100th Anniversary
1876 - 1976

The Analyst

The Analytical Journal
of The Chemical Society

A monthly International publication dealing
with all branches of analytical chemistry

Volume 101 No 1200 Pages 145–224 March 1976

THE ANALYST

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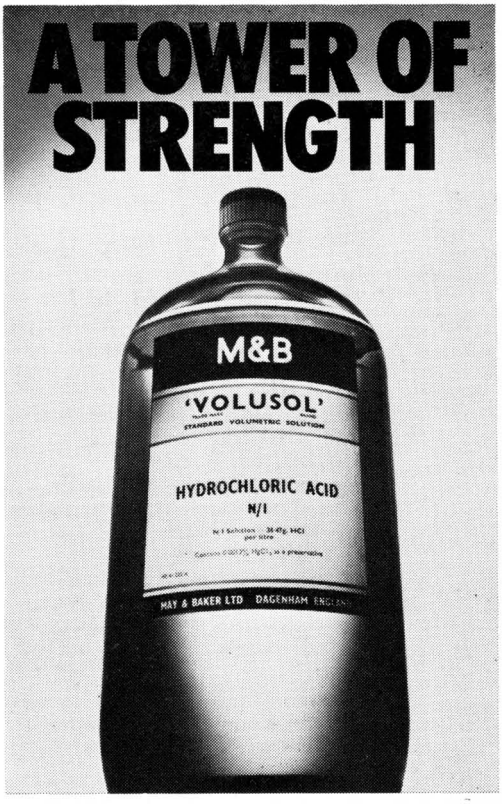

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A Review

Summary of Contents

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HERBERT WEISZ

Lehrstuhl für Analytische Chemie, Chemisches Laboratorium der Universität, 78 Freiburg im Breisgau, West Germany.

Analyst, 1976, **101**, 152–160.

Fungicide Residues

Part V. Determination of Residues of Chloraniformethan in Grain and Cucumbers by Gas Chromatography

A method for determining residues of chloraniformethan {*N*-[2,2,2-trichloro-1-(3,4-dichloroanilino)ethyl]formamide} in grain and cucumbers is presented. After extraction with methanol and preliminary partitioning with hexane, chloraniformethan is extracted into dichloromethane, separated from interfering co-extractives by preparative thin-layer chromatography on silica gel and determined by electron-capture gas - liquid chromatography.

R. A. HOODLESS and M. SARGENT

Department of Industry, Laboratory of the Government Chemist, Cornwall House, Stamford Street, London, SE1 9NQ.

Analyst, 1976, **101**, 161–166.

The Determination of Chlorhydroxyquinoline in Medicated Pig Feeds
Part II. Ultraviolet Spectrophotometric Batching Assay and Gas-chromatographic Assay for Mono- and Dichloro Components

Two procedures have been developed for determining chlorhydroxyquinoline (halquinol) in pig feed medicated at a level of 120 p.p.m. The ultraviolet spectrophotometric procedure can be used to determine halquinol and the gas - liquid chromatographic procedure to determine specifically the 5,7-dichloro- and the combined 5- and 7-chloroquinolin-8-ol components. Halquinol was found to react with copper during the initial extraction with chloroform, thus preventing formation of the silyl ether prior to gas - liquid chromatography. Partitioning the chloroform extract with dilute mineral acid so as to cause dissociation of the chelate and adding EDTA before back-extraction into dichloromethane overcame this problem.

T. COWEN and W. F. HEYES

Squibb International Development Laboratory, Moreton, Wirral, Merseyside.

Analyst, 1976, **101**, 167–173.

A Simple Method for Monitoring Excessive Levels of Lead in Whole Blood Using Atomic-absorption Spectrophotometry and a Rapid, Direct Nebulisation Technique

A method for monitoring excessive levels of lead in whole blood, using a rapid, direct nebulisation, atomic-absorption technique is described. The blood sample is diluted with an equal volume of 0.2% *m*/*V* Triton X-100 solution and immersed in an ultrasonic bath for 10 min. A 200-μl aliquot of the resulting solution is then nebulised and the peak height of the transient output pulse is monitored. Some preliminary results are also reported for cadmium and selenium in blood using the single-pulse nebulisation atomic-fluorescence technique.

K. C. THOMPSON and R. G. GODDEN

Shandon Southern Instruments Limited, Frimley Road, Camberley, Surrey, GU16 5ET.

Analyst, 1976, **101**, 174–178.

Solvent Mediator Studies on Barium Ion-selective Electrodes Based on a Sensor of the Tetraphenylborate Salt of the Barium Complex of a Nonylphenoxypoly(ethyleneoxy)ethanol

The barium ion-selective electrode sensor based on the neutral carrier complex of a nonylphenoxypoly(ethyleneoxy)ethanol containing 12 ethylene oxide units and 2 mol of tetraphenylborate ion per mole of Ba^{2+} (12 EOU.Ba.2TPB) requires a more viscous solvent mediator than 4-nitroethylbenzene for long-life poly(vinyl chloride) (PVC) matrix-membrane barium ion-selective electrodes. Both 2-nitrophenyl octyl ether and di-2-nitrophenyl ether used in conjunction with the sensor in a PVC matrix membrane give functional barium ion-selective electrodes, but those with di-2-nitrophenyl ether mediator are far superior with lifetimes of about 30 d. Barium ion-selective electrodes with the sensor and mediator in liquid membranes can be made for a wider range of nitro-aromatic solvent mediators.

Barium ion-selective electrodes made from discs taken from a master membrane containing 0.40 g of a saturated solution of Antarox CO-880.Ba.-2TPB in di-2-nitrophenyl ether can be used as indicator electrodes for the potentiometric titration of SO_4^{2-} with Ba^{2+}. A role for a potentiometric titration finish in the determination of sulphur in organic compounds by the oxygen-flask method is recommended.

A. M. Y. JABER, G. J. MOODY and J. D. R. THOMAS

Chemistry Department, University of Wales Institute of Science and Technology, Cardiff, CF1 3NU.

Analyst, 1976, **101**, 179–186.

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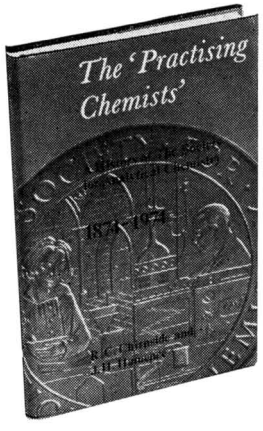

The 'Practising Chemists'

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Society for Analytical Chemistry
1874–1974

By R. C. CHIRNSIDE and
J. H. HAMENCE

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MARCH 1976 Vol. 101 No. 1200

The Analyst

100th Anniversary of *The Analyst*

With this issue, No. 1200, *The Analyst* celebrates 100 years of continuous publication. To mark the occasion, we publish here an Editorial by Dr. F. A. Robinson, President of The Chemical Society, followed by a brief historical survey by Dr. G. W. C. Milner, President of the Analytical Division. In addition, some extracts from the first issue, published in March, 1876, are reproduced, including the title page and an introduction that sets out the original aims of the journal.

Editorial

The Centenary of *The Analyst*

It is a pleasure and a privilege to be asked to contribute this Editorial to mark the Centenary of *The Analyst*, for I was once an analytical chemist myself, a member for 30 years of the Society of Public Analysts and Other Analytical Chemists (later the Society for Analytical Chemistry), an occasional contributor to the journal and, between the years 1938 and 1968, the writer of the bulk of the abstracts relating to biochemistry that appeared in *The Analyst* and subsequently in *Analytical Abstracts*—or so it seemed to me at the time! *The Analyst* underwent a great many changes both before my time and after, and has now become one of the primary journals of The Chemical Society, taking its rightful place alongside the Society's other journals as the proper medium for the publication of original papers in the rapidly expanding field of analytical chemistry.

The Analyst was originally founded "primarily, as the organ of the Society of Public Analysts and, secondly, as the representative of Analytical Chemists in general." Its contents were by no means restricted to descriptions of analytical methods, for it also published "all cases of prosecution for adulteration, and such parliamentary and other proceedings as may appear to touch the interest of Analysts in general." At times it must have been a very lively journal, quick to defend the interests of the new science and those who practised it, especially Public Analysts, who saw themselves as the first "Practising Chemists" in contrast to the "amateur and theoretical chemists" whom they clearly despised! In fact, however, the first "Practising Chemists" must surely have been those arch-enemies of the Public Analyst, the chemists of the Inland Revenue Laboratory at Somerset House, since this was founded in 1841, only a year after the foundation of The Chemical Society; it became eventually the Laboratory of the Government Chemist. Somerset House and the Public Analysts appear to have been in an almost perpetual state of conflict and the pages of *The Analyst* were frequently enlivened with editorial comments and correspondence that "displayed a command of language which many might envy today. Sarcasm, irony, hyperbole and mild invective were all employed on occasion."

By the end of the century, however, the "Practising Chemists" obviously felt that they had established themselves, and more and more attention was given in the journal to scientific papers and abstracts. During the War of 1939–45 there was a revival of interest in legal matters, as a steady stream of Food Orders was issued and consideration had to be given to food standards and food substitutes. Other materials besides foods also came under the control of analysts and new techniques, such as microchemical analysis, a variety of physical methods and microbiological assays, were developed to solve new types of problems. More recently, attention has had to be directed to the detection and determination of trace amounts of, for example, metals, pesticides and medicinal chemicals, often using absurdly small amounts of sample.

Such new and challenging developments inevitably led to a great expansion in research and the need to make the results available in a specialist journal. So now, at the end of its first

century, *The Analyst* has become one of the primary journals of The Chemical Society, with which the former Society for Analytical Chemistry amalgamated in 1975. My wish for *The Analyst* is that it will continue to develop in the second century of its existence with the same vigour and initiative that it displayed during its first century, and that its new status will make it better known to chemists in other fields whose own research work is likely to be helped by new developments in analytical chemistry.

F. A. ROBINSON
President, The Chemical Society

The Analyst: 100 Years of Development

Following the formation of the Society of Public Analysts in August, 1874, its Proceedings were first published, with the co-operation of Sir William Crookes, in *Chemical News* but, with the termination of that arrangement at the end of 1875, the Society resolved to publish its own journal and the first issue appeared in March, 1876, under the title it still bears today, *The Analyst*. The subscription price then was 3s. 6d. (17½p) per annum, and the first issue contained 16 pages.

Originally, the journal was heavily weighted towards legislative aspects of the work of Public Analysts, and for many years was concerned particularly with adulteration. Notable features of early issues were provocative and often highly critical Editorials and correspondence, reflecting the embryonic state of the science of analytical chemistry at that time. Over the years, the papers and the abstracts published in *The Analyst* have consistently reflected the developments that took place in analytical chemistry, not only as applied to foods and drugs, but also to the wider areas of water, inorganic and mineral analysis and later to biochemistry and related topics. In 1885, for example, the sub-title of the journal was "A monthly journal devoted to the advancement of the analysis of foods and drugs and of general analytical and microscopical research," and the changes since then would seem amply to justify the present sub-title of "A monthly international journal dealing with all branches of analytical chemistry."

The aim of those responsible for the publication of *The Analyst* has always been to maintain the highest possible standards, and there is no doubt that this aim has been achieved. In the course of 100 years, many papers of fundamental importance to the development of analytical chemistry have appeared in its pages. The Reinsch test for the detection of arsenic was described by Chaston Chapman and A. H. Allen, and the Gutzeit test was described for the first time at a Society meeting; the Marsh - Berzelius test was recommended in an SPA/SCI report in 1902. In 1916, T. E. Wallis applied the microscope to quantitative analysis by employing lycopodium powder as the quantifying agent. F. Twyman, in 1920, found that many drugs showed distinctive absorption spectra and pointed out the potential of the technique for the non-destructive detection of trace amounts of active constituents, and one of the first papers on the determination of vitamins in foods was published by A. F. Watson in 1922. In the period 1924–36, a major series of 31 papers on the analytical chemistry of tantalum and niobium by W. R. Schoeller and co-workers appeared in *The Analyst*; the first two parts had been published in the *Journal of the Chemical Society* in 1921. With the advent of newer techniques, of major importance were the papers on partition chromatography by R. L. M. Synge (who was later awarded the Nobel Prize jointly with A. J. P. Martin for this work) in 1946 and gas chromatography by A. J. P. Martin and A. T. James in 1952. Papers on fundamental aspects are encouraged in *The Analyst*, and a notable example from the past is the paper on the metal complexing properties of aminopolycarboxylic acids and the analytical application of complexones published by G. Schwarzenbach in 1955. If space permitted, it would be possible to cite many other examples.

The format of the journal has changed in various ways during its history, particularly when some of its contents were split off into other journals. In 1950, abstracts, which had appeared in *The Analyst* since its earliest years, were published separately in *British Abstracts C*, which had been expanding for several years, but that journal ceased publication in 1953 and the SAC inaugurated *Analytical Abstracts* as a new journal in 1954. In 1964, the detailed affairs of the Society, which had been published regularly in *The Analyst* since its inception,

were removed to form the basis of another new journal, *Proceedings of the Society for Analytical Chemistry*. Other less obvious changes have involved regular revision of the layout, typography and covers so as to maintain a modern image.

The Society has been concerned with standardisation for over 50 years; in 1935 these activities were brought under the newly formed Analytical Methods Committee, and its recommended methods of analysis have appeared regularly in *The Analyst*.

Papers from many major conferences have been published in *The Analyst*. In 1952, the November and December issues were devoted to the Proceedings of the First International Congress on Analytical Chemistry, held in Oxford under the patronage of IUPAC. In 1965, the Society initiated its own conferences, and papers presented at these SAC Conferences have been a regular feature of *The Analyst*. In December, 1974, probably the most important issue in its history was published, containing the Centenary, Plenary and Keynote Lectures presented at the Centenary Celebrations of the Society for Analytical Chemistry.

An important feature of the journal since the late 1950s has been the publication of critical reviews of various aspects of analytical chemistry, and about 60 have appeared to date. A more recent innovation has been the acceptance of brief, urgent communications on topics of immediate interest to analytical chemists, and these appear in print in as little as 5 weeks after receipt.

This brief historical survey illustrates the prominent role played by *The Analyst* in the development of analytical chemistry over the past 100 years. During the first 99 years, its progress was fostered initially by the Society of Public Analysts and then by the Society for Analytical Chemistry but, consequent upon the amalgamation of the SAC with The Chemical Society in January, 1975, it is now one of the primary journals of The Chemical Society and has thus entered a new phase in its existence. Within such an organisation, a journal with the traditions and merits of *The Analyst* must continue to flourish, and it is my belief that it will continue in the forefront of analytical chemistry and in the wider field of chemistry in general, where it has such an important contribution to make.

G. W. C. MILNER
President, Analytical Division

The reproductions on the next four pages are taken from the first issue of The Analyst, *published in March,* 1876.

The Analyst,

INCLUDING THE PROCEEDINGS OF

THE "SOCIETY OF PUBLIC ANALYSTS."

[No. 1.] 31st MARCH, 1876. [PRICE 6d. *Or to Subscribers 3s. 6d. per annum. Free by Post.*]

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THE ANALYST.

THE ANALYST appears, primarily, as the organ of the "Society of Public Analysts," and, secondly, as the representative of Analytical Chemists in general.

The Society of Public Analysts is still in its infancy, but at a very early period of its existence it became manifest that a literary organ of some kind was essential to its success.

Under these circumstances, and in the very early days of the existence of Public Analysts as a corporate body, an experiment (purely temporary) was made of utilising the columns of an established Chemical Journal, for the purpose of reporting the proceedings of the Society.

It was found, however, as the Society enlarged its borders, that as Public Analysts unfortunately could not entrench themselves within the quietude which ought to obtain in a Laboratory, but had occasionally to appear in Police Courts, a merely technical journal did not supply a sufficiently expansive vehicle for the communication of matter which, though not scientific, was of vital interest to Public Analysts as such.

Hence the object of THE ANALYST is not only to present to its readers the latest and best authenticated processes of analysis as they are perfected, but to publish all cases of prosecution for adulteration, and such parliamentary and other proceedings as may appear to touch the interests of Analysts in general.

This is, at all events, the task we propose to ourselves, relying upon the loyal co-operation of all Analysts.

SOCIETY OF PUBLIC ANALYSTS.

On the 15th Inst. an Ordinary Meeting of the above Society was held at Cannon Street Hotel.

Mr. Wanklyn, Vice-President, occupied the Chair.

There was a numerous attendance of members, and the interest of the Meeting was enhanced by the presence of an unusally large number of visitors.

After the ordinary routine business had been transacted the following resolution was put from the Chair :—

"That the name of Professor A. G. Anderson be removed from the roll of members "of this Society, and that the Secretaries be directed to inform him of such removal, "and announce the fact in the Society's journal."

A ballot was taken and the Resolution was carried unanimously.

The Scrutineers appointed to examine the voting papers, announced that the following gentlemen had been elected.

Member—Mr. J. W. Thomas.

Associates—Messrs. S. T. Clothier, Francis Heron, L. de Koningh, E. Lapper, H. G. D'Arcy Power.

A Paper on the Determination of Quinine was read by Mr. Allen, and another on the Analysis of Butter was read by Dr. Muter.

Each Paper led to a lengthened discussion, and the Meeting did not terminate till a late hour, three other Papers being held over.

The announcement by Mr. Wigner of the early appearance of the first number of THE ANALYST was received with applause.

in the ash of flour and bread. It is, therefore, to no purpose to make estimations of the sulphuric acid in the ash of alumed bread.

From some experiments recently made in my laboratory, I have been led to seek for the sulphuric acid in the cold aqueous extract of flour.

The major part of the mineral matter of flour goes into the cold aqueous extract, whilst the total weight of the extract is only some five per cent. of the flour. Before determining the sulphuric acid in the extract I coagulate the soluble gluten and remove it by filtration.

RETAILING MILK IN THE STREETS.

The question has arisen in more than one instance recently, whether an itinerant vendor of milk can be fined for refusing to serve an inspector under the Food and Drugs Act, or, in other words, whether a milkwalk may be considered either "premises," or "a shop," or "stores," and whether a house-to-house delivery of milk involves "exposure for sale."

To those interested in the question, we commend the following extracts from two letters, addressed respectively by the Home Office, and the Local Government Board, to the Wandsworth Board of Works, in reply to an application from that body for an authoritative interpretation of the law.

Mr. Cross, says, that "having consulted the chief magistrates of the Police Courts "of the Metropolis, he is of opinion that Sec. 7, of the Act 38 and 39. Vic., cap. 63, "applies when a vendor 'exposes to sale,' *anywhere*, or *has on sale* by retail in any shop "or stores. 'He,' however, recommends that a case be stated for the opinion of one "of the High Courts of Justice."

The Local Government Board, referring to a case in point, say: "If the milk was "retailed at the corner of the street to all passers by, it was exposed for sale, and such "exposure may perhaps be held to bring the case within the statute, but if it was being "delivered from house-to-house, the case may be different."

The Board, however recommend, that "before any amendment of the existing law is proposed, the judgment of the High Court of Justice should be obtained."

THE PREPARATION OF THE FERROUS PHOSPHATE OF THE PHARMACOPŒIA.

By Rees Price.

(Pharmaceutical Journal, 3rd Series, No. 297. 1876.)

Mr. Rees Price's experiments, appear to show that the Pharmacopœia process of making ferrous phosphate by means of ferrous sulphate, sodio phosphate, and sodic acetate, is one which may be attended with a loss of one-fourth of the phosphate of iron, and that by using instead of acetate of soda, or ascetic acid, an excess of phosphate of sodium no loss occurs. He therefore suggests as a substitute for the process of the British Pharmacopœia the following:—

Granulated Ferrous Sulphate	224 grains
Sodic Phosphate	660
Cold distilled water	12 ounces.

A. W. B.

DR. LETHEBY.

We have to announce with extreme regret—a regret which will be shared by our readers—the death, somewhat suddenly, of Dr. Letheby. He had been unwell for some weeks, his complaint being we believe, inflammation of the lungs; but he was expected to be present as a scientific witness in a case at the Richmond Petty Sessions on the 29th inst. At the last moment, however, a telegram was received notifying his decease.

Dr. Letheby was too well known in the chemical world, to require any lengthened obituary notice at our hands.

We may however, mention, that he was an early Member of the Chemical Society; that he took his M.B. degree in 1843, became Ph.D., and M.A. in 1858; that amongst the numerous appointments which he had held, were those of Medical Officer of Health, and Public Analyst for the City of London; and that he was the author of numerous scientific and hygienic works. He died in his sixtieth year.

A NOVEL READING OF THE SALE OF FOOD AND DRUGS ACT.

At Westminster, Henry Fielding, a milkman, of 15, Lower Symonds Street, Chelsea, appeared in answer to an adjourned summons, charging him with selling, to the prejudice of the purchaser, some milk which was not of the nature, substance, and quality demanded. Mr. Pemberton, barrister, appeared for the prosecution; and Mr. Symth, solicitor, for the defendant. The adulteration was not in dispute, but the summons being under the 6th section of the Adulteration of Food Act, and the inspector admitting that he purchased it solely for the purposes of analysis, it was asked whether the sale was to the prejudice of the purchaser. Mr. Pemberton said that if he proved that the article was not only different from the article demanded, but inferior in quality, the purchaser was prejudiced and an offence committed. Mr. Smyth said it was his duty to submit that the complainants had not proved their case. The proceeding was under a penal statute, and he was quite sure the magistrate would look at the statute strictly and give effect to it strictly. The word "prejudice," now appeared for the first time in an Adulteration of Food statute. He contended that it was introduced to enable any person buying food *bona fide* to have it analyzed, and to prevent an army of informers springing up. Mr. Pemberton contended that the purpose for which the article was bought was no part of the inquiry. Mr. Arnold said he could not tell what the Legislature intended by the words; but, as they were in the Act, they must have some meaning. Mr. Pemberton said that if any one paid more for an article than it was worth, it was to his "prejudice." Mr. Arnold said it was a very nice point and required consideration. He had read the Act very carefully since he had adjourned the case, but he should like to consider the matter further, as he was much struck by Mr. Pemberton's argument that the prejudice of the purchaser must mean a loss to the pocket of the purchaser. He should adjourn the case and consider his judgment. Another summons, in which the same principle was involved, was also adjourned.—*Times*.

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Recent Advances in the Ring Oven Technique

A Review*

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Summary of Contents

Introduction

The first paper on the ring oven technique was published in 1954.[1] This technique is basically a special type of spot analysis carried out on filter-paper in which, however, the substances to be identified or determined are concentrated in the form of sharply developed, well defined circular lines as a result of the heat-barrier action of the edge of the central bore-hole of the heating block of the ring oven. Originally, this method was devised as a separation technique for extremely minute samples (*e.g.*, in investigations of works of art). From the two basic aspects—the concentration of substances on filter-paper in the form of well defined lines and the possibility of carrying out separations on a single drop—all of the applications of this technique in many fields that have since been published in more than 300 papers were derived.

In 1961 a monograph, "Microanalysis by the Ring Oven Technique," was published and in 1970 the Second Edition[2] appeared (subsequently referred to in this paper as the monograph) in which all of the then known applications of the method were surveyed. Since then, about 100 further publications dealing with the ring oven method have been published. Most of them are discussed in this review, although some can be mentioned only very briefly.

The Apparatus and Its Use

The basic form of the ring oven itself has not been altered since its first development. The ring oven is usually made of aluminium, but various other metals have been used. In the monograph[2] ring ovens made of glass are mentioned. Within the last few years, similar glass ring ovens have been described by Chiba[3] and co-workers; one of them is a portable oven, obviously designed for field work.[4–6] The "Nichrome-wire ring chamber" of Hashmi *et al.*[7] is a glass ring oven with a heating wire installed in it, in a manner similar to that in the usual metal ring ovens. Improvised home-made ring ovens that are simple and inexpensive have also been described.[8,9]

Instead of the edge of the hot ring oven for concentrating substances on filter-paper in narrow zones, a brief mention has been made of the possibility of using a hot stream of air[10] or nitrogen[11] for the purpose of providing a "heat barrier."

The dependence on the quality of various filter-papers of the limits of identification in several reactions for anions has been investigated.[12]

* Reprints of this review will be available shortly. For details, see summaries in advertisement page s

Gertner and Grdinić carried out some theoretical investigations on the process of precipitation, complexing and transporting ions to the ring zone.[13–15] Autoradiography has been used in a number of these experiments (*e.g.*, using labelled silver ions).[13,16] The determination of the solubility of poorly soluble silver salts (such as halides, phosphate, sulphide and chromate) on filter-paper has been carried out using ring oven colorimetry; it has been found that solubilities are much lower on paper than directly in water.[16] Ackermann and Gressmann had already made similar studies some time ago.[17] By using the same technique, the solubilities of a number of other salts (*e.g.*, sulphides of various metals) have likewise been investigated.[18]

Qualitative Analysis of Metal Ions

Identification reactions for almost all metal ions have been adapted for use with the ring oven method, but it is beyond the scope of this review to record all of the new developments that have been described in recent years. For some of them, unfortunately, not enough detailed information has been provided (*e.g.*, identification limits, interferences, precise reaction conditions). Many of the reactions used for quantitative analysis can also be applied for qualitative purposes; therefore, the reactions mentioned in the following section should also be considered from the viewpoint of qualitative analysis.

The identification of a number of metals using the fluorescence of their morinates has been described, beryllium and aluminium having already been identified with morin (2',3,4',-5,7-pentahydroxyflavone)[2]; the reactions of arsenic, calcium, caesium, lanthanum, lead, lithium, magnesium, strontium and zinc are described, although several of the reactions have poor identification limits (*e.g.*, caesium and arsenic, 15 μg).[19] A brief study on the sensitivity of detection of some metals, alone and in defined mixtures, has been carried out.[20]

Ghose and Dey devised systematic separation schemes for 20 common metals[21] and for fourteen "less common" metals (gold, beryllium, thallium, cerium, titanium, zirconium, thorium, vanadium, molybdenum, tungsten, uranium, selenium, tellurium and platinum),[22] by combining solvent-extraction procedures and the ring oven; some further examples of extraction procedures can be found in the monograph.[2]

Two analytical schemes for all six platinum metals, using solvent extraction directly on the filter-paper, have been published recently.[23,24] An example of the application of potassium thiocarbonate as a reagent for the separation of metal ions is provided by a systematic scheme for 20 common metals.[25] The separation of some metals using dithiooxamide, and a study on the separation efficiency by use of radioactive isotopes of the corresponding metal ions (silver, cobalt, zinc, iron, barium), have been described.[26] Du and Gutbier described an interesting scheme for the simultaneous separation of some anions (SO_4^{2-}, Cl^-, NO_3^-) and cations (iron, nickel, aluminium, cadmium, copper); a ring oven with three different bore-hole diameters (17, 22 and 28 mm) was used.[27]

To close this section, two practical applications of the ring oven method can be mentioned. Locke and Riley (Guggenheim Museum, New York) published a systematic scheme for the analysis of paint samples from valuable paintings.[28] This work is of particular interest to the present author, because more than 20 years ago the ring oven technique was originally devised with investigations of objets d'art in mind. A number of such applications to metal specimens can be found in the monograph.[2] Thomas and Lee used the ring oven method for the analysis of trace amounts of metal particles (iron, zinc, manganese) in intravenous solutions (injections).[29]

Determination of Cations and Anions

The comparison of the colour intensities of unknown rings with those of standard rings, a procedure that has been called "ring colorimetry," has found many applications in the analysis of metal ions, anions and organic substances. In fact, most papers on the ring oven technique deal with the quantitative aspects of the method. It should be mentioned that there has been some discussion about whether "semi-quantitative" or "quantitative" is the most appropriate description for ring colorimetry. West[30] may be quoted here: "If the ring oven is semi-quantitative at these levels then so are such methods as polarography, emission spectroscopy and colorimetry." In this connection, it might be of interest that some workers have compared results achieved by the ring oven method with results obtained

by other well established analytical techniques, such as polarography,[3] spectrophotometry[5,31] and atomic-absorption spectroscopy[32–34]; in all instances the results were in good agreement.

Cations

Table I lists newer applications of the ring oven method to the determination of metal ions.

In the method for manganese,[35] the resulting benzidine blue is not very stable and changes colour with time. In order to obtain a series of permanent standard rings for comparison, a coloured crayon of a suitable colour (matching that of benzidine blue) was used.

TABLE I

DETERMINATION OF METAL IONS

Ion	Reaction	Reference
Mn	Benzidine	35
Be	Morin (crayon) in UV	36
Cd	$[Fe(\alpha,\alpha'\text{-bpy})_3][CdI_4]$	37
Cu	Bathocuproin	3
Fe	Bathophenanthroline	3
V	Schiff base (anthranilic acid + salicylaldehyde)	38
Se	2,3-Diaminonaphthalene in UV	39
Ca	Di(2-hydroxyphenylimino)ethane	40
Pb	Potassium chromate	33, 41
Pb	Dithizone	32
Pb	As $[PbCl_4]^{2-}$ in UV	42
Pb	Molybdate → molybdenum blue	43

In the determination of beryllium with morin, West and Jungreis[36] used a crayon in which the morin is incorporated in a suitable solid medium (monoglyceryl stearate and paraffin wax) instead of a reagent solution. A number of other morinates have likewise been used for the determination of the corresponding metals; the qualitative applications of these morinates have been cited above.[19] Fluorescent iron morinate rings have been used as a common standard scale for the determination of several metals (iron, copper, cobalt, nickel, manganese).[44] This is a further example of the possibility of using a universal standard scale instead of preparing an individual standard scale for every substance to be determined. Earlier examples can be found in the monograph.[2] The determination of platinum metals had been described, but unfortunately without sufficient details.[24]

The rapid separation and determination of uranium(VI) using DEAE-cellulose anion-exchange paper and potassium hexacyanoferrate(II) has been described.[45]

Johri and co-workers developed three practical applications for ring colorimetry, namely the determination of arsenic in animal feeds (utilising the reaction $As_2S_3 \rightarrow Ag_2S$),[46] calcium in mineral waters[40] and some trace metals (iron, zinc, manganese, copper) in milk.[47] Du and Gutbier[48] determined trace amounts of copper in nickel and cobalt salts with dithiooxamide, first using a spot of lead sulphide on the paper in order to locate the trace metal.

Many other determinations have been carried out in connection with air pollution studies, and are considered later.

