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

ELSEVIER PUBLISHING COMPANY
AMSTERDAM

Anal. Chim. Acta, Vol. 46, No. 2, 165–328, July 1969
Published monthly
Completing Volume 46

Provisional Publication Schedule for 1969

In the interests of rapid publication it has been found necessary to schedule 5 volumes for appearance in 1969. Since monthly publication will be maintained, this implies that 2 of the volumes will each consist of three issues, while 3 of the volumes will each consist of only 2 issues. The following provisional schedule applies:

Vol. **44**, No. **1**	January 1969	
Vol. **44**, No. **2**	February 1969	**(completing Vol. 44)**
Vol. **45**, No. **1**	March 1969	
Vol. **45**, No. **2**	April 1969	
Vol. **45**, No. **3**	May 1969	**(completing Vol. 45)**
Vol. **46**, No. **1**	June 1969	
Vol. **46**, No. **2**	July 1969	**(completing Vol. 46)**
Vol. **47**, No. **1**	August 1969	
Vol. **47**, No. **2**	September 1969	
Vol. **47**, No. **3**	October 1969	**(completing Vol. 47)**
Vol. **48**, No. **1**	November 1969	
Vol. **48**, No. **2**	December 1969	**(completing Vol. 48)**

Subscription price: $ 17.50 or Dfl. 63.— per volume plus postage. Total subscription price for 1969: $ 87.50 or Dfl. 315.— plus postage. Additional cost for copies by airmail available on request. For subscribers in the U.S.A. and Canada, 2nd class postage paid at Jamaica, N.Y. For advertising rates apply to the publishers.

Subscriptions should be sent to:

ELSEVIER PUBLISHING COMPANY P.O. Box 211, Amsterdam, The Netherlands

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)

Reprints

Fifty reprints will be supplied free of charge. Additional reprints (minimum 100) can be ordered at quoted prices. They must be ordered on order forms which are sent together with the proofs.

SUMMARIES OF PAPERS PUBLISHED IN ANALYTICA CHIMICA ACTA

Vol. 46, No. 2, July 1969

DETERMINATION OF IMPURITIES IN TITANIUM AND TITANIUM DIOXIDE BY NEUTRON ACTIVATION ANALYSIS

PART II. DETERMINATION OF 27 TRACE CONSTITUENTS IN TITANIA POWDER

A procedure for the determination by neutron activation of the most important impurities in titania is described. It is based on reactor irradiation of 50-mg amounts of sample. The activities are separated in six subgroups mostly by anion exchange. The method allows the determination of antimony, arsenic, barium, calcium, chromium, cobalt, copper, gallium, gold, hafnium, indium, iron, manganese, molybdenum, nickel, potassium, rare earths, silver, sodium, tantalum, thorium, tin, tungsten, uranium and zinc. The reproducibility and the accuracy of the analytical results are satisfactory. The sensitivity ranges from $5 \cdot 10^{-4}$ μg (10 p.p.b. in 50 mg of sample) for arsenic and gold up to 5 μg for tin.

R. Neirinckx, F. Adams and J. Hoste,
Anal. Chim. Acta, 46 (1969) 165–178

A THIN-LAYER CHROMATOGRAPHIC METHOD FOR THE DETERMINATION OF PLANT PIGMENTS IN SEA WATER AND CULTURES

A combined thin-layer chromatographic–reflection densitometric procedure is described for the determination of chlorophylls and carotenoid pigments in marine particulate matter. A comparison of various methods of filtration showed that optimum recoveries of plant pigments are obtained with Whatman GF/C glass fibre filters covered with a layer of magnesium carbonate. Plant pigments are extracted by means of acetone and methanol and the combined extracts are evaporated under reduced pressure. Thin-layer chromatography is carried out on silica gel G. Chlorophyll *c* remains at the starting point and is separated from the origin spot by a subsequent development with a more polar solvent mixture. The separated pigments are determined on the plate by photodensitometry in the reflectance mode. Chlorophylls *a*, *b* and *c*, carotene, many of the individual xanthophylls, and certain degradation products of the chlorophylls can be determined. The complete analysis can be performed in under 1 h. The sensitivity of the method for chlorophyll *a* is *ca.* 0.12 μg and the precision for most pigments is $\pm$ 5% or better at the 0.5-μg level.

C. Garside and J. P. Riley,
Anal. Chim. Acta, 46 (1969) 179–191

SIMPLEX OPTIMIZATION OF THE RESPONSE FROM CHEMICAL SYSTEMS

An experiment design based on the simplex configuration offers an efficient approach to determining which levels of the controlling variables (or factors) give the maximum response from a system. This paper presents details for applying simplex optimization to chemical problems. A design matrix is given for locating the vertices of the initial simplex for up to 10 factors and a form is included for calculating the new points. A graphical technique is described for the two-factor case.

D. E. Long,
Anal. Chim. Acta, 46 (1969) 193–206

Elsevier Titles in Chemistry

INORGANIC CHEMISTRY

A Guide to Advanced Study
Third, completely revised edition

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**

5½×8½, 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.

INTRODUCTION TO NUCLEAR CHEMISTRY

by **D. J. Carswell**

5½×8½″, ix+279 pages, 23 tables, 69 illus., 1967, Dfl. 32.50, 70s.

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**

6×9″, x+280 pages, 57 tables, 74 illus., 357 lit. refs., 1967, Dfl. 60.00, £7.0.0.

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.
Appendices: The language of group theory. The spin hamiltonian. Calculation of *g*-values. Determination of spin-density distribution and bond angles. Analysis of electron spin resonance spectra. Index.


Elsevier Publishing Company

Amsterdam London New York

THE DETERMINATION OF TIN BY ATOMIC FLUORESCENCE SPECTROSCOPY, WITH AN ELECTRONICALLY MODULATED ELECTRODELESS DISCHARGE TUBE AS SOURCE

The atomic fluorescence characteristics of tin in Ar/H_2, $Ar/O_2/H_2$ and air/C_2H_2 flames have been investigated and optimal conditions have been established for the determination of tin. An electronically modulated electrodeless discharge tube excited at 2450 MHz is used as source. Measurement of the fluorescence emission at 303.4 nm in a nitrogen-separated $Ar/O_2/H_2$ flame gives a detection limit of 0.1 p.p.m. under almost entirely interference-free conditions. Good analytical curves are obtained in the range 0.5–250 p.p.m. Sn. Of 34 elements examined at 100-fold concentrations, only Ca, Mg, Mo, Sr and U interfered.

R. F. BROWNER, R. M. DAGNALL AND T. S. WEST,
Anal. Chim. Acta, 46 (1969) 207–216

DETERMINATION OF ALUMINUM IN VANADIUM METAL BY ATOMIC ABSORPTION SPECTROPHOTOMETRY

The determination of aluminum in vanadium metal over the range 100 p.p.m. to 1% aluminum was investigated by atomic absorption spectrophotometry. Commercially available equipment with a nitrous oxide–acetylene burner was used. High concentrations of vanadium were found to influence the aluminum absorption in a variable manner depending upon certain conditions. Reproducible results could be obtained only by carefully matching the matrix and acid concentrations in the standards to that in the actual sample.

R. K. HANSEN, R. Z. BACHMAN, J. W. O'LAUGHLIN AND CH. V. BANKS,
Anal. Chim. Acta, 46 (1969) 217–223

A RAPID FUSION METHOD FOR DECOMPOSITION AND COMPREHENSIVE ANALYSIS OF SILICATES BY ATOMIC ABSORPTION SPECTROPHOTOMETRY

A method is proposed for the determination of silicon, aluminium, titanium, iron, calcium, magnesium, sodium and potassium in various silicates by atomic absorption spectrophotometry. The samples are fused with a mixture of equal amounts of lithium carbonate and boric acid and dissolved in hydrochloric acid, the complete procedure requiring about 15 min. Interferences are eliminated by adding lanthanum to samples and standards.

S. H. OMANG,
Anal. Chim. Acta, 46 (1969) 225–230

DETERMINATION OF POLYMER STRUCTURE BY HIGH-RESOLUTION NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY

High-resolution nuclear magnetic resonance spectroscopy gives considerable information about composition, structure, and stereoconfiguration of polymer chains. Proton resonance spectra of a polycarbonate, polyamide, poly(vinyl chloride), and a poly(vinyl chloride)–poly(vinyl acetate) copolymer, which were obtained at 60 MHz, 100 MHz, and 220 MHz are discussed. Spectra obtained with the 220-MHz superconducting solenoid spectrometer approach first order, permitting more detailed information to be obtained and, in most cases, eliminating the need for spin-decoupling experiments in stereoconfiguration determinations.

R. S. SUDOL,
Anal. Chim. Acta, 46 (1969) 231–237

THE MASS SPECTRA OF ORGANIC MOLECULES

by J. H. Beynon, R. A. Saunders and A. E. Williams, Research Department, Imperial Chemical Industries Ltd., Manchester, Great Britain

7 x 10″, ix + 510 pages, 20 tables, 181 illus., 547 lit. refs., 1968, Dfl. 97.50

Contents: 1. The principles and methods of mass spectrometry. 2. Types of ions in the mass spectra of organic compounds. 3. The mass spectra of hydrocarbons. 4. The mass spectra of oxygenated compounds. 5. The mass spectra of nitrogen compounds. 6. The mass spectra of sulphur compounds. 7. The mass spectra of halogenated compounds. 8. The mass spectra of boron compounds. 9. The mass spectra of phosphorus compounds. 10. The mass spectra of silicon compounds. 11. Examples of structure determination from mass spectra. Appendix 1. Peaks commonly encountered in the mass spectra of organic compounds. Appendix 2. The masses and abundances of nuclides commonly encountered in the mass spectra of organic compounds. References. Indexes.

MASS SPECTROMETRIC ANALYSIS OF SOLIDS

edited by A. J. Ahearn, Member of Technical Staff, Bell Telephone Laboratories, Inc., Murray Hill, New Jersey, U.S.A.

5½ x 8½″, viii + 175 pages, 13 tables, 46 illus., 242 lit. refs., 1966, Dfl. 30.00

Contents: 1. Introductory survey. 2. The production of ions from solids. 3. Photographic emulsions as ion detectors in quantitative mass spectrography. 4. Analysis of special samples. 5. Mass spectrographic microprobe analysis. Indexes.

ATOMIC-ABSORPTION SPECTROSCOPY

and Analysis by Atomic-Absorption Flame Photometry

by J. Ramírez-Muñoz, Principal Applications Chemist at Beckman Instruments Inc. and Scientific Research Collaborator of the C.S.I.C., Spain

6 x 9″, xii + 493 pages, 23 tables, 156 illus., 950 lit. refs., 1968, Dfl. 80.00

Contents: *Part I: Fundamentals.* 1. Origins of the method and nomenclature. 2. General principles and characteristics. 3. Absorption and emission. 4. The literature of atomic-absorption spectroscopy. 5. Theory. *Part II: Instrumental Systems.* 6. Instrumental systems. 7. Emission systems. 8. Absorption system. 9. Selection system. 10. Photometric system. 11. Instruments. *Part III: Range and Limitations of Atomic Absorption Methods.* 12. Determinable elements. Choice of lines. 13. Sensitivity. 14. Limitations in atomic absorption. *Part IV: Experimental Methods.* 15. Experimental process. 16. Standard solutions. 17. Preparation of the sample. 18. Experimental measurements and calibration. *Part V: Applications.* 19. Applications. Appendix. Bibliography.

Still available:

MASS SPECTROMETRY AND ITS APPLICATIONS TO ORGANIC CHEMISTRY

by J. H. Beynon

7 x 10″, xii + 640 pages, 11 tables, 185 illus., 2213 lit. refs., 1960, reprinted 1964 and 1967, Dfl. 85.00

TABLE OF META-STABLE TRANSITIONS FOR USE IN MASS SPECTROMETRY

by J. H. Beynon, R. A. Saunders and A. E. Williams

10 x 7″, xix + 392 pages, 1965, Dfl. 50.00

MASS AND ABUNDANCE TABLES FOR USE IN MASS SPECTROMETRY

by J. H. Beynon and A. E. Williams

10 x 7″, xxi + 570 pages, 1963, Dfl. 60.00


Elsevier Publishing Company Amsterdam London New York

408 E

2,3-NAPHTHOTRIAZOLE AS A GRAVIMETRIC, SPECTROPHOTOMETRIC, AND FLUORIMETRIC REAGENT FOR THE DETERMINATION OF SILVER

2,3-Naphthotriazole is suitable for the determination of macro- and microquantities of silver. The gravimetric (for 5–100 mg Ag) and spectrophotometric ($\varepsilon = 1{,}000$; 1–30 μg Ag/ml) methods are rapid and free of halide and foreign ion interferences; excellent selectivity is attained by means of masking agents. The fluorimetric method (0.025–0.1 μg Ag/ml) is much more sensitive than the spectrophotometric method but is more subject to interferences.

G. L. WHEELER, J. ANDREJACK, J. H. WIERSMA AND P. F. LOTT,
Anal. Chim. Acta, 46 (1969) 239–245

MOLECULAR WEIGHT MEASUREMENTS OF POLYCARBOXYLIC ACIDS IN WATER BY VAPOR PRESSURE OSMOMETRY

Number-average molecular weight determinations of polycarboxylic acids in water by vapor pressure osmometry may yield erroneous results owing to dissociation. Based on experimentally determined values of $\overline{Mn}$ and pH, a correction system is described which makes possible calculations of accurate $\overline{Mn}$ values. The applicability of the correction system is demonstrated for 15 known aliphatic and aromatic compounds, containing between one and six carboxyl groups per mole and to water-soluble humic compounds of widely differing molecular weights, containing relatively high concentrations of carboxyl groups. This method may be especially useful for naturally occurring water-soluble organic compounds which are insoluble or only very slightly soluble in organic solvents.

E. H. HANSEN AND M. SCHNITZER,
Anal. Chim. Acta, 46 (1969) 247–254

THE POLARIMETRIC DETERMINATION OF MANNITOL AND SORBITOL AS THEIR MOLYBDATE COMPLEXES

(in French)

A polarimetric method for the determination of sorbitol and mannitol as their molybdate complexes is described. Sugars which interfere with the periodate method can be tolerated. A detailed study of the optimal concentrations of molybdic acid and nitric acid is described. Citric acid and cerium(III) prevent interferences when biological samples are analysed.

M. HAMON, C. MORIN AND R. BOURDON,
Anal. Chim. Acta, 46 (1969) 255–261

FORMATION CONSTANTS OF IODINE–IODIDE COMPLEXES IN WATER–ACETONITRILE AND WATER–ETHANOL. SOLVATION COEFFICIENTS OF THE I_3^- ANION

(in French)

Formation constants of I_3^- and I_5^- complexes have been determined in water–ethanol and water–acetonitrile mixtures by potentiometry. Solvation coefficients (or medium effect coefficients) of this species were evaluated by solubility measurements of iodine in the different solvent mixtures. The solvation coefficient values of the complex anion I_3^- were then deduced by means of formerly determined values of solvation coefficients of the iodide anion *(Bull. Soc. Chim. France*, (1968) 3421). The results were not in agreement with the hypothesis that the iodine molecule and I_3^- anion have the same solvation coefficients.

C. BARRAQUÉ, J. VEDEL AND B. TRÉMILLON,
Anal. Chim. Acta, 46 (1969) 263–269

DIFFRACTION OF X-RAYS BY CHAIN MOLECULES

by B. K. VAINSHTEIN

Foreword by M. F. PERUTZ

6 x 9″, xiii + 414 pages, 3 tables, 258 illus., 256 lit.refs., 1966, Dfl. 65.00, £7.10.0.

Contents: 1. Principles of the theory of X-ray diffraction. 2. Structures of chain molecules and assemblies. 3. Diffraction by an isolated chain molecule. 4. Scattering intensity and structure of object. 5. Properties of the distribution and interference functions. 6. Diffraction by assemblies of parallel chain molecules. 7. Diffraction by assemblies with nonparallel packing of chain molecules and by amorphous polymers. Subject index.

INFRA RED INSTRUMENTATION AND TECHNIQUES

by A. E. MARTIN

5½ x 8½″, x + 180 pages, 13 tables, 94 illus., 86 lit.refs., 1966, Dfl. 32.50, 75s.

Contents: 1. Historical. 2. Modern infra-red spectrometers. 3. Miscellaneous instruments. 4. Interferometric spectrometers. 5. Accessories. 6. Experimental methods and techniques. Index.

ENERGY TRANSFER IN RADIATION PROCESSES

Chemical, Physical and Biological Aspects

Proceedings of the International Symposium held in Cardiff, 1965

edited by G. O. PHILLIPS

5½ x 8½″, xvi + 182 pages, 10 tables, 81 illus., 273 lit.reft., 1966, Dfl. 32.50, 75s.

Contents: Introductions to the sections by F. S. Dainton, G. F. J. Garlick and Tikvah Alper. Invited papers by E. J. Bowen, Jett C. Arthur, N. Riehl, R. Mason. Contributed papers.

MASS SPECTROMETRIC ANALYSIS OF SOLIDS

edited by A. J. AHEARN

5½ x 8½″, viii + 175 pages, 13 tables, 46 illus., 242 lit.refs., 1966, Dfl. 30.00, 70s.

Contents: 1. Introductory survey. 2. The production of ions from solids. 3. Photographic emulsions as ion detectors in quantitative mass spectrography. 4. Analysis of special samples. 5. Mass spectrographic microprobe analysis. Subject index.

RADIOCHEMICAL SURVEY OF THE ELEMENTS

Principal characteristics and applications of the elements and their isotopes

by M. HAISSINSKY
and
J.-P. ADLOFF

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

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

Elsevier Publishing Company

P.O. Box 211, Amsterdam — Ripple Rd., Barking, Essex, London — 52 Vanderbilt Ave. N.Y. 10017, New York

DUAL-WAVELENGTH SPECTROPHOTOMETRY

PART I. GENERAL METHOD

The application of dual-wavelength spectrophotometry in inorganic chemical analysis is discussed. The method has been successfully applied to the determination of trace amounts of metals and the determination of metals in the presence of diverse ions without separation. Three basic methods for the choice of the two wavelengths to be used for a particular system are proposed.

S. SHIBATA, M. FURUKAWA AND K. GOTO,
Anal. Chim. Acta, 46 (1969) 271–279

ACTIVATION ANALYSIS OF PAPER CHROMATOGRAMS

The use of activation analysis in conjunction with paper chromatography, and particularly activation after chromatography, is reviewed. Irradiation facilities, the use of markers, internal standards and general activation conditions are discussed, and detailed attention is given to the behaviour of various types of paper on activation, with especial reference to stability and impurities. Practical applications dealing with phosphorus, oxygen, iodine, arsenic and various metals are reviewed.

A. Z. BUDZYŃSKI AND J. Z. BEER,
Anal. Chim. Acta, 46 (1969) 281–306

THE ANALYTICAL CONCENTRATION OF TRACES OF DISSOLVED ORGANIC MATERIALS FROM SEA WATER WITH AMBERLITE XAD-1 RESIN

(Short Communication)

J. P. RILEY AND D. TAYLOR,
Anal. Chim. Acta, 46 (1969) 307–309

COULOMETRIC GENERATION OF MANGANESE(III)

(Short Communication)

G. F. ATKINSON AND G. A. BRYDON,
Anal. Chim. Acta, 46 (1969) 309–311

THE DETECTION OF BARIUM BY INDUCED PRECIPITATION

(Short Communication)

J. W. HAMYA AND A. TOWNSHEND,
Anal. Chim. Acta, 46 (1969) 312–313

FORMATION OF ZINC CHELATES OF NITROSO-SCHÄFFER'S ACID AND NITROSO-C ACID IN AQUEOUS SOLUTION

(Short Communication)

O. MÄKITIE AND H. SAARINEN,
Anal. Chim. Acta, 46 (1969) 314–318

DIRECT POTENTIOMETRIC DETERMINATION OF CYANIDE IN BIOLOGICAL SYSTEMS

(Short Communication)

B. GYÖRGY, L. ANDRÉ, L. STEHLI AND E. PUNGOR,
Anal. Chim. Acta, 46 (1969) 318–321

COORDINATION CHEMISTRY REVIEWS

Editor: A.B.P. LEVER (Downsview, Ont., Canada)

This international journal offers rapid publication of relatively short review articles in the field of coordination chemistry. The term "coordination chemistry" is interpreted broadly, but does not include "organometallic chemistry". In general the reviews published fall into the following categories:

- surveys of developments in a particular area during the last few years
- surveys and/or discussions of the results obtained with a particular technique during the last few years
- general or philosophical discussions of some specific aspects of coordination chemistry

Articles dealing with the application of physical techniques are also included, as well as those on the theory or practice of the coordination chemistry of transition or non-transition metals. The main language of the journal is English, although reviews in French or German are also published.

CONTENTS Volume 4, No. 1, February 1969

CONTENTS Volume 4, No. 2, April 1969

Publication is in one volume of four issues per year. Subscription price for 1969 is Dfl. 70.00 plus Dfl.. 3.50 postage or equivalent (US$19.50 plus US$1.00). A free specimen copy will be sent on request.
Vol. 1 (1966), Vol. 2 (1967); Vol. 3 (1968); Vol. 4 (1969).

Please send your order to your usual supplier or to the publisher.

Elsevier

P.O. Box 211
Amsterdam - The Netherlands

Analytica Chimica Acta
Elsevier Publishing Company, Amsterdam
Printed in The Netherlands

DETERMINATION OF IMPURITIES IN TITANIUM AND TITANIUM DIOXIDE BY NEUTRON ACTIVATION ANALYSIS

PART II. DETERMINATION OF 27 TRACE CONSTITUENTS IN TITANIA POWDER

R. NEIRINCKX, F. ADAMS AND J. HOSTE
Institute for Nuclear Chemistry, Ghent University, Ghent (Belgium)
(Received March 15th, 1969)

Titania is a widely used, high-quality, white pigment, whose qualities are strongly affected by the presence of trace elements. The deterioration of the whiteness, caused by chromium and vanadium was studied by STONHILL[1], who pointed out that 10 p.p.m. of these elements had a noticeable effect on the quality of the pigment, whereas several p.p.m. of tungsten had little influence. Furthermore, the effect of niobium, antimony, tantalum and tungsten was investigated by WEYL AND FÖRLAND[2], while MCTAGGART AND BEAR[3] examined the influence of Fe, Cr, Ni, Mn, Co, Pr, Y, Nd and Cu. Impurities in titania may be also important if it is used for the production of titanium dioxide single crystals or in the pharmaceutical industry.

The classical analytical procedures start mostly with a dissolution in a sulfuric acid medium and removal of the matrix. The determinations of the trace elements are often carried out by emission spectrometry. Pulse polarography was used by LAGROU *et al.*[4] who separated antimony, copper and lead from titania solutions by coprecipitation of their sulfides on cadmium sulfide. Titania analyses were made by spark-source mass spectroscopy by JACKSON AND WHITEHEAD[5]. Neutron activation methods were used by BROWNLEE[6] for the determination of niobium in rutile, by SANKAR[7] for the determination of uranium in ilmenite, and by BROOKSBANK[8] and ALIMARIN AND YAKOVLEV[9] for the non-destructive determination of vanadium.

In the present work a procedure for the simultaneous determination of many metallic trace impurities in titania is described. The method is based on activation analysis and γ-spectroscopy with a high-resolution Ge(Li) detector, after suitable separations in different groups. In the choice of the separation procedure care was taken to restrict the volumes to a minimum, as this allows the measurement with the Ge(Li) detector in favourable geometrical conditions.

Nuclear data and interferences

Pertinent nuclear data for reactor irradiation of titanium were summarized in Part I of this series[10]. The only radioactive titanium isotopes produced by reactor irradiation of pure titanium are ^{45}Ti and ^{51}Ti. Because of the short half-life of ^{51}Ti this isotope can only interfere with the determination of short-lived isotopes. The cross-section for the (n, 2n) reaction on ^{46}Ti with the fast neutron flux is too small to cause any interference. ^{46}Sc, ^{47}Sc and ^{48}Sc are the major activities after a reactor irradiation and have to be removed. A previously published procedure, based on

anion exchange was used for the analysis of titanium foil and titanium sponge[10]. This procedure did not give satisfactory results with most of the titania samples as they turned out to have a relatively high antimony content (0.01%). A simple and fast separation of many impurities from scandium and antimony was thus required. The nuclear data of the radionuclides, produced from trace constituents of titania by reactor irradiation, are shown in Table I. A list of the γ-rays used for the quantitative determinations of these elements is included[11].

TABLE I

IMPURITIES IN TITANIA: NUCLEAR DATA

Target nuclide and abundance (%)	*Order of magnitude (p.p.m)*	*Reaction product*	*Activation cross-section (barn)*	*Half-life*	*γ-Radiation used (MeV)*
^{109}Ag(48.2)	10^{-2}	^{110m}Ag	2.8	270 d	0.6578; 0.8845; 0.9372
^{75}As(100)	10^{-1}	^{76}As	4.3	26.5 h	0.5592; 0.6570; 1.2158
^{197}Au(100)	10^{-2}	^{198}Au	96	2.7 d	0.4118
^{130}Ba(0.10)	10	^{131}Ba	8.8	11.6 d	0.1242; 0.2161; 0.3731
^{46}Ca(0.0033)	10^{3}	^{47}Ca	0.3	4.7 d	0.1600; 1.2969
^{140}Ce(88.5)	10^{2}	^{141}Ce	0.5	32.5 d	0.1454
^{59}Co	10^{-2}	^{60}Co	36	5.2 y	1.1731; 1.3324
^{50}Cr(4.31)	1	^{51}Cr	11	27.8 d	0.3200
^{63}Cu(69.1)	1	^{64}Cu	3.0	12.8 h	0.5110; 1.3455
^{151}Eu(47.8)	10^{-2}	^{152}Eu	1,700	13 y	0.1218; 0.3442; 1.4074
^{58}Fe(0.33)	10	^{59}Fe	0.9	45.1 d	1.0986; 1.2915
^{71}Ga(39.6)	1	^{72}Ga	3.4	14.2 h	0.6301; 0.8341; 2.2014
^{180}Hf(35.2)	10	^{181}Hf	10	44.6	0.1331; 0.3457; 0.4822
^{115}In(95.7)	1	^{116m}In	150	54 min	0.4170; 1.0971; 1.2934
^{41}K(6.9)	10^{3}	^{42}K	1.1	12.4 h	1.5247
^{139}La(99.9)	10	^{140}La	8.9	40.2 h	0.3286; 0.4868; 1.5954
^{55}Mn(100)	1	^{56}Mn	13.3	2.58 h	0.8469; 1.8107; 2.1128
^{98}Mo(23.8)	10	^{99}Mo	0.13	67 h	0.1406; 0.1809; 0.7399
^{23}Na(100)	10^{2}	^{24}Na	0.54	14.8 h	1.3684; 2.7536
^{58}Ni(67.9)	1	^{58}Co	0.105	71 d	0.5110; 0.8103
^{121}Sb(57.2)	10^{2}	^{122}Sb	3.7	2.8 d	0.5640; 0.6925; 1.1405
^{112}Sn(0.96)	10^{2}	^{113}Sn $\xrightarrow{\text{EC}}$ ^{113m}In	1.3	112 d (^{113}Sn)	0.3914 (^{113m}In)
^{181}Ta(99.99)	10^{2}	^{182}Ta	19	111 d	0.0677; 0.1003; 1.1212; 1.2216
^{232}Th(unstable $T_{\frac{1}{2}} = 1.4 \cdot 10^{10}$y)	1	^{233}Th $\xrightarrow{\beta^-}$ ^{233}Pa	7.5	27.4 d (^{233}Pa)	0.2999; 0.3118; 0.3403 (^{233}Pa)
^{238}U(99.3)	10	^{239}U	2.7	23.5 min	0.0747
^{186}W(28.4)	1	^{187}W	34	23.9 h	0.1343; 0.4793; 0.6857
^{64}Zn(48.9)	10	^{65}Zn	0.25	254 d	0.5110; 1.1154

Separation scheme

The separation scheme is shown in Fig. 1. The sample is dissolved in hydrofluoric acid, diluted to 1 *M* hydrofluoric acid and placed on a Dowex 1-X8 column. The elements with small K_D values in this medium are eluted, namely, Na, K, Ca, Ba, Fe, Co, Ni, Zn, As, Ga, Cu, Cr, Ag and In (fraction I). A subsequent elution with 22 *M* hydrofluoric acid removes Ti, Sc, Sn, Hf, Pa and U (fraction II). Tin cannot be conveniently detected in this fraction as a result of the large ^{46}Sc, ^{47}Sc and ^{48}Sc activities. A sensitive determination for this element can however be based on the

^{113m}In daughter activity, eluted with fraction I. Moreover, the sensitivity for the determination of tin can be enhanced by introducing a sufficient waiting period to allow reequilibration of ^{113}Sn–^{113m}In. After ingrowth of ^{113m}In a new fraction of 1 *M* hydrofluoric acid can be eluted (fraction Ib). This fraction contains ^{113m}In of

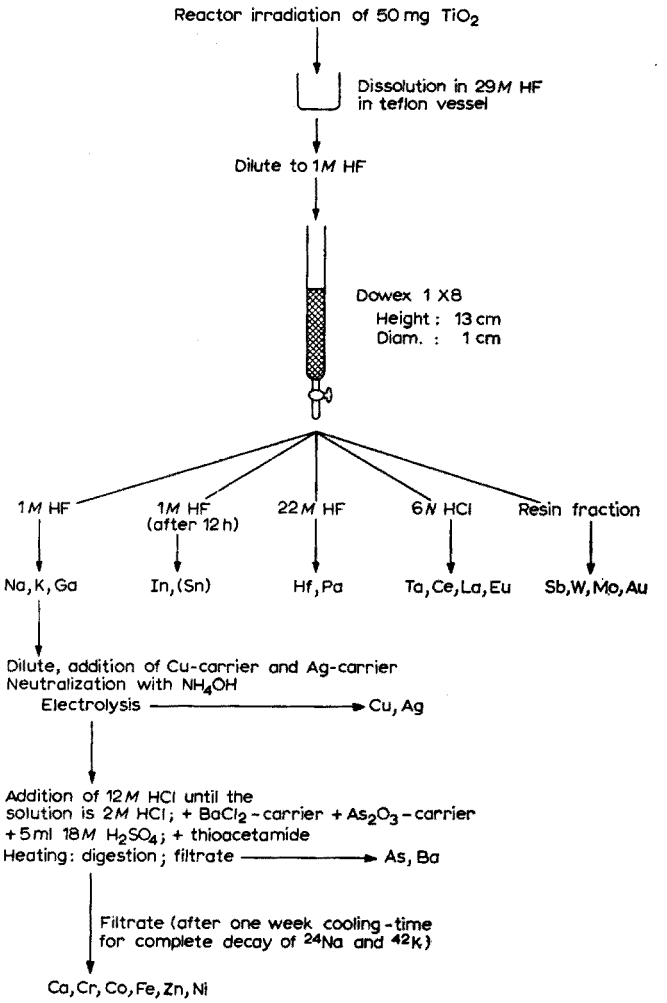


Fig. 1. Separation scheme for titania powder.

sufficient purity to allow its measurement with a NaI(Tl) detector. Protactinium and hafnium are not collected completely in the 22 *M* hydrofluoric acid fraction. A residual amount is eluted with 6 *M* hydrochloric acid. Uranium, also present together with the scandium isotopes in fraction II can conveniently be determined by the measurement of a fission product, *e.g.* ^{140}Ba (fraction I). Alternatively, a short irradiation can be performed, allowing a determination based on the ^{239}U radiation at 79 keV or by means of the ^{239}Np activity in fraction II. Hydrochloric acid (6 *M*)

TABLE II

ELUTIONS WITH 1 *M* AND 22 *M* HYDROFLUORIC ACID AND 6 *M* HYDROCHLORIC ACID FROM DOWEX 1-X8

Fraction	*Element*	*Tracer used*	*Carrier amount (mg)*	*% in fraction*
1 *M* HF	Na	^{24}Na	0.5	>99.8
(30 ml)	K	^{42}K	5	>99.4
	Ca	^{47}Ca	2	>98.3
	Ba	^{131}Ba	20	>98
	Fe	^{59}Fe	3	>99
	Co, Ni	^{60}Co	0.05	>99.7
	Zn	^{65}Zn	2	>99.6
	As	^{76}As	1	>99
	Ga	^{72}Ga	1	>98
	Cu	^{64}Cu	0.1	>99.6
	Cr	^{51}Cr	0.5	>99.4
	Ag	^{110m}Ag	1	>96
	In	^{116m}In	1	>99.3
	Mn	^{56}Mn	10	99.2
22 *M* HF	Sc	^{46}Sc	1	>99.99
(50 ml)	Ti	None	50	>99.9
	Th	^{233}Pa	1	90
	Sn	^{117m}Sn	10	—
	U	^{239}U	1	>99.3
	Hf	^{181}Hf	0.1	90
	Sb	^{124}Sb	1	1.0
6 *M* HCl	Hf	^{181}Hf	0.1	10
(50 ml)	Ta	^{182}Ta	1	94
	Th	^{233}Pa	1	10
	Ce	^{141}Ce	1	99.5
	Sm	^{153}Sm	1	99.7
	La	^{140}La	0.1	>98
	Eu	^{152m}Eu	1	>98.5
Resin	Sb	^{124}Sb	1	99
fraction	Au	^{198}Au	0.1	>98.5
	W	^{187}W	0.1	>99
	Mo	^{99}Mo	10	>97.5
	Ta	^{182}Ta	1	6

allows the elution of tantalum and the rare earths (fraction III). The elements antimony, gold, tungsten and molybdenum are retained on the resin. The main activity of this fraction is due to ^{122}Sb and ^{124}Sb, so that the sensitivity for gold, tungsten and molybdenum is rather low and strongly dependent on the antimony content of the sample.

The separation scheme was tested with radioactive tracers added to 50 mg of titania. The results are summarized in Table II. The determination of all the elements present in the 1 *M* hydrofluoric acid fraction was possible by Ge(Li) spectrometry. However, for many titania samples, the high ^{24}Na and ^{42}K activities mask most of the lower-energy photopeaks. A cooling time of *ca.* 5 days allows the quantitative determination of the other elements (Fig. 2). The relatively short half-lives of ^{64}Cu (12.8 h) and ^{76}As (26.5 h) do not allow such a long cooling period. An additional separation of these elements, immediately after the elution with 1 *M* hydrofluoric acid, is therefore required.

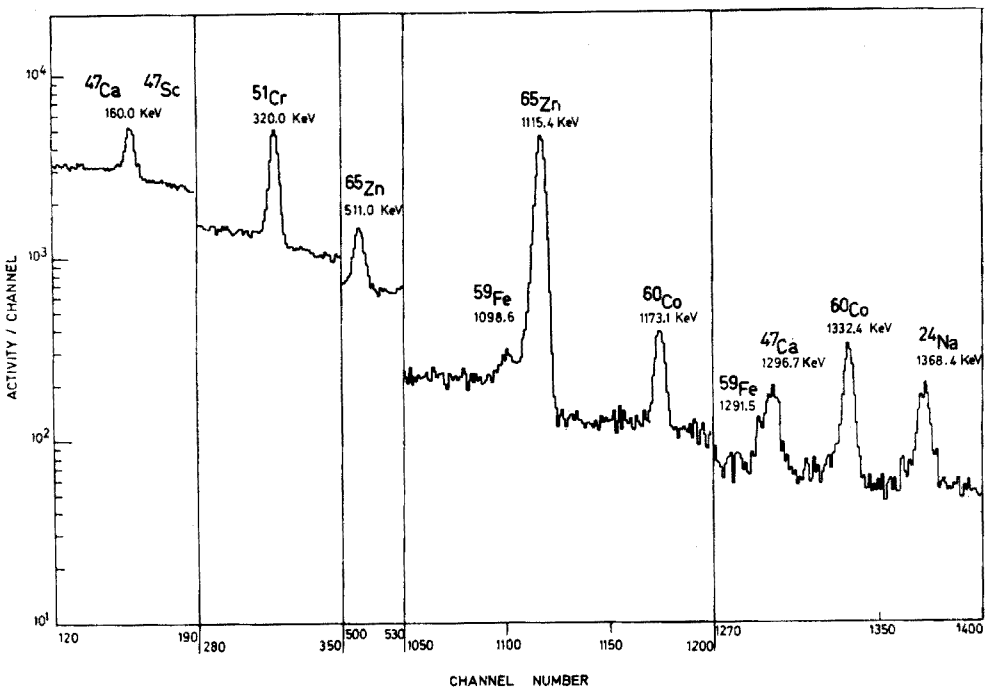


Fig. 2. Ge(Li) spectrum of the long-lived isotopes in the 1 *M* hydrofluoric acid fraction.

TABLE III

RESULTS OF TRACER EXPERIMENTS ON COPPER ELECTROLYSIS AND ARSENIC(III) SULFIDE PRECIPITATION

Element	*Tracer used*	*Amount of carrier (mg)*	*% Electrolyzed*	*% Precipitated*
Ag	^{110m}Ag	1	98	> 99.1
As	^{76}As	15	< 0.2	> 99.2
Ba	^{131}Ba	20	< 1	> 99.4
Ca	^{47}Ca	2	< 1	< 0.7
Co	^{60}Co	0.05	< 0.5	< 0.4
Cr	^{51}Cr	0.5	< 0.9	< 2
Cu	^{64}Cu	0.1	> 99	—
Fe	^{59}Fe	3	< 0.4	< 0.6
K	^{42}K	5	< 0.3	< 0.15
Na	^{24}Na	0.5	< 0.01	< 0.1
Zn	^{65}Zn	2	< 0.25	< 1.5

Electrolysis of the copper, followed by precipitation of arsenic(III) sulfide is used, when a high sodium or potassium content does not allow a determination of these elements. The results of the tracer experiments, which were carried out for this copper electrolysis and arsenic sulfide precipitation are shown in Table III. Silver is electrolyzed together with copper. Barium coprecipitates with the arsenic(III) sulfide, because of the addition of sulfuric acid to form the very slightly soluble sulfate.

For the measurement of the long-lived isotopes in the 1 *M* hydrofluoric acid fraction (see Fig. 2), the filtrate from the arsenic(III) sulfide precipitation is evaporated to a volume of 50 ml.

The short-lived ^{56}Mn and ^{116m}In isotopes were used for the determination of manganese and indium in titania. A 3-h irradiation in the "Thetis" reactor was used. After a cooling time of 15 min, a rapid separation and measurement of the 1 *M* hydrofluoric acid fraction on the Ge(Li) detector is carried out.

EXPERIMENTAL

Instrumental

Either an 18-cm^3 and a 40-cm^3 Ge(Li) detector was used with a Tennelec (TC 200) amplifier, an Intertechnique 20 MHz ADC (CA 13) and a BM 96 4096-channel memory unit. All measurements were performed at counting rates of less than 10,000 counts/sec to avoid photopeak smearing and to insure proportionality of photopeak with source intensity. A doubly differentiated pulse form was used. Although the peaks remained reasonably symmetrical up to count rates of 30,000 counts/sec, errors could be introduced as a result of random pulse pile-up and inadequate compensation of the dead time. The first of these effects cannot be completely avoided since the choice of the pulse form is dictated by other characteristics, namely charge collection and the suppression of detector and preamplifier noise. In the applied experimental conditions, this effect could be neglected.

Preparation of standards

In view of the large number of standards to be irradiated with the samples, they were divided into groups. After irradiation, the standards within one group should be easily removable from the quartz ampoules. Only the standards of the elements belonging in the same fraction of the separation scheme and soluble in a common solvent, were irradiated together. With these considerations, the standards were divided into 8 groups (Table IV). For each group of standards a solution was made, containing a well-balanced concentration of the elements to insure that the peaks of the main γ-rays were of comparable count-rate: 10 μl was pipetted into a quartz tube and evaporated to dryness. The concentration of each element is shown in Table IV. After irradiation, the content of each tube was dissolved and measured on the Ge(Li) detector in the same geometrical conditions as the corresponding fractions of the separated titania samples.

The standards of the elements manganese, indium and uranium, which were determined separately by short irradiations, were placed in separate quartz tubes (see Table IV). After irradiation they were dissolved and measured in the same geometrical conditions as the corresponding fraction of the separated titania sample.

Irradiation conditions

Samples of titania (50 mg) were enclosed in quartz tubes. For the long-lived isotopes, the irradiations were carried out in the BR-2 reactor at the S.C.K. (Mol), either at a flux of 10^{14} n cm^{-2} sec^{-1} during 15 h, or at a flux of $5 \cdot 10^{12}$ n cm^{-2} sec^{-1} during one week. The separations were started about 24 h after the end of the irradiation. This long waiting period, caused by the transfer of the samples from Mol to

TABLE IV

CONCENTRATION OF THE STANDARD SOLUTIONS

Element	*Compound*	*Weight (μg)*	*Solvent*	*Fraction with which it has to be compared*
Na	Na_2CO_3	1	1 *M* HNO_3	1 *M* HF
K	K_2CO_3	10	—	—
Ag	$AgNO_3$	10^2	—	Cu-electrolysis
As	As_2O_3	1	6 *M* NH_4OH	As_2S_3-precipitate
Co	Co–Al	10	6 *M* HCl	1 *M* HF
Zn	ZnO	10	—	—
Ga	Ga_2O_3	1	—	—
Cu	CuO	10	—	Cu-electrolysis
Cr	Cr	10	—	1 *M* HF
Sn	Sn	10	—	Indium-fraction
Ca	$CaCO_3$	10^3	—	1 *M* HF
Ba	$BaCO_3$	10^3	—	Precipitation as $BaSO_4$
Fe	Fe	10^3	—	1 *M* HF
Ni	NiO	10^3	—	—
Eu	Eu_2O_3	10^{-2}	6 *M* HNO_3	6 *M* HCl
Sm	Sm_2O_3	10^{-1}	—	—
La	La_2O_3	1	—	—
Ce	CeO_2	10	—	—
Ta	Ta_2O_5	10	Oxalic acid after $KHSO_4$-fusion	6 *M* HCl
W	WO_3	1	6 *M* NH_4OH + NH_4-tartrate	Resin-fraction
Mo	$(NH_4)_6Mo_7O_{24}\cdot 4H_2O$	10	—	—
Sb	Sb_2O_3	10	—	—
Au	Au	10^{-1}	Aq. regia	Resin-fraction
Th	$Th(NO_3)_4$	10	H_2O	22 *M* HF
Mn	$MnSO_4\cdot H_2O$	10^2	H_2O	1 *M* HF
In	In	1	6 *N* HCl	1 *M* HF
U	$UO_2(NO_3)_2\cdot 6H_2O$	10^2	H_2O	22 *M* HF

Ghent, prevented the determination of short-lived species. For the short-lived isotopes (^{239}U, ^{56}Mn, ^{116m}In) the samples were irradiated for 3 h in the "Thetis" reactor of Ghent University. In this case the separations started about 15 min after irradiation.

Procedure

Transfer the sample to a teflon reaction vessel and dissolve in 0.5 ml of 29 *M* hydrofluoric acid. Use a water-cooled polythene condenser to avoid losses of volatile compounds. Dilute to 15 ml to obtain 1 *M* hydrofluoric acid and transfer to a Dowex 1-X8 200–400 mesh column with a diameter of 1 cm and a length of 13 cm.

Elute with 1 *M* hydrofluoric acid at a rate of 0.5 ml per minute until a fraction of 30 ml is collected. Optionally, introduce a waiting period of 12 h and elute an

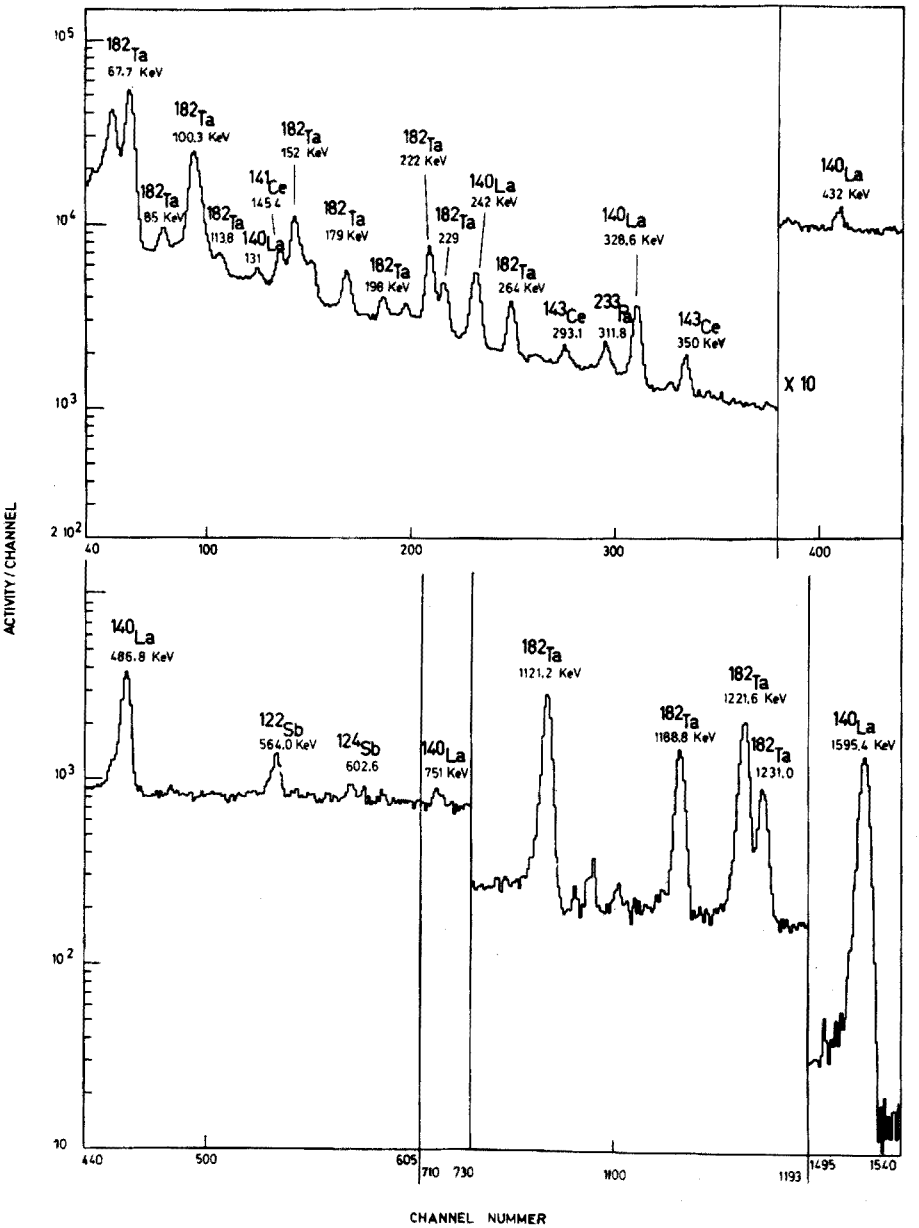


Fig. 3. Ge(Li) spectrum of the 6 *M* hydrochloric acid fraction.

additional 30 ml of 1 *M* hydrofluoric acid (indium-fraction). Elute at the same rate with 50 ml of 22 *M* hydrofluoric acid and with 50 ml of 6 *M* hydrochloric acid. Count the first 1 *M* hydrofluoric acid fraction on the Ge(Li) detector for ^{24}Na, ^{42}K and ^{72}Ga. If the sensitivity for arsenic and copper is unsatisfactory, dilute this fraction to 100 ml, add 20 mg of copper nitrate tetrahydrate and 2 mg of silver nitrate and

neutralize with 6 *M* ammonia solution until the blue color of the copper complex appears. Insert two Bertiaux electrodes and electrolyze the solution at 1.5 V and 0.1 A for 1 h. Dissolve the copper deposit in 6 *M* nitric acid, add 20 mg of copper nitrate tetrahydrate and 2 mg of silver nitrate to the original solution and repeat the electrolysis. Measure the combined nitric acid solutions on a NaI(Tl) detector,

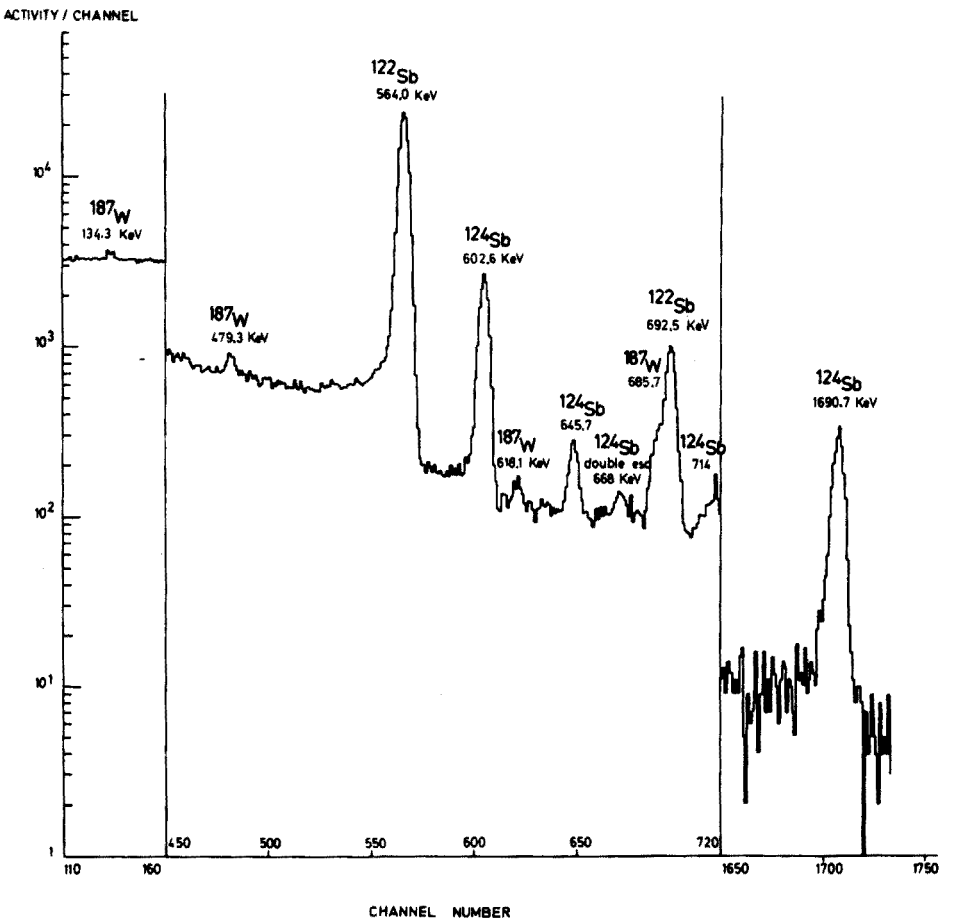


Fig. 4. Ge(Li) spectrum of the resin fraction.

coupled to a 400-channel analyzer. For the precipitation of arsenic(III) sulfide, add 20 mg of arsenic(III) oxide, and 20 mg of barium chloride dihydrate, but no carrier for the other elements. Add 5 ml of 18 *M* sulfuric acid, followed by 12 *M* hydrochloric acid to bring the solution to 2 *M* hydrochloric acid. Add 0.5 g of thioacetamide, heat the solution until boiling and digest for 1 h. Filter off and count for ^{76}As and ^{131}Ba.

Finally, measure the 22 *M* hydrofluoric acid fraction, the 6 *M* hydrochloric acid fraction (Fig. 3) and the resin fraction (Fig. 4) on the Ge(Li) detector.

Calculation of the results

The concentrations of the trace elements were calculated from the ratios of

the most intense photopeak areas for samples and standards. The background was obtained by graphical extrapolation of the channels neighbouring the photopeak.

For the calculation of the calcium concentration two different methods were used. The determination on the ^{47}Sc isotope, which is the daughter of ^{47}Ca, is more sensitive but has the inconvenience that there may be interferences from incomplete separation of ^{47}Sc, formed by $^{47}Ti(n,p)^{47}Sc$. Furthermore, the exact time of separation of the sample and measurement must be known. As the ^{47}Ca standard is not separated from ^{47}Sc, the amount of ^{47}Sc formed during the irradiation and after the end of the irradiation must be taken into account. All this leads to the equation:

$$\begin{array}{llll} {}^{46}Ca \xrightarrow{(n,\gamma)} {}^{47}Ca & \xrightarrow{\beta^-} {}^{47}Sc & \xrightarrow{\beta^-} {}^{47}Ti & \\ \varphi N_1 & N_2 & N_3 & \text{stable} \\ \sigma_1 & T_{\frac{1}{2}}=4.8\ \text{d} & T_{\frac{1}{2}}=3.43\ \text{d} & \end{array}$$

$$N_{3t,t'} = \frac{N_1\sigma_1\varphi}{\lambda_3-\lambda_2}(1-e^{-\lambda_2 t})\,e^{-\lambda_2 t'} + \frac{N_1\sigma_1\varphi}{\lambda_3-\lambda_2}(e^{-\lambda_3 t}-1)\,e^{-\lambda_3 t'} - \frac{N_1\sigma_1\varphi}{\lambda_3}(e^{-\lambda_3 t}-1)\,e^{-\lambda_3 t'} \quad (1)$$

= number of ^{47}Sc nuclei present after an irradiation time t, and a waiting time t' in the unseparated standard.

$$N_{3,t,t'',t'''} = \frac{N_1\sigma_1\varphi}{\lambda_3}(1-e^{-\lambda_2 t})\,(1-e^{-\lambda_3 t''})\,e^{-\lambda_2 t'''} \quad (2)$$

= number of ^{47}Sc nuclei present after an irradiation time t, a waiting time t'' between separation and measurement and a waiting time t''' between irradiation and separation.

Taking the weights of the calcium standard and the titania samples into account, one calculates the concentrations of calcium in titania by comparison of the results from eqns. (1) and (2). The accuracy of the determination obviously depends on the decontamination factor of calcium from ^{47}Sc, formed from the titanium. Reasonably reproducible results for the calcium determinations were obtained.

However, the concentration of calcium is very high, of the order of 0.1%, and it is not certain that a much lower concentration of calcium could be determined by this procedure. Under the irradiation conditions and with a decontamination factor of 99.99% for scandium isotopes, the errors introduced in the calcium determinations are smaller than 3 p.p.m., *i.e.* 0.3% of the calcium concentration found. For much lower concentrations, however, a more specific calcium–scandium separation would be necessary. Even in this case, one has to deal with interferences from the $^{50}Ti(n,\alpha)^{47}Ca$ threshold-reaction. This gives rise, in the irradiation conditions, to an apparent calcium concentration of *ca.* 300 p.p.m. The results of the calcium determination should be interpreted regarding this interference.

Calculation of lower limits

The proposal of CURRIE[12] is accepted. For full-energy peaks with areas smaller than $C_D = 4.65\ \sigma_B$ ($\sigma_B = \sqrt{B}$), the lower limit was set equal to $4.65\ \sigma_B$. For a full-energy peak with a count-rate higher than $4.65\ \sigma_B$, the concentration of the corresponding elements and the standard deviation were calculated.

RESULTS

Three titania samples were analyzed for Ag, As, Au, Ba, Ca, Ce, Co, Cr, Cu, Eu, Fe, Ga, In, K, La, Mn, Mo, Ni, Sb, Sn, Ta, Th, U, W and Zn. Spectra of some of the fractions are shown in Figs. 2–4. In a sample of commercial titania, nineteen elements were detected, while for eight others lower limits were calculated. The results are shown in Table V.

TABLE V

CONCENTRATION OF IMPURITIES IN TITANIA I

Element	*Concentration (p.p.m.)*				*σ* %
	1	*2*	*3*	$\bar{x}$	
Ag	< 0.04	< 0.03	< 0.06		
As	0.077	0.064	0.056	0.066	9
Au	< 0.02	< 0.02	< 0.02		
Ba	25.5	27.5	21.9	25.0	6
Ca 1296.6 keV	2,800	2,300	2,900	2,700	7
Ca 160.0 keV	—	2,800	2,200	2,500	
Ce	346	296	333	325	5
Co	0.082	0.097	0.098	0.092	5
Cr	3.0	2.6	3.5	3.0	10
Cu	5.7	5.9	5.6	5.7	2
Eu	0.064	0.055	0.082	0.067	12
Fe	13.3	15.1	19.6	16.0	11
Ga	< 0.6	< 0.6	< 0.6		
Hf	5.4	6.0	5.4	5.6	4
In	< 0.5	< 0.5	< 0.5		
K	1,070	1,072	969	1,037	3
La	19.1	16.8	19.9	18.6	5
Mn	0.96	0.88	0.96	0.93	3
Mo	< 5	< 5	< 5		
Na	154	161	146	154	
Ni	< 6	< 6	< 12		
Sb	261	282	269	270	2.5
Sn	< 200	< 300	< 200		
Ta	66.1	52.9	62.8	60.6	7
Th	2.20	2.20	2.46	2.29	4
U	7.9	6.7	7.1	7.2	5
W	—	5.5	4.9	5.2	10
Zn	39.2	38.9	35.7	37.9	3

Fourteen elements were detected in a sample of rutile. For twelve others the lower limits were calculated. The results, together with those of other laboratories, are shown in Table VI.

In an anatase-sample twelve elements were detected and for fourteen others the lower limits were calculated. The results are shown in Table VII, together with

results from other laboratories. All the analyses were done in triplicate.

The lower-limit determinations suffer from more important fluctuations owing to variations in the Compton continuum, as the measurements were made at different times after the end of the irradiation and during different measuring times.

