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Determination of Residues of Organophosphorus Pesticides in Foods

A Review*

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Summary of contents

Methods for the determination of residual organophosphorus insecticides were thoroughly reviewed in 1961 by Chilwell and Hartley.[1] This has recently been supplemented by a special review by Bache and Lisk[2] of the application of emission spectroscopy to organophosphorus pesticide residue determinations, while biennial reviews on pesticide residue analysis have been published by Egan,[3,4] and Abbott and Thomson.[5] The purpose of the present paper is to supplement these reviews in the light of the considerable developments in residue analysis and of the introduction of new compounds in the intervening 6 years. British Standard names of pesticides are used wherever appropriate.[6] Some of the new compounds have been introduced specifically to replace certain of the organochlorine insecticides and acaricides, residue methods for which were reviewed recently by Beynon and Elgar.[7] There have also been several general works on residue analysis in recent years; these represent a new feature in the modern literature of residue analysis.

As pointed out in the 1961 review, the choice of analytical methods will, to some extent, depend on the circumstance in which the residue information is required. The three principal circumstances are the evaluation of the residue situation for a new pesticide for clearance in official safety approval procedure; the examination of a sample of food specifically for an individual pesticide; and the general examination of foods of unknown agricultural history for undesirable residues of any pesticide. The principal development in residue analysis in the past 6 years has been the establishment of multi-detection systems, wherein residues of all or any of a number of substances within a group, or even within a number of different groups, can be both separated and determined by a single chromatographic procedure. Such methods were first developed for the persistent organochlorine compounds, although the possibility of the extension of gas - liquid chromatographic methods to organophosphorus compounds was clearly recognised by Goodwin, Goulden and Reynolds[8] at an early stage. Some general practical considerations for the application of gas - liquid chromatography to organophosphorus compounds were described by Egan, Hammond and Thomson.[9] The application of paper chromatography to the determination of organophosphorus pesticide residues has been reviewed by Getz,[10] and thin-layer chromatographic methods for the determination of residues of pesticides (including organophosphorus compounds) have been thoroughly reviewed by Abbott and Thomson.[11]

The general multi-detection systems of analysis are of special value in organophosphorus pesticide residue problems, as not only do they enable more than one compound to be handled

* Reprints of this paper will be available shortly. For details see Summaries in advertisement pages.

but they also allow a fuller understanding of the nature and extent of the intermediate and terminal breakdown compounds that may be present. Indeed, the evaluation of the significance of breakdown compounds is one of the chief difficulties in organophosphorus residue analysis and whereas to some extent it can be taken into account by the older total-phosphorus methods by adjustment of the initial conditions of sample extraction, this is an arbitrary procedure. A closer understanding is required of the stability and toxicological significance, especially to mammals, of the breakdown products, as without this knowledge residue measurement for regulatory control purposes may itself also be largely arbitrary. This subject has been considered by the International Union of Pure and Applied Chemistry, which has appointed a commission to deal with problems arising.

Barry, Hindley and Johnson[12] have reviewed chromatographic systems developed by Getz[13,14] and Kovacs[15]; Kovacs, for example, described a thin-layer system responding only to sulphur-containing esters, the lower limits of detection for which range from 0·1 to 0·5 μg. But although there has been considerable progress in the development of general screening methods for the detection of organophosphorus residues in foods, a complete quantitative multi-detection system is not yet available.

In the discussion of general techniques and of methods for individual pesticides which follows, the literature has been reviewed to January, 1967.

Extraction procedures

The extraction of residues of organophosphorus pesticides and their metabolites from various sample substrates poses several difficulties. Not only does the nature of the sample under examination govern the process to be applied, but the wide range of polarities encountered in these compounds makes it essential to use a general solvent for preliminary extraction if residues of unknown compounds are being sought. Such solvents also remove considerable amounts of co-extractives that are apt to complicate clean-up stages. The examination of samples from field trials of experimental pesticides is easier as solvents suitable for the known compounds may often be chosen so as to limit such interferences.

It is probably fair to say that more analyses for residues of organophosphorus pesticides have been performed on samples of vegetable tissue than on any other type of material. Extraction solvents are usually chosen from amongst benzene,[16,17] chloroform,[18] dichloromethane,[19] acetonitrile,[20] ketone - hexane mixtures,[9] ethyl acetate,[21] acetone[22] and methanol,[23] often in the presence of anhydrous sodium sulphate to aid in the liberation of the more water-soluble compounds. The chopped or shredded sample is usually treated with the chosen solvent, under vigorous agitation, in a top-drive macerator or similar apparatus; frequently centrifugal action is required to break the emulsion formed and filtration of the solvent extract may also be essential. Deep-freezing of the produce, followed by rapid thawing in the presence of solvent, may be used to shatter the cell structure, thus giving ready release of the contents which may hold systemic pesticide residues.[24] A more efficient clean-up technique may, subsequently, be required when such methods are used. When surface residues of relatively non-polar compounds are under examination the extraction procedure may be greatly simplified merely by rinsing the produce with hexane or benzene, thus severely limiting the amount of co-extractives released.

The extraction of pesticide residues from soil and water offers fewer problems in general. Soils are best dehydrated by trituration with anhydrous sodium sulphate before Soxhlet extraction with benzene or dichloromethane, although some polar compounds may cling tenaciously to certain types of soil. For water samples, direct extraction with hexane or benzene may be suitable for non-polar compounds, acetonitrile, dimethylformamide or ethyl methyl ketone being used as general extractants. Animal tissue has been rather neglected as a sample substrate, with the exception of those pesticides used in veterinary practice. These tend to be non-polar in character and often behave similarly, as far as extraction procedures are concerned, to the widely used organochlorine pesticides. Gentle maceration or Soxhlet extraction with hexane[25] followed by partition clean-up procedures yields suitable extracts for further examination by most end-methods. Milk has been extracted with acetone and benzene for detecting residues of azinphos-methyl.[26] Bunyan and Taylor[27] have described the preparation of avian tissue extracts for the starch-gel electrophoretic evaluation of phorate residues.

Clean-up procedures

A useful review of clean-up procedure in pesticide residue analysis has been prepared by McKinley, Coffin and McCully.[28] It is unusual for a simple sample extract to be immediately suitable for quantitative examination. Usually 1 or 2 preliminary clean-up stages are required to ensure a sufficient degree of purity for meaningful results to be obtained. Chromatographic purification through columns or layers of absorbents, solvent-partition procedures and distillation techniques are frequently used for this purpose. The columnar chromatographic clean-up procedure of Laws and Webley,[19] often used allied to a total-phosphorus determination end-method, was also of use for thin-layer or gas-chromatographic methods. The dichloromethane extract was first partitioned between light petroleum and aqueous methanol (15 per cent. v/v), which separated the organophosphorus pesticides into petroleum-soluble and water-soluble fractions. Transfer of the former fraction to an alumina column (Brockman V) and elution with light petroleum gave clean extracts of such compounds as disulfoton, diazinon, parathion and phorate; further elution with a solution of diethyl ether in light petroleum (15 per cent.) liberated the more polar compounds, malathion and azinphos-methyl. The water-soluble fraction was cleaned-up by elution with chloroform from a carbon column; demeton-methyl, dimethoate, mevinphos and phosphamidon may thus be obtained substantially free from organic phosphorus of plant origin. Clean-up in this way served also as a partial classification step; a similar procedure has been used by Blinn.[29] Several other column clean-up materials have been used, including Florisil,[17,30,31] magnesium oxide[9,22] and carbon - Celite mixtures.[20,32,33] Thin-layer chromatography was often used as a clean-up procedure, but as this was usually closely allied with quantitative and identification purposes, this aspect of clean-up will be dealt with later. Fischer and Klingelhöller[34,35] showed an original approach to the extraction and clean-up problem. They extracted samples with hot ethanolic potassium hydroxide, thereby saponifying both the fatty materials present and the organophosphorus pesticides under examination. The latter were then identified by the thin-layer chromatographic patterns given by the fragments, up to four spots being observed from some compounds.

Solvent partition between hexane and acetonitrile,[36] dimethylformamide[25] or nitromethane[37] has been as useful for the clean-up of organophosphorus pesticides extracted from fatty samples, as it was for organochlorine residues.[36,38] Chloroform - water partition systems have been used for extracts of vegetable tissue.[39,40]

The weakly basic properties of menazon have been used to obtain excellent clean-up by means of absorption on to a cation-exchange resin, subsequent elution with ammonium acetate solution yielding the pesticide free from naturally occurring phosphorus compounds.[23] For thermally stable, fairly volatile pesticides, the sweep or co-distillation clean-up proposed by Storherr and Watts[41] offered considerable advantages. This took only 20 minutes and did not need specialised absorbents or equipment, large volumes of expensive solvents or numerous transfers. The procedure was designed to be used before microcoulometric gas-chromatographic determination but should be adaptable to other end-methods.

Methods of detection and determination

Spectroscopic and allied methods—

Procedures involving the determination of residues of organophosphorus pesticides by total-phosphorus measurements have been long established, and are still of great use, particularly when known compounds are under examination. When residues of unknown materials are being sought, stringent extraction and clean-up techniques are essential in order to exclude adventitious or naturally occurring non-pesticidal phosphorus. The procedure described by Laws and Webley[19] has been widely accepted as generally applicable in this latter case. The columnar clean-up has already been described; phosphorus was determined as a molybdenum-blue complex after extraction of the phosphomolybdic acid into an isobutanol - benzene mixture. Although this method divided the original extract into three parts before final determination, thus giving some measure of differentiation, additional identifying procedures must be used for valid results to be ensured. Brewerton[42] has preferred to use perchloric acid alone for the combustion of concentrated extracts, instead of the mixed acids used by Laws and Webley.[19] Following a similar column clean-up, Blinn[33] used a Schöniger-flask combustion of the extract before a molybdenum-blue end-method, with 1-amino-

2-naphthol-4-sulphonic acid as reductant. This essential combustion stage has also been improved by Getz,[43] who used a solution of ammonium persulphate, thus shortening the reaction time, avoiding transfer losses and eliminating the handling of the strong acids used in conventional digestion procedures. The molybdenum-blue determination of phosphorus lent itself to automated techniques quite well, as has been described by Winter and Ferrari.[44] Unfortunately, the laborious sample preparation, homogenisation, extraction and clean-up stages of a residue analysis were far less amenable to automatic applications. The sensitivity of the molybdenum-blue procedures could be enhanced by a factor of over 10, by determining the molybdenum content of the phosphomolybdic acid instead of reducing it in the usual way,[45,46] although the time required for each analysis was necessarily lengthened.

The use of 4-(*p*-nitrobenzyl)pyridine has been advocated[47] for the rapid quantitative colorimetric determination of organophosphorus residues. The reaction occurred within 10 minutes at between 175° and 180° C, giving complexes with absorption maxima at about 520 mμ and sensitivities around 2 μg. Little clean-up was required for strawberries, potatoes or apples at a 1 p.p.m. level, but carrots, spinach and lettuce required thorough clean-up to avoid interference. For certain individual organophosphorus pesticides it was possible to devise more specific colorimetric procedures. Azinphos-methyl has been determined by hydrolysis to anthranilic acid, diazotisation and coupling with *N*-1-naphthylethylenediamine[30]; a collaborative study of this procedure[48] has been described. A version of this method involving the use of direct coupling with the amine offers some advantages in speed of analysis and improved recoveries. Alkaline hydrolysis of extracted fenitrothion residues to yield the yellow 3-methyl-4-nitrophenate has been used[49] for the determination of this pesticide in milk. Similarly, hydrolysis to the corresponding phenols was applicable[50] to residues of fenchlorphos and dichlofenthion, 4-aminophenazone being used as a reagent.

Considerable attention has been directed to colorimetric procedures for residues of dimethoate; Enos and Frear[39] extracted the pesticide from hexane solutions with hydrobromic acid, boiled to hydrolyse, with resulting liberation of hydrogen sulphide, and then converted this to methylene blue for colorimetric measurement. A complex formed between dimethoate and 1-chloro-2,4-dinitrobenzene in the presence of methanolic sodium hydroxide was claimed by George, Walker, Murphy and Giang[51] to have a greater degree of specificity and to be less subject to interference from co-extractives than several other methods.

Because it required a more stringent clean-up technique, infrared spectroscopy was generally less useful than visible spectrophotometry. The uses of spectral measurement in these regions had been reviewed by Blinn,[52] who concluded that their advantages lie in that very low concentrations of a compound may often be used and that the measurements were essentially non-destructive and specific. The infrared spectra of some organophosphorus pesticides were described earlier by McCaulley and Cook,[53] who underlined some of the difficulties encountered in the interpretation of spectra and the necessity for the specimens examined to be as pure as possible. To achieve this latter standard, Crosby and Laws[54] have used semi-preparative gas chromatography to prepare the specimens. This technique has been extended and combined with thin-layer chromatography[55] to form a system for the detection, identification and determination of organophosphorus pesticide residues. Further details of this work are given later in the relevant sections. Mention might also be made here of the use of spectrophotofluorometric measurements for the determination of azinphos-methyl residues.[26]

The application of polarography to residue analysis has not been widely pursued, probably because of difficulties induced by the need for excellent prior clean-up for results to be fully meaningful. A useful review of this subject has been given by Gajan.[56] Bates[57] has used polarography for determining residues of azinphos-methyl, and other organophosphorus pesticides have been studied by Gajan.[58,59]

Paper chromatography—

As in almost every other field of micro or trace analysis, paper chromatography brings useful attributes to the study of residues of organophosphorus pesticides.[10] As an analytical tool its chief benefits stem from its simplicity, efficiency, specificity, flexibility and relative cheapness. Comparatively little laboratory space is required and these advantages make it an attractive procedure for screening numerous samples of unknown treatment history. Before being able to use this method fully, a very clean extract is essential. Combinations

of solvent partition with columnar-elution techniques are usually sufficient for samples of vegetable tissue, although remaining co-extractives from fatty samples sometimes cause interference. Extraction from leafy vegetables with acetonitrile, followed by clean-up on a carbon - Celite mixture eluted with chloroform, was found suitable by Getz[20] for examination by the paper-chromatographic procedures of Mitchell.[60] These procedures included 1 and 2-dimensional chromatography on Whatman No. 1 papers in both aqueous and non-aqueous solvent systems. The aqueous solvent consisted of 10 per cent. heavy mineral oil in diethyl ether as immobile solvent and 50 per cent. dimethylformamide in water as mobile solvent. The non-aqueous systems comprised 2,2,4-trimethylpentane as mobile solvent with either 20 per cent. dimethylformamide in diethyl ether, or 10 or 30 per cent. formamide in acetone as the immobile solvent. By suitable combinations of these systems 11 organophosphorus pesticides could be separated from one another. Pesticide spots were made visible by examination under ultraviolet light, by treatment with bromine and fluorescein, or by spraying with ammoniacal silver nitrate and irradiating with ultraviolet light. *N*-Bromosuccinimide with fluorescein and 2,6-dibromo-*p*-benzoquinone-4-chlorimine were used as colour-forming agents by MacRae and McKinley[61] for the identification of some organophosphate insecticides. Whatman No. 1 papers were impregnated with 8 per cent. mineral oil in diethyl ether and developed with ammoniacal methanol. Acetylated papers were also used, being developed with acetone - water (7+3) after impregnation with 2 per cent. mineral oil in diethyl ether.

Paper-chromatographic procedures were used by Bates[22] in devising a general scheme for the determination of organophosphorus pesticide residues in foodstuffs. The pesticide, together with important metabolites, was extracted from the crop with acetone, and after the addition of water, was partitioned into chloroform. The solvent was removed, the extract taken up in acetone and the fats and waxes frozen out at −80°C; further clean-up was achieved by column chromatography. Solvent systems were described for polar and non-polar compounds. For the former group a formamide-impregnated paper was developed twice with hexane, followed by development with a benzene - chloroform (9 + 1) mixture in a direction perpendicular to the first two developments. Non-polar pesticides were separated on papers impregnated with dimethylformamide and developed first with hexane and then with aqueous dimethylformamide (1 + 1), in two directions at right angles. The observed R_F values are used to characterise the pesticide or metabolite. After being made visible with bromine - 4-methylumbelliferone or silver nitrate - bromophenol blue, the excised spots were subjected to wet digestion with mixed perchloric, nitric and hydrochloric acids or to oxygen-flask combustion and total-phosphorus determination by a molybdenum-blue method. Eichenberger and Gay[62] have used aqueous mobile solvents for the semi-quantitative determination of insecticide residues in vegetable tissue by spot size comparison.

THIN-LAYER CHROMATOGRAPHY—

In recent years the advantages of versatile thin-layer chromatographic procedures have been explored fully, and have largely replaced paper chromatography in pesticide residue analysis. The intrinsic clean-up properties of the absorbents most frequently used, silica gel and alumina, render minimal the amount of pre-purification of the extract required. Thin-layer chromatography is tolerant of vegetable co-extractives, but fatty or waxy materials may affect the observed R_F values of pesticides. For waxy or animal tissue samples it is therefore often advisable to use a solvent partition clean-up stage,[36,38] although subsequent alumina or Florisil column clean-up may usually be omitted. General reviews of the application of thin-layer chromatography to pesticide residue analysis have been prepared by Conkin[63] and by Abbott and Thomson.[11]

Clean-up—Generally it is the total load of material that is to be applied to the chromatoplate at one point that governs the thickness of the layer required for efficient chromatographic clean-up. For clean extracts the usual layer thickness of about 250 μ is adequate; for preparative and clean-up purposes 500-μ or 1-mm thickness is more usual. The amount of previous clean-up required may therefore be governed, in part, by the availability of layering apparatus capable of producing layers of various thicknesses.

When leaves that have been sprayed with Colep were washed with benzene to remove residues, the amount of natural materials simultaneously removed by this simple process was shown by Conkin[63] to be sufficient to reduce the R_F values on chromatoplates by from

0·05 to 0·1 unit. Possible interference of this kind may be countered in two ways. The addition of a dyestuff of similar migratory properties could introduce an internal standard by means of which corrected R_F values might be calculated, it being assumed that the pesticides and the dye were held back proportionately by the interfering materials. Butter yellow, Sudan red and Sudan yellow have been found to be suitable marker dyestuffs for use with pesticidal compounds. The alternative possibility required that the extract be divided into two equal portions, to one of which was added a known specimen of the suspected pesticide. After developing and making visible, in an appropriate manner, the presence of a single spot in the "loaded" sample tended to confirm the identity of the compound, while the appearance of two spots showed their dissimilarity.

Multi-band chromatoplates,[64] in which 2 or 3 layers of different absorbents are spread on to 1 plate, have shown excellent clean-up properties. Dimethoate[65] and diazinon[66] have been determined in vegetable tissue on chromatoplates of this nature. Melchiorri, Maffei and Siesto[67] stated that by using 2-dimensional chromatographic separation, larger samples of crude extracts of vegetable oils could be examined for diazinon and still yield adequate resolution that was not found with 1-dimensional development. Fenitrothion has been separated from coloured co-extractives by thin-layer chromatography before photometric determination of its hydrolysis product.[68]

Although most of the published work on thin-layer chromatography of organophosphorus pesticides has been carried out with 20 × 20-cm chromatoplates, coated microscope slides (25·4 × 76·2 mm) have been advocated by Stanley.[69] An economical and rapid method is thus available as these micro chromatoplates require comparatively less preparation time, and cleaning is dispensed with as used slides may be discarded without undue expense. Shorter development times are required, and little mobile solvent is used, staining jars being convenient development chambers. Chromatoplates of 15 × 7·5 cm have been used in a screening test for residues in vegetable tissue,[70] final determination being made by a rapid total-phosphorus method.

Colour-forming reagents—Fluorescein, either built-in or spray-applied, had been used by Salo, Salminen and Fiskari[71] for organophosphorus pesticides; exposure to bromine vapour showed the yellow spots on a red background. Walker and Beroza[72] added silver nitrate to the fluorescein - bromine combination, and gave limits of detection ranging from 0·5 to 10 μg for 59 pesticidal compounds; for the organochlorine compounds 7 minutes' irradiation with ultraviolet light was also required. Dichlorofluorescein has also been found useful for organophosphorus pesticides.[55] Kovacs[15] described the use of a mixture of tetrabromophenolphthalein, silver nitrate and citric acid in the sensitive detection of organophosphorus residues in strawberry, kale and lettuce extracts. Among the more specific colour-forming agents, palladium(II) chloride has been found preferable for organophosphorus compounds by Bäumler and Rippstein,[73] Blinn[29] and Steller and Curry.[74] 2,6-Dibromo-*p*-benzoquinone-4-chlorimine has been used by Braithwaite[75] to detect from 0·1 to 0·2 μg of several organic phosphorothioates and phosphorodithioates on silica-gel plates.

Abbott, Crosby and Thomson[55] studied the properties of several indicator compounds, belonging in the main to the azo and triphenylmethane classes of dyestuffs, when applied as colour-forming agents for pesticidal compounds. Brilliant green (C.I. No. 42040) showed useful reactions and good sensitivity. With this material it was possible to locate organochlorine, organophosphorus and triazine compounds and to distinguish between them. On spraying the silica-gel chromatoplate with a 0·5 per cent. solution of Brilliant green in acetone, organochlorine pesticides were observed as pale yellow spots on a green background and might be readily marked. On placing the sprayed plate, still damp with acetone, into an atmosphere of bromine vapour the green background and the spots of organochlorine pesticide disappeared, but triazine herbicides were located as semi-transient green spots on white, and organophosphorus pesticides appeared as permanent dark green or yellow spots. 4-(*p*-Nitrobenzyl) pyridine was used by Watts[76] to detect both thio-organophosphorus and non-thio-organophosphorus compounds. This reagent gave blue spots with most of these compounds but did not react to any extent with crop co-extractives.

Among the more specific colour-forming methods mention must be made of the use of esterase-inhibition procedures for the detection of organophosphorus compounds and their metabolites. The sensitivity of this procedure varies markedly with the compound studied; the anti-cholinesterase activity of phorate oxygen analogue sulphone, for example, is 1000

times that of the parent phorate, the limit of detection being correspondingly lower. Methods used on paper chromatograms (Getz and Friedman[14] and McKinley and Johal[77]) have been similarly applied by Bunyan[78] and El Rafai and Hopkins[79] to thin-layer separations. The developed paper or plate was briefly exposed to bromine vapour and placed in contact with a sheet of filter-paper that had been impregnated with out-dated human plasma. After incubation for 10 to 20 minutes at 35° to 40° C the paper was removed and sprayed with acetylcholine bromide and bromothymol blue. After 5 to 10 minutes the transferred spots appeared in blue on the yellow background. By using techniques of this nature sensitivities down to about 10 mg have been obtained from developed thin-layer chromatograms. Liver extract has been used as the source of esterase with an ester of a substituted umbelliferone as substrate, to give indication of organophosphorus pesticides on the silica-gel chromatoplate itself.[11]

Quantitative evaluation of chromatograms—The techniques that have been applied to the quantitative evaluation of thin-layer chromatograms fall readily into 2 classes. The one more widely used by pesticide-residue analysts comprised those procedures in which the chromatographed compound was separated from the layer adsorbent before the application of standard microchemical analytical methods, such as spectrophotometry or gas - liquid chromatography. The alternative technique involved the relationships that existed between the weight of compound contained in the located spot and its size, density of coloration (visual or photometric) or radioactivity. The methods based upon the measurement of spot area avoid the difficulties associated with elution of the material from the adsorbent and the possibilities of further pollution of the purified sample. Seher[80] simultaneously chromatographed samples of the unknown and a series of standards applied to the chromatoplate in equal volumes of solution. After rendering the developed chromatogram visible, the areas occupied by the standard sample spots were determined and plotted against the corresponding weight of material. Reference of the area of the unknown sample spot to this curve gave a measure of the material present. Purdy and Truter[81] showed that the square root of the area of the spot was a linear function of the logarithm of the weight of the material it contained. Statistical evaluation[82] showed that this relationship was preferable to those of area against logarithm of the weight, or logarithm of the area against logarithm of the weight. Planimetric means were used by Aurenge, Degeorges and Normand[83] to determine spot areas; graphs of area squared against weight of material were linear. They also traced the chromatograms, cut out the traced spots and weighed them (the weight per square centimetre of the tracing paper being known) to determine the area of the spot more accurately.

Separatory systems—Paper-chromatographic procedures generally found suitable for the separation of organochlorine pesticides were less applicable to the more hydrophilic organophosphorus compounds, although several systems have been proposed.[20,22,60] Thin-layer chromatography, because of its greater versatility, has proved more useful and indeed more attention appears to have been given to its application to organophosphorus compounds than to any other of the main groups of pesticides. Fischer and Klingelhöller[34,35] used both paper and thin-layer chromatography to identify residues of several organophosphorus pesticides extracted from animal tissue by hot ethanolic potassium hydroxide solutions. The degradation products from each compound gave a different spot pattern; for example, malathion yielded four spots, of R_F 0·32, 0·44, 0·58 and 0·66, on silica gel G developed with methanol - dichloromethane - 10 per cent. ammonia solution (20 + 80 + 3). The separation of 7 pesticides on silica-gel chromatoplates with hexane - acetone (4 + 1) as mobile solvent was reported by Bäumler and Rippstein,[73] although parathion and demeton-methyl were incompletely resolved by this sytem. In association with the use of bio-assays for pesticide residue determination, Salo, Salminen and Fiskari[71] identified parathion, demeton-methyl and malathion by chromatography on silica gel G, developed with toluene for 20 minutes. Parathion-methyl could not be distinguished from parathion in this way.

A comprehensive survey of the thin-layer chromatography of pesticides was carried out by Walker and Beroza,[72] who included many organophosphorus compounds among the 62 materials examined. Of the 19 solvent systems they described, those based on benzene or chloroform showed the most promise for general use. Uchiyama and Okui[84] studied the thin-layer chromatography of several organophosphorus pesticides on silica gel developed with hexane - acetone (4 + 1). Residues of demeton-methyl in soybean oil and thiometon in tea leaves were identified in this way. Fenitrothion was isolated from olive oil with almost 100 per cent. recovery by eluting the observed pesticides from the developed chromatoplate

with aqueous ethanol. In the review by Conkin[63] separation of Colep from its major metabolites was described, 7 mobile solvents being applied to silica-gel chromatoplates. A reversed-phase technique was advised for the separation of Colep, EPN, parathion-methyl, parathion, *p*-nitrophenol and phenol; silica-gel layers impregnated with either a mineral oil or, preferably, silicone fluids were developed with ethanol - acetone - water (1 + 1 + 2) for 2 hours, the phenols being used as reference materials. Steller and Curry[74] used thin-layer chromatography on air-dried, 500-μ layers of silica gel G - silica gel HF (1 + 1) developed with acetone - chloroform (3 + 1) to separate dimethoate and its oxygen analogue extracted from apples, alfalfa and green tomatoes. Quantitative measurements were made by total-phosphorus determination on pesticides eluted from the adsorbent with dilute nitric acid.