In a number of papers, further examples of the combination of thin-layer and paper chromatography with the ring oven technique have been discussed: various groups of metals (*e.g.*, the six platinum metals,[49] other noble metals[50] and alkali metals[51]) are separated by one of the two chromatographic techniques and are then dissolved from the chromatographic support using methods described in the monograph[2] and determined with the aid of suitable ring colorimetric methods. In some instances, previously known methods are used for the final determination, while in some of the papers insufficient information is provided.[52–58]

The question of whether photometric measurement of the intensity of the rings could make ring colorimetry more accurate was considered long ago; the monograph[2] (p. 71) should be consulted on this point. Recently, two contributions have dealt with this possibility again: Friedrichs and Grover[59] evaluated the intensity of the rings by light reflectance measurement for the determination of iron as Prussian blue in air pollution studies; Du *et al.*[60] measured the light absorption of the rings, cellulose acetate filters being used instead of ordinary filter-paper. In order to achieve even better transparency of the supporting matrix, the filters were moistened with paraffin oil before the final photometric measurement, and in this way

iron (Prussian blue), nickel (dimethylglyoxime) and copper (benzoin α-oxime) were determined with the aid of a microscope photometer.

A completely new approach to quantitative determinations, the use of catalysed reactions and of unstable reaction products, is discussed later under New Applications of the Ring Oven Technique.

Anions

Very little can be reported about new contributions to the determination of anions. West *et al.*[61] determined sulphuric acid in air pollution studies. Potassium bromide is washed to the ring zone together with the acid, sodium fluorescein and potassium bromate is being added to the ring zone. The red eosin ring formed is compared with a standard scale. By this method, total protons can be measured likewise by adding all three reagents at once to the centre sample spot and transferring the eosin formed to the ring zone.

Phosphate, silicate, arsenate and germanate have been determined with the aid of the well known molybdenum - benzidine blue reaction.[62] Because all four of these ions form heteropoly acids with molybdate, all of which can be converted into benzidine blue, a common standard scale can be applied; this is also an example of a "universal standard scale."

A few other methods for the determination of anions are considered in the last section of this review.

Organic Substances (Herbicides, Vitamins, etc.)

Although the ring oven method has found many applications with inorganic substances, the number of analytical applications with organic substances is still rather limited; it is to be hoped that future developments will result in more applications in this area, perhaps with the aid of the newly developed adsorption barrier technique (see the last section).

In addition to the examples of alkaloids already mentioned in the monograph,[2] some further determinations of this group of substances have been reported. Shah and Hussain[63] determined ajmaline with *p*-dimethylaminobenzaldehyde in a number of pharmaceutical products. Chiba[6] reported the detection and determination of some alkaloids (morphine, codeine, cocaine, caffeine, quinine, ephedrine, strychnine and papaverine) by the ring oven technique, mostly using suitable known colour reagents, *e.g.*, picric acid, iron(III) chloride, molybdophosphoric acid, molybdate and hexacyanoferrate(II). Ergosterol has been determined with 2,3,5-triphenyltetrazolium chloride and potassium hydroxide, forming a red reaction product that was stabilised with copper nitrate (0.03–1 $\mu g\ \mu l^{-1}$).[64]

In the monograph,[2] some methods are mentioned for determining a number of insecticides. The first application of the ring oven technique to herbicide analysis was described by Čoha and Vojinović[65]; amiben was determined by spraying the rings first with sodium nitrite, followed by *N*-1-naphthylethylenediamine dihydrochloride (red), while diquat and paraquat rings were made visible with methanolic sodium hydroxide solution (brown and bluish green, respectively). Čoha and Kljajić[66] subsequently described the separation of five herbicides (amiben, paraquat, diquat, CDAA and CIPC) by thin-layer chromatography, followed by their determination. The extraction of trace amounts of the herbicides diquat and paraquat from water and soil, using column chromatography on a cation-exchange resin for their separation and the ring oven technique for their determination, was described by Čoha.[67] It is certainly of great interest to provide methods for determining small amounts of pesticides now that environmental problems are becoming increasingly important.

Several fats and oils of mineral and vegetable origin have been determined, using the phenomenon that these substances render filter-paper translucent. These translucent rings are always compared with the corresponding translucent standard scale prepared with hard paraffin wax.[68]

Hashmi and co-workers described the determination of a number of organic substances, *e.g.*, amino-acids[69] and vitamins,[70] with the "Nichrome-wire ring chamber," using a somewhat different approach ("sensitivity scale") that had previously been discussed in principle by Wenger *et al.* ("extinction method").[71]

Radiochemical Applications

The application of the ring oven method to radiochemical analytical procedures has been discussed extensively in the monograph[2] and here only some of the newer developments are

mentioned. The use of radioactive tracers for the study of precipitation, complexing reactions and for following the efficiency of separations on filter-paper have already been mentioned above in the sections on The Apparatus and Its Use[13,14,16] and Qualitative Analysis of Metal Ions.[26]

Klockow and co-workers carried out a series of studies on radiochemical separations by the ring oven technique. The genetically related radionuclides cerium-144 and praseodymium-144 could be separated on filter-paper impregnated with hydrated manganese oxides; the praseodymium-144 is transferred to the ring zone with trichloroacetic acid, whereas the cerium-144 remains in the centre of the filter-paper; a carrier-free solution of cerium-144 was applied.[72] Silver-109m could be separated from its parent nuclide cadmium-109 on carboxymethylcellulose paper using thiosulphate as a complexing agent for the silver. For the separation of cadmium-109 from a silver matrix, another possibility was described.[73] The silver is retained on paper impregnated with copper(II) sulphide and the cadmium (together with released copper ions) is washed into the ring zone with acetic acid.[73] The selective isolation of caesium-137, zirconium-95 - niobium-95, ruthenium-106 and yttrium-90 plus cerium-144 - praseodymium-144 from a mixture containing long-lived fission products has been reported.[74] Suitable combinations of impregnated filter materials and solvents with and without complexing agents were applied.

Kriváň[75] published a method for the determination of copper in the presence of several other metals involving the release of radioactivity through an exchange reaction. A spot of cadmium sulphide, labelled with cadmium-109, is prepared in the centre of a filter-paper, the test solution (copper) is added and an amount of cadmium equivalent to the copper present is released and transferred to the ring zone. The activity of the radioactive cadmium in the ring is measured. If a standard amount of copper is treated likewise, it is easy to calculate the amount to be determined from the ratio of the two measured activities. This method is generally applicable and should permit the determination of a number of other metal ions.

Air Pollution Studies

The ring oven method has been used very frequently in recent years to study and control air pollution. This question has been discussed particularly in two recent books[76,77] and in some summarising papers (*e.g.*, reference 8). In the monograph,[2] contributions to this special field up to 1970 are cited.

Samples collected by impaction or electrostatic precipitation can be dissolved and drops of the solution obtained can be subjected to the usual ring oven procedures. In most instances, however, samples of airborne particulate matter are collected on filter-paper or other suitable filter media (membrane filters, glass-fibre filters, etc.). When a filter-paper is applied as the collecting medium, the sample collected can be concentrated directly into the ring zone and made visible there for comparison purposes. If the samples are collected on other filtration media, they must be transferred on to ordinary filter-paper and concentrated there. These methods for the transfer, concentration and determination of collected airborne particulate matter are described extensively in the monograph[2] and in an earlier publication.[78]

If the collected sample is washed directly on the ring oven into the ring zone, only one ring is available for the comparison instead of the usual three rings in ring colorimetry, and this is accepted in many published practical examples. A possible means of obtaining three rings is to dissolve the collected sample in a suitable solvent and to prepare three sample rings, but much more of the material to be determined needs to be collected than is actually used in preparing the three rings. We proposed some time ago an alternative procedure,[79] in which three circular pieces of different but well defined sizes are cut from the filter used for sampling, the areas of the three cuttings being in a suitable ratio to each other, and the collected material is transferred from these small pieces to the ring zones of three separate filter-papers, thus giving three rings (corresponding to three rings with different known drop numbers). This procedure was verified by Chiba in the determination of nickel and copper in air.[5,31]

In Table II, most of the determinations of polluting material by the ring oven method known to date are summarised. Table II lists not only newer contributions but also determinations that were mentioned in the monograph[2] in order to present as complete as possible a survey of this field of analysis.

Chinese workers have discussed the determination of phosphorus, sulphur, selenium, mercury, lead and cadmium in air,[89] but unfortunately no information on the details of the methods used is available to the reviewer.

An investigation on water pollution has been described,[34] copper, cadmium and zinc being determined after their concentration by extraction procedures.

TABLE II

DETERMINATION OF AIRBORNE PARTICULATES

Ion	Reaction	Reference
Sb	Molybdophosphoric acid	80
Cu	Dithiooxamide crayons	81
Cu	Bathocuproin	5
Cd	$[Fe(\alpha,\alpha'\text{-bpy})_3][CdI_4]$	37
Pb	Molybdate + diphenylcarbazide	43
Pb	Chromate	33, 41
Pb	Dithizone	32
Pb	As $[PbCl_4]^{2-}$ in UV	42
Fe	Potassium hexacyanoferrate(II)	78
Fe	Potassium hexacyanoferrate(II) (reflectance)	59
Fe	Bathophenanthroline	4
Al	Morin	78
Mn	Benzidine	35
Ni	Dimethylglyoxime	31
Zn	*o*-Mercaptothenalaniline crayons	82
Be	Morin crayon	36
Se	3,3'-Diaminobenzidine	83
PO_4^{3-}	*o*-Dianisidine molybdate	84
PO_4^{3-}	Molybdate + benzidine	85
SO_4^{2-}, SO_3^{2-}	$BaCl_2$ + $KMnO_4$ + oxalic acid (co-precipitation)	86
H_2SO_4	BrO_3^- + Br^- + fluorescein	61
Sulphur	Copper(I) sulphate → colloidal Cu_2S	87
Caffeine	Acetylacetone + *p*-dimethylaminobenzaldehyde	88

Combination with Other Analytical Techniques

The combination of electrographic sampling, involving anodic dissolution of metals, with the ring oven technique was described by Stephen.[90] Two more recent papers on this combination of techniques for the analysis of various alloys have been published.[91,92]

Combinations with chromatographic techniques have already been mentioned in this review for mixtures of metal ions[49–53, 55–58] and for organic substances (herbicides).[66] As an example of the combination with circular chromatography, Frei and Stockton[93] developed a method for the determination of trace metals (cobalt, copper, iron, nickel) in natural samples (tap water, sea water, algae).

The use of ion exchange in connection with the analysis of metal ions,[45] herbicides[67] and radioactive substances[73,74] has already been mentioned in other sections of this review.

As a further example of the combination with titrimetric analysis, the complexometric titration of aluminium after its separation from other metals by the ring oven method has been described.[94]

The ring oven technique has been used to concentrate metal samples (silver, palladium, rhodium, platinum, gold, copper, iron) on filter-paper for X-ray fluorescence analysis, giving higher sensitivities in the determination of these metals.[95]

New Applications of the Ring Oven Technique

Adsorption Barrier

All of the applications of the ring oven method that have been described so far are based on the use of a heat barrier, and substances that are not sufficiently stable at the temperature of the heating block cannot be analysed. A means of avoiding this difficulty while still being able to concentrate substances in a narrow outlined ring zone is to use an adsorption barrier instead of a heat barrier, the ring oven itself being used only for the preparation of a

narrow ring zone, consisting of a suitable adsorbent. The actual separation and concentration of the sample are carried out on this prepared filter-paper without the heated ring oven.[96,97] In an example of this technique, blood peroxidase was washed from the centre of a filter-paper with sodium chloride solution on to a magnesium hydroxide adsorption barrier, where the enzyme was retained; a sharply outlined blue ring appeared on spraying the filter-paper with benzidine - hydrogen peroxide - acetic acid solution.

It now seems to be possible to apply the ring oven technique to biological specimens; preliminary work in our laboratory has given promising results. A number of binary mixtures of dyestuffs have been separated on the basis of their different adsorption behaviour on various adsorbents (aluminium, magnesium and beryllium hydroxides).[98]

This technique has also been used for the determination of fluoride and oxalate. These anions release chloranilate from a lanthanum chloranilate spot prepared in the middle of a filter-paper that also bears an adsorption barrier ring consisting of aluminium and magnesium hydroxides. The chloranilate released, which is not stable to heat and would otherwise change colour, is transferred to the adsorption zone with methyl Cellosolve; a violet ring appears.[99]

Catalysed Reactions

Tests based on the use of catalysed reactions are very sensitive, because the final product to be observed is not the result of a stoicheiometric reaction between the reagent and the substance to be identified; the latter serves only as a catalyst and, consequently, a much greater amount of the end-product is formed. If the catalyst to be identified is concentrated in the form of a sharply outlined ring zone, such reactions can be made even more sensitive. Some catalysed identification reactions have been described[100] in which the catalyst is washed into the ring zone together with one of the reactants and sprayed with the second reactant.

It is not practicable to apply the same technique to semi-quantitative determinations because the amount of reaction product formed is obviously dependent on the time that has elapsed since the start of the reaction, which makes it impossible to use a stable standard scale.

The observation of simultaneously started catalysed reactions with unknown and known concentrations of the catalyst enabled us to apply such reactions to the determination of catalysts. This is, in effect, an application of the "simultaneous comparison method" to the ring oven technique[101]: one drop of the unknown catalyst sample solution and of two different standard solutions are spotted at three concentric points around the middle of a round filter-paper, thus forming an equilateral triangle. These three drops are washed in the usual manner on the ring oven from the centre to the ring zone; the three different amounts of the catalyst are thereby concentrated in the form of three sharply outlined circular segments. The filter-paper is then sprayed with a solution of the two reactants of the catalysed reaction and the changes in colour in the three segments are observed. The order of these changes shows whether the concentration of the sample is higher or lower than the two standard concentrations or if it lies between them. In further steps, the sample drop and two drops of suitably chosen standard solutions (six different standard concentrations are sufficient) are placed on a new filter-paper and the whole procedure is repeated. In most instances, after the third comparison step one can establish the standard concentration to which the unknown concentration corresponds best. For higher accuracy it is advisable to carry out a second determination with a different number of drops of the unknown solution. Several catalysts have been determined in this manner, *e.g.*, vanadium (*p*-phenetidine + bromate), molybdenum (iodide + hydrogen peroxide), copper (*p*-phenylenediamine + hydrogen peroxide), cobalt (pyrocatechol + hydrogen peroxide), silver (Leucomalachite Green + $S_2O_8^{2-}$), L-cystine (iodine - azide reaction). Hexacyanoferrate(II) inhibits the catalytic action of cobalt in the above reaction and so can be determined indirectly.[102] It is possible to apply this technique to the determination of some enzymes.[97]

Unstable Reaction Products

If a chemical reaction yields coloured products that are unstable, it cannot be used in ring colorimetry in the usual way because it is impossible to have a stable standard scale. The "segment technique" mentioned above offers the possibility of using even reactions such as

these in ring colorimetry. The technique is the same as described above; in catalysed reactions, products are formed that change with time, *i.e.*, that are also unstable. In this instance also, six standard solutions are used instead of a standard scale. A number of methods for direct and indirect determinations in this manner have been described, *e.g.*, iron (thiocyanate), vanadium (3,3'-dimethylnaphthidine), chromate (*o*-tolidine) and formaldehyde (*o*-dianisidine). Lead and EDTA were determined indirectly by using the reaction of chromate with *o*-tolidine.[103]

Nanogram amounts of nitrate and nitrite have been determined alone and in admixture by this method with the well known Griess - Ilosvay reaction; nitrate has first to be reduced on a zinc-coated iron plate.[104]

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Fungicide Residues

Part V.* Determination of Residues of Chloraniformethan in Grain and Cucumbers by Gas Chromatography

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A method for determining residues of chloraniformethan {*N*-[2,2,2-trichloro-1-(3,4-dichloroanilino)ethyl]formamide} in grain and cucumbers is presented. After extraction with methanol and preliminary partitioning with hexane, chloraniformethan is extracted into dichloromethane, separated from interfering co-extractives by preparative thin-layer chromatography on silica gel and determined by electron-capture gas - liquid chromatography.

Chloraniformethan {*N*-[2,2,2-trichloro-1-(3,4-dichloroanilino)ethyl]formamide} is a recently introduced systemic fungicide.[1,2] In the United Kingdom,[3] recommendations have been issued for its safe use on cereals and hops and, provisionally, on leaf brassicas, swedes and turnips. It is included in the list of Approved Products and Their Uses for Farmers and Growers[4] as a protection of barley and roses against powdery mildew. Its use on cucumbers has also been reported.[5,6]

The only published method for residue analysis[6] is based on the hydrolysis of chloraniformethan to give 3,4-dichloroaniline, which is determined colorimetrically by diazotisation and coupling with 2-aminoethyl-1-naphthylamine. This method does not enable chloraniformethan to be distinguished from several other widely used pesticides that also yield dichloroaniline on hydrolysis. In the method described below, thin-layer and gas chromatography are used for the determination of chloraniformethan at residue levels.

Fortified samples of barley, wheat and cucumber were used to obtain the results for recovery recorded here.

Experimental

The work was carried out on samples with unknown history, all recovery tests being made on artificially fortified chopped samples. The samples were extracted with methanol using either a homogeniser or a Soxhlet extraction apparatus. The extracts were diluted with water and given a preliminary wash with hexane in order to remove some of the highly coloured co-extractives. Partitioning into dichloromethane was used so as to provide a concentration step, followed by clean-up on preparative thin-layer chromatographic plates and determination by gas - liquid chromatography with an electron-capture detector.

Chloraniformethan is relatively involatile but appears to be unstable at temperatures above about 200 °C. Thermal analysis showed a slow loss of mass between about 200 and 300 °C, indicating slow decomposition, followed by a more rapid loss above 300 °C as vaporisation of the decomposition products occurred. Its reproducible gas-chromatographic determination thus presents some difficulty. The most satisfactory results were obtained with a column of 5% neopentyl glycol succinate on Gas-Chrom Q operated at 180 °C. This column gave a sharp, symmetrical peak for chloraniformethan, which was reproducible provided that care was taken to avoid conditions that favour decomposition (use of clean silanised glass columns and clean sample extracts and avoidance of contamination of the stationary phase, particularly in the injection region of the column).

It was not possible to confirm the identity of the gas-chromatographic peak by mass spectrometry as the chloraniformethan appeared to be lost or had decomposed either at the silicone rubber gas-chromatographic - mass spectrometric interface or in the heated metal

* For Part IV of this series, see *Analyst*, 1975, **100**, 249.

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transfer line. However, it seems probable that when decomposition occurs, a major residual product under these conditions would be 3,4-dichloroaniline. With the standard gas-chromatographic conditions used in the method, chloraniformethan gave a main peak at 8.3-min retention time with very small peaks at 0.3, 0.5, 1.0, 3.0, 6.1 and 6.7 min, whereas 3,4-dichloroaniline has a retention time of 6.0 min. During preliminary work and while attempting to obtain satisfactory chromatograms using different column packings and higher injection temperatures, a major peak with the same retention time as 3,4-dichloroaniline was observed.

The gas-chromatographic determination of chloraniformethan could be applied to sample extracts only after a rigorous clean-up. It was found that column chromatography gave a "clean" sample, as indicated by the electron-capture detector, but remaining co-extractives still interfered, apparently by causing breakdown on injection. Silica gel, alumina and polyamide columns were tried with a variety of eluting solvents without success. Preparative-scale thin-layer chromatography was found to give a much better clean-up without undue difficulty and also greatly facilitated the separation of chloraniformethan from other potentially interfering pesticides. A range of different commercially available pre-prepared thin-layer chromatographic plates was tested. Silica gel was chosen because it gave narrower bands and (with some sample extracts) lower levels of background material than alumina, cellulose or polyamide layers and was much easier to handle during the quantitative application and removal of samples; a 1000-μm thick layer gave the best compromise between sample capacity and ease of use. Calcium sulphate was a satisfactory binding agent but layers that incorporated an organic polymer binder were found to be unsuitable.

The R_F values of chloraniformethan were compared with those of the main co-extractive bands obtained from barley samples, which were found to require the most exacting clean-up of the crops tested. The values obtained with a number of solvent systems are shown in Table I, the best compromise between good separation and convenient running conditions being given by a 1 + 1 mixture of 2,2,4-trimethylpentane and butan-2-one. The R_F values of potentially interfering pesticides were then measured for this system. Those compounds which were not readily detected on the fluorescent thin-layer chromatographic plates were rendered visible by spraying with either silver nitrate or Dragendorff's reagent.

Samples were applied to commercial 20 × 20 cm thin-layer chromatographic plates, which contained an inorganic binder and inorganic fluorescent (254 nm) additive, by using a commercial streak applicator. No application problems were experienced provided that the sample solution was completely dry and the application was carried out slowly. Control spots of a concentrated chloraniformethan solution were applied at either side of the streak so as to allow the band of interest to be located (by observation of the spots under ultraviolet light) after running the plate. The band was removed simply by scoring around it and then scraping the silica from the plate. It was found to be important to use acetone rather than methanol for washing the sample from this silica gel. The latter solvent is widely recommended for this purpose but caused irreproducible results, apparently as a result of interference by dissolved silica gel during gas chromatography.

Method

Reagents

Analytical-reagent grade materials should be used unless otherwise indicated.

Acetone, redistilled.

Dichloromethane, redistilled.

Hexane. Redistil laboratory-reagent grade hexane from sodium hydroxide.

Methanol, redistilled.

Sodium chloride solution, saturated.

Sodium sulphate, anhydrous, granular.

Mobile phase. Add 1 volume of 2,2,4-trimethylpentane to 1 volume of butan-2-one.

Chloraniformethan. Technical grade material (about 95% pure) was purified by recrystallisation twice from benzene (m.p. 132–133 °C, literature value[7] 134–135 °C) and the product used to prepare a standard 1 mg ml^{-1} stock solution in acetone. Working standards and solutions for fortification of samples were prepared from this solution by further dilution with acetone as required.

Apparatus

Grinder. A domestic coffee grinder is suitable.

Homogeniser or Soxhlet extraction apparatus.

Rotary evaporator.

Thin-layer plates. A 1000-μm thick layer of silica gel GF with fluorescent indicator on 20 × 20 cm glass plates (Anachem). Remove a 1-cm strip of silica gel from each side before use.

Thin-layer chromatographic applicator (Burkard) with 1-ml *syringe (Agla).*

Glass tanks for thin-layer chromatography. The tanks were lined with Whatman No. 1 filter-paper along their longer sides and at their base. Efficient sealing of the lids was ensured without the use of grease by using spring clamps and a PTFE gasket.

Gas chromatographs. Two Pye 104 instruments fitted with ^{63}Ni electron-capture detectors were used isothermally. One detector was operated in the pulsed mode at a fixed frequency using the standard Pye 104 supply unit (pulse interval set to 150 μs) and amplifier. The other detector was operated in the pulse mode at constant current using a combined supply - amplifier unit of the type supplied with Pye GCV gas chromatographs. Both detectors were used at a temperature of 250 °C without a flow of purge gas. The stationary phase was 5% neopentyl glycol succinate on Gas-Chrom Q (supplied by Phase Separations Ltd.) packed into a silanised glass column, 0.9 m × 4 mm i.d. The column was operated at 180 °C with a nitrogen carrier gas flow-rate of 40 ml min^{-1}. The temperature of the injection port was also maintained at 180 °C.

Procedure

Fortification

Samples were fortified for use in recovery experiments by the addition of a standard acetone solution to the ground or chopped sample. After allowing the acetone to evaporate, the sample was sealed and stored overnight at −20 °C before analysis as described below.

Extraction

For the Soxhlet extraction, place 20 g of ground or chopped sample in the paper thimble and extract it for 4 h with 200 ml of methanol and a rate of heating such that the solvent cycles about twenty-five times per hour. Alternatively, samples can be homogenised three times with methanol in a 150-ml vortex beaker, the mixture being filtered on a Büchner funnel fitted with a glass sinter and washed with methanol after each operation. A total of 200 ml of methanol is used.

Analysis

Dilute the methanol extract with distilled water (40 ml for grain samples and 20 ml for cucumber samples) and add 10 ml of saturated sodium chloride solution. Extract twice with 50-ml portions of hexane and discard the extracts. Add a further 100 ml of water and extract with two 50-ml portions of dichloromethane. (It is essential that these extracts are completely dried before proceeding further.) Dry the extracts by shaking them with anhydrous sodium sulphate and then, in order to ensure that they are completely dry, pass them through a further 20 g of sodium sulphate in a 20 mm diameter glass column. Combine the extracts and any washings in a 250-ml round-bottomed flask. Concentrate the solution to a volume of about 1 ml using the rotary evaporator (under reduced pressure with the water-bath at 40–50 °C). Carefully wash the extract out of the flask with dichloromethane, making the solution up to volume in a 2-ml calibrated flask. This step can be carried out successfully only if the dichloromethane solution is completely dry.

Fill the syringe from the applicator using about 1 ml of the concentrated solution of the extract. Apply 0.50 ml of this solution to a thin-layer chromatographic plate in the form of a 10-cm streak about 3 cm from the bottom of the plate using twenty-five applications of 20 μl each. Ensure that a narrow band of extract is obtained by drying carefully with a stream of cold air between each application. Apply a 5-μl spot of standard chloraniformethan solution (a 1 mg ml^{-1} solution in acetone) either side of the streak of extract. Score a line across the plate 10 cm ahead of the front of the extract band. Run the plate in the development solvent immediately, ensuring that the solvent is allowed to run up to the scored line. Keep the tank in the dark.

After development, dry the plate in a current of cold air, again in the dark if possible. Very briefly examine the plate under ultraviolet light and mark the two chloraniformethan spots. Remove a 10 × 1-cm band of silica gel from the plate between the spots (it may be more satisfactory to remove a 1.5-cm band if the original streak has spread to any extent). Pack the silica gel into a small glass column plugged with silanised glass-wool and dry it further by passing a flow of cold air through the column. Elute the column with acetone, collecting the eluate in a 10-ml calibrated flask, and make the volume up to the mark with acetone. Inject 5 μl of this solution into the gas chromatograph and compare the peak height with those obtained for 5-μl injections of standard solutions. When using the conventional (fixed frequency) electron-capture detector, ensure that the peak heights of the sample and standard solutions are directly comparable.

Results

Chloraniformethan was found to give excellent sensitivity on both electron-capture detector systems used. With a signal to noise ratio of 3:1 the detection limits were 0.05 ng with constant-current operation and 0.15 ng with fixed pulse frequency operation. This difference is not significant and may be partly explained by minor differences that exist between the two gas chromatographs and their columns. Linearity of calibrations was observed from the detection limit up to about 1 ng with the fixed pulse frequency detector and to greater than 2.5 ng with the constant-current detector. The detection limits for chloraniformethan in sample extracts are slightly poorer and vary to some extent with individual samples. However, detection limits (as defined above or, when co-extractive peaks coincide, as four times the height of any interfering peak) in the samples investigated are estimated to be 0.1 mg kg^{-1} in barley, 0.05 mg kg^{-1} in wheat and 0.05 mg kg^{-1} in cucumber.

The R_F values of chloraniformethan for eighteen different thin-layer chromatographic solvent systems are shown in Table I. This table also shows the R_F values for three co-

TABLE I

R_F VALUES OF CHLORANIFORMETHAN AND BARLEY CO-EXTRACTIVES ON SILICA GEL GF PREPARATIVE THIN-LAYER CHROMATOGRAPHIC PLATES

Solvent	R_F value			
	Chloraniformethan	Barley		
		(*i*)	(*ii*)	(*iii*)
2,2,4-Trimethylpentane - acetone - diethyl ether (3 + 1 + 1)	0.19	0.21	0.14	0.05
2,2,4-Trimethylpentane - tetrahydrofuran (1 + 1)	0.41	0.40	0.11	—
2,2,4-Trimethylpentane - acetone - diethyl ether (5 + 3 + 2)	0.31	0.29	0.17	0.09
2,2,4-Trimethylpentane - butan-2-one (1 + 1)	0.50	0.34	0.15	0.11
2,2,4-Trimethylpentane - ethanol (1 + 1)	0.64	0.72	0.40	0.20
2,2,4-Trimethylpentane - dichloromethane - ethyl acetate (3 + 4 + 3)	0.30	0.30	0.17	0.08
Methanol - diethyl ether (1 + 99)	0.39	0.35	0.18	0.09
Acetone - diethyl ether (1 + 99)	0.35	0.68	0.33	0.58
Ethyl acetate - diethyl ether (7 + 3)	0.50	0.75	0.42	0.18
Ethyl acetate - diethyl ether (7 + 13)	0.58	0.87	0.51	0.25
Butan-2-one - diethyl ether (1 + 2)	0.70	0.58	0.40	0.17
Butan-2-one - benzene (1 + 3)	0.20	0.20	0.09	—
Ethyl acetate - diethyl ether (1 + 1)	0.67	0.79	0.51	0.16
Ethyl acetate - dichloromethane (3 + 7)	0.58	0.45	0.31	0.21
Acetone - hexane (1 + 1)	0.43	0.54	0.40	0.21
Ethyl acetate	0.92	0.96	0.08	—
Propan-2-ol	0.71	0.69	0.21	0.13
Chloroform	0.11	0.11	0.06	0.03

extractive bands [(*i*)–(*iii*)] obtained from barley. In each system these bands are the three most interfering bands present and do not necessarily represent the same co-extractive in each instance. Barley was used, as our main aim was to develop a method for this crop, but the solvent system selected on the basis of these data was also suitable for wheat and cucumber samples. The mixture 2,2,4-trimethylpentane - butan-2-one (1 + 1) was chosen as the solvent because as well as giving good separation of chloraniformethan from co-

extractives, it gave clear, reproducible chromatograms, was easy to use and gave a reasonable development time (about 30 min). Fig. 1 shows the chromatograms obtained with barley, wheat and cucumber samples taken through the standard procedure described above.