TABLE VI

CONCENTRATION OF IMPURITIES IN RUTILE RLL 16 E 66

Element	*Concentration (p.p.m.)*				σ %	*Lab. A*	*Lab. B*
	1	*2*	*3*	$\bar{x}$			
Ag	< 0.07	< 0.04	< 0.07	—	—	—	—
As	< 0.02	< 0.01	< 0.02	—	—	—	—
Al	—	—	—	—	—	38	53
Au	< 0.01	< 0.01	< 0.01	—	—	—	—
Ba	14.1	15.7	15.7	15.2	3	50	—
C	—	—	—	—	—	200	100
Ca (1296.6 keV)	1,800	1,900	1,700	1,800	3	470	—
Ca (160.0 keV)	—	1,900	2,200	2,050	—	—	—
Ce	< 6	< 5	< 5	—	—	—	—
Co	0.022	0.015	0.025	0.021	14	—	—
Cr	1.2	1.7	1.9	1.6	12	2.0	3
Cu	2.86	3.04	3.05	2.98	2	2.8	3
Eu	< 0.03	< 0.02	< 0.02	—	—	—	—
Fe	29.3	29.6	27.3	28.7	3	31	28
Ga	< 0.6	< 0.6	< 0.6	—	—	—	—
Hf	8.0	8.1	8.5	8.2	2	—	—
In	< 0.1	< 0.5	—	—	—	—	—
K	1,504	1,394	1,392	1,430	2	—	—
La	< 0.04	< 0.03	< 0.03	—	—	—	—
Mg	—	—	—	—	—	39	40
Mn	0.41	0.48	0.34	0.41	10	0.5	7
Mo	< 2	< 2	< 2	—	—	—	—
Na	142	148	153	147	2	—	—
Nb	—	—	—	—	—	80	90
Ni	< 14	< 14	< 14	—	—	20	11
P	—	—	—	—	—	300	—
Pb	—	—	—	—	—	10	12
S	—	—	—	—	—	700	—
Sb	83.3	82.7	88.6	84.9	2	90	90
Si	—	—	—	—	—	160	100
Sn	< 200	< 150	< 170	—	—	10	20
Ta	7.44	7.91	7.57	7.64	2	—	—
Th	< 0.2	< 0.1	< 0.1	—	—	—	—
V	—	—	—	—	—	9.6	15
W	2.39	2.18	—	2.28	2	100	—
Zn	77.3	101.4	78.3	85.6	10	80	80
Zr	—	—	—	—	—	270	260

TABLE VII

CONCENTRATION OF IMPURITIES IN ANATASE DK 3 G 66

Element	*Concentration (p.p.m.)*				σ%	*Lab. A*	*Lab. B*
	1	*2*	*3*	$\bar{x}$			
Ag	< 0.04	< 0.03	< 0.06			—	—
As	< 0.01	< 0.02	< 0.02			—	—
Al	—	—	—			21	65
Au	< 0.012	< 0.015	< 0.020			—	—
Ba	< 19	< 14	9.1	9.1		50	—
C	—	—	—			100	100
Ca(160.0 keV)	1,300	1,600	1,600	1,500	7	420	
Ce	< 5	< 4	< 9			—	—
Co	0.015	0.012	0.015	0.014	7	—	—
Cr	1.9	1.2	1.8	1.6	12	2.1	3
Cu	2.2	2.5	2.4	2.4	4	2.1	2
Eu	< 0.03	< 0.03	< 0.03			—	—
Fe	24.0	17.3	26.9	22.7	12	39	40
Ga	< 0.4	< 0.5	< 0.6			—	—
Hf	5.2	6.4	7.4	6.3	10	—	—
In	< 0.1	< 0.3	< 0.9			—	—
K	2,820	2,930	3,000	2,920	2	3,000	—
La	< 0.03	< 0.04	< 0.04			—	—
Mg	—	—	—			49	100
Mn	1.94	1.94	1.54	1.81	7	0.9	2
Mo	< 3.5	< 4.5	< 4.7			—	—
Na	48.7	51.0	52.6	50.8	2	—	—
Nb	—	—	—			70	80
Ni	< 6	< 5	< 12			20	9
P	—	—	—			1,600	—
Pb	—	—	—			10	13
S	—	—	—			270	—
Sb	229.0	258.7	280.0	255.9	7	300	630
Si	—	—	—			110	100
Sn	< 100	< 300	< 200			10	20
Ta	3.21	3.73	3.77	3.57	4	—	—
Th	< 0.07	< 0.11	< 0.13			—	—
V	—	—	—			9.5	6
W	< 1.3	< 1.6	< 3.3			100	—
Zn	26.8	21.5	28.1	25.5	8	32	80
Zr	—	—	—			230	230

Thanks are due to the "Instituut voor Aanmoediging van het Wetenschappelijk onderzoek in Nijverheid en Landbouw" for financial support to one of us (R.N.)

SUMMARY

A procedure for the determination by neutron activation of the most important impurities in titania is described. It is based on reactor irradiation of 50-mg amounts of sample. The activities are separated in six subgroups mostly by anion exchange. The method allows the determination of antimony, arsenic, barium, calcium, chromium, cobalt, copper, gallium, gold, hafnium, indium, iron, manganese, molybdenum, nickel, potassium, rare earths, silver, sodium, tantalum, thorium, tin, tungsten, uranium and zinc. The reproducibility and the accuracy of the analytical results are satisfactory. The sensitivity ranges from $5 \cdot 10^{-4}$ μg (10 p.p.b. in 50 mg of sample) for arsenic and gold up to 5 μg for tin.

RÉSUMÉ

On décrit une méthode pour le dosage par activation neutronique des principales impuretés de l'oxyde de titane. Elle est basée sur l'irridiation au réacteur d'échantillons de 50 mg. Les activités sont séparées en six sous-groupes principalement par échangeur d'anions. Cette méthode permet le dosage des éléments suivants: antimoine, argent, arsenic, baryum, calcium, chrome, cobalt, cuivre, étain, fer, gallium, hafnium, indium, manganèse, molybdène, nickel, or, potassium, sodium, tantale, terres rares, thorium, tungstène, uranium et zinc. La reproductibilité et la précision des résultats sont satisfaisantes. La sensibilité, de l'ordre de $5 \cdot 10^{-4}$ μg pour arsenic et or (10 p.p.b. dans des échantillons de 50 mg), s'élève à 5 μg pour l'étain.

ZUSAMMENFASSUNG

Es wird ein Verfahren zur Bestimmung der wichtigsten Verunreinigungen in Titan und Titandioxid mit Hilfe der Neutronenaktivierungsanalyse beschrieben. Dazu werden 50 mg-Proben im Reaktor bestrahlt, und anschliessend die Aktivitäten vorwiegend mit Anionenaustauschern in 6 Untergruppen aufgeteilt. Diese Methode erlaubt die Bestimmung von Antimon, Arsen, Barium, Calcium, Chrom, Cobalt, Kupfer, Gallium, Gold, Hafnium, Indium, Eisen, Mangan, Molybdän, Nickel, Kalium, Seltene Erden, Silber, Natrium, Tantal, Thorium, Zinn, Wolfram, Uran und Zink. Die Reproduzierbarkeit und Richtigkeit der analytischen Ergebnisse sind befriedigend. Die Empfindlichkeit reicht von $5 \cdot 10^{-4}$ μg (10 p.p.b. in 50 mg-Proben) für Arsen und Gold bis zu 5 μg für Zinn.

REFERENCES

1 L. G. STONHILL, *Chemist Analyst*, 47 (1958) 68.
2 W. A. WEYL AND T. FÖRLAND, *Ind. Eng. Chem.*, 42 (1950) 257.
3 F. K. MCTAGGART AND J. BEAR, *J. Appl. Chem.*, 5 (1955) 643.
4 A. LAGROU, J. VANHEES AND F. VERBEEK, *Z. Anal. Chem.*, 224 (1967) 310.
5 P. F. S. JACKSON AND J. WHITEHEAD, *Analyst*, 91 (July 1966) 418.
6 J. BROWNLEE, *AECU-4438*, 1959, p. 63.
7 M. SANKAR, *ORA Project 04997*.
8 W. A. BROOKSBANK, *ORNL-1880*, 1955, p. 14.
9 I. P. ALIMARIN AND YU. V. YAKOVLEV, *AEC-Tr-4497*, p. 169.
10 R. NEIRINCKX, F. ADAMS AND J. HOSTE, *Anal. Chim. Acta*, 43 (1968) 369.
11 R. DAMS AND F. ADAMS, *Radiochim. Acta*, 10 (1968) 1.
12 L. CURRIE, *Anal. Chem.*, 40 (1968) 587.

Analytica Chimica Acta
Elsevier Publishing Company, Amsterdam
Printed in The Netherlands

A THIN-LAYER CHROMATOGRAPHIC METHOD FOR THE DETERMINATION OF PLANT PIGMENTS IN SEA WATER AND CULTURES*

C. GARSIDE AND J. P. RILEY
Department of Oceanography, The University of Liverpool, P.O. Box 147, Liverpool L69 3BX (England)

(Received April 2nd, 1969)

One of the most urgent requirements in biological oceanography is the acquisition of data on marine primary productivity, to enable maps of the most productive areas of the world's oceans to be prepared. Estimates of the biomass, and hence of productivity, can be conveniently made from measurements of the concentrations of the photosynthetic pigments – chlorophylls *a*, *b*, and *c* in the water. Although total chlorophylls can be determined directly by fluorimetry[1,2], most determinations of these pigments are carried out by modifications of the extractive spectrophotometric procedure of RICHARDS[3]. In these methods the separated particulate matter is extracted with 90% acetone and the extract is examined spectrophotometrically at the absorption maxima of the three chlorophylls. A survey of the method has been published by PARSONS[4] who has drawn attention to the many factors which must be taken into account before a standard method for pigment analysis can be recommended.

An investigation carried out under the aegis of UNESCO has done little to clarify the situation, and the report on this work[5] is largely inconclusive and at times self-contradictory. Even if the difficulties associated with the filtration of the water sample and the quantitative extraction of the pigments are disregarded, the method has several drawbacks: (i) the absorption bands of the chlorophylls overlap seriously and the precision attainable in the determination of minor chlorophylls is therefore very poor; (ii) the sensitivity is relatively low, and this necessitates the use of large water samples; (iii) no information is obtained about the presence of chlorophyll degradation products, and in fact certain of these are determined along with their parent chlorophylls; (iv) only a very approximate figure can be obtained for the total xanthophyll concentration; (v) the individual xanthophylls cannot be identified, although they can often give a useful indication of the species of phytoplankton present in the water sample. Many of these difficulties could be overcome by separating the pigments before determining them.

Separation of the plant pigments can be readily achieved by chromatography. Paper chromatography has been used[6,7] to separate the pigments of marine algae grown in culture. However, it is too insensitive to be used conveniently with sea water samples. Much greater sensitivity can be attained by means of thin-layer chromatography, and several workers[8–11] have used this technique for the qualitative examination of the pigments of marine organisms. JEFFREY[12] has devised a comprehensive

* Presented in part at the International Symposium on Analytical Chemistry, Birmingham, July, 1969.

scheme for the separation and quantitative determination of a wide variety of marine phytoplankton pigments. In this, the majority of pigments were separated by two-dimensional thin-layer chromatography on sucrose containing 5% of cornflour. Chlorophyll *c* and certain chlorophyll degradation products could not be separated in this way and were resolved on thin layers of cellulose or polyethylene. The pigments were determined by eluting the spots with suitable solvents followed by spectrophotometry. However, the method is too time-consuming to be used for routine work, and the very low pigment capacity of the plates makes it difficult to obtain eluates with sufficiently high absorbances for accurate measurement of major, let alone minor, pigments. The present paper describes a quantitative method for the routine determination of plant pigments in sea water and cultures. In this procedure pigments are separated as described by RILEY AND WILSON[9] and determined on the thin layer plate by reflective densitometry. This technique has a sensitivity approximately ten times greater than RICHARD's method[3], and permits the determination of not only chlorophylls, but also carotene, many individual xanthophylls and some chlorophyll degradation products.

The analysis is carried out in five stages—filtration, extraction, removal of solvent, thin-layer chromatography and densitometry. To simplify the discussion the development of each of these stages will be considered separately.

Filtration of samples

In this stage it is essential not only that the filtration should be rapid and efficient, but also that the filter should be insoluble in the polar solvents used to extract the pigments, to prevent interference in the chromatographic stage. The latter criterion rules out the use of cellulose ester filters such as Millipore HA which are generally used in the extractive spectrophotometric procedure. The choice of filters is therefore restricted to those composed of reconstituted cellulose (*e.g.* Sartorius Cella) or glass fibre (*e.g.* Whatman GF/C). There is some difference of opinion about the suitability of Whatman GF/C filters for this purpose. SPENCER[13] found them to be very suitable for plant pigment analysis. However, HUMPHREY AND WOOTON[14] observed that they only retained *ca.* 75% of the chlorophylls held by a Millipore HA filter, whereas the retentitivity of Sartorius Cella filters was similar to that of the Millipore HA.

Tests were therefore carried out to compare the efficiencies of Cella, Oxoid and GF/C filters with that of the Millipore HA filter. Aliquots (1 l) of dilute cultures of *Phaeodactylum tricornutum* (Plymouth No. 100), *Olisthodiscus sp.* (Plymouth No. 239) and *Coscinodiscus granii* (Plymouth No. 1050) were filtered separately through 5-cm discs of the above filters at a vacuum of *ca.* 250 torr. The flow rates of all the membrane filters were *ca.* 120 ml/min, with the exception of the Oxoid filter which was much slower (*ca.* 25 ml/min). The GF/C filters had a flow rate of *ca.* 500 ml/min. The filters were extracted with 15 ml of 90% aqueous acetone for 10 min with ultrasonic agitation. The absorbances of the extracts were measured at 663 nm after centrifugation. The Oxoid, Cella and Millipore filters appeared to behave identically. However, the spread between replicate measurements with the same filter amounted to a few percent and this made it difficult to draw any firm conclusions. The average results obtained with the GF/C filters were usually 5–10% below the average of those obtained with other filters, but did lie within the spreads of their experimental measurements. This suggested that the glass fibre filters were allowing particulate matter to pass

through. In order to check whether this was the case, the filtrates from the above experiments were examined with a Coulter counter. It was found that the cultures after filtration through cellulose and cellulose acetate filters contained no detectable particles having diameters within the range of the instrument (2–100 μm). As had been anticipated the GF/C filters did not retain the phytoplankton quantitatively, but allowed some of the cells to pass through; the proportion ranging from $< 1\%$ of the particles of diameter 25 μm to *ca.* 5% of those having a diameter of 4 μm. However, if the filters were covered with a 1–2 mm layer of magnesium carbonate the particles were retained as efficiently as with a Millipore filter.

An appreciable proportion of the organic particulate matter in the sea has a diameter smaller than 1.5 μm— the smallest size which can be counted with precision with the Coulter counter. Fluorimetric measurements[15] were therefore made on sea water filtrates obtained with the various filters to determine whether they were allowing any detectable amounts of chlorophyll to pass. No traces ($< 0.5\%$ of the total chlorophyll) could be detected in the filtrates from the Millipore HA, Oxoid, Cella or from the Whatman GF/C filter if it was covered with a layer of magnesium carbonate. However, the filtrate from the uncovered GF/C filter still contained *ca.* 5% of the chlorophyll present in the original sample. The Whatman GF/C filter covered with magnesium carbonate was finally selected for the present work, on account of its efficiency, and also because of the ease with which the plant pigments could be extracted off it. In contrast, the Cella filters, although efficient, were flimsy and it was difficult to extract the pigments off them, perhaps because of penetration of the particulate matter into the body of the filter.

Extraction of pigments

Methanol is a much more effective solvent for the extraction of phytoplankton pigments than aqueous 90% acetone, which is usually used for this purpose[16]. However, it suffers from the disadvantage that rapid degradation of chlorophylls to phaeophytins frequently occurs in it, probably because it does not deactivate chlorophyllases. Experiments showed that if a preliminary extraction is made with a small volume of acetone, and the extraction is completed with methanol, the combined extract is stable.

The phytoplankton pigments are comparatively labile and in choosing an extraction technique it is necessary to ensure that in attaining complete extraction no degradation occurs. Overnight digestion with 90% acetone[3] has been found to give incomplete extraction [14]. Although HUMPHREY AND WOOTON[14] have found that mechanical grinding increases the efficiency of extraction, other workers[17] have shown that degradation occurs if the grinding lasts longer than 1 min. Ultrasonic agitation has been used by NELSON[18] and by LAESSØE AND HANSEN[16] to accelerate the extraction of phytoplankton pigments. This technique has been employed in the present investigation, the extraction being carried out for 15 min with methanol. Tests showed that no detectable decomposition of any phytoplankton pigments occurred even when the ultrasonic extraction was continued for a further hour. Only one out of over 50 species of phytoplankton examined in these laboratories was not extracted quantitatively in 15 min. The exception—*Chlorella salina* (Plymouth No. 309) — required grinding and prolonged ultrasonic agitation before it could be extracted completely. Contrary to the suggestion in the UNESCO report[5], freeze-drying did not make plank-

ton easier to extract and this process was without effect on the extraction of the *Chlorella sp.* No difficulties were usually experienced in the chromatography stage from substances, other than pigments, extracted from the plant cells by acetone and methanol (but see *Procedure*, p. 187).

Removal of solvent from extracts

Before the pigments can be applied to the thin-layer plates, it is necessary to concentrate them from the solvent extracts. In most previous work this has been done by adding sodium chloride solution and then extracting with ether, after which the ether is evaporated in a stream of nitrogen. This process is time-consuming and suffers from the disadvantages that it is difficult to make quantitative, and that the pigments which it yields are damp. In order to accelerate and simplify the recovery of pigments from the acetone–methanol extracts tests were made with a rotary vacuum evaporator. The evaporation of the extract could be completed in 5 min with a water bath temperature not exceeding 50°. Under these conditions no decomposition of the chlorophylls or carotenoids could be detected. This rapid method was used in all subsequent work; the pigments recovered in this way were dissolved in ether for application to the thin-layer plates.

Thin-layer chromatography

The following criteria were borne in mind in selecting the thin-layer procedure for the separation of phytoplankton pigments: (i) it should be possible to separate the individual chlorophylls, their degradation products and the carotenoids; (ii) the stationary phase should have a sufficiently high carrying capacity to enable minor pigments to be identified and determined; (iii) for simplicity and speed only unidimensional development should be needed; (iv) the stationary phase should not decompose the pigments; (v) it should be possible to remove the xanthophylls quantitatively from the thin layer to confirm their identity by co-chromatography and spectrophotometry.

The choice of the correct adsorbent is of crucial importance in satisfying these requirements. A survey of the literature showed that the following stationary phases have been used in the thin-layer chromatography of plant pigments; silica gel G[9,10,19,20], glucose[8], sucrose[12,20–22] and cellulose[23,24]. Tests showed that the above organic adsorbents, particularly cellulose, had only *ca.* 1/5–1/10 of the carrying capacity of silica gel; this puts restrictions on the use of spectrophotometric procedures for the confirmation of the identity and determination of the pigments. In addition, the separations which could be achieved on these materials by unidimensional chromatography were considerably inferior to those obtained with silica gel G[9]. Not only did the various pigment spots lie close together, but they were generally more diffuse. There are various reports in the literature that silica gel, because of its acidic nature, may cause degradation of chlorophylls[19,23] and some carotenoids[15,26]. However, we have been unable to detect any decomposition of even the more labile pigments on silica gel under the basic conditions used by RILEY AND WILSON[9]. It was therefore decided to adopt the latter method, but it was thought advisable to determine whether any other type of silica gel would give separations superior to silica gel G. Merck pre-coated silica gel G plates gave performances much inferior to hand-coated plates. Tests showed that excellent separations of the pigments and in particular of the in-

dividual xanthophylls, were obtained with Merck silica gel PF254. This adsorbent was used in all subsequent work with a slight modification of the developing solvent used by RILEY AND WILSON[9].

The routine identification of the pigments was carried out on the basis of their R_p values relative to chlorophyll *a* (see below). In order to obtain reproducible values the chromatographic tank was maintained at 20.0 ± 0.3°, and great care was taken to ensure that the atmosphere in the tank was completely saturated with solvent vapour. In general the individual xanthophylls were resolved and could be readily identified. However, if there was any doubt about the identity or homogeneity of any spot, it was eluted and examined spectrophotometrically. Further information about the pigment could be obtained by chromatography on thin-layer plates coated with a 1:1 mixture of glucose and sucrose, which resolves xanthophylls better than the separate sugars.

RILEY AND WILSON'S[9] solvent system is not sufficiently polar to develop chlorophyll *c* away from the origin spot. This is not a serious disadvantage with the pigments from vigorously growing cultures, that are free from degradation products, and therefore give little contamination of this spot. However, senescent cultures and some sea water samples may contain enough of these materials to produce serious errors in the chlorophyll *c* determination. For this reason a search was made for a solvent system which could be used to resolve chlorophyll *c*. It was found that a good separation of chlorophyll *c* could be achieved by carrying out a further development of the plate with a mixture of light petroleum, ethyl acetate and formdimethylamide in the ratio 1:1:2 by volume. The efficiency of this separation stage was tested by carrying out a chromatographic run on the mixed pigments from a senescent culture of *Phaeodactylum tricornutum*. The chlorophyll *c* spot was eluted with methanol and its absorption curve in 90% acetone was plotted. The curve so obtained was identical with published curves for this pigment, showing that separation from coloured impurities had been achieved, and that the comparatively labile chlorophyll *c* had not been degraded. This development stage was therefore adopted for the working method, and was carried out after photometry of the carotenoids and other chlorophylls had been performed.

Although it is preferable to measure the plates immediately, this may not always be possible. The developed plates can be stored for at least a month at −20° if the thin layer is covered with a sheet of glass. Under these conditions only carotene showed any signs of decomposition, its amount falling by *ca.* 20% in the first week and thereafter remaining constant.

Photometry

Previous methods for the determination of phytoplankton pigments after thin-layer chromatography have involved eluting the spots and carrying out spectrophotometry on the eluates[12,9]. This procedure is very time-consuming, if many pigments are present in the samples, and its lack of sensitivity is a very important limitation. Thus, 1 μg of carotene ($E^{1\%}_{1cm}$ = 2500) dissolved in 3 ml of light petroleum will give an optical density of but 0.075 in a 1-cm cuvette and similar concentrations of chlorophylls will give readings of only 1/3 of this. It is therefore difficult to see how JEFFREY[12] can claim to determine minor pigments accurately when the *total* pigment loading of her plates was only 0.5–4 μg.

Measurement of the reflectance or absorbance of the pigment spots *in situ*

is an alternative means of determining phytoplankton pigments separated by thin-layer chromatography. This technique has the advantages over spectrophotometry that it is faster and about ten times as sensitive. It has the disadvantage that it does not provide wavelength *vs.* absorption curves for confirmation of the identities of xanthophylls, which are therefore deduced solely on the basis of their R_p values (see below). However, this is not a serious drawback since if there is any doubt about the identity, it is a simple matter to elute the pigment from the spot and examine it by co-chromatography on sucrose–glucose thin-layers.

A Joyce Loebl Chromoscan fitted with a thin-layer scanner was used to investigate the potentialities of this alternative approach. This instrument provides a graphical record of light absorbance or reflectance along the length of the thin-layer plate. A mechanical integrator is fitted which measures the areas under the peaks on this record and enables the pigment to be determined quantitatively.

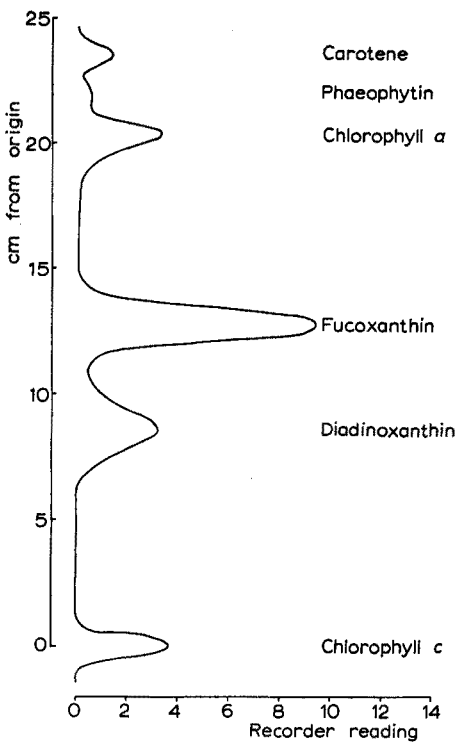


Fig. 1. Chromoscan trace for thin-layer chromatogram of pigments from senescent *Phaeodactylum tricornutum*.

Preliminary tests with the instrument showed that it gave a higher sensitivity and more constant base line when reflection was used rather than transmission. A 10 × 1 mm slit was placed in the scanning light beam and this enabled the whole width of the spot to be scanned. The best overall response for all pigments was obtained when the plate was scanned with light which had been filtered through an Ilford No. 601 filter (maximum transmission at 430 nm). The pigment spots generally gave symmetrical peaks on the recorder chart (Fig. 1). The identification of a pigment spot

was carried out by measuring the distance on the record between its peak and that of the origin, and dividing this by the distance moved by the chlorophyll *a* peak. Comparison of the ratio thus obtained (R_p) with those obtained from authentic pigments enabled the identification to be completed (see Table I). The R_p values are much less affected by small variations in the chromatographic conditions than are the more conventional R_F values; even so, if pigments are to be identified accurately these conditions should be controlled carefully. To check the reproducibility of the R_p values, 6 replicate runs were carried out on a pigment extract from a mixture of phytoplankton species. They showed (Table I) that the R_p values were satisfactorily reproducible. It should be stressed that the R_p values for the various pigments should be redetermined for each fresh batch of silica gel, as they may vary slightly.

TABLE I

AVERAGES AND STANDARD DEVIATIONS FOR R_p VALUES AND CALIBRATION VALUES FOR PIGMENTS

Pigment	R_p	*ng of pigment/unit integrator reading*[a]
Chlorophyll *a*	1	47.4 ± 0.4
Chlorophyll *b*	0.82 ± 0.02	14.5 ± 0.7
Chlorophyll *c*	0.00	22.4 ± 0.5
Phaeophytin *a*	1.07 ± 0.04	42.8 ± 0.4
β-Carotene	1.17 ± 0.04	11.8 ± 0.4
Fucoxanthin	0.64 ± 0.03	29.1 ± 1.0
Lutein	0.55 ± 0.02	33.6 ± 0.9
Violaxanthin	0.41 ± 0.01	23.8 ± 1.2
Diadinoxanthin + dinoxanthin	0.44 ± 0.02	19.2 ± 0.9
Neoxanthin	0.21 ± 0.02	26.7 ± 0.7

[a] On the most sensitive setting of the instrument.

The reproducibility of the integrator of the Chromoscan was tested by carrying out a series of 6 replicate runs on the same chromatogram of a mixture of phytoplankton pigments. It was observed that the integrator readings for any pigment had a spread of less than ± 2%. An estimate of the reproducibility, from one plate to another, was obtained from the integrator readings from 6 replicate chromatograms. It was found that the percentage of the total integrated area represented by a particular pigment never varied by more than ±1%.

In order to calibrate the Chromoscan, silica gel plates were spotted with known μl volumes of extracts of a range of phytoplankton species. After development, the plates were scanned with the instrument at its highest sensitivity. The amount of pigment in each spot was determined by extracting it with 90% acetone, diluting to a suitable volume and determining the absorbance at the appropiate wavelength. The following $E^{1\%}_{1cm}$ values were employed in calculating the weights of pigments: chlorophyll *a*, 911 at 663 nm[27]; *b*, 525 at 645 nm[27]; *c*, 1400 at 446 nm[28]; phaeophytin *a*, 1310 at 409 nm[28]; β-carotene, 2505 at 451 nm[29]; fucoxanthin 1040 at 449 nm[30]. Because reliable data were not available for the other xanthophylls the value of 1000 at the absorption maximum was assumed for these pigments. It was found that for each of the pigments the integrator reading was a linear function of the amount of pigment, up to a recorder reading of about 80% of full scale deflection (Fig. 2). The

average calibration values for a range of pigments, expressed as ng of pigment/unit integrator reading are presented in Table I. If an integrator reading of 3 is taken as significant, then the sensitivity of the method ranges from 0.03 μg for β-carotene to *ca.* 0.14 μg for chlorophyll *a*. The maximum loading of the plate should not exceed 30 μg of total pigments if reliable results are to be obtained. It was thought that even

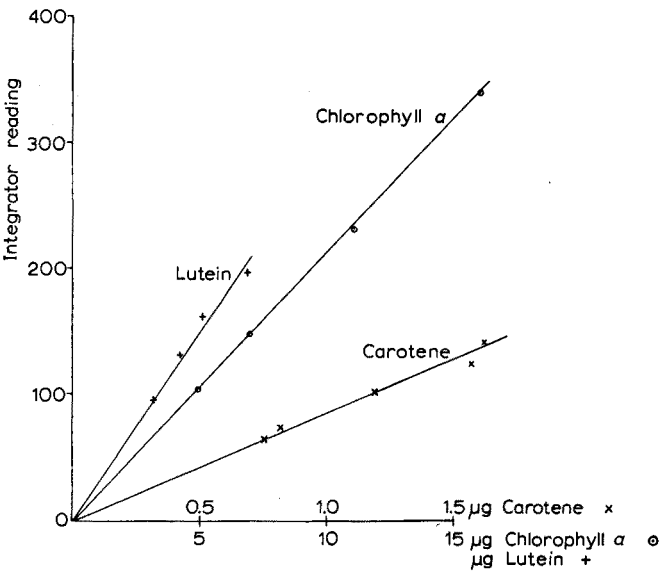


Fig. 2. Calibration curves for determination of some phytoplankton pigments.

higher sensitivities for chlorophylls might be attained by scanning the plate with ultraviolet radiation and examining the red fluorescent light which they emit. It was found that chlorophyll spots invisible to the eye could be readily detected in this way, the ultimate sensitivity being *ca.* 0.3 ng of chlorophyll *a*. This technique may be of value for the determination of small quantities of these pigments. However, the relationship between the intensity of the excited light and amount of chlorophyll was only linear up to 10 ng. Absorption of the UV and excited lights by the pigment spot prevented the use of the method for the determination of greater amounts of chlorophyll.

EXPERIMENTAL

Reagents

Magnesium carbonate suspension. Add *ca.* 3 g of finely powdered magnesium carbonate (light) to 100 ml of distilled water. Shake immediately before use.

First developing solvent. Prepare from redistilled solvents a mixture containing light petroleum (b.p. 60–80°), ethyl acetate and diethylamine in the ratio 55:32:13 by volume.

Second developing solvent. Prepare from redistilled solvents a mixture containing light petroleum (b.p. 60–80°), ethyl acetate and formdimethylamide in the ratio 1:1:2 by volume.

Developing solvent for use with glucose–sucrose plates. Mix light petroleum (b.p.

60–80°) with 0.5–5% of its volume of *n*-propanol according to the xanthophylls to be separated.

Thin-layer plates

Silica gel. Coat 6 × 20 cm glass plates with a 0.25-mm layer of Merck silica gel PF254. Dry the coated plates in an oven at 100° for several hours and allow to cool in the laboratory (relative humidity 50–60%) for 30 min before use.

Glucose–sucrose plates. Shake a mixture of equal weights of glucose and sucrose (both ground to pass a 40-mesh sieve) with ethyl acetate using glass beads to assist the mixing process. Spread the resultant slurry as a 0.5-mm layer on 6 × 20 cm glass plates using a Desaga spreader and allow the ethyl acetate to evaporate at room temperature.

Procedure

All work on the pigment and its extracts must be carried out as rapidly as possible in subdued light. If it should be necessary to store pigments they should be dissolved in peroxide-free ether containing 1% of diethylamine, blanketed with nitrogen and refrigerated at −20°.

Place a 7-cm diameter Whatman GF/C glass fibre filter in a filter holder and pour onto it sufficient magnesium carbonate suspension to give a layer 1–2 mm in thickness. Fit a 200-mesh stainless steel gauze into the filter holder about 1 cm above the magnesium carbonate to remove most of the zooplankton (this filter is not necessary if cultures are being examined). Filter a known volume of the sample, sufficient to provide *ca.* 12 μg of chlorophyll *a* (normally 0.5–5 l of sea water or 10–100 ml of culture) using a vacuum not exceeding 250 torr. As soon as the last drops of liquid have passed through, transfer the filter and magnesium carbonate to a test tube, add 3–5 ml of acetone and gently break up the filter with a glass rod. Place the test tube in an ultrasonic bath for 4–5 min, add 10 ml of methanol, mix and continue the ultrasonic agitation for a further 10 min. Remove the test tube from the bath and decant the solvent mixture. Pass it through a drawn out glass tube containing a 2-cm layer of anhydrous sodium sulphate retained by a plug of glass fibre filter. Collect the dry extract in a 25-ml pear-shaped flask. Wash the residue and sodium sulphate with a few ml of methanol and diethyl ether and combine the washings with the extract. The residue from the plankton should now be practically colourless. In the rare event that some pigment remains in the residue, repeat the ultrasonic extraction with further 5-ml aliquots of methanol until the extracts are no longer coloured.

Evaporate the combined extracts *in vacuo* with a rotary evaporator, the bath temperature of which should not exceed 50°. Immediately the solvent has evaporated, dissolve the residue in 1–2 ml of diethyl ether containing 1% (v/v) of diethylamine. Gradually apply the whole of this solution (or a known fraction of it) as a small spot *ca.* 2 cm from the end of a 6 × 20 cm plate coated with silica gel. Scratch a mark at the side of the plate 10 cm further up the plate from the centre of this spot. While the rotary evaporation stage is proceeding, allow a chromatographic tank (maintained at 20°) containing *ca.* 0.6 cm of the first developing solvent to come to equilibrium.

The extracts from some very low productivity waters yield a white or yellow residue which interferes in the chromatographic separation. This interference can be prevented by carrying out a preliminary development with light petroleum (b.p.

60–80°) to the top of the plate. As soon as possible transfer the spotted plate to the tank and develop until the centre of the chlorophyll *a* spot is level with the scratch on the plate. Remove the plate from the tank and allow the solvent to evaporate for 2 min. Scan the plate with the Chromoscan in the reflectance mode using light filtered through an Ilford No. 601 filter (maximum transmission at 430 nm). Note the integrator reading for each peak and calculate the R_D values relative to chlorophyll *a*. If the identity of any of the xanthophyll spots is in doubt, scrape off the spot and extract it with methanol. Evaporate the extract in a rotary evaporator, take up the pigment in 1–2 ml of *n*-hexane and determine its absorption spectrum using 4-cm microcells. Further confirmation of the identity of the pigment can be obtained by co-chromatography with authentic pigments on glucose–sucrose plates with a mixture of light petroleum containing *n*-propanol as developing solvent.

During the initial chromatography on silica gel, chlorophyll *c* remains at the origin. Carry out the chromatographic separation of this pigment from the base line spot with the second developing solvent. Develop in the same direction as before, unless xanthophyll spots have been removed for identification, when the development should be made at right angles to the previous direction. Conclude the development when the chlorophyll *c* spot has been separated from the origin spot by *ca.* 1 cm. Allow the solvent to evaporate for 5 min and scan the chlorophyll *c* spot with the Chromoscan using an Ilford No. 601 filter. The blank for the method is normally negligible, but should be determined each time batches of reagents are changed, by extracting an unused filter and carrying out the whole procedure.

To standardize the method, prepare a mixture of plankton species containing chlorophylls *a*, *b* and *c*, carotenes, and the xanthophylls expected in the samples. Extract the pigments and carry out the chromatography as described above. Measure the plate with the Chromoscan. Scrape off the spots and transfer each quantitatively to drawn-out pieces of 8-mm bore tubing containing glass fibre plugs. Moisten the powder with a few drops of methanol and then elute with *ca.* 4.5 ml of 90% acetone, collect the eluate in a 5-ml graduated flask and dilute to volume with the same solvent. Measure the absorbance of each pigment solution at the appropriate absorption maximum. Minor xanthophylls may not give sufficiently high absorbances for precise standardization. For these pigments a preparative run should be made by streaking the extract of mixed pigments on a thin-layer plate coated with silica gel PF254, and developing with the first developing solvent. The spots of the xanthophylls in question are then eluted and run as described above.

RESULTS

In order to assess the precision of the method, 6 replicate analyses were carried out on 100-ml portions of a composite phytoplankton culture. The mean weights and standard deviations found for the various pigments were: chlorophyll *a* 7.87 ± 0.33 μg; chlorophyll *b* 1.68±0.05 μg; chlorophyll *c* 0.76±0.04 μg; phaeophytin *a* 1.41± 0.13 μg; carotene 1.04±0.04 μg; lutein 4.67±0.17 μg; violaxanthin 0.67±0.05 μg; neoxanthin 1.23±0.11 μg. Thus, for the chlorophylls and major xanthophylls the coefficient of variation of the method did not exceed 5%.

Three cultures of marine phytoplankton (including a senescent one of *Phaeodactylum tricornutum*) were examined by the proposed method. The pigments were

TABLE II

COMPARISON BETWEEN PIGMENT ANALYSES CARRIED OUT BY THE PRESENT METHOD, THOSE OBTAINED BY SPECTROPHOTOMETRY OF THE ELUTED SPOTS AND BY THE POLYCHROMATIC METHOD

Pigment	*Present method*		*Elution and photometry*[9]		*Polychromatic method*[15,31]
	μg/100 ml	*Percentage of total carotenoid*	*μg/100 ml*	*Percentage of total carotenoids*	*μg/100 ml*
	Phaeodactylum tricornutum[a] (Plymouth No. 100)				
Chlorophyll *a*	2.13		2.07		1.96
Chlorophyll *b*	0.00		0.00		0.24
Chlorophyll *c*	0.99		0.72		0.87
Phaeophytin *a*	0.17		0.16		—
Carotene	0.18	3.4	0.17	3.2	
Fucoxanthin	3.82	72.8	3.77	73.4	4.56
Diadinoxanthin	1.25	23.8	1.20	23.4	
Ratio of chlorophyll *a*:*c*	1:0.46		1:0.34		1:0.44
	Dunaliella primolecta (Plymouth No. 81)				
Chlorophyll *a*	0.94		0.92		0.87
Chlorophyll *b*	0.26		0.25		0.26
Chlorophyll *c*	0.00		0.00		0.05
Carotene	0.11	1.9	0.12	2.2	
Lutein	4.06	72.8	3.99	74.4	5.15
Violaxanthin	1.03	18.6	0.94	17.6	
Neoxanthin	0.31	5.7	0.31	5.8	
Ratio of chlorophyll *a*:*b*	1:0.28		1:0.27		1:0.33
	Olisthodiscus sp. (Plymouth No. 239)				
Chlorophyll *a*	2.32		2.11		2.01
Chlorophyll *b*	0.00		0.00		0.25
Chlorophyll *c*	1.23		1.13		2.21
Carotene	0.59	18.1	0.57	16.1	
Fucoxanthin	2.07	63.6	2.41	68.2	7.28
Diatoxanthin	0.60	18.3	0.55	15.7	
Ratio of chlorophyll *a*:*c*	1:0.53		1:0.53		1:1.10

[a] A senescent culture was used.

also determined spectrophotometrically in 90% acetone extracts of the individual spots[9]. In addition, the original cultures were examined by the polychromatic method[15], and their pigment contents were evaluated by means of the equations of PARSONS AND STRICKLAND[31]. The results of these comparative studies are presented in Table II. They show that there is reasonably close agreement between the two thin-layer methods. The polychromatic method gives results for chlorophyll *a* in reasonable accord with those found by the other procedures. However, the figures for other pigments by this method are of variable quality, and there is a tendency for it to give completely spurious results for chlorophyll *b*. To conclude, the thin-layer densitometric method is suitable for shipboard use and provides quantitative data not only on the photosynthetic pigments, but also on the individual xanthophylls. It has 5–10 times the sensitivity of the polychromatic method and is rapid — a complete analysis can be performed in 1 hour.

SUMMARY

A combined thin-layer chromatographic–reflection densitometric procedure is described for the determination of chlorophylls and carotenoid pigments in marine particulate matter. A comparison of various methods of filtration showed that optimum recoveries of plant pigments are obtained with Whatman GF/C glass fibre filters covered with a layer of magnesium carbonate. Plant pigments are extracted by means of acetone and methanol and the combined extracts are evaporated under reduced pressure. Thin-layer chromatography is carried out on silica gel G. Chlorophyll *c* remains at the starting point and is separated from the origin spot by a subsequent development with a more polar solvent mixture. The separated pigments are determined on the plate by photodensitometry in the reflectance mode. Chlorophylls *a*, *b* and *c*, carotene, many of the individual xanthophylls and certain degradation products of the chlorophylls can be determined. The complete analysis can be performed in under 1 h. The sensitivity of the method for chlorophyll *a* is *ca.* 0.12 μg and the precision for most pigments is $\pm 5\%$ or better at the 0.5-μg level.

RÉSUMÉ

On décrit une méthode combinée, chromatographie sur couche mince–densitométrie par réflexion, pour le dosage des pigments chlorophylles et caroténoïde dans des eaux de mer. Après comparaison de diverses méthodes de filtration, les auteurs ont adopté les filtres en fibres de verre Whatman GF/C recouverts d'une couche de carbonate de magnésium. Les pigments végétaux sont extraits au moyen d'acétone et de méthanol, les extraits mélangés sont évaporés sous pression réduite. La chromatographie sur couche mince est effectuée sur gel de silice G. Une analyse complète peut se faire en moins d'une heure. La sensibilité de la méthode pour la chlorophylle *a* est d'environ 0.12 μg; la précision pour la plupart des pigments est de $\pm 5\%$, ou meilleure encore pour 0.5 μg.

ZUSAMMENFASSUNG

Zur Bestimmung von Pflanzenpigmenten in Wasser-Kulturen wird ein kombiniertes Verfahren aus Dünnschichtchromatographie und Reflexionsdensitometrie beschrieben. Für die Filtration der Pflanzenpigmente eignet sich am besten ein Whatman GF/C-Glasfiberfilter, welches mit einer Schicht von Magnesiumcarbonat bedeckt ist. Die Pigmente werden mit Aceton und Methanol extrahiert und die vereinigten Extrakte unter vermindertem Druck verdampft. Die Dünnschichtchromatographie wird auf Silicagel G durchgeführt. Chlorophyll *c* verbleibt beim Startpunkt und wird von diesem durch nachfolgende Behandlung mit einer mehr polaren Lösungsmittelmischung abgetrennt. Die abgetrennten Pigmente werden dann auf der Platte mit der reflektierenden Photodensitometrie bestimmt. Die Chlorophylle *a*, *b* und *c*, Caroten, zahlreiche einzelne Xanthophylle und bestimmte Abbauprodukte des Chlorophylls können bestimmt werden. Die vollständige Analyse kann in weniger als 1 Stunde durchgeführt werden. Die Empfindlichkeit der Methode für Chlorophyll beträgt 0.12 μg und die Richtigkeit für die meisten Pigmente ist $\pm 5\%$ oder besser im 0.5 μg-Bereich.

REFERENCES

1 K. KALLE, *Dtsch. Hydrogr. Zeitschr.*, 4 (1951) 92.
2 C. J. LORENZEN, *Deep Sea Res.*, 13 (1966) 223.
3 F. A. RICHARDS WITH J. G. THOMPSON, *J. Marine Res. (Sears Found. Marine Res.)*, 2 (1952) 150.
4 T. R. PARSONS, *J. Fish. Res. Board Can.*, 18 (1961) 1017.
5 *UNESCO Monographs on Oceanographic Methodology No. 1*, Determination of photosynthetic pigments in sea water, 1966.
6 S. W. JEFFREY, *Biochem. J.*, 80 (1961) 336.
7 S. W. JEFFREY AND M. B. ALLEN, *J. Gen. Microbiol.*, 36 (1964) 277.
8 J. C. MADGWICK, *Deep Sea Res.*, 12 (1965) 233.
9 J. P. RILEY AND T. R. S. WILSON, *J. Marine Biol. Assoc. U.K.*, 45 (1965) 583.
10 J. P. RILEY AND T. R. S. WILSON, *J. Marine Biol. Assoc. U.K.*, 47 (1967) 351.
11 J. P. RILEY AND D. SEGAR, *J. Marine Biol. Assoc. U.K.*, in press.
12 S. W. JEFFREY, *Biochim. Biophys. Acta*, 162 (1968) 271.
13 C. P. SPENCER, *J. Conseil, Conseil Perm. Intern. Exploration Mer.*, 28 (1964) 327.
14 G. F. HUMPHREY AND M. WOOTON, in *UNESCO Monographs on Oceanographic Methodology No. 1*, 1966, p. 38.
15 T. R. PARSONS AND J. D. H. STRICKLAND, *A Practical Handbook of Seawater Analysis*, Fisheries Research Bureau of Canada, 1968.
16 A. LAESSØE AND V. K. HANSEN, *Cons. internat. Explor. Mer. Plankton Committee Report. No. 143*, 1961.
17 D. KERR AND D. V. S. RAO, in *UNESCO Monographs on Oceanographic Methodology No. 1*, 1966, p. 66.
18 D. J. NELSON, *Science*, 132 (1960) 351.
19 K. H. SCHALTEGGER, *J. Chromatog.*, 19 (1965) 75.
20 D. Y. C. LYNN CO AND S. H. SCHANDERL, *J. Chromatog.*, 26 (1967) 442.
21 B. COLMAN AND W. VISHNIAC, *Biochim. Biophys. Acta*, 82 (1964) 616.
22 D. NUTTING, M. VOET AND R. BECKER, *Anal. Chem.*, 37 (1965) 445.
23 M. F. BACON, *J. Chromatog.*, 17 (1965) 322.
24 H. A. W. SCHNEIDER, *J. Chromatog.*, 21 (1966) 448.
25 H. H. STRAIN, J. SHERMA AND M. GRANDOLFO, *Anal. Chem.*, 39 (1967) 926.
26 J. W. NELSON AND A. L. LIVINGSTON, *J. Chromatog.*, 28 (1967) 465.
27 L. P. VERNON, *Anal. Chem.*, 32 (1960) 1144.
28 J. D. H. STRICKLAND, in J. P. RILEY AND G. SKIRROW, *Chemical Oceanography*, Vol. 1, Academic Press, New York, 1965, p. 477.
29 B. H. DAVIES, in T. W. GOODWIN, *Chemistry and Biochemistry of Plant Pigments*, Academic Press, New York, 1965, p. 489.
30 C. GARSIDE AND J. P. RILEY, *Deep Sea Res.*, 15 (1968) 627.
31 T. R. PARSONS AND J. D. H. STRICKLAND, *J. Marine Res. (Sears Found. Marine Res.)*, 21 (1963) 21.

Analytica Chimica Acta
Elsevier Publishing Company, Amsterdam
Printed in The Netherlands

SIMPLEX OPTIMIZATION OF THE RESPONSE FROM CHEMICAL SYSTEMS*

DUANE E. LONG
Chemical Division, Dugway Proving Ground, Utah 84022 (U.S.A.)
(Received March 4th, 1969)

The optimization of a chemical system is the process of adjusting the controlling variables so that some result achieves the best possible level (within the limitations of the attainable modifications of the system). The primary aim can be the maximization of the yield of a reaction, the improvement of the stability of a product in solution, or the minimization of a complex function such as one related to overall cost. Such goals are usually attained by determining how each of the pertinent variables (termed *factors*) affects the final results. The classical experimental approach is to change one factor at a time while holding the others constant. This is better than a random search for the optimum combination of factors, but other techniques can give more information with less work and allow results not normally attainable by the classical method[1]. Of the several optimization techniques potentially useful for chemical work, the design based on a class of figures called the simplex is probably the most efficient in terms of the number of experiments and the ease of calculation. The theoretical proposal of simplex optimization has been given by SPENDLEY *et al.*[2]. The purpose of the present paper is to express the simplex approach with sufficient information to facilitate its employment in chemical investigations. The effectiveness of the simplex method when applied to chemical reactions has been demonstrated by the less than two days apiece required to increase the sensitivity of the *p*-rosaniline method for sulfur dioxide by a factor of seven[3], and of the molybdenum-blue method for phosphate by a factor of thirteen[4]. The experience gained from these applications shows that besides optimizing a reaction, the process generates other information which gives valuable insight into the behavior of a system.

SEEKING THE OPTIMUM

In general, any given system will react to changes in the magnitudes of its controlling factors, and the term *response* pertains to the numerical observation of the results of an experiment in which these factors are purposely held at some particular level. Running experiments at enough combinations of levels gives responses whose magnitudes can be used to graphically depict a response surface. This surface often resembles a mountain, with the maximum response lying at the summit. A response surface can represent criteria such as yield or the sensitivity of an analytical reaction, where the factors are important components of the reagent and the response is the quantity of product, the net absorbance, etc.

* Presented in part at the 155th ACS meeting, San Francisco, California, April, 1968.

Figure 1 shows contour diagrams for the important varieties of two-factor response surfaces, the contour lines representing *loci* of constant response. The type of surface is determined by the relation between variables (*e.g.* by the class of reaction) but the exact shape (the steepness of the slopes, the elongation of the ridges, etc.) depends upon the relative scales used to draw the figures or to space the experiments. The contours can represent minima as well as maxima, then depicting valleys or depressions.

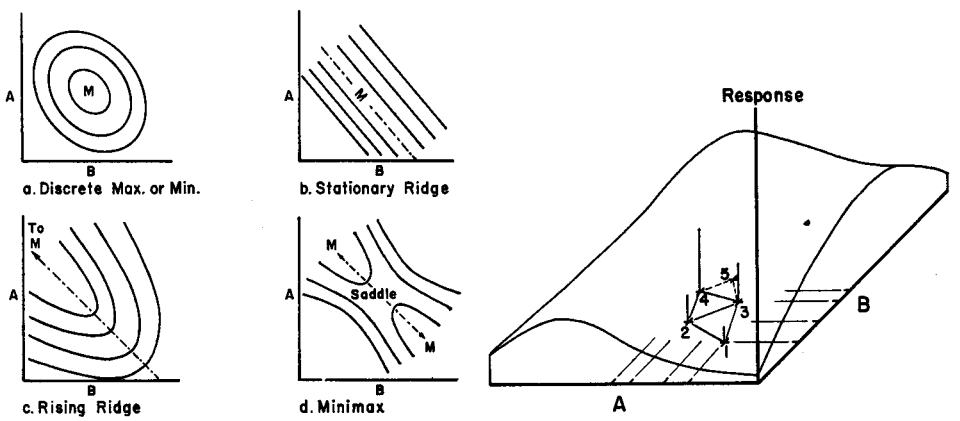


Fig. 1. Two-factor contour diagrams[5]. (M) Maximum or minimum.

Fig. 2. Simplex optimization[3].

To maximize a response, the problem is to locate the coordinates of the summit of the response surface, these coordinates being the optimum magnitude of the factors affecting the response. To accomplish this goal, the factors governing the conditions in a set of experimental runs are given different levels according to a definite plan. Comparing the responses resulting from a correctly planned set allows one to predict the direction in factor space for experimental conditions capable of even higher response.

Although the Fibonacci search[6] for a maximum along a line is the best method where the response depends upon only one factor, for two or more factors the simplex is probably the most efficient easily applied procedure. (A survey of other optimization techniques is given by KOEHLER[7].) A two-factor simplex is formed by running three experimental points having relative positions such that they describe the vertices of an equilateral triangle. The responses from these three experiments are compared and the point giving the poorest result is eliminated, replacing it by a fourth sample having a position at the mirror image of the eliminated point (see Fig. 2). A second simplex is thus formed by the new point and the two retained points. After the fourth experiment is run, the three responses in the new simplex are compared and the worst experimental value is again eliminated. The fifth point is then located and the process repeated successively with the responses increasing as the simplex series proceeds directly towards the optimum. After the maximum on the response surface is passed the values decrease, so at the highest response the step size (the length of the edge of the simplex, which determines the separation between successive levels of the factors)

is made smaller and the progress continued in the immediate neighborhood until the maximum is more exactly located.

SIMPLEX OPTIMIZATION

The details of the optimization procedure are categorized below in the form of steps. It will be seen that many of the points can be considered as refinements which may be omitted with only a slight effect on the overall efficiency. These may be picked up as experience is gained, and additional possibilities will appear as the method is applied to a particular problem.

Define the quantity to be optimized

The decision as to which criteria should make up an optimum will depend upon the use intended for the final system. It may be a straightforward case of optimizing only yield, stability, linearity, or cost, or it may be a complex function relating several such variables. An experiment often gives several responses of interest, and a consideration of the auxiliary responses can shift the resulting optimum from the point which is maximum for a single principal response. A practical procedure is to follow the most important response with the simplex series, meanwhile recording all the auxiliary responses for each experimental point. After the maximum for the principal criterion has been reached, judgement of the optimum can be based upon a weighting of the relative importance of the responses (*e.g.*, it may be desirable to sacrifice color yield in favor of better stability by going to a different portion of the response surface). If a more systematic relation is desired between several coexisting responses, a desirability coefficient may be derived by a weighting procedure[8] and the resulting function maximized. However, even if this procedure is used, the individual responses should be recorded for probable later consideration in terms of a response surface (as will be discussed later).

Select the factors

To simplify the optimization it is usually preferable to choose only the most important factors. The importance is determined by the comparative change in response caused by a change in level of each one of the factors. This may be based on a prior knowledge of the system or upon preliminary experimentation.

Factorial experiments are a good way of judging the relative significance of the possible factors and they give a quantitative measure of the contribution of each factor to the overall response. (Unfortunately, most chemists are not trained in their use. A clear explanation is given by BROWNLEE[9]. Meanwhile, to choose the important factors, classical experimentation or past experience with similar systems must be relied on.) A factorial design specifies the proper combination of variables in each sample of the experiment. Two-level factorials are adequate and their application is a well established procedure which has been treated in detail[10,11]. It is possible to include every conceivable variable in a factorial experiment for evaluation. The required number of samples increases exponentially as 2^k, where k is the number of factors, but this can be reduced by fractional replication[12–15], if interaction between certain factors can be assumed absent. For such screening, it is even possible to investigate k factors with as few as $k+1$ samples, by means of main-effect factorial designs[16–18].

Because for the simplex only those factors of obvious practical significance are generally of interest, it usually is unnecessary to perform a statistical analysis of variance on the data resulting from the factorial experiments. Instead, after the experiments employing the treatment combinations dictated by the design have been run, the magnitude of the effects can be calculated by a standard method and their relative importance judged by inspection.

In determining which factors give the highest effects, it must be realized that the apparent changes in response are not an absolute measure of effect but depend upon the scales and differences in levels selected for the experiments (*i.e.* upon the step size). It therefore may sometimes be desirable to normalize the responses by relating them to the total range possible for a factor or to the ease and precision of controlling its level. If there is doubt about the significance of a factor, it can always be included in the simplex to assure that its effect is accounted for. However, when first gaining familiarity with the simplex procedure it is recommended that only two factors be included because a graphical method may be used to locate the new simplex. This experience assists in visualizing the optimization of greater numbers of factors, where calculations are used instead. The graphical approach can be employed if only two factors are important or if the next factors in rank give very much lower effect. Regardless of the number of factors included in the simplex, any remaining factors are held constant. This is usually easily accomplished by maintaining uniform experimental conditions except for those factors which are systematically varied according to the simplex sequence.

Factors purposely omitted from the simplex are usually those of lesser importance. However, in the optimum region reached by the simplex sequence, the slopes of the response surface can differ considerably from those in the region of factor space checked by the first factorial. This means that the magnitudes of the effects due to a given change in level will differ and so could the relative order of importance of the factors. So to locate the exact optimum, after the conditional maximum has been reached, re-run a factorial to choose all the currently important factors and include these in a final multi-dimensional simplex. (The presence of interaction between factors in two sets of experiments would make it desirable to include all potentially significant factors in the original experiment, but these will not generally be known at the outset.)

In optimizing an analytical reagent, the concentration of the substance being analyzed should not be taken as a variable for the simplex, since the maximum response will proceed in the direction of increasing concentration. Instead, a constant concentration is chosen at a low enough value so that the range of the instrumentation will not be exceeded by the increase in response resulting from optimization.

For the case where two factors are interdependent with respect to the levels attainable (as in a reagent having two solvents, both of which affect the response but whose fractions in a mixture must of necessity always total unity), their combination may be treated as a single factor with possible levels ranging, *e.g.* from a mixture composed of fractions zero A, one B at one end of the scale, to one A, zero B at the other.

Choose the step size

Scales must be assigned to the factors being optimized and the spacing between

successive experimental levels decided. The choice of the step size is arbitrary but it is some advantage if the step for each factor causes a comparable change in response. This effect of a factor upon the response determines the slope of the response surface and it usually changes as the maximum is approached. If one factor gives an effect which is small compared to the effects of other factors, it may be that the base level chosen for it is near a conditional maximum (for that region of factor space), the system is relatively independent of its level, or the unit adopted for it is disproportionately small. This can be checked by changing to a larger step.

An initial large step size is usually an advantage, since the maximum is approached more rapidly and error will have a proportionally smaller influence. The step size should be large enough that experimental error is a sufficiently small proportion of the total change in response due to making the step.

Too large a step is bad if it causes excessive overshoot of the maximum when applied to a process already nearly optimized. If a peak is very sharp in comparison to the step size, it may be missed. This can be checked (if suspected) by running the midpoint of the simplex, and if it gives a higher response than all of the apexes, the center region must be explored with smaller steps. A large step may also make it difficult to maneuver between constraints or to stay on a high yield portion of a steep slope, but a reduction in size can be made after these problems are encountered.

If the levels of a factor cannot be assigned a definite ordering (as by relative magnitudes), the factor is considered as qualitative in nature and separate optimization of the quantitative factors may be required for each version of the qualitative factor. The results of the separate optimizations are then compared to determine the best version for the qualitative factor.

Identify the constraints for the system

Constraints are boundaries on the response surface which cannot or should not be crossed into regions of disallowed levels for one or more factors. These may be dictated by pressure or temperature limitations of the equipment, solubility or miscibility problems, or the obvious inability to put less than zero quantity of a component into a mixture. Undesirable results such as instability of product, inadequate reaction rate, etc., can also be treated as constraints. If possible, these decisions should be made before the series is started, although many become apparent only during the actual experimentation. The region lying within the constraints may be termed the "experimental region"[19], and the simplex will be seeking the best response available within this allowable portion of factor space.

Locate the initial simplex

To establish the first simplex in the series, it is necessary to decide upon the beginning values for each of the factors. These often will be the factor levels in accepted use prior to the current investigation, unless preliminary experimentation has indicated a better region of factor space for starting the simplex series. Then, with the desired step size, the experimental points defining the initial simplex are located by choosing values for the factors such that the points lie at the vertices of a regular simplex of the required dimensionality. For a two-factor simplex, the figure is an equilateral triangle, and therefore requires three points. Since two factors can be followed graphically, all that is necessary is to lay out the two factors as the x and

y coordinates and locate the vertices of the simplex on the graph. The initial experimental orientation of the triangle does not significantly affect the efficiency, but it is advantageous to orient it with one side parallel to the axis of factor A if more factors are to be added in later stages.

