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 fur alle Gebiete der analytischen Chemie

ELSEVIER SCIENTIFIC PUBLISHING COMPANY
AMSTERDAM

Anal. Chim. Acta, Vol. 76, No. 1, 1–236, May 1975
Published monthly

Publication Schedule for 1975

Vol. **74**, No. **1**	January 1975	
Vol. **74**, No. **2**	February 1975	**(completing Vol. 74)**
Vol. **75**, No. **1**	March 1975	
Vol. **75**, No. **2**	April 1975	**(completing Vol. 75)**
Vol. **76**, No. **1**	May 1975	
Vol. **76**, No. **2**	June 1975	**(completing Vol. 76)**
Vol. **77**, No. **1**	July 1975	
Vol. **77**, No. **2**	August 1975	**(completing Vol. 77)**
Vol. **78**, No. **1**	September 1975	
Vol. **78**, No. **2**	October 1975	**(completing Vol. 78)**
Vol. **79**, No. **1**	November 1975	
Vol. **79**, No. **2**	December 1975	**(completing Vol. 79)**

Subscription price: Dfl. 570.00 plus Dfl. 54.00 postage, US$ 265.53 inclusive of postage. Subscribers in the U.S.A. and Canada receive their copies by airmail. Additional charges for airmail to other countries are available on request. For advertising rates apply to the publishers.

Subscriptions should be sent to:
Elsevier Scientific 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 B15 2TT (Great Britain)

Reprints

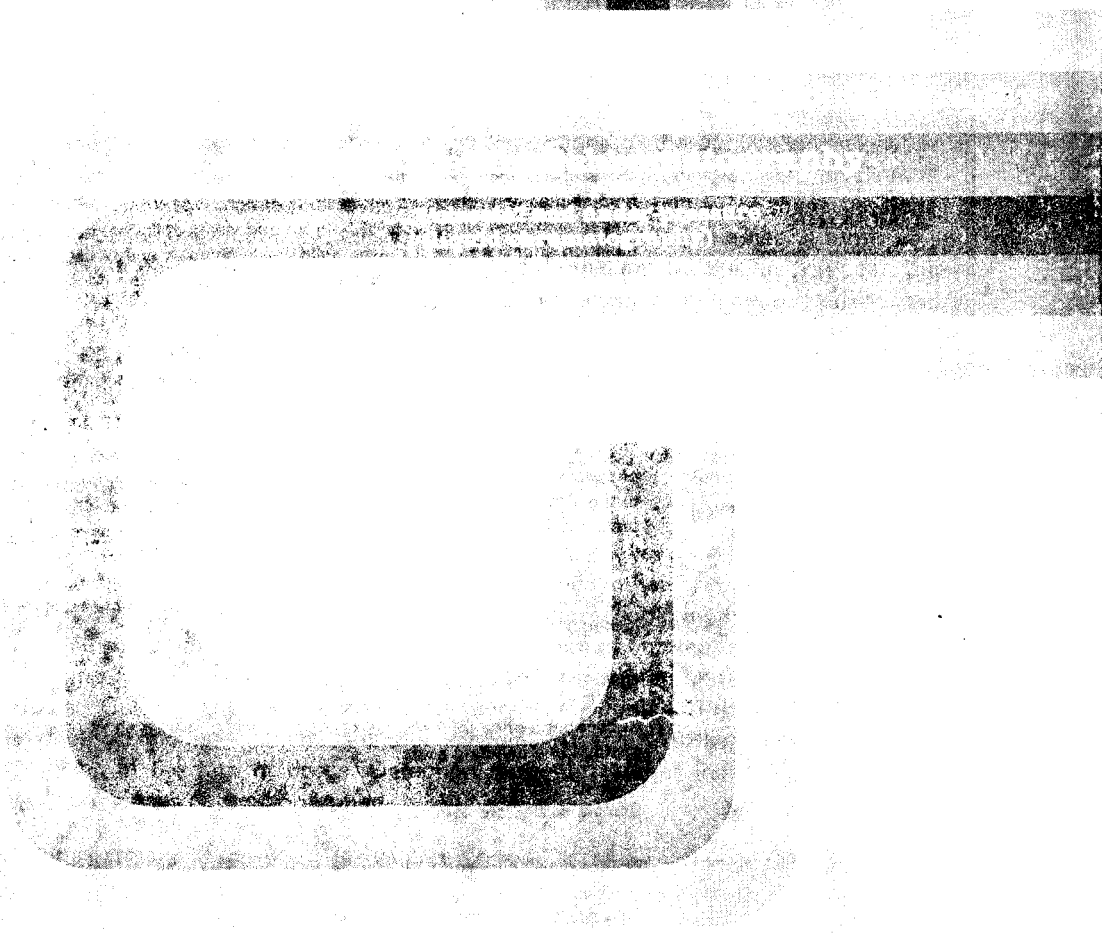
Fifty reprints will be supplied free of charge. Additional reprints (minimum 100) can be ordered at quoted prices. They must be ordered on order forms which are sent together with the proofs.

PRINTED IN THE NETHERLANDS

Reagents

MERCK

The right way to optimal separations



Silica gels 40, 60, 100 (Å pore diameter)
Macroporous Silica gels, Merckogel® SI (max. 25000 Å)
Silanised Silica gel (hydrophobic)

Aluminium oxide type E and T
Kieselguhr · Cellulose · Cellulose ion exchangers
Merckosorb® · Perisorb® · Merckogel® OR-PVA

Please ask for our special brochures

75-EÜ

E. Merck, Darmstadt Germany

The Science of the Total Environment

Pharmaceutical applications of Thin-Layer and Paper chromatography

edited by **KAREL MACEK,** *Medical Faculty, Charles University, Prague*

1972, xvi + 744 pages, Dfl. 250.00
ISBN 0-444-40939-4

Chromatography is the most widely used modern procedure in analytical chemistry today. With an increasing awareness of the importance of the applications of paper and thin-layer chromatography in the fields of pharmaceutical research, production and control, it has become necessary to survey the possibilities of these methods with their associated literature, and to present this information in a useful form.
With this in mind, the editor provides an introduction to the techniques, the evaluation, and the applications of paper and thin-layer chromatography. Selected procedures such as the preparation of samples, detection methods, choice of solvent systems and sorbents, and the principles of quantitative analysis, are discussed generally and the book is richly tabulated. An appendix outlines the preparation of more than 150 detection reagents. On the basis of these data, readers with an understanding of the principles of the techniques described can solve any analytical problems they may encounter in drug analysis.

CONTENTS: Introduction. Techniques of paper and thin-layer chromatography. Radioactive compounds. Combination of TLC and PC with other chromatographic techniques. Combination of PC and TLC with some spectroscopic methods. Identification of organic compounds by PC and TLC. Documentation of chromatograms. Laboratory for PC and TLC. The tasks of paper and thin-layer chromatography. Synthetic drugs. Steroids. Cardiac glycosides and their genins. Saponins. Peptide and protein hormones. Alkaloids. Vitamins. Antibiotics. Plant extracts. Auxiliary compounds. Investigation of the fate of drugs. Detection reagents. Author index. Subject index. List of substances chromatographed.

CONTRIBUTORS: V. Betina, J. Davídek, I. M. Hais, K. Hiller, J. Janák, G. Katsui, B. P. Lisboa, M. Luckner, K. Macek, L. Nover, V. Rábek, G. Székely, H. D. Woitke.

Elsevier

BOOK DIVISION, P.O. BOX 211
AMSTERDAM - THE NETHERLANDS

181Ea

ANALYTICA CHIMICA ACTA
Vol. 76 (1975)

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 SCIENTIFIC PUBLISHING COMPANY
AMSTERDAM

Anal. Chim. Acta, Vol. 76, No. 1, 1–236, May 1975

PRINTED IN THE NETHERLANDS

Analytica Chimica Acta, 76 (1975) 1–26

THE DETERMINATION OF MERCURY BY NON-FLAME ATOMIC ABSORPTION AND FLUORESCENCE SPECTROMETRY

A REVIEW

A. M. URE

The Macaulay Institute for Soil Research, Craigiebuckler, Aberdeen AB9 2QJ (Scotland)

(Received 15th November 1974)

The current expansion in the literature dealing with the determination of mercury stems largely from its importance in the field of environmental pollution although there has been a perennial interest in the determination of mercury particularly as a guide to mineral prospecting. This expansion has been made possible by the development of non-flame atomic absorption and fluorescence analytical methods, now available to most laboratories. These non-flame methods are in principle simple and sensitive but in practice the accurate determination of mercury in natural samples is difficult, and this has contributed a great deal to the growth of the literature on analytical techniques.

The present review is principally concerned with analytical aspects of non-flame atomic absorption and fluorescence methods for the determination of mercury; cataloguing applications of these techniques is subsidiary to that main theme. A critical review is made difficult by the volume of the literature and by the problem of assessing the analytical accuracy of the techniques. Such assessment is as difficult for the analyst as for the reviewer, because of the almost complete absence of authenticated standard samples—an absence reflecting the problems of compositional stability and contamination.

The first experimental investigations of the phenomenon of atomic absorption and fluorescence in mercury vapour were made from about 1910 onwards by Wood[1,2]. More detailed investigations were made by Goos and Meyer[3] and Hughes and Thomas[4], while Müller[5], Müller and Pringsheim[6] and Woodson[7–9] made the first practical analytical use of atomic absorption for the determination of mercury in air and gases. The early history of the method has been reviewed by Woodson[7] and others[10–12].

The non-flame atomic absorption technique has been discussed in reviews of general analytical methods[11–19], of methods applicable to waters[20–22], biological materials[23] and foodstuffs[24], and is the principal subject of the reviews in refs. 25–31.

Atomizers of the electrically heated, miniature graphite or metal filament type applicable to the determination of mercury and other elements have recently been reviewed[32]. A full account of the analytical chemistry of mercury up to 1960 is available[33] and extraction methods for water samples have been surveyed[34]. A comprehensive[35] and a shorter[36] bibliography on mercury in the environment

both contain considerable analytical information, as does a bibliography of non-English references[37].

For the purpose of this review, techniques for the determination of mercury by non-flame atomic absorption or fluorescence can conveniently be assigned to one of three groups according to the method used to provide elemental monatomic vapour for the analysis. In the first, the mercury is already in vapour form, *i.e.* the sample is air or gas containing mercury vapour, and sample treatment is not required, or is minimal. In the second, mercury volatilized from solid or liquid samples by heat is then determined in the vapour phase directly or after a preliminary concentration in an absorbing agent. In the third, mercury in solution is separated by a variety of physical and chemical means such as that of reduction to elemental mercury followed by aeration to release the monatomic mercury vapour. These categories are not mutually exclusive and many techniques using combinations of these various procedures are quoted.

After a brief account of the use of atomic fluorescence methods, there are sections devoted to the problems of sample preparation and contamination, loss of mercury from solution and the analytical accuracy of the techniques. Finally, applications of non-flame atomic absorption and fluorescence to various sample types are listed.

DIRECT DETERMINATION OF MERCURY IN AIR AND GASES

The early mercury vapour meters such as that of Woodson[7] consisted simply of a tube to contain the mercury vapour through which light from an ultraviolet lamp passed to a photoelectric cell from whose output the absorption by mercury was determined. Simple single-beam[38–41] and double-beam instruments for mercury in gases have been designed[42,43]. Other pieces of apparatus for mercury in air have been described[44–46]. Commercial mercury vapour monitors[47] and meters[30,48–53] are available and have been modified for[54], or applied to[55–59] the determination of mercury in gases.

The atomic absorption measurement is usually made at 253.7 nm, and although the 184.9-nm line is some 30 times more sensitive[60], it can be used only when absorption by atmospheric constituents such as oxygen is obviated[61,62]. A spectral interference effect from the Co 253.65-nm line has been reported, but the effect is small[63].

Since all the techniques reviewed here for non-gaseous samples make the mercury determination by atomic absorption in the vapour phase, most of them can be applied or adapted to the determination of mercury in air and other gases, as has been shown by many workers[41,55,62,64–72]. Most commercial equipment is suitable for gaseous samples[73–78].

Combined mercury in gases has been converted to elemental form by furnace pretreatment[65,66,79] or by passage over heated carbon rods[61,62] which eliminates the ultraviolet absorption of atmospheric oxygen by converting it to non-absorbing carbon monoxide and so allows the more sensitive 184.9-nm resonance line to be used.

All determinations of mercury by atomic absorption in the vapour phase are subject to overestimation caused by molecular absorption by organic and

inorganic species or by light-scattering loss from smoke, dust and particulate material. These effects can be important for naturally gaseous samples, and even more so in the determination of mercury in the vapours evolved from solid samples by heat treatment (see following Section). The effects are further considered below (Pp. 4, 5). References to applications of the methods to air and gases are summarized on p. 15.

DETERMINATION OF MERCURY FOLLOWING ITS RELEASE BY HEATING

In the methods described in this section, the release of mercury from a sample by heating may be followed directly by its determination in a one-stage process, or by an intermediate concentration step (p. 4).

Pyrolytic methods, non-oxidative

Direct methods. Simple single-beam mercury vapour meters have been applied to the determination of mercury directly in the vapour evolved from geological materials and soils by pyrolysis[80–83]. The pyrolysis, in an inert gas or in the absence of sufficient air for oxidation, of geological materials containing organic matter or sulphides produces smoke and vapour which interfere by the effects of light scattering and molecular absorption and make these single-beam instruments inaccurate[84–86]. Interfering vapour can be removed by traps[87–89] but it is probably preferable to use a double-beam method to compensate for its effect or to use a preconcentration technique (see below).

Pyrolysis under reducing conditions has been achieved with hydrogen[90], iron powder and activated carbon[79] and carbon[61,62]. Sublimation temperatures for mercury in different compounds have been studied[91–94] and temperature programming has been used to distinguish different mercury compounds[91,95].

Micro-furnace methods. Miniature and micro-furnace techniques including carbon and graphite rod or tube atomizers and wire loop or metallic ribbon atomizers for non-flame atomic absorption and fluorescence analysis applicable to the determination of mercury have been surveyed[32]; their potentialities, sensitivities and detection limits have been discussed[96–99]; and applications to the determination of mercury in biological materials[100], minerals[101] and graphite powder[102,103] have been described. Micro-furnace atomizers are available as accessories for most commercial atomic absorption instruments. They have the advantage that they require only very small samples and have consequently very low absolute detection limits. A fast, pulse-heated atomizer shows potential discrimination between organic and inorganic mercury[104].

Combustion methods

The advantage of combustion techniques lies in their ability to oxidize the interfering smokes, tars and other organic vapours liberated on pyrolysis. Combustion techniques are better suited to biological materials than pyrolytic methods. Many of these combustion methods use apparatus similar to that for non-oxidative pyrolysis; indeed the same furnace can be employed by substituting oxygen for the nitrogen stream over the sample[105]. Oxygen-flask combustion methods which provide solutions for analysis are discussed in the following Section.

The vapour from the oxidizing furnace[106–109] or from a sample burned in a nebulizer burner[110] is passed to the atomic absorption cell by way of traps to remove oxidation products[106–109].

REDUCTION OF VAPOUR PHASE INTERFERENCE

The chief difficulty in determining mercury by atomic absorption in air or gases directly, or in the vapour produced by pyrolytic or combustion furnace techniques, is the enhancing interference produced by molecular absorption from organic or inorganic species or by the light scattering losses produced by smokes and particulates. The problem is particularly severe in non-oxidative pyrolytic techniques with samples of high organic or sulphide content. Two approaches aimed at reducing these interferences are considered below.

Instrumental methods

Methods of correcting for such errors include the use of double-beam instruments which measure mercury atomic absorption together with molecular absorption and light-scattering loss in one beam and, in the other, measure the interfering molecular absorption and light-scattering loss in sample vapour from which the mercury has been removed by a palladium chloride absorber[84,111] or a gold amalgamation trap[112,113]. Other instruments measure non-atomic absorption by using as a light source the unabsorbed wings of a pressure[114,115] or Lorentz[116–118] broadened mercury line transmitted by a mercury vapour cell. Use has also been made of Zeeman-effect line-splitting for the reference beam source[78,119]. Non-atomic absorption and light-scattering effects can also be measured at adjacent spectral lines of aluminium[120], cobalt[118] and silicon[121]. A continuous source was first used by Ballard[122] to make this measurement, and hydrogen and deuterium lamps have been widely used, particularly in commercial double-beam atomic absorption spectrometers[25,27,61,123–126].

Preconcentration and collection methods

Another method of avoiding vapour-phase interference effects is to separate the mercury from the interfering vapours by collecting it, before the determination, by one of the concentration methods given below. Concentration methods for mercury in solutions are considered on p. 10.

The earliest methods used for the collection of mercury after pyrolysis were those of Vaughn and McCarthy[127] and Williston and Morris[128], who used a gold wire amalgamator, and Williston[112], who used activated charcoal. Mercury was then determined in the vapour evolved from the traps by heat. The amalgamation trap method has been the basis for most subsequent pyrolytic techniques[41,68,83,84,94,105,129,130]. In some, two stages of amalgamation and volatilization have been used[131,132]. These non-oxidative pyrolytic methods have been applied mostly to geological samples such as rocks, ores and minerals[84,90,94,127,129–131], soils[84,88,105,127], coal[129] and sediments[90]. For organic and carbonaceous samples, it is preferable to use combustion techniques[105,129] to which similar amalgamation methods of collection have been applied[95,105,129,133–141].

When amalgamation traps are employed, pyrolytic, and to a lesser extent

combustion, methods may still be subject to positive errors caused by molecular absorption and light-scattering effects, and to negative errors as a result of occlusion of the noble metal surface of the trap by deposited organic materials. While in fortunate cases these errors may partially cancel each other[105], it may be preferable for samples with high organic content to use a totally different method, such as that of wet oxidative digestion followed by reduction–aeration[105], which can largely eliminate such interferences (see below).

Collection techniques for mercury in the vapour-phase have included simple amalgamation in noble metal traps[65–68,79,142,143] and amalgamation in traps of heated gold[85,134] or silver[79] which decompose organic mercury compounds. Other concentration procedures have used activated charcoal[113,144], magnesium sulphate[144], iodine–potassium iodide-treated filter paper[56], and a cold finger technique with liquid nitrogen[145,146]. An acetone–ice-cooled finger has also been used[147] but has been criticized for incomplete collection[145,146]. Most of these collection methods use heat treatment to volatilize mercury for measurement.

Collection in acidic potassium permanganate solution, which is quantitative for elemental or inorganic mercury, but not for organically bound mercury[148], has been used for mercury in gases[55,149] and for the gaseous products of combustion procedures[71,137,150–152]. The potassium permanganate solution can either be analyzed by reduction–aeration methods, (see below) and refs. 141, 150–152) or can be further treated by extraction with dithizone in chloroform[153,154].

The Schöniger oxygen-flask combustion technique[155,156] is a particularly useful combination of combustion treatment followed by collection in solution. It was first used for trace mercury determination with a dithizone extraction and colorimetric procedure[157]. It has been recommended for the determination of mercury by reduction and aeration of the resulting solution[16] and applied with hydrochloric acid collecting solutions[123,158,159]. Incomplete collection of the mercury by some of these solutions[160] or incomplete combustion, which requires an oxidizing collecting solution such as chlorine water[161] or acidic potassium permanganate[160,162], may partly account for low recoveries of mercury reported where an oxidizing collector is not used[123,158,163]. In the oxygen flask procedure, the commonly employed platinum sample holder should be replaced by one of tantalum because of interference by platinum[160] in the reduction–aeration procedure[123,160] which is described in detail below. Solutions prepared by oxygen flask combustion can be analyzed by reduction–aeration methods (see below, and refs. 120, 123, 160, 162, 164) or by one of the concentration procedures outlined on p. 10 ff. The sample size is limited by the size of the combustion flask[120] but at least 1 g of peat, for example, can be burned in a 5-l flask[160]. The method is rapid and combines the advantages of almost complete combustion and freedom from interference by organic materials with quantitative collection of mercury. Biological, organic and carbonaceous samples[165–167] can be burned alone; soils or rocks require admixture with a combustible material such as cellulose powder[160].

These concentration techniques, as well as providing a means of eliminating or reducing interferences, offer significant improvements in detection limits.

REDUCTION–AERATION METHODS FOR DETERMINATION OF INORGANIC AND ORGANIC MERCURY IN SOLUTION SAMPLES

The principal disadvantage of simple pyrolytic and combustion furnace methods is the difficulty of preventing organic vapours or other volatile products from interfering with the determination. An alternative method, discussed below, uses acid digestion of the sample to dissolve the mercury and wet oxidation to decompose organic compounds. Dissolution of a sample ash is not feasible since dry ashing even at low temperature with excited oxygen causes loss of mercury[137,168,169], but solutions can be prepared by the oxygen-flask combustion method (see above). The mercury can be volatilized from the solution by the reduction–aeration method used by Kimura and Miller[148] in a dithizonate colorimetric method and developed by Poluektov and Vitkun[170] for atomic absorption determination.

The reduction–aeration technique

In the method of Hatch and Ott[171], which has formed the basis for most subsequent work, the solution obtained by digestion of a rock sample with sulphuric acid is oxidized with hydrogen peroxide and potassium permanganate, treated with a mixed solution of sodium chloride and hydroxy ammonium sulphate and finally reduced with tin(II) sulphate solution. Air is bubbled through the solution and passed through a magnesium perchlorate drier into the atomic absorption cell, whence the mercury-laden air is recirculated through the system. This method of equilibrium partitioning by continuous recirculation and aeration has been used by many workers[34,163,172–174] and has several advantages over an alternative system[160,175] in which the mercury-laden air from the aeration vessel passes through the measuring cell to an exhaust. These are, firstly, that the recorded absorption reaches a plateau which is easier to measure than the asymmetrical peak obtained by the latter method. Secondly, with recirculation larger samples can be accepted without the severe loss of sensitivity which occurs in the exhaust method as a consequence of dilution by the larger volume of air required to liberate the mercury. Large samples however require longer times to reach equilibrium and recirculation is more likely to suffer from memory effects because of the complicated air-train employed. The effect on the absorption peak height and area of sample volume[176–178] and other variables such as temperature[160,177] and air flow-rate[176,177,179] have been discussed. Other carrier gases such as nitrogen[180] have been used in place of air. Instrumental performance has been discussed[181] and hollow-cathode lamps have been compared with vapour-discharge lamps[61,182].

Mercury can also be partitioned between sample solution and a fixed volume of air by agitation in a closed sample vessel for times from 10 s (ref. 183) to 2 min (refs. 160, 184), after which the mercury-laden air is blown directly from the vessel, without recourse to bubbling, into the measurement cell[160,183–192]. In an elegant use of the method[183,188,191,192], the sample is partitioned in the syringe used to inject the mercury-laden air into the atomic absorption cell. Partitioning by agitation has several advantages over aeration by bubbling, although it has been less widely applied. First, the absorption peak height is somewhat

greater[160], is of shorter duration and therefore is more suitable for peak height measurement. Secondly, no spray is generated and driers between the sample vessel and the absorption cell are unnecessary[121,160,193] since the errors caused by the residual water vapour are usually negligible. This is important since driers such as magnesium perchlorate[34,72,171,194,195], anhydrous calcium sulphate[194] or silica gel[160] not only need frequent changing[173,194] but can cause contamination[194] or variable losses of mercury[160,121] particularly if the condensate in the filter is acidic[196]. Thirdly, there is no problem of foaming which in the bubbling method can cause low mercury values[160,197] and may even prevent analysis[189] especially in samples which have not been completely freed of organic components[189,193,198]. The use of anti-foaming agents is not a complete answer, since most of these depress the absorption peak height[57,179,189]. Heated atomic absorption cells have been used to prevent condensation on windows and walls[120,180,194,199].

Solution samples can be reduced directly with tin(II), as can digests[120] particularly where no potassium permanganate is used. Where it has been employed, most workers have followed Hatch and Ott and used a mixture of hydroxyammonium sulphate and sodium chloride[171] or hydroxyammonium chloride alone[160,173,200] to reduce the excess of permanganate and manganese oxides before the reduction to elemental mercury by tin(II). The presence of chloride ion as well as hydroxylamine is essential for the retention of mercury in solution[160,200,201]. Ascorbic acid[202] has also been used instead of hydroxylamine while other reducing agents such as hypophosphorous acid[171,194] have been used with limited success. The evolution of mercury by aeration without the addition of a reducing agent has also been described[203,204].

Apparatus that has been designed specifically for reduction–aeration analysis[125,180,205–208] includes ancillary equipment for adaptation of commercial atomic absorption instruments[209–212], some of these being available in kit form[213–222]. Automated analytical methods have also been described[47,198,223–226].

The sensitivity of the reduction–aeration method depends on the apparatus, on the sample volume and on the concentration, but in general, for most sample types determination of mercury, contents down to a few ng g^{-1} are attainable with absolute detection limits of about 1 ng (refs. 18, 125).

Digestion and wet oxidation procedures

The reduction–aeration method depends on the ability of a reducing agent such as tin(II) chloride to reduce in solution, inorganic and a limited number[33] of organic mercury compounds to metallic mercury. It is the function of the digestion and oxidation procedures to dissolve inorganic mercury compounds and decompose organic compounds. The complete destruction of organic material by wet chemical methods is tedious and difficult[227], especially in view of the temperature restrictions imposed by the volatility of mercury and some of its compounds. Many of the digestion and oxidation procedures used in practice fail to destroy organic material completely and their effectiveness must be validated for the particular sample type involved. Digestion procedures which have been developed in connection with other methods of analysis and have been reviewed[33,227–231], are relevant to non-flame atomic absorption methods. Methods suitable for atomic absorption determination have also been discussed and reviewed by many authors[18,25,27,147,232–234], and

sample decomposition by nitric and sulphuric acids under reflux is described in official procedures[235,236] and has been thoroughly investigated[237].

Many acid-digestion and oxidation combinations have been used, and selected examples of these are listed below. They differ in the time and temperature used, as well as whether the digestion is carried out under reflux, with simple air condensers or long-necked flasks, or in open flasks or tubes with no condenser.

Digestion with nitric acid has been used for foods[178] and soils[175], red fuming nitric acid for fish and plant material[238] and chromic acid for urine[238]. Mixtures of nitric and sulphuric acids have been employed for fish[25,27], waste water and effluents[189], in combination with vanadium pentoxide for biological materials[147,191] or followed by oxidation with potassium permanganate for waters[173,239], biological material[240] and soils, rocks and plant materials[160,175]. Acid digestion mixtures have been supplemented by oxidation with hydrogen peroxide for fish[223,241] or by potassium persulphate for fish[240], soils[175,234], water[173,200,242], rocks and sediments[242] and moss[243]. Nitric and sulphuric acid treatment has been followed by oxidation with perchloric and nitric acid for fish[172] and other samples[120]. Potassium permanganate and persulphate have been used for the preparation of water samples[223]. Procedures with sulphuric acid and potassium permanganate have been used for fish[214], foods[214,244], human tissue[245], urine[177,246,247], biological materials[55,214,174], water[244,248], effluents and other liquids[154,244], sewage[244], chemicals[244] and eyedrops[249]. Sulphuric acid digestion with oxidation by potassium permanganate and hydrogen peroxide has been used for fish[183,241], soils[171] and water[250]. Aqua regia has been used to dissolve paper and pulp[179,251], rock samples[252] and soils[184] and a mixture of nitric acid and hydrobromic acid for biological materials[253]. Perchloric acid has been used with nitric acid for hair, fruit, fish and foods[254] and for fish and blood[198] and in conjunction with nitric and sulphuric acids for fish[172] and various samples[120], but the possibility of losses of volatile mercury halides should be borne in mind[18,255].

Comparisons of permanganate and hydrogen peroxide with permanganate and persulphate oxidation in nitric and nitric–sulphuric acid digests respectively have been made[241]. Treatment of soils with nitric acid is claimed to be as effective as a mixture of nitric and sulphuric acids with potassium permanganate oxidation[256] but other workers[160,175] have found lower values in soils with nitric acid digestion alone.

Decomposition with hydrofluoric–nitric acid[257] in a sealed Teflon bomb at 150°C followed by boric acid treatment to complex the hydrofluoric acid has been applied to geological materials. A similar technique has been used for fish with nitric acid[158] and for fish and seaweed with a nitric and sulphuric acid mixture[259]. A stainless-steel bomb has been used for coal[137] and cereals[260,261]. Difficulties caused by losses on opening the bomb have been reported[137] and decontamination of the bomb lining is difficult; a platinum-lined stainless steel bomb has since been employed[262]. A sealed ampoule digestion procedure has been described[263]. Oxygen flask and other combustion methods used to provide solutions for the reduction–aeration technique have been described in an earlier Section.

Other wet oxidation procedures include the liberation of mercury from organic compounds by bubbling ozone-enriched oxygen through aqueous solutions[264] and adding bromine to drinking water with the excess of bromine

being reduced by hydroxyammonium chloride[53]. Photo-oxidation by ultraviolet radiation of organic complexes with the liberation of metal ions[265] has been applied to determinations of total mercury in waters[22,223].

Methods for organic mercurials

Reduction–aeration methods can estimate the content of organic mercury by determining separately the total mercury and the inorganic mercury—the organic mercury content being found by difference[57,266–270]. Inorganic mercury is determined by complexing the mercury with cysteine in acidic solution, from which, on the addition of tin(II) chloride and making the solution alkaline with sodium hydroxide, inorganic mercury is released. If a cadmium[266] or a copper[267] salt is also added, mercury is liberated from both organic and inorganic compounds. Discrimination of different organomercury compounds has been obtained by making use of the varying lability of methoxyethylmercury, phenylmercury and ethylmercury in treatments with and without cysteine and with and without digestion[271]. Aeration with air or oxygen in alkaline solution after reduction with tin(II) liberates mercury from ethylmercury but not methylmercury while bubbling nitrogen only releases inorganic mercury[272].

Interference effects

Interference by organic compounds. Although the absorption of many organic vapours has been shown to be small compared to that of mercury[38,144], and simple aliphatic alcohols and ketones have a negligible effect[173,273,274], unsaturated and aromatic organic compounds often absorb in the 253.7 nm region[18]. Acid digestion destroys simple alcohols and acetone[177], removes interference from halogenated hydrocarbons such as CCl_4 and $CHCl_3$ (refs. 144, 173), and reduces that from aromatic compounds[173,177]. No interference is shown by EDTA in digestion–reduction–aeration procedures[177]. In few applications of reduction–aeration to practical analysis have any vapour phase interference effects from organic compounds been reported, but the possibility of such effects exists and must be allowed for where necessary by one of the correction methods already described (p. 4) or reduced by purging the sample with air before the reducing agent (Sn^{2+}) is added[173].

Interference by inorganic species. The effects of anions of the common acids is quite small[275,276]. Depressive interferences by nitrate, perchlorate, sulphate, oxalate, phosphate[120], sulphite[275], sulphide[267], thiosulphate[177,267,274], bromide and iodide[120,176,177,253,275,276] have been reported and the effects of digestion on bromide and iodide interferences have been discussed in terms of possible errors from this source in fish and marine samples[253]. The elimination of bromide interference in the determination of mercury in lithium bromide has been described[276]. A preliminary dithizone extraction does not eliminate the possibility of iodide interference since iodide inhibits the mercury extraction[277]. The depression by chloride has been measured[120]; and, especially at hydrochloric acid concentrations above 4 *M*, has been attributed[34,278] to the formation of mercury–chloride complexes[279]. For most samples, these effects are not serious provided that proper standardization procedures are used.

Depressive interference effects caused by the presence of Pd (refs. 160, 177,

199), Pt (refs. 160, 177, 199, 205, 274), Au (refs. 160, 177, 199, 205, 274, 280), Ag (refs. 177, 267), Cu (refs. 171, 177) and Zn (ref. 150) have been reported, although the effects of Pd, Pt, Au and Ag in most geological samples[257] and soils[160] is negligible. Interference by copper is generally negligible[190,199,277] except in the analysis of copper metal[171]. The magnitude of the effects of these elements is probably in the order Pd > Pt > Au > Ag > Cu > Zn.

Serious depression by selenium and tellurium has been reported[199,205,274,280], although other authors find the effect of selenium to be much smaller[160] or nonexistent even at a Se/Hg ratio of 1000 (ref. 177). With dithizone extraction followed by reduction–aeration, Pt, Au, Mo and W have been shown to depress the apparent mercury content[34,278,281] but Cd, Cu^{2+}, Co, Ni, Pb, Zn, Fe^{2+}, Fe^{3+} have no effect[34].

Matrix interference effects can be reduced by the use of standards of similar composition to that of the samples[90]; such standards may be prepared by spiking the sample solutions themselves after the original mercury content has been removed[282] generally by reduction and aeration[193,253,283]. The use of a potassium permanganate solution to collect the products of a preliminary reduction and aeration can provide a standard matrix for sample and standard solutions. This is discussed in the following Section.

METHODS OF CONCENTRATION FROM SOLUTION

Methods of concentrating or collecting mercury from the vapour phase have already been considered (p. 4), largely in terms of reducing interference effects in the pyrolytic and combustion preparative techniques. The following discussion is concerned with concentrating mercury from solutions to increase sensitivity or to reduce matrix and other interference effects.

Reduction–aeration methods

Various concentration procedures involving the reduction–aeration technique have been used. These include the collection in acidic potassium permanganate solution of the mercury volatilized by reduction and aeration, from digests of liquid[152] or solid samples[160] and from natural waters[284,285] followed by its determination by further reduction–aeration.

Collection in acidic permanganate solution not only achieves large concentration factors but reduces the possibility of matrix interference effects by providing a standard matrix for the final determination[160,284]. This concentration method has been thoroughly studied[204,285]. Vapour-phase amalgamation on gold or silver has been used to collect mercury evolved from digests of geological materials[202,280], sediments, water and other samples by reduction–aeration[286,287].

Charcoal[288] has also been used to collect mercury from a reduction–aeration procedure.

Dithizone extraction methods

Dithizone extraction of sea water[289,290], blood[232,291], digests of human tissues[245], biological materials[153], fish[292] and urine[246] has been followed by evaporation of the solvent and volatilization of the mercury by heat into the

atomic absorption cell. This technique has been applied, following a combustion or a digestion and reduction–aeration treatment, with a high sensitivity instrument[154]. Mercury volatilized from a dithizone-in-chloroform extract has been amalgamated with gold[277].

The reduction–aeration technique has been applied to dithizone extracts after back-extraction into aqueous solution with hydrochloric acid[34,278] and has been used with the syringe partitioning method[192].

Amalgamation methods

Displacement reactions in acid solution (spontaneous amalgamation) based on the classical method of Reinsch[293] can deposit and amalgamate mercury on to wire or foils of platinum, gold, silver or copper[56,141,159,161,185,186,275,294–298], from which the mercury is then volatilized by heat for the atomic absorption measurement. Similar methods based on electrolytic deposition have also been described[296,299–305]. Interferences from sulphide[275,295], bromide and iodide[275] have been reported with amalgamation methods.

Other methods

Cadmium sulphide has been used to collect mercury[64,122] from various solution samples[55,306,307], from oxygen-flask combustion solutions[158,308], from resin column eluates[309] and from sea water by a colloid flotation technique[283]. Coprecipitation of mercury with cadmium sulphide has been applied in solutions of potassium bromide and carbonate[310] and in water samples[248]. In the latter case the precipitate is redissolved in aqua regia and the mercury determined by the reduction–aeration technique but, in general, the mercury is liberated from cadmium sulphide collectors for atomic absorption analysis by heat. A sulphide-treated polyurethane foam[250] and a starch–xanthate cationic polymer[311] have been used to extract mercury from waters. Ion-exchange resin columns have been used to collect mercury[22,309,312].

ATOMIC FLUORESCENCE METHODS

While atomic absorption methods have been the most widely used for determining mercury, atomic fluorescence has also been employed, principally with microfurnace or carbon filament techniques[242,313–316] where the small size of the vapour cloud lends itself to this technique. Selective impulse volatilization methods have been used with atomic fluorescence[102,103] and applied to the determination of impurities in graphite[103]. Platinum wire loop atomizers have also been used at 184.9 nm instead of the more usual 253.7 nm (ref. •242). The reduction–aeration procedure has also been used with atomic fluorescence[200,317,318]. Organic vapour interference is claimed to be lower than in the atomic absorption determination but particle scattering effects are higher[317,318]. Comparisons of atomic fluorescence with atomic absorption[200,317] have been made in air and argon[98] and various analytical applications of atomic fluorescence have been described[242,319–323].

PROBLEMS OF SAMPLE PREPARATION

The determination of mercury is made more difficult by the problem of

obtaining a representative sample without losses of volatile mercury or its compounds and without contamination during sampling, storage or laboratory preparation.

As most non-flame methods use small samples, typically of the order of 1 g, rocks, soils, coals and geological materials[91,137] must usually be dried and ground to a fine powder to obtain a reproducible subsample of the bulk material. Considerable losses have been reported on drying sediments and soils at 60–80°C (refs. 91, 169, 175), therefore drying at room temperature is advisable[91,324]. Geological materials have been ground wet[202] and soils can be analyzed wet[175] but the reproducibility of sampling such wet unground soils is poor. Negligible losses are likely in grinding soils[91] but in grinding rocks of about 10-mm size down to *ca.* 20 mesh (0.841 mm) the mercury content found increased by almost 100% falling again steeply with a ca. 200 mesh (0.074 mm) sample[325]. Special soil samplers have been described[326].

Sampling dried biological materials is also difficult because they are less easily reduced to homogeneous powders. Errors caused by discrete particles of eel skin, for example, have been reported[238]. Homogenization procedures with sodium hydroxide have been used for biological tissues before subsampling[100,139,198,238,254,266,327].

In drying biological materials by freeze drying, no loss of mercury(II)[328,329] or phenylmercury acetate[329] occurs, but losses of 50% or more of other forms of organically combined mercury[169,328,329] have been found. Vacuum desiccation and freeze drying have given identical mercury contents[308]. The absence of sulphydryl groups may contribute to losses of mercury in urines or sediments[329]. Losses from frozen fish stored in plastic bags are negligible[330] and even 200 year-old museum fish appear to have held their mercury[330].

Losses as a result of bacterial action in sea water can be reduced by the addition of bacteriostatic compounds such as chloroform, penicillin, streptomycin etc[331] although acetic acid and formaldehyde[331] are ineffective. Nutrients such as glucose increase this bacterial loss which occurs partly by volatilization and partly by adsorption on the vessel[331] but the presence of acid permanganate can prevent this adsorption[248]. Methylation of inorganic mercury can occur in soils and the subsequent decomposition of organic mercury compounds by micro-organisms such as chlamydomonas[332] may be one of the principal sources of volatilization from soils[326].

CONTAMINATION OF SAMPLES AND APPARATUS

The cross-contamination of geological samples is a major problem[333] and its avoidance requires the segregation of samples known or suspected to be widely different in mercury content[91]. The presence of mercury vapour in buildings[69] and in the laboratory atmosphere[149] is a hazard because of its absorption by samples, apparatus and reagents. Its presence in soil laboratories has been noted[58]. The possibilities of contamination from McLeod vacuum gauges have been investigated[334] and should be considered when these are fitted to vacuum or freeze-drying equipment.

Reagents themselves may be contaminated[194] and must be tested and selected

or purified by purging with air[257]. If reduction and aeration is used as a concentration procedure, contamination must be avoided by removing the mercury from the aerating gas[284,285]. Unstoppered bottles of acidic potassium permanganate used for many digestion procedures readily collect mercury from contaminated laboratory air (unpublished work).

Contamination by adsorbed mercury on glass[120,257,332,335–337], on polyethylene[337,338], polyvinyl chloride and polytetrafluoroethylene[337] have all been documented. Mercury can diffuse into and through polyethylene[330,339] and this has been used to prepare mercury-free matrix solutions for the preparation of standard solutions[282]. In freeze-dried fish samples, however, no adsorption of mercury was found in the polyethylene storage bags[169].

Most workers use simple acid and water washing[25,27] of vessels, but soaking in detergent followed by acid washing[160], acid potassium permanganate washing[237,284] and even washing with sulphuric acid and dichromate followed by oven drying[337] has been used to remove adsorbed mercury.

LOSSES OF MERCURY FROM SOLUTION

Losses of mercury from solution samples such as sea water have been discussed on p. 12. In the following, losses from solutions prepared from samples will be reviewed.

Almost all the mercury can be lost from solutions of mercury salts as a result of the continuous reduction, by traces of reducing agents, of mercury(II) to mercury(I) which then disproportionates to mercury(II) and mercury with a resulting liberation of mercury vapour at room temperatures[340]. This can be prevented by the presence of potassium permanganate or another agent of higher oxidation potential than that of the reaction of $Hg^{2+} \rightarrow Hg^{+}$ (ref. 340); for this reason permanganate has frequently been added to sample and standard solutions[25,27,200,244,248,253,283,284]. After the addition of potassium permanganate, acidic standard solutions can be preserved for up to 6 months[283]. Acid–permanganate may also reduce adsorption losses on vessel walls[244], but this may be caused by the acidity[341] rather than by the permanganate. Acidifying solutions with nitric acid[341,342] appears to be partly effective in reducing losses[25,27,204,343] and is most effective if the nitric acid is in the vessel before a sample is added[282,343]. Losses can occur on transfer of solutions from vessel to vessel[120,163,344], or on filtration[290], but the incorporation of sodium chloride, it is claimed, reduces these transfer losses[344].

Nitric acid acidification of natural water samples may in some cases increase the possibility of loss since, over a period of time, mercury dissolved from particulate matter may then volatilize[342]. Adsorption losses on most vessels are also possible and adsorption half-lives for glass, polyvinyl chloride and polyethylene have been calculated and losses in polytetrafluoroethylene vessels reported[337].

ANALYTICAL ACCURACY

Validation of analytical methods for mercury is difficult because only a few reference materials are available: wheat and corn flour[345–347], kale[348,349], orchard

leaves, liver and coal[120,350]. Gelatin has been suggested as a matrix for reference materials[351]. Their paucity is at least partly due to the impermanence of the mercury content of natural materials. Only the NBS orchard leaves, liver and coal standard samples[120,350,352] have authenticated mercury contents; for the remainder too few determinations have been reported for definitive mercury contents to be assigned to them. Inconsistent results for mercury have been obtained for Bowens' kale[348] and inhomogeneity has been reported for mercury in the U.S. Geological Survey Standard Rocks[353].

Most workers therefore have attempted to standardize their method by using alternative methods of analysis or by addition and recovery experiments. Such comparisons have been made of non-flame atomic absorption techniques with flame emission and atomic absorption[354,355], spectrographic emission[257,356], neutron activation[138,169,191,200,244,256,259,277,351,354,357,358], x-ray fluorescence[89], isotope dilution mass spectrometry[120] and molecular absorption[13,16,68,138,244,257,357,359] methods. Other comparative analyses on an interlaboratory basis have been made[138,200,273,355,357,359], improved precision being obtained when a standardized analytical method is used[360].

There is little doubt that measurement of the atomic absorption of mercury in the vapour phase can be carried out accurately and precisely, and that in practical analysis most of the errors arise from incomplete destruction or removal of organic compounds, or from loss of or contamination by mercury. The most useful comparisons are those in which radically different sample preparative methods are used irrespective of the final measurement technique. These include the comparison of digestion with oxygen flask combustion preparative methods[120,147,160,194], with furnace combustion[105] and with pyrolytic methods[361].

Adequate recoveries of added inorganic and organic mercury have been reported for most types of sample treated by digestion methods and determined by the reduction–aeration technique. These include, for example, 97% recovery in diets at the 0.001 and 0.01 p.p.m. level[193], 95–102% in foods[178], around 100% in biological materials[147] and soils[160,234], effluents[362], 98–104% in urine[197], 93–94% in waters[192] and sea water[283]. With fish samples moderately good recoveries of added mercury have been reported for digestion procedures[188,224], but difficulties due to the presence of fats in fish and other biological tissues[362,363] have been encountered. Since the fat is often low in mercury content[253] it may be removed after digestion[147,253] or extracted with chloroform before digestion[364]. Some workers[172,241] have obtained low recoveries with fish using a digestion technique; others have abandoned this in favour of combustion[139] because of low and erratic results with fish samples. In an inter-laboratory comparative analysis, the results by several methods were more consistent for fish with low fat contents[354].

Recoveries of added mercury from solutions prepared by oxygen-flask methods have often been low[159,160], probably partly on account of inadequate collection of mercury from the flask by simple acid solutions, the presence of sufficient oxidizing agent such as potassium permanganate being essential for complete collection[160,365]. Quantitative recovery has been obtained with acidic permanganate[160,162] and chlorine water[161] collecting solutions.

APPLICATIONS

A wide variety of sample types has been analyzed by non-flame atomic absorption and fluorescence techniques and the summary given below includes references already discussed as well as cataloguing other applications of the various techniques reviewed.

Air and gases

References 7, 14, 38–44, 46, 47, 55, 56, 58, 59, 61, 62, 65–72, 113–116, 126, 134, 142, 144, 149, 291, 319, 360, 366–376.

Biological materials

References 55, 57, 119, 129, 133, 147, 159, 174, 186, 187, 190, 191, 196, 214, 216, 226, 240, 245, 247, 253, 254, 259–262, 266, 268–270, 277, 327, 329, 377–384.

Blood

References 13, 67, 100, 133, 196, 198, 225, 232, 254, 262, 266, 268, 291, 309, 374, 385–387.

Detectors for gas chromatograph

References 388–390.

Chemicals

References 119, 126, 171, 189, 244, 273, 276, 310, 360, 389, 391–394.

Coal

References 129, 134, 137, 150, 166, 225, 281.

Effluents

References 47, 176, 189, 244, 362, 395.

Eye drops

Reference 249.

Fats

Reference 129.

Fish

References 15, 119, 123, 134, 139, 140, 145, 162, 163, 172, 186, 190, 198, 213, 216, 217, 224, 225, 238, 240, 241, 253, 254, 258, 259, 262, 266, 292, 296, 298, 308, 354, 371, 373, 381, 384, 396–407.

Flour

References 147, 163, 242, 345, 346.

Foods

References 72, 133, 134, 147, 178, 188, 193, 213, 244, 254, 308, 355, 362, 383, 408–411.

Gelatin
References 134, 351

Hair
References 72, 167, 134, 254, 262, 412–414.

Ice
Reference 415.

Isotope composition
Reference 416.

Minerals
References 83, 85, 89, 91, 101, 108, 131, 195, 205, 295, 417

Mosses
References 243, 248, 418.

Oils
Reference 161.

Optical coatings
Reference 419.

Paints
References 69, 119, 132, 304.

Paper and wood pulp
References 110, 119, 132, 304.

Plant material
References 72, 110, 140, 147, 148, 158, 163, 165, 194, 238, 243, 253, 259–262, 270, 381, 418, 420–422.

Plastics and rubber
References 149, 189.

Radioactive materials
Reference 423.

Rocks
References 28, 82, 84–86, 90–95, 127, 129, 130, 135, 136, 160, 171, 195, 200, 202, 242, 252, 257, 280, 325, 349, 417, 424–429.

Seaweed
References 253, 259, 381.

Sediments

References 72, 83, 90, 140, 175, 200, 242, 256, 276, 286, 290, 298, 358, 360, 403, 428, 430–433.

Soils

References 81–83, 86, 88, 114, 127, 130, 134–136, 148, 160, 171, 175, 191, 234, 256, 280, 324, 326, 424, 425, 429.

Urine

References 23, 55, 67, 70, 100, 110, 126, 133, 177, 196–198, 238, 254, 300, 302, 305, 307, 310, 320, 371, 373, 374, 385, 386–387, 399, 434–438.

Water

Drinking. References 53, 250, 362, 391.

Natural. References 20, 22, 34, 67, 70–72, 75, 129, 141, 176, 180, 190, 192, 198, 200, 205, 216, 223, 239, 242, 244, 248, 250, 275, 278, 285, 286, 295, 298, 311, 359, 360, 371, 373, 374, 399, 400, 410, 418, 422, 428, 439–442.

Sea and brine. References 22, 121, 192, 216, 225, 248, 282–285, 289, 290, 312, 342, 360, 415, 428.

SUMMARY

Analytical aspects of the determination of mercury by non-flame atomic absorption and fluorescence spectrometry are comprehensively reviewed. Numerous applications are listed.

REFERENCES

1 R. W. Wood, *Physical Optics*, Macmillan, New York, 3rd edn., 1934, p. 587.
2 R. W. Wood, *Phil. Mag.*, 23 (1912) 689.
3 F. Goos and H. Meyer, *Z. Phys.*, 35 (1926) 803.
4 A. L. Hughes and A. R. Thomas, *Phys. Rev.*, 30 (1927) 466.
5 K. Müller, *Z. Phys.*, 65 (1930) 739.
6 K. Müller and P. Pringsheim, *Naturwissenschaften*, 18 (1930) 364.
7 T. T. Woodson, *Rev. Sci. Instrum.*, 10 (1939) 308.
8 T. T. Woodson, *Safe Pract. Bull., Pa. Dep. Labor Ind.*, 9 (1939)
9 T. T. Woodson, *U.S. Patent No. 2227117*, 1939.
10 L. R. Biggs, *J. Ind. Hyg. Toxicol.*, 20 (1938) 161.
11 I. R. Jonasson, *Geol. Surv. Can. Pap., 70–57*, 1970.
12 F. N. Ward, *U.S. Geol. Surv. Prof. Pap.*, 713 (1970) 46.
13 L. Vignoli, R. Badre, M. C. Morel and J. Ardorino, *Chim. Anal. (Paris)*, 45 (1963) 53.
14 R. A. Baker and M. D. Luh, *Ind. Water Wastes*, 16 (3) (1971) 21.
15 Anonymous, *Arch. Fischereiwiss.*, 23 (1972) 7.
16 C. G. Lamm and J. Růžička, in *Mercury Contamination in Man and his Environment, Tech. Rep. Ser. Int. At. Energy Ag.*, 137, 1972, Ch. 9.
17 R. G. Smith, in R. Hartung and B. D. Dinman (Eds.), *Environmental Mercury Contamination Int. Conf. Michigan 1970*, Ann Arbor, 1972, p. 97.
18 R. S. Reimers, W. D. Burrows and P. A. Krenkel, *J. Water Pollut. Contr. Fed.*, 45 (1973) 814.
19 A. V. Holden, *J. Food Technol.*, 8 (1973) 1.
20 P. N. Cheremisinoff and Y. H. Habib, *Water Sewage Works*, 119 (1972) 73.

21 M. J. Fishman and D. E. Erdmann, *Anal. Chem.*, 45 (1973) 361R.
22 W. B. Fitzgerald and W. B. Lyons, *Nature (London)*, 242 (1973) 452.
23 W. Slavin, *Occup. Health Rev.*, 17 (1965) 9.
24 K. Kojima, M. Fujita and M. Ohno, *Soda To Enso*, 23 (1972) 199.
25 J. D. Brooks and W. E. Wolfram, *Chem. Eng. News.*, 48 (1970) 37.
26 D. C. Manning, *At. Absorption Newslett.*, 9 (1970) 97.
27 J. D. Brooks and W. E. Wolfram, *Amer. Lab.*, 3 (1971) 54.
28 V. G. Feklichev and V. P. Pashutin, in E. I. Semenov (Ed.), *Metod. Mineral. Issled.*, Nauka, Moscow, U.S.S.R., 1971, p. 38.
29 F. Yamauchi and Y. Katsuno, *Soda To Enso*, 22 (1971) 303.
30 D. P. Hubbard (Ed.), *Annual Reports on Analytical Atomic Spectroscopy, Vol. 1*, The Society for Analytical Chemistry, London, 1971.
31 D. P. Hubbard (Ed.), *Annual Reports on Analytical Atomic Spectroscopy, Vol. 2*, The Society for Analytical Chemistry, London, 1972.
32 G. F. Kirkbright, *Analyst (London)*, 96 (1971) 609.
33 J. F. Coetzee, in I. M. Kolthoff, P. J. Elving and E. B. Sandell (Eds.) *Treatise on Analytical Chemistry, Part II, Vol. 3*, Interscience, New York, 1961, p. 231.
34 Y. Chau and H. Saitoh, *Environ. Sci. Technol.*, 4 (1970) 839.
35 D. Taylor, *Mercury as an Environmental Pollutant. A Bibliography*, ICI Brixham Laboratory, England, 3rd edn., 1973.
36 M. E. Barnes (Ed.), *Mercury Contamination in the Natural Environment*, PB 192910, US Department of the Interior, Washington DC, 1970.
37 E. A. Jenne and W. Sanders, *J. Water Pollut. Contr. Fed.*, 45 (1973) 1952.
38 V. F. Hanson, *Ind. Eng. Chem., Anal. Ed.*, 13 (1941) 119.
39 V. A. Ipatov and L. P. Pakhomov, *Prib. i Tekh. Eksp.*, 2 (1958) 91.
40 N. I. Petrova, N. B. Shleikhev and A. Z. Razyapov, *Gig. Sanit.*, 33 (1968) 44.
41 G. Thilliez, *Chim. Anal. (Paris)*, 50 (1968) 226.
42 D. J. Troy, *Anal. Chem.*, 27 (1955) 1217.
43 O. Aksnes, *Patent, German Offen., 2017241*, 26 Nov. 1970.
44 T. S. Ely, *U.S. At. Energy Comm. WASH-744*, 1957.
45 C. S. McMurray and J. W. Redmond, *U.S. At. Energy Comm., Y-1188*, 1958.
46 A. B. Belozovskii, E. V. Zhuravlev, V. I. Tarasov and Yu. V. Subbotin, *Gig. Sanit.*, 11 (1972) 78.
47 I. A. Capuano, *U.S. Patent 3, 713, 776*, 30 Jan. 1973.
48 R. O. R. Brooks and A. Holmes, *The Control of Mercury Metal in the Laboratory, At. Energy Res. Est., MED/R-2350*, Harwell, Berks., 1958.
49 *Lab. Pract.*, 15 (1966) 1171.
50 *Lab. Pract.*, 20 (1971) 607.
51 *Anal. Chem.*, 43 (*4*) (1971) 121A.
52 *Anal. Chem.*, 43 (*14*) (1971) 81A.
53 T. Kempf and J. Kneisel, *Beckman Rep.*, 1 (1972) 32.
54 M. M. Schachter, *J. Ass. Offic. Anal. Chem.*, 49 (1966) 778.
55 J. L. Monkman, P. A. Maffett and T. F. Doherty, *Amer. Ind. Hyg. Ass. J.*, 17 (1956) 418.
56 W. C. L. Hemeon and G. F. Haines, Jr., *Amer. Ind. Hyg. Ass. J.*, 22 (1961) 75.
57 L. Magos and A. A. Cernik, *Brit. J. Ind. Med.*, 26 (1969) 144.
58 H. D. Fisher and J. W. Cary, *Soil Sci. Soc. Amer., Proc.*, 34 (1970) 701.
59 E. Mayz, M. Corn and G. Barry, *Amer. Ind. Hyg. Ass. J.*, 32 (1971) 373.
60 R. M. Dagnall, J. M. Manfield, M. D. Silvester and T. S. West, *Nature (London), Phys. Sci.*, 235 (1972) 156.
61 J. W. Robinson, P. J. Slevin, G. D. Hindman and D. K. Wolcott, *Anal. Chim. Acta*, 61 (1972) 431.
62 J. W. Robinson and P. Loftin, *Int. Conf. At. Abs. Spectrosc., Sheffield*, Abstract B1, Hilger, London, 1969.
63 D. C. Manning and F. Fernandez, *At. Absorption Newslett.*, 7 (1968) 24.
64 A. E. Ballard and C. D. W. Thornton, *Ind. Eng. Chem., Anal. Ed.*, 13 (1941) 893.
65 G. A. Hamilton and A. D. Ruthven, *Lab. Pract.*, 15 (1966) 995.
66 J. H. McCarthy, Jr., W. W. Vaughn, R. E. Learned and J. L. Meuschke, *U.S. Geol. Surv. Circ. 609*, 1969.
67 L. A. Krause, R. Henderson, H. P. Shotwell and D. A. Culp, *Amer. Ind. Hyg. Ass. J.*, 32 (1971) 331.
68 D. L. Chase, D. L. Sgontz, E. R. Blosser and W. M. Henry, *U.S. Nat. Tech. Inform. Serv.*,

PB Rep. No. 210822, 1972.
69 R. S. Foote, *Science (Wash. DC)*, 177 (1972) 513.
70 J. Y. Hwang and Y. Hashimoto, *IUPAC Int. Congr. Anal. Chem. 4, Kyoto, Japan, 1972*, Jap. Soc. for Anal. Chem., Book of Abstracts, Abstr. No. A0611.
71 E. Kunkel, *Fresenius' Z. Anal. Chem.*, 258 (1972) 337.
72 M. A. Bombace, L. C. Rossi, G. F. de Clemente, G. Z. Labellarte, M. Allegrini and E. Lanzola, *Preprint, Eur. Colloq. Comm. Eur. Commun., Luxembourg 1973*, No. 3.
73 L. P. Morgenthaler, *McKee-Pederson Instrum. Appl. Notes*, 5 (1970) 25.
74 Mercury Analysis Down to 0.01 μg, *Instrum. News*, 21 (1971) 13.
75 Data Control Inc., *Int. Lab.*, (July/Aug. 1971) 67.
76 G. M. Farren, O. C. Karpathy and M. M. Chamran, *Pittsburgh Conf., Anal. Chem. Appl. Spectrosc.*, Abstr. No. 99, 1971.
77 L. P. Morgenthaler, *Anal. Chem.*, 43 (7) (1971) 89A.
78 Mercury analysis systems, *Lab. Equip. Dig.*, 10 (11) (1972) 42.
79 Imperial Chemical Industries, *Fr. Demande 2, 024, 596*, 28 August 1970.
80 H. E. Hawkes and S. H. Williston, *Mining Congr. J.*, 48 (1962) 30.
81 R. W. Boyle, *Geol. Surv. Can. Pap., 66–46*, 1966.
82 A. Sutherland Brown, *West. Miner*, (Feb. 1966) 39.
83 L. M. Azzaria and G. R. Webber, *Can. Inst. Mining Met. Bull.*, 62 (1969) 521.
84 C. H. James and J. S. Webb, *Trans. Inst. Mining Met.*, 73 (1964) 633.
85 L. M. Azzaria, *Geol. Surv. Can. Pap., 66–54*, 1967, p. 13.
86 A. Sutherland Brown, *Geol. Surv. Can. Pap., 66–54*, 1967, p. 73.
87 J. A. Wenninger, *J. Ass. Offic. Agr. Chem.*, 48 (1965) 826.
88 H. V. Warren and R. E. Delavault, *Oikos*, 20 (1969) 537.
89 H. G. Henry, K. R. Stever, W. L. Barry and H. H. Heady, *Appl. Spectrosc.*, 26 (1972) 288.
90 S. R. Aston and J. P. Riley, *Anal. Chim. Acta*, 59 (1972) 349.
91 M. Koksoy, P. M. D. Bradshaw and J. S. Tooms, *Trans. Inst. Mining Met., Sect. B*, 76 (1967) B121.
92 J. A. Goleb, *Appl. Spectrosc.*, 25 (1971) 522.
93 J. A. Goleb, *XVI Colloq. Spectrosc. Int., Heidelberg*, 1 (1971) 23.
94 V. Z. Fursov, *Proc. Acad. Sci., USSR*, 204 (1972) 184.
95 V. Z. Fursov and I. I. Stepanov, *Razved Okhr. Nedr.*, 37 (1971) 38.
96 B. V. L'vov and A. D. Khartsyzov, *Zh. Prikl. Spektrosk.*, 10 (1969) 413.
97 B. V. L'vov, *Spectrochim. Acta, Part B*, 24 (1969) 53.
98 Yu. I. Belyaev and T. A. Koveshnikova, *Acta XVII Colloq. Spectrosc. Int., Florence*, 1 (1973) 62.
99 R. M. Dagnall, J. M. Manfield, M. D. Silvester and T. S. West, *Spectrosc. Lett.*, 6 (1973) 183.
100 J. W. Hurd, H. M. Stahr, W. Hyde and A. Anderson, *Pittsburgh Conf. Anal. Chem. Appl. Spectrosc. Paper, 130* (1972).
101 S. H. Omang, *Kjemi*, 31 (1971) 13.
102 A. M. Pchelintsev, Yu. I. Belyaev, A. V. Karyakin and T. A. Koveshnikova, *Zh. Anal. Khim.*, 26 (1971) 1355.
103 A. M. Pchelintsev, Yu. I. Belyaev, A. V. Karyakin and T. A. Koveshnikova, *3rd. Int. At. Abs. Fluoresc. Spectrosc. Congr., GAMS Paris, 1971*, Hilger, 1973, p. 28.
104 R. Stephens, *Anal. Lett.*, 5 (1972) 851.
105 B. G. Weissberg, *Econ. Geol.*, 66 (1971) 1042.
106 Yu. N. Kuznetsov and L. P. Chabovskii, *Uch. Zap. Tsent. Nauch. Issled. Inst. Olovyannoi Prom.*, (1964) 75.
107 Yu. N. Kuznetsov and L. P. Chabovskii, *Zavod. Lab.*, 31 (1965) 1085.
108 L. P. Chabovskii and Yu. N. Kuznetsov. *Zavod. Lab.*, 34 (1968) 1549.
109 Yu. N. Kuznetsov, V. M. Konovalov, I. I. Stepanov and L. P. Chabovskii, *Prikl. Spektrosk. Mater. Soveshch., 16th, 1965*, 1 (1969) 105.
110 O. Lindström, *Anal. Chem.*, 31 (1959) 461.
111 R. L. Windham, *Anal. Chem.*, 44 (1972) 1334.
112 S. H. Williston, *U.S. Patent, No. 3, 281, 596*, Oct. 1966.
113 S. H. Williston, *J. Geophys. Res.*, 73 (1968) 7051.
114 A. R. Barringer, *Trans. Inst. Mining Met. (Lond.) Sect B*, 75 (1966) 120.
115 A. R. Barringer, *U.S. Patent, No. 3, 449, 565*, 10 June 1969.
116 C. Ling, *Anal. Chem.*, 39 (1967) 798.

117 C. Ling, *Anal. Chem.*, 40 (1968) 1876.
118 C. Ling, *U.S. Patent, No. 3, 413, 482*, 26 Nov. 1968.
119 T. Hadeishi and R. D. McLaughlin, *Science (Wash. DC)*, 174 (1971) 404.
120 T. C. Rains and O. Menis, *J. Ass. Offic. Anal. Chem.*, 55 (1972) 1339.
121 R. A. Carr, J. B. Hoover and P. E. Wilkniss, *Deep Sea Res.*, 19 (1972) 747.
122 A. E. Ballard, D. W. Stewart, W. O. Kamm and C. W. Zuehlke, *Anal. Chem.*, 26 (1954) 921.
123 C. A. Bache, D. Gutenmann and D. J. Lisk, *Science*, 172 (1971) 951.
124 H. L. Kahn, *At. Absorption Newslett.*, 7 (1968) 40.
125 D. C. Manning, *At. Absorption Newslett.*, 9 (1970) 109.
126 M. Olivier, *Fresenius' Z. Anal. Chem.*, 257 (1971) 187.
127 W. W. Vaughn and J. H. McCarthy, Jr., *U.S. Geol. Surv. Prof. Pap.*, 501D (1964) 123.
128 S. H. Williston and M. H. Morris, *U.S. Patent No. 3, 173, 016*, 1965.
129 O. I. Joensu, *Appl. Spectrosc.*, 25 (1971) 526.
130 J. Marinenko, I. May and J. I. Dinnin, *U.S. Geol. Surv. Prof. Pap.*, 800B (1972) 151.
131 W. W. Vaughn, *U.S. Geol. Surv. Circ.*, 540 1967.
132 J. A. Wenninger and J. H. Jones, *J. Ass. Offic. Agr. Chem.*, 46 (1963) 1018.
133 V. Lidums and U. Ulfvarson, *Acta Chem. Scand.*, 22 (1968) 2150.
134 D. H. Anderson, J. H. Evans, J. J. Murphy and W. W. White, *Anal. Chem.*, 43 (1971) 1511.
135 K. Kato, *Chishitsu Chosasho Geppo*, 22 (1971) 437.
136 K. Kato, A. Ando and T. Kishimoto, *Bunseki Kagaku*, 21 (1972) 1057.
137 J. V. O'Gorman, N. H. Suhr and P. L. Walker, Jr., *Appl. Spectrosc.*, 26 (1972) 44.
138 M. D. Schlesinger and H. Schultz, *Rep. Invest. U.S. Bur. Mines, 7609*, 1972.
139 R. J. Thomas, R. A. Hagstrom and E. J. Kuchar, *Anal. Chem.*, 44 (1972) 512.
140 W. A. Willford, R. J. Hesselberg and H. L. Bergman, *J. Ass. Offic. Anal. Chem.*, 56 (1973) 1008.
141 E. Kunkel, *3rd. Int. At. Fluoresc. Spectrosc. Congr., GAMS Paris, 1971*, preprint 503.
142 J. Scullman and G. Widmark, *Int. J. Environ. Anal. Chem.*, 2 (1972) 29.
143 S. J. Long, D. R. Scott and R. J. Thompson, *Anal. Chem.*, 45 (1973) 2227.
144 C. M. Christian and J. W. Robinson, *Anal. Chim. Acta*, 56 (1971) 466.
145 A. Schulert, A. Zeind, J. Davis and D. G. Nicholson, in D. D. Hemphill's (Ed.) *Trace Subst. Environ. Health-5, Proc. Univ. Misouri Annu. Conf. 5th, 1971*, 1972, p. 413.
146 A. R. Schulert, J. T. Davis and D. G. Nicholson, in R. Hartung and B. D. Dinman (Eds.), *Environmental Mercury Contamination, Int. Conf., Michigan 1970*, Ann Arbor, 1972, 153.
147 M. Malaiyandi and J. P. Barrette, *Anal. Lett.*, 3 (1970) 579.
148 Y. Kimura and V. L. Miller, *Anal. Chim. Acta*, 27 (1962) 325.
149 R. O. Scott and A. M. Ure, *Proc. Soc. Anal. Chem.*, 9 (1972) 288.
150 B. W. Bailey and F. C. Lo, *J. Ass. Offic. Anal. Chem.*, 54 (1971) 1447.
151 V. Lidums, *Chem. Scr.*, 2 (1972) 159.
152 P. Jones and G. Nickless, *Proc. Soc. Anal. Chem.*, 10 (1973) 269.
153 Y. Taguchi, *Nippon Eiseigaku Zasshi*, 25 (1971) 553.
154 H. Makabe, F. Kawashima and K. Kawasaki, *Shimadzu Hyoron*, 28 (1971) 71.
155 W. Schöniger, *Mikrochim. Acta*, (1955) 123.
156 W. Schöniger, *Mikrochim Acta*, (1956) 869.
157 W. H. Gutenmann and D. J. Lisk, *J. Agric. Food Chem.*, 8 (1960) 306.
158 E. G. Pappas and L. A. Rosenberg, *J. Ass. Offic. Anal. Chem.*, 49 (1966) 782.
159 I. Okuno, R. A. Wilson and R. E. White, *J. Ass. Offic. Anal. Chem.*, 55 (1972) 96.
160 A. M. Ure and C. A. Shand, *Anal. Chim. Acta*, 72 (1974) 63.
161 M. E. Hinkle, *U.S. Geol. Surv. Prof. Pap.*, 750-B (1971) 171.
162 Y. Ukishima, T. Nagai, T. Nagano, T. Unno, S. Terada and S. Hattori, *Eisei Kagaku*, 18 (1972) 270.
163 J. F. Uthe, F. A. J. Armstrong and M. P. Stainton, *J. Fish. Res. Board Can.*, 27 (1970) 805.
164 K. Katsumura, M. Ishizaki, A. Sasamoto, T. Sasamoto and S. Ueno, *Shokuhin Eiseigaku Zasshi*, 14 (1973) 137.
165 C. A. Bache, W. H. Gutenmann, L. E. St. John, Jr., R. D. Sweet, H. H. Hatfield and D. J. Lisk, *J. Agric. Food Chem.*, 21 (1973) 607.
166 E. N. Pollock, *166th Natl. Meet. Am. Chem. Soc., Chicago IU, USA, Aug. 26–31, 1973.*
167 J. J. Silvin and D. J. Lisk, *Bull. Environ. Contam. Toxicol.*, 9 (1973) 318.
168 C. E. Mulford, *At. Absorption Newslett.*, 5 (1966) 135.
169 K. K. S. Pillay, C. C. Thomas, Jr., J. A. Sondel and C. M. Hyche, *Anal. Chem.*, 43 (1971) 1419.

