# ANALYTICA CHIMICA ACTA

*International monthly devoted to all branches of analytical chemistry*
*Revue mensuelle internationale consacrée à tous les domaines de la chimie analytique*
*Internationale Monatsschrift für alle Gebiete der analytischen Chemie*

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Published monthly

ANALYTICA CHIMICA ACTA

# GENERAL INFORMATION

### *Languages*

Papers will be published in English, French or German.

### *Submission of papers*

Papers should be sent to: Prof. Philip W. West, Coates Chemical Laboratories, College of Chemistry and Physics, Louisiana State University, Baton Rouge 3, La. 70803 (U.S.A.)
or to
Dr. A. M. G. Macdonald, Department of Chemistry, The University, P.O. Box 363, Birmingham 15 (Great Britain)

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Authors should submit two copies in double-spaced type with adequate margins on pages of uniform size. Acknowledgements, summary and references should be placed at the end of the paper.

Tables should be typed on separate pages and numbered in Roman numerals in the order in which they are mentioned in the text. All Tables should have descriptive titles. The use of chemical formulae and conventional abbreviations is encouraged in Tables and Figures but chemical formulae should not be used in the text unless they are necessary for clarity. Units of weight, volume, etc., when used with numerals should be abbreviated and unpunctuated (*e.g.*, 2%, 2 ml, 2 g, 2 μl, 2 μg, 2 ng, 2 cm, 200 mμ).

Figures should be drawn in Indian ink on drawing or tracing paper with all lettering in **thin** pencil. Standard symbols should be used in line drawings; the following are available to the printers:

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References should be given at the end of the paper and should be numbered in the order of their appearance in the text (**not** arranged alphabetically). Abbreviations of journal titles should conform to those adopted by the *Chemical Abstracts List of Periodicals*, 1961 Edition and supplements. The recommended form for references to journal papers and books is as follows:

1. J. J. Lingane and A. M. Hartley, *Anal. Chim. Acta*, 11 (1954) 475.
2. F. Feigl, *Spot Tests in Organic Analysis*, 7th Ed., Elsevier, Amsterdam, 1966, p. 516.

For multi-author references, **all** authors must be named, and initials given, in the reference list, although the use of, for example, Smith *et al.*, is desirable in the text.

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# ANALYTISCHE INTERFEROMETRIE

**(Technisch-physikalische Monographien, Band 24)**

Von Dr. W. NEBE, Jena

1970. 173 Seiten mit 79 Abbildungen und 15 Tabellen. 14,7 cm x 21,5 cm
Leinen 35,- M

Die analytische Interferometrie befasst sich mit der Bestimmung der Konzentration von Gas- und Flüssigkeitsgemischen durch Interferenzmessung. In der Monographie werden einleitend die hierzu erforderlichen theoretischen Grundlagen und die gebräuchlichen Messgeräte beschrieben. Dann werden grundsätzliche methodische Fragen dieses Analysenverfahrens, wie Geräteichung, Lichtquellen usw., behandelt. Die Methode der interferometrischen Gasanalyse wird für Zwei- und Mehrstoffgemische diskutiert; anschliessend geht der Verfasser auf die Besonderheiten der interferometrischen Flüssigkeitsanalysen ein. Umfangreiche Tabellen, in denen die für dieses Analysenverfahren wichtigsten Daten zusammengestellt sind, ergänzen das Werk.

**AKADEMISCHE VERLAGSGESELLSCHAFT**
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DDR-701 Leipzig, Sternwartenstrasse 8

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# *Practical Manual of Gas Chromatography*

edited by **JEAN TRANCHANT**, Laboratoire Central des Poudres, Paris

authors: J. Buzon, N. Guichard, J. Lebbe, A. Prévôt, J. Serpinet, J. Tranchant
6 x 9", xix + 387 pages, 13 tables, 133 illus., 1611 lit. refs., 1969, Dfl. 85.00, £ 10.0.0,
SBN 444-40677-8

**Contents:** Preface (P. Chovin). Foreword and general remarks. List of symbols. 1. Principles and retention values (J. Tranchant). 2. Isothermal-isobaric chromatography (J. Tranchant). 3. Programmed chromatography (J. Tranchant). 4. Apparatus (J. Lebbe). 5. Columns (A. Prévôt). 6. Detectors (N. Guichard and J. Buzon). 7. Qualitative analysis; separation and identification (J. Tranchant). 8. Quantitative analysis (J. Lebbe). 9. Application and techniques (J. Serpinet). Index.

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# SUMMARIES OF PAPERS PUBLISHED IN ANALYTICA CHIMICA ACTA

Vol. 51, No. 1, July 1970

## THE LIQUID-STATE ION-SENSITIVE ELECTRODE

### THEORY AND EXPERIMENTS WITH METAL DITHIZONATES

A new type of generally applicable ion-selective electrode is described. The electrodes are based on solvent extraction systems, the organic phase being supported on graphite rod. A series of electrodes based on metal dithizonates is discussed and a theory to explain their behaviour is proposed. The higher the extraction constant of the metal dithizonate, the greater is the selectivity of the electrode prepared from it for the particular metal involved in the extraction system, and the more sensitive is the electrode for the particular metal ion. The new electrodes are robust and versatile in application.

J. Růžička and J. Chr. Tjell,
*Anal. Chim. Acta,* 51 (1970) 1–19

## FLUORIMETRIC DETERMINATION OF TRACE NITRATES

Nitrate is determined by reacting it with fluorescein in solutions of high sulfuric acid concentrations. Since fluorescein fluoresces and the reaction product does not, nitrate can be determined down to 0.01 $\mu$g ml$^{-1}$ in the final reaction solution by measurement of the fluorescence suppression. The method is easy to use and has good reproducibility. Suggestions are offered to eliminate potential interfering ions.

H. D. Axelrod, J. E. Bonelli and J. P. Lodge, Jr.,
*Anal. Chim. Acta,* 51 (1970) 21–24

## pH DEPENDENCE OF THE N.M.R. LINE BROADENING OF WATER BY PARAMAGNETIC EDTA CHELATES

The effect of pH and temperature upon the n.m.r. line width of solutions of the ethylenediaminetetraacetic acid (EDTA) chelates of copper(II), nickel(II) and iron(III) has been studied. The concentration of these chelates required to give a line width of 10 Hz is only a factor of about two greater than the hydrated metal ions. This result indicates rapid exchange of the solvent from a coordination site on the chelated metal ion. The temperature dependence of the n.m.r. spectra shows that in each case the line width is controlled by the relaxation time of the proton in the paramagnetic environment of the coordinated metal ion rather than the rate of solvent exchange. The pH dependence of the solvent line width is related to the formation of protonated and hydroxy complexes of the chelates as confirmed by potentiometric titration.

J. F. Whidby and D. E. Leyden,
*Anal. Chim. Acta,* 51 (1970) 25–30

## INTRODUCTION TO NUCLEAR CHEMISTRY

by D. J. Carswell

**ix + 279 pages, 23 tables, 69 illus., 1967, Dfl. 32.50, 70s.**

**Contents:** 1. The development of nuclear chemistry. 2. Fundamental particles and nuclear structure. 3. Nuclear reactions and radioactivity. 4. Properties of nuclear radiations. 5. The detection and measurement of nuclear radiation. 6. Nuclear instrumentation. 7. Radiation chemistry. 8. Isotope measurement and separation methods. 9. Charged particle accelerators, neutron sources, production and properties of the actinide elements. 10. Uses of isotopes. 11. Experimental nuclear chemistry (including 16 selected experiments). Index.

## STATISTICAL THERMODYNAMICS

*An Introduction to its Foundations*

H. J. G. Hayman

**ix + 256 pages, 14 illus., 1967, Dfl. 47.50, 110s.**

**Contents:** Preface. Nomenclature. 1. An introductory survey. 2. Some simple partition functions. 3. The microcanonical assembly. 4. The second law of thermodynamics. 5. The canonical assembly. 6. The third law of thermodynamics. 7. Dilute gases. 8. The grand canoinical assembly. 9. Fermi-Dirac, Bose-Einstein and imperfect gases. 10. The partition function method applied to Fermi-Dirac, Bose-Einstein and photon gases. 11. Classical statistical thermodynamics. 12. The relationship between classical and quantum statistics.
Appendices: 1. The probability integral. 2. Stirling's formula for ln *n*!. 3. The method of variation of constants. 4. The dynamic equilibrium of a microcanonical assembly. 5. The adiabatic principle. 6. Liouville's theorem. Index.

## THE STRUCTURE OF INORGANIC RADICALS

*An Application of Electron Spin Resonance to the Study of Molecular Structure*

by P. W. Atkins and M. C. R. Symons

**x + 280 pages, 57 tables, 74 illus., 357 lit. refs., 1967, Dfl. 60.00, £7.0.0.**

**Contents:** 1. Introduction. 2. An introduction to electron spin resonance. 3. Formation and trapping of radicals. 4. Trapped and solvated electrons. 5. Atoms and monatomic ions. 6. Diatomic radicals. 7. Triatomic radicals. 8. Tetra-atomic radicals. 9. Penta-atomic radicals. 10. Summary and conclusions.
Appendices: 1. The language of group theory. 2. The spin Hamiltonian. 3. Calculation of *g*-values. 4. Determination of spin-density distribution and bond angles. 5. Analysis of electron spin resonance spectra. Index of data. Subject index.

## FUNDAMENTALS OF METAL DEPOSITION

by E. Raub and K. Müller

**viii + 265 pages, 10 tables, 138 illus., 245 lit. refs., 1967, Dfl. 60.00, £7.5.0.**

**Contents:** 1. Chemical and electrochemical principles. 2. Electrode processes. 3. The cathodic discharge of ions. 4. The structure of electrolytic metal deposits. 5. Physical and chemical properties of electrolytic metal deposits. 6. Distribution of electrolytic metal deposits on the cathode. Index.

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# AN AUTOMATIC PROCEDURE FOR THE DETERMINATION OF BORON IN SEA WATER

The curcumin method of Uppström for the determination of boron in sea water has been adapted to an open system for automatic analysis. The equipment used is a module system called Autolab. The concentration range investigated is 0.1–6 mg of boron per litre and the sample standard deviation was found to be 1.5% in the middle of the range. The necessary modifications for the adaptation of the manual method as well as the equipment used are described.

P. Hulthe, L. Uppström and G. Östling,
*Anal. Chim. Acta*, 51 (1970) 31–37

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# THE SYNERGIC EXTRACTION OF TERNARY COMPLEXES OF EUROPIUM(III)

It has been shown that the complex $Eu(HSal)_3$ from europium(III) and salicylic acid ($H_2Sal$) can be extracted from aqueous solutions into isoamyl alcohol. In contrast, extraction by non-hydroxylic solvents (*e.g.* benzene, hexane) does not occur over the pH range 2–5 unless a formally neutral oxygen donor is present; ternary compounds of the composition $Eu(HSal)_3L_2$ (where L = tributylphosphate, triphenylphosphine oxide or triphenylarsine oxide) are then extracted. Synergic extraction of $Eu(HSal)_3$ also occurs in the presence of 4-methylpyridine-N-oxide or methyl isobutyl ketone.

H. M. N. H. Irving and S. P. Sinha,
*Anal. Chim. Acta*, 51 (1970) 39–45

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# A SPECTROPHOTOMETRIC DETERMINATION OF CARBANIONS WITH TETRANITROMETHANE

Tetranitromethane has been employed as a reagent for determining the rates of ionization and the dissociation constants of C-acids. The intensely yellow by-product, nitroformate, provides the basis for a convenient spectrophotometric assay at 350 nm ($\varepsilon$=14,400).

P. Christen and J. F. Riordan,
*Anal. Chim. Acta*, 51 (1970) 47–52

## TREATISE ON ELECTROCHEMISTRY

Second, completely revised edition

by G. Kortüm

*Professor of Physical Chemistry, University of Tübingen, Germany*

7 × 10″, xxii + 637 pages, 71 tables, 151 illust., 882 lit.refs., 1965, Dfl. 85.00, £ 10

**Contents:** 1. Definitions and fundamental laws. 2. Fundamental principles of thermodynamics. 3. The solvation of ions. 4. Weak and strong electrolytes. 5. Theory of ionic interaction. 6. Association and incomplete dissociation of strong electrolytes. 7. The results and applications of conductance measurements. 8. Electromotive forces. 9. Practical applications of potentiometric measurements. 10. Acids and bases. 11. Potential differences at phase boundaries. 12. Electrical polarization and the kinetics of electrode processes. 13. Applications of electrochemical processes. Appendix. Subject index.

## OXIDATION AND COMBUSTION REVIEWS

edited by C. F. H. Tipper,

*Department of Inorganic, Physical and Industrial Chemistry, University of Liverpool, Great Britain*

Volume 1

6 × 9″, viii + 344 pages, 29 tables, 45 illus., 815 lit.refs., 1965, Dfl. 40.00, 110s.

**Contents:** 1. Application of the theory of branched chain reactions in low temperature combustion. 2. Oxidation reactions induced by ionising radiation. 3. Gas phase photo-oxidation. 4 Oxidation reactions involving nitrogen dioxide. 5. Oxidative degradation high polymers. 6. The heterogeneous selective oxidation of hydrocarbons. Author and subject indexes.

Volume 2

6 × 9″, viii + 301 pages, 19 tables, 64 illus., 311 lit.refs., 1967, Dfl. 60.00, 150s.

**Contents:** 1. Thermal explosion theory. 2. Some fundamentals of combustion instability 3. The use of adiabatic compression and mass spectrometry in the study of combustion. 4. Trapped radicals and combustion. Author and subject indexes.

*Volume 3 and subsequent volumes are published as a review journal under the same title*

## ATMOSPHERIC OXIDATION AND ANTIOXIDANTS

by G. Scott,

*Head of Works Research and Development Department, Dyestuffs Division, Imperial Chemical Industries Ltd., Grangemouth, Stirlingshire, Great Britain*

7 × 10″, x + 528 pages, 172 tables, 174 illus., 1206 lit.refs., 1965, Dfl. 72.50, £ 8.10.0

**Contents:** 1. The historical development of antioxidants. 2. Peroxides. 3. Autoxidation. 4. Antioxidants: radical chain-breaking mechanisms. 5. Antioxidants: Preventive mechanisms. 6. Measurement of oxidative deterioration. 7. Oxidative deterioration of saturated oils and polymers. 8. Oxidation of olefinic oils, fats and polymers. 9. Degradation of vulcanised rubber. 10. Mechano-oxidation of polymers. Index.

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## RAPID EXTRACTION AND SPECTROPHOTOMETRIC DETERMINATION OF NICKEL(II) WITH ISONITROSOACETOPHENONE

A rapid separation and spectrophotometric determination of nickel based on extraction with HINAP into chloroform is described. Over the pH range 7.2–8.0, the percentage extraction of 1–3 mg of nickel is 99.6% nickel. Interference of cyanide, thiocyanate, citrate, tartrate and EDTA is removed by pH control and by masking agents; masking agents are also used to prevent interferences of ions such as Co, Cu, Pd, Cd, Mn, Fe, Zn, Au, Hg and Zr. Chloroform is the most efficient solvent. The separation of nickel from other elements in cupronickel and steel is rapid and quantitative. The color of the chloroform extract measured at 340 nm obeys Beer's law in the range 0.1–3.0 $\mu$g of nickel per ml ($\varepsilon = 22{,}000$). The colored extracted species is a neutral complex, $Ni(INAP)_2$.

U. B. Talwar and B. C. Haldar,
*Anal. Chim. Acta*, 51 (1970) 53–59

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## SPECTROSCOPIC STUDIES OF ORGANIC COMPOUNDS INTRODUCED INTO A RADIOFREQUENCY INDUCED PLASMA

### PART II. HYDROCARBONS

The spectra of hydrocarbons introduced into a plasma torch have been studied. The background of the plasma torch is much simpler than that of a flame and higher temperatures are obtained. The plasma has fair sensitivity towards carbon: *ca.* 2 $\mu$g sec$^{-1}$ for CN (3883 Å) and 10 $\mu$g sec$^{-1}$ for $C_2$ (5165 Å). The torch may be operated with an "inert" oxidizing or reducing atmosphere. The discharge, almost completely fragments organic compounds and thus in this form is not suitable for the production of the molecular spectra of larger molecules.

D. Truitt and J. W. Robinson,
*Anal Chim. Acta*, 51 (1970) 61–67

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## THE SUBSTOICHIOMETRIC DETERMINATION OF CHROMIUM IN ALUMINIUM ALLOYS

Chromium in aluminium-based alloys has been determined by activation analysis and by isotope dilution using the substoichiometric principle. Chromium, separated by oxidation to dichromate followed by solvent extraction, was substoichiometrically extracted with tetraphenylarsonium chloride into 1,2-dichloroethane and assayed by measurement of its $\gamma$-emission.

N. K. Baishya and R. B. Heslop,
*Anal. Chim. Acta*, 51 (1970) 69–76

# ADHESION AND ADHESIVES

## 2nd, completely revised, edition

**edited by R. Houwink and G. Salomon**

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## Adhesives

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The introductory chapter of this first volume is a concise guide to the scientific background of all adhesive processes. Surface science and bulk properties of solids, as related to adhesive bonding, are discussed and illustrated by recent examples from the technology of metals, glass, rubber and plastics. An annotated bibliography is provided for the reader who wants access to more highly specialized works. The further nine chapters, making up the volume, cover the basic materials used in adhesion technology. Greatest attention is paid to synthetic adhesives, especially the classical thermohardening and the new epoxy resins. Fields previously examined in the first edition have been extended, and a brief contribution added on the adhesion of glass and on glass-to-metal bonding.

CONTENTS:

1. Adhesion. 2. Animal glue and related protein adhesives. 3. Vegetable adhesives. 4. Synthetic organic adhesives. 5. Bituminous binders and coatings. 6. Rubbers. 7. Glass, enamels and ceramics. 8. Inorganic adhesives and cements. Part A: Soluble silicates. 9. Inorganic adhesives and cements. Part B: Miscellaneous inorganic materials. 10. Metallic adhesives. Appendix: Historical table. Subject index.

**Volume 2**

## Applications

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In this second volume the main trends i industrial application are outlined by sixtee specialists. Assessment of surface geome try, choice of surface treatment, and selec tion of the most economical bonding proces — the correct decision in each case is th basis for any successful engineering process The introductory chapter deals with thi general background; it links the two volume and extends the annotated bibliography t 1966. Two compact "adhesives charts' facilitate the choice of a candidate adhesiv and of processing methods. The work als contains a Subject Index to Volume 2 and a Author Index to both volumes.

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Introduction. 11. Surface texture. 12. Surface treatmen of adherends. 13. Adhesive bonding processes. 14 Wood, reconstituted wood and glued laminated struc tures. 15. Adhesive-bonded metal structures. 16. Bonde composite structures. 17. Pressure-sensitive adhesiv tapes. 18. Rubber-textile structures. 19. The tack o printing inks. 20. Adhesion in paint technology. 21 Miscellaneous applications. 22. Testing of adhesive 23. Mechanical testing of bonded joints. Appendix Adhesives charts. Subject index. Author index t Volumes 1 and 2.

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# COLLECTION OF TRACES OF SILVER ON POWDERED DITHIZONE

## APPLICATION OF ULTRASONICS

Microgram quantities of silver can be collected on 5 mg of finely ground dithizone powder from 25 ml of 0.01–1 *M* nitric acid solution within 15 min by the use of ultrasonics. This separation method is successfully applied to the photometric determination of a few p.p.m. of silver in high-purity lead metal.

K. Fukuda and A. Mizuike,
*Anal. Chim. Acta*, 51 (1970) 77–82

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# EFFECT OF PESTICIDES ON LIVER CHOLINESTERASES FROM RABBIT, PIGEON, CHICKEN, SHEEP AND PIG

The effect of twelve different pesticides, including carbamates, chlorinated hydrocarbons and organophosphorus compounds, on liver enzymes isolated from rabbit, pigeon, chicken, sheep, and pig is reported. The cholinesterases from these sources were inhibited at very low concentrations by the organophosphorus pesticides DDVP, Paraoxon, Parathion, and methyl Parathion. None of these enzymes was inhibited by any of the chlorinated pesticides, and pigeon and sheep liver cholinesterase were not inhibited by Sevin. Some selectivity of the enzyme systems for the determination of pesticides is possible.

G. G. Guilbault, M. H. Sadar, S. Kuan and D. Casey,
*Anal. Chim. Acta*, 51 (1970) 83–93

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# COMPUTER CALCULATION OF CONCENTRATIONS OF FLAME GAS SPECIES AND METAL ATOMS IN THE NITROUS OXIDE–ACETYLENE AND AIR–ACETYLENE FLAMES

A computer method based on the principle of the minimisation of free energy is used to calculate the concentrations of both flame gas and condensed species in flames in thermal equilibrium. The degrees of atomisation of aluminium and silicon are calculated for various fuel-lean, stoichiometric and fuel-rich nitrous oxide–acetylene and air–acetylene flames over a range of temperatures and are found to be both critically dependent on the carbon: total oxygen ratio. When this ratio exceeds unity, the degrees of atomisation are a maximum. The concentrations of 43 species and their dependence on the concentrations of atomic aluminium and silicon are considered, and a comparison is made with the nitrous oxide–acetylene and air–acetylene premixed flames used in practice.

J. E. Chester, R. M. Dagnall and M. R. G. Taylor,
*Anal. Chim. Acta*, 51 (1970) 95–107

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### INORGANIC CHEMISTRY

*A Guide to Advanced Study*

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by **R. B. Heslop** and **P. L. Robinson**

6×9″, viii+774 pages, 155 tables, 400 illus., 227 lit. ref., 1967, Dfl. 32.50, 65s.

Contents: Modern inorganic chemistry. The atomic nucleus: genesis of the elements. Radiochemistry. Electronic structures of atoms. The periodic table. Valency; nature and classification of chemical bonding. Structure and shape of molecules. Bonding and structure in compounds of non-transition elements. Bonding in transition-metal complexes. The solid state. Oxidation-reduction: redox reactions. Acids and bases. Hydrogen. The hydrides. The noble gases. The alkali metals. Beryllium, magnesium and the alkaline earth metals. Boron and aluminium. Gallium, indium and thallium. Carbon and silicon. Organometallic compounds. Germanium, tin and lead. Nitrogen and phosphorus. Arsenic, antimony and bismuth. Oxygen, sulphur, selenium, tellurium and polonium. The oxides. Peroxides and peroxo-compounds. The halogens. The halides and pseudohalides. The transition metals. Complex or co-ordination compounds and ions. Substitution reactions of metal complexes. The lanthanides, scandium and yttrium The actinides. Titanium, zirconium and hafnium. Vanadium, niobium and tantalum. Chromium, molybdenum and tungsten. Manganese, technetium and rhenium. Iron, cobalt and nickel. The platinum metals. Copper, silver and gold. Zinc, cadmium and mercury. Index.

### INTRODUCTION TO THE ATOMIC NUCLEUS

Volume 3 in a collection of monographs on "*Topics in Inorganic and General Chemistry*" edited by P. L. Robinson

by **J. G. Cuninghame**

$5\frac{1}{2}\times 8\frac{1}{2}$, xi+220 pages, 3 tables, 58 illus., 170 lit. refs., 1964, Dfl. 15.00, 35s.

Contents: Historical introduction. General definitions and properties. Nuclear forces. Stable nuclides. Radioactivity. Nuclear models. Nuclear reactions. Fission. Alpha-decay. Beta-decay. Gamma-emission. Interaction of particles and rays with matter. Index.

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by **D. J. Carswell**

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Contents: The development of nuclear chemistry. Fundamental particles and nuclear structure. Nuclear reactions and radioactivity. Properties of nuclear radiations. The detection and measurement of nuclear radiation. Nuclear instrumentation. Radiation chemistry. Isotope measurement and separation methods. Charged particle accelerators, neutron sources, production and properties of the actinide elements. Uses of isotopes. Experimental nuclear chemistry. Index.

### RADIOCHEMICAL SURVEY OF THE ELEMENTS

*Principal Characteristics and Applications of the Elements and their Isotopes*

by **M. Haïssinsky** and **J.-P. Adloff**

6×9″, ix+177 pages, 1965, Dfl. 32.50, 75s.

Contents: Introduction. The elements in alphabetical order. Element 102. Element 104.

### THE STRUCTURE OF INORGANIC RADICALS

*An Application of Electron Spin Resonance to the Study of Molecular Structure*

by **P. W. Atkins** and **M. C. R. Symons**

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Contents: Introduction. An introduction to electron spin resonance. Formation and trapping of radicals. Trapped and solvated electrons. Atoms and monatomic ions. Diatomic radicals. Triatomic radicals. Tetra-atomic radicals. Penta-atomic radicals. Summary and conclusions.
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## STUDIES ON AQUEOUS SOLUTIONS OF ESSENTIAL OILS

The aqueous solutions of 12 essential oils were studied. A level of stable concentration, unchanged for at least 20 days, was obtained for elemicin, eugenol, isoeugenol, estragole, safrol and myristicine. Anethole, isomyristicine, eugenol methyl ether, isoeugenol methyl ether, asarone and isosafrol were only partially studied. Stable aqueous solutions filtered through UF fritted glass filters are sterile and can be tested in tissue culture. Propenyl isomers have lower solubility than the allyl counterparts. Changes occurred in several oils after various periods of standing, as observed by spectrophotometric absorption spectra. Millipore membranes of the MF type, and possibly polypropylene tubes were found to remove the oils from solution at concentrations which are otherwise stable.

M. Samuelly and W. Keup,
*Anal. Chim. Acta*, 51 (1970) 109–116

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## AN INFRARED TECHNIQUE FOR STUDYING THE SOLID-STATE PHOTOLYSIS OF COORDINATION COMPOUNDS

*(Short Communication)*

G. Lohmiller and W. W. Wendlandt,
*Anal. Chim. Acta*, 51 (1970) 117–119

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## VITAL PARAMETERS IN THE DETERMINATION OF RHODIUM BY ATOMIC ABSORPTION

*(Short Communication)*

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## REFERENCE ELECTRODE FOR ELECTROCHEMICAL STUDIES IN DIMETHYLFORMAMIDE

*(Short Communication)*

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*Anal. Chim. Acta*, 51 (1970) 124–126

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by A. B. P. LEVER, Associate Professor of Chemistry,
York University, Toronto, Canada

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J. L. Roberts and G. Conway,
*Anal. Chim. Acta*, 51 (1970) 128–130

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J. L. Jones and J. Gastfield,
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# THE LIQUID-STATE ION-SENSITIVE ELECTRODE

## THEORY AND EXPERIMENTS WITH METAL DITHIZONATES

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(Received January 25th, 1970)

The development of ion-sensitive electrodes has made a great impact on various branches of analytical chemistry[1,2] and the new types of electrodes are being used in many laboratories. Our first work in this field was concerned with application of commercially available calcium-sensitive electrodes in continuous-flow analysis[3]. It became clear that there was a correlation between extraction data and electrode function and an attempt was made to verify this idea experimentally with organic solvents containing metal dithizonates and diethyldithiocarbamates. For this purpose the body of the Orion liquid–liquid membrane electrode was used, but this combination did not prove to be very practical. Further work resulted then in development of the liquid-state electrode[4–6]. This new type of ion-sensitive electrode is formed by a thin layer of organic liquid which is adsorbed on the surface of a conducting material. The organic phase must not be miscible with water and the conducting material should be sufficiently organophilic that the organic phase covers the surface of it continuously, thus preventing the direct contact of the material with the aqueous solution to be measured. Apart from this "passive" function, the organic phase also plays an active role in establishing the electrode potential. If the organic solvent is inert, *e.g.* carbon tetrachloride, toluene, benzene, xylene, mesitylene, hydrocarbons, etc., no definite or reproducible potential will be established. If, however, an organic reagent which forms extractable chelates, is dissolved in the solvent, the electrode starts functioning as described below. (There are, of course, also other possibilities than employing metal chelate extraction systems. For example, iodide-sensitive electrodes can be made by means of iodine solution in benzene[6], a pH electrode by dissolving quinhydrone in isopentanol[5], etc.).

In discussing the properties of the conducting material, which serves as a support for the organic phase, it should be mentioned here that even a polished stainless steel wire might behave as, *e.g.*, a copper-sensitive electrode, if covered by a thin layer of copper dithizonate dissolved in an organic solvent. After a while, however, the organic phase will break and the potential as well as the electrode function will change abruptly. Porous organophilic conductors such as graphite are much more suitable for this purpose because they serve not only as supports, but also as reservoirs of the organic phase, which, by slowly diffusing from within them, forms a continuously renewed ion-sensitive surface.

The main purpose of this paper is to present a theory which would allow us to predict electrode properties from extraction data. Therefore, metal dithizonates dissolved in various solvents were chosen as the extractants and hydrophobized carbon as the porous contact material. This combination of the electrode materials appeared to give the best chance of correlating theory and experimental data.

## THEORETICAL PART

If the surface of the liquid-state electrode consists only of a pure inert solvent (*e.g.* carbon tetrachloride), no definite potential is established, and consequently, there is no possibility of observing any definite response either to cations or to anions. This inert electrode, however, can be converted to a hydrogen ion-sensitive or a metal ion-sensitive one, by dissolving a pure reagent HA or a metal chelate $MA_n$ in the solvent. It can therefore be concluded that the electroactive species of the type HA, $MA_n$, $M'A_n$, etc., are solely responsible in determining the electrode function. The potential arising at the interface between the organic and the aqueous phase is therefore due to a difference in chemical potentials of the species in the organic and the aqueous phase

$$E = E_0' + \frac{RT}{nF} \ln \frac{(a)_w}{(a)_o} \tag{1}$$

where $(a)_w$ is the activity of the ionic species ($H^+$, $M^{n+}$ etc.) in the aqueous phase, and $(a)_o$ is the activity of these ionic species present in an extractable form (HA, $MA_n$ etc.) in the organic phase; $n, R, F, T$, have the usual meaning[2,7].

If the discussion is confined to dilute solutions of ionic species in the aqueous phase as well as to dilute solutions of extractable species in the organic phase, activities can be replaced by concentrations and the theory of metal chelate solvent extraction[8] can be applied for evaluation of electrode properties by means of extraction data. By defining the conditions for the formation of the hydrogen ion-sensitive electrode and of the metal ion-sensitive electrodes, it will be possible to describe pH and pM–potential curves and also the selectivities of individual types of liquid-state electrodes.

### *Hydrogen ion-sensitive electrode*

If the organic phase forming the electrode surface, contains a weak organic acid, HA, the electrode potential will be

$$E = E_o + \frac{RT}{F} \ln \frac{[H^+]}{[HA]_o} \tag{2}$$

where $E_0$ is the standard potential valid for $[H^+]/[HA]_o = 1$, $[H^+]$ is the hydrogen ion concentration in the measured aqueous water phase, and $[HA]_o$ is an equilibrium concentration of weak organic acid in the organic phase.

After a rearrangement we obtain:

$$E = E_0 - \frac{RT}{F} \ln [HA]_o + \frac{RT}{F} \ln [H^+] \tag{3}$$

If now the *original* concentration of organic acid, $c_{HA}$, in the organic solvent of volume $V_0$ is chosen as the starting amount of the electroactive species contained in the electrode, to which we shall always refer, we can write

$$c_{HA} = [HA]_0 \tag{4}$$

where $[HA]_0$ is the *equilibrium* concentration of organic acid during the measurement. In such a case eqn. (3) can be rewritten to

$$E = E_0 - \frac{RT}{F} \ln c_{HA} + \frac{RT}{F} \ln [H^+] \tag{5}$$

and, if the first two terms of this equation are constant then:

$$E = \text{const}_{HA} - 0.0591 \text{ pH} \qquad (\text{at } 25°) \tag{6}$$

From these equations it follows that

(a) *the electrode will function as hydrogen electrode with a slope of 59.1 mV/pH;*

(b) *the potential will be more negative, the higher the concentration of organic acid in the organic phase.*

An increase of pH, however, will affect of value of $[HA]_0$ because increased dissociation of organic acid will lead to its transfer from the organic to the aqueous phase:

$$HA_0 \rightleftharpoons H + A^-$$

As a result of this, the electrode does not contain the original quantity of organic acid and consequently also $\text{const}_{HA}$ has changed. The pH value at which the above process starts to influence the electrode potential can be calculated from the theory of solvent extraction[8] by means of the following equations:

$$K_{HA} = \frac{[H^+][A^-]}{[HA]} \quad (7) \qquad p_{HA} = \frac{[HA]_0}{[HA]} \tag{8}$$

and

$$V_0 c_{HA} = V_0[HA]_0 + V[HA] + V[A^-] \tag{9}$$

where $K_{HA}$ is the dissociation constant of the weak organic acid, $p_{HA}$ is the partition coefficient of HA between the organic and water phase, [ ] denote concentrations in water, $[\ ]_0$ denote concentrations in organic phase, and $V$, $V_0$ denote volumes of aqueous and organic phase.

Because an organic acid, which is much more soluble in organic solvent than in water is always chosen (so that $p_{HA} \geqslant 100$), we can calculate (from eqns. (7), (8), (9)) *the highest permissible pH up to which the electrode will function according to eqn. (6):*

$$\text{pH} = \text{p}K_{HA} + \log p_{HA} + (\log V_0/V) - 2 \tag{10}$$

The deviation at this pH will still be negligible ($\Delta E = +0.41$ mV), but at higher pH values condition (4) is no longer fulfilled and the slope of the potential–pH curve will decrease, because the decrease of $[HA]_0$ will cause an increase of electrode potential. The bend in the potential–pH curve will appear at $\text{pH} = \text{p}K_{HA} + \log p_{HA} + \log V_0/V$. Here the deviation ($\Delta E$) will already be +41.0 mV. This value is only approximate because the volume of the organic phase is not sufficiently defined. Furthermore, in practice a porous conducting material (see EXPERIMENTAL) which has been impregnated with a solution of HA in organic solvent is used. Therefore, the depletion of the surface layers of the liquid-state electrode will cause a concentration gradient within the porous material and subsequently the transfer of HA across it from the inside of the electrode to the electrode surface and then into the measured aqueous solution. The latter factor then mainly determines the slope of a potential–pH curve in very alkaline solutions.

It can be concluded that the use of the liquid-state hydrogen ion-sensitive electrode is limited on the alkaline side by eqn. (10). Ideally, the organic acid used should have a very low $K_{\mathrm{HA}}$ value, a sufficiently high $p_{\mathrm{HA}}$, and, for the reasons given below, it should not form any stable chelates with metal ions.

*Metal ion-sensitive electrode*

If the aqueous phase contains not only hydrogen ions but also metal ions, $M^{n+}$, which form extractable chelates, $MA_n$, with reagent HA, the following reaction applies:

$$\mathrm{M}^{n+} + n(\mathrm{HA})_o \rightleftharpoons (\mathrm{MA}_n)_o + n\mathrm{H}^+ \tag{11}$$

The equilibrium constant of this process is:

$$K = \frac{[\mathrm{MA}_n]_o\,[\mathrm{H}^+]^n}{[\mathrm{M}^{n+}]\,[\mathrm{HA}]_o^n} \tag{12}$$

The $K$ value is the extraction constant which is well established for a number of extraction systems[8]. By combining eqns. (2) and (12):

$$E = E_0 + \frac{RT}{nF}\ln K\,\frac{[\mathrm{M}^{n+}]}{[\mathrm{MA}_n]_o} \tag{13}$$

$$E = E_0 - \frac{RT}{nF}\ln\,[\mathrm{MA}_n]_o + \frac{RT}{nF}\ln K + \frac{RT}{nF}\ln\,[\mathrm{M}^{n+}] \tag{14}$$

If the $K$ value is sufficiently high, all the reagent is converted to the form of a metal chelate, consequently:

$$c_{\mathrm{HA}} = n[\mathrm{MA}_n]_o \tag{15}$$

and

$$E = E_0 - \frac{RT}{nF}\ln c_{\mathrm{HA}} + \frac{RT}{nF}\ln n + \frac{RT}{nF}\ln K + \frac{RT}{nF}\ln\,[\mathrm{M}^{n+}] \tag{16}$$

This overall expression can be simplified if we write that

$$\mathrm{const}_{\mathrm{MA}n} = E_0 - \frac{RT}{nF}\ln c_{\mathrm{HA}} + \frac{RT}{nF}\ln n + \frac{RT}{nF}\ln K \tag{17}$$

so that the final expression for potential–metal ion concentration dependence is:

$$E = \mathrm{const}_{\mathrm{MA}n} - \frac{0.0591}{n}\,\mathrm{pM}^{n+} \tag{18}$$

In other words, there will be a change of potential of 59.1, 29.5 and 19.7 mV per tenfold change of the metal ion concentration if $n$ equals to 1, 2 or 3 respectively (at. 25°C). Further, from the nature of $\mathrm{const}_{\mathrm{MA}n}$ it follows that:

(a) *the higher the extraction constant K, the more positive is the potential;*

(b) *the higher the concentration of the reagent $c_{\mathrm{HA}}$ in the organic phase, the more negative is the potential.*

Assuming a fair constancy of $E_0$, the latter factor is the only one which could affect the stability of the electrode potential ($\mathrm{const}_{\mathrm{MA}_n}$). Because the surface of the liquid-state electrode changes with the composition of the measured aqueous phase, losses of metal chelate which might occur in very alkaline and heavily complexing media, must always be avoided. In practice, however, the precaution of filling the electrode with metal chelate solution in the organic phase (rather than with pure reagent HA) is always taken and therefore the electrode will always tend to reestablish the original potential, corresponding to the value of $\mathrm{const}_{\mathrm{MA}_n}$. Clearly, the higher the extraction constant of metal chelate, the more difficult it will be to affect the composition of the organic phase.

### *Influence of pH on the metal-ion sensitive electrode*

In contrast to other types of electrodes, the composition of the surface of the liquid-state electrode changes immediately with the composition of measured aqueous phase. Thus a metal-ion sensitive electrode will change to a $H^+$ sensitive electrode if used in a very acidic solution and *vice versa*. The electrode potential can then be described by the following set of equations:

Originally:

$$E = E_0 - \frac{RT}{nF}\ln\,[\mathrm{MA}_n]_o + \frac{RT}{nF}\ln K + \frac{RT}{nF}\ln\,[\mathrm{M}^{n+}] \qquad (14)$$

This is, however, not true in a very acidic solution, where part of the reagent is converted back to the HA form:

$$c_{\mathrm{HA}} = n[\mathrm{MA}_n]_o + [\mathrm{HA}]_o \qquad (19)$$

($p_{\mathrm{HA}}$ is assumed to be greater than $10^2$ and $[\mathrm{A}^-] \leqslant [\mathrm{HA}]_o + [\mathrm{MA}_n]_o$)
Then:

$$E = E_0 - \frac{RT}{nF}\ln c_{\mathrm{HA}} + \frac{RT}{nF}\ln\left(n + \frac{[\mathrm{HA}]_o}{[\mathrm{MA}_n]_o}\right) + \frac{RT}{nF}\ln K + \frac{RT}{nF}\ln\,[\mathrm{M}^{n+}] \qquad (20)$$

From the value of $K$ (eqn. (12)) it follows that:

$$\frac{[\mathrm{HA}]_o}{[\mathrm{MA}_n]_o} = \frac{[\mathrm{H}^+]^n}{[\mathrm{M}^{n+}]}\,\frac{1}{K[\mathrm{HA}]_o^{\,n-1}} \qquad (21)$$

Thus finally:

$$E = E_0 - \frac{RT}{nF}\ln c_{\mathrm{HA}} + \frac{RT}{nF}\ln K + \frac{RT}{nF}\ln\left(n + \frac{[\mathrm{H}^+]^n}{[\mathrm{M}^{n+}]}\,\frac{1}{K\,[\mathrm{HA}]_o^{\,n-1}}\right) + \frac{RT}{nF}\ln\,[\mathrm{M}^{n+}] \qquad (22)$$

which is the overall expression for the potential–pH dependance of an $\mathrm{M}^{n+}$-sensitive electrode. The magnitude of the terms in round brackets determine the influence of pH. Clearly, if

$$\frac{[\mathrm{H}^+]^n}{[\mathrm{M}^{n+}]}\,\frac{100}{K[\mathrm{HA}]_o^{\,n-1}} \leqslant n \qquad (23)$$

there will be no influence of hydrogen ions as eqn. (22) simplifies to eqn. (18). It can also be seen that:

(a) *the higher the value of K, the smaller is the influence of hydrogen ions;*

(b) *the lower the metal ion concentration, the higher must be the pH, in order to avoid the interference of hydrogen ions.*

More precisely, for a case of $n=1$ and the same interference limit as above

$$\text{pH} \geqslant \text{pM} - (\log K) - 2 \tag{24}$$

$$\text{pM} \leqslant \text{pH} + (\log K) - 2 \tag{25}$$

in which case the deviation will be equal to or less than +0.19 mV. On the other hand, hydrogen and metal ions will contribute equally to the electrode potential if

$$\text{pH} = \text{pM} - \log K \tag{26}$$

which is the point where the slope changes in the potential–pH curve. Here, the deviation of potential, caused by hydrogen ions will already be +19.7 mV. Thus, for example, a liquid-state electrode made of silver dithizonate dissolved in carbon tetrachloride ($\log K = 7.18$) will have a limit of detection at pH 5 equal to $6.6 \cdot 10^{-11}$ *M* $Ag^+$. The change of the slope of the potential–pH curve should occur at pH 3. The mercury-sensitive liquid-state electrode made of mercury dithizonate in carbon tetrachloride will be even more sensitive and less influenced by hydrogen ions (see Table I). On the other hand, electrodes for zinc(II) and lead(II) made of the same reagent will be easily affected by change of pH.

TABLE I

THE POTENTIALS OF THE LIQUID-STATE METAL DITHIZONATE ELECTRODES AND CORRESPONDING EXTRACTION CONSTANTS[a]

| *Electrode* | *Organic phase*[b] | *pH* | *E(mV)* | $n^{-1}$ *log* $K$[b] |
|---|---|---|---|---|
| $Hg^{2+}$ | $Hg(HDz)_2$ | 3 | +475 | 13.4 |
| $Ag^+$ | AgHDz | 5 | +355 | 7.18 |
| $Cu^{2+}$ | $Cu(HDz)_2$ | 5 | +250 | 5.2 |
| $Zn^{2+}$ | $Zn(HDz)_2$ | 5 | + 50 | 1.1 |
| $Pb^{2+}$ | $Pb(HDz)_2$ | 5 | + 60 | 0.4 |
| $H^+$ | $H_2Dz$ | 5 | − 50 | |

[a] Solvent $CCl_4$.

[b] *K* values from ref. 8.

### *Influence of pH and of foreign metal ions*

If a liquid-state electrode of the type $MA_n$ is immersed in a solution containing not only the ion of interest $M^{n+}$ but also foreign ions $H^+$ and $M'^{n'+}$, the potential of the electrode will still correspond to eqn. (14); condition (15), however, must be expanded to

$$c_{HA} = n[MA_n]_o + [HA]_o + n'[M'A_{n'}]_o \tag{27}$$

as $M'^{n'+}$ will also be present in the organic phase (as chelate $M'A_{n'}$ in a quantity corresponding to its concentration in the aqueous phase and to the value of the extraction constant $K'$. Therefore:

$$[MA_n]_o = \frac{c_{HA}}{\left(n + \dfrac{[HA]_o}{[MA_n]_o} + n'\dfrac{[M'A_{n'}]_o}{[MA_n]_o}\right)} \tag{28}$$

Now the values of $\frac{[HA]_0}{[MA_n]_0}$ and $\frac{[M'A_{n'}]_0}{[MA_n]_0}$ can be expressed by means of the corresponding extraction constants $K$, $K'$ (see eqns. (12), (21)), so that the denominator of eqn. (28) is:

$$\left(n + \frac{[H]^n}{[M^{n+}]}\frac{1}{K[HA]_0^{n-1}} + n'\frac{K'[M'^{n'+}]\,[HA]_0^{n'-n}}{K[M^{n+}]\,[H^+]^{n'-n}}\right) \tag{29}$$

The general expression is then obtained by combining eqns. (14), (28) and (29):

$$E = E_0 - \frac{RT}{nF}\ln c_{HA} + \frac{RT}{nF}\ln K + \frac{RT}{nF}\ln\left(n + \frac{[H^+]^n}{[M^{n+}]}\frac{1}{K[HA]_0^{n-1}}\right.$$
$$\left. + n'\frac{K'[M'^{n'+}]\,[HA]_0^{n'-n}}{K[M^{n+}]\,[H^+]^{n'-n}}\right) + \frac{RT}{nF}\ln[M^{n+}] \tag{30}$$

If

$$\frac{[H^+]^n}{[M^{n+}]}\frac{100}{K[HA]^{n-1}} \leqslant n \text{ and if } n'\frac{K'[M'^{n'+}]\,[HA]_0^{n'-n}}{K[M^{n+}]\,[H^+]^{n'-n}}\,100 \leqslant n$$

then eqn. (30) reduces to:

$$E = \text{const}_{MA_n} - \frac{0.0591}{n}\,\text{p}M^{n+} \tag{18}$$

as there is no interference from either $H^+$ or $M'^{n'+}$ ions.

However, if (*a*) the values of $H^+$ $M'^{n'+}$ or $K'$ are too high, and/or (*b*) the values of $M^{n+}$ or $K$ are too low, so that the expressions in brackets cannot be neglected compared to $n$, the ions $H^+$ and $M'^{n'+}$ will affect the electrode potential according to eqn. (30) and will interfere in the direct potentiometric measurement of $M^{n+}$ ions.

The use of eqn. (30) can also be shown by the following simplified example. If the influence of hydrogen ions is avoided (by choosing pH according to the conditions (24) and (25)) and consider a case when $n = n'$ (only divalent or only monovalent ions), eqn. (30) simplifies to:

$$E = E_0 - \frac{RT}{nF}\ln c_{HA} + \frac{RT}{nF}\ln K + \frac{RT}{nF}\ln n\left(1 + \frac{K'[M'^{n+}]}{K[M^{n+}]}\right) + \frac{RT}{nF}\ln[M^{n+}] \tag{31}$$

and further to:

$$E = \text{const}_{MA_n} + \frac{RT}{nF}\ln\left(1 + \frac{K'[M'^{n+}]}{K[M^{n+}]}\right) + \frac{RT}{nF}\ln[M^{n+}] \tag{32}$$

and finally to

$$E = \text{const}_{MA_n} + \frac{RT}{nF}\ln\left([M^{n+}] + \frac{K'}{K}[M'^{n+}]\right) \tag{33}$$

This expression has already been used in direct potentiometry for the evaluation of the selectivities of liquid–liquid membrane electrodes[2]. It is therefore not surprising that also the condition (35) which is derived from it, defines the position of the "break-point" (PUNGOR AND TÓTH[9], SHATKAY[10]) for the potential–foreign metal ion concentration curve. It can be seen that:

(a) *there will be no interference caused by a foreign metal ion if:*

$$\log[M^{n+}] \geqslant \log K' - \log K + \log[M'^{n'+}] + 2 \tag{34}$$

(b) *the change of slope at the potential–foreign ion concentration curve will occur when:*

$$\log\,[M'^{n+}] = \log\,[M^{n+}] + \log K - \log K' \qquad (35)$$

Clearly, *the higher the extraction constant of the metal chelate* MA, *the more selective will the* $M^{n+}$ *ion-sensitive electrode be.* Therefore, for example, the selectivities of the metal dithizonate electrodes will decrease in the following order:
gold(III) > mercury(II) > silver(I) > copper(II) > zinc(II) > lead(II) > hydrogen(I), in fact in the same manner as the extractibility of the corresponding metal dithizonates[8,11]. This picture will, of course, change again whenever hydrolysis or masking of the $M^{n+}$ takes place.

