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Volume 94, No. 1116 **March, 1969**

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Summaries of Papers in this Issue

Spectrophosphorimeter Microscopy: An Extension of Fluorescence Microscopy

This paper describes how a conventional spectrofluorimeter, or a spectrophosphorimeter with synchronously driven choppers, can be combined with a microscope to give an instrument capable of measuring the fluorescence or phosphorescence spectra of specimens with sizes down to 1 μm. Factors governing the performance of such an instrument are discussed and experimental details of its use are included. The technique has considerable potential in the fields of micro-analytical chemistry, forensic science, biological and biochemical research and photochemistry. Examples illustrating its possibilities are described.

C. A. PARKER

Royal Naval Scientific Service, Admiralty Materials Laboratory, Holton Heath, Poole, Dorset.

Analyst, 1969, **94**, 161–176.

The Selection of Exciting Energy in Radioisotope X-ray Fluorescence Analysis

The use of exciting X-rays with energies either just above or far above the X-ray absorption edge of the wanted element has been proposed for radioisotope X-ray fluorescence analysis. The performance of these two methods is compared in terms of limits of detection, reduction of heterogeneity effects and compensation for matrix effects.

K. G. CARR-BRION

Warren Spring Laboratory, Stevenage, Herts.

Analyst, 1969, **94**, 177–181.

3,5′-Bis(dicarboxymethylaminomethyl)-4,4′-dihydroxy-*trans*-stilbene as a Selective Spectrofluorimetric Reagent for Cadmium

3,5′-Bis(dicarboxymethylaminomethyl)-4,4′-dihydroxy-*trans*-stilbene forms fluorescent complexes with aluminium, beryllium, magnesium, calcium, strontium, barium, zinc, cadmium, yttrium, lanthanum, gadolinium and lutetium. All the metal complexes have a metal-to-ligand stoicheiometry of 2:1. Magnesium, calcium, strontium and barium exhibit maximal fluorescence at pH 10·9, with excitation - fluorescence maxima at 360 and 440 nm. Zinc, cadmium, yttrium, lanthanum, gadolinium and lutetium show maximal fluorescence at pH 7·9, with excitation - fluorescence maxima at 360 and 440 nm, and 360 and 430 nm for yttrium and the lanthanons. Aluminium and beryllium show maximal fluorescence at pH 5·2 and 6·4, respectively, with excitation - fluorescence maxima at 345 and 410 nm and 360 and 405 nm, respectively. The formation of the cadmium complex provides a sensitive and selective determination of cadmium in the range 0·5 to 25·0 μg. A simple separation procedure is described in which 3,5′-bis-(dicarboxymethylaminomethyl)-4,4′-dihydroxy-*trans*-stilbene can be used for the determination of cadmium in the presence of most other metals except lead.

B. BUDESINSKY and T. S. WEST

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

Analyst, 1969, **94**, 182–188.

Investigation of a Rapid and Non-destructive Fast-neutron Activation Analysis for Many Elements by Using a Semi-conductor Detector

Investigations in the field of fast-neutron activation analysis with a small neutron generator have established the fact that oxygen in various materials can be determined by the rapid, non-destructive method. The present study has been carried out to investigate the applicability of the method with a germanium (lithium) γ-ray detector and data processing by electronic computer, for many other elements. More than sixty elements were activated under the conditions: bombarding time 400 seconds; neutron output 5×10^{10} neutrons second^{-1}; counting time 500 seconds after cooling; cooling time 10 seconds after bombardment, and their sensitivities under these conditions were calculated. According to the experimental results obtained by the mathematical method, many elements, in addition to oxygen, can be determined by this method without chemical separation.

I. FUJII, T. INOUYE, H. MUTO, K. ONODERA
Central Research Laboratory, Tokyo Shibaura Electric Co. Ltd., Kawasaki, Japan
and A. TANI
NAIG Nuclear Research Laboratory, Kawasaki, Japan.

Analyst, 1969, **94**, 189–197.

Trace Determination of Mercury, Thallium and Gold with Crystal Violet

A spectrophotometric method for the determination of small amounts of mercury, thallium and gold is presented. The sample is wet ashed with a nitric acid - hydrochloric acid mixture, and interferences are eliminated by adding ethylene glycol monomethyl ether and EDTA. Thallium interference is eliminated by adding sodium metabisulphite for mercury and gold, whereas excess oxidant interference is eliminated by adding hydroxylamine for thallium and gold. Because gold interferes in the determination of thallium and mercury, it is determined separately after heating the sample to volatilise the mercury. Iodide forms complexes with mercury and gold, whereas bromide forms complexes with thallium and gold. Crystal violet produces a toluene-extractable compound with these complexes in an acidic medium. A single extraction step suffices to determine 0·1 μg of each element in a 1-cm cell with a standard spectrophotometer at 605 nm; Beer's law is followed up to absorbance of unity. The method has been tested with urine, air, vegetation, water, soil, rocks and sediments.

E. L. KOTHNY
529 Florence Drive, Lafayette, California 94549, U.S.A.

Analyst, 1969, **94**, 198–203.

The Use of a Special Intermittent Nebulisation Technique to Suppress the Background in Flame-emission Spectra

A submerged oscillator - capillary technique is described, which produces a frequency and phase-stable intermittent fluid supply at 50 Hz to the flame of a flame spectrophotometer. With this technique the background radiation from the flame can be suppressed.

The cooling effect of the nebulised water on the flame is overcome by the choice of a suitable organic solvent. The usefulness of the technique is demonstrated with typical spectral curves.

K. RÜDIGER, B. GUTSCHE, H. KIRCHHOF and R. HERRMANN
Department of Medical Physics, Univ.-Hautklinik Gießen, Western Germany.

Analyst, 1969, **94**, 204–208.

The Determination of Sodium in High Purity Water with Sodium-responsive Glass Electrodes

A detailed investigation has been made of the accuracy of sodium-responsive glass electrodes for determining sodium (1 to 50 μg per litre) in high purity waters (*e.g.*, condensed steam and boiler feed-water) from power stations. The electrode potential can be made to follow the Nernst equation down to a sodium concentration of about 1 μg per litre by controlling the pH of the sample and by using a continuous flow of the sample past the electrode. Octadecylamine seriously affected the response of the electrodes, but other impurities likely to be present in power-station waters caused no significant effects. The standard deviation of analytical results varied from 0·4 to 1·2 μg per litre at concentrations of 2 and 26 μg of sodium per litre. Details of a recommended analytical procedure for discrete samples are given.

H. M. WEBBER and A. L. WILSON

Central Electricity Research Laboratories, Cleeve Road, Leatherhead, Surrey.

Analyst, **1969**, **94**, 209–220.

The Rapid Determination of Diazinon and its Oxygen Analogue in Animal Tissues by Gas Chromatography

A rapid method for the simultaneous gas-chromatographic determination of diazinon and its oxygen analogue (diazoxon) in blood, fat, liver, muscle and brain is described. The use of a selective thermionic phosphorus detector makes clean-up unnecessary. Preparation of the sample before injection consists in trituration with sand and sodium sulphate, elution with methanol or ether and concentration. Quantitative measurements are made by comparison with an internal standard. The method is satisfactory for the determination of 0·05 p.p.m. of diazinon and 0·2 p.p.m. of diazoxon in a 0·1-g sample.

A. F. MACHIN and M. P. QUICK

Ministry of Agriculture, Fisheries and Food, Central Veterinary Laboratory, New Haw, Weybridge, Surrey.

Analyst, **1969**, **94**, 221–225.

The Separate Determination of Xanthine and Hypoxanthine in Urine and Blood Plasma by an Enzymatic Differential Spectrophotometric Method

The enzymatic spectrophotometric determination of oxypurines (hypoxanthine *plus* xanthine) in urine and blood plasma has been extended by the use of differential spectrophotometry at 280 and 292 nm to enable the separate determination of hypoxanthine and xanthine to be carried out. The method retains the high degree of accuracy and specificity of the determination of total oxypurines, and has shown good recoveries and reproducibility when applied to aqueous solutions and to urine. Although less precise when applied to plasma, the method is the only simple method at present available that enables the determination of hypoxanthine and xanthine to be carried out on this material.

RONALD A. CHALMERS and R. W. E. WATTS

The Medical Professorial Unit, St. Bartholomew's Hospital, West Smithfield, London, E.C.1.

Analyst, **1969**, **94**, 226–233.

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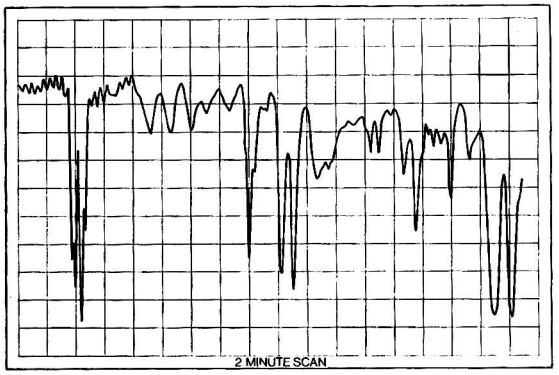

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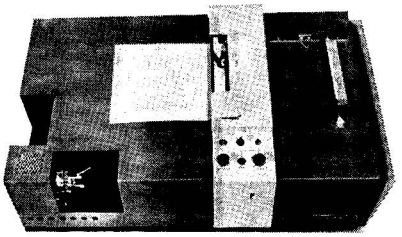

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Precise Manual Enthalpimetric Titrations

A procedure for high precision enthalpimetric titrimetry is described, in which titrant is added in small increments at regular intervals in the equivalence-point region of the titration, with temperature measurements being made after each addition. Many of the inaccuracies associated with the widely used continuous titration procedure are eliminated and relative standard deviations of as low as 0·1 per cent. can be achieved.

M. W. BROWN, K. ISSA and A. G. SINCLAIR

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Analyst, 1969, **94**, 234–235.

Automatic Counter for Radioactive Deposits on Planchets

An apparatus is described that automatically end-window counts the radioactive deposits on each of twenty-four planchets. Each sample is introduced in turn into a lead castle and counted for a pre-determined time to give a printed record of both the radioactivity and the sample number. The recorder previously described for recording radioactivity in chromatographic-column eluates is incorporated in the apparatus.

C. P. LLOYD-JONES and E. J. SKERRETT

University of Bristol, Department of Agriculture and Horticulture, Research Station, Long Ashton, Bristol.

Analyst, 1969, **94**, 236–241.

Apparatus for Vapour-phase Kinetic Studies of Organic Compounds

An apparatus is described that has proved to be particularly useful in studying the vapour-phase kinetics of pyrolysis of high-boiling liquid or solid organic compounds. The design includes a stainless-steel reaction chamber, a spring-operated closing valve, a hypodermic-needle injection assembly and a null-point pressure sensing device consisting of a stainless-steel diaphragm. The pressure change is automatically recorded on a strip-chart recorder. Reproducible results (to within ± 2 per cent.) are readily obtainable with either a packed or unpacked seasoned reactor.

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Department of Chemistry, Utah State University, Logan, Utah 84321, U.S.A.

Analyst, 1969, **94**, 242–245.

Notice to Authors

THE Editor welcomes papers on all aspects of the theory and practice of analytical chemistry, fundamental and applied, inorganic and organic, including chemical, physical and biological methods. Papers are submitted to the Editorial Committee, who decide on their suitability for publication.

Intending authors should consult the current Notice to Authors, last published in full in *The Analyst*, 1968, **93**, 269, reprints of which can be obtained on application to The Editor, *The Analyst*, 9/10, Savile Row, London, W.1. All papers submitted will be expected to conform to the recommendations there laid down, and any that do not may be returned for amendment.

THE ANALYST

Spectrophosphorimeter Microscopy: An Extension of Fluorescence Microscopy

By C. A. PARKER

(Royal Naval Scientific Service, Admiralty Materials Laboratory, Holton Heath, Poole, Dorset)

This paper describes how a conventional spectrofluorimeter, or a spectrophosphorimeter with synchronously driven choppers, can be combined with a microscope to give an instrument capable of measuring the fluorescence or phosphorescence spectra of specimens with sizes down to 1 μm. Factors governing the performance of such an instrument are discussed and experimental details of its use are included. The technique has considerable potential in the fields of micro-analytical chemistry, forensic science, biological and biochemical research and photochemistry. Examples illustrating its possibilities are described.

The area of exciting beam from a typical high-sensitivity spectrofluorimeter or spectrophosphorimeter is about 10 to 100 mm^2 and, to make full use of the exciting light, a sample of about 1-ml volume is required. The beam can, in principle, be concentrated into a much smaller area so that a much smaller total weight of specimen can be measured with corresponding increase in sensitivity. A spectrofluorimeter or spectrophosphorimeter can be converted for such micro measurements by replacing the usual sample compartment with a microscope. The apparatus can then be used to examine inhomogeneous microscopic specimens by transmitted, reflected or fluorescence light, and measurements made of the fluorescence or phosphorescence spectra from selected small areas. Details of specially constructed apparatus for the measurement of the absorption and fluorescence spectra of microscopic specimens have been given by various authors. For example, micro-absorption spectrophotometers have been described by Thorell,[1] and by Barnes and Thomson,[2] who measured the absorption spectra of small crystals at high optical densities. Olson[3] and Loeser[4] have described rapid scanning microspectrofluorimeters, and Rigler[5] used prism monochromators on both the excitation and emission sides of an instrument used to characterise intracellular nucleic acids and nucleoproteins stained with acridine orange. More complex absorption and fluorescence instruments, based on television scanning, have been described by Loeser and co-workers,[6,7,8] and by Freed and Engle.[9] Porter and Strauss[10] used a micro-flash photolysis apparatus to investigate the transient triplet absorption and fluorescence of chloroplasts.

Micro apparatus incorporating a phosphorimeter has not previously been described, and the object of the present paper is to show how a spectrophosphorimeter, based on the synchronously driven chopper arrangement of Parker and Hatchard,[11] can be conveniently combined with a conventional microscope to give an apparatus capable of isolating the phosphorescence of a microscopic specimen from its fluorescence and of measuring the spectra of both types of emission. The combined instrument is referred to as a spectrophosphorimeter microscope, whichever type of emission is measured. If, however, the phosphorimeter facility is not required, the same principle can be applied to combine a conventional spectrofluorimeter with a microscope.

DESCRIPTION OF APPARATUS—

The optical arrangement is shown in Fig. 1. The parts enclosed by the two rectangles represent the excitation and emission sides of a macro spectrophosphorimeter based on the synchronously driven chopper arrangement.[11] The remainder of the equipment consisted of a Gillett and Sibert "Conference" microscope, together with some accessories to be described.

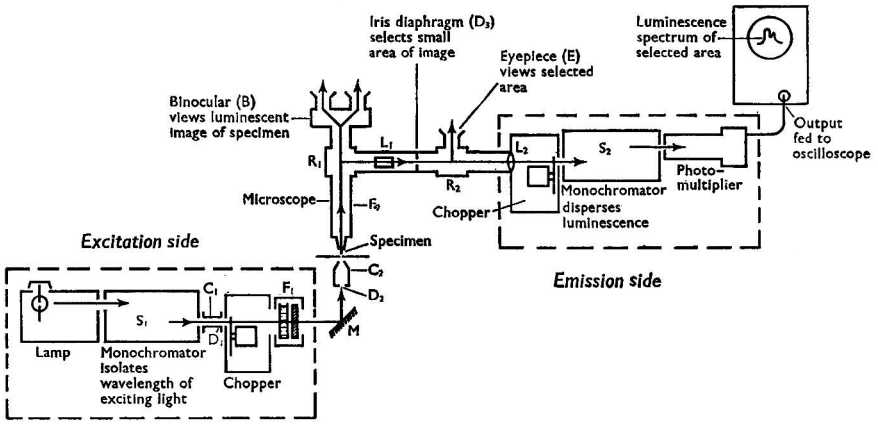

Fig. 1. Details of the spectrophosphorimeter microscope

Light from a 200-W extra-high pressure mercury lamp or a 150-W xenon lamp was dispersed by a Bausch and Lomb high-intensity grating monochromator, S_1, and the beam from the exit slit focused by means of the quartz - fluorite condenser, C_1, fitted with iris diaphragm, D_1, on to the sub-stage iris diaphragm, D_2, of the microscope condenser, C_2. For this purpose a front-surface aluminised mirror, M, was placed in the well of the microscope. (This mirror and its adjustable stand were designed for this purpose by the manufacturers, and were removed when the specimen was to be viewed by transmitted light from the built-in quartz - iodine lamp of the microscope.) The microscope condenser was adjusted to focus an image of the field iris, D_1, on the specimen, *i.e.*, the microscope was set up for Kohler illumination. For dark-ground illumination with low and medium power objectives, a patch stop was placed in the filter holder of the condenser, C_2, and the latter re-focused slightly (see later section). For high power objectives a dark-ground condenser was used and this was oiled to the slide with pure liquid paraffin. For frontal illumination the sub-stage condenser was replaced by a patch stop and the exciting light passing through the microscope slide was focused on the specimen by means of the Gillett and Sibert ×4·7 mirror objective. The various methods of illumination are discussed in a separate section. When a high degree of purity of the exciting light was required, additional glass or liquid filters were inserted in the beam at F_1.

The image of the specimen could be viewed by the binocular at B, or the light could be deflected by the 3-way cube, R_1, through a lens system, L_1, which produced a magnified image on the iris diaphragm, D_3. This image could be viewed by the eyepiece, E, and the light from a very small area of the specimen could be selected by closing the iris diaphragm, D_3. After selection of the chosen area, the light from this area was passed by means of the 3-way cube, R_2, to the lens, L_2, which focused the beam to a very small spot on the entrance slit of the analysing monochromator, S_2. Secondary filters could be inserted in the microscope tube at F_2, but these were only required to facilitate viewing the fluorescence when exciting with visible light. They were not required when the fluorescence spectrum was measured because the double-prism analysing monochromator had a high rejection efficiency for stray light. To ensure protection of the eyes at all times, the binocular, B, and the differentiating eyepiece, E, were fitted with pale yellow glass filters that had very low ultraviolet transmission, and the microscope stage and sub-stage mirror were surrounded by a curtain of black cloth.

The analysing system consisted of a Hilger D.284 quartz-prism double monochromator, S_2, with an E.M.I. 9558 photomultiplier. The output from the latter was passed through a 47-MΩ resistor shunted by a 0·002-μF capacitor and the resulting voltage applied to the Y amplifier (50 mV per cm) of a storage oscilloscope via an operational amplifier. The time constant of the measuring circuit was about 0·1 second and the spectrum could thus be scanned from 2·9 μm^{-1} (345 nm) to 1·25 μm^{-1} (800 nm) in 6 seconds, with a resolution varying from 0·02 μm^{-1} (2·4 nm) to 0·035 μm^{-1} (2·2 nm), *i.e.*, approximately equal to the limiting resolution of the monochromator with 0·5-mm slits. The horizontal scan of the oscilloscope was operated directly from a potentiometer driven by the wavelength drum of the monochromator so that the latter could be operated by hand if desired. In practice, the scans were generally made in 30 to 60 seconds, depending on the amount of noise level on the trace. To obtain the highest precision the double-beam pen-recording system,[12] with a 1-second time constant could, of course, be used, but for the rapid scanning of microscopic specimens the single-beam system with oscilloscope gave adequate precision, provided that the mercury lamp was operated from a d.c. supply.[13] The fluorescent-screen quantum counter,[14] together with the double-beam recording system, could be used to measure an approximate excitation spectrum.

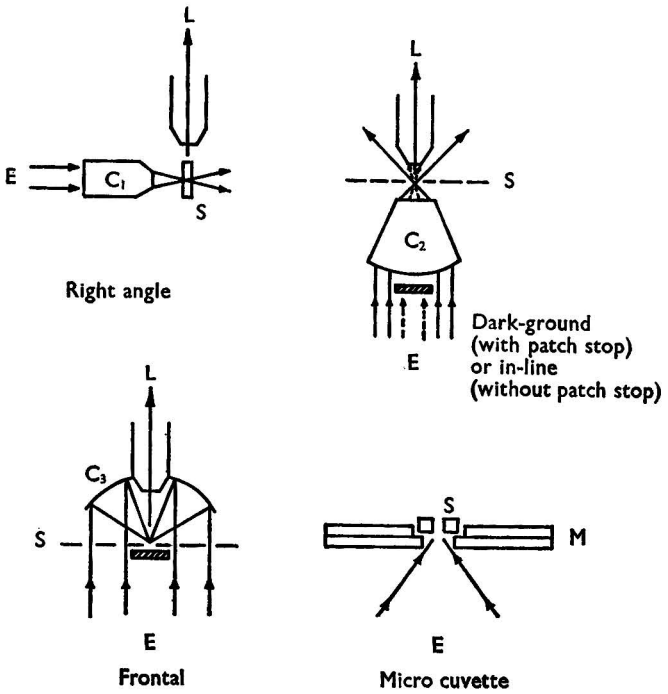

Fig. 2. Modes of illumination and viewing: E, exciting light; L, luminescence; S, specimen; C_1, 16-mm microscope objective used as condenser; C_2, aplanatic glass or silica Abbe condenser; C_3, mirror objective. The micro cuvette can be used with dark-ground, in-line or frontal illumination

MODES OF ILLUMINATION AND VIEWING—

The advantages and limitations of the three basic types of specimen arrangement used in conventional macro spectrofluorimetry have been discussed in detail elsewhere.[15] The corresponding (or nearly corresponding) arrangements that can be used with the spectrophosphorimeter microscope are shown in Fig. 2. Strict right-angle illumination can only be used at relatively low magnifications because of the difficulty of focusing sufficient light on a thin specimen. The right-angle arrangement shown in Fig. 2 refers to a sample cuvette

consisting of a short length of synthetic silica capillary tube, of 0·5-mm bore, and containing a 3 to 4-mm column of specimen solution. The solution is illuminated by exciting light focused by a 16-mm microscope objective and is viewed from above by using the microscope with a low power objective (×5 or less). For thinner specimens the nearest approach to right-angle illumination is obtained by some form of dark-ground illumination. If the specimen is mounted directly on the microscope slide (as is unavoidable with very small specimens) this arrangement leads to a greater "blank" than with the conventional right-angle method unless special precautions are taken. This is because the photomultiplier sees the illuminated part of the slide as well as the specimen, and greater care is necessary to separate scattered exciting light and to avoid fluorescent slides. If relatively large volumes of liquid are to be examined, the microcuvette, shown in Fig. 2, overcomes this difficulty. It is constructed from a 1-mm slice of synthetic silica tube (5 mm o.d., 0·7 mm i.d.) that has its faces polished with fine carborundum followed by rouge. Small volumes of solution (about 0·3 μl) in a non-volatile solvent can be held within the bore by surface tension. The cuvette rests in a well that is constructed from two microscope slides (M in Fig. 2) with holes bored in them, one slightly larger and the other slightly smaller than the outside diameter of the cuvette. The central area of the illuminated liquid is viewed by a ×5 objective. The liquid can, alternatively, be illuminated from above by a mirror objective, but for use at short wavelengths the outer parts of the microscope slides must then be cut away (or silica slides used) to allow passage of the ultraviolet exciting light to the mirror.

Micro-luminescence spectrometry is of greatest potential value for the examination of inhomogeneous specimens, *e.g.*, sections or whole amounts of botanical or biological material (perhaps after application of a suitable reagent or fluorochrome as in fluorescence microscopy), minute crystals, dust, debris and fibres. Frequently the dark-ground or in-line modes of illumination (Fig. 2) can be used because the thin specimens required for high power observation generally transmit sufficient of the luminescence. For opaque specimens, when the selected area has a diameter of not less than about 20 μm, a mirror objective (*e.g.*, Gillett and Sibert, ×4·7) is suitable. For frontal illumination at high magnifications a special objective must be used, in which the exciting light is fed down the periphery of the objective and focused on the specimen. Alternatively, the exciting light can be focused by the objective itself via a partially reflecting plate fitted in the microscope tube (as in many metallurgical microscopes). However, for short wavelength excitation, the objective must then be of quartz - fluorite and fluorescence of the objective can give rise to a large blank value.

For objective powers up to about ×20, satisfactory dark-field illumination was obtained with an aplanatic or Abbe condenser and patch stop. For objective powers of ×40 and greater, a dark-ground condenser was used. Alternatively, and to obtain the highest intensity of exciting light, an Abbe condenser of synthetic silica, or a three-lens aplanat of glass, was used without patch stop, *i.e.*, to give the in-line arrangement. With this arrangement a large proportion of the exciting light passes into the microscope objective; at short wavelengths it can cause fluorescence of the latter, and at all wavelength settings of the excitation monochromator any stray light in the exciting beam will pass through directly to the analysing system. With this mode of illumination it is, therefore, necessary to insert efficient primary filters in the beam from the excitation monochromator (F_1 in Fig. 1) to remove stray light. If a single monochromator is used in the analysing system it is essential to prevent the direct beam of exciting light entering the monochromator by inserting a suitable filter at some point between the fluorescing specimen and the entrance slit. With a double monochromator the secondary filter is not necessary. The choice of primary and secondary filters and the problems to which they can give rise are fully discussed elsewhere.[16]

METHOD OF FOCUSING—

The sub-stage condenser was first set up for visible light by removing the patch stop, setting the excitation monochromator to about 500 nm and focusing an image of the field iris on the specimen. The sub-stage iris was then fully opened, the patch stop inserted and the sub-stage condenser adjusted slightly until a uniform circle of diffracted light appeared on the specimen. When the wavelength setting was changed to the ultraviolet region the focus of the sub-stage condenser varied appreciably and a further slight adjustment was necessary to obtain optimum illumination of the specimen. With semi-permanent or permanent mounts sealed around the edges of the cover-slip with shellac varnish, the latter

provided a convenient fluorescent screen on which to focus the ultraviolet beam. (It fluoresced strongly orange - yellow in violet or ultraviolet light.) A useful fluorescent screen can be made from anthracene. Anthracene was allowed to crystallise slowly from ethanol to give thin plates, several millimetres square and a fraction of a millimetre thick. Several of these crystals were permanently mounted under a cover-slip and, although the anthracene slowly decomposed under intense illumination, the mount could be used for many months as a fluorescent screen because a fresh area of a crystal could be selected after one had decomposed. Once focused, the condenser setting was found to be sufficiently reproducible from slide to slide, provided that microscopic slides of the same nominal thickness were used.

The procedure generally adopted for the examination of microscopic specimens was as follows. The specimen was first examined in visible light, either by removing the sub-stage mirror and using the built-in quartz - iodine lamp, or by setting the excitation monochromator to about 500 nm. The field of interest was selected and the wavelength drum set to the required excitation wavelength. With appropriate primary and secondary filters in position the specimen was viewed by its fluorescence or phosphorescence emission. When the feature of interest emitted strongly it was possible to see the ten-times magnified image in the differentiating eyepiece, E. Weakly emitting areas could not be seen at the higher magnification. For these, and for emission in the violet and ultraviolet regions, it was necessary to re-set to visible light to focus the feature of interest in the centre of the differentiating iris. The latter was then closed to the required diameter, the excitation monochromator re-set to the excitation wavelength and the fluorescence or phosphorescence emission scanned. The procedure adopted for measuring excitation spectra with the xenon lamp was similar; the sub-stage condenser was set to optimum focus at a wavelength in the middle of the region scanned. Methods of correcting the excitation spectrum are discussed in a later section.

The drum operating the differentiating iris diaphragm was calibrated in five arbitrary divisions. For drum settings 1, 2 and 3, almost the whole of the light passed the entrance slit of the analysing monochromator when it was set at 0·5 mm, corresponding to a band width of 0·024 μm^{-1} (3·8 nm) at 2·5 μm^{-1} (400 nm). The diameters of the area of the specimen viewed with each of these settings and each of the objectives available are shown in Table I. With the low power objectives these diameters were measured by simply counting the number of divisions of a stage micrometer (1 division $\equiv$ 10 μm) that appeared in the field of view of the differentiating eyepiece when the iris was closed to the required position. With the highest power objectives the diatom *Synedra Fulgens* was used as a scale; the striations on this diatom are 0·66 μm apart.[17]

TABLE I

DIAMETERS OF FIELDS ISOLATED BY DIFFERENTIATING IRIS DIAPHRAGM

Objective (magnification/NA)	Field diameter at following iris settings, μm, No. 1	No. 2	No. 3
×4·7/0·12	26	74	112
×5/0·18	23	65	99
×10/0·28	11	32	48
×16/0·35	7·4	21	32
×40/0·85	2·8	7·9	12
×100/1·3 (with liquid paraffin) ..	1·2	3·5	5·3

INTENSITY OF EXCITING LIGHT—

The performance of a conventional spectrofluorimeter can be conveniently discussed by considering the efficiency of its two main parts, *viz.*, the light source, excitation monochromator and associated optics on the one hand, and the analysing monochromator, photodetector and recording equipment on the other.[18] The same method is adopted in discussing the performance of the micro instrument.

To obtain a measure of the efficiency of the excitation optics, the total flux of 366-nm radiation was determined with the ferrioxalate actinometer[19] at three points in the system, *viz.*, at the exit slit of the excitation monochromator (S_1 in Fig. 1), at the fully open field iris (D_1 in Fig. 1) and at the specimen position when using the glass aplanatic condenser or the

silica Abbe condenser. In the first two positions the ferrioxalate solution was exposed in the normal way by using a silica cuvette. In the specimen position the solution was exposed in a silica dish, with an optically flat bottom, and placed on a microscope slide to intercept the whole of the beam. The results are shown in Table II. The loss of light between the exit slit and the field iris (more than 50 per cent.) mainly arises because the condenser, C_1, accepted light only from the central region of the exit slit. Between the field iris and the specimen, by using either the glass or silica condenser without patch stop, the light flux was reduced by a factor of about 5. This was caused by three main factors: only part of the beam was collected by the sub-stage mirror, only part of the reflected light was collected by the sub-stage condenser and, finally, some light was lost by reflection in the sub-stage condenser and at the slide, which was not oiled to the condenser. Insertion of the patch stop reduced the intensity to one half with the glass condenser, and to about two thirds with the silica. Although the total fluxes passed by the silica Abbe condenser were greater than those passed by the glass aplanat, this was more than offset by the greater area of illumination produced by the former so that the intensities at the specimen were less.

TABLE II

INTENSITIES OF ILLUMINATION

Lamp and wavelength, nm	Position and conditions	Total light flux, einstein second^{-1}	Area of beam, mm^2	Intensity, einstein second^{-1} mm^{-2}
Hg 366	Exit slit	15×10^{-8}	30	$0{\cdot}5 \times 10^{-8}$
Hg 366	Iris D_1	$6{\cdot}3 \times 10^{-8}$	300	$2{\cdot}1 \times 10^{-10}$
Hg 366	C_2/glass (no patch)	$1{\cdot}2 \times 10^{-8}$	0·07	$1{\cdot}7 \times 10^{-7}$
Hg 366	C_2/glass (patch)	$0{\cdot}6 \times 10^{-8}$	0·07	$0{\cdot}9 \times 10^{-7}$
Hg 366	C_2/mirror	$2{\cdot}0 \times 10^{-8}$	0·5	$0{\cdot}4 \times 10^{-7}$
Hg 366	C_2/DG	$1{\cdot}0 \times 10^{-10}$	0·005	$0{\cdot}2 \times 10^{-7}$
Quartz - iodine 310–390	C_2/glass (no patch)	$1{\cdot}6 \times 10^{-9}$	0·8	$0{\cdot}2 \times 10^{-8}$
Quartz - iodine 310–390	C_2/glass (patch)	$0{\cdot}8 \times 10^{-9}$	0·8	$0{\cdot}1 \times 10^{-8}$
Hg 366	C_2/silica (no patch)	$1{\cdot}4 \times 10^{-8}$	0·3	$0{\cdot}5 \times 10^{-7}$
Hg 366	C_2/silica (patch)	$1{\cdot}0 \times 10^{-8}$	0·3	$0{\cdot}3 \times 10^{-7}$
Hg 436	Iris D_1	$5{\cdot}3 \times 10^{-8}$	—	—
Hg 405	Iris D_1	$3{\cdot}9 \times 10^{-8}$	—	—
Hg 313	Iris D_1	$4{\cdot}3 \times 10^{-8}$	—	—
Hg 250	Iris D_1	$0{\cdot}6 \times 10^{-8}$	—	—

The light fluxes and intensities obtained at the specimen position with the dark-ground condenser and the mirror objective are also included in Table II. It was not convenient to apply the chemical actinometer with these two modes of illumination, and the intensities of exciting light were measured relative to that obtained with the aplanatic condenser by comparing the intensities of fluorescence observed through the microscope from a glycerol - gelatin mount. The total flux obtained from the mirror objective was about three times greater than that obtained from the glass condenser with patch stop, but this flux was distributed over a considerably larger area, and the mean intensity with the mirror objective was less than with the patch-stop method. Although the total flux obtained from the dark-ground condenser was very low, it was concentrated in a very small area so that the intensity was still reasonably high. It is interesting that the intensities of near ultraviolet light isolated (by means of 3 mm of Chance - Pilkington OX9A glass) from the quartz - iodine lamp run at full power were some 80 to 90 times less than the corresponding intensities obtained from the mercury lamp, in spite of the losses with the latter arising from use of a monochromator.

The light fluxes issuing from the field iris, D_1, when the monochromator was set to some of the other principal mercury lines are also shown in Table II. These were simply determined by comparison with the flux at 366 nm, by using the fluorescent-screen quantum counter.[14] They give an indication of the corresponding intensities at the specimen, but the latter are not precisely proportional to the former because of the change in focal length of the silica condenser with wavelength. Some data on sources are given elsewhere,[20] from which approximate fluxes with other arrangements can be deduced.

SENSITIVITY OF THE ANALYSING SYSTEM—

The sensitivity of the analysing system is discussed only where it is affected by the use of a microscope in place of the normal cell compartment of a spectrofluorimeter. The amount of luminescence collected from a specimen, small enough to be viewed by the differentiating iris, is proportional to the square of the numerical aperture of the objective, a value which increases rapidly with increase in objective power (see column 3 of Table III). Obviously it is not possible to use a high power objective to view a large specimen, but when a choice is possible it is better to use the objective of higher power with a large iris aperture than one of lower power with a correspondingly smaller iris aperture. It is readily seen that the sensitivity, expressed in terms of the minimum *weight* of material detectable, is greatly increased by the use of a high power objective. Thus with a volume, v, of solution containing a luminescent solute at low concentration, c, illuminated by an intensity of exciting light, I_0, the total rate of emission of luminescence is proportional to the product I_0vc and, as the fraction of this luminescence collected by the analysing system is proportional to the square of the numerical aperture (NA), the response, R, of the system follows the relationship—

$$R \propto I_0vc\,(\mathrm{NA})^2 \propto I_0w\,(\mathrm{NA})^2,$$

where w is the weight of solute in the volume, v. For a given intensity of exciting light the minimum detectable weight of solute will, therefore, vary in inverse proportion to the numbers shown in column 3 of Table III. On this basis even the objective of lowest power is more sensitive than the analysing monochromator used alone. In practice the situation is even more favourable because the intensities of exciting light produced by the sub-stage condenser or mirror objective are considerably greater than that available in a macro cuvette. For example, the intensity obtained with the glass condenser without patch stop was $1{\cdot}7 \times 10^{-7}$ einstein second^{-1} mm^{-2}, compared with $0{\cdot}5 \times 10^{-8}$ einstein second^{-1} mm^{-2}, at the exit slit of the excitation monochromator (see Table II). These values of I_0 have been used to calculate the over-all theoretical weight sensitivities shown in column 4 of Table III. It will be observed that the weight sensitivity with the ×100 objective should, in principle, be 5800 times greater than that obtainable with the analysing monochromator alone. In practice this factor is reduced somewhat by losses in the microscope.

TABLE III

CALCULATED RELATIVE SENSITIVITIES OF ANALYSING SYSTEM

Optics of analysing system	Numerical aperture (NA)	100 (NA)^2	Relative weight sensitivity 10^9 (NA)^2 I_0	Relative concentration sensitivity 10^{14} (NA)^2 I_0v
×4·7 (mirror objective) ..	0·12	1·44	2·4	0·4
×5	0·18	3·24	5·5	0·5
×10	0·28	7·84	13	0·15
×16	0·35	12·3	21	0·07
×40	0·85	72·3	120	0·02
×100	1·3	169	290	0·004
Monochromator alone ..	*ca.*0·1	1·0	0·05	5000

NOTE—

The value of I_0 (see Table II) for the first six items is that obtained with the glass condenser without patch stop; that for the last item is the value observed at the exit slit of the excitation monochromator. The values of v for the first six items are calculated from column 4 of Table I, assuming a cubical specimen; that for the last item is taken as 1 ml.

Expressed in terms of the minimum detectable *concentration*, the microscope gives a lower sensitivity than the conventional macro arrangement. On this basis the relevant parameter is $I_0v\,(\mathrm{NA})^2$. Values of this parameter, based on values of v calculated from the diameters shown in column 4 of Table I, are given in column 5 of Table III. The volume of solution required for the macro measurement was assumed to be 1 ml. The concentration sensitivity with the ×100 objective is a million times less than that obtainable with the analysing monochromator alone, and there is, clearly, no point in using the microscope arrangement when large amounts of a *homogeneous* specimen are available. If, however, the weight of specimen is limited, it is advantageous to dissolve it in a very small volume of solvent and utilise the high weight sensitivity of the spectrophosphorimeter microscope. This

has the added advantage of greatly reducing the blank due to solvent impurities and Raman scatter. Admittedly the manipulation of small volumes of solvent presents difficulties, but this restriction does not apply to the microscopic specimens for which the technique is most suited; a minute crystal, a single cell of biological material, a pollen grain or a fibre do not need a cuvette but are simply mounted in a suitable medium.

As with conventional spectrofluorimeters, the spectral sensitivity of the analysing system is the product of three factors,[21] *viz.*, the spectral sensitivity of the photomultiplier, the band width of the monochromator and the transmission of the monochromator (including its entrance-slit optics). With the present micro instrument the entrance-slit optics consisted of a microscope with glass optics. The approximate transmission curve of the microscope (curve C in Fig. 3) was determined by comparing the spectral distribution of light from a xenon lamp before and after passage through the microscope. It will be seen that the measurement of luminescence emission with this arrangement is limited by the glass cut-off to wavelengths longer than about 340 nm. The complete spectral sensitivity curve of the analysing system can be determined, approximately, by comparing the observed spectra of solutions of standard fluorescent substances[22] placed on the microscope stage with the known corrected spectra of the substances. For many of the purposes for which the spectrophosphorimeter microscope is likely to be used, such correction is not necessary, and the emission spectra reproduced in this paper have not been so corrected.