Table I can be used to construct simplexes of up to ten factors. For the initial location of each vertex, the Table specifies fractions of the step sizes, which are to be taken as the distances from the experimental origin. A triangular simplex for factors A and B will require vertices 1, 2, and 3. A three-factor simplex is a tetrahedron, so factors A, B, and C require vertices 1, 2, 3 and 4. The four-factor simplex is an analogous figure in four-factor space, therefore including factor D requires the addition of

TABLE I

INITIAL LOCATION OF VERTICES

Vertex no.	*Factor*									
	A	*B*	*C*	*D*	*E*	*F*	*G*	*H*	*I*	*J*
1	0	0	0	0	0	0	0	0	0	0
2	1.000	0	0	0	0	0	0	0	0	0
3	0.500	0.866	0	0	0	0	0	0	0	0
4	0.500	0.289	0.817	0	0	0	0	0	0	0
5	0.500	0.289	0.204	0.791	0	0	0	0	0	0
6	0.500	0.289	0.204	0.158	0.775	0	0	0	0	0
7	0.500	0.289	0.204	0.158	0.129	0.764	0	0	0	0
8	0.500	0.289	0.204	0.158	0.129	0.109	0.756	0	0	0
9	0.500	0.289	0.204	0.158	0.129	0.109	0.094	0.750	0	0
10	0.500	0.289	0.204	0.158	0.129	0.109	0.094	0.083	0.745	0
11	0.500	0.289	0.204	0.158	0.129	0.109	0.094	0.083	0.075	0.742

vertex 5. Vertex 1 has coordinates of zero and so is located at the point chosen as the starting levels for the factors. This first vertex is the experimental origin and generally corresponds to the factor levels employed before the current attempt to optimize. For the remaining vertices, the step size for each factor is multiplied by the appropriate fraction in Table I. Each result is then added to the value which that factor has at the origin, *i.e.* at Vertex 1. Doing this for each vertex gives the set of experimental coordinates needed for the initial simplex. (The Table gives the correct shape of the simplex and the position with respect to the experimental origin, but its actual location in factor space is determined by what is picked as the starting point.) The coordinates for each vertex specify the magnitudes of the factors to be combined as that sample or experiment.

Search for the optimum response with simplex designs

After the first simplex has been run, the worst experimental value is eliminated and a new vertex is located by reflecting the simplex into factor space in the direction opposite from the undesirable result. (A single new vertex is required to form a new simplex when taken together with the retained vertices.) For the two-factor case, this may be done by employing a triangular mask cut to the size of the initial simplex and laying it on the graph with two vertices coinciding with the two retained points. The third vertex will then be positioned opposite the eliminated point, at the location for the next experiment. Optionally with two, but necessarily with three or more factors, the coordinates for the new point are found by a simple calculation. First, the

coordinates of the k retained vertices (from a simplex of $k+1$ vertices) are tabulated, then these are summed over each factor, and each sum is multiplied by $2/k$. Finally, the coordinates of the discarded point are subtracted to obtain the coordinates for the new point. A convenient form for the calculations is given in Table II.

If a given vertex is retained after $k+1$ successive simplexes, it is possible that it was a spurious value caused by error, so the value should be replaced by a new observation at that point.

TABLE II

CALCULATION OF SUCCEEDING POINT[20]

Vertex no.	Factor A	B	C	--	k
(k retained vertices)	(Coordinates of retained vertices)				
Sums of retained coordinates					
$\frac{2}{k}$ (Sums)					
Coordinates of discarded vertex . . .					
Coordinates of new vertex					

If the response at a new point is also the lowest value in the simplex, causing it to reflect back onto the previous position, then the second lowest reading should be eliminated instead of the lowest. This will allow progress up a ridge which is being straddled, or if a peak has been reached, this will cause the simplex to circle the maximum, so that the cessation of progress will be verified and the region around the maximum defined.

To locate the maximum more exactly, the step size of the simplex can be decreased to perhaps 0.25 or 0.10 of the previous step. (A large step should be used first, to decrease the effort required to reach the general region of the maximum, but then a smaller step should be used to increase the resolution in finding the exact position.) To accomplish this, the coordinates of the simplex giving the highest value are taken as the new experimental origin, and the remaining vertices are located according to the design matrix by applying the smaller step. For the two-factor graphical method, a smaller triangle is merely cut for use. Decreasing the step size will allow the maximum to be approached as closely as desired, although errors in adjusting small increments of the factors may prove limiting.

Simplex optimization refers not only to maximizing a response but also can be applied to the minimization of an impurity, reaction time, cost, etc., by rejecting the

largest value from each simplex. The worst value is rejected whatever the criteria, so that simplex optimization can be applied to situations assessed on a non-numerical basis or used when a quantitative measure cannot be applied to the response (*i.e.* when it is subjective in nature).

Considering the previously discussed steps, a reasonable scheme of investigation can be outlined as follows:

1. Choose the most important variables for use in the simplex (perhaps using a factorial experiment) and hold the remainder constant.

2. Sequentially apply the simplex designs until the region of the optimum is located, then reduce the step size and circle the optimum.

3. If the exact location is required, re-run a factorial experiment to determine the variables of interest in this region of factor space, then formulate a new simplex and continue to the optimum.

The simplex series will approach the optimum in spite of most operating errors (although a few more experiments may be required if errors occur or steps are misapplied). This is a particular advantage while learning and the procedure is simple enough that technicians can carry on most of the optimization.

A TWO-FACTOR EXAMPLE

An example of a two-factor investigation is shown in Fig. 3 for the optimization of a *p*-rosaniline agent for determining sulfur dioxide[3]. Factorial experimentation had shown that the two components in the reagent having the greatest effect on sensitivity were formaldehyde and hydrochloric acid, hence they were varied while the other factors were held constant. A solvent blank and a single concentration of sulfur dioxide in sodium hydroxide–glycerine were analyzed by each reagent represented by a vertex. The first simplex was started at a region somewhat removed from the formerly employed reagent (point zero). (Although in this particular example, the base of the simplex is inclined to the x axis, it is preferable to keep it parallel to ease the later addition of more factors and the decreasing of the step size with the design matrix.) Employing reagents corresponding to the three points of the initial simplex showed that point 2 gave the lowest response, so point 4 was located opposite

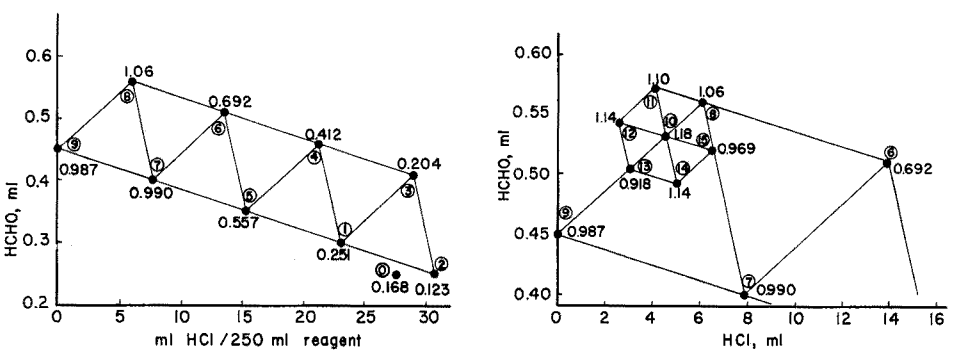


Fig. 3. Optimization of SO_2 reagent[3]. (1 (encircled)) Apex number; (0.251) response, absorbance units.

Fig. 4. Exploration of maximum[3].

2 by a triangular mask. After continuing the sequence, a decrease in response was noted in progressing from point 8 to point 9. (This portion of the graph is enlarged in Fig. 4.) A smaller simplex was then started from point 8 and running points 10 and 11. At point 13 where the response again decreased, the point giving the second lowest response (reagent 12) was instead eliminated to prevent the simplex from reflecting back on itself. This allowed the simplex to advance and then to start circling, which helped to define the shape of the response curve around the maximum. The maximum appears closest to point 10 and could have been more exactly located by a further decrease in step size, but already the formaldehyde was being measured in increments of 0.01 ml and could not be more accurately controlled in the practical application of the analysis. At this stage more factors could also have been added and the investigation continued by the method of calculating the new vertices. The origin for such a set of experiments would have been located in factor space at the point determined by the overall experimental conditions for point 10.

RESPONSE SURFACES

A knowledge of the responses at the vertices in a simplex series allows contours to be drawn depicting the approximate shape of the local response surface. In the example of Fig. 4, contour intervals of 0.10 A were chosen and their position between vertices estimated by linear interpolation. The resulting pairs of points were joined by contoured lines as pictured in Fig. 5. Additional experimental points could have been

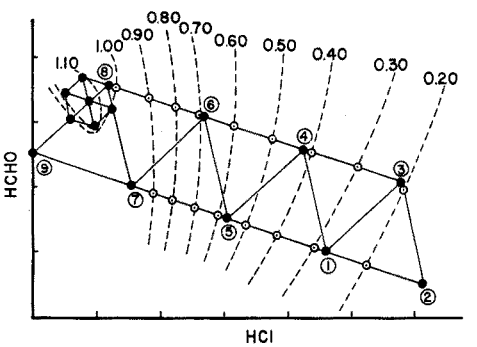


Fig. 5. Contours representing response surface[3].

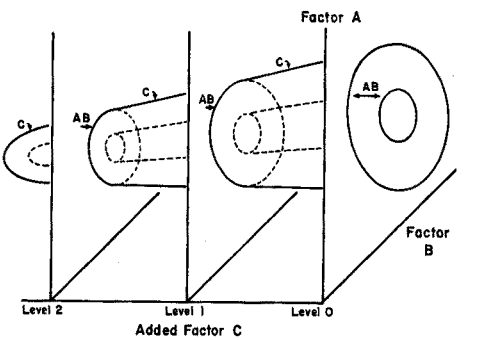


Fig. 6. Three-factor contour diagram. (AB) Contours due to factors A and B; (C) contours due to the addition of factor C.

run if the shape of the surface was of basic interest in the study. The color stability was carried as an auxiliary response and was seen to be better on the slopes to the right of the peak. In applications requiring that the color be more stable than at the point of maximum response, it would be necessary to move off and compromise the sensitivity, the new location constituting the practical optimum. Similarly, in an application for the determination of high concentrations, so that too many dilutions are required when full sensitivity is used, a reagent corresponding to some other region of factor space could be chosen. Such compromises are plainly understood by applying the response surface concept.

A two-factor contour diagram can also be considered as being determined at some given level of a third factor. A change in level of this added factor could result in a different set of contours in a plane parallel to the first set, proceeding along the z axis. A series of such experiments at different levels of the third factor may then be used to construct a contour diagram for the three-factor case, connecting the two-factor contour lines between sets to obtain a surface as each contour in three-factor space (Fig. 6). In this way it is possible to represent the responses caused by different combinations of levels for the three factors. The response surface for greater numbers of factors exists in higher dimensional factor space but a direct geometrical representation is not possible. Contours for up to three of the factors must instead be shown at chosen constant levels for the remaining factors. (This picturing of the response surface is only an aid to visualizing some of the relations between the variables and is not essential to a successful optimization.)

The drawing of contours between experimental points or the formation of a surface between contours is an interpolation process. Similarly, the depiction of the pertinent part of the response surface given by the optimization series, especially if supplemented by some additional experimental points, allows the estimation of changes in response caused by variations in the factors, even for intermediate regions which are not investigated directly. Interpolations based on slope make it possible to specify, for example, that a change of $x\%$ in one reagent will cause a $y\%$ change in sensitivity and a $z\%$ change in color stability (if stability was followed as an auxiliary response). It can be inferred that certain types of analytical errors may be greater near cliffs. A day-to-day change in sensitivity may be due to shifts in factor values (*i.e.* along the axes) rather than due to the action of an additional, undefined factor, hence if day-to-day reproducibility is required (*e.g.* if standards cannot be run daily and a permanent standard curve is unavoidable) the accuracy and/or precision (depending upon the circumstances) will be improved by specifying a reagent away from steep portions of the response surface.

Quantitative functions representing the contours *within* a simplex can be derived by running points along the edges of the simplex and fitting equations to the resulting contours[21].

The max. on a two-factor response surface can be a unique point analogous to a mountain peak or it could be a line along a ridge. Ridges are relatively common in chemical work[22]. A ridge-line corresponds to a region of constant effect for one factor if the line is level and parallel to an axis, *i.e.* it represents an extended maximum where a range of experimental conditions give the same yield. In three-factor space, instead of just a line there may be a whole surface of maximum yield corresponding to a constant level of some factor. This surface can be a plane at a region where two factors give a constant effect over a range of levels. Similarly, four factors can yield a maximum which is a point, a line, a surface, or a volume in four-dimensional factor space. In general, a level ridge inclined to the axes occurs when the factors have compensating effects, and the direction of the ridge indicates how much of a change in one factor is required to compensate for a given change in the other in order to maintain a constant response. A falling ridge shows that the response will decrease from the attainable maximum if some operating condition must be changed but it indicates the direction for the best compromise, since going down along the ridge line decreases the response less than cutting steeply across contours.

EFFECT OF ERROR

The observed value of the response obtained for each experimental point differs from a statistically correct value by a quantity dependent upon the variance in the actual reading. The location of an experimental point in factor space is also uncertain by a quantity dependent upon errors in measuring or controlling the factors. The overall error in response for the intended point identified in factor space by the simplex coordinates will depend upon the sum of these variances, but for purposes of ranking the magnitudes of the responses, only the actual observation error (*i.e.* not including error in preparing the specified treatment combination) contributes to the uncertainty of rank. If the precision is poor enough and the two lowest values in a simplex are sufficiently close in magnitude that the true second lowest value is observed as the lowest by chance error, then an incorrect direction will be chosen for the next simplex. However, this only temporarily affects the direction of progress and even if recurrent, merely imparts a wobbling which reduces the efficiency in terms of the total number of experimental points required to attain the optimum.

Replication of the individual experiments is not desirable as a means of straightening an error-ridden course because it reduces the advance made per total effort expended[23]. Rather than to double or triple the total number of samples by replicating, it is more efficient to allow some wrong decisions as to which is the lowest point and run correspondingly more vertices, since the average direction is always correct. Errors in attaining the specified factor levels cause an error in determining the actual location of a point in factor space and therefore cause error in defining the exact shape of the response surface; however, as long as the response change per unit change in the factors exceeds the magnitude of the errors in measuring the actual response, the simplex will still continue to progress toward a true optimum response.

The value obtained for a response differs from a true value for the system by an amount dependent upon experimental error. If it is necessary to have the best estimate of the numerical value of the optimum (as distinguished from the true values of the factors which locate this response) and its precision, the mean and its experimental error must be determined by running at least three replicates of the vertex corresponding to this experimental point. Previous estimates of error probably will not hold in the new region of factor space. If three samples are run, an estimate of the standard deviation can be obtained by multiplying[24] their range by 0.59. If four samples are used, the range is multiplied by 0.49, and if five samples, multiply by 0.43. The average of the replicates will be a better estimate of the actual maximum response, and the 95% confidence limits on this mean can be estimated[24] by multiplying the range of three samples by 1.47, the range of four samples by 0.77, or the range of five samples by 0.53.

SHIFTS IN THE OPTIMUM

Simplex optimization can be applied when a sequential approach is feasible. If the wait for making an observation is too long (as in a study of long-term stability), it may instead be better to run a large number of samples simultaneously, as in a factorial design, and subject the resulting data to an exhaustive analysis to determine the best direction to move for the next set of experiments[25,26].

Trends caused by changes correlated with time can make sequential experimentation difficult. The amplitude of the variations may often be minimized by keeping experimental conditions as constant as possible during an investigation. If a significant variation is an unavoidable characteristic of the system, a control sample (*e.g.* consisting of experimental point no. 1) can be re-run along with each succeeding point and their difference employed as the quantity to be optimized. An experimental series carried on over several days can also be compensated for day-to-day variations by re-running the last simplex of the previous day and applying any difference as a correction factor.

Short-term variations may be caused by errors in preparing the treatment combinations or in measuring the responses and can affect the precision of a determination. Variations of an intermediate period can cause a wobbling of the course of the simplex and increase the number of simplexes required to attain the optimum. The longer-term variations may be due to the effect of some unidentified factor which is not being controlled and which causes the response to vary and/or the position of the optimum to shift in factor space. If the unknown factor cannot be controlled experimentally (*e.g.* if it occurs to a varying extent depending upon the batch of chemical, or appears as a reagent ages), it may be necessary to readjust the values for the known factors in an attempt to follow the current optimum as it moves. This search can even be maintained after an initial optimization by carrying the simplex procedure on through the normal application of the optimized process (*e.g.* through the production runs). The conditions from run to run can be varied to form a simplex whose vertices still yield acceptable product. The sequence will give a series of simplexes which follow the movement of the optimum. This application on a continuing basis is a form of Evolutionary Operation and is also a means of compensating for uncontrolled or unidentified factors.

Because of this ability to compensate for omitted factors and to follow a shift in the optimum, simplex can be used to adapt an analysis from a test-tube method to one using an automated analyzer, to adjust for differences in reagent lots, to scale up from laboratory to pilot plant, or even for peaking an analytical method for use in a laboratory different from that in which it was developed. Factors which affect the response may not have been explicitly identified while they remained constant, but at another time, place, with another operator, etc., they may change and have the effect of shifting the optimum. To achieve this correction for the effect of unidentified or unsuspected variables, start under the new conditions with a simplex located at the previously optimized factor magnitudes and investigate the immediate neighborhood with small steps.

IMPROVING THE BASIC SYSTEM

The maximum response attained by simplex optimization is very probably the optimum for the specific system and conditions investigated. However, it is not unlikely that a basically different chemical or physical approach could yield a response with a yet higher maximum, so that research along these lines may still be desirable. Simplex can assist to an extent in the search for a better system by showing in which direction to extend the experimental region. This is most easily seen when a constraint is encountered by the progressing simplex.

When the simplex approaches a constraint, one is normally forced to move along the constraint to find the highest response available within the restrictions given. However, it can be considered that the simplex is pointing to an optimum lying beyond the boundaries of a constraint, and that removing the constraint will allow higher yield. This often can be done by such expedients as improving the physical capability of the apparatus, by attempting to prevent precipitation, by changing the dielectric constant of the solution, or by some other means suggested by the nature of the particular constraint under consideration. Such changes in the system may place other factors in force and lead even further into unexplored regions.

It can be seen from the preceding applications that the simplex approach is a most valuable experimental aid which systematizes the course of an investigation and allows a direct pursuit of the optimum conditions, yet does not require extensive training or a theoretical knowledge of the system being studied.

J. S. HUNTER is thanked for his helpful criticism of the manuscript.

SUMMARY

An experiment design based on the simplex configuration offers an efficient approach to determining which levels of the controlling variables (or factors) give the maximum response from a system. This paper presents details for applying simplex optimization to chemical problems. A design matrix is given for locating the vertices of the initial simplex for up to 10 factors and a form is included for calculating the new points. A graphical technique is described for the two-factor case.

ZUSAMMENFASSUNG

Die Planung eines Experimentes, welche nach der Simplex-Methode geschieht, bietet einen wirksamen Weg, um die variablen Faktoren zu bestimmen, welche eine maximale Antwort für ein System ergeben. Diese Arbeit beschreibt Einzelheiten für die Anwendung der Simplex-Optimalisierung auf chemische Probleme. Eine Plan-Matrix zur Festlegung der Vertikalen wird angegeben bis hin zu 10 Faktoren einschliesslich der Angabe zur Berechnung neuer Punkte. Eine graphische Technik des Zwei-Faktor-Falles wird beschrieben.

REFERENCES

1 G. E. P. BOX, *Biometrics*, 10 (1954) 27.
2 W. SPENDLEY, G. R. HEXT AND F. R. HIMSWORTH, *Technometrics*, 4 (1962) 441.
3 D. E. LONG, *N. W. Regional ACS Meeting, June, 1967.*
4 D. E. LONG, Dugway Proving Ground, unpublished work, 1967.
5 Adapted from G. E. P. BOX, *Biometrics*, 10 (1954) 36.
6 A. H. BOAS, *Chem. Eng.*, (3) 70 (1963) 107.
7 T. L. KOEHLER, *Chem. Eng.*, (25) 67 (1960) 146.
8 C. W. LOWE, *Trans. Inst. Chem. Engrs. (London)*, 45 (1967) T3.
9 K. A. BROWNLEE, *Industrial Experimentation*, 4th Edn., Chemical Publ. Co., New York, 1953.
10 O. L. DAVIES (Editor), *Design and Analysis of Industrial Experiments*, Hafner Publ. Co.. New York, 1960, p. 247.
11 O. L. DAVIES (Editor), *Design and Analysis of Industrial Experiments*, Hafner Publ. Co., New York, 1960, p. 625.

12 O. L. DAVIES (Editor), *Design and Analysis of Industrial Experiments*, Hafner Publ. Co., New York, 1960, p. 483.
13 G. E. P. BOX AND J. S. HUNTER, *Technometrics*, 3 (1961) 311.
14 G. E. P. BOX AND J. S. HUNTER, *Technometrics*, 3 (1961) 449.
15 S. ADDELMAN, *Technometrics*, 4 (1962) 47.
16 S. ADDELMAN AND O. KEMPTHORNE, *Orthogonal Main-Effect Plans*, Aeronautical Research Lab. Technical Report 79, 1961.
17 S. ADDELMAN, *Technometrics*, 4 (1962) 21.
18 D. E. LONG, M. J. HOULE AND R. L. POWELL, *N. W. Regional ACS Meeting, June, 1967.*
19 G. E. P. BOX, *Biometrics*, 10 (1954) 29.
20 Adapted from W. SPENDLEY, G. R. HEXT AND F. R. HIMSWORTH, *Technometrics*, 4 (1962) 447.
21 J. W. GORMAN AND J. E. HINMAN, *Technometrics*, 4 (1962) 463.
22 G. E. P. BOX AND P. V. YOULE, *Biometrics*, 11 (1955) 297.
23 W. SPENDLEY, G. R. HEXT AND F. R. HIMSWORTH, *Technometrics*, 4 (1962) 451.
24 Calculated from data given by K. A. BROWNLEE, *Industrial Experimentation*, 4th Edn., Chemical Publ. Co., New York, 1953, p. 60.
25 T. L. KOEHLER, *Chem. Eng.*, (25) 67 (1960) 147.
26 O. L. DAVIES (Editor), *Design and Analysis of Industrial Experiments*, Hafner Publ. Co., New York, 1960, p. 506.

Analytica Chimica Acta
Elsevier Publishing Company, Amsterdam
Printed in The Netherlands

THE DETERMINATION OF TIN BY ATOMIC FLUORESCENCE SPECTROSCOPY, WITH AN ELECTRONICALLY MODULATED ELECTRODELESS DISCHARGE TUBE AS SOURCE

R. F. BROWNER, R. M. DAGNALL AND T. S. WEST
Chemistry Department, Imperial College, London, S.W.7 (England)
(Received March 8th, 1969)

Tin has proved rather insensitive to determination by flame spectroscopic techniques and reported detection limits compare unfavourably even with those obtainable for some of the more refractory oxide-forming elements. Conventional flame emission techniques have been particularly poor in this respect, but recent advances, especially in the use of hot pre-mixed laminar flames have allowed detection limits in some cases below the 1 p.p.m. level to be achieved. SKOGERBOE *et al.*[1], measuring the atomic emission at 243.0 nm from a turbulent oxyhydrogen flame on a total consumption burner, quote a detection limit of 0.5 p.p.m. FASSEL AND GOLIGHTLY[2], using a pre-mixed acetylene–oxygen flame and measuring emission at 303.4 nm, found detection limits of 9 p.p.m. when measuring in the primary zone of the flame and 4 p.p.m. in the interconal zone. The last-mentioned authors indicate that their quoted detection limits probably err on the ungenerous side. In both cases[1,2] it was necessary to spray ethanolic or aqueous ethanolic solutions.

In a pre-mixed nitrous oxide–acetylene flame, PICKETT AND KOIRTYOHANN[3] found a detection limit of 0.3 p.p.m. at 284.0 nm. The molecular emission of the SnH species has been reported from this laboratory[4] in a cool argon–hydrogen flame at 609.5 nm with a detection limit of 1.5 p.p.m.

No details of interferences are quoted by any of the above authors, but it is to be expected that determinations in the hotter flames, *i.e.* acetylene with oxygen or nitrous oxide, would be largely free from interference—at least from that due to depletion of the atomic population by compound formation.

Atomic absorption techniques applied to tin[5–7] have shown poor sensitivity when an air–acetylene flame is used. But, it has been shown[7–9] with hollow-cathode sources, that, rather surprisingly, the greatest sensitivity is achieved in a very fuel-rich air–hydrogen flame at 224.6 nm. Similar behaviour has been reported[10,11] with electrodeless discharge tubes as sources. The best detection limit achieved by any of these authors was 0.1 p.p.m.[7] with a prototype hollow-cathode lamp. However, as intimated by AMOS AND WILLIS[9] and discussed below, the determination is very prone to interference under the most sensitive flame conditions. In order largely to remove these interferences, the fuel/air ratio must be decreased to an extent which reduces the sensitivity approximately three-fold. The corresponding optimal detection limit under these conditions is *ca.* 0.3 p.p.m.

A weak fluorescence signal for tin at 286.3 nm in an air–hydrogen flame has

also been reported[12], with a high-intensity hollow-cathode lamp as source, the quoted detection limit being 100 p.p.m.

EXPERIMENTAL

The atomic fluorescence readings were made with a Perkin-Elmer 290 atomic absorption spectrophotometer modified for fluorescence work as described previously[13]. In order to improve transmission in the low UV region, the quartz lenses in the monochromator were replaced with Spectrosil Grade "B" lenses. Spectra were recorded on a Hitachi-Perkin-Elmer 159/0–11 mV recorder at a scanning speed of 9.0 nm min^{-1} and with a time constant of 1 sec in the external circuit. When samples were sprayed at a fixed wavelength, the time constant was set at 15 sec. The slits were kept at 1.25 mm (2.0-nm spectral bandpass) except when a spectrum was scanned, when 0.42 mm slits (0.7-nm spectral bandpass) were used.

Electrodeless discharge tube source

The preparative conditions for tin electrodeless discharge tubes have been given elsewhere[10]. However, subsequent work showed that in order to obtain the really intense source necessary for fluorescence work, the ratio of tin to iodine is critical and must lie between the limits of 2.5:1 and 3.5:1 by weight. The discharge tube used in this study after more than seven hundred hours running over a period of two years shows no noticeable deterioration or loss in intensity whatsoever.

Power to the discharge was supplied by a Microtron 200 microwave generator with an output continuously variable from 20 to 200 W at a frequency of 2450 ± 25 MHz. The efficiency of coupling with the source was monitored with a reflected power meter (essentially a directional coupling device) mounted in line between the generator and the source. The discharge was initiated with a "Tesla" high-frequency vacuum tester.

Superimposed on the e.h.t. of the magnetron valve was a 50-Hz component from a modulator unit which allowed variable modulation of the microwave source between zero and 100%, in phase with the tuned amplifier of the spectrophotometer.

The types of experimental arrangement used for comparative purposes were (i) with a mechanical chopper between the flame and the monochromator and the source not electronically modulated; (ii) with a chopper between the source and the flame, again with no electronic modulation of the source; and (iii) with electronic modulation of the source only. After preliminary examinations, electronic source modulation (iii) was adopted for all further work.

The conditions which produce maximum light output from the source compatible with good stability have been previously described[11] and were achieved by running the discharge tube in a $\frac{1}{4}$-wave cavity (Electro Medical Supplies 214L) at 40 W (7-W reflected power) with *ca.* 35% modulation. Slight air cooling of the base of the tube was necessary to avoid an excessive build up of vapour pressure.

Flames

All flames were burned on a circular Unicam air–acetylene burner head fitted to the burner chamber *via* a drilled stainless steel adaptor. When separation of the

flame was required, a nitrogen sheathing device[14] was incorporated around the burner head.

Hydrogen to the argon–oxygen–hydrogen flame was supplied at a pressure of 15 p.s.i. through the normal "fuel" regulator on the instrument, but the supplies of argon to the nebuliser (at 30 p.s.i.) and oxygen to the "auxiliary air" port in the burner chamber (at 5 p.s.i.) were controlled with needle valve and rotameter assemblies as supplied with the Perkin-Elmer Model 303 atomic absorption spectrometer.

Tin solutions were made by dissolving pure analytical reagent-grade tin in the minimum of concentrated analytical reagent-grade hydrochloric acid (with a platinum wire to catalyse the reaction) and diluting to the required volume with 0.1 *M* hydrochloric acid, in order to prevent precipitation of tin as the oxychloride.

RESULTS AND DISCUSSION

*Electronic source modulation**

In atomic fluorescence spectroscopy, measurement of the total of all contributions to the radiative emission from the flame at the wavelength of interest is feasible either by mechanical chopping between the flame and monochromator[11] or in conjunction with a d.c. amplification system only when the element to be determined, or any other species present, gives little thermal emission and also when the wavelength of measurement does not correspond with a region of high flame background emission. These criteria are of particular importance in atomic fluorescence spectroscopy where, as in flame emission spectroscopy, the analytical signal is due to the release of a very small amount of energy, with a consequent need for a large amplification factor.

The most sensitive fluorescence lines for the determination of tin lie under the 306.4-nm OH band system and, with the usual unseparated flame, the intensity of this at the band head exceeds the fluorescence emission for low concentrations of tin by at least two orders of magnitude even in a cool air–hydrogen flame. Separation of the flame with either a silica tube or a flow of nitrogen[14] around the central flame improves the situation, but still leaves a large signal to be backed off and one which will alter considerably both with fluctuations in the gas supply and with changes in the sensitivity of the system to cover different ranges of solution concentration.

Electronic modulation in conjunction with a tuned amplification circuit provides a simple means of overcoming these problems and is also better adapted to the needs of atomic fluorescence than mechanical chopping of the source emission.

There is, for example, no obstruction of light reaching the atom reservoir by the rotating sector and no light is scattered from the blade edges to bypass the flame and produce a high background reading. Moreover, if necessary, the source may be used very close to the flame without focussing so that reduction of the fluorescence intensity is minimal.

A comparison of detection limits achieved for tin at 303.4 nm in an unseparated argon–oxygen–hydrogen flame with source focussing (thus further minimising the inverse-square law effect) but with the three types of arrangement for modulation

* The general advantages of electronic source modulation for atomic fluorescence spectroscopy have been discussed elsewhere[15].

(Table I) shows that the detection limit achieved with electronic source modulation is 25% better than with mechanical source modulation and 2.5 times better than when the fluorescence is measured on top of the OH emissions by chopping between the flame and the monochromator.

With electronic source modulation, the noise level of the base line over and above the electronic noise from the amplifier, and "pick up" in the leads, is proportional to the square root of the intensity of the OH emission from the flame and so may be attributed almost entirely to the effect of photomultiplier shot noise.

TABLE I

EFFECT OF DIFFERENT MODULATION SYSTEMS ON DETECTION LIMIT

(4 p.p.m. tin solution sprayed in an argon–oxygen–hydrogen flame, measuring the fluorescence at 303.4 nm)

Type of modulation	*Fluorescent signal (relative)*	*Noise*	*Detection limit (S:N = 1)*
Mechanical 1[a]	52	6	0.45
Mechanical 2[b]	51	2	0.15
Electronic[c]	65	2	0.12

[a] Mechanical chopper between flame and monochromator.
[b] Mechanical chopper between source and flame.
[c] Electronic modulation (*ca.* 35%) of source only.

Sensitivities in different flames

The relative fluorescence signals from all the tin lines observed in argon–oxygen–hydrogen and argon–hydrogen flames were measured under conditions of maximum sensitivity for the particular flame. However, in the air–acetylene flame optimal signals were obtained under conditions so fuel-rich that the resultant extreme luminosity produced large signal fluctuations with a consequent loss of precision. For this flame, therefore, readings were taken under leaner conditions, with signals *ca.* 10% below their optimal value.

Fluorescence signals were observed from three of the four tin lines arising from the ground state, but no signal was found at 207.31 nm, probably because of the low sensitivity of the photomultiplier (R.C.A. I.P. 28) at this wavelength. The lines are arranged (Table II) so that the first in each group arises from resonance radiation to the ground state and the others in the group result either from direct line or step-wise transitions from the same excited state.

Only one direct line (235.5 nm) and one step-wise line (242.9 nm) transition arising from the 224.6-nm resonance line were observed; the others were too weak to be detected. The 224.6-nm resonance transition itself was not observed in the argon–hydrogen flame, nor was the 235.5-nm line. Similarly, only the direct line transition at 326.2 nm arising from the 254.7-nm inter-combination line was seen in any flame. However, all the lines originating from the 6s $^3P_1^0$ upper state of the 286.3-nm resonance line were seen and the strongest line in each flame was one coming from this state. The most sensitive line in the argon–oxygen–hydrogen and air–acetylene flames was the step-wise transition at 303.4 nm. It should be noted, however, that this contains a component from a resonance fluorescence transition at 303.4 nm (as will be discussed under *Filtering studies*) due to thermal population of the low lying $5p^2\ ^3P_1$ state.

TABLE II

FLUORESCENT INTENSITIES FOR TIN IN DIFFERENT FLAMES[a]

Wavelength (nm)	*Transition*		*Spectral term values (K)*		*Relative source intensity*	*Relative fluorescent signal*		
	Lower state	*Upper state*	*Lower*	*Upper*		*Argon–oxygen–hydrogen*	*Argon–hydrogen*	*Air–acetylene*
224.65 (R)[b]	$^3P_0$	$^3D_1^0$	0	44,509	1.00	3.70	—	2.15
235.48	$^3P_1$	$^3D_2^0$	1692	44,145	32.1	3.61	—	3.00
242.95	$^3P_2$	$^3D_3^0$	3428	44,576	54.3	7.42	6.30	3.54
254.66 (I)[c]	$^3P_0$	$^1P_1^0$	0	39,257	46.0	5.92	4.75	2.55
326.23	$^1D_2$	$^1P_1^0$	8613	39,257	570	8.14	35.5	17.0
270.65 (R)	$^3P_1$	$^3P_2^0$	1692	38,629	380	81.5	58.5	27.0
284.00	$^3P_2$	$^3P_2^0$	3428	38,629	500	215	58.5	72.0
333.00	$^1D_2$	$^3P_2^0$	8613	38,629	725	37.0	4.74	8.06
286.33 (R)	$^3P_0$	$^3P_1^0$	0	34,914	330	237	122	36.5
300.91	$^3P_1$	$^3P_1^0$	1692	34,914	420	200	94.0	31.4
303.41	$^3P_1$	$^3P_0^0$	1692	34,641	620	519	130	91.2
317.50	$^3P_2$	$^3P_1^0$	3428	34,914	780	482	213	64.4
380.10	$^1D_2$	$^3P_1^0$	8613	34,914	718	66.7	39.6	12.1

[a] Test solution of 100 p.p.m. tin sprayed, with same instrumental settings for all fluorescent signals between 25 and 519, but gain turned up for lines weaker than this. These readings have been scaled down accordingly. Each value is an average of five.
[b] R = Resonance line.
[c] I = Inter-combination line.

In the argon–hydrogen diffusion flame, the direct line fluorescence at 317.5 nm is most sensitive. At their respectively most sensitive lines, the maximum signal from an argon–oxygen–hydrogen flame is 5.7 times more intense than the maximum signal from an air–acetylene flame and 2.4 times more intense than that from an argon–hydrogen flame.

Replacement of the argon in an argon–oxygen–hydrogen flame by nitrogen reduces the intensity at 303.4 nm 2.2 times. The drop is the same if an air–hydrogen flame is used instead. Replacing the argon in an argon–hydrogen diffusion flame with nitrogen reduces the intensity at 317.5 nm three-fold. This differing change can be almost exactly accounted for by the greater proportion by volume of argon to total fuel gases in the diffusion flame compared with the pre-mixed flame.

A fluorescence spectrum of tin in an argon–oxygen–hydrogen flame is shown in Fig. 1.

Fuel flows

In an argon–oxygen–hydrogen flame, the oxygen flow rate is quite critical (Fig. 2) and the flows for optimal fluorescence are: H_2, 8.0 l min^{-1}; O_2, 1.1 l min^{-1} and Ar, 7.7 l min^{-1}. The argon flow is fixed and results from the maintenance of a pressure differential of 30 p.s.i. at the nebuliser. However, under these conditions the determination is very prone to interference and in order to overcome this, the oxygen flow must be increased to 1.8 l min^{-1} with a three-fold reduction in sensitivity. The optimum flows in the argon–hydrogen flame are: H_2, 4.5 l min^{-1}; Ar 7.7 l min^{-1}, and are much less critical than in the oxygen-containing flame (Fig. 2B). For air–acetylene, best sensitivity was obtained with flows of: C_2H_2, 2.2 l min^{-1}; air, 8.0 l min^{-1}.

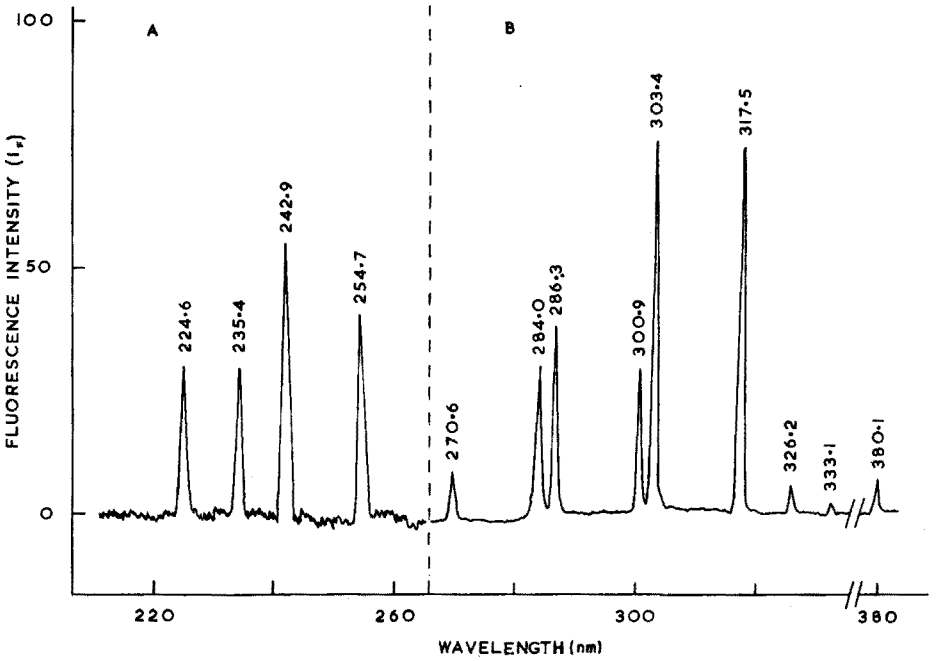


Fig. 1. Tin atomic fluorescence spectrum in argon–oxygen–hydrogen flame. Solution of 100 p.p.m. tin sprayed, with scanning rate of 9.0 nm min^{-1} and spectral bandpass of 0.7 nm. The ordinate of (B) is attenuated fifty-fold with respect to (A).

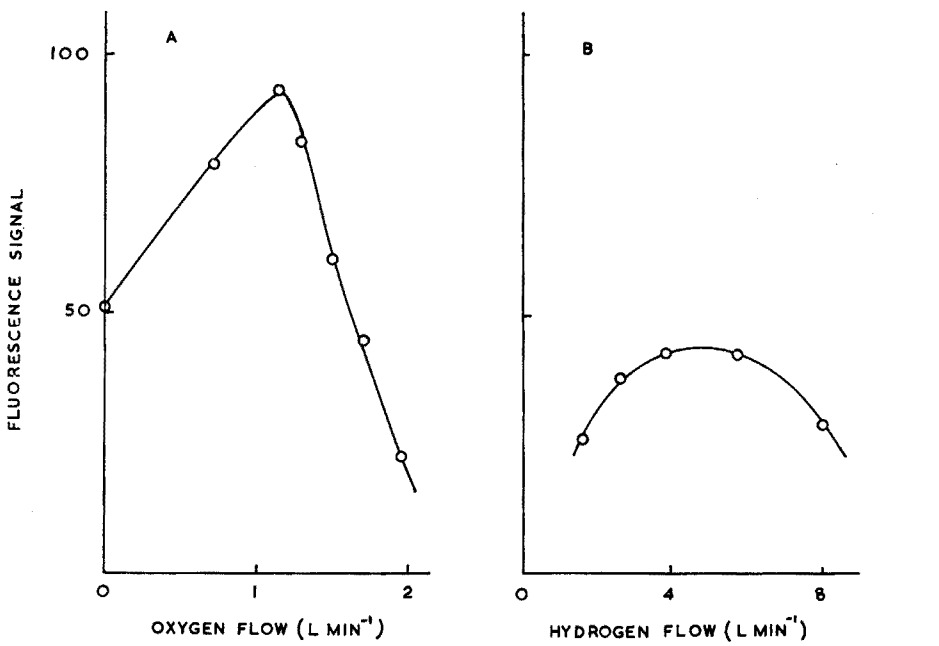


Fig. 2. (A) Variation of fluorescence signal at 303.4 nm with oxygen flow in the argon–oxygen–hydrogen flame. Argon and hydrogen flows fixed at 7.7 l min^{-1} and 9.2 l min^{-1} respectively. (B) Variation of fluorescence signal at 317.5 nm with hydrogen flow in the argon–hydrogen diffusion flame. Argon flow fixed at 7.7 l min^{-1}. Test solution of 100 p.p.m. Sn in both cases.

In all flames, with the optical arrangement used, the height of measurement was not critical and best values were obtained 2.5 cm above the burner head.

Filtering studies

In order to examine in more detail the fluorescence processes occurring in the argon–oxygen–hydrogen and argon–hydrogen flames, the 286.3-nm system was selected, as the lines were bright enough to be easily detected, even when the source was filtered, and the line of greatest analytical interest (303.4 nm) was part of the system. The change in intensity of the fluorescent lines relative to the 286.3 nm line with and without a filter between source and flame is shown in Table III. The

TABLE III

FILTERING STUDIES

(100 p.p.m. tin as test solution. 0.7-nm spectral bandpass used)

Wavelength (nm)	*Unfiltered signal relative to 286.3 nm*	*Filter % transmission*	*Filtered signal relative to 286.3 nm*	*% change in signal relative to 286.3 nm*
Argon–oxygen–hydrogen flames				
286.3	1.00	3.00	1.00	—
300.9	0.84	10.2	0.95	+13
303.4	2.19	14.3	2.30	+5
317.5	1.62	32.3	1.62	0
380.1	0.28	41.5	0.28	0
Argon–hydrogen flames				
286.3	1.00	3.00	1.00	—
300.9	0.77	10.2	0.82	+6
303.4	1.06	14.3	3.45	+225
317.5	1.75	32.3	1.75	0
380.1	0.33	41.5	0.33	0

air–acetylene flame could not be examined similarly as the signal-to-noise ratio became too unfavourable under these conditions. The results show an appreciable population of tin atoms to exist in the $5p^2\ ^3P_1$ state which lies only 0.210 eV above the ground state. These then undergo a process of "thermally assisted resonance fluorescence" with the 303.4 and 300.9 nm lines emitted by the source. The relative populations caused by thermal excitation at the estimated temperatures of the argon–oxygen–hydrogen flame used (1440°K)[16] and of the argon–hydrogen flame ($\leqq$ 1140°K) are calculated to be 0.7:1 and $<$ 0.6:1. The unexpectedly high ratio of the intensity of the 303.4 to 286.3 nm line in the argon–hydrogen flame implies that the $^3P_1$ state is populated by a quasi luminescent process, probably involving chemical reduction of the tin salt.

Detection limits and analytical curve

The detection limit at 303.4 nm (defined as the solution concentration which gives a signal as great as the background noise fluctuations) with an unsheathed argon–oxygen–hydrogen flame under the most sensitive conditions is 0.12 p.p.m. In a leaner flame, with virtually interference-free conditions, the detection limit is 0.36 p.p.m. However, this lean flame may be efficiently separated with a nitrogen

sheath (the very fuel-rich flame cannot) with a 3.5-fold reduction in background noise from the lower OH emission. This gives a detection limit of 0.10 p.p.m. in aqueous solution, whereas a value of 0.75 p.p.m. was achieved on the same instrument in its conventional atomic absorption mode with a very fuel-rich air–hydrogen flame maintained on a 5-cm slot burner (measured at 224.6 nm, the most sensitive absorption line). However, as in the case of fluorescence, the flame conditions giving maximum sensitivity were very interference-prone and the detection limit found for the leaner flame which was necessary to eliminate the interferences, was 2 p.p.m.

The analytical curve at 303.4 nm was linear from 0.5 to 250 p.p.m. with increasing curvature beyond this, becoming almost asymptotic to the concentration axis at about 1000 p.p.m. of tin. The relative standard deviation (on twelve consecutive runs) at the 5-p.p.m. level was 2.1%.

Interferences

Under the most sensitive conditions, even zinc, thallium and potassium interfere with the determination of tin, but interferences become slight in a leaner flame. The effect of 500 p.p.m. of interfering ion on a 5-p.p.m. solution of tin was examined in the case of thirty-four cations and anions (Table IV). Of these, only Ca, Mg, Mo(VI), Sr and U(VI) actually interfered. An explanation of the interferences experienced in argon–oxygen–hydrogen flames in terms of a probable mechanism for the production of tin atoms under these conditions will form the basis of a later publication.

TABLE IV

INTERFERENCE STUDIES

No interference[a]	*Interference signal depression (%)*	
Al, Ba, Cd, Co, Cs	Ca	12
Cu, Fe(III), Hg, K, Li	Mg	6
Mn, Na, Ni, NH_4, Pb	Mo(IV)	5
Rb, Ti(IV), Tl, Zn, Zr	Sr	5
Br^-, citrate, Cl^-, HPO_4^-,	U(VI)	10
I^-, MnO_4^-, NO_3^-, PO_4^{2-}, SO_4^{2-}		—

[a] Producing a change in signal less than twice the standard deviation at this tin concentration.

CONCLUSIONS

Atomic fluorescence spectroscopy with an electronically modulated electrodeless discharge tube, used in conjunction with a nitrogen-separated argon–oxygen–hydrogen flame provides an unusually sensitive flame spectroscopic method for the determination of traces of tin. This is felt to be most encouraging when the early stage of development of the technique is considered, because the direct dependence of the analytical signal on the intensity of light reaching the flame lends itself to improvement in ways which atomic absorption and thermal emission spectroscopy do not. By means of refinements in the optical system, which are currently being considered, it is hoped to achieve even better sensitivity.

We are grateful to Dr. K. C. THOMPSON of this Department for one of the electrodeless discharge tubes used in this study. Thanks are also due to the Science Research Council for the provision of an SRC (CAPS) studentship to one of us (R.F.B.) and to the Perkin Elmer Corporation (Beaconsfield) U.K. for the loan of the instrumentation used in this work.

SUMMARY

The atomic fluorescence characteristics of tin in Ar/H_2, $Ar/O_2/H_2$ and air/C_2H_2 flames have been investigated and optimal conditions have been established for the determination of tin. An electronically modulated electrodeless discharge tube excited at 2450 MHz is used as source. Measurement of the fluorescence emission at 303.4 nm in a nitrogen-separated $Ar/O_2/H_2$ flame gives a detection limit of 0.1 p.p.m. under almost entirely interference-free conditions. Good analytical curves are obtained in the range 0.5–250 p.p.m. Sn. Of 34 elements examined at 100-fold concentrations, only Ca, Mg, Mo, Sr and U interfered.

RÉSUMÉ

On a examiné les caractéristiques de fluorescence atomique de l'étain dans les flammes Ar/H_2, $Ar/O_2/H_2$ et air/acétylène; et on a établi les conditions optima pour le dosage de l'étain. On utilise comme source un tube à décharge sans électrode électroniquement modulé, excité à 2450 MHz. La mesure d'émission de fluorescence à 303.4 nm dans une flamme $Ar/O_2/H_2$ fournit une limite de détection de 0.1 p.p.m. dans des conditions presque complètement exemptes d'interférences. De bonnes courbes analytiques sont obtenues entre 0.5 et 250 p.p.m. Sn. Des 34 éléments examinés à des concentrations 100 fois supérieures, seuls Ca, Mg, Mo, Sr et U interfèrent.

ZUSAMMENFASSUNG

Die Bestimmung von Zinn mit der Flammenabsorptionsspektroskopie unter Verwendung von Ar/H_2-, $Ar/O_2/H_2$- und $Luft/C_2H_2$-Flammen wurde näher untersucht und optimale Bedingungen aufgestellt. Eine elektronisch modulierte mit 2450 MHz angeregte elektrodenlose Entladungsröhre wurde als Quelle verwendet. Die Messung der Fluoreszenzemission bei 303.4 nm in einer stickstoffgetrennten $Ar/O_2/H_2$-Flamme ergab eine Nachweisgrenze von 0.1 p.p.m. unter nahezu völlig störungsfreien Bedingungen. Gute analytische Kurven wurden im Bereich von 0.5 bis 250 p.p.m. Sn erhalten. Von 34 Elementen, die in hundertfacher Konzentration geprüft wurden, störten nur Ca, Mg, Mo, Sr und U.

REFERENCES

1 R. K. SKOGERBOE, A. T. HEYBEY AND G. H. MORRISON, *Anal. Chem.*, 38 (1968) 1821.
2 V. A. FASSEL AND G. W. GOLIGHTLY, *Anal. Chem.*, 39 (1967) 466.
3 E. E. PICKETT AND S. R. KOIRTYOHANN, *Spectrochim. Acta*, 23B (1968) 4, 235.
4 R. M. DAGNALL, K. C. THOMPSON AND T. S. WEST, *Analyst*, 93 (1968) 518.
5 J. E. ALLAN, *Spectrochim. Acta*, 18 (1968) 259.

6 B. M. GATEHOUSE AND J. B. WILLIS, *Spectrochim. Acta*, 17 (1968) 710.
7 L. CAPACHO-DELGADO AND D. C. MANNING, *Spectrochim. Acta*, 22 (1968) 1505.
8 J. E. ALLAN, *4th Australian Spectroscopy Conference, 1963*.
9 M. D. AMOS AND J. B. WILLIS, *Spectrochim. Acta*, 22 (1966) 1325.
10 R. M. DAGNALL, K. C. THOMPSON AND T. S. WEST, *Perkin Elmer Atomic Absorption Newsletter*, 6 (1967) 117.
11 R. F. BROWNER, R. M. DAGNALL AND T. S. WEST, *Anal. Chim. Acta*, 45 (1969) 163.
12 D. C. MANNING AND P. HENEAGE, *Perkin Elmer Atomic Absorption Newsletter*, 7 (1968) 80.
13 R. F. BROWNER, R. M. DAGNALL AND T. S. WEST, *Talanta*, 16 (1969) 75.
14 R. S. HOBBS, G. F. KIRKBRIGHT, M. SARGENT AND T. S. WEST, *Talanta*, 15 (1968) 997.
15 R. F. BROWNER, R. M. DAGNALL AND T. S. WEST, paper presented at *10th Eastern Analytical Symposium, 1968, New York, U.S.A.*
16 E. M. BULEWICZ, C. G. JAMES AND T. M. SUGDEN, *Proc. Roy. Soc.*, A235 (1956) 89.

Analytica Chimica Acta
Elsevier Publishing Company, Amsterdam
Printed in The Netherlands

DETERMINATION OF ALUMINUM IN VANADIUM METAL BY ATOMIC ABSORPTION SPECTROPHOTOMETRY*

R. K. HANSEN, R. Z. BACHMAN, J. W. O'LAUGHLIN AND CHARLES V. BANKS
Institute for Atomic Research and Department of Chemistry, Iowa State University, Ames, Iowa 50010 (U.S.A.)

(Received March 21st, 1969)

The atomic absorption method described below was developed to fill the need for a more rapid and accurate method than that presently used[1] for the determination of aluminum in vanadium metal at levels below 1%. Aluminum is used in the reduction of vanadium oxide to the metal, and its determination in vanadium produced by this process is of considerable importance.

It has been reported that atomic absorption spectrophotometry is not entirely free from chemical and spectral interferences[2-6], and these interferences are more likely when highly concentrated solutions of a given matrix are analyzed for a trace constituent. The premixed nitrous oxide–acetylene flame employed in the present work has been successfully applied to the determination of aluminum[7-11], and RAMAKRISHNA *et al.*[7] reported observing no interference for the determination of 20 μg aluminum/ml in the presence of 103 μg vanadium/ml. Work performed in developing this method indicated that high concentrations of vanadium could interfere with the aluminum determination under certain conditions. The important parameters which influenced the interference were flame stoichiometry, acid concentration, and aluminum concentration.

EXPERIMENTAL

Apparatus

A Perkin-Elmer Model 303 atomic absorption spectrophotometer and nitrous oxide burner head were used in this work. The experimental conditions are shown in Table I. The wavelength chosen for this work was 3961.5 Å rather than 3092.7 Å because a scan of the spectrum of the nitrous oxide–acetylene flame[12] showed slightly less emission in the region of 3961.5 Å. Because the reported sensitivities[8] of the two lines are nearly equal, it was thought that a better signal-to-noise ratio might be possible with the 3961.5 Å line. The burner slot of 0.048 cm was used in place of the standard 0.038-cm slot to decrease the clogging of the burner by the concentrated solutions. A Perkin-Elmer hollow-cathode lamp was used as radiation source.

* Work was performed in the Ames Laboratory of the U.S. Atomic Energy Commission. Contribution No. 2337. The paper was presented in part at the 19th Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy, March 3–8th, 1968, Cleveland, Ohio.

TABLE I

OPERATING CONDITIONS FOR DETERMINATION OF ALUMINUM IN VANADIUM

Wavelength	3961.5 Å
Slit	0.3 mm
Bandpass	2.0 Å
Source current	26 mA
N_2O flow setting at 30 psig for maximum Al sensitivity	7.0 (15.9 l/min)
C_2H_2 flow setting at 5 psig for maximum Al sensitivity	6.0 (8.4 l/min)
Sensitivity	2 μg/ml/1% absorption
Observation height	Center of light beam lies 0.8 cm above burner top
Burner slot	0.048 cm × 5.08 cm

Reagents

A stock aluminum solution containing 1.237 mg/ml was prepared by dissolving Alcoa aluminum wire (99.99%) in dilute hydrochloric acid containing trace mercury as catalyst, and diluting with deionized water. A stock vanadium solution containing 0.025 g/ml was prepared by dissolving 28.75 g of ammonium metavanadate (reagent grade) in 150 ml of 1:1 sulfuric acid and diluting to 500 ml with deionized water. These stock solutions were stored in polyethylene bottles, and more dilute working solutions were prepared each day during the course of this work.

All other chemicals used were reagent grade.

RESULTS AND DISCUSSION

Suppression of ionization

Potassium, added as potassium chloride, was used to suppress ionization of aluminum in all experiments conducted. Figure 1 shows the effect of increasing potassium concentration on the absorption of a solution containing 5μg aluminum/ml. Similar results were obtained for solutions containing 5 μg aluminum/ml, 4,000 μg vanadium/ml and varying potassium concentration. Unless otherwise indicated, sufficient potassium chloride was added so that the final solution contained 3,350 μg potassium/ml.

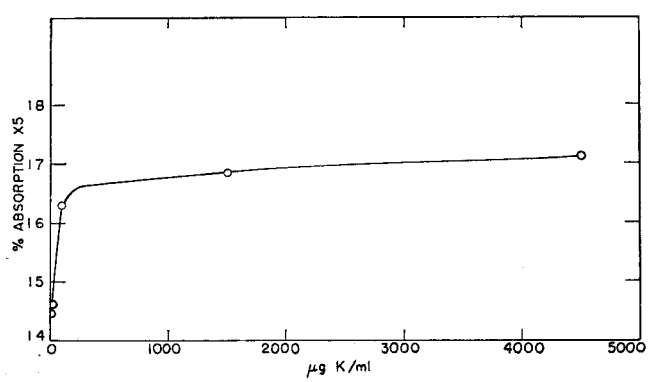


Fig. 1. Effect of potassium on the atomic absorption of a solution containing 5 μg Al/ml.

Effect of acids

BOWMAN AND WILLIS[13] reported that 0.3 *N* sulfuric acid enhanced aluminum absorbance by as much as 20%, depending on the height in the flame at which the measurement was made. This enhancement by sulfuric acid was not observed under the conditions used in this work. On the contrary, sulfuric acid was found to depress the aluminum absorption at an observation height at which BOWMAN AND WILLIS observed considerable enhancement. The effect of varying sulfuric concentration was checked on solutions containing 50 μg aluminum/ml. The depression was 2% at 0.3 *N* sulfuric acid, 3% from 0.6 *N* to 1.7 *N* and 5% at 3.4 *N*. It should be noted that BOWMAN AND WILLIS used different instrumentation and means of sample preparation for their experiment.

The sulfuric acid concentration was also found to influence the degree of interference by vanadium on aluminum absorption. Under the same conditions of observation height and flame stoichiometry, 3,750 μg vanadium/ml in 0.8 *N* sulfuric acid enhanced the absorbance of 186 μg aluminium/ml by 6% and depressed the absorbance by 8% when the sulfuric acid concentration was raised to 2.1 *N*.

The effect of nitric acid was determined on solutions containing 50 μg aluminum/-ml in the presence of 10,000 μg vanadium/ml and 2.1 *N* sulfuric acid. The aluminum absorption was not affected by nitric acid up to 0.4 *N*. At 0.6 *N* the aluminum absorption decreased by 0.8% and continued to decrease in a linear manner with increasing nitric acid concentration up to a 5% decrease with 2.5 *N* nitric acid. The use of a minimum amount of nitric acid in the dissolution of vanadium samples will result in a final nitric acid concentration of the sample solutions sufficiently low that nitric acid can be excluded from the standard aluminum solutions with no sacrifice in accuracy.