The use of thin-layer and gas - liquid chromatography in the detection, infrared identification and determination of organophosphorus pesticide residues extracted from samples of vegetable tissue has been described by Abbott, Crosby and Thomson.[55] Thin-layer chromatography was used both as a preliminary tentative identification procedure, and for clean-up purposes before the gas-chromatographic preparation of a pure specimen for confirmation of identity by infrared spectroscopy. For this purpose, 500-μ layers of silica gel G were developed with hexane - acetone (9 + 1) for 40 minutes, the resolved pesticide spots being eluted from the adsorbent with dichloromethane. Other chromatographic systems were recommended for further confirmatory purposes, should there be insufficient material for positive infrared examination. Better resolution of the pesticides was obtained with a mobile solvent composed of hexane - acetone (19 + 1) but clean-up was not so good. The use of mixtures of equal parts of kieselguhr G with either silica gel G or alumina G gave chromatoplates with useful properties; these were developed with 4 different mobile solvents. These mixed-composition chromatoplates effected separations that were not possible on silica gel alone, *e.g.*, carbophenothion from fenchlorphos, and parathion from thiometon.

GAS - LIQUID CHROMATOGRAPHY—

With the acceptance of gas chromatography as a reliable analytical technique, its advantages were rapidly realised by residue analysts. The simultaneous qualitative and quantitative measurements that it can give showed many advantages over earlier spectrophotometric methods, although the apparatus required is comparatively expensive. The degree of clean-up is dependent in part on the mode of detection used, but is generally less than that needed for infrared spectroscopy. By using a phosphorus-specific detector, quite crude extracts may be examined, although column performance rapidly deteriorates. Microcoulometric detection methods[85] give a good selectivity but the sensitivity of this system is not good. Burchfield *et al.*[86,87] have described the simultaneous and selective determination of phosphorus, sulphur and halogen in pesticide residues by microcoulometric gas chromatography after reduction to the corresponding hydrides.

The introduction of electron-capture detection revolutionised the analysis of residues of organochlorine pesticides. For some organophosphorus compounds this mode has proved to be equally effective,[9,88] particularly for those compounds containing chlorine-substituted aryl rings. Sensitivities for other phosphorus-containing compounds are generally much lower, however; Cook, Stanley and Barney[89] have studied the correlation of the chemical structure with response in electron-capture detectors.

The greatest recent advance in the detection of organophosphorus compounds stems from the description by Karmen[90,91,92] of a flame-ionisation system, sensitive only to halogens and phosphorus. He showed that the presence of a phosphorus-containing compound in the flame greatly increased the rate of volatilisation of sodium, or other metal, vapour from a heated screen thus increasing the observed current. This sytem was the "sodium thermionic detector" of Giuffrida,[93] who has explored its application to residue analysis in several publications.[32,94,95,96] A conventional hydrogen flame-ionisation detector collector electrode was coated with a fused sodium salt in early models. Later, other alkali-metal salts were used to obtain greater degrees of sensitivity and selectivity. Schmit, Wynne and Peters[97] obtained improved stability of response by fitting over the flame jet a wire coil coated with a mixture of boric acid, copper(II) nitrate and silver solder; a normal platinum collector electrode was used. A similar system was used by Coahran,[98] who placed a ceramic tube over the jet and filled the cup so formed with sodium sulphate. A useful life of several weeks was claimed, although a systematic decrease in response was observed. Hartmann[99,100] has

described the development of an extremely sensitive, stable detector of this "thermionic" type. A cylindrical pellet was formed under high pressure from a mixture of caesium bromide with a filler such as Celite. The base was shaped to fit over a flame-ionisation detector jet and a central hole was drilled, at the top of which the small hydrogen flame burned. The background current from such a detector was much higher (3×10^{-9} amp) than that given in the normal flame-ionisation mode (10^{-11} amp). All of these "sodium thermionic" detectors were extremely sensitive to flow-rates of hydrogen, air and carrier gas. The characteristics and operational parameters of this type of detector have been described by Beckmann and Gauer.[101] As the detector was not sensitive to bleeding stationary phase, provided flow-rates could be controlled, the use of temperature programming was far more useful than with electron-capture detectors of similar sensitivity to pesticides.

Two other types of specific detectors have been described recently. Photometric detection of flame emission of phosphorus compounds burning in a hydrogen - air flame has been used by Brody and Chaney.[102] A narrow band-pass interference filter isolated the phosphorus emission at 526 mμ, and a linear response over a wide concentration range was claimed. An emission spectrometric detector, in which the intensity of the 2535·65 Å atomic phosphorus line was used for quantitative, as well as selective qualitative, purposes has been applied[103] to organophosphorus pesticide residue analysis on samples of vegetables and animal tissue at levels from 0·03 to 0·6 p.p.m.

No matter how selective or sensitive the detection system may be, it is of little value without a suitable gas-chromatographic system behind it. Microcoulometric detection has been used by Challacombe and McNulty[85] to study chromatographic parameters, as its absolute nature allowed quantitative evaluation of column and system performance. Relative retention times were given of 12 organophosphorus pesticides on a silicone-oil column. Nelson[104] studied gas-chromatographic methods for screening tests for several sulphur-containing organophosphorus pesticide residues on fruit and vegetables. An acetonitrile - petroleum partition procedure provided adequate clean-up for the microcoulometric detection system used.

The practical conditions found to be suitable for the general application of electron-capture gas chromatography to organophosphorus residue analysis have been described by Egan, Hammond and Thomson.[9] Extraction with ethyl methyl ketone - hexane (3 + 2) was followed by a hexane - aqueous sodium sulphate solution partition and alumina or magnesia column clean-up. Columns of silicone elastomer E301 or Apiezon L were used, in each case a little Epikote 1001 was added to minimise decomposition on the copper tubing. Relative retention volumes and detection sensitivities were quoted. A similar silicone - Epikote column has been used[37] to determine fenitrothion in cocoa; retention times for parathion, paraoxon and chlorthion were also quoted. Stainless-steel columns were favoured by Abbott, Crosby and Thomson[55] for the semi-preparative gas-chromatographic separation of organophosphorus pesticides. Apiezon L (2 or 4 per cent.) with Epikote 1001 was supported on Celite or glass ballotini; flame-ionisation detection was used quantitatively on 5 per cent. of the gas stream, the remainder being tapped in solvent for subsequent infrared identification of eluted peaks.

Fenchlorphos residues in animal tissues and milk have been determined at 0·0005 and 0·001 p.p.m. levels, respectively.[105] Chlorfenvinphos can be used as a soil or foliar insecticide, and also for the control of maggot-fly strike on sheep. Gas-chromatographic procedures for residues of this compound in crops and soils,[31] sheep,[25] animal tissue and milk[106] have recently been reported.

ANTI-ESTERASE ACTIVITY TECHNIQUES—

Many of the organophosphorus pesticides and their major metabolic products inhibit acetylcholinesterase action, and this process has often been turned to analytical benefit; some of the problems of the methods have been discussed by McKinley.[107] These procedures are of greatest use when the active agent present can be confidently identified, as the anti-esterase activity of different members of this class of compounds varies between wide extremes. Such esterase-activity measurements are, however, useful as screening tests, provided negative results are obtained; and they also have specific properties for making the spots visible on paper and thin-layer chromatograms, as already described.

McKinley and Read[108] described the use of esterase-inhibition techniques for the detection of organophosphorus pesticides. A simplified version for routine checking or screening

purposes involved the use of beef liver as a source of cholinesterase, prepared homogenates being stable for up to 6 months. Paper chromatography on Whatman No. 1 papers impregnated with mineral oil and developed with acetone - ethanol - water (1 + 1 + 2) or acetone - water (1 + 9) was used to separate the pesticides studied. Exposure to bromine vapour was sometimes necessary to convert the compounds into active enzyme inhibitors before applying a spray of liver homogenate. After incubation for 20 minutes a spray with 1-naphthyl acetate and Azoene Fast Blue RR salt showed the pesticides as yellow spots on a dark brown background. A similar procedure[14] involved the use of pooled out-dated human serum as a source of esterase, and acetylcholine bromide as substrate. Spraying a treated paper chromatogram with bromothymol blue showed blue areas of inhibition against a background rendered yellow by liberated acetic acid. The inherent dangers of drawing too rapid conclusions from such methods have been underlined by Menn, McBain and Dennis,[109] who used these procedures to detect naturally occurring cholinesterase inhibitors in untreated potatoes, sugar-beet, oranges and apples. Application of this procedure to an agar - agar diffusion process has been described[110]; precision of result was not very good but the simplicity of the test lent itself to use for screening purposes.

As an example of the use of esterase inhibition to determine residues of known compounds, the spectrophotometric procedure for mevinphos may be described.[18] The chloroform-extracted residue was incubated with standardised acetylcholinesterase solution at 35° C for 30 minutes. After further incubation with standard acetylcholine bromide solution for 60 minutes the unhydrolysed acetylcholine was combined with iron(III) chloride to give a red complex, the optical density of which was measured spectrophotometrically at 540 mμ An automatic analysis version of this procedure has also been described.[44]

Methods for individual pesticides

The following notes should be read in conjunction with those given in the previous review,[1] which they supplement in so far as they relate to compounds which were then considered. As indicated above, there has been substantial progress in the application of multi-detection methods to organophosphorus pesticide residue analysis since 1961. Although methods, both specific and otherwise, continue to be of use for residues of individual compounds, the identity of which is not known at the time of applications, such methods are now being replaced by multi-detection systems for survey and regulatory purposes. Problems in residue analysis are being considered by I.U.P.A.C.[111]

Attention should, however, be drawn to several textbooks on pesticide residue analysis, some of which incorporate useful chapters or monographs on organophosphorus pesticides. These include Volume 2 of Zweig's book,[112] which contains chapters on azinphos-methyl, carbophenothion, coumaphos, demeton, demeton-methyl, diazinon, dichlorvos, dimethoate, disulfoton, ethion, fenchlorphos, fenthion, malathion, mevinphos, parathion, phenkapton, phorate and phosphamidon. Methods for several individual pesticides are also detailed in the second volume of the U.S. Food and Drug Administration's Pesticides Analytical Manual.[12] A number of methods of analysis of commercial origin, not easily obtainable elsewhere, are included: a brief guide to these and other methods is given in Volume 2 of a comprehensive guide to methods of residue analysis compiled for the U.S. Public Health Service by Burchfield, Johnston and Storrs.[113] Gudzinowicz[114] has published a practical guide to the use of the electron-capture detector in residue analysis.

Azinphos-methyl (Guthion)—

S-(3,4-Dihydro-4-oxobenzo[*d*]-[1,2,3]-triazin-3-ylmethyl) dimethyl phosphorothiolothionate.

Several papers on azinphos-methyl residue determination have appeared since the previous review.[1] In the 1965 A.O.A.C. Official First Action method,[115] azinphos-methyl residues were initially extracted with acetone. After clean-up on a magnesia column the residue was hydrolysed to anthranilic acid and this was diazotised and coupled with *N*-1-naphthylethylenediamine to form a coloured compound. If the hydrolysis stage was omitted, interfering compounds (other than azinphos-ethyl) could be detected. Miles[30] has briefly reviewed methods up to 1963, and used the diazotisation colorimetric method examined on a collaborative basis earlier by Cox[115]; the method has been applied to azinphos-methyl, azinphos-ethyl and their oxygen analogues for residues on fruit and vegetables. Bates[57] used a cathode-ray polarographic method for residues extracted from cucumbers and tomatoes,

following magnesium oxide clean-up, sensitive to 0·1 p.p.m. Gutenmann and Lisk[116] have studied bromination and methylation following alkaline hydrolysis to anthranilic acid; the 2-amino-3-5-dibromobenzoic acid so formed was then examined by gas chromatography. Adams and Anderson[26] have developed a sensitive fluorimetric method based on anthranilic acid, for residues of both azinphos-methyl and its oxygen analogue, for milk (0·005 p.p.m.) and for most animal tissues (0·02 p.p.m.). Following alkaline hydrolysis, anthranilic acid was extracted into benzene, the solution irradiated at 340 mμ and the fluorescence measured at 400 mμ.

BIDRIN—

3-(Dimethoxyphosphinyloxy)-*NN*-dimethyl-*cis*-crotonamide.

Elgar and MacDonald[117] have described a cholinesterase method for determining residues in crops, sensitive to 0·05 p.p.m. Stevens and van Middelem[118] extracted cabbage with dichloromethane and measured the iodoform produced, following reaction of the extracted bidrin with hypoiodite, by electron-capture gas chromatography (0·01 p.p.m.). Murphy, Gaston and Gunther[119] determined residues in various crops and in citrus pulp[120] following distillation clean-up by hydrolysis of bidrin to dimethylamine, which is determined colorimetrically with copper and carbon disulphide as the dimethyl dithiocarbamate complex (0·2 p.p.m.). Sun, Lau and Johnson[121] have described a specific bio-assay method for determining residues in fruit and vegetables.

BROMOPHOS—

4-Bromo-2,5-dichlorophenyl dimethyl phosphorothionate.

Bracha and Bonard[122] used a 4-aminophenazone-coupling colorimetric method to determine bromophos residues in mud walls. Bromophos was separated from its hydrolytic products by thin-layer chromatography; this method was also used quantitatively for determining bromophos residues in urine.

CARBOPHENOTHION (Trithion)—

S-(4-Chlorophenylthiomethyl) diethyl phosphorothiolothionate.

Barry, Hindley and Johnson[12] described clean-up conditions for the elimination of interfering inhibitors, and other improvements to the method of Patchett and Batchelder[123] discussed in the earlier review.[1] Coffin[124] has studied residues of carbophenothion and its 5 possible oxidation products on lettuce. The samples were extracted with acetonitrile, eluted from polythene-coated alumina with 40 per cent. acetonitrile, partitioned between water and chloroform and successively eluted from a magnesium oxide column with different solvents. Determination was by paper chromatography, involving the use of an enzymatic-detection technique.

CHLORFENVINPHOS (Supona)—

2-Chloro-1-(2,4-dichlorophenyl)vinyl diethyl phosphate.

Robinson, Malone and Bush[25] described a gas - liquid chromatographic method for determining chlorfenvinphos residues in sheep fat and organs, by using dimethylformamide partition followed by Florisil clean-up. The method was sensitive to 0·005 p.p.m. and could also be used for residues of the principal metabolite, trichloroacetophenone. Beynon, Davies, Elgar and Stoydin[31] described a cholinesterase-inhibition method with gas - liquid chromatography for residues in soil and crops (0·02 p.p.m.).

CRUFORMATE (Ruelene)—

O-4-t-Butyl-2-chlorophenyl methyl methylphosphoramidate.

Cruformate residues in milk have been determined by paper chromatography by Leahy and Taylor[125] with a limit of detection of 0·05 p.p.m.

DEMETON (Systox)—

A 65 + 35 mixture of demeton-*O*[diethyl 2-(ethylthio)ethyl phosphorothionate] and demeton-*S*[diethyl *S*-[2-(ethylthio)ethyl] phosphorothiolate].

Several paper-chromatographic methods have been published since the previous review. Coffin and McKinley[126] have examined demeton, diazinon and phosdrin residues on lettuce by using 3 different solvent systems. Both isomers were found to disappear rapidly, with the formation of the corresponding sulphones and sulphoxides. Adams, Anderson and MacDougall[127] determined demeton in the presence of other residues in foods by paper chromatography (0·3 p.p.m.); a similar sensitivity was obtained by the cholinesterase method of MacDougall described in Zweig.[112] The behaviour of electron-capture gas-chromatographic detectors towards demeton has been described in detail by Barney, Stanley and Cook,[128] who noted some anomalous responses. Giang and Schechter[129] described an infrared residue method for demeton and its sulphone and sulphoxide in plants, with 80 per cent. recovery at the 20 μg level.

DEMETON-METHYL—

A 70 + 30 mixture of demeton-*O*-methyl[2-(ethylthio)ethyl dimethyl phosphorothionate] and demeton-*S*-methyl[*S*-(2-ethylthio)ethyl] dimethyl phosphorothiolate].

In addition to the general methods above and those reviewed earlier,[1] Woggon, Spranger and Ackermann[130] have examined paper and thin-layer chromatographic systems, and have found toluene - isopropyl alcohol - methanol - acetonitrile - water (40 + 16 + 16 + 20 + 9) to be a useful, rapidly developing solvent.

DIAZINON—

Diethyl 2-isopropyl-6-methyl-4-pyrimidinyl phosphorothionate.

The methylene blue - sulphur methods described in the previous review[1] have been supplemented by some specific paper-chromatographic studies, including that of Getz[20] who used acetonitrile extraction. Diazinon was included in the study of Coffin and McKinley[126] referred to under demeton, whereas Gilmore and Cortes[66] have used multi-band thin-layer chromatography for the clean-up of plant extracts for diazinon residue determinations.

DICHLORVOS (DDVP)—

2,2-Dichlorovinyl dimethyl phosphate.

Dichlorvos is the de-hydrochlorination product of trichlorphon. Mustafa, Sidky, el-Darawy and Kamel[131] have examined methods for distinguishing residues of these colorimetrically, as the violet - blue colour formed between the phosphite obtained, after reduction, and 3,5-dinitrobenzoic acid. Hughes[132] has measured residues of dichlorvos and the breakdown product dichloroacetaldehyde in air colorimetrically, following reaction with alkali and 2,4-dinitrophenylhydrazine. Heuser and Scudamore[133] have described a rapid method for dichlorvos vapour. Büchler and Heizler[134] conducted a systematic study of the colour reaction of dichlorvos with alkaline resorcinol and described a sensitive method for the measurement of residues in solution (0·05 μg per ml) or in air (5 μg per m^3) based on this.

DIMEFOX—

NNN'N'-Tetramethylphosphorodiamidic fluoride.

In addition to the general residue methods reviewed earlier,[1] Lloyd and Tweddle[135] have used a copper - dithiocarbamate colorimetric method for the determination of dimefox concentrations in air.

DIMETHOATE (Rogor)—

Dimethyl *S*-(*N*-methylcarbamoylmethyl) phosphorothiolothionate.

Residue methods have been reviewed by de Pietri-Tonelli, Bazzi and Santi[136] and by Smart.[137] Smart also made a comparative practical study of the methods of Laws and Webley,[19] Chilwell and Beecham[138] and Giang and Schechter,[129] concluding that the latter gave variable results. Abbott, Bunting and Thomson[65] used multi-band thin-layer chromatography for

clean-up of vegetable extracts, and determinations were made by spot area measurement. Steller and Curry[74] also used a thin-layer chromatographic clean-up followed by a molybdenum-blue phosphorus determination. The sulphur was reduced, by Enos and Frear,[39] to hydrogen sulphide with hydrobromic acid, distilled off, converted to methylene blue and measured colorimetrically. The same authors[139] have also described semi-quantitative paper-chromatographic separation for residues of dimethoate in milk (0·01 p.p.m.). Faderl[140] also used a paper-chromatographic method for the separation of dimethoate from other residues in plant tissue; determination was by total-phosphorus determination, following oxygen-flask combustion. George, Walker and Murphy[51] converted the residue extracted from milk or vegetable tissue to a coloured product by reaction with methanolic sodium hydroxide and 1-chloro-2,4-dinitrobenzene. This method was used earlier by van Middlelem and Waites,[141] who compared it with a gas-chromatographic method involving the use of an electron-capture detector. More recently Bache and Lisk[142] have applied gas chromatography to the determination of dimethoate and phorate residues in soil, by use of the sensitive, selective emission-spectroscopic detector described by McGormack, Tong and Cooke.[143] The sensitivities for dimethoate and its oxygen analogue were each 0·02 p.p.m. Klisenko[144] has described colorimetric methods for determining dimethoate in air by coupling with diazotised sulphanilic acid or total-phosphorus determination.

DIOXATHION (Delnav)—

1,4-Dioxan-2,3-diyl bis-(*OO*-diethyl phosphorothiolothionate).

A practical evaluation of the colorimetric method of Dunn,[145] described in the previous review,[1] has been published by Barry, Hindley and Johnston.[12]

ETHION—

Tetraethyl *SS'*-methylene bis-(phosphorothiolothionate).

Graham and Orwoll[146] hydrolysed residues to the thiophosphoric acid and determined this colorimetrically as the complex copper salt (0·05 p.p.m.). An alternative cholinesterase method was described by Barry, Hindley and Johnston.[12]

FENCHLORPHOS (Ronnel)—

Dimethyl 2,4,5-trichlorophenyl phosphorothionate.

Claborn and Ivey[105] have described a method for the determination of fenchlorphos residues in milk and animal tissue to a level of 0·001 p.p.m. Hexane extraction of the sample was followed by acetonitrile partition, Florisil column chromatography and electron-capture gas - liquid chromatography. The same authors have also described a colorimetric method (0·05 p.p.m.) based on hydrolysis to 2,4,5-trichlorophenol and reaction with 4-aminophenazone.

FENITROTHION (Sumithion)—

Dimethyl 3-methyl-4-nitrophenyl phosphorothionate.

Yuen[147] has adapted the Averell and Norris[148] method for determining fenitrothion residues in cocoa beans. Dawson, Donegan and Thain[37] have described a gas - liquid chromatographic method applicable to cocoa beans. Residues in fruit and vegetables can be determined after thin-layer chromatographic clean-up by ultraviolet spectrophotometry of the hydrolysis product 3-methyl-4-nitrophenol, as described by Kovac and Sohler[68]; a similar method was used by Franz and Kovac[49] for residues in milk.

MALATHION—

S-[1,2-Di(ethoxycarbonyl)ethyl] dimethyl phosphorothiolothionate.

The 1965 A.O.A.C. Official First Action method was based on the evaluation by Conroy[149] of the Norris, Vail and Averell[150] copper colorimetric method reported in the previous review.[1] By using a similar analytical method, Koivistoinen, Karinpaa, Kononen and Vanhaven[16] have shown that residues of malathion were stripped from fruits more efficiently by simple tumbling with benzene, without maceration, than by various blending or maceration processes. Rowlands *et al.*[151,152,153] have described the column clean-up conditions required for the examination of various crops, difficulty having been experienced earlier by McFarlane[154] in some instances.

MENAZON—

S-(4,6-Diamino-1,3,5-triazin-2-ylmethyl) dimethyl phosphorothiolothionate.

Menazon residues were extracted from plant tissue by Calderbank and Turner[23] with methanol, the extract diluted and made slightly acid and run through an ion-exchange column to separate the residue from natural phosphorus-containing compounds. The residue was then determined by a colorimetric phosphorus method with an over-all sensitivity of about 0·05 p.p.m.

MEVINPHOS (Phosdrin)—

2-Methoxycarbonyl-1-methylvinyl dimethyl phosphate.

Like the methods reviewed previously,[1] the 1965 A.O.A.C. Official First Action method for mevinphos residues was non-specific. It was based on water-soluble cholinesterase-inhibiting substances, as described by Blumen in a collaborative study.[18] In this, apples, tomatoes and cabbage were extracted with chloroform; after esterase treatment, unhydrolysed acetylcholine was reacted with alkaline hydroxylamine and an iron(III) salt to give a red complex, which was measured at 540 mμ. Mean recoveries of about 95 per cent. at the 0·15 to 0·3 p.p.m. level were obtained. Mevinphos residues were also studied in the chromatographic system used by Coffin and McKinley,[126] described under demeton.

PARATHION—

Diethyl 4-nitrophenyl phosphorothionate.

The 1965 A.O.A.C. Official Final Action method for parathion residues was based on that of Averell and Norris,[148] as described in the previous review.[1] Lausen[155] has described the use of titanium(III) chloride in the reduction stages, and George[156] has described the application of the method to residues of aromatic pesticides other than parathion. van Middelem has reviewed information on residues of parathion on leafy vegetables[157] and has made a special study of extraction and clean-up procedures for these.[158] Getz[13] included parathion in the clean-up and paper-chromatographic identification system that he examined, as also did Coffin and McKinley,[159] who obtained 87 to 100 per cent. recoveries of parathion added to lettuce, apples and strawberries. An improved clean-up of brassica for parathion residue analysis, in which Florisil was used, has been described by Beckman, Bevenue, Gauer and Erro.[17] These authors used the colorimetric method but they also examined gas-chromatographic systems for the separation of parathion and organochlorine residues. Dawson, Donegan and Thain[37] have studied the gas-chromatographic determination of parathion and similar compounds in cocoa beans (0·1 p.p.m.). Ott and Gunther[160] have studied the polarography of low concentrations of parathion in the presence of malathion but the procedure has not been applied to residue analysis.

PARATHION-METHYL—

Dimethyl 4-nitrophenyl phosphorothionate.

Further studies of the application of the Averell and Norris[148] method have been made by George.[156] Coffin and McKinley[159] included parathion-methyl in their clean-up study for residues on fruit and vegetables.

PHENKAPTON—

S-(2,5-Dichlorophenylthiomethyl) diethyl phosphorothiolothionate.

An account of methods for determining phenkapton residues in biological material by Stammbach, Delley, Suter and Szekely[161] supplemented the information in the previous review[1]: fuller information on the thio-indigo-derivative method was given.

PHORATE (Thimet)—

Diethyl *S*-(ethylthiomethyl) phosphorothiolothionate.

Several papers on phorate residue determinations have appeared since the previous review.[1] A cholinesterase method for residues on potatoes and sugar-beet (0·01 p.p.m.) has been described by Archer, Zweig, Winterton and Francis.[162] Winnet and Katz[163] reduced the extracted residue with hydrobromic acid and determined sulphur colorimetrically as

methylene blue. Getz has studied the degradation of residues on kale[13]; 8 different products were identified, although not all of these were present at any one time. The isolation and identification of residues of phorate and its oxidation products on plant material have also been studied by Blinn,[29] by using thin-layer chromatography, infrared spectrophotometry and visual spectrophotometry (following chromotropic acid reaction). It should be possible to develop this work on a fully quantitative basis. Waldron, Pasarela, Woollard and Ware[164] have improved the formaldehyde - chromotropic acid method of Giang and Schechter,[165] following a study of clean-up procedure for potatoes and tomatoes. Bache and Lisk[143] have applied gas chromatography to the determination of phorate and dimethoate residues in soil by using an emission-spectroscopic detector: phorate sulphone, sulphoxide and its oxygen analogue, and their corresponding derivatives were also separated by thin-layer chromatography. Bunyan and Taylor[27] have worked with avian tissue with specific esterase inhibition following starch-gel electrophoresis.

PHOSPHAMIDON—

2-Chloro-2-diethylcarbamoyl-1-methylvinyl dimethyl phosphate.