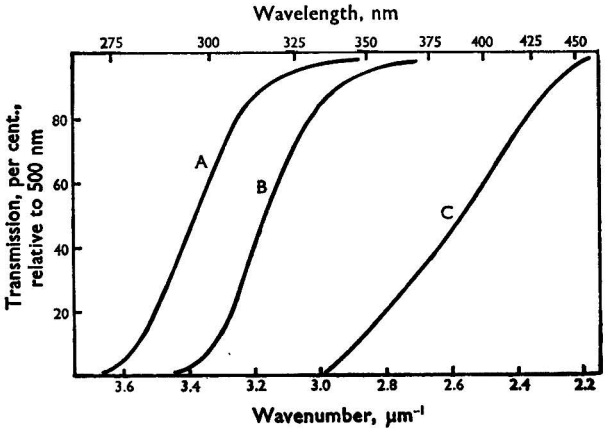

Fig. 3. Transmission of optics: curve A, 0·13-mm glass cover-slip; curve B, 0·88-mm microscope slide; curve C, microscope optics including 3-way cubes

METHOD OF MOUNTING THE SPECIMEN—

For qualitative examination of small solid objects, *e.g.*, crystals, the specimen can simply be mounted in air under a cover-slip. A glass slide and cover-slip are often satisfactory for excitation at long wavelengths, but for work at shorter wavelengths both the absorption and the fluorescence of the microscope slide and the cover-slip must be considered. Typical transmission curves of these two items are shown in Fig. 3 (curves A and B). With the exciting light coming from below, a glass slide cannot be used for excitation much below 3·2 μm^{-1} (313 nm) because little or no exciting light will pass through it to reach the specimen. If high sensitivity is required the glass may not be suitable, even at longer wavelengths, because its fluorescence may mask that from the specimen. The transmission of the cover-slip at the wavelength of the exciting light is of no consequence in all those modes of illumination in which the exciting light enters from below. It is sufficient that the cover-slip transmits the luminescence from the specimen. The transmission of the cover-slip extends to 3·5 μm^{-1}, *i.e.*, to below 300 nm (see curve A in Fig. 3), and at all excitation wavelengths its fluorescence will be negligible compared with that from a microscope slide of the same glass. However, with top-surface illumination at wavenumbers greater than 3·4 μm^{-1} a cover-slip of silica must be used.

Ideally, the specimen should be mounted in a medium of the same refractive index to avoid distortion of the magnified image and to resolve fine detail. The medium must, of course, be reasonably transparent at the wavelength of the exciting light used, and should show low fluorescence. This excludes many conventional media entirely, *e.g.*, Canada balsam.

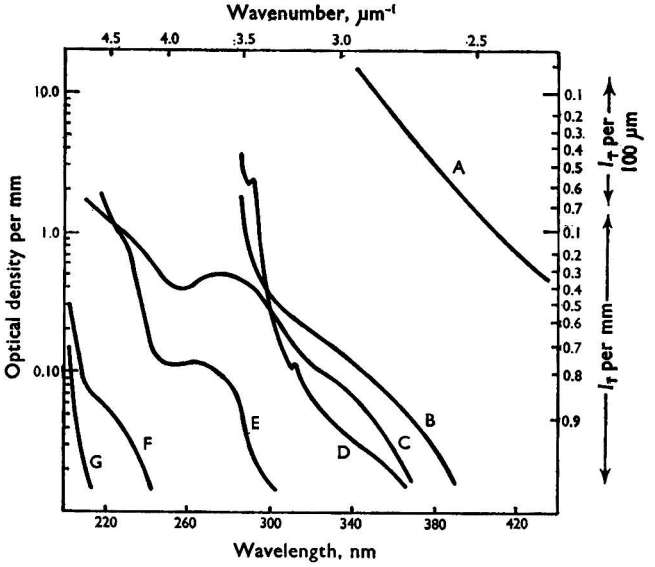

Fig. 4. Absorption - transmission curves of mounting media: curve A, dried balsam film; curve B, "glygel" containing 160 ml of glycerol, 30 g of gelatin, 2g of phenol and 120 ml of water; curve C, 25 per cent. w/v poly(vinyl alcohol) in water; curve D, 10 g of polystyrene in 30 ml of xylene; curve E, liquid paraffin after passage over alumina; curve F, vacuum-distilled glycerol; and curve G, vacuum-distilled propylene glycol

The suitability of mounting media for excitation at various wavelengths in the visible and ultraviolet regions can be judged by the absorption spectra given in Fig. 4, and the fluorescence data in Fig. 5. The absorption spectra are plotted on a logarithmic scale (left-hand ordinates) as optical density per millimetre. The right-hand scale indicates the fractions of light transmitted (I_T) for the indicated thicknesses. Thus propylene glycol and vacuum-distilled glycerol transmit more than 70 per cent. per millimetre at all wavelengths down to 210 nm. Liquid paraffin without special purification (not shown in Fig. 4) has absorption bands between 270 and 280 nm (optical density 0·3 per mm), which are reduced in intensity by passage over activated alumina (see curve E in Fig. 4). Glycerol - gelatin and polystyrene have an absorption cut-off starting at about 300 nm, while poly(vinyl alcohol) remains reasonably transmissive down to short wavelengths. All of these three mountants have relatively low absorption above 300 nm but they show appreciable fluorescence, even at 366 nm (see Fig. 5), and this can be objectionable when weakly fluorescing specimens have to be measured in thick mounts. The glycerol also gives quite strong fluorescence, probably due to slight aerial oxidation on standing after distillation. Thus for high sensitivity work with thick mounts, and for all work at short wavelengths, it is necessary to use a liquid mountant, such as water, propylene glycol or liquid paraffin. Undoubtedly the fluorescence of media such as poly(vinyl alcohol), glycerol - gelatin or polystyrene, could be reduced by purification but it is generally simpler and better to use one of the other liquid media, even with strongly luminescent specimens and long wavelength excitation, unless a permanent mount is essential.

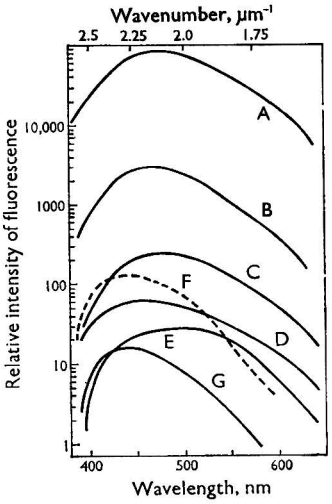

Fig. 5. Fluorescence of mounting media with 366 nm excitation: (for details see legend to Fig 4.) curve A, balsam; curve B, "glygel"; curve C, poly(vinyl alcohol); curve D, polystyrene; curve E, liquid paraffin; curve F, glycerol; and curve G, propylene glycol. (The lower curves have been corrected for the main Raman band of the solvent)

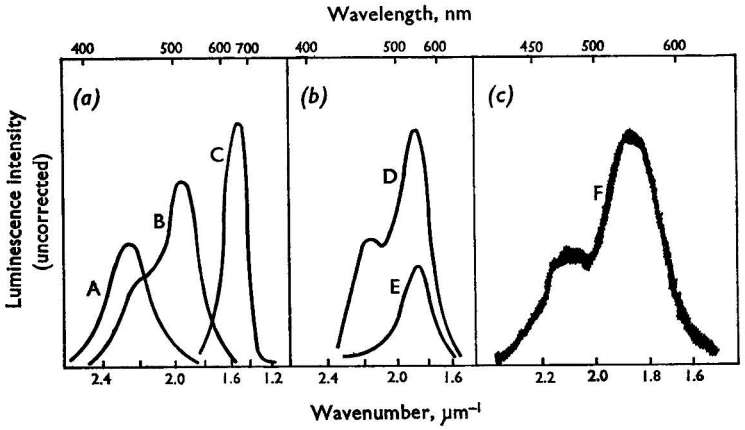

Fig. 6. Luminescence of zinc sulphide crystals excited by 366 nm. (*a*) Mixed phosphors in strewn mount in air: curve A, silver-activated (blue); curve B, copper-activated (green); and curve C, cadmium-activated (red), at ×5 sensitivity. Diameter of the field was 26 μm with ×4·7 mirror objective. (*b*) Cadmium, copper-activated in air mount: curve D, total luminescence; and curve E, long-lived luminescence through 100-Hz choppers at greater sensitivity. Diameter of the field was 26 μm with × 4·7 mirror objective. (*c*) Cadmium copper-activated, finely divided and mounted in liquid paraffin: crystal size and diameter of the field was 1·9 μm: glass condenser without patch stop; × 100 objective oiled with liquid paraffin

EXAMINATION OF MICROCRYSTALS—

Measurement of crystal photoluminescence with a conventional spectrofluorimeter frequently has to be carried out with a polycrystalline mass because of the difficulty of growing sufficiently large single crystals. The use of the microscope technique overcomes this limitation and also makes possible the location of microscopic variations in the emission over the surface or through the body of the crystal, or the detection of mixtures of crystals with different emission spectra.

Some examples of results obtained with zinc sulphide crystals are shown in Fig. 6. The left-hand section shows the emission spectra observed from small crystals (about 26 μm in diameter) that luminesced blue, green and red, and were strewn as a mixture on a microscope slide. Fig. 6 (*b*) indicates the capability of distinguishing between short-lived and long-lived emissions with the spectrophosphorimeter microscope. The specimen was a crystal of copper-activated zinc cadmium sulphide with which the measurements were made on an area, 26 μm in diameter. The spectrum of the long-lived luminescence (observed with the choppers out of phase, see curve E) indicated that the latter consisted almost entirely of the green emission band and contained none of the blue emission band present in the total luminescence spectrum (curve D). The decay was non-exponential, as is frequently the case with crystal phosphors. To demonstrate the spatial resolution of the spectrophosphorimeter microscope, this same specimen was finely ground and the powdered material dispersed on a slide in liquid paraffin. A fragment, 1·9 μm in diameter, was selected for measurement by using the oil immersion objective. The instrument was operated at high sensitivity and the extent of the noise level is indicated in curve F. The weight of specimen was about 10 pg.

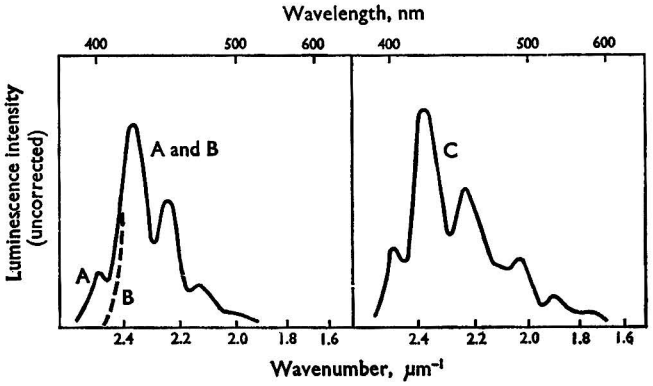

Fig. 7. Diagram of self-absorption and impurity effects: anthracene crystals excited at 366 nm: curve A, zone-refined crystal observed by frontal illumination; curve B, same crystal as curve A observed by in-line or dark-ground illumination; and curve C, recrystallised blue-fluorescent anthracene observed by frontal illumination, showing bands due to impurity

The results obtained with anthracene crystals are shown in Fig. 7. The crystals were relatively thick and curves A and B illustrate the artifacts that can arise from self-absorption of the fluorescence. With in-line or dark-ground illumination, the emission originated from the lower surface of the crystal. As a result a considerable proportion of the short wavelength part of the emission spectrum was absorbed by passage through the crystal, and a distorted spectrum was recorded (see curve B). This inner filter error was considerably less when the frontal illumination mode was used (see curve A). Comparison of curves A and C in Fig. 7 illustrates the sensitivity of the technique for detecting trace impurities in crystals. Curve A was obtained from zone-refined anthracene and curve C from twice recrystallised anthracene of "blue fluorescent" quality. The latter shows some additional weak bands in the long wavelength tail due to traces of naphthacene impurity.

Although the microscope optics were of glass the instrument could be used to measure the spectra of materials emitting in the near ultraviolet region, as indicated by the emission spectrum of a small phenanthrene crystal shown in Fig. 8. The spectrum extended to about 2·8 μm^{-1}, and was similar in shape to that observed from a mass of crystals of the same specimen measured with a conventional spectrofluorimeter in which all-silica optics were used. The intensities of the high wavenumber peaks were reduced somewhat relative to those at lower wavenumber, because of absorption by the microscope optics (see curve C in Fig. 3), but this distortion was not sufficient to interfere with the identification of the spectrum, and the latter could, of course, be corrected if desired.

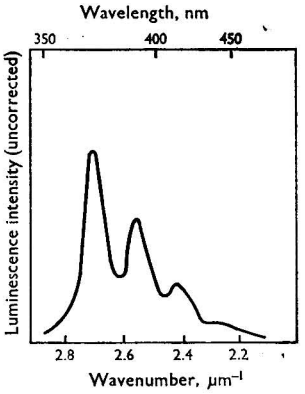

Fig. 8. Fluorescence emission in the near ultraviolet region: excitation of zone-refined phenanthrene at 313 nm: single crystal of diameter 26 μm with × 4·7 mirror objective

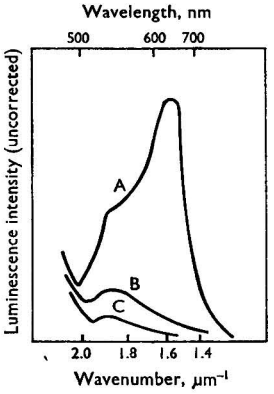

Fig. 9. Measurement of cell nuclei. Human epithelium cell treated with aqueous coriphosphine 0 and mounted in polystyrene. Excitation at 436 nm through aplanatic glass condenser without patch stop: diameter of the field about 5 μm with × 40 objective: curve A, nucleus; curve B, protoplasm; and curve C, background

Examination of biological material—

In biological and biochemical work, and in forensic science, the spectrophosphorimeter microscope can be regarded as an extension of the well established technique of fluorescence microscopy. It overcomes the difficulty and tedium of visual observation of weak fluorescence, makes possible the observation of invisible ultraviolet fluorescence and provides an objective measurement of colour or mixture of colours caused by the presence of several fluorescent components. It can, of course, be applied to specimens treated with fluorochromes and extends the choice of the latter to those emitting in the violet or ultraviolet regions. Thus the fluorescence spectrum of the nucleus of a human epithelium cell fluorochromed with coriphosphine 0 (see Fig. 9) was readily measured and distinguished from that of the surrounding protoplasm.

Another example that takes advantage of the spatial resolution of the instrument is shown in Fig. 10 (*a*). This refers to a single dyed woollen fibre, in which the dyestuff was distributed non-uniformly. The fibre diameter was about 30 μm, and the appearance, in visible light, varied from colourless to red over a short distance. The fluorescence was isolated from 30-μm lengths of fibre and the results indicated the presence of some dyestuff even on the apparently colourless portions. The blue - green emission (about 2·2 μm^{-1}) is due to the natural components of the wool. In comparison with wool the wood-pulp fibre of paper tissue [see Fig. 10 (*b*)] gave a very intense emission showing vibrational structure that was probably due to the presence of carbonyl groups in the cellulose polymer. On prolonged irradiation the intensity of the emission decreased because of photochemical reactions.

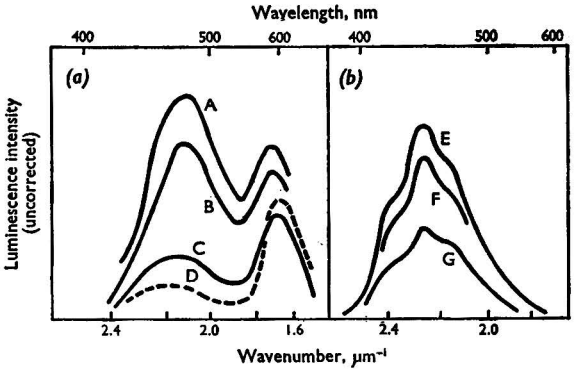

Fig. 10. Fluorescence of fibres: excitation at 366 nm by aplanatic glass condenser and patch stop. (*a*) Dyed woollen fibre (about 30-μm diameter) in air mount. Spectra refer to positions along the fibre separated by 100-μm intervals. Appearance in visible light varied from colourless (curve A) to red (curve D): diameter of the field was about 30 μm. (*b*) Wood-pulp fibre from paper tissue in air mount. Diameter of the field was 20 μm. Curves F and G refer to same field as curve E, but after 3 and 15 minutes' irradiation

The pollens of many plants contain fluorescent pigments, and spectral measurements can be made on a single pollen grain as shown in Fig. 11. The spectra are dependent on the mounting medium and frequently undergo changes on irradiation. Undoubtedly the spectra arise from the presence of several fluorescent components that are affected to different degrees by the treatments.

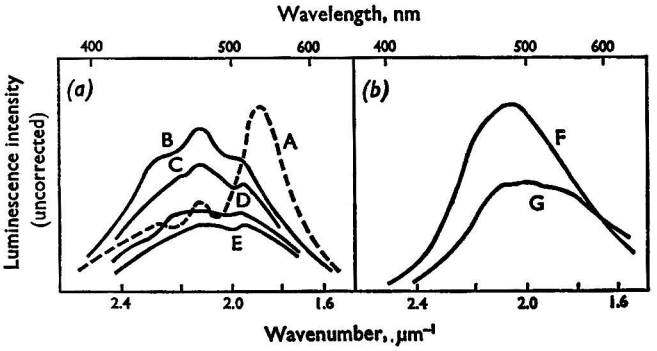

Fig. 11. Fluorescence of pollen grains. Excitation at 366 nm with aplanatic glass condenser and patch stop. Diameters of the field were about 20μm for (*a*) and about 30μm for (*b*). (*a*) Pollen of dandelion: curve A, in air; curves B, C, D and E in glycerol-gelatin before, and after, 5, 10 and 15 minutes' irradiation. (*b*) Pollen of apple blossom in air before (curve F), and after, 10 minutes' irradiation

PHOTOCHEMICAL REACTIONS—

In fluorescence microscopy the fluorochromed specimen is often observed to fade during irradiation, and similarly in spectrophosphorimeter microscopy the very high intensities of exciting light available at the specimen can be an embarrassment in the investigation of labile and weakly fluorescent specimens. With strongly fluorescent specimens the difficulty

can generally be overcome by reducing the intensity of the exciting light and increasing the sensitivity setting of the detection system. The high light intensities can be turned to good account in the investigation of photochemical reactions on the micro scale. For example, crystals of 9-methylanthracene were found to decompose very rapidly when exposed to the full intensity of the spectrophosphorimeter microscope. The original green emission attributed to the excited dimer disappeared within a few seconds and was replaced by a blue fluorescence with a structured spectrum (see Fig. 12). The main course of the reaction appeared to be the formation of an insoluble photo-dimer, and the blue emission was attributed to residual isolated monomer molecules in the photo-dimer matrix. To avoid changes occurring during the spectral measurements, the latter were made with filters in the beam of exciting light to reduce its intensity by a factor of about 30.

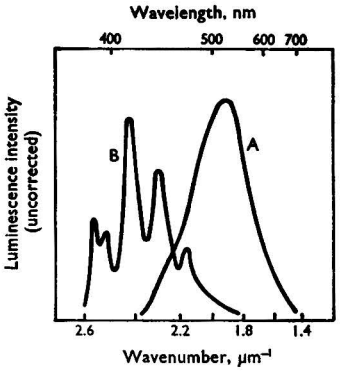

Fig. 12. Photo reaction of crystalline 9-methylanthracene. Excitation at 366 mn with aplanatic glass condenser and patch stop, and intensity reduced 30 times. Area of field about 15 μm: curve A, before irradiation; curve B, after irradiation at full intensity. The change was substantially complete within 10 to 20 seconds, depending on the thickness of the crystal

FLUORESCENCE - EXCITATION SPECTRA—

Corrected fluorescence - excitation spectra can, in principle, be measured by using the double-beam recording system and fluorescence quantum counter.[14] If a silica Abbe microscope condenser is used, the spectra are only approximately corrected because the area of illumination at the microscope slide will vary with wavelength as the focal length of the condenser varies. Fully corrected spectra can be obtained by using a mirror objective, but this must have a front-surface aluminised mirror. The usual mirror objectives have the reflecting coating on the back surface, and this gives rise to large errors in the ultraviolet region arising from absorption by the glass of the mirror.

With a single-beam recording system, *e.g.*, an oscilloscope, the uncorrected spectrum is first recorded by simply scanning the excitation monochromator with the emission monochromator tuned to the appropriate fluorescence band. The quantum distribution of the excitation system is then recorded from the output of a fluorescence quantum counter in the excitation beam. The corrected spectrum is then derived by dividing the ordinates of the first recording by those of the second. The three excitation spectra of zinc sulphide crystals shown in Fig. 13 were obtained in this way.

The interpretation of the excitation spectra of microscopic specimens presents special problems because the fraction of exciting light absorbed at various wavelengths is generally not known. If this fraction is small at all wavelengths the corrected excitation spectrum is

proportional to the product $\epsilon\phi_f$.[23] For solutions, the fluorescence efficiency, ϕ_f, is often independent of wavelength of excitation and the excitation spectrum is then a replica of the absorption spectrum (ϵ). On the other hand, if the exciting light is almost completely absorbed at all wavelengths the excitation spectrum reflects the variation of ϕ_f with wavelength, so that if ϕ_f is constant the spectrum consists simply of a horizontal straight line.[24] Microscopic specimens frequently correspond to the first condition in the long wavelength region of excitation and to the second in the short wavelength region. The presence of other strongly absorbing substances can produce additional gross distortion of the excitation spectrum and adds to the difficulties of interpretation.

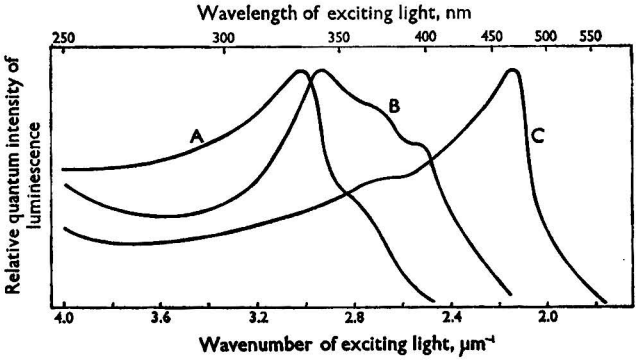

Fig. 13. Corrected excitation spectra of zinc sulphide crystals. Mixed phosphors in strewn mount in liquid paraffin on silica slide: diameter of the field was 40 μm with × 5 objective: analysing monochromator set to maxima of emission spectra: band width of excitation monochromator was 7 nm: curve A, silver-activated (blue); curve B, copper-activated (green); and curve C, cadmium-activated (red). The emission spectra in air of crystals from the same specimens are shown in Fig. 6(*a*)

FUTURE DEVELOPMENTS—

The use of a micro cuvette, holding about 0·3 μl of solution, was discussed earlier. If smaller micro cuvettes are required, the use of diatom skeletons is worth considering. These can, in principle, be used as porous, transparent and substantially non-fluorescent cuvettes with diameters from a few microns to a few hundred microns, depending on the species. The technique of chromatography can be adapted to the micro scale by using a thin layer of silica gel or alumina, about 1 mm wide and 20 to 30 mm long, deposited on a microscope slide. After development, the chromatogram can be scanned under the microscope and the spectra of sub-nanogram amounts of adsorbates located and their fluorescence-emission spectra measured. The scale of operations can, in principle, be reduced considerably by chromatography on single fibres. Thus the minimum amount of dyestuff observed in the spectra shown in Fig. 10 (*a*) was estimated to be about 1 pg in the section of fibre actually observed.

The spectrophosphorimeter microscope is capable of distinguishing between long-lived photoluminescence and prompt fluorescence, even when the intensity of the latter is many times greater. This facility is especially valuable for the examination of microscopic specimens at low temperature when many substances emit phosphorescence at high intensity. Under these conditions the spectrum of prompt fluorescence, the spectrum of phosphorescence and the lifetime of phosphorescence all provide criteria for identification. Moreover, the phosphorescence of almost all organic compounds is situated at wavenumbers greater than 3·0 μm^{-1}, and hence a microscope with glass optics is quite adequate (see Fig. 3). By inserting the second chopper between the objective and the viewing eyepiece it is possible to view the specimen by phosphorescence alone (with the choppers out of phase), or by both fluorescence and phosphorescence (with the choppers in phase). With the choppers out of phase the

problem of choosing primary and secondary filters disappears because the choppers themselves act as a 100 per cent. efficient filter for the exciting light and a completely black field is obtained in the absence of a phosphorescent specimen, even with no primary or secondary filters.

I thank Mr. C. G. Hatchard for carrying out the ferrioxalate actinometry and measuring the absorption and emission spectra of the mountants, and Mr. N. I. Hendey for providing a specimen of *Synedra Fulgens*. This paper is published with the permission of the Navy Department, Ministry of Defence.

References

1. Thorell, B., *I.R.E. Trans. Med. Electron.*, ME–7, No. 3, 1960, 119.
2. Barnes, J. C., and Thomson, A. J., *J. Scient. Instrum.*, 1967, **44**, 577.
3. Olson, R. A., *Rev. Scient. Instrum.*, 1960, **31**, 844.
4. Loeser, C. N., *Ibid.*, 1966, **37**, 237.
5. Rigler, R., *Acta Physiol. Scand.*, 1966, **67**, Suppl. 267.
6. Loeser, C. N., *in* Waelsch, H., *Editor*, "Ultrastructure and Cellular Chemistry of Neural Tissue," Cassell and Co., London, 1957, p. 224.
7. West, S. S., Loeser, C. N., and Schoenberg, M. D., *I.R.E. Trans. Med. Electron.*, ME–7, No. 3, 1960, 138.
8. Loeser, C. N., and West, S. S., *Ann. New York Acad. Sci.*, 1962, **97**, 346.
9. Freed, J. J., and Engle, J. L., *Ibid.*, 1962, **97**, 412.
10. Porter, G., and Strauss, G., *Proc. Roy. Soc. A*, 1966, **295**, 1.
11. Parker, C. A., and Hatchard, C. G., *Analyst*, 1962, **87**, 664.
12. Parker, C. A., "Photoluminescence of Solutions," Elsevier Publishing Company, Amsterdam, London and New York, 1968, p. 217.
13. ——, *op. cit.*, p. 178.
14. ——, *op. cit.*, p. 204.
15. ——, *op. cit.*, p. 220.
16. ——, *op. cit.*, p. 186.
17. Hendey, N. I., "An Introductory Account of the Smaller Algae of British Coastal Waters: Part V, Bacillariophyceae (Diatoms)," H.M. Stationery Office, London, 1964, p. 163.
18. Parker, C. A., *op. cit.*, p. 404.
19. ——, *op. cit.*, p. 208.
20. ——, *op. cit.*, p. 158.
21. ——, *op. cit.*, p. 252.
22. ——, *op. cit.*, p. 256.
23. ——, *op. cit.*, p. 247.
24. ——, *op. cit.*, p. 226.

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The Selection of Exciting Energy in Radioisotope X-ray Fluorescence Analysis

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The use of exciting X-rays with energies either just above or far above the X-ray absorption edge of the wanted element has been proposed for radioisotope X-ray fluorescence analysis. The performance of these two methods is compared in terms of limits of detection, reduction of heterogeneity effects and compensation for matrix effects.

One of the main advantages of radioisotope X-ray fluorescence analysis[1] over the conventional technique is the ability to select the energy of the exciting X-rays. However, the energy requirements to achieve one desirable feature, such as a low limit of detection, may differ from those required for reducing heterogeneity effects. The choice will also depend on the type of energy discrimination or selection used. The selection of exciting energy will be considered in the light of new and published data.

Experimental

Instrumental conditions—

Measurements were made with a Philips, 400-channel, pulse height analyser, with a Telsec pre-amplifier and the detectors listed in Table I. Concentric, source-detector geometry was used with a standard live counting time of 100 seconds. The proportional counter resolution for copper K radiation was 20 per cent. with xenon, and 18 per cent. with argon.

Table I

Limits of detection by using different exciting energies

Oxide	Source		Filter	Detector	S/N ratio for 1 per cent. of oxide	Limit of detection, per cent. of oxide
SnO_2	Barium target 10-mCi ^{241}Am	~32 keV	Silver foil	Sodium iodide scintillator	0·61	0·005
SnO_2	10-mCi ^{241}Am	~60 keV	None	Calcium fluoride scintillator	0·56	0·006
PbO	10-mCi ^{238}Pu	~15 keV	None	Ar Proportional	1·85	0·004
PbO	1-mCi ^{109}Cd	~22 keV	None	Ar Proportional	3·22	0·004
PbO	1-Ci ^{147}Pm* Aluminium bremsstrahlung		None	Ar Proportional	0·85	0·010
ZnO	Germanium target 1-mCi ^{109}Cd	~10 keV	Copper foil	Ar Proportional	2·13	0·013
ZnO	10-mCi ^{238}Pu	~15 keV	None	Ar Proportional	8·12	0·002
ZnO	1-mCi ^{109}Cd	~22 keV	None	Ar Proportional	26·0	0·001
ZnO	1-mCi ^{109}Cd	~22 keV	None	Xe Proportional	6·52	0·002
ZnO	1-mCi ^{109}Cd	~22 keV	Copper foil	Ar Proportional	2·37	0·005
ZnO	1-Ci ^{147}Pm* Aluminium bremsstrahlung		None	Ar Proportional	4·70	0·003
Fe_2O_3	10-mCi ^{238}Pu	~15 keV	None	Ar Proportional	7·04	0·003
Fe_2O_3	1-mCi ^{109}Cd	~22 keV	None	Ar Proportional	5·24	0·004
Fe_2O_3	1-Ci ^{147}Pm* Aluminium bremsstrahlung		None	Ar Proportional	3·11	0·006
TiO_2	10-mCi ^{238}Pu	~15 keV	None	Ar Proportional	2·35	0·011
TiO_2	1-mCi ^{109}Cd	~22 keV	None	Ar Proportional	1·28	0·020
TiO_2	2-mCi ^{55}Fe	~ 6 keV	TiO_2 - polythene	Ar Proportional	0·37	0·022
TiO_2	10-mCi ^{238}Pu	~15 keV	TiO_2 - polythene	Ar Proportional	0·65	0·030

* As a crude approximation, the effective exciting energy of a bremsstrahlung source can be taken to be close to the X-ray absorption edge of the wanted element.

MATERIALS—

The compounds used were of analytical-reagent grade. All of the mineral particle-size fractions were chemically assayed by standard methods and shown to be of constant composition. Pure, powdered boric acid was used as the suspending medium, which has X-ray absorption coefficients and density similar to water.

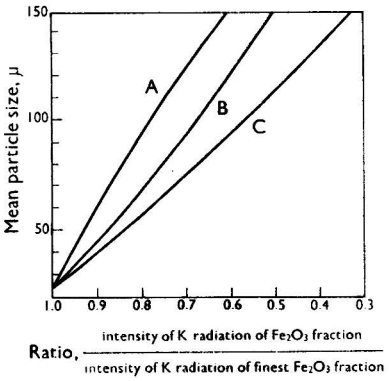

Fig. 1. Graphs showing the variation of iron X-ray intensity with particle size: curve A, ^{109}Cd excitation; curve B, ^{238}Pu excitation; and curve C, ^{3}H - Zr excitation

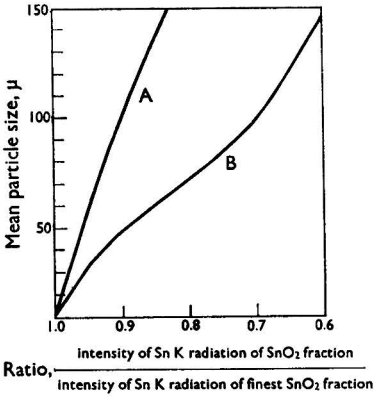

Fig. 2. Graphs showing the variation of tin X-ray intensity with particle size: curve A, 60-keV excitation from ^{241}Am; curve B, Ba - ^{241}Am source - target assembly excitation

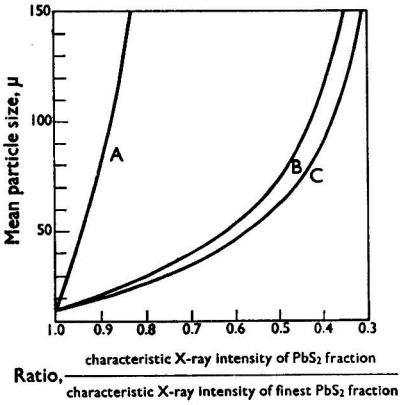

Fig. 3. Graphs showing the variation of lead X-ray intensity with particle size: curve A, Pb K radiation excited by ^{147}Pm - Al; curve B, Pb L radiation excited by ^{109}Cd; and curve C, Pb L radiation excited by ^{238}Pu

RESULTS

In Table I, the limits of detection found for certain elements in the equivalent of an aqueous suspension are summarised. This limit is defined as the concentration of the oxide that gives a detector output equal to twice the standard deviation of the detector output for the pure matrix in a measuring time of 100 seconds. Figs. 1, 2 and 3 show the effect of increasing the exciting energy on the change of characteristic intensity with particle size for hematite, cassiterite and galena, again in the equivalent of an aqueous suspension. Fig. 3 also shows the effect when using lead K, rather than lead L, radiation.

Discussion of results

Limits of detection—

The limit of detection depends on the ratio of the characteristic to non-characteristic X-ray intensities measured by the system, hereafter called the S/N ratio, and also on the magnitude of these intensities.[2] The maximum fluorescent yield is obtained when the exciting energy is just greater than the absorption edge of the wanted element, the so-called "efficient" excitation. However, the use of X-rays of this energy does not necessarily give the lowest limit of detection. Because of the lack of suitable radioisotopes, the range of exciting sources is limited and source - target assemblies[3] have to be used to obtain the X-rays of optimum energy for most elements. Because of the inefficiency of these source - target assemblies, much lower effective outputs are obtained from them than from primary X-ray sources of the same activity. Thus, lower characteristic intensities are obtained, offsetting in part the increase in fluorescence yield. If proportional or scintillation counters are used to measure the X-rays, their energy resolving power is insufficient to separate the back-scattered primary and the characteristic X-rays when efficient excitation is used. A suitable X-ray filter must be used to remove the back-scattered primary X-rays, but the fluorescent X-rays from the filter material limit the increase in the S/N ratio, because these X-rays cannot be resolved from the characteristic radiation.

The alternative method of obtaining a low limit of detection with proportional or scintillation counters is to use an almost mono-energetic source emitting X-rays which, when back-scattered, are clearly separated from the characteristic X-rays by the energy resolving power of the detector.[4] In practice, this requires the use of X-rays of energy well above the absorption edge of the wanted element, which results in a poor fluorescent yield. This lack of characteristic intensity is offset, in part, by the higher exciting intensities obtained from sources, as opposed to source - target assemblies. Providing X-ray filters are not used, a high S/N ratio is obtained and low limits of detection result. If filters have to be used, for instance, to separate characteristic radiation from adjacent elements, the fluorescent X-rays from the filter reduce the S/N ratio and give poorer limits of detection. Examples of these effects are given in Table I.

If a lithium-drifted, solid-state detector operated at the temperature of liquid nitrogen is used, the back-scattered primary and characteristic X-rays can be resolved when the exciting X-ray energy still results in a high fluorescent yield. Thus, a filter is not required and a high S/N ratio is obtained.[5] At present, the apertures of such detectors are rather small, typically 25 to 50 mm^2, which result in lower count-rates and hence offset to some extent the effect of the increased S/N ratio.

Bremsstrahlung sources, such as promethium-147 - aluminium, which emit a wide range of X-ray energies, generally give a relatively poor limit of detection, because only a portion of their output efficiently excites the characteristic radiation. The radiation back-scattered from the sample contains a component that is identical in energy with the characteristic X-rays, thereby limiting the S/N ratio obtainable, even with a high resolution detector.

Heterogeneity effects—

The simple equations used to predict X-ray fluorescent intensities apply only when the system is homogeneous. As soon as any degree of heterogeneity is present, deviations from the expected behaviour are found, which can cause appreciable errors in analytical results. For convenience, these may be classified into particle-size and particle-composition effects. With the former, the characteristic intensity depends on the size of the particles containing the wanted element and also on that of the rest of the matrix; with the latter, the intensity depends on the composition of the particles containing the wanted element. For any system a range of particle sizes, usually called the transition zone, exists where the characteristic intensity is markedly dependent on the particle size; above and below this zone only a limited dependence is shown.

Claisse and Samson's[6] simplified theory to account for these effects, predicts that the magnitude of the changes in characteristic intensity can be reduced for a given system, either by proceeding from a lower to a higher energy characteristic radiation, for example, in determining tungsten by its K rather than its L rays,[7] or by using inefficient excitation. The use of the K radiation of elements with middle and higher atomic numbers is particularly attractive in radioisotope X-ray fluorescence analysis, because suitable sources, *e.g.* cobalt-57

and gadolinium-153, exist that can excite these X-rays with a high S/N ratio. This is especially so if solid-state detectors are used because, in this region, the best of these detectors show an energy resolving power superior to that of most X-ray spectrometers. For a given characteristic X-ray, the largest effect is found when the energy of the exciting radiation is just greater than the absorption edge of the wanted element. These predictions for the collimated geometry of an X-ray spectrometer were confirmed by Claisse and Samson[6] and, subsequently, by many other workers. The results given in Figs. 1, 2 and 3 show a similar effect with the broad beam geometry used in radioisotope X-ray fluorescence analysers. It is interesting to note that an identical relationship between relative intensity and particle size was found for an X-ray spectrometer operating at 32 keV and the barium source - target assembly used to obtain the results given in Fig. 2.

Thus, when heterogeneity effects limit the accuracy achieved, and the normal solutions of fusion or reproducible grinding are not possible, the use of a higher energy characteristic X-ray or inefficient excitation may reduce the effects to an acceptable level. However, both of these actions will also shift to larger values the range of particle size in which these effects occur. If the analysed material is mainly in the resultant size range, an increase in the observed effect could occur, although the over-all magnitude is reduced.