Effect of vanadium

Experiments were conducted which showed that vanadium could enhance or depress the aluminum absorption depending upon certain conditions. The effect of varying vanadium concentration on the aluminum absorption was studied. The absorbances of 2.1 *N* sulfuric acid solutions containing 99 μg aluminum/ml were compared with absorbances of solutions containing the same aluminum concentration but varying vanadium concentration from 10 μg/ml to 10,000 μg/ml. Blank corrections were made to compensate for any trace aluminum in the reagents added. These absorbance comparisons were made for four different flame stoichiometries, and the measurements were taken at observation heights corresponding to the point of maximum sensitivity for aluminum (no vanadium present) in the flame of a given stoichiometry. A nitrous oxide to acetylene mole ratio of 1.89 gave the highest sensitivity for aluminum. The total nitrous oxide flow rate was 15.9 l/min. The results are shown in Table II. Small concentrations of vanadium appear to have little measurable effect on aluminum absorbance under these conditions. The small enhancement observed at the level of 1,000 μg vanadium/ml was most noticeable in a moderately fuel-rich flame. Apparently, the depressive effect of the high matrix concentration at the level of 10,000 μg vanadium/ml overwhelms any enhancing effect, and a depression of absorbance results there.

The enhancing effect of vanadium on aluminum absorption was more readily observed at a nitrous oxide:acetylene mole ratio of 1.89 by choosing a solution with

TABLE II

EFFECT OF VANADIUM AND FLAME STOICHIOMETRY ON THE ATOMIC ABSORPTION OF SOLUTIONS CONTAINING 99 μg Al/ml AND 2.1 *N* IN SULFURIC ACID

V concn. (μg/ml)	*N_2O:C_2H_2 mole ratio*							
	2.09		*1.89*		*1.73*		*1.59*	
	% Change in absorbance due to V							
10	+0.7[a]	−2.4[b]	+0.5[a]	+1.2[b]	+1.9[a]	−1.4[b]	−0.3[a]	+1.2[b]
100	+1.0	−0.4	+0.1	+1.9	+2.1	−0.2	−0.1	+0.4
1,000	+0.6	−1.8	+3.6	+2.5	+3.6	+5.2	+1.3	+3.8
10,000	−14.3	−9.2	−16.3	−11.2	−9.0	−6.0	−14.3	−7.0

[a] Sample uptake rate = 3.3 ml/min.
[b] Same solution as "a" but sample uptake rate = 5.0 ml/min.

a moderately high vanadium concentration (3,750 μg/ml) and a low acid concentration (0.8 *N* in sulfuric acid). Under these conditions, enhancements of 4% – 8% were observed for 186 μg aluminum/ml and 371 μg aluminum/ml.

In addition to the 3961.5 Å line, aluminum lines at 3082.16 Å, 3092.7 Å and 3944 Å showed similar enhancements. Spectral interference is a factor in the case of the 3082.16 Å line which lies close to a vanadium line at 3082.11 Å[14]. The potassium chloride plus vanadium blank absorption was much larger at that wavelength relative to line sensitivity than the blank values obtained at the other three lines checked. This spectral interference has been noted by other workers[6].

Because the data on the effect of varying vanadium concentration on aluminum were obtained at optimum observation heights, which varied somewhat with changes in flame stoichiometry, data were taken which showed the effect of vanadium on aluminum absorption at a constant observation height for various flame stoichiometries. The observation height chosen yielded maximum sensitivity for 186 μg aluminum/ml at a nitrous oxide:acetylene mole ratio of 1.89. Changes in flame stoichiometry were done by varying the fuel flow while keeping the total oxidant flow constant at 15.9 l/min. The results are shown in Table III. The data show that, in general, increasing the fuel-rich character of the flame caused increased enhancement

TABLE III

EFFECT OF 3,750 μg V/ml ON THE ABSORBANCE OF 186 μg Al/ml AT VARIOUS FLAME STOICHIOMETRIES AND CONSTANT OBSERVATION HEIGHT

N_2O to C_2H_2 mole ratio	*% Change in absorbance*					
	0.8 N in H_2SO_4 (a)			*2.1 N in H_2SO_4 (b)*		
2.09	+1.3	+3.1	+4.2	−1.1	−1.4	+2.7
1.89	+3.6	+4.6	+4.9	−1.2	−2.4	+2.4
1.73	+7.6	+9.7	+7.8	+0.6	+1.9	+3.0
1.59	+15.5	+14.7	+17.6	+0.3	+7.9	+10.7

of aluminum absorbance by the vanadium. Again, the trend was not so clearly observed with the solutions 2.1 *N* in sulfuric acid because of the depressive effect of the higher sulfuric acid concentration. Small changes in gas flow settings changed the flame stoichiometry sufficiently so that one had difficulty reproducing the enhancement effects from day to day with any good degree of precision.

Another factor to be considered is that of the aluminum concentration in the solution. When operating conditions were held constant, vanadium depressed the absorbance of small concentrations of aluminum and enhanced the absorbance of large concentrations of aluminum. This depressive effect of vanadium on low concentrations of aluminum is shown in Table IV. The data under column "a" can be directly compared with the enhancements shown under column "a" in Table III. The data under column "b" of Table IV show that the depressive effect could not be eliminated by operating at optimum observation heights.

TABLE IV

EFFECT OF 3,750 μg V/ml ON 18.6 μg Al/ml IN 0.8 *N* SULFURIC ACID AT VARIOUS FLAME STOICHIOMETRIES

N_2O to C_2H_2 mole ratio	*% Change in absorbance*				
	a			*b*	
2.09	−9.0	−12.1	−10.2	−16.1	−8.2
1.89	−12.6	−12.5	−8.6	−8.5	
1.73	−9.6	−14.3	−10.0	−7.3	
1.59	−9.6	−8.8	−10.0	−5.4	

[a] Data obtained at constant observation height which was the same as in Table II.
[b] Data obtained at optimum observation heights for each flame stoichiometry.

Another difference was observed between the behavior of 18.6 μg aluminum/ml and 186 μg aluminum/ml. In 0.8 *N* sulfuric acid, maximum sensitivity was achieved in a given flame stoichiometry at a point higher in the flame for the solution of higher aluminum concentration than for the solution of lower aluminum concentration. No vanadium was present during this experiment. It was also determined that 3,750 μg vanadium/ml did not significantly change the point of maximum sensitivity for 18.6 μg aluminum/ml in a flame with a nitrous oxide:acetylene mole ratio of 1.89.

Precision of recommended procedure

The precision obtained for replicate aliquots with the recommended procedure is shown in Table V. For 300 p.p.m. aluminum in vanadium metal, the coefficient of

TABLE V

PRECISION OF THE DETERMINATION OF ALUMINUM IN VANADIUM BY ATOMIC ABSORPTION

Aliquot no.	*Sample no. 1*[a,b] *p.p.m. Al in V metal*	*Sample no. 2*[a] *% Al in V metal*
1	302	1.02
2	324	1.01
3	315	1.02
4	329	1.02
5	352	1.00
Ave.	324	1.01
Coefficient of variation	±6%	±1%

[a] Samples contain 0.25 g V in 25 ml of solution.
[b] Data obtained by use of 5× scale expansion.

variation is ±6%, and for 1% aluminum in vanadium metal, the coefficient of variation is ±1%.

RECOMMENDED PROCEDURE

The recommended procedure involves matching the matrix concentration and acid concentration of the standards to that of the samples. Dissolve vanadium metal samples of nominal weight 0.25 g in 2 ml of concentrated nitric acid and 7.5 ml of 20% sulfuric acid. Prepare standard aluminum solutions to bracket the aluminum concentration of the samples. Add sufficient stock vanadium and sulfuric acid to the standards to simulate sample composition closely. Add potassium chloride to standards and samples so that the final solutions contain 3,350 μg potassium/ml. Dilute to 25 ml with deionized water. It is recommended that the samples be bracketed between standards and read in the following order: standard, sample, standard, etc. This will serve to increase the precision of the method while minimizing changes in flame character caused by burner clogging and other factors. Blank corrections are applied to the standards by subtracting any absorbance due to the matrix materials. Generally, the blank was found to give less than 1% absorption.

SUMMARY

The determination of aluminum in vanadium metal over the range 100 p.p.m. to 1% aluminum was investigated by atomic absorption spectrophotometry. Commercially available equipment with a nitrous oxide–acetylene burner was used. High concentrations of vanadium were found to influence the aluminum absorption in a variable manner depending upon certain conditions. Reproducible results could be obtained only by carefully matching the matrix and acid concentrations in the standards to that in the actual sample.

RÉSUMÉ

On examine le dosage de l'aluminium dans le vanadium métal (100 p.p.m. à 1% d'aluminium) par spectrophotométrie par absorption atomique. On utilise un équipement conventionnel avec brûleur oxyde nitreux-acétylène. On constate que de fortes concentrations en vanadium influencent l'absorption de l'aluminium, de façons différentes suivant les conditions. On peut obtenir des résultats reproductibles en utilisant pour les étalons des matrices et des concentrations d'acide similaires.

ZUSAMMENFASSUNG

Die Bestimmung von Aluminium in Vanadinmetall im Bereich von 100 p.p.m. bis 1% Aluminium wurde mit der Flammenabsorptionspektralanalyse untersucht. Ein kommerzielles Gerät mit einem Stickstoffoxid–Acetylen-Brenner wurde verwendet. Es wurde gefunden, dass hohe Konzentrationen von Vanadin die Aluminiumabsorption auf verschiedenste Weise beeinflussen. Reproduzierbare Ergebnisse konnten nur erhalten werden durch sorgfältige Zusammenstellung der Matrix und der Säurekonzentration in den Standards ebenso wie in den Proben.

REFERENCES

1 R. J. HYNEK AND L. J. WRANGELL, *Anal. Chem.*, 28 (1956) 1520.
2 C. TH. J. ALKEMADE, *Anal. Chem.*, 38 (1966) 1252.
3 S. R. KOIRTYOHANN AND E. E. PICKETT, *Anal. Chem.*, 38 (1966) 1087.
4 S. R. KOIRTYOHANN AND E. E. PICKETT, *Anal. Chem.*, 38 (1966) 585.
5 D. C. MANNING AND L. CAPACHO-DELGADO, *Anal. Chim. Acta*, 36 (1966) 312.
6 V. A. FASSEL, J. O. RASMUSON AND T. G. COWLEY, *Spectrochim. Acta*, 23B (1968) 579.
7 T. V. RAMAKRISHNA, P. W. WEST AND J. W. ROBINSON, *Anal. Chim. Acta*, 39 (1967) 81.
8 M. D. AMOS AND J. B. WILLIS, *Spectrochim. Acta*, 22 (1966) 1332.
9 F. R. HARTLEY AND A. S. INGLIS, *Analyst*, 92 (1967) 622.
10 L. CAPACHO-DELGADO AND D. C. MANNING, *Analyst*, 92 (1967) 553.
11 R. J. JAWOROWSKI, R. P. WEBERLING AND D. J. BRACCO, *Anal. Chim. Acta*, 37 (1967) 284.
12 D. C. MANNING, *Perkin-Elmer Corp. Atomic Absorption Newsletter*, 5 (1966) 127.
13 J. A. BOWMAN AND J. B. WILLIS, *Anal. Chem.*, 39 (1967) 1214.
14 W. F. MEGGERS, C. H. CORLISS AND B. F. SCRIBNER, *Tables of Spectral Line Intensities. Part II*, NBS Monograph 32-Part II, U. S. Government Printing Office, Washington, D.C., 1961.

Analytica Chimica Acta
Elsevier Publishing Company, Amsterdam
Printed in The Netherlands

A RAPID FUSION METHOD FOR DECOMPOSITION AND COMPREHENSIVE ANALYSIS OF SILICATES BY ATOMIC ABSORPTION SPECTROPHOTOMETRY*

SVERRE H. OMANG
Central Institute for Industrial Research, Oslo 3 (Norway)
(Received February 10th, 1969)

A complete silicate analysis by chemical methods is usually carried out in two steps. In the first step, the sample is decomposed, generally by means of acid dissolution or fusion, followed by leaching with water or mineral acids; in the second step, the sample solution is analyzed for the different elements present. This can be achieved by a great variety of methods, ranging from gravimetry and titrimetry to spectrography, spectrophotometry and other instrumental methods, or a combination of these.

A fairly complete scheme for an atomic absorption spectrophotometric determination based on dissolution in hydrofluoric acid has been described by LANGMYHR AND PAUS[1] and by BERNAS[2]. However, the need for a supplement to this method is obvious, especially since these authors experienced difficulties with certain minerals which required high temperatures and thus special equipment to achieve dissolution, and also with samples containing organic matter.

Several authors have reported the use of fluxes to achieve decomposition of silicate materials and have obtained clear, stable solutions after dissolution of the melt in mineral acids, but few have combined this way of decomposition with atomic absorption analysis. No complete scheme including the determination of silica has, to the author's knowledge, appeared in the literature.

WANG[3] suggested the use of lithium tetraborate as a flux in X-ray spectrography and emission spectrography and molten fluorides were suggested by CHEAD AND SMITH[4]. BISKUPSKY[5] recommended the use of a boric acid–lithium fluoride flux mixture as a general method for the decomposition of silicates and obtained fusion in 5–10 min. However, melting with fluorides removes silica, which makes such a flux unsuited for complete analysis from a single sample solution. SUHR AND INGAMELLS[6], studying different methods of decomposition suitable for emission spectrography, recommend a lithium metaborate fusion followed by nitric acid dissolution. Based on this technique, SHAPIRO[7] published an atomic absorption spectrophotometric method for calcium, magnesium, sodium, potassium and manganese in rocks and minerals; a complete decomposition required 3–4 h. VAN LOON AND PARISSIS[8] applied the same method for the atomic absorption determination of silica in rocks and minerals, achieving fusion and leaching in about 1 h.

* Presented in part at the Thirteenth Nordic Chemistry Meeting in Copenhagen, Denmark, August, 1968.

The method described below was worked out in order to obtain a rapid fusion method applicable to all sorts of silicates, in conjunction with atomic absorption spectrophotometry to complete the analysis. Various fusion agents were tried in order to find the most effective one. Based on work by DÜMECKE AND WIEGMANN[9], who used a mixture of lithium carbonate, lead carbonate and boric acid as flux, a mixture of two of these compounds, lithium carbonate and boric acid, was found to be the most suitable.

The present paper describes the use of the lithium carbonate–boric acid mixture as flux, followed by dissolution in dilute hydrochloric acid and analysis by means of atomic absorption spectrophotometry. Several interference phenomena encountered are eliminated by adding lanthanum to samples and standards. Complete decomposition, dissolution and determination of 8 elements in one sample are accomplished in less than 2.5 h. Results for feldspar, cement, clay, limestone and bauxite are reported.

Interferences

In developing the method, several chemical interference phenomena had to be taken into consideration. Of special interest to the present procedure, where all the sample constituents including silicon are present in a single solution, are the interferences encountered in the determinations of aluminium, silicon and titanium, which have not been fully examined before. VAN LOON[10] has examined aluminium and reported chemical interference from calcium, magnesium, potassium, sodium, titanium and hydrochloric acid. Interference from silicon was not examined since this element was removed during the dissolution step. All other interferences, except the acid dependence, were removed by making the solutions and standards 1% in lanthanum.

The results of VAN LOON were verified in the present work and it was also found that 100 p.p.m. of silicon gave rise to a positive deviation of 4 relative % in the absorption measurements of a 25-p.p.m. aluminium solution. Additions of lanthanum or fluoride to samples and standards eliminated this phenomenon. However, the fluoride addition did not remove the serious calcium interference, hence this compound was unsuitable for releasing purposes. LANGMYHR AND PAUS[1], who used hydrofluoric acid for decomposition, added calcium to the aluminium standards in order to overcome this difficulty, since lanthanum cannot be added in the presence of fluoride. The addition of 1% lanthanum to samples and standards was found satisfactory in the present work.

Interference in silicon determinations with high-temperature flames has not been reported earlier, the reason probably being that hydrofluoric acid usually has been present or that silicon has been the only major element present in solution. The present investigations showed a 7% enhancement of the absorption when 100 p.p.m. of aluminium was added to a 200-p.p.m. silicon solution. This interference, however, was also removed by adding lanthanum or fluoride to samples and standards (Table I).

Quite recently the determination of titanium in silicates was treated in detail by VAN LOON AND PARISSIS[11]. It appears that serious inter-elemental interferences are present; among these, aluminium and hydrochloric acid are of most interest in the proposed method, each increasing the absorption by up to 45 relative %. In the

present work, the absorption of a 5-p.p.m. solution of titanium was found to be enhanced by 6% when 100 p.p.m. of silicon was added, and by 19% when 100 p.p.m. of aluminium was added. However, even in this case, lanthanum was found to be effective as a releasing agent. Since lanthanum is also known to remove interferences

TABLE I

EFFECT OF INTERFERENCE ON SILICON ABSORPTION

Substance added	*Concentration*	*Deviation (rel %)*
HCl	1.2 *N*	−4
HCl	3 *N*	−10
Li_2CO_3/H_3BO_3	0.5 g/100 ml	0
Na^+ (as NaCl)	350 p.p.m.	0
Ca^{2+}	500 p.p.m.	0
Al^{3+}	20 p.p.m.	+1.5
Al^{3+}	100 p.p.m.	+7
La^{3+}	1%	+10
NH_4F	160 mg/100 ml	−7
NH_4F and Al^{3+}	160 mg NH_4F/100 ml 200 p.p.m. Al^{3+}	0[a]
La^{3+} and Al^{3+}	1% La, 200 p.p.m. Al^{3+}	0[b]

[a] Measured relative to a standard containing the same amount of fluoride.
[b] Measured relative to a standard containing the same amount of lanthanum.

in the determinations of calcium and magnesium[7], it was decided to use this agent throughout and also to apply constant acidity to all solutions. Alkali interference in the determinations of sodium and potassium is usually eliminated by adding caesium to samples and standards[1]. However, the lithium ion already present in a fusion solution has been shown to have the same effect[7]. No further addition of chemicals is thus necessary, unless dilutions are employed.

EXPERIMENTAL

Equipment and reagents

A Perkin-Elmer Model 303 atomic absorption spectrophotometer equipped with an automatic recorder readout accessory and a Hitachi-Perkin-Elmer Recorder Model 159 together with a nitrous oxide burner, a three-slot Boling burner head, and regular hollow-cathode lamps were used. Instrument settings were the same as recommended in Perkin-Elmer Analytical Methods Book.

The lithium carbonate and lanthanum oxide used were of puriss quality and were not purified further. Other chemicals were of reagent-grade quality.

Silicon reference standard solution (1000 p.p.m.). This was prepared by mixing 534.76 mg of spectrographically pure silica and 2 g of sodium carbonate, fusing for 30 min in a platinum crucible over a Meker Burner and dissolving in 250 ml of distilled water.

Lanthanum solution. A 5% solution in 1.5 *N* hydrochloric acid was prepared by moistening 29.3 g of lanthanum oxide (Fluka) with a few ml of distilled water and adding 67.5 ml of hydrochloric acid (37%) rather slowly, before dilution to 500 ml with distilled water.

Other standard solutions were prepared according to the Analytical Methods

Book. All working standards for aluminium, silicon, titanium, iron, calcium and magnesium contained 1% lanthanum and 0.6 *N* hydrochloric acid.

In the determinations of calcium, magnesium, sodium and potassium, various degrees of dilution were employed. Standards and sample solutions had to contain the same amounts of lanthanum and lithium carbonate–boric acid as the diluted samples for these methods.

All elements were measured against at least two standards, one high and one low, but both close to the expected sample value.

PROCEDURE

Weigh 100–200 mg of the finely ground sample into a 25-ml platinum crucible and mix well with 250 mg of lithium carbonate and 250 mg of boric acid. Fuse the mixture over a Meker burner, cautiously for the first 3 min to reduce spatter, and then more vigorously for another 2–3 min until a clear melt is obtained. Swirl the crucible gently to bring the melt up on the walls of the container before cooling. This is done to dissolve spatter and to avoid a thick bead which will be slow to dissolve. When cold, add 5 ml of 6 *N* hydrochloric acid and 20 ml of distilled water and mount the crucible under a stirring motor equipped with a glass propeller dipping into the solution. After less than 10 min at moderate speed, transfer the clear solution to a 100-ml volumetric flask, add 20 ml of 5% lanthanum solution in 1.5 *N* hydrochloric acid and dilute to volume with distilled water.

Determine the various components from this solution and dilute if necessary to give a concentration suitable for absorption measurements.

Aspirate sample solutions and standards for silicon, aluminium, and titanium, into a nitrous oxide–acetylene flame, and for iron, calcium, magnesium, potassium and sodium, into an air–acetylene flame.

In the case of bauxite, reduce the quantity of water to the crucible during dissolution or add more hydrochloric acid to avoid a cloudy precipitate of titanium dioxide.

RESULTS AND DISCUSSION

The proposed method was tested on a series of National Bureau of Standards standard samples (Table II). For alumina and silica, an accuracy of 1.0% relative or better was achieved, with the exception of limestone and cement which gave results 2.5% and 3.6% low in alumina, respectively. This could not be explained on the basis of interference measurements.

VAN LOON AND PARISSIS[8] have reported consistently high initial values for silica, the reason being a residue of undissolved polysilicic acid in the standards; to overcome this, a depolymerizing agent such as calcium oxide was added before fusion. In the present method of standards preparation with sodium carbonate as a fusing agent, no such effect was encountered.

The hydrofluoric acid dissolution technique has some disadvantages which can be overcome by using an alkali borate fusion. Under favorable circumstances, a total hydrofluoric acid decomposition and dissolution of silicates require 15–30 min, while a fusion and dissolution ordinarily are accomplished in 15–20 min, so that for

TABLE II

PERCENTAGE RESULTS FOR FELDSPAR, CEMENT, CLAY, LIMESTONE AND BAUXITE

Sample		*Soda Feldspar NBS 99*	*Cement NBS 1016*	*Plastic Clay NBS 98*	*Argillaceous Limestone NBS 1a*	*Bauxite NBS 69a*
SiO_2	present[a]	68.66	21.05	59.11	14.11	6.01
	found	68.28	21.26	59.22	14.13	5.94
Al_2O_3	present	19.06	4.97	25.54	4.16	55.0
	found	19.13	4.79	25.45	4.06	55.47
Fe_2O_3	present	0.067	3.71	2.05	1.63	5.82
	found	0.069	3.70	2.08	1.65	5.88
TiO_2	present	0.017	0.34	1.43	0.16	2.78
	found	0.018	0.33	1.43	0.16	2.75
Na_2O	present	10.73	0.55	0.28	0.39	—
	found	10.71	0.552	0.300	0.376	—
K_2O	present	0.41	0.04	3.17	0.71	—
	found	0.39	0.034	3.19	0.738	—
CaO	present	0.36	65.01	0.2	41.32	0.29
	found	0.33	64.84	0.201	41.20	0.22
MgO	present	0.05	0.42	0.72	2.14	0.02
	found	0.054	0.39	0.71	2.18	0.024

[a] Values established by U. S. National Bureau of Standards.

many samples, the times required differ only slightly. However, if boric acid does not dissolve the precipitated fluorides cold, an additional treatment on a waterbath for 15–30 min will be necessary. Materials such as limestone and raw mix containing organic matter must, in any case, be fused before dissolution. In bauxite analysis, a teflon-covered bomb and higher temperatures are necessary, and for clay 3–4 h are required to achieve complete decomposition in hydrofluoric acid[12].

BERNAS[2] mentions several objections to the use of metal crucibles and fluxes containing anions, but none of the disadvantages mentioned were encountered in the method described here. On the contrary, distinct advantages were obtained when the lithium-containing flux was used, since this cation effectively removes interference in the determination of the other alkali metals, as well as ionization in the determination of calcium when a nitrous oxide–acetylene flame is used.

The reasons for the advantages of this mixture compared to lithium metaborate are not quite clear. The evolution of carbon dioxide together with the slightly more alkaline character of the mixture probably makes it a more effective and faster-acting fusion agent than lithium metaborate. An important detail in this connection is the alkalinity of the mixture, which is determined by the relative proportions of the two compounds and thus can be adjusted to the desired degree. It is, however, necessary not to make the flux too alkaline, as the platinum crucibles will then be seriously attacked.

This work was supported by A/S Dalen Portland-Cementfabrik, Brevik, Norway, and the Royal Norwegian Council for Scientific and Industrial Research.

SUMMARY

A method is proposed for the determination of silicon, aluminium, titanium, iron, calcium, magnesium, sodium and potassium in various silicates by atomic absorption spectrophotometry. The samples are fused with a mixture of equal amounts of lithium carbonate and boric acid and dissolved in hydrochloric acid, the complete procedure requiring about 15 min. Interferences are eliminated by adding lanthanum to samples and standards.

RÉSUMÉ

Une méthode est proposée pour le dosage des éléments suivants: silicium, aluminium, titane, fer, calcium, magnésium, sodium et potassium dans divers silicates par spectrophotométrie par absorption atomique. Les échantillons sont fondus avec un mélange, en proportions égales de carbonate de lithium et d'acide borique, puis dissous dans l'acide chlorhydrique. L'analyse complète demande environ 15 min. Les interférences sont éliminées par addition de lanthane aux échantillons et aux étalons.

ZUSAMMENFASSUNG

Es wird eine Methode vorgeschlagen zur Bestimmung von Silicium, Aluminium, Titan, Eisen, Calcium, Magnesium, Natrium und Kalium in verschiedenartigen Silicaten mit Hilfe der Flammenabsorptionsspektralphotometrie. Die Proben werden in einer Mischung von gleichen Mengen Lithiumcarbonat und Borsäure geschmolzen und in Salzsäure gelöst. Das gesamte Verfahren dauert etwa 15 Min. Störungen werden durch Zugabe von Lanthan zu den Proben und den Standard eliminiert.

REFERENCES

1 F. J. Langmyhr and P. E. Paus, *Anal. Chim. Acta*, 43 (1968) 397, 506, 508; 44 (1969) 445.
2 B. Bernas, *Anal. Chem.*, 40 (1968) 1682.
3 M. S. Wang, *Appl. Spectry.*, 16 (1962) 141.
4 A. C. Chead and G. F. Smith, *J. Am. Chem. Soc.*, 53 (1931) 483.
5 V. S. Biskupsky, *Anal. Chim. Acta*, 32 (1965) 165.
6 N. H. Suhr and C. O. Ingamells, *Anal. Chem.*, 38 (1966) 730.
7 L. Shapiro, *U. S. Geol. Surv. Bull.*, 187B (1967).
8 J. C. Van Loon and C. Parissis, *Anal. Letters*, 1 (1968) 519.
9 G. Dümecke and J. Wiegmann, *Silikat Technik*, 16 (1965) 287.
10 J. C. Van Loon, *Perkin Elmer Atomic Absorption Newsletter*, 7 (1968) 3.
11 J. C. Van Loon and C. Parissis, *Anal. Letters*, 1 (1968) 249.
12 T. C. Rains and T. A. Rush, *Natl. Bur. Std. (U.S.), Tech. Note*, 454 (1968) 32.

Analytica Chimica Acta
Elsevier Publishing Company, Amsterdam
Printed in The Netherlands

DETERMINATION OF POLYMER STRUCTURE BY HIGH-RESOLUTION NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY*

ROBERT S. SUDOL
Plastics Department, E. I. du Pont de Nemours and Co., Inc., Experimental Station, Wilmington, Del. 19898 (U.S.A.)

(Received February 3rd, 1969)

High-resolution NMR spectroscopy of polymers in solution is an effective means of determining their structure and stereochemical configurations. If a suitable solvent can be found for the particular polymer being investigated, high-resolution NMR techniques can be applied to determine the polymer's structure in much the same way as for any other large organic molecule. With the availability of cheaper deuterated solvents, thermostatically controlled variable-temperature accessories, high-resolution frequency-swept spectrometers, such as the Varian HA-100, and higher field spectrometers, such as the Varian 220-MHz superconducting solenoid instrument, it is possible to analyze polymers with molecular weights greater than 25,000. Previously, these were difficult (and, in some cases, even impossible) to analyze at lower frequencies. In this paper, 60-MHz, 100-MHz, and 220-MHz proton resonance spectra of well-known polymers illustrate how high-resolution NMR spectroscopy can be used in determining the structure of polymers. In particular, it is shown how the 220-MHz superconducting solenoid spectrometer permits more detailed information to be obtained, without the need of specialized techniques such as spin decoupling. By increasing the magnetic field strength, chemical shift differences expressed as frequencies increase proportionately, but spin–spin couplings, and line widths remain unaffected. Therefore, in many cases, high field spectra approach first order, and enable rapid straightforward analyses to be made on polymers with complex spin–spin coupling patterns and small chemical shift differences. In most cases, the need for spin decoupling is eliminated.

EXPERIMENTAL

The NMR spectra were recorded using Varian A-60, HA-100, and 220-MHz high-resolution spectrometers (Varian Associates, Palo Alto, California, U.S.A.). The 220-MHz spectrometer uses a superconducting solenoid operating at 51.7 kilogauss, which is the field required for protons at 220 MHz. Chemical shift measurements were made using tetramethylsilane (TMS) as an internal reference for ambient probe temperature, and hexamethyldisiloxane (HMDS), which absorbs 0.06 p.p.m. to low field of TMS, as an internal reference for elevated temperatures. All spin-decoupling experiments were done with the Varian HA-100, with Muirhead-Wigan (Muirhead & Co. Limited) decade audio oscillators to provide the irradiating radio-frequency

* Presented at the 156th National ACS Meeting, Atlantic City, N. J., in September, 1968.

field. Commercial polymers—a polycarbonate from bisphenol A, nylon 66, poly(vinyl chloride), and a poly(vinyl chloride)–poly(vinyl acetate) copolymer—were dissolved in suitable solvents at 5%–10% concentration by weight. All NMR sample tubes, and solvents used were of the highest quality commercially available.

RESULTS AND DISCUSSION

In Fig. 1 the high-resolution spectrum of bisphenol-A polycarbonate obtained at 60 MHz is a simple, two-line pattern with an 8:6 integral ratio which supports the proposed structure. Theoretically, a molecule containing four magnetically non-equivalent nuclei forming two different pairs of symmetrically equivalent nuclei

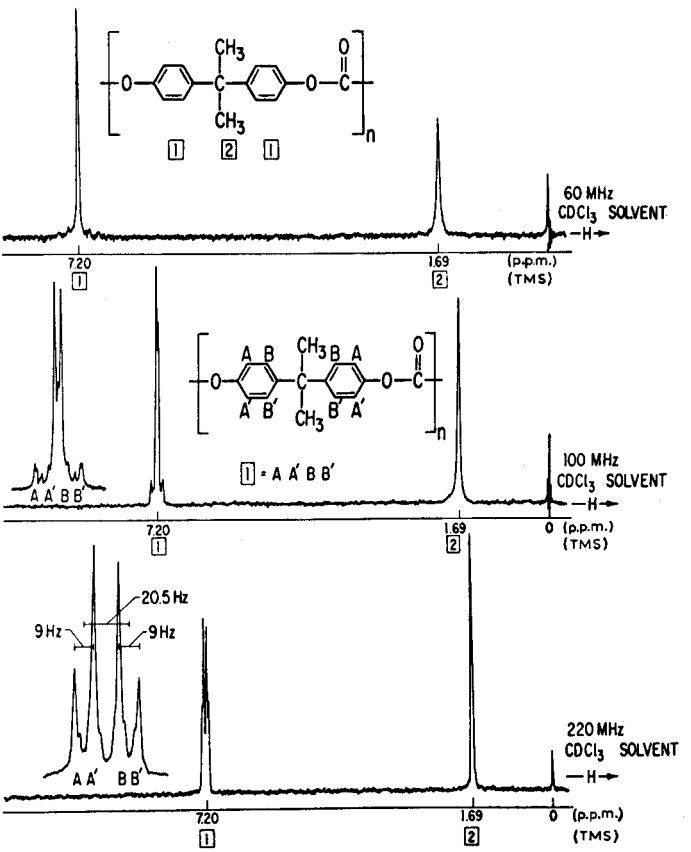


Fig. 1. High-resolution spectra of bisphenol-A polycarbonate resin.

separated from each other by a chemical shift, which is of the same order of magnitude as the coupling constants involved, is an AA'BB' system. Such a system should have a symmetrical pattern of 24 lines for the aromatic protons of bisphenol-A polycarbonate similar to the spectrum obtained of *o*-dichlorobenzene. Rarely is the 24-line AA'BB' pattern resolved in the case of polymers; normally only a four-line (weak, strong, strong, weak) pattern is seen. The anticipated four-line pattern was

never completely resolved until a 100-MHz spectrometer was used. At 220 MHz the pattern is clear and substantially better resolved than at 60 MHz or 100 MHz.

Proton magnetic resonance in solid polyamides has been studied by many workers in the field of polymer chemistry. Although many references can be found relating to wide-line investigations[1,2], there appears to be a lack of high-resolution measurements on polyamides in the literature; and, to date, no reference spectra of nylon 66 have been found. This may be due to the solubility problems and the occurrence of broad resonance bands which often hinder a detailed analysis of the resonance spectrum. By preparing a 5% (w/v) solution of nylon 66 in hexafluoroacetone deuterate (HFAD; $(CF_3)_2CO$:1.6 D_2O) and examining the solution at 100°, well resolved, high-resolution spectra were obtained (Fig. 2). If it is assumed that nearest

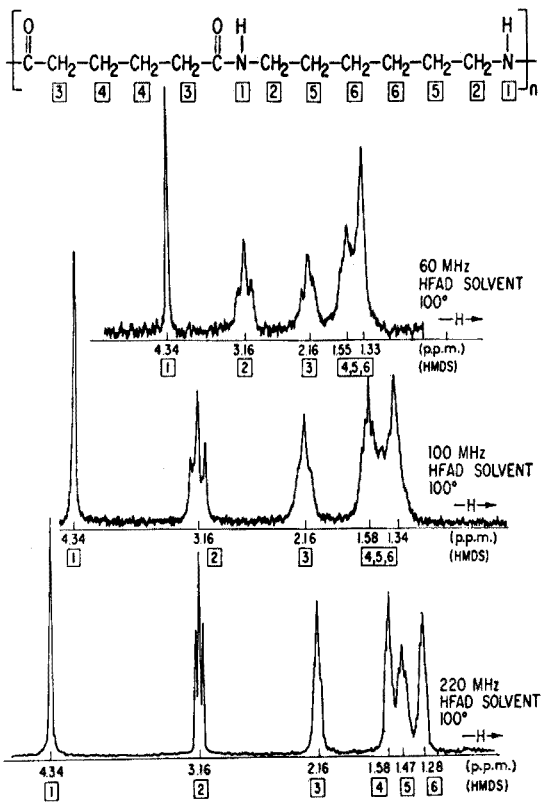


Fig. 2. High-resolution spectra of nylon 66.

neighbor effects are reflected in the chemical shifts, the spectra show once again, as in the case of bisphenol-A polycarbonate (Fig. 1), that the 220-MHz results are substantially more revealing than those obtained using the A-60 or HA-100 spectrometers. All proton resonance groups are clearly separated from each other in the spectrum, integral measurements (not shown) were consistent with group assignments and easily obtained, and spin–spin coupling patterns are clearly resolved (Fig. 3). With the HA-100 spectrometer in the frequency-sweep mode of operation and two

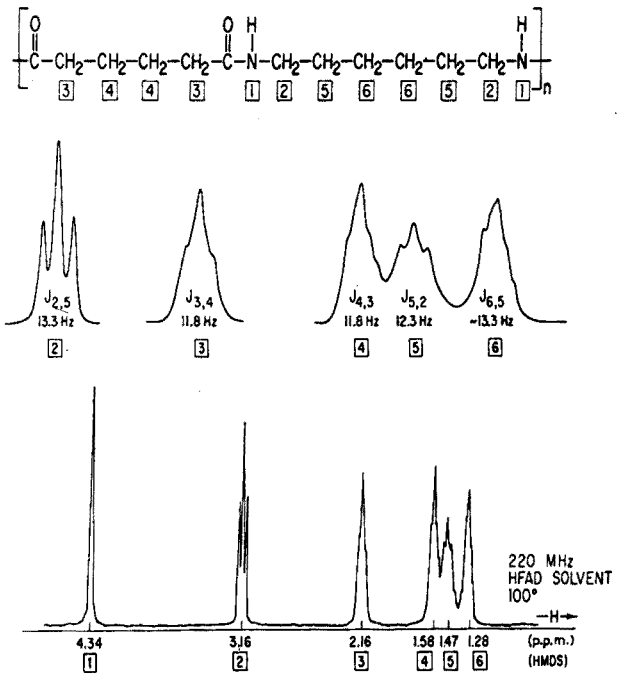


Fig. 3. High-resolution spectra of nylon 66.

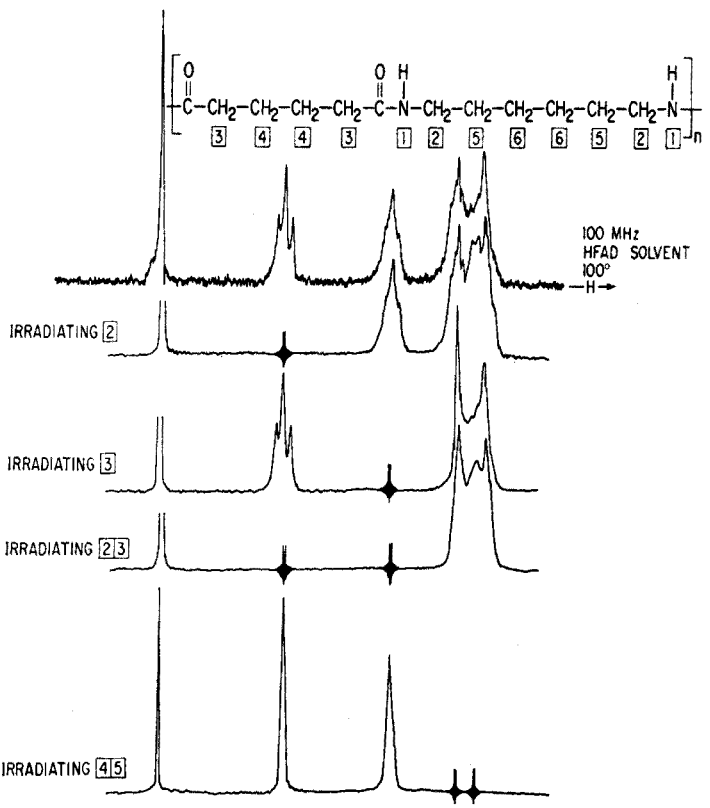


Fig. 4. Spin-decoupling experiments with nylon 66.

Muirhead-Wigan decade oscillators, spin-decoupling experiments were performed to produce further supporting evidence of the resonance assignments (Fig. 4).

Probably the most interesting application of high-resolution NMR spectroscopy is the determination of the regularity of the stereochemical configuration of polymer chains, *i.e.* their tacticity. The first tacticity determinations by NMR were carried out by BOVEY AND TIERS on polymethyl methacrylate[3]. Later, BOVEY *et al.*[4] used the spin-decoupling technique and a model polymer made from isotopic-substituted α-deuterovinyl chloride to determine the tacticity of poly(vinyl chloride). In this work, BOVEY defined *ddd* or *lll* monomer units as isotactic sequences; *dld* or *ldl* as syndiotactic sequences; and *ldd*, *dll*, *ddl* or *lld* as heterotactic sequences. This has paved the way for a better understanding of poly(vinyl chloride), but the time required and the errors which accompany spin-decoupling techniques sometimes make the analysis impractical. With the 220-MHz spectrometer, a routine method is available in which the spectrum requires no spin decoupling for most tacticity determinations; and the spectrum can be obtained and integrated in 5 min (Fig. 5).

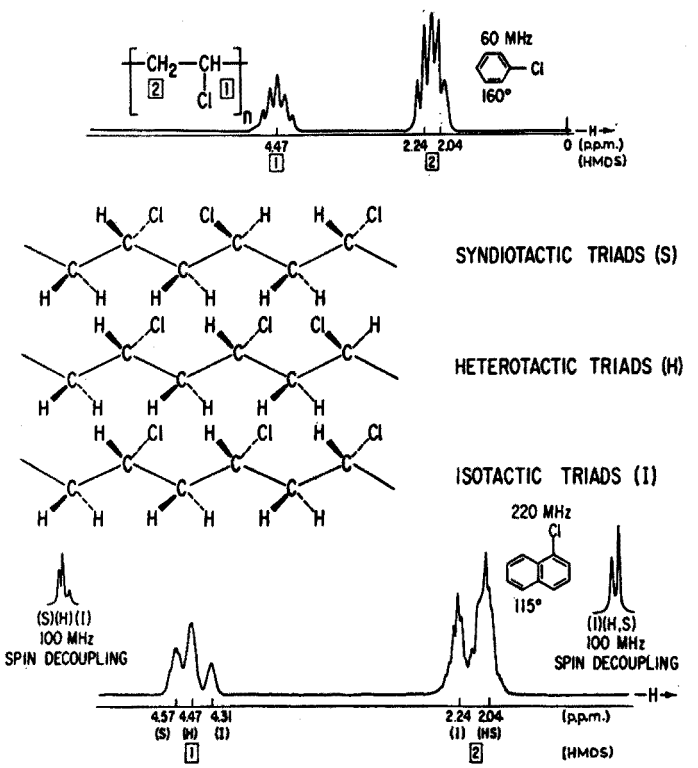


Fig. 5. Spectra of poly(vinyl chloride).

Because of the straightforward approach that can be made with the 220-MHz spectrometer to determine monomer content and tacticity, an investigation of a commercial poly(vinyl chloride) copolymer was undertaken. The 220-MHz spectrum (Fig. 6) indicated that it was a poly(vinyl chloride)–poly(vinyl acetate) copolymer

with a large amount of plasticizer. The poly(vinyl chloride) was in long blocks, giving rise to tacticity patterns similar to Fig. 5. From this one 220-MHz spectrum the types of monomers present, degrees of tacticity, and amount of plasticizer can readily be calculated. This same sample would have been difficult to analyze at 100 MHz or 60 MHz.

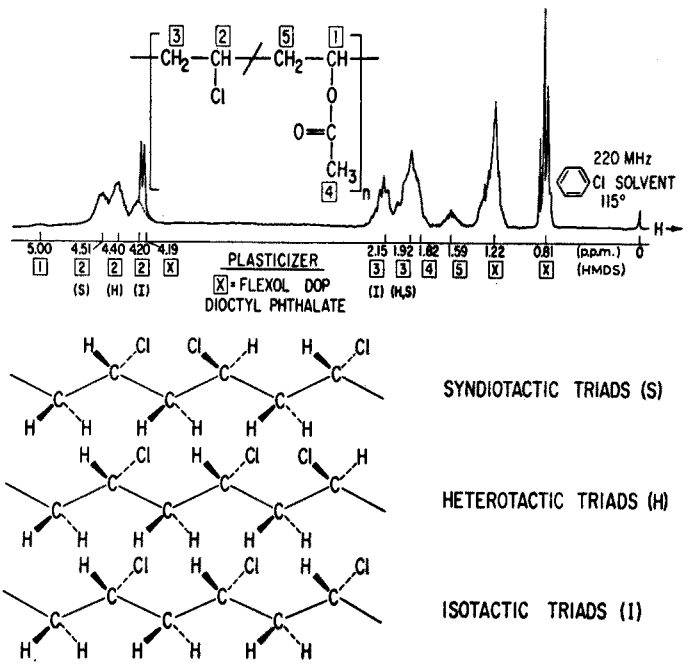


Fig. 6. The 220-MHz spectrum of poly(vinyl chloride)–poly(vinyl acetate) copolymer.

The assistance of my colleagues in the Du Pont Company who have reviewed and commented upon this paper is appreciated. I am indebted to Mr. ROBERT S. WALLACE for obtaining the 220-MHz spectra.

SUMMARY

High-resolution nuclear magnetic resonance spectroscopy gives considerable information about composition, structure, and stereoconfiguration of polymer chains. Proton resonance spectra of a polycarbonate, polyamide, poly(vinyl chloride), and a poly(vinyl chloride)–poly(vinyl acetate) copolymer, which were obtained at 60 MHz, 100 MHz, and 220 MHz are discussed. Spectra obtained with the 220-MHz superconducting solenoid spectrometer approach first order, permitting more detailed information to be obtained and, in most cases, eliminating the need for spin-decoupling experiments in stereoconfiguration determinations.

RÉSUMÉ

La spectroscopie à résonance magnétique nucléaire à haute résolution fournit

des renseignements intéressants sur la composition, la structure et la stéréoconfiguration des chaînes polymères. On examine les spectres de résonance protonique d'un polycarbonate, polyamide, poly(vinylchlorure) et copolymère poly(vinylchlorure)–poly(vinylacétate), obtenus à 60 MHz, 100 MHz et 220 MHz. Des spectres obtenus avec spectromètre solénoïde superconducteur 220 MHz permettent d'obtenir des informations plus détaillées, dans la plupart des cas, éliminant la nécessité d'essais de spin-découplage lors des déterminations de stéréoconfiguration.

ZUSAMMENFASSUNG

Die hochauflösende Kernmagnetischeresonanz ergibt weitgehende Informationen über die Zusammensetzung, die Struktur und die Stereokonfiguration polymerer Ketten. Die Protonenresonanzspektren von Polycarbonat, Polyamid, Poly-(vinylchlorid) und einem Polyvinylchlorid–Polyvinylacetat-Copolymeren, welche mit 60 MHz, 100 MHz und 220 MHz aufgenommen wurden, werden diskutiert. Spektren, welche mit einem 220 MHz superleitenden Solenoid-Spektrometer von nahezu 1. Ordnung erhalten wurden, erlauben eine ausführlichere Information und in den meisten Fällen sind Spinentkopplungs-Experimente bei der Bestimmungen von Stereokonfigurationen nicht erforderlich.

REFERENCES

1 W. P. SLICHTER, *J. Polymer Sci.*, 36 (1959) 259.
2 D. W. JONES, *J. Polymer Sci.*, 59 (1962) 271.
3 F. A. BOVEY AND G. V. D. TIERS, *J. Polymer Sci.*, 44 (1960) 173.
4 F. A. BOVEY, D. W. ANDERSON AND D. C. DOUGLASS, *J. Chem. Phys.*, 39 (1963) 1199.

Analytica Chimica Acta
Elsevier Publishing Company, Amsterdam
Printed in The Netherlands

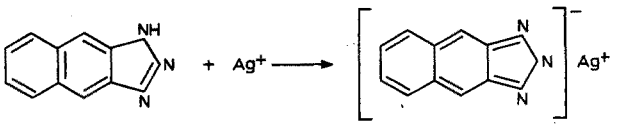
2,3-NAPHTHOTRIAZOLE AS A GRAVIMETRIC, SPECTROPHOTOMETRIC, AND FLUORIMETRIC REAGENT FOR THE DETERMINATION OF SILVER

GARRY L. WHEELER, JOHN ANDREJACK, JAMES H. WIERSMA AND PETER F. LOTT
Department of Chemistry, University of Missouri, Kansas City, Mo. 64110 (U.S.A.)
(Received March 4th, 1969)

Gravimetric and titrimetric methods for the determination of silver with benzotriazole and its derivatives have been reviewed by KODAMA[1] while spectrophotometric methods for the determination of silver have been recently reviewed by EL-GHARMY AND FREI[2].

In an investigation of the properties of the benzotriazoles and naphthotriazoles, it was found that benzotriazole did not fluoresce under ultraviolet light, that 1,2-naphthotriazole fluoresced slightly when examined with short wavelength UV light, and that 2,3-naphthotriazole showed a very strong fluorescence which could be quenched by silver ions. Accordingly, it was decided to investigate the use of 2,3-naphthotriazole as a reagent for silver.

The addition of an ammoniacal solution of 2,3-naphthotriazole to a solution containing silver ions and EDTA, resulted in a precipitate corresponding to the formula $AgC_{10}H_6N_3$.



A study of the determination of macro and trace amounts of silver with 2,3-naphthotriazole in the presence of foreign ions is reported below. The reagent has been found suitable for the direct determination of milligram amounts of silver gravimetrically, while submilligram and trace amounts of silver are determined by spectrophotometric or fluorimetric methods.

EXPERIMENTAL

Apparatus

Absorption spectra were obtained with a Bausch and Lomb 505 recording spectrophotometer. Absorbance measurements were made with a Beckman DU and a Model B spectrophotometer using 1.00-cm quartz cells. Fluorescence spectra were also obtained from a Bausch and Lomb 505 recording spectrophotometer and fluorescence accessory[3]. Relative fluorescent intensities were determined with a Farrand Model A-2 Fluorimeter, and fluorimetric titrations were performed with this instrument according to the procedure of WIERSMA AND LOTT[4].

Reagents

2,3-Naphthotriazole was prepared by dissolving 5.0 g of 2,3-diaminonaphthalene (Aldrich Chemical Co., Milwaukee, Wisc.) in 200 ml of glacial acetic acid, diluting to 800 ml with water then to 1000 ml with ice. When the ice was completely melted, 25 ml of aqueous 1.2 *M* sodium nitrite was added rapidly while stirring briskly. The precipitate, 2,3-naphthotriazole, was recrystallized twice from boiling water containing decolorizing carbon. The average yield was 60% of the theoretical yield and the product had a melting range of 194°–197°. The material is quite insoluble in cold water and acidic solutions (except concentrated sulfuric acid). It is very soluble in basic solution and is soluble to the extent of 0.15 g/100 ml in boiling water.

For the gravimetric procedure, a solution of 2,3-naphthotriazole was prepared by dissolving 2.50 g of 2,3-naphthotriazole in 30 ml of concentrated aqueous ammonia, diluting to 100 ml with water and filtering through a Reeve-Angel glass fiber filter (grade 934 AH) until no residue was left on the filter.

For the spectrophotometric and fluorimetric procedure, 100 mg reagent was dissolved in 100 ml of hot water or 100 ml of 0.025 *M* sodium hydroxide. Reagent solutions containing 10.0 mg/ml concentration or greater were prepared daily, because considerable decomposition occurred within a week; less concentrated solutions were stable for a month when stored in the dark.

Standard silver solution (5.00 mg/ml) was prepared by dissolving 0.7875 g of reagent-grade silver nitrate in a 100-ml volumetric flask containing 1 ml of nitric acid and diluting to volume with water. Fresh silver nitrate solution was prepared daily.

A masking solution, 0.05 *M* in EDTA and 0.05 *M* in sodium tartrate, was prepared by dissolving 18.6 g of the disodium salt of EDTA and 11.5 g of sodium tartrate in 1 l of water.

Gravimetric procedure

To a solution containing 5–100 mg of silver, add 25 ml of masking solution, adjust to *ca.* pH 11 with aqueous ammonia, and add 2–20 ml of stock 2,3-naphthotriazole solution depending upon the amount of silver in the sample. A 50% excess of reagent is sufficient to precipitate the silver completely. Digest the samples at 60°–70° for 15 min and allow to cool to room temperature. Then filter through a weighed Coors Gooch crucible and Reeve-Angel glass fiber filter (grade 934 AH), wash twice with 20–30 ml of hot water (as near 100° as possible), and dry at 110° for 1–2 h to constant weight. The conversion factor is 0.3908.

Spectrophotometric procedure

Silver in the range 1–30 μg/ml can be determined by measurement of the absorbance of the silver triazole complex in basic solution.

Prepare samples by adding to a series of 100-ml volumetric flasks, 0–3 mg silver, 5 ml of a solution of 2,3-naphthotriazole solution (10 mg/ml) in 0.025 *M* sodium hydroxide, 10 ml of the masking agent solution and 10 ml of pH 10.5 buffer. As ammonium hydroxide must be excluded to prevent precipitation of the silver complex, a metheneamine–sodium hydroxide buffer was prepared by adding 6 g of metheneamine to 1 l of water and sufficient sodium hydroxide to adjust the solution to pH 10.5 as shown by a pH meter; the pH is critical (see below). Allow the yellow

color of the silver–naphthotriazolate complex to develop for 3–5 min, and then measure the absorbance at 436, 416, or 390 nm against a reference standard containing all reagents except silver. Measurements at 436 nm are preferred as most metal–EDTA complexes do not absorb light of this wavelength.

Direct fluorimetric procedure

Silver in the range 0.025–0.100 μg/ml can be determined by measurement of the fluorescence intensity of samples relative to a reference blank containing all reagents except silver. The direct fluorimetric procedure uses the same reagents as the spectrophotometric procedure, but no masking agents, and is applicable to lower silver concentrations. Since silver quenches the fluorescence of 2,3-naphthotriazole, the fluorescence intensity decreases with increasing silver concentration; measurements were made after a standing time of 5 min.

RESULTS AND DISCUSSION

Gravimetric method

The results of several determinations of silver with the reagent, in the absence and presence of foreign metals are shown in Table I. Figure 1 shows qualitative interference tests conducted by adding 10 ml of masking solution, 2 ml of concentrated ammonia solution and 5 ml of 0.05 *M* 2,3-naphthotriazole solution to 10 ml of 0.01 *M* solution of foreign metal ion. The reagent is not specific for silver; however, the combination of the reagents, EDTA and sodium tartrate, make the method highly selective for silver. Of the ions tested, as shown in Fig. 1, only antimony and iodide interfered. The hydroxides of tin, titanium, beryllium and manganese are

TABLE I

GRAVIMETRIC DETERMINATION OF SILVER IN THE PRESENCE AND ABSENCE OF METAL NITRATES

Metal added[a]	*Ag taken (mg)*	*Ag found (mg)*
—	5.00	5.18, 5.29
—	10.00	10.20, 10.32
—	15.00	15.02, 15.28
—	20.00	19.82, 19.81
Al(III)	15.00	15.06, 15.02
Cd(II)	15.00	14.98, 14.94
Co(II)	10.00	10.08, 10.27
Cr(III)	15.00	15.28, 15.28
Cu(II)	10.00	10.32, 10.28
Fe(III)	20.00	19.98, 19.94
Hg(II)	20.00	19.94, 20.02
Mn(II)	20.00	19.99, 20.02
Ni(II)	10.00	10.16, 10.36
Pb(II)	15.00	14.94, 14.94
Zn(II)	10.00	10.28, 10.16
Mixture consisting of Al(III), Cd(II), Co(II), Cu(II), Fe(III), Mn(II), Ni(II), Pb(II), Pd(II), Zn(II)	19.50	19.19, 19.42, 19.50

[a] 0.1 g of each metal nitrate added.

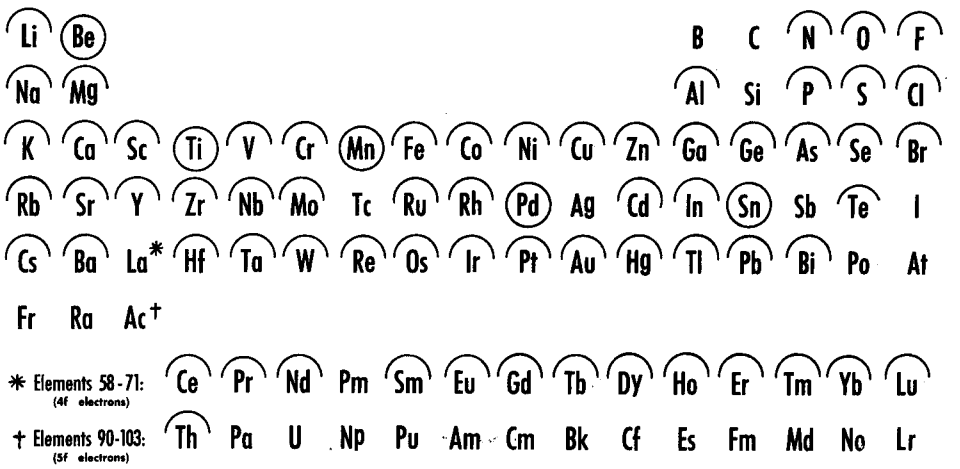
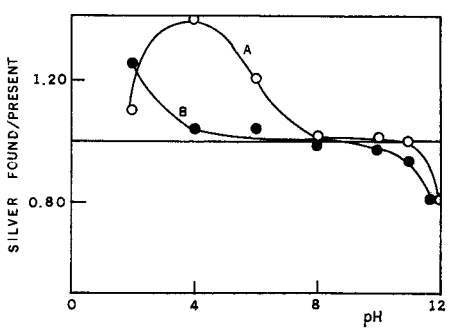


Fig. 1. Effect of foreign ions. (⌒) Ions non-interfering or masked by EDTA; (o) ions masked by tartrate.

insoluble even in the presence of EDTA, therefore tartrate must be used to form soluble complexes. Tartrate also masks palladium.

The reagent and silver form an insoluble complex with a 1:1 molar ratio, thus confirming the theoretical gravimetric factor of 0.3908. It was found that temperatures of 100°–140° were suitable for drying the precipitate. The precipitate is neither hygroscopic nor light-sensitive. The silver complex is insoluble in most organic solvents (no solvent was found which would extract the complex from the aqueous layer). The complex is soluble in concentrated nitric and sulfuric acids. Cyanide readily dissolves the precipitate. The pH range is critical for the precipitation of the silver complex of naphthotriazole owing to the insolubility of the reagent in acidic solutions. Aqueous ammonia tended to coagulate the precipitate. Figure 2 shows the effect of pH on precipitation of silver naphthotriazolate.

The average error was less than 0.2 mg in samples containing 5–100 mg of silver and 100 mg of foreign metals. No interference was found from nitrate, sulfate,



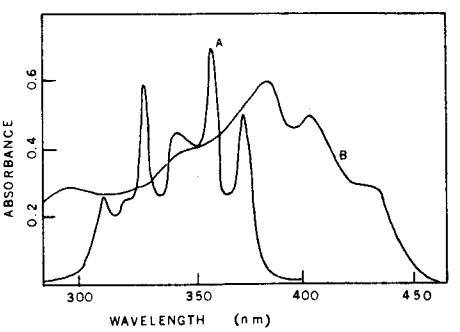


Fig. 2. Effect of pH and hot water washing on recovery of silver. (A) Precipitate not washed with hot water; (B) precipitate washed with hot water.

