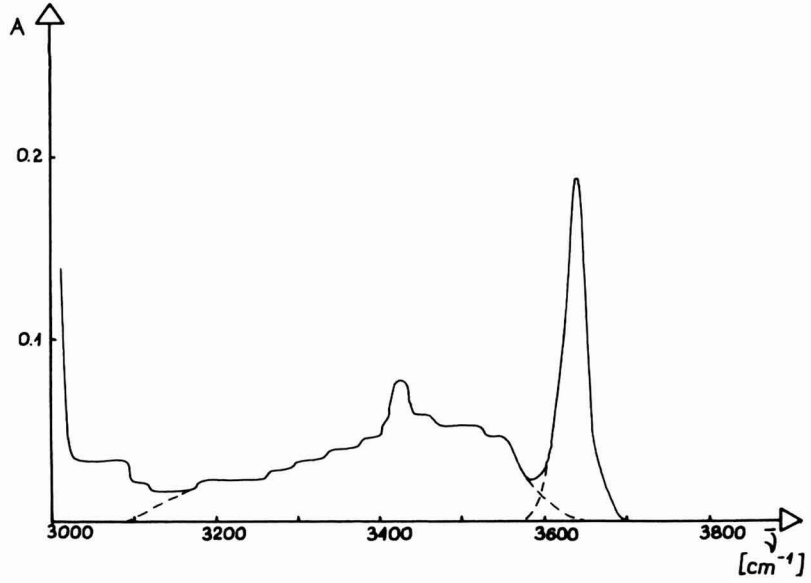

FIG. 3. The ir spectrum of the 0.1 *M* CCl_4 solution of 1,5-pentanediol in the range of 3800–3000 cm^{-1}; measuring temperature, 30°C.

TABLE 1
THE INTEGRAL INTENSITY VALUES OF THE VALENCY ABSORPTION BANDS WITH FREE (b) AND BONDED (B) HYDROXYL GROUPS OF THE 0.1 *M* CCl_4 SOLUTIONS OF THE SELECTED GLYCOLS, AND THE b/B RATIO VALUES AT 30°C

Substance	*b* (cm^{-1})	*B* (cm^{-1})	*b/B*
1,3-Propanediol	6.4	21.2	0.30
1,4-Butanediol	17.2	14.8	1.16
1,5-Pentanediol	6.4	17.4	0.37

the free form, then we will find a certain lack of tenacity inside the considered fraction of the homologous glycol series. Thus the worst pronounced tendency toward appearing in the free form can be observed with hydroxyls belonging to 1,3-propanediol, the better one with those of 1,5-pentanediol, and the best one in the case of 1,4-butanediol. It seems that the above formulated remark could be explained on the basis of differentiated ability of the discussed glycols to form intramolecular hydrogen bonds and not with the phenomena of intermolecular interactions. Namely when it comes to intermolecular hydrogen bonds one has to state that all the discussed glycols contain but the first-order hydroxyl groups, which should be reflected in a similar or a monotonously diminishing tendency to form linear multimers, caused with the increasing shielding effect of a given hydroxyl with the increasing volume of the remaining part of a molecule.

When considering a possibility of forming intramolecular hydrogen bonds the before mentioned lack of tenacity inside the glycol homologous series seems to be well grounded. To explain this phenomenon we took advantage of the schemes given in Fig. 4. They illustrate the selected conformers of 1,2-ethanediol, 1,3-propanediol, 1,4-butanediol, and 1,5-

TABLE 2
THE SPOT AREAS[a] OF THE SELECTED GLYCOLS ON CHROMATOGRAPHIC PAPERS WHATMAN 1–4

Substance	The spot area (mm^2) on Whatman paper:			
	1	2	3	4
1,2-Ethanediol	223	211	130	216
1,3-Propanediol	250	231	143	224
1,4-Butanediol	303	477	225	342
1,5-Pentanediol	265	225	200	244

Note. The spotted amounts of substances: 5 μl.

[a] The presented number values are mean values taken from 10 separate determinations.

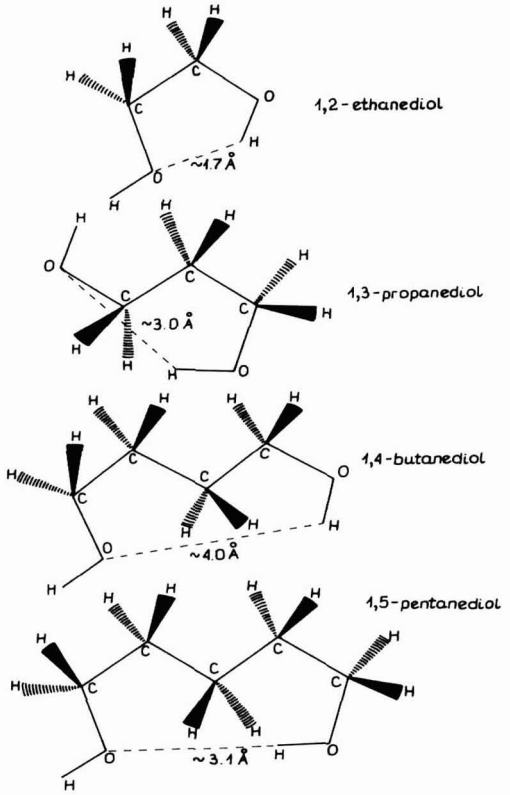

FIG. 4. The selected conformers of 1,2-ethanediol, 1,3-propanediol, 1,4-butanediol, and 1,5-pentanediol.

pentanediol, i.e., such cases, in which all the carbon atoms and the hydroxyl oxygen and hydrogen atoms appear at the same plane. The presented drawings preserve basic proportions of the discussed molecules, such as bond lengths and angles (C–C 1.54 Å; C–O 1.43 Å; O–H 0.96 Å; ∢ CCC 109°; ∢ COH 106°).

As it comes out from the structures given in Fig. 4, with 1,2-ethanediol the distance between the hydrogen atom from one hydroxyl and the oxygen atom from the other one is the comparatively shortest one and equals ~1.7 Å. The similar distance increases when shifting toward 1,3-propanediol (~3.0 Å) and 1,5-pentanediol (~3.1 Å), and achieves its maximum with 1,4-butanediol (~4.0 A). Remembering the numerous other conformers of the considered glycols it can be stated that their ability to form intramolecular hydrogen bonds remains in a reverse proportion to the distance between certain elements of the both functional groups, as it was confirmed with the spectroscopic results.

Comparing of the results presented in Tables 1 and 2 (in correlation with

the data given in Fig. 4) it is suffice to state that independently from the type of chromatographic paper used the results of spectroscopic investigations are fully reflected in the spot surface areas obtained on those sorbents. Thus in the case of the greatest tendency to form both intra- and intermolecular hydrogen bonds (1,2-ethanediol) those areas were the smallest ones, while in the case of the worst pronounced tendency to give intramolecular hydrogen bonds the areas were the greatest ones (1,4-butanediol). On this basis and in accordance with our previous observations concerning low-active sorbents, e.g., chromatographic papers (*1*, *2*), one can conclude that the higher is the concentration of free hydroxyls able to occupy active centers of a sorbent, the greater are the obtained spot areas. With glycols one can suspect that intramolecular hydrogen bonds lower ability of one functional group per molecule to intermolecularly interact with a sorbent to such an extent, that the whole mechanism of sedimentation of a given molecule on a sorbent surface chages (Fig. 5).

The example quoted in this paper gives evidence of high usefulness of a low-active cellulose sorbent employed for the relative and purely qualitative comparison of substances in respect of their ability to self-associate. The proposed method omits the very process of chromatographic developing, which is also of certain advantage.

Additionally it can be stated that the chromatographic paper Whatman 2 seems to be the most suitable sorbent, revealing the greatest differences in the spot surfaces (even if the order obtained with 1,3-propanediol and 1,5-pentanediol slightly changed in comparison with the spectroscopic and other sorbent results). It seems that in purpose to express the differentiated tendency of the discussed glycols to self-associate a semiquantitative "index of self-association" can be introduced (reflecting simultaneously interactions with a sorbent itself),

$$i.s. = \frac{1000 - \bar{S}}{\bar{S}},$$

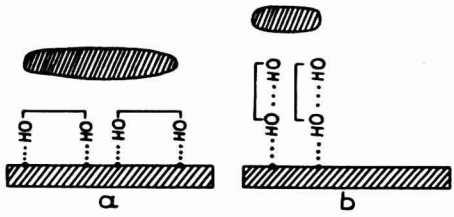

FIG. 5. Schematic comparison of the spot surface areas on a cellulose sorbent in the case of adsorption of the molecules without an intramolecular hydrogen bond (a) and with such a bond (b).

TABLE 3
THE SPOT SURFACE AREAS OF THE EXAMINED GLYCOLS ON THE CHROMATOGRAPHIC PAPER WHATMAN 2 AND THE CALCULATED INDEXES OF SELF-ASSOCIATION

Substance	$\bar{S}$ (mm^2)	*i.s.*
1,2-Ethanediol	211	3.7
1,3-Propanediol	231	3.3
1,4-Butanediol	477	1.1
1,5-Pentanediol	225	3.4

where $\bar{S}$ is the number value (in mm^2) of the spot surface area. The obtained indexes of self-association values dealing with the examined glycols on the Whatman 2 surface are given in Table 3.

The higher is the index number value (see Table 3), the greater is supposed to be the tendency of a given glycol to form intramolecular hydrogen bonds and vice versa. Thus an attempt to give a number illustration of structural differences and—consequently—of a differentiated possibility to form intramolecular hydrogen bonds succeeds in the organization of the selected substances, e.g., those giving a homologous series in accordance with the index of self-association number values.

SUMMARY

A comparison was performed of the selected glycols, i.e., 1,2-ethanediol, 1,3-propanediol, 1,4-butanediol, and 1,5-pentanediol, in respect of their ability to form intramolecular hydrogen bonds. The following analytical techniques were employed: ir absorption spectroscopy and a new method taking advantage of chromatographic paper.

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Use of Phenyl Acetic Acid in Determination of Iron

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INTRODUCTION

In a previous communication Adam and Přibil have pointed out the possibility of using phenyl acetic acid (PAA) for the determination of some metals by extraction (*3*, *4*). This possibility has been utilized for the polarographic determination of uranium in lead (*2*) and titrimetric determination of zinc and copper (*1*).

In our paper we want to show one effective complexometric method of iron determination by separating iron from other metals as a salt of PAA in chloroform medium.

In a traditional analysis system an excess of EDTA solution is added to the sample to form a complex of Fe and Al (Ti). Free EDTA is then titrated with a Zn or Pb solution. After this NH_4F is added to mask Al (Ti) and the released EDTA is again titrated with a Zn (Pb) solution. The Fe content is then calculated from the difference of consumptions of the Zn standard solution.

The proposed extraction method is a selective one. Though aluminium has a tendency to come partly into extract, it can be masked by adding a small quantity of ammonium fluoride.

MATERIALS AND METHODS

Reagents and apparatus. 1 *M* solution of phenyl acetic acid (PAA) prepared by dissolution of 68.1 g of PAA in 500 ml of chloroform; 5×10^{-2} *M* solutions of metals prepared from the salts (sulfate, chloride, nitrate) and checked complexometrically; 5×10^{-2} *M* EDTA solution standardized

[1] The paper was submitted when B. B. Sinha was at Charles University, Prague, Czechoslovakia, under a UNESCO scheme.

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with 5×10^{-2} *M* lead nitrate, with Xylenol Orange 1% solution in water as indicator; 1.10^{-1} *M* water solution of ammonium fluoride; 3 *M* hydrochloric acid; saturated solution of hexamethylenetetramine (hexamine) in water.

Practical standard samples.

Serpentinit (Věžná u Rožně, Czechoslovakia).

G-1 USA standard silicate.

W-1 USA standard silicate.

Magnezit (Košice, Czechoslovakia).

Magnezit K II 6-02-002 (Czechoslovakia).

Magnezit O II 6-02-001 (Czechoslovakia).

For pH measurement a PHM 64 Research pH meter Radiometer was used, with a glass calomel combined electrode from the same firm.

RESULTS AND DISCUSSION

Procedure

Metal solutions (5×10^{-2} *M*) of various volumes (1 to 15 ml) were pipetted out for separate measurements, in a 250-ml separating funnel and diluted with 150 ml water followed by the addition of 10 ml 1 *M* PAA in chloroform and, after a short shaking, saturated hexamine solution was added to maintain pH 5.5. The mixture was thoroughly shaked for a reasonable period (30 sec to 1 min) and left for settling and separation for at least 5 min. The chloroform phase was separated in another separating funnel of 100 ml capacity. To the previous water phase an additional 10 ml of PAA solution was added, and the extract was collected with the first collection in the same way. Finally to the water phase another 5 ml of PAA solution was added, and the extract was collected in the same way as the first and second collection.

These three collections together were reextracted with 20 ml 3 *M* HCl. The organic phase was removed and the water phase was collected in a 100-ml beaker, and the solution was diluted to about 100 ml with water and EDTA solution added in excess. The pH was maintained at 5.5 by adding hexamine and five drops of Xylenol Orange were added. The excess of EDTA was titrated with standard zinc solution.

Effect of Extraction

In the above mentioned procedure it has also been determined that more than 99% extraction is possible by only a single stage extraction.

Influence of Cations

In the previous work by Adam and Přibil (*4*) we have seen that with iron other metals such as In, Pb, Cu, and U can also be extracted efficiently.

But our present interest is to apply this method to analysis of silicates where all the above mentioned metals may exist in trace or not exist at all. We therefore tried to see the possibilities of extraction of other metals present in silicates which can interfere during the extraction of iron. It was observed that aluminium can be partly extracted which should be masked. It has been found that ammonium fluoride can mask aluminium selectively. It was also observed that Ca, Mg, and Mn are not extracted.

As in other silicate analysis silica should be removed by hydrofluorization while bringing the silicate into solution.

In our experiments we have noted that the higher concentration of NH_4F may decrease the extraction of iron. By adding NH_4F quantitatively it was found by trial that the optimum concentration of NH_4F to mask aluminium is 2.5 ml of 0.1 *M* for masking 2 ml of 5×10^{-2} *M* Al.

Although our experience indicates that Ca, Mg, and Mn are not extracted in the same condition, still by deliberately adding the standard solution of these elements we have seen that they do not interfere with the extraction of iron.

The results of the determination of iron from standard iron solution by extraction are shown in Table 1 and in the presence of interfering elements are shown in Table 2.

Influence of Anions

It has been found that sulfates, chlorides, and nitrates are without negative influence on the extraction process. On the other hand some compounds, e.g., EDTA, DCTA, EGTA, and others, such as oxalic, citric, and tartaric acid, have a negative influence even in a trace quantity.

TABLE 1
DETERMINATION OF IRON(III)

Fe given (mg)	Fe found (mg)	Difference	
		mg	%
3.07	3.14	0.07	+2.3
3.07	3.10	0.03	+0.9
6.14	6.17	0.03	+0.5
6.14	6.10	0.04	−0.6
15.36	15.52	0.16	+1.0
15.36	15.30	0.06	−0.4
30.72	30.69	0.03	−0.1
30.72	30.75	0.03	+0.1
46.08	45.49	0.59	−1.3
46.08	46.15	0.07	+0.1

TABLE 2
DETERMINATION OF IRON IN THE PRESENCE OF Al, Mn, Ca, AND Mg

Metals in mg given						Difference	
Al	Mn	Ca	Mg	Fe	Fe in mg found	mg	%
1.07	—	—	—	15.36	15.15	0.21	+1.4
5.39	—	—	—	15.36	15.86	0.50	+3.3
5.39	—	—	—	15.36	15.43	0.07	+0.4
—	1.09	—	—	15.36	15.50	0.14	+0.9
—	2.19	—	—	15.36	15.30	0.06	−0.4
—	4.40	—	—	15.36	15.35	0.01	−0.06
—	—	2.0	0.48	15.36	15.55	0.19	+1.2
—	—	4.0	1.94	15.36	15.30	0.06	−0.4
—	—	8.0	7.77	15.36	15.35	0.01	−0.06
5.39	4.4	4.0	1.94	15.36	15.35	0.01	−0.06
5.39	1.04	2.0	7.77	15.36	15.43	0.07	+0.4

Analytical Applications

For analytical applicability to the study of this method we used two types of samples containing iron: (a) silicate rocks and (b) carbonate rocks.

TABLE 3
DETERMINATION OF Fe_2O_3 IN STANDARD ROCKS

Standard and composition (%)	Fe_2O_3 (% declared)	Fe_2O_3 (% found)
Serpentinit-Ostrava, ČSSR		
SiO_2, 39.80; TiO_2, 0.16; Al_2O_3, 1.82; Cr_2O_3, 0.25; MgO,		8.78
37.49; CaO, 1.61; MnO, 0.12; NiO, 0.28; Na_2O, 0.16;		8.68
K_2O, 0.03; P_2O_5, 0.024; CO_2, 0.50; H_2O, 9.77	8.82	8.80
W-1 (USA)		
SiO_2, 52.46; Al_2O_3, 15.03; MgO, 6.62; CaO, 10.96; Na_2O,		11.14
2.07; K_2O, 0.64; H_2O, 0.67; TiO_2, 1.07; MnO, 0.16;		11.53
P_2O_5, 0.14; CO_2, 0.05	11.16	11.13
G-1 (USA)		
SiO_2, 72.41; Al_2O_3, 14.27; MgO, 0.41; CaO, 1.39; Na_2O,		1.89
3.32; K_2O, 5.45; H_2O, 0.40; TiO_2, 0.26; P_2O_5, 0.09;		1.94
MnO, 0.03; CO_2, 0.08	1.96	1.94
Magnezit K II 6-02-002 (ČSSR)		
SiO_2, 0.41; Al_2O_3, 0.48; CaO, 0.84; MgO, 43.94		3.62
Loss by ignition, 49.64	3.72	3.70
		3.40
Magnezit O II 6-02-001 (ČSSR)		
SiO_2, 1.64; Al_2O_3, 0.21; CaO, 3.56; MgO, 41.30		4.15
Loss by ignition, 49.12	4.22	4.30
		4.19

Procedure

(a) To 0.5 g of sample of powdered silicate rock (72 BS) in a basin of platinum or Teflon are added 10 ml HF and 4 ml $HClO_4$, heated at 60°C overnight and then evaporated to dryness on a sand bath and cooled; the residue is dissolved in 2 ml $HClO_4$ followed by dilution with water, filtering, and washing with hot water until it is acid free. The filtrate is collected in a volumetric flask, the volume made up while cooled. An aliquot quantity was taken for extraction, reextraction, and subsequent titration as described above.

(b) To 0.5 g of sample of powdered carbonate rock (72 BS) are added 10 ml H_2O and 10 ml HCl, heated on a sand bath with a glass cover until effervescence ceases. The cover is removed and the sample is heated further until it is a paste and then cooled, diluted with water, filtered, washed with hot water until acid free, cooled, and a volume made up. An aliquot portion was taken and we proceeded as in (a).

Some carbonates rich in silicate should be treated as silicate and processed accordingly (as in some magnesit and others).

The results of practical samples with detailed composition are summarized in Table 3.

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Ion Flotation of Zinc Using Ethylhexadecyldimethylammonium Bromide

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INTRODUCTION

A number of adsorptive bubble separational methods has evolved and received increased research activity in recent years. The methods describe techniques for separating dissolved or suspended materials by means of adsorption or attachment at the surface of bubbles rising through aqueous solutions. The adsorptive bubble separational techniques are generally divided into two major classes; foam separational techniques which require the generation of foam or froth to remove the undersired substances and nonfoaming techniques in which no froth is generated. Ion flotation is one of the foam separational techniques which has received a significant amount of this increased research activity recently.

The first mention of ion flotation is credited to Langmuir and Schaefer who studied the effect of metal ions on steric acid monolayers (*2*). Sebba was the first to use the ion flotation technique for the removal and separation of surface inactive inorganic ions (*8*). There are very few flotation methods for removing zinc in the literature. Oikawa *et al.* reported that zinc ions can be removed from nonferrous mine wastewater by ion flotation using *n*-monodecanoyl diethylenetraiamine (*6, 7*).

Ethylhexadecyldimethylammonium bromide (hereafter referred to as EHDABr for brevity) has been used in a number of adsorptive separational methods. Grieves investigated its utilization for the removal of a number of anions from aqueous solutions including orthophosphate, phenolate, and dichromate (*1*). Moore and Phillips determined formation constants for the reaction between the surfactant EHDABr and various anions (*5*). In our laboratory, we have used EHDABr to develop ion flotation methods for the removal of copper (*3*) and cadmium (*4*) from aqueous solutions. This research paper describes an ion flotation method for the removal of zinc ions from aqueous solutions using EHDABr.