170 N. S. Poluektov and R. A. Vitkun, *Zh. Anal. Khim.*, 18 (1963) 37.
171 W. R. Hatch and W. L. Ott, *Anal. Chem.*, 40 (1968) 2085.
172 R. K. Munns and D. C. Holland, *J. Ass. Offic. Anal. Chem.*, 54 (1971) 202.
173 J. F. Kopp, M. C. Longbottom and L. B. Lobring, *J. Amer. Water Works Ass.*, 64 (1972) 20.
174 G. Höllerer and J. Hoffman, *Z. Lebensm.-Unters.-Forsch.*, 150 (1973) 277.
175 I. K. Iskandar, J. K. Syers, L. W. Jacobs, D. R. Keeney and J. T. Gilmour, *Analyst (London)*, 97 (1972) 388.
176 R. Osland, *Spectrovision*, 24 (1970) 11.
177 G. Lindstedt, *Analyst (London)*, 95 (1970) 264.
178 W. L. Hoover, J. R. Melton and P. A. Howard, *J. Ass. Offic. Anal. Chem.*, 54 (1971) 860.
179 D. C. Lee and C. W. Laufmann, *Anal. Chem.*, 43 (1971) 1127.
180 T. R. Gilbert and D. N. Hume, *Anal. Chim. Acta*, 65 (1973) 461.
181 P. A. Ulluci, J. Y. Hwang and A. L. Malenfant, *Pittsburgh Conf. Anal. Chem. Appl. Spectrosc.*, Abstr. 28, 1971.
182 M. E. Roth, *Flame Notes*, 2 (1967) 98.
183 M. P. Stainton, *Anal. Chem.*, 43 (1971) 625.
184 J. W. Hamm and J. W. B. Stewart, *Commun. Soil Sci. Plant Anal.*, 4 (1973) 233.
185 G. Cumont, *Chim. Anal. (Paris)*, 53 (1971) 634.
186 G. Cumont, *Ann. Fals. Expert. Chim.*, 64 (1971) 115.
187 G. Cumont, *3rd. Int. At. Absorption Fluoresc. Spectrosc. Congr., GAMS Paris, 1971*, p. 610.
188 V. A. Thorpe, *J. Ass. Offic. Anal. Chem.*, 54 (1971) 206.
189 K. H. Nelson, W. D. Brown and S. J. Staruch, *Int. J. Environ. Anal. Chem.*, 2 (1972) 45.
190 G. Cumont, M. B. Dagorn and H. Lelievre, *Preprint, Eur. Colloq. Comm. Eur. Commun., Luxembourg, 1973*, No. 17.
191 F. D. Deitz, J. L. Sell and D. Bristol, *J. Ass. Offic. Anal. Chem.*, 56 (1973) 378.
192 J. A. J. Thompson and F. T. McComas, *Environ. Lett.*, 5 (1973) 189.
193 G. B. Collins, *Preprint, Eur. Colloq. Comm. Eur. Commun., Luxembourg, 1973*, No. 11.
194 T. C. Rains, in *Nat. Bur. Stand. (U.S.) Tech. Note*, 584, 1971, p. 108.
195 R. B. Brand and N. M. Wilkinson, *J. Geochem. Explor.*, 1 (1972) 195.
196 W. J. Hermann, Jr., J. W. Butler and R. G. Smith, in F. W. Sunderman (Ed.), *Laboratory Diagnosis of Diseases Caused by Toxic Agents: Proc. Appl. Semin.*, W. H. Green, St. Louis, Missouri, 1970, p. 379.
197 A. O. Rathje, *Amer. Ind. Hyg. Ass. J.*, 30 (1969) 126.
198 I. Skare, *Analyst (London)*, 97 (1972) 148.
199 I. R. Jonassen, J. J. Lynch and L. J. Trip, *U.S. Geol. Surv. Can. Pap., 73-21*, 1973.
200 V. I. Muscat and T. J. Vickers, *Anal. Chim. Acta*, 57 (1971) 23.
201 Y. Kimura and V. L. Miller, *Anal. Chem.*, 32 (1960) 420.
202 P. C. Head and R. A. Nicholson, *Analyst (London)*, 98 (1973) 53.
203 W. H. Gutenmann, D. J. Lisk and N. Grier, *Bull. Environ. Contam. Toxicol.*, 8 (1972) 138.
204 S. Oki and M. Watanabe, *Bunseki Kagaku*, 20 (1971) 1204.
205 A. M. Igoshin and L. N. Bogusevich, *Razved Okhr. Nedr.*, 34 (9) (1968) 55.
206 M. H. Mittelhauser, *At. Absorption Newslett.*, 9 (1970) 134.
207 P. E. Coffey, *U.S. Patent, No. 3, 711, 248*, 16 Jan. 1973.
208 Mercury determination, *Anal. Chem.*, 45 (1973) 754A.
209 J. Y. Hwang, P. A. Ullucci and S. B. Smith, Jr., *Amer. Lab.*, 3 (*8*) (1972) 41.
210 J. W. Ball and E. A. Jenne, *Bull. Environ. Contam. Toxicol.*, 7 (1972) 369.
211 W. A. Klemm and N. R. Fetter, *At. Absorption Newslett.*, 11 (1972) 108.
212 D. R. Wolber and R. E. Bosshart, *Anal. Chem.*, 44 (1972) 1546.
213 Mercury detection, *Anal. Chem.*, 42 (*14*) (1970) 101A.
214 R. Braun and A. P. Husbands, *Spectrovision*, 26 (1971) 2.
215 H. L. Kahn, *At. Absorption Newslett.*, 10 (1971) 58.
216 J. Ramirez-Munoz, *Beckman Instrum., Inc., Appl. Res. Tech. Rep.*, No. 556, 1971.
217 R. Scobbie, *Varian Techtron Tech. Top.*, January, 1971, p. 3.
218 R. Stux and E. Rothery, *Varian Techtron Tech. Top.*, January 1971, p. 1.
219 Mercury analyzer, *Anal. Chem.*, 44 (*3*) (1972) 97A.
220 Kit for detection of low mercury levels, *Lab. Equip. Dig.*, 10 (*1*) (1972) 8.
221 The A 3300 Atomic Absorption/Flame Emission Spectrometer, *G-I-T Fachzeitschrift für das*

Laboratorium, 6 (1972) 746.
222 H. Hein, *Chem. Labor. Betr.*, 24 (1973) 348.
223 P. D. Goulden and B. K. Afghan, *Tech. Bull. Dept. Energy, Mines, Resourc. Inland Waters Br. (Canada)*, No. 27, 1970.
224 F. A. J. Armstrong and J. F. Uthe, *At. Absorption Newslett.*, 10 (1971) 101.
225 B. W. Bailey and F. C. Lo, *Anal. Chem.*, 43 (1971) 1525.
226 G. Lindstedt and I. Skare, *Nord. Hyg. Tidskr.*, 52 (1971) 45.
227 T. T. Gorsuch, *The Destruction of Organic Matter*, Pergamon, Oxford, 1970.
228 E. E. Campbell and B. M. Head, *Amer. Ind. Hyg. Ass. Quart.*, 18 (1955) 275.
229 M. G. Ashley, *Analyst (London)*, 84 (1959) 692.
230 Analytical Methods Committee, *Analyst (London)*, 85 (1960) 643.
231 N. A. Smart, *Residue Rev.*, 23 (1968) 1.
232 M. B. Jacobs, S. Yamaguchi, L. J. Goldwater and H. Gilbert, *Amer. Ind. Hyg. Ass. J.*, 21 (1960) 475.
233 G. D. Christian, *Anal. Chem.*, 41 (1) (1969) 24A.
234 J. R. Melton, W. L. Hoover and P. A. Howard, *Soil Sci. Soc. Amer., Proc.*, 35 (1971) 850.
235 Association of Official Agricultural Chemists, *Official Methods of Analysis of the Association of Official Agricultural Chemists*, 10th edn., 1965.
236 Association of Official Analytical Chemists, *Official Methods of Analysis of the Association of Official Analytical Chemists*, 11th edn., 1970.
237 Analytical Methods Committee, *Analyst (London)*, 90 (1965) 515.
238 A. Bouchard, *At. Absorption Newslett.*, 12 (1973) 115.
239 A. Montiel, *Analusis*, 1 (1972) 66.
240 M. T. Jeffus, J. S. Elkins and C. T. Kenner, *J. Ass. Offic. Anal. Chem.*, 53 (1970) 1172.
241 R. T. Barber, A. Vijayakumar and F. A. Cross, *Science (Lond.)*, 178 (1972) 636.
242 V. I. Muscat, T. J. Vickers and A. Andren, *Anal. Chem.*, 44 (1972) 218.
243 D. S. Yeaple, *Nature (London)*, 235 (1972) 229.
244 S. H. Omang, *Anal. Chim. Acta*, 53 (1971) 415.
245 M. M. Joselow, L. J. Goldwater and S. B. Weinberg, *Arch. Environ. Health*, 15 (1967) 64.
246 G. Devoto, *Rass. Med. Sarda. Suppl.*, 71 (1968) 289.
247 G. Lindstedt and I. Skare, *Analyst (London)*, 96 (1971) 223.
248 A. M. Igoshin and L. N. Bogusevich, *Gidrokhim. Mater.*, 47 (1968) 150.
249 A. Hofer and R. Heidinger, *Fresenius' Z. Anal. Chem.*, 264 (1973) 412.
250 M. A. Mazurski, A. Chow and H. D. Gesser, *Anal. Chim. Acta*, 65 (1973) 99.
251 J. Marton and T. Marton, *Tappi.*, 55 (1972) 1614.
252 V. M. Novikov and S. Ya. Gol'dapel, *Ezhegodnik. Inst. Geokhim. Sib., Otd Akad. Nauk SSSR 1968*, 1969, p. 297.
253 S. H. Omang, *Anal. Chim. Acta*, 63 (1973) 247.
254 G. Lindstedt and I. Skare, *3rd Int. At. Abs. Fluoresc. Spectrosc. Congr., GAMS Paris, 1971*, p. 603.
255 T. T. Gorsuch, *Analyst (London)*, 84 (1959) 135.
256 R. L. Jones and T. D. Hinesly, *Soil Sci. Soc. Amer., Proc.*, 36 (1972) 921.
257 S. H. Omang and P. E. Paus, *Anal. Chim. Acta*, 56 (1971) 393.
258 W. Holak, B. Krinitz and J. C. Williams, *J. Ass. Offic. Anal. Chem.*, 55 (1972) 741.
259 P. E. Paus, *At. Absorption Newslett.*, 11 (1972) 129.
260 R. Tkachuk, *54th Can. Chem. Conf. Exhib., Halifax, Nova Scotia, Canada, 1971.*
261 R. Tkachuk and F. D. Kuzina, *J. Sci. Food Agric.*, 23 (1972) 1183.
262 T. Ukita, T. Osawa, N. Imura, M. Tonomura, Y. Sayato, K. Nakamuro, S. Kanno, S. Fukui, M. Kaneko *et al.*, *Eisei Kagaku*, 16 (1970) 258.
263 A. Carisano, M. Riva and A. Daghetta, *Ind. Conserve (Parma)*, 47 (1972) 115.
264 L. Lopez-Escobar and D. N. Hume, *Anal. Lett.*, 6 (1973) 343.
265 F. A. J. Armstrong, P. M. Williams and J. D. H. Strickland, *Nature (London)*, 211 (1966) 481.
266 L. Magos, *Analyst (London)*, 96 (1971) 847.
267 Y. Umezaki and K. Iwatomoto, *Bunseki Kagaku*, 20 (1971) 173.
268 L. Magos and T. W. Clarkson, *J. Ass. Offic. Anal. Chem.*, 55 (1972) 966.
269 L. Magos, *Biochem. J.*, 130 (1972) 63P.
270 L. Magos and A. M. Jawad, *J. Sci. Food Agric.*, 24 (1973) 1305.
271 J. C. Gage and J. M. Warren, *Ann. Occup. Hyg.*, 13 (1970) 115.
272 J. C. Gage, in R. Hartung and B. D. Dinman (Eds.), *Environmental Mercury Contamination, Int.*

Conf. Michigan, 1970, Ann Arbor, 1972, p. 155.
273 N. S. Poluektov, R. A. Vitkun and Yu. V. Zelukova, *Spektral Anal. Geol. Geokhim. Mater. Sib. Soveshch. Spektrosk. 2nd Irkutsk., USSR, 1963*, 1967, p. 200.
274 N. S. Poluektov, R. A. Vitkun and Yu. V. Zelyukova, *Zh. Anal. Khim.*, 19 (1964) 937.
275 M. E. Hinkle and R. E. Learned, *U.S. Geol. Surv. Prof. Pap., 650-D*, 1969, p. 251.
276 T. A. Rush and T. C. Rains, *Nat. Bur. Stand. (US) Tech. Note*, 584 (1971) 113.
277 U. Ulfvarson, *Acta Chem. Scand.*, 21 (1967) 641.
278 Y. K. Chau and H. Saitoh, *Effluent Water Treat. J.*, 11 (1971) 135.
279 T. Moeller, *Inorganic Chemistry. An advanced text book*, Wiley, New York, 1952.
280 C. Huffman, Jr., R. L. Rahill, V. E. Shaw and D. R. Norton, *U.S. Geol. Surv. Prof. Pap., 880-C*, 1972, p. 203.
281 F. N. Kudsk, *Scand. J. Clin. Lab. Invest.*, 17 (1965) 171.
282 J. Olafsson, *Anal. Chim. Acta*, 68 (1974) 207.
283 D. Voyce and H. Zeitlin, *Anal. Chim. Acta*, 69 (1974) 27.
284 G. Topping and J. M. Pirie, *Anal. Chim. Acta*, 62 (1972) 200.
285 E. Harsányi, L. Pólos and E. Pungor, *Anal. Chim. Acta*, 67 (1973) 229.
286 G. W. Kalb, *At. Absorption Newslett.*, 9 (1970) 84.
287 K. Eichner, *Mitteilungsbl. Ges. Deut. Chem.-Fachgruppe Lebensmittelchem. Gerichtl. Chem.*, 26 (1972) 240.
288 A. E. Moffitt, Jr. and R. E. Kupel, *At. Absorption Newslett.*, 9 (1970) 113.
289 R. Chester, D. Gardner, J. P. Riley and J. Stoner, *Mar. Pollut. Bull.*, 4 (1973) 28.
290 D. Gardner and J. P. Riley, *Nature (London)*, 241 (1973) 526.
291 M. B. Jacobs, L. J. Goldwater and H. Gilbert, *Amer. Ind. Hyg. Ass. J.*, 22 (1961) 276.
292 G. Wobeser, N. O. Nielsen, R. H. Dunlop and F. M. Atton, *J. Fish. Res. Board Can.*, 27 (1970) 830.
293 H. Reinsch, *J. Prakt. Chem.*, 24 (1841) 244.
294 J. J. Barakso and C. Tarnocai, *Can. Inst. Mining Met. Bull.*, 63 (1970) 501.
295 M. J. Fishman, *Anal. Chem.*, 42 (1970) 1462.
296 H. Möhler, *XVI Colloq. Spectrosc. Int., Heidelberg*, 1 (1971) 240.
297 R. A. Nicholson and J. D. Smith, *Lab. Pract.*, 21 (1972) 638.
298 F. E. Hoggins and R. R. Brooks, *N.Z. J. Mar. Freshwater Res.*, 7 (1973) 125.
299 H. Brandenberger and H. Bader, *Helv. Chim. Acta*, 50 (1967) 1409.
300 H. Brandenberger and H. Bader, *At. Absorption Newslett.*, 6 (1967) 101.
301 H. Brandenberger and H. Bader, *Chimia*, 21 (1967) 597.
302 H. Brandenberger and H. Bader, *At. Absorption Newslett.*, 7 (1968) 53.
303 P. E. Doherty and R. S. Dorsett, *Anal. Chem.*, 43 (1971) 1887.
304 J. K. Duffer, *J. Paint Technol.*, 43 (1971) 67.
305 K. H. Schaller, P. Strasser, R. Woitowitz and D. Szadkowski, *Fresenius' Z. Anal. Chem.*, 256 (1971) 123.
306 C. W. Zuehlke and A. E. Ballard, *Anal. Chem.*, 22 (1950) 953.
307 W. T. McBryde and F. Williams, *U.S. Atomic Energy Commission*, Y-1178, 1957.
308 E. G. Pappas and L. A. Rosenberg, *J. Ass. Offic. Anal. Chem.*, 49 (1966) 792.
309 T. Y. Toribara and C. P. Shields, *Amer. Ind. Hyg. Ass. J.*, 29 (1968) 87.
310 R. E. Mansell and E. J. Hunemorder, *Anal. Chem.*, 35 (1963) 1981.
311 C. L. Swanson, R. E. Wing, W. M. Doane and C. R. Russell, *Environ. Sci. Technol.*, 7 (1973) 614.
312 W. F. Fitzgerald, C. D. Hunt and W. B. Lyons, in *Studies of Pollutants in the Marine Environment (Heavy Metals, Halogenated Hydrocarbons and Petroleum), Brookhaven. Natl. Lab.*, 1972.
313 M. P. Bratzel, R. M. Dagnall and J. D. Winefordner, *Anal. Chim. Acta*, 48 (1969) 197.
314 R. G. Anderson, I. S. Maines and T. S. West, *Anal. Chim. Acta*, 51 (1970) 355.
315 A. F. King and T. S. West, *Proc. Soc. Anal. Chem.*, 10 (1973) 279.
316 S. Shimomura and R. Hiroto, *Anal. Lett.*, 6 (1973) 613.
317 K. C. Thompson and G. D. Reynolds, *Analyst (London)*, 96 (1971) 771.
318 K. C. Thompson, *Lab. Pract.*, 21 (1972) 645.
319 J. W. Robinson and Y. E. Araktingi, *Anal. Chim. Acta*, 63 (1973) 29.
320 T. J. Vickers and S. P. Merrick, *Talanta*, 15 (1968) 873.
321 R. A. Vitkun, N. S. Poluektov and Yu. V. Zelyukova, *Zh. Anal. Khim.*, 25 (1970) 474.
322 S. D. Fihn, S. W. Subber and C. D. West, *Abstr. Pittsburgh Conf. Anal. Chem. Appl. Spectrosc.*,

Paper 134, 1972.
323 A. W. Andren and R. C. Harriss, *Nature (London)*, 245 (1973) 256.
324 A. J. Maclean, B. Stone and W. E. Cordukes, *Can. J. Soil Sci.*, 53 (1973) 130.
325 G. M. Crosby, in *Quart. Colo. Sch. Mines*, 64 (1969) 169.
326 P. Poelstra, M. J. Frissel, N. vander Klugt and D. W. Bannink, *Neth. J. Agr. Sci.*, 21 (1973) 77.
327 V. Lidums and U. Ulfvarson, *Acta Chem. Scand.*, 22 (1968) 2379.
328 K. K. S. Pillay, in R. Hartung and B. D. Dinman (Eds.), *Environmental Mercury Contamination Int. Conf. Michigan 1970*, Ann Arbor Science Publishers, 1972, p. 137.
329 P. D. la Fleur, *Anal. Chem.*, 45 (1973) 1534.
330 H. M. Benedict, J. N. Bishop, C. A. Bruns, W. S. Ferguson, J. C. Gage, W. S. Lyon, B. B. Mesman, J. F. Shea and R. G. Smith, in R. Hartung and B. D. Dinman (Eds.), *Environmental Mercury Contamination Int. Conf., Michigan 1970*, 1972, p. 161.
331 E. D. S. Corner and F. H. Rigler, *J. Mar. Biol. Ass. U.K.*, 36 (1957) 449.
332 D. Ben-Bassat, G. Shelef, N. Gruner and H. I. Shuval, *Nature (London)*, 240 (1972) 43.
333 W. D. Ehmann and J. F. Lovering, *Geochim. Cosmochim. Acta*, 31 (1967) 357.
334 C. J. Carstens, C. A. Hord and D. H. Martin, *Rev. Sci. Instrum.*, 43 (1972) 1385.
335 E. B. Sandell, *Colorimetric Determination of Traces of Metal*, Interscience, New York, 2nd edn., 1950.
336 P. Benes, *Collect. Czech. Chem. Commun.*, 35 (1970) 1349.
337 R. M. Rosain and C. M. Wai, *Anal. Chim. Acta*, 65 (1973) 279.
338 P. Benes and I. Rajman, *Collect. Czech. Chem. Commun.*, 34 (1969) 1375.
339 V. P. Gunn, *Mercury Contamination in Man and his Environment, Tech. Rep. Int. At. Energy Agency*, 137 (1972) 87.
340 T. Y. Toribara, C. P. Shields and L. Koval, *Talanta*, 17 (1970) 1025.
341 H. V. Weiss and K. Chew, *Anal. Chim. Acta*, 67 (1973) 444.
342 R. A. Carr and P. E. Wilkniss, *Environ. Sci. Technol.*, 7 (1973) 62.
343 R. V. Coyne and J. A. Collins, *Anal. Chem.*, 44 (1972) 1093.
344 D. Polley and V. L. Miller, *Anal. Chem.*, 27 (1955) 1162.
345 A. Andersson, *Oikos. Suppl.*, 9 (1967) 9.
346 D. Merten and A. Tugsavul, *Nuclear Techniques for Studying Pesticide Residue Problems, Panel Proc., Ser Int. At. Energy Agency*, STI/PUB/252 (1970) 39.
347 Atomic Energy Agency, Vienna, Reference Materials for Mercury available from International Atomic Energy Agency, Kärntuer Ring 11, P.O. Box 590, A-1011, Vienna, Austria.
348 H. J. M. Bowen, *Analyst (London)*, 92 (1967) 124.
349 M. Fleischer, *U.S. Geol. Surv. Prof. Pap., 713*, (1970) p. 53.
350 *Nat. Bur. Stand. (U.S.) Spec. Publ.*, 260 (1973) 43, 46.
351 D. H. Anderson, J. J. Murphy and W. W. White, *Anal. Chem.*, 44 (1972) 2099.
352 T. C. Rains and O. Menis, *Appl. Spectrosc.*, 27 (1973) 407.
353 J. M. McNeal, N. H. Suhr and A. W. Rose, *Chem. Geol.*, 10 (1972) 307.
354 J. F. Uthe, F. A. J. Armstrong and K. C. Tam, *J. Ass. Offic. Anal. Chem.*, 54 (1971) 866.
355 W. Cuisa, M. Giacco and G. Ottombrini, *Ann. Chim. (Rome)*, 62 (1972) 426.
356 M. Dall'aglio and D. Visibelli, *Preprint XVI Colloq. Spectrosc. Int., Heidelberg*, 1971.
357 M. D. Schlesinger and H. Schultz, *Tech. Progr. Rept. U.S. Bur. Mines, 43*, 1971.
358 D. R. Young, J. N. Johnson, A. Soutar and J. D. Isaacs, *Nature (London)*, 244 (1973) 273.
359 A. E. Greenberg, J. Thomas and B. R. Tamplin, *J. Amer. Water Works Ass.*, 65 (1973) 552.
360 Bur. Internat. Technique du Chlore, *Anal. Chim. Acta*, 72 (1974) 37.
361 F. E. Hoggins and R. E. Brooks, *J. Ass. Offic. Anal. Chem.*, 56 (1973) 1306.
362 A. Cavallaro and G. Elli, *Boll. Lab. Chim. Prov. (Bologna)*, 22 (1971) 168.
363 J. M. Wood, *Chem. Eng. News.*, 49 (1971) 22.
364 J. G. Saha and Y. W. Lee, *Bull. Environ. Contam. Toxicol.*, 7 (1972) 301.
365 J. Růžička and C. G. Lamm, *Talanta*, 16 (1969) 157.
366 M. B. Jacobs and R. Jacobs, *Amer. Ind. Hyg. Ass. J.*, 26 (1965) 261.
367 A. R. Barringer, *U.S. Patent, No. 3, 571, 589*, 23 Mar. 1971.
368 J. A. Razumov and I. S. Fishman, *Zh. Prikl. Spektrosk.*, 10 (1969) 710.
369 A. R. Barringer, *Abstr., 3rd Int. Geochem. Symp., Toronto, 1970*, p. 14.
370 V. Z. Fursov, *Dokl. Akad. Nauk. SSSR*, 194 (1970) 1421.
371 J. Y. Hwang and P. A. Ullucci, *Amer. Lab.*, 2 (1970) 50.
372 V. Z. Fursov and I. I. Stepanov, *Spektrosk. Anal. Geol.*, (1971) 254.

373 J. Y. Hwang, P. A. Ullucci and A. L. Malenfant, *Can. Spectrosc.*, 16 (1971) 100.
374 R. R. Lauwerys and J. P. Buchet, *Arch. Environ. Health*, 26 (1973) 65.
375 Minnesota Mining and Manufacturing Co., *German Patent, No. 2, 263, 399*, 28 June 1973.
376 B. Z. Siegel, S. M. Siegel and F. Thorarinsson, *Nature (London)*, 241 (1973) 526.
377 W. E. Evans, *Mar. Poll. Bull.*, 2 (1971) 68.
378 E. J. Hough and M. E. Zabik, *J. Sci. Food Agric.*, 24 (1973) 107.
379 A. E. Moffit, Jr. and R. E. Kupel, *Appl. Spectrosc.*, Abstract 224, 25 (1971) 151.
380 S. Opalka and E. Mizikowska, *Med. Pracy.*, 22 (1971) 633.
381 A. M. Jones, Y. Jones and W. D. P. Stewart, *Nature (London)*, 238 (1972) 164.
382 S. E. Bryan and J. E. Bright, *Toxicol. Appl. Pharmacol.*, 26 (1973) 109.
383 H. C. Freeman and D. A. Horne, *Bull. Environ. Contam. Toxicol.*, 10 (1973) 172.
384 Y. A. Greichus, A. Greichus and R. J. Emerick, *Bull. Environ. Contam. Toxicol.*, 9 (1973) 321.
385 R. E. Keiser, G. M. Farren, J. A. Grach and J. J. Linn, *Clin. Chem.*, 17 (1971) 661.
386 N. Kubasik, M. T. Volosin and H. Sine, *Clin. Chem.*, 18 (1972) 716.
387 J. Angerer, M. Bohlmann, D. Szadkowski and G. Lehnert, *Preprint 19, Eur. Colloq., Comm. Eur. Commun, Luxembourg, 1973.*
388 H. Hey, *Fresenius' Z. Anal. Chem.*, 256 (1971) 361.
389 J. E. Longbottom, *Anal. Chem.*, 44 (1972) 1111.
390 G. E. Penketh, W. R. McLean and D. L. Stanton, *Euroanalysis International Conference Heidelberg, 1972.*
391 M. S. Dill, *U.S. At. Energy Comm., Y-1572, CFSTI*, 1967.
392 N. S. Poluektov and Yu. V. Zelyukova, *Zavod. Lab.*, 35 (1969) 186.
393 A. J. Barnard, Jr., E. F. Joy and F. W. Michelotti, *Clin. Chem.*, 17 (1971) 841.
394 M. I. Ovrutskii, N. S. Kozachuk and D. V. Isaev, *Metody Anal. Khim. Reaktivov Prep.*, 18 (1971) 112.
395 K. S. Shater, A. F. Eremina, A. I. Semeryakova, V. V. Smirnov and A. V. Efremov, *Zavod Lab.*, 36 (1970) 1470.
396 M. Giaccio, *Quad. Merceol.*, 9 (1970) 209.
397 J. Steensrud and D. Sandoz, *Applications Tips, (Varian), 66*, 1970.
398 A. Carisano, M. Riva and A. Daghetta, *Ind. Conserve (Parma)*, 46 (1971) 194.
399 J. Y. Hwang, P. A. Ullucci, A. L. Malenfant and Miss Morane, *3rd Int. At. Absorption Fluoresc. Spectrosc. Congr. GAMS, Paris*, 1971, p. 219.
400 A. L. Malenfant, J. Y. Hwang and S. B. Smith, *Amer. Ind. Hyg. Ass. J.*, 32 (1971) 30.
401 D. Pirati, P. Pirazzoli, S. Gherardi and G. Guidi, *Ind. Conserve (Parma)*, 46 (1971) 258.
402 J. F. Uthe, *Intern. Symp. Identification Measurement Environ. Poll., Ottawa, Canada, 1971.*
403 C. S. Annett, M. P. Fadow, F. M. D'Itri and M. E. Stephenson, *Mich. Acad.*, 4 (1972) 325.
404 E. A. Childs, J. N. Gaffke and D. L. Crawford, *Bull. Environ. Contam. Toxicol.*, 9 (1973) 276.
405 A. V. Holden, *Pestic. Sci.*, 4 (1973) 399.
406 M. Hussain and E. L. Bleiler, *Mar. Poll. Bull.*, 4 (1973) 44.
407 J. H. Martin and G. A. Knauer, *Geochim. Cosmochim. Acta*, 37 (1973) 1639.
408 R. Sawyer, *Process. Biochem.*, 7 (2) (1972) 37.
409 Working Party on the Monitoring of Foodstuffs for Mercury and Other Heavy Metals, 1st Report, *Survey of Mercury in Food*, HMSO, London, 1971.
410 C. R. Parker and R. L. Stux, *Symp. Anal. Chem., Roy. Aust. Chem. Inst., Adelaide, 1971.*
411 Working Party on the Monitoring of Foodstuffs for Heavy Metals, 3rd Report, *Survey of Mercury in Food*, (Supplementary Report), HMSO London, 1973.
412 E. A. Eads and C. E. Lambdin, *Environ. Res.*, 6 (1973) 247.
413 W. H. Gutenmann, J. J. Silvin and D. J. Lisk, *Bull. Environ. Contam. Toxicol.*, 9 (1973) 318.
414 P. J. Nord, M. P. Kadaba and J. R. J. Sorensen, *Arch. Environ. Health*, 27 (1973) 40.
415 R. A. Carr and P. E. Wilkniss, *Science (Wash. D.C.)*, 181 (1973) 843.
416 K. R. Osborn and H. E. Gunning, *J. Opt. Soc. Amer.*, 45 (1955) 552.
417 V. M. Novikov, E. S. Rossinskaya and S. Ya. Gol'dapel, *Ezheg Rab. 1970g, Inst. Geokhim. Sib. Otd. Akad. Nauk SSSR*, 1971, p. 412.
418 F. Dietz, *Schriftenreihe G.W.F. (München)*, 113 (1972) 269.
419 V. A. Razumov and T. P. Utkina, *Spektrosk. Tr. Sib. Soveshch 4th, 1965*, 1969, p. 291.
420 W. H. Smith, *Science (Wash. D.C.)*, 176 (1972) 1237.
421 C. Galoppini, G. Lotti and P. Pelosi, *Agr. Ital. (Pisa)*, 71 (1971) 302.
422 L. Kiekens, M. Verloo and A. Dhaese, *Meded. Fac. Landbouwwet Rijkuniv. Gent*, 38 (1973) 8.

423 J. A. Wheat, *U.S. At. Energy Comm., DP-1164*, 1968.
424 M. Boucetta and J. Fritsche, *Dosage Elem. Etat. Traces Roches Autres Subst. Miner. Natur., Actes Colloq. 1968*, Ed. Cent. Nat. Rech. Sci., Paris, 1970, p. 263.
425 G. Friedrich and M. Kulms, *Erzmetall*, No. 2 (1969) 74.
426 I. I. Stepanov, A. A. Rudkovskii and V. Z. Fursov, *Izv. Akad. Nauk Kaz. SSR, Ser. Geol.*, 26 (3) (1969) 84.
427 R. A. Cadigan, *U.S. Geol. Surv. Prof. Pap., No. 713*, 1970, p. 17.
428 R. E. Cranston and D. E. Buckley, *Environ. Sci. Technol.*, 6 (1972) 274.
429 R. W. Hatch, *Can. Res. Develop.*, 5 (6) (1972) 13.
430 D. S. Cronan, *Can. J. Earth Sci.*, 9 (1972) 319.
431 Y. Yamane, M. Miyazaki, K. Sakai, N. Murakami, Y. Furuta, H. Kishi, E. Ishikawa, C. Shintani, R. Nagata *et al.*, *Eisei Kagaku*, 17 (1971) 274.
432 J. P. Vernet and R. L. Thomas, *Eclogae Geol. Helv.*, 65 (1972) 293.
433 S. R. Aston, D. Bruty, R. Chester and R. C. Padgham, *Nature (London)*, 241 (1973) 450.
434 A. Taylor and V. Marks, *Brit. J. Ind. Med.*, 30 (1973) 293.
435 H. Hayes, J. Muir and L. M. Whitby, *Ann. Occup. Hyg.*, 13 (1970) 235.
436 D. Szedkowski, G. Lenhert and H. G. Essing, *Med. Welt.*, 28 (1970) 1298.
437 A. E. Moffitt, Jr., P. M. Quinn and L. P. Limtiaco, *Amer. Lab.*, 3 (8) (1972) 8.
438 Instrumentation Laboratory Inc., *Clinical Applications of Atomic Absorption/Emission Spectroscopy*, Rev. Ed., Instrumentation Laboratory Inc., Mass., U.S.A., 1972.
439 J. D. Smith, R. A. Nicholson and P. J. Moore, *Nature (London)*, 232 (1971) 393.
440 E. A. Voege, *Symp. Mercury in Man's Environment*, Royal Soc. Canada, 1971.
441 E. A. Jenne, *Mercury in Waters of the United States, 1970–71*, U.S. Dept. Interior Geol. Surv. Water Resources Div., Menlo Park, Calif., 1972.
442 L. J. Williams, J. C. Joyce and J. T. Monk, Jr., *J. Amer. Water Works Ass.*, 65 (1973) 275.

Analytica Chimica Acta, 66 (1973) 27–36

THE DETERMINATION OF OXYGEN IN COPPER BY α-PARTICLE ACTIVATION ANALYSIS

C. VANDECASTEELE*, F. ADAMS and J. HOSTE

Institute for Nuclear Sciences, Rijksuniversiteit Gent, Proeftuinstraat 86, B-9000 Gent (Belgium)

(Received 13th October 1974)

The electrical and mechanical properties (*e.g.* electrical conductivity) of copper are strongly influenced by the presence of oxygen[1]. Copper with a low oxygen content (a few $\mu g\ g^{-1}$) is frequently used for special mechanical and electrical applications. Methods developed so far for the determination of oxygen in copper are usually not sensitive enough to determine such low concentrations with a reasonable precision. Reducing fusion[2] and 14-MeV neutron activation[3,4], two techniques that allow a routine determination of oxygen are at their detection limits in the $\mu g\ g^{-1}$ range. Moreover, the former method requires a correction for surface oxygen amounting to about 0.3 $\mu g\ cm^{-2}$ for a carefully lathed sample[5], while the latter requires a very rapid post-irradiation etching[3,6]. Detection limits for the hydrogen reduction method are lower[2], since large samples (up to 100 g) can be analysed, so that surface contamination also becomes less important. Spark-source mass spectrometry provides excellent sensitivity, but can yield accurate results only when suitable standard reference materials are available.

In principle, activation with charged particles (helium-3 and α-particles) should yield lower detection limits than the foregoing methods and can be used as an "absolute method", *i.e.* with a standard not the same as the sample. According to a recent monograph[7], only helium-3 activation has been applied[8] for the determination of the oxygen in copper at a concentration of 550 $\mu g\ g^{-1}$. At this level, the post-irradiation removal of the surface oxide layer and the radiochemical purification of the ^{18}F formed from oxygen are not very critical. The authors claim that a non-destructive determination is possible with helium-3 particles of 6.3 MeV, *i.e.* with a range of 15 μm. It is obvious that this non-destructive method and probably also the alternative method, based on precipitation of the interfering gallium, zinc and copper activities as the hydroxides followed by the precipitation of fluoride as PbF_2, are applicable only for fairly high oxygen concentrations. At this level, other and easier analytical methods can also be applied.

In the present paper, a method is described, whereby a thickness of copper of about 120–200 μm is analysed. After irradiation with α-particles, the samples are carefully etched to remove the ^{18}F produced from surface oxygen. Particular attention is also paid to the chemical separation, so that a high decontamination of ^{18}F from interfering activities is obtained. This allows the determination of oxygen at the 1 $\mu g\ g^{-1}$ level or below.

---

* Aspirant of the N.F.W.O.

As part of a program on the determination of gases in non-ferrous metals, organised by the Eurisotop Office, samples were distributed to several laboratories to be analysed by different analytical techniques. The purpose was to obtain low oxygen copper standard reference materials with a certified oxygen concentration. Results for these samples are reported in the present paper. The accuracy of the standardization used in charged particle activation analysis was studied in some detail by comparing different methods. Although these copper samples have a low oxygen content, they are industrial products containing various other impurities. The influence of some of these impurities was also studied.

Nuclear data

When copper is irradiated with 45-MeV α-particles, a number of nuclear reactions take place. The most important are summarized in Table I. Figure 1 shows a Ge(Li) γ-ray spectrum of a copper sample irradiated for 30 min at a 0.5-μA intensity and counted 116 min after irradiation for 10 min at a distance of about 80 cm from the Ge(Li) detector. The sample was etched after irradiation. The detection efficiency for the annihilation photons under these conditions is about 350 times lower than in the normal counting geometry for analysis.

TABLE I

MAIN NUCLEAR REACTIONS OF 45-MeV α-PARTICLES WITH COPPER AND SOME OF ITS IMPURITIES

Reaction	*Isotope formed*		
	$T_{\frac{1}{2}}$	*γ-ray energy (keV)*	*% Annihilation radiation*
$^{63}Cu(\alpha, \alpha 2n)^{61}Cu$	3.41 h	67(5%); 283(13%); 373(2%); 656(10%); 1185(4%)	120
$^{63}Cu(\alpha, pn)^{65}Zn$	244 d	1115(51%)	3.4
$^{63}Cu(\alpha, n)^{66}Ga$	9.45 h	833(6%); 1039(39%); 1919(3%); 2190(6%); 2752(25%); 4295(4%)	114
$^{65}Cu(\alpha, 2n)^{67}Ga$	78.1 h	93(70%); 184(21%); 300(15%); 393(4%)	—
$^{65}Cu(\alpha, n)^{68}Ga$	68.3 min	1077(3%)	176
$^{75}As(\alpha, 3n)^{76}Br$	16.1 h	559(63%); 657(19%); 750(6%); 850(7%); 1210(13%); 1370(5%); 1470(7%); 1860(11%); 2100(7%); 2390(4%); 2780(5%); 2970(8%); 3570(2%)	133
$^{75}As(\alpha, 2n)^{77}Br$	57 h	239(27%); 297(8%); 521(23%); 579(7%); 757(2%); 818(3%); 1005(2%)	2
$^{121}Sb(\alpha, 2n)^{123}I$	13.3 h	159(83%)	—
$^{121}Sb(\alpha, n)^{124}I$	4.2 d	603(67%); 646(12%); 723(14%); 1369(5%); 1500(4%)	50
$^{123}Sb(\alpha, 3n)^{124}I$		1691(14%); 2091(2%); 2294(1%)	

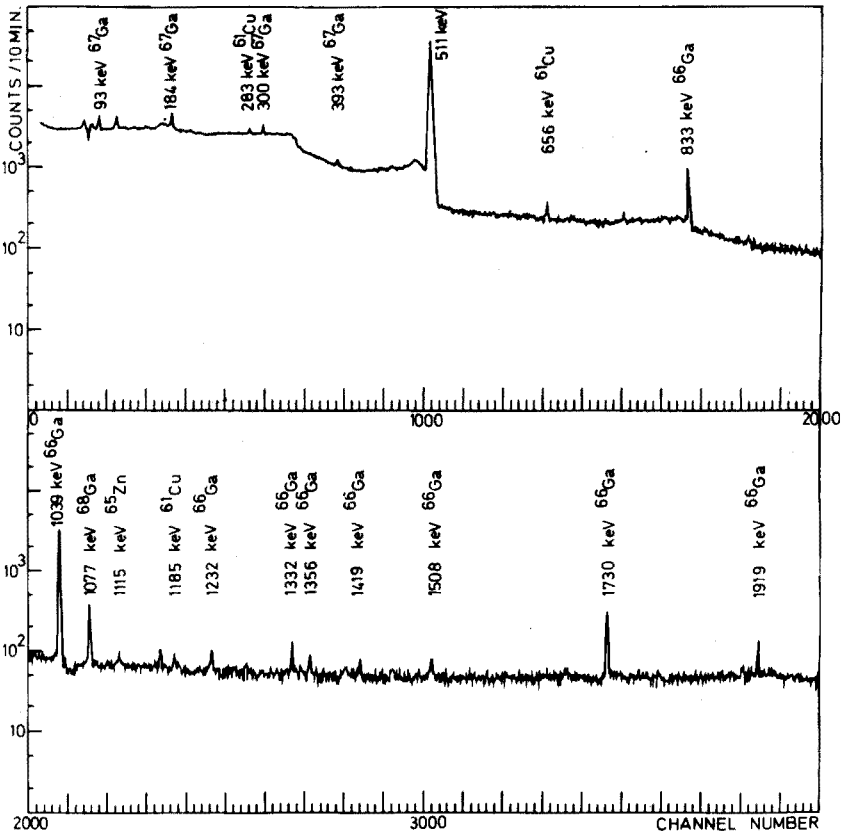


Fig. 1. Ge(Li) γ-ray spectrum of a copper sample irradiated with 45 MeV α-particles. $T_{irr}=30$ min; 0.50 μA intensity; counting started 116 min after irradiation.

From the oxygen in the sample, ^{18}F, a pure β^+-emitter with a 109.8-min half-life, is produced. The nuclear reactions and the shape of the excitation function have previously been described[9]. For a copper sample containing 1 $\mu g\ g^{-1}$ of oxygen, the annihilation radiation measured 2 h after irradiation is about $1.60 \cdot 10^5$ times more intense than that from the pure, chemically separated ^{18}F fraction measured 5 h after irradiation. If one accepts that 5% of the annihilation radiation may be due to interfering isotopes, the chemical separation of ^{18}F should yield a total decontamination of about $3 \cdot 10^6$. As will be explained in detail below, this is achieved by steam-distillation of fluosilicic acid, followed by precipitation of gallium as the hydroxide and of the fluoride as lead chlorofluoride. In the precipitate thus obtained, γ-rays of isotopes formed from impurities in copper can be identified. Some data on their decay properties are also summarized in Table I.

As has been shown by Engelmann[10], the elements N(250), F(0.58) and Na(23) also produce ^{18}F, when irradiated with 40-MeV α-particles; the figures in parentheses give the element to oxygen ratio that results in a 100% error on the oxygen concentration. The interference of fluorine through $^{19}F(\alpha, \alpha n)^{18}F$ should thus be expected. In a recent paper, Segebade and Dudzus[11] determined fluorine in copper of low

oxygen content by photon activation. They reported a concentration of about 0.025 $\mu g\ g^{-1}$. For the samples analysed in the present paper, this would yield an error of *ca.* $+0.04\ \mu g\ g^{-1}$ oxygen.

Standardization methods

In charged particle activation analysis, several methods for flux monitoring have been applied. In a recent paper[9] on the determination of oxygen in silicon, the present authors used a few thin mica foils, irradiated in front of the sample. The method yields an exact correction for flux variations during the irradiation. It has, however, the disadvantage that the sample surface becomes heavily contaminated by recoil ^{18}F nuclei from the monitor foils. This contamination can be removed by careful surface etching. For silicon, where a non-destructive determination of oxygen can be applied, the thickness to be removed can be determined experimentally. This is impossible for copper, because of the high interfering activities.

As appears from Fig. 1, during the α-irradiation of copper a high activity of ^{66}Ga is formed. A thin copper foil placed in front of the sample can thus serve as a flux monitor (Method I), whereby the 1039-keV peak is counted. In this case, the sample is only contaminated by activities formed from copper, so that the post-irradiation etch must only remove the ^{18}F activity produced from the surface oxide. In practice, a stack of muscovite foils is irradiated as a standard, with a copper foil placed in front of it. Each of the mica foils is counted, thus yielding simultaneously the experimental activation curve for the production of ^{18}F from oxygen in mica and, after summation of the induced annihilation peak, the activity in the standard. A sample is irradiated behind a flux monitor of equal thickness and the final precipitate is counted. The flux monitors of sample and standard are counted at an equal low geometry. The oxygen concentration in the sample can then be calculated from

$$C_x = C_s \cdot \frac{A_x}{A_s} \cdot \frac{I_s}{I_x} \cdot \frac{S_s}{S_x} \cdot \frac{e_s}{e_x} \cdot \frac{1}{R} \cdot G \qquad (1)$$

where C_x, C_s = oxygen concentration ($\mu g\ g^{-1}$) in sample and mica standard; A_x, A_s = measured ^{18}F activity in sample and standard corrected for decay; I_x, I_s = relative beam intensities during the irradiation of the sample and the standard. They are obtained from the 1039-keV photopeak of ^{66}Ga, corrected for saturation and decay; S_x, S_s = saturation factors for ^{18}F production; e_x, e_s = equivalent thickness ($g\ cm^{-2}$) of the sample and the standard. For the sample, the equivalent thickness refers to the energy after traversing the flux monitor and a copper layer equal to the depth of etching; for the standard, to the energy after traversing the flux monitor. The equivalent thicknesses are deduced from the experimental activation curve in mica and from the range-energy data for mica and copper[12]; R = yield of the chemical separation; G = ratio of the counting efficiency for the activity in a standard foil to that in the PbClF precipitate, taking into account the different counting geometry and the 511-keV γ-ray absorption in the precipitate.

Some samples were also irradiated without a copper foil in front of them. The current I'_x on the sample was then directly measured (Method II). The ratio K of the ^{66}Ga 1039-keV activity at the end of the irradiation induced in the flux monitor to the measured current, divided by the saturation factor for ^{66}Ga production

is determined in a separate experiment. This allows I_x in eqn. (1) to be replaced by KI'_x, while I_s is calculated as before. In this way, differences in the emission of secondary electrons from mica and copper have no influence. They can influence the final result, when the current on the mica standard, measured without a specially designed Faraday cup, is used directly in eqn. (1). Indeed, the current falling on the same material, namely pure copper, is measured, both when K is determined and when the samples are irradiated.

Finally, for one sample, the same flux monitoring and standardization methods were applied as for oxygen in silicon[9] (Method III).

EXPERIMENTAL

Samples and standards

The samples were obtained from the cylindrical copper blocks ($h=9$ mm, diameter = 26 mm) made available by Eurisotop. After turning off a surface layer, they were cold-rolled to a thickness of about 0.7 mm. Discs of 21 mm diameter were punched out of the copper sheets thus obtained.

As a standard, a stack of 35 muscovite foils of 21-mm diameter and about 15-μm thick was used. The oxygen content was 47.4%. The flux monitors were 25-μm thick discs of spectrographic copper, also of 21-mm diameter.

Irradiation

The samples were irradiated under vacuum ($< 10^{-4}$ torr) for 20–30 min with 45-MeV α-particles at an intensity of about 1 μA. They were placed in a water-cooled copper sample holder, behind an aluminium tube (18 mm diameter, 5 cm long). This reduced the loss of secondary electrons. In front, a copper collimator (16 mm diameter, 10 cm long) was placed. During the irradiation, the beam current on the sample was read on a digital voltmeter or integrated by means of a current digitizer followed by a scaler.

The standard mica foils were irradiated for 10 min at 0.1 μA in the same experimental configuration.

Chemical treatment

After irradiation, etch the sample twice for 3–8 min in a solution of 6 M nitric acid at room temperature. This removes a surface layer varying from 25 to 80 μm, as determined precisely with a micrometer. The energy of the α-particles after traversing the etched copper layer varies between about 42.5 and 37.5 MeV, when no flux monitor is used, and between about 40 and 34.5 MeV, when a 25-μm thick copper flux monitor is placed before the sample.

Dissolve the sample directly in 20 ml of 14 M nitric acid, in the apparatus to be used for the steam-distillation. Add about 300 mg of NaF carrier, 5 ml of a zinc nitrate solution (5 mg ml^{-1} in 2 M nitric acid) and 5 ml of a Ga_2O_3 solution (10 mg ml^{-1} in 2 M nitric acid) before the dissolution. As soon as the sample has dissolved, add 70 ml of distilled water and 100 ml of 85% phosphoric acid and some glass wool. Increase the temperature to 125°C, and collect *ca.* 70 ml of distillate. As soon as this temperature is reached, introduce steam and maintain the temperature at 125°C. Continue the distillation until a total of 350 ml is collected. After addition of 10 ml

of Ga_2O_3 solution, bring the distillate to pH 8 by adding 14 *M* ammonia solution. Filter the $Ga(OH)_3$ precipitate on a 47-mm diameter, 5-μm membrane filter. Adjust the pH to about 5 and add 20 ml of a lead nitrate solution (250 g l^{-1}) and 5 ml of a sodium chloride solution (160 g l^{-1}), so that lead chlorofluoride precipitates. Filter the precipitate on a similar membrane filter as before, wash with 20 ml of lead nitrate solution, wrap in an aluminium foil and place it between two 1-mm thick aluminium annihilator discs in a plastic container. The chemical treatment takes between 2 and 3 h.

An alternative chemical separation method based on cation exchange followed by lead chlorofluoride precipitation was also used. After dissolution of the sample, bring the pH to 1 by adding 14 *M* ammonia solution. Transfer the solution to the top of a 2.8 × 22-cm column filled with Dowex 50W-X8 cation exchanger. Elute the column with 250 ml of 0.1 *M* nitric acid. Afterwards, precipitate lead chlorofluoride as described above.

Counting

Countings were made on a Philips Ge(Li) detector with a detection efficiency of 15% of that of a 3 × 3-in. NaI(Tl) coupled to a 4000-channel analyser. Although a γ, γ-coincidence counting system yields a detection efficiency about twice higher, the Ge(Li) detector was preferred. Indeed, it allows the purity of the precipitate to be checked each time with a negligible loss in sensitivity. During 10–16 h, counts of 50 min were made every h starting 4–5 h after the irradiation. The copper flux monitors were measured in an 80 times lower geometry, for 5 min, 12–20 h after the irradiation. The standard mica foils were placed between two 1-mm aluminium discs in a plastic container and counted in the low counting geometry.

The 511-keV peak in the spectra was manually integrated and the background was obtained by linear interpolation from the lower and higher energy side. After subtraction of the 511-keV peak in the natural background (about 1 c.p.m.), the decay curve was analysed by the method of least squares based on the CLSQ computer program[13]. The iterative process was started with half-lives of 109.8 min, 16.1 h and 4.2 days. Afterwards, as a qualitative criterion for ^{18}F, iterations were made for the half-life of the first component.

Determination of the chemical yield

To avoid errors caused by coprecipitation and an inexact stoichiometry of the lead chlorofluoride, the yield was determined from an irradiation in an ^{227}Ac–Be isotopic neutron source[14] with a total neutron output of 10^8 n s^{-1}. The $^{19}F(n,\alpha)$-^{16}N reaction was used, counting the γ-rays of 7.14-s ^{16}N above 4.5 MeV. The sample was irradiated for 20 s, counted for 20 s, starting 10 s after irradiation, and compared to a sodium fluoride standard. About 350 counts were obtained for the precipitate per cycle. Each sample was analysed 5 times.

RESULTS AND DISCUSSION

Figure 2 shows a Ge(Li) γ-ray spectrum of the final lead chlorofluoride precipitate for a low-oxygen copper sample. The prominent peaks in the spectrum of Fig. 1, especially of ^{66}Ga, ^{67}Ga, ^{68}Ga and ^{61}Cu, can no longer be detected. From the

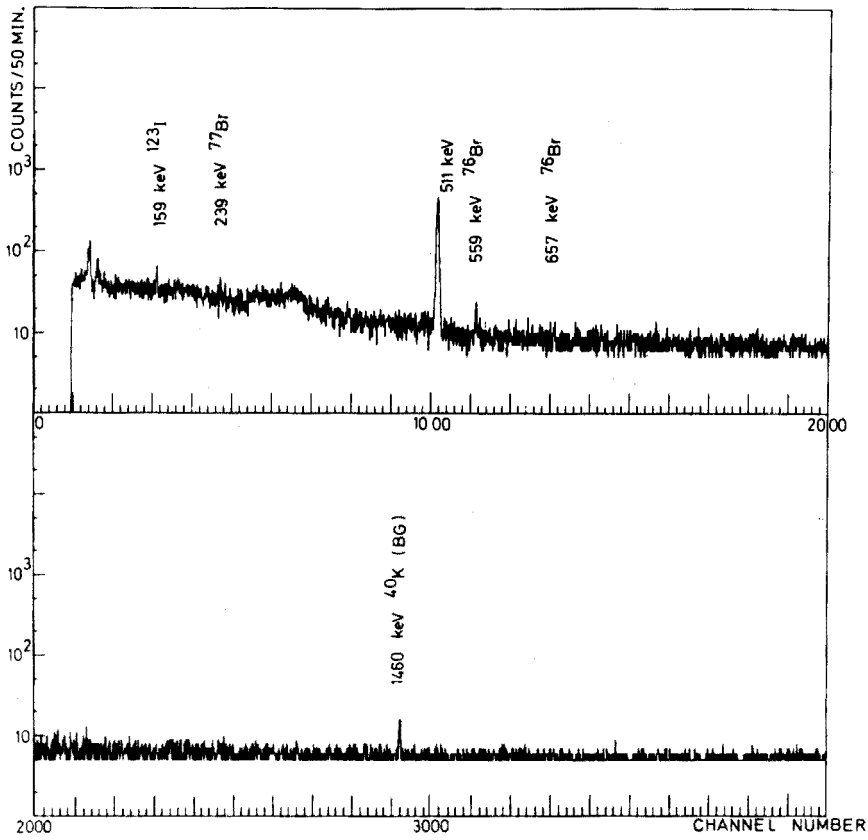


Fig. 2. Ge(Li) γ-ray spectrum of the PbClF precipitate for a low oxygen copper sample, $T_{irr} = 30$ min; 1.00 μA intensity; counting started 328 min after irradiation.

data in Table I and the spectra of the lead chlorofluoride precipitates, and when the peak efficiences as a function of energy are considered, it can be deduced that the contributions of ^{66}Ga and ^{61}Cu to the 511-keV activity are, respectively, less than 2 and 5% of the ^{18}F contribution from 1 μg g^{-1} of oxygen. From the relative peak intensities of ^{66}Ga (1039 keV) and ^{68}Ga(1077 keV) as deduced from Fig. 1, from the nuclear data of Table I, and from the efficiency curve of the Ge(Li) detector, the contribution of ^{68}Ga to the 511-keV peak, 5 h after irradiation, can be estimated to be about 1.5 times larger than the 1039-keV peak of ^{66}Ga *i.e.* less than 0.5% of the ^{18}F activity in a sample containing 1 μg g^{-1} of oxygen.

Measurements of the activities after the different steps of the radiochemical separation showed that the intensity of the 1039-keV ^{66}Ga peak was lowered during the steam-distillation by a factor of 20000–150000. During the precipitation of gallium hydroxide and of fluoride as lead chlorofluoride, its intensity was further reduced by a factor of more than 20–100. In each case, the ^{66}Ga activity was lowered by more than $3 \cdot 10^6$ during the entire chemical separation process. For copper, the corresponding factor was in excess of $3 \cdot 10^4$. The average yield for the separation was 70 ± 4%. During the separation by ion exchange, similar decontamina-

tion and yield were obtained.

Instead of the isotopes formed from the copper matrix, the 159-keV peak of ^{123}I, the 239-keV peak of ^{77}Br, the 559- and 657-keV peaks of ^{76}Br and sometimes the 603-keV peak of ^{124}I appeared in the spectrum. As shown in Table I, ^{123}I is not a β^+-emitter, and ^{77}Br emits only to a negligible extent, whereas ^{76}Br and ^{124}I may contribute significantly to the 511-keV activity. Analysis of the decay curve allows correction for these interferences. Owing to the relatively short total measuring time, it is generally not possible to identify the individual contributions of ^{76}Br and ^{124}I and the small possible contribution of ^{66}Ga and ^{61}Cu. Usually their overall activity is attributed to one component with an apparent 16.1-h half-life. The relative importance of this long-lived "component" varies, 5 h after irradiation, from 3 to 8% of the ^{18}F activity produced by 1.2 μg g^{-1} of oxygen. As can be deduced from the peak areas of the 559- and 603-keV peaks and from the data on the relative β^+ intensities in Table I, the ^{76}Br contribution exceeds several times that of ^{124}I. The error caused by the improper resolution of the decay curve can thus be neglected. Figure 3 shows a decay curve for a low-oxygen copper sample. After iteration, half-lives for ^{18}F with an experimental error varying from 2 to 7 min and lying between 100 and 120 min, were always obtained.

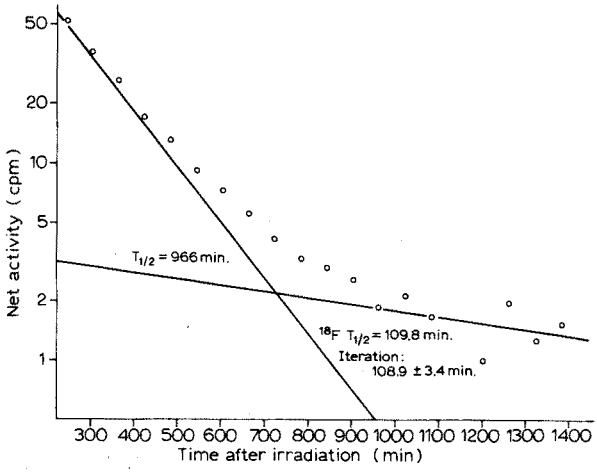


Fig. 3. Decay of the net 511-keV activity in the PbClF precipitate from an α-irradiated low-oxygen copper sample. T_{irr} = 20 min; 1.00 μA intensity.

The standardization with the copper flux monitor was repeated twice. Values of $(I_s \cdot S_s \cdot e_s)/(A_s \cdot e_x)$, as deduced from the two experiments and for e_x corresponding to the same energy between 42 and 34 MeV agreed within less than 3%. For the standardization with measurement of the beam current, the determination of K was repeated 4 times. The results showed a standard deviation of 2%.

Some analytical results are given in Table II. The first two results are for p.a. electrolytic copper and were obtained by the two chemical separation methods described. The accuracy of the method was tested by analysing a reference material (copper deoxygenated with phosphorus) recently certified[15] to contain 70 ± 7 μg O g^{-1}; the fair agreement between these values shows the accuracy of the applied

TABLE II

RESULTS FOR OXYGEN IN COPPER

Sample	*Standardization*	*Result* ($\mu g\ g^{-1}$)	*Average* $\pm s$
Electrolytic (p.a.)	Method II	320	335±20
		350[a]	
Reduced with P	Method I	67	
	Method II	65	
Low oxygen[b]	Method I	1.35	1.22±0.15
		1.05	
		1.35	
		1.15	
	Method II	1.08	1.10±0.09
		1.17	
		1.09	
		0.95	
		1.19	
	Method III	1.30	

[a] Chemical separation by cation exchange.
[b] Overall mean value (10 results): 1.17 $\mu g\ g^{-1}$; $s = 0.13\ \mu g\ g^{-1}$.

standardization procedures. The other results in Table II are all for the proposed low-oxygen copper standard reference material distributed by Eurisotop Office. The t-test showed no significant difference at the 90% confidence level between the 3 standardization methods. The overall standard deviation amounted to 11%.

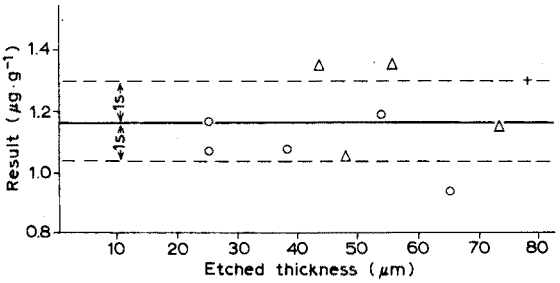


Fig. 4. Analytical results for a copper sample with low oxygen content as a function of the depth of etching. (Δ) Method I, (○) method II, (+) method III.

As shown in Fig. 4, the results obtained were not correlated to the thickness removed by the post-irradiation etch and were thus free of any surface contamination effect.

Detection limits are not easy to define for the present procedure. Indeed, the sensitivity is in part determined by the presence of other impurities (such as As and Sb). For a sample with the same concentration of these impurities as the analysed low-oxygen copper samples, a good estimate of the detection limit can be obtained by setting the ^{18}F activity equal to the 511-keV activity due to ^{76}Br and ^{124}I. This yields a limit of about 60 ng g^{-1}.

Grateful acknowledgement is made to R. Kieffer for valuable technical assistance, to the Comité de gestion du cyclotron of Louvain-La-Neuve (U.C.L.) for the use of the cyclotron time, to the operating staff of the cyclotron and the scientists of the laboratoire de chimie nucleaire for their kind cooperation, to Dr. J. Van Audenhove (BCMN-Geel) and Dr. J. Pauwels (Eurisotop Office) for preparing and providing the samples and to the "Nationaal Fonds voor Wetenschappelijk Onderzoek" for financial support.

SUMMARY

The determination of oxygen in copper by activation analysis with 45-MeV α-particles is described. The chemical separation of ^{18}F produced from oxygen consists of steam distillation as fluosilicic acid, followed by precipitation of the interfering gallium activities as the hydroxide and of the fluoride as lead chlorofluoride or of cation exchange followed by lead chlorofluoride precipitation. This allows a total decontamination from matrix radiation in excess of 10^6. As standard, mica foils are used, while, for the purpose of flux monitoring, the activity induced in a thin copper or mica foil, placed before the sample is counted. Alternatively, the intensity of the particle beam is measured. The method, which allows determination of oxygen at the 1 $\mu g\ g^{-1}$ level with a precision of about 10%, is applied to the determination of a concentration of 1.2 $\mu g\ g^{-1}$. Analysis of a reference material containing 70 $\mu g\ O\ g^{-1}$ proves the accuracy of the standardization methods applied.

REFERENCES

1 G. Kraft, *L'Importance technologique et économique du dosage de l'oxygène dans les métaux non ferreux, Eurisotop Office Information Booklet 59*, Eurisotop, Brussels, 1972.
2 G. Kraft, *Chemische Verfahren zur Bestimmung von Sauerstoff in NE-metallen, Eurisotop Office Information Booklet 71*, Eurisotop, Brussels, 1972.
3 C. Vandecasteele, A. Speecke and J. Hoste, *The Determination of Oxygen in non-ferrous Metals by 14-MeV Neutron Activation Analysis, Eurisotop Office Information Booklet 68*, Eurisotop, Brussels, 1972.
4 S. Taczanowski and J. Janczyszyn, *Radiochem. Radioanal. Lett.*, 6 (1971) 101.
5 P. Boisot, M. Cuypers, G. Kraft, J. Pauwels, L. Quaglia and G. Weber, *The Influence of the Surface Treatment of Samples on Oxygen Assays in non-ferrous Metals, ITE Report no. 76*, Eurisotop, Brussels, 1972.
6 F. Dugain, M. André and A. Speecke, *Radiochem. Radioanal. Lett.*, 4 (1970) 35.
7 C. Engelmann, *The Use of Activation Analysis by γ-Photons and charged Particles to determine the Oxygen Content in non-ferrous Metals, Eurisotop Office Information Booklet 74*, Eurisotop, Brussels, 1973.
8 D. Lee, C. Stauffacher and S. Markowitz, *Anal. Chem.*, 42 (1970) 994.
9 C. Vandecasteele, F. Adams and J. Hoste, *Anal. Chim. Acta*, 71 (1974) 67.
10 C. Engelmann, *J. Radioanal. Chem.*, 7 (1971) 281.
11 C. Segebade and T. Dudzus, *Radiochem. Radioanal. Lett.* 16 (1974) 137.
12 C. Williamson, J. Boujot and J. Picard, *Report CEA-R3042*, Paris, 1966.
13 J. Cumming in G. O'Kelley (Ed.), *Applications of Computers to Nuclear and Radiochemistry, Nuc. Sci. Ser. Rep.*, NAS-NS 3107, p. 25.
14 L. Alaerts, J. Op de Beeck and J. Hoste, *Anal. Chim. Acta*, 69 (1974) 1.
15 G. Kraft and J. Hoste, *The Certification of Oxygen in non-ferous Metals. Part 2: Oxygen in P-deoxygenated Copper, ITE Report No. 80*, Eurisotop, Brussels, 1974.

Analytica Chimica Acta, 76 (1975) 37–46

HIGH-PRECISION ENERGY-DISPERSIVE X-RAY FLUORESCENCE ANALYSIS OF MANGANESE IN FERROMANGANESE

R. JANSSENS*, W. MAENHAUT** and J. HOSTE

Institute for Nuclear Sciences, Rijksuniversiteit Gent, Proeftuinstraat 86, B-9000 Gent (Belgium)

(Received 23rd October 1974)

For the determination of manganese in ferromanganese, accurate and highly precise methods are necessary. Usually a wet chemical analysis based on the pyrophosphate method or the bismuthate method is applied[1,2]. Alaerts *et al.*[3] developed a method based on radioactivation with an isotopic neutron source. In this work, the possibility of applying isotope-excited energy-dispersive x-ray fluorescence is investigated. During the past five years, this technique has achieved appreciably increased utilization, mainly because of progress in semiconductor detector technology. Compared to wavelength-dispersive spectrometry, the energy-dispersive technique has the merit of instrumental simplicity.

^{109}Cd was chosen as an excitation source, the Ag K x-rays emitted allowing an efficient excitation of manganese. For sample preparation, pressed powder pellets were chosen, as this technique is simpler than fusion techniques.

EXPERIMENTAL

Instrumentation

Si(Li) semiconductor detector and preamplifier. A Nuclear Semiconductor top hat Si(Li) detector with an active surface area of 30 mm^2 and a sensitive depth of 3.0 mm was used. The detector entrance window consisted of a 200-nm thick gold contact and a 0.3-μm thick silicon dead layer. The beryllium window of the vertical dipstick cryostat was 25-μm thick. The preamplifier was a cooled input FET charge sensitive amplifier employing pulsed optical feedback. The detector energy resolution was 165 eV for the 5.90-keV Mn Kα line when operating at an amplifier pulse shaping time constant of 12 μs and at a count rate of 1000 c.p.s.

Amplifier. The NSI 511 L amplifier system used included an active base-line restorer, a pulse stretcher, a linear gate, a biased amplifier, a fast discriminator, a pile-up and low-level pulse rejecter and a live-time controller. The live-time controller corrected for pulses rejected by the pulse pile-up rejector by extending the analyser live-time. The amplifier was connected to a NS 700 multi-channel analyser (1024 channels, 10^6 count capacity, 100-MHz A.D.C. (analog digital con-

* Research fellow of the "Instituut tot Aanmoediging van het Wetenschappelijk Onderzoek in Nijverheid en Landbouw".

** Research associate of the "Nationaal Fonds voor Wetenschappelijk Onderzoek".

vertor) frequency). The data from the analyser were read out with an ASR 33 teletype.

Because of the high precision required, the live-time controller had to be optimized and checked for reliability in the following way. The 4-μs time-constant board was placed within the amplifier and the amplifier gain controls were set as follows: coarse gain 2; fine gain 0.00. A weak ^{55}Fe source was placed at a fixed distance from the detector, so that the count rate amounted to about 1000 c.p.s. By means of a strong ^{109}Cd source, placed at a variable distance, the count rate was increased up to 20,000 c.p.s. The live-time trim on the time-constant board was adjusted so that the Mn Kα+Kβ net peak area from the ^{55}Fe source remained constant as a function of the count rate. The setting of the live-time trim was then kept unchanged, and the same type of experiment was carried out with other fixed and variable sources and for different settings of the amplifier gain. As another fixed source, an ^{241}Am source was chosen. For this source the 13.93-keV Np Lα and 59.56-keV γ-ray peak areas were determined as a function of the count rate. It was shown that the area of the reference peak from the fixed source could only be kept within $\pm 1\%$ if the same amplifier gain and variable source were used as for the adjustment of the live-time trim. Accurate live-time correction when a variable source other than ^{109}Cd was used, could only be obtained by adequate change of the amplifier gain. Consequently, the live-time controller appears to be energy-dependent. Precise correction is thus only possible for a well defined spectrum.

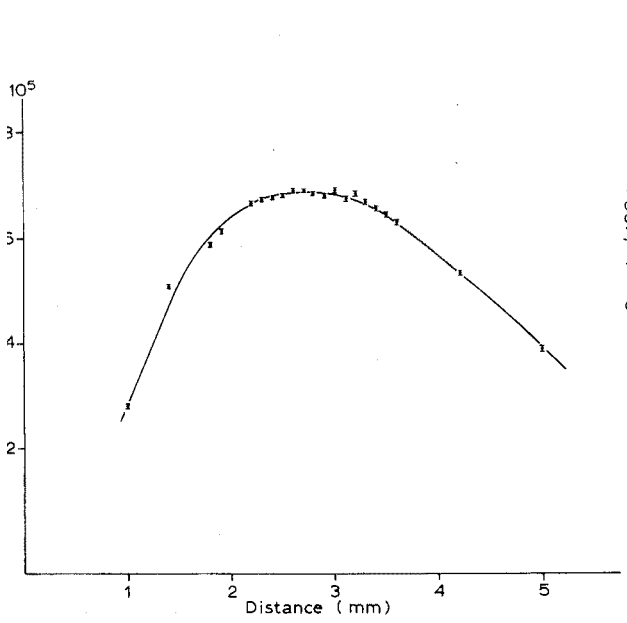
In order to optimize the live-time correction for the analysis of ferromanganese, the following procedure was adopted. It was first assumed that accurate live-time correction over the entire spectrum was carried out when the pure ^{109}Cd source was measured, and the proper amplifier gain setting (coarse gain, 2; fine gain, 0.00) was used. The ^{109}Cd source was positioned at different distances from the detector and for each position the ratio output count rate/input count rate was determined. The input count rate was observed with a scaler connected to the fast discriminator of the amplifier; the output count rate was derived from the spectrum obtained with the multi-channel analyser. It appeared that the ratio output count rate/input count rate was linearly dependent on the input count rate. The slope of the straight line was about $2.0 \cdot 10^{-6}$/input count. Final adjustment for the analysis of ferromanganese was performed by exciting a ferromanganese pellet at different amplifier settings and recording the ratio output count rate/input count rate as a function of the input count rate. The ferromanganese pellet was prepared and excited as described later. The count rate was varied by raising or lowering excitation source and pellet. For each amplifier setting, the slope of the straight line, showing the dependence of the ratio output count rate/input count rate upon input count rate, was calculated. It appeared that the slope could be matched with the value of $2.0 \cdot 10^{-6}$/input count obtained for the pure ^{109}Cd source, when the amplifier coarse gain 8 was used. Evaluation of the accuracy of the live-time correction for this optimal gain was, however, quite difficult. As the count rate obtained in the analysis of ferromanganese amounted only to some 3,500 c.p.s., the live-time correction was restricted to 20%.

The stability of the counting equipment was tested by taking 100 different countings of the ^{109}Cd source over a 24-h period. The Ag Kα+Kβ net peak area showed a relative standard deviation on one measurement of 0.09%, while from

counting statistics a value of 0.08% was expected.

Source–sample–detector assembly. An annular ^{109}Cd sealed source (New England Nuclear), was used in the direct excitation mode. ^{109}Cd has the advantage that practically all photons emitted lie in a narrow energy range between 22.1 and 25.5 keV. The source activity was *ca.* 5 mCi.

The source, sample and detector were used at a 180° reflection geometry. The source was placed as close as possible to the Si(Li) detector. In order to optimize the assembly the Fe $K\alpha+K\beta$ peak area from a 1-mm thick iron disk with a diameter of 33.0 mm was counted as a function of the distance between disk and top face of the source. The results are presented in Fig. 1; between 2.4 and 3.2 mm, a plateau was obtained. For a distance of 3.0 mm the influence of the diameter of the iron disk was investigated; the results are shown in Fig. 2.



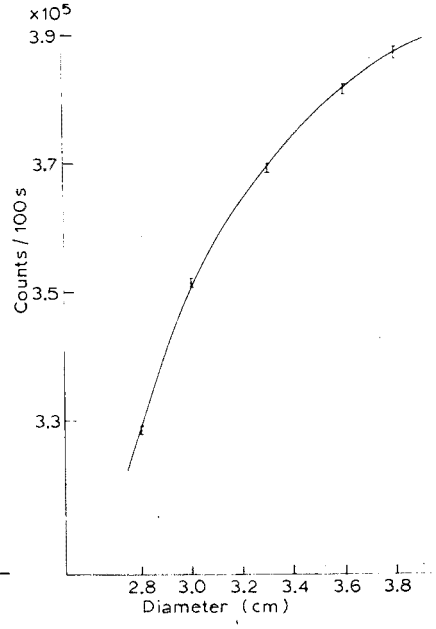


Fig. 1. Relationship of the distance between the iron disk and the top face of the ^{109}Cd source, with the Fe $K\alpha+K\beta$ net peak area.

Fig. 2. Relationship between the diameter of the iron disk and the Fe $K\alpha+K\beta$ net peak area.

The source–sample–detector assembly chosen for all further measurements is shown in Fig. 3. The reproducibility of the positioning of the sample in this counting assembly was investigated by exciting the iron disk of 33.0 mm diameter ten times. The relative standard deviation for the Fe $K\alpha+K\beta$ net peak area was 0.10% and was only slightly higher than could be expected from counting statistics (0.09%).

Sample preparation

Samples of the ferromanganese were taken by standard procedures, and ground by means of two different breakers and a disk mill. The particle size of

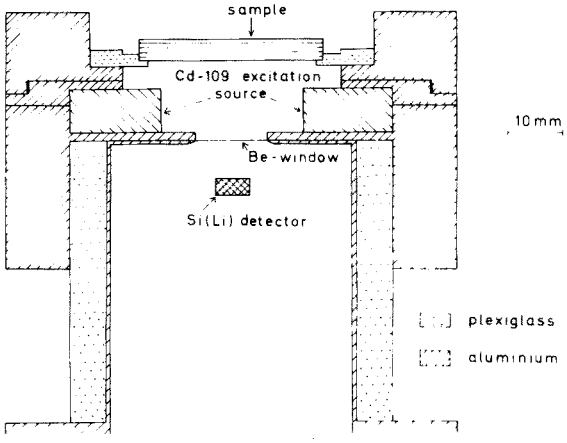


Fig. 3. Source–sample–detector assembly.

the powder finally obtained was smaller than 150 μm. The particle size distribution was determined by sieving with 170, 200 and 325 mesh sieves and weighing each of the four fractions obtained. Averaged over all samples, the fractions contained the following weight percentages: f_1 (particle size: 0–45 μm), 54.5%; f_2 (45–75 μm), 15.1%; f_3 (75–90 μm), 9.9%; and f_4 (90–150 μm), 20.5%. The relative standard deviations on the weight percentages, as calculated from the individual results for the different samples, were smaller than 0.2%.

The ferromanganese pellets for the x-ray analysis were prepared as follows: 14.1 g of ferromanganese powder together with 0.9 g of wax (Hoechst-Wachs C Pulver) were transferred to a 20-ml glass vial. The mixture was thoroughly shaken for 30 s, quantitatively transferred to a die of 33.0-mm inner diameter and pelletized under a hydraulic pressure of 5,600 kg cm^{-2}. The pellets thus obtained had identical cylindrical shape and dimensions: 33.00 ± 0.02 mm diameter and 3.75 ± 0.07 mm height.

The effect of ferromanganese particle size on fluorescent x-ray intensity was investigated. The results are shown in Table I. Because of the better mechanical properties of pellets prepared with ferromanganese powder of particle size smaller than 45 μm, it was decided to use this fraction for further pelleting.

In order to investigate the reproducibility of pelleting, ten replicate pellets of one sample were prepared and excited. The Mn Kα+Mn Kβ+Fe Kα+Fe Kβ net peak area was determined. The relative standard deviation on one measurement was 0.13%. The standard deviation expected from counting statistics was 0.11%.

TABLE I

EFFECT OF PARTICLE SIZE ON FLUORESCENT X-RAY INTENSITY

Particle size (μm)	0–45	45–75	75–90	90–150
Mn Kα+Mn Kβ+Fe Kα+Fe Kβ net peak area[a]	100	97.78	95.75	94.05

[a] Normalized to peak area obtained for 0–45-μm particles.

Precise Mn Kα peak area integration

A spectrum obtained for a ferromanganese pellet is shown in Fig. 4. As the Mn Kβ and Fe Kα lines overlap, only the Mn Kα line was used for the determination of manganese. However, this Mn Kα line was not fully resolved from the Mn Kβ–Fe Kα doublet, so that special care had to be taken for the integration of the Mn Kα peak area.

With the aid of Boekelheide's method[4], it was found that the shape of the Mn Kα peak agreed closely with a pure Gaussian function. Therefore, it was decided to fit the data around the peak maximum with eqn. (1), wherein the quadratic function accounts for the background:

$$y(x_i) = a_1 \exp\left[-\frac{1}{2}\left(\frac{x_i - a_2}{a_3}\right)^2\right] + a_4 + a_5 x_i + a_6 x_i^2 \qquad (1)$$

where x_i is the channel number; $y(x_i)$ the data calculated for channel x_i; a_1 the net height of the Gaussian peak; a_2 the centroid of the Gaussian peak; a_3 the standard deviation of the Gaussian (f.w.h.m. $= 2.354 \times a_3$); and a_4, a_5, a_6 are parameters of the quadratic function.

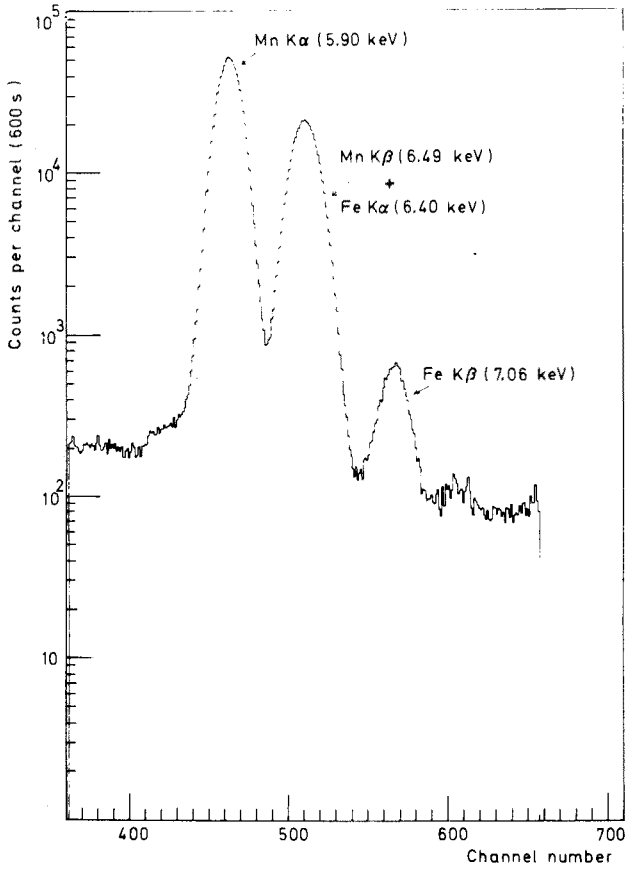


Fig. 4. Spectrum obtained for a ferromanganese pellet.

For the determination of the six parameters a_i, computer program SIDONI was used. In this program, which was based on the "grid search" method described by Bevington[5], the initial chosen values for a_i were varied, until a minimum was obtained for χ^2, defined by eqn. (2):

$$\chi^2 = \sum \left\{ \frac{1}{\sigma_i^2} [y_i - y(x_i)]^2 \right\} \tag{2}$$

where y_i are the data of channel x_i, and σ_i is the standard deviation of $y_i (\sigma_i^2 = y_i)$. Fast convergence of χ^2 and adequate values of the parameters a_i were obtained for the data of the following channels; 360–364, 456–477 and 620–624, where the channels 360–364 and 620–624 were representative of the pure background. The agreement between the original and calculated data is shown in Fig. 5. From the parameters a_1 and a_3, the net area of the Gaussian peak was calculated, according to eqn. (3):

$$S = (2\pi)^{\frac{1}{2}} a_1 a_3 \tag{3}$$

Although this area possibly differed from the true one, no errors were introduced if standard and unknown samples were treated in the same manner.

In order to check the reproducibility of the peak area integration, a pellet of a ferromanganese sample was excited ten successive times. For each spectrum obtained, the six parameters a_i and the Mn Kα net peak area were calculated.

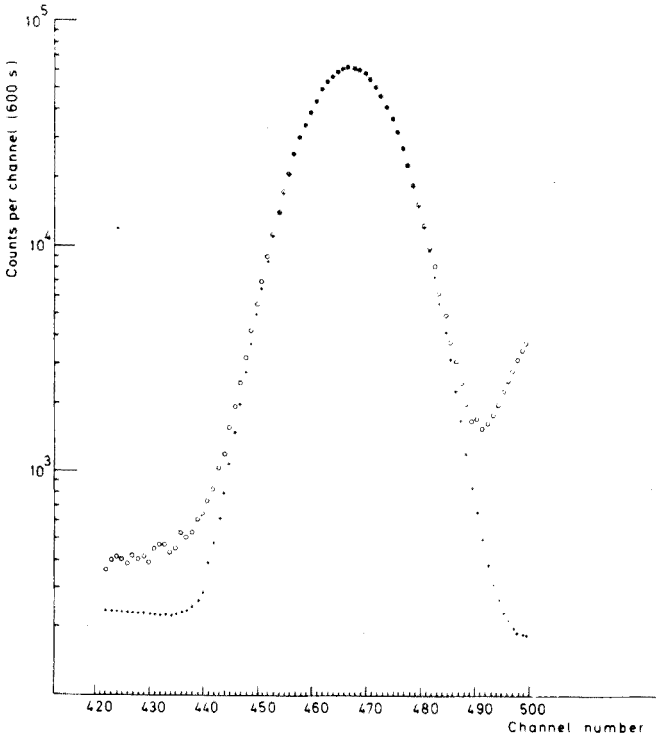


Fig. 5. Experimental (○) and fitted (●) points for the Mn Kα peak.

The average peak area was 1,087,085 counts, whereas the standard deviation on one individual peak area was 2,500 counts.

To evaluate the relative importance of the standard deviation from peak area integration and that from irreproducible pelleting, two or three different pellets were prepared from each ferromanganese sample available. Each pellet was excited three times during 600-s live-time, and for each excitation period the Mn Kα net peak area was calculated. The average peak area over all measurements was 1,102,508 counts, whereas all peak areas were comprised in the range between 1,083,266 and 1,114,750 counts. Therefore, it was assumed that the standard deviations of peak area integration and of pelleting irreproducibility were the same for all samples. A variance analysis was carried out[6], the result of which is shown in Table II. Applying the F-test to the mean squares of the table, one obtains:

$$F = \frac{\text{mean square between pellets}}{\text{mean square within pellets}} = 1.098$$

For 15 and 52 degrees of freedom, the 0.1 value of F is 1.63. The ratio observed being only 1.098, it was concluded that the variance from irreproducible pelleting is completely negligible.

TABLE II

VARIANCE ANALYSIS OF THE Mn Kα PEAK INTENSITIES OBTAINED FOR THE FERROMANGANESE PELLETS

Source of variation	*Sum of squares*	*Degrees of freedom*	*Mean square*	*Quantity estimated by the mean square*[a]
Between pellets	106,045,791	15	7,069,719	$\sigma_0^2+3\sigma_1^2$
Within pellets	334,710,657	52	6,436,743	σ_0^2

[a] σ_0^2: Variance due to peak area integration; σ_1^2: variance due to irreproducible pelleting.

Standardization

Most of the standardization problems were overcome thanks to the fact that six of the ferromanganese samples had been analysed by Alaerts *et al.*[3]. These authors determined the manganese concentration of an "ultimate standard" with the aid of the bismuthate method and relative to this standard, the five other samples were analysed by means of neutron activation analysis. The data are shown in Table III. The standard deviations for the results obtained by neutron activation analysis do not include the standard deviation on the ultimate standard. Since the concentrations of the different samples analysed lay in a narrow concentration range, the standard deviation for the neutron activation technique can be considered as constant. Therefore, from the individual standard deviations in the Table, a better estimate for the standard deviation of the neutron activation technique was calculated. A value of 0.127% was obtained.

Because of the narrow concentration range of manganese in ferromanganese, it was assumed that in the region of interest a linear functional relationship holds between the Mn Kα peak intensity and the manganese concentration. In fitting the calibration line, however, it had to be considered that neither the manganese

TABLE III

FERROMANGANESE STANDARD SAMPLES

Sample code	*Number of analyses*	*Mean Mn concentration (%)*	*s (%)*
Ultimate standard[a]	5	76.51	0.084
0570/297[b]	3	76.94	0.04
0370/047[b]	3	77.70	0.09
0171/450[b]	3	77.75	0.16
0371/070[b]	3	78.22	0.16
0870/444[b]	3	78.28	0.14

[a] Analysed by the bismuthate method.
[b] Analysed by n.a.a.

TABLE IV

REGRESSION ANALYSIS OF MANGANESE CONCENTRATION AND Mn Kα PEAK INTENSITY[a]

Standard sample	*y, Mn concentration (%)*	*x, average Mn Kα net peak area*
Ultimate standard	76.51	1,087,430
0570/297	76.94	1,089,654
0370/047	77.70	1,099,126
0171/450	77.75	1,102,905
0371/070	78.22	1,104,632
0870/444	78.28	1,106,756

[a] Number of observations $n=6$; $\bar{y}=77.567$; $\bar{x}=1{,}098{,}417$; $\Sigma(x-\bar{x})^2=3.265\cdot10^8$. Regression coefficient, $b=0.8499\cdot10^{-4}$. Standard error about regression, $s=0.145$.

concentrations of the standard samples, nor the Mn Kα peak intensities were free from error. Since the true manganese concentrations of the unknown samples were to be derived from the peak intensities, the linear regression line of concentration upon Mn Kα intensity was needed[7–9]. The least squares regression was carried out with the aid of the computer program LESSQR. For each sample, the mean Mn Kα peak intensity of six measurements of 600 s (either three replicate measurements of two pellets or two replicate measurements of three pellets) was used, so that the variance was equal to 6,436,743/6 = 1,072,791 for all samples. The data used for the regression analysis and the results obtained are given in Table IV. The standard deviation about regression, *s*, being only slightly higher than the standard deviation on the standard concentrations, the supposition of a linear functional relationship between Mn Kα peak intensity and manganese concentration is fully confirmed.

Calculation of results

From the regression parameters and the Mn Kα peak intensities, the manganese concentrations of the unknown samples were calculated from:

$$y_i = \bar{y} + b(x_i - \bar{x}) \tag{4}$$

where y_i is the concentration of sample i, and x_i is the average peak intensity of six measurements of sample i. The standard deviation on each concentration was obtained from:

$$s_i = \left\{ \left(s_{ult} \times \frac{y_i}{y_{ult}} \right)^2 + s^2 \left[\frac{1}{n} + \frac{(x_i - \bar{x})^2}{\Sigma (x - \bar{x})^2} \right] + b^2 s_x^2 \right\}^{\frac{1}{2}} \quad (5)$$

In this equation, the second term on the right-hand side is the variance of a point on the regression, *i.e.* it is the variance of the mean value of the concentrations for a given Mn Kα peak intensity. This variance takes into account that the concentrations of the standard samples are not free from error and that the same peak area can be obtained for samples of different manganese concentration. The third term on the right side of eqn. (5), $b^2 s_x^2$, reflects the fact that the concentration sought is not for a given peak area but for a given sample. Indeed, for each sample an error s_x is associated with the average peak area obtained. Finally, the first term on the right side of eqn. (5) takes into account that all concentrations of the standard samples are based on the same ultimate standard, the concentration of which is known with a precision $s_{ult} = 0.084\%$.

RESULTS AND DISCUSSION

The results are given in Table V. The mean relative standard deviation over all unknown samples is 0.20%. For four of the five unknowns, the manganese concentration was higher than that of the most concentrated standard sample. In order to check if extrapolation of the regression equation for those high concentrations was permissible, the manganese content of unknown 0871/83 was determined by means of the bismuthate method. A concentration of $78.90 \pm 0.12\%$ (five determinations) was found. This value agrees closely with that obtained from the regression.

TABLE V

ANALYSIS OF UNKNOWN FERROMANGANESE SAMPLES

Sample code	*Mn Kα net peak area*[a]	*Mn concentration (%)*	*s (%)*
0771/94	1,094,447	77.26	0.14
0671/81	1,106,861	78.29	0.15
1171/126	1,108,447	78.42	0.16
0471/812	1,110,377	78.59	0.17
0871/83	1,112,832	78.80	0.18

[a] Average of 6 measurements.

X-ray fluorescence analysis of manganese in ferromanganese with the aid of a ^{109}Cd isotopic source appears to be a fast and highly precise technique. The precision is limited only by the Mn Kα peak area integration and by the precision of the standards available. Errors arising from pelleting, sample positioning and instrumental instability are negligible.

The financial support of the "Interuniversitair Instituut voor Kernwetenschappen", the "Instituut tot Aanmoediging van het Wetenschappelijk Onderzoek in Nijverheid en Landbouw" and the "Nationaal Fonds voor Wetenschappelijk Onderzoek" is gratefully acknowledged. Thanks are also due to Dr. D. Desoete of Sidmar N.V. for providing the ferromanganese samples and to Mr. J. De Donder for preparing the computer programs SIDONI and LESSQR.