A paper-chromatographic method for determining phosphamidon residues in plant tissue has been published by Anliker and Menzer[166] in which they used a blue tetrazolium dye as a colour-forming agent. Total-phosphorus determination on eluted spots gave a sensitivity for the method of about 0·3 p.p.m.

THIONAZIN (Zinaphos)—

Diethyl *O*-2-pyrazinyl phosphorothionate.

Kiigemagi and Terriere[167] have determined residues of thionazin and its oxygen analogue in soil and crops spectrofluorimetrically after hydrolysis to 2-pyrazinol (0·05 p.p.m.).

TRICHLORPHON (Dipterex)—

Dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate.

Several new methods were available to supplement the cholinesterase methods for trichlorphon residues considered in the previous review.[1] Hirano, Tanaka and Tamura[168] have studied the colorimetric method, formerly reported by Arthur and Casida,[169] in which chromic acid oxidation of the residue to formaldehyde was followed by a modified Fujiwara alkaline pyridine technique. Cerna[170] has used a similar method for residues in apples, cherries and lettuce. Mustafa, Sidky, el-Darawy and Kamel[131] utilised the other half of the molecule for residue determination, measuring the violet - blue colour formed by the dialkyl phosphite, after hydrolysis, with 3,5-dinitrobenzoic acid and alkali.[95] A microcoulometric gas-chromatographic method, sensitive to 0·1 p.p.m., with clean-up from animal tissue by dialysis, was described by Barry, Hindley and Johnston,[12] together with modified procedures for crops. el-Rafai and Giuffrida[95] have reported a gas - liquid chromatographic method for determining trichlorphon residues in the presence of dichlorvos residues, with a sodium thermionic detector. Anderson, Anderson and Olson[171] used electron-capture gas chromatography for the detection and determination of residues of trichlorphon and its breakdown products in plant and animal tissues (0·1 p.p.m.). A bio-assay method involving the use of *Aedes aegyptii* for residues in milk was used by Wickham and Flanagan[172]; the sensitivity obtained was about 0·05 p.p.m.

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Determination of Chlorinated Pesticides in Aqueous Emulsions

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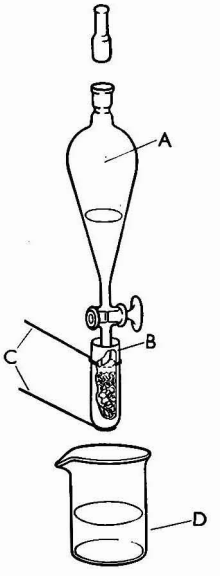
A method is described for the determination of chlorinated pesticides that involves their adsorption on to granulated carbon, and their subsequent extraction and degradation by the Stepanow method. The inorganic chlorine is then determined potentiometrically.

A method has been devised for the determination of chlorinated pesticides in aqueous suspension; it was intended in the first instance for use in the assessment of emulsion stability, but it could be applied to any insecticidal wash based on a halogenated active ingredient. Several methods currently in use to determine emulsion stability are based on visual observation of separated cream or oil after certain specified periods.[1,2,3,4,5,6,7] Such methods are in many instances satisfactory but there is an underlying assumption that the active principle separates wholly with the oil or cream. Clearly this need not always be so. The method devised is based on the determination of the pesticide in a portion of the aqueous phase at an appropriate time after mixing. Active material is absorbed from the aqueous phase on to granulated carbon contained in a Whatman extraction thimble supported on wire stirrups (Fig. 1). The carbon is dried and then placed in a suitable extraction apparatus. The



A = 125-ml Separating funnel
B = 22 x 80-mm Whatman extraction thimble
C = Wire supports for thimble
D = 250-ml Beaker

Fig. 1. Ground-glass jointed extraction assembly

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pesticide is removed from the carbon by continuous extraction with an isopropyl alcohol - benzene mixture containing metallic sodium. There is thus simultaneous extraction and reaction with sodium to produce inorganic chloride, which may be determined by any standard method. The method has been successfully applied to aqueous emulsions prepared from dieldrin, aldrin, endrin, DDT and lindane concentrates at about 0·2 per cent. level of active ingredient. Lower concentrations of active ingredient can be determined by increasing the volume of emulsion used (500 ml of emulsion containing 0·005 per cent. of active ingredient would provide sufficient chlorine, on the basis of a 60 per cent. content, to give a final titre of about 5 ml of 0·1 N silver nitrate solution). It may also be used for chlorinated phenoxy-acetic acids. The results in Table I show the recoveries that have been obtained.

TABLE I

RECOVERY OF CHLORINATED PESTICIDES FROM AQUEOUS EMULSION

	Operator 1		Operator 2		Operator 3	
Sample	Added, g	Found, g	Added, g	Found, g	Added, g	Found, g
Dieldrin ..	0·196	0·190	0·191	0·191	0·0985	0·0980
Endrin ..	0·214	0·207	—	—	0·105	0·107
Aldrin ..	0·320	0·313	—	—	0·157	0·152
DDT ..	0·251	0·253	0·250	0·249	0·121	0·123
Lindane ..	0·197	0·197	0·195	0·192	0·0982	0·0994

METHOD

APPARATUS—

Extraction assembly—Ground-glass jointed, with 250-ml flask and straight tube extractor.
Separating funnel, 125-ml capacity.
Beaker, 250-ml capacity.
Measuring cylinder, 100 ml.
Whatman extraction thimble, 22 × 80 mm.
Wire supports for thimble.
Vacuum desiccator.
Potentiometric titration apparatus with mercury(I) sulphate and silver electrodes.

REAGENTS—

Light petroleum, boiling-range 40° to 60° C.
Benzene.
Isopropyl alcohol.
Sodium sulphate, anhydrous.
Sodium metal.
Silver nitrate solution, 0·1 N.
Phosphorus pentoxide.
Nitric acid, 50 per cent.
Granulated carbon—Nuchar C–190, fraction on 30 mesh. Obtainable from Kodak Limited, Kirkby, Liverpool.

PROCEDURE—

Fill a 22 × 80-mm extraction thimble to two-thirds of its volume with granulated carbon and fit the thimble in an upright position into the wire supports. Place a volume of emulsion, containing sufficient active material to give a final titre of between 5 and 50 ml of 0·1 N silver nitrate solution, into the 125-ml separating funnel and add to it anhydrous sodium sulphate at the rate of 1·5 g per 10 ml of emulsion. Shake the funnel vigorously to dissolve the sodium sulphate. Clamp the separating funnel above the thimble and run the emulsion through the carbon at a rate of about 5 ml per minute. Collect the eluate in a clean beaker. Use a small volume of eluate to wash out the separating funnel and elute the washings through the carbon. Transfer the combined eluates to the separating funnel, wash out the beaker with 25 ml of light petroleum and add this to the combined eluates. Shake the funnel and allow it to stand. Retain the light petroleum layer.

After the thimble has been thoroughly drained, place it on wads of filter-paper in a vacuum desiccator over phosphorus pentoxide. Evacuate the desiccator and leave the

thimble to dry for at least 3 hours. Transfer the thimble to the extraction tube, run 25 ml of isopropyl alcohol through the carbon, followed by the light petroleum from the separating funnel and 20 ml of benzene. Carefully add 3 g of sodium metal to the solvent mixture in the flask and reflux the mixture for $2\frac{1}{2}$ hours. Cool the flask, add sufficient 50 per cent. isopropyl alcohol to dissolve unreacted sodium and then place the flask on a steam-bath to remove hydrocarbon solvent. Wash the mixture into a 250-ml beaker with 50 ml of water, neutralise with 50 per cent. nitric acid and add 10 ml in excess. Cool the solution and titrate it potentiometrically with 0·1 N silver nitrate solution by using mercury(I) sulphate and silver electrodes.

1 ml of 0·1 N silver nitrate solution ≡ 0·003546 g of chlorine.

Determinations were made by operators 1 and 2 on 100 ml of emulsion prepared from 1 ml of concentrate and by operator 3 on 50 ml of emulsion containing 0·5 ml of concentrate. The measured volumes of concentrate were weighed to determine the actual weights of active ingredients added. The contents of active material in the original unemulsified concentrates were determined by the method described, omitting the carbon-adsorption stage.

The method has been accepted by the Ministry of Agriculture, Fisheries and Food for inclusion in the next issue of Technical Bulletin No. 1, Specifications and Methods of Analysis for Pesticides.

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The Determination of Malathion in Formulations by a Method Based on Cleavage by Alkali

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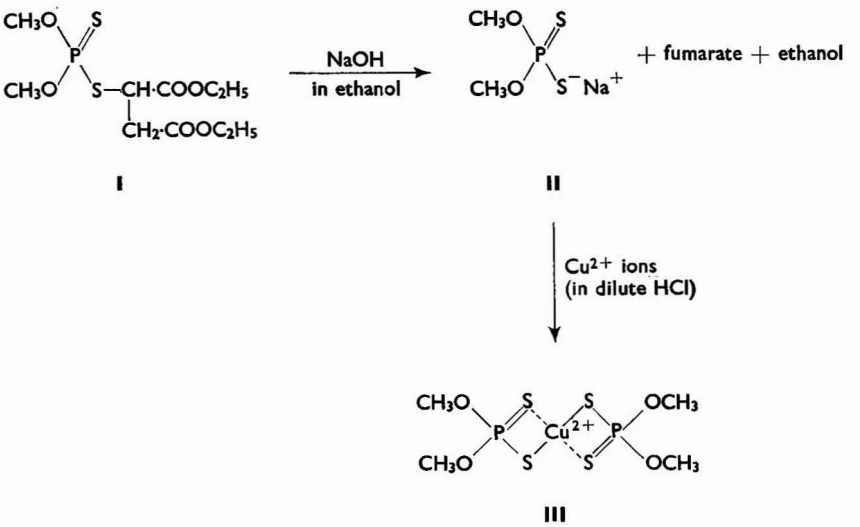
A method for the determination of malathion in formulations is described. The malathion dissolved in methanol is hydrolysed at room temperature by sodium hydroxide in the presence of phenol to give *OO*-dimethylphosphorodithioic acid. The latter is converted into its yellow copper complex by reaction with copper(II) sulphate in acetate buffer solution, and the complex extracted with chloroform. The copper(II) sulphate remaining in the aqueous layer is then determined iodimetrically. The free *OO*-di methylphosphorodithioic acid in the sample, and other materials that are likely to interfere, are accounted for in a parallel determination, which omits the hydrolysis stage. Hence the amount of malathion in the sample can be calculated.

Colorimetric methods for the determination of malathion that have been reported to date are all based on the following scheme of reactions—



I II III

The malathion, **I**, in ethanol is converted to sodium *OO*-dimethylphosphorodithioate, **II**, by treatment with alkali. The latter is then reacted with copper(II) ions in dilute hydrochloric acid to give a yellow-coloured complex, **III**, which can be extracted into various organic solvents.

In the original colorimetric method,[1] which is intended for the determination of malathion residues, the malathion (0·25 to 2·5 mg) is dissolved in a mixture of ethanol (25 ml) and carbon tetrachloride (100 ml), and is treated with 1 ml of 6 N sodium hydroxide for 1 minute. The solution is then shaken with 2 per cent. aqueous sodium chloride (75 ml) for 1 minute, the layers allowed to separate and the carbon tetrachloride layer discarded. The aqueous layer is washed with 25 ml of carbon tetrachloride, acidified with 1 ml of 7 N hydrochloric acid and washed once more with 25 ml of carbon tetrachloride. Finally, the

* On loan to the Department from "Shell" Research Limited, Woodstock Agricultural Research Centre, Sittingbourne, Kent, England, through the Colombo Plan.

aqueous layer is shaken for 1 minute with exactly 25 ml of carbon tetrachloride, together with 2 ml of 1 per cent. copper sulphate solution, and the optical density of the yellow copper complex in the carbon tetrachloride layer is measured at 418 mμ. The amount of malathion in the sample is found by comparing the optical density measurement against a standard graph prepared with various known amounts of pure insecticide.

Upham[2] reported a modification of the original method that is intended for the determination of malathion in formulations. In this modification the use of acetonitrile was introduced for the extraction of solid formulations, and the carbon tetrachloride replaced by cyclohexane as extractant for the yellow complex to improve colour stability. Ware,[3,4] in a further modification, eliminated the standard graph by carrying a standard through the procedure with the sample, and also replaced the washing stages by a single "ferric-oxidation" step (first mentioned by Upham[2]) to remove materials likely to interfere by reducing copper(II) to copper(I) ions. Orloski,[5] on behalf of a further collaborative study group, reported a more restrictive version of the method reported by Ware.

Despite the considerable amount of collaborative work that has been carried out on the colorimetric method, none of the versions reported to date appears to be entirely satis factory. The World Health Organisation Expert Committee on Insecticides, in its Fifteenth Report,[6] noted that methods for the assay of malathion often give faulty results when applied to poor quality products and that, in particular, methods based on the cleavage by alkali of the molecule might be greatly improved if the mechanism of this cleavage were known. The Committee recommended that this mechanism should be investigated. Double[7] noted that colorimetric methods of malathion determination were unsatisfactory for commercial dust formulations containing malathion, DDT and lindane; reproducible results could not be obtained.

In January 1964, a reliable method was urgently required to check the toxicant contents of malathion formulations being produced in Pakistan. The most recent version of the colorimetric method[3,4] available was tried, and gave erratic results. Neither the polarographic[8] nor the infrared spectrophotometric method[2,9] could be used owing to non-availability of the necessary equipment, and attempts were therefore made to improve the colorimetric method.

A consideration of the colorimetric method, particularly in relation to the purpose for which it was required, suggested some modifications that might improve its reliability and, at the same time, would make it more economic in routine use. Firstly, it was thought that the use of methanol, instead of ethanol, as the medium in which to carry out the cleavage by alkali of the malathion molecule, would afford some degree of protection to the CH_3O–P bonds in both malathion and *OO*-dimethylphosphorodithioic acid against cleavage by alkali. This protection would be expected to act through a concentration (mass action) effect. Secondly, at least 500 ml of ethanol, an expensive solvent in Pakistan, was required to process each sample through the procedure. Thirdly, the formation and extraction of the yellow complex was being carried out from hydrochloric acid solution, and thus under conditions that could be conducive to the observed instability of the yellow complex. In view of these considerations it was decided to adopt the use of methanol, instead of ethanol, as the medium for the cleavage by alkali and to use sodium acetate - acetic acid buffer solution to terminate the alkali treatment, so that the yellow complex would be formed and extracted under milder conditions of acidity.

Initially, work was carried out with the intention of incorporating these features into the colorimetric method. However, this eventually led to the development of a method with a titrimetric finish, which is reported below.

Method

Scope of the method—

The method is intended for use in the determination of *S*-(1,2-dicarbethoxyethyl)-*OO*-dimethylphosphorodithioate in malathion insecticide and its formulations.

Compounds in these materials, which, like *S*-(1,2-dicarbethoxyethyl)-*OO*-dimethylphosphorodithioate are converted to *OO*-dimethylphosphorodithioic acid by the alkali treatment, will interfere with the method described. Known compounds of this type[1] are *OOOO*-tetra methylpyrophosphorotrithioate and bis(dimethoxyphosphorothiono) disulphide. Either one or both of the half-esters of *S*-(1,2-dicarboxyethyl)-*OO*-dimethylphosphorodithioate, namely

S-[(1-carboxy-2-carbethoxy)ethyl]-*OO*-dimethylphosphorodithioate and *S*-[(1-carbethoxy-2-carboxy)ethyl]-*OO*-dimethylphosphorodithioate, are also converted into *OO*-dimethylphosphorodithioic acid. The dicarboxylic acid, *S*-(1,2-dicarboxyethyl)-*OO*-dimethylphosphorodithioate, is not converted into *OO*-dimethylphosphorodithioic acid by alkali treatment and does not interfere.

The method has not been applied to formulations that contain mixtures of malathion with other pesticides. Gusathion, Imidan, ekatin, ethion, dimethoate and other dialkylphosphorodithioate insecticides will certainly interfere, and so also will captan and dithiocarbamate fungicides. However, it is considered probable that the method will be applicable to malathion formulations containing DDT, methoxychlor, perthane, heptachlor, aldrin, dieldrin, endrin, BHC (technical), lindane, toxaphene or DDVP.

Apparatus—

Measuring flasks—50 and 100-ml capacity.
Separating funnels—250-ml capacity, with close-fitting stoppers.
Pipettes—1, 10 and 25-ml capacity.
Graduated measuring cylinders—25 and 50-ml capacity.
Conical (or iodine) flasks—250-ml capacity, with glass stoppers.
Burette—Of at least 25-ml capacity, calibrated in 0·1-ml divisions.

Reagents—

Sodium hydroxide (carbonate-free), 3 N.
Methanol—General-purpose reagent grade, containing not more than 0·25 per cent. w/w of water.
Phenol solution, 30 per cent. w/v in methanol.
Chloroform—General-purpose reagent "A" grade.
Potassium iodate solution, 0·05 N.
Sodium thiosulphate solution, 0·02 N—Dissolve 10·00 g of sodium thiosulphate pentahydrate in 2 litres of water that has been freshly distilled from alkaline potassium permanganate. Keep in a dark bottle. Add 20 mg of mercury(II) iodide to the solution to protect it from possible deterioration by *Thiobacillus thioparus*. These precautions[10] are necessary to ensure good storage stability. Standardise against 0·05 N potassium iodate in the conventional manner.
Copper(II) sulphate solution, 0·02 N.
Buffer solution—Dissolve 48 g of sodium acetate trihydrate and 12 ml of glacial acetic acid in distilled water and make up to 1 litre.
Potassium iodide—Iodine-free, general-purpose reagent grade.
Potassium thiocyanate—General-purpose reagent grade.
Starch solution, 1 per cent. w/v in water.

Sample preparation—

Technical malathion and emulsifiable concentrates—No sample preparation is necessary. Weigh accurately directly into a 50-ml measuring flask an amount of technical malathion or emulsifiable concentrate that contains 0·75 to 0·90 g of pure malathion. Dissolve it in methanol and make up to the mark. Mix thoroughly. This is the sample solution.

Wettable powders and dust concentrates (25 and 50 per cent.)—Weigh accurately into a 50-ml beaker an amount of the formulation that contains 0·75 to 0·90 g of pure malathion. Disperse it in 20 ml of methanol and filter through a fine porosity paper, collecting the filtrate directly in a 50-ml measuring flask. Wash the beaker and filter-paper with 10-ml portions of methanol, allowing each wash to percolate through the filter before adding the next. Adjust the extract to the 50-ml mark and mix thoroughly. This is the sample solution.

Procedure—

(*a*) *Determination of malathion* plus *free* OO-*dimethylphosphorodithioic acid and any interfering materials*—Transfer by pipette a 10-ml aliquot of the sample solution into a 250-ml separating funnel containing a mixture of 1 ml of 3 N sodium hydroxide and 1 ml of 30 per cent. phenol solution, each solution being added by pipette. Stopper the funnel, mix the solutions by tilting the funnel and by gentle swirling (not by shaking), and allow to stand for 45 minutes. Then add, with a graduated cylinder, 25 ml of buffer solution and 50 ml

of chloroform, followed by 25 ml, added by pipette, of 0·02 N copper (II) sulphate solution. Stopper the funnel and shake it *vigorously* for at least 2 minutes (see Note 1). Allow the layers to separate and drain off the yellow chloroform layer into a 100-ml measuring flask. Extract the blue aqueous layer three times with 10-ml portions of chloroform, collecting these washings in turn in the 100-ml measuring flask. If the third wash with chloroform shows any yellow colour (as has been found with some emulsifiable concentrate formulations), carry out further washings with 10-ml portions of chloroform until the washings are colourless (see Note 2).

Drain the blue aqueous layer into an iodine flask and wash out the separating funnel with two 10-ml portions of distilled water. Add 2·0 g of potassium iodide to the contents of the flask, dissolve it by swirling, and titrate the liberated iodine with sodium thiosulphate solution. Continue until the yellow colour fails to return. Then add 1·0 g of potassium thiocyanate and titrate until the yellow colour of the aqueous dispersion becomes faint. Finally, add 3 ml of 1 per cent. starch solution and titrate dropwise to the end-point (blue to colourless). Record the volume, T, of sodium thiosulphate used in the titration.

(*b*) *Determination of free* OO-*dimethylphosphorodithioic acid and any interfering materials*—Transfer by pipette a 10-ml aliquot of the sample solution into a separating funnel containing a mixture of 1 ml of 3 N sodium hydroxide, 1 ml of 30 per cent. phenol solution (each solution added by pipette) and 25 ml of buffer solution. Add 50 ml of chloroform, followed by 25 ml, added by pipette, of 0·02 N copper(II) sulphate solution. Stopper the funnel and shake it *vigorously* for at least 2 minutes (see Note 1). Allow the layers to separate and drain the slightly yellow chloroform layer three times with 10-ml portions of chloroform, collecting the chloroform washings in the 100-ml flask (see Note 2).

Drain the blue aqueous layer into a 250-ml iodine flask and wash out the separating funnel with two 10-ml portions of distilled water.

Proceed with the iodimetric titration of the copper(II) sulphate remaining in the aqueous solution as described in section (*a*) above. Record the volume, T_b, of sodium thiosulphate used in the titration.

NOTES—

1. The instructions given for shaking should be followed to ensure that the *OO*-dimethylphosphorodithioic acid is quantitatively reacted with the copper sulphate.

2. The chloroform extracts may be kept for measurement of their optical densities (if this is desired). The chloroform may be recovered (90 per cent.) by distillation from a flask containing 10 g of calcium oxide for each litre of chloroform extract being distilled.

CALCULATION OF RESULTS—

Calculate the percentage of malathion in the sample taken for analysis from the following formula—

$$\text{Malathion, per cent. w/w} = \frac{B\,(T_b - T) \times 13{\cdot}23 \times 100}{W}$$

where B is the weight in grams of copper(II) sulphate pentahydrate to which 1 ml of the sodium thiosulphate solution is equivalent;

T_b is the titre in millilitres of sodium thiosulphate solution required for the copper(II) sulphate remaining after reaction with the unhydrolysed malathion sample;

T is the titre in millilitres of sodium thiosulphate solution required for the copper(II) sulphate remaining after reaction with the hydrolysed malathion sample;

and W is the original weight in grams of malathion sample taken for analysis.

The figure 13·23 takes into account that one molecule of copper(II) sulphate pentahydrate reacts with two molecules of *OO*-dimethylphosphorodithioic acid derived from two molecules of malathion, and that only one-fifth of the malathion weighed is used in each determination.

ACCURACY AND PRECISION OF RESULTS—

A statistical analysis of twelve consecutive results obtained with a fresh sample of 99·6 per cent. malathion showed—

Average result, 99·57 per cent.; range, 1·7 per cent.; standard deviation, 0·61 per cent.; "95 per cent. error," 1·22 per cent.; and standard error, 0·18 per cent.

DIFFERENCES BETWEEN THE PRESENT METHOD AND FORMER METHODS—

Although the present method is based on the same principle as the colorimetric method originally described by Norris, Vail and Averell,[1] and later modified by Upham,[2] Ware[3,4] and Orloski,[5] it differs considerably in practice, particularly in the following respects.

Conversion of the malathion into *OO*-dimethylphosphorodithioic acid is carried out by using equivalent amounts of sodium hydroxide and phenol in methanol for the hydrolysis stage, instead of using sodium hydroxide in ethanol.

Conversion of the *OO*-dimethylphosphorodithioic acid into its copper complex and extraction of the complex from the aqueous layer are carried out at pH 4·8 to 5·0 in the presence of acetate buffer solution, instead of in hydrochloric acid solution.[1,2,3,4,5] Also, chloroform is used as extractant for the complex, instead of carbon tetrachloride[1] or cyclohexane.[2,3,4,5]

Compounds such as thiols and mercaptans, which are likely to interfere in the method by reducing copper(II) to copper(I) ions, are accounted for in a parallel determination, which omits the hydrolysis stage, instead of being eliminated during the procedure by a "ferric-oxidation step."[2,3,4,5]

The procedure is finished titrimetrically instead of colorimetrically.

The yellow complex, as formed under the conditions of the method, is apparently much more stable than that obtained with former methods.

Since mid-1964, the method has been successfully applied to the determination of *S*-(1,2-dicarbethoxyethyl)-*OO*-dimethylphosphorodithioate in malathion imported from the U.S.A., as well as to 50 to 60 samples of malathion formulations produced in Pakistan. Toxicant contents determined by the method did not differ by more than 2 per cent. (absolute) from contents stated by producers in supply contracts.

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The Consecutive Determinations of Perchlorate and Nitrate Ions

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A method is described for the determination of nitrate and perchlorate ions in admixture; it is applicable to amounts of each ion in the range 1 to 25 mg. The method is based on the initial precipitation and gravimetric determination of tetraphenylphosphonium perchlorate. The filtrate from this precipitation is treated with the new nitrate precipitant, *N*-(4-chlorobenzyl)-1-naphthylmethylamine and the corresponding nitrate salt is filtered off, and determined gravimetrically or titrimetrically. The method is satisfactory for ratios of nitrate to perchlorate of 1:4·8 to 8·3:1, the widest range tested.

Recently, we have described[1] investigations of a series of *N*-substituted 1-naphthylmethylamines as a source of possible new organic precipitants for the nitrate ion in aqueous solution. In particular, it has been shown that several substituted *N*-benzyl-1-naphthylmethylamines possess analytically useful properties as precipitants for the nitrate ion. Organic precipitants for anionic radicals offer greater flexibility in methods for completing the determination than inorganic precipitants (*cf.* benzidine hydrochloride and barium chloride), but for the nitrate ion only organic precipitants are available. The present series of reagents behaves similarly to 1,4-diphenyl-3,5-endanilo-4,5-dihydro-1,2,4-triazole (nitron) in that a crystalline nitrate salt of the organic base is formed in dilute acidic solutions. This can be filtered off, washed free from contaminants, dried and weighed, or alternatively the moist precipitate can be titrated directly with standard alkali solution.

The new reagents have distinct advantages over the conventional nitron reagent. They have appreciably better precipitation sensitivities towards the nitrate ion, the theoretical conversion factors can be applied and the more convenient titrimetric finish can be used in place of the gravimetric procedure, if desired. The reagents are easily synthesised from available materials and, as straightforward derivatives of dimethylamine, they form stable hydrochloride salts and equally stable reagent solutions.

Three compounds have been proposed as satisfactory reagents. These are *N*-benzyl-1-naphthylmethylamine, *N*-(4-methylbenzyl)-1-naphthylmethylamine and *N*-(4-chlorobenzyl)-1-naphthylmethylamine. The limiting concentrations of nitrate ion for observable precipitates to form with these reagents are 1:14,000, 1:50,000 and 1:70,000, respectively.