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MATERIALS AND METHODS

Apparatus. A Perkin-Elmer Model 360 atomic absorption spectrophotometer was used to analyze the solutions for zinc. The accessories included a standard cadmium hollow cathode lamp and a Houston Instruments Model 5110 recorder. An ion flotation apparatus was constructed consisting of an air filter assembly capable of removing the particulate matter in the laboratory compressed air line down to 0.6 μm or less, a Pyrex glass flotation column (3.8 × 122 cm), a medium porosity Pyrex dispersion tube, and a Rodger Gilmont flow meter assembly. A Corning Model 199 pH meter with digital display was used for the pH measurements.

Reagents. Technical grade ethylhexadecyldimethylammonium bromide (EHDABr) was obtained from the Eastman Kodak Company and used without further purification. A 0.10 *M* EHDABr stock solution was prepared by dissolving the appropriate quantity of the reagent in the desired volume of deionized water. Aqueous standard zinc (1000 ppm) for atomic absorption spectrophotometric analysis was obtained from the J. T. Baker Company.

Evaluation procedure. A typical flotation procedure involves mixing 12.5 ml of 100 ppm zinc with 42 ml of 12 *M* HCl in a 250-ml volumetric flask and diluting the resulting solution to volume with deionized water. A 15-ml aliquot is removed and analyzed for zinc at 213.9 nm with the atomic absorption spectrophotometer. The air flow rate is adjusted to the desired value prior to transferring the aqueous zinc solution to the flotation column. Flow rates of 40 to 50 ml/min proved to be optimum with the zinc solution in the column; 6 ml of 0.10 *M* EHDABr is injected into the column with a syringe to initiate the metal removal process. The flotation process is allowed to proceed for 150 min after which a 15-ml aliquot is removed and analyzed for zinc with the atomic absorption spectrophotometer at 213.9 nm.

RESULTS

A number of variables which affect the efficiency by which zinc ions are floated from aqueous solution using EHDABr were studied by use of the evaluation procedure described in the preceding section. The efficiency of zinc removal can be conveniently expressed by the following equation:

$$F = \frac{C_b - C_a}{C_b} \times 100$$

where F is the percentage zinc floated, C_b is the zinc concentration before flotation, and C_a is the zinc concentration after flotation.

A study was undertaken to determine the flotation efficiency of zinc as

TABLE 1
EFFECT OF HCl CONCENTRATION ON THE FLOTATION OF ZINC

HCl concentration (M)	Efficiency of removal (%)
1.0	63.89
1.5	89.67
2.0	95.03
2.5	90.21
3.0	91.30

Note: EHDABr = 2.5×10^{-3} M; time = 150 min; Zn^{2+} = 5 ppm; flow rate = 50 ml/min.

a function of acid concentration. The results shown in Table 1 indicate that the metal ions is floated efficiently only in highly acidic solutions. A 2.0 M HCl solution was used in the evaluation procedure.

The study of the effect of surfactant concentration is shown in Table 2. The results indicate that a 2.1×10^{-3} M EHDABr solution is needed to remove more than 90% of the zinc from the solution in 150 min. This represents a surfactant to zinc ratio of 27 to 1. Higher EHDABr to zinc ratios do not increase the flotation efficiency significantly.

A study of the effect of time on flotation of zinc is shown in Table 3. It indicates that 91.7% of the zinc is removed after 90 min at a flow rate of 50 ml/min. By increasing the time to 150 min while keeping the flow rate constant, the percentage flotation is increased to 94.8%. Longer time periods did not improve the flotation efficiency. A 150-min time period was chosen for the evaluation procedure.

An investigation was undertaken to determine the optimum flow rate for flotation of zinc. The total volume of air passed through the solutions was kept constant at 6 liters while the time and flow rate were varied. The results shown in Table 4 indicated that there is a very small difference in

TABLE 2
EFFECT OF THE CONCENTRATION OF EHDABr ON THE FLOTATION OF ZINC

Concentration of EHDABr (M)	Efficiency of removal (%)
4.26×10^{-4}	45.10
8.51×10^{-3}	63.29
1.28×10^{-3}	80.46
1.70×10^{-3}	83.08
2.13×10^{-3}	91.23
2.55×10^{-3}	95.23
2.98×10^{-3}	94.29

Note: Cl^- = 2 M; time = 150 min; Zn^{2+} = 5 ppm; flow rate = 50 ml/min.

TABLE 3
EFFECT OF TIME ON THE FLOTATION OF ZINC

Time (min)	Efficiency of removal (%)
30	57.73
60	65.68
90	91.72
120	92.08
150	94.80

Note: EHDABr = 2.5×10^{-3} *M*; Zn^{2+} = 5 ppm; Cl^- = 2 *M*; flow rate = 50 ml/min.

flotation efficiency of only 4% for flow rates of 20 to 100 ml/min. It is advantageous to use high flow rates to conserve time. Flow rates of 40 and 50 ml/min were chosen for the evaluation procedure although higher flow rates can be used without a significant loss in flotation efficiency.

An interference study was undertaken to determine if a number of metallic ions which often occur with zinc in nature would lower its flotation efficiency. The results shown in Table 5 indicate that none of the copper and lead ions was removed under the experimental conditions. Some 97.5% of the cadmium was removed with very little effect on the flotation of zinc.

DISCUSSION

A mechanism for the formation of the surface active zinc sublate is as follows:

$$EHDABr \rightleftharpoons EHDA^+ + Br^-$$
$$Zn^{2+} + 4Cl^- \rightleftharpoons ZnCl_4^{2-}$$
$$ZnCl_4^{2-} + 2EHDA^+ \rightleftharpoons (EHDA)_2(ZnCl_4)$$

TABLE 4
EFFECT OF FLOW RATE ON THE FLOTATION OF ZINC AT CONSTANT TOTAL VOLUME

Flow Rate (ml/min)	Time (min)	Volume of air (ml)	Efficiency of removal (%)
20	300	6000	91.90
30	200	6000	93.62
40	150	6000	96.01
50	120	6000	93.63
60	100	6000	93.35
80	75	6000	93.66
100	60	6000	92.74

Note: EHDABr = 2.5×10^{-3} *M*; Zn^{2+} = 5 ppm; Cl^- = 2 *M*.

TABLE 5
EFFECT OF FOREIGN METAL IONS ON THE FLOTATION OF ZINC

Foreign ion present	Foreign ion concentration (ppm)	Foreign ion removed (%)	Efficiency removal (%) of zinc
None	—	—	95.98
Cd^{2+}	5.00	97.60	92.51
Cu^{2+}	5.00	0.00	94.54
Pb^{2+}	5.00	0.00	94.03
Ni^{2+}	5.00	5.22	92.75

Note: EHDABr = 2.5×10^{-3} *M*; time = 150 min; Cl^- = 2 *M* flow rate = 40 ml/min.

The investigation shows that zinc ions can be floated efficiently from aqueous solutions using EHDABr. The method is simple and rapid if high flow rates are utilized. However, the high concentration of HCl necessary for efficient flotation limits the method's potential for industrial applications for removal of zinc ions from polluted wastewater. However, it can be considered for removal of both cadmium and zinc ion from industrially generated wastewater containing brine.

SUMMARY

Zinc ions react with the surfactant ethylhexadecyldimethylammonium bromide (EHDABr) to form a surface active sublate which can be removed from aqueous chloride solutions by ion flotation. A typical ion flotation procedure involves passing air through a 235-ml solution containing 5 ppm Zn^{2+}, 2.0 *M* HCl, and 2.5×10^{-3} *M* EHDABr at a flow rate of 40 ml/min for 150 min. The procedure is simple and rapid. Cadmium, copper, lead, and nickel ions cause reductions of zinc flotation efficiencies of less than 2.5% under the experimental conditions.

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Identification of Glucan Synthetase Glycosidically Bound to Amylopectin from Cotton Leaf Starch

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INTRODUCTION

Two forms of glucan synthetase, soluble (*3, 10–13, 22, 23*) and starch-granule bound (*1, 4, 17, 20*), have been reported. Starch-granule bound glucan synthetase in these preparations (*17, 20*) was isolated by homogenizing leaves or seeds in cold water followed by repeated sedimentation of starch granules by differential centrifugation at about 350*g*. Possible contamination of starch granules with soluble glucan synthetase however was not elucidated. Recently Chang (*7*) has successfully isolated starch-granule bound glucan synthetase, which is free from soluble glucan synthetase. Glucan synthetase bound to starch granules strongly resisted successive extractions with 0.1 *M* Na-phosphate buffer at pH 7.5. The mode of binding of this enzyme bound to starch granules, however, was not investigated (*1, 4, 7, 17, 20*).

The purposes of the present communication were (a) to clarify the association of glucan synthetase proteins with starch components and (b) to identify the type of linkages between enzyme proteins and glucan moieties in cotton starch.

MATERIALS AND METHODS

Glandless cotton plants (*Gossypium hirsutum* L. cv. Coker 100) were grown in a growth chamber as previously described (*8*).

Starch-granule bound glucan synthetase was isolated by the procedure of Chang (*7*). Glucan synthetase was assayed according to Tanaka and Akazawa (*23*) with minor modifications. Standard reaction mixture contained (in μmoles except amylopectin and enzyme): glycine–NaOH buffer, pH 8.5, 25; EDTA, 0.2; glutathione, 2.5; KCl, 5; ADP-glucose or UDP-glucose, 0.25; amylopectin, 0.25 mg, and enzyme suspension or solution, 0.1 ml in a total of 0.25 ml. After incubation at 30°C for 10 min

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the reaction was terminated by immersing the mixture in a boiling water bath for 1 min. The amount of ADP or UDP liberated was determined by the pyruvate kinase method (*17*).

Alkaline hydrolysis of starch-granule bound glucan synthetase was conducted according to the procedure of Butler and Cunningham (*5*) with minor modifications. Starch granules from 16 g of fresh cotton leaves were dissolved in 2 ml of 2 *N* NaOH by constant stirring and hydrolyzed for 2 hr at 90°C in a test tube covered with a glassbead. The mixture was neutralized with 2 *N* HCl. The hydrolysate was dialyzed overnight against 2 liters of 0.01 *M* Na-phosphate buffer at pH 7.2 containing 2.5 m*M* EDTA. The material was cleared by centrifugation at 12,100*g* for 10 min at room temperature. The clear supernatant was saved for Con A-Sepharose column chromatography.

Separation of the alkaline hydrolysate into amylose, amylopectin, and proteins was carried out by use of a Con A-Sepharose as previously described (*6*) with minor modifications. Amylopectin-bound protein was eluted with 0.1 *M* borate buffer at pH 6.2 or 0.1 *M* MMP followed by 1 *M* NaCl.

Acid hydrolysis of amylopectin-bound proteins from alkaline hydrolysate was conducted in 2 *N* HCl according to the procedure of Butler and Cunningham (*5*).

Amylolysis of starch-granule bound glucan synthetase was carried out by suspending the freshly isolated starch granules in 2.0 ml of 0.06 *M* KH_2PO_4 solution containing one drop of 0.1 *M* $CaCl_2$ and 5.0 mg of α-amylase (Calbiochem[1]). The mixture was incubated at room temperature by shaking for 20 min. The hydrolysate was cleared by centrifugation at 20,200*g* for 25 min. The clear supernatant was saved for chromatography on a column of Sephadex G-25 for separation of sugars and proteins.

RESULTS AND DISCUSSIONS

As shown in Fig. 1, the alkaline hydrolysate of unwashed starch granules was resolved into two fractions of proteins and two regions of glucans (amylose and amylopectin). The protein eluted in the earlier fraction (tubes 37–50) was partially separated from amylose (tubes 23–52), but the protein eluted in the later fraction (tubes 87–120) completely overlapped amylopectin. To determine the effects of salt washings on the polymers, the starch granules were extracted repeatedly with buffer containing 0.9% NaCl (w:v) and hydrolyzed in 2 *N* NaOH. The chromatographs of alkaline hydrolysates were characterized by decreasing protein

[1] Mention of a trade name, proprietary product, or specific equipment does not constitute a guarantee or warranty by the USDA and does not imply its approval to the exclusion of other products that may be suitable.

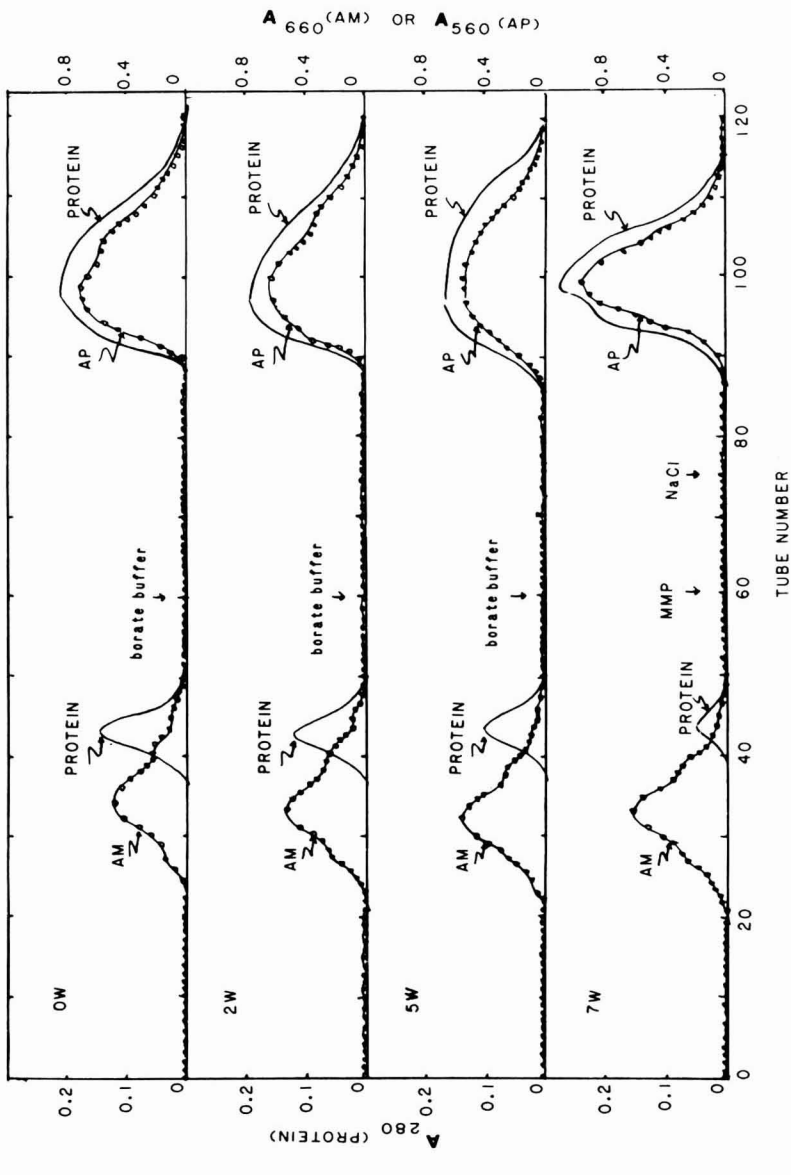

FIG. 1. Separation of a mixture containing amylose (AM), amylopectin (AP), and proteins by use of a column (2.2 × 22 cm) of Con A-Sepharose. Each of four aliquots of starch granules was prepared from 16 g of fresh cotton leaves according to the standard procedure (*7*). This was designated as unwashed starch granules (0W). The second aliquot (2W) was treated by washing the unwashed starch granules (0W) by centrifugation (12,000*g* for 5 min) twice with 0.02 *M* Na-phosphate buffer at pH 7.2 containing 0.9% NaCl (w:v). Similarly the third and fourth aliquots were washed five times (5W) and seven times (7W), respectively. Residual starch granules from the washings were hydrolyzed with NaOH as described in Materials and Methods. A sample containing about 65 A_{660} units was loaded on the column. The column was eluted to tube 60 with 0.9% NaCl (w:v) in 0.02 *M* Na-phosphate buffer (pH 7.2) and was then eluted with 0.1 *M* Na-borate buffer at pH 6.2 (0W, 2W, 5W) or 0.1 *M* **MMP** (methyl-α-D-manophyranoside) followed by 1 *M* NaCl (7W). The column was run at room temperature, the flow rate was 0.35 ml/min and each fraction contained 2.0 ml. Amylose and amylopectin in each tube were identified by the procedure of Kovacs and Hill (*16*). Protein was identified by the A_{280} on the chart (0.25 A/span and 0.1 in./min).

content in tubes 37–50 accompanied by apparently constant amounts of amylose. Also the tube 34 containing maximum amylose was widely separated from the tube 43 containing maximum protein in each chromatograph (2W, 5W, and 7W). This demonstrated that the proteins (tubes 37–50) were ionically bound to starch granules and not covalently linked with amylose. Therefore this linear glucan was not retained to the column under the conditions (the first elution buffer in Fig. 1) which were known to lead to adsorption of branched chain amylopectin as previously observed (*6*). In contrast, the binding of protein to amylopectin (OW) survived repeated washings with NaCl and resisted alkaline hydrolysis (2W, 5W, and 7W). Therefore, this complex was retained to the column under the conditions favorable for adsorption of branched polysacchardies and discharged from the column with borate buffer.

As shown in Fig. 2, the protein and amylopectin which were eluted as two overlapping regions (Fig. 1) were completely separated into two independent polymers by chromatography on the column of Con A-Sepharose after acidic hydrolysis. This indicated that the two polymers were linked with bonds (Fig. 1), which, though resistant to alkaline hydrolysis, were labile to acidic hydrolysis. The protein was eluted in the earliest fractions following the void volume, whereas amylopectin formed an insoluble complex with the lectin and was desorbed with borate buffer.

As shown in Fig. 3, the amylolytic hydrolysate of glucan synthetase-bound starch granules was resolved into soluble carbohydrates (including reducing sugars) and proteins by chromatography on the column of Sephadex G-25. The regions of starch degradation products were free from proteins. Also, glucan synthetase activity was successfully identified within the area of protein peak, although it was contaminated with α-amylase protein. This indicated that the proteins associated with amylopectin (Fig. 1) were certainly glycosidically linked and contained glucan synthetase activity.

As shown in Table 1, more protein was extracted as the wash number increased (2E, 5E, and 7E). This protein was free from glucans and did not contain glucan synthetase activity. Therefore, the protein (tubes 37–50 in Fig. 1) did not contain glucan synthetase. In contrast, the residual starch granules (0R, 2R, 5R, and 7R) contained nearly constant amounts of protein, glucan synthetase activity, and glucan regardless of repeated washings. Therefore, the proteins associated with amylopectin (0W, 2W, 5W, and 7W in Fig. 1) resisted the salt washings and were only proteins which contained glucan synthetase activity.

Concanavalin A (Con A), a lectin (phytohemagglutinin), is known to interact specifically with branched chain polysaccharides to form an insoluble complex (*15*). This affinity chromatography also successfully

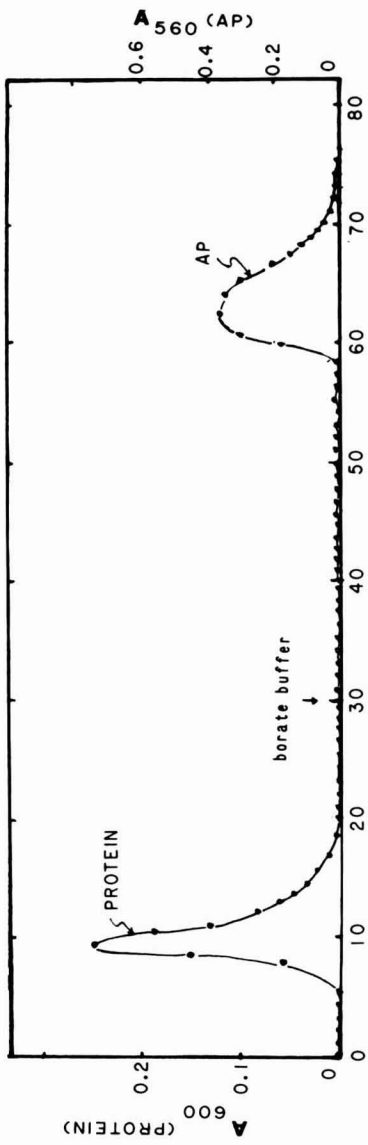

FIG. 2. Fractionation of amylopectin (AP) and protein by use of a column (1.2 × 12 cm) of Con A-Sepharose following acid hydrolysis. The region containing proteins bound with amylopectin (tubes 87–120, 0W in Fig. 1) was lyophilized and dissolved in 2 ml of 2 *N* HCl. The mixture was hydrolyzed at 110°C for 30 min in a test tube covered with a glassbead (*5*). The hydrolysate was neutralized and loaded on the column. The column was eluted to tube 30 with 0.02 *M* Na-phosphate buffer at pH 7.2 containing 0.9% NaCl and was then eluted with 0.1 *M* Na-borate buffer at pH 6.2. Other column conditions and polymer identification procedures were referred to Fig. 1 except that protein was identified by the procedure of Lowry *et al.* (*19*).