Matrix effects—

Characteristic X-rays, generated in the sample by elements other than the wanted one, can enhance the latter's characteristic intensity. This effect can be eliminated if the exciting energy is chosen so that the interfering characteristic X-rays are not excited.

Changes in the concentration of elements other than the wanted one alter the X-ray absorption coefficients of the sample and hence the characteristic intensity from the wanted element. Various methods have been used to overcome this effect, and the choice of exciting energy can be of importance with some of them.

The Compton back-scattered component of the exciting radiation varies in a similar manner to the characteristic radiation, as the mass-absorption coefficients change. If efficient excitation is used, the ratio of the two intensities can be taken to give an output almost independent of variations in the matrix.[5] However, this is normally only possible with radioisotope X-ray fluorescence analysis when a high resolution, solid-state detector is used. Lubecki, Wasilewska and Gorski[8] have proposed the use of the Compton back-scattered component when inefficient excitation is used. Here a nomogram is used, and the method depends on the absence of elements with an absorption edge lying between the characteristic and exciting X-ray energies.

Rhodes, Ahier and Poole[4] suggested measuring the intensity of the exciting X-rays transmitted through a pellet of the sample. The greater penetrating power of the X-rays when inefficient excitation is used permits the use of thicker samples, thus facilitating the preparation of the pellet. Here again, elements with an absorption edge lying between the exciting and characteristic X-ray energies interfere, and the possibility of such an element being present increases as the difference between the two energies increases. When an interfering element is known to be present, its effect can be overcome by reducing the energy of the exciting X-rays below that of the absorption edge of the interfering element. Carr-Brion[9] suggested the measurement of the characteristic X-ray intensity obtained from a block of the wanted element placed behind a layer of the material being analysed. This compensates for variations in both exciting and characteristic absorption coefficients, and elements with absorption edges between these no longer interfere. The use of inefficient excitation is preferred with this method, because not only does it permit the use of a thicker sample layer but heterogeneity effects, which are the chief limitation on the use of this method with a conventional X-ray spectrometer,[10] would be markedly reduced.

Conclusions

The selection of exciting X-ray energy is of great importance in radioisotope X-ray fl uorescence analysis. When adequate intensities can be obtained, the lower limit of detection is achieved when the exciting X-rays can just be completely separated from the characteristic X-rays of the wanted element by the energy resolving power of the detector. For reduction of heterogeneity effects, the use of the highest energy characteristic X-rays and inefficient

excitation is generally preferred. The choice of energy for compensating for matrix effects depends on the method used. Generalisations are obviously impossible, and selection of the optimum energy for any analytical problem must take into account the factors outlined in this paper.

References

1. Rhodes, J. R., *Analyst*, 1966, **91**, 683.
2. Jenkins, R., and de Vries, J. L., "Practical X-ray Spectrometry," Philips, Eindhoven, 1967, p. 160.
3. Watt, J. S., *Int. J. Appl. Radiat. Isotopes*, 1964, **15**, 617.
4. Rhodes, J. R., Ahier, T. G., and Poole, D. O., *U.K. Atomic Energy Authority Research Report*, A.E.R.E., R.4474, 1964, H.M. Stationery Office, London.
5. Rhodes, J. R., "Proceedings of 2nd Symposium on Low Energy X and Gamma Sources and Applications," *U.S. Atomic Energy Commission Report*, ORNL–11C–10, Austin, Texas, 1967, p. 442.
6. Claisse, F., and Samson, C., *Adv. X-ray Analysis*, 1962, **5**, 335.
7. Carr-Brion, K. G., and Payne, K. W., *Analyst*, 1968, **93**, 441.
8. Lubecki, A., Wasilewska, M., and Gorski, L., *Spectrochim. Acta*, 1967, **23A**, 831.
9. Carr-Brion, K. G., *Analyst*, 1964, **89**, 346.
10. ——, *Ibid.*, 1965, **90**, 9.

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3,5′-Bis(dicarboxymethylaminomethyl)-4,4′-dihydroxy-*trans*-stilbene as a Selective Spectrofluorimetric Reagent for Cadmium

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3,5′-Bis(dicarboxymethylaminomethyl)-4,4′-dihydroxy-*trans*-stilbene forms fluorescent complexes with aluminium, beryllium, magnesium, calcium, strontium, barium, zinc, cadmium, yttrium, lanthanum, gadolinium and lutetium. All the metal complexes have a metal-to-ligand stoicheiometry of 2:1. Magnesium, calcium, strontium and barium exhibit maximal fluorescence at pH 10·9, with excitation - fluorescence maxima at 360 and 440 nm. Zinc, cadmium, yttrium, lanthanum, gadolinium and lutetium show maximal fluorescence at pH 7·9, with excitation - fluorescence maxima at 360 and 440 nm, and 360 and 430 nm for yttrium and the lanthanons. Aluminium and beryllium show maximal fluorescence at pH 5·2 and 6·4, respectively, with excitation - fluorescence maxima at 345 and 410 nm and 360 and 405 nm, respectively. The formation of the cadmium complex provides a sensitive and selective determination of cadmium in the range 0·5 to 25·0 μg. A simple separation procedure is described in which 3,5′-bis-(dicarboxymethylaminomethyl)-4,4′-dihydroxy-*trans*-stilbene can be used for the determination of cadmium in the presence of most other metals except lead.

4,4′-Diamino-*trans*-stilbene-*NNN′N′*-tetra-acetic acid is well known as a highly sensitive metallofluorescent indicator for EDTA titrations.[1,2] The stilbene skeleton appears to be a good carrier of fluorescence. For these reasons, we have prepared such a reagent based on the stilbene skeleton, *viz.*, 3,5′-bis-(dicarboxymethylaminomethyl)-4,4′-dihydroxy-*trans*-stilbene (BDDS) and have investigated the fluorescence properties of its metal complexes.

Experimental

Apparatus—

Fluorescence measurements were made with a double monochromating spectrofluorimeter (Farrand Optical Co., Catalogue No. 104244), fitted with a 150-watt xenon arc lamp (Hanovia Division, Catalogue No. 901 C–1) and RCA IP28 photomultiplier, and equipped with a Honeywell-Brown Recorder. Fused quartz cells (10 × 20 × 50 mm) were used throughout. To obtain the maximum sensitivity compatible with good definition of maxima, 10-nm band-width slits were used in the exciting and analysing monochromators. Fluorescence was measured at right angles to the incident light so that the mean solution path length of exciting radiation was 5 mm and of the fluorescent emission, 10 mm.

Spectrophotometric measurements were performed with a Unicam SP800 ultraviolet spectrophotometer, and 1-cm fused quartz cells were used throughout.

The pH measurements were made with a Vibron pH meter, model 39A (Electronic Instruments Ltd., Richmond, Surrey, England).

PREPARATION OF 4,4′-DIHYDROXY-*trans*-STILBENE—

4,4′-Diamino-*trans*-stilbene dihydrochloride (2·83 g, 0·01 mole) was dissolved by warming to 80° to 90° C in 50 ml of 5 M sulphuric acid. The solution was cooled to 0° C and a solution of sodium nitrite (1·40 g, 0·02 mole, in 20 ml of water) was added while stirring the solution and maintaining the temperature at between 0° and 5° C. The solution of the diazonium salt thus formed was filtered from the undissolved residue and added dropwise to a solution of sodium sulphate, which consisted of 15 g of the decahydrate dissolved in 10 ml of water and 11 ml of concentrated sulphuric acid, and heated to 125° to 130° C. The addition of the diazonium salt solution was regulated by the decrease in the nitrogen evolution and the disappearance of the yellow colour. When all the diazonium salt solution had been added heating of the reaction mixture was continued for an additional 20 minutes. It was then cooled to room temperature and extracted three times with 50-ml portions of diethyl ether. The collected ether layers were washed four or five times with 30-ml portions of water until the aqueous phase no longer showed a strongly acidic reaction (final pH 4 to 5). The ether layer was then dried overnight with anhydrous sodium sulphate, filtered from the sodium sulphate and evaporated *in vacuo*. The residue was dissolved in 20 ml of hot ethanol and about 0·1 g of charcoal was added. After 10 minutes, the charcoal was removed by filtration and the filtrate allowed to crystallise at room temperature for 2 to 3 days. The white precipitate was collected by suction and dried at 100° C for 12 hours. The yield was 0·7 to 0·8 g of product that melted at 282° to 284° C (according to the literature[3] the melting-point is 284° C).

PREPARATION OF 3,5′-BIS(DICARBOXYMETHYLAMINOMETHYL)-4,4′-DIHYDROXY-*trans*-STILBENE—

4,4′-Dihydroxy-*trans*-stilbene (0·63 g, 0·003 mole) and imino-acetic acid disodium salt (1·17 g, 0·006 mole) were dissolved by warming to 60° to 70° C in 25 ml of glacial acetic acid. The solution was cooled to room temperature and 1 ml of 38 per cent. formaldehyde solution added. The flask was stoppered and heated to 60° to 70° C, with occasional shaking, for 12 hours. The solution was then poured, with constant stirring, into 250 ml of absolute ethanol and the mixture allowed to stand for 3 hours. The white precipitate formed was collected by suction, washed with 20 ml of absolute ethanol, dried at 70° C for 5 hours, dissolved in 25 ml of water and re-precipitated by the addition of 2 ml of 6 M hydrochloric acid. After 12 hours, the white precipitate was collected by suction, washed with 10 ml of water and dried at 105° C for 3 hours. It was then recrystallised from hot water and dried for 6 hours at 105° C to obtain the final crystalline BDDS product. The yield was 0·3 to 0·4 g of free acid. Elemental analysis for $C_{24}H_{26}N_2O_{10}$ (molecular weight 502·484) is as follows: calculated, 57·3 per cent. of carbon, 5·22 per cent. of hydrogen and 5·58 per cent. of nitrogen; found, 57·42 per cent. of carbon, 5·31 per cent. of hydrogen and 5·43 per cent. of nitrogen.

REAGENTS—

BDDS solution, $1{\cdot}00 \times 10^{-4}$ M, and analytical-reagent grade salts of metal ions.

pH ADJUSTING SOLUTIONS—

Perchloric acid, 0·50 M, *plus* M hexamine *plus* 0·50 M sodium perchlorate for pH 1·10 to 7·44; M hexamine *plus* 0·50 M sodium perchlorate *plus* 0·50 M sodium hydroxide for pH 7·44 to 13·00; and 10 M sodium hydroxide for pH 13·00 to 15·80. The ionic strength was kept constant (=0·10 N) in the pH range 1·10 to 13·00 throughout stability constant measurements.

DETERMINATION OF CADMIUM—

(*a*) *In admixture with other metal ions*—Transfer a solution containing 0·5 to 25·0 μg of cadmium to a separating funnel. Add 5 ml of 20 per cent. sodium potassium tartrate, then add sufficient concentrated ammonia solution to bring the pH to 11·0 (± 0·1), 2 g of potassium cyanide, 1 ml of 0·2 per cent. aqueous sodium diethyldithiocarbamate solution and 10 ml of carbon tetrachloride. Stopper and shake for 1 minute, then wash the tetrachloride layer with two 20-ml portions of water. Extract the cadmium back into an aqueous phase by shaking with 10 ml of 0·2 M hydrochloric acid and adjust to pH 7 with a pH meter. Add 5 ml of M hexamine, 5 ml of $1{\cdot}00 \times 10^{-4}$ M BDDS and make up to 25 ml with water. Measure

the fluorescence at 440 nm with the excitation monochromator set at 360 nm, then construct the calibration graph for the given range of amounts of cadmium with the same conditions; a straight-line graph should be obtained.

(*b*) *In admixture with zinc only*—Adjust the pH of a solution containing 0·5 to 25 μg of cadmium and 0 to 6 μg of zinc to 7 by using a pH meter. Add 5 ml of M hexamine and 5 ml of $1{\cdot}00 \times 10^{-4}$ M BDDS, and make up to 25 ml with water. Measure the fluorescence at 440 nm with the excitation monochromator set at 360 nm. Construct the calibration graph for the given range of cadmium amounts with the same conditions.

Results and discussion

Sensitivity of determination—

To counteract variations in the intensity of the xenon arc source and the response of the detector - amplifier system, all fluorescence intensities were expressed as the ratio of fluorescence of a freshly prepared standard quinine sulphate solution (8×10^{-4} g of quinine per litre in 10^{-3} M sulphuric acid) measured at 450 nm, with the excitation monochromator set at 350 nm, in the same cell. Relative molar fluorescences, according to the molar absorptivity, were expressed as the calculated ratio of fluorescence of a M sample solution (metal concentration) to that of a M quinine solution (in 10^{-3} M sulphuric acid).

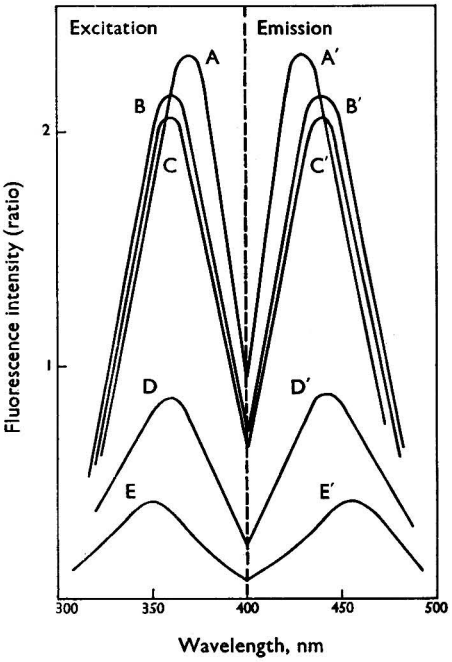


Fig. 1. Excitation and emission spectra of lanthanum A, A', pH 7·9; cadmium, B, B', pH 7·9, and calcium, C, C', pH 10·9 complexes with BDDS, and of BDDS alone, D, D', pH 10·9; E, E', pH 7·9. $C_M = C_L = 2{\cdot}00 \times 10^{-5}$ M

Spectral characteristics—

The spectral characteristics of the lamp and photomultiplier used have been given in previous papers from this laboratory.[4,5] The excitation and emission spectra of the calcium, cadmium and lanthanum complexes are plotted in Fig. 1. These are uncorrected for the variations in spectral response of the source, monochromator and detector system. It will be seen that the fluorescence characteristics are those of the fluorophore rather than of the metal involved.

INFLUENCE OF pH AND ADHERENCE TO LAMBERT - BEER'S LAW—

The dependence of fluorescence intensity of the reagent and its calcium, cadmium and lanthanum complexes on pH is given in Figs. 2 and 3. A strong increase in the reagent fluorescence appears above pH 11 and is connected with the formation of the totally deprotonated ligand L^{6-}. A low fluorescence occurs in the range pH 8 to 10 and corresponds

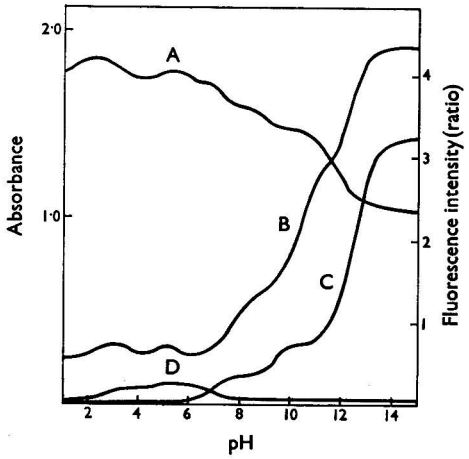


Fig. 2. pH dependence of *absorbance*: A, 300 nm; B, 370 nm and of *fluorescence*, C, 360 nm and 440 nm; D, 340 nm and 390 nm of BDDS alone. $C_L = 2{\cdot}00 \times 10^{-5}$ M

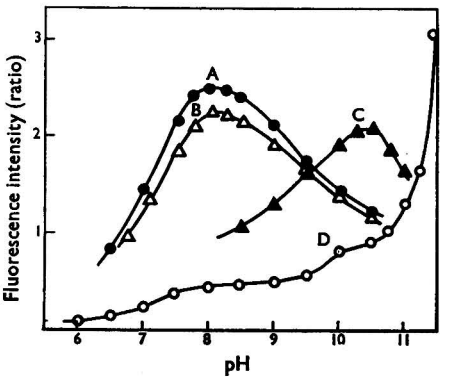
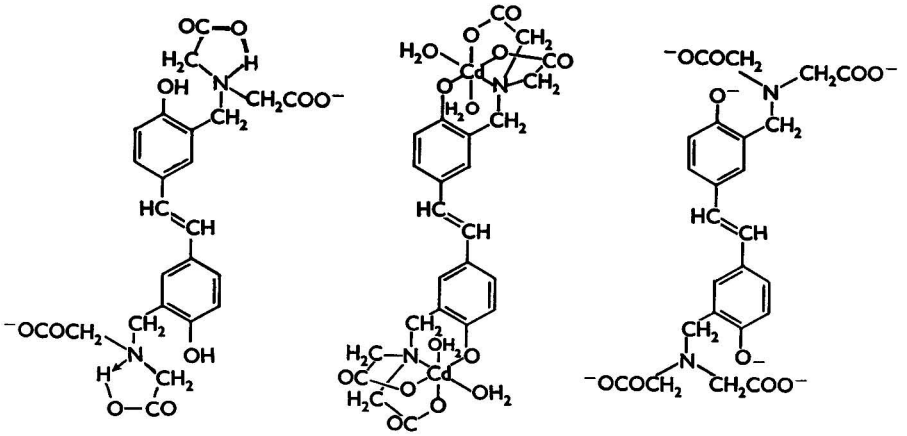


Fig. 3. pH dependence of fluorescence of lanthanum, A, 360 nm and 430 nm, cadmium, B, 360 nm and 440 nm and calcium, C, 360 nm and 440 nm complexes with BDDS and of BDDS alone, D, 360 nm and 440 nm. $C_M = C_L = 2{\cdot}00 \times 10^{-5}$M

to the formation of the species H_2L^{4-} and H_3L^{3-}. A shift of fluorescence maximum from 445 to 390 nm appears at pH 6 and is connected with formation of the H_4L^{2-} species. The formulae of the H_4L^{2-} (I), the calcium complex (II) and the L^{6-} (III) species of BDDN are as follows—



I II III

It will be seen from Fig. 4 that there is a linear relationship between fluorescence signal and cadmium concentration in the range $1{\cdot}00 \times 10^{-6}$ to $2{\cdot}00 \times 10^{-5}$ M Cd^{2+}.

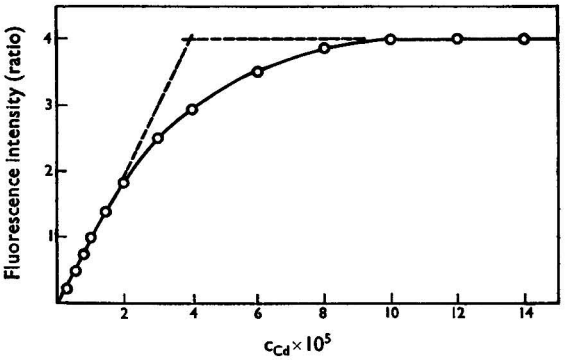


Fig. 4. Dependence of fluorescence on the concentration of cadmium. Excitation 360 nm, emission 440 nm, pH 7·9, $C_L = 2{\cdot}00 \times 10^{-5}$M

INFLUENCE OF TIME AND TEMPERATURE—

No considerable change of fluorescence was observed during the period up to 60 minutes after preparation of the solution. After 3 hours the decrease of fluorescence is about 10 per cent. In all experiments, the temperature was 20° ± 3° C. No significant variation of fluorescence with temperature of development was noted, but no further specific study of the temperature effect was made.

EFFECT OF FOREIGN IONS ON THE DETERMINATION OF CADMIUM—

The effect of twenty-seven cations and fifteen anions on the cadmium determination was investigated. The limiting value of the concentration of a foreign ion was taken as that which caused an error of ±5 per cent. in the determination of 10 μg of cadmium. The results obtained are summarised in Table I. Interfering ions were 2 μg of lead, 10 μg of thallium (I), 400 μg of EDTA and 500 μg of DPTA. These results were obtained by the procedure given under (*a*) in Experimental. Procedure (*b*) can be used for cadmium in the presence of up to 6 μg of zinc. If larger amounts of zinc are present, procedure (*a*) must be applied.

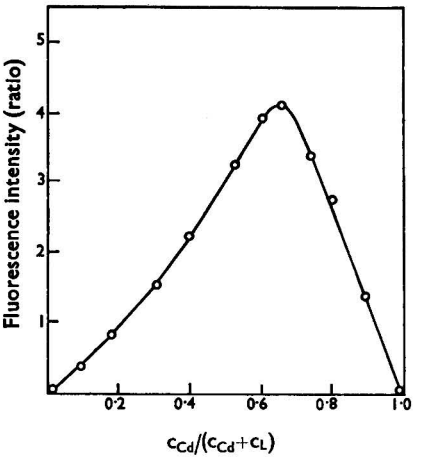


Fig. 5. Continuous variation in isomolar solution. $C_{Cd} + C_L = 4{\cdot}00 = 10^{-5}$M, pH 7·9, excitation 360 nm, emission 440 nm

TABLE I

ANALYSIS OF CADMIUM SOLUTIONS TREATED AS UNKNOWN SAMPLES

Cadmium, μg		
Present	Found	Foreign ions present, μg
0·5	0·5*	—
5·0	4·8*	—
10·0	9·8*	—
15·0	15·1*	—
25·0	24·7*	—
5·0	4·8*	Zn 2
10·0	9·6*	Zn 3
20·0	19·2*	Zn 5
25·0	23·5*	Zn 7
10·0	9·9†	Fe(III) 1000, Cu(II) 1000, Co(II) 1000, Ni 1000
10·0	10·1†	Zn 500, Hg(II) 1000, Ag 1000, Sn(II) 1000
10·0	10·2†	Mn(II) 1000, Pd(II) 1000, Bi 500
20·0	20·3†	Y 1000, La 1000, U(VI) 1000, Th 1000
20·0	19·1†	Zr 1000, Hf 1000, Al 1000, In 1000
10·0	9·8†	V(V) 1000, Nb(V) 1000, Ta(V) 1000
10·0	9·8†	Cr(III) 1000, Mo(VI) 1000, W(VI) 1000
10·0	9·8†	Pb(II) 1
10·0	9·4†	Pb(II) 2
10·0	9·7†	Tl(I) 5
10·0	9·4†	Tl(I) 10
20·0	19·5†	PO_4^{3-} 1000, F^- 1000, AcO^- 1000, Cl^- 1000
20·0	19·4†	CN^- 1000, NO_3^- 1000, Br^- 1000, I^- 1000
20·0	19·8†	ClO_4^- 1000, AsO_4^{3-} 1000, SO_4^{2-} 1000, SCN^- 1000
20·0	19·9†	Citrate 5000
10·0	9·8†	EDTA 200
10·0	9·6†	EDTA 300
10·0	9·4†	EDTA 400
10·0	9·8†	DPTA 200
10·0	9·9†	DPTA 300
10·0	9·6†	DPTA 400
10·0	9·3†	DPTA 500

* Procedure (*a*).
† Procedure (*b*).

PRECISION DATA—

These were obtained by multiple analyses of a series of solutions containing 10 μg of cadmium. The precision of the fluorescence intensity measurement for cadmium is ±3·2 per cent., corresponding to 0·32 μg of cadmium.

STRUCTURE OF THE CADMIUM COMPLEX—

The molar ratio of metal to ligand in the complex formed was investigated by the method of continuous variation in isomolar solutions. The corrected curve is given in Fig. 5. A 2:1 ratio of cadmium to BDDS was found.

The stability constants of proton complexes of the BDDS ligand were determined in the acidity area from pH 1 to 13 by ultraviolet spectrophotometry.[6] Values found are as follows: log $K_{1,1}$, 12·23 ± 0·33; log $K_{2,1}$, 9·62 ± 0·40; log $K_{3,1}$, 7·36 ± 0·37; log $K_{4,1}$, 5·80 ± 0·20; log $K_{5,1}$, 4·68 ± 0·09; log $K_{6,1}$, 3·48 ± 0·12; log $K_{7,1}$. 1·45 ± 0·10; when $K_{a,1} = [H_aL][H_{a-1}L]^{-1}[H]^{-1}$.

The composition and over-all stability constant of the calcium, cadmium and lanthanum 2:1 complexes were determined by the method described elsewhere.[7] The log $K_{\beta 2}$ values found were $Ca_2L = 16{\cdot}47$; $Cd_2L = 21{\cdot}46$; and $La_2L = 22{\cdot}63$.

The structure of the fluorescent cadmium complex formed may possibly be of the CdL^{2-} form shown, **II**.

FLUORESCENCE OF OTHER METAL IONS—

BDDS gives fluorescent complexes with several other ions. This property of the reagent is summarised in Table II, which also shows the optimal pH values for each fluorescence, and gives their intensities relative to that of a quinine sulphate solution of equal molarity.

TABLE II

RELATIVE MOLAR FLUORESCENCE OF SEVERAL METAL COMPLEXES OF BDDS

Metal ion	pH optimum	Molar ratio of metal to ligand	Wavelength, nm		Relative molar fluorescence*
			Excitation	Emission	
Al	5·2	—	345	410	0·10
Be	6·4	—	360	405	0·14
Mg	10·9	—	360	440	0·14
Ca	10·9	2:1	360	440	0·21
Sr	10·9	—	360	440	0·16
Ba	10·9	—	360	440	0·15
Zn	7·9	—	360	440	0·01
Cd	7·9	2:1	360	440	0·22
Y	7·9	—	360	430	0·26
La	7·9	2:1	360	430	0·24
Gd	7·9	—	360	430	0·16
Lu	7·9	—	360	430	0·21

* Relative to quinine sulphate.

CONCLUSION

BDDS is shown to be a selective spectrofluorimetric reagent for the determination of cadmium in the presence of zinc. The reagent also forms fluorescent complexes with lanthanum, gadolinium and lutetium, while the complexes of other lanthanons are not fluorescent. The behaviour is similar to that of 1-di(carboxymethylaminoethyl)-2-hydroxy-3-naphthoic acid[8] and there appears to be a close connection between the ability to fluoresce and the occupation of the 4f electron shell in those metals—lanthanum $4f^0$, gadolinium $4f^7$ and lutetium $4f^{14}$. The reagent can be used for the determination of these elements in admixture with other lanthanons if an excess of reagent is used. The phenomenon appears to be general for all metallofluorescent reagents containing the chelating system of phenolic hydroxyl adjacent to a di(carboxymethyl)aminomethyl group.

An advantage of BDDS compared with other such reagents, *e.g.*, BDDN,[9] is its good stability in aqueous solution. It has a lower pH value for optimal fluorescence of its magnesium, calcium, strontium and barium complexes because of the values of the stability constants of the proton complexes, $\log K_{HL} = 12{\cdot}2$ and $\log K_{H_2L} = 9{\cdot}6$, being lower than those for BDDN. The pH 10·9 corresponds to the maximal concentration of the species HL^{5-}. BDDS is, however, less suitable for determination of metal ions in acidic solution than BDDN, as the reagent alone is also fluorescent under these conditions. With respect to the parent compound, 4,4′-diamino-*trans*-stilbene, the substitution of amino groups by hydroxyl groups produces a marked decrease in fluorescence intensity. The BDDS reagent is, however, chiefly remarkable for the fact that it forms a strongly fluorescent cadmium complex and a relatively weakly fluorescing zinc complex. By means of the simple procedure described here it can be used to determine microgram amounts of cadmium in the presence of most other metals except lead.

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REFERENCES

1. Kirkbright, G. F., Rees, D. I., and Stephen, W. I., *Analytica Chim. Acta*, 1962, **27**, 558.
2. Kreingold, S. U., Bozhevolnov, E. A., Lastovski, R. P., and Sidorenko, V. V., *Zh. Analit. Khim.*, 1963, **18**, 1356.
3. Sah, P. P. T., and Anderson, H. H., *J. Clin. Chem. Soc.*, 1946, **13**, 84; *Chem. Abstr.*, 1947, **41**, 5869f.
4. Kirkbright, G. F., West, T. S., and Woodward, C., *Analyt. Chem.*, 1965, **37**, 137.
5. ——, ——, ——, *Talanta*, 1965, **12**, 517.
6. Budesinsky, B., and Haas, K., *Acta Chim. Hung.*, 1963, **39**, 7.
7. ——, ——, *Z. analyt. Chem.*, 1965, **210**, 263.
8. Budesinsky, B., and West, T. S., *Analytica Chim. Acta*, 1968, **42**, 455.
9. ——, ——, *Talanta*, in the press.

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Investigation of a Rapid and Non-destructive Fast-neutron Activation Analysis for Many Elements by Using a Semi-conductor Detector*

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Investigations in the field of fast-neutron activation analysis with a small neutron generator have established the fact that oxygen in various materials can be determined by the rapid, non-destructive method. The present study has been carried out to investigate the applicability of the method with a germanium (lithium) γ-ray detector and data processing by electronic computer, for many other elements. More than sixty elements were activated under the conditions: bombarding time 400 seconds; neutron output 5×10^{10} neutrons second^{-1}; counting time 500 seconds after cooling; cooling time 10 seconds after bombardment, and their sensitivities under these conditions were calculated. According to the experimental results obtained by the mathematical method, many elements, in addition to oxygen, can be determined by this method without chemical separation.

The fast-neutron activation technique, in which a small neutron generator is used, has been widely investigated by many groups of workers,[1,2,3,4,5] who have taken advantage of the fact that activation analysis can be used in laboratories without access to a reactor. The particular difficulties in the determination of oxygen by conventional methods and the high specificity of fast-neutron activation of oxygen under convenient experimental conditions have prompted us to study the rapid and non-destructive method used for the determination of oxygen. This method is widely used for the determination of oxygen in metals, particularly in iron and steel, in routine work in industries in Japan.[6,7]

However, its use for the determination of many elements, except oxygen and a few others such as phosphorus, silicon, aluminium, fluorine, praseodymium and neodymium, has not been reported. One of the major problems currently associated with this method of activation analysis is centred around the lack of resolving power of the conventional γ-ray detectors (for example, sodium iodide and caesium fluoride detectors) and the large numbers of results accumulated in an experiment. These difficulties have been either substantially reduced or eliminated by using a high resolution germanium (lithium) γ-ray detector and processing the results by electronic computer. This study was carried out to investigate the applicability of this rapid fast-neutron activation method to the determination of many elements.

Experimental

Apparatus—

With the use of a neutron generator (Toshiba NT-200), neutrons of 14-MeV energy were obtained by bombarding a tritiated titanium target (prepared by NAIG Co., Japan) with a beam current of 200-keV deuterons. The total neutron output was measured with a silicon surface barrier detector (Toshiba M8811A-50), which was set at a definite position in the extension tube of the generator, the output of which was fed via a 200-channel pulse height analyser (200-CH.P.H.A.) at a multi-scalar mode. The neutron generator produces about 5×10^{10} 14-MeV neutrons per second under ordinary operating conditions.

* Paper presented at the Second SAC Conference 1968, Nottingham.

After the irradiation, samples were rapidly transferred to the standard position near a co-axial type germanium (lithium) detector (ORTEC, Model No. 8102–20, total active volume 22 cm^3) through a pneumatic system. A pre-amplifier (ORTEC 118A) and a linear amplifier (ORTEC 410) were used, the output of which was fed via a 800-channel pulse height analyser (800-CH.P.H.A.).

SAMPLE—

To investigate effectively the feasibility of the fast-neutron activation method for many elements, pure elements or compounds were chosen as target materials; the chemical forms of these materials are shown in Table I. The target materials were packed tightly in the cylindrical polythene rabbit case. To keep the geometrical conditions of neutron bombardment and of measurement of γ-rays constant from sample to sample, all samples in polythene rabbit cases were of the same shape and size, being 12·0 mm in diameter and 10·0 mm in length.

TABLE I

SENSITIVITY

Element	Nuclear reaction	Radio-nuclide produced	Half-life	Energy of emitted γ-ray, keV	Amount of target material, g	Chemical form of target material	Sensitivity, μg per 10 counts
Nitrogen ..	(n,2n)	^{13}N	10·05 minutes	511	0·9945	$(NH_2)_2CO$	11·0
Fluorine.. ..	(n,p)	^{19}O	29·4 seconds	200	1·0497	LiF	13·4
				1336			276
	(n,2n)	^{18}F	112 minutes	511			14·9
Sodium	(n,p)	^{23}Ne	40·2 seconds	436	1·4399	Na_2CO_3	57·93
	(n,α)	^{20}F	10·7 seconds	1640			176·3
Magnesium ..	(n,α)	^{23}Ne	40·2 seconds	440	0·4610	MgO	369
	(n,2n)	^{23}Mg	11·9 seconds	511			760
	(n,p)	^{24}Na	14·97 hours	1370			328
Aluminium ..	(n,p)	^{27}Mg	9·45 minutes	842	3·2069	Al	11·2
				1010			37·3
	(n,α)	^{24}Na	14·97 hours	1370			487
	(n,γ)	^{28}Al	2·27 minutes	1780			1056
Silicon	(n,p)	^{28}Al	2·27 minutes	1780	2·6344	Si	13·22
Phosphorus ..	(n,2n)	^{30}P	2·5 minutes	511	1·6748	$Ca(H_2PO_4)_2$-$.H_2O$	15·98
	(n,α)	^{28}Al	2·27 minutes	1780			11·20
Chlorine.. ..	(n,2n)	^{34m}Cl	32·4 minutes	1160	0·9240	NH_4Cl	3769
				2100			472
Potassium ..	(n,2n)	^{38}K	7·7 minutes	511	1·2577	K_2CO_3	51·75
				2160			506·1
	(n,p)	^{41}A	110 minutes	1290			1990
Scandium ..	(n,2n)	^{44m}Sc	2·44 days	271	0·8702	Sc_2O_3	210
	(n,2n)	^{44}Sc	3·92 hours	511			20·2
Vanadium ..	(n,α)	^{51}Ti	5·79 minutes	323	0·6045	V_2O_5	14·49
Chromium ..	(n,2n)	^{49}Cr	41·9 minutes	511	5·4902	Cr	668
	(n,p)	^{52}V	3·77 minutes	1433			43·5
Manganese ..	(n,α)	^{52}V	3·77 minutes	1433	1·5806	$MnCO_3$	49·0
Iron	(n,p)	^{56}Mn	2·576 hours	845	8·6296	Fe	135
				1810			1280
				2130			2525
	(n,2n)	^{53}Fe	8·9 minutes	511			604
Cobalt	(n,2n)	^{58}Co	71·3 days	511	2·6703	Co_3O_4	2107
	(n,α)	^{56}Mn	2·576 hours	845			372
				1810			5478
				2130			8327
Nickel	(n,2n)	^{57}Ni	36 hours	511	9·8921	Ni	4556
	(n,p)	^{62}Co	13·9 minutes	1170			1653
Copper	(n,2n)	^{62}Cu	9·73 minutes	511	9·6814	Cu	2·208
				1170			1095
Zinc	(n,2n)	^{63}Zn	38·3 minutes	511	4·2880	Zn	18·4
				670			557
				960			1145
	(n,p)	^{66}Cu	5·1 minutes	1040			1080
Gallium	(n,2n)	^{68}Ga	68 minutes	511	7·5465	Ga	5·49
				1078			537
	(n,2n)	^{70}Ga	21·1 minutes	1040			561
Arsenic	(n,p)	^{75m}Ge	48 seconds	139	2·6551	As	78·72
	(n,p)	^{75}Ge	82 minutes	264			1052
	(n,α)	^{72}Ga	14·3 hours	834			7085

TABLE I—*continued*

Element	Nuclear reaction	Radio-nuclide produced	Half-life	Energy of emitted γ-ray, keV	Amount of target material, g	Chemical form of target material	Sensitivity, μg per 10 counts
Selenium ..	(n,2n)	^{77m}Se	17·5 seconds	162	3·1113	Se	14·16
Bromine ..	(n,2n)	^{80}Br	7·6 minutes	620	2·0701	KBr	19·5
Rubidium ..	(n,2n)	^{84m}Rb	23 minutes	239	1·8414	Rb_2CO_3	3·09
				463			10·36
	(n,2n)	^{86m}Rb	1·02 minutes	560			14·91
Strontium ..	(n,2n)	^{87m}Sr	2·80 hours	388	1·9786	$Sr(NO_3)_2$	42·16
Yttrium.. ..	(n,α)	^{86m}Rb	1·02 minutes	560	2·3375	Y_2O_3	979
	(n,n')	^{89m}Y	16 seconds	913			41·7
Zirconium ..	(n,2n)	^{89m}Zr	4·4 minutes	588	2·3808	ZrO_2	15·4
				1530			636
Niobium ..	(n,α)	^{92}Y	3·6 hours	210	7·6653	Nb	877
				480			3021
Molybdenum ..	(n,2n)	^{91m}Mo	66 seconds	511	2·5900	Mo	18·39
				658			1032
Ruthenium ..	(n,p)	^{101}Tc	14·0 minutes	307	0·9526	Ru	150·4
	(n,p)	^{103}Tc	4·5 minutes	470			309·3
	(n,2n)	^{95}Ru	1·65 hours	340			28·2
Silver	(n,2n)	^{106}Ag	24 minutes	511	8·3174	Ag	6·60
	(n,2n)	^{108}Ag	2·3 minutes	620			1060
Cadmium ..	(n,2n)	^{111m}Cd	48·6 minutes	150	5·0000	Cd	13·84
				246			15·59
Indium	(n,2n)	^{112m}In	20·7 minutes	155	1·4683	In_2O_3	138
	(n,α)	^{112}Ag	3·2 hours	618			1263
	(n,2n)	^{114}In	72 seconds	511			153
	(n,2n)	^{115m}In	4·50 hours	335			369
	(n,2n)	^{116m}In	53·99 minutes	406			784
				1085			1134
				1274			726
Tin	(n,2n)	^{123}Sn	39·5 minutes	153	4·1981	Sn	23·4
	(n,2n)	^{111}Sn	35 minutes	511			390·5
Antimony ..	(n,2n)	^{120}Sb	16·4 minutes	511	3·6728	Sb	4·23
				1180			41·2
	(n,2n)	^{122}Sb	3·5 minutes	70			21·0
Iodine	(n,2n)	^{126}I	13·3 days	390	1·5002	KI	2050
				668			3798
Caesium.. ..	(n,2n)	^{132}Cs	6·2 days	670	2·5685	$CsNO_3$	517·4
Barium	(n,2n)	^{137m}Ba	2·6 minutes	662	1·3011	$Ba(OH)_2$-.$8H_2O$	3·20
Cerium	(n,2n)	^{139m}Ce	55 seconds	740	2·9434	CeO_2	8·06
Praseodymium..	(n,2n)	^{140}Pr	3·4 minutes	511	1·1452	Pr_6O_{11}	1·00
	(n,γ)	^{142}Pr	19·2 hours	1570			722
Neodymium ..	(n,2n)	^{141m}Nb	64 seconds	760	1·0176	Nd_2O_3	31·3
Europium ..	(n,α)	^{150}Pm	2·7 hours	340	1·7354	Eu_2O_3	3329
	(n,2n)	^{152m}Eu	9·3 hours	511			3492
				960			4383
Gadolinium ..	(n,2n)	^{159}Gd	18·0 hours	364	1·8672	Gd_2O_3	711·8
Hafnium ..	(n,2n)	^{179m}Hf	19 seconds	215	2·4772	HfO_2	36·06
Gold	(n,2n)	^{196}Au	5·55 days	354	21·265	Au	1432

MEASUREMENT OF THE γ-RAY SPECTRUM—

The samples, in the chemical forms shown in Table I, were activated under the fixed experimental conditions and the activities measured by using the measuring system shown in Fig. 1. The efficiency of the separation of photopeaks in a γ-ray spectrum and intensities of radioactivities in photopeaks were investigated.