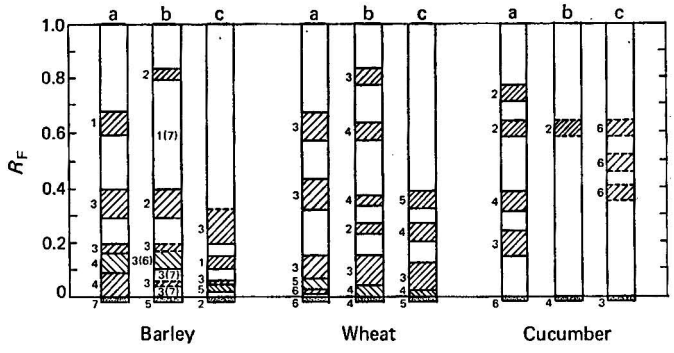

Fig. 1. Thin-layer chromatograms obtained with barley, wheat and cucumber samples taken through the procedure described in the text. R_F values are measured from the front of the 2 mm wide sample streak. Chloraniformethan gives an R_F value of 0.50. Broken lines indicate unsharp edges of bands.

a Bands visible by white light		b Bands visible by absorption at 254 nm		c Bands visible as fluorescence at 366 nm	
1	Very faint yellow	1	Very faint	1	Bright grey
2	Faint yellow	2	Faint	2	Bright blue - grey
3	Light yellow	3	Light	3	Light blue
4	Dark yellow	4	Dark	4	Moderately bright blue
5	Light brown	5	Very dark	5	Bright blue
6	Dark brown	(6)	Yellow coloration	6	Very faint pink
7	Very dark brown	(7)	General background coloration		

The R_F values for some pesticides that may be used under similar circumstances to those which require the use of chloraniformethan and thus represent a possible interference are shown in Table II. Organochlorine pesticides (DDT and BHC) were not included as they are removed by the preliminary wash with hexane. It can be seen from Table II that only

TABLE II

R_F VALUES OF POTENTIALLY INTERFERING PESTICIDES FOR STANDARD THIN-LAYER CHROMATOGRAPHIC CLEAN-UP SYSTEM

Pesticide	R_F value	Pesticide	R_F value
Chloraniformethan	0.50	Dinoseb	0.20
2,3,6-TBA	0.00	Dimethoate	0.21
MCPA	0.00	Triforine	0.34
Mecoprop	0.01	Demeton-S-methyl	0.36
2,4-D	0.01	Formothion	0.51
Oxydemeton-methyl	0.01	Carboxin	0.52
Dichlorprop	0.02	Linuron	0.54
Dicamba	0.02	Fenitrothion	0.60
2,4-DB	0.05	Barban	0.62
Ioxynil	0.07	Thiometon	0.66
Bromoxynil	0.09	Tridemorph	0.73 + 0.65
Ethirimol	0.16	Trithion	0.70

three of the compounds included are likely to be removed from the thin-layer chromatographic plate with chloraniformethan, namely, formothion, carboxin and linuron. These compounds were found to be well separated from chloraniformethan by the gas-chromatographic system: linuron had a much shorter retention time, formothion a far longer retention time and carboxin gave satisfactory separation (about 12 min compared with 8.3 min for chloraniformethan) but in any event the response was more than fifty times less than that for chloraniformethan.

The recoveries obtained for fortified samples of barley, wheat and cucumber are shown in Table III.

TABLE III

RECOVERY OF CHLORANIFORMETHAN FROM FORTIFIED SAMPLES

Sample	Extraction method	Chloraniformethan added/mg kg^{-1}	Chloraniformethan recovered, %
Barley	Soxhlet	5	91, 90, 90
	Macerated	5	75, 70, 72, 92, 87, 110, 82, 88
	Soxhlet	2	85, 85, 92, 91
	—	1	76, 70, 85, 74, 76, 90, 83, 82
	—	0.5	92, 90, 83, 90, 93, 95, 93
Wheat	Soxhlet	5	85, 78, 85, 79
	—	0.5	65, 57, 67, 71
Cucumber	Macerated	5	73, 83, 72, 77
	—	0.5	79, 70
	Soxhlet	0.5	66, 76

The authors thank the Government Chemist for permission to publish this paper.

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The Determination of Chlorhydroxyquinoline in Medicated Pig Feeds

Part II.* Ultraviolet Spectrophotometric Batching Assay and Gas-chromatographic Assay for Mono- and Dichloro Components

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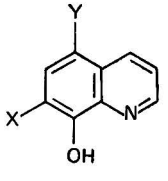
Two procedures have been developed for determining chlorhydroxyquinoline (halquinol) in pig feed medicated at a level of 120 p.p.m. The ultraviolet spectrophotometric procedure can be used to determine halquinol and the gas - liquid chromatographic procedure to determine specifically the 5,7-dichloro- and the combined 5- and 7-chloroquinolin-8-ol components. Halquinol was found to react with copper during the initial extraction with chloroform, thus preventing formation of the silyl ether prior to gas - liquid chromatography. Partitioning the chloroform extract with dilute mineral acid so as to cause dissociation of the chelate and adding EDTA before back-extraction into dichloromethane overcame this problem.

Halquinol (chlorhydroxyquinoline), **I**, a mixture of chlorinated quinolin-8-ols that has antibacterial activity, is added to feeds in order to promote growth and control scours in growing pigs. The mixture contains three structurally related components as shown below.



I
(Halquinol)

X	Y	% *m/m*
Cl	Cl	57–74
H	Cl	23–40
Cl	H	Not more than 3

The spectrofluorimetric determination of halquinol in medicated pig feeds was described in an earlier paper.[1] This procedure was developed specifically for application to feeds containing 600 p.p.m. of halquinol; when it was applied to feeds containing 120 p.p.m. of halquinol, the high fluorescence values for blank feeds led to interferences of the order of 30%. In order to overcome this interference we sought to develop an alternative extraction procedure.

The most convenient way to separate halquinol from co-extracted material proved to be by partitioning the original chloroform extract with dilute mineral acid, thus causing the halquinol to be extracted into the aqueous layer in its protonated form. The latter extract was sufficiently free from interfering substances to permit ultraviolet spectrophotometric assay, whereas measurement of fluorescence required chelation of the halquinol with magnesium, a procedure that could not be carried out directly on the acid extract. Consequently, ultraviolet spectrophotometry, rather than spectrofluorimetry, was adopted as a batching assay procedure.

A method was also required for determining quantitatively the individual components of halquinol. Use has been made of several gas - liquid chromatographic procedures for separating substituted quinolinols in order to evaluate the isomeric purity of synthesised material.[2–5] Silylation of halquinol leads to a separation of the main components at 175 °C on a column of 5% JXR on Gas-Chrom Q. The procedure enabled the monochloroquinolinols to be separated from dichloroquinolinol, but it was not possible to distinguish between the 5- and 7-chloro isomers. As the 7-chloro isomer is present in small amounts (usually 1–3%)

* For details of Part I of this series, see reference list, p. 173.

in halquinol, the determination of the total monochloroquinolinol content was considered to be adequate for our purposes.

Experimental

A common additive to feeds that is intended to promote the growth and fattening of pigs is copper sulphate, which is usually added at a level equivalent to 200 p.p.m. of copper. Other metals such as zinc, calcium and iron are also added as a mineral supplement. These metals readily form chelates with halquinol in solution. Of the metals present in an animal feed, copper is the most strongly chelated and this chelate is predominantly formed during solvent extraction of the feed. It is therefore essential that the solvent used to extract a feed for assay purposes be that in which both halquinol and its copper chelate are appreciably soluble. Only chloroform meets this requirement, but it also extracts various interfering materials from animal feedstuffs; further extraction is required in order to produce a solution that is free from co-extracted feed substances.

Partitioning a chloroform extract of a feed containing halquinol with dilute sulphuric acid caused the halquinol and its copper chelate to be extracted into the aqueous layer as the protonated forms of the chloroquinolinols, leaving the interfering substances in the chloroform layer. Subsequent difficulty due to the formation of emulsions was overcome by evaporating the chloroform and dissolving the residue in hexane before partitioning the organic phase with dilute acid. Unfortunately, it was not possible to extract the feed directly with hexane or with dilute acid because of the insolubility of the halquinol - copper chelate in the former and the slow dissolution of solid halquinol in the latter.

The extraction of samples of blank feed spiked with known levels of halquinol, by adding chloroform and shaking the mixtures at room temperature, resulted in an average 80% recovery of active material. In order to obtain satisfactory recoveries (95% or more), it was necessary to reflux the feed with chloroform for 2 h.

In developing the gas - liquid chromatographic assay, we aimed to make the initial preparation of the sample as simple as possible; in this context we attempted direct extraction of a feed with chloroform. After silylation, aliquots of the solution were injected into the chromatograph, but from the large number of peaks observed it was obvious that some form of clean-up procedure would be required. In addition, some of the halquinol present in the feed was extracted as the copper chelate, in which the O—H bond of the hydroxyl group of the quinolinol is replaced by an O—Cu bond. The chelate would not be expected to react with silylating reagents prior to the chromatography, and an apparent loss of halquinol would result during extraction. In order to overcome these difficulties, we applied the same extraction procedure as that used in the spectrophotometric assay. Protonated halquinol in the aqueous layer was then back-extracted into an organic solvent after adjustment of the pH to 7. EDTA solution was added prior to the pH adjustment so as to prevent any recombination of halquinol with the copper ions.

After evaporation of the solvent on a rotary evaporator, a silylating reagent was added to the solution before gas - liquid chromatography. The discovery that the copper chelate dissociated more rapidly in dilute perchloric acid than in dilute sulphuric acid led to the use of perchloric acid for subsequent extractions during the development of the gas - liquid chromatographic assay. Application of this proposed procedure to a blank feed demonstrated the presence of a small amount of co-extracted material, the retention time of which showed it to be completely resolved from the mono- and dichloroquinolinols, and therefore no interference with the assay was expected.

Several assays of a medicated feed by gas - liquid chromatography gave low and erratic recoveries of halquinol, the poor reproducibility being traced to the rotary evaporation stage of the extraction procedure. Halquinol is slightly volatile at the temperature used for the evaporation of chloroform. The assay results were improved by adding silylating reagent before evaporating the solvent and by substituting a more volatile solvent (dichloromethane) for the original back-extraction solvent (chloroform), so that rotary evaporation could be carried out at a lower temperature.

Although the use of dilute perchloric acid resulted in a more rapid dissociation of the halquinol - copper chelate and was incorporated in the gas - liquid chromatographic assay procedure so as to improve the efficiency of the partitioning, we retained the use of dilute sulphuric acid in the spectrophotometric batching assay mainly for economic reasons.

Spectrophotometric Batching Assay

Reagents

Asbestos fibre. Micro-analytical grade, acid washed.
Halquinol. A sample used to medicate the feed, or a suitable reference standard.
Chloroform. AnalaR grade.
Hexane. Fraction from petroleum (BDH Chemicals Ltd.).
Sulphuric acid, 1.0 M.

Apparatus

All absorbance measurements were made on a Hilger and Watts H700 spectrophotometer.

Extraction of Sample

Weigh accurately about 10 g of the feed into a 250-ml round-bottomed flask, add 100 ml of chloroform and reflux the mixture for 2 h. Filter it immediately through a No. 4 sintered funnel pre-coated with a 0.5-in bed of asbestos fibre. The sinter is pre-coated by adding the asbestos as a chloroform slurry. Use a further 50 ml of chloroform in order to complete the transfer and to wash the feed residue. Evaporate the filtrate to about 1 ml on a rotary evaporator and then gently remove the remaining chloroform with a stream of nitrogen.

Using two consecutive 60-ml volumes of hexane, transfer the residue quantitatively into a 250-ml separating funnel, then extract the halquinol from the hexane phase with five consecutive 95-ml volumes of 1.0 M sulphuric acid. The first two portions of acid should be used to complete the transfer of halquinol from the round-bottomed flask into the separating funnel.

Collect the extracts in a 500-ml calibrated flask and dilute to volume with 1.0 M sulphuric acid. Filter an aliquot of the extract through a Whatman No. 42 filter-paper, discarding the first 25 ml of filtrate.

Preparation of Standard

Weigh accurately about 25 mg of halquinol into a 100-ml calibrated flask. Dissolve the sample in and dilute to volume with 1.0 M sulphuric acid. Pipette 5 ml of this solution into a 500-ml calibrated flask and again dilute to volume with 1.0 M sulphuric acid.

Measurement of Absorbance

Measure the absorbance of the sample and standard solutions against 1.0 M sulphuric acid in 1-cm silica cells at 258 nm. Repeat the absorbance measurements on the samples alone at 290 nm. Subtraction of the sample absorbance at 290 nm from that at 258 nm corrects for the background absorbance of co-extracted substances. The concentration of halquinol in the sample of medicated feed can then be calculated.

Gas-chromatographic Assay for Mono- and Dichloro Components

Reagents and Materials

Asbestos fibre, chloroform and hexane as before.
Carbon disulphide. AnalaR grade.
Dichloromethane. Analytical-reagent grade.
5-*Chloroquinolin*-8-*ol.* A sample of material that was shown to contain no dichloroquinolinol.
5,7-*Dichloroquinolin*-8-*ol.* A sample of material that was shown to contain no monochloroquinolinols.
Perchloric acid, 1.0 M.
Sodium hydroxide solution, 10.0 M.
EDTA solution, 5% *m/V.*
Buffer solution, pH 7. Dissolve 27.2 g of potassium dihydrogen orthophosphate in about 700 ml of distilled water. Adjust the solution to pH 7.0 with sodium hydroxide solution, then dilute to 1 l with distilled water.
Internal standard. Dissolve 25 $\pm$ 1 mg of eicosane (analytical-reagent grade, BDH Chemicals Ltd.) in dichloromethane and dilute the solution to 50 ml with dichloromethane.

Stationary phase. JXR (Applied Science Laboratory Supplies).
Gas-Chrom Q, 100–120 *mesh.*
Column packing. Dissolve 0.5 g of JXR in toluene. Add 10 g of Gas-Chrom Q to the solution and evaporate off the solvent with a rotary evaporator. The final traces of solvent can be removed by drying the packing in an open dish at 60 °C under vacuum.
Silylating reagent [*NO-bis(trimethylsilyl)acetamide, BSA*]. Laboratory-reagent grade (BDH Chemicals Ltd.).

Apparatus

All of the experimental work described was carried out with a Pye, Series 104, gas chromatograph, equipped with a flame-ionisation detector, and single-column operation with injection heater and on-column injection.
Column. A glass column, 5 foot long and of 4 mm i.d., was packed with the prepared column packing of 5% JXR on 100–120-mesh Gas-Chrom Q. Conditioning was then carried out according to the schedule described by Vandenheuvel and Court.[6]
The following operating conditions were used. The measured gas flow-rates were: carrier gas, oxygen-free nitrogen, 80 ml min^{-1}; hydrogen, 80 ml min^{-1}; and air, 600 ml min^{-1}. An oven temperature of 178–180 °C, together with an injection heater temperature of about 200 °C (setting 5), produced satisfactory chromatograms.

Preparation of Standard

Weigh accurately on a microbalance about 4 mg of 5-chloroquinolin-8-ol and 7 mg of 5,7-dichloroquinolin-8-ol into the same 50-ml calibrated flask. Dissolve the samples in chloroform and dilute the solution to volume with chloroform. Pipette 5 ml of this solution into a 100-ml separating funnel containing 50 ml of hexane. Continue as described below under Extraction Procedure.

Preparation of Sample

Weigh accurately about 10 g of feed into a 250-ml round-bottomed flask, add 40 ml of chloroform and reflux the mixture for 2 h. Cool and filter the sample through a No. 4 sintered funnel, pre-coated with a 0.5-in bed of asbestos fibre (the sinter is pre-coated by adding the asbestos as a chloroform slurry). Use a further 50 ml of chloroform to complete the transfer and to wash the feed residue. Evaporate the filtrate to about 1 ml on a rotary evaporator and transfer the residue quantitatively into a 100-ml separating funnel, using four 10-ml portions of hexane. Finally, rinse the round-bottomed flask with two 4-ml portions of chloroform and add these to the separating funnel. Continue as described below under Extraction Procedure.

Extraction Procedure

Extract the halquinol from the hexane phase with four consecutive 15-ml volumes of 1.0 M perchloric acid and collect the extracts in a 250-ml beaker.
Add 10 ml of 5% EDTA solution to the acid in the beaker, then adjust the pH to 7 (with a pH meter) with 10.0 M sodium hydroxide solution. Add 25 ml of pH 7 buffer and transfer the contents of the beaker quantitatively into a 250-ml separating funnel. Wash the beaker with two 10-ml portions of distilled water and two 5-ml portions of dichloromethane, transferring each wash into the 250-ml separating funnel. Shake the funnel well for 2 min, allow the layers to separate, then run the lower layer into a 50-ml calibrated flask. Extract the halquinol with three further 10-ml portions of dichloromethane, collecting the extracts quantitatively in the flask. Add, by pipette, 2 ml of internal standard solution to the flask, then dilute to volume with dichloromethane.
After mixing the flask contents, pipette 25 ml of this solution into a 50-ml pear-shaped flask, add 0.25 ml of silylating reagent and evaporate the solution to dryness on a rotary evaporator at room temperature. Add to the flask a further 0.2 ml of silylating reagent, followed by 1 ml of carbon disulphide. This solution of the silyl derivative is fairly stable but should not be stored overnight.

Gas - Liquid Chromatographic Procedure

Inject 2.5-μl aliquots of sample and standard solutions on to the column and, from the resulting traces, determine the peak height ratio of each component to internal standard for each injection. The contents of 5-chloro- and 5,7-dichloroquinolin-8-ol in the feed can then be calculated.

A typical trace is shown in Fig. 1.

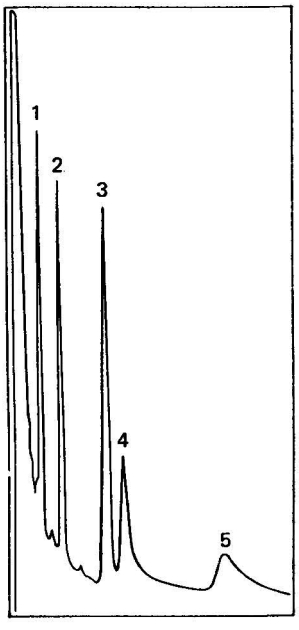


Fig. 1. Typical gas - liquid chromatographic trace of halquinol in feed extract: 1, 5-chloroquinolin-8-ol (R_t = 2 min); 2, 5,7-dichloroquinolin-8-ol (R_t = 3.5 min); 3, internal standard (eicosane, R_t = 7 min); and 4 and 5, co-extracted matter from feed (R_t = 8.5 and 17 min).

Discussion and Results

Correction for Background Absorbance

The ultraviolet spectrophotometric batching assay described above involves absorbance measurements at two wavelengths, namely 258 and 290 nm. This procedure corrects for the interference of co-extracted material, which is typically of the order of 10%. By this means, assay of a blank feed is not required. The ultraviolet absorbance spectrum of a blank feed shows the absorbance to be constant between 253 and 300 nm. As the absorbance of the standard solution is virtually zero at 300 nm, an absorbance measurement at this wavelength allows a correction to be made for the contribution by the blank in an extracted sample.

Obviously this procedure applies only to the particular feeds used in the development of the assay. It is possible that other manufacturers' feeds may not yield extracts that have the same characteristics.

Effect of Silylation

Although the gas - liquid chromatographic procedure described does not enable the in-

dividual monochloro components of halquinol to be assayed, the assay of the total monochloroquinolinol content was considered to be adequate, for reasons explained earlier. All three components of halquinol can be separated with a column of 10% OV-17 on Gas-Chrom Q at a temperature of 200 °C, but only if halquinol is chromatographed without prior silylation.[7] Formation of the silyl ether prior to chromatography results in the two monochloro derivatives being eluted at the same time. However, we considered it to be essential to silylate the halquinol at the expense of separating the two monochloro isomers, for reasons that concern the chelating properties of quinolinols. The presence of any metal surface, for example, a syringe needle or parts of the flame-ionisation detector, especially at elevated temperatures, would result in a loss of chlorhydroxyquinoline by chelate formation with the metal surface, thereby introducing further sources of error into the method.

In addition, evidence was obtained indicating that unsilylated halquinol is not completely eluted from the column during chromatography. Injection of solvent alone results in the production of small peaks that have the same retention times as the halquinol components, a phenomenon that does not occur when the silyl ether of halquinol is chromatographed.

Applicability

Attempts to apply the assay procedures to feeds containing 600 p.p.m. of halquinol were unsuccessful because a halquinol - copper chelate precipitated during extraction, as had been expected from earlier work on the solubility of the chelate. Although reduction of the amount of medicated feed used in the assay averted the precipitation of a chelate, it resulted in problems associated with the homogeneity of the feed. No limitations are anticipated with feeds that contain less than 120 p.p.m. of halquinol. The assay procedures are therefore recommended for use only when the level of active substance is not greater than 120 p.p.m.

A number of other common medicinal feed additives that may possibly be present have been examined for their effect on the assay procedures described. Nitrovin, arsanilic acid and Eskalin (virginiamycin) at levels of 10, 250 and 10 p.p.m., respectively, do not interfere. Dimetridazole, at a level of 200 p.p.m. does cause interference and should be absent.

Validity

The validity of the above procedures was ascertained by preparation of samples of feed spiked at a level of 120 g of halquinol per ton of feed. Spiking was accomplished by adding aliquots of a solution of halquinol in chloroform to 10 g of unmedicated feed (Messrs. Tyrell, Byford & Pallet Ltd., Norfolk), the chloroform being subsequently removed by rotary evaporation. Halquinol was added in this manner to achieve maximum adsorption of active substance by the feed. Table I shows the recovery of halquinol from the feed by use of the procedures described above. In all instances, the recovery values were considered to be acceptable for an assay of animal feed containing a very low level of active substance.

TABLE I

RECOVERY OF HALQUINOL FROM SPIKED FEEDS (120 g ton^{-1})

	Halquinol			5-Chloro component			5,7-Dichloro component		
	Added, p.p.m.	Recovered, p.p.m.	Recovery, %	Added, p.p.m.	Recovered, p.p.m.	Recovery, %	Added, p.p.m.	Recovered, p.p.m.	Recovery, %
Ultraviolet spectrophotometric assay	126	118	94						
	125	118	94						
	123	114	93						
	124	117	94						
	126	118	94						
	110	105	95						
	110	105	95						
	110	107	97						
	110	107	97						
	102	96	94						
	102	99	97						
Gas - liquid chromatographic assay				42	41	98	78	73	94
				42	42	100	78	72	92
				43	45	105	80	77	96
				43	46	107	80	75	94
				43	45	105	80	73	91
				43	46	108	80	76	95

Application of the procedures to manufactured batches of medicated feeds stored for periods as long as 6 months at temperatures between ambient and 40 °C showed good agreement between the results of the ultraviolet spectrophotometric assay and gas - liquid chromatographic determination of the individual components. In Table II the results obtained with four batches of medicated feeds are compared, the two procedures being carried out independently on separate extracts.

TABLE II

COMPARISON OF ULTRAVIOLET SPECTROPHOTOMETRIC AND GAS - LIQUID CHROMATOGRAPHIC ASSAY RESULTS

		Gas - liquid chromatographic assay		
Sample	Ultraviolet spectrophotometric assay total, p.p.m.	Monochloro components, p.p.m.	Dichloro components, p.p.m.	Total, p.p.m.
1	110	30	85	115
2	105	29	78	107
3	112	39	77	116
4	94	34	58	92

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NOTE—Reference 1 is to Part I of this series.

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A Simple Method for Monitoring Excessive Levels of Lead in Whole Blood Using Atomic-absorption Spectrophotometry and a Rapid, Direct Nebulisation Technique

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A method for monitoring excessive levels of lead in whole blood, using a rapid, direct nebulisation, atomic-absorption technique is described. The blood sample is diluted with an equal volume of 0.2% *m/V* Triton X-100 solution and immersed in an ultrasonic bath for 10 min. A 200-μl aliquot of the resulting solution is then nebulised and the peak height of the transient output pulse is monitored. Some preliminary results are also reported for cadmium and selenium in blood using the single-pulse nebulisation atomic-fluorescence technique.

The detection of excessive cadmium or lead levels in whole blood has been attempted by various atomic-absorption techniques.[1–8] However, very few methods[9,10] have made use of direct nebulisation into a flame because of problems associated with blockage of the nebuliser and the burner slot. Single-pulse nebulisation techniques,[11–14] in which discrete samples (typically 50–250 μl in volume) are nebulised, have been successfully used for the analysis of solutions with a high content of dissolved solids. A typical example of this technique is the analysis of 5–10% *m/V* steel solutions.[11,14] It has been found possible to monitor excessive levels of lead in whole blood rapidly by using a simple, single-pulse nebulisation absorption technique with a conventional air - hydrogen flame. Some preliminary studies are also reported for cadmium and selenium using the single-pulse nebulisation atomic-fluorescence technique.

Experimental

A Shandon Southern Instruments A3400 atomic-absorption - atomic-fluorescence spectrophotometer and Autograph pen recorder were used. A conventional lead hollow-cathode lamp source was used for the absorption measurements and electronically modulated cadmium and selenium microwave discharge lamp sources[15,16] were used for the atomic fluorescence measurements. A wide-bore nebuliser with a 0.70 mm bore capillary was constructed by drilling out the end orifice of the standard A3400 nebuliser with a 1.32 mm diameter drill; the internal constriction of the nebuliser body was drilled out using a 1.04 mm diameter drill and a 0.70 mm bore capillary (19 s.w.g.) was then inserted. The natural uptake rate for water at an air pressure of 250 kN m^{-2} (36 p.s.i.g.) was 9 ml min^{-1}. This nebuliser showed no sign of suffering a blockage with the 1 + 1 diluted ultrasonically treated blood. A wide slot (120 × 0.76 mm) A3424 air - acetylene/air - hydrogen burner was used for making the absorption measurements. Blockage problems were rarely encountered with this burner if fresh blood samples with no sign of clotting were used. When a partial blockage did occur the height of the peaks decreased, the widths increased and markedly asymmetrical peak shapes were observed. For the fluorescence measurements a nitrogen-shielded air - acetylene/air - hydrogen burner with 10 holes (2.0 mm in diameter) in a rectangular (20 × 10 mm) pattern was used. This burner did exhibit some problems with slight blockage after 50–60 blood samples had been run, but could be easily removed for cleaning. A drill (1.98 mm in diameter) was used to remove any residue in the burner grid holes. The burner was then immersed in a hot detergent solution (5% *V/V* Decon 90) for 5 min and finally thoroughly washed out with distilled water.

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Optimisation of Operating Conditions

Treatment of the blood sample

A 1-ml sample of blood was placed in a small test-tube, diluted with 1 ml of 0.2% *m/V* Triton X-100 and placed in an ultrasonic bath (MEL Ltd., 2-l bath, operating at 70 W and 22 kHz) for 10 min. During this period cold water was passed through the bath in order to prevent undue heating of the diluted sample. The 1 + 1 diluted, ultrasonically treated blood solution is more stable with respect to clot formation than the original blood and can be stored at 4 °C in a refrigerator for up to 1 week. A 1-ml sample of blood was found to be sufficient for nine discrete measurements. The ultrasonic treatment resulted in efficient haemolysis of the red cells. The diluted blood samples were always shaken prior to nebulisation.

Blank solution

The blank solution contained 0.1% *m/V* Triton X-100 and 0.01% *V/V* silicone anti-foaming agent (BDH Chemicals Ltd.) and was nebulised continuously between the pulse nebulisations of the diluted blood samples. Without anti-foaming agent excessive foaming occurred in the drain tube and this hindered drainage of the spray chamber.

Pulse nebulisation technique

The optimum pulse nebulisation volume with respect to the signal to noise ratio and freedom from burner slot blockage was 200 μl. Initially, the nebuliser uptake tube was transferred from the blank solution and immediately placed in a 200-μl aliquot of the 1+1 diluted blood sample; when the sample has been nebulised (which required 1.3 s) the uptake tube was immediately transferred back to the blank solution. It was essential to effect these transfers as rapidly as possible.

A much better procedure, which was used for all of the work reported in this study, was to insert the end of a shortened MLA No. 9025 polypropylene disposable pipette tip into the end of the silicone rubber nebuliser uptake tube (Fig. 1). The total volume of the cut-down pipette tip was approximately 200 μl. By inserting the (wide) end of this tip below the surface of the sample and removing it immediately the sample reached the start of the silicone rubber uptake tube, it was possible to nebulise discrete (approximately 200 μl) aliquots directly from the 1+1 diluted blood solution. After the tip had been removed from the sample it was replaced in the blank solution after a delay of approximately 1–2 s. The delay prevented the blood from draining back into the blank solution. This pipetting technique

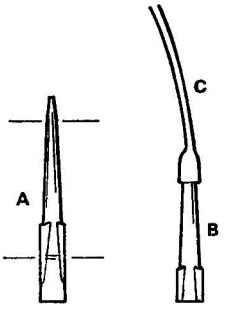

Fig. 1. Pulse nebulisation uptake device. A, Standard MLA No. 9025 polypropylene disposable pipette tip; B, as A but modified for pulse nebulisation technique; C, silicone rubber nebuliser uptake tube. The horizontal lines on A represent the points at which the pipette is cut.

considerably simplified the analysis and resulted in a considerable saving in pipette tips and polystyrene beakers. The precision of the nebulisation technique was not found to be a limiting factor.

Wavelengths, spectral band passes and damping

Table I lists the wavelengths, spectral band passes and degree of damping that were used in this work.

TABLE I

OPERATING PARAMETERS

In order to attain the optimum signal to noise ratio, the A3400 spectrophotometer was operated at a time constant of 2 s.

Element	Technique*	Wavelength/nm	Spectral band pass/nm
Lead	AA	217.0	0.6
Cadmium	AA	228.8	0.6
Cadmium	AF	228.8	3.0
Selenium	AF	196.1	3.0

* AA = atomic absorption; AF = atomic fluorescence.

Choice of flame

Absorption measurements. The air - acetylene flame was used for the initial measurements. The flow-rate of acetylene was set so that minimum alteration in the base-line level occurred when the uptake tube to the nebuliser was removed from the blank solution (air then being passed through the nebuliser). This setting almost corresponded to the maximum transparency of the flame at 217.0 nm and was very critical. The air - hydrogen flame, which was used for all of the work reported, exhibited much less flame gas absorption at 217.0 nm than the air - acetylene flame. With the optimum flame conditions, the change in the base-line level on removal of the uptake tube from the blank solution was very small and was not very dependent on the hydrogen flow-rate. The air and hydrogen flow meters were set to readings of 6.0 and 5.8, respectively. If an aliquot of the blank solution was substituted for a blood sample (see *Pulse nebulisation technique*) the change in the base-line level observed was below the detection limit for lead. The burner height was set 2–3 mm lower than the optimum position for aqueous lead standards.

Fluorescence measurements. A nitrogen-shielded air - hydrogen flame was used. This flame exhibited a background emission at 228.8 nm that was seven times lower than that of the nitrogen-shielded air - acetylene flame. The air and hydrogen flow meters were set to readings of 6.0 and 5.2, respectively, and the flow-rate of the nitrogen shield gas was 5 l min^{-1}. The burner height adjustment was set to the lowest position.

Results

Absorption Measurements

Measurements were carried out with the sensitivity set to a scale expansion of 40 times (f.s.d. $\equiv$ 0.025 absorbance unit). The 2σ base-line noise, expressed as the concentration of lead in whole blood, was 5 μg per 100 ml of blood. The average background absorption (for 10 blood samples) as measured at the 220.4-nm lead non-resonance line was equivalent to 22 μg of lead per 100 ml of blood. The standard deviation was 1.8 μg per 100 ml and the calibration graph was linear up to 200 μg per 100 ml; higher levels were not studied. Calibration was carried out by use of the method of standard additions. Table II shows a comparison of some results obtained by using the pulse nebulisation technique with those obtained using the punched disc, electrothermal atomisation technique. It can be seen that the pulse nebulisation method appears to be satisfactory as a method for rapidly monitoring excessive levels of lead in blood. It is not as precise or sensitive as the various flameless methods. Fig. 2 shows a typical trace for lead. The relative standard deviation (10 measurements) for a blood sample containing 65 μg of lead per 100 ml was 4.9%.