In common with the other known nitrate precipitants, these reagents form slightly soluble salts with several other anionic species. However, the investigations referred to above have shown that the selectivity of these reagents as precipitants is closely related to their sensitivity towards the nitrate ion. As this increases, their selectivity decreases, and it does not appear that truly selective reagents of this type can be developed without loss of their analytically important function to precipitate quantitatively the nitrate ion. At best, one can choose the most selective reagent for a determination compatible with the sensitivity requirements of the analysis.

The addition of aqueous solutions of the three above-mentioned amines, in the form of their hydrochlorides, to the ions Br^-, Cl^-, IO_3^-, BrO_3^-, SO_4^{2-}, $H_2PO_4^-$, acetate and hydrogen tartrate at concentrations of 5 mg per ml leads to no precipitation of the corresponding amine salts, except that very faint turbidities are shown by the 4-chlorobenzyl reagent with chloride and bromide ions. When these tests are repeated with ClO_3^-, ClO_4^-, $Fe(CN)_6^{3-}$, $Fe(CN)_6^{4-}$, I^-, NO_2^-, MnO_4^-, SCN^-, NO_3^-, MoO_4^{2-}, $C_2O_4^{2-}$ (oxalate), ReO_4^- and TcO_4^-, all three reagents give rise to quite appreciable precipitates. This behaviour is very similar to that of nitron acetate, and the procedures described[2] for the removal of interfering ions when using this reagent would have to be used with the new reagents. These procedures do not, however, deal with the problem of interference by the perchlorate ion, the effect of which on the recommended gravimetric procedure[1] with the new reagents is shown in Table I.

TABLE I

RECOVERIES OF NITRATE IN THE PRESENCE OF PERCHLORATE WITH THE *N*-BENZYL-1-NAPHTHYLMETHYLAMINE REAGENTS

(No attempt was made to remove the perchlorate ion)

Nitrate reagent	Nitrate present, mg	Perchlorate present, mg	Nitrate found, mg	Error, mg
N-Benzyl-1-naphthylmethylamine hydrochloride	10·0	9·0	10·32	+0·32
	10·0	6·0	10·06	+0·06
N-(*p*-Methylbenzyl)-1-naphthylmethylamine hydrochloride	10·0	9·0	11·63	+1·63
	10·0	6·0	11·18	+1·18
N-(*p*-Chlorobenzyl)-1-naphthylmethylamine hydrochloride	10·0	9·0	11·75	+1·75
	10·0	6·0	11·51	+1·51

Although these reagents are considerably more selective than nitron (which has, in fact, been recommended for the determination of the perchlorate ion), it is apparent that as the sensitivity of the reagents to the nitrate ion increases, recoveries of nitrate in the presence of perchlorate become correspondingly greater. Because the perchlorate ion cannot readily be removed from a solution of the nitrate ion without the concomitant removal (or destruction) of the latter ion, special attention has been given to this particular combination of ions, and the present paper describes how this difficulty has been avoided by the development of a method for the consecutive determinations of these two ions.

The proposed method is based on the fact that although these present reagents, and, in general, all other organic reagents for the precipitation of the nitrate ion, react to a greater or lesser extent with the perchlorate ion, there are reagents for the perchlorate ion that are unaffected by appreciable amounts of the nitrate ion. In particular, the tetraphenylarsonium and tetraphenylphosphonium cations, described by Willard and Smith[3] and Willard and Perkins[4] for the gravimetric and titrimetric determination of the perchlorate ion, offer distinct possibilities for application in the present investigation.

Tetraphenylphosphonium chloride has been selected in preference to its arsonium analogue because of its ease of preparation and comparative cheapness. The tetraphenylphosphonium salts are, in general, rather more soluble than the corresponding arsonium salts, and thus enable a more concentrated reagent solution to be used. The differences in solubility of their perchlorate salts are too small to be of particular significance in the analytical procedures for the determination of the perchlorate ion.

The procedure depends essentially on the treatment of the aqueous mixture of nitrate and perchlorate ions first with a solution of tetraphenylphosphonium chloride. The precipitate of the corresponding perchlorate salt is quantitatively removed by filtration, and the nitrate ion in the filtrate is then precipitated with one of the new nitrate reagents.

Any difficulty in applying this procedure to the quantitative precipitation of these two ions might be expected to arise in the precipitation of the nitrate ion in the presence of the excess of tetraphenylphosphonium chloride. Recoveries of nitrate ion in the presence of this salt show little variation from those obtained in its absence, and it is apparent that this is not a significant factor in the successful development of a method for the consecutive determinations of these two ions. In the following procedures, only *N*-(4-chlorobenzyl)-1-naphthylmethylamine is applied because this is the most suitable reagent for the amounts of nitrate ion examined. The other reagents are less sensitive and are only really applicable for amounts of nitrate of 20 mg or more.

EXPERIMENTAL

REAGENTS—

N-(4-*Chlorobenzyl*)-1-*naphthylmethylamine hydrochloride*—Prepare a saturated aqueous solution of amine hydrochloride by dissolving 1 g of the hydrochloride in 100 ml of warm water containing 1 ml of about 2 N hydrochloric acid. Filter the warm solution to remove any insoluble material. The preparation of the amine hydrochloride is described in detail elsewhere.[1]

N-(4-*Chlorobenzyl*)-1-*naphthylmethylamine nitrate*—Prepare a saturated aqueous solution. Suspend an excess of the freshly precipitated amine nitrate in warm (50° C) water. Shake well; allow to cool to room temperature and filter.

Tetraphenylphosphonium chloride—Prepare a solution of phenylmagnesium bromide from 160 g of bromobenzene and 20·9 g of magnesium in 600 ml of dry ether. Agitate the mixture with a constant stream of dry nitrogen, and to the ice-cold Grignard reagent add slowly a solution of 18 g of phosphorus trichloride in 50 ml of ether. Reflux the mixture for 1 hour and cool it in ice - water. Pass dry oxygen through the cold solution at a rate of about 250 ml per minute for 1½ hours; maintain vigorous stirring throughout this procedure and a temperature of 0° C (salt - ice coolant). Treat the resultant mixture with 250 g of crushed ice and acidify it with 80 ml of concentrated hydrochloric acid. Separate and discard the ether layer. Add water to the aqueous layer (and the product that separates out as an oil) to give a total volume of 1 litre, and heat the mixture until almost all of the oily material has dissolved. Add about 5 g of Norit to the hot solution and filter. Treat the hot filtrate with 250 g of sodium chloride and cool the mixture in ice - water. Filter the crude product (tetraphenylphosphonium bromide) that crystallises out, wash it with small amounts of ether and redissolve it in 2 litres of distilled water. Pass this solution successively through two columns, each containing 250 g of Amberlite IR–4–B resin, fully charged with chloride ion, and concentrate the eluate to a volume of about 500 ml. Add 150 g of sodium chloride to the solution, cool the mixture and filter off the precipitate of tetraphenylphosphonium chloride. Dissolve the well drained solid in 50 ml of ethanol by warming, and concentrate the solution to about 20 ml. Precipitate the phosphonium salt from this solution by the addition of 500 ml of ether and re-crystallise it from the minimum volume of water.

The yield of pure tetraphenylphosphonium chloride (melting-point 266° C) is 40 g (about 80 per cent.). This preparation is an improved version of the basic preparation described by Willard, Perkins and Blicke.[5] (Triphenylphosphine is formed intermediately, but preparations with commercial triphenylphosphine as starting material were less successful than the method described above.[5])

Tetraphenylphosphonium chloride solution, 0·03 M, aqueous—Dissolve 11·25 g of tetraphenylphosphonium chloride in water and dilute to 1 litre.

Sodium hydroxide, 0·05 N—Carbonate-free. Standardise immediately before use.

Bromothymol blue - phenol red indicator—Mix 0·4 per cent. ethanolic solutions of the indicator acids in the ratio 2 : 3.

Paper pulp—Macerate quantitative filter-paper clippings in boiling water until the paper is entirely disintegrated.

(A). PROCEDURE FOR THE DETERMINATION OF PERCHLORATE—

Heat the test solution containing 1 to 30 mg of perchlorate ion and 1 to 25 mg of nitrate ion in not more than 20 ml of water to 80° to 90° C, and add a measured amount of 0·03 M tetraphenylphosphonium chloride solution (Note 1). Stir the contents of the flask at this temperature for several minutes until the precipitate has coagulated. Cool to room temperature and after 2 hours filter off the precipitate on a weighed No. 3 sintered-glass crucible and retain the filtrate in a clean flask. Wash the precipitate with three 2-ml portions of ice-cold distilled water, drain it well and dry the crucible and contents at 105° C in an air-oven until constant weight is obtained.

$$1 \text{ mg of } (C_6H_5)_4P^+ClO_4^- \equiv 0{\cdot}2266 \text{ mg of } ClO_4^-.$$

(B). PROCEDURE FOR THE DETERMINATION OF NITRATE—

Heat the filtrate from procedure (A) to 80° to 90° C and add sufficient hot 1 per cent. aqueous solution of *N*-(4-chlorobenzyl)-1-naphthylmethylamine hydrochloride (Note 2). Set aside the mixture for a period of 5 to 24 hours (Note 3), and filter off the precipitate on a weighed No. 3 sintered-glass crucible, portions of the filtrate being used to complete the transference of the precipitate from the flask to the filter. Wash the precipitate successively with one 2-ml portion of distilled water, two 2-ml portions of saturated amine nitrate solution and, finally, three 2-ml portions of distilled water. Dry the precipitate to constant weight in an air-oven at 105° C.

$$1 \text{ mg of } (C_{18}H_{17}ClNH_2)^+NO_3^- \equiv 0{\cdot}1800 \text{ mg of } NO_3^-.$$

Alternative titrimetric finish to procedure (B)—Precipitate the amine nitrate as described in procedure (B), but filter off the precipitate on a pulp pad (1 cm thick, three layers) prepared in the usual way on a Witt plate contained within a small glass filter funnel. Transfer the precipitate to the filter-pad and rinse the flask with three 2-ml portions of distilled water,

each rinsing being used to wash the precipitate on the filter. Then wash the precipitate with two 5-ml portions of saturated amine nitrate solution and, finally, with two 5-ml portions of distilled water. Remove the water in the funnel by gentle suction and transfer the precipitate and pulp into the original precipitation vessel. Rinse the inside of the funnel thoroughly with hot distilled water to remove all adhering particles of precipitate, and collect this wash liquid in the flask with the precipitate to give a total volume of 40 to 50 ml. Break up the pulp by vigorous stirring and boil the resultant mixture for 1 to 2 minutes. Add 3 to 4 drops of indicator solution and titrate the hot solution with 0·05 N sodium hydroxide solution to the indicator colour change. Carry out a blank determination with distilled water in place of the nitrate solution and subtract the value obtained from the volume of titrant in each determination.

1 ml of 0·05 N sodium hydroxide $\equiv$ 3·1 mg of NO_3^-.

NOTES—

1. For amounts of perchlorate less than 10 mg, add 5 ml of 0·05 M tetraphenylphosphonium chloride; for amounts of perchlorate in the range 10 to 20 mg, add 8 ml of reagent; for larger amounts, add proportionately more.

2. The use only of the *N*-(4-chlorobenzyl)-1-reagent is described here. This reagent is ideal for amounts of nitrate ion in the range 1 to 25 mg in 20 ml of test solution. For amounts of nitrate ion in the range 1 to 10 mg, use 10 ml of reagent solution. For amounts in the range 11 to 25 mg, increase the volume of reagent accordingly. The reagent solution may deposit crystals of the amine hydrochloride on standing and cooling. These must be redissolved by warming and the hot solution used for the precipitation of the nitrate ion. When the nitrate ion is present in amounts greater than 20 mg, the *N*-benzyl-1-naphthylmethylamine, or better, *N*-(4-methylbenzyl-1-naphthylmethylamine reagent,[1] can be used.

3. When more than 10 mg of nitrate ion are present, it is permissible to filter the precipitate after 5 hours. For smaller amounts, it is necessary to store the precipitates overnight before filtration is commenced.

RESULTS AND DISCUSSION

The procedure described for the precipitation of the perchlorate ion differs appreciably from that described by Willard and Perkins in that sodium chloride is not present. The very high chloride-ion concentration (2·0 to 3·0 M) recommended by these workers makes it impossible to apply the subsequent procedure for the precipitation of the nitrate ion because of the resultant co-precipitation of the amine hydrochloride with the amine nitrate. The presence of chloride ion in such concentrations, as recommended by Willard and Perkins, certainly leads to a more crystalline precipitate of the perchlorate salt being formed, but in the present study, a similar effect has been achieved by the use of a stronger reagent solution and carrying out the precipitation at elevated temperatures. The titrimetric procedure for perchlorate developed by Willard and Perkins, in which the excess of precipitant is titrated with standard iodine solution, is, in general, less satisfactory than the direct gravimetric procedure, and it cannot, of course, be applied to the consecutive determinations now described.

The results shown in Table II obtained with the two gravimetric procedures give an indication of the suitability of the reagents for this particular analysis. The sensitivity of

TABLE II

THE CONSECUTIVE DETERMINATIONS OF PERCHLORATE AND NITRATE

Nitrate taken, mg	Perchlorate taken, mg	Nitrate-to-perchlorate ratio	Weight of nitrate precipitate, mg	Nitrate found, mg	Error, mg	Weight of perchlorate precipitate, mg	Perchlorate found, mg	Error, mg
2·50	6·00	1 : 2·4	13·5	2·42	−0·08	26·7	6·05	+0·05
2·50	12·00	1 : 4·8	13·8	2·48	−0·02	53·3	12·08	+0·08
5·00	10·00	1 : 2	27·9	5·02	+0·02	44·3	10·04	+0·04
5·00	12·00	1 : 2·4	28·1	5·06	+0·06	52·7	11·94	−0·06
10·00	15·00	1 : 1·5	56·0	10·08	+0·08	66·0	14·96	−0·04
12·50	3·00	4·2 : 1	69·6	12·53	+0·03	13·4	3·04	+0·04
12·50	30·00	1 : 2·4	69·3	12·47	−0·03	131·7	29·84	−0·16
15·00	18·00	1 : 1·2	83·5	15·03	+0·03	80·4	18·22	−0·22
20·00	20·00	1 : 1	112·0	20·16	+0·16	88·6	20·08	+0·08
25·00	3·00	8·3 : 1	138·6	24·95	−0·05	13·5	3·06	+0·06

the precipitation reactions is in both instances unusually great so that concentrations of about 0·05 per cent. of the two anions may be readily determined.

It must, however, be remembered that neither reagent is particularly selective in its precipitation reactions. Tetraphenylphosphonium chloride forms sparingly soluble salts with several anionic species (*cf.* tetraphenylarsonium chloride), and it may appear that the present analytical method has been developed to deal with an essentially artificial problem. The occasions when these two ions occur together may certainly be rare, but the fact remains that the perchlorate ion constitutes the most serious interference in the determination of the nitrate ion with any organic precipitant. The present study not only shows how this interference can be eliminated, but has the additional advantage of providing consecutive determinations of these two ions.

With the range of new organic precipitants now available for the determination of varying amounts of the nitrate ion,[1] there is no reason to suppose that the procedures now described could not be readily adapted to deal with larger amounts of these two troublesome ions.

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Molecular-emission Spectroscopy in Cool Flames

Part I. The Behaviour of Sulphur Species in a Hydrogen - Nitrogen Diffusion Flame and in a Shielded Air - Hydrogen Flame

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A method is described for the determination of sulphur that involves simple measurement of the S_2 band emission at 384 mμ in a nitrogen-diluted hydrogen diffusion flame burning in air, or in a pre-mixed air - hydrogen flame burning inside a cooled sheath. Sulphuric acid may be determined by this technique in the range 6·4 to 500 p.p.m. of sulphur in the latter flame, or dissolved sulphur dioxide in the range 3·2 to 320 p.p.m. in either flame. The temperature of the flame most suitable for conversion of sulphur species into S_2 is established as 390° C.

The analytical method proposed for sulphates is simpler than the indirect flame-photometric methods hitherto described, and means are suggested whereby the method may be used for the determination of any form of sulphur species. The analytical signal for sulphur varies linearly with the square of the sulphate-ion concentration in the test solution.

The twin techniques of atomic-absorption spectroscopy and atomic-fluorescence spectroscopy, which have been much used recently for the determination of traces of metals, are both based on the absorption of resonance radiation by free atoms produced in flames by the thermal dissociation of molecular species. A comparison has been made recently of atomic-absorption spectroscopy and atomic-fluorescence spectroscopy with the corresponding molecular techniques of absorption spectrophotometry and fluorescence-emission spectrophotometry, both of which probably have a much wider range of application, but are subject to many more interferences.[1] It is the specificity of determination available from these atomic techniques that renders them so useful for analytical purposes. Although most metals may now be determined by atomic-absorption spectroscopy and atomic-fluorescence spectroscopy, including, latterly, those which form molecular species with oxygen that do not dissociate very easily,[2] there are elements that remain beyond the range of both techniques, as currently practised. For example, sulphur, phosphorus and the halogens have their principal resonance lines in the vacuum ultraviolet region of the spectrum, where the background absorption of the oxygen in the atmosphere, and also of the burning flame gases, is excessively high. It appeared to us that any pre-mixed flame with an oxygen support would always lead to great difficulties for these elements, and while one solution would be to use atom reservoirs other than flames, *e.g.*, the atomic-furnace device of L'vov,[3] another possible solution would be to utilise the absorption and emission characteristics of simple molecular species, such as S_2. We have therefore sought to obtain simple molecular species in cool diffusion flames that burn at lower temperatures than pre-mixed flames. To reduce the temperature of these flames even further, we have diluted the fuel gases with an inert gas, such as argon or nitrogen. This not only reduces the temperature of the flame drastically, but concomitantly reduces enormously the background emission of species such as OH, and also severely restricts the ingress of oxygen, thus minimising combination of the desired element with oxygen in the inner region of the flame. We have examined many such diluted diffusion flames, and in the present paper we report some of the results obtained for the determination of sulphur in a hydrogen - nitrogen diffusion flame burning in air.

The burner head used in these studies was the standard 1·8 × 7·5-cm air - acetylene emission head supplied with the Unicam SP900A flame spectrometer. The steel head of this burner is perforated near one end by a 1-cm square pattern of 13 holes, and is well suited to the construction of this Unicam flame spectrometer as it brings the flame close up to the entrance

slit of the monochromator. However, any other suitable emission burner head could be substituted for it.

By aspirating the nebulised test solution on nitrogen rather than on air, practically any gas mixture may be used without risk of explosive flash-back into the expansion chamber of the nebuliser. A third gas may be introduced via a second jet in the burner base, as described by Mackison,[4] to permit the study of mixtures of two other gases with the fuel gas. Mixtures investigated in this study included: propane - oxygen - nitrogen; propane - nitrous oxide - nitrogen; acetylene - oxygen - nitrogen; acetylene - nitrous oxide - nitrogen; hydrogen - oxygen - nitrogen; hydrogen - nitrous oxide - nitrogen; hydrogen - nitrogen; and hydrogen - argon. The last-mentioned binary mixtures were found to be the most useful.

THE HYDROGEN - NITROGEN FLAME—

Nitrogen was used as the nebulising gas at an optimised pressure of 15 p.s.i. by using the conventional SP900A nebulising system, and hydrogen was introduced at the base of the burner in the usual way at a pressure sufficient to prevent the flame from lifting off. These optimised values were obtained by monitoring the emission at 384 mμ of a solution of sulphur dioxide sprayed into the flame. The flame itself is completely colourless, apart from occasional flashes produced by the entrance of particles of airborne dust. With these settings of gas pressure, the hydroxyl emission at about 300 to 320 mμ was very weak, being about 40 times less intense than in a pre-mixed air - hydrogen flame on the same burner head. Similarly, the emission at 589 mμ of a 2 p.p.m. sodium solution was about 50 times less than in an air - hydrogen flame.

The effect of introducing air into the flame through a third jet[4] was to increase the flame temperature, and the emissions from sodium and the hydroxyl band, while drastically decreasing the S_2 emission at 384 mμ. With nitrous oxide, no emission at 384 mμ could be detected. The emitting S_2 species are congregated in the cool inner regions of the flame, and it was observed that the dark blue emission was most marked about 3 cm above the burner top for $\leqslant 10^{-2}$ F sulphur dioxide solution and spread down to the top of the burner head for stronger solutions. Subsequent experiments revealed that the temperatures in the outer regions of the flame were much higher than those in the sharply defined blue-emitting zone, while those immediately above the burner head were too low to produce appreciable concentrations of the S_2 species.

A total-consumption burner was found to be unsatisfactory because it produced too hot a flame.

IDENTITY OF NEBULISED SPECIES—

All of the inorganic sulphur-containing species examined showed varying degrees of the characteristic blue emission at 384 mμ. These were sulphuric acid, ammonium sulphate, sodium and potassium sulphite, potassium metabisulphite, sodium thiosulphate, ammonium thiocyanate, sodium dithionite, sulphur dioxide, hydrogen sulphide and also thioglycollic acid. The maximum response per mole of sulphur present was obtained from sulphur dioxide and hydrogen sulphide solutions. The order of emissive response in unbuffered solutions was found to be sulphide $>$ sulphite $>$ thiosulphate $\gg$ ammonium sulphate $\simeq$ sulphuric acid $\gg$ alkali-metal sulphates. The emission from alkali sulphites and sulphides was found to increase about 100-fold upon acidification, presumably, because of the release of sulphur dioxide and hydrogen sulphide. Thiosulphates did not show the same marked increase, however, because of their tendency to deposit sulphur before entry into the flame. Sulphates do not give increased response upon acidification and the emission of sulphuric acid is about 600 times less than that of an equimolar solution of sulphur dioxide. Further, the optimum height for emission from sulphates is higher up in the flame, about 4 cm above the burner head, thus indicating that they require a higher temperature for the production of S_2 species. On the other hand, these species are not produced in any appreciable concentration, even from sulphates, in the hotter pre-mixed air - hydrogen flame. This is most probably due to dissociation and perhaps oxidation of the S_2 molecules to sulphur monoxide in such a milieu. A compromise between the requirements of higher temperature and the breakdown of the S_2 species was subsequently obtained by shielding a pre-mixed hydrogen - air flame with a cooled borosilicate glass or quartz sheath. This device and the results obtained are described subsequently.

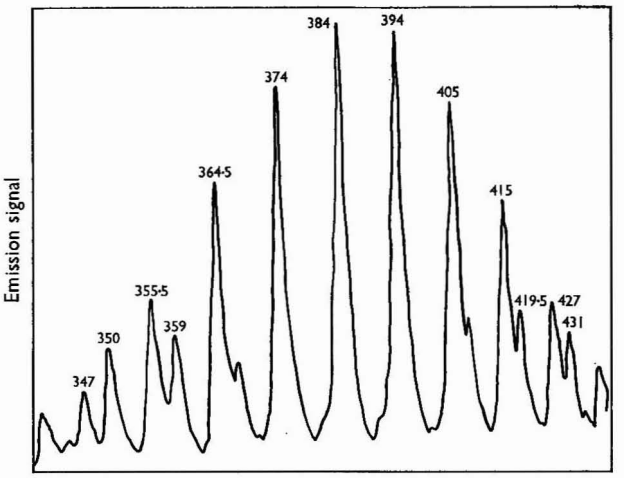

Fig. 1. Emission spectrum of S_2 species obtained by nebulising a 5×10^{-3} M aqueous solution of sulphur dioxide into a hydrogen - nitrogen diffusion flame and measuring with a Unicam SP900A spectrophotometer under the following conditions: slit-width, 0·018 mm; gain, 2·0; band-width, 1; hydrogen-pressure reading, 10 cm on a butyl phthalate filled manometer; nitrogen pressure, 15 p.s.i.; and top of burner head, 3 cm below centre of analysing monochromator slit

In the unsheathed diffusion flame, however, a spectral scan of the emission obtained by spraying a 5×10^{-3} F aqueous sulphur dioxide solution, with a 0·02-mm slit-width, yielded the results shown in Fig. 1. The only emission due to sulphur corresponds to the S_2 species,[5] as follows—

Wavelength, mμ	431	427	419·5	415	405	394	384	374
Relative intensity	3	4	4	6	9	10	10	9
Wavelength, mμ	364·5	359	355·5	350	347	342	337	
Relative intensity	7	3	4	3	2	1	0·5	

The relative intensities of these lines are not corrected for variations in the spectral response of the monochromator and photomultiplier.

Other possible species such as SO, which has a band peaking at 320 mμ, and SH with two bands at 324 and 328 mμ, were not detected. Similar results were obtained with a hydrogen - argon flame.

The intensity of the emission does not exhibit a linear response with the concentration of sulphur dioxide in the nebulised solution. This was first of all suspected to be because of the ease of oxidation of sulphur dioxide in aqueous solution, but careful control of conditions to eliminate this possibility, up to the point of entry into the flame, revealed that while oxidation could lead to appreciable diminutions in signal, the non-linear response was inherent in the system, and investigation showed that the signal was, in fact, proportional to the square of the concentration. The signal was also found to vary linearly with the square of the monochromator slit-width. In this latter respect, the behaviour strongly resembles that observed in the measurement of flame background.[6]

Linear calibration graphs were obtained over the range 10^{-4} to 10^{-2} F solution of sulphur dioxide, *i.e.*, 3·2 to 320 p.p.m., by using the dependence of signal on the square of the concentration. A detection limit of 5×10^{-5} F (1·6 p.p.m.), *i.e.*, corresponding to signal-to-noise ratio of 1 : 1, was observed. Special care must be taken to prevent oxidative degradation of the very dilute solution of sulphur dioxide.

DETERMINATION OF SULPHATES BY S_2 EMISSION—

Because it is difficult to convert most sulphur-containing species quantitatively into sulphites and to stabilise the sulphite ion, but relatively easy to convert them into sulphates

which are very stable, an effort was made to improve the production of S_2 species from sulphate ion by using the flame-shielding arrangement described briefly above.

A borosilicate-glass tube, 15 cm long by 2·4 cm i.d., was placed over the 1·75 cm diameter burner stem of a conventional (circular) Unicam propane-emission burner head and attached at the lower end by a rubber stopper. By systematic variation of conditions it was found that optimum stability and sensitivity for S_2 emission from sulphate solutions was obtained, as before, when the nebulising gas pressure was 15 p.s.i. and the hydrogen flow-rate slightly more than sufficient to prevent strike-back. When the shielded flame is lit, a penetrating whine is heard that dies out after about 1 minute when the shield has warmed up and condensation has ceased. If the hydrogen pressure is too high the whine will persist. These experiments also revealed that the tube must be vertical to allow even diffusion and removal of water vapour, and that the best results were obtained with the glass tube projecting about 10 cm above the burner head, with the latter situated about 0·5 cm above the bottom of the entrance slit of the monochromator. Because of the higher temperature of this flame only low emission was observed from S_2 species, but when the outer surface of the screening tube was cooled by a current of cold air from a piece of pressure tubing (0·65 cm bore) placed about 1 cm away from the wall of the tube, level with the entrance slit of the monochromator, and at right angles to the line between the slit and the tube, good S_2 emission signals were obtained. The screening tube should be allowed to warm up for 1 minute before switching on the cooling air, as this minimises problems that may otherwise arise from condensation of water vapour. The glass slide placed over the entrance slit prevents dust being blown into the monochromator. With the arrangement described, condensation was observed only on cooling very strongly, and even then only in the lower regions of the tube below the burner head.