Fig. 3. Spectra. (A) 2,3-Naphthotriazole, 10 μg/ml, pH 10; (B) silver complex of naphthotriazole, 10 μg/ml, pH 10.

phosphate, acetate, oxide, fluoride, chloride or bromide in the gravimetric procedure, because silver in the sample was kept in solution by the presence of ammonia.

Spectrophotometric method

Figure 3 shows the ultraviolet–visible absorbance spectra of the reagent and the complex. Spectrophotometric results for silver in the presence of foreign metals, with a 50 μg/ml reagent are shown in Table II. Silver (1 μg/ml) can be determined in the presence of a mixture containing 5,000 μg/ml each of copper(II), cobalt(II), nickel(II), manganese(II), and iron(III); these five ions are the most highly colored EDTA complexes. Since the absorbance spectrum shows three peaks, further selectivity can be achieved by proper wavelength selection.

TABLE II

SPECTROPHOTOMETRIC DETERMINATIONS OF SILVER IN THE PRESENCE OF OTHER IONS

	Ag present (p.p.m.)	*Ag found (p.p.m.)*
A[a]	0.5	0.5
	1.0	1.0
	3.0	3.3
	5.0	5.2
	10.0	10.0
B[b]	1.0	0.9
	5.0	5.0
	10.0	10.0
	20.0	20.0
C[c]	1.0	1.0
	2.5	2.4
	5.0	5.0
	10.0	10.0

[a] 50 p.p.m. each Cu(II), Co(II), Ni(II), Mn(II), Fe(III) nitrates present.
[b] 100 p.p.m. each Cu(II), Co(II), Ni(II), Pb(II), Zn(II) nitrates present.
[c] 5,000 p.p.m. each Cu(II), Co(II), Ni(II), Mn(II), Fe(III) nitrates present.

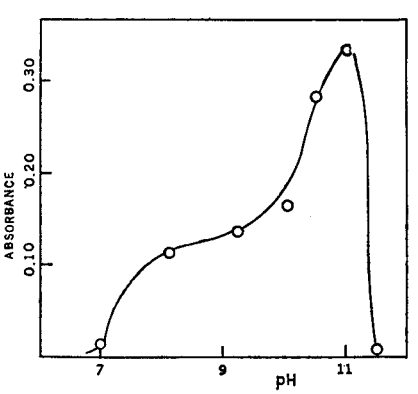


Fig. 4. Effect of pH on absorbance (50 μg Ag^+/ml; wavelength 436 nm).

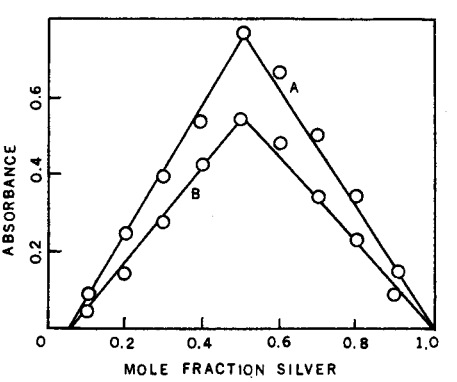


Fig. 5. Mole ratio study. (A) 0.14 mM reagents; (B) 0.11 mM reagents.

The pH is critical for maximal color development and stability (Fig. 4).

A Job study (Fig. 5) showed that the ratio of silver to 2,3-naphthotriazole in the complex was 1:1. The molar absorptivity of the complex is 1,000.

Fluorimetric method

Table III shows the results obtained in the direct fluorimetric determination of silver in the absence of foreign metal nitrates but in the presence of chloride; a reagent solution containing 0.05 μg/ml was used. Chloride, bromide and iodide ions were found to interfere with the procedure when present in the sample in excess of 1 p.p.m. Masking agents such as EDTA, triethanolamine, and tartrate were found to complex silver sufficiently to prevent complete quenching of the fluorescence of the 2,3-naphthotriazole when silver was added.

TABLE III

DIRECT FLUORIMETRIC DETERMINATIONS OF SILVER

	Ag present (p.p.b.)	*Ag found (p.p.b.)*
A[a]	25	25, 24
	50	49, 50
	75	75, 75
	100	103
	125	125
B[b]	25	25
	50	50
	75	76
C[c]	25	24
	50	52
	75	74

[a] No interfering ions.
[b] 60 p.p.b. Cl^- present.
[c] 600 p.p.b. Cl^- present.

Filter selections for the maximum excitation and fluorescence of the reagent were determined from the spectra recorded on the Bausch and Lomb 505. The excitation maximum was observed at 362 nm and the fluorescence maximum at 406 nm. Accordingly, Corning filters number CS 7-55 and CS 3-73 were used as excitation and transmission filter, respectively.

Fluorimetric titrations were also performed, with the same filters in the Farrand fluorimeter and a recorder output[4], for the determination of 0.1–2.0 μg of silver per ml (pH 10.5 metheneamine buffer) with a 0.1 mg/ml solution of 2,3-naphthotriazole as the titrant. The end-point was indicated by the increase in the fluorescence intensity when excess of reagent was added. The minimum amount of silver which could be determined titrimetrically by this procedure was about 10 times larger than the amount which could be determined by the direct fluorimetric procedure; since the titrant was added continuously, there was no time of standing to permit the reaction to go "to completion". Both fluorimetric procedures are capable of detecting lower quantities of silver than the spectrophotometric method.

Attempts to increase the sensitivity of the spectrophotometric and fluorimetric methods by means of extraction were unsuccessful, since no suitable organic solvent could be found.

SUMMARY

2,3-Naphthotriazole is suitable for the determination of macro- and microquantities of silver. The gravimetric (for 5–100 mg Ag) and spectrophotometric ($\varepsilon = 1{,}000$; 1–30 μg Ag/ml) methods are rapid and free of halide and foreign ion interferences; excellent selectivity is attained by means of masking agents. The fluorimetric method (0.025–0.1 μg Ag/ml) is much more sensitive than the spectrophotometric method but is more subject to interferences.

RÉSUMÉ

Le 2,3-naphtotriazole convient au dosage de macro- et de microquantités d'argent. Les méthodes gravimétriques (pour 5 à 100 mg Ag) et spectrophotométriques ($\varepsilon = 1{,}000$; 1 à 30 μg Ag/ml) sont rapides. Les halogénures et ions étrangers ne gênent pas; on peut obtenir une excellente sélectivité au moyen de réactifs de masquage. La méthode fluorimétrique est beaucoup plus sensible (0.025–0.1 μg Ag/ml) que la méthode spectrophotométrique; cependant elle présente plus d'interférences d'ions étrangers.

ZUSAMMENFASSUNG

2,3-Naphthotriazol ist zur Bestimmung von Makro- und Mikromengen Silber geeignet. Die gravimetrische (5–100 mg Ag) und spektralphotometrische ($\varepsilon = 1{,}000$; 1–30 μg Ag/ml) sind schnell und frei von Störungen durch Halogenide und andere Fremdionen. Eine ausgezeichnete Selektivität wird mit Hilfe von maskierenden Reagenzien erzielt. Die fluorimetrische Methode (0.025–0.1 μg Ag/ml) ist sehr viel empfindlicher als die spektralphotometrische Methode, aber auch störanfälliger gegenüber Fremdionen.

REFERENCES

1 K. KODAMA, *Methods of Quantitative Inorganic Analysis*, Interscience, New York, 1963.
2 M. T. EL-GHARMY AND R. W. FREI, *Anal. Chem.*, 40 (1968) 1986.
3 J. H. WIERSMA AND P. F. LOTT, *Chemist-Analyst*, 55 (1966) 20.
4 J. H. WIERSMA AND P. F. LOTT, *Analytical Letters*, 1 (1968) 603.

Analytica Chimica Acta
Elsevier Publishing Company, Amsterdam
Printed in The Netherlands

MOLECULAR WEIGHT MEASUREMENTS OF POLYCARBOXYLIC ACIDS IN WATER BY VAPOR PRESSURE OSMOMETRY*

E. H. HANSEN** AND M. SCHNITZER
Soil Research Institute, Canada Department of Agriculture, Ottawa, Ontario (Canada)
(Received February 24th, 1969)

Vapor pressure osmometry is used increasingly for measuring number-average molecular weights ($\overline{Mn}$) of a variety of organic compounds. This method provides rapid molecular weight determinations at different temperatures in water as well as in organic solvents. When measured in water, $\overline{Mn}$ values may, however, be erroneous because of dissociation of acidic functional groups. Thus, the authors recently found that molecular weights of a number of aliphatic and aromatic polycarboxylic acids were considerably lower than the theoretical values. Although the dissociation can be minimized by doing measurements in organic solvents, many naturally occurring polycarboxylic compounds are, however, insoluble or only slightly soluble in these media, so that water is the solvent of choice. Based upon the experimentally determined values of $\overline{Mn}$ and pH, a correction system was developed which makes possible calculations of accurate $\overline{Mn}$ values in aqueous solutions. The purpose of this paper is to describe this correction system and to demonstrate its applicability to a number of representative known organic compounds, containing between one and six carboxyl groups per mole, and to water-soluble humic compounds of widely differing molecular weights, containing relatively high concentrations of carboxyl groups.

THEORETICAL

As vapor pressure osmometry is based on a colligative property, it depends only upon the *number* of molecules, ions, atoms or other dissolved particles per unit weight of solvent but not on their *nature*. Assuming that no dissociation or association takes place, the number-average molecular weight $\overline{Mn}$ can be expressed as follows[1]:

$$\overline{Mn} = \lim_{a \to 0} \overline{Mn(a)} = \lim_{a \to 0} \frac{K}{\Delta R/a} \qquad (1)$$

where K is a calibration constant which must be determined for each solvent to be used; ΔR is the instrument readout for the sample solution, in ohms; a is the weight of sample per 1000 g of solvent; and $\overline{Mn(a)}$ is the number-average molecular weight at concentration a as determined by eqn. (1).

The following is a simple correction system which permits calculations of accurate number-average molecular weights for water-soluble samples, and which is based upon the experimentally determined values of $\overline{Mn(a)}$ and pH.

* Contribution No. 300.
** Postdoctorate Fellow of the National Research Council of Canada.

The dissociation of a polybasic acid H_nX can, at equilibrium, be described by the equation

$$H_nX \rightleftharpoons nH^+ + X^{n-} \tag{2}$$

$$c(1-\alpha) \rightleftharpoons nc\alpha + c\alpha \tag{3}$$

If the initial concentration of H_nX is c mol/l, the concentrations at equilibrium are expressed by eqn. (3), where α is the degree of dissociation and n is defined by

$$n = \frac{\text{molecular weight}}{\text{equivalent weight}} = \frac{\overline{\text{Mn(corr)}}}{\text{EW}} = \frac{\lim_{a\to 0} \overline{\text{Mn}(a)_{\text{corr}}}}{\text{EW}} \tag{4}$$

The hydrogen-ion concentration is thus given by

$$nc\alpha = [H^+] = 10^{-\text{pH}} \tag{5}$$

By substituting $n = \overline{\text{Mn}(a)_{\text{corr}}}/\text{EW}$ and $c = a/\overline{\text{Mn}(a)_{\text{corr}}}$, where a is measured in g/kg ($\simeq$g/l), eqn. (5) can be written

$$10^{-\text{pH}} = \frac{\overline{\text{Mn}(a)_{\text{corr}}}}{\text{EW}} \cdot \frac{a}{\overline{\text{Mn}(a)_{\text{corr}}}} \cdot \alpha$$

or

$$10^{-\text{pH}} = \frac{\alpha}{\text{EW}} \cdot a \tag{6}$$

Let $\alpha/\text{EW} = y$, then $y = 10^{-\text{pH}}/a$
or

$$\log y = -\text{pH} - \log a \tag{7}$$

The total number of particles (*i.e.* undissociated molecules and ions) present is, from eqn. (3),

$$c(1-\alpha) + nc\alpha + c\alpha = c(1+n\alpha) = \frac{a}{\overline{\text{Mn}(a)_{\text{corr}}}} \cdot \left(1 + \frac{\overline{\text{Mn}(a)_{\text{corr}}}}{\text{EW}} \cdot \alpha\right)$$

Using the experimentally determined value of $\overline{\text{Mn}(a)}$, the total number of particles can also be expressed as $a/\overline{\text{Mn}(a)}$; thus we have the identity

$$\frac{a}{\overline{\text{Mn}(a)_{\text{corr}}}}\left(1 + \frac{\overline{\text{Mn}(a)_{\text{corr}}}}{\text{EW}} \cdot \alpha\right) = \frac{a}{\overline{\text{Mn}(a)}}$$

which can be reduced to $1/\overline{\text{Mn}(a)_{\text{corr}}} + y = 1/\overline{\text{Mn}(a)}$
or

$$\overline{\text{Mn(corr)}} = \lim_{a\to 0} \overline{\text{Mn}(a)_{\text{corr}}} = \lim_{a\to 0} \frac{\overline{\text{Mn}(a)}}{1 - y \cdot \overline{\text{Mn}(a)}} \tag{8}$$

EXPERIMENTAL

Reagents

All known organic compounds were of the highest purity available commercially.

The humic compound was a water-soluble fulvic acid which originated from a Podzol Bh horizon; methods of extraction and purification of this material as well as a number of its analytical characteristics have been reported[2]. The purified fulvic acid was fractionated, with water as eluent, on Sephadex gels of various fractionation ranges as described previously[3]. Briefly, to prepare chromatographically homogeneous fractions, 6.0 g of fulvic acid was first fractionated on Sephadex G-50. Material excluded by this gel was collected, while fractions retarded on it were subsequently eluted and then separated on Sephadex G-25. The material excluded by the G-50 gel was concentrated and re-fractionated on the same gel. This procedure was repeated several times until no further separation occurred and all of the material was eluted as 'void' volume. The same method was then used on G-25, G-15 and G-10 gels. The fractionation procedure is illustrated in Fig. 1.

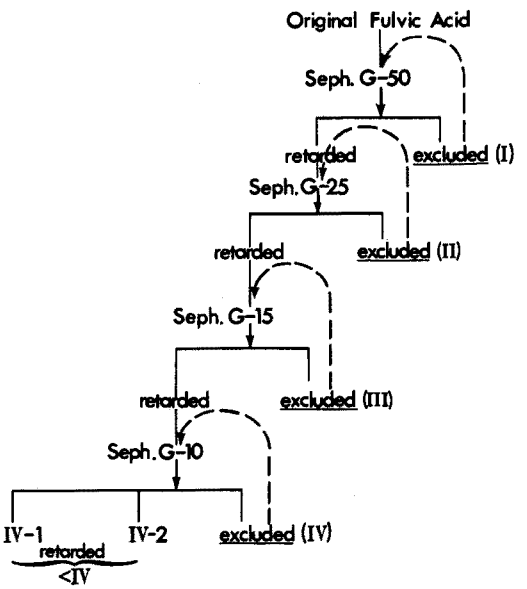


Fig. 1. Fractionation of fulvic acid on Sephadex gels. Roman numerals refer to fractions.

Apparatus

All molecular weight measurements were made on a model 302 Hewlett-Packard vapor pressure osmometer. The apparatus was calibrated, with 0.02–0.17 *M* aqueous potassium bromide solutions. Under these conditions the calibration constant *K* was 70.75 kg ohms/g.

Values of pH were measured to three decimal places on a Beckman Research pH-Meter which was standardized with a Beckman buffer of pH = 7.413 at 25°.

Procedure

The method outlined in the operational manual was used[1]. The concentrations of the compounds analyzed ranged from 0.1 to 2.5% (w/v), depending on the solubility of the compound in water. All measurements were made in distilled water at 37°.

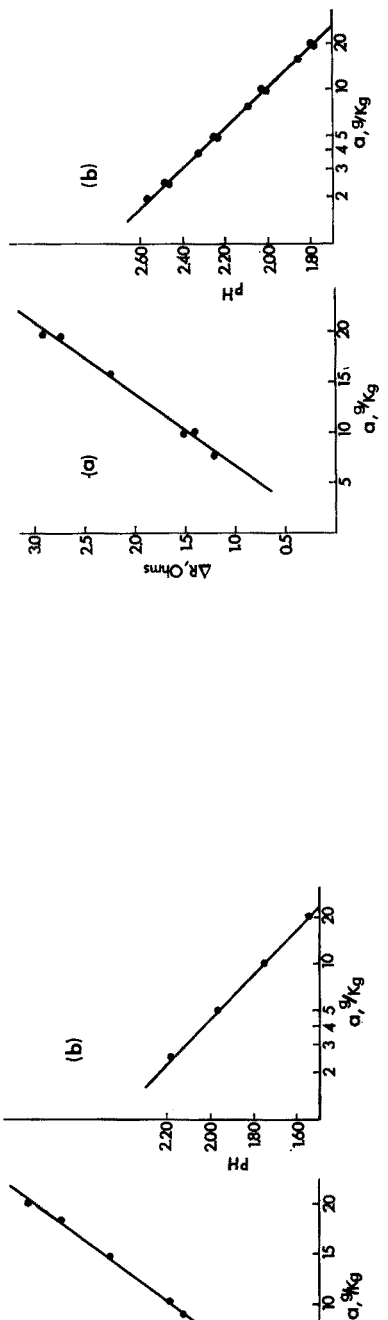


Fig. 2. Benzenepentacarboxylic acid. Relationship between (a) ΔR and a; (b) pH and log a; (c) $\overline{\mathrm{Mn}(a)}$ and a; and (d) $\overline{\mathrm{Mn}(a)_{\mathrm{corr}}}$ and a.

Fig. 3. Fulvic acid. Relationship between (a) ΔR and a; (b) pH and log a; (c) $\overline{\mathrm{Mn}(a)}$ and a; and (d) $\overline{\mathrm{Mn}(a)_{\mathrm{corr}}}$ and a.

RESULTS

Calculation of results

The detailed procedure that was ultimately adopted for the molecular weight measurements is described below, with benzenepentacarboxylic acid (Fig. 2) and fulvic acid (Fig. 3) as examples:

(*a*) The experimentally determined values of ΔR are plotted against a (Figs. 2a and 3a); if necessary, the method of least squares is used.

(*b*) The experimentally determined values of pH are plotted against log a (Figs. 2b and 3b).

(*c*) From corresponding values of ΔR and a, values of $\overline{\mathrm{Mn}(a)}$ are calculated by eqn. (1). Similarly, values of y are calculated from eqn. (7). From the corresponding values of $\overline{\mathrm{Mn}(a)}$ and y, a series of values of $\overline{\mathrm{Mn}(a)_{\mathrm{corr}}}$ are calculated by eqn. (8). See Tables I and II.

TABLE I

CALCULATIONS OF $\overline{\mathrm{Mn}(a)}$ AND $\overline{\mathrm{Mn}(a)_{\mathrm{corr}}}$ FOR BENZENEPENTACARBOXYLIC ACID

a	ΔR	$\Delta R/a$	pH	$y \cdot 10^3$	$\overline{Mn(a)}$	$\overline{Mn(a)_{corr}}$
20.000	7.22	0.361	1.552	1.403	196	270
17.000	6.16	0.363	1.602	1.472	195	274
15.000	5.46	0.364	1.638	1.535	194	276
12.000	4.42	0.368	1.704	1.648	192	281
10.000	3.72	0.372	1.760	1.738	190	284
8.000	3.02	0.378	1.827	1.862	187	288

TABLE II

CALCULATIONS OF $\overline{\mathrm{Mn}(a)}$ AND $\overline{\mathrm{Mn}(a)_{\mathrm{corr}}}$ FOR FULVIC ACID

a	ΔR	$\Delta R/a$	pH	$y \cdot 10^3$	$\overline{Mn(a)}$	$\overline{Mn(a)_{corr}}$
20.000	2.854	0.1427	1.772	0.8453	496	854
17.000	2.442	0.1436	1.830	0.8710	493	863
15.000	2.167	0.1445	1.870	0.8995	490	877
12.000	1.755	0.1462	1.945	0.9462	484	893
10.000	1.480	0.1480	2.006	0.9863	478	904
8.000	1.205	0.1506	2.085	1.029	470	910

(*d*) By plotting $\overline{\mathrm{Mn}(a)}$ against a and extrapolating to infinite dilution ($a=0$), $\overline{\mathrm{Mn}}$ is determined (Figs. 2c and 3c). Similarly, a plot of $\overline{\mathrm{Mn}(a)_{\mathrm{corr}}}$ against a, extrapolated to $a=0$, yields the corrected number-average molecular weight $\overline{\mathrm{Mn}(\mathrm{corr})}$ (Figs. 2d and 3d).

Molecular weight measurements of known compounds

Theoretical, experimental and corrected molecular weights for a number of aliphatic and aromatic carboxylic acids are shown in Table III. For the corrected molecular weights the mean deviation from the theoretical values was 0.92%. As

TABLE III

THEORETICAL, EXPERIMENTAL AND CORRECTED MOLECULAR WEIGHTS FOR KNOWN ALIPHATIC AND AROMATIC CARBOXYLIC ACIDS

Acid	*Molecular weight*			*% Deviation from theor. value*
	Theor.	*Exp.*	*Corr.*	
Aliphatic				
Oxalic	90	57	89	−1.11
Malonic	104	92	105	+0.96
Succinic	118	117	119	+0.85
1,4-Butanedicarboxylic	146	139	144	−1.39
2-Hydroxy-1,2,3-propane-tricarboxylic·H_2O	210	180	207	−1.43
1,2,3-Propanetricarboxylic	176	162	174	−1.15
1,2,3,4-Butanetetracarboxylic	234	202	231	−1.30
Aromatic				
2,5-Dihydroxybenzoic	154	155	155	+0.65
3,4,5-Trihydroxybenzoic·H_2O	188	186	186	−1.16
Benzoic	122	122	122	0.00
1,2-Benzenedicarboxylic	166	134	165	−0.60
1,2,4-Benzenetricarboxylic	210	166	213	+1.43
1,2,4,5-Benzenetetracarboxylic	254	161	251	−1.20
Benzenepentacarboxylic	298	182	299	+0.34
Benzenehexacarboxylic	342	178	341	−0.29

TABLE IV

EXPERIMENTAL AND CORRECTED MOLECULAR WEIGHTS FOR FULVIC ACID

Compound	*Molecular weight* ($M\bar{n}$)		*Weight fraction* f_x
	Exp.	*Corr.*	
Fulvic acid	460	951	1.0000
Fraction I	910	2110	0.0668
II	675	1815	0.1088
III	570	1181	0.5670
IV	449	883	0.1079
IV-1	207	311	0.0939
IV-2	257	275	0.0149

indicated by the data in Table III, dissociation could cause experimentally determined molecular weights to be up to 50% lower than the theoretical ones.

Molecular weights of fulvic acid and fractions

Table IV shows experimental and corrected molecular weights for fulvic acid and for a number of fractions derived from it. The corrected molecular weights ranged from 275 to 2110. Since at the present time no generally accepted method for molecular weight determinations of these substances is available, it was not possible to check these data by an independent method. However, attempts were made to ascertain the reliability of the corrected experimental molecular weights by calculating

the molecular weight of the fulvic acid from the following relationship[4]:

$$\overline{Mn} = \Sigma M_x V_x = 1/\Sigma f_x/M_x$$

where V_x is the number of fractions and f_x is the weight fraction of molecules of size x (Table IV). The $\overline{Mn}$ obtained in this manner was 952, which is in very good agreement with the $\overline{Mn(corr)}$ value of 951 found for the unfractionated fulvic acid.

DISCUSSION

The concentration range within which the proposed correction system is applicable is determined by several factors. While the upper limit of the concentration a depends on the solubility of the particular compound in water, the lower limit is largely determined by the sensitivity of the vapor pressure osmometer. With the Hewlett-Packard instrument, the mean deviation on individual ΔR-measurements was found to be 0.03 ohms. This agrees well with the information supplied by the manufacturer, according to which a reproducibility of repetitive readings is better than 1%, except at very low readings (less than 3 ohms). Although many carboxylic acids have limited solubility in water, the $\overline{Mn}$ determinations of compounds with molecular weights of less than 500 do not present any problems, as ΔR-readouts usually are above 3 ohms. With increasing molecular weights, however, ΔR-readings might often have to be taken in the below 3 ohms region. For the sake of reliability it may thus be advisable to perform series of at least 6–10 measurements for the plotting of ΔR *vs.* a curves.

With increasing values of a, ΔR *vs.* a curves often exhibit slightly decreasing slopes. This tendency becomes more prominent with increasing acidity and with increasing molecular weight; a straight line relationship between ΔR and a is therefore found only above $a \approx 5$ g/kg. Furthermore, as the molecular weight increases, pH will at very low concentrations, of course, cease to be a linear function of log a.

Thus, with a practical lower concentration limit of $a \approx 5$ g/kg and with an upper limit determined by the solubility of the individual compounds in water, only a fairly narrow range of a may be applicable. However, as the results in Tables III and IV indicate, this is not a serious limitation of the method.

As most of the ΔR-readings for the fulvic acid and the fractions derived thereof were below 3 ohms, it is estimated that the mean deviation is higher than that for the known compounds and is likely of the order of 2–3%.

The method proposed may be especially useful for measuring number-average molecular weights of naturally occurring water-soluble organic compounds which are insoluble or only slightly soluble in organic solvents.

The authors gratefully acknowledge the technical assistance of S. I. M. SKINNER.

SUMMARY

Number-average molecular weight determinations of polycarboxylic acids in water by vapor pressure osmometry may yield erroneous results owing to dissociation. Based on experimentally determined values of $\overline{Mn}$ and pH, a correction system is described which makes possible calculations of accurate $\overline{Mn}$ values. The

applicability of the correction system is demonstrated for 15 known aliphatic and aromatic compounds, containing between one and six carboxyl groups per mole and to water-soluble humic compounds of widely differing molecular weights, containing relatively high concentrations of carboxyl groups. This method may be especially useful for naturally occurring water-soluble organic compounds which are insoluble or only very slightly soluble in organic solvents.

RÉSUMÉ

Des déterminations de poids moléculaires d'acides polycarboxyliques dans l'eau par osmométrie à pression de vapeur peuvent fournir des résultats erronés, en raison de la dissociation. On décrit un système de correction, basé sur les valeurs de $\overline{Mn}$ et du pH permettant de calculer $\overline{Mn}$ avec précision. L'utilité de ce système de correction est démontré pour 15 composés aliphatiques et aromatiques connus, renfermant entre un et six groupes carboxyle et pour des composés humiques solubles dans l'eau de poids moléculaires très différents, renfermant relativement beaucoup de groupes carboxyle. Cette méthode peut être spécialement utile pour des composés organiques solubles dans l'eau et insolubles ou seulement très légèrement solubles dans des solvants organiques.

ZUSAMMENFASSUNG

Molekulargewichtsbestimmungen von Polycarbonsäuren in Wasser durch Dampfdruck-Osmose ergeben aufgrund der Dissoziation fehlerhafte Resultate. Auf der Grundlage experimentell bestimmter Werte von $\overline{Mn}$ und pH wird ein Korrektursystem beschrieben, welches die Berechnung genauer $\overline{Mn}$-Werte ermöglicht. Die Anwendbarkeit des Korrektursystem wird für 15 bekannte aliphatische und aromatische Verbindungen beschrieben, welche zwischen einer und sechs Carboxyl-Gruppen pro Mol enthalten und für wasserlösliche Huminverbindungen mit sehr unterschiedlichen Molekulargewichten, welche relativ hohe Konzentrationen an Carboxylgruppen enthalten. Diese Methode ist besonders für natürlich auftretende, wasserlösliche organische Verbindungen geeignet, die in organischen Lösungsmitteln unlöslich oder nur sehr wenig löslich sind.

REFERENCES

1 HEWLETT-PACKARD, F & M SCIENTIFIC DIVISION, *Model 302 Vapor Pressure Osmometer, Operating and Service Manual.*
2 M. SCHNITZER AND S. I. M. SKINNER, *Soil Sci.*, 96 (1963) 86.
3 M. SCHNITZER AND S. I. M. SKINNER, *Proc. 2nd Symposium on the Use of Isotopes and Radiation in Soil Organic Matter Studies*, I.A.E.A., Vienna, 1968, p. 41.
4 S. R. RAFIKOV, S. A. PAVLOVA AND I. I. TVERDOKHLEBOVA, *Determination of Molecular Weights and Polydispersity of High Polymers*, Israel Program for Scientific Translation, Jerusalem, 1964, p. 5.

Analytica Chimica Acta
Elsevier Publishing Company, Amsterdam
Printed in The Netherlands

DOSAGE POLARIMETRIQUE DU MANNITOL ET DU SORBITOL A L'ETAT DE COMPLEXE MOLYBDIQUE

M. HAMON, C. MORIN ET R. BOURDON
Laboratoire de Chimie Analytique, Faculté de Pharmacie de Paris, Paris 6e (France)
(Reçu le 13 mars, 1969)

Le dosage des hexitols, sorbitol et mannitol se heurte, dans les milieux complexes, à quelques difficultés. Ainsi, la présence d'oses, simples ou condensés, ne permet pas l'utilisation de la classique periodimétrie puisque oses et hexitols sont simultanément oxydables. Aussi a t-on envisagé d'appliquer à des fins quantitatives la formation bien connue de complexes de ces hexitols, tant avec l'acide borique qu'avec l'acide molybdique[1-3].

Les complexes molybdiques, contrairement aux polyols libres, présentent un pouvoir rotatoire spécifique élevé, supérieur à celui des dérivés boriques. Nous limiterons notre propos à l'étude des seuls complexes molybdiques. Dans les conditions optimales que nous préciserons, le pouvoir rotatoire spécifique du complexe molybdique du mannitol est voisin de $+145°$; celui du sorbitol, de $+105°$. Cette exaltation du pouvoir rotatoire permet de différencier ces deux hexitols linéaires. Nous avons pu vérifier que le méso-inositol ne conduit pas à un composé optiquement actif dans les conditions de la mesure, de même que certains polyols ne possédant pas de carbone asymétrique, comme le glycérol ou le pentaérythritol. Enfin, fait encore plus intéressant, les oses et leurs polymères (glucose, dextran) conservent leur pouvoir rotatoire spécifique initial en présence d'acide molybdique.

PARTIE EXPÉRIMENTALE

Deux techniques sont décrites en fonction de la sensibilité du polarimètre utilisé et de la composition du milieu.

Technique I adaptée à l'emploi d'un polarimètre optique permettant d'apprécier une minute d'angle

Dans une fiole jaugée de 20 cm³, introduire soit 4 cm³ d'une solution de molybdate de sodium à 40% et 1.2 cm³ de l'acide nitrique pur ($d = 1.33$), soit 5 cm³ d'une solution nitromolybdique préparée à l'avance*. Ajouter 5–6 cm³ d'eau distillée puis une prise d'essai de l'ordre de 300 mg du polyol (ou volume de solution correspondant à ce poids). Compléter à 20 cm³ avec de l'eau distillée et agiter. La lecture polarimétrique ne varie pas dans le temps et peut être faite immédiatement sous une épaisseur de 1 ou 2 dm.

* Cette solution est obtenue en mélangeant: 100 cm³ d'une solution de molybdate de sodium à 40% et 30 cm³ de l'acide nitrique pur ($d = 1.33$). Elle se conserve pendant deux semaines sans aucun dépôt.

Technique II adaptée à l'utilisation d'un polarimètre électronique permettant d'apprécier 0.002° (milieux biologiques)

Préparer une solution contenant 2 g de molybdate de sodium, 1.5 ou 2 cm^3 d'acide nitrique pur ($d=1.33$), 0.25 g d'acide citrique, 0.25 g de nitrate céreux, et compléter avec l'eau distillée à 100 cm^3. Mélanger x cm^3 de la solution précédente et $x/10$ cm^3 d'une solution de polyol de concentration inférieure à 8%. Agiter et lire la déviation polarimétrique sous épaisseur convenable.

DÉTERMINATION DES CONDITIONS OPÉRATOIRES OPTIMALES

Choix de l'acide

L'acide molybdique nécessaire à la complexation est obtenu par acidification d'une solution de molybdate de sodium. Le choix de l'acide utilisé est important; les conditions optimales sont définies par un pH compris entre 1 et 2 (alors que le pK de l'acide molybdique est voisin de 5), il est donc nécessaire de choisir un acide fort. Le désir d'obtenir des solutions aussi peu colorées que possible—même en présence de certains ions métalliques comme fer(III), nous a conduits à préférer l'acide nitrique aux acides chlorhydrique et sulfurique, utilisés par d'autres auteurs[4,5].

Choix du molybdate

Le molybdate de sodium, sel de l'acide molybdique normal H_2MoO_4, doit être préféré au sel d'ammonium[5], beaucoup plus difficile à redissoudre en milieu nitrique. Cette observation est à rapporter au fait que le sel ammoniacal dérive d'un acide paramolybdique $H_6Mo_7O_{24}$.

Rôle du pH

La concentration en protons joue, comme on le sait[4,6], un rôle majeur. KIRSTEN ET NILSSON[4] préconisent l'ajustage à pH 1.2 pour obtenir la déviation polarimétrique maximale. En fait, la détermination du pH n'est guère compatible avec la conduite de dosages en série, moins encore avec les exigences des méthodes automatiques. Aussi, nous sommes-nous attachés à étudier systématiquement la variation du pouvoir rotatoire en fonction du rapport molaire entre l'acide nitrique et le molybdate de sodium.

Paramètres influençant le pouvoir rotatoire spécifique

Le rapport molaire $[HNO_3]/[H_2MoO_4]$. A la lumière des travaux antérieurs, il est nécessaire d'opérer en présence d'un excès d'acide molybdique; aussi, avons-nous défini des conditions opératoires telles que le rapport molaire Na_2MoO_4/polyol soit au moins égal à 3. Nous avons alors fixé la concentration en molybdate de sodium à 75 mM par litre. En ajoutant des quantités croissantes d'acide nitrique, on observe que le pouvoir rotatoire spécifique passe par un maximum pour des valeurs de $R=[HNO_3]/[Na_2MoO_4]$ comprises entre 1.6 et 2.5 selon qu'il s'agit de mannitol ou de sorbitol. Les variations de $[\alpha]$ sont en fait peu sensibles pour des variations de R comprises entre 2 et 4.

La concentration en molybdate de sodium. En élevant la concentration en molybdate de sodium, on observe des évolutions comparables en fonction du rapport R. La valeur maximale de $[\alpha]$ est sensiblement constante, quel que soit l'excès de

TABLEAU I

CONSTANCE DE [α] MAXIMUM

Acide molybdique (mM °/₀₀)	*Mannitol (mM °/₀₀)*	$[\alpha]_D$ (°)	*Acide molybdique (mM °/₀₀)*	*Sorbitol (mM °/₀₀)*	$[\alpha]_D$ (°)
75	15	144 ± 2	75	15	106 ± 2
330	80	143 ± 2	330	80	107 ± 2
410	80	143 ± 2	410	80	105 ± 2
820	80	143 ± 2	820	80	108 ± 2

TABLEAU II

VARIATIONS COMPARÉES DE $[\alpha]_D$ EN FONCTION DE R POUR DIVERSES CONCENTRATIONS EN ACIDE MOLYBDIQUE DANS LE CAS DU SORBITOL ET DU MANNITOL

Polyol	*Acide molybdique (mM °/₀₀)*	$[\alpha]_D$ *pour* $R = 4$	$[\alpha]_D$ *pour* $R = 5$
Sorbitol	75	106.5	93.5
	165	87	70
	330	53	36
	410	48	30
Mannitol	75	144.5	139
	165	135.5	128
	410	109.5	83

molybdate par rapport à la quantité de polyol (Tableau I). La zone en plateau de la courbe $[\alpha]=f(R)$ se rétrécit au fur et à mesure que la concentration en molybdate augmente. La décroissance de $[\alpha]$, en fonction de R, est d'autant plus rapide que la concentration en molybdate est plus élevée (Tableau II). Cette observation doit être rapportée soit à la diminution de l'activité des ions molybdates, soit à leur polymérisation. La pente des courbes $[\alpha]=f(R)$ varie également, selon que l'on s'adresse, à concentration constante en molybdate, à l'un ou l'autre des deux polyols, sorbitol ou mannitol. Après le maximum, $d[\alpha]/dR$ présente toujours une valeur inférieure dans le cas du mannitol. Ici réside le principe d'une différenciation des deux polyols qui fera l'objet d'un travail particulier.

La concentration en molybdate et la valeur du rapport R. Les remarques des paragraphes précédents conduisent à représenter les valeurs de $[\alpha]$ par une surface $[\alpha]=f(R)\ f(H_2MoO_4)$. Plus simplement, nous nous sommes limités à projeter sur le plan $[\alpha]$–R, quelques valeurs remarquables de $[H_2MoO_4]$. Lorsque le rapport R est fixé à l'optimum, la déviation polarimétrique observée ne dépend que de la quantité de polyol tant que le réactif molybdique est en large excès (Fig. 1). Ainsi, sont réalisées les conditions d'un étalonnage linéaire; le réactif nitro-molybdique adéquat peut être préparé à l'avance; la méthode devient alors applicable au dosage en continu qui a été décrit par l'un d'entre nous dans une application biologique récente[7].

Il résulte également des précédentes observations, qu'il faut choisir le réactif le plus dilué compatible avec les conditions de l'expérience (concentration en polyol), car, plus la solution est diluée, moins $[\alpha]$ dépend de R, donc de la concentration en acide. Toutefois, le pouvoir rotatoire mesuré diminue aussi avec la dilution et la limite est également imposée par les possibilités de l'appareillage.

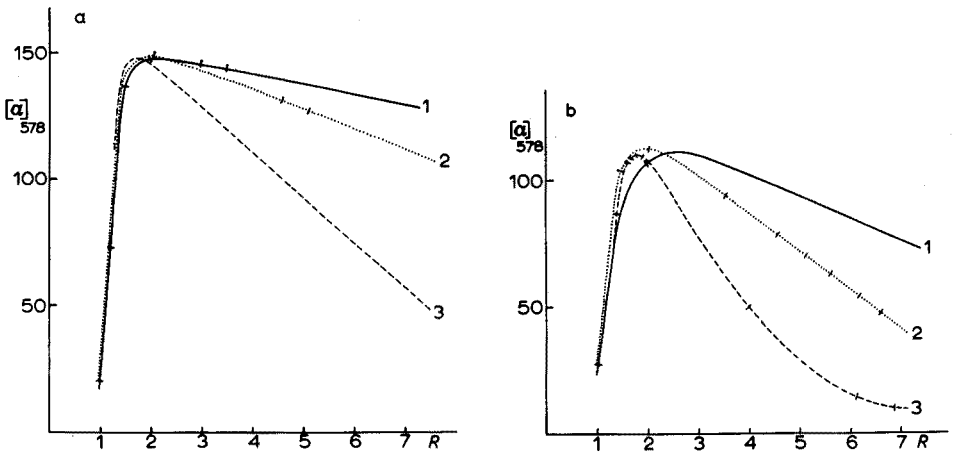


Fig. 1. Variations de $[\alpha]_{578}$ en fonction de R à diverses concentrations molybdiques. (a) Mannitol; (b) sorbitol. (1) concentration du réactif molybdique = 75 mmoles/l; (2) concentration du réactif molybdique = 165 mmoles/l; (3) concentration du réactif molybdique = 410 mmoles/l.

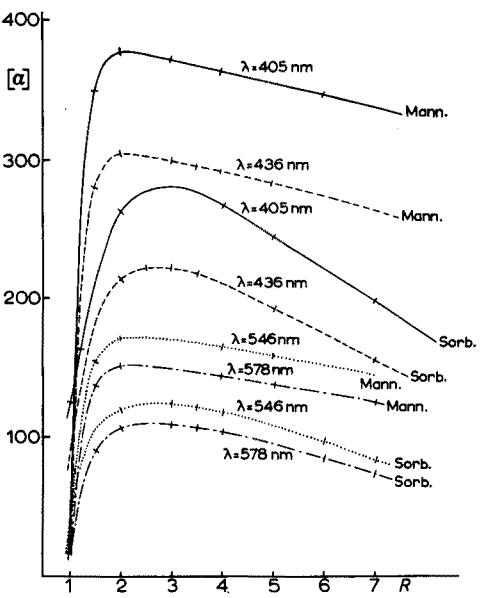


Fig. 2. Variations de $[\alpha]$ en fonction de R à diverses longueurs d'onde.

La longueur d'onde. La Fig. 2 traduit les variations du pouvoir rotatoire aux diverses longueurs d'onde utilisées, en fonction du rapport R et en présence d'une concentration en acide molybdique de 370 mM %. Remarquons que les échantillons commerciaux, principalement dans le cas du mannitol, conduisent à des valeurs de $[\alpha]$ un peu supérieures (Tableau III).

Le Tableau IV traduit pour sa part l'influence de la concentration en molybdate à R constant et égal à 5. Par ailleurs, la courbe $\alpha = f(1/\lambda^2)$ n'est pas une droite.

TABLEAU III

VALEURS DE [α] MAXIMUM À DIVERSES LONGUEURS D'ONDE

λ	[α] *Mannitol (°)*		[α] *Sorbitol (°)*	
	Purissime	*Pur commercial*	*Purissime*	*Pur commercial*
589	136	—	106	—
578	141	152	109	110
546	162	172	124	125
436	294	306	224	224
405	363	381	278	281

TABLEAU IV

INFLUENCE DE LA CONCENTRATION EN ACIDE MOLYBDIQUE À R CONSTANT ET ÉGAL À 5

λ	[α] *Mannitol (°)*		[α] *Sorbitol (°)*	
	Acide molybdique 370 mM °/₀₀	*Acide molybdique 2 M* °/₀₀	*Acide molybdique 370 mM* °/₀₀	*Acide molybdique 2 M* °/₀₀
578	139	72.5	93.5	23
546	159	83	106.5	26
436	286	155	194	52
405	356	196	246	66

En dépit des résultats de HONNELAITRE[8], cette observation permet de penser que la complexation n'est pas simple et que plusieurs complexes sont susceptibles de se former[6,8].

Elimination des perturbations dues aux substances réductrices ou aux complexes aisément réductibles

L'existence de tels systèmes risque de provoquer, même en milieu acide et à froid, le bleuissement lent, mais net, du milieu réactionnel. L'absorption de la lumière incidente diminue considérablement l'énergie transmise au récepteur et rend très imprécise la lecture polarimétrique, quel que soit le type d'appareil.

Dans la plupart des cas, il est possible d'empêcher cette réduction en introduisant une petite quantité de nitrite de sodium[4]. Cette addition doit être extemporanée, aussi avons-nous cherché à remplacer cet anion par un réactif stable en milieu acide.

Addition d'acide citrique. L'acide citrique complexe les dérivés du molybdène-(VI) et cette complexation permet de pallier les inconvénients pouvant résulter de la présence d'acide molybdique excédentaire non complexé par le polyol. Cet excès peut réagir avec divers anions, tels les phosphates, pour donner des composés beaucoup plus réductibles. L'addition d'acide citrique évite la formation de tels complexes; il est alors possible de pratiquer le dosage en présence simultanée de phosphates et de réducteurs.

Toutefois, la stabilité du complexe acide citrique–molybdène(VI) est telle que le polyol et l'acide citrique peuvent entrer en compétition pour complexer l'acide molybdique, entraînant ainsi une diminution de [α] (voir Tableau V).

Par contre, si la quantité d'acide citrique varie proportionnellement à celle

TABLEAU V

VARIATIONS DU POUVOIR ROTATOIRE SPÉCIFIQUE À CONCENTRATION MOLYBDIQUE CONSTANTE EN PRÉSENCE DE QUANTITÉS CROISSANTES D'ACIDE CITRIQUE

(75 mM/‰ d'acide molybdique–15 mM/‰ de mannitol)

Acide citrique (mM ‰)	12	24	36	48	72	96
$[\alpha]_{578}$ (°)	140	128	84	53	2.5	1.5

TABLEAU VI

STABILITÉ DE $[\alpha]$ LORSQUE $[H_2MoO_4]$/[AC. CITRIQUE] EST CONSTANT

Acide citrique (mM ‰)	*Acide molybdique (mM ‰)*	*Mannitol (mM ‰)*	$[\alpha]_{578}$ (°)
12	75	15	139 ± 2
24	151.5	30	137.5 ± 2
35.5	227	45	143 ± 2
47	303	60	141 ± 2

du complexe polyol–acide molybdique, le pouvoir rotatoire ne change pas (Tableau VI).

Addition de nitrate céreux. Dans certains milieux biologiques complexes, peuvent apparaître un léger trouble et une coloration jaune pâle, même en présence d'acide citrique. Nous avons essayé d'ajouter au milieu des sels métalliques de terres rares[9], susceptibles de donner avec l'acide molybdique des complexes suffisamment imparfaits pour ne pas gêner ensuite l'association avec le polyol.

L'addition de nitrate de lanthane ou de nitrate cérique provoque l'apparition d'un volumineux précipité. Par contre, le nitrate céreux nous a permis d'obtenir la clarification et la décoloration totale du milieu.

CONCLUSION

L'étude des divers paramètres de la réaction de complexation entre sorbitol et mannitol d'une part, acide molybdique d'autre part, permet de conclure que le pouvoir rotatoire spécifique du complexe polyol–molybdène conserve une valeur pratiquement constante, sous réserve que:

1. le rapport $\frac{[\text{a. molybdique}]}{[\text{polyol}]}$ soit au moins égal à 3;

2. le rapport $R = \frac{[HNO_3]}{[H_2MoO_4]}$ soit compris entre 2 et 2.5;

3. le pH soit voisin de 1.5.

Ces conditions sont aisément réalisables; le pouvoir rotatoire mesuré est alors directement proportionnel à la concentration en polyol.

RÉSUMÉ

Les auteurs décrivent les fondements d'une méthode polarimétrique de dosage du sorbitol et du mannitol sous forme de complexes molybdiques. Cette méthode est insensible à la présence des oses usuels et peut, de plus, être utilisée pour la mise en évidence du mannitol dans le sorbitol et réciproquement.

SUMMARY

A polarimetric method for the determination of sorbitol and mannitol as their molybdate complexes is described. Sugars which interfere with the periodate method can be tolerated. A detailed study of the optimal concentrations of molybdic acid and nitric acid is described. Citric acid and cerium(III) prevent interferences when biological samples are analysed.

ZUSAMMENFASSUNG

Es wird eine polarimetrische Methode zur Bestimmung von Sorbitol und Mannitol in Form ihrer Molybdat-Komplexe beschrieben. Zucker, die die Perjodat-Methode stören, können toleriert werden. Eine detaillierte Untersuchung der optimalen Konzentrationen der Molybdän- und Salpetersäure wird beschrieben. Zitronensäure und Cer(III) verhindern Störungen, wenn biologische Proben analysiert werden.

BIBLIOGRAPHIE

1 C. TANRET, *Bull. Soc. Chim. France*, 29 (1921) 670.
2 M. FREREJACQUE, *Compt. Rend.*, 200 (1935) 1410.
3 E. PILSKO, *Chem. Zvesti*, 12 (1958) 312.
4 W. J. KIRSTEN ET S. K. NILSSON, *Anal. Chim. Acta*, 27 (1962) 345.
5 A. M. MANZONE ET C. JACOBELLI TURI, *Ann. Chim. (Rome)*, 51 (1961) 220.
6 E. J. BOURNE, D. H. HUTSON ET H. WEIGEL, *J. Chem. Soc.*, 35 (1961).
7 R. BOURDON, A. NICAISE ET J. POLLET, *Ann. Biol. Clin. (Paris)*, sous presse 1969.
8 A. HONNELAITRE, *Ann. Chim. (Paris)*, [10] 3 (1925) 5.
9 A. ROSENHEIM ET H. SCHWER, *Z. Anorg. Chem.*, 89 (1914) 224.

Analytica Chimica Acta
Elsevier Publishing Company, Amsterdam
Printed in The Netherlands

CONSTANTES DE FORMATION DES COMPLEXES IODE–IODURE DANS LES MELANGES EAU–ACETONITRILE ET EAU–ETHANOL. COEFFICIENTS DE SOLVATATION DE L'ANION I_3^-

CHRISTIAN BARRAQUÉ, JACQUES VEDEL ET BERNARD TRÉMILLON
Faculté des Sciences de Paris, Laboratoire de Recherches de Chimie Analytique, E.N.S.C.P., 11, rue Pierre et Marie Curie, Paris, Vième (France)

(Reçu le 21 mars, 1969)

Lors d'une étude précédente[1] nous avons déterminé les "coefficients de solvatation" Γ_{X^-} (ou coefficients d'effet de milieu[2], ou coefficients de partage[3], ou coefficients d'activité dégénérés[4] ...) des anions halogénures et thiocyanate dans l'acétonitrile, l'éthanol, l'acétone, le tétrahydrofurane, le N,N-diméthylformamide et leurs mélanges avec l'eau. Ces coefficients constituent une mesure des différences de potentiel chimique standard des espèces dissoutes dans l'eau pure, d'une part, et dans un solvant différent S, d'autre part, différences auxquelles ils sont reliés par l'expression:

$$(\mu_i^\circ)_S - (\mu_i^\circ)_{eau} = RT \ln \Gamma_i \qquad (1)$$

Ils traduisent ainsi, pour un solvant donné, l'influence de la solvatation sur l'activité a_i de l'espèce i. Lorsque l'espèce dissoute est plus solvatée dans le solvant étudié que dans l'eau, Γ est inférieur à l'unité; dans le cas contraire, Γ est naturellement supérieur à l'unité.

La connaissance de ce coefficient permet de relier l'échelle d'activité d'une espèce chimique i dans un solvant S à l'échelle correspondante en solution aqueuse, c'est-à-dire de se ramener à un même état de référence pour tous les solvants: l'état dans lequel se trouve le soluté lorsqu'il est infiniment dilué dans l'eau. En effet, à l'activité unité dans le solvant S correspond une activité dans l'eau:

$$a_{eau} = \Gamma \qquad (2)$$

Par exemple, l'origine de l'échelle de pH dans un solvant autre que l'eau correspond à une valeur de pH en solution aqueuse égale à $-\log \Gamma_{H^+}$ [5–8].

La connaissance des coefficients de solvatation permet également de prévoir le déplacement des équilibres lorsqu'on change de solvant et, par suite, d'établir des corrélations entre les propriétés chimiques des solutés dans différents milieux. Si l'on considère, par exemple, un équilibre de dissociation du type simple:

$$AB \rightleftharpoons A + B \qquad (3)$$

les valeurs de la constante d'action de masse K_e et K_S de cet équilibre en solution aqueuse et dans S sont reliées entre elles par l'expression:

$$K_e = K_S \frac{\Gamma_A \cdot \Gamma_B}{\Gamma_{AB}} \qquad (4)$$

Nous avons déjà exposé, dans un précédent mémoire[1], de quelle manière il était possible d'accéder expérimentalement à un ordre de grandeur d'un certain nombre de coefficients de solvatation. Pour un soluté moléculaire, des mesures de solubilité sont suffisantes, à la condition que leurs valeurs ne soient pas trop élevées; Γ est alors égal au rapport des solubilités dans l'eau et dans le solvant S. Mais, pour un soluté ionique, il est nécessaire de poser des hypothèses extra-thermodynamiques concernant la valeur relative des coefficients de solvatation de certaines espèces de référence, et dont on ne peut s'assurer de la validité autrement que par des confrontations de résultats. Nous avons principalement décrit la méthode que nous avons exploitée: méthode électrochimique utilisant comme référence de potentiel le couple ferrocène–cation ferricinium[9], et revenant à faire l'hypothèse que ces deux espèces conservent le même coefficient de solvatation quel que soit le solvant dans lequel elles se trouvent[5]. Cette hypothèse est différente de celle exploitée par PARKER *et al.*[10] pour des déterminations similaires, ce qui rend possibles des confrontations intéressantes.

Dans l'étude évoquée au début, nous avons d'abord déterminé les valeurs de Γ_{Ag^+}; puis, en mettant à profit l'insolubilité des sels d'argent et la détermination de leurs produits de solubilité K_s, nous avons atteint les valeurs des coefficients de solvatation des anions Cl^-, Br^-, I^- et SCN^- d'après la relation:

$$\log \Gamma_{X^-} = \log \frac{(K_s)_{eau}}{(K_s)_S} - \log \Gamma_{Ag^+} \qquad (5)$$

Nous nous proposons ici d'exposer les résultats obtenus à la suite de cette première étude, concernant la stabilité des complexes formés entre l'iode et l'iodure dans les mélanges d'acétonitrile ou d'éthanol et d'eau. Nous avons cherché à déterminer les constantes de formation de ces complexes, puis les valeurs du coefficient de solvatation de la molécule d'iode. Ayant déjà obtenu[1] par ailleurs les valeurs de Γ_{I^-}, nous en avons déduit finalement les valeurs du coefficient de solvatation de l'anion complexe I_3^-.

RÉSULTATS EXPÉRIMENTAUX

Mesures de solubilité de l'iode et coefficient de solvatation de I_2

Des cinq familles de mélanges-solvants dans lesquelles ont été déterminés les coefficients de solvatation de l'anion I^-, il n'y en a que deux—eau–acétonitrile et eau–éthanol—où la solubilité de l'iode reste inférieure à 1 mole l^{-1}; elle est très élevée dans l'acétone et dans le tétrahydrofurane; dans le diméthylformamide, des réactions explosives peuvent se produire[12]. Puisque le coefficient de solvatation doit correspondre à des solutions diluées, nous avons donc écarté ces trois derniers solvants.

Les mesures de solubilité d'iode dans chaque milieu utilisé ont été effectuées, par titrage potentiométrique au moyen de thiosulfate, après avoir saturé la solution par dissolution d'iode à la température de 30° suivie d'un refroidissement et d'une décantation lente à la température finale de $25 \pm 0.1°$, qui est celle pour laquelle toutes les mesures sont valables. La valeur de la solubilité de l'iode dans l'eau pure à 25° ($1.3 \cdot 10^{-3}$ *M*) est celle que nous avons utilisée dans un travail antérieur[13]. Le Tableau I rassemble les résultats obtenus. Pour le calcul des coefficients de solvatation, *toutes les concentrations ont été exprimées en fractions molaires, rendant Γ adimensionnel.*

TABLEAU I

SOLUBILITÉ ET COEFFICIENTS DE SOLVATATION DE L'IODE DANS LES MÉLANGES EAU–ÉTHANOL ET EAU–ACÉTONITRILE À 25 ± 0.1°

Solvant organique (% en poids)	*Solubilité de l'iode*		$\log \Gamma_{I_2} = \log \frac{(s)_{eau}}{(s)_s}$
	g/l	*Fraction molaire*	
0	0.33	$2.36 \cdot 10^{-5}$	
Ethanol			
50	4.2	$4.4 \cdot 10^{-4}$	−1.3
70	35	$4.76 \cdot 10^{-3}$	−2.35
90	105	$1.92 \cdot 10^{-2}$	−2.95
100	202	$4.31 \cdot 10^{-2}$	−3.9
Acétonitrile			
40	4.75	$4.6 \cdot 10^{-4}$	−1.3
60	15.2	$1.75 \cdot 10^{-3}$	−1.9
80	36.4	$5.33 \cdot 10^{-3}$	−2.4
95	58	$9.71 \cdot 10^{-3}$	−2.65
100	64.5	$1.15 \cdot 10^{-2}$	−2.7

Constantes de formation des complexes iode–iodure

En solution aqueuse, ont été mis en évidence principalement le complexe I_3^-, mais aussi I_5^-, I_6^{2-}, I_7^- et I_9^- [14,15]. Pour I_3^-, la constante de formation déterminée[16] est:

$$\log K_e = \log \frac{[I_3^-]}{[I^-][I_2]} = 2.87 \pm 0.02 \text{ à } 25° \qquad (6)$$

(concentrations exprimées en mol l^{-1}).

Nous avons cherché à déterminer les valeurs de cette constante dans les mélanges eau–acétonitrile et eau–éthanol, précédemment utilisés. Nous avons opéré en mesurant le potentiel d'équilibre d'une électrode de platine plongeant dans les solutions contenant un mélange d'iode et d'iodure de potassium dans un rapport de concentrations variable. L'interprétation des mesures a été effectuée en exploitant la méthode du logarithme-limite de BENT ET FRENCH[17] et de MOORE ET ANDERSON[18]. D'une part, lorsque l'iodure est en grand excès par rapport à l'iode, le potentiel doit varier selon la relation théorique, en admettant que l'iode est alors sous la forme complexe I_3^-:

$$E = E_0° + 0.029 \log [I_3^-] - 0.088 \log [I^-] \qquad (7)$$

Si l'on ne fait pas varier la concentration d'iode, la variation de E en fonction de $\log [I^-]$ doit suivre une droite de pente −88 mV. D'autre part, lorsque l'iode est au contraire en excès par rapport à l'iodure et en admettant que ce dernier est alors sous la forme I_3^-, le potentiel devrait varier selon la relation théorique:

$$E = E_1° - 0.059 \log [I_3^-] + 0.088 \log [I_2] \qquad (8)$$

A concentration invariable d'iodure, la variation de E en fonction de $\log [I_2]$ devrait suivre une droite de pente 88 mV.

Les valeurs des potentiels normaux $E_1°$ et $E_2°$ conduisent à la valeur de la constante de formation de I_3^-:

$$\log K = \frac{E_1^\circ - E_0^\circ}{0.088} \tag{9}$$

Compte tenu de la correction de dilution, la relation (7) a été vérifiée dans tous les cas étudiés, conduisant aux valeurs de E_0°. En revanche, la relation (8) n'a pu être vérifiée que dans l'acétonitrile pur. Dans ce solvant, nous avons obtenu les valeurs suivantes (intervalles d'incertitude correspondant à une limite de confiance de 95%):

$$E_0^\circ = 0.478 \pm 0.001 \text{ V}$$

$$E_1^\circ = 1.071 \pm 0.002 \text{ V}$$

Par suite:

$$\log K = 6.85 \pm 0.05$$

(concentrations exprimées en mol l^{-1}). Cette valeur diffère de celle déterminée par DESBARRES[19], qui est de 7.4, mais elle est identique à celle donnée récemment par PARKER ET ALEXANDER[11], qui est de 6.8. Nous pensons que la valeur de DESBARRES correspond à un solvant mieux déshydraté, car nous avons constaté que la constante de formation de I_3^- diminue lorsque la teneur en eau de l'acétonitrile augmente.