*The influence of hydrolysis and complexation*

The main factor influencing the potential of the liquid-state, metal chelate electrode in alkaline solutions is the decrease in the concentration of the metal ion $M^{n+}$, caused by hydrolysis. As, according to eqn. (18), the electrode responds only to "free" metal ion concentration $[M^{n+}]$, which decreases with increasing pH (while the total metal concentration $[M_{tot}]$ remains constant), the electrode potential will decrease accordingly. Using the Ringbom's concept of conditional constants and side-reaction coefficients[11], eqn. (18) can be written:

$$E = \text{const}_{MA_n} + \frac{0.0591}{n}\log\frac{[M_{tot}]}{\alpha_{M^{n+}}} \qquad (36)$$

because $\alpha_{M^{n+}} = [M_{tot}]/[M^{n+}]$. Thus, for example, hydrolysis of mercury(II) and silver(I) can be described by the relevant values of log $\alpha$ (Table II).

TABLE II

LOGARITHMIC VALUES OF SIDE-REACTION COEFFICIENTS $\alpha$ FOR HYDROXIDES[11]

| pH | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Ag(I) | | | | | | | | | | 0.1 | 0.5 |
| Hg(II) | | 0.5 | 1.9 | 3.9 | 5.9 | 7.9 | 9.9 | 11.9 | 13.9 | 15.9 | 17.9 |

It can be seen that the first decrease of potential of the silver electrode, due to hydrolysis of silver ions should occur around pH 10 and its slope should increase with increasing pH. Mercury, on the other hand, starts to hydrolyse around pH 3 and the slope of the potential–pH curve above this value should be constant, namely, −59 mV/pH.

From this and from previous considerations we can conclude that:

(*a*) each potential–pH curve is composed of three main parts. On the acid side there is a slope due to the influence of the hydrogen ions. Then comes a plateau, the potential of which corresponds to eqn. (14), and on the alkaline side (where the electrode still responds correctly to $M^{n+}$ another decrease of potential is observed, which is due to hydrolysis (eqn. (36)).

(*b*) The plateau on this curve is a result of two parameters of the extraction constant and of the hydrolysis of the metal ion. The higher the extraction constant of the metal chelate and the lower the stability constant of the hydroxo complex of $M^{n+}$, the longer the plateau will be and *vice versa*.

Complexing agents will have a similar influence on the electrode potential as the hydroxyl ions because in their presence the free metal ion concentration will decrease. This influence can be evaluated either by eqn. (36), or perhaps more precisely, from the values of stability constants and solubility products[12]. The calculated values of the potentials of the silver dithizonate electrode in solutions containing equal total concentrations of silver in absence and presence of various amounts of chloride and bromide can serve as an example (Table III).

TABLE III

SILVER ION-SENSITIVE LIQUID-STATE ELECTRODE[a]

| *Concentrations (moles/l)* | | | | *Potentials (mV)* | | *Deviations (mV)* |
|---|---|---|---|---|---|---|
| *Silver total* $[Ag_{tot}]$ | *Chloride total* $[Cl_{tot}]$ | *Bromide total* $[Br_{tot}]$ | *Silver ion*[b] $[Ag^+]$ | *Calculated*[c] | *Measured (at 20°)* | |
| $1 \cdot 10^{-2}$ | 0 | 0 | $1 \cdot 10^{-2}$ | +446 | +439 | −7 |
| $1 \cdot 10^{-3}$ | 0 | 0 | $1 \cdot 10^{-3}$ | +388 | +388 | 0 |
| $1 \cdot 10^{-4}$ | 0 | 0 | $1 \cdot 10^{-4}$ | +330 | +323 | +7 |
| $1 \cdot 10^{-5}$ | 0 | 0 | $1 \cdot 10^{-5}$ | +272 | +269 | −3 |
| $1 \cdot 10^{-3}$ | $2 \cdot 10^{-3}$ | 0 | $10^{-6.6}$ | +180 | +200 | +20 |
| $1 \cdot 10^{-3}$ | $1.1 \cdot 10^{-3}$ | 0 | $10^{-7.6}$ | +122 | +135 | +13 |
| $1 \cdot 10^{-3}$ | 0 | $2 \cdot 10^{-3}$ | $10^{-9.2}$ | +29 | +50 | +21 |
| $1 \cdot 10^{-3}$ | 0 | $1.1 \cdot 10^{-2}$ | $10^{-10.2}$ | −29 | −11 | +18 |

[a] Silver dithizonate in *o*-xylene used as organic phase.
[b] Based on solubility products $L_{AgCl} = 10^{-9.6}$, $L_{AgBr} = 10^{-12.2}$.
[c] Based on measured value for $1 \cdot 10^{-3}$ *M* $Ag^+$ (+388 mV) and slope 58 mV/pAg.

## EXPERIMENTAL

### *Apparatus*

*The liquid-state electrode.* This is made of Teflon tubing (approx. 10 cm long, walls 2 mm thick, internal diameter 5.9 mm) precisely machined so that inside the lower end can be fitted very tightly a 25-mm long and 6.3-mm thick carbon rod. This spectral-grade porous carbon rod is first hydrophobized and impregnated with the pure organic solvent ($CCl_4$, $CHCl_3$, xylene, benzene, etc.), and then mounted into the electrode body in such a way that its flat end is even with the end of the Teflon tube. This is best done by placing the tube in an electric oven (adjusted to about 150°) so that it expands a little, and then while the tube is still hot, inserting the cold carbon rod into it by pressing both against a flat firm surface. The Teflon tube constricts on cooling thus sealing the carbon rod securely into the correct position. (It is easy to replace the used carbon with a new rod when the electrode body is heated again).

After the electrode has cooled, its open end is connected to a slight vacuum and the other end, now closed with the carbon rod, is immersed in a solution of metal dithizonate (or dithizone) in the organic solvent with which the carbon rod was originally impregnated. After 10–15 min, the vacuum is disconnected, the electrode is removed from the organic solution and the excess of solution is poured off the elec-

electrode cavity. (If more than about one quarter of the tube is filled with organic phase during evacuation, it is possible that the carbon rod does not fit the tube tightly enough. The organic phase will then leak out during use, form a drop hanging on the sensitive surface and given erroneous results.) The connection to the potentiometer is made by means of a stainless steel wire (*ca.* 10 cm long and 2 mm thick), which is screwed into the carbon and leads through a plastic cap by means of which the other end of the Teflon tube is closed.

The organic phase forms at the carbon surface a nearly invisible layer which is water-repellent and may be renewed either by dipping the electrode in the organic phase, by scratching off the electrode surface or by a combination of both. The removal of the surface layers improved the slope for electrodes made with dithizone in various solvents. This improvement is probably due to the removal of the super saturated surface layers of carbon, the pores of which were clogged by precipitated dithizone.

*Electrodes* used for pH control and recording were the glass electrode (type G 202 B) together with the reference calomel electrode (K 401). When chloride ions had to be avoided, a potassium nitrate bridge (B 350) was used.

All potential–pH curves were recorded automatically by means of the following instruments. For pH control: Titrator TTT 1 a, Titrigraph SBR 2 b, Autoburette ABU 13 (equipped with 2.5-ml piston chamber). For pH and potential recording: two pH meters 26 and two-pen recorder RE 520 (Servogor Goertz Austria). All electrodes and instruments, except for the last were manufactured by Radiometer A/S, Copenhagen.

### *Reagents*

Reagents and chemicals were of A.R. grade; double-distilled water was used in all experiments.

*Dithizone and metal dithizonates.* Dithizone[8,13], dissolved in various solvents such as carbon tetrachloride, chloroform, benzene and xylene, was purified in the usual manner by solvent extraction[13]. After purification, the dithizone concentration was adjusted to $1 \cdot 10^{-4}$ *M* and various metal dithizonates were prepared from it by solvent extraction, with an excess of metal ion in the aqueous phase. The conditions of preparation such as pH, molar excess and time of shaking were chosen[8,14] so that more than 99% of dithizone was converted to the required metal dithizonate. Although primary dithizonates were prepared and used for the electrodes, the possibility that at least one of them (copper dithizonate) was converted to the secondary dithizonate during the use of the electrode, cannot be ruled out.

### *Recording of potential-pH curves*

A solution (100 ml) of $1 \cdot 10^{-3}$ *M* nitric acid containing a precisely known quantity of metal ion $M^{n+}$ (in the case of the dithizone electrode, only nitric acid solutions were used), was placed in a glass beaker, and mixed well with a Teflon-covered magnetic stirring bar. The electrodes (liquid-state, calomel and two glass electrodes, see Fig. 1) were immersed in the solution and both the pH and the potential of the liquid-state electrode were measured and registered by means of a two-pen recorder. When the potential of the liquid-state electrode became stable, which required from several seconds to a few minutes depending on the type of the organic phase, the Titrigraph was started. The glass electrode connected to the Titrator which functions as a potentiostat, controls the pH of the solution. The function of the Titrigraph is to provide an additional potential between the calomel electrode and the Titrator. The potential is changed linearly and synchronously with the movement of the recording paper in the two-pen recorder. The overall effect of this arrangement is that the Titrator activates the Autoburette whenever the Titrigraph produces a potential difference. The Autoburette then adds, in small increments, sufficient titrant (0.25 *M* sodium hydroxide), which makes the solution more alkaline. Although the potential is changing continuously while the titrant is added stepwise, it is possible to obtain good control of pH and fairly reproducible curves. The rate at which the pH was changed was the same for all experiments, 0.5 pH unit per min. This required efficient stirring and careful positioning of the tip of the autoburette outlet, so that it was supplying sodium hydroxide in the vicinity of both glass electrodes. Otherwise larger variations of pH (occurring in these unbuffered solutions mainly between pH 5 to 9) were experienced. In all the cases presented here (Figs.

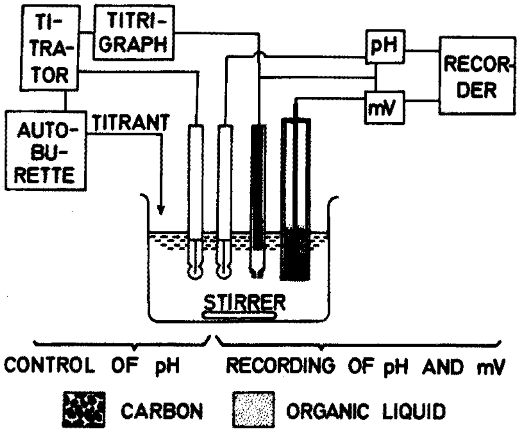


Fig. 1. Apparatus used for automatic recording of potential–pH curves. The liquid-state electrode, made of porous carbon impregnated with organic phase, is the first on the right. Next is the calomel electrode, used as reference and then two glass electrodes.

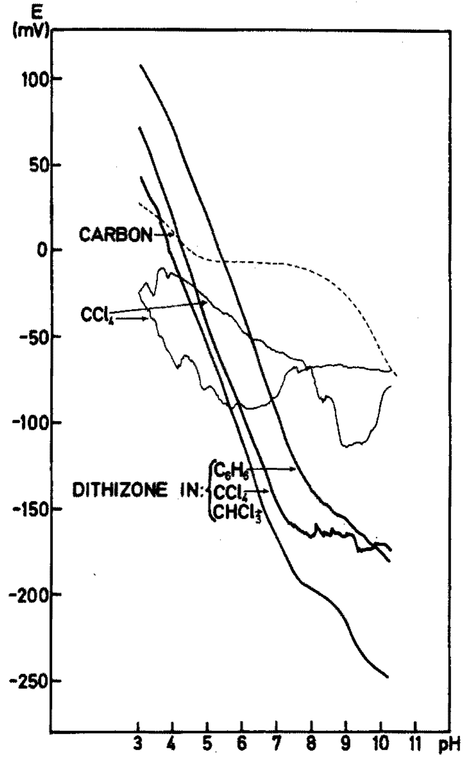
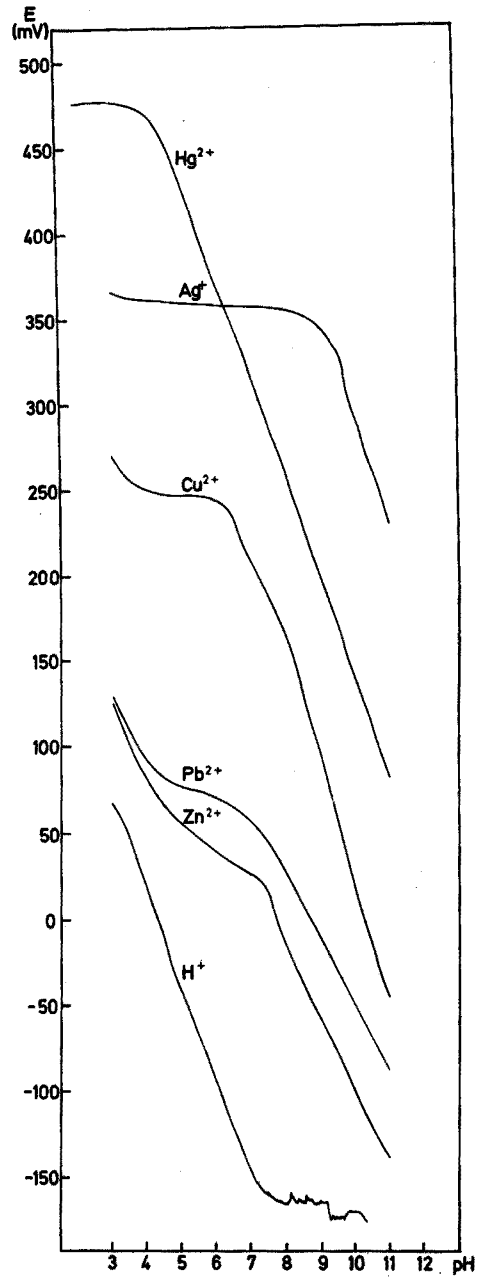


Fig. 2. Potential–pH curves recorded with (a) bare carbon (dotted line), (b) carbon impregnated with pure $CCl_4$ (two runs as shown by thin lines), (c) liquid state electrodes impregnated with solutions of dithizone in benzene, carbon tetrachloride and chloroform.



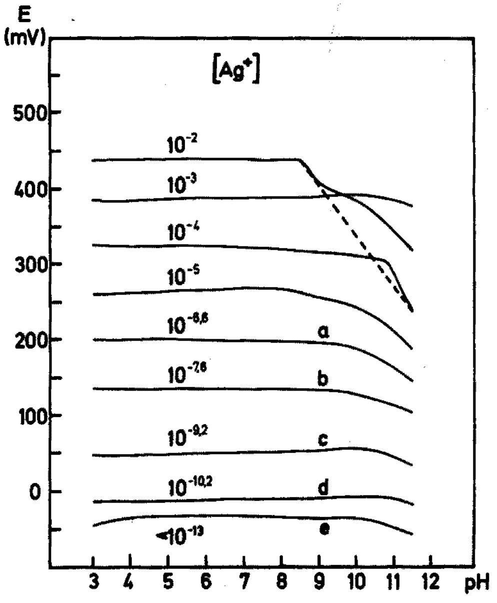


Fig. 3. The potential–pH curves recorded in solutions containing $1 \cdot 10^{-3}$ *M* $Hg^{2+}$ (top curve), $1 \cdot 10^{-3}$ *M* $Ag^{+}$ (second curve), $1 \cdot 10^{-3}$ *M* $Cu^{2+}$, $1 \cdot 10^{-3}$ *M* $Pb^{2+}$, $1 \cdot 10^{-3}$ *M* $Zn^{2+}$ and $1 \cdot 10^{-3}$ *M* $H^{+}$ by means of various liquid-state electrodes. The electrode used for each curve was made from the relevant metal dithizonate in carbon tetrachloride, the curve for $1 \cdot 10^{-3}$ *M* $H^{+}$ being recorded with pure dithizone in the solvent.

Fig. 4. The liquid-state silver ion-sensitive electrode. Xylene was used as the organic solvent. Curves a–e were recorded in solutions "buffered" with silver chloride, bromide or iodide (see also Table III).

2–4), the second pen of the recorder (connected to the glass electrode) drew a straight line with variations not exceeding ±0.05 pH unit. Therefore, when the records were redrawn this line was omitted and replaced by the horizontal axis showing pH units. To ensure that the readings of the two glass electrodes did not differ from each other as well as from the correct pH scale by more than 0.1 pH unit, the apparatus was frequently checked by standard buffers.

## RESULTS AND DISCUSSION

### *The hydrogen ion-sensitive electrode*

At first the potential–pH curve was recorded with the electrode made of bare carbon, then with a liquid-state electrode the surface of which consisted of pure carbon tetrachloride, and finally with liquid-state electrodes made of solutions of dithizone in carbon tetrachloride, chloroform and benzene (Fig. 2).

The electrode made of bare carbon (dotted line) exhibited a potential which was to a certain extent pH-dependent, which behaviour has already been described[15]. However, when the same grade of carbon was made hydrophobic and impregnated with carbon tetrachloride so that its surface became continuously covered by a very thin, almost invisible layer of the solvent, the electrode provided no stable potential even after immersion for several hours in $10^{-3}M$ nitric acid. When the potential–pH curves were recorded, no dependence on pH was found and repeated runs were completely irreproducible (Fig. 2). In contrast electrodes impregnated with a solution of dithizone in carbon tetrachloride, reached a fairly stable potential in a few minutes, and the recorded potential–pH curves were reproducible. Because dithizone is a weak organic acid ($pK_{HA}=4.5$), a dependence of potential on the acidity of the measured solution was expected, and in fact, the potential–pH curves for electrodes made with solutions of dithizone in carbon tetrachloride, chloroform and benzene had almost the theoretical slope (58 mV/pH) up to pH 7 (Fig. 2). The position of the curves on the potential axis was found to be a function of concentration of dithizone in the organic solvent; the higher this concentration, the more the curve was shifted to negative potentials and *vice versa*. Moreover, if the electrode was used above pH 8 and then a new recording was made, the starting potential in a fresh solution of nitric acid (at pH 3) was the higher, the more the electrode had been depleted of dithizone in the previous experiment. If this depletion was not excessive, the electrode potential reached the original value as the concentration of dithizone at the electrode surface was restored by diffusion of dithizone from within the electrode. If the electrode was not used in alkaline solution, the same starting potential at pH 3 was always obtained. These observations confirm that the concentration of the electroactive species at the surface of the electrode affects the electrode potential, and indicate that the values of $c_{HA}$ and $[HA]_0$ have the correct meanings as used in the theoretical part. Unfortunately, it was not possible to measure this effect more precisely with exact concentrations of dithizone in the organic phase, because dithizone (as well as metal dithizonates) are adsorbed at the carbon surface. When solutions of dithizone in an organic solvent were sucked through the porous carbon, pure solvent appeared first on the other side of the rod and then gradually the concentration of dithizone in the "eluate" increased. The result of this "chromatography" on the electrode material is that the solution of dithizone present in the pores at the external surface of the carbon rod (which is later in contact with the measures solution) is more concentrated than the original solution used for impregnation. It is even possible to produce a super saturated electrode which exhibits less than the theoretical slope even in very acidic medium, because the value of $p_{HA}$ (which is related to the ratio of solubilities in organic and water phase) becomes smaller than the equilibrium value. This can be overcome by cutting off 0.5–1 mm of the carbon surface, thus removing the super saturated surface layers. The curves shown in Fig. 2. were obtained with electrodes treated in this way and they exhibited a slope of about 57–59 mV/pH. The value of pH at which the change of slope occurs, has also a theoretical significance, as shown by eqn. (10). If tabulated values of $pK_{HA}+\log p_{HA}$ for dithizone[8] in carbon tetrachloride (8.9), chloroform (10.5) and benzene (9.1) are used and the value of $V_0/V$ is estimated to be $10^{-2}$–$10^{-3}$, the change should occur at pH 6–7, 7.5–8.5 and 6–7, respectively. In practice (Fig. 1), all the bends are located between pH 7 and 8. This discrepancy between theory and experiment occurs because the value of $V_0$ is not well defined, and also because the electrodes might have been still slightly "supersaturated" with dithizone.

The dithizone electrode is, of course, not at all suitable for pH measurements and its significance is only theoretical. It is not practical, not only because it ceases to function even in neutral solutions, but also because the hydrogen of dithizone is very easily replaced by various metal ions[8,13]. Consequently, the dithizone electrode is easily "poisoned" even by traces of metal ions present in measured solutions and becomes sensitive to them rather than to hydrogen.

### *The metal ion-sensitive electrode*

According to the previous theory, the liquid-state electrode should be sensitive to a metal ion in solution, if a chelate of the particular metal is present in the organic phase. To prove this, solutions of the metal dithizonates of mercury, silver, copper, lead or zinc in carbon tetrachloride were prepared and used as the organic phase in various liquid-state electrodes, which were then used to record potential–pH curves in aqueous solutions containing different known concentrations of various metal ions.

The mercury(II)-sensitive electrode was made by shaking $1\cdot10^{-4}$ *M* dithizone solution (in $CCl_4$) with a tenfold molar excess of mercury(II) nitrate in 0.1 *M* nitric acid. The orange coloured organic phase was separated and twice filtered in order to remove droplets of water, and the carbon rod was charged with it. The potential of the prepared electrode was usually 475 mV (S.C.E.) in $1\cdot10^{-2}$ *M* nitric acid–$1\cdot10^{-3}$ *M* mercury(II) nitrate solution, and a freshly prepared electrode reached this value in about 1 min. With solutions of $1\cdot10^{-2}$ *M* nitric acid containing known concentrations of mercury(II) nitrate and with the calomel electrode equipped with the salt bridge, a series of potential–pH curves was recorded. The one corresponding to $1\cdot10^{-3}$ *M* mercury(II) is shown in Fig. 3. Its shape and position on the potential axis are close to theory. Because the extraction constant of mercury dithizonate is very high (Table I), hydrogen ions do not affect the electrode potential in acidic medium and the curve starts with a plateau, the potential of which is the highest of the metal dithizonates, as is the extraction constant. The decrease of potential with increasing pH starts when hydrolysis of mercury occurs (compare Table II and eqn. (36)) and its linear decrease over 7 pH units (4–11) indicates the decrease of mercury(II) concentration over exactly 14 orders of magnitude, thus following the theoretical curve describing hydrolysis of mercury (ref. 11, Fig. II. 10). The position of the plateau on the potential axis changed according to the original concentration of mercury(II) nitrate in solution. A change of −28 mV per tenfold decrease of mercury(II) concentration was observed down to $10^{-5}$ *M*, but it was found impossible to reach higher sensitivity, probably because of adsorption of mercury on the tip of the salt bridge and the surfaces of the glass vessel and electrodes.

A silver(I)-sensitive electrode was prepared similarly, by shaking $1 \cdot 10^{-3}$ *M* dithizone in carbon tetrachloride with a 10-fold molar excess of silver nitrate in $10^{-3}$ *M* nitric acid, and using the organic phase to impregnate the carbon rod. The potential of this electrode reached a stable value of 360 mV (S.C.E.) in $1 \cdot 10^{-3}$ *M* nitric acid and $1 \cdot 10^{-3}$ *M* silver nitrate. Potential–pH curves were recorded as described for mercury, with various known quantities of silver nitrate in diluted nitric acid. The shape of the curve and its position on the potential axis were close to theory. Again, owing to the high value of the extraction constant, hydrogen ions did not affect the potential, and the curve started with the plateau at pH 3. Because silver does not hydrolyse until pH 9–10, the plateau continues to this value, being longer than that of mercury. In even more alkaline solutions silver hydrolyses and the potential of the electrode decreases. When solutions containing different concentrations of silver nitrate were used for recording potential–pH curves the potential of the plateau changed −58 mV with a tenfold decrease of silver(I) concentration (see also Fig. 3 and Table III).

The copper(II)-sensitive electrode was again prepared by using a solution produced by solvent extraction; $1 \cdot 10^{-4}$ *M* dithizone (in $CCl_4$) was shaken with a 2-fold molar excess of copper(II) sulphate at pH 4. The electrode containing copper(II) dithizonate reached a potential of about 250 mV (S.C.E.) at pH 5 in $1 \cdot 10^{-3}$ *M* copper(II) nitrate. This potential changed −25 to −30 mV with a 10-fold decrease of copper(II) concentration, but sometimes an even greater change of potential was observed. This irregularity is believed to be due to the formation of the secondary dithizonate[13]. Because the extraction constant of copper dithizonate is lower than that of mercury and silver, the potential of the plateau is also lower and consequently hydrogen ions affect the electrode potential even at pH 3. The potential–pH curve thus starts with a slope, then at pH 4 the effect ceases and the plateau proceeds up to pH 6 when copper starts to hydrolyse. In more alkaline solutions the electrode still functions well, but the copper(II) concentration decreases owing to the formation of various copper hydroxides.

The lead(II)- and zinc(II)-sensitive electrodes were prepared in the usual way with lead and zinc dithizonates, which were made by shaking $1 \cdot 10^{-4}$ *M* dithizone solution with an aqueous solution containing a 10-fold molar excess of lead and zinc at pH about 6. Owing to the low solubility of lead dithizonate ($2.5 \cdot 10^{-5}$ *M* in $CCl_4$), the organic extract had to be filtered to remove excess of precipitated metal chelate. The potentials of the zinc and lead dithizonate electrodes were found not to be reproducible and it usually requires several minutes before they reached the values shown in Table I. The potential–pH curves recorded with these electrodes showed a strong influence of hydrogen ions up to pH 5 (lead) and nearly 7 (zinc). Because of this and also because of hydrolysis, the plateau of the lead electrode was very short, and that of zinc was only rudimentary. Although both electrodes were found to be sensitive to zinc(II) and lead(II) ions (above pH 5), they are not suitable for practical use. The operational range is narrow and the potential is unstable; moreover, the electrodes are sensitive to traces of $Hg^{2+}$, $Ag^+$ and $Cu^{2+}$ ions which easily replace zinc or lead bound to dithizone, thus "poisoning" the electrode. These observations are in agreement with theory, which also indicates how to choose more suitable materials for making zinc- and lead-sensitive electrodes.

In Fig. 2, the curve for an electrode made of pure dithizone is also shown. Its shape and slope have already been discussed, but in this connection it is interesting to mention its position on the potential axis. It lies in the lowest part of the graph, lower than any metal dithizonate electrode, thus representing a limiting value. This curve in fact borders from the acid side the area in which other metal dithizonate electrodes can function, because it shows the pH at which metal dithizonates are being converted to dithizone.

Closer comparison of the potentials of the individual liquid-state electrodes shows that although they decrease in the same manner as the extraction constants, the differences between them are not precisely related to the differences in the $K$ values (eqn. (14) and Table III). This is most obvious in the case of the mercury electrode, the potential of which should be about 250 mV higher, and also in the case of the lead electrode, the potential of which should nearly be 70 mV lower. However, it is not known whether $E_0$ is the same in the case of dithizone and of the different dithizonates. Further, the effective concentration ($[MA_n]_o$) of dithizonate in the pores of the electrodes is also unknown. The only statement that can definitely be made is that the individual $\text{const}_{MA_n}$-values (eqn. (18)) mainly depend on the extraction constants.

In order to demonstrate the performance of the liquid-state electrode in a slightly different manner, and also to show how complexation affects the electrode potential, a series of curves was recorded with a silver-sensitive electrode made with silver dithizonate in xylene (Fig. 3). The inert solvent was changed for the following reasons. The solvent extraction of metal dithizonates into carbon tetrachloride has been fully described[8,13], and was therefore used in the above experiments. However, the volatility of carbon tetrachloride (b. p. 76–77°), and its low viscosity and photosensitivity are unfavourable properties for the present purpose; phosgene and chlorine, which are formed by its decomposition, destroy metal dithizonates and dithizone as well. Although it was possible to use electrodes made with carbon tetrachloride for a few days without renewing the organic phase, xylene, benzene, toluene and mesitylene were found to be superior. For example, a silver(I)-sensitive electrode, prepared in the usual way, but with xylene instead of carbon tetrachloride, had a potential of 388 mV (S.C.E.) at pH 3 in $1 \cdot 10^{-3}$ $M$ silver nitrate and could be used for several weeks. The potential–pH curves recorded with it in a series of solutions containing $1 \cdot 10^{-3}$ $M$ nitric acid and $1 \cdot 10^{-2}$–$1 \cdot 10^{-13}$ $M$ silver(I), are shown in Fig. 4. The solutions containing $1 \cdot 10^{-3}$ $M$ $Ag^+$ to $1 \cdot 10^{-5}$ $M$ $Ag^+$ were prepared from $1 \cdot 10^{-2}$ $M$ silver nitrate in $1 \cdot 10^{-3}$ $M$ nitric acid by further dilution with the acid in the usual manner. However, it was impossible to prepare more dilute solutions and to use them directly for the recording of potential–pH curves, because silver, like mercury, was adsorbed on all the available surfaces. This is not surprising as it is known that up to 1 $\mu$g of silver can be adsorbed on 1 cm$^2$ of a glass surface. In order to overcome this difficulty, $1 \cdot 10^{-3}$ $M$ silver nitrate solution was mixed with known quantities of sodium chloride, bromide or iodide. The solutions obtained, "buffered" with silver chloride, bromide or iodide (Table III) were then used for the recording of potential–pH curves. From these curves as well as from the "plateau potentials" summarized in Table III, it can be seen that the liquid-state silver dithizonate electrode behaves according to theory within the pH range 3–9 down to $10^{-10}$ $M$ $Ag^+$.

Although the above results support our theory to a large extent, several important points remain unclear. What is the nature of $E_0$ (and consequently also of $\text{const}_{MA_n}$) and how does this value vary from one metal chelate to an other? The change of potential of the electrode on replacing the carbon support of the organic phase with a nickel support should be studied, as well as the effect of other ligands such as cupferron, diethyldithiocarbamate, etc. Another question is connected to the amount of electroactive species with which the organic phase is doped. Although it was confirmed that an increase of dithizone (metal dithizonate) concentration makes the potential more negative and *vice versa*, more precise evaluation of this variable was not carried out for the reasons already mentioned. The use of porous graphite is also connected with the following problem. Because most of the organic phase is contained within the pores, most probably only that very thin layer of organic phase, which separates the aqueous solution from carbon particles, should be regarded as an "active" volume. Clearly, values of $V_0$ and $c_{HA}$ are not well defined and difficult to measure. Last but not least, the kinetic factors remain an unsolved problem. In all cases, the response of the electrode was found to be very fast, faster than that of the glass electrode. The curves presented in this paper were not affected by a relatively fast change of pH. However, this does not mean that equilibrium states were reached in the measurements, although equilibrium data were used in the theoretical part of the present paper.

Future experiments should further elucidate the behaviour of the electrodes. In the meantime, the theory, as put forward here may serve as a guide in choosing suitable metal chelates from extraction data and as a means of explaining the electrode behaviour.

## CONCLUSION

A new type of generally applicable ion-sensitive electrode is proposed and an attempt has been made to describe how it functions. It is shown that the inert organic solvent forming the electroactive surface of the liquid-state electrode can be "doped" by an electroactive compound which then mainly determines the electrode function. The important properties of this electroactive compound are: a higher solubility in organic solvent than in an aqueous sample to be measured, and an ability to dissociate (preferably to a small extent) to give ions identical with those to be determined in the aqueous sample. From a number of suitable species ranging from elementary iodine (iodide-sensitive electrode) to organic compounds, liquid ion-exchangers and biochemically active substances (such as, *e.g.*, valiomycin for potassium-sensitive electrode) metal dithizonates were selected for the verification of this concept of the liquid-state electrode. On the basis of this extraction system, it has been shown that the higher the extraction constant, then the higher the selectivity, the better the stability, and the more positive the potential of the electrode. Moreover, the higher the extraction constant, the higher the sensitivity that can be achieved and the more acidic the solution that can be used for the measurements. It has further been shown that the potential–pH curves have three main parts and their shape and position in potential–pH coordinates is characteristic for each liquid-state electrode and for the composition of the organic and aqueous phase. There is no doubt that the latter property can be used for quantitative determination of ionic species in aqueous solutions, but it is also suggested that this phenomenon could be used for the analysis of the organic phase itself.

The possibilities and practical uses of the liquid-state electrode still have to be explored. Its simplicity, robustness and versatility should make it possible for the electrode to be accepted in analytical laboratories.

## SUMMARY

A new type of generally applicable ion-selective electrode is described. The electrodes are based on solvent extraction systems, the organic phase being supported on graphite rod. A series of electrodes based on metal dithizonates is discussed and a theory to explain their behaviour is proposed. The higher the extraction constant of the metal dithizonate, the greater is the selectivity of the electrode prepared from it for the particular metal involved in the extraction system, and the more sensitive is the electrode for the particular metal ion. The new electrodes are robust and versatile in application.

The authors express their sincere thanks to Professors N. HOFMAN-BANG and F. WOLDBYE for their advice and interest in this work.

## RÉSUMÉ

Un nouveau type d'électrode sélective est proposé. Ces électrodes sont basées sur des systèmes d'extraction dans un solvant; la phase organique étant supportée par une baguette de graphite. On décrit une série d'électrodes à dithizonates métalliques; une théorie est donnée pour expliquer leur comportement. Plus grande est la constante d'extraction du dithizonate métallique meilleures sont la selectivité et la sensibilité de l'électrode. Ces nouvelles électrodes sont robustes et versatiles dans leur application.

## ZUSAMMENFASSUNG

Es wird ein neuer Typ einer allgemein anwendbaren ionenselektiven Elektrode beschrieben. Die Elektroden bestehen aus Solventextraktionssystemen, wobei die organische Phase auf einem Graphitstab aufgetragen ist. Es wird eine Reihe von Metalldithizonat-Elektroden diskutiert und eine Theorie zur Erklärung ihres Verhaltens vorgeschlagen. Je höher die Extraktionskonstante des Metalldithizonats ist, desto grösser ist die Selektivität der daraus hergestellten Elektrode für das einzelne im Extraktionssystem enthaltene Metall, und desto empfindlicher ist die Elektrode für das einzelne Metallion. Die neuen Elektroden sind robust und vielseitig in der Anwendung.

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# FLUORIMETRIC DETERMINATION OF TRACE NITRATES

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One of the most widely used methods for the determination of trace nitrates involves the nitration of aromatic compounds to form colored products. HOLLER AND HUCH[1] studied the nitration of many xylenols and determined that 3,4-xylenol was best. TARAS[2] outlined a procedure using phenoldisulfonic acid as the compound to be nitrated; ANDREWS[3] used 2,6-xylenol. These methods can determine 1 $\mu$g or less of nitrate per milliliter, but they usually require neutralization, steam distillation or solvent extraction of the product before analysis.

The above methods as well as others were spectrophotometric; none has used the technique of fluorescence. Fluorescein is known to react with nitrate in concentrated sulfuric acid[4] to produce a di-nitrated dye. This reaction has been developed by us to determine nitrates to 0.01 $\mu$g ml$^{-1}$ (in the reacted solution) without the use of heat, distillation, or solvent extraction procedures. The reaction is direct, requiring but one step in the analysis procedure.

## EXPERIMENTAL

### *Apparatus*

Fluorescence measurements were made with a Perkin-Elmer Model 203 spectrofluorimeter equipped with a continuous-spectrum Xenon lamp source. The excitation wavelength was set at 435 nm and the emission was measured at 485 nm. Quartz cells (1 cm) were used with all analyses and measurements were made in an air-conditioned room at 23°.

### *Reagents*

All chemicals were reagent-grade, and the water used was first passed through an ion-exchange column and then distilled. Nitrate samples were prepared from potassium nitrate. Disodium fluorescein was obtained from Eastman Kodak Co., and Solvent Orange 16 was obtained through the courtesy of G. Siegle & Co., Stuttgart-Feuerbach, West Germany.

### *Procedure*

To concentrated (*ca.* 95%) sulfuric acid containing $5 \cdot 10^{-7}$ *M* disodium fluorescein, a nitrate sample is added. The volume of aqueous sample should not reduce the acid concentration below 87%. The sample is allowed to react for 45 min and then the fluorescence is measured, with the standards treated in a manner identical to the

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samples, including acidity dilution. (The reaction time and acid concentration can be varied as indicated in later sections.)

## RESULTS AND DISCUSSION

### *Working curve*

Figure 1 shows a typical working curve for $5 \cdot 10^{-7}$ *M* disodium fluorescein reacting with nitrate in 90% sulfuric acid. The reaction product has no fluorescence, eliminating any possible interference from it. However, the nitrate concentrations might not be exactly as indicated, because the sulfuric acid can contain nitrate as a trace impurity. The fluorescence method will not indicate this, because there are no blanks without nitrate for comparison. From this curve, one appears to be able to determine $2 \cdot 10^{-7}$ *M* (~0.01 μg ml$^{-1}$) nitrate in the final analysis solution.

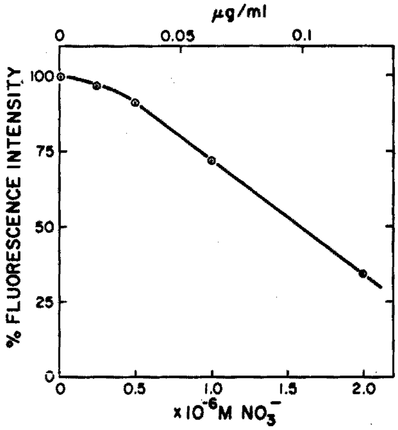
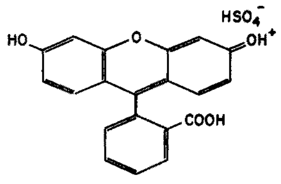


Fig. 1. Standardization of $5 \cdot 10^{-7}$ *M* disodium fluorescein with nitrate in 90% sulfuric acid.

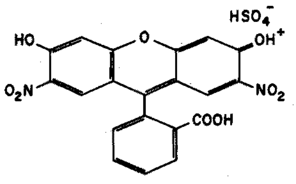
West and Ramachandran[5] developed a colorimetric nitrate method with chromotropic acid. With their procedure and the technique of standard additions and extrapolation of the working curve, the nitrate concentration in our concentrated sulfuric acid was estimated to be no higher than 0.03 μg ml$^{-1}$. This would indicate that the concentrations in Fig. 1 are close to actual. The fluorescence of fluorescein can be easily measured in concentrations lower than $5 \cdot 10^{-7}$ *M*, but the reaction will not occur with similarly diluted nitrate. Analyses can also be made with higher concentrations of fluorescein and nitrate, but the slope of the working curve is much steeper.

### *Reaction product*

The reaction product does not have any fluorescence, and it would be difficult to determine its structure. According to the Colour Index[6] fluorescein reacts with nitrate in concentrated sulfuric acid to form Solvent Orange 16. This dye has no fluorescence and is possibly the reaction product. Whether one nitrate ion per fluorescein molecule is sufficient to suppress all fluorescence is not known.



FLUORESCEIN



SOLVENT ORANGE 16

*Reaction time*

The reaction time for nitrate concentrations about comparable to fluorescein is *ca.* 15 min. For higher nitrate levels, however, the reaction time for completion is about 45 min with some evidence of a very slow continuing reaction (10% $h^{-1}$). Nevertheless, if one treats the standards and the samples alike, an accurate determination can be made. Analysis reproducibility of the one sample was ±5% for nitrate concentrations below 1 $\mu$g $ml^{-1}$.

Heating the samples in a water bath at 60° reduced the reaction time by 50%, but this procedure is unnecessary for most analyses.

*Concentration of sulfuric acid*

The ultimate reaction sensitivity can be obtained when the sulfuric acid concentration is greater than 89%. The sensitivity decreases with decreasing acidity. For example, the reaction occurs in 80% sulfuric acid, but the dye and nitrate concentrations must be higher than in 90% sulfuric acid.

*Interferences*

Potential interferents were tested by combining them with nitrate and analyzing the solution in the manner previously described. For a $10^{-6}$ *M* nitrate–$5 \cdot 10^{-7}$ *M* fluorescein solution with $10^{-3}$ *M* additions of sulfite, acetate or phosphate, no interference was observed. Solutions containing $10^{-4}$ *M* ammonium, magnesium(II), copper(II) and iron(III) showed a 5% positive interference.

Chloride, bromide and iodide interfered. Potentially, the interference from the most common halogen, chloride, can be eliminated by using the antimony(III) method of WEST AND RAMACHANDRAN[5]. Chloride in 100-fold amounts compared to nitrate totally eliminated all nitrate by forming nitrosyl chloride. The antimony(III) technique removed this interference.

Similary, iron(II) in 100-fold mole excess over nitrate eliminated it from the sample. Sample preparation with oxidants, however, could oxidize any iron(II) present to iron(III), and thus prevent interference.

A ten-fold mole excess of nitrite over nitrate did not interfere. Larger excesses, however, did cause errors. This problem was eliminated by the addition of sulfamic acid in a two-fold excess over the nitrite concentration as recommended by ANDREWS[3]. The sulfamic acid did not react with the nitrate or interfere with dye fluorescence. WEST AND RAMACHANDRAN[5] used a urea–sulfite solution to remove nitrite interferences in the chromotropic acid–nitrate reaction. In the present fluorescence reaction, however, the urea reacted with both the nitrite and nitrate.

Formaldehyde, although it does not react with nitrate, was found to enhance the fluorescence of the dye, causing a negative error. A 200-fold mole excess of formaldehyde over $5 \cdot 10^{-7}$ $M$ fluorescein enhanced the fluorescence by 50%. Since the formaldehyde does not inhibit the nitrate–fluorescein reaction, formaldehyde could be added to samples, standards, and blanks to eliminate the interference of any trace formaldehyde contaminations. A similar experiment with acetaldehyde did not result in any enhancement.

## SUMMARY

Nitrate is determined by reacting it with fluorescein in solutions of high sulfuric acid concentrations. Since fluorescein fluoresces and the reaction product does not, nitrate can be determined down to 0.01 $\mu$g ml$^{-1}$ in the final reaction solution by measurement of the fluorescence suppression. The method is easy to use and has good reproducibility. Suggestions are offered to eliminate potential interfering ions.

## RÉSUMÉ

Les nitrates sont dosés par réaction avec la fluorescéine en solution très concentrée en acide sulfurique. On peut ainsi analyser jusqu'à 0.01 $\mu$g ml$^{-1}$ de nitrate en mesurant la diminution de fluorescence. Cette méthode est facile et présente une bonne reproductibilité.

## ZUSAMMENFASSUNG

Nitrat wird bestimmt durch Reaktion mit Fluorescein in Lösungen hoher Schwefelsäurekonzentration. Da Fluorescein, nicht aber das Reaktionsprodukt fluoresziert, kann Nitrat bis zu 0.01 $\mu$g ml$^{-1}$ herab in der Reaktionslösung durch Messung der Fluoreszenzverminderung bestimmt werden. Die Methode ist leicht anwendbar und hat eine gute Reproduzierbarkeit. Es werden Vorschläge zur Eliminierung möglicherweise störender Ionen gemacht.

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# pH DEPENDENCE OF THE N.M.R. LINE BROADENING OF WATER BY PARAMAGNETIC EDTA CHELATES

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The rate of water molecule exchange between the coordination sphere of cations in aqueous solution and the solvent water has been of considerable interest[1-5]. A convenient technique for the study of this process is the broadening of the solvent n.m.r. line as a result of a decrease in the nuclear relaxation time of a nucleus in a molecule coordinated to a paramagnetic metal ion. Rapid exchange of the molecule between a coordinated state and bulk solvent leads to the observed broadening. PEARSON *et al.*[6,7] have shown that for most systems of one paramagnetic environment, the n.m.r. line width at half height is given by:

$$1/T_2 = 1/T_{2a} + \frac{P_b}{P_a}\left(\frac{1}{T_{2b}+\tau_b}\right) \qquad (1)$$

where $1/T_2$ is the experimental line width, $T_{2a}$ is the nuclear relaxation time in the diamagnetic environment, $T_{2b}$ is the relaxation time in the paramagnetic environment, $\tau_b$ is the mean lifetime of the nucleus in the paramagnetic environment, and $P_a$ and $P_b$ represent the mole fraction of the proton in the diamagnetic and paramagnetic environment respectively. Analytical applications of this phenomenon have been proposed[8,9]. The actual magnitude of the broadening may be determined by one of several possible conditions[10].

(*a*) The relaxation rate is controlled by the large chemical shift difference between the coordinated state and the bulk solvent and relaxation is very rapid ($\tau_b \gg T_{2b}$).

(*b*) The rate of relaxation is controlled by the change in resonance frequency, and the chemical exchange is rapid. This case is not covered by eqn. (1).

(*c*) The relaxation is controlled by the lifetime of the nuclear spin state and the line width is controlled by the rate of chemical exchange ($\tau_b \gg T_{2b}$).

(*d*) The line width is controlled by the nuclear spin relaxation in the complex ($T_{2b} \gg \tau_b$).

In cases in which the line width is determined by $\tau_b$, the exchange process can be measured directly. However, when the line width is controlled by $T_{2b}$, one can state only that $\tau_b < T_{2b}$ and thereby set a lower limit on the rate of exchange. SWIFT AND CONNICK[3] have demonstrated the usefulness of temperature variation to determine whether $\tau_b$ or $T_{2b}$ control the n.m.r. line width. If $\tau_b$ controls the line width, a plot of log $(1/T_2)$ *versus* $1/T$ (temperature) is expected to yield a conventional Arrhenius plot with a negative slope. On the other hand, the slope of an analogous plot for a system in which the line width is controlled by $T_{2b}$ is expected to be small and positive. In this study, the above properties of solutions of chelates of paramagnetic ions with ethylenediaminetetraacetic acid (EDTA) are applied to the investigation of the nature of the exchange of water molecules between a coordinated state and the bulk solution. Although the study did not yield quantitative information about the rate of exchange, there were interesting qualitative results which are useful in the discussion of the structure of certain metal–EDTA chelates in aqueous solution.

## EXPERIMENTAL

All spectra were obtained using a Varian Associates HA-100 high-resolution nuclear magnetic resonance spectrometer equipped with a variable temperature probe. The line width values reported are the average of several measurements. Estimated precision is ±0.1 Hz. A sweep rate of 0.1 Hz $sec^{-1}$ was employed and the radio-frequency power was adjusted for maximum signal-to-noise ratio. The spectra were recorded at an ambient probe temperature of 29°. The pH measurements were made at room temperature with a Leeds and Northrup Model 7403 pH meter which was standardized with NBS buffer solutions. The metal–EDTA chelates were prepared from reagent-grade disodium EDTA and the nitrates of nickel (II) and copper(II). Ammonium iron(III) sulfate was used in the preparation of the iron chelate. The compounds were obtained by combination of 0.5 moles of the metal salt and 0.5 moles of EDTA in 500 ml of water and boiling the solution for 2 h. The solution was cooled and the crystals were filtered and washed with cold water. The compounds were dried at 110° for 1 h. Samples for obtaining the n.m.r. spectra were prepared by adjusting the pH of a solution of the desired compound with concentrated potassium hydroxide.

## RESULTS AND DISCUSSION

In the study of the exchange of a ligand between a paramagnetic site and a diamagnetic site by means of n.m.r. line width measurements, it is first necessary to establish which factor controls the line width. In the present system the metal–EDTA complex is the paramagnetic site and water is the exchanging ligand. Figure 1

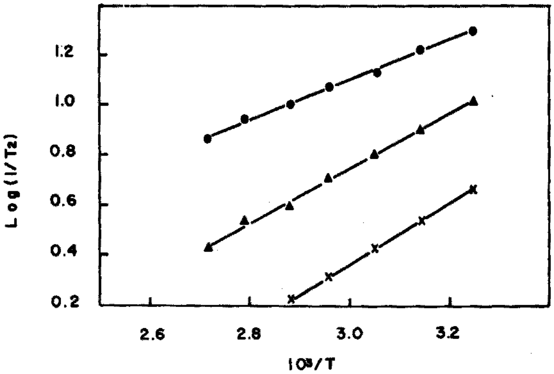

Fig. 1. Log($1/T_2$) *versus* $1/T$ for metal–EDTA solutions. (●) Fe(III)–EDTA, pH 5.0; (▲) Cu(II)–EDTA, pH 7.0; (×) Ni(II)–EDTA, pH 7.4.

shows some representative plots of log($1/T_2$) of the solvent resonance *versus* $1/T$ for solutions of Cu(II), Ni(II) and Fe(III) chelates of EDTA. The positive slopes of these and other plots made throughout the pH range 2–13 strongly indicate that the width of the water line is controlled by $T_{2b}$ in all cases. Under these conditions $\tau_b < T_{2b}$. Therefore, the rate of exchange of the solvent between a coordinated state and the bulk solvent can be established only as greater than or equal to $1/T_{2b}$. The values obtained at pH 6.0 are $1.4 \cdot 10^4$, $4.5 \cdot 10^3$ and $9.9 \cdot 10^4$ sec$^{-1}$ respectively for the EDTA chelates of Cu(II), Ni(II), and Fe(III). These may be compared with $2 \cdot 10^8$ and $3 \cdot 10^4$ sec$^{-1}$ for Cu(II) and Ni(II) respectively for the exchange of the water of hydration of the aquo complexes[3].