SUMMARY

A high-precision x-ray fluorescence method for the determination of manganese in ferromanganese is described. The method involves excitation of the sample with a ^{109}Cd isotopic source and measurement with a high-resolution Si(Li) detector. To preserve the optimal energy resolution even at high count rates, the system incorporates a pulsed optical feedback preamplifier and a pulse pile-up rejector. The rejected pulses are corrected for by means of an adequate live-time correction circuit. Processing of the spectra is accomplished with the aid of a digital computer. The relative precision of the method is approximately 0.2%.

REFERENCES

1 N. H. Furman (Ed.), *Scott's Standard Methods of Chemical Analysis, Vol. II*, Van Nostrand, Princeton, 6th edn., 1962, p. 645.
2 W. F. Hillebrand, G. E. F. Lundell, H. A. Bright and J. E. Hoffmann, *Applied Inorganic Analysis*, Wiley, New York, 2nd. edn., 1953, p. 52.
3 L. Alaerts, J. P. Op de Beeck and J. Hoste, *J. Radioanal. Chem.*, 15 (1973) 601.
4 I. F. Boekelheide, *Rev. Sci. Instrum.*, 31 (1960) 1001.
5 P. R. Bevington, *Data Reduction and Error Analysis for the Physical Sciences*, McGraw-Hill, New York, 1969.
6 O. L. Davies and P. L. Goldsmith (Eds.), *Statistical Methods in Research and Production*, Oliver and Boyd, Edinburgh, 4th edn., 1972.
7 D. V. Lindley, *J. Roy. Statist. Soc., Supp.*, 9 (1947) 218.
8 C. Eisenhart, *Ann. Math. Statist.*, 10 (1939) 162.
9 A. Mandansky, *J. Amer. Statist. Assoc.*, 54 (1959) 173.

Analytica Chimica Acta 76 (1975) 47–55

RAPID DETERMINATION OF CHROMIUM IN NATURAL WATERS BY CHEMILUMINESCENCE WITH A CENTRIFUGAL FAST ANALYZER

J. L. BOWLING* and J. A. DEAN

Department of Chemistry, University of Tennessee, Knoxville, Tennessee 37916 (U.S.A.)

G. GOLDSTEIN and J. M. DALE

*Analytical Chemistry Division, Oak Ridge National Laboratory**, Oak Ridge, Tennessee 37830 (U.S.A.)*

(Received 4th October 1974)

As a result of heightened public awareness and interest there is an increasing demand for analytical measurements related to the evaluation of environmental quality. In particular, methodology is required which will enable rapid, accurate, and inexpensive assays of trace contaminants on a routine basis. Because it appears that many of these requirements can be achieved with the centrifugal fast analyzer, an instrument developed at Oak Ridge National Laboratory for clinical analyses[1–5] and extensively used for that purpose, a program was initiated to apply the instrument to environmental problems. Previous applications include the determination of sulfate[6], phosphate[7], zinc[8], and selenium[9] in natural waters.

Currently, there is considerable interest in analysis for chromium in natural waters not only for routine evaluation of water quality but also to identify the chemical species which are present. Chromium is commonly released to waterways in the highly toxic hexavalent form from cooling tower effluents containing chromate as a corrosion inhibitor, and also from industrial wastes. A limit of 50 p.p.b. in potable water supplies has been established. Very little is known of the ultimate fate of chromium(VI) in natural waters. Chromium(III), while much less toxic, can exist in a variety of complexed and hydrolyzed species, or associated with particulate matter. The environmental consequences of long-term exposure to elevated concentrations of such substances is also uncertain.

Recently, a chemiluminescence method has been suggested for determination of chromium(III), based on catalysis of the oxidation of luminol (5-amino-2,3-dihydrophthalazine-1,4-dione) by hydrogen peroxide in basic solution[10]. Although extremely sensitive, the method is not very selective for chromium(III) —ions such as Cu(II) (ref. 11, 12), Co(II) (ref. 13, 14), and Fe(II) (ref. 15) also catalyze the reaction; however, EDTA can be employed to complex interfering ions, taking advantage of the fact that, while most metal–EDTA complexes form very rapidly, chromium(III) complexation is kinetically very slow[16]. This study also found that neither chromium(VI) nor complexed forms of chromium(III) acted as catalysts. This technique therefore offers the possibility, not only of a

---

* Present address: Department of Pathology, Walter Rèed Army Medical Center, Washington, D.C.

** Oak Ridge National Laboratory is operated by Union Carbide Corporation for the U.S. Atomic Energy Commission.

sensitive determination of chromium(III), but also of learning something about the chemical form of trace quantities of chromium in environmental samples. Moreover, chemiluminescent methods in general are becoming increasingly more important in analytical chemistry[17,18]. Consequently, the centrifugal fast analyzer system was adapted for luminescence measurements by making appropriate hardware and software modifications to the conventional instrument. This paper describes the determination of chromium(III) and, after reduction, chromium(VI) in natural waters.

EXPERIMENTAL

Apparatus

The centrifugal fast analyzer used for this work, and its operation, have been described in detail elsewhere[19]. Basically, the instrument consists of a rotor assembly with 15 cuvettes in the outer rim. A teflon transfer disk with wells drilled into it to hold samples and reagents is installed as the inner part of the rotor assembly. When the rotor is accelerated, the samples and reagents are mixed and discharged by centrifugal force through a channel in the transfer disk to the cuvettes. In normal use for absorbance measurements, a perpendicular light beam from a monochromator is passed through the cuvettes to a photomultiplier assembly. As each cuvette passes over the stationary light beam, the photomultiplier signal is amplified, digitized, and stored by means of a small dedicated computer – a Digital Equipment Corporation PDP 8/I – which also performs the necessary calculations. Since the present purpose is to detect light emission rather than absorption, the following modifications are necessary.

The rotor assembly, photomultiplier, and computer interface were the same as previously described for absorbance measurements, but a light-emitting diode (Monsanto Commercial Products Co., Type MV5222) was installed in place of the monochromator. When operated at constant current, the light-emitting diode provides a stable light source of about the same intensity as chemiluminescence signals to check the performance of the system and the software, and also serves as a reference source to monitor day-to-day variations in the system response. The diode is turned off when sample measurements are made.

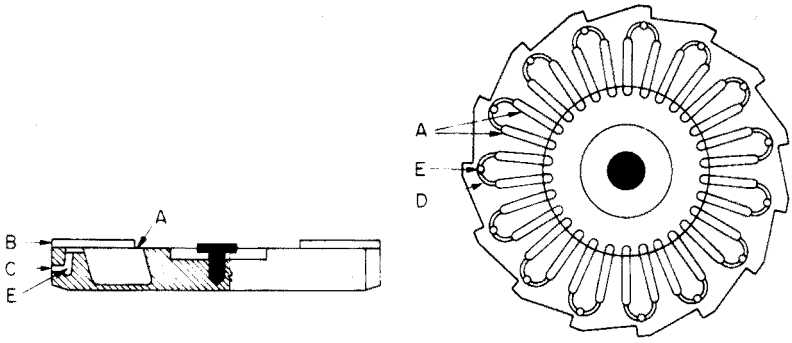


Fig. 1. Parallel-mixing transfer disk for chemiluminescence analyses with the centrifugal fast analyzer. (A) Sample chamber; (B) lid; (C) channel to cuvette; (D) channel to mixing chamber; (E) mixing chamber.

Because of the short duration of chemiluminescent light pulses, usually less than 5 s, the reagents and samples must be mixed very rapidly, preferably in the first 0.25 s after starting the rotor. A parallel-mixing transfer disk was designed and fabricated for this purpose[20] and is shown in Fig. 1. In this design, the two solutions to be mixed are placed in the sample chambers (A) cut along the radii of the disk. These chambers are covered with a transparent lid (B) to prevent solution crossover. A small area near the center is left open for filling. When the rotor is accelerated, the solutions are forced through the curved channels (D) into a mixing chamber (E) near the edge of the disk. The mixed solutions then exit to the cuvettes through a channel (C) at the bottom of the mixing chamber. Tests have shown that the solutions are completely mixed when they enter the cuvette.

Software

Since measurements are made on each of the 15 cuvettes in the rotor in sequence, light emission cannot be monitored continuously, hence it is necessary to take a sufficient number of discrete readings during the light pulse to define the shape of the pulse. The shortest reading interval on any individual cuvette is obviously the time required for one rotor revolution, and in the data collection routine, a specified number of sets of observations are taken for each cuvette at intervals defined in terms of rotor revolutions (every revolution, every second revolution, etc.). In practice, the rotor is started from a stationary state aligned so that the first cuvette is the first to pass the photomultiplier, and accelerated at a maximum rate to 1000 r.p.m.; this requires about 2 s. A clock is started at the beginning of the second revolution and the first set of emission readings is taken for each of the 15 cuvettes. Each cuvette is read 16 times during a single pass under the photomultiplier, and the readings for each are averaged, stored, and treated as a single observation. After the specified number of rotor revolutions, a clock reading is again taken followed by the second set of light emission readings. As many as 21 sets of data can be taken in this way, each set consisting of a clock reading taken at the beginning of data collection and the averaged readings for each of the 15 cuvettes. A dark current reading is taken after all the data have been collected, the individual cuvette observations are corrected for dark current, and the corrected sets of observations for each cuvette are added to integrate the light-pulse signal. The data printed out consist of a dark current reading, a table of times for the beginning of each data set, the data set for each cuvette, and the sum of the observations for each cuvette. Finally, the sums for the cuvettes containing standards are used to prepare a calibration curve by a non-linear least-squares fit to the data. Based on this calibration curve, the concentration in each of the other cuvettes is calculated and printed out. Peak emission can also be used in place of summed readings.

Reagents

0.12 M borate buffer, pH 10.3. Dissolve 3.7 g of boric acid in 450 ml of deionized water. Adjust the pH to 10.3 with 0.1 *M* potassium hydroxide, and dilute to 500 ml with water.

0.37 M hydrogen peroxide solution. Dilute 4 ml of 30% (w/w) hydrogen peroxide to 100 ml with water.

0.0022 M luminol–0.01 M EDTA solution. Dissolve 39 mg of luminol (J. T. Baker Chemical Company, Phillipsburg, N.J.) and 372 mg of disodium EDTA dihydrate in 100 ml of borate buffer.

0.1 M chromium(III) stock solution. Dissolve 4 g of chromium(III) nitrate nonahydrate in 100 ml of deionized water. Standardize by oxidizing the chromium-(III) with ammonium peroxydisulfate, then reducing the chromium(VI) formed with excess of iron(II) and back-titrating the excess with standard potassium permanganate solution[21].

0.0155 M chromium(VI) stock solution. Dissolve 0.300 g of potassium chromate in 100 ml of deionized water.

Prepare less concentrated Cr(III) and Cr(VI) solutions by appropriate dilution of the stock solutions.

Procedure

Analysis for chromium(III). Pipet 0.3 ml of a standard or sample containing 50–600 p.p.b. of chromium(III), plus 0.1 ml of hydrogen peroxide solution into one chamber of a parallel-mixing pair; pipet 0.1 ml of luminol–EDTA solution into the other. A reagent blank is always placed in the first position in the disk and at least 3 standards in following positions. Align the rotor so that the first cuvette will be the first to pass the photomultiplier tube, and then accelerate to 1000 r.p.m. Initiate data collection on the second revolution of the rotor, taking light emission readings every second revolution for a total of 21 sets of readings.

Analysis for chromium(VI). Take an appropriate sample (about 15 ml) and reduce Cr(VI) to Cr(III) by adding 0.1 ml of 30% (w/w) hydrogen peroxide and 0.05 ml of 1 *M* hydrochloric acid. After 5 min, heat the solution to near boiling for 30 min to remove the excess of peroxide. Then cool, dilute to a known volume, and follow the procedure for chromium(III).

RESULTS AND DISCUSSION

Effect of reagent concentrations

Initially, the reagent concentrations recommended by Seitz *et al.*[10] for measuring the steady-state light emission in a flowing system were adopted. However,

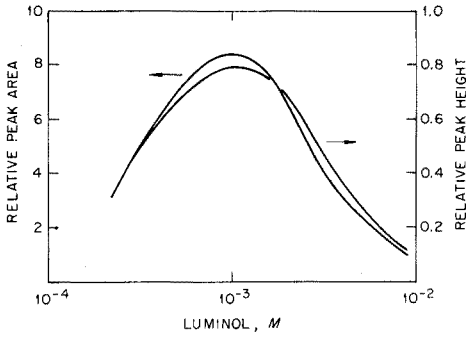


Fig. 2. Effect of luminol reagent concentration on total and peak light emission. Final solution contained 0.3 ml of 0.26 p.p.m. Cr(III) solution, 0.1 ml of 0.37 *M* H_2O_2, and 0.1 ml of luminol–EDTA solution.

the characteristics of the light pulse emitted from rapidly mixed solutions were not suitable and it was necessary to optimize conditions for measurements with the centrifugal fast analyzer. All tests were made in a borate buffered medium, pH 10.3.

The hydrogen peroxide and luminol concentrations influence both the intensity and duration of the chemiluminescent light pulse. Figure 2 shows the effect of luminol concentration on the total light emission measured for 2.5 s.

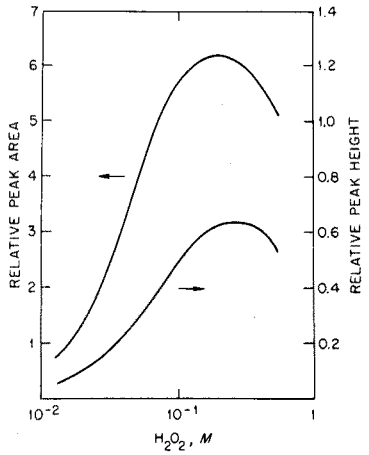


Fig. 3. Effect of hydrogen peroxide concentration on total and peak light emission. Final solution contained 0.3 ml of 0.26 p.p.m. Cr(III) solution, 0.1 ml of $2.2 \cdot 10^{-3}$ *M* luminol–EDTA solution, and 0.1 ml of hydrogen peroxide solution.

Maximum total light emission and peak emission were obtained with about 10^{-3} *M* luminol but, for reasons that will be brought out later, $2.2 \cdot 10^{-3}$ *M* luminol was selected. Tests were also made with various concentrations of hydrogen peroxide with the results shown in Fig. 3; optimal concentration for maximum total light emission was 0.2–0.3 *M* hydrogen peroxide. The shape and duration of the light pulse were considered as well as total light emission; Fig. 4 shows the light pulse observed with three combinations of reagent concentrations. Ideally, the light pulse should have (1) a slow rise time, so that the solutions will be well mixed and the data collection routine initiated before an appreciable fraction of the light has been emitted, and (2) a rapid decay, so that an extended data collection time is not needed to observe the last few percent of the signal. Based on these criteria, $2.2 \cdot 10^{-3}$ *M* luminol and 0.37 *M* hydrogen peroxide concentrations were selected, although neither is optimal for maximum light output.

Effect of EDTA

Since the chromium(III) and EDTA-containing solutions are mixed virtually at the instant that the chemiluminescent measurement is started, it is unlikely that chromium(III)–EDTA complex formation is significant. This was confirmed by tests that showed only a 10% decrease in chemiluminescence when chromium(III) and EDTA solutions were mixed 15 min before the luminol and peroxide solutions were added.

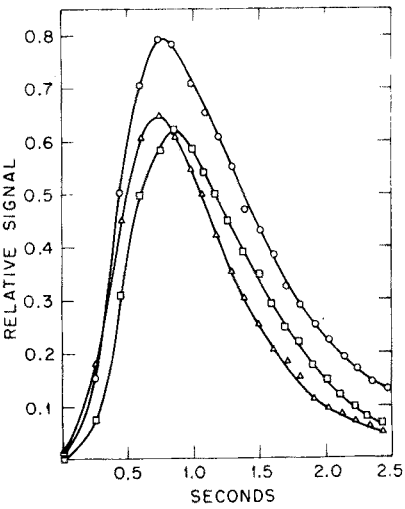


Fig. 4. Effect of reagent concentrations on the intensity and duration of the light pulse. (○) $9.0 \cdot 10^{-4}$ *M* luminol, 0.37 *M* H_2O_2; (△) $2.2 \cdot 10^{-3}$ *M* luminol, 0.37 *M* H_2O_2; (□) $2.2 \cdot 10^{-3}$ *M* luminol, 0.18 *M* H_2O_2. In all cases 0.3 ml of 0.26 p.p.m. Cr(III) solution was used and 0.1 ml each of the luminol EDTA and H_2O_2 solutions.

Sensitivity

In the measurement of light emission with a photomultiplier detector followed by digital conversion, the sensitivity, linear range, and precision are a function of photomultiplier voltage, signal amplification, and the voltage range of the analog-to-digital converter. Since the primary purpose was to investigate chromium pollution in surface waters, instrumental conditions were adjusted so that the drinking water standard for hexavalent chromium (50 p.p.b.) was the lower detection limit. Under these conditions a typical calibration curve for chromium(III) is shown in Fig. 5. No attempt was made to ascertain the minimal detectable quantity of chromium(III) but considerably greater sensitivity is possible. Seitz *et al.*[10] have shown a detection

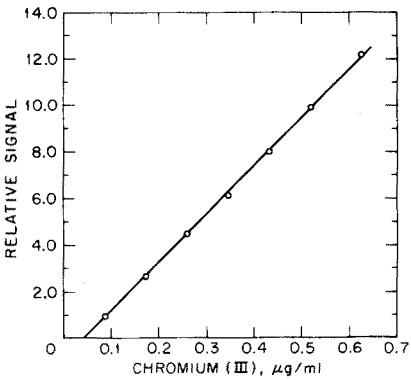


Fig. 5. Calibration curve for the chemiluminescent determination of chromium. Conditions as given in the recommended procedure.

limit of 0.025 p.p.b. with their apparatus. At a fixed photomultiplier voltage and signal amplification, the upper limit for measurement is determined by the highest voltage that the analog-to-digital converter will accept.

Precision and accuracy

Precision and accuracy were determined by replicate analyses of separate aliquots of standard solutions. The precision data thus include normal pipetting errors as well as system noise. In each case, a series of standards was run in the same rotor and used to construct a calibration curve from which the recoveries were calculated. Results for both chromium(III) and chromium(VI) solutions are shown in Table I. Relative standard deviations were 1–2% for both chromium(III) and chromium(VI) with recoveries ranging from 96% to 104%. The essentially quantitative recoveries of chromium(VI), as measured with chromium(III) standards, indicates that reduction was complete and that chromium(III) complexes were not formed.

TABLE I

PRECISION AND ACCURACY OF CHEMILUMINESCENCE ANALYSIS FOR TRIVALENT AND HEXAVALENT CHROMIUM

Cr(III) ($\mu g\ ml^{-1}$)		S_r (%)	*Cr(VI)* ($\mu g\ ml^{-1}$)		S_r (%)
Taken	*Found*[a]		*Taken*	*Found*[a]	
0.087	0.085	1.2	0.166	0.177	1.3
0.173	0.166	1.4	0.248	0.251	1.2
0.260	0.253	1.6	0.331	0.327	0.9
0.347	0.332	1.6	0.414	0.410	0.7
0.434	0.437	1.7	0.497	0.492	1.0
0.520	0.524	1.4	0.581	0.578	1.0
0.624	0.629	1.2			

[a] Average of 10 replicates; Cr(III) standards used.

Analysis of natural waters

Six natural water samples, taken from various points in a watershed which receives cooling tower effluents containing chromate, were analyzed for both chromium(III) and chromium(VI) by the recommended procedures. No chromium(III) ion was detected in any of the samples by this method. Results for chromium(VI) are shown in Table II along with total soluble chromium analyses by atomic absorption spectrometry and chromium(VI) determinations by differential pulse polarography[22]. In the case of sample 1, chromium(VI) determinations by chemiluminescence and differential pulse polarography are in agreement but lower than the total chromium determined by atomic absorption spectrometry. Thus some chromium(III) must be present. Since none was detected by chemiluminescence, it was probably in the form of a complex ion which could not catalyze the luminol–peroxide reaction. For example, lower light emission was observed for chromium(III) solutions prepared from sulfate salts or by dissolving chromium metal in hydrochloric acid than from chromium nitrate solutions, and Seitz *et al.*[10] list several ligands which decrease light emission. In samples 4–6, the

TABLE II

ANALYSIS FOR CHROMIUM IN NATURAL WATERS

Sample	*Total Cr (μg ml^{-1})*	*Cr (VI) (μg ml^{-1})*	
	by A.a.s.[a]	*C.l.*[a]	*D.p.p.*[a]
1	2.50	2.06	2.14
2	0.08	0.05	[b]
3	0.09	0.05	[b]
4	2.79	2.32	2.80
5	0.12	0.05	0.11
6	0.05	0.04	0.061

[a] A.a.s., Atomic absorption spectrometry; c.l., chemiluminescence; d.p.p., differential pulse polarography.
[b] Not analyzed.

total chromium and chromium(VI) analyses by differential pulse polarography are in agreement indicating that no chromium(III) was present. Chemiluminescent results, however, were somewhat low. This could be due to the presence of complexing agents in the water. Although the aquo-complex of chromium(III) tends to react very slowly with ligands, chromium(III) complexes are formed instantaneously by reduction of Cr(VI) to Cr(III) in complexing media, and would cause low results for chromium(VI) determinations.

SUMMARY

The centrifugal fast analyzer was adapted for luminescence measurements by designing an improved transfer disk which mixes solutions more efficiently and by reprogramming the dedicated computer for rapid data acquisition. The modified analyzer system was applied to the chemiluminescent determination of chromium(III) and, after reduction, chromium(VI), by catalysis of the luminol–peroxide reaction in basic medium. From 50 to 600 p.p.b. of chromium in water samples were determined with a relative standard deviation of 1–2%. Sensitivity can be increased if necessary. The method is very rapid—as many as 11 samples can be analyzed in 2.5 s—and only a small sample, 0.3 ml, is required. Since this method is selective for uncomplexed chromium(III) ions, comparison of results with analyses for total chromium and chromium(VI) by other methods provides information concerning the chromium species present in a sample and the presence or absence of complexing agents.

REFERENCES

1 N. G. Anderson, *Anal. Biochem.*, 28 (1969) 545.
2 N. G. Anderson, *Anal. Biochem.*, 32 (1969) 59.
3 N. G. Anderson, *Science*, 166 (1969) 317
4 D. N. Mashburn, R. H. Stevens, D. D. Willis, L. H. Elrod and N. G. Anderson, *Anal. Biochem.*, 35 (1970) 98.
5 C. D. Scott and C. A. Burtis, *Anal. Chem.*, 45 (3) (1973) 327A.
6 R. L. Coleman, W. D. Shults, M. T. Kelley and J. A. Dean, *Anal. Chem.*, 44 (1972) 1031.

7 R. L. Coleman, J. A. Dean, W. D. Shults and M. T. Kelley, *Anal. Lett.*, 4 (1971) 169.
8 G. Goldstein, W. L. Maddox and M. T. Kelley, *Anal. Chem.*, 46 (1974) 485.
9 J. L. Bowling, J. A. Dean and G. Goldstein, *Anal. Lett.*, 7 (1974) 205.
10 W. R. Seitz, W. W. Suydam and D. M. Hercules, *Anal. Chem.*, 44 (1972) 957.
11 A. K. Babko and L. I. Dubovenko, *Zavod. Lab.*, 30 (1964) 1325.
12 A. K. Babko and L. I. Dubovenko, *Z. Anal. Chem.*, 200 (1964) 428.
13 A. K. Babko and N. M. Lukovskaya, *Zavod. Lab.*, 29 (1963) 404.
14 A. K. Babko and V. N. Danilova, *Zavod. Lab.*, 30 (1964) 1198.
15 W. R. Seitz and D. M. Hercules, *Anal. Chem.*, 44 (1972) 2143.
16 R. E. Hamm, *J. Amer. Chem. Soc.*, 75 (1953) 5670.
17 W. R. Seitz and M. P. Neary, *Anal. Chem.*, 46 (2) (1974) 188A.
18 U. Isacsson and G. Wettermark, *Anal. Chim. Acta*, 68 (1974) 339.
19 R. L. Coleman, J. A. Dean, W. D. Shults and M. T. Kelley, *Anal. Lett.*, 5 (1972) 169.
20 W. L. Maddox, R. L. Coleman and W. D. Shults, *U.S. Patent 3,744,974*, July 10, 1973.
21 American Society for Testing and Materials, *Annual Book of ASTM Standards. Part 23. Water; Atmospheric Analysis*, ASTM, Philadelphia, Pa., 1972, p. 402.
22 S. T. Crosmun and T. R. Mueller, *Anal. Chim. Acta*, 75 (1975) 199.

Analytica Chimica Acta, 76 (1975) 57–64

FLUORESCENCE DERIVATIZATION FOR TRACE DETERMINATION OF SOME ALKALOIDS AND ADRENALINE*

F. NACHTMANN and H. SPITZY

Department of General- and Radiochemistry, Institute of Technology, Graz (Austria)

R. W. FREI**

Analytical Research and Development, Pharmaceutical Department, Sandoz Limited, Basle (Switzerland)

(Received 13th September 1974)

The high pharmacological activity of alkaloids prompts the need for very sensitive methods to determine traces of these substances. One means of improving the sensitivity of analytical methods is the introduction of fluorophores by fluorescence derivatization. If high selectivity is required, for example in the analysis of these compounds and their metabolites in biological samples, a combination of the derivatization technique with liquid chromatography can be valuable. Many compounds of biological interest have been studied by means of such labelling reactions[1–3] and recently combinations of fluorescence derivatization and high-speed liquid chromatography (h.s.l.c.) have proved useful in connection with pesticide and other analyses[4–7]. Phenolic groups can be derivatized with 1-dimethylaminonaphthalene-5-sulfonyl chloride (dans-Cl) almost as easily as primary and secondary amino groups. In this study, it was intended to extend the technique to adrenaline and to a group of alkaloids commonly used in pharmaceutical preparations.

EXPERIMENTAL

Preliminary investigation by a titration procedure

The alkaloids (Table I; Sandoz Ltd.) were dried at 110°C for 5 h before weighing. Other reagents were sodium hydroxide (0.01 *M*, Titrisol, Merck), acetone (analytical grade, Merck) and dans-Cl (0.2% in acetone; Merck).

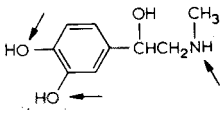
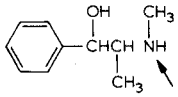
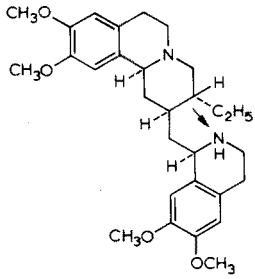
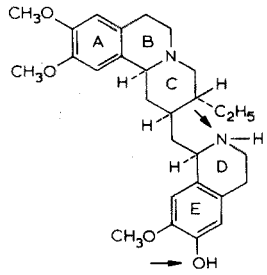
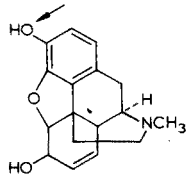
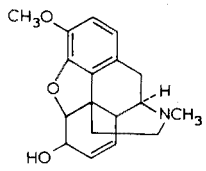
Titrations were carried out with an automatic titrator TTT 2 and an autoburet ABU 13 (Radiometer). The buret volume was 2.500 ml. No thermostating was used during the titration. Before titration, the alkaloids were dissolved in 10 ml of water in a 100-ml beaker (0.2 ml of 0.1 *M* hydrochloric acid had to be added to dissolve adrenaline). Acetone (10 ml) was added and the desired pH (8.5–10) obtained by addition of 0.01 *M* sodium hydroxide by the titrator. Then 10 ml of the dans-Cl solution were added and the titration was carried out until completion of the labelling reaction.

* This paper is dedicated to Prof. Dr., DDr.h.c. M. K. Zacherl on the occasion of his 70th birthday.

** To whom correspondence should be addressed.

TABLE I

LIST OF COMPOUNDS INVESTIGATED

Compounds investigated	*Structure*	*Reactive groups*		*Expected derivative*
		Phenolic OH	*=NH*	
Adrenaline		2	1	Tri-dans-adrenaline
Ephedrine·HCl		—	1	Mono-dans-ephedrine
Emetine·2 HBr		—	1	Mono-dans-emetine
Cephaeline·2 HBr		1	1	Di-dans-cephaeline
Morphine·HCl		1	—	Mono-dans-morphine
Codeine·HCl		—	—	None

Derivatization for trace analysis

As a result of the optimization studies the following procedure is recommended: 10 μl of aqueous alkaloid solution containing not more than 30 nmole of reactive groups are mixed with 50 μl of dans-Cl (0.1% in acetone) and 50 μl of 0.1 *M* sodium carbonate. All solutions are pipetted with Oxford samplers into 10-ml centrifuge tubes. A blank solution contains 10 μl of water instead of the alkaloid solution. The centrifuge tubes are tightly closed, shaken and dipped into a water-bath with exclusion of light. The reaction is complete after 20 min at 45°C. After cooling to room temperature in the dark, 5 ml of benzene are added and the tubes shaken vigorously for 2 min. The phases are separated by centrifugation at 5000 r.p.m. for 3 min. A portion (3.5 ml) of the benzene layer is pipetted into a 1-cm cuvette for measurement.

The composition of the reaction mixture can be varied somewhat, but it is important that the amount of water does not exceed 60%. The volume of buffer solution should not be less than that of the dans-Cl solution, and there should always be at least a 6–7-fold excess of dans-Cl.

Measurement of spectra

A Zeiss PMQ II Spectrophotometer equipped with the ZFM 4 fluorescence attachment was used for fluorescence measurements. The mercury line at 365 nm was used for excitation. The Zeiss F 53 fluorescence standard served to calibrate the instrument at 530 nm. The measurements were carried out without cuvette covers.

Quantitative measurements

Calibration curves were obtained for all investigated compounds under the same conditions; analytical-grade reagents were used throughout. Since the blank depends on the quantity of dans-Cl and buffer, different reaction conditions are recommended for concentrations below 2 nmole. Conditions for 2–10 nmole concentrations: 10 μl of aqueous alkaloid solution; 50 μl of dans-Cl 0.1%; 50 μl of buffer. Conditions for concentrations < 2 nmole: 20 μl of buffer; 10 μl of dans-Cl, 0.1%; 10 μl of alkaloid solution in acetone.

Alkaloid solutions in acetone should be added to the buffer solution, otherwise the acetone will rise on the glass walls and the labelling reaction may be incomplete.

Thin-layer chromatography of the derivatives

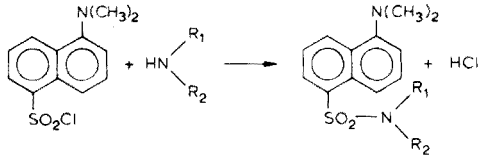
Separations were carried out by ascending chromatography on silica gel plates activated for 1 h at 110°C. The plates were hand-coated by a Camag coating apparatus with Merck silica gel G. The best separation was achieved with a benzene:methanol (3:1) system. After drying in a cold air stream, the spots were detected under a Camag u.v. lamp TL-900 with the 350-nm mercury line.

Extraction of the zones was performed by scraping off the spots into a 10-ml centrifuge tube, followed by extraction with 5 ml of a mixture of benzene and triethylamine (19:1). An equal area of adsorbent was extracted for estimation of the blank.

RESULTS AND DISCUSSION

Study of the derivatization by continuous titration

The reaction of dans-Cl with amines or phenols occurs according to the general equation:



With each mole of dans-Cl reacting, one mole of HCl is produced. Side reactions, with the exception of hydrolysis of the reagent, are negligible. If the pH is kept constant by continuously neutralizing the hydrochloric acid formed, the derivatization reaction can easily be followed. It should also be possible with this titrimetric approach to determine the ratio of reagent to reactant.

For the titrations, some technical difficulties must be considered. The amount of reactant has to be such that an easily measurable amount of sodium hydroxide is used up in the titration step. Dans-Cl should be in excess, but the hydrolysis of dans-Cl to the sulfonic acid, which also produces hydrochloric acid, has to be taken into account. This rate of hydrolysis increases with pH, but remains constant during the titration period, since the amount of dans-Cl reacting is relatively small in comparison with the total amount present. Carbon dioxide, which is introduced during stirring, also influences the amount of alkali used, particularly at higher pH values.

By keeping the experimental conditions constant (amounts and ratio of solvents, pH, stirring speed), these factors can be checked and necessary corrections made. For example, by just titrating a solution of 10 ml of water, 10 ml of acetone and 10 ml of a 0.2% dans-Cl solution with 0.01 *M* sodium hydroxide, (see Fig. 1), a linear curve is obtained, which runs parallel with the upper part of the actual titration plot, and which can be extrapolated to zero titration time. Typically, at pH 8.5 about 600 μl of alkali and at pH 10 about 720 μl are consumed.

From the corrected volumes of titrant (total volume consumed minus blank), the mole ratios of reagent to reactant can easily be computed.

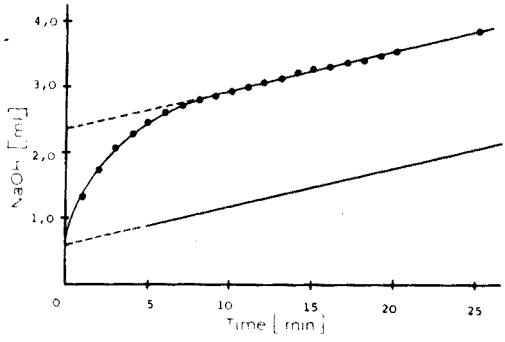


Fig. 1. Monitoring of the dansylation process of emetine by continuous titration with 0.01 *M* NaOH.

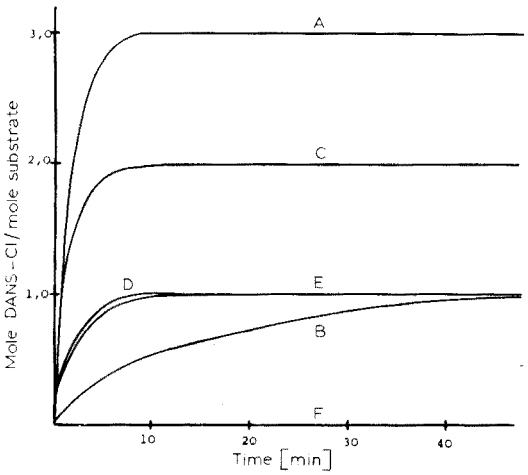


Fig. 2. Corrected mole ratios obtained by continuous titration monitoring of the dansylation reaction. (A) Adrenaline; (B) ephedrine; (C) cephaeline; (D) emetine; (E) morphine; (F) codeine.

In Fig. 2 the results for all the compounds investigated are shown. The results agree perfectly with the expected reactive phenolic and amino groups which are summarized in Table I. As expected, codeine did not react. Since reaction conditions were kept similar for all compounds, a relative comparison of the reaction kinetics was possible. Ephedrine obviously reacts more slowly than the other compounds investigated, which can be attributed to a steric hindrance on the secondary amino group (Table I).

Optimization of the derivatization reaction

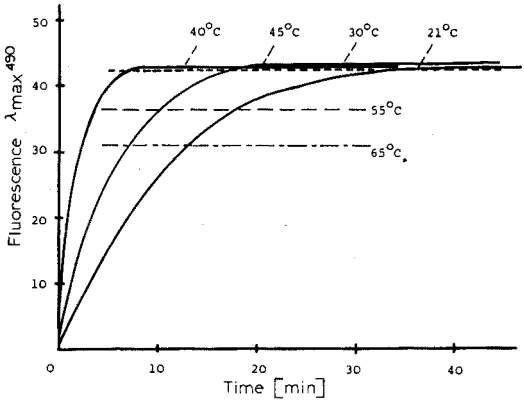
Optimal concentrations of buffer and reagents. Because hydrochloric acid is released during the reaction, buffering is required. For amines or phenols, saturated hydrogencarbonate or carbonate solutions are recommended[1]. In this study, 0.1 *M* sodium carbonate solutions were used. With 25 μl of this buffer solution, 7.5 nmoles of cephaeline (in 10 μl) could be derivatized quantitatively, but an excess of buffer was needed to hydrolyze excess of dans-Cl to the sulfonic acid; otherwise, dans-Cl was co-extracted with the derivatives and increased the fluorescence of the blank. The optimal ratio of reagent to reactant was also tested. In order to reduce the fluorescence of the blank to a minimum, but still guarantee a quantitative derivatization, a 6–18-fold stoichiometric excess (6-fold for adrenaline, 18-fold for ephedrine, emetine and morphine) was required. This agrees with observations made earlier[8].

For extraction of the derivatives, benzene was found to be most suitable.

Influence of temperature and time. Figure 3 shows a typical study for ephedrine. Similar results were obtained for the other compounds studied. The curves have been corrected for blank fluorescence. Optimal reaction temperatures are 45°C for ephedrine, 55°C for emetine and cephaeline, and 60°C for adrenaline and morphine. At higher temperatures the fluorescence yield decreases (see Fig. 3) probably because of faster hydrolysis of the dans-Cl. After a reaction time of 20 min, even ephedrine is completely derivatized. The derivatives are quite stable to temperature changes; the fluorescence intensity remains constant even after prolonged heating in the dark.

Fluorescence properties of the derivatives

Spectra. In order to compare fluorescence emission spectra and relative intensities, all measurements had to be converted to fluorescence intensities of 10 nmole of dans–alkaloid and then corrected for blank fluorescence. The resulting emission spectra in benzene are shown in Fig. 4, and could be used for characterization of the derivatives. The relative intensities do not quite correspond to the ratios of dans-Cl to reactant as determined in Fig. 2. Particularly exceptional is adrenaline which with three substituents should have the highest intensity, but is in fact the second lowest. This could be attributed to steric interference of the three bulk dansyl moieties which would distort the derivative out of the planar configuration and hence reduce the fluorescence yield.



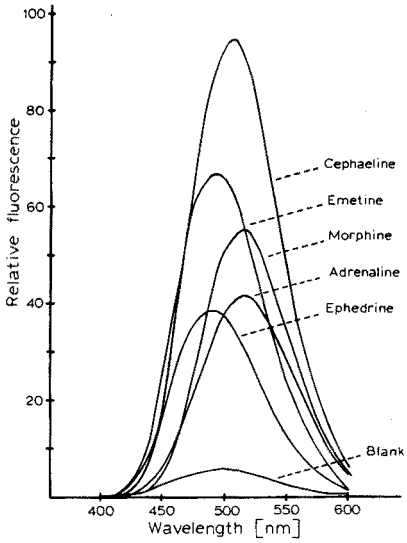


Fig. 3. Derivatization of ephedrine as a function of time and temperature.

Fig. 4. Fluorescence emission spectra of the dans-derivatives recorded with the Zeiss PMQ 2 with fluorescence attachment ZFM 4. Excitation by mercury line at 365 nm. Emission maxima were as follows: cephaeline, 505 nm; emetine, 490 nm; morphine, 510 nm; adrenaline, 515 nm; ephedrine, 490 nm.

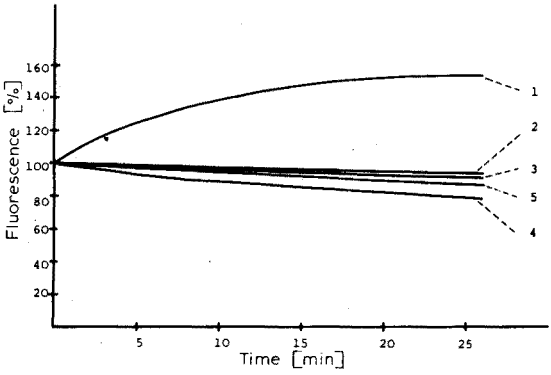


Fig. 5. Influence of the excitation radiation (365 nm) on the fluorescence stability of the dans-derivatives. (1) Tri-dans–adrenaline; (2) mono-dans–ephedrine; (3) mono-dans–emetine; (4) di-dans–cephaeline; (5) mono-dans–morphine.

Influence of u.v.radiation. Dansyl derivatives are light-sensitive[9]. The derivatives were therefore exposed to the excitation radiation of the spectrometer for various lengths of time (Fig. 5). A slight linear decrease was observed for ephedrine, emetine and morphine. About 20% loss of fluorescence was noted for cephaeline. The adrenaline derivative behaved abnormally, in that the fluorescence yield increased drastically on irradiation. It is conceivable that one dansyl molecule is lost, releasing some of the strain on the derivative and hence permitting the derivative to assume a fluorigenically more favourable planar configuration. Studies on this subject are in progress.

Reproducibility and calibration curves

The reproducibility of the method was tested with morphine, by a series of seven assays, each derivatized under identical conditions. The relative standard deviation obtained varied between 1.4 and 3.8% depending on the concentration of morphine. (8.8 nmoles≙1.4%; 4.4 nmole≙2.5%; 0.9 nmole≙3.0%; 0.3 nmole≙3.8%) Similar results were obtained for the other compounds investigated. The limiting factor for the reproducibility is, without doubt, the derivatization step.

The calibration curves for the five derivatives are shown in Fig. 6. All curves extend through zero with the exception of dans–ephedrine, where a small portion remains in the aqueous phase after a single extraction step. The linear concentration range should extend still further than indicated in the graph. Detection limits in 5 ml of benzene were measured at a 2:1 signal-to-noise ratio and were found to be 51 ng for adrenaline, 73 ng for ephedrine, 84 ng for emetine, 57 ng for cephaeline and 60 ng for morphine.

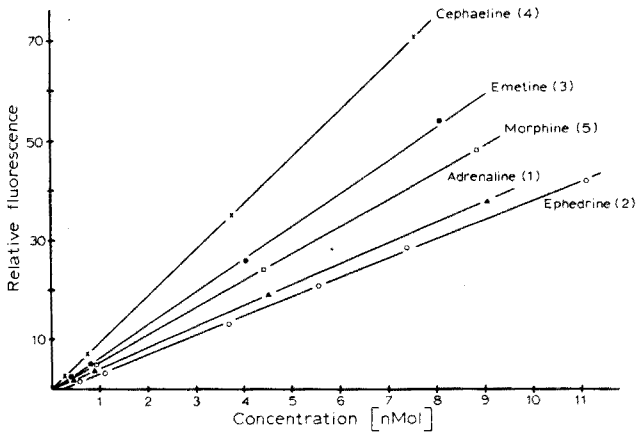


Fig. 6. Calibration curves for the various dansyl derivatives. Curves are numbered as in Fig. 5.

Thin-layer chromatography of the derivatives

A complete separation of the derivatives was possible in one run with a solvent mixture of benzene:methanol (3:1, v/v). The following R_F values were found (average of 5 chromatograms): tri-dans–adrenaline, 0.72; mono-dans–ephedrine, 0.65; di-dans–cephaeline, 0.58; mono-dans–emetine, 0.51; mono-dans–morphine, 0.32. After elution of the spots and fluorescence measurement, a lowering of

the detection limit of about seven-fold was noticed, owing to elimination of interferences causing background fluorescence.

CONCLUSIONS

The titrimetric approach to an initial study of the derivatization reaction furnished valuable data at low instrumental cost. For investigators with no mass spectrometer or n.m.r., it can give information on the composition of derivatives, and can indicate roughly the kinetics of the labelling reactions. Similar techniques could be generally useful for preliminary investigations of other new derivatization procedures with other systems.

In this study it was shown that the investigated compounds can be derivatized quantitatively, and analyzed in trace amounts with a ten- to hundred-fold increase in sensitivity compared to u.v. or colorimetric methods. The derivatization step also serves to increase selectivity. The method is applicable to the determination of active principles in pharmaceutical formulations and could conceivably be utilized for the analysis of body fluids. The selectivity and potential of this derivatization approach is considerably improved in combination with chromatographic separation techniques. The evaluation of t.l.c. spots could be further improved by using the Camag Eluchrom instrument or by fluorescence densitometric measurements[4]. An interesting approach would be the adoption of high-speed liquid chromatography to the determination of these derivatives. Studies along this line are in progress.

Dipl. Ing. R. Aigner is thanked for helpful suggestions.

SUMMARY

Derivatization with dans-Cl was studied for adrenaline, cephaeline, codeine, emetine, ephedrine and morphine. Titrimetry was used to study the derivatization step and to determine the ratios of reagent to reactant. Only codeine did not react. The other five compounds formed single derivatives with emission maximum wavelengths between 490 and 515 nm. Reactions were quantitative after 20 min at 45°C with a 6–7-fold excess of reagent. Fluorescence was measured after extraction into benzene. Detection limits between 0.12 and 0.44 nmole/5 ml were observed. The relative standard deviation at 2 nmole/5 ml was $\pm 2\%$. Derivatives could be separated by t.l.c. to eliminate fluorescence or quenching interferences; fluorescence was then measured after elution of the spots. About a 7-fold lowering of the detection limit was achieved.

REFERENCES

1 N. Seiler and W. Wiechman, in A. Niederwieser and G. Pataki (Eds.), *Progr. Thin-Layer Chromatogr. Relat. Methods, Vol. 1*, Ann Arbor Science Publishers, 1970, p. 95.
2 N. Seiler, *J. Chromatogr.*, 63 (1971) 97.
3 A. A. Boulton, *Methods Biochem. Anal.*, 16 (1968) 327.
4 J. F. Lawrence and R. W. Frei, *J. Chromatogr.*, 98 (1974) 253.
5 R. W. Frei and J. F. Lawrence, *J. Chromatogr.*, 83 (1973) 321.
6 R. W. Frei, J. F. Lawrence, J. Hope and R. M. Cassidy, *J. Chromatogr. Sci.*, 12 (1974) 40.
7 R. M. Cassidy, S. S. Le Gay and R. W. Frei, *J. Chromatogr. Sci.*, 12 (1974) 85.
8 M. Frei-Häusler, R. W. Frei and O. Hutzinger, *J. Chromatogr.*, 79 (1973) 211.
9 J. F. Lawrence and R. W. Frei, *J. Chromatogr.*, 66 (1972) 96.

Analytica Chimica Acta, 76 (1975) 65–69

DETERMINATION OF TRACE AMOUNTS OF ANTIMONY IN GEOLOGICAL MATERIALS BY ATOMIC ABSORPTION SPECTROMETRY

E. P. WELSCH and T. T. CHAO
U.S. Geological Survey, Denver, Colorado 80225 (U.S.A.)
(Received 4th October 1974)

The U.S. Geological Survey needs a rapid, sensitive, and interference-free method of determining trace amounts of antimony in geological samples for use in geochemical exploration. The colorimetric rhodamine B method[1] currently in use, though sensitive and relatively free from interference, is rather time-consuming. An atomic absorption method developed by Yanagisawa *et al.*[2] provides a swifter means of antimony determination by extracting antimony from 6 *M* hydrochloric acid into MIBK (methyl isobutyl ketone). However, owing to the complexity of matrices encountered in geological materials, MIBK extraction alone is not adequate to deal with the high concentration of some interfering ions; a conspicuous example is iron. Burke's[3] work with aluminum-, iron- and nickel-base alloys has demonstrated that antimony can be quantitatively extracted into a 5% solution of trioctylphosphine oxide (TOPO) in MIBK from 10% hydrochloric acid in the presence of iodide and ascorbic acid. Ascorbic acid maintains the dissolved iron as iron(II), thereby minimizing its extraction into the organic layer. Hence, the TOPO–MIBK extraction may be adaptable to the determination of antimony in geological materials.

Both Nicholas[4], and Schweinsberg and Heffernan[5] have recommended the volatilization of antimony from geological samples by heating with ammonium iodide before analysis by atomic absorption. The volatilization of antimony as iodide allows its isolation from the sample matrix. The method reported in this paper combines the volatilization of antimony triiodide with TOPO–MIBK extraction for antimony determination. The optimal conditions for sample heating and solvent extraction of the released antimony have been determined.

EXPERIMENTAL

Apparatus

A Perkin–Elmer 103 atomic absorption spectrophotometer was used with an IL (Instrumentation Laboratory) antimony hollow-cathode lamp and a Boling burner with an air–acetylene flame. The instrument was operated with a lamp current of 10 mA, slit width of 0.2 nm and monochromator setting at 217.6 nm.

A Pyropot heating device (Pyroco Products, Margat, Queensland, Australia 4019), similar to the one used by Heffernan *et al.*[6] but accommodating 10 test tubes, provided a controlled heat source for the iodide fusion. When the test tubes are inserted into the holders, only the bottom 3–4 cm is exposed to the

desired temperature, thereby leaving the upper portions of the tubes cool enough to allow condensation of SbI_3 vapors.

Reagents and standards

Iodide reagent. Prepare daily by dissolving 30 g of potassium iodide and 5 g of ascorbic acid in 100 ml of 10% hydrochloric acid.

TOPO (trioctylphosphine oxide)–MIBK (methyl isobutyl ketone). Dissolve 4 g of TOPO in 100 ml of MIBK.

Antimony stock and standard solutions. Prepare a 100 μg Sb ml^{-1} stock solution by dissolving 0.137 g of potassium antimony tartrate in 500 ml of 6 *M* hydrochloric acid. Combine 10 ml of this solution with 5 ml of concentrated hydrochloric acid in a 100-ml volumetric flask and dilute to volume with demineralized water. The resulting 10 μg Sb ml^{-1} solution is stable for at least a week.

Antimony working standard solutions in MIBK (0, 1, 5, and 10 μg Sb ml^{-1}). Add 1.0 g of potassium iodide and 1.5 g of ascorbic acid to each of four 125-ml separatory funnels. (Standard solutions prepared with the same iodide concentration as sample solutions yielded identical absorbance values.) Pipette 100 ml of 10% hydrochloric acid into the first two funnels, and 95 and 90 ml, respectively, into the remaining two. Shake to dissolve the salt and organic acid. Transfer 0, 1, 5, and 10 ml of the 10 μg Sb ml^{-1} solution to the four funnels. Measure 10 ml of TOPO–MIBK into each funnel and shake for 1 min. Discard the aqueous phases by draining, and store the organic layers in screw-cap test tubes. They remain stable for 2 days.

With a 0.5-g sample taken for analysis, the range of antimony concentrations in the sample that can be determined with the above standard solutions is 0–40 p.p.m. For samples containing more than 40 p.p.m., the MIBK extract may be diluted, or a set of high-antimony working standards may be prepared by using 0, 5, 10, and 50 ml of the 10 μg Sb ml^{-1} solution (corresponding to 0, 5, 10, and 50 μg Sb ml^{-1} in MIBK). This set of standards will cover antimony concentrations in the sample up to 200 p.p.m. Still higher concentrations of antimony can be determined by reducing sample weight.

Procedure

Weigh 0.5 g of 100-mesh soil, rock, or stream sediment sample into a 25 × 200 mm screw-cap test tube along with 2 g of powdered ammonium iodide, and mix thoroughly. Dry the mixture overnight at 105°C in an electric oven. Allow to cool and then heat the sample for 10 min in the Pyropot, which has been pre-heated to 350°C. After the sample has cooled, add 14 ml of 10% hydrochloric acid, mix well on a tube vibrator, and place in a boiling water bath for 15 min. Mix again while hot, cool, add 6 ml of the iodide reagent, and mix again. Measure 2 ml of TOPO–MIBK into each sample, secure the screw cap, and shake for 1 min. Decant the contents into a 16 × 150 mm test tube, centrifuge, and aspirate the organic layer into the air–acetylene flame of the spectrophotometer. Determine the antimony concentration of the sample by comparing with a calibration curve prepared from antimony working standard solutions containing 0, 1, 5, and 10 μg Sb ml^{-1} in MIBK.

As many as 80 samples per man-day may be analyzed by this method.

RESULTS AND DISCUSSION

Sample decomposition

It should be emphasized that thorough dehydration, by heating the mixture of geological sample and ammonium iodide before sample decomposition, is essential for the complete volatilization of antimony triiodide. Suppose, for instance, that antimony is present in the sample as Sb_2O_3 and the volatilization of SbI_3 proceeds as follows.

$$Sb_2O_3 + 6\ NH_4I \rightleftharpoons 2\ SbI_3 + 6\ NH_3 + 3\ H_2O$$

Any moisture in the sample or ammonium iodide will tend to reverse this reaction.

The temperature and duration of heating in the Pyropot are quite critical and must be controlled in order to obtain consistent results. A heating temperature of 350°C was found to be optimal for the samples used, compared to the temperature of 300°C suggested by Schweinsberg and Heffernan[5] for the determination of antimony in ores. The larger sample size and larger test tubes used here probably help account for this difference.

Sensitivity

The 103 atomic absorption meter reads 35 divisions (0.35 absorbance) without expansion when a 10 μg Sb ml^{-1} standard (in MIBK) is aspirated. With the scale expanded roughly 3 times, one scale division is equivalent to 0.1 μg Sb ml^{-1}. For practical purposes, a 0.25 μg Sb ml^{-1} solution in MIBK is a more reasonable lower limit that can be readily determined. This value corresponds to 1.0 p.p.m. Sb when a 0.5-g sample is extracted into 2 ml of TOPO–MIBK. Best results were obtained with the flame as fuel-rich as possible but without any traces of yellow.

TOPO–MIBK affects the aspiration rate and thus the sensitivity adversely, owing to the clogging effect of TOPO. For this reason, pure MIBK is aspirated during the warm-up and between all readings. The 103 meter is then expanded until the 10 μg Sb ml^{-1} standard reads 100 divisions of the scale. The meter is set at zero with the reagent-blank MIBK (0 μg Sb ml^{-1} standard) and once more adjusted to 100 scale divisions with the 10 μg Sb ml^{-1} standard.

Greater sensitivity for antimony determination by atomic absorption may be achieved with better fuel–air regulation or more sophisticated instruments equipped with automatic zero correction devices. The newer electrodeless discharge lamps may also be helpful.

When the 0, 5, 10, and 50 μg Sb ml^{-1} standard solutions (in MIBK) are used, the burner should be turned slightly out of line to reduce sensitivity, as the 50 μg Sb ml^{-1} standard would otherwise be off-scale at zero expansion. Likewise, for this set of antimony standards, an additional calibration curve should be constructed.

Analytical data

Reproducibility data for antimony content in seven geological samples determined by the proposed method are shown in Table I. The samples were analyzed on five consecutive days. The relative standard deviation of replicate analysis ranges

TABLE I

RESULTS OF FIVE REPLICATE DETERMINATIONS OF ANTIMONY ON CONSECUTIVE DAYS

Sample no.	*Material*	*Range (p.p.m.)*	*Mean (p.p.m.)*	s_r (%)
GXR-1	Jasperoid	92–108	97.6	5.6
GXR-2	Soil	40–46	43.6	4.5
GXR-3	Spring deposit	21–23	21.9	3.7
GXR-4	Copper mill tailings	4.8–5.4	5.1	5.3
GXR-5	Soil	1.4–1.8	1.6	7.9
GXR-6	Soil	2.4–3.2	2.8	10.3
Universal-1	Gossan	52–56	53.6	3.7

TABLE II

RECOVERY OF ANTIMONY ADDED TO ROCKS AND SOILS[a]

Sample no.	*Sb in sample (μg)*	*Sb added (μg)*	*Total Sb recovered (μg)*	*Recovery (%)*
1	23.5	10	34.0	105
2	2.4	2	4.3	95
3	1.3	2	3.2	95
4	1.1	1	2.1	100
5	30.5	10	41.0	105

[a] Each result is the average of duplicates.

between 3.7% and 10.7% for antimony concentrations in samples varying from a few p.p.m. to about 100 p.p.m. As expected, the poorer precision occurs at the lower levels of concentration. If the replicate determinations were performed on the same day, better precision would be obtained.

Recovery of known amounts of antimony added to five samples is presented in Table II. The recovery of added antimony ranged from 95 to 105%.

Interferences

In the proposed method of antimony determinations, interferences caused by foreign ions are minimized in three ways. The heating at 350°C under the working conditions allows the isolation of antimony from the sample matrix. When 10% hydrochloric acid is used to dissolve the released antimony, smaller amounts of potential interfering ions are put into solution than in the commonly used fusion or concentrated strong-acid digestion methods. The extraction of antimony into TOPO–MIBK is also advantageous.

Iron is one of the commonest interfering ions in the analysis of geological materials, owing to its ubiquitous abundance in rocks and soils. The equivalent of 1% iron in the sample extracted into solution causes considerable enhancement of antimony values. Ascorbic acid in the iodide reagent reduces iron to iron(II) and eliminates its interference. An equivalent of 20% iron in the sample was

added to antimony standard solutions, which were then analyzed according to the above procedure. No iron interferences were found. It should be pointed out that a sample containing 20% iron would only partially dissolve in 20 ml of 10% hydrochloric acid. Therefore, it would require a sample of much higher iron content to put the equivalent of 20% iron actually into solution.

The 1 and 10 μg Sb ml^{-1} standard solutions spiked with 500 μg ml^{-1} of Cu, Pb, Zn, Sn, As, or Hg gave the same meter readings as those without the added metals. A 0.5-g sample can, therefore, tolerate up to 2000 p.p.m. of each of these six metals. Interference is unlikely in normal samples.

The proposed method, which combines the volatilization of antimony triiodide from the sample with TOPO–MIBK extraction of antimony for atomic absorption measurement, is rapid, has a sensitivity and limit of determination adequate for geochemical exploration, and is not susceptible to interference by iron and other related metals.

The authors thank H. W. Lakin, U.S. Geological Survey, for furnishing the six GXR samples used in this study.

SUMMARY

For the determination of traces of antimony in rocks and soils, dried samples are heated with ammonium iodide to volatilize antimony triiodide, which is then taken up with 10% hydrochloric acid and extracted into TOPO–MIBK. Analysis is completed by atomic absorption spectrometry. The range is 1.0–40 p.p.m. Sb, the relative standard deviation being about 10–4%. Up to 20% iron and 2000 p.p.m. Cu, Pb, Zn, Sn, As or Hg do not interfere.

REFERENCES

1 F. N. Ward and H. W. Lakin, *Anal. Chem.*, 26 (1954) 1168.
2 M. Yanagisawa, M. Susuki and T. Takeuchi, *Anal. Chim. Acta*, 47 (1969) 121.
3 K. E. Burke, *Analyst (London)*, 97 (1972) 19.
4 D. J. Nicolas, *Anal. Chim. Acta*, 55 (1971) 59.
5 D. P. Schweinsberg and B. J. Heffernan, *Proc. Australas. Inst. Mining Met.*, No. 241 (1972) 87.
6 B. J. Heffernan, R. O. Archibald and T. J. Vickers, *Proc. Aust. Inst. Min. Met.*, No. 223 (1967) 65.

Analytica Chimica Acta, 76 (1975) 71–78

DETERMINATION OF TRACE ELEMENTS IN PLANT MATERIALS BY INDUCTIVELY COUPLED PLASMA OPTICAL EMISSION SPECTROMETRY

R. H. SCOTT and A. STRASHEIM
National Physical Research Laboratory, C.S.I.R., Pretoria (South Africa)
(Received 23rd October 1974)

The inorganic analysis of plant materials for certain trace elements is frequently used for diagnostic purposes in agricultural studies. Most common of the analytical methods used are colorimetric, flame atomic emission and absorption and x-ray spectrometry. The d.c. arc and spark techniques have also been used for such determinations in emission spectroscopy[1,2].

The purpose of the present study was to investigate the suitability of inductively coupled plasmas as optical emission sources for the direct analysis of some important trace elements in solutions of plant materials.

Orchard leaves of the following varieties were used as samples in this investigation: apple, apricot, grape, pear, peach and plum. The elements which were determined were Fe, Mn, Cu, Al, B and Zn. The six leaf varieties were received as finely ground, well mixed powders which had been analyzed by a number of independent laboratories by one or more of the various analytical techniques listed above. Thus a comparison of results was possible.

EXPERIMENTAL

Apparatus

The inductively coupled plasma source, the monochromator and the readout instrumentation have been described previously[3]. Table I lists the instrumentation and the operating parameters used in the present study. It should be noted that although a monochromator was used, simultaneous determinations of all the elements are possible since no changes were made to the instrumental conditions during this study. For example, no optimization of experimental conditions for any one element was carried out. Parameters were chosen to produce a minimum of matrix interference effects as had been generally established previously[4,5].

The spectral lines used in this study are listed in Table II.

Preparation of standards

One composite aqueous standard solution was prepared for the analyses. Reagent-grade chemicals were used. The standard solution contained only the analytical elements in solution, except for the final zinc determination (see Discussion).

Sample dissolution procedure

Place the plant material in open dishes in an oven at 90°C for a minimum of

TABLE I

INSTRUMENTATION AND OPERATING PARAMETERS

Spectrometer	
Ebert mount 0.5 m	0.5 m focal length (Jarrel-Ash Division, Fisher Scientific Co., Model No. 82000)
Unilateral slit assembly	25 μm entrance slit width, 5 mm slit length 25 μm exit slit width
Grating	1180 ruling mm^{-1}, blazed at 300 nm, 1st order
Reciprocal linear dispersion	1.6 nm mm^{-1}, 1st order
Photomultiplier	Hamamatsu type R106, S-19 photo-cathode response
Optics	
Plasma imaged in 1:1 ratio onto entrance slit by 16-cm focal length × 5-cm diameter fused quartz lens. Height of observation, 20 mm above coil to center of slit	
Photomultiplier power supply	
High-voltage supply (Keithley Instruments, Model No. 244)	
Amplifier	
Linear picoammeter with built-in pre-amplifier, zero suppression and variable damping (Keithley Instruments, Model No. 417)	
Readout	
Digital voltmeter (Systron Donner, Model No. 7004)	
Plasma source	
Radio-frequency generator	27-MHz, 4-kW max. power output (International Plasma Corp., Model No. 140-27)
Stabilized r.f. power to coupling unit	1 kW forward, < 10 W reflected
Coupling unit	Tuneable capacitor, $1\frac{1}{2}$ turns coil (water cooled)
Plasma torch assembly[3]	Fused quartz, tangential coolant flow, capillary injector
Gas supply	
Argon coolant	9.5 l min^{-1}
Argon aerosol transport	1.0 l min^{-1} (stabilized flow)
Nebulizer and sample chamber	
Teflon and glass pneumatic nebulizer[6]	Solution uptake rate *ca.* 3 ml min^{-1}
Sample chamber as in Ref. 3	
No desolvation unit	

TABLE II

SPECTRAL LINES USED

Element	Fe	Mn	Cu	B	Al	Zn
λ *(nm)*	371.99	403.31	327.39	249.77	308.21	213.85

24 h and then allow to cool in a desiccator. Clean the silica crucibles by placing in (1 + 1) nitric acid and heating to boiling point. Then transfer the crucibles to a muffle furnace and fire at 500°C. After cooling, weigh 2 g of each variety of orchard leaf sample into the crucibles. Place the crucibles in a muffle furnace, increase the temperature slowly to 500°C and maintain for 8 h. Remove the crucibles and allow to cool. Wet the ash by a few drops of distilled water, and add 1 ml of (1 + 1) nitric acid (Suprapur; 65%) dropwise. Transfer the crucibles to a water bath. After the contents have dried, place the crucibles in the muffle furnace for a further

2 h at 500°C, and then allow to cool. Prepare the sample solutions by dissolving the contents of each of the crucibles in 4 ml of (1 + 1) nitric acid (Suprapur) whilst warming gently, transferring to 50-ml Grade A volumetric flasks, and bringing to volume with distilled water.

RESULTS

Linearity of response

Dilutions of the composite standard were prepared; the responses of the readout instrumentation were found to be linear over the working range of concentrations for all the elements investigated. The spectral background intensity measurement at the wavelengths of the various analysis lines (determined by spraying distilled water) could be zeroed with the aid of the zero suppression facility on the amplifier. The instrumentation could thus easily be calibrated to read out actual concentrations in the plant material. Once the linearity of response had been ascertained, calibration was carried out with only one composite standard solution. This is undoubtedly a time-saving and thus highly advantageous characteristic of the plasma technique.

Analysis of samples

A total of 7 dry ashings were performed for each plant material. The results of the present analysis for Fe, Mn, Cu and Al are compared graphically to averages of the results of independent investigators in Figs. 1–4, respectively. Standard deviations of both sets of results are shown. A recent atomic absorption comparative value is given for each element. Since the values for boron did not cover an

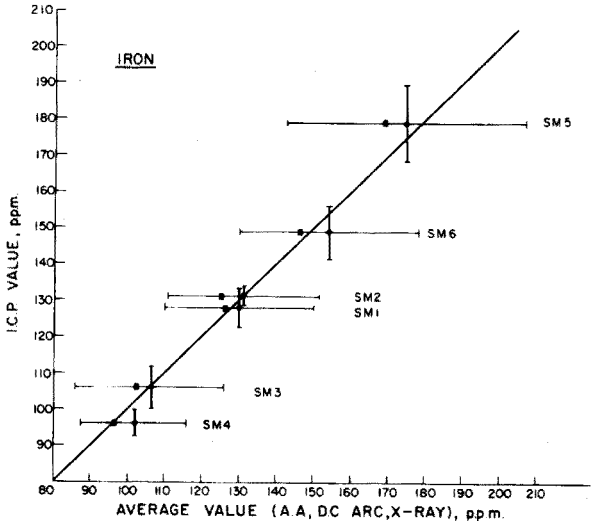


Fig. 1. Comparison of results for iron in orchard leaves. The "average value" for each sample is the average of the values obtained by a number of independent laboratories using one or more of the techniques given in parenthesis. The standard deviation of these values is given for each sample, as well as the standard deviation of the results obtained in the present study for 7 dissolutions. A recent atomic absorption value is also given, indicated by (■).

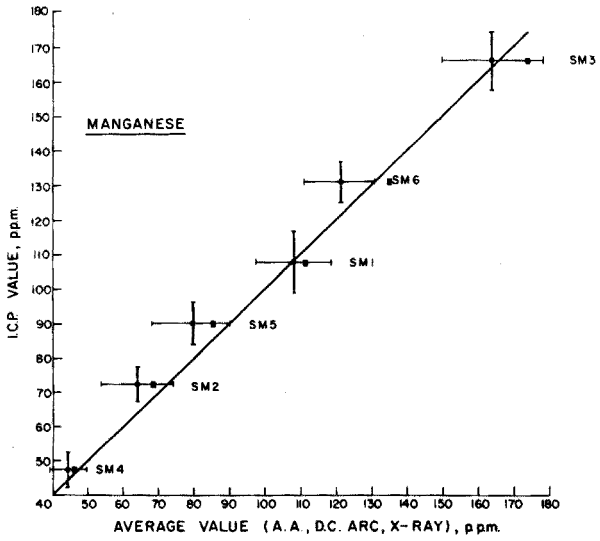


Fig. 2. Comparison as in Fig. 1, for manganese.

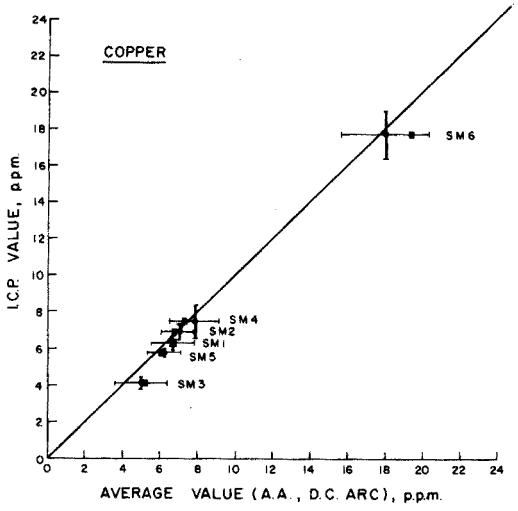


Fig. 3. Comparison as in Fig. 1, for copper.

appreciable range, these were unsuitable for graphic comparison and are therefore shown in Table III; recent colorimetric values obtained for boron here are also given.

For all the above elements, the plasma results compare well with those obtained by other analytical methods. Although the plasma results for aluminium were all slightly lower than the comparative average values, they were within the standard deviation for each sample and therefore acceptable; the atomic absorption results were also slightly lower than the average values. Thus slight characteristic biases for the various analytical techniques appeared to exist. This can also be seen in the results for manganese.

The plasma values for zinc in the orchard leaves were between 3 and 7 p.p.m.

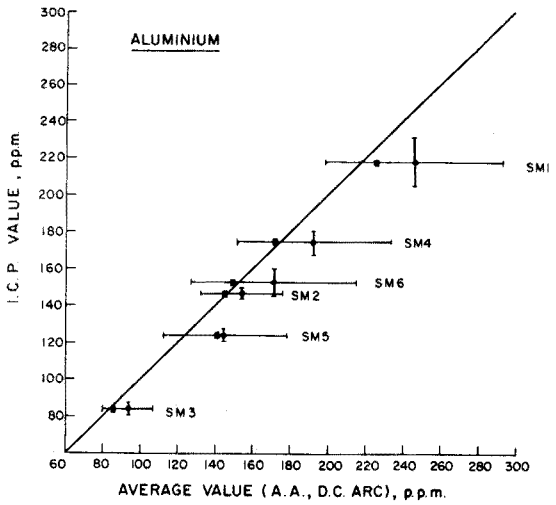


Fig. 4. Comparison as in Fig. 1, for aluminium.

TABLE III

COMPARISON OF RESULTS FOR BORON

Sample no.	*Present value (p.p.m.)*	*Average value*[a] *(p.p.m.)*	*Colorimetric value (p.p.m.)*
SM1	26±3	25±1	25
SM2	26±4	24±3	27
SM3	27±3	25±4	26
SM4	22±3	22±1	22
SM5	29±3	27±3	30
SM6	26±3	26±2	28

[a] D.c. arc and colorimetric methods used in other laboratories.

lower than the comparative average values (depending on the sample) and these results were therefore considered to be unsatisfactory. A more detailed study showed that the cause of the low values was a decrease in the plasma spectral background intensity at 213.8 nm mainly owing to the presence of fairly high concentrations of potassium and calcium in the leaf solutions. (Typical concentrations are K: 500–2000 p.p.m.; Ca: 500–1000 p.p.m.). The spectral background intensity depended on the fairly high concentrations of these and various other concomitants, in particular those elements with relatively low ionization potentials. Table IV shows the intensity values obtained (in arbitrary units) at 213.8 nm for a few solutions compared to the value for distilled water; the value for a 1 p.p.m. zinc solution is also given for evaluating the extent of these interferences.

This dependence of the plasma spectral background intensity on the presence of easily ionized elements existed in certain other regions of the spectrum as well. The bands of NO (with bandheads at 214.3 and 247.1 nm) and of NH (bandhead at 336.0 nm) are examples. The intensities of the bandheads decreased by 3–7% when

TABLE IV

PLASMA SPECTRAL INTENSITY AT 213.8 nm

Sprayed solution	*Intensity*[a]	*Sprayed solution*	*Intensity*[a]
Distilled water	100	100 p.p.m. Ca	99
100 p.p.m. K	97	500 p.p.m. Ca	98
500 p.p.m. K	95	1000 p.p.m. Ca	97
1000 p.p.m. K	95	1000 p.p.m. Mg	98
2000 p.p.m. K	94	1 p.p.m. Zn	120

[a] Arbitrary units; precision approximately 1%.

a solution of 1000 p.p.m. lithium was sprayed compared to distilled water. A corresponding slight increase in the reflected r.f. power meter reading of about 5 W was monitored. This indicated that the power coupling efficiency changed slightly, and therefore a variation in the plasma temperature and/or electron number density distribution may have occurred[5-7], which could account for the observed change in the spectral background intensity.

Since the standard solution that was used for calibration purposes for the orchard leaf analyses contained no ionization buffer[8], the plasma temperature and/or electron number density distribution that existed when the above solution was sprayed could have differed from those prevailing when the analytical solutions were sprayed. Even though a small relative change in the background intensity at 213.8 nm resulted, the absolute change in intensity was significant enough to produce erroneous results for zinc, where the line-to-background intensity ratio was rather small, being only 0.2 for a 1-p.p.m. solution. The addition of an ionization buffer to the standard solution (and blank solution) was thought to be unnecessary because of the relatively high ionization potentials of the trace elements of

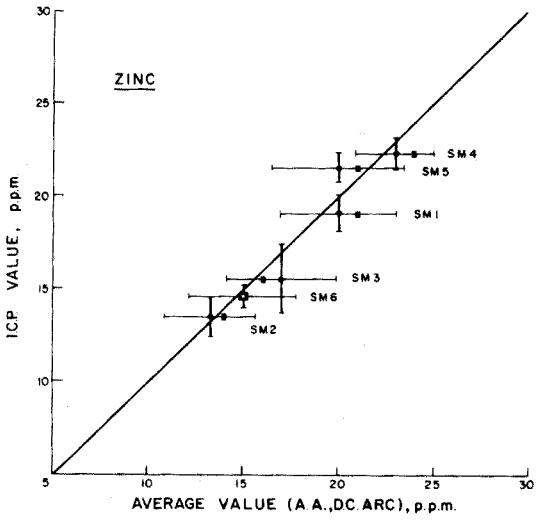


Fig. 5. Comparison as in Fig. 1, for zinc. In this case, synthetic orchard leaf calibration standards were used for the plasma study. See text for details.

interest in the orchard leaves. The good agreement between the plasma values and average values by other analytical techniques for most of these elements tended to support this contention. However, as has been shown above, the presence of reasonably high concentrations of easily ionized elements in the sample solutions may alter the intensity of the spectral background at the analytical wavelength, leading to erroneous measurement of the true line intensity in direct reading spectrometry.

For the zinc determination, the use of calibration standards which contained the major elements of the orchard leaves, namely K, Ca and Mg, at the appropriate concentrations, gave acceptable results. Table IV shows that the spectral background intensity at 213.8 nm did not decrease significantly with increase in potassium concentration from 500 p.p.m. to 2000 p.p.m. and that the influence of Ca and Mg was less than that of potassium. Accordingly, standard and blank solutions were prepared as follows: standard solution, 1 p.p.m. Zn, 1000 p.p.m. K, 1000 p.p.m. Ca, 300 p.p.m. Mg; blank solution, 1000 p.p.m. K, 1000 p.p.m. Ca and 300 p.p.m. Mg. The plasma results for zinc are given in Fig. 5. It can be seen that the values are all within one standard deviation of the average comparative values and seem therefore to be of acceptable accuracy.

CONCLUSION

This study has demonstrated that a number of important trace elements in orchard leaf samples can be satisfactorily determined by using an inductively coupled plasma source in emission spectrometry. An interference effect was observed in the case of zinc which requires further study before definitive conclusions can be made; a relatively small change in the spectral background intensity is caused by reasonably high concentrations of certain elements in the sample solution. It is particularly noticeable when trace quantities of the analyte have to be determined since the spectral line to background intensity ratio in such cases is small.

It was established that the intensities of certain other regions of the background spectrum are affected in a similar manner to that described above. Thus the interference is not necessarily confined to zinc. A satisfactory accuracy could be attained by using synthetic standard solutions for calibration purposes. These synthetic solutions contained the major elements of orchard leaves in approximately the same concentrations as found in the leaf samples.

The authors wish to thank Dr W. J. Pienaar of the Fruit Research Institute, Stellenbosch, for providing the orchard leaf samples as well as the comparative values of the trace element concentrations. The assistance of H. Fischer is also appreciated.

SUMMARY

The use of inductively coupled plasmas as spectrometric emission sources for the determination of Fe, Mn, Cu, Al, B and Zn in orchard leaves is investigated. The plasma is shown to be sufficiently sensitive for the direct determination of all of the above elements in solutions of the plant materials after a dry ashing procedure. Comparative values for the trace element concentrations by other analytical methods are given.

REFERENCES

1 A. J. Bedrosian, R. K. Skogerboe and G. H. Morrison, *Anal. Chem.*, 40 (1968) 854.
2 M. H. Chaplin and A. R. Dixon, *Appl. Spectrosc.*, 28 (1974) 5.
3 R. H. Scott and M. L. Kokot, *Anal. Chim. Acta*, 75 (1975) 257.
4 G. F. Larson, V. A. Fassel, R. H. Scott and R. N. Kniseley, *Anal. Chem.*, 48 (1975).
5 R. H. Scott, *Inter-element Effects in an Inductively Coupled Plasma*, Rep. Fis. 51, National Physical Research Laboratory, CSIR, Pretoria, South Africa, 1974.
6 R. N. Kniseley, H. Amenson, C. C. Butler and V. A. Fassel, *Appl. Spectrosc.*, 28 (1974) 285.
7 P. W. J. M. Boumans, H. Wagenaar and F. J. de Boer, *XVII Colloquium Spectrosc. Int., Lecture reprint, Vol. 1*, 1973, p. 114.
8 J. W. Robinson, *Handbook of Spectroscopy, Vol 1*, CRC Press, Cleveland, 1974, p. 830.

Analytica Chimica Acta, 76 (1975) 79–83

MICRODOSAGE COLORIMÉTRIQUE DE L'ACIDE PERIODIQUE PAR L'ACIDE 2,2'-AZINO-DI(3-ETHYLBENZOTHIAZOLE-6-SULFONIQUE)

G. MAHUZIER

Laboratoire de Chimie Analytique, Faculté Française de Médecine et de Pharmacie, Beyrouth (Liban)

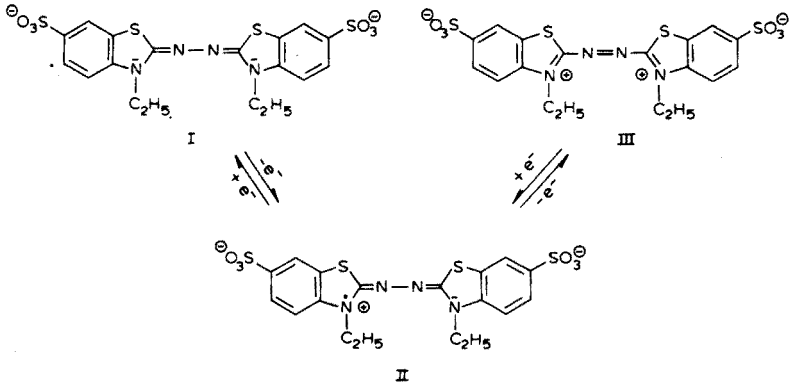
B. S. KIRKACHARIAN et C. HARFOUCHE-OBEIKA

Laboratoire de Chimie Analytique-U.E.R. de Chimie Thérapeutique, Centre Pharmaceutique de Chatenay Malabry, 92 290 (France)

(Reçu le 29 juillet 1974)

Les travaux de Hünig *et al.*[1–3] concernant l'étude de l'oxydation des azines hétérocycliques ont montré que les 2,2'-azino-benzothiazolines substituées ou non étaient susceptibles de s'oxyder en composés intensément colorés. Selon les conditions opératoires, deux substances ont pu être isolées et identifiées: en milieu très acide et en présence d'un oxydant puissant, un composé auquel a été attribué la structure d'un cation azodiquaternaire (III); et en milieu neutre ou peu acide un composé auquel a été attribué la structure d'un cation radicalaire (II).

Les spectres d'absorption de ces composés sont très différents et peuvent ainsi se prêter à des fins analytiques. C'est ainsi que le sel d'ammonium de l'acide 2,2'-azino-di(3-éthylbenzothiazoline-6-sulfonique) ou ABTS (I) a été successivement utilisé comme indicateur d'oxydo réduction en cérimétrie[4] et pour le microdosage de l'eau oxygénée en présence de peroxydase[5].