The rabbit case containing the target material was transferred through a pneumatic system, which was constructed with polythene tubing, with a 600-watt tandem-type blower motor. When the rabbit case arrived at the bombarding area, neutron bombardment of the sample was automatically started by the deuteron beam shutter, which was set in motion by the signal from the sample arrival indicator; at the same time, the 200-channel pulse height analyser (multi-scalar mode, 2 seconds per channel) by which the neutron output was recorded and the scalar timer for the beam shutter were also started by this signal.

After a lapse of 400 seconds from the start of neutron bombardment, the rabbit case containing the target material was driven back from the bombardment area to the counting

system and, at the same time, a pre-set timer for the cooling period was started; 10 seconds after the end of bombardment, the 800-channel pulse height analyser was started by the pre-set timer and the γ-rays emitted from the sample were measured during 500 seconds.

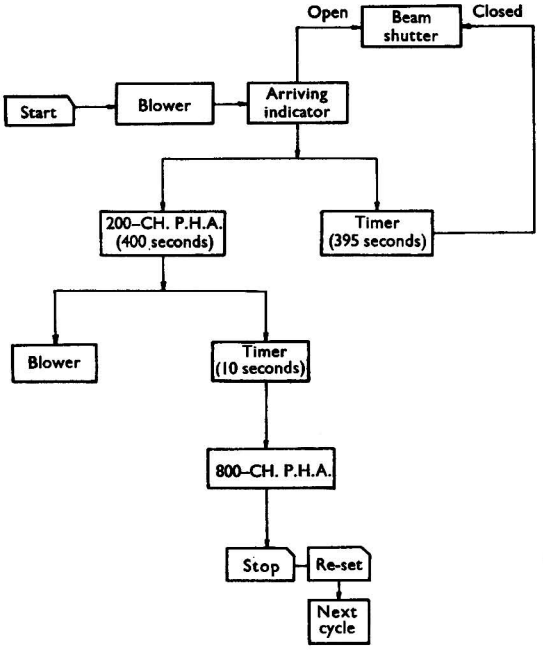

Fig. 1. Automatic γ-ray measuring system

TREATMENT OF RESULTS—

One of the authors has developed a new mathematical method[8,9] for the analysis of complex γ-ray spectra and prepared a computer code for the automatic reduction of results. The computer code includes calculations of all of the necessary corrections for neutron-output fluctuations. With this computer code, each complex spectrum can be processed within 10 seconds by using a GE-635 computer.

The main mathematical procedures used in this method are as follows: smoothing of the spectra; determination of the background distribution and subtraction of this component from the smoothed spectrum; and sorting of the peaks.

Smoothing of the spectra—For smoothing the spectra, the Fourier method of analysis is used. The 800-channel pulse height distribution is converted into the Fourier transformed space, and a Gaussian-type filter function is multiplied to cut off the higher frequency range. This modulated frequency distribution is then inversely transformed into the original energy space. An example of a smoothed spectrum obtained, the original results for which are shown in Fig. 2 (*a*), is given in Fig. 2 (*b*).

Background subtraction—It is assumed that the background distribution is a very slowly varying function that connects almost all of the minima of the spectrum. In this calculation, a criterion is used to avoid identifying genuine troughs between peaks as points belonging to the background distribution. For this purpose, if a smoothed spectrum has successively located minima at E_1 and E_{i+1}, then the slope of the line that connects these adjacent minima is checked. If this value is higher than that given in the input data for this comparison, then E_{i+1} is not taken into account for the points that construct the background distribution.

By determining the background distribution by this method and subtracting this component from the smoothed spectrum, the background-subtracted distribution, g(E), is obtained. Fig. 2 (*c*) shows the spectrum without background thus obtained, the original results for which are shown in Fig. 2 (*a*).

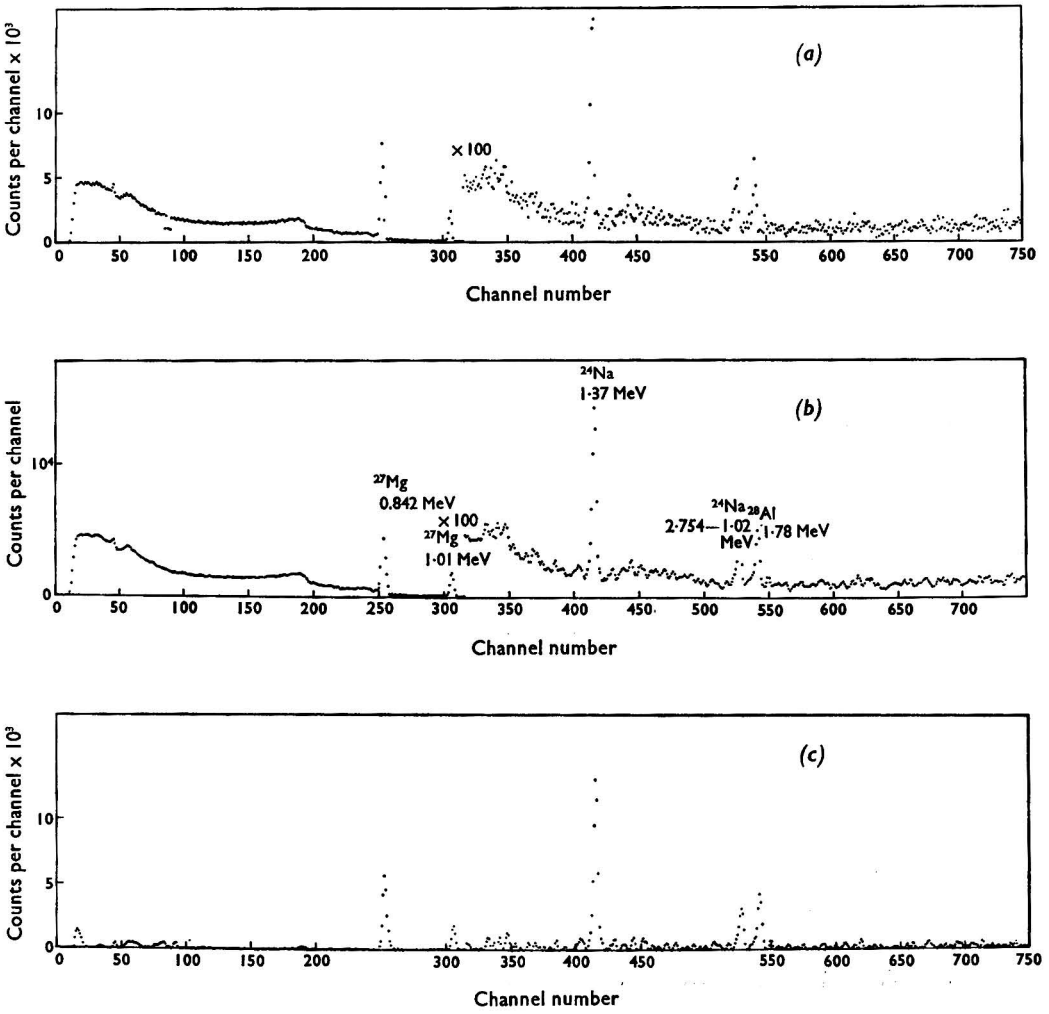

Fig. 2. γ-Ray spectra for aluminium, with germanium (lithium) detector, co-axial type, 22 cm³, and energy scale 3·26 keV per channel: (*a*), original results; (*b*), smoothed results; and (*c*) background-subtracted results

Peak sorting—Each peak is separated by locating a connected region where the background-subtracted distribution, g(E), has a positive value. The centre position of the peaks is determined by locating the part where the slope of g(E) is zero. The area under the peak is simply calculated by integrating the area between adjacent zero points of g(E). If the peak has several points where the curve has a downwards convex shape, then the number of components of the peak is examined up to the 3-fold instance. The centre positions and the areas of these multiplets are determined by locating also the slope zero positions, and the whole peak is constructed by superimposing Gaussian functions of standard peak widths at these energies.

The computer code applies the correction for the neutron-generator output by calculating the value given by the following equation—

$$J(\lambda) = (\omega/\lambda)\ \{1 - \exp(-\lambda T)\}/\int_0^T \phi(t) \exp\{-\lambda (T - t)\}\, dt \quad .. \quad .. \quad (1)$$

where ω is the neutron flux under the standard conditions, $\phi(t)$ the monitored neutron flux fluctuation, λ the decay constant of radionuclides produced by the neutron bombardment

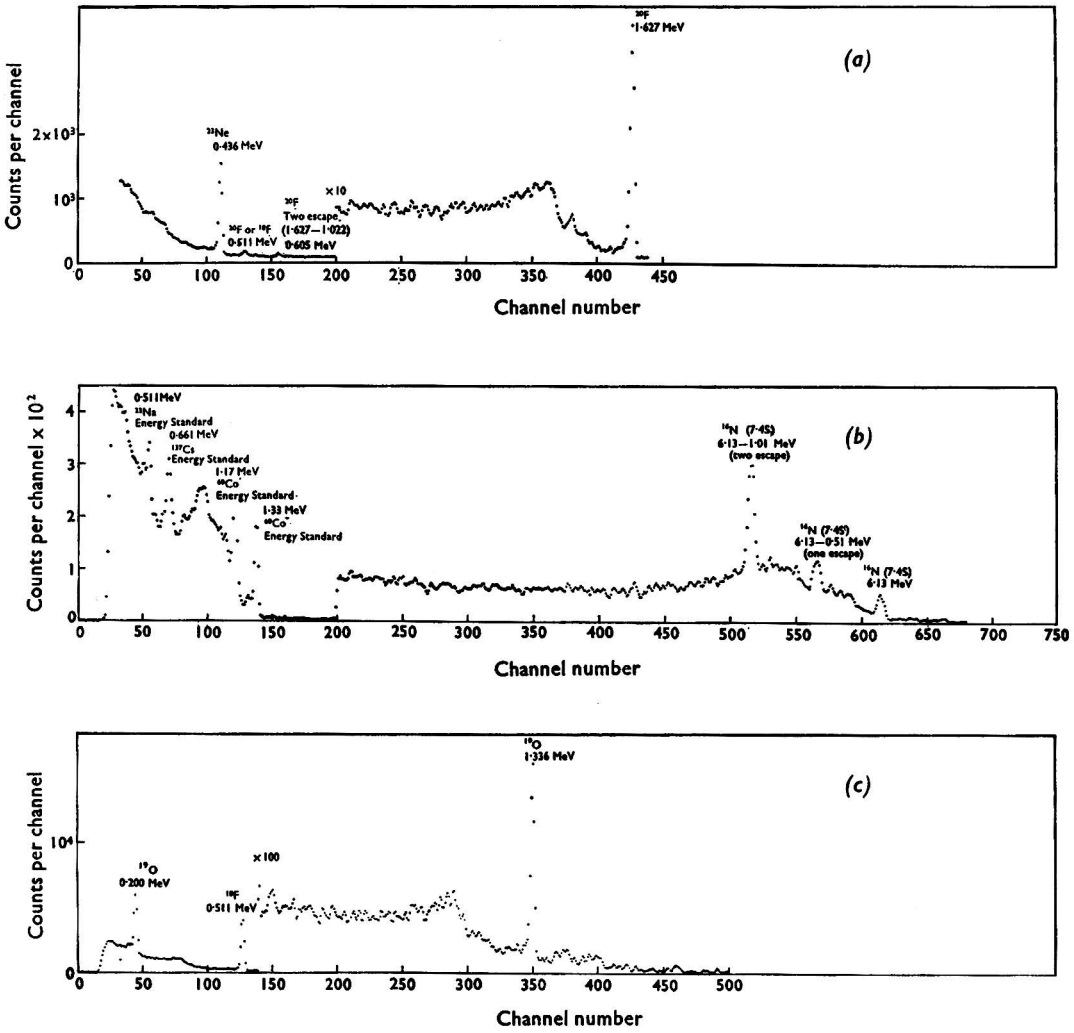

Fig. 3. γ-Ray spectra (smoothed): (*a*), for sodium carbonate with germanium (lithium) detector, co-axial type, 22 cm³, and energy scale 3·77 keV per channel; (*b*), for calcium carbonate with germanium (lithium) detector, co-axial type, 22 cm³, and energy scale 10·0 keV per channel; and (*c*), for lithium fluoride with germanium (lithium) detector, co-axial type, 22 cm³, and energy scale 3·73 keV per channel

and T the bombardment time. This programme prints out the energy, channel number, total counts per peak and normalised counts obtained by multiplying the value given by equation (1).

Results

The smoothed spectra for sodium, oxygen and fluorine are shown in Fig. 3. The resolving power of the detection system used in our experiments was 5·9 keV (fwhm) at 661 keV, and this value was excellent in comparison with that of the sodium iodide (thallium) detection system.

The area of the photopeaks, under the normalised conditions, in the spectra of many elements was determined by dividing the area of the photopeaks obtained in peak sorting by the value of $J(\lambda)$ in equation (1). The normalised conditions used were as follows: bombarding time 400 seconds; neutron output 5×10^{10} neutrons per second; counting time 500 seconds from the end of cooling; and cooling period 10 seconds from the end of bombardment. The

normalised results are shown in Fig. 4, with the energies of the γ-rays emitted by the radionuclides produced.

For several elements, such as lanthanum, samarium, terbium, dysprosium, holmium, erbium, thulium, ytterbium, lutetium, germanium, rhodium, iridium, osmium, rhenium, tellurium, tungsten, lead, platinum and palladium, the assignment of photopeaks in their γ-ray spectra was difficult.

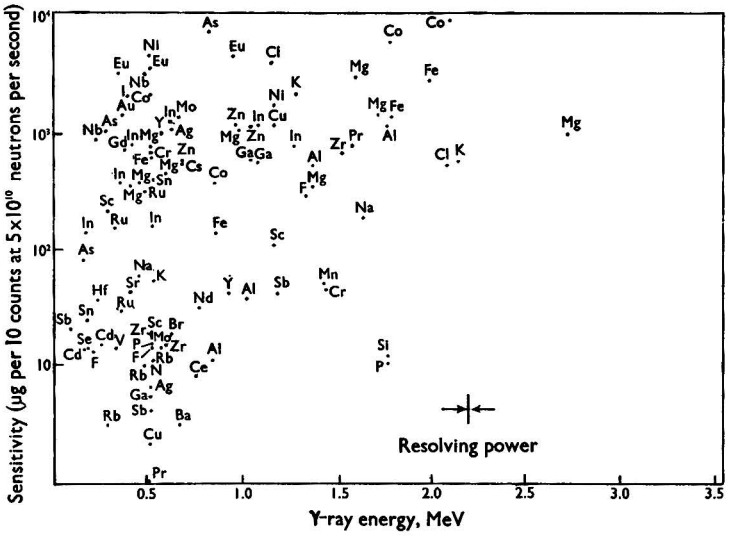

Fig. 4. Sensitivity

Under the conditions used in our experiments, elements could be classified into three groups from the viewpoint of sensitivity as follows: the first group (less than 10 μg per 10 counts), copper, gallium, rubidium, silver, antimony, barium, cerium and praseodymium; the second group (10 to 100 μg per 10 counts), nitrogen, fluorine, sodium, aluminium, silicon, phosphorus, potassium, scandium, vanadium, chromium, manganese, zinc, arsenic, selenium, bromine, strontium, yttrium, zirconium, molybdenum, ruthenium, cadmium, tin, neodymium and hafnium; and the third group (100 to 1000 μg per 10 counts), magnesium, chlorine, iron, cobalt, nickel, niobium, indium, iodine, caesium, europium, gadolinium and gold.

For the elements lithium, boron, carbon, sulphur, calcium, bismuth and rare earth elements, except cerium, praseodymium, neodymium, gadolinium and europium, no prominent γ-ray photopeaks available for the activation analysis were found under our experimental conditions.

Discussion

As the resolving power of a germanium (lithium) detector is much superior to that of a sodium iodide (thallium) detector, it is clear that many elements can be determined non-destructively by the use of the former detector. All of the elements shown in Fig. 4 produced radionuclides available for the activation analysis, but some elements gave nuclides emitting γ-rays with identical energy, for instance, annihilation γ-rays, or with slightly different energy. In such an instance adjustment of the bombarding and cooling times may decrease the interference.

When the semi-conductor detector is used, the two problems of counting efficiency and resolving power arise. The main amplifier (ORTEC, Model 410) has integrating and differentiating circuits, the time constants of which are variable. It has also a double-delay line cripping circuit for pulse shaping. According to our experimental results, to obtain a good resolving power it was necessary to use the time constant of more than 2 μseconds or the delay line. It was found preferable to use the latter because both the energy-resolving power and the counting efficiency were greater, even with higher counting rates, by using the delay line.

The maximum resolving power obtained was 0·91 per cent. (fwhm) for γ-rays of 661 keV at a counting rate of about 1000 counts per second. The maximum allowable counting rate of the input pulses was thought to be about 10,000 counts per second as the resolving power of the detector used at 10,000 counts per second became more than twice that at 1000 counts per second, as shown in Fig. 5.

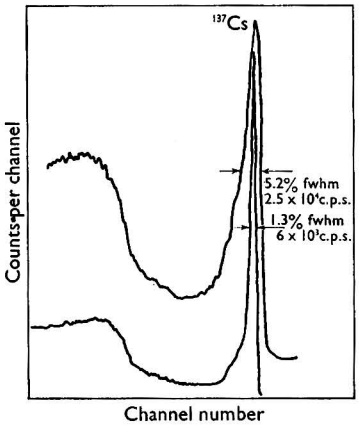

Fig. 5. γ-Ray spectrum

Counting rate, counts per second	Full width half maximum (fwhm), per cent.	Gain (channel number)
$2{\cdot}5 \times 10^4$	5·2	378
$1{\cdot}1 \times 10^4$	2·4	381
$7{\cdot}9 \times 10^3$	1·7	382
$5{\cdot}9 \times 10^3$	1·3	383
$3{\cdot}1 \times 10^3$	1·04	383
$1{\cdot}1 \times 10^3$	0·91	383

Liquid nitrogen for detector cooling was readily supplied at least every 2 weeks.

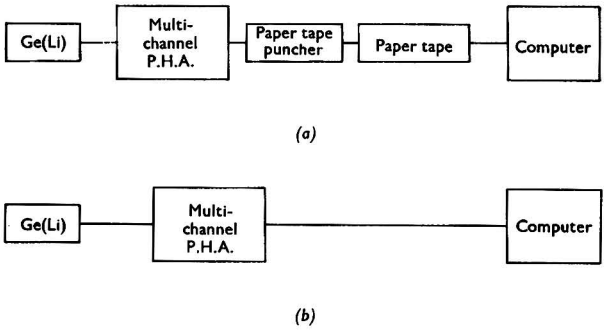

Fig. 6. Methods for connecting a germanium (lithium) detector system to an electronic computer

Some methods for connecting a germanium (lithium) detector system to an electronic computer were considered, as shown in Fig. 6 (*a*) and (*b*). In our experiments, the computer was used as an off-line system, as shown in Fig. 6 (*a*). However, in future experiments it will be used as an on-line system as in Fig. 6 (*b*). In the arrangement shown in Fig. 6 (*a*), the work of tape punching is troublesome and time consuming. In that of 6 (*b*) the resolving

time of the measuring system is determined by that of the multi-channel pulse height analyser and is shorter than when using a separate analog-to-digital converter and a computer, unless the latter is especially designed to reduce the resolving time. Also in Fig. 6 (*b*), the multi-channel pulse height analyser memory is connected to the computer memory through the interface, and the analyser and computer are used for data accumulation and handling, respectively. Programmes for data accumulation are thus eliminated.

In the fast-neutron activation method for determining oxygen, γ-rays emitted from ^{16}N produced by the ^{16}O (n,p) ^{16}N reaction have a much higher energy than those emitted from almost any of the other radionuclides. Therefore, as is well known, the matrix effect could be eliminated by the pulse height analysis, although the resolving power of the conventional type of γ-ray detector was poor. On the other hand, it is clear that the separation of photopeaks of ^{16}N from the others in the spectrum obtained with the germanium (lithium) detector is more satisfactory than in that obtained with conventional detectors. However, the efficiency of the germanium (lithium) detector is not sufficient to keep the sensitivity practical unless a large volume detector is available. Consequently, it is concluded that, at the present stage, the sodium iodide (thallium) detector is more suitable than the germanium (lithium) detector for the determination of oxygen by the activation method.

Conclusion

A quality control system consists of the successive collection and handling of data. However, the time required for the former is generally much longer than for the latter. Therefore, from an industrial point of view, a rapid, non-destructive data collection system must be developed. The method described here is an attempt to meet this requirement.

Activation analysis is one of the most useful methods in the field of rapid, non-destructive testing, but it has long been recognised as a very sensitive technique for assaying certain elements. However, our efforts have been directed towards the development of a new technique of fast-neutron activation analysis, and have included the use of a rapid, non-destructive method with an electronic computer, a small neutron generator and a germanium (lithium) detector system for determining macro amounts of many elements in a sample. This new method has many applications in the field of industrial quality control.

References

1. Coleman, R. F., and Perkin, J. L., *Analyst*, 1959, **84**, 233.
2. Veal, D. J., and Cook, C. F., *Analyt. Chem.*, 1962, **34**, 178.
3. Steel, E. L., and Meinke, W. W., *Ibid.*, 1962, **34**, 185.
4. Fujii, I., Muto, H., Ogawa, K., and Tani, A., *J. Atom. Energy Soc. Japan*, 1963, **5**, 455.
5. Fujii, I., Muto, H., and Miyoshi, K., *Japan Analyst*, 1964, **13**, 249.
6. Fujii, I., Miyoshi, K., Muto, H., and Shimura, K., *Analytica Chim. Acta*, 1966, **34**, 146.
7. Fujii, I., and Muto, H., *Ibid.*, 1967, **39**, 329.
8. Inouye, T., and Rasmussen, N. C., *Trans. Amer. Nucl. Soc.*, 1967, **10**, No. 1.
9. Inouye, T., Harper, T., and Rasmussen, N. C., *Nucl. Instrum. Meth.*, in the press.

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Trace Determination of Mercury, Thallium and Gold with Crystal Violet

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A spectrophotometric method for the determination of small amounts of mercury, thallium and gold is presented. The sample is wet ashed with a nitric acid - hydrochloric acid mixture, and interferences are eliminated by adding ethylene glycol monomethyl ether and EDTA. Thallium interference is eliminated by adding sodium metabisulphite for mercury and gold, whereas excess oxidant interference is eliminated by adding hydroxylamine for thallium and gold. Because gold interferes in the determination of thallium and mercury, it is determined separately after heating the sample to volatilise the mercury. Iodide forms complexes with mercury and gold, whereas bromide forms complexes with thallium and gold. Crystal violet produces a toluene-extractable compound with these complexes in an acidic medium. A single extraction step suffices to determine 0·1 μg of each element in a 1-cm cell with a standard spectrophotometer at 605 nm; Beer's law is followed up to absorbance of unity. The method has been tested with urine, air, vegetation, water, soil, rocks and sediments.

SMALL mercury contents in biological and other natural products are a useful guide for locating sources of contamination, for health surveys[1,2] and for geochemical prospecting.[3,4] Available micro methods for mercury, gold and thallium are generally time consuming, and small amounts in biological material cannot be determined without pre-treatment with instrumental techniques. Small amounts of mercury in inorganic samples, for example, can be determined by atomic absorption,[5] but other techniques applied to any of these elements[6,7] generally involve many steps or bulky experiment that cannot be applied successfully in the field.

Crystal violet has been described as a useful reagent for the colorimetric determination of gold,[8] thallium[6,9] and mercury.[10] The procedure described below has been developed by working out the most favourable conditions for colour development by the interferences encountered in the method for mercury in urine and air.[10]

Analytical procedure for thallium and mercury

Reagents—

All reagents were of analytical-reagent grade.

Hydrochloric acid, 6 N—Mix equal volumes of hydrochloric acid (sp.gr. 1·18) and water. Add 2 per cent. of $KCr(SO_4)_2.12H_2O$ and 1 per cent. of $FeCl_3.6H_2O$.

Hydrogen peroxide, 30 *and* 50 *per cent.*

Nitric acid, 10 N—Mix one volume of nitric acid (sp.gr. 1·40) with 0·5 volume of water, and add 1 per cent. of chromium trioxide.

Potassium permanganate, 5 *per cent.*—Dissolve 50 g of potassium permanganate in 1 litre of water.

Hydrochloric acid, 5 and 2 N.

Nitric acid—Fuming (sp.gr. 1·48) and concentrated (sp.gr. 1·40).

Chromium - iron solution—Dissolve 3 g of CrO_3 and 2 g of iron(III) chloride in a minimum amount of water and dilute to 5 ml.

Acid mixture—Mix 40 ml of hydrochloric acid (sp.gr. 1·18), 10 ml of nitric acid (sp.gr. 1·40) and 1 g of chromium trioxide and dilute to 100 ml.

Mercury standard solution—Dissolve 179 mg of mercury(II) bromide in 150 ml of water. Add 1 ml of 5 N hydrochloric acid and make up to 200 ml (1 ml ≡ 0·05 mg of mercury). Dilute 1 ml to 50 ml before use (1 ml ≡ 10 μg of mercury).

Thallium standard solution—Dissolve 124 mg of thallium sulphate in 150 ml of water and dilute to 200 ml (1 ml ≡ 0·5 mg of thallium). Dilute 1 ml to 50 ml before use (1 ml ≡ 10 μg of thallium).

Gold standard solution—Dissolve 100 mg of gold (powder) in 3 ml of hydrochloric acid (sp.gr. 1·18) and 1 ml of nitric acid (sp.gr. 1·40) by warming. Add 10 ml of water, boil to remove the brown vapours, then add 0·2 g of sodium bromide, 50 mg of potassium bromate and 20 ml of 5 N hydrochloric acid, and make up to 200 ml (1 ml ≡ 0·5 mg of gold). Dilute 1 ml to 50 ml before use (1 ml ≡ 10 μg of gold).

Toluene, sulphur free.

Sodium metabisulphite, $Na_2S_2O_5$, 20 per cent. solution.

Potassium iodide, 2·5 per cent. solution.

Sodium bromide, 5 per cent. solution.

Hydroxylammonium chloride, 10 per cent. solution.

Ethylenediaminetetra-acetic acid, disodium salt, 5 per cent. solution.

Crystal violet, 0·1 and 1 per cent. solutions—Dissolve 1 g of hexamethylpararosaniline (Crystal violet) in 100 ml of ethylene glycol monomethyl ether. This 1 per cent. solution can be used to prepare the 0·1 per cent. solution by diluting 10 ml with 90 ml of water.

Absorbing solution, 0·015 M—Solution A. Dissolve 1·12 g of sodium iodide, 0·83 g of potassium bromate and 2·6 g of sodium bromide in 300 ml of water and make up to 500 ml with ethylene glycol monomethyl ether. Mix equal volumes of solution A and 2N hydrochloric acid before use.

ORGANIC SAMPLE PREPARATION—

Satisfactory techniques for the wet ashing of organic samples and urine have been described by Sandell,[6] and Miller and Swanberg,[11] respectively. The sample is digested with 10 per cent. of 6 N hydrochloric acid, containing chromium and iron as catalysts, and is refluxed; the digestion is completed by the addition of 50 per cent. hydrogen peroxide. The overnight oxidation of urine with acidified permanganate was proposed by Monkman, Moffet and Doherty[2] and Jacobs and Singerman.[12] The acid-reflux method for vegetation described by Ward and McHugh[13] has been adapted for the present method by neutralising some of the acid. In principle, the combustion-flask technique can also be applied to recover the volatile elements.[14] The results shown have been obtained with the following method.

Cut the vegetation into about 3-cm pieces. Fresh samples can be used, but without the roots. Alternatively, whole plants are washed with distilled water and dried in an oven at 50° C. Weigh 10 g of fresh material or 5 g of dried sample into a 250-ml covered beaker and add 15 ml of 10 N nitric acid with 1 per cent. of chromium trioxide and simmer for 1 hour. Add 15 ml of water and filter by suction, washing sparingly. Add 40 ml of 5 per cent. potassium permanganate to the filtrate and bring to the boil, then simmer for 30 minutes. Add 30 per cent. hydrogen peroxide to dissolve the manganese dioxide and 1 ml in excess, then simmer for 1 hour. Compare the colour of the digest with a blank prepared from the reagents. Add a further 1 ml of hydrogen peroxide if the colour of the digest is yellowish, and repeat the simmering. Transfer to a 100-ml graduated flask, add 10 ml of 5 N hydrochloric acid and make up to the mark with water, then transfer 50-ml portions into two separating funnels.

ALTERNATIVE RAPID WET-ASHING METHOD—

The sample is prepared as in the previous method.

Add 10 ml of fuming nitric acid (sp.gr. 1·48) to the sample and heat for about 1 to 2 minutes in a water-bath until the mass liquifies. Add 50 per cent. hydrogen peroxide, dropwise, until the liquid becomes colourless. Observe the evolution of brown gases and adjust the addition of hydrogen peroxide as required. Then add 1 drop of chromium - iron solution and simmer for 5 minutes. Dilute with 30 ml of water, filter and wash the precipitate. Transfer the filtrates to a 100-ml graduated flask and fill to the mark with water, then transfer 50-ml portions into two separating funnels.

INORGANIC SAMPLE PREPARATION—

Silicates are usually analysed for adsorbed or easily dissolved metals. If lattice-bound elements are required to be determined, general methods with hydrofluoric - nitric - sulphuric (perchloric) acids mixtures can be used. In this study concentrated acid leachings were

prepared as follows: grind air-dried rocks, soils or sediments and sift through a 80 to 100-mesh nylon screen; digest 10 g with 10 ml of acid mixture for 5 to 10 minutes in a boiling water bath, then add 30 ml of water, filter by suction, wash the residue and transfer the filtrate to a 100-ml graduated flask, and make up to the mark with water, then transfer 50-ml portions into two separating funnels.

PREPARATION OF SOILS OF HIGH ORGANIC CONTENT—

Digest for 5 to 10 minutes with 10 ml of acid mixture in a loosely stoppered Erlenmeyer flask contained in a boiling water bath. Add 5 ml of nitric acid (sp.gr. 1·40) and leave in the water-bath for a further 15 to 20 minutes. Add 30 ml of water, filter by suction, wash the residue and transfer the filtrate to a 100-ml graduated flask, and make up to the mark with water. Transfer 50-ml portions into two separating funnels.

ANALYTICAL PROCEDURE FOR GOLD

PREPARATION OF SAMPLES—

Dry ash the sample in a crucible at 550° to 650° C until no carbon particles or dark zones are visible. Weigh the ashes to relate the amounts of the other elements to ash content. Treat the ashes with nitric acid, heat in a water-bath and filter as described previously for silicates, but use half the amount of reagents. Make the volume of the filtrates up to about 50 ml and transfer to a separating funnel.

METHOD OF ANALYSIS

CALIBRATION GRAPHS—

Construct a calibration graph for each of the three elements by spiking a blank with increasing amounts of mercury, thallium or gold standard solution. Always use the same volume of aqueous solution and toluene when constructing the calibration graphs and when determining the concentration of the sample solution (because of partition effects).

ANALYSIS OF SAMPLE SOLUTION—

The three separating funnels contain mercury, thallium and gold. Add 1 ml of a 2·5 per cent. potassium iodide solution to the mercury and gold. Reduce the free iodine by adding 20 per cent. sodium metabisulphite solution, dropwise, then add 5 drops in excess. Add 1 ml of the 5 per cent. sodium bromide solution to the separating funnel containing thallium. Reduce the free bromine by adding, dropwise, a 10 per cent. solution of hydroxylammonium chloride and then 5 drops in excess. Add to the three funnels 2 ml of a 5 per cent. disodium EDTA solution, and mix well. Add 5 ml of a 0·1 per cent. Crystal violet solution in ethylene glycol monomethyl ether and mix. The colour of the solution should become green. If it is blue or dark green, add 2 to 5 ml of 5 N hydrochloric acid, then add 5 ml of toluene and shake gently ten times, repeating the shaking after the coalescence of the drops. After total coalescence transfer the toluene extract to a folded 7-cm No. 40 Whatman filter-paper and collect the filtrate in a test-tube or directly in a spectrophotometric cell. Take the reading within 20 minutes at a wavelength of 605 nm. Subtract the absorbance value of gold from that of mercury and of thallium and compare with a standard curve. If results are outside the given range, then the filtered solution can be diluted with toluene to bring them within the range.

ANALYSIS OF NATURAL WATER FOR THALLIUM AND GOLD—

Add 10 per cent. v/v of 5 N hydrochloric acid and 1 per cent. of 5 per cent. potassium permanganate when collecting the sample at the spring or well. Add 1 per cent. of a 2·5 per cent. potassium iodide solution for mercury or add 1 per cent. of a 5 per cent. sodium bromide solution for thallium, then add 1 per cent. of 20 per cent. sodium metabisulphite solution to reduce the free iodine, or add 2 per cent. of 10 per cent. hydroxylammonium chloride solution to reduce the free bromine. Transfer 570 ml (equivalent to 500 ml of original sample) to a 1-litre funnel. Add 40 ml of 0·1 per cent. Crystal violet solution in ethylene glycol monomethyl ether, then swirl and add 5 or 10 ml of toluene and shake it gently 10 times, then wait until the droplets start to coalesce before repeating the procedure twice. Separate the toluene extract, filter and measure on the spectrophotometer as described in Analysis of sample solution.

ANALYSIS OF AIR AND GASES FOR MERCURY—

Flush air, which has been filtered through a glass-fibre filter, at a rate of 1 to 2 litres per minute through a midget impinger with 10 ml of absorbing solution; 10 to 50 litres are usually necessary. Add 5 drops of 20 per cent. sodium metabisulphite solution and transfer to a 50-ml separating funnel. Add 4 drops of 1 per cent. Crystal violet solution in ethylene glycol monomethyl ether and extract with 5 ml of toluene. Separate the toluene, filter and proceed as described in Analysis of sample solution.

RESULTS

Although no significant losses occurred when the acids were simmered in a covered beaker, boiling of the acidic solutions was, however, avoided.

The filtered, dry toluene extract was stable for about 30 minutes.

TABLE I

RECOVERY OF THALLIUM AND MERCURY FROM NATURAL SAMPLES

Mercury present, μg	Thallium present, μg	Mercury added, μg	Thallium added, μg	Mercury found, μg	Thallium found, μg	Recovered, per cent.
10·5*	—	—	—	10·9	—	104
7·0	—	3·0	—	10·0	—	100
1·0	1·0	1·0	—	2·0	—	100
0·53	—	0·33	—	0·83	—	96
0·35	1·0	—	1·0	0·35	—	100
0·35	1·0	1·0	—	1·35	—	100
0·73†	0·22	0·2	—	0·91	—	98
0·73‡	0·22	—	0·2	—	0·32	76
0·35	1·0	—	1·0	—	2·10	105
0·20	0·53	—	1·0	—	1·56	102
1·0	1·0	—	2·0	—	2·9	96
0·44§	0·35‖	—	—	0·39‖	—	89

* 10 μg of gold added.
† The manganese dioxide was filtered off.
‡ The manganese dioxide was filtered off. Note adsorption of thallium.
§ By neutron activation,[17] mean of twenty-three values.
‖ Present method, mean of four values.

TABLE II

RECOVERY OF MERCURY FROM 100 ml OF SPIKED URINE

Mercury added, μg	Mercury recovered, μg	Per cent.
1	0·73	73
3	2·4	80
4	3·5	88
5	4·2	84
8	7·4	93
10	9·5	95
12	11·0	92
15	14·4	96
20	18·0	90

TABLE III

ANALYSES OF NATURAL PRODUCTS

Product	Mercury, p.p.m.	Thallium, p.p.m.	Gold, p.p.m.
Serpentinite from Napa Co., California (0·49 per cent. Ni)	0·00	0·00	0·08
Andesitic soil, Sonoma Co., California	0·25	0·00	0·04
Vein quartz from Morgan Hill, California	0·88	0·00	0·08
Silica - carbonate soil from Mercuryville, California	8·0	2·7	0·02
Kale sample, dry	0·15	0·14	0·002*
Water from Mercuryville, California	5×10^{-3}	14×10^{-3}	—
Laboratory air	0·3 to 3 μg per m³ of mercury		

* Neutron activation.[17]

The recovery of added mercury and thallium is shown in Table I; the recovery of mercury added to urine in Table II; and the analysis of natural products in Table III.

The glass-fibre filter for air analysis was necessary to remove the dust and cinnabar particles. Less than 0·01 μg of mercury passed through the filter from a 20-litre cinnabar dust laden air sample. The absorption efficiency for the midget impinger and the absorbing solution was from 99·0 to 99·5 per cent. for a 2 litre per minute air stream containing 30 to 300 μg per m^3 of mercury vapour.

The accuracy of the procedures depended on the amount of sample, the manipulator's skill and the instrument used. The relative error observed with a Beckman D spectrophotometer ranged from 3 to 10 per cent. when determining 10 to 0·5 μg of element in 10 ml of solution.