The above flame is colourless inside the protective tube, but is shown up as a separated flame upon spraying a sodium solution into it. Within the tube the signal obtained from a 2 p.p.m. solution of sodium was about 15 times less than that for an unprotected pre-mixed hydrogen - air flame and about three times greater than that of the hydrogen - nitrogen diffusion flame. The signal obtained from an ammonium sulphate solution in this flame was about 30 times greater than in the hydrogen - nitrogen diffusion flame, while the emission from a sulphur dioxide solution was virtually identical. The ratio for the S_2 signals from ammonium sulphate and sulphur dioxide of the same sulphur content is about 1:20 for the protected flame and about 1:600 for the diffusion flame. Varying signals are obtained for different salts. Thus, where a standard solution of sulphuric acid gave an S_2 emission at 384 mμ, corresponding to 21 scale-divisions, equimolar solutions of ammonium sulphate, sodium sulphate and potassium sulphate gave 23, 12 and 12, respectively. This problem of differing response with different accompanying cations may readily be resolved, however, by passing these salts over a cation-exchange resin in the hydrogen-ion form, so that all forms of sulphate are converted into sulphuric acid. In this way the ease with which most inorganic sulphur species may be converted into sulphate, and also organic compounds via the oxygen-flask (or some other simple combustion process), may provide a simple and reasonably sensitive procedure for the determination of all sulphur species.

As before, the method shows a linear dependence of signal upon the square of the concentration of the sulphate ion. The detection limit (signal-to-noise ratio = 1) for sulphuric acid corresponded to 10^{-4} F (about 3 p.p.m. of sulphur) and linear calibration graphs were obtained over the range 6·4 to 500 p.p.m. For concentrations of sulphur greater than 100 p.p.m. the diffusion flame gives adequate sensitivity and is simpler to use.

FLAME TEMPERATURES—

Whereas the temperatures of most flames can only be determined satisfactorily by spectroscopic techniques such as line reversal, the diluted diffusion flame and the cooled, separated flame used in these experiments give temperatures of only a few hundred degrees centigrade, and they can thus be measured fairly reliably by inserting a calibrated thermocouple probe into the flame. In these studies, the end of the ceramic cover of an industrial pyrometer was removed so that the small area of the exposed thermocouple junction did not greatly affect the flow of the flame gases. While it was recognised that the presence of hydrogen might give rise to catalytic effects that could cause spuriously high temperatures, the temperatures at the centre of the flame were found to be only about 300° to 400° C, and

TABLE I

APPROXIMATE FLAME TEMPERATURES IN THE HYDROGEN - NITROGEN DIFFUSION FLAME DURING ASPIRATION OF DISTILLED WATER

Distance above burner head, cm	Temperature, °C: Centre of flame (minimum)	Temperature, °C: Edge of flame (maximum)
1	280	810
2	330	830
3	370	840
4	410	850
5	450	850
6	480	850

no catalytic glow could be detected. The thermocouple was calibrated in a heating-bath up to 360° C by using a standardised 0° to 360° C thermometer. It will be seen from Table I that the temperature varies from top to bottom of the hydrogen - nitrogen diffusion flame and from the inner to the outer regions of the flame. The temperatures recorded at the centre of the flame are minimum temperatures obtained by lateral movement of the probe at a constant height, while those recorded at the outer edge are maximum temperatures similarly obtained. The flame conditions used to obtain the temperatures recorded in Table I were those used under the conditions recommended for the determination of sulphur dioxide, with distilled water aspirating through the flame. The temperature at the centre of the flame, at the position most favourable for analysis, corresponds to 390° C. These temperatures are recorded only to the nearest 10° C because of the temperature gradient across the flame. Without water aspirating through the flame the centre temperatures at about 3 cm above the burner head increased by 30° C, while the outside temperatures increased by only 5° C. Aspiration of acetone and methanol caused a slight temperature decrease in the centre of the flame and a more marked temperature increase at the outer edge of the flame.

Temperature measurements were made in the hydrogen - air shielded flame by exposing a further length of the probe from its ceramic sheath and inserting it into the flame from the base of the tube via a slit cut in the rubber bung. Temperature measurements were made with the probe situated mid-way between the edge of the burner head and the flame shield. The flame conditions were those used for the sulphuric acid determination, with distilled water spraying through the flame. In this intance, the temperature measurements recorded in Table II were obtained with, and without, air cooling of the outer sheath. Without water

TABLE II

APPROXIMATE FLAME TEMPERATURES IN THE LOWER PART OF THE SHIELDED AIR - HYDROGEN FLAME DURING ASPIRATION OF DISTILLED WATER

Distance above burner head, cm	Temperature at outer edge of flame, °C: Cooled sheath	Temperature at outer edge of flame, °C: Uncooled sheath
0·1	280	420
0·3	320	440
1·0	360	520

Temperatures at centre of flame > 1400° C.

aspirating in this type of flame the temperature increased by 10° to 20° C. When the wires leading to the thermocouple were bent so that the thermocouple junction was over the centre of the burner head, inner flame temperatures in excess of 1400° C were reached. As the thermocouple was uncalibrated in this region, this latter temperature can only be regarded as approximate.

INTERFERENCES—

Interference studies were carried out with a 5×10^{-3} F solution of sulphuric acid and optimised flame conditions, with the shielded air - hydrogen flame. The following did not interfere when present in 10-fold molar excess (*i.e.*, concentrations of 5×10^{-2} F), hydrochloric, perchloric, boric, acetic, oxalic, hydrobromic, nitric and orthophosphoric acids, and hydrogen peroxide. In addition, 40-fold excesses of hydrochloric and perchloric acids, and hydrogen peroxide (*i.e.*, 2×10^{-1} F concentrations) could be tolerated. Above 10-fold

excess, orthophosphoric, nitric and hydrobromic acids caused a decrease in the S_2 signal, while acetic and oxalic acids caused an increase in signal. These effects are probably caused by viscosity and surface tension, respectively, which modify the nebuliser efficiency. In all instances, however, good calibration graphs for sulphur may be obtained in the presence of any of these acids. The enhancing or depressive action of individual acids may, therefore, be accounted for by dilution or by making up to a pre-determined strength. Cationic interferences were not investigated because the method proposed here would operate best following ion-exchange separation of the sulphuric acid.

Discussion

The above experiments show how useful progress may be made in molecular-emission studies in relatively cool flames, despite the steady trend in atomic-absorption spectroscopy and flame-emission spectroscopy for hotter flames to be used to produce greater populations of refractory metal atoms and thus to improve signals. In this paper we have discussed the behaviour of sulphur species and have shown how an analytical method for sulphur may be devised, irrespective of the original species, by oxidation to sulphate, conversion to sulphuric acid and measurement of S_2 emission in a hydrogen - nitrogen diffusion flame or, more sensitively, in a shielded pre-mixed hydrogen - air flame, in which the sheath is cooled to prevent dissociation of the S_2 species produced in the hot lower region of the enclosed flame. The sensitivity of the method for sulphur dioxide is considerably greater than that for sulphuric acid in either flame, and shows greater and more extensive emission than that observed by Crider[7] in a shielded hydrogen - air pre-mixed flame without cooling.

The method proposed here appears to have considerable promise in relation to previously described flame (atomic)-emission methods that have depended on depressive effects on the emission of elements such as barium[8] or strontium,[9] or on indirect methods involving the precipitation of barium sulphate[10] and determination of the excess of barium. Because of the square law dependence on sulphate-ion concentration, the method suggested here is most attractive for a narrow range of sulphur concentrations. Work is now in progress on the application and adaptation of the principle of the procedure to the analysis of technical materials and will be reported in a subsequent paper. Meanwhile, it is apparent that the sulphur content of most organosulphur compounds could be readily obtained directly by the above procedure, following rapid decomposition by an oxygen-flask[11] technique. Although the method based on sulphite is considerably more sensitive than that based on sulphate, the ease with which most organic and inorganic sulphur species may be oxidised quantitatively to sulphate, and when necessary converted into sulphuric acid by ion exchange, suggests that the sulphuric acid procedure may be generally more useful for most analytical problems.

Experimental

Apparatus—

A Unicam SP900A flame-emission - absorption spectrophotometer, fitted with standard air - acetylene (rectangular) and air - propane (circular) burner heads, was used. Flame shielding for the pre-mixed flame was as described in the text.

Fuel gas—Hydrogen, from a cylinder.

Diluent gas—Nitrogen, from a cylinder.

Supporting gas—Air, from a compressor unit.

Reagents—

Sulphuric acid, 10^{-1} F—Prepare by diluting analytical-reagent grade sulphuric acid, standardising and subsequently adjusting the solution to exactly 10^{-1} F.

1 ml of 10^{-1} F sulphuric acid $\equiv$ 3200 μg of sulphur.

Sulphur dioxide solution, 10^{-2} F—Prepare by passing gaseous sulphur dioxide into de-aerated distilled water. A sample of this stock solution was oxidised by hydrogen peroxide (neutralised) and standardised alkalimetrically. An aliquot of the stock solution was then diluted with de-aerated distilled water to be exactly 10^{-2} F in sulphur dioxide.

1 ml of 10^{-2} F sulphur dioxide $\equiv$ 320 μg of sulphur.

PROCEDURES—

Calibration graph for sulphuric acid (**32** *to* 160 *p.p.m. of sulphur*) *in air - hydrogen flame*—Transfer by pipette 1 to 5-ml aliquots of 10^{-1} F sulphuric acid into a series of 100-ml standard flasks and dilute to volume with distilled water. Assemble the sheathed burner unit, as described previously, and arrange it so that the burner head is opposite and projecting 0·5 cm above the level of the bottom of the entrance slit of the monochromator, and in such a position that a stream of cooling air may be directed on the glass shielding tube at right angles to the plane it subtends with the slit, and at the same level.

Set the air pressure at 15 p.s.i. and the hydrogen flow-rate to about 16 cm on the butyl phthalate filled manometer of the fuel-meter, and light the flame. Allow the shield to warm up for 1 minute before switching on the cooling air. Set the monochromator at 384 mμ, select the emission mode of the flame spectrophotometer and select the band-width setting No. 3. Spray the calibration solution into the flame, allowing sufficient time for equilibrium to be reached and record the emission intensity at 384 mμ. The background signal, which is very low (about 1·5 divisions, with a slit of 0·05 mm and an instrumental gain of 3·3), should be deducted from the observed signal. The calibration graph, prepared by plotting emission intensity against the square of the sulphur concentration, is virtually linear in this range. For higher concentrations (above 100 p.p.m. of sulphur) of sulphuric acid the procedure with the hydrogen - nitrogen diffusion flame is simpler to operate and possesses adequate sensitivity.

Calibration graph for sulphurous acid (3·2 *to* 16 *p.p.m. of sulphur*) *in the hydrogen - nitrogen diffusion flame*—Transfer by pipette 1 to 5-ml aliquots of the freshly prepared 10^{-2} F sulphur dioxide solution into a series of 100-ml standard flasks and dilute to volume with freshly de-aerated water. Set up the air - acetylene burner head so that the head-plate is about 3 cm below the centre of the entrance slit of the monochromator. Set the nitrogen pressure to 15 p.s.i. and the hydrogen flow-rate to about 10 cm on the manometer, and light the flame. Clean the spray unit and burner head by aspirating distilled water through it and by using the same optical settings on the instrument as above, nebulise the sulphur dioxide solutions as expeditiously as possible to avoid aerial oxidation of the dissolved sulphur dioxide. Prepare calibration graphs of signal against the square of sulphur concentration, as before.

Calibration graphs can be conveniently prepared only over a 5-fold concentration range because of the square law relationship. When sulphuric acid solutions containing more than 100 p.p.m. of sulphur are being sprayed into the nitrogen - hydrogen diffusion flame, the burner head should be situated about 4 cm below the centre of the slit to allow for the maximum S_2 signal being obtained higher up in the flame for sulphuric acid than for sulphurous acid. A convenient working range for sulphuric acid in the diffusion flame corresponds to 160 to 500 p.p.m. of sulphur.

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A Field Method for Determining 4,4′-Di-isocyanatodiphenylmethane in Air

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A method has been developed for determining up to 0·04 p.p.m. v/v of di-isocyanatodiphenylmethane (MDI) in a 5-litre sample of air. The test atmosphere is drawn through 0·4 M hydrochloric acid, which hydrolyses the isocyanate to the corresponding amine. This is then diazotised and coupled with 3-hydroxy-2-naphthanilide. After acidification of the solution the resulting pink compound is extracted into chloroform and compared visually with inorganic colour standard solutions. The preparation of three standards is described, corresponding to 0·01, 0·02, 0·04 p.p.m. of MDI, the recommended threshold limit value being 0·02 p.p.m. v/v. There is no interference from the tertiary-amine catalysts that are used with MDI in the production of polyurethanes.

4,4′-Di-isocyanatodiphenylmethane (MDI) is used for the production of rigid and semi-rigid polyurethane foams that have wide applications for thermal insulation, and as packing and building materials. It is also a constituent of surface-coating lacquers.

The recommended threshold limit value for MDI[1] is 0·02 p.p.m. v/v. Because of its low vapour pressure it is unusual under normal conditions of use for atmospheric concentrations of MDI vapour to exceed this value. However, if equipment for dispensing rigid foam in bulk is incorrectly operated, or if the ventilation on spraying equipment is inadequate, then droplets of material containing free MDI can escape into the general atmosphere, where they constitute a hazard.

In an earlier method[2] the test atmosphere was drawn through a solution of sodium acetate and hydrochloric acid. The hydrolysis product was coupled with *p*-nitrodiazobenzene solution to give a yellow azo compound that was extracted into chloroform and matched visually against inorganic colour standards. This suffered from two disadvantages. One, that the standard colours were rather pale and difficult to match, particularly in artificial light, and the other, that interfering orange - red colours were given by some of the tertiary amines that were used as catalysts in certain applications.

The method described in this paper avoids both of these disadvantages; the test atmosphere is drawn through dilute aqueous hydrochloric acid, and MDI is hydrolysed to 4,4′-diaminodiphenylmethane. This is then diazotised by treatment with sodium nitrite, and the excess of nitrous acid is destroyed with sulphamic acid. The resulting solution is coupled with an alkaline solution of 3-hydroxy-2-naphthanilide to give a pinkish-orange azo compound. After acidification, this is extracted into chloroform and the solution compared visually with inorganic colour standard solutions. These solutions are deeper in colour and much more easily seen than those described.[2] There is no interference from any of the common tertiary-amine catalysts.

Method

Apparatus—

Sampling pump—Capable of drawing air at 1 litre per minute through the absorber.
All-glass absorber—Of the type shown in Fig. 1.
Flat-bottomed specimen tubes—2 × $\frac{3}{8}$ inches (see Notes).
Glass-stoppered separating funnel—25-ml capacity, with its stem cut off $\frac{3}{4}$ inch below the tap.

REAGENTS—

Dilute hydrochloric acid—Dilute 40 ml of concentrated hydrochloric acid (sp.gr. at 20° C, 1·18) to 1 litre with water.

Sodium nitrite solution, 0·7 per cent. w/v.

Sulphamic acid solution, 10 per cent. w/v.

Sodium hydroxide solution, N.

Brenthol AS suspension—Weigh 0·5 g of Brenthol AS (3-hydroxy-2-naphthanilide) (obtainable from I.C.I. Ltd.) into a bottle, and add 50 ml of water. Shake vigorously immediately before use.

Dilute sulphuric acid—Dilute 16·5 ml of concentrated sulphuric acid (sp.gr. at 20° C, 1·84) to 100 ml with water.

Chloroform—Analytical-reagent or B.P. grade.

Cobalt(II) chloride colorimetric solution[3]—Prepare a solution in 0·25 M hydrochloric acid containing 59·5 mg per ml of analytical-reagent grade cobalt(II) chloride, $CoCl_2.6H_2O$ (purity not less than 97·5 per cent.).

Iron(III) chloride colorimetric solution[3]—Prepare a solution in 0·25 M hydrochloric acid containing 45·05 mg of iron(III) chloride per ml.

Colour standard solutions—Mix the volumes of the cobalt(II) chloride and iron(III) chloride colorimetric solutions specified in Table I, and dilute to 500 ml with 0·25 M hydrochloric acid. These solutions have the same quality and depth of shade as the test solutions (containing the equivalent amounts of 4,4′-di-isocyanatodiphenylmethane) prepared as described below. Store in clean glass bottles with tightly fitting glass stoppers.

TABLE I

COMPOSITION OF COLOUR STANDARD SOLUTIONS

Cobalt(II) chloride solution, ml	Iron(III) chloride solution, ml	MDI, p.p.m.
13·0	15·0	0·01
23·0	10·0	0·02
42·0	9·0	0·04

PROCEDURE—

Draw 5·0 litres of the test atmosphere, at a rate of about 1 litre per minute, through the absorber containing 3·0 ml of dilute hydrochloric acid. Detach the air-inlet tube, allowing any liquid in it to drain into the body of the absorber and remove the last drops by blowing gently. Add 0·10 ml (3 drops) of sodium nitrite solution, stopper with a B19 glass stopper, mix the contents by inverting the tube and allow it to stand for 1 minute. Add 0·2 ml (6 drops) of sulphamic acid solution, making sure that the joint is wetted by the reagent. Stopper, shake well, and allow the tube to stand for 1 minute. Pour the contents into the 25-ml separating funnel containing 2 ml of N sodium hydroxide and 0·2 ml (6 drops) of Brenthol AS suspension, shaking the bottle containing the latter immediately before use. Stopper, mix well, and allow the mixture to stand for 1 minute. Add 1 ml of dilute sulphuric acid and 3·0 ml of chloroform. Stopper the funnel, shake it vigorously for 1 minute, allow the chloroform layer to separate and run it into a 2 × $\frac{3}{8}$-inch flat-bottomed specimen-tube. Fill similar tubes to the same depth with the three colour standard solutions. Compare the chloroform extract visually with the three standards in turn by viewing vertically through the depth of the liquids when the tubes are held side by side above a sheet of white paper in a good light, preferably daylight. Assign the appropriate MDI concentration to the extract, interpolating a value between two of the standards, if necessary.

NOTES—

1. Flat-bottomed tubes have been recommended for comparison of the colour, because with round-bottomed test-tubes the appearance of the bottoms of similar tubes containing chloroform and water is different and can cause difficulty in colour matching.

2. An alternative means of comparing the colours obtained is to use the Lovibond Special Purposes Comparator. A disc containing glass standards, equivalent to the above colour standard solutions, for use with this instrument is available from Tintometer Scales Limited, Waterloo Road, Salisbury, Wiltshire. A special narrow 20-mm cell is also needed and is obtainable from the same source.

CHOICE OF ABSORPTION SOLUTION AND OF STANDARD MATERIAL—

An established absorption solution for isocyanates is 0·4 M aqueous hydrochloric acid. Mixtures of dilute hydrochloric acid and acetic acid have also been used,[4] but it has been found that the addition of acetic acid to hydrochloric acid does not improve the degree of trapping of MDI (see "Absorption of MDI from the Atmosphere" below) or give an increased colour yield from a given weight of MDI, and 0·4 M hydrochloric acid has therefore been adopted.

Under the conditions used, MDI and 4,4′-diaminodiphenylmethane (DADPM) give equivalent colour yields (see "Comparison of Calibration Graphs obtained from MDI and DADPM" below) and, in establishing the preferred conditions of diazotisation, coupling and colour matching, DADPM has been used as standard because it is much more easily dissolved than MDI in dilute hydrochloric acid.

SCALE OF EXPERIMENTS—

To obtain the maximum intensity of colour in the final chloroform extract, the volumes of reagents were kept as small as possible, *i.e.*, 3 ml of 0·4 M hydrochloric acid and 3 ml of chloroform. This volume of chloroform was sufficient for visual colour matching but insufficient for the spectrophotometric measurements that were used to establish the quantitative basis of the method. Therefore, the scale was increased 5-fold to 15 ml of 0·4 M hydrochloric acid and 15 ml of chloroform for many of the tests.

CHOICE OF COUPLING AGENT—

Four coupling agents were tried, *viz.*, *N*-1-naphthylethylenediamine dihydrochloride, 2-naphthol-3,6-disulphonic acid, sodium salt (R-salt), 3-hydroxy-2-naphthoic acid and 3-hydroxy-2-naphthanilide (Brenthol AS). The last two have the advantage that they produce dyestuffs that can be extracted into chloroform. This is important because field tests showed that the coupled test solution can be turbid owing to spray-borne polyurethane foam or lacquer, and this makes colour matching of the aqueous solution difficult. When the dyestuff was extracted into chloroform the turbidity was left in the aqueous layer.

3-Hydroxy-2-naphthoic acid was about 25 per cent. more sensitive than Brenthol AS but produced a fairly strong yellow colour in the chloroform extract, which, although it did not interfere with spectrophotometric measurement of the pink colour due to MDI, made visual matching rather difficult. 3-Hydroxy-2-naphthanilide (Brenthol AS) gave only a pale yellow blank and was therefore preferred as a coupling agent. For most of the subsequent investigation the following conditions were used, with results as shown in Table II.

TABLE II

EFFECT OF CHANGING METHOD OF ADDING 3-HYDROXY-2-NAPHTHANILIDE

Method of adding 3-hydroxy-2-naphthanilide—	Solution					Suspension		
DADPM, μg	3·8	5·5	7·6	10·3	15·2	7·5	12·5	17·5
Optical density at 540 mμ, with 4-cm cells	0·197	0·305 0·310	0·394	0·570 0·560	0·800	0·342	0·677	0·905

Fifteen millilitres of a solution containing 5 to 30 μg of DADPM in 0·4 M hydrochloric acid were treated with 0·5 ml of 0·5 N sodium nitrite solution. After 1 minute, 1·0 ml of sulphamic acid solution was added, and after a further minute the solution was poured into a separating funnel containing 10 ml of a 0·1 per cent. solution of Brenthol AS in N sodium hydroxide. After 1 minute, 5 ml of dilute sulphuric acid and 15 ml of chloroform were added and the mixture was shaken for 1 minute. The chloroform extract was filtered directly into a dry 4-cm spectrophotometer cell and the optical density measured at 540 mμ, with a reagent blank as the comparison solution.

TIMES OF DIAZOTISATION AND COUPLING WITH 3-HYDROXY-2-NAPHTHANILIDE—

Tests showed that 1-minute periods were sufficient for the diazotisation and coupling stages.

EFFICIENCY OF SINGLE EXTRACTION—

This was checked by four tests on 5·5-μg amounts of DADPM. In two of the tests, single extractions with 15·0 ml of chloroform were carried out; in the other tests, three

separate extractions with 5·0 ml of chloroform were carried out. The combined extracts were diluted to 15·0 ml, filtered and their optical densities measured with the following results—

Number of extractions per 15·0 ml of total extract	1	1	3	3
Optical density at 540 mμ, with 4-cm cells	0·305	0·310	0·315	0·325

These results show that about 95 per cent. of the colour is removed by a single extraction.

COMPARISON OF CALIBRATION GRAPHS OBTAINED FROM MDI AND DADPM—

As MDI is not easily soluble in dilute aqueous hydrochloric acid, a solution was prepared in dry dioxan, the solvent having been dried by refluxing with sodium, followed by distillation. The solution used contained 52·5 μg of distilled MDI per ml. Aliquots of this solution were diluted to 15·0 ml with 0·4 M hydrochloric acid, diazotised without delay and then coupled with 3-hydroxy-2-naphthanilide, as described under "Choice of Coupling Agent" above; similar tests were also carried out on a solution of DADPM in dry dioxan containing 35·5 μg per ml, the results of which are shown in Tables III and IV. They show that DADPM and equivalent weights of MDI give closely similar optical density levels, indicating that MDI is hydrolysed rapidly and completely to DADPM by 0·4 M hydrochloric acid at room temperature. The level of optical densities obtained from DADPM in the presence of dioxan was lower than that obtained in its absence (see Table II).

TABLE III

CALIBRATION POINTS FOR DADPM IN THE PRESENCE OF DIOXAN

DADPM solution, ml	0·1	0·2	0·3	0·4	0·5
DADPM, μg	3·55	7·10	10·65	14·2	17·75
Optical density at 540 mμ, with 4-cm cells	0·140	0·275	0·395	0·500	0·600

TABLE IV

CALIBRATION POINTS FOR MDI IN THE PRESENCE OF DIOXAN

MDI solution, ml	0·1	0·2	0·3	0·4
MDI, μg	5·25	10·5	15·75	21·0
DADPM, equivalent μg assuming complete hydrolysis ..	4·2	8·4	12·6	16·8
Optical density at 540 mμ, with 4-cm cells	0·165	0·300	0·460	0·535
DADPM, μg equivalent to optical density (from Table III) ..	4·3	7·9	12·8	15·6
Percentage recovery of original MDI as DADPM	102	94	102	93

ABSORPTION OF MDI FROM THE ATMOSPHERE—

Because of its low volatility and the very low concentrations involved, attempts to prepare accurately known atmospheric concentrations of MDI vapour were unsuccessful. Steady concentrations were, however, prepared by passing 1 litre of pure nitrogen per minute from a cylinder through a flow-meter and a bubbler containing 15 ml of Suprasec DN, which was immersed in a water-bath. By raising or lowering the temperature of the water-bath, the MDI concentration in the nitrogen leaving the bubbler was increased or reduced. Two bubblers of the type shown in Fig. 1, each containing 3 ml of 0·4 M hydrochloric acid, were connected in series to the outlet of the bubbler containing the MDI. Glass-to-glass contact between the three bubblers inside rubber sleeves was ensured. After each test the contents of the bubblers were diluted separately to 15·0 ml, and diazotised and coupled with 3-hydroxy-2-naphthanilide, as described under "Choice of Coupling Agent" above. Results of the tests (Table V) show that an average of 75 per cent. of the total MDI vapour trapped was con-

TABLE V

RECOVERY OF MDI VAPOUR FROM THE ATMOSPHERE

Test number	1	2	3	4	5	6	7
Temperature of water-bath, °C	20	20	20	27	44	35	42
Time of bubbling, minutes ..	85	90	101	96	67	120	75
Optical density, 1st bubbler ..	0·325	0·400	0·430	0·345	0·835	0·975	0·550
Optical density, 2nd bubbler ..	0·135	0·095	0·145	0·140	0·250	0·290	0·190
MDI, μg, 1st bubbler (see Table II for calibration points)	7·6	9·35	10·1	8·05	19·7	23·1	12·9
MDI, μg, 2nd bubbler	3·15	2·3	3·4	3·3	5·9	6·8	4·4
MDI, total μg	10·75	11·65	13·5	11·35	25·6	29·9	17·3
MDI, p.p.m.	0·013	0·013	0·013	0·012	0·038	0·025	0·022
Percentage of total MDI in in 1st bubbler..	71	80	75	71	77	77	75

tained in the first bubbler. Attempts were made to improve this by incorporating either acetic acid or a surface-active agent (Lissapol NX) but little, if any, improvement resulted.