If background correction measurements were made at 217.0 nm using a deuterium hollow-cathode lamp, the background absorption was 30% higher than that at 220.4 nm. This

TABLE II

COMPARISON OF RESULTS FOR LEAD IN BLOOD

Lead concentration in blood/μg per 100 ml		Lead concentration in blood/μg per 100 ml	
Punched disc electrothermal atomisation method	Pulse nebulisation* method	Punched disc electrothermal atomisation method	Pulse nebulisation* method
10	9	29	34
12	10	41	36
16	15	52	44
17	17	66	61
25	20	81	75

* Average of three single-pulse nebulisations.

difference was attributed to absorption by iron at its 216.5-nm absorption line (normal blood contains about 500 μg ml^{-1} of iron), which was substantiated by the fact that if a 1000 μg ml^{-1} solution of iron was pulse nebulised into the flame the background absorption at 217.0 nm was 50% greater than at 215.0 or 220.4 nm. Also, if the wavelength was accurately set to 217.0 nm and an iron lamp brought on to the optical axis, the pulse nebulisation of 1000 μg ml^{-1} iron solution gave appreciable absorption peaks (about 0.3 absorbance unit). Over-correction for background absorption when using a deuterium lamp has previously been observed in the determination of low levels of aluminium and tin in steels.[14]

The atomic-absorption detection limit for cadmium using the air - acetylene or air - hydrogen flame was about 1 μg of cadmium per 100 ml of blood. This was not considered adequate for screening excessive cadmium levels. The flame conditions were identical with those used for the lead determination.

Fluorescence Measurements

The use of atomic fluorescence in conjunction with the pulse nebulisation technique resulted in a considerable improvement in the sensitivity to cadmium (Fig. 3). With the nitrogen-shielded air - hydrogen flame the 2σ base-line noise level, expressed as a concentration of cadmium in whole blood, was 0.15 μg per 100 ml. The scatter contribution from the matrix to the observed signal is difficult to determine for cadmium. However, the pulse nebulisation

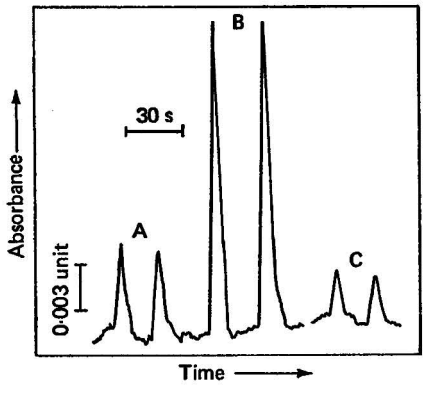

Fig. 2. Typical lead atomic-absorption trace. A, Blood sample measured at 217.0 nm (nominal lead level 20 μg per 100 ml); B, as A but with added lead, equivalent to an addition of 100 μg per 100 ml of blood (referred to the whole blood sample); C, background absorption from sample matrix measured at the 220.4 nm lead non-resonance line.

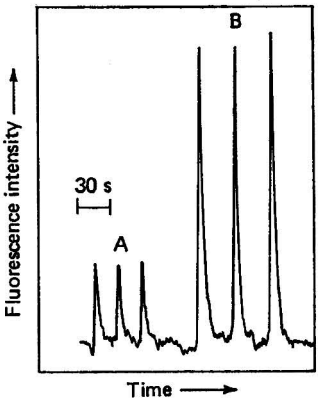

Fig. 3. Typical cadmium atomic-fluorescence trace. A, Blood sample; B, as A but with added cadmium, equivalent to an addition of 2 μg per 100 ml of blood (referred to the whole blood sample).

of a solution containing 1 650 μg ml^{-1} of sodium and 250 μg ml^{-1} of iron(III) gave a signal equivalent to about 0.15 μg of cadmium per 100 ml of blood (this could even have been due to cadmium impurities in the reagents).

The possibility of determining selenium in a similar manner was investigated. The scatter contribution from the matrix to the observed signal was roughly estimated by comparing the fluorescence and incident light intensities at 196.1 and 207.5 nm and was found to be of the order of 10 μg of selenium per 100 ml of blood. The 2σ base-line noise level was 5 μg per 100 ml.

Conclusions

The single pulse nebulisation of 1+1 diluted blood samples for monitoring excessive levels of lead by use of atomic-absorption spectrophotometry would appear to be a feasible method. It should be stressed that the atomic-absorption instrument must be operated at the maximum usable sensitivity (0.025 absorbance unit $\equiv$ f.s.d.) and that all of the various operating parameters (*e.g.*, burner position and height, impact ball position, hydrogen flow-rate) must be carefully optimised in order to obtain adequate sensitivity. The determination of excessive levels of cadmium in blood by use of atomic-fluorescence spectrophotometry would also appear to be feasible.

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Solvent Mediator Studies on Barium Ion-selective Electrodes Based on a Sensor of the Tetraphenylborate Salt of the Barium Complex of a Nonylphenoxypoly(ethyleneoxy)ethanol*

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The barium ion-selective electrode sensor based on the neutral carrier complex of a nonylphenoxypoly(ethyleneoxy)ethanol containing 12 ethylene oxide units and 2 mol of tetraphenylborate ion per mole of Ba^{2+} (12 EOU.Ba.2TPB) requires a more viscous solvent mediator than 4-nitroethylbenzene for long-life poly(vinyl chloride) (PVC) matrix-membrane barium ion-selective electrodes. Both 2-nitrophenyl octyl ether and di-2-nitrophenyl ether used in conjunction with the sensor in a PVC matrix membrane give functional barium ion-selective electrodes, but those with di-2-nitrophenyl ether mediator are far superior with lifetimes of about 30 d. Barium ion-selective electrodes with the sensor and mediator in liquid membranes can be made for a wider range of nitro-aromatic solvent mediators.

Barium ion-selective electrodes made from discs taken from a master membrane containing 0.40 g of a saturated solution of Antarox CO-880.Ba.-2TPB in di-2-nitrophenyl ether can be used as indicator electrodes for the potentiometric titration of SO_4^{2-} with Ba^{2+}. A role for a potentiometric titration finish in the determination of sulphur in organic compounds by the oxygen-flask method is recommended.

Liquid membranes based on tetraphenylborate (TPB) salts of barium complexes of poly-(ethylene glycol) derivatives dissolved in 4-nitroethylbenzene give ion-selective electrodes with high selectivity for barium over calcium, magnesium and other cations.[1] Thus, an electrode with an ion exchanger made from a TPB salt of the barium complex of a nonylphenoxypoly(ethyleneoxy)ethanol (Igepal CO-880) in 4-nitroethylbenzene gives a fast, stable and reproducible response at barium chloride concentrations from 10^{-1} to 10^{-5} M with a long-term drift of only 1–2 mV d^{-1}.[1] In view of the promising nature of this electrode, the present study was undertaken in order to determine the effect on the selective ion-sensing properties of different solvent mediators in the presence of the barium complex of a nonylphenoxypoly(ethyleneoxy)ethanol (NP) in liquid-membrane and in poly(vinyl chloride) (PVC) matrices. The possible extension to the PVC matrix system is justified by the long lifetimes of valinomycin - PVC sensing matrices[2] and the known compatibility of PVC with nitroaromatic solvent mediators.[3,4]

Experimental

Chemicals

The chemicals used were of analytical-reagent grade, except for gifts of NPs (Antarox CO non-ionic surfactants) from GAF (Great Britain) Ltd., Manchester, and of 2-nitrophenyl octyl ether from Professor W. Simon, Zürich, Switzerland. Additional 2-nitrophenyl octyl ether was synthesised.[5]

Neutral Carrier Complexes

Barium - NP complex solutions were prepared[1] by adding barium chloride solution to an aqueous solution of a nonylphenoxypoly(ethyleneoxy)ethanol [$C_9H_{19}.C_6H_4.O(CH_2CH_2O)_{29}.-C_2H_5OH$] known as Antarox CO-880. The oxonium ion formed was precipitated with excess of aqueous sodium TPB and the filtered precipitate washed well with water and vacuum dried at 35 °C.

*Presented at the International Reference and Ion-selective Electrode Conference held at the University of Newcastle upon Tyne, January 7–9th, 1976.

The "ion exchangers" used in the electrode membranes were saturated solutions of the precipitated complex in solvent mediator.

Physical Constants of Solvent Mediators

Boiling-points were determined at atmospheric pressure and dynamic viscosities at 25 °C. Relative permittivities were also measured at 25 °C using a Calvert dielectric bridge.

Electrodes

Liquid-membrane electrodes were based on an Orion Research Inc. liquid-membrane electrode (type 92–20) assembled in the normal way.[6] The outer chamber contained the "ion exchanger" and the internal reference chamber a 0.1 M solution of barium chloride saturated with silver chloride.

PVC matrix-membrane electrodes were assembled according to standard practice[3,4,6,7] with master membranes prepared by adding 0.40 g of "ion exchanger" to a solution of 0.17 g of PVC in 6 cm^3 of tetrahydrofuran followed by controlled evaporation of the tetrahydrofuran solvent.

Electrode Evaluation

Electrodes were evaluated by studying their behaviour in various test solutions using a Corning (No. 476 109) ceramic plug-type calomel reference electrode containing 4 M potassium chloride solution. The e.m.f.s of such cells were recorded with a Beckman research pH meter or a Radiometer (PHM 64) digital pH meter to 0.1 mV in conjunction with a Servoscribe, Model RE 4541, potentiometric recorder for measuring response times. E.m.f. measurements were performed in stirred solutions at 25 ± 0.1 °C. The ceramic plug-type calomel reference electrode used had a minimum leakage rate corresponding to just 4×10^{-5} mol h^{-1} of potassium chloride. Remembering that the volumes of the test solutions were between 10 and 20 cm^3, the barium ion-selective electrode would be subjected to less than 10^{-6} mol of potassium chloride, even after 1 h, an amount that is several orders of magnitude below that which has the slightest effect on the performance of the barium ion-selective electrode.

Results and Discussion

The nonylphenoxypoly(ethyleneoxy)ethanol (Antarox CO-880) that formed the basis of this investigation corresponds to the Igepal CO-880 used by Levins.[1] This material has 30

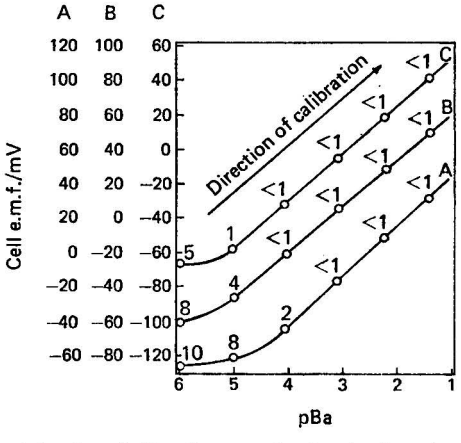

Fig. 1. Calibration graphs for barium ion-selective electrodes with Antarox CO-880.Ba.-2TPB sensor at 25 °C. A, liquid membrane with sensor in 4-nitroethylbenzene; B, PVC membrane containing sensor and 4-nitroethylbenzene; and C, PVC membrane containing sensor and di-2-nitrophenyl ether.

ethylene oxide units (EOU) per mole and it has been established[8] that such materials with 10–40 EOU form complex precipitates with 12 EOU and 2 mol of TPB^- per mole of Ba^{2+} (12 EOU.Ba.2TPB). Elemental analyses of Antarox CO-880 gave C 59.5, H 9.8 and O (difference) 30.7% [required for $C_9H_{19}.C_6H_4.O(CH_2CH_2O)_{29}.C_2H_5OH$: C 58.5, H 9.4 and O 32.2%] and of the complex with barium, C 68.3, H 7.3 and Ba 9.3% {required for $[C_9H_{19}.C_6H_4.O(CH_2CH_2O)_{29}.C_2H_5OH.Ba_{2.5}][(C_6H_5)_4B]_5$: C 67.3, H 7.2 and Ba 9.9%}.

Preliminary Investigation

The preliminary investigation was aimed at confirming the response of the Antarox CO-880.Ba.2TPB precipitate in ion-selective electrode membranes and, as can be seen from Fig. 1, the material used in conjunction with 4-nitroethylbenzene mediator in the liquid membrane (curve A) and in the PVC matrix membrane (curve B) gives electrodes with the type of long, near-Nernstian response range described by Levins[1] for liquid-membrane electrodes based on Igepal CO-880.Ba.2TPB sensor with 4-nitroethylbenzene mediator. Table I summarises the specifications of the electrodes, together with those of Levins' electrode.

The main difference between the two liquid-membrane electrodes concerns selectivity, Levins' electrode being marginally superior, but the different supports for the liquid mem-

TABLE I

SPECIFICATIONS OF VARIOUS BARIUM ION-SELECTIVE ELECTRODES WITH MEMBRANES CONTAINING A BARIUM COMPLEX OF NONYLPHENOXYPOLY(ETHYLENEOXY)ETHANOL IN 4-NITROETHYLBENZENE

	Electrode		
Property	Liquid membrane with Igepal CO-880.Ba.2TPB sensor[1,9]	Liquid membrane with Antarox CO-880.Ba.2TPB sensor (this work)	PVC matrix membrane with Antarox CO-880.Ba.-2TPB sensor (this work)
Effective linear calibration range/M	$>10^{-1}$–10^{-5}	10^{-1}–5×10^{-5}	10^{-1}–10^{-5} at first, but soon becomes 10^{-1}–10^{-4}
Slope at 25 °C/mV per decade	26.6	27, but increases for example to 30 by about day 25	26.5–29.5, depending on electrode
Static response time (dilute concentrated solutions)/s	10–180	<60 for 10^{-1}–10^{-3} M but 120–480 for 10^{-4}–10^{-5} M	<60 for 10^{-1}–10^{-4} M but 240–900 for 10^{-5} M
Reproducibility/mV	±0.2	±0.5	±0.5
Long-term drift/mV d^{-1}	1–2	2–3	5–6
Static response at 10^{-6} M Ba^{2+}	Slow, erratic	Slow (~10–30 min)	Slow (~15 min)
Operational lifetime/d	Not given	>30	~2. End of useful life characterised by drop in slope and lengthening response time
Selectivity coefficients (separate solution method):			
$K_{Ba\ H}$	2×10^{-4}	—	Electrode deteriorates too quickly for selectivity determinations to be made
$K_{Ba\ Li}$	2×10^{-4}	—	
$K_{Ba\ Na}$	2×10^{-4}	2.3×10^{-4}	
$K_{Ba\ K}$	8×10^{-3}	8.0×10^{-3}	
$K_{Ba\ NH_4}$	6×10^{-4}	—	
$K_{Ba\ Mg}$	$<1 \times 10^{-4}$	1.4×10^{-3}	
$K_{Ba\ Ca}$	$<1 \times 10^{-4}$	3.3×10^{-4}	
$K_{Ba\ Sr}$	2×10^{-3}	2×10^{-3}	
$K_{Ba\ Fe^{2+}}$	$<1 \times 10^{-4}$	—	
$K_{Ba\ Co}$	$<1 \times 10^{-4}$	—	
$K_{Ba\ Ni}$	$<1 \times 10^{-4}$	3.3×10^{-4}	
$K_{Ba\ Cu}$	Slow poisoning	—	

branes may be responsible for this effect. Also, the selectivity data (reference 9 and Table I) were determined by the separate solution method and do not typify the conditions of the more analytically analogous mixed solution systems.[6,10,11] Nevertheless, the two liquid-membrane electrodes were sufficiently similar for Antarox CO-880.Ba.2TPB sensor with 4-nitroethylbenzene mediator to form a reference comparison ion-selective electrode system in the present investigation.

Attempts to develop an ion-selective electrode with the sensor - 4-nitroethylbenzene mediator "liquid ion exchanger" trapped in a PVC matrix membrane were disappointing in that the electrode, although its response characteristics were initially promising, quickly deteriorated (Table I). This breakdown of the electrode is connected with loss of 4-nitroethylbenzene mediator from the membrane surface, for after momentarily dipping the electrode membrane in fresh 4-nitroethylbenzene solvent, good electrode behaviour, as depicted in Fig. 1 (curve B), was immediately, but unfortunately only temporarily, restored. The regeneration - breakdown cycle can be repeated many times. This effect suggests that simple leaching of the mediator occurs, but there must be a more subtle reason as electrodes assembled from the same membranes that had previously been soaked for up to 4 d in either water or 0.1 M barium chloride solution behaved in the manner depicted by curve B (Fig. 1), even on first calibration, followed by characteristic breakdown. Such experiences with the PVC matrix membranes gave the incentive for this study in order to determine a PVC - sensor - mediator system with the advantages of the previously described PVC matrix-membrane ion-selective electrodes.[3,4,7,12]

Compatibility of Antarox CO-880.Ba.2TPB and Solvent Mediator with Each Other and with PVC

Attempts to make PVC matrix-membrane electrodes with the plasticising solvent mediators, dioctyl phenylphosphonate and dinonyl phthalate, used with dialkyl phosphate sensors[12] were unsuccessful because of the insolubility of the Antarox CO-880.Ba.2TPB precipitate. Efforts were therefore concentrated on nitroaromatic solvents and it can be seen from Table II that although all of the solvent mediators had moderate permittivities, functional electrodes

TABLE II

BARIUM ION-SELECTIVE ELECTRODE CHARACTERISTICS OF SOLVENT MEDIATORS USED IN MEMBRANES OF PVC CONTAINING ANTAROX CO-880.Ba.2TPB SENSOR

Solvent mediator	Boiling-point of solvent at atmospheric pressure/°C	Dynamic viscosity of solvent at 25 °C/mN s m^{-2}	Relative permittivity of solvent at 25 °C	Electrode slope to Ba^{2+}/mV	Linear calibration range/M	Lifetime/d
4-Nitroethylbenzene	245	1.99	24.1	26.5–29.5, depending on electrode	10^{-1}–10^{-5} at first	<2; decrease in slope and lengthening response time
2-Nitrotoluene	221	1.93	26.6	29	10^{-1}–10^{-4}	1; rapid decrease in slope
3-Nitrotoluene	231	1.89	26.4	29	10^{-1}–10^{-4}	1; rapid decrease in slope
Nitrobenzene	211	1.68	34.9	—	—	Responds for only 1 or 2 solutions
2-Nitro-4-isopropyltoluene	259	3.24	17.7	27	10^{-1}–10^{-5} at first	<2; decrease in slope by day 2
2-Nitro-3-methyltoluene	224	2.37	16.5	27	10^{-1}–10^{-4} at first	<2; decrease in slope by day 2
3-Nitro-2-methyltoluene	240	3.19	20.7	26	10^{-1}–10^{-5} at first	2; decrease in slope by day 2
2-Nitrophenylbutyrate	Chars at 252	10.1	19.0	27	10^{-1}–10^{-5}	5 for fresh membrane; mediator appears to decompose in membranes, for replicate electrodes from master membrane lead to progressively decreasing lifetimes according to the age of the membrane
2-Nitrophenyl octyl ether	Chars at 290	12.8	23.5	21–27	10^{-1}–10^{-4}	15–21; erratic slopes, vary between calibrations within 21–27-mV range
Di-2-nitrophenyl ether	Chars at 280	16.1	28.3	28	10^{-1}–9 × 10^{-6}	~30; steady slope and fast response. Best of the membrane systems studied
Dioctyl phenyl-phosphonate	Chars at 200	16.3	6.2	—	—	Antarox CO-880.Ba.2TPB insoluble in solvent

with serviceable lives of more than 1 or 2 d are associated with mediators of high boiling-point and high viscosity. Although these specifications were met by 2-nitrophenyl butyrate, 2-nitrophenyl octyl ether and di-2-nitrophenyl ether, the last compound gave electrodes of the highest quality.

In addition to the breakdown - regeneration character noted above for PVC matrix-membrane electrodes based on 4-nitroethylbenzene with Antarox CO-880.Ba.2TPB sensor, master membranes containing solvent mediators of low viscosity shrink during storage. This shrinkage is clearly detrimental to the utility of such solvent mediators in PVC matrix-membrane electrodes, although as shown in Table III acceptable liquid-membrane electrodes are obtainable in many instances.

TABLE III

SPECIFICATIONS OF BARIUM ION-SELECTIVE ELECTRODES WITH LIQUID ION-EXCHANGER MEMBRANES COMPOSED OF ANTAROX CO-880.Ba.2TPB SENSOR AND NITROAROMATIC SOLVENT MEDIATOR

	Solvent mediator						
Property	4-Nitroethyl-benzene	2-Nitro-toluene	2-Nitro-3-methyl-toluene	2-Nitro-4-isopropyl-toluene*	2-Nitrophenyl butyrate	2-Nitrophenyl octyl ether*	Di-2-nitro-phenyl ether
Effective linear calibration range/M	10^{-1}–5×10^{-5}	10^{-1}–10^{-4}	10^{-1}–10^{-4}	10^{-1}–10^{-4}	10^{-1}–10^{-4}	10^{-1}–~10^{-4}, depending on fabrication batch	10^{-1}–10^{-4}
Slope at 25 °C/mV per decade	27, but increases to 30 by day 30	29 for >20 d	29, but increases to 32 by day 20	27–29	28, but increases to 31 by day 14	~22, depending on fabrication batch	28, but increases to 31 by day 30
Response time/min (values near detection limit in parentheses)	<1 (2–8)	<1 (>10)	<1 (>10)	2–6 (10)	<1 (>10)	2–8	<1 (~10)
Reproducibility/mV	±0.5	±0.5	±0.5	±0.5	±0.5	±1	±0.5
Long-term drift/mV d^{-1}	2–3	2–3	2–3	2–4	~2	Erratic response	~1
Operational lifetime/d	>30	>20	>20	>20	>14	~15	>30
Selectivity coefficient (mixed solution method at interferent level of 10^{-1} M):							
$K_{Ba\,Na}$	2.3×10^{-3}	6.0×10^{-3}	2.0×10^{-3}	1.1×10^{-1}	1.6×10^{-3}	—	7.7×10^{-3}
$K_{Ba\,K}$	2.0×10^{-1}	3.0×10^{-1}	6.5×10^{-1}	1.0	4.8×10^{-1}	—	2.0×10^{-2}
$K_{Ba\,Mg}$	8.0×10^{-4}	9.0×10^{-4}	5.3×10^{-4}	9.0×10^{-4}	6.6×10^{-4}	—	2.4×10^{-4}
$K_{Ba\,Ca}$	8.0×10^{-4}	1.8×10^{-3}	1.6×10^{-3}	1.2×10^{-3}	1.2×10^{-3}	—	6.0×10^{-4}
$K_{Ba\,Sr}$	6.5×10^{-3}	8.3×10^{-3}	9.0×10^{-3}	1.0×10^{-2}	9.4×10^{-3}	—	4.4×10^{-3}

* Antarox CO-880.Ba.2TPB is poorly soluble in these solvents, especially 2-nitrophenyl octyl ether.

Variations in the relative permittivities of the mediators do not appear to lead to conclusive differences in electrode selectivity, but low solubility of the sensor in the solvent mediator can lead to poor response, even for liquid-membrane electrodes with sensor plus 2-nitrophenyl octyl ether membranes, and worthwhile selectivity assessments could not be made (Table III). In this respect, it is interesting that a liquid-membrane electrode with a membrane of 2-nitrophenyl octyl ether alone gives a linear response with a reproducible slope of 27–28 mV per decade at concentrations of barium ions between 10^{-1} and 10^{-4} M, although the reproducibility between calibrations is poor (±6 mV). On the other hand, an electrode assembled with a liquid membrane of di-2-nitrophenyl ether alone is very erratic, but when the membrane contains a saturated solution of Antarox CO-880.Ba.2TPB sensor in the di-2-nitrophenyl ether solvent mediator, barium ion-selective electrodes of both the liquid-membrane and PVC matrix-membrane varieties of superior quality are readily obtained (Tables II, III and IV).

A final comment on the liquid-membrane electrodes concerns the effect of different batches of Millipore filter support membranes used in the Orion 92–20 body. Thus, earlier batches of the Orion 92–20–04 support membrane intended for calcium electrodes gave marginally better lower linear calibration detection limits than did later batches. The effective linear calibration ranges quoted in Table III refer to the more modest data obtained with the later batches.

TABLE IV

SPECIFICATIONS OF BARIUM ION-SELECTIVE ELECTRODES WITH ANTAROX CO-880.Ba.2TPB SENSOR AND EITHER 2-NITROPHENYL OCTYL ETHER OR DI-2-NITROPHENYL ETHER MEDIATOR

	Solvent mediator				
	Liquid ion-exchange membrane			PVC matrix membrane	
Property	4-Nitroethylbenzene (reference system)	2-Nitrophenyl octyl ether*	Di-2-nitrophenyl ether	2-Nitrophenyl octyl ether*	Di-2-nitrophenyl ether
Effective linear calibration range/M	10^{-1}–5×10^{-5}	10^{-1}–~10^{-4}, depending on fabrication batch	10^{-1}–10^{-4}	10^{-1}–10^{-5}	10^{-1}–9×10^{-6}
Slope at 25 °C/mV per decade	27, but increases to 30 by day 30	~22, depending on fabrication batch	28, but increases to 31 by day 30	21–27 depending on occasion	28–29, remains steady to day ~30 and then becomes noisy in response
Response time/min	<1	2–8	<1	2–4	<1
Reproducibility/mV	±0.5	±1	±0.5	±1	±0.5
Long-term drift/mV d^{-1}	2–3	Erratic	~1	Erratic	1–2
Response time towards 10^{-6} M Ba^{2+}/min	10–30	Erratic	~10	~10	~5
pH range at 10^{-3} M Ba^{2+}	2–10	—	2.5–10	Steady fall in response of 10 mV over pH range 4–9	1.5–10
Operational lifetime/d	>30	~15	>30	15–21	~30
Selectivity coefficient (mixed solution method at interferent level of 10^{-1} M unless stated otherwise in parentheses):					
$K_{Ba,Li}$	9.0×10^{-3}	Too erratic for worthwhile selectivity studies	4.4×10^{-3}	1.8×10^{-3}	1.8×10^{-3}
$K_{Ba,Na}$	2.3×10^{-3}		7.7×10^{-3}	3.6×10^{-3}	3.0×10^{-3}
$K_{Ba,K}$	2.0×10^{-1}		2.0×10^{-3}	6.0×10^{-2}	9.5×10^{-3}
$K_{Ba,Rb}$	4.5×10^{-1}		6.0×10^{-3}	9.5×10^{-3}	1.8×10^{-2}
$K_{Ba,Cs}$	4.8×10^{-1}		2.0×10^{-1}	3.0×10^{-1}	9.0×10^{-2}
$K_{Ba,Be}$ (10^{-3} M Be^{2+})	7.3×10^{-3}		6.0×10^{-3}	1.0×10^{-3}	2.6×10^{-3}
$K_{Ba,Mg}$	8.0×10^{-4}		2.4×10^{-4}	1.7×10^{-4}	2.2×10^{-4}
$K_{Ba,Ca}$	8.0×10^{-4}		6.0×10^{-4}	4.4×10^{-4}	2.3×10^{-4}
$K_{Ba,Sr}$	6.5×10^{-3}		4.4×10^{-3}	4.4×10^{-3}	2.8×10^{-3}
$K_{Ba,Ni}$	8.0×10^{-4}		3.0×10^{-4}	2.2×10^{-4}	1.2×10^{-4}
$K_{Ba,Cu}$†	1.0×10^{-3}		2.2×10^{-3}	2.6×10^{-4}	3.6×10^{-3}

* Antarox CO-880.Ba.2TPB is poorly soluble in these solvents.
† A long recovery time is required after the electrodes have been in contact with Cu^{2+} ions.

Electrodes with Antarox CO-880.Ba.2TPB Sensor and Di-2-nitrophenyl Ether Solvent Mediator in PVC

PVC matrix-membrane electrodes with Antarox CO-880.Ba.2TPB sensor and the solvent mediators of 2-nitrophenyl octyl ether and di-2-nitrophenyl ether were selected for detailed study and compared with corresponding liquid-membrane electrodes, including that with the sensor in conjunction with 4-nitroethylbenzene used as a reference comparison (Table IV and Fig. 1). Those electrodes with 2-nitrophenyl octyl ether as solvent mediator are generally too erratic to be useful and are therefore not considered further. The remaining electrodes listed in Table IV work well and each has a possible application for measuring barium-ion activities.

The PVC matrix-membrane electrodes based on trapped saturated solutions of Antarox CO-880.Ba.2TPB in di-2-nitrophenyl ether are clean and easy to assemble and use, and are also economical in sensor materials. They have long linear calibration ranges of steady but slightly sub-Nernstian slope, rapid responses and a lifetime of about 30 d. The selectivity is slightly better for the PVC matrix-membrane electrode than for either of the liquid-membrane electrodes (Table IV), but unless the offending species are present in large amounts, interference is not especially serious and because of the valence advantage[13] the effect of the apparently higher K_{ij} values for the univalent alkali-metal ions is diminished.

The PVC matrix-membrane electrode can be used over the same barium activity range in ethanol - water mixtures that contain up to 33.3% of ethanol. After allowing for the effect

of ethanol on activity coefficients in solutions that contain 33.3% of ethanol, there is a small decrease in slope of 1–2 mV per decade, the linear calibration being up to 15 mV more positive than in purely aqueous solution. The differences are, of course, less marked for solutions that contain smaller proportions of ethanol.

Application of the PVC Matrix-membrane Electrode to the Determination of Sulphur in Organic Compounds

Among the possible applications of the PVC matrix-membrane barium ion-selective electrode with the barium adduct as sensor and di-2-nitrophenyl ether as mediator is the potentiometric titration of sulphate with barium ions. This was confirmed by the titration of 10^{-1} and 10^{-2} M solutions of sodium sulphate and sulphuric acid with barium chloride to a potentiometric end-point using the electrode with a calomel reference electrode, the coefficient of variation corresponding to the theoretical end-point being 0.1%. The application studied here in greater detail concerns the titration stage of the oxygen-flask method for determining sulphur in organic compounds, for which a recommended finishing stage is based on titration with barium ions to a thorin indicator end-point.[14–17] The visual end-point is not easy to observe, even with methylene blue screening, so that an alternative finish can be helpful to the analytical chemist.