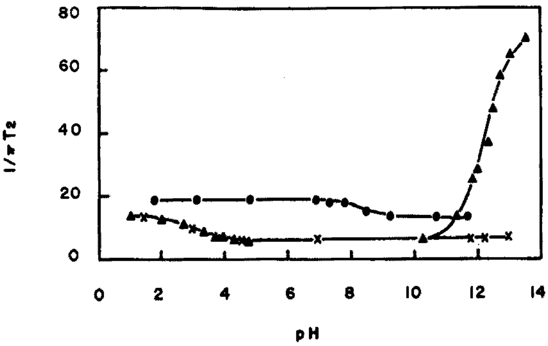

Fig. 2. $1/\pi T_2$ *versus* pH for metal–EDTA solutions. (●) 0.013 *M* Fe(III)–EDTA; (▲) 0.053 *M* Cu(II)–EDTA; (×) 0.070 *M* Ni(II)–EDTA.

In order to obtain information concerning the various metal–EDTA species which exist in the pH range of 1–13, a plot of $1/\pi T_2$ *versus* pH was made as shown in Fig. 2. The acid–base equilibria represented by eqns. (2)–(5) were studied by potentiometric titrations.

$$H_2MY \rightleftarrows HMY + H^+ \qquad K_1 = \frac{[H^+][HMY]}{[H_2MY]} \tag{2}$$

$$HMY \rightleftarrows MY + H^+ \qquad K_2 = \frac{[H^+][MY]}{[HMY]} \tag{3}$$

$$MY \rightleftarrows M(OH)Y + H^+ \qquad K_3 = \frac{[H^+][M(OH)Y]}{[MY]} \tag{4}$$

$$M(OH)Y \rightleftarrows M(OH)_2Y + H^+ \qquad K_4 = \frac{[H^+][M(OH)_2Y]}{[M(OH)Y]} \tag{5}$$

TABLE I

$pK_a$ VALUES FOR METAL–EDTA SPECIES

| *Metal ion* | $pK_1$ | $pK_2$ | $pK_3$ | $pK_4$ |
|---|---|---|---|---|
| Cu(II) | 2.5 | 3.3 | 12.2 | — |
| Ni(II) | 2.0 | 3.3 | 12.2 | — |
| Fe(III) | — | 3.0 | 8.0 | 12.0 |

The p$K_a$ values of the equilibrium constants represented in these equations are given in Table I. The ionic charge on the species is omitted for convenience. Inflections in the various curves shown in Fig. 2 correspond well with the p$K_a$ values reported for the respective compounds. In some cases such as the iron(III)–EDTA complex there is no inflection of the n.m.r. data whereas a dissociation constant was obtained from the potentiometric titration data.

Because the values of $\tau_b$ are assumed small in all cases compared to $T_{2b}$, the values of $T_{2b}$ reported earlier should approximate this quantity. Comparison of these values for nickel(II)–EDTA and $T_{2b}$ calculated from the data of DESAI *et al.*[5] for nickel(II)–ethylenediamine systems shows agreement to better than an order of magnitude. A more critical comparison would likely not be valid. In the pH ranges in which the predominant species are $H_2MY$ and HMY, one explanation of the solvent line broadening is that an effective relaxation mechanism is the dipole–dipole relaxation of protons undergoing rapid exchange from the protonated carboxyl groups on the ligand. The effect of the relaxation is transmitted to the bulk of the solvent by the proton exchange between the solvent and the carboxyl groups. An increase in pH in this region results in a slight decrease in the broadening efficiency. However, a very efficient broadening mechanism is in effect in the pH range 4–10. A relaxation mechanism which is more effective than the dipole–dipole interaction is the contact interaction which requires unpaired electron spin density at the proton undergoing relaxation; this requires coordination of a molecule containing the proton to the metal ion. It is interesting to compare data given by DAY AND REILLEY[8], who report the concentrations of aquocopper(II) and aquoiron(III) complexes required to give a water line width of 10 Hz, with the corresponding data in Fig. 2. The concentrations given for the aquo complexes are only about 25–50% less than those required for the metal–EDTA chelates. This observation has a strong implication that the metal chelates of EDTA are almost as efficient at solvent line broadening as the aquo complexes of these ions. Unlike the above comments, an explanation of this observation must allow coordination of water molecules to the metal ion. All of the metal–EDTA chelates discussed here are known to contain a coordinated water molecule. Nickel(II)–EDTA and copper(II)–EDTA contain quinquedentate EDTA with a water molecule in an equatorial position where it is *cis* to one nitrogen atom and *trans* to the other[11]. According to X-ray data, iron(III)–EDTA contains hexadentate EDTA with a water molecule coordinated to the metal in a seven-coordinate structure[12]. These water molecules coordinated to the chelated metal ions must exchange more rapidly (per molecule) than those in the aquo complexes because similar broadening efficiencies are observed. Substitution of ethylenediamine in the coordination sphere of nickel has been shown to enhance the rate of water exchange from the remaining aquated sites[5]. An excess of EDTA present in the solution results in a sharpening of the n.m.r. line of the solvent. The effect is undoubtedly a result of the intense competition of EDTA for the coordination site occupied by the water. Earlier data indicate that manganese(II)–EDTA behaves in much the same way as the compounds reported here[8].

The most dramatic result shown in Fig. 2 is that for copper(II)–EDTA in the pH range 11–13. Increasing pH in this range results in the formation of a copper–hydroxy–EDTA complex as confirmed by potentiometric titration. A hydroxy complex of the nickel chelate also forms in this pH region, whereas the monohydroxy complex of the iron chelate forms in the pH range 7–9. There is a significant enhancement of the line broadening in the pH region 11–13 for the copper chelate with no analogous effect in the case of the nickel chelate. The iron chelate becomes less effective in this pH range. One explanation of this observation is the lability of the complex. Ligand-exchange studies of metal chelates of (+) 1,2-propylenediaminetetraacetic acid ((+)PDTA) with (−)PDTA show that no exchange of iron(III)–PDTA and nickel(II)–PDTA occurs after three days in neutral solution[13]. However, copper(II)–(+)PDTA is racemized after 16 h. Each chelate is racemized in minutes in acidic solutions. If this relative lability is applied to the hydroxy complexes of the EDTA chelates, the line broadening by the copper chelate in the high pH region can be explained on the basis of a much more rapid exchange of the hydroxyl groups between the coordination site and the bulk water followed by proton exchange in the solvent. Investigation utilizing $^{17}O$ n.m.r. is required to confirm this assumption.

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## SUMMARY

The effect of pH and temperature upon the n.m.r. line width of solutions of the ethylenediaminetetraacetic acid (EDTA) chelates of copper(II), nickel(II) and iron(III) has been studied. The concentration of these chelates required to give a line width of 10 Hz is only a factor of about two greater than the hydrated metal ions. This result indicates rapid exchange of the solvent from a coordination site on the chelated metal ion. The temperature dependence of the n.m.r. spectra shows that in each case the line width is controlled by the relaxation time of the proton in the paramagnetic environment of the coordinated metal ion rather than the rate of solvent exchange. The pH dependence of the solvent line width is related to the formation of protonated and hydroxy complexes of the chelates as confirmed by potentiometric titration.

## RÉSUMÉ

On a examiné l'influence du pH et de la température sur la largeur des raies r.m.n. de solutions de chélates de l'EDTA avec cuivre(II), nickel(II) et fer(III). La concentration de ces chélates, nécessaire pour avoir une largeur de raie de 10 Hz n'est que le double de celle des ions métalliques hydratés. L'influence de la température sur les spectres r.m.n. montre que dans chaque cas la largeur de raie est controlée par le temps de relaxation du proton dans l'environnement paramagnétique de l'ion métallique coordiné, plutôt que par la vitesse d'échange de solvant. L'influence du pH sur la largeur de raie du solvant est due à la formation de complexes (protonique et hydroxy) des chélates; ceci a été confirmé par titrage potentiométrique.

## ZUSAMMENFASSUNG

Der Einfluss des pH-Wertes und der Temperatur auf die n.m.r.-Linienbreite von Lösungen der Äthylendiamintetraessigsäure(EDTA)-Chelate von Kupfer(II),

Nickel(II) und Eisen(III) ist untersucht worden. Die Konzentration dieser Chelate, die für die Erzeugung einer Linienbreite von 10 Hz erforderlich ist, ist nur um einen Faktor von etwa zwei grösser als bei den hydratisierten Metallionen. Dieses Ergebnis weist auf einen schnellen Lösungsmittelaustausch an einer Koordinationsstelle des chelatisierten Metallions hin. Die Temperaturabhängigkeit der n.m.r.-Spektren zeigt, dass in jedem Fall die Linienbreite mehr durch die Relaxationszeit des Protons in der paramagnetischen Umgebung des koordinierten Metallions bestimmt wird als durch die Geschwindigkeit des Lösungsmittelaustausches. Die pH-Abhängigkeit der Lösungsmittel-Linienbreite hängt mit der Bildung von protonisierten und Hydroxykomplexen zusammen, wie durch potentiometrische Titration bestätigt wird.

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# AN AUTOMATIC PROCEDURE FOR THE DETERMINATION OF BORON IN SEA WATER

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If the boron content of a large number of sea water samples is to be analysed on board ship, an automatic system is desirable. By such means preservation, as well as transport and storage of samples, is avoided, while the results of the analyses are available immediately and can be stored directly with other data, *e.g.* depth, salinity, temperature and location. Furthermore, discrepancies can be detected while the ship is still on the sampling station, providing an opportunity to repeat the analyses if necessary. Automatic on-board analysis with direct read-out is also advantageous in the detection of short-time variations. Finally, with an automatic procedure, errors depending on differences in manual operation are eliminated.

The application of automatic systems in analytical chemistry, in general, has been discussed by DIJKSTRA[1] and MALISSA[2,3]. Problems concerning automatic analysis in chemical oceanography have been studied by LEE[4], WEICHART[5], BERNHARD AND MACCHI[6], GRASSHOFF[7,8], HENRIKSEN[9,10], BREWER AND RILEY[11,12], CHAN AND RILEY[13] and ARMSTRONG *et al.*[14], amongst others. These authors used continuous closed systems for their analyses, that is, systems in which the samples as well as the reagents are pumped through plastic tubes, the different samples sometimes being segmented by air bubbles. An alternative system, which has not been used very much, so far, in the chemical analysis of sea water, is the so-called "open system", in which the discrete samples are transported in open glass or plastic tubes and the reagents are delivered by dispensers.

The closed system of most widespread usage is the so-called Technicon "Auto-Analyzer". A technical report on the practical aspects of the use of the AutoAnalyzer in sea water nutrient analyses has been published by HAGER *et al.*[15]. With the use of viscous fluids, strong acids and bases and some organic chemicals, difficulties arise particularly in connection with the pumping system. These difficulties can easily be overcome by using an open system employing suitable materials such as glass or teflon. Reactions in which gases are evolved are more easily dealt with in an open system, which also normally has a higher speed of operation. The "carry-over" effect of the AutoAnalyzer is strongly dependent on the rate of analysis, as shown by PEHRSSON[16]. This is not the case with the open system. PEHRSSON has also shown that in an open system the drift is due to the photometer unit alone, whilst in the Auto-Analyzer, drift also results from changes in the internal diameter of the flow tubes.

## AUTOMATIC METHODS FOR DETERMINING BORON

Automatic procedures for the determination of boron as boric acid in aqueous solutions by means of the AutoAnalyzer have been developed by DEMMIT[17] and by JAMES AND KING[18] for reactor water control. DEMMIT[17] investigated an indirect method using carminic acid, in which the samples were buffered with ammonia–ammonium chloride solution at a pH of 8.6–8.7. Metals forming insoluble hydroxides at this pH will interfere. JAMES AND KING[18] used the chromotropic acid method described by KUEMMEL AND MELLON[19]. This method can be used in the presence of nitrates but is sensitive to light, which causes some trouble. Both methods mentioned have the advantage of a very short response time, less than 5 min. A specially constructed apparatus called "SENA Boron meter" has been described by FLETCHER[20]. The method is colorimetric and utilizes the specific reaction of phthalein violet with aqueous boric acid solutions. Recently BASSON *et al.*[21] have published an automatic procedure for the determination of boron in plants based on the reaction with azomethine H previously investigated by CAPELLE[22].

The method proposed by DEMMIT[17] cannot be used for the determination of boron in sea water for two reasons. In the buffer range required by the method there is a risk that magnesium hydroxide may be precipitated. Secondly, a reproducibility of ±0.2 p.p.m. in the 0–10 p.p.m. boron range is not good enough. The reproducibility of the method of JAMES AND KING[18] is better, but the sensitivity of the chromotropic acid reagent to light makes the method rather inconvenient. The phthalein violet reagent used by FLETCHER[20] has been investigated by PATROVSKÝ[23], who pointed out that the reduction of the color by boron is sensitive to many ions (even sodium and potassium chloride). The azomethine H method has the draw-back of slow development of the color especially in the presence of Na+ and K+, which prevents its use for sea water analysis with the apparatus used here.

In this work the boron determination method devised by UPPSTRÖM[24], previously found to be satisfactory for sea water analysis, has been automated. Briefly, this method consists of four steps. Water is first eliminated with propionic anhydride, the boron remaining in homogenous solution. The reaction is catalyzed by oxalyl chloride and the water is eliminated according to the following reactions:

$$(COCl)_2 + H_2O \rightarrow 2H^+ + 2Cl^- + CO + CO_2 + \Delta$$

$$(CH_3CH_2CO)_2O + H_2O \xrightarrow{\Delta,\ H^+} 2CH_3CH_2COOH$$

The next step is the reaction between boron and curcumin, after the addition of sulphuric acid, to form the complex rosocyanin. This is a highly sensitive and specific reaction. The third step is the neutralisation of the interfering proton complex of excess curcumin with an acetate buffer solution. Finally, the orange coloured solution is analysed spectrophotometrically at 540 nm.

In the concentration range of interest in sea water, including brackish and estuary waters, *i.e.* 0.1–6 mg of boron per litre, a reproducibility of ±0.05 mg must be achieved. As viscous sulphuric–acetic acid solutions are used, and hydrogen chloride, carbon monoxide and carbon dioxide, as well as heat, are evolved, an open system is to be preferred. For the automation an open equipment called Autolab, which is described in this paper, has been used. The equipment has previously been used in clinical chemical work by ÅBERG[25].

## EXPERIMENTAL

### *Apparatus*

An Autolab (Linson Instrument AB, Stockholm, Sweden, marketed by AB Lars Ljungberg & Co, Stockholm, Sweden) was used. Tubes of glass or polythene containing the water samples are placed in holders linked together forming an outer chain. A pipetting device automatically transfers a given volume into boron-free glass tubes in an inner chain, allowing a final volume of 8 ml. The sets of tube holders can be connected to form a chain of any length, and thus function as a time regulator in the water bath or on a board. The chain passes the modules chosen for the analyses. Such modules are dispenser units with syringes and stirrers, thermostated water baths, boards for time delay and an interference filter photometer, in which the reaction mixture is sucked up into a glass tube in the light path (see Fig. 1). The operation scheme (Fig. 2) shows how the equipment is arranged for the boron analysis. The hydrochloric acid and carbon monoxide formed in the water elimination procedure are removed by suction through a specially made device which is shown in Fig. 3. The absorbance in arbitrary units is recorded on a printer and simultaneously displayed in

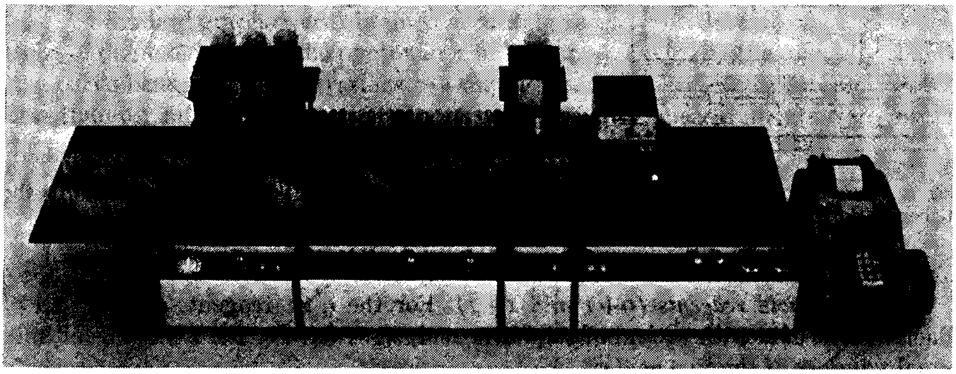

Fig. 1. View of the Autolab showing the different modules available. For the boron determination modules are combined as in Fig. 2.

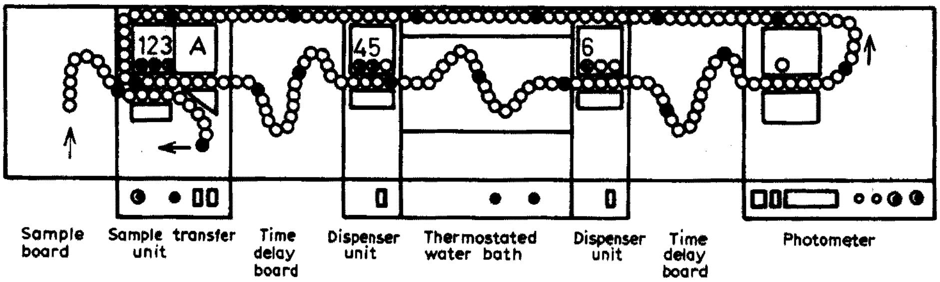

Fig. 2. Operation scheme for the boron determination with Autolab showing the sample pipette (1) and the reagent syringes for (9 + 1) acetic acid (2) propionic anhydride (3), (1 + 1) acetic acid (4), curcumin (5) and buffer (6).

digital form on the front of the apparatus. The set of dispensers for one analysis can be replaced by a new set for another, within a few minutes, before the boron analysis is complete. Up to 240 samples per hour can be processed with Autolab.

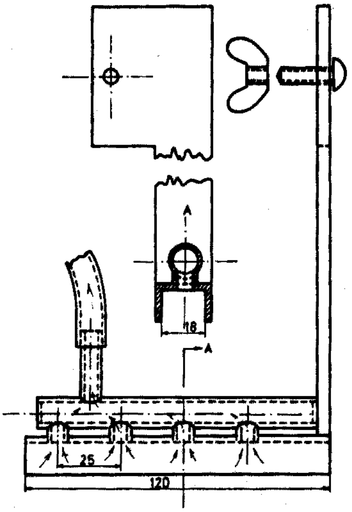

Fig. 3. Device for suction of gases formed at the water elimination process. It is mounted on the dispenser unit marked A in the operation scheme in Fig. 2.

### *Reagents*

*Acetic acid reagents (9+1 and 1+1).* For the 9+1 reagent, mix 900 ml of glacial acetic acid with 100 ml of sulphuric acid, s.g. 1.84. For the 1+1 reagent, mix equal volumes of the two acids.

*Propionic anhydride reagent.* Add 20 ml of oxalyl chloride to 500 ml of propionic anhydride (>97%; British Drug Houses Ltd) in an open flask. After some minutes a gas-developing reaction commences owing to the residual water in propionic anhydride. Allow the flask, provided with a stopper containing a small hole to allow additional gases to escape, to stand overnight in a hood. Check that the solution is free from gas bubbles before use.

*Curcumin–acetone reagent.* Dissolve 5 g of curcumin (B.D.H.) in 1 l of acetone and filter to obtain a clear solution.

*Buffer solution.* Mix 90 ml of ethanol (96%), 180 g of ammonium acetate and 135 ml of glacial acetic acid, and dilute to 1 l with water. If not otherwise stated all the reagents used are of analytical grade from Merck AG, Darmstadt.

### *The automated procedures*

The samples (3–5 ml) are placed in tubes in the outer chain. There is practically no limit to the number of samples which can be accommodated, but an ordinary sampler table carries about 150 tubes. The sampler pipette removes 0.1 ml of the aqueous sample containing 0–6 mg of boron per litre. The sample is then washed down with 1.0 ml of the (9+1) acetic acid reagent into a tube in the inner chain. The next syringe in the first dispenser unit adds 1.0 ml of propionic anhydride reagent. The solution is then stirred and allowed to react for 10 min, after which 0.7 ml of the (1+1) acetic acid reagent and 0.4 ml of curcumin–acetone reagent are added from separate syringes in the next dispenser unit. After stirring, the sample solution is allowed to pass through a water bath at 40° with a time delay of 12 min before 5 ml of the buffer solution is added from a syringe in the third dispenser unit to give a final volume of 8 ml. The solution is then stirred again and after a further 5 min, the absorbance is measured at 540 nm and printed out in arbitrary units proportional to the absorbance. The unit can be adjusted so as to give the concentration of boron in the sample directly in mg B/l. It is advisable to set the calibration reading for two concentrations in the range, *e.g.* 100 and 400 for 1 and 4 mg B/l respectively. The straight line relationship must, of course, be checked for the whole range of boron concentrations.

## RESULTS AND DISCUSSION

### *Calibration curves and standard deviations*

Standard graphs were obtained according to the procedure above, with a glass tube of diameter 1 cm in the photometer unit (Fig. 4). Owing to the high absorbance, the curve bends for concentrations >4 mg of boron per litre. Since sample volumes less than 0.1 ml can only be reproduced with difficulty, measurements were instead made in a cell with a diameter of 0.5 cm whereupon a straight line was obtained. The 0.5-cm cell does not belong to the standard equipment. The sample standard deviation was calculated for 50 samples containing 3 mg of boron per litre and found to be ±0.045 mg B/l; the detection limit is approximately 0.01 mg B/l (see Fig. 4).

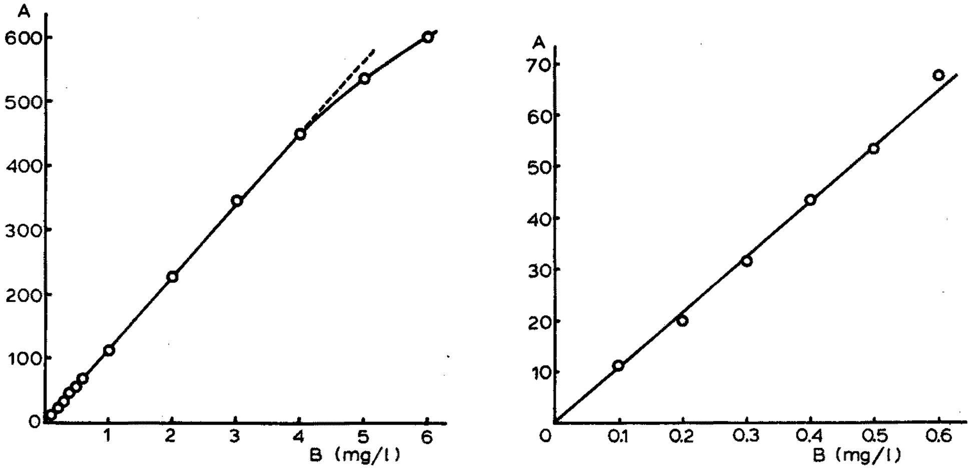

Fig. 4. Calibration curves obtained with glass tubes of 1 cm diameter as photometer cell. For concentrations >4 mg boron per litre, the relationship between absorbance and boron concentration is no longer linear and a photometer glass tube 0.5 cm in diameter is therefore recommended.

### *Adaptation of the manual method*

Some changes were necessary to bring down the final volume for analysis. The curcumin is dissolved in acetone which allows a more concentrated solution than acetic acid. Thus a smaller amount of sulphuric acid reagent is needed. In the manual method, the reaction between curcumin and boric acid takes place at room temperature. To shorten the time of reaction the tubes pass through a water bath at 40°.

The separate addition of oxalyl chloride as in the manual method is avoided because it reacts with atmospheric moisture thereby forming crystals of oxalic acid which may clog the dispenser tubings. To improve the water elimination, some sulphuric acid is added to the acetic acid used to dissolve the propionic anhydride in the water sample. In the propionic anhydride reagent the sulphuric acid acts together with the remaining oxalyl chloride as a catalyst.

*Technical aspects*

A 2-ml syringe was found to be the most suitable for delivering the 0.1-ml sample. The reason for this is that the 0.3-ml syringe has a long stroke which causes the sample to be ejected in the form of drops leading to irregularities since the whole sample is not always washed down with the acetic acid. Similar results were obtained by PEHRSSON[16]. The sampling pipette was rinsed twice, before and after dipping into the sample. Ethanol (99%) was used instead of distilled water to avoid introducing more water in the reaction.

No carry-over effect arising from the teflon stirrer could be observed. This is probably due to the high rotation velocity and the hydrophobic surface. During transportation, the glass tubes drop into the water bath and this may cause breakage and splashing. It is therefore advisable to allow the tubes to slide down into the bath on an aluminium plate.

As the inner tube chain (for this analysis) is of considerable length, the driving force is not strong enough to carry filled tubes all the way around. The holders must therefore be unloaded immediately after passage through the photometer unit. This can easily be achieved by allowing the tubes to drop down through a hole. Owing to the nature of the chemicals used in the analysis, corrosive effects must be diminished by suction and protective coatings.

We are indebted to Professor DAVID DYRSSEN, head of the department, for valuable advice. The Autolab was purchased with a grant from the Knut and Alice Wallenberg's Foundation, which we gratefully acknowledge. This work is part of a research program on the analysis of sea water constituents which is supported by the Swedish Natural Science Research Council. Thanks are also due to Dr. SUSAN JAGNER for revising the English text of this paper and to Linson Instrument AB for pleasant cooperation.

## SUMMARY

The curcumin method of UPPSTRÖM for the determination of boron in sea water has been adapted to an open system for automatic analysis. The equipment used is a module system called Autolab. The concentration range investigated is 0.1–6 mg of boron per litre and the sample standard deviation was found to be 1.5% in the middle of the range. The necessary modifications for the adaptation of the manual method as well as the equipment used are described.

## RÉSUMÉ

La méthode au curcumin de UPPSTRÖM pour le dosage du bore dans l'eau de mer a été adaptée à un système d'analyse automatique. L'équipement utilisé est un système de module appelé Autolab. Les concentrations en bore sont de 0.1 à 6 mg par litre, avec une déviation standard de 1.5% pour les valeurs moyennes. On décrit les modifications nécessaires pour adapter la méthode manuelle de même que l'équipement.

## ZUSAMMENFASSUNG

Die Curcumin-Methode von UPPSTRÖM für die Bestimmung von Bor in Seewasser ist einem offenen System für automatische Analyse angeglichen worden. Der untersuchte Konzentrationsbereich ist 0.1–6 mg Bor pro Liter; die Proben-Standardabweichung wurde in der Mitte des Bereichs zu 1.5% gefunden. Die für die Angleichung der manuellen Methode notwendigen Änderungen werden ebenso wie die verwendete Ausrüstung beschrieben.

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# THE SYNERGIC EXTRACTION OF TERNARY COMPLEXES OF EUROPIUM(III)

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In a previous paper[1] it was shown that europium(III) can be extracted into isoamyl alcohol from aqueous solutions containing salicylic acid ($H_2Sal$) and that the extractable species has the composition $Eu(HSal)_3$. If isoamyl alcohol is replaced by a non-hydroxylic solvent such as benzene or hexane, no extraction occurs over the pH range 2–5. However, the addition of tributylphosphate (TBP) to the system has a striking effect; Table I shows how the distribution ratio

$$D_{Eu} = \frac{\text{total concentration of europium in the organic phase}}{\text{total concentration of europium in the aqueous phase}}$$

varies with pH for various fixed total concentrations of tributyl phosphate $[TBP]_{org}$ in benzene. The concentration of salicylic acid ($H_2Sal$) was kept constant at $5.34 \cdot 10^{-2}$ $M$ and low concentrations of europium(III) labelled with $^{152,154}Eu$ were used. Over 98% of europium was extracted over the pH range 5.2–5.4.

During preliminary experiments the value of $D_{Eu} = 10^{0.2}$ was found for the extraction by TBP from an aqueous phase containing 0.1 $M$ sodium perchlorate at

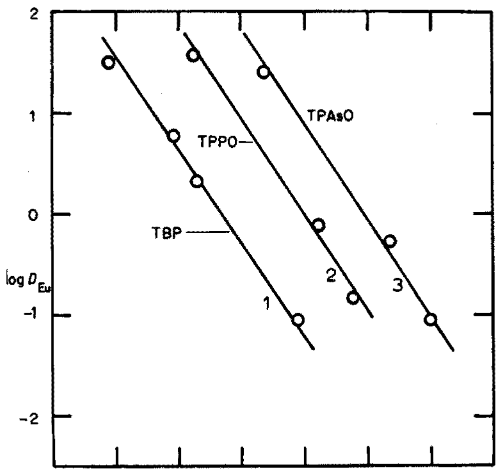

Fig. 1. Determination of the composition of the adducts $Eu(HSal)_3L_n$. Plots of log $D_{Eu}$ against log $[L]_{org}$ at a fixed pH = 4.6 for TBP (curve 1), 4.0 for TPPO (curve 2) and 4.0 for TPAsO (curve 3). The lines drawn are all of slope 2.

TABLE I

SYNERGIC EXTRACTION OF EUROPIUM(III)–SALICYLATE BY SOLUTIONS OF TBP IN BENZENE

($[H_2Sal)]_{tot} = 5.34 \cdot 10^{-2}$ *M*; $^{152,154}$Eu ~ $10^{-7}$ *M*)

| *pH* | *log* $D_{Eu}$ | *pH* | *log* $D_{Eu}$ |
|---|---|---|---|
| Total $[TBP]_{org} = 1.099 \cdot 10^{-2}$ *M* | | Total $[TBP]_{org} = 1.099 \cdot 10^{-1}$ *M* | |
| 3.54 | −1.412 | 3.95 | −0.150 |
| 3.90 | −1.378 | 4.40 | 0.246 |
| 4.06 | −1.252 | 4.85 | 0.884 |
| 4.35 | −1.116 | 5.12 | 1.028 |
| 4.60 | −1.074 | 5.50 | 0.748 |
| 4.75 | −0.984 | 5.72 | 0.592 |
| 4.94 | −0.982 | 7.00 | 0.229 |
| 6.71 | −1.462 | | |
| Total $[TBP]_{org} = 7.330 \cdot 10^{-2}$ *M* | | Total $[TBP]_{org} = 3.664 \cdot 10^{-1}$ *M* | |
| 3.42 | −1.218 | 3.96 | 0.844 |
| 4.45 | 0.035 | 4.20 | 1.102 |
| 4.60 | 0.263 | 4.31 | 1.221 |
| 4.98 | 0.477 | 4.70 | 1.519 |
| 5.36 | 0.525 | 4.90 | 1.578 |
| 5.47 | 0.353 | 5.38 | 1.811 |
| 5.82 | 0.048 | 6.14 | 1.212 |
| 6.82 | −0.298 | 7.10 | 0.131 |

TABLE II

SYNERGIC EXTRACTION OF EUROPIUM(III)–SALICYLATE BY A SOLUTION OF TPPO IN BENZENE

($[H_2Sal]_{tot} = 5.34 \cdot 10^{-2}$ *M*; $^{152,154}$Eu ~ $10^{-7}$ *M*)

| *pH* | *log* $D_{Eu}$ | *pH* | *log* $D_{Eu}$ |
|---|---|---|---|
| Total $[TPPO]_{org} = 7.682 \cdot 10^{-4}$ *M* | | Total $[TPPO]_{org} = 7.682 \cdot 10^{-3}$ *M* | |
| 3.50 | −2.456 | 2.90 | −1.223 |
| 4.05 | −2.181 | 3.25 | −0.889 |
| 4.22 | −2.032 | 3.72 | −0.386 |
| 4.50 | −1.879 | 4.30 | 0.103 |
| 5.01 | −1.724 | 4.64 | 0.533 |
| 5.58 | −1.337 | 4.70 | 0.551 |
| 5.90 | −1.276 | 5.70 | 0.880 |
| 6.94 | −1.886 | 5.82 | 1.078 |
| | | 6.70 | 0.468 |
| Total $[TPPO]_{org} = 4.285 \cdot 10^{-3}$ *M* | | Total $[TPPO]_{org} = 7.682 \cdot 10^{-2}$ *M* | |
| 3.36 | −1.439 | 3.00 | 0.119 |
| 3.77 | −1.080 | 3.40 | 0.867 |
| 4.21 | −0.712 | 4.02 | 1.532 |
| 5.12 | 0.003 | 4.38 | 1.594 |
| 5.84 | 0.807 | 4.95 | 1.609 |
| 6.56 | −0.296 | 5.18 | 1.628 |
| | | 5.64 | 1.630 |
| | | 5.90 | 1.726 |
| | | 6.16 | 1.554 |
| | | 6.64 | 1.226 |

pH 2. YOSHIDA[2] has shown that some lanthanides are extracted when perchlorate is the anion and the value $D_{Eu} = 10^{-0.5}$ was reported for the above conditions because of the extraction of (probably) $Eu(ClO_4)_3 \cdot 6TBP$. For this reason the presence of perchlorate ions was avoided in subsequent experiments in the work described here.

From the experimental results for the extraction of europium(III) given in Table I, the values of $D_{Eu}$ corresponding to various fixed total concentrations of the oxygen-donor ligand $C_{L,org}$ (where L = TBP) were read off at a series of pH values and are represented on a log–log plot in Fig. 1. The linear plot has an integral slope of two.

If the extraction of the adduct follows the equation

$$Eu(HSal)_3 + nL_{org} \rightarrow (Eu(HSal)_3L_n)_{org}$$

governed by an extraction constant

$$K_{ex} = \frac{[Eu(HSal)_3L_n]_{org}}{[Eu(HSal)_3][L]^n_{org}} \qquad (1)$$

it follows that

$$D_{Eu} = [Eu(HSal)_3L_n]_{org} / \sum_0 Eu(HSal)_j \qquad (2)$$

provided that the concentration of the adduct (or adducts), $[Eu(HSal)L_n]$, in the aqueous phase is negligible compared to those of the europium–salicylate complexes. Since the species $Eu(HSal)_3$ has been shown not to be extracted into benzene, the term $[Eu(HSal)_3]_{org}$ does not appear in the numerator. By introducing the expression for $K_{ex}$ and the various stability constants defined by $\beta_j = [Eu(HSal)_j]/[Eu][HSal]^j$, eqn. (2) reduces to:

$$D_{Eu} = K_{ex}\beta_3[HSal]^3[L]^n_{org} / \sum_0 \beta_j[HSal]^j$$

whence $\log D_{Eu} = \log K_{ex}\beta_3 + 3 \log [HSal] - n \log [L]_{org} - \log \sum_0 \beta_j[HSal]^j$ (3)

(Charges on ions are omitted for simplification.)

At a fixed pH, [HSal] will be constant and hence the plot of $\log D_{Eu}$ against $\log [L]_{org}$ should be linear and of integral slope $n$, provided that only one adduct species is extracted under the prevailing experimental conditions. The present experi-

TABLE III

SYNERGIC EXTRACTION OF EUROPIUM(III)–SALICYLATE BY A SOLUTION OF TPAsO IN BENZENE

($[H_2Sal]_{tot} = 5.34 \cdot 10^{-2}$ $M$; $^{152,154}Eu \sim 10^{-7}$ $M$)

| *pH* | *log* $D_{Eu}$ | *pH* | *log* $D_{Eu}$ |
|---|---|---|---|
| Total $[TPAsO]_{org} = 1.005 \cdot 10^{-3}$ $M$ | | Total $[TPAsO]_{org} = 2.01 \cdot 10^{-2}$ $M$ | |
| 3.71 | −1.453 | 3.58 | 1.012 |
| 4.32 | −0.605 | 4.35 | 1.696 |
| 5.06 | 0.338 | 4.80 | 1.895 |
| 5.90 | 0.797 | 5.00 | 2.327 |
| 6.65 | 0.837 | 5.20 | 2.555 |
| | | 6.24 | 2.177 |
| | | 6.80 | 1.891 |
| Total $[TPAsO]_{org} = 2.01 \cdot 10^{-3}$ $M$ | | | |
| 3.20 | −1.242 | | |
| 3.83 | −0.722 | | |
| 4.28 | 0.094 | | |
| 4.45 | 0.339 | | |
| 5.40 | 1.185 | | |
| 6.75 | 1.173 | | |

ments show that $n=2$. Since only very small amounts of adduct are formed, it is legitimate to put $[L] \equiv C_{L,org}$.

The total amount of salicylic acid is given by the mass-balance:

$$[H_2Sal]_{tot} = [H_2Sal]_{org} + [H_2Sal] + [HSal] + 2[(H_2Sal)_2] + 3[Eu(HSal)_3L_2]_{org}$$

$$= [HSal]\ \{(p_s+1)([H^+]/K_1)+1\} + 2K_{dim}\ \{[HSal]p_s[H^+]/K_1\}^2 \qquad (4)$$

for at tracer levels the term involving europium can be neglected. Here $p_s = [H_2Sal]_{org}/[H_2Sal]$, $K_{dim} = [(H_2Sal)_2]_{org}/[H_2Sal]^2_{org}$ and $K_1 = [H^+][HSal^-]/[H_2Sal]$. It is shown elsewhere[3] that $p_s = 1.7$ and $K_{dim} = 35$, and by taking[1] $pK_1 = 2.85$, the value of [HSal] can be calculated for each pH value used. These are then introduced into eqn. (3) together with the values $\beta_1 = 10^{2.59}$, $\beta_2 = 10^{4.21}$ and $\beta_3 = 10^{4.86*}$ obtained previously[1] to give the consistent values for the extraction constant shown in Table IV. The average value of $K_{ex}$(TBP) is $3.68 \pm 0.14$.

*Synergic effect of triphenylphosphine oxide*

The results of measurements of the extraction of europium(III) salicylate by solutions of triphenylphosphine oxide (TPPO) in benzene are given in Table II. TPPO proves to be a more effective synergist than TBP, for a concentration as low as $7.68 \cdot 10^{-2}$ $M$ can extract upwards of 97% of europium from an aqueous solution containing $5.36 \cdot 10^{-2}$ $M$ salicylate at pH 4. The broad pH range for maximum extraction is also noteworthy and recalls similar differences in the synergic extraction of uranyl nitrate by TBP and TBPO[4]. Plots of log $D_{Eu}$ against log $[TPPO]_{org}$ at various pH values gave straight lines of slope 2, confirming the composition of the extractable species as $Eu(HSal)_3 \cdot 2TPPO$. The curve for pH 4 is shown in Fig. 1.

The calculations of the value of the extraction constant (Table IV) lead to $K_{ex}(TPPO) = 5.33 \pm 0.35$.

TABLE IV

EVALUATION OF EXTRACTION CONSTANTS $K_{ex}$ FOR THE SYSTEMS Eu(III)–SALICYLATE–L (L = TBP, TPPO, TPAsO)

| *log $D_{Eu}$* | *−log [L]* | *log $K_{ex}$* | *Average* |
|---|---|---|---|
| *L = TBP* | | | |
| −1.023 | 1.959 | 3.87 | |
| 0.301 | 1.135 | 3.55 | 3.68 ± 0.14 |
| 0.778 | 0.959 | 3.67 | |
| 1.472 | 0.436 | 3.62 | |
| *L = TPPO* | | | |
| −1.820 | 3.115 | 5.39 | |
| −0.320 | 2.368 | 5.39 | |
| 0.480 | 2.115 | 5.69 | 5.33 ± 0.35 |
| 1.640 | 1.115 | 4.85 | |
| *L = TPAsO* | | | |
| −0.26 | 2.998 | 6.71 | |
| 0.50 | 2.697 | 6.87 | 6.64 ± 0.28 |
| 1.96 | 1.697 | 6.33 | |

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* This corrects a typographical error in ref. 1.

### *Synergic effect of triphenylarsine oxide*

There is again an improvement in the synergic extraction of europium salicylate when TPPO is replaced by triphenylarsine oxide, TPAsO (Table III); with $2.07 \cdot 10^{-2}$ *M* TPAsO the percentage extraction reaches 99.7% at pH 5.2. At pH 4 and 5, the plot of log $D_{Eu}$ against log $[TPAsO]_{org}$ is linear (*cf.* Fig. 1) and of slope 2, establishing the composition of the extractable species as $Eu(HSal)_3 \cdot 2TPAsO$. The extraction constant (Table IV) is $K_{ex}(TPAsO) = 6.64 \pm 0.28$.

Compared with TBP, TPPO and TPAsO, 4-methylpyridine-N-oxide (MPNO) proved to be a poor synergist (*cf.* Table V) and less than 4% extraction was achieved at pH 4 even with a high total concentration (*ca.* 0.1 *M*) of $[MPNO]_{org}$. Since the value of $D_{Eu}$ could not be varied over a wide enough range, the value of $K_{ex}$ (MPNO) could not be determined accurately.

### *Extraction into methyl isobutyl ketone*

The extraction of europium into isoamyl alcohol as $Eu(HSal)_3$ has already been described[1]. Similar measurements of the extraction of europium(III) from aqueous salicylates by hexone (methyl isobutyl ketone) are reported in Table VI. Extraction from the lowest concentrations of salicylic acid is slightly higher in this case but the

TABLE V

EXTRACTION OF EUROPIUM(III) FROM $5.367 \cdot 10^{-2}$ *M* AQUEOUS SALICYLIC ACID BY A SOLUTION OF 4-METHYLPYRIDINE-N-OXIDE IN BENZENE

| *pH* | *log* $D_{Eu}$ | *pH* | *log* $D_{Eu}$ |
|---|---|---|---|
| Total $[MPNO]_{org} = 4.231 \cdot 10^{-2}$ *M* | | Total $[MPNO]_{org} = 1.028 \cdot 10^{-1}$ *M* | |
| 3.32 | −2.666 | 3.45 | −2.153 |
| 3.66 | −2.487 | 3.51 | −2.060 |
| 3.68 | −2.467 | 3.58 | −1.984 |
| 3.92 | −1.837 | 3.60 | −1.920 |
| 4.02 | −1.507 | 3.75 | −1.777 |
| 4.40 | −1.575 | 4.00 | −1.437 |
| 4.52 | −1.666 | 4.50 | −1.504 |

TABLE VI

THE EXTRACTION OF EUROPIUM(III) FROM AQUEOUS SALICYLIC ACID INTO HEXONE

| *pH* | *−log [HSal]*[a] | *log* $D_{Eu}$ |
|---|---|---|
| 2.90 | 3.734 | −2.757 |
| 2.96 | 3.674 | −2.616 |
| 3.60 | 3.040 | −1.281 |
| 4.00 | 2.651 | −0.546 |
| 4.62 | 2.215 | 0.057 |
| 5.30 | 1.603 | 0.447 |
| 5.60 | 1.499[b] | 0.568 |
| 5.75 | 1.408 | 0.559 |
| 6.28 | 1.351[b] | 0.516 |
| 6.40 | 1.308 | 0.615 |

[a] The value of [HSal] was calculated by eqn. (4) with $K_1 = 1.413 \cdot 10^{-3}$, $p_s = 324$ and $K_{dim} = 0^5$.

[b] The concentration of $[H_2Sal]_{tot}$ in the aqueous phase $= 5.0 \cdot 10^{-2}$ *M*. For the rest of the experiments $[H_2Sal]_{tot} = 5.367 \cdot 10^{-2}$ *M*.

initial slope of the plot of log $D_{Eu}$ against $-\log [H_2Sal]$ is closer to 2 than to the value 3 expected for the partition of a species with the composition ratio Eu:HSal = 1:3.

It may be that hexone, by virtue of its carbonyl group, is acting both as a pure solvent and as a coordinated component of the extractable species but the subject was not further investigated since the use of hexone offered no special advantages.

## EXPERIMENTAL

Distribution ratios for europium(III) were measured radiometrically by using $^{152,154}$Eu as described previously[1]. Various concentrations of sodium salicylate were used in the aqueous phase which was adjusted to the desired pH, and the synergist was added to the benzene phase. No attempt was made to maintain constant ionic strength. To avoid volume changes on equilibration, each phase was presaturated with the other one. After equilibration of equal volumes of the two phases by shaking in stoppered test-tubes for 2–4 h at room temperature, the system was centrifuged to effect phase separation and aliquot portions of each phase were removed to a well-type scintillation counter. Purified laboratory specimens of TBP, TPPO, TPAsO and MPNO were used.

The partition of europium(III) between aqueous solutions of salicylic acid at various pH values and hexone (methyl isobutyl ketone) was investigated by the method previously described for isoamyl alcohol[1]. Great difficulty was encountered in obtaining reproducible results with the higher concentrations of salicylic acid and at the higher pH values, as opaque organic phases were obtained even after long standing and centrifugation.

## DISCUSSION

Since europium(III) is not significantly extracted by solutions of TBP, TPPO, TPAsO or MPNO in benzene under the conditions of the present experiments if salicylic acid is absent from the aqueous phase, the pronounced increases in extractability reported above present a clear case of synergism. The bis-adducts, $Eu(HSal)_3L_2$, are presumably bulkier and less hydrophilic than the parent chelated complex $Eu(HSal)_3$, thus favouring their partition into an organic phase[4].

If the distribution coefficient for the neutral complex $Eu(HSal)_3$ between benzene and water is defined by $p_C = [Eu(HSal)_3]_{org}/[Eu(HSal)_3]$, it follows from eqn. (1) that the formation constant for the bis-adducts *in the organic phase* is given by

$$K^*(Eu(HSal)_3L_2) = [Eu(HSal)_3L_2]_{org}/[Eu(HSal)_3]_{org}[L]_{org}^2 = K_{ex}/p_c \qquad (5)$$

The order of increasing extraction constants,

$$K_{ex}(TBP) = 10^{3.68} < K_{ex}(TPPO) = 10^{5.33} < K_{ex}(TPAsO) = 10^{6.64}$$

is therefore a measure of the increasing tendency to form a donor–acceptor adduct. The 40-fold improvement in replacing $(BuO)_3P{:}O$ by $(C_6H_5)_3P \rightarrow O$ thus reflects the higher donor capacity of the oxygen in the more polar phosphine oxide. The further 20-fold improvement on replacing phosphorus by arsenic is probably due to the greater polarizability of the larger atom with the result that the group $\dot{:}As \rightarrow O$ is a better coordinating group than $\dot{:}P \rightarrow O$, and a stronger adduct is formed. Although the system involving 4-methylpyridine-N-oxide was not investigated fully, the present results establish the order of synergic efficiency as MPNO < TBP < TPPO < TPAsO.

## SUMMARY

It has been shown that the complex $Eu(HSal)_3$ from europium(III) and salicylic acid ($H_2Sal$) can be extracted from aqueous solutions into isoamyl alcohol. In contrast, extraction by non-hydroxylic solvents (*e.g.* benzene, hexane) does not occur over the pH range 2–5 unless a formally neutral oxygen donor is present; ternary compounds of the composition $Eu(HSal)_3L_2$ (where L = tributylphosphate, triphenylphosphine oxide or triphenylarsine oxide) are then extracted. Synergic extraction of $Eu(HSal)_3$ also occurs in the presence of 4-methylpyridine-N-oxide or methyl isobutyl ketone.