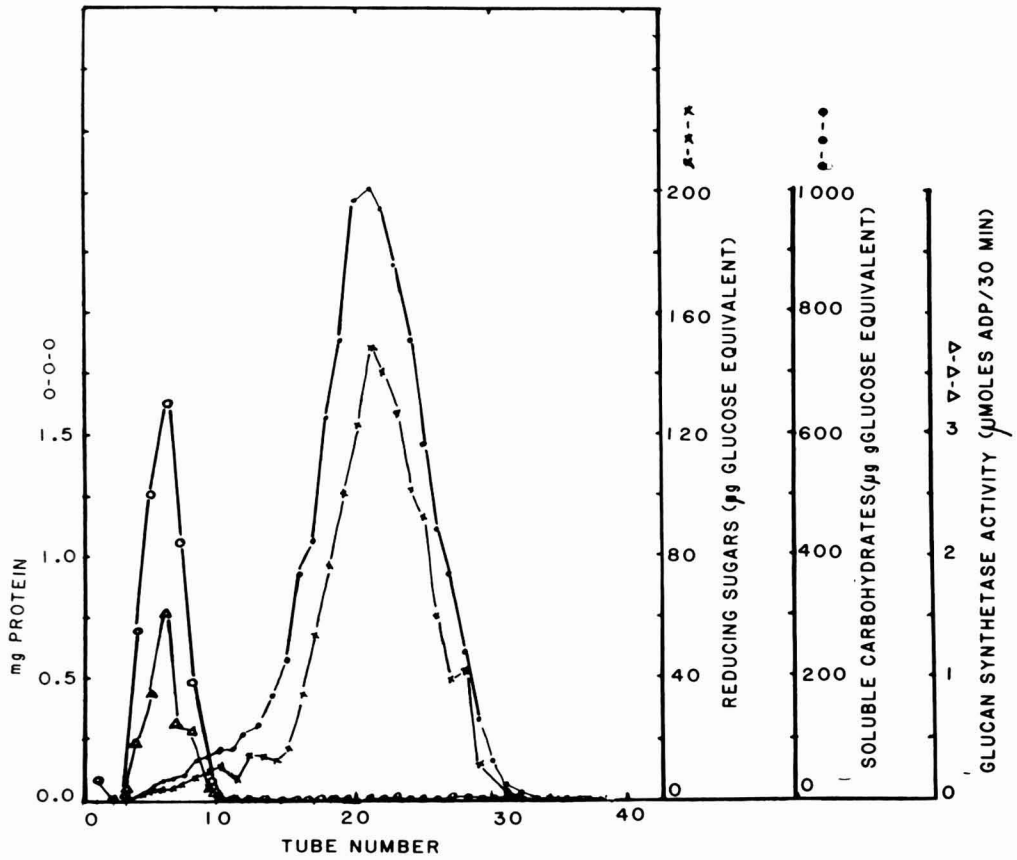

FIG. 3. Separation of soluble carbohydrates, reducing sugars, and glucan synthetase protein by use of a column (2.2 × 22 cm) of Sephadex G-25. The hydrolysate of starch-granule bound glucan synthetase incubated with α-amylase (Calbiochem[1]) was prepared as described in Materials and Methods. The sample equivalent to about 62 A_{660} was loaded on the column, which was then eluted with water. Total soluble carbohydrates, reducing sugars, and proteins were identified by the procedures of Hodge and Hofreiter (*14*), Nelson (*21*), and Lowry *et al.* (*19*), respectively. The procedure of Tanaka and Akazawa (*23*) was used to liberate ADP or UDP in glucan synthetase assay. The amount of ADP or UDP was determined by the procedure of LeLoir and Goldemberg (*18*). The column was operated at about 4°C, the flow rate was 0.40 ml/min, and each fraction contained 2.0 ml.

separated amylopectin (*6*) and glycoproteins (*2*) from amylose. The associated proteins and amylopectin eluted by chromatography on this similar column material (Fig. 1) are almost certainly the amylopectin–protein complex. It is also well-known that some of the proteins glycosidically bound to carbohydrates are resistant to alkaline hydrolysis, but labile to acids (*5*). Similar properties were demonstrated by the proteins bound to amylopectin in cotton starch (Figs. 1 and 2).

TABLE 1

PROTEIN, GLUCAN SYNTHETASE ACTIVITIES, AMYLOSE (AM), AND AMYLOPECTIN (AP) FROM IONIC EXTRACTS OF STARCH GRANULES AND RESIDUAL STARCH GRANULES[a]

Extraction (E) and residue (R) No.	Protein (mg)	Glucan synthetase activity (μmoles ADP released/10 min)	AM (mg)	AP (mg)
0E	0	0	0	0
2E	0.85	0	0	0
5E	1.72	0	0	0
7E	3.10	0	0	0
0R	13.92	3.32	35	115
2R	13.61	3.19	37	111
5R	14.01	3.35	37	118
7R	13.80	3.29	32	105

[a] Four aliquots of starch granules were prepared and washed as described in Fig. 1. The unwashed starch granules (0E) were washed twice and the extracts were combined (2E). Similarly, the combined extracts from five- and seven-time washed starch granules were prepared and designated as 5E and 7E, respectively. The residual starch granules corresponding to 0E, 2E, 5E, and 7E were also designated as 0R, 2R, 5R, and 7R, respectively. Amylose and amylopectin were determined as previously described (*8*).

α-Amylase was reported to degrade starch granules from many sources (*24*). The similar action of this enzyme on cotton leaf starch granules also was found in our laboratory. This enzyme specifically hydrolyzes α-(1-4)-glycosidic linkages (*9*). The protein bound to amylopectin (Fig. 1) and the glucan synthetase activity associated with amylopectin (Table 1) were released from starch-degradation products by α-amylase action in the experiments shown in Fig. 3.

From these experimental results, it is concluded that in cotton leaf starch, amylopectin is the only glucan glycosicically linked with protein and this protein has glucan synthetase activity. The amylose component is free of covalently bound proteins and, therefore, contains no bound glucan synthetase.

SUMMARY

Starch-granule bound glucan synthetase was isolated from cotton leaves by a sucrose density technique. Starch granules were successively washed with 0.02 *M* Na-phosphate buffer containing 0.9% NaCl. The alkaline hydrolysate of washed materials and acid hydrolysate of protein-bound amylopectin were separated by use of Con A-Sepharose. Amylolytic hydrolysate of starch granules also was carried out with α-amylase and fractionated by chromatography on Sephadex G-25. Amylopectin was the only glucan glycosidically linked with proteins, in which glucan synthetase activity was identified. The amylose component was free from covalently bound proteins.

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N,N'-Dichloro-*p*-nitrobenzenesulfonamide (Dichloramine-N) as a New Oxidimetric Reagent: Synthesis and Analytical Application

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INTRODUCTION

The availability of potential reagents in nonaqueous and partially aqueous redox titrimetry is limited. Recently, aromatic sulfonyl haloamines have drawn the attention of analytical chemists as redox titrants. The diverse nature of the chemistry of *N*-haloamines is a consequence of their ability to act as sources of species such as halonium cations, hypohalites, and *N*-anions which act as bases, nucleophiles, and nitrenoids. Consequently these reagents react with a wide variety of functional groups effecting an array of molecular transformations (*4*).

The dihaloamines such as dichloramine-T and dichloramine-B have been used as redox titrants in nonaqueous and partially aqueous media for the oxidimetric determination of a variety of compounds and their metal complexes (*6, 7, 11, 13*). The present paper reports the preparation of a new member of the series, namely, dichloramine-N (*N,N'*-dichloro-*p*-nitrobenzenesulfonamide, *p* $O_2N-C_6H_4-SO_2NCl_2$; hereafter abbreviated as DCN) which can be employed as a potential redox titrant in acetic acid medium. Determinations of some typical, yet diverse, reductants such as ascorbic acid, glutathione, methionine, indigocarmine, sodium sulfite, and sodium arsenite have been carried out with this new redox titrant by visual end point method and these results are also presented in this paper. Another significance of introducing this novel oxidant DCN is that the presence of electron withdrawing nitro group in the *para* position of the phenyl ring could facilitate those oxidation reactions involving electron-deficient transition states.

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MATERIALS AND METHODS

Dichloramine-N

DCN was prepared by the chlorination of *p*-nitrobenzenesulfonamide in alkaline medium. Pure chlorine gas was bubbled through an aqueous solution of *p*-nitrobenzenesulfonamide (10 g) in 100 ml of 4 M sodium hydroxide for over a period of about 30 min at 65°C with constant stirring of the solution. The light yellow precipitate of DCN formed was filtered under suction, thoroughly washed with water, and dried. This material was further purified by dissolving in carbon tetrachloride and cooling the filtrate to get crystals of DCN. The yield of the compound was 9.4 g (79%). The sample was stored in amber-colored bottles. The dry sample melted at 144–146°C (uncorrected) with decomposition. Thin layer chromatography (TLC) of DCN in silica gel plates (Uniplate, Analtech, Inc., Newark, DE) using the solvent system petroleum ether:chloroform:*n*-butanol = 1:1:0.5 (v/v) gave R_f = 0.60 whereas the *p*-nitrobenzenesulfonamide had R_f = 0.37.

Elemental analyses of carbon, hydrogen, nitrogen, sulfur, and chlorine and the molecular weight determination were performed by Huffman Laboratories, Inc., CO.

Found: Carbon 26.55, hydrogen 1.52, nitrogen 10.31, sulfur 11.89, and chlorine 26.35%.

Calculated: Carbon 26.60, hydrogen 1.49, nitrogen 10.33, sulfur 11.82, and chlorine 26.15%.

The active chlorine present in DCN determined iodometrically was 25.90% which is in close agreement with the data obtained by elemental analysis. The molecular weight of DCN determined by Knaur Vapor Pressure Osmometry is 272 at 30°C in 1,1-dichloromethane. This is further confirmed by the mass spectral data.

Spectral Characteristics of DCN

Ultraviolet. The ultraviolet spectrum of the compound in ethanol was obtained with a Beckman DK-2A ratio recording dual beam spectrophotometer (Fig. 1). DCN has a λ_{max} at 255 nm (ξ_{max} = 9151).

Infrared. The infrared spectrum (KBr) was recorded on a Perkin-Elmer 298 grating IR spectrophotometer. The IR spectrum (*1–3, 5, 9, 10*) of DCN (Fig. 2) shows bands (in cm^{-1}) at 3100 (medium, aromatic ν-CH), 1930 (weak, ν-NC1), 1600 (strong, phenyl ring), 1508 (strong, ν_{sym}-NC1; ν_{asym}-NO_2), 1338 (strong, ν_{sym}-NO_2), 1304 (shoulder, ν_{asym}-SO_2), 1162 (strong, ν_{sym}-SO_2), 1075 (medium, aromatic CH in plane bending), 1004 (weak, ν-SN), 848 (strong, νC-N), 764, 728 (doublet, C–N–O bending and out of plane CH bending of two adjacent aromatic H atoms), and 670 (strong, in plane ring deformation).

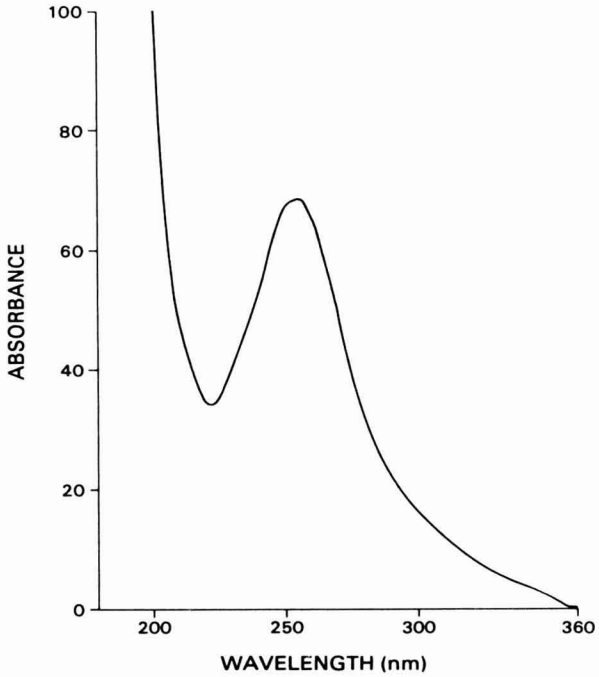


FIG. 1. Ultraviolet spectrum of *N, N'*-dichloro-*p*-nitrobenzenesulfonamide (20 μg/ml) in ethanol at room temperature.

Nuclear magnetic resonance. The nuclear magnetic resonance (NMR) spectra of DCN were carried out in $CDCl_3$ solvent using tetramethylsilane (TMS) as the internal standard. The ^1H spectrum (Varian 60 MHz Nuclear Magnetic Resonance Spectrometer) shows a quadruplet centered around 8.4δ with coupling constant $J = 8.0$ Hz, thus indicating the presence of *ortho* and *meta* protons. However, the ^1H-Fourier transformation spectrum (Varian 80 MHz FT-80A Nuclear Magnetic Resonance Spectrometer) shows a fine structure indicating an A_2B_2 pattern.

The ^{13}C-FT-NMR spectrum (Varian 80 MHz FT-80A Nuclear Magnetic Resonance Spectrometer) shows signals (ppm) at: 145.62 (C-1 atom attached to S atom), 132.91 (C-2,6), 134.66 (C-4 atom attached to N atom of the NO_2 group), and 124.30 (C-3,5). In this ^{13}C spectrum, the deshielding effect of the substituent nitro group is evident as all carbon resonances occur at lower fields in comparison to other haloamines.

Mass spectrometry. The electron impact mass spectrum of DCN was obtained on a DuPont 21-291 mass spectrometer using 70 eV electrons with source and probe temperatures at 250 and 50°C, respectively. It has peaks at *m/e* 270 (23%, M^+), 235 (96, M^+-Cl), 200 (100, M^+-Cl_2), 186 (100, M^+-NCl_2), 148 (100, $Cl_2NSO_2^+$), and 122 (100, $O_2NC_6H_4^+$).

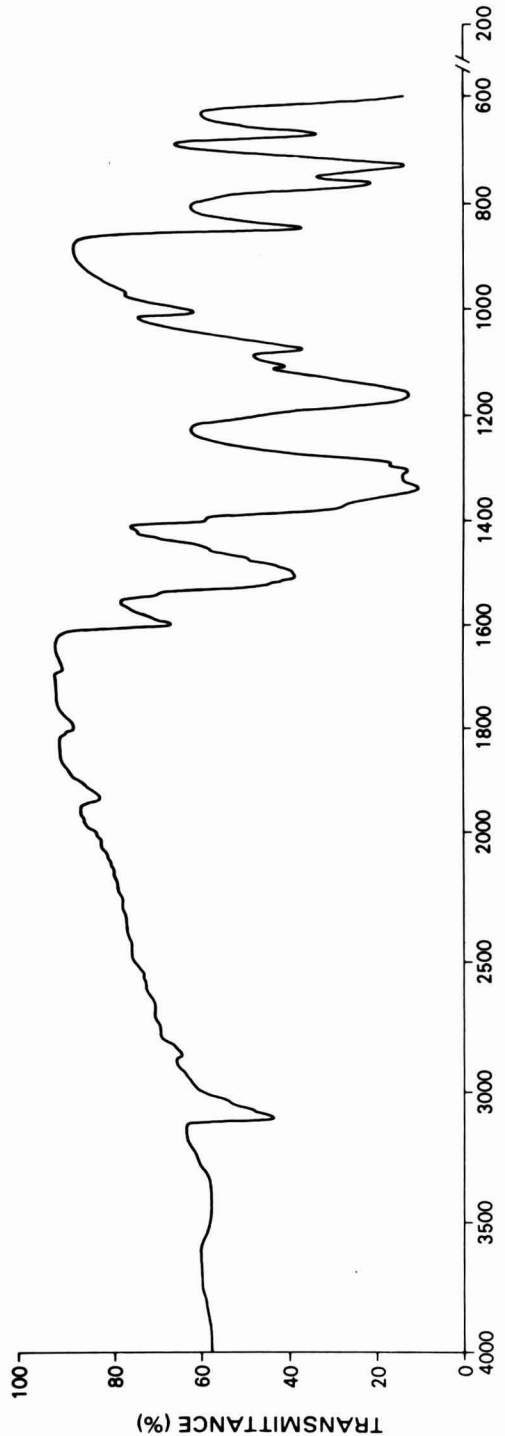


FIG. 2. Infrared spectrum of *N*,*N*′-dichloro-*p*-nitrobenzenesulfonamide in KBr at room temperature.

Preparation, Standardization, and Stability of Stock Solutions of DCN

Although DCN is sparingly soluble in water, it is fairly soluble in common organic solvents such as glacial acetic acid. Solubilities in water and glacial acetic acid were 0.103 and 47.72 g per kg of the solvent at 30°C, respectively.

Approximately 0.025 *M* (~0.1 *N*) solutions of DCN were prepared by dissolving 6.78 g of the sample in a liter of glacial acetic acid containing 5% acetic anhydride. DCN solutions were found to decompose slightly when stored in colorless bottles exposed to light or kept in the dark and hence they were preserved in amber-colored bottles. These solutions were found to be fairly stable. The normality of a typical stock solution over a period of 16 days is given in Table 1. Approximately 0.005 *M* (~0.02 *N*) solution of DCN was prepared by the appropriate dilution of the above stock solution with glacial acetic acid containing 5% acetic anhydride. Standardization of DCN solutions was carried out by adding excess amount of 10% aqueous KI solution to known volumes of the oxidant and titrating the liberated iodine with a standard thiosulfate solution to a starch end point.

Reductant Solutions

The compounds used were of acceptable analytical reagent grade. Solutions of reduced glutathione (Aldrich Chemical Co.) and *d*-isoascorbic

TABLE 1
STABILITY OF DICHLORAMINE-N SOLUTIONS IN ACETIC ACID MEDIUM

	Number of days							
	0	1	2	3	4	6	8	16
Normality of solution (kept in amber-colored bottle in dark)	0.1047	0.1047	0.1047	0.1047	0.1047	0.1047	0.1047	0.1047
Normality of solution (kept in amber-colored bottle in daylight)	0.1047	0.1047	0.1047	0.1047	0.1047	0.1046	0.1047	0.1047
Normality of solution (kept in colorless bottle in dark)	0.1047	0.1042	0.1042	0.1038	0.1034	0.1029	0.1021	0.0994
Normality of solution (kept in colorless bottle in daylight)	0.1047	0.1042	0.1042	0.1038	0.1029	0.1025	0.1012	0.0973

acid (Eastman Kodak Co.) (~4 mg/ml), and DL-methionine (~3 mg/ml) (Pfaltz and Bauer, Inc.) were prepared by dissolving the solids in distilled water. Solutions (~4 mg/ml) of sodium sulfite (Baker Chemical Co.) and sodium arsenite (Fisher Scientific Co.) were prepared in deaerated (saturated with nitrogen) distilled water. Aqueous solution of indigocarmine (~0.5 mg/ml) (Aldrich Chemical Co.) was prepared and its strength checked by the chloramine-T and dichloramine-T methods (*7*).

Aqueous solutions of $NaHCO_3$ (1 *M*), KI (10%), KBr (10%), and starch (1%) were prepared from compounds of acceptable grades of purity.

Recommended Determination Procedure

To an aliquot of the aqueous reductant solution (ascorbic acid or glutathione) were added 0.5 ml of KI solution (10%), 1 ml of starch (1%), and enough distilled water to give a total volume of 50 ml. The solution was titrated with 0.02 *N* DCN to the appearance of a permanent pale blue color. This procedure has to be modified suitably for the determination of sulfite and arsenite.