Dans les autres milieux, nous avons attribué la non-vérification de la relation (8) à la formation simultanée avec le complexe I_3^- d'un autre complexe I_5^-, comme en solution aqueuse; ce complexe est plus riche en iode que I_3^- et n'apparaît donc que lorsque le rapport de concentrations de l'iode à l'iodure est élevé. Il faut alors envisager l'intervention des deux couples oxydo-réducteurs I_2/I_5^- et I_5^-/I_3^-, conduisant aux expressions du potentiel d'équilibre:

$$E = E_2^\circ + 0.029 \log \frac{[I_5^-]^3}{[I_3^-]^5} = E_3^\circ + 0.029 \log \frac{[I_2]^5}{[I_5^-]^2} \tag{10}$$

Le potentiel normal E_1°, nécessaire au calcul de la constante K (relation (9)), est déductible des valeurs de E_2° et E_3°, selon la relation:

$$E_1^\circ = \frac{6\,E_3^\circ + 4\,E_2^\circ}{10} \tag{11}$$

L'exploitation de la courbe expérimentale de variation du potentiel E obtenue en maintenant invariable la concentration totale de l'iode, à la valeur c_1, et en ajoutant progressivement de l'iodure, concentration totale c_2 ($< c_1$), a été effectuée en résolvant mathématiquement par la méthode de LAGRANGE (au moyen d'un calculateur automatique programmable EMD-8-48), l'équation suivante:

$$[I_5^-] + 10^{(E-E_3^\circ)/0.145} [I_5^-]^{2/5} = c_1 - c_2 \tag{12}$$

qui résulte de la combinaison des relations (10) et de:

$$c_1 = [I_2] + 2[I_5^-] + [I_3^-] \tag{13}$$

$$c_2 = [I_5^-] + [I_3^-] \tag{14}$$

De la résolution de (12), on tire $[I_5^-]$ et E_3°, puis $[I_3^-]$ et E_2°.

Les résultats de l'ensemble de ces déterminations sont rassemblés dans le Tableau II.

TABLEAU II

CONSTANTES DE FORMATION DES COMPLEXES I_3^- ET I_5^- DANS LES MÉLANGES EAU–ACÉTONITRILE ET EAU–ÉTHANOL À 25° ± 0.1°

Solvant organique (% en poids)	$\log K = \log \frac{[I_3^-]}{[I_2]\,[I^-]}$ (± 0.05) (*concentrations exprimées en mol* l^{-1})	$\log \beta_2 = \frac{[I_5^-]}{[I_2]^2[I^-]}$ (± 0.3)
0	2.87 ± 0.02[15]	
Ethanol		
50	3.68	2.4
70	3.77	1.8
90	4.05	1.7
100	4.13	1.8
Acétonitrile		
40	4.26	1.7
60	5.01	1.7
80	5.16	1.55
95	6.14	2.1
100	6.85	—

CONCLUSION

Après transposition des valeurs des constantes ci-dessus ($\log K$) dans l'échelle des fractions molaires, et en utilisant les valeurs précédemment déterminées des coefficients de solvatation de l'iode et de l'anion iodure, nous pouvons calculer les valeurs du coefficient de solvatation de l'anion complexe I_3^-:

$$\log \Gamma_{I_3^-} = (\log K)_S - (\log K)_{eau} - \log \Gamma_{I_2} - \log \Gamma_{I^-} \qquad (15)$$

Ces valeurs sont rassemblées dans le Tableau III.

TABLEAU III

COEFFICIENTS DE SOLVATATION DE I_2, I^- ET I_3^- DANS LES MÉLANGES EAU–ACÉTONITRILE ET EAU–ÉTHANOL À 25° (DANS L'ÉCHELLE DES FRACTIONS MOLAIRES)

Solvant organique (% en poids)	$\log \Gamma_{I_2}$	$\log \Gamma_{I^-}$ *(d'après réf. 1)*	$\log \Gamma_{I_3^-}$
Ethanol			
50	−1.3	2.4	1.75
70	−2.35	2.9	1.2
90	−2.95	3.6	1.4
100	−3.9	4.3	1.15
Acétonitrile			
40	−1.3	1.2	1.15
60	−1.9	1.9	1.9
80	−2.4	2.95	2.55
95	−2.65	3.9	4.1
100	−2.7	4.2	5.0

Un examen de ce Tableau conduit aux quelques conclusions suivantes.

En premier lieu, il apparaît que la solvatation de l'anion complexe I_3^-—tout comme celle de l'anion simple I^-, mais au contraire de celle de la molécule d'iode I_2—se trouve très nettement diminuée par l'adjonction du solvant organique, surtout de l'acétonitrile. I_3^- obéit donc à la règle générale de solvatation des anions minéraux, comme les halogénures et SCN^-, qui ont une préférence marquée pour l'eau. Toutefois, lors de l'addition à l'eau d'éthanol, l'affinité de ce solvant organique pour l'iode, manifestée par les très petites valeurs de Γ_{I_2} provoque tout de même après une diminution notable un accroissement de la solvatation de I_3^-; celle-ci doit ainsi passer par un minimum pour un mélange eau–éthanol de composition intermédiaire.

Ces constatations viennent infirmer l'hypothèse de PARKER ET ALEXANDER[11] —selon laquelle une espèce moléculaire volumineuse aurait le même coefficient de solvatation qu'un gros anion similaire—dans le cas du couple iode–I_3^-, rendant erronés les résultats de détermination de coefficients de solvatation sur la base de cette hypothèse. C'est plutôt le couple I_3^-–I^- qui pourrait être considéré comme référence dans les mélanges eau–acétonitrile, où les coefficients de solvatation de ces deux anions semblent rester du même ordre du même ordre de grandeur.

Il faut enfin souligner que les variations observées pour la stabilité du complexe I_3^- ne peuvent être imputées aux variations de constante diélectrique, pratiquement sans influence sur l'équilibre iso-ionique $I_3^- \rightleftharpoons I_2 + I^-$, mais doivent nécessairement l'être à des modifications de solvatation des constituants.

RÉSUMÉ

Les constantes de formation des complexes I_3^- et I_5^- dans les mélanges eau–éthanol et eau–acétonitrile ont été déterminées potentiométriquement. Les mesures de solubilité de l'iode dans ces différents mélanges-solvants ont conduit aux valeurs du coefficient de solvatation (ou coefficient d'effet de milieu) de ce soluté. En utilisant ensuite les valeurs du coefficient de solvatation de l'anion iodure, obtenues lors d'une étude précédente (*Bull. Soc. Chim. France*, (1968) 3421), les valeurs du coefficient de solvatation de l'anion complexe I_3^- ont pu être déduites. Les résultats ont infirmé l'hypothèse selon laquelle la molécule d'iode et l'anion I_3^- posséderaient les mêmes coefficients de solvatation.

SUMMARY

Formation constants of I_3^- and I_5^- complexes have been determined in water–ethanol and water–acetonitrile mixtures by potentiometry. Solvation coefficients (or medium effect coefficients) of this species were evaluated by solubility measurements of iodine in the different solvent mixtures. The solvation coefficient values of the complex anion I_3^- were then deduced by means of formerly determined values of solvation coefficients of the iodide anion (*Bull. Soc. Chim. France*, (1968) 3421). The results were not in agreement with the hypothesis that the iodine molecule and I_3^- anion have the same solvation coefficients.

ZUSAMMENFASSUNG

Die Bildungskonstanten von J_3^-- und J_5^--Komplexen wurden in Wasser-

Äthanol- und Wasser-Acetonitril-Mischungen mit Hilfe der Potentiometrie bestimmt. Die Solvatationskoeffizienten dieser Spezies wurden durch Löslichkeitsmessungen von Jod in den verschiedenen Lösungsmischungen ermittelt. Die Werte des Solvatationskoeffizienten des komplexen Anions I_3^- wurden dann mittels früher bestimmter Werte für das Jodid-Anion abgeleitet. Die Ergebnisse stehen nicht in Übereinstimmung mit der Hypothese, dass das Jodmolekül und das Iod_3^--Anion dieselben Solvatationskoeffizienten besitzen.

BIBLIOGRAPHIE

1 C. BARRAQUÉ, J. VEDEL ET B. TRÉMILLON, *Bull. Soc. Chim. France*, (1968) 3421.
2 R. G. BATES, *Determination of pH*, Wiley, New York, 1964.
3 I. M. KOLTHOFF, J. J. LINGANE ET W. D. LARSON, *J. Am. Chem. Soc.*, 60 (1938) 2512.
4 E. GRUNWALD ET J. BERKOWITZ, *J. Am. Chem. Soc.*, 73 (1951) 4939.
5 J. VEDEL, *Ann. Chim. (Paris)*, (1967) 335.
6 S. GUIOT ET B. TRÉMILLON, *J. Electroanal. Chem.*, 18 (1968) 261.
7 C. MADIC ET B. TRÉMILLON, *Bull. Soc. Chim. France*, (1968) 1634.
8 A. E. PUCCI, J. VEDEL ET B. TRÉMILLON, *J Electroanal. Chem.*, à paraître.
9 H. M. KOEPP, H. WENDT ET H. STREHLOW, *Z. Elektrochem.*, 64 (1960) 483; H. STREHLOW, dans J. J. LAGOWSKI, *The Chemistry of Non-Aqueous Solvents*, Vol. 1, Academic Press, New York, 1966, p. 129.
10 R. ALEXANDER, E. C. F. KO, Y. C. MAC ET A. J. PARKER, *J. Am. Chem. Soc.*, 89 (1967) 3705; R. ALEXANDER ET A. J. PARKER, *J. Am. Chem. Soc.*, 89 (1967) 5549.
11 A. J. PARKER ET R. ALEXANDER, *J. Am. Chem. Soc.*, 90 (1968) 3313.
12 C. SINICKI ET M. BREANT, *Bull. Soc. Chim. France*, (1967) 3080.
13 C. BARRAQUÉ ET B. TRÉMILLON, *Bull. Soc. Chim. France*, (1965) 1674.
14 H. WELLS ET M. WHEELER, *Am. J. Sci.*, 43 (1892) 475.
15 L. ABBEGG ET K. HAMBURGER, *Z. Anorg. Chem.*, 50 (1906) 403.
16 H. D. MURRAY, *J. Chem. Soc.*, 127 (1925) 882.
17 H. E. BENT ET C. E. FRENCH, *J. Am. Chem. Soc.*, 63 (1941) 568.
18 R. L. MOORE ET R. C. ANDERSON, *J. Am. Chem. Soc.*, 67 (1945) 167.
19 J. DESBARRES, *Bull. Soc. Chim. France*, (1961) 502.

Analytica Chimica Acta
Elsevier Publishing Company, Amsterdam
Printed in The Netherlands

DUAL-WAVELENGTH SPECTROPHOTOMETRY

PART I. GENERAL METHOD

SHOZO SHIBATA, MASAMICHI FURUKAWA AND KAZUO GOTO
Government Industrial Research Institute, Nagoya (Japan)

(Received January 27th, 1969)

In 1951 Chance[1] proposed a new technique "dual-wavelength spectrophotometry" for measurements of turbid samples in biochemical analysis, and several valuable methods based on this technique have been reported[2-4]. In this method, two light beams of different wavelength from two gratings are passed through a sample solution and the difference between the absorbances, ΔA, at wavelengths λ_1 and λ_2 is measured (Fig. 1).

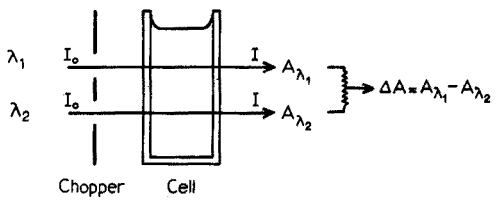


Fig. 1. Schematic representation of the method $A = \log I_0/I$.

In the classical method (single wavelength method), it is usual to measure the absorbance of the sample solution, in which the colour has been developed, against water or a blank solution in a reference cell set to read zero. In the dual-wavelength method, only one cell is used, hence many errors which are caused by the cells, *e.g.* cell position, cell constant, and by differences between sample and reference solution, such as turbidity and concentration, can be completely eliminated. Accordingly, much better precision and sensitivity can be attained by the use of this technique, which does not seem to have been used previously for chemical analysis.

In the present paper, the possibilities of the dual-wavelength method for the determination of inorganic ions by spectrophotometric analysis are discussed fundamentally. The optical diagrams of the dual-wavelength method are shown in Fig. 2. A detailed description of the instruments with a discussion of their use, mainly for turbid preparations, is available in the literature[5-8]. Instruments of this type can be used easily both for the classical "split-beam method" and for the "dual-wavelength method". The schematic diagrams are shown in Fig. 3; mode A is useful for the determination of the absorption spectra of turbid samples.

Quite generally, the special merits of dual-wavelength spectrophotometry are as follows.

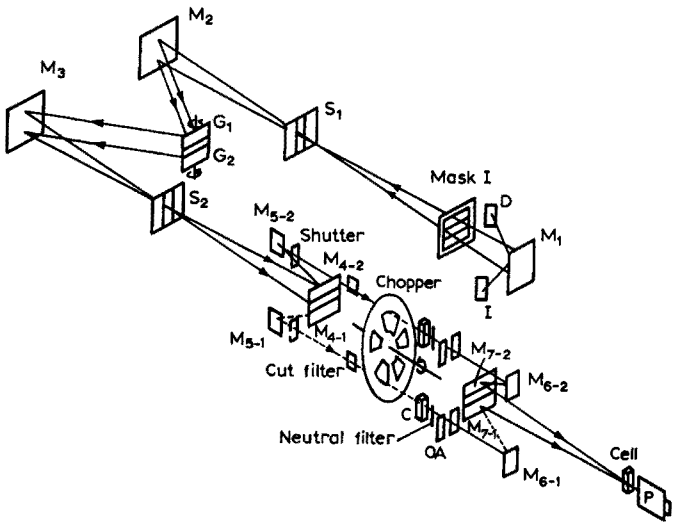


Fig. 2. Optical diagram. (G_1, G_2) Grating; (M_2) collimating mirror; (M_3) camera mirror; (M_1–M_6 concave mirror; (S_1, S_2) slit; (D) deuterium discharge lamp; (I) tungsten–iodide lamp; (P) photomultiplier; (OA) optical attenuator; (C) sub cell compartment.

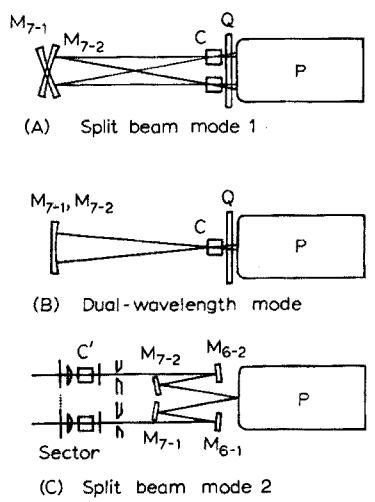


Fig. 3. Illustration of conversion of the instrument from "split beam" to "dual wavelength". (P) photomultiplier; (Q) quartz diffuser plate; (C) cell; (C') sub cell compartment.

1. Extremely small variations in absorbance can be detected in a sample having comparatively high absorbance and scattering characteristics. This small variation can be detected only by taking the balance between the absorbance values at two different wavelength λ_1 and λ_2.

2. It will enable colorimetry, without reference, with a sample whose absorbance is very low. Usually the full-scale absorbance is 0.00–0.010 for the smallest measurement range.

3. Derivative spectra can be produced by means of wavelength scanning with

λ_1 and λ_2 values set closely to one another, so that many materials can be determined.

4. A special relative spectrum can be obtained by means of wavelength scanning against a fixed λ_1.

5. Simultaneous reactions in a sample can be recorded with two pen recorders at λ_1 and λ_2.

EXPERIMENTAL

Reagents

Arsenazo III solution. Dissolve 100 mg of Dotite arsenazo III (2,7-di-(*o*-arsonophenylazo)-1,8-dihydroxynaphthalene-3,6-disulfonic acid, disodium salt) in 100 ml of water.

Carboxyarsenazo solution. Dissolve 100 mg of Dotite carboxyarsenazo (2-(2-carboxyphenylazo)-7-(2-arsonophenylazo)-1,8-dihydroxynaphthalene-3,6-disulfonic acid, disodium salt) in 100 ml of water.

Oxine solution. Dissolve 1 g of pure oxine in 5 ml of glacial acetic acid and dilute to 100 ml with water.

Dysprosium and thorium standard solutions. These solutions were prepared from the pure metal oxide (99.99%; Johnson-Matthey, London).

Nickel and cobalt standard solutions. These solutions were prepared from high-purity metals as the perchlorates.

Organic solvents were purified by usual methods and other reagents were prepared from the analytical-grade or purified materials.

All solutions were prepared with redistilled water.

Apparatus

All measurements were made with a Model 356 Hitachi dual-wavelength/double-beam recording spectrophotometer with 1-cm cells. The optical lay-out is shown in Fig. 2.

A Yanagimoto 42A type pH meter was used.

RESULTS AND DISCUSSION

Linearity of calibration curve (adherence to Beer's law)

Generally, the choice of the combination of two wavelengths is unlimited. In the present paper, in order to determine the two wavelengths, three basic methods were found suitable for the determination of a colour system in a transparent solution by the dual-wavelength method.

Methods for the selection of the wavelengths λ_1 and λ_2 for concentrated or turbid samples will be discussed in subsequent papers.

In order to determine the optimal wavelengths for the measurement of the absorbances at λ_1 and λ_2 in the dual-wavelength method, three basic methods were found suitable.

Method I. $A_{\lambda 1}$ (isosbestic point) – $A_{\lambda 2}$ (absorbance maximum of the complex). This method is the most fundamental; as an example, the determination of dysprosium with carboxyarsenazo[9-11] is shown in Figs. 4 and 5. Generally, in the colour reaction of suitable chromogenic reagents with metal ions, it is possible to obtain one or more

isosbestic point. It should be noted that if the isosbestic point cannot be determined accurately, measurements at full scale, $A = 0.01$, are not possible because the error caused by the uncorrected wavelength for the isosbestic point may be significant for such a small absorbance. In the case of the determination of dysprosium with carboxyarsenazo an isosbestic point can be determined accurately (Fig. 4). Therefore, even if the measurements are made at full scale, $A = 0.01$, the calibration graph is linear and p.p.b. amounts of dysprosium can be determined directly. Recently, many sensitive chromogenic reagents or colour systems for metals have been reported, and the dual-wavelength method can be very useful for the determination of trace amounts of metals in conjunction with these colour reactions. Carboxyarsenazo is not selective for dysprosium but it provides an extremely sensitive method, which is useful to illustrate the procedure.

Method II. $A_{\lambda 1}$ *(absorbance maximum of the complex)* $- A_{\lambda 2}$ *(a wavelength on a foot of the absorbance spectrum).* If no isosbestic point is available for a particular

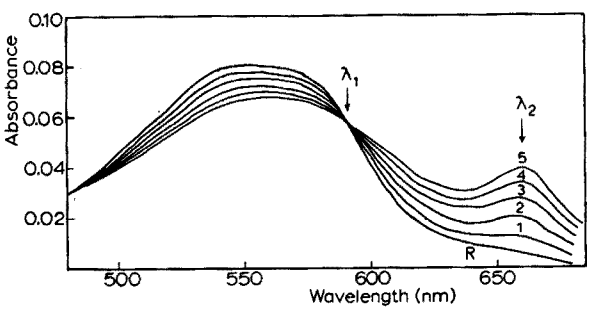


Fig. 4. Isosbestic point of the dysprosium–carboxyarsenazo system (1) 1 μg, (2) 2 μg, (3) 3 μg, (4) 4 μg and (5) 5 μg dysprosium, respectively. Carboxyarsenazo, $6.5 \cdot 10^{-5} M$ (R). pH 4.0 with acetic acid–sodium acetate buffer; aqueous 25% acetone solution; total volume 25 ml; measured against water.
$\lambda_1 = 590$ nm ($\lambda_1 = 467$ nm), $\lambda_2 = 660$ nm.

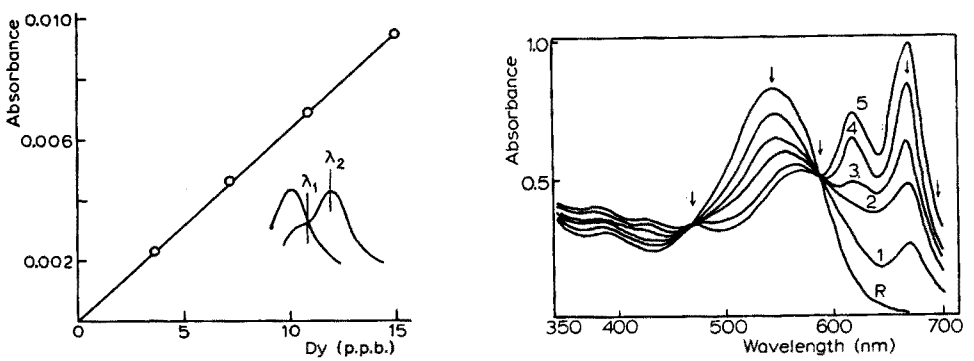


Fig. 5. Calibration graph for the dysprosium–carboxyarsenazo system. Absorbance measured at 590 and 660 nm. Conditions as for Fig. 4.

Fig. 6. Absorbance spectra of the thorium–arsenazo III system. $\lambda_1 = \lambda_2$, 8N hydrochloric acid solution. Thorium: (1) 0.4 p.p.m., (2) 1.0 p.p.m., (3) 1.3 p.p.m., (4) 1.7 p.p.m., (5) 2.2 p.p.m., respectively.

colour system, or if it cannot be measured accurately, the other wavelength of illumination to be used can usually be chosen on a foot of the absorbance curve of the coloured complex. Typical results for this method are shown in Figs. 6 and 7. The absorbance spectra of the thorium–arsenazo III system[12,13] are shown in Fig. 6, and the calibration graph is shown in Fig. 7.

Method III. $A_{\lambda 1}$ (absorbance maximum of the reagent) – $A_{\lambda 2}$ (absorbance maximum of the complex). In this case, the measured absorbance will be the sum of the absorbances caused by the decrease in the absorbance of the reagent and by the increase in the absorbance of the complex; thus, the apparent molar absorptivity of the coloured complex can be increased appreciably. Typical results for this method are shown in Fig. 8.

Instrumental masking of interferences

A metal in the presence of other diverse ions can readily be determined by the dual-wavelength method. In this case, the absorbance maximum of the metal to be determined is taken as λ_1 and it may be desirable to make the second absorbance

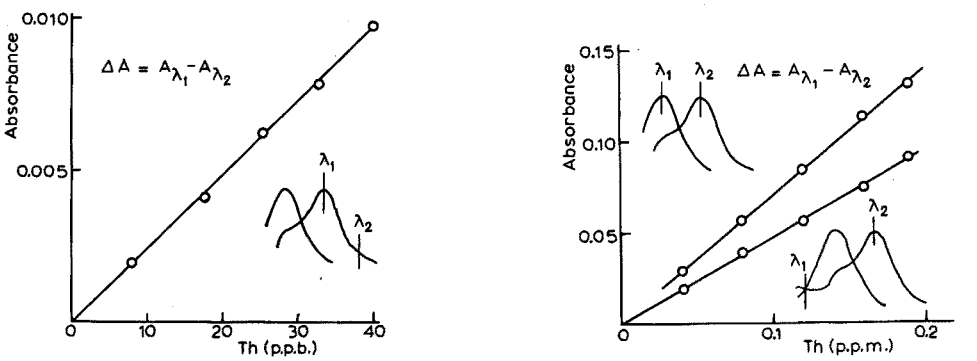


Fig. 7. Calibration graph for the thorium–arsenazo III system by method II. Absorbance measured at 667 and 682 nm.

Fig. 8. Calibration graphs for the thorium–arsenazo III system by method III. (1) Absorbance measured at 540 and 667 nm; (2) absorbance measured at 467 and 667 nm.

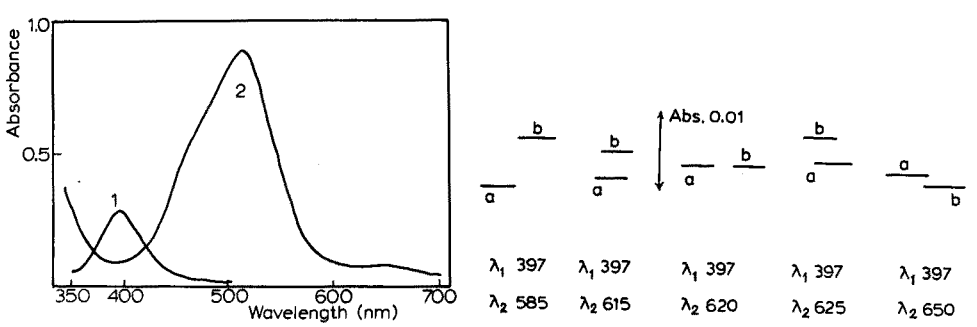


Fig. 9. Absorbance curves of nickel and cobalt(II) in 2 *N* perchloric acid solution. (1) Nickel. 0.05 *M*, (2) cobalt, 0.17 *M*. $\lambda_1 = \lambda_2$; scanning speed, medium; chart speed 60 mm/min.

Fig. 10. Determination of λ_1 and λ_2 for the cobalt(II)–nickel(II) system.

measurement at some wavelength on the absorbance curve of the diverse metal. If the ratio of the absorbances at wavelengths λ_1 and λ_2 is known, the absorbance errors caused by changes in the concentration of the diverse metal will be eliminated electrically. If this ratio is not available, the most suitable λ_1 or λ_2 can be determined experimentally by using artificial mixed solutions containing both metals and solutions of the diverse ion. The amounts of diverse ion that can be tolerated may depend on the selection of λ_1 and λ_2.

Determination of nickel in the presence of cobalt

When nickel is to be determined, λ_1 is set to the absorbance maximum for the nickel ion, 397 nm (Fig. 9) and λ_2 is changed several times until a constant level is obtained when solutions containing suitable amounts of cobalt are used. The preliminary testing for the determination of nickel in the presence of cobalt as perchlorate is shown in Fig. 10.

From these results, the most suitable combination of λ_1 and λ_2 is seen to be 397 and 620 nm; with this combination, ΔA for cobalt is zero or negligible, so that the interference of cobalt can be eliminated completely.

Procedure. To a slightly acidic solution containing 5 mg of nickel and various amounts of cobalt, add 5 ml of 70% perchloric acid, and dilute to 25 ml with water. Measure the absorbance at 397 *vs.* 620 nm in a 1-cm cell. Some results are shown in Table I.

TABLE I

DETERMINATION OF NICKEL IN THE PRESENCE OF COBALT

(A_{397}–A_{620})

Ni taken (mg)	*Co added (mg)*	*Ni found (mg)*	*Error (mg)*
5.8	20	5.8	±0.0
5.8	40	5.9	+0.1
5.8	85	6.0	+0.2
5.8	100	6.1	+0.3
5.8	125	5.9	+0.1
5.8	150	6.0	+0.2
5.8	170	6.2	+0.4

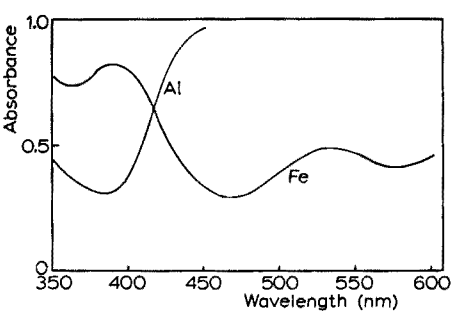


Fig. 11. Absorption spectra of aluminum(III) and iron(III) oxinate in chloroform. Iron 5 p.p.m., aluminum 2 p.p.m., against chloroform. $\lambda_1 = \lambda_2$.

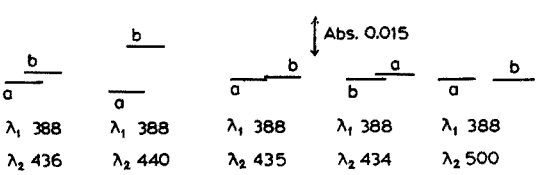


Fig. 12. Determination of λ_1 and λ_2 for the aluminum(III)–iron(III) system.

Determination of aluminum in the presence of iron with oxine–chloroform extraction

Another example is shown by the determination of aluminum[14] in the presence of iron[15]. The absorbance curves of aluminum and iron(III) oxinate in chloroform are shown in Fig. 11.

The preliminary testing for the determination of λ_1 and λ_2 is shown in Fig. 12.

Procedure. Buffer the mixed aluminum and iron solution to a pH of 5 and add 3 ml of 1% (w/v) 8-hydroxyquinoline solution in 2 *N* acetic acid. Mix well and dilute to about 50 ml with water in a 100-ml separatory funnel. Add 10 ml of chloroform and shake for 3 min. Run the chloroform extract into a small stoppered flask containing 1 g of anhydrous sodium sulfate and shake to remove droplets of water. Measure the absorbance at 388 *vs.* 500 nm in a 1-cm cell. Prepare the standard curve by taking appropriate amounts of aluminum and applying the above procedure.

A typical calibration curve is shown in Fig. 13, and some results are given in Table II.

TABLE II

DETERMINATION OF ALUMINUM IN THE PRESENCE OF IRON

(A_{388}–A_{500})

Al taken (μg)	*Fe added* (μg)	*Al found* (μg)	*Error* (μg)
5.0	—	5.0	±0.0
5.0	95.2	5.1	±0.1
10.0	—	10.0	±0.0
10.0	25.0	9.9	−0.1
15.0	—	15.2	+0.2
15.0	72.0	15.1	+0.1
20.0	—	20.1	+0.1
20.0	48.0	20.3	+0.3

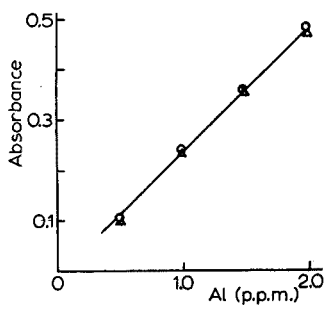


Fig. 13. Calibration graph for the aluminum–oxinate system. (○) With standard aluminum solution; (△) in the presence of large amounts of iron. Absorbance measured at 388 and 500 nm.

Derivative absorbance spectrum

If λ_1 and λ_2 are set to very close wavelengths, it is possible to obtain a differential absorption curve and from these results a very critical shoulder of the spectrum can be determined; this method may be useful for the detection and determination of

some component in the presence of others. For example, the derivative absorbance spectrum of the neodymium glass filter is shown in Fig. 14 and the normal absorbance spectrum is shown in Fig. 15. The possibilities of this derivative method will be discussed in a subsequent paper.

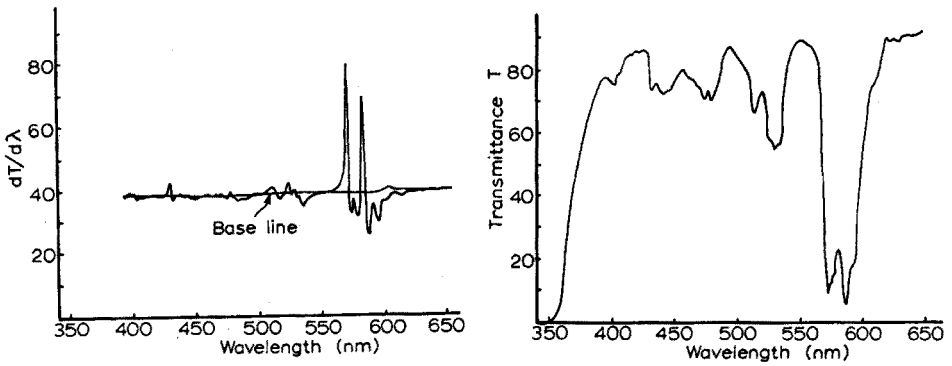


Fig. 14. Derivative transmission spectrum of neodymium glass filter: $\lambda_1 - \lambda_2 = 1$ nm.

Fig. 15. Transmission spectrum of neodymium glass filter: $\lambda_1 = \lambda_2$.

CONCLUSION

The dual-wavelength method can be seen from the above results to be suitable as a colorimetric method for inorganic analysis. The method is useful in trace analyses, as well as for the direct determination of a metal ion in the presence of other diverse ions.

Many other useful applications may be expected; for example, ions could be determined in the presence of a precipitate without separation, highly concentrated samples could be analysed, two components could be determined simultaneously with two recorders, etc. Such further studies with mathematical treatments for precision and error, are now in progress.

SUMMARY

The application of dual-wavelength spectrophotometry in inorganic chemical analysis is discussed. The method has been successfully applied to the determination of trace amounts of metals and the determination of metals in the presence of diverse ions without separation. Three basic methods for the choice of the two wavelengths to be used for a particular system are proposed.

RÉSUMÉ

On examine l'application de la spectrophotométrie à double longueur d'onde pour l'analyse chimique inorganique. Cette méthode a pu être utilisée avec succès pour le dosage de traces de métaux, ainsi que pour le dosage de métaux en présence de divers ions, sans séparation. On propose trois méthodes fondamentales pour le choix des deux longueurs d'onde, pour un système particulier.

ZUSAMMENFASSUNG

Die Anwendung der Spektralphotometrie unter Verwendung zweier Wellenlängen in der anorganisch-chemischen Analyse wird diskutiert. Die Methode wurde erfolgreich angewendet zur Bestimmung von Spuren von Metallen und zur Bestimmung von Metallen in Gegenwart zahlreicher Ionen ohne vorhergehende Trennung. Drei grundsätzliche Methoden zur Auswahl der 2 Wellenlängen, welche für ein besonderes System benötigt werden, werden vorgeschlagen.

REFERENCES

1 B. CHANCE, *Rev. Sci. Instr.*, 22 (1951) 634; *Science*, 120 (1954) 767.
2 B. CHANCE AND G. WILLIAMS, *Advan. Enzymol.*, 17 (1956) 65.
3 B. CHANCE AND L. MELA, *J. Biol. Chem.*, 241 (1966) 4588; *Proc. Natl. Acad. Sci. U.S.*, 55 (1966) 1243.
4 T. ONISHI AND S. EBASHI, *J. Biochem. (Tokyo)*, 54 (1963) 506; 55 (1964) 599.
5 R. RIKMENSPOEL, *Appl. Opt.*, 3 (1964) 351; *Rev. Sci. Instr.*, 36 (1965) 497.
6 T. ONISHI, B. HAGIHARA AND K. OKUNUKI, *J. Biochem. (Tokyo)*, 54 (1963) 287.
7 T. ONISHI, *J. Biochem. (Tokyo)*, 55 (1964) 172.
8 J. C. COWLES, *J. Opt. Soc. Am.*, 55 (1965) 690.
9 S. B. SAVVIN AND YU M. DEDKOV, *Zh. Analit. Khim.*, 19 (1964) 21.
10 T. HIRAI AND S. SHIBATA, *Nagoya Kogyo Gijutsu Shikensho Hokoku*, 15 (1966) 122.
11 O. KAMMORI AND I. TAGUCHI, *Dotite News Letter*, 14 (1966) 2 (Dozin Chem., Kumamoto, Japan).
12 S. B. SAVVIN, *Talanta*, 8 (1961) 673; *Arsenazo III*, Atomizdat, Moscow, 1966.
13 I. KUZNETSOV AND S. B. SAVVIN, *Radiokhimiya*, 3 (1961) 79.
14 A. CLAASSEN, L. BASTINGS AND J. VISSER, *Anal. Chim. Acta*, 10 (1954) 373.
15 K. MOTOJIMA, *Nippon Kagaku Zasshi*, 76 (1955) 903.

Analytica Chimica Acta
Elsevier Publishing Company, Amsterdam
Printed in The Netherlands

REVIEW

ACTIVATION ANALYSIS OF PAPER CHROMATOGRAMS

A. Z. BUDZYŃSKI AND J. Z. BEER
Department of Radiobiology and Health Protection, Institute of Nuclear Research, Warsaw 91 (Poland)

(Received November 7th, 1968)

Activation analysis is based on the measurement of radioactivity induced in analysed samples or emitted in the course of a nuclear reaction, after bombardment with elementary particles, ions or photons. The extreme sensitivity of this technique greatly exceeds those of most other analytical methods and this is one of the principal reasons for the numerous applications of activation analysis in various branches of science and technology. Most of the activation methods are specifically designed as auxiliary tools in geochemistry, cosmochemistry, metallurgy and biochemistry.

The principles of activation analysis are relatively simple but its potentialities closely depend on various technical refinements and on combinations with other analytical methods, in particular with separation techniques. A multitude of analytical methods is based on various combinations of activation analysis and chromatographic or electrophoretic procedures, the separating potentialities of which have been widely exploited for rapid and efficient processing of activated samples. However, with one of the common supporting materials, it becomes possible also to combine the two techniques in the reverse order, *i.e.* direct activation of chromatographically or electrophoretically separated samples. Such exceptional possibilities are connected with the application of cellulose, especially in the form of chromatographic paper, which contains only trace amounts of elements other than carbon, hydrogen and oxygen. These three elements, under the commonly applied activation conditions, are not easily transformed into radioisotopes which might interfere with the radiometric determination of other elements located in bands or spots.

Activation of paper chromatograms was first introduced in 1952 by WINTERINGHAM *et al.*[1] for the detection of chlorine and bromine in metabolic products from insects treated with specific toxic compounds containing these elements. Shortly afterwards, SCHMEISER AND JERCHEL[2,3] and SCHMEISER[4] activated chlorine, bromine, phosphorus and sulphur on paper chromatograms with fast neutrons. Subsequently, this analytical method became adopted by many laboratories working on divergent problems, the solution of which would have been very difficult, or even impossible, by other means.

When substances are activated after their separation on paper, the analytical methods developed are largely based on operations that do not involve radioactivity. This makes it possible to avoid difficulties caused by the fact that treatment of the mixture to be analysed before separation, even with small doses of elementary particles or photons, can alter the chromatographic or electrophoretic properties of various substances[5].

In biochemistry, activation analysis of paper chromatograms offers the important possibility of obtaining radioactive paper chromatograms and electrophoretograms when the mixture studied cannot be conveniently labelled *in vivo*. This possibility is also of importance for forensic applications of activation analysis.

With the simple procedures of activation analysis of paper chromatograms, many problems connected with the radiochemical isolation of the analysed compound from the activated sample can be avoided, and the risk of contamination in the laboratory can be reduced. The radioactivity defines the distribution of the spots and thus allows identification of the components of the mixture analysed as well as their quantitative determinations.

Basic literature on activation analysis

Detailed descriptions of the rapidly developing technique of activation analysis are beyond the scope of this review. Theoretical principles and technical details have been fully discussed in many specialized monographs by KOCH[6], SCHULZE[7], ALBERT[8], ATKINS AND SMALES[9], BOWEN AND GIBBONS[10], RAKOVIČ[11], LYON[12], and TAYLOR[13]. Useful references to publications containing nomograms and tabulated data which can aid the calculations necessary for the choice of appropriate activation conditions and for interpretation of the results of the analyses can be found in a review article by GIRARDI[14], and in the tables of neutron activation constants compiled by BAUMGÄRTNER[15]. A recent review article by COLEMAN AND PIERCE[16], concentrates on work published during 1963–66 and includes a brief theoretical outline of the subject. BOWEN[17] has recently reviewed the main problems of activation analysis.

Applications of activation analysis for the evaluation of paper chromatograms have been previously reviewed by RAKOVIČ[18].

IRRADIATION FACILITIES

Most of the analytical procedures based on activation use neutrons for the activation of the sample. The reactions employed are usually of the (n, γ) type and are induced by thermal neutron capture. Thermal neutrons with energies of about 0.025 eV at 15° are most effective. As the neutron energy increases, the capture cross-section for the (n, γ) reaction decreases as the inverse of the neutron velocity. However, a very effective activation may often occur over a small energy range, the so-called resonance peak.

Nuclear reactors are the main source of neutrons for activation analysis, and a considerable number of graphite reactors are in use throughout the world. These reactors produce thermal neutron fluxes accompanied by relatively small amounts of fast neutrons and γ-radiation, and are, therefore, particularly suitable for activation analysis of paper chromatograms. However, swimming-pool reactors with power of 100 keV up to several megawatts are now commonest. These reactors can produce fluxes of 10^{12}–10^{14} n cm^{-2} sec^{-1}, the neutrons possessing widely varying energies from thermal neutrons of *ca.* 0.02-eV energy up to fast neutrons of more than 10-MeV energy. Higher fluxes can be obtained for periods of a few milliseconds in pulsed reactors. The production of thermal neutrons in a nuclear reactor depends on slowing down by collision mainly in light or heavy water or graphite. Usually, no special

effort is made to select neutrons of a defined energy for irradiation of samples. However, in many cases it is important to have a knowledge of the energy spectrum.

The flux intensity may vary in different sections of the reactor channels used for activation, and the flux gradient may be quite steep in small reactors. In these cases, it is best to spin the samples during activation to ensure uniform bombardment. The temperature of the reactor channels varies considerably depending on the cooling system applied. Channel temperatures in the range 20–30° can be maintained by efficient cooling, but higher temperatures are not uncommon and sometimes the temperature exceeds 100°. The channel temperature affects the behaviour of many substances during activation so that knowledge of the temperature is essential for appropriate setting up of the irradiation conditions.

The activation services of nuclear centres enable other laboratories to perform the activations at a relatively low cost. For example, the cost of routine irradiation of a sample with a neutron flux of 10^{12} n cm^{-2} sec^{-1} for one week is *ca.* \$ 20.

In order to allow applications of activation analysis in laboratories without access to a reactor, and also to extend the range of the technique, other sources of neutrons have been studied. A number of isotopic neutron sources are commercially available; these sources are based on combinations of α-emitters and γ-emitters with light elements, such as: ^{124}Sb–Be, ^{210}Po–Be, ^{228}Th–Be, ^{241}Am–Be, ^{242}Cm–Be, or ^{228}Th–^{18}O, ^{241}Am–^{18}O, and they can usually produce fluxes of *ca.* 10^6 or 10^7 n cm^{-2} sec^{-1}. HENNELLY[19] devised an antimony–beryllium source of neutrons containing 1000 Ci of ^{124}Sb; this source can yield a nearly mono-energetic 25-keV flux of 10^{10} n cm^{-2} sec^{-1}. It is expected that ^{252}Cf emitting 10^9 n sec^{-1} mg^{-1} will be available in the near future.

Isotopic neutron sources are not extensively used in activation analysis because of their low neutron flux, but there are problems to which they can be applied. For example, sources which can give useful fluxes of 10^6 n cm^{-2} sec^{-1} or more, may be used in activation analyses for sodium, bromine and iodine[20].

Considerable progress has been made recently in the construction of accelerator sources of neutrons. Useful neutron fluxes of high energy are produced in accelerators mainly by deuteron bombardment of tritium or beryllium targets. Deuteron bombardment of tritium targets produces 14-MeV neutrons which can be used in (n, 2n), (n, p) and (n, α) reactions with almost all elements, in particular with the atoms of the first two periods of the periodic system. Cockroft-Walton small accelerators are often used for this purpose. Deuteron bombardment of beryllium produces a broad energy spectrum of neutrons up to above 6 MeV. All types of accelerators can be used as sources of thermal neutrons by surrounding the target with a moderator.

Appropriate choice of neutron energy may improve the selectivity of the activation analysis. For this purpose new sources of mono-energetic neutrons are being developed, in which targets are bombarded with positively charged ions from the accelerator (*cf.* LENIHAN[21]).

Accelerated charged particles such as protons, deuterons or α-particles can also be used in activation analysis, although their short ranges of penetration put significant limits to their utility for the activation of paper chromatograms. The generation of intense fluxes of charged particles usually requires powerful accelerators and is rather expensive. Lower fluxes of α-particles may be obtained by the decay of suitable nuclides.

TABLE I

EFFECTS OF THERMAL NEUTRONS ON CONSTITUENTS OF CELLULOSE, IMPURITIES OF CHROMATOGRAPHIC PAPERS AND ATOMS OF POTENTIAL APPLICATIONS IN ACTIVATION ANALYSIS OF PAPER CHROMATOGRAMS

(The cross-section values, essentially for a neutron velocity of 2200 m sec^{-1}, are taken from HUGHES AND SCHWARTZ[26]. *A* before the cross-section value denotes absorption cross-section used for illustration of the processes in which no radioactive isotopes are formed)

Element	*Isotope activated*	*Natural abundance (%)*	*Nuclear reaction*		*Radionuclide formed*			
			Type	*Cross-section (barns)*	*Isotope formed*	*Half-life*	*Approx. time of decay of*	
							90%	*99%*
$_{1}H$	^{1}H	99.985		*A* 0.332				
$_{6}C$	^{12}C	98.892	n, γ	0.0033	^{13}C[a]	∞	—	—
	^{13}C	1.108	n, γ	0.0009	^{14}C	5760 y	$2\cdot10^{4}$ y	$4\cdot10^{4}$ y
$_{7}N$	^{14}N	99.63	n, p	1.75	^{14}C	5760 y	$2\cdot10^{4}$ y	$4\cdot10^{4}$ y
$_{8}O$	^{16}O	99.76		< 0.00002	^{17}O[a]	∞	—	—
$_{9}F$	^{19}F	100	n, γ	0.009	^{20}F	11 sec	35 sec	1 min
$_{11}Na$	^{23}Na	100	n, γ	0.536	^{24}Na	15.0 h	2 d	4 d
$_{12}Mg$	^{24}Mg	78.6		*A* 0.034				
	^{25}Mg	10.11		*A* 0.280				
	^{26}Mg	11.29	n, γ	0.027	^{27}Mg	9.6 min	30 min	1 h
$_{13}Al$	^{27}Al	100	n, γ	0.21	^{28}Al	2.3 min	7 min	15 min
$_{14}Si$	^{28}Si	92.28		*A* 0.080				
	^{29}Si	4.67		*A* 0.28				
	^{30}Si	3.05	n, γ	0.111	^{31}Si	2.62 h	8 h	17 h
$_{15}P$	^{31}P	100	n, γ	0.19	^{32}P	14.3 d	46 d	95 d
$_{16}S$	^{32}S	95.06	n, α	*A* 0.0018				
	^{33}S	0.74	n, p	0.015	^{33}P	25 d	80 d	167 d
	^{34}S	4.18	n, γ	0.26	^{35}S	87.1 d	278 d	1.5 y
$_{17}Cl$	^{35}Cl	75.4	n, γ	30	^{36}Cl	$3.1\cdot10^{5}$ y	$9.8\cdot10^{5}$ y	$2\cdot10^{6}$ y
			n, p	0.19	^{35}S	87.1 d	278 d	1.5 y
	^{37}Cl	24.6	n, γ	0.56	^{38}Cl	37.3 min	2 h	4 h
$_{19}K$	^{39}K	93.1	n, γ	3	^{40}K	$1.3\cdot10^{9}$ y	$4\cdot10^{9}$ y	$8\cdot10^{9}$ y
	^{41}K	6.9	n, γ	1.1	^{42}K	12.45 h	1.6 d	3.5 d
$_{20}Ca$	^{40}Ca	96.92		*A* 0.22				
	^{44}Ca	2.13	n, γ	0.67	^{45}Ca	164 d	1.4 y	3 y
$_{25}Mn$	^{55}Mn	100	n, γ	13.3	^{56}Mn	2.58 h	8 h	17 h
$_{26}Fe$	^{54}Fe	5.90	n, γ	2.5	^{55}Fe	2.94 y	8 y	17 y
	^{56}Fe	91.52		*A* 2.7				
	^{58}Fe	0.33	n, γ	0.98	^{59}Fe	45 d	141 d	294 d
$_{27}Co$	^{59}Co	100	n, γ	20	^{60}Co	5.25 y	17 y	35 y
$_{29}Cu$	^{63}Cu	69.09	n, γ	4.3	^{64}Cu	12.8 h	1.6 d	3.5 d
$_{33}As$	^{75}As	100	n, γ	5.4	^{76}As	26.5 h	3.5 d	7.3 d
$_{34}Se$	^{74}Se	0.96	n, γ	26	^{75}Se	121 d	1 y	2 y
	^{80}Se	49.96	n, γ	0.030	^{81m}Se	57 min	3.3 h	7 h
				0.5	^{81}Se	18 min	1 h	2 h
$_{35}Br$	^{79}Br	50.57	n, γ	2.9	^{80m}Br	4.4 h	14 h	29 h
				8.5	^{80}Br	18 min	1 h	2 h
	^{81}Br	49.43	n, γ	3.1	^{82}Br	36 h	4.6 d	10 d
$_{47}Ag$	^{109}Ag	48.65	n, γ	3.2	^{110m}Ag	253 d	2 y	4 y
				113	^{110}Ag	24 sec	1.5 min	3 min
$_{53}I$	^{127}I	100	n, γ	5.6	^{128}I	25 min	80 min	2.6 h
$_{55}Cs$	^{133}Cs	100	n, γ	0.017	^{134m}Cs	2.9 h	9 h	19 h
				30	^{134}Cs	2.19 y	7 y	15 y
$_{76}Os$	^{190}Os	26.4	n, γ	8	^{191m}Os	14 h	44 h	4 d
					^{191}Os	15.0 d	47 d	100 d
	^{192}Os	41.0	n, γ	1.6	^{193}Os	3.1 h	4 d	9 d
$_{82}Pb$	^{204}Pb	1.37	n, γ	0.7	^{205}Pb	$3\cdot10^{7}$ y	10^{8} y	$2\cdot10^{8}$ y
	^{208}Pb	51.55	n, γ	0.0006	^{209}Pb	3.32 h	10.5 h	22 h
$_{92}U$	^{235}U	0.714	n, f	582	Fission products			

[a] Stable isotopes which are the products of neutron activation of the constituents of cellulose.

Of course, the simultaneous occurrence of several different nuclear reactions which lead to a variety of products is a feature of activation by charged particles. This complexity compares unfavorably with thermal-neutron activation where frequently only one reaction occurs. However, several techniques have been described in which bombardment with charged particles and paper chromatography are used, sometimes in very ingenious combinations[22-24].

High-energy γ-photons can also induce nuclear reactions suitable for activation analysis. Most γ-ray activation techniques are based on the (γ, n) reaction, and important light elements such as carbon, nitrogen and oxygen can be determined[25].

ACTIVATION OF ELEMENTS WITH THERMAL NEUTRONS

Out of 81 stable elements, 75 can be converted into radioactive isotopes by bombardment with thermal neutrons. These particles, however, are of little value for the activation of light elements with $Z < 10$. Thermal neutron activation processes selected from the point of view of activation analysis of paper chromatograms are listed in Table I, which shows the main characteristics of the nuclear reactions of these isotopes that have significant natural abundances and high cross-sections and are converted into sufficiently long-lived radioisotopes. Also included are the reactions of certain elements which are of spectral analytical importance and of some elements which occur as paper impurities. Certain reactions are given in which no radionuclides are formed, in order to illustrate the behaviour of high natural-abundance isotopes. Data for hydrogen, carbon and oxygen illustrate the behaviour of the main constituents of chromatographic paper.

Special attention should be given to uranium-235 which has a remarkably high cross-section. The atoms of this element, upon irradiation with thermal neutrons, do not undergo a specific nuclear reaction but are disrupted in a fission process to produce various radioactive products in a reaction similar to that occurring in a nuclear explosion of a uranium charge. Although the natural abundance of uranium-235 is rather low, its high cross-section indicates very interesting perspectives for application of uranium compounds in activation analyses of paper chromatograms.

Rare elements are not represented in Table I, although they are readily activated. However, activation of these elements directly on paper has not yet been exploited in paper radiochromatographic or radioelectrophoretic techniques.

Activatable markers

Various materials contain no atoms that can be conveniently transformed by activation into radionuclides. These substances can be labelled by physical or chemical additon of activatable elements. For example, admixtures of rare earth elements have been suggested for labelling certain products such as gunpowder or narcotics[21] in order to facilitate criminological studies.

A paper-chromatographic technique called "derivative activation chromatography" has been proposed by STEIM AND BENSON[27,28], for the determination of certain organic compounds that do not possess activatable atoms. Mixtures of such compounds are converted to their bromine- or mercury-containing derivatives, separated by paper chromatography, and activated to produce readily detectable radionuclides in measurable quantities.

Anal. Chim. Acta, 46 (1969) 281–306

TABLE II

EFFECTS OF NEUTRON IRRADIATION OF FILTER PAPER

Type of paper	*Reactor*	*Reactor type*	*Neutron flux ($n\ cm^{-2}\ sec^{-1}$)*	*Time of irrad. (h)*	*Paper after irradiation*	*Reference*
Whatman No. 1	WWR-C, (EWA), Warsaw, Poland	Tank type, light water moderated and cooled	10^{12}	24	Still workable, but longer irradiation impossible	37
Whatman No. 1, Schleicher and Schüll No. 589	MNR (Mc Master), Hamilton, Canada	Pool type, light water moderated and cooled	$5 \cdot 10^{12}$	4	Still workable, but longer irradiation impossible	35
Eaton, Dikeman, Grade 301	CP-5 Argonne, Ill., U.S.A.	Tank type, heavy water moderated and cooled, heavy water and graphite reflected	10^{12}	48	Badly discolored (brown), very brittle, could not be unrolled without crumbling	36
				12	No obvious effect on the physical properties	
			10^{10}	100	Paper breaks during unrolling	34
Various commercial grades of chromatographic paper	BEPO, Harwell, U K.	Graphite moderated and reflected, air cooled	$7 \cdot 10^{12}$ (temp. 150°)	?	Extremely brittle and impossible to unwrap	38
	HERALD, Aldermaston, U.K.	Pool type, light water moderated and cooled	$2.5 \cdot 10^{11}$	336	Condition fairly good	
Whatman No. 1, HCl washed cut out spots	JEEP-1, Kjeller, Norway	Tank type, heavy water moderated and cooled, graphite reflected	$2 \cdot 10^{12}$	120	Exposure to higher doses results in the destruction	39
Whatman No. 3	ASTRA, Seibersdorf, Austria	Pool type, light water moderated and cooled	$2 \cdot 10^{12}$	0.33	Yellow, tensile strength diminished by about 60%	40
				ca. 3	Original shape lost	
				Longer in-core irradiations	Converted into a tarry mass	

A similar procedure has been suggested by SPENCER AND BRODY[29], who exposed the samples containing organic compounds to non-radioactive halogens and then subjected them to neutron bombardment. Free halogens can add to unsaturated bonds, or substitution for hydrogen can be achieved with $SOCl_2$, PCl_5, PBr_3 or other similar reagents.

PAPER AS SUPPORT IN ACTIVATION ANALYSIS

Filter paper is an excellent support for radiochemical work. Its good mechanical properties and highly developed surface make it possible to handle various radioactive samples in a convenient form without danger of loss. Owing to the chemical resistance of paper, many chemical reactions can be carried out directly *in situ*. The low content in cellulose of elements other than carbon, hydrogen and oxygen is of particular importance for neutron activation of samples deposited on paper. Thus, not only methods for the activation analysis of paper chromatograms will be described below, but also several examples will be given of techniques based on the activation of unseparated samples, simply deposited on paper. BOWEN AND CAWSE[30], determined in this manner as little as 10^{-7} g of potassium and phosphorus in biological materials. KYRIAZOPOULOS *et al.*[31] devised a technique for the activation of meteorological samples deposited on filter paper (*cf.* page 303). TOWLE AND FARRAND[32] recommend the use of samples of phosphoric acid or ammonium phosphate deposited on paper for the measurement of thermal neutron flux intensity; 1-μl aliquots of diluted solutions placed on 1 cm^2 of paper can be conveniently used for this purpose.

Effects of neutron flux on filter paper

The energy of thermal neutrons is not sufficiently high to break the covalent bonds of cellulose molecules and to destroy the fibres which constitute filter paper. However, these effects are brought about during activation by γ-radiation and high-energy neutrons accompanying thermal neutrons. Partial disintegration of the paper on bombardment with elementary particles can be a limiting factor in performing certain analyses. Deterioration of the mechanical properties of the paper is particularly undesirable, if the chromatogram is to be subsequently scanned in a device through which the paper is passed under tension[33].

Brief activation of a paper leads to minor changes in its mechanical properties, but on prolonged activation the paper darkens, becomes more and more fragile, and finally disintegrates or is converted to a tarry mass. In the experience of the authors, it is not possible to handle paper chromatograms after exposure to a neutron flux of *ca.* 10^{12} n cm^{-2} sec^{-1} for longer than 24 h. BOCK-WERTHMANN AND SCHULZE[34] have reported that paper strips break during unrolling after irradiation for 100 h in a neutron flux of 10^{10} n cm^{-2} sec^{-1}. ROBINSON[35] irradiated paper strips with a neutron flux of $5 \cdot 10^{12}$ n cm^{-2} sec^{-1} at a γ-dosage of *ca.* 10^7 R/h for 4 h and found the paper still in a workable condition, but longer irradiations required special plastic protectors (see below). SHERMA AND STRAIN[36] found that although 12-h irradiation with 10^{12} n cm^{-2} sec^{-1} caused no obvious changes in the physical properties of the paper, 48-h irradiations led to extensive breakdown of the paper. Observations of this kind are summarized in Table II.

Damage to the paper does not vary greatly among the common commercial

TABLE III

IMPURITIES OF LINTERS PAPERS

(Extreme values are given, obtained in determinations of the impurities in various sorts of chromatographic and other filter papers)

Component	*p.p.m.*	*References*
Mineral		
Total content	600–700	
Ca + Mg salts	240–280	44
Si	120–210	
Na	30– 85	30, 45, 46
Fe	6– 14	
Al, Cu, Pb, Mn	Traces	35, 44
Cl	40–170	41, 45
Organic		
Cotton wax; this fraction contains alcohols, fatty acids, hydrocarbons and phospholipids	200–500	44
Nitrogen; this fraction contains peptides, cysteic acid, glycine, proline, leucine, arginine and tyrosine	100–150	47
Aspartic acid, glutamic acid, alanine, valine		47, 48
Sugars, as components of hemicelluloses	Various amounts	44

TABLE IV

INORGANIC IMPURITIES IN WHATMAN PAPERS[49]

Type of paper	*Maximal content (μg cm⁻²)*		
	Ash[a]	*Fe*	*Cu*
Chromatographic, thin	5	0.1	0.02
Chromatographic, thick	10	0.2	0.04
Ashless, thin	1	0.05	0.01
Hardened, thin	0.7	0.05	0.01

[a] Largely sodium sulphate, sodium carbonate and silica in the chromatographic grades.