## RÉSUMÉ

Il est possible d'extraire le complexe $Eu(HSal)_3$, obtenu à partir d'europium(III) et d'acide salicyclique ($H_2Sal$) dans l'alcool isoamylique. Par contre une extraction par solvant non hydroxylé (ex. benzène, hexane) ne s'effectue pas en dehors des pH 2–5 sans qu'un donneur d'oxygène neutre soit présent. Des composés ternaires de composition $Eu(HSal)_3L_2$ (où L = tributylphosphate, oxyde de triphénylphosphine) sont extraits. Une extraction de $Eu(HSal)_3$ se fait également en présence de méthyl-4-pyridine-N-oxyde ou de méthylisobutylcétone.

## ZUSAMMENFASSUNG

Es ist gezeigt worden, dass der Komplex $Eu(HSal)_3$ aus Europium(III) und Salicylsäure ($H_2Sal$) aus wässriger Lösung mit Isoamylalkohol extrahiert werden kann. Dagegen findet eine Extraktion durch Lösungsmittel ohne Hydroxylgruppen (z.B. Benzol, Hexan) im pH-Bereich 2–5 nicht statt, ausser wenn ein formal neutraler Sauerstoffdonor zugegen ist; ternäre Verbindungen der Zusammensetzung $Eu(HSal)_3L_2$(L = Tributylphosphat, Triphenylphosphinoxid oder Triphenylarsinoxid) werden dann extrahiert. Eine synergetische Extraktion von $Eu(HSal)_3$ findet ebenfalls in Gegenwart von 4-Methylpyridin-N-oxid oder Methylisobutylketon statt.

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# A SPECTROPHOTOMETRIC DETERMINATION OF CARBANIONS WITH TETRANITROMETHANE*

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Various organic reactions such as electrophilic substitutions[1] and analogous enzyme-catalyzed reactions[2,3] are known to involve carbanion intermediates. Detection of carbanions and especially measurements of the rates of deprotonation of C-acids are most commonly carried out by procedures that involve hydrogen isotope exchange or halogenation[4]. In principle, any electrophile which reacts selectively with carbanions may be suitable for determination of carbanion, provided that it reacts rapidly enough for the ionization of the C-acid to be rate-determining. However, the value of such an analytical reagent depends in large measure on how readily its reaction can be followed quantitatively.

In the present study, tetranitromethane has been used as a reagent for carbanions. It reacts rapidly with carbanions with the concomitant formation of an intensely yellow by-product, nitroformate, which provides the basis for a convenient spectrophotometric assay.

## EXPERIMENTAL

### *Materials*

Tetranitromethane, nitromethane, nitroethane, malononitrile, and methylsulfone were obtained from Aldrich Chemical Company. 1,1-Dinitroethane was purchased from the same company as a 30% solution in ethylene chloride which was removed before use in a rotary evaporator under reduced pressure. 2,4-Pentanedione (Eastman Organic Chemicals), acetonitrile (J. T. Baker Chemical Company), acetone (Merck), Dowex anion exchanger AG 1-X2, 200–400 mesh (Biorad Laboratories) and 1,2-dichloroethane (Fisher Scientific Company) were also used.

### *Instrumentation*

A Unicam SP-800 recording spectrophotometer equipped with an automatic cell changer and repetitive reading program was used for determining the rate of nitroformate production. The cell-holder was thermostatted at 25°. Absorbance at discrete wavelengths was measured with a Zeiss PMQ II spectrophotometer. Absorption spectra were obtained with a Cary 15 recording spectrophotometer and infrared

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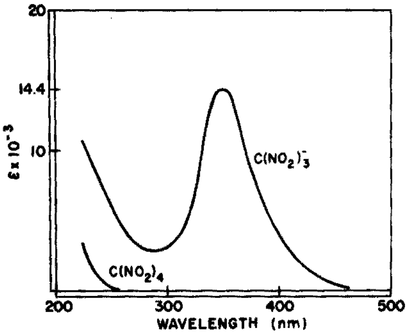
spectra with a Beckman IR 20 spectrophotometer. Hydrogen ion concentration was determined with a general-purpose combined glass–calomel electrode (GK 2021 C) attached to a Radiometer model 22 pH-meter.

*Determination of carbanions*

The procedure is based on the following reactions:

$$\begin{aligned} &HA + H_2O \underset{k_2}{\overset{k_1}{\rightleftarrows}} A^- + H_3O^+ \\ &A^- + C(NO_2)_4 \xrightarrow{k_3} [NO_2A] + C(NO_2)_3^- \end{aligned} \qquad (1)$$

where HA denotes the C-acid, $A^-$ the carbanion, $C(NO_2)_4$ is tetranitromethane, and $[NO_2A]$ indicates the reaction product. The by-product nitroformate, $C(NO_2)_3^-$, is used as a quantitative reaction indicator. If $k_2[H_3O^+] \ll k_3[TNM] \gg k_1$, the apparent rate of nitroformate production, $k_3'$, will equal $k_1$, ionization being rate-determining. Nitro-



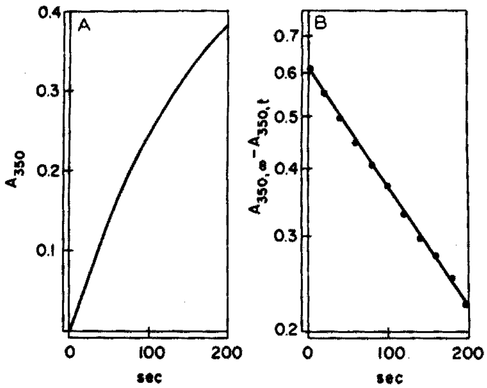


Fig. 1. Absorption spectra of tetranitromethane and nitroformate (0.05 *M* Tris chloride–0.3 *M* NaCl, pH 8.0).

Fig. 2. Tetranitromethane and nitroethane. (A) Nitroformate production *vs.* time, (B) first-order plot of data from Fig. 2A. Conditions as in Table I.

TABLE I

RATES OF NITRATION ($k_3'$) AND IONIZATION RATES ($k_1$) OF C-ACIDS

| *C-acid* | *M* | $k_3'$ *(min*$^{-1}$*)*[a] | $k_1$ *(min*$^{-1}$*)*[b] |
|---|---|---|---|
| $CH_3NO_2$ | $6.2 \cdot 10^{-5}$ | $7 \cdot 10^{-6}$ | $2.6 \cdot 10^{-6}$ |
| $C_2H_5NO_2$ | $4.2 \cdot 10^{-5}$ | $6 \cdot 10^{-6}$[c] | $2.2 \cdot 10^{-6}$ |
| $CH_3SO_2CH_3$ | 0.133 | $3 \cdot 10^{-10}$ | $2 \cdot 10^{-10}$ |
| $CH_3CN$ | 0.63 | $6 \cdot 10^{-11}$ | $4 \cdot 10^{-12}$ |
| $CH_3COCH_3$ | 0.46 | $1 \cdot 10^{-9}$ | $2.8 \cdot 10^{-8}$ |

[a] In 0.1 *M* glycine, 0.1 *M* NaCl, $2.8 \cdot 10^{-4}$ *M* tetranitromethane, pH 11.0, 25°. The values of $k_3'$ are corrected for hydroxide ion catalysis (see text).

[b] Data of PEARSON AND DILLON[4].

[c] From Fig. 2.

formate, in contrast to tetranitromethane, absorbs strongly at 350 nm[5] ($\varepsilon = 14{,}400$; Fig. 1). For determination of reaction rates, the concentrations of C-acids were chosen according to the rates of ionization (*cf.* Table I). Fast ionizing C-acids were used at low concentrations with an excess of tetranitromethane and the increase in $A_{350}$ was treated as a first-order reaction. Slowly ionizing compounds were added to an excess of TNM and the data were plotted as zero-order reactions. Correction was made for the spontaneous production of nitroformate from tetranitromethane at each pH employed. Zero-order rates were calculated assuming mononitration, and no product other than nitroformate absorbing at 350 nm. In order to promote its dissolution, tetranitromethane was employed as a 0.03 *M* solution in 95% ethanol.

## RESULTS

The rates and products of the reaction of tetranitromethane with nitroethane were studied in order to delineate the basic features of the tetranitromethane–carbanion reaction. The general feasibility of determination of carbanions by this reaction was examined by comparing the rates of ionization of a number of C-acids obtained by the present procedure with those obtained by other methods.

### *Determination of the rate of ionization of nitroethane*

Tetranitromethane reacts readily with nitroethane at pH 11 as judged from the production of nitroformate (Fig. 2A). The pseudo first-order rate of nitroformate production (Fig. 2B), corrected for hydroxide ion catalysis of ionization, is $5.8 \cdot 10^{-6}$ min$^{-1}$. This value is close to the rate of ionization, measured by bromination or iodination[6] ($2.2 \cdot 10^{-6}$ min$^{-1}$). The correction for hydroxide ion catalysis assumes the same ratio $k_{H_2O}/k_{OH}$ as for acetone[7], *i.e.* $k_{H_2O} = k_{pH11}/5.2 \cdot 10^4$. Under the present conditions, catalysis of the ionization by cations and by anions other than hydroxide

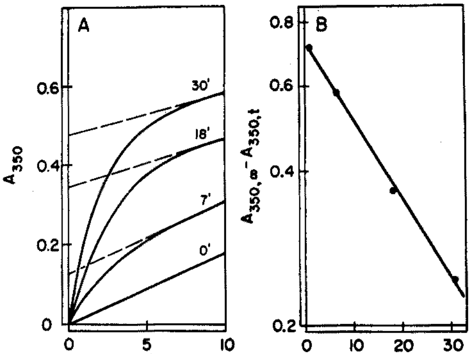


Fig. 3. Reaction of tetranitromethane with nitroethane stored for different times at pH 10. (A) Nitroformate production *vs.* time; $4.2 \cdot 10^{-5}$ *M* nitroethane, 1.12 m*M* tetranitromethane in universal buffer, pH 10[10]. (B) First-order rate plot of the extrapolated values taken from the ordinate of Fig. 3A.

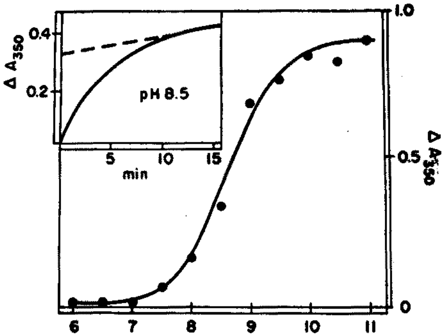


Fig. 4. Reaction of tetranitromethane and nitroethane at different pH values. Nitroethane, $4.2 \cdot 10^{-5}$ *M*, was pretreated for 48 h in universal buffer[10] of the indicated pH. Then tetranitromethane (final concentration 1.12 m*M*) was added and $A_{350}$ was recorded. The inset shows a typical tracing obtained at pH 8.5. The magnitude of the 350-nm burst (intercept on ordinate in inset) is plotted *vs.* pH.

is negligible[8]. The same rate of ionization was also obtained by an indirect assay where nitroethane was stored at pH 10 and the progress of ionization was measured on aliquots withdrawn at intervals by means of the tetranitromethane reaction (Fig. 3A). The increase in $A_{350}$ on addition of tetranitromethane reveals a fast reaction ($k_3 \cdot$[TNM] limiting), superimposed on a much slower reaction ($k_1$ limiting). Extrapolation of the indicator reaction to zero time gives the $A_{350}$ value corresponding to the amount of nitroethane anion present after the indicated period at pH 10. At pH 10 the rate of protonation, $k \cdot [H_3O^+]$, is negligible[6] and ionization may be treated as a first-order reaction (Fig. 3B). The derived rate constant of $6.3 \cdot 10^{-6}$ min$^{-1}$ is in good agreement with the value determined in the direct assay (Fig. 2).

*Determination of the dissociation constant of nitroethane*

Addition of tetranitromethane to a solution of nitroethane, pretreated at various pH values to allow ionization equilibrium, results in a rapid increase in absorbance at 350 nm followed by a slower increase (inset, Fig. 4). Extrapolation to zero time indicates the equilibrium concentration of nitroethane anion at each pH value. A plot of $\Delta A_{350}$ *versus* pH (Fig. 4) coincides with the theoretical titration curve for a group with a p$K$ of 8.6, the value reported for nitroethane[9].

*Identification of the reaction product*

The product of the reaction of tetranitromethane with nitroethane, designated as [$NO_2A$] in scheme 1, was identified as 1,1-dinitroethane. Nitroethane ($3 \cdot 10^{-3}$ $M$) was treated with 2 l of 0.05 $M$ glycine buffer, pH 10.0, for 6 h and an equimolar amount of tetranitromethane was then added. Reaction was complete after 30 min at room temperature and the absorbance at 350 nm did not increase further. The bright yellow solution was pumped onto a Dowex AG 1-X2 anion-exchange column (200–400 mesh, chloride form, 18 × 0.8 cm). Nitroformate remained bound as a yellow-brown zone at the top of the column. The other reaction product migrated slowly as a yellow zone during the application of the 2-l sample and was easily eluted with 1 $M$ ammonium acetate, pH 4.0. Aliquots of each fraction were added to 6 $N$ sodium hydroxide in order to locate the product. Those fractions which gave a yellow color by this procedure

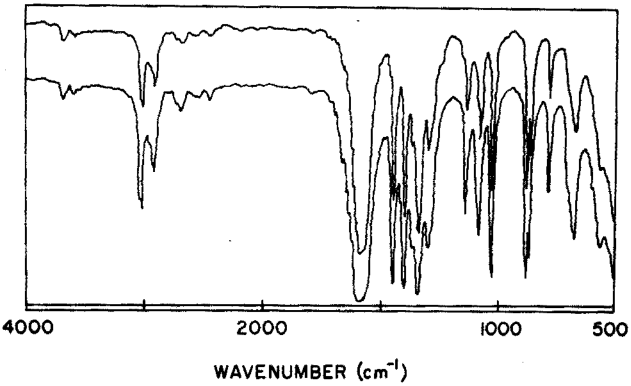


Fig. 5. Infrared spectra of isolated tetranitromethane–nitroethane reaction product (upper curve) and authentic 1,1-dinitroethane (lower curve).

were pooled (190 ml) and acidified with acetic acid to pH 3.0. This solution was extracted with three 10-ml portions of 1,2-dichloroethane which was subsequently removed under reduced pressure on a rotary evaporator. The residue, a yellow oil, was identical with authentic 1,1-dinitroethane on the basis of the following criteria: absorption spectrum with $\lambda_{max}=380$ nm and $\varepsilon_{380}=16{,}700$, p$K_a$ of 5.0 as determined by spectrophotometric titration, and infrared spectra (Fig. 5). The identification of 1,1-dinitroethane as the reaction product of nitroethane and tetranitromethane is perfectly consistent with scheme 1.

### *C-acids with various electron-withdrawing substituents. Rates of nitration and rates of ionization*

The general applicability of tetranitromethane as a carbanion reagent is demonstrated in Table I. Carbon acids substituted by nitro, sulfonyl, cyano, or ketonic carbonyl groups react readily with tetranitromethane. With all these compounds, the rate of nitration, determined as in Fig. 2 for nitroethane, agrees within an order of magnitude with the reported value of the rate of ionization.

## DISCUSSION

Tetranitromethane can be employed to determine carbanions by a convenient spectrophotometric method as demonstrated by detailed kinetic and analytical studies of the reaction between tetranitromethane and nitroethane (Figs. 2–5), and by successful determination of the ionization rate constants of a number of C-acids with different electron-withdrawing substituents (Table I). The method is based on spectrophotometric measurements of the production of the colored reaction by-product nitroformate ($\varepsilon=14{,}400$).

In all systems where the rate of carbanion formation is much lower than the rate of the tetranitromethane reaction, this indicator reaction can be employed to determine both the *rate* of carbanion formation, *e.g.* the ionization rate of C-acids (Table I) and the absolute *concentration* of carbanion, *e.g.* the equilibrium concentration as a function of pH (Fig. 4).

In systems where the generation of carbanions is too fast to be rate-limiting, tetranitromethane can be employed successfully as a trapping agent for this reactive species. The *rate* of nitroformate production will then be proportional to the *concentration* of carbanion, and will provide a relative measure of the equilibrium or steady-state concentration of this reactive species under different conditions of the carbanion-generating system. Such a situation (*i.e.* $k_1>>k_3$ (TNM) in scheme 1) will be encountered with rapidly ionizing C-acids and in certain enzyme-catalyzed reactions. It has already been possible by this procedure to demonstrate carbanion intermediates in the reaction pathways of three carbon–carbon lyases, *i.e.* fructose 1,6-diphosphate aldolase from rabbit muscle[11], yeast fructose 1,6-diphosphate aldolase[12] and yeast 6-phosphogluconate dehydrogenase.

We would like to thank CHARLOTTE HART for her excellent technical assistance. The constant advice and encouragement of B. L. VALLEE is gratefully appreciated. One of us (P.C.) thanks the University of Zürich and the American–Swiss Foundation for Scientific Exchange for financial support.

## SUMMARY

Tetranitromethane has been employed as a reagent for determining the rates of ionization and the dissociation constants of C-acids. The intensely yellow by-product, nitroformate, provides the basis for a convenient spectrophotometric assay at 350 nm ($\varepsilon = 14{,}400$).

## RÉSUMÉ

Le tétranitrométhane est utilisé comme réactif pour déterminer les vitesses d'ionisation et les constantes de dissociation des acides organiques. Le nitroformiate jaune intense qui se forme permet une analyse spectrophotométrique à 350 nm ($\varepsilon = 14{,}400$).

## ZUSAMMENFASSUNG

Tetranitromethane ist gebraucht worden, um die Geschwindigkeitskonstanten der Ionisation und die Dissoziationskonstanten von C-Säuren zu bestimmen. Das intensiv gelbe Nebenprodukt Nitroformat erlaubt einfache spektralphotometrische Bestimmungen.

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# RAPID EXTRACTION AND SPECTROPHOTOMETRIC DETERMINATION OF NICKEL(II) WITH ISONITROSOACETOPHENONE

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Isonitrosoacetophenone (HINAP) has been employed for the separation and spectrophotometric determination of palladium(II)[1]. It has been reported[2] that it can extract nickel(II) into chloroform from alkaline solution but its use in the extraction, separation and spectrophotometric estimation of nickel(II) has not previously been investigated. A detailed study is presented in this paper.

## EXPERIMENTAL

### *Apparatus*

Activity measurements were taken on a $\gamma$-ray spectrometer provided with a single-channel analyzer and a well-type 3.8 × 3.8 cm NaI (Tl) crystal detector. Radioisotopes emitting $\beta$-rays only were counted on a thin end-window type G.M. counter operated with a high voltage power supply and a decade scaler. A Hilger UV spectrophotometer and a Unicam SP 300 colorimeter (1-cm glass cells) were used for absorbance measurements. pH Measurements were taken with an Elico pH-meter Ll 10 (Electronic and Industrial Instruments Co., Hyderabad).

### *Reagents*

A.R.-grade chemicals were used throughout. All standard and carrier solutions were prepared in double-distilled water. The stock solution of nickel was prepared by dissolving nickel sulfate or chloride in water and diluting the solution to a known volume. It was analysed for nickel by the dimethylglyoxime method[3]. HINAP was prepared as described by WELCHER[4]. Solutions required for the interference studies were prepared by dissolving appropriate salts in water. The strength of each solution was determined by known methods[3].

### *Procedure for extraction and separation of nickel*

Treat 1 ml of solution containing 1–3 mg of nickel with 2 ml of a 2% solution of HINAP in 0.1 *M* sodium hydroxide. Adjust the pH to 7.2–8.0 with hydrochloric acid or sodium hydroxide, using a pH meter. Dilute to 10 ml with water and transfer to a 25-ml graduated separatory funnel, rinsing the beaker twice with two 4-ml portions of organic solvent. Make the organic phase up to 10 ml and shake for 2 min.

In preliminary studies an aliquot of each phase was analysed for nickel by the dimethylglyoxime method[5]. The pH of the equilibrated aqueous phase was measured. In the study of the effects of other ions, 1 ml of solution containing the ion of interest was added before the pH was adjusted; the extraction of other ions was followed by measuring the activity of the radioisotopes used, corrections for background, coincidence, self-absorption and self-scattering being applied when necessary. Total activity always agreed with the activity taken within ±2%. In separation experiments, any masking agents were added to the aqueous solution before the pH was adjusted to 7.6. Nickel was extracted with two 5-ml portions of chloroform, re-extracted into the aqueous phase by equilibrating the combined chloroform phase twice with 5 ml of *M* hydrochloric acid and determined colorimetrically by the dimethylglyoxime method.

### *Procedure for spectrophotometric determination of nickel*

To a suitable aliquot of solution containing 1.0–30.0 $\mu$g of nickel, add 1 ml of a 1% (w/v) solution of HINAP in 0.1 *M* sodium hydroxide, adjust the pH of the solution to 7.6, keeping the total volume of the solution to 10 ml, and pour the solution into a separatory funnel. Rinse the beaker with 5 ml of chloroform, adding it to the separatory funnel. Shake the two phases for 2 min, allow the phases to separate and collect the chloroform phase in a 10-ml measuring flask. Repeat the extraction with 5 ml of chloroform and transfer the organic phase into the measuring flask. Make up the volume to 10 ml, if necessary, with chloroform. Measure the absorbance either at 340 nm with a spectrophotometer or with a colorimeter and a blue filter. Calculate the amount of nickel from a calibration curve.

### *Procedure for the separation of nickel from other elements in cupronickel alloy and stainless steel*

Dissolve a sample of known weight (30 mg of stainless steel or 10 mg of cupronickel alloy) in 5 ml of aqua regia. Add 0.05 ml of *M* EDTA solution (disodium salt), ammonium acetate and/or sodium pyrophosphate, followed by 5 ml of 5% HINAP on 0.1 *M* sodium hydroxide. Adjust the pH to 8.0–9.8, transfer to a separatory funnel, dilute to 20 ml with water of pH 8.0–9.8, and extract twice with 5 ml of chloroform. Back-extract the nickel into 10 ml of *M* hydrochloric acid and determine by the dimethylglyoxime method.

## RESULTS AND DISCUSSION

The extraction of nickel(II) with HINAP into chloroform was studied over the pH range 2.5–10.0 (Table I). In the pH range 7.2–8.0, 99.6% of the nickel could be extracted. The extraction of nickel was rapid and independent of shaking time over the period 2–30 min. Two extractions sufficed to achieve quantitative extractions. Nickel could be re-extracted into the aqueous phase by shaking the chloroform solution with 10 ml of *M* hydrochloric acid. Chloroform proved to be the most efficient extractant (Table I).

The extractions of $Cr^{3+}$, $Mo^{6+}$, $W^{6+}$, $Re^{7+}$, $Os^{6+}$, $Ce^{3+}$, $Ir^{4+}$, $Sn^{4+}$, $Sb^{3+}$, $Ag^{+}$, $Cs^{+}$, $As^{3+}$, $As^{5+}$, $P^{5+}$, $Br^{-}$ and $Cl^{-}$ were less than 0.5%. $Mn^{2+}$, $Zn^{2+}$, $Ru^{3+}$, $Au^{3+}$ and $Hg^{2+}$ were extracted to the extent of 1–7.5%, while the extractions of iron(III) and cadmium(II) were 10% and 37.5%, respectively. The percentage extraction of co-

TABLE I

EXTRACTION COEFFICIENTS OF NICKEL(II)–ISONITROSOACETOPHENONE BETWEEN ORGANIC SOLVENTS AND AQUEOUS SOLUTION

(Conditions as in separation procedure)

| *pH* | *Solvent* | *Extraction coefficient (E)* | *% Ni extracted into organic solvent* |
|---|---|---|---|
| 2.5 | Chloroform | No extraction | — |
| 5.5 | Chloroform | 5.5 | 84.6 |
| 6.0 | Chloroform | 75.0 | 98.7 |
| 6.7 | Chloroform | 166.1 | 99.4 |
| 7.2 | Chloroform | 250.0 | 99.6 |
| 7.6 | Chloroform | 250.0 | 99.6 |
| 8.0 | Chloroform | 250.0 | 99.6 |
| 8.2 | Chloroform | 180.0 | 99.4 |
| 8.5 | Chloroform | 106.0 | 99.1 |
| 9.0 | Chloroform | 65.0 | 98.5 |
| 7.5 | Methyl ethyl ketone | 80.0 | 98.7 |
| 7.5 | Benzene | 54.0 | 98.2 |
| 7.5 | Isoamyl alcohol | 3.6 | 78.2 |
| 7.5 | Ethyl acetate | 0.18 | 15.3 |

balt(II), copper(II) and palladium(II) was better than 98%. However, cobalt was not back-extracted when the organic phase was stripped with *M* hydrochloric acid and its extraction in the presence of sodium sulfite did not exceed 0.2%. The extraction of copper was reduced to 0.6%, when nickel was extracted at pH 9.8 from aqueous solution containing $Na_2EDTA$ and 5 ml of 5% HINAP solution in 0.1 *M* sodium hydroxide. Potassium iodide suppressed the extractions of palladium(II) and cadmium(II) to 2.4% and 0.01% respectively. The extraction of manganese(II) was lowered to 0.1% in the presence of phosphate. Pyrophosphate reduced the extraction of iron(III) and zinc(II) to 0.54% and 0.27%, respectively. In the presence of potassium bromide, gold(III) and mercury(II) were reduced to 0.31% and 0.11%, respectively. Thiourea lowered the extraction of ruthenium(III) from 7.5 to 1.8%. Zirconium(IV) was kept in solution by sodium fluoride.

Cyanide, thiocyanate, citrate, tartrate and EDTA hindered the extraction of nickel into chloroform. The interference by citrate and tartrate was removed by

TABLE II

SEPARATION FACTORS FOR DIFFERENT ELEMENTS

(100 μg of Ni and 40 mg of HINAP in 10 ml of aqueous solution at pH 7.6; extraction with 10 ml of $CHCl_3$ for 10 min. Extraction coefficient of Ni = 250.0)

| *Separation factor* | *Ions* |
|---|---|
| $10^6$ | $Cl^-$, $Br^-$, $As^{3+}$, $Mo^{6+}$, $Re^{7+}$, $Cs^+$, $Cd^{2+}$ [a], $Sn^{4+}$ [a] |
| $10^5$ | $P^{5+}$, $Cr^{3+}$, $Sb^{3+}$, $Ce^{3+}$, $W^{6+}$, $Ir^{4+}$, $Mn^{2+}$ [a], $Co^{2+}$ [a], $Zr^{4+}$ [a], $Hg^{2+}$ [a] |
| $10^4$ | $Ag^+$, $Os^{6+}$, $Fe^{3+}$ [a], $Cu^{2+}$ [a,b], $Zn^{2+}$ [a], $Ru^{3+}$ [a], $Pd^{2+}$ [a], $Au^{3+}$ [a] |

[a] In presence of masking agent (see text).
[b] Extraction at pH 9.8.

extracting nickel in the presence of sodium molybdate. Cyanide, thiocyanate and EDTA did not interfere, when nickel was extracted from aqueous solution at pH 9.8 containing a large excess of HINAP. The extraction of nickel was not affected by the masking agents employed in the present investigation.

The values for the separation factor (*S*), defined by the relationship (extraction coefficient of nickel)/(extraction coefficient of other element), are given in Table II. These values show that nickel can be efficiently separated from many elements by extraction with HINAP into chloroform. This conclusion is confirmed by the results (Table III) obtained for the separation of nickel from other elements in synthetic mixtures, cupronickel alloy and stainless steel. The recovery of pure nickel was 100±2%.

TABLE III

SEPARATION OF NICKEL FROM OTHER ELEMENTS

| *Sample* | *Composition* | *Masking agents added* | *Ni recovered* | *Amount of other elements in recovered Ni* |
|---|---|---|---|---|
| Synthetic mixture | 25 µg $Ni^+$<br>10 mg Ag | — | 24.5 µg | Less than 0.7 µg |
| Synthetic mixture | 25 µg $Ni^+$<br>10 mg Co | — | 25.0 µg | Not detectable |
| Synthetic mixture | 25 µg $Ni^+$<br>10 mg Fe(III) | $Na_4P_2O_7$ | 24.5 µg | Less than 0.8 µg |
| Synthetic mixture | 25 µg $Ni^+$<br>10 mg Cu | $Na_2EDTA$ | 24.5 µg | Less than 0.7 µg[c] |
| Cupro-nickel alloy[a] | Ni 25%,<br>Cu 74.5%,<br>Mn and Zn traces | $Na_2EDTA$ | 25.54%[b] | Less than 0.7 µg Cu[c] |
| Stainless steel | 0.08% Ni,<br>18.8% Cr,<br>81.0% Fe | Ammonium acetate + $Na_4P_2O_7$ | 0.08%[b] | Cr not detectable,<br>Fe less than 0.8 µg |

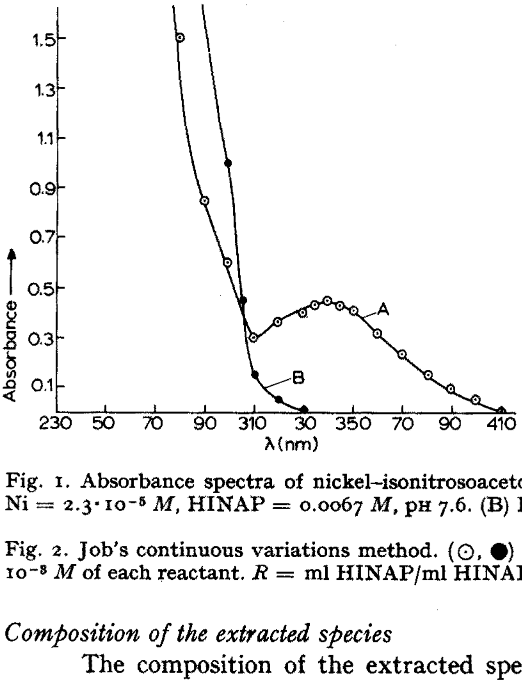
[a] Government of India Mint, Bombay.
[b] Duplicate analysis (nickel was determined by the dimethylglyoxime method and also by the isonitrosoacetophenone method).
[c] Extraction carried out at pH 9.8.

*Spectrophotometric determination of nickel*

The absorbance spectrum (Fig. 1) of the nickel–HINAP complex extracted into chloroform from aqueous solution at pH 7.6 exhibited a strong maximum at 340 nm, whereas an extract from a solution without nickel showed no absorbance at this wavelength. Beer's law was obeyed in the range 0.1–3.0 µg of nickel per ml. The molar absorptivity of the colored species at 340 nm was 22,000 evaluated on the basis of total nickel.

The effect of variation in the concentration of HINAP from 0.01% to 10% was studied, keeping other factors constant. Not more than 1 ml of 1% reagent solution was required for the extraction and the color development with 1–30 µg of nickel in 10 ml of chloroform. Excess of the reagent did not affect the intensity of the color in chloroform. The absorbance of the chloroform solution remained unchanged between 5 min and 5 h after extraction; it then decreased. Temperature variation from 10° to 35° caused no significant change in the absorbance. The following ions, when present in the milligram amounts noted in parentheses, did not interfere with the colorimetric determination: $V^{5+}$(20), $Cr^{3+}$(2), $Mn^{2+}$(20), $Zn^{2+}$(20), $Mo^{6+}$(20), $W^{6+}$(20), $Au^{3+}$(10), $Hg^{2+}$(10), $Pt^{4+}$(20), $Pt^{2+}$(20), $Ir^{4+}$(20), $U^{6+}$(20), $Ce^{3+}$(10), $La^{3+}$(20), $Os^{8+}$(20), $Se^{4+}$(20), $Pb^{2+}$(20), $As^{5+}$(20), $As^{3+}$(20), $Ca^{2+}$(20), $Sr^{2+}$(20), $Ba^{2+}$(20), $Mg^{2+}$(20), $Li^{+}$(20), $Re^{7+}$(20).

$P_2O_7^{4-}$, $SO_3^{2-}$(20), $NO_3^{-}$, $SO_4^{2-}$, $F^-$, $Cl^-$, $Br^-$, $I^-$, and urea did not interfere. The interference by the ions $Cd^{2+}$, $Pd^{2+}$, $Ru^{3+}$, $Fe^{3+}$, $Cu^{2+}$, $Zr^{4+}$, $CN^-$, $SCN^-$, citrate, tartrate and EDTA could be removed by extracting nickel in the presence of the appropriate reagents mentioned earlier under extraction experiments. Thorium(IV) and beryllium(II) could be masked with fluoride.



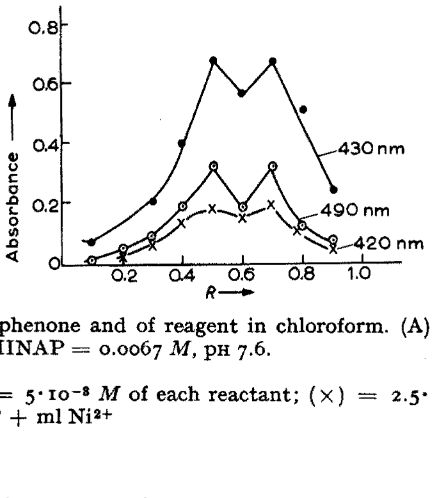


Fig. 1. Absorbance spectra of nickel–isonitrosoacetophenone and of reagent in chloroform. (A) Ni = $2.3 \cdot 10^{-5}$ *M*, HINAP = 0.0067 *M*, pH 7.6. (B) HINAP = 0.0067 *M*, pH 7.6.

Fig. 2. Job's continuous variations method. (⊙, ●) = $5 \cdot 10^{-3}$ *M* of each reactant; (×) = $2.5 \cdot 10^{-3}$ *M* of each reactant. $R$ = ml HINAP/ml HINAP + ml $Ni^{2+}$

*Composition of the extracted species*

The composition of the extracted species was studied by Job's continuous variations method[6,7]. With equimolar solutions, the Job's curves (Fig. 2) showed two maxima at ratios of Ni:HINAP equal to 1:1 and 1:2; these were independent of the concentrations of reactants and the wavelength of light employed. The reaction of nickel with HINAP may, therefore, be formulated as:

$$Ni^{2+} + HINAP \rightleftharpoons Ni(INAP)^{+} + H^{+}$$

$$Ni(INAP)^{+} + HINAP \rightleftharpoons Ni(INAP)_2 + H^{+}$$

The color extracted into chloroform from solutions containing nickel and excess of HINAP is attributed to a neutral complex $Ni(INAP)_2$.

*Precision and accuracy*

The precision and accuracy of the spectrophotometric method was tested by analyzing samples containing known amounts of nickel. With solutions containing

2.6 μg of nickel per ml, the average of 12 determinations was 2.62 μg ± 0.02 μg at the 95% confidence limit. The results (Table III) of analyses of cupronickel and stainless steel samples by the proposed method agree well with the accepted values.

In conclusion it may be stated that the extraction of nickel(II) with HINAP into chloroform permits rapid separation and spectrophotometric determinations of microgram amounts of nickel. Furthermore, as much as 3 mg of nickel can be extracted into 10 ml of chloroform.

This work was supported by a PL 480 grant, sponsored by the U.S. National Bureau of Standards.

## SUMMARY

A rapid separation and spectrophotometric determination of nickel based on extraction with HINAP into chloroform is described. Over the pH range 7.2–8.0, the percentage extraction of 1–3 mg of nickel is 99.6%. Interference of cyanide, thiocyanate, citrate, tartrate and EDTA is removed by pH control and by masking agents; masking agents are also used to prevent interferences of ions such as Co, Cu, Pd, Cd, Mn, Fe, Zn, Au, Hg and Zr. Chloroform is the most efficient solvent. The separation of nickel from other elements in cupronickel and steel is rapid and quantitative. The color of the chloroform extract measured at 340 nm obeys Beer's law in the range 0.1–3.0 μg of nickel per ml ($\varepsilon = 22{,}000$). The colored extracted species is a neutral complex, $Ni(INAP)_2$.

## RÉSUMÉ

On décrit une séparation rapide et un dosage spectrophotométrique du nickel. On effectue une extraction à l'aide d'isonitrosoacétophénone (HINAP) dans le chloroforme. Au pH 7.2–8.0 le pourcentage d'extraction de 1–3 mg de nickel est de 99.6% Ni. Les interférences des cyanures, thiocyanates, citrates, tartrates et EDTA sont éliminées par contrôle de pH et par des agents masquants; ceux-ci sont également utilisés pour masquer des ions tels que Co, Cu, Pd, Cd, Mn, Fe, Zn, Au, Hg et Zr. Le chloroforme est le solvant qui convient le mieux. La séparation du nickel d'avec d'autres éléments dans le cupronickel et l'acier est rapide et quantitative. Le complexe extrait correspond à la formule $Ni(INAP)_2$. On effectue les mesures à 340 nm; la loi de Beer s'applique de 0.1 à 3.0 μg Ni/ml ($\varepsilon = 22{,}000$).

## ZUSAMMENFASSUNG

Es wird eine schnelle Abtrennung und spektrophotometrische Bestimmung von Nickel durch Extraktion mit Isonitrosoacetophenon (HINAP) in Chloroform beschrieben. Im pH-Bereich 7.2–8.0 beträgt die prozentuale Extraktion von 1–3 mg Nickel 99.6%. Störungen durch Cyanid, Thiocyanat, Citrat, Tartrat und EDTA werden durch pH-Kontrolle und Maskierungsmittel unterbunden; zur Vermeidung von Störungen durch Co, Cu, Pd, Cd, Mn, Fe, Zn, Au, Hg und Zr werden ebenfalls Maskierungsmittel verwendet. Das wirkungsvollste Lösungsmittel ist Chloroform. Die Trennung von Nickel von anderen Elementen in Kupfernickel und Stahl ist

schnell und quantitativ. Die bei 340 nm gemessene Färbung des Chloroformextrakts gehorcht dem Beerschen Gesetz im Bereich 0.1–3.0 μg Nickel/ml ($\varepsilon = 22{,}000$). Die extrahierte gefärbte Verbindung ist ein neutraler Komplex, Ni(INAP)$_2$.

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# SPECTROSCOPIC STUDIES OF ORGANIC COMPOUNDS INTRODUCED INTO A RADIOFREQUENCY INDUCED PLASMA

## PART II. HYDROCARBONS

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Organic compounds were introduced into a radiofrequency plasma discharge. The ultraviolet emission spectra were examined and evaluated as a tool of analytical chemistry. Preliminary results have already been reported[1].

This section is devoted to the studies made on the ultraviolet–visible emission obtained when hydrocarbons were introduced into the radiofrequency plasma torch. The equipment used was the same that was reported previously[1].

Several hydrocarbons were introduced into the radiofrequency plasma and their emission spectra studied. The spectrum emitted from the tail flame by a typical hydrocarbon is shown in Fig. 1. Four molecular fragments were identified as sources to emission signals. These were elemental carbon, elemental hydrogen and the fragments, $C_2$ and CN. Carbon gave rise to a concentration-dependent signal coincident with the 2479 Å carbon line. Lines coincident with the Balmer hydrogen series were found, though often they were weak. The two strongest and best developed series were a series coincident with the Swan $C_2$ system (5635, 5585, 5540, 5165, 5129, 4737, 4715, and 4697 Å) and (CN) cyanogen system (4216, 4127, 3883, and 3586 Å). The

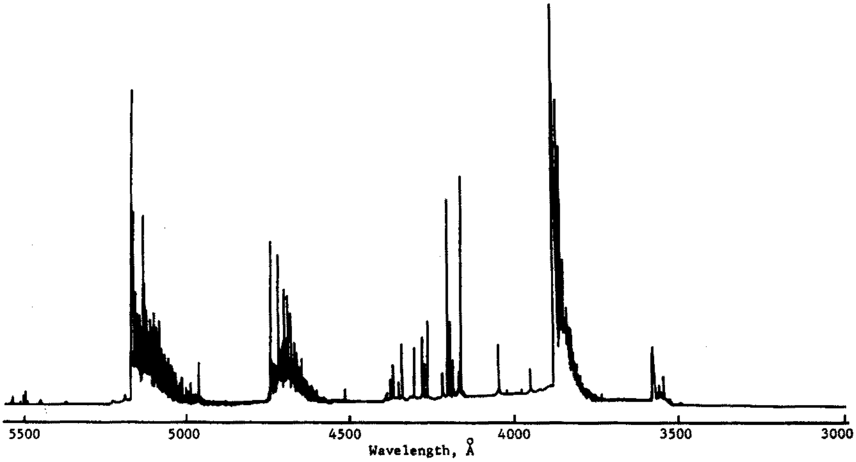

Fig. 1. Spectrum of typical hydrocarbon.

other lines and bands expected were also present. The spectrum obtained from the plasma when no organic sample was being introduced was also recorded and this represents the spectral background. The background emission was mainly argon lines, OH bands, and a continuum. The cyanogen system was presumably generated by the carbon in the hydrocarbon and the nitrogen from impurities present.

## EXPERIMENTAL

### *Point of injection*

Samples can be injected into the plasma torch in different places: (*a*) the coolant stream, (*b*) the center stream, and (*c*) the tail flame via a side arm. As can be seen from examination of Table I, the part of introduction of the sample had little effect on the intensity of the emission observed from the tail flame. When the emission signal was observed from the core or secondary region the cyanogen band system and atomic carbon line were stronger relative to the $C_2$ band system than in the tail flame.

TABLE I

EFFECT OF POINT OF SAMPLE INTRODUCTION AND ADDED GAS ON SEVERAL SPECTRAL FEATURES FOR *n*-HEPTANE

| *Carrier gas (0.2 l/min)* | *Point of injection* | *Intensity of emission signals* | | | | | |
|---|---|---|---|---|---|---|---|
| | | *2478 Å (C)* | *3089 Å (OH)* | *3371 Å ($N_2$)* | *3883 Å (CN)* | *4201 Å*[a] *(Ar)* | *5165 Å ($C_2$)* |
| Argon | Blank | 0 | 0 | 0 | 0 | 2.4 | 0 |
| | Center | 0.4 | 0 | 0 | 10.3 | 2.4 | 11.5 |
| | Coolant | 0.2 | 0 | 0 | 10.4 | 2.4 | 11.2 |
| | Tail flame | 0.1 | 0.2 | 0 | 10.5 | 2.5 | 11.5 |
| Helium | Blank | 0 | 6.2 | 0 | 0 | 1.9 | 0 |
| | Center | 0.5 | 0.7 | 0 | 10.2 | 1.9 | 10.1 |
| | Coolant | 0.4 | 0.6 | 0 | 10.0 | 1.9 | 10.2 |
| | Tail flame | 0.1 | 0.8 | 0 | 10.3 | 1.9 | 10.2 |
| Nitrogen | Blank | 0.1 | 0 | 0.6 | 7.4 | 3.0 | 0 |
| | Center | 0.3 | 0 | 0.9 | 17.0 | 5.8 | 0.4 |
| | Coolant | 0.2 | 0 | 0.8 | 15.0 | 5.6 | 0.6 |
| | Tail flame | 0.1 | 0 | 0.7 | 15.0 | 5.6 | 0.9 |
| Oxygen | Blank | 0 | 0.3 | 0 | 0 | 2.8 | 0 |
| | Center | 0.2 | 0.7 | 0 | 10.0 | 2.2 | 8.4 |
| | Coolant | 0.1 | 0.6 | 0 | 11.0 | 2.2 | 9.6 |
| | Coolant[b] | 0.1 | 9.0 | 0 | 0 | 2.8 | 0 |
| | Tail flame | 0.1 | 3.5 | 0 | 10.1 | 2.8 | 9.6 |
| Air | Blank | 0 | 0.1 | 0.2 | 0.4 | 2.4 | 0 |
| | Center | 0.2 | 0.1 | 0.3 | 6.8 | 2.7 | 2.5 |
| | Coolant | 0.1 | 0.1 | 0.4 | 7.4 | 2.8 | 2.8 |
| | Tail flame | 0 | 0.1 | 0.4 | 7.6 | 2.9 | 2.7 |

[a] Possibly overlaps 4216 Å CN band.

[b] Concentration of oxygen increased 4 times.

### *Carrier gas*

Various gases were added to the argon stream along with the sample in order to modify the plasma. Nitrogen, oxygen and air representing a reactive carrier gas, and helium which under the conditions used appeared to be essentially a diluent, were examined (Table I). Along the same line, the coolant stream of argon was replaced with air, nitrogen or helium. With the added carrier gases (Table I), the addition of nitrogen caused an increase in cyanogen band intensities and a decrease in intensity of the carbon line and $C_2$ band intensities thus indicating CN formation by reaction. The decrease in intensity of the CN and $C_2$ bands when oxygen was added probably indicates oxidation of most of the available carbon to carbon monoxide or dioxide. It should be noted that these emission bands were not observed when carbon monoxide or carbon dioxide was introduced into the plasma. At higher oxygen flow rates only the OH band intensity reflected a dependence on hydrocarbon concentration. The addition of helium generally reduced the intensity of all the spectrum except the OH bands and the continuum. The use of these other carrier gases as coolant gases gave similar results with a much higher continuum level and CN and $C_2$ bands were observed only at high hydrocarbon concentrations except in the case of nitrogen where the CN bands broadened to such an extent that it was difficult to determine where the continuum and CN bands meet. In addition, the signal-to-noise ratio decreases as the concentration of hydrocarbon increases.

*The effect of radiofrequency power on the emission spectra*

The emission intensity of several bands and lines were studied as a function

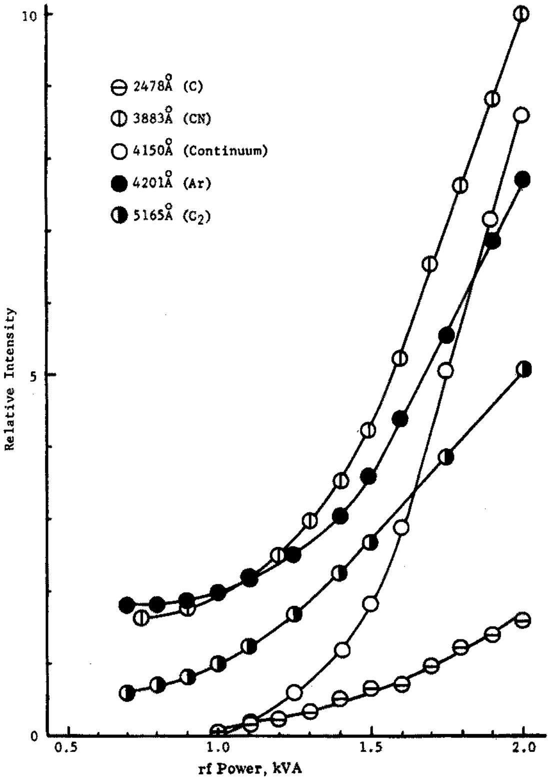

Fig. 2. Effect of r.f. power on emission intensity for *n*-octane.

of radiofrequency power. All of the bands in the cyanogen (CN) and $C_2$ band system increased in intensity with increasing radiofrequency power, but so did the continuum, and the line-to-background ratio was reduced rapidly at power levels above 1.4 kVA. The intensity of the carbon line increased more slowly with increasing power levels at an approximately constant line to background ratio. These data are shown in Fig. 2. It is interesting to note that there was a rapid increase in the slope of the emission intensity for CN, $C_2$, and argon as the power reaches the 1.3–1.5 kVA region. This probably denotes a change in the distribution of the argon plasma proper and its circulation pattern rather than any mechanistic change.