Oxidation of Sulfite

The procedure was completely analogous except that the solution of sulfite was bubbled with nitrogen and the bubbling was continued during the addition of starch-KI indicator solution and deaerated distilled water and also during the reaction.

Oxidation of Arsenite

The procedure was analogous to that for sulfite except that 50 ml of 1 *M* $NaHCO_3$ is added to the arsenite solution instead of distilled water which maintains the pH around 4.3 during the course of the reaction.

Oxidation of Indigocarmine

The starch–iodide method failed for indigocarmine. To a known volume of an aqueous solution of indigocarmine, 1 ml of 10% potassium bromide, and 10–20 ml of water were added and the solution was then titrated with 0.02 *N* DCN until the color completely turns yellow, indicating its conversion to isatinsulfonate.

Oxidation of Methionine

To an aliquot of the aqueous solution of methionine, ~1 ml of 10% potassium bromide, 0.1–0.2 ml of indigocarmine indicator (0.05%), and enough water to give 50 ml total volume were added. The resultant solution was titrated with 0.02 *N* DCN to the appearance of a pale yellow

color. Blank was run concurrently with the same amount of the indicator solution.

The amount (X mg) of the reductant in the sample solution is given by $X = NVM/E$ where N and V are the normality and volume of the DCN solution respectively, M is the molecular weight of the reductant, and E is the electron-change per mole of the reductant ($E = 2$ for ascorbic acid, methionine, sulfite, and arsenite; 1 for glutathione and 4 for indigocarmine).

Product Analyses

The reaction mixture of each reductant (ascorbic acid, methionine, glutathione, and indigocarmine) was analyzed for the products as described below.

In the case of ascorbic acid ($R_f = 0.55$), the products dehydroascorbic acid ($R_f = 0.72$) and *p*-nitrobenzenesulfonamide ($R_f = 0.89$) were identified by TLC in ethanol: 10% acetic acid = 90:10 (v/v) using silica gel plates (Uniplate, Analtech, Inc., Newark, DE). These R_f values of ascorbic and dehydroascorbic acids compare well with those reported in the literature (*12*).

Paper chromatography was carried out to detect the presence of oxidized glutathione (disulfide) (*8*) and methionine sulfoxide (*6*) in the reaction mixtures from glutathione and methionine, respectively. Phenol saturated with water was used as the solvent system for the identification of oxidized glutathione ($R_f = 0.088$) whereas benzyl alcohol saturated with water served as the solvent system for methionine sulfoxide ($R_f = 0.67$). In both the cases, the spray reagent employed was ninhydrin.

The reaction mixture of indigocarmine had yellow color with $\lambda_{max} = 410$ nm confirming the presence of isatinsulfonate as the product formed (*7*). The blue solution of indigocarmine has a $\lambda_{max} = 605$ nm.

RESULTS AND DISCUSSION

Table 2 gives some typical results showing the consumption of DCN per mole of the reductant. The deviation was calculated from four replicates. The results of range studied and percentage error in recovery have been presented in Table 3.

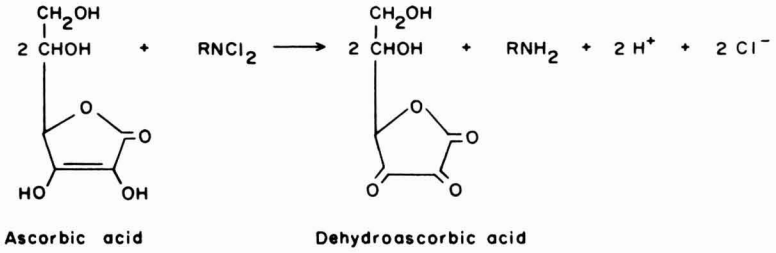
It follows from these Tables 2 and 3 that these oxidations proceed quantitatively and stoichiometrically with the consumption of two equivalents of the oxidant per mole of each of ascorbic acid, methionine, sulfite, and arsenite giving dehydroascorbic acid, methionine sulfoxide, sulfate, and arsenate, respectively. The stoichiometries of these reactions are represented by Eqs. (1)–(4).

TABLE 2
OXIDATION OF SOME REDUCTANTS WITH DICHLORAMINE-N

Reductant	Reductant taken (mmole)	DCN consumed (meq)	Reductant found[a] (mmole)	Standard error[b] (μmole)
Ascorbic acid	0.0733	0.1467	0.0733	0.09
Methionine	0.1018	0.2042	0.1021	0.27
Sulfite	0.1488	0.2983	0.1491	0.26
Arsenite	0.1587	0.3181	0.1590	0.33
Glutathione	0.1350	0.1357	0.1357	0.64
Indigocarmine	0.0248	0.0989	0.0247	0.12

[a] Calculated based on the assumption that the numbers of equivalents of DCN consumed per mole of the reductant are 1 for glutathione, 2 for each of ascorbic acid, methionine, sulfite, and arsenite, and 4 for indigocarmine.

[b] Calculated for four replicates.



(1)

$$CH_3SR' + \tfrac{1}{2}\,RNCl_2 + H_2O \rightarrow CH_3S(=O)R' + \tfrac{1}{2}\,RNH_2 + H^+ + Cl^- \quad (2)$$

$$2\,Na_2SO_3 + RNCl_2 + 2\,H_2O \rightarrow 2\,Na_2SO_4 + RNH_2 + 2\,H^+ + 2\,Cl^- \quad (3)$$

$$2\,Na_3AsO_3 + RNCl_2 + 2\,H_2O \rightarrow 2\,Na_3AsO_4 + RNH_2 + 2\,H^+ + 2\,Cl^- \quad (4)$$

where $R = p\text{-}O_2N\text{-}C_6H_4\text{-}SO_2\text{-}$ and $R' = -CH_2-CH_2-\underset{\displaystyle NH_2}{\underset{|}{CH}}-COOH$.

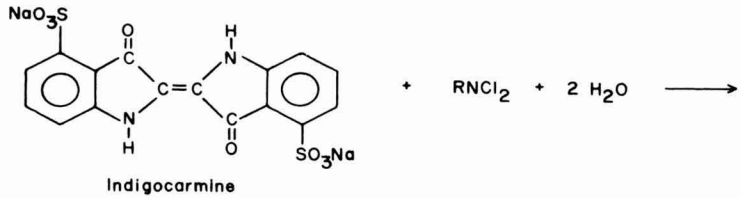
The oxidation of glutathione (GSH) to the disulfide and indigocarmine to isatinsulfonate under these conditions involves one-electron and four-electron changes, respectively, which can be stoichiometrically represented as in Eqs. (5) and (6).

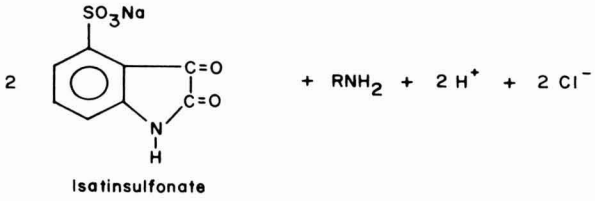
$$4\,GSH + RNCl_2 \rightarrow 2\,GSSG + RNH_2 + 2\,H^+ + 2\,Cl^- \quad (5)$$

TABLE 3
DETERMINATION OF SOME REDUCTANTS WITH DICHLORAMINE-N

Reductant	Range studied[a] (mg)	Percentage error in recovery
Ascorbic acid	4.3–107.5	0.4–0.1
Methionine	4.5–76.0	0.5–0.2
Sulfite	3.8–82.7	0.5–0.1
Arsenite	8.2–103.0	0.9–0.2
Glutathione	12.5–103.8	0.8–0.1
Indigocarmine	3.9–19.3	1.3–0.5

[a] About 8 aliquots of the reductant solution were taken in the range.





where G = HOOC–CH(NH$_2$)–CH$_2$–CONH–CH(CH$_2$–)–CONH–CH$_2$–COOH. (6)

In the case of sulfite and arsenite it is necessary to carry out the reaction in an inert atmosphere, as these compounds are susceptible to aereal oxidation which results in decrease in the amount of the compound recovered. In addition, as the end point in the arsenite titration is not sharp, addition of a large excess of $NaHCO_3$ becomes necessary to get a clear end point. Addition of $NaHCO_3$ seems to create a buffer medium (pH around 4.2) suitable for the reaction to proceed instantaneously.

Although DCN directly interacts with ascorbic acid, glutathione, sulfite, and arsenite, it is likely that in the case of methionine and indigocarmine the actual oxidizing species is bromine formed *in situ* by the reaction of added bromide with the oxidant.

$$RNCl_2 + 4\ H^+ + 4\ e^- \rightarrow RNH_2 + 2\ H^+ + 2\ Cl^- \quad (7)$$

$$RNCl_2 + 4\ H^+ + 4\ Br^- \rightarrow RNH_2 + 2\ Br_2 + 2\ H^+ + 2\ Cl^- \quad (8)$$

In all these reactions, DCN is reduced to *p*-nitrobenzenesulfonamide.

SUMMARY

A new oxidimetric reagent, dichloramine-N (*N,N'*-dichloro-*p*-nitrobenzenesulfonamide; DCN) has been synthesized and its structure elucidated from the spectral data and elemental analyses. The ultraviolet, infrared, nuclear magnetic resonance, and mass spectral data have been presented. Analytical applications of DCN as a potential redox titrant in the determination of diverse reductants such as ascorbic acid, methionine, sulfite, arsenite, glutathione, and indigocarmine have been described.

ACKNOWLEDGMENTS

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Thermal Analysis of Neuroleptic Drugs and Vitamins

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INTRODUCTION

Differential thermal analysis (DTA), differential scanning calorimetry (DSC), thermogravimetry (TG), and differential TG (DTG) have found wide-spread use in pharmacy (*1,4*). Recently, these techniques have also been used in pharmaceutical analysis. Wendlandt *et al.* suggested utilization of the DTA, DSC, and TG curves for identification of the particular powders, capsules, and tablets containing analgesics (*11*), antacids (*10*), and vitamins (*2*). Margomenou-Leonidopoulou *et al.*, on the basis of the DTA, TG, and DTG curves of thermal decomposition of *N*-butylscopolamine and novalgine, suggested the possibility of quantitative analysis of both components in their mixtures (*5, 6*). In a similar manner, Paulik *et al.* determined mechanically bound and crystallization water in model powders and granulates which contained lactose, gelatin, and/or caffeine, amidopyrine, and phenacetin (*8*). On the other hand, Otto determined the content of γ-hexachlorocyclohexane in a Delitex powder by measuring DTA peak areas and by isothermal TG (*7*). Talc did not decompose over the temperature range studied.

Radecki *et al.*, based on the analysis of the thermal decomposition of a variety of drugs, active components, and vehicles contained in them and used for their manufacture, showed the possibility of utilizing the DTA, TG, and DTG curves for identification and qualitative and quantitative control of the composition of solid and soft drug formulations (*9, 12*). These studies also revealed those thermal processes which enabled the determination of the active component.

In this paper, the results of these studies in reference to drugs containing acetylsalicylic acid, 5-pyrazolone derivatives, components stimulating the central nervous system, the Aviomarin, Glutamic acid, and Laxative chocolate tablets, and vitamins are presented.

MATERIALS AND METHODS

Reagents and materials. The drugs used in this study were manufactured by Pharmaceutical Works "Polfa" (manufacturer given)—pulver

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Vibovit (Kutno), tablets Antineuralgiae, Calcium pantothenicum (25 and 100 mg), Coffan (Jelenia Góra), Asprocol, Laxative chocolate (Rzeszów), Aviomarin, Vitaminum B_6 (Kraków), Calcipiryna, Polopiryna, Polopiryna S, Pyralginum, Tabulettae antidolorosae, Tabulettae anti-influenze (Starogard Gd.), Carbromalum, Cyclobarbitalum Calcium, Reladorm (Tarchomin), Calcio-Cardiamid, Cardenosinum, Cardiamid-Coffein, Cofedon, Gardan, Pabialgin, Pyramidonum (0.1 and 0.3 g) (Pabianice), Isalgin (Warszawa), Vitaminum B_1 (3 and 25 mg) (Grodzisk Maz.), Vitaminum PP (50 and 200 mg) (Poznań), dragees Isochin, Vitaminum B compositum (Warszawa), Vitaminum B_2, Vitaminum C (0.1 and 0.2 g) (Kraków), suppositories Pabialgin, Pyramidonum (Rzeszów), ampouls D-Panthenolum (Jelenia Góra); Pharmaceutical Cooperatives "Galena" (Wrocław)—tablets Coffein Natrium benzoicum; Galenic Laboratory "Cefarm" (Gdańsk)—tablets Glutamic acid.

Both the active components and vehicles of these drugs, with regard to purity, conformed to criteria for pharmaceutical substances (*3*).

Apparatus and procedure. Thermal decomposition of the drugs and materials was carried out on a F. Paulik, J. Paulik, and L. Erdey OD-130 derivatograph (MOM, Budapest). All measurements were accomplished under identical conditions: 100-mg samples were placed in Pt crucibles (9.5 mm in diameter), and heated in a furnace under atmospheric pressure at a rate of temperature increase of 5°C min^{-1} up to 600–1000°C. As the reference material, α-Al_2O_3 was used. The sensitivities used were DTA, 1/3; TG, 100; and DTG, 1/5.

All drugs, with the exception of powders and ointments, were finely powdered before analysis. One sample was made up of three suppositories, tablets, and dragees or 1–3 g of granulate. In the case of ampouls, for analysis was submitted a dry residue left after evaporation on a water bath of the solvent from three ampouls.

To detect transient decomposition products, the heating was interrupted at a predetermined temperature and the contents of the crucible were analyzed by thin-layer chromatography, infrared spectroscopy, and elemental analysis. The interruption occurred at temperatures corresponding either to horizontals or distinct inflections in the TG and DTG curves, which indicated completion of a given decomposition step. Owing to the complexity of the drugs, the results obtained in this way gave only preliminary information as to composition of the residues, and were not taken into account in further considerations.

RESULTS AND DISCUSSION

Drugs comprising acetylsalicylic acid, 5-pyrazolone derivatives, the components stimulating the central nervous system, the Aviomarin, Glutamic acid, and Laxative chocolate tablets and vitamins have complicated

chemical composition. About 49% of the drugs were found to consist of 6–11 components, whereas 51% of the drugs contained 3–5 components. It must also be mentioned that dragees contained additionally 6–11 components of the dragee mass of unknown composition. This mass constitutes as much as 38–53% of the total mass of the coated tablets.

The analysis of the composition of these drugs shows that particular drugs are physical mixtures of several or more than 10 chemical compounds differing in elemental composition, chemical structure, molecular weight, and physicochemical properties. Particular components of these mixtures occurred in a ratio ranging from 1:100 to 1:250. Occasionally, the ratio was 1:2000.

Frequently, an active principle is the main component of a drug. It is very convenient for analysis that 59% of the studied drugs contained only one active component, 17% of the drugs two active components, whereas in 24% of the drugs three or more active components were found. These facts facilitate the identification of an active component in spite of considerable influence of the remaining ingredients of the mixtures on the shape of the DTA, TG, and DTG curves of its thermal decomposition. In this situation, the obtained curves were the resultant of the complexity and differentiation in the composition of the studied drugs.

Qualitative Analysis of Drugs

The acetylsalicylic acid drugs contain organic compounds whose thermal decomposition occurs in the same temperature range. This fact made the identification of their thermal decomposition steps difficult, especially the stage due to the loss of acetic acid from the molecule of acetylsalicylic acid. This step is well developed only on thermal decomposition curves of the Polopiryna, Polopiryna S, Calcipiryna, Tabulettae anti-influenze, and Tabulettae antidolorosae tablets. The endothermic DTA peak due to this process is very useful in the identification of acetylsalicylic acid. In the Tabulettae anti-influenze, Tabulettae antidolorosae, and Antineuralgiae tablets, this peak is deformed by that of phenacetin which melts over the same temperature range.

Thermal effects due to the decomposition of caffeine, quinine hydrochloride, and carbromal were not reflected on the DTA, TG, and DTG curves of thermal decomposition of drugs containing them due to their low content. Close temperature ranges of thermal decomposition of both the acetylsalicylic and aminoacetic acids as well as the components of the Isochin dragees, distorted the TG and DTG curves of thermal decomposition of both drugs, thus making impossible the identification of any of them based on these curves. In this case, endothermic DTA peaks may only be of certain value.

However, the decarboxylation of calcium carbonate, the active

component of the Polopiryna S and Calcipiryna tablets, is distinctly marked. On the TG and DTG curves of thermal decomposition the loss in weight is observed which is accompanied by an endothermic DTA peak (Fig. 1).

Endothermic DTA peaks of drugs containing 5-pyrazolone derivatives are very useful for the identification of the particular components. Together with the TG and DTG curves of thermal decomposition of pyramidon, they allowed confirmation of its presence in the Pyramidonum (0.1 and 0.3 g), Pabialgin, Isalgin, and Gardan tablets. In a similar manner, novalgine can be identified in the Gardan and Pyralginum tablets. The identification is facilitated by the stage due to its dehydration, which is particularly distinct on the TG and DTG curves of thermal decomposition of both drugs. On the other hand, the identification of the active components contained in the Cofedon tablets is difficult due to the fact that three of them have very similar content. In view of the fact that the vehicles also undergo thermal decomposition in the same temperature range, it is difficult to confirm the presence of either of the active components based on the endothermic DTA peaks and the TG and DTG curves of thermal decomposition of the drugs, even in spite of distinct differences in their melting points.

Thermal decomposition of the suppository bases makes it practically impossible to confirm the presence of pyramidon in the Pyramidonum and Pabialgin suppositories. It is also impossible to identify allobarbital,

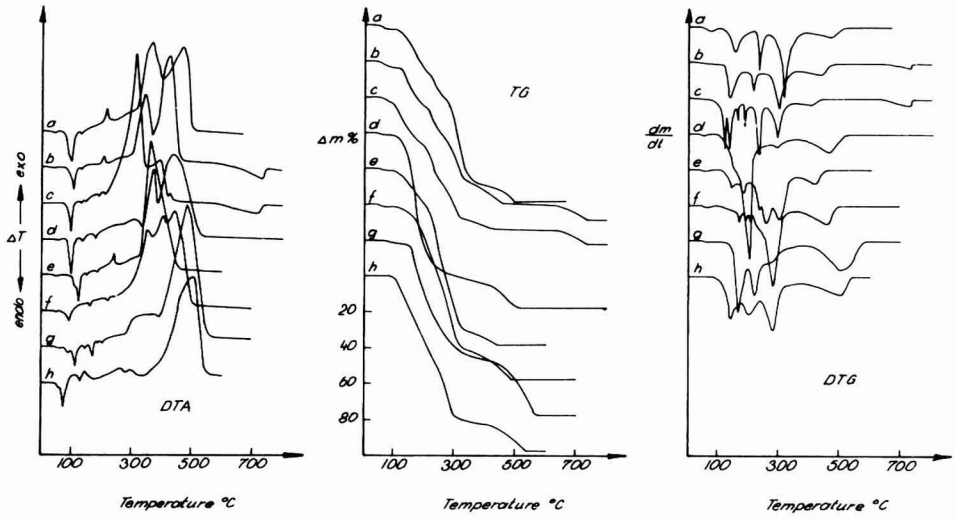

FIG. 1. DTA, TG, and DTG curves of the thermal decomposition of drugs containing acetylsalicylic acid: (a) Polopiryna (T); (b) Polopiryna S (T); (c) Calcipiryna (T); (d) Asprocol (T); (e) Tabulettae anti-influenze (T); (f) Tabulettae antidolorosae (T); (g) Isochin (D); (h) Antineuralgiae (T). For symbols in parentheses see Table 1.