Des essais préliminaires ayant montré que l'acide périodique oxyde l'ABTS de manière régulière en milieu convenablement tamponné, alors que l'acide iodique est sans action, nous avons étudié les conditions dans lesquelles le dosage spectrophotométrique de cet oxydant peut être réalisé.

La méthode que nous proposons est très simple et de bonne sensibilité. Elle permet de doser sans précautions particulières des quantités d'acide périodique comprises entre 0,02 et 0,1 μmol ml^{-1} et est utilisable lors des études cinétiques d'oxydation.

PARTIE EXPÉRIMENTALE

Réactifs

Solution tampon pH 7,6. Ajouter une solution aqueuse de phosphate monopotassique (0,1 *M*, 128 ml) à une solution aqueuse de phosphate disodique (0,1 *M*, QSP, 100 ml).

Solution d'ABTS. Dissoudre 0,137 g d'ABTS (Boehringer, Mannheim) dans 100 ml de la solution tampon pH 7,6.

Solution d'acide périodique 0,1 M. Dissoudre 22,79 g de l'acide périodique dihydraté dans 1 l de l'eau distillée. Cette solution est diluée au moment de l'étalonnage de façon à obtenir des solutions étalons renfermant des quantités croissantes d'acide périodique comprises entre 0,02 et 0,1 μmol ml^{-1}.

Technique proposée

À 1 ml de la solution à doser contenant des quantités d'acide périodique comprise entre 0,02 et 0,1 μmol ml^{-1}, ajouter 2 ml de la solution d'ABTS. Après agitation et repos de 30 min à l'obscurité, l'absorbance est lue à 420 nm dans des cuves de 1 cm par rapport à un blanc dans lequel la solution à doser est remplacée par de l'eau distillée.

L'étalonnage est réalisé parallèlement en remplaçant la solution inconnue par des solutions étalons d'acide périodique.

RÉSULTATS ET DISCUSSION

Étude spectrale de la réaction

Le spectre de la solution d'ABTS oxydé par l'acide periodique, Fig. 1, est rigoureusement identique à celui obtenu lors de l'oxydation par l'eau oxygénée en présence de peroxydase[5]. Il présente un maximum à 420 nm. La coloration verte observée peut être attribuée à la formation du cation radicalaire car la formation du cation azodiquaternaire radicalaire semble exclue. En effet, ce dernier présente une large bande d'absorption entre 480 et 570 nm et ne peut être obtenu dans les conditions opératoires que nous indiquons: seul un très large excès d'acide periodique en milieu sulfurique concentré nous a permis de le mettre en évidence. Ce composé n'est d'ailleurs stable qu'en milieu très acide, toute dilution aqueuse entraînant le déplacement de l'équilibre de la réaction vers la forme semi oxydée ou radicalaire.

Influence de la nature du milieu réactionnel

La nature de la substance utilisée pour tamponner le milieu réactionnel et maintenir ainsi reproductibles les conditions opératoires a été choisie après avoir essayé des solutions tampons de natures différentes. La solution de phosphates 0,1 *M* nous a semblé la plus appropriée car, à pH égal, elle permet le développement maximum de la coloration (Tableau I).

TABLEAU I

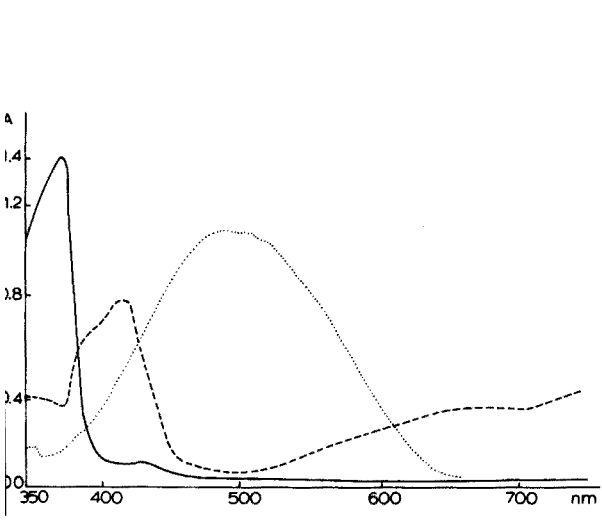
INFLUENCE DE LA NATURE DES SOLUTIONS TAMPONS

Solutions tampons de pH 7,6	*Absorbance en présence de 0,1 µmol d'acide périodique*
Solutions de phosphates, 0,1 *M*	0,880
Solution de véronal sodique et HCl	0,582
Solution d'acide citrique, phosphate monopotassique, véronal et d'acide borique 0,028 *M*	0,101
Solution d'acide citrique 0,1 *M* et de phosphate disodique 0,2 *M*	0,098
Solution de tampon TRIS 0,2 *M*	0,004

Influence du pH et de la concentration en ions phosphates

La valeur du pH du milieu réactionnel influe de manière importante sur l'intensité de la coloration. En utilisant des solutions tampons de même molarité en ions phosphates, mais de pH différents, nous constatons que l'intensité de la coloration croit rapidement pour des valeurs de pH comprises entre 6,5 et 7,5, puis beaucoup plus faiblement de 7,5 à 8,5. Dans ce dernier intervalle les fluctuations du pH sont donc moins importantes et l'emploi d'une solution tampon dont le pH est voisin de 7,6 a été retenu.

La molarité en ions phosphates intervient également sur la vitesse, la stabilité et l'intensité de la réaction. C'est ainsi que plus la concentration en ions phosphates est faible plus la coloration se developpe rapidement et plus intense est la réaction; mais, inversement la stabilité dans le temps apparaît plus



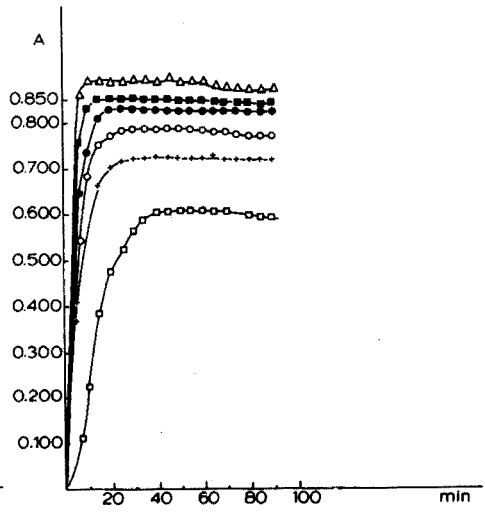


Fig. 1. Spectres des differentes forme d'ABTS: (—) forme réduite; (----) forme semi oxydée ou radicalaire; (...) forme entièrement oxydée ou azodiquaternaire.

Fig. 2. Variation de l'intensité de la coloration en fonction du temps et de la molarité en ions phosphates. Molarité en ions, phosphates: (□) 0,2; (×) 0,1; (○) 0,067; (●) 0,05; (■) 0,033; (△) 0,017.

courte (Fig. 2). Pour concilier ces différents facteurs avec le maintien d'une capacité tampon suffisante, nous avons choisi d'opérer en milieu phosphate 0,1 *M*.

Détermination de la concentration optimale en ABTS

Hünig a signalé que dans certaines conditions la forme radicalaire a tendance à se dismuter (II⇌I + III), mais ce phénomène peut être évité en déplaçant l'équilibre grâce à la présence d'un large excès de la forme réduite[5].

Afin d'éviter une absorption trop intense du témoin blanc réactif, nous nous sommes arrêtés à des concentrations telles qu'il y ait toujours au minimum 50 molécules d'ABTS pour une molécule d'acide périodique. Cette proportion nous permet ainsi d'avoir un témoin qui ne dépasse pas 0,03 d'absorbance.

Sensibilité, reproductibilité, précision

L'absorption à 420 nm est linéairement proportionnelle à la concentration pour des quantités d'acide périodique comprises entre 0,02 et 0,1 μmol ml^{-1} (Fig. 3). Mais cette linéarité n'est plus observée pour des quantités inférieures.

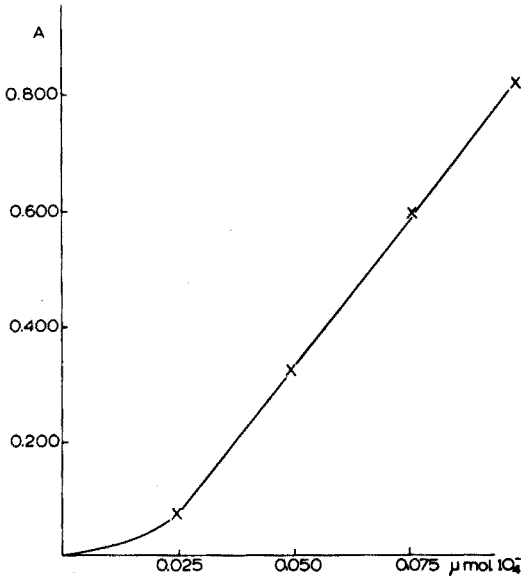


Fig. 3. Variation de l'absorbance à 420 nm avec la concentration en acide périodique.

Cette constatation peu satisfaisante est dans la pratique peu gênante, car la majorité des dosages fait appel à un excès d'acide périodique et c'est la disparition par réduction de quantités relativement faibles de cet oxydant qui doit être évalué.

Des essais de reproductibilité et de précision ont été effectués sur 15 déterminations de la concentration de solutions de sorbitol et de mannitol: 0,02 μmol de polyol ont été oxydées par une quantité d'acide périodique théoriquement double de celle nécessaire pour obtenir une dégradation complète; l'acide périodique n'ayant pas réagi est dosé par la méthode proposée. Les résultats sont consignés dans le Tableau II. Ils montrent que la reproductibilité est bonne

ABLEAU II

SSAIS DE REPRODUCTIBILITÉ ET DE PRÉCISION DE LA MÉTHODE PROPOSÉE

Dosage répétés de solutions de D-mannitol et de D-sorbitol.)

	$\bar{x}$ (μmol)	s	s_r (%)	Théorie (g)	Trouvé (g)	Erreurs (%)	Trouvé méthode de Fleury et Lange[6]	Erreur (%)
	Reproductibilité (15 essais)			*Exactitude*				
-mannitol	0,0506	±0,0015	2,96	0,364	0,366	+0,5	0,373	+2
-sorbitol	0,0521	±0,0012	2	0,3824	0,380	−0,6	0,385	+0,7

et que la limite d'erreur reste inférieure à 1% et meilleure que celle obtenue selon la méthode iodométrique habituelle de Fleury et Lange[6].

En conclusion la méthode proposée permet de doser des quantités d'acide périodique très petites de l'ordre de 0,1 μM; ceci est particulièrement intéressant dans les microdosages de polyols et a pu même être appliqué au niveau cellulaire.

RÉSUMÉ

L'acide périodique oxyde en milieu convenablement tamponné l'acide 2,2'-azino-di-(3-éthyl-benzothiazole-6-sulfonique) avec production d'un composé qui présente une très forte absorption à 420 nm. Cette réaction permet un microdosage colorimétrique extrêmement simple de l'ion périodique pour des quantités comprises entre 0,02 μM et 0,1 μM. Son application au dosage de polyols comme le sorbitol et le mannitol montre l'exactitude et la reproductibilité de la méthode proposée.

SUMMARY

Periodic acid oxidizes 2,2'-azino-di-(3-ethylbenzothiazole-6-sulfonic acid) in suitable media, to form a compound which absorbs strongly at 420 nm. The reaction provides a very simple spectrophotometric determination of periodate in the range 0.02–0.1 μM. The application of the method to the analysis of sorbitol and mannitol shows its accuracy and precision.

BIBLIOGRAPHIE

1 S. Hünig, *Justus Liebigs Ann. Chem.*, 676 (1964) 32.
2 S. Hünig, H. Balli, H. Conrad et A. Schott, *Justus Liebigs Ann. Chem.*, 676 (1964) 36.
3 S. Hünig, H. Balli, H. Conrad et A. Schott, *Justus Liebigs Ann. Chem.*, 676 (1964) 52.
4 H. Lang, H. Hunel et H. Hann, *Z. Anal. Chem.*, 201 (1964) 321.
5 W. Werner, H. G. Rey et H. Wielinger, *Z. Anal. Chem.*, 252 (1970) 224.
6 P. Fleury et J. Lange, *J. Pharm. Chim.*, (8) 17 (1933) 107, 196.

Analytica Chimica Acta, 76 (1975) 85–90

SPECTROPHOTOMETRIC DETERMINATION OF NITROGEN IN VANADIUM, TITANIUM AND URANIUM WITH THYMOL AFTER ALKALI FUSION

HIROSHI HASHITANI, HIDEYO YOSHIDA and TAKEO ADACHI

Analytical Chemistry Laboratory, Japan Atomic Energy Research Institute, Tokai, Ibaraki (Japan)

(Received 15th October 1974)

A sensitive spectrophotometric method for the determination of ammonia nitrogen with thymol[1,2] has been successfully applied to determine down to 3 p.p.m. of nitrogen in uranium and its compounds[3], and in zirconium alloys[4] without separation. In each case, nitrogen was converted to ammonia without loss during the dissolution of the sample, and the major constituent elements were kept in solution with citrate during the color development.

However, this method was not successful in analyzing vanadium and its alloys because of the difficulty of the dissolution. Thus, alkali fusion was investigated to separate nitrogen as ammonia before the photometric determination. The method established was used for the separation of nitrogen from titanium and uranium also.

EXPERIMENTAL

Reagents

Standard nitrogen stock solution (100 μg N ml^{-1}). Dissolve 0.3821 g of ammonium chloride in water and dilute to 1 l. This solution was diluted with water before use.

Buffer solution (pH 10). Dissolve 22 g of sodium carbonate and 5 g of sodium hydrogencarbonate in water and dilute to 1 l.

Sodium hypochlorite solution (0.3% active chlorine). Dilute a commercially available solution (10% chlorine) and store in an amber glass bottle. The dilute solution can be used for at least a month if stored in the refrigerator.

Thymol solution (5%). Dissolve 5 g of thymol in 50 ml of acetone. Mix with an equal volume of 1 *M* sodium hydroxide before use. Before mixing, cool the two solutions to avoid excessive increase in temperature.

Apparatus

A Hitachi–Horiba M 3 pH meter with glass electrode was used for pH adjustment. A Shimadzu QV 50 spectrophotometer with 10-mm glass cells was used for absorbance measurement. A split-type electric furnace, a reaction tube and a nickel boat as shown in Fig. 1 were used for the pyrolytic separation.

Procedure

Pack the sample (1 g) into a nickel boat with 15 g of potassium hydroxide

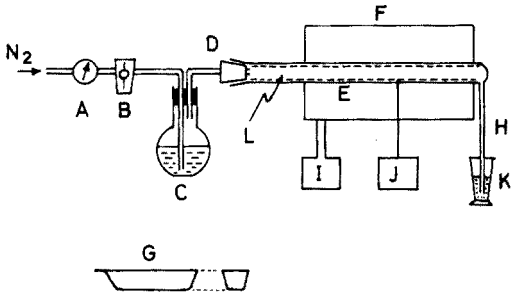


Fig. 1. Apparatus for pyrolytic separation with KOH. (A) Regulator; (B) manometer; (C) water tower; (D) plug, silicone rubber; (E) pyrolytic reaction tube, fused silica, 42 mm i.d., 580 mm long; (F) split-type furnace, 470 mm long; (G) sample boat, nickel, 150 mm long, 25 mm wide, 20 mm high; (H) delivery tube, fused silica, 10 mm i.d., 250 mm long; (I) variable transformer; (J) thermocouple (alumel–chromel) and pyrometer; (K) pharmaceutical flask, 50 ml graduate; (L) nickel lining tube, 0.3 mm thick.

pellets, and push to the center of the furnace. Carry out the fusion at 500–600°C for 20 min in a stream of nitrogen (0.5 l min^{-1}). Absorb the produced ammonia in 20 ml of 0.05 *M* hydrochloric acid, and determine photometrically as follows.

Adjust the pH of the solution, containing 3–80 μg of nitrogen, to 9.8–10.3 with 2 *M* sodium hydroxide and 2.0 ml of buffer solution (pH 10). Then, add 0.5 ml of the sodium hypochlorite solution with stirring; 20–30 s after addition of the hypochlorite, add 2.5 ml of thymol solution, and adjust the pH of the solution to 11.5–11.9 with 2 *M* sodium hydroxide. Dilute the resultant solution to exactly 50 ml with water. After allowing to stand for 1 h in darkness, measure the absorbance of the colored solution at 660 nm using a reagent blank as reference.

RESULTS AND DISCUSSION

Preparation of a vanadium sample for investigation

A vanadium flake metal manufactured by the Oregon Metallurgical Corporation, with a nominal nitrogen content of 0.006%, was found to contain only 15 p.p.m. of nitrogen by the proposed method. In order to investigate conditions for separation of nitrogen as described below, vanadium metal with a high nitrogen content was prepared as follows: 20 g of the above-described metal, fabricated to 0.1 mm plate thickness, was sealed into a silica tube (16 mm i.d. × 130 mm) with 0.2 torr of nitrogen gas at room temperature, and the tube was heated to 800°C for 7.5 h. The metal obtained contained about 400 p.p.m. of nitrogen.

Recovery of nitrogen in vanadium

In preliminary experiments, vanadium metal was so easily fused with sodium hydroxide in a nickel crucible that the nitrogen present as nitride was expected to be recovered quantitatively as ammonia. However, the recovery was not quantitative with sodium hydroxide in experiments with the apparatus shown in Fig. 1. As shown in Fig. 2, potassium hydroxide was more effective than sodium hydroxide for the recovery of nitrogen in vanadium. The nickel boat was not attacked

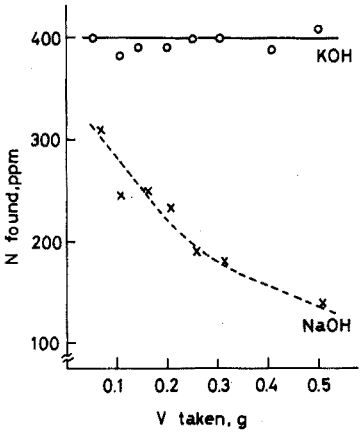


Fig. 2. Comparison of KOH and NaOH as fusing agents for vanadium (7 g of alkali were used).

appreciably by potassium hydroxide, and could be used repeatedly by washing with water between determinations.

An inert gas must be used for collecting the evolved ammonia because a violent reaction occurred in the reaction tube when the fusion was carried out in a stream of air. Nitrogen gas does not react with vanadium metal at 500°C at atmospheric pressure. Although the nitrogen gas used was moistened by passing through a water tower in the procedure, dry nitrogen gas gave the same value (414 ± 8.4 p.p.m. N, $n=4$) for vanadium metal as the value obtained with the moist nitrogen gas (416 ± 9.0 p.p.m. N, $n=4$). Potassium hydroxide seemed to contain enough water to convert nitride to ammonia. However, the dry nitrogen was not preferable because a white substance, which could be dissolved in water, was occasionally produced inside the delivery tube. The vanadium metal was fused in 5 min with potassium hydroxide at 500–600°C, but recovery tests showed the necessity of prolonged fusion for 15 min or more to obtain reproducible values. Higher flow rates of nitrogen gas gave low results because dilute hydrochloric acid could not quantitatively absorb the evolved ammonia (Table I).

TABLE I

EFFECT OF FLOW RATE OF NITROGEN GAS[a]

Flow rate ($l\ min^{-1}$)	*No. of detn.*	*N found (p.p.m.)* (*av.* $\pm\sigma$)
0.25	5	405 ± 7
0.5	4	401 ± 7
0.75	5	394 ± 15
1.0	5	385 ± 15
1.3	3	395 ± 9
1.6	5	378 ± 19

[a] V metal taken, *ca.* 0.13 g.

Photometric determination of ammonia nitrogen

This photometric method is considered to be based on a reaction between monochloramine and thymol. The monochloramine formed is not stable in alkaline solution. The pH of the solution before the addition of hypochlorite (9.8–10.3), the time interval between the addition of hypochlorite and that of thymol (within 30 s), and the pH of the colored solution (11.5–11.9) are important factors[2]. In the method established, no effect of temperature was observed in the range 5–30°C. Maximal color intensity was attained after 60 min and remained constant for at least 5 h when the solution was stored in the dark.

With the further addition of acetone, the absorbance was increased, but the solution should be allowed to stand for 1.5 h to obtain maximal color intensity. The molar absorptivity for nitrogen was $1.16 \cdot 10^4$ l mol^{-1} cm^{-1} at 660 nm in the proposed procedure.

The validity of the method

Because vanadium metal does not dissolve in mineral acids except nitric acid, determination of nitrogen by the usual Kjeldahl method is difficult. Standard samples for nitrogen in vanadium are not available at present. Therefore, some other standard samples were analyzed to verify the validity of the proposed method. As shown in Table II, the analytical values for titanium and its alloys were in good agreement with the certified values.

TABLE II

DETERMINATION OF NITROGEN IN STANDARD SAMPLES OF TITANIUM AND ITS BASE ALLOYS

Sample	*Content of other elements (%)*	*N found (p.p.m.)*	*Certified value (%)*
NBS 173	5.4 Al, 4.1 V, 0.16 Fe	163±5 (n=9)	0.018
NBS 173A	6.7 Al, 4.1 V, 0.15 Fe	187±4 (n=8)	0.018
NBS 174	4.3 Al, 4.6 Mn, 0.18 Fe	122±3 (n=7)	0.01_2
NBS 176	5.2 Al, 2.5 Sn, 0.07 Fe	106±3 (n=7)	0.01_0
H-1[a]	0.03 Fe	78, 75	0.007
H-2[a]	0.05 Fe	68, 65	0.006
H-3[a]	0.11 Fe	75, 72	0.007

[a] Produced by the Japan Analytical Chemistry Research Institute.

Determination of nitrogen in uranium and its compounds

A photometric method based on thymol for the determination of nitrogen in uranium and its compounds without separation has been reported[3]. In this method, dissolution of the sample to convert nitrogen quantitatively to ammonia and prevention of precipitation of uranium are important. The method was successfully used for determining nitrogen in the metal, dioxide, carbide, nitride and carbonitride. However, a non-stoichiometric sesquinitride prepared either directly from the metal or through the hydride[5], was hard to dissolve in mineral acids. After those samples had been dissolved in hydrochloric acid–fluoboric acid, insoluble matter was observed and low results were obtained. Thus, the pyrolytic separation

TABLE III

ANALYTICAL RESULTS FOR NON-STOICHIOMETRIC URANIUM SESQUINITRIDE (U_2N_{3+x})

Sample	*N:U ratio obtained by*		
	Proposed method	*Dumas method*	*Calculation*
1	1.52 (8.23% N) 1.52 (8.20% N) 1.54 (8.33% N)	1.66	1.655
2	1.54 (8.32% N)	1.61	1.595

with potassium hydroxide was tried. As can be seen in Table III, the obtained N:U ratio of two samples was 1.52–1.54, but was lower than the calculated values which were obtained from the difference of the weights of metal and product. It has been reported that the 1.54 N:U ratio pertains to a uranium-rich phase boundary of the non-stoichiometric sesquinitride[5–7]. The excess nitrogen beyond the 1.54 N:U ratio was considered to be evolved as nitrogen gas by the fusion with potassium hydroxide. The values obtained by the Dumas method agreed with the calculated values.

From these experimental results, it is concluded that traces of nitrogen present as an impurity in uranium metal, oxide and carbide, and the constituent nitrogen in uranium nitride and carbonitride, can readily be recovered as ammonia either by dissolution or fusion. However, interstitial nitrogen as in the case of non-stoichiometric sesquinitride cannot be determined.

TABLE IV

ANALYTICAL EXAMPLE FOR DETERMINATION OF NITROGEN IN METALS AND THEIR COMPOUNDS

Sample	*N found (p.p.m.)*
V, plate	31.6 ± 3.5 ($n=6$)
V–Ti (20%), plate	65.9 ± 1.9 ($n=6$)
U, turnings, 99.9%[a]	42, 40
Ti, sponge, 99.7%	31, 32
Ti, block (NBS 352, for H)	206, 206
TiO_2, reagent grade	31, 24
Al, plate, 99.99%	<1, <1
In, block, 99.95%	3, 3
Mg, block, 99.99%	<1, <1
Mn, plate, 99.99%	25, 20
Sb, block, 99.99%	2, 3
Sn, chip, 99.999%	2, 3
Ta, sponge, 99%	23, 21
Ta, powder, 99.9%	50.2 ± 1.9 ($n=24$)
Zn, block, 99.99%	<1, <1

[a] Value obtained by the direct thymol photometry[2] and distillation-Nessler photometry was 40 p.p.m. N.

Application to other metals

In general, it is considered that nitrogen present as impurity in metal and alloy can be quantitatively recovered as ammonia, if the sample is fused with potassium hydroxide and has a melting point not lower than 400°C. If the sample melts before the fusion, nitrogen gas may be produced but not ammonia (m.p. of KOH is 380°C).

Aluminium, Be, In, Mg, Si, Sb, Sn, Ta, Ti, U, V, W and Zn were completely fused, but iron was not completely fused, and Ni, Nb and Zr were not fused. The analytical results for these metals and their compounds which could be fused are shown in Table IV. Except for uranium, the validity of the analytical values has not been fully ascertained yet.

Part of this work was presented at the International Congress on Analytical Chemistry, IUPAC, Kyoto, 1972.

SUMMARY

Vanadium metal packed into a nickel boat with potassium hydroxide, is fused at 500–600°C, and the volatilized ammonia is absorbed in dilute hydrochloric acid. The ammonia is converted to monochloramine with sodium hypochlorite at pH 9.8–10.3, and reacted with thymol at pH 11.5–11.9 for 1 h, and the absorbance is measured at 660 nm. The proposed method was also used for the determination of nitrogen in titanium and uranium. Nitrogen in some other metals such as Al, Be, In, Mg, Mn, Si, Sb, Sn, Ta, V, W and Zn which can be fused with potassium hydroxide and have melting points above 400°C may also be determined.

REFERENCES

1 R. Th. Roskam and D. de Langen, *Anal. Chim. Acta*, 30 (1964) 56.
2 H. Hashitani and H. Yoshida, *Bunseki Kagaku*, 17 (1968) 1136.
3 H. Hashitani and H. Yoshida, *Bunseki Kagaku*, 19 (1970) 1081.
4 H. Hashitani and H. Yoshida, *Bunseki Kagaku*, 19 (1970) 1564.
5 H. Tagawa, *J. Nucl. Mater.*, 41 (1971) 313.
6 J. Bugl and A. A. Bauer in J. T. Waber, P. Chicotti and W. N. Miner (Eds.), *Compounds of Interest in Nuclear Reactor Technology, IMD Special Report No. 13, Metallurgy Soc. AIME*, Edwards, Ann Arber, Mich., 1964, p. 215.
7 H. Tagawa and N. Masaki, *J. Inorg. Nucl. Chem.*, 36 (1974) 1099.

Analytica Chimica Acta, 76 (1975) 91–96

SPECTROPHOTOMETRIC DETERMINATION OF DI(2-ETHYLHEXYL), DI(4-OCTYLPHENYL) AND MONO(4-OCTYLPHENYL)PHOSPHORIC ACIDS WITH RHODAMINE-B

K. BHATTACHARYYA and T. K. S. MURTHY

Chemical Engineering Division, Bhabha Atomic Research Centre, Trombay, Bombay 400085 (India)

(Received 23rd September 1974)

Di(2-ethylhexyl)phosphoric acid (DEHPA), di(4-octylphenyl)phosphoric acid (DOPPA) and mono(4-octylphenyl)phosphoric acid (MOPPA) are used for the liquid–liquid extraction of uranium from sulphuric acid leach liquors obtained from ores[1,2] and from commercial wet-process phosphoric acid[3–5]. The need for the determination of small amounts of extractants in raffinates has been recognized[6]. A spectrophotometric method has recently been reported for the determination of DEHPA[7] based on the formation of an ion-association complex with rhodamine-B and its extraction into 1,2-dichloroethane. In an attempt to extend this method for the determination of DOPPA and MOPPA, it was found that the procedure can be simplified and the extraction step avoided by carrying out the reaction in an organic medium, preferably in 1,2-dichloroethane.

The present method is based on the observation that from an aqueous solution of moderate to high pH, rhodamine B is extracted into some organic solvents in the colourless lactone form (eqn. 1). If DEHPA, DOPPA or MOPPA is added to such a solution, the characteristic colour of the dye is restored with ion-pair formation and simultaneous conversion of the dye to the quinonoid form (eqn. 2). The equilibria involved are:

$$\underset{\text{(Coloured quinonoid form)}}{(RBH^+H_2PO_4^-)_{aq}} \overset{\text{pH 10}}{\rightleftharpoons} \underset{\text{(Colourless lactone form)}}{(RB^0)_{DCE}} + (H_3PO_4)_{aq} \quad (1)$$

$$(RB^0)_{DCE} + (HA)_{DCE} \rightarrow \underset{\text{(Coloured quinonoid form)}}{(RBH^+A^-)_{DCE}} \quad (2)$$

where HA = DEHPA, DOPPA or MOPPA, and RB = rhodamine-B. The organophosphoric acid can be determined in an aqueous solution by extraction into 1,2-dichloroethane and addition of the nearly colourless dye solution in the same solvent to the extract.

EXPERIMENTAL

Reagents

Rhodamine-B. Prepare a 0.04% solution of rhodamine-B (B.D.H. Laboratory

reagent-grade) in 0.1 *M* phosphate buffer of pH 10 and extract with an equal volume of 1,2-dichloroethane. Collect the organic phase after allowing it to settle for about 30 min. Prepare this reagent daily.

DEHPA solution. Prepare a 10 μg ml^{-1} solution by dissolving a weighed amount of B.D.H. Laboratory reagent-grade DEHPA in 1,2-dichloroethane.

DOPPA and MOPPA. These two compounds were prepared in a pure state from "Acid octylphenyl phosphate" (Mobil Chemicals, U.S.A.), which is essentially a mixture of DOPPA and MOPPA. Dissolve 100–150 g of this mixture in about 60 ml of n-heptane and extract thrice with an equal volume of monoethylene glycol. Preserve the combined glycol layer for recovery of MOPPA. Keep the heptane layer at 0–5°C for 2–3 days, collect the solid DOPPA and purify by repeated crystallization from n-heptane (m.p. 99–100°C). A potentiometric titration in ethanolic medium with sodium hydroxide showed only the single sharp inflection characteristic of a strong monobasic acid.

For recovery of MOPPA, dilute the glycol fraction with about 800 ml of 4 *M* hydrochloric acid. Collect the viscous material that separates on standing. Determine the MOPPA content by titrating a known amount potentiometrically in ethanol with sodium hydroxide.

Prepare standard solutions containing 10 μg ml^{-1} of DOPPA and MOPPA in 1,2-dichloroethane, using these separated compounds.

Solvents. Use reagent grade solvent after washing with 5% (w/v) sodium carbonate solution and water.

Apparatus

For spectrophotometric measurements a Beckman Model-B spectrophotometer with 10.0-mm glass cells was used.

Recommended Procedure

DEHPA and DOPPA. Pipette 10.0 ml of the sample (sulphate liquor for DEHPA and wet-process phosphoric acid for both) into a separating funnel and extract with 10.0 ml of 1,2-dichloroethane for 2–3 min. Allow the phases to separate for 15 min. In the case of the sulphate liquor, collect the organic layer as such; in the case of the phosphoric acid, run down the acid, wash the organic layer with an equal volume of 0.2 *M* phosphoric acid, and collect the organic layer after the phases have separated.

Pipette an aliquot of 1,2-dichloroethane extract containing 10–20 μg of DEHPA or DOPPA into a 10.0-ml volumetric flask, add the rhodamine-B reagent in 1,2-dichloroethane (5.0 ml for DEHPA and 1.0 ml for DOPPA) and make up the volume with 1,2-dichloroethane. Measure the absorbance at 560/565 nm against a reagent blank. Determine the amount of DEHPA/DOPPA from calibration curves obtained by treating known aliquots of standard solutions with the same amount of reagent as in the case of samples.

MOPPA in phosphoric acid. For the recovery of MOPPA from phosphoric acid (6 *M*) solution, extract three times with an equal volume of chloroform. Evaporate the solvent from the combined extract and take up the residue in 10.0 ml of 1,2-dichloroethane. Proceed with the spectrophotometric determination of MOPPA in this solution as in the case of DOPPA.

RESULTS

Choice of solvent for recovery and colour development

The solvent to be used in this method must satisfy three conditions: (a) it should extract quantitatively the organophosphate from aqueous raffinates; (b) it should extract the dye from an aqueous solution of appropriate pH effectively in the colourless lactone form; (c) it should facilitate ion-pair formation when the reagent and the organophosphoric acids are mixed. Of a number of solvents tried, 1,2-dichloroethane was found to be the most satisfactory in all aspects except for the recovery of MOPPA from phosphoric acid solution. For this purpose, chloroform was found suitable. However, in chloroform medium the colour formation was not satisfactory, hence this solvent has to be removed before the final spectrophotometric determination.

Preparation of rhodamine-B reagent

When the solid reagent (RB-chloride) was dissolved in 1,2-dichloroethane, a highly coloured solution was obtained. However when an aqueous solution was extracted with the same solvent, most of the dye was transferred into the organic phase in a colourless form. Hence this method of preparation of the reagent was considered more suitable for obtaining low blank absorbance. To study the effect of pH of the aqueous solution on the extraction of the dye, 0.1 *M* phosphoric acid containing 0.04% dye was adjusted to pH 1.5–10 and extracted with an equal volume of 1,2-dichloroethane. With increasing pH, the absorbance of both aqueous and organic phases decreased. The transfer of the dye into the organic phase was almost quantitative (distribution ratio >500) for pH 3 and above. However, a pH of 10 was preferred as the organic phase then showed minimal absorbance.

TABLE I

EFFECT OF AQUEOUS pH ON COLOUR DEVELOPMENT

Aqueous pH	1.5	2.0	3.0	4.0	6.0	8.0	10.0
Blank absorbance (B) (565 nm)	>2	0.960	0.470	0.380	0.320	0.300	0.240
Sample absorbance (S)	>2	1.190	0.710	0.610	0.555	0.520	0.480
Difference (S−B)	—	0.230	0.240	0.230	0.235	0.220	0.240

The extraction pH had little effect on the intensity of the colour formed when a 10-μg aliquot of DOPPA was added to 10.0 ml of the reagent prepared as above. The results are shown in Table I. Similar results were obtained with DEHPA and MOPPA.

Absorption spectra

The absorption spectrum of the DEHPA–rhodamine-B complex in 1,2-dichloroethane had a maximum[7] at 560 nm, the DOPPA and MOPPA complexes at 565 nm. These two wavelengths were used for the respectice absorbance measurements.

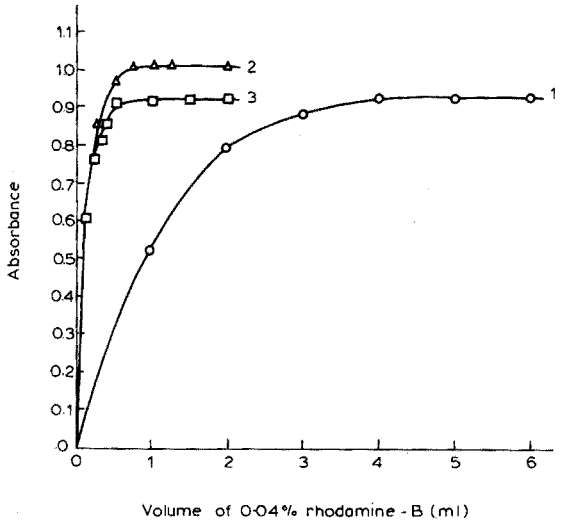


Fig. 1. Effect of reagent concentration on the colour intensity of the DEHPA, DOPPA or MOPPA–Rhodamine-B complex in 1,2-dichloroethane: (1) 30 μg DEHPA; (2) 40 μg DOPPA; (3) 25 μg MOPPA. (Absorbances are measured at 560–565 nm and corrected for blank values.)

Effect of reagent concentration

To known aliquots of DEHPA, DOPPA and MOPPA, varying amounts of the reagent were added and the total volumes were made up to 10.0 ml with 1,2-dichloroethane. The absorbances of the solutions were measured at 560–565 nm and corrected for blank absorbance from reagent. The results (Fig. 1) show that a higher reagent concentration was needed for maximal colour formation with DEHPA as compared to DOPPA and MOPPA. In all subsequent work, 5.0 ml of 0.04% rhodamine-B was used for DEHPA and 1.0 ml for the other two esters.

Calibration graph

The calibration graphs were linear for all three phosphate esters up to 4 p.p.m. in the final 1,2-dichloroethane solution. The molar absorptivities were calculated as 10^5 for DEHPA and DOPPA and $0.94 \cdot 10^5$ for MOPPA.

Effect of additives in the solvent

Neutral compounds like tri-n-butyl phosphate, di-n-butyl-butyl phosphonate and tri-n-octyl phosphine oxide are sometimes added to the organic phase as synergic agents or phase modifiers in the extraction process. These compounds were found to cause no interference in the determination of DEHPA, DOPPA and MOPPA when present in 200-fold exces.

Separation and determination of DEHPA from sulphate solution

A synthetic sulphate solution simulating a uranium ore leach liquor with the following composition (g l^{-1}) was prepared: Fe(III) 3; Al(III) 3; Mn(II) 2.5; Cu(II) 1.0 ; Ni(II) 1.0; SO_4^{2-} 30. To 10.0-ml aliquots of this solution, 0–100 μg of DEHPA was added and dispersed well. It was then extracted into 10.0 ml of 1,2-

dichloroethane and the DEHPA was estimated by the procedure given above. The recovery of added DEHPA was 95–100%.

Separation and determination of DEHPA and DOPPA from phosphoric acid

To 10.0-ml aliquots of wet-process phosphoric acid (6 *M*), 10–50 μg of these esters were added and the recovery and determination were carried out as described above. The experimentally determined values were within ±1 μg of the added amounts.

During the recovery of the phosphate esters, traces of phosphoric acid which extracted into the 1,2-dichloroethane phase, interfered seriously with the spectrophotometric determination, giving erratic absorbance readings. Washing of the extract with dilute (0.2 *M*) phosphoric acid eliminated this trouble.

Recovery and determination of MOPPA from phosphoric acid

It was found that the recovery of MOPPA from phosphoric acid was not quantitative when 1,2-dichloroethane was used for extraction. Different solvents were then tried for this purpose. In each case 50 μg of MOPPA was added to 10.0 ml of 6 *M* phosphoric acid and extracted into 40 ml of solvent. The organic layer was evaporated after separation and the residue dissolved in 10.0 ml of 1,2-dichloroethane. The MOPPA content in this solution was determined by mixing a known aliquot with rhodamine-B reagent in 1,2-dichloroethane. The results in Table II show that chloroform is the most suitable solvent.

In subsequent experiments, three extractions with equal volumes of chloroform were carried out for recovery of MOPPA from phosphoric acid. Under these conditions the recovery of known amounts of the ester added to commercial phosphoric acid was better than 95%.

TABLE II

RECOVERY OF MOPPA FROM 6 *M* PHOSPHORIC ACID

Solvent	*MOPPA extracted (%)*
n-Heptane	13
Petroleum ether (70–80°C)	25
Methyl isobutyl ketone	75
1,2-Dichloroethane	82
Chloroform	98

DISCUSSION

The proposed method for the determination of DEHPA, DOPPA and MOPPA is based on the formation of coloured ion-pairs of the type RBH^+A^- in 1,2-dichloroethane when the colourless lactone form of the dye is mixed with the acids. In this respect the method is similar to that used by Palit and co-workers[8–10] who prepared acid-sensitive reagents in benzene with basic dyes. However, they observed that dyes of the rhodamine-B class were useless for this purpose[11]. This is not in line with the highly sensitive reaction obtained in the present work, when this

dye was used with DEHPA, DOPPA and MOPPA. The difference appears to be due to the solvent as well as the nature of the acids. For example, in benzene medium the reactions were less sensitive with all the three phosphate esters.

The extractive spectrophotometric method reported earlier for DEHPA[7] could be applied for the determination of traces of DOPPA and MOPPA from commercial phosphoric acid after a preliminary extraction into dichloroethane or chloroform. However, it was found that the overall sensitivity was lower ($\varepsilon = 75{,}000$ instead of 10^5) and the absorbance of the blank higher in that method. Moreover, the present method eliminates one extraction step.

The empirical composition of the coloured complex formed between the phosphate esters and the dye was established by the molar ratio method and Job's continuous variations method in isomolar solutions, to be RB·HA.

SUMMARY

A spectrophotometric method for the determination of di(2-ethylhexyl), di(4-octylphenyl) and mono(4-octylphenyl) phosphoric acids is described. The method was applied for their determination in aqueous raffinates from uranium extraction processes where they are employed as extractants. The reagent was a dichloroethane extract of rhodamine-B prepared from an aqueous phosphate buffer of pH 10. The organophosphoric acids were separated from the aqueous solution by extraction with dichloroethane and this organic extract was mixed with rhodamine-B reagent. The intense colour produced had maximal absorbance at 560/565 nm. The molar absorptivity was about 10^5 in all cases.

REFERENCES

1 C. A. Blake, K. B. Brown and C. F. Coleman, *U.S. Atomic Energy Comm., Report ORNL-1903*, 1955.
2 K. B. Brown, C. F. Coleman, D. J. Crouse, C. A. Blake and A. D. Ryon, *Proc. 2nd U.N. Int. Conf. Peaceful Uses At. Energy, Geneva*, 3 (1958) 478.
3 F. J. Hurst, D. J. Crouse and K. B. Brown, *U.S. At. Energy Comm., Report ORNL-TM 2522*, 1969.
4 T. K. S. Murthy, V. N. Pai and R. A. Nagle, *Proc. of a Symposium on the Recovery of Uranium, Sao Paulo, 17–20 August, 1970*, I.A.E.A., Vienna, 1971, p. 341.
5 Oak Ridge National Laboratory, *Annual Progress Report, ORNL-4883*, 1973, p. 56.
6 A. W. Ashbrook, *Anal. Chim. Acta*, 58 (1972) 123.
7 K. Bhattacharyya and T. K. S. Murthy, *Anal. Chim. Acta*, 71 (1974) 107.
8 S. R. Palit, *Chem. Ind. (London)*, (1960) 1531.
9 S. R. Palit, *Anal. Chem.*, 33 (1961) 1441.
10 P. Ghosh and S. R. Palit, *J. Indian Chem. Soc.*, 41 (1964) 567.
11 P. Ghosh and S. R. Palit, *J. Indian Chem. Soc.*, 41 (1964) 570.

Analytica Chimica Acta, 76 (1975) 97–106

THE ANALYTICAL CONCENTRATION OF HUMIC SUBSTANCES FROM NATURAL WATERS

R. F. C. MANTOURA and J. P. RILEY

Department of Oceanography, University of Liverpool, P.O. Box 147, Liverpool, L 69 3 BX (England)

(Received 12th November 1974)

There is an increasing interest in the dissolved humic materials in natural waters, including sea water, because of their probable roles as chelating agents for trace metals and as growth promoters for phytoplankton, and also because of their involvement in the organic geochemical cycle. These materials are extremely complex chemically, and their composition varies according to the origin and nature of the water in which they are found. Thus, those found in river and lake waters consist of mixtures of polyelectrolytic macromolecules such as humic, fulvic and hymatomelanic acids derived from, or modified from, those present in the soils of the watershed area. More than 90% of the dissolved organic material present in sea water is present as complex polymeric fluorescent material which has been termed "Gelbstoff"[1], or "water humus"[2]. The origin of this latter material is uncertain. It seems unlikely that that which is present in offshore waters contains a significant proportion of terrestrial humic or fulvic acids since these are known to precipitate as their calcium and magnesium derivatives when they enter the sea. It is thought that it may result from the decomposition of dead organisms, and/or from reactions involving extracellular metabolites of algae[3].

The analytical separation of the humic compounds from natural waters has usually been carried out by methods involving adsorption. Many workers have favoured inorganic adsorbents such as activated carbon[4,5], alumina[4,6,7], silica gel[4,7,8], magnesia[4,7] and calcium carbonate[4,9]. However, recoveries with these tend to be low, partly because of the inefficiency of the adsorption stage, but also because of the difficulty of eluting the humic acids from the stronger adsorbents. Furthermore, it has been reported[4] that chemical alteration of these compounds may occur on activated carbon. It has been claimed that higher recoveries can be achieved by the use of organic adsorbents, such as nylon[10,11], polyamide (nylon) powder[10] and microporous polystyrene beads (Amberlite XAD-1 or XAD-2[12,13]). Because of this, a detailed study of the adsorptive properties of these materials has been made as a preliminary to an examination of the humic materials present in natural waters. Optimal conditions have been evaluated for the efficient recovery of both humic and fulvic acids with adsorbent columns.

Initial experiments were directed towards a comparison of the above organic adsorbents, the following criteria being borne in mind: (1) the need for high adsorption and elution efficiencies; (2) the necessity for preventing denaturization of the adsorbate; (3) the practical need for high flow rates consequent on the low concentration of humic complexes present in natural waters.

Attempts were made to use the method described by Sieburth and Jensen[10] for the recovery of Gelbstoff from coastal waters. In these, 1-l aliquots of water spiked with 3 mg of humic acid were allowed to flow through columns packed with nylon stockings which had been treated to remove dye[11]. It was found that although 71% of the humic acid was adsorbed at pH 3.5, the overall recovery of humic acid on elution with 0.2 *M* sodium hydroxide was only 54%. The overall recovery was not significantly improved when the pH was lowered to 2.2 to further repress ionization of the carboxylic acid groups and thus increase the hydrophobic character of the humic molecule. This contrasts with the overall yield of 68% claimed by Sieburth and Jensen[10] using the same conditions. It seems likely that this discrepancy arises as a result of failure of the latter workers to free the nylon from dye with the result that the latter was eluted along with their Gelbstoff and interfered with the subsequent photometric determination. It seems likely that the failure to desorb the humic acid completely may be explained by the reaction of its quinoid groups with the free amino groups present in the nylon[14]. Because of the poor recoveries which were obtained with this polyamide, studies were directed towards the use of Amberlite XAD-2, which had already been shown to have potentialities[12]. The objective of this investigation was not only to determine the optimum conditions for the use of the resin but also to carry out a fundamental study of the physical chemistry of the adsorption process.

EXPERIMENTAL

Materials

Amberlite XAD-2 is a macroreticular resin based on polystyrene; it has a porosity of 0.43, an average specific surface area of 310 $m^2\ g^{-1}$ and a mean pore diameter of 9 nm (ref. 15). Before use, the resin (20–50 mesh) was washed free from chloride with distilled water and extracted with acetone in a soxhlet apparatus for 2 h. After washing with water, the resin was packed into a column of the appropriate dimensions, washed with ten bed volumes of 0.5 *M* sodium hydroxide and finally with water. If it was necessary to store the resin for more than a few hours it was treated with 0.02% sodium azide solution.

Buffers

TRIS buffer (0.5 M). The buffer was prepared by dissolving 60.6 g of tris-(hydroxymethyl)aminomethane in about 800 ml of water and titrating it with 5 *M* hydrochloric acid to pH 7.0. The solution was finally diluted to 1 l.

Phthalate buffer (0.1 M) was prepared by dissolving 20.4 g of potassium hydrogenphthalate in water and diluting to 1 l with water.

Humic and fulvic acids

Humic and fulvic acids were extracted from "garden" peat by means of the alkali extraction procedure described by Schnitzer and Khan[16], with the exception that desalting was carried out by exhaustive dialysis. The yields, expressed on a dry weight basis (dried in vacuum over phosphorus pentoxide), were 15.4% for humic acid and 5.2% for fulvic acid. Elemental analysis (on an ash-free basis) showed for humic acid 54.4% C, 3.6% H, 1.9% N and 40.1% O; and for fulvic acid 49.4% C, 1.5%

H, 2.4% N and 46.7% O. A portion of the humic acid was fractionated by fractional precipitation at 21°C with ammonium sulphate at pH 7 (ref. 17) in order to obtain 14 fractions having narrow ranges of polydispersity.

Solution of algal decay products

Two species of phytoplankton (*Hemiselmis rufescens* and *Dunaliella primolecta*) were grown in culture. After the conclusion of the growth phase the cells were allowed to decay in the dark for *ca.* 9 months. The dead cells were removed by filtration on a glass fibre filter (Whatman GF/C) and the filtrate was retained for use in adsorption experiments.

STUDY OF ADSORPTION OF HUMIC MATERIALS ON AMBERLITE XAD-2

In order to obtain an insight into the physical chemistry of the adsorption process, the isotherms for the adsorption of humic acid were determined. Solutions of humic acid (number average molecular weight 21,100±300) ranging in concentration from 0.1 to 22 μM were acidified to pH 2.20±0.05 with hydrochloric acid. Aliquots (100 ml) of these solutions were shaken at 21±1°C with a known weight (*ca.* 0.4 g) of the purified resin for 24 h, which kinetic experiments had indicated was more than sufficient for the attainment of equilibrium. The absorbances of the initial and final solutions were then measured at 250 nm and 460 nm in order to estimate the extent of adsorption of humic acid by the resin. The results from these experiments were then fitted to the Langmuir isotherm represented by the equation

$$\frac{1}{\Gamma_{HA}} = \left(\frac{1}{K\Gamma_\infty}\right)\frac{1}{a_{HA}} + \frac{1}{\Gamma_\infty}$$

where Γ_{HA} is the adsorption density of the humic acid on the resin (moles g^{-1}), Γ_∞ is the theoretical adsorptive capacity at saturation, K is the equilibrium adsorption constant ($mole^{-1}$ l), and a_{HA} is the activity of the humic acid solution. In fitting the data to the equation it was necessary to assume that a_{HA} was identical with the molar concentration (c_{HA}); this is probably a reasonable approximation in view of the extreme dilution of the humic acid and the low ionic strength of the medium ($\mu = <0.001$). In agreement with the Langmuir isotherm model, a linear relationship was observed when Γ_{HA}^{-1} was plotted against c_{HA}^{-1} (Fig. 1). The equilibrium adsorption constant, K, deduced from the slope of the plot was found to be $6.0 \cdot 10^4$ $mole^{-1}$ l. From the ordinate intercept a value of $4.5 \cdot 10^{-6}$ mole g^{-1} was calculated for the theoretical adsorptive capacity.

In order to obtain data on the thermodynamics of the adsorption process, the adsorption experiments were repeated at temperatures of 33°C, 42°C, 51°C and 64°C. The standard enthalpy of adsorption, ΔH_a^0 was evaluated from the expression[15]

$$\log K = \frac{-\Delta H_a^0}{R}\left(\frac{1}{T}\right) + \text{constant}$$

by plotting log K as a function of $1/T$ where T is the absolute temperature and R is the gas constant. This gave a linear relationship (Fig. 2) from the slope of which ΔH_a^0 was calculated to be -5.4 kJ $mole^{-1}$. The standard free energy of adsorption ΔG_a^0 was evaluated from the expression

$$\Delta G_a^0 = -RT \ln 55.5\ K$$

and was found to be -36.4 kJ mole^{-1} at 21°C. The standard entropy of adsorption ΔS_a^0 calculated from the second law of thermodynamics

$$\Delta S_a^0 = (\Delta H_a^0 - \Delta G_a^0)/T$$

amounted to 103 J mol^{-1} K^{-1} at 21°C.

Adsorption isotherms were also determined at two ionic strengths for fulvic acid and some humic acid fractions which had been isolated by fractional precipitation with ammonium sulphate. These experiments showed that there was a

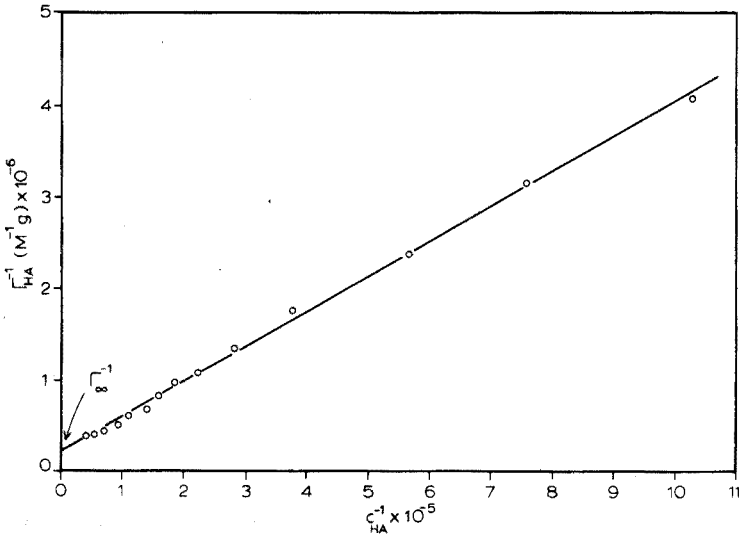


Fig. 1. Langmuir plot for adsorption of humic acid on Amberlite XAD-2 (at pH 2.2; 21 C; μ *ca.* 0.001).

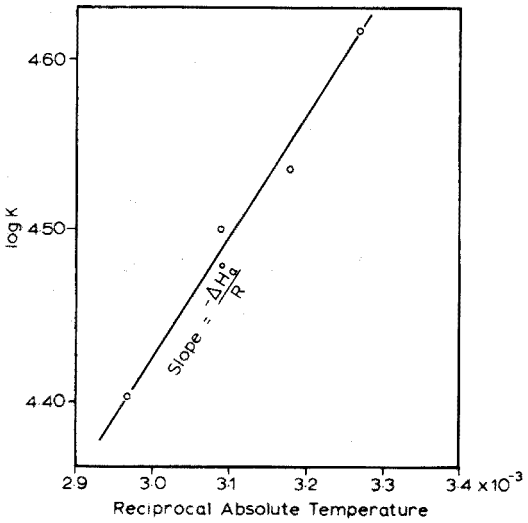


Fig. 2. Plot showing the temperature dependency of the equilibrium adsorption constant for humic acid on Amberlite XAD-2.

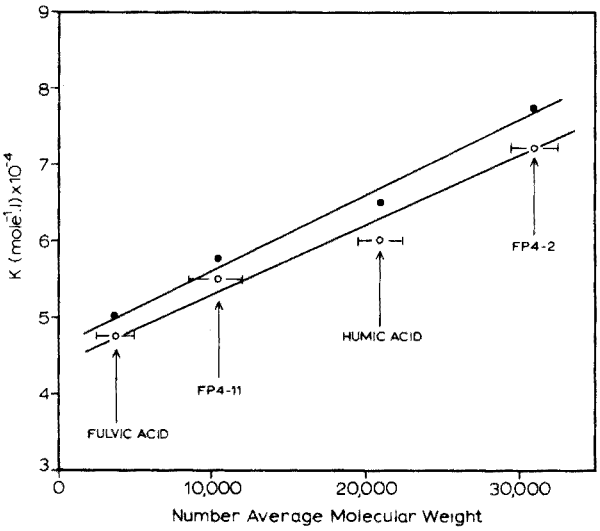


Fig. 3. Relationship between equilibrium adsorption constant and number average molecular weight of fulvic and humic acids and two humic acid fractions (F.P. 4-2 and F.P. 4-11): (●) $\mu = 0.67$ (NaCl); (○) $\mu = 0$.

roughly linear relationship between the equilibrium adsorption constant and the number average molecular weight determined by osmometry (Fig. 3).

STUDY OF THE USE OF AMBERLITE XAD-2 IN COLUMN OPERATION

Several variables have to be taken into account in the extension of the adsorption to column operation with a view to concentrating humic acids from large volumes of natural waters. Parameters which must be taken into account during adsorption include pH, flow rate, column geometry, and the degree of saturation of the resin with the adsorbate. In the subsequent desorption stage it is only necessary to consider the nature and concentration of the eluant and its flow rate. The effects of variation of these parameters have been investigated.

Adsorption

The influence of pH on the uptake of humic acid by Amberlite XAD-2 was studied by acidifying 1-l aliquots of solutions of ionic strengths 0.01 and 0.67 (NaCl) containing 10 mg of humic or fulvic acid per litre with hydrochloric acid to a series of pH values in the range 1.5–3.5. These solutions were allowed to flow through a bed of the resin 10.0 cm $\times$ 0.8 cm^2 at a rate of 40 bed volumes per h. The absorbances of the initial solutions and the percolates were then measured at 250 nm in order to assess the efficiency of adsorption. The results of these experiments (Fig. 4) showed that efficient recoveries of both humic and fulvic acids could be achieved at pH values of 2.2 and below. Since the risk of denaturization increases with acidity and as the adsorption efficiency only increases by *ca.* 2% when the pH is lowered from 2.2 to 1.5, it was decided to employ a pH of 2.20 ± 0.05 in all subsequent work. The efficiency of adsorption was found to be somewhat enhanced by increase in ionic strength of the medium (Fig. 4).

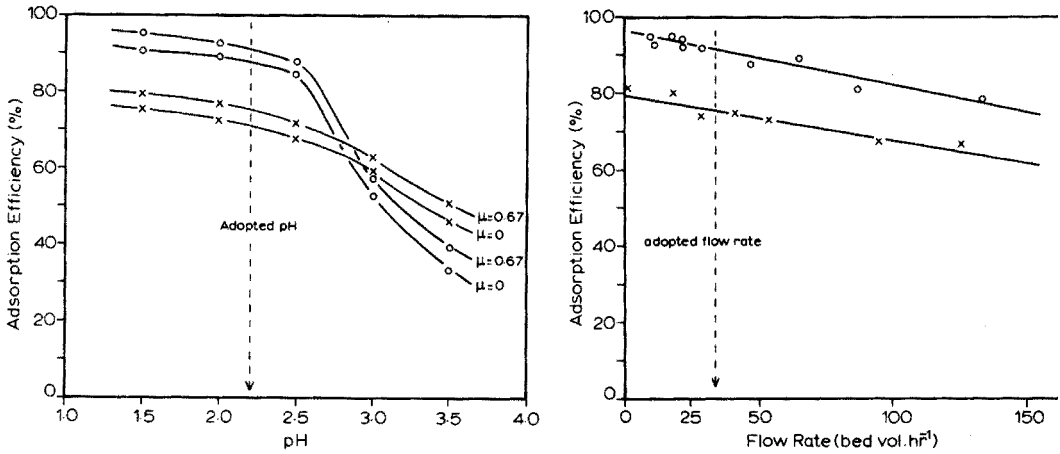


Fig. 4. Efficiency of adsorption of humic and fulvic acids on Amberlite XAD-2 as a function of pH: (○) humic acid; (×) fulvic acid.

Fig. 5. Efficiency of adsorption of humic and fulvic acids at pH 2.2 on Amberlite XAD-2 as a function of flow rate (μ *ca.* 0.005): (○) humic acid; (×) fulvic acid.

The effect of flow rate on retention of humic and fulvic acids at pH 2.2 was determined under similar conditions to those described above, except that the rate of flow of the sample was controlled in the range 5–130 bed volumes per hour by means of a peristaltic pump. These experiments showed (see Fig. 5) that the percentage adsorption of both classes of compound decreased approximately linearly as the flow rate increased. Acceptable uptakes (>92% for humic acid and >75% for fulvic acid) could be achieved with flow rates of less than 35 bed volumes per hour. This flow rate was adopted in all further work. An investigation was also made of the effect of variation of the column height to cross-section ratio on the adsorption efficiency for the same volume (8 cm^3) of resin. This showed that the maximum uptake occurred when the ratio was *ca.* 14:1; at ratios greater than this there was a gradual decline in adsorption efficiency. The cause of this is uncertain, but a similar phenomenon has been observed for adsorption of polychlorinated biphenyls on the same resin[19]. Finally, experiments were carried out to study the effect of variation of the degree of saturation of the resin on the uptake of humic acid. It was found that it was only feasible to employ about 20% of the theoretical adsorptive capacity of the resin, corresponding to *ca.* 20 mg g^{-1}. At greater loadings than this significant leakage (>5%) occurred.

Desorption

The elution of adsorbed humic and fulvic acids from 12 cm × 0.8 cm^2 columns of Amberlite XAD-2 was studied with a variety of both aqueous and organic media. It was found that with aqueous media the pH was the principal parameter controlling the efficiency of desorption (see Table I). The maximum recovery was achieved by the use of 0.2 *M* sodium hydroxide, 40 ml of which at a flow rate of 3 ml min^{-1} eluted *ca.* 95% of the adsorbed humic and fulvic acids. Slightly greater recoveries (*ca.* 98%) could be achieved if the eluant was allowed to stand in the

TABLE I

ELUTION OF ADSORBED HUMIC AND FULVIC ACIDS FROM AMBERLITE XAD-2

Eluant	*Molarity*	*pH*	*Percentage desorption*	
			Humic acid	*Fulvic acid*
Potassium hydrogenphthalate	0.1	4.0	57	63
Tris buffer	0.4	7.0	71	75
Ammonium hydroxide	1.0	10.6	83	89
Sodium hydroxide	0.2	13.3	95	96
Methanol	—	—	35	38
Ethanol	—	—	21	27
Acetone	—	—	33	35
Methanol–ammonia[a]	1.0	—	91	95

[a] 1:1 (vol) mixture of methanol and 2 *M* ammonium hydroxide.

column overnight. Because of the low solubility of humic compounds in organic solvents, desorption with such eluants was incomplete. However, a comparatively good recovery (*ca.* 91%) was obtained by the use of an aqueous methanol eluant which was 1 *M* with respect to ammonia. This reagent has the advantage that it can be readily removed with a rotary evaporator leaving the humic and fulvic acids as the ammonium salts relatively free from inorganic salts.

DISCUSSION

The successful use of adsorption for the concentration of naturally occurring macromolecules from the aquatic environment requires an understanding of the basic physical chemistry of the process. Insight into the mechanism of adsorption can be most readily acquired from an examination of the adsorption isotherm. The adsorption of humic substances at dilutions similar to those in natural waters can be treated in a manner similar to that used in the study of specific adsorption of a gas from a mixture of gases, provided that the adsorption is restricted to a mono-layer[18]. It was found that the adsorption of the humic compounds closely fitted a Langmuir isotherm. The low value of the enthalpy of adsorption ($\Delta H_a^0 = -5.4$ kJ mol^{-1}) is evidence that the interaction between the adsorbed humic molecule and the Amberlite involves primarily hydrophobic bonding. It is noteworthy that chemisorptive and ion-exchange bonding, typical of adsorption of such compounds on inorganic adsorbents such as alumina and activated carbon, have ΔH_a^0 values in excess[14] of 40 kJ mol^{-1}, and this offers a probable explanation for the difficulty which is experienced in desorbing organic compounds from these media[4–7]. Thus, 85% of the Gibbs free energy of adsorption ΔG_a^0 (the driving force of the adsorption) is provided by the favourable entropy change ΔS_a^0 of +103 J mol^{-1} K^{-1}. This large gain in entropy is associated with the displacement of the polar water molecules from the non-polar surface of the resin, as well as with the breakdown of the cluster structure of the neighbouring water molecules. Similar behaviour has been observed[15] for the adsorption of sodium anthraquinone sulphonate on Amberlite XAD-2.

In any process involving humic acids, there is always a possibility that chemical or biological alteration may occur. However, experiments using gel filtration on Sephadex G-75 Superfine showed that the process of acidification to pH 2.2, adsorption onto Amberlite XAD-2 and elution with 0.2 *M* sodium hydroxide does not denaturize the overall molecular structure of the humic acid. There is an approximately linear relationship between the equilibrium adsorption constant and the number average molecular weight (Fig. 3). This is in keeping with Traube's Rule which attributes the increase in strength of adsorption to the increase in hydrophobicity as the molecular weight rises. With environmental samples containing polydisperse humic acids, this may lead to a slight selectivity of uptake in favour of the compounds of higher molecular weight. However, under the optimized conditions developed for column operation, this effect is unlikely to change significantly the molecular weight distribution of the final humic acid concentrate. This phenomenon can be turned to advantage during the elution stage if the desorption is carried out with a series of eluants having progressively greater pH values (Fig. 6). Under these conditions, it is possible to achieve fractionation of the humic and fulvic acids on a molecular weight basis, the smallest molecules being eluted first (see Fig. 6). If such fractionation is not required, quantitative elution of adsorbed humic compounds can be most readily achieved by using 4 bed volumes of 0.2 *M* sodium hydroxide.

The efficiency of adsorption of humic and fulvic acids increases by 11 and 7% respectively as the ionic strength of the sample is increased from *ca.* 0 to 0.67. This enhanced adsorption is probably the result of the concomitant decrease in dipole and coulombic repulsion of the hydrophilic groups of adjacent adsorbed humic molecules. This behaviour is typical of amphipathic and surface-active molecules[18].

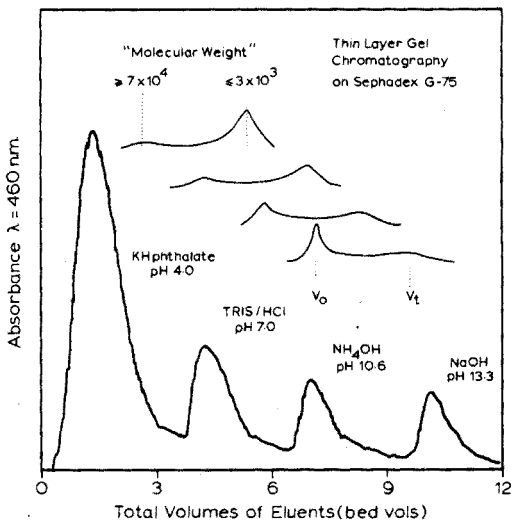


Fig. 6. Fractional desorption of a mixture of humic and fulvic acids from Amberlite XAD-2. The inset illustrates the molecular weight dispersion of the fractions as demonstrated by thin layer gel filtration chromatography.

TABLE II

ADSORPTION AND RECOVERY OF AQUEOUS HUMICS AND PRODUCTS OF ALGAL DECAY

Sample	*No. of samples*	*% Adsorption*	*Eluant*	*% Recovery*[a]
R. Tamar (Devon)	10	87±2	NaOH	82±3
R. Mersey (Lancashire)	3	85±3	NaOH	81±4
Lake Bala (Merionethshire)	6	89±2	NaOH	87±4
Lake Celyn (Merionethshire)	8	90±1	NaOH	87±4
Hemiselmis sp.		92	NH_3–CH_3OH	85
Dunaliella sp.		86	NH_3–CH_3OH	82

[a] Mean result and standard deviation.

The column adsorption procedure has been applied to the examination of samples of river and lake waters as well as to waters in which phytoplanktonic algae had been allowed to decay. The extent of adsorption of the humic acids at pH 2.2 was determined by difference from the decrease in absorbance at 460 nm which occurred on passage of the sample through the column. The results of these experiments are summarized in Table II. They show that overall recoveries of 82–87% of light absorbing species could be achieved. Since light absorbing species other than humic acids will undoubtedly be present in the samples of natural waters, the yields of humic and fulvic acids will almost certainly be greater than these figures imply.

SUMMARY

The thermodynamics of the adsorption of humic and fulvic acids on the macroreticular polystyrene resin Amberlite XAD-2 have been investigated with a view to optimizing the conditions for its application to the analytical concentration of these compounds. Under the optimal conditions, recoveries of humic and fulvic acids of above 92 and 75%, respectively, were achieved. It has been shown that these compounds can be fractionated on a molecular weight basis during the desorption stage by serial elution at selected pH values.

REFERENCES

1 K. Kalle, *Ann. Hydrog.*, 66 (1938) 1.
2 B. A. Skopintsev, *Oceanology (USSR)*, 11 (1972) 775.
3 E. K. Duursma in J. P. Riley and G. Skirrow (Eds.), *Chemical Oceanography*, Academic Press, London, 1965.
4 L. M. Jeffrey and D. W. Hood, *J. Mar Res.*, 17 (1958) 247.
5 A. Momzikoff, *Cah. Biol. Mar.*, 10 (1969) 221.
6 J. R. Moed, *Limnol. Oceanogr.*, 16 (1971) 140.
7 P. M. Williams and A. Zirino, *Nature (London)*, 204 (1964) 462.
8 Y. V. Larianov and B. A. Skopintsev, *Biol. Vnutr Vodv; Inform. Byul.*, 15 (1972) 63; C.A. (1973) 151480t.
9 A. Otsuki and R. G. Wetzel, *Limnol. Oceanogr.*, 18 (1973) 490.
10 J. McN. Sieburth and A. Jensen, *J. Exp. Mar. Biol. Ecol.*, 2 (1968) 174.
11 L. W. Lewis Jr., M.Sc. Thesis, U.S. Navy, National Technical Information Service No. 745-172.

12 J. P. Riley and D. Taylor, *Anal. Chim. Acta*, 46 (1969) 307.
13 D. H. Steurmer and G. R. Harvey, *Nature (London)*, 250 (1974) 480.
14 L. R. Snyder, *Principles of adsorption Chromatography*, Arnold, London, 1968.
15 R. L. Gustafson and J. Paleos in S. F. Faust and J. V. Hunter (Eds.), *Organic compounds in aquatic environments*, Dekker, New York, 1971.
16 M. Schnitzer and S. U. Khan, *Humic substances in the environment*, Dekker, New York, 1972.
17 B. K. G. Theng, J. R. H. Wake and A. M. Posner, *Plant Soil*, 29 (1968) 305.
18 A. W. Adamson, *Physical Chemistry of Surfaces*, Interscience, New York, 2nd edn., p. 398.
19 G. R. Harvey, *Adsorption of Chlorinated Hydrocarbons from Sea Water by Cross Linked Polymers*, Woods Hole Oceanographic Institute, Technical Report No. WHOI-72-86, 1972.

Analytica Chimica Acta, 76 (1975) 107–112

PLASTICIZED OPEN-CELL POLYURETHANE FOAM AS A UNIVERSAL MATRIX FOR ORGANIC REAGENTS IN TRACE ELEMENT PRECONCENTRATION

PART III. COLLECTION OF COBALT TRACES ON 1-NITROSO-2-NAPHTHOL AND DIETHYLDITHIOCARBAMATE FOAMS

T. BRAUN and A. B. FARAG*

Institute of Inorganic and Analytical Chemistry, L. Eötvös University, P.O. Box 123, 1443 Budapest (Hungary)

(Received 10th October 1974)

Recent work in this laboratory has been concerned with the preparation of plasticized open-cell polyurethane foam immobilizing various organic reagents for the detection, semiquantitative determination, quantitative collection and preconcentration of several metal ions. The collection and preconcentration of silver[1] and mercury[2] on plasticized zinc dithizonate foam have already been described. The detection and semiquantitative determination of zinc and lead on dithizone foam, and of copper and cobalt on rubeanic acid and Amberlite LA-1 foams, respectively, have also been examined[3].

The present work is a continuation of the subject with the aim of preparing plasticized 1-nitroso-2-naphthol and diethyldithiocarbamate foams suitable for trace element preconcentration. As a model the collection and preconcentration of cobalt was investigated.

EXPERIMENTAL

Reagents and materials

All chemicals used were of analytical-reagent grade unless otherwise specified. Tri-n-butyl phosphate (TBP) was purified as described by Hamlin *et al.*[4]. Polyurethane foam, a polyether of open-cell type, was supplied by the North Hungarian Chemical Works, Sajóbábony, Hungary. 1-Nitroso-2-naphthol and diethylammonium diethyldithiocarbamate solutions were prepared by dissolving 0.5 g of the solid reagent in 50 ml of TBP or chloroform. Cobalt(II) chloride solutions were spiked with carrier-free ^{58}Co (Institute of Isotopes, Budapest). The universal buffer was prepared from a mixture of citric acid, boric acid, diethylbarbituric acid, potassium dihydrogenphosphate and various amounts of sodium hydroxide solution[5].

---

* Present address: National Research Centre, Dokki, Cairo, Egypt.

Instrumentation

For activity measurement a NaI(Tl) well-crystal and an energy-selective counting device (type NK-107/B, Gamma, Budapest) were employed.

Preparation of 1-nitroso-2-naphthol and diethyldithiocarbamate foams

1-Nitroso-2-naphthol and diethylammonium diethyldithiocarbamate foams were prepared by equilibration of the washed, dried foam cubes (5 mm edge) with the TBP solution of the reagent, the procedure being similar to that previously described[1,2].

Column preparation

The dried reagent foam (2 g) was packed in glass columns (15-mm diameter and 12-cm length) by the vacuum method[6].

COLLECTION OF TRACES OF COBALT(II) ON 1-NITROSO-2-NAPHTHOL FOAM

1-Nitroso-2-naphthol has been widely used in liquid–liquid extraction for the separation[7] of cobalt(II). It has been suggested[8] that this reagent first forms a complex with cobalt(II) which later decomposes to a cobalt(III) complex. In a recent publication, Rakovic and Glagolicova[9] separated radioactive cobalt by isotope exchange with stable cobalt(III)—1-nitroso-2-naphthol granules packed in a chromatographic column. They claimed that ^{60}Co can be separated in high yield (97%) when this column method is used at a flow-rate near 0.04 ml min^{-1}.

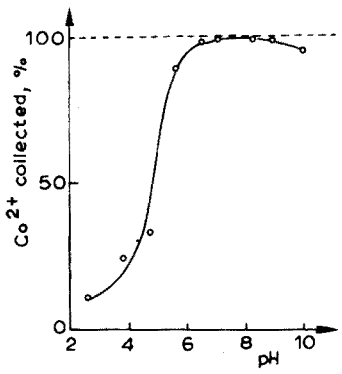
The possibility of using 1-nitroso-2-naphthol foam for the rapid and quantitative collection of traces of cobalt(II) was investigated and the effect of different factors was studied.

Effect of pH on the collection of cobalt(II)

In batch experiments, 10-ml aliquots of aqueous solution containing 0.1 μg of cobalt(II) were shaken with 0.1 g of TBP-plasticized 1-nitroso-2-naphthol foam at various pH values. The pH of the aqueous solution was adjusted by a universal buffer mixture[5]. The percentage of cobalt extracted by the reagent foam was plotted against the pH value (Fig. 1). As can be seen, cobalt is completely collected from aqueous solutions having pH values ranging between 6.6 and 9.0. At lower pH values, the percentage collection of cobalt sharply decreased, while at higher pH values the percentage collection of cobalt(II) slightly decreased (*cf.* Fig. 1).

Effect of plasticizer on the collection of cobalt(II)

The collection rate of cobalt(II) with 1-nitroso-2-naphthol foam, which had been previously loaded with the reagent solution in TBP or chloroform, was determined with an aqueous solution of cobalt at pH 6.8 (phosphate buffer) in batch experiments[1,2]. The curves of Fig. 2 represent the results obtained for the percentage collection of 0.1 μg of cobalt(II) from 10 ml of the aqueous solution with 0.1 g of plasticized or unplasticized reagent foam (curves a and b, respectively). It is clear from these curves that the collection rate of cobalt(II) with the plasticized foam material is better than that with the unplasticized one (chloroform). These results are in agreement with those previously obtained with silver and mercury on dithizone foam[1,2].



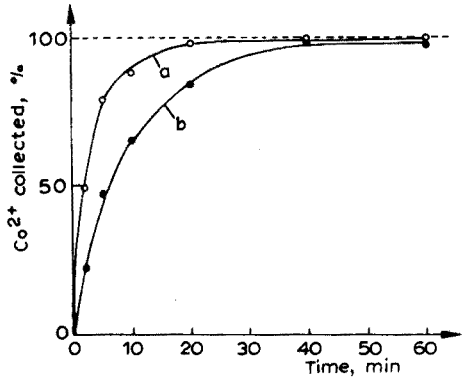


Fig. 1. Effect of pH on the collection of cobalt(II) with TBP-plasticized 1-nitroso-2-naphthol foam.

Fig. 2. Effect of plasticizer on the collection rate of cobalt(II). (a) TBP; (b) $CHCl_3$.

TABLE I

COLLECTION OF COBALT(II) ON COLUMNS PACKED WITH TBP-PLASTICIZED 1-NITROSO-2-NAPHTHOL FOAM

(Flow-rate, 5–6 ml cm^{-2} min^{-1}.)

Amount of Co taken (μg)	*Average[a] Co retained on foam ($\bar{X}$, %)*	*Relative accuracy of the mean (%)*	*Standard deviation (s)*	*Confidence limit[b] ($\bar{X} \pm ts/\sqrt{n}$) t 0.95*
Carrier-free	98.4	−1.6	0.551	98.4±0.9
1.0	98.3	−1.7	0.753	98.3±1.2
10.0	98.2	−1.8	1.384	98.2±2.2
100.0	99.0	−1.0	0.751	99.0±1.2
1000.0	98.7	−1.3	0.785	98.7±1.2

[a] Average of 4 determinations.
[b] See reference 10.

Collection of cobalt(II) on columns packed with TBP-plasticized 1-nitroso-2-naphthol foam

Cobalt concentrations ranging between 1 and 1000 μg were quantitatively collected from aqueous solutions (pH 6.8) on 1-nitroso-2-naphthol foam columns at a flow-rate of 5–6 ml cm^{-2} min^{-1} (Table I). Also, carrier-free cobalt-58 was successfully collected on the proposed reagent foam columns (Table I).

COLLECTION OF TRACES OF COBALT ON DIETHYLAMMONIUM DIETHYLDITHIOCARBAMATE FOAM

Dithiocarbamates react with many metals to give strongly coloured chelate compounds[7]. Sodium diethyldithiocarbamate is extensively used for the separation of a large number of metal ions in liquid–liquid extraction systems. In recent publications[11–13], zinc diethyldithiocarbamate solution (chloroform) loaded on

polytetrafluoroethylene (Floroplast PF-4) has been used for the substoichiometric displacement of various metals in column operations.

In the present work, the preparation of diethyldithiocarbamate foam was first tried with the sodium salt, but the low solubility of this reagent salt in organic solvents did not allow the preparation of diethyldithiocarbamate foam with reasonable capacity. Attention was then directed towards the preparation of this reagent foam from diethylammonium diethyldithiocarbamate. This reagent is considered a useful alternative to the sodium salt because it is appreciably soluble in various organic solvents.

Polyurethane foam loaded with a 1% (w/v) solution of diethylammonium diethyldithiocarbamate in TBP was evaluated for the collection of traces of cobalt(II).

Effect of pH on the collection rate of cobalt(II)

In separate experiments, 10-ml aliquots of aqueous solution containing 0.1 μg of cobalt(II) were shaken with 0.1 g of the reagent foam at various pH values. The pH of the aqueous solution was adjusted with dilute perchloric acid solution because the use of the universal buffer mixture for the adjustment of the pH affected the retention

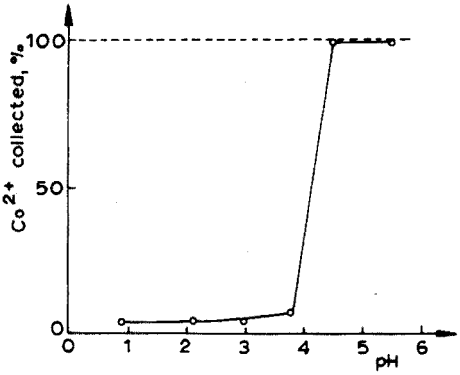


Fig. 3. Effect of pH on the collection of cobalt(II) with TBP-plasticized diethylammonium diethyldithiocarbamate foam.