TABLE V

TRACE ELEMENTS IN WHATMAN NO. 3 CHROMATOGRAPHIC PAPER[40]

Radionuclide detected	*Half-life*	*Content (p.p.m.)*	*Radionuclide detected*	*Half-life*	*Content (p.p.m.)*
^{28}Al	2.3 min	7	^{133}Ba	7.5 y	[a]
^{80}Br	17.6 min	[a]	^{51}Cr	27.8 d	1
^{38}Cl	37 min	6	^{124}Sb	60.0 d	3
^{56}Mn	2.58 h	0.2	^{46}Sc	84 d	[a]
^{64}Cu	12.8 h	7	^{75}Se	120 d	[a]
^{24}Na	15.4 h	9	^{65}Zn	245 d	27
^{198}Au	2.7 d	0.003	^{110m}Ag	253 d	[a]
^{131}Ba	12.0 d	[a]	^{60}Co	5.27 y	1
^{139}Ba	84 min	[a]			

[a] Nuclide present, but confirmed only qualitatively.

grades of chromatographic paper with the exception of DEAE-paper which is affected more than the unmodified forms[38]. Naturally, increased temperatures in the irradiation facilities may cause melting of media made of such materials as cellulose acetate.

The basic elements constituting cellulose (carbon, hydrogen and oxygen) are not activated by thermal neutrons (*cf.* Table I). Neutron fluxes in reactors are contaminated with other particles but, in practice, the nuclear reactions of the atoms of cellulose do not contribute significantly to the radioactivity of activated paper chromatograms, and it should be emphasized that the main limitations for the activation of material in spots on paper are connected with radiolytic degradation of paper.

According to BENSON *et al.*[41] the paper damage is caused mainly by the γ-radiation associated with the neutron field and the allowable exposure to γ-rays is close to 10^8 R. This finding has been confirmed by ROBINSON[35].

ROBINSON[35,42] applied the polymer preparation, Chrome Guard (Canadian Tire Corp.), to minimize the mechanical disintegration of activated paper chromatograms during handling after irradiation. In spite of this and similar possibilities of improving the mechanical properties of irradiated paper, the activation of atoms which produce long-lived isotopes is rarely possible on paper.

Impurities of filter paper

Chromatographic paper is produced almost exclusively from cotton cellulose. The shorter cotton fibres, so-called linters, represent the purest natural cellulose. During the paper production, the linters are interwound and form a structure with a highly developed surface. The marked chemical resistance of filter paper is due to the high content of crystalline component in the cellulose fibres of the cotton linters. The amorphous regions of the cellulose fibres are more susceptible to the action of various chemical agents[43,44]. To understand certain properties of filter paper, it is important to know that the linters are hollow. The structure of mature cotton fibre consists of primary wall, secondary wall and central canal. The thin primary wall is only a few tenths of a micron thick and consists largely of waxy and pectic substances plus a small amount of cellulose; this layer is removed by treatment with alkaline solutions during the paper manufacture. The principal chemical constituent of cotton is the cellulose occurring in the secondary wall. The central canal represents up to 17% of the linter's cross-section.

Chromatographic papers contain 98–99% of α-cellulose, 0.3–1% of β-cellulose and 0.4–0.8% of pentosanes. Papers made from linters contain amino acids and peptides. According to GRÜNE[44], the total nitrogen content of chromatographic papers varies from 10 to 15 mg per 100 g of paper. The total amount of substances that can be extracted with ethyl ether varies from 20 to 50 mg per 100 g of paper. Chromatographic papers contain 0.06–0.07% of inorganic impurities. More detailed data on the nature and concentration of impurities in filter paper are given in Table III; the impurity content of various types of Whatman paper is shown in Table IV. Further data[40] on the trace elements contained in Whatman No. 3 paper are given in Table V; the results were obtained by neutron activation of the paper and γ-spectrometry.

Radioactive background of neutron-activated paper chromatograms

Organic impurities in chromatographic paper present no problem from the

point of view of activation with thermal neutrons. However, the presence of inorganic impurities introduces a distinct background along the chromatograms, which, immediately after the activation, is high enough to flood the radioactivity of the spots.

It is known that the specific activity of an activated element is, *inter alia*, inversely related to the half-life of the radioisotope formed. For this reason, very short-lived isotopes contribute substantially to the background activity of the strip immediately after the activation; decay of these isotopes is a matter of hours. An idea about the further decay of the radioactivity can be derived from the data presented in Fig. 1, which shows the distribution of radioactivity on a neutron-activated paper strip containing a spot of a phosphorus compound; the strip was scanned twice—one day after the activation and then two days later. It can be seen that high activity is distributed along the whole strip even after 28 h of "cooling", but that after 3 days a sufficiently uniform background is formed and the required peak can be distinguished.

The nature of the radionuclides that contribute mainly to the background

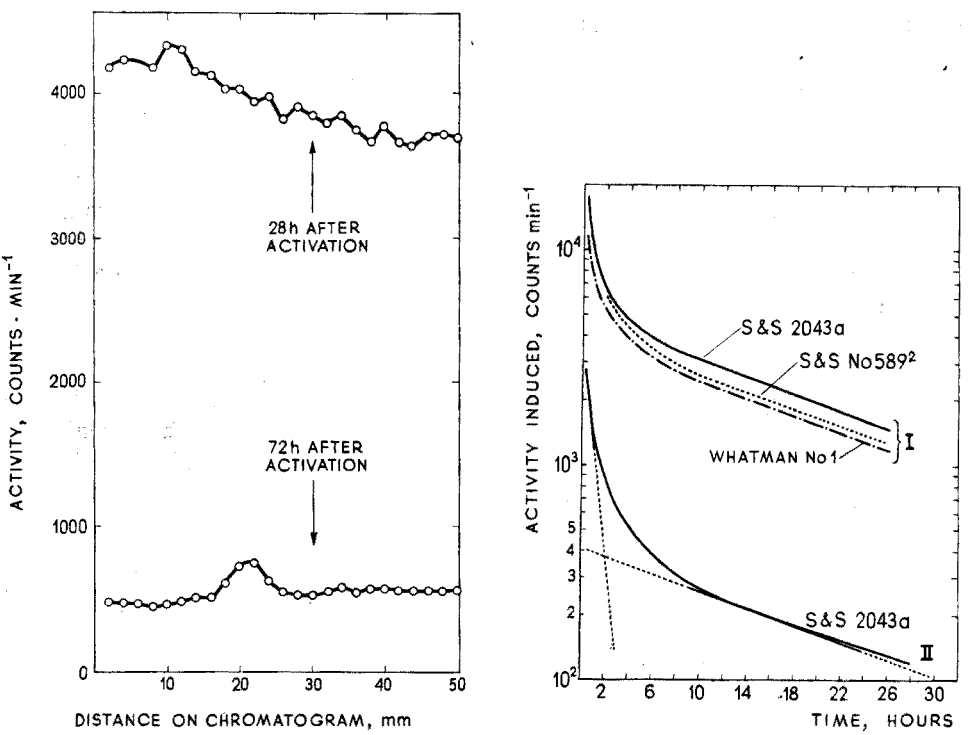


Fig. 1. Neutron activation of phosphorus on paper. Tri-*n*-butyl phosphate (2.5 μg = 0.25 μg of phosphorus) was placed on a silica-coated Whatman No. 3 paper strip prewashed with dilute hydrochloric acid and water. The strip was then activated in a neutron flux of *ca.* $9 \cdot 10^{11}$ n cm^{-2} sec^{-1} for 24 h. The strip was scanned 28 and 72 h after the activation as indicated[37].

Fig. 2. Kinetics of decay of induced radioactivity on various chromatographic papers after 25-min bombardment with a flux of $5 \cdot 10^{12}$ n cm^{-2} sec^{-1}, according to BORN AND STÄRK[45]. The broken straight lines represent decays of ^{38}Cl and ^{24}Na, respectively. (I) untreated papers, (II) acid-washed paper.

activity of irradiated paper may be deduced from the kinetics of the background decay and on the basis of their radiation spectrometry. Figure 2 shows the decay of induced background radioactivity in several sorts of chromatographic papers. Analysis of the decay curves indicates that chlorine and sodium are the major activatable impurities of chromatographic papers[45,50]. γ-Spectrometric measurements have also established manganese as one of the main activatable contaminants of filter paper[35]. These three elements are activated by thermal neutrons to ^{24}Na, ^{38}Cl and ^{56}Mn, respectively. The activities of ^{38}Cl ($T_{\frac{1}{2}}=37$ min) and of ^{56}Mn ($T_{\frac{1}{2}}=2.5$ h) disappear almost completely after several hours and then the radioactivity remaining on the paper is emitted almost exclusively by ^{24}Na. The activity of ^{24}Na ($T_{\frac{1}{2}}=15.1$ h) decays after 3 days to a level which allows counting of the activities of the spots; 99% of ^{24}Na decays in 4 days.

The radioactivities induced in different types of papers vary substantially as

TABLE VI

COMPARISON OF RADIOACTIVITIES INDUCED IN VARIOUS WHATMAN GRADES OF FILTER PAPER BY NEUTRON ACTIVATION, DETERMINED AT DIFFERENT TIMES AFTER IRRADIATION[32]

Whatman paper No.	*Description*	*Activity (counts min^{-1} cm^{-2})*		
		3 days	*7 days*	*11 days*
3 MM[a]	Untreated as received	16920	483	223[b]
3 MM[a]	HCl washed	2020	135	83[b]
3 MM[a]	HF washed	1932	113	72[b]
3 MM[a]	HF and HCl washed	1856	143	84[b]
1 (chromatographic)		10210	385	170
1	A rapid, qualitative non-acid paper	4370	445	408
2	A medium, qualitative non-acid paper	4010	201	183
4	A rapid, qualitative non-acid paper	6130	384	271
5	A fine, non-acid paper	9480	308	168
7	A medium, non-acid paper	1656	148	133
30	A fine-medium, HCl washed paper	1176	132	111
31	A rapid, HCl washed paper	3240	191	119
40	A medium, HCl–HF washed paper	1280	181	107
41	A rapid, HCl–HF washed paper	1157	127	96
42	A fine, HCl–HF washed paper	1331	164	111
50	A fine, hardened (HNO_3 washed) paper	1084	168	120
52	A medium, hardened (HNO_3 washed) paper	1628	195	129
54	A rapid, hardened (HNO_3 washed) paper	1294	209	121

[a] 3 MM paper is approx. twice as thick as most of the other papers shown. Thus, its respective count rates should be halved to compare them with other papers.

[b] These count rates are reduced by *ca.* 40% when a 20 μg cm^{-2} Al absorption filter is used to eliminate ^{35}S activity. Pure ^{32}P activity is only reduced by *ca.* 5% with this filter.

can be seen from Table VI. The activity 3 days after neutron activation consists primarily of ^{24}Na, whereas the activities at 11 days are probably[32] nearly all either ^{35}S or ^{32}P.

PATEK AND SORANTIN[40] have reported recently that the trace elements in Whatman No. 3 paper are not distributed homogeneously in the paper, as can be seen from Fig. 3; hence they recommend the use of larger paper samples, whenever possible, especially for blanks. SIUDA[51] irradiated Whatman No. 1, 2, 3, and 3 MM chromatographic papers with thermal neutrons and also found considerable fluctuations in the background activity. He found that careful washing of these grades of paper with 2% EDTA (disodium salt) not only reduced the background but also improved its homogeneity.

Fig. 3. Autoradiogram of Whatman No. 3 chromatographic paper irradiated for 15 min at $5 \cdot 10^{12}$ n cm^{-2} sec^{-1}, cooled for 72 h and exposed to Kodirex film for 24 h[40].

Removal of impurities from filter paper

Activatable impurities can be partially removed from the paper by appropriate washing (*cf.* Fig. 2 and Table VI). Several washing agents mostly diluted acids, have been used to remove the mineral impurities from filter papers. According to GRÜNE[43], washing with hydrochloric or hydrofluoric acid can reduce the concentration of mineral impurities from 0.05–0.07% to 0.007–0.01%; water-soluble impurities can be removed completely, but certain small fractions of calcium and magnesium remain in the paper, whereas iron and silicon are only slightly removed, possibly because of the formation of iron(III) silicate. BENSON *et al.*[41] activated chromatograms prepared on paper washed with hydrochloric acid. LUŠTINEC[52] recommends prewashing of paper with 1 *N* hydrochloric acid and 0.5% ethylenediaminetetraacetic acid. Dilute nitric acid was used by BORN AND STÄRK[45], who found that nitric acid can reduce the chlorine and sodium contents of the paper five and ten times, respectively. Nitric acid was also used by ROBINSON[35], NĂŞCUŢIU[53] and SHERMA AND STRAIN[36]. The chromatographic paper is usually washed either by dipping it into the wash solution or by a technique similar to descending chromatography.

JOHNSON *et al.*[33] found that the treatment of chromatographic paper with formaldehyde[54] followed by overnight washing with water, effectively removed activatable impurities; paper prepared in this way is suitable for the separation of phospholipids.

The chemicals used for the pretreatment should be removed from the paper with water or with the chromatographic solvent. This final wash should be performed under the same conditions as the wash with the special solutions. It is essential that water used for preparation of the wash solutions and the final wash contains no activatable cationic or anionic impurities. Treatment with Dowex 50 (H^+) is generally sufficient for this purpose.

In certain cases, the impurities can be removed from the paper simultaneously with the main chromatographic process; this can be achieved by application of solvents containing agents used for pretreatment of the paper. NĂŞCUŢIU[55] applied this modification for analysis of mixtures of thorium and rare-earth elements. The chromatograms to be activated were developed with a mixture of ethanol and 2-,4 - or 6-*M* nitric acid in order to elute the impurities with the solvent front.

In practice, even exhaustive washing cannot completely remove trace amounts of impurities, largely because of the hollow structure of the linters constituting the filter paper. It should also be remembered that a prolonged action of various agents can degrade cellulose, modifying the surface of the cellulose fibres and causing changes in the chromatographic and electrophoretic properties of the papers. These changes may affect separations in particular cases.

Certain mixtures can be separated on papers containing less impurity, such as commercially available prewashed so-called "ashless" papers (*e.g.* Whatman No. 40, 41 and 42). Unfortunately, these paper grades are rarely used in chromatographic practice.

Elimination of the radioactive background by subtraction

The delay necessary for "cooling" of the background radioactivity presents a serious obstacle when the radionuclides induced in the spots decay rapidly or when rapid results are required. There is, however, a technique by which the radioactive background can be eliminated without delay. This is the complement-subtraction method of LEE[56], which was originally devised for direct estimation of γ-ray abundances in radionuclide mixtures. The method consists of simultaneous measurement of the radioactivities of the sample analysed and of the standard isotope sample under the same conditions. The result of the measurements is subtracted directly from pulse-height data stored in a 256-channel analyser. The correct end-point is determined by visual inspection of the subtraction effect on the monitoring screen. With this technique an isotope's share in the complex γ-spectrum can be estiminated and the method has proved to be especially useful in activation analysis, for results can be obtained immediately after bombardment of the samples.

The complement-subtraction method has not been applied to the activated paper chromatograms but it may prove to be particularly useful for elimination of the sodium and chlorine contributions to the radioactive background of chromatograms evaluated soon after irradiation.

ACTIVATION CONDITIONS

Calculation of bombardment time and the radioactivity expected

Primary evaluation of the applicability of activation analysis for particular problems consists in calculation of the radioactivity which may be induced in the

sample by irradiation. This calculation is also necessary for the choice of the irradiation conditions and an estimate of its duration.

The amount of radioactivity induced in any nuclide depends on its cross-section, abundance and atomic weight, as well as on the flux intensity, the energy of the particles used for the activation, and the activation period. The radioactivity of an activated sample may be conveniently adjusted by varying the activation period and the delay between activation and counting. In many cases, setting the activation period equal to the half-life of the nuclide of interest may give an analytical sensitivity close to the optimum.

The activity A (disintegrations per sec) of the radioisotope induced can be found from the following formula:

$$A = f \cdot \sigma \cdot \frac{W \cdot \Phi}{M} \cdot (1 - e^{-0.693 t/T_{\frac{1}{2}}}) \cdot e^{-0.693 T/T_{\frac{1}{2}}} \cdot N_A$$

where f = flux of bombarding particles (particles $cm^{-2}\,sec^{-1}$)

σ = activation cross-section for the reaction applied

$$\left(\frac{\text{barn}}{\text{atom}} = \frac{10^{-24}\ cm^2}{\text{atom}}\right)$$

W = weight of the element present (g)

Φ = fractional abundance of the nuclide

M = gram-atom of the element activated ($g\,mole^{-1}$)

$T_{\frac{1}{2}}$ = half-life (sec)

t = time of irradiation (sec)

T = time between irradiation and counting (sec)

N_A = Avogadro's number ($6.02 \cdot 10^{23}$ atom $mole^{-1}$)

Naturally, in order to find the total radioactivity of an irradiated element, this formula must be applied to each isotope of the element in turn.

A numerical example may be instructive about the usage of the above formula. Let us consider the results of irradiation of 1 μg of phosphorus with a flux of 10^{12} n $cm^{-2}\,sec^{-1}$ for 4 h. From Table I, phosphorus occurs in nature solely in the form of ^{31}P, therefore its fractional natural abundance is 1; the cross-section of this isotope to thermal neutrons is $0.19 \cdot 10^{-24}$ cm^2 $atom^{-1}$. Assuming that the sample will be measured 24 h after the irradiation the expected total activity of the sample is:

$$A = 10^{12}\ \text{n}\ cm^{-2}\,sec^{-1} \times 0.19 \cdot 10^{-24}\ cm^2\,atom^{-1} \times \frac{10^{-6}\ g \cdot 1}{31\ g\ mole^{-1}}$$

$$\times (1 - 2.72^{-(0.693 \cdot 4\ h)/(14.3\ d \cdot 24\ h/d)}) \times 2.72^{-(0.693 \cdot 1\ d)/(14.3\ d)}$$

$$\times 6.02 \cdot 10^{23}\ \text{atom}\ mole^{-1} = 28.5\ \text{disintegrations}\ sec^{-1}.$$

If the activity of the sample is measured with a simple Geiger–Müller (G–M) counter tube with an efficiency of 10% then the counting rate will be:

$$\frac{28.5 \cdot 10 \cdot 60}{100}\ \text{counts}\ min^{-1} \cong 171\ \text{counts}\ min^{-1}$$

The result of this calculation indicates that the expected activity is well above the average background value and is easily measurable.

Effects of accompanying radiation on substances in spots

Some energy quanta in the neutron fluxes are higher than the energies of chemical bonds. Therefore, certain proportions of the substances in the spots undergo degradation upon activation. Moreover, the atoms resulting from the nuclear reactions have higher energies than atoms in the normal state. The excited atoms, so-called hot atoms, are exceptionally mobile and can even liberate themselves from the molecules. The chemical reactions that occur during activation can alter the physical properties of substances in spots on paper chromatograms or electrophoretograms and, in some cases, the substances may even volatilize. These factors must be taken into consideration when the results of activation analyses of paper chromatograms are evaluated.

Internal standards

Flux densities at various irradiation positions, even relatively similar or close to each other, are distinctly different. In practice, it is very difficult to reproduce the irradiation conditions in subsequent experiments. Accordingly, when quantitative evaluation of activated chromatograms is required, it is essential to add, before irradiation, one or more spots containing known weights of the element to be activated. The activities induced in such internal standards serve not only as a basis for quantitative analysis but also as a check on the homogeneity of the activating flux.

The arrangement of the internal standards is dictated by the target size and shape. Single strips rolled concentrically form very compact targets and in such cases one or two standard spots are sufficient. Quite often, these are placed at both ends of the paper strip. Two-dimensional chromatograms usually are rolled to form long cylinders and then a row of standard spots is spaced along the length of the sheet[35,41,42,57].

Salts containing only one type of atom that can be activated to a radionuclide are ideal internal standards for the activation analysis of paper chromatograms. Ammonium dihydrogen phosphate, used by ROBINSON[35,42] as a standard for neutron activation of phospholipids on paper, is an excellent example of such substance. Lithium salts can also be used for this purpose, since lithium is transformed to tritium, the radioactivity of which can be easily discriminated.

In accelerator irradiations, an alternative practice to the inclusion of internal standards is often adopted. Because of the limited region of the high neutron flux, sample and standard cannot be simultaneously irradiated, and the change in neutron flux is monitored and normalized by measuring the flux with boron trifluoride counters, fission chambers, a plastic scintillator or determination of the cooling water activity[16].

Canning of samples for irradiation

Several materials can be used for packaging the samples to be activated; most common are aluminium, silica and polyethylene. Aluminium withstands high neutron fluxes and high temperatures. However, the metal is activated by thermal neutrons to ^{28}Al ($T_{\frac{1}{2}}=2.3$ min) and contains appreciable amounts of sodium which gives ^{24}Na ($T_{\frac{1}{2}}=15$ h); since both these radioisotopes are strong γ-emitters, aluminium cannot be used when the sample is to be measured shortly after irradiation. In all

other cases, it is a very convenient material, in particular in the form of commercially available foils of various thicknesses which may be easily shaped and folded.

Silica is also resistant to prolonged activation and high temperatures. It is activated to ^{31}Si ($T_{\frac{1}{2}}=2.6$ h), a β-emitter with no γ-component. Silica is one of the purest canning materials and is often recommended for long irradiations. Disadvantages may arise sometimes from its poor mechanical properties.

Polyethylene softens at *ca.* 110° and becomes brittle[17] after exposure to more than $2\cdot10^{17}$ n cm^{-2}. It can be obtained in very pure forms and is ideal for short irradiations.

TOWLE AND FARRAND[32] used titanium for packaging of filter paper samples and found that this material had many advantages. It is activated to ^{51}Ti ($T_{\frac{1}{2}}=$ 5.8 min) and is available in a very pure form containing iron (60 p.p.m.) as the only significant impurity. These authors also tested the suitability of other materials such as household-grade aluminium foil, Mylar and polyethylene foils, Scotch cellophane and masking tapes, and coatings of Krylon, "Q Dope", and pure polystyrene, for packaging of the paper samples.

Procedure for activation analysis of paper chromatograms

It cannot be overemphasized that, for success in activation analysis of chromatograms, a very high standard of cleanliness in handling must be observed before irradiation. Rusty metal clips, finger prints, dust, activatable ions in the solvents and other contaminants must be scrupulously avoided, in order to prevent high background and misleading artefacts.

In the following paragraphs is described a typical sequence of operations in neutron analysis of paper chromatograms, based on that described by ROBINSON[42] and illustrated by Fig. 4. This particular example refers to an activation analysis for phosphorus compounds in mouse liver extracts separated on paper chromatograms.

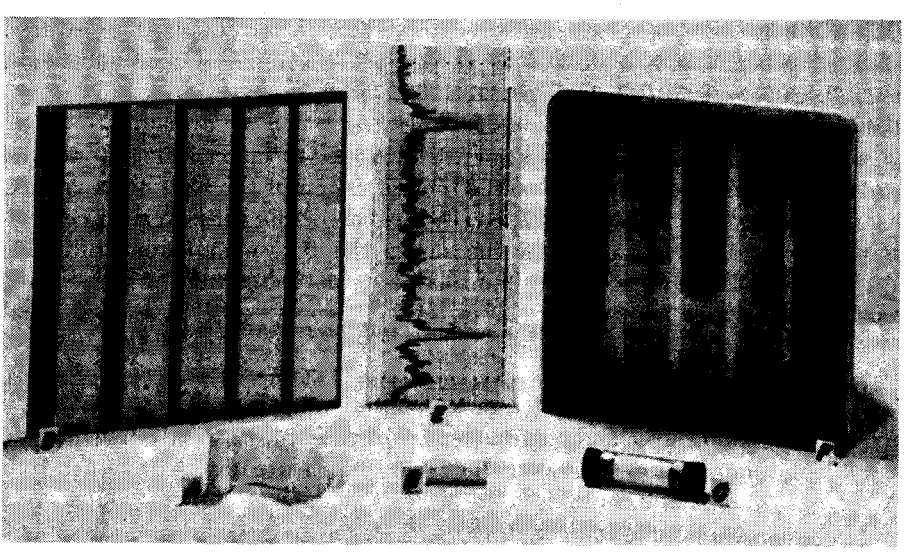


Fig. 4. Essential stages in activation analysis of paper chromatograms (for details see text)[42].

Acid-washed paper was used for separation of the mixtures which were chromatographed in usual manner. The "calibration spots" were applied to the developed, dried chromatograms; these spots were located ahead of the solvent front and contained 0.1, 0.01 and 0.001 μg of phosphorus as ammonium dihydrogen phosphate (analytical-reagent grade). The calibration spots were formed by placing 5 μl of the appropriate solutions to form *ca.* 1-cm^2 spots. These spots acted as standards for comparison with sample spots on the same chromatograms and as a check on the homogeneity of the neutron flux throughout the sample. Subsequently, the strips were hung vertically and coated on one side with a protective spray ("Chrome Guard" solution, *cf.* p. 289). For activation, as many as five chromatograms laminated with wax paper strips of the same size (A, Fig. 4) were rolled to form a cylinder (B) which was wrapped in wax paper with the ends tamped flat. The rolled sample was placed in a protective vessel (C) and irradiated.

Immediately on receipt of the irradiated sample, it was placed for a few hours in a beaker over water or very lightly steamed to improve flexibility and thus aid the unrolling of the activated chromatograms. The wax paper was discarded and the radioactive chromatograms were placed between two thick Perspex sheets and left for 5–6 days to "cool". An alternative method of flattening the rolled paper sheets is a gentle treatment with a household steam iron.

Cooled chromatograms can be scanned or evaluated by autoradiography. Part E of Fig. 4 shows the pattern obtained by scanning an activated strip with an end-window G–M counter tube, and F shows the developed autoradiograms which can be also seen in Fig. 5.

A detailed procedure for neutron activation of phosphorus compounds on paper chromatograms has also been described by BENSON[57].

Determination of radioactivity on activated paper chromatograms

A number of scanning techniques can be applied for the determination of the radioactivity distribution on activated paper chromatograms. Detailed descriptions of these techniques are beyond the scope of this review, but it should be noted that the scanning techniques available can be divided into three main groups: (1) scanning by fragmentation of the chromatogram and determination of the radioactivities of individual segments; (2) stepwise scanning; (3) continuous scanning.

The technique of scanning by fragmentation is suitable for the evaluation of all kinds of chromatograms since it consists in dividing a paper strip or sheet into a series of samples which can then be measured in a standard manner on planchettes, by a scintillation technique, or after combustion, in an internal-filling gas counter.

A great variety of gadgets and instruments has been designed for automatic or semi-automatic, stepwise or continuous scanning of radiochromatograms. Some of these have been described in a review by POCCHIARI AND ROSSI[58]. Many types of radiochromatogram scanners suitable for both one- and two-dimensional chromatograms are commercially available.

Autoradiography of activated paper chromatograms

Autoradiography offers a precise method of localization of radioactive substances on chromatograms, especially in the case of α- and low-energy β-emitters. Unfortunately, this technique requires a relatively long time for exposure of the

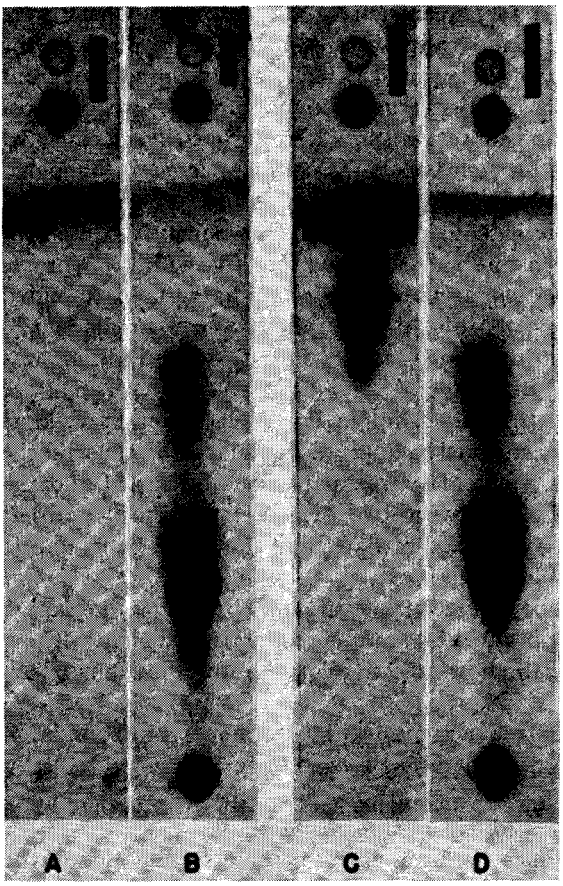


Fig. 5. Autoradiograms of activated chromatograms containing separated extracts of mouse liver. (A) and (B) chloroform and water extracts, respectively, of untreated sliced mouse liver showing the phosphorus compounds normally present. (C) and (D) chloroform and water extracts, respectively, of sliced mouse liver treated with an organophosphorus compound. Above the solvent front can be seen the calibration spots[42].

photographic material, so that final results can rarely be obtained until several days have passed, and quite often several weeks are required.

On Fig. 4 (A–D) paper chromatograms can be seen mounted on a wooden board for autoradiographic exposure; the chromatograms should be covered with thin plastic foil, in order to avoid direct contact of radioactive substances with the film. A suitable film, usually an X-ray type, should be pressed with a weight or a spring system against the board during the exposure. For many radioisotopes, good results can be obtained with single coated X-ray film exposed with the emulsion side away from the chromatogram. The reduction in background activity compensates for losses in time of exposure of the film. The calibration spots at the top of each autoradiogram can be seen in Fig. 5.

Autoradiograms of activated paper chromatograms can be conveniently identified on the film by writing in pencil on the paper before irradiation[33]. For

several days after irradiation the resulting radioactive pencil marking is clearly recorded by X-ray film after a few hours of exposure to the chromatogram. Naturally, any other pencil marks on the paperogram must be avoided.

APPLICATION OF PAPER CHROMATOGRAPHY AND ELECTROPHORESIS FOR THE SEPARATION OF PREVIOUSLY ACTIVATED SAMPLES

Although problems connected with the separation on paper of activated samples do not really fall within the scope of this review, it seems appropriate to devote a few paragraphs to this neighbouring and important field.

Separations of activated samples by means of paper have proved to be particularly valuable in studies of the Szilard–Chalmers effect. Complex mixtures of compounds containing the same radionuclide can be conveniently separated on paper by chromatography or electrophoresis. Various authors have applied these procedures after neutron bombardment of the samples, and have been able to elucidate certain problems of chemical reactions depending on nuclear transformations. This field has been reviewed by ADLOFF[59].

Considerably more difficult situations occur when different radionuclides are induced in the molecules of various compounds in the irradiated material. The individual components of such mixtures frequently occur at concentrations that differ by several orders of magnitude, so that γ-spectrometry, which is the very versatile and basic tool of activation analysis, may be incapable of yielding the results required. The difficulties arising from the complexity of samples can be overcome by the application of suitable separation techniques. In such cases, the aim is to separate the radioactive species to be determined, either in a radiochemically pure form or into several groups which can then be further examined by γ-spectrometry.

Chromatography and electrophoresis carried out on paper can provide valuable radiochemical separation techniques, and have been widely applied in analyses of complicated mixtures formed by bombardment of various inorganic and organic compounds with different kinds of elementary particles or photons. The results of such analyses have made it possible to elucidate the nature of various chemical effects associated with nuclear transformations. Chromatographic and electrophoretic techniques have been applied, *inter alia*, in studies on the reactions of recoil atoms, and on the transformations that occur during neutron activation of metal complexes.

Separation and examination of the mixtures formed after neutron activation of uranium compounds and reactor fuel materials has made it possible to identify and determine fission products and accompanying impurities[60–64]. The difficulties connected with high activities of the irradiated samples, which after irradiation contain ^{239}Np and various products of ^{238}U and ^{235}U fission, have been overcome in methods recently elaborated by ÖRDÖGH *et al.*[65–67]. These methods are based on the separation of uranium from impurities by means of paper chromatography by the descending overflow technique and with anhydrous ether containing 5 vol % of nitric acid, as solvent. Most of the uranium is carried away whereas the impurities remain on the starting line. Thorium can be also removed by this procedure, although not as quickly as uranium. The zones containing impurities are then ashed and activated; final differentiation and determination of particular elements is based on paper chromatography, chemical separation and γ-ray spectrometry. The techniques

developed allow the determination of the following impurities of uranium: Na, Np, Cr, Ni, Co, Mn, Cu, Mo, Cd, Au, Fe, Ag, P and Si.

Neutron activation followed by paper chromatographic separations simplifies routine analyses for numerous elements in geological samples. Of particular interest are the possibilities of determining trace elements in rocks, soils, water and in plants. Simultaneous determinations of Mn, Cu, Co, Ga, W, Ta, Th, U, Sc, Ca and Rb can be carried out by γ-ray spectrometry of radiochromatograms followed by computer analysis[68,69]. Similar procedures were applied in geochemistry and in metallurgy for determination of trace elements[70,71].

Many other examples of methods based on activation followed by separation on paper are listed in the bibliographies of neutron activation by BOCK-WERTHMANN[72] published at intervals by the A.E.D. Information Service. These bibliographies include element and matrix indexes and provide an excellent reference source.

PRACTICAL APPLICATIONS OF ACTIVATION ANALYSIS OF PAPER CHROMATOGRAMS

Phosphorus

The activation analysis of paper chromatograms has found widespread application for the determination of molecules containing phosphorus, and many investigations of phosphorus compounds have been aided by this technique. Phosphorus is readily activated by thermal neutrons and can be detected on activated radio-chromatograms in amounts as low as 10^{-3} μg^{35}, *i.e.* at a level two orders of magnitude lower than the limit of sensitivity of colorimetric methods. Thus, activation analysis may be conveniently applied when labelling with ^{32}P is impossible or when only very small samples are available.

Various compounds of phosphorus are highly important constituents of living matter. Phospholipids are among these essential biological molecules, and the complexity of phospholipid fractions and their chemical and physical properties make their determination one of the more difficult problems of biochemical analysis. BENSON *et al.*[41], and later BENSON[57], have shown that characterization of phospholipid mixtures can be conveniently carried out on neutron-activated paper chromatograms. They determined the composition of phospholipids extracted with hot ethanol from bovine blood serum, bovine spermatozoa and plant material (*Chlorella* and *Scenedesmus*). The samples were chromatographed, sealed in polyethylene tubes (150 cm long, 20–32 mm internal diameter) and irradiated in a $5 \cdot 10^{11}$ n cm^{-2} sec^{-1} flux for 7 h; 6 days later autoradiograms were prepared. The same authors have also demonstrated the possibilities which exist when the fate of a molecule containing both ^{14}C and non-radioactive phosphorus is followed; they activated paper chromatograms with spots of photosynthetic 3-phosphoglycerate-^{14}C, calculated its specific ^{14}C radioactivity and, on this basis, determined the original concentration of this compound within the cell.

Phospholipids have been determined by activation analysis of paper chromatograms in various mammalian cell fractions[73] and in crude extracts of corn lipids[33,74]. BLOMSTRAND AND NAKAYAMA[75] used this technique to determine phospholipids in human liver biopsy samples for the first time.

It should be noted that activation of phospholipids leads to major modifications of their properties. HÖLZL[76] has shown that irradiation of a sample of phospho-

lipids with a neutron flux of $2 \cdot 10^{13}$ n cm^{-2} sec^{-1} for 300 min renders the sample completely soluble in all typical lipid solvents.

Nucleic acid research is another field of biochemistry in which the potentialities of phosphorus activation have been exploited. MURRAY AND OFFORD[38] have described a technique for the characterization of small amounts of nucleic acids; they digested enzymatically 100–200 μg of nucleic acids, separated the oligonucleotides formed by a two-dimensional ionophoretic technique on DEAE–cellulose paper, activated the ionopherograms with thermal neutrons, and then examined them by autoradiography. This technique may be especially valuable for the estimation of nucleic acids from sources that cannot conveniently be labelled biosynthetically. About 100 mg of tissue is sufficient for the examination of nucleic acids from plants, animals or humans. The application of this technique is suggested for characterization of tumour materials.

FLIKKE AND STEINNES[39] determined nucleotide ratios in small samples of ribonucleic acid after paper electrophoretic separation of an alkaline hydrolysate. They cut out the individual spots, sealed them in small polyethylene envelopes and irradiated for 5 days at a neutron flux of $2 \cdot 10^{12}$ n cm^{-2} sec^{-1}; after 7 days, the β-radioactivity of the samples was measured with a G–M-counter tube, screened with 50 mg cm^{-2} of aluminium foil to discriminate the activity of low-energy β-emitters.

By means of electrophoretic migration in moist paper, non-radioactive phosphorus compounds, mainly various phosphoric acids, have been separated and located by neutron activation[5]. Dry paper chromatograms wrapped in polyethylene and aluminium foil were placed in the thermal column of the reactor at *ca.* 10^{12} n cm^{-2} sec^{-1} for a week and then left for 7–14 days to reduce the radioactivity of the paper itself. Detection of radioactive zones was carried out with a screened proportional G–M-counter tube through a 1-in slit.

Phosphoric acid, tributyl phosphate, dibutyl phosphate and monobutyl phosphate have been neutron-activated on paper by TOWLE AND FARRAND[32].

In the course of studies on the chemical effects of the ^{31}P (n, γ) ^{32}P reaction in organic media, SIUDA[77] developed paper chromatographic and paper electrophoretic methods for the separation of several inorganic and phenyl derivatives of phosphorus. Among other methods, detection of the spots was carried out by neutron activation of the paperograms, in the thermal column of the reactor EWA at $3 \cdot 10^{12}$ n cm^{-2} sec^{-1} for 5 h; after cooling for about 10 days, the strips were scanned between two end-window G–M-counter tubes with 0.5-cm wide slits in the diaphragm.

HALMANN AND KUGEL[78] separated and analysed mixtures containing methylphosphonic acid, methylphosphinic acid, dimethylphosphonic acid, trimethylphosphine oxide and several inorganic phosphorus oxyacids. The spots containing phosphorus were detected after bombardment of the chromatograms with $2.5 \cdot 10^{12}$ n cm^{-2} sec^{-1} for 30 sec; after about one week the strips were counted with an end-window proportional counter.

WINTERINGHAM *et al.*[79] developed an interesting technique for the estimation of non-radioactive ^{31}P in the presence of ^{32}P on paper chromatograms. The radioactive phosphorus is counted on the chromatograms, which are then left for several half-lives to allow decrease of ^{32}P radioactivity to a very low level. The chromatogram is then exposed to a neutron flux so that the total amount of ^{31}P is activated and can be determined by radiometry.

Oxygen

Various activation procedures may ease the difficulties that arise when a technique is required to follow the metabolic or other fate of oxygen. FOGELSTRÖM-FINEMAN *et al.*[24] applied the stable heavy isotope of oxygen, ^{18}O, in a method which was then exploited in studies on photosynthesis by CALVIN *et al.*[23]. The method was based on the reaction $^{18}O(p, n)^{18}F$, the product being a 0.64-MeV positron emitter with a half-life of 1.8 h. In order to eliminate the interference of the oxygen contained in the cellulose (the natural abundance of ^{18}O is 0.2%), the paper chromatograms were provided with 1.5-mm wide teeth on one side and eluted sidewise in a special device with the teeth just touching a heated reactor-grade tantalum sheet. The sheet was then rolled up to form a cylinder and irradiated in a cyclotron (1–10.5 μA, 4.5 MeV protons, 3–4 min) under rotation and cooling with a jet of air. As little as 0.1–1 μg of ^{18}O could be easily detected by this method.

AMIEL AND NIR[22] detected the same oxygen isotope directly on paper chromatograms by means of the nuclear reaction $^{18}O(\alpha, n)^{21}Ne$. A radioisotope α-source was located on one side of the chromatogram and the neutrons emitted from the spots were counted by a $^{10}BF_3$ proportional counter placed on the other side of the chromatogram.

Iodine

Naturally occurring ^{127}I can be easily activated by thermal neutrons to short-lived radioactive ^{128}I ($T_{\frac{1}{2}}=25$ min). This nuclear reaction has been exploited for the determination of iodine compounds separated by paper chromatography. Activation of chromatograms allows the determination of as little as 10^{-3}–10^{-4} μg of iodine per cm^2 of paper[80]. Various isomers of monoiodobenzene and *o*-diiodobenzene have been determined in this manner after paper chromatography and irradiation with a thermal neutron flux of $5 \cdot 10^{12}$ n cm^{-2} sec^{-1} for 10 min[81].

Several other workers have reported similar analyses of iodine-containing thyroid hormones[80], iodine compounds of human plasma[82] and iodo-derivatives of amino acids in human serum[83–86]. In the latter analyses the spots containing iodine compounds were cut out from the chromatograms, irradiated and burned in oxygen. After acidification, free iodine was distilled and precipitated as $Ag^{128}I$. Such radiochemical separation requires about 1 h. Iodine can be separated from other more abundant radioisotopes by this procedure.

Arsenic

During an investigation of radioactive compounds formed as a result of nuclear transformations of arsenic in organic medium, SIUDA[87] developed both paper chromatographic and paper high-voltage electrophoretic techniques for the separation of several phenyl and inorganic derivatives of arsenic. Spots containing arsenic were detected by neutron activation via the reaction $^{75}As\ (n, \gamma)\ ^{76}As$; irradiation at $2 \cdot 10^{12}$ n cm^{-2} sec^{-1} was carried out for 5 h. After 4 days, the strips were scanned between two end-window G–M-counter tubes through 0.5-cm wide collimator slits.

Metals

SHERMA AND STRAIN[36] have studied the possibilities of neutron activation in combination with paper electrochromatography for the analysis of alkaline metal

cations. For sodium and potassium with short irradiation periods and γ-ray spectrometry, this technique offers some advantages over flame photometry; with longer irradiation periods plus "cooling" and γ-spectrometry, an extremely sensitive method for rubidium and caesium becomes available. The relationships between the activities induced and the amounts of these ions are linear in the range 0.0001–0.001 mmole.

Cobalt and vanadium, trace elements of biological importance, have been determined in plant material by measurement of radioactivity induced on paper chromatograms[41,88]. Ash from 10 g of plant material was solubilized by heating with mineral acids and hydrogen peroxide, and then iron was extracted with ether, vanadium with cupferron in chloroform, and cobalt with diethyldithiocarbamate in chloroform. The vanadium and cobalt extracts were chromatographed on paper, and the chromatograms were irradiated with thermal neutrons at $5 \cdot 10^{11}$ n cm^{-2} sec^{-1} for 15 min. The reactions $^{51}V(n,\gamma)^{52}V$ ($T_{\frac{1}{2}}=3.7$ min) and $^{59}Co(n,\gamma)^{60m}Co$ ($T_{\frac{1}{2}}=10.7$ min) allow the determination of 0.1 μg of cobalt or vanadium on the chromatograms.

Sodium, copper, magnesium and bromine have been activated in meteorological samples labelled with sodium nitrate, copper nitrate, magnesium nitrate and ammonium bromide, and deposited on paper[31]. Powders or solutions of these non-radioactive compounds were injected into clouds, and then samples were collected with paper sheets and a high-volume air sampler. No chromatographic or electrophoretic separations were carried out. The samples were irradiated with a thermal neutron flux of 10^{12} n cm^{-2} sec^{-1} for 10 min, and, 5 min after irradiation, the γ-ray spectra of both sample and clean filter paper were measured. Less than 10^{-8} mole of sodium, copper, magnesium or bromine could be detected by this method.

The authors thank Dr. ANDRZEJ SIUDA for critical review of the manuscript and for his valuable suggestions.

SUMMARY

The use of activation analysis in conjunction with paper chromatography, and particularly activation after chromatography, is reviewed. Irradiation facilities, the use of markers, internal standards and general activation conditions are discussed, and detailed attention is given to the behaviour of various types of paper on activation, with especial reference to stability and impurities. Practical applications dealing with phosphorus, oxygen, iodine, arsenic and various metals are reviewed.

RÉSUMÉ

On passe en revue les possibilités d'emplois de l'analyse par activation combinée avec la chromatographie sur papier, en particulier l'activation après chromatographie. On examine les possibilités d'irradiation, l'emploi de marqueurs, les étalons internes et les conditions générales d'activation. Un intérêt tout particulier est porté au comportement de divers types de papiers sur l'activation, en prenant en considération la stabilité et les impuretés. On décrit des applications pratiques pour phosphore, oxygène, iode, arsenic et divers métaux.

ZUSAMMENFASSUNG

Es wird über die Verwendung der Aktivierungsanalyse in Verbindung mit der Papierchromatographie berichtet, besonders über die Aktivierung nach der Chromatographie. Das Bestrahlungsverhalten, die Verwendung von Markierungsmitteln, interner Standards und die allgemeinen Aktivierungsbedingungen werden diskutiert. Besondere Aufmerksamkeit wird dem Verhalten der verschiedenen Papiertypen bei der Aktivierung gewidmet. Unter Berücksichtigung der Stabilität und der Verunreinigungen. Praktische Anwendungen für Phosphor, Sauerstoff, Jod, Arsen und verschiedene Metalle werden angegeben.

REFERENCES

1 F. P. W. Winteringham, A. Harrison and R. C. Bridges, Radioactive tracer techniques in paper chromatography, *Nucleonics*, 10 (1952) 53.
2 K. Schmeiser and D. Jerchel, Quantitativer Nachweis von Schwefel, Chlor und Brom enthaltenden Verbindungen auf Papierchromatogrammen mit Hilfe induzierter Radioaktivität, *Angew. Chem.*, 65 (1953) 366.
3 K. Schmeiser and D. Jerchel, Phosphor-Bestimmung durch Neutron-Aktivierung in Papierelektropherogrammen, *Angew. Chem.*, 65 (1953) 490.
4 K. Schmeiser, Auswertung von Papierchromatogrammen mit Hilfe induzierter Aktivität, *Angew. Chem.*, 66 (1954) 107.
5 P. A. Sellers, T. R. Sato and H. H. Strain, Neutron irradiation of some crystalline salts of phosphoric, phosphorous and hypophosphorous acids in vacuum, *J. Inorg. & Nucl. Chem.*, 5 (1957) 31.
6 R. C. Koch, *Activation Analysis Handbook*, Academic Press, New York, 1960.
7 W. Schulze, *Neutronenaktivierung als analytisches Hilfsmittel*, F. Enke Verlag, Stuttgart, 1962.
8 P. Albert, in Albert de Visscher, *L'Analyse par Radioactivation*, Gauthier-Villars, Paris, 1964.
9 D. H. F. Atkins and A. A. Smales, Activation analysis, *Advan. Inorg. Chem. Radiochem.*, 1 (1959) 315.
10 H. J. M. Bowen and D. Gibbons, *Radioactivation Analysis*, Clarendon Press, Oxford, 1963.
11 M. Raković, Aktivačni analysa, *Chem. Listy*, 55 (1961) 893.
12 W. S. Lyon, Jr., *Guide to Activation Analysis*, D. Van Nostrand, Princeton, N.J., 1964.
13 D. Taylor, *Neutron Irradiation and Activation Analysis*, G. Newnes, London, 1964.
14 F. Girardi, Some recent developments in radioactivation analysis. A review of improvements in the analytical technique, *Talanta*, 12 (1964) 1017.
15 F. Baumgärtner, *Table of Neutron Activation Constants*, Verlag Karl Thiemig KG, Munich, 1967.
16 R. F. Coleman and T. B. Pierce, Activation analysis, *Analyst*, 92 (1967) 1.
17 H. J. M. Bowen, Neutron activation analysis, *Chimia (Aarau)*, 21 (1967) 113.
18 M. Raković, Kombinace neutrové aktivačni analysy s chromatografii na papiře, *Chem. Listy*, 58 (1964) 1141.
19 E. J. Hennelly, Intense Sb–Be sources make 10^{10} n/sec, *Nucleonics*, 19/3 (1961) 124.
20 F. P. W. Winteringham, Radioisotopes in microbiochemical research, *Proc. Intern. Symp. Microchem.*, Pergamon Press, London, 1958.
21 J. M. A. Lenihan, Activation analysis, *Nature*, 207 (1965) 347.
22 S. Amiel and A. Nir, Analysis of ^{18}O based on the reaction $^{18}O(\alpha, n)^{21}Ne$, *Proc. of the Symposium on Radiochemical Methods of Analysis, Salzburg*, Vol. 1, I.A.E.A., Vienna, 1965, p. 287.
23 M. Calvin, J. M. Anderson, J. A. Bassham, U. Blass, O. Holm-Hansen, V. Moses, N. G. Pon, P. B. Sogo and G. Tollin, Photosynthesis, *Proc. Sec. U.N. Intern. Conf. Peaceful Uses At. Energy, Geneva*, 24 (1958) 25.
24 I. Fogelström-Fineman, O. Holm-Hansen, B. M. Tolbert and M. Calvin, A tracer study with ^{18}O in photosynthesis by activation analysis, *Intern. J. Appl. Radiation Isotopes*, 2 (1957) 280.
25 C. Engelmann, Dosage de l'oxygène, du carbone, de l'azote et de quelques autres impurités dans le béryllium, le calcium, le sodium et le bore par activation aux rayons γ, *Proc. of the Symposium on Radiochemical Methods of Analysis, Salzburg*, Vol. I., I.A.E.A., Vienna, 1965, p. 341.
26 D. J. Hughes and R. B. Schwartz, *Neutron Cross Sections*, 2nd Edn., U.S. At. Energy Comm., New York, 1958.
27 J. M. Steim, Neutron activation chromatography, *Dissertation Abstr.*, 23 (1963) 3622.

28 J. M. STEIM AND A. A. BENSON, Derivative activation chromatography, *Anal. Biochem.*, 9 (1964) 21.
29 R. P. SPENCER AND K. R. BRODY, Detection and assay of compounds of biological interest by use of radiohalogenation, *Proc. of the Symposium on Radioisotope Sample Measurement Techniques in Medicine and Biology, Vienna, 24–28 May, 1965*, I.A.E.A., Vienna, 1965, p. 237.
30 H. J. M. BOWEN AND P. A. CAWSE, Determination of sodium, potassium and phosphorus in biological material by radioactivation, *Analyst*, 86 (1961) 506.
31 B. D. KYRIAZOPOULOS, G. C. LIVADAS AND P. N. DIMOTAKIS, A new tracer technique in clouds research, *Intern. J. Appl. Radiation Isotopes*, 17 (1966) 663.
32 L. H. TOWLE AND R. S. FARRAND, *Radiation Stability of Organic Liquids*, TID-6186, June, 1960.
33 P. JOHNSON, E. J. WEBER, H. E. CARTER AND M. S. KROBER, Detection of phospholipids on paper chromatograms by neutron activation, *J. Lipid Res.*, 6 (1965) 425.
34 W. BOCK-WERTHMANN AND W. SCHULZE, A paper chromatographic separation method for activation analysis, *Proc. Intern. Conf. on Modern Trends in Activation Analysis*, 1965.
35 J. R. ROBINSON, Neutron activation of phosphorus on paper chromatograms, *Can. Nucl. Technol.*, 1/5 (1962) 25.
36 J. SHERMA AND H. H. STRAIN, Separation of the alkali metal cations from mixtures of various cations by electrochromatography, *Anal. Chem.*, 34 (1962) 76.
37 J. Z. BEER AND A. Z. BUDZYŃSKI, unpublished results.
38 K. MURRAY AND R. E. OFFORD, Use of neutron activation in the characterization of small quantities of nucleic acids, *Nature*, 211 (1966) 376.
39 M. FLIKKE AND E. STEINNES, Determination of base ratios in small samples of ribonucleic acid by neutron activation paper chromatographic analysis, *Arch. Biochem. Biophys.*, 118 (1967) 82.
40 P. PATEK AND H. SORANTIN, Determination of trace elements in chromatographic paper by neutron activation and γ-spectrometry, *Anal. Chem.*, 39 (1967) 1458.
41 A. A. BENSON, B. MARUO, R. J. FLIPSE, H. W. YUROW AND W. W. MILLER, Application of the nuclear reactor in biochemical analysis: neutron activation chromatography, *Proc. Sec. U.N. Intern. Conf. Peaceful Uses At. Energy, Geneva*, 24 (1958) 289.
42 J. R. ROBINSON, A technique for neutron activation of phosphorus compounds on paper chromatograms, *Can. J. Biochem. Physiol.*, 40 (1962) 1460.
43 A. GRÜNE, *Über chromatographische und elektrophoretische Filterpapiere*, C. Schleicher und Schüll, Dassel, undated.
44 A. GRÜNE, Cellulosepapiere für die Chromatographie, in K. MACEK and I. M. HAIS, *Stationary Phase in Paper and Thin-layer Chromatography*, Elsevier, Amsterdam, 1965, p. 15.
45 H. J. BORN AND H. STÄRK, Quantitative Bestimmung von Jod und jodhaltigen Verbindungen auf Papierchromatogrammen durch Neutronenaktivierung, *Atomkernenergie*, 4 (1959) 286.
46 M. RAKOVIČ AND H. TALPOVÁ, Příprava standardů pro neutronovou aktivační analysu, *Chem. Listy*, 57 (1963) 1183.
47 V. WYNN, A peptide-like contaminant of filter paper, *Nature*, 164 (1949) 445.
48 M. S. CATLETT, R. GIUFFRIA, A. T. MOORE AND M. I. ROLLINS, Microscopical observations on the contents of the cotton fiber lumen, *Textile Res. J.*, 21 (1951) 880.
49 *Whatman Papers for Chromatography and Electrophoresis*, Technical Bulletin C1, H. Reeve Angel, London, January 1965.
50 M. RAKOVIČ, Ozařování biologických tkání a jiných vzorků neutrony. I. Aktivace biologických tkání v reaktoru, *Sb. Lekar.*, 64 (1962) 216.
51 A. SIUDA, personal communication.
52 J. LUŠTINEC, The application of neutron activation chromatography to the determination of the purity of phosphate preparations, *Biol. Plant., Acad. Sci. Bohemoslov.*, 2 (1960) 67.
53 T. NĂŞCUŢIU, Analiza substanţelor anorganice prin radioactivare pe hîrtie cromatografică, *Studii Cercetari Chim. Acad. Rep. Populare Romine*, 10 (1962) 275.
54 L. HÖRHAMMER, H. WAGNER AND G. RICHTER, Zur papierchromatographischen Auftrennung von Phosphatiden. I. Mitteilung, Biochem. Z., 331 (1959) 155.
55 T. NĂŞCUŢIU, The analysis of inorganic substances by radioactivation on chromatographic paper. II. Thorium and rare earths, *Rev. Roumaine Chim.*, 9 (1964) 283.
56 W. LEE, Direct estimation of γ-ray abundances in radionuclide mixtures. Complement-subtraction method, *Anal. Chem.*, 31 (1959) 800.
57 A. A. BENSON, Neutron activation chromatography of phosphorus compounds, in S. P. COLOWICK AND N. O. KAPLAN, *Methods in Enzymology*, Academic Press, London, 6 (1963) 881.
58 F. POCCHIARI AND C. ROSSI, Quantitative radiopaper chromatography, *J. Chromatog.*, 5 (1961) 377.
59 J. P. ADLOFF, Application of chromatographic and electrophoretic methods to the study of the Szilard–Chalmers effect, *Chromatog. Rev.*, Vol. 7, Elsevier, Amsterdam, 1965, p. 52.
60 V. KNOBLOCH AND Z. URBANEC, Die Trennung einer Fraktion von seltenen Erden aus den

Spaltprodukten von ^{235}U, *Compte-rendu du XXXIe Congres International de Chimie Industrielle, Liège, 1958*, p. 1.
61 N. GETOFF, Papierchromatographische Trennung einiger Uranspaltprodukte, *Oesterr. Chemiker-Ztg.*, 62 (1961) 1.
62 N. GETOFF AND H. BILDSTEIN, Chromatographische Trennung einiger Uranspaltprodukte und des Urans mittels Ionenaustauschpapier, *Oesterr. Chemiker-Ztg.*, 66 (1965) 72.
63 N. GETOFF, H. BILDSTEIN, V. PFEIFER, H. SORANTIN AND H. TITZE, Analysis of nuclear fuel elements. III. Separation of some uranium fission products by paper chromatograph, *Atompraxis*, 12 (1966) 563.
64 P. N. DIMOTAKIS AND D. C. PERRICOS, Two-dimensional paper chromatography of fission products of natural uranium, *Radiochim. Acta*, 8 (1967) 15.
65 M. ÖRDÖGH AND M. CSAJKA, Neutronenaktivierungsanalyse von Verunreinigungen. Kobalt und Nickel in Uranverbindungen nuklearer Reinheit, *Mikrochim. Acta*, (1966) 606.
66 M. ÖRDÖGH, E. SZABÓ AND D. HEGEDÜS, Determination of impurities in reactor-grade uranium oxide, *Radiochemical Methods of Analysis*, Vol. I, I.A.E.A., Vienna, 1965, p. 175.
67 M. ÖRDÖGH AND V. UPOR-JUVANCZ, Analysis of contaminants in uranium compounds by post-irradiation paper chromatography, *J. Chromatog.*, 25 (1966) 464.
68 R. COULOMB, Dosage de quelques oligoéléments dans les matériaux géologiques par irradiation neutronique et chromatographie sur papier, *Compt. Rend.*, 254 (1962) 4328.
69 R. COULOMB AND J. C. SCHILTZ, Chromatographie sur papier et calcul éléctronique en irradiation neutronique: application au dosage en grande série des oligoéléments dans les matériaux géologiques, *Proc. of the Symposium on Radiochemical Methods of Analysis, Salzburg*, Vol. II, I.A.E.A., Vienna, 1965, p. 177.
70 J. C. SCHILTZ AND C. COQUEMA, L'activation neutronique en géochimie: méthode de dosage de Ta, Th, Co et Sc, *Bull. Soc. Franc. Mineral. Crist.*, 87 (1964) 156.
71 H. A. DAS AND J. G. RAAPHORST, Some applications of paper-chromatographic separation in activation analysis of inorganic substances, *Proc. of the Meeting: Journées Hellènes de Séparation immédiate et de Chromatographie*, Athens, 1965, p. 71.
72 W. BOCK-WERTHMANN, Atomkernenergie-Dokumentation beim Gmelin Institut, AED-C-14-01, 1961, AED-C-14-02, 1962, AED-C-10-03, 1964.
73 E. H. STRICKLAND AND A. A. BENSON, Neutron activation–paper chromatographic analysis of phosphatides in mammalian cell fractions, *Arch. Biochem. Biophys.*, 88 (1960) 344.
74 F. NAKAYAMA AND R. BLOMSTRAND, Neutron activation paper chromatographic analysis of phospholipids in human liver and bile, *Acta Chem. Scand.*, 15 (1961) 1595.
75 R. BLOMSTRAND AND F. NAKAYAMA, Application of neutron activation–paper chromatographic analysis of phospholipids in clinical investigation, *Scand. J. Clin. & Lab. Invest.*, 14 (1962) 28.
76 J. HÖLZL, Über die Neutronaktivierung der Phosphatide, *Naturwissenschaften*, 54 (1964) 241.
77 A. SIUDA, Separation of some inorganic and phenyl phosphorus compounds by paper chromatography and paper electrophoresis, *Nukleonika*, 10 (1965) 459.
78 M. HALMANN AND L. KUGEL, Paper chromatography and paper electrophoresis of methylphosphonic, methylphosphinic and dimethylphosphinic acids and of trimethylphosphine oxide, *Bull. Res. Council Israel*, 10A (1961) 124.
79 F. P. W. WINTERINGHAM, P. M. BRIDGES AND G. C. HELLYER, Mode of insecticidal action studied with labelled systems. Phosphorylated compounds in the muscle of the adult housefly, *Musca domestica L.*, *Biochem. J.*, 59 (1955) 13.
80 H. STÄRK AND D. KNORR, Zur Bestimmung von Schilddrüsenhormonen auf Papierchromatogrammen durch Neutronaktivierung, *Atomkernenergie*, 6 (1961) 408.
81 F. BAUMGÄRTNER AND A. SCHÖN, Eine papierchromatographische Trennung der isomeren Dijodbenzole und ihr aktivierungsanalytischer Nachweis, *J. Chromatog.*, 13 (1964) 266.
82 A. DIMITRIADOU, T. R. FRASER, J. D. H. SLATER AND P. C. R. TURNER, Staining for iodide in chromatograms of human plasma: an artefact due to thiourea or thiouracils, *Nature*, 187 (1960) 691.
83 A. DIMITRIADOU, R. FRASER AND P. C. R. TURNER, Iodotyrosine-like substances in human serum, *Nature*, 201 (1964) 575.
84 A. DIMITRIADOU, P. C. R. TURNER AND T. R. FRASER, Activation analysis of paper chromatograms for iodine ($^{127}I \rightarrow {}^{128}I$), *Nature*, 197 (1963) 446.
85 A. DIMITRIADOU, P. C. R. TURNER AND R. FRASER, Activation analysis of paper chromatograms for iodine, *Nature*, 198 (1963) 576.
86 A. DIMITRIADOU, P. C. R. TURNER, J. D. H. SLATER AND R. FRASER, Iodotyrosine-like material in serum detected by analysis for ^{127}I but not ^{131}I, *Biochem. J.*, 82 (1962) 20P.
87 A. SIUDA, Paper chromatography and paper electrophoresis of some inorganic and phenyl arsenic compounds, *Nukleonika*, 10 (1965) 393.
88 H. W. YUROW, Determination of vanadium and cobalt in plants by neutron activation analysis of paper chromatograms, *Dissertation Abstr.*, 21 (1960) 46.