The procedure adopted for the combustion stage was standard, the gaseous products being absorbed in 15 cm³ of water plus 0.5 cm³ of 100-volume hydrogen peroxide. The liquid contents were washed into a calibrated flask containing 10 cm³ of ethanol and the volume was made up to 50 cm³. Aliquots (10 cm³) diluted with 5 cm³ of water were titrated with standard 0·02 M barium perchlorate in 80% ethanol to a potentiometric end-point with the PVC matrix-membrane barium ion-selective indicator electrode and a calomel reference electrode. A typical titration curve is shown in Fig. 2, while results for a selection of sulphur-containing organic compounds are summarised in Table V.

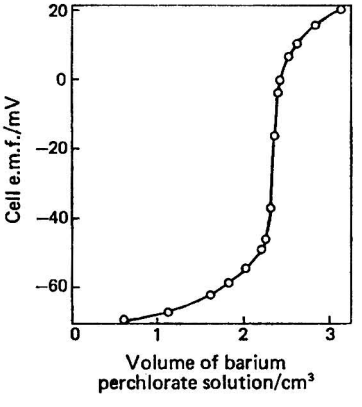

Fig. 2. Typical potentiometric titration curve for 10 cm³ of oxygen-flask absorption solution (containing 1.56 mg of sulphur) plus 5 cm³ of water with 2.083 × 10^{-2} M barium perchlorate solution in 80% ethanol. Electrode pair used: Corning (No. 476109) ceramic plug-type calomel reference electrode and barium indicator electrode with PVC membrane containing Antarox CO-880.Ba.2TPB sensor and di-2-nitrophenyl ether mediator.

Barium perchlorate in 80% ethanol has previously been recommended[15] for low concentrations of sulphate and, following preliminary experiments in this work, was chosen as it gave potentiometric titration curves superior to those obtained with aqueous barium chloride.

The sulphur recoveries averaged 100.25%, confirming this technique as a possible application for the barium ion-selective electrode. The recovery was also satisfactory for the phosphorus-containing materials, which necessitated a separation with magnesium oxide.[17]

TABLE V

SULPHUR FOUND IN ORGANIC COMPOUNDS

Sample	Elements present	Sample mass/mg	Theoretical sulphur content, %	Sulphur content found, %
Dibenzyl disulphide	C, H, O and S	28.6	26.0	25.9
		30.2	26.0	26.0
S-Benzylthiouronium chloride	C, H, N, Cl and S	33.7	15.8	15.7
Dibenzyl disulphide + aniline phosphate (5 + 3)	C, H, N, O, P and S	39.6	26.0	25.7
Triphenylphosphonic sulphide	C, H, P and S	31.6	10.9	11.0
		34.0	10.9	11.2

Conclusion

A sensor based on the tetraphenylborate salt of a barium complex with nonylphenoxypoly(ethyleneoxy)ethanol gives functional barium ion-selective electrodes when present in liquid membranes of its saturated solution in several nitroaromatic solvent mediators. However, the use of a PVC matrix membrane demands the use of the high-viscosity di-2-nitrophenyl ether as mediator for the sensor in order to obtain electrodes with long lives. This PVC matrix-membrane electrode has a life of 30 d with good selectivity and can be used in ethanol - water mixtures that contain up to 33.3% of ethanol.

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A Semi-automated Method for the Determination of Inorganic, Organic and Total Phosphate in Sediments

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A simple, rapid and semi-automated method for the determination of inorganic, organic and total phosphorus in lake and river sediments is described. Total phosphorus is extracted from sediments with 1 N hydrochloric acid after ignition at a high temperature (550 °C) or by digestion with sulphuric acid - potassium persulphate at 135 °C in a sealed PTFE-lined Parr bomb. Organic phosphorus is determined by the difference in phosphorus content of the 1 N hydrochloric acid extract measured before and after ignition of the dry sediments at 550 °C. In all instances the orthophosphate is determined by using standard Technicon AutoAnalyzer II techniques. The interferences caused by silica and variable acid concentrations on the determination of phosphorus have been studied. Freedom from interferences under the chosen experimental conditions as well as the good results obtained for recovery and precision indicate that the methods are suitable for monitoring inorganic, organic and total phosphorus in sediments.

A simple, rapid and reasonably accurate method of extraction of total and inorganic phosphorus from sediments, as well as a rapid automated detection system, is required for large-scale monitoring of lake and river systems. The role of phosphorus, either deposited or originating in sediments, is recognised by environmentalists[1–4] as a major factor that reflects the ageing or eutrophication of natural waterways. The state in which phosphate occurs in sediments has received considerable attention, especially in that field which is concerned with the detailed fractionation of phosphorus species.[5,6] Much of this work has been an adjunct of studies on soils in which a wealth of information relating to sediments can be found.[7–9] Detailed fractionation schemes are worthwhile in themselves but for large monitoring programmes the cost and time involved can be limiting factors and therefore a simple screening procedure is required.

For the determination of total phosphorus several approaches using various methods of extraction are possible.[7–9] Fusion with sodium carbonate and digestion with perchloric acid are the two most common methods that are adaptable from techniques of soil analysis.[7–9] The use of perchloric acid was ruled out owing to the hazards entailed, while the fusion procedure was deemed to be unsuitable for routine work. The use of hydrofluoric acid for extraction purposes was considered but it was rejected because of the need for special handling and the potential hazards involved; furthermore, its use for the dissolution of the silica lattice does not seem to be necessary as the phosphorus constituents of sediments[10] are not intimately associated with the silica lattice. Digestion with sulphuric acid - nitric acid[11] or with persulphate has been reported, but because of possible inconvenience in applying them to routine use, these methods also were not adopted. It is perhaps clear that numerous methods for extraction exist.

An excellent review outlining 77 methods for all combinations of water, soil and sediments has been given by Olson.[4] For routine work the method decided on was ignition at 550 °C for 2 h followed by extraction with 1 N hydrochloric acid for 16 h at room temperature. It is recognised that compared with fusion or other such extraction procedures, it is not comprehensive,[12] and in fact gives up to an 8 per cent. lower recovery of the total phosphate. Discussion of its application to phosphate in sediment in a similar programme has been presented by Wildung *et al.*,[13] and its applicability to soil phosphorus has been reported by Hesse.[8]

The method chosen for the determination of inorganic phosphorus was that in which the phosphate released by extraction of the unignited dry sediment with 1 N hydrochloric acid

for 16 h is determined. Although this parameter is operationally defined, it provides a very good estimate[14] of all but the most inert forms of inorganic phosphorus. The alternative use of 1 N sulphuric acid for the extraction has also been reported.[12]

The organic phosphorus was determined from the difference between the phosphorus extracted with 1 N hydrochloric acid before and after igniting the sediment for 1 h at 550 °C. The merits of this procedure have been discussed by Sommers *et al.*[15] and have been outlined by others.[7–9]

In accordance with our present programme[16] for the analysis of contaminants in sediments, an alternative procedure for the determination of total phosphorus was studied, which involved the digestion of a 0.2–0.5-g aliquot of a sediment with 3 g of potassium persulphate and 5 ml of concentrated sulphuric acid in a PTFE bomb.[17] In this instance the use of hydrochloric acid was avoided because of its volatility. Persulphate was chosen as the oxidising agent because of the success reported[18] in the oxidation of organic matter as well as of the organic phosphorus.

The following study is perhaps exceptional in that the analysis of phosphate extracts from sediments is carried out by methods currently in use for waters and waste waters. The application of a bomb technique for the extraction of total phosphate increases the capabilities of the analyst who at present may be employing the bomb method only in analysis for heavy metals.

Experimental

Apparatus

All measurements of orthophosphate in sample extracts were made on the AutoAnalyzer II system (Technicon Instruments Corporation, Tarrytown, New York). The assembly included a sampler IV with two sample probes, pump IV, heating bath (Research Cartridge A), colorimeter, dual-pen recorder and dual-channel digital printer, all of the components being supplied by Technicon. All mixing coils for the two manifolds were mounted on the heating bath.

Measurements of the silicon content in sample extracts were made on a Perkin-Elmer 503 atomic-absorption spectrophotometer by direct aspiration and using the conditions outlined in the Perkin-Elmer Methods manual.

Reagents

For both detection methods employed, orthophosphate standards were prepared from certified potassium dihydrogen orthophosphate (Fisher Scientific). A standard stock solution containing 100 μg ml^{-1} of phosphorus was prepared by dissolving 0.4394 g of the reagent in a small volume of distilled water, adding 1 ml of concentrated sulphuric acid and then diluting the solution to 1 l. More dilute standards were prepared by serial dilution of the stock solution. For the extractions with 1 N hydrochloric acid the standards were prepared in 0.1 N hydrochloric acid and for the extractions by the bomb method in 1.0 per cent. *V/V* sulphuric acid.

Solutions for the detection system referred to as Method I were prepared as follows. Dissolve 17.6 g of L-ascorbic acid (Fisher Scientific; reagent grade) in a small volume of distilled water. To this solution add 0.50 ml of Levor IV (Technicon No. T 21-0332) wetting agent and then dilute to 1 l and mix thoroughly; the solution was found to be stable for about 1 week. The ammonium molybdate solution was prepared by mixing 10 g of the ammonium salt [$(NH_4)_6Mo_7O_{24}.4H_2O$ (Fisher Certified)] with about 500 ml of distilled water and 62 ml of concentrated sulphuric acid. Considerable shaking was required in order to effect dissolution and when completed the mixture was diluted to 1 l with distilled water. No filtration was required. This molybdate solution is 2.2 N in sulphuric acid. For the preparation of molybdate solutions that were 1.1 N in sulphuric acid, 31 ml of the concentrated acid were used.

The mixed reagent required for Method II was prepared by mixing the following reagents in the order given: 50 ml of 4.9 N H_2SO_4 (136 ml l^{-1} of concentrated sulphuric acid), 15 ml of ammonium molybdate solution [40 g l^{-1} of $(NH_4)_6Mo_7O_{24}.4H_2O$], 30 ml of ascorbic acid solution (18 g l^{-1}) and 5.00 ml of an antimony potassium tartrate solution [3.0 g l^{-1} of $K(SbO).C_4H_4O_6.\frac{1}{2}H_2O$]. Prior to mixing, each of the above reagents (Fisher Certified) was

diluted to 1 l with distilled water. The mixed reagent prepared as above is stable for not more than 6–8 h.

For the extractions by the bomb method, AnalaR-grade potassium persulphate was used. The sulphuric and hydrochloric acids were reagent grade (Baker Chemicals Ltd.). For silica interference studies, Fisher Scientific atomic-absorption standards (1000 μg ml^{-1} of silicon) were used. The source of the silica was sodium silicate (Na_2SiO_3) and for atomic-absorption analysis the standards were diluted appropriately to give solutions that were 0.1 N in hydrochloric acid and contained 1 per cent. V/V of sulphuric acid.

Extractions

The sediment samples used in this comparative study were obtained from Lake Ontario and Lake Erie sediment core samples. Each sediment was freeze-dried and then crushed to a fine powder (20–50 g of the dry powder, 100 mesh).

Extraction with 1 N *hydrochloric acid*

Aliquots (0.3–0.5 g) of dry sediment were accurately weighed and then transferred into 10-cm^3 Coors alumina crucibles. The uncovered crucibles, contained in a suitable tray, were placed into a warm muffle furnace (Lindberg, Heavy Duty, SIB) and ignited at 550 °C. The rate of heating of the furnace ($\sim$100 to 550 °C) was not controlled. The temperature indicator on the muffle furnace showed that the ballistic heating attained a constant temperature of 550 °C within 1 h. The samples were maintained at 550 °C for an additional 1.5 h, then removed, allowed to cool to room temperature and finally transferred into 100-ml calibrated flasks; 50.0 ml of 1.0 N hydrochloric acid were then added to the flasks. The mixtures were next shaken for 14–18 h (overnight) on a shaker bath (Precision Scientific Ltd.). The water temperature was not controlled but left at ambient room temperature (22–25 °C).

For determinations of 1 N hydrochloric acid extractable inorganic phosphate an identical aliquot of sediment was used except that no ignition was performed. After extraction, aliquots of the ignited and non-ignited mixtures were transferred into 15-ml test-tubes and centrifuged at 2000 rev min^{-1} for about 5 min. The clarified extracts were finally diluted ten times and analysed by the two automated Technicon procedures described below.

Extraction by the bomb method

For the extraction of total phosphate using the Parr PTFE bomb (Parr Instrument Co., Moline Ill.) an accurately weighed aliquot of sediment (0.3–0.5 g), together with 3 $\pm$ 0.1 g of potassium persulphate and 5.00 ml of concentrated sulphuric acid, was added to the bomb, which was then heated in an oven at 135 $\pm$ 5 °C for 2 h. The contents of the bomb were then transferred quantitatively into a 500-ml calibrated flask. After dilution to volume with distilled water, the extract (containing 1 per cent. V/V of sulphuric acid) was analysed for total phosphate by the automated procedure outlined below.

Detection Systems

The determination of the orthophosphate was carried out by using the automated systems described by the Technicon Instruments Corporation. The manifolds used are shown in Fig. 1. The procedures referred to below as Methods I and II are Technicon industrial methods Nos. 94–70W and 155–71W, respectively. Method I includes ascorbic acid alone for the reduction of the molybdophosphoric acid whereas in Method II the mixed reagents[3,4,19] ascorbic acid, sulphuric acid, ammonium molybdate and antimony potassium tartrate are used. Method I is intended for use for high levels of phosphorus (up to 10 μg ml^{-1}) and Method II for low levels (less than 0.5 μg ml^{-1}). The wetting agent (Levor IV), used in order to obtain a smooth bubble pattern, is present in the ascorbic acid reagent line for Method I whereas it is added externally (see Fig. 1) in the water line (0.5 ml l^{-1} of Levor) in Method II.

Results and Discussion

Detection Systems

The molybdenum-blue procedures for the determination of orthophosphate in water are well established and the current literature on this subject is voluminous.[3,4] All of these methods depend on the formation of molybdophosphoric acid, which is reduced by a variety of reagents

to the heteropoly molybdophosphoric acids. Of the potential systems available, the two that were chosen involved the use of ascorbic acid as reductant, which was convenient as the equipment and technique have been described by Technicon Instrument Corporation. The advantages and disadvantages of these systems have already been discussed at length elsewhere.[1,3,4]

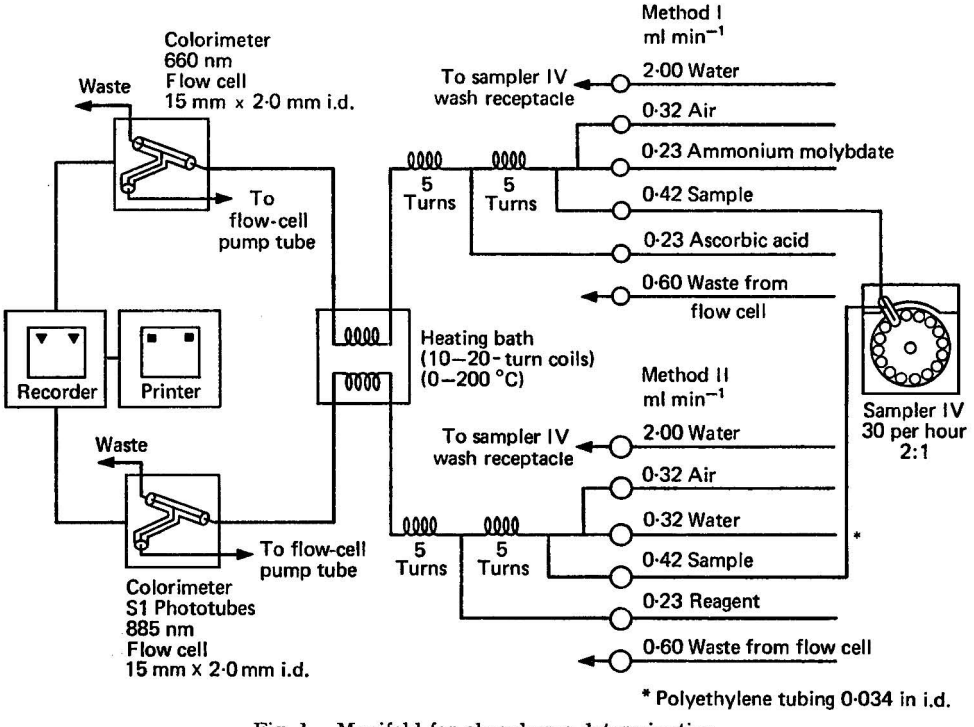

Fig. 1. Manifold for phosphorus determination.

In the present study, these two methods were chosen because the combination gives convenient flexibility in analysing sediment extracts over a wide concentration range. For instance, results obtained by Method I were found to be linear over the range 0.5–5 μg ml^{-1} of phosphorus and by Method II from 0.05 to 1 μg ml^{-1}. As solution extracts (see under Experimental) contain 1 mg ml^{-1} of sediment, the above concentration ranges allow direct analyses of sediments containing from 100 to 5000 p.p.m. (μg g^{-1}) of phosphorus to be made, which encompasses the entire range of sediment phosphate levels expected.

An alternative detection system and an automated method using the Technicon Auto-Analyzer technique were considered. The method involved the use of tin(II) chloride as reductant and had been optimised by other workers[20] for the determination of a very low level of phosphate (1 μg l^{-1} of phosphorus) as well as being modified to accommodate higher levels (50–500 μg l^{-1} of phosphorus). Unfortunately, at these high concentrations the response is non-linear, a factor that produces uncertainties if considerable care is not exercised. It is possible to dilute the solution but for large-scale operations this step was ruled out as being unsatisfactory. Consequently, and in view of comments made by other workers,[3,4] it was decided to use ascorbic acid as the reductant in orthophosphate detection.

Influence of acidity

After deciding which detection system should be used for sediment analysis, it became necessary to assess a few pertinent variables in these methods because the above two Technicon procedures were designed for the detection of orthophosphate in waste waters and sea waters and not for use with highly acidic sediment extracts. The influence of the acid content in the sample extracts was therefore checked and the results obtained are given in

Fig. 2. By using Method I (ascorbic acid alone), with 2.2 N sulphuric acid in the ammonium molybdate reagent line, a plateau extending from 0.5 to 2 per cent. *V/V* of sulphuric acid is revealed. It is evident that no serious loss or enhancement in sensitivity will occur if the sample (containing 1 per cent. *V/V* of sulphuric acid or 0.1 N in hydrochloric acid) has an incorrect amount of acid brought about by a small technical error. In fact, an extension of the plateau occurs if the amount of acid (sulphuric acid) in the molybdate solution is reduced (see Fig. 2). In all instances in this study the data obtained were for 1 μg ml^{-1} phosphorus solutions. This concentration of orthophosphate is itself a variable and determines in part the magnitude of the acid plateau, and has been discussed in the literature.[4,7]

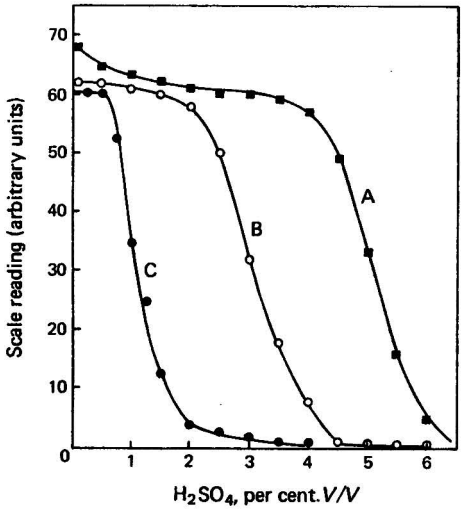

Fig. 2. Effect of concentration of sulphuric acid (per cent. *V/V*) in sample solution on the response for 1 μg ml^{-1} of phosphorus. A, Method I (1.1 N H_2SO_4*); B, method I (2.2 N H_2SO_4*); and C, method II.
*Concentration of sulphuric acid in the ammonium molybdate reagent solution used.

With regard to the extension of the acid plateau (*i.e.*, using 1·1 N sulphuric acid) it would be advantageous to use less sulphuric acid in the molybdate reagent as the influence of the variable acid content in the sample extract would not affect the sensitivity for orthophosphate. This advantage would accrue in the bomb method of extraction because considerable care is needed in order to prepare accurately a 1 per cent. *V/V* sulphuric acid solution for the sample extract. Unfortunately, the advantage is offset by interference from silica, an aspect that is discussed later.

Regarding Method II, the data in Fig. 2 reveal a response to the sulphuric acid content similar to that obtained with Method I. However the rapid fall in sensitivity occurs with much less acid present in the sample solution, the consequence of which is that for the bomb method of extraction (solution extracts contain 1 per cent. *V/V* of sulphuric acid) very careful attention must be given to preparing solutions so as to contain exactly 1.00 per cent. *V/V* of sulphuric acid. However, the extractions with hydrochloric acid that give rise to concentrations of 0.1 N of this acid in the solutions on dilution are less problematical because by Method II the solution contains an equivalent of about 0.4 per cent. *V/V* of sulphuric acid and the analysis is then performed on the plateau.

The dependence of sensitivity on acid concentration in Method II can be partly overcome by diluting the sulphuric acid in the mixed reagent (see Fig. 1 and Experimental). However, such studies were not attempted as the onset of silica interferences and possible hydrolysis of the metal salts were suspected.

Interferences and influence of temperature

As indicated under Experimental, the technique adopted was the direct application of the manifolds described in the Technicon manual,[21] with, however, one alteration. This was the insertion of the variable temperature water-bath for acceleration of the colour development once all of the reagents were mixed. The enhancement of colour development (for Method I; ascorbic only) was initially considered to be desirable as it could allow the analysis of both low (0.1–1.0 μg ml^{-1}) and high (1–10 μg ml^{-1}) levels of phosphorus in the sample extracts. However, as will be seen later this approach proved unsuccessful because increased temperatures, especially with low concentrations of sulphuric acid in the molybdate solution, gave rise to serious errors caused by reactive silicates. The interference of silicates is not uncommon and has been extensively discussed in the literature.[3,4,22–26]

In the present study, silica standards containing up to 50 μg ml^{-1} of silicon and with the appropriate acid (1 per cent. V/V sulphuric acid) gave basically the same response as was given elsewhere,[25] namely, that insufficient acid results in interference from silica. The use of 2.2 N sulphuric acid in the molybdate reagent (Method I) is essential in order to suppress the interferences by silica at 32 °C. If 1.1 N sulphuric acid is used in the molybdate reagent line, a negligible response at 32 °C occurs for 50 μg ml^{-1} of silicon compared with that at 63 °C, the consequence of which is strict adherence to the recommended acid concentrations outlined in the Technicon manual as well as maintaining a reduced temperature in the water-bath.

The complexity of the reactions of phosphate with ammonium molybdate[27–29] and the temperature - time - ionic strength relationship for silica have recently been examined. It should be emphasised that the influence of temperature and acid concentration were studied more exhaustively for Method I as this method was initially adopted for routine studies. As Method II is inherently the same as Method I, the antimony potassium tartrate acting as a catalyst,[3,19] it was considered to be only an alternative method of detection for determinations of low level phosphates that were encountered in some routine samples.

With regard to silica and the temperature effects, initial studies on sediment extracts gave some unexpected results, which are shown in Table I and Fig. 3. In this study only Method I was used and, as is evident from Table I, the very high values occur only if the molybdate reagent is low in sulphuric acid. Method II was later run simultaneously with Method I, using identical samples and temperatures. In this instance, the concentration of phosphorus did not change with temperature, presumably because the sulphuric acid content in the final mixture (*i.e.*, mixing coils) was high enough to suppress the silica interference.

In order to confirm that silica was in fact responsible for the increases at high temperatures

TABLE I

INFLUENCE OF TEMPERATURE AND NORMALITY OF SULPHURIC ACID IN THE MOLYBDATE SOLUTION ON THE DETERMINATION OF PHOSPHORUS IN SEDIMENTS BY METHOD I (BOMB METHOD OF EXTRACTION*)

Results for phosphorus are expressed in p.p.m.

	Temperature/°C				
	32	63	63	63	
	Sulphuric acid/N				
Sample	2.2	2.2	1.1	0.56	Silicon in extract/ μg ml^{-1}
1	1870	1880	2040	5600	3.6
2	1122	1120	1220	2860	4.5
3	915	890	1048	2950	3.6
4	650	655	820	2650	5.0
5	1100	1100	1320	3820	7.0
6	1370	1380	1550	3930	6.5
7	1050	1040	1220	3340	6.1
8	1150	1120	1270	3700	4.5
9	845	840	975	2570	4.5
10	1000	1040	1145	2800	5.3
11	965	940	1100	3050	5.0
12	700	710	875	2980	4.5

* Extracts by bomb method contain 1 mg of sediment per ml of solution (1 per cent. V/V H_2SO_4).

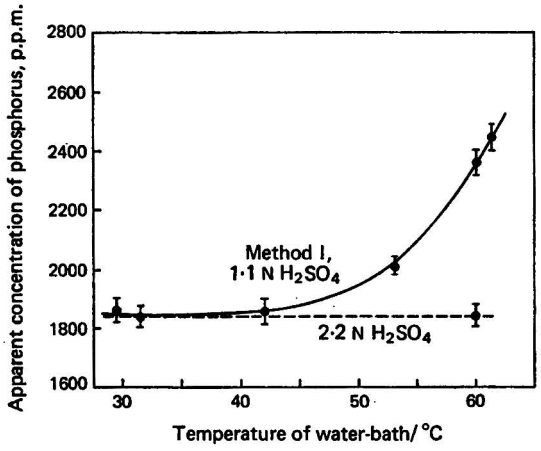

Fig. 3. Effect of temperature of heating water-bath on the apparent concentration of phosphorus. The 1.1 N H_2SO_4 refers to the concentration of sulphuric acid in the ammonium molybdate reagent solution.

the sample extracts were analysed for total silicon by flame atomic-absorption spectroscopy. For the extracts by both the bomb method and with hydrochloric acid the silicon content ranged from 4 to 8 μg ml^{-1} in the solutions for 1 mg of sediment used per ml of solution. Silicon standards (1–50 μg ml^{-1} of silicon) prepared with the same acids as those used for the samples were then analysed by Methods I and II at the temperatures used for the samples.

Although the results obtained are not shown, the response for silica (using 1.1 N sulphuric acid in the molybdate reagent line) reflected the enhanced apparent concentration of phosphate and in fact an estimate of the increase would require the presence of about 2–4 μg ml^{-1} of silicon in the solutions, which tends to confirm the silica content measured by atomic-absorption methods. In order to give more credence to the phosphate values obtained at low temperatures with 2.2 N sulphuric acid, a graph of the apparent phosphate concentration as a function of temperature was prepared, the results being given in Fig. 3. It is clear that at temperatures below 40 °C the concentration levels off, indicating that silica is not responding. In summary, in order to avoid interference from silica (less than 50 μg ml^{-1} of silicon), the sediment extracts must be analysed at low temperatures and with adequate sulphuric acid present.

No major studies were carried out on interferences other than that of silica. Considerable information in this field is, however, available from related studies on water analysis.[1–4] Constituents such as arsenic, germanium and bismuth have been reported[3,29,30] but none of these are major components of normal lake or river sediment compared with phosphorus. Dilution of extracts for phosphate determinations brings these constituents to levels that are not detectable. By the methods outlined in this paper, silica can interfere but by careful control of the acid concentration and temperature its influence can be minimised.

Extractions

The results for phosphorus concentrations in some sediments processed by the two extraction techniques are given in Table II. Each sediment was analysed in duplicate by both procedures and the orthophosphate in the extracts was measured simultaneously by the two detection systems. In view of the influence of sulphuric acid on sensitivity, the results obtained for the extracts by the bomb method using Method II are less reliable than those by Method I. Evidence indicating that no serious loss of phosphorus by volatilisation occurs if the time of ignition is extended to 16 h at 550 °C is included in Table II. The compatibility of these data with the results for ignition for 2 h at 550 °C and the results for the bomb method of extraction indicates that the oxidation of organic phosphorus to inorganic orthophosphate is similar in the two procedures.

TABLE II

COMPARISON OF RESULTS FOR PHOSPHORUS (p.p.m.) OBTAINED BY DETECTION WITH METHODS I AND II

Sediment sample	Bomb method of extraction		Extraction with 1 N HCl after ignition		Extraction with 1 N HCl before ignition, Method I
	Method I	Method II	Method I	Method II	
1	1800	—	1850	1800	1400
	1870	1770	1820*	—	
2	1080	1030	1090	1100	810
	1120	1070	1090*	1180	
3	870	800	860	860	640
	920	860	860*	950	
4	650	(570)	605	620	495
	680	610	755*	815	
5	1060	1000	1060	1030	805
	1120	1070	1080*	1150	
6	1390	1360	1230	1220	820
	1370	1300	1310*	1390	
7	1040	990	1130	1080	840
	1060	1000	1020*	1100	
8	1130	1080	1200	1130	880
	1160	1080	1150*	1070	
9	—	—	855	810	750
	850	780	820*	890	
10	1020	990	980	970	750
	1060	970	930*	1010	
11	970	930	910	900	690
	970	900	930*	1010	
12	690	640	675	660	525
	730	660	675*	730	

* Sample ignited for 16 h at 550 °C (normal time is 2 h at 550 °C).

Precision and some recovery data for the extractions are given in Tables III and IV. Standard addition studies were made on sample 1 (see Table II) for both extractions. In each instance the standard addition graphs were virtually linear and recoveries were 98–100 per cent. The dry sediments were fortified with an appropriate potassium dihydrogen orthophosphate standard and the extracts were analysed for orthophosphate by Method I. Precision data for fortified sediments using the bomb method of extraction are given in Table III. Although the precision is very good, further studies on this sediment conducted over a 5-month period resulted in a precision of greater significance for all of the extractions; Table IV shows that the two extraction techniques gave the same values.

TABLE III

RECOVERY AND PRECISION DATA FOR EXTRACTIONS BY THE BOMB METHOD

No. of tests	Phosphorus in unfortified sample, p.p.m.*	Amount of phosphorus added†/μg	Expected value, p.p.m.	Average value found, p.p.m.	Recovery, per cent.	Coefficient of variation, per cent.
5	1767	0	—	1767	—	1.7
5	1767	500	3434	3416	99.4	0.8
5	1767	1000	5100	5050	99.0	1.3
5	1767	1500	6767	6750	99.7	1.2

* Micrograms of phosphorus per gram of sediment.
† KH_2PO_4 solution was added to 0.300 g of sediment in order to fortify it to the levels indicated.