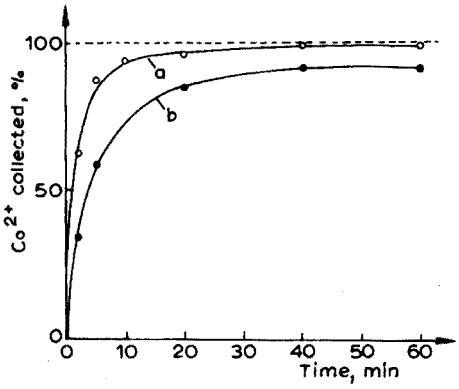


Fig. 4. Effect of plasticizer on the collection rate of cobalt(II). (a) TBP; (b) $CHCl_3$.

of cobalt(II) on the reagent foam. Figure 3 shows a plot of the percentage of cobalt collected on the foam *vs.* pH value. As is clear from this curve, the collection of cobalt is quite complete from aqueous solutions of pH value 4.5–5.5. Consequently, aqueous solutions of cobalt(II) adjusted to pH 5.5 were used in the subsequent investigations.

Effect of plasticizer on the collection rate of cobalt(II)

The percentage collection of 0.1 μg of cobalt(II) from 10 ml of aqueous solution (pH 5.5) on 0.1 g of diethylammonium diethyldithiocarbamate foam (plasticized with TBP or unplasticized) was determined as a function of shaking time. The effect of plasticizer on the collection rate of cobalt(II) is obvious from the curves of Fig. 4. The collection rate of cobalt(II) on the proposed reagent foam is enhanced by the plasticizer.

Collection of cobalt(II) on columns packed with TBP-plasticized diethylammonium diethyldithiocarbamate foam

The analytical applicability of the proposed TBP-plasticized diethylammonium diethyldithiocarbamate foam was tested by using the reagent foam in column experiments for the collection of various concentrations of cobalt(II). Table II shows the results obtained for short columns of the reagent foam at a flow-rate of 5–6 ml cm^{-2} min^{-1}.

TABLE II

COLLECTION OF COBALT(II) ON COLUMNS PACKED WITH TBP-PLASTICIZED DIETHYLAMMONIUM DIETHYLDITHIOCARBAMATE FOAM

(Flow-rate, 5–6 ml cm^{-2} min^{-1}.)

Amount of Co taken (μg)	*Average[a] Co retained on foam ($\bar{X}$, %)*	*Relative accuracy of the mean (%)*	*Standard deviation (s)*	*Confidence limit[b] ($\bar{X} \pm ts/\sqrt{n}$) t 0.95*
Carrier-free	97.7	−2.3	1.544	97.7±2.5
1.0	98.6	−1.4	0.532	98.6±0.8
10.0	99.6	−0.4	0.250	99.6±0.4
100.0	99.3	−0.7	0.655	99.3±1.0
1000.0	99.4	−0.6	0.512	99.4±0.8

[a] Average of 4 determinations

[b] See reference 10.

SUMMARY

The preparation of 1-nitroso-2-naphthol and diethylammonium diethyldithiocarbamate foams is described. The effect of pH, plasticizer and shaking time on the collection efficiency of cobalt(II) on these reagent foams has been investigated. The collection of various concentrations of cobalt(II) on the proposed reagent foams packed in columns is quantitative at flow-rates of 5–6 ml cm^{-2} min^{-1}.

REFERENCES

1 T. Braun and A. B. Farag, *Anal. Chim. Acta*, 69 (1974) 85.
2 T. Braun and A. B. Farag, *Anal. Chim. Acta*, 71 (1974) 133.
3 T. Braun and A. B. Farag, *Anal. Chim. Acta*, 73 (1974) 301.
4 A. G. Hamlin, B. J. Roberts, W. Loughlin and S. G. Walker, *Anal. Chem.*, 33 (1961) 1547.
5 A. I. Vogel, *Quantitative Inorganic Analysis*, Longmans, London, 3rd edn., 1961.
6 T. Braun and A. B. Farag, *Anal. Chim. Acta*, 61 (1972) 265.
7 A. K. De, S. M. Khopkar and R. A. Chalmers, *Solvent Extraction of Metals*, Van Nostrand–Reinhold, 1970.
8 I. M. Kolthoff and E. Jacobsen, *J. Amer. Chem. Soc.*, 79 (1957) 3677.
9 M. Rakovic and A. Glagolicová, *Radiochem. Radioanal. Lett.*, 15 (1973) 387.
10 *Anal. Chem.*, 45 (1973) 630.
11 Yu. V. Yakovlev and O. V. Stepanets, *Zh. Anal. Khim.*, 25 (1970) 578.
12 O. V. Stepanets, Yu. V. Yakovlev and I. P. Alimarin, *Zh. Anal. Khim.*, 25 (1970) 1906.
13 I. P. Alimarin, Yu. V. Yakovlev and O. V. Stepanets, *J. Radioanal. Chem.*, 11 (1972) 209.

Analytica Chimica Acta, 76 (1975) 113–119

A RAPID SOLVENT EXTRACTION METHOD FOR THE DETERMINATION OF RADIO-IODINE IN SEA WATER

W. W. FLYNN

Australian Atomic Energy Commission Research Establishment, Lucas Heights, N.S.W. 2232 (Australia)

(Received 21st October 1974)

Iodine isotopes are produced during nuclear fission and the routine monitoring of sea water for radio-iodine is necessary where reactor effluent is discharged to coastal waters. Various separation methods have been proposed for the determination of iodine isotopes in aqueous solutions, but very few methods have been published for the determination of radio-iodine in sea water.

One of the commonest methods for the assay of individual iodine isotopes is γ-ray spectrometry, because it does not usually require a prior separation procedure. However, environmental samples contain very low concentrations of radio-iodine and accurate analysis can be difficult, owing to interference from other radionuclides and the low counting efficiency of γ-ray spectrometers. The World Health Organisation (WHO)[1] gives the detection limit for iodine-131 in liquids by γ-ray spectrometry as $2.0 \cdot 10^{-3}$ μCi l^{-1} (depending on shielding, crystal size, etc.). As the maximum permissible levels for radio-iodine in estuarine waters (derived from figures set by the International Commission for Radiological Protection (ICRP)[2]), are lower than this detection limit for γ-ray spectrometry, other methods must be considered.

Yerric and Ross[3] used toluene in an extraction procedure to determine iodine-125 and -129 in biological samples. The high colour of the final toluene–iodine solution was eliminated by adding 2-methyl-1-butene and irradiating with u.v. light, to cause the iodine to add to the olefinic double bond and form a colourless compound suitable for counting by liquid scintillation. They gave no decontamination factors for other radionuclides and very few details concerning various aspects of the method.

In a compendium of radiochemical procedures for the determination of fission products in sea water, Lai and Goya[4] determined iodine-131 by extracting several times with carbon tetrachloride, before finally precipitating with carrier as silver iodide for counting and recovery. No decontamination factors were given and recovery of iodine was only 90% with a carrier recovery of 70%.

Loveridge and Gordon[5] used a similar extraction method for the determination of radio-iodine in Harwell effluent, but employed toluene for the final extraction. The solution was decolorized with 2-methyl-1-butene before liquid scintillation counting. Decontamination factors for a number of nuclides were greater than 10^5 and carrier recovery, determined colorimetrically, was generally higher than 80%. Counting efficiencies were: iodine-131, 89%; iodine-129, 72%, and iodine-126, 42%.

The American Society for Testing Materials (ASTM) lists three procedures[6] for the determination of radioactive iodine in industrial water and waste water containing more than 100 pCi l^{-1}. These are ion exchange, distillation of iodine, and solvent extraction with carbon tetrachloride. In all cases, a final precipitation of carrier as silver iodide for counting and determination of recovery is recommended. These methods are inadequate for determination of the low levels of radio-iodine usually found in sea water.

In developing a method for routine use, the main factors to be considered are: the complexity of the method, the time required for completion, the sensitivity required, and the amount of sample necessary to detect the low quantities of radio-iodine in sea water. A survey of the various methods showed that a combination of the toluene extraction method of Yerric and Ross[3], with the alkaline oxidation procedure of Loveridge and Gordon[5] might give promising results.

With the method developed here, good recoveries could be obtained by one extraction with 100 ml of toluene from 1.0 l of sea water. A final extraction into a small volume of toluene was necessary for counting and colorimetric determination of carrier recovery.

The high efficiency of the liquid scintillation spectrometer allowed greater sensitivity than the maximal permissible levels for estuarine waters set by the ICRP for the various iodine isotopes. The method is easy to use and rapid; six samples can be processed for counting in about 4 h. Interference from many common ions is negligible and decontamination factors are satisfactory for most radionuclides expected to interfere. Recovery of carrier averaged 83%.

EXPERIMENTAL

Apparatus

An Ansitron liquid scintillation spectrometer with a background of 15 c.p.m., a Hilger-Spekker absorptiometer, and a M.S.E. centrifuge were used.

Reagents

^{131}I standard solution. The isotope was obtained (Isotope Division, AAEC) as an aqueous solution of iodide in sodium thiosulphate. This was diluted to give a working solution of about 5000 dis. min^{-1} ml^{-1}.

Iodide carrier stock solution (10 mg I^- ml^{-1}). Dissolve 13 g of A.R. grade potassium iodide in demineralized water containing a few milligrams of sodium hydrogen-carbonate, and dilute to 1.0 l.

Standardize by pipetting 5.0 ml into a 100-ml beaker, add 50 ml of demineralized water and 1.0 ml of 6 *M* nitric acid, and then heat to near boiling. Add 5 ml of 0.1 *M* silver nitrate solution dropwise with continuous stirring, digest for 1 min and filter quantitatively onto a weighed sintered glass crucible. Wash three times with 5 ml of demineralized water and three times with 5 ml of alcohol. Dry at 110°C for 10 min, cool and weigh as silver iodide.

Iodide carrier working solution (about 2.0 mg I^- ml^{-1}). Dilute 50 ml of standardized stock solution to 250 ml.

2-Methyl-1-butene (Koch-Light Laboratories, England) and Liquid Scintillant NE 220 (Nuclear Enterprises, Scotland) were also used.

Recommended procedure

Collect sea-water samples in polythene containers to which sodium hydroxide has been added. Allow 5 ml of 20% (w/v) sodium hydroxide solution per litre.

To a 1.0-l aliquot of sea water, add 4.0 ml of iodide carrier working solution, 5 ml of 5% potassium permanganate solution and 20 ml of 20% sodium hydroxide solution. Heat to boiling for 30 min, and then cool. Acidify to pH 1.5–2.0 with about 10 ml of 16 *M* nitric acid, stir and slowly add 10% sodium sulphite solution until the sea water is colourless, plus 1.0 ml in excess.

Transfer to a 2-l separating funnel, add 2 ml of 10% sodium nitrite solution and 100 ml of toluene. Shake for 2 min and allow to settle. If the organic phase is not deep red, add more sodium nitrite solution and shake again. Discard the aqueous phase. Wash the organic phase with 25 ml of 1.0 *M* nitric acid.

Strip the radio-iodine and carrier from the solvent by shaking for 1 min with 1 ml of 10% sodium sulphite solution and 25 ml of 1.0 *M* nitric acid. After separation, transfer the aqueous phase to a 100-ml separating funnel and discard the organic phase.

To the aqueous phase, add 1 ml of sodium nitrite solution and 20 ml of toluene, shake for 1 min and allow to separate. Discard the aqueous phase. Wash the organic phase with 20 ml of 1% urea solution.

Run the toluene into a 40-ml centrifuge tube, centrifuge for 5 min, and then remove the small amount of water with a Spitzer pipette. Transfer to a 25-ml volumetric flask and dilute to volume with toluene. Pipette 10.0 ml of the toluene solution into a glass liquid scintillation counting cell and add 1.0 ml of 2-methyl-1-butene. Lightly stopper, irradiate with u.v. light until the solution is colourless, and then cool.

Fill the cell with liquid scintillant, mix well and allow 30 min for the phosphorescence to decay. Count in a low-background liquid scintillation spectrometer. Measure the absorbance of the toluene–iodine solution using 0.5-cm cells at 485 mm. Determine the iodine concentration by comparison with a standard curve.

DISCUSSION

The principal iodine nuclides of concern in monitoring sea water are iodine-126, -129 and -131, all of which are low-energy β-emitters with half-lives of 13 days, $1.59 \cdot 10^7$ years, and 8.04 days respectively. The maximal permissible levels in estuarine waters (derived from ICRP figures)[2] for these particular isotopes have been established as: iodine-126 and -131: $2.1 \cdot 10^{-7}$ μCi ml^{-1}; iodine-129: $4.3 \cdot 10^{-8}$ μCi ml^{-1}.

Using a liquid scintillation spectrometer, Loveridge and Gordon[4] established counting efficiencies for seven iodine nuclides. With the same type of counter, the efficiency for iodine-131 was found to be the same as that quoted by Loveridge and Gordon[5] (89%). It was therefore assumed that the efficiencies for counting iodine-126 and -129 were similar to those previously reported by Loveridge and Gordon[5].

In determining a radiobiological hazard, it is preferable that the result be over-estimated, rather than under-estimated. With the liquid scintillation counting

method, the value for the iodine isotope having the lowest counting efficiency should be used when the iodine isotopes present are not known. Loveridge and Gordon's figure of 42% efficiency for counting iodine-126 would give a safety factor of two if all the radioiodine were iodine-131, and a safety factor of about 1.7 if it were all due to iodine-129.

With a 1.0-l aliquot of sea water and a liquid scintillation counter with a background including reagents of 50 c.p.m., the minimal detectable limit was less than $5.0 \cdot 10^{-9}$ μCi ml^{-1} for a counting time of 2 h. This is well within the lowest limit derived from ICRP figures[2] for radio-iodine of $4.3 \cdot 10^{-8}$ μCi ml^{-1} for iodine-129.

Toluene was chosen as the solvent for extraction because the results obtained by Yerric and Ross[3] were promising. There was no need to use carbon tetrachloride in the same method. Extraction of iodine-131 from sea water at various pH values showed that maximal recovery was obtained at pH 1.7 (Fig. 1); from pH 1.1 to 2.3, recoveries were greater than 90%. When the amount of carrier was greater than 1.0 mg, the recovery of iodine-131 was essentially complete by the recommended procedure. The possibility of organic material being present in the sea water led to the adoption of Loveridge and Gordon's method[5] for the destruction of algae with alkaline permanganate. When the pH exceeded 12, no loss of radio-iodine or carrier was observed during boiling for as long as 60 min. Adding an excess of 4 g of sodium hydroxide had no effect on recovery if the pH was properly adjusted before extraction.

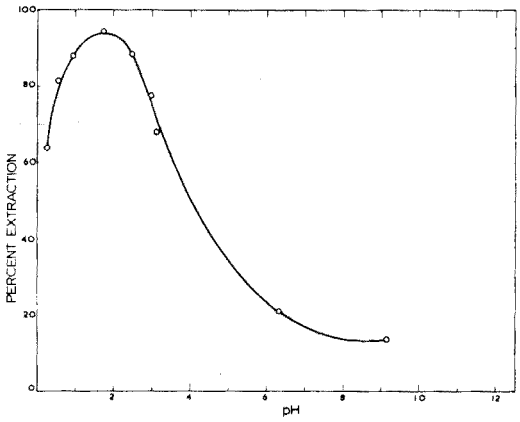


Fig. 1. Effect of pH on extraction of ^{131}I from 500 ml of sea water containing 4.0 mg of iodide and 1.0 ml of 10% $NaNO_2$ solution, with 50 ml of toluene.

To obtain over 90% recovery from a litre of sea water, 100 ml of toluene was necessary for extraction; with the same amount of solvent, the recovery was 65% from 2 l of sea water.

One wash with 25 ml of 1.0 *M* nitric acid removed any gross interferences. After the iodine had been reduced to iodide with sodium sulphite, the radio-iodine and carrier were stripped from the organic phase with a further 25 ml of 1.0 *M* nitric acid. Reoxidation of the iodide to iodine with sodium nitrite was

necessary before the final extraction into a small volume of toluene for the assay of the radioactivity and the determination of carrier recovery. Colorimetric determination of carrier showed that the absorbance of the toluene–iodine solution increased steadily with time. Washing the organic phase with a urea solution stabilized the absorbance for 24 h.

Large quantities of foreign ions were added without affecting the recovery (Table I), and a study of some of the radionuclides likely to be present in reactor effluent gave decontamination factors in excess of 10^3 (Table II). Recovery of iodine-131 from samples of sea water spiked with a standard solution and left for eight weeks in polythene bottles under varying conditions showed that the method was essentially quantitative (Table III).

The total amount of iodine in the sea water used for experiments was 43 $\mu g\ l^{-1}$; this was determined by refluxing with sulphuric acid and potassium permanganate, followed by oxidation of the distilled iodine to iodate for colorimetric determination by the starch–iodide method. Both γ-well scintillation and β-liquid

TABLE I

EFFECT OF VARIOUS ELEMENTS ON ^{131}I RECOVERY FROM 1.0 l OF SEA WATER

Foreign ion	*Amount (mg)*	*Added as*	*Recovery (%)*	
			I_2	^{131}I
Ag^+	5	$AgNO_3$	37.5	98.1
	1.0		80.5	98.0
Al^{3+}	100	$Al_2(SO_4)_3$	81.8	100.5
As^{3+}	100	As_2O_3	85.0	99.5
Bi^{3+}	100	$Bi(NO_3)_3$	76.0	96.4
Br^-	100	KBr	77.0	105.1
Ca^{2+}	100	$CaCl_2$	79.5	100.6
Co^{2+}	100	$Co(NO_3)_2$	80.0	97.5
Cr^{6+}	100	K_2CrO_4	84.1	98.1
Cu^{2+}	100	$CuCl_2$	81.0	99.8
Fe^{3+}	100	$FeCl_3$	81.5	98.8
Hg^{2+}	50	$HgCl_2$	48.4	101.4
	10		75.9	101.7
In^{2+}	100	$InCl_2$	89.5	95.9
Mg^{2+}	100	$MgCl_2$	80.0	99.5
Mn^{2+}	100	$MnCl_2$	80.0	99.5
Ni^{2+}	100	$NiSO_4$	80.0	101.0
Pb^{2+}	100	$Pb(NO_3)_2$	85.3	98.0
Sb^{3+}	100	$SbCl_3$	87.2	96.3
Sn^{4+}	100	$SnCl_4$	90.5	96.9
Te^{4+}	100	K_2TeO_3	86.8	96.3
U^{6+}	100	$UO_2(NO_3)_2$	84.9	96.3
Zn^{2+}	100	$ZnCl_2$	80.5	98.9
Acetate	100	$NH_4C_2H_3O_2$	84.9	99.9
Ascorbic acid	100		81.6	96.9
Boric acid	100		83.2	100.5
Oxalic Acid	100		83.2	99.0
Tartaric Acid	100		84.8	98.7

TABLE II

DECONTAMINATION FACTORS FOR VARIOUS RADIONUCLIDES

Radionuclide	*Decontamination factor*	*Radionuclide*	*Decontamination factor*
^{207}Bi	> 4000	^{54}Mn	> 10000
^{82}Br	> 10000	^{22}Na	> 10000
^{144}Ce	> 5000	^{147}Pm	> 1000
^{60}Co	> 10000	^{106}Ru	> 10000
^{51}Cr	> 2000	$^{90}Sr-^{90}Y$	> 40000
^{134}Cs	> 50000	$^{95}Zr-^{95}Nb$	> 5000
^{59}Fe	> 8000		

TABLE III

RECOVERY FROM 1.0 l OF SEA WATER SPIKED WITH $^{131}I^{a}$ AND STORED FOR EIGHT WEEKS IN POLYTHENE BOTTLES UNDER VARYING CONDITIONS

Alkali or acid added	*Iodide added (mg)*	*I^- Carrier recovery (%)*	*^{134}I recovery (%)*
0	0	71.2[b]	99.6
0	0	74.4[b]	100.7
NaOH 200 mg	0	72.6[b]	104.1
1000 mg	0	80.1[b]	98.0
0	5.0	77.4	100.0
0	5.0	78.0	100.0
10 *M* HCl 2 ml	0	81.2[b]	101.4
16 *M* HNO_3 2 ml	0	82.5[b]	97.0
11 *M* $HClO_4$ 2 ml	0	82.5[b]	86.6

[a] 100,000 d.p.m.
[b] 8.0 mg added before extraction.

scintillation counting procedures were used to determine recovery of iodine-131 and no significant differences were observed. The time taken for the analysis of six samples, excluding counting, is about 4 h.

SUMMARY

A solvent extraction procedure for the determination of radio-iodine in sea water is described. The water is treated with alkaline permanganate to remove algae, and, after removal of permanganate, the iodide is oxidized to iodine for extraction into toluene. The radio-iodine with carrier is stripped from the solvent then re-extracted into a smaller volume of toluene for liquid scintillation counting and colorimetric determination of carrier recovery as iodine. 2-Methyl-1-butene is used to decolorize the toluene–iodine solution under u.v. light and avoid colour quenching during counting. Samples spiked with iodine-131 showed essentially quantitative recovery from 1.0 l of sea water with a typical recovery of 80–85% of carrier. The method is applicable in the presence of high concentrations of

many foreign ions and the decontamination factor for a number of radionuclides is greater than 10^3. The limit of detection is less than $5.0 \cdot 10^{-9}$ μCi ml^{-1}.

REFERENCES

1 WHO, *Technical Report Series 173*, 1959.
2 *ICRP Report of Committee II on Permissible Dose for Internal Radiation*, 1959.
3 R. E. Yerric and H. H. Ross, *USAEC Rep. TID7688*, 1963, p. 20.
4 M. G. Lai and H. A. Goya, *USNRDL Rep. TR912*, 1965, p. 33.
5 B. A. Loveridge and M. S. Gordon, *UKAEA Rep. AERE R5406*, 1967.
6 *ASTM Standard Methods, D2334-68*, 1973, p. 604.

Analytica Chimica Acta, 76 (1975) 121–130

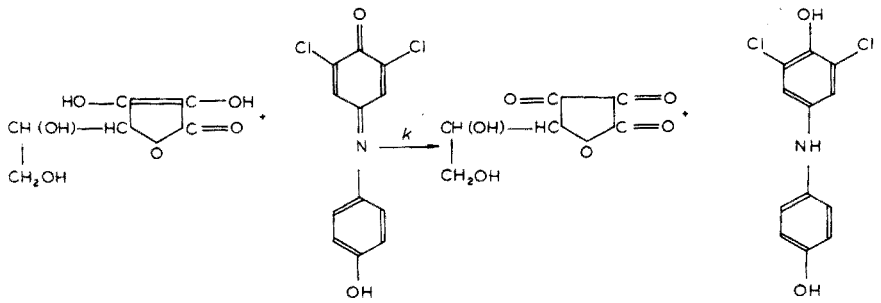
KINETIC DETERMINATION OF ASCORBIC ACID BY THE 2,6-DICHLOROPHENOLINDOPHENOL REACTION WITH A STOPPED-FLOW TECHNIQUE

M. I. KARAYANNIS

Laboratory of Analytical Chemistry, University of Athens, Athens-144 (Greece)

(Received 16th September 1974)

Ascorbic acid can be determined titrimetrically by a method, first proposed by Tilmans[1], which is based on the very fast reaction of ascorbic acid with 2,6-dichlorophenolindophenol (DCPI); ascorbic acid is oxidized to dehydroascorbic acid, while DCPI is reduced to its leuco compound[2]. This reaction is quite widely used for the titrimetric assay of vitamin C in food products and biological samples, but it has many drawbacks and is time-consuming[3,4]. The concentration of DCPI must be checked frequently against standard quantities of vitamin C and adjusted according to the concentration of vitamin C in the sample to be analysed. The stoichiometry of the reaction is given by the equation:



A complete bibliography on the methods used for the assay of vitamin C in various samples has been given by Strohecker and Henning[3].

In the work described here, a kinetic method was developed for the determination of vitamin C in aqueous solutions, which is very sensitive, accurate and reproducible. The method applies stopped-flow techniques and is based on a kinetic investigation of the reaction of ascorbic acid and DCPI, which will be published elsewhere.

THEORY

In many reactions applied for kinetic determinations, there is a trend to create pseudo-first order conditions for the species determined and to transform the signal of the transducer used to a quantity which is proportional to the concentration of the species[5].

If we consider a bimolecular reaction,

$$A + B \xrightarrow{k} C + D \tag{1}$$

which can be described by second-order kinetics; the rate law is given by

$$-\frac{d[A]}{dt} = -\frac{d[B]}{dt} = k[A]_t[B]_t \tag{2}$$

where k, the rate constant, applies to a particular set of reaction conditions such as temperature, ionic strength, pH and complexing anion concentrations; $[A]_t$ and $[B]_t$ are the momentary concentrations of the reactants A and B. Equation (2) is generally applied for the determination of A or B under appropriate conditions. The most popular technique is the initial rate method, where the derivative dP/dt of a parameter P proportional to A or B at the very beginning of the reaction is measured manually or automatically. If a spectrophotometric method is used and the transmittance T of the reacting mixture is monitored as the reaction progresses the following mathematical expressions are valid, provided that reactant B is the only absorbing species at the wavelength used:

$$\log T = -\varepsilon_B \cdot b \cdot [B]_t \tag{3}$$

$$\log(V/V_0) = -\varepsilon_B \cdot b \cdot [B]_t \tag{4}$$

where ε_B = molar absorptivity of B (in 1 mol^{-1} cm^{-1}); b = light path in cm; V_0 = output signal of the photomultiplier at transmittance $T = 1$ (in the present work $V_0 = 800$ mV); and V = output signal of the photomultiplier at transmittance T. The derivative of V with respect to time can be expressed as

$$\frac{dV}{dt} = \frac{dV}{d[B]} \cdot \frac{d[B]}{dt} \tag{4a}$$

From eqn. (4):

$$\frac{dV}{d[B]} = -\frac{V_0 \cdot \varepsilon_B \cdot b}{0.43} \exp\left\{-\frac{\varepsilon_B \cdot b \cdot [B]_t}{0.43}\right\} = \frac{\varepsilon_B \cdot b}{0.43} \cdot V \tag{4b}$$

and combination of eqns. (4a) and (4b) leads to:

$$\frac{dV}{dt} = -\frac{\varepsilon_B \cdot b}{0.43} V \frac{d[B]}{dt} \tag{5}$$

which with eqn. (2) gives:

$$\frac{dV}{dt} = k \frac{\varepsilon_B \cdot b \cdot [B]}{0.43} \cdot V \cdot [A]_t \tag{6}$$

and finally,

$$\frac{dV}{dt} = k \frac{A_t \cdot V}{0.43} \cdot [A]_t \tag{7}$$

where A_t is the momentary absorbance of the reaction mixture. From the stoichiometry of the reaction, $[A]_t = ([A]_0 - [B]_0 + [B]_t)$, where $[A]_0$ and $[B]_0$ are the starting concentrations of the reactants. Replacement of $[A]_t$ in eqn. (7) by

$([A]_0-[B]_0+[B]_t)$ gives:

$$\frac{dV}{dt} = k\frac{A_t \cdot V}{0.43} \cdot [A]_0 \cdot \left\{1 - \frac{[B]_0}{[A]_0} + \frac{[B]_t}{[A]_0}\right\} \quad (8)$$

Equation (8) allows calculation of $[A]_0$ if the slope dV/dt is measured manually, or automatically, at any point of the reaction curve $V=f(t)$. Equation (8) is only applicable for analytical purposes if the following prerequisities are achieved:

(1) the quantity $Q=A_t \cdot V/0.43$ must remain constant within experimental error; (2) the quantity $Y=(1-[B]_0/[A]_0+[B]_t/[A]_0)$ must remain constant within experimental error; (3) there must be a suitably long linear part in the reaction curve, especially when a manual method is applied, for the measurement of dV/dt.

If these demands are met, then eqn. (8) can be written as:

$$\frac{dV}{dt} = k \cdot Q \cdot Y \cdot [A]_0 \quad (9)$$

and if $k \cdot Q \cdot Y$ is replaced by S:

$$\frac{dV}{dt} = S \cdot [A]_0 \quad (10)$$

The proportionality factor S in eqn. (10) is the slope of the analytical curve for the determination of $[A]_0$. In real cases, the quantity Q is not constant, but the analytical conditions can be adjusted so that Q remains independent of the value of V. It is known that the function,

$$A \cdot T = f(T) \quad (11)$$

goes through a maximum at $T=0.37$ (or $A=0.43$). Since the experimental conditions are set for $T=V/V_0$, the expression for the quantity Q takes the form,

$$Q = \frac{A \cdot T \cdot V_0}{0.43} \quad (12)$$

and is maximal for $T=0.37$ ($A=0.43$), *i.e.* $Q=294.30$ mV. The values of Q for different values of T (or its equivalent V) can be calculated from eqn. (12).

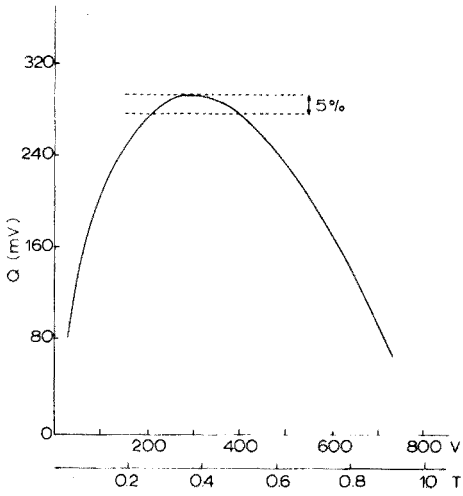
Table I shows the results; column 4 gives the molar concentrations of B for $\varepsilon_B=8600$ l mol^{-1} cm^{-1} and $b=2$ cm, while column 6 gives the relative deviation of Q from its maximal value of 294.30 mV. From Fig. 1, it is evident that Q remains constant within 5% of its maximal value for the 200–400-mV range of the reaction curve. For the same range, the concentration of B changes about 50%. The constancy of Q for this range of V signifies the existence of a real linear part in the reaction curve for the same range.

Figure 2 shows theoretical curves of the reaction of A and B for three different starting concentrations of B, giving output signals of 140 mV (curve b), 20 mV (curve c) and 545 mV (curve a), respectively, at zero time after mixing. Analytical conditions giving curves (c) or (b) are suitable for the determination of A in the reacting system. The analytical concentration of B giving the form of curve (b) has the additional advantage that at the point where the slope dV/dt is measured (immediately after mixing) the change of concentration of B is very

TABLE I

Q VALUES AND CORRESPONDING B CONCENTRATIONS AS A FUNCTION OF V AND T

V (mV)	$T = V/V_0$	*Absorbance*	$[B]_t$ ($\cdot 10^{-5}$ M)	Q (mV)	*Rel. error* (%)
80	0.1000	1.0000	5.81	184.20	37.41
100	0.1250	0.9031	5.25	207.94	29.30
200	0.2500	0.6021	3.50	277.26	6.10
240	0.3000	0.5228	3.00	288.95	1.80
280	0.3500	0.4559	2.65	293.95	0.12
300	0.3750	0.4260	2.52	294.25	0.02
320	0.4000	0.3979	2.31	293.21	0.37
340	0.4250	0.3716	2.16	290.92	1.15
360	0.4500	0.3468	2.02	287.46	2.32
380	0.4750	0.3233	1.88	282.88	3.88
400	0.5000	0.3010	1.75	277.26	5.79
440	0.5500	0.2596	1.51	263.05	10.62
480	0.6000	0.2218	1.29	245.19	16.69
520	0.6500	0.1871	1.09	224.00	23.90
560	0.7000	0.1549	0.90	199.74	32.10
600	0.7500	0.1249	0.73	172.61	41.40
640	0.8000	0.0969	0.56	142.80	51.50
680	0.8500	0.0706	0.41	110.51	62.40
720	0.9000	0.0458	0.27	75.86	74.22



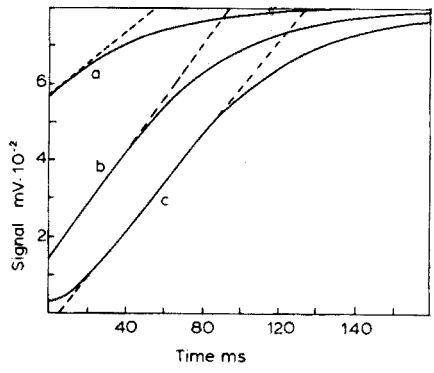


Fig. 1. Dependence of Q on the transmittance T, and on the signal level V.

Fig. 2. Theoretical curves for the reaction $A + B \xrightarrow{k}$ Products, $k = 51.7 \cdot 10^3$ s^{-1} mol^{-1}; $[A]_0 = 5.0 \cdot 10^{-4}$ M; $[B]_0 = 0.878 \cdot 10^{-5}$ M (a); $4.37 \cdot 10^{-5}$ M (b); $8.75 \cdot 10^{-5}$ M (c).

small and thus the ratios $[B]_0/[A]_0$ and $[B]_t/[A]_0$ of the quantity Y are almost equal and cancel each other.

Although curve (c) has a good linear part in agreement with the above theory, the slope dV/dt at 300 mV is smaller than in curve (b), because in this case dV/dt is measured at a point where the reaction has progressed and the ratios $[B]_0/[A]_0$ and $[B]_t/[A]_0$ are significantly different, so that the constancy of Y and S are affected. The value of $[B]_0/[A]_0$ is also decisive for the lower limit of concentration of A which can be determined by this technique. For $[B]_0 \ll [A]_0$, the value of Y remains constant and equal to unity as the reaction progresses, thus ensuring linearity between dV/dt and $[A]_0$ for a wide range of concentration. As the ratio $[B]_0/[A]_0$ approaches unity, the value of Y approaches $[B]_t/[A]_0$ and, depending on the point where dV/dt is measured, may deviate significantly from unity. Accordingly, the conditions for applying eqn. (8) for the kinetic determination of A are: (1) a large value of k in order to achieve high sensitivity; (2) a very large value of ε_B in order to achieve the conditions for the constancy of S. This requirement ensures that the limit of determination of A lies at very low concentrations.

The curved part between 20 mV and 200 mV in curve (c) of Fig. 2, can be explained by referring to eqn. (9) and Fig. 1. According to eqn. (9), the slope dV/dt of the function $V=f(t)$ at each point depends on the value of Q, which is changing drastically at this range of signal V.

EXPERIMENTAL

Apparatus

The stopped-flow spectrophotometer D-131 of the Durrum Company was used without modification. The course of the reaction was displayed on the screen of a storage oscilloscope (Tektronix R 564 B) and photographed with a polaroid camera (Tektronix C-12).

The pH was measured with a Research Model pH-meter (Beckman, U.S.A.).

A 5-ml burette and class A glassware were used for standardizations.

Reagents

All solutions used were prepared in distilled water from p.a. reagents.

Vitamin C ($1.000 \cdot 10^{-1}$ *M*). Dissolve 17.6130 g of vitamin C (Merck) in 1 l of 0.05 *M* oxalic acid solution. Standardize against 0.05 *M* iodine solution with starch as indicator. The stock solution was stable for over two months if kept in a refrigerator; storage at room temperature entailed a concentration loss of about 3.5% per month. Solutions of different concentrations in vitamin C were prepared from the stock solution by diluting with 0.05 *M* oxalic acid.

2,6-Dichlorophenolindophenol ($1 \cdot 10^{-3}$ *M*). Dissolve about 60 mg of DCPI powder in 200 ml of a solution containing 210 mg of $NaHCO_3$ per liter. The pH of the resulting solution was about 7.5. Standardize by titration with freshly prepared vitamin C solution pre-standardized against iodine. Solutions of different concentrations in DCPI were prepared from the stock by diluting with a solution of $NaHCO_3$ of the above concentration.

Procedure

Switch the unit on at least 20 min before the measurements are started, and

turn the lamp selector knob to the TUNGSTEN position. Set the wavelength selector knob at the 330–1000 nm position and the filter slide control at the 360–550 nm position. Turn the wavelength dial to 522 nm and the slit-width control to 0.3 mm. Calibrate the instrument for 0 and 100% transmittance with water in the observation cell, as follows: (1) with the (+) and (−) INPUT switches at GND, adjust the oscilloscope vertical position control to set the trace on the bottom horizontal line of the graticule; (2) set the oscilloscope vertical V/DIV control to 0.1, switch the vertical amplifier (−) INPUT switch to DC and adjust the photomultiplier supply voltage to position the highest trace excursion on the top horizontal line of the graticule. This is the 100% transmission setting for the instrument. In all measurements, the light path was 2 cm.

Fill the two 20-ml reservoir syringes and the two 2-ml drive syringes with the ascorbic acid and DCPI solutions. Push the ACTUATE button twice to see if the trace on the scope is reproducible. Take the picture with the polaroid camera and measure the slope of the linear part of the trace at 300 mV and express it in mV s^{-1}.

All measurements were performed at 522 nm, which is the isosbestic point for DCPI at various pH values[6]. The molar absorptivity of DCPI at 522 is 8600 l mol^{-1} cm^{-1}. All concentrations of ascorbic acid and DCPI given in the Tables and Figures are the actual concentrations in the reacting mixture at zero time after mixing. The pH of the reacting mixture remains below 1.5 during the reaction. The reaction rate constant k does not change for pH values smaller than 2 and equals $(51.7 \pm 0.5) \cdot 10^3$ s^{-1} mol^{-1}. This is the apparent value and is not corrected for second-order errors.

RESULTS AND DISCUSSION

Figures 3 and 4 show typical oscilloscope traces which describe the reaction

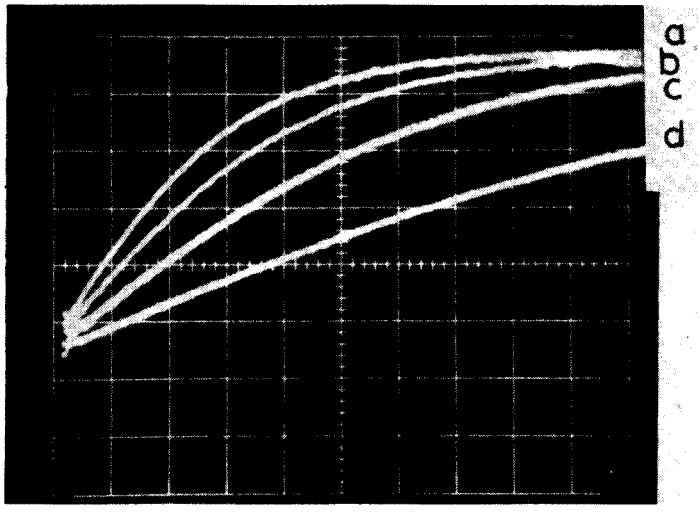


Fig. 3. Oscilloscope picture of the reaction of ascorbic acid and DCPI in 0.025 M oxalic acid. DCPI, $2.93 \cdot 10^{-5}$ M. Ascorbic acid: (a) $2.0 \cdot 10^{-3}$ M; (b) $1.5 \cdot 10^{-3}$ M; (c) $1.0 \cdot 10^{-3}$ M; (d) $5.0 \cdot 10^{-4}$ M. Base line is 0.0 mV. Ordinate scale 100 mV/div; abscissa scale 5 ms/div.

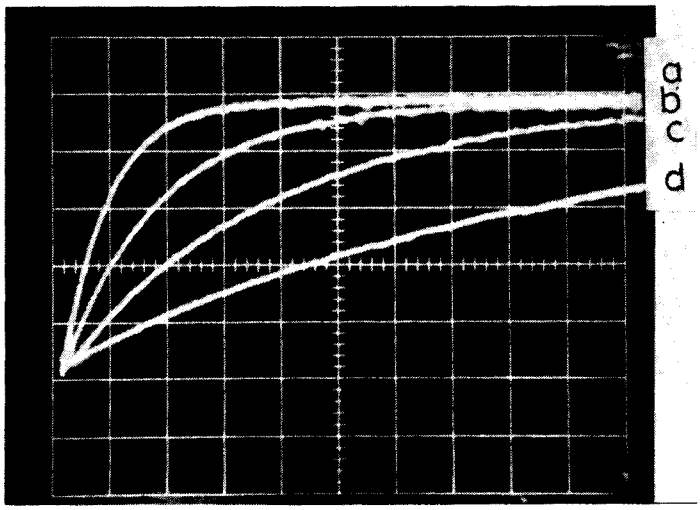


Fig. 4. Oscilloscope picture of the reaction of ascorbic acid and DCPI in 0.025 M oxalic acid. DCPI, $9.74 \cdot 10^{-6}$ M. Ascorbic acid: (a) $5.0 \cdot 10^{-4}$ M; (b) $2.5 \cdot 10^{-4}$ M; (c) $1.25 \cdot 10^{-4}$ M; (d) $5.0 \cdot 10^{-5}$ M. Base line voltage 435 mV. Ordinate scale 50 mV/div; abscissa scale 50 ms/div.

of the ascorbic acid and DCPI. The slope dV/dt was measured manually from such curves for different concentrations of ascorbic acid, but at the same level of the signal for all concentrations. Table II shows the results for two different sets of experimental conditions; in the first set, the concentration of DCPI was $2.93 \cdot 10^{-5}$ M, which allows the determination of pure solutions of ascorbic acid in 0.05 M oxalic acid in the range $5.0 \cdot 10^{-4}$–$1.0 \cdot 10^{-2}$ M. The slopes were measured at 320 mV, where the value of Q, according to Table I, is 293.21 mV (this value

TABLE II

KINETIC DETERMINATION OF ASCORBIC ACID

Ascorbic acid		*Error (%)*	*Slope (mV s^{-1})*		*Y*
Taken	*Found*		*Experimental*	*Theoretical*	
Concentration: $\cdot 10^{-4}$ M			*Measured at 320 mV*		
5.00	4.92	−1.66	7500	7625	0.9876
10.00	10.16	+1.60	15500	15250	0.9938
15.00	14.82	−1.20	22600	22875	0.9959
20.00	20.13	+0.66	30700	30500	0.9969
30.00	30.17	+0.55	46000	45750	0.9979
40.00	40.20	+0.49	61400	61000	0.9985
Concentration: $\cdot 10^{-5}$ M			*Measured at 600 mV*		
2.5	2.24	−10.30	200	223	0.9026
5.0	4.71	−5.80	420	446	0.9513
12.5	12.33	−1.35	1100	1115	0.9805
25.0	25.24	+0.94	2250	2229	0.9903
40.0	39.25	−1.88	3400	3567	0.9939
50.0	49.30	−1.32	4400	4459	0.9951

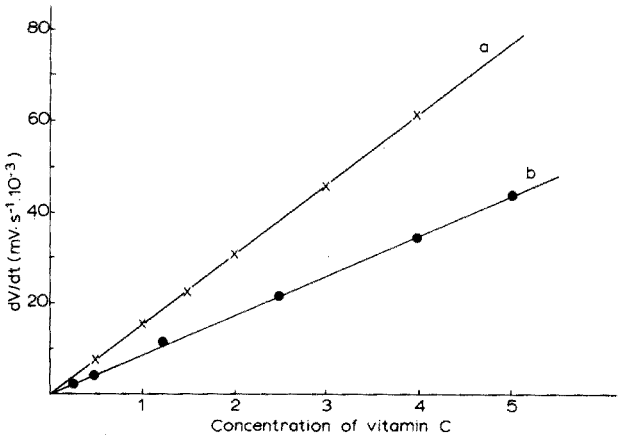


Fig. 5. Working curves for the determination of ascorbic acid. (a) DCPI: $2.93 \cdot 10^{-5}$ *M*; concentration scale, 10^{-3} *M*. (b) DCPI: $9.74 \cdot 10^{-6}$ *M*; concentration scale, 10^{-4} *M*.

is 0.37% lower than $Q_{max} = 294.3$ mV). Figure 5(a) shows the calibration curve obtained; the slope calculated by the least-squares method, was $S_{exp} = 152.50 \cdot 10^5$ mV s^{-1} mol^{-1}, in very good agreement with the theoretical value $151.47 \cdot 10^5$ mV s^{-1} mol^{-1} calculated from the equation $S = k \cdot Q \cdot Y$ with $Y = 1$. The accuracy of the determination of ascorbic acid, expressed as the mean relative error, was 1.00%. For lower concentrations of ascorbic acid, the error was larger because of the error introduced from the parameter Y of eqn. (8); column 6 in Table II gives the values of Y calculated for $[B]_0 = 2.93 \cdot 10^{-5}$ *M* and $[B]_t = 2.31 \cdot 10^{-5}$ *M*, which were the starting concentration and the concentration at $V = 320$ mV of B, respectively. As the Table shows, under these conditions, less than 10^{-3} *M* vitamin C

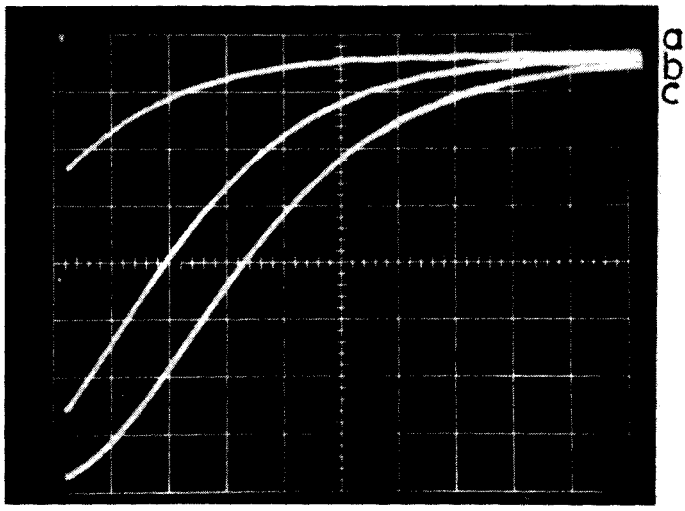


Fig. 6. Experimental curves of the reaction of DCPI and ascorbic acid in 0.025 *M* oxalic acid. Ascorbic acid, $5.0 \cdot 10^{-4}$ *M*. DCPI: (a) $0.878 \cdot 10^{-5}$ *M*; (b) $4.37 \cdot 10^{-5}$ *M*; (c) $8.75 \cdot 10^{-5}$ *M*. Ordinate scale 100 mV/div; abscissa scale 20 ms/div.

cannot be determined with an accuracy better than 2%; the error for concentrations of $1.5 \cdot 10^{-4}$ *M* is about 9.0%. For this range of concentrations, the measured slopes must be corrected for *Y*. The reproducibility of the method is better than 1.0% for the $2.0 \cdot 10^{-3}$ *M* samples.

The second set of measurements, shown in Table II and Fig. 5(b) was done with $9.74 \cdot 10^{-6}$ *M* DCPI. This concentration allows the determination of vitamin C in the range $2.0 \cdot 10^{-5}$–$1.0 \cdot 10^{-3}$ *M*. The slopes in this case were measured at 600 mV. The value of *Q* at this level was 172.61 mV. The slope of the analytical curve was $S_{exp} = 88.0 \cdot 10^5$ mV s^{-1} mol^{-1} which is in very good agreement with the theoretical value $89.17 \cdot 10^5$ mV s^{-1} mol^{-1}. The mean relative deviation calculated from Fig. 5(b) was 2.3%, which is larger than in the first set of data, because the value of *Q* (172.61 mV) was 41.4% less than its maximal value, and because the slope measurement at this level of *V* was less reproducible. From Fig. 1, it is evident that the value of *Q* for this level changes very rapidly with *V*. Figure 6 shows a group of reaction curves, taken with three different concentrations of DCPI; the concentration of vitamin C was kept constant at $5.0 \cdot 10^{-4}$ *M* in 0.05 *M* oxalic acid. The slopes dV/dt were measured at 600 mV for curve (a), and at 300 mV for curves (b) and (c). The values obtained were 4474 mV s^{-1}, 7370 mV s^{-1} and 6870 mV s^{-1}, respectively. The theoretical values of the slopes were calculated by applying eqn. (9) with $k = 51.7 \cdot 10^3$ s^{-1} mol^{-1} and with values of *Q* taken from Table I. These values were corrected for the parameter *Y* and are given in Table III. There is good agreement between the experimental and theoretical values.

TABLE III

EXPERIMENTAL AND THEORETICAL SLOPES FOR THREE DIFFERENT CONCENTRATIONS OF DCPI

DCPI (10^{-5} *M*)	*Ascorbic acid* (10^{-5} *M*)	*Level of measurement of dV/dt* (*mV*)	*Q* (*mV*)	*Y*	*Slopes* (*mV* s^{-1})	
					$(dV/dt)_{exp}$	$(dV/dt)_{theor}$[a]
0.878	50.0	600	172.61	0.997	4470	4445
4.370	50.0	300	294.25	0.962	7370	7312
8.750	50.0	300	294.25	0.875	6870	6650

[a] Values given were calculated from eqn. (9).

The proposed method has several advantages compared with the classical method. It is fast, even when the slope is measured manually from the pictures. The slope can also be measured automatically with electronic slope-measuring systems. It is also sensitive and accurate. The method can be applied successfully for the determination of vitamin C in aqueous solutions, in biological systems and in the presence of other reducing materials.

The author thanks Dr. G. Papageorgiou for constructive criticism of the manuscript, and acknowledges the assistance of Miss Lela Nanou and Miss Dina Athanasopoulou during the experimental work.

SUMMARY

The reaction of ascorbic acid with 2,6-dichlorophenolindophenol is applied for the kinetic determination of ascorbic acid in 0.05 *M* oxalic acid. Stopped-flow techniques are used; the concentrations of the reactants of the second-order reaction can be adjusted so that the transmittance signal remains nearly invariant for a wide range of voltage. Theoretical and experimental results are in very good agreement. Analytical working curves are presented for the determination of ascorbic acid in the ranges $5.0 \cdot 10^{-4}$–$1.0 \cdot 10^{-2}$ *M* and $5.0 \cdot 10^{-5}$–$1.0 \cdot 10^{-3}$ *M* with errors of 1.0% and 2.2%, respectively. The method is simple, fast and sensitive.

REFERENCES

1 J. Tilmans, *Z. Lebensm.-Unters. Forsch.*, 54 (1927) 33.
2 R. Strohecker and R. Vaubel, *Angew. Chem.*, 49 (1936) 666.
3 R. Strohecker and H. M. Henning, *Vitamin Assay*, Verlag Chemie, 1966, pp. 227–253.
4 H. J. Loeffler, *Ind. Eng. Chem., Anal. Ed.*, 14 (1942) 846.
5 H. V. Malmstadt, C. J. Delaney and E. A. Cordos, *Critical Reviews in Analytical Chemistry*, 2 (*4*) (1972) 559.
6 J. McD. Armstrong, *Biochim. Biophys. Acta*, 86 (1964) 194.

Analytica Chimica Acta, 76 (1975) 131–141

ANODIC STRIPPING VOLTAMMETRY WITH THE FLORENCE MERCURY FILM ELECTRODE. DETERMINATION OF COPPER, LEAD AND CADMIUM IN SEA WATER

WALTER LUND and MAGNE SALBERG
Department of Chemistry, University of Oslo, Blindern, Oslo 3 (Norway)
(Received 30th September 1974)

It has for the last decade been amply demonstrated that anodic stripping voltammetry is eminently suited for trace analysis of heavy metals such as bismuth, copper, lead, cadmium and zinc at the sub p.p.b. level. Owing to the increasing need for improved detection limits in trace analysis the classical hanging mercury drop electrode (h.m.d.e.) has recently been replaced by the mercury film electrode (m.f.e.), which may increase the sensitivity of the anodic stripping voltammetric technique by a factor of more than one hundred. Of the various mercury film electrodes suggested in the literature, the rotating glassy carbon electrode, mercury plated *in situ*—in this paper named the Florence electrode after the originator[1]—seems to be the electrode of choice. This electrode seems to possess most of the virtues of an ideal electrode for anodic stripping voltammetry. Very high sensitivity and selectivity are obtained, owing to the formation of a very thin mercury film, which is easily formed *in situ*. The glassy carbon seems to be an excellent support material, which gives a low background current as well as a wide accessible potential range. The rotation of the disc electrode gives effective, reproducible mass transport to the electrode. However, so far there are few authors except Florence[1–5] who have reported successful results with this particular electrode. Since various problems are often encountered when film electrodes are used[6,7], a study of the Florence electrode appeared to be of interest.

The determination of trace metals in sea water is an important application of the stripping voltammetric technique. The analysis of copper, lead and cadmium in sea water was therefore chosen as a useful model system for the critical evaluation of the technique. The analyses were carried out at a pH of 8, in the absence of added chemicals, in contrast to the investigations by Florence, which were carried out on acidified samples. The results with the film electrode are compared with results obtained by using the classical hanging mercury drop electrode.

EXPERIMENTAL

Apparatus

A versatile solid-state voltammeter built in this laboratory, and a Hewlett Packard 7030 AM XY recorder were used for the stripping voltammetric experiments.

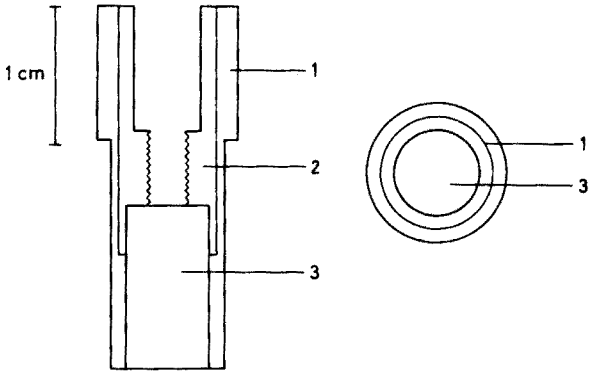


Fig. 1. Glassy carbon electrode. 1, Teflon; 2, brass; 3, glassy carbon rod, 6 mm diameter.

The electrolytic cell was a Metrohm EA 880–20 vessel with a thermostatted jacket, in which water at 25.0°C was circulating. The working electrode was a disc with a diameter of 0.6 cm (electrode area 0.283 cm^2), made of glassy carbon, which was pressed into Teflon. The glassy carbon was of GC-A grade (Tokai Electrode Mfg. Co. Ltd., Tokyo). The construction of the electrode is shown in Fig. 1. The disc was polished by fine emery paper, followed by solutions of aluminium hydroxide with particle size 5 and 0.3 μm. The electrode was rotated by means of a Beckman rotating unit, consisting of a variable speed drive unit (188501) and a rotating electrode body (188551). The hanging mercury drop electrode used was of the type Metrohm E 410. When this electrode was used, the solution was stirred by a three-edged Teflon stirrer connected to the Beckman rotating unit. The reference electrode, a Metrohm EA 427 saturated Ag/AgCl electrode, was placed inside a salt bridge filled with the solution to be analyzed. All potentials are referred to the Ag/AgCl electrode. A platinum spiral served as counter electrode. Dissolved oxygen was removed from the solution by passing highly purified nitrogen through the cell. The electrolytic cell was treated with a silicone repellent, dimethyldichlorsilane, to prevent adsorption of metals on the glass walls. The cell was also equilibrated with sea water for at least 30 min before the measurements.

Reagents and solutions

Mercury(II) nitrate solution (0.1 *M*) was prepared from highly purified mercury oxidized in nitric acid (Suprapur, Merck). The metal solutions were prepared from analytical-grade nitrate or sulphate salts. Solutions with a concentration below 10^{-3} *M* were prepared just before use. The water used was deionized with an ion-exchange resin and distilled.

Sea water

The sea water was collected from the Oslofjord, filtered through a 0.45-μm Millipore filter and stored in a 20-l polyethylene bottle which had been cleaned with nitric acid and rinsed with distilled water and sea water. The concentration of metals in this batch of sea water did not change during the time of the investigation (three months). The pH of the raw sea water was 7.3. When nitrogen

was passed through the cell to remove oxygen during the analysis, the pH increased slightly owing to the removal also of dissolved carbon dioxide. However, after 15 min the pH reached a steady value of 8.0.

All experiments described in this paper were carried out at a pH of 8.0 ± 0.1. In this pH region the hydrogencarbonate present in sea water acts as a buffer[8]. This is to be preferred to the addition of chemicals, which will usually contaminate the samples, and which may also change the chemical composition of the samples.

Procedure for mercury film electrode

To 25 ml of sea water add 0.2 ml of $5 \cdot 10^{-3}$ *M* mercury(II) nitrate, and sparge the solution with nitrogen for 15 min while the electrode is rotating to prevent gas bubbles adhering to its surface. Condition the electrode by a 3-min deposition at -0.9 V, followed by a linear sweep to 0 V, where the potential is kept for 5 min. Then start the determination by depositing at -0.9 V for 10 min. At the end of the deposition period, record the stripping voltammogram while the potential is scanned to 0 V at a speed of 3 V min^{-1}. Keep the potential at 0 V for 5 min before a new deposition is carried out. Rotate the electrode at 70 r.p.s. during the deposition and stripping steps. Remove the mercury film with a soft paper tissue only when a new aliquot is to be analyzed. The concentration of the metals in the sea water sample is determined by the standard addition method, by adding 100 μl of a standard solution to the sea water sample. The standard solution contained $1\text{–}8 \cdot 10^{-6}$ *M* of the metals in question.

Procedure for hanging mercury drop electrode

Deaerate 25 ml of the sea-water sample with nitrogen for 15 min, and then carry out the deposition for 60 min at -0.9 V. During the deposition period, stir the solution with a synchronous motor at a rotation speed of 40 r.p.s. After a rest period of 30 s, record the stripping voltammogram while the potential is scanned to 0 V at a speed of 0.6 V min^{-1}. Maintain the potential at 0 V for 2 min, and discard an extra mercury drop before any new deposition is carried out on a fresh mercury drop. The diameter of the mercury drop was 1.0 mm.

RESULTS AND DISCUSSION

A typical voltammogram of the sea-water sample, obtained with the Florence electrode, is shown in Fig. 2. The voltammogram exhibits three well defined peaks, which correspond to the oxidation of copper, lead and cadmium, respectively. The peak potentials were observed at -0.25 V (Cu), -0.47 V (Pb) and -0.65 V (Cd) *vs.* Ag/AgCl. The peaks were well separated, as would be expected when a thin mercury film is used. The voltammogram was recorded while the electrode was rotating. Owing to the thin film, no rest period between the deposition and stripping steps was needed. The rotating electrode did not generate noise problems during the recording of the voltammogram.

The data from five independent determinations of copper, lead and cadmium are given in Table I. The concentration of the metals was determined by the method of standard addition. The results are somewhat high, particularly for cadmium, indicating that the sea-water sample was probably contaminated. However,

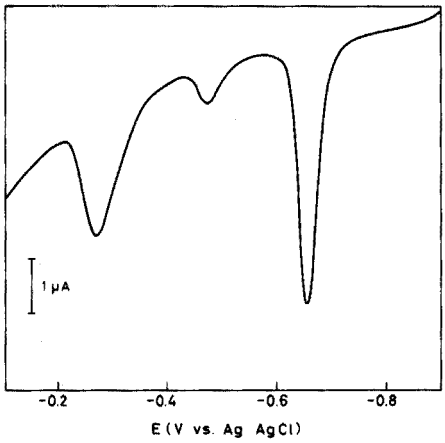


Fig. 2. Stripping voltammogram of the sea-water sample, with the rotating glassy carbon electrode mercury plated *in situ*. The three peaks correspond to the oxidation of copper, lead and cadmium, respectively. Deposition time 10 min, scan rate 3 V min^{-1}, deposition potential −0.9 V.

TABLE I

ANALYTICAL RESULTS FOR COPPER, LEAD AND CADMIUM OBTAINED WITH THE FLORENCE ELECTRODE

(i_1 and i_2 represent the peak current before and after the addition of standard)

Aliquot no.	*Copper*			*Lead*			*Cadmium*		
	i_1 *(μA)*	i_2 *(μA)*	*Conc. (p.p.b.)*	i_1 *(μA)*	i_2 *(μA)*	*Conc. (p.p.b.)*	i_1 *(μA)*	i_2 *(μA)*	*Conc. (p.p.b.)*
1	2.55	5.15	1.98	0.60	1.75	0.43	4.40	7.80	1.15
2	2.60	5.10	2.10	0.70	1.80	0.52	4.40	8.10	1.06
3	2.70	4.85	2.52	0.70	1.85	0.50	4.95	8.50	1.24
4	2.40	4.65	2.15	0.50	1.40	0.46	4.90	7.95	1.43
5	2.60	4.85	2.33	0.55	1.55	0.45	4.95	8.35	1.30
Mean			2.22			0.47			1.24
s_r (%)			10			8			11

in this investigation the sea water was only used as a model system for studying the mercury film technique, and thus the actual concentration of the metals in the sample was of minor interest.

Reproducibility and electrode material

The relative standard deviation of the data given in Table I is *ca.* 10%, which is a satisfactory reproducibility, the low concentration levels being taken into consideration. The reproducibility of four successive measurements, made on the same aliquot, is given in Table II. In this case a standard deviation of *ca.* 3% was obtained. The mercury film was not removed between the measurements indicated in Table II. The first scan, which conditions the electrode, is not included

TABLE II

REPRODUCIBILITY OF MEASUREMENTS MADE ON THE SAME ALIQUOT

(The data represent peak currents, given in μA)

Measurement no.	*Copper*	*Lead*	*Cadmium*
1	2.75	0.75	4.70
2	2.70	0.70	4.90
3	2.85	0.70	4.95
4	2.70	0.70	4.90
Mean	2.75	0.71	4.86
s_r (%)	2.5	3.5	2.5

in the Table. In agreement with the results obtained by Florence[1], we also found that the first scan always gave lower peak heights than the following scans.

The rotating glassy carbon electrode mercury plated *in situ* probably gives more reproducible results than stationary mercury-coated graphite electrodes, where the stripping response may deteriorate with time[6,7]. The glassy carbon material, with its very low porosity and great hardness, seems to be a more suitable electrode material than graphite, which must be impregnated with paraffin wax to behave properly. Also the rotating glassy carbon disc provides an effective and reproducible mass transport to the electrode, with no need for additional stirring of the solution. However, the need for conditioning the electrode with a separate scan before the determination, indicates that also for this electrode the surface structure is of some importance. This is not surprising if it is taken into consideration that the mercury film which is formed is not in fact a homogeneous film, but consists of small mercury droplets[6,9]. Even so, Florence[5] reports that his glassy carbon electrodes have not been repolished for five years! The conclusion that glassy (or vitreous) carbon is a superior material for mercury film electrodes is supported by the recent findings of Copeland *et al.*[10], whereas Clem *et al.*[7] seem to find no basis for selecting glassy carbon over graphite.

Effect of instrumental parameters

According to Roe and Toni[11] the peak current of an m.f.e. is given by:

$$i_p = e^{-1} n^2 F^2 R^{-1} T^{-1} AlvC_A \qquad (1)$$

where c_A is the concentration of the metal in the mercury, A and l are the area and thickness, respectively, of the mercury film, v the voltage scan rate, e the base of natural logarithms, and n, F, R and T have the usual meaning. By Faraday's law:

$$C_A = \frac{it}{nFAl} \qquad (2)$$

where t is the depositon time, and i is the reduction current of the metal in question, disregarding the current arising from the reduction of mercury(II) ions when a Florence electrode is used. When this particular electrode is used, i can be calculated from the equation for a rotating disc electrode[12]:

$$i = 0.88\pi^{\frac{1}{2}} nFD^{\frac{2}{3}} p^{-\frac{1}{6}} N^{\frac{1}{2}} AC_B \quad (3)$$

where C_B is the concentration in mol l^{-1} of the metal ion in the bulk solution being analyzed, N is the number of revolutions of the electrode per s, p is the kinematic viscosity ($cm^2\ s^{-1}$) and D is the diffusion coefficient ($cm^2\ s^{-1}$).

Combining eqns. (1)–(3), the desired equation for the peak current is obtained:

$$i_p = 0.88\pi^{\frac{1}{2}} e^{-1} n^2 F^2 R^{-1} T^{-1} D^{\frac{2}{3}} p^{-\frac{1}{6}} N^{\frac{1}{2}} AvtC_B \quad (4)$$

or

$$i_p = kN^{\frac{1}{2}} AvtC_B \quad (5)$$

where k is a constant.

According to Roe and Toni[11], the peak potential E_p is given by

$$E_p = E^0 + \frac{2.3\ RT}{nF} \log \frac{nF\delta lv}{RTD}$$

where E^0 is the formal electrode potential, and δ is the thickness of the diffusion layer.

Although the equations given by Roe and Toni are based on some simplifying assumptions, they seem to be in fairly good agreement with the numerical results obtained from an exact mathematical treatment[13], provided that thin films (< 10 μm) and slow scan rates (< 1 V min^{-1}) are used.

According to eqn. (4), the peak current is independent of the mercury film thickness (see also reference 13). This fact is of particular importance when a Florence electrode is used, as the mercury film is not normally removed between each measurement made on the same aliquot. This gives rise to an increase in film thickness with the number of measurements. The effect of the mercury film thickness is illustrated in Table II, where the data from four successive measurements, made on the same aliquot, are given. No increase in peak heights was observed in these experiments, when the sequence of deposition and stripping was repeated four times. The film thickness was increased by *ca.* 0.1 μm per measurement (10 min deposition time, $4 \cdot 10^{-5}$ *M* mercury(II) nitrate).

The effect of the mercury film thickness was also studied by varying the concentration of mercury(II) nitrate. The results are given in Table III. Each of

TABLE III

RELATIONSHIP BETWEEN PEAK CURRENT AND MERCURY CONCENTRATION

(The peak currents given represent mean values of three measurements)

Concn. $Hg(NO_3)_2$ (mol l^{-1})	*Peak current (μA)*		
	Copper	*Lead*	*Cadmium*
$2 \cdot 10^{-6}$	2.05	0.60	2.75
$5 \cdot 10^{-6}$	2.40	0.80	3.90
$1 \cdot 10^{-5}$	2.50	0.80	4.30
$2 \cdot 10^{-5}$	2.50	0.80	4.70
$4 \cdot 10^{-5}$	2.40	0.80	4.90

the values given is the mean of three measurements. In contrast to the results of Florence[1], no increase in peak height was observed for copper and lead, when the concentration of mercury(II) ions was increased from $5 \cdot 10^{-6}$ M to $4 \cdot 10^{-5}$ M. Only for cadmium was a slight increase in peak height observed. However, the most reproducible results were obtained with a concentration of mercury(II) ions of $4 \cdot 10^{-5}$ M, and this concentration was therefore preferred. Florence also used this concentration of mercury(II) for the analysis of sea water[3]. Excessive concentrations of mercury(II) should be avoided, as this would lead to the formation of large mercury droplets, which would fall off the rotating electrode.

The effect of the electrode rotation speed was also studied. The peak heights of copper and cadmium were found to be proportional to the square root of the rotation speed, in the range 10–100 r.p.s., as predicted by eqn. (4). Furthermore, in accordance with eqn. (4), the peak heights were found to be directly proportional to the deposition time.

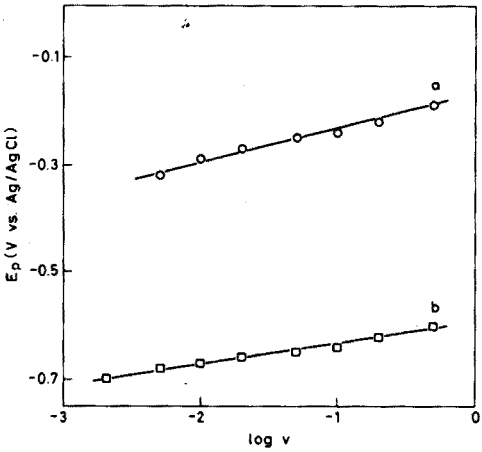


Fig. 3. Variation in peak potentials with scan rate for copper (a) and cadmium (b).

The variation in peak current and potential with scan rate was studied in the range 0.12–30 V min^{-1}. The results are given in Table IV and Fig. 3. Because the total deposition time depends on scan rate, the peak currents had to be corrected by a factor of $t/(t+t_s)$, where t_s is the time taken to scan from the deposition potential to the peak potential in question. The increases in the corrected peak heights for copper and cadmium with increasing scan rate were found to be less than predicted by eqn. (4), (direct proportionality) even at scan rates below 1 V min^{-1}. The increases were also somewhat less than those observed by Florence[1]. This behaviour would be expected for thicker films (for a h.m.d.e. the peak current is proportional to the square root of the scan rate[14]). However, our film thicknesses were usually well below 1 μm. Also the observed shift in the peak potentials with scan rate is indicative of a thin film (for a h.m.d.e. the peak potentials are independent of the scan rate[14]). The shift towards less negative potentials was found to be a linear function of the logarithm of the scan rate (see Fig. 3), in accordance with eqn. (6).

TABLE IV

EFFECT OF SCAN RATE ON PEAK CURRENT AND PEAK POTENTIAL

Scan rate, v ($V\,s^{-1}$)	*log v*	E_p (V)		Corr. i_p (μA)		$i_p v^{-1}$	
		Cu	*Cd*	*Cu*	*Cd*	*Cu*	*Cd*
0.002	−2.699	—	−0.70	—	0.29	—	145
0.005	−2.301	−0.32	−0.68	0.29	0.69	58	138
0.01	−2.000	−0.29	−0.67	0.54	1.30	54	130
0.02	−1.699	−0.27	−0.66	1.00	2.45	50	127
0.05	−1.301	−0.25	−0.65	2.45	4.85	49	97
0.10	−1.000	−0.24	−0.64	4.65	8.00	47	80
0.20	−0.699	−0.22	−0.62	7.20	11.40	36	57
0.50	−0.301	−0.19	−0.60	10.50	17.00	21	34

In contrast to the results for lead in potassium nitrate[1], the deposition potential was found to affect the height of the stripping peaks. When the deposition potential was changed from −0.8 to −1.0 V, the peak height for copper increased from 2.00 to 2.50 μA, and the peak height for cadmium from 4.60 to 5.25 μA.

When the *in situ* technique is used, the mercury film is formed at the deposition potential chosen for the metals in question. In most cases, this potential will be in the region of −0.5 to −1.3 V, and thus the problems encountered when low overpotentials are used for the mercury deposition are avoided[6]. However, the effect of the deposition potential may not be too critical, as even a deposition potential of −0.25 V has recently been used, without causing difficulties[4].

Except for the increase in peak current with scan rate, the Florence electrode seems to behave in accordance with the theory of rotating thin film electrodes. This is indeed interesting, as it has recently been shown[6,9] that the electrode, in fact, is not covered with a homogeneous mercury film, but consists of small mercury droplets.

Calibration curves and chemical species

The variation in peak height with the concentration of metal was studied by adding metals to the sea-water sample. A linear relationship was observed for both copper, lead and cadmium, as can be seen from Fig. 4. The first point on each of the calibration curves corresponds to the concentration of the metal in the sea-water sample. From Fig. 4 it can also be seen that the slope of the calibration curves is markedly different for the three metals (note that the concentration is given in μmol l^{-1}). There may be various reasons for this variation in slope. The shape of the stripping peaks (Fig. 2) is somewhat different for the three metals, the peak width for cadmium being smaller than that of copper. This will make the slope of the calibration curve somewhat larger for cadmium than for copper. However, the presence of complex-forming ligands, such as humic acids, may also have a marked effect on the slope. It has been shown that humic acids form stronger complexes with copper than with cadmium[15], which results in markedly smaller peak currents for copper than for cadmium, in accordance with the results indicated in Fig. 4. However, sea-water samples taken from different areas may contain

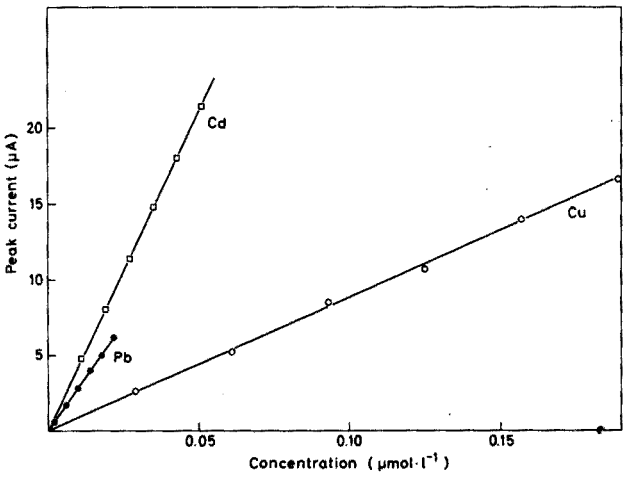


Fig. 4. Calibration curves for copper, lead and cadmium in the sea-water sample, pH 8.0.

different amounts and types of such ligands. This may be the reason why Smith and Redmond[16] found that the sensitivity (or the slope of the calibration curve; A mol^{-1} l) decreased in the sequence lead, cadmium, copper at pH 8.1, whereas Zirino and Healy[17] found that the sensitivity was higher for cadmium than for lead at pH 8.3. A h.m.d.e. was used as working electrode in these investigations[16,17], but we also obtained curves similar to those given in Fig. 4 when a h.m.d.e. was used.

Even so, all the metals present in solution as hydrated ions or as labile complexes will be determined by using the standard addition technique, or a calibration curve made from a representative sea-water sample, provided that the complex-forming ligands are present in an excess in the solution, and an equilibrium is attained in the solution after the addition of standard[17].

Comparison with the h.m.d.e.

A typical voltammogram obtained with a h.m.d.e. is shown in Fig. 5. The peaks are less well separated, compared to Fig. 2, even though a lower scan rate (0.6 V min^{-1}) was used in Fig. 5. A deposition period of 60 min was used, in order to obtain satisfactory peak heights. The peak potentials were −0.10 V (Cu), −0.40 V (Pb) and −0.55 V (Cd). The data from three independent analyses of copper, lead and cadmium are given in Table V. The concentrations were determined from calibration curves, similar to those given in Fig. 4. A t-test showed that there was agreement between the results obtained with the Florence electrode (Table I) and the h.m.d.e. (Table V) for lead and cadmium (no significant difference even at the 90% level). However, the difference between the copper values in Table I and Table V is significant even at the 99.9% level. This may be due to contamination of the sample by copper from the glass walls of the cell during the long deposition times necessary for the h.m.d.e. case (60 min).

It is obvious that mercury film electrodes, such as the Florence electrode, have many advantages, compared to the h.m.d.e. As expected, the Florence electrode

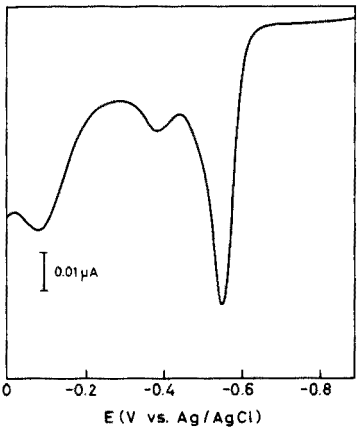


Fig. 5. Stripping voltammogram of the sea-water sample, with a hanging mercury drop electrode. Deposition time 60 min, scan rate 0.6 V min^{-1}, deposition potential −0.9 V.

TABLE V

ANALYTICAL RESULTS FOR COPPER, LEAD AND CADMIUM OBTAINED WITH A HANGING MERCURY DROP ELECTRODE

	Peak current (nA) for aliquot				*s_r (%)*	*Concn. (p.p.b.)*
	1	*2*	*3*	*Mean*		
Copper	41	49	42	44	10	3.8
Lead	4	5	5	4.7	12	0.5
Cadmium	73	80	83	79	7	1.2

gave good resolution of neighboring peaks even at relatively fast scan rates. It also produced a dramatic increase in sensitivity (*i.e.* peak current per concentration unit, for a given deposition time). Thus, when the peak currents in Tables I and V are compared, and the six times longer deposition period for the h.m.d.e. is considered, it is found that the sensitivity for copper and cadmium is increased by a factor of 350. This is primarily due to the fact that the concentration of metals is much higher in the film than in the mercury drop. However, other parameters also contribute to the improved sensitivity of the m.f.e.; thus a higher rate of mass transport can be used in combination with a m.f.e. than when a h.m.d.e. is used; with the h.m.d.e., the mercury drop tends to fall off if the solution is stirred too vigorously. Furthermore, a faster scan rate can be used for an m.f.e. than for a h.m.d.e., without losing the good resolution of the peaks. Also the peak current is directly proportional to the scan rate for an m.f.e., whereas it is proportional only to the square root of the scan rate for a h.m.d.e. Lastly, the peak current for an m.f.e. is proportional to the area of the electrode, whereas for a h.m.d.e. it increases with the radius of the mercury drop[14]. The radius of a mercury drop can hardly be increased much above 0.5 mm, whereas areas as large as 2.0 cm^2 have been employed for film electrodes[6]. However, as the charging current is also

directly proportional to the scan rate and electrode area[14], the detection limit of a m.f.e., which depends on the magnitude of the residual current, cannot be improved by increasing the scan rate or electrode area.

We are grateful to Dr. Arne Thorvin Andersen at the Institute of Marine Biology and Limnology, University of Oslo, for providing the sea water samples.

SUMMARY

The use of the rotating glassy carbon electrode mercury plated *in situ* for anodic stripping voltammetry has been investigated. The choice of electrode material is discussed. The effect of instrumental parameters on the stripping response for copper, lead and cadmium in sea water is studied, the results being in accordance with the theory of thin film electrodes. The variation in the observed sensitivity for the three metals in sea water is discussed in terms of complex-forming ligands. Lastly the performance of the film electrode is compared to that of the hanging mercury drop electrode.

REFERENCES

1 T. M. Florence, *J. Electroanal. Chem. Interfacial Electrochem.*, 27 (1970) 273.
2 T. M. Florence, *J. Electroanal. Chem. Interfacial Electrochem.*, 26 (1970) 293.
3 T. M. Florence, *J. Electroanal. Chem. Interfacial Electrochem.*, 35 (1972) 237.
4 T. M. Florence, *J. Electroanal. Chem. Interfacial Electrochem.*, 49 (1974) 255.
5 T. M. Florence and Y. J. Farrar, *J. Electroanal. Chem. Interfacial Electrochem.*, 51 (1974) 191.
6 D. N. Hume and J. N. Carter, *Chem. Anal. (Warsaw)*, 17 (1972) 747.
7 R. G. Clem, G. Litton and L. D. Ornelas, *Anal. Chem.*, 45 (1973) 1306.
8 W. Lund and B. V. Larsen, *Anal. Chim. Acta*, 72 (1974) 57.
9 M. Stuliková, *J. Electroanal. Chem. Interfacial Electrochem.*, 48 (1973) 33.
10 T. R. Copeland, J. H. Christie, R. A. Osteryoung and R. K. Skogerboe, *Anal. Chem.*, 45 (1973) 2171.
11 D. K. Roe and J. E. A. Toni, *Anal. Chem.*, 37 (1965) 1503.
12 R. N. Adams, *Electrochemistry at solid electrodes*, Dekker, New York, 1969, p. 80.
13 W. T. DeVries, *J. Electroanal. Chem. Interfacial Electrochem.*, 9 (1965) 448.
14 W. D. Ellis, *J. Chem. Educ.*, 50 (1973) A131.
15 T. A. O'Shea, H. E. Allen and K. H. Mancy, Paper Presented at the 2nd Trace Metals Symposium, The University of Illinois, Champaign, Ill., Nov. 1971.
16 J. D. Smith and J. D. Redmond, *J. Electroanal. Chem. Interfacial Electrochem.*, 33 (1971) 169.
17 A. Zirino and M. L. Healy, *Environ. Sci. Technol.*, 6 (1972) 243.