*Plasma profile of emission intensity*

In the plasma torch, as in the case of flames, the distribution of emitting species as a function of height can shed some light on the processes involved. This is shown in Fig. 3 for several spectral features. The continuum and argon emission appears to have this origin in the core and then decrease in intensity with increasing height. The carbon line, and $C_2$ and CN band intensity appear to arise in both the core and secondary region. All of the species appear to decrease drastically at the interface between the core and secondary region which probably reflects a decrease in argon ion concentration. The intensities then level off throughout the tail flame

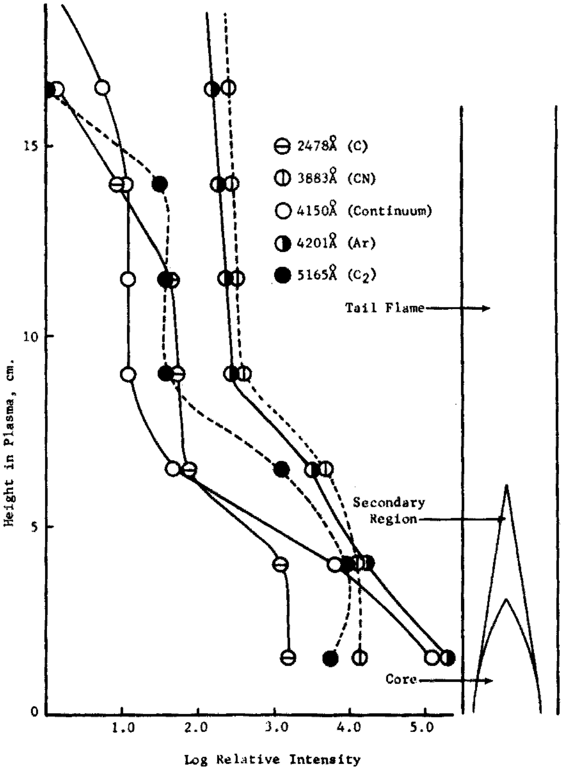

Fig. 3. Plasma profile of emission for *n*-octane.

region, with the carbon line and $C_2$ bands disappearing rapidly just beyond the coolant tube, and argon lines and CN bands persisting a short distance into the after flame.

## DISCUSSION

### *The effect of number of carbons on the emission spectra*

All the compounds containing carbon which were investigated gave bands characteristic of $C_2$ and CN. It has been suggested that the CN bands occur owing to reaction with nitrogen which originates either in the argon support gas or organic compounds. The presence of $C_2$ bands from compounds containing only one carbon such as methane, carbon tetrachloride, methyl iodide, chloroform, carbon monoxide, and carbon dioxide indicates that there is, to a limited extent at least, some $C_2$ produced by reaction. In order to spread some light on this problem methane, ethane, and propane were run under identical conditions. The intensities of $C_2$, CN bands and the 2479 Å carbon line are shown in Fig. 4. It was interesting to note that bands from methane, ethane, and propane appeared suddenly when a threshold concentration was reached. For example, with the instrument set to its most sensitive position and taking the noise level as the unit of intensity, the lowest intensity reading

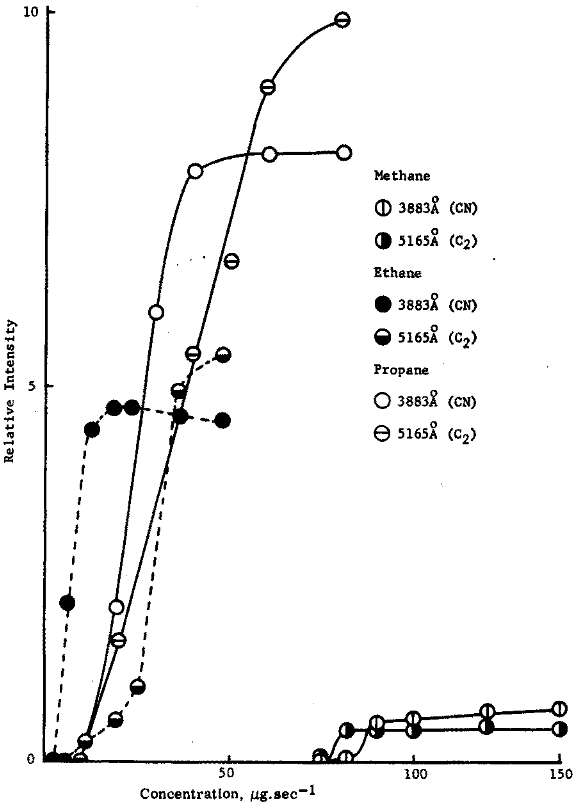

Fig. 4. Effect of concentration and number of carbon atoms on emission intensity.

obtainable for a $C_2$ band in propane was of the order of $10^5$ times greater than the noise level. Thus at concentrations greater than a certain threshold point a change in concentration of less than 1% produced a change in intensity of at least $10^5$. This threshold phenomenon appeared to indicate that the spectra were generated by molecular collision between sample molecules. The levelling of the curves at higher concentrations appeared to be due to formation of elemental carbon in increasing amounts.

### *Available methods of energy transfer*

The basic plasma itself consists of an equal mixture of positive argon ions and electrons and in addition a large concentration of excited neutral atoms. The energy available from the excited neutral argon atoms is up to 14.5 eV. Also associated with the plasma is a high thermal temperature. The tip of the secondary region melts quartz, various steels and nickel, but not tungsten. With this experimental equipment, placing objects into the core also places them in the field of the coil so that metallic objects, which couple much better than the plasma, are heated directly by the fields. It is of passing interest to note that tungsten placed in the core within the coil showed evidence that the metal had flowed and was heated to a white color at power levels of the order of 2 kVA. The plasma produces a large quantity of light; protection for the eyes was necessary at all times. In addition to those argon lines emitted in the visible and near-ultraviolet region, line spectra should also be produced in the vacuum ultraviolet since transitions from the metastable state to the ground state (first resonance argon lines 1067 and 1048 Å) would produce lines in these regions. Further to the above-mentioned characteristics there is one further associated with the plasma but not actually part of the plasma discharge and that is the hot quartz coolant tube which contains the plasma. In the neighbourhood of the core and coil, the temperature of the coolant is probably of the order of 400–500°. The tube does not glow significantly but will melt borosilicate glass on contact.

Considering all of these features of the plasma discharge, there are several available methods of energy transfer available. The simplest would be direct kinetic energy transfer by collision, transfer of greater energy by collision with an ion, possible energy transfer by absorption of infrared radiation, possible absorption of high energy radiation or photolysis, direct interaction with the radiofrequency field or reaction on the surface of the quartz tube which could easily be catalyzed by the hot tube.

### *Emission bands not found*

Several bands which one might expect to observe were not found. The CH bands which are most prevalent when hydrocarbons are introduced into flames were not found under any circumstances in the plasma discharge. These bands were not found even when air or oxygen was introduced along with the organic sample. CO and $CO_2$ bands were not found from this source although their absence was not surprising as they are almost exclusively found in low pressure discharges. However, owing to the effect of added oxygen or air on the $C_2$ and CN band system intensities and carbon line intensity, the existence of these radicals in the plasma must be postulated.

No emission from larger molecules like water or aromatics like benzene, was observed either by emission or absorption. Even addition of large quantities of nitrogen with the sample and injection into the tail flame yielded only the emission mentioned before.

## SUMMARY

The spectra of hydrocarbons introduced into a plasma torch have been studied. The background of the plasma torch is much simpler than that of a flame and higher temperatures are obtained. The plasma has fair sensitivity towards carbon: *ca.* 2 $\mu$g sec$^{-1}$ for CN (3883 Å) and 10 $\mu$g sec$^{-1}$ for $C_2$ (5165 Å). The torch may be operated with an "inert" oxidizing or reducing atmosphere. The discharge almost completely fragments organic compounds and thus the method is not suitable for the production of the molecular spectra of larger molecules.

## RÉSUMÉ

On a examiné les spectres d'hydrocarbures introduits dans une torche de plasma. On peut obtenir ainsi des températures plus élevées. Le plasma présente une bonne sensibilité pour le carbone: env. 2 $\mu$g sec$^{-1}$ pour CN (3883 Å) et 10 $\mu$g sec$^{-1}$ pour $C_2$ (5165 Å). On peut opérer en atmosphère inerte, oxydante ou réductrice. On ne peut pas envisager la production de spectres de grosses molecules.

## ZUSAMMENFASSUNG

Die Spektren von Kohlenwasserstoffen in einem Plasmabrenner sind untersucht worden. Der Untergrund des Plasmabrenners ist viel einfacher als der einer Flamme, und es werden höhere Temperaturen erhalten. Das Plasma zeigt eine gute Empfindlichkeit gegenüber Kohlenstoff: *ca.* 2 $\mu$g sec$^{-1}$ für CN (3883 Å) und 10 $\mu$g sec$^{-1}$ für $C_2$ (5165 Å). Der Brenner kann mit einer "inerten" oxydierenden oder reduzierenden Atmosphäre betrieben werden. Die Entladung zerstört organische Verbindungen fast vollständig und ist daher in dieser Form für die Erzeugung von Molekülspektren und Spektren grösserer Moleküle nicht geeignet.

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# THE SUBSTOICHIOMETRIC DETERMINATION OF CHROMIUM IN ALUMINIUM ALLOYS

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The determination of chromium by neutron-activation analysis has been described by several authors. JERVIS AND MACKINTOSH[1] used activation followed by $\gamma$-spectrometry to determine chromium in high-purity aluminium, and chromium has also been determined in aluminium alloys and silicon[2], and in magnesium[3]. SMALES[4] suggested a procedure in which the metal was extracted as the blue peroxo complex for determination of chromium in sodium–potassium alloys. The determination of chromium in various metals[5] and of several impurities including chromium in zirconium and its alloys[6] has also been described. Granitic rocks[7,8] and stainless steel micro-spheres[9] have also been analysed for chromium by neutron activation. In these later methods, chromium-51 was separated in a radiochemically pure state with an inactive carrier, and the chemical yield was determined. The separation is tedious, and the extraction into ether of the blue peroxo compound is critically dependent on temperature, the pH of the aqueous phase and the concentration of hydrogen peroxide. The application of the substoichiometric principle[10] can lead to simpler and quicker techniques of both activation analysis and isotope dilution analysis. RŮŽIČKA AND STARY[11] described methods for the determination of many metals based on this principle, and suggested the possibility of determining chromium by the formation of the stable chromium(III)–EDTA complex. ALIMARIN AND PEREZHOGIN[12], who studied the extraction of large anions with the tetraphenylarsonium cation, suggested the possibility of the substoichiometric separation of chromium(VI) by extraction of its tetraphenylarsonium salt into chloroform or 1,2-dichloroethane. HALA *et al.*[13] also reported good extraction of chromium(VI) with triphenyltetrazolium, tetraphenylarsonium and *n*-propyl-triphenylphosphonium cations into chloroform.

In the work described here the substoichiometric determination of chromium with EDTA was investigated. Results were unsatisfactory because many other metals interfered, and the ion-exchange separation of unreacted chromium(III) ion from chromium(III)–EDTA complex proved extremely difficult. Complexes formed from chelating reagents used for colorimetric determination of chromium, such as diphenylcarbazide and 2-thenoyltrifluoroacetone, could not be satisfactorily extracted into organic solvents. However, extraction of chromium(VI) with a substoichiometric amount of tetraphenylarsonium chloride into 1,2-dichloroethane was found to be reproducible, making possible the determination of chromium both by neutron activation and isotope dilution. Chromium(VI) was produced by oxidation of chromium(III) with cerium(IV) sulphate[14]. The method, which is simple, accurate and quick, was used for the analysis of chromium in aluminium alloys.

## EXPERIMENTAL

### *Reagents*

*Chromium carrier solution.* A 0.1 *M* stock solution of chromium(III) chloride was prepared by dissolving 0.52 g of 99.9% electrolytic chromium in 5 ml of twice-distilled hydrochloric acid and then diluting to 100 ml with distilled water.

*Active chromium solution.* A 10-ml aliquot of 0.1 *M* chromium(III) chloride solution was labelled with 20 $\mu$Ci chromium-51 and was diluted to 100 ml with distilled water.

*Hydrochloric acid.* Hydrochloric acid (s.g. 1.18) was distilled twice from all-glass apparatus.

*Cerium(IV) solution.* An approximately 10% solution of cerium(IV) sulphate was prepared by dissolving technical-grade cerium(IV) sulphate in 3 *M* sulphuric acid and decanting the supernatant liquid from the insoluble residue.

*Tetraphenylarsonium chloride solution.* A 0.005 *M* solution of tetraphenylarsonium chloride was prepared by dissolving an appropriate amount of the A.R. grade salt in distilled water. Other compounds used to study the interferences were: $NH_4Fe(SO_4)_2 \cdot 12H_2O$, $CuSO_4 \cdot 5H_2O$, $CoSO_4 \cdot 7H_2O$, $NiSO_4 \cdot 6H_2O$, $ZnSO_4 \cdot 7H_2O$, $MnSO_4 \cdot 4H_2O$, $Al_2(SO_4)_3 \cdot 16H_2O$, $TiCl_4$, $ZrO_2$, $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, $NH_4VO_3$, $Na_2WO_4 \cdot 2H_2O$, NaBr, KI, HCl, $HNO_3$, $HClO_4$, $H_3PO_4$ and $H_2SO_4$.

### *Standard and test samples for neutron irradiation*

Electrolytic chromium (99.9% pure) was used as the standard. The metal was cut into small pieces, washed with dilute hydrochloric acid to remove any surface contamination introduced during cutting, washed with distilled water, and dried. The metal (30–40 mg) was sealed in a thoroughly cleaned and dried silica tube. Test samples of B.C.S. No. 300 aluminium alloy, No. 263/1 5% magnesium–aluminium alloy (Bureau of Analysed Samples Limited) and sheet-aluminium (*ca.* 100 mg) were sealed similarly in different silica tubes and irradiated in the same aluminium can.

### *Irradiation conditions and counting equipment*

The standard and samples were irradiated in the Universities Research Reactor, Risley, Warrington at a neutron flux of about $3.6 \cdot 10^{12}$ n cm$^{-2}$ sec$^{-1}$ for 7–8 h.

The $\gamma$-emission from chromium-51 was counted by means of a Panax scintillation counter and a P7102 scaler. Experiments were designed to give at least 10,000 counts so that the standard deviation did not exceed 1%.

### *Preliminary experiments on the reproducibility of substoichiometric extraction of chromium*

*Extraction of chromium from solutions of different concentration with the same substoichiometric amount of tetraphenylarsonium chloride reagent.* Four different aliquots (1.0, 1.5, 2.0 and 2.5 ml) of aqueous 0.01 *M* chromium(III) chloride, labelled with $^{51}$Cr, were measured into 25-ml conical flasks. A 10% solution of cerium(IV) sulphate (2.0 ml) was added to each, and the mixture was boiled for about 10 min. The cold solution was made 1 *M* in hydrochloric acid[14] and was shaken with 10 ml of isobutyl methyl ketone for 3 min. It was found that in the absence of hydrochloric acid the extraction was much less efficient. In each case, the organic layer was washed three times with *M* hydrochloric acid. The dichromate was back-extracted by shaking three times with water, the total volume of the aqueous extract being kept to about 10 ml. The aqueous extract, in each case, was transferred to another separating funnel and to it was added 0.5 ml of *M* sulphuric acid followed by 1.0 ml of 0.005 *M* tetraphenylarsonium chloride solution. The aqueous solution was shaken for 1 min with 6.0 ml of 1,2-dichloroethane. A count-rate was taken on 5.0 ml of the organic extract (Table I).

TABLE I

EXTRACTION OF DIFFERENT CONCENTRATIONS OF CHROMIUM WITH THE SAME SUBSTOICHIOMETRIC AMOUNT OF TETRAPHENYLARSONIUM CHLORIDE REAGENT

| | | | | |
|---|---|---|---|---|
| Labelled 0.01 *M* $CrCl_3$ solution (ml) | 1.0 | 1.5 | 2.0 | 2.5 |
| Activity of Cr isolated (counts/min) | 1592 | 1545 | 1561 | 1555 |

*Extraction of chromium from solutions of different concentration with different substoichiometric amounts of tetraphenylarsonium chloride reagent.* A second identical series of chromium(III) solutions were oxidised and extracted with isobutyl methyl ketone, and the dichromate was back-extracted as described above. To the aqueous solution was added 0.5 ml of *M* sulphuric acid followed by 1.0, 1.5, 2.0 and 2.5 ml of 0.005 *M* tetraphenylarsonium chloride solution, respectively. Each solution was shaken for 1 min with 6.0 ml of 1,2-dichloroethane. In each case, the count-rate was taken on 5.0 ml of the organic layer (Table II).

TABLE II

EXTRACTION OF CHROMIUM FROM SOLUTIONS OF DIFFERENT CONCENTRATION WITH DIFFERENT SUBSTOICHIOMETRIC AMOUNTS OF TETRAPHENYLARSONIUM CHLORIDE REAGENT

| | | | | |
|---|---|---|---|---|
| Labelled 0.01 *M* $CrCl_3$ solution (ml) | 1.0 | 1.5 | 2.0 | 2.5 |
| 0.005 *M* $(C_6H_5)_4AsCl$ solution added (ml) | 1.0 | 1.5 | 2.0 | 2.5 |
| Activity of Cr isolated (counts/min) | 1594 | 2401 | 3170 | 3960 |
| Activity corresponding to 1.0 ml of reagent solution | 1594 | 1600 | 1585 | 1584 |

*Extraction of chromium from solutions of increasing concentration with a fixed volume of tetraphenylarsonium chloride solution.* Aqueous 0.01 *M* $CrCl_3$ (10.0 ml), labelled with $^{51}Cr$, was oxidised by boiling with 10.0 ml of 10% cerium(IV) solution for 10 min, and the dichromate formed was extracted into isobutyl methyl ketone and back-extracted as described above. The aqueous extract was diluted to 50.0 ml in a standard flask; 9 solutions were made from it containing 1.0–9.0 ml, diluted to 10.0 ml with water. Aqueous *M* sulphuric acid (0.5 ml) was added, followed by 2.0 ml of 0.005 *M* tetraphenylarsonium chloride solution. The resulting solutions were shaken with 6.0 ml of 1,2-dichloroethane for 1 min. The count-rates from 5.0 ml of the organic extract were measured (Table III). A plot of activity against the volume of 0.002 *M* chromium(VI) is shown in Fig. 1.

TABLE III

EXTRACTION OF CHROMIUM FROM SOLUTIONS OF INCREASING CONCENTRATION WITH A FIXED VOLUME OF TETRAPHENYLARSONIUM CHLORIDE REAGENT

| Labelled *ca.* 0.002 *M* $CrCl_3$ solution (ml) | 1.0 | 2.0 | 3.0 | 4.0 | 5.0 | 6.0 | 7.0 | 8.0 | 9.0 |
|---|---|---|---|---|---|---|---|---|---|
| Activity of Cr isolated (counts/min) | 576 | 1111 | 1678 | 2231 | 2794 | 3082 | 3107 | 3087 | 3107 |

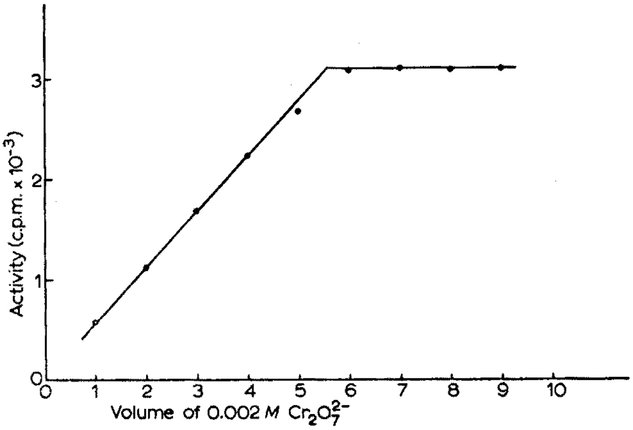

Fig. 1. Reproducibility of substoichiometric extraction of chromium(VI) with tetraphenylarsonium chloride into 1,2-dichloroethane.

### *Preliminary experiments on isotope dilution analysis*

To four solutions, each containing 2.0 ml of 0.01 *M* chromium(III) chloride, labelled with $^{51}Cr$, were added respectively 0.0, 1.0, 2.0 and 4.0 ml of inactive 0.01 *M* chromium(III) chloride solution. Chromium(III) in each solution was oxidised and extracted with isobutyl methyl ketone, and chromium(VI) was back-extracted into water as described above. To each aqueous extract was then added 0.5 ml of *M* sulphuric acid followed by 2.0 ml of 0.005 *M* tetraphenylarsonium chloride solution, and chromium(VI) was extracted and counted as indicated above (Table IV).

TABLE IV

EFFECT OF CARRIER ON EXTRACTION OF CHROMIUM

(2.0 ml of $^{51}Cr$-labelled 0.01 *M* $CrCl_3$ solution was taken in each case)

| 0.01 *M* inactive $CrCl_3$ solution added (ml) | 0.0 | 1.0 | 2.0 | 4.0 |
|---|---|---|---|---|
| Activity of Cr isolated (counts/min) | 2634 | 1728 | 1323 | 853 |

### *Interferences of other ions in the extraction of chromium(VI)*

The effect of various cations was examined for $^{51}Cr$-labelled 0.01 *M* chromium (III) chloride solutions to which an equal volume of 0.1 *M* solutions of $Fe^{3+}$, $Cu^{2+}$, $Co^{2+}$, $Ni^{2+}$, $Al^{3+}$, $Zn^{2+}$, $Mn^{2+}$, $Ti^{4+}$, $Zr^{4+}$ or $V^{5+}$ was added. The solutions were oxidised, extracted and back-extracted before the tetraphenylarsonium chloride extraction, exactly as described above. In no case was the count-rate influenced by the presence of the metal other than chromium, when results were compared with those obtained for pure chromium(III) solutions. When the metal ion other than chromium was added after the oxidation to chromate, there was again no effect on the final count-rates.

The effect of several anions was investigated as follows. A 10.0-ml aliquot of $^{51}$Cr-labelled 0.01 *M* chromium(III) chloride solution was oxidised, extracted and then back-extracted as described above, and the final aqueous solution was diluted to 100.0 ml. To 5.0-ml samples was added 1.0 ml of a 0.05 *M* solution of HCl, NaBr, KI, $HClO_4$, $H_3PO_4$, $HNO_3$, $H_2SO_4$, $NH_4VO_3$, $(NH_4)_2MoO_4$ or $Na_2WO_4$, followed by 0.5 ml of 0.005 *M* $(C_6H_5)_4AsCl$ solution. The mixture was extracted with 1,2-dichloroethane, and the extract counted as described above. The results were compared with that from a 5.0-ml sample to which no interfering anion had been added. The ions $I^-$, $ClO_4^-$, $NO_3^-$, $MoO_4^{2-}$, and $WO_4^{2-}$ interfered considerably, $Br^-$ and $PO_4^{3-}$ interfered moderately, and $VO_3^-$, $Cl^-$ and $SO_4^{2-}$ did not interfere (Table V).

TABLE V

INTERFERENCE OF ANIONS IN THE SUBSTOICHIOMETRIC SEPARATION OF CHROMIUM(VI) WITH TETRAPHENYLARSONIUM CHLORIDE

(Molar ratio of anion/Cr(VI) was 10 in each case)

| *Anionic species added* | *Activity in the extract (counts/min)* | *% Interference* |
|---|---|---|
| None | 1853 | — |
| $Cl^-$ | 1897 | — |
| $Br^-$ | 1578 | 15 |
| $I^-$ | 211 | 88 |
| $ClO_4^{-a}$ | 22 | 99 |
| $PO_4^{3-}$ | 1696 | 8 |
| $NO_3^-$ | 1025 | 45 |
| $SO_4^{2-}$ | 1875 | — |
| $VO_3^-$ | 1847 | — |
| $MoO_4^{2-a}$ | 5 | 100 |
| $WO_4^{2-a}$ | 116 | 94 |

[a] The addition of $(C_6H_5)_4AsCl$ reagent produced a precipitate which disappeared on shaking with 1,2-dichloroethane.

### *The substoichiometric determination of chromium by activation analysis and by isotope dilution*

*Activation analysis.* Accurately weighed, irradiated chromium (about 30 mg) was dissolved in 3.0 ml of twice-distilled hydrochloric acid and the solution was diluted to 100.0 ml with distilled water. A 10.0-ml aliquot of this solution was further diluted to 100.0 ml with distilled water for use as standard in activation analysis.

Different weighed samples of irradiated alloys (B.C.S. No. 300 and No.263/1) and of sheet-aluminium were dissolved in 8 *M* hydrochloric acid in 25-ml conical flasks. Each solution was evaporated to fumes with 2.0 ml of 50% aqueous sulphuric acid. To the cold solutions, 1.0 ml of inactive 0.01 *M* chromium(III) chloride solution was added, followed by 2.0 ml of 10% cerium(IV) sulphate solution. Solutions were boiled for about 10 min and then cooled. The cold solutions were made 1 *M* in hydro-

TABLE VI

SUBSTOICHIOMETRIC DETERMINATION OF CHROMIUM IN ALUMINIUM AND ALUMINIUM-BASED ALLOYS

| *Samples* | *Weight of sample (mg)* | *Activity from sample (counts/min)*[a] | *Activity from standard (counts/min)*[a] | *Amount of Cr in standard (mg)* | *Cr found (%)* | *Range of certified analyses(%)* |
|---|---|---|---|---|---|---|
| *By neutron activation analysis* | | | | | | |
| B.C.S. No. 300[b] | 15.6 | 3431 | 4044 | 0.0305 | 0.166 | |
| | 20.7 | 4448 | | | 0.162 | 0.14–0.16 |
| | 28.2 | 6063 | | | 0.162 | |
| B.C.S. No. 263/1[c] | 11.9 | 3907 | 3939 | 0.0305 | 0.254 | |
| | 14.4 | 4656 | | | 0.250 | 0.23–0.25 |
| | 15.5 | 4841 | | | 0.242 | |
| Al metal | 57.2 | 956 | 3558 | 0.0305 | 0.0143 | |
| | 72.4 | 1191 | | | 0.0141 | |
| | 113.5 | 1840 | | | 0.0139 | |
| *By isotope dilution analysis* | | | | | | |
| B.C.S. No. 300[b] | 57.6 | 2815 | 3470 | 0.38 | 0.154 | |
| | 82.9 | 2557 | | | 0.164 | 0.14–0.16 |
| | 111.0 | 2365 | | | 0.160 | |
| B.C.S. No. 263/1[c] | 62.3 | 2367 | 3300 | 0.38 | 0.240 | |
| | 85.5 | 2161 | | | 0.234 | 0.23–0.25 |
| | 105.9 | 1937 | | | 0.252 | |
| Al metal | 69.2 | 3165 | 3251 | 0.38 | 0.0149 | |
| | 104.7 | 3128 | | | 0.0142 | |
| | 118.6 | 3106 | | | 0.0149 | |

[a] The activity expressed above is the average of three different counts on the sample corrected for background.

[b] This aluminium alloy contains Cu 1.28%, Mg 2.76%, Zn 5.98%, Si 0.16%, Fe 0.30%, Mn 0.41%, Cr 0.15%, Ti 0.15%.

[c] This 5% magnesium–aluminium alloy contains Mg 4.92%, Cu 0.09%, Si 0.12%, Fe 0.35%, Mn 0.36%, Ti 0.038%, Zn 0.05%, Cr 0.24%, Be 0.004%.

chloric acid and shaken with 10.0 ml of isobutyl methyl ketone for 3 min. The organic layer in each case was washed with *M* hydrochloric acid, and the dichromate was back-extracted by shaking the organic layer three times with water, the total volume of the aqueous extract being kept to 10.0 ml. To each solution, 0.5 ml of *M* sulphuric acid was added followed by 1.0 ml of 0.005 *M* tetraphenylarsonium chloride solution. Solutions were shaken for 1 min with 6.0 ml of 1,2-dichloroethane and the count-rates from 5.0 ml of the organic extracts were measured.

The standard solution (1.0 ml) together with 1.0 ml of inactive chromium(III) chloride was treated in exactly the same way. The count-rates from 5.0 ml of the organic extracts were measured (Table VI).

*Isotope dilution analysis.* Weighed samples of the alloys and aluminium metal were dissolved in 8 *M* hydrochloric acid in 25-ml conical flasks and then evaporated to fumes with 2.0 ml of 50% sulphuric acid. An appropriate aliquot (1.0 ml) of standard active chromium solution (containing 0.38 mg/ml) was added to each, followed by 2.0 ml of 10% cerium(IV) sulphate solution, and the solutions were boiled for about 10 min. The cold solutions were made 1 *M* in hydrochloric acid, and chromium was extracted with isobutyl methyl ketone followed by back-extraction to water, as before. The aqueous extract, in each case, was treated with 0.5 ml of *M* sulphuric acid followed by 0.5 ml of 0.005 *M* tetraphenylarsonium chloride solution. The mixtures were shaken with 6.0 ml of 1,2-dichloroethane, and the count-rate from 5.0 ml of the organic extract was measured.

The standard active chromium chloride solution (1.0 ml) was treated in exactly the same way, and the count-rate from 5.0 ml of the organic extract was measured (Table VI).

## DISCUSSION

Chromium in small amounts in metals and alloys can be determined both by neutron activation analysis and by isotope dilution analysis by measuring the $\gamma$-emission from chromium-51, produced by the neutron irradiation of chromium. Chromium can be readily separated from most other metals in a radiochemically pure form by oxidation with cerium(IV) followed by extraction with isobutyl methyl ketone from 1 *M* hydrochloric acid solution. Although hydrochloric acid had to be added to the solution after the oxidation process in order to obtain efficient extraction, a large volume of the acid could not be tolerated during the oxidation because it was oxidised to chlorine and cerium(IV) was lost. For this reason the solutions of aluminium alloys in hydrochloric acid had to be fumed with sulphuric acid. The function of the hydrochloric acid which had to be added to the cold solution after oxidation of Cr(III) to Cr(VI) has not been established, and in view of the relative redox potentials it would be expected to be entirely oxidised to chlorine. Nevertheless, its presence raised the efficiency of extraction into the ketone from 30% to 93%.

Reproducible extraction of 97% of chromium with tetraphenylarsonium chloride into 1,2-dichloroethane was obtained. The presence of sulphate and chloride ions in 10 times the concentration of dichromate (0.001 *M*) did not interfere, but with fairly large concentrations of these ions (above *M*) and with much longer shaking times, the percentage extraction of chromium(VI) fell. Under the same conditions chloroform extracted only 94% of chromium as tetraphenylarsonium dichromate. A shaking time of 1 min with 1,2-dichloroethane as solvent was found to be suitable for extraction of chromium(VI) from an approximately 0.05 *M* sulphuric acid solution.

Of ten metallic ions used, none affected the extraction of $(C_6H_5)_4As$–Cr(VI) ion pairs. Although $Br^-$, $I^-$, $ClO_4^-$, $PO_4^{3-}$, $NO_3^-$, $MoO_4^{2-}$ and $WO_4^{2-}$ interfered, these interferences can be removed through the oxidation and solvent extraction of chromium as described.

With the proposed method, four or five samples can be analysed for chromium, once the irradiation is done, in 3 h. The precision of the neutron activation method is marginally better than that of the isotope dilution method. The activation method should allow determination of chromium in aluminium down to the 10-p.p.m. level in a sample of 100 mg, irradiated for 8 h at a neutron flux of $10^{12}$n cm$^{-2}$ sec$^{-1}$.

We thank the Manager and Staff of the Universities Research Reactor, Risley, Warrington for carrying out the irradiations and for helpful advice on the preparation of samples for irradiation.

## SUMMARY

Chromium in aluminium-based alloys has been determined by activation analysis and by isotope dilution using the substoichiometric principle. Chromium, separated by oxidation to dichromate followed by solvent extraction, was substoichiometrically extracted with tetraphenylarsonium chloride into 1,2-dichloroethane and assayed by measurement of its $\gamma$-emission.

## RÉSUMÉ

Un dosage substoechiométrique du chrome dans des alliages à base d'aluminium est décrit; on utilise l'analyse par activation et la dilution isotopique. Le chrome séparé par oxydation en dichromate est extrait au moyen de chlorhydrate de tétraphénylarsonium dans le dichloro-1,2-éthane; on mesure ensuite son émission $\gamma$.

## ZUSAMMENFASSUNG

Chrom in Aluminium-Legierungen wurde durch Aktivierungsanalyse und durch Isotopenverdünnung nach dem substöchiometrischen Prinzip bestimmt. Nach Abtrennung durch Oxidation zu Dichromat und anschliessende Solventextraktion wurde das Chrom mit unterstöchiometrischem Tetraphenylarsoniumchlorid in 1,2-Dichloräthan extrahiert und durch Messung der $\gamma$-Emission bestimmt.

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# COLLECTION OF TRACES OF SILVER ON POWDERED DITHIZONE

## APPLICATION OF ULTRASONICS

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Organic gathering precipitates or collectors used for separating traces of inorganic ions from an aqueous solution are usually generated in the sample solution *in situ*. However, the addition to the sample solution of separately prepared collectors is advantageous in some cases, especially when the formation of the proper collectors in the sample solution is difficult. The favorable properties of preformed voluminous *p*-dimethylaminobenzylidenerhodanine precipitates for the collection of traces of silver ion from dilute nitric acid solutions have been reported previously[1]. The present paper describes the use of finely ground dithizone powder for the same purpose. An ultrasonic field has been applied effectively to accelerate the collection. The proposed method has two advantages compared with the conventional coprecipitation method with newly forming dithizone precipitates[2]: greater concentration factors of silver with respect to matrices and easier filtration. The application to the separation and determination of low p.p.m. of silver in high-purity lead[3–5] is also described.

## EXPERIMENTAL

### *Apparatus*

A Fujitsu well-type NaI(Tl) scintillation counter. A Hirama model V photoelectric filter photometer with matched 2- and 5-cm micro cells. An ultrasonic generator (250 kHz, 5 W) with a piezoelectric transducer.

### *Reagents*

*Dithizone.* Grind 0.1 g of dithizone to *ca.* 100–200 mesh powder in an agate mortar, moistening with 5 ml of ethanol in small portions, and allow to air-dry.

*Silver-110m solutions (0.2–100 μg of silver per ml).* Add silver-110m (The Radiochemical Centre; silver nitrate in 0.1 *M* nitric acid; specific activity, 0.25 mCi per mg) and silver nitrate to 0.1 *M* nitric acid.

All the reagents used were of reagent grade. Water purified by distillation and ion exchange was used throughout the work.

### *Procedure*

Place 25 ml of sample solution (0.01–1 *M* acidity) containing 0.2–20 μg of silver in a 30-ml flask (*ca.* 0.3 mm thick at the bottom), and add 5–20 mg of dithizone. Stir the solution and apply ultrasonics using the arrangement shown in Fig. 1. After the prescribed time, filter off the dithizone on a filter paper of 3 cm diameter placed on a sintered-glass disc, and wash with three 3-ml portions of 0.1 *M* nitric acid. Immerse the filter paper with dithizone in 5 ml of concentrated nitric acid until the dithizone has completely dissolved. Remove the filter paper, rinse thoroughly with 3 ml of water, and combine these solutions.

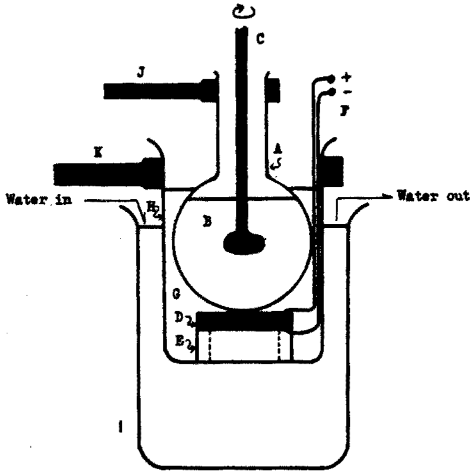

Fig. 1. Experimental arrangement. (A) 30-ml round bottomed glass flask, (B) sample solution, (C) stirrer (150–300 rpm), (D) piezoelectric transducer, (E) support (polyvinyl chloride cylinder), (F) leads to the ultrasonic generator, (G) xylene, (H) 50-ml glass beaker, (I) 100-ml glass beaker, (J, K) clamp.

### *Tracer experiments*

The recoveries of silver were determined by $\gamma$-activity measurement, with coefficients of variation of 1–3%.

## RESULTS AND DISCUSSION

### *Effect of ultrasonics on collection of silver*

Figure 2 shows the effect of ultrasonics on collection of 2 $\mu$g of silver from 25 ml of 0.2 *M* nitric acid with 5 mg of dithizone. It is clear that the sound field accelerates the collection remarkably. The silver recovery reaches about 98% after 15 min of ultrasonic irradiation, remains nearly constant between 15 and 30 min, and then decreases rapidly. The effect of temperature is practically insignificant over the range 20–60°. Therefore, experiments were carried out at room temperature, unless otherwise stated. Even after 30 min of ultrasonic irradiation, the temperature change was less than 2°.

Since dithizone is soluble in dilute nitric acid, the dissolution of dithizone during the collection step was investigated photometrically. A 1-ml aliquot of the solution was taken and filtered through a 3-cm filter paper placed on a sintered-glass disc, the combined filtrate and washings (1 ml of water) were adjusted to pH 8.5 with sodium hydroxide and shaken with 10 ml of chloroform, and the absorbance of the organic layer was measured in a 2-cm cell at 610 nm. The results illustrated in Fig. 3 correspond with those in Fig. 2. Even after 45 min of ultrasonic irradiation, no change in the size and shape of dithizone powder was observed under an optical microscope (90 ×).

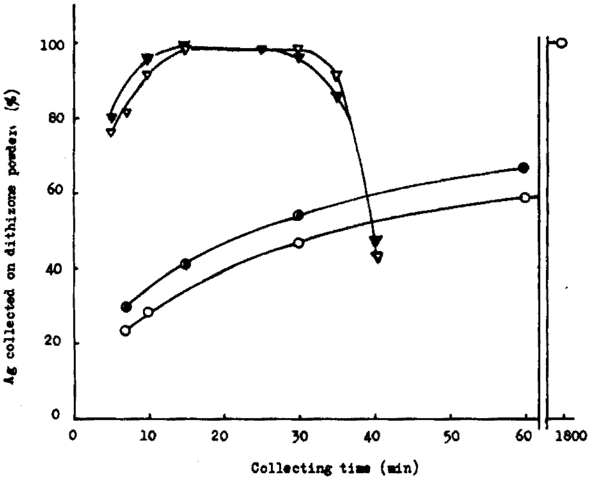

Fig. 2. Effect of ultrasonics on silver recovery. Ag present, 2 μg. Solution volume, 25 ml. $HNO_3$ acidity, 0.2 *M*. Dithizone, 5 mg. (▽) With sound field, 20°, (▼) with sound field, 60°, (○) no sound field, 20°, (⊙) no sound field, 60°.

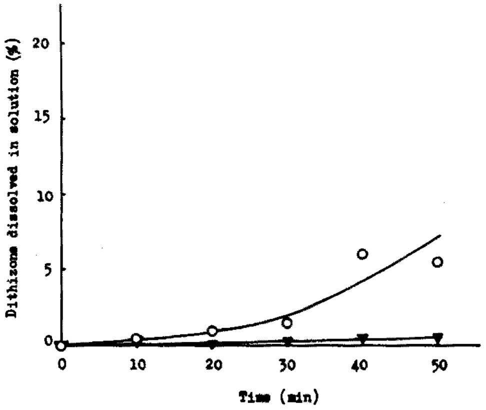

Fig. 3. Dissolution of dithizone in 0.2 *M* nitric acid. Solution volume, 25 ml. Dithizone, 5 mg. (○) With sound field, (▼) no sound field.

*Required quantity of dithizone*

The required quantity of dithizone for collecting 0.2–100 μg of silver from 25 ml of 0.2 *M* nitric acid is shown in Table I. Five mg of dithizone assures almost perfect recoveries for *ca.* 2 μg or less of silver. However, more dithizone is required for collecting larger amounts of silver.

### *Effect of acidity on silver recovery*

The effect of the acidity of the sample solution on silver recovery is shown in Table II. The proposed method is applicable to 0.01–1 $M$ nitric acid solutions.

TABLE I

COLLECTION OF SILVER ON DITHIZONE POWDER FROM 0.2 $M$ NITRIC ACID

(Solution volume, 25 ml. Collecting time, 15 min)

| *Ag present (μg)* | *Dithizone used (mg)* | *Ag recovered (%)* |
|---|---|---|
| 0.2 | 1 | 63 |
| | 5 | 99 |
| 2 | 1 | 55 |
| | 5 | 98 |
| | 20 | 100 |
| 20 | 5 | 46 |
| | 20 | 96 |
| 100 | 20 | 23 |
| | 50 | 92 |

TABLE II

EFFECT OF ACIDITY ON SILVER RECOVERY

(Ag present, 2 μg. Solution volume, 25 ml. Dithizone, 5 mg. Collecting time, 15 min)

| $HNO_3$ ($M$) | 0.01 | 0.2 | 0.5 | 1 | 3 | 5 |
|---|---|---|---|---|---|---|
| Ag recovery (%) | 98 | 98 | 97 | 95 | 91 | 43 |

TABLE III

SEPARATION OF SILVER FROM MATRIX ELEMENTS IN NITRATE SOLUTIONS

(Solution volume, 25 ml. Dithizone, 5 mg. Collecting time, 15 min)

| *Present* | | | *Acidity (M)* | *Ag recovered (%)* | *Matrix element accompanying Ag*[a] *(μg)* |
|---|---|---|---|---|---|
| *Cu(g)* | *Pb(g)* | *Ag(μg)* | | | |
| 0.5 | — | 2 | 0.2 | 97 | 25[b] |
| — | 0.5 | 2 | 0.2 | 98 | 10 |
| — | 1.0 | 5 | 0.2 | 97 | 30[c] |

[a] Determined by EDTA titration.
[b] Conventional coprecipitation method 0.5 mg.
[c] Conventional coprecipitation method 1.3 mg.

### *Separation of silver from matrix elements*

Table III shows that p.p.m. amounts of silver in copper and lead matrices can be separated in greater than 97% yields at an acidity of 0.2 $M$ by the proposed method. The concentration factors of silver with respect to the matrices are (2–5)$\cdot 10^4$. Concentration factors of *ca.* $10^3$ are attained by the conventional coprecipitation method[2], in which 2 ml of acetonic 0.25% (w/v) dithizone solution was added to a sample solution to give dithizone precipitates *in situ*.

## DETERMINATION OF SILVER IN HIGH-PURITY LEAD

The proposed method was applied to the photometric determination of silver in high-purity lead metal. The presence of *ca.* 100 μg of lead interferes with the determination of a few μg of silver in the photometric *p*-dimethylaminobenzylidenerhodanine method[6]. The recommended procedure is as follows.

### *Recommended procedure*

Transfer a weighed sample (2 g) to a 50-ml conical flask, add 5 ml of nitric acid (1:1), and heat to dissolve. Cool the solution, transfer to a 50- or 100-ml volumetric flask, and dilute to the mark. Take a 25-ml aliquot, and separate silver as described under *Procedure* using 5 mg of dithizone. Add 1 ml each of concentrated sulfuric acid and 10% ammonium persulfate solution to the resulting solution, and evaporate just to dryness on a water bath. Repeat the wet oxidation once more. Cool to room temperature, add 3 ml of a buffer solution (8 g of ammonium acetate in 100 ml of 33% (v/v) acetic acid) to dissolve the residue, and add 2 ml of a 0.01% (w/v) acetic acid solution of *p*-dimethylaminobenzylidenerhodanine. After 5 min, measure the absorbance of the solution in a 5-cm micro cell at 575 nm against water. Determine the silver concentration by reference to a calibration curve prepared with standard silver nitrate solutions.

With regard to the photometry, the calibration curve is linear up to at least 12 μg of silver, and the error is *ca.* 0.2 μg in this range. No interference results from the presence of 1 mg of copper, 500 μg of bismuth, 100 μg of iron, and 50 μg of lead. Table IV shows the silver content of a sample of commercial high-purity lead metal determined by the proposed method. The blank value through the entire procedure was 0.0 μg of silver. The time required for a determination is about 2 h.

The proposed method may also be applied to the separation of traces of gold, mercury, and palladium.

TABLE IV

DETERMINATION OF SILVER IN HIGH-PURITY LEAD

| *Sample taken (g)* | *Ag found (μg)* | *Ag in lead (p.p.m.)* |
|---|---|---|
| 0.5[a] | 3.4 | 2.8 |
| 1.0 | 2.3 | 2.3 |
| 1.0[b] | 3.1 | 2.1 |
| | | (av.) 2.4 |

[a] 2.0 μg of silver was added immediately after dissolution of the sample.
[b] 1.0 μg of silver was added immediately after dissolution of the sample.

The authors wish to thank Prof. Y. MIYAHARA and Dr. H. NOMURA of Nagoya University for the loan of the ultrasonic apparatus.

## SUMMARY

Microgram quantities of silver can be collected on 5 mg of finely ground dithizone powder from 25 ml of 0.01–1 *M* nitric acid solution within 15 min by the use of ultrasonics. This separation method is successfully applied to the photometric determination of a few p.p.m. of silver in high-purity lead metal.

## RÉSUMÉ

Des microquantités d'argent peuvent être concentrées sur 5 mg de poudre à base de dithizone, à partir de 25 ml de solution, en milieu acide nitrique 0.01 à 1 *N*. La durée de cette réaction est de 15 min, en utilisant des ultrasons. Cette méthode de séparation a pu être appliquée avec succès au dosage photométrique de quelques p.p.m. d'argent dans un plomb très pur.

## ZUSAMMENFASSUNG

Mikrogramm-Mengen von Silber können innerhalb von 15 min auf 5 mg feinverriebenem Dithizonpulver aus 25 ml 0.01–1 *N* salpetersaurer Lösung unter Anwendung von Ultraschall gesammelt werden. Diese Trennungsmethode wird bei der photometrischen Bestimmung von wenigen p.p.m. Silber in hochreinem Blei-Metall mit Erfolg angewendet.

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# EFFECT OF PESTICIDES ON LIVER CHOLINESTERASES FROM RABBIT, PIGEON, CHICKEN, SHEEP AND PIG

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The increasing use of various pesticides (organophosphorus, carbamate and chlorinated) for insect control has attracted much attention to basic studies of cholinesterase, and extensive investigations on the mode of toxic action of pesticides towards this enzyme have been conducted[1-7]. In a recent paper, GUILBAULT *et al.*[8] described a simple method for the purification of cholinesterases from honey bees and boll weevils and studied the inhibitory effect of different pesticides toward these purified preparations.

Because various animals have been known to behave in different ways to different pesticides[9], it was believed that the *in vitro* inhibition of cholinesterases isolated from different animals might be useful for the highly sensitive and selective assay of these pesticides. A simple method for the purification of cholinesterases from animal livers is described, and the inhibitory effect of different pesticides on these partially purified preparations is presented.

Enzymic activity was assayed by means of the fluorogenic substrate, N-methylindoxyl acetate, which is cleaved by cholinesterase to the highly fluorescent, N-methylindoxyl[10]. The rate of production of fluorescence is proportional to the concentration of enzyme as affected by the pesticide present.

## EXPERIMENTAL

### *Reagents*

*Substrate.* A $10^{-2}$ *M* solution (0.0191 g/10 ml of solvent) of N-methylindoxyl acetate was prepared in methyl cellosolve. The substrate is available commercially (Isolab, Inc., Elkhart, Ind.).

*Pesticides.* Stock solutions of various concentrations were prepared in dioxane. The pesticides were of $99^{+}$% purity (Polysciences Corp., Evanston, Ill.). Paraoxon was provided by American Cyanamid. The Parathion was assayed for the percent Paraoxon impurity by i.r. (P→O bond) and thin-layer chromatography, and was found to contain about 1% of Paraoxon.

*Buffers.* 0.1 *M* Phosphate buffer was prepared by dissolving sodium dihydrogenphosphate in triply distilled water. The pH was adjusted to 7.0 with sodium hydroxide.

*Purification of liver cholinesterases*

Unless otherwise stated, all operations were performed at 0–4° and all centrifugations were carried out in a Servall RC2-B refrigerated centrifuge. The purification procedure is described below.

*Preparation of crude extracts.* Frozen sliced sheep liver (2 kg) obtained commercially was homogenized in 2 l of 0.05 *M* phosphate buffer, pH 7.0, at room temperature in a Waring blender at high speed for 5 min. The crude homogenate was allowed to stand for 4 h in the refrigerator and then centrifuged at 6000 *g* for 20 min. The precipitate was discarded and the supernate was decanted and filtered through glass wool. The filtered supernate was kept in the refrigerator for further purification. The crude extracts of chicken, rabbit, pig, and pigeon liver cholinesterases were prepared from their acetone powders (Sigma, St. Louis); 10 g of acetone powder was immersed in 400 ml of 0.05 *M* phosphate buffer and then dispersed thoroughly in a Branson sonic oscillator at moderate power until a homogeneous suspension was obtained. The suspension was kept in the cold room for 4 h with occasional stirring. The suspension was centrifuged and treated in the same manner as described above.