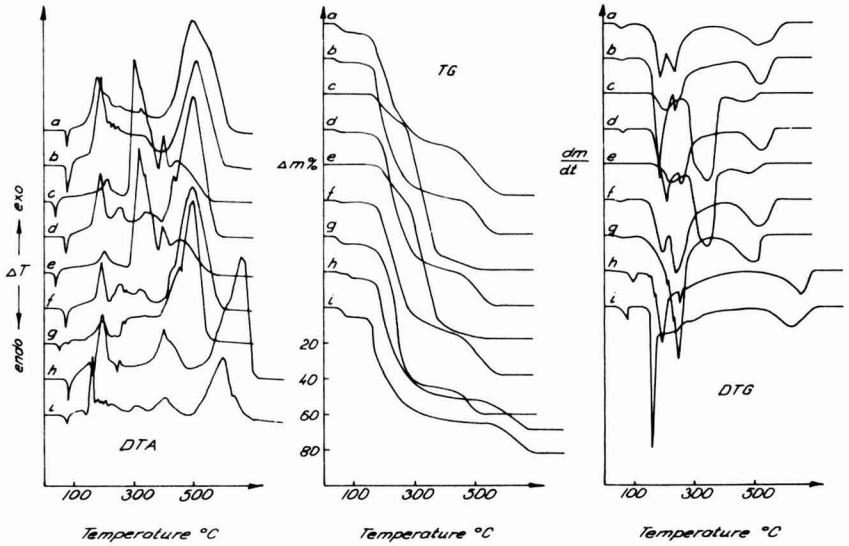

FIG. 2. DTA, TG, and DTG curves of the thermal decomposition of drugs containing 5-pyrazolone derivatives: (a) Pyramidonum (0.1 g) (T); (b) Pyramidonum (0.3 g) (T); (c) Pyramidonum (S); (d) Pabialgin (T); (e) Pabialgin (S); (f) Isalgin (T); (g) Cofedon (T); (h) Gardan (T); (i) Pyralginum (T). For symbols in parentheses see Table 2.

phenobarbital, papaverine hydrochloride, and caffeine in drugs containing them. Subtle thermal effects due to their decomposition are completely overlapped by the DTA, TG, and DTG curves of thermal decomposition of other constituents of the drugs (Fig. 2).

In the group of drugs containing the central nervous system stimulants, results of the studies on the Aviomarin tablets used as antivomiting agent, the Glutamic acid tablets—amino acid and the Laxative chocolate tablets, are also presented.

The content of the active components in these drugs (with the exception of the Coffan and Laxative chocolate tablets) exceeds 40% of the total mass. This is distinctly reflected on the DTA, TG, and DTG curves of their thermal decomposition. Particular decomposition stages of the active components are distinctly reflected on the TG and DTG curves, thus facilitating in each case identification of the active component in drugs containing them. Moreover, endothermic DTA peaks are also well developed due to melting of carbromal and glutamic acid, dehydration of calcium cardiamide, and decarboxylation of calcium carbonate, the product of decomposition of calcium cyclobarbital. It is worth noting that the shape of the DTA curves over the remaining temperature ranges reflected is very similar to that of the corresponding DTA curves of thermal decomposition of the pure active components.

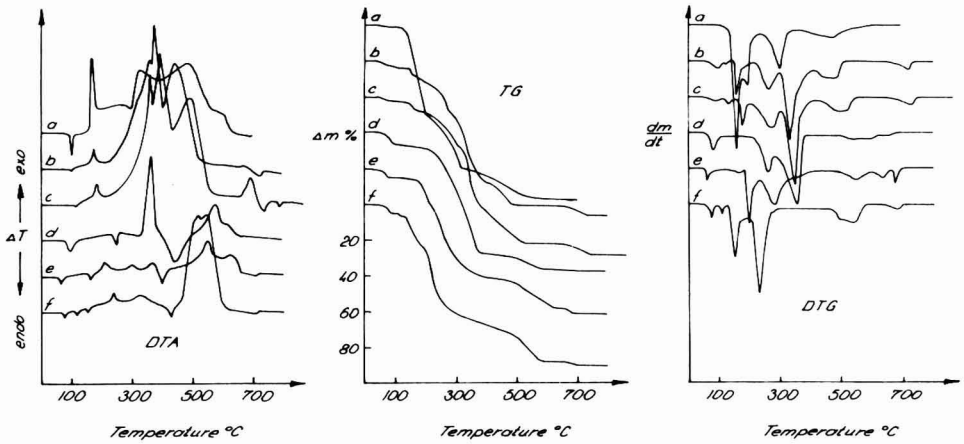

FIG. 3. DTA, TG, and DTG curves of the thermal decomposition of drugs containing components stimulating the central nervous system: (a) Carbromalum (T); (b) Cyclobarbitalum Calcium (T); (c) Reladorm (T); (d) Calcio-Cardiamid (T); (e) Cardenosinum (T); (f) Cardiamid-Coffein (T). For symbols in parentheses see Table 3.

Thermal effects due to decomposition of the active components, occurring at low percentage in drugs, are not practically observed on the curves of thermal decomposition of the drugs. For this reason, the presence of caffeine and isacen in the Coffan and Laxative chocolate tablets could not be detected. On the other hand, the identification of vehicles of these drugs was possible on the basis of endothermic DTA peaks (Figs. 3 and 4).

Vitamin-containing drugs are the most complex dosage forms. The

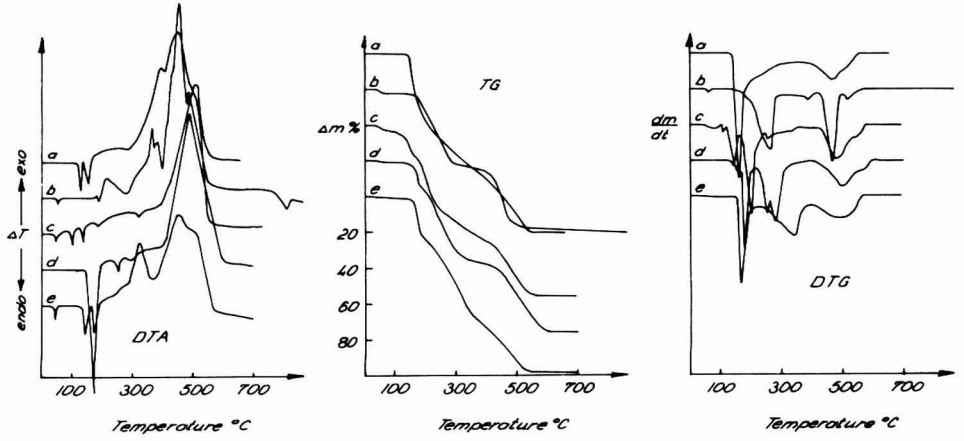

FIG. 4. DTA, TG, and DTG curves of the thermal decomposition of drugs containing components stimulating the central nervous system: (a) Coffan (T); (b) Coffeinum Natrium benzoicum (T); (c) Aviomarin (T); (d) Glutamic acid (T); (e) Laxative chocolate (T). For symbols in parentheses see Table 3.

majority of them contain at least six components, four of them being dragees. Owing to the fact that in only three dosage forms does the content of the active components exceed 40% of the total mass, it is relatively easy to substantiate the low usefulness of the DTA, TG, and DTG curves of thermal decomposition of these drugs for qualitative analysis of their composition.

It was shown that the identification of the active component was possible only in the case of the Vitaminum PP (50 and 200 mg), Calcium pantothenicum (25 and 100 mg) tablets, Vitaminum C (0.1 and 0.2 g) dragees as well as D-Panthenolum ampouls. In these drugs the identification of vitamins was carried out on the basis of sharp and narrow endothermic DTA peaks due to their melting. Individual stages of thermal decomposition of these drugs, poorly developed on the TG and DTG curves, were difficult to assign to the decomposition of active components or vehicles contained in them. However, in the case of the Calcium pantothenicum (25 and 100 mg) tablets, the stage due to decarboxylation of calcium carbonate was utilized. This stage is distinct and well developed on the TG and DTG curves of thermal decomposition of both drugs, and on the DTA curve it is confirmed by an endothermic peak.

On the basis of the shape of the DTA, TG, and DTG curves of thermal decomposition of the remaining vitamin-containing drugs, it is only possible to identify some of the vehicles contained in them. It was found that the Vitaminum B_1 (3 and 25 mg), Vitaminum B_6, Calcium pantothenicum (25 mg), tablets and Vitaminum B_2 dragees contained sucrose, lactose, or both. The thermal decomposition of glucose, the major component of the Vibovit powder, overlapped all thermal effects due to decomposition of the vitamin complex contained in the powder. However, based on the shape of the curves of thermal decomposition of the Vitaninum B compositum dragees, it is difficult to detect any of the components (Figs. 5 and 6).

Quantitative Analysis of Drugs

The analysis of the drugs showed that in 51% of them the content of the main active component exceeded 50% of the total mass, in 27% of the drugs it was 25%, whereas in only 22% of the drugs was the content of the active components lower than 25% of the total mass. However, the very complicated and differentiated physical and chemical properties of the composition of the drugs makes difficult the quantitative analysis of their composition and practically makes it impossible in the case of dragees.

The content of the active components in drugs was determined exclusively on the basis of the loss in weight as indicated by the TG curves. The DTG curves enabled distinct differentiation of successive stages of the thermal decomposition. By reading out from the TG curves the losses in

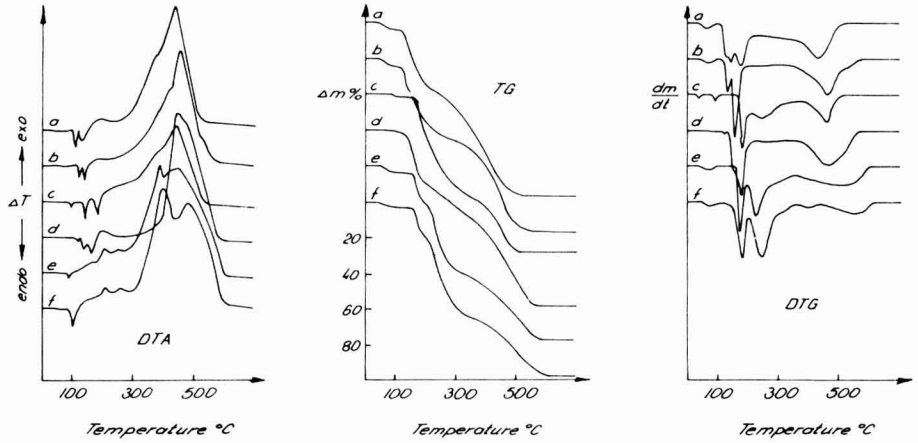

FIG. 5. DTA, TG, and DTG curves of the thermal decomposition of vitamin-containing drugs: (a) Vitaminum B_1 (3 mg) (T); (b) Vitaminum B_1 (25 mg) (T); (c) Vitaminum B_2 (D); (d) Vitaminum B_6 (T); (e) Vitaminum PP (50 mg) (T); (f) Vitaminum PP (200 mg) (T). For symbols in parentheses see Table 4.

weight of the active component of a drug on one hand, and analogous losses in weight of the active component neat, on the other, the content of the latter could easily be calculated. The percentage of the residue left in the crucible was read out directly from the TG curve. This procedure is reported in detail elsewhere (*9*, *12*).

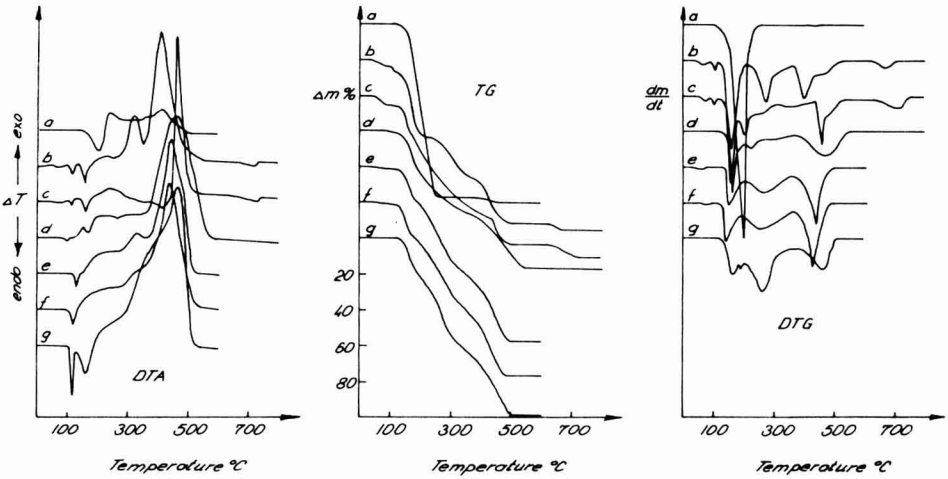

FIG. 6. DTA, TG, and DTG curves of the thermal decomposition of vitamin-containing drugs: (a) D-Panthenolum (A); (b) Calcium pantothenicum (25 mg) (T); (c) Calcium pantothenicum (100 mg) (T); (d) Vitaminum B compositum (D); (e) Vitaminum C (0.1 g) (D); (f) Vitaminum C (0.2 g) (D); (g) Vibovit (P). For symbols in parentheses see Table 4.

The values obtained in this way are shown as the "Found" values in Tables 1–4, whereas those denoted as "Theoretical" were calculated based on the manufacturer's information.

The temperature intervals listed in Tables 1–4 refer to the losses in weight recorded on the TG and DTG curves of the thermal decomposition of drugs, shown in Figs. 1–6, based on which the active components were determined.

In the determination, use was made of the stages of thermal decomposition associated with dehydration of novalgine in the Gardan and Pyralginum tablets as well as calcium cardiamide in the Calcio-Cardiamid, Cardenosinum, and Cardiamid-Coffein tablets. A relatively greater error is associated with dehydration of calcium cardiamide. Moreover, the stage due to decarboxylation of calcium carbonate was used for the determination of its content in the Polopiryna S and Calcipiryna tablets. Calcium carbonate is also formed by combustion of organic anions of calcium cyclobarbital and calcium pantothenate. Based on its decarboxylation, quantitative determination of both salts in the Cyclobarbitalum Calcium, Reladorm, and Calcium pantothenicum (25 and 100 mg) tablets was possible.

The content of acetylsalicylic acid in the Polopiryna, Polopiryna S, Calcipiryna, Tabulettae anti-influenze, and Tabulettae antidolorosae tablets was determined based on the thermal decomposition stage due to the dissociation of ester linkage and the releasing of acetic acid from the molecule. This stage was not associated with the formation of a horizontal, only with well-marked inflections on the TG and DTG curves. Well-defined loss in weight due to the releasing of caffeine from the caffeine–sodium benzoate complex was used in the determination of the content of the active component in the Coffeinum Natrium benzoicum tablets. On the other hand, the content of carbromal and glutamic acid in the tablets was determined based on the thermal decomposition stage due to formation of transient products of the active components. Their composition and chemical structure could not be determined. These stages are well marked on the TG and DTG curves of the thermal decomposition of both drugs. The lack of such stages on the thermal decomposition curves of other drugs makes impossible the determination of the active components contained in them.

CONCLUSIONS

This study confirmed the possibility of employing the DTA, TG, and DTG curves of thermal decomposition of drugs containing acetylsalicylic acid, 5-pyrazolone derivatives, components stimulating the central nervous system, the Aviomarin, Glutamic acid, and Laxative chocolate tablets, and vitamins for the identification of particular dosage forms and

TABLE 1

RESULTS OF ANALYSIS OF THE THERMAL DECOMPOSITION OF DRUGS CONTAINING ACETYLSALICYLIC ACID

Trade name	Drug form	Active component	Figure	Temperature intervals (°C)	Weight loss (%)	Active component (%)		Residue in crucible (%)	
						Found	Theoretical	Found	Theoretical
Polopiryna	T[a]	Acetylsalicylic acid	1a	90–220	26.0	81.3	83.3	2.0	1.7
Polopiryna S	T	Acetylsalicylic acid	1b	90–170	16.0	50.0	50.0	10.0	9.5
		Calcium carbonate		600–730	7.3	16.6	16.7		
Calcipiryna	T	Acetylsalicylic acid	1c	70–170	22.0	68.8	64.1	18.0	15.9
		Calcium carbonate		600–730	8.3	18.9	19.2		
Asprocol	T	Acetylsalicylic acid	1d				62.5	2.0	2.5
		Aminoacetic acid					25.0		
Tabulettae anti-influenze	T	Acetylsalicylic acid	1e	80–175	13.5	42.2	45.0	1.0	2.0
		Phenacetin					30.0		
		Caffeine					6.0		
Tabulettae antidolorosae	T	Acetylsalicylic acid	1f	80–210	17.5	54.7	50.0	3.0	1.7
		Phenacetin					16.7		
		Caffeine					8.3		
Isochin	D[a]	Acetylsalicylic acid	1g				46.2	2.5	1.7[b]
		Caffeine					4.6		
		Quinine hydrochloride					2.3		
Antineuralgiae	T	Acetylsalicylic acid	1h				41.7	2.5	2.0
		Phenacetin					41.7		
		Carbromal					8.3		

[a] T, Tablets; D, dragees.

[b] It was not possible to calculate the weight of a residue left after combustion of the organic matter of a dragee mass due to the lack of information about coating composition.

TABLE 2

RESULTS OF ANALYSIS OF THE THERMAL DECOMPOSITION OF DRUGS CONTAINING 5-PYRAZOLONE DERIVATIVES

Trade name	Drug form	Active component	Figure	Temperature intervals (°C)	Weight loss (%)	Active component (%)		Residue in crucible (%)	
						Found	Theoretical	Found	Theoretical
Pyramidonum (0.1 g)	T[a]	Pyramidon	2a				55.0	2.5	3.3
Pyramidonum (0.3 g)	T	Pyramidon	2b				82.0	0.5	0.0
Pyramidonum	S[a]	Pyramidon	2c				15.0	1.0	0.0
Pabialgin	T	Pyramidon	2d				73.3	0.5	0.0
		Allobarbital					10.0		
Pabialgin	S	Pyramidon	2e				22.0	0.0	0.0
		Allobarbital					3.0		
Isalgin	T	Pyramidon	2f				71.4	1.0	0.1
		Phenobarbital					4.3		
		Papaverine hydrochloride					5.7		
Cofedon	T	Phenacetin	2g				30.0	0.5	0.1
		Pyramidon					20.0		
		Caffeine					10.0		
		Phenobarbital					3.0		
Gardan	T	Novalgine	2h	60–100	2.5	50.0	50.0	11.0	11.1
		Pyramidon					33.3		
Pyralginum	T	Novalgine	2i	60–110	4.2	84.0	86.2	20.0	19.2

[a] T, Tablets; S, suppositories.

TABLE 3

RESULTS OF ANALYSIS OF THE THERMAL DECOMPOSITION OF DRUGS CONTAINING COMPONENTS STIMULATING THE CENTRAL NERVOUS SYSTEM

Trade name	Drug form	Active component	Figure	Temperature intervals (°C)	Weight loss (%)	Active component (%)		Residue in crucible (%)	
						Found	Theoretical	Found	Theoretical
Carbromalum	T[a]	Carbromal	3a	100–220	51.5	87.3	87.6	2.5	3.0
Cyclobarbitalum Calcium	T	Calcium cyclobarbital	3b	600–740	5.2	57.7	57.1	14.5	17.5
Reladorm	T	Calcium cyclobarbital	3c	630–740	5.5	61.1	58.8	11.0	14.1
		Diazepane					5.9		
Calcio-Cardiamid	T	Calcium cardiamide	3d	50–100	5.5	83.3	80.0	22.5	25.6
Cardenosinum	T	Calcium cardiamide	3e	50–80	4.25	64.4	63.2	18.5	21.5
		Theophylline					14.6		
		Adenosine					0.2		
Cardiamid-Coffein	T	Calcium cardiamide	3f	100–130	2.5	37.9	40.0	9.5	14.9
		Caffeine					10.0		
		Strychninum nitrate					0.05		
Coffan	T	Caffeine	4a				10.0	0.0	0.0
Coffeinum Natrium benzoicum	T	Caffeine-sodium benzoate	4b	140–280	36.5	96.1	90.9	19.5	17.9
Aviomarin	T	8-Chlorotheophylline-benzhydramine	4c				50.0	5.0	4.1
Glutamic acid	T	Glutamic acid	4d	160–180	10.0	83.3	83.3	5.5	3.6
Laxative chocolate	T	Isacen	4e				0.5	0.5	0.0

[a] T, Tablets.