Analytica Chimica Acta, 76 (1975) 143–147

THE USE OF VITREOUS CARBON AND CARBON FIBRE ELECTRODES FOR THE COULOMETRIC GENERATION OF IODINE

V. J. JENNINGS, A. DODSON and R. J. EASTMAN
Lanchester Polytechnic, Priory Street, Coventry CV1 5FB (England)
(Received 23rd July 1974)

Previous papers have described the use of vitreous or glassy carbon as generating electrodes in coulometric bromine titrations[1,2] and coulometric acid–base titrations[3]. The present paper reports on the use of vitreous carbon and carbon fibre in coulometric iodine titrations.

Carbon fibre[4] is similar in manufacture and properties to vitreous carbon and is available as a tow containing up to 10000 parallel filaments each of about 8 μm in diameter. Thus a tow length of 1 cm should provide an active surface area of about 25 cm^2 for an electrochemical reaction. Since limiting currents are dependent on the surface area of the electrode and high limiting currents are desirable for high current efficiencies in coulometric titrations, an electrode of carbon fibre presents some promise.

It is known that electrode reactions involving the iodine system vary with different electrode materials. Zittel and Miller[5] have reported that the chronopotentiograms for the oxidation of iodide at pyrolytic graphite and vitreous carbon electrodes showed three steps, for which they postulated three reactions:

$$I = \tfrac{1}{2} I_2 + e^- \quad (1)$$

$$\tfrac{1}{2} I_2 + H_2O = IO^- + 2 H^+ + e^- \quad (2)$$

$$IO^- + 2 H_2O = IO_3^- + 4 H^+ + 4 e^- \quad (3)$$

In the present work, the potentiometric titration of arsenic(III) was examined with coulometrically generated iodine at a vitreous carbon electrode in phosphate buffered solution of pH 7; these results were compared to those obtained by titrating the same arsenic(III) solution in acidic media with bromine. A pH of 7 has been recommended by Marinenko and Taylor[6] and others as the optimal pH for arsenic(III) titrations with iodine. The current–voltage curves obtained at vitreous carbon, carbon fibre and platinum electrodes for the generation of iodine are also described. Finally, titrations of sodium thiosulphate by iodine generated at vitreous carbon, carbon fibre and platinum electrodes are reported.

EXPERIMENTAL

Potentiometric titration of arsenic(III) with coulometrically generated iodine

The preparation of the 0.001 *M* arsenic(III) solution and the cell used have

been described previously[1]. A Thorn coulometric titrator (TE110) provided the current. Since the arsenic(III) solution was acidified to pH 1.48 to keep it in a stable oxidation state and the quantity of phosphate buffer that could be used was limited, it was necessary to add sufficient (about 15 ml 0.5 *M*) sodium hydroxide solution to neutralize (to about pH 7) the 25 ml of arsenic(III) solution pipetted into the coulometric cell. The blank titre was obtained on 150 ml of 0.025 *M* disodium hydrogenphosphate–0.025 *M* potassium dihydrogenphosphate in which 1.6 g of solid potassium iodide was dissolved to give a solution 0.05 *M* in iodide. The potentiometric end-points for both blank and arsenic(III) titrations were taken when the platinum indicator electrode reached a potential of 175 mV *versus* the saturated calomel reference electrode. After the blank titre had been found (the mean value was 39 mC; about 0.4% of the arsenic(III) value of 10 C), the 25 ml of arsenic(III) solution was added together with the amount of sodium hydroxide solution required for neutralization. The constant current used was 4.990 mA and the active area of the vitreous carbon electrode[1] was 1 cm^2.

Current–voltage curves for iodine generation at vitreous carbon, carbon fibre and platinum electrodes

Current–voltage curves were obtained as described previously for bromine generation[1]. The carbon fibre electrode was made by sealing a length of carbon fibre into a pyrex glass tube with an epoxy resin at both ends. A 2-cm length

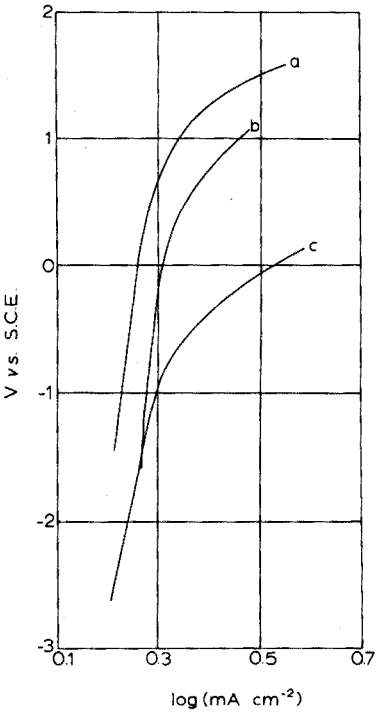


Fig. 1. Effect of current density on working electrode potential; (a) platinum, (b) vitreous carbon, and (c) carbon fibre.

was dipped into the electrolyte solution and a slight pressure contact was made at the other end. It was found that it was easy to break the fibres by strong pressure. It was necessary to seal the fibre into the glass tube in order to prevent the solution "climbing" up the fine fibres by capillary action.

The active geometrically measured surface areas for the vitreous carbon and platinum working electrodes were 2.7 cm^2 and 0.9 cm^2, respectively. The cell used contained 30 ml of a solution 0.167 *M* in both disodium hydrogenphosphate and potassium dihydrogenphosphate, to which was added, in the anolyte compartment, 2.5 g of solid potassium iodide. The solution was stirred magnetically until the iodide dissolved to give a solution 0.5 *M* in iodide. The limiting current for this concentration should be 250 mA at a 1-cm^2 platinum electrode[7]. The catholyte compartment (capacity 3 ml) containing a platinum auxiliary electrode was separated from the analyte solution by a sintered-glass disc, porosity 4.

Figure 1 is a graphical plot of log current density against the potential of the working electrode measured against a saturated calomel reference electrode. Current–voltage curves were obtained for other concentrations of iodide as well as 0.5 *M* and showed the expected effect of increasingly positive potentials required for lower iodide concentrations to maintain a constant current. With 0.5 *M* iodide it was possible to obtain a maximum current of about 32 mA from the Thorn coulometer. Attempts to increase the output current above this figure gave a non-linear response.

Titrations of sodium thiosulphate with coulometrically generated iodine at vitreous carbon, carbon fibre and platinum electrodes

Titrations of 1-ml aliquots of 0.1 *M* standardized sodium thiosulphate solution were carried out in a titration cell and electrolyte solution similar to that described for the current–voltage curves. The pipette was calibrated and found to deliver 1.002 ml at 20°C. The end-point was found by the addition of 1 ml of 1% starch solution as indicator. The constant current from the Thorn coulometric titrator was 25 mA and titration times were about 380 s. To reduce the possibility of oxidation of the iodide by dissolved air, pure nitrogen was bubbled through the buffer solution for 10 min before the solid iodide was added and an atmosphere of nitrogen was maintained above the surface of the solution during the titration.

RESULTS AND DISCUSSION

Table I shows that the coulometric generation of iodine at a vitreous carbon anode gives results of similar accuracy and precision to those obtained in coulometric bromine titrations which were done as described previously[1]. It was expected that the addition of sodium hydroxide to neutralize the acid present in the arsenic(III) solution might interfere with the accuracy and/or precision, but any effects were too small to be detected.

The log current density *versus* electrode potential plots (Fig. 1) show that vitreous carbon has an over-voltage of about 50 mV compared to platinum. This value can be compared to that of 100 mV found previously for the generation of bromine[1]. The 2.3 RT/nF slope factor (for linear segments of the graphs) is about the same for both platinum and vitreous carbon, 40–35 mV, respectively.

TABLE I

COMPARISON OF VITREOUS CARBON WORKING ELECTRODES USED IN THE COULOMETRIC GENERATION OF IODINE AND BROMINE FOR THE TITRATION OF ARSENIC(III)

	Iodine	*Bromine*
Arsenic(III) taken (μeq)	100.1	100.1
Arsenic(III) found (mean value) (μeq)	100.3	100.3
Number of results	7	7
Relative standard deviation (%)	0.1	0.2
Titration error (%)	0.2	0.2

Speculatively this suggests an electrochemical reaction with an n value of 1.5 or more but below 2. However, for the direct formation of iodine n should be 1, and for hypoiodite n should be 2.

After a period of use the vitreous carbon electrode showed some change in the current–potential curves, the electrode potential becoming more positive for a given applied current. It was possible to reproduce the current–potential curve by cleaning either in an ultrasonic bath with toluene, or by wiping the surface with tissues moistened with methanol and chloroform.

The carbon fibre electrode showed similarities to the vitreous carbon electrode though the slope factor was about 50 mV. The surface area calculation of 50 cm^2 is very approximate, since the very thin fibres had a tendency to break.

The thiosulphate titration results reported in Table II suggest that either vitreous carbon or carbon fibre may replace platinum though there is some indication that the accuracy is decreased; a slight positive error is present for both materials compared to platinum. It is possible that both electrodes adsorb the iodine species produced electrochemically on the surface. In addition, with the carbon fibre, the individual filaments tended to mat and it was necessary to maintain vigorous stirring around the end-point to avoid over-titration. Some potentiometric titrations showed that there was a difference amounting to about 0.2 s between the potentiometric end-point and starch end-point. The latter is said

TABLE II

COULOMETRIC TITRATION OF SODIUM THIOSULPHATE WITH IODINE GENERATED AT VITREOUS CARBON, PLATINUM AND CARBON FIBRE ANODES

(1.002 ml of 0.1000 M $Na_2S_2O_3$ was taken; this requires a generating current of 25.000 mA for a time of 386.7 s)

Anode material	*Mean*[a] *(s)*	*% Error of mean*	s_r *(%)*
Vitreous carbon	388.5	0.5	0.2
Carbon fibre	388.2	0.4	0.3
Platinum	387.5	0.2	0.5

[a] Mean of 8 results.

to require a concentration of $2.5 \cdot 10^{-5}$ *M* iodine before the starch–iodine blue coloured complex is formed. The potentiometric end-points would require an excess concentration of iodine of perhaps one tenth of this value. However, since the starch–iodine end-points were obtained by difference between total times minus blank times, the positive errors of 1–2 s obtained with vitreous carbon and carbon fibre probably indicate some iodine adsorption effect which does not occur with platinum.

We thank the Carbon Fibres Unit of Courtaulds Ltd., Coventry for their gift of samples of Grafil AU carbon fibre.

SUMMARY

Vitreous carbon and carbon fibre can be used as working electrodes for the generation of iodine in coulometric titrations. Results are presented comparing the behaviour of these two materials with platinum in arsenic(III) and/or thiosulphate titrations.

REFERENCES

1 V. J. Jennings, A. Dodson and A. M. Atkinson, *Analyst (London)*, 97 (1972) 923.
2 V. J. Jennings, A. Dodson and A. Harrison, *Analyst (London)*, 99 (1974) 145.
3 V. J. Jennings, A. Dodson and G. Tedds, *Talanta*, 20 (1973) 681; 21 (1974) 21.
4 R. M. Gill, *Carbon Fibres in Composite Materials*, The Plastics Institute, London, 1972, p. 14.
5 H. E. Zittel and F. J. Miller, *J. Electroanal. Chem. Interfacial Electrochem.*, 13 (1967) 193.
6 G. Marinenko and J. K. Taylor, *Anal. Chem.*, 39 (1967) 1568.
7 J. J. Lingane, *Electroanalytical Chemistry*, Interscience, New York, 2nd edn., 1958, p. 490.

Analytica Chimica Acta, 76 (1973) 149–154

A METHOD FOR THE DETERMINATION OF THE DISSOCIATION CONSTANTS OF ACIDS WITH AN UNCALIBRATED GLASS ELECTRODE

M. BOS and W. LENGTON

Department of Chemical Technology, Twente University of Technology, Enschede (The Netherlands)

(Received 7th October 1974)

In the development of acid–base titrations in non-aqueous media, the determination of acid–base dissociation constants and the establishment of the pH scale in the solvent of interest is very important. By potentiometry with either the hydrogen or glass electrode in cells with or without a liquid junction, these dissociation constants can be determined over a wide range[1–3]. It is generally assumed[1] that the hydrogen or glass electrode should first be calibrated in solutions of known pH, prepared from either strong acids or from weak acids with known dissociation constant.

To establish a pH scale in some solvents, it is thus necessary to determine at least one acid dissociation constant by means of another technique. For this purpose differential vapor pressure measurements, conductivity measurements or spectrophotometry are often used. In this paper it is shown that dissociation constants of weak acids can also be determined directly with an uncalibrated glass electrode.

THEORY

The dissociation constant of a weak acid in a solvent in which no ion pairs are formed, can be expressed by:

$$K_{HX} = a_{H^+} a_X / a_{HX} \tag{1}$$

If the activity coefficient of the uncharged species is assumed to be unity and the degree of dissociation is low, then the activity of the solvated proton is given by:

$$a_{H^+} = (K_{HX} C_{HX})^{\frac{1}{2}} \tag{2}$$

For a solution of a mixture of the acid and its salt (which is assumed to be completely dissociated), the activity of the solvated proton can be calculated from:

$$a_{H^+} = K_{HX} \frac{a_{HX}}{a_X} = K_{HX} \frac{C_{HX}}{C_X} \frac{1}{f_X} \tag{3}$$

With the use of equimolar amounts of acid and its salt this reduces to:

$$a_{H^+} = K_{HX}/f_X \tag{4}$$

The response at 20 °C of a glass electrode–reference electrode set following Nernst's law can be written as:

$$E_{mV} = E'_0 + 58 \log a_H \quad (5)$$

E'_0 contains the standard potential of the glass electrode, the potential of the reference electrode and the liquid junction potential, which is assumed to be constant. Combination of eqns. (2) and (5) gives for the potential of the glass electrode in a solution of pure acid, HX:

$$E_{acid} = E'_0 - 29\ pK_{HX} + 29 \log c_{HX} \quad (6)$$

Combination of eqns. (4) and (5) gives

$$E_{buffer} = E'_0 - 58\ pK_{HX} - 58 \log f_{X^-} \quad (7)$$

Subtraction of eqns. (6) and (7) finally gives:

$$E_{acid} - 29 \log c_{HX} - E_{buffer} - 58 \log f_{X^-} = 29\ pK_{HX} \quad (8)$$

If f_{X^-} is estimated by the limiting Debye–Hückel law, then all quantities on the left-hand side of eqn. (8) are known and pK_{HX} can be calculated. Now also E'_0 can be calculated, which calibrates the glass electrode set.

EXPERIMENTAL

Solvents

Acetonitrile containing less than 0.01% water was obtained from Eastman. Dimethylsulfoxide (Merck, reagent grade) was heated to 60 °C with barium oxide and potassium permanganate for 1 h; the mixture was then filtered and the DMSO was kept for 24 h over activated molecular sieve (3A, Union Carbide). Further purification of the DMSO was performed by vacuum distillation (3 mm, 42 °C), during which the middle 80% fraction was kept. Finally the DMSO was kept over molecular sieve for 24 h and vacuum-distilled again. The water content of the end-product was less than 0.01% (Karl Fischer titration). N,N-dimethylformamide (Merck, reagent grade) was kept for 24 h over molecular sieve (3A, Union Carbide) and then vacuum-distilled (18 mm, 52 °C); the middle 80% fraction was used in the experiments.

Acids, bases and salts

Picric acid (Merck, reagent grade), tetraethylammonium perchlorate, TEAP (Eastman), 2,6-dinitrophenol (Merck, indicator grade), 2,4-dinitrophenol (Merck, indicator grade), 2-nitrophenol (Fluka, puriss.) and 3-nitrophenol (Fluka, puriss) were recrystallized from ethanol and dried *in vacuo* at 30 °C. 1,1,3,3-Tetramethylguanidine (Eastman), benzoic acid (UCB, reagent grade), salicylic acid (ACF, reagent grade), sodium hydroxide (Merck, titrisol), tetraethylammonium iodide (Merck, für die polarographie), and tetramethylammonium bromide (Merck, für die Polarographie) were used as received.

Apparatus

A Radiometer pH-meter type PHM 26 was used in the expanded-scale mode for the determination of the e.m.f. values of the glass electrode to ± 0.2 mV. As reference electrode in the organic solvents a silver chloride-coated silver wire

immersed in 0.01 *M* tetramethylammonium chloride in the solvent of interest was used. This electrode was connected to the solution via a 0.1 *M* tetraethylammonium perchlorate salt bridge. Separation between reference electrode, salt bridge and solution was by glass frits.

In aqueous solution, a Radiometer 3 *M* KCl calomel electrode, type K 411, was used.

In all cases the cell was thermostatted to (20.0±0.05) °C.

Procedure

A sample of a standardized solution of the acid was placed in the cell and mV readings of the glass electrode set were taken at 5-min intervals until a stable reading (within ±0.5 mV) was obtained. This usually occurred within 10–20 min in the solvents used, except for DMF, for which 60–80 min were necessary. Then a calculated amount of base was added to give an equimolar amount of acid and its salt and mV measurements were continued.

In some instances buffer solutions were prepared separately from the acid and its salt. It was noticed, however, that reproducibility was then less good, probably because the electrode set has to be transferred from one solution to another, which can change its E'_0 value[3]. When not in use, the glass electrode was stored in aqueous buffer of pH 7.

RESULTS AND CONCLUSIONS

The results of the e.m.f. measurements with the glass electrode in water are given in Table I. Tables II, III and IV show the data obtained with the glass electrode in DMSO, DMF and acetonitrile. From a comparison of the

TABLE I

POTENTIOMETRY WITH THE GLASS ELECTRODE IN WATER

Compound	*e.m.f. (mV)*	$f_{\pm}$[a]	pK_a	E'_0 *(mV)*
Benzoic acid, 10^{-2} *M*	182.0			
Benzoic acid–Na benzoate, 10^{-3} *M*	120.8	0.95	4.15	360.5
Benzoic acid, 10^{-3} *M*	153.0			
Benzoic acid–Na benzoate, 10^{-3} *M*	120.8	0.95	4.15	360.5
Acetic acid, 0.1224 *M*	195.8			
Acetic acid–Na acetate, 0.0490 *M*	95.0	0.77	4.62	356.2
Acetic acid, 0.01224 *M*	168.0			
Acetic acid–Na acetate, 0.00490 *M*	92.8	0.92	4.58	356.3
2,5-Dinitrophenol, $5.82 \cdot 10^{-4}$ *M*	122.8			
2,5-Dinitrophenol–Na salt, $1.16 \cdot 10^{-3}$ *M*	67.0	0.95	5.17	366.5
2-Nitrophenol, 5.10^{-3} *M*	88.0			
2-Nitrophenol–Na salt, 10^{-3} *M*	−49.0	0.95	7.04	357.7
3-Nitrophenol, 0.100 *M*	68.0			
3-Nitrophenol–Na-salt, $2.67 \cdot 10^{-3}$ *M*	−110.5	0.94	8.20	364.0

[a] Calculated with Debye–Hückel limiting law: $-\log f = 0.5010\,(\mu)^{\frac{1}{2}}$.

TABLE II

POTENTIOMETRY WITH THE GLASS ELECTRODE IN DMSO

Compound	*e.m.f. (mV)*	$f_{\pm}$[a]	pK_a	E'_0 *(mV)*
2,6-Dinitrophenol, 0.100 *M*	248.0			
2,6-Dinitrophenol–TMG salt, 0.0342 *M*	160.0	0.62	4.45	405.9
2,4-Dinitrophenol, 0.100 *M*	243.0			
2,4-Dinitrophenol–TMG salt, 0.0339 *M*	149.5	0.62	4.64	406.4
Salicylic acid, 0.100 *M*	198.0			
Salicylic acid–TMG salt, 0.0339 *M*	65.0	0.62	6.00	400.9
Benzoic acid, 0.100 *M*	30.0			
Benzoic acid–TMG salt, 0.0342 *M*	−192.5	0.62	9.08[b]	322.4

[a] Calculated with Debye–Hückel limiting law: $-\log f = 1.11\,(\mu)^{\frac{1}{2}}$.
[b] pK_a (benzoic acid) = 10.3 calculated from buffer potential with $E'_0 = 403$ mV.

TABLE III

POTENTIOMETRY WITH THE GLASS ELECTRODE IN DMF

Compound	*e.m.f. (mV)*	$f_{\pm}$[a]	pK_a	E'_0 *(mV)*
2,4-Dinitrophenol, 0.100 *M*	375.0			
2,4-Dinitrophenol–TMG salt, 0.0333 *M*	235.8	0.53	6.36	588.4
2,6-Dinitrophenol, 0.100 *M*	372.0			
2,6-Dinitrophenol–TMG salt, 0.0333 *M*	239.3	0.53	6.13	578.9

[a] Calculated with Debye–Hückel limiting law: $-\log f = 1.53\,(\mu)^{\frac{1}{2}}$.

TABLE IV

POTENTIOMETRY WITH THE GLASS ELECTRODE IN ACETONITRILE

Compound	*e.m.f. (mV)*	$f_{\pm}$	pK_a	E'_0 *(mV)*
Hydriodic acid, 0.0834 *M*	981.0			
Hydriodic acid–TEA iodide, 0.0668 *M*	935.0	0.59[a]	3.12	1102.9
Hydrobromic acid 0.1997 *M*	907.3			
Hydrobromic acid–TMA bromide, 0.0100 *M*	784.0	0.70[b]	5.26	1080.1
Bromophenol blue, 0.0100 *M*	651.5			
Bromophenol blue TMG salt, $4.73 \cdot 10^{-3}$ *M*	399.7	0.79[b]	10.90[c]	1025.4

[a] Calculated with extended Debye–Hückel expression: $-\log f = \dfrac{1.53\,(\mu)^{\frac{1}{2}}}{1+2.82\,(\mu)^{\frac{1}{2}}}$.
[b] Calculated with Debye-Hückel limiting law: $-\log f = 1.53\,(\mu)^{\frac{1}{2}}$.
[c] $pK_a = 12.0$ calculated with buffer potential and $E'_0 = 1092$.

obtained pK_a values and the literature values (Table V), it can be seen that the direct potentiometric method gives results which are generally accurate to ± 0.2 pK units. Exceptions are the experiments for the determination of pK_a values above 10; probably this is due to impurities in either the solvent or acid used. Basic impurities in the solvent, even in low concentrations, cause deviations from

TABLE V

COMPARISON OF pK_a VALUES FROM THE POTENTIOMETRIC METHOD AND LITERATURE VALUES

System	*pK_a found*	*pK_a lit.*	*Ref.*
Benzoic acid–water	4.15	4.19	4
Acetic acid–water	4.60	4.75	4
2,5-Dinitrophenol–water	5.17	5.15	4
2-Nitrophenol–water	7.04	7.17	4
3-Nitrophenol–water	8.20	8.28	4
2,6-Dinitrophenol–DMSO	4.45	4.9	5
2,4-Dinitrophenol–DMSO	4.64	5.2	6
Salicylic acid–DMSO	6.00	6.9	5
Benzoic acid–DMSO	9.08/10.3[a]	11.0	5
2,4-Dinitrophenol–DMF	6.36	6.3	7
2,6-Dinitrophenol–DMF	6.13	5.7/6.18	7
Hydriodic acid–acetonitrile	3.12	—	
Hydrobromic acid–acetonitrile	5.26	5.5	8
Bromophenol blue–acetonitrile	10.90/12.0[a]	12.0	9

[a] Determined from buffer potential with E'_0 from the other experiments.

the expression $a_{H^+} = (KC_{HX})^{\frac{1}{2}}$ for solutions of the acid. This problem has also been encountered in connection with the conductance method[1], but the potentiometric method compares favourably with this method as much higher concentrations of the acid can be used.

The authors wish to thank Prof. Dr. Ir. E. A. M. F. Dahmen for his support and Mrs. B. Verbeeten-v. Hettema for preparing the manuscript.

SUMMARY

A method is presented for the determination of absolute pK_a values of acids in solvents with a high dielectric constant by potentiometry with an uncalibrated glass electrode; in the determination, the glass electrode becomes calibrated. The method has the advantage that it is rapid and simple. Moreover, the range of pK_a values that can be determined by this method is about 3 pK units greater than for the direct conductivity method.

REFERENCES

1 E. J. King in A. K. Covington and T. Dickinson (Eds.), *Phys. Chem. Org. Solvent Systems*, Plenum Press, London, 1973, p. 331.
2 R. F. Cookson, *Chem. Rev.*, 74 (1974) 5.
3 A. Albert and E. P. Serjeant, *The determination of ionization constants*, Chapman and Hall, London, 2nd edn., 1971, p. 10.
4 R. C. Weast, S. M. Selby and C. D. Hodgman, *Handbook of Chemistry and Physics*. The Chemical Rubber Co., 45th edn., 1964, p. D77.
5 I. M. Kolthoff, M. K. Chantooni and S. Bhowmik, *J. Amer. Chem. Soc.*, 90 (1968) 23.

6 B. W. Clare, D. Cook, E. C. F. Ko, Y. C. Mac and A. J. Parker, *J. Amer. Chem. Soc.*, 88 (1966) 1911.
7 I. M. Kolthoff, M. K. Chantooni and H. Smagowski, *Anal. Chem.*, 42 (1970) 1622.
8 I. M. Kolthoff, S. Bruckenstein and M. K. Chantooni, *J. Amer. Chem. Soc.*, 83 (1961) 3927.
9 I. M. Kolthoff, S. Bhowmik and M. K. Chantooni, *Proc. Nat. Acad. Sci. U.S.A.*, 56 (1966) 1370.

Analytica Chimica Acta, 76 (1975) 155–164

PREPARATION AND PROPERTIES OF A CARBONATE ION-SELECTIVE MEMBRANE ELECTRODE

H. B. HERMAN* and G. A. RECHNITZ

Department of Chemistry, State University of New York, Buffalo, New York 14214 (U.S.A.)

(Received 21st October 1974)

The measurement of carbonate and hydrogen carbonate concentration levels is important to several disciplines. Previous attempts to prepare hydrogen carbonate ion-selective membrane electrodes have resulted in electrodes which have a relatively low hydrogen carbonate to chloride selectivity[1,2]. A procedure[3] with 1-decanol as solvent also yields an electrode with a low hydrogen carbonate to chloride selectivity, although a recent patent[4], based on different solvents and exchangers, has claimed higher selectivity. A successful gas sensing electrode for carbon dioxide has been described[5]. A preliminary communication[6] has been published concerning a carbonate-selective membrane electrode; to our knowledge, no other reports have appeared.

The present paper reports data on a liquid membrane electrode which is highly selective to carbonate over chloride, sulfate, phosphate and hydrogen carbonate. The electrode exhibits Nernstian response over a wide range of carbonate concentrations. In order that other laboratories can utilize this electrode, its preparation and properties are described in detail.

EXPERIMENTAL

Preparation of electrode

The electrode was constructed with an Orion series 92 electrode body and a Millipore VC cellulose acetate support membrane with 0.1-μm average pore size and 4-mm diameter. The support membrane is not silylanized and is therefore not hydrophobic. However, if the membrane is first soaked with the liquid ion exchanger before the aqueous inner solution is added, a stable interface is formed. Commercially available hydrophobic Orion calcium membranes appeared to give identical response. The electrode was stored in air when not being used.

The active liquid phase consisted of a quaternary ammonium salt, Aliquat 336 (General Mills), dissolved in the organic solvent. The salt was made up at 1% (v/v) except for some early studies at 10% (v/v). The salt was used as received in chloride form or after conversion to the hydrogen carbonate form. A mixture of sodium chloride and sodium hydrogen carbonate, both 0.1 *M*, was used as an inner reference solution. Solutions to be measured were thermostated at 25°C and stirred from above with a Corning model LM-2 stirrer.

---

* On leave from the University of North Carolina at Greensboro, Greensboro, North Carolina 27412

Preparation of special solvent

The organic solvent used to form the liquid membrane, trifluoroacetyl-*p*-butylbenzene, was synthesized by a Friedel–Crafts acetylation of butylbenzene with trifluoroacetic anhydride and anhydrous aluminum chloride catalyst. Introduction of the trifluoroacetyl group into various compounds has been described[7]. However, details of the preparation of this compound have not appeared and are summarized here. A mixture of butylbenzene (60 g) and carbon disulfide (200 ml) was cooled to −5°C with an ice–salt mixture in a 500-ml three-neck round-bottom flask. Anhydrous aluminum chloride (120 g) was then added with the aid of a powder funnel. The flask was fitted with a sealed stirrer, dropping separatory funnel and condenser. Trifluoroacetic anhydride (100 g), stored at 0°C, was added dropwise over a 2-h period from the funnel. The mixture gradually darkened during the addition. The temperature should be allowed to rise as little as possible. If the reaction becomes too vigorous there is the possibility of foaming boil-over. The reaction was continued for about 2 h after the addition, until hydrogen chloride gas ceased to be evolved. The dark, two-phase mixture was slowly and carefully added to ice (150 g) and hydrochloric acid (150 ml) in a beaker. The carbon disulfide layer was separated, and washed three times with a total of 300 ml of 10% hydrochloric acid. Simple distillation at atmospheric pressure (to 150°C) was used to remove most of the remaining carbon disulfide in the unextracted material. The product (b.p. *ca.* 242°C at 760 torr) was collected by vacuum distillation (at 20 torr) with a 8.5-cm Vigreaux column. Most of the product was collected at 120°C although the temperature was allowed to rise to 123°C before the distillation was halted. The yield was estimated to be about 44%.

Gas chromatographic analysis was carried out with a Hewlett-Packard F and M model 5750 Research Chromatograph and Hewlett-Packard model 3370A integrator. A 2.4 m × 0.6 cm column packed with 20% SE30 on Chromosorb W (acid-washed and treated with dimethylchlorosilane) was used. Conditions were: initial temperature 170°C, programming rate 6°C min^{-1}, helium flow rate 100 ml min^{-1}, thermal conductivity detector. A mass spectrogram was taken with a Hitachi Perkin–Elmer RMU-6 spectrometer set for a 70-eV ionization potential. An n.m.r. spectrum was taken with a 100-MHz Jelco model MH-100NMR spectrometer; the sample was dissolved in carbon tetrachloride (25% (v/v)) + TMS. An i.r. spectrum was taken on a neat sample between salt plates on a Perkin–Elmer model 727 spectrophotometer.

Behavior of electrodes

The electrode behavior appeared typical of liquid membrane electrodes. The response times ranged from about 30 s to a few minutes, the slowest response being found at the lowest carbonate levels. In most carbonate solutions the electrical noise did not exceed 0.2 mV, and a reproducibility of about 0.5 mV from sample to sample was readily achieved. Electrode lifetime in routine use will depend on factors such as the specific support membrane and solutions conditions but appears to be consistent with other reports[2]. Some of the data reported here were taken with an electrode more than one month old.

Millivolt readings for the carbonate and glass electrodes were taken with an Orion model 801 pH meter and model 605 electrode switch. Buffer solutions for

calibrating the glass electrode were made as recommended by Bates[8]. Reagent-grade chemicals, dried if necessary, were used for all experiments.

RESULTS AND DISCUSSION

Characterization of the organic solvent

Since the properties of the electrode discussed here seem to be determined by the solvent used to form the liquid membrane, it was felt important to characterize fully the synthesized material, in order to aid other laboratories in preparing satisfactory electrodes.

The distilled product was a stable colorless liquid with a density of 1.107 g ml^{-1} at 25°C. The gas chromatographic results are shown in Fig. 1. In addition to the air peak and one major component, seven minor compounds can be distinguished. Integrated areas and retention times are shown in Table I. The major component at 90.4% was identified as trifluoroacetyl-*p*-butylbenzene by the results below. If peaks 3 and 5 correspond to the *o*- and *m*-isomers, then the synthesized material is at least 96% a mixture of the isomers. From previous studies on solvent mixtures, it is believed that further purification would not appreciably change the properties of a liquid membrane electrode made with this solvent. Redistillation and/or a longer, more efficient distillation column should substantially improve the purity.

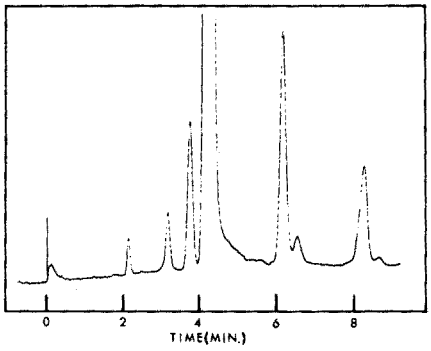


Fig. 1. Gas chromatogram of trifluoroacetyl-*p*-butylbenzene and minor impurities. Conditions discussed in Experimental section.

The major peaks in the mass spectrum of the distilled product are shown in Table II. The parent peak at 230*m/e* is correct for a trifluoroacetyl-substituted butylbenzene. The base peak at 160*m/e* can be explained by loss of an HCF_3 neutral fragment. A decomposition releasing fluoroform has been observed[7] for similar compounds in solution reactions in the presence of 10% potassium hydroxide. No peaks higher than 230*m/e* were observed for this material, but earlier runs on less pure mixtures had noticeable peaks at 286, 268 and 246 *m/e*. Apparently the side reactions which tend to reduce the yield also produce compound(s) with higher molecular weights than the parent compound. The i.r. spectrum of the product is

TABLE I

MAJOR COMPONENTS IN THE GAS CHROMATOGRAPHIC ANALYSIS OF TRIFLUOROACETYL-*p*-BUTYLBENZENE AND MINOR IMPURITIES[a]

Peak	*Relative area (%)*	*Uncorrected Retention time (min)*
1	0.45	2.0
2	0.79	3.0
3	2.20	3.5
4	90.40	4.0
5	4.11	5.8
6	0.20	6.2
7	1.84	7.7
8	0.03	8.1

[a] Air peak at 0.35–0.36 min. Conditions discussed in Experimental section.

TABLE II

MAJOR PEAKS IN THE MASS SPECTRUM OF TRIFLUOROACETYL-*p*-BUTYLBENZENE[a]

m/e	*% of base peak*	*m/e*	*% of base peak*	*m/e*	*% of base peak*
231	1.5	130	2.6	102	4.7
230	11.3[b]	118	2.8	92	2.8
186	3.4	117	11.0	91	29.2
161	12.8	114	3.0	90	10.6
160	100[c]	104	4.9	89	5.4
158	4.5	103	4.9	77	7.1
132	4.6				

[a] Conditions discussed in the Experimental section.
[b] Parent peak.
[c] Base peak.

shown in Fig. 2. The peak at 1727 cm^{-1} clearly shows that a carbonyl group had been incorporated into the starting material, butylbenzene. The peaks centered around 1180 cm^{-1} probably can be attributed to the trifluoromethyl group.

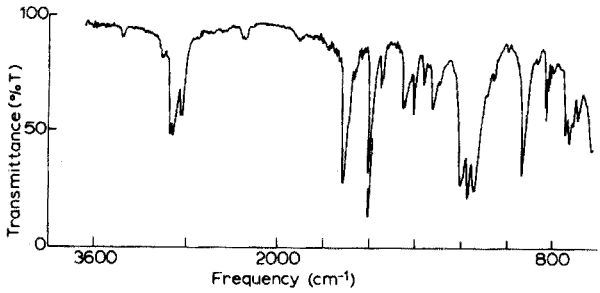


Fig. 2. I.r. spectrum of trifluoroacetyl-*p*-butylbenzene. Conditions discussed in Experimental section.

The n.m.r. spectrum (Fig. 3) shows an AB pattern characteristic of *para* substitution for the aromatic protons. The integrated intensities, correcting for the small amount of impurities, have the ratio 3.9:2:3.9:3. Prediction from the structure of trifluoroacetyl-*p*-butylbenzene would give 4:2:4:3 for the intensities and the observed splitting pattern if, as would be expected, the middle methylene protons of the butyl group would be unresolved.

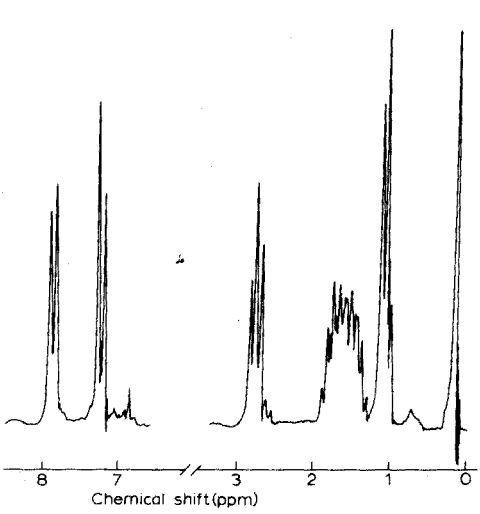


Fig. 3. N.m.r. spectrum of trifluoroacetyl-*p*-butylbenzene. Conditions discussed in Experimental section.

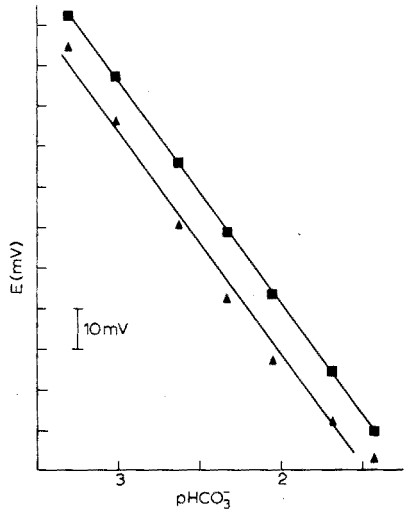


Fig. 4. Apparent response of two different liquid membrane electrodes to hydrogen carbonate: (■) liquid membrane made with 1-decanol; (▲) liquid membrane made with trifluoroacetyl-*p*-butylbenzene. Both use Aliquat 336 as the exchanger salt. Lines shown have slope −54.8 mV/decade.

Potentiometric behavior

Since earlier work[2,4] led us to expect hydrogen carbonate response for a liquid exchanger consisting of a quaternary ammonium salt dissolved in an organophilic solvent, the observed carbonate response of the present electrode had to be tested in detail. Indeed, when a liquid membrane electrode is constructed with an organic phase of 10%(v/v) Aliquat 336 in 1-decanol, its response (Fig. 4) is almost Nernstian (slope, −54.8 mV/decade; r, −0.99994). This electrode is not useful for "real" systems since its selectivity for hydrogen carbonate over chloride ion is very low ($1/K = 0.59$). When trifluoroacetyl-*p*-butylbenzene is substituted for 1-decanol, the observed "bicarbonate" response becomes sigmoidal (Fig. 4).

One plausible explanation for a decrease in slope at high concentration is simultaneous cationic solubilization, similar to the anionic solubility observed recently with neutral carriers[9] which can give diminished response or even peaks. This would not, however, explain an observation of super-Nernstian behavior. It is possible to attribute small deviations from Nernstian response to the liquid junction potential at the reference electrode–solution interface[9]. However, the deviations observed here appear too large to be accounted for by this possibility.

If the electrode responds to hydrogen carbonate ion, it may be possible to estimate the hydrogen carbonate/carbonate selectivity by adding the interferent to a background level of the main ion. The response curve for such an experiment involving addition of sodium carbonate to a solution of sodium hydrogen carbonate is shown in Fig. 5. The activity of carbonate ion is calculated from the moles of sodium carbonate per liter and the ionic strength of the solution. It is possible to calculate an apparent selectivity from these data. However, the initial portion of the curve, including the first point where *only* sodium hydrogen carbonate is present, becomes linear if the activity of carbonate ion is calculated from the equation

$$a_{CO_3^{2-}} = (K_1)_a(K_2)_a\gamma_{CO_3^{2-}} C_T/(a_{H^+}^2\gamma_{CO_3^{2-}} + (K_1)_a a_{H^+}\gamma_{CO_3^{2-}}/\gamma_{HCO_3^-} + (K_1)_a(K_2)_a) \quad (1)$$

where $a_{CO_3^{2-}}$ is the activity of carbonate ion; $(K_1)_a$ and $(K_2)_a$ are the first and second ionization constants of carbonic acid; $\gamma_{CO_3^{2-}}$ and $\gamma_{HCO_3^-}$ are the activity coefficients of carbonate and hydrogen carbonate calculated from the modified Davies equation[10]; C_T is the total concentration of carbonate species; and a_{H^+} is the activity of hydrogen ion.

Carbonate response

At this point an explanation for the s-shaped response to hydrogen carbonate ion can be hypothesized; namely the electrode actually responds to changes in the carbonate ion activity. Figure 6 shows the response of the electrode over several decades of carbonate activity. The response is linear from 10^{-7} to 10^{-2} *M*

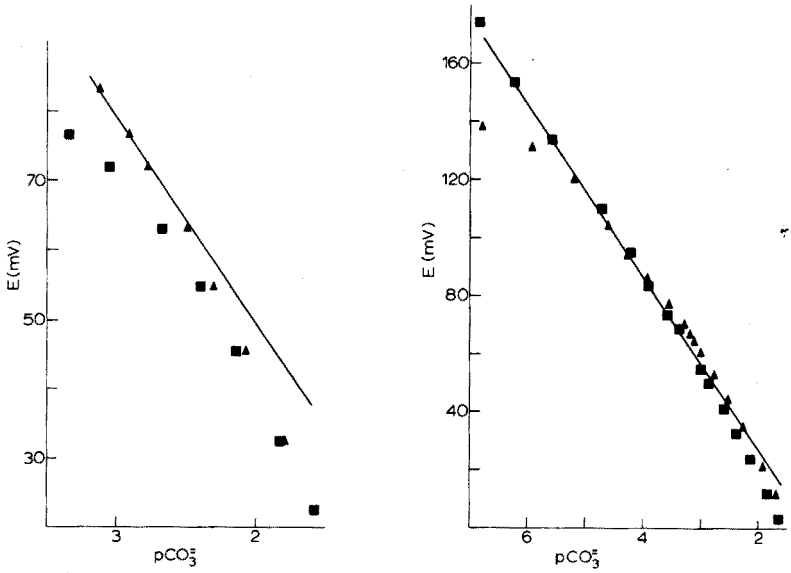


Fig. 5. Apparent response of a liquid membrane electrode to carbonate: (■) calculations based on addition of carbonate to a background level of hydrogen carbonate; (▲) calculations based on addition of carbonate to a background level of carbonate. Nernstian slope shown (−29.6 mV/decade).

Fig. 6. Response of the carbonate ion selective membrane electrode: (■) water solution; (▲) 0.1 *M* sodium chloride solution. Nernstian slope shown (−29.6 mV/decade).

carbonate. The carbonate activity was calculated from eqn. (1) with the values (ref. 11) $(K_1)_a = 4.45 \cdot 10^{-7}$, and $(K_2)_a = 4.69 \cdot 10^{-11}$ and the modified Davies equation[10]. The curve shown is a composite constructed with solutions of sodium hydrogen carbonate alone and sodium hydrogen carbonate–sodium carbonate mixtures. The potential begins to increase significantly at high pH (>9.4 at 50 m*M* C_T) where hydroxide response might be expected. The lower limit of response can be estimated from where the extrapolated response curve for carbonate intersects the potential measured in pure water. The apparent $a_{CO_3^{2-}}$ at this lower limit was calculated to be $10^{-8.9}$. From this, solutions of carbonate more concentrated than about 10^{-8} *M* would be expected to exhibit Nernstian behavior if no other significant interference were present.

The apparent selectivity for carbonate over chloride ion was very good. A response curve in the presence of 0.1 *M* sodium chloride is shown in Fig. 6. There is a negligible effect on the response when the activity of carbonate is greater than 10^{-5} *M*. The selectivity for carbonate over chloride ($1/K$) was calculated to be $7.5 \cdot 10^3$, by a non-linear least-squares procedure[12] from the equation:

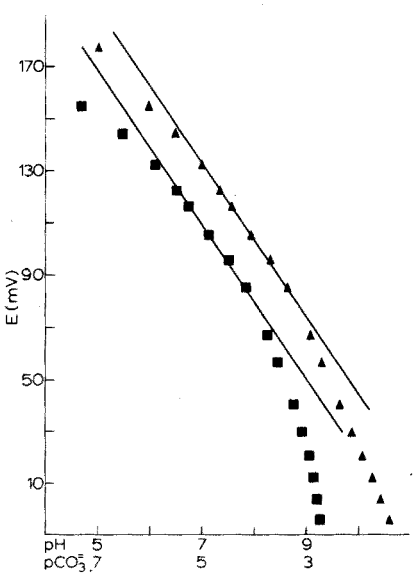
$$E_{obs} = E_{const} + \text{slope} \log(a_{CO_3^{2-}} + K a_{Cl^-}^2) \quad (2)$$

where E_{obs} is the observed electrode potential; $a_{CO_3^{2-}}$ and a_{Cl^-} are the activities of carbonate and chloride; and E_{const}, slope and K are experimentally determined values. The response time in high background concentrations of chloride was slower than in carbonate alone but still typical of electrodes of this type. The curves shown in Fig. 6 represent two different electrodes and show a small difference in E_{const} (approximately 4 mV); however, the day-to-day changes observed with the same electrode are much smaller.

The hypothesis of carbonate response for the electrode was tested further in experiments where a solution of sodium carbonate was titrated with hydrochloric acid. When the mV data were plotted *versus* pH or $pa_{CO_3^{2-}}$ (Fig. 7), a considerable linear region was observed extending more than two logarithmic units. This pH behavior would be predicted between the pK values of carbonic acid if the electrode were responding to carbonate ion. The slope began to increase, at about pH 9.0 (4 m*M* C_T) again indicating some hydroxide response. At low pH (<6) where the carbonate concentration was calculated to be less than 10^{-6} *M*, Nernstian behavior was no longer observed. At that point, the chloride ion activity has increased sufficiently to become important. A reversal of slope was even observed if enough hydrochloric acid was added (not shown on the graph).

Effect of pH

The electrode response to hydroxide is indicated by the following two experiments. First, a sodium hydroxide solution was added to water and the electrode response noted. Second, a sodium carbonate solution was added to water and the electrode response again noted. The data are plotted in Fig. 8; it can be seen that the response at these high pH values was essentially identical for the two experiments. It can, therefore, be concluded that the electrode response at high pH is due to hydroxide alone. Note that the response to hydroxide ion below pH 10 is not enough to explain the measured values in carbonate-containing solutions. However, hydroxide ion is a possible interference and the potential user should be aware of this fact.



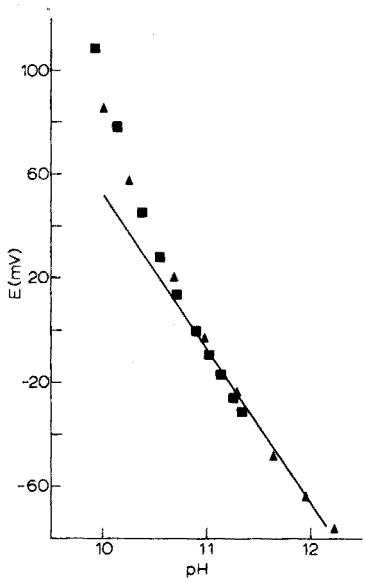


Fig. 7. Response of the carbonate electrode during the titration of sodium carbonate with hydrochloric acid; (■) *versus* $p_{CO_3^{2-}}$; (▲) *versus* pH. Nernstian slopes shown (−29.6 mV/decade).

Fig. 8. Response of the carbonate electrodes in solutions of high pH: (■) sodium carbonate solutions; (▲) sodium hydroxide solutions. Nernstian slope shown (−59.15 mV/decade).

The response to hydroxide alone is sigmoidal before breaking into the Nernstian slope above pH 10.5.

Table III summarizes the information necessary for proper use of the electrode. The potential observed in pure hydroxide solutions is converted to an equivalent $pa_{CO_3^{2-}}$. At a given pH, Nernstian behavior would be observed at about one unit less than the value for $a_{CO_3^{2-}}$ in Table III.

Selectivity properties

The apparent selectivity with respect to other ions was also investigated. The potential of an ion in a solution of a pure interferent ion was compared with the potential in a carbonate solution (10^{-3} *M*). The selectivity[13] was calculated from:

$$1/K = (a^{2/z}/a_{CO_3^{2-}}) \exp[2.3(E_{int} - E_{CO_3^{2-}})/29.6] \quad (3)$$

where a_{int} and $a_{CO_3^{2-}}$ are the activities of interferent and carbonate, Z is the charge on the interferent ion, and E_{int} and $E_{CO_3^{2-}}$ are the potentials measured in solutions of interferent and carbonate.

TABLE III

EQUIVALENT ACTIVITIES OF CARBONATE AT VARIOUS pH VALUES[a]

pH	8.6	8.8	9.0	9.2	9.4	9.6	9.8
$pa_{CO_3^{2-}}$	8.3	7.6	7.2	6.5	4.9	4.3	3.5

[a] At a given pH, Nernstian behavior will be observed at $pa_{CO_3^{2-}}$ values about one unit less than in this Table.

Apparent selectivities for various ions are listed in Table IV. These results indicate that useful buffers can be made from TRIS and ammonia (chloride anions) or phosphate mixtures if pH control is important. The selectivity for carbonate over hydrogen carbonate was too high to be measured. No apparent deviation from Nernstian behavior was observed even when the concentration of hydrogen carbonate was about 1000 times that of carbonate. Selectivity constants determined from eqn. (3) assume that the interferent responds in a Nernstian manner. This assumption was only investigated for chloride ion and found to hold approximately. The selectivity values in Table IV may, therefore, be concentration-dependent. The values should, however, give an idea of the potential for interference by the ion at its indicated concentration.

TABLE IV

APPARENT SELECTIVITY VALUES OF THE CARBONATE ELECTRODE FOR DIVERSE IONS[a]

Ion	*1/K*	*Interferent concn. (M)*
CO_3^{2-}	1	10^{-3}
Cl^-	$5.4 \cdot 10^3$	10^{-1}
Acetate	39	10^{-1}
SO_4^{2-}	$6.7 \cdot 10^3$	10^{-1}
NO_3^-	3.4	10^{-1}
ClO_4^-	0.040	10^{-1}
Borate	21	10^{-2}
Hydrogen phthalate	0.012	$0.5 \cdot 10^{-1}$
HPO_4^{2-}	$3.8 \cdot 10^3$	$0.25 \cdot 10^{-1}$

[a] It was assumed that the response of the electrode was due only to the listed ion.

Conclusion

The proposed electrode seems to be a potentially useful device, with Nernstian behavior over a wide concentration range (10^{-2}–10^{-7} *M* carbonate). Under most conditions, it has excellent response and low noise level; because of its small response to chloride, hydrogen carbonate, phosphate and sulfate, it should have applications as a probe in physiological, biological and chemical studies.

We gratefully acknowledge support by a grant from the Environmental Protection Agency.

SUMMARY

A liquid membrane ion-selective electrode has been prepared for the measurement of carbonate ion activities in solutions containing high levels of hydrogen carbonate and chloride. The electrode exhibits Nernstian response in the approximate range 10^{-2}–10^{-7} *M* carbonate, and has a fast response and low noise level under most conditions. In the physiologically important pH region, hydroxide ion does not interfere. Detailed information is given on the composition and properties of the active membrane phase.

REFERENCES

1 A. L. Grekovich, E. A. Materova and N. V. Garbuzovz, *Zh. Anal. Khim.*, 28 (1973) 1206.
2 J. W. Ross, in R. A. Durst (Ed.), *Ion-Selective Electrodes*, Nat. Bur. Stand.(U.S.), Spec. Publ. 314, 1969, Chap. 2.
3 C. J. Coetzee and H. Freiser, *Anal. Chem.*, 41 (1969) 1128.
4 W. M. Wise, *U.S. Patent No. 3,723,281*, March 27, 1973.
5 J. W. Ross, J. H. Riesman and J. A. Krueger, *Pure Appl. Chem.*, 36 (1973) 473.
6 H. B. Herman and G. A. Rechnitz, *Science*, 184 (1974) 1074.
7 J. H. Simons, W. T. Black and R. F. Clark, *J. Amer. Chem. Soc.*, 75 (1953) 5621.
8 R. G. Bates, *Determination of pH*, Wiley, New York, 2nd edn., 1973.
9 J. H. Boles and R. P. Buck, *Anal. Chem.*, 45 (1973) 2057.
10 C. W. Davies, *Ion Association*, Butterworths, London, 1962, p. 41.
11 H. C. Helgeson, *J. Phys. Chem.*, 71 (1967) 3121.
12 W. E. Wentworth, *J. Chem. Educ.*, 42 (1965) 96, 162.
13 K. Srinivasan and G. A. Rechnitz, *Anal. Chem.*, 41 (1969) 1203.

Analytica Chimica Acta, 76 (1975) 165–176

AN EXAMINATION OF SOME ACTIVE ORGANOMETALLIC SUBSTANCES FOR ION-SELECTIVE ELECTRODES

MICHAEL SHARP

Department of Analytical Chemistry, University of Umeå, S-901 87 Umeå (Sweden)

(Received 14th October 1974)

Potentiometric methods of analysis have been greatly revitalized during the past few years by the introduction of electrodes which show selective response to certain ionic species. A number of reviews and conference proceedings[1] provide summaries of the present state of development of such sensors and include discussions of the relevant theoretical and experimental aspects as well as fields of application. It is primarily because of their utility in widely different analytical problems that extensive efforts are currently being made to construct new electrodes or to improve upon those already in existence. The preparation of new electrodes essentially involves choosing suitable electroactive substances which may be incorporated in either liquid-membrane or solid-state assemblies. Such a choice remains, however, largely empirical with only a few, mainly practical, requirements serving as guides[2]. It is clear that greater understanding at the molecular level of electrode mechanisms and the factors which influence selectivity are needed both for interpreting the behaviour of available sensors and for preparing new ones with much greater efficiency. Few satisfactory correlations between basic electrochemical processes and acceptable electrode function have yet appeared. Accordingly, the present article surveys the examination of several different organic materials which were chosen on the basis of the criteria mentioned above. Brief descriptions of the systems are given below. These experiments concluded a search for new electroactive compounds among substances which had not earlier been considered.

Successful calcium ion-selective electrodes have been prepared by introducing lipophilic groups, *e.g.*, hydrocarbon chains, into an anion such as phosphate which is known to form a stable, water-insoluble salt with calcium ions. A solution of the calcium organo-phosphate salt can subsequently be employed as a liquid-membrane in a sensor which shows selective response towards calcium ions over an activity range of 10^{-1}–10^{-5} M[3]. Liquid–membrane electrodes for nitrate, perchlorate, chloride, tetrafluoroborate and thiocyanate ions have been prepared by a similar method with tetraalkylammonium– or metal–phenanthroline cations as lipophilic species[3,4]. To extend this approach further a number of organometallic salts was synthesized from metal ions which had been rendered lipophilic by converting them to suitable organometallic derivatives and which, in their inorganic chemistry, form strong, water-insoluble complexes with certain anions. In particular, derivatives of lead and thallium were considered to be of special interest.

Since lead ions form sparingly soluble precipitates with sulphate, chromate and carbonate ions, attempts were directed to the construction of liquid-membrane

electrodes for these important anions for which no acceptable electrochemical sensor is available. Several hundreds of organolead compounds containing the relatively stable cations, R_3Pb^+ or R_2Pb^{2+}, where R represents an alkyl or aryl group, are known[5]. In general they are solid, crystalline substances of definite salt-like character which are easily synthesized. They are, furthermore, only slightly soluble in water but dissolve readily in organic solvents depending on the size and nature of both the organic ligands attached to the metal and the anion. Detailed information on methods of preparation and general chemical properties may be found in the literature[6].

Organothallium salts containing the linear cation, R_2Tl^+, may be isolated as well defined, stable substances[7]. The dicyclohexylthallium ion, $(C_6H_{11})_2Tl^+$, in particular, has been proposed as a gravimetric reagent for nitrate, the precipitate formed being less soluble and more easily filtered, dried and weighed than alternative solids, *e.g.*, that derived from nitron[8]. Despite its apparently straightforward synthesis[9] this reagent has not found widespread use. Although many other anions such as halides, oxalate, carbonate, sulphide, nitrite and permanganate also form insoluble salts with $(C_6H_{11})_2Tl^+$ cations, the acetate, fluoride, sulphate and perchlorate salts are water-soluble. Most of the likely interfering ions in a nitrate determination can be removed, however, by pretreatment of the acidified sample solution with, say, excess of silver acetate. Such a procedure could form the basis of a potentiometric method for the determination of nitrate provided that the water-insoluble organothallium nitrate can be dissolved in a suitable organic medium and a liquid–membrane electrode thereby constructed. Efforts were made to prepare a nitrate responsive sensor in this way.

The steric and electrostatic interactions which operate in the formation of strong chelate complexes between multidentate organic ligands and different metallic cations are often of a highly selective nature. Many such substances can be obtained as insoluble solids, some of which are electrically semiconducting. These materials might be expected to function in solid-state electrodes which respond selectively to the metal ion complexed provided that sufficiently rapid ion-exchange processes occur across the solid-solution interface. The metal–phthalocyanines (Fig. 1), offer a series of water-insoluble complexes where the electrical conductivity, although very low, is adequate for constructing electrodes by means of the "Selectrode" technique[10]. A summary of phthalocyanine chemistry is given in a recent monograph[11]. Several metal–phthalocyanines were tested in the present study.

Tetracyanoethylene (TCNE) polymers[12], which are parquet-type polymers containing the same arrangement of complexing atoms as phthalocyanine (Fig. 2), were also chosen for examination. The unsaturated character of the organic ligand matrix, into which various metal ions can be complexed, is reflected by relatively low electrical resistivities and these materials can be used in solid-state electrodes without difficulty. In addition, some differences in chelating behaviour from that observed in metal–phthalocyanines are to be expected because of substantially greater charge delocalization throughout the solid.

One further class of materials, the coordination polymers, was also investigated. Aromatic molecules containing suitably positioned coordinating groups such as –OH, $-NH_2$, –SH, =O, =N– etc., readily form solid, semiconducting polymeric materials with transition metal ions. A typical structural arrangement is

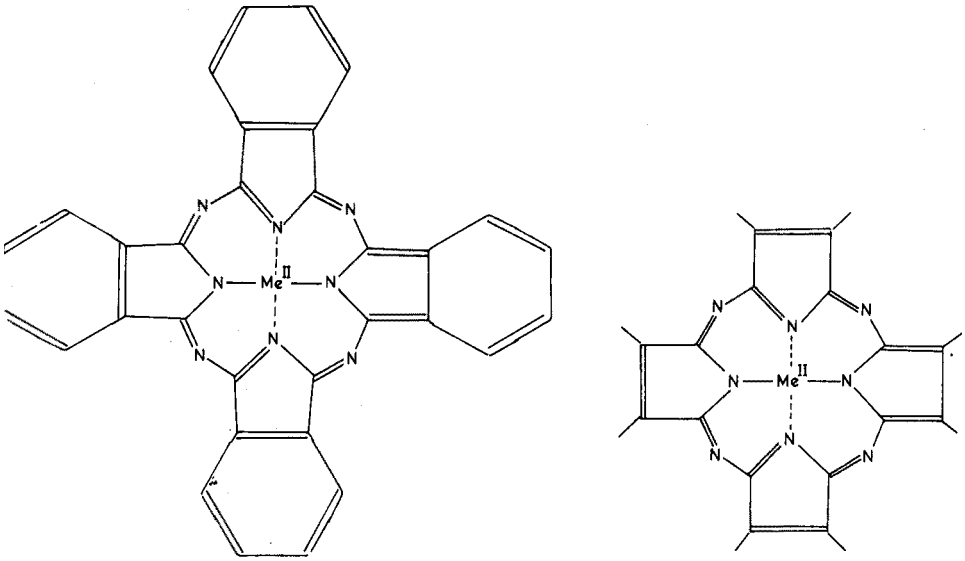


Fig. 1. Structural formula of metal-substituted phthalocyanine.

Fig. 2. Structure of metal–TCNE polymer unit.

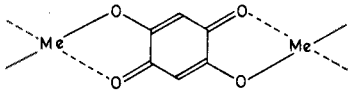


Fig. 3. Structural unit of linear coordination polymer derived from 2,5-dihydroxy-*p*-benzoquinone.

shown in Fig. 3. Conduction in these systems occurs by electron transmission through the solid across the ligand–metal–ligand bridges. Substances of this type might yield electrodes which respond to the metal ions contained in them. Two examples from this class of materials were therefore studied as the active components of solid-state electrodes. Further details of the preparative methods and properties of these substances are available[13].

EXPERIMENTAL

Organolead salts

The triphenyllead salts, $(Ph_3Pb)_2X$, used were prepared by first converting commercially available Ph_3PbBr (Research Organic/Inorganic Chemical Corp., California) to the corresponding hydroxide through reaction with sodium hydroxide[14]. Metathesis reactions between Ph_3PbOH and appropriate sodium salts in ethanol or ethanol–water mixtures afforded the required sulphate, chromate and carbonate salts. $(Ph_3Pb)_2SO_4$ and $(Ph_3Pb)_2CO_3$ were obtained as white, water-insoluble solids which dissolved in organic solvents such as *o*-dichlorobenzene, chloroform, dichlorodiethyl ether or nitrobenzene to give solutions which could be used as liquid membranes. $(Ph_3Pb)_2CrO_4$ was obtained as a yellow, highly insoluble

solid for which no solvent could be found. Examination of the liquid-membrane electrode behaviour of this compound was thus not possible.

Organothallium salts

Attempts were made to synthesize dicyclohexylthallium nitrate, $(C_6H_{11})_2TlNO_3$, by the method of Hartmann and Bäthge[15]. Repeated efforts did not, however, yield the required product. Failure in synthesis was also reported by DiGregorio *et al.*[16]. Diphenylthallium salts, which were obtained commercially, were also tested but no satisfactory organic solvent could be found.

Metal–phthalocyanines

Lithium phthalocyanine, Li_2Pc, was prepared by the addition of 30 g of phthalonitrile to a solution of 2 g of lithium in 150 ml of amyl alcohol and was isolated as a blue solid[17]. Lead phthalocyanine, PbPc, and copper phthalocyanine, CuPc, were prepared from Li_2Pc through double decomposition reactions performed in ethanol solution, the required products being precipitated[17]. Magnesium phthalocyanine, MgPc, and iron(II) phthalocyanine, FePc, were obtained commercially from Eastman Kodak Co.

Tetracyanoethylene (TCNE) polymers

A parquet-type polymer containing magnesium was prepared[18] by suspending 3 g of TCNE (Eastman Kodak Co.) and 0.57 g of finely divided magnesium in 20 ml of nitrobenzene and refluxing for approximately 10 h. The black, infusible solid product was filtered off, dissolved in concentrated sulphuric acid and reprecipitated by adding the solution to an ice–water mixture. After a second filtration the product was dried in an oven at 60–70 °C. Polymers containing copper, nickel and zinc were similarly prepared. The electrical resistivities of these substances are of the order 10^6–10^8 ohm cm.

Coordination polymers

Polymers of this type were synthesized by heating the appropriate mixture of chloranil (1 mole), *o*-phenylenediamine (2 moles) and a metal acetate (1 mole) to 250 °C under a dry nitrogen atmosphere for a period of 30 min[19]. The resulting product (Fig. 4) was crushed and washed with large quantities of hot

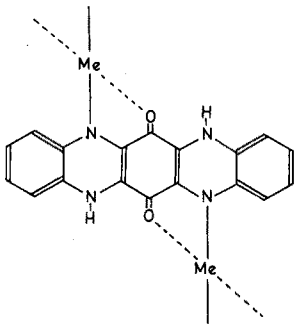


Fig. 4. Structural unit of coordination polymer obtained from chloranil, *o*-phenylenediamine and metal acetate.

ethanol and water. Polymers containing copper, cadmium and a mixture of cadmium and iron(III) were prepared. The cadmium-containing polymer was found to decompose upon treatment with water. An attempt was made to reduce this structural breakdown by introducing iron(III) into the polymer synthesis in addition to cadmium. The cross-linking (50%) which resulted provided a more stable solid. Electrical resistivities in the megohm range have been reported for these polymers[20].

Electrodes

Tests with $(Ph_3Pb)_2SO_4$ and $(Ph_3Pb)_2CO_3$ were carried out with Orion series 92 liquid–membrane electrode bodies together with Orion nitrate porous membrane supports. The internal reference systems comprised an Ag–AgCl electrode immersed in aqueous solutions containing $NaCl(10^{-3}\ M) + Na_2SO_4(10^{-3}\ M)$, and $NaCl(10^{-3}\ M) + Na_2CO_3$–$NaHCO_3(10^{-3}\ M)$, respectively. The liquid–membrane solutions were prepared with *o*-dichlorobenzene, the concentrations being 9.77 g l^{-1} for $(Pb_3Pb)_2SO_4$ and 1.86 g l^{-1} for $(Ph_3Pb)_2CO_3$.

Measurements for the metal–phthalocyanines and for the different polymeric materials were carried out by the Selectrode method.

Measuring procedure

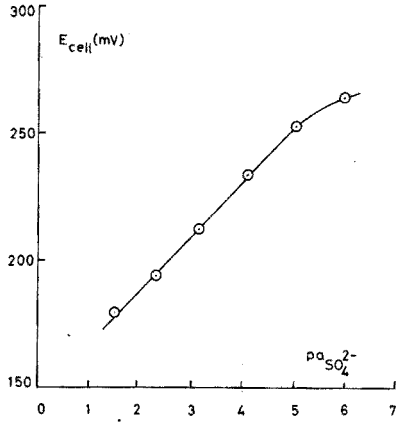
Cell potentials for the system: reference electrode–test solution–indicator electrode, were recorded at 25 °C with an Orion model 701 digital pH meter connected to an Orion model 751 digital printer. Orion model 90–02 double liquid-junction electrodes or Radiometer K401 saturated calomel electrodes served as external reference. No corrections for liquid-junction potentials were applied to the cell potentials observed. Single-ion activities were evaluated from the extended Debye–Hückel relation with the ion-size parameters of Kielland[21]. In general, solutions of metal nitrate salts were employed for testing metal ion response whilst sodium salt solutions were used for examining the response to anions.

RESULTS

Organolead salts

Figure 5 shows the variation of cell potential with sulphate ion activity for a freshly prepared $(Ph_3Pb)_2SO_4$–*o*-dichlorobenzene liquid-membrane electrode. Linear response was observed over the activity range 10^{-1}–10^{-5} *M* sulphate but the slope of 22 mV decade^{-1} was appreciably less than Nernstian. Continued usage, however, revealed a steady loss of electrode function, the reproducibility and sensitivity towards sulphate becoming very poor. Such selectivity determinations as could be performed indicated that many common anions including halides, nitrate and hydroxide, seriously interfered with the sulphate response. Quantitative selectivity measurements were not possible.

Figure 6 shows the response of the $(Ph_3Pb)_2CO_3$–*o*-dichlorobenzene liquid-membrane electrode to changes in carbonate ion activity at about pH 10. Some response to carbonate was evident, but the linearity of the calibration curve was limited to a region between 10^{-1}–10^{-3} *M* carbonate. The lower limit of detection was extremely sensitive to the pH of the test solution, more alkaline solutions



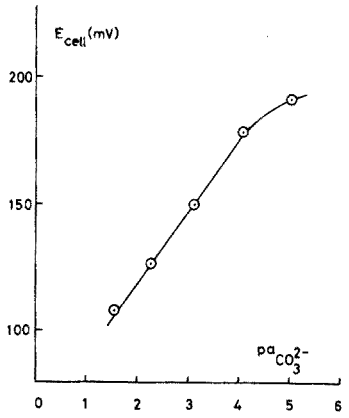


Fig. 5. Response of $(Ph_3Pb)_2SO_4$–*o*-dichlorobenzene liquid–membrane electrode in Na_2SO_4 solutions.

Fig. 6. Response of $(Ph_3Pb)_2CO_3$–*o*-dichlorobenzene liquid–membrane electrode in Na_2CO_3 solutions at about pH 10.

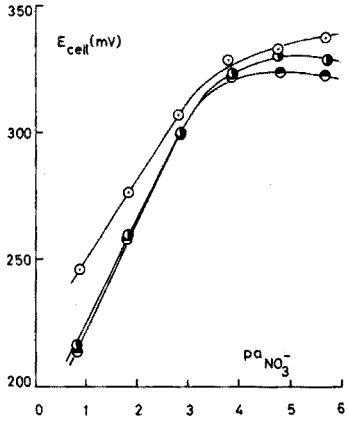
giving much narrower regions of carbonate response. Qualitative observations again showed severe interference by halides and nitrate.

Organothallium salts

The failure to find a suitable solvent for the organothallium compounds chosen precluded any examination of these materials for electrode function.

Metal–phthalocyanines

Solid-state electrodes prepared from magnesium, lead, copper and iron(II) phthalocyanines were studied. For the first three of these compounds the response



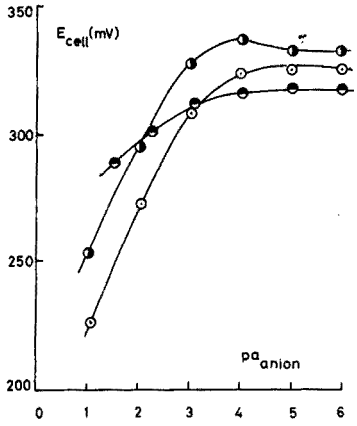


Fig. 7. Response curves of three MgPc solid-state electrodes in $Mg(NO_3)_2$ solutions.

Fig. 8. Response of an MgPc solid-state electrode in $NaNO_3$ (⊙), NaCl(◑) and Na_2SO_4 (◓) solutions.

curves measured in the respective metal nitrate solutions were characteristic of anion rather than cation sensitivity. The results for MgPc are typical. Figure 7 shows the response curves obtained for three MgPc electrodes. It is apparent that sensitivity to nitrate activity variations prevails. Figure 8 illustrates the response of a MgPc electrode measured in sodium nitrate solutions. A close correspondence between the curves of Figs. 7 and 8 is clear. Additional measurements in sodium chloride and sulphate solutions, included in Fig. 8, were also characteristic of anion response, and comparisons between the nitrate, chloride and sulphate curves reflected some degree of anion selectivity. Somewhat poorer reproducibility and selectivity was observed for the PbPc and CuPc systems.

The results obtained for FePc electrodes were so irreproducible that no definite trends in response behaviour or selectivity characteristics could reliably be discerned.

TCNE polymers

The solid-state electrode prepared from the Mg–TCNE polymer showed a response towards magnesium ions as shown in Fig. 9. The slope of the linear portion of the response curve between 10^{-1}–10^{-4} *M* Mg^{2+} was approximately 33 mV decade^{-1}. The potentials recorded were both reproducible (± 2 mV) and rapidly established (< 2 min). Little or no selectivity for Mg^{2+} over Ca^{2+}, Na^{+} and K^{+} ions was found. Exposure to zinc, nickel and copper solutions showed that these ions strongly interfered with the magnesium ion response.

Subsequent measurements with an electrode made from the Cu–TCNE polymer confirmed the expected response towards copper(II) ions over the activity range 10^{-1}–10^{-5} *M* Cu^{2+} as shown in Fig. 10. The slope of the linear region of the response curve was 29 mV decade^{-1} as required by the Nernst equation.

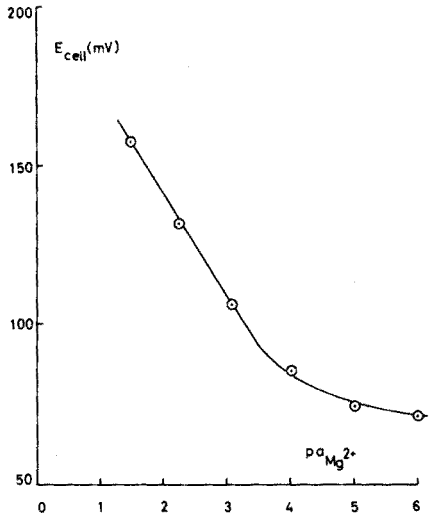


Fig. 9. Response of Mg–TCNE polymer electrode in $Mg(NO_3)_2$ solutions.

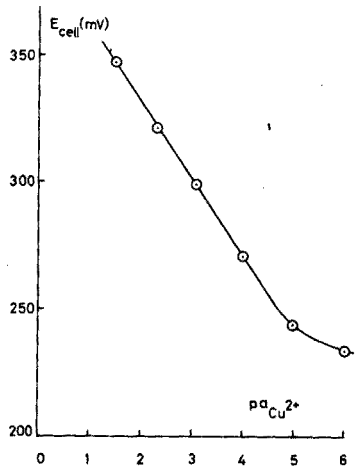


Fig. 10. Response of Cu–TCNE polymer electrode in $Cu(NO_3)_2$ solutions.

The potentials observed were reproducible and rapidly attained and selectivity towards Cu^{2+} ions over Ni^{2+}, Zn^{2+}, Mg^{2+} and K^+ ions was found.

The Ni–TCNE and Zn–TCNE polymers were also tested. The electrode response ranges were found to be narrow, 10^{-1}–10^{-3} M M^{2+}, and the response times somewhat longer than those encountered for the Cu–TCNE electrode. The slopes of the response curves were significantly less than those required by the Nernst relation. Figure 11 illustrates the observed behaviour.

Coordination polymers

Figure 12 shows the response of the solid-state electrode prepared from the

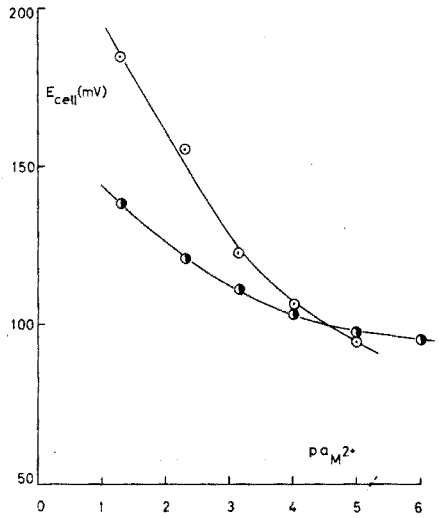


Fig. 11. Response of Ni–TCNE polymer (⊙), and Zn–TCNE polymer (◑) electrodes in $Ni(NO_3)_2$ and $Zn(NO_3)_2$ solutions respectively.

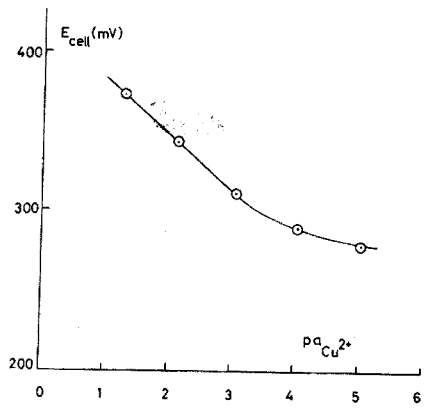


Fig. 12. Response of copper-containing coordination polymer electrode in $Cu(NO_3)_2$ solutions.

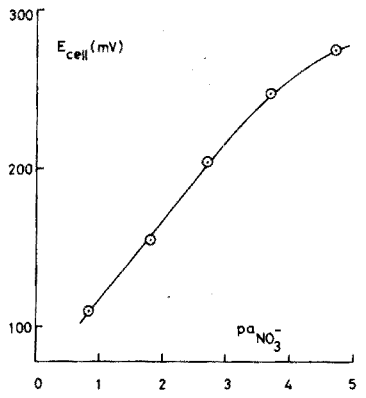


Fig. 13. Response of Cd–Fe(III) coordination polymer electrode in $Cd(NO_3)_2$ solutions.

coordination polymer derived from copper acetate, *o*-phenylenediamine and *o*-chloranil. The curve was linear over the range 10^{-1}–10^{-3} *M* Cu^{2+} with a slope of 33 mV decade^{-1}. The cell potentials were reproducible and the response times normally less than 2–3 min. Some selectivity for copper(II) ions over nickel(II) and alkali metal cations was observed.

Quite different behaviour was shown by the electrode made from the Cd–Fe coordination polymer. As shown in Fig. 13, response to anions rather than to cations was evident in cadmium nitrate solutions. Further measurements with sodium nitrate, bromide, iodide, perchlorate and sulphate solutions confirmed the response to nitrate ions and indicated some selectivity for nitrate over the other ions studied. Figure 14 summarizes these observations. The cell potentials were fairly reproducible (± 3 mV) and response times were of the order of 5–10 min.

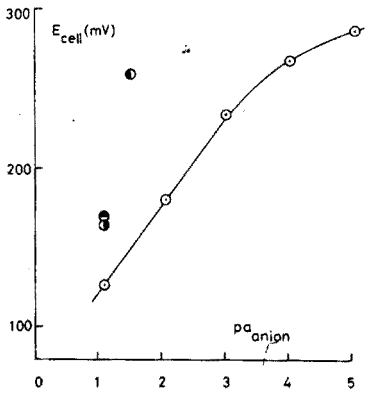


Fig. 14. Response of Cd–Fe(III) coordination polymer electrode in $NaNO_3$ (⊙), $NaClO_4$ (◑), NaI and NaBr (◓), and Na_2SO_4 (◑) solutions.

DISCUSSION

The results obtained with the organolead compounds did not support the idea of constructing sulphate, chromate and carbonate ion selective electrodes through the use of lipophilized lead ions. The gradual loss of response observed with $(Ph_3Pb)_2SO_4$ electrodes is consistent with extraction data obtained by Schweitzer and McCarty[29], who found that, in contrast to many other anions, sulphate could not be extracted into organic solvents from aqueous media by Ph_3Pb^+ ions. The behaviour shown by the electrodes tested thus probably reflects an exchange process whereby sulphate ions are replaced by other ions such as hydroxide or chloride in the organic membrane phase. From the order of the selectivities observed, the Ph_3Pb^+ ions in the organic medium would seem to function simply as large, mobile, ion-exchange sites which exert very little selective influence on their counter-ions. In such cases selectivity is controlled by the hydrophobic character of the individual anions as suggested by recent studies[23].

The severe interference by hydroxide ions on the carbonate response of the

$(Ph_3Pb)_2CO_3$ electrode necessarily limits the practical utility of this sensor. Since carbonate determinations must be made in alkaline solutions with pH $\geqslant 10$, the lower limit of measurement for carbonate ions becomes fixed at between 10^{-3} and 10^{-4} *M*. It is conceivable that modification of the organic ligands attached to the lead atom might enhance the selectivity between carbonate and hydroxide so that lower detection limits might be attained. Such possibilities have not, however, been fully explored.