Recovery tests were also carried out on spray-borne MDI. Pairs of bubblers were mounted close to the spraying head of a machine producing rigid foam-laminated building board. Each pair consisted of an absorber of the type used in the method, containing 3·0 ml of 0·4 M hydrochloric acid, followed by a sintered-plate absorber containing 15 ml of 0·4 M hydrochloric acid, the two being joined together by glass-to-glass joints inside rubber sleeves.

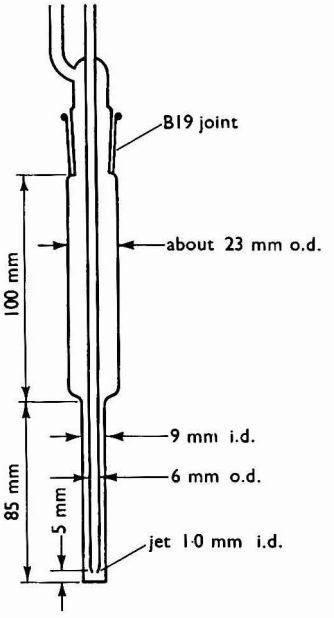

Fig. 1. All-glass absorber

For each test air was drawn through the absorbers at 1 litre per minute for times ranging from 2 to 5 minutes. After sampling, the contents of the first absorber were diluted to 15·0 ml with 0·4 M hydrochloric acid, and the contents of both absorbers were diazotised and coupled with 3-hydroxy-2-naphthanilide, etc., as described under "Choice of Coupling Agent" above. The results are given in Table VI. The average percentage recovery was 76 per cent., *i.e.*, close to that for vapour, although the spread was greater.

TABLE VI

RECOVERY OF MDI FROM SPRAY IN THE ATMOSPHERE

Test number	1	2	3	4	5	6
Time of sampling, minutes	3·0	8·0	2·0	5	4·5	4·25
Optical density at 540 mμ, with 4-cm cell, 1st absorber	0·355	1·13	0·445	0·355	0·600	0·390
Optical density at 540 mμ, with 4-cm cell, 2nd absorber	0·100	0·470	0·145	0·095	0·260	0·070
MDI, μg, 1st absorber	8·2	26·4	10·4	8·2	14·0	9·1
MDI, μg, 2nd absorber	2·3	11·0	3·4	2·2	6·1	1·6
MDI, total μg	10·5	37·4	13·8	10·4	20·1	10·7
MDI, p.p.m.	0·35	0·47	0·69	0·21	0·44	0·25
Percentage of total MDI in 1st absorber	78	71	75	79	70	85

STABILITY OF 3-HYDROXY-2-NAPHTHANILIDE SOLUTION AND ITS USE INSTEAD OF AN AQUEOUS SUSPENSION—

In all of the work described previously, freshly prepared solutions of 3-hydroxy-2-naphthanilide were used, which were pale yellow initially, but darkened. Old solutions produced much deeper blank solutions in the test procedure than fresh ones, even when they were kept in darkness. This was of little importance when the final colour was being measured

on a spectrophotometer, but in the usual matching procedure the use of solutions of 3-hydroxy-2-naphthanilide more than 4 hours old resulted in colours that were too yellow to be matched with the inorganic colour standard solutions. It would be very inconvenient to have such an unstable solution when testing atmospheres that are remote from laboratory facilities.

This problem was overcome by using a 1 per cent. w/v aqueous suspension of 3-hydroxy-2-naphthanilide. The coupling solution was prepared shortly before use by adding to the separating funnel 10 ml of N sodium hydroxide and 1·0 ml of the 1 per cent. aqueous suspension of 3-hydroxy-2-naphthanilide. Table II shows calibration points obtained by using either this suspension or the solution; all the points lie close to the same curve when plotted on a graph.

PRODUCTION OF PERMANENT INORGANIC COLOUR STANDARD SOLUTIONS—

It was desired to prepare inorganic colour standards that would match the chloroform extracts obtained when the method was applied to MDI concentrations of 0·01, 0·02 and 0·04 p.p.m. v/v.

In establishing the standards, colours were developed with DADPM and the amounts of DADPM corresponding to the desired MDI concentrations were calculated, assuming that only 75 per cent. of the MDI is trapped in a single absorber, but that the trapped MDI gives the same colour as an equivalent weight of DADPM. Thus 0·01, 0·02 and 0·04 p.p.m. v/v of MDI on a 5-litre sample (at 20° C and 760 mm of mercury) correspond to 0·31, 0·62 and 1·24 μg of DADPM, respectively. For convenience in handling, the preparation was scaled-up 5 times to that 15 ml of chloroform extract could be obtained.

From a solution of DADPM, containing 5·0 μg per ml in 0·4 M hydrochloric acid, 0·31, 0·62 and 1·24-ml portions were taken and were diluted to 15·0 ml with 0·4 M hydrochloric acid and diazotised, coupled and extracted with chloroform, as described under "Choice of Coupling Agent" above. Portions of each extract were run into 2 × $\frac{3}{8}$-inch flat-bottomed specimen tubes. Similar tubes were filled with coloured solutions made by diluting mixtures of standard iron(III) and cobalt(II) chloride solutions[3] with 0·25 M hydrochloric acid. The proportions of the standards were varied by trial and error until an exact match was obtained when the tubes containing chloroform extract and standard were held side by side above a sheet of white paper and viewed vertically through the depth of the liquid in good daylight, but not bright sunlight. Table I shows the volumes of cobalt(II) and iron(III) chloride solutions, diluted to 500 ml with 0·25 M hydrochloric acid, which will produce standards equivalent to 0·01, 0·02 and 0·04 p.p.m. v/v of MDI in a 5-litre sample when tested by the procedure described above. The validity of these standards was checked by tests on a second standard DADPM solution.

STRENGTH OF NITRITE SOLUTION REQUIRED FOR DIAZOTISATION—

In some of the tests described under "Production of Permanent Inorganic Colour Standard Solutions" above, the chloroform extracts were much yellower than in others, making matching very difficult. This was traced to the failure to destroy completely with sulphamic acid all of the excess of nitrous acid left after the diazotisation. The difficulty was overcome by reducing the strength of the nitrite used from 0·5 to 0·1 N and by ensuring that the ground surfaces of the joint and stopper of the bubbler tube were wetted with sulphamic acid when the latter was added. Reduction in the strength of the nitrite did not affect the colour of the final chloroform extracts, as shown when the calibration points with 0·1 N sodium nitrite given below are plotted on the same graph as those in Table II.

DADPM, μg	3·15	6·3	9·45	12·6
Optical density at 540 mμ, with 4-cm cells ..	0·185	0·350	0·465	0·620

INTERFERENCE FROM TERTIARY-AMINE CATALYSTS—

Several tertiary-amine catalysts are used to promote the formation of polyurethanes and it is possible that atmospheric concentrations of these might arise together with MDI.

Six commercial catalysts were tested as follows: 1·0 ml of a 0·1 per cent. w/v solution of distilled MDI in dry dioxan was diluted to 100 ml with 0·4 M hydrochloric acid and 5·0 ml of this solution were further diluted to 50 ml with 0·4 M hydrochloric acid. Five-millilitre volumes of this solution were mixed in turn with 10 mg of each catalyst and diluted to 15 ml

with 0·4 M hydrochloric acid. Each solution was then diazotised and coupled with 3-hydroxy-2-naphthanilide, as described under "Choice of Coupling Agent" and the results are shown in Table VII. In no instance did the optical density in the presence of the catalyst differ significantly from that obtained with MDI alone.

TABLE VII

INTERFERENCE FROM TERTIARY-AMINE CATALYSTS

Name of catalyst	Optical density at 547 mμ, with 4-cm cells
NN-Dimethylcyclohexylamine	0·210
Di-azabicyclo-octane	0·216
Dimethyl-β-phenylethylamine	0·203
N-Ethylmorpholine	0·209
NN-Dimethylbenzylamine	0·225
NN-Dimethylaminoethanol	0·225
None, *i.e.*, 5 μg of MDI only	0·215

DISCUSSION OF RESULTS

The work described above presents the experimental basis of a method for determining MDI in air at concentrations in the region of the recommended threshold limit value of 0·02 p.p.m. v/v. Conditions for sample collection, colour development and colour matching have been established. It has been shown that the colours produced can be reproduced artificially by stable reproducible inorganic coloured solutions, thus facilitating visual comparisons in the field. The colours are stronger and, being pink, more easily matched than the pale yellow colours given by the previous method.[2]

The range of inorganic standards chosen is sufficient for field use in checking the level of safety in an industrial atmosphere. It represents from half to twice the threshold limit value.

The equipment required is portable and independent of laboratory facilities. With the exception of the sampling pump or aspirator, it can all be packed into a wooden carrying-case 19 × 9 × 8 inches. A determination can be completed in 12 to 15 minutes, including sampling time.

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The Polarographic Determination of Dimetridazole in Animal Feeding Stuffs

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It has been found that the reduction wave associated with dimetridazole in saturated borax solution is enhanced by copper, and that this enhancement is dependent upon the amount of copper in solution. A method is described in which this effect is eliminated.

The polarographic determination of dimetridazole, 1,2-dimethyl-5-nitroimidazole, has been described by Kane[1] and by Daftsios.[2] Kane examined polarographically feed extracts in saturated borax solution from −0·3 to −1·0 volt with a silver-wire anode. He reported that unmedicated feed did not contain any interfering substances but that electro-inactive material depressed the diffusion current by 30 to 35 per cent. Rectilinearity of the diffusion-current - concentration relationship was, however, maintained. Recoveries were better than 90 per cent.

Daftsios used methanol extracts from feed samples, which, after chromatographic separation, were dissolved in acid solution and examined polarographically from −0·3 to −1·05 volts, with a mercury-pool anode. Chromatographic purification was effective in removing those materials which caused the depression of diffusion currents reported by Kane, and Daftsios reported that the peak heights from samples extracted from medicated feeds were the same as those of pure standards of the same concentration. Polarograms for unmedicated feeds showed no response. Recoveries by the method were good, averaging 98·5 per cent., with a standard deviation of 0·98, on the three feeds tested.

In spite of the slightly lower recoveries, Kane's method has the advantage of greater speed and simplicity and this method was chosen for further study by the Prophylactics in Animal Feeds Sub-committee of the Analytical Methods Committee. Three test samples of feed were examined and although agreement was good on two samples, one sample gave consistently high results in certain laboratories. In the authors' laboratories, recoveries of 200 per cent. of dimetridazole were repeatedly obtained with a Davis cathode-ray polarograph. The phenomenon reported by Kane was also observed, *i.e.*, the suppression of peak height in feed extract solutions, compared to the peak height observed in simple aqueous solution. Solutions examined were saturated with borax and determined polarographically from −0·5 to −1·0 volt. The peak potential was −0·85 volt and the peak height in feed solution was 22,000 units compared to a height of 62,000 units for the same concentration of dimetridazole in water.

The effect of metal ions on the wave was investigated in an attempt to find the interference that led to high results. By chance, copper was the first element selected for examination and it was found that the element had a marked effect, not only on peak height, but also on the background to the peak. The former was considerably increased and the latter was changed from a flat or, at most, gently rising line to a sharply rising and sometimes stepped slope. The effect of these two interferences on the quantitative determination of dimetridazole in the presence of copper is shown in Figs. 1 and 2. Fig. 1 shows the observed peak heights at various concentrations of dimetridazole in feed extract solutions saturated with borax, in the presence of 0, 10, 30 and 40 p.p.m. of copper. Fig. 2 shows the effect of increasing concentration of copper on a single concentration of dimetridazole and the suppression of peak height in passing from water to feed extract solution. The parallelism of the curves in Fig. 1 indicates that the interference of copper at a particular level is constant,

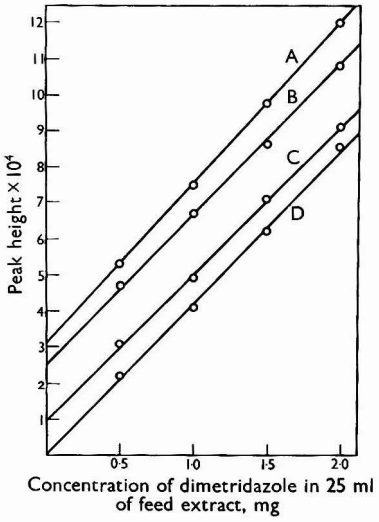

Fig. 1. The effect of A, 40 p.p.m. of copper; B, 30 p.p.m. of copper; C, 10 p.p.m. of copper; and D, no copper on the peak height of dimetridazole

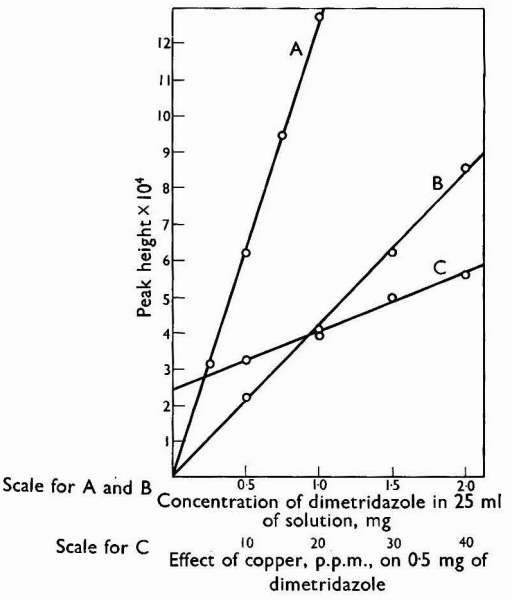

Fig. 2. Graph A, relationship between peak height and amount of dimetridazole in aqueous borax solution; graph B, relationship between peak height and amount of dimetridazole in feed extract saturated with borax; and graph C, effect of increasing amounts of copper on the peak height of a constant amount of dimetridazole

and suggested that copper itself produced a peak at a similar potential to dimetridazole. Examination of copper alone in borax-saturated feed extract solutions showed that, in fact, it did not do so, but merely produced a sharply rising background. In simple aqueous solution saturated with borax, *i.e.*, in the absence of feed extracts, copper was without effect on the peak height.

It was apparent from these results that whatever substances were causing the suppression of the dimetridazole wave, copper had the opposite effect and that, in the presence of these two opposing effects, simple polarography of feed extract solutions was an uncertain basis for a quantitative method. Subsequently, iron, nickel and zinc were investigated but were found to be without effect on the dimetridazole wave. Other elements present in the feeds would be extracted in variable amounts by the acid extraction process and it was assumed that if the effect were general, greater variations in recovery of dimetridazole would have been observed. The effect of copper could be overcome either by removing the feed extract substances responsible for the suppression of the dimetridazole wave or by removing the copper itself. Essentially the former solution would be to return to the more time-consuming method described by Daftsios. Removal of copper from solution could not be achieved without loss of speed and simplicity. Fig. 3 shows the effect of potassium cyanide on 20 and 100 p.p.m. of copper in borax-saturated feed extract solutions containing dimetridazole. It is clear that the copper interference has been eliminated. The copper concentrations, from 20 to 100 p.p.m. in solution, cover concentrations up to about 1000 p.p.m. of soluble copper in the feeds themselves. In the presence of potassium cyanide the peak potential was shifted to −0·35 volt.

To test the general applicability of the method it was tried on several feeding stuffs, with and without added copper, in the presence and absence of cyanide. Results are shown in Table I. All of the samples listed are commercial feeds sold for poultry of one sort or another. Sample number 1 contained dimetridazole as the figures in the second and third columns show. Dimetridazole and copper were added to the feed extract solutions in

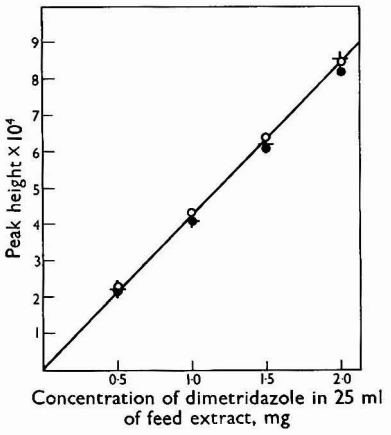

Fig. 3. Peak height of dimetridazole in borax-saturated feed-extract solution containing copper complexed with potassium cyanide: +, no copper; ○, 20 p.p.m. of copper; and ● 100 p.p.m. of copper

the form of 1 mg per ml solutions. Each solution was made up to 25 ml before saturating with borax and finally adding 1 ml of 5 per cent. potassium cyanide solution. To maintain constant volumes of feed extract solutions throughout, water was added, in appropriate volume, when dimetridazole and copper solutions were not. When dimetridazole was added, 1 mg was used, and copper was added in 2-mg amounts.

TABLE I

THE EFFECT OF COPPER AND POTASSIUM CYANIDE ON THE PEAK HEIGHT (×1000) ASSOCIATED WITH THE REDUCTION OF DIMETRIDAZOLE

Sample	Borax solution	With KCN	Added dimetridazole	Added dimetridazole + KCN	Added dimetridazole + Cu	Added dimetridazole + Cu + KCN
1	17·6	15·0	55·0	49·0	103·5	51·0
2	0	0	40·2	39·0	75·0	40·8
3	0	0	42·6	39·0	96·0	40·2
4	0	0	39·6	38·4	70·0	38·4
5	0	0	38·4	36·6	56·0	38·4
6	0	0	62·0	39·6	140·0	40·8

The figures in the sixth column indicate that copper has a marked effect in all cases. Expressed directly in terms of dimetridazole these figures represent recoveries ranging from 145 to 365 per cent. The figures in the seventh column, however, are the same as those in the fifth, and indicate that in all cases the interference has been eliminated. Falls in the values of peak heights between the fourth and fifth columns, for samples 1 and 6, and to a lesser extent for samples 3 and 5, suggested that copper was present in the feeds themselves. The soluble copper content of these samples was determined by atomic-absorption spectrophotometry, and they were found to contain 25, 320, 5 and 5 p.p.m. of soluble copper. Soluble in this context means extracted by the acid-extractant solution and retained in solution after saturation with borax. The lowest level capable of producing a significant effect is probably 5 p.p.m. of copper.

On the basis of these results it is considered that Kane's method is applicable to the examination of feeding stuffs containing dimetridazole provided potassium cyanide solution is added to complex any copper that may be present.

Method

Reagents—

Potassium cyanide solution, 5 per cent. w/v.

Sodium tetraborate.

Dimetridazole standard solution—Dissolve 0·1 g in 200 ml of water containing 5 ml of diluted hydrochloric acid.

Hydrochloric acid, 0·65 per cent. w/v—Dilute 17·6 ml of concentrated hydrochloric acid (sp.gr. 1·18) to 1 litre with water.

Procedure—

To 40 g of feeding stuff in a 500-ml flask add 320 ml of 0·65 per cent. w/v hydrochloric acid and stir the mixture for 2 hours. Allow to settle for 1 hour. Transfer by pipette 1 ml of water and 1 ml of standard dimetridazole solution, separately, into two 25-ml flasks. Make up to volume with feed extract solution. Transfer the solutions to two dry centrifuge tubes and shake them with 3 g of borax. Add 1 ml of 5 per cent. w/v potassium cyanide solution and shake the tubes again. Spin them in a centrifuge at 3000 to 4000 r.p.m. for about 3 minutes.

Transfer sufficient solution from the two tubes to dry polarographic cells and de-oxygenate the solution with oxygen-free nitrogen. Record the polarograms of the solutions from −0·05 to −0·55 volt, or over a suitable potential range to measure the peak height at −0·35 volt. Calculate the dimetridazole content of the sample solution from the difference between the peak heights of the two solutions.

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The Determination of Water in Organic Liquids

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The modified Karl Fischer method, described in a previous report by Archer and Jeater, has been developed further. It has been established that the main cause of end-point drift is pick-up of water from the surroundings. A totally enclosed titration cell that prevents pick-up of water has been devised. A complete apparatus incorporating the cell is described. Water can be determined in samples containing amounts from several per cent. down to a few parts per million with this apparatus.

In work on the determination of water by the Karl Fischer method, described in a previous report,[1] some of the difficulties in determining small amounts of water were overcome. *N*-Ethylpiperidine[2] was used as a catalyst to increase the rate of reaction. End-points were detected by using a polarised electrode system in which changes of voltage across the electrodes were measured. The increased sensitivity in making voltage, in preference to current, measurements has been pointed out by other authors.[3,4] The method was subject to a considerable end-point drift; a system was devised to correct for this.

Other authors have stressed the necessity for excluding moisture pick-up from the surroundings,[5,6] and it seemed most probable that end-point drift was mainly due to this cause.

This view was confirmed by H. L. Evans of the Chemical Inspectorate, Woolwich, who with the authors is currently serving on a British Standards Institute committee investigating methods for determining small amounts of water. By using a cell similar to the one previously described,[1] he was able to reduce drift to a very low level by enclosing the cell in a Perspex "dry box" containing silica gel. Similar results were obtained in this laboratory with a totally enclosed titration cell. This cell has now been incorporated into a general purpose apparatus capable of dealing with samples containing amounts of water from several per cent. down to a few parts per million.

A compact transistorised electronic unit, replacing the pH meter previously used, is described. Although other systems have not been extensively studied it is thought that the circuit offers one of the most sensitive methods for the detection of Karl Fischer titrations. Near the null point the addition of reagent equivalent to 4 μg of water gives a voltage change of 0·01 volt.

Experimental

Description of apparatus—

Cell and titration assembly—The cell and titration assembly are shown in Fig. 1. The 10-ml syringe and micrometer movement are held in the special holder shown in Fig. 2. (If the apparatus is to be used exclusively for the determination of water in the p.p.m. range the 10-ml syringe can be replaced with a standard "Agla" 0·5-ml syringe assembly.)

The cell is of about 30-ml capacity. The silicone rubber connection is normally stoppered with a piece of glass rod. The PTFE tap and the burette piston are lightly greased with silicone high vacuum grease. The connection from the syringe assembly is standard 2-mm i.d. polythene tubing. The platinum electrodes are similar to those previously described[1]; they are sealed into soft glass and held in a PTFE plug machined to fit a standard B10 socket. The reagent reservoir is connected to the two-way tap with black neoprene rubber. The

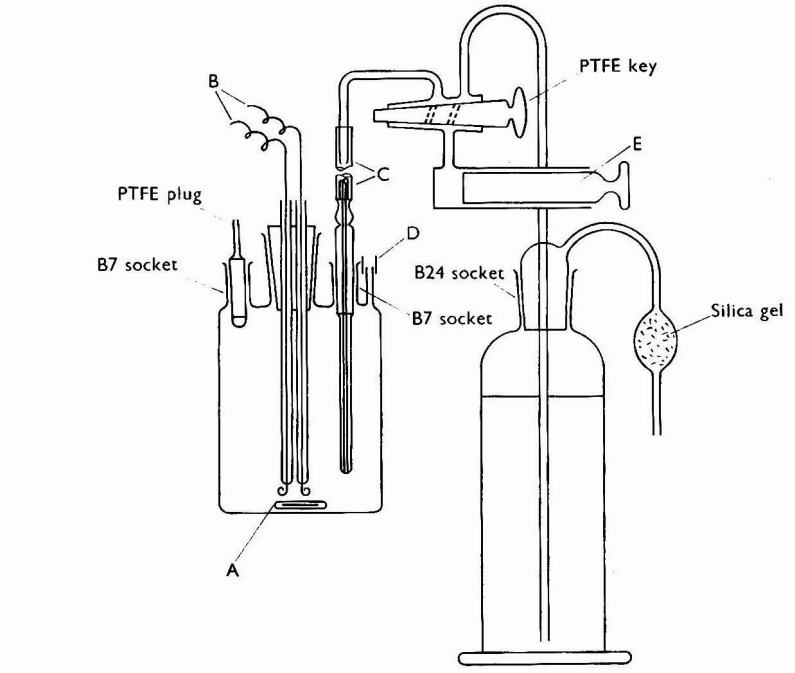

Fig. 1. Cell and titration assembly

connection from the syringe to the two-way tap is made in the position shown so that air bubbles can be easily purged from the system during filling operations, without tilting the apparatus. If samples are to be added to the cell with either a dropping bottle or a pipette they can be admitted by removing the B7 stopper. Samples can be introduced from a hypodermic syringe by removing the glass stopper in the silicone rubber connection.

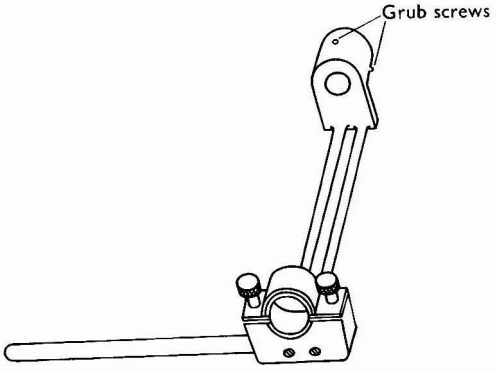

Fig. 2. Holder for syringe and micrometer movement

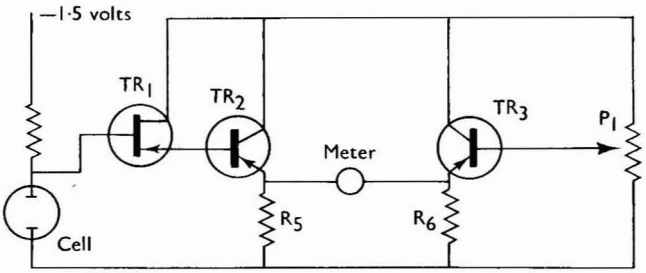

Fig. 3. Details of the basic circuit

Transistorised electronics unit—The basic electrical circuit is as previously described, but as drift has been almost eliminated the recorder is no longer necessary.

The unit comprises a transistorised high impedance voltmeter, a power supply and ballast resistor for the electrode circuit and an internal means for checking the state of the battery. The basic circuit for the high impedance voltmeter is shown in Fig. 3, and the completed circuit is shown in Fig. 4.