*Ammonium sulfate fractionation.* A calculated amount of ammonium sulfate was added to the supernate to bring it up to the desired saturation. After the ammonium sulfate had completely dissolved, the solution was left in the refrigerator for 30 min then centrifuged at 6000 *g* for 20 min. Precipitates from various saturations were dissolved in small amounts of 0.003 *M* phosphate buffer. Compared with that of ammonium sulfate, the precipitate of low density in some fractions rose to the surface on standing. After centrifugation at 6000 *g* for 20 min, the separated top layer was filtered through glass wool and this active precipitate was collected and dissolved in a small amount of 0.003 *M* phosphate buffer, pH 7.0, for dialysis.

*Dialysis.* Different fractions of enzyme solution from ammonium sulfate fractionation were dialyzed against 10 volumes of 0.003 *M* phosphate buffer, pH 7.0, for 3 h. The dialysis was repeated with two successive portions of fresh phosphate buffer at 3-h intervals. Dialyzed enzyme solutions were assayed for both enzyme activity and protein concentration. Fractions of high specific activity were combined for chromatography.

*Sephadex G-100 chromatography.* The preparation of gel, column packing, sample application, column elution and fraction collection were the same as described previously[8]. The chromatographic patterns of these cholinesterases from different sources were very similar. The protein was eluted as a broad asymmetrical peak, whereas the enzyme activity always eluted as a narrow symmetrical peak falling in the front edge of the protein peak. A typical elution profile, that of sheep liver, is illustrated in Fig. 1. Fractions of high specific activity were combined and then employed in all of the inhibitory studies described below. If the combined fraction was too dilute for inhibition studies, it was concentrated in the freeze dryer until the desired concentration was reached.

*Procedures*

*Measurement of enzyme activity.* Cholinesterase activity of different preparations for various studies was assessed either fluorimetrically, colorimetrically or titrimetrically. The inhibitory effect of pesticides on enzyme activity was determined fluorimetrically by the method of GUILBAULT AND SADAR[10] with N-methylindoxyl

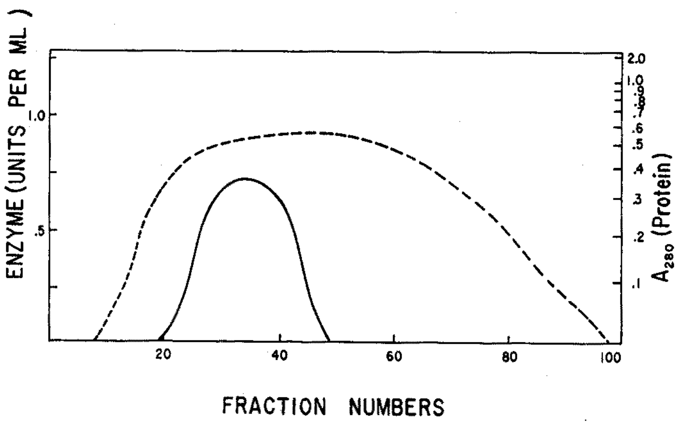

Fig. 1. Chromatography of partially purified sheep liver cholinesterase on Sephadex G-100. (-----) Protein; (——) enzyme.

acetate as substrate. The substrate preference studies were accomplished titrimetrically by continuous titration with a Metrohm 3M titrator. As a rule, the total volume of the reaction mixture was 10 ml. Generally 3 ml of 0.2 *M* magnesium chloride, 1 ml of 0.05 *M* TRIS buffer, 4 ml of distilled water and 1 ml of properly diluted enzyme solution were pipetted into the reaction vessel and brought to pH 7.4 with sodium hydroxide. Substrate (1 ml) was adjusted to pH 7.4 with sodium hydroxide and then added to start the reaction. The amount of sodium hydroxide consumed *vs.* time was recorded after a constant rate had been attained. The enzymic hydrolysis of acetylcholine was spot-checked by the modified method of Simpson *et al.*[11]. One enzyme unit is reported as 1 $\mu$mole substrate hydrolyzed per min for all the methods used in this experiment.

*Electrophoresis.* Disc electrophoresis was accomplished by the method of Davis[12] with acrylamide gel cast in glass tubes. The applied current was maintained at 3 mA per column until the dye front approached 3 mm from the end of the column. Cholinesterase activity was detected by the method of Hunter and Burstone[13] with $\alpha$-naphthylacetate as substrate and Fast Blue as the coupler. After electrophoresis protein was demonstrated as described by Chrambach *et al.*[14]. For inhibition studies after electrophoresis, the gel cylinders were incubated in $10^{-5}$ *M* eserine for 40 min, and then the enzyme activity was demonstrated as described.

*Estimation of protein.* Protein concentrations in different preparations were estimated by the method of Folin–Ciocaltau as outlined by Litwack[15] with egg albumin as reference. The specific activity of the preparation was calculated based on the enzyme units/mg protein obtained by this method.

*Assay of pesticides.* To 3 ml of phosphate buffer pH 7.0, and 0.1 ml of $10^{-2}$ *M* N-methylindoxyl acetate was added 0.1 ml of dioxane (the solvent used for the pesticides). The fluorescence was adjusted to zero and then 0.1 ml of the appropriate enzyme stock solution was added. The rate of change in fluorescence with time, $\Delta F$/min, was recorded. The rate was labelled as the blank rate or the rate with no pesticide present. To 3.0 ml of buffer was added 0.1 ml of a solution of the pesticide to be assayed and 0.1 ml of the enzyme solution. The resulting solution was then incubated for a period of time (usually 3 min). Then 0.1 ml of the substrate solution was added and $\Delta F/\text{min}$ was recorded. The percent inhibition was calculated:

$$\%\ \text{inhibition} = \frac{(\Delta F/\text{min})_{\text{no inhibitor}} - (\Delta F/\text{min})_{\text{inhibitor}}}{(\Delta F/\text{min})_{\text{no inhibitor}}} \times 100$$

The concentration of pesticide was determined from a calibration plot of % inhibition *vs.* concentration of the pesticides.

## RESULTS AND DISCUSSION

### *Purity of isolated enzymes*

To determine the relative purity of the preparations after chromatography, *ca.* 0.3 mg of enzyme protein was electrophorized in acrylamide gel and both protein and enzyme activity were detected as described previously[8]. The active preparation appeared to be essentially homogeneous since only a single broad enzyme band was found. This enzyme band was sensitive to eserine and represented cholinesterase. A broad major protein band was also present in the corresponding gel with several trace contaminants indicating the presence of trace amounts of contaminating proteins. The contaminating bands became more prominent as the protein used for electrophoresis was increased, but no cholinesterase could be found in these contaminating bands despite the appreciable amounts of stainable material present.

### *Characterization of purified preparations*

Liver cholinesterases from different sources were characterized by their hydrolysis rate against various substrates[8]. The enzymic hydrolysis of acetylcholine and butylcholine by these preparations is given in Table I. As can be seen from this Table, most of the liver cholinesterases hydrolyzed butylcholine faster than acetylcholine, with the exception of chicken liver cholinesterase which cleaved both substrates at the same rate. Rabbit liver enzyme hydrolyzed butylcholine three times faster than acetylcholine indicating the presence of a large amount of pseudocholinesterase. Again, the purified preparation did not show marked substrate inhibition with both substrates up to a concentration of 0.1 *M*. The results confirm that the cholinesterases purified from animal livers are different from those previously obtained from insects, and are predominantly pseudocholinesterases.

TABLE I

RELATIVE ACTIVITY OF LIVER CHOLINESTERASES FROM DIFFERENT SOURCES AGAINST ACETYL- AND BUTYLCHOLINE ESTERS

| *Source of enzyme* | *Protein (mg/ml)* | *μM of choline-ester hydrolyzed/min* | | *Relative specific activity* | |
|---|---|---|---|---|---|
| | | *Acetyl* | *Butyryl* | *Acetyl* | *Butyryl* |
| Sheep | 0.44 | 1.0 | 1.6 | 2.27 | 3.64 |
| Pig | 0.67 | 1.9 | 2.82 | 2.83 | 4.21 |
| Pigeon | 1.10 | 2.62 | 3.26 | 2.38 | 2.96 |
| Chicken | 0.68 | 1.98 | 1.98 | 2.91 | 2.91 |
| Rabbit | 0.56 | 0.67 | 2.50 | 1.20 | 4.46 |

*Stability of enzyme preparations*

Data on the stability of the cholinesterase preparations are presented in Fig. 2. The stability was determined by noting the rate of hydrolysis of N-methylindoxyl acetate. The enzymes from rabbit, sheep, pig and pigeon were very stable, losing no activity in 15 days of storage at 4°. These enzymes lost only about 10% of their activity after 24 days' storage. The chicken liver cholinesterase was slightly more unstable, losing about 6 and 9% of its activity after 15 and 24 days, respectively.

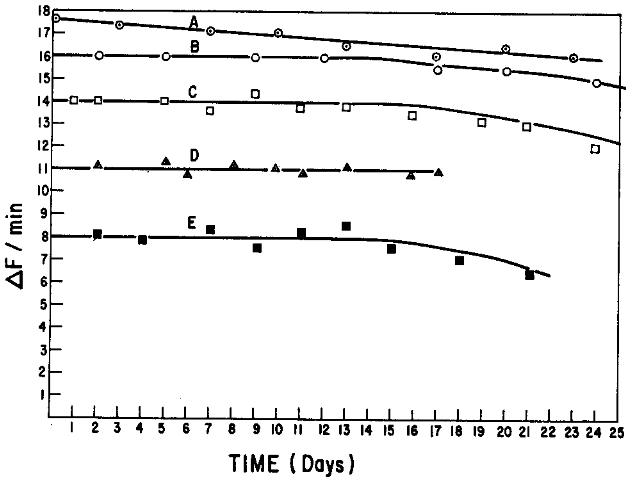

Fig. 2. Stability of purified liver cholinesterase preparations with time. Rate of hydrolysis of N-methylindoxyl acetate, $\Delta F$/min, by the enzyme preparation used as measure of enzymic activity. (A) Chicken, (B) rabbit, (C) sheep, (D) pig, (E) pigeon.

*Inhibition by pesticides*

The pure enzymes from different animal sources were studied for inhibition by different pesticides. The inhibition of these animal cholinesterases was also compared to that of horse serum cholinesterase, which is the most widely used enzyme in the assay of pesticides.

In a previous study[10], N-methylindoxyl esters were found to be the best substrates for the assay of cholinesterase, from considerations of stability, blank rate, rate of enzymic hydrolysis and lowest detectable enzyme concentration. A $10^{-2}$ $M$ solution of N-methylindoxyl acetate was prepared in methyl cellosolve and used as substrate for each cholinesterase. The other conditions for assay (buffer, pH, etc.) were the same as previously found optimal[10]. The rate of production of the highly fluorescent N-methylindoxyl from the non-fluorescent ester, N-methylindoxyl acetate was measured at an excitation wavelength, of 430 nm and an emission wavelength of 501 nm. The concentration of each enzyme was selected with respect to its activity and the rate of increase of fluorescence. All concentrations were, however, taken from the linear portion of the curve drawn by plotting $\Delta F/\Delta t$ *vs.* enzyme concentration (Fig. 3). The blank rate, *i.e.*, rate without any pesticide present, was always taken by adding 0.1 ml of dioxane, the solvent for pesticides.

The effect of incubation on the inhibition of a typical enzyme, rabbit liver cholinesterase, is indicated in Fig. 4. DDVP (Vapona) and Paraoxon maximally inhibited after a period of only 2 min, whereas 3–4 min was necessary for Sevin and about 5 min for (ethyl) Parathion and methyl Parathion.

With a 3-min preincubation time some of the results obtained for the inhibition of sheep, chicken, rabbit, pigeon and pig liver cholinesterase by various pesticides

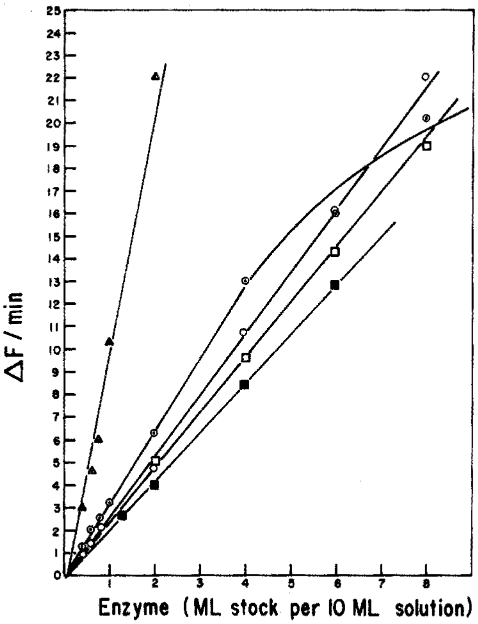

Fig. 3. Plot of rate of enzymic hydrolysis of N-methylindoxyl acetate, $\Delta F$/min, *vs.* concentration of liver enzyme present. N-Methylindoxyl acetate, $3.3 \cdot 10^{-4}$ $M$; phosphate buffer, 0.1 $M$, pH 7.0. (△) Pig, (⊙) pigeon, (○) sheep, (□) rabbit, (■) chicken.

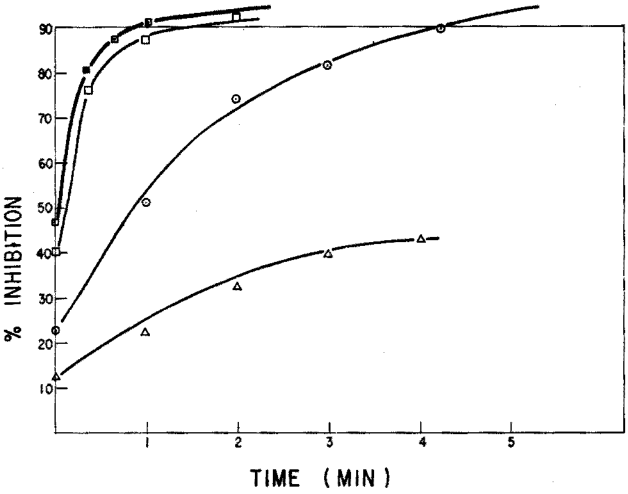

Fig. 4. Effect of preincubation on the inhibition of rabbit liver cholinesterase by four organophosphorus insecticides ($6 \cdot 10^{-6}$ $M$). (⊡—⊡) Paraoxon, (□—□) DDVP, (⊙—⊙) Parathion, (△—△) Sevin.

are indicated in Figs. 5–9 respectively. All five enzymes were inhibited by DDVP, Parathion and methyl Parathion, in that order. Chicken, rabbit and pig were inhibited by Sevin, generally at a concentration higher than methyl Parathion. Rabbit was also inhibited by Lindane, though the inhibition never exceeded 25%. Other chlorinated pesticides had no effect at all on any of the liver cholinesterases.

The $I_{50}$ values (concentration of pesticide necessary to cause a 50% inhibition

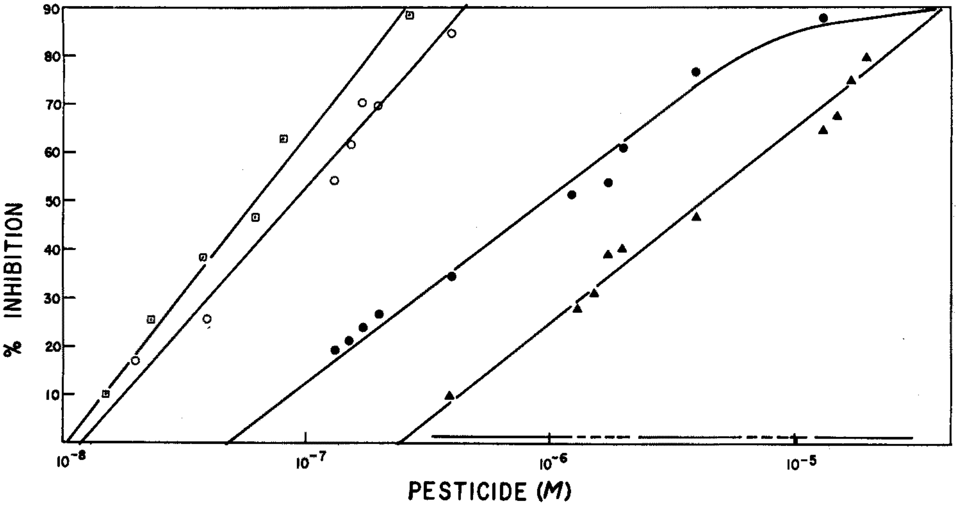

Fig. 5. Plot of % inhibition of sheep liver cholinesterase by various pesticides. 3-min preincubation. Enzyme = 0.02 unit/ml of total solution. (⊡—⊡) Paraoxon, (○—○) DDVP, (●—●) Parathion, (▲—▲) Methyl Parathion, (------) Aldrin, Dieldrin, DDT, Lindane, 2,4-D, Captan, Heptachlor, Methoxychlor, Mirex, Dalapon, Sevin.

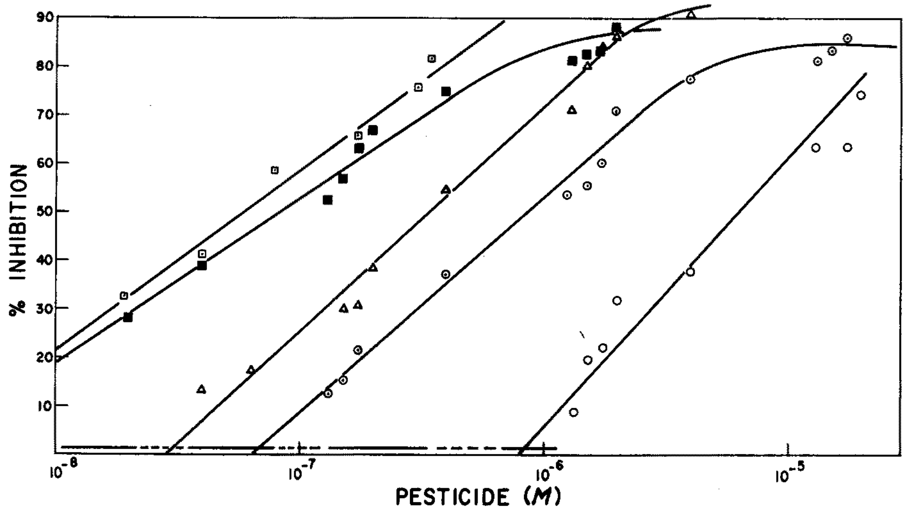

Fig. 6. Plot of % inhibition of chicken liver cholinesterase by various pesticides. 3-min preincubation. Enzyme = 0.02 unit/ml of total solution. (⊡—⊡) Paraoxon, (■—■) DDVP, (△—△) Parathion, (⊙—⊙) Methyl Parathion, (○—○) Sevin, (------) Aldrin, Dieldrin, DDT, Lindane, 2,4-D, Captan, Heptachlor, Methoxychlor, Mirex, Dalapon.

of the enzyme activity) of various liver cholinesterases are listed in Table II. Values obtained with horse serum cholinesterase (Sigma, activity 2.85 units) under identical conditions, are included for comparison. Rabbit cholinesterase was most sensitively inhibited by Paraoxon and DDVP, as little as $2.4 \cdot 10^{-8}$ and $8.4 \cdot 10^{-8}$ *M* pesticide producing a 50% inhibition, respectively. Chicken cholinesterase was also sensitively inhibited by Parathion and methyl Parathion, as little as $5.3 \cdot 10^{-7}$ *M* and $9.3 \cdot 10^{-7}$ *M*

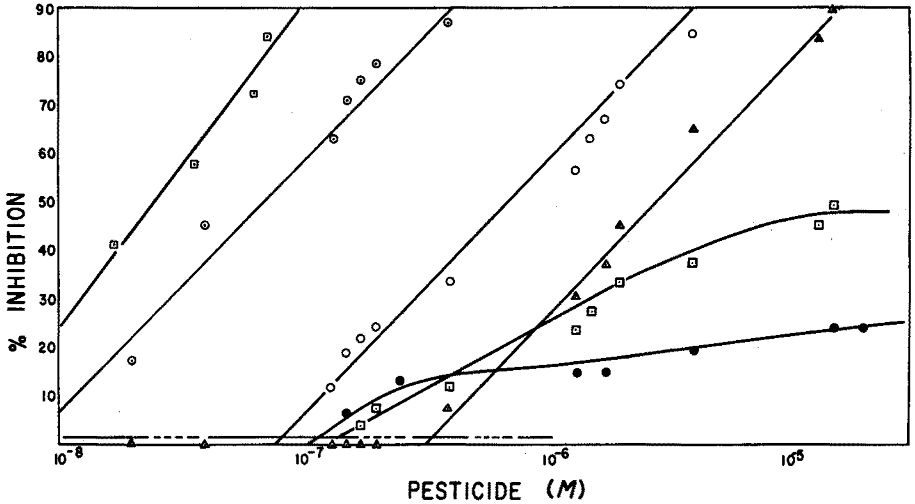

Fig. 7. Plot of % inhibition of rabbit liver cholinesterase by various pesticides. 3-min preincubation. Enzyme = 0.02 unit/ml of total solution. (⊡—⊡) Paraoxon, (⊙—⊙) DDVP, (○—○) Parathion, (△—△) Methyl Parathion, (⊡—⊡) Sevin, (●—●) Lindane, (———----——) Aldrin, Dieldrin, DDT, 2,4-D, Captan, Heptachlor, Methoxychlor, Mirex, Dalapon.

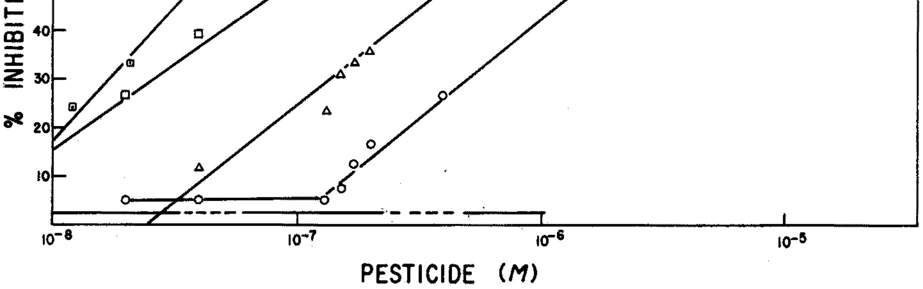

Fig. 8. Inhibition of pigeon liver cholinesterase by various pesticides. 3-min preincubation time. Enzyme = 0.02 unit/ml of total solution. (⊡—⊡) Paraoxon, (□—□) DDVP, (△—△) Parathion, (○—○) Methyl Parathion, (—·—) Sevin, Lindane, Aldrin, Dieldrin, DDT, 2,4-D, Captan, Heptachlor, Mirex, Methoxychlor, Dalapon,

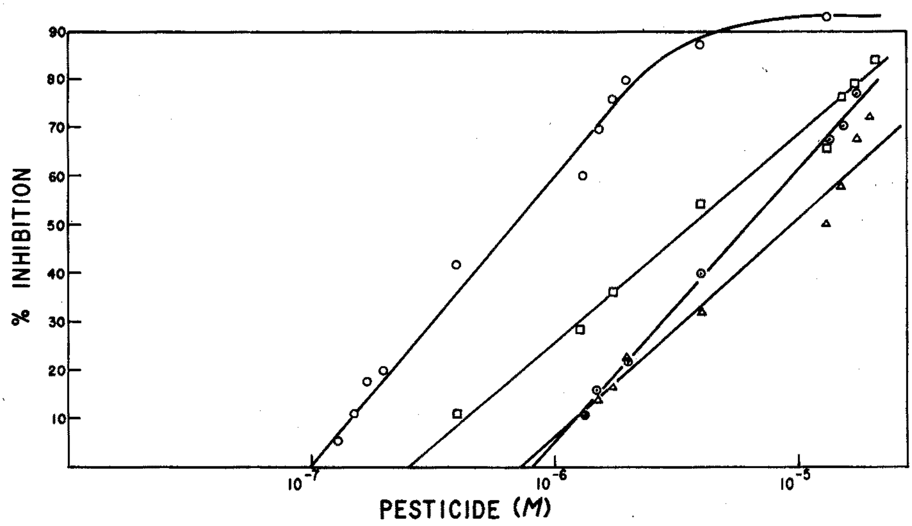

Fig. 9. Inhibition of pig liver cholinesterase by various pesticides. 3-min preincubation. Enzyme = 0.02 unit/ml of total solution. (○—○) DDVP, (□—□) Parathion, (△—△) Methyl Parathion, (⊙—⊙) Sevin.

TABLE II

COMPARISON OF $I_{50}$ OF HORSE, RABBIT, PIGEON, CHICKEN, SHEEP, AND PIG LIVER CHOLINESTERASE BY VARIOUS INHIBITORS—PREINCUBATION MAXIMUM

| *Pesticide* | *$I_{50}(M)$*[a] | | | | | |
|---|---|---|---|---|---|---|
| | *Horse* | *Rabbit* | *Pigeon* | *Chicken* | *Sheep* | *Pig* |
| Paraoxon | $1.5 \cdot 10^{-6}$ | $2.4 \cdot 10^{-8}$ | $5.2 \cdot 10^{-8}$ | $7.5 \cdot 10^{-8}$ | $6.0 \cdot 10^{-8}$ | |
| DDVP | $4 \cdot 10^{-6}$ | $8.4 \cdot 10^{-8}$ | $9.8 \cdot 10^{-8}$ | $9.2 \cdot 10^{-8}$ | $9.5 \cdot 10^{-8}$ | $8.3 \cdot 10^{-7}$ |
| Parathion | $3.0 \cdot 10^{-5}$ | $8.4 \cdot 10^{-7}$ | $6.4 \cdot 10^{-7}$ | $5.3 \cdot 10^{-7}$ | $9.7 \cdot 10^{-7}$ | $5.6 \cdot 10^{-6}$ |
| Me Parathion | $1 \cdot 10^{-5}$ | $4.5 \cdot 10^{-6}$ | $1.8 \cdot 10^{-6}$ | $9.3 \cdot 10^{-7}$ | $6.2 \cdot 10^{-6}$ | $9.7 \cdot 10^{-6}$ |
| Sevin | $8.2 \cdot 10^{-6}$ | $1.8 \cdot 10^{-5}$ | — | $8.0 \cdot 10^{-6}$ | — | $7.9 \cdot 10^{-6}$ |

[a] All $I_{50}$ values were obtained by noting the concentration of pesticide that effected a 50% decrease in the rate of hydrolysis of N-methylindoxyl acetate (overall concentration $3 \cdot 10^{-4}$ *M*). The rate was calculated by measuring the change in fluorescence after 2 min.

pesticide producing 50% inhibition. Sevin inhibited pig cholinesterase at the lowest concentration. All of these enzymes were inhibited by the organophosphorus and carbamate pesticides at much lower concentrations than is horse serum cholinesterase, the enzyme most widely used for assay of pesticides.

With rabbit liver cholinesterase, $1 \cdot 10^{-9}$–$2 \cdot 10^{-7}$ *M* Paraoxon or DDVP could be assayed with an accuracy of about 5%. With chicken liver cholinesterase, $6 \cdot 10^{-9}$–$7 \cdot 10^{-7}$ *M* Paraoxon or DDVP could be assayed with an accuracy of about 5%. Thus chicken liver cholinesterase is the optimum enzyme for assay of Paraoxon and DDVP; Parathion and methyl Parathion interfere. Interference from Sevin was eliminated by dilution to very low concentrations.

Chicken liver cholinesterase is also the best for assay of methyl Parathion, $9 \cdot 10^{-8}$–$6 \cdot 10^{-6}$ *M* being determinable with an accuracy of about 5%. Pigeon liver was inhibited by Parathion in the range $5 \cdot 10^{-8}$–$7 \cdot 10^{-6}$ *M*, compared to a range of

$7.5 \cdot 10^{-8}$–$2 \cdot 10^{-6}$ $M$ with chicken liver. Essentially comparable results (5% accuracy) were obtained with either enzyme. Pigeon is the preferable enzyme, since Sevin does not inhibit this enzyme and will not interfere.

Rabbit liver cholinesterase is the best enzyme for assay of Sevin, $3 \cdot 10^{-7}$–$2 \cdot 10^{-5}$ $M$ being determinable with an accuracy of about 5%. The other 3 organophosphates interfere.

From the results of this study it can be concluded that animal liver cholinesterases offer promise of selectivity and sensitivity for the assay of DDVP, Paraoxon and carbamate pesticides.

It is known that phosphorothionates are not strong inhibitors of esterases but that their oxygen analogs are very potent inhibitors[2]. Since both Parathion and methyl Parathion showed strong inhibition, as is clear from the $I_{50}$ values, the presence of oxygen analogs (Paraoxon) as impurity was suspected. The i.r. spectra of both Parathion and methyl Parathion had very distinct peaks corresponding to P=O. Separation on TLC showed less than 1% impurity but $I_{50}$ values were only about 10-fold less for pure Paraoxon. Hence it was concluded that inhibition is not only due to Paraoxon as impurity, but to Parathion as well. Since both Parathion and methyl Parathion, available commercially and in common use, are of 99% purity, the data of inhibition by these pesticides have been included above. Such data can be useful in qualitative and quantitative work. Moreover, it is very difficult to obtain absolutely pure Parathion or to purify this compound to remove the minute traces of Paraoxon impurity.

We would like to thank DAVID HACKNEY who synthesized the substrate used in this study, and GERRY TULLY and MAYA WALDBERG who helped isolate the enzymes used. Financial support through the Louisiana Community Studies Pesticides Project, Louisiana State University Medical Center, which is supported by the Department of Health, Education and Welfare, and by the National Institutes of Health, is gratefully acknowledged.

## SUMMARY

The effect of twelve different pesticides, including carbamates, chlorinated hydrocarbons and organophosphorus compounds, on liver enzymes isolated from rabbit, pigeon, chicken, sheep, and pig is reported. The cholinesterases from these sources were inhibited at very low concentrations by the organophosphorus pesticides DDVP, Paraoxon, Parathion, and methyl Parathion. None of these enzymes was inhibited by any of the chlorinated pesticides, and pigeon and sheep liver cholinesterase were not inhibited by Sevin. Some selectivity of the enzyme systems for the determination of pesticides is possible.

## RÉSUMÉ

On a examiné l'influence de douze pesticides différents, comprenant carbamates, hydrocarbures chlorés et composés organiques phosphorés, sur les enzymes du foie de lapins, pigeons, poulets, moutons et porcs. Les cholinestérases sont inhibées à très faibles concentrations par des pesticides organophosphorés DDVP, Paraoxon, Parathion et Méthylparathion. Aucune de ces enzymes n'est inhibée par des pesticides chlorés. Une certaine sélectivité est possible pour le dosage de pesticides.

## ZUSAMMENFASSUNG

Es wird über den Einfluss von zwölf verschiedenen Pesticiden, einschliesslich Carbamaten, chlorierten Kohlenwasserstoffen und organischen Phosphorverbindungen, auf Leberenzyme von Kaninchen, Taube, Huhn, Schaf und Schwein berichtet. Auf Cholinesterasen dieser Herkunft wirkten die Organophosphorpesticide DDVP, Paraoxon, Parathion und Methylparathion bei sehr niedrigen Konzentrationen als Inhibitoren. Keines dieser Enzyme wurde durch irgendein chloriertes Pesticid inhibiert; Sevin war kein Inhibitor für die Cholinesterasen von Taube und Schaf. Eine gewisse für die Bestimmung von Pesticiden anwendbare Selektivität der Enzymsysteme ist möglich.

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# COMPUTER CALCULATION OF CONCENTRATIONS OF FLAME GAS SPECIES AND METAL ATOMS IN THE NITROUS OXIDE–ACETYLENE AND AIR–ACETYLENE FLAMES

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Conventional flame emission and atomic absorption spectroscopic techniques rely on the presence of an atomic vapour of the element to be determined. For elements such as zinc and cadmium this is readily achieved with cool flames, *e.g.* air–acetylene. However, significant atomisation of elements that are refractory in nature is achieved only in hot, reducing atmospheres such as the fuel-rich nitrous oxide–acetylene[1] and oxy–acetylene flames[2].

For simple flames, equilibrium concentrations of flame gas species may be calculated from the equilibrium constant data for the various flame reactions. In the case of more complicated systems, such as those under consideration in this paper, this method is inapplicable because insufficient thermodynamic data are available and the flame reactions have not all been characterised. For this reason the technique of free energy minimisation is used in this study for the calculation of equilibrium concentrations of both flame gas and condensed species.

The method of free energy minimisation was proposed initially by WHITE *et al.*[3] for vapour-phase equilibria and later extended to include condensed phases by KUBERT AND STEPHANOU[4]. The method has more recently been applied via the use of a digital computer program to determine the composition of oxy–acetylene flames[5]. A modified version of this program has been used in this paper to calculate the equilibrium concentrations of various gas and condensed species present in the nitrous oxide–acetylene and air–acetylene flames. The method has been previously used to evaluate the analytical usefulness of the premixed nitrous oxide–hydrogen flame in atomic spectroscopy[6].

The fundamental criterion for the technique used is that in a system in chemical equilibrium the free energy is a minimum. The theory of the method has been described in detail[3,4], hence only a brief summary will be given here. The basic assumptions which must be made are that the rate of the chemical reactions involved is high compared with the residence time of the species considered, all gaseous species must exhibit perfect gas behaviour and condensed species must be immiscible.

The following steps are involved in the derivation of the computer calculation for application to flames:

(*a*) An expression is developed for the total free energy of the system (at 1 atmosphere) as a function of the free energy of the individual components for a mixture of known composition.

(*b*) The equilibrium composition (unknown) is expressed in terms of the assumed composition and of unknown increments representing the changes required to bring the composition to the final equilibrium composition. This is achieved by using the first two terms of a Taylor's expansion.

(*c*) The expanded function is minimised subject to mass balance constraints using Lagrange multipliers.

(*d*) As a result, a system of linear simultaneous equations is generated and then solved by the method of Gaussian elimination with pivoting to yield a new approximation to the composition corresponding to minimum free energy.

(*e*) The process is repeated until successive solutions agree to the desired accuracy.

(*f*) The values of the concentrations are then stored and used as initial guesses for the calculation at the next temperature.

## SUMMARY OF PROGRAM

The computer program used in the oxy–acetylene study[5] was simplified, modified and converted to Fortran IV for use with an IBM 7094 computer using a PUFFT (Purdue University Fast Fortran Translator) compiler. The modified program consists of a main routine in which the thermodynamic data are read and the values of F/RT (molar standard free energy function) are calculated. From this a subroutine THERMO is called in which the problem data are read and the system of equations is set up and solved by calling subroutine GPIVOT (Gaussian elimination with pivoting). The solutions are tested for convergence and the process is repeated until the problem converges (difference between successive solutions $\leqslant 10^{-6}$). The solutions at each temperature are then stored in a two-dimensional array and used as initial guesses for the calculation at the next temperature. The concentrations are calculated in moles per mole of feed and those of the gaseous species in volume per cent. The computer used can handle real numbers to as small as approximately $10^{-38}$ and concentrations smaller than this are set at zero. However, values less than the reciprocal of the Avogadro number (*ca.* $1.6 \cdot 10^{-24}$) are clearly meaningless. If the problem fails to converge, a message to this effect is printed out together with the current values of the concentrations and the temperature.

The amounts of the elements present in any system are calculated by the computer as follows,

Let the flame reaction be $2\ N_2O + X\ C_2H_2 + 2 \times 0.0625\ H_2O \rightarrow$ products where $X$ is the number of moles of acetylene (1 at stoichiometry) and 0.0625 is the number of moles of water reaching the flame per mole of nitrous oxide. The values used are typical of those measured in the authors' laboratory using conventional analytical premixed flames, *e.g.* aqueous solution uptake rate *ca.* 5 ml min$^{-1}$, *ca.* 5% nebuliser efficiency and total nebuliser gas flow rate of *ca.* 5 l min$^{-1}$.

$$\mathrm{DIV} = 2.0 + X + 0.125$$

(where DIV is the normalising factor to bring the feed to one mole).

and carbon $= 2X/\mathrm{DIV}$
hydrogen $= (2X + 0.25)/\mathrm{DIV}$
oxygen $= 2.125/\mathrm{DIV}$
nitrogen $= 4.0/\mathrm{DIV}$

Thus, by setting $X$ at 1.0 and increasing to 1.50 in steps of 0.05 a range of flames from stoichiometric to 50% fuel-rich were studied in one calculation. The solution at the final temperature for each flame is used as the initial guess for the first temperature of the succeeding flame. The fuel-lean ($3N_2O+C_2H_2$) flame was investigated in a separate study by setting $X$ at 0.6667 and the concentrations of all the flame species were also calculated without the introduction of water. Various elements were introduced into the system at 0.0001 g atom per mole of feed and the percentage degree of atomisation is given by concentration of free atoms $\cdot 10^6$.

A similar study of the air–acetylene flame was made based on the flame reaction:

$6\ N_2+1.5\ O_2+X\ C_2H_2+7.5\times 0.05\ H_2O \rightarrow$ products, where 0.05 is the number of moles of water reaching the flame per mole of air. This value was estimated from the experimental data given above for the nitrous oxide–acetylene flame and the difference in the molecular weight of the nebulising gas.

Thus,

DIV $=7.5+X+0.375$
and carbon $=2X/\mathrm{DIV}$
hydrogen $=(2X+0.75)\mathrm{DIV}$
oxygen $=3.375/\mathrm{DIV}$
nitrogen $=12.0/\mathrm{DIV}$

Flames from stoichiometric to 100% fuel-rich were investigated by setting $X$ at 1.0 and increasing to 2.0 in steps of 0.1. The flames were studied without water and also with addition of certain elements as before.

Each separate flame is identified on the computer output by printing the flame type, for example air–acetylene, the number of moles of acetylene ($X$) and the amount of each element present. (The authors will be pleased to supply full details of the computer program and sample outputs on request.)

### *Summary of data*

The data consist of two sections: the thermodynamic and problem data. The thermodynamic data consist of sets of ten cards for each species punched with the species title, temperature, $[(F\text{-}H_{298})/T]$ and the enthalpy of formation at 298°K. The temperature is in ascending order in increments of 100°K. The last two functions were obtained from the JANAF Thermochemical Tables[7]. The first card of the problem data contains the number of systems to be studied. This is followed by a card containing the numbers of elements, gaseous and condensed species, the initial temperature and the total pressure. There then follows a set of cards, one for each species, containing the species title, the initial guess of the concentration and the numbers of atoms of each element present in the species. The order of species must clearly be the same in both the thermodynamic and problem data.

The program in use at present is capable of handling forty species at each of ten temperatures. The number of species may be increased at will with the reservation that there must be fewer condensed species than elements otherwise the matrix will be singular.

### *Limitations of the method*

Chemical equilibrium is assumed for those flames investigated; in view of the agreement between the calculated adiabatic flame temperatures (based on equilibrium calculations)[8] and those measured in practice[9–11], this is considered to be a reasonable approximation. In addition, all gaseous species are assumed to exhibit perfect gas behaviour and solid species to be immiscible.

No account is taken of air entrainment into the flame because no measurements of this are currently available and complete mixing does not occur until well above the analytically useful flame regions. Thus the system may be considered as approximating to the conditions prevailing in the interconal zone of a flame-shielded premixed flame[12] or the central regions of a conventional circular premixed flame.

The absence of data for the $C_2H$ species in the available JANAF thermochemical tables and hence its omission from the system, is not considered to have much influence on the relative concentrations of the other flame components. The effect of omitting certain minor species was investigated and found to be negligible. Because flame temperatures are not known to a great degree of accuracy for all the various flame compositions studied, the isothermal concentrations of the species were plotted in all instances against flame composition.

For these reasons the absolute values of the species concentrations are not strictly comparable with results obtained in practice. However, the trends are indeed indicative of those which are usually observed in practice.

## RESULTS AND DISCUSSION

The results clearly show that the degrees of atomisation of both aluminium and silicon in the nitrous oxide–acetylene and air–acetylene flames are markedly

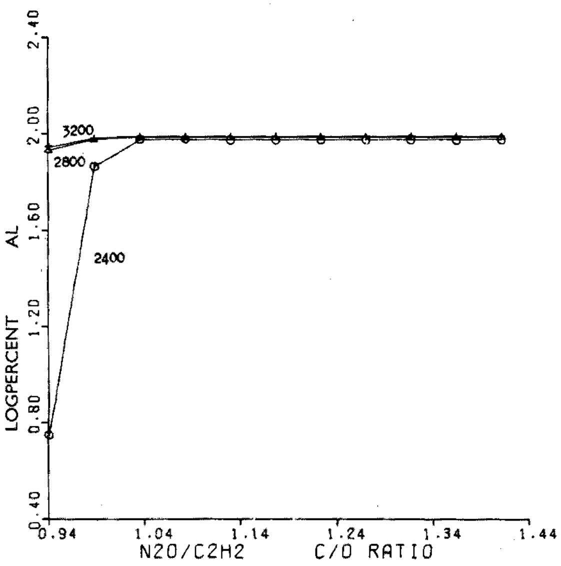

Fig. 1. * Log % atomisation Al *vs.* C:O ratio in $N_2O/C_2H_2$.

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* All figures are computer-generated ("Calcomp") and depict log per cent of species under consideration *vs.* the carbon: total oxygen ratio in either $N_2O/C_2H_2$ or air/$C_2H_2$ flames.
The dependence is shown at temperatures of 2400 (○), 2800 (△) and 3200 (+)°K in the $N_2O/C_2H_2$ flame and 2000 (○), 2300 (△) and 2600 (+)°K in the air/$C_2H_2$ flame.

dependent on flame composition (Figs. 1–4). In addition, the degrees of atomisation of both these elements are inversely related to the concentration of atomic oxygen and the hydroxyl radical (Figs. 5–8), as would be expected from consideration of reactions of the type:

$$M+O \rightarrow MO$$

$$M+OH \rightarrow MO+\tfrac{1}{2}H_2$$

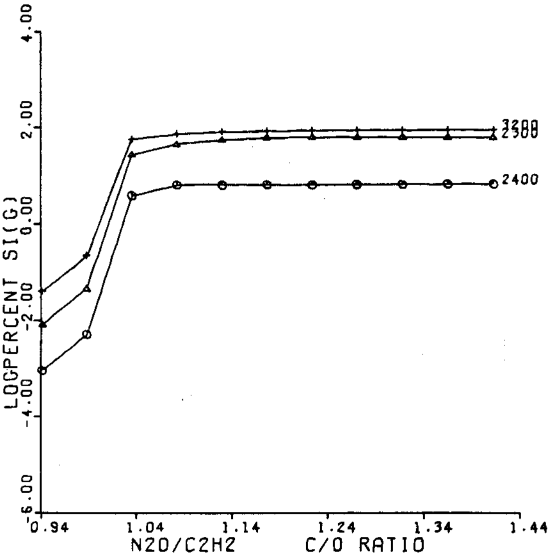

Fig. 2. Log % atomisation Si *vs.* C:O ratio in $N_2O/C_2H_2$.

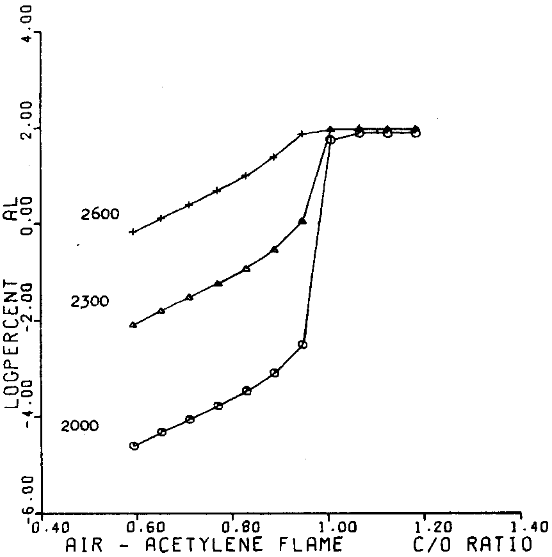

Fig. 3. Log % atomisation Al *vs.* C:O ratio in air/$C_2H_2$.

The concentrations of aluminium and silicon in turn show a critical dependence on the ratio of carbon-to-oxygen in the flame. In all instances when the carbon: total oxygen ratio (oxidant plus water) is greater than unity (at. 6.25% fuel-rich $N_2O/C_2H_2$ and 68.75% fuel-rich air/$C_2H_2$), the isothermal concentrations of atomic oxygen and the hydroxyl radical are correspondingly low and approximately constant, and the degrees of atomisation of the metal ions involved are a maximum and also approximately constant.

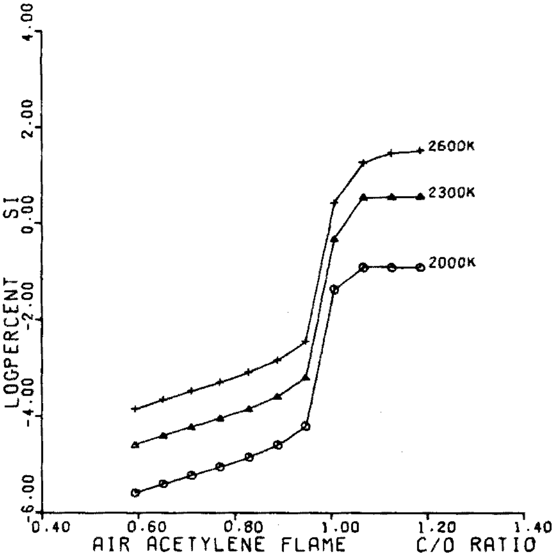

Fig. 4. Log % atomisation Si *vs.* C:O ratio in air/$C_2H_2$.

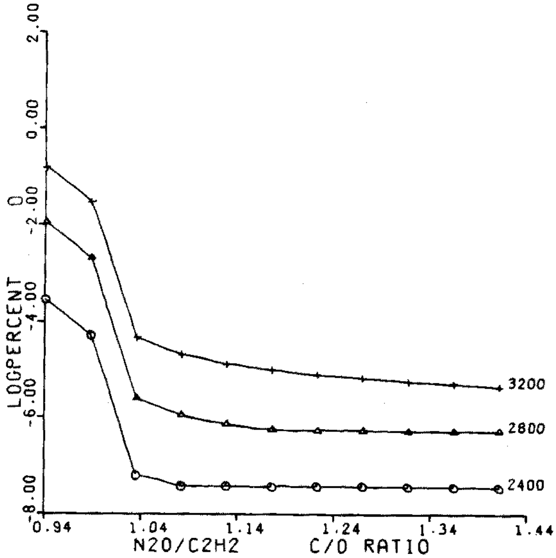

Fig. 5. Log % O *vs.* C:O ratio in $N_2O/C_2H_2$.

The degrees of atomisation of aluminium and silicon also exhibit a direct relationship to the concentrations of CN and atomic C (Figs. 9–12) which in turn are inversely related to the atomic oxygen and hydroxyl concentrations. This latter relationship may be due to reactions of the type:

$$CN + O \rightarrow CO + \tfrac{1}{2}N_2$$

$$C + O \rightarrow CO$$

$$CN + OH \rightarrow CO + \tfrac{1}{2}N_2 + \tfrac{1}{2}H_2$$

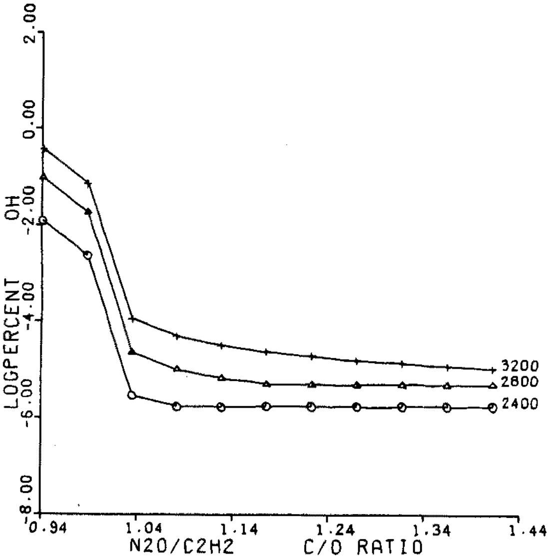

Fig. 6. Log % OH *vs.* C:O ratio in $N_2O/C_2H_2$.