The behaviour of the metal–phthalocyanine electrodes, which exhibited anion rather than cation response, cannot easily be explained. The different sensitivities of the MgPc electrode, for example, to various anions may reflect selective anion adsorption at the electrode surface. Equilibria between anions adsorbed at non-labile metallic ion sites and free anions in solution may then be responsible for the electrode function observed. Anion determinations with such electrodes, however, do not appear to offer a practical method of measurement.

Much more promising were the observations made with the metal–TCNE semiconducting polymer electrodes. All the electrodes studied showed response to the metal ions contained in the polymer employed and, in some cases, reasonable selectivities were obtained. Electrode response was generally both reproducible and fast. Surface ion-exchange processes appear to be much more facile in these systems, in spite of the close similarity of the complexing site to that found in the phthalocyanines. The latter show wide variations in metal exchange capability depending on the ionic character of the metal–ligand bonding[24]. Alkali metals are readily exchanged while transition metals such as copper show considerably less lability. Increased ionic character in the bonding of the metal–TCNE polymers compared with that found in the corresponding metal–phthalocyanines may thus account for the differences in electrode behaviour observed between the two types of material, *cf.* MgPc and Mg–TCNE polymer sensors. Polymerization of TCNE, which is a highly electronegative molecule, creates a structure where charge delocalization possibilities are improved with respect to the monomer and where negatively charged configurations are consequently stabilized. In this respect, it is to be expected that the metal–TCNE linkages will be largely ionic in character. From a practical viewpoint, the electrodes tested do not offer sufficient advantages over those based on metal suphides or selenides to warrant continued studies.

Electrodes prepared from the coordination polymers investigated did not yield satisfactory results. The copper-containing material showed some response to copper(II) ions but over a very limited activity range. The potential-controlling equilibrium at the solid–solution interface is most likely a solubility one involving both the metal ion and the organic ligand. Electrode response is thus expected to be strongly influenced at low pH values by protonation of the phenolate groups in the ligand and at high pH values by metal hydroxide formation. Because of the narrow response range and poor selectivity it must be concluded that acceptable copper ion-selective devices cannot be made from this substance.

Surprising results were obtained for the Cd–Fe-containing polymer. The response in cadmium nitrate solutions was characteristic of the anion rather than the cation. As in the case of the metal–phthalocyanines, it seems likely that selective adsorption of anions occurs at non-labile metal ion sites, possibly iron atoms

bearing residual charges, and the resulting ion-exchange with free anions in the solution is responsible for the observed electrode behaviour. Nevertheless, the range of nitrate response and the degree of anion selectivity shown by this material eliminated it from serious consideration as a useful electrode constituent.

The author wishes to thank Prof. Gillis Johansson for his continued interest and advice, Mrs. K. Jiewertz-Palmgren for technical assistance, and the Swedish Natural Science Research Council for financial support.

SUMMARY

Examinations of a number of possible electroactive substances for use in both liquid membrane and solid-state ion-selective electrodes were carried out. Liquid membrane electrodes incorporating organometallic salts of lead and thallium were considered as constituents of sulphate, chromate, carbonate and nitrate responsive sensors. No practically useful device was, however, found. Several electrically semiconducting metal–phthalocyanines, metal–tetracyanoethylene (TCNE) polymers and metal–coordination polymers were also synthesized and investigated with solid-state electrode constructional techniques. Metal–phthalocyanine electrodes were found to be responsive to anions rather than to cations and some anion selectivity was observed. Metal–TCNE polymer electrodes showed response to metal ions identical with those contained in the polymer, and some good selectivities, operational activity ranges and response times were found. Electrodes made from coordination polymers incorporating copper showed a limited response to copper ions whilst inclusion of cadmium and iron(III) in the polymer matrix produced an electrode with anion reponse and slight anion selectivity.

REFERENCES

1 J. Koryta, *Anal. Chim. Acta*, 61 (1972) 329; A. K. Covington, *CRC Critical Reviews in Anal. Chem.*, 1974, p. 355; *Plenary Lectures of the IUPAC International Symp. on Selective Ion Sensitive Electrodes, Cardiff, 1973*, Butterworths, London, 1973.
2 A. Shatkay, *Anal. Chem.*, 39 (1967) 1056.
3 J. W. Ross, *Ion Selective Electrodes*, NBS Special Publ. 314, Washington, 1969, Ch. 2.
4 G. J. Moody and J. D. R. Thomas, *Selective Ion Sensitive Electrodes*, Merrow, Watford, England, 1971.
5 R. W. Leeper, L. Summers and H. Gilman, *Chem. Rev.*, 54 (1954) 101.
6 L. C. Willemsens and G. J. M. Van der Kerk, *Investigations in the Field of Organolead Chemistry*, International Lead Zinc Research Organization, 1965.
7 G. E. Coates, *Organometallic Compounds*, Methuen, London, 2nd edn., 1960, p. 155.
8 A. J. Nutten, *Chem. Prod.*, 19 (1956) 264.
9 H. Hartmann and G. Bäthge, *Angew. Chem.*, 65 (1953) 107.
10 J. Ruzicka and C. G. Lamm, *Anal. Chim. Acta*, 54 (1971) 1.
11 F. H. Moser and A. L. Thomas, *Phthalocyanine Compounds, ACS Monograph 157*, Reinhold, New York, 1963.
12 J. E. Katon, *Organic Semiconducting Polymers*, Dekker, New York, 1968, p. 226
13 J. E. Katon, *Organic Semiconducting Polymers*, Marcel Dekker, New York, 1968, p. 229.
14 L. C. Willemsens and G. J. M. Van der Kerk, *Investigations in the Field of Organolead Chemistry*, International Lead Zinc Research Organization, 1965, p. 72.
15 H. Hartman and G. Bäthge, *Angew. Chem.*, 65 (1953) 107.
16 J. S. DiGregorio and M. D. Morris, *Anal. Chem.*, 42 (1970) 94.

17 P. A. Barrett, D. A. Frye and R. P. Linstead, *J. Chem. Soc., London*, (1938) 1157.
18 A. A. Berlin, N. G. Matveeva, A. I. Sherle and N. D. Kostrova, *Vysokomol. Soedin.*, 4 (1962) 860.
19 B. A. Bolto and D. E. Weiss, *Aust. J. Chem.*, 15 (1962) 653.
20 J. E. Katon, *Organic Semiconducting Polymers*, Dekker, New York, 1968, p. 231.
21 J. Kielland, *J. Amer. Chem. Soc.*, 59 (1937) 1675.
22 G. K. Schweizer and S. W. McCarty, *J. Inorg. Nucl. Chem.*, 27 (1965) 191.
23 R. E. Reinsfelder and F. A. Schultz, *Anal. Chim. Acta*, 65 (1973) 425.
24 F. H. Moser and A. L. Thomas, *Phthalocyanine Compounds, ACS Monograph 157*, Reinhold, New York, 1963, p.38.

Analytica Chimica Acta, 76 (1975) 177–182

DETERMINATION OF TRI- AND TETRAVALENT IONS WITH A DIVALENT ION-SELECTIVE ELECTRODE

FU CHUNG CHANG* and K. L. CHENG
Department of Chemistry, University of Missouri–Kansas City, Kansas City, Mo. 64110 (U.S.A.)
(Received 22nd August 1974)

The development of ion-selective electrodes for the determination of polyvalent ions has so far proved unsuccessful[1]; even if the problems of preparation could be overcome, the electrodes would suffer from poor precision.

A new method is proposed here for the determination of tri- and tetravalent ions indirectly with a divalent ion-selective electrode. EDTA forms complexes with different metal ions. In general, the stability constants of M(III)–EDTA complexes are much larger than those of M(II)–EDTA complexes. For example, the stability constant is $10^{8.7}$ for Mg–EDTA and $10^{22.9}$ for Bi–EDTA[2]. If both bismuth(III) and Mg–EDTA exist in the solution, the amount of bismuth(III) can be determined by measuring the amount of magnesium(II) displaced. Zn–EDTA can also be used as a displacement reagent in place of Mg–EDTA.

EXPERIMENTAL

Apparatus

An Orion Model 92-32 divalent ion electrode and a Model 90-01 reference electrode coupled with a Corning Model 10 expanded scale pH meter were employed. A Sargent-Welch automatic constant-rate burette Model C was used for all titrations.

Reagents

Reagent-grade Mg–EDTA was purified by recrystallization in aqueous solution at pH 4 and dried at 110°C. Zn–EDTA, reagent grade, was used without further purification. Other solutions were prepared from appropriate salts of reagent-grade quality.

Procedure

Pipette 10.00 ml of 0.040 *M* Mg–EDTA or Zn–EDTA solution into a 150-ml beaker, and add the sample solution containing the metal ion to be determined. Adjust the pH to a value between 7 and 9 when using Mg–EDTA and between 5.5 and 7.0 when using Zn–EDTA. Transfer the solution to a 100-ml volumetric flask, dilute to the mark, and then transfer back to the original beaker. Place the electrodes into the solution, and stir at a constant speed with a magnetic stirrer. After equilibrium has been reached, record the potential.

* Present address: Institute of Nuclear Energy Research, Atomic Energy Council, Lung-Tan, Taiwan, Republic of China.

RESULTS AND DISCUSSION

Determination of tri- and tetravalent metal ions

Some of the calibration curves for M(II) and M(IV) ions are shown in Fig. 1. In the displacement reaction, 0.0040 *M* Mg–EDTA or Zn–EDTA solution was used as the divalent metal complex. Suitable concentration ranges for the

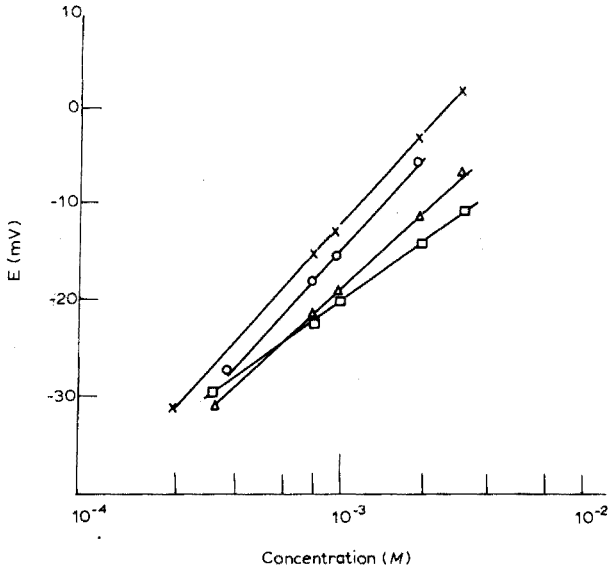


Fig. 1. Calibration curves for La(III), Fe(III), Bi(III) and Zr(IV) in the displacement of Mg–EDTA (0.004 *M*): (×) Bi(III), Fe(III); (○) La(III); (△) Zr(IV); (□) Ce(III).

TABLE I

OPTIMAL CONCENTRATION RANGES OF METAL IONS FOR THE DISPLACEMENT REACTION IN THE PRESENCE OF $4 \cdot 10^{-3}$ *M* Mg–EDTA OR Zn–EDTA

Metal ion	*Concentration (M)*	
	Mg–EDTA	*Zn–EDTA*
La(III)	$4 \cdot 10^{-4}$–$2 \cdot 10^{-3}$	$1 \cdot 10^{-4}$–$3 \cdot 10^{-3}$
Fe(III)	$2 \cdot 10^{-4}$–$3 \cdot 10^{-3}$	$1 \cdot 10^{-4}$–$3 \cdot 10^{-3}$
Bi(III)	$2 \cdot 10^{-4}$–$3 \cdot 10^{-3}$	$1 \cdot 10^{-4}$–$3 \cdot 10^{-3}$
Ga(III)	$2 \cdot 10^{-4}$–$2 \cdot 10^{-3}$	$1 \cdot 10^{-4}$–$3 \cdot 10^{-3}$
In(III)	$3 \cdot 10^{-4}$–$3 \cdot 10^{-3}$	$5 \cdot 10^{-4}$–$3 \cdot 10^{-3}$
Tl(III)	$5 \cdot 10^{-4}$–$3 \cdot 10^{-3}$	$5 \cdot 10^{-4}$–$3 \cdot 10^{-3}$
Sm(III)	$3 \cdot 10^{-4}$–$3 \cdot 10^{-3}$	$5 \cdot 10^{-4}$–$3 \cdot 10^{-3}$
Al(III)	$2 \cdot 10^{-4}$–$2 \cdot 10^{-3}$	—[a]
Ce(III)	$3 \cdot 10^{-4}$–$3 \cdot 10^{-3}$	—[a]
Th(IV)	$4 \cdot 10^{-4}$–$2 \cdot 10^{-3}$	$2 \cdot 10^{-4}$–$3 \cdot 10^{-3}$
Zr(IV)	$3 \cdot 10^{-4}$–$3 \cdot 10^{-3}$	$2 \cdot 10^{-4}$–$3 \cdot 10^{-3}$
VO(II)	$3 \cdot 10^{-4}$–$3 \cdot 10^{-3}$	$2 \cdot 10^{-4}$–$3 \cdot 10^{-3}$

[a] No reaction.

M(III) and M(IV) ions to be determined are shown in Table I. Lanthanum(III) ion cannot displace zinc(II) from Zn–EDTA, because the stability constant of La–EDTA ($10^{15.5}$) is lower than that of Zn–EDTA ($10^{16.5}$). The same is true for Al and Ce(III) ions. Thallium(I) does not react with EDTA, but thallium(III) forms a strong complex with EDTA; thus thallium(I) ion could be oxidized by adding nitric acid and saturated bromine water dropwise until a definite yellow coloration appeared[3], after which the pH was adjusted and the measurement made in either Mg–EDTA or Zn–EDTA solution.

In the measurement of aluminium(III), the displacement reaction occurs below pH 5 at a temperature of 80°C; however, a turbidity forms when the aluminium-(III) concentration is higher than 0.002 *M*, because the Al–EDTA complex is not strong enough to prevent the formation of $Al(OH)_3$. At pH 7, thorium(IV) and zirconium(IV) are very easy to hydrolyze. When these metals are determined in Mg–EDTA solution at pH 7, there is a competition reaction between the formation of Th–EDTA and $Th(OH)_4$. However, one can successfully measure these metal ions in Zn–EDTA solution at lower pH rather than in Mg–EDTA solution. Chromium(III) forms a complex with EDTA at pH 3–5 on heating to 80°C until the color changes from light green to purple. The differences in potential at different concentrations of chromium(III) in Mg–EDTA solution were very small, which implies that displacement was incomplete. When Zn–EDTA was used instead of Mg–EDTA, the results were generally better.

V(IV) ion reacted like a divalent ion because it forms a complex with oxygen, VO(II), which can be directly measured by the divalent ion-selective electrode. It was also possible to measure the VO(II) ion indirectly by the displacement reaction with Mg–EDTA because of the greater stability of its complex with EDTA.

Optimal concentration ranges in the determination of M(III) and M(IV)

In the presence of 0.0040 *M* Mg–EDTA or Zn–EDTA solution, most of the tri- and tetravalent ions can be determined at a maximum concentration of 0.003 *M*. If the concentration of M^{z+} was below 0.0002 *M* in the displacement reaction with Mg–EDTA, the potential did not follow a Nernstian relationship. This phenomenon may be caused by the slight solubility of Mg–EDTA. At pH 7, the conditional stability constant[4] is $10^{5.6}$, and the free magnesium(II) in solution is $2 \cdot 10^{-4}$ *M* which is larger than the amount of M^{z+} ion being added, so that M^{z+} cannot be determined below this concentration level. When a 0.004 *M* Zn–EDTA solution is used, the optimal concentration range of M^{z+} for the displacement of Zn–EDTA lies in the range 0.0001–0.0003 *M*. This is because the stability constant of Zn–EDTA is $10^{16.5}$, and at pH 5.5 the free zinc(II) in the solution is only $2 \cdot 10^{-7}$ *M*. If a more concentrated or more dilute Mg–EDTA or Zn–EDTA solution is used in the displacement reaction, the optimal concentration range of tri- and tetravalent ions may be increased or decreased. Certainly, this will be limited by the solubility of Mg–EDTA or Zn–EDTA and the ionic strength of the solution.

Optimal pH range for the potential measurements

The proposed method of determining tri- and tetravalent ions is based on two steps: the first is the displacement reaction and the second is the potential measurement of free magnesium or zinc ion with a divalent electrode. It is desirable

to select an optimal pH for the two steps. The optimal pH ranges for determining various ions are shown in Table II.

In Mg–EDTA solution, magnesium(II) forms a stable complex with EDTA above pH 7. When the pH is below 7, the Mg–EDTA complex tends to dissociate, resulting in an increase in free magnesium(II) which, in turn, increases the potential and hence interferes with the measurement. Above pH 10, magnesium(II) forms $Mg(OH)^+$ and $Mg(OH)_2$ which are not measured by the divalent electrode. Therefore, the optimal pH range was between 7 and 10 when Mg–EDTA was used.

TABLE II

OPTIMAL pH RANGES FOR DISPLACEMENT FROM Mg–EDTA OR Zn–EDTA, AND SLOPES OF CALIBRATION CURVES

Metal ion	*pH Value*		*Slope (mV decade^{-1})*	
	Mg–EDTA solution	*Zn–EDTA solution*	*Mg–EDTA*	*Zn–EDTA*
La(III)	7.0–8.5	—[a]	28.5	—[a]
Fe(III)	6.5–9.3	5.5–6.5	29.6	29.4
Bi(III)	6.5–8.7	5.5–7.0	29.9	29.5
Ga(III)	6.5–9.0	6.0–6.5	29.7	29.2
In(III)	7.0–9.0	5.5–7.0	29.2	29.3
Tl(III)	6.5–9.5	5.5–7.5	29.1	29.0
Sm(III)	6.5–10.0	5.5–7.0	30.1	30.3
Al(III)	6.5–6.8	—[a]	29.3	—[a]
Ce(III)	6.5–10.0	—[a]	22.2	—[a]
Th(IV)	6.5–9.0	5.5–7.0	16.0	28.3
Zr(IV)	6.5–7.5	6.0–7.0	27.1	28.5
VO(II)	6.5–9.5	—[a]	29.5	—[a]
Cr(III)	—[a]	5.5–7.0	8.8	29.3

[a] No reaction.

In Zn–EDTA solution, zinc(II) forms a stable complex with EDTA at pH 4–7; however, hydrogen ion interferes with the potential measurement below pH 5.5 when the divalent ion electrode is used, and zinc(II) tends to hydrolyze above pH 7.5. Accordingly, measurements of zinc(II) should be done between pH 5.5 and 7.0.

Slope of calibration curves for metal ions

Whether or not the displacement is complete can be established from the potential change in different concentration of M^{2+} ion, according to the Nernst equation. The theoretical slope, 2.3 $RT/2F$, should be equal to 29.4 at room temperature. When nearly the same slope is obtained in the sample solution, displacement can be regarded as complete. The slopes obtained in the displacement reactions for various ions with Mg–EDTA and Zn–EDTA are shown in Table II. Ce, Cr, Th and Zr have low slope values when Mg–EDTA is used; apparently, the displacements are incomplete owing to hydrolyses. Samarium(III) has a relative higher slope value, probably because samarium also reacts at the divalent electrode to some extent in the displacement reaction.

Determination of metal ions in presence of foreign ions

In the determination of M^{3+} ions, there is no interference from monovalent ions. The presence of divalent ions such as copper(II), cadmium(II) and lead(II) can be masked with cyanide[5]. Other polyvalent ions will interfere unless they can be effectively complexed.

Determination of ion concentrations in mixtures

Titrimetric methods are recommended in the determination of mixed ions concentrations because no calibration curves are necessary and no ionic strength problems need be considered. In Fe–Cr mixtures, owing to the slow reaction between chromium(III) and EDTA at room temperature, iron(III) can be determined by back-titration of the excess of EDTA with standard zinc(II) solution. Then the solution is treated with excess of EDTA again, and heated to boiling for the formation of the Cr–EDTA complex, and the amount of chromium(III) is determined by back-titration of excess EDTA. In mixtures of copper(II) and M^{3+}, the total amount

TABLE III

BACK-TITRATION DATA OF IRON AT DIFFERENT VOLUME PERCENTAGES OF METHANOL

(0.2500 mmole of EDTA and 0.1565 mmole of iron(III) titrated with 0.025 *M* zinc(II). Theoretical equivalence point is at 3.74 ml.)

Zn added (0.025 M)	*E (mV) at Vol.(%) CH_3OH of:*				
	0	*10*	*20*	*30*	*40*
0.00	−74.6	−70.5	−66.4	−60.1	−52.0
2.00	−73.4	−69.7	−65.5	−59.4	−51.3
3.00	−72.6	−69.1	−65.2	−58.9	−51.4
3.60	−67.3	−63.7	−63.1	−57.0	−49.5
3.70	−56.5	−53.2	−51.1	−44.4	−43.2
3.80	−51.8	−50.1	−47.4	−41.8	−40.4
3.90	−48.7	−47.8	−5.2	−39.7	−38.6
4.00	−46.5	−46.0	−43.0	−37.7	−37.3
4.20	−42.8	−42.8	−39.7	−35.0	−34.6

TABLE IV

DETERMINATION OF IONS IN MIXTURES

M^{z+}	*M^{z+} Added (mole)*	*M^{z+} Found (mole)*
M^{z+}	$1.70 \cdot 10^{-5}$	$1.69 \cdot 10^{-5}$
Cr^{3+}	$6.00 \cdot 10^{-5}$	$5.80 \cdot 10^{-5}$
Cu^{2+}	$1.29 \cdot 10^{-4}$	$1.30 \cdot 10^{-4}$
La^{3+}	$1.47 \cdot 10^{-4}$	$1.40 \cdot 10^{-4}$
Cu^{2+}	$1.29 \cdot 10^{-4}$	$1.28 \cdot 10^{-4}$
Gd^{3+}	$1.02 \cdot 10^{-4}$	$1.02 \cdot 10^{-4}$
U^{2+}	$1.29 \cdot 10^{-4}$	$1.30 \cdot 10^{-4}$
Al^{3+}	$1.47 \cdot 10^{-1}$	$1.45 \cdot 10^{-1}$

of the unknown can be determined by back-titration of the excess of EDTA with standard magnesium(II) solution at pH 8–9. Then cyanide[5] is added and the released EDTA, which is equal to the amount of copper(II) masked, is titrated with magnesium(II) solution. M^{3+} can be obtained from the difference of the two titrations. The titration end-point can be obtained more precisely in 20–30% (v/v) methanol solution. Table III shows that the potential change for each addition of titrant before the end-point is small. Table IV gives some results of the titration of mixed ions.

Precision

In the direct calibration method, the precision was found to be the same as that for the measurement of divalent ions, being $\pm 2\%$ in the range $2 \cdot 10^{-4}$–$2 \cdot 10^{-3}$ *M*.

In the titrations, since EDTA forms complexes with most metal ions in an equimolar ratio, the inflection point in the titration curve should correspond to the theoretical equivalence point. The precision can be estimated as follows: if the titrant is $3 \cdot 10^{-2}$ *M*, and 0.1-ml increments are added near the end-point, the error should be less than $3 \cdot 10^{-6}$ mole, and so the relative error depends on the amount of sample taken; $\pm 2\%$ is reasonable for an ion-selective electrode, and 10^{-3}–10^{-4} mole of sample is recommended for this method. Of course, reducing the concentration of titrant or the volume in each addition may reduce the error, but the potential difference should be big enough to identify the equivalence point. From the titration data in Table III, establishment of the end-point is not difficult.

The assistance of Dr. H. T. Tsai and S. C. Wu, Institute of Nuclear Energy Research, Atomic Energy Council, is gratefully acknowledged.

SUMMARY

A method of determining tri- and tetravalent ions with a divalent ion-selective electrode is proposed. The determination is based on displacement of the divalent metal ion from the Mg–EDTA or Zn–EDTA complex. The approximate ranges for direct measurement are $3 \cdot 10^{-3}$–$3 \cdot 10^{-4}$ *M*. Titrimetric methods are recommended for analysis of mixtures of metal ions. Optimal pH ranges and precision are discussed.

REFERENCES

1 B. H. Jack, *Anal. Chem.*, 41 (1969) 1459.
2 G. Schwarzenbach and H. Flaschka, *Complexometric Titrations*, Methuen, London, 1969, p. 10.
3 K. L. Cheng, *Microchem. J.*, 8 (1964) 22.
4 A. J. Ringbom, *J. Chem. Educ.*, 35 (1958) 282.
5 D. D. Perrin, *Masking and Demasking of Chemical Reactions*, Wiley, New York, 1970, p. 33.

Analytica Chimica Acta, 76 (1975) 183–192

NITRATE AND AMMONIUM ION-SELECTIVE ELECTRODES AS SENSORS

PART II. ASSAY OF NITRATE ION AND NITRATE AND NITRITE REDUCTASES IN STATIONARY SOLUTIONS AND UNDER FLOW-STREAM CONDITIONS

W. R. HUSSEIN* and G. G. GUILBAULT

Department of Chemistry, University of New Orleans, New Orleans, Louisiana 70122 (U.S.A.)

(Received 6th October 1974)

In a previous paper[1], the induction and isolation of nitrate and nitrite reductases from *Escherichia coli* were reported. The dissimilatory (respiratory) formate–nitrate reductase, which reduces nitrate to nitrite (eqn. 1) in the presence of a hydrogen donor, was induced and isolated from *E. coli* strain B. Also, nitrite reductase (NADH-nitrite oxidoreductase EC 1.6.6.4.) which reduces nitrite to ammonia (eqn. 2) was induced and isolated from the mutant *E. coli* strain Bn. Nitrate and ammonium ion-selective electrodes were used as continuous sensors during the growth procedure for verification of the various phases of the growth curves.

$$NO_3^- \xrightarrow[\text{reductase}]{\text{Nitrate}} NO_2^- \qquad (1)$$

$$NO_2^- + 6e^- + 8H^+ \xrightarrow[\text{reductase}]{\text{Nitrite}} NH_4^+ + 2H_2O \qquad (2)$$

According to eqns. (1) and (2), the enzymes nitrate and nitrite reductases catalyze the formation of nitrite and ammonium ions from nitrate, respectively, in the presence of suitable hydrogen donors and under optimal conditions. Spectrophotometry is almost the only technique applied for assay of the enzyme activities[2–4]. The decrease in nitrate concentration, or the increase in nitrite or ammonium ion concentrations, can be monitored for assay of enzyme activities.

In the present investigations, nitrate and ammonium ion-selective electrodes were applied as new analytical techniques for the assay of the enzyme activities. Nitrate ion-selective electrodes were used to follow the decrease in nitrate ion concentration (eqn. 1) both in stationary solutions and under flow-stream conditions. The ammonium ion-selective electrode, was used to follow the increase in ammonium ion concentration (eqn. 2). By application of the flow-stream technique with two nitrate ion-selective electrodes as reference and as indicating electrodes, the sensitivity was increased tenfold. This is due to the prefixed reaction time and elimination of the dilution factor when the enzyme is injected into the substrate solution under stationary conditions, and also to compensate for the additional response exerted

* Present Address: Department of Chemistry, University of Oklahoma, Norman, Oklahoma.

by non-enzymatic proteins at the sensitive membrane of the nitrate electrode. The results obtained by both techniques were compared with those from standard spectrophotometric methods.

Finally, a method for the assay of nitrate ion, based on its enzymatic reduction to ammonium ion, which is then assayed by the ammonium ion-selective electrode, was developed.

EXPERIMENTAL

Instrumental

All measurements were made with the Orion Digital pH-meter (Model 801) and the signal was displayed on a recorder (Sargent, Model SRLG).

For potentiometric measurements in stationary solutions, the Orion nitrate ion-selective electrode (liquid type – Model 92-07) was used (Fig. 1, A). For measurements in flow streams, the electrodes were modified by incorporating the liquid ion exchanger into a polyvinyl chloride matrix to form a semi-solid membrane[5]. Also the normal electrode cap was replaced by another threaded flow-through cap with two stainless steel inlet and outlet capillaries. The two capillaries opened internally into a reaction chamber of approximately 0.1-ml volume (Fig. 1, B).

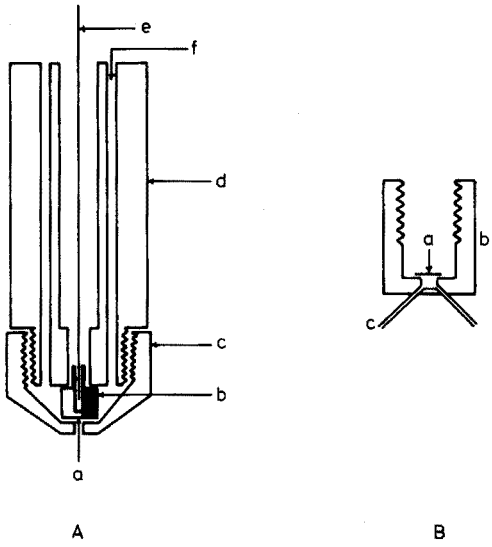


Fig. 1. Modification of the Orion nitrate electrode Model 92-07. A, Original design; (a) membrane, (b) membrane spacer, (c) cap, (d) electrode body, (e) internal reference, (f) injection opening. B. Modified design; (a) membrane, (b) modified cap, (c) stainless steel capillaries.

The flow system, shown in Fig. 2, consisted of a Sage peristaltic pump (Model 375) with variable tubing size and adjustable flow rates (d, e), two nitrate electrodes as reference and indicating electrodes (a', a), a mixing chamber (b), and a delay coil (c). The enzyme and substrate solutions were injected separately into the mixing chamber where proper mixing took place. A reaction time of 1 min was permitted through the delay coil (c) leading to the nitrate indicating electrode (a).

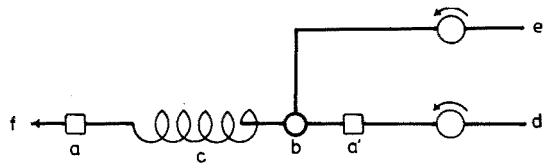


Fig. 2. Flow-diagram for continuous assay of enzyme activity: (a, a'), indicating and reference nitrate electrodes, respectively; (b) mixing chamber; (c) delay coil; (d) substrate solution at 1.5 ml min^{-1}; (e) enzyme at 0.5 ml min^{-1}; (f) waste.

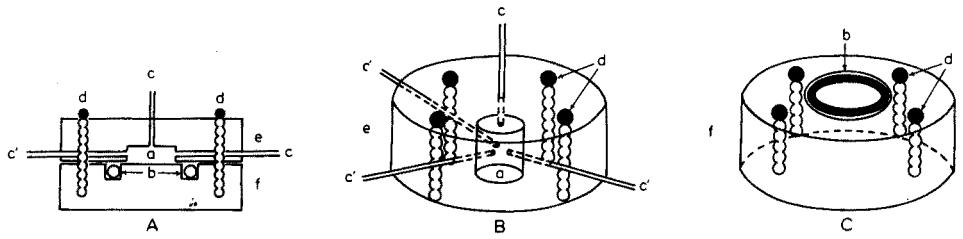


Fig. 3. Mixing chamber: (a) flow-through chamber (volume 1 ml); (b) O-ring; (c, c')) vertical and horizontal capillaries, respectively; (d) screws; (e) upper disc; (f) lower disc. A side view; B, upper disc; C, lower disc.

Since the mixing chamber was located after the first electrode (reference), the changes in substrate concentration arising from the enzymatic reaction were detected by the second electrode (indicating). A detailed diagram of the mixing chamber is shown in Fig. 3. The delay coil was kept in controlled-temperature bath and the outflow was gravity-fed to waste.

Since two nitrate reference and indicating electrodes, with relatively high impedance, were used in the potentiometric measurements in flow-streams, a separate differential amplifier with two high-impedance inputs[6] was built in the laboratory.

The ammonium-selective electrode was prepared in this laboratory. The sensitive membrane consisted of the antibiotic nonactin (SW 15859-Batch No. LBH-3599-99-10; Squibb Institute for Medical Research) imbedded in a silicone rubber matrix (No. 3140 RTV, Dow-Corning Corp., Midland, Mich.)[7]. The paste, obtained by mixing nonactin (300 mg) and silicone rubber (500 mg), was spread into a thin sheet and allowed to dry. Small discs were cut from the rubber elastic membrane containing the nonactin and fitted on one end of an open glass tubing. A silver wire in 0.1 *M* ammonium chloride was used as the internal reference electrode.

For spectrophotometric measurements, a Beckman DB spectrophotometer was used with an attached Sargent recorder (Model SRLG).

All chemicals used were reagent grade and the dissimilatory formate–nitrate reductase and nitrite reductase isolated from E. coli strains B and Bn respectively[1], were used as the stock solutions. The enzyme extracts were frozen until use.

Analytical Techniques

Potentiometric measurements. For measurements in stationary solutions, the Orion nitrate-selective electrode was used with a saturated calomel electrode to

follow the decrease in nitrate concentration. For measurements under flow-stream conditions, two modified PVC nitrate-sensitive membranes were used for the reference and the indicating electrodes. The nonactin ammonium-selective electrode was used with a s.c.e. to follow the increase in ammonium ion concentration.

Spectrophotometric measurements. Nitrate, nitrite, ammonium, and proteins were assayed spectrophotometrically according to the standard procedures[8] for comparison with data obtained potentiometrically. The brucine-sulfanilic method[9] was used for assay of nitrate, the Griess method[9] was used for assay of nitrite, and the phenol–sodium nitroprusside method[10] was used for ammonium ion measurements. The Folin–Ciocalteu method[11] was used for the determination of proteins.

RESULTS AND DISCUSSION

Potentiometric measurements in stationary solutions

A study of optimum parameters for the dissimilatory nitrate reductase was done potentiometrically with a nitrate-selective indicating electrode and a standard reference saturated calomel electrode. In all measurements, the decrease in nitrate concentration as indicated by the potential changes was taken as the measure of the enzyme activity. The reaction rates were determined by the slope method (mV min^{-1}). In all measurements, 20 ml of 10^{-3} *M* nitrate solution in 0.5 *M* phosphate buffer at pH 7.25 plus 1 ml of 1 *M* formate as the hydrogen donor were used, except for substrate studies; 3 ml of the enzyme preparation (1.35 I.U.) were used (one specific activity unit (I.U.) equals one μmole of nitrite produced or nitrate reduced per min. per mg of protein).

Optimal formate concentration. In order to determine the optimal concentration of sodium formate, the reaction rate was measured with various formate concentrations. The results obtained are shown in Table I; a formate concentration of $8 \cdot 10^{-2}$ *M* gave the maximum reaction rate. This formate concentration was used in all studies performed on nitrate reductase.

TABLE I

OPTIMAL FORMATE CONCENTRATION

Formate concn. ($\cdot 10^{-1}$ *M*)	0.4	0.8	1.2	1.6
ΔE (*mV* min^{-1})	8.12	14.15	9.10	7.60

Effect of temperature, pH, and buffer. Studies of optimal temperature, pH, and buffer type are essential for development of methods for assay of enzyme activity. Figure 4 shows that an increase in enzyme activity was observed with an increase in temperature; maximum activity was attained at about 45°C, which agrees very well with the reported values[2]. At such high temperatures, however, the error in measurement of the electrode response increases. Therefore, an incubation temperature of 38°C was used in all studies.

Studies on pH and buffer effects, showed that TRIS buffer gave the lowest enzyme activity; therefore, detailed studies were performed with phosphate and

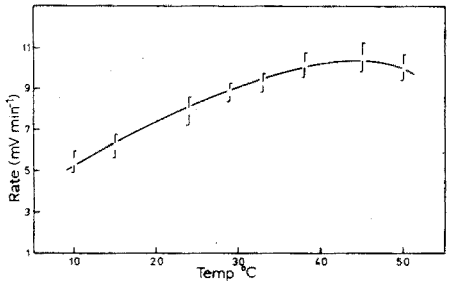


Fig. 4. Potentiometric measurements of the temperature effect on nitrate reductase activity in stationary solution.

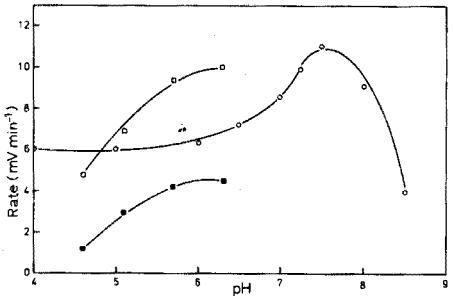


Fig. 5. Potentiometric measurements of pH and buffer effects on nitrate reductase activity in stationary solution. (○) phosphate buffer; (□) Acetate–LiOH buffer; (■) Acetate–KOH buffer.

acetate buffers. A series of 10^{-3} *M* nitrate solutions were prepared in 0.5 *M* phosphate buffers in the pH range 4–8.5. Solutions with acetate buffer were limited to the pH range 4.5–6.2. Two sets of acetate buffer solutions were prepared by using lithium hydroxide and potassium hydroxide for pH adjustment. Figure 5 shows the effect of pH and buffer on the nitrate reductase activity; clearly, phosphate buffer at about pH 7.5 provided optimal conditions. Above pH 7.5, a rapid enzymatic deactivation was observed, but at lower pH, the enzyme proved to be fairly stable. The enzyme activity remained almost constant in the pH range 4–6. Studies with acetate buffer showed that lithium enhanced the reaction rate. A maximum activity was obtained at a pH slightly above 6; at this pH, the buffering capacity of acetate buffer is not very good. Therefore, phosphate buffer was chosen for further studies.

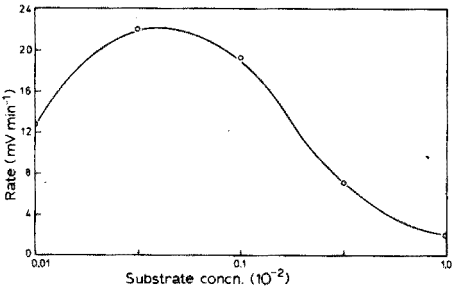


Fig. 6. Potentiometric measurements of substrate concentration effect on nitrate reductase activity in stationary solution.

Substrate concentration. For optimal concentration studies, nitrate solutions over the concentration range 10^{-2}–10^{-4} *M* were prepared in 0.5 *M* phosphate buffer at pH 7.25. In the assay, an enzyme activity of 1.35 I.U. was used. Figure 6 shows the changes in reaction rate with substrate concentration. An optimal substrate concentration was found to be between 10^{-3} and $5 \cdot 10^{-4}$ *M* nitrate. At substrate concentrations higher than 10^{-3} *M*, a sharp decrease in the rate was observed which could be due to enzyme inactivation. At lower substrate concentrations, the decrease in electrode response can be due to either the decrease in electrode sensitivity, or because the formation of the enzyme–substrate complex becomes a limiting factor at such low substrate concentrations.

Potentiometric measurements in flow-streams

Certain difficulties were encountered in potentiometric measurements in stationary solutions, such as the dilution effect when the enzyme was injected into the substrate solution. Stirring of the enzyme–substrate mixture in the open atmosphere can possibly cause oxidation of the enzyme. It was also found that proteins gave a certain electrode response. A more serious error that can be introduced is in the calculation of the slope. Because of the logarithmic electrode response, any slight deviation can introduce a relatively large error, particularly when small changes take place. Therefore, similar studies were carried out under flow-stream conditions.

The flow-system already described (Fig. 2), was used for nitrate reductase studies; two nitrate electrodes prepared in PVC membranes were used as indicating and reference electrodes.

Calibration of PVC nitrate electrodes. Since the flow-through cap is closed to the outer solution and cannot be replaced without destroying the electrode identity, the electrodes were calibrated under flow-stream conditions rather than in stationary solution. In the calibration procedure, a saturated calomel reference electrode was immersed in a beaker where the final waste of the flow system (f of Fig. 2) was collected. This insured electrical contact between the indicating nitrate and the reference calomel electrode. The two nitrate electrodes were calibrated separately against the calomel electrode over the nitrate concentration range 10^{-1}–10^{-4} *M* in 0.5 *M* phosphate buffer at pH 7.25.

Figure 7 shows the calibration curves for the two nitrate electrodes *versus* the calomel electrode under flow-stream conditions. The two electrodes did not give the

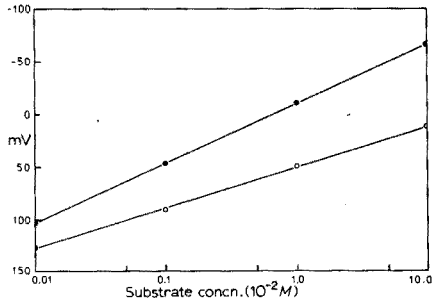


Fig. 7. Calibration of nitrate electrodes (PVC membrane) in flow-stream *vs.* s.c.e. (●) indicating nitrate electrode, (○) reference nitrate electrode.

same slopes (52 mV and 45 mV), which can be attributed to minor differences in homogeneity during the fabrication of the membrane. However, this does not cause any analytical problems in measurements.

Effect of pH and buffer. A 10^{-3} *M* nitrate and $8 \cdot 10^{-2}$ *M* formate solution was prepared in 0.5 *M* phosphate and acetate–LiOH buffers over the pH range 4–8.5. The substrate and the enzyme solutions were introduced into the mixing chamber at flow rates of 1.5 and 0.5 ml min^{-1}, respectively. The results obtained are shown in Fig. 8. Comparing these results with those shown in Fig. 5, the similarity in the trend is noticeable, but the broad peak obtained with phosphate buffer in the case of stationary solution measurements was much sharper in the case of flow streams with a maximum at pH 7.2. In certain enzyme systems, the optimal pH range is very narrow and in such cases the pH of the substrate becomes very critical.

Substrate concentration. Nitrate solutions over the concentration range $5 \cdot 10^{-2}$–10^{-4} *M* in 0.5 *M* phosphate buffer with a formate concentration of $8 \cdot 10^{-2}$ *M* were prepared for the determination of optimal substrate concentration. A 0.25% egg albumin solution was used for base-line determination and washing of the enzyme Figure 9 shows an optimal substrate concentration of 10^{-3}–$5 \cdot 10^{-4}$ *M* nitrate, which agrees with the results obtained in stationary solution (Fig. 6).

Nitrate reductase assay

The nitrate electrode response towards nitrate reductase activity was calibrated under optimal conditions, both in stationary solution and in flow-stream.

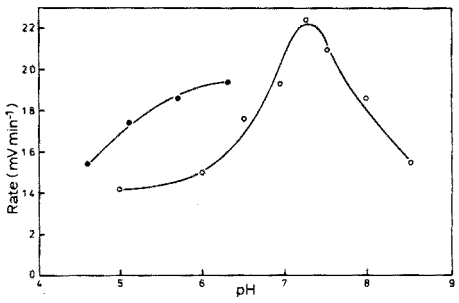


Fig. 8. Potentiometric measurements of pH and buffer effects of nitrate reductase activity in flow-stream. (○) Phosphate buffer, (●) Acetate–LiOH buffer.

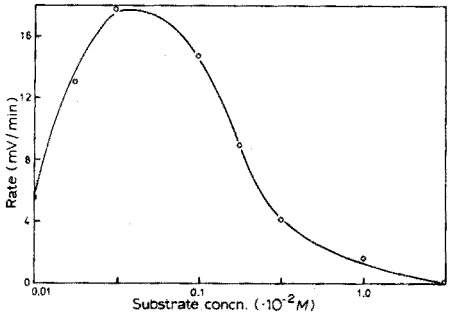


Fig. 9. Potentiometric measurements of the effect of substrate concentration on nitrate reductase activity in flow-stream.

Stationary solutions. A 10^{-3} *M* nitrate and $8 \cdot 10^{-2}$ *M* formate solution was prepared in 0.5 *M* phosphate buffer at pH 7.25. Aliquots of 20 ml of the substrate plus 5 ml of various enzyme solutions were used in the calibration study. The enzyme concentration range was 0.07–2.4 I.U. The calibration curve (Fig. 10) was constructed by plotting the enzyme activity units against the corresponding changes in mV min^{-1} detected by the indicating nitrate electrode as nitrate was reduced enzymatically to nitrite. The sensitivity limit was found to be 0.07 activity units. The activity units of the stock nitrate reductase preparation were determined potentiometrically in stationary solution and compared with those determined spectrophotometrically[1]. The results obtained showed excellent agreement between the techniques.

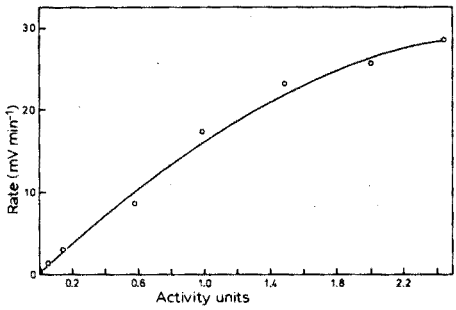


Fig. 10. Calibration of electrode response towards nitrate reductase activity in stationary solution.

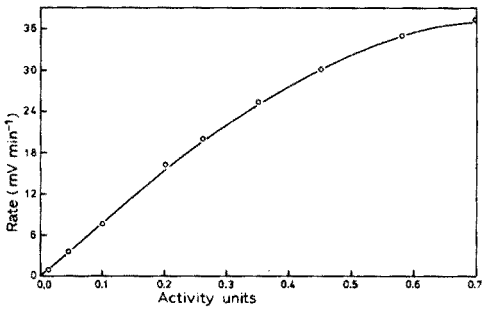


Fig. 11. Calibration of electrode response towards nitrate reductase activity in flow-stream.

Flow-stream conditions. Similarly, a 10^{-3} *M* nitrate and $8 \cdot 10^{-2}$ *M* formate solution in 0.5 *M* phosphate buffer at pH 7.25 was used for calibration of the electrode response under dynamic conditions. The ratio of the substrate to enzyme flow-rates was 1.5:0.5 ml min^{-1}. Figure 11 shows the enzyme activity calibration curve obtained. The enzyme concentration range was 0.007–0.7 units. By application of the flow-stream technique, the sensitivity was increased tenfold. The detection limit was 0.007 unit, compared with a detection limit of 0.07 unit obtained by the stationary solution technique. The scattering of the data obtained was much smaller, owing to the fixed reaction time and elimination of the dilution factor as well as compensation for the extra response exerted by the proteins at the nitrate electrode surface.

Analytical applications

One analytical application of the nitrate and nitrite reductases is in the construction of a new nitrate-sensitive electrode. Nitrate is reduced to nitrite by the action of nitrate reductase, while nitrite is reduced further to ammonium ion in the presence of nitrite reductase. The ammonium ion produced is quantitatively related to the nitrate concentration in solution.

In this study, the nonactin ammonium-selective electrode was used to monitor the increase in ammonium concentration as a result of enzymatic reduction of nitrate. The partially purified dissimilatory (respiratory) formate–nitrate reductase plus the partially purified NADH–nitrite reductase were used to carry through the reduction of nitrate to ammonium.

The calibration graph of the nonactin ammonium-selective electrode showed a linear response over the concentration range 10^{-1}–$5 \cdot 10^{-4}$ *M* with a linear slope of 40 mV/decade. The electrode was stable over a period of several months with some loss in sensitivity.

Figure 12 shows an elementary study of the effect of substrate concentration on the enzyme-system activity. The nitrate solutions over the concentration range of 10^{-2}–10^{-4} *M* were prepared in 0.1 *M* NaH_2PO_4–LiOH buffer. Potassium was eliminated because of its serious interference at the ammonium electrode (selectivity coefficient $6.5 \cdot 10^{-2}$ for ammonium over potassium) and the sodium concentration was kept at a minimum. The reaction rate (mV min^{-1}) increased with increase in nitrate concentration. A maximum was obtained at 10^{-3} *M* nitrate. The decrease in electrode response at lower substrate concentration is due to the non-linearity of the electrode behavior at concentrations lower than $5 \cdot 10^{-4}$ *M*. The decrease in response at high substrate concentrations is due to substrate inhibition of the enzymatic reaction.

The enzymes isolated at this stage of the work had relatively low activity and were isolated in small quantities. Improvements in the yields are still necessary. For the construction of a nitrate electrode (with the nitrate and nitrite reductases as catalysts to reduce nitrate to ammonium ions, and an ammonium-selective membrane as a sensor), it is necessary to have the enzyme mixture entrapped in a solid matrix at the surface of the ammonium-selective electrode, *i.e.*, the enzymes have to be immobilized. Immobilization techniques, either chemical or physical, can be applied. Experience indicates that chemical immobilization is preferred to physical entrapment.

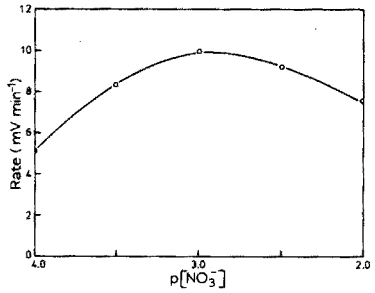


Fig. 12. Utilization of an ammonium-selective electrode in the enzymatic reduction of nitrate to ammonium.

The necessity of two enzyme systems, namely, nitrate and nitrite reductases, which are isolated separately and accordingly will have different relative activities, requires intensive studies for optimization of the appropriate ratios. The presence of more than one enzyme system with more than one chemically different active groups, makes it rather difficult to apply a certain immobilization technique without destroying any of these active groups.

More basic studies have to be pursued for the enzyme–nitrate electrode. Various findings such as the stability of the electrode, the linear range of response, interferences and performance limitations, have to be established. This electrode could have certain advantages over the liquid ion-exchanger nitrate electrode; mainly, the interferences in the enzyme electrode should be less. The only serious interference should be potassium ion which gives a response at the ammonium-selective electrode. Nitrite would introduce a positive interference since it can be reduced enzymatically to ammonium ion.

CONCLUSIONS

As a new, accurate and sensitive technique, nitrate and ammonium-selective electrodes were used as sensors for the assay of nitrate and nitrite reductase systems. Measurements based on the decrease in nitrate concentration in flow-streams, proved to be more sensitive than those applied in stationary solutions.

The financial assistance of the National Science Foundation (Grant No. GI-32995) and the National Institute of Health (Grant No. ES 00426 A1) is gratefully acknowledged.

SUMMARY

Nitrate ion-selective electrodes were successfully applied both in stationary solutions and in flow streams as new analytical techniques for the assay of nitrate reductase activity. An ammonium-selective electrode was similarly used to measure the increase in ammonium ion concentration during the enzymatic reduction of nitrate to ammonium. Preliminary studies on a true enzyme electrode for assay of nitrate ion are discussed.

REFERENCES

1 W. Hussein and G. G. Guilbault, *Anal. Chim. Acta*, in press (1974).
2 A. L. McNamara, *J. Agr. Food Chem.*, 19 (1971) 229.
3 E. G. McNall and D. E. Atkinson, *J. Bacteriol.*, 72 (1956) 226.
4 J. D. Kemp and D. E. Atkinson, *J. Bacteriol.*, 92 (1966) 628.
5 J. Davies, G. Moody and J. Thomas, *Analyst.*, 97 (1972) 87.
6 M. J. Brand and G. A. Rechnitz, *Anal. Chem.*, 42 (1970) 616.
7 G. G. Guilbault and G. Nagy, *Anal. Chem.*, 45 (1973) 417.
8 S. P. Colwick and N. O. Kaplan, *Methods in Enzymology, Vol. XXII, Part A*, Academic Press, New York and London, 1971.
9 F. J. Welcher, *Standard Methods of Chemical Analysis, Vol. 2, Part B*, Van Nostrand, New York, 1968.
10 N. W. Tietz, *Fundamentals of Clinical Chemistry*, Saunders, Philadelphia, 1970.
11 G. Litwack, *Experimental Biochemistry*, Second Edition, 1963, Wiley, New York.

Analytica Chimica Acta, 76 (1975) 193–198

SHORT COMMUNICATION

Application of resonance Raman spectrometry to the determination of vitamin B_{12}

CHENG-WEN TSAI* and MICHAEL D. MORRIS
Department of Chemistry, University of Michigan, Ann Arbor, Michigan 48104 (U.S.A.)
(Received 4th October 1974)

The resonance Raman effect has been known for many years[1]. Its potential for trace analysis was pointed out by Brandmüller[2] in 1959. However, full exploitation of resonance Raman spectrometry did not begin until the development of high power, multiline gas lasers as Raman excitation sources. The recent application of tunable dye lasers as excitation sources[3] promises even broader applications of resonance Raman spectrometry.

The resonance Raman effect is extremely sensitive. Rimai *et al.*[4] have observed resonance Raman spectra from β-carotene solutions as dilute as 10^{-7} *M*. Moreover, Raman bands are typically quite narrow, 10–20 cm^{-1} half-width. This width corresponds to 0.3–0.6 nm at 500 nm. Thus, while resonance Raman spectrometry has a sensitivity comparable to that of spectrophotometry, it offers a selectivity which spectrophotometry cannot match.

In the present work, resonance Raman spectrometry was investigated as an alternative to spectrophotometry for the determination of vitamin B_{12}. The standard spectrophotometric determination at 360 nm offers good sensitivity, but suffers from many potential interferences[5]. Microbiological methods have detection limits approaching 10^{-9} *M*, but require very careful control of experimental conditions in order to achieve reproducibility[6].

Recently, Hester *et al.*[7–9] have examined the resonance Raman spectra of vitamin B_{12}, cyanocobalamin. Similar, less extensive work has been reported by George and Mendelsohn[10]. Wozniak and Spiro[11] have shown that various cobalt corrins have resonance Raman spectra similar to that of cyanocobalamin.

Although several bands of the cyanocobalamin Raman spectrum are resonance-enhanced, the strongest is a ring stretching vibration at 1504 cm^{-1}. This band can be strongly enhanced using either the 488.0 nm or 514.5 nm line of an Ar^+ laser. Both of these lines lie in the α–β band system of the absorption spectrum of cyanocobalamin.

Previous researchers have worked with solutions in the 10^{-4}–10^{-3} *M* range. Such concentrations yield excellent spectra in which all the minor bands

* Present Address: Department of Chemistry, Texas Technological University, Lubbock, Texas 79409, U.S.A.

are clearly visible. In the present paper, it is shown that the resonance Raman spectrum of cyanocobalamin is detectable at submicromolar concentrations, and the effect of pH and of other water-soluble vitamins on the major band is described.

Experimental

All Raman spectra were obtained on a Spex "Ramalog" spectrometer, with a slit width of 9 cm^{-1} (400 μm) and a scan speed of 25 cm^{-1} min^{-1}. A cooled (−30°C) RCA C31034 photomultiplier was used as the detector. Both photon counting and d.c. current measurements were employed with chart recorder display. The excitation source was the 488.0-nm line from a Coherent Radiation CR 5 argon ion laser. Laser power was limited to 2 W at the laser head to avoid sample destruction. Standard 1.8-mm o.d. melting point capillaries were used as sample cells.

Cyanocobalamin, thiamine hydrochloride, pyridoxine hydrochloride, riboflavin and ascorbic acid were all U.S.P. grade and were used as received. Niacin (Diamond Shamrock, assay 99.5%) and folic acid (Calbiochem) were used without further purification. All other reagents were A.C.S. reagent grade. Distilled water was used to prepare all solutions.

Most experiments were run in acetic acid–sodium acetate buffers of pH 5. Buffers based on hydrochloric acid, phthalic acid, tris(hydroxymethyl)aminomethane, carbonate, phosphate and boric acid were used as required for control of pH. Riboflavin-containing solutions were made 0.001 *M* in mercury(II) ion and exposed to laser light (488 nm) for 10 min before measurements were made. This procedure reduced riboflavin fluorescence to tolerable levels.

The vitamin B_{12} 1504-cm^{-1} line was examined by scanning the spectrum from *ca.* 1450 to 1540 cm^{-1} and measuring the peak intensity relative to a baseline at 1530 cm^{-1}. The 1050-cm^{-1} line of 0.1 *M* nitrate was employed as an internal standard. Data are reported as the ratio of scattering intensity at 1504 cm^{-1} to intensity at 1050 cm^{-1}. For solution concentrations above about 10^{-6} *M*, the data are reproducible to about ±2%.

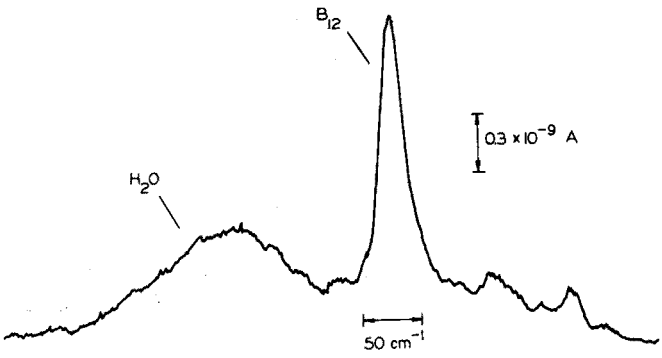


Fig. 1. Resonance Raman Band of vitamin B_{12} at 1504 cm^{-1}. Excitation at 488 nm. Resolution 9 cm^{-1}. $5 \cdot 10^{-6}$ *M* vitamin B_{12}.

Results and discussion

The 1504-cm^{-1} band of vitamin B_{12} is shown in Fig. 1. Also visible is the broad, weak water band at 1645 cm^{-1}. The full width at half-height of the 1504-cm^{-1} band is 20 cm^{-1}, about 0.6 nm. This width is independent of pH, concentration and laser frequency or power.

The pH dependence of the relative intensity of the 1504 cm^{-1} line ($6 \cdot 10^{-5}$ *M* Vitamin B_{12}) is shown in Fig. 2. In acidic solution the benzimidazole group of vitamin B_{12} is protonated and the benzimidazole–cobalt bond is broken. The change in relative intensity occurs around pH 3, where deprotonation of benzimidazole and formation of the cobalt–ligand bond is known to occur[12]. The inflection at *ca.* pH 8 apparently corresponds to deprotonation of the co-ordinated water molecule[12].

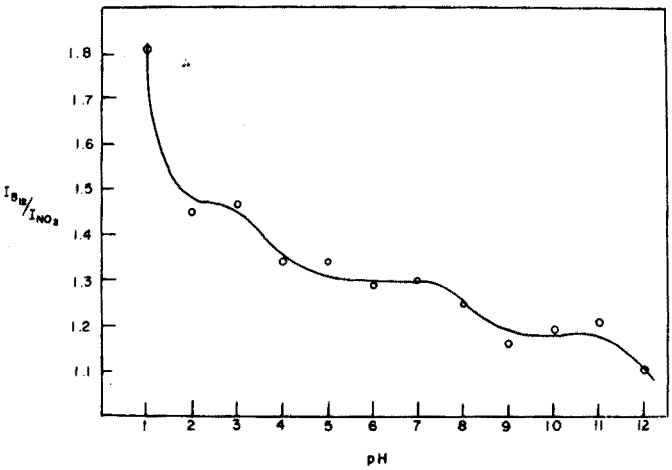


Fig. 2. pH dependence of 1504-cm^{-1} intensity. $6 \cdot 10^{-5}$ *M* vitamin B_{12}. Excitation at 488 nm. Resolution 9 cm^{-1}.

The causes of the large rise at pH 1 and the further dip in intensity at pH 12 are not known. These intensity changes are reproducible and reversible. Adjustment of the solution pH to any given value produces the same relative intensity, regardless of the prior history of the solution. This suggests that hydrolyses of the phosphate ester linkage and other irreversible reactions involving break-up of the molecule did not occur.

Because pH 5 is in a flat region of the intensity–pH curve, this pH was chosen for further experiments.

Table I shows the concentration dependence of the intensity of B_{12} scattering at pH 5. Scattering intensity is linear with concentration over the range $2 \cdot 10^{-6}$–$2 \cdot 10^{-5}$ *M*. Above $2 \cdot 10^{-5}$ *M*, reabsorption of scattered light causes negative deviations from linearity. Below $2 \cdot 10^{-6}$ *M* scattering intensity (per unit concentration) appears to increase. However, the signals in this region are sufficiently small that systematic errors in the photon-counting system and in baseline measurement techniques may be significant and partly responsible for the increase. Below

TABLE I

CONCENTRATION DEPENDENCE OF VITAMIN B_{12} SCATTERING AT 1504 cm^{-1}

$c_{B_{12}}$ ($\cdot 10^{-6}$ M)	$I_{B_{12}}/I_{NO_3}$ [a]
80	2.00
60	1.50
40.0	1.07
20.0	0.54
9.0	0.260
8.0	0.240
7.0	0.210
5.00	0.144
4.00	0.120
2.00	0.057
1.00	0.038
0.40	0.017
0.20	0.10

[a] 0.1 *M* KNO_3, pH = 5, photon counting.

$2 \cdot 10^{-7}$ *M* signals are too small (less than twice background fluctuations) to allow measurement. The background appears to be due to the presence of residual fluorescent impurities and to scattering by water. Lower detection limits are not obtained by increasing laser power.

The effect of other water-soluble vitamins on the vitamin B_{12} resonance Raman signal is shown in Table II. With the exception of riboflavin, the presence of at least a ten-fold excess of any given vitamin has only a small effect, usually a slight depression of less than 10%.

In order to reduce the intense fluorescence of riboflavin to manageable levels, mercury(II) ion was added to the solution to complex the riboflavin. After exposure to laser light for about 10 min, the fluorescence background decays to a signal whose intensity is about equal to that from $5 \cdot 10^{-5}$ *M* vitamin B_{12}. In the absence of mercury(II) nitrate, riboflavin fluorescence is *ca.* 1000 times stronger than the Raman scattering from $5 \cdot 10^{-5}$ *M* vitamin B_{12} and makes the measurement of that signal impractical if not impossible. The orange color persists, since only a very small volume of the solution is in the laser beam and subject to photodecomposition. The remaining fluorescence is due to a mixture of photodecomposition products and free and complexed riboflavin supplied by diffusion from the remainder of the solution. Since the undecomposed riboflavin and its complex have absorption spectra which overlap the B_{12} emission, these species can reabsorb the scattered light and attenuate the B_{12} signal. This reabsorption accounts for the strong attenuation of the B_{12} signal in the presence of even small amounts of riboflavin and is a serious impediment to the use of resonance Raman spectrometry in the analysis of vitamin B_{12} in multivitamin preparations.

An attempt was made to use resonance Raman spectrometry to assay vitamin B_{12} in multivitamin preparations, including standard decavitamin tablets. However, the concentration of riboflavin is sufficiently high and the concentration of vitamin B_{12} is so low (*ca.* $1 \cdot 10^{-6}$ *M* after extraction and dilution to volume) that

TABLE II

EFFECT OF OTHER WATER-SOLUBLE VITAMINS ON VITAMIN B_{12} SCATTERING

Added vitamin[a]	*Concentration (M)*	*Relative signal*
None	—	1.00
Thiamine hydrochloride	$0.51 \cdot 10^{-4}$	0.93
	$1.01 \cdot 10^{-4}$	0.93
	$2.02 \cdot 10^{-4}$	0.93
	$4.04 \cdot 10^{-4}$	0.93
Pyridoxine hydrochloride	$0.30 \cdot 10^{-4}$	0.94
	$1.60 \cdot 10^{-4}$	0.95
	$3.2 \cdot 10^{-4}$	0.96
	$6.4 \cdot 10^{-4}$	0.91
Folic acid	$1.13 \cdot 10^{-6}$	0.93
	$2.25 \cdot 10^{-6}$	0.87
	$9.0 \cdot 10^{-6}$	0.88
Niacin	$0.200 \cdot 10^{-4}$	0.93
	$0.50 \cdot 10^{-4}$	0.93
	$0.99 \cdot 10^{-4}$	0.87
	$1.98 \cdot 10^{-4}$	0.87
Riboflavin	$1.00 \cdot 10^{-6}$	0.78
	$2.50 \cdot 10^{-6}$	0.70
	$5.0 \cdot 10^{-6}$	0.71
	$10.0 \cdot 10^{-6}$	0.62

[a] Vitamin $B_{12} = 5.5 \cdot 10^{-6}$ *M*, pH = 5.0, 0.1 *M* KNO_3 internal standard.

residual fluorescence and reabsorption obscure any vitamin B_{12} signal.

Several groups have attempted time resolution of fluorescence emission from Raman scattering[13–15]. The technique gives only modest improvements in signal quality and is not capable of rejecting fluorescence signals which are orders of magnitude larger than the Raman signal. Practical application of resonance Raman scattering to analysis of vitamin B_{12} will have to await either improved fluorescence rejection techniques or a simple, highly efficient separation of vitamin B_{12} and riboflavin.

We wish to thank Ms. Betty Silver, Diamond Shamrock Chemical Company for samples of several vitamins.

REFERENCES

1 J. Behringer, in H. A. Szymanski (Ed.), *Raman Spectroscopy*, Plenum, New York, 1967, pp. 168–223.
2 J. Brandmüller, *Z. Anal. Chem.*, 170 (1959) 29.
3 T. C. Strekas and T. G. Spiro, *J. Raman Spectrosc.*, 1 (1973) 387.
4 L. Rimai, R. G. Kilponen and D. Gill, *J. Amer. Chem. Soc.*, 92 (1970) 382.
5 M.-U.-L. Hashmi, *Assay of Vitamins in Pharmaceutical Preparations*, Wiley, London, 1973, pp. 243–245.

6 M.-U.-L. Hashmi, *Assay of Vitamins in Pharmaceutical Preparations*, Wiley, London, 1973, pp. 267–276.
7 E. Mayer, D. J. Gardiner and R. E. Hester, *Biochim. Biophys. Acta*, 297 (1973) 568.
8 E. Mayer, D. J. Gardiner and R. E. Hester, *Mol. Phys.*, 26 (1973) 783.
9 E. Mayer, D. J. Gardiner and R. E. Hester, *J. Chem. Soc., Trans. Farad. Soc. Part II*, (1973) 1350.
10 W. O. George and R. Mendelsohn, *Appl. Spectrosc.*, 27 (1973) 390.
11 W. T. Wozniak and T. G. Spiro, *J. Amer. Chem. Soc.*, 95 (1973) 3402.
12 J. M. Pratt, *The Inorganic Chemistry of Vitamin* B_{12}, Academic Press, New York, 1972, pp. 130–153.
13 P. P. Yaney, *J. Opt. Soc. Amer.*, 62 (1972) 1297.
14 R. P. Van Duyne, D. L. Jeanmaire and D. F. Shriver, *Anal. Chem.*, 46 (1974) 213.
15 F. E. Lytle and M. S. Kelsey, *Anal. Chem.*, 46 (1974) 855.

Analytica Chimica Acta, 76 (1975) 199–203

SHORT COMMUNICATION

Direct spectropolarimetric determination of molybdenum(VI) with D-(−)-1,2-propylenediaminetetraacetic acid

R. A. GIBBS and R. J. PALMA Sr.
Department of Chemistry, Midwestern University, Wichita Falls, Texas 76308 (U.S.A.)
(Received 14th October 1974)

Several indirect titrimetric methods have been described for the determination of molybdenum with EDTA, DTPA and CDTA[1,2], but there are very few direct complexiметric methods for molybdenum[3–6] and they usually have difficult end-points.

The use of the optically active and stereospecific compounds, D-(−)-1,2-propylenediaminetetraacetic acid (D(−)PDTA) and D-(−)-*trans*-1,2-cyclohexanediaminetetraacetic acid (D(−)CDTA) in titrimetry has enjoyed considerable recent success[8–11], because of the introduction and improvement[12] of commercial photoelectric polarimeters. The method has proved to be simple, sensitive, rapid and versatile. The titrant and complexes formed serve as self-indicators, thus permitting the maximum quantitative pH range of the metal complexes to be utilized. The end-point is little influenced by high electrolyte concentrations and is therefore suitable with many separation schemes. D(−)PDTA was selected as the optically active titrant because of its chelating strength and intrinsic optical activity. The stability constants of most metal complexes of PDTA are slightly higher than those of EDTA complexes[13].

Experimental

Apparatus. A Perkin-Elmer Model 241 Photoelectric Polarimeter was used to monitor continuously the optical rotation of solutions. A 1-dm flow-through glass polarimeter cell with optically inactive end-plates and a 1-dm quartz o.r.d. cell (Perkin-Elmer) were used. The titrimetric apparatus was as previously described[7], except that the magnetic stirbar pump was replaced with a peristaltic pump for faster response. A Leeds and Northrup Research Model 7415 pH meter was used for all pH measurements. All pipets, burets and volumetric flasks were calibrated.

Reagents. All chemicals were analytical-reagent grade except as noted. All solutions were prepared with distilled demineralized water and stored in polyethylene bottles. D-(−)-1,2-propylenediaminetetraacetic acid monohydrate (*ca.* 0.25 mole) and 0.50 mole of sodium hydroxide pellets were dissolved in sufficient water to prepare 500 ml of solution; after filtration through a fine sintered glass filter, this stock Na_2–D(−) PDTA solution was standardized against a standard zinc solution by the usual methods employed for EDTA. More dilute solutions were prepared

fresh daily by serial dilution of the stock solution. A $9.820 \cdot 10^{-2}$ *M* $Na_2D(-)$-PDTA solution had $[M]_{578}^{22} = -129$ in a sodium acetate–acetic acid buffer of pH 4.78. The titer and optical rotation of the stock solution remained constant for 4 months. The standard sodium tungstate solution was prepared by dissolving 15.9834 g of dried 99.9% pure $Na_2WO_4 \cdot 2H_2O$ (Fisher) in water and diluting to 500 ml. The standard sodium molybate solution was prepared by dissolving 12.0255 g of $Na_2MoO_4 \cdot 2H_2O$ in 1 l of water and was then standardized[14] gravimetrically as lead molybdate. The various acetate buffers were prepared by adding sufficient glacial acetic acid to 1.2 *M* sodium acetate to attain the desired pH. The ammonia buffer was prepared by adding sufficient ammonia liquor to 2.0 *M* ammonium nitrate to attain the desired pH.

Recommended general procedure. Pipet a suitable aliquot of the metal ion solution into the titration vessel and add 10 ml of acetate buffer of appropriate pH. Add sufficient water to attain an exact final volume between 100 and 150 ml. This is needed to apply volume corrections to the observed rotation. Commence pumping and discharge all air bubbles from the polarimeter cell and tubing. With the spectropolarimeter set at 365 nm, zero the instrument against the prepared sample solution. (All readings were taken in the 5-s integration mode.) Titrate at ambient temperature with *ca.* 0.5 *M* Na_2–D(−)PDTA solution from a microburet readable to ±0.001 ml. Correct rotational readings as needed, by multiplying by the factor $(V_i+v)/V_i$, where V_i is the initial volume and v is the volume of titrant added, and determine the end-point graphically in the usual way.

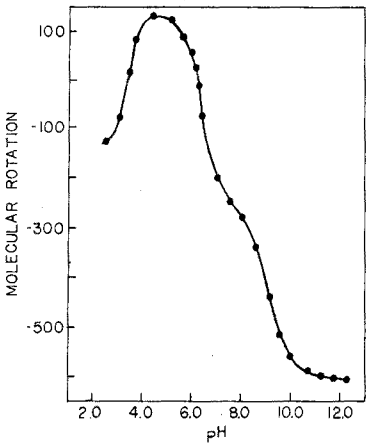
Results and discussion

pH studies were performed in order to determine the quantitative pH range for analysis, and to establish the maximum difference in rotation between the metal complex and ligand itself. This was accomplished by preparing a solution $5.10 \cdot 10^{-2}$ *M* in the metal ion and Na_2–D(−)PDTA, adjusting the pH to 12.5 with 0.1 *M* sodium hydroxide, titrating this solution in the flow-through cell with 0.1 *M* nitric acid, and correcting the observed rotation for dilution. Figure 1 shows the pH profile of the molybdenum(VI) complex; a fine white precipitate occurred at pH 2.5, which indicated dissociation of the complex and formation of the metal oxide. The shoulder at *ca.* pH 7.0 corresponds to a large maximum in the pH profile of the ligand itself caused by protonation–deprotonation of two of the acetate groups[7]; this indicates that the complex formed at pH 7.0 probably has at least two unbonded acetate groups. The maximum difference in optical rotation, and thus maximum interaction, between the metal complex and the ligand occurred over the pH range 4–6, in which the rotation of the ligand itself is essentially constant[7]; accordingly, molybdenum(VI) was titrated at pH 4.80.

TABLE I

MOLECULAR ROTATIONS OF THE MOLYBDENUM(VI) COMPLEX OF D(−)PDTA

pH	*589 nm*	*578 nm*	*546 nm*	*436 nm*	*365 nm*
4.78	+41	+43	+48	+78	+129
10.21	−211	−220	−250	−415	−620



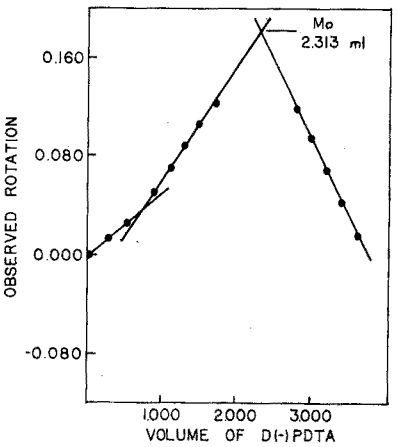


Fig. 1. Effect of pH on the $[M]^{22}_{365}$ of molybdenum(VI)–D(−)PDTA complex.

Fig. 2. Spectropolarimetric titration of $9.941 \cdot 10^{-2}$ *M* molybdenum(VI) at pH 4.8 and 365 nm.

The molecular rotations of the metal complex at various wavelengths are given in Table I. Measurements were made with 100 ml of solution which was $5 \cdot 10^{-2}$ *M* in metal ion and in ligand and contained 10 ml of the appropriate acetate or ammoniacal buffer. This plain Drude o.r.d. behavior was expected, since the complex did not absorb significantly in these regions. Because of the favorable rotation, a wavelength of 365 nm was chosen for analysis.

Figure 2 shows a typical titration of molybdenum(VI) with *ca.* 0.5 *M* D(−)PDTA at 365 nm. The two distinct end-points at 2:1 and 1:1 molar ratios of metal to ligand suggest that molybdenum(VI) forms two different complexes with D(−)PDTA, the first undergoing a rearrangement with excess of ligand. This is in contrast to EDTA, which forms only a 2:1 Mo(VI)–EDTA chelate[15] and a 2:1 Mo(V)–EDTA chelate[16], the former having a stability constant[3] of 11.7. The possibility that the two end-points resulted from slow kinetics was partly ruled out by boiling solutions containing 1:1 and 2:1 molar ratios of metal to ligand; there was no noticeable change in the molecular rotation after boiling. The structure of the Mo(VI)–D(−)PDTA and Mo(V)–D(−)PDTA complexes is currently being studied.

Tungsten(VI) and molybdenum(VI) are frequently isolated together in many separations, and tungsten(VI) normally interferes with the complexiметric titration of molybdenum(VI)[17]. It therefore seemed attractive to develop a simple titration for molybdenum(VI) in the presence of tungsten(VI) and, hopefully, a sequential titration for both ions. Mixtures of molybdenum(VI) and tungsten(VI) were titrated over the pH range 4–6 to determine the optimal pH; the best end-point was obtained at pH 5.2 but it was still so indistinct that sequential titration for tungsten-(VI) was impracticable. The molybdenum(VI) end-point however, was sharp and reproducible.

A typical spectropolarimetric titration of nearly equal molar quantities of tungsten(VI) and molybdenum(VI) with *ca.* 0.5 *M* D(−)PDTA at 365 nm is shown in Fig. 3. The molybdenum(VI) is complexed first, and the equivalence point was

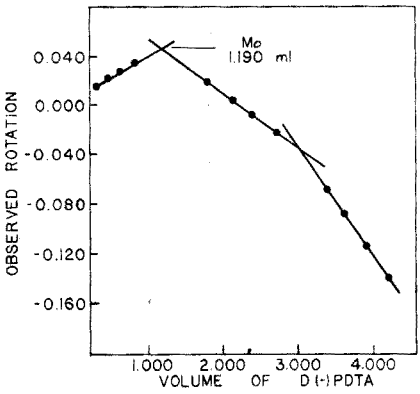


Fig. 3. Spectropolarimetric titration of $9.941 \cdot 10^{-2}$ *M* molybdenum(VI) in the presence of $9.690 \cdot 10^{-2}$ *M* tungsten(VI) at pH 5.2 and 365 nm.

TABLE II

RECOVERIES OF MOLYBDENUM(VI)

	Added[a] *(mg)*	*Found (mg)*	*Mean deviation (%)*	*Standard deviation*
Mo(VI)	95.36	95.66	+0.32	0.017
	19.07	19.15	+0.42	0.031
Mo(VI)[b]	47.68	47.55	+0.27	0.022

[a] Average of at least five titrations.
[b] In presence of added tungsten(VI).

taken at the 1:1 molar ratio of ligand to metal. The poor tungsten(VI) end-point occurred at a 2:1 ratio. It was therefore possible to determine molybdenum(VI) in the presence of tungsten(VI) without loss of accuracy. Table II gives the results of all the titrations. Higher concentrations of both metal ions were determined, but no detailed study of linear response was performed.

We are grateful to the Petroleum Research Fund administered by the American Chemical Society for support of this research (PRF-7577-B3), and to Dr. Karl H. Pearson, Cleveland State University, for helpful criticisms and the D(−)PDTA.

REFERENCES

1 W. T. Elwell and D. Wood, *Analytical Chemistry of Molybdenum and Tungsten*, Pergamon, Oxford, 1971, pp. 71–74.
2 H. Flaschka and A. J. Barnard Jr., *Chelates in Analytical Chemistry*, Dekker, New York, 1967, pp. 316–318.
3 I. Sajo, *Z. Anal. Chem.*, 199 (1963) 16.
4 S. Hayashi, *Jap. Anal.*, 11 (1962) 438, 951.

5 F. Bermejo-Martinez and M. Gimenez-Saiz, *Quim. Ind. Bilbao*, 15 (1968) 147.
6 J. Kinnunen and B. Wennerstrand, *Chemist–Analyst*, 47 (1958) 38.
7 R. J. Palma, Sr., P. E. Reinbold and K. H. Pearson, *Anal. Chem.*, 42 (1970) 47; *Anal. Lett.*, 2 (1969) 553.
8 R. J. Palma, Sr., and K. H. Pearson, *Anal. Chim. Acta*, 49 (1970) 497.
9 D. L. Caldwell, P. E. Reinbold and K. H. Pearson, *Anal. Chim. Acta*, 49 (1970) 505; *Anal. Chem.*, 42 (1970) 416; *Anal. Lett.*, 3 (1970) 93.
10 K. W. Street, Jr. and K. H. Pearson, *Anal. Chim. Acta*, 63 (1973) 107.
11 J. R. Baker and K. H. Pearson, *Anal. Chim. Acta*, 50 (1970) 255.
12 P. E. Reinbold and K. H. Pearson, *Anal. Chem.*, 43 (1971) 293.
13 *Stability Constants of Metal–Ion Complexes*, The Chemical Society, London, 2nd edn., 1964.
14 A. I. Vogel, *Quantitative Inorganic Analysis*, Wiley, New York, 3rd edn., 1961, p. 507.
15 R. D. Pecsok and D. E. Sawyer, *J. Amer. Chem. Soc.*, 78 (1958) 5496.
16 D. E. Sawyer and J. McKinnie, *J. Amer. Chem. Soc.*, 82 (1960) 4191.
17 E. Lassner and R. Scharf, *Chemist–Analyst*, 50, (1961) 6.