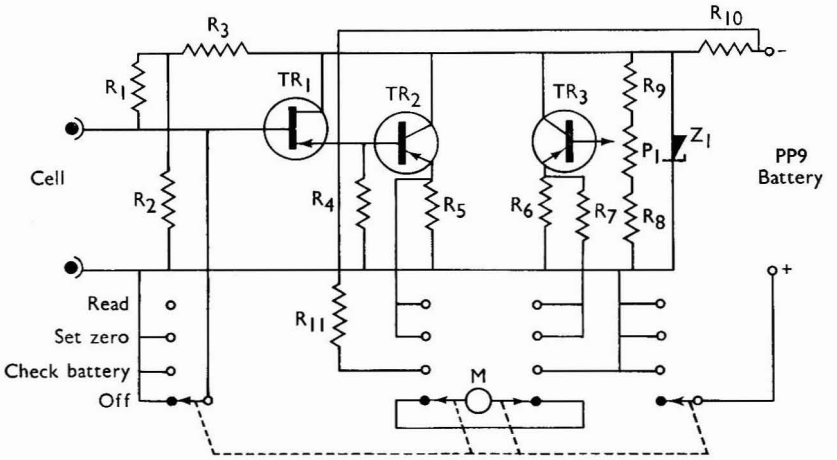

Fig.4. Details of the complete circuit

A field effect transistor is used to provide an input impedance which is high compared with the cell impedance. Two types have been found suitable, the junction-gate transistor giving an input impedance greater than 10^9 ohms, and the insulated-gate transistor giving an input impedance greater than 10^{12} ohms. The former is preferred as it is more robust and much cheaper.

The voltage appearing across the cell is applied to the gate of the field effect transistor operating as a source follower. As the output impedance of the field effect transistor is still too great to operate a meter, a further emitter-follower stage is required in which a normal silicon transistor (TR2) is used. The standing voltage across R1 when the cell is effectively a short circuit is backed off by the voltage across R2, which can be set up by the potentiometer. The symmetry of TR2 and TR3 provide a measure of temperature compensation.

Thus the potential across the electrodes is indicated on the meter which is calibrated from 0 to 1 volt. A change in the ambient temperature from 20° to 60° C causes a zero shift of less than 7 mV per °C (the set zero control can accommodate the zero shift over this range). The same change in ambient temperature causes a sensitivity change of less than 1 per cent.

The internal meter can be used as a 10-volt meter to check the P9 battery (9 volts nominal). It is recommended that the battery is changed when the reading drops to 7 volts.

The electrodes are connected to the unit with long flexible leads so that the cell can be readily inverted in the preliminary cell conditioning operations.

EXPERIMENTS ON END-POINT DRIFT—

An Agla 0·5-ml syringe assembly was used in order to achieve as high a precision as possible. In a typical experiment to investigate "drift," 10-ml of dry methanol *plus* 2 ml of *N*-ethylpiperidine were added to the titration cell, and Karl Fischer reagent was then added until the needle of the electrometer swung over to the left, and remained there for 15 seconds. Reagent was then added as necessary to maintain the needle at this position (the null point), and the amounts added over successive 5-minute intervals were recorded and calculated to their water equivalent.

	Water equivalent of reagent addition, μg
1st 5 minutes	39·2
2nd 5 minutes	30·8
3rd 5 minutes	21·8
4th 5 minutes	20·4
5th 5 minutes	18·3
6th 5 minutes	19·0
7th 5 minutes	20·4

In a further experiment the cell contents were titrated to the null point. Then, taking advantage of the flexible lead from the burette, the cell was inverted so that the liquid came in contact with all the inner surfaces. Again the contents were titrated to the null point, and the drift-rate measured as before. It was found that the cell immediately settled down to a similar steady rate of drift as eventually found in the first experiment. Several replicate experiments confirmed this finding.

Thus, once traces of water in the titration cell have been absorbed, the drift settles down to a rate equivalent to about 4 μg of water per minute. Hence, except perhaps for amounts of water in a sample less than about 10 p.p.m., drift can, for all practical purposes, be ignored. It seems certain that the small residual drift is caused by chemical reaction at the end-point.

METHOD

Set up the titration cell and 10-ml syringe assembly as shown in Fig. 1, introduce 10 ml of dry methanol and 2 ml of *N*-ethylpiperidine into the cell and titrate to the null point. Traces of water within the cell are absorbed by inverting the cell as described above. Introduce the sample and titrate to the null point, noting the difference in micrometer reading.

It is convenient to calibrate the Karl Fischer reagent in terms of the microgram equivalent of water per small scale reading of the micrometer. A satisfactory calibration procedure is to take 10 μl of water with a Hamilton syringe. With this procedure, replicate standardisations gave results of 9·48, 9·53, 9·47 and 9·50 μg of water per small division of the micrometer scale. The actual delivery of the Hamilton syringe should be checked by weighing. Replicate weighings were found to differ by less than ±0·5 per cent.

CONCLUSIONS

A previously described method[1] has been further simplified by eliminating the necessity for assessing end-point drift. The method can now be regarded as a general method capable of determining both high and very low concentrations of water in organic liquids.

Appendix

LIST OF COMPONENTS

R_1	= 1-megohm resistor
R_2	= 1500-ohm resistor
R_3	= 3900-ohm resistor
R_4*	= Selected resistor
R_5, R_6	= 1000-ohm resistors
R_7†	= Selected resistor
R_8	= 3900-ohm resistor
R_9	= 15,000-ohm resistor
R_{10}	= 150-ohm resistor
R_{11}	= 100,000-ohm resistor
P_1	= 5000-ohm potentiometer
TR_1	= 2N2386 F.E. transistor
TR_2, TR_3	= 2N3703 transistors
Z_1	= OAZ200 Zener diode
M	= Ammeter, 100 μA full-scale deflection

* R_4 is selected with P_1 in mid-position and switch to "set zero" to give meter reading of zero.

† R_7 is selected to give full scale on meter when 2 megohm resistor substituted for cell on switch "read" position.

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Fast-neutron Activation Analysis of Silicon in Sputum

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A method for a relatively rapid and accurate determination of silicon in sputum by activation analysis is described. The method has been developed to identify and separate the cases of silicosis from those of pulmonary tuberculosis.

The sputum is digested by trypsin, then spun in a centrifuge three times at room temperature, and once after heating with 5 per cent. trichloroacetic acid to remove phosphorus which interferes in the activity measurement. For irradiation, a neutron generator with a fast-neutron yield of 2×10^9 n per cm^2 per second is used. After exposing for 2·3 minutes and then waiting for 1 minute the activity measurements are made by using a multi-channel analyser for 4·6 minutes. The silicon content is evaluated from the aluminium-28 activity produced by the reaction ^{28}Si (n,p) ^{28}Al with a photopeak at 1·78 MeV.

The results show that the silicon content in the sputum of patients suffering from silicosis is, on the average, four times that of the samples used for reference.

It is a current problem in tuberculosis dispensaries to distinguish cases of silicosis from those of pulmonary tuberculosis, as the X-ray photographs in the two cases are often quite similar. Considering its nature, it seems reasonable to assume that the sputum of patients suffering from silicosis contains much more silicon than that of patients with tuberculosis. The determination of silicon with conventional techniques being lengthy, laborious and inaccurate, investigations have been made to develop a method for the neutron-activation analysis of silicon in sputum.

Neutron-activation analysis has been already carried out for several elements present in biological substances.[1] Silicon has not been included in these experiments mainly because it is not an essential biological constituent. Another reason for this is that silicon-31, produced by the reaction ^{30}Si (n,γ) ^{31}Si in silicon exposed to thermal neutrons in a reactor, being a β-emitter of 2·6-hours half-life, cannot be identified by gamma spectroscopy, so that complicated and lengthy chemical procedures have to be used for the separation of silicon.

The recent availability[2] of neutron generators producing fast neutrons with energies of 14 MeV offers the possibility of making use of the reaction ^{28}Si (n,p) ^{28}Al, the cross-section of which is 220 mb. Considering the 92·17 per cent. abundancy of silicon-28, the 2·3-minute half-life and the 1·78-MeV photopeak of aluminium-28 activity for the transition $^{28}\text{Al} \rightarrow {}^{28}\text{Si}^*$ and $^{28}\text{Si}^* \xrightarrow{E = 1{\cdot}78\text{MeV}} {}^{28}\text{Si}$ that one 1·78-MeV gamma photon is emitted per β-particle,[3 to 9] the product of this reaction lends itself well to gamma spectroscopic measurement after simple separation from interfering activities.

Fast-neutron activation analysis of silicon in inorganic substances has already been extensively dealt with in the literature.[10,11,12,13,14] The method is rapid, selective and of high sensitivity, *e.g.*, 0·4 μg per 100 d.p.m. for a fast-neutron flux of 10^9 n per cm^2 per second. An accuracy of 1 per cent. has been achieved by Martin, Mathur and Morgan[12] for a 2-g sample with a 1 per cent. silicon concentration.

Although fast-neutron activation analysis by the use of neutron generators has not yet been applied to biological substances, considering the above, the method also seems promising for their analysis.

Experimental

Preparation of samples—

The sputum of various patients was collected during 3 to 6 days, depending on the individual rate of secretion.

REAGENT—

Tris(hydroxymethyl)aminomethane - formic acid buffer solution—This consists of a mixture of 50 ml of 0·2 M tris(hydroxymethyl)aminomethane (24·2 g dissolved in 1 litre of distilled water) and 26·8 ml of 0·2 M formic acid made up to 100 ml with distilled water.

DIGESTION—

Trypsin was dissolved in 0·1 M tris(hydroxymethyl)aminomethane - formic acid buffer to give a 1 mg per ml concentration and the solution added to the sputum collected over 24 hours in amounts equal to that of the measured sputum. The well mixed solutions were digested for 24 hours at 37° C, during which period the viscous sputum became fully digested.

REMOVAL OF PHOSPHORUS—

To eliminate any interference from aluminium-28 produced in phosphorus by the reaction ^{31}P (n,α) ^{28} Al, with a cross section of 130 mb, this element had to be removed from the samples before activation. This is achieved by spinning the solutions three times in a centrifuge after acidifying them to 5 per cent. with 100 per cent. trichloroacetic acid. The opalescent supernatant liquid is discarded each time after spinning for 30 minutes and the precipitate suspended in 10 to 15 ml of 5 per cent. trichloroacetic acid. To remove the inorganic phosphorus bonded to the macromolecule, the precipitate obtained in the third spin is hydrolysed with 5 per cent. trichloroacetic acid for 30 minutes at 80° C before spinning it a fourth time, the precipitate being suspended each time in 20 ml of ethanol, which removes not only the phosphoric acid released by the hydrolysis from the macromolecule, but also the phosphorus bonded to the lipoids of the cellular fragments, as well as the trichloroacetic acid. The final precipitate is evaporated to dryness. In the samples prepared by this method no phosphorus could be determined by the Fiske - Subba Row test.[15] The detection limit for this test is 0·5 μg of phosphorus. In the samples prepared without the addition of trichloroacetic acid, the phosphorus concentration, checked by the Fiske - Subba Row method, was found to vary from 10 to 80 μg per 100 mg of dry substance. By "spiking" the sample with 1 mg of silicon dioxide we determined that after the phosphate separation the recovery of silicon was 95 per cent.

IRRADIATION—

The dried samples are put into 2 × 2-cm polythene bags and transferred in a cylindrical polythene holder to the neutron generator by means of a vacuum facility. Their arrival at the place of irradiation is checked by photo diodes, and causes the automatic start of the assembly that measures the neutron yield, and of the timer that times the operation of the relay and valve by which the sample is returned to the place of measurement after the pre-set time for irradiation.

The irradiations were carried out in the KFKI NA-1-type neutron generator producing 14-MeV neutrons at a maximum rate of 10^{10} n per second.[16] The neutron flux was measured by Gamma Production, Emmerich-type plastic phosphor fast-neutron detector and 2 to 3 minutes' irradiation times were applied with a neutron flux of about 2×10^9 n per cm^2 per second.

ACTIVITY MEASUREMENT—

Two different assemblies were used for the activity measurements. Assembly A consisted of a 3·5 by 2·75-inch sodium iodide - thallium crystal detector in a lead chamber constructed from 10-cm thick lead bricks and a KFKI-type 128 channel analyser. The irradiated samples could be measured immediately after irradiation. The samples were left to cool for 1 minute to remove the 7·3-second half-life, 6 to 7-MeV nitrogen-16 activity produced by the ^{16}O (n,p) ^{16}N reaction.

Assembly B consisted of a 6 by 4-inch sodium iodide - thallium crystal detector in an iron chamber of 15-cm wall thickness and Raduga-type 100 channel analyser. This assembly was located farther from the neutron generator, so that the activity measurement could not be started until 5 to 6 minutes after irradiation. It proved to have a lower background and a higher counting efficiency than assembly A. The measuring times were 4·6 minutes.

RESULTS AND DISCUSSIONS

The silicon concentration was evaluated from the 1·78-MeV peak activity measured in the repeatedly calibrated energy-channel width of 1·78 $\pm$ 0·11 MeV. As the measurements

TABLE I

MEASURED SILICON ACTIVITIES AND CONCENTRATIONS IN FAST-NEUTRON ACTIVATION ANALYSIS OF SPUTUM FOR SILICOTIC AND NON-SILICOTIC PATIENTS

The values are normalised for 1·78 ± 0·11 MeV to 100 mg of dry material, 2·3 minutes' activation, 1 minute's decay, 4·6 minutes' measuring times and given neutron flux

		A_{st}, counts per minute		Concentration of silicon in sputum, p.p.m.	
		Assembly A	Assembly B	Assembly A	Assembly B
Background		91 ± 10·5	89 ± 9·3	—	—
Silicosis No.	1	68 ± 13·4	253 ± 34·5	17·6 ± 2·1	18·7 ± 4·1
Samples	2	82 ± 20·2	222 ± 29·2	18·2 ± 2·8	15·6 ± 1·8
	3	99 ± 18·3	190 ± 25·3	14·7 ± 3·2	17·2 ± 3·2
	4	80 ± 12·5	210 ± 37·2	20·1 ± 2·3	21·3 ± 1·9
Control	5	27 ± 8·6	49 ± 14·0	3·9 ± 1·5	4·2 ± 1·7
	6	15 ± 7·0	36 ± 9·8	2·8 ± 0·9	1·8 ± 0·8
	7	25 ± 6·7	92 ± 15·2	5·1 ± 1·7	3·7 ± 0·43

were carried out under somewhat different conditions, the measured activities, A_m, were normalised to 100-mg amples, 2·3 minutes' activation, 1 minute's waiting time and 4·6 minutes' measuring time with a half-life of 2·3 minutes for the given reading on the meter measuring the neutron yield. The normalised activity values were taken as standard and have been calculated from the formula—

$$A_{st} = 100\, A_a/\pi\eta.f.w$$

where A_a is the measured activity A_m *minus* background counts; $\pi\eta$ is the total time coefficient and equals $\eta_a.\eta_w.\eta_m$, the subscripts a, w and m denoting the time coefficients of the activation, waiting and measuring times, respectively; f is the reading of the neutron yield, meter counts; and w is the weight of the sample in milligrams.

The average values of the standard activity obtained by using either of the assemblies are seen to be much higher for silicosis than for the samples used for the check (Table I): for assembly A, 83·3 counts as compared with 22·2 and for assembly B, 226 as compared with 59. Even the minimum values measured for silicosis are found to be more than twice the maximum value found for the control samples. The results thus seem definitely to support the diagnostic value of the silicon analysis in sputum.

Samples from which the phosphorus had not been removed were also measured. The results obtained spectacularly confirmed the need for the separation from phosphorus.

In view of the limited number of cases investigated to date, no definite conclusions can be drawn, but the results are promising enough for the experiments to be continued on a more extensive scale and under increasingly uniform conditions.

We thank Professor M. Böszörményi, Director of the "Korányi" National Institute for Tuberculosis, for suggesting the problem and his continued interest, and Miss Lucy Gonda for assistance with the experiments.

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Modification of the Curcumin Method for Low Level Boron Determination

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A simple modification of the curcumin spectrophotometric determination of boron has been developed in which the acid required to develop the colour is destroyed with ammonium acetate, thus eliminating the need for dilution to large volumes. Provided suitable precautions are taken to minimise contamination, the method is suitable for direct determinations of boron down to 0·1 mg of boron per litre.

The most sensitive colorimetric method for determining small amounts of boron is probably the curcumin method. In the direct determination of boron in aqueous samples by Hayes and Metcalfe's method,[1] the lower limit, for accurate results, is obtained with 1-cm cells when 1 mg of boron per litre is present. This limit can be lowered, as these authors suggest, by using longer cells; by a precipitation method; or by concentrating the sample before determination. In a variation reported by Thierig and Umland,[2] a solvent-extraction step is used to concentrate the coloured species before colour determination. The need to determine boron rapidly at fairly low levels in samples, such as ground waters or sea water, led us to vary this method, so that the simplicity of the original method is retained, but the sensitivity is substantially increased.

Originally an aqueous sample of up to 0·25 ml is mixed with 3 ml of curcumin reagent and 3 ml of acid reagent. After a period of colour development, the solution is diluted to 100 ml with ethanol, to reduce the acidity and eliminate the intense colour of the excess of reagent in acidic solution. We have found that neutralisation of the acid by a weak base allows the volume of the final solution to be kept small and the colour intensity correspondingly high. We have used acetate salts for this purpose, because the reaction product with sulphuric acid produces acetic acid, which is already present in the system in excess; a slight excess of acetate ion does not render the solution sufficiently basic to destroy the coloured complex; and also, concentrated aqueous solutions of acetate salts are readily prepared as reagents. The ammonium salt was found to be most suitable because the ammonium sulphate formed upon neutralisation is the most soluble of the common sulphates.

Experimental

To determine the amount of ammonium acetate required for complete neutralisation of the excess of acid, blank solutions were prepared according to Hayes and Metcalfe's procedure, but only carried up to the point of dilution with ethanol. Instead of ethanol, increasing volumes of 7·8 M ammonium acetate were added, *plus* 40 to 50 per cent. acetic acid to reach a volume of 18·2 ml. The absorbance at 555 mμ was then determined in 1-cm cells by using a reagent blank. As the results in Table I show, the absorbance becomes constant at about 5·6 ml of ammonium acetate.

Table I

Effect of ammonium acetate on absorbance

0·5 ml of distilled water, 3·0 ml of curcumin reagent, 3·0 ml of acid reagent; ammonium acetate added as shown, *plus* aqueous acetic acid to give a final volume of 18·2 ml. Fifty per cent. acetic acid is used except when indicated by an asterisk, when 40 per cent. is used

7·8 N Ammonium acetate, ml	3·8	4·0*	4·4	5·0	5·0*	5·6	5·8	6·0	6·0*
Absorbance	0·128	0·134	0·103	0·082	0·084	0·075	0·080	0·077	0·077

The stoicheiometric volume of acetate required to neutralise both protons of the sulphuric acid is 6·9 ml, indicating that neutralisation of about half of the second proton is required. The residual absorbance represents the reagent blank and is believed to be caused primarily by boron in the sulphuric acid.

Further experiments showed that with 5·5 ml of ammonium acetate the addition of at least 25 ml of 50 per cent. acetic acid produced no effect on the coloured complex, *i.e.*, the absorbance times the final volume of the solution was constant.

APPLICATION TO ANALYSIS—

On the basis of the above results the final procedure developed is as follows.

Introduce by pipette 0·50 ml of aqueous sample, containing 0·1 to 3 mg of boron per litre, into a 60-ml plastic bottle. Add 3·00 ml of curcumin reagent (0·125 g in 100 ml of glacial acetic acid). Add 3·00 ml of acid reagent (equal volumes of 96 per cent. sulphuric acid and glacial acetic acid). Mix by swirling and allow to stand ½ to 4 hours. Add 15·0 ml of ammonium acetate - acetic acid reagent (250 g of ammonium acetate *plus* 300 ml of glacial acetic acid in sufficient water to make 1 litre). Read the absorbance at 555 mμ in 1-cm cells against a blank prepared with distilled water. The amount of acetate - acetic acid reagent used is equivalent to 6 ml of 7·8 M of ammonium acetate and 9 ml of 50 per cent. acetic acid in the above experiments. In the final solution the volume is therefore about 21 ml, about one-fifth of that in the Hayes and Metcalfe procedure.

For solutions containing 1 to 2 mg of boron, or less, per litre, replacement of all glassware by polythene equipment is necessary to obtain consistent and reproducible results and low blanks. With these precautions, and by choosing a low boron reagent sulphuric acid, the absorbance of reagent blanks is consistently in the range 0·02 to 0·03 against distilled water.

Boron standards are prepared in three media: distilled water, a synthetic solution approximating the composition of sea water and a solution representative of brackish ground water. The standard graphs are shown in Fig. 1. The "sea" water contains 0·6 M sodium

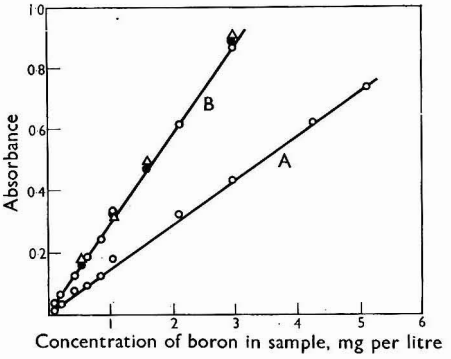

Fig. 1. Standard graphs for boron determination: graph A, 0·25-ml sample; graph B, 0·50-ml sample; ○, distilled water samples; ●, synthetic sea water; and △, synthetic ground water

chloride and 0·05 M magnesium chloride; the "ground" water contains 0·3 M sodium sulphate and 0·007 M each of calcium and magnesium chlorides. Boron in the form of boric acid is added to each. In the distilled-water systems the results are represented quite well by straight lines drawn through the origin, and show the method to be applicable to boron levels down to 0·1 mg of boron per litre. The results for the two simulated solutions indicate that the presence of these inorganic species exerts little, if any, effect on the colour, and that the method could be used for a variety of such solutions without preparation of separate standards for each.

The increase in sensitivity over the Hayes and Metcalfe method is about a factor of 4. For example, a sample containing 1 μg of boron results in an absorbance of 0·60, with the former procedure an absorbance of about 0·15 is found.

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Received *January* 1st, 1967

The Detection of Ephedrine in Biological Material by Ultraviolet Spectrophotometry

By S. L. TOMPSETT

(*Department of Clinical Chemistry, The Royal Infirmary, Edinburgh* 3)

Several toxicologically important substances exhibit ultraviolet spectra of low sensitivity and of a non-specific nature. In some instances, identification and determination can be improved by conversion into a derivative. This has been successfully accomplished in the case of ethchlorvynol[1] (1-chloro-3-ethylpent-1-en-4-yn-3-ol), dextropropoxyphene[2] (α-(+)-4-dimethylamino-3-methyl-1,2-diphenyl-2-propionyloxybutane) and diphenhydramine[3] (benadryl, *NN*-dimethyl-2-diphenylmethoxyphenylamine), and some related substances.

Cases involving overdosage by ephedrine [(−)-2-methylamino-1-phenylpropan-1-ol hemihydrate] are admitted to hospital quite frequently but are not usually fatal. In such cases, only low concentrations of ephedrine may be encountered in body fluids and hence, as a result of a lack of specific chemical reactions, and, in particular, an ultraviolet spectrum of low specificity and sensitivity, the chemical confirmation of ephedrine overdosage has been extremely difficult.

Conditions are, however, improved by the conversion of the separated ephedrine to benzaldehyde in the presence of periodate and ammonia. This technique has been described for the determination of the metanephrines in urine by their conversion to the aldehyde, vanillin.[4] This is a general reaction and is applicable to substances containing the groupings $-CHOH.CH_2NH_2$ and $-CHOH.CH_2NH.CH_3$.

The materials available in such cases are a sample of blood withdrawn at admission and a sample of urine collected for 8 hours following admission.

Method

Procedure—

To 10 ml of urine (or 10 ml of blood serum) in a 50-ml glass-stoppered measuring cylinder are added 1 ml of 10 N sodium hydroxide and 40 ml of chloroform (AnalaR). The mixture is shaken vigorously for 2 minutes. After separation of the phases, the aqueous layer is removed and discarded. Sufficient anhydrous sodium sulphate (AnalaR) is added to the chloroform extract to effect clarification and 30 ml of the filtered chloroform extract are measured into a glass-stoppered measuring cylinder, followed by 10 ml of N sulphuric acid. The mixture is shaken vigorously for 2 minutes. The acidic extract is separated for examination and, as a preliminary, it is examined in a recording spectrophotometer against a comparable blank over the range 350 to 200 mμ.

Conversion to benzaldehyde is carried out as follows: 4-ml aliquots of the acidic extract are measured into test-tubes A and B, and 1 ml of ammonia solution (sp.gr. 0·88) (AnalaR) is added to each. To tube A are added 0·25 ml of 10 per cent. w/v sodium metabisulphite solution and, after mixing, 0·25 ml of 2 per cent. w/v potassium periodate solution. To tube B are added 0·25 ml of potassium periodate solution, followed 5 minutes later by 0·25 ml of sodium metabisulphite solution. The contents of tube B are examined without delay in a Unicam SP800 Recording Spectrophotometer, with the contents of tube A as blank, over the range 350 to 200 mμ.

The above conditions result in optimum production of benzaldehyde from ephedrine. For quantitative purposes, measurements are made in a Unicam SP500 Spectrophotometer at a wavelength of 254 mμ (peak for benzaldehyde).

Biological fluids contain substances related to ephedrine, which react with periodate to produce aldehydes, *e.g.*, the metanephrines. Such substances contain substituted hydroxyl groups in the benzene ring, and are not extractable by chloroform under the conditions applicable to ephedrine.

Standards—Standards containing 50, 100, 200 and 500 μg of ephedrine are prepared, the complete procedure being carried out. The distribution coefficient of ephedrine is unaffected when urine or serum is substituted for water.

Phenylephrine [(−)-1-(3-hydroxyphenyl)-2-methylaminoethanol]—

This substance is a constituent of some pharmaceutical preparations and may be recognised by the above procedure, *m*-hydroxybenzaldehyde being the resultant product. It cannot be recovered from biological fluids by solvent extraction, but may, however, be recovered by the use

of cation-exchange resins.[4] Extracts obtained will produce, in addition to *m*-hydroxybenzaldehyde, varying amounts of vanillin and *p*-hydroxybenzaldehyde when the periodate reaction is applied.

The periodate reaction cannot be applied to compounds containing a catecholic grouping, owing to the liberation of free iodine.

In four cases of overdosage by ephedrine the results of testing 100 ml of serum and 100 ml of urine were: 140 μg and 4·6 mg; 280 μg and 8·4 mg; 300 μg and 8·8 mg; and 320 μg and 9·5 mg, respectively.

In all of the cases, undistorted ultraviolet absorption curves corresponding to benzaldehyde were obtained.