TABLE 4

RESULTS OF ANALYSIS OF THE THERMAL DECOMPOSITION OF VITAMIN-CONTAINING DRUGS

Trade name	Drug form	Active component	Figure	Temperature intervals (°C)	Weight loss (%)	Active component (%)		Residue in crucible (%)	
						Found	Theoretical	Found	Theoretical
Vitaminum B_1 (3 mg)	T[a]	Thiamine hydrochloride	5a				3.0	2.5	5.3
Vitaminum B_1 (25 mg)	T	Thiamine hydrochloride	5b				25.0	3.5	3.6
Vitaminum B_2	D[a]	Riboflavin	5c				1.4	12.5	0.5[b]
Vitaminum B_6	T	Pyridoxine hydrochloride	5d				48.8	2.5	2.0
Vitaminum PP (50 mg)	T	Nicotinamide	5e				24.2	2.5	2.5
Vitaminum PP (200 mg)	T	Nicotinamide	5f				39.3	2.5	1.2
D-Panthenolum	A[a]	Pantothenic acid	6a				94.3	0.0	0.0
Calcium pantothenicum (25 mg)	T	Calcium pantothenate	6b	620–690	2.5	26.0	25.0	6.5	7.8
Calcium pantothenicum (100 mg)	T	Calcium pantothenate	6c	620–730	6.25	65.0	62.5	10.5	11.8
Vitaminum B compositum	D	Vitamin complex	6d				21.5	18.0	1.4[b]
Vitaminum C (0.1 g)	D	Ascorbic acid	6e				25.0	3.0	1.3[b]
Vitaminum C (0.2 g)	D	Ascorbic acid	6f				30.8	3.0	1.6[b]
Vibovit	P[a]	Vitamin complex	6g				5.8	1.5	1.2

[a] P, Powders; T, tablets; D, dragees; A, ampouls.

[b] It was not possible to calculate the weight of a residue left after combustion of the organic matter of a dragee mass due to the lack of information about coating composition.

the qualitative and quantitative monitoring of their composition. The identification of individual components is most conveniently accomplished on the basis of simultaneously recorded DTA, TG, and DTG curves of their thermal decomposition, using temperature ranges, peak areas, and peak shapes of the corresponding DTA peaks as well as the loss in weight from the TG curves. In the quantitative determination, only the TG and DTG curves were used because they permitted discrimination of individual stages due to the thermal decomposition of an active component and precise determination of the loss in weight.

On the basis of the shapes of the TG and DTG curves of thermal decomposition, the content of active components was determined in 17 out of 41 decomposed drugs without the necessity of separation of vehicles. Moreover, in the Polopiryna S and Calcipiryna tablets the content of two active components was determined simultaneously due to the fact that their thermal decomposition took place over different temperature ranges. The results obtained in this study are in good agreement with those calculated from the manufacturer's information.

The quantitative determination by this method of those active components which occur in low content (these are frequently very strongly acting drugs) is practically impossible due to lack of thermal effects on the curves associated with their decomposition.

It appears also reasonable to recommend the possibility of using the DTA, TG, and DTG curves of the decomposition of the drugs for the control of technological processes during the manufacture of drugs as well as for the quality control of the manufactured products.

SUMMARY

The thermal decomposition of 41 drugs containing acetylsalicylic acid, 5-pyrazolone derivatives, the components stimulating the central nervous system, the Aviomarin, Glutamic acid, and Laxative chocolate tablets, and vitamins has been studied by employing the differential thermal analysis (DTA), thermogravimetry (TG), and differential TG (DTG) techniques. The possibility has been demonstrated to employ the DTA, TG, and DTG curves of their thermal decomposition for the identification of particular dosage forms and for the qualitative and quantitative monitoring of their composition. Based on the stages due to dehydration, decarboxylation, and formation of intermediate decomposition products, the content of the active components was determined in 17 of the drugs. The results of the determinations were in good agreement with those calculated from information supplied by the manufacturers.

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Alternating Current Polarography of Metal Carbonyl Complexes in Nonaqueous Solvents

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INTRODUCTION

The electrochemical analysis of transition metal carbonyl complexes and related compounds have been studied by techniques such as direct current (dc) polarography, cyclic voltammetry, and controlled potential electrolysis (*4–7*). Due to the solubility properties and, to some extent, the reactivity of these molecules with water, the use of nonaqueous solvents is required. It has been reported (*2*) that alternating current (ac) polarography is better suited to resolve waves than dc polarography, particularly when a small amount of one substance is in the presence of a large amount of another. The purpose of this study was to develop a method for obtaining $E_{1/2}$ values and the quantitative analysis of organometallic substances in nonaqueous solvents using ac polarography. Although it was expected that difficulties due to the high cell resistances would be encountered when using nonaqueous solvents, the feasibility of analyzing pharmaceutical substances in acetonitrile solution by ac polarography has been reported (*10*). Other workers (*9*) had previously attempted ac polarography in methanolic solution without notable success, perhaps because LiCl was used, an electrolyte which we found to be less suitable than tetrabutylammonium perchlorate. In searching for optimum conditions (supporting electrolyte, solvents, cell design, etc.), which would provide the basis for a new analytical technique, it was discovered that even though well-shaped ac polarograms could be obtained, neither peak potential (E_p) nor peak height was reproducible. The trouble was traced to the solid auxiliary electrode which was used to apply the ac potential (a three-electrode cell geometry is normally employed to overcome high cell resistances) (*7*). If the electrode is "poisoned" or coated by absorption or reaction with a reduction product of the electroactive species, proper cleaning of the electrode surfaces between runs should allow one to otain successive, identical polarograms on the same solution. Although every method known to this labora-

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tory for obtaining a fresh electrode surface was used (sanding with emery cloth, cleaning in concentrated acids, etc.) it was not possible to obtain reproducible results with any of the solid auxiliary electrodes employed (W, Ag, Pt, Au). It is possible that some electroactive species may bind irreversibly to these solid electrodes, thus changing their characteristics slightly with each successive run.

The next logical alternative was to apply the ac potential via a platinum wire immersed in a mercury pool. A mercury pool has been used as the cathode in place of the dropping mercury electrode in ac polarography (*1*) and has also been used as a condenser reference electrode (*3*). No reference has been found of its use as an auxiliary electrode in this particular solvent system in ac polarography. Using this electrode it was possible to obtain reproducible polarograms of the same substance in successive runs. In most cases, the reproducibility of peak potential was within ± 10 mV, a realistic figure for the precision of the instrumentation. It is interesting to note that if one polarogram is taken immediately following another on the same solution, without disturbing the pool, the same variations in peak potential and height are observed as with a solid electrode, but to a lesser extent. However, if the polarographic cell and its contents are disturbed between runs (by bubbling argon purge gas through the solution, for example), the two polarograms will be identical.

EXPERIMENTAL

Polarograms were recorded with a Metrohm Polarecord E261 equipped with the following Metrohm accessories: ac modulator E393, ir compensator E446 (used for dc polarograms only), and polarography stand with drop controller E354. All polarographic equipment was purchased from Brinkman Instruments, Inc., Westbury, N.Y. A 20-ml-capacity Metrohm EA876 polarographic vessel was used, (single compartment) fitted with a dropping mercury electrode, a $Ag/AgClO_4$ (0.001 *M*), TBAP 0.1 *M* reference electrode, an argon degassing tube, and an auxiliary electrode. The $Ag/AgClO_4$ (0.001 *M*), TBAP 0.1 *M* reference electrode was used when the solvent was methanol, dimethylformamide, and acetonitrile. The reference electrode was connected to the solutions studied via a salt bridge containing TBAP dissolved in CH_3OH. It was also found that $Ag/AgClO_4$ rapidly decomposes in dimethylformamide solution, when this combination is used as the reference. The reference electrode contains either a commercially available metal wire sealed in glass, a platinum foil electrode, or a platinum wire completely submerged in a mercury pool. The supporting electrolyte is tetrabutylammonium perchlorate (TBAP) obtained from Alfa Products, Inc., Danvers, Mass., dissolved in either Baker Photrex reagent acetonitrile, Baker Insta-Analyzed *N,N*-dimethylformamide (DMF), or Baker Insta-Analyzed methanol. These

solvents were obtained from J. T. Baker Chemical Co., Phillipsburg, N.J., and were used without further purification. The compounds used in this study were purchased from Alfa Products Inc., Danvers, Mass., and used without purification. Triple-distilled mercury was used for both the pool and the dropping mercury electrode (DME).

The applied dc potential was introduced into the cell between the reference electrode and the dropping mercury electrode. The applied ac current of 10 mV was introduced between the dropping mercury electrode and the auxiliary mercury pool.

Solutions were purged with argon which was saturated with the appropriate supporting electrolyte (to avoid evaporation of sample) to remove oxygen from the system. This was done for 20 min initially and for 5 min between successive runs on the same solution. Alternating current polarography is noted for its insensitivity to dissolved oxygen, because the reduction of oxygen on mercury is highly irreversible. However, degassing was necessary because oxygen produced products which in themselves affected the overall reactions within the system (*8*). A controlled drop time of 0.17 sec was used with an applied ac potential of 10 mV root mean square (RMS) at 60 Hz. The scan rate was 500 mV/min (8.3 mV/sec). The scan range was from 0 to −3 V. All runs were made at 21 ± 1°C.

Sample preparation was accomplished with the minimum amount of sample handling. Solid samples, approximately 6 to 25 mg, were weighed accurately into a 25-ml volumetric flask. Samples were dissolved and diluted to volume with supporting electrolyte. The solution was degassed as described above.

RESULTS AND DISCUSSION

The Auxiliary Electrode

Various conventional platinum, silver, tungsten, and gold electrodes were employed in turn as the auxiliary electrode, in the three-electrode system. Platinum foil electrodes with a relatively large surface area were also used. Although the ac polarograms obtained initially appeared satisfactory in the case of each of the above electrodes, it was found that it was usually not possible to successfully replicate a particular polarogram with regard to its peak potential (E_p) and peak height (I_p). Figure 1 illustrates this point; polarograms of the same manganese carbonyl solution using a tungsten auxiliary electrode, cleaned and polished by various abrasive and nonabrasive methods did not give reproducible peak potentials. The E_p values were as follows, in succession, −1.93, −1.73, and −1.69 V. Although one would like to draw inferences concerning the anodic trend of these values, in other similar cases no correlation or even a reversal to this trend was found.

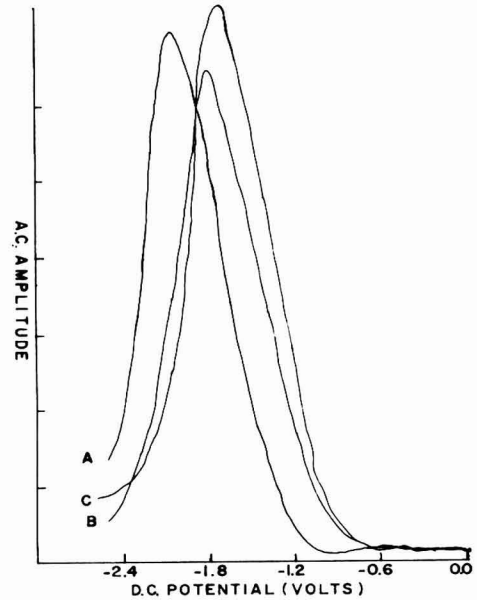

FIG. 1. Nonreproducible polarograms of 10^{-4} *M* $Mn_2(CO)_{10}$, due to poisoning of the Pt auxiliary electrode; A, B, and C are −1.93, −1.73, and −1.67 dc V, respectively. Applied constant ac voltage = 10 mV.

On the other hand, as shown in Fig. 2, use of a mercury pool electrode provides successive polarograms which are virtually superimpossible with E_p values of −1.55 V. Figure 2 further illustrates the linear correlation of peak height and concentration of the tested solution. The use of the

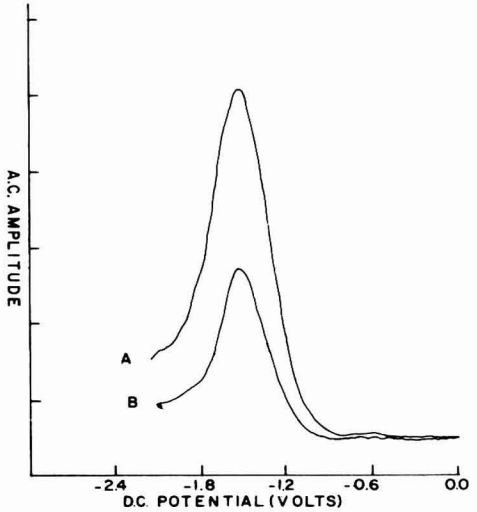

FIG. 2. Amplitude (ac) as a function of concentration of $Mn_2(CO)_{10}$ at constant 10 mV applied ac voltage. A, 2×10^{-5} *M*; B, 1×10^{-5} *M*.

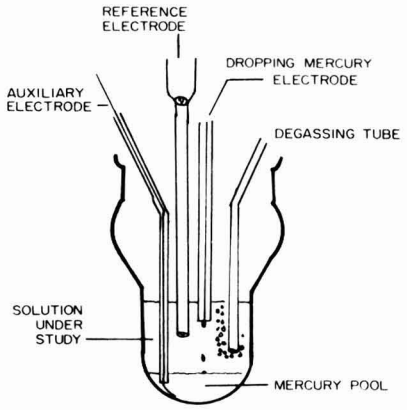

FIG. 3. Placement of electrodes in experimental polarographic cell.

Hg pool as the auxiliary electrode is a logical choice if one suspects the counterelectrodes are too small or if "poisoning" is a problem.

Since high cell resistances were encountered, every way to reduce these had to be investigated, including cell design. A two-compartment cell was employed initially, typical for dc polarography. However, again high cell resistances were encountered which prevented the collection of any useful data. Therefore, the one compartment cell was tried (see Fig. 3). Initial results were encouraging. However, only when the mercury pool electrode was used in conjunction with the methanol/tetrabutylammonium perchlorate electrolyte were satisfactory operating conditions obtained.

Effect of Solvent

Table 1 compares values of E_p obtained for $Mn_2(CO)_{10}$ and $CpMo(CO)_3$ (Cp = cyclopentadiene) in several different solvents using the auxiliary mercury pool electrode.

TABLE 1
COMPARISON OF E_p VALUES IN DIFFERENT SOLVENTS

Compound	Solvent	$-E_p$ (volts)
$Mn_2(CO)_{10}$	Dimethylformamide	−0.79
	Methanol	−1.55
	Acetonitrile	−1.60
$CpMo(CO)_3$	Methanol (with 10% water)	−1.08
	Methanol (anhydrous)	−1.22
	Methanol/tetrahydrofuran	−1.60
	Tetrahydrofuran	−2.05
	Dimethylformamide	−1.50

Aside from the differences in E_p values for each solvent, polarograms which were superior in shape of peak and flatness of baseline were consistently obtained when methanol was used as the supporting electrolyte solvent. Other solvents, such as *N,N*-dimethylformamide and acetonitrile, were also used but were found to give poor results, possibly due to the higher cell resistances encountered. The quality of the data obtained as well as the decreased cell resistance, which allows this data to be recorded, can mainly be attributed to the choice of solvent system (electrolyte) as well as the control of temperature, degassing, and drop times.

The explanations for the occurrence of shifts in E_p, for a particular material in different solvent systems, is not fully understood. It is possible that solvent reactions with the reduced species could generate new electroactive species. Presently, there are no data to confirm this, but work in this area will be pursued.

TABLE 2
SUMMARY OF AC POLAROGRAPHIC DATA OBTAINED WITH MERCURY POOL ELECTRODE IN METHANOL SOLUTION

Compound	E_p (volts)	I_p (Ma/Molarity)
$Cr(CO)_6$	−2.80	460
$Mn_2(CO)_{10}$	−1.55	700
$Fe_2(CO)_9$	−2.24	—
$Mo(CO)_6$	−2.80	—
$W(CO)_6$	−2.40	650
$Re_2(CO)_{10}$	−2.16	680
$CpCr(CO)_3$	−2.80	1200
$CpMn(CO)_3$	−2.59	4900
$CpFe(CO)_{2\ 2}$	−1.75	—
	−2.77	—
$CpMo(CO)_{3\ 2}$	−0.85	670
	−2.51	1300
Cp_2TiCl_2	−0.40	480
	−0.80	220
	−1.57	220
	−1.78	270
	−2.70	large
Cp_2VCl_2	−2.60	3700
Cp_2ZrCl_2	−1.46	1070
	−1.78	300
	−2.21	260
	−2.89	4660
$CpMo(CO)_3Cl$	−0.94	440
	−2.50	22,400
$(C_6H_5)_3PMo(CO)_5$	−2.87	1060
	−3.02	1120
$Mn(CO)_5Br$	−0.13	120
	−0.89	530

Results of Sample Studies

Table 2 shows data recorded on various metal complexes in solution. All data in this table were recorded using the mercury pool auxiliary electrode in the methanol/TBAP supporting electrolyte. This table summarizes values obtained for peak potentials (E_p) and in some cases comparative values of peak height (I_p).

Sample sizes were mainly confined to small milligram quantities. The technique requires very small concentrations of sample in solution for accurate quantitative measurements to be obtained. The typical concentration range was from 10^{-2} to 10^{-8} *M*.

SUMMARY

Using commercially available instrumentation, a supporting electrolyte and electrode system was devised which permits ac polarography of organometallic compounds, transition metal complexes, and other substances in nonaqueous solvents such as methanol, *N,N*-dimethylformamide, or acetonitrile. The first two solvents mentioned were found to be far superior to the latter. Tetrabutylammonium perchlorate electrolyte (0.1 *M* or less) in methanol, for example, affords a fairly flat baseline in the applied dc potential range of 0 to −3 V with well-shaped voltammetric peaks for most reducible substances. A unique feature of the method, which permits one to easily obtain replicable polarograms and peak potentials, is the use of a mercury pool auxiliary electrode rather than the usual platinum or tungsten electrodes.

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Thin-Layer Chromatographic Separation of Serotonin from Epinephrine and Norepinephrine

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INTRODUCTION

Previous publications have reported a number of thin-layer chromatographic procedures for the separation of epinephrine and norepinephrine from each other. Thus, Waldi (*7*) separated epinephrine on Kieselgel G using chloroform–methanol (9:1) as the solvent system while Halmekoski (*2*) also used Kieselgel G-coated plates for the separation of epinephrine from norepinephrine employing *n*-butanol–acetic acid–water (4:1:5), *n*-pentanol–acetic acid–ethanol–water (4:1:1:5), or *n*-butanol–propanol–acetic acid–water (4:1:1:5) as solvent systems. He further reported the use of aqueous sulfur dioxide in the place of water to decrease tailing. Nishimoto and Toyoshima (*4*) reported the chromatographic separation of epinephrine from norepinephrine on silica gel G and employing acetone–formic acid–water (7:1:2) as a solvent system. Considerable tailing was observed due to the presence of metallic elements in the adsorbent but such tailing was markedly reduced by pretreatment of the plates with ethylenediaminetetraacetic acid. Sapira (*5*) separated epinephrine and its analogs on polyamide thin layers using isobutyl alcohol–anhydrous acetic acid–cyclohexanone (80:7:10) or isopropyl alcohol–aqueous ammonia (4:1) as solvent. Walicka (*8*) separated epinephrine from norepinephrine on cellulose MN300 employing *n*-butanol–acetic acid–0.2% aqueous sodium hydrogen sulfite (8:8:3), *n*-pentanol–acetic acid–methanol–0.2% aqueous sodium hydrogen sulfite (8:2:2:3), or *n*-butanol–n-propanol–acetic acid–0.2% aqueous sodium hydrogen sulfite (8:2:2:3) as developing solvents. Thielemann (*6*) separated epinephrine from norepinephrine on Fertigfolien UV 254 sheets or Kieselgel G using acetone–formic acid–water (7:1:2) as developing solvent.

Review of the literature showed that very little work has been reported on the thin-layer chromatographic separation of either serotonin alone or in the presence of epinephrine and norepinephrine and none on the

[1] Taken from a thesis submitted in partial fulfillment of the M.S. degree.

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separation of all three. In one study, reported by Bancher *et al.* (*1*), a two-dimensional thin-layer chromatographic separation of the dansyl derivatives of serotonin, dopamine, epinephrine, and norepinephrine on Kieselgel was successfully done with ethyl acetate–cyclohexane (3:2) and benzene–ethylamine (5:1) as developing solvents in the first and second dimensions, respectively. A method was also reported by Hansen and Hedeskov (*3*) for the separation of serotonin, dopamine, noradrenaline, and adrenaline on micropolyamide F1700 plates using 3% formic acid followed by a second development with benzene–acetic acid (9:1) perpendicular to the first and by a third development in *n*-propanol–pyridine–water in the same direction as the first.

In this paper a thin-layer chromatographic method of separation of serotonin from epinephrine and norepinephrine on silica gel G plates pretreated with ethylenediaminetetraacetic acid is presented.

MATERIALS AND METHODS

Apparatus

Thin-layer chromatographic equipment. A developing tank (27 × 27 × 7.5 cm) with glass top.

Syringe. Ten-microliter syringe (Hamilton & Company).