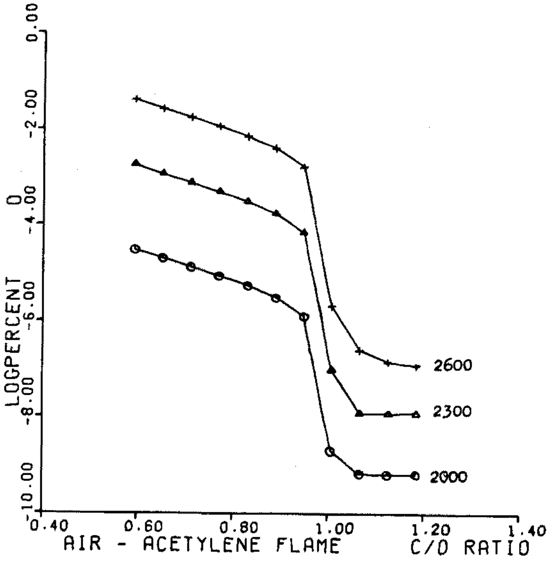

Fig. 7. Log % O *vs.* C:O ratio in air/$C_2H_2$.

$$C + OH \rightarrow CO + \tfrac{1}{2}H_2$$
$$C + MO \rightarrow CO + M$$
$$CN + MO \rightarrow M + CO + \tfrac{1}{2}N_2$$

It is not known which is the principal reducing species although it is likely to be carbonaceous. The concentration of CN in all nitrous oxide–acetylene and air–acetylene flames studied is at least two orders of magnitude greater than that of

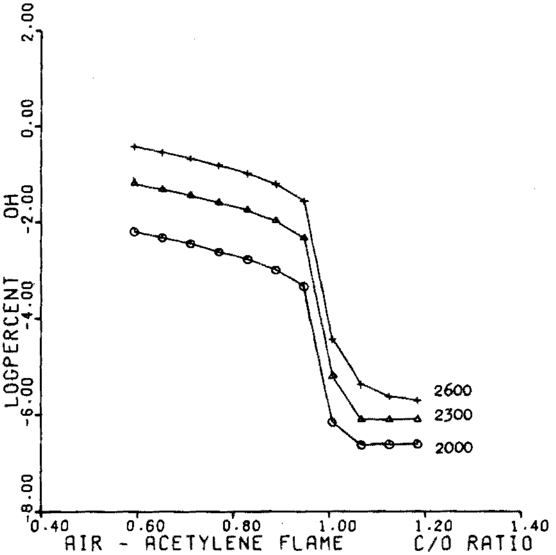

Fig. 8. Log % OH *vs.* C:O ratio in air/$C_2H_2$.

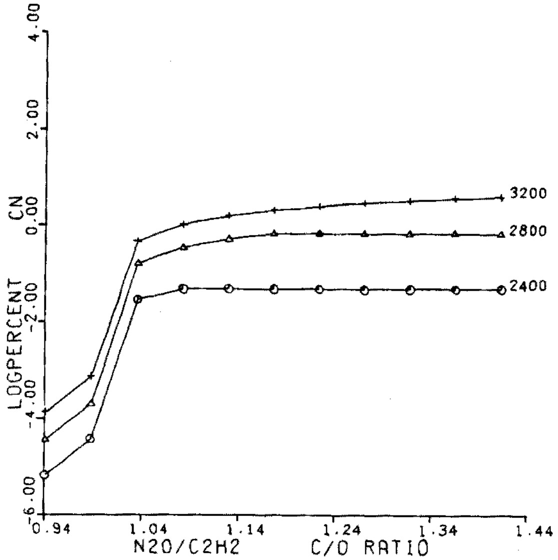

Fig. 9. Log % CN *vs.* C:O ratio in $N_2O/C_2H_2$.

atomic C. In addition, the computer calculations indicate the presence of significant concentrations of HCN (Figs. 13 and 14). This species appears not to have been previously considered in flame gas processes, but its reactions may be assumed to be similar to those of CN. In all the flames studied the concentration of HCN is at least 10-fold that of the CN concentration and hence its presence in the inter-conal regions cannot be ignored.

It is unlikely that hydrogen species such as atomic H are a major reducing

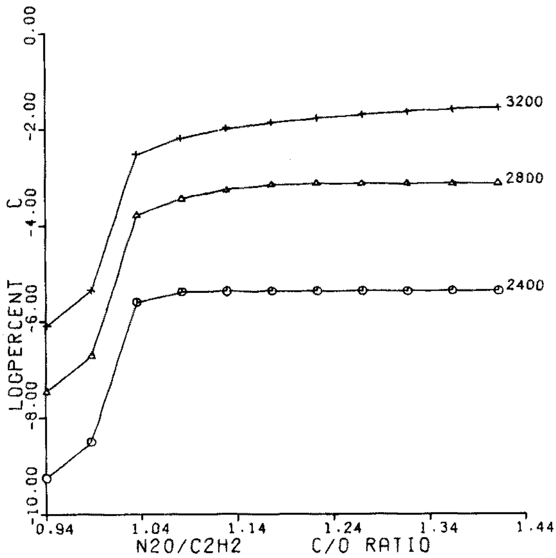

Fig. 10. Log % C *vs.* C:O ratio in $N_2O/C_2H_2$.

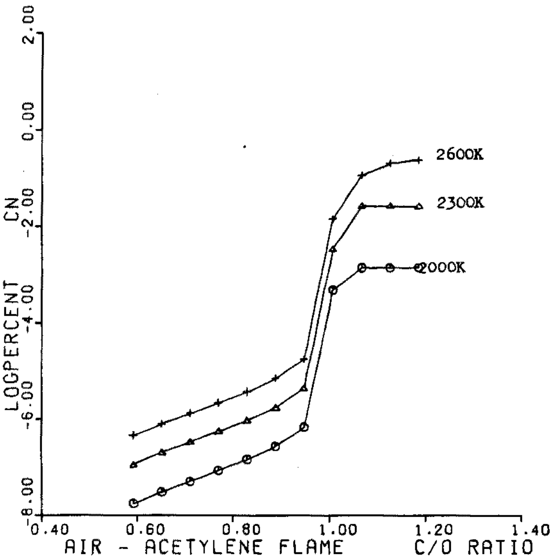

Fig. 11. Log % CN *vs.* C:O ratio in air/$C_2H_2$.

species, despite their apparent large concentrations, because they can only form products with oxygen which are still oxidising in nature, namely OH and $H_2O$.

As would be expected, the effect of water addition is to make the flame less fuel-rich because there is an increase in the total oxygen content. Thus, the concentration of CO increases at the expense of other carbon species such as CN. This effect is especially marked in the stoichiometric and slightly fuel-rich flames where the increase in oxygen content due to the water reduces the carbon: oxygen ratio considerably.

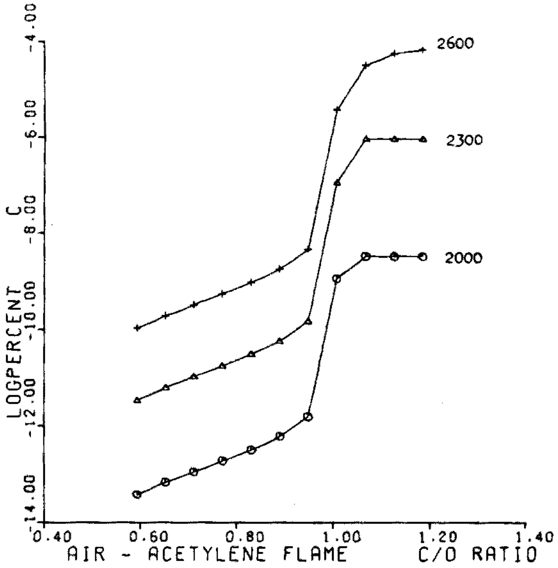

Fig. 12. Log % C *vs.* C:O ratio in air/$C_2H_2$.

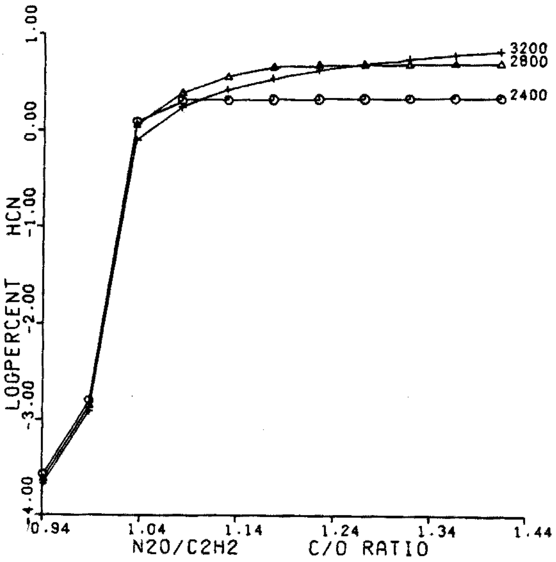

Fig. 13. Log % HCN *vs.* C:O ratio in $N_2O/C_2H_2$.

It was observed that in the lower temperature ($< 2700°K$) stoichiometric nitrous oxide–acetylene flames* considered in this study and also in those flames in which the carbon:oxygen ratio is less than unity, the principal aluminium species is $Al_2O_3$(solid). Above 2700°K (melting point of $Al_2O_3$) and in all flames of both nitrous oxide–acetylene and air–acetylene in which the carbon:oxygen ratio is greater than unity the principal aluminium species is atomic Al; other important species include AlO and AlH.

In all air–acetylene flames the principal silicon species was found to be gaseous SiO. This is largely a result of the comparatively low flame temperature because even when the carbon:oxygen ratio exceeds unity the maximum degree of atomisation of silicon is less than 20%. The species SiO predominates in those nitrous oxide–acetylene flames in which the carbon:oxygen ratio is less than unity; this is especially apparent at the lower temperatures considered. However, in fuel-rich nitrous oxide–acetylene flames atomic silicon is the main silicon species even at low temperatures. The species $Si_2$ and $Si_3$ were not found to be present to any appreciable extent ($< 10^{-10}\%$).

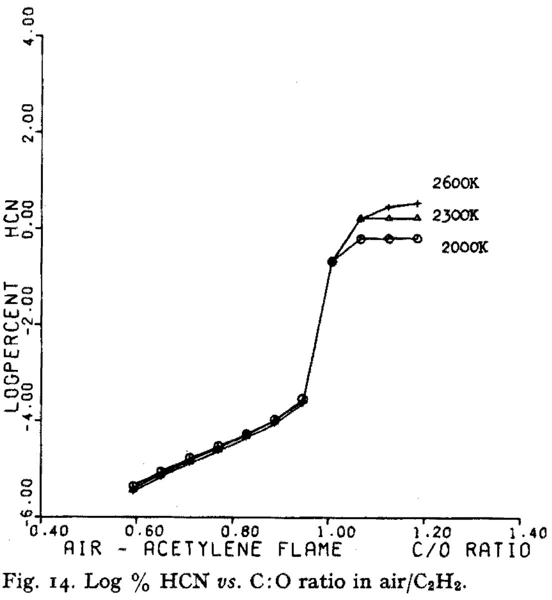

Fig. 14. Log % HCN vs. C:O ratio in air/$C_2H_2$.

An anomalously high isothermal concentration of atomic aluminium was found over the temperature range 2400–3300°K in the stoichiometric and even fuel-lean nitrous oxide–acetylene flames examined. This is a result of the absence of entrained oxygen from the air in these "ideal" systems which in practice resemble flame-shielded flames. Similarly, the computer calculations indicate that the degrees of atomisation of both aluminium and silicon in fuel-rich ($>70\%$) air–acetylene flames (the lowest temperature studied was 2000°K) is sufficiently high for these elements to be determined by techniques such as atomic absorption spectroscopy. However, as

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* The maximum calculated temperatures[8,9] for the $N_2O/C_2H_2$ and air/$C_2H_2$ flames are *ca.* 3230°K and *ca.* 2500°K respectively. The maximum experimentally determined temperatures of analytically useful fuel-rich $N_2O/C_2H_2$ [10,11] and air/$C_2H_2$ flames are *ca.* 2900°K and 2450°K respectively.

before, such flames are considerably more fuel-rich than their practical counterparts using the same oxidant and fuel gas flow rates. The high acetylene flow rate required in practice to produce the above "ideal" fuel-rich ( >70%) air–acetylene flames would result in a low flame temperature (< 2000°K). This in turn would probably result in a negligible degree of atomisation of these elements.

TABLE I

SPECIES CONSIDERED IN THE COMPUTER CALCULATION

| *Flame gas species* | *Flame gas species* | *Flame gas species* | *Metal species* | *Metal species* |
|---|---|---|---|---|
| C | $CH_2O$ | $O_2$ | Al | $Al_2O_3$[b] |
| C(Solid) | CO | OH | AlH | Si |
| $C_2$ | $CO_2$ | N | AlO | SiO |
| $C_3$ | CN | $N_2$ | AlC | $SiO_2$ |
| CH | $C_2N_2$[a] | NH | AlN | $SiO_2$ (liquid) |
| $CH_2$ | H | $NH_2$ | $Al_2O$ | $Si_2$ |
| $CH_3$ | $H_2$ | NO | AlOH | $Si_3$ |
| $C_2H_2$ | HCN | $N_2O$ | $AlO_2H$ | SiC |
| CHO | $H_2O$ | $NO_2$ | $Al_2O_2$ | |
| | O | | | |

[a] Not considered in air–acetylene flames.
[b] Solid below 2700°K, liquid above.

The absolute values obtained as a result of the calculations presented in this study are not strictly comparable with results obtained in practice for those reasons stated above. However, it is considered that the results obtained by this computer method show both qualitative and quantitative trends and general comparisons between these "ideal" flames and many analytically useful premixed flames are possible and are of value. Work is proceeding on the evaluation of a number of flame types with respect to the degrees of atomisation of several elements introduced together into the flames burning under a variety of different conditions.

Two of us (J.E.C. and M.R.G.T.) are indebted to the Welding Institute and the Ministry of Technology respectively for financial support to carry out this work.

## SUMMARY

A computer method based on the principle of the minimisation of free energy is used to calculate the concentrations of both flame gas and condensed species in flames in thermal equilibrium. The degrees of atomisation of aluminium and silicon are calculated for various fuel-lean, stoichiometric and fuel-rich nitrous oxide–acetylene and air–acetylene flames over a range of temperatures and are found to be both critically dependent on the carbon:total oxygen ratio. When this ratio exceeds unity, the degrees of atomisation are a maximum. The concentrations of 43 species and their dependence on the concentrations of atomic aluminium and silicon are considered, and a comparison is made with the nitrous oxide–acetylene and air–acetylene premixed flames used in practice.

## RÉSUMÉ

Une méthode "computer" basée sur le principe de la minimisation d'énergie libre est utilisée pour calculer les concentrations des gaz des flammes gazeuses et des particules condensées dans des flammes, en équilibre thermique. Les degrés d'atomisation de l'aluminium et du silicium ont pu être déterminés pour divers mélanges (oxyde nitreux–acétylène, air–acétylène).

## ZUSAMMENFASSUNG

Ein Computer-Verfahren, das auf dem Prinzip der kleinsten freien Energie beruht, wird benutzt, um sowohl Flammengas- als auch kondensierte Teilchen-Konzentrationen in Flammen im thermischen Gleichgewicht zu berechnen. Die Atomisierungsgrade von Aluminium und Silicium werden für verschiedene brenngasarme, stöchiometrische und brenngasreiche Lachgas–Acetylen- und Luft–Acetylenflammen über einen Temperaturbereich berechnet; sie erweisen sich beide als kritisch abhängig vom Kohlenstoff:Gesamt-Sauerstoff-Verhältnis. Wenn dieses Verhältnis eins überschreitet, sind die Atomisierungsgrade maximal. Die Konzentrationen von 43 Teilchen und deren Einfluss auf die Konzentrationen von atomarem Aluminium und Silicium werden behandelt, und vorgemischte Lachgas–Acetylen- und Luft–Acetylenflammen, wie sie in der Praxis benutzt werden, werden miteinander verglichen.

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# STUDIES ON AQUEOUS SOLUTIONS OF ESSENTIAL OILS

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Various biological effects have been described after accidental or experimental intake of essential oils, both in man and animals[1-8]. A study of the toxic effects of various essential oils on preparations in tissue culture was therefore planned. Aqueous solutions are readily incorporated into the medium, and are distributed uniformly to the cells. This eliminates the use of organic solvents or carriers for the preparation of test solutions which might interfere with processes of growth and maturation of the tissues in culture. However, a careful search of the literature did not produce any definite values on the degree of water solubility of these substances, and were therefore established in this laboratory.

## EXPERIMENTAL

### *Materials and methods*

The essential oils used were either obtained from commercial sources or prepared for research (see Table II).

### *Preparation of solutions*

Oil and distilled water were mixed by shaking in a separatory funnel. Oil was in excess and separated grossly from the aqueous phase on standing for 16 h or more. The aqueous phase was centrifuged for 30 min at 2500 rpm at room temperature in an IEC centrifuge. Floating oil droplets were removed and the supernatant solution was transferred to clean tubes and recentrifuged under the same conditions twice more. For oils lighter than water (anethole and estragole) an additional 1-h funnel separation was done before the last centrifugation.

Experiments were conducted in which hot distilled water and preheated separatory funnels were used. Since no differences in results were found, this procedure was discontinued. Preparations made by the above-mentioned method had an oil content above the stable concentration level. The following methods were tried in order to eliminate the excess.

1. Adsorption on glass beads. Three passages over 6.5 g of glass beads of 2.5-mm diameter. This was done routinely for all solutions except eugenol and isoeugenol.

2. Centrifugation. (*a*) Spinco Centrifuge, not refrigerated, at 40,000 rpm for

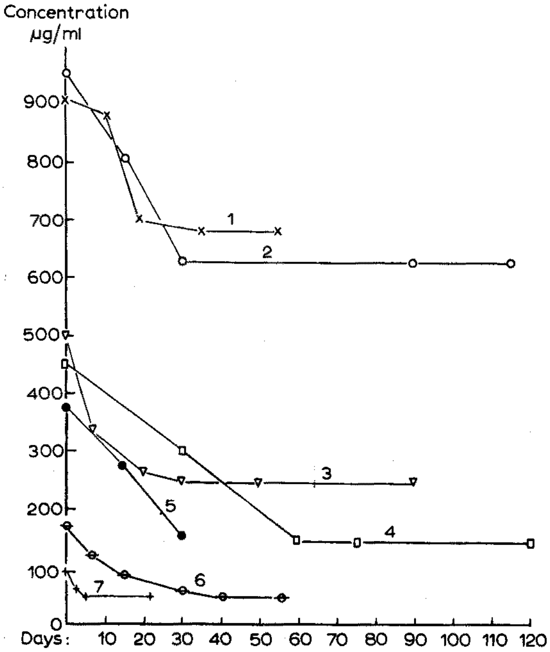
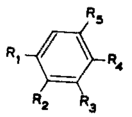


Fig. 1. Concentration range of essential oil aqueous solutions. (1) Elemicin; (2) eugenol, diluted 1:2; (3) estragole; (4) isoeugenol, diluted 1:2; (5) isoeugenol, diluted 1:3; (6) myristicine, (7) safrol.

TABLE I

CONCENTRATION RANGES FOR AQUEOUS SOLUTIONS OF ESSENTIAL OILS

| *Essential oil* | | | | | | *Level of stable concentration*[b] | | | *Concentration range and period of standing* | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Name* | *Structure*[a]  | | | | | | | | *Rubber stoppered tubes* | | *Glass stoppered tubes* | |
| | $R_1$ | $R_2$ | $R_3$ | $R_4$ | $R_5$ | $\bar{x}$ | $s$ | $v$ | *Concn. (μg ml$^{-1}$)* | *Time (days)* | *Concn. (μg ml$^{-1}$)* | *Time (days)* |
| Estragole | Al | — | — | MeO | — | 245.5 | 18.3 | 7.44 | 500–230 | 20 | 280 | 180 |
| Anethole | Pr | — | — | MeO | — | | | | 59–2.5 | 90 | 52 | 80 |
| Eugenol methyl ether | Al | — | MeO | MeO | — | | | | 630–100 | 75 | 340 | 165 |
| Isoeugenol methyl ether | Pr | — | MeO | MeO | — | | | | 350– 83 | 63 | 336 | 145 |
| Elemicin | Al | — | MeO | MeO | MeO | 681.3 | 38.6 | 5.66 | 913–675 | 30 | 837 | 195 |
| Asarone | Pr | MeO | — | MeO | MeO | | | | 933–700 | 55 | 670 | 100 |
| Safrol | Al | — | –$OCH_2O$– | | — | 54 | 4.5 | 8.25 | 92– 50 | 5 | 84 | 16 |
| Isosafrol | Pr | — | –$OCH_2O$– | | — | | | | 33.4– 2.2 | 30 | 22 | 180 |
| Myristicine | Al | — | –$OCH_2O$– | | MeO | 48 | 3.4 | 7.15 | 166– 42.5 | 40 | 150 | 90 |
| Iso-myristicine | Pr | — | –$OCH_2O$– | | MeO | | | | 61– 6.25 | 50 | 59 | 160 |
| Eugenol | Al | — | MeO | OH | — | 627 | 38.8 | 6.19 | 2000–600 | 115 | 1800 | 155 |
| Isoeugenol | Pr | — | MeO | OH | — | 147.5 | 6.9 | 4.7 | 1200–141 | 120 | | |

[a] Al = allyl: $-CH_2-CH=CH_2$, Pr = propenyl: $-CH=CH-CH_3$, MeO = methoxy.
[b] $\bar{x}$ = average, $s$ = standard deviation, $v$ = coefficient of variance.

1 h, in polypropylene or polyallomer tubes. (*b*) IEC Centrifuge, Model UV, at 1500 rpm for 30 min in Filterfuge stainless steel tubes with 2 Millipore filters of the MF type with pore size of 0.22 μm, diameter 25 mm.

3. Filtration. (*a*) Millipore filter as above, in Micro Syringe holder; the volume filtered was 2 ml. (*b*) Fritted Glass, F (fine) and UF (ultrafine) filters, 2–5 cm diameter; the volumes filtered were 2–5 ml.

Gravimetric solutions were prepared as follows. Oil in weighed amounts was mixed in glass-stoppered flasks with the necessary volume of distilled water to make solutions of the desired concentration, and then shaken on a wrist-action mechanical shaker for 30 min.

Absorbance curves were recorded for each oil, on a Beckman Model DK 2 Spectrophotometer, at the beginning of the experiments and after various intervals of standing, with and without access of air.

### *Determination of concentration*

Equal volumes of the aqueous solution of oil and carbon tetrachloride were shaken in separatory funnels with teflon stopcocks. After separation, the carbon tetrachloride fraction containing the heavy oil was collected. The absorbance was read on a Beckman Model DU Spectrophotometer in ultraviolet light at the wavelength of peak absorbance for each oil, and the concentrations were calculated from standard curves.

## RESULTS

The solutions prepared by the above-described method still contained oil in excess. The excess separated from the solution either by sedimentation of oil droplets or by volatilization and escape through the rubber stoppers covering the tubes, or both during relatively long periods of standing. The process was greatly prolonged by minimizing volatilization in glass stoppered tubes. Figure 1 shows the decrease in concentration (μg $ml^{-1}$) of the solutions stored in rubber-stoppered glass tubes as a function of time (days). The high values represent the average concentrations after preparation (see *Methods*). Elemicin, eugenol, isoeugenol, estragole, safrol and myristicine reached a concentration level which remained stable for 20 days or more. For the other oils, the experiments were interrupted before the solutions reached a stable level, and the concentrations continued to decrease at a slow rate.

Table I contains the concentration range, *i.e.* the highest average concentrations (μg $ml^{-1}$) after preparation of the solution and the lowest average values reached, either at the stable concentration level or at the termination of experiments. The average period of time (days) to reach the stable concentration level or the lowest values mentioned is also indicated. Standard deviations and coefficients of variance were calculated for the oils which reached a stable concentration level. Concentrations maintained for 100 days or more in glass-stoppered tubes are mentioned in order to indicate the effect of volatilization.

Table II includes the expected and actually obtained concentrations of gravimetric solutions. The solutions of asarone were unsatisfactorily cloudy and unstable above 50 μg $ml^{-1}$, although the solutions made by the usual procedure were clear at markedly higher concentrations. Changes in absorbance curves were observed in 6

TABLE II

CONCENTRATION OF GRAVIMETRIC SOLUTIONS

| *Essential oil* | *Source*[a] | *Gravimetric solutions* | | | *Peak wave-length (nm)* | *Changes in absorbance curves* |
|---|---|---|---|---|---|---|
| | | *Expected ($\mu$g ml$^{-1}$)* | *Obtained ($\mu$g ml$^{-1}$)* | *(%)* | | |
| Estragole | (K) | 50 | 46.5 | 93.0 | 279 | Wide peak with max. absorbance at 268 nm after 70 days but occasionally in fresh solutions |
| | | 100 | 92.5 | 92.5 | | |
| | | 150 | 142.5 | 95.0 | | |
| Anethole | (F) | 2 | 1.97 | 98.5 | 262 | Unchanged 70 days. Same shape but peak at higher wave-length (266 nm) after 150 days |
| | | 2.5 | 2 | 80.0 | | |
| Eugenol methyl ether | (F) | 250 | 236 | 94.0 | 283 | No change |
| | | 400 | 394 | 98.5 | | |
| Isoeugenol methyl ether | (F) | 30 | 28 | 94.5 | 260 | Blunt depression at 301 nm after 3 days. Peaks at 270 nm and 301 nm after 100 days |
| | | 60 | 55.1 | 92.0 | | |
| Elemicin | (Se) | 250 | 249 | 99.0 | 259 | No change |
| | | 500 | 488 | 97.0 | | |
| | | 650 | 675 | 104.0 | | |
| Asarone | (F) | 50 | 55 | clear | 259 | No change in shape or peaks for at least 73 days. At 99 days, one peak present at 300 nm |
| | | 100 | 123 | cloudy | | |
| | | 150 | 185 | cloudy | | |
| | | 700 | 613 | cloudy | | |
| Safrol | (C) | 20 | 18.1 | 90.5 | 290 | New small peak at 255 nm after 2 weeks. Gradual decrease in 290 nm peak, increase in 255 nm peak At 90 days, only peak at 255 nm |
| | | 40 | 38 | 95.0 | | |
| Isosafrol | (F) | 1.5 | 1.4 | 93.0 | 261 | No change |
| Myristicine | (S) | 10 | 9.7 | 97.0 | 256 | No change |
| | | 25 | 24.2 | 97.0 | | |
| Isomyristicine | (Se) | 4 | 3.75 | 94.0 | 271 | No change |
| | | 6 | 5.3 | 88.0 | | |
| Eugenol | (Fi) | 200 | 189.5 | 95.0 | 283 | No change |
| | | 400 | 382.5 | 95.5 | | |
| Isoeugenol | (F) | 70 | 62.3 | 89.0 | 261 | Blunt depression at 300 nm after 65 days. Main peak at 270 nm and depression at 300 nm after 140 days |
| | | 100 | 86 | 86.0 | | |

[a] (S) Dr. A. T. Shulgin, Dow Chem. Co.; (Se) Dr. T. A. Seto, Brooklyn State Hospital; (Fi) Fischer Sci.Co.; (F) Fritzsche Bros.; (K) K & K Labs. Inc.; (C) Cal. Biochem. Co.

of the 12 oils used after various periods of standing. Safrol and estragole presented occasionally a changed "aged" (spectrographically) appearance from the first day of preparation. Also mentioned in Table II are the sources of the oils used and the peak wave length at which the concentration readings were made.

Adsorption on glass beads reduced the concentration by 10 to 40%. The adsorption was great mainly with the low solubility oils, when the concentrations were far above the solubility level. In all the cases the excess was reduced only by a fraction, leaving the concentrations above the level of stable concentration (high values of the concentration range in Table I and Fig. 1).

Centrifugation in the Spinco centrifuge reduced the concentration to a certain extent. The reduction was inconsistent and did not reach the stable concentration level. Visible sediment was never found. The effect of the procedure also depended upon the type of tubes used. Polypropylene tubes grossly changed their appearance after use with elemicin solution; the reduction in concentration was striking and reached a level much below the stable concentration level (from 675 to 275 $\mu$g ml$^{-1}$). The tubes could not be cleaned and had to be discarded. A reduction below the stable concentration level occurred also for myristicine (60 to 26 $\mu$g ml$^{-1}$) and estragole (255 to 175 $\mu$g ml$^{-1}$), but to a lesser degree. The use of polypropylene tubes was then discontinued. With polyallomer tubes, the concentrations were reduced inconsistently but reached only to or above the values of the stable concentration, never below.

Centrifugation in Filterfuge tubes was made in relatively few instances, since it was found to parallel the results obtained by filtration through Millipore filters (see below) without presenting additional advantages.

Filtration through Millipore membrane, MF type, was effective in reducing the concentration to or below the stable concentration level, independent of the level of saturation. Serious doubts that the reduction in concentration was due to the filtration process itself were raised by the fact that agitation in a test tube containing the membrane and oil solution was sufficient to lower the concentration to the same values as filtration, or even more.

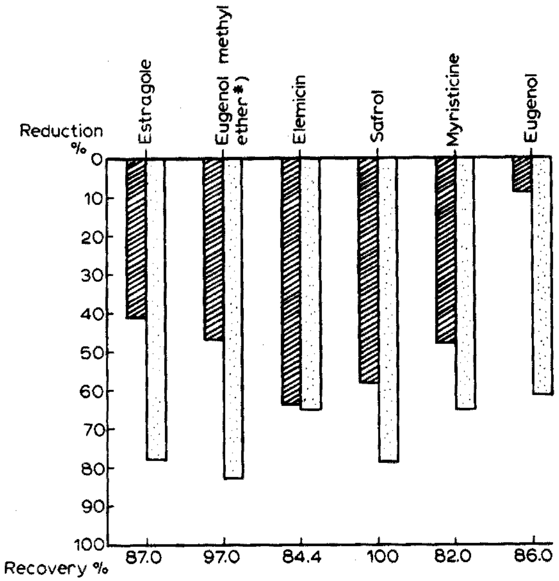


Fig. 2. Percentage of reduction in concentration by use of Millipore filters MF close to the stable concentration level (filtration or shaking), and of recovery from the Millipore filters. Shaded column, filtration; dotted column, shaking.
* From level of 240 $\mu$g ml$^{-1}$.

Figure 2 shows the percentage reduction in concentration by filtration and by shaking for 1 h. At the beginning of the experiments the solutions were close to the stable concentration level and the process reduced the concentration much below that level. The reduction was usually greater by shaking (62 to 80%) than by filtration (9 to 64%); only in the case of elemicin was the reduction identical with both processes.

The Millipore membranes, which were shaken for 1 h with the oil solutions, were blotted and shaken for 1 h with carbon tetrachloride. The oil thus recovered from the Millipore membrane amounted to 82–100% of the oil "lost" by the previous contact of the solution with the filter (Fig. 2).

Filtration through fritted glass filters reduced the concentrations inconsistently at high concentration. At the stable concentration level, filtration and/or shaking with the filter did not reduce the concentrations below that level in any of the 12 oils tested. The solutions filtered through the UF fritted glass filters are sterile, and thus can be incorporated into media for tissue culture.

## DISCUSSION

The aim of this work was to obtain and study saturated aqueous solutions of essential oils. Twelve oils were studied; for elemicin, eugenol, isoeugenol, estragole, safrol and myristicine, the study was pursued to completion. For asarone, eugenol methyl ether, isoeugenol methyl ether, isomyristicine, anethole and isosafrol, only partial results were obtained.

It was found that after the initial preparation of the solutions, a prolonged process of equilibration takes place, in which separation of fine oil droplets or volatilization, or both, are effective. An indication of the relative importance of either process was obtained by using glass-stoppered tubes which minimized volatilization; the concentrations remained unchanged for extensive periods of time in the case in which volatilization was the main means of escape (myristicine, elemicin, safrol, etc.) and reduction to a certain extent was observed where droplet formation was effective (*e.g.* asarone, eugenol methyl ether, eugenol, etc.) (Table I). The time necessary to reach a level of stable concentration was *ca.* 30 days for elemicin, estragole, myristicine; considerably shorter for safrol (3 days), and longer for eugenol and isoeugenol (over 100 days, if undiluted) (Fig. 1).

Several methods of reducing the excess of oil at an accelerated rate were tried. Centrifugation and filtration as such were both unsuccessful. From high concentrations, there was a reduction to relatively lower values by either method, but the rate was unpredictable, unreliable and it proved to be due to contact adsorption by the tube (glass, polyallomer, polypropylene) or the filter (fritted glass, Millipore). At stable concentration levels, glass and polyallomer tubes, as well as fritted glass filters were ineffective in further lowering the concentration, even by prolonged contact, shaking or filtration. In contrast, contact with the Millipore membrane of MF type (mixed cellulose esters) was effective in further reducing the concentration below the stable level by removing the oil from the solution. As demonstrated, the removal was not due to the filtration process (Fig. 2). For elemicin, filtration (short contact) was sufficient and as efficient as one hour of contact by shaking. For eugenol, filtration reduced the concentration by only 9% and shaking lowered it by 62% below solubility level. For eugenol methyl ether, estragole, safrol and myristicine, filtration reduced the concentration by 40–50% and shaking lowered it by 70–80%. Recovery of the oil from the Millipore membrane which extracted it, was possible by shaking the filter for 1 h with carbon tetrachloride with a recovery rate of 82–100%. No further studies were made to determine the nature of this process. There are indications that polypropylene might also have a similar effect, but its use was discontinued and not studied.

During long periods of standing, changes occurred in several of the oils in solution; these were detected by changes in their spectrographic absorption curves (Table II). A similar phenomenon was also observed by other methods by TRUITT *et al.*[9] and ZACKSO-SZASZ *et al.*[10].

Correlating structure and solubility in the 12 oils studied, it appears that in any pair of isomers of the allyl-propenyl structure, the propenyl compound has a lower solubility than the allyl isomer.

We wish to thank Dr. T. A. SETO for his valuable suggestions and for the supply of elemicin and isomyristicine. We are also indebted to Dr. A. T. SHULGIN for supplying myristicine.

## SUMMARY

The aqueous solutions of 12 essential oils were studied. A level of stable concentration, unchanged for at least 20 days, was obtained for elemicin, eugenol, isoeugenol, estragole, safrol and myristicine. Anethole, isomyristicine, eugenol methyl ether, isoeugenol methyl ether, asarone and isosafrol were only partially studied. Stable aqueous solutions filtered through UF fritted glass filters are sterile and can be tested in tissue culture. Propenyl isomers have lower solubility than the allyl counterparts. Changes occurred in several oils after various periods of standing, as observed by spectrophotometric absorption spectra. Millipore membranes of the MF type, and possibly polypropylene tubes were found to remove the oils from solution at concentrations which are otherwise stable.

## RÉSUMÉ

Un niveau stable de concentration qui n'a pas changé pendant au moins 20 jours fut obtenu avec l'élémicine, l'eugénole, l'isoeugénole, l'estragole, le safrole et la myristicine. L'anéthole, l'isomyristicine, l'eugénole méthyle éther, l'isoeugénole méthyle éther, l'asarone et l'isosafrole ont été étudiées seulement partiellement. Les solutions aqueuses stables, filtrées à travers le filtre de verre fritté UF sont stériles et peuvent être essayées dans la culture de tissus. Les isomères propényliques sont moins solubles que les isomères allyliques. On a put observer des changements dans plusieurs huiles, après des périodes de temps différentes, comme le prouvent les spectres d'absorption spectrophotométriques. Les membranes Millipores du type MF et probablement les tubes de polypropylène peuvent retirer les huiles d'une solution, même si les solutions sont à des concentrations stables.

## ZUSAMMENFASSUNG

Die wässrigen Lösungen von 12 ätherischen Ölen sind untersucht worden. Für Elemizin, Eugenol, Iso-eugenol, Estragol, Safrol und Myristizin wurde eine Höchstkonzentration ermittelt, die für wenigstens 20 Tage stabil blieb. Anethol, Iso-myristizin, Eugenol methyläther, Iso-eugenol methyläther, Asaron und Iso-safrol wurden nur teilweise untersucht. Stabile wässrige Lösungen ,die durch UF Glasfritten filtriert werden, sind steril und können in Gewebekulturen zu Testzwecken verwendet

werden. Propenyl-isomere haben eine niedrigere Löslichkeit als ihre Allyl Analoge. Nach verschiedener Zeitdauer des unberührten Abstehens fanden in mehreren Ölen Veränderungen statt, die sich in spektrophotometrischen Absorptionsänderungen ausdrückten. Millipore Membrane (MF Typ) und möglicherweise Polypropylen-Zentrifugenröhren führten zur Entfernung der ätherischen Öle aus der Lösung in Konzentrationen, die unter anderen Umständen stabil waren.

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# SHORT COMMUNICATIONS

## An infrared technique for studying the solid-state photolysis of coordination compounds

The use of reflectance spectroscopy to study the solid-state photolysis of coordination compounds was introduced by STEMBRIDGE AND WENDLANDT[1] in 1965. Since the photolysis reaction is essentially a heterogeneous surface-type reaction, the resulting reflectance curve gave information concerning the extent of the photochemical dissociation and also the identification of the photolyzed product or products. Although the method has been used in other investigations[2–5], it is rather limited for the identification of the photolysis products. White or colorless products cannot be identified because they lack a reflectance spectrum in the visible wavelength region plus the fact that the unphotolyzed substance sometimes obscures the spectra of the reaction products.

In an attempt to determine the reaction stoichiometry of the photolysis reaction, SIMMONS AND WENDLANDT[2] photolyzed a slurry of the compound contained in ethanol. Wet chemical analysis of the resulting products was used to determine the amount of each product present in the reaction. This method gave good results but it was rather time-consuming. More recently, D'ASCENZO AND WENDLANDT[5] irradiated coordination compound samples in a rotating quartz tube. Again, wet chemical analysis was used to determine the extent of the photolysis reaction.

A method that has been found to yield fast and accurate results is to irradiate samples of coordination compounds contained in potassium chloride pellets. Generally, all of the photolysis products as well as the initial compounds absorb in the infrared wavelength region. Even the presence of gaseous products such as carbon dioxide, which is trapped in the pellet, can be detected. The method is far less time-consuming than the wet chemical technique and it is much more sensitive for the identification of products than is diffuse reflectance spectroscopy.

### *Experimental*

Potassium chloride pellets, 1 cm in diameter, were prepared containing 0.5–1.5% by weight of the coordination compound. The pellets were irradiated for various time periods by a 200 V mercury lamp (George W. Gates Co.). Infrared spectra of the irradiated and non-irradiated pellets were recorded on a Perkin-Elmer Model 457 spectrometer.

To prevent secondary photolysis of the reaction products from taking place, a Pyrex glass filter was employed during the irradiation process.

*Preparation of* $K_3[Mn(C_2O_4)_3]\cdot 3H_2O$. This compound was prepared and analyzed by the method previously described[2].

### *Results and discussion*

The KCl pellet technique was applied to the solid-state photolysis of $K_3[Mn(C_2O_4)_3]\cdot 3H_2O$. This compound has previously been investigated by SIMMONS AND

WENDLANDT[2] who found that the photochemical dissociation reaction proceeded according to the equation:

$$2K_3[Mn(C_2O_4)_3 \cdot 3H_2O(s) \xrightarrow{h\nu} 2K_2[Mn(C_2O_4)_2](s) + K_2C_2O_4(s) + 2\,CO_2(g) + 6H_2O(g)$$

The photolysis reaction is well known and produces two gaseous and two solid products. Hence, it is an ideal system in which to illustrate the use of the KCl pellet technique.

The infrared spectra of pellets containing $K_3[Mn(C_2O_4)_3] \cdot 3H_2O$ and photolyzed $K_3[Mn(C_2O_4)]_3 \cdot 3H_2O$ are given in Fig. 1. The major absorption bands for each of these species plus the dissociation products are listed in Table I.

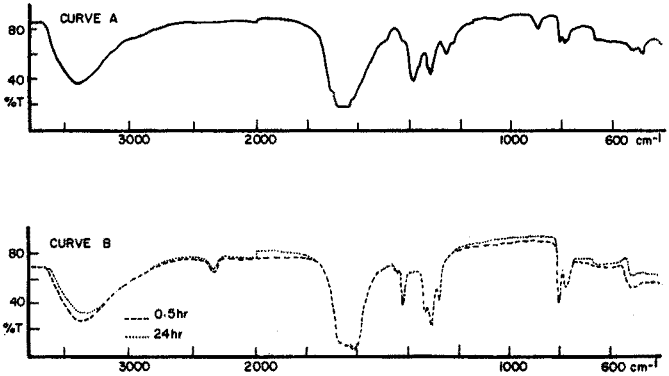

Fig. 1. (A) Infrared spectrum of $K_3[Mn(C_2O_4)_3] \cdot 3H_2O$ in a KCl pellet. (B) Infrared spectrum of an irradiated KCl pellet containing $K_3[Mn(C_2O_4)_3] \cdot 3H_2O$; irradiation times, indicated.

TABLE I

INFRARED ABSORPTION BAND FREQUENCIES OF COMPOUNDS IN KCl PELLETS

| *$K_3[Mn(C_2O_4)_3] \cdot 3H_2O$* | | *$K_2[Mn(C_2O_4)_2]$* | *$K_2C_2O_4 \cdot H_2O$* | *$CO_2$* |
|---|---|---|---|---|
| *Pure* | *Irradiated* | | | |
| | 2340[a] | | | 2344[b] |
| 1710 | 1690 | | | |
| 1676, 1653 | 1655, 1610 | 1660, 1615 | 1610 | |
| 1385 | 1420 | 1425 | | |
| 1310 | 1340, 1310 | 1340, 1310 | 1310 | |
| 1260 | 1288 | 1288 | | |
| 896 | | | | |
| 812, 793 | 803, 785 | 808, 785 | | |
| 775 | 772 | 770 | 772 | |
| | | 615 | 605 | |
| 529 | 525 | 523 | 525 | |
| 490 | | | | |

[a] All values in $cm^{-1}$.

[b] Reference 6.

The irradiation of pellets containing $K_3[Mn(C_2O_4)_3] \cdot 3H_2O$ results in shifts of the major absorption bands, owing to the formation of $K_2[Mn(C_2O_4)_2]$; the latter is a result of the photolytic reduction of manganese(III) to manganese(II) by the oxalate ion. Comparison of the spectrum of the photolyzed compound with the absorption bands of $K_2C_2O_4$ shows that this compound is also present. An absorption band at 2340 $cm^{-1}$ in the spectra of the irradiated pellet correlates nicely with the asymmetric stretching frequency of carbon dioxide, indicating that it is formed during the photolysis reaction and is trapped in the KCl pellet. Long periods of photolysis result in the photolysis of the decomposition products and perhaps interaction between the potassium chloride and the products, unless a Pyrex filter is employed to filter out the shorter wavelengths from the mercury lamps.

The pellet technique was also used to study the solid-state photolysis of $K_3[Co(C_2O_4)_3]\cdot 3H_2O$. This compound had previously been studied[3] by a slurry technique. Again, all of the decomposition products were present in the irradiated pellets, including carbon dioxide.

The KCl pellet technique is a simple, sensitive, and rapid method for the investigation of solid-state photolysis processes of coordination compounds. The reactions observed are the same as were found by reflectance spectroscopic techniques or by other wet chemical methods.

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# Vital parameters in the determination of rhodium by atomic absorption

In contrast to other spectroscopic methods, atomic absorption is relatively free from interference between the spectral lines of the elements; an exception is the interference of calcium hydroxide bands in the determination of barium[1,2]. Only atoms in the ground state are capable of absorbing radiation at the resonant wavelengths normally used in atomic absorption. Therefore interferences caused by the transfer of energy between atoms in the excited state are of consequence only when a large proportion of the atoms of the element to be determined is ionized. Ionized atoms are unable to absorb the resonance lines of the element of interest. Ionization can be suppressed by the presence of cations of low ionization potentials; the enhancement of the barium absorption by alkali metals is a typical example[3].

Anion interference is experienced in atomic absorption when compounds formed in the flame fail to dissociate into free atoms, *e.g.* the interference of phosphate in the determination of calcium and strontium.

In the case of rhodium absorption, certain enhancement and depressing effects which cannot be explained by the above phenomena, have been observed in this Laboratory. Since not much has been published on the effect of various cations and anions on the atomic absorption spectrophotometry of rhodium in a premixed air–acetylene flame, the tests described below were carried out. In these tests a 303 Perkin Elmer apparatus was connected to a 10-mV Sargent Recorder. Sample uptake in all cases was 3.5 ml $min^{-1}$.

## *Experimental*

*Fuel–oxidant mixture.* In line with the observations of HENEAGE[4], it was noticed that the absorption of the rhodium was substantially decreased when a fuel-rich mixture was used with a 3-slot laminar flow burner or a 1-slot nitrous oxide burner. It significantly increased when the flame was as lean and oxidizing as possible. Nitrous oxide–acetylene mixtures consistently gave low absorbance values, even in the presence of ionization-depressing alkali salts (Table I).

TABLE I

EFFECT OF OXIDANT–FUEL RATIO ON RHODIUM ABSORPTION AT 3435 Å[a]

(20 μg Rh $ml^{-1}$; 10% HCl medium)

| *Burner* | *Oxidant ($l\ min^{-1}$)* | *Acetylene ($l\ min^{-1}$)* | *Rhodium absorbance* |
|---|---|---|---|
| Three slot | Air 19.9 | 2.7 | 0.204 |
| Three slot | Air 19.9 | 3.1 | 0.164 |
| Three slot | Air 19.9 | 4.3 | 0.108 |
| One slot | Air 19.9 | 2.5 | 0.221 |
| One slot | Air 19.9 | 3.0 | 0.165 |
| One slot | Air 19.9 | 3.6 | 0.055 |
| One slot | $N_2O$ 3.18 | 6.3 | 0.062 |
| One slot | $N_2O$ 3.18 | 6.6 | 0.063 |
| One slot | $N_2O$ 3.18 | 7.2 | 0.062 |

[a] Similar observations were made at 3397 Å and 3692 Å.

*Burner height.* In agreement with the data reported by Deiley[5], it was also noticed that the distance between the bottom of the light beam and the top of the burner was critical. Thus the absorbance of 20 $\mu$g of rhodium as chloride per ml varied between 0.086 and 0.143 (fuel-rich flame) and from 0.17 to 0.21 (lean flame) when the burner height was varied from 1/8 to 1 in below the light beam.

The above observations allow the conjecture that rhodium is easily converted in a reducing environment to the metal (b.p. $>3700°$). It can also be assumed that this reduction could be prevented or at least minimized by the formation of stable rhodium salts.