Variables such as the amount of persulphate and the total time required for the bomb digestions were also studied. A comparison of the results for the digestion of sediments by the bomb method (see under Experimental) for 2 and 7 h did not reveal significantly different

phosphate levels. Digestion for 2 h was chosen as it was more convenient when analysing ten sediment samples per day using ten bombs. The variation in results was also found to be insignificant when 1–5 g of potassium persulphate were employed; the use of excessive amounts of this reagent is to be avoided owing to the limitation of the bomb size. Furthermore, a small response, possibly due to orthophosphate, was observed when blanks (5 g of potassium persulphate per 100 ml of 1 per cent. *V*/*V* sulphuric acid) were analysed. For routine samples, no response for reagent blanks was detectable at concentrations of 3 g of persulphate per 500 ml of 1 per cent. *V*/*V* sulphuric acid. The successful use of persulphate as an oxidising agent in phosphate determinations has recently been reviewed.[18]

TABLE IV

LONG-TERM STATISTICAL DATA FOR EXTRACTIONS

The sediment used for this study was prepared in bulk for the purpose of an in-house standard. Sample 1 in Tables I and II and the sample used to prepare Table III were sub-samples of this original bulk sediment.

Method of extraction	No. of tests*	Average value for phosphorus found†, p.p.m.	Standard deviation, p.p.m.	Coefficient of variation, per cent.
With 1 N HCl before ignition	12	1417	35	2.5
With 1 N HCl after ignition	13	1837	47	2.6
Bomb method (H_2SO_4 - $K_2S_2O_8$)	18	1844	64	3.5

* Number of tests refer to data generated over a 5-month period.
† The phosphorus concentrations were determined by Method I.

It must be emphasised that in the present study the persulphate did not remove all of the colour in the concentrated (undiluted) bomb extract. Therefore, perchloric acid was used to check if this acid would completely oxidise all colour-producing agents and if it would yield higher phosphate values for the sediments. The colour was successfully removed but the phosphate values were not significantly higher than those for the sulphuric acid - potassium persulphate method. In order to check the background colour of the extract by the latter method the molybdate reagent was removed (see Fig. 1) and blank measurements on extracts were made. Insignificant responses were recorded, thus indicating that dilution of 5 ml of the extracts to 500 ml reduced the colour to non-detectable levels.

Some parameters in the ignition technique prior to extraction with 1 N hydrochloric acid were also shown to have no effect in reducing the losses of organic phosphates by volatilisation. Such losses are known to occur in sewage analysis[18,31] but for the sediments analysed there appears to be an adequate amount of basic ash-forming material to prevent loss of the volatile phosphorus(V) oxide that may be formed during oxidation. In fact, a series of studies using dry sediments fortified with magnesium chloride solution, equivalent to 10 000 $\mu g\ g^{-1}$ of magnesium in the sediment, showed no appreciable increase in total phosphorus when analysed by the ignition procedures.

Difficulties experienced in the determination of orthophosphate are discussed in the literature.[1] One particular problem of interest in sediment analysis is the potential loss of phosphate by co-precipitation with salts that precipitate on dilution (decreased acid content) or on standing (1–5 d). Precipitation of phosphorus with iron hydroxides has been reported[32] when the acid extracts are adjusted to pH 4. However, in the present study such losses did not occur as both the hydrochloric and sulphuric acids are maintained at relatively high concentrations. In fact, the diluted sediment extract showed no change in the phosphate level after storage for 5 weeks.

The results of this study are given in Table V and indicate little change, with the exception of possible increases in orthophosphate levels in unignited sediments due to hydrolysis of organic phosphates. Also in Table V is a comparison of the results obtained by the tin(II) chloride and ascorbic acid methods of phosphate detection. The former method[20] employed in this study involved the use of the Technicon AutoAnalyzer I technique, which gave rise to considerable curvature of the calibration graph at the high level of orthophosphate deter-

mined. This curvature makes routine application of the tin(II) chloride method more difficult to adapt than the ascorbic acid methods. No attempts were made to achieve linearity or to extend the operating range of this detection system to the determination of high levels of phosphate.

TABLE V

COMPARISON OF DETECTION SYSTEMS

Samples 1 to 5 are those shown in Table II. The phosphate determination using tin(II) chloride has been described elsewhere.[30] For this comparison the same phosphate standards (in 0.1 N HCl) were used throughout.

	Phosphorus, p.p.m.		
	Original value	5-week-old solutions	
Sample	(Method I)	Method I	$SnCl_2$
Extraction with 1 N *HCl before ignition—*			
1	1395	1430	1390
2	810	820	800
3	640	660	660
4	495	528	490
5	805	860	825
Extraction with 1 N *HCl after ignition—*			
1	1830	1840	1600
2	1090	1080	1030
3	855	860	830
4	650	605	570
5	1080	1040	1010

Conclusions

The extraction of phosphorus from sediments by the application of a sealed PTFE bomb method of digestion using sulphuric acid - potassium persulphate or by the more traditional ignition method followed by extraction for 16 h with 1 N hydrochloric acid gives comparable results for total phosphorus. The organic phosphorus can be determined by the difference between extractable phosphate obtained before and after ignition. The bomb method is a practical method when only a few samples of sediment are to be analysed, whereas the ignition procedure is more applicable for a large-scale programme involving hundreds of samples.

The orthophosphate content in sediment extracts (0.1 N hydrochloric acid or 1 per cent. *V/V* sulphuric acid) has been shown to be conveniently measured by using the two automated procedures that are normally used for waste waters. Adherence to a specific acid content is necessary in order to avoid interference from silica.

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Determination of Polyoxyethylene Alkylphenyl Ether Non-ionic Surfactants in Waters

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A method is proposed for the determination of polyoxyethylene alkylphenyl ethers, $RO(CH_2CH_2O)_nH$ (where R is an alkylphenyl group), at the 1–0.1 mg l^{-1} level in waters. The method is based on the spectrophotometric determination of the complexes of the polyethers with the sodium cation after extraction as picrates into 1,2-dichloroethane. The number-average degree of polymerisation ($\bar{n}$) is evaluated by thin-layer chromatography or by gas chromatography.

The method was tested on commercial compounds that have R = *p-tert*-nonylphenyl ($3.3 \leqslant \bar{n} \leqslant 21.5$). For typical surfactants ($6.5 \leqslant \bar{n} \leqslant 21.5$) the proposed method is about eight times more sensitive than that based on the ammonium tetrathioisocyanatocobaltate(II) reagent and it gives results for the absolute concentration with an accuracy ranging from −6 to +8 per cent. in the indicated interval of $\bar{n}$. The precision was evaluated from the analysis of waste waters. A few interferences are discussed.

Many spectrophotometric methods for the determination of polyoxyethylene non-ionic surfactants[1–6] are based on the co-ordination reactions between polyoxyethylene oligomers and metal cations.[7] These methods give only relative concentration values with respect to a polyoxyethylene surfactant taken as a standard, because the apparent specific absorptivity of the extracted complexes varies with the nature of the surfactants and with the reaction variables.[5,8,9]

We therefore searched for a more sensitive procedure for the determination of commercial polyoxyethylene non-ionic surfactants, $RO(CH_2CH_2O)_nH$ (where R is an alkylphenyl group and $\bar{n}$ is the number-average degree of polymerisation), that would give absolute results, at least within a certain range of $\bar{n}$ values, below the 1 mg l^{-1} level in aqueous solutions. The method devised involves the extraction of the complexes formed by the polyoxyethylene chain with the sodium cation in the form of an ion-pair with picrate anion into an organic phase, followed by spectrophotometric determination of the absorbance at 378 nm. An example of the use of the method finally adopted is the determination of *p-tert*-nonylphenyl-polyoxyethylene non-ionic surfactants in waste water.

Method

Apparatus

A Unicam SP500 ultraviolet - visible light spectrophotometer was used with matched silica cells of various path lengths for the measurement of the absorbance of the solutions.

Reagents

Chloroform. AnalaR grade.

Diethyl ether. AnalaR grade.

1,2-*Dichloroethane.* Carlo Erba, RP grade, freshly distilled.

Sodium hydroxide solution, 10 M. Prepare this solution with AnalaR grade sodium hydroxide.

Sodium nitrate - picrate reagent solution. In a 100-ml calibrated flask, dissolve 56.6 g of sodium nitrate plus 0.92 g of picric acid [dried under vacuum over phosphorus(V) oxide in a desiccator] in 0.1 M sodium hydroxide solution and make the volume up to the mark.

Standard solutions of surfactants. Aqueous solutions were prepared by using the polydisperse surfactants that had values of $\bar{n}$ of 3.3, 5.4, 6.5, 7.5, 8.6, 9.7, 10.8, 12.9, 15.0 and 21.5 (Chemische Werke Hüls). All surfactants were previously dried at 50 °C under vacuum (1 mmHg) for 2 h. The value of $\bar{n}$ was checked by vapour pressure osmometry at 37 °C in 1,2-dichloroethane with a Hewlett-Packard Mechrolab vapour pressure osmometer.

Procedures

Extraction of the complexes

With a calibrated pipette, transfer 50.0 ml of the water sample into a 250-ml separating funnel that has a PTFE stop-cock, add 50.0 ml of sodium nitrate - picrate reagent, mix the solution and allow it to stand for 1 h. Then add 5.0 ml of 1,2-dichloroethane and shake the mixture vigorously for 3 min. Transfer the organic layer, dropwise, into a conical 10-ml centrifuge tube fitted with a polyethylene stop-cock and centrifuge it for 10 min at 2000 rev min^{-1}. Prepare a blank extract with 50.0 ml of distilled water.

Determination of the surfactant by absorption spectrophotometry

Measure the absorbance of the clear organic extract at 378 nm in a 1-cm (or 2-cm) cell against the blank. Calculate the concentration of surfactant in the water sample (c mg l^{-1}) by use of the expression

$$c = f \times \frac{A}{0.360\ b} \qquad (\text{for } 3.3 \leqslant \bar{n} \leqslant 21.5)$$

where A is the absorbance at 20 °C and b cm is the cell path length. If $6.5 \leqslant \bar{n} \leqslant 21.5$, then f (the multiplication factor) = 1. If $3.3 \leqslant \bar{n} \leqslant 6.5$, evaluate f from Fig. 1.

Evaluation of $\bar{n}$ by gas - liquid and thin-layer chromatography

Add 2.0 ml of 10 M sodium hydroxide solution to 100 ml of sample solution containing about 1 mg l^{-1} of surfactant and extract with 20 ml (twice) and 10 ml (once) of diethyl ether. Combine the extracts, evaporate the solution to dryness in a small conical centrifuge tube, add to the residue 0.1 ml of chloroform and use the solution obtained (approximately 0.1 per cent. m/m concentration) for the analysis by gas - liquid or thin-layer chromatography, coupled with direct spectrophotometry of the spots on the thin layer.[10–12]

Calculate the number average degree of polymerisation of the surfactant ($\bar{n} = \Sigma\, x_n\, n$) from the observed distribution of the molar fraction, x_n, for various values of n.

Discussion of the Method

Extraction of the Complexes and Calibration Graphs

The absorption spectrum of an extract shows a maximum at 378 nm, which characterises the picrate chromophore in 1,2-dichloroethane. Distinct advantages of the picrate reagent over the classical ammonium tetrathioisocyanatocobaltate(II) reagent are the higher absorptivity and the stability with time and towards the extraction solvent. 1,2-Dichloroethane is a particularly suitable solvent for the extraction of polyoxyethylene complexes but it reacts slowly with isothiocyanate ions.[9] Extracts with picrate are stable for several days.

In the procedure the following experimental conditions at 20 °C are adopted: organic to aqueous phase ratio 5:100 V/V; and concentrations of the reagents in the aqueous phase, sodium nitrate 3.33 M, picric acid 0.02 M and sodium hydroxide 0.05 M (total Na^+ concentration 3.38 M). A thorough study of the two-phase extraction systems with the various surfactants shows that the absorbance at 378 nm tends to a constant maximum when the concentration of sodium nitrate in the aqueous phase is 3 M and that of picric acid is at least 0.015 M (surfactant concentration, 1.00 mg l^{-1} for $3.3 \leqslant \bar{n} \leqslant 15.0$). With a value of $\bar{n}$ of 21.5 the maximum absorbance is reached only at a picric acid concentration of 0.05 M.

For every surfactant considered, Beer's law ($A = \bar{a}bc$) at 378 nm holds at least for the concentration range 1–0.1 mg l^{-1} of surfactant in the aqueous phase.

The variation of the apparent mean absorptivity ($\bar{a}$) with $\bar{n}$ is indicated in Fig. 1. In the range $6.5 \leqslant \bar{n} \leqslant 12.9$ (typical surfactants for the detergent formulation), $\bar{a}$ varies linearly with $\bar{n}$, but the slope is so small ($\Delta\bar{a}/\Delta\bar{n} = 0.007$) that the mean value obtained from the linear regression ($\bar{a} = 0.360$, corresponding to the surfactant with a value of $\bar{n}$ of 9.7) appears to be acceptable in an averaging procedure, because the systematic error is only −5.9 per cent. when the value of $\bar{n}$ is 6.5 and +7.7 per cent. when it is 12.9 or 15.0. With the proposed procedure the calibration line for an $\bar{n}$ value of 21.5 does not differ significantly from that obtained for a value of 6.5. Therefore, within the range $6.5 \leqslant \bar{n} \leqslant 21.5$, a mean value of $\bar{a} = 0.360$ can be used, the consequential error being not more than 8 per cent.

In the range $3.3 \leqslant \bar{n} \leqslant 6.5$, determination of the surfactant is also possible by means of

an experimentally determined multiplication factor (f), which corrects the systematic error arising from the lower absorbance response according to Fig. 1.

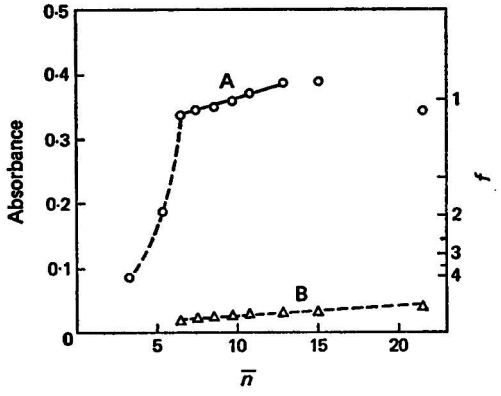

Fig. 1. Absorbance at 20 °C (b = 1 cm) of extracts in 1,2-dichloroethane with sodium picrate reagent at 378 nm (A) and ammonium tetrathioisocyanatocobaltate(II) reagent at 620 nm (B) as a function of the number average degree of polymerisation ($\bar{n}$) of surfactants. Absorbances have been calculated from $\bar{a} = A/bc$ values determined by linear regression.[13] Ratio of organic to aqueous phase 1:20 V/V; concentration of surfactant in aqueous phase 1.00 mg l^{-1}. In the range $6.5 \leqslant \bar{n} \leqslant 12.9$, the points of the graph A are interpolated by continuous straight line $A = 0.290 + 0.0075\,\bar{n}$. In the range $3.3 \leqslant n \leqslant 12.9$, the factor f can be evaluated by means of the right-hand scale corresponding to the broken line.

Extraction of the Surfactant and Evaluation of $\bar{n}$

Gas - liquid chromatography is an accurate method for determining the value of $\bar{n}$ up to 6.5.[11] When coupled with thin-layer chromatography, this limit increases to 9.7.[12] The determination of the value of $\bar{n}$ up to 12.5 is also possible by means of multiple-elution thin-layer chromatography.[14] As an example, Table I shows the results obtained by gas - liquid

TABLE I

GAS - LIQUID CHROMATOGRAPHIC ANALYSIS OF POLYOXYETHYLENE *p-tert*-NONYLPHENYL ETHERS ($\bar{n}$ = 5.4)

Degree of polymerisation (n)	Molar fraction of the n-polymer (x_n)	
	Untreated	Extracted
1	0.005	0.003
2	0.013	0.027
3	0.109	0.143
4	0.216	0.217
5	0.230	0.244
6	0.171	0.172
7	0.118	0.105
8	0.072	0.061
9	0.037	0.021
10	0.019	0.007
11	0.010	Trace
12	Trace	—
$\bar{n}_{GLC}$*	5.43	5.09

* Number-average degree of polymerisation calculated from gas - liquid chromatographic data.

chromatographic fractionation of surfactant with an $\bar{n}$ value of 5.4 for both the untreated compound and that extracted with diethyl ether from an aqueous solution containing 1 mg l^{-1} of surfactant, comparison of which indicates that diethyl ether appears to be an acceptable extraction solvent for these surfactants. With the proposed procedure, the extraction efficiency ranges between 90 ($\bar{n}$ = 5.4) and 80 ($\bar{n}$ = 15.0) per cent. The alkaline aqueous phase permits the selective extraction of non-ionisable solutes, thus minimising interferences from acidic organic substances, which are usually present in waste waters.

TABLE II

PRECISION OBTAINED IN THE ANALYSIS OF WASTE WATER SAMPLES CONTAINING SURFACTANTS ($\bar{n}$ = 7.5 AND 9.7)

Methylene blue active substances (MBAS) expressed as sodium dodecyl sulphate (mg l^{-1}).

	Concentration of surfactant/mg l^{-1}			
	$\bar{n}$ = 7.5		$\bar{n}$ = 9.7	
Analysis No.*	Sample A (MBAS 0.4)	Sample B (MBAS 0.5)	Sample A (MBAS 3.3)	Sample B (MBAS 1.2)
1	0.92	0.11	0.86	0.38
2	0.93	0.14	0.86	0.40
3	0.90	0.12	0.90	0.41
4	0.94	—	0.83	0.43
5	—	—	0.84	0.39
$\bar{x}$	0.922	0.123	0.858	0.402
s	±0.017	±0.016	±0.027	±0.019
100 ($s/\bar{x}$)	±1.8	±13	±3.1	±4.7

* $\bar{x}$, average; s, root mean square deviation; 100 ($s/\bar{x}$), coefficient of variation, per cent.

Precision and Accuracy of the Method

Table II shows the precision that can be obtained in the repeated analysis of waste water samples that have different levels of surfactants, the concentrations of which were determined on the clear liquid obtained after separation of the settlable solids down to 5 μm equivalent spherical diameter.

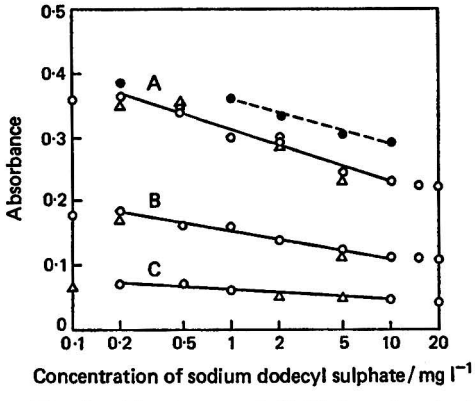

Fig. 2. Absorbances at 20 °C (b = 1 cm) of extracts in 1,2-dichloroethane as a function of the concentration of sodium dodecyl sulphate. Results marked with open circles ($\bar{n}$ = 9.7) and triangles ($\bar{n}$ = 8.6) were obtained with the proposed procedure. Closed circles indicate results observed with a picrate concentration of 5 × 10^{-3} M. The concentration of the non-ionic surfactant is shown over the set of points: A, 1.0 mg l^{-1}; B, 0.5 mg l^{-1}; and C, 0.2 mg l^{-1}. In the range indicated, straight segments were obtained by linear regression.

The sensitivity of the proposed method is about eight times higher than that obtained with the ammonium tetrathioisocyanatocobaltate(II) reagent[9] in the same organic to aqueous phase ratio (see Fig. 1). With the latter reagent the averaging procedure is not acceptable, because it would give rise to a systematic error of −30 per cent. for a surfactant that has an $\bar{n}$ value of 6.5 and +27 per cent. for a surfactant that has an $\bar{n}$ value of 15.0.

Interferences

Anionic surfactants are usually present in waste water and interfere in the determination of non-ionic surfactants. Systematic errors observed with the proposed method are illustrated in Fig. 2. The error is reduced by increasing the picrate concentration, and this fact indicates that the colourless surfactant anion competes with the yellow picrate in establishing the equilibrium of the two-phase extraction system. At 378 nm Beer's law for the non-ionic surfactants is obeyed in the presence of interfering anionic surfactants at concentrations of up to at least 10 mg l^{-1}.

With concentrations of sodium dodecyl sulphate (Serva, Heidelberg, West Germany) in the range 0.2–10 mg l^{-1} in the aqueous phase, the absorbance decreases linearly with $\log_{10} c_A$, where c_A mg l^{-1} is the concentration of the anionic surfactant. When c_A is less than 0.2 mg l^{-1}, no interference is detectable and the absorbance values do not differ significantly from that observed at $c_A = 0$. As the determination of anionic surfactants (as MBAS, methylene blue active substances expressed as sodium dodecyl sulphate) is mandatory in water control, the absorbance at $c_A = 0$ can be evaluated by means of the diagram in Fig. 2.

Soaps (as sodium stearate) and hydrocarbons (aliphatic compounds up to C_{12} and benzene) do not interfere at concentrations of up to at least 10 mg l^{-1}. Cationic surfactants interfere[15] and the method is not applicable when they are present. In coloured water samples, the possible extraction of organic substances that may absorb in the same region as the picrate chromophore must be checked by a preliminary extraction with a sodium nitrate reagent prepared as indicated above but free from picric acid.

The authors are grateful to Professor B. Stancher for his assistance with the gas - liquid chromatographic analysis. Thanks are due to Chemische Werke Hüls, Marl, Kreis Recklinghausen, West Germany, for supplying the surfactant samples.

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Catalymetric - Thermometric Titration of Some Derivatives of Barbituric Acid

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The compound barbitone, and the related compounds amylobarbitone, butobarbitone, hexobarbitone, phenobarbitone and quinalbarbitone, have been determined by catalymetric - thermometric titration involving an acid - base reaction in the analyte followed by a base-catalysed indicator reaction. The base-catalysed reactions used were the dimerisation of acetone and the polymerisation of acrylonitrile with dimethylformamide as a matrix diluent. The effects of excipients are reported, and a method of assay of dosage forms is proposed.

Since the introduction of barbital, in 1903, a vast number of derivatives of barbituric acid have been synthesised and studied both pharmacologically and clinically. The range of derivatives in widespread use is, however, fairly restricted and standard methods of assay are generally sufficient to deal with the various derivatives encountered. In order to obtain the requisite amounts of active ingredients for assay, most previously reported methods require the crushing and mixing together of a number of the tablets used as dosage forms. Because most of the dosage forms contain excipients it is usually necessary to extract the active ingredients in order to obtain an analytically acceptable size of sample. For example, the official method of the British Pharmacopoeia[1] recommends the use of 20 tablets and an ether extraction procedure, followed by a gravimetric finish. Garratt[2] recommends a somewhat tedious and time-consuming extraction process for the assay of the elixir containing barbitones, preceding a gravimetric finish. In these processes it is possible to obtain erroneously high results because of the presence of other ether-extractable components in the sample.

A proposed photometric determination[3] requires an extraction in order to remove the effects of insoluble excipients. Similarly, some ultraviolet spectrophotometric methods[4] require separation of the active constituents by chromatography prior to their determination. A method using gas - liquid chromatography in order to separate and determine barbiturates in tablets has been reported[5] but this also requires the separation, by solvent extraction, of the active ingredient of the dosage form. The non-aqueous titration of barbituric acid, and of some commercial preparations containing it, has been reported by several workers.[6,7] The end-point can be determined visually[6] or potentiometrically.[7] A coulometric method has been reported[8] using a supporting electrolyte of sodium perchlorate in a 20% V/V acetone in water medium. In order to obtain acceptable reproducibility, this method also requires the removal of the excipients of the dosage form.

The use of thermometric titrimetry for the determination of the active ingredients of several pharmaceutical and foodstuff samples has been reported previously.[9,10] The advantages of thermometric analysis for reaction systems in which the excipients of pharmaceutical preparations are present, and are chemically inert with respect to the reactions involved, have been discussed previously.[11] Several systems with low specific heats and involving thermometric methods in which the solvent system is utilised in a thermal indication system have been reported for the titration of some acid - base systems.[12–15] Such reactions, in which the equivalence point of the analyte reaction is marked by the onset of a catalysed reaction involving the bulk of the solvent, are designated catalymetric reactions. The main advantage of such systems is that the relatively massive enthalpy change of the secondary, or catalysed, reaction allows the use of less precise thermostatically controlled reaction systems and of recorders of lower sensitivity. This factor results in a titration system of simpler design and lower cost, without any accompanying loss in precision and accuracy.

The present investigation involved a detailed study of the applicability of the catalymetric - thermometric titration technique to acid - base reactions in acrylonitrile - dimethylformamide

and acetone - chloroform mixtures. Dimethylformamide has been used to accentuate the acidic strength of barbiturates, and the polymerisation of acrylonitrile, catalysed by alcoholic potassium hydroxide solution, has been employed for the indication of the end-point.[13,16–18]

Vaughan and Swithenbank[12] had previously reported the use of acetone as an indicator solvent in the titration of organic acids with alkaline hydroxides. They suggested that while acetone could be used as the solvent, if sodium hydroxide in propan-2-ol were used as the titrant, then, in many instances, when other components were introduced into the solvent system or into the sample a significant alteration in the sensitivity of the reaction would occur, especially at or near to the equivalence point of the analyte reaction and thus to the onset of the catalysed reaction. It was therefore considered necessary to investigate such effects, as previous experiences with tablet and encapsulated forms of pharmaceutical preparations had indicated that acetone alone was not the best solvent for many of the active ingredients; the use of propan-2-ol also had some disadvantages. However, as many of the formulations contain both polar and non-polar constituents, it was considered desirable that the solvent used should be miscible with aqueous solutions, capable of dissolving most, if not all, of the non-polar components, have a low specific heat and, more essentially, be capable of taking part in a reaction catalysed by the hydroxide ion or some other strongly basic ion. General considerations indicated that of the commonly available solvents, acetone satisfies many of the above criteria and hence would be a suitable solvent in the reactions under investigation.

Experimental

Apparatus

Details of the apparatus have been described previously.[15,19] The motor-driven syringe pump was used to deliver the titrant to the titrand at a nominal rate of 1.0 ml min^{-1}; the actual delivery rate was monitored by use of gravimetry. The titrand solution was stirred using a magnetic stirrer and follower. The reaction vessel was thermally insulated in an expanded polystyrene block. The temperature changes were recorded as the imbalance voltage from a Wheatstone bridge containing a 22-kΩ thermistor as one of the arms. The titrant and the titrand were both initially at room temperature.

Materials

Solvents

Propan-2-ol and ethanol. Laboratory-reagent grade propan-2-ol and absolute ethanol were separately dried by refluxing for several hours over an excess amount of freshly ignited calcium oxide and the individual products were distilled through a fractionating column.

Dimethylformamide and acrylonitrile. Laboratory-reagent grade dimethylformamide and acrylonitrile were dried by passing them through separate columns of molecular sieve (Linde, type 4A) before use.

Acetone. Dry acetone was prepared by passing laboratory-reagent grade acetone through a column of molecular sieve (Linde, type 4A), and then distilling the effluent in an all-glass apparatus fitted with a Perkin triangle in order to give a reflux ratio of not less than 1:4.

Chloroform. Laboratory-reagent grade chloroform was passed over a column of molecular sieve (Linde, type 4A) and then distilled. The fraction boiling between 60 and 61 °C at atmospheric pressure was collected.

Titrant solutions

Separate solutions, 0.15 and 1.0 M, of potassium hydroxide (AnalaR grade) in dry propan-2-ol and dry ethanol, respectively, were prepared by dissolving the appropriate amounts of potassium hydroxide pellets in the dry solvent. The solutions were standardised against zone-refined benzoic acid, using the catalymetric - thermometric method. They were also standardised by the classical procedure, using phenolphthalein and thymol blue as visual indicators.

Procedure for the Assay of Some Barbiturates Using Acrylonitrile

An aliquot (2 ml) of the barbiturate solution (containing 0.1 mmol) was pipetted into the titration vessel containing 5.0 ml of acrylonitrile. The total volume of solution was adjusted to 10.0 ml with dimethylformamide. The titration vessel was covered with a polyethylene

cap through which a hole, just large enough to house the thermistor, had been drilled. This hole was kept small in order to minimise the interference effects of atmospheric moisture and carbon dioxide. The titration vessel was next placed in the block of insulation material and the thermistor inserted through the hole in the cap; it was immersed in the analyte solution, which was then stirred magnetically for 1–2 min until thermal equilibrium was attained. (The attainment of thermal equilibrium was indicated by the presence of a constant trace on the recorder when set at 100 mV.) The barbiturate solution was then titrated against the standardised 0.15 M potassium hydroxide solution, using a motor-driven syringe to deliver the titrant. The end-points were located at the point of intersection of the extrapolations of the two linear portions of the enthalpogram (see Fig. 1, A). The amount of barbiturate present could then be calculated.

Procedure for the Assay of Some Barbiturates Using Acetone

A known amount of barbiturate solution (containing 0.1 mmol) in dry acetone was pipetted into the titration vessel. The volume of solution was adjusted to 9.0 ml with dry acetone and then 1.0 ml of chloroform was added. The tip of the titrant delivery tube and thermistor were submerged beneath the surface of the mixture, which was kept agitated with a PTFE-coated magnetic stirrer so that the solution attained thermal equilibrium with its surroundings, as indicated by the presence of a constant trace on the recorder when set at 10 mV. Once equilibrium had been established, the 1.0 M titrant was delivered at constant rate until there was an indication of the end-point (see Fig. 1, B). The mixture was efficiently stirred so that optimum heat transfer was achieved through the solution to the thermistor.

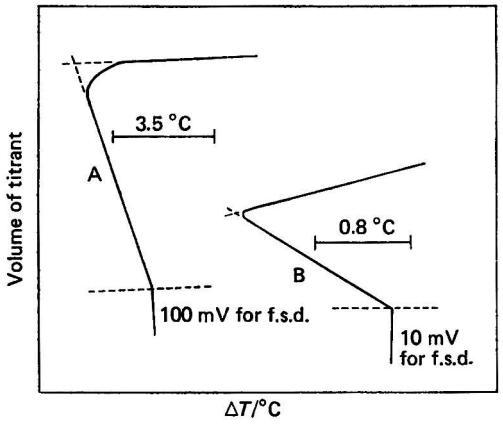

Fig. 1. Typical enthalpograms: A, with acrylonitrile as indicator solvent; and B, with acetone as indicator solvent.

Results

The results are summarised in Table I. The reproducibility of the results obtained has been evaluated by performing several replicates. The mean and standard deviations of the replicates are given whenever possible.