Electric drier.

Spraying bottle.

Samples. Samples of epinephrine, norepinephrine, and serotonin were prepared in 50% acetic acid to give 0.4% solutions.

Thin-layer chromatographic plates. Silica gel G-coated plates. Eastman Kodak Chromatogram plates with fluorescence, Catalog No. 6060. These plates were first treated with 0.1% ethylenediaminetetraacetic acid (EDTA) solution for 30 sec and then dried for 30 min at 70°C in an oven before use.

Recommended Procedure

The plates were spotted, using a 10-μl syringe containing the relevant samples individually and as mixtures. The amount plated was 8 μg contained in 2 μl. The diameter of the spots was about 5 mm. The plates were dried using an electric drier. Then they were placed in a developing tank containing the solvents *n*-butanol–ethanol–acetic acid–water (8:2:1:3). The tank was covered with a glass plate top and 45 min was allowed for prior equilibration, after which the plates were developed by placing them in the tank for 15 hr. After the solvent had migrated to about 13 cm, the plates were removed and dried in a forced-air oven for 15 min at 90°C until the acetic acid disappeared. Clearly visible brown–orange spots were self-indicated after drying.

TABLE 1

Mobile solvent systems	Ratio of solvent components	Maximum number separable[a]
Acetic acid		0
Acetone		0
Methanol		0
Ethanol		1 (S)
Acetic acid–water	1:1	0
Acetic acid–ethanol	1:1	0
Acetic acid–*n*-propanol	1:1	0
Acetic acid–butanol	1:1	0
Acetic acid–ethyl acetate	1:1	0
n-Butanol–methanol	1:1	0
n-Butanol–ethanol	1:1	1 (S)
n-Butanol–ethanol–acetic acid	1:1:1	0
n-Butanol–acetic acid–water	1:1:1	0
n-Butanol–ethanol–acetic acid–water	1:1:1:1	2 poorly
n-Butanol–ethanol–acetic acid–water	1:1:1:2	0
n-Butanol–ethanol–acetic acid–water	1:1:1:3	0
n-Butanol–ethanol–acetic acid–water	2:1:1:1	0
n-Butanol–ethanol–acetic acid–water	2:2:1:1	0
n-Butanol–ethanol–acetic acid–water	2:1:3:1	0
n-Butanol–ethanol–acetic acid–water	3:2:3:3	2 poorly (E) and (N)
n-Butanol–ethanol–acetic acid–water	3:3:1:1	0
n-Butanol–ethanol–acetic acid–water	3:3:3:2	0
n-Butanol–ethanol–acetic acid–water	4:1:1:1	2 poorly (E) and (S)
n-Butanol–ethanol–acetic acid–water	4:1:1:2	2 poorly (N) and (S)
n-Butanol–ethanol–acetic acid–water	4:1:1:3	2 poorly (E) and (S)
n-Butanol–ethanol–acetic acid–water	4:2:1:2	1 poorly (S)
n-Butanol–ethanol–acetic acid–water	5:1:1:1	2 poorly (E) and (S
n-Butanol–ethanol–acetic acid–water	5:1:1:2	2 poorly (N) and (S)
n-Butanol–ethanol–acetic acid–water	5:1:1:3	3 poorly
n-Butanol–ethanol–acetic acid–water	5:1:2:3	0
n-Butanol–ethanol–acetic acid–water	5:1:3:1	0
n-Butanol–ethanol–acetic acid–water	5:1:3:2	0
n-Butanol–ethanol–acetic acid–water	6:1:1:1	2 poorly (E) and (N)
n-Butanol–ethanol–acetic acid–water	6:1:1:2	2 poorly (E) and (N)
n-Butanol–ethanol–acetic acid–water	6:1:1:3	2 poorly (E) and (N)
n-Butanol–ethanol–acetic acid–water	6:2:1:3	3 poorly with tailing
n-Butanol–ethanol–acetic acid–water	7:1:1:3	0
n-Butanol–ethanol–acetic acid–water	7:2:1:1	0
n-Butanol–ethanol–acetic acid–water	7:2:1:3	2 (E,N), (S)
n-Butanol–ethanol–acetic acid–water	8:1:1:3	2 (E,N), (S)
n-Butanol–ethanol–acetic acid–water	8:2:1:3	3
n-Propanol		1 (S)

[a] Abbreviations: (E) Epinephrine, (N) norepinephrine, (S) serotonin.

RESULTS AND DISCUSSION

Table 1 lists some mobile solvent systems and the respective ratios which were used to effect a separation of serotonin from epinephrine and norepinephrine on silica gel G. Good resolving power is exhibited by *n*-propanol and ethanol for serotonin only. The resolving power of solvent mixtures containing ethanol–acetic acid is unsatisfactory as is that of *n*-butanol and water alone. However, the addition of specified amounts of acetic acid to *n*-butanol–ethanol–water mixtures gives the most promising results. Indeed, small variations in the composition of the various solvent systems containing *n*-butanol–ethanol–acetic acid–water produce marked changes in the degree of separation of the three biogenic amines. The proportion of acetic acid to water is of especially great importance. Furthermore, the results of Table 1 demonstrate that the most suitable composition of the solvent mixture which separates serotonin from epinephrine and norepinephrine is *n*-butanol–ethanol–acetic acid–water (8:2:1:3).

Table 2 records the R_f values for the individual biogenic amines employing the *n*-butanol–ethanol–acetic acid–water (8:2:1:3) solvent system on silica gel G plates. These results demonstrate that serotonin is well separated from both epinephrine and norepinephrine. However, epinephrine and norepinephrine are not well separated from each other as is indicated by their slightly different R_f values and consequential overlapping of their spots.

Table 3 records the results of the simultaneous separation of serotonin from epinephrine and norepinephrine in mixtures containing all three. Once again, serotonin is effectively separated from both epinephrine and norepinephrine. On the other hand, the separation of epinephrine from norepinephrine is improved in the mixture, with the overlap being less and the two spots barely coalescing, thus rendering all three components detectable in the mixture.

During the course of these studies, it was found unnecessary to use any detection methods by way of uv fluorescence or spray reagents, since,

TABLE 2

R_f VALUES OF SINGLE-COMPONENT SAMPLES IN SOLVENT SYSTEM CONTAINING *n*-BUTANOL:ETHANOL:ACETIC ACID:WATER (8:2:1:3) USING SILICA GEL G PLATES WITH FLUORESCENT INDICATOR AND TREATED WITH 0.1% EDTA SOLUTION

Compound	R_f
Epinephrine	0.34
Norepinephrine	0.33
Serotonin	0.61

TABLE 3

R_f VALUES OF SEPARATED COMPONENTS FROM A MIXTURE CONTAINING EPINEPHRINE, NOREPINEPHRINE, AND SEROTONIN IN SOLVENT SYSTEM CONTAINING *n*-BUTANOL: ETHANOL: ACETIC ACID: WATER (8:2:1:3) USING SILICA GEL G PLATES WITH FLUORESCENT INDICATOR AND TREATED WITH 0.1% EDTA SOLUTION

Compounds	R_f
Epinephrine	0.34
Norepinephrine	0.32
Serotonin	0.60

under the conditions of the procedure, detection was self-indicating with brown spots developing automatically. It was also found that the use of ethylenediaminetetraacetic acid to pretreat the plates was a necessary requirement since in its absence serotonin showed considerable tailing.

SUMMARY

A thin-layer chromatographic procedure is reported for the separation of serotonin from epinephrine and norepinephrine. The method involves the use of ethylenediaminetetraacetic acid-impregnated silica gel G plates and *n*-butanol–ethanol–acetic acid–water (8:2:1:3) as developer. Serotonin is well separated from both epinephrine and norepinephrine. Epinephrine and norepinephrine overlapped slightly but were both detectable. The procedure is thus applicable to the separation of all three biogenic amines in spite of the absence of an absolutely clear separation of epinephrine from norepinephrine.

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Some Analytical Applications of Aromatic Sulfonyl Haloamines: Estimation of Indigocarmine by Chloramine-B, Bromamine-T, and Dibromamine-T

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INTRODUCTION

Recently, aromatic sulfonyl haloamines have attracted the attention of analytical chemists as versatile redox titrants. The diverse chemical behavior of *N*-haloamines is attributed to their ability to act as halonium cations, hypohalites, and *N*-anions which act as both bases and nucleophiles and as nitrenoids in limited cases. As a result, these compounds react with a wide range of functional groups, effecting an array of molecular transformations.

Although the well-known members of this class of aromatic sulfonyl haloamines, namely, chloramine-T, chloramine-B, and bromamine-T, are soluble in water, dichloramine-T and dibromamine-T are employed as redox titrants in nonaqueous or partially aqueous media.

Indigocarmine is used as a redox indicator in analytical chemistry. The determination of indigo and indigocarmine (IC) is of considerable interest in view of their commercial importance. A variety of oxidants such as cerium (IV) (*8*), sodium vanadate (*9*), potassium dichromate (*7*), chloramine-T (*3*), and dichloramine-T (*3*) have been shown to oxidize IC quantitatively under a prescribed set of experimental conditions. The present communication reports our investigations on the oxidimetric estimation of IC in solution with three sulfonyl haloamines, sodium *N*-chlorobenzenesulfonamide (chloramine-B, CAB), sodium *N*-bromo-*p*-toluenesulfonamide (bromamine-T,BAT), and *N,N'*-dibromo-*p*-

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toluenesulfonamide (dibromamine-T,DBT). The back titration methods described were elegant, rapid, and simple for assay of the reductant in solution.

MATERIALS AND METHODS

Indigocarmine. About 2 m*M* solutions of analar indigocarmine (E. Merck) were prepared by dissolving the required amount of the compound in appropriate solvents and buffers.

Chloramine-B. CAB was prepared (*1*) by passing pure chlorine gas through benzenesulfonamide dissolved in 4 *M* NaOH over a period of 1 hr at 70°C. The mass obtained was filtered, dried, and recrystallized from water. The purity of the compound was checked by the iodometric estimation of active chlorine present in the compound and by recording its Fourier transform-^{1}H- and ^{13}C-NMR spectra (obtained on a Bruker WH 270-MHz nuclear magnetic resonance spectrometer).

^{1}H spectrum: δ (relative to TMS); 7.86 (doublet corresponding to H *ortho* to the heteroatom); 7.72 (multiplet corresponding to H *meta* to the heteroatom). The latter integrates to three protons. The coupling constant $J_{o,m}$ is 8.0 Hz.

^{13}C spectrum: (ppm relative to TMS); 142.48 (C-1 attached to S atom); 134.39 (C-4, *para* to the heteroatom); 131.26 (C-2,6); and 129.37 (C-3,5).

Dibromamine-T. DBT was prepared by the bromination (*5*) of chloramine-T (CAT) solutions. About 4 ml of liquid bromine was added dropwise from a microburet to a solution of ~20 g of CAT in 400 ml of water with constant stirring of the solution at room temperature. DBT separated out was filtered under suction, washed thoroughly with water till all the bromine adsorbed on the compound was completely eliminated, and then dried in a vacuum desiccator for 24 hr. The purity of the compound was checked by the iodometric determination of the active bromine content of the compound and by recording its FT-^{1}H- and ^{13}C-NMR spectra.

^{1}H spectrum: δ (relative to TMS); 2.52 (singlet, corresponding to $-CH_3$); 8.01 (doublet for *ortho* H); 7.46 (doublet for *meta* H). The coupling constant $J_{o,m}$ is 8.0 Hz. ^{13}C spectrum: (ppm relative to TMS); 147.04 (C-1); 126.86 (C-4); 131.41 (C-2,6); 129.86 (C-3,5).

Bromamine-T. BAT was obtained by dissolving DBT in 4 *M* NaOH (*6*). About 20 g of DBT was dissolved with stirring in ~30 ml of 4 *M* NaOH at room temperature and the resultant aqueous solution was cooled in ice. Pale yellow crystals of BAT formed were filtered under suction, washed quickly with the minimum quantity of cold water, and dried over phosphorus pentoxide. The purity of BAT was checked iodometrically and by recording its NMR spectra (obtained on a Varian 60-MHz nuclear magnetic resonance spectrometer).

^1H spectrum: δ (relative to TMS); 2.4 (singlet corresponding to $-CH_3$) 7.8 (doublet for *ortho* H); 7.4 (doublet for *meta* H). The coupling constant $J_{o,m}$ is 8.0 Hz.

^{13}C spectrum: (ppm relative to TMS); 145.39 (C-1); 140.50 (C-4); 131.75 (C-2,6); 129.40 (C-3,5); and 23.0 (methyl carbon).

Approximately 0.025 *M* (~0.05 *N*) solutions of CAB and BAT were prepared in distilled water and standardized by the iodometric method. About 0.0125 *M* (~0.05 *N*) solution of DBT was prepared in glacial acetic acid and standardized iodometrically.

Buffer solutions. The following buffer solutions were prepared according to the standard methods reported in the literature (*2*): pH 1 and 2 (HCl + KCl); pH 3 (citric acid + Na_2HPO_4); pH 4–6 (acetate + acetic acid); and pH 7–10) (borax + boric acid + NaCl).

Compounds of acceptable grades of purity were used in preparing other solutions.

Absorbance measurements on oxidized IC solutions were carried out on a Beckman Model DB spectrophotometer.

PRELIMINARY STUDIES

With CAB. An aliquot of aqueous IC solution was added to a known volume of the oxidant solution containing enough acid, base, or buffer in an iodine flask. The reaction mixture was shaken and set aside for different intervals of time. Then 10 ml of (2 NH_2SO_4) and 10 ml of 10% KI were added and the liberated iodine was titrated to a starch endpoint with standard sodium thiosulfate. A blank titration was carried out with CAB under identical conditions.

With BAT. A known excess of the oxidant solution was added to an aliquot of aqueous IC solution and the rest of the procedure was identical to that of CAB.

With DBT. An aliquot of IC solution prepared in glacial acetic acid was added to a known excess of 0.05 *N* DBT. The mixture was shaken and set aside for different intervals of time. Then 10 ml of 10% KI was added and the liberated iodine was titrated against standard thiosulfate. A blank titration was carried out under identical conditions.

Table 1 shows the extent of oxidation of indigocarmine in various media. It is seen that with CAB, oxidation is stoichiometric with a four-electron change within 30 min in 0.1–1.0 *N* HCl, 0.1–1.0 *N* H_2SO_4, and pH 1 buffer. Stoichiometric oxidation occurs with BAT in 0.01–0.10 *N* NaOH within the same period. A similar behavior is noted with DBT (Table 2) in glacial acetic acid solutions.

The standard deviation and coefficient of variance for six trials are: σ (%*V*), 0.08 (0.02) for CAB; 0.81 (0.32) for BAT; 0.00 (0.00) for DBT.

TABLE 1
EXTENT OF OXIDATION OF INDIGOCARMINE WITH CHLORAMINE-B AND BROMAMINE-T IN VARIOUS SOLVENT MEDIA

Medium	μmol of CAB consumed / μmol of IC taken	μmol of BAT consumed / μmol of IC taken
0.1 *N* $HClO_4$	1.50	3.43
1.0 *N* $HClO_4$	1.43	—
0.05 *N* HCl	2.08	—
0.10 *N* HCl	1.99	5.42
1.00 *N* HCl	1.99	—
0.05 *N* H_2SO_4	2.08	—
0.10 *N* H_2SO_4	1.99	3.24
1.00 *N* H_2SO_4	1.99	—
pH 1	2.02	6.85
pH 2	2.65	5.37
pH 3	3.16	4.37
pH 4	3.28	4.75
pH 5	3.41	5.12
pH 6	3.28	4.64
pH 7	2.90	4.23
pH 8	2.71	4.13
pH 9	2.53	3.92
pH 10	2.46	3.60
0.001 *N* NaOH	—	2.85
0.01 *N* NaOH	—	1.99
0.03 *N* NaOH	—	1.99
0.05 *N* NaOH	—	1.99
0.08 *N* NaOH	—	1.99
0.10 *N* NaOH	—	2.01

Note. IC taken: 21.0 μmol; CAB taken: 1140 μmol; BAT taken: 606 μmol; time: 30 min.

RECOMMENDED PROCEDURE

With CAB. Add aliquots of IC solution (2–54 μmol) to 25 ml of 0.05 *N* CAB containing enough HCl or H_2SO_4 to give an overall normality of 0.1–1.0 *N* (or enough pH 1 buffer). After 30 min, estimate the unreacted CAB by the iodometric titration.

With BAT. Add 25 ml of 0.05 *N* BAT to aliquots of IC solution (2–54 μmol) containing enough NaOH to give an overall normality of 0.01–0.10 *N*. After 30 min, estimate the unreacted BAT iodometrically.

With DBT. Add 25 ml of 0.05 *N* DBT to aliquots of IC in glacial acetic acid solution (1–21 μmol). After 30 min, add 10 ml of 10% KI and titrate the liberated iodine with standard thiosulfate.

The amount (x μmol) of IC present in the sample solution is given by $x = 10^3 N (V_1 - V_2)/4$, where N is the normality of the thiosulfate solution, V_1 and V_2 are volumes of the thiosulfate solution for the blank and the test

TABLE 2
EXTENT OF OXIDATION OF INDIGOCARMINE WITH DIBROMAMINE-T IN GLACIAL ACETIC ACID MEDIUM

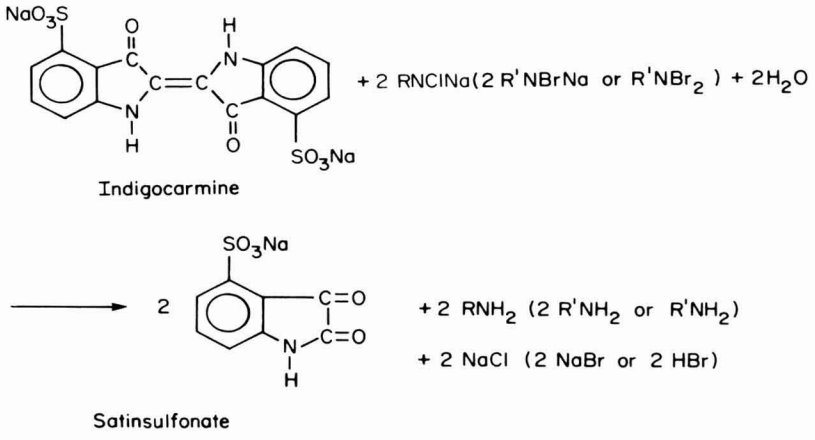
Time (min)	DBT consumed (μmol)	μmol of DBT consumed / μmol of IC taken
1	0.287	0.36
5	0.416	0.52
15	0.508	0.64
30	0.802	1.00
45	0.802	1.00
60	0.802	1.00

Note. IC taken: 0.802 μmol; DBT taken: 426.0 μmol.

sample titrations, respectively, and 4 is the number of electron change per mole of IC.