*Effect of anions.* To establish optimum conditions for the formation of such stable rhodium salts which could be dissociated into free atoms, rhodium solutions were introduced in various media into the acetylene–air flame of a 3-slot laminar-flow burner in the absence of other cations. The data in Table II indicated that in most instances, in a fuel-lean flame, the various anions tested had comparatively little effect on the rhodium absorption. In all cases, however, depression of the absorption

TABLE II

ABSORBANCE VALUES FOR RHODIUM IN VARIOUS MEDIA AT 3435 Å

(Three-slot laminar burner; 20 $\mu$g Rh ml$^{-1}$)

| *Medium* | *Acetylene 2.7 l min*$^{-1}$ *Air 19.9 l min*$^{-1}$ | *Acetylene 3.1 l min*$^{-1}$ *Air 19.9 l min*$^{-1}$ | *Acetylene 4.3 l min*$^{-1}$ *Air 19.9 l min*$^{-1}$ |
|---|---|---|---|
| 1% HCl | 0.210 | 0.167 | 0.110 |
| 10% HCl | 0.204 | 0.164 | 0.108 |
| 3% HCl | 0.207 | 0.153 | 0.110 |
| 2% KCN | 0.206 | 0.164 | 0.120 |
| 2% $H_3PO_4$ | 0.185 | 0.117 | 0.052 |
| 2% $HNO_3$ | 0.212 | 0.164 | 0.140 |
| 2% $HClO_4$ | 0.215 | 0.167 | 0.136 |

TABLE III

ABSORBANCE VALUES FOR RHODIUM IN THE PRESENCE OF OTHER METAL CHLORIDES

(Three-slot laminar burner; 20 $\mu$g Rh ml$^{-1}$; 10% HCl medium)

| *Compound added (1 mg ml*$^{-1}$*)* | *Absorbance* | | |
|---|---|---|---|
| | *Acetylene 2.7 l min*$^{-1}$ *Air 19.9 l min*$^{-1}$ | *Acetylene 3.1 l min*$^{-1}$ *Air 19.9 l min*$^{-1}$ | *Acetylene 4.3 l min*$^{-1}$ *Air 19.9 l min*$^{-1}$ |
| — | 0.204 | 0.164 | 0.108 |
| $CuCl_2$ | 0.192 | 0.167 | 0.104 |
| $Cu(NO_3)_2$ | 0.154 | 0.134 | 0.096 |
| $NiCl_2$ | 0.149 | 0.132 | 0.084 |
| $CoCl_2$ | 0.147 | 0.136 | 0.084 |
| $FeCl_3$ | 0.196 | 0.167 | 0.104 |
| $ZnCl_2$ | 0.202 | 0.167 | 0.112 |
| NaCl | 0.200 | 0.165 | 0.124 |
| KCl | 0.200 | 0.167 | 0.122 |
| $MgCl_2$ | 0.196 | 0.160 | 0.126 |
| $CaCl_2$ | 0.194 | 0.161 | 0.120 |
| $CrCl_3$ | 0.197 | 0.162 | 0.128 |
| $AlCl_3$ | 0.204 | 0.163 | 0.129 |
| $K_2Cr_2O_7$ | 0.365 | 0.304 | 0.216 |
| $LaCl_3$ | 0.372 | 0.312 | 0.264 |
| $UO_2Cl_2$ | 0.390 | 0.342 | 0.302 |

became very pronounced in a fuel-rich flame, particularly in the case of phosphoric acid.

*Effect of cations.* The effect of various mono-, di- and multivalent cations, added to the hydrochloric acid solution of rhodium, was studied (Table III). In all but one case, cations had no, or only a slight, depressing effect on the rhodium absorption. The significant enhancement caused by potassium dichromate, lanthanum chloride and uranium chloride is probably due to a mechanism similar to that described below. Again, it was noted that in all cases the rhodium absorption was greatly enhanced by use of a fuel-lean flame.

*Effect of sulfates.* When various cations were introduced into the rhodium chloride solutions as sulfates, striking enhancement effects were observed in the case of the alkali metals, aluminum, and zinc, less enhancement in the case of the alkaline earths, and widely varying effects in the case of other cations (Table IV).

TABLE IV

ABSORBANCE VALUES FOR RHODIUM IN THE PRESENCE OF VARIOUS METAL SULFATES

(20 $\mu$g Rh ml$^{-1}$; 10% HCl medium)

| *Sulfate added (% w/v)* | | *Acetylene 2.7 l min$^{-1}$ Air 19.9 l min$^{-1}$* | *Acetylene 3.1 l min$^{-1}$ Air 19.9 l min$^{-1}$* | *Acetylene 4.3 l min$^{-1}$ Air 19.9 l min$^{-1}$* |
|---|---|---|---|---|
| | | 0.204 | 0.164 | 0.108 |
| $KHSO_4$ | 0.1 | 0.334 | 0.326 | 0.322 |
| $KHSO_4$ | 1 | 0.380 | 0.374 | 0.370 |
| $KHSO_4$ | 3 | 0.396 | 0.392 | 0.388 |
| $KHSO_4$ | 6 | 0.392 | 0.392 | 0.392 |
| $NaHSO_4$ | 3 | 0.396 | 0.394 | 0.392 |
| $Li_2SO_4$ | 3 | 0.398 | 0.397 | 0.396 |
| $(NH_4)_2SO_4$ | 3 | 0.232 | 0.228 | 0.134 |
| $ZnSO_4$ | 2 | 0.392 | 0.394 | 0.396 |
| $SrSO_4$ | 2 | 0.340 | 0.342 | 0.338 |
| $MgSO_4$ | 2 | 0.340 | 0.336 | 0.328 |
| $CaSO_4$ | 2 | 0.345 | 0.344 | 0.346 |
| $NiSO_4$ | 2 | 0.130 | 0.122 | 0.080 |
| $CoSO_4$ | 2 | 0.135 | 0.114 | 0.090 |
| $CuSO_4$ | 2 | 0.324 | 0.306 | 0.224 |
| $FeSO_4$ | 2 | 0.352 | 0.320 | 0.068 |
| $Fe_2(SO_4)_3$ | 2 | 0.334 | 0.284 | 0.060 |
| $Cr_2(SO_4)_3$ | 1 | 0.375 | 0.368 | 0.290 |
| $Al_2(SO_4)_3$ | 2 | 0.375 | 0.364 | 0.356 |

### *Discussion*

No ready answer can be offered at this time to explain the synergetic effect of alkali metals and sulfate. The enhancement is possibly connected with the formation of a well-defined rhodium alum, *e.g.* $K_2SO_4 \cdot Rh(SO_4)_3 \cdot 24H_2O$[6]. It could be conjectured that such products are formed when the rhodium solution is dried in the flame before dissociation. The rapid dissolution of rhodium in molten sodium or potassium hydrogen sulfate (among the platinum metals only rhodium and palladium are attacked) may be connected with both the rhodium alum formation and the stability of the latter in the flame. It can be assumed that the enhancement of the rhodium absorption by the alkaline earths, aluminum, and zinc is due to similar mechanisms. The stability of the rhodium compounds formed can be deduced from the fact that in the presence of the alkali metals, alkaline earths, aluminum, and zinc, differences in temperature and oxidation–reduction characteristics of flame (rich *vs.* lean flame) do not affect the rhodium absorption.

Nickel and cobalt sulfates have a strong depressing effect on the rhodium absorption, irrespective of the flame temperature, while copper sulfate enhances the rhodium absorption moderately. At low temperatures (fuel-rich flame) both iron(II) and iron(III) sulfate have a strong depressing effect. However, when an extremely hot oxidizing flame is used, the rhodium absorption is enhanced by a factor of almost six. It would appear that iron, either introduced into the flame as an iron(II) salt, or reduced in the flame from iron(III) to iron(II), has a strong reducing effect on the rhodium. On the other hand, in a hot oxidizing flame, iron, either introduced into the flame as an iron(III) salt, or oxidized by the flame from iron(II) to iron(III), protects the rhodium salt from reduction.

TABLE V

ELIMINATION OF DEPRESSING EFFECTS BY ADDITION OF HYDROGEN SULFATE

(20 $\mu$g Rh ml$^{-1}$; 10% HCl–3% $NaHSO_4$ medium)

| *Compound present* | *Acetylene 2.7 l min$^{-1}$ Air 19.9 l min$^{-1}$* | *Acetylene 4.3 l min$^{-1}$ Air 19.9 l min$^{-1}$* |
|---|---|---|
| — | 0.396 | 0.396 |
| $NiCl_2$ | 0.396 | 0.394 |
| $NiSO_4$ | 0.392 | 0.394 |
| $CoSO_4$ | 0.396 | 0.392 |
| $CuCl_2$ | 0.398 | 0.392 |
| $CuSO_4$ | 0.394 | 0.394 |
| $FeSO_4$ | 0.394 | 0.396 |
| $Fe_2(SO_4)_3$ | 0.392 | 0.394 |

The addition of potassium and/or sodium hydrogen sulfate not only enhances the rhodium absorption but effectively eliminates the depressing effects of other cations (Table V) and that of phosphoric acid. The effect of the platinum metals on the rhodium absorption is complex and will be covered in another paper.

The enhancing effect of potassium hydrogen sulfate on the absorption of rhodium was confirmed by the following: Mr. ANTRIM H. JONES of General Motors Research Laboratory, Detroit (Perkin Elmer 303), Mr. JOSEPH IACONETTI of Walker & Whyte, Inc., New York, N.Y. (Perkin Elmer 303) and Mr. LEWIS CASABONA of Isotopes Inc., Teledyne Co., Westwood, N.J. (Jarrel Ash 82-500). The cooperation of these gentlemen is acknowledged with thanks.

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## Reference electrode for electrochemical studies in dimethylformamide*

Dimethylformamide (DMF) has frequently been employed as a polarographic solvent both for organic and inorganic solutes. Although several electrodes have been suggested[1-3], there is still no standard reference electrode for this solvent. Recently, Marple[4] stated that a cadmium–cadmium chloride electrode should make a good reference electrode, but he did not report on its behavior in an actual polarographic analysis. In this laboratory the cadmium electrode was tested and modified to permit easy use with a variety of electrochemical methods. The electrode was successfully employed in a polarographic study of vanadium in DMF. The electrode was found to be stable and to give reproducible potential readings.

### *Reagents and apparatus*

The DMF was "Baker Analyzed" and was used without further purification. Cadmium metal, sodium chloride, and cadmium chloride were "Baker Analyzed" reagents. Triple-distilled mercury (Metalsalts Corp., Hawthorne, N.J.) and tetraethylammonium perchlorate (TEAP; Eastman Kodak) supporting electrolyte were used.

The cadmium chloride monohydrate was heated to 110° before use. The TEAP was recrystallized twice from water, then dried in a vacuum oven at 60°.

Polarographic measurements were made with a Sargent Model XXI Polarograph that had been modified for three-electrode polarography. Potential measurements were made with a Rubicon Precision Potentiometer.

Potential measurements were made in a modified H-cell. The cross-piece of this cell, which contained micro glass frits at either end, was provided with inlet and outlet tubes so that the contents of the salt bridge could be changed. For most studies a 3% agar-saturated potassium chloride salt bridge was employed. One compartment of the H-cell contained an aqueous saturated calomel electrode (S.C.E.); the other compartment held a solution of TEAP in DMF. The cadmium electrodes were placed in the DMF solution when potential measurements were made.

A cadmium amalgam half-cell was constructed according to the directions of Marple[4]. The most successful design of the modified electrode consisted of a piece

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* Taken in part from the Ph.D. Dissertation of Charles W. Manning, University of Maryland, 1970.

of ¼-inch diameter "thirsty glass" (porous Vycor, Corning Glass Co., Corning, N.Y.) forced into the end of plastic tubing that was unaffected by DMF. A platinum wire sealed in glass was used for electrical connection. The half-cell filling recommended by MARPLE proved unacceptable. The liquid mercury was found to coalesce and form a pool at the bottom of the electrode assembly. Under these conditions the cadmium amalgam and the cadmium ions in the bulk of the electrode were not in the intimate contact required to maintain a constant potential. To remedy this situation, enough cadmium was added to the mercury to make a thick slurry without producing a solid. Into this slurry was mixed sodium chloride and cadmium chloride, and the mixture was placed in the electrode assembly over a piece of glass wool. The platinum contact was then forced into this slurry. A saturated solution of cadmium chloride and sodium chloride in DMF was added and the plastic tube was sealed at the top around the glass tubing. The electrode was then allowed to equilibrate in a DMF solution of TEAP.

### *Results and discussion*

The potential of the MARPLE electrode was measured against an S.C.E. under working conditions. The S.C.E. and the cadmium electrode were connected through a 680 kΩ resistor which allowed 1 μA of current to flow. At this current level, the potential of the cadmium electrode drifted about 3.4 mV in 15 min. Runs on different days produced slightly different cadmium half-cell potentials. The average value of the potential was −0.7866 V with a standard deviation of 3.59 mV.

The modified electrode was tested in two days, by measuring its potential against an S.C.E. and by observing for six months the consistency of the polarographic half-wave potential of vanadium in DMF when the cadmium electrode was employed as the reference. Table I shows the potentials for the modified cadmium electrode measured 10, 13, and 16 days after fabrication. The large variation between days is due to different liquid-junction potentials between the S.C.E. and the cadmium half-cell on different days. The standard deviation between days is much larger than between electrodes and corresponds to the standard deviation between days found with the MARPLE electrode. Since all potential measurements were made in a short period of time without disturbing the H-cell contents, the liquid-junction potential should remain constant for a single day but could vary from one day to the next.

TABLE I

POTENTIAL OF THE MODIFIED CADMIUM ELECTRODE *vs.* S.C.E.

| | *Potential at 10 days (V)* | *Potential at 13 days (V)* | *Potential at 16 days (V)* |
|---|---|---|---|
| | −0.7405 | −0.7316 | −0.7336 |
| | −0.7415 | −0.7334 | −0.7334 |
| | −0.7460 | −0.7370 | −0.7340 |
| | −0.7404 | −0.7327 | −0.7350 |
| Average | −0.7421 | −0.7337 | −0.7340 |
| s.d. (mV) | 2.65 | 2.34 | 0.71 |
| Average between days: −0.7366 V | | | |
| s.d. between days: 4.76 mV | | | |

The modified electrode proved most satisfactory as a reference in a polarographic study of vanadium in DMF. The half-wave potentials were consistent and agreed closely with the potentials given by MICHLMAYR AND GUTMANN[5], who reported a half-wave potential of $-2.03$ V *vs.* S.C.E. Over a six-month period, the half-wave potential for the same reduction against the cadmium reference electrode was $-1.31$ V with a standard deviation of 14 mV. Conversion to an S.C.E. reference gave a half-wave potential value of $-2.05$ V.

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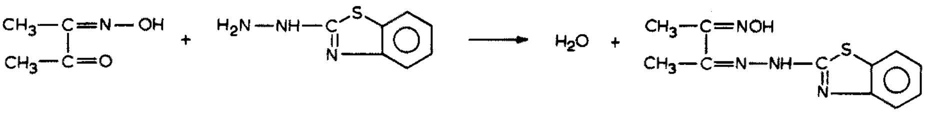
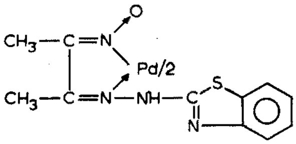
## New specific reagent for palladium

In spite of the large number of good reagents for the identification of palladium[1], a new reagent deserves description because it approaches very closely to an ideal reagent. This new reagent is a colorless compound which is easily obtained by the condensation of diacetylmonoxime and 2-hydrazinobenzothiazole:



It reacts sensitively with palladium over a surprisingly wide range of pH—from concentrated hydrochloric acid to concentrated ammonia solution—yielding a red-violet complex compound which can be extracted into chloroform.

In analogy to nickel-dimethylglyoxime, it seems likely that the inner complex salt presents the formula:



Solutions of the following ions were treated with an alcoholic solution of the reagent in alkaline and acid medium: aluminum, antimony, arsenic, barium, beryllium, bismuth, cadmium, calcium, cerium, cesium, chromium(III), cobalt, copper, gallium, germanium, gold, indium, iron, lead, lanthanum, lithium, magnesium, manganese, mercury, molybdenum, nickel, niobium, platinum, potassium, rhodium, ruthenium, silver, sodium, strontium, tantalum, thallium, thorium, tin, titanium, tungsten, uranium, vanadium, zinc and zirconium. Disregarding the formation of hydroxide precipitates, only cobalt in alkaline medium produces a red compound (limit of identification 0.5 $\mu$g); in acidic solution none of the above ions interferes.

*Reagents*

An alcoholic 0.1% solution of diacetylmonoxime–benzothiazolehydrazone is used. The reagent may be obtained by heating for 15 min equimolar solutions of 2-hydrazinobenzothiazole and diacetylmonoxime. The precipitate formed is filtered and washed with distiled water.

*Procedure*

A drop of alkaline or acid solution is treated in a micro test tube with 3–4 drops of the reagent. In presence of palladium a red-violet compound is formed which can be extracted with 5 drops of chloroform.

*Limit of identification.* 0.25 $\mu$g palladium.

*Limit of dilution.* 1:200.000.

In the presence of cobalt the reaction must be made in acidic media. Fluoride, tartrate, citrate and oxalate do not interfere; only cyanide prevents the reaction.

It is possible to detect palladium in alloys by treating a small amount of the sample with 2–3 drops of aqua regia and then with a few drops of the reagent.

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# Evaluation of light-scattering data from dilute protein solutions

Light-scattering, although less used at present than ultracentrifugation, is an elegant method for determining the molecular weight of proteins. Two methods are available: (*a*) ZIMM's method[1], which requires extrapolation to zero angle of measurements of scattered light at several angles, and (*b*) the asymmetry method[2] in which the turbidity (a function of the molecular weight) is calculated from measurements of scattered light at 0° and 90° to the incident beam and corrected by use of the particle scattering factor (a function of molecular asymmetry) which is obtained from the ratio of scattered light measured at 45° to that measured at 135°.

The problem of correction for the light scattered by the solvent is encountered in both methods. The procedure to be described for making this correction can be used with either method, although the following description is of the asymmetry method.

The molecular weight of a protein as calculated from light-scattering data obtained from dilute solutions of the protein is very sensitive to small errors in the values of the turbidity of the solvent and the protein solution. This is because the molecular weight is calculated from the function

$$Hc/(\tau_s - \tau_d)$$

where $H$ is a constant, $c$ is concentration of protein, $\tau_s$ is the turbidity of the protein solution, and $\tau_d$ is the turbidity of the solvent. At low protein concentrations, $\tau_d$ is nearly equal to $\tau_s$, so that small errors in either will have a large effect on the difference between them. To detect and eliminate the effect of errors in $\tau_d$ on the value calculated for the molecular weight, it is helpful to plot $\tau_s$ against concentration. Extrapolation of this plot to zero concentration gives an independent estimate of $\tau_d$. From this plot, one can also determine whether or not the concentration dependence of $Hc/(\tau_s - \tau_d)$ is compatible with that of $\tau_s$.

To illustrate the comparison of the concentration dependence of the two functions and of the extrapolation, $\tau_s$ is plotted in Fig. 1A and $Hc/(\tau_s - \tau_d)$ is plotted in Fig. 1B. The values of $\tau_s$ are for solutions of myosin from dog hearts. The preparation of the solutions and the technique of measurement have been described[3]. Attention is called to the fact that after each protein solution was made up to the desired concentration, it was clarified by filtration or centrifugation before being placed in the light-scattering cell for measurement. After the scattered light had been measured, the protein concentration was determined from the absorbancy at 280 nm. The technique described herein is applicable to data from experiments in which this scheme is followed.

First, the value of $\tau_d$ obtained by extrapolating the plot of $\tau_s$ to zero concentration (Fig. 1A) is lower than the measured values (X on ordinate Fig. 1B). This approach is similar to that used by FESSENDEN AND STEIN[4] to determine the turbidity of water. After this, more exhaustive clarification was performed on the solvent samples in later experiments. It was then possible to obtain by direct measurement values of $\tau_d$ in agreement with those obtained by extrapolation.

Also the plot of $\tau_s$ is linear and $d\tau_s/dc$ is constant over the concentration range studied, indicating that the molecular weight is also constant over this range. Because of this relationship, it follows that a plot of $Hc/(\tau_s - \tau_d)$ based on the value of $\tau_d$ obtained by extrapolation of the values of $\tau_s$, will also be linear and will be consistent with the values of $\tau_s$, for it will also indicate a constant molecular weight over the concentration range studied. However, this is not the case for the measured values of $\tau_d$ (Fig. 1B). They each give values of $Hc/(\tau_s - \tau_d)$ which represent a non-linear function of concentration and are thus inconsistent with the values of $\tau_s$. Such values of $Hc/(\tau_s - \tau_d)$ increase as concentration decreases and this could give an erroneous impression that molecular dissociation is taking place.

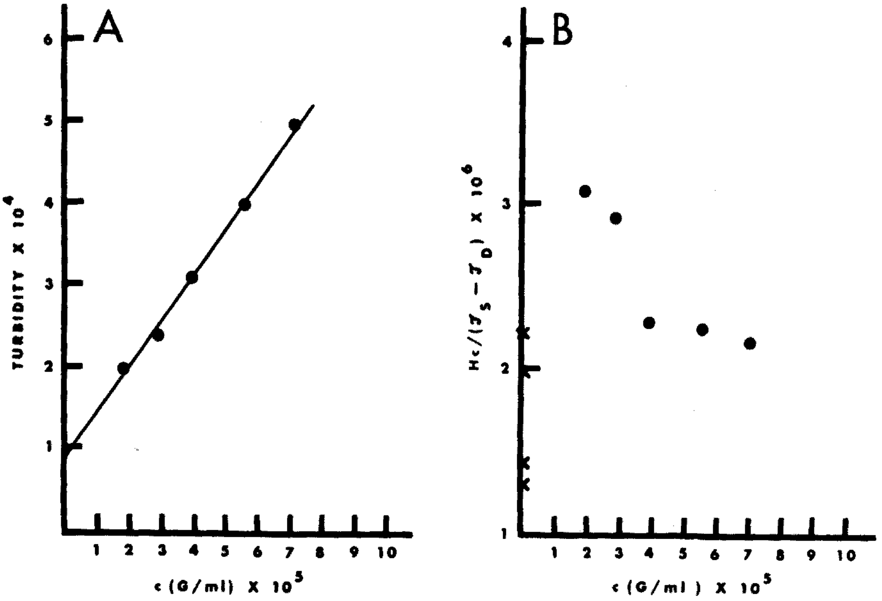

Fig. 1. In A, turbidities ($\tau_s$) of protein solutions are plotted against concentration. In B, the function $Hc/(\tau_s - \tau_d)$ is plotted against concentration. Here $(\tau_s - \tau_d)$ represents the net turbidity obtained by subtracting the measured turbidity ($\tau_d$) of the companion solvent from the turbidity ($\tau_s$) of the solution. Four measured values of $\tau_d$ are shown by X on the ordinate.

If $\tau_s$ were a markedly non-linear function of concentration, then the analysis for consistency would not be as direct or conclusive as the one made here, the extrapolation would be more hazardous, and if the extrapolation were made from data near an *inflection point*, the results could be very misleading.

In connection with the comparison of $Hc/(\tau_s - \tau_d)$ with $d\tau_s/dc$, one notes that as concentration approaches zero

$$\lim_{c \to 0}.\ [Hc/(\tau_s - \tau_d)] = H(dc/d\tau_s)$$

and thus if a reliable estimate of $dc/d\tau_s$ (or $d\tau_d/dc$) at zero concentration is available, then it is not essential to know the value of $\tau_d$.

Finally, when a statistical analysis of light-scattering data from dilute solutions of proteins is to be done, it will often be simpler from a statistical point of view to work with $\tau_s$ as a function of concentration rather than with the function $Hc/(\tau_s - \tau_d)$. In doing such an analysis of $\tau_s$, it should be kept in mind that the error will usually be a function of concentration and will also be biased toward positive values.

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# Spectrophotometric determination of cobalt with furildioxime

Reagents for the colorimetric determination of cobalt include nitroso-R salt[1], 1-nitroso-2-naphthol[2], thiocyanate ion[3], and rubeanic acid[4]. The use of 1-nitroso-2-naphthol entails a preliminary oxidation of the cobalt(II) to cobalt(III) in order to obtain reproducible results. The thiocyanate–cobalt equilibrium is not very favorable and the use of 50% acetone as solvent is essential to increase sensitivity by enhancing formation of the blue tetrathiocyanatocobaltate(II) ion.

Furildioxime was proposed as a reagent for the detection and determination of nickel by SOULE[5] in 1925. The interference of cobalt in the colorimetric determination of nickel suggested the use of furildioxime as a reagent for cobalt. A preliminary survey showed that quantitative reaction occurred at the 0–4 p.p.m. level to give a yellow color. There is no need to oxidize the metal to cobalt(III). In fact it is by oxidation of cobalt(II) that interference is avoided in nickel determinations[6]. This suggests that furildioxime is selective for the lower oxidation state of cobalt. The reagent has the advantage that it is considerably more soluble in water than rubeanic acid. The sensitivity of the proposed method is essentially the same as that which uses nitroso-R salt[2].

### *Apparatus*

Absorbance measurements were made with a recording Beckman DB spectrophotometer. A Corning Model 5 pH meter was used to determine pH.

### *Reagents*

*Standard 51.4 p.p.m. cobalt stock solution.* This was prepared from reagent-grade cobalt(II) sulfate, free of nickel and iron (G. Frederick Smith Chemical Co.).

*Furildioxime* (Eastman No. 3308) was used without purification to prepare a 1% (w/v) solution in 95% ethanol.

*Buffer solution.* This was prepared by adding sufficient 85% phosphoric acid dropwise to 0.10 *M* sodium monohydrogen phosphate to give a pH of 8.0.

Deionized water and reagent grade salts were used throughout the investigation.

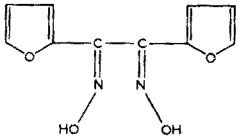
### *Procedure*

Pipet an aliquot of the sample containing 1.25–100 μg of cobalt into a 25-ml volumetric flask. Pipet 1 ml of furildioxime solution and 5 ml of buffer solution into the flask. Make up to volume with deionized water and mix well. Prepare a blank in the same manner but omit the cobalt solution. After 10 min read the absorbance of the sample at 350 nm in a 1-cm quartz cell. The calibration graph is linear in the range 0–4 p.p.m. of cobalt.

### *Results and discussion*

Preliminary results showed that the ligand (I) gave a yellow color with small amounts of cobalt. The principal absorption is in the ultraviolet with a broad tail extending into the visible.

The chemistry of the system was studied by the systematic investigation of time, pH, and ligand concentration. The amount of pH 8.0 buffer solution is not critical but the concentration of ligand must be carefully controlled in the blank because absorbance readings are taken on the foot of the wave exhibited by the ligand itself. The absorbance of the complex increases slightly during the first few minutes after mixing but readings are constant after 10 min.



(I)

Figure 1 shows that the pH should be kept within 0.5 pH unit of the value selected for use in order to insure satisfactory reproducibility. At pH values below 7 the yellow color of the complex was diminished markedly. This is attributed to insufficient active ligand which is probably an ionized form of the dioxime. As the pH is increased above 10 or 11, traces of a red-brown flocculent precipitate begin to form. This is attributed to metal hydroxide formation.

Generally, cobalt(II) complexes, especially those containing nitrogen donors, are very susceptible to air oxidation. The more alkaline the solution, the more this reaction is promoted[7]. It is noteworthy that absorbance readings were constant in this case.

By using the method of continuous variations[8] it was found that the complex is of the type Co(Ligand)$_2$. Considering the size and nature of the ligand, the metal is probably bound in a square planar configuration like cobalt(II) phthalocyanine[9] and cobalt(II) dimethylglyoxime[10]. This is speculative, however, because it is known that the $d^7$ configuration can assume either square planar ($dsp^2$) or tetrahedral ($sp^3$) symmetry depending on the environment. Proposal of an equilibrium mixture may be plausible in some cases. A distorted octahedral configuration has also been suggested as a possibility[9]. Such a structure would require promotion of a d electron to an outer 5s or 4d orbital. Two solvent molecules could then conceivably occupy the two, elongated, weakly-bonding sites of the metal. Magnetic moment measure ments would possibly aid in the elucidation of the structure.

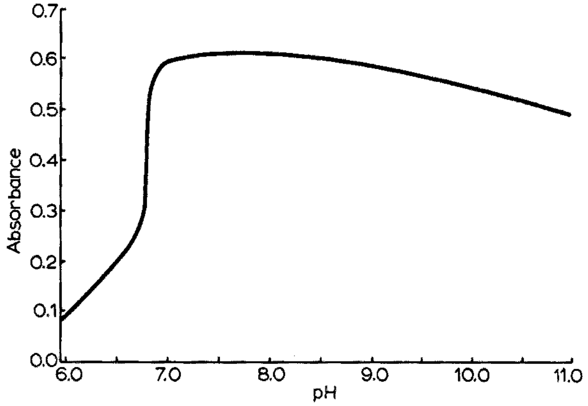


Fig. 1. Influence of pH on absorbance of cobalt(II)–furildioxime complex.

With the conditions described it was determined that Beer's law held over the range 0–4 p.p.m. The relative standard deviation of five determinations at the 0.2 p.p.m. level was ± 1.26% with a mean of 0.207 p.p.m. and at the 2 p.p.m. level was ± 0.70% with a mean of 2.02 p.p.m. The molar absorptivity at 350 nm is $1.84 \cdot 10^4$ l mole$^{-1}$ cm$^{-1}$.

Up to 700 p.p.m. of sodium, potassium, magnesium, and chloride, can be tolerated with negligible effect at a concentration of 2 p.p.m. cobalt. Ammonium ion at all concentrations above 5 p.p.m. gave a faint turbidity but the results in terms of cobalt analysis were very satisfactory up to an ammonium content of 700 p.p.m. The spectrum of one of these samples was essentially unchanged from that obtained in the absence of ammonium ion. The usual interferences from copper, nickel, iron(II), lead, and silver were encountered. In large part these problems arose from the generation of precipitates formed with the ligand or hydrous oxides caused by the necessity of working at pH 8.0. This problem can be overcome by a preliminary selective extraction of the cobalt with dithizone with carbon tetrachloride[11] at pH 5. Alternatively, extraction of cobalt from the interfering ions as thiocyanate may be used[12].

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# The determination of dysprosium in borate glasses*

Optically excited fluorescence of rare earths in glasses has already been described for the determination of gadolinium[1], samarium[2] and europium[3]; the fluorimetric method provides a rapid, non-destructive analysis. Small amounts of rare earths can be excited efficiently in glasses by monochromatic light. The optimal conditions for analytical determination of dysprosium in glasses were found from a detailed study of the excitation and emission spectra.

### *Apparatus*

The spectrofluorimeter used for the measurements of excitation and emission spectra was constructed in this laboratory[4]. It consists of a 500-W xenon arc (Mazda) lamp, interchangeable with a mercury medium-pressure 100-W lamp and a mercury high-pressure lamp which was introduced especially for the $Dy^{3+}$ experiments, an excitation monochromator (Bausch and Lomb, 250 mm focal length), a sample compartment, an analysing monochromator (Bausch and Lomb, 500 mm focal length) an EMI 9558QB photomultiplier connected through a 16 MΩ resistor to an EIL Vibron electrometer, and a Kipp and Zonen recorder.

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* This work was performed under NBS contract No NBS (G) 83.

Slow drifts in intensity were corrected by frequent measurements of a reference glass, a borate glass containing 1% (w/w) dysprosium. The fluorescence was viewed from the front (illuminated) side of the glass at an angle of 35°. The photomultiplier was cooled to −20° for all measurements, thus decreasing the dark current by about two orders of magnitude. The apparatus for the measurement of decay times was described earlier.[4]

### *Reagents*

Sodium tetraborate and boric acid, 99.5% purity (Baker Analysed Reagents) and dysprosium chloride (Research Chemical Nuclear Corporation of America, 99.9% purity) were used.

### *Preparation of glasses*

Dry dysprosium chloride, and borax and boric acid (123 g:62 g) were mixed in an electric vibrator in glass containers. The mixtures contained dysprosium in concentrations ranging from 10 to 20,000 p.p.m. Homogeneity was obtained after shaking for 10 min. These mixtures melted at temperatures lower than 1000° but clear transparent solutions resulted only after heating at 1100° for a least 15 min in platinum crucibles. Drops of the hot melt were allowed to fall directly on to a clean white glazed ceramic surface. The drop was placed inside the circumference of a 1-mm thick spacer ring and pressed with a second ceramic tile so as to obtain glasses of constant 1-mm thickness. A standardized procedure was followed for the preparation because variations of the annealing conditions affected the luminescence properties. The glasses were inserted into a holder with a circular opening of 12 mm diameter and their excitation and emission spectra were measured at room temperature.

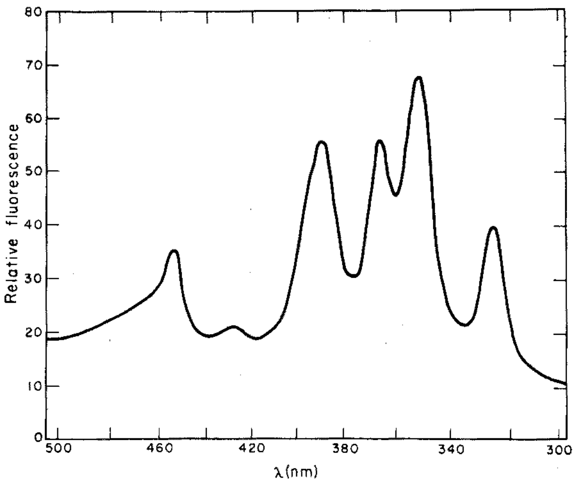

Fig. 1. Excitation spectrum for emission at 575 nm with a xenon arc.

*Excitation spectra*

The excitation spectrum of dysprosium(III) with xenon excitation (Fig. 1) consists of lines peaking at 325, 352, 366, 390, 428 and 454 nm. When the excitation was measured at 485 nm, the lines at 428 and 454 nm were obscured by the reflection of the 485-nm fluorescence. The line at 352 nm is the strongest. Table I shows the relative fluorescence and the experimental lines compared to the centres of gravity of dysprosium(III) energy levels in aqueous solutions. Assignment of the levels was taken from the work of CARNALL *et al.*[5] who correlated the experimentally determined band intensities with the theoretical expression in the S′L′J′ coupling scheme. The excitation spectrum for dysprosium in glasses obtained in this work is similar to that obtained by OZAWA AND NISHIKAWA[6] for dysprosium in yttria.

TABLE I

EXCITATION SPECTRUM AT 575 nm FLUORESCENCE AND THE ENERGY LEVELS OF DYSPROSIUM(III)[a]

| *Assignments of the levels*[5] | *Wave nos.*[b] *(cm⁻¹)* | *Wave nos.*[c] *(cm⁻¹)* | *Wavelength (nm)* | *Relative fluorescence* |
|---|---|---|---|---|
| $^6H_{15/2} \rightarrow {}^6P_{3/2}$ | 30803 | 30769 | 325 | 2650 |
| $^6H_{15/2} \rightarrow {}^6P_{7/2}$ | 28551 | 28409 | 352 | 5450 |
| $^6H_{15/2} \rightarrow {}^4M_{19/2}$ | 27219 | 27322 | 366 | 4300 |
| $(^4P,{}^4D)_{3/2}$ | 27254 | | | |
| $^6P_{5/2}$ | 27503 | | | |
| $^6H_{15/2} \rightarrow {}^4F_{7/2}$ | 25754 | 25641 | 390 | 4200 |
| $^4I_{13/2}$ | 25919 | | | |
| $^6H_{15/2} \rightarrow {}^4G_{11/2}$ | 23321 | 23364 | 428 | 800 |
| $^6H_{15/2} \rightarrow {}^4I_{15/2}$ | 22293 | 22026 | 454 | 2200 |

[a] The entrance/exit slits were 1.0 and 1.0 mm for excitation and 1.0 and 0.5 mm for emission. The applied voltage was 1250 V.

[b] Literature values[5].

[c] This work.

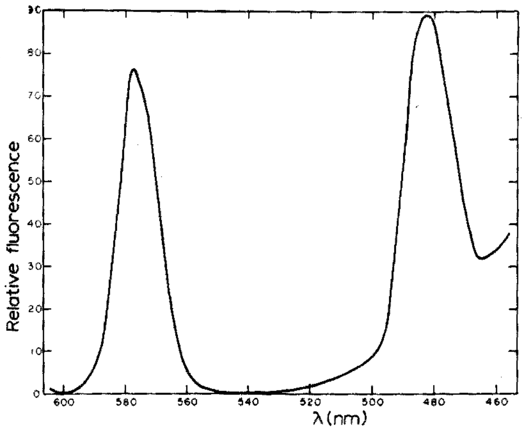

Fig. 2. Emission spectrum for excitation at 365 nm (mercury source).

*Emission spectra*

The emission spectrum of dysprosium(III) with excitation at 365 nm (mercury lamp) is presented in Fig. 2. With xenon excitation at the peak excitation wavelength of 352 nm, an identical spectrum was obtained with emission lines peaking at 485 and 578 nm. Table II gives the assignments of the emission spectrum measured at 365 nm (mercury source). This spectrum is similar to that obtained by Faria and Palumbo[7] for yttrium vanadate–dysprosium emission. The decay time of the 578-nm fluorescence was 1.2 msec for concentrations up to 1% dysprosium. This value decreased at higher concentrations, indicating quenching of fluorescence; this result is consistent with measurements of continuous fluorescence.

TABLE II

EMISSION SPECTRUM OF DYSPROSIUM(III) MEASURED AT 365 nm (Hg SOURCE)[a]

| | | |
|---|---|---|
| Wavelength (nm) | 485 | 578 |
| Wave nos. (cm$^{-1}$) | 20619 | 17301 |
| Assignment | $^4F_{9/2} \rightarrow {}^6H_{15/2}$ | $^4F_{9/2} \rightarrow {}^6H_{13/2}$ |

[a] The entrance/exit slits were 2.0 and 2.0 mm for excitation and 2.0 and 2.0 mm for emission. The applied voltage was 1250 V.

TABLE III

RELATIVE FLUORESCENCE AS FUNCTION OF CONCENTRATION MEASURED AT 578 nm AFTER EXCITATION AT 365 nm

| | | | | | |
|---|---|---|---|---|---|
| Concn. (p.p.m.)[a] | 11 | 55 | 109 | 546 | 1090 |
| Rel. fluor. | 6 | 18 | 40 | 168 | 500 |
| Concn. (p.p.m.)[b] | 546 | 1090 | 2110 | 4200 | 5000 |
| Rel. fluor. | 8 | 18 | 28 | 58 | 60 |
| Concn. (p.p.m.)[b] | 8290 | 10250 | 13460 | 17540 | 20430 |
| Rel. fluor. | 76 | 96 | 126 | 103 | 90 |

[a] The entrance/exit slits were 3.0 and 3.0 mm for excitation and 3.5 and 4.0 mm for emission. The applied voltage was 1250 V. High-pressure mercury source.

[b] The entrance/exit slits were 1.0 and 1.0 mm for excitation and 1.0 and 1.5 mm for emission. The applied voltage was 1400 V. Medium-pressure mercury source.

The concentration dependence of fluorescence with different lamps is presented in Table III; the high-pressure source was more sensitive and was used for the lower concentration range. A linear concentration dependence was observed in the range 10–13,000 p.p.m. Above these values quenching of the fluorescence took place.

TABLE IV

EMISSION LINES OF RARE EARTHS IN BORATE GLASSES

| *Rare earth ion* | *Wavelength (nm)* |
|---|---|
| Eu$^{3+}$ | 495, 577, 590, 600, 617, 655 |
| Sm$^{3+}$ | 560, 565, 601, 605, 647 |
| Tb$^{3+}$ | 488, 543, 547, 584[a], 627[a] |
| Dy$^{3+}$ | 485, 578 |
| Gd$^{3+}$ | 312 |

[a] Very weak lines.

*Conclusion*

In conclusion, the best procedure for analytical determination of dysprosium(III) in glasses, in the absence of other interfering rare earths such as $Tb^{3+}$, $Sm^{3+}$ and $Eu^{3+}$ which emit in the vicinity of 578 nm (Table IV) is the fluorimetric determination at this wavelength. The 485-nm fluorescence can be used if elements interfering with the 578-nm line are present. When terbium is present it can be determined also by excitation at 232 nm and the dysprosium concentration measured by difference.

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*Anal. Chim. Acta*, 51 (1970) 133–137

# ERRATA

LEO HARJU AND ANDERS RINGBOM, Titrations with Complexing Agents Forming Mononuclear and Binuclear Complexes with Metals, *Anal. Chim. Acta*, 49 (1970) 205–219.

Page 217, Fig. 12: To avoid misinterpretation, it should be mentioned that the two curves do not intersect; both curves have a break point at 3.76 ml.

LEO HARJU AND ANDERS RINGBOM, Compleximetric Titrations with Triethylenetetraminehexaacetic Acid, *Anal. Chim. Acta*, 49 (1970) 221–230.

Page 224, line 7 from below: for "back-titration with a *lead or zinc* salt solution" read "back-titration with a *lanthanum* solution".

Page 228, second and third line under the heading *Lead*: for $pPb_{eq} \approx 7.6$ read $pPb_{eq} \approx 6.0$.

H. RABER AND M. DIELACHER, Eine indirekte bromometrische Bestimmung von Harnsaüre–Xanthingemischen, *Anal. Chim. Acta*, 49 (1970) 198–200.

The third line from the bottom on p. 199 of the above paper should read:
% Harnsäure $= -693.0\ V_1/E_1 + 182.5$

# BOOK REVIEWS

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Alexis Volborth, *Elemental Analysis in Geochemistry. Part A. Major Elements*, Elsevier, Amsterdam, 1969, vii + 373 pp., price £ 7.

The present book is the eighth in the well-known Elsevier series on methods in geochemistry and geophysics. According to the author's preface it is intended to be a compilation of the basic and modern instrumental "macro" methods, with some emphasis on trace element determination. Trace elements are to be covered in detail in a second volume.

The treatment of the subject is very uneven, and several topics which might have been expected to be discussed in some detail have been omitted. Thus, the possibility of determining the major elements by the rapid photometric schemes of Shapiro and Brannock and others is not even mentioned. Indeed, the author seems to go to some lengths to avoid the use of photometric methods even for copper, nickel and zinc, preferring instead gravimetric procedures. Flame photometry and atomic absorption spectrophotometry are only discussed perfunctorily — the omission of the determination of calcium and strontium by the latter method is rather surprising. In contrast, the forty pages devoted to estimation of silicon and oxygen by fast neutron activation seems rather out of proportion to the general importance of this procedure.

The classical gravimetric and volumetric scheme of silicate analysis developed by Hillebrand and Washington occupies about one-third of the book. This covers not only the determination of the major elements but also that of a few minor elements, such as copper, nickel, zinc and fluoride. The methods described for the latter elements are more suitable for the analysis of ores than for trace analysis, although this is not stated! There are a few concessions to modernity in this section, for example, the use of EDTA titration for the determination of calcium and magnesium, the determination of potassium as its tetraphenylboron derivative, and the use of ion exchange as a substitute for the gravimetric separation scheme. The sections on the determination of each element are preceded by a trite and often inaccurate description of its occurrence and chemical properties. Thus, the reader will be surprised to learn (p. 110) that carbon is the main constituent of carbonate rocks!

A rather elementary chapter on the nature and origin of electro-magnetic radiation serves as an introduction to a 42-page chapter on X-ray fluorescence analysis. These two chapters and the excellent index are perhaps the best features of the book.

The printing and binding of the book is well up to the high standards set by Elsevier publications. However, the author's cumbersome and often pedantic literary style makes it heavy to read. Furthermore, the text abounds not only in printing mistakes, but also in errors of fact, particularly in the section on classical methods. For example all silver halogenides are said to be easily soluble in ammonia (p. 138); palau is not gold-plated platinum, but an alloy of palladium and gold (p. 268); alkaline earth metals do not hydrolyse in dilute hydrochloric acid medium (p. 200). The reviewer would be failing in his duty if he did not draw the readers' attention to the risk of explosion involved in heating ethyl, or butyl alcohols with perchloric acid (p. 95) — incidentally the analyst cannot expect rapid results for his analyses if he is to evaporate

6 ml of perchloric acid on the water bath! In view of all the above deficiencies it is only possible to give this book a lukewarm recommendation.

J. P. RILEY (Liverpool)

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R. N. ADAMS, *Electrochemistry at Solid Electrodes*, M. Dekker, New York, 1969, xiii+402 pp.

In this pleasant book, the art of the application of solid electrodes in the recording of current–voltage curves has found its master of description of practical problems and practical interpretation of the experimental results. The author belongs to the rare breed of electrochemists who do not forget that chemistry is an essential part of their trade. The book is based on the idea that adsorption is *not* the single or most important part of *every* electrode reaction, an idea with which the reviewer is in total agreement.

After a brief introduction, the author discusses the scope and limitations of solid electrodes and then turns to problems of mass transfer to stationary electrodes in quiet and flowing solutions and to periodically moving electrodes. Current–voltage curves are discussed in Chapter 5 for simple oxidation systems as well as those accompanied by chemical reactions. For slow voltage sweeps, rotated electrodes are recommended; for rapid voltage scanning, single sweep curves are preferred for analytical purposes, whereas cyclic voltammetry is recommended for electrochemical mechanistic studies. Chronopotentiometry is then dealt with, followed by a discussion of the effects of electrode history on electrode properties.

The second part of the book is devoted to experimental results. First, the techniques used in the elucidation of an organic electrode process are discussed, and the limitations of electrodes, cells and instruments are described. Individual organic systems are discussed in the last chapter, which shows that just as the dropping mercury electrode is used predominantly for reduction processes, so the solid electrodes have predominated in oxidation studies, in accordance with the available potential ranges for each type of electrode. Some of the mechanisms indicated might be disputed and it is also arguable that confusion may arise from the use of the term polarographic curves for current–voltage curves obtained under non-potentiostatic conditions with solid electrodes. The historical introduction somehow misses the effect that the introduction of the dropping mercury electrode had on studies of $i$–$E$ curves with solid electrodes.

This book can be highly recommended to analytical chemists interested in determinations of compounds (predominantly organic) which can undergo electrooxidation.

P. ZUMAN (Potsdam, N.Y.)

*Ion-Selective Electrodes*, Edited by R. A. DURST, National Bureau of Standards Special Publication No. 314, U.S. Government Printing Office, Washington, D.C. 20402, 1969, xxii + 452 pp., price $3.50.

Ion-selective electrodes are providing indubitably the most exciting developments in analytical chemistry at the present time. Although special glass electrodes for monovalent cations have been available for some time, it is only about 4 years ago that the first of the new breeds of electrode came on the market. Since then, reactions to their usage have varied from the ecstatic to the disillusioned. Some electrodes, such as the single-crystal lanthanum fluoride electrode, have confirmed their initial promise whereas others, such as the original calcium liquid exchanger electrode, have been superseded.

The book under review, which comprises the proceedings of a Symposium organized by the Analytical Chemistry Division of the National Bureau of Standards, may mark the point in time when ion-selective electrodes 'came of age'. Here is a realistic appraisal of the present state of the art as well as hints of future developments.

In the first chapter, G. EISENMAN deals with the theory of membrane electrodes and discusses the theoretical treatment of selectivity with reference to the newer types of electrode. J. W. ROSS then describes the performance of solid-state and liquid ion-exchanger membrane electrodes. Heterogeneous membrane electrodes, based on a silicone-rubber matrix as suggested by PUNGOR, are reviewed by A. K. COVINGTON, who also discusses reference electrodes; the importance of properly functioning reference electrodes for accurate potential measurements can scarcely be over-emphasized. The use of ion-selective electrodes in thermodynamic studies is reviewed by J. N. BUTLER. The difficulties encountered in the establishment of activity standards are dealt with by R. G. BATES. Biochemists and clinical chemists will find a wealth of useful information in a chapter on the application of calcium-selective electrodes to biological fluids, by E. W. MOORE. Biomedical studies with other electrodes are discussed by R. N. KHURI. Various studies on complex formation and reaction kinetics are described by G. A. RECHNITZ. The application of various electrodes in industrial analysis is assessed by T. S. LIGHT and, finally, R. A. DURST reviews the available analytical procedures and applications. The book ends with a very interesting report of the symposium discussions.

Ion-selective electrodes have not yet reached the stage where a simple direct measurement yields an unequivocal result in any type of solution; the emphasis must be on ion-selective rather than ion-specific. However, this volume provides a great deal of information on how to obtain optimal performance from the present generation of electrodes. It can be very warmly recommended to all analytical chemists.

A. M. G. MACDONALD (Birmingham)

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