Discussion

The results recorded in Table I indicate that dimethylformamide and acetone are satisfactory solvents for the determination of weakly acidic barbiturates. For the systems investigated in this work the end-points consist of a change in the slope of the enthalpogram, brought about by the use of a secondary polymerisation reaction catalysed by the excess of titrant at the equivalence point. Typical enthalpograms for the systems studied are illustrated in Fig. 1. The ease of location of the end-point depends upon the sharpness of the change in

slope. The rounding of the end-point is a factor of the change in free energy of the system and the rate of completion of the reaction.

The end-points of the curves presented are all slightly rounded; however, extrapolation permits precise and reproducible location of the end-point. Bark and Bark[11] have previously discussed the basic theory of the extrapolation technique, but apart from these fundamental considerations, it became apparent during this work that trace amounts of water could contribute to curvature; Vaughan has previously commented on this in his work on acetone, and Forman and Hume[14] suggest that the interference from water is associated with its amphoteric character and its high dielectric constant, which result in high heats of mixing and dilution.

TABLE I

CATALYMETRIC - THERMOMETRIC DETERMINATION OF VARIOUS BARBITURATES

Where two amounts have been recorded, these represent different runs, carried out at different times (up to 6 months apart) on different apparatus.

	Acrylonitrile matrix				Acetone matrix			
Compound	Amount taken/ mg*	Average amount recovered/ mg	Maximum mass difference/ mg	Deviation from mean, %	Amount taken/ mg	Average amount recovered/ mg	Maximum mass difference/ mg	Deviation from mean, %
Barbitone	36.8	36.8	0.5	1.35	46.1	46.3	0.1	0.2
	27.6	27.6	0.1	0.35				
Amylobarbitone	57.6	58.1	0.8	1.4	34.0	33.95	0.3	0.8
	67.9	67.8	0.2	0.3	45.3	45.4	0.5	1.1
Butobarbitone	63.7	63.0	0.8	1.2	31.8	31.9	0.3	0.95
					42.5	42.55	0.2	0.48
Hexobarbitone	70.9	70.85	0.5	0.7	47.3	47.3	0.0	0.0
Pentobarbitone	45.3	45.5	0.5	1.1	56.6	56.6	0.0	0.0
	67.9	67.9	0.0	0.0	67.9	68.06	0.4	0.6
Phenobarbitone	58.1	58.05	0.4	0.7				
	69.7	70.1	1.4	2.0				
Quinalbarbitone	59.6	60.0	1.2	2.0	35.8	35.85	0.2	0.55
					47.7	47.7	0.0	0.0

* Not less than 5 readings at each mass; maximum number, 10 readings.

In the course of this investigation it became apparent that the curvature at the end-point relates partly to the over-all sensitivity of the potentiometric recorder and is not only concerned with the concentration of water present. With judicious selection of the sensitivity of the recorder, it is possible to reduce the curvature to an analytically tolerable level.

With acrylonitrile, the titration products are soluble in dimethylformamide and this fact enhances the sensitivity of the end-point detection as the end-point is marked by a sudden and massive heat rise, which is produced from the polymerisation of acrylonitrile catalysed by the excess of the titrant. However, one of the problems encountered during the course of the work in the acetone system was the formation of highly voluminous and gelatinous precipitates, these being the potassium salts of barbituric acid derivatives. This precipitation resulted not only in the tendency for the titre to drift to a higher value but the end-point was noted to be occasionally erratic and ill-defined, possibly as a result of low thermal conductivity through the lumpy precipitate that often coated the tip of the thermistor and thereby increased its response time. The problem was overcome by the addition of 1 ml of chloroform to the sample solution before commencing the titration; it was then possible to obtain good reproducibility.

This last effect demonstrates the striking changes in the sensitivity of the reaction upon addition of solvents of low dielectric constant, which enhance the sharpening of the end-points by rendering the titration product soluble. Chloroform, being a non-polar solvent, does not form solvates with either acids or bases, and consequently does not influence the equilibrium states of neutralisation reactions. Thus, in theory, any amount of chloroform can be used in a titration. However, in practice, if too much of the inert solvent is added, the resulting decrease in the polarity of the solvent mixture (chloroform - acetone) may interfere with the thermometric end-point detection.

The lower limit of barbiturate concentration for an analytically useful titration is partly determined by the molarity of the titrant and the temperature change obtained at the end-point. The upper limit of determination is, however, unrestricted except by thermal capacity considerations and the solubility of the titrand in the fixed volume of solvent system.

The investigation was carried further in order to assess the influence of excipients on the proposed methods of assay. The excipients were present in amounts far in excess of their normal occurrence in tablets.

TABLE II

EFFECT OF EXCIPIENTS ON THE ASSAY OF THE BARBITURATES

A nominal amount (50 mg) of each of the compounds was mixed with nominal amounts of the excipients and the barbiturate was assayed as before.

Excipient	Nominal amount per 50 mg of barbiturate/mg
Lactose	2000
Magnesium stearate	150
Starch	1000
Talc	1000

Acetone matrix
Lowest recovery: pentobarbitone, 98.7%, with 1000 mg of talc present
Highest recovery: quinalbarbitone, 101.7%, with 1000 mg of starch present

Acrylonitrile matrix
Lowest recovery: hexobarbitone, 98.65%, with 1000 mg of talc present
Highest recovery: phenobarbitone, 102.0%, with 2000 mg of lactose present

It is evident from the findings reported in Table II that a judicious selection of solvents could virtually eliminate the adverse influence of excipients and permit quantitative assay of the active components. The results further indicate that the technique has a potential use in the routine assay of single dosage forms of barbiturates. This additional feature helps to enhance the versatility and convenience of the proposed methods.

Mixtures of Barbiturates

Although many dosage forms of the barbiturates contain only one active ingredient, there are those tablets and capsules which contain more than one barbiturate. While a discussion of the reasons for having mixtures of barbiturates is outside the bounds of this paper, it is pertinent to note that it would be useful in such instances to have available a simple method for the serial determination of these mixtures.

Although the catalymetric method cannot normally be used for serial titrations of analytes before the onset of the catalysed reaction, it was thought appropriate to consider the shapes of the enthalpograms obtained when different barbiturates were used.

Although there is a clear change in the slope at the end-point that depends upon the strength of the acid used, the variation in the strengths of the acids (phenobarbitone > barbitone > quinalbarbitone > amylobarbitone > hexobarbitone) is not sufficient to allow sequential analysis, even when the differences in acid strengths are enhanced in the solvent systems used. The differences in ΔH values for the acid - base reactions are not sufficiently great to result in any significant change in the slope of the enthalpogram as the acids are serially titrated. It is therefore considered that this method should be restricted to the assay of the total barbiturate content of a sample.

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A Method for the Detection of Microgram Amounts of Hydroxamic Acids

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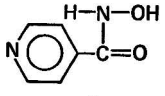
A simple and rapid method for the detection of trace amounts of hydroxamic acids is presented. It consists in extraction of the coloured complex formed by the reaction of hydroxamic acids (5–30 μg) with vanadium into one drop of isobutyl methyl ketone or a water-immiscible alcohol. The applicability of the method was tested with a variety of hydroxamic acids. It was established that carboxylic acids, their chlorides or esters, and hydroxylamines do not interfere.

In the course of studies on heterocyclic hydroxamic acids it was observed that the method reported by Agrawal[1] for the detection of microgram amounts of hydroxamic acids failed to detect certain hydroxamic acids, the identities of which were subsequently established by other methods. Agrawal's method consists in adding one drop of a solution of the sample in chloroform to a mixture of one drop of ammonium metavanadate solution (2.3×10^{-4} M) and two drops of concentrated hydrochloric acid contained in a micro-scale test-tube and shaking the tube vigorously. It was considered that with this test the presence of hydroxamic acid in the sample solution would cause a violet colour to develop in the organic phase. Isonicotinohydroxamic acid (**I**) is one of the many hydroxamic acids that failed to respond to the above test, although the test was claimed to be applicable to all hydroxamic acids. With isonicotinohydroxamic acid, for example, the chloroform layer remained colourless.



I

Considering the great importance of hydroxamic acids in analytical[2] and medicinal[3–5] chemistry, and the fact that many workers are engaged in synthesising these compounds, it was decided to investigate the reason for the failure of some of them to respond to this test.

Agrawal's method is based on two assumptions, namely, that all hydroxamic acids are soluble in chloroform and that the violet complex, which all of the hydroxamic acids form with vanadium(V), can be extracted into chloroform. Neither of these assumptions is universally valid. A search of the literature showed that although a large number of hydroxamic acid - vanadium complexes[6–13] can be extracted into chloroform, some of them, including the benzohydroxamic acid complex,[14] are not soluble in this solvent. The complexes that are insoluble in chloroform are generally those formed by vanadium(V) with hydroxamic acids that are soluble in water, *e.g.*, benzo-, nicotino-, isonicotino- and 5-sulphosalicylohydroxamic acids. It also seemed that oxygen-containing organic solvents such as isobutyl methyl ketone or the higher alcohols might prove to be more widely applicable than chloroform as extraction solvents for the vanadium - hydroxamic acid complexes. Appropriate experiments were therefore carried out and the results obtained are discussed below.

Experimental

Reagents

Hydroxamic acids

These acids were prepared by the general method of Blatt.[15] They were then recrystallised repeatedly until a sharp, constant melting-point was obtained. Their identity and purity were established by microanalysis, gas - liquid chromatography and infrared and ultraviolet spectroscopy.

Standard vanadium solution, approximately 10^{-4} M. A solution containing 12 mg l^{-1} of analytical-reagent grade ammonium metavanadate was prepared and standardised titri-

metrically against potassium permanganate solution.[16] This stock solution, which was kept for more than 2 weeks, was oxidised before use by dropwise addition of dilute potassium permanganate solution until a faint pink colour persisted.

Chloroform. AnalaR grade.

Hydrochloric acid. AnalaR grade.

Isobutyl methyl ketone, 3-methylbutan-1-ol and butan-1-ol were laboratory grade chemicals and were used after purification by standard methods.[17]

Procedure

Mix one drop of vanadium solution with two drops of concentrated hydrochloric acid in a micro-scale test-tube. Allow the solution, which becomes slightly warm, to cool, then add one drop of the solution of the sample in isobutyl methyl ketone, 3-methylbutan-1-ol or butan-1-ol and shake the contents of the test-tube for 30 s. If a hydroxamic acid is present the organic phase develops a violet or reddish violet colour.

Discussion

The above method was applied to hydroxamic acids that differed widely in respect of the nature of the groups associated with the functional –N–OH and –C=O groups, *viz.*, those which had as substituents (with examples in parentheses) aliphatic groups (aceto-), aromatic groups (benzo-), saturated rings (cyclohexanecarbo-), heteroaromatic groups (nicotino-), amino-acids (aminoaceto-), hydroxy acids (lacto-) and dicarboxylic acids (phthalo-). Disubstituted acids (*e.g.*, *N*-phenylquinaldino- and *N*-*m*-tolyl-*o*-methoxybenzohydroxamic acid) and unsaturated acids (*e.g.*, *N*-phenylcinnamohydroxamic acid) were also tested. Positive results were obtained for all of the above acids. Hydroxamic acids are generally prepared by the reaction of acid chlorides or esters of carboxylic acids with hydroxylamines. It was observed that the presence of various amounts of these parent substances did not cause interference in the detection of hydroxamic acids, the detection limits of which are within the range 5–30 μg.

The wide applicability of the proposed method is attributable to the choice of oxygen-containing solvents such as isobutyl methyl ketone, 3-methylbutan-1-ol and butan-1-ol instead of chloroform as extraction solvents for the vanadium - hydroxamic acid complexes. It appears that they are better "solvating solvents"[18] for vanadium - hydroxamic acid complexes than chloroform, owing to their greater efficiency in replacing co-ordinated water molecules from the unchelated sites of vanadium(V), thus rendering the complex hydrophobic. The extraction into the solvents used in the present study is also more rapid than into chloroform; the colour appears in the organic phase even before the shaking is begun.

Although the above method was found to be applicable to a large number of hydroxamic acids, it is suggested that oxygen-containing solvents, chloroform and benzene should be tried, in that order, as extraction solvents before confirming the absence of hydroxamic acids.

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A Raman Spectral Data Search System

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A computer-based storage and retrieval system has been devised for Raman spectral data. Unknown compounds can be identified by comparison of their spectra with the indexed library of data by means of a simple computer program. The method is illustrated by an example.

With the advent of gas lasers as excitation sources, Raman spectrometry has been increasingly applied in chemical and analytical investigations. Much work has been done on the use of computers for searching spectral-data files but to our knowledge there is no system in general use for the storage and search of Raman spectroscopic data. The IUPAC Commission I.5 on Molecular Structure and Spectroscopy has considered methods for the presentation of Raman spectra in permanent data collections. Their recommendations have been published recently,[1] but do not, of course, affect the subsequent handling of the data.

The present rate of accumulation of Raman spectroscopic data is impressive. Spectra were obtained from 300 compounds in the first year of operation of an instrument in these laboratories. In addition the American Petroleum Institute collection[2] contains 531 spectra, the Manufacturing Chemists' Association collection[3] contains 54 spectra and Schrader and Meier[4] have produced a systematic collection of the Raman and infrared spectra of about 1 000 compounds. Sadtler Research Laboratories are publishing a selection of Raman spectra[5] and to date four volumes, each containing 400 spectra, have been produced; the rate of accumulation of Sadtler spectra has been stated to be 2 000 per year. Thus at present there is access to about 3 500 spectra.

In the application of Raman spectroscopy as an analytical tool to aid the identification of unknown compounds there is a need for a system with which to handle this increasing volume of data. Further, in order to keep pace with the rapid rate of accumulation of spectra, it is desirable that such a system should be devised at an early stage. This paper describes a simple and effective computer system. The programs have been written in Fortran IVG language and the system is based on one for the retrieval of infrared spectral data,[6] which is itself based on the ASTM and Wyandotte Chemical Classification.[7]

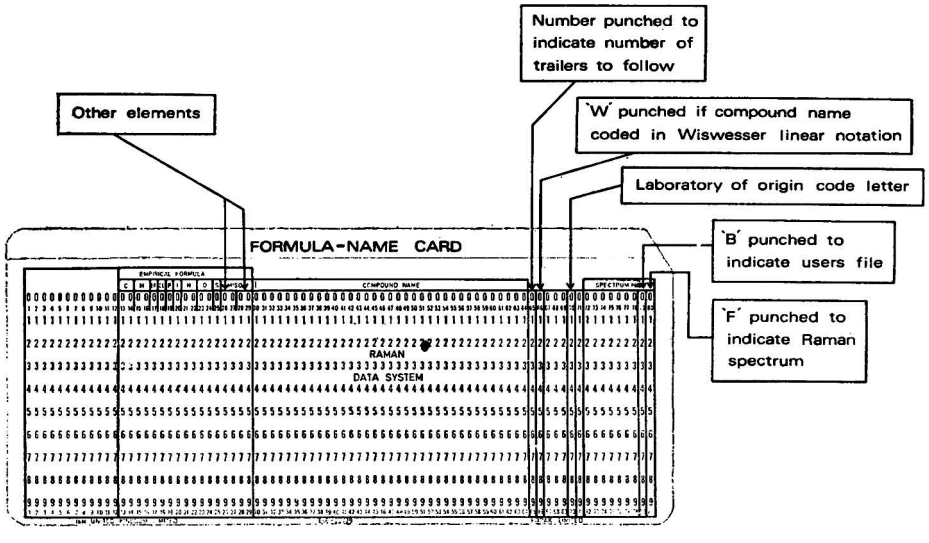


Fig. 1. Specimen Raman formula-name card.

Method

The description of the method is divided into two parts: the storage of data and the search of the data file.

Data-storage system

For each compound a minimum of three standard IBM 80 column-punched cards are used in order to code the data.

The first card, the formula-name card, is shown in Fig. 1 and is similar to that used in the Wyandotte system.[7] The spectrum number identifies each set of cards. In addition, two elements can be coded in columns 26–29 by using their international symbol but it is not possible to code the number of atoms. Thus, sodium would be coded as N in columns 26–27 and A in columns 28–29. A maximum number of three trailer cards can be used in order to complete a compound name, using columns 30–64 on each card, the number of trailer cards necessary being coded in column 65. If the name is coded in the Wiswesser Linear Notation[8] (WLN) a W is coded in column 66. By using this notation one can select classes of compounds with common functional groups so as to facilitate structure - spectra correlation studies. Table I shows the code used to identify the origin of the spectra.[7]

TABLE I

CODES FOR THE IDENTIFICATION OF SPECTRA IN COLUMN 79 OF THE FORMULA-NAME CARD

Code	Originator
A	American Petroleum Institute
B	User's file
C	Sadtler Research Laboratories
D	NBS-National Research Council
E	American Society for Testing and Materials abstracted
F	Documentation of Molecular Spectroscopy
G	Coblentz
H	Manufacturing Chemists' Association
J	Infrared Data Committee of Japan

The second and third cards contain the spectral data (Table II) and are used to record the wavenumbers of the most intense peaks in the spectrum within the range 200–2 700 cm^{-1}. Well resolved shoulder peaks can also be recorded. Values for peaks below 200 cm^{-1} are not included because many of the reference spectra have been recorded on double monochromator instruments. In these instances strong Rayleigh scattering, which obscures the Raman

TABLE II

LAYOUT OF SPECTRAL-DATA CARDS

Frequency values are recorded in cm^{-1} as integral values: the second data card must be present and coded with the serial number even if no spectral data are coded on it.

Columns	Data
First data card—	
1–6	Serial number
7–10	Blank
11–15	1st peak frequency value
16–20	2nd peak frequency value
21–25	3rd peak frequency value
76–80	14th peak frequency value
Second data card—	
1–6	Serial number
7–10	Blank
11–15	15th peak frequency
16–20	16th peak frequency
61–65	25th peak frequency
66–80	Blank

spectrum, is evident at low wavenumbers. Above 2 700 cm^{-1} the CH stretching band has little diagnostic value as this is present in the spectrum of almost all organic compounds. The peaks, a maximum of 25, can be recorded in random order although it is convenient to list them in order of frequency or intensity. The reference data relates only to Stokes Raman shifts.

After data punching, the computer is programmed to translate the data for storage on file. A flow chart for the computer program used in this operation is shown in Fig. 2. Empirical formula, (Fig. 3) and spectrum number indexes can be generated from this data file.

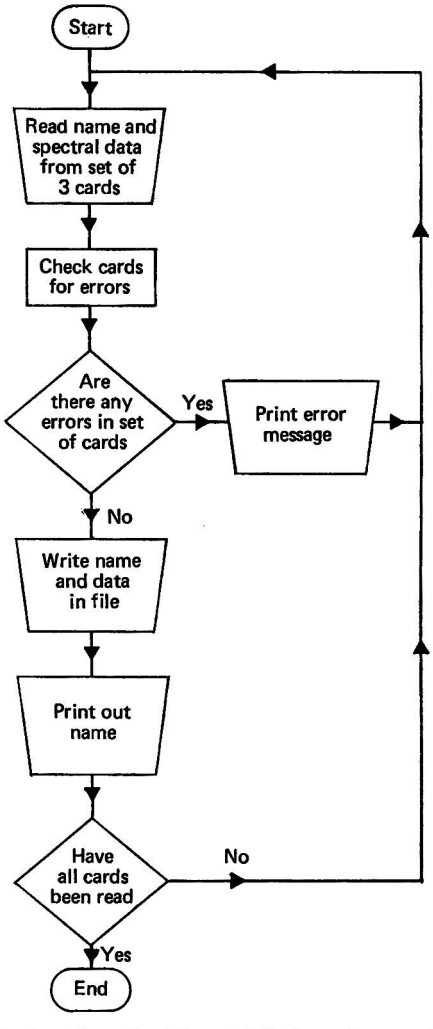


Fig. 2. Flow chart for establishing reference file.

Table III shows a tentative suggestion for the layout of an instrumental-data card. Clearly in the type of system described here this card is not essential.

Search Procedure

The method of searching involves selecting the n strongest peaks ($n \leqslant 25$) of the non-polarised Raman spectrum (usual range 2 700–200 cm^{-1}) of the sample. The strongest peak in the range is expanded to the full ordinate scale of the chart and peaks suitable for coding should

EMPIRICAL FORMULA									NAME	SPEC.NO.
C	H	BR	CL	F	I	N	O	S	MISC	
5	10	0	0	0	0	0	2	0		
									2-METHOXYETHYL VINYL ETHER	707CF
5	10	0	0	0	0	0	3	0		
									ACETIC ACID+2-METHOXYETHYL ESTER	91CF
5	10	0	0	0	0	0	3	0		
									CARBONIC ACID+DIETHYL ESTER	36CF
5	10	0	0	0	0	0	3	0		
									LACTIC ACID+ETHYL ESTER	86CF
5	10	0	0	0	0	0	4	0		
									GLYCEROL+1-ACETATE	488CF
5	10	0	0	0	0	0	5	0		
									L-(+)-ARABINOSE	644CF
5	10	0	0	0	0	2	0	0		
									3-(DIMETHYLAMINO)PROPIONITRILE	155CF
5	11	0	0	0	0	1	0	0		
									PIPERIDINE	242CF
5	11	0	0	0	0	1	1	0		
									2-PENTANONE+OXIME	690CF
5	11	0	0	0	0	1	2	0		
									DL-2-AMINO-3-METHYL-BUTYRIC ACID	426CF
5	11	0	1	0	0	0	0	0		
									1-CHLORO-3-METHYLBUTANE	317CF
5	11	0	1	0	0	0	0	0		
									1-CHLOROPENTANE	75%CF
5	11	1	0	0	0	0	0	0		
									1-BROMOPENTANE	776CF
5	11	1	0	0	0	0	1	0		
									4-BROMOBUTYL METHYL ETHER	717CF
5	12	0	0	0	0	0	0	1		
									2-METHYL-2-BUTANETHIOL	350AF
5	12	0	0	0	0	0	0	1		
									2-THIAHEXANE	207AF
5	12	0	0	0	0	0	0	1		
									3-METHYL-2-BUTANETHIOL	219AF
5	12	0	0	0	0	0	0	1		
									3,3-DIMETHYL-2-THIAHUTANE	303AF
5	12	0	0	0	0	0	1	0		
									1-PENTANOL	182CF
5	12	0	0	0	0	0	1	0		
									2-METHYL-2-BUTANOL	647CF
5	12	0	0	0	0	0	1	0		
									2-PENTANOL	636CF
5	12	0	0	0	0	0	1	0		
									2,2-DIMETHYL-1-PROPANOL	605CF
5	12	0	0	0	0	0	1	0		
									3-METHYL-1-HUTANOL	604CF
5	12	0	0	0	0	0	1	0		
									3-PENTANOL	637CF
5	12	0	0	0	0	0	2	0		
									3-METHOXY-1-BUTANOL	199CF
5	12	0	0	0	0	0	3	0		
									ORTHOACETIC ACID+TRIMETHYL ESTER	450CF
5	12	0	0	0	0	0	3	0		
									2-(2-METHOXYETHOXY)ETHANOL	621CF
5	12	0	1	0	0	1	2	0		
									BETAINE HYDROCHLORIDE	401CF
5	13	0	0	0	0	1	1	0		
									3-(DIMETHYLAMINO)-1-PROPANOL	196CF
6	2	0	0	4	0	0	0	0		
									1,2,4,5-TETRAFLUOROBENZENE	195AF
6	2	0	0	4	0	0	0	0		
									1,2,4,5-TETRACHLOROBENZENE	761CF
6	2	0	4	0	0	0	0	0		
									1,2,4,5-TETRABROMOBENZENE	779CF
6	3	0	0	0	0	3	6	0		
									1,3,5-TRINITROBENZENE	737CF
6	3	0	0	3	0	0	0	0		
									1,3,4-TRIFLUOROBENZENE	194AF
6	3	0	0	3	0	0	0	0		
									1,3,5-TRIFLUOROBENZENE	235AF
6	3	0	1	0	0	2	4	0		
									1-CHLORO-2,4-DINITROBENZENE	746CF
6	3	0	3	0	0	0	0	0		
									1,2,4-TRICHLOROBENZENE	322CF
6	4	0	0	0	2	2	5	0		
									2,4-DINITROPHENOL	669CF

Fig. 3. Print-out of reference compounds in order of their empirical formulae.

TABLE III

LAYOUT OF INSTRUMENTAL-DATA CARD

Columns	Data
1–6	Serial number
7–10	Wavelength of excitation (nm), *e.g.*, Kr^+, 647.1
11–14	Power of source (mW) measured at the sample
15–16	Geometry at sample, *e.g.*, 0 90° 1 180° 2 glancing angle 3 axial 4 transverse 5 capillary 6 liquid cell 7 gas cell 8 solid cavity 9 crystallite
17–20	Spectrometer make and model
17–18	Letter code used to describe the manufacturer, *e.g.*, CA = Cary, SP = Spex
19–20	Numerical code used to describe the model, *e.g.*, CA82 = Cary 82, SP4 = Spex Ramalog 4
21–22	Spectral slit width (in cm^{-1}) from 1–99 cm^{-1} where the limiting values describe 1 or <1 and 99 or >99 cm^{-1}
23–24	Pen period for the range 1–99 s with the same conditions affecting limiting values as for columns 21, 22
25–27	Monochromator scan speed (cm^{-1} min^{-1}) with a range 1–999 cm^{-1} min^{-1} where the lower limit describes 1 or <1
29–31	Chart speed (cm^{-1} min^{-1}) with range 1–999 cm^{-1} min^{-1}
32–33	Sensitivity of detector assuming the use of pulse-rate (photon counting) only: column 32 is the numerical pulse-rate (counts s^{-1}) and column 33 is the value of n in the multiplier 10^n
34–35	Grating blaze (nm), *e.g.*, Column 34 . . . 7 Column 35 . . . 5 × 10 = 750 nm
36–68	Numerical depolarisation ratios. Three columns each, *i.e.*, three decimal places, are used to describe 11 values. The values are punched in chronological order of the appearance of the bands at increasing shift ($\Delta\nu$) from the exciting lines. The Raman shifts for the depolarisation ratios that are recorded are marked on the Raman chart
69–80	These columns are identical with those on the other cards. Column 69 is used to punch a "T" for Trailer card if additional information is required

then have intensity values greater than 5% of that of the strongest peak. Shoulder peaks are not included unless they are well resolved.

A ± 10 cm^{-1} tolerance has been built into the search program unless otherwise specified. Thus, peaks with variations of up to ± 10 cm^{-1} can be matched with the corresponding peaks in the stored data, although a wider tolerance limit may be set in a preliminary search, *e.g.*, ± 25 cm^{-1}, because of the possibility of slight differences in reference spectra from various sources. All peaks selected for use in the search must match those in the reference spectrum within the tolerance limit.

It is possible to use negative sorting, which can reduce the number of matching spectra. Thus, a region of the spectrum where no absorption occurs is coded as a negative frequency value. In order that a negative value may cover a reasonable frequency range the tolerance is increased to that specified for a positive frequency plus 10 cm^{-1}. The total number of positive and negative frequencies must not exceed 25.

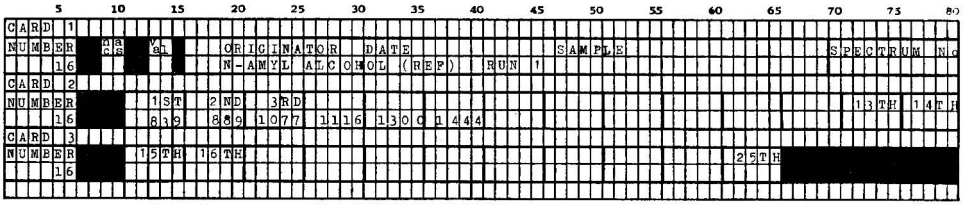


Fig. 4. Raman retrieval request form. CARD 1: NUMBER, search request serial number; NACS, number of answer compounds required (if left blank, 25 are given); VAL, value of tolerance required (if left blank, value is ± 10 cm^{-1}); NAME, name of originator, date, sample details; SPECTRUM NO, spectrum number of sample. CARD 2: NUMBER, as for CARD 1; 14 peak values on this card (coded as far to the right as possible). CARD 3: NUMBER, as for CARD 1; 11 peak values on this card (coded as far to the right as possible); this card must be included.

The search request is simplified by using a pre-printed coding form, (Fig. 4) which is self-explanatory. The input consists of the title card, which must be followed by two data cards. In the example, the Raman spectrum of pentan-1-ol (Fig. 5) has been coded by using six peaks, which are coded for convenience in order of frequencies. The search program is used to print out matching spectral curve numbers, chemical names and empirical formulae for the first 25 answers, and the total number of matching spectra is indicated. A maximum of 99 answers can be requested.

A flow chart for the computer program operating the search routine is shown in Fig. 6.

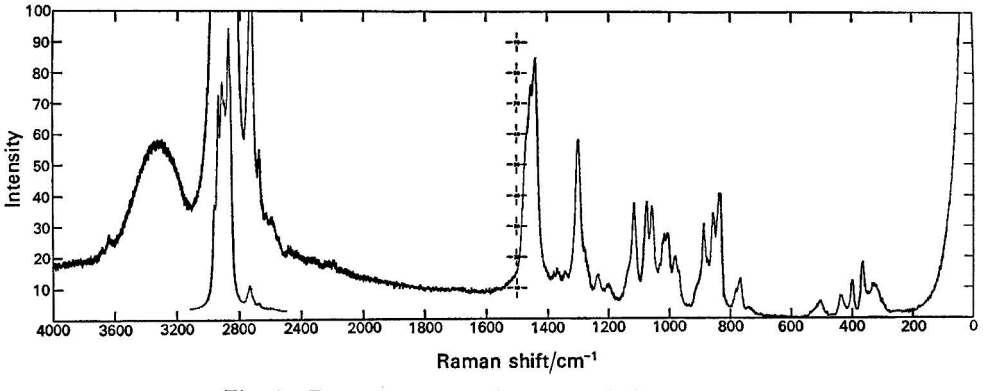


Fig. 5. Raman spectrum of test sample (pentan-1-ol).

Results and Discussion

The Raman spectrum of pentan-1-ol (Fig. 5), obtained on a Cary 82 Raman spectrometer in these laboratories, was used as a test of the whole system. This is a challenging test

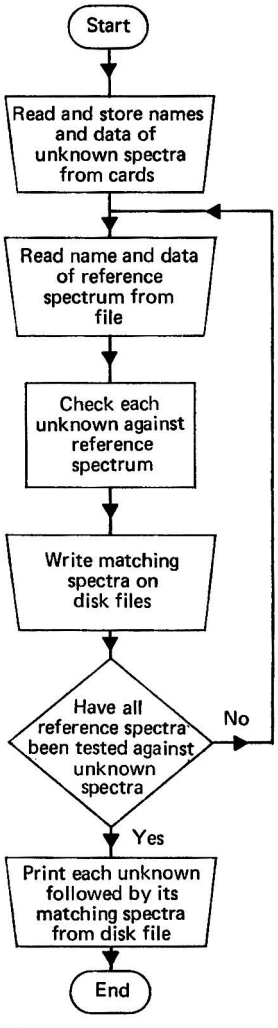


Fig. 6. Flow chart for search routine.