RESULTS AND DISCUSSION

Table 3 shows some of the results obtained for the estimation of IC with CAB, BAT, and DBT. The error in recovery of the sample is within 1%. The stoichiometry of oxidation of IC to isatinsulfonate could be represented as follows:



(1)

In these reactions the oxidimetric reagents, CAB and BAT, undergo a two-electron change while DBT undergoes a four-electron change. In each case the oxidant is reduced to the respective sulfonamide

$$RNClNa + 2\,H^+ + 2\,e^- \longrightarrow RNH_2 + Na^+ + Cl^-, \quad (2)$$

$$R'NBrNa + 2\,H^+ + 2\,e^- \longrightarrow R'NH_2 + Na^+ + Br^-, \quad (3)$$

$$R'NBr_2 + 4\,H^+ + 4\,e^- \longrightarrow R'NH_2 + 2\,H^+ + 2\,Br^-; \quad (4)$$

TABLE 3

ESTIMATION OF INDIGOCARMINE WITH CHLORAMINE-B, BROMAMINE-T, AND DIBROMAMINE-T

Chloramine-B								
pH1			0.1 *N* HCl			0.1 *N* H_2SO_4		
IC taken (μmol)	IC found (μmol)	% error in recovery	IC taken (μmol)	IC found (μmol)	% error in recovery	IC taken (μmol)	IC found (μmol)	% error in recovery
6.44	6.45	0.16	2.32	2.30	0.86	2.32	2.30	0.86
10.73	10.67	0.56	6.95	6.89	0.86	6.95	6.89	0.86
21.46	21.46	0.00	11.59	11.48	0.95	11.59	11.48	0.86
32.19	32.28	0.28	23.18	22.96	0.95	23.18	23.22	0.17
42.92	42.94	0.05	34.76	34.70	0.17	34.76	34.96	0.58
53.65	53.73	0.15	46.35	46.44	0.19	46.35	46.18	0.37
—	—	—	57.94	57.94	0.00	57.94	58.18	0.41
Bromamine-T						Dibromamine-T		
0.01 *N* NaOH			0.1 *N* NaOH			Acetic acid		
IC taken (μmol)	IC found (μmol)	% error in recovery	IC taken (μmol)	IC found (μmol)	% error in recovery	IC taken (μmol)	IC found (μmol)	% error in recovery
2.10	2.10	0.00	2.36	2.38	0.85	2.62	2.62	0.00
6.33	6.33	0.00	7.08	7.15	0.99	4.38	4.40	0.46
10.55	10.52	0.28	11.80	11.74	0.51	8.76	8.67	1.03
21.12	21.09	0.14	23.61	23.48	0.55	13.13	13.13	0.00
31.65	31.63	0.06	35.40	35.47	0.20	17.51	17.51	0.00
42.21	42.19	0.05	47.21	47.21	0.00	21.89	22.00	0.50
52.77	52.73	0.08	59.01	58.95	0.10	—	—	—

TABLE 4

SPECTROPHOTOMETRIC ESTIMATION OF ISATINSULFONATE (ISN) OBTAINED BY THE OXIDATION OF INDIGOCARMINE WITH CHLORAMINE-B, BROMAMINE-T, AND DIBROMAMINE-T

	ISN (CAB)		ISN (BAT)		ISN (DBT)		
IC taken (μmol)	Calculated (μmol)	Found (μmol)	Calculated (μmol)	Found (μmol)	IC taken (μmol)	Calculated (μmol)	Found (μmol)
2.1	4.20	4.52	4.20	4.15	0.80	1.60	1.68
6.2	12.40	12.40	12.40	12.50	2.40	4.80	4.62
10.5	21.00	21.00	21.00	21.00	4.00	8.00	7.98
21.0	42.00	42.10	42.00	42.00	8.00	16.00	15.96
31.5	63.00	63.05	63.00	63.05	12.00	24.00	23.94
42.0	84.00	84.00	84.00	84.21	16.00	32.00	31.92
52.5	105.00	105.10	105.00	105.00	20.00	40.00	39.90

R = $C_6H_5SO_2-$ for CAB; R′ = p-$CH_3-C_6H_4SO_2-$for BAT and DBT.

The yellow isatinsulfonate solution has a λ_{max} at 410 nm (log ϵ_{max} =2.8). It was of interest to estimate the amount of this product formed in solutions of IC oxidized by the sulfonyl haloamines. A calibration graph was prepared with isatinsulfonate and Beer's law was found to apply up to 25 μmol. A solution of IC was treated with the oxidant and the excess oxidant was destroyed by thiosulfate through the liberation of iodine. It was then made up to a known volume and absorbance was measured. Checks made for interference by other products of oxidation were found to be negative. The results of spectrophotometric analysis are shown in Table 4. It is seen that the experimental values obtained are in good agreement with those calculated based on the assumption that 2 mol of isatinsulfonate are formed per mole of IC.

Benzenesulfonamide formed in the reaction with CAB was detected (*10*) by TLC. A mixture of petroleum ether, chloroform, and n-butanol (2:2:1 v/v) was the solvent, with iodine as the detection reagent (R_f = 0.88). p-Toluenesulfonamide, the reduced product of both BAT and DBT, was detected (*4*) by paper chromatography with benzyl alcohol saturated with water as the solvent and 0.5% vanillin in 1% HCl in ethanol as the spray reagent (R_f = 0.91).

SUMMARY

Sodium N-chlorobenzenesulfonamide (chloramine-B, CAB), sodium N-bromo-p-toluenesulfonamide (bromamine-T, BAT) and N,N'-dibromo-p-toluenesulfonamide (dibromamine-T, DBT) are employed as analytical reagents for estimating indigocarmine (IC) in solution. A four-electron stoichiometry is observed within 30 min in the following media: CAB, 0.1−1.0 N HCl or H_2SO_4 and pH 1 buffer; BAT, 0.01−0.10 N NaOH; DBT, glacial acetic acid. The back titration methods are fairly rapid, simple, and accurate. The product of oxidation, isatinsulfonate, was estimated by spectrophotometric measurements at 410 nm. The sulfonamides formed by reduction of the haloamines are detected by TLC and paper chromatography.

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BOOK REVIEWS

Physical Methods in Modern Chemical Analysis, Volume 1. Edited by THEODORE RUWANA. Academic Press, New York, 1979. x + 320 pp. $28.00.

As the instruments and methodology available to the analytical chemist become more and more sophisticated, so do the analytical problems faced by the chemist. These include the need to measure compounds at lower and lower levels (down to femtograms) and to measure compounds found in extremely complex mixtures. The purpose of this volume, and the series of which it is a part, is to present information on new and modified methods at a level appropriate to those analytical chemists who wish to expand and update their working knowledge of today's methods.

The volume includes five monographs, a subject index, and a list of contributors. The first monograph presents new information on a widely used methodology: gas chromatography. The second and third monographs discuss the instrumentation and applications of mass spectrometry, a methodology which has continually changed and expanded. Atomic fluorescence and atomic absorption spectroscopy methodologies are discussed in the fourth monograph. The last monograph presents information about flame and plasma emission analytical methods.

The editor and contributors have done a fine job in this first volume of an important new series. These monographs provide useful information and should be closely examined by both analytical chemists and by graduate students preparing to become analysts.

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Tables of Standard Electrode Potentials. By GUILIO MILAZZO, SERGIO CAROLI, AND V. K. SHARMA. Project of the Electrochemistry Commission of the International Union of Pure and Applied Chemistry. Wiley-Interscience, New York, 1978. xvi + 421 pp. $48.25.

This book is a collection of the existing data on electrode potentials obtained from more than 10,000 papers. The extended tables contain the following information: electrochemically conducting phase, intermediate species, composition of the solution, solvent, temperature, pressure, measuring method, comparison electrode, liquid junction, electrode reaction, standard value, uncertainty, temperature coefficient, notes, references, and electrode reference number.

Forty-six solvents, five measuring methods, and forty electrodes are included.

The tables are divided into 10 groups, there being separate tables for the lanthanides and the actinides.

In general, this is a monumental piece of work and should be of interest to those working in the field. It is pointed out, however, that no evaluation of the data has been made; the

values shown are merely those collected from the literature cited. The authors followed the philosophy that "it is better to know something than nothing."

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Monitoring Toxic Substances. Edited by DENNIS SCHUETZLE. American Chemical Society, Washington, D.C., 1979. xi + 289 pp., $26.50.

It is time that we take care of our living environment. It is time that we examine, measure, and determine the toxic substances or chemicals distributed in our environment affecting our health conditions and living atmospheres. The recently legislated Toxic Substances Control Act (January 1, 1977) has emphasized the need for evaluating the effect of chemicals on the environment. A significant part of this risk assessment consists of identifying the nature, concentration levels, sources, and biological effects of chemicals or toxic substances released into the environment as a result of human activities. This volume, consisting of 16 papers, brings together many environmental scientists, engineers, and chemists to discuss the sensitive analytical methodology and rapid and accurate instrumental techniques for monitoring toxic substances in environmental and biological systems. It is the Proceedings of a Symposium sponsored by the Division of Industrial and Engineering Chemistry of the American Chemical Society and held at Chicago, Illinois, August 31, 1977. It constitutes the ACS Symposium Series 94. A great many up-to-date references and a complete index are provided.

The beginning chapter by B. N. Ames describes the so-called "Ames Test" or the "*Salmonella*/microsome assay" for the rapid biological screening of chemical substances for potential mutagenicity and carcinogenicity. The new developments in cell culture *in vitro* assay tests for mutagenicity and carcinogenicity are presented by W. G. Thilly and J. G. Deluca in Chapter 2. Versatile indicator cells for many forms of chemically induced genetic damage of human lymphoblasts are of primary concern in this paper.

In Chapter 3, the phased approach developed by J. A. Dorsey *et al.* of the EPA Group for environmental assessments, which has been successfully implemented on a number of programs, is reviewed. It is a viable and cost effective concept for identifying potential environmental problems associated with discharge from industrial and energy processes. Chapter 4, also contributed by T. A. Bellar *et al.* of the EPA Group, reports organic analytical methodology and procedures of solvent extraction and GC/MS techniques for identification and measurement of volatile organic compounds in aqueous environmental samples. The purge and trap method with GC/MS is particularly demonstrated.

Chapter 5 by R. A. Hites reviews the sample isolation methods which are used with GC/MS and demonstrates two case studies on the organic compounds in industrial wastewaters and river systems, Brackish River and Delaware River. Some 123 compounds found in wastewater, river water, and sediment are listed and about 99 compounds found in the Delaware River are tabulated with the concentration range in parts per million and parts per billion levels.

The adsorbent accumulation techniques which are applicable to bioassay requirements and the preliminary results of bioassays performed on organic mixtures accumulated from drinking water are reported in Chapter 6 by B. A. Glatz *et al.*

Chapter 7 by P. Barrett and T. R. Copeland considers trace metal monitoring by atomic absorption spectrophotometry (AAS). Over 60 metals and metalloids are amenable to AAS

analysis at concentration levels ranging from milligrams to femtograms per milliliter, depending on the element, atomizer, and conditions employed. In this paper, analyses of water and air samples for some 25 elements by flame AAS and flameless AAS are summarized. The following paper by F. N. Abercrombie and R. B. Cruz discusses the determination of trace inorganic toxic substances by inductively coupled plasma-atomic emission spectroscopy (ICP–AES). ICP–AES is a challenging, sensitive, and accurate technique for simultaneous multi-element analysis. Instrumentation, operating principles, sample dissolution, applications to environmental samples, interferences, accuracy, precision, and detection limits, and data acquisition and processing are comprehensively reviewed.

Modern methods of surface microanalytical techniques for the chemical characterization of atmospheric particulates are illustrated by R. W. Linton in the ninth paper. These methods provide valuable insights into the chemical specification, mechanisms of formation, sources, reactivity, and potential toxicity of pollutant species. Chapter 10 by P. D. Maker *et al.* describes the method of high-resolution Fourier transform mid-IR absorption spectroscopy for the analysis of trace constituents in the atmosphere. In the eleventh paper, a relatively new technique, "Opto-Acoustic Spectroscopy," applied to the detection of low molecular weight gaseous pollutants is introduced by C. K. N. Patel. These techniques are simple and practical and will play increasingly important roles in pollution monitoring.

Chemical ionization mass spectrometry (CIMS) has been found to be an effective and powerful tool for the analysis of a variety of air pollution samples. The CIMS coupled with the computer techniques provide a selective ionization and sensitive analysis of air pollutants. Both positive and negative CIMS methods are presented in Chapter 12 by T. M. Harvey *et al.* The results are well interpreted.

For the analysis of semivolatile brominated organics in ambient air with GLC/electron capture detector using a glass fiber filter and a high-volume air sampler is found more sensitive than with GC/MS/Comp and is compared in Chapter 13 by R. A. Zweidinger.

Chapter 14 by W. E. Rich and R. A. Wetzel recommends the use of a new technique, ion chromatography, for the rapid measurement of ionic species in both air and water pollutants. It is expected that this method, after being well developed and modified, will replace a number of classical techniques currently used for the analysis of ionic species in environmental samples.

The particular toxic substances associated with carcinogenesis such as nitrosoamines have been brought to public attention since the last decade. The development of a specific detector for monitoring of such hazardous substances and review of their formation and sources are described in Chapter 15 by D. H. Fine.

The closing paper contributed by S. R. Heller and G. W. Milne introduces the use of the NIH/EPA Chemical Information Computer System in support of the Toxic Substances Control Act. It is a collection of chemical and biological data bases equipped with interactive searching. When complete, it will provide a highly accessible body of information on the monitoring of toxic substances.

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Drug Design, Vol. VIII. Edited by E. J. ARIENS. Academic Press, New York, 1979. xvii + 420 pp., $42.00.

Drug Design, Volume 11 in a continuing series of monographs on medicinal chemistry, is itself a subseries of which this is Volume VIII.

Chapter 1 discusses Advances in the Methodology of Quantitative Drug Design. Among the topics explored are the alternatives available in exploiting an original lead, which physical properties should be related to potency, mathematical methods and the analysis of QSAR.

Chapter 2 examines the Application of Pattern Recognition to Drug Design. Using the now available programmable small computers it is possible to study individual features of molecules in terms of their location and accessibility. Since this is a comparatively unexplored area, much of the chapter is devoted to historical and theoretical background.

Chapter 3 deals with the Design of Controlled Delivery Systems. This report takes the conventional timed or sustained release oral dosage forms to implanted drugs. All of this new technology is clearly presented with theoretical and practical aspects detailed.

Receptor Binding as a Tool in the Development of New Bioactive Steroids is the subject of Chapter 4. The receptor–ligand interaction is a key step in eliciting biological response. Intensive study of the receptor provides new insights. Coupled with the economy of such experiments compared to animal studies we may look forward to further expansion of this field. This chapter presents characterization of steroid receptors, screening methodology, and results as well as correlations between binding sites and biological activity.

Chapter 5 surveys the Design of Synthetic Sweeteners. It covers the history, mechanism of sweet taste response, specific details of the structural features of known sweeteners, the measurement of sweetness, inhibitors, and several models for an ideal sweetener.

Chapter 6 addresses the problem of the Assessment of Environmental Effects of Chemicals. It identifies the problem, describes the tests to be carried out, the setting up of test programs, and areas of future work.

The final chapter covers the Design of Selective Ion Binding Macrocyclic Compounds and Their Biological Applications. The rationale, history, nomenclature, biological applications, synthesis, reactions, structural relationships, ion selectivities, and potential drugs are all discussed.

Each chapter has a substantial bibliography and the volume has an index. The book is well printed with ample charts, diagrams, and tables. The reviewer looks forward to each new volume.

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Multichannel Image Detectors. Edited by YAIR TALMI. American Chemical Society Symposium Series, Vol. 102, Washington, D.C., 1979. XII + 351 pp., $30.00.

With increasing requirements on sensitivity of analytical methods, a small size of the sample and a detection and determination of signals by short-lived species, spectroscopic methods faced problems of measurements of small signals superimposed on large backgrounds (unfavorable signal-to-noise ratio) and measurement of signals over a short period of time. Spectroscopic measurements in the visible and uv region under the above-mentioned conditions were made possible by introduction of parallel multichannel detectors. These optoelectronic image devices (OID) are usually constructed on a single silicon crystal wafer. Twenty-five-nanometer linear detectors with up to 2000 discrete light sensors are commercially available, as well as two-dimensional image devices with several hundred thousand sensors. These detectors can be used typically in the range between 190 and 1000 nm, their dynamic range (real time) can be as high as 10^5:1 and they show highly linear transfer characteristics. To bring to the attention of potential users the state of art in this

area, the American Chemical Society at its 176th National Meeting, at Miami Beach in September 1978, sponsored a symposium devoted to the discussion of basic properties of OID and their applications in chemistry, mostly analytical. This reviewed volume brings out in a remarkably short time after the meeting both the reviews and original papers presented before the symposium. Contributions are divided into three groups—analytical spectroscopic applications (which include not only spectrophotometric measurements in the uv and visible region, but also atomic absorption and emission, fluorescence analysis, detectors for HPLC and stopped-flow measurements) and spectroscopic measurements over extremely short periods of time (in laser spectroscopy, fluorescence measurements, Raman spectroscopy, and other measurements on a picosecond time scale). In the third group, some less conventional applications are described. The quality of papers is excellent, better than in most symposium volumes, perhaps because this is such a rapidly growing field which attracted numerous first class analytical and physical chemists. It will be an extremely useful source of information for a beginner or specialist in this area.

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Laboratory Handbook of Chromatographic and Allied Methods. Edited by O. MIKEŠ. Ellis Horwood Ltd., Chichester, and Halsted Press (Wiley), New York, 1979. 764 pp., $89.50 (library rate).

This book is an English translation of the original Czech edition published by the Czechoslovak Academy of Sciences and is a completely rewritten successor to the 1966 edition of the "Laboratory Handbook of Chromatographic Methods." It is divided into thirteen chapters. The first two are general in that they are discussions of the fundamental types of chromatography and the theory of chromatography. The next ten chapters are specific descriptions of paper chromatography, adsorption column chromatography, ion-exchange chromatography, gel chromatography, automation and mechanization of column operations in liquid chromatography (high-performance liquid chromatography), thin-layer chromatography, gas chromatography, countercurrent distribution, and electromigration methods. Each chapter contains a discussion of the method and its relation to the theory given in Chapter 2, a description of the materials, apparatus, and techniques involved and some specific examples from the recent literature. The literature coverage seems to be through about 1974 with a few references in 1975. The final chapter is devoted to an update of the monographic literature through mid-1978. The translation is superb.

The most striking characteristic of the book is the balance which has been struck between the various chromatographic methods. Scientific investigators have a tendency to embrace only one or two of the various techniques and to attempt to do everything with them and, furthermore, to extend and defend them fiercely against all others. In this book, the advantages and disadvantages of each of the techniques are clearly given with little prejudice. A second striking characteristic is the care with which the literature references have been chosen. Actually, relatively few references are cited (76 for thin-layer chromatography and 89 for gas chromatography, for example). The major ones relating to the method are used, of course, but the specific examples chosen seem to be from quite recent literature (1973) and are carefully chosen to illustrate a specific point or difficulty.

One might think from the timing of this publication and the fact that literature coverage is through 1974 that high-performance liquid chromatography (HPLC), the newest of the many methods, might be slighted. This does not seem to be true. HPLC is discussed in relation to classical column chromatography, as it should be, in Chapter 4, and the various equipment

and instrumental methods are described separately in Chapter 8. Another chapter of special interest is included on countercurrent distribution, a sort of discontinuous chromatographic method which is rarely discussed today but which is extremely useful for the preparative separation of complex mixtures.

All in all, this is an excellent book; the presentation is lucid and pertinent; the introduction and theory are well given; and the various industrial sources for equipment and supplies are quite complete. It would make a good textbook for a course in chromatography were it not for the excessive price. As it is, the book should be available for general consultation in any scientific library or laboratory where chromatography is carried out.

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Kirk–Othmer Encyclopedia of Chemical Technology, Vol. 6, 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, 1979. xxiii + 869 pp., $120.00, subscription price, $95.00 per volume.

This volume is a continuation of the material started with the first five volumes, reviewed earlier in this *Journal* (*Microchem. J.* **24**, 389 (1979); **25**, 141 (1980)). The present volume is a continuation of subjects beginning with the letter "C" and covering the subjects "Chocolate and Cocoa," "Choline," "Cholinesterase Inhibitors," "Chromatography, Affinity," "Chromium and Chromium Alloys," "Chromium Compounds," "Chromogenic Materials," "Cinnamic Acid, Cinnamaldehyde, and Cinnamyl Alcohol," "Citric Acid," "Clathration," "Clays," "Coal," "Coal Conversion Processes," "Coated Fabrics," "Coating Processes," "Coatings, Industrial," "Coatings, Marine," "Coatings, Resistant," "Cobalt and Cobalt Alloys," "Cobalt Compounds," "Coffee," "Color," "Colorants for Ceramics," "Colorants for Foods, Drugs, and Cosmetics," "Colorants for Plastics," "Color Photography," "Color Photography, Instant," "Composite Materials," "Computers," "Contact Lens," "Contraceptive Drugs," "Coordination Compounds," "Copolymers," and "Copper."

Forty-eight contributors furnished the material for the volume.

Like the other volumes in the series, it is a monumental piece of work and belongs in every library serving chemists.

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Modern Organic Elemental Analysis. By T. S. MA AND ROBERT C. RITTNER. Dekker, New York, 1979. xiv + 518 pp., $45.00.

The purpose of the book is to organize in a single volume the methods of organic elemental analysis applicable to different sample sizes and the techniques for the determination of all of the elements in organic materials. The subjects covered are: carbon and hydrogen, nitrogen,

oxygen, the halogens, sulfur, selenium, tellurium, phosphorus, arsenic, antimony, bismuth, boron, silicon, mercury, metallic elements, simultaneous determinations, ultramicroanalysis, and trace analysis.

The authors are well qualified for the undertaking and the book is rich in illustrations and references. Those working in the field of organic analysis will find the book to be of great value.

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(Signed) Roselle Coviello, Senior Vice President