Analytica Chimica Acta
Elsevier Publishing Company, Amsterdam
Printed in The Netherlands

SHORT COMMUNICATIONS

The analytical concentration of traces of dissolved organic materials from sea water with Amberlite XAD-1 resin

There is widespread interest in the dissolved organic components of sea water. This stems partly from the role that many of them (*e.g.* vitamins or essential amino acids) play in the marine food chain, and partly from a growing appreciation of the hazards arising from contamination of the sea with toxic chemicals, such as pesticides and to a lesser extent detergents. Owing to the high concentrations of inorganic ions and to the low concentrations of dissolved organic components, the latter are often difficult to determine in sea water. Unless bio-assay techniques are used it is almost always necessary to carry out a preconcentration step. Only comparatively few techniques are suitable for this purpose[1], and for some types of compound no efficient methods of concentration are available. Recently, the Rohm and Haas Company have produced a cross-linked polystyrene resin—Amberlite XAD-1—which they claim[2] has the ability to adsorb dissolved organic molecules, such as surfactants and fatty acids, from aqueous solutions. This resin is in the form of beads (20–50 mesh) each of which is a conglomerate of a large number of microbeads. The hydrophobic portions of the organic molecules are adsorbed to the surfaces of these microbeads primarily by van der Waals forces, while their hydrophilic portions are oriented in the aqueous phase occupying the space between the microbeads. This note describes an investigation into the utility of this material for the concentration of traces of dissolved organic compounds from sea water.

Adsorption studies were carried out with 1-cm diameter ion-exchange columns which had been packed to a depth of 7 cm with Amberlite XAD-1 resin. Before use the resin columns were washed first with distilled water until free from chloride, then with 200 ml of ethanol, and finally with 200 ml of water. Aliquots (1 l) of sea water (filtered through a 0.5 μ filter) were spiked with appropriate amounts of the substance under investigation and adjusted to the required pH value. The spiked sea water was allowed to percolate through the columns at a rate of *ca.* 5 ml/min. The columns were washed with water of the same pH value and eluted with successive 10-ml aliquots of an appropriate eluant. The eluates, and where possible the percolates, were analyzed for the added substance by some suitable method; radiochemical techniques were employed when radioactive compounds were available.

The uptake of more than 30 different materials was investigated over the pH range 2–9. It was found that at levels of *ca.* 2–5 μg/l, none of the carbohydrates (arabinose, fructose, glucose, mannose), amino acids (alanine, arginine, aspartic acid, cystine, methionine, valine, mixed amino acids of *Chlorella*), protein (from *Chlorella*), or phenols (phenol, resorcinol) examined were taken up to a detectable extent by the resin at any pH value in this range. Inorganic cations and anions did not appear to be taken up at all. Several classes of organic compounds were taken up quantitatively from sea water and could be recovered completely by elution with a suitable reagent (see Table I). The quantitative concentration of members of each of the three classes

TABLE I

COMPOUNDS QUANTITATIVELY ADSORBED BY AMBERLITE XAD-1 RESIN FROM SEA WATER

Compound	*Concn. (μg/l)*	*Optimum pH*	*Percent retention*	*Eluting agent*	*Eluant vol. (ml)*	*Total % recovery*	*Analytical method*
n-Heptanoic acid	5.0	2.0	100	2 *N* NH_4OH	100	100	^{14}C-counting
n-Heptadecanoic acid	5.0	2.0	100	1 *N* alc. KOH	100	100	^{14}C-counting
4-Ketoglutaric acid	0.5	7.6	100	C_2H_5OH	50	100	^{14}C-counting
Cholesterol	10.0	2.0	100	C_2H_5OH	100	100	Fluorimetric[3]
Pregnenalone	1.0	2.0	100	C_2H_5OH	50	100	^{14}C-counting
Pyridostygmine	1.0	5.0	35	2 *N* HNO_3	50	35	^{14}C-counting
Vitamin B_2	2.5	2.0	100	C_2H_5OH	50	100	Fluorimetric[4]
Vitamin B_{12}	2.0	7.6	100	C_2H_5OH	50	100	^{57}Co-counting
Surfactants							
Teepol[a]	300	2.0	100	C_2H_5OH	50	100	Photometric[5]
Hyamine 2389[b]	5000	2.0	100	C_2H_5OH	50	100	Photometric[6]
Triton X-100[c]	5000	2.0	100	C_2H_5OH	50	100	Photometric[7]
Nonidet P80[d]	1000	2.0	100	C_2H_5OH	50	100	Photometric[7]
Insecticides							
Lindane[e]	1.0	2.0	100	C_2H_5OH	50	100	^{14}C-counting
D.D.T.[f]	1.0	2.0	100	C_2H_5OH	100	100	GLC[8]
Endrin[g]	10.0	2.0	75	C_2H_5OH	50	75	GLC[8]
Malathion[h]	10.0	2.0	100	C_2H_5OH	50	100	GLC[8]
Dyes							
Rhodamine B	200	7.6	100	C_2H_5OH	100	100	Photometric
Methylene blue	100	7.6	100	2 *N* HNO_3	150	100	Photometric
Humic acids							
Humic acids (peat)	—	2.0	100	0.2 *N* KOH	100	—	Photometric
Humic acids (river water)	—	2.0	100	0.2 *N* KOH	100	—	Photometric
Humic acids (sea water)	—	2.0	100	0.2 *N* KOH	100	—	Photometric

[a] Anionic type—alkylbenzene sulphonate.
[b] Cationic type—methyldodecylbenzyltrimethylammonium chloride + methyldodecylxylylenebistrimethylammonium chloride.
[c] Nonionic—condensed iso-octylphenoxypolethoxyethanol with ethylene oxide.
[d] Nonionic—condensed dioctylphenol with ethylene oxide.
[e] γ-Benzene hexachloride.
[f] Dichlorodiphenyltrichloroethane.
[g] 1,2,3,4,10,10-Hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4,5,8-*endo-endo*-dimethanonaphthalene.
[h] O,O-Dimethyl S-1,2-di(ethoxycarbonyl)ethyl phosphorodithioate.

of surfactants is of particular interest since these materials are notoriously difficult to determine at low concentrations. At present, the determination of fatty acids and insecticides at low levels in sea water is generally carried out after preconcentration by solvent extraction. The extraction of the large volumes of water which must be taken for the analysis is difficult and the use of the adsorption technique for the preconcentration is much simpler and more efficient.

The resin is very effective for the removal of humic substances from acidified (pH 2) natural waters. Fractionation of these materials can be obtained by successive

elution of the column with water at pH 7, 1 *N* ammonia solution and 0.2 *N* potassium hydroxide. The adsorbed humic compounds of both river water and sea water (*Gelbstoff*) give similar elution patterns.

Department of Oceanography,
The University,
P.O. Box 147,
Liverpool L69 3BX (England)

J. P. RILEY
D. TAYLOR

1 J. P. RILEY, in J. P. RILEY AND G. SKIRROW, *Chemical Oceanography*, Vol. 2, Academic Press, London, 1965.
2 *Rohm and Haas Export Department Technical Bulletin EX-IE-12*, 1966.
3 D. B. MCDOUGAL AND H. S. FARMER, *J. Lab. Clin. Med.*, 50 (1957) 485.
4 ASSOCIATION OF VITAMIN CHEMISTS, *Methods of Vitamin Assay*, Interscience, 1947.
5 J. LONGWELL AND W. D. MARRIENCE, *Analyst*, 80 (1955) 167.
6 J. AUERBACK, in J. M. ROSEN AND H. A. GOLDSMITH, *Systematic Analysis of Surface Active Agents*, Interscience, 1960.
7 N. T. CRABB AND H. E. PERSINGER, *J. Am. Chem. Soc.*, 41 (1964) 752.
8 E. S. GOODWIN, R. GOULDON AND J. G. REYNOLDS, *Analyst*, 86 (1967) 697.

(Received April 2nd, 1969)

Anal. Chim. Acta, 46 (1969) 307–309

Coulometric generation of manganese(III)

The coulometric generation of manganese(III) from sulfuric acid solution has been studied by several workers[1–3]. Although FENTON AND FURMAN[3] included small amounts of phosphoric acid in their generating electrolytes, no studies have been made in a pure phosphoric acid medium. This report discusses the current generation efficiency of manganese(III) from phosphoric acid and a wide range of aqueous phosphoric–sulfuric acid mixtures.

Reagents

Manganese(II) perchlorate ($Mn(ClO_4)_2 \cdot 6H_2O$) and iron(II) ethylenediammonium sulfate (Oesper's reagent; $FeC_2H_4(NH_3)_2(SO_4)_2 \cdot 6H_2O$) were supplied by the G. Frederick Smith Chemical Co. Oesper's reagent was assayed by potentiometric titration with standard potassium dichromate solution.

Sulfuric and phosphoric acids, reagent grade, were used as received. All water was deionized.

Experimental

Coulometric experiments were performed with a Sargent Model IV Coulometric Current Source, and a coulometric titration cell kit, Leeds and Northrup Co. #7961. The coulometer was calibrated against a precision resistor and potentiometer. A current level of 9.64 mA was used, corresponding to a current density on the platinum anode of 0.4 mA/cm^2. The end-point detection system of COOKE, REILLEY AND

FURMAN[4] was employed, incorporating a saturated calomel electrode as reference electrode, and a voltage source previously described[5].

Current generation efficiencies were determined by generating manganese(III) from 0.1 *M* manganese(II) perchlorate, followed by the addition of a weighed quantity

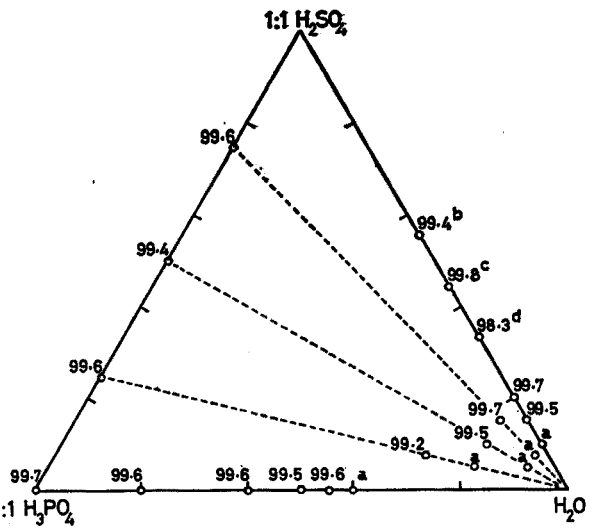


Fig. 1. Current generation efficiencies of Mn(III) in solutions of sulfuric acid, phosphoric acid, and their mixtures. The points at which decreasing efficiencies begin (see text) are denoted by a; the values at b, c and d are taken from the literature (see references 1, 2 and 3 respectively).

of Oesper's reagent 1–2% in excess, and completion of the titration. Titration efficiencies were determined in the usual way, the iron(II) salt being added at the outset. Purified nitrogen was used to deaerate the solutions initially, and for blanketing during the titrations.

Results

The current generation efficiencies of manganese(III) in different phosphoric acid–sulfuric acid–water mixtures are summarized in Fig. 1. The values given are the average of at least four determinations, and have an average deviation of not more than 0.2%. Determinations in solutions of higher acid concentrations were eventually limited by the insolubility of manganese(II) perchlorate and of Oesper's reagent.

Titration efficiency was determined in 7.4 *M* phosphoric acid to be 100.07±0.04%, and in 1.48 *M* phosphoric acid as 99.24±0.08%, each solution being 0.1 *M* in manganese(II) perchlorate. The data in Fig. 1 show that there is a broad range of acid concentrations in which generation efficiency is essentially constant. The lower limit of acidity depends on the acid present; it is lowest for sulfuric acid solutions, highest for phosphoric acid solutions, and intermediate for mixtures of the two acids. In all cases there is a short range of concentrations between those at which high current efficiencies (>99%) exist and those at which efficiencies decrease linearly with amount of charge passed. Figure 2 illustrates this behaviour for two such cases. The gradual decrease is accompanied by slow plating of the platinum anode, usually with concomitant evolution of oxygen. The nature of this electrode

deposit and its appearance in relation to the solution composition are presently under investigation.

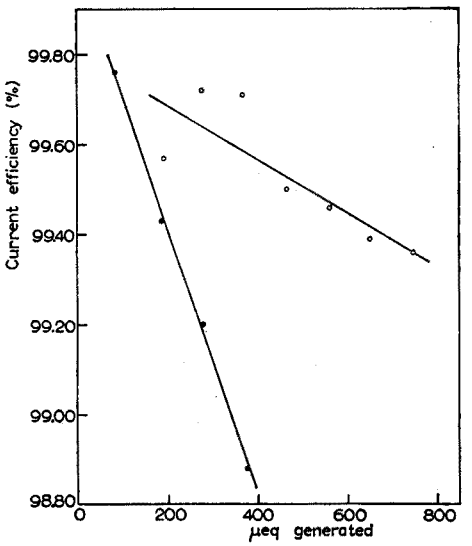


Fig. 2. Decrease in current generation efficiency of Mn(III) with successive determinations. (○) 20% (v/v) H_3PO_4. (●) 3:1 H_3PO_4–H_2SO_4 diluted 1:9.

The authors wish to express their gratitude to the National Research Council of Canada for financial support and to the Province of Ontario for the award of a fellowship to one of us (G.A.B.).

Department of Chemistry,
University of Waterloo,
Waterloo, Ontario (Canada)

G. F. ATKINSON
G. A. BRYDON

1 R. G. SELIM AND J. J. LINGANE, *Anal. Chim. Acta*, 21 (1959) 536.
2 R. P. BUCK, *Anal. Chem.*, 35 (1963) 592.
3 A. J. FENTON AND N. H. FURMAN, *Anal. Chem.*, 32 (1960) 745.
4 W. D. COOKE, C. N. REILLEY AND N. H. FURMAN, *Anal. Chem.*, 24 (1952) 205.
5 G. F. ATKINSON, *J. Chem. Educ.*, 42 (1965) 656.

(Received January 27th, 1969)

Analytica Chimica Acta
Elsevier Publishing Company, Amsterdam
Printed in The Netherlands

The detection of barium by induced precipitation

The alkaline earth metals have been reported to induce the precipitation of lead sulphate from an ammonium acetate solution of that salt[1]. This induction effect has been used to detect as little as 0.4 μg of barium in 0.05 ml. A modified version was developed recently in which ethanol was added to a lead sulphate–ammonium acetate solution to cause supersaturation[2]; the reliable detection of 0.1 μg of barium or 0.2 μg of strontium per ml was then possible. The induction was explained by the formation of sub-microscopic barium or strontium sulphate particles acting as nuclei for the subsequent bulk precipitation of the lead salt from the supersaturated solution.

FEIGL's original procedure[1] is simpler than the modified version[2] in that the reproducible addition of ethanol is not necessary. However, the mechanism of the original test is not clear. It is difficult to understand why lead sulphate should dissolve in ammonium acetate to give a supersaturated solution which will later reprecipitate lead sulphate. An attempt to clarify the mechanism is described below.

Tests showed that 1.4 g of lead sulphate was just completely dissolved in 31 ml of water by adding 12 g of ammonium acetate. However, all solutions in which more than 7 g of ammonium acetate were used to dissolve part or all of the lead sulphate in 31 ml, deposited crystals after a certain time (a few min if 12 g was used, 2 days if 7 g was used). When less than 5 g was used, the resulting solution was stable for at least a month, but precipitation could be induced by adding μg amounts of barium. A solution containing 5 g of ammonium acetate was used subsequently since spontaneous precipitation did not occur. Temperature measurements showed that the supersaturation did not arise from the cooling of an initially warm reagent solution.

FEIGL's reagent solution[1] was prepared by adding sulphuric acid to lead acetate solution to precipitate lead sulphate, which was subsequently dissolved in the supernate by addition of ammonium acetate. Tests with sodium sulphate and sulphuric acid showed that an equimolar concentration of acid and salt gave more immediate induced precipitation. Moreover, dissolution of the lead sulphate precipitate by adding 5 g of ammonium acetate and 5 g of sodium acetate also accelerated the onset of induced precipitation, without affecting the stability of the reagent. Sodium acetate alone also dissolved the precipitate, but induced precipitation was much slower. The optimal preparation of the reagent solution is given below.

This reagent allows the detection of as little as 0.1 μg of barium in 0.4 ml by noting the appearance of a visible turbidity within 30 sec. Barium sulphate alone does not give a visible turbidity at this concentration. The sensitivity is 30 times greater than that of the original test[1], and is almost as great as that of the modified version[2]. Moreover, the test has a reduced sensitivity to strontium and calcium. The detection limit for strontium is 12 μg and for calcium is 500 μg. Lead (>120 μg) and iron(II) or silver (100 μg) interfered by giving precipitates, but 100 μg of Co^{2+}, Ni, Zn, Hg^{2+}, Al, Mn^{2+}, Cr^{3+}, Cd, Cu^{2+}, Cu^{+}, I^{-} or Cl^{-} were without effect. Iron(III) gave a deep red colour with the reagent.

The induced precipitate consisted of hexagonal plates of various degrees of perfection, whereas lead sulphate forms orthorhombic crystals[3]. Moreover, the crystals contained ammonia as well as lead and sulphate, but were free of acetate.

Analysis showed that the precipitate was not homogeneous, so that its exact composition could not be established; the results, however, agreed reasonably well with those calculated for $(NH_4)_2Pb(SO_4)_2$. Dissolution of this precipitate in water or dilute acid results in the precipitation of lead sulphate. Crystals of $(NH_4)_2Pb(SO_4)_2$ are reported to be hexagonal[4], and have been formed by crystallisation from a solution of lead sulphate containing ammonium acetate and ammonium sulphate[4].

It is possible, therefore, to deduce a mechanism for the induced precipitation. Lead sulphate dissolves in the ammonium acetate–sulphate solution to give a supersaturated solution of lead ammonium sulphate. If the supersaturation is not too great, this solution is stable for at least a month. Addition of barium, however, produces minute barium sulphate particles which act as nuclei for the precipitation of the lead ammonium sulphate, so that precipitation can then occur rapidly. It would seem that lead sulphate is not an efficient nucleating agent.

Experimental

Preparation of reagent solution. Add to a 10% lead acetate solution (15 ml) a mixture of *M* sulphuric acid (7.8 ml) and *M* sodium sulphate solution (7.8 ml). Add ammonium acetate (5 g) and sodium acetate (5 g), both as their hydrated salts, and swirl for a few min to dissolve the lead sulphate partially. Filter through Whatman No. 42 paper.

Detection of barium. To a small test tube, add 4 drops of test solution followed by 4 drops of reagent solution. Shake. The appearance of a turbidity in the bulk of the solution within 30 sec indicates the presence of barium. Sensitivity: 0.1 μg of Ba; dilution limit: $1:4\cdot10^6$.

Precautions. All glassware, especially the bottle used for storing the reagent, should be thoroughly washed with EDTA and then with distilled water before use. Contamination of the walls of glassware can function as nucleation centres and lead to crystal growth on the walls. Experiments in which this occurs should be rejected.

Analysis of precipitate. Found: C=0.1%, H=3.4%, N=6.3%, $PbSO_4$=72% (all means of 2 results); calculated for $(NH_4)_2Pb(SO_4)_2$, C=0.0%, H=1.8% N=6.4%, $PbSO_4$=70%.

The authors thank Professor R. BELCHER for his valuable suggestions and encouragement. J. W. H. thanks the Association of Commonwealth Universities for a Commonwealth Scholarship.

Department of Chemistry,
The University, P.O. Box 363,
Birmingham 15 (England)

JAMES W. HAMYA
A. TOWNSHEND

1 F. FEIGL, *Spot Tests in Inorganic Analysis*, 5th Edn., Elsevier, Amsterdam, 1958, p. 218.
2 A. VAUGHAN AND A. TOWNSHEND, *Anal. Chim. Acta*, 43 (1968) 134.
3 F. C. PHILLIPS, *An Introduction to Crystallography*, Longmans Green, London, 1956, p. 68.
4 C. K. MØLLER, *Acta Chem. Scand.*, 8 (1954) 81.

(Received March 17th, 1969)

Formation of zinc chelates of nitroso-Schäffer's acid and nitroso-C acid in aqueous solution

The metal-chelating properties of nitroso-Schäffer's acid (1-nitroso-2-naphthol-6-sulphonic acid) are similar to those of nitroso-R acid (1-nitroso-2-naphthol-3,6-disulphonic acid). The sodium salt of nitroso-R acid (nitroso-R salt) is a well-known analytical reagent[1]. Nitroso-C acid (2-nitroso-1-naphthol-5-sulphonic acid) resembles the better known 2-nitroso-1-naphthol-4-sulphonic acid. Its sodium salt has also been used in analysis for certain metal ions, particularly for cobalt(II) ions[2]. The names used for these water-soluble sulpho derivatives of *o*-nitrosonaphthols in the present work were derived from the parent naphtholsulphonic acids: nitroso-Schäffer's acid from Schäffer's acid (2-naphthol-6-sulphonic acid) and nitroso-C acid from C-acid (1-naphthol-5-sulphonic acid). They were prepared from the parent naphtholsulphonic acids by nitrosation.

Although the *o*-nitrosonaphthol groupings in these compounds are the same as in nitroso-R acid and in 2-nitroso-1-naphthol-4-sulphonic acid, they differ from these in the extent of naphtholic dissociation, and thus the chelates formed with a metal differ in stability. The values of the stability constants of several metal chelates (including zinc chelates) of nitroso-R acid and 2-nitroso-1-naphthol-4-sulphonic acid were reported earlier[3]. The results of a potentiometric study on the formation reactions of the zinc chelates of nitroso-Schäffer's acid and nitroso-C acid in aqueous solutions are presented below.

Experimental

The sodium salt of nitroso-Schäffer's acid was prepared from the sodium salt of 2-naphthol-6-sulphonic acid (purified, B.D.H., Ltd) and the sodium salt of nitroso-C acid from the sodium salt of C-acid (purified, B.D.H., Ltd). The nitrosation reactions were carried out by acidifying cooled aqueous solutions of these salts containing equivalent amounts of sodium nitrite with hydrochloric acid. The precipitated nitroso salts were recrystallized twice from water as dihydrates. The purities of the preparations were controlled by neutralization titrations.

The zinc salt was zinc perchlorate. Potassium chloride was added as neutral salt to increase the ionic strengths of the solutions.

A Radiometer PHM 4c potentiometer equipped with a Beckman glass electrode and an open-bridge potassium chloride reference electrode was used for potentiometric titrations which were carried out in a nitrogen atmosphere in a thermostatted system at 25°. Apparent activity coefficient values taken from NÄSÄNEN *et al.*[4] were used to calculate the hydrogen ion concentrations in the solutions.

The dissociation constants of the acids relating to the equilibrium

$$HL^- \overset{K_2}{\rightleftharpoons} L^{2-} + H^+ \qquad (1)$$

were calculated by the usual method[3].

The formation constants of the first and second zinc chelates relating to the successive chelation equilibria

$$HL^- + Zn^{2+} \overset{K_1}{\rightleftharpoons} ZnL + H^+ \qquad (2)$$

$$HL^- + ZnL \overset{K_{II}}{\rightleftharpoons} ZnL_2^{2-} + H^+ \quad (3)$$

were evaluated by a method described earlier[3] from potentiometric titration data for solutions of zinc perchlorate containing ligand in excess.

The equations of the Debye–Hückel type assumed to express the dependence of the equilibrium constants on ionic strength were

$$pK_2 = pK_2^0 - 2.036\sqrt{\mu}/(1+\alpha\sqrt{\mu}) + B\cdot\mu \quad (4)$$

for the dissociation reactions, and

$$pK_I = pK_I^0 + 2.036\sqrt{\mu}/(1+\alpha\sqrt{\mu}) \quad (5)$$

$$pK_{II} = pK_{II}^0 - 2.036\sqrt{\mu}/(1+\alpha\sqrt{\mu}) \quad (6)$$

for the chelation reactions. The parameters α and B of these equations and the thermodynamic values of the equilibrium constants (pK^0's) at 25° were computed by fitting the equations to the data by the method of least squares.

Results and discussion

The values of the second dissociation constants of nitroso-Schäffer's acid and nitroso-C acid were determined from data from several titrations of solutions of varying ionic strength (Table I). The value $pK_2 = 7.22$ for nitroso-Schäffer's acid at ionic strength 0.1 (KCl) was calculated from eqn. (4) with the parameter values $\alpha = 1.87$ and $B = 0.24$. Nitroso-C acid is a slightly stronger acid than nitroso-Schäffer's

TABLE I

DISSOCIATION CONSTANTS OF NITROSO-SCHÄFFER'S ACID AND NITROSO-C ACID AT DIFFERENT IONIC STRENGTHS AND 25°

(The calculated values were obtained by means of eqn. (4))

Nitroso-Schäffer's acid			*Nitroso-C acid*		
$\sqrt{\mu}$	pK_2		$\sqrt{\mu}$	pK_2	
	Observed	*Calculated*		*Observed*	*Calculated*
0.083	7.471	7.459	0.083	7.170	7.169
0.103	7.427	7.431	0.103	7.137	7.138
0.139	7.384	7.384	0.139	7.095	7.088
0.240	7.274	7.280	0.240	6.966	6.974
0.327	7.213	7.216	0.327	6.896	6.902
0.501	7.133	7.136	0.503	6.824	6.815
0.705	7.107	7.101	0.706	6.790	6.793
0.980	7.128	7.125			
1.366	7.258	7.260			

acid. The value $pK_2 = 6.91$ ($\mu = 0.1$) was obtained for nitroso-C acid (eqn. (4), $\alpha = 1.34$ and $B = 0.42$). Also the acid strength of the naphtholic hydroxyl group is lower in nitroso-Schäffer's acid than in nitroso-R acid ($pK_3 = 6.88$, $\mu = 0.1$, KCl[5]). The same applies to nitroso-C acid and the previously studied 2-nitroso-1-naphthol-4-sulphonic acid ($pK_2 = 6.10$, $\mu = 0.1$, KCl[3]).

It is interesting to compare the acid strengths of the naphtholic hydroxyl groups of nitroso-Schäffer's acid and nitroso-C acid with those of the nitrosonaphthols and the parent naphtholsulphonic acids. The following values at ionic strength 0.1 and 25° have been reported: $pK=7.63$ for 1-nitroso-2-naphthol, $pK=7.24$ for 2-nitroso-1-naphthol (both values in 0.1 *M* $NaClO_4$)[6], $pK_2=8.72$ for 2-naphthol-6-sulphonic acid (Schäffer's acid) and $pK_2=8.80$ for 1-naphthol-5-sulphonic acid (C-acid)[7]. Extrapolated values at zero ionic strength obtained in the present study were $pK_2^0=7.60$ for nitroso-Schäffer's acid and $pK_2^0=7.32$ for nitroso-C acid (Table III).

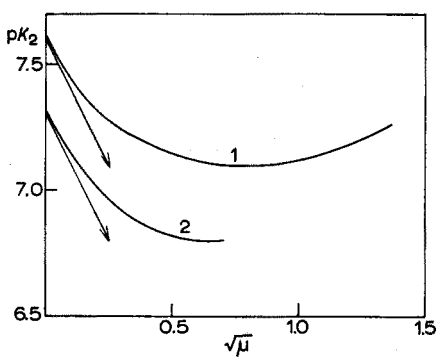
Data from titrations of the sodium salts of nitroso-Schäffer's acid and nitroso-C acid with 0.1 *M* sodium hydroxide solution in the presence of zinc perchlorate are shown in Table II. The formation of the first and second zinc chelates was assumed to occur in accordance with eqns. (2) and (3). Titrations of solutions of higher ionic strength were possible up to a neutral salt concentration of *ca.* 0.5 *M*, above which precipitation occurred when the formation of the second chelate was almost complete, particularly in the case of nitroso-C acid.

TABLE II

FORMATION CONSTANTS OF ZINC CHELATES AT DIFFERENT IONIC STRENGTHS

(KCl as neutral salt)

Nitroso-Schäffer's acid			*Nitroso-C acid*		
μ (mean)	$pK_I(ZnL)$	$pK_{II}(ZnL_2^{2-})$	*μ (mean)*	$pK_I(ZnL)$	$pK_{II}(ZnL_2^{2-})$
0.008	2.73	3.96	0.008	3.03	4.51
0.116	3.04	3.63	0.116	3.34	4.17
0.256	3.21	3.53	0.255	3.50	4.01
0.504	3.40	3.49	0.503	3.70	3.87



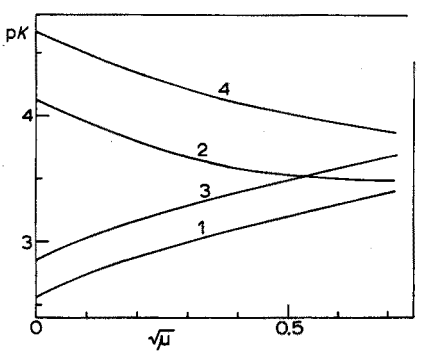


Fig. 1. The effect of ionic strength on the dissociation of (1) nitroso-Schäffer's acid and (2) nitroso-C acid.

Fig. 2. The formation constants of zinc chelates as functions of the square root of ionic strength. (1) ZnL and (2) ZnL_2^{2-} of nitroso-Schäffer's acid and (3) ZnL and (4) ZnL_2^{2-} of nitroso-C acid.

The curves in Figs. 1 and 2 show the p*K* values as functions of ionic strength. Computed values of α in the Debye–Hückel equations for the zinc chelates are given in Table III.

TABLE III

COMPUTED VALUES OF THE PARAMETERS OF THE DEBYE–HÜCKEL EQUATIONS AND THE THERMODYNAMIC CONSTANTS AT 25°

	Equation	α	B	pK^0
Nitroso-Schäffer's acid	(4)	1.87	0.24	$pK_2^0 = 7.60$
Chelate ZnL	(5)	1.02		$pK_I^0 = 2.55$
Chelate ZnL_2^{2-}	(6)	1.81		$pK_{II}^0 = 4.09$
Nitroso-C acid	(4)	1.34	0.42	$pK_2^0 = 7.32$
Chelate ZnL	(5)	1.03		$pK_I^0 = 2.84$
Chelate ZnL_2^{2-}	(6)	1.11		$pK_{II}^0 = 4.67$

TABLE IV

STABILITY CONSTANTS OF THE ZINC CHELATES AT IONIC STRENGTH 0.1 (KCl)

	$pK_2(HL^-)$	$\log \beta_1$	$\log \beta_2$
Nitroso-Schäffer's acid	7.22	4.19	7.73
Nitroso-C acid	6.91	3.58	6.29

The constants $\beta_1=[ZnL]/[Zn^{2+}][L^{2-}]$ and $\beta_2=[ZnL_2^{2-}]/[Zn^{2+}][L^{2-}]^2$ were calculated from the expressions $\beta_1=K_I/K_2$ and $\beta_2=K_IK_{II}/K_2^2$ (Table IV).

Nitroso-Schäffer's acid thus forms stronger zinc chelates than nitroso-C acid. The stability of the first zinc chelate of nitroso-Schäffer's acid is, however, slightly lower than that of the first zinc chelate ZnL^- of nitroso-R acid ($\log \beta_1=4.46$, $\mu=0.1$)[3], both of which acids have the same 1-nitroso-2-naphthol grouping. The order of stabilities of the first zinc chelates of the 2-nitroso-1-naphthol derivatives, nitroso-C acid (2-nitroso-1-naphthol-5-sulphonic acid) and 2-nitroso-1-naphthol-4-sulphonic acid ($\log \beta_1=3.06$, $\mu=0.1$, for ZnL)[3], is the opposite.

The logarithms of the stability constants of the zinc chelates of nitroso-Schäffer's acid at zero ionic strength and 25° are $\log \beta_1^0=5.05$ and $\log \beta_2^0=8.56$, and those of the zinc chelates of nitroso-C acid $\log \beta_1^0=4.48$ and $\log \beta_2^0=7.13$ (Table III).

The stabilities of the zinc chelates, which chelates in this case are of medium strength, characterize the abilities of metal-chelate formation in general. As one would expect, both of the studied reagents, nitroso-Schäffer's acid and nitroso-C acid, form coloured, strong chelates with divalent cobalt, palladium, iron and copper ions.

A grant awarded by the National Research Council for Sciences (Finland) is gratefully acknowledged.

Department of Inorganic Chemistry,
University of Helsinki,
Helsinki 17 (Finland)

OSMO MÄKITIE
HEIKKI SAARINEN

1 H. S. VAN KLOOSTER, *J. Am. Chem. Soc.*, 43 (1921) 746.
2 L. A. SARVER, *Ind. Eng. Chem., Anal. Ed.*, 10 (1938) 378.
3 O. MÄKITIE, *Agrogeol. Publ. (Institute of Soil Science, Tikkurila, Finland)*, 79 (1961) 61 (in English).

4 R. NÄSÄNEN, P. LUMME AND A.-L. MUKULA, *Acta Chem. Scand.*, 5 (1951) 1199.
5 O. MÄKITIE, *Suomen Kemistilehti*, B 33 (1960) 207.
6 D. DYRSSEN AND E. JOHANSSON, *Acta Chem. Scand.*, 9 (1955) 763.
7 O. MÄKITIE, *Suomen Kemistilehti*, B 35 (1962) 1; B 37 (1964) 17.

(Received February 22nd, 1969)

Anal. Chim. Acta, 46 (1969) 314–318

Direct potentiometric determination of cyanide in biological systems

The determination of cyanide is frequently needed for practical purposes, and may be very important in agricultural chemistry and in plant biochemistry. Cyanide has been found in as many as 360 species of plants belonging to 140 genera and to 41 families. Glucosides containing cyanide are also common in many cultivated plants; the glucoside content is inherent but its concentration varies according to the different parts and the age of the individual plant, and it may be altered by soil, fertilization practices, etc. Although the glucoside itself, of which the cyanide is a constituent, is not toxic to human or animal organisms, its hydrolysis produces free hydrocyanic acid of high toxicity. A very important fact should be considered, however, namely, that the glucoside-splitting enzyme occurs in the same tissues of the plant; hence in order to start hydrolysis the cellular structure of the plant must be destroyed. If the plant is fed immediately to livestock, the quantity of cyanide arising by hydrolysis may be critical. Moreover, the raw material of many products in food technology contains cyanide-developing glucosides. Sudan grass, sorghum and linseed as fodder, almonds and the distilled products of certain fruits are the most important materials for the determination of cyanide.

Methods for the chemical determination of cyanide in plants have been reviewed by SEIFERT[1]. The most important methods for quantitative work are the argentimetric method, and colorimetric methods based either on the Berlin blue reaction[2] or on picric acid as the reagent[3]. For cyanide analysis, it is necessary to split the glucoside molecule; this can be done either by chemical or autoenzymatic hydrolysis, but it has been shown by repeated analyses that chemical hydrolysis may not be complete. It has been stated by HANSSEN AND STURM[4] for amygdaline and by LÜDTKE[5] for linamarine (glucoside of *Linum*), that the pH dependence of the cyanide hydrolysis is very favourable, because hydrolysis may be practically completed between pH 4 and 7. The free cyanide generally must be removed by steam distillation or aeration with nitrogen.

Application of the pCN electrode for measuring CN-ion activity

As a new technique for the determination of cyanide, the use of a membrane electrode selective for cyanide ions is proposed. Cyanide selectivity is a property of any membrane electrode containing silver halide. The cyanide-selective electrode can be applied either for direct measurement or as an indicator electrode in the titration of cyanide with silver nitrate. The electrochemical behaviour of the electrode may be expressed by the following equation[6]:

$$E = E_0 + \frac{RT}{F} \cdot \ln (a_X + K \cdot a_{CN}^2)$$

where a_X is the activity of the halide exchanged by cyanide in the swollen layer of the electrode; K is the cyanide selectivity constant of the electrode against the appropriate halide ion; a_{CN} is the activity of the cyanide ion in the bulk solution; and E_0 is the normal potential of the appropriate electrode.

According to this formula, the potential of the electrode increases with the values of a_X, which depend on the amount of cyanide penetrating the swollen layer of the membrane. The electrode indicates cyanide ion but not hydrocyanic acid, hence the pH value of the solution must be constantly higher than the value of the dissociation constant (pK) for hydrocyanic acid, and should not be changed during measurement. Extensive studies in this work showed that linear calibration graphs cannot be achieved unless pH exceeds pCN.

In plant biochemistry, direct measurements of pCN should be of major interest because distillation is not needed and because it is possible to make direct readings after hydrolysis in the alkaline substrate.

Experimental

The results of measurements obtained by the cyanide electrode and by the traditional method were compared on plant material containing amygdaline as a known source of cyanide.

TABLE I

COMPARISON OF METHODS OF CYANIDE DETERMINATION IN BITTER ALMOND DISTILLATES

Nr.	*HCN content (p.p.m.) calculated from*	
	Argentimetric titration	*Measurement by the pCN electrode*
1	265	275
2	280	272
3	243	284
4	267	288
5	271	270
6	248	290
7	246	274
8	263	283
9	277	280
10	255	282
11	244	277
12	280	280
13	280	284
14	281	272
15	249	278
16	241	288
17	255	280
18	240	276
19	244	281
20	271	286
	Av. 260	Av. 280
	s = 15.38 p.p.m.	s = 5.75 p.p.m.

After the hydrolysis of amygdaline, cyanide was determined by the cyanide-selective electrode and also by titration with silver nitrate on 20 different distillates (Table I); the mean error of the averages was calculated. The electrochemical measurements were carried out with a saturated calomel reference electrode and the pCN electrode was dipped into the solution of unknown cyanide content.

The results indicate that the measurements with the pCN electrode are better than those of titrations with silver nitrate in the concentration interval occurring in

TABLE II

DETERMINATION OF CYANIDE IN VARIOUS MATERIALS BY DIRECT POTENTIOMETRIC MEASUREMENT WITH THE pCN ELECTRODE

Type of material	*Designation or variety*	*Cyanide content (p.p.m.)*	*Type of material*	*Designation or variety*	*Cyanide content (p.p.m.)*
Bitter almond		280	Peach leaves	Alexander	39
Spicy almond	Bt 13/55	92		Sunbeam	63
	Bt 13/62	95		May Flower	72
	Bt 13/65	98		Mariska	74
	Bt 13/66	86		Champion	76
Sweet almond	Bt 70	22		Elberta	91
	Bt 1	31		Ford	120
	Bt 11	54			
Sudan grasses[a]	1.A	298	Sudan grasses	5.A	130
	B	86		B	49
	C	89		C	16
	2.A	27		6.A	71
	B	14		B	45
	C	2		C	36
	3.A	312		7.A	123
	B	70		B	62
	C	12		C	34
	4.A	17		8.A	175
	B	27		B	70
	C	2		C	1

[a] Sudan grass altered by the fertilization practices (sample numbers) and successive developmental stages (capital letters) of the plants in the respective sample.

TABLE III

CYANIDE CONTENT OF FRUIT BRANDIES DETERMINED IN PARALLEL BY DIFFERENT METHODS

Designation of the brandy	*Argentimetric standard method (p.p.m.)*	*Direct potentiometry[a] (p.p.m.)*
Cherry	26.3	31.1
Apricot I	4.1	3.2
Apricot II	5.8	5.2
Sour Cherry I	6.1	10.8
Sour Cherry II	11.7	15.3
Blue plum I	40.8	44.5
Blue plum II	6.3	6.8
Blue plum III	14.3	4.0
Blue plum IV	2.6	2.6

[a] Direct immediate measurement with the pCN electrode at pH 11.

this study. The direct potentiometric method is less time-consuming and errors are smaller. During this work, the cyanide concentration in enzymatic auto-hydrolysates of bitter almonds with and without steam distillation were compared; results from the same sample were 240 and 230 p.p.m., respectively.

Studies performed in order to ascertain the effect of ethanol on the measurements indicated that in solutions containing 0–60% ethanol, the change in the normal potential was negligible, *i.e.* it did not exceed the error: pCN = 0.15. It is entirely feasible to determine the cyanide content of fruit brandies without preliminary isolation of the cyanide from alcohol.

On the basis of the above results, various plant materials as well as fruit brandies, were examined. The results obtained are presented in Tables II and III. It is clear that the determination of cyanide by direct use of the pCN electrode is eminently suitable for the rapid routine analysis of plants and products. After enzymatic hydrolysis, the substrate must be adjusted to pH 10–11 and then direct readings of pCN are at once possible. In order to obtain more accurate analytical data, a previous distillation from alkaline medium is advisable.

Institute of Horticulture BEA GYÖRGY
and Department of Analytical Chemistry, L. ANDRÉ
University of Chemical Industries, L. STEHLI
Veszprém (Hungary) E. PUNGOR

1 PL. SEIFERT, in PEACH-TRACEY, *Moderne Methoden der Pflanzenanalyse*, Bd. IV, Springer-Verlag, Berlin, 1955, pp. 676–688.
2 E. BERL AND I. M. DELPY, *Ber. Deut. Chem. Ges.*, 43 (1910) 1430.
3 A. GÜNTHER AND R. C. BLINN, *Analysis of Insecticides and Acaricides*, New York, 1955, p. 401.
4 E. HANSSEN AND W. STURM, *Z. Lebensm. Untersuch.-Forsch.*, 134 (1967) 69.
5 M. LÜDTKE, *Biochem. Z.*, 323 (1953) 428.
6 E. PUNGOR AND K. TÓTH, in preparation.

(Received March 17th, 1969)

Analytica Chimica Acta
Elsevier Publishing Company, Amsterdam
Printed in The Netherlands

BOOK REVIEWS

I. M. KOLTHOFF, P. J. ELVING ET E. B. SANDELL, *Treatise on Analytical Chemistry. Part I. Theory and Practice*, Vol. 8, Interscience Publishers–J. Wiley & Son, Inc., New York, 1968, 4869–5384 pp., price 178 s.

Ce volume comprend la suite des méthodes physico-chimiques classiques: *la mesure du temps* dont l'importance en chimie analytique ne cesse de croître depuis que se développent les méthodes catalytiques basées sur les vitesses de réaction et toutes les méthodes chronoélectroanalytiques. Les principaux chapitres sont: l'instrumentation, le calibrage, les erreurs et les applications à la chimie analytique. Si l'on fait abstraction des photographies des appareils qui n'ont pas d'intérêt, ni du point de vue didactique, ni du point de vue documentaire (car dans ce domaine, l'évolution est trop rapide), les renseignements apportés sont précieux et on ne les trouve pas dans les autres ouvrages de chimie analytique.

Une deuxième partie (section D-5) a pour objet les méthodes thermiques. Après une introduction (Eléments de thermodynamique chimique), les principaux chapitres sont: Principes de thermométrie (mesure des températures), Cryoscopie, Ebullioscopie, Calorimétrie, Titration enthalpique, Analyse différentielle thermique. Cette partie est, d'une façon générale, remarquablement présentée, après une introduction concise, précise et claire, ne donnant que l'essentiel.

Les auteurs traitent de la thermométrie; le chimiste analyste peut se renseigner rapidement sur le choix d'un thermomètre, sur la façon de le calibrer et de l'utiliser, sur les possibilités et la précision des mesures.

J'ai aussi beaucoup apprécié le chapitre des titrations enthalpiques, car il est fort bien fait et de conception originale. Il est question de la signification analytique des chaleurs de réaction, des équations mathématiques auxquelles elles obéissent, des courbes de titration, de l'appareillage, des titrations différentielles et dérivées, de l'utilisation des enthalpogrammes en analyse quantitative, de la sensibilité, de la précision et de l'exactitude de cette méthode. Un certain nombre d'applications sont proposées. Chapitre très complet mais sans détails superflus, illustré de nombreux graphiques.

Il n'est pas possible d'étudier ici chaque chapitre, mais il est certain que ce volume est remarquable en toutes ses parties, tant par son originalité que par sa présentation. Il est facile à consulter et a été vraiment conçu pour les chimistes analystes.

D. MONNIER (Genève)

Instrumentation in Gas Chromatography, Edited by J. KRUGERS, Centrex Publishing Company, Eindhoven, 1968, xi+245 pp., price 83 s.

Few texts are available which provide the practicing analytical chemist with a thorough practical survey of a particular technique backed by an adequate theoretical treatment. This volume provides both the experienced and the aspiring gas chromatographer with a wealth of just those practical details which he needs to obtain the best results from his equipment. Indeed, much of the information is of the type otherwise derived only from extensive first-hand experience, and possession of this book will save much time spent in reference to often obscure apparatus manuals or theoretical texts.

After an introduction to basic concepts and system components, chapters are devoted to sampling techniques, carrier gases, ovens, columns, detectors and electronics. Preparative-scale applications are discussed as are pyrolytic techniques although the treatment of the latter is rather limited. Programmed temperature applications do not occupy a separate chapter but are considered under various other headings.

Seldom in these days of escalating book prices can a wholehearted endorsement be given, but this volume should prove an excellent investment for anyone involved in the practice of gas chromatography.

Advances in Analytical Chemistry and Instrumentation. Vol. 6. Progress in Gas Chromatography, Edited by J. H. PURNELL, Interscience Publishers–J. Wiley and Son, Inc., New York, 1968, vii+392 pp., price 140 s.

This volume departs from the previous format of this well-established series in being devoted entirely to a single analytical technique. Since it consists of a collection of review articles of a relatively specialized nature, it is rather more a source book for the experienced chromatographer than a basic text for the general reader.

The distinguished group of authors contribute critical and comprehensive reviews both in well-established areas and in regions of probable future development. Among the former, peak identification, selective stationary phase design and preparative gas chromatography are treated in considerable depth. An extensive theoretical discussion of liquid–surface effects in GLC is included, and a very useful article on physical measurement by gas chromatography surveys a field which is often neglected by the analyst. The review of optimum conditions in gas chromatographic analysis is of considerable practical value. Shorter reviews of flow-programming and on the use of modified solids in gas–solid chromatography indicate promising areas of development.

P. C. UDEN (Birmingham)

A. D. GEL'MAN, A. I. MOSKVIN, L. M. ZAITSEV AND M. P. MEFOD'EVA, *Complex Compounds of Transuranides*, Translated from the Russian by J. SCHMORAK, Israel Program for Scientific Translations, Jerusalem, 1968, viii+152 pp., price $ 10.25.

This book is another in the series of translations made by I.P.S.T. of Russian scientific literature. The original Russian text was published as a monograph in 1961 as individual chapters and prepared for publication by A. D. GEL'MAN. The monograph consists of five chapters each followed by an extensive bibliography. The first three chapters deal with the complexes of neptunium and plutonium for different valency states with anions of mono, di, and polybasic acids in aqueous solution, together with some solid compounds which are formed. Chapter 4 deals with complex formation of some of the transplutonium compounds in solution. The last chapter deals with applications of complex compounds for the separation of transuranium elements.

The translation, on the whole, is well done; only a few sentences are ambiguous or make no sense at all. "Structure" has been used many times to mean both physical state and chemical form, and words such as "ponderable" have been used to describe amounts of material. However, the editing of the translation leaves much to be desired. The most serious criticism is that many of the references have errors making them very difficult to find, and are wrongly subscripted in the text. Duplication of most of the figures in the original Russian diminutive format makes them of very little value.

It is doubtful now whether this translation is going to prove to be a useful addition to this sphere of work. During the seven years which have elapsed between the original book and the publication of the translation many significant advances have been made in this field. In any case, the original Russian approach to this topic was outdated, being very much in the classical style. No attempt has been made to discuss or interpret any of the complexes in terms of modern chemical bonding theories. In this respect the publication is merely a catalogue of data and no attempt has been made at being critical. Most of the complexes described in any detail are those studied in connection with intended separation procedures, and these have been made to seem unduly important.

The only obvious usefulness that the book has is that it contains many references to Russian work published in journals which have not been translated into English.

A. J. FUDGE (Harwell)

Analytica Chimica Acta
Elsevier Publishing Company, Amsterdam
Printed in The Netherlands

ANNOUNCEMENTS

SPECTROSCOPY SOCIETY OF CANADA

S.S.C. REFERENCE STANDARDS

Spectrographic analyses at the Geological Survey of Canada of two replacement lots of syenite rock revealed that both were of interesting composition, and the previous decision to issue only SY-3 (higher radioactive content) was reversed. Approximately 500 pounds of each has been prepared and bottled, and distribution has commenced on the basis of the provisional analyses given below. Most of the results were obtained by the Spectrochemical Laboratory, Geochemistry, Mineralogy and Economic Geology Division of the Geological Survey of Canada; the remainder (marked by an asterisk*) by the Analytical Chemistry Section, Mineral Sciences Division, Mines Branch. As was the case with standard SY-1, recipients are being requested to report their analytical results to:

Dr. A. H. GILLIESON,
c/o Mineral Sciences Division,
Mines Branch,
555 Booth Street,
Ottawa 4, Ontario.

to whom all requiries regarding all standards (both non-ferrous and non-metallic) and their supply should be addressed.

REFERENCE STANDARD SAMPLES AVAILABLE

The S.S.C. standards currently available and their cost, inclusive of packaging and postage, are:

Phosphor-Bronze Disc $2^1/_4$ by $^3/_8$ in, nominal tin content 5% — $15.00 ea.
Phosphor-Bronze Disc $2^1/_4$ by $^3/_8$ in, nominal tin content 7% — $15.00 ea.
Phosphor-Bronze Disc $2^1/_4$ by $^3/_8$ in, nominal tin content 10% — $15.00 ea.
4 Copper Rods (Commercial Purity) 12 in long by $^5/_{16}$ in diameter.

The impurities are at the p.p.m. level and their total excluding oxygen is approximately the same in each of the four standards ($35.00 each).

Sulphide Ore, SU-1, $^1/_4$ lb bottle $ 5.00.
Syenite Rock, SY-2, 100 g bottle $10.00.
Syenite Rock, SY-3, 100 g bottle $10.00.

PROVISIONAL ANALYSIS OF NEW SYENITE ROCK STANDARDS

Constituent	*SY-2*	*SY-3*
	Percentage Major Constituents	
SiO_2	60.8*	60.3*
Al_2O_3	11.3	11.0, 11.5*
Fe (total)	5.0	4.9
MgO	1.99	2.49, 2.60*
CaO	9.65	8.26, 8.00*
Na_2O	4.22*	3.88*
K_2O	4.15*	3.85*
H_2O (moisture)	0.21*	0.76*
TiO_2	0.14	0.13
P_2O_5	Not determined	0.59*
MnO	0.34	0.32
CO_2	Not determined	$<$ 0.10*
SO_4	Not determined	0.13*
U_3O_8	0.033*	0.073*
ThO_2	0.030*	0.102*

* Determinations by Mines Branch.

Constituent	*SY-2*	*SY-3*
	Minor Constituents in p.p.m.	
Sr	270	300
Ba	430	410
Cr	< 20	Not found
Zr	280	260
V	< 30	< 30
Ni	< 20	Not found
Ce	< 500	1900
Cu	< 8	18
Y	160	870
Nb	Not found	130
Co	< 20	Not found
La	< 100	1700
Pb	64	120
Sc	< 10	12
Yb	17	69
Be	16	16
Ag	< 0.05	0.054
Zn	200	180
Ga	33	43
Sn	2.5	4.8
B	35	45
Ge	1.0	1.1
Mo	0.99	0.90
Tl	2.0	2.2
Bi	Not found	0.58

Other elements not found include As < 30, Sb < 10, Cd < 2, In < .2, Au < 1 and Te < 10 p.p.m.

Anal. Chim. Acta, 46 (1969) 325–326

Analytica Chimica Acta
Elsevier Publishing Company, Amsterdam
Printed in The Netherlands

THIRTEENTH CONFERENCE ON ANALYTICAL CHEMISTRY IN NUCLEAR TECHNOLOGY

Mountain View Hotel and Motor Lodge, Gatlinburg, Tennessee 37830
September 30, October 1 and 2, 1969

The Thirteenth Conference on Analytical Chemistry in Nuclear Technology will be held in Gatlinburg, Tennessee, September 30, October 1 and 2, 1969, under the sponsorship of the Analytical Chemistry Division, Oak Ridge National Laboratory.

All sessions of the Conference will be held in the Mountain View Hotel. Registration will begin on September 29 at 4 p.m. in the hotel lobby and continue each day for the duration of the Conference. Sessions will begin at 9 a.m. each day.

TECHNICAL PROGRAM

Papers up to 25 minutes in length, that describe original, unpublished work related to the following topics are solicited by the Program Committee:

Analytical Chemistry for Fast Breeder Reactor Programs
1. Determination of major components, oxygen to metal ratios, and trace impurities in such fuels as uranium and/or plutonium oxides, nitrides and carbides.
2. The analysis of alkali metal coolants for trace constituents, such as carbon, hydrogen, oxygen, nitrogen and other impurities.

Activation and Radiochemical Analysis
1. Non-destructive radionuclide analysis by the use of lithium-drifted germanium detectors. Papers dealing with this topic and the application of computers to the processing of data from such systems are especially solicited.
2. Radioactive Standards, present uses and future needs—a general open discussion will follow the presentation of papers.
3. Analytical applications of nuclear techniques such as neutron, photon, or charged particle activation and of nuclear devices such as radioisotope sources.

Automated Methods of Analysis
1. In process control.
2. In the detection and control of environmental pollution.
3. In bioanalytical and clinical applications.

EXHIBITION OF MODERN ANALYTICAL INSTRUMENTS AND LABORATORY EQUIPMENT

Information concerning exhibit space is available from H. R. BEATTY, Oak Ridge National Laboratory, P. O. Box X, Oak Ridge, Tennessee 37830.

GENERAL INFORMATION

Abstracts. Those who wish to make contributions should submit an abstract of 200–500 words not later than June 20, 1969. The title, name of the author, an estimate of the time that will be required for the presentation, and in case of multiple authorship, an indication of the name of the speaker should accompany the abstract.

Abstracts of papers and inquiries concerning the Conference, including requests for programs, should be directed to the Oak Ridge National Laboratory, P. O. Box X, Oak Ridge, Tennessee 37830, Attention: L. J. BRADY, Chairman.

Registration Fee. The registration fee, except for full-time students, is $5.00 per person.

Tape Recordings. In lieu of published proceedings, tape recordings will be made of all papers and copies will be available on a loan basis after the Conference. Speakers who do not wish recordings made of their papers should so inform the committee.

Reservations. Reservations for lodging should be made by direct communication with: The Mountain View Hotel and Motor Lodge, Gatlinburg, Tennessee 37738.

ANALYTICA CHIMICA ACTA, VOL. 46 (1969)

AUTHOR INDEX

ANALYTICA CHIMICA ACTA, VOL. 46 (1969)

SUBJECT INDEX

CONTENTS

(continued on page 4 of cover)

(contents, continued from page 3 of cover)