The calibration graphs were linear up to absorbance of unity and passed through the origin. For a ratio of toluene to solution of 1 : 20 the molar absorptivity for mercury was 55 $\times$ 10^3; for a ratio of 1 : 2 the molar absorptivity for mercury was 62 $\times$ 10^3, and for gold and thallium 70 $\times$ 10^3.

Discussion

Because of the remote possibility of encountering gold (the earth's crust abundance is 0·001 to 0·02 p.p.m.), the correction in the analysis for mercury and thallium can be neglected in most determinations. Gold can be removed by tellurium co-precipitation directly from the metabisulphite - iodide medium without affecting the mercury results (observe first line, Table I), or from the bromide medium by adding telluric acid and tin(II) chloride.[6] In this instance, after filtration of the precipitate, bromine water must be added in order to re-oxidise Tl^+ to Tl^{3+} and Sn^{2+} to Sn^{4+}. Gold can also be separated by copper powder cementation,[8] but the technique of running a separate dry-ashed sample required less time than any other analytical procedure. An approximate determination of gold can be carried out, however, by adding sulphite to a bromide medium. After 10 seconds to 3 minutes, the dye is added and the gold complex extracted. Under these conditions thallium is reduced about 200 times more rapidly than gold and 5 to 20 times as much thallium gives only a 10 per cent. error. This estimate may have useful applications in geochemical analysis, when low concentrations of thallium are present. Because of the volatility of mercury, the sample solution cannot be concentrated by evaporation. However, Merodio,[15] studying separation of mercury by distillation, found that no mercury was lost from oxidising acid mixtures unless halogens were present.

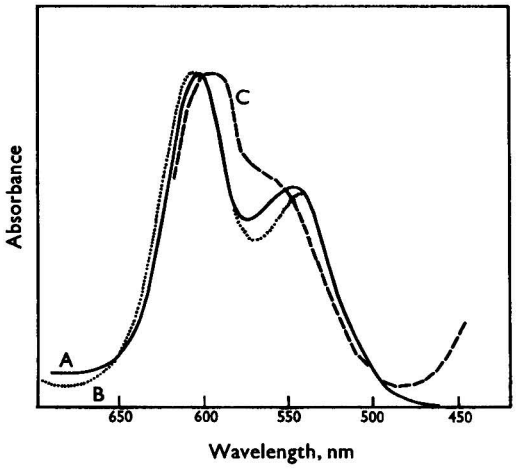

Fig. 1. The spectra of Crystal violet complexes: curve A, mercury *plus* thallium; curve B, gold; and curve C, organic

Some organic and soil samples high in organic compounds may give residual products that interfere seriously (Fig. 1). In this case additional concentrated nitric acid and hydrogen peroxide and a longer digestion time are needed. The addition of catalysts such as chromium and iron seems to hasten the destruction of nitrogenated compounds.

Sublimation of mercury from soils high in organic compounds by pyrolysis has given low recoveries. This was found to be caused by the trapping of mercury by tarry or sulphur-containing pyrogens.

The wet-ashing procedure recommended by Down and Gorsuch[16] may have some merit for quick wet ashing of thallium-containing substances. Gold and cinnabar have been tested on a macro scale, but were not dissolved unless a small amount of chloride was added. Because the wet-ashing method is still a time-limiting factor, an improvement in this sense will be welcome.

Small amounts of other elements do not interfere in the analytical method presented. A detailed discussion has been presented earlier.[10] Iodine interferes in the bromide medium giving a different hue. This hue given by free iodine in toluene can be eliminated by careful addition of hydrazine and shaking. However, an excess of hydrazine slowly destroys the gold and thallium complexes; therefore, a quick manipulation is necessary in order to read the absorbance before fading occurs. If molybdenum or tungsten give visible precipitates in the aqueous layer, they may absorb large amounts of the complexes of mercury, thallium or gold. Not more than 100 mg per litre of molybdenum and 10 mg per litre of tungsten can be tolerated. High concentrations of antimony, arsenic, bismuth and cadmium interfere in the presence of iodide by giving insoluble complexes that adsorb some mercury. Thirty milligrams per litre of antimony or arsenic and 50 mg per litre of bismuth or cadmium can be tolerated in the 0·5 N hydrochloric acid solution. Copper and cobalt have been found to give coloured colloidal precipitates that remain in suspension in the toluene layer, thus making the quantitative determination of mercury difficult, but filtration of the toluene eliminates this source of error. Insufficient sodium metabisulphite is a cause of heavy precipitates and highly coloured extracts, generally produced by formation of complexes of $(ICl_4)^-$, $(FeCl_4)^-$ and other elements (vanadium, manganese) with Crystal violet, which form during violent shaking. However, not more than 0·5 per cent. of sodium metabisulphite in excess should be added, because for every 1 per cent. in excess results are 5 to 10 per cent. lower. Thiocyanate and sulphide interfere seriously with zinc, molybdenum and other metals, because of the formation of a toluene-soluble complex or by removing metals as sulphides. Tin(II) is an interference that is destroyed during the preparation of the sample, but it may be introduced accidentally for gold *plus* tellurium precipitation.

The addition of ethylene glycol monomethyl ether acts as a coagulant of colloidal precipitates of cobalt, copper, bismuth and molybdenum, thus de-sorbing the complexes of interest. It also breaks emulsions.

To prevent contamination, only acid-washed glassware is recommended for the procedure.

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References

1. Joselow, M. M., Goldwater, L. J., and Weinberg, S. B., *Arch Envir. Hlth*, 1967, **15**, 64.
2. Monkman, J. L., Moffett, P. A., and Doherty, T. F., *Amer. Ind. Hyg. Ass. J.*, 1956, **17**, 418.
3. Warren, H. V., Delavault, R. E., Barasko, John, *Econ. Geol.*, 1966, **61**, 1010.
4. Williston, S. H., *Engng Min. J.*, 1964, **165**, 98.
5. Vaughn, W. W., U.S. Geological Survey Report 540, 1965, p. 8.
6. Sandell, E. B., "Colorimetric Determination of Traces of Metals," Third Edition, Interscience Publishers, New York and London, 1959.
7. Ehmann, W. D., *Geochim. Cosmochim. Acta*, 1967, **31**, 357.
8. Panchev, B., *Bulg. Akad. Nauk, Izv. Geol. Inst., Sof.*, 1965, **14**, 231.
9. Perrin, D. D., "Organic Complexing Reagents," Interscience Publishers, a division of John Wiley & Sons, New York, 1964.
10. Kothny, E. L., "A Micromethod for Mercury," Paper presented at the Society of Applied Spectroscopy, American Chemical Society, Anaheim, California, October–November, 1967, to be published.
11. Miller, V. L., and Swanberg, Frank, jun., *Analyt. Chem.*, 1957, **29**, 391.
12. Jacobs, M. B., and Singerman, Ana, *J. Lab. Clin. Med.*, 1967, **59**, 871.
13. Ward, F. N., and McHugh, J. B., *Prof. Pap. U.S. Geol. Surv.*, 501 D, D128/30, 1964.
14. Cuthbert, Margaret, and Ward, F. N., *Ibid.*, 501 C, C154/56, 1964.
15. Merodio, J. C., *An. Asoc. Quim. Argent.*, 1961, **49**, 225.
16. Down, J. L., and Gorsuch, T. T., *Analyst*, 1967, **92**, 398.
17. Bowen, H. J. M., *Ibid.*, 1967, **92**, 124.

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The Use of a Special Intermittent Nebulisation Technique to Suppress the Background in Flame-emission Spectra

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A submerged oscillator - capillary technique is described, which produces a frequency and phase-stable intermittent fluid supply at 50 Hz to the flame of a flame spectrophotometer. With this technique the background radiation from the flame can be suppressed.

The cooling effect of the nebulised water on the flame is overcome by the choice of a suitable organic solvent. The usefulness of the technique is demonstrated with typical spectral curves.

The liquid sample is converted into an aerosol by a nebuliser, which is used with conventional flame-spectrophotometric methods. The droplets are continuously introduced into a flame, in which they evaporate, and the salt particles that remain vaporise and dissociate. The free atoms or the incompletely dissociated molecular fragments are then excited to give a steady emission in the hot flame. The emission intensity of the selected analytical line or the band of the molecular fragment used for analysis is a measure of the concentration of the sample.[1] If this method is used for analytical lines that have characteristic radiation within the bands of the OH–, C_2– and CH– molecules, it is difficult to distinguish between the background emission of the flame and the analytical line of interest, especially if apparatus with low spectral resolution is used. The flame-photometric method would be improved if the flame background could be successfully suppressed.

In earlier work[2] we used a peristaltic pump to produce the intermittent nebulisation. In this way the fluid supply to the nebuliser is continuously interrupted at a frequency of 10 Hz. The flame background appears in the first order in both half-periods independently of whether nebulisation is, or is not, used, and independently of the technique, while the line of analytical interest only appears in the half-phases associated with nebulisation. Therefore, a d.c. signal from the flame background and a superimposed a.c. signal from the analytical line appear in the output of the photomultiplier. If an a.c. amplifier is connected to the photomultiplier, signals corresponding to the line intensities will pass the amplifier, and the d.c. signal of the background will be suppressed in the first order.

The disadvantages of the earlier technique with the peristaltic pump were the short lifetime of the tubes at high frequencies and the changes in their internal diameters during the measurement, and, hence, changes in the fluid supply.

We overcame these by elongating the suction capillary of a nebuliser - burner with a plastic tube, and dipping the end of the tube periodically into the sample (dipping method).[3] In this way liquid droplets and small air bubbles travel to the nebuliser nozzle through the plastic tube and the capillary.

There are, however, other disadvantages; the frequency and phase stability of this sytem are not very good, because of the interpolation of the air bubbles, which permits individual columns of liquid to oscillate about their mean positions in a relatively smooth manner, the effect being rather like the movement of an accordion, depending on the forces acting in the system.

There is also a risk that the liquid columns may be broken up and the signal sequences disturbed by the forced vibration of the tube. This technique can only be used in conjunction with an internal standard and a synchronised amplifier.

This paper describes the development of a technique (the submerged oscillator - capillary technique) that eliminates these disadvantages, and gives some of our experiences with the technique.

Principle of the method

The suction capillary of a conventional nebuliser - burner is elongated downwards by a 15-cm plastic tube and a 5-cm metal capillary both having the same internal diameter as that of the nebuliser capillary, *viz.*, 0·045 cm (see Fig. 1).[4] While the sample is being drawn in, the bottom capillary is moved upwards with a velocity v_K, which is, at first, greater than the velocity of the liquid, v_F, that would apply for continuous nebulisation in a stationary tube.

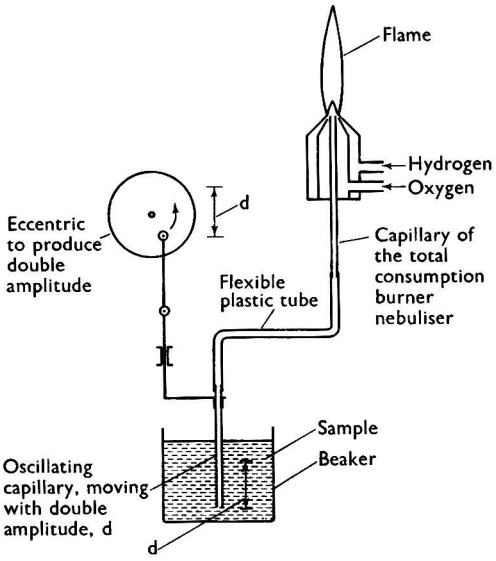

Fig. 1. Arrangement for the intermittent nebulisation

There is no liquid transport through the capillary if $v_K \geqslant v_F$ and both have the same sign. If capillary and liquid move towards one another, the velocity of the liquid through the capillary will be greater than in a stationary tube ($v_K = 0$). When the lower suction capillary is given a sinusoidal movement in the sample by means of an eccentric drive with a throw of 2 H and frequency $\omega = 2\pi\nu$, provided the lower edge remains in the liquid, the velocity v_K is given by the equation—

$$v_K = H \,.\, \omega \sin \omega \,.\, t.$$

The velocity v_F can be varied by the correct choice of the working pressure in the nebuliser (about 0·3 to 1 kp cm^{-2} with a flow-rate of 1 to 5 ml minute^{-1}). Fig. 2 shows the dependence of the flow per unit time on the threshold frequency with a fixed throw of 2 H. If the condition $v_K \geqslant v_F$ is not satisfied, there is no intermittent nebulisation.

Experimental

Apparatus—

We used the Carl Zeiss spectral photometer, type PM4 Q III, with the flame attachment FA 1. The nebuliser - burner combination was fed with hydrogen and compressed air. We used the wavelength drive of the apparatus with the adjustments 0·05 to 0·2. With the adjustment 0·05 speeds of 1·2 nm minute^{-1} at 285 nm, 1·5 nm minute^{-1} at 307 nm and 2·3 nm minute^{-1} at 348 nm are obtained (if the adjustment 0·2 is used the speeds will be four times these values).

A "normal" spectrum was recorded with a swinging shutter (chopper) and continuous nebulisation, and this was followed by recording a spectrum over the same range with the intermittent nebulisation technique. A sewing-machine mechanism was used as the eccentric drive for the lower capillary, the sewing-needle being replaced by the lower capillary. The lower end of this metal capillary moves in the sample with a throw of 10 mm. The machine is driven by a synchronous 50-Hz motor, which was adjusted to be in phase with the syn-

chronous amplifier in the apparatus. A lock-in-amplifier, Model RJB, available from Electronics, Missiles and Communications Inc., was used.

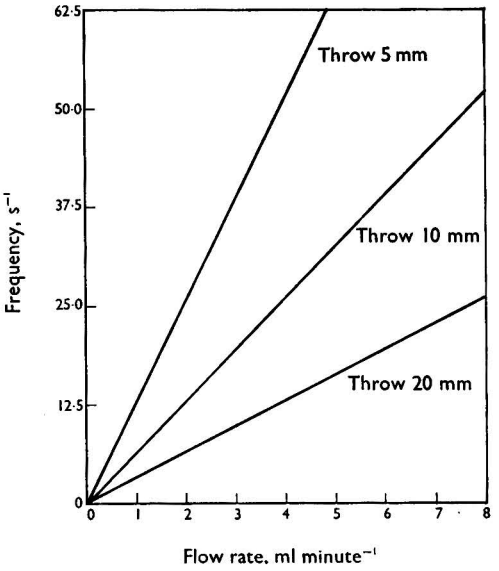

Fig. 2. The dependence of the flow per minute through the capillary on the threshold frequency. The parameter is a throw of 2H of the sinusoidal movement

Results

Fig. 3 shows the flame spectrum from 340 to 355 nm obtained by using the conventional method with the chopper and steady nebulisation of 100 p.p.m. of nickel as nickel nitrate dissolved in water - isopropyl alcohol (25 + 75 per cent. v/v), in a flame of hydrogen and compressed air. In Fig. 4 the intermittent nebulising technique was used under the same conditions (submerged oscillator - capillary technique) without the chopper.

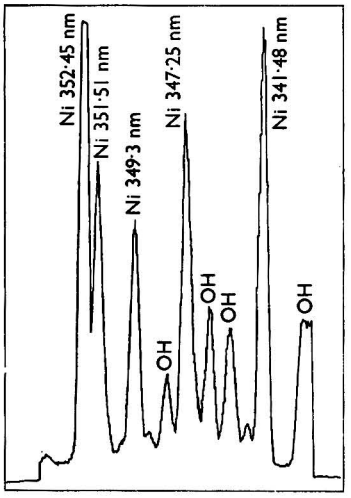

Fig. 3. Spectrum for nickel between 340 and 355 nm, with a chopper

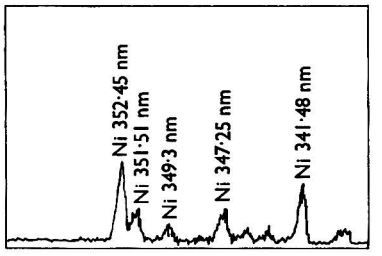

Fig. 4. Intermittent nebulisation (same conditions as for Fig. 3)

The nickel lines can be clearly seen in both spectra, and the OH-band lines of the background disappear when the intermittent technique is used. The pulse duty factor used (the relation between the time of nebulisation and the average pulse spacing) results in the amplitude being smaller with the intermittent technique.

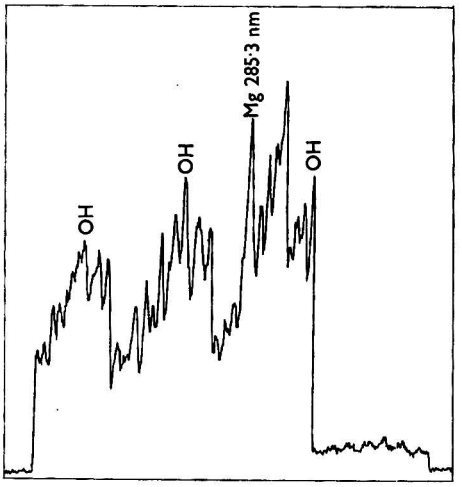

Fig. 5. Spectrum for magnesium between 275 and 300 nm, with a chopper

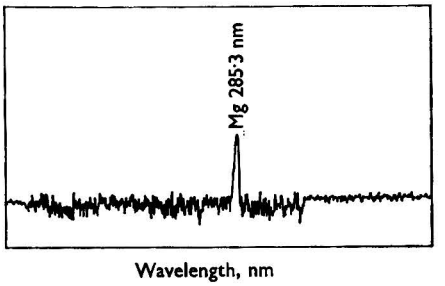

Fig. 6. Intermittent nebulisation (same conditions as for Fig. 5)

Figs. 5 and 6 show corresponding results for the magnesium line at 285·3 nm, also surrounded by OH-bands. A solution of 50 p.p.m. of magnesium was used, with the same isopropyl alcohol content as before.

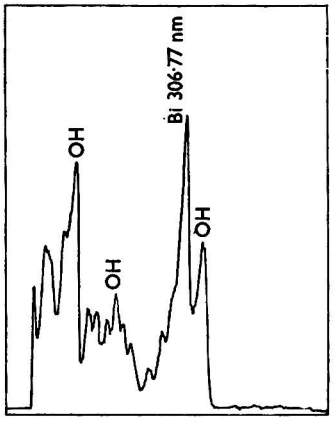

Fig. 7. Spectrum for bismuth between 306 and 309 nm, with a chopper

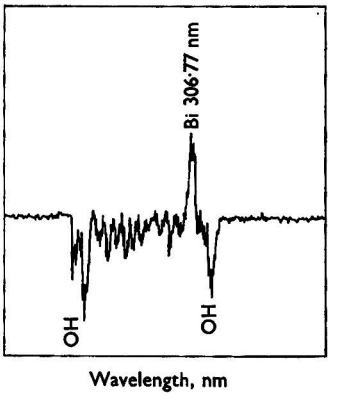

Fig. 8. Intermittent nebulisation (same conditions as for Fig. 7)

Figs. 7 and 8 show corresponding curves for the bismuth line at 306·77 nm, which lies in a very strong OH-band. A solution of 5000 p.p.m. of bismuth as nitrate in aqueous solution was used (with the same flame as before).

Discussion

When the intermittent nebulisation technique is used, instead of continuous nebulisation with a swinging shutter, it can be seen from the figures that the line intensities are reduced by a factor of 1·5, under the experimental conditions used. This may be because the pulse duty factor is not optimal or because the synchronisation procedure of the amplifier or of the intermittent nebuliser is inaccurate. It can also be deduced from the figures that, at the points at which the intermittent nebulisation suppresses the stronger OH-bands or equivalent ones, the background noise appears greater than in those parts of the spectrum in which such band lines do not exist. This leads to the question arising as to whether the detection limit can be improved by this intermittent method.

Detection limits

The magnesium line intensity was measured against the background twenty-five times at 285·3 nm (with the chopper). The detection limits have been calculated according to Kaiser's[4] recommendations, and are found to be 2 p.p.m. for continuous nebulisation with the chopper in use. With intermittent nebulisation this technique cannot be used because the blank is almost zero. We tried to eliminate this drawback by using the statistical fluctuations of a weak magnesium line at a low magnesium concentration (10 p.p.m.) as a value for the background, when the detection limit was found to be almost the same (2 p.p.m.).

Final discussion

It can be shown that in contrast with experimental arrangements tested earlier, the apparatus described here allows results to be recorded over long periods of time with a simple synchronised 50-Hz amplifier. The flame signals obtained with this intermittent nebulisation are stable in frequency and in phase. The sample influx does not vary with time, provided that the sample level is kept constant in the sample container. The lifetime of the arrangement is almost unlimited. The disadvantage of this simple fundamental principle, however, is that with this technique the flame signals are also modulated. The nebulisation of water reduces the flame temperature, while it is raised during the period in which the blank is being measured. Theoretically this should lead to a modulation of the flame signals that were to be suppressed by this arrangement of intermittent nebulisation. This disadvantage is important only in some circumstances. The background radiation is reduced considerably when the simple technique without organic solvents is used, but it does not disappear completely. The disadvantage of the temperature modulation of the flame background can be avoided by adding to the solvent used (water) an organic solvent (in this case 75 per cent. of isopropyl alcohol). We used a suitable amount to compensate for the reduction of the flame temperature caused by the presence of water, so that, apart from the noise, the background disappeared. However, a certain amount of noise, *i.e.*, some fluctuation of the baseline, remains in this region of the spectrum, even when stronger bands of the flame have been electronically suppressed by this method; this cannot be avoided. We hope, however, to achieve a better signal-to-noise ratio by the choice of a more suitable frequency for the intermittent nebulisation. It might then be possible to improve the detection limits.

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References

1. Herrmann, R., and Alkemade, C. Th. J., *translated by* Gilbert, P. T., "Flame Photometry," Interscience Publishers, New York, 1963.
2. Herrmann, R., and Lang, W., *Z. analyt. Chem.*, 1964, **203**, 1.
3. Neu, W., Herrmann, R., and Kirchhof, H., *Messtechnik*, 1968, **4**, 154.
4. Kaiser, H., *Z. analyt. Chem.*, 1966, **216**, 80.

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The Determination of Sodium in High Purity Water with Sodium-responsive Glass Electrodes

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A detailed investigation has been made of the accuracy of sodium-responsive glass electrodes for determining sodium (1 to 50 μg per litre) in high purity waters (*e.g.*, condensed steam and boiler feed-water) from power stations. The electrode potential can be made to follow the Nernst equation down to a sodium concentration of about 1 μg per litre by controlling the pH of the sample and by using a continuous flow of the sample past the electrode. Octadecylamine seriously affected the response of the electrodes, but other impurities likely to be present in power-station waters caused no significant effects. The standard deviation of analytical results varied from 0·4 to 1·2 μg per litre at concentrations of 2 and 26 μg of sodium per litre. Details of a recommended analytical procedure for discrete samples are given.

The determination of minute concentrations (1 to 10 μg per litre) of sodium in water has become of great importance in high pressure power stations. The purity of the steam must be rigorously controlled to minimise corrosion and the formation of deposits in superheaters and turbines. One of the aims is, therefore, to ensure that the concentration of sodium in steam does not exceed 10 μg per litre. Determination of similar concentrations in condensate and boiler feed-water may also be of great value in detecting ingress of cooling water via leaks in the condenser, and is also required in checking and controlling the performance of de-ionisation plant for purifying condensate. For all of these purposes, a method is required, capable of giving results with a standard deviation of not greater than 1 μg of sodium per litre or 10 per cent. of the concentration (whichever is the greater).

Two techniques have been used in power stations for determining such small concentrations of sodium, *i.e.*, flame photometry[1,2] and the use of sodium-responsive glass electrodes.[3,4,5] Neutron-activation and atomic-absorption spectrophotometric techniques were not considered as the former was thought to be unsuitable for routine use in power stations, and the latter appeared to have no advantages over flame photometry. The flame-photometric technique is well established, but the use of sodium-responsive glass electrodes is a more recently developed technique that appeared to have potential advantages for power-station applications. A detailed account of the electrochemistry of cation-sensitive glass electrodes is given by Eisenman,[6] and a paper by Mattock[7] provides a shorter, but useful, introduction to the development and use of sodium-sensitive glass electrodes. Two instruments for on-line analysis with these electrodes are commercially available, and both are claimed by the manufacturers to measure sodium concentrations as low as 1 μg per litre.

Virtually no quantitative information on the performance of these electrodes at concentrations of less than 100 μg per litre was available when our work started. The purpose of our work was, therefore, to obtain quantitative results on sources of error so that the value of the technique for power stations could be more accurately assessed. Results are given for the relationship between sodium concentration and electrode potential; the effects of other substances; the effect of certain experimental parameters; and the rate of response of the electrode to changing concentrations. As a result of these tests, an analytical procedure for discrete samples has been devised, and tests made to determine the precision and bias of analytical results. This work is described in the following sections.

Since completion of our work, Hawthorn and Ray[3] have reported the results of their investigations on the use of sodium-responsive glass electrodes for analysing water. They obtained good precision but did not investigate several of the factors tested in the present paper. We think that their paper and ours complement each other well, and provide strong evidence for the usefulness of sodium-responsive electrodes in analysing high purity water.

BASIS OF TECHNIQUE—

The technique is essentially the same as that used for measuring the pH value of a solution with a glass electrode. When a sodium-responsive glass electrode is immersed in a solution, it assumes a potential, determined by the activity of sodium ions in the solution. Thus, by measuring its potential against a reference electrode of constant potential, an estimate of sodium activity (and hence concentration) can be obtained. If the potential of the sodium electrode is affected only by the activity of sodium ions, it should follow the Nernst equation—

$$E = E^\circ + \frac{2{\cdot}3026RT}{F} \log a_{Na^+}$$

where E is the potential of the electrode, a_{Na^+} is the sodium-ion activity, R is the gas constant, T is the absolute temperature, F is the Faraday constant and E° is a zero term. Provided the activity coefficient of the sodium ions is constant, the equation can be re-written—

$$E = E^{\circ\prime} + \frac{2{\cdot}3026RT}{F} \log C_{Na^+}$$

where C_{Na^+} is the molar concentration of sodium ions.

Mattock[7] has shown that these equations are obeyed for concentrations down to about 10^{-3} M, but increasingly large deviations from Nernstian response occur as the sodium concentration is decreased further. These deviations appear to be caused largely by the effect of other ions on the potential of the sodium electrode. Mattock[7] showed that hydrogen, potassium, ammonium and lithium ions could all affect the potential. He suggested that the ratio of the molar concentrations of sodium and hydrogen ions should exceed 10^3 for the effect of hydrogen ions to be negligible, while Hanss, de Heaulme and Morin[8] suggested that a ratio of 10^4 is required. For sodium concentrations of 2·3 μg per litre (10^{-7} M) these results indicate that the pH of the solution should be in the range 10 to 11. Two commercial instruments (Beckman Instruments Ltd. and Electronic Instruments Ltd.), in which sodium-responsive glass electrodes are used for continuous analysis, have achieved this condition by the addition of ammonia to samples of high purity water. This technique seemed most suitable, and was used throughout our work. For normal laboratory analyses, ammonia solutions were considered more convenient than cylinders of ammonia gas. The addition of ammonia was, therefore, made as shown in Fig. 1; this technique was devised by Electronic Instruments Ltd.

To obtain significant results at sodium concentrations of 1 μg per litre, it is necessary to minimise contamination of the solution with ions affecting the potential of the electrode. With the exception of the sodium electrode, glass can be eliminated from the apparatus, but there is still the possibility of contamination from plastic materials and from ingress of potassium chloride used in the reference electrode. Therefore, it was considered essential to make measurements on flowing, rather than static, solutions, and with the reference electrode downstream of the sodium electrode. The flow cell designed by Electronic Instruments Ltd. seemed suitable, and was used throughout our work. This flow cell incorporates a magnetic stirrer for mixing the ammonia with the sample. We found this stirrer unnecessary; adequate mixing was obtained by passing the combined ammonia - sample stream through a coil of Tygon tubing (100 cm long of 2·5 mm bore) placed before the flow cell. The magnet, therefore, was removed from the flow cell, and the Tygon coil used throughout.

EXPERIMENTAL

REAGENTS, APPARATUS AND TECHNIQUE—

All experimental conditions and techniques were exactly as described under Method, except when otherwise stated. The types of instrumentation used in our work were as follows.

Pump—A standard peristaltic pump supplied by Technicon Instruments Ltd. was used to pump the sample and the ammonia - air mixture; the desired flow-rates were achieved by combining streams from the appropriate clear Tygon pump tubes.

pH Meter—A Beckman Research pH meter was used.

Sodium-responsive glass electrode—Type GEA 33 (Electronic Instruments Ltd.) was used.

Calomel reference electrode—Type RS 23 (Electronic Instruments Ltd.) was used with a remote junction and a saturated solution of potassium chloride in the salt-bridge.

Whenever necessary, the concentration of sodium in the de-ionised water (used to prepare standard solutions) was determined as described by Webber and Wilson.[9] The de-ionised water was produced by re-circulation (in an all-plastic system) of distilled water through a laboratory-scale, mixed-bed de-ionisation unit. The sodium content of this water was between 1 and 2 μg per litre.

All chemicals were of analytical-reagent grade, except cyclohexylamine and morpholine, both of which were of fine-chemical grade.

During this work, the temperature in the laboratory varied between 18° and 25° C.

Effect of ammonia concentration and flow-rate—

Variations in the concentration of ammonia in the solution might affect the potential of the sodium electrode. Also, variations in the flow-rate of the solution past the electrode might affect the transfer of sodium ions between the solution and the glass surface; this transfer might, in turn, also affect the electrode potential. Tests were, therefore, made to determine the magnitudes of these possible effects.

The effect of ammonia concentration was checked by pumping only de-ionised water (to which various additions of ammonia solution had been made) through the flow cell. The ammonia solution was prepared by isopiestic distillation in polythene apparatus. When the ammonia concentration in the sample was increased from 0·025 to 0·25 M, the apparent sodium concentration increased by 1·5 μg per litre. Further tests with other solutions containing greater concentrations of sodium showed that the effect of the same changes in ammonia concentration was negligibly small.

The combined effects of flow-rate and ammonia concentration were determined by analysing each of two solutions (1·4 and 13 μg of sodium per litre) as described under Method, but with different flow-rates for the solution (1·2 and 4·0 ml per minute) and the ammonia - air mixture (3·2 and 9·6 ml per minute). Each of the four combinations of flow-rates was tested for each of the two solutions. The results obtained had ranges of 1·2 and 0·8 μg of sodium per litre for the solutions containing 1·4 and 13 μg of sodium per litre, respectively. The possible variations in flow-rates and ammonia concentrations are much smaller than those tested, and, therefore, no important errors are expected from these sources.

Response time of the sodium-responsive electrode—

Following a stepwise change in the concentration of sodium in the sample solution, 3 to 4 minutes elapsed before the first detectable change in electrode potential was observed. This initial time delay is equivalent to the time required for the new solution to displace the previous solution in the flow cell and tubing leading to it. The delay was decreased by using greater flow-rates. However, the time taken for the potential to reach the new equilibrium value was almost independent of flow-rate, and was largely determined by the concentration of sodium in the new solution. At a solution flow-rate of 2 ml per minute the times to equilibrium response were about 10 minutes for 230 μg of sodium per litre, 30 minutes for 23 μg of sodium per litre and at least 120 minutes for 2·3 μg of sodium per litre. However, in the last case, after only 20 minutes the reading was usually within the equivalent of about 1 μg of sodium per litre of the equilibrium value. These results indicated that samples should be pumped past the sodium electrode for at least 30 minutes if their concentrations are in the range 1 to 50 μg of sodium per litre.

Mattock[10] reports that, in a flow cell of very different design from that used by us, the response time of electrodes is increased if air is allowed to flow past the electrode during the change from one sample to the next. In our work, the continuous stream of sample always contains air bubbles, and a few tests were made to check whether they caused any marked increase in response time. The tests showed no significant difference in response time whether the ammonia was added to the sample as a solution (air absent) or as a gas mixed with air.

Method

Apparatus—

A schematic diagram of the apparatus is shown in Fig. 1. With the exception of the electrodes, all parts of the apparatus that come into contact with samples or standard sodium solutions should be made of plastic materials. Tygon and poly(vinyl chloride) tubing and

polythene and Perspex apparatus were found suitable. It is advisable to clean all containers by soaking them for several days in the de-ionised water.

Polythene aspirators—Screw-capped polythene aspirators are convenient for storing samples and standard solutions. Aspirators fitted with spigot-type polythene taps are recommended, because the delivery tube (fitted with a plastic connector) to the pump can easily be transferred from one aspirator to another, with little chance of contamination. Check the aspirators for contamination by filling them with de-ionised water, setting them aside overnight, and analysing the water for sodium as described under Procedure. If the water from any of the aspirators contains a significantly higher concentration of sodium than freshly de-ionised water, discard the suspected aspirators or clean them again, and repeat the test. During determinations, the cap of each aspirator must remain loosely in place to minimise contamination from the atmosphere.

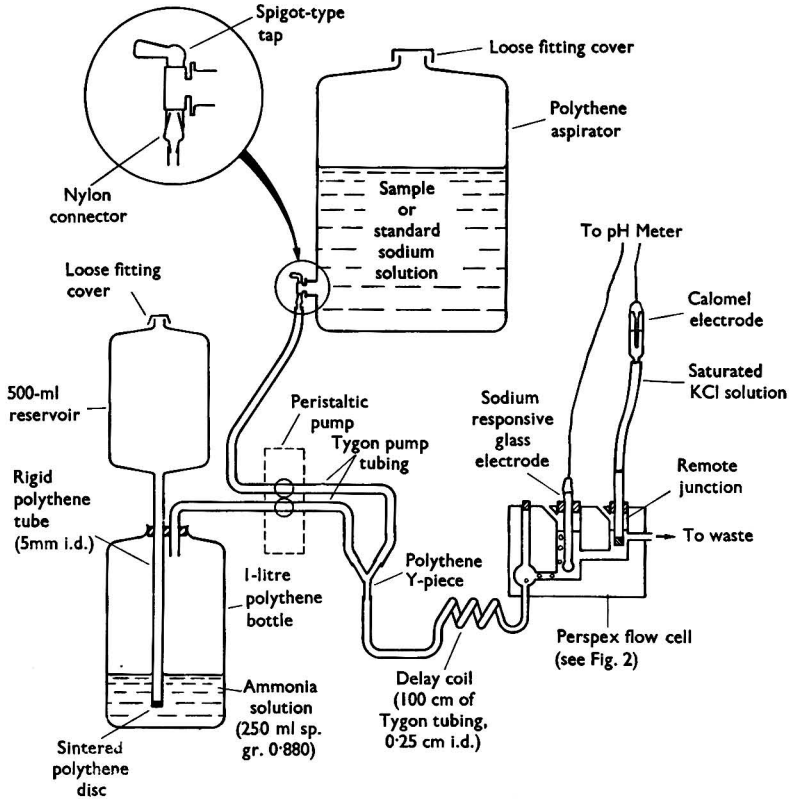

Fig. 1. Apparatus for determination of sodium

Polythene bottle for ammonia solution—Use a 1-litre polythene bottle for the ammonia solution (see Fig. 1). The bottle should contain about 250 ml of ammonia solution (sp.gr. 0·88), so that it covers the sintered polythene disc.

Check the concentration of ammonia in the solution leaving the electrode flow cell, daily, by titration with hydrochloric acid. When the concentration falls below 0·1 N, replace the ammonia solution in the bottle.

Electrode flow cell—The flow cell is made of Perspex with the dimensions given in Fig. 2. Clean the cell initially by washing it thoroughly with de-ionised water.

Pump—To our knowledge, any peristaltic pump can be used, provided it is capable of giving continuous flows of 2 ml per minute for the sample or standard solutions, and 10 ml per minute for the ammonia - air mixture. Check the flow-rates periodically, and renew the pump tubing whenever necessary.

Sodium-responsive glass electrode—Our work showed that Type GEA 33 electrodes (Electronic Instruments Ltd.) are suitable. Detailed tests of other manufacturers' electrodes have not yet been made. Place the electrode in de-ionised water for at least 48 hours before initial use. Between analyses, leave the electrode in the flow cell with its tip in dilute ammonia solution (about 0·1 N). Do not allow the bulb of the electrode to become dry. If the apparatus is used regularly, better accuracy is obtained if the equipment is in continuous use; when samples or standards are not being analysed, de-ionised water should be analysed continuously.

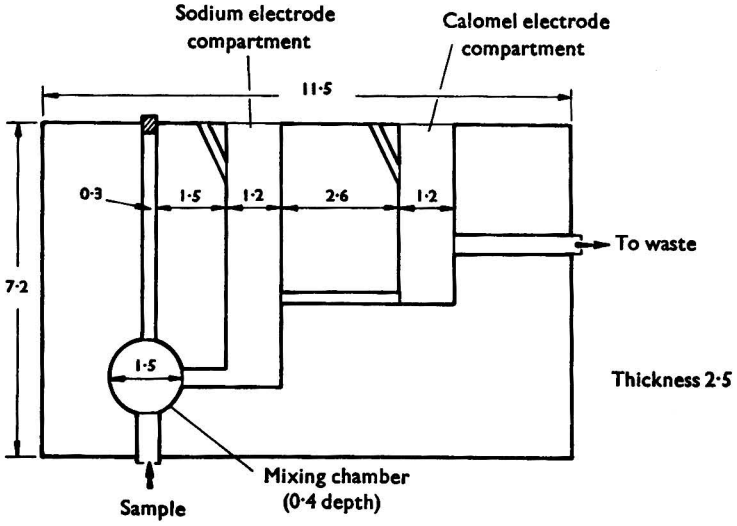

Fig. 2. Perspex flow cell (all dimensions are in cm)

Reference electrode—Use a calomel electrode with a remote liquid junction and a saturated solution of potassium chloride between the junction and the electrode. Type RS 23 electrodes (Electronic Instruments Ltd.) were found to be suitable. When solution is not passing through the flow cell, remove the remote junction from the flow cell.

pH Meter—Any meter capable of discriminating to within 0·01 pH units should be suitable, but the meter should, preferably, be fitted with a temperature-compensation device. If such a device is not present in the meter, ensure that the temperatures of the sample and standard solutions are within 1° C of each other during analyses.

REAGENTS—

Ammonia solution, sp.gr. 0·88—Analytical-reagent grade.

Water—For preparing standard solutions and final washing of apparatus, use distilled water that has been passed through a mixed-bed de-ionisation unit. It is preferable to re-circulate the de-ionised water through the mixed bed (with an all-plastic re-circulation system) for some time before withdrawing any water. Water containing between 1 and 2 μg of sodium per litre can be conveniently obtained in large amounts by this technique.

Standard sodium solution A—Dry sodium chloride (analytical-reagent grade) at 250° to 350° C for 1 to 2 hours. Dissolve 0·117 g in water, and dilute with water to 2 litres in a calibrated flask. Store in a polythene bottle. This solution was found to be stable for at least 6 months.

1 ml of solution A $\equiv$ 23 μg of sodium.

Standard solution B—Weigh 4·95 kg of water in a pre-weighed 5-litre polythene aspirator. Add 50·0 g of standard sodium solution A, and mix. This solution was stable for at least 8 weeks.

1 ml of solution B $\equiv$ 0·23 μg of sodium.

PROCEDURE—

Sample collection—Use a clean, screw-capped polythene aspirator, and collect at least 250 ml of sample. Great care should be taken to avoid contamination during sampling. The screw-cap should be partially unscrewed to allow an inflow of air while the sample is being pumped out during analysis. Immediately before analysis, wash the inside of the tap of the aspirator by running out about 50 ml of sample to waste, and wash the outside of the tap with de-ionised water.

In adequately clean bottles, samples have been found to be stable for many days but it is advisable to complete the analyses without undue delay after sampling.

Analysis of samples—If the remote junction of the reference electrode has been removed from the flow cell, wash the tip of the junction with water to remove any solid potassium chloride, and insert it in the flow cell.

Connect an aspirator containing standard sodium solution B to the appropriate tube of the pump (see Fig. 1). Open the tap of the aspirator, and begin pumping the solution and ammonia - air mixture through the flow cell. Measure the temperature, t° C, of the solution leaving the flow cell, and adjust the temperature-compensation device (if present) of the pH meter accordingly. After 30 minutes, adjust the pH meter so that a reading of 5·00 is obtained on the pH scale.

With the pump still operating, disconnect the pump tube from the tap of the aspirator, and then connect the tube to the aspirator containing the sample. Open the tap of the sample aspirator so that the sample is pumped through the flow cell. This transfer should be carried out as rapidly as possible; 5 to 15 seconds is sufficient. If the pH meter does not have a temperature-compensating device, measure the temperature of the solution leaving the cell; it must be within the range $t \pm 1$° C. After 30 minutes, note the reading, R, of the pH meter. For pH meters with a temperature-compensating device, measure the temperature of the solution leaving the flow cell and adjust the compensator, if necessary, just before taking the reading, R.

CALCULATION OF RESULTS—

(*i*) For pH meters with temperature compensation, the concentration, C, of sodium in the sample is given by the expression—

$$C = 23 \times 10^6 \times \text{antilog}_{10}\,(-R)\ \mu g \text{ of sodium per litre.}$$

(*ii*) For pH meters without temperature compensation, correct the value of R for temperature from the expression—

$$R' = 5{\cdot}00 + (R - 5{\cdot}00)\,(1 - k\,[t - 20])$$

where the factor, k, can be taken as 0·0034 for temperatures between 10° and 30° C. Calculate the concentration, C, of sodium in the sample from the expression—

$$C = 23 \times 10^6 \times \text{antilog}_{10}\,(-R')\ \mu g \text{ of sodium per litre.}$$

NOTES—

1. The recommended procedure is intended mainly for analyses in the range 1 to 50 μg of sodium per litre. The concentration of standard sodium solution B has been chosen on this basis, but the choice of this concentration is discussed in detail under Calibration of the electrode/measuring system in the Discussion.

2. The recommended method of calculating results assumes that the potential of the electrode is determined by a Nernstian-type equation. It is difficult to show, conclusively, that this is true for the range 1 to 50 μg of sodium per litre (see Calibration of the electrode/measuring system in the Discussion), but the following tests will give an indication that the electrode is behaving correctly, and these tests at least should be carried out before attempting to analyse samples for the first time.

Checking the slope of the calibration—Carry out the procedure for analysing a sample, but use a standard sodium solution (2·3 μg of sodium per ml) in place of the sample. The change in reading of the pH meter should be 1·00 ± 0·02.

Time for electrode potential to reach equilibrium—The 30 minutes recommended in the procedure, before making a measurement, did not always allow sufficient time for the electrode

to reach its equilibrium potential when a change in concentration occurred. The error introduced was only about 1 μg of sodium per litre, and this is considered tolerable. It is desirable to check the time necessary for this bias to become acceptably small for each experimental system that is used. For this purpose, prepare a batch of de-ionised water (retaining sufficient for the next test described below), and standardise the pH meter as described under Analysis of samples. Replace the standard sodium solution B with the de-ionised water, and take readings of the pH meter at 10-minute intervals until the difference between successive readings is less than 0·02. The potential of the electrode when this condition is reached can be regarded as the equilibrium potential. The results can then be used to determine the time allowed for the analysis of solutions.

Checking the consistency of the electrode response at low concentrations of sodium—Use the de-ionised water from the previous test to prepare 2 litres of a solution containing 23 μg of added sodium per litre. Analyse this solution as described under Analysis of samples, but take the reading from the pH meter at the equilibrium time found in the previous test. Calculate the sodium concentration, C, of the de-ionised water from the results of the previous test, and hence calculate the sodium content of the other solution, *i.e.*, $C + 23$ μg of sodium per litre. Compare this result with the concentration calculated from the observed reading of the pH meter for this solution. The electrode can be assumed to be behaving consistently if the two results agree to within about 2 μg of sodium per litre.

Results

Relationship between electrode potential and concentration—

To the best of our knowledge, no convincing demonstration had been made that the electrode response followed a Nernstian-type equation to concentrations as low as 1 μg of sodium per litre (about 4×10^{-8} M). We therefore attempted to confirm this in the following manner.

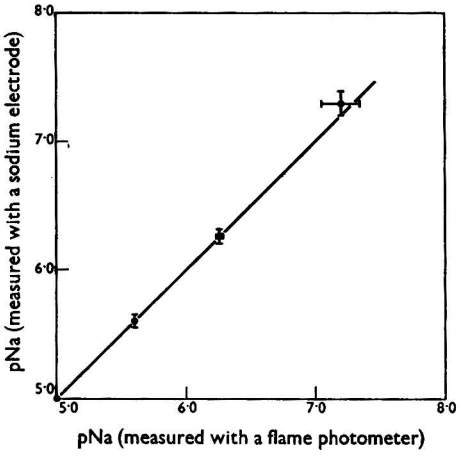

Fig. 3. Calibration of the sodium electrode. Lines through the points are the 95 per cent. confidence limits: horizontal marks, results obtained with the flame photometer; and vertical marks, results obtained with the sodium electrode

Solutions containing about 230, 60 and 12 μg of sodium per litre were prepared by diluting a standard solution with de-ionised water. The sodium contents of the water and the two lower standard solutions were determined by flame photometry.[9] Each of these four solutions was then analysed in turn by the recommended procedure, except that analysis was continued until the equilibrium reading for each solution had been taken. Each solution was analysed four times, and the mean result for the solution containing 230 μg of sodium per litre was used to calculate the sodium concentrations of the other three solutions, assuming that the

Nernst equation was obeyed. The results obtained are given in Table I and Fig. 3, which show that the sodium concentrations measured flame photometrically and potentiometrically agree within experimental error. The average change in electrode potential for a 10-fold change in sodium concentration was 58·6 ± 1·0 mV (95 per cent. confidence limits) as compared with the theoretical value of 58·56 mV at 22° C.

TABLE I

COMPARISON OF ANALYSES BY FLAME PHOTOMETRY AND SODIUM-RESPONSIVE GLASS ELECTRODE

Concentration of sodium added to water, μg per litre	Mean concentration of sodium found* by— Flame photometry		Sodium electrode (assuming Nernstian behaviour)	
	μg per litre	pNa†	pNa†	μg per litre
0	1·47 ± 0·5	7·19 ± 0·15	7·29 ± 0·09	1·20 ± 0·25
12	13·1 ± 0·5	6·25 ± 0·02	6·26 ± 0·06	12·7 ± 2·0
60	59·0 ± 0·5	5·59 ± 0·00	5·60 ± 0·05	57·6 ± 6·1
230	—	5·00	5·00	230

* The figures after the ± signs are the 95 per cent. confidence limits.

† $pNa = \log \frac{1}{Na}$, where Na is the molar concentration of sodium.

A second sodium-responsive electrode of the same type was used to analyse the same four solutions; the average difference between results obtained by the two electrodes was only 0·02 pNa units.

PREPARATION AND STABILITY OF STANDARD SODIUM SOLUTIONS—

The equivalents of 0, 0, 11·5 and 23 μg of sodium per litre were added to four portions of de-ionised water, and the solutions were then analysed as described under Procedure, except that readings were not taken until equilibrium had been attained. This series of determinations was repeated 10 days later, and the results are given in Table II.

TABLE II

STABILITY OF STANDARD SODIUM SOLUTIONS

Date of analysis	Concentration found, μg of sodium per litre, for solutions with added sodium concentrations of— 0 μg per litre	0 μg per litre	11·5 μg per litre	23 μg per litre
9.9.66	1·90	2·05	17·2	26·3
19.9.66	1·56	1·58	14·2	26·1

The result of 17·2 obtained on 9.9.66 seems suspect, although no specific reason is known for its rejection. If this result is ignored, Table II shows that solutions containing 2 and 26 μg of sodium per litre were stable to within 0·5 μg per litre over a period of 10 days. Further tests on the latter solution showed that it was stable to within 1 μg of sodium per litre over a period of 8 weeks. The results for the two blank solutions show that replicate solutions can be prepared with little difference in their concentrations of sodium. Finally, the measured concentrations of the other two solutions are within about 1 μg of sodium per litre of the values expected from the sodium content of the de-ionised water and the amount of sodium added. It appears, therefore, that such dilute standard solutions can be prepared and handled without unduly large errors.

PRECISION AND BIAS OF ANALYTICAL RESULTS—

An aliquot of each of the four solutions used in the previous section was analysed on each of ten occasions (all between the two sets of tests in the previous section) exactly as described under Method. At the beginning of each batch of measurements, the standard solution (pNa = 5) was passed through the cell until a steady reading was obtained on the pH meter. The other four solutions were then analysed in random order, readings of their apparent pNa values being taken after 20 and 30 minutes. Finally, the standard solution was passed through the cell, and the apparent pNa value read after 20 minutes.

A summary of the results is given in Table III. On three occasions during these tests, the apparatus was used immediately after it had been shut down overnight (normally the apparatus was left operating on a blank solution when not in use). On these occasions, the apparent sodium contents of all the solutions were greater than on the other seven occasions. The mean results for these seven occasions are also given in Table III.

TABLE III

PRECISION AND BIAS OF ANALYTICAL RESULTS

Concentration* of sodium, μg per litre	Number of batches of analyses	Mean concentration of sodium found,† μg per litre: Readings after 20 minutes	Readings after 30 minutes
232	10	234 (12)	—
	7	229 (10)	—
26·2	10	28·6 (2·3)	28·1 (2·3)
	7	27·5 (1·2)	27·3 (1·2)
14·2	10	16·6 (2·9)	16·4 (2·5)
	7	15·1 (0·9)	15·1 (0·9)
1·73	10	3·31 (0·89)	3·12 (0·72)
	7	2·92 (0·61)	2·78 (0·50)
1·82	10	3·08 (0·57)	2·95 (0·52)
	7	2·77 (0·38)	2·69 (0·36)

* Mean of the results from Table II.
† The figures in brackets are the standard deviations of individual results.

EFFECT OF OTHER SUBSTANCES—

Other substances present in samples could cause interference by affecting the activity coefficient for sodium ions or by a direct effect on the electrode. The concentrations of impurities in condensates and steam are insufficient to cause the first type of interference. Of the substances likely to be present in samples, it was thought that only calcium, magnesium, sulphate, chloride, hydrazine, cyclohexylamine and morpholine might affect the electrode potential directly, and that metallic hydroxides and octadecylamine might form films on the electrode surface affecting its response to sodium ions. The effects of these substances were tested, and the results are given in Table IV. Flame-photometric analysis of the solutions, with and without the substances under test, showed that these substances contributed less than 0·5 μg of sodium per litre.

TABLE IV

EFFECT OF OTHER SUBSTANCES

Substance	Concentration of substance, p.p.m.	Apparent* sodium concentration, μg per litre, at sodium concentrations of— 1·4 μg per litre	24·4 μg per litre
Ca^{2+}	1	3·7	29·4
Mg^{2+}	1		
SO_4^{2-}	4		
Cl^-	2		
Fe^{3+}	2	1·9	—
Hydrazine	1	3·0	23·7
Cyclohexylamine	1		
Morpholine	1		
Octadecylamine	10	16·3	—

* If the other substances caused no effect, the results would be expected to be within the ranges 1·4 ± 0·25 and 24·4 ± 3·0 μg of sodium per litre (95 per cent. confidence limits).

DISCUSSION OF THE METHOD

CALIBRATION OF THE ELECTRODE/MEASURING SYSTEM—

The results in Table I and Fig. 2 show that the potential of the sodium-responsive glass electrode was proportional to the pNa value of a solution, for concentrations of sodium

between 230 and 1·4 μg per litre. The validity of these tests is not dependent on any assumption concerning the relationship between electrode potential and concentration. Our average results show that the electrode potential varied by 58·6 mV per pNa unit (22° C), and this agrees well with the theoretical value (from the Nernst equation) of 58·56 mV per pNa unit. To our knowledge, this is the first time that unequivocal proof of the Nernstian behaviour of these electrodes in this concentration range has been obtained.

When it is desired to analyse samples with sodium contents in the range 1 to 50 μg per litre, calibration of the apparatus is complicated by the fact that it is exceedingly difficult to prepare standard sodium solutions with accurately known pNa values in the range 8 to 6 pNa units. This arises because of the apparent impossibility of preparing (with certainty) water with a negligibly small sodium content (say, less than 0·1 μg per litre). Thus, the calibration technique normally used for pH determinations is not directly applicable when the pNa value of high purity water is to be determined. The method of calibration used in the method, therefore, requires special consideration.

The careful work of Hawthorn and Ray[3] and the present work indicate that it is, at least, extremely difficult (and perhaps impracticable) to prepare water containing less than 1 μg of sodium per litre. In both investigations the lowest sodium contents were between 1 and 2 μg per litre. It seems likely, therefore, that in routine analysis, sodium contents of up to 5 μg per litre may be encountered. If no allowance is made for the sodium content of the water used to prepare standard solutions, large errors may result. For example, a nominal standard of 10 μg per litre could actually contain 15 μg per litre. An error of 50 per cent. would, therefore, result in the analysis of samples at this concentration, and still larger errors would be caused at smaller concentrations. There appears to be no method of determining the sodium content of the water used to prepare standard solutions without either making assumptions about the calibration of the electrode or using an independent method of analysis. The first leads to a circular argument, and the second is often not available in power-station laboratories. In these circumstances, the only approach is to use standard solutions (for calibration) of such concentration that the sodium content of the water used to prepare them causes negligible error in the pNa values of the standards. For our purposes, the lowest concentration feasible appears to be about 200 μg of sodium per litre.

When a Nernstian slope for the calibration can be assumed, it is adequate to use only one standard solution for calibration. Our experience, and that of Hawthorn and Ray,[3] shows that this approach was adequate in tests made over a number of days, and this technique has, therefore, been made the normal method of calibration in the proposed method. In the concentration range with which this paper is concerned, there has, as yet, been too little precise investigational work reported to decide on the possibility of non-Nernstian response of these electrodes. The suggested procedure (see Note 2 of the Method) for checking the slope of the calibration provides some indication of the true response of the electrode, and is considered a useful check to make periodically. This test is open to the objection that the calibration may depart from Nernstian slope at concentrations appreciably lower than those of the standard solutions (230 and 2300 μg of sodium per litre). We have insufficient experience to estimate this possibility. If it is assumed to be sufficiently possible to require experimental confirmation, the only unequivocal method known to us is that described in the section Relationship between electrode potential and concentration.

It is important to note that these problems of calibration would be considerably simplified if it were possible to prepare and handle water containing not more than 0·1 μg of sodium per litre, routinely and consistently, and to guarantee, without test, that one had done so. Under these conditions, a standard solution of nominal concentration 5 μg per litre could be prepared with negligible error. The extrapolation of the calibration to 1 μg per litre would then be unlikely to lead to unacceptable error, even if the response deviated from Nernstian behaviour. The results in Table II indicate the errors likely to arise in handling solutions in this concentration range.

PRECISION AND BIAS—

The results in Table III show clearly that more precise and less biased results were obtained when solutions were continually passed through the electrode flow cell. This procedure is, therefore, included in the proposed method. The effect presumably arises because of changes in the electrode surface or solution double layer, or both, that take place in static solution.

The results in Tables II and III indicate that the equilibrium electrode potential was not reached within 30 minutes, for the solutions containing between 1 and 26 μg of sodium per litre. For each of these four solutions the result obtained after 30 minutes, by the proposed method, was about 1 μg per litre greater than the estimated true value. As a compromise between analytical time and bias, it was decided to recommend measurement after 30 minutes, but the bias could be reduced by allowing more time for equilibration with each sample.

Under the conditions of the proposed method, Table III shows that the standard deviations of analytical results varied between about 0·4 and 1·2 μg per litre for sodium concentrations of about 2 to 26 μg per litre. These standard deviations are similar to those found by Hawthorn and Ray[3] at similar concentrations, *i.e.*, 0·5 to 1·5 μg per litre. This completely independent confirmation indicates that such precision should be fairly readily attainable in any laboratory. The precision is also quite adequate for the analysis of high purity waters.

The results in Table III show an average bias of 1·0 μg of sodium per litre in the concentration range 1·7 to 26 μg per litre, for the proposed method. It appears that a constant allowance could be made for this error because it varied little with concentration. For example, if 1·0 μg per litre were subtracted from each of the mean results, the largest deviation from the expected value would be about 0·1 μg per litre. The observed bias does not prevent small changes in concentration from being detected.

The results in Table IV show that small effects were caused by, at least, some of the following: calcium, magnesium, sulphate, chloride, iron(III), hydrazine, cyclohexylamine and morpholine. However, as the concentrations of these substances that were used are much larger than those normally present in condensates and steam, the effects are considered to be unimportant. The effect caused by octadecylamine was larger and apparently caused by adsorption of the amine on the surface of the sodium-responsive electrode, because subsequent tests showed that the electrode gave erroneous results for solutions containing only sodium chloride. Also, the original correct response to standard solutions was obtained after the electrode had been washed with ethanol. Although the concentration of octadecylamine was much greater than would normally be present in samples, there is the possibility that analysis of a series of samples containing octadecylamine may lead to progressively increasing errors because of cumulative adsorption on the electrode. The effect could, perhaps, be eliminated by washing the electrode with ethanol after each sample, but this has not been investigated. Accordingly, considerable care is necessary in ensuring that valid results are obtained for samples containing octadecylamine. The problem would be expected to be much greater if sodium-responsive electrodes were used for the continuous analysis of a sample stream containing the amine.

Comparison of proposed method with flame photometry—

We have used the proposed method and a flame-photometric technique[9] for determining the sodium content of water in the concentration range 1 to 50 μg per litre, and a comparison of the two methods is useful.

First, the flame-photometric method was less precise in the range 1 to 25 μg per litre, the standard deviation varying from about 1 to 2 μg per litre as compared with corresponding values of about 0·5 and 1 μg per litre with the sodium-responsive electrode. The precision of the two techniques was similar at a concentration of 50 μg per litre. The poorer precision of flame photometry at the lower concentrations is probably associated with its greater susceptibility to contamination. The electrode system is almost completely sealed so that the chances of contamination are reduced. This is an advantage especially for routine analysis in power-station laboratories.

Secondly, the flame-photometric method appears to be more critically dependent on experimental parameters than the sodium electrode technique. More-experienced analysts are needed to carry out the former than the latter.

Thirdly, results can be obtained more rapidly by the flame-photometric technique. This advantage is partly offset by the much smaller amount of attention required from the analyst when the sodium electrode is used. For example, determinations of copper in samples of feed-water can be carried out by the same analyst, simultaneously with the sodium determinations.

Fourthly, the type of flame photometer suitable for analysis of high purity water is considerably more expensive than the equipment needed when sodium-responsive electrodes are used.

Finally, the flame-photometric technique allows an absolute determination of sodium concentration without recourse to any other methods of analysis. This is not so for the sodium electrode technique, if the possibility of deviations from Nernstian behaviour is conceded.

We therefore prefer the technique in which sodium-responsive glass electrodes are used for routine analysis in the concentration range 1 to 50 μg of sodium per litre.

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References

1. American Society for Testing Materials, "1967 Book of A.S.T.M. Standards," American Society for Testing and Materials, Philadelphia, 1967, Part **23**, p. **354**.
2. Crandall, W. A., and Nacovsky, W., *Proc. Amer. Pwr. Conf.*, 1958, **20**, 726.
3. Hawthorn, D., and Ray, N. J., *Analyst*, 1968, **93**, 158.
4. Knoedler, E. L., and Perier, C. H., *Mater. Prot.*, 1965, **4**, 56.
5. Ammer, H. G., *Energie Münch.*, 1966, **18**, 433.
6. Eisenman, G., *in* Reilley, C. N., *Editor*, "Advances in Analytical Chemistry and Instrumentation," Volume 4, Interscience Publishers, New York, London and Sydney, 1965, p. 213.
7. Mattock, G., *Analyst*, 1962, **87**, 930.
8. Hanss, M., de Heaulme, M., and Morin, P., *Bull. Soc. Chim. Fr.*, 1963, **1**, 2658.
9. Webber, H. M., and Wilson, A. L., *Analyst*, 1969, **94**, in the press.
10. Mattock, G., *Chimia*, 1967, **21**, 209.

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The Rapid Determination of Diazinon and its Oxygen Analogue in Animal Tissues by Gas Chromatography

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A rapid method for the simultaneous gas-chromatographic determination of diazinon and its oxygen analogue (diazoxon) in blood, fat, liver, muscle and brain is described. The use of a selective thermionic phosphorus detector makes clean-up unnecessary. Preparation of the sample before injection consists in trituration with sand and sodium sulphate, elution with methanol or ether and concentration. Quantitative measurements are made by comparison with an internal standard. The method is satisfactory for the determination of 0·05 p.p.m. of diazinon and 0·2 p.p.m. of diazoxon in a 0·1-g sample.

THE previously published methods for determining organophosphorus pesticides in animal tissues and fluids by gas chromatography have usually involved extensive clean-up of the sample.[1,2,3] Jain, Fontan and Kirk[4] described the gas-chromatographic determination of some chlorinated and phosphorus pesticides in blood after solvent extraction without further clean-up, but indicated that their method was not necessarily suitable for other tissues. As they used electron-capture detection, the method would be rather insensitive to many organophosphorus compounds.

This paper describes the rapid determination, without clean-up, of diazinon and its oxygen analogue, diazoxon, in small samples of blood and animal tissues. (Although diazoxon is unlikely to be found in practice in the tissues studied, its greater polarity makes it a useful model compound for judging the usefulness of the method for determining other organophosphorus pesticides.) The thermionic detector described by Hartmann,[5] which is available commercially in the Aerograph gas chromatograph, is used.

EXPERIMENTAL

Preliminary work showed that the detection of both compounds was extremely sensitive and that there was remarkably little interference from other material in the tissues. Roughly quantitative recoveries of both were attained from fortified blood, fat, brain, liver and muscle by grinding the sample with anhydrous sodium sulphate, packing the mixture into a small column and eluting with diethyl ether or methanol. Diazinon could then be satisfactorily recovered after evaporation of the eluate to dryness under nitrogen, but serious losses of diazoxon occurred if evaporation was complete, even when care was taken to stop the flow of nitrogen as soon as the solvent had gone. These losses were avoided by concentrating the solution to a volume of not less than about 0·1 ml, and this procedure was convenient provided an internal standard was used. Tri-isobutyl phosphate was a suitable internal standard and could be concentrated to the same extent as diazoxon without loss. Both methanol and ether were effective eluants, but methanol was better for fat because it extracted less unwanted material. Ether was more suitable for brain, liver and muscle, however, as chromatograms of methanolic extracts of these tissues sometimes showed a peak that was imperfectly resolved from the tri-isobutyl phosphate peak, making measurement of the latter difficult.

To assess the effectiveness of the extraction procedure, rats were dosed orally with diazinon, and their fat, liver, muscle and brain extracted by four different methods: (*i*) the

procedure described above; (*ii*) a similar elution after grinding with a sand - sodium sulphate mixture; (*iii*) Soxhlet extraction with ether after grinding with a sand - sodium sulphate mixture; and (*iv*) homogenisation with methanol in a Potter - Elvehjem apparatus. Relative recoveries of diazinon by the four methods are shown in Table I, from which it appears that there is some advantage in using sand and sodium sulphate, but none in Soxhlet extraction or homogenisation. No diazoxon was found in any of the tissues.

TABLE I

RELATIVE RECOVERIES OF DIAZINON FROM TISSUES OF DOSED RATS BY FOUR EXTRACTION PROCEDURES

		Diazinon found, per cent. of that found by method (*i*)			
Tissue		Method (*i*) elution from sodium sulphate	Method (*ii*) elution from sodium sulphate *plus* sand	Method (*iii*) Soxhlet extraction with ether	Method (*iv*) Potter - Elvehjem homogenisation
Fat	Rat 1 ..	100	108	116	108
	Rat 2 ..	100	128	106	—
	Rat 3 ..	100	86	103	—
Liver	Rat 1 ..	100	122	114	119
	Rat 2 ..	100	89	84	72
	Rat 3 ..	100	106	122	—
Muscle	Rat 1 ..	100	107	109	86
	Rat 2 ..	100	118	105	63
	Rat 3 ..	100	92	103	71
Brain	Rat 1 ..	100	112	103	93
	Rat 2 ..	100	113	118	74
	Rat 3 ..	100	120	106	—

Gas chromatography on SE-30 silicone stationary phase was unsatisfactory as the peaks from both compounds, especially diazoxon, showed marked tailing. This was largely overcome and sensitivity much improved by using XE-60 (cyanoethyl methyl silicone) as stationary phase.

METHOD

APPARATUS—

Gas chromatograph—The Aerograph, Model 204–1B, with phosphorus detector was used. This was fitted with a 5 feet × ⅛ inch o.d. glass column, packed with 1·5 to 2 per cent. XE-60 on 100 to 120-mesh Aeropak 30 (acid-washed, silanised Chromosorb W).

The temperature of the column was 170° C, and of the detector and injection port 200° C. The flow-rate of nitrogen carrier gas was 15 ml per minute, that of air 170 ml per minute, and of hydrogen about 15 ml per minute, finally adjusted to give a background current of 1·5 to 3×10^{-9} A.

Extraction tubes—These were made from 15 cm × 6 mm i.d. tubing, drawn out at one end to 1 cm × 1 mm i.d.

Small agate pestle and mortar.

Conical centrifuge tubes—These were 10-ml graduated tubes, with stoppers.

REAGENTS—

Methanol and diethyl ether—Dry analytical-reagent grade solvents with anhydrous sodium sulphate and distil. Test by gas chromatography after concentration to one hundredth of the original volume.

Sodium sulphate—Extract analytical-reagent grade anhydrous sodium sulphate with ether, dry and heat at 200° C for several hours.

Sodium sulphate - sand mixture—Extract and dry the sand (acid-washed) by the same method as for sodium sulphate and mix equal weights of sodium sulphate and sand.

Internal standard solution—Tri-isobutyl phosphate ("Pure," Koch-Light Laboratories Ltd.) in methanol, 0·25 μg per ml.

PROCEDURE—

Plug the constriction in an extraction tube with glass-wool, add about 0·5 g of sodium sulphate and proceed as follows.

Blood—Add 0·1 ml evenly to 2·5 g of sodium sulphate in a small beaker and stir with a glass rod to make the mixture as homogeneous as possible. Pour the mixture into the extraction tube, wash the beaker with methanol and use this methanol to elute the mixture. Collect 1 ml of eluate in a centrifuge tube containing 0·2 ml of internal standard solution, concentrate to about 0·2 ml in a stream of nitrogen, and inject duplicate 0·5-μl aliquots into the gas chromatograph. If the peak height of either test compound is less than 5 per cent. of full scale at the highest sensitivity (range EC1 and attenuation $\times$ 1 on the Aerograph instrument), inject a larger volume and attenuate the tri-isobutyl phosphate peak. It is usually possible to inject 5 μl without interference from tissue components. Measure the ratios of the heights of the diazinon and diazoxon peaks to the tri-isobutyl phosphate peak, and compare them with the corresponding ratios obtained from standard solutions of the pure compounds. The approximate retention time for tri-isobutyl phosphate is 1·5 minutes, for diazinon 3·9 minutes, and for diazoxon 5·7 minutes.

Fat—Grind 0·1 g of sample with 3 g of sodium sulphate - sand mixture in a small agate mortar. Transfer the powder to the extraction tube and continue as for blood.

Liver, muscle and brain—Grind 0·1 g of sample with 3 g of sodium sulphate - sand mixture and transfer to the extraction tube. Elute with ether, collecting 5 ml of eluate in a centrifuge tube containing 0·2 ml of internal standard solution. Concentrate to 0·2 ml under nitrogen, warming the tube just sufficiently to prevent condensation, then continue as for blood.

RESULTS

CALIBRATION—

Solutions for calibration contained 0·01 to 10 μg per ml of diazinon and 0·025 to 20 μg per ml of diazoxon, dissolved in methanol containing 0·25 μg per ml of tri-isobutyl phosphate. The range of concentration was covered by ten solutions and each was injected in triplicate. Both compounds showed a linear relationship between concentration and ratio of peak height to the tri-isobutyl phosphate peak over the whole range. The equations of the two lines, with the standard errors of their slopes, were as follows—

$$\text{Diazinon: } R = 1{\cdot}654\ (\pm 0{\cdot}0084)\ C - 0{\cdot}0014$$

$$\text{Diazoxon: } R = 0{\cdot}536\ (\pm 0{\cdot}0070)\ C - 0{\cdot}031$$

where R = ratio of peak heights, and C = concentration, μg per ml.

RECOVERIES—

Tables II and III show the mean recoveries of diazinon and diazoxon from blood and tissues, fortified at various levels, together with the range and the number of samples analysed in each group. The standard error of the mean for all tissues at each level and the pooled standard deviation for all tissues at all levels are also shown. There were no consistently significant differences between different tissues or between different levels.

In the fortification experiments, the tissues were ground with sodium sulphate and sand immediately before the addition of diazinon and diazoxon. This procedure avoided losses of diazoxon by reaction with enzymes in liver and blood samples.

DISCUSSION

The calibration equations show that the detector responds linearly to both compounds, and is more than three times as sensitive to diazinon as to diazoxon, over the 1000-fold range of concentrations examined. As both equations have negative constant terms, sensitivity would decrease at lower concentrations, the decrease being more rapid for diazoxon, and this has been found on other occasions. For quantitative work, peak heights should not be less than 5 per cent. of full scale, and this height is given by about 10 pg of diazinon and 50 pg of diazoxon.

Tables II and III show that recoveries from fortified tissues are satisfactory over a range of 0·05 to 1 p.p.m. for diazinon and 0·2 to 2 p.p.m. for diazoxon. The few results obtained at other levels suggest that these ranges can be extended to 0·02 to 10 p.p.m. and 0·1 to 2 p.p.m., respectively. In practice, levels of both compounds above 1 p.p.m. are probably best determined by diluting the solution before injection.

TABLE II

RECOVERY OF ADDED DIAZINON FROM TISSUES

		Diazinon recovered, per cent., from—							
	Tissue	0·02 p.p.m.	0·05 p.p.m.	0·1 p.p.m.	0·25 p.p.m.	0·5 p.p.m.	1 p.p.m.	2 p.p.m.	10 p.p.m.
Fat	Mean ..	—	97·5	109·4	86·0	85·2	102·4	—	—
	Range ..	—	83 to 114	93·5 to 119	76 to 94	75 to 94	96 to 108	—	—
	Number ..	—	4	5	7	5	2	—	—
Liver	Mean ..	—	98·7	84·3	91·9	96·9	89·5	—	—
	Range ..	—	81 to 128	80 to 88	70 to 110	81 to 107	—	—	—
	Number ..	—	5	3	10	5	1	—	—
Muscle	Mean ..	—	100·0	103·2	95·3	85·3	90·7	—	—
	Range ..	—	80 to 120	88 to 118·5	77 to 116	75 to 115	84 to 97	—	—
	Number ..	—	2	6	6	5	2	—	—
Brain	Mean ..	—	100·8	78·4	99·0	90·7	—	—	—
	Range ..	—	90 to 108	73 to 82	85 to 112	76 to 102	—	—	—
	Number ..	—	3	4	7	5	—	—	—
Blood	Mean ..	98·3	107·4	100·5	110	—	90·6	92	96
	Range ..	—	107 to 108	89 to 117	—	—	79 to 108	—	—
	Number ..	1	2	15	1	—	7	1	1
All tissues	Mean ..	98·3	100·0	98·2	93·4	89·6	92·5	92	96
	± S.E.M...	—	±2·91	±2·03	±2·09	±2·61	±3·36	—	—
	Number ..	1	16	33	31	20	12	1	1

Pooled standard deviation (115 values) = 5·21.

TABLE III

RECOVERY OF ADDED DIAZOXON FROM TISSUES

		Diazoxon recovered, per cent., from—				
	Tissue	0·1 p.p.m.	0·2 p.p.m.	0·5 p.p.m.	1 p.p.m.	2 p.p.m.
Fat	Mean	—	98·4	88·5	86·9	94·4
	Range	—	81 to 110	79 to 113	76 to 100	88 to 99
	Number ..	—	5	6	6	3
Liver	Mean	—	112	87·7	68	81
	Range	—	—	72 to 116	—	—
	Number ..	—	1	6	1	1
Muscle	Mean	93·5	99·0	78·7	107·5	80·8
	Range	—	94 to 102	77 to 81	107 to 108	78 to 83·5
	Number ..	1	3	3	2	2
Brain	Mean	68	73·2	87·8	97·0	—
	Range	—	68 to 79	66 to 109	85 to 109	—
	Number ..	1	2	2	2	—
Blood	Mean	—	90·8	—	—	83·9
	Range	—	72 to 108	—	—	83 to 84·5
	Number ..	—	5	—	—	2
All tissues	Mean	80·8	93·8	86·4	90·8	86·7
	± S.E.M. ..	—	±3·18	±3·08	±3·83	±4·49
	Number ..	2	16	17	11	8

Pooled standard deviation (54 values) = 12·71.

Good chromatograms were obtained from all the tissues. At maximum sensitivity the base-line wanders only slightly and noise is negligible. None of the chromatograms has shown peaks from tissue components, although a peak with a retention time of about

2·5 minutes sometimes appears in sheep blood that has been stored for some days before analysis: it has not been found in fresh blood. The indications are that the method would be satisfactory, with minor alterations, for the determination of several other organophosphorus pesticides and in fact it has been used successfully to determine chlorfenvinphos in sheep blood and cruformate in cattle blood. It should be particularly useful for phosphorus compounds of low electron affinity, for which the electron-capture detector response is poor.

It is concluded that the method is adequate for the determination of diazinon and diazoxon in animal tissues and body fluids at low levels. The method has the advantages of speed, the small amounts of samples and reagents required and the simplicity of the apparatus. Because so little material is injected, columns last several months without noticeable deterioration.

The authors thank the Director of the Central Veterinary Laboratory for permission to publish this work, and Miss C. N. Hebert for statistical analysis of the results. They are grateful to Fisons Pest Control Ltd. for samples of diazinon and diazoxon.

References

1. Claborn, H. V., and Ivey, M. C., *J. Agric. Fd Chem.*, 1965, **13**, 353.
2. Robinson, J., Malone, J. C., and Bush, B., *J. Sci. Fd Agric.*, 1966, **17**, 309.
3. Watts, R. R., and Storherr, R. W., *J. Ass. Off. Analyt. Chem.*, 1967, **50**, 581.
4. Jain, N. C., Fontan, C. R., and Kirk, P. L., *J. Pharm. Pharmac.*, 1965, **17**, 362.
5. Hartmann, C. H., *Bull. Env. Contam. & Toxicol.* (*U.S.*), 1966, **1**, 159.

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The Separate Determination of Xanthine and Hypoxanthine in Urine and Blood Plasma by an Enzymatic Differential Spectrophotometric Method

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The enzymatic spectrophotometric determination of oxypurines (hypoxanthine *plus* xanthine) in urine and blood plasma has been extended by the use of differential spectrophotometry at 280 and 292 nm to enable the separate determination of hypoxanthine and xanthine to be carried out. The method retains the high degree of accuracy and specificity of the determination of total oxypurines, and has shown good recoveries and reproducibility when applied to aqueous solutions and to urine. Although less precise when applied to plasma, the method is the only simple method at present available that enables the determination of hypoxanthine and xanthine to be carried out on this material.

AN enzymatic spectrophotometric method for the determination of the "oxypurine" (hypoxanthine *plus* xanthine) content of urine and blood plasma was reported previously,[1] and this paper describes an extension of the method that enables hypoxanthine (6-oxypurine) and xanthine (2,6-dioxypurine) to be measured separately. The term "oxypurine" is used in the present context for hypoxanthine and xanthine only.

EXPERIMENTAL

The stages of the procedure are essentially as previously described.[1] The oxypurines are separated from uric acid, and in the case of plasma from proteins also, by column chromatography to yield an aqueous oxypurine solution.

The present method includes measurement at 280 and 292 nm during the enzymatic reactions, no additional steps or cuvettes being required. The hypoxanthine present in the final solution is determined by measuring the change in extinction at 280 nm (E_{280}) that occurs when the oxypurine is oxidised to uric acid by xanthine oxidase (xanthine: oxygen oxidoreductase, E.C.1.2.3.2.), and the total oxypurine concentration is determined as before by measuring the extinction change at 292 nm (E_{292}) that occurs when the uric acid formed from the oxypurines, is oxidised to allantoin by uricase (urate: oxygen oxidoreductase, E.C.1.7.3.3.). The total oxypurine concentration is corrected for any residual uric acid remaining in the solution after the chromatography, and the concentration of xanthine is determined by the difference between the total oxypurine (hypoxanthine *plus* xanthine) and the hypoxanthine concentrations.

EXPLANATION OF THE METHOD—

The absorption spectra of hypoxanthine, xanthine and uric acid in 0·05 M Tris buffer (pH 8·2), measured with the buffer in the reference cuvette, are shown in Fig. 1.

It can be seen from the figure that xanthine and uric acid have isosbestic points at 249 and 280 nm, while hypoxanthine and uric acid have an isosbestic point at 270 nm. If xanthine oxidase is added to a solution containing the oxypurines the change in extinction at 249 and 280 nm would be caused solely by the hypoxanthine present; as the oxypurines are oxidised to uric acid the extinction changes because of the loss of xanthine and hypoxanthine and the formation of uric acid. The change caused by the loss of xanthine is equalled by a change of opposite sign because of the formation of uric acid; the over-all change in extinction can, therefore, be attributed to the hypoxanthine present. Similarly, the extinction change at 270 nm during a similar reaction is caused solely by the xanthine present in the solution. Thus by measuring the extinction changes at the isosbestic points, the oxypurines can be determined separately.

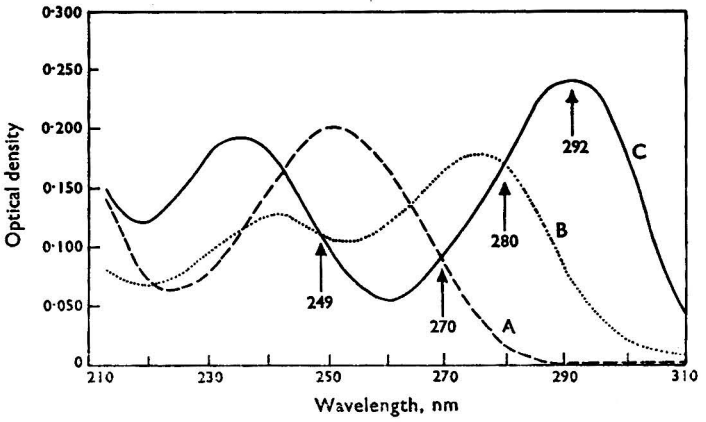

Fig. 1. The absorption of A, hypoxanthine; B, xanthine; and C, uric acid (20 μM concentrations), in 0·05 M Tris buffer (pH 8·2), showing the isosbestic points

Hypoxanthine could be determined in a mixture of the oxypurines by measurement of the extinction changes at 249 nm, but many substances present in biological fluids absorb strongly at this wavelength, so that measurement at this point is not practicable. At 270 nm, where xanthine could be determined in a mixture, an overlap occurs between the xanthine and uric acid spectral absorption curves; enzymatic oxidation of a mixture of xanthine and hypoxanthine at 270 nm is accompanied by a decrease caused by loss of xanthine, and an increase caused by the formation of uric acid from the xanthine; the over-all change in E_{270} is thus less than expected and xanthine cannot be directly determined at 270 nm. The effect of this overlap is similar to that between xanthine and uric acid at 292 nm discussed previously.[1]

Xanthine and uric acid have an isosbestic point at 280 nm where there is a small overlap between hypoxanthine and uric acid, and it has been shown experimentally that the determination of hypoxanthine at this wavelength is subject to minimal interference. This wavelength was therefore chosen for the present differential method. The specificity of the determination of total oxypurine (hypoxanthine *plus* xanthine) discussed earlier has been retained in the present method, and the xanthine concentration is determined by difference between the total oxypurine concentration and that of hypoxanthine.

MICROMOLAR EXTINCTION CHANGES—

Repeated measurements on a standard solution of hypoxanthine gave values for the micromolar extinction changes for the enzymatic conversion of hypoxanthine into uric acid by xanthine oxidase at 280 and 292 nm as follows—

$$\Delta E^{1\mu M}_{280\,nm} = +0{\cdot}0074$$

$$\Delta E^{1\mu M}_{292\,nm} = +0{\cdot}0120.$$

From these micromolar extinction changes, the extinction change at 292 nm from hypoxanthine (HX) alone, in a mixture with xanthine, can be determined—

$$\Delta E^{HX}_{292\,nm} = \Delta E^{HX}_{280\,nm} \times \frac{0{\cdot}0120}{0{\cdot}0074}$$

$$= \Delta E^{HX}_{280\,nm} \times 1{\cdot}62.$$

Multiplication of the extinction change of hypoxanthine, at 280 nm, by 1·62, therefore, gives the corresponding extinction change at 292 nm. The concentration of the hypoxanthine can thus be calculated directly as uric acid by using the same factor (0·0745) as used previously.[1] The xanthine concentration is determined by the difference between this value and the specifically determined total oxypurine concentration.

Similar measurements at 270 and 292 nm with a standard solution of xanthine confirmed that the overlaps referred to above lead to erroneous micromolar extinction changes for xanthine, and measurement at 270 nm cannot be used for the differential analysis.

Method

Apparatus, reagents, collection and preservation of samples are as described previously.[1]

Procedure and calculation—

The preliminary chromatographic separation of the oxypurines and the preparation of the final oxypurine-containing solutions have been described earlier.[1] The modified measuring procedures for urine and plasma, which form the only variations in the method, are described below.

Urine—

The hypoxanthine content of the final solution is determined as described below, by measuring the change in extinction at 280 nm that occurs when the oxypurines are oxidised to uric acid by xanthine oxidase; the total oxypurine content (hypoxanthine *plus* xanthine) is then determined in the same cuvette by measuring the change in extinction at 292 nm when the uric acid formed from the oxypurines is oxidised to allantoin by uricase. Cuvettes additional to the assay cuvette are required as before to correct for change in extinction that results from factors other than the enzymatic reactions.

(*a*) *Assay cuvettes*—To 2·8 ml of 0·05 M Tris buffer (pH 8·2), in a silica cuvette, add 0·1 ml of the test solution, mix by covering with Parafilm and inverting several times, and read the optical density of the solution at 280 nm [E_{280}, reading (i)]. Add 0·1 ml of dilute xanthine oxidase solution, mix, and read E_{280} at 1 to 2-minute intervals until the reaction is complete (5 to 10 minutes), recording the final value of E_{280} [reading (ii)].

Re-set the spectrophotometer at 292 nm and record the reading of the assay cuvette at this wavelength [E_{292}, reading (iii)]. Add 0·05 ml of dilute uricase, mix and read the value of E_{292} after 5 minutes, 30 minutes, and every 10 minutes thereafter, until the reaction is complete (usually 30 to 40 minutes). Record the final value of E_{292} [reading (iv)].

(*b*) *Enzyme blank cuvette*—Mix 2·9 ml of Tris buffer and 0·1 ml of dilute xanthine oxidase and read the value of E_{280} at the same time intervals as the assay cuvette during the xanthine oxidase reaction, recording the final value [reading (v)].

Record the optical density of the contents of the cuvette at 292 nm, when the spectrophotometer has been re-set [reading (vi)], add 0·05 ml of dilute uricase, mix and read at the same time intervals as the assay cuvette during the uricase reaction, recording the final reading of E_{292} [reading (vii)].

(*c*) *Reference cuvette*—Use Tris buffer to set the spectrophotometer to zero at both 280 and 292 nm.

Concurrently determine the residual uric acid content, if any, of the test solution by the following modifications of the method of Liddle, Seegmiller and Laster.[2]

(*a*) *Assay cuvette*—Mix 2·9 ml of Tris buffer and 0·1 ml of the test solution and record the value of E_{292} [reading (viii)]. Add 0·05 ml of dilute uricase and read the value of E_{292}

every 10 minutes until the reaction (if any) is complete, recording the final value of E_{292} [reading (ix)].

(*b*) *Enzyme blank cuvette*—Mix 3·0 ml of Tris buffer and 0·05 ml of dilute uricase and read the value of E_{292} at the same time intervals as the assay cuvette, recording the final value of E_{292} [reading (x)].

(*c*) *Reference cuvette*—This is the same as for the oxypurine determination.

CALCULATION—

(*i*) Residual uric acid in cuvette—

$$\Delta E_{292} = \text{reading (viii)} - [\text{reading (ix)} - \text{reading (x)}] = A.$$

(*ii*) Total oxypurines + residual uric acid—

The true initial reading for uricase reaction = reading (iii) − reading (vi) = a.

The true final reading for uricase reaction = reading (iv) − reading (vii) = b.

$\therefore \Delta E_{292}$ (oxypurines + uric acid) = (a − b) = B.

(*iii*) Total oxypurine (hypoxanthine *plus* xanthine) in cuvette—

ΔE_{292} (oxypurines) = $B - A$.

Therefore, oxypurines in cuvette (≡ 0·1 ml of urine) = $\left[\frac{(B - A) \times 3{\cdot}05}{0{\cdot}0745}\right]$ μg, as uric acid.

(*iv*) Hypoxanthine—

$$\Delta E^{HX}_{280} = \text{reading (ii)} - [\text{reading (i)} - \text{reading (v)}] = C.$$

$$\Delta E^{HX}_{292} = \Delta E^{HX}_{280} \times 1{\cdot}62 = C \times 1{\cdot}62.$$

Therefore, hypoxanthine in cuvette (≡0·1 ml of urine) = $\left[\frac{C \times 1{\cdot}62 \times 3{\cdot}00}{0{\cdot}0745}\right]$ μg, as uric acid.

(*v*) Xanthine—

Subtraction of the concentration of hypoxanthine in the cuvette, expressed as micrograms of uric acid, from the concentration of the total oxypurines (hypoxanthine *plus* xanthine), expressed in the same units, gives the concentration of the xanthine in the cuvette (≡0·1 ml of urine), also as micrograms of uric acid.

The results can be converted into the usual units (milligrams per 24 hours) by the use of the appropriate factors.

PLASMA—

The procedure for the determination of the oxypurines in the final solution obtained from plasma is similar to that described for urine, with the cuvettes below.

(*a*) *Assay cuvette*—To 1·9 ml of 0·05 M Tris buffer in a silica cuvette add 1·0 ml of the test solution, mix and read the optical density of the solution in the cuvette at 280 nm [reading (i)]. Add 0·1 ml of dilute xanthine oxidase, mix and proceed as described for urine, obtaining reading (ii) at 280 nm and readings (iii) and (iv) at 292 nm.

The procedures for (*b*), enzyme blank and (*c*), reference cuvette are the same as those described for urine.

CALCULATION—

The total oxypurine, hypoxanthine and xanthine concentrations in the cuvette, from 1·0 ml of the test solution (≡1·0 ml of plasma), are calculated as described in paragraphs (*i*) to (*v*) above for urine, as micrograms of uric acid.

From these results the oxypurine concentration of the plasma can be calculated as milligrams per 100 ml, as described earlier, and the values can be converted into milligrams of hypoxanthine and xanthine per 100 ml of plasma by multiplication by the appropriate factors.

Results

The extraction procedures for obtaining the oxypurines from urine and plasma have already been tested and satisfactory results obtained.[1] It was, therefore, only required to test the reading method at 280 nm for the recoveries of hypoxanthine from mixtures with xanthine in the presence of urine and plasma extracts.

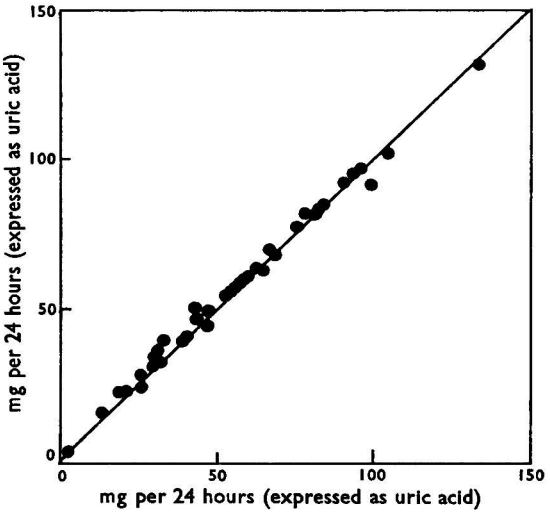

Fig. 2. The reproducibility of the method for the determination of hypoxanthine at 280 nm in urine. Each point represents one analysis in duplicate

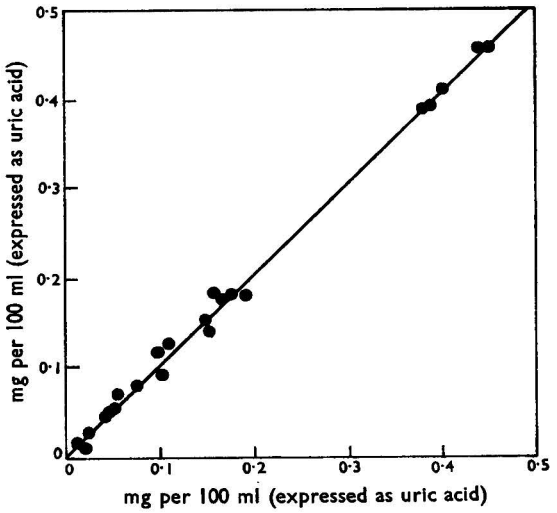

Fig. 3. The reproducibility of the method for the determination of hypoxanthine at 280 nm in plasma. Each point represents one analysis in duplicate

Recoveries of both hypoxanthine and xanthine from aqueous solutions of the oxypurines in varying mixtures, both alone and in the presence of 0·1 ml of urine extract or 1·0 ml of plasma extract, were determined and are shown in Table I. In the table, the results are

TABLE I

RECOVERIES OF HYPOXANTHINE AND XANTHINE FROM STANDARD AQUEOUS SOLUTIONS ALONE, AND IN THE PRESENCE OF EXTRACTS OF URINE AND PLASMA

Each figure represents one duplicate analysis. Results are corrected where necessary for the original oxypurine concentration of the urine or plasma

	Oxypurines added to cuvette, expressed as μg of uric acid			Oxypurines found in cuvette, expressed as μg of uric acid			Recovery, per cent. w/w		
Experiment	Hypoxanthine	Xanthine	Total oxypurines	Hypoxanthine, by measurement at 280 nm	Xanthine, by difference	Total oxypurines, specifically determined at 292 nm	Hypoxanthine	Xanthine	Total oxypurines
Aqueous solution—									
1	2·53	2·53	5·06	2·57	2·38	4·95	101·6	94·1	97·8
2	5·10	5·10	10·2	5·22	4·58	10·2	102·4	89·8	100·0
3	10·2	10·2	20·4	10·4	10·4	20·8 (5)	102·0	102·0	102·4
4	10·2	5·1	15·3	10·4	4·96	15·4	102·0	97·3	100·6
5	5·1	10·2	15·3	5·28	9·95	15·2	103·5	97·5	99·5
6	2·53	10·1	12·6	2·20	10·2	12·4	87·0	101·0	98·4
7	10·1	2·53	12·6	10·1	2·60	12·7	100·0	102·7	100·8
8	1·01	1·01	2·02	1·12	1·02	2·14	111·0	101·0	106·0
9	5·05	2·53	7·58	5·09	2·41	7·50	100·8	95·3	98·9
10	2·53	5·05	7·58	2·55	4·96	7·50	100·8	98·0	98·9
						Mean	101·1	97·9	100·3
Urine—									
1	5·05	5·05	10·1	5·54	4·36	9·90	109·7	86·3	98·0
2	5·05	10·1	15·15	5·54	9·96	15·5	109·7	98·6	102·3
3	10·1	10·1	20·2	10·5	9·60	20·1	104·0	95·0	99·5
4	10·1	5·05	15·15	10·7	4·20	14·9 (5)	106·0	83·2	98·7
5	2·53	10·1	12·6	2·67	9·97	12·6	105·5	98·7	100·0
6	10·1	2·53	12·6	10·5	2·15	12·6 (5)	104·0	85·0	100·4
7	2·53	2·53	5·05	2·42	2·53	4·95	95·7	100·0	98·0
8	5·05	2·53	7·58	5·22	2·36	7·58	103·4	93·4	100·0
9	2·53	5·05	7·58	2·68	4·90	7·58	106·0	97·0	100·0
						Mean	105·0	93·0	99·7
Plasma—									
1	1·01	1·01	2·02	0·80	1·17	1·97	79·2	115·8	97·5
2	2·02	2·02	4·04	1·76	2·33	4·09	87·1	115·4	101·4
3	5·05	5·05	10·1	4·33	5·98	10·3	85·7	118·3	102·0
4	5·05	2·02	7·07	4·55	2·54	7·09	89·9	125·7	100·2
5	2·02	5·05	7·07	1·56	5·48	7·04	77·2	108·5	99·5
6	10·1	10·1	20·2	8·78	11·6	20·4	87·0	114·9	101·0
						Mean	84·3	116·5	100·3

corrected where necessary for the original urine and plasma oxypurine concentrations. Urine analyses were carried out on an extract, from a 4-hour collection of urine from a normal subject, containing 10 mg of uric acid per 25·0 ml and 0·6 mg of oxypurine per 25·0 ml expressed as uric acid. Hypoxanthine and xanthine concentrations were 0·29 and 0·31 mg per 25·0 ml, respectively, expressed as uric acid. Plasma analyses were carried out on extracts from plasma from a normal subject. The plasma was collected with the precautions previously enumerated,[1] and contained 6·5 mg of uric acid per 100 ml, 65 mg of protein per ml (determined by the method of Warburg and Christian)[3] and 0·04 mg of total oxypurines per 100 ml, expressed as uric acid. Hypoxanthine and xanthine concentrations were 0·01 and 0·03 mg per 100 ml, respectively, expressed as uric acid. The subject's diet was not restricted during the collection periods.

The reproducibility of the methods for total oxypurine have been evaluated previously for plasma and urine.[1] The reproducibility of the methods for the determination of hypoxanthine at 280 nm in urine and in plasma are shown graphically in Figs. 2 and 3. The standard deviations (Note 1) for the duplicate determinations on urine and plasma for the determination of hypoxanthine at 280 nm were 1·30 mg per 24 hours, with a standard error of the mean of $\pm$0·14 (forty-two paired observations in the range 2·3 to 133·6 mg per 24 hours, as uric acid), and 0·0092 mg per 100 ml, with a standard error of the mean of $\pm$0·0013 (twenty-four paired observations in the range 0·01 to 0·88 mg per 100 ml, as uric acid), respectively.

NOTE 1—

Standard deviation $= \sigma = \sqrt{\frac{\Sigma d^2}{2n}}$, where d is the difference between duplicate determinations, and n is the number of pairs of determinations.[4] Standard error of the mean $= \frac{\sigma}{\sqrt{2n}}$, where σ is the standard deviation.

DISCUSSION

Enzymatic spectrophotometric methods for the separate determination of xanthine and hypoxanthine in urine were described by Petersen, Jørni and Jørgensen[5] and by Klinenberg, Goldfinger, Bradley and Seegmiller,[6] but neither of these groups of workers was able to extend its method to the analysis of plasma. As in the present investigation, Petersen, Jørni and Jørgensen[5] based their method on the changes of E_{280} that occur when hypoxanthine is oxidised to uric acid by xanthine oxidase (xanthine: oxygen oxidoreductase, E.C.1.2.3.2.), and on the fact that 280 nm is an isosbestic point for xanthine and uric acid, with only slight end absorption from hypoxanthine occurring. The values that these workers obtained for the micromolar extinction changes for hypoxanthine agree closely with the values reported in the present paper. The amount of uric acid formed from the oxypurines during the assay is measured specifically by the decrease in E_{292} that occurs when uric acid is oxidised by uricase (urate: oxygen oxidoreductase, E.C.1.7.3.3.), as previously.[1]

The recoveries of hypoxanthine were low for plasma and slightly high for urine. Xanthine is determined by difference, so that the recoveries of this oxypurine were correspondingly high for plasma and slightly low for urine. The low recovery of hypoxanthine from plasma cannot be caused by the extinction of this purine at 280 nm (Fig. 1) because it did not occur in the recovery experiments with aqueous solutions (Table I). However, it could be explained by the occurrence of an unidentified second reaction that decreases E_{280} during the xanthine oxidase reaction. The slightly high recovery of hypoxanthine from the urine might also be caused by the oxidation of another xanthine oxidase substrate influencing the change in E_{280}. Urine may contain 1-methylxanthine, which is of dietary origin and derived mainly from beverages such as tea and coffee. Xanthine oxidase oxidises 1-methylxanthine to 1-methyluric acid, which has a similar absorption spectrum to uric acid but is not, however, a substrate for uricase. A period of 5 days on a repetitive purine-free diet is allowed for equilibrium before any definitive determinations of plasma and urinary purines are made in human metabolic studies. This ensures that the excretion rates reflect the patient's endogenous pattern of purine production and that interfering substances of dietary origin have been eliminated. It has been shown that allopurinol [4-hydroxypyrazolo(3,4-*d*)pyrimidine] and its metabolite oxipurinol [4,6-dihydroxypyrazolo(3,4-*d*)pyrimidine], which are xanthine

oxidase inhibitors *in vitro* and *in vivo*, and may be present in blood and urine samples submitted for analysis, do not interfere with the enzymatic reactions in the determination. No interference in the reactions at 292 nm was detected in the earlier work,[1] and in the present work full recovery of both hypoxanthine and xanthine added to the urine of a xanthinuric patient undergoing treatment with allopurinol and oxipurinol was effected, thus indicating little or no interference at either 280 or 292 nm.

The present method is only slightly more complex than the determination of the total oxypurines,[1] and although subject to the limitations discussed above, it is the only potentially routine method that can be applied to plasma as well as to urine.

Conclusion

The enzymatic spectrophotometric determination of the oxypurines (hypoxanthine and xanthine) in urine and plasma has been extended to their separate determination by differential spectrophotometry. The method gives good recoveries and reproducibilities for the determination in urine, but the determination in plasma is somewhat less precise. Little alteration in procedure from that described earlier for the total oxypurine determination is required and little additional working time is involved.

We thank the Governors of St. Bartholomew's Hospital for their generous research grants and Professor E. F. Scowen for his continued interest. We also thank the staff of our Metabolic Ward for their help in the collection of the numerous samples for this investigation.

References

1. Chalmers, R. A., and Watts, R. W. E., *Analyst*, **1968**, **93**, 354.
2. Liddle, L., Seegmiller, J. E., and Laster, L., *J. Lab. Clin. Med.*, **1959**, **54**, 903.
3. Warburg, O., and Christian, W., *Biochem. Z.*, **1941**, **310**, 384.
4. Youden, W. J., "Statistical Methods for Chemists," John Wiley and Sons Inc., New York; Chapman and Hall Ltd., London, **1951**, p. 16.
5. Petersen, B. B., Jørni, J., and Jørgensen, S., *Scand. J. Clin. Lab. Invest.*, **1965**, **17**, 454.
6. Klinenberg, J. R., Goldfinger, S., Bradley, K. H., and Seegmiller, J. E., *Clin. Chem.*, **1967**, **13**, 834.

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Precise Manual Enthalpimetric Titrations*

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A procedure for high precision enthalpimetric titrimetry is described, in which titrant is added in small increments at regular intervals in the equivalence-point region of the titration, with temperature measurements being made after each addition. Many of the inaccuracies associated with the widely used continuous titration procedure are eliminated and relative standard deviations of as low as 0·1 per cent. can be achieved.

Although the technique of enthalpimetric titrimetry has been extensively studied during the past 10 years, the precision achieved by almost all investigators has generally not been better than 0·5 to 1·0 per cent. in terms of relative standard deviation.[1] As this technique is applicable principally to the determination of macro amounts, the degree of precision is unsatisfactory.

It seems that the imprecision is attributable to the experimental procedure generally adopted. This procedure involves the continuous addition of titrant from a constant-rate burette, coupled with continuous monitoring of titrand temperature with a thermistor bridge - millivolt chart arrangement. There are several potential sources of error in such a procedure, including variation in burette and recorder speeds; variation in initiation period of the reaction (*e.g.*, the time required to reach critical supersaturation in a precipitation titration); and difficulty in locating exactly the start and the equivalence-points of the titration on thermograms that generally show curvature at both these points because of significant mixing and reaction times and response times of the recording system.

A continuous enthalpimetric titration procedure is thus inherently imprecise. In an attempt to improve the precision of enthalpimetric titrimetry it was decided to abandon the continuous titration technique in favour of a procedure in which titrant, in the region near the equivalence-point, was added in small increments, with temperature measurements being made after each addition. (An incremental addition technique has been described by Goyan, Johnson and Blackwood.[2] These authors, however, followed the entire course of the titration; this required long titration times and the precision was about 1 per cent.)

Experimental

Apparatus—

Burette—A 20-ml Metrohm syringe burette, manually operated.

Thermistor bridge circuit—A standard Wheatstone bridge arrangement in which the F23 thermistor was supplied by Standard Telephones and Cables Ltd. When set at maximum sensitivity a temperature change of 0·05° C results in a full-scale deflection on a 1-mV galvanometer.

Titration flask—A magnetically stirred 200-ml Dewar flask.

Strip-chart recorder—Control Instruments Ltd. "Hi-Speed" recorder that gives full-scale deflection for 1 mV.

Procedure—

The titrand and essential reagents are placed in the reaction vessel and diluted to about 100 ml. The thermistor and burette tips are placed below the stirred liquid surface. About 90 per cent. of the titrant required is added rapidly from the burette. The thermistor circuit is switched on, the sensitivity is set so that a nearly full-scale deflection will occur during the final part of the titration, and the recorder is set to zero. Further additions of titrant are then made in increments of 0·10 ml at regular intervals of 10 or 15 seconds, until well past the equivalence-point, with temperature measurements being made after each addition. These measurements are facilitated by use of the recorder: after each addition of titrant the chart is advanced so that the pen crosses one of the half-inch spaced cross-lines. Each crossing point thus marks the temperature for a known volume of added titrant. Extrapolations

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can then be made directly on the chart. Fig. 1 shows a typical extrapolated thermogram. A titration takes less than 5 minutes to complete.

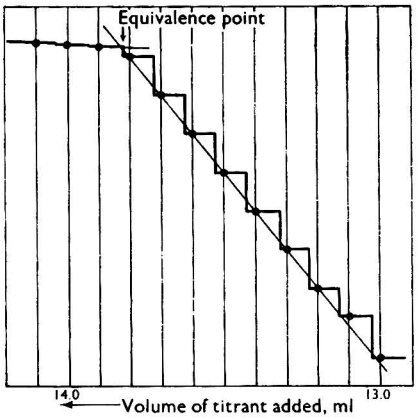

Fig. 1. A typical thermogram in which the vertical lines are the cross-lines on the chart, and the heavy stepped line is the chart pen-trace

Results and discussion

The proposed technique has two major advantages over the continuous titration procedure in that, firstly, extrapolations can be made with much greater accuracy so that titres can readily be measured to 0·01 ml, and secondly the problem of calculating the titre from the thermogram is entirely eliminated as each point on the thermogram represents an exactly known volume of added titrant. Several titrations were performed by using the procedure described; the results are given in Table I.

Table I

Precision results for enthalpimetric titrations

Titrand	Titrant	Number of titrations	Mean titre, ml	Standard deviation, ml	Relative standard deviation, per cent.
0·025 M I^-	0·50 M Ag^+	10	4·98	0·006	0·13
0·0375 M Cl^-	0·50 M Ag^+	10	7·50	0·019	0·25
0·025 M Ce(IV)	0·50 M Fe(II)	9	5·00	0·011	0·21
0·025 M NH_4^+	0·27 M OCl^-	10	13·75	0·013	0·09
0·0125 M I^-	0·27 M OCl^-	7	13·71	0·020	0·15
0·0125 M $S_2O_3^{2-}$	0·27 M OCl^-	5	18·15	0·011	0·06

Note—
The hypochlorite titrations were carried out in bicarbonate - bromide solution.

In each instance the thermograms were ideal, *i.e.*, perfectly linear on either side of the equivalence point. The optimum amount of titrand was in the range 1 to 5 mmoles in 100 ml of solution, giving a temperature rise of about 0·1° C during the final stage of the titration.

The results show that, provided the titration reaction is satisfactory, standard deviations of 0·02 ml or less are obtained. These deviations can probably be attributed more to error in the use of the syringe burette than to the method of equivalence-point detection. As the standard deviation is independent of the total titre, and as titres of up to 20 ml can conveniently be used, relative standard deviations of as low as 0·1 per cent. can be achieved.

References

1. Tyrrell, H. J. V., and Beezer, A. E., "Thermometric Titrimetry," Chapman and Hall Ltd., London, 1968.
2. Goyan, F. M., Johnson, R. D., and Blackwood, R. H., *J. Amer. Pharm. Ass.*, **1961**, **50**, 773.

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Automatic Counter for Radioactive Deposits on Planchets

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An apparatus is described that automatically end-window counts the radioactive deposits on each of twenty-four planchets. Each sample is introduced in turn into a lead castle and counted for a pre-determined time to give a printed record of both the radioactivity and the sample number. The recorder previously described for recording radioactivity in chromatographic-column eluates is incorporated in the apparatus.

The measurement of the radioactivity of many samples can be tedious and time consuming, and requires frequent attention in order to record the results and change the samples. It would often be advantageous to use the overnight period for the assay of several samples previously prepared. The apparatus, shown in Fig. 1, was designed to use an end-window Geiger - Müller detector, a standard scaler and the digital print-out recorder previously described to give, automatically, a printed record of the radioactivity on each of twenty-four planchets.

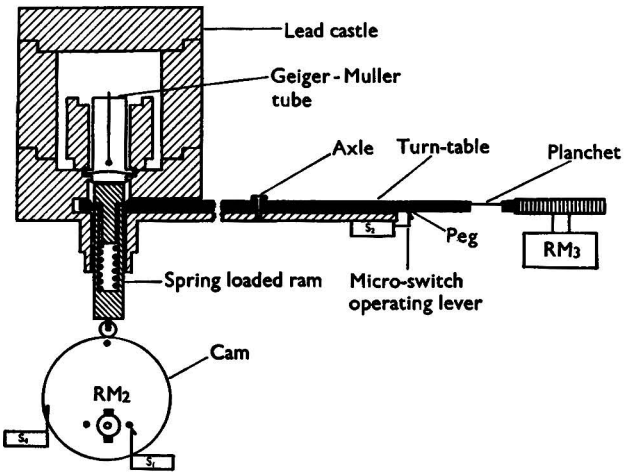

Fig. 2. Turn-table and lead castle

Principle of the method—

One planchet is placed in each of the twenty-four circumferential holes of the turn-table (Fig. 2). When rotation of the turn-table brings a planchet directly under the Geiger - Müller detector a ram raises the planchet out of the turn-table and accurately positions it against a collar beneath the Geiger - Müller tube. The pulses from the Geiger - Müller tube pass initially through a conventional scaler, and are then diverted to an interrogation circuit for 30 seconds. Depending on the count-rate, the circuit selects pulses from either the X1, X10 or X100 outputs of the scaler, thus bringing the count-rate within the range of the

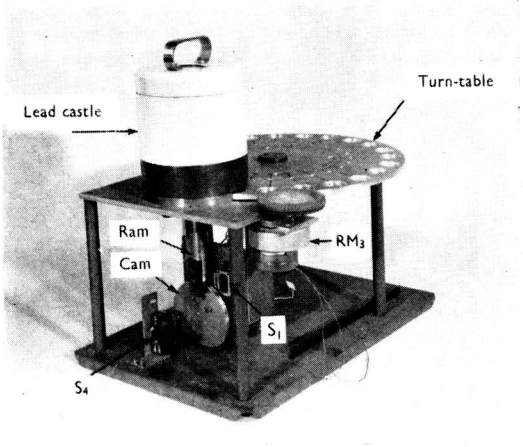

Fig. 1. Diagram of apparatus

To face page 236]

electromechanical print-out device. The counts from the selected output are accumulated during a pre-set period in the print-out device previously described.[1] When the pre-set time interval has elapsed the total counts and the number of the sample are printed out, the recorder re-set and the planchet lowered into the turn-table, which then rotates to bring the next planchet into position under the detector.

SEQUENCE OF OPERATIONS—

(*i*) On completion of a count the total count is printed out, together with the planchet number, and the recorder re-sets.

(*ii*) The ram lowers the planchet into the turn-table.

(*iii*) The turn-table rotates to align a planchet beneath the counting head.

(*iv*) The ram raises the planchet into the counting position and the timer and scaler are re-set.

(*v*) Counts pass to the interrogate circuit for 30 seconds. If this circuit receives a pulse from X100 output, a relay selects X10 output from the scaler for connection to the recorder. If, subsequently, a pulse is received from X1000 output, the X100 is connected to the recorder; asterisks are simultaneously printed on the final paper record.

(*vi*) Pulses from the selected decade of the scaler are accumulated in the recorder for a time pre-set on a seconds counter.

DESCRIPTION OF CIRCUIT—

The system (shown in Fig. 3) for determining the length of time each sample is counted is based on a synchronous motor RM_1 continuously connected to the mains supply, with an output shaft running at 1 r.p.s., and a pre-determining counter RLE. In the latter any number up to 9999 can be manually pre-set and, on receipt of impulses, the device then counts downwards towards zero. When zero is reached a contact RLE/1 is made. A subsequent re-set pulse applied to its auxiliary coil L3 returns the counter to the pre-set number. (This timing method, as in most commercial scalers, is dependent on the stability of the mains frequency.)

A disc with a 36° slot in it is mounted on the axle of the motor RM_1 so as to lie in the path of the light beam from the bulb on to the photo-conductive cell PEC_1.* The dark resistance of the cell is high (greater than 1 MΩ) and the voltage across R_{25} is insufficient to cause base current to flow into Tr_1. When illuminated, the resistance of PEC_1 drops, the voltage across R_{25} rises and base current flows into Tr_1, thus Tr_1 and hence Tr_2 pass into full collector current. Thus the counter RLE is energised for about 100 ms every second until it reaches zero when contact RLE/1 is made. When RLE/1 makes, C_{10} charges via D_{11}, R_{15}, R_{13} and R_{12} and applies a positive pulse to the trigger of SCR_1, thus making the SCR conduct and discharge C_9 through the uniselector coil DM_1; the wiper then steps from contact 1 to 2. The relay RLB is thus energised while relay RLA releases. When RLA releases, the count input to the recorder is disconnected (RLA/2), and the contact RLA/1 is made, which starts the recorder on its print-out and re-set cycle.

RLB/1 energises the relay RLF in the EKCO N530 scaler, which re-sets the scaler through contact RLF/1. With RLB energised, RLB/2 opens to disconnect the anode supply to SCR_2 and SCR_3, which de-energises relays RLG and RLH if they have previously operated. RLB/3 is made and the motor RM_2 lowers the ram until the cam operates the microswitch S_1. During this process an additional peg on the cam closes the microswitch S_4, which re-sets the "seconds" counter through its auxiliary coil L3. C_9 re-charges and when S_1 makes, a positive pulse is applied to the trigger of SCR_1 via D_{12}, R_{17} and R_{13}, which causes SCR_1 to conduct, discharging C_9 through the uniselector and moving the latter to position 3. Relay RLC is thus energised via 3. RLC/1 connects the motor RM_3 to the mains supply, which drives the turn-table until the next one of twenty-four pegs inserted at equal intervals in a circle under the turn-table operates the microswitch S_2 (C_9 having re-charged). This causes stepping of the uniselector to position 4 by means of SCR_1.

* Initially a cam mounted on the motor shaft was used to operate a microswitch once every second. However, in protracted use the microswitch became unreliable in operation.

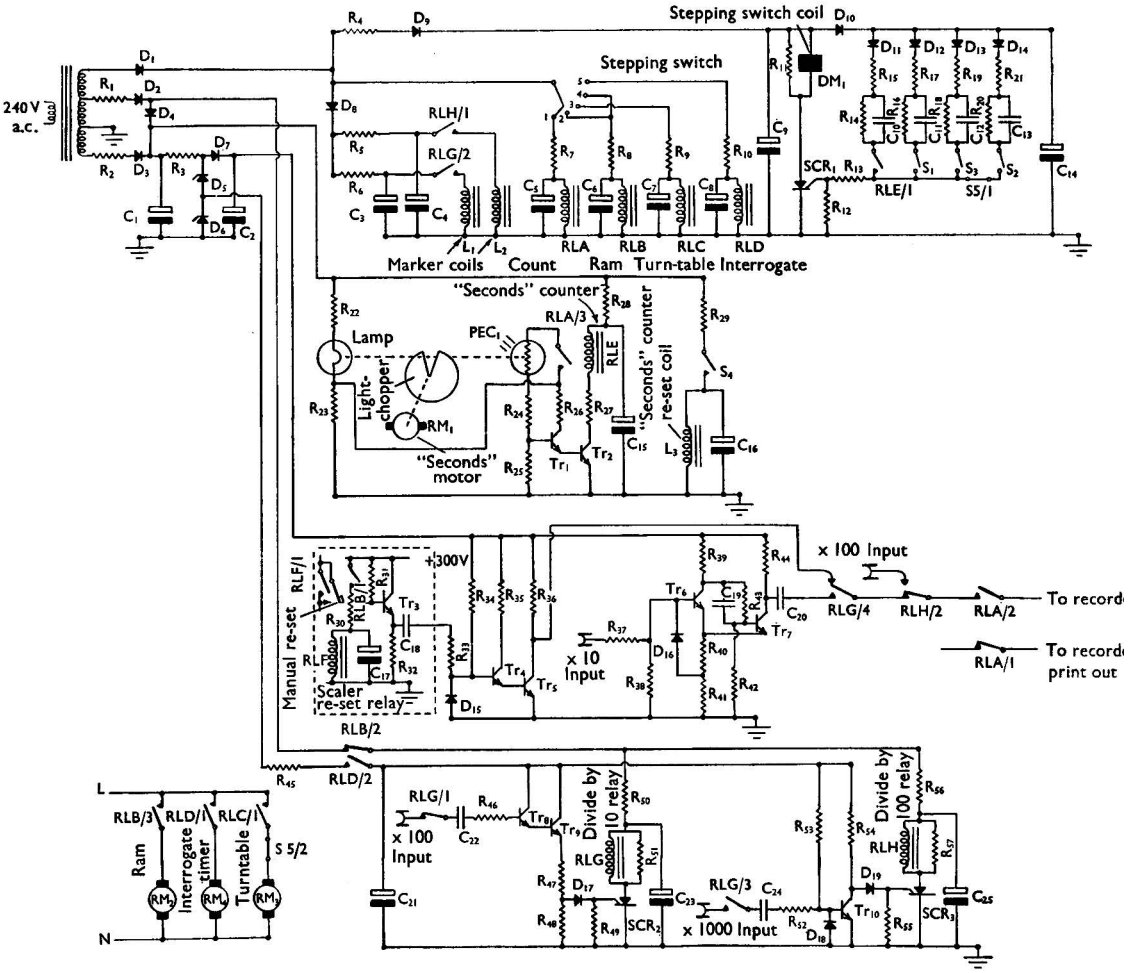

Fig. 3. Diagram of circuit.
(For list of components, see Appendix, p. 239)

RLB is thus re-energised, RLB/3 makes, and the motor RM_2 raises the ram and moves the stud from S_1; S_1 opens and C_{11} discharges through R_{16}. When S_1 makes, SCR_1 triggers by way of D_{12}, R_{17}, R_{13} and C_{11} and the uniselector steps to position 5. Relay RLD now makes, connecting the motor RM_4 to mains by means of RLD/1 and applying power to the "divide" circuit through RLD/2. RLG/1 connects the X100 output from the scaler to C_{14}. If a positive pulse is received, the transistor pair emitter follower Tr_8 and Tr_9 delivers a positive pulse to the trigger of SCR_2, which conducts and stays in the conducting state because the current through RLG and R_{51} is greater than its maintaining current. RLG/2 discharges C_3 through marker coil L_1, RLG/3 connects the X1000 output to point C_{24} and RLG/4 changes over. If a negative output pulse is received transistor Tr_{10} stops conducting. The resultant positive pulse fires SCR_3, which then stays on because the current through RLH and R_{57} is greater than its maintaining current. RLH/1 makes and discharges C_4 through the marker coil L_2. After 30 seconds, the cam on the motor RM_4 operates the microswitch S_3 and SCR_1 is fired by means of D_{13}, R_{19}, R_{13} and C_{13}. The uniselector steps to position 1. Relay RLA is then made and pulses are fed into the recorder via RLA/2. If neither of the "divide" relays RLG and RLH has operated, pulses from the emitter follower Tr_3 pass through the inverter circuit Tr_4 and Tr_5 and thence via the normally closed positions of

RLG/4 and RLH/2 into the recorder via RLA/2. If relay RLG has operated, pulses from the X10 output which have been lengthened by the pulse-shaping circuit Tr_6 and Tr_7 pass through C_{20} and the unenergised position of RLH/2 to the recorder. If RLH has operated, pulses from the X100 decade of the scaler pass via RLA/2 to the recorder.

The switch S_5 permits interruption of the automatic cycling as soon as the counting process finishes and the planchet has been returned to the turn-table. The turn-table can then be manually rotated for re-loading and, as soon as S5 returns to its "make" positions, the automatic process recommences.

TABLE I

REPRODUCIBILITY OF RESULTS OBTAINED WITH THE APPARATUS

Sample	Planchet No.	Total counts in 2000 seconds		
		Count 1	Count 2	Count 3
C-14 standard	1	703 × 100	6909 × 10	691 × 100
Alanine-C14, 0·1 ml	2	6575 × 100	6545 × 100	6494 × 100
	3	6459 × 100	6409 × 100	6379 × 100
	4	6669 × 100	6651 × 100	6619 × 100
Background	5	263	373	330
Alanine-C14, 0·1 ml	6	1183 × 100	1177 × 100	1167 × 100
	7	1104 × 100	1107 × 100	1102 × 100
	8	1117 × 100	1121 × 100	1112 × 100
Background	9	283	343	314
Glutamine-C14, 0·1 ml	10	5664 × 100	5643 × 100	5658 × 100
	11	6422 × 100	6432 × 100	6424 × 100
	12	5995 × 100	6002 × 100	6037 × 100
*Code No. CS7, 0·5 ml from 17 ml	13	4316 × 10	4332 × 10	4351 × 10
	14	4095 × 10	4091 × 10	4162 × 10
	15	4259 × 10	4241 × 10	4283 × 10
*Code No. CS8, 0·5 ml from 20 ml	16	1628 × 10	1674 × 10	1698 × 10
	17	1728 × 10	1703 × 10	1694 × 10
	18	1748 × 10	1784 × 10	1747 × 10
*Code No. CS9, 0·5 ml from 18·5 ml	19	2225 × 10	2243 × 10	2255 × 10
	20	2304 × 10	2267 × 10	2270 × 10
	21	2245 × 10	2207 × 10	2313 × 10
*Code No. CS10, 0·5 ml from 18·0 ml	22	3314 × 10	3309 × 10	3416 × 10
	23	3457 × 10	3431 × 10	3435 × 10
	24	3328 × 10	3326 × 10	3382 × 10

* Samples obtained during experimental work on movement of labelled amino-acids in apple shoots.[2]

The reproducibility of results obtained with this apparatus is shown in Table I, which gives measurements on typical samples encountered in practice. The values for replicates show good agreement, comparable with that achieved with individual manually recorded measurements with a conventional scaler.

The authors thank Mr. R. S. Cole for constructing the turn-table and lead castle assembly.

Appendix

LIST OF COMPONENTS USED IN THE CONSTRUCTION OF THE APPARATUS

R_1 and R_2	= 10-ohm, 5-watt resistors
R_3	= 2500-ohm, 5-watt resistor
R_4	= 22,000-ohm, 2-watt resistor
R_5 and R_6	= 47,000-ohm, 2-watt resistors
R_7, R_8	= 3300-ohm, 2-watt resistors
R_9, R_{10}	= 6800-ohm, 2-watt resistors
R_{11}	= 10,000-ohm, 2-watt resistor
R_{12}	= 1000-ohm, ½-watt resistor
R_{13}	= 2200-ohm, ½-watt resistor
R_{14}, R_{16}, R_{18}, R_{20}	= 22-megohm, ½-watt resistors
R_{15}, R_{17}, R_{19}, R_{21}	= 2200-ohm, ½-watt resistors
R_{22}, R_{23}	= 1000-ohm, 3-watt resistors
R_{24}	= 10,000-ohm, ½-watt resistor
R_{25}	= 2200-ohm, ½-watt resistor
R_{26}	= 8200-ohm, ½-watt resistor

R_{27} = 220-ohm, ½-watt resistor
R_{28} = 5000-ohm, 5-watt resistor
R_{29} = 24,000-ohm, 5-watt resistor
R_{30} = 10,000-ohm, 1-watt resistor
R_{31} = 6800-ohm, ½-watt resistor
R_{32} = 1-megohm, ½-watt resistor
R_{33} = 220,000-ohm, ½-watt resistor
R_{34} = 10-megohm, ½-watt resistor
R_{35} = 10,000-ohm, ½-watt resistor
R_{36} = 22,000-ohm, ½-watt resistor
R_{37} = 4·7-megohm, ½-watt resistor
R_{38} = 4·7-megohm, ½-watt resistor
R_{39}, R_{40}, R_{44} = 2200-ohm, ½-watt resistors
R_{41} = 3300-ohm, ½-watt resistor
R_{42} = 150,000-ohm, ½-watt resistor
R_{43} = 100,000-ohm, ½-watt resistor
R_{45} = 470-ohm, ½-watt resistor
R_{46} = 3·9-megohm, ½-watt resistor
R_{47} = 2200-ohm, ½-watt resistor
R_{48} = 4700-ohm, ½-watt resistor
R_{49} = 820-ohm, ½-watt resistor
R_{50} = 3300-ohm, 2-watt resistor
R_{51} = 10,000-ohm, ½-watt resistor
R_{52} = 3·9-megohm, ½-watt resistor
R_{53} = 470,000-ohm, ½-watt resistor
R_{54} = 15,000-ohm, ½-watt resistor
R_{55} = 820-ohm, ½-watt resistor
R_{56} = 3300-ohm, 2-watt resistor
R_{57} = 10,000-ohm, ½-watt resistor

All ½-watt resistors were 5 per cent. accuracy HYSTAB type (Radiospares Ltd.); all others were 10 per cent. accuracy.

C_1 = 300-μF capacitor, 275-volt electrolytic working
C_2 = 500-μF capacitor, 50-volt electrolytic working
C_3, C_4 = 64-μF capacitor, 450-volt electrolytic working
C_5, C_6, C_7, C_8 = 8-μF capacitor, 150-volt electrolytic working
C_9 = 16-μF capacitor, 450-volt electrolytic working
C_{10}, C_{11}, C_{12}, C_{13} = 0·047-μF capacitor, 400-volt paper working
C_{14} = 4-μF capacitor, 500-volt electrolytic working
C_{15} = 100-μF capacitor, 275-volt electrolytic working
C_{16}, C_{17} = 8-μF capacitor, 275-volt electrolytic working
C_{18}, C_{19}, C_{20} = 1000-pF capacitor, 500-volt paper working
C_{21} = 50-μF capacitor, 50-volt electrolytic working
C_{22} = 100-pF capacitor, 500-volt paper working
C_{23}, C_{25} = 8-μF capacitor, 275-volt electrolytic working
C_{24} = 470-pF capacitor, 500-volt paper working
D_1, D_8, D_9, D_{10}, D_{11}, D_{12}, D_{13}, D_{14} = Mullard BYX 10
D_2, D_3, D_4 = Mullard BYX 22–400
D_5, D_6 = Mullard BZY 95–C20
D_7 = Mullard BYX 22–200
D_{15}, D_{16}, D_{17}, D_{18}, D_{19} = Mullard OA 200
Tr_1, Tr_2 = Motorola MJE 340 transistors
Tr_3 = Mullard BFX 85 transistor
Tr_4, Tr_5 = Mullard SN 930 2N 930 transistors
Tr_6, Tr_7 = Motorola MPF 105 transistors
Tr_8, Tr_9 = Mullard GFY 51 BFY 51 transistors
Tr_{10} = Mullard BFY 52 transistor
SCR_1 = Mullard BTY 79–400R
SCR_2, SCR_3 = Thorn - A.E.I. 106 B1
Transformer
Secondaries { 0 to 150-volt, 150 mA; 100 to 0 to 100-volt, 150 mA }
Lamp 50-volt, 2·5-watt

RLA = P.O. 3000 type 6000-ohm coil
RLA/1 5A silver, break
RLA/2, RLA/3 twin gold, make

RLB = P.O. 3000 type, 6000-ohm coil
RLB/1, RLB/3 5A silver, make
RLB/2 twin gold, break

RLC = P.O. 3000 type, 10,000-ohm coil
RLC/1 5A silver, make

RLD = P.O. 3000 type, 10,000-ohm coil
RLD/1 5A silver, make
RLD/2 twin gold, make

RLF = P.O. 3000 type, 10,000-ohm coil
RLF/1 5A silver, make

RLG = P.O. 3000 type, 6000-ohm coil
RLG/1 twin gold, break
RLG/2 5A silver, make
RLG/3 twin gold, make
RLG/4 twin gold, change-over

RLH = P.O. 3000 type, 10,000-ohm coil
RLH/1 5A silver, make
RLH/2 twin gold, change-over

RLE = Pre-determing counter (type No. 1506, Counting Instruments Ltd., Boreham Wood), 0 to 9999 counts, 24-volt count coil. Integral re-set coil (L_3) for 24-volt operation

L_1, L_2 = Solenoids (type SCM/HOR/50/50/2, Webber Bros. Ltd., Bristol), mounted inside the Elmeg counter[1] to print asterisks on recording paper.

DM_1 = Rotary stepping switch, 24-volt d.c., 24-ohm coil, 1 level, 10 non-bridging outlets. Outlets 1 and 6, 2 and 7, 3 and 8, 4 and 9, 5 and 10, connected in pairs to give 1 level 5 outlets (Thorn Electrical, type 40)

RM_1 = Synchronous motor 240-volt, 50 Hz, 1 r.p.s. (type Q1M/60/RMC400, Smiths Instruments Ltd.)

RM_2 = Asynchronous motor 240-volt, 50 Hz, 4 r.p.m. (type 955, Crouzet)

RM_3 = Synchronous motor 240-volt, 50 Hz, $\frac{1}{2}$ r.p.m. (type 875, Crouzet)

RM_4 = Synchronous motor 240-volt, 50 Hz, 2 r.p.m. (type Q1M/2/RMC200, Smiths Instruments Ltd.)

S_1, S_2, S_3, S_4 = Bulgin microswitches

S_5 = Bulgin two-pole change-over switch

References

1. Lloyd-Jones, C. P., and Skerrett, E. J., *Analyst*, 1964, **89**, 782.
2. Hill-Cottingham, D. G., and Lloyd-Jones, C. P., *Nature*, 1968, **220**, 389.

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Apparatus for Vapour-phase Kinetic Studies of Organic Compounds*

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An apparatus is described that has proved to be particularly useful in studying the vapour-phase kinetics of pyrolysis of high-boiling liquid or solid organic compounds. The design includes a stainless-steel reaction chamber, a spring-operated closing valve, a hypodermic-needle injection assembly and a null-point pressure sensing device consisting of a stainless-steel diaphragm. The pressure change is automatically recorded on a strip-chart recorder. Reproducible results (to within ±2 per cent.) are readily obtainable with either a packed or unpacked seasoned reactor.

Kinetic studies of vapour-phase reactions are generally restricted to compounds sufficiently volatile to be distilled into the hot zone without thermal decomposition. By using the apparatus described in this paper a study of the vapour-phase kinetics of a wide variety of organic liquids and solids of low volatility was carried out without any difficulty in introducing the sample or in measuring the pressure change during the pyrolysis. Samples are introduced into an evacuated hot zone as liquids or in a suitable solvent with a hypodermic needle.

The design includes automatic pressure measuring and recording equipment for relatively fast reactions that involves the use of a null-point gauge capable of handling high temperatures, adjustable sensitivity and a pressure transducer that activates a strip-chart recorder. The reaction chamber is designed so that introduction of packing materials for studies involving surface-to-volume ratios is simple; the rapidity of action of the spring-operated closing valve necessitates the use of only a small amount of reactant; the system is leak-proof. A scale drawing is shown in Fig. 1, a list of components is given in the Appendix (p. 244), and details of construction, sources of materials not considered standard and operational details are available on request from the authors.

The equipment is easy to operate, essentially trouble free, and the kinetic results are reproducible to within ±2 per cent. The correlation coefficient for the Arrhenius plots of thirty-six different esters was better than 0·988 and, because of the incorporation of automatic pressure measuring equipment, rapid reactions can be studied. For example, some of the reactions studied have been completed in less than 4 minutes ($k = 4 \times 10^{-2}$ second^{-1}), which is considered a fast reaction in a static reactor. Surface-to-volume ratio studies can also be readily made with this design in investigations to determine the homogeneity of the reaction.

The reaction chamber is cylindrical, 5 cm o.d. × 10 cm long, with a volume of about 200 ml; at each end is a mounting block 2 cm deep (items 9 and 11, Fig. 1). The valve head (item 33, Fig. 1) is a tapered unit, which is sealed into a copper seat that also acts as a gasket to seal the chamber from the valve supporting system. The hollow stem of the valve emerges through a Viton O-ring seal (item 28, Fig. 1); a spring-loaded closing device (item 16, Fig. 1) capable of being held open in two positions, one for ready evacuation of the chamber and the other, which partially seals off the evacuation system, is the position preferred for injection. Attached to the end of the stem is the septum holder that forms an air-tight seal (when provided with a silicone rubber strip). The hole in the centre of the stem emerges through the valve head in a position which can be sealed to the outside of the chamber when the valve is closed. The septum, which is placed at the outside end of the hollow rod, is a strip of silastic silicone rubber which can be readily moved along after each sample injection.

At the other end of the cylinder is the null-point sensing device for the pressure monitoring system (A, Fig. 1), consisting of a thin 0·076-mm stainless-steel diaphragm (item 34, Fig. 1) with three to four concentric indentations for greater uniform flexibility. The diaphragm is silver soldered into the body of the mounting block, and silver soldered in the centre of

* Summary of the Ph.D. thesis presented by J. A. Kirby to the Utah State University in June, 1967.

the outside of the diaphragm is a 6-mm diameter smooth polished disc of brass, which serves as one of a pair of electrical contacts. The other contact is a threaded steel rod (item 6) with a 60° tapered point. Although brass and stainless steel have different thermal expansion this has not caused any difficulty. The steel rod can be screwed in and out, thus the sensitivity of the null-point gauge can be adjusted so that the greater the gap between the two contacts the greater the pressure required to close the circuit, and *vice versa*. After years of use some corrosion occurs, necessitating cleaning of the two contacts with concentrated hydrochloric acid, followed by thorough washing with sodium hydrogen carbonate solution, water and acetone. The null-point gauge, once adjusted, is sensitive to a pressure change of 0·05 mm.

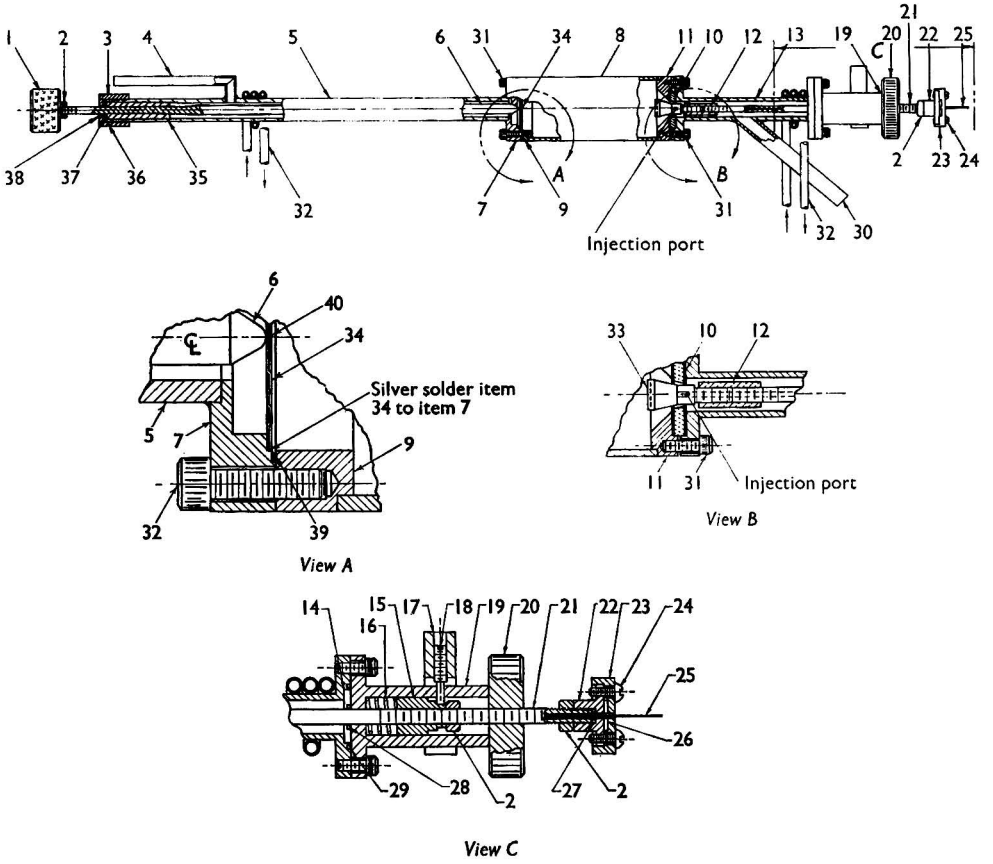

Fig. 1. Apparatus assembly for vapour-phase kinetic studies. Details of the diaphragm; view *A*, item 34; view *B*, injection port; and view *C*, valve system

The pressure monitoring system consists of a relay circuit actuated by the null-point contacts at the diaphragm (A, Fig. 1), already discussed. This circuit operates a solenoid valve that controls the inlet of nitrogen to the outside of the diaphragm. When a sufficiently large amount of nitrogen enters, the pressure is sufficient to cause the contacts to open. The amount of nitrogen introduced is subject to the pressure in the large ballast and the setting on the needle valve. The pressure is monitored by a pressure transducer, the output of which is plotted on a strip-chart recorder.

The output of the transducer is linear to within ± 1 per cent., as certified by the manufacturer and also as determined by Blau.[1] The valve is 0 to 30 mV over the full operating range, 0 to 1600 mm of mercury. By using a back e.m.f. system, a 2-mV recorder measures only the last 2-mV output of the transducer. The cold-water copper cooling coils (item 32, Fig. 1) protect the nylon support and Viton O-ring, and permit handling of the valve end of the reactor.

The stainless-steel reactor is placed in the centre of a large (12-inch diameter), well lagged, aluminium block, which is heated by two 450-watt immersion heaters activated by a carefully regulated voltage.[2,3] The temperature is measured by two chromel-alumel thermocouples wired in series to an ice - water junction and calibrated against a Bureau of Standards calibrated platinum resistance thermometer every month. Each week the calibration is also accomplished by determining the rate of pyrolysis of the 1-phenylethyl acetate used as a standard of comparison, and whose rate constant has been determined by several workers immediately following the calibration of the thermocouples. The results in Table I illustrate some of the recent kinetic results obtained with this equipment on both liquid and solid compounds.[4 to 12]

TABLE I

RATE CONSTANTS IN THE THERMAL DECOMPOSITION OF ESTERS

Ester	Temperature, °C	$k \times 10^3$	k average $\times 10^3$
ACETATES—			
$(CH_3)_3COAc$	295·6	3·43, 3·23, 3·32, 3·38, 3·39	3·31
$C_6H_5CHCH_3OAc$	391·3	7·82, 7·92, 7·84	7·86
o-$CH_3C_6H_4CHCH_3OAc$	374·9	12·4, 11·9, 12·1	12·1
cyclo-$C_3H_5C(CH_3)_2OAc$	285·3	10·0, 10·1, 10·0, 10·1	10·05
$CH_2=CHCH_2C(CH_3)_2OAc$	292·3	4·21, 4·23, 4·20	4·21
$CH_3CH=CHCHCH_3OAc$	376·0	20·1, 20·3, 20·0, 20·2	20·1
$(CH_3)_2CH(CH_3)_2OAc$	275·9	2·79, 2·76, 2·60	2·73
$C_6H_5C(CH_3)_2OAc$	294·3	1·31, 1·28, 1·26	1·28
$CH_2=CHC(CH_3)_2OAc$	295·3	8·43, 8·43, 8·36	8·41
cyclo-$C_3H_5CH(CH_3)OAc$	373·0	20·3, 20·7, 20·5, 20·4	20·5
BENZOATES—			
$C_6H_5CHCH_3OCOC_6H_5$	385·9	2·82, 2·86, 2·81, 2·81	2·83
o-$CH_3OC_6H_4CHCH_3OCOC_6H_5$ (solid dissolved in cyclohexane)	358·3	14·3, 14·5, 14·8, 14·6	14·6
o-$ClC_6H_4CHCH_3OCOC_6H_5$	370·7	6·92, 7·14, 7·15, 7·09	7·09
CARBONATES—			
p-$CH_3OC_6H_4CHCH_3OCO_2CH_3$	326·4	18·9, 18·2, 17·9, 18·2	18·3
m-$NO_2C_6H_4CHCH_3OCO_2CH_3$	364·9	11·9, 12·6, 12·6	12·4
$C_6H_5CHCH_3OCO_2CH_3$	359·6	29·3, 28·9, 28·8, 28·8	28·9

We thank the National Science Foundation, No. GP6006, and the University Research Council, Utah State University, No. U–202, for the grants received, and Mr. George Eddington, machinist at Utah State University, for his assistance.

Appendix

LIST OF COMPONENTS

Item		Item	
1	Contact adjusting knob	23	Septum cap
2	Lock nut	24	Round head machine screw
3	Cap	25	Needle (hypodermic)
4	Nitrogen inlet	26	Rubber septum
5	Housing	27	Rubber septum seal
6	Contact rod	28	O-Ring in spring valve assembly ($\frac{3}{8}$ inch o.d., $\frac{1}{16}$-inch wall)
7	Diaphragm mounting block		
8	Reacting chamber	29	O-Ring in spring valve assembly ($1\frac{1}{8}$ inch o.d., $\frac{1}{16}$-inch wall)
9	Mounting block		
10	Valve seat	30	Vacuum outlet
11	Mounting block	31	Socket head cap screw
12	Nipple	32	Cooling coils (copper)
13	Exhaust housing	33	Valve head
14	Washer	34	Diaphragm
15	Valve positioning nut	35	Nylon support
16	Spring	36	O-Ring Parker contact adjusting assembly ($\frac{1}{4}$ inch o.d., $\frac{1}{16}$-inch wall)
17	Valve release		
18	Retaining pin	37	O-Ring Parker contact adjusting assembly ($\frac{3}{8}$ inch o.d., $\frac{1}{16}$-inch wall)
19	Spring housing		
20	Valve lock wheel	38	Nylon bushing insulator
21	Valve stem	39	Copper gasket
22	Septum mount	40	Electrical terminal silver solder

References

1. Blau, S. E., M.S. Thesis, Utah State University, Logan, Utah, 1963.
2. Smith, G. G., and Bagley, F. D., *Rev. Scient. Instrum.*, 1961, **32**, 703.
3. Smith, G. G., and Jones, D. A. K., *J. Org. Chem.*, 1963, **28**, 3496.
4. Smith, G. G., Bagley, F. D., and Taylor, R., *J. Amer. Chem. Soc.*, 1961, **83**, 3647.
5. Taylor, R., Smith, G. G., and Wetzel, W. H., *Ibid.*, 1962, **84**, 4817.
6. Smith, G. G., and Blau, S. E., *J. Phys. Chem.*, 1964, **68**, 1231.
7. Smith, G. G., and Taylor, R., *Chem. & Ind.*, 1961, 949.
8. Smith, G. G., and Yates, B. L., *J. Chem. Soc.*, 1965, 7242.
9. ——, ——, *J. Org. Chem.*, 1965, **30**, 434.
10. Smith, G. G., Pospisil, J., and Kirby, J. A., Abstract of Papers, American Chemical Society Meeting, September, 1966, New York, N.Y., S–100.
11. Smith, G. G., Lum, K. K., Abstract of Papers, American Chemical Society Meeting, San Francisco, California, April 1, 1968, P–035.
12. Kirby, J. A., Ph.D. Thesis, Utah State University, 1967.

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Book Reviews

KIRK-OTHMER ENCYCLOPEDIA OF CHEMICAL TECHNOLOGY. Volume 15. Second Edition. PETROLEUM (REFINERY PROCESSES) TO POISONS, ECONOMIC. Edited by HERMAN F. MARK, JOHN J. MCKETTA, JUN., DONALD F. OTHMER and ANTHONY STANDEN. Pp. xiv + 923. New York, London, Sydney and Toronto: Interscience Publishers, a division of JohnWiley & Sons, Inc. 1968. Price £23 10s.; price per volume for subscribers to the complete set of 18 volumes, £17 10s.

By far the most important series of monographs (see also *Analyst*, 1963, **88**, 899 *et seq.*) in this latest issue of this Encyclopedia is contained in the 111 pages dealing with Petroleum (Refinery Processes), Petroleum (Products) and Petroleum Waxes. The first part, as may be expected, is largely engineering in character; and the petroleum products are dealt with separately from petrochemicals which, presumably, were disposed of in an earlier volume. It is estimated that about 2500 products are currently produced, wholly or partly, from petroleum, in addition to 3000 petrochemicals. The petroleum waxes are an important member of the petroleum products group and, therefore, merit a monograph of their own. The principal groups dealt with are paraffin waxes, microcrystalline waxes and petrolatum. The series makes a highly interesting and informative monograph, but with few analytical implications.

Pharmaceuticals, another important monograph, occupies only 21 pages and is concerned chiefly with compounds involved in the Food & Drug Administration legislation of the United States. An interesting section of this monograph deals with the use of the computer, for example, in the design of fractionation columns and processes. Also classifiable among industrial processes is the section on photochemical technology, which starts with a theoretical introduction and applies the information so derived to commercial photochemical synthetic processes. Examples of these are mercaptan formation and the production of polymer systems. Batch and continuous reactors for carrying out photochemical reactions, particularly chlorination, are described, and provide some normally inaccessible information on a recent method of technology.

The monographs on Pilot Plants (33 pages), Piping Systems (29 pages) and Plant Layout and Plant Location (31 pages) have, of course, a strong industrial bias. It is, however, pleasing to be able to record that the rôle of analytical work is fully appreciated; thus (p. 615), "In chemical-process development work there is hardly anything more important than chemical analysis. Chemists must develop procedures for these analyses, and, if at all possible, should make them simple enough for them to be used by pilot-plant personnel in the plant. Pilot-plant managers must balance the greater utility for chemical analysis of a close physical location of analytical laboratory and pilot-plant against the greater freedom in design and operation that a plant location gives to the pilot plant." There is more in this strain, and it sets the trend for the whole of the monograph.

Plastics Technology (21 pages) is the only other monograph with a highly industrialised slant, but following this is a 21-page monograph on Plastics Testing, which is of great interest, although it is, of course, not confined to purely chemical methods. Determinations of molecular-weight distribution and of molecular weight itself provide an important section of this monograph, and there is a useful (if short) section on the identification of plastics from simple tests such as behaviour on burning. Gel-permeation chromatography, which is particularly useful for molecular-weight distribution work, is a more sophisticated aspect of this particular branch of the study. The evaluation of the non-polymeric additives is less specialised, and the same applies to additives such as inorganic or fibrous solids. The monograph on Plasticizers deals with the analytical aspects of the subject less than with methods of deciding which is the best plasticiser for use in any particular circumstances. This latter approach is very thorough and is enhanced by tables of properties and compatibilities.

Organic chemistry has been well represented under every letter of the alphabet, and this also applies in the present instance. Phenol itself has a 13-page monograph, but its derivatives, such as Phenolic Aldehydes, Phenolic Ethers and Phenolic Resins, cover, in all, some 60 pages. All these compounds are of industrial importance, and this aspect of their utilisation is given

priority. However, the analytical methods are confined to those which assist the industrial operations, and are not dealt with in any great depth. Phthalocyanine compounds and Pigments are adjacent alphabetically, and have a great deal in common; they occupy 17 pages, which is not too much for such important compounds. The latter covers a wide field, as it deals with both inorganic and organic pigments as well as with dispersed pigment concentrates. China clay, which is perhaps the most important of all white pigments, is treated quite briefly and no mention is made of the important developments by which poor quality clays can be up-graded for use in high-grade products, in which fineness, particle size and whiteness are important. Work of this nature is opening up fresh fields in developing countries where unexploited deposits occur.

The chemical elements appearing in this particular issue are only Phosphorus (with, of course, its compounds, including the phosphorus acids and phosphates); Platinum Group Metals and their compounds; and Plutonium. The last (17 pages) is of particular interest as it is described as "the strangest and most fascinating element known." When one gram of plutonium undergoes a fission reaction, the energy released is equivalent to that produced by the combustion of 3 tons of coal. It is estimated that in 1970 the world's production of plutonium will be approximately 20 metric tons; as 6 kilograms of plutonium, apparently, suffice to make an atomic weapon, the implications are disturbing—to say the least.

Miscellaneous monographs of the "unexpected" type in the present monograph include Photography, a 41-page monograph with a strong theoretical bias; Phonograph Record Compositions, which traces the art from the shellac era to the present use mainly of vinyl copolymers, and the requirements of records for stereophonic sound; Plywood, which is dealt with from a production aspect; and what are described as Poisons, Economic, which are, in fact, "chemicals intended for the control, suppression, or destruction of plants or animals which are of economic significance as pests." In other words—Pesticides!

It will be seen that this present issue of the series offers the usual variety of interest and instruction; and its standard is certainly commensurate with that of the earlier volumes.

JULIUS GRANT

ANNUAL REVIEW OF NMR SPECTROSCOPY. Edited by E. F. MOONEY. Volume 1. Pp. x + 353. London and New York: Academic Press. 1968. Price 95s.; $14.

This volume introduces a new series to supplement the two existing series entitled "Advances in Magnetic Resonance" (also Academic Press) and "Progress in N.M.R. Spectroscopy" (Pergamon Press). That the commercial publishers should feel there is need for a third edited series of volumes appearing at frequent intervals, with separate chapters by different authors, shows the extent to which magnetic resonance (predominantly high resolution n.m.r.) has pervaded all chemistry research laboratories. This essentially British volume is a little more comprehensive, and similar to an Annual Report, but the distinction is not clear-cut and several of these articles would not have been out of place in the other series. The problem also arises of finding authors with time for such writing. It is noticeable that the authors here are not well represented among the 1200 different references to published work. Of the eight authors, two have no such reference even in their own articles, five have from one to four references and the eighth has twelve. It seems that a class of review authors may soon arise to interpret the essential features of research papers to the general practitioner.

These comments are not intended to imply that there is anything amiss with the seven valuable articles in this volume. The chapters are as follows: Review of proton resonance by R. A. Y. Jones covers a wide range of material published in 1966 and early in 1967; N.M.R. and Conformational Analysis by W. A. Thomas covers this special field quite fully, especially for 1965–66; Interpretation of Spectra by E. O. Bishop covers well trodden ground but exemplifies modern practice; Heteronuclear double resonance by W. McFarlane deals with an important area that is currently very active; N.M.R. of Polymers by P. R. Sewell includes only high resolution aspects, and it is surprising how informative the spectra can be even with moderately broad lines; Signal to noise enhancement by G. E. Hall includes all aspects, not merely computer averaging, but in 12 pages of text this is largely an intelligent guide to the 70 references; Fluorine n.m.r. by E. F. Mooney and P. H. Winson is largely an account of work published in 1966, a prolific year, or very early in 1967, and forms a companion to the first chapter.

Any group active in these fields should be able to avoid several man-hours of literature searching and duplication of experiments by use of this book, so that its purchase could be economically justified.

D. H. WHIFFEN

MANUEL PRATIQUE DE CHROMATOGRAPHIE EN PHASE GAZEUSE. Edited by JEAN TRANCHANT. Contributions by J. BUZON, N. GUICHARD, J. LEBBE, A. PRÉVOT, J. SERPINET and J. TRANCHANT. Pp. xii + 361. Paris: Masson & Cie. 1968. Price F 80.

The book is designed as a good reference source for the chemist who wishes to use gas chromatography as a tool for the general analytical laboratory. It has a well balanced mixture of theoretical principles, practical advice and a review of the many types of problems for which gas chromatography may provide an answer.

The earlier chapters deal with the theoretical principles generally considered necessary for a working knowledge of the process of chromatography. The authors then proceed to consider the essential features of each working part of a gas chromatograph, and how techniques such as temperature programming and preparative chromatography fit into the general purpose of the technique.

A large and well written review section is devoted to columns of all types. This section is comprehensive, as can be seen by the use of over 230 references for this chapter alone. These are as up to date as one can obtain in a textbook. This section and that dealing with detection have obviously been written by chemists with a sound working knowledge of the problems encountered in real life analysis.

When the fairly short chapter dealing with separation and identification is considered in conjunction with the range of application discussed, the whole is a very useful addition to many chemists' bookshelves. There are many classes of products considered and, for each class, there is a list of references, so that one can easily check the details.

Over-all the book should prove to be useful in many laboratories. Its price may deter some, but it is good value for money. It is well produced with clear diagrams and photographs, and has that important feature for a book designed to be well used, a substantial binding. L. S. BARK

Errata

JANUARY (1969) ISSUE, p. 68, 5th line of text. *After* ". . . check this content." *add* "The restriction on cyclohexylamine content of cyclamates laid down in the Soft Drinks Regulations 1964 (S.I. 1964, No. 760) gives no indication of the sensitivity of the procedure, but this is deemed to be well above 100 p.p.m."

IBID., p. 69, 2nd line under "Method." *After* ". . . 100-ml separating funnel," *add* "(Cyclamic acid must be neutralised before proceeding further. This is conveniently carried out by the controlled addition of potassium carbonate solution in the presence of a small piece of litmus paper)."

LECTURES AND COURSES

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Further details from Dr. D. Thorburn Burns, Department of Chemistry.

Loughborough. Leicestershire.

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Clerk of the County Council

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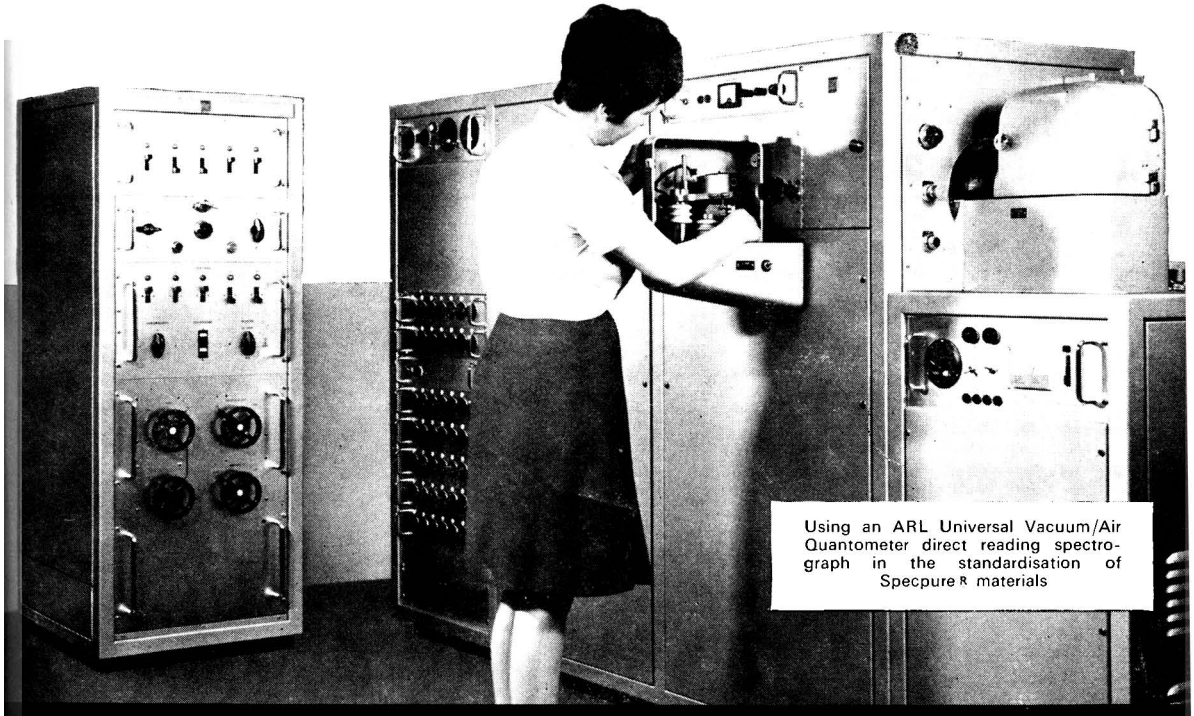

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