Analytica Chimica Acta, 76 (1975) 204–207

SHORT COMMUNICATION

Extraction–spectrophotometric determination of silver with thiodibenzoylmethane

R. R. MULYE and S. M. KHOPKAR
Department of Chemistry, Indian Institute of Technology, Bombay 400 076 (India)
(Received 12th September 1974)

The thio derivative of dibenzoylmethane was synthesized by Chaston *et al.*[1]. Berg and Reed[2] later isolated the complexes of cobalt, nickel, etc. and characterized their spectral and thermal properties. Uhlemann and Muller[3,4] first explored this reagent for the extraction and spectrophotometric determination of cobalt, nickel and copper. They extended their studies to the extractive photometric determination of copper[5], thallium, mercury[6] and cadmium[7]; the simultaneous extraction and direct spectrophotometric determination of cobalt and nickel[8] was possible in alkaline solutions. The work described here shows that silver can be quantitatively extracted at pH 6–7.5 with 10^{-3} *M* reagent in benzene as a pale yellow colored complex, and can be measured spectrophotometrically at 420 nm.

Amongst the β-diketones, benzoylacetone and dibenzoylmethane have been used[9] for the extraction of silver but these extractions are not quantitative and the equilibration time exceeds 1 h. Thiothenoyltrifluoroacetone has been successfully utilized for the extraction and colorimetric determination of silver[10]. Other methods for extraction of silver have been summarized[10,11] recently.

The method proposed here is simple and selective. With a low concentration of the reagent, it is possible to achieve the extraction–spectrophotometric determination of silver at tracer concentrations. Silver can be separated from moderate amounts of gold, cadmium, thallium, selenium etc., with which it may be associated in fission products.

Experimental

Apparatus. The equipment used was as follows: SF-4 quartz spectrophotometer with 10-mm matched silica cells, FEK-57 filter photometer, Cambridge pH meter with glass electrode and calomel reference electrode, and wrist-action flask shaker.

Reagents. Thiodibenzoylmethane was prepared from dibenzoylmethane (Koch-Light) by the usual procedure[2]. A 10^{-3} *M* solution was prepared in benzene. Buffer solution pH 6.0 was prepared by dissolving 77 g of ammonium acetate in 1 l of water and acidifying with acetic acid to pH 6.0. Buffer solution pH 12 was prepared by mixing 10 ml of 0.05 *M* borax with 12.65 ml of 0.1 *M* sodium hydroxide.

A stock solution of silver nitrate was prepared by dissolving 1.764 g of silver nitrate (BDH) in 500 ml of distilled water. The solution (2.240 mg Ag ml^{-1}) was

standardized titrimetrically[12]; solutions of lower concentration were prepared by appropriate dilution.

General procedure. Take an aliquot of silver(I) solution containing 5–100 μg of silver. Add 10 ml of buffer solution pH 6.0. Dilute with distilled water to 25 ml, and transfer the solution to a separatory funnel. Shake with 10 ml of 10^{-3} *M* thiodibenzoylmethane in benzene for about 10 min. Allow the layers to separate. After carefully removing the aqueous phase, shake the intense yellow colored organic phase with 10 ml of buffer solution pH 12 for about 2 min, to remove excess of reagent from the organic phase. Remove the alkaline aqueous phase. Transfer the organic phase to a 10-ml volumetric flask and dilute to the mark with benzene. Measure the absorbance of the complex at 420 nm against a reagent blank prepared similarly. Calculate the amount of silver from a calibration curve.

For studies of pH effect, the pH of the silver(I) solution was adjusted with 0.01 *M* ammonia solution or 0.01 *M* nitric acid to the required value.

Results and discussion

Absorption spectra. The absorption spectrum of the silver–thiodibenzoylmethane complex against the reagent blank is shown in Fig. 1; the reagent spectrum is also shown. Measured against the reagent blank, the complex shows maximal absorbance at 420 nm. The molar absorptivity of the complex was $1.069 \cdot 10^4$; the sensitivity according to Sandell's definition was 0.010 μg cm^{-2}. The excess of reagent had to be removed with alkaline buffer, otherwise the difference between the complex and the reagent was slight. This procedure has been used for removal of excess of reagent by other workers[3,13].

Effect of pH. The extraction of silver was studied in the pH range of 1–12 (Fig. 2); extraction was quantitative in the pH range 6–7.5.

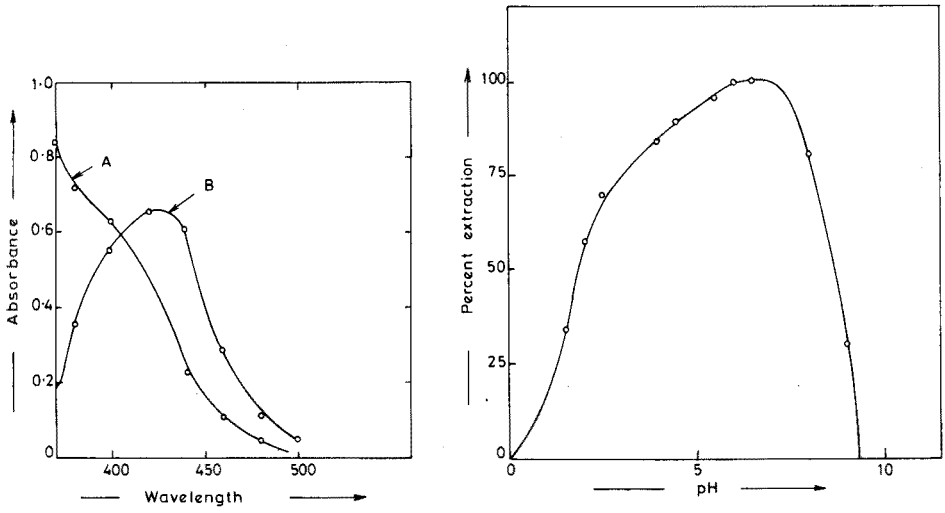


Fig. 1. Absorption spectra. (A) Reagent blank against benzene. (B) Silver complex against reagent blank. Ag = $5.178 \cdot 10^{-5}$ *M*; reagent = $1 \cdot 10^{-3}$ *M*; pH 6.0.

Fig. 2. Extraction as a function of pH. Silver(I), 56 μg; 10^{-3} *M* reagent.

Beer's Law. Different amounts of silver (0.7–9.8 μg ml^{-1}) were extracted at pH 6.0. The absorbance of the complex was measured at 420 and 450 nm. Beer's law was obeyed over the concentration range 0.5–10 μg Ag ml^{-1} at 420 nm.

Stability of the complex. The absorbance of the silver–thiodibenzoylmethane was stable for 24 h if stored in the dark. The complex remained stable for more than 4 h when exposed to diffuse light.

Effect of reagent concentration and time. The silver was extracted with different concentrations and volumes of reagent. The concentration was varied from 10 ml of $2.5 \cdot 10^{-4}$ *M* to 10 ml of $1 \cdot 10^{-3}$ *M* reagent. The extraction was also carried out with varying volumes of $1 \cdot 10^{-3}$ *M* reagent. The results showed that a single extraction with 10 ml of 10^{-3} *M* reagent provided quantitative extraction of silver. The extraction was incomplete with smaller volumes or smaller concentrations of the reagent; higher reagent concentrations showed no improvement in the extraction.

Extraction was done for times varying from 3 to 15 min. The extraction was quantitative within 5–10 min, and a time of 10 min is therefore recommended.

Effect of salting-out agents. The nitrates of sodium, potassium, ammonium, calcium and magnesium were used as salting-out agents to study the effect on extraction of silver with 10^{-3} *M* thiodibenzoylmethane at pH 6.0. None of these agents had any significant effect on the extraction.

TABLE I

EFFECT OF DIVERSE IONS

(28 μg Ag; pH 6.0; 10^{-3} *M* reagent.)

Tolerance limit (mg)	*Ion*
2.5	Li^+, Na^+, K^+
2.0	Al^{3+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, Mg^{2+}, WO_4^{2-}, ASO_3^{3-}, VO_3^-, cit^{3-}, $tart^{3-}$, $malonate^{2-}$, F^-, SO_3^{2-}, SO_4^{2-}
1.0	Au^{3+}, Be^{2+}, $Mo_7O_{24}^{6-}$, SeO_3^{2-}, TeO_3^{2-}, $EDTA^{4-}$, $C_2O_4^{2-}$, NO_2^-
0.75	Zr^{4+}, Th^{4+}
0.50	Tl^+, Ti^{4+}
0.25	Cd^{2+}

Effect of salting-out agents. The nitrates of sodium, potassium, ammonium I); the tolerance limit was calculated as described earlier[14]. Most of the elements were tolerated in high ratios (1 : 100); some ions were tolerated in moderate amounts (1 : 10). Ions showing strong interference were uranium, copper, cobalt, cadmium, nickel and mercury. Interferences of some ions were eliminated by masking agents[15]; for example, titanium, beryllium and thallium were masked by sodium fluoride, whereas cadmium was masked with EDTA.

From ten determinations of 56 μg of silver, the absorbance was found to be 0.560 ± 0.01. The relative standard deviation was about $\pm 1.0\%$. The total operation required about 30 min.

REFERENCES

1 H. S. Chaston, S. E. Livingstone, T. N. Locleyer, V. A. Pickles and J. S. Shannon, *Aust. J. Chem.*, 18 (1965) 673.
2 E. W. Berg and K. P. Reed, *Anal. Chim. Acta*, 36 (1966) 372.
3 E. Uhlemann and H. Muller, *Anal. Chim. Acta*, 48 (1969) 115.
4 E. Uhlemann and H. Muller, *Anal. Chim. Acta*, 41 (1968) 311.
5 E. Uhlemann, B. Schuknecht, K. D. Busse and V. Pohl, *Anal. Chim. Acta*, 56 (1971) 185.
6 E. Uhlemann and B. Schuknecht, *Anal. Chim. Acta*, 69 (1974) 79.
7 B. Schuknecht, G. Rubisch and E. Uhlemann, *Anal. Chim. Acta*, 69 (1974) 329.
8 E. Uhlemann and B. Schuknecht, *Anal. Chim. Acta*, 63 (1973) 236.
9 J. Stary and E. Haldky, *Anal. Chim. Acta*, 28 (1963) 227.
10 K. R. Solanke and S. M. Khopkar, *Mikrochim. Acta*, (in press).
11 A. K. De, S. M. Khopkar and R. A. Chalmers, *Solvent Extraction of metals*, Van Nostrand–Reinhold, London, 1970.
12 A. I. Vogel, *Quantitative Inorganic Analysis*, Longmans–Green, London, 1962, p. 260.
13 H. Hashitani and K. Katsuyama, *Jap. Anal.*, 19 (1970) 356.
14 K. R. Solanke and S. M. Khopkar, *Anal. Chim. Acta*, 66 (1973) 307.
15 D. D. Perrin, *Masking and Demasking of Chemical Reactions*, Wiley–Interscience, New York, 1970.

Analytica Chimica Acta, 76 (1975) 208–212

SHORT COMMUNICATION

A single digestion procedure for rapid manual determinations of Kjeldahl nitrogen and total phosphorus in natural waters

KENNETH H. NICHOLLS
Department of Zoology, College of Biological Sciences, University of Guelph, Guelph, Ontario (Canada)
(Received 21st June 1974)

During a limnological investigation of the Holland River and Cook Bay of Lake Simcoe (Ontario, Canada)[1,2], it was realized that methods presently recommended for manual determination of Kjeldahl nitrogen[3–5] are not well suited to routine analyses of large numbers of samples. A manual method was developed for Kjeldahl nitrogen in natural waters with a sulphuric acid–hydrogen peroxide digestion followed by a phenol–hypochlorite determination of ammonia. The method is especially sensitive for low levels of organic nitrogen and is much less tedious than presently recommended methods involving digestion and distillation of ammonia.

The improved phenol–hypochlorite method[6,7] for the direct determination of ammonia in natural waters, utilizing nitroprusside as a catalyst and citrate to complex interfering alkaline earth ions, has the advantages over direct nesslerization of greater sensitivity and freedom from interferences. Furthermore, the time and cost involved in distillation makes the distillation procedures much less desirable than the phenol–hypochlorite method for routine limnological investigations.

Several authors have automated the Kjeldahl digestion of aqueous samples and determined the resulting ammonium ion as an indophenol blue complex by automation of the phenol-hypochlorite method[8–10]. No manual Kjeldahl methods have taken advantage of the phenol–hypochlorite method for the determination of ammonia in the digested sample. The manual method outlined here utilizes the phenol–hypochlorite method of Solorzano[7] to determine the ammonia in the digested sample, following the digestion procedure of Harwood *et al.*[11] for total phosphorus determination. Analytical reagent quality 50% hydrogen peroxide has several advantages over other oxidants for the destruction of organic matter[12] and when combined with sulphuric acid, forms an effective digestion mixture for the determination of Kjeldahl nitrogen in plant material[13]. Furthermore, the use of a strong oxidant such as hydrogen peroxide in the digestion mixture prevents any danger of reduction of nitrate to ammonia[14]. Copper and mercury when used as catalysts in the digestion procedures interfere with the final phenol–hypochlorite determination of ammonia and no catalyst is used in the digestion step of the present method.

Experimental

Reagents. All reagents must be of analytical-reagent quality. The stock sulphuric acid and hydrogen peroxide solution should be the lowest possible in ammonia. Water used for solution of reagents and dilution of samples must be distilled and deionized. All glassware must be cleaned initially by refluxing with sulphuric acid in a fume hood.

Procedure. Place 25 ml of the water samples in 50-ml volumetric long-necked pyrex flasks (Note a). Add 0.5 ml of concentrated sulphuric acid (*d* 1.82). Add 2–3 anti-bumping granules (Note b), Boil the sample at about 200°C in a fume hood until all the water has been evaporated and white fumes of sulphur trioxide are evolved. The acid should reflux down the neck of the flask. If much organic matter is present in the sample, it will be charred at this stage. Remove the flasks from the hot plate and add 4 drops (0.5 ml) of 50% hydrogen peroxide. Return the flasks to the hot plate and heat to fumes of SO_3. If the digest is not now clear, repeat the addition of 50% hydrogen peroxide. Reflux the acid for a further 5 min; all the peroxide must be destroyed.

Add about 25 ml of deionized water to the digest. Add one drop of 0.4% (w/v) phenolphthalein solution in (1+1) water–ethanol. Add 10 *M* sodium hydroxide solution dropwise until the sample turns pink. Without delay add dilute (1+4) sulphuric acid solution dropwise until the sample just turns colourless. Top up to the 50-ml mark with deionized water. Empty the samples from the 50-ml digestion flasks into 125-ml Erlenmeyer flasks and determine ammonia as described by Solorzano[7].

Standards are prepared with ammonium sulphate. Standards and deionized water blanks receive the same treatment as outlined for samples.

All analyses were carried out with a model 101 Hitachi Coleman spectrophotometer and 1-cm cells. Prepared standards yielding concentrations of ammonia nitrogen greater than 0.6 mg l^{-1} were diluted with deionized distilled water after formation of the indophenol blue complex.

Notes. (a) A 25-ml sample diluted to 50 ml after digestion will contain sufficient nitrogen to cover the range 0.05–1.5 mg N l^{-1}. For water with higher nitrogen contents, dilution will be necessary. It is advisable to use a constant volume for all samples, standards and blanks. If phosphorus as well as ammonia are to be determined on the digested sample, 100-ml volumetric flasks and a correspondingly larger sample volume will be necessary. To determine phosphorus, the single solution method of Murphy and Riley[15] may be used directly on the digested sample after neutralization and dilution[11]. (b) Fisher's "Boileasers" work well but should be boiled in acid solution, thoroughly rinsed with deionized water, and oven-dried before use.

Results and discussion

When Solorzano's method was used, Beer's law was obeyed over the range 0.005–2.0 mg N l^{-1} in undigested standards prepared with ammonium sulphate (Fig. 1). Somewhat lower absorbances at 640 nm on digested standards compared to undigested standards (Fig. 1) may have been brought about by some oxidation of ammonia by peroxide. These apparent losses are quite small and can be considered negligible as long as samples and standards are treated alike.

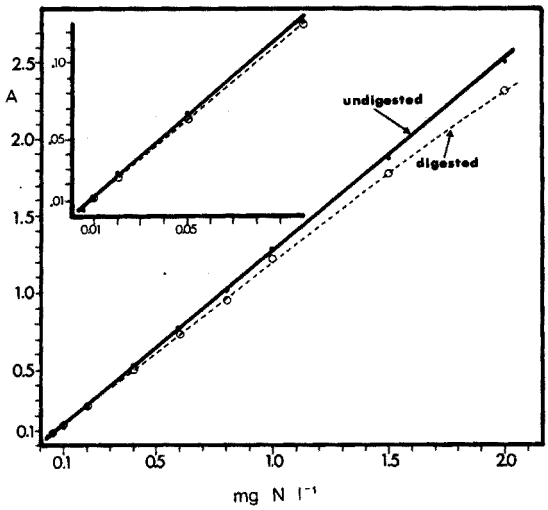


Fig. 1. Absorbance of indophenol blue complex on undigested and digested standards of ammonium sulfate by Solorzano's [7] phenol–hypochlorite method.

Losses of ammonia during neutralization of the digest were found to be unimportant. Ten samples, each containing 1.0 mg $N \cdot l^{-1}$, as ammonium sulfate, were digested as outlined above. Five of the samples were neutralized without cooling, and after 5 min the solution was diluted to volume and the reagents for formation of the indophenol were added. The other 5 samples were neutralized in an ice bath and diluted to volume, and the reagents were added without delay. There was no apparent loss of ammonia during the 5-min delay in the first treatment; the average ammonia nitrogen concentrations were 0.94 ± 0.019 and 0.95 ± 0.017 mg $N\ l^{-1}$, respectively. Losses of ammonia during neutralization may occur if sodium hydroxide solution is added in excess, so that a hot solution of high pH results. It is therefore important that the acid-to-sample volume ratio recommended above be used and that the alkali be added dropwise during neutralization.

Nearly complete recovery of nitrogen from prepared standards of urea and *l*-cystine were obtained (Table I). The recovery of nitrogen from pyridine and its

TABLE I

RECOVERY OF NITROGEN FROM THREE ORGANIC NITROGEN COMPOUNDS

Compound	*Concentration (mg N l^{-1})*	*Number of samples*	*Mean recovery ($\pm s_r$) (%)*
Urea	0.20	5	98 ± 3.8
	2.00	8	97 ± 2.6
l-Cystine	0.20	5	96 ± 6.2
	2.00	5	96 ± 4.6
2-Aminopyridine	0.20	8	69 ± 7.0
	2.00	5	59 ± 3.6

derivatives at low concentrations by Kjeldahl digestion is most often incomplete[16]. The proposed method yielded 60–70% of the nitrogen in 2-aminopyridine (Table I), which is comparable to the recoveries obtained from such compounds by other methods[9,16]. No nitrogen was recovered from 2-aminopyridine when peroxide was omitted from the digest.

Excellent recoveries of *l*-cystine from spiked samples of stream and lake water were achieved (Table II).

TABLE II

RESULTS OF AMMONIA NITROGEN AND TOTAL KJELDAHL NITROGEN ANALYSES OF NATURAL WATERS

(mg N l^{-1}; arithmetic mean of 10 subsamples ± standard deviation)

		NH_3 nitrogen	*Total Kjeldahl nitrogen*
Sample No. 1[a]		0.03 ± 0.004	0.44 ± 0.009
Sample No. 2[b]		<0.010	1.32 ± 0.021
Sample No. 1 + *l*-cystine spike			
	theoretical	0.03	0.60
	determined	0.03 ± 0.005	0.59 ± 0.008
	recovery	100%	98%
Sample No. 2 + *l*-cystine spike			
	theoretical	<0.010	1.23
	determined	<0.010	1.25 ± 0.023
	recovery	—	102%

[a] Upper West Branch of the Holland River, York Co., Ontario, July 5, 1972.
[b] South Cook Bay of Lake Simcoe, Simcoe Co., Ontario, July 5, 1972.

Conclusions

The proposed manual method for Kjeldahl nitrogen determinations in natural waters is sufficiently simple to be performed routinely. One person can complete 30–40 analyses per day. Concentrations as low as 20 μg N l^{-1} can be determined reproducibly. Another advantage of the proposed method is that the digested sample may be analyzed for total phosphorus after neutralization. Harwood *et al.*[11] showed that the acid–hydrogen peroxide digestion used here can replace the widely used acid–persulphate digestion for total phosphorus in natural waters. The method described above should find application in laboratories which are not equipped for automated analyses, yet where total nitrogen and phosphorus are being regularly determined in water samples.

REFERENCES

1 K. H. Nicholls and H. R. MacCrimmon, *J. Environ. Quality*, 3 (1974) 31.
2 K. H. Nicholls and H. R. MacCrimmon, *Int. Rev. Gesamten Hydrobiol.*, (1975) in press.
3 *Standard Methods for the Examination of Water and Waste Water*, American Public Health Association, 1971.
4 H. L. Golterman (Ed.), *Methods for Chemical Analysis of Fresh Waters*, Blackwell, Oxford, 1969.
5 J. D. H. Strickland and T. R. Parsons, *A Practical Handbook of Sea Water Analysis*, Bull. Fish. Res. Bd. Canada, No. 167, 1968.

6 J. E. Harwood and A. L. Kuehn, *Water Res.*, 4 (1970) 805.
7 L. Solorzano, *Limnol. Oceanogr.*, 14 (1969) 799.
8 J. E. Harwood and D. J. Huyser, *Water Res.*, 4 (1970) 539.
9 P. A. Kammerer, M. G. Rodel, R. A. Hughes and G. F. Lee, *Environ. Sci. Technol.*, 1 (1967) 340.
10 W. H. McDaniel, R. N. Hemphill and W. T. Donaldson, *Technicon Symposia—Automation in Analytical Chemistry*, Mediad, New York, 1967, p. 363.
11 J. E. Harwood, R. A. van Steenderen and A. L. Kuhn, *Water Res.*, 3 (1969) 425.
12 Analytical Methods Committee, *Analyst*, 92 (1967) 403.
13 R. L. Thomas, R. W. Sheard and J. R. Moyer, *Agron. J.*, 59 (1967) 240.
14 S. Vitula, *Agrochemia (Bratislava)*, 11 (1971) 150.
15 J. Murphy and J. P. Riley, *Anal. Chim. Acta*, 27 (1962) 31.
16 R. B. Bradstreet, *The Kjeldahl Method for Organic Nitrogen*, Academic Press, New York, 1965.

Analytica Chimica Acta, 76 (1975) 213–214

SHORT COMMUNICATION

Some pitfalls in high-pressure liquid chromatography

W. J. CHAMBERLAIN, D. B. WALTERS and O. T. CHORTYK

Richard B. Russell Agricultural Research Center, Agricultural Research Service, United States Department of Agriculture, Athens, Ga. 30604 (U.S.A.)

(Received 22nd July 1974)

This report calls attention to certain pitfalls in the methodology of high-pressure liquid chromatography (h.p.l.c.), as used in studies with natural products or complex organic mixtures such as cigarette smoke condensate.

In h.p.l.c., column temperature is usually not an important consideration[1]. However, temperature does effect separations in reversed-phase techniques, which require an apolar stationary phase with a polar mobile phase (generally methanol–water). Recent studies[2] on polycyclic aromatic hydrocarbons (PAH) from cigarette smoke condensate have shown that the column temperature has a significant effect on retention time and resolution of these biologically active compounds. For example, the separation of pyrene and benzo(a)pyrene (BaP) at ambient column temperature is significantly different from the separation at 60°C (Table I). Also, temperature change significantly alters the flow rate, so that the pressure must be adjusted to maintain a constant flow of 1.5 ml min^{-1}.

TABLE I

EFFECT OF COLUMN TEMPERATURE ON RETENTION TIME (t_R) AND RESOLUTION (R_s)[a]

Sample	*Temp. (°C)*	t_R *(min)*	R_s
Pyrene	20	3.25	0.88
BaP	20	10.75	
Pyrene	60	2.5	1.00
BaP	60	7.25	

[a] An octadecylsilane column with a mobile phase of methanol–water (60:40, v/v) was used in a DuPont 820 HSLC, equipped with a constant-pressure pumping system.

Another difficulty in h.p.l.c. involves injection technique, specifically the injection of samples at high pressures (1200–2000 p.s.i.). The use of high-pressure syringes containing special seals that allow injection with inlet pressure up to 300 atm is recommended. An alternative involves the use of a stop-flow technique, which stops the flow of mobile phase either with a flow control valve or by stopping the pump, allowing the use of regular syringes. Effects of variation in

TABLE II

EFFECT OF INJECTION TECHNIQUE ON RETENTION TIME (t_R) AND PEAK HEIGHT

Type of syringe	*Injection technique*	t_R *(min)*	*Peak height range (cm)*[a]
Regular	Regular	6.0	4.8–10.8
High-pressure	Regular	6.0	18.5–19.3
Regular	Stop-flow	6.6	15.8–17.0
High-pressure	Stop-flow	6.3	15.6–15.7

[a] Range for four determinations, with a flow rate of 1.1 ml min^{-1}.

injection techniques are illustrated for pyrene in Table II. The results show that losses are considerable when regular, instead of high-pressure, syringes are used to inject under pressure. The flow-stop method, apparently, is the easiest injection technique, but requires a short time lag to regain the desired pressure. This lag is reflected in the increased retention times. This increase in retention time is due to re-equilibration of flow rate and should be taken into account. The problem of reproducibility with different types of syringes has been eliminated by the recent development of a septum-less injection system, while the use of constant-flow pumps reduces the problem of a changing flow-rate during analysis[3].

Another serious problem in h.p.l.c. of natural product extracts or fractions of cigarette smoke condensate is column blockage. Extraneous materials, soluble in the injecting solvent but insoluble in the mobile phase, are deposited on the column. This gradually reduces the flow rate in a constant-pressure system, necessitating washing the column with an appropriate solvent or series of solvents. Continuous day-to-day examination of the data is necessary to insure that the flow rate has not decreased because of column blockage. The metal frit at the entrance of the column, which first becomes blocked, may be drilled out and replaced with glass wool or another frit. (Kits for replacing frits are available.) This does not eliminate the problem but is helpful. Solubility of the injected material in the mobile phase may also be a problem when working at a maximum solubility limit of a particular compound. For example, BaP may be precipitated out on the column after injection in an organic solvent, that is diluted with the mobile methanol–water phase. Increased column temperature is often helpful in eliminating this problem.

Thus, continued development of sensitive analytical instruments, such as the liquid chromatograph, also required recognition of methodological shortcomings that could result in serious analytical errors.

The authors thank Scott Pioreck and Joey Futch for their assistance.

REFERENCES

1 J. J. Kirkland, *Modern Practice of Liquid Chromatography*, Wiley, New York, 1971.
2 D. B. Walters, W. J. Chamberlain, M. E. Snook and O. T. Chortyk, *Anal. Chim. Acta*, 73 (1974) 190.
3 *Varian Instrument Bulletin No. SEP-1827A.*

Analytica Chimica Acta, 76 (1975) 215–218

SHORT COMMUNICATION

Effects of quaternary ammonium bases on valence-saturated but coordination-unsaturated chelates. Part III. Extraction of nickel– and cobalt–thenoyltrifluoroacetone chelates

SHINICHIRO NORIKI

Analytical Chemistry Division, Department of Chemistry, Faculty of Fisheries, Hokkaido University, Hakodate 040 (Japan)

(Received 2nd October 1974)

Thenoyltrifluoroacetone (TTA) forms valence-saturated (for the central atom) but coordination-unsaturated chelates, $M(TTA)_2 \cdot 2H_2O$, with nickel(II) and cobalt(II)[1]. The chelates can be extracted into some oxygen-containing solvents such as alcohols and ketones, or into inactive solvents such as benzene and 1,2-dichloroethane by means of synergism[2–6]. Such chelates, however, are hardly extracted directly into inactive solvents because of the water molecules involved.

In previous papers[7,8], it has been reported that coordination-unsaturated chelates can be changed to coordination-saturated chelates and extracted into inactive solvents by the action of quaternary ammonium base. This paper shows that nickel(II)– and cobalt(II)–TTA chelates can be extracted into 1,2-dichloroethane, an inactive organic solvent, in the presence of tetradecyldimethylbenzylammonium chloride (zephiramine) or cetyltrimethylammonium bromide (CTMAB).

Experimental

TTA solution, $5 \cdot 10^{-3}$ M. Dissolve 0.555 g of thenoyltrifluoroacetone in 500 ml of 1,2-dichloroethane.

Quaternary ammonium base solutions, $5 \cdot 10^{-3}$ M. Dissolve 0.920 g of tetradecyldimethylbenzylammonium chloride or 0.911 g of cetyltrimethylammonium bromide in 500 ml of water.

Buffer solution. The pH of a solution was adjusted with phthalate (pH 4–6), phosphate (pH 6–8) or borate (> pH 8) buffer.

Metal solutions. Prepare a $1 \cdot 10^{-2}$ *M* solution of nickel and a $5 \cdot 10^{-3}$ *M* solution of cobalt, by dissolving nickel sulfate and cobalt chloride. These solutions were standardized with EDTA.

All the chemicals used were of analytical-reagent grade.

General procedure. Place the metal ion, buffer and quaternary ammonium base solutions into a separatory funnel, and dilute to 20 ml with water. Add the TTA solution in 1,2-dichloroethane (10 ml) and shake the funnel for 5 min. Dry the organic phase with anhydrous sodium sulfate, and measure the absorbance against water as reference. Make any necessary blank corrections.

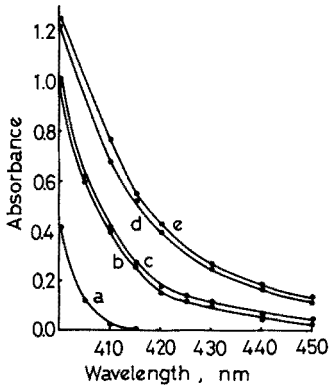


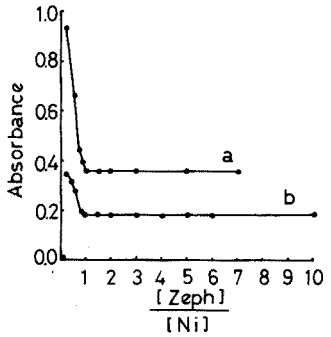
Fig. 1. Absorption spectra. $[Me]_{aq} = 1.25 \cdot 10^{-4}$ *M*, $[TTA]_{org} = 5 \cdot 10^{-3}$ *M*, $[QAB]_{aq} = 5 \cdot 10^{-4}$ *M*, pH 7.0. (a) Reagent blank; (b) Ni and CTMAB; (c) Ni and zeph; (d) Co and CTMAB; (e) Co and zeph. $V_{org} = 10$ ml and $V_{aq} = 20$ ml.

Absorption spectra

The absorption spectra of Ni– and Co–TTA chelates formed in the presence of the quaternary ammonium base were measured without correction for reagent blanks (Fig. 1). As can be seen, the effects of zephiramine and CTMAB on the extractions are the same for nickel and cobalt, respectively. Although the spectra have no peaks in this region, the wavelength of 420 nm was used hereafter, because the reagent blank showed no absorption.

Conditions for extraction

The effects of pH on the extractions in the presence of quaternary ammonium base were examined by changing the pH of the solutions from 4 to 9. Since constant maximal absorbances at 420 nm were obtained in the pH range 5–9 for nickel and cobalt, pH 7.0 was chosen for further experiments. Shaking times from



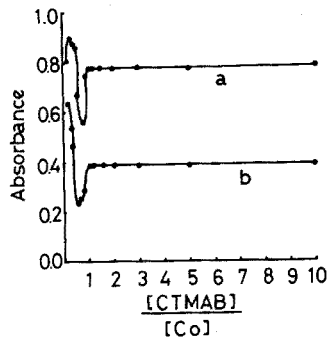


Fig. 2. Effect of zephiramine on Ni–TTA chelate. $[TTA]_{org} = 5 \cdot 10^{-3}$ *M*; pH 7.0. (a) $[Ni]_{aq} = 2.5 \cdot 10^{-4}$ *M*; (b) $[Ni]_{aq} = 1.25 \cdot 10^{-4}$ *M*.

Fig. 3. Effect of CTMAB on Co–TTA chelate. $[TTA]_{org} = 5 \cdot 10^{-3}$ *M*; pH 7.0. (a) $[Co]_{aq} = 2.5 \cdot 10^{-4}$ *M*; (b) $[Co]_{aq} = 1.25 \cdot 10^{-4}$ *M*.

2 to 10 min had no effect on the extractions, and the extracted chelates were stable at least for 1 h. Molar concentrations of TTA more than 10-fold or 6-fold that of nickel or cobalt, respectively, gave constant absorbances.

The addition of the quaternary ammonium bases caused strange absorbance patterns (Figs. 2 and 3) because of turbidity in the 1,2-dichloroethane phase, but clear solutions with constant absorbances were observed at molar concentration ratios above one, for both nickel and cobalt.

Composition of the extracted chelates

The metal: TTA molar ratios of the chelates extracted in the presence of the quaternary ammonium bases were determined by the continuous variations method; the results for the nickel–TTA–zephiramine system are shown in Fig. 4; the graph for the cobalt–TTA–CTMAB system was of exactly the same form; the limiting value method was also used (Fig. 5). Both methods showed that the nickel and cobalt chelates contain a metal: TTA ratio of 1:3. Similarly to the cases mentioned previously[7,8], the ordinary $M(TTA)_2 \cdot 2H_2O$ chelates change to the coordination-saturated chelates, the two molecules of water being expelled by the action of the quaternary ammonium base. It is reasonable to consider that the $M(TTA)_3$ chelates carry a single negative charge owing to higher chelation, and that the chelates are extracted into the inactive solvent after coupling with the positively charged quaternary ammonium base. Figures 2 and 3 show that a 1:1 molar ratio of quaternary ammonium bases to the metal is sufficient for complete extraction.

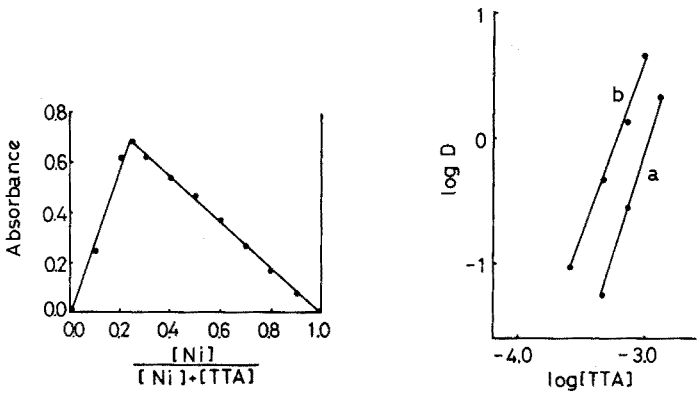


Fig. 4. Continuous variations method for Ni–TTA chelate in the presence of zephiramine. [Ni]+[TTA]$=5 \cdot 10^{-3}$ *M*; $[zeph]_{aq}=7.5 \cdot 10^{-4}$ *M*; pH 7.0.

Fig. 5. Relationship between log [TTA] and log *D* in the presence of QAB $[M]_{aq}=1.25 \cdot 10^{-4}$ *M*; pH 7.0. (a) Slope is for Ni–TTA chelate in the presence of $[zeph]_{aq}=5 \cdot 10^{-4}$ *M*. (b) Slope is for Co–TTA chelate in the presence of $[CTMAB]_{aq}=5 \cdot 10^{-4}$ *M*.

Thus, the extracted chelates appear to have the compositions $Ni(TTA)_3$(zephiramine) and $Co(TTA)_3$(CTMAB).

Quaternary ammonium bases have two effects on the extraction: one is to form a highly coordinated chelate, and the other is to couple with the formed highly coordinated chelate.

REFERENCES

1 J. Starý, *The Solvent Extraction of Metal Chelates*, Pergamon, Oxford, 1964, p. 70.
2 H. Kawamoto and H. Akaiwa, *J. Inorg. Nucl. Chem.*, 31 (1969) 1141.
3 H. Akaiwa, H. Kawamoto and T. Saito, *Nippon Kagaku Zasshi*, 91 (1970) 980.
4 A. K. De and A. K. Majumdar, *Anal. Chim. Acta*, 27 (1962) 153.
5 A. K. De and M. S. Rahaman, *Anal. Chim. Acta*, 27 (1962) 591.
6 W. M. Jackson and G. I. Gleason, *Anal. Chem.*, 45 (1973) 2125.
7 M. Nishimura, S. Noriki and S. Muramoto, *Anal. Chim. Acta*, 70 (1974) 121.
8 S. Noriki and M. Nishimura, *Anal. Chim. Acta*, 72 (1974) 339.

Analytica Chimica Acta, 76 (1975) 219–223

SHORT COMMUNICATION

A precise method for locating the end-points of segmented titration curves

LOUIS MEITES
Department of Chemistry, Clarkson College of Technology, Potsdam, New York 13676 (U.S.A.)
JOHN G. McCULLOUGH*
Department of Chemistry, Grand Valley State Colleges, Allendale, Michigan 49401 (U.S.A.)
(Received 10th October 1974)

When a titration curve consists of two straight lines, as in the case of photometric, amperometric, and conductometric titrations, the end-point is usually estimated as the intersection of two extrapolated straight lines fitted to the data. It is well known that the precision of locating the end-point is severely limited if the two lines are fitted visually, while fitting by least-squares regression analysis may be made quite inaccurate by a single wild data point. One of us introduced a combined graphical and analytical method for locating such end-points, which seems to be an improvement over the graphical and least-squares methods, and applied it to titration curves of a particular shape[1]. The method is extended here for the general case of any titration curve consisting of two straight lines. In some cases, the method involves performing a great deal of arithmetic, and for doing this chore a FORTRAN computer program has been written.

Several points on a schematic segmented titration curve are shown in Fig. 1. The instrumental signal A, which may be a conductance, absorbance, or current, is plotted against V, the volume or weight of titrant delivered. The values of A may have either sign, and the branches of the curve may have any slope. To begin, the titration curve is plotted, and the approximate location of the end-point is found graphically. An even number N of points is chosen, half on either side of the estimated end-point. (If an odd number of points is chosen, the middle point is ignored by this procedure and by the computer program.) The method consists in finding analytically the straight line that passes through the first two points, and the straight line that passes through the last two points. Then VEP_2, the value of V at the intersection of these lines, is taken as one estimate of the end-point volume. Then the straight lines passing through, respectively, the first and third points and the Nth and $(N-2)$th points are found, and VEP_3, the value of V at their intersection, is found. Thus $(N/2)-1$ values of VEP are found.

What is here called the derived plot is a plot of VEP_K against V_K or V_{N-K+1}. The values of V to be used should be those of the branch of the titration curve having the greater slope. If the data were perfect, the derived plot would be a horizontal

* Present address: Union Carbide Corporation, Tarrytown, New York 10591, U.S.A.

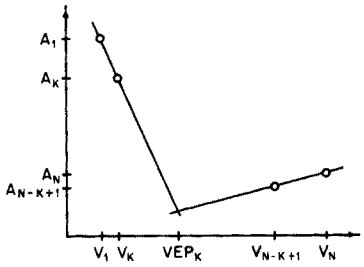


Fig. 1. Schematic segmented titration curve: A, instrumental signal, *vs.* V, volume of titrant delivered.

straight line. The values of VEP_K are extrapolated to the line $VEP = V$, and this intersection is taken as the end-point. It is shown in several examples below that, when this extrapolation is made, several common defects in the data can be recognized and allowed for.

The equations needed are derived as follows, with reference to Fig. 1. The straight line $A(V)$ passing through the points (V_1, A_1) and (V_K, A_K) is

$$A(V) = A_1 + m_K(V - V_1) \tag{1}$$

where

$$m_K = (A_K - A_1)/(V_K - V_1)\,. \tag{2}$$

The straight line $A'(V)$ passing through the points (V_N, A_N) and (V_{N-K+1}, A_{N-K+1}), which are symmetrically placed with respect to the first pair, is

$$A'(V) = A_N + m'_K(V - V_N) \tag{3}$$

where

$$m'_K = (A_{N-K+1} - A_N)/(V_{N-K+1} - V_N)\,. \tag{4}$$

Where these lines intersect,

$$A(V) = A'(V) \tag{5}$$

and

$$V = \mathrm{VEP} \equiv \mathrm{VEP}_{N-K+1} \tag{6}$$

where VEP_K is an estimate of the end-point based on these four pairs of data. The solution of eqns. (1), (3), (5) and (6) is

$$(m'_K - m_K)\mathrm{VEP}_K = A_1 - A_N + m'_K V_N - m_K V_1 \tag{7}$$

The solution of eqns. (2), (4) and (7) for VEP_K is a relatively simple expression only if the values of the signal, A, are all zero after the end-point, as in the case previously considered[1]. In every other case, including that where the values of the signal are zero before the end-point, the solution is a formidably long expression, and solving it manually for several values of VEP_K would take a very long time. Therefore a FORTRAN program has been written to perform this arithmetic and several other computations. The program will multiply the values of the signal A by the instrumental range setting, if that is supplied. Provision can be made, if desired, for the program to convert the values of resistance to conductance, or transmittance to absorbance, or pH to 10^{-pH}. The signals are then corrected for dilution, if desired, and the solutions of eqns. (2), (4) and (7) are computed. Values of V, A (both raw

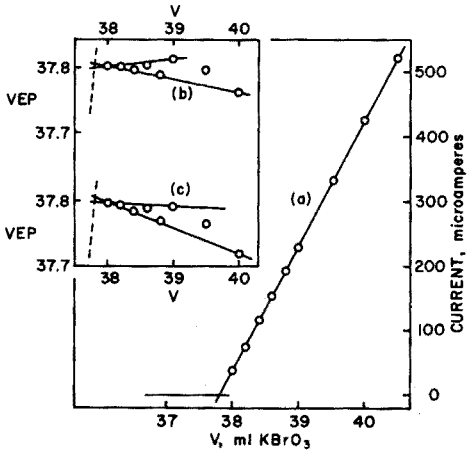


Fig. 2. Amperometric titration of arsenic(III) with potassium bromate in 1 *M* HCl–4 *M* NaBr, for a rotating platinum electrode at +0.35 V *vs.* s.c.e.: (a) conventional plot; (b) derived plot; (c) derived plot without correcting currents for dilution.

and treated), and VEP are then tabulated*.

The utility of this method was shown for a titration curve the second branch of which was zero[1], and is further illustrated by the following examples. Figure 2(a) is a portion of an amperometric titration curve of arsenic(III) with potassium bromate in an acidic bromide solution. A rotating platinum electrode was held at +0.35 V *vs.* s.c.e., where only excess of titrant yields a current. Values of current were corrected for dilution. Since the values of the signal were essentially zero before the end-point, the volume axis was expanded and only the second branch of the curve was plotted. In this way, VEP was estimated very precisely as 37.79 ml. However, this expedient works only for a titration curve of this particular shape; *i.e.*, where the first branch is zero. Figure 2(b) shows the derived plot for this titration. Even with the wildest possible extrapolations, as shown, the end-point is estimated as 37.802 ± 0.002 ml. Although the apparent precision of this result exceeds the linearity and accuracy of the Class A buret used, it would be significant if the titrant had been dispensed more accurately, *e.g.*, by weight instead of volume. Figure 2(c) is the derived plot without correction for dilution, from which the end-point is estimated as 37.802 ± 0.004 ml. Omitting to correct the data for dilution does less harm to the derived plot, whose slope is thereby increased, than to the titration curve, where curvature would be introduced. Nevertheless, Fig. 2(b) is more useful than Fig. 2(c) and data should be corrected for dilution whenever possible.

Figure 3 is a plot of the conductometric titration of 150 ml of approximately 33 m*M* sodium acetate with 0.2 *M* hydrochloric acid. The values of conductance were measured much less precisely than the currents of Fig. 2, and consequently the points in the derived plot show considerable scatter, and the end-point is no more precisely located than by the ordinary graphical method. The points on the derived plot, corrected for dilution, are randomly scattered about 25.4 ml with a mean

* A copy of the program may be obtained by sending a self-addressed 9 × 12 in. envelope to the second author.

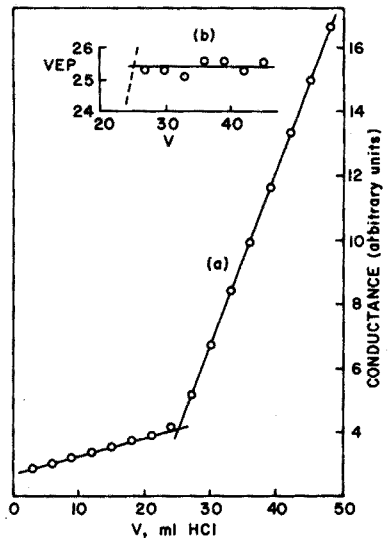


Fig. 3. Conductometric titration of 33 m*M* sodium acetate with 0.2 *M* hydrochloric acid: (a) conventional plot; (b) derived plot.

deviation of 0.2 ml. Probably in this case, where errors appear to be random, least-squares line fitting would yield results as good as obtained by the present method. This titration shows that the method will yield high precision only with good data, and was not worth using in this case. However, the quality of this titration curve could not be judged well until the derived plot was made.

Figure 4(a) illustrates the amperometric titration of iron(II) with potassium bromate in acidic bromide solution containing phosphoric acid. The potential of the rotating platinum electrode was +0.55 V *vs.* s.c.e. (The phosphoric acid greatly

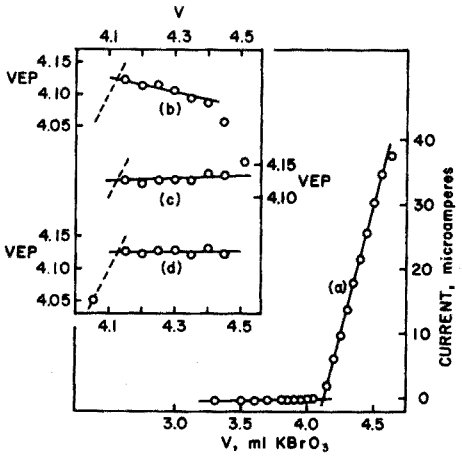


Fig. 4. Amperometric titration of iron(II) with potassium bromate in 1 *M* HCl–4 *M* NaBr containing phosphoric acid, for a rotating platinum electrode at +0.55 V *vs.* s.c.e.: (a) conventional plot; derived plots (b) using all the points, (c) omitting the first and last points, (d) omitting the last two points.

depressed the currents originating from the presence of iron(II) and iron(III) and made the titration feasible.) The data are not as good as those of Fig. 2, possibly because the titration reaction is slow[2]. The last point is obviously in error, and causes the derived plot, Fig. 4(b), to be curved; nonetheless, the end-point is easily and precisely located. After the wild point was discovered, it and the first point were discarded, and the derived plot, shown in Fig. 4(c), became more nearly straight and horizontal; still, the estimate of the end-point was hardly affected. The derived plot of Fig. 4(d) was obtained by omitting the last two points of Fig. 4(a), thus deliberately making an incorrect preliminary estimate of the end-point. Since the computer program assigned equal numbers of points to the two branches, as always, one estimate of VEP is greatly different from the others and lies on the line VEP$=V$, thus clearly revealing the error.

It is concluded that, with the method described here, the end-point of a segmented titration curve can be located as precisely as that of a potentiometric titration, provided that the branches of the curve are strictly linear. This requires that the signal measured be composed of contributions of which each is strictly proportional to the concentration of a species involved in the titration and responsible for that contribution, that the titration reaction has reached equilibrium by the time each value of the signal is measured, and that if the reaction is not complete, points in the curved region near the end-point on the ordinary plot will in general correspond to outliers on the derived plot. For poorly defined titration curves, the method is of little or no use. However, it will extract the maximum precision from good data, because it uses fully the precision of data near the end-point. When data are taken near an end-point that is a minimum, the instrumental scale should be expanded to measure the small signals as precisely as possible. The fact that such expansion was made with the data of Fig. 2 no doubt accounts for the reduction in scatter in the derived plot as the end-point is approached. The derived plot should be made with the VEP scale expanded just enough to show the scatter in the data; the length of the V scale need only be great enough to separate the points. Very little graph paper is required; usually, a corner of the main plot, as in Fig. 3, suffices.

We thank Victoria A. Spendel and Wolfgang U. Spendel for performing many titrations, and Robert R. McQueen for help in writing the computer program.

REFERENCES

1 L. Meites, *Polarographic Techniques*, Interscience, New York, 2nd edn., 1965, pp. 482–4.
2 G. F. Smith, *J. Amer. Chem. Soc.*, 53 (1931) 4291.

Analytica Chimica Acta, 76 (1975) 224–227

SHORT COMMUNICATION

Polarographic determination of stability constants of 2-, 3- and 4-hydroxybutyrate complexes of copper(II)

I. FILIPOVIĆ, I. PILJAC, B. GRABARIĆ and B. MAYER

Laboratory of Inorganic Chemistry, Faculty of Technology and Institute of Inorganic and Analytical Chemistry, University of Zagreb, POB 179, Zagreb, Croatia (Yugoslavia)

(Received 23rd September 1974)

In previous papers, the stability constants of the 2-, 3- and 4-hydroxybutyrate complexes of copper(II) have been determined by spectrophotometric[1] and potentiometric[2] measurements as a part of investigations carried out with different bivalent cations and these three ligands. In order to obtain more reliable values for the complex stability constants, the same systems should also be examined by polarographic methods. An improved technique for the determination of complex stability constants from polarographic data of reversible electrode processes has already been described[3]. Essentially the same technique has been used on the moderately quasi-reversible cathodic reduction of copper(II) in the presence of unsubstituted butyrate ion, and a numerical evaluation of stability constants for such electrode processes has been proposed[4].

In this communication, the same technique and method have been applied in investigating hydroxy-substituted butyrate complexes of copper(II); the polarographically obtained stability constants are compared with those obtained by two other essentially different methods.

Experimental

Apparatus. All polarograms were recorded with a controlled-potential polarographic instrument with operational amplifiers[4]; the polarographic cell used was the same as in earlier work[3].

Chemicals. Sodium salts of 2-, 3- and 4-hydroxybutyric acids were recrystallized three times from ethanol. All other chemicals were prepared as described previously[4].

Procedures. Measurements were carried out in buffer solutions having a constant concentration of the appropriate hydroxybutyric acid[5] (0.01 mol dm^{-3}). Metal ion concentrations were 0.4 mmol dm^{-3} and the ionic strength of the buffer solutions was kept constant at the value 2 mol dm^{-3} by adding sodium perchlorate. The capillary constant ($m^{\frac{2}{3}}t^{\frac{1}{6}}$) was 1.79 $mg^{\frac{2}{3}}$ $s^{-\frac{1}{2}}$ and all measurements were carried out at a constant temperature of (298.2±0.1) °K.

Results and discussion

The procedure of evaluating the half-wave potential ($E_{\frac{1}{2}}$) and diffusion

current (i_d) of copper(II) in the absence of ligand and in the presence of various ligand concentrations, from polarographic data has been described previously[4]. It was shown that the values of $E^s_{\frac{1}{2}}$ and i^s_d in the absence of complex formation must be taken with care, because of the pH influence on these values. The "best" values of $E^s_{\frac{1}{2}}$ and i^s_d were those recorded in copper(II) solution with 1 mmol dm^{-3} of perchloric acid and a constant ionic strength of 2 mol dm^{-3} ($NaClO_4$), *i.e.* $E^s_{\frac{1}{2}}=(42.5\pm0.1)$ mV and $i^s_d=(4.70\pm0.04)$ μA obtained as mean values from three measurements of three independently prepared solutions.

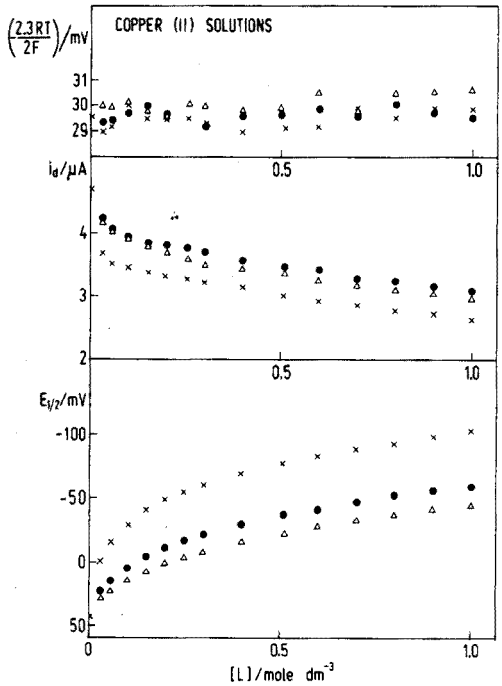


Fig. 1. Experimental polarographic data for Cu(II) complexes: (×) 2-hydroxybutyrate; (●) 3-hydroxybutyrate; (△) 4-hydroxybutyrate.

The $E_{\frac{1}{2}}$ and i_d values of copper(II) in the presence of different ligand concentrations (up to *ca.* 1 mol dm^{-3}) together with the computed estimates of the Nernst slope ($-2.3RT/2F$) are presented in Fig. 1. The ligand which forms more stable complexes causes more negative $E_{\frac{1}{2}}$ shifts and greater decreases of i_d, hence the results presented in Fig. 1 indicate that the order of stability of copper(II) complexes would be 2- >3- >4-hydroxybutyrate. The values of the computed estimates of the Nernst slope, also given in Fig. 1, show that the evaluation of $E_{\frac{1}{2}}$ and i_d was carried out from a part of the polarogram where electrode reduction of copper(II) was still sufficiently reversible.

From the values given in Fig. 1 using the DeFord and Hume[6] expression, one can obtain the function $F_0=1+\Sigma_n\beta_n[L]^n$, where β_n are the cumulative stability constants of n complex species and [L] is the free ligand concentration. Leden's graphical extrapolations[7] showed that in all the investigated systems there was a

maximum of four successive copper(II) complexes. By applying the weighted least-squares fitting of F_0 polynomials described previously[3], the computed refined values of the complex stability constants with their 95% confidence intervals were evaluated. The logarithmic stability constants are given in Table I, together with the values obtained for the same systems under identical experimental conditions by spectrophotometric[1] and potentiometric[2] methods. The pK_A values of the respective monocarboxylic acids as a measure of ligand basicity are quoted in the same Table. Good agreement between the stability constants obtained by the three different methods confirms that the treatment of their evaluation from polarographic data of quasi-reversible electrode processes suggested previously[4] is correct.

TABLE I

STABILITY CONSTANTS OF COPPER(II) COMPLEXES

Ligand[a]	*Method*	*log* β_1	*log* β_2	*log* β_3	*log* β_4	pK_A[b]
	Pol.	2.68±0.06	4.45±0.02	4.57±0.09	4.89±0.05	
2	Pot.	2.63±0.01	4.31±0.01			3.80
	Spe.	2.67±0.02	4.71±0.01			
	Pol.	1.93±0.05	3.07±0.06	3.12±0.18	3.14±0.18	
3	Pot.	1.86±0.02	3.12±0.01			4.53
	Spe.	1.83±0.02	2.82±0.02			
	Pol.	1.72±0.04	2.62±0.09	2.71±0.21	2.73±0.19	
4	Pot.	1.80±0.03	2.63±0.01			4.85
	Spe.	1.77±0.02	2.25±0.04			

[a] n-Hydroxybutyrate.
[b] Results obtained potentiometrically at ionic strength of 2 mol dm^{-3} ($NaClO_4$) and at (298.2±0.1) °K.

Previous investigations[8–11] on different unsubstituted monocarboxylate complexes show that the stability of these complexes is governed mainly by the basicity of the ligand, *i.e.* the ligand with greater basicity forms more stable complexes. The basicity of the investigated ligands expressed as pK_A values follows the order 4- >3- >2-. The stability of the investigated copper(II) complexes, however, follows the opposite order. Clearly, when hydroxy-substituted monocarboxylate ligands are used, the central metal ion can coordinate through the hydroxy-group, too, so that the stability of the complexes is increased. The position 2 of the hydroxy-group in the hydroxybutyrate ligands is the most favourable for such coordination. The farther positions 3 and 4 are less favourable for such bidentate coordination with copper(II) ion, but this influence on stability still predominates over the effect of ligand basicity.

REFERENCES

1 B. Grabarić, B. Mayer, I. Piljac and I. Filipović, *J. Inorg. Nucl. Chem.*, 36 (1974) 3809.
2 B. Grabarić, B. Mayer, I. Piljac and I. Filipović, submitted for publication to *Electrochim. Acta.*

3 I. Piljac, B. Grabarić and I. Filipović, *J. Electroanal. Chem. Interfacial Electrochem.*, 42 (1973) 433.
4 B. Grabarić, M. Tkalčec, Vl. Simeon, I. Piljac and I. Filipović, *Anal. Chim. Acta*, 74 (1975) 147.
5 I. Filipović and I. Piljac, *Croat. Chem. Acta*, 36 (1964) 181.
6 D. D. DeFord and D. N. Hume, *J. Amer. Chem. Soc.*, 73 (1951) 5321.
7 I. Leden, *Z. Phys. Chem. (Leipzig)*, 188A (1941) 160.
8 B. Grabarić and I. Filipović, *Croat. Chem. Acta*, 42 (1970) 479.
9 I. Filipović, I. Piljac, A. Medved, J. Savić, A. Bujak, B. Bach-Dragutinović and B. Mayer, *Croat. Chem. Acta*, 40 (1968) 131.
10 I. Filipović, A. Bujak and V. Vukićević, *Croat. Chem. Acta*, 42 (1970) 493.
11 I. Filipović, T. Matusinović, B. Mayer, I. Piljac, B. Bach-Dragutinović and A. Bujak, *Croat. Chem. Acta*, 42 (1970) 541.

Analytica Chimica Acta, 76 (1975) 228–229

SHORT COMMUNICATION

A comment on the ratio of consecutive stepwise conditional stability constants

G. J. VAN ROSSUM and G. DEN BOEF

Laboratory for Analytical Chemistry, University of Amsterdam, Nieuwe Achtergracht 166, Amsterdam (The Netherlands)

(Received 10th October 1974)

Conditional stability constants adequately describe complex formation equilibria in analytical chemistry. The concept of the conditional stability constant was introduced by Ringbom for ligands such as EDTA that form 1:1 complexes with metal ions. It is equally suitable in the case of stepwise complex formation.

Recently[1,2], the theory of complexiметric titrations based on 1:2 complexation reactions (formation of ML and ML_2) has been described. The ratio of the two consecutive conditional stability constants K'_1/K'_2 appeared to play an important role in the shape of the titration curve.

In general, the ratio of consecutive stability constants in stepwise complex formation $K_1/K_2 > 1$. At first sight this might be completely different in the case of the ratio of the corresponding conditional stability constants. Introducing the relevant side-reaction coefficients for the particles involved in the following 1:2 complex formation reactions

$$M + L \rightleftharpoons ML \text{ and } ML + L \rightleftharpoons ML_2$$

we get

$$\frac{K'_1}{K'_2} = \frac{K_1}{K_2} \frac{\alpha_{ML}^2}{\alpha_{ML_2}\alpha_M}$$

Assuming that side reactions occur to a considerable extent with the metal ion M, *e.g.* by reactions with the buffer or with hydroxyl ions, but only to a negligible extent with ML and ML_2, experimental conditions could be expected for which $K'_2 \gg K'_1$, whereas $K_1 > K_2$. This, however, is not so in practice, obviously because the incomplete coordination of ML always leads to large values of α_{ML} as well under such conditions.

As is well known, the ratio of two consecutive stability constants is to a certain degree determined statistically. It can easily be seen that the statistical contribution to this ratio is independent of the presence of an ion giving a side reaction with M.

Following the same reasoning as originally presented by Bjerrum[3] for a monodentate ligand, the tendency of a particle ML_n to take up a ligand is proportional to $N-n$, where N is the coordination valency of M, whereas the tendency of the resulting particle ML_{n+1} to split off a ligand is proportional to $n+1$.

Therefore from statistical considerations K_{n+1} will be proportional to

$$\frac{N-n}{n+1}$$

and

$$\frac{K_n}{K_{n+1}} = \frac{n+1}{n} \cdot \frac{N-n+1}{N-n}$$

This reasoning is the same for the reaction of a ligand with a hydrated metal ion, as for the reaction of the same ligand with a metal ion undergoing a so-called side reaction with *e.g.* a substance from the buffer solution. So, from a statistical point of view, the ratio of conditional consecutive stepwise stability constants would not differ from the ratio of the corresponding absolute stability constants.

Complex formation reactions involving polydentate ligands may be considered in a similar way. A general formula for the statistical contribution to the ratio of consecutive stability constants cannot be given here, as the value will depend on the symmetry of the complexes. It is obvious, however, that in this case also side reactions will hardly influence this statistical contribution.

The electrostatic effect on the ratio of consecutive stability constants, if present, always works in the same direction as the statistical effect. So, with the exception of some well known cases, where $K_2 > K_1$ it may in general be stated that for stepwise complex formation

$$K'_1 > K'_2 > K'_3 > \ldots$$

The same applies to the dimensionless quantities Z which have been introduced in the mathematical treatment of titration curves[1,2]. This is important for the classification of possible titration curves involving 1:2 metal–ligand complex formation, as it limits the number of possible cases considerably. In these titrations the shape of the titration curve depends not only on the absolute values of the different constants but also on their relative magnitude with respect to each other.

This property of conditional stepwise stability constants will be used in a subsequent paper on complexiметric back-titrations involving 1:2 metal–ligand complex formation[4].

REFERENCES

1 F. Freese, G. den Boef and G. J. van Rossum, *Anal. Chim. Acta*, 58 (1972) 429.
2 F. Freese and G. den Boef, *Anal. Chim. Acta*, 60 (1972) 131.
3 J. Bjerrum, *Metal ammine formation in aqueous solution*, P. Haase and Son, Copenhagen, 1941.
4 G. J. van Rossum and G. den Boef, *Anal. Chim. Acta*, to be published.

Analytica Chimica Acta, 76 (1975) 230–233

BOOK REVIEWS

F. Kasler, *Quantitative Analysis by n.m.r. spectroscopy* (Analysis of Organic Materials Vol. 2), Academic Press, London 1973, viii + 190 pp., price £4.50.

N.m.r. spectroscopy has been discussed in innumerable books and reviews, but detailed information on its quantitative aspects has been remarkably difficult to come by. This monograph now makes available the necessary information in an easily assimilable form.

In the first part of the book, general aspects of n.m.r. work are considered, particularly the origin of the signal, interactions of nuclei with their environment, and instrumentation. In the second part, practical considerations such as optimal conditions, sensitivity enhancement and quantitative measurements are described. The third part contains the applied methods: properties such as molecular weight and isotopic composition, functional groups such as alcoholic amine and methyl groups, and some organic elements can be determined very rapidly though not with very high sensitivity. In the final chapters, quantitative methods for substances such as steroids, relaxant drugs, paint solvents, pesticides and polymers are discussed.

Expensive instrumentation, such as n.m.r. spectrometers, is generally held to be better employed in structural elucidation than in quantitative work. With the current development of cheaper instruments, quantitative n.m.r. spectrometry seems more feasible as a routine procedure. Kasler's book provides an excellent survey for anyone interested in the quantitative possibilities of the technique.

T. R. Crompton, *Chemical Analysis of Organometallic Compounds Vol 1, Elements of Groups I-III* (Analysis of Organic Materials, Vol. 4), Academic Press, 1973, x + 258 pp, price £5.80.

Organometallic compounds are now of considerable importance in many areas of chemistry, so that books describing their analysis must be welcomed. This monograph deals with compounds of the first three groups of the Periodic Table. Since aluminium and zinc compounds have been discussed in an earlier monograph by the same author, the present text deals predominantly with organocompounds of lithium, beryllium, magnesium, mercury and boron; little has been done on organic compounds of the other relevant elements. Refreshingly, in most cases, organometallic compounds are discussed, rather than metal-organic complexes, so that the monograph covers a wide and difficult field of analysis.

The methods described—many with full experimental detail—vary from classical gravimetry to n.m.r. spectrometry; a good deal of attention is given to gas chromatography. Not only determinations of the heteroelement or compound but determinations of other elements in the organometallic compounds are described. For the latter analyses, the present state of knowledge is more sophisticated than is indicated, but there is still much useful information to be gleaned from this text.

In some respects the rapid advance of analytical progress has left this text outdated already—for example, in the discussion of gas–chromatographic and flameless atomic-absorption method for mercury. But on the whole, the book will be of great usefulness to chemists who have to contend with analysis of materials containing these less conventional compounds.

Y. A. Gawargious, *The Determination of Nitro and Related functions* (Analysis of Organic Materials, Vol. 5), Academic Press, London, 1974, viii + 154 pp, price £3.50.

This monograph covers quantitative analytical procedures for the following nitrogen-containing functional groups: nitro, nitroso, azo, azide, nitrate and nitramine. The emphasis is heavily weighted towards classical titrations with reducing agents. Techniques such as spectrophotometry and polarography can be applied in many cases but these are only briefly surveyed.

In addition to the literature reviews, detailed procedures are given for many determinations, so that recourse to the original papers is unnecessary. However, these procedures are entirely titrimetric or gasometric so that the final picture which emerges is not well balanced. The surveys of titrimetric methods are thorough, and these parts may save lengthy literature searches for workers who seek classical procedures.

A. M. G. Macdonald (Birmingham)

J. W. Robinson, *Undergraduate Instrumental Analysis*, Dekker, New York, 2nd Edn., 1973, xvii + 379 pp., price $12.75.

Several texts dealing with undergraduate analytical chemistry in the conventional manner have appeared recently; Professor Robinson's book is concerned almost wholly with instrumental analysis and so provides a useful counter-weight. While it remains true that students of chemistry must be drilled in qualitative, gravimetric and titrimetric analysis if they are to understand the fundamental reactions and techniques of analytical chemistry, instrumental methods seem to be regarded as more relevant by the modern student. Unhappily, by the time the necessary physicochemical background to an instrumental method has been discussed, and the functions of the assorted bits and pieces of machinery have been explained, there is never sufficient space or time to explain that everything is not quite so routinely simple as it may seem, that one must look sceptically at the dial or chart or digital reading that comes out at the end, and that indeed the application of most instrumental methods to the analysis of real samples should provoke sufficient chemical thought to keep even the classical analyst happy.

The present text comprises introductory material on spectroscopy and chapters on n.m.r., i.r., u.v., a.a.s., spectrophotometry, polarimetry, flame photometry, emission and x-ray spectrometry, chromatography (mainly g.l.c.) thermal analysis, mass spectrometry and electrochemistry. The treatment of the last three topics is not very satisfactory, whereas the various spectrometric methods are well done—or appear to be so, for the review copy of this book had many blank pages in the n.m.r. and i.r. sections. At the end of most chapters, some experiments are

suggested and problem questions are set. Even allowing for the difficulties of producing experimental procedures which will work on instrumentation from many different manufacturers, the experimental suggestions seem inadequate and will do little to indicate to the student that chemistry is basically a practical subject intimately connected with most aspects of everyday life.

However, this book is written at a breathless pace which should hold the attention of students, and it should provoke thought and interest from anyone concerned with teaching analytical chemistry.

A. M. G. Macdonald (Birmingham)

K. Cammann, *Das Arbeiten mit ionenselektiven Elektroden*, Springer–Verlag, Berlin, 1973, xii+226 pp., price DM 56,—.

Developments in the range and applications of ion-selective electrodes during the past 7 years have not been matched by the appearance of books on the subject. Even now, when these electrodes are an accepted part of the analyst's equipment and development has slowed, books must be out of date before they are printed. Nevertheless, books on new techniques are an essential part of the orderly transition of a method from the research bench to routine usage, and the present book is therefore welcome.

It starts with chapters on the fundamentals of potentiometry and potential measurements. Subsequent chapters describe the different kinds of ion-selective electrodes, the measuring techniques and analytical techniques necessary for best performance, and finally their applications in a variety of fields. Useful appendices cover activity coefficients, temperature effects, and evaluation factors. Few errors appear in the text; some early 1973 references are included.

As an introduction to the proper use of ion-selective electrodes this is a good book, and an up-dated English version would find a large market.

H. Hachenberg, *Industrial Gas Chromatographic Trace Analysis*, Heyden, London, 1973, viii+217 pp., price £5.50.

Prospective buyers should not be deterred by the meaningless blurb on the cover of this book. The author is chief chemist of a large German industrial laboratory, and his treatment of the subject is more down to earth than is achieved in most gas–chromatographic texts.

The first part summarizes the essential instrumentation with useful notes on limitations and advantages of different detectors, columns etc. The second, larger part is concerned with applications to trace analysis. This covers the analysis of gases, ranging from traces of inorganic gases to head space analysis of fruit flavourings; analysis for liquid hydrocarbons; the determination of traces of water; sulphur compounds in fuels and applications in synthetic polymer production. Analyses of gases and polymers are given most emphasis.

The practical outlook of the text is to be welcomed. The translation is good,

and the book can be recommended to anyone who wishes to obtain a realistic picture of analytical gas chromatography before delving more deeply into the subject.

Electrophoresis and Isoelectric Focusing in Polyacrylamide Gel, Edited by R. C. Allen and H. R. Maurer, de Gruyter, Berlin 1974, 315 pp., price DM 105,—.

This volume contains the Proceedings of the first Conference of the Blue Fingers held in October 1972 in Tübingen, the aims of the Conference being to provide exchanges of information between the Blue Fingers and to tell the White Fingers how not to get blue. In both these aims, the text succeeds admirably.

The 30 lectures are grouped under the headings: physicochemical properties of the gel; theory and practice of optimization and standardization; evaluation as separation method; isolectric focussing and isotachophoresis; quantitative pattern evaluation; preparative methods; micro methods; biochemical applications; and clinical applications. The problems encountered with these techniques on polyacrylamide gels, and the difficulties involved in obtaining useful intelligible results in real applications are exposed in clear terms, and requirements for further progress are outlined. Biochemists and clinical chemists should find much of interest in these Proceedings, which have been arranged in excellent fashion.

D. Ceausescu, *Tratarea statistica a datelor chimico-analitice*, Editura Technica, Bucharest, 1973, 239 pp.

This book written in Romanian, is concerned with the criteria for evaluating the accuracy and precision of a complete analytical method. To this end, the usual statistical evaluation—variance and factorial analysis, detection of systematic errors, dependent variables, etc.—are discussed; some 30 useful examples of complete calculations are included.

Methods of Biochemical Analysis, Vol. 21, Edited by D. Glick, Interscience Publishers–J. Wiley & Sons, Inc., New York, 1973, viii and 572 pp., price £11.50.

The topics chosen for the latest volume in this series are again widely varied. Two of the articles deal with nucleic acids and related compounds: C. Horvath writes on the rapid analysis of nucleic acid constituents at the subnanomole level using high-performance ion-exchange chromatography with narrow-bore columns and P. J. Elving, J. E. O'Reilly and C. O. Schmakel contribute an extensive account of the polarography and voltammetry of nucleosides and nucleotides and their parent bases as an analytical and investigative tool. Three articles are concerned with aspects of enzymology: D. Zakim deals with techniques for the characterization of UDP glucuronyltransferase, glucose 6-phosphatase and other tightly-bound microsomal enzymes, J. Okuda and I. Miwa discuss newer developments in enzymic

determination of D-glucose and its anomers and K. G. Oldham describes radiometric methods of enzyme assay. J. R. Majer and A. A. Boulton discuss chemical and biological applications of the integrated ion-current (IIC) technique of quantitative mass spectrometric analysis and O. E. Olson, I. S. Palmer and E. I. Whitehead review methods for the determination of selenium in biological materials. As in all previous volumes each article contains a comprehensive list of references.

H. G. Bray (Birmingham)

D. Hellwinkel, *Die systematische Nomenklatur der organischen Chemie: Eine Gebrauchsanweisung*, (Heidelberger Taschenbücher Band 135) Springer–Verlag, Berlin, 1974, viii + 170 S, Geheftet DM 14,80 US $6.10).

In English, the I.U.P.A.C. nomenclature recommendations are readily available, and alternative practical approaches such as that published recently by the American Chemical Society have also been offered. German chemical nomenclature has not been systematically treated in the same way. The present volume fills this gap very well. It offers a rational approach based on I.U.P.A.C. proposals, but includes some alternatives, and is presented in a more readable style, although the approach is not so exhaustively rigorous. Well chosen examples abound.

K. A. Connors, *Reaction Mechanisms in Organic Analytical Chemistry*, John Wiley and Sons, New York, 1973, xiii + 634 pp., price £9.25.

It is always stimulating and refreshing to find an author with something new to say or with a novel approach to an old problem, as long as he does not lose all essential contact with realism in the process. Goethe came close to expressing the essential requirement with his "Dear friend-theory is all gray, And the golden tree of life is green".

Professor Connors believes that looking at organic analytical reactions from the reaction mechanism viewpoint, rather than in terms of methods and/or techniques, is a more effective and enjoyable way of teaching and learning the essentials of organic analysis. He may well be correct. But his book is unlikely to be accepted by modern students as their salvation, and his claim that analytical research may be stimulated by this approach appears to involve some wishful thinking.

Professor Connors does succeed, however, in his secondary aim, i.e. to introduce many of the methods and concepts of physical organic chemistry; he has selected for discussion chemical equilibria, reaction rates, extrathermodynamic relationships, electrophilic and nucleophilic substitution, addition to carbon-carbon and to carbon-heteroatom multiple bonds etc. This book certainly serves as a good source of information on named organic reactions and reagents, although many of these are so exotic and relatively little used that students who have not heard of them previously have not missed much in terms of modern analytical procedures and problems.

This book, produced by the direct reproduction process, contains clear formulae and diagrams, and there are very few misprints. The idealistic approach may appeal to some readers who are theoretically and ideally orientated, rather than practically motivated.

D. M. W. Anderson (Edinburgh)

C. H. Vincent, *Random Pulse Trains, their Measurement and Statistical Properties.* IEE Monograph Series 13, Peter Peregrines Ltd., London, 1973, 264 pp., price £7.50.

Random Pulse Trains, their Measurement and Statistical Properties is a work which has arisen out of Dr. Vincent's long experience in the field of electronic instrumentation in nuclear physics. However, while applications to measurement of nuclear events are covered fully, the subject is treated in a sufficiently generalised manner to find application in all fields where random pulse trains are measured.

General statistical ideas and principles are developed fully in the first chapter and applied in specific areas in later chapters. Topics covered include linear and logarithmic rate measurement, dead time estimation and derandomisation, period meters and excursion detection, generation of random and pseudorandom sequences, ending with a section on special problems including radioactive decay analyses and deconvolution of channel spreading.

The mathematical treatment of each subject is rigorous, pitched at graduate level in physics or electrical engineering. In each case, however, useful approximations are derived together with a clear indication as to the limit of their application. A consistent set of symbols is used throughout and ample references are given to original work. A short section in each chapter expanding on the range of fields to which the theory could be applied would have been a valuable addition.

This book will make a valuable addition to the library of any researcher or graduate student faced with the problem of making ever more sophisticated measurements of fluctuating phenomena.

L. G. Earwaker (Birmingham)

Nuclear Magnetic Resonance Spectroscopy of Nuclei Other Than Protons, Edited by T. Axenrod and G. A. Webb, Wiley–Interscience, New York, 1974, xiii + 407 pp., price £10.00.

Readers who expect this book to be a comprehensive work on the n.m.r. spectra of other nuclei may be surprised to find that it contains a collection of specific articles based on papers given at the Advanced Study Institute on *Nuclear Magnetic Resonance Spectroscopy of Nuclei Other than Protons* held in Italy during 1972. The book contains twenty-five chapters by no less than twenty-nine contributors. Six of these deal with various aspects of carbon-13 n.m.r., five are concerned with nitrogen n.m.r. (^{14}N and ^{15}N), three with fluorine-19, and others on deuterium and tritium, oxygen-17, phosphorus-31, and silicon-29. In addition, there are chapters on Fourier transform techniques, multiple resonance, paramagnetism, and orientated molecules. The breadth of coverage is very variable, for example the

general chapter (by G. E. Maciel) on pulsed Fourier transform n.m.r. of metal nuclei contrasts with another (by J. W. Akitt) on the very specific topic of the aqueous tetrafluoroborate anion. Some chapters are extremely brief; thus the carbon-13 n.m.r. spectra of organo–transition metal complexes is dealt with by B. E. Mann in only four pages and is limited to metal carbonyls and platinum compounds. The article by Gill and Geraldes should be read by all organic chemists attempting to correlate $^{1}J(^{13}C–H)$ with "the hybridization state" of molecules.

This book does not do justice to the rather general title as it only deals with selected aspects. However, many chapters do make rather interesting reading and the presentation is very good. The book will appeal mainly to n.m.r. spectroscopists, but it may be useful to other chemists in suggesting how n.m.r. spectroscopy of nuclei other than protons might assist in their research work.

W. B. Jennings (Birmingham)

C. Liteanu and S. Gocan, *Gradient Liquid Chromatography*, (Ellis Horwood Series in Analytical Chemistry, Edited by R. A. Chalmers), Ellis Horwood Ltd, Chichester (distributed by Wiley and Sons, Chichester), 1974, xii+338 pp., price £10.50.

This volume is a technical triumph for those concerned with the production of the Rumanian authors' manuscript. The English is good; only a few minor misprints were detected. Although the many Tables and mathematical expressions are well set, some of the Figures have been reduced to a size so small that the lettering (which appears to be freehand in places) is difficult to read.

The book starts well, with an interesting dedication "to those who conceive and build scientific equipment thus contributing to the progress of science no less than those who conceive and build scientific theory". The text is divided into two parts—(1) The Fundamental Problems of Chromatography (kinetics of eluent and zone migration; factors influencing the reproducibility of R_f values; the estimation of separation efficiency; and the optimization of the chromatographic process), and (2) The Use of Gradients (mobile-phase, stationary-phase, environmental, and combined gradients). There are three appendices, and a subject index.

This is a stolid and thorough text, emphasizing the mathematical aspects of chromatography and failing to achieve a desirable blend of theory with practice. Although there are plenty of details of apparatus, theoretical elution curves, and plots of mathematical functions, there is too little direct analytical interest. More serious, however, is the fact that the text is not really up-to-date. There are a few references to work published in 1970 and 1971, but only one general reference to 1972 could be detected, although several references are made to the authors' own publications in 1972 and 1973. Taken from the viewpoint of its worth as a general review, the text is distinctly 1960-ish in flavour. This is one for the chromatographic specialist and/or theoretician rather than for the general analytical practitioner, particularly at the price asked.

D. M. W. Anderson (Edinburgh)

(Continued from page 4 of cover)

VOL. 76 NO. 1 ANALYTICA CHIMICA ACTA MAY 1975

CONTENTS

(Continued on inside page of cover)