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Received *January* 14*th*, 1966

The Use of Porous Polymer Beads in the Gas-chromatographic Separation of Glycols and Glycol Ethers

BY J. F. PALFRAMAN AND E. A. WALKER

(*Ministry of Technology, Laboratory of the Government Chemist, Cornwall House, Stamford Street, London, S.E.*1)

THE strongly polar nature of glycols and their ethers gives rise to problems in separation by gas chromatography on conventional packed columns. Peak asymmetry caused by tailing severely restricts the ability to carry out quantitative investigations. Even when using a relatively inert support such as silanised Chromosorb "G" coated with polar liquid phases, such as PEGA, loss of sample by adsorptive effects is still evident.

In recent papers Hollis[1,2] has described the use of porous polymer beads for the separation of polar materials, and our own requirements for a satisfactory method of determining small amounts of other glycols as impurities in ethyl digol [diethylene glycol monoethyl ether, 2-(2-ethoxy-ethoxy) ethanol] prompted an investigation of the use of Porapak (a commercially available form) for this purpose. Synthesised by cross-linking styrene with divinylbenzene, these polymers, originally produced for gel-permeation chromatography, are designed to provide a material with both good mechanical properties and large surface area, in a suitably graded bead form.

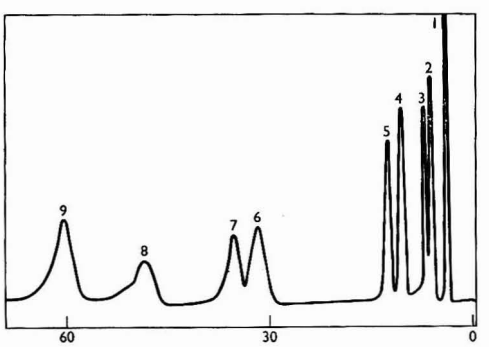

Fig. 1. Chromatogram of a mixture of glycols, glycol ethers and pentan-1-ol

The instrument used was a Pye Argon Gas Chromatograph containing a 4 ft × 4-mm o.d. glass column filled with 100 to 120-mesh Porapak S (obtained from Waters Ass. Ltd., Stockport, Cheshire), conditioned overnight at 225° C in a stream of argon. With a flow-rate of 50 ml per minute and a temperature of 200° C, a mixture of glycols, glycol ethers and pentan-1-ol was applied to the column, and the resulting chromatogram is shown in Fig. 1. (Pentan-1-ol was included as it is being used as an internal standard in the development of a specific method, which it is hoped will be published at a later date, for the determination of ethanediol in ethyl digol.)

The results of replicate analyses of the mixture carried out over a period of several weeks indicated that the column was stable under the operating conditions.

The spread of the individual retention times (Table I), in our experience, compares favourably with the results obtained when using diatomaceous supports with conventional stationary phases.

TABLE I

RELATIVE RETENTION TIMES OF SEVERAL GLYCOLS AND GLYCOL ETHERS (PENTAN-1-OL = 1·000) DEMONSTRATING COLUMN STABILITY AT 200° C

Peak No.	Components	Number of days after column conditioning						
		3	6	10	13	17	20	28
1	Ethylene glycol monomethyl ether (2-methoxyethanol)	0·425	0·412	0·421	0·426	0·416	0·423	0·429
2	Ethanediol	0·626	0·624	0·628	0·626	0·632	0·628	0·630
3	Ethylene glycol monoethyl ether (2-ethoxyethanol)	0·726	0·725	0·725	0·727	0·731	0·728	0·720
4	Pentan-1-ol	1·000	1·000	1·000	1·000	1·000	1·000	1·000
5	Ethylene glycol monoisopropyl ether (2-isopropoxy ethanol)	1·17	1·18	1·17	1·16	1·17	1·17	1·17
6	Ethylene glycol monobutyl ether (2-butoxyethanol)	3·04	3·00	3·01	2·99	3·00	3·06	3·02
7	Diethylene glycol monomethyl ether [2-(2-methoxyethoxy)ethanol]	3·39	3·34	3·35	3·34	3·38	3·35	3·37
8	2-Methylpentane-2,4-diol	4·73	4·70	4·71	4·65	4·79	4·69	4·72
9	Diethylene glycol monoethyl ether [2-(2-ethoxyethoxy)ethanol]	5·87	5·84	5·84	5·82	5·88	5·84	5·88

This spread would, of course, be reduced by using a more suitable internal standard (*e.g.*, one more centrally placed in the chromatogram).

The behaviour of these materials on four other modifications of this type of column packing (*viz.*, Porapak P, Q, R and T) is also being carried out.

We wish to thank Dr. D. T. Lewis, the Government Chemist, for permission to publish this paper.

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2. ——, *J. Gas Chromat.*, 1966, **4**, 235.

Received *February 17th*, 1967

Review of Techniques in Gas Chromatography. Part I. Choice of Solid Supports—Explanatory Note

BY J. F. PALFRAMAN AND E. A. WALKER

(*Ministry of Technology, Laboratory of the Government Chemist, Cornwall House, Stamford Street, London, S.E.*1)

THE authors' attention has been drawn to a possible misunderstanding of their purpose in including in the Appendix, beginning on p. 79, to their review paper (*Analyst*, 1967, **92**, 71) an illustrated classification of the many types of support available. This was not intended to be a comprehensive list, and did not imply that the authors recommended any specific manufacturers' products.

Analyst, August, 1967, Vol. 92, *pp.* 537–538

Book Reviews

PURIFICATION OF LABORATORY CHEMICALS. By D. D. PERRIN, W. L. F. ARMAREGO and DAWN R. PERRIN. Pp. viii + 362. Oxford, London, Edinburgh, New York, Toronto, Paris and Braunschweig: Pergamon Press. 1966. Price 70s.

That the authors have been courageous in attempting a work of this nature there can be little doubt, and the value of their publication will depend on one's interest in the purification of laboratory chemicals. From a commercial manufacturer's point of view it must be stated that the book is of little interest. Yields and specifications for the purified products are largely absent, and the information as to the methods adds little to the data collected in the course of manufacture.

The first 50 pages of the book give details of common laboratory techniques used in purification methods. Various methods of distillation, such as vacuum, steam and azeotropic are described, and chapters on recrystallisation, drying, chromatography, ion-exchange, solvent extraction and derivative formation are included.

Under "Recent and More Specialised Methods," molecular sieves, ion-exchange celluloses, vapour-phase chromatography, zone refining and the use of metal hydrides are briefly featured, together with relevant references.

By far the largest portion of the work, some 230 pages, deals with the purification of some 3000 individual organic items, and a shorter chapter deals with inorganic and metallo-organic substances. A final chapter deals with purification methods for the various classes of compounds.

There is no question that this book will prove of infinite value in many universities and research laboratories where a quick reference to the possible and suitable solvents for purifying the extensive range of organic compounds given is required.

The extent to which the methods quoted improve the compounds is open to some doubt, largely because melting-points are given as the main criteria of purity. It is, perhaps, unfortunate that the authors have not given details of the method by which this factor has been determined, as it is well known that the rate of heating, temperature of insertion and the point taken as the melt have a material bearing on the figure obtained.

The authors can also be criticised for not making further use of zone refining as a quick, easy and effective method of materially improving organic compounds. Simple apparatus is commercially available for treating small amounts of material, but it is not always easy to obtain references to the compounds to which this process is applicable.

Some of the items included have well defined carcinogenic properties, and although it can be argued that if the material is already in use in the laboratory concerned this property should be known, it would have been helpful if a note at the end of the monograph drew attention to the need of the special precautions required for handling these compounds, as often it will be a junior member of the staff who will be carrying out the purification. A. G. HILL

BENZENOID-METAL COMPLEXES. STRUCTURAL DETERMINATIONS AND CHEMISTRY. By H. ZEISS, P. J. WHEATLEY and H. J. S. WINKLER. Pp. vi + 101. New York: The Ronald Press Company. 1966. Price $7.00.

In a novel field it is difficult to decide when a sufficient amount of undisputed information has been established to warrant the publication of a survey of the existing state of knowledge in that field. It seems possible that a revision of this book may soon be necessary in view of the many compounds cited whose structures are still undetermined. However, as some considerable time may elapse before such problems are solved, the authors have written a clear account of transition metal complexes, in which a benzenoid or arene group is π-bonded to the metal.

Chromium predominates in this connection, although manganese, nickel, cobalt, molybdenum, vanadium, etc., have not been neglected. Methods of preparation and properties of the complexes are given in fair detail together with an extensive account of the varied physical and physico-chemical techniques used in the investigation of their reactions. These comprise spectroscopic measurements (visible, infrared, ultraviolet, X-ray, electron-spin resonance), radiochemical methods involving the use of carbon-14, magnetic and dipole moments, polarographic measurements, etc. The mechanism of the exchange reactions undergone by these compounds and the relevance of electrophilic and nucleophilic substitution in this connection are fully discussed.

The book is printed on good quality paper, and the structural formulae are designated clearly. In addition to adequate author and subject indexes, extensive tables of compounds with literature references as recent as 1965 are provided. To sum up, this volume will be most valuable to any workers in this field, although it does not disclose anything of interest to analysts in general. The price, inevitably, is rather high.

F. G. ANGELL

XXTH INTERNATIONAL CONGRESS OF PURE AND APPLIED CHEMISTRY, CONGRESS LECTURES PRESENTED IN MOSCOW, U.S.S.R., 12–18 JULY, 1965, UNDER THE AUSPICES OF THE INTERNATIONAL UNION OF PURE AND APPLIED CHEMISTRY AND THE ACADEMY OF SCIENCES OF THE U.S.S.R. Pp. vi + 293–657. London: Butterworth & Co. (Publishers) Ltd. 1965. Price 75s.

The contents of this book appear in Pure and Applied Chemistry, *Volume* 10, *Number* 4 (1965).

This volume contains the twenty-two plenary lectures that were delivered at the XXth Congress of I.U.P.A.C. in Moscow, in 1965. The largely unrelated topics cover the physical chemistry of surface phenomena and disperse systems, radiation, cosmic, inorganic and analytical chemistry, and finally, principles of chemical technology. The fame of the plenary lecturers guarantees that the contents of the volume are largely worth while. In the section devoted to analytical chemistry, there are lectures on organic reagents (F. Feigl), triple complexes in photometric analysis (A. K. Babko), separation and enrichment of trace components (J. Minczewski) and new excitation sources in spectrochemical analysis (B. F. Scribner).

Granted that the lectures themselves served their various purposes excellently, their reproduction in this hardback form following their initial publication in the journal, *Pure and Applied Chemistry*, 1965, **10**, (4), seems unwarranted. Who will buy this book? The unwary librarian who has already subscribed to the journal? The general reader whose casual interest would be better served by cheap paperbacks? Plenary lectures are excellent in their proper place—the lecture theatre, enhanced by the aura of the great occasion; on paper, they appear too general for the specialist and too specialised for the rest.

These are, of course, general criticisms of publications of this nature, to which policy I.U.P.A.C. seems to have committed itself. The present volume suffers from more particular defects—the editing appears to have been cursory and the proof-reading poor. Elegant courtesies to the host country are essential at international congresses, but their direct transcription to print in a scientific volume seems out of place. The final point is one of nomenclature. The International Union is the arbiter in matters of chemical nomenclature and, from time to time, it issues recommendations on naming and symbols. But in this volume, U-238 is used beside ^{124}Xe, Fe^{II} beside Fe^{2+} and Fe(II), and strange names such as vanadium phosphoro molybdate, acido-complex and diantipyrilemethane appear. If official I.U.P.A.C. publications ignore official I.U.P.A.C. recommendations, then who *will* pay any attention to them? Must one conclude that the time and effort spent in formulating these recommendations is entirely wasted?

Without introduction to tell one what the congress was about, and without index to allow ready reference, this book leaves one asking, in a truly international language: *cui bono*?

A. M. G. MACDONALD

Errata

MARCH (1967) ISSUE, p. 187, 12th line. *For* "solution,[3]" *read* "solution,[4]".

IBID., p. 187, 19th line. *For* "sulphide.[3]" *read* "sulphide.[4]"

IBID., p. 190, 6th line. *For* "Whatman GP/A" *read* "Whatman GF/A."

APRIL (1967) ISSUE, p. 232, last formula. *For* "7-(2-Sulpho . . .)" *read* "7-(4-Sulpho. . . .)".

MAY (1967) ISSUE, p. 297, caption to Fig. 4. *For* "A, blank; B, 25 μg of tin sample; and C after correction for the blank" *read* "A, 25 μg of tin sample; B, after correction for the blank; and C, blank."

Summaries of Papers in this Issue

Determination of Residues of Organophosphorus Pesticides in Food

A Review

SUMMARY OF CONTENTS

D. C. ABBOTT and H. EGAN

Ministry of Technology, Laboratory of the Government Chemist, Cornwall House, Stamford Street, London, S.E.1.

Analyst, 1967, **92**, 475–492.

Determination of Chlorinated Pesticides in Aqueous Emulsions

A method is described for the determination of chlorinated pesticides that involves their adsorption on to granulated carbon, and their subsequent extraction and degradation by the Stepanow method. The inorganic chlorine is then determined potentiometrically.

P. J. COOPER, R. E. S. ANDREWS and P. W. HAMMOND

Ministry of Technology, Laboratory of the Government Chemist, Cornwall House, Stamford Street, London, S.E.1.

Analyst, 1967, **92**, 493–495.

The Determination of Malathion in Formulations by a Method Based on Cleavage by Alkali

A method for the determination of malathion in formulations is described. The malathion dissolved in methanol is hydrolysed at room temperature by sodium hydroxide in the presence of phenol to give *OO*-dimethylphosphorodithioic acid. The latter is converted into its yellow copper complex by reaction with copper(II) sulphate in acetate buffer solution, and the complex extracted with chloroform. The copper(II) sulphate remaining in the aqueous layer is then determined iodimetrically. The free *OO*-dimethylphosphorodithioic acid in the sample, and other materials that are likely to interfere, are accounted for in a parallel determination, which omits the hydrolysis stage. Hence the amount of malathion in the sample can be calculated.

A. C. HILL, M. AKHTAR, M. MUMTAZ and J. A. OSMANI

Department of Locust Warning and Plant Quarantine, Malir Halt, Karachi, Pakistan.

Analyst, 1967, **92**, 496–500.

The Consecutive Determinations of Perchlorate and Nitrate Ions

A method is described for the determination of nitrate and perchlorate ions in admixture; it is applicable to amounts of each ion in the range 1 to 25 mg. The method is based on the initial precipitation and gravimetric determination of tetraphenylphosphonium perchlorate. The filtrate from this precipitation is treated with the new nitrate precipitant, *N*-(4-chlorobenzyl)-1-naphthylmethylamine, and the corresponding nitrate salt is filtered off, and determined gravimetrically or titrimetrically. The method is satisfactory for ratios of nitrate to perchlorate of 1:4·8 to 8·3:1, the widest range tested.

R. C. HUTTON and W. I. STEPHEN

Department of Chemistry, The University, P.O. Box 363, Birmingham.

Analyst, 1967, **92**, 501–505.

Molecular-emission Spectroscopy in Cool Flames

Part I. The Behaviour of Sulphur Species in a Hydrogen - Nitrogen Diffusion Flame and in a Shielded Air - Hydrogen Flame

A method is described for the determination of sulphur that involves simple measurement of the S_2 band emission at 384 mμ in a nitrogen-diluted hydrogen diffusion flame burning in air, or in a pre-mixed air - hydrogen flame burning inside a cooled sheath. Sulphuric acid may be determined by this technique in the range 6·4 to 500 p.p.m. of sulphur in the latter flame, or dissolved sulphur dioxide in the range 3·2 to 320 p.p.m. in either flame. The temperature of the flame most suitable for conversion of sulphur species into S_2 is established as 390° C.

The analytical method proposed for sulphates is simpler than the indirect flame-photometric methods hitherto described, and means are suggested whereby the method may be used for the determination of any form of sulphur species. The analytical signal for sulphur varies linearly with the square of the sulphate-ion concentration in the test solution.

R. M. DAGNALL, K. C. THOMPSON and T. S. WEST

Chemistry Department, Imperial College, London, S.W.7.

Analyst, 1967, **92**, 506–512.

A Field Method for Determining 4,4′-Di-isocyanatodiphenylmethane in Air

A method has been developed for determining up to 0·04 p.p.m. v/v of di-isocyanatodiphenylmethane (MDI) in a 5-litre sample of air. The test atmosphere is drawn through 0·4 M hydrochloric acid, which hydrolyses the isocyanate to the corresponding amine. This is then diazotised and coupled with 3-hydroxy-2-naphthanilide. After acidification of the solution the resulting pink compound is extracted into chloroform and compared visually with inorganic colour standard solutions. The preparation of three standards is described, corresponding to 0·01, 0·02, 0·04 p.p.m. of MDI, the recommended threshold limit value being 0·02 p.p.m. v/v. There is no interference from the tertiary-amine catalysts that are used with MDI in the production of polyurethanes.

D. A. REILLY

Imperial Chemical Industries Limited, Dyestuffs Division, Hexagon House, Blackley, Manchester 9.

Analyst, 1967, **92**, 513–519.

The Polarographic Determination of Dimetridazole in Animal Feeding Stuffs

It has been found that the reduction wave associated with dimetridazole in saturated borax solution is enhanced by copper, and that this enhancement is dependent upon the amount of copper in solution. A method is described in which this effect is eliminated.

P. J. COOPER and R. A. HOODLESS

Ministry of Technology, Laboratory of the Government Chemist, Cornwall House, Stamford Street, London, S.E.1.

Analyst, 1967, **92**, 520–523.

The Determination of Water in Organic Liquids

The modified Karl Fischer method, described in a previous report by Archer and Jeater, has been developed further. It has been established that the main cause of end-point drift is pick-up of water from the surroundings. A totally enclosed titration cell that prevents pick-up of water has been devised. A complete apparatus incorporating the cell is described. Water can be determined in samples containing amounts from several per cent. down to a few parts per million with this apparatus.

E. E. ARCHER, H. W. JEATER and J. MARTIN

B.P. Chemicals (U.K.) Ltd., Great Burgh, Yew Tree Bottom Road, Epsom, Surrey.

Analyst, 1967, **92**, 524–528.

Fast-neutron Activation Analysis of Silicon in Sputum

A method for a relatively rapid and accurate determination of silicon in sputum by activation analysis is described. The method has been developed to identify and separate the cases of silicosis from those of pulmonary tuberculosis.

The sputum is digested by trypsin, then spun in a centrifuge three times at room temperature, and once after heating with 5 per cent. trichloroacetic acid to remove phosphorus which interferes in the activity measurement. For irradiation, a neutron generator with a fast-neutron yield of 2×10^9 n per cm^2 per second is used. After exposing for 2·3 minutes and then waiting for 1 minute the activity measurements are made by using a multi-channel analyser for 4·6 minutes. The silicon content is evaluated from the aluminium-28 activity produced by the reaction ^{28}Si (n,p) ^{28}Al with a photopeak at 1·78 MeV.

The results show that the silicon content in the sputum of patients suffering from silicosis is, on the average, four times that of the samples used for reference.

A. SÁRDI

Central Research Institute of Physics, Budapest, XIII, Váci ut 34, Hungary.

and A. TOMCSÁNYI

"Koráni," National Institute for Tuberculosis, Budapest, Hungary.

Analyst, 1967, **92**, 529–531.

Modification of the Curcumin Method for Low Level Boron Determination

A simple modification of the curcumin spectrophotometric determination of boron has been developed in which the acid required to develop the colour is destroyed with ammonium acetate, thus eliminating the need for dilution to large volumes. Provided suitable precautions are taken to minimise contamination, the method is suitable for direct determinations of boron down to 0·1 mg of boron per litre.

ROBERT R. GRINSTEAD and SIGRID SNIDER

The Dow Chemical Company, 2800 Mitchell Drive, Walnut Creek Research Laboratories, Walnut Creek, California 94598.

Analyst, 1967, **92**, 532–533.

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S. L. TOMPSETT

Department of Clinical Chemistry, The Royal Infirmary, Edinburgh 3.

Analyst, 1967, **92**, 534–535.

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J. F. PALFRAMAN and E. A. WALKER

Ministry of Technology, Laboratory of the Government Chemist, Cornwall House, Stamford Street, London, S.E.1.

Analyst, 1967, **92**, 535–536.

Review of Techniques in Gas Chromatography Part 1. Choice of Solid Supports—Explanatory Note

J. F. PALFRAMAN and E. A. WALKER

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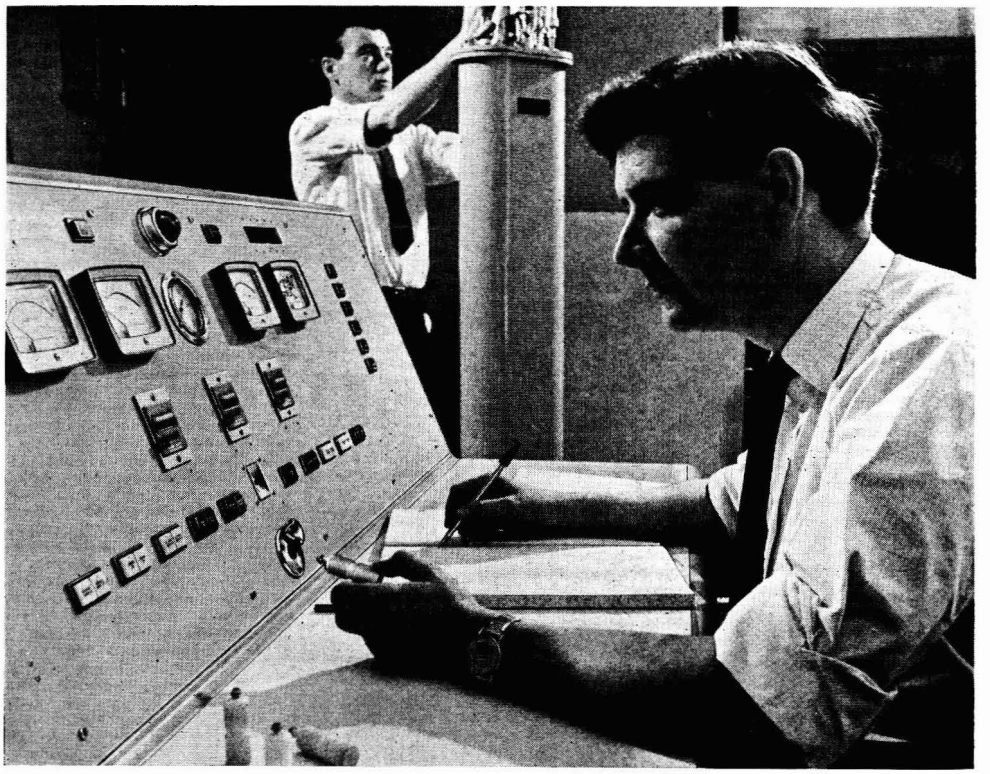
Analyst, 1967, **92**, 536.



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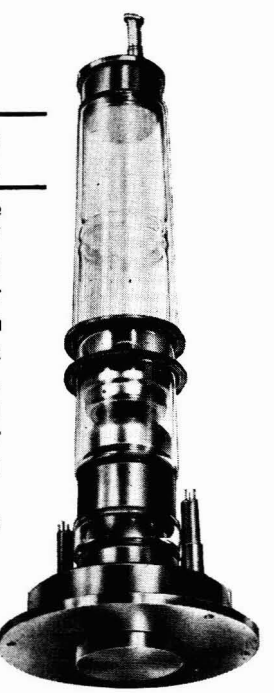

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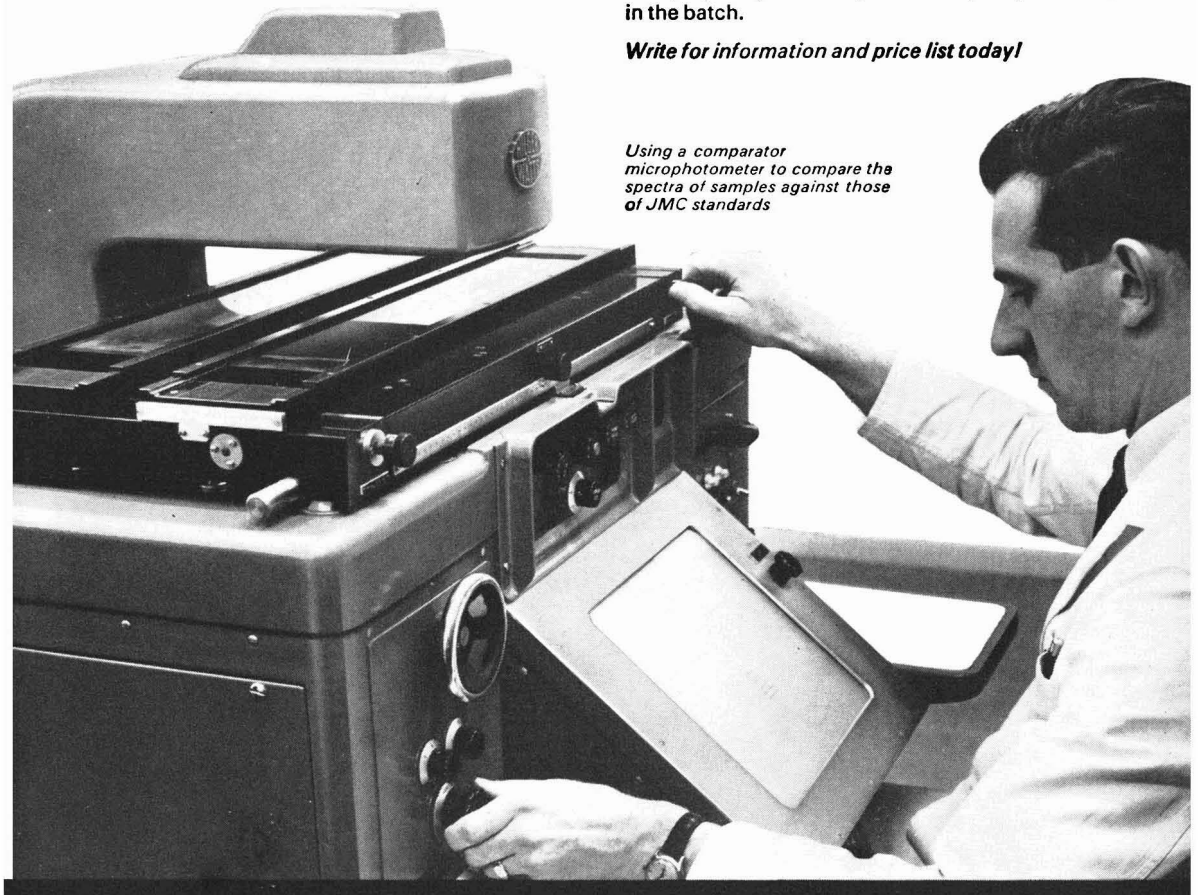

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