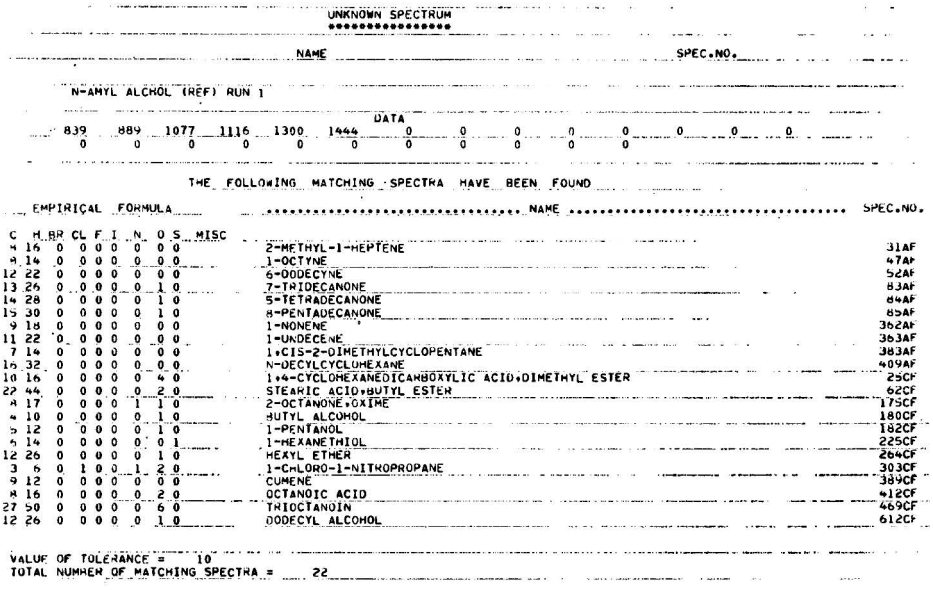
because the OH and CH groups are not strongly Raman active. The dominant peaks in the spectrum are due to aliphatic CH stretching vibrations and no other peaks present can be interpreted in terms of functional groups. Further, the spectra of the short-chain aliphatic alcohols are all similar and are difficult to distinguish from those of the corresponding ethers.

The search was carried out as shown in Fig. 4, using six relatively weak peaks, and with a tolerance of ± 10 cm^{-1}. For this search the prominent shoulder peaks near 1 050 cm^{-1} and 850 cm^{-1} have been omitted. The result of the search is shown in Fig. 7. Pentan-1-ol is among the 22 answers and the reference spectrum from the Sadtler collection was used for positive identification. Pentan-1-ol is also found in the empirical formula listing shown in Fig. 3.

When the search was repeated with the additional coding of ten negative peaks, only two matching spectra were obtained (Fig. 8): the spectra of pentan-1-ol and butanol are remarkably similar, differing only in relative peak intensities, which most search systems do not take into account.

The two variables in the search procedure are the tolerance and the number of peaks coded. It has often been found advantageous to submit a number of searches on the same spectrum

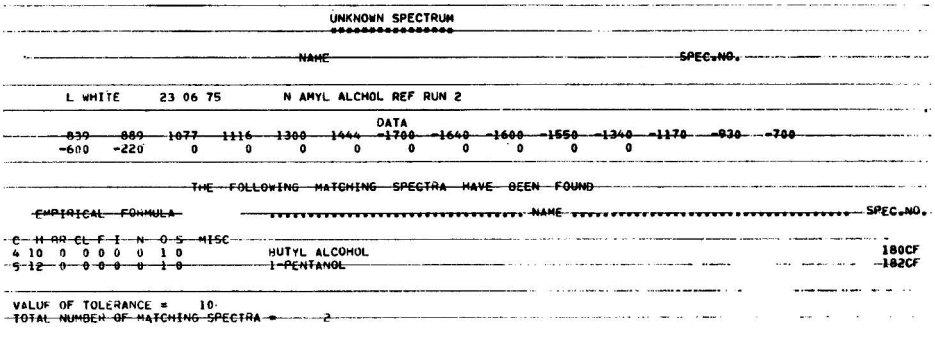
simultaneously, by means of varying these two parameters. The concordance in the different sets of answers may then reduce the number of spectra to be examined.



```
                                UNKNOWN SPECTRUM
                                ****************

NAME                                                                SPEC.NO.

 N-AMYL ALCHOL (REF) RUN 1

                                      DATA

  839   889  1077  1116  1300  1444     0     0     0     0     0     0     0     0
    0     0     0     0     0     0     0     0     0     0     0     0

                THE FOLLOWING MATCHING SPECTRA HAVE BEEN FOUND

 EMPIRICAL FORMULA                    NAME                                SPEC.NO.

 C  H BR CL F  I  N  O S  MISC
 8 16  0  0 0  0  0  0 0                2-METHYL-1-HEPTENE                    31AF
 8 14  0  0 0  0  0  0 0                1-OCTYNE                              47AF
12 22  0  0 0  0  0  0 0                6-DODECYNE                            52AF
13 26  0  0 0  0  0  1 0                7-TRIDECANONE                         83AF
14 28  0  0 0  0  0  1 0                5-TETRADECANONE                       84AF
15 30  0  0 0  0  0  1 0                8-PENTADECANONE                       85AF
 9 18  0  0 0  0  0  0 0                1-NONENE                              362AF
11 22  0  0 0  0  0  0 0                1-UNDECENE                            363AF
 7 14  0  0 0  0  0  0 0                1+CIS-2-DIMETHYLCYCLOPENTANE          383AF
16 32  0  0 0  0  0  0 0                N-DECYLCYCLOHEXANE                    409AF
10 16  0  0 0  0  0  4 0                1+4-CYCLOHEXANEDICARBOXYLIC ACID+DIMETHYL ESTER   25CF
22 44  0  0 0  0  0  2 0                STEARIC ACID+BUTYL ESTER              62CF
 8 17  0  0 0  0  1  1 0                2-OCTANONE+OXIME                      175CF
 4 10  0  0 0  0  0  1 0                BUTYL ALCOHOL                         180CF
 5 12  0  0 0  0  0  1 0                1-PENTANOL                            182CF
 6 14  0  0 0  0  0  0 1                1-HEXANETHIOL                         225CF
12 26  0  0 0  0  0  1 0                HEXYL ETHER                           264CF
 3  6  0  1 0  0  1  2 0                1-CHLORO-1-NITROPROPANE               303CF
 9 12  0  0 0  0  0  0 0                CUMENE                                389CF
 8 16  0  0 0  0  0  2 0                OCTANOIC ACID                         412CF
27 50  0  0 0  0  0  6 0                THIOCTANOIN                           469CF
12 26  0  0 0  0  0  1 0                DODECYL ALCOHOL                       612CF

 VALUE OF TOLERANCE =    10
 TOTAL NUMBER OF MATCHING SPECTRA =    22
```

Fig. 7. Print-out of answers for test search for pentan-1-ol, coding six peaks.

The simplicity of the method has enabled many successful searches to be completed by a number of personnel. The initial data file consists of 1 300 spectra, and the total search and print-out times are usually less than 1 min each. The method cannot be applied to the spectra of mixtures. The greatest restriction of any spectral matching system is usually the insufficient number of spectra in the data store. However, in the authors' experience, even with a limited store, if an exact match is not obtained the system may indicate the chemical class and structural type of the unknown compound.



```
                                UNKNOWN SPECTRUM
                                ****************

NAME                                                                SPEC.NO.

 L WHITE     23 06 75        N AMYL ALCHOL REF RUN 2

                                      DATA

  839   889  1077  1116  1300  1444 -1700 -1640 -1600 -1550 -1340 -1170  -930  -780
 -600  -220     0     0     0     0     0     0     0     0     0

                THE FOLLOWING MATCHING SPECTRA HAVE BEEN FOUND

 EMPIRICAL FORMULA                    NAME                                SPEC.NO.

 C  H BR CL F  I  N  O S  MISC
 4 10  0  0 0  0  0  1 0                BUTYL ALCOHOL                         180CF
 5 12  0  0 0  0  0  1 0                1-PENTANOL                            182CF

 VALUE OF TOLERANCE =    10
 TOTAL NUMBER OF MATCHING SPECTRA =     2
```

Fig. 8. Print-out of answers for test search for pentan-1-o coding six peaks and ten negative peaks.

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Communication

Material for publication as a Communication must be on an urgent matter and be of obvious scientific importance. Rapidity of publication may preclude the use of diagrams, but tables and formulae can be included. Communications should not be simple claims for priority: this facility for rapid publication is intended for brief descriptions of work that has progressed to a stage at which it is likely to be valuable to workers faced with similar problems. A fuller paper may be offered subsequently, if justified by later work.

Manuscripts are not subjected to the usual examination by referees and inclusion of a Communication is at the Editor's discretion.

A Novel Approach to the Elimination of Matrix Interferences in Flameless Atomic-absorption Spectroscopy Using a Graphite Furnace

Matrix interference effects of the inter-element type are known to occur in flameless atomic-absorption spectroscopy, but little is known about the exact physico-chemical processes involved. However, at least two main mechanisms may be postulated: (*i*) a depletion of the atomic vapour owing to the formation of molecular species, leading to a reduction in the height of the absorption peak, and (*ii*) a delay in the formation of the atomic vapour, leading to both height reduction and broadening of the absorption peak. Campbell and Ottaway[1,2] in their study of carbon furnaces, indicated that the carbon tube itself plays a dominant role in the formation of the atomic vapour by assisting in the reduction of the metal oxide to metal.

In this study, it was considered that the thermal destruction of an aqueous sample solution containing a water-soluble organic material would produce a "molecular mixture" of carbon and sample, which would assist in the efficient formation of the atomic vapour and also help in reducing matrix interference effects. The value of this novel approach in substantially removing inter-elemental interference effects in the systems quoted is summarised here.

The carbon furnace used was a Perkin-Elmer HGA72, installed in a 403 atomic-absorption spectrophotometer fitted with the optical modification. Background correction was used throughout the study. A transient recorder and a conventional chart recorder were used simultaneously in order to permit peak shapes to be studied. Ascorbic acid, tartaric acid and sucrose were chosen as suitable water-soluble materials. The separate additions of 1% of ascorbic acid, tartaric acid and sucrose to single-element solutions of 0.1 μg ml^{-1} of lead (as nitrate), 0.02 μg ml^{-1} of copper (as sulphate) and 1.0 μg ml^{-1} of gallium (as nitrate) in either 0.1% hydrochloric or nitric acid, gave rise to enhancements of the absorption peak height and area. The magnitude of the enhancement depended on both the element investigated and the organic material used. Ascorbic acid was found to give the greatest enhancements of peak height, ranging from a factor of about 1.4 for lead to 1.95 for copper and 20 for gallium. No significant blanks were obtained from ascorbic acid.

A short study was then carried out on the analysis of lead. Volumes of 50 μl were subjected to the following programme: drying at 100 °C for 45 s, thermal destruction at 450 °C for 30 s and atomisation at 2 080 °C for 7.5 s as shown on the instrument temperature indicator. The gas-stop facility was not used. Solutions of 0.1 μg ml^{-1} of lead (as nitrate) in 0.1% nitric acid or hydrochloric acid were analysed singly and in the presence of 100 μg ml^{-1} concentrations of calcium, magnesium, strontium and barium, and any interferences were noted. The interferents were added as their nitrates to the nitric acid solutions and as their chlorides to the hydrochloric acid solutions. The analyses were also carried out on similar solutions containing 1% of ascorbic acid in order to determine its effectiveness in reducing inter-element interferences. The results obtained are shown in Table I. It can be seen that the addition of ascorbic acid at the 1% level almost completely overcame the interferences observed. Even in the worst case, that of magnesium in the hydrochloric acid system, suppression was reduced from 88 to 7% on the addition of ascorbic acid. Increasing the ascorbic acid concentration to 2% did not overcome this suppression further.

The examination of peak shapes showed two types of suppression. For example, calcium in the nitrate system resulted in lower but broader peaks, whereas in the chloride system the peaks were

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TABLE I

COMPARISON OF PEAK HEIGHTS* FROM 0.1 μg ml^{-1} OF LEAD WITH AND WITHOUT 1% OF ASCORBIC ACID IN SOLUTIONS CONTAINING 100 μg ml^{-1} OF OTHER METALS

	0.1% Hydrochloric acid		0.1% Nitric acid	
Sample	No ascorbic acid	1% Ascorbic acid	No ascorbic acid	1% Ascorbic acid
Pb	100	141	100	144
Pb + Ca	64	142	83	142
Pb + Mg	12	131	133	137
Pb + Sr	77	138	86	139
Pb + Ba	83	143	99	145

* Mean of at least four injections; coefficient of variation better than 5%.

lower but of "normal" shape. The former could possibly be overcome by the use of peak area measurements, but the latter, obviously, would not be amenable to such treatment. The use of ascorbic acid on lead plus interferent systems, however, restores peak shapes to that of lead plus ascorbic acid without interferent. Fig. 1 illustrates the peak shapes obtained for lead plus calcium in hydrochloric acid.

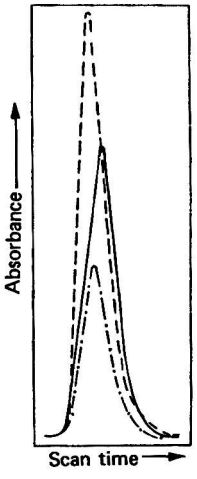

Fig. 1. Peak shapes obtained from 0.1 μg ml^{-1} lead solutions, showing the interference effects of 100 μg ml^{-1} of calcium in 0.1% hydrochloric acid and the influence of 1% m/V of ascorbic acid on this system: ———, 0.1 μg ml^{-1} of Pb in 0.1% HCl; –·–·–, 0.1 μg ml^{-1} of Pb + 100 μg ml^{-1} of Ca in 0.1% HCl; and – – – –, 0.1 μg ml^{-1} of Pb + 100 μg ml^{-1} of Ca + 1% m/V of ascorbic acid in 0.1% HCl.

The direct determination of lead in six natural waters, with calcium and magnesium concentrations ranging from 3 to 100 and 1 to 30 μg ml^{-1}, respectively, was successfully carried out by addition of 1% of ascorbic acid to both the samples and standards using the conditions described above. Additions of 0.05 μg ml^{-1} of lead gave apparent values of 0.008–0.039 μg ml^{-1} for untreated waters and of 0.048–0.051 μg ml^{-1} for waters treated with ascorbic acid. The results for "natural" lead present in these samples also increased by the appropriate factors. In the course of these analyses, it was noted that the enhancement of lead varied between 10 and 40%, depending on the tube used. However, for each analytical determination performed on the same tube, the enhancement was constant. In any event, the magnitude of the enhancement did not influence the effectiveness of ascorbic acid in substantially reducing interferences.

Further studies will be carried out on other elements and matrices to determine the extent and range of the "releasing" action of ascorbic acid in flameless atomic-absorption spectroscopy generally.

References

1. Campbell, W. C., and Ottaway, J. M., *Talanta*, 1974, **21**, 837.
2. Campbell, W. C., and Ottaway, J. M., *Proc. Soc. Analyt. Chem.*, 1974, **11**, 161.

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Book Reviews

ENVIRONMENTAL CHEMISTRY. Volume 1. *Specialist Periodical Report.* Senior Reporter: G. EGLINTON. Pp. xii + 199. London: The Chemical Society. 1975. Price £7.

This report is sub-titled "A review of the recent literature concerning the organic chemistry of environments" and includes material published up to mid-1973. Eleven authors contribute to this volume, covering organic material in water and sediments in rivers, lakes, bogs, marshes, swamps, oceans, fjords and anoxic basins; biological cycling is studied using stable isotopes; and hydrocarbons, DDT and PCBs in the marine environment and 2,4-dichlorophenoxyacetic acid are also discussed. The treatment is comprehensive, readable and illuminating and contains much material from the literature before the 1970s in order to give a more complete picture. It is intended for chemists and non chemists engaged in environmental studies, and thus contains more elementary detail (tables of amino-acids, formulae of monosaccharides, etc.) than is usual in a Specialist Periodical Report. The Report serves to emphasise the large amount of information that is available on the vast number of organic compounds present in the aqueous environment, at the same time indicating the many areas in which information is lacking. All of the studies rely heavily on suitable analytical procedures, a fact that is acknowledged in three of the articles. Only in the chapter on 2,4-D, however, is there more than a cursory discussion of analytical aspects. Perhaps this will be remedied in a future volume.

The Report is exceptionally valuable in that it brings together information from a wide variety of sources, and summarises them in a logical manner. It will therefore be very useful as a source book to all who are engaged in environmental studies—research workers, doomwatchers and students alike.

A. TOWNSHEND

ANNUAL REPORTS ON ANALYTICAL ATOMIC SPECTROSCOPY, REVIEWING 1974. Volume 4. Pp. xii + 268. London: The Chemical Society. 1975. Price £12.

There is no doubt that this publication continues to provide a valuable condensation of the year's activity in the field of analytical atomic spectroscopy. That it is effective in providing up to date information is the result of the continuing activities of the correspondents in obtaining information from symposia and conferences, frequently well in advance of the publication of the material in scientific journals. This is a procedure which is of considerable benefit to those who through occupation or geography are unable to meet regularly with fellow scientists and thus obtain early news of developments.

In the reviews themselves the presentation is generally professional and leaves little or nothing to be desired in terms of clarity or comprehensiveness. The only minor reservation that I have is that in the interests of brevity the reviewers have occasionally not included what I judge to be sufficient data from the less accessible references; perhaps my own geographical isolation makes me more conscious of this problem.

Notwithstanding that this is already an excellent publication, which I recommend to all analytical spectroscopists, it seems to me that the incoming Editor might consider for future volumes the inclusion of a brief summary of the year's activities, outlining the areas in which progress appears to be most promising and perhaps those in which progress needs to be made. Opinions stated in this summary could be of assistance to both developers and users, the former by identifying real research problems, and the latter by indicating the relevance of new developments.

JOHN AGGETT

NANOGEN INDEX. A DICTIONARY OF PESTICIDES AND CHEMICAL POLLUTANTS. Edited by KINGSLEY PACKER. Pp. 256. Freedom, California: Nanogens International. 1975. Price £13·50; $32 (hardback); £10; $24·50 (softback).

The Nanogen Index lists all known chemicals that are used as pesticides, and control chemicals and chemical pollutants, with about 10 000 entries covering information up to the beginning of 1975. The compounds are listed alphabetically within nine sections: organic pesticides; organometallic pesticides; inorganic agricultural chemicals; experimental pesticides; degradation products and metabolites; drugs used in agriculture and food production; other agricultural and food processing materials; other environmental contaminants; and recently introduced control chemicals.

There is also a functional group directory, a numerical index, an alphabetical index and an index of new control chemicals. An updating service providing details of recently introduced control chemicals is available.

For each material, the following information is given: code (a three-letter system for coding each chemical unequivocally); common names (names officially approved by designated bodies, *e.g.*, BSI and ISO, are given, together with other names recommended or used in other countries, *e.g.*, Germany, Russia, Italy, South Africa); chemical names; structure; "details" (includes occurrence, properties, whether registered or approved for specific uses, cross-references to metabolites, general information); other names (trivial names and trade names); and uses. No information is given on toxicities, chemical properties or methods of analysis.

Many of the compounds are available from Nanogens International as analytical standards at concentrations of 1, 10, 100 or 1000 p.p.m., usually in solution in benzene or cyclohexane, and all such compounds are indicated.

The information is clearly laid out in tabular form. This comprehensive dictionary will provide indispensable reference data for all workers involved with the chemistry and analysis of these substances, especially for those who are unfamiliar with the complex nomenclature and the profusion of common names.

P. C. WESTON

EXTRACTION CHROMATOGRAPHY. Edited by T. BRAUN and G. GHERSINI. *Journal of Chromatography Library, Volume* 2. Pp. xviii + 566. Amsterdam, London and New York: Elsevier Scientific Publishing Company. 1975. Price Dfl130; $54·25.

The application of organic reagents that are commonly used in liquid - liquid extraction to chromatographic separation by retaining them on a solid support has been investigated in depth only within the last 20 years, but over that period some hundreds of papers have been published about the technique, now called extraction chromatography. While the method has, perhaps, not yet been accepted into the mainstream of experimental usage for analytical separations, sufficient valid applications have been reported to demonstrate the value of this type of procedure. Indeed, those laboratories which have applied extraction chromatography on a regular basis for over a decade to difficult separations, such as that of individual rare earths, have demonstrated that the method is well able to complement other, more familiar, two-phase separations, such as ion exchange and solvent extraction. It is therefore timely that a book should be produced which considers the present state of the art, and this publication contains 14 chapters by various authors describing different aspects of the development and application of extraction chromatography. These cover theoretical aspects, capabilities of stationary phases and characteristics of inert supports, application to separation of metallic and non-metallic ions, actinides, lanthanides and fission products. The use of the technique in radiotoxicology, for trace-metal pre-concentration and separation, is also considered and a bibliography is included.

This is a useful book, which collects together a great deal of information about the design and application of extraction chromatography and it is likely to be of interest both to those wishing to obtain background information about the capabilities of the technique and to those wanting to apply it. While some chapters perhaps do not compare critically enough the merits of extraction chromatography with other competitive separation techniques, the over-all usefulness of the method is clearly demonstrated.

T. B. PIERCE

THE INTERPRETATION OF INFRARED SPECTRA—A PROGRAMMED INTRODUCTION. By R. R. HILL and D. A. E. RENDELL. Pp. xii + 196. London, New York and Rheine: Heyden and Son Ltd. 1975. Price £4·40; $12; DM36.

This book has undergone an objective validation programme and has been revised three times through the co-operation of students and supervisors in 10 teaching establishments. The authors claim that their text enables the average student, with little or no prior knowledge of the subject, to increase his test performance from 15–40 to 85–90 per cent. The text is clearly written, and ways have been found to eliminate the need, common in this type of book, for a considerable proportion of pages to carry only a few lines of text.

The book is divided into five parts: "Basic Principles," "Experimental Considerations," "Characteristic Group Frequencies and the Empirical Method," "Infrared Absorption Characteristics of the Common Classes of Organic Compounds" and "Problems." In addition to the pages occupied by "Answers," "List of Compounds," and "Subject Index," a large sheet showing five correlation tables fits into a pocket in the back cover.

The clear diagrams and formulae, the excellent reproductions of model infrared spectra and the wide range of suggestions for further reading, are all features of this book. The text is not completely free of misprints, but those which were noticed did not appear likely to cause confusion in the minds of students. The last page of this programmed text asks students to appreciate that it provides an introduction to the uses of infrared spectroscopy and covers only the most commonly occurring correlations. As such, this book appears to be sensible and sound; the price is reasonable, the quality of production is high and it is to be hoped that generations of students will benefit by using it intelligently. D. M. W. ANDERSON

ALDEHYDES—PHOTOMETRIC ANALYSIS. Volumes 1 and 2. By EUGENE SAWICKI and CAROLE R. SAWICKI. *The Analysis of Organic Materials, Number* 9. Volume 1: pp. xxviii + 283. Volume 2: pp. xiv + 344. London, New York and San Francisco: Academic Press. 1975. Price: Volume 1, £10.50; Volume 2, £10.80.

This series (Volume 3 is in preparation) deals with the spectrophotometric analysis of aldehydes and their precursors. It is stated that ultraviolet and visible spectrophotometry is now by far the most widely used analytical technique. Aldehydes yield a vast assortment of derivatives with their own absorption, fluorescence or even phosphorescence spectra, and mixtures can often be separated by one or another form of chromatography.

Acrolein affords a convenient illustration of the treatment. Ultraviolet absorption maxima with $m\epsilon$ values are recorded in different solvents. The various reagents used in the analysis are tabulated, giving spectroscopic data, determination limits, analysis times, interferences and references. In this instance a fluorescence method based on the product formed by the interaction of 3-aminophenol and acrolein is very sensitive, with crotonaldehyde as the main interfering substance. Several other reagents are noted and detailed procedures are described.

A section on aliphatic aldehydes contains much information (254 references), including spectral properties of parent substances and derivatives. A colorimetric procedure applied to dinitrophenylhydrazones permits the simultaneous determination of alkanals, alk-2-enals and alk-2,4-dienals and several tested chromatographic applications are fully described, with practical examples.

A similar section on aromatic aldehydes reviews a complex literature and later sections cover in detail benzaldehyde, cinnamaldehyde, coniferaldehyde and related substances. Other compounds covered are citral, chloral, crotonaldehyde, formaldehyde and aldosterone.

Volume 2 has substantial sections on furfural, glyceraldehyde, glycolaldehyde and related compounds. There are interesting treatments of malonaldehyde, pyruvaldehyde, succinaldehyde and their derivatives. Various benzaldehyde derivatives (including hydroxyaldehydes) are fully surveyed. The authors supply much useful information about retinal and pyridoxal, assembled neatly, while the treatment of $\alpha\beta$-unsaturated aldehydes and of vanillin is convincing. Short sections deal with naphthaldehydes, streptomycin and pyrene-1-aldehyde as well as with two indolealdehydes.

The authors have combed the literature assiduously and have marshalled the data with skill and good judgement. The fact that a third volume is promised in the near future inhibits criticism based on omissions. Retinal was discussed without reference to the use of manganese(IV) oxide in converting unsaturated alcohols into aldehydes. The procedure is a versatile one, has been reviewed and deserves full discussion.

A justifiable element of repetition makes it likely that the value of these two volumes will "snowball" for those who use them most. The claim is made that the range of methods described affords "a cutting edge" of investigations ranging from environmental pollution via general plant and animal biochemistry to liver damage, ageing and even cancer. It can be agreed that analysts who follow the general approach and master the technical aspects of one or two of the areas discussed will be equipped to cope with new issues as they arise. R. A. MORTON

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A Semi-automated Method for the Determination of Inorganic, Organic and Total Phosphate in Sediments

A simple, rapid and semi-automated method for the determination of inorganic, organic and total phosphorus in lake and river sediments is described. Total phosphorus is extracted from sediments with 1 N hydrochloric acid after ignition at a high temperature (550 °C) or by digestion with sulphuric acid - potassium persulphate at 135 °C in a sealed PTFE-lined Parr bomb. Organic phosphorus is determined by the difference in phosphorus content of the 1 N hydrochloric acid extract measured before and after ignition of the dry sediments at 550 °C. In all instances the orthophosphate is determined by using standard Technicon AutoAnalyzer II techniques. The interferences caused by silica and variable acid concentrations on the determination of phosphorus have been studied. Freedom from interferences under the chosen experimental conditions as well as the good results obtained for recovery and precision indicate that the methods are suitable for monitoring inorganic, organic and total phosphorus in sediments.

K. I. ASPILA, HAIG AGEMIAN and A. S. Y. CHAU

Canada Centre for Inland Waters, Water Quality Laboratory, P.O. Box 5050, 867 Lakeshore Road, Burlington, Ontario, L7R 4A6, Canada.

Analyst, 1976, **101**, 187–197.

Determination of Polyoxyethylene Alkylphenyl Ether Non-ionic Surfactants in Waters

A method is proposed for the determination of polyoxyethylene alkylphenyl ethers, $RO(CH_2CH_2O)_nH$ (where R is an alkylphenyl group), at the 1–0.1 mg l^{-1} level in waters. The method is based on the spectrophotometric determination of the complexes of the polyethers with the sodium cation after extraction as picrates into 1,2-dichloroethane. The number-average degree of polymerisation ($\bar{n}$) is evaluated by thin-layer chromatography or by gas chromatography.

The method was tested on commercial compounds that have R = *p-tert*-nonylphenyl ($3.3 \leqslant \bar{n} \leqslant 21.5$). For typical surfactants ($6.5 \leqslant \bar{n} \leqslant 21.5$) the proposed method is about eight times more sensitive than that based on the ammonium tetrathioisocyanatocobaltate(II) reagent and it gives results for the absolute concentration with an accuracy ranging from −6 to +8 per cent. in the indicated interval of $\bar{n}$. The precision was evaluated from the analysis of waste waters. A few interferences are discussed.

L. FAVRETTO and F. TUNIS

Istituto di Merceologia, Università di Trieste, 34100 Trieste, Italy.

Analyst, 1976, **101**, 198–202.

Catalymetric - Thermometric Titration of Some Derivatives of Barbituric Acid

The compound barbitone, and the related compounds amylobarbitone, butobarbitone, hexobarbitone, phenobarbitone and quinalbarbitone, have been determined by catalymetric - thermometric titration involving an acid - base reaction in the analyte followed by a base-catalysed indicator reaction. The base-catalysed reactions used were the dimerisation of acetone and the polymerisation of acrylonitrile with dimethylformamide as a matrix diluent. The effects of excipients are reported, and a method of assay of dosage forms is proposed.

L. S. BARK and O. LADIPO

Ramage Laboratories, University of Salford, Salford, M5 4WT.

Analyst, 1976, **101**, 203–208.

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A Method for the Detection of Microgram Amounts of Hydroxamic Acids

A simple and rapid method for the detection of trace amounts of hydroxamic acids is presented. It consists in extraction of the coloured complex formed by the reaction of hydroxamic acids (5–30 μg) with vanadium into one drop of isobutyl methyl ketone or a water-immiscible alcohol. The applicability of the method was tested with a variety of hydroxamic acids. It was established that carboxylic acids, their chlorides or esters, and hydroxylamines do not interfere.

SHAHID ABBAS ABBASI

Department of Chemistry, Indian Institute of Technology, Powai, Bombay-400 076, India.

Analyst, 1976, **101**, 209–211.

A Raman Spectral Data Search System

A computer-based storage and retrieval system has been devised for Raman spectral data. Unknown compounds can be identified by comparison of their spectra with the indexed library of data by means of a simple computer program. The method is illustrated by an example.

I. A. DEGEN, Miss L. BIRMINGHAM and G. A. NEWMAN

Research Division, Kodak Limited, Headstone Drive, Harrow, Middlesex, HA1 4TY.

Analyst, 1976, **101**, 212–219.

A Novel Approach to the Elimination of Matrix Interferences in Flameless Atomic-absorption Spectroscopy Using a Graphite Furnace

Communication

J. G. T. REGAN and J. WARREN

Department of Industry, Laboratory of the Government Chemist, Cornwall House, Stamford Street, London, SE1 9NQ.

Analyst, 1976, **101**, 220–221.

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