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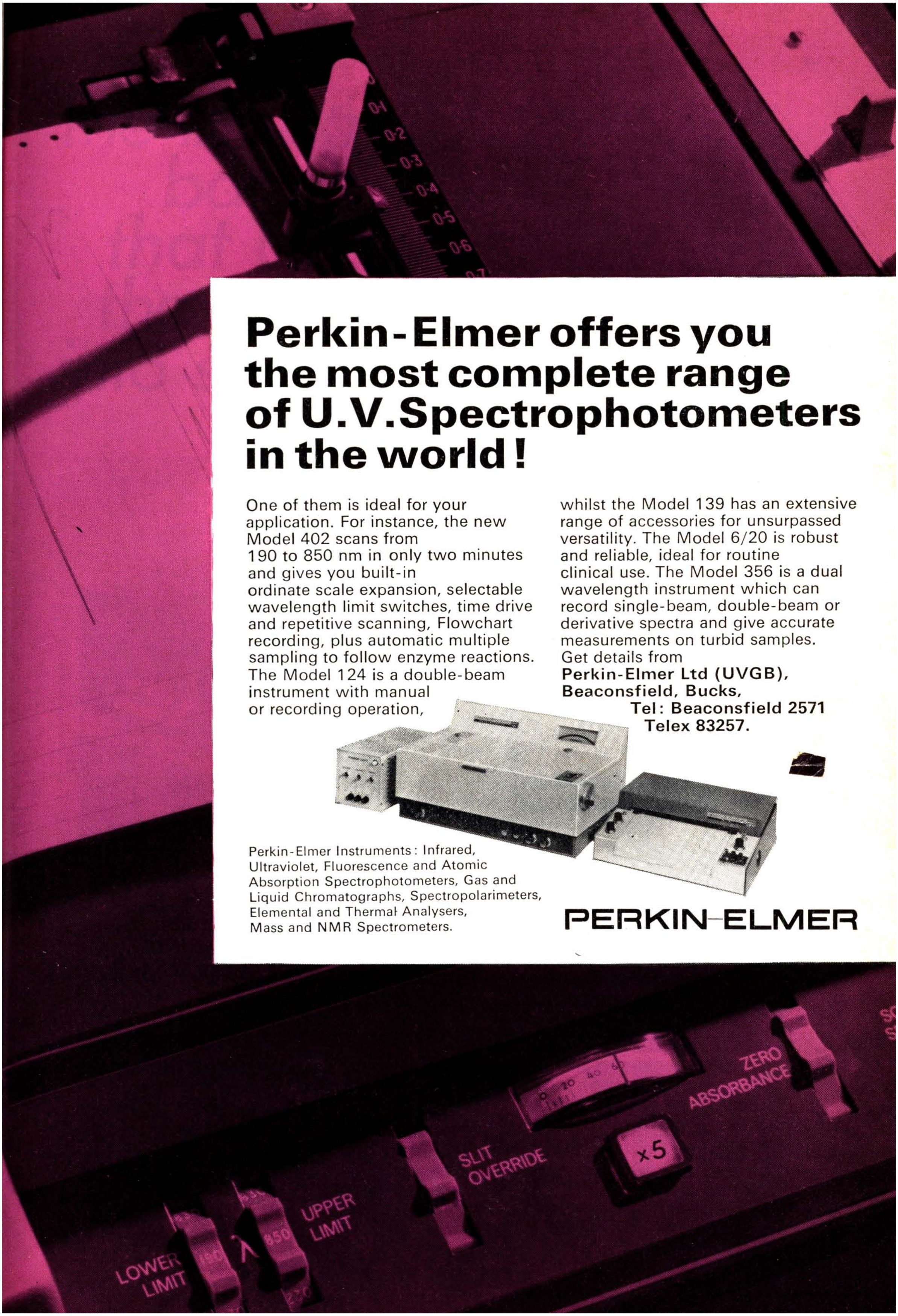

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X-ray Fluorescence Analysis

A Review

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K. G. CARR-BRION and K. W. PAYNE
Warren Spring Laboratory, Stevenage, Hertfordshire.

Analyst, 1970, **95**, 977–991.

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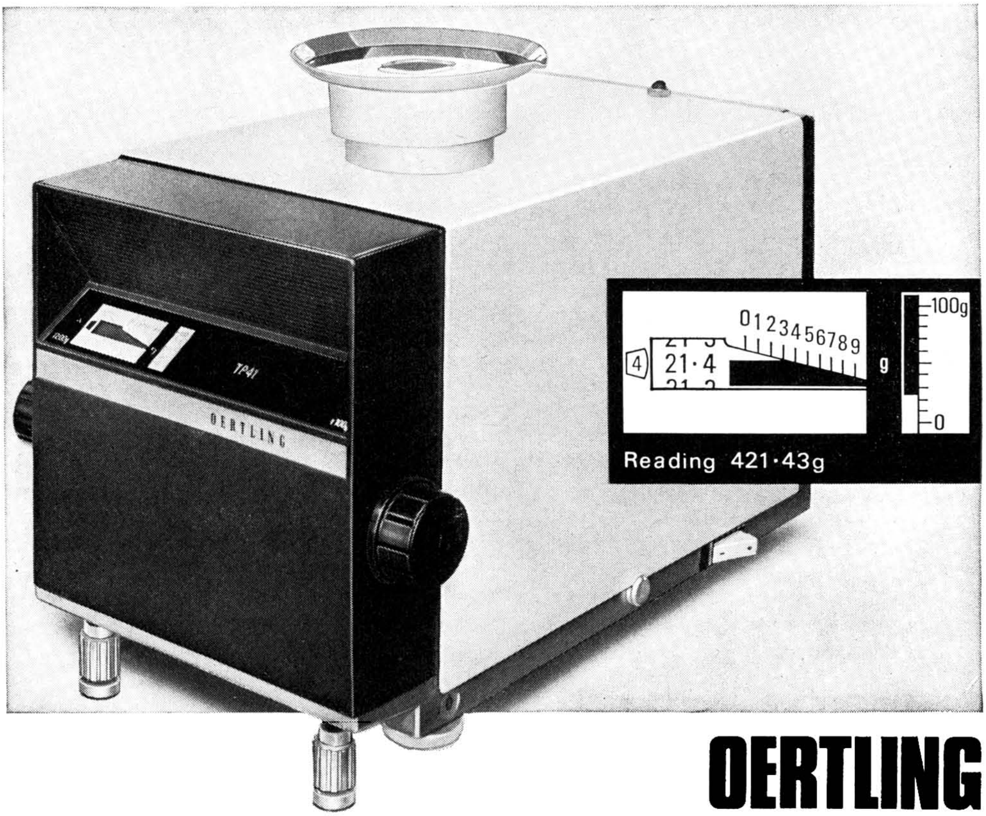
The X-ray Fluorescence Determination of Light Elements in Volatile Liquids *in Vacuo* with a Newly Designed Sample Cell

A sample cell that permits volatile liquids to be studied in a vacuum-path X-ray spectrograph is described. Details of the construction of the cell are given as well as experimental results of studies on sulphur and chlorine-bearing materials.

D. J. HUGHES and J. E. DAVEY
The Gas Council, London Research Station, Michael Road, London, S.W.6.

Analyst, 1970, **95**, 992–994.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Simultaneous Determination of Copper and Manganese in Plants by Neutron-activation Analysis

By using the radioactivation technique, copper and manganese have been determined simultaneously in plant leaves.

A simplified and rapid procedure for the determination of both elements in plant leaves is presented. The copper and manganese in irradiated samples are separated by solvent extraction, the former being extracted with α-benzoinoxime - pyridine - chloroform and the latter with 8-hydroxyquinoline - chloroform.

The time required for radiochemical separation from the end of irradiation to counting is less than 1 hour for ten samples.

The precision of the method in the range of 0·1 to 0·2 p.p.m. is about ±5 per cent. The limits of detection calculated from the analysis of standard specimens are about 0·01 μg for copper and 0·001 μg for manganese.

S. OHNO, M. SUZUKI

National Institute of Radiological Sciences, 9–1, 4-chome, Anagawa, Chiba-shi, Japan.

and M. YATAZAWA

Faculty of Agriculture, Nagoya University, Furo-cho, Chikusa-ku, Nagoya, Japan.

Analyst, 1970, **95**, 995–999.

A Routine Method for the Determination of Low Caesium-137 Activities in Biological Samples

A routine method for the determination of low fall-out activities of caesium-137 in biological materials is described. Radiocaesium is extracted by adsorption on ammonium molybdophosphate. The usual radiochemical purification of the alkaline ammonium molybdophosphate solution by anion exchange is omitted and caesium precipitated directly with bismuth tri-iodide in the presence of citric acid as complexing agent. A caesium chloroplatinate source is prepared for β-counting.

A. MIRNA

Federal Institute for Meat Research, Kulmbach, Germany.

Analyst, 1970, **95**, 1000–1002

The Spectrophotometric Determination of Hydroxyproline: An Analytical Investigation

Thirty-eight methods for the spectrophotometric determination of hydroxyproline have been tested. The sensitivity of twenty-seven of these under routine conditions and their applicability for determining hydroxyproline in synthetic and natural mixtures have been examined. Most procedures were suitable for use in the presence of other amino-acids, but sugars, urea and ammonium chloride caused considerable analytical losses. No single method was both sensitive and reproducible for use with synthetic mixtures and biological materials. The best results with any method were obtained by the identical treatment of all samples and by use of internal standards.

A. D. MITCHELL and I. E. P. TAYLOR

Botany Department, University of British Columbia, Vancouver 8, B.C., Canada.

Analyst, 1970, **95**, 1003–1011.


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A Critical Study of Brilliant Green as a Spectrophotometric Reagent: The Determination of Perrhenate and Gold

The spectrophotometric methods for determining rhenium and gold with Brilliant green have been re-examined in an investigation of the effects of purity of reagent and acidity on the precision and accuracy of results. The perrhenate - Brilliant green ion-association complex is extracted from a solution at pH 6, and is unaffected by reagent purity. The extraction of the tetrachloroaurate(III) - Brilliant green ion-association complex is carried out from 0·5 M hydrochloric acid solution, however, and an impure reagent gives recoveries. Procedures are recommended for the determination of rhenium and gold, and the purification of the reagent is discussed.

A. G. FOGG, C. BURGESS and D. THORBURN BURNS
Department of Chemistry, University of Technology, Loughborough, Leicestershire.

Analyst, 1970, **95**, 1012–1017.

Spectrophotometric Determination of Molybdenum with Gallein and Cetyltrimethylammonium Bromide

A ternary complex system between molybdenum, gallein and cetyltrimethylammonium bromide is proposed for the determination of microgram amounts of molybdenum. The complex has an absorbance maximum at 618 nm. The sensitivity of the reaction is 0·0026 μg cm^{-2} of molybdenum for $\log \frac{I_0}{I} = 0{\cdot}001$. The development of this method for the determination of molybdenum included a study of the effect of pH, temperature, reagent concentration, rate of complex formation and conformity to Beer's law.

C. L. LEONG
Geological Survey Federal Headquarters, P.O. Box 1015, Ipoh, Perak, Malaysia.

Analyst, 1970, **95**, 1018–1022.

The Application of Polarography and Related Electroanalytical Techniques to the Determination of Tetramethylthiuram Disulphide

Methods have been evaluated for the determination of tetramethylthiuram disulphide by d.c. and a.c. polarography. Linear calibration graphs were obtained over the concentration range 10^{-4} to 10^{-3} M by d.c. polarography, and 10^{-6} to 10^{-4} M by a.c. polarography. The electrode reaction was studied by cyclic voltammetry and evidence obtained to support the postulated mechanism of reduction of the S—S bond to give two dimethyldithiocarbamate molecules.

M. J. D. BRAND and B. FLEET
Chemistry Department, Imperial College, London, S.W.7.

Analyst, 1970, **95**, 1023–1026.

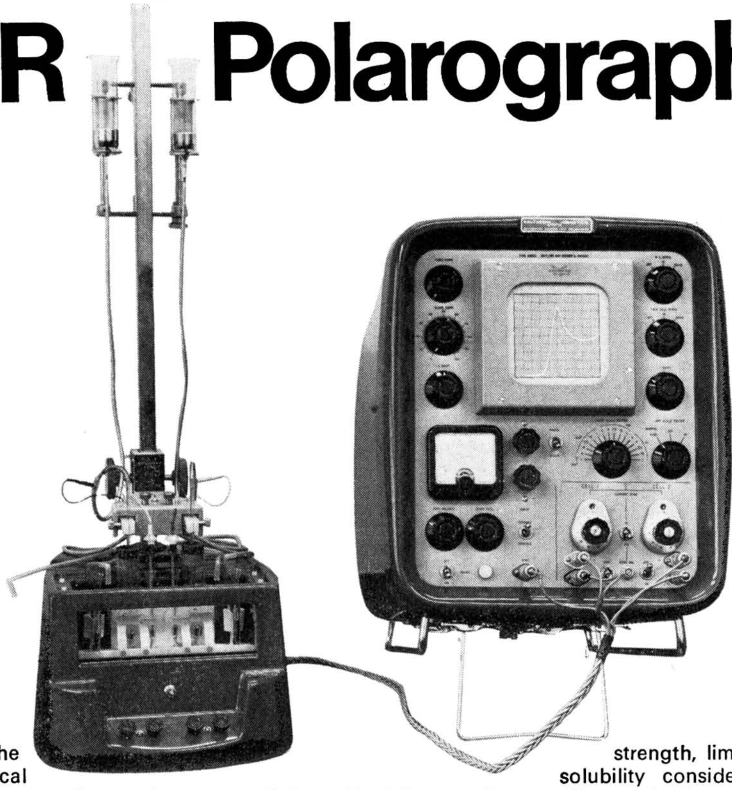
The Application of Anion-selective Membrane Electrodes in Pharmaceutical Analysis

Part I. Halogenated Pharmaceutical Compounds

Anion-selective membrane electrodes have been applied to pharmaceutical analysis. Twenty-four halogenated pharmaceutical compounds were determined by both the direct and indirect potentiometric methods. The compounds investigated represented a wide range of pharmaceutical compounds used extensively in pharmacy and medicine. Determinations of the dissociated halides were carried out directly with no previous treatment but the undissociated halides were determined after applying Schöniger's method. Comparison of the results obtained with the direct and indirect methods shows that they are in agreement. The direct method can be adopted for rapid routine pharmaceutical analysis it is easy to carry out.

YEHIA M. DESSOUKY, K. TÓTH and E. PUNGOR
Department of Analytical Chemistry, University of Chemical Industries, Veszprém, Hungary.

Analyst, 1970, **95**, 1027–1031.



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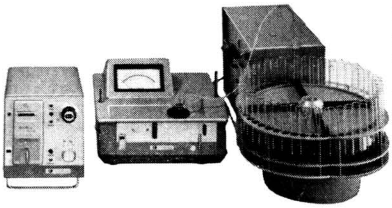

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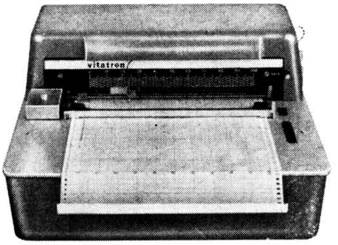

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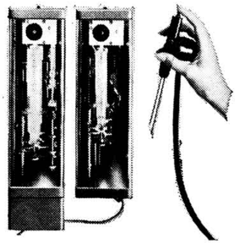

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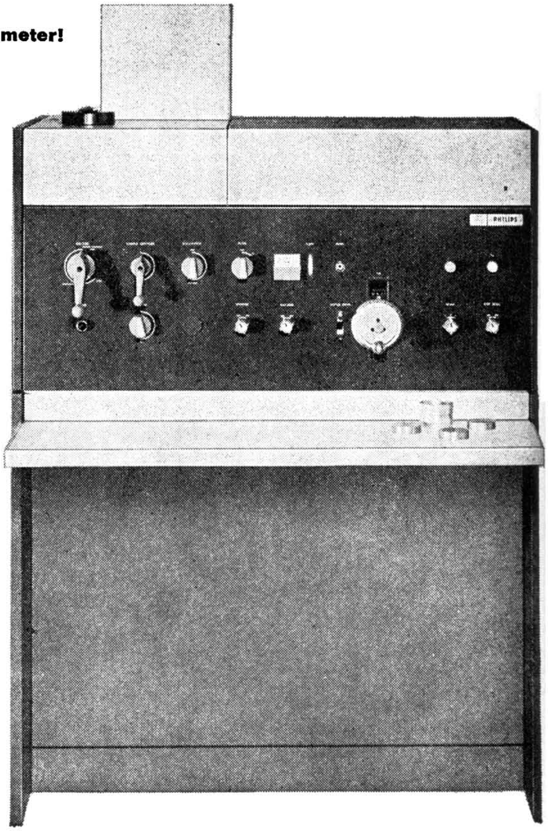

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THE ANALYST

X-ray Fluorescence Analysis

A Review*

BY K. G. CARR-BRION AND K. W. PAYNE
(*Warren Spring Laboratory, Stevenage, Hertfordshire*)

SUMMARY OF CONTENTS

WHEN electrons are ejected from the inner orbitals of an atom their replacement results in the emission of X-rays.[1] Those caused by filling vacancies in the innermost orbital are called K X-rays, those by filling vacancies in the next orbital L X-rays, and so on. Each element emits X-rays at certain characteristic energies that are equal to the energies of the corresponding electron transitions.[2] Thus if a substance is suitably excited so that electrons are ejected, the elements composing it can be determined and, as the X-ray intensity at each characteristic energy level depends on the concentration of the element emitting it, both qualitative and quantitative analysis become possible (Fig. 1). If X-rays from a tube or radioisotope are used for excitation the technique is known as X-ray fluorescence analysis. However, as techniques involving electron[2] and other particle excitation perform functions similar to those in which X-ray excitation is used, they have been included in this review paper.

The existing range of commercial instruments permits the detection of all elements except hydrogen and helium. If sealed X-ray tube excitation is used the range of elements detected is generally limited to those with atomic numbers higher than oxygen because of the difficulties associated with the excitation of the characteristic X-rays from the lighter elements. Most elements can be determined at concentrations varying from a few parts per million to major amounts, although the limit of detection deteriorates with low atomic numbers.

The simplest type of instrument for X-ray fluorescence analysis is the portable unit[3] with radioisotope sources, which is used for mineral prospecting or shop-floor testing. In these instruments use is normally made of X-ray filters to select the required characteristic

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

X-rays. Next are laboratory or base-camp type instruments, in which radioisotope excitation and semi-conductor detectors[4] are used for separating the characteristic X-rays, which enable many elements to be determined simultaneously and are ideal for rapid qualitative or quantitative analysis. The least complex of the instruments in which crystal spectrometers are used to select the characteristic X-rays make use of sequential manual selection of the required diffraction angles. This type of instrument is most suited to the general analytical laboratory because it combines the best in instrumental performance with maximum flexibility, and if several different routine analyses are required an automated version of it increases sample throughput and reduces operator error. When a fixed number of elements needs to be determined routinely with maximum speed, an instrument with a number of crystal monochromators (usually between 5 and 25), each set to determine a particular element, would be the preferred choice. These are also suitable for on-stream analysis as are the simpler radioisotope units with conventional detectors.

X-RAY EXCITATION

X-RAY TUBES—

The sealed-off X-ray tube is the most usual excitation source for elements whose atomic number is higher than 10. The fundamental design has remained unchanged, but recently the power, stability and reliability have been greatly improved. Many commercial spectrometers are equipped with 2 or 3-kW tubes, with operating potentials of 100 kV, which enable the K spectra of all elements except thorium and uranium to be excited. Anode materials commonly used include tungsten, gold, platinum, molybdenum, rhodium, palladium, silver and chromium. The first four of these targets are the most effective for the excitation of elements with wavelengths less than about 0·3 nm and the following three anode elements give moderate excitation efficiency over a wider wavelength range. Chromium anodes, besides providing more effective excitation for the lighter elements, cause fewer electrons to be back-scattered and hence reduce heating of the beryllium window of the X-ray tube, which enables windows as thin as 0·2 mm to be used, with consequent reduced attenuation of the X-ray beam. The count-rate for aluminium is about five times greater for a thin-window chromium-anode tube than for a tube with a heavy-element anode.[5] Dual anode tubes, such as tungsten and chromium, combine some advantages for heavy and light element excitation but necessarily require thicker windows.[6] Tubes with the normal electrical potentials reversed virtually eliminate window heating, and very thin windows can be used with currents up to 100 mA. These tubes are a standard part of one manufacturer's equipment.[7]

De-mountable tubes with low atomic number anodes and ultra-thin windows can increase the intensity of light elements by one order of magnitude, but at the cost of lowered stability and convenience.[8,9] Specialised developments of these tubes permit the determination of elements of atomic number down to boron.[10,11] Another development is the windowless tube, which combines X-ray excitation with that due to electrons scattered from the target.[12]

The spectral distribution of the X-ray tube is an important feature in the use of some mathematical approaches to quantitative analysis.[13]

It is sometimes desirable to reduce the characteristic radiation arising from the tube target to remove interference with the spectral lines of the analysed elements, and this is effected by placing a filter between the tube window and the sample. The filter is usually made from metal foil of an element the absorption edge of which is of slightly longer wavelength than the relevant tube line.

ELECTRON AND PROTON EXCITATION—

Instruments are available commercially which perform functions identical with those of true X-ray fluorescence instruments, but which involve the use of an electron beam to excite the characteristic X-rays.[14,15] Electrons are particularly effective for exciting low energy characteristic X-rays for which existing sealed X-ray tubes become less efficient. The increased intensities obtained at these energies are offset by poorer characteristic-to-background ratios caused by bremsstrahlung ("white" X-rays produced as the electrons decelerate in the sample material) of similar energies. However, improved limits of detection with much shorter counting times are achieved for elements of atomic number lower than that of sulphur.[14,16] Electrons penetrate the sample to much less extent than a primary X-ray beam, hence the characteristic X-rays originate at levels closer to the surface of the sample, which increases the importance of the need for the outermost surface to be representative of the bulk material but reduces the magnitude of absorption effects on the secondary X-rays. Much of the energy of the electron beam is dissipated as heat; overheating of the sample is avoided by allowing the beam to scan over a fairly large area, typically 1 cm^2. With electrically non-conducting samples the sample must be prepared in a form that enables charge build-up to be avoided.[17]

Protons are also valuable for exciting low energy X-rays, the efficiency of excitation increasing as the energy of the characteristic X-ray decreases.[18,19,20] Bremsstrahlung is not produced, as the particles decelerate giving high signal-to-background ratios. The depth of penetration of protons into the sample is more limited than that of electrons, thus making surface preparation even more critical (this fact can be of use in determining the composition of surface films). The efficiency of excitation is reduced as the proton energy decreases, because accelerating voltages in the range 25 to 200 keV have been used.

RADIOISOTOPE SOURCES—

α-, β-, γ- and X-radiation from radioisotopes are capable of exciting characteristic X-rays.[21] However, with practicable activities the intensities available are 10^6 to 10^7 times less than those obtained with the type of X-ray tubes used in analytical spectrometers. In spite of the disadvantage, sealed radioisotope sources[22] have become of increasing importance in recent years because of their compactness, low cost, stability and special emission characteristics. Their small size makes them ideal for incorporation in instruments designed for field use or remote on-stream sensing of elemental concentrations in process control.

Like protons, α-particles excite low energy X-rays with high efficiency and without accompanying bremsstrahlung,[23] which makes them especially useful in determining low atomic number elements. However, α-emitters are highly toxic, and it has proved difficult to manufacture sealed sources that have windows sufficiently thin to permit the escape of the particles but able to resist the radiation damage caused by the particles and the accidental wear and tear associated with general analytical work. β-Particle excitation[24] is accompanied by bremsstrahlung, which again results in poor signal-to-background ratios, and the design of safe sources emitting low energy β-particles also presents some safety problems. However, wide use is made of the bremsstrahlung for excitation, by using sources that consist of a β-emitter intimately mixed with a suitable target material. Tritium - zirconium and tritium - titanium sources are useful for exciting elements in the range silicon to zinc, while promethium - aluminium or promethium - ceramic sources are of use as general-purpose sources for the range of elements from about iron to barium (while these sources can excite the K X-rays of the heaviest elements, the signal-to-background ratio is too poor to make this of much value for analytical work).

The most effective radioisotope sources are those emitting X-rays or low energy γ-rays at certain isolated energies such as iron-55, plutonium-238, cadmium-109, americium-241 and cobalt-57. Because these emissions are confined to narrow energy bands they make little contribution to background intensities at other energies, unlike the bremsstrahlung sources described previously. If the back-scattered primary and required characteristic radiations can be separated by the energy-resolving power of the detector used, these sources give the largest signal-to-background ratios obtained with radioisotope excitation for medium and high atomic number elements. When a radioisotope emitting a suitable energy is not available, use can be made of a "source target" assembly,[25] with which characteristic X-rays of suitable energy are excited by a primary X-ray or γ-ray source; these characteristic X-rays are used in turn to excite the element being determined.

The limits of detection obtained with even the best radioisotope X-ray systems are at present one or two orders of magnitude poorer than those obtained with conventional X-ray tube spectrometers. An important advantage of radioisotopes is that, by suitable choice of source, primary X-rays that can efficiently excite the element being determined are excluded.[26] This results in an appreciable reduction in heterogeneity effects, which can be major sources of error in the analysis of powders, slurries and other multiphase systems.

MEASUREMENT OF X-RAYS

DETECTORS—

X-ray detectors convert the incident X-ray into electrical pulses suitable for subsequent counting. The types of detector in common use today are the scintillation counter, in which a phosphor and photomultiplier combination is used, the gas-flow proportional counter and the semi-conductor detector, the last of which is of more value as an energy discriminator and will be discussed later. Both the scintillation and proportional counters also produce pulses that are proportional to the energy of the incident X-ray, thus allowing them to be used to discriminate between X-rays of different energies. However, the resolution of the scintillation counter (typically 50 per cent.) is poor when compared with that of the proportional counter (typically 20 per cent.).

The scintillation counter is the most effective detector for radiations up to about 0·15 nm. The phosphor in the counter is usually thallium-activated sodium iodide, but a calcium iodide - europium system has given improved resolution at low exciting voltage.[27]

The gas-flow proportional counter, although requiring more attention than a sealed proportional counter and being more susceptible to pulse amplitude shifts,[28] has a great advantage in effective wavelength range in the medium to soft region. It has good sensitivity and energy resolution, although the latter is impaired by contamination of the counter wire by impurities in the argon - methane gas normally used. The use of thicker wire and of lower noise - higher gain pre-amplifiers eliminates the shift of pulse height distribution caused by variation in the counting rate.[29,30] In the soft X-ray region flow proportional counters fitted with very thin windows of polypropylene or polycarbonate are used to measure radiations up to 5 nm.[11,31,32]

Because of the low mass absorption coefficient of the counter gas for its own fluorescent X-rays, many of these will escape detection, giving rise to lower energy escape peaks. The difference in energy between the main and escape peaks will be equal to the energy of the fluorescent X-rays. Argon and krypton counter gases are particularly susceptible to escape peaks. Pulse height discrimination can be used to reduce interference caused by this effect, or, in more serious instances, the counter gas should be replaced with neon.[33] Scintillation counters can also give rise to escape peaks resulting from iodine radiation in the phosphor.

ELECTRONICS—

X-ray intensities are normally measured by counting the number of pulses from the detector, a nucleonics system of pulse amplifier, single-channel pulse height analyser, scaler-timer (for quantitative analysis) and rate meter (for qualitative analysis, Fig. 1) being required. For convenience outputs are recorded by printer tape punch and pen recorder. The performance required for X-ray spectrometry is well within the capabilities of present solid-state modular[34] systems, provided mains-frequency independent timing is used.

Conversion of X-ray intensities into elemental concentrations can be carried out automatically by using an off- or on-line computer.[35] With the former, punched tape output is used, which is subsequently processed, thus causing a delay in the availability of the results. An on-line computer (usually a miniature one with 4 to 8- K store) allows instant data processing and has the additional advantage of being able to control the instrument to a variable pre-set program. The value of such a system increases with the amount of routine work required of the instrument.

ENERGY SELECTION

MONOCHROMATORS—

The selection of a specific X-radiation is achieved in most conventional spectrometers with an analysing crystal from which the X-rays are diffracted according to the well known Bragg relationship. When a flat crystal is used the fluorescent X-rays pass first through a primary collimator and, after diffraction, are again collimated in their path to the detector. Finely spaced collimators will give better resolution but lower intensities, and are best suited to measuring the narrow peaks of the heavier elements, while for converse reasons widely spaced collimators are more suitable for the less sensitive light elements. In curved-crystal spectrometers the monochromator usually consists of a bent and ground crystal (Johansson type)[36] and primary and secondary slits that are aligned on the focusing circle of the crystal. Although this system may have advantages in increased intensity or resolution, or both, over

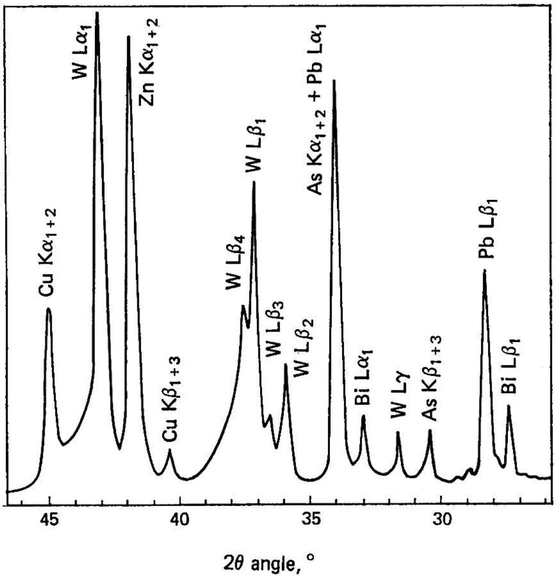

Fig. 1. X-ray fluorescence chart record of part of the spectrum of a sulphide ore. Instrumental settings: crystal, LiF (200); counters, scintillation and flow proportional in series; tube, tungsten anode; excitation, 60 kV; and current, 32 mA

flat-crystal collimator systems, the focusing arrangement usually confines its use to the fixed geometry systems used in multi-channel spectrometers.

Several crystals with various lattice spacings are required to cover the X-ray spectrum. The lithium fluoride (200) crystal, 2d spacing 0·4028 nm, is outstandingly the best choice for high reflectivity coupled with good resolution for wavelengths between about 0·04 and 0·38 nm. For higher wavelengths, up to about 0·84 nm aluminium Kα, the pentaerythritol crystal is generally the most suitable. Graphite gives high intensity in this region, but resolution is poor.[37] The potassium hydrogen phthalate crystal is suitable for the measurement of magnesium and sodium, and can be used for fluorine determination if thin counter windows are fitted to the instrument.[38] Reverting to the short wavelength region, lithium fluoride (220), 2d spacing 0·2848 nm, gives much better intensity than the similarly spaced topaz, and is useful for separating the K spectra of the lanthanides.[39]

In the soft X-ray region multi-layer films of a soap, *e.g.*, lead stearate, are used in the 1 to 8-nm range.[10,11,40,41] Gratings compete with crystals for optimum intensity in this region but require more precise instrumentation.[42] A novel approach, at present in the development stage, is the use of a "mirror" of evaporated paraffin as dispersant.[43]

Very high resolving power has been achieved with a spectrometer in which two crystals are arranged to give a dispersion that is the sum of their separate dispersions.[44] However, even if nearly perfect crystals are used the intensity is about one quarter of that obtained with a single-crystal spectrometer.

Several publications relating wavelength to 2*θ* angles for various crystals are available.[45,46]

PULSE HEIGHT SELECTION—

In conventional spectrometers, radiation received by a proportional counter is amplified by a linear amplifier and passed into a discriminator that selects a specified range of pulse amplitudes to be passed on the scaler units.[47] This method (in contrast to that described in the following section) gives a relatively crude energy separation, but is extremely useful for the partial elimination of higher order interferences and for the reduction of background radiation and interference from fluorescent radiation in the analysing crystal. In automatic spectrometers pulse height selection is automated by varying the electronic amplification sinusoidally with the diffracting angle of the crystal.[48]

SEMI-CONDUCTOR DETECTORS—

In the last few years, semi-conductor detectors (Figs. 2 and 3) made from lithium-drifted silicon or germanium and operated under vacuum at liquid nitrogen temperature have become of increasing importance for selecting and measuring characteristic X-rays.[49,50] The statistical spread in pulse amplitudes resulting from X-rays of a single energy is much less than that found with proportional counters. Half-height energy peak widths as narrow as 190 eV in the 6-keV region have been reported from commercial detectors and narrower values can be expected as both detectors and pre-amplifiers are improved. The separation of the Kα X-rays of adjacent elements varies from about 200 eV at sodium through 600 eV at copper to 2 keV at tungsten. However, Kα - Kβ, K - L and other overlaps still occur, which are unlikely to be separated by the energy-resolving power of these detectors. In these instances resort must then be made to "stripping" techniques, which are well established in γ-ray spectrometry, and is facilitated by the high pulse amplitude stability of these detectors.

The main advantage of semi-conductor detectors is that they permit complete flexibility in the choice of excitation, which can be with low power X-ray tubes, secondary X-ray targets,[51] particles or radioisotope sources, thus giving the benefits previously outlined. Other advantages are their ability to determine simultaneously a large number of elements and to measure the K X-rays from the heaviest elements with higher efficiency and resolution than can be achieved with existing commercial X-ray spectrometers. The instrument, of which the detector forms a part, is compact and requires no special installation facilities, unlike conventional X-ray spectrometers. Operation under vacuum and at liquid nitrogen temperature presents little real difficulty: the vacuum surround is sealed and the required pressure maintained by an ion pump or molecular sieve; and the liquid nitrogen Dewar flask requires to be replenished at 5 to 25-day intervals, depending on its size. However, an adequate self-contained cooling system for remote operation in a process control environment is not yet commercially available.

The main limitation of these detectors, apart from their poor energy resolution at lower energies when compared with a crystal spectrometer, is their small area, the highest resolution detectors being now between 12·5 and 30 mm^2 compared with about 1000 mm^2 for a typical proportional counter. Their smaller areas result in reduced count-rates,[52] which to a certain extent offset the advantage gained by the increased signal-to-background ratios they exhibit. However, larger-area detectors with equivalent resolution should become available in the next few years. The use of low powered tubes rather than radioisotope X-ray sources has been proposed to offset the poor geometrical efficiency of these detectors, but existing pre-amplifier - amplifier combinations are not capable of handling count-rates much higher than those obtained with radioisotope sources without appreciable loss in resolution; again, the improved designs becoming available should remove this difficulty.

X-RAY FILTERS—

X-ray filters make use of "absorption edges," *i.e.*, the immediate increase in the absorbing power of an element when the X-ray energy is raised to a level just sufficient to eject electrons from one of the orbitals of the atom,[53,54] usually the K orbital for most filters. The absorbing power of two adjacent elements varies similarly above and below their absorption edges and, within the energy region between the two edges, one element absorbs very strongly while the other absorbs weakly. Therefore, the difference in intensities measured when two such filters of suitable thickness are placed successively in an X-ray beam corresponds to the intensity within that energy region. If the filters are selected so that the required characteristic X-rays lie within that region, the characteristic intensity can be isolated and measured, with only a limited dependence on intensity variations at other energies. The "resolution" achieved with the so-called balanced-filter technique varies from 300 eV at 1·5 keV through 700 eV at 10 keV to 2 keV at 70 keV, which is inferior to that obtained with semi-conductor detectors. While the large area of filters enables high count-rates to be obtained even with radioisotope sources, the filter pairs are difficult to balance for samples of widely

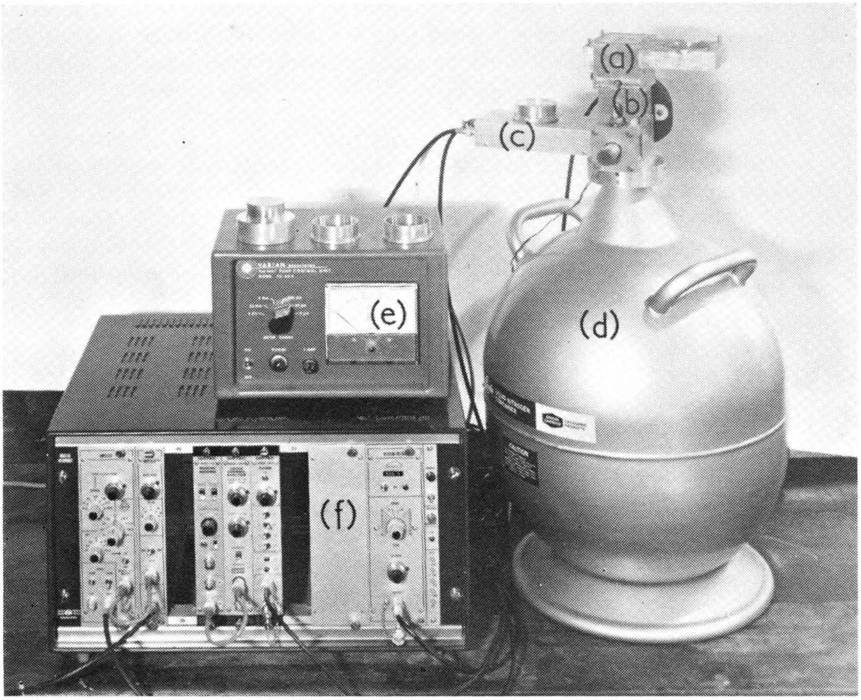

Fig. 2. Semi-conductor X-ray detector: (*a*), sample presenter with holder; (*b*), detector housing; (*c*), pre-amplifier; (*d*), crystal; (*e*), ion pump power supply; and (*f*), nucleonics

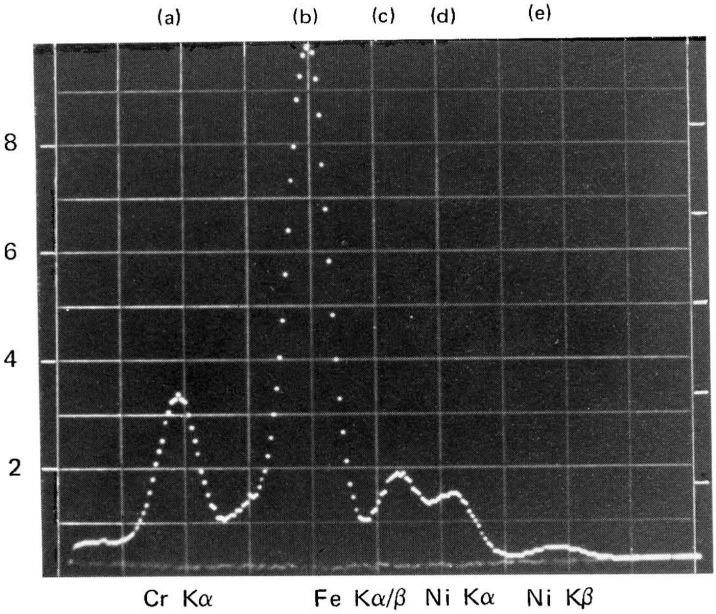

Fig. 3. Spectrum from stainless steel, ^{238}Pu excitation: (*a*) chromium Kα; (*b*), iron Kα; (*c*), iron Kβ; (*d*), nickel Kα; and (*e*), nickel Kβ

varying composition, and fluorescent X-rays from the filters themselves limit the signal-to-background ratio. Most filters consist either of thin metal foils or powders suspended in a suitable plastic base such as polyethylene. They are used when intensity, size or cost is limited, and have been widely used in portable radioisotope X-ray analysers for prospecting work[3] and in on-stream analysers[55] for process control.

An allied technique is that of selective excitation,[56] in which the sample is successively excited by X-rays of energy slightly greater then slightly less than that of the absorption edge of the element being determined. As the two exciting radiations are of almost identical energy, they are scattered similarly by all elements with the exception of the element being determined, which emits fluorescent X-rays with one and not the other. Thus the difference in the two back-scattered intensities gives the required characteristic intensity. This technique has been used in on-stream analysis, in which the sample flows past the two sources sequentially.

EFFECT OF SAMPLE ON X-RAY INTENSITY

INTER-ELEMENT EFFECTS—

The X-ray fluorescent intensity of an element depends on all of the other elements present in the sample. In this section samples free from heterogeneity effects will be considered. The ways in which extraneous elements can influence an analysis can be sub-divided into (*a*) interference from the spectra of other elements, and (*b*) absorption or enhancement by other elements of the element being determined.

Although X-ray spectra are much simpler than optical-emission spectra, complex samples may give rise to interferences that are too close together to be resolved. In the K series the Kβ line of an element of atomic number Z often lies close to the Kα line of element (Z + 1), (Z + 2) or (Z + 3); well known examples are the partial overlap of vanadium Kβ with chromium Kα and of cadmium Kβ with antimony Kα. In the middle wavelength region interferences can occur between K and L lines, as shown in Fig. 1 between arsenic Kα and lead Lα, while at long wavelengths M lines can give additional interference. Another type of interference is that of higher or lower order crystal reflections of other elements present in the sample.

In most instances it is possible to overcome interferences by one or more of the following methods:

Use of another spectrum line of the element.

Reducing the exciting potential below that of the interfering element.

Selection of other instrumental settings, *viz.*, crystal, counter, collimator and pulse height discriminator.

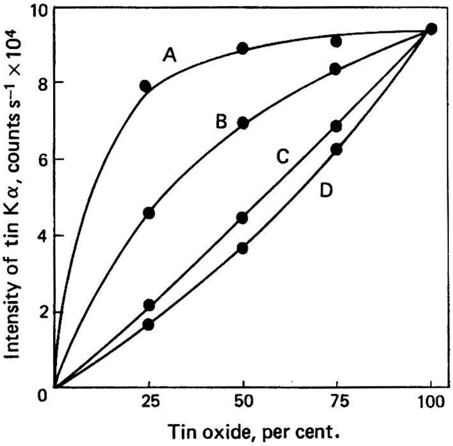

Fig. 4. Intensity - concentration relationship for tin(IV) oxide in different matrices: A, silica; B, iron(III) oxide; C, tungsten trioxide; and D, lead(IV) oxide

Unwanted secondary radiation can also be reduced by placing a suitable filter between the crystal and the detector, an application of the principles described in a previous section.

Samples inevitably absorb X-rays because the primary X-ray beam penetrates the sample to a finite depth in producing the secondary radiation. The extent of the absorption, primary and secondary, is proportional to the mass absorption coefficient at the wavelength concerned, which in turn is derived from the sum of the products of the individual absorptions of the elements and their mass fractions. Absorption maxima occur at wavelengths just shorter than the absorption edges (K, L and M) of the elements involved.

When the sum of the primary and secondary mass absorption coefficients of an element is equal to that of the matrix, a straight-line relationship between intensity and concentration applies. If the element has a greater mass absorption than the matrix, the intensity - concentration curve will deviate towards the intensity axis and *vice versa* (Fig. 4). Generally the component of total absorption arising from primary absorption has a smaller effect on resulting intensity than that from secondary absorption. Absorption effects for electron excitation are less than those for X-ray excitation because of the smaller depth of penetration of the electrons into the sample.[57]

Enhancement effects occur when the fluorescent X-rays generated in the sample from an extraneous element are re-absorbed by the element under analysis, resulting in an increased emission of that element. The effect is most pronounced when the extraneous element is of slightly higher energy than the absorption edge of the element being determined, but even in this situation the enhancement is unlikely to exceed 15 per cent. of the intensity.

For samples such as ferrous alloys, with which various elemental lines and absorption edges are in close juxtaposition, the intensity - concentration relationship is a complex combination of absorption and enhancement effects.[58] The methods of correction are described in a subsequent section.

Methods for measuring mass absorption coefficients have been described,[59,60,61,62,63] and several tabulations of the values of the secondary coefficients have been produced.[64,65,66]

HETEROGENEITY EFFECTS—

In the X-ray fluorescence analysis of a heterogeneous material such as a multiphase alloy or powder, the relationship between the characteristic intensity and concentration depends on the distribution and X-ray absorption coefficients of the individual components of the material, in addition to the effects already outlined in the previous section. Distribution includes such factors as size, shape and packing density. Theoretical treatments of these effects in real systems are generally too complex to be of practical value, but correlation of theories based on simplified systems with experimental results shows that the main effects caused by variations in heterogeneity are understood.[67,68,69] The practical value of such work is that it allows prediction for any system of the conditions that would minimise the effect of expected changes in the degree of heterogeneity. These effects will now be considered in greater detail.

Changes in the grain size of the component containing the element to be determined and also other components in a heterogeneous material can affect the characteristic intensity from a given concentration of the element. Generally, if the X-ray absorption coefficients of the grains containing the element are larger than those of the remainder of the material, the intensity will increase as the grain size of the component is reduced; if the absorption coefficients are smaller the converse will apply. This effect is caused by local absorption in the grain. If the grain size increases, it will eventually exceed the depth from which characteristic X-rays originate. The fluorescent X-ray intensity then depends on the area fraction of the grains containing the element, provided that the grains are randomly positioned and the sample surface is sufficiently flat to make shadowing effects negligible. Shadowing effects, which are complex and difficult to predict, generally result in a lowering of intensities and are reduced by increasing the incidence and take-off angles of the exciting and fluorescent X-rays.

An allied effect on the characteristic intensity is found if the composition of the grains containing the element to be determined changes, even if this change has a negligible effect on the over-all absorption coefficients of the material.[70,71] This is particularly important in the analysis of geological materials; for example, a change from a sulphide to an oxide ore will result in an increase in the characteristic intensity caused by a reduction in the local absorption of X-rays. The effect is reduced by decreasing the grain size and eventually disappears, providing the effect of changes in the over-all absorption coefficients of the material is compensated for.

Variation in the packing density of a heterogeneous sample affects the characteristic intensity, two factors being mainly responsible. Firstly, increased packing density generally reduces irregularities in the surface of the sample exposed to the X-ray beam, thus reducing shadowing effects. Secondly, if the spaces between the grains are considered as another component, its grain size will decrease as the packing density increases, and this will also affect the characteristic intensity.

SAMPLE SURFACE—

Irregularities in the surface of a sample presented to the X-ray beam will cause variations in the path lengths of primary and secondary X-rays, and produce shadowing effects, hence giving variations in intensity.[72] The effect of surface finish is pronounced for the measurement of light elements, for high mass absorption coefficient matrices and when light-element tube anodes are used. It is also specially critical for electron and other particle excitation.[16,73]

It is important that the surface finish should be consistent between samples and between samples and standards. The method of achieving a good surface finish depends on the type of sample material; for hard metals grinding, milling or lapping methods are usually used, and care must be taken not to introduce contamination during the preparation.[74] In the analysis of polymers an admixture of polyethylene gives a much improved surface finish.[75] Soft alloys, such as solder or leaded brass, may be susceptible to surface smearing of one constituent,[76] and a spark erosion method has been found effective in the preparation of these materials.[77]

QUANTITATIVE ANALYSIS

STANDARDS—

Like most other instrumental methods of analysis, X-ray spectrometry depends on the reliability of comparative standards. Even so-called absolute methods require a basis of at least one standard.

In methods in which the sample is prepared as a liquid or solid solution use can be made of pure metals or chemical reagents for standardisation, but this is not always as simple as it appears. The high degrees of purity stated by manufacturers often ignore the presence of adsorbed or combined gases in metals, and for this reason massive metal is preferable to powder. Reagent chemicals may not conform to the stated formula, and a preliminary treatment to obtain a reliable stoicheiometric form is sometimes necessary.

Reasonably good ranges of standards are available commercially for the commoner alloys and are often fabricated in sizes to fit X-ray specimen holders. The analyst who requires standards for the analysis of ores, rocks, refractories and similar material in powdered form is less well suited. A limited selection is available from British Chemical Standards and the U.S. National Bureau of Standards, but analysts are often forced to depend on chemical assays carried out within their own organisation. Even internationally recognised standards show a disconcerting spread of results between the co-operating analysts, which contributes to the error of the dependent X-ray analysis.

METHODS OF OVERCOMING HETEROGENEITY—

It is essential in quantitative analysis to eliminate as largely as possible the heterogeneity effects described in a previous section. Particle-size effects in powders can be reduced by fine grinding, although the size required to eliminate the effect for the lighter elements is below that obtained with mechanical grinders.[67] However, when assays are restricted to one type of material, accurate results can be obtained by a standardised grinding procedure that enables all samples and standards to be reduced to a virtually constant particle size; this method is successfully applied in a large number of geological[78] and industrial laboratories. The use of higher energy exciting radiation will reduce the primary mass absorption coefficient and hence the particle-size effect. Powder dilution is successful in reducing these effects only if the mass absorption coefficient of the diluent is about equal to that of the particles containing the element to be determined.[79] Mathematical means of correcting for particle size have also been used.[80,81]

Particle-size effects are removed completely by preparing the sample as a liquid[82,83,84] or solid solution (glass).[85 to 92] This method also has several other advantages that will be discussed later, but some samples are not amenable to dissolution and inevitably more preparation time is required, and sensitivity is reduced by the dilution.

METHODS OF OVERCOMING INTER-ELEMENT EFFECTS—

Dilution of the sample, particularly in a moderately heavy absorbing medium, reduces absorption effects, as the mass absorption coefficient then tends towards that of the diluent, which would be common to all samples and standards.[85,88,89] Absorption effects can also be eliminated by determining the element in two different dilutions of the sample and applying a simple calculation.[92,93,94,95,96]

Dilution, by powder, liquid or glass, can be conveniently combined with internal standardisation.[97,98,99,100] This method consists in adding to the sample an element, not present in the sample, which has an energy similar to that of the element being determined; in some applications the internal standard takes the form of a solid suspended in a liquid undergoing analysis.[101,102] Because the absorption for the element and its internal standard are similar, their intensity ratio will compensate for absorption differences between samples. Internal standards need to be carefully chosen for optimum effectiveness.[103] The instance of the absorption edge of another element present in appreciable amount in the sample falling between those of the element to be determined and the internal standard must particularly be avoided.[104]

The method of standard additions is useful for unusual samples and trace-element determinations. A known amount of the element concerned is added to a portion of the sample and the concentration deduced from the count-rates before and after addition.[94,105,106]

Massive samples do not readily lend themselves to dilution, internal standardisation or additive methods because preparatory work is usually too lengthy, so chemically analysed samples are used instead to provide calibration graphs and correction procedures. Increase in computerisation during the last few years has led to a spate of mathematical methods, both "empirical" and "fundamental."[107] In empirical or partially empirical methods, use is made of the relationship linking the intensity of an element to the concentrations of all of the elements present in the sample, and hence the mutually corrected intensities of the elements.[81,86,108 to 115] Several of these methods involve the measurement of the elemental intensities in a series of multi-component standards, thereby providing simultaneous equations that can be solved for correction coefficients. Fundamental methods of correction are based on equations relating the elemental intensity to mass absorption coefficient and fluorescent yield, and require only one standard or a pure element as a basis for computation.[13,116,117,118,119] An iterative procedure is then used in which successive approximations of concentration are calculated until no practical difference is achieved by further iteration. For samples with slight variation in composition assumptions can be made to reduce the computations, thus enabling a desk-top computer to be used.[120]

The thin-film technique virtually eliminates absorption effects because, in a thin specimen, absorption is negligible and elemental concentration is directly proportional to intensity. The method has been applied both to samples occurring as thin films, such as alloys deposited on other metals or glass,[121] and to samples pre-treated by spreading or evaporating them on to a supporting medium.[122,123] The method is also applied for measuring film thickness.[124,125]

Corrections for absorption by the sample have also been made by using transmission methods.[126,127] The sample is prepared in the form of a thin disc that will transmit X-rays, which is placed either in the sample holder with a target containing the element behind it, or in the path of the secondary X-rays immediately before the detector.[79]

Enhancement effects are often treated as negative absorption. It has been found in practice that absorption correction methods are also valid for the correction of enhancement effects.

ON-STREAM ANALYSIS—

X-ray fluorescence is in many ways particularly suitable for on-stream analysis[128] as it has a large sample throughput, permits the examination of powders, slurries, liquids and moving sheets without pre-treatment and has high elemental selectivity over wide concentration ranges. Outputs are in the form of true digital signals, and are ideal for handling by digital computers.

The first basic component of an on-stream X-ray fluorescence analyser is the sample presenter, which produces continuously a smooth sample surface at a constant distance from the X-ray head. This head contains the X-ray source, provision for separating the characteristic X-rays, and X-ray detectors for measuring these intensities and others such as back-scatter from a transmission through the sample stream, which are required for absorption corrections on the fluorescent intensities and for calculating variables such as sample density. Finally, there are the necessary nucleonics and digital means of carrying out the necessary conversion of the X-ray intensities into elemental concentrations, with correction for matrix and heterogeneity effects, which can be either a separate low-cost or central-control computer.

Almost invariably X-ray tube on-stream analysers are multi-channel instruments capable of measuring simultaneously the characteristic intensities from a dozen or more elements, with crystal monochromators to isolate the required energies.[129] The X-ray head can be mounted so that it can move across and sequentially measure several process streams brought to it from various points in the plant. It is normal practice to site the X-ray head and generator in a special room that is isolated from the full rigours of the plant environment. Radioisotope X-ray analysers operating at a control point are also available, but this technique is best suited for use with remote sensing heads, each placed at the required point in the plant, with the signals being returned along low voltage cables to a central data processor and control unit.[130]

If a large sample throughput and rapid response time are to be retained, normal sample preparation is not possible in true on-stream X-ray fluorescence analysis, and the sample has to be examined as received. Hence with heterogeneous samples such as slurries or powders, grinding or fusion methods cannot be used to overcome effects caused by variation in grain size or composition. If these effects cause a significant reduction in accuracy they must either be reduced to an acceptable level by suitable choice of the exciting X-ray beam energy or corrected by a direct or indirect measurement of the grain size. Radioisotope analysers are particularly useful for reduction of heterogeneity effects, which can be an important factor in their choice for on-stream analysis. The alternative to on-stream analysers is the use of fully automatic off-stream X-ray analysers that grind, pellet or fuse small samples and present them to a laboratory-type instrument. They have the disadvantages that samples are small and delay in obtaining the results of analysis is increased, and are potential sources of breakdown in the complex mechanical and electrical devices required to carry out these operations.

On-stream X-ray fluorescence analysers are used in the processing of ores of cobalt,[131] nickel, copper and zinc,[132,133] and molybdenum and lead and also in the cement[134,135] and plating industries.[136] Potential areas of application include other ore processing, paper making, petrochemicals, pigment and extractive metallurgical industries.

LIMITS OF DETECTION—

As the characteristic X-ray intensity merges into the background X-ray intensity, the probability that the signal measured originates from the selected element decreases progressively. To assess the value of different methods and techniques of analysis the arbitrary concept of limit of detection has been introduced,[137] and is normally expressed as that concentration of the element which would produce a signal equal to one or more standard deviations found for the measurement of the background signal in the absence of the element. The ultimate limit of detection in X-ray fluorescence analysis is determined by the random counting error, which can be expressed in simple mathematical formulae. Other sources of variation, which can make the limit of detection appreciably greater than that calculated from counting statistics, include instrumental drift in the source, energy selecting system or detector, and lack of knowledge of the true background intensity. This intensity is rarely measured directly as it would entail the use of a blank sample with a composition identical with that of the sample under examination, but not containing the element being determined, and with an identical degree of heterogeneity and surface finish. Assumptions often made include linearity and constancy of rate of change of background intensity with energy on scanning across an X-ray peak or when proceeding from one sample to another of different composition.

The limit of detection can be reduced instrumentally either by increasing the signal-to-background ratio, or by increasing the precision with which the background intensity can be measured. Techniques used for increasing the signal-to-background ratio include optimising the exciting X-ray energy by selecting tube anode and voltage,[138] primary filter[139] or radioisotope,[140] increasing the dispersion or degree of collimation in the spectrometer and by eliminating background intensity of other energies by the use of energy-selective detectors. The limit of detection decreases as the reciprocal of the signal-to-background ratio, provided that the precision with which the background intensity can be measured is not affected. In many instances random counting error is the major source of uncertainty in determining the background intensity, and may be reduced by increasing the measuring time or the background intensity. If the counting time is progressively increased a point is reached when instrumental drift becomes the major source of error. Intensity can be increased by using higher output tubes or more active radioisotope sources, more efficient excitation energies, electron excitation, closer coupling of source and sample, broader collimation, selection and surface treatment of the analysing crystal and by increasing the efficiency of the detector by the use of thinner entrance windows. All of these factors are particularly important in the determination of the lighter elements for which a spectacular increase in intensity has been achieved over the past few years. Some of the techniques used, such as broader collimation, result in a poorer signal-to-background ratio, and care must be taken to check that it does not offset the gain in precision in measuring the background.

The limit of detection obtained with given instrumental conditions varies with the composition of the sample. An adjacent, not fully resolved, emission from another element may increase the background intensity and also variations in that intensity as the concentration of the interfering element changes. As the X-ray absorption coefficients of the sample increase, both the intensities and the signal-to-background ratios decrease, thus giving poorer limits of detection. The lowest limits are found with low atomic number matrices such as hydrocarbons, for which limits of detection below 1 p.p.m. have been widely reported.[141] In some instances the scattered background radiation originates from depths greater than the characteristic X-rays. The signal-to-background ratio can be increased with these samples by reducing the specimen thickness to about the critical depth, reduction below which results in lower intensities with no gain in signal-to-background ratios.

If the limit of detection under optimum instrumental conditions is still inadequate, then X-ray fluorescence becomes just one of several analytical techniques for determining elements after chemical concentration,[142] which involves dissolution of solid samples as an initial step followed by solvent extraction and precipitation or concentration on a suitable ion-exchange resin. All chemical concentration methods nullify two of the main justifications for X-ray fluorescence analysis, *viz.*, examination of the sample as received and speed of analysis. However, its selectivity, flexibility and wide working ranges are retained, and these may be of decisive importance when selecting the technique for determining the concentrated elements.

As the limit of detection of X-ray fluorescence for liquid samples is inferior to that obtained by techniques such as atomic absorption, solvent extraction is of little value, except for laboratories that have only a limited range of techniques available. Limits of detection can be reduced by evaporating the extract and determining the amount of element in the solid residue, but this procedure is tedious for routine analysis and prone to losses or contamination in the evaporation stage. With precipitation methods X-ray fluorescence has the advantage that the solid can be examined directly after removal of the liquid. If a "blanket" method is used,[143] it also permits the simultaneous determination of many elements and the incorporation, if required, of a suitable internal standard. Ion-exchange methods have similar advantages in that the element can be determined on the resin, thus eliminating the need for elutriation required for use with solution techniques. Ion-exchange[144] papers (and membranes) are of particular value because the element can be concentrated in a sample depth close to the critical depth, thus giving high signal-to-background ratios. Ion-exchange techniques coupled with X-ray fluorescence determination have been successfully used in the determination of trace elements in various metals,[145,146] water, petroleum[147] and biological materials.[144]

COUNTING STRATEGY

The main causes of loss of precision in an X-ray spectrographic analysis lie in the sample preparation, instrumental faults and counting errors. Of these variables the counting error is the easiest to control and make quantitative. According to the laws of variance

$$\sigma_{\text{total}} = \sqrt{\sigma^2_{\text{preparation}} + \sigma^2_{\text{instrument}} + \sigma^2_{\text{counting}}}$$

where σ denotes the standard deviation.

When the total number of X-ray counts on a sample is N, a large number, the random distribution of X-ray quanta approximates to a Gaussian distribution of which the standard deviation is for practical purposes equal to $\sqrt{N}$. The well known properties of this distribution enable the precision due to counting to be calculated; for example, the probability that the count will lie between $\pm 2\sigma$ of the observed value is 95·4 per cent. If a measurement includes a background, then

$$\sigma_{(\text{peak} - \text{background})} = \sqrt{\sigma^2_{\text{peak}} + \sigma^2_{\text{background}}}$$

When ratio methods are used, especially if these involve background measurements, the mathematics of determining counting error become complicated.[148]

The choice of "fixed time" or "fixed count" modes of operation on the spectrometer depends on the application, taking into account the precision required and the available instrumental time. It is occasionally useful to subdivide the available time between peak and background measurements in a proportion to give maximum precision,[148,149] but this requires a prior knowledge of the approximate respective count-rates.

A selected list of books,[150 to 158] bibliographies[159,160] and review papers[161,162,163] is included at the end of the specific references. These are not exhaustive but have been selected on the basis of value to the practising X-ray spectrographer.

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The X-ray Fluorescence Determination of Light Elements in Volatile Liquids *in Vacuo* with a Newly Designed Sample Cell

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A sample cell that permits volatile liquids to be studied in a vacuum-path X-ray spectrograph is described. Details of the construction of the cell are given as well as experimental results of studies on sulphur and chlorine-bearing materials.

THE X-ray determination of light elements is hampered by the need to reduce absorption effects of air on the relatively long-wavelength radiation associated with light elements.

Numerous papers have been published on the subject of hydrogen or helium-path determinations,[1,2,3,4] but for most modern vacuum spectrometers the modifications required and the volumes of gas involved make this approach impractical. Little literature is available about determinations on volatile materials in vacuum-path spectrometers.

Arnaud, Romand and Courty[5] published details of the determination of light elements in volatile liquids; they used a sample cell designed by Grubis[6] and constructed on a bellows principle. Carr-Brion and Payne[7] described a polythene cap that would fit commercial sample cells, a small pin-hole being incorporated in the cap to allow for expansion of the sample vapour and air. We have found this system unsatisfactory as a small hole will not allow sufficient vapour to escape and causes the windows to distort; a hole that is too large enables the liquid to boil and thus vitiates the effect of the cap.

As volatile materials evaporate rapidly or boil at ambient temperatures under reduced pressure, it is necessary to isolate them from the surrounding vacuum; we therefore considered the use of a totally sealed cell. However, the window of such a cell would have to be strong enough to withstand the vapour pressure of the sample yet sufficiently thin to keep absorption of the X-rays to a minimum.

We found a simple sealed cell to be wholly inadequate because of the bulging or, in extreme cases, rupturing of the window caused by expansion of the liquid or evolution of dissolved gases. Bulging of the window is undesirable, as it cannot be reproducible and so leads to loss of accuracy in analyses.

DESCRIPTION OF CELL—

To overcome these difficulties, consideration was given to the possibility of using a double-cell construction consisting of an open cell, containing the liquid, within an outer sealed cell.

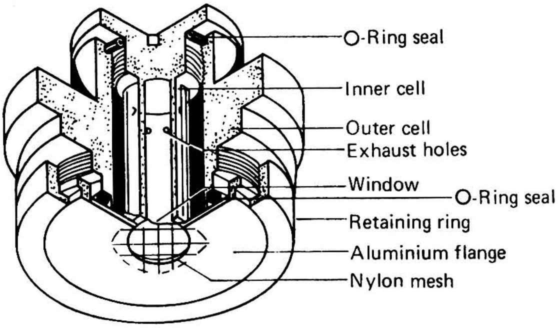

Fig. 1. Section of sample cell

Such an arrangement would equalise the pressure on both sides of the window of the inner cell, thus allowing it to remain flat. Only the outer window is likely to distort; nevertheless the amount of vapour contained between the two windows should not be sufficient to leave a marked effect on the fluorescent intensity from the liquid.

We experimented with various designs of cell constructed from both steel and PTFE, and finally decided on that shown in Fig. 1; a section of the assembled cell is shown in Fig. 2. The main body of the cell is made from stainless steel with O-rings incorporated to maintain vacuum seals. The inner window is supported by a push-fit PTFE ring, while the outer window is held in place with an aluminium flange. The inner cell is filled with a hypodermic syringe through one of the exhaust holes, which allow the vapour in the inner cell to expand into the outer cell.

It is necessary that the outer window should not distend to such an extent that its separation from the inner window becomes so great as to create a substantial path length for absorption of secondary X-rays by the vapour. Further, the free space between the base of the cell and the lower surface of the sample changer in the Philips PW1220 spectrometer is such that even a moderate degree of swelling causes the window to make contact with the sample changer. By weaving a mesh nylon thread across the outer face of the window, distortion was reduced to a minimum without undue absorption of X-rays and the window remained intact.

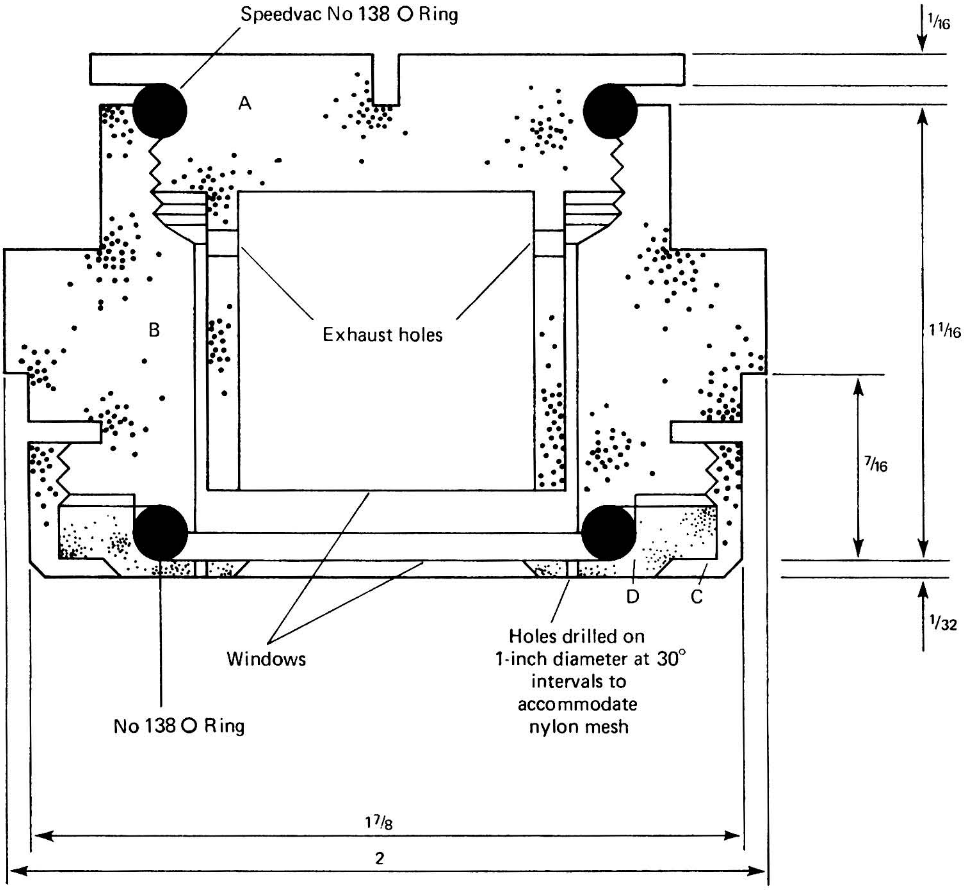

Fig. 2. Section of assembled cell. Parts A, B and C in stainless steel and part D in aluminium (all measurements are in inches)

Initial tests were performed with 6-μm thick Mylar windows. Further tests were carried out by using 2-μm thick polycarbonate for both inner and outer windows. The outer window burst during most of these tests but on those that were successful there was an increase of 50 per cent. in the sulphur Kα intensity from a dilute solution of thiophene in heptane. A compromise with a 6-μm thick Mylar outer window and a 2-μm thick polycarbonate inner window was completely reliable and still gave an improved count-rate over the double Mylar system.

RESULTS AND DISCUSSION

Our analytical problems in this field were concerned mainly with the determination of chlorine in aqueous media and of chlorine and sulphur in naphthas, condensates, etc.

Series of standards were prepared by adding measured amounts of butanethiol and 1,2-dichloroethane in heptane to give solutions of up to about 1000 p.p.m. of sulphur and 800 p.p.m. of chlorine. These were investigated by using the double cell on a Philips PW1220 X-ray spectrograph with a chromium-target tube operated at 50 kV and 36 mA. Intensities of the chlorine and sulphur Kα peaks were measured with a methane - argon flow proportional counter and pentaerythritol analysing crystal by counting for 100 s on both peak and background. The calibration graphs for chlorine and sulphur are illustrated in Figs. 3 and 4, respectively. Detection limits in both instances were estimated to be about 10 p.p.m., but these can be improved by using thinner window materials.

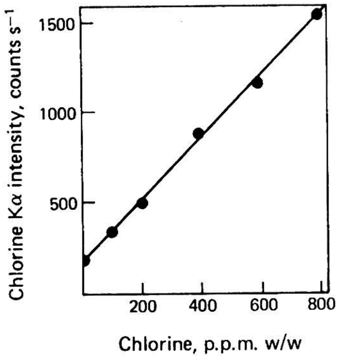

Fig. 3. Calibration graph for chlorine

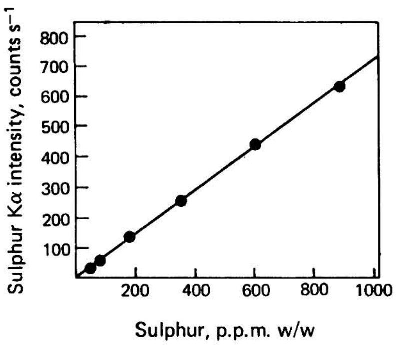

Fig. 4. Calibration graph for sulphur

The determinations of the sulphur contents of a series of naphthas carried out with the cell compared favourably with those obtained by chemical methods.

We have found that the main disadvantage of the cell is the time taken to dismantle, refill and re-assemble it, particularly if the outer window has to be changed. However, the whole operation should not take more than about 2 minutes with practice.

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Simultaneous Determination of Copper and Manganese in Plants by Neutron-activation Analysis

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By using the radioactivation technique, copper and manganese have been determined simultaneously in plant leaves.

A simplified and rapid procedure for the determination of both elements in plant leaves is presented. The copper and manganese in irradiated samples are separated by solvent extraction, the former being extracted with α-benzoinoxime - pyridine - chloroform and the latter with 8-hydroxyquinoline - chloroform.

The time required for radiochemical separation from the end of irradiation to counting is less than 1 hour for ten samples.

The precision of the method in the range of 0·1 to 0·2 p.p.m. is about ±5 per cent. The limits of detection calculated from the analysis of standard specimens are about 0·01 μg for copper and 0·001 μg for manganese.

COPPER is one of the important metal ions and manganese an essential element in the medical and biological fields.[1] Hence, the determination of copper and manganese in biological materials has so far frequently been carried out by radioactivation analysis, as both elements can be determined with high sensitivity by thermal-neutron activation analysis.

With this technique, Souliotis[2] has determined copper and zinc in plants, while Malvano, Rosa and Grosso[3] determined copper and other elements in human serum albumin. Bowen[4] has also reviewed the methods of analysis for the trace elements in kale powder and compared the results of radioactivation analysis with those of other analytical methods.

In the radiochemical method for the separation of copper and manganese in irradiated samples, an ion-exchange method and precipitation technique have, in general, been used by several workers.[5,6,7,8] Although most workers have published radiochemical methods involving the repeated precipitation of suitable copper and manganese compounds, these procedures cannot be applied to the simultaneous analysis of several samples because considerable time is needed to carry out the separation procedure.

As reported by Healy and Bate,[9] α-benzoinoxime has been investigated as an extractant for molybdenum, tungsten and other elements, and also as an extractant for copper. However, because of the conditions of extraction, it appears that the induced radionuclides generally involved in biological samples, particularly sodium-24 activated by thermal neutrons, are extracted with the copper.

In this work a simplified and accurate activation-analysis method is presented for the simultaneous determination of copper and manganese in a series of samples of plant leaves after irradiation.

EXPERIMENTAL

APPARATUS—

A TMC 400-channel γ-ray spectrometer with a 3 × 3-inch NaI (thallium) crystal was used.

REAGENTS—

All reagents were of recognised analytical grade.

Copper carrier solution—Prepare a stock solution by dissolving 1·0 g of copper metal in concentrated nitric acid and diluting with distilled water to give a solution containing 10 μg ml^{-1} of copper.

Manganese carrier solution—Prepare a stock solution by dissolving 0·45 g of manganese nitrate in distilled water to give a solution containing 10 μg ml^{-1} of manganese.

α-Benzoinoxime - pyridine - chloroform solution—Dissolve 1 g of α-benzoinoxime in 50 ml of pyridine and pour the solution into 450 ml of chloroform. Store it in a coloured bottle.

8-Hydroxyquinoline - chloroform solution—Dissolve 1 g of 8-hydroxyquinoline in 500 ml of chloroform. Store the solution in a coloured bottle.

IRRADIATION—

The plant-leaf samples were dried in a drying oven at 110° C for 24 hours and were then ashed in a muffle furnace at 450° C for 5 hours. The standard specimens were prepared by placing together on a 3 × 3-cm polythene sheet, with a pipette, 100-μl aliquots of the copper and manganese carrier solutions and evaporating them carefully to dryness under an infrared lamp.

The ashed sample (0·1 g) and 0·1 g of ashed sample to which were added suitable amounts of each standard were placed in a small polythene bag, which was then heat-sealed.

Ten samples and two standard specimens were packed closely together and irradiated in a thermal neutron flux of about $4{\cdot}4 \times 10^{12}$ neutrons cm^{-2} s^{-1} for 1 hour in the Triga Mark II reactor at the Musashi Institute of Technology.

RADIOCHEMICAL PROCEDURE—

Open the polythene bag containing the irradiated samples and transfer them into 100-ml glass beakers. Introduce 10 μg of copper and manganese carrier into the beaker containing the sample and dissolve the latter in a few millilitres of concentrated hydrochloric acid solution. Evaporate the solution to dryness by heating the beaker on a sand-bath, dissolve the residue in a few drops of concentrated hydrochloric acid, add 15 ml of distilled water and then about 1 ml of 10 per cent. hydroxylamine. Filter the solution into a separating funnel, add to it 5 ml of 5 per cent. sodium tartrate solution and adjust the pH to between 6·0 and 7·0 with 0·5 N ammonia solution, with bromothymol blue as indicator; then extract the copper with 20 ml of α-benzoinoxime - pyridine - chloroform (repeating this extraction step) by shaking the mixture for 30 s. Retain the aqueous phase for the determination of manganese. Transfer the organic phase into another separating funnel and back-extract the copper with 20 ml of 3 N nitric acid solution. Transfer, with a pipette, 5·0 ml of this solution to the counting vial for γ-ray spectrometry.

To extract manganese with 8-hydroxyquinoline - chloroform[10] after extracting the copper, transfer the aqueous phase into another separating funnel and adjust the pH value to between 8·0 and 9·0 with 0·5 N ammonia solution, with phenolphthalein as indicator. Extract the manganese with 20 ml of 0·2 per cent. 8-hydroxyquinoline - chloroform and then back-extract the manganese with 20 ml of 3 N nitric acid. Transfer, with a pipette, 5 ml of this solution to the counting vial for γ-ray spectrometry.

ACTIVITY MEASUREMENT—

The radiochemical purities of the separated copper and manganese were checked by γ-ray spectrometry and by following the decay curve.

For quantitative measurements the photopeaks of copper-64 at 0·51 MeV and the photopeak of manganese-56 at 0·84 MeV mainly were selected and counted for 1 to 5 minutes, depending on the activity level.

RESULTS AND DISCUSSION

The separation of copper and manganese from the radioisotopes induced from other elements has been considered. The percentage extraction of copper-64 and manganese-56, and other radioisotopes, with 0·2 per cent. α-benzoinoxime - pyridine - chloroform from the media selected for separation is shown in Table I, the optimum acidity for the selective separation of copper-64 and manganese-56 with α-benzoinoxime - pyridine - chloroform and 8-hydroxyquinoline - chloroform being also investigated. It is well known that copper and manganese can be extracted with α-benzoinoxime and 8-hydroxyquinoline, respectively, in chloroform from a weakly acidic solution.[9]

TABLE I

EXTRACTION OF COPPER-64, MANGANESE-56 AND OTHER ISOTOPES WITH α-BENZOINOXIME - PYRIDINE - CHLOROFORM AND WITH 8-HYDROXYQUINOLINE AFTER EXTRACTION OF COPPER-64

	Percentage extraction		
	α-Benzoinoxime - pyridine - chloroform		
Isotope	pH 6 to 7, without 5 per cent. sodium tartrate	pH 6 to 7, containing 5 per cent. sodium tartrate	8-Hydroxyquinoline
Manganese-56	10·2	—	96·6
Copper-64	85·8	86·1 to 87·6	—
Molybdenum-99	1	—	—
Zinc-65	1	—	—
Cobalt-60	—	—	—
Tungsten-187	3	—	—
Iron-59	—	—	—
Sodium-24	20·6	6·5	—

However, because of the conditions of extraction, *i.e.*, of the effects of pH and the presence of a masking reagent, it appears that the other induced radionuclides usually involved in biological samples are co-extracted with the copper. The results obtained are shown in Table II, from which it is evident that quantitative extraction with α-benzoinoxime - pyridine - chloroform from a hydrochloric acid solution at a pH between 6·0 and 7·0 containing 5 ml of 5 per cent. sodium tartrate solution was achieved while more than 82 per cent. of the copper-64 could be extracted. It can also be seen that sodium-24 is partially co-extracted with the copper-64 by this reagent from such a solution (Table I).

TABLE II

EFFECT OF ACIDITY ON EXTRACTION OF COPPER-64 AND MANGANESE-56 WITH α-BENZOINOXIME - PYRIDINE - CHLOROFORM

	α-Benzoinoxime - pyridine - chloroform			
	Without 5 per cent. sodium tartrate		Containing 5 per cent. sodium tartrate	
pH value	Copper-64	Manganese-56	Copper-64	Manganese-56
2	23·8	6·6	22·1	—
3	40·1	11·9	43·8	—
4	57·4	16·1	56·9	—
5	71·8	25·3	74·5	—
6	83·7	45·1	82·9	—
7	87·3	78·1	85·0	—
8	71·6	89·7	72·4	—
9	56·4	93·4	54·6	—

Extraction with 8-hydroxyquinoline will clearly separate manganese-56 in a radiochemically pure state as the amount of copper, the only other element extracted with this reagent, in biological materials is negligible.

TABLE III

RESULTS (P.P.M. ASH WEIGHT) OBTAINED FOR COPPER AND MANGANESE IN ASHED PLANT LEAVES

	Concentration, p.p.m.			
Sample	Copper	Mean value	Manganese	Mean value
Allium cepa Linn.	16·0, 15·8, 16·1	15·9	557·1, 550·7, 564·6	557·4
Spinacia olercea Linn.	18·7, 18·3, 18·0	18·3	738·1, 740·8, 752·0	743·6
Brassica pekinensis Rupr.	8·7, 8·8, 8·2	8·5	237·6, 229·7, 231·6	232·9
Raphanus sativus Linn.	6·6, 6·2, 6·0	6·2	287·9, 300·1, 295·8	294·6
Brassica olercea var. *capitata* Linn.	9·0, 8·7, 8·4	8·7	222·1, 218·1, 231·9	224·0

In Table III results are shown for the determination of copper and manganese in plant leaves. The accuracy of the procedure was tested by the addition method. Five samples were prepared by placing aliquots of 0·5 and 1·0 ml of sample solution (equivalent to 0·5 and 1·0 mg of plant-leaf-ash sample), spiked with 0·05 and 0·25 µg of copper and manganese, and 0·1 and 0·5 µg of copper and manganese, respectively, on a 3 × 3-cm polythene sheet and evaporating them carefully to dryness under an infrared lamp. After irradiation, they were submitted to the chemical separation and counting as described above. The results obtained (triplicate determinations on each sample) are shown in Table IV, from which it can be seen that the average precision for the determination of copper and manganese by this method is about ±5 per cent. for a concentration of 0·1 p.p.m.

TABLE IV

RESULTS OBTAINED FOR COPPER AND MANGANESE BY STANDARD ADDITION METHOD

Sample No.	Amount, mg	Amount of standard added, µg: Copper	Amount of standard added, µg: Manganese	Activity of copper-64, counts minute^{-1}	Activity of manganese-56, counts minute^{-1}	Found in ash, µg mg^{-1}: Copper	Found in ash, µg mg^{-1}: Manganese
1	0·5	—	—	88·3 ± 12·6	10 487·9 ± 177·3	—	—
		0·05	0·05	596·3 ± 32·7	12 636·2 ± 194·7	0·015	0·534
		0·25	0·25	2 709·8 ± 69·8	21 818·7 ± 225·8	0·016	0·556
2	1·0	—	—	173·7 ± 17·6	20 739·6 ± 249·4	—	—
		0·1	0·1	1 231·1 ± 47·0	24 409·0 ± 270·6	0·016	0·563
		0·5	0·5	5 167·4 ± 96·4	41 523·0 ± 352·9	0·017	0·547

The main source of error can be attributed to interfering nuclear reactions induced by fast neutrons. In the present work, the ^{64}Zn (n,p) ^{64}Cu and the ^{56}Fe (n,p) ^{56}Mn reactions are the only fast-neutron reactions that can result in significant interference. The iron content of some plant leaves was determined by the *o*-phenanthroline method and found to be in the range 50 to 150 µg mg^{-1} in the ashed leaves. The activity of 1 µg of iron is estimated to be equivalent to that of 0·003 µg of manganese. Therefore, calculation showed that the activity from the latter reaction contributed notably to the total manganese-56 activity. The correction required for this contribution amounts to about ±3 per cent. However, with the ^{64}Zn (n,p) ^{64}Cu reaction only the fast-neutron reactions can result in significant interference. The activity of copper-64 from this reaction could be neglected because the cross-section of the ^{64}Zn (n,p) ^{64}Cu reaction is much smaller than that of the ^{63}Cu (n,γ) ^{64}Cu reaction and, moreover, the amount of zinc occurring in plants[11] is usually less than 100 µg mg^{-1} of ash.

TABLE V

RECOVERIES OF COPPER AND MANGANESE IN ADMIXTURE

Mixture	Added, µg: Copper	Added, µg: Manganese	Activity of copper-64, counts minute^{-1}	Activity of manganese-56, counts minute^{-1}	Found, µg: Copper	Found, µg: Manganese
A	0·1	0·1	1 001·5 ± 42·45	3 492·4 ± 79·28	0·085	0·096
B	0·5	0·5	4 913·6 ± 94·04	16 862·0 ± 174·21	0·42	0·471
C	1·0	1·0	9 727·3 ± 132·32	34 503·2 ± 249·2	0·83	0·954
D	2·0	2·0	19 319·2 ± 186·47	67 105·1 ± 347·5	1·64	1·810
E	0·1	—	1 173·6 ± 45·96	—	0·099	—
F	—	0·1	—	3 623·6 ± 80·76	—	0·099

As shown in Table V, the chemical recovery was in the range from 82 to 85 per cent. for copper and from 90·5 to 96 per cent. for manganese. The chemical separation time, including the time from the end of irradiation to counting, of the proposed method was less than 1 hour for ten samples.

The limits of detection were calculated from analyses of standard specimens to be about 0·01 µg for copper and 0·001 µg for manganese. The precision of the method is about ±6 per cent.

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A Routine Method for the Determination of Low Caesium-137 Activities in Biological Samples

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A routine method for the determination of low fall-out activities of caesium-137 in biological materials is described. Radiocaesium is extracted by adsorption on ammonium molybdophosphate. The usual radiochemical purification of the alkaline ammonium molybdophosphate solution by anion exchange is omitted and caesium precipitated directly with bismuth tri-iodide in the presence of citric acid as complexing agent. A caesium chloroplatinate source is prepared for β-counting.

IN feeding experiments with guinea pigs and hogs the influence of natural clay minerals on the caesium-137 fall-out contamination was studied. Measurement of low levels of caesium-137 present in small samples requires β-counting, with carefully purified sources. For preliminary concentration, the absorption of caesium on ammonium molybdophosphate is widely used. Kamath *et al.*[1] and Mercer *et al.*[2] subject the alkaline ammonium molybdophosphate solution to anion-exchange treatment to remove the molybdophosphate; these procedures are, however, time consuming in routine work. According to Grüter[3] the use of citric acid as a complexing reagent for molybdophosphate prevents side reactions when precipitating caesium with sodium tetraphenylboron in moderately acidic solutions. It was found that caesium can also be precipitated with bismuth tri-iodide in the presence of citric acid, which not only prevents re-precipitation of ammonium molybdophosphate but also prevents side reactions with the excess of precipitant.

Whether co-precipitation of other activities can be avoided in the presence of citric acid was not specifically investigated but, in the proposed procedure, polyvalent ions are removed by previous treatment with sodium hydroxide - sodium carbonate. The decontamination effect of this procedure was proved with samples spiked with zirconium-95 and niobium-95; less than 1 per cent. of the added activity was found to be present in the caesium fraction. This separation factor seems to be sufficient with older fall-out mixtures.

REAGENTS—

Ammonium molybdophosphate reagent—Dissolve 8 g of disodium hydrogen orthophosphate in 250 ml of water and mix this solution with a solution containing 10 g of ammonium nitrate in 50 ml of water *plus* 30 ml of concentrated nitric acid. Stir the mixture, heat it to about 80° C, and slowly add a solution containing 70 g of ammonium molybdate [$(NH_4)_6Mo_7O_{24}.4H_2O$] in 250 ml of water. Filter off the yellow precipitate by suction and wash it with 100 ml of 1 per cent. nitric acid and then with methanol. Ammonium molybdophosphate that has been dried in a vacuum desiccator can be stored in a dark bottle for several months without any loss of ion-exchange properties.

Bismuth tri-iodide reagent—Dissolve 40 g of bismuth tri-iodide in a solution containing 40 g of sodium iodide in 96 ml of water *plus* 4 ml of glacial acetic acid. Stir the solution frequently, filter if necessary and store in a dark bottle in a refrigerator.

Sodium hydroxide, 50 per cent. w/v and 2 N *solutions.*

Sodium carbonate, saturated solution.

Citric acid solution, 30 *per cent. w/v.*

Acetic acid, 30 *per cent. v/v.*

Nitric acid, 1 *per cent. v/v, and concentrated.*

Chloroplatinic acid, 10 *per cent. w/v.*

Hydrochloric acid, concentrated and 10 *per cent. v/v.*

Thymol blue indicator solution—Dissolve 50 mg of thymol blue in 100 ml of 20 per cent. v/v methanol.

Ethanol, 96 *per cent.*

Collodion, 1 *per cent. w/v, in ethanol - diethyl ether* (1 + 1).

PROCEDURE—

The weighed biological material is carefully ashed in a stainless-steel tray for 12 hours below 450° C to destroy organic matter and an aliquot portion of the resultant carbonaceous ash used for the analysis. (The results of the measurements are based on the weight of fresh material taken.) After adding 40 mg of caesium carrier, the sample is leached by heating with a mixture of 200 ml of water and 20 ml of concentrated hydrochloric acid. The carbonaceous residue is filtered off, washed with water and ashed in a small quartz crucible at 450° C. The ash is dissolved in 20 ml of 10 per cent. hydrochloric acid and the solution combined with the former solution for subsequent radiochemical treatment.

Neutralise the solution of the ash with 50 per cent. sodium hydroxide until the thymol blue indicator turns blue, add 10 ml of saturated sodium carbonate, heat for 10 minutes in a boiling water bath, centrifuge, and collect the supernatant solution. Wash the precipitate with 100 ml of water, centrifuge the mixture again and combine the supernatant solutions. Neutralise the resultant solution with concentrated nitric acid until the thymol blue indicator turns a slight reddish colour, then add sufficient concentrated nitric acid to make the solution approximately 0·5 N. Add 1 g of ammonium molybdophosphate reagent and stir for 1 hour to absorb the caesium. After the molybdophosphate has settled, decant the supernatant solution, then suspend the molybdophosphate in 50 ml of 1 per cent. nitric acid and again, after it has settled, decant the supernatant liquid. Dissolve the ammonium molybdophosphate reagent in 10 ml of 2 N sodium hydroxide, heat the solution on a hot-plate for 5 minutes, cool and add 5 ml of 30 per cent. citric acid. Continue evaporating until the volume is about 5 ml. Filter the solution into a 50-ml centrifuge bottle and wash the filter with water until a total volume of 10 ml is obtained.

After cooling the solution in ice - water, add 5 ml of bismuth tri-iodide reagent and continue cooling for 10 minutes, stirring frequently to facilitate precipitation of caesium bismuth tri-iodide. Centrifuge the mixture, discard the supernatant solution and wash the precipitate with 10 ml of bismuth tri-iodide solution diluted 1 + 9 with 30 per cent. acetic acid. Centrifuge again and discard the wash solution. Decompose the caesium bismuth tri-iodide by heating it with 5 ml of 30 per cent. nitric acid in a boiling water bath for 2 minutes. Filter off the iodine and wash the filter with 7 ml of 30 per cent. nitric acid. Continue heating the filtrate to remove all of the iodine and filter as before (see Note).

NOTE—

In the simultaneous treatment of several samples cross-contamination by splashing is likely to result when boiling off the iodine by direct heating. Therefore, it seems more convenient to remove iodine by heating in a water-bath than on a hot-plate.

Cool the solution in ice - water and precipitate the caesium with 1 ml of 10 per cent. chloroplatinic acid solution; continue cooling for 30 minutes. Centrifuge the mixture, discard the supernatant solution, re-suspend the precipitate in cold ethanol, then filter it off on a tared filter-paper and wash it twice with cold ethanol. Dry the precipitate to constant weight at room temperature to determine the recovery of caesium. Attach the filter-paper on a planchet with a few drops of 1 per cent. collodion solution to prepare a source for β-counting.

COUNTING—

An Intertechnique RA12 low level β-counter, with an automatic sample changer and a background of 0·65 count minute^{-1} was used. Corrections for self-absorption, back-scattering and for the counting efficiency were derived from a series of carefully prepared caesium-137 sources.

RESULTS

From synthetic solutions containing 40 mg of caesium carrier, 2 g of potassium chloride, 2 g of disodium hydrogen orthophosphate and 2 g of ammonium chloride in 400 ml of water, the chemical recovery for caesium was about 70 per cent.; from biological samples (muscle tissue, bone and skin) about 60 per cent. The recovery from synthetic solutions spiked with caesium-137 amounted to 96 per cent. of the added activity. All sources were free from potassium. Residual potassium in the caesium sources was checked by flame photometry with a spectrophotometer after decomposing the chloroplatinate with magnesium in dilute hydrochloric acid by the method of Fresenius and Jander.[4]

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The Spectrophotometric Determination of Hydroxyproline: An Analytical Investigation

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Thirty-eight methods for the spectrophotometric determination of hydroxyproline have been tested. The sensitivity of twenty-seven of these under routine conditions and their applicability for determining of hydroxyproline in synthetic and natural mixtures have been examined. Most procedures were suitable for use in the presence of other amino-acids, but sugars, urea and ammonium chloride caused considerable analytical losses. No single method was both sensitive and reproducible for use with synthetic mixtures and biological materials. The best results with any method were obtained by the identical treatment of all samples and by use of internal standards.

INTEREST in the quantitative determination of hydroxyproline has increased in recent years. Many biological materials, *e.g.*, collagen, urine, leather and some plant proteins, contain this imino-acid.

Two colorimetric reagents have been used in the determination of hydroxyproline. The reaction with ninhydrin[1] has found wide application in automatic amino-acid analysers,[2] but is not satisfactory for determination of microgram amounts.

The more specific reagent, *p*-dimethylaminobenzaldehyde, has been preferred by most workers in the field. All published procedures have involved oxidation of hydroxyproline and subsequent condensation with *p*-dimethylaminobenzaldehyde. Lang[3] used sodium hypochlorite as the oxidant, but this was found to be unsatisfactory by Waldschmidt-Leitz and Akabori,[4] and Wiss.[5] McFarlane and Guest[6] used sodium peroxide in the presence of copper(II) ions as the oxidant, and the modification by Neuman and Logan,[7] in which sodium peroxide was the oxidant, has been widely used. The method has been modified[8 to 25] for specific purposes but with varying degrees of success. Different concentrations of reagents, various heating times, use of different agents for destruction of excess of oxidant, extraction of the oxidation products[16,21] or distillation products[22,23,25] before colorimetry, and reversion to the use of sodium peroxide[20] have been recommended by different authors.

Muting[26] first used sodium hypobromite instead of hypochlorite.[3] Other methods involving the use of this oxidant were developed by Kivirikko and Liesmaa,[27] Kivirikko[28] and Lamport (private communication, 1968).

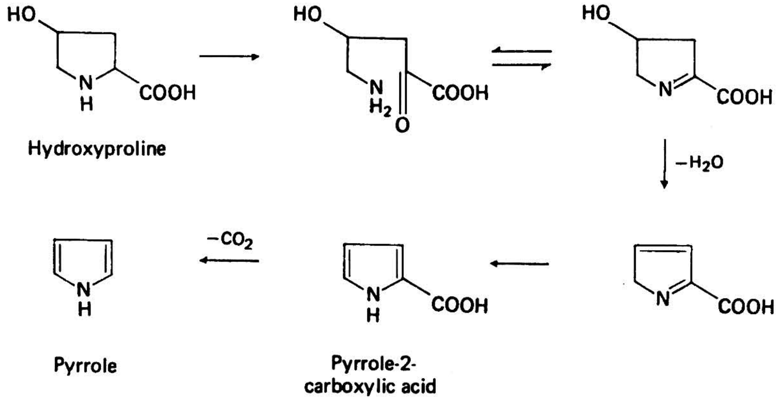

TABLE I: SUMMARY OF PROCEDURES, SENSITIVITY, ABSORBANCE DATA AND TIME

	Oxidation			Colour development			
Oxidant	Temperature, °C	Time, minutes	Reducing agent	Aldehyde concentration in test solution, per cent. w/v	Temperature, °C	Time, minutes	Other information
Chloramine T	Room	4	Perchloric acid	5·90	60	21	Pre-mixed reducing agent and aldehyde
	20	20	Perchloric acid	4·30	60	15	Pre-mixed reducing agent and aldehyde
	Room	20	Perchloric acid	4·00	60	20	Separate reduction and aldehyde
	Room	20	Perchloric acid	1·67	60	15	Separate reduction and aldehyde
	Room	25	Thiosulphate	1·42	Room	30	Separate reduction and aldehyde Sample saturated with potassium chloride
	17 to 21	4	Perchloric acid	9·60	60	25	Pre-mixed reducing agent and aldehyde
	Room	20	Thiosulphate	1·43	Room	15	Separate reduction and aldehyde Sample saturated with potassium chloride
Sodium hypobromite	0	5	3 per cent. sodium sulphite	1·41	90	5	5 minutes at 0° C after heating
	0	3 to 10	3 per cent. sodium sulphite	1·64	100	2·5	3 minutes at 0° C after heating
	0	5 to 10	3 per cent. sodium sulphite	1·64	100	2·5	3 minutes at 0° C after heating
	0	2	20 per cent. sodium sulphite	0·65	100	2	No chilling treatment
Hydrogen peroxide *plus* sodium hydroxide	Room	5	Heat at 70° C for 10 minutes	2·50	70	40	—
	Room	5	Heat at 80° C for 5 minutes	1·00	80	30	—
	Room	5	Shake at room temperature	1·00	70	16	—
	Room	5	Heat at 80° C for 5 minutes	1·00	80	30	—
	Room	5	Warm to 40° C for 15 minutes	1·00	70	30	—
	Room	5	Heat at 80° C for 5 minutes	1·00	70	20	Sodium peroxide oxidising agent
	Room	5	Heat at 80° C for 5 minutes	1·00	70	16	—
	Room	5	Add 0·05 M iron(II) sulphate	0·91	70	16	—
	40	10	—	1·11	70	16	Hydrogen peroxide added at 40° C
	Room	5	Heat at 80° C for 5 minutes	1·00	70	16	—

Chloramine T (sodium toluene-*p*-sulphonchloroamide) was used first by Stegemann[29] and there have been numerous modifications[30 to 39] of this procedure, including one for automatic analysis.[40]

All oxidation - condensation procedures are based on the same operating principles. The oxidation of hydroxyproline is ended by chemical or physical destruction of the oxidant. The colour reagent is added and the solution acidified and heated, after which the red complex forms. This complex has an absorption maximum at about 560 nm.

TAKEN FOR COLORIMETRIC DETERMINATIONS OF HYDROXYPROLINE

Sensitivity (absorbance given by 1 μg of hydroxyproline)	Reproducibility of analysis (over-all range of results)* per cent.	Observed absorption maximum, nm	Wavelength for measurement, nm	Time for analysis, minutes†	Reference number
0·116	± 4	568	562	70	39
0·078	± 2	562	550	70	36
0·070	± 2	561	557	70	31
0·027	± 5	560	560	75	29
0·026	± 5	562	560	225	37‡
0·018	± 2·5	560	558	120	33
0·012	± 3·5	561	560	200	30‡
0·067	± 1·5	560	560	70	Lamport's method
0·040	± 2	560	560	70	28
0·028	±10	560	560	75	27
0·012	±10	560	560	75	26
0·031	1·5	553	560	110	19
0·028	1	558	555	110	18
0·021	3·5	557	560	75	15
0·019	4	558	560	100	13
0·019	4	558	560	110	14
0·018	3	558	550	90	20
0·018	2	559	560	90	7
0·018	2	557	560	90	8
0·017	4·5	560	555	90	17
0·015	5	559	540	200	16‡

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* Reproducibility was calculated from the range of absorbance values obtained by repeated analyses of samples containing 1 μg ml^{-1} of hydroxyproline.

† The time given is that required to perform a calibration analysis consisting of twenty-five samples (triplicate samples at eight concentrations *plus* one reagent blank) from addition of first reagent to completion of spectrophotometry.

‡ These procedures include an extraction step.

The detailed pathway and mechanism of the reaction are not known. Some workers[23,41] postulated that the oxidation reaction is as follows. Hydroxy-D-proline or allohydroxy-D-proline is converted by oxidation into α-keto-γ-hydroxy-δ-aminovaleric acid, which is in equilibrium with Δ^1-pyrroline-4-hydroxy-2-carboxylic acid. Loss of water gives a structure which, on rearrangement, gives pyrrole-2-carboxylic acid. During heating pyrrole-2-carboxylic acid is converted into pyrrole by decarboxylation and the pyrrole condenses with *p*-dimethylaminobenzaldehyde to form the red complex (see reaction scheme, p. 1003).

Bergman and Loxley[32,38] suggested that the oxidation products varied with the oxidant. They disputed the reaction shown because of their findings that pyrrole-2-carboxylic acid (the postulated product) was destroyed by chloramine T (the oxidant), and that the reaction between pure pyrrole-2-carboxylic acid and *p*-dimethylaminobenzaldehyde was slower than that in the analytical system. Similar conclusions were reached by Blomfield and Farrar.[24]

The work reported here was undertaken to establish the most suitable procedures for multiple determinations of microgram amounts of hydroxyproline in the biological materials that are known to contain this imino-acid. Of thirty-eight methods tested initially, sixteen were found to be unsuitable. The early procedures[3,4,5,6] were not sufficiently sensitive and those requiring repeated solvent extraction[21,22,23] or distillation[25] were unsuitable for routine use. Several other methods[9,10,11,21,24,34,35,38] were found to give results similar to those given by previously published procedures.

MATERIALS AND METHODS

Twenty-two methods[7,8,12 to 20,26 to 33,36,37,39] and Lamport's, making twenty-three in all, were tested by using each procedure exactly as published. The methods were studied first by analysis of pure solutions of hydroxyproline and calibration graphs were obtained for each procedure. Analyses on test solutions were performed in triplicate by using eight concentrations within the limits set by the respective authors up to a maximum of 15 μg ml^{-1}.

Other test solutions were of 1 ml containing: a synthetic amino-acid mixture with 5 μg each of alanine, aspartic acid, arginine, cystine, glutamic acid, glycine, histidine, hydroxyproline, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine and valine; 5 mg of urea *plus* 5 μg of hydroxyproline; 5 mg of ammonium chloride *plus* 5 μg of hydroxyproline; 5 mg of glucose *plus* 5 μg of hydroxyproline; 5 mg of mannitol *plus* 5 μg of hydroxyproline; hydrolysed collagen; hydrolysed collagen *plus* 5 μg of hydroxyproline; hydrolysed human urine; hydrolysed human urine *plus* 5 μg of hydroxyproline; hydrolysed plant-seed coat extract; hydrolysed plant-seed coat extract *plus* 5 μg of hydroxyproline; and 5 μg of hydroxyproline in 0·2 M citrate buffer at a pH of 3·25.[2]

Spectrophotometric determinations were performed on a Beckman DU spectrophotometer and on a Unicam SP800 recording spectrophotometer. The same pair of matched cells with a 1-cm light path was used throughout.

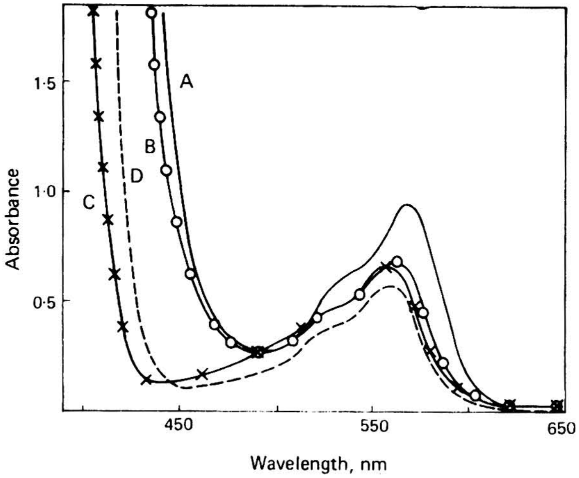

Fig. 1. Absorption spectra of reaction products from four different analyses for hydroxyproline: A, 8 μg ml^{-1}, reference 39; B, 6 μg ml^{-1}, reference 36; C, 10 μg ml^{-1}, Lamport's method; and D, 15 μg ml^{-1}, reference 19

Hydrolysis of collagen—Rat-tail collagen (a gift from Dr. Robert L. Coupe) was hydrolysed with 6 N hydrochloric acid for 16 hours at 110° C *in vacuo*. The acid was removed in a vacuum desiccator and the residue dissolved in de-ionised water.

Hydrolysis of urine—Human urine was hydrolysed and treated by the method of Prockop and Udenfriend.[30]

PREPARATION AND HYDROLYSIS OF PLANT-SEED COAT EXTRACT—

Coats of bean (*Vicia faba* L.) seeds were ground in a mill. The meal (10 g) was stirred with 100 ml of M sodium hydroxide solution for 4 hours at room temperature. The filtrate was neutralised with concentrated hydrochloric acid and the resulting precipitate removed by centrifugation. The supernatant liquid was then dialysed against running tap water for 48 hours, lyophilised and hydrolysed with 6 N hydrochloric acid under reflux for 24 hours. The hydrolysate was treated with activated charcoal[42] to remove coloured substances.

p-Dimethylaminobenzaldehyde was recrystallised[19] and stored under nitrogen. The stock solution of hydroxyproline (20 μg ml^{-1}) was stored at 4° C in 0·25 N hydrochloric acid[17] and was stable for 3 months.

All other chemicals were of the highest quality available. De-ionised water was used for all solutions.

RESULTS

The sensitivity and reproducibility of each procedure were determined from calibration graphs. The results are presented in Table I. Most methods were reproducible to within ±5 per cent. up to a concentration of 15 μg ml^{-1}. Four procedures in which chloramine T[31,36,39] or sodium hypobromite (Lamport's method) were used were found to be most sensitive for pure solutions of hydroxyproline; however, the methods that we found to be least sensitive[26,30] also involved these oxidants. The latter two procedures were not examined further. Only one method[33] was found unsatisfactory, as we obtained no reproducible results in spite of several attempts with different conditions.

Absorption spectra of the reaction products were obtained for all analyses and those from four (three[19,36,39] and Lamport's) different procedures are shown in Fig. 1. The maxima ranged from 558 to 568 nm. The forms of the graphs were generally similar but the colour of the solutions ranged from deep orange[39] to purple (in Lamport's method).

The effects of some common physiological compounds[38] on the quantitative recovery of hydroxyproline were studied by determining the absorbance of 5 μg of hydroxyproline in the presence and absence of the individual compounds. The results are given in Table II.

TABLE II

RECOVERIES OF ADDED HYDROXYPROLINE IN PURE SOLUTIONS OF COMMONLY OCCURRING CONTAMINANTS*

	Percentage recovery of hydroxyproline in presence of					
Oxidant	Amino-acids	Urea	Ammonium chloride	Glucose	Mannitol	Reference number
Chloramine T	103·0	78·0	68·0	95·0	99·0	39
	95·0	82·5	70·0	98·5	100·0	36
	97·2	62·8	94·2	98·0	102·8	31
	99·0	71·0	75·7	99·0	103·6	29
	101·0	105·0	106·0	101·0	101·0	37
	100·0	97·8	63·8	97·8	95·8	32
Sodium hypobromite	95·0	50·1	50·1	103·7	97·8	Lamport's method
	95·0	47·8	50·0	102·0	83·0	28
	100·0	80·7	37·5	141·2	140·0	27
Hydrogen peroxide	87·6	62·2	93·2	97·7	88·2	19
	85·6	71·5	89·3	77·2	71·5	18
	99·5	81·7	95·2	98·3	88·9	15
	100·0	90·0	92·3	92·3	86·5	13
	101·3	88·0	92·6	91·7	92·6	14
	46·5	37·5	91·2	41·2	21·5	20
	91·0	66·6	89·8	81·6	83·8	7
	99·0	72·7	96·6	99·0	106·0	8
	96·2	83·4	97·3	95·1	86·1	17
	100·0	100·0	23·4	118·0	56·3	16

* Compositions of solutions are given under Materials and methods.

The presence of protein amino-acids had least influence on hydroxyproline determinations. All of the chloramine T and sodium hypobromite procedures, and some with hydrogen peroxide, were satisfactory, only small correction factors being required to determine the actual amounts present. The presence of tyrosine with or without tryptophan accounted for less than 2 per cent. of the hydroxyproline colour.

Only three procedures[16,32,37] were found suitable (recovery 100 ± 5 per cent.) for hydroxyproline assay in the presence of pure urea (0·083 M). The presence of ammonium chloride (0·093 M) in test solutions caused less colour development, particularly with sodium hypobromite as the oxidant. The chloramine T and peroxide procedures showed a wide range of behaviour in the presence of both urea and ammonium chloride.

The presence of glucose or mannitol had little or no effect on the colour developed when chloramine T was the oxidant. The recoveries varied widely in the methods in which sodium hypobromite or hydrogen peroxide was used.

The efficiency of hydroxyproline determination in complex solutions was examined with preparations of hydrolysed samples of collagen, human urine and plant-seed coat extract. The results are shown in Table III. Seven methods[13,15,16,17,27,29,32] gave good recoveries of hydroxyproline from collagen but none was a highly sensitive procedure. No procedure gave satisfactory (100 ± 5 per cent.) recoveries for urine and in some instances[20,31] (including Lamport's method) there was very little reaction. Only four procedures gave satisfactory recoveries from plant-seed coat extract; in all of these hydrogen peroxide was the oxidant and all were low sensitivity methods.

TABLE III

DETERMINATION OF ADDED HYDROXYPROLINE IN HYDROLYSATES OF COLLAGEN, URINE AND PLANT-SEED COAT EXTRACT*

	Percentage recovery of added hydroxyproline in presence of			
Oxidant	Hydrolysed collagen	Hydrolysed urine	Hydrolysed plant-seed coat extract	Reference number
Chloramine T	94·0	89·0	78·0	39
	64·5	45·4	67·0	36
	73·5	20·0	45·8	31
	100·0	28·5	72·0	29
	81·3	92·3	85·0	37
	100·0	74·6	85·3	32
Sodium hypobromite	90·8	10·0	62·8	Lamport's method
	71·4	38·1	84·3	28
	100·0	46·6	60·0	27
Hydrogen peroxide	78·0	48·6	67·0	19
	78·7	62·8	85·7	18
	99·0	67·3	91·0	15
	95·8	45·8	100·0	13
	93·2	56·0	90·2	14
	42·8	22·0	100·0	20
	90·0	57·8	77·3	7
	75·0	54·4	68·6	8
	95·5	86·6	100·0	17
	100·0	51·4	100·0	16

* Compositions of solutions are given under Materials and methods.

Hydroxyproline is resolved from other substances by elution from a cation-exchange resin in an amino-acid analyser system.[1,2] The procedure described by Baily[18] was designed for the determination of hydroxyproline in an amino-acid analyser eluate at a pH of 3·25. The recovery of hydroxyproline in all the hydrogen peroxide and sodium hypobromite systems tested was between 60 and 80 per cent. However, no reaction was obtained with chloramine T because the buffers used in chloramine T procedures were not sufficiently concentrated to bring the pH within the range for oxidation. If phenol were present in the amino-acid analyser buffer there was interference with the reaction[43]; the colour developed was unstable and had an absorption maximum at 500 nm with a small shoulder at 560 nm as shown by the recording spectrophotometer. The presence of methanol in the buffer had no detectable effect on normal colour development.

Activated charcoal has been used by many workers[42] to remove substances that interfered with colour development. The possibility that hydroxyproline was adsorbed on to the activated charcoal was examined. Activated charcoal (1 g) was stirred with 100 ml of a 20 μg ml^{-1} solution of hydroxyproline for 10 minutes. The recovery of hydroxyproline was 90 ± 2 per cent.

DISCUSSION

Many procedures for the spectrophotometric determination of hydroxyproline were devised for specific purposes. One procedure[40] has been adapted for use with an automatic analyser, but in many laboratories a simple, short, sensitive and accurate method may be required to perform a few analyses at irregular intervals. In this context we report the time required to obtain results for a calibration graph (Table I). We do not know of any previous attempt to survey available methods, nor to assess their applicability to materials other than those for which they were devised.

Three different oxidising agents have been used by various workers. Our results (Table I and Fig. 1) indicate that the use of either chloramine T or sodium hypobromite can provide the greatest sensitivity. A wide range of chemical and physical systems has been examined (Table I) but optimal conditions have not been defined, and at present we can assess a method or oxidant only by its effectiveness in the determination of the material to be studied. For example, sodium hypobromite reacts with urea and ammonium chloride, causing the evolution of nitrogen, which may explain the low recoveries of hydroxyproline in solutions containing these contaminants (Tables II and III). Certain procedural improvements have been made, for example, in modifications of Stegemann's method[29] by Bergman and Loxley.[32,38,39] One small, but important, change in procedure was the addition of the reducing agent (perchloric acid) and the aldehyde at the same time, and not 5 minutes apart as originally described. This achieved a 4-fold increase in sensitivity (Table I), apparently because of the avoidance of the rapid destruction of the oxidation product, as measured by colour development, by the reducing agent. The rapid and identical treatment of samples after addition of oxidising agent was essential to obtain reproducible results.

Although the identities of the oxidation products are in dispute, and the absorption maxima after reaction with the aldehyde varied, the forms of the spectra were strikingly similar (Fig. 1). The peak at about 560 nm and the shoulder at about 525 nm were always present but the wavelength of minimum absorbance between 440 and 500 nm varied, and may be related to the oxidising agent used or to the nature of salts in the test solution. The use of chloramine T and the accompanying buffer apparently resulted in an orange - purple solution with an absorbance minimum at 560 nm, whereas the other oxidants that were not used with organic buffers gave purple solutions in which the absorbance minima were between 440 and 460 nm. The broad apex about each absorption maximum presumably accounted for the range of wavelengths (540 to 562 nm) chosen for measurement by various workers (Table I), but we cannot explain why Mitoma, Smith, Davidson, Udenfriend, DaCosta and Sjoerdsma[16] selected 540 nm. Many questions concerning optimal analytical conditions remain unanswered.

The presence of interfering substances in biological mixtures poses many problems. In some procedures attempts were made to remove these by extraction into either toluene[30,37] or diethyl ether[15,16] before colour development. Stegemann and Stalder[36] suggested extraction into 1,2-dichlorobenzene after colour development to give increased sensitivity. It was our experience that this extraction step made the procedure very cumbersome when large numbers of analyses were to be performed. Variation in the time of extraction led to wide discrepancies in the results, presumably because of decay of the oxidation product. Koevoet and Baars[44] reported that while most interference occurred during the colour development phase, there was some during oxidation. This was not removed by a post-oxidative extraction step. Some workers[38,42,45] found that activated charcoal preferentially removed tyrosine and certain pigments, but also removed some hydroxyproline. We found that there was a loss of about 10 per cent. in this pre-treatment. In hydrolysates containing much humin the step was necessary to eliminate variable interference with the colorimetry. With the present uncertainty we recommend that, when possible, purification of the sample be carried out before oxidation.

Methods for preliminary separation of hydroxyproline by ion-exchange chromatography have been described.[1,43,46,47,48] Direct analysis of eluates from the ion-exchange columns required that the oxidant be in a buffer solution that was sufficiently concentrated to counter the elution buffer. The final solution during oxidation had to be at a pH of approximately 6·0, and none of the chloramine T procedures was suitable for the direct analysis of column eluate. Our lack of understanding of the mechanism and optimum conditions for the reaction make preliminary removal of interfering substances desirable. The use of ion-exchange chromatography for removal of contaminants before analysis is highly recommended whenever conditions permit, but great care should be exercised when using activated charcoal.

The choice of a spectrophotometric procedure is part of the over-all problem of carrying out a quantitative analysis for hydroxyproline. Preparation of samples for analysis is also a major step. Most determinations are performed after hydrolysis and great care is needed in this step. The importance of corrections for destruction of amino-acids during hydrolysis was discussed by Tristram and Smith.[49] These corrections are particularly important when either carbohydrates or salts, or both, are present, as failures to notice and compensate for losses are frequent and serious sources of analytical errors in routine analysis. The use of internal standards[39] is essential for accurate analysis.

We conclude that at present no single analytical procedure can provide accurate results at high sensitivity for all types of hydroxyproline determination. The presence of various contaminating substances resulted in poor recoveries of added hydroxyproline (Tables II and III). Interference by the amino-acids of a protein hydrolysate was generally small and the high sensitivity methods of Lamport, Woessner,[31] Stegemann and Stalder,[36] and Bergman and Loxley[39] were suitable. Recoveries of added hydroxyproline from hydrolysates of collagen and plant-seed coat extract were lower than from the synthetic amino-acid mixture, and apparently other substances interfered in the analyses of the biological materials.

Urea, ammonium chloride, glucose and mannitol were reported to be the main interfering substances in urine.[44] The results in Table II support this view to some extent and only the procedure of Kivirikko, Laitinen and Prockop[37] appeared unaffected by these substances. However, hydrolysed whole urine[30] contained other substances that interfered with colour development in this procedure although interference was less than in other methods. In some methods the total interference of urea, ammonium chloride, glucose and mannitol approximated to that of urine, but in most cases the interference of the urine was much greater.

In our experience the most adaptable procedures were those of Kivirikko, Laitinen and Prockop[37] and Bergman and Loxley,[32,39] which gave satisfactory recoveries from urine hydrolysates. Sodium hypobromite procedures were unsatisfactory for urine, as were most peroxide methods, although many of them gave high recoveries from the glycoprotein hydrolysates. It is remarkable that none of the most sensitive procedures gave satisfactory recoveries from the hydrolysates. This is not too serious a problem when material is readily available and samples can be concentrated, but it may lead to difficulties in, for example, sequence studies on proteins with very low hydroxyproline content.

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A Critical Study of Brilliant Green as a Spectrophotometric Reagent: The Determination of Perrhenate and Gold

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The spectrophotometric methods for determining rhenium and gold with Brilliant green have been re-examined in an investigation of the effects of purity of reagent and acidity on the precision and accuracy of results. The perrhenate - Brilliant green ion-association complex is extracted from a solution at pH 6, and is unaffected by reagent purity. The extraction of the tetrachloroaurate(III) - Brilliant green ion-association complex is carried out from 0·5 M hydrochloric acid solution, however, and an impure reagent gives low recoveries. Procedures are recommended for the determination of rhenium and gold, and the purification of the reagent is discussed.

IN a previous paper[1] the determination of antimony with Brilliant green (C.I. 42040) was examined critically, and an improved procedure was suggested. It was shown that Brilliant green deteriorates rapidly in the 2 M hydrochloric acid solution from which the hexachloroantimonate(V) - Brilliant green ion-association complex is extracted, and it is probable that this deterioration results simply from the formation of a much less highly coloured form of Brilliant green (the RH^{2+} form). Consequently it is necessary to extract the antimony as rapidly as possible into the toluene after the addition of the Brilliant green. Unfortunately, several other factors, including the purity of reagent and the need to oxidise antimony(III) to antimony(V), may also contribute to the unreliability of the procedure, and it was not possible to state categorically that the change in the Brilliant green in 2 M hydrochloric acid was the only significant factor.

Methods exist for the determination of rhenium[2] and gold[3] with Brilliant green, in which perrhenate is extracted from an aqueous solution at pH 4·2 and gold from 0·5 M hydrochloric acid solution. It was considered that a further study of these procedures would give more information about the reliability and optimum use of Brilliant green as a reagent. In the determination of rhenium no change of oxidation state is necessary and, as the extraction is made from a nearly neutral solution, the possibility of a change in the form of the Brilliant green does not arise. The gold system was selected for study because it is of intermediate complexity between the rhenium and antimony systems; no change of oxidation state is necessary but extraction from acidic solution is required.

EXPERIMENTAL

BRILLIANT GREEN SAMPLES—

The four samples of Brilliant green from four different batches (labelled A, B, C and D) supplied by Hopkin and Williams Limited and used in our previous study of the determination

TABLE I

ANALYSIS OF BRILLIANT GREEN SAMPLES

	Brilliant green ($C_{27}H_{34}N_2O_4S$), per cent. w/w by—	
Brilliant green sample	Macro-Kjeldahl nitrogen assay	Titanium(III) chloride method
A	84·4	80·2
B	86·0	68·1
C	94·0	89·4
D	83·3	81·7
E	93·4	89·7
E purified by Soxhlet extraction	—	100·5
Residue of E remaining in Soxhlet	—	94·8
B.P. grade	—	98·2

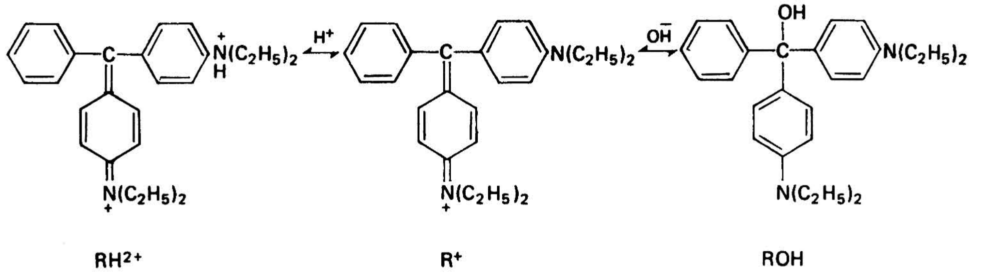
of antimony were used in the present work. Two further samples, one from a further batch (labelled E) and the other of B.P.[4] grade material, were also examined.

Methods of purifying samples of Brilliant green have been suggested by Burke and Menis[5] and by Kerr and Gregory.[6] In the present work satisfactory results were obtained simply by extracting the impure sample with acetone in a Soxhlet extractor. The Brilliant green crystallises in the Soxhlet flask as the acetone becomes saturated, the crystals being filtered off from the acetone; analysis indicated that they were of high purity (Table I). As the residue in the Soxhlet thimble was also pure, it would appear that the main impurities are very soluble in acetone and remain in the saturated acetone solution.

In general, the Brilliant green contents of the samples, as determined by the macro-Kjeldahl and titanium(III) chloride methods (Table I), were not in good agreement. The results obtained with the more specific titanium(III) chloride method were expected to be more reliable, and this was confirmed later (Table IV; see p. 1016) by the close correlation between the recovery of gold and the standard of purity given by this method for any particular sample.

EFFECT OF ACID ON BRILLIANT GREEN—

In the solid state and in neutral solution, Brilliant green exists in the R^+ form, the form in which it is also used to extract anions in the analytical procedures for antimony, rhenium, gold and other metals. In more acidic solutions the RH^{2+} form is present, and in alkaline solution the colourless carbinol base is produced. The acid-dissociation constant of the R^+ form (to the carbinol)[7] is given as 7·9, but no reliable value is available for the dissociation $RH^{2+} \rightarrow R^+$. Difficulty is experienced in studying these systems because of the relative slowness with which equilibrium is reached.



In the present work the Brilliant green acid - base equilibria were studied semi-quantitatively by allowing Brilliant green to equilibrate in various buffers, and measuring the absorbance of the buffer solution at the wavelength of maximum absorption for the R^+ form (615 nm). The results shown in Fig. 1 (*a*) were obtained after 5 hours. It is apparent that

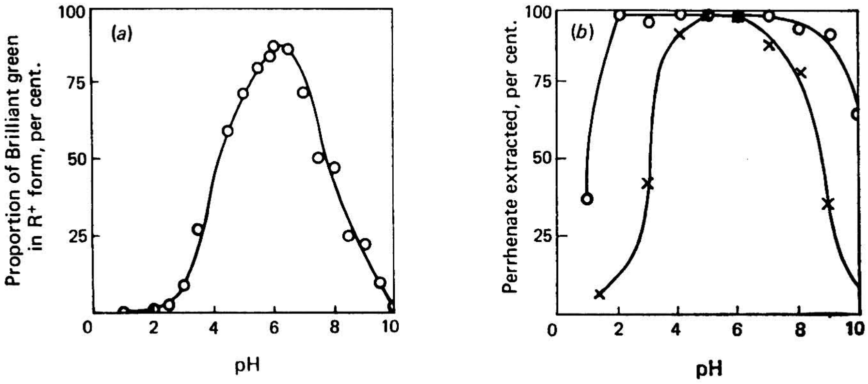


Fig. 1. Effect of pH on the stability of the R^+ form of Brilliant green and on the extractability of perrhenate: (*a*), variation of R^+ with pH after 5 hours; and (*b*), extractability of perrhenate from buffer solutions after o, 1 minute and x, 5 hours

the analytically useful R^+ ion is not fully formed and is present in appreciable amounts only over a narrow pH range in the neutral region.

EXTRACTABILITY OF PERRHENATE AS A FUNCTION OF pH AND TIME—

The extraction of perrhenate with Brilliant green might be expected to take place with greatest efficiency in the pH region in which the R^+ form of the dye is present in maximal amount, and was shown to do so when the perrhenate - Brilliant green solution was allowed to equilibrate for 5 hours in the buffer prior to extraction (Fig. 1 *b*). Nevertheless, because equilibrium is reached only slowly it is possible to effect extraction of perrhenate over a wider pH range (pH 2 to 7) as shown in Fig. 1 (*b*), in which the amount extracted after mixing for 1 minute is shown.

DETERMINATION OF PERRHENATE

In our previous determination of antimony it appeared that significantly different absorbances were obtained when different batches of Brilliant green were used. When the procedure for determining perrhenate outlined below was used reproducible results were obtained and there was little significant difference between the results given by the different batches of dye (Table II); the result given by the most impure batch (B) was only 3 per cent. lower than that by B.P. grade material. The molar absorptivity based on 100 per cent. extraction of the perrhenate (100,000) is similar to that indicated earlier for the antimony extract (103,000). The usual precautions were taken when using benzene; unfortunately, low recoveries of perrhenate were obtained when toluene was used instead of benzene.

TABLE II

SENSITIVITY AND PRECISION OF THE RECOMMENDED PROCEDURE FOR PERRHENATE

Brilliant green sample	A	B	C	D	B.P. grade
Mean absorbance*	0·430	0·425	0·436	0·429	0·437
Standard deviation	0·005	0·004	0·003	0·005	0·002
Apparent molar absorptivity ..	100,100	99,000	101,500	99,900	101,800

* Six determinations at the 20-μg rhenium level.

REAGENTS—

Brilliant green solution, 0·05 per cent. w/v, in ethanol.

Buffer solution, pH 6—Dilute 500 ml of 0·1 M potassium dihydrogen phosphate solution and 56 ml of 0·1 M sodium hydroxide solution to 1 litre with water in a calibrated flask.

Standard rhenium solution, 5 μg ml^{-1}—Dissolve 0·1553 g of potassium perrhenate in water and dilute the resulting solution to 1 litre with water in a calibrated flask. Dilute 5 ml of this solution to 100 ml with buffer solution in a calibrated flask.

PREPARATION OF CALIBRATION GRAPH—

By pipette, introduce aliquots of the standard potassium perrhenate solution (0 to 10 ml) into a 100-ml separating funnel and add buffer solution from a measuring cylinder to make the total volume up to 10 ml. Add 1 ml of Brilliant green solution and extract the Brilliant green - perrhenate ion-association complex with 10, 5 and 5-ml portions of benzene, filtering each extract through a No. 31 Whatman filter-paper into a 25-ml calibrated flask. Make the solution up to volume with benzene, mix the solution thoroughly, and measure its absorbance at 640 nm in a 1-cm cell against benzene.

PRELIMINARY INVESTIGATION OF THE DETERMINATION OF GOLD WITH BRILLIANT GREEN

Stanton and McDonald[3] determined gold in soils with Brilliant green. The final aqueous solution containing the gold is made 0·5 M in hydrochloric acid and the gold extracted with exactly 5 ml of a 0·05 per cent. w/v solution of Brilliant green in toluene. After discarding the aqueous solution the toluene is washed with 50 ml of 0·5 M hydrochloric acid containing Brilliant green. The toluene extract is filtered through a filter-paper to remove water and the absorbance of the extract measured at 650 nm.

The results shown in Table III were obtained in the present study by using the method of Stanton and McDonald, and it is evident that different batches of Brilliant green give widely different absorbances with high standard deviations.

TABLE III

RESULTS OBTAINED FOR THE DETERMINATION OF GOLD WITH THE PROCEDURE OF STANTON AND MCDONALD

Brilliant green sample	A	B	C	D	E
Mean absorbance*	0·395	0·210	0·614	0·276	0·667
Standard deviation	0·040	0·051	0·005	0·011	0·007
Apparent molar absorptivity	44,700	23,800	69,600	31,300	75,500
Recovery of gold, per cent.†	44·2	23·5	68·8	31·0	74·7

* Six determinations at the 8·7-μg gold level.
† Based on ϵ = 101,000.

For comparison purposes it was advisable that the procedure developed for gold should be as similar as possible to that described previously for antimony. Accordingly, the Brilliant green, in ethanolic solution, was added last, after the addition of toluene and immediately before the extraction. It soon became apparent that one extraction with toluene did not extract all of the gold; two 10-ml volumes of toluene were used subsequently. Further, the absorbance maximum occurs at 643 nm, and not at 650 nm as stated by Stanton and McDonald, and subsequent measurements were made at 643 nm.

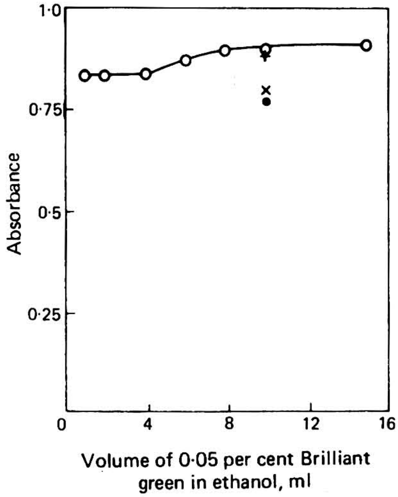


Fig. 2. Effect of Brilliant green concentration on the apparent recovery of gold: o, sample E and B.P. grade; +, sample C; x, samples A and D; and ●, sample B

EFFECT OF BRILLIANT GREEN CONCENTRATION AND THE PURITY OF THE REAGENT—

By using the procedure outlined below, except that only the first addition of Brilliant green was made (in varying amounts), the results shown in Fig. 2 were obtained. Two conclusions can be drawn from these results: first, that impure samples of Brilliant green give lower absorption readings and second, that low recoveries can be expected even with pure samples if less than about 8 ml of 0·05 per cent. Brilliant green solution is used.

Results obtained with 10 ml of 0·05 per cent. solution are given in Table IV. The pure samples of Brilliant green give molar absorptivities of about 97,000, based on 100 per cent. extraction of gold, a value that is similar to that obtained previously with perrhenate (100,000) and antimony[1] (103,000). It appeared, therefore, that the pure batches of Brilliant green were giving good recoveries.

EFFECT OF LENGTH OF TIME OF EQUILIBRATING THE GOLD - BRILLIANT GREEN SOLUTIONS PRIOR TO EXTRACTION—

The effect of leaving Brilliant green in contact with 0·5 M hydrochloric acid solution containing 43·5 μg of gold for various periods of time, before addition of the toluene and

TABLE IV

RESULTS OBTAINED FOR THE DETERMINATION OF GOLD (*i*) WITH A SINGLE ADDITION OF BRILLIANT GREEN AND (*ii*) WITH THE RECOMMENDED PROCEDURE

	Brilliant green sample	A	B	C	D	Purified E	E	B.P. grade
(*i*)	Absorbance*	0·770	0·747	0·866	0·775	0·871	0·851	0·862
	Recovery of gold, per cent.†	86·3	83·7	97·1	86·9	97·6	95·5	96·7
(*ii*)	Absorbance*	0·831	0·825	0·875	0·828	0·885	—	—
	Recovery of gold, per cent.†	93·2	92·5	98·1	92·8	99·2	—	—

* 44-μg gold level.
† Based on ϵ = 101,000.

extraction of the gold, was studied. The results are shown in Fig. 3. It is apparent that, as with antimony,[1] the gold must be extracted as soon as possible after the addition of the Brilliant green.

As in this procedure two extractions with toluene are carried out, it was considered that a further addition of Brilliant green before the second extraction might have beneficial results. As can be seen in Fig. 4, which also shows the effect of hydrochloric acid concentration, this view proved to be correct. Nevertheless, it is still advisable to extract the gold as rapidly as possible after each addition of Brilliant green. The molar absorptivity obtained with the procedure below was 101,000.

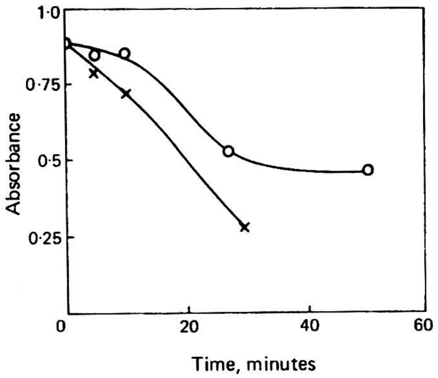


Fig. 3. Effect of equilibrating 0·5 M hydrochloric acid solution containing gold before addition of toluene and extraction of the gold; 1 ml of 0·5 per cent. w/v Brilliant green (purified sample E): o, in ethanol; and x, in water

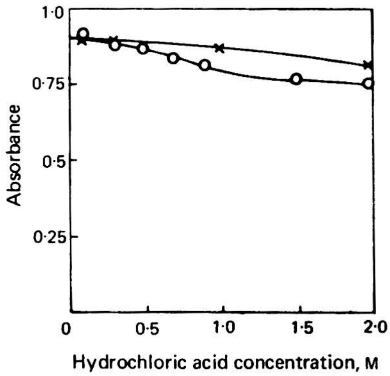


Fig. 4. Effect of hydrochloric acid concentration on the extraction of the Brilliant green - tetrachloroaurate(III) complex: o, with a single addition of Brilliant green; and x, with the recommended procedure

DETERMINATION OF GOLD WITH BRILLIANT GREEN

REAGENTS—

Brilliant green solution, 0·5 per cent. w/v, in ethanol.

Standard gold solution, about 10 μg ml^{-1}, in 0·5 M hydrochloric acid solution—Weigh accurately the contents of a nominal 1-g ampoule of sodium tetrachloroaurate(III) dihydrate and dissolve them in 0·5 M hydrochloric acid. Dilute the solution to 1 litre with 0·5 M hydrochloric acid in a calibrated flask. By pipette, introduce 10·0 ml of this solution into a 500-ml calibrated flask and dilute to volume with 0·5 M hydrochloric acid.

Toluene—Analytical-reagent grade toluene, treated with potassium dichromate solution to remove traces of reducing agents.[3]

PREPARATION OF CALIBRATION GRAPH—

With a pipette, introduce an aliquot of the standard gold solution, containing up to 50 μg of gold, into a 100-ml separating funnel, and make the volume up to 50 ml with 0·5 M hydrochloric acid. Add 10 ml of toluene and 1 ml of Brilliant green solution, immediately shake the mixture for 30 s and, as soon as separation has occurred, filter the toluene extract through a No. 31 Whatman filter-paper into a 25-ml calibrated flask. Add a further 10 ml of toluene and 0·5 ml of Brilliant green solution to the aqueous layer. Shake the mixture for 30 s and, as soon as separation has occurred, filter the extract through the filter-paper into the flask. Wash the filter-paper with toluene and dilute the extract to 25 ml with toluene. Mix the solution thoroughly and measure its absorbance at 643 nm in a 1-cm cell against toluene.

The results obtained with this procedure with different batches of Brilliant green are given in Table IV. The mean absorbance of twelve determinations at the 26-μg gold level with purified sample E was 0·533, with a standard deviation of 0·005.

Discussion

The present investigations of the rhenium - Brilliant green and gold - Brilliant green systems were carried out because these systems are simpler than the antimony - Brilliant green system investigated previously.[1] The optimum pH for the extraction of the perrhenate ion has been shown to be 6, the pH at which the R^+ form of Brilliant green is most fully formed. The acid - base equilibria of Brilliant green are reached only slowly, however, so if perrhenate is extracted within 1 minute of adding the Brilliant green, perrhenate can be determined quantitatively by extraction from solutions of pH between 2 and 7.

Gold is extracted with Brilliant green from 0·5 M hydrochloric acid solution, at which acid concentration the latter is converted almost completely into its RH^{2+} form when equilibrium is reached. To extract $R^+AuCl_4^-$ quantitatively, one would expect the extraction to be carried out rapidly and before much of the R^+ form has been further protonated to RH^{2+}, which has been shown to be the case. In the recommended procedure two extractions with toluene are made; the addition of further Brilliant green (in the R^+ form) before the second extraction ensures quantitative recovery of the gold.

In our previous work with antimony[1] there was some indication that lower recoveries of antimony were being obtained with the less pure batches of Brilliant green but the evidence was inconclusive. With gold there is no doubt that much lower recoveries are obtained with the less pure batches. The mechanism of the interference by the impurities in the Brilliant green must be associated with the acidity of the solution, because the purity of the Brilliant green has little effect on the recoveries of ReO_4^- at pH 6.

With regard to extractions with Brilliant green from acidic solutions two conclusions are apparent from the present work. First, the extractions must be carried out as rapidly as possible after the addition of the Brilliant green, and second, a pure dye sample must be used. Samples guaranteed to be of B.P.[4] grade (assay not less than 96 per cent.) can be used directly without further purification. For other grades the purification procedure described in the present paper is recommended.

The impurity that interferes in the gold procedure, and also probably in the antimony procedure, has not been identified, and to eliminate the possibility that it is simply the RH^{2+} form of the dye, the following test was made. The acidified gold solution was equilibrated with 0·5 ml of 0·5 per cent. w/v Brilliant green solution for 40 minutes before resuming the analytical procedure at the stage when the first 10 ml of toluene and 1 ml of Brilliant green solution are added and the ion-association complex is extracted. Quantitative recovery of the gold was again obtained. Apparently the RH^{2+} form of Brilliant green does not interfere directly.

We thank Mr. J. A. Clark of Hopkin and Williams Limited for providing samples and for helpful discussion and encouragement, and Professor R. F. Phillips for providing facilities.

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Spectrophotometric Determination of Molybdenum with Gallein and Cetyltrimethylammonium Bromide

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A ternary complex system between molybdenum, gallein and cetyltrimethylammonium bromide is proposed for the determination of microgram amounts of molybdenum. The complex has an absorbance maximum at 618 nm. The sensitivity of the reaction is 0·0026 μg cm^{-2} of molybdenum for $\log\frac{I_0}{I} = 0{\cdot}001$. The development of this method for the determination of molybdenum included a study of the effect of pH, temperature, reagent concentration, rate of complex formation and conformity to Beer's law.

IN 1956, Wood used gallein to determine small amounts of tin in soils.[1] Elements that were found to interfere with tin included molybdenum, titanium, antimony, iron(III) and tungsten. Of these, the most serious interference was that due to molybdenum.

It was found that on addition of cetyltrimethylammonium bromide (cetrimide) to molybdenum and gallein (replacing catechol violet in the method of Bailey, Chester, Dagnall and West[2]), a blue complex, soluble in water, was formed. As the reaction appeared to be very sensitive, a detailed study was carried out in this laboratory with the aim of developing a suitable sensitive spectrophotometric method for the determination of minute amounts of molybdenum.

EXPERIMENTAL

REAGENTS—

Molybdenum(VI), 10^{-2} M stock solution—Dissolve 1·766 g of dry ammonium heptamolybdate tetrahydrate in distilled water and make up to 1 litre. Dilute as required.

Molybdenum(VI), 1000 p.p.m. stock solution—Dissolve 1·840 g of dry ammonium heptamolybdate tetrahydrate in distilled water and make up to 1 litre. Dilute as required.

Gallein, 10^{-3} M solution—Dissolve 91·1 mg of recrystallised gallein in 250 ml of ethanol.

Cetyltrimethylammonium bromide, 0·1 per cent. solution—Dissolve, with warming, 0·1 g of cetyltrimethylammonium bromide in 100 ml of distilled water.

Hydrochloric acid solution, pH 1·0—Adjust the solution to pH 1·0 with a pH meter.

APPARATUS—

A Beckman B spectrophotometer with 1-cm glass cuvettes and a Cambridge Instruments direct-reading pH meter were used.

RESULTS AND DISCUSSION

SPECTRAL CHARACTERISTICS—

In Fig. 1 curves A and B show the absorption spectra of gallein and its molybdenum complex at pH 1·0 while curve C shows the effect of the addition of 0·02 per cent. of cetrimide to the complex as represented in curve B. It will be noted that the formation of the pure blue complex is accompanied by a marked increase in the absorbance and a considerable bathochromic shift in the absorption maximum of the complex from 460 to 618 nm. Further, it will be noted that the absorbance of the reagent is small at the wavelength of maximum absorption of the molybdenum - gallein - cetrimide complex (618 nm), thus providing nearly ideal conditions for analytical measurements.

OPTIMUM CONDITIONS FOR COLOUR DEVELOPMENT—

Effect of pH—The effect of pH on the absorption of the complex was studied at 618 nm with solutions containing 1 ml of 10^{-3} M molybdenum solution, 5 ml of 10^{-3} M gallein solution

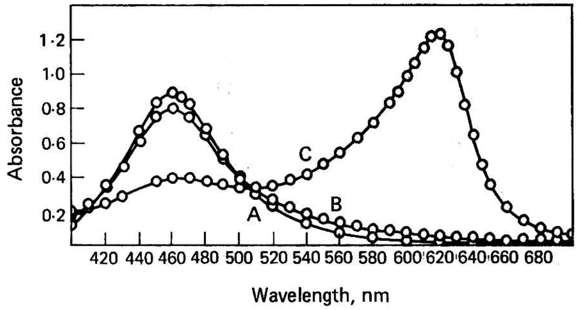

Fig. 1. Absorption spectra of molybdenum(VI) - gallein - cetrimide system: curve A, 2 ml of 10^{-3} M gallein solution diluted to 50 ml at pH 1·0, measured against distilled water; curve B, as A but with the addition of 1 ml of 10^{-3} M molybdenum(VI) before dilution to 50 ml; and curve C, as B but with further addition of 10 ml of 0·1 per cent. cetrimide

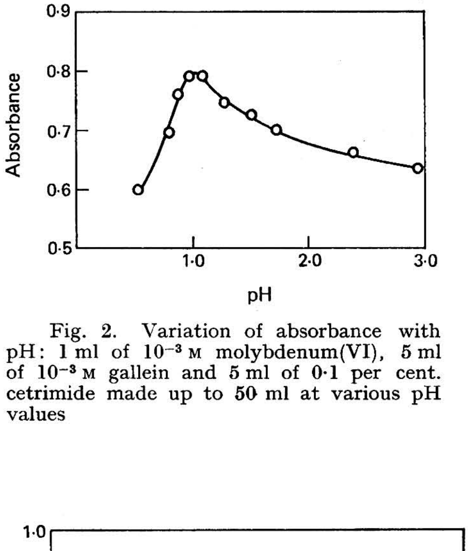

Fig. 2. Variation of absorbance with pH: 1 ml of 10^{-3} M molybdenum(VI), 5 ml of 10^{-3} M gallein and 5 ml of 0·1 per cent. cetrimide made up to 50 ml at various pH values

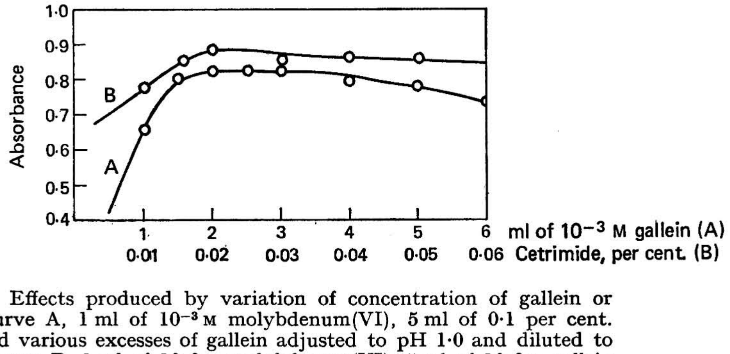

Fig. 3. Effects produced by variation of concentration of gallein or cetrimide: curve A, 1 ml of 10^{-3} M molybdenum(VI), 5 ml of 0·1 per cent. cetrimide and various excesses of gallein adjusted to pH 1·0 and diluted to 50 ml; and curve B, 1 ml of 10^{-3} M molybdenum(VI), 5 ml of 10^{-3} M gallein and various excesses of 0·1 per cent. cetrimide adjusted to pH 1·0 and diluted to 50 ml

and 5 ml of 0·1 per cent. cetrimide solution. No buffer was used in the study, the solutions being adjusted carefully with dilute hydrochloric acid to the required pH before absorbance measurements were taken and the pH values being checked again afterwards.

A graph was plotted of absorbance values against pH (Fig. 2) and showed that the absorption of the complex is dependent on the pH, the maximum absorbance being obtained between pH 0·95 and 1·1. A pH of 1·0 was chosen for all further work.

Effect of reagent concentration—Maximum absorbance was obtained with a 2-fold molar excess of gallein relative to molybdenum (curve A, Fig. 3). The effect of using a greater concentration of gallein was to reduce the sensitivity of the reaction slightly. Nevertheless, it was decided to use a larger excess of gallein, *viz.*, a 5-fold molar excess, in order to be on the flat part of the absorbance curve.

Variation of the cetrimide concentration in the range 0·01 to 0·05 per cent. (curve B) revealed that maximum absorbance was obtained at about 0·02 per cent. However, a 0·03 per cent. cetrimide concentration was decided upon as being the best concentration.

Effect of time and order of addition of reagents—The stability of the colour was studied at an average temperature of 23° C by measuring the absorbance at regular time intervals. Maximum absorbance was obtained after 15 minutes and the absorbance remained constant for at least 1 hour. After 48 hours, the absorbance had decreased by about 3 per cent.

The order of addition of the reagents was found to be important. Gallein had to be added to the molybdenum first, before the cetrimide. When this order was not followed a marked decrease in the intensity of the colour was observed.

Effect of temperature—The effect of temperature on the absorbance of the complex was studied between 10° and 40° C. The graph depicted in Fig. 4 shows that the absorbance is dependent on temperature. Between 20° and 30° C, the absorbance is constant.

Solvent extraction study—Various organic solvents were used in an attempt to extract the complex from aqueous solution. The complex was not extracted by the following solvents: chloroform, carbon tetrachloride, toluene, dichlorobenzene, propanol, butanol, pentanol, isopentyl alcohol, di-isopropyl ether, ethyl acetate, isobutyl methyl ketone, and cyclohexanone.

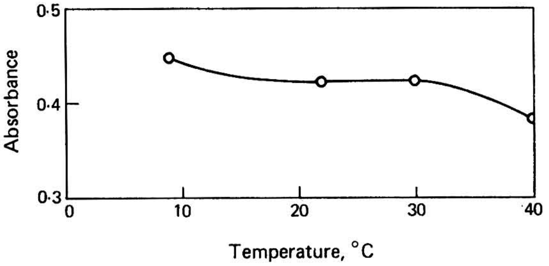

Fig. 4. Variation of absorbance with temperature: 5 ml of 10 μg ml^{-1} molybdenum(VI), 1 ml of 10^{-3} M gallein and 10 ml of 0·1 per cent. cetrimide at temperatures in the range 10 to 40° C

PREPARATION OF CALIBRATION GRAPH—

The calibration graph was prepared by transferring from 0·5 to 7 ml of 10 μg ml^{-1} molybdenum(VI) standard solution and 2·5 ml of 10^{-3} M gallein solution into a series of 50-ml beakers; 15 ml of 0·1 per cent. cetrimide solution were then added and the solution was diluted to about 25 ml with distilled water. The pH of each solution was adjusted to 1·0 with dilute hydrochloric acid by using a pH meter. The solutions were then transferred to 50-ml calibrated flasks and diluted to the mark with pH 1·0 dilute hydrochloric acid solution. After 15 minutes the absorbances were measured in 1-cm cuvettes against a reagent blank at 618 nm.

BEER'S LAW AND SENSITIVITY OF REACTION—

The calibration graph was found to obey Beer's law in the range 0 to 25 μg of molybdenum in 50 ml of solution. The optimum range for absorbance measurements on the spectrophotometer is 0·2 to 0·8 unit. With the recommended procedure these values correspond to 25 to 70 μg of molybdenum in 50 ml of solution.

According to Sandell's expression of sensitivity, *i.e.*, μg cm^{-2} of molybdenum for $\log \frac{I_0}{I} = 0{\cdot}001$, the sensitivity was found to be 0·0026 μg cm^{-2} of molybdenum at 618 nm and 23° C.

PRECISION OF METHOD—

To determine the precision of the proposed method in pure molybdenum solutions, the same amount of molybdenum (25 μg) was determined twelve times. The average absorbance was 0·148 and the standard deviation 0·005, or 3·3 per cent.

INTERFERENCES STUDY—

The selectivity of the proposed method was investigated by the determination of 25 μg of molybdenum in the presence of a series of other ions. The results are shown in Table I.

TABLE I

EFFECT OF SOME INTERFERING IONS

Ion added	Weight excess relative to molybdenum	Absorbance at 618 nm	Interference, per cent.	Ion added	Weight excess relative to molybdenum	Absorbance at 618 nm	Interference, per cent.
Mo(VI) (standard)	—	0·148	—	Ni	×100	0·140	−5
Ag	×100	0·161	+9	Sb(III)	× 10	0·148	0
Al	×100	0·148	0	Sn	×100	0·065	−56
Au*	×100	—	—	Ti	×100	0·152	+4
Bi*	×100	—	—	V(V)	×100	0·048	−67
Cd	×100	0·138	−7	W	× 15	0·149	0
Co(II)	×100	0·126	−15	Zn	×100	0·146	−2
Cr(VI)*	×100	—	—	Acetate	×100	0·154	−4
Cu(II)	×100	0·143	−3	Citrate	×100	0·143	−3
Fe(III)	×100	0·037	−74	EDTA	×100	0·142	−4
Hg*	×100	—	—	Fluoride	×100	0·120	−19
K	×100	0·149	0	Oxalate	×100	0·046	−69
Mn(II)	×100	0·142	−4	Tartrate	×100	0·149	0
Na	×100	0·152	+4	Thiocyanate	×100	0·162	+9

* Indicates turbid solution.

The limiting value of the concentration of a foreign ion was taken as that which caused an error in absorbance corresponding to twice the standard deviation of the absorbance of the pure molybdenum solution. The major interferences were caused by elements that were known to form gallein complexes, *viz.*, vanadium, tin, antimony, tungsten, iron(III), and those that caused precipitation. Tungsten can be tolerated up to a 15-fold excess by weight. Attempts were made to eliminate the interference due to iron(III) by reduction to iron(II) with iodide. Unfortunately, precipitation of cetrimide occurred, probably because of the small amounts of iodide or iodine remaining in solution. Attempts were also made to mask some of the interfering ions with EDTA, citrate and tartrate, but without success.

DISCUSSION

The number of chromogenic reagents for molybdenum is relatively small. Of these, thiocyanate and dithiol are generally used. More recently the reagent 2-amino-4-chlorobenzenethiol[3] has been proposed and the reaction has a sensitivity of 0·0054 μg cm^{-2} of molybdenum in chloroform.

In 1968, Bailey, Chester, Dagnall and West[2] proposed the ternary system molybdenum - catechol violet - cetrimide for molybdenum. This reaction has a molar absorptivity of $4{\cdot}6 \times 10^4$ in water.

With a sensitivity of 0·0026 μg cm^{-2} of molybdenum, the proposed method is about twice as sensitive as those based on thiocyanate or dithiol, and is among the most sensitive for molybdenum to date. The major disadvantage of the proposed method is the dependence of the colour reaction on pH, especially when there is no buffer operating in that pH range. In common with other methods, the proposed method lacks specificity to a certain extent. It appears that a prior separation of molybdenum before the determination may be necessary to improve the specificity of the method. A separation of molybdenum by solvent extraction (*e.g.*, with thiocyanate) may be applicable.

Ion-exchange separation of molybdenum may also be applicable. Molybdenum can be separated from iron, copper, vanadium, lead, nickel and chromium in the presence of citrate ions.[4]

When used in conjunction with an ion-exchange procedure, the proposed method for determining molybdenum may be useful in determining the molybdenum content of some steel alloys.

I thank the Director, Geological Survey Malaysia, for permission to publish this paper.

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The Application of Polarography and Related Electroanalytical Techniques to the Determination of Tetramethylthiuram Disulphide

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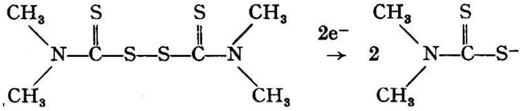
Methods have been evaluated for the determination of tetramethylthiuram disulphide by d.c. and a.c. polarography. Linear calibration graphs were obtained over the concentration range 10^{-4} to 10^{-3} M by d.c. polarography, and 10^{-6} to 10^{-4} M by a.c. polarography. The electrode reaction was studied by cyclic voltammetry and evidence obtained to support the postulated mechanism of reduction of the S—S bond to give two dimethyldithiocarbamate molecules.

IN a previous paper[1] fungicides related to dithiocarbamic acid were divided into three classes. The application of polarography and related electroanalytical techniques to the first of these classes of compound, *i.e.*, simple dithiocarbamic acid salts, was considered. In this paper the work is extended to include the second class, the thiuram disulphides. Although several compounds of this type have been suggested as fungicides,[2] only the tetramethyl derivative (thiram) has found wide application. The tetra-ethyl derivative is used as an anti-oxidant for rubber and as a drug (Antabuse) for the treatment of alcoholism. It has been postulated[3] that thiram shows fungitoxicity because of its breakdown to form the dimethyldithiocarbamate anion.

The polarographic behaviour of thiram differs from that of sodium diethyldithiocarbamate in that no anodic waves are observed. Nangniot[4] studied the polarographic behaviour of this class of compound; he observed a cathodic wave at −0·43 V, which was attributed to the two-electron reduction of the S—S bond—



$$(CH_3)_2N-\overset{S}{\overset{\|}{C}}-S-S-\overset{S}{\overset{\|}{C}}-N(CH_3)_2 \xrightarrow{2e^-} 2\ (CH_3)_2N-\overset{S}{\overset{\|}{C}}-S^-$$

The polarographic reduction of tetra-ethylthiuram disulphide has been examined by cathode-ray polarography and a linear peak current - concentration relationship was found to exist in the range 1·5 to 15 p.p.m.[5] Earlier, two waves had been observed[6] by d.c. polarography for the reduction of tetra-ethylthiuram disulphide and the more negative one was classified as an adsorption wave. Comparison with the anodic waves of the diethyldithiocarbamate anion led Gregg and Tyler to the conclusion that the tetra-ethylthiuram disulphide - diethyldithiocarbamate anion system formed a redox couple that showed Nernstian reversibility. It is apparent, in the light of more recent work,[7] that anodic polarographic waves observed for diethyldithiocarbamate anions arise through formation of insoluble mercury salts, rather than true oxidation. The essential condition for thermodynamic reversibility, *i.e.*, chemical reversibility of the system, is not fulfilled in this case.

In the present work the techniques of d.c. and a.c. polarography have been applied to the determination of thiram and the electrode reaction has been studied by cyclic voltammetry.

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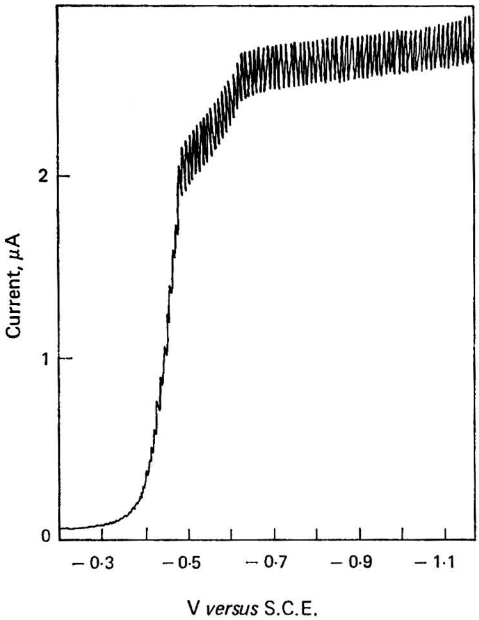

Fig. 1. Direct current polarogram of a 5×10^{-4} M solution of tetramethylthiuram disulphide in 0·1 M NaOH containing 50 per cent. of ethanol; h = 65 cm, t = 3·8 s at 0·0 V *versus* S.C.E.

EXPERIMENTAL

The thiram sample used was a commercial product. All other reagents were of analytical-reagent grade.

Stock solutions of thiram and tetra-ethylthiuram disulphide were prepared in absolute ethanol but electrochemical studies were made in solutions containing 50 per cent. ethanol. Both the apparatus and the techniques used have been previously described.[1]

PROCEDURE—

Dilute an aliquot containing 1 to 10 μM of thiram to 5 ml with ethanol. Add 1 ml of M ammonia - M ammonium nitrate buffer solution and dilute the mixture to 10 ml with water. Transfer the solution to the polarographic cell, de-oxygenate the cell with nitrogen for 3 minutes and record the polarogram between —0·2 and —0·8 V *versus* S.C.E.

RESULTS AND DISCUSSION

DIRECT CURRENT POLAROGRAPHY—

The d.c. polarographic behaviour of thiram and its tetra-ethyl analogue was in accordance with that reported by Nangniot.[4] Two cathodic waves were observed (Fig. 1), the more

TABLE I

RELATIONSHIP BETWEEN WAVE HEIGHT AND CONCENTRATION FOR THIRAM

Concentration of thiram, M	Current, μA			
	Direct current polarography		Alternating current polarography	
	I	II	I	II
2×10^{-4}	0·13	0·35	5·71	—
4×10^{-4}	0·13	0·94	8·06	3·88
6×10^{-4}	0·13	1·50	8·74	4·94
8×10^{-4}	0·13	2·07	9·07	5·76
1×10^{-3}	0·13	2·65	9·26	6·58

I, adsorption wave. II, diffusion wave.

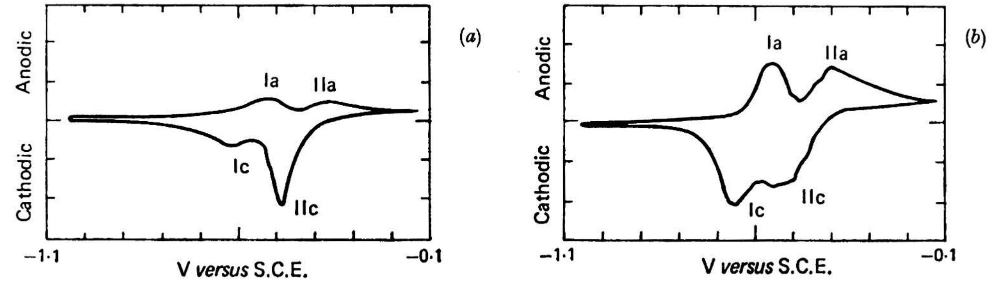

Fig. 2. Cyclic voltammogram of (*a*) 5×10^{-4} M sodium diethyldithiocarbamate in 50 per cent. ethanol at pH 11. Potential sweep rate 1 V s^{-1}, vertical sensitivity 10 µA per division; and (*b*), 5×10^{-4} M tetra-ethylthiuram disulphide in 50 per cent. ethanol at pH 11. Potential sweep rate 1 V s^{-1}, vertical sensitivity 10 µA per division

positive of which (I) was dependent on concentration while the more negative wave (II) was found to be independent of concentration over the range 10^{-4} to 10^{-3} M (Table I). From the dependence of the height of both waves on the mercury reservoir height wave I was confirmed as being diffusion-controlled while wave II was a typical Brdička adsorption wave and corresponded to reduction of thiram adsorbed on the electrode surface.

Comparison of the wave height for thiram with the height of the wave for an equimolar solution of benzoquinone (n = 2) indicated that the reduction involved two electrons and corresponded to the mechanism postulated by Nangniot, namely a fission of the S—S bond to form two dimethyldithiocarbamate molecules.

CYCLIC VOLTAMMETRY—

Confirmation of the above was obtained from cyclic voltammetric measurements of thiram and of sodium diethyldithiocarbamate (Fig. 2 *a* and *b*). Both compounds showed identical anodic peaks due to the formation of the mercury dithiocarbamate complex. On scanning the potential anodically (Fig. 2 *b*) two anodic peaks are obtained corresponding to the formation of mono and bulk layers of the insoluble mercury complex.[1] On the reverse cathodic scan the two peaks observed (IIc and Ic) are due to the stripping of these surface layers.

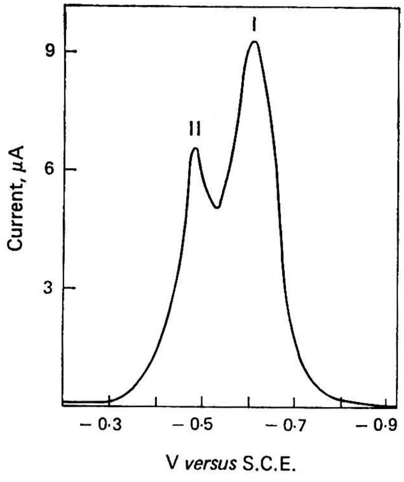

Fig. 3. Alternating current polarogram measured with the Univector polarograph, 1×10^{-3} M tetramethylthiuram disulphide in M NaOH containing 50 per cent. of ethanol

In the case of thiram the two anodic peaks are observed but the cathodic stripping peak IIc is partially obscured by the large cathodic peak (Ic) due to the fission of the S—S bond.

ALTERNATING CURRENT POLAROGRAPHY—

Thiram was observed to give a single a.c. polarographic peak (Fig. 3, peak I) while at higher concentrations a second, poorly defined, peak (II) appeared. As peak height in this case is proportional to concentration it is difficult to obtain an analytically useful relationship between peak current and concentration. Below 1×10^{-4} M, however, the height of the single peak (I) was dependent on concentration and a rectilinear calibration graph was obtained for the concentration range 10^{-6} to 10^{-4} M.

The course of the reduction of thiram is not typical of processes that give rise to a.c. waves. In general, well developed a.c. waves are only observed for thermodynamically reversible systems. Although the reduction of thiram is not reversible in this sense, a corresponding anodic reaction due to mercury complex formation with the diethyldithiocarbamate ion exists and it is most probably this reaction sequence that is responsible for the a.c. current.

CATHODIC STRIPPING VOLTAMMETRY—

Although the limit of detection attainable with a.c. polarography of 10^{-6} M is adequate for residue analysis, it is possible to extend the level of detection by the use of stripping voltammetry after a prereduction of the thiram to dimethyldithiocarbamate. The dimethyldithiocarbamate is subsequently preconcentrated at a mercury-film electrode and then stripped off by the application of a cathodic voltage sweep.

The reduction of the thiram can be carried out electrochemically, by controlled-potential electrolysis at a mercury pool (−1·0 V *versus* S.C.E.) or, more conveniently, by chemical reduction with ascorbic acid. Some preliminary experiments to investigate the feasibility of this approach showed that thiram could be determined down to 10^{-8} M after the addition of an excess of ascorbic acid. The pre-electrolysis was carried out at −0·1 V *versus* S.C.E. to overcome interference from oxidation of the ascorbic acid and from residual chloride ion in solution.

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The Application of Anion-selective Membrane Electrodes in Pharmaceutical Analysis

Part I. Halogenated Pharmaceutical Compounds

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Anion-selective membrane electrodes have been applied to pharmaceutical analysis. Twenty-four halogenated pharmaceutical compounds were determined by both the direct and indirect potentiometric methods. The compounds investigated represented a wide range of pharmaceutical compounds used extensively in pharmacy and medicine. Determinations of the dissociated halides were carried out directly with no previous treatment but the undissociated halides were determined after applying Schöniger's method. Comparison of the results obtained with the direct and indirect methods shows that they are in agreement. The direct method can be adopted for rapid routine pharmaceutical analysis as it is easy to carry out.

MANY pharmaceutical preparations contain bound halogens, the determination of which is often a problem. The strongly coloured medicinal dyes, such as acriflavine, are assayed with respect to their chloride contents by a gravimetric method.[1] Few results have been published about the analysis of anti-cancer (cytostatic) compounds. As most of these agents are halogenated compounds, they are analysed by determining their halogen contents with the Volhard method.[2,3]

The most important methods for assaying antibiotics are the microbiological methods.[4,5] However, because these methods are time consuming and need special precautions, chemical methods are often useful.

Andrey and Mirimanoff[6] determined the chloride content of chloramphenicol in formulations by Volhard's method. The error they found was within ±6 per cent. An electrochemical method was reported by Mukherjee and Dutta based on potentiometric titration with silver nitrate solution as titrant.[7]

Baker measured the iodine content of liothyronine hydrochloride by its catalytic effect on the cerium(IV) - arsenite system,[8] although official methods for the determination are based on iodimetric methods.[9,10] Alsos determined liothyronine by neutron-activation analysis of the iodine.[11]

Dicophane (DDT) is assayed by Volhard's method.[12] A complicated method has been reported for the determination of iodochlorohydroxyquinoline.[13] Recently the oxygen-flask method has been used.[14]

Thiamine hydrochloride, one of the most important members of the vitamin B group, was determined by Pijck and Claeys[15] with hypobromite in alkaline solution but the official method[16] was the determination of the total chloride content by Volhard's method. However, in the 1968 British Pharmacopoeia this was revised, and a potentiometric end-point detection was used.[17]

In the last decade new potentiometric sensors, *e.g.*, ion-selective electrodes, have been developed.[18] The application of ion-selective electrodes in analytical chemistry is of importance[19] and the use of halide ion-selective electrodes makes possible the determination of halide compounds without chemical separation of many undesirable ions that may exist in the solution. This application has opened a new way for the determination of numerous pharmaceutical compounds containing halides either in dissociated or undissociated form and also intermediates produced or used in pharmaceutical manufacturing.

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In this direct determination the ion-selective electrodes measure only the activity of the halide ions and, therefore, the dissociated halide salts can be measured directly without chemical preparation in an appropriate solution.[20] To avoid the effect of the mean activity coefficient it is important to work with dilute solutions or with solutions in which the ionic strength is maintained at a constant value.

The titration of halides can also be carried out potentiometrically with ion-selective electrodes as indicator electrodes.

If the halide is covalently bound to the skeleton of the molecule it can only be determined after digestion or combustion.

The aim of the work described here is to apply the ion-selective electrodes to the determination of pharmaceutical compounds. This paper contains results for dissociated halide salts and undissociated halide compounds in which the halide was liberated after combustion by using the Schöniger method.[21]

EXPERIMENTAL

COMPOUNDS INVESTIGATED—

All pharmaceutical compounds used were of pharmaceutical grade within the pharmacopoeial limits, and all reagents used were of analytical grade.

Group A, "Medicinal dyes"—

1. Acriflavine hydrochloride (3,6-Diamino-10-methylacridinium chloride hydrochloride).
2. Crystal violet (Hexamethyl-*p*-rosaniline chloride).
3. Fuchsine base (*p*-Rosaniline chloride).
4. Malachite green (Tetramethyl-di-*p*-aminotriphenylcarbinol anhydride chloride).
5. Methylene blue [3,7-Bis(dimethylamino) phenazathionium chloride].
6. Rose Bengal (Tetra-iodotetrachlorofluorescein).

Group B, "Anti-cancer or cytostatic compounds"—

1. Chlorambucil 4-{*p*-[Bis(chloroethyl)aminophenyl]butyric acid}.
2. Cyclophosphamide {2-[Bis(2-chloroethyl)amino]tetrahydro-1,3,2-oxazaphosphorine-2-oxide}.
3. Degranol [1,6-Bis(2-chloroethylamino)-1,6-dideoxy-D-mannitol dihydrochloride].
4. Mustine [Bis(2-chloroethyl)methylamine hydrochloride].
5. Myelobromol (1,6-Dibromo-1,6-dideoxy-D-mannitol).

Group C, "Antibiotics"—

1. Chloramphenicol [D-*threo*-*N*-(β-Hydroxy-α-hydroxymethyl-*p*-nitrophenethyl)-2,2-dichloroacetamide].
2. Chlortetracycline hydrochloride (7-Chloro-4-dimethylamino-1,4,4a,5,5a,6,11,12a-octahydro-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxonaphthacene-2-carboxyamide hydrochloride).
3. Griseofulvin [(+)-7-Chloro-2′,4,6-trimethoxy-6′-methylspiro(2,3-dihydrobenzofuran-2,1′-cyclohex-2′-ene)-3,4′-diene].
4. Oxytetracycline hydrochloride (4-Dimethylamino-1,4,4a,5,5a,6,11,12a-octahydro-3,5,6,10,12,12a-hexahydroxy-6-methyl-1,11-dioxonaphthacene-2-carboxyamide hydrochloride).
5. Tetracycline hydrochloride (4-Dimethylamino-1,4,4a,5,5a,6,11,12a-octahydro-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxonaphthacene-2-carboxyamide hydrochloride).

Group D, Official pharmaceutical compounds, their molecules containing both dissociated and undissociated halogen—

1. Chloropyraminum hydrochloride [*NN*-Dimethyl-*N*-(*p*-chlorobenzyl)-*N*-(2-pyridyl)-ethylenediamine hydrochloride].
2. Chlorpromazine hydrochloride [2-Chloro-10-(3-dimethylaminopropyl)phenothiazine hydrochloride].
3. Mepacrine hydrochloride [3-Chloro-9-(4-diethylamino-1-methylbutylamino)-7-methoxyacridine dihydrochloride].

(Chlortetracycline hydrochloride, degranol and mustine can also be included.)

Group E, Official pharmaceutical compounds, their molecules containing both dissociated and undissociated different halogens—

1. Liothyronine hydrochloride [L-4-(4-Hydroxy-3-iodophenoxy)-3,5-di-iodophenylalanine hydrochloride].

Group F, Official pharmaceutical compounds, their molecules containing only undissociated halogen—

1. Dicophane (DDT) [1,1,1-Trichloro-2,2-bis(4-chlorophenyl)ethane].

Group G, Official pharmaceutical compounds, their molecules containing undissociated different halogens—

1. Iodochlorhydroxyquinoline (5-Chloro-8-hydroxy-7-iodoquinoline). (Rose Bengal can also be included.)

Group H, Miscellaneous pharmaceutical compounds—

1. Pethidine hydrochloride (1-Methyl-4-phenyl-4-carbethoxypiperidine hydrochloride).
2. Thiamine hydrochloride [3-(4-Amino-2-methylpyrimidin-5-ylmethyl)-5-(2-hydroxyethyl)-4-methylthiazolium chloride hydrochloride].

APPARATUS

A blood pH meter, type OP-203, (Radelkis, Budapest, Hungary) with halide ion-selective membrane electrodes (System Pungor *et al.*, type OP-711, Radelkis, Hungary) and a saturated calomel electrode were used for all potentiometric measurements. Before use, all ion-selective electrodes were pre-treated in a 10^{-3} M solution of the appropriate halide salt.

PROCEDURE FOR DIRECT METHOD—

A calibration graph was plotted with standard halide solutions. The ionic strength of the solutions was maintained at 0·1 with sodium nitrate in most cases. The results were evaluated by use of a calibration graph.

Before potentiometric measurements were taken, the undissociated halogen was dissociated by the Schöniger method.[19] After ionisation of the halogen the sample was neutralised with 2 N nitric acid, with methyl red as indicator, and made up to a known, calculated volume with distilled water. Solutions were usually prepared with a concentration of 10^{-3} M. The ionic strength of each solution was calculated and adjusted to be approximately equal to that of the solution used for preparing the calibration graph.

If the compound studied contained different halogen ions, such as iodochlorohydroxyquinoline, liothyronine hydrochloride and Rose Bengal, an iodide-selective membrane electrode was used for the direction determination of iodide ions. In this case, as the chloride ions are in the solution, they do not interfere in the measurement of the iodide ions. Before the direct determination of chloride any traces of iodide ions should be removed or de-activated in the solution. We effected this by taking an aliquot, acidifying it with a few drops of concentrated nitric acid, then adding a few drops of potassium permanganate solution until a pink colour appeared. The solution was neutralised with potassium hydroxide solution, diluted to a known volume and its chloride content measured directly with a chloride-selective membrane electrode.

This is a rapid, routine method, the accuracy of which is not as high as that of the titration method. The values found by this method are within 2 to 3 per cent. of those found by the titration method, provided that the electrodes are correctly calibrated(Table I).

PROCEDURE FOR TITRATION METHOD—

A known aliquot of the sample was made slightly acidic and titrated potentiometrically with silver nitrate solution, with an ion-selective membrane electrode as the indicator electrode. Naturally the titration of compounds containing undissociated halogens could only be carried out after converting the halogens into halides by Schöniger's method.

If the solution contained different halide ions, *e.g.*, chloride and iodide ions, the titration was carried out with an iodide-indicator electrode. The evaluation of the results was carried out on the basis of the inflection points.

TABLE I

DETERMINATION OF THE HALIDE CONTENT OF VARIOUS PHARMACEUTICAL COMPOUNDS

Compound	Solution used for calibration	Concentration in pX		△ pX	Method used for the preparation of the solution
		direct method	titration method		
Group A—					
1. Acriflavine	KCl	2·84	2·80	0·04 pCl	Dissolved in distilled water
hydrochloride		3·84	3·80	0·04 pCl	Dissolved in distilled water
2. Crystal violet	KCl	2·92	2·88	0·04 pCl	Dissolved in distilled water
	KCl	3·88	3·89	0·01 pCl	Dissolved in distilled water
3. Fuchsine base	KCl	2·06	2·02	0·04 pCl	Dissolved in distilled water
	KCl	3·07	3·04	0·03 pCl	Dissolved in distilled water
	KCl	4·06	4·04	0·02 pCl	Dissolved in distilled water
4. Malachite green	KCl - KNO_3	2·79	2·74	0·05 pCl	Dissolved in distilled water
	KCl - KNO_3	3·77	3·74	0·03 pCl	Dissolved in distilled water
5. Methylene blue	KCl - KNO_3	2·08	2·04	0·04 pCl	Dissolved in distilled water
	KCl - KNO_3	3·08	3·04	0·04 pCl	Dissolved in distilled water
	KCl - KNO_3	4·06	4·04	0·02 pCl	Dissolved in distilled water
6. Rose Bengal	KCl - KNO_3	3·05	3·07	0·02 pCl	After Schöniger
	KI - KNO_3	3·03	3·03	0·00 pI	After Schöniger
Group B—					
1. Chlorambucil	KCl - KNO_3	3·00	3·00	0·00 pCl	After Schöniger
2. Cyclophosphamide	KCl - KNO_3	3·00	3·00	0·00 pCl	After Schöniger
3. Degranol	KCl - KNO_3	1·64	1·63	0·01 pCl	Dissolved in distilled water
	KCl - KNO_3	2·62	2·63	0·01 pCl	Dissolved in distilled water
	KCl - KNO_3	3·60	3·63	0·03 pCl	Dissolved in distilled water
	KCl - KNO_3	2·56	2·60	0·04 pCl	After Schöniger
4. Mustine	KCl - KNO_3	2·00	2·00	0·00 pCl	Dissolved in distilled water
	KCl - KNO_3	3·00	3·00	0·00 pCl	Dissolved in distilled water
	KCl - KNO_3	4·00	4·00	0·00 pCl	Dissolved in distilled water
	KCl - KNO_3	2·86	2·86	0·00 pCl	After Schöniger
5. Myelobromol	KBr - KNO_3	3·00	3·01	0·01 pBr	After Schöniger
Group C—					
1. Chloramphenicol	KCl - KNO_3	3·01	3·02	0·01 pCl	After Schöniger
2. Chlortetracycline	KCl - KNO_3	2·01	2·01	0·00 pCl	Dissolved in distilled water
hydrochloride	KCl - KNO_3	3·01	3·01	0·00 pCl	Dissolved in distilled water
	KCl - KNO_3	4·00	4·01	0·01 pCl	Dissolved in distilled water
	KCl - KNO_3	3·02	3·00	0·00 pCl	After Schöniger
3. Griseofulvin	KCl - KNO_3	2·99	3·01	0·02 pCl	After Schöniger
4. Oxytetracycline	KCl - KNO_3	2·04	2·02	0·02 pCl	Dissolved in distilled water
hydrochloride	KCl - KNO_3	3·04	3·02	0·02 pCl	Dissolved in distilled water
	KCl - KNO_3	4·04	4·02	0·02 pCl	Dissolved in distilled water
5. Tetracycline	KCl - KNO_3	2·02	2·00	0·02 pCl	Dissolved in distilled water
hydrochloride	KCl - KNO_3	3·02	3·00	0·02 pCl	Dissolved in distilled water
	KCl - KNO_3	4·02	4·00	0·02 pCl	Dissolved in distilled water
Group D—					
1. Chloropyraminum	KCl - KNO_3	1·97	2·00	0·03 pCl	Dissolved in distilled water
hydrochloride	KCl - KNO_3	2·97	3·00	0·03 pCl	Dissolved in distilled water
	KCl - KNO_3	3·97	4·00	0·03 pCl	Dissolved in distilled water
	KCl - KNO_3	2·76	2·74	0·02 pCl	After Schöniger
2. Chlorpromazine	KCl	2·02	2·00	0·02 pCl	Dissolved in distilled water
hydrochloride	KCl	3·00	3·00	0·00 pCl	Dissolved in distilled water
	KCl	4·02	4·00	0·02 pCl	Dissolved in distilled water
	KCl - KNO_3	2·79	2·80	0·01 pCl	After Schöniger
3. Mepacrine	KCl	2·74	2·70	0·04 pCl	Dissolved in distilled water
hydrochloride	KCl	3·71	3·70	0·01 pCl	Dissolved in distilled water
	KCl - KNO_3	2·74	2·74	0·00 pCl	After Schöniger
Group E—					
Liothyronine	KCl - KNO_3	3·44	3·40	0·04 pCl	After Schöniger
hydrochloride	KI - KNO_3	3·00	3·01	0·01 pI	After Schöniger
Group F—					
Dicophane	KCl - KNO_3	2·70	2·70	0·00 pCl	After Schöniger
Group G—					
Iodochlorhydroxy-	KCl - KNO_3	2·94	2·96	0·02 pCl	After Schöniger
quinoline	KI - KNO_3	2·94	2·94	0·00 pI	After Schöniger

TABLE 1—*continued*

Compound	Solution used for calibration	Concentration in pX		Δ pX	Method used for the preparation of the solution
		direct method	titration method		
Group H—					
1. Pethidine	KCl - KNO_3	2·02	2·01	0·01 pCl	Dissolved in distilled water
hydrochloride	KCl - KNO_3	3·02	3·01	0·01 pCl	Dissolved in distilled water
	KCl - KNO_3	4·02	4·01	0·01 pCl	Dissolved in distilled water
2. Thiamine	KCl - KNO_3	1·72	1·69	0·03 pCl	Dissolved in distilled water
hydrochloride	KCl - KNO_3	2·72	2·69	0·03 pCl	Dissolved in distilled water
	KCl - KNO_3	3·72	3·69	0·03 pCl	Dissolved in distilled water

RESULTS AND DISCUSSION

The results of the analyses of twenty-four compounds are shown in Table I. This table also gives details of the salts used for plotting the calibration graph, the results of the direct and indirect potentiometric determinations, the difference between the results determined with the direct and indirect methods and the method of preparation of the sample. The concentrations are given in pX ($-\log C_X$; X is any halide ion) for easy comparison. The standard deviation of the results obtained by the direct method is 0·56 per cent., which was calculated after converting the results into arithmetical values.

From the results it can be concluded that the direct potentiometric method described in this paper is suitable for the determination of the halide content of various pharmaceutical preparations. It can be carried out within a few minutes and can therefore be used for quick, routine analyses. For determinations in which greater accuracy is required, the potentiometric titration method with ion-selective electrodes is suggested.

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A Simple Diffusion Method for the Determination of Fluoride

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A method is described for the determination of sub-microgram amounts of inorganic fluoride. Diffusion on to a thin layer of solid alkali is used to separate fluoride from any contaminants. The diffused fluoride may be determined with an ion-selective fluoride electrode or by spectrophotometric methods.

Phosphate and sulphate do not interfere. The interference of halides, carbonate, nitrate and acetate is discussed. Organic material was found not to interfere with the recovery of fluoride except in determinations involving human serum. High-temperature ashing of organic samples was found to lead to loss of fluoride.

When used with the ion-selective electrode the method is applicable over the range 0·05 to 200 μg of fluoride.

THE advent of the ion-selective fluoride electrode has almost removed the need for diffusion procedures. Frant and Ross[1] described a method for the determination of fluoride with a total ionic strength adjustment buffer containing acetic acid, sodium hydroxide, sodium chloride and sodium citrate.

More recently Duff and Stuart[2] and Edmond[3] have developed suitable procedures for the direct determination of fluoride in calcium phosphate, which have a wide general application. However, it is still frequently desirable to separate fluoride from various contaminants. Originally, high temperature distillation was used by Willard and Winter.[4] This has largely been superseded by diffusion techniques, but the need for a simple, reliable method, applicable over a fairly wide range of concentrations from sub-microgram amounts to several hundred micrograms, has long been recognised. Several diffusion methods have been described for use with spectrophotometric reagents. Wharton[5] described a method by which amounts down to 0·2 μg could be determined by using zirconium - SPADNS [4,5-dihydroxy-3-(*p*-sulphophenylazo)-2,7-naphthalenedisulphonic acid, trisodium salt]. Leach and Griffiths[6] used a similar method with the zirconium - Eriochrome cyanine R reagent of Singer and Armstrong,[7] and claimed an error of only 0·02 μg on samples containing more than 0·1 μg of fluoride. It is possible that this accuracy may only be approached under ideal conditions. The remaining purely chemical method for the sub-microgram determination of fluoride appears to be that of Hall,[8] which involves trapping hydrogen fluoride on filter-paper treated with magnesium succinate and determining the fluorine by the direct colour development of the lanthanum - alizarin fluorine blue complex. This technique has been modified[9] with sodium hydroxide as the trapping agent. Although accurate, the method undoubtedly requires considerable care because there are several stages between trapping the fluoride and the final quantitative determination during which loss, or contamination, of fluoride may occur. This technique could be adapted for use with the fluoride ion-selective electrode, but would require prior extraction of the sodium hydroxide from the filter-paper. All of the spectrophotometric methods have the disadvantage of being effective only over a narrow range of fluoride concentrations.

Rowley and Farrah[10] and Marshall and Wood[11] have described similar methods in which a thin layer of solid sodium hydroxide is used for trapping hydrogen fluoride. Both methods, however, involve conventional transfer of solutions to calibrated glassware, and neither is really suitable for use at sub-microgram levels of fluoride. The present work describes a process in which the entire fluoride determination is carried out in a single chamber, with a thin layer of alkali to trap the diffused hydrogen fluoride. Quantitative transfer of solutions is avoided, thus minimising experimental errors. The final fluoride determination can be carried out either by using a fluoride ion-selective electrode or by spectrophotometric methods.

METHOD

DIFFUSION CELL—

This consists of a 5-ml capacity heavy-walled, screw-capped, polythene sample bottle supplied by Xlon Ltd., inside which is placed a standard-size polypropylene test-tube cap (Oxoid Ltd.).

ELECTRODE—

The pF values were obtained on a Radiometer PHM53 ion-selective meter with an Orion type 94–09 solid-state fluoride electrode in conjunction with a calomel reference electrode.

SPECTROPHOTOMETRIC DETERMINATIONS—

These were carried out with a Unicam SP500 spectrophotometer.

REAGENTS—

All reagents used should be of analytical-reagent grade.

Standard fluoride stock solution—A solution containing 1000 μg ml^{-1} of fluoride, made by dissolving 2·205 g of sodium fluoride in 1 litre of de-ionised water.

Standard fluoride experimental solutions—Solutions containing 500, 100, 10, 5, 1·0, 0·5 and 0·1 μg ml^{-1} of fluoride are prepared from the stock solution. All except the two most dilute will keep indefinitely if stored in polythene containers in a cool place.

Sodium hydroxide solution, 0·1 M—Dissolve 4·0 g of sodium hydroxide in 1 litre of de-ionised water.

Perchloric acid - silver sulphate solution—Dissolve 5 g of silver sulphate in 100 ml of 70 to 72 per cent. w/w perchloric acid.

Acetate buffer solution, pH 5·2—Add 443 ml of de-ionised water to 57 ml of glacial acetic acid. Adjust the pH to 5·2 with 5 M sodium hydroxide solution.

Spectrophotometric reagent A, SPADNS (4,5-*dihydroxy*-3-(p-*sulphophenylazo*)-2,7-*naphthalenedisulphonic acid, trisodium salt*)—Dissolve 0·632 g in 500 ml of de-ionised water.

Spectrophotometric reagent B, zirconyl chloride octahydrate—Dissolve 0·133 g in 50 ml of de-ionised water, add 350 ml of concentrated hydrochloric acid and make the volume up to 500 ml with de-ionised water.

Final spectrophotometric reagents—For a low level of fluoride (0 to 0·5 μg) take one volume of reagent A, one volume of reagent B and two volumes of de-ionised water. For a high level of fluoride (0·5 to 5·0 μg) take one volume of reagent A and one volume of reagent B only.

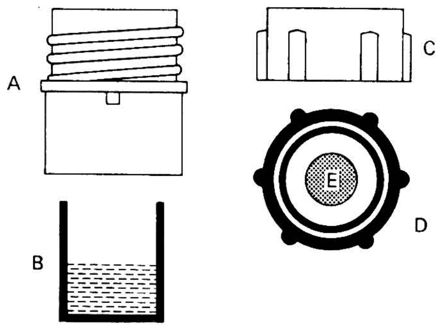

Fig. 1. Sectional diagram of the diffusion cell used: A, body of cell; B, polypropylene inner unit; C, cap; D, internal structure of cap; E, alkali trapping agent

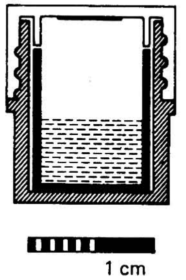

Fig. 2. The completed diffusion cell

PROCEDURE—

Details of the apparatus are shown in Fig. 1, and a view of the apparatus when assembled is shown in Fig. 2.

Drop 0·11 ml of the 0·1 M sodium hydroxide solution, by pipette, on to the centre of the inside of the cap (E) of the diffusion unit (D). Evaporate the solution to dryness in a vacuum desiccator over sodium hydroxide.

Place the sample (ashed or otherwise) in the polypropylene inner unit (B) and add 1 ml of perchloric acid - silver sulphate solution. Then place the inner unit inside the body (A) of the cell. Replace the cap containing the dried alkali layer and leave the assembled cell overnight in an oven at 60° C (16 to 20 hours).

The procedure after diffusion varies, depending on whether an ion-selective electrode or spectrophotometric finish is being used.

(*a*) *For use with an ion-selective electrode*—

Remove the cap, add 1·00 ml of acetate buffer to the alkali layer by pipette and briefly stir the mixture. Transfer this (not necessarily quantitatively) to an Orion micro sample dish (ref. 94.00.02) and measure the pF. Find the fluoride concentration from a calibration graph obtained by plotting log p.p.m. of fluorine *versus* electrode potential (mV) for similarly treated standards. A typical calibration graph is shown in Fig. 3.

(*b*) *For use with spectrophotometric reagents*—

Drop 2 ml of the appropriate reagent from a pipette over the alkali layer on the cap and mix with the layer, which is left to stand for 30 minutes. Then measure the optical density in 1-cm glass cells at 590 nm or the observed $\lambda_{max.}$ against a blank set at 0·300 (for low fluoride levels) or 0·500. Find the fluoride concentration from a calibration graph obtained from similarly prepared standards. Fig. 4 shows typical calibration graphs.

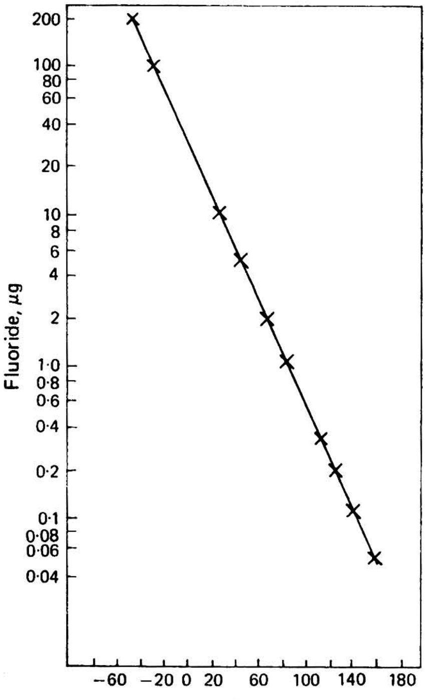

Fig. 3. Typical calibration graph from diffused samples with 1·0 ml of acetate buffer

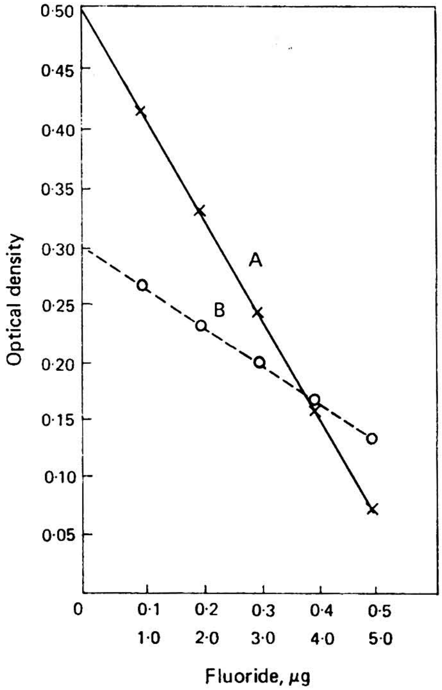

Fig. 4. Calibration graph for fluoride concentration by spectrophotometric method; high and low concentrations are plotted with SPADNS: A, 0 to 5·0 μg of fluoride; and B, 0 to 0·5 μg of fluoride

TABLE I

EFFECT OF CONTAMINANTS ON THE RECOVERY OF FLUORIDE

		Ion-selective fluoride electrode											SPADNS				
Diffusion medium		HClO$_4$						HClO$_4$ and Ag$^+$					HClO$_4$ and Ag$^+$				
Fluoride added, µg		0·00	0·14	0·29	1·4	10·0	100	0·00	0·14	0·29	10·0	100	0·00	0·14	0·29	2·9	4·0
Contaminant	Per cent. w/v (aqueous solution)	Fluoride recovered, µg															
None	—	0	0·14	0·29	1·4	10·0	100	0	0·14	0·29	10·0	100	0	0·14	0·29	2·8	3·8
Glucose	0·1	0	0·15	0·28	1·45	10·1	100	0	0·14	0·30	10·0	100	0	0·14	0·30	3·0	4·0
	1·0	0	0·13	0·29	1·50	9·9	100	0	0·15	0·31	10·1	100	0	0·14	0·29	2·8	3·8
	10	0	0·15	0·29	1·40	10·5	100	0	0·15	0·29	10·0	100	0	0·15	0·29	2·9	3·8
KH$_2$PO$_4$	0·1	0	0·136	0·295	1·41	9·8	100	0	0·14	0·29	10·0	100	0	0·14	0·29	2·9	4·0
	1·0	0	0·14	0·29	1·4	10·0	100	0	0·14	0·29	10·0	100	0·3	0·14	0·29	2·9	4·2
	10	0	0·14	0·29	1·42	10·1	98	0	0·14	0·28	10·1	98	0·15	—	0·29	2·9	4·2
NaBr	0·1	—	0·14	0·28	1·35	10·1	100	0	0·15	0·30	10·0	98	0·1	0·15	0·29	3·0	—
	1·0	0·1	0·15	0·31	1·5	10·2	98	0	0·14	0·27	9·8	100	0·2	0·16	0·29	3·1	—
	10	*	*	*	*	*	*	0	0·14	0·28	9·8	98	0·4	0·18	0·31	3·1	—
Na$_2$CO$_3$	0·1	—	—	—	—	—	—	—	—	—	—	—	0	0·13	0·29	3·0	—
	1·0	—	—	—	—	—	—	—	—	—	—	—	0	0·13	0·27	2·9	—
	10	*	*	*	*	*	*	0·1	0·15	0·30	10·2	105	0	0·12	0·27	3·0	—
KCl	0·1	0	—	—	1·4	10	100	0	—	—	10·0	100	0	0·14	0·29	—	3·9
	1·0	—	—	—	—	—	—	—	—	—	—	—	0	0·14	0·30	—	4·0
	10	*	—	—	*	*	*	0	—	—	10·0	100	0·05	0·15	0·31	—	4·0
NaNO$_3$	1·0	*	*	*	*	*	*	0·07	0·15	0·3	11	105	0·05	0·15	0·31	—	4·0
	10·0	*	*	*	*	*	*	—	0·15	0·31	11	—	0·05	0·15	0·33	—	4·1
CuCl$_2$	0·1	—	—	—	—	—	—	—	—	—	—	—	0	0·15	0·32	—	—
	1·0	—	—	—	—	—	—	—	—	—	—	—	0	0·17	0·31	—	—
	10	—	—	—	—	—	—	—	—	—	—	—	0·05	0·12	0·31	—	—
Total ionic strength adjustment buffer, 0·5 ml		*	*	*	*	*	*	0	0·135	0·29	9·8	95	—	—	—	—	—
Na$_2$SO$_4$	10	0·0	0·15	0·30	1·4	10	100	0·0	0·14	0·29	10	100	—	—	—	—	—

* Indicates observed value less than control.
Results are the mean of five samples, each of 0·1 ml.

Fluoride contents up to 10 μg can be determined by dissolving the alkali layer in deionised water, transferring this to a 25-ml calibrated flask to which 2 ml of a (1 + 1) SPADNS - zirconyl chloride solution have been added and making the volume up to 25 ml. At this level loss of fluoride on transfer is negligible.

RESULTS AND DISCUSSION

Several buffer solutions were tried for use with the electrode. The total ionic strength adjustment buffer of Frant and Ross[1] was excellent. The acetate buffer finally chosen was Frant and Ross' buffer *minus* sodium chloride and sodium citrate, which were found to be unnecessary with diffused samples. One disadvantage of this buffer is that it is difficult to re-diffuse the fluoride.

There are times when it is desirable to compare results, both diffused and undiffused, on the same sample. In such circumstances it is found that Sorensen's phosphate buffer,[12,13] pH 6·8, is ideal.

SELECTIVITY AND REPRODUCIBILITY—

Attempts to diffuse fluoride from perchloric acid alone showed that many contaminants interfered. The addition of a 5 per cent. w/v solution of silver sulphate was found to prevent interference in many cases.

The use of silver sulphate has already been discussed by Hall.[8] The action may be based on the formation of a stable complex with the contaminant. This interference was observed whether the fluoride was determined with an ion-selective electrode or spectrophotometrically, which suggests that it is caused by saturation of the alkali layer with diffused contaminant, thus preventing reaction with the diffusive hydrogen fluoride. Support for this is given by the fact that diffusion of fluoride on to the alkali layer followed by re-diffusion with contaminant alone, with perchloric acid but in the absence of a silver salt, still gave full recovery of fluoride with the electrode. Also, neither acetate nor chloride ion interferes with the electrode performance as both are present in the total ionic strength adjustment buffer of Frant and Ross.

Table I gives the results of a blind trial carried out on solutions containing various contaminants and shows the effect of contaminants on the recovery of fluoride diffused from perchloric acid alone and from perchloric acid - silver sulphate solution.

Various methods have been used for the determination of fluoride in unashed bone. Hardwick correlated the results of various methods but has yet to publish his findings. A selection of results is given in Table II, which shows that the present method gives a result near the median of diffusion methods and has a lower standard deviation.

TABLE II

FLUORIDE CONTENT OF UNASHED BONE

Method	Reagent	Fluoride recovered, p.p.m.
Hall[9]	Lanthanum - alizarin fluorine blue complex	715 ± 61
Wharton[5]	SPADNS	788 ± 162
Leach and Griffiths[6] ..	Zirconium - Eriochrome cyanine R ..	686 ± 45
Present	Acetate buffer - fluoride electrode	739 ± 26

ELECTRODE RESPONSE—

A drawback with the ion-selective electrode is its relatively slow response time. Elimination of contaminants by diffusion appears to improve the response time slightly.

USE WITH SPECTROPHOTOMETRIC REAGENTS—

As shown in Table I the method gives satisfactory results when used with SPADNS reagent.

The choice of SPADNS in preference to alizarin - fluorine blue complex was difficult. Crosby, Dennis and Stevens[14] have reviewed various reagents and, like Hall,[8,9] find alizarin to be the most satisfactory. However, the use of alizarin would remove one of the most attractive features of the present method by requiring quantitative transfer of solutions and several manipulations between trapping fluoride and its final determination. The use of SPADNS obviates this.

On the other hand, SPADNS tends to give poor reproducibility with blanks, especially when used for low-level fluoride determination. Generally, it was felt that the convenience of SPADNS outweighed its disadvantages. Alizarin was, however, used during investigations on unashed bone with results similar to those with Hall's method.

It may be argued that diffusion is superfluous when used with the ion-selective electrode, and it is true that for acid-soluble samples with fairly high fluoride contents, the direct methods of Edmond[3] and Duff and Stuart[2] are preferable. However, the lower limit of detection by direct methods is about 0·7 μg of fluoride. Diffusion concentrates the fluoride into a volume of 1·00 ml and thus enables fluoride determination to be carried out at levels down to 0·05 μg.

Also, with single samples of unknown fluoride content the much wider sensitivity range (0·05 to 200 μg of fluoride) of the electrode makes its use preferable to spectrophotometric methods with their narrow ranges.

Edmond[3] in his work on phosphate rock noted that total ionic strength adjustment buffer invariably gives lower recoveries of fluoride than citrate buffer. This has been confirmed on analysis of ground human teeth for which the mean recoveries by the direct method with citrate buffer, total ionic strength adjustment buffer, and diffusion followed by acetate buffer, were 245, 230 and 226 p.p.m. of fluoride, respectively.

TABLE III

RECOVERY OF ADDED FLUORIDE FROM ORGANIC MATERIAL

Assay method: fluoride electrode

		Fluoride, p.p.m.		
Sample	No. of samples	Observed in sample	2·0 p.p.m. added	40 p.p.m. added
Human serum	12	0·095	1·4	—
Tea leaves (mixed) ..	3	16·6	—	56·6
Urea	6	0	2·0	—
Casein	4	5·6	—	46·0

ORGANIC MATERIAL—

The method is not satisfactory when used with organic material. Although full recovery of added fluoride is usually obtained from unashed samples (Table III), human serum showed a 30 per cent. loss. High temperature ashing (*i.e.*, 500° C) often leads to a fairly high loss. Addition of calcium oxide[4,15] does not seem to improve matters.

Table IV gives some typical results with organic material. When tea leaves were heated with 2 ml of 5 M sodium hydroxide solution, then ashed at 800° C, the indicated fluoride content was increased from 17·0 to 49·2 ± 7 p.p.m. of fluoride.

TABLE IV

TYPICAL RESULTS WITH ORGANIC MATERIALS

Assay method	SPADNS		Fluoride electrode	
Sample	No. of samples	Mean fluoride content, p.p.m.	No. of samples	Mean fluoride content, p.p.m.
Casein batch I	8	6·0	6	5·6
Casein ashed at 500° C	4	4·5	4	4·9
Casein ashed *plus* 25 mg of CaO ..	—	—	3	5·1
Casein batch II	3	4·4	—	—
Casein ashed at 500° C *plus* 25 mg of CaO	4	4·1	—	—
Casein batch III	4	5·3	—	—
Dried milk	29	9·4	—	—
Dried milk ashed at 500° C	2	9·0	—	—
Trufood batch I	4	9·8	—	—
Trufood ashed at 500° C *plus* CaO ..	2	10·0	—	—
Trufood batch II	4	8·3	—	—
Human serum	6	0·08	4	0·095
Human serum ashed at 500° C ..	4	0·05	—	—
Human serum ashed *plus* 25 mg of CaO	2	0·045	—	—
Tea leaves (mixed)	—	—	5	16·6
Tea leaves dried at 90° C	—	—	7	17·0
Tea leaves ashed at 500° C	—	—	3	14·9
Complan batch I	5	6·8	—	—
Complan ashed at 500° C	2	6·7	—	—
Complan batch II	4	6·8	—	—

CLEANING OF UNITS—

Trouble was experienced at first because the caps of the units tended to split during diffusion. This was overcome by annealing them before initial use.

Annealing and cleaning of units can be carried out simultaneously and conveniently by warming the separated units to 70° C in 10 per cent. perchloric acid. After 20 minutes at this temperature they are allowed to cool slowly to room temperature, washed twice with distilled and de-ionised water and dried at 60° C. The polypropylene inner units are boiled with 10 per cent. perchloric acid for 10 minutes before being washed with distilled and de-ionised water and dried as above.

After this initial cleaning it is usually only necessary to rinse the main units in distilled and de-ionised water and dry them before re-use. New inner units are used each time.

CONCLUSION

A method has been described which presents a practicable way of combining the advantages of the ion-selective fluoride electrode together with diffusion for separation of interfering substances. As described it is available over the range 0·05 to 200 μg of fluoride, but there is no reason why this range should not be greatly increased by using larger amounts of alkali trapping agent and acetate buffer solution. It is also a useful method for use with spectrophotometric reagents.

I thank Professor J. L. Hardwick for his interest and encouragement and also for allowing his results on the fluoride content of unashed bone to be used.

Dr. E. J. Duff and Dr. R. W. C. Broadbank were kind enough to read through the manuscript and make several useful comments. I also thank Miss C. J. Martin for preparing the solutions for use in the blind trials.

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An Improved Synthesis of Alizarin Fluorine Blue

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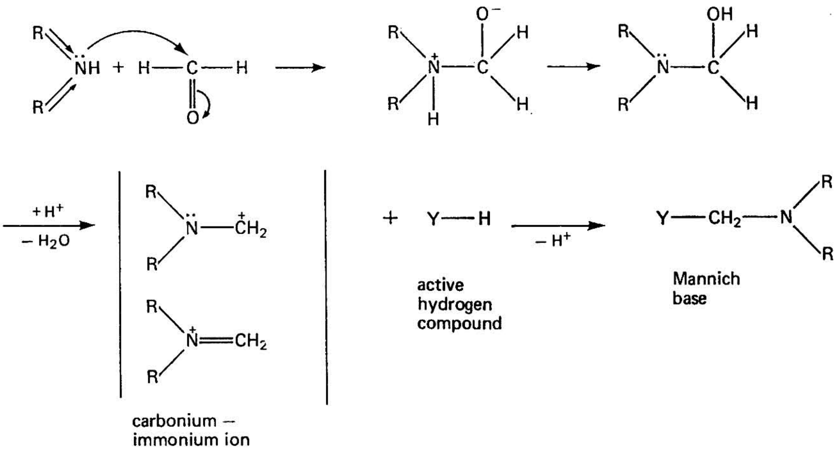
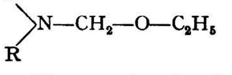
The ideas of Hellmann and Opitz concerning optimum conditions for aminomethylation reactions are applied to the synthesis of alizarin fluorine blue. The yield is increased from 13 to 61 per cent. by using an aqueous ethanolic solvent of minimum alkalinity.

THE compound alizarin fluorine blue (1,2-dihydroxyanthraquinon-3-ylmethylamine-*NN*-diacetic acid) has achieved considerable use as a reagent for the detection of fluoride and certain metal ions, and as a metallochromic indicator.[1,2] The synthesis formerly suggested by Leonard and West consisted of a Mannich-type condensation between alizarin, formaldehyde and iminodiacetic acid under strongly alkaline, solely aqueous conditions. The yield of the poorly crystalline product was about 13 per cent.

Study of the excellent paper by Hellmann and Opitz[3] concerning the optimum conditions required for aminomethylation showed that the Mannich condensation most probably proceeds in this manner:



We therefore repeated the synthesis with pre-formation of the carbonium - immonium intermediate and with aqueous ethanol as the solvent, which enabled a satisfactory alizarin concentration to be used under minimum conditions of alkalinity (the vital cationic intermediate requiring a proton for its formation). Under these conditions the *N*-ethoxymethyl compound



$$R_2N{-}CH_2{-}O{-}C_2H_5$$

may be formed, but this also reacts with a proton to give the active carbonium - immonium intermediate.

The total yield by this method of synthesis was 61 per cent.

METHOD OF SYNTHESIS

Dissolve 1 g of alizarin (1,2-dihydroxyanthraquinone, recrystallised from ethanol) in 50 ml of ethanol and add 4 ml of N sodium hydroxide solution dropwise with swirling. Separately dissolve 3·25 g of iminodiacetic acid disodium salt monohydrate in 25 ml of ethanol - 4 per cent. sodium hydroxide mixture (11 + 9), add 1 ml of 36 per cent. formaldehyde solution and maintain at 5° C for 12 hours. Mix the two solutions at 20° C in a 100-ml flask fitted with stirrer, reflux condenser and nitrogen inlet. Displace the air in the apparatus with nitrogen and thereafter maintain a slow flow over the surface of the mixture. Heat the mixture to 78° C and maintain it at this temperature for a total of 10 hours, adding further 0·7-ml portions of 36 per cent. formaldehyde solution after 3 and 7 hours.

Dilute the mixture to 250 ml with de-ionised water, heat to 50° C, then add 3 N hydrochloric acid until the pH is 1·8. After 5 hours filter off the precipitate, wash it sparingly with warm water, then dissolve it in 200 ml of water containing 8 ml of N sodium hydroxide solution. Heat the resulting mixture to 50° C and then add dilute acetic acid to bring the pH to 5·5. Maintain it at 50° C for 10 minutes, then filter off the small residue of alizarin or non-carboxylated polymer. Cool the filtrate to 20° C and extract with diethyl ether until the extracts are virtually colourless. Acidify the aqueous phase to pH 1·8, allow it to stand overnight, filter off the clear yellow - orange precipitate, wash it with water followed by a mixture of ethanol - diethyl ether in the ratio (1 + 1) and finally dry under vacuum at 80° C over phosphorus(V) oxide. We obtained a yield of 0·98 g or 61 per cent. by this method.

ELEMENTAL ANALYSIS—

	Percentage of carbon	Percentage of hydrogen	Percentage of nitrogen
Theoretical	59·20	3·92	3·63
Found	59·13	4·07	3·53

MASS SPECTROMETRY—

The mass spectrum showed no parent ion peak at 385 but gave peaks at 326 (10 per cent. abundance) and 254 (100 per cent. abundance; high molecular weight end only) indicating the loss of $-CH_2COOH$ and $-N(CH_2COOH)_2$ radicals from the product.

CONCLUSIONS

Although the product obtained by this method is no purer than rigorously purified material obtained with the old method, a good quality product is much easier to obtain because the conversion yield of alizarin into alizarin fluorine blue is much greater. The products of the old and new methods are identical with respect to thin-layer chromatography (both yielding only one spot), electrophoresis, mass spectrometry, melting-point and reactivity of the lanthanum(III) chelate towards fluoride.

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Mixed-solvent Systems for the Flame Analysis of Petroleum Materials

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Flame methods (emission, fluorescence and atomic-absorption spectroscopy) are particularly valuable in the petroleum industry because they permit petroleum products in organic solvent systems to be analysed directly, although the benefits of these methods for oil analysis have been partly offset by various difficulties. In this paper we indicate how three of these difficulties can be avoided by selecting appropriate solvent mixtures.

ONE problem with the use of organic solvents in oil analysis by flame methods is to provide standard solutions against which samples can be compared. Up to the present time it has been necessary to use organic salts of metals, *e.g.*, naphthenates and hexanoates, to obtain standard solutions. Unfortunately, organic salts of metals are much less readily available than inorganic salts and are more expensive (or require accurate preliminary analyses that make them expensive) and their solutions are less stable. However, we have found that mixed-solvent systems, in which water and oil are compatible, allow the use of inorganic salts for standardisation.

After a number of trials we have found that a mixture of 5 volumes of cyclohexanone, 3 volumes of butanol and 2 volumes of industrial methylated spirit is generally satisfactory. This mixture can accommodate about 10 ml of aqueous solution and 0·3 g of oil in each 100 ml of solvent. It possesses the additional merits of having good spraying and burning characteristics and of giving relatively stable solutions.

The mixed-solvent system is also useful for oil samples containing water or water-soluble salts. For example, if it is required to measure sea-water contamination of crude oils, direct flame methods appear to be much simpler than the standard method.[1] With some solvents (*e.g.*, isopropyl alcohol - white spirit to B.S. 245 : 1956) the results are erratic because of sample heterogeneities (water globules or salt particles), but the mixed-solvent system of cyclohexanone, butanol and industrial methylated spirit gives very good results. An interesting aspect of this work is that atomic-absorption readings with some solvent systems (*e.g.*, isopropyl alcohol - white spirit) are negative; the sodium light emitted by the flame is modulated by sample irregularities and registers on the a.c. detector as an addition to the modulated signal from the hollow-cathode lamp.

In lubricating-oil blending installations it is common practice to use simple rather than mixed solvents in the analysis of blend samples and to use as standards chemically analysed blends appropriate for each sample type. This is because the metallic additives blended into lubricating oils can vary widely in composition and physical form (the basic ones are often present as colloidal suspensions). If standards and samples are dissimilar, then the differences can affect flame measurements and lead to systematic errors. For the flame determination of certain metallic additives the mixed-solvent system as described does not improve this situation. However, the addition of strong mineral acid to the mixed-solvent system (we use 1 volume of concentrated hydrochloric acid to 20 volumes of solvent) virtually removes these errors, as shown by the figures in Table I, which were obtained with an oil containing a highly basic calcium additive.

We intend to test further applications of mixed solvents and report them more fully in due course.

TABLE I

EXAMPLE OF ERRORS IN THE DETERMINATION OF CALCIUM

Determination	Calcium, per cent. w/w
By a chemical method[2]	0·230
By atomic-absorption spectroscopy against naphthenate standards (with cyclohexanone as solvent for both sample and standards)	0·108
By atomic-absorption spectroscopy against calcium chloride (with mixed solvents containing hydrochloric acid)	0·242

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A Method for Decreasing the Atomic-absorption Sensitivity of Calcium by Using the Germanium 422·657 nm Line

By K. C. THOMPSON

(*Southern Analytical Ltd., Frimley Road, Camberley, Surrey*)

A method for decreasing the atomic-absorption sensitivity of calcium by using the overlap of the germanium 422·657 nm non-resonance line profile with the calcium 422·673 nm resonance line profile is described.

Almost linear calibration graphs were obtained with concentrations of up to 1500 μg ml^{-1} in the air - acetylene flame (sensitivity 8·3 μg ml^{-1}), and up to 1000 μg ml^{-1} in the nitrous oxide - acetylene flame (sensitivity 6·0 μg ml^{-1}).

It is often desirable to determine relatively high levels of calcium by atomic-absorption spectroscopy. The main calcium resonance line is at 422·673 nm. With this line, the sensitivity for most commercial instruments is about 0·1 μg ml^{-1}. Rotation of the burner reduces the sensitivity 10 to 20 times. Thus solutions containing more than 100 to 200 μg ml^{-1} of calcium will require dilution before nebulisation. The calcium resonance line at 239·856 nm was found[1] to give a sensitivity of 10 μg ml^{-1}. This line is emitted only weakly by most hollow-cathode lamps, thus causing the determination at this wavelength to be noisy.

Germanium has a non-resonance line at 422·657 nm,[2] which is 0·016 nm from the main calcium resonance line at 422·673 nm. Previous studies[3,4] have shown that the iodine 206·163 nm non-resonance line appreciably overlaps the bismuth 206·170 nm resonance line ($\Delta\lambda$ = 0·007 nm), and that the arsenic 228·812 nm non-resonance line weakly overlaps the cadmium 228·802 nm resonance line ($\Delta\lambda$ = 0·01 nm). For germanium and calcium, it appears that the wavelength separation is too great for appreciable overlap. From Döppler and collisional broadening considerations, Parsons, McCarthy and Winefordner[5] have shown that the total line half-width of the calcium 422·673 nm resonance line is about 3·5 times greater than that of the cadmium 228·802 nm resonance line. It therefore appears feasible that overlap of the calcium and germanium line profiles should occur. There are several other examples of spectral overlap in atomic-absorption spectroscopy.[6,7,8]

EXPERIMENTAL

A Southern Analytical A3000 atomic-absorption spectrophotometer, modified to incorporate a modulated microwave source, was used in this study.[9]

The source was a Southern spectral sources germanium electrodeless discharge lamp, which was excited in a Southern spectral sources tuneable microwave cavity. The microwave power was supplied by a 2450-MHz magnetron (Electro-Medical Supplies Limited). There was no emission attributable to calcium from the germanium lamp.

The germanium lamp was operated at 30 W with moderate air cooling, and electronically modulated at 300 Hz.[9]

A 12-cm air - acetylene and a 5-cm nitrous oxide burner were used.

RESULTS

FLAME CONDITIONS—

A fuel-rich flame for both the air and nitrous oxide flames was found to give optimum results. The burner was set with the top of the burner 2 mm below the bottom of the entry slit for both flames.

EFFECT OF SOURCE POWER—

The calcium absorbance was almost independent of the germanium source power over the range 20 to 50 W. This indicates that the total line width of the calcium absorption line profile is much greater than that of the germanium emission line.

SPECTRAL BAND PASS—

The absorbance of a 1000 μg ml^{-1} calcium solution was almost independent of the spectral band pass over the range 0·18 to 1·2 nm. A spectral band-pass of 0·3 nm was used.

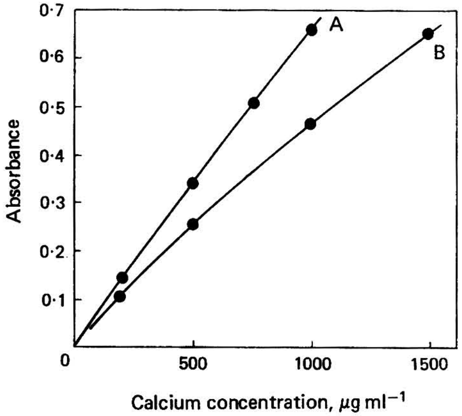

Fig. 1. Calibration graph for calcium with a germanium line source. (All solutions contained 5000 μg ml^{-1} of potassium to suppress ionisation.): A, nitrous oxide - acetylene flame; and B, air - acetylene flame: spectral band-pass 0·3 nm

CALIBRATION GRAPHS—

The 422·657 nm germanium line is a *raie ultime*[2] and is very intense; a low photomultiplier gain can, therefore, be used. This minimises the noise from the thermal emission of calcium at 422·673 nm. (After switching over to the emission mode, the thermal emission of a 1000 μg ml^{-1} calcium solution in the nitrous oxide flame at 422·673 nm was 80 per cent. as intense as the germanium source at 422·657 nm.)

The calibration graphs are shown in Fig. 1, and it can be seen that fairly good linearity was observed with concentrations of up to 1500 and 1000 μg ml^{-1} in the air and nitrous oxide flames, respectively. The sensitivities were 8·3 and 6 μg ml^{-1} in the air and nitrous oxide flames, respectively.

CONCLUSIONS

The use of the germanium 422·657 nm non-resonance line appears to be a feasible method for determining high levels of calcium directly (100 to 1500 μg ml^{-1}) and is preferred to the use of the very weak calcium line at 239·856 nm.

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Book Reviews

An Introduction to Spectroscopic Methods for the Identification of Organic Compounds. Volume 1. Nuclear Magnetic Resonance and Infrared Spectroscopy. Edited by F. Scheinmann. Oxford, New York, Toronto, Sydney and Braunschweig: Pergamon Press. 1970. Price (hard-cover) 45s., $7.00; (flexi-cover))30s., $4.75.

Both n.m.r. and infrared spectroscopy require fairly expensive equipment and special measurement and interpretational techniques, so that it is not easy, or necessarily appropriate, for universities and colleges to give each annual class a thorough training in these fields. Consequently they are happy grounds for what modern jargon calls "post-experience courses," that is, the fortnight's residential course for graduates, and also for special M.Sc. courses. This is a class book for such short courses in n.m.r. or in infrared spectroscopy; and when supplemented by the promised Volume 2, on mass, ultraviolet and e.s.r. spectroscopy and combined techniques, would be appropriate for the M.Sc. course. The book is based on a series of such courses at Salford University and the content and coverage seem reasonable for the intended readership. One might wish that Raman spectroscopy had been more thoroughly covered in a book for the seventies, as this is still a growing technique. Quantitative analysis is not considered.

The most useful parts are probably the correlation tables, problems and answers compiled by P. W. Hickmott and O. Meth-Cohn for n.m.r. and by R. K. Smalley and B. J. Wakefield for the infrared, which together occupy half the space. These are well done with sufficient, but not excessive, detail.

The remaining chapters, by authors well known in their subject areas, are an introduction to n.m.r. by J. Feeney and S. M. Walker, applications of n.m.r. by J. A. Elvidge, theory of the infrared by M. St. C. Flett and applications of the infrared by G. Eglinton. They are all fairly appropriate and satisfactory and the application of n.m.r. in particular has interesting examples. They could all have benefited from a tighter editorial hand as a few diagrams have no real scale attached (*e.g.*, Fig. 5 on p. 47), some equations require units (*e.g.*, $\nu = 4{\cdot}125\sqrt{(k/\mu)}$ on p. 114), the structure $CF_2 = CH_2$ is broken after the double bond by the end of a line (p. 25) and there is the curious note (p. 124) that "The term 'frequency' is used in this chapter . . . for the reciprocal of frequency, waves per (cm^{-1})".

To summarise, this volume contains some useful tables and problems and appropriate textual introduction, and is a book that should be available where applied n.m.r. and infrared spectroscopy are taught. But it is not essential reading for a wider audience.

D. H. Whiffen

Gas Chromatography in Biology and Medicine. A Ciba Foundation Symposium. Edited by Ruth Porter. Pp. x + 213. London: J. & A. Churchill Ltd. 1969. Price (Limp) 30s.; (Cloth) 60s.

This book is an account of a Ciba Symposium held in 1969. It is introduced by A. J. Martin, who outlined his development of the principles and practices of partition chromatography. This outstanding achievement was acknowledged by several speakers throughout the symposium.

The book presents the experiences of specialists in the design and function of gas-chromatographic equipment, and the application of the technique to respiratory and forensic medicine and pharmacology. The last section forecasts how its facility for separation and detection will be further extended with spectrometric identification and computerised automation.

The most appropriate conditions for the use of the three principal types of detector are enumerated; briefer mention is made of selective detectors. There is discussion on the potential value to anaesthetic and forensic medicine of the W-value detector, which allows the measurement of oxygen and anaesthetic vapours in a column-free system independently of other respiratory gases and of temperature, pressure and flow changes.

Conditions for attaining ideal efficiencies of gas-chromatographic columns and modifications of column components to improve selectivity are presented with relevant references. In the discussion suggestions are given for attaining maximum theoretical selectivity by preliminary treatment of the extract to be analysed and by adjusting column lengths and relative polarities of single and mixed stationary phases. The description of an apparatus designed to analyse respired air as an entire expiration or as a fractionated series of that expiration bridges the second and third sections of the book. Technical difficulties inherent in analysis of alveolar plateaux or expanding dead-space during automated anaesthesia are enumerated to emphasise the necessary compromise between the use of ingenious and simple mechanisms as presented, and more accurate and expensive analyses provided by mass spectrometry. Standard gas-chromatographic equipment involving two columns calibrated in parallel and series, and fed from a semi-automatic extraction unit, is proposed for serial determinations of blood gases. The use of alternative sampling and detecting techniques and the problems inherent in determining nitrogen tensions are indicated in the discussion. There follows an interesting account of closed or semi-closed anaesthesia automated through on-line computer analysis of partition coefficients determined by gas-chromatographic analysis of anaesthetic and respired gases. The corrected partition coefficients were accepted as indicating solubility of anaesthetic agents in body fluids and tissues, and hence anaesthetic potency.

The more general sections on pharmacology and forensic medicine weigh desirable and possible analyses against the limitations imposed by availability, size and environmental contamination of samples. Screening procedures to provide indications of the groups of compounds and separate components within the groups are outlined. As in other parts of the discussion emphasis is laid on the urgent need for commercial development of equipment more flexible and adaptable to laboratory and field requirements.

An evaluation of future developments in this field emphasises how the versatility of standard gas-chromatographic practice allows the volatile eluent, already separated, detected and, if necessary, concentrated, to be coupled to infrared, n.m.r. or mass spectrometers for identification against computerised reference scans. The discussion suggests that n.m.r., which offers the advantage of structural identification, may soon supersede mass spectrometry as the range of sizes of chromatographic samples and requirements for n.m.r. spectrometry approach a point of compatibility.

The final chapter deals with the use of computers for automatic evaluation of resolved peaks, for separation and compensation of unresolved peaks, and for the automated control of processes monitored from recordings of gas chromatograms.

The Symposium was attended by international experts in the field; throughout, the discussion was relevant and illuminating, and reveals the rapid extension of the technique into many scientific disciplines. Each reader will doubtless wish to find fuller treatment of his particular interest and one might hope for at least an entire paper on the application of gas chromatography to the problems of routine clinical chemistry, but within the restrictions imposed by its size the publication amply fulfils the policy of the Ciba Foundation to promote international co-operation in medical and chemical research.

C. H. GRAY

A FIRST COURSE IN QUANTITATIVE ANALYSIS. By RAY U. BRUMBLAY. Pp. x + 420. Reading, Massachusetts, Menlo Park, California, London and Don Mills, Ontario: Addison-Wesley Publishing Company. 1970. Price 65s.

There are 272 pages of theory in this well bound, elegant book, followed by twenty-four exercises with comprehensive instructions. The final 36 pages of directions for preparing or obtaining samples for analysis, tables of ionisation constants, activities and electrode potentials, answers (with occasional explanations) and a competent index complete an unusually arresting book of elementary quantitative analysis. Diagrams and graphs are attractively sketched, there is a step-wise development of relevant theory, abundantly illustrated by numerical exercises and calculations to be attempted, and the text shows in an unmistakable way its evolution from laboratory teaching. The careful presentation of theory and the structure of the practical chapters reflect the problems of teaching classes with large numbers of students. Of immediate interest to teachers and students in the preliminary classes of polytechnics and universities, the book will find much more than occasional use in the reference libraries of schools and technical colleges for students at about advanced level.

Even the armchair analyst will find the theoretical part of the book stimulating, although units are not quoted for equilibrium and allied constants, concentration units are not always included, formulae are used frequently as shorthand and both normalities and molarities appear. Treatment at this level is in depth. The mathematics of analysis data is examined. The principles of gravimetric and volumetric analyses are developed. Electroanalysis and photometry in their several aspects, complexation analysis and methods of separation are explained at length. A distinguishing feature of both theoretical and practical chapters lies in the numbered statements summarising the position reached, or listing first the advantages and then the disadvantages of adopting a particular procedure.

In the Practical part, calibration of weights and apparatus, and main aspects of gravimetric and volumetric analyses lead to the polarographic determination of cadmium, the application of flame spectrophotometry for sodium, potassium and calcium in water, ion-exchange separations and the Kjeldahl nitrogen analysis. Directions are clear for undertaking both the experimental and written work. Reactions, reagents and solutions to be used are listed. Possible errors to be expected are mentioned. A detailed description of procedure follows and each chapter is completed by specific instructions on the method of calculation.

Brumblay is a sound investment, and on examination may well prove to be just the book students and teachers have been looking for. It is strongly recommended. B. J. MOODY

MICROANALYSIS BY THE RING-OVEN TECHNIQUE. By DR. HERBET WEISZ. Second Edition. Pp. x + 170. Oxford, New York, Toronto, Sydney and Braunschweig: Pergamon Press. 1970. Price 60s.; $8.00.

Those unfamiliar with the ring oven may like to know that it is a simple apparatus which, by control of application and evaporation of solvent, enables drops of solution applied to a filter-paper to be concentrated into a narrow ring on the paper. The sensitivity of spot reactions is thereby increased, and by cutting the ring into segments various identification tests can be applied. Separations are achieved by using precipitating agents, and separated components may be transferred for further treatment. Various simple ancillary techniques and separation schemes have been devised for inorganic and organic compounds.

The method is at its best with very small amounts; it is rapid, and its reproducibility lends itself to semi-quantitative work.

The ring oven is a simple tool in the analyst's armoury. What it achieves can usually be done by other means, but seldom with the same combination of speed, simplicity and certainty.

Those who have used the ring oven will be interested in the new material in this edition. The qualitative and quantitative analyses of metals and anions have been considerably extended. Organic and radiochemical applications are virtually new, and combinations with other techniques are described. There is a chapter on applications to various fields, including *objets d'art*, air pollution and toxicology.

The first edition described a new technique and listed some twenty-five references to its use; with the same clarity and completeness this edition deals with a well-tried method that has attracted more than a hundred additional direct references. D. W. WILSON

PRINCIPLES AND PRACTICE OF X-RAY SPECTROMETRIC ANALYSIS. By EUGENE P. BERTIN. Pp. xxiv + 679. New York and London: Plenum Press. 1970. Price £17 10s.; $37.50; D.M. 150.

As the bulk of the basic theory and practice of X-ray spectrometry is well established, the time appears ripe for the production of a detailed standard reference work for practising X-ray analysts. This the author has attempted to achieve. Unfortunately the mass of detail tends to obscure much of the relevant information contained in the book. Too much space is devoted to material which can only be considered of historical value, whereas such important subjects as heterogeneity effects and the use of X-ray analysis for process control are examined only briefly. The addition of a chapter on the electron probe is largely superfluous, as it cannot hope to cope with a technique requiring a volume of its own. The absorption coefficient data given were published in 1960 and are of doubtful value, especially in the long wavelength region.

This book contains a great deal of useful information. However a shorter, more selective book with adequate references and up-to-date data would have been of greater value.

K. G. CARR-BRION

ANALYTICAL METHODS USED IN SUGAR REFINING. Edited by R. W. PLEWS, A.R.I.C., A.C.T. (Liv.). Pp. viii + 234. Amsterdam, London and New York: Elsevier Publishing Co. Ltd. 1970. Price 110s.

This book deals with the analysis of sugars, molasses and syrups, with the use of methods that have been the result of much research by members of I.C.U.M.S.A. and by refinery chemists.

The method of polarisation of raw sugar is described in detail and is a method of I.C.U.M.S.A. that is accepted internationally.

A chapter of general interest is the Determination of Inorganic Non-sugars and Trace Elements, which again gives methods that are the result of much co-operative research among chemists. The methods are described in detail and the analyst should obtain accurate results if the procedures are carefully followed.

Another chapter is devoted to Microbiological Examination of Sugars, which is of extreme importance, and again the tests are well set out.

This book is of great value to the sugar chemist and is, indeed, a publication that I am sure will be of much interest throughout the food industry. J. H. DEFRATES

TRAVAUX PRATIQUES DE CHIMIE MINERALE PRECIS D'ANALYSE QUALITATIVE. By G. DOUBLE, G. FERRONI and Mlle. A. PETROCCHI. Pp. x + 213. Separate analysis scheme, pp. 217–284. Paris: Dunrod. 1970. Price Fr. 34.

This manual of inorganic qualitative analysis will be of interest to all teachers and students concerned with qualitative analysis in schools, technical colleges and universities. The analysis schemes are new but the systematic presentation of the properties and reactions of the cations and anions of a wide range of elements is particularly striking and likely to be of considerable value. Acknowledging the work of West and Vick, and Belcher and Weisz, as their starting-point, the authors, who teach at the University of Marseilles, have used oxidation - reduction processes, organic reagents and the formation of complexes as features of the analytical operations. Sulphide separation has been avoided. The use of small volumes (*ca.* 2 ml) and weak concentrations (1 to 2 per cent.) reduces costs, especially in the analysis of less common elements. The presentation is systematic, and a chemist with only a modest acquaintanceship with the language will find this a stimulating book.

Rules of nomenclature (I.U.P.A.C., 1957) and a summary of relevant elementary theory occupy the first 40 pages. The latter comprises solvent theory, partition law, aspects of ionic equilibria, the constitution, formation, stability and use of complexes, oxidation and reduction, and colloids, suspensions and emulsions. The remainder of the book presents a systematic study of the relevant properties and reactions of the ions of metals and non-metals. Cyanides are described separately and profusely. Under each heading, sections are devoted to insoluble compounds (with solubility products expressed as pK values) and complexes, followed by the principal separation and characterisation reactions. Each introductory section lists oxidation states and unusual properties. There is finally a space for notes. When there are several important oxidation states, the chapters are sub-divided accordingly.

The analysis schemes are presented in a separate booklet, the first, roughly at Advanced level standard, used for the Diploma of University Studies in Science (Second Year University) and for Higher grade Technician Qualifications, and the second, for the fourth year examinations at university. The schemes describe the systematic separation and characterisation of anions and cations. Metals are separated by successive addition of hydrochloric acid, saturated ammonium chloride solution containing ammonia (pH = 9), ammonium fluoride, and sodium hydroxide, leaving zinc in solution. The more advanced scheme includes tungsten, thorium, cerium, lanthanum, zirconium, beryllium, gallium, uranium, vanadium, titanium, gold and platinum. Finally there are tables giving information about reagents and how to analyse a solid.

The schemes have been used by thousands of students at Marseilles and by graduate teachers from that University in other institutions. Whether snags appear, and there must be some, can only be discovered by extended use. Whatever the final verdict, there is no doubt that this practical text can be strongly recommended for reference purposes. B. J. MOODY

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LECTURES AND COURSES

University of Salford Department of Chemistry and Applied Chemistry

A symposium on "Inorganic Aspects of Chromatography" is to be held at the University of Salford on 29 and 30 March 1971.

Plenary lectures are to be given by

Dr C S G Phillips	(University of Oxford)
Dr G Nickless	(University of Bristol)
Dr R Paterson	(University of Glasgow)
Dr R Stock	(Nottingham Regional College of Technology)
and Mr A Iveson	(UKAEA Capenhurst)

Short original papers are invited for presentation at the above meeting. Titles and short abstracts of proposed contributions should be forwarded before 15 January 1971 to the Symposium Secretaries, Mr W E Sharples and Mr J A Noble, Department of Chemistry and Applied Chemistry, University of Salford, Salford M5 4WT, Lancashire.

Other information can be obtained from the Administrative Assistant, (Short Courses), Room 111, The University of Salford, Salford M5 4WT. Please quote reference E/4.

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Symposia of the Faraday Society No. 3

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International Union of Pure and Applied Chemistry in conjunction with The Society of Chemical Industry

Proceedings of the International Symposium on Surface Area Determination held in Bristol 1969. The proposal to hold the Symposium whose proceedings are reported in this volume was made by the IUPAC Commission on Colloid and Surface Chemistry at its meeting in Prague in August 1967. The objective was to take a first step towards reaching international agreement on methods of determining the surface area of solids of different kinds, acceptance of which would make feasible the subsequent goal of the establishment of a set of Standards of Surface Area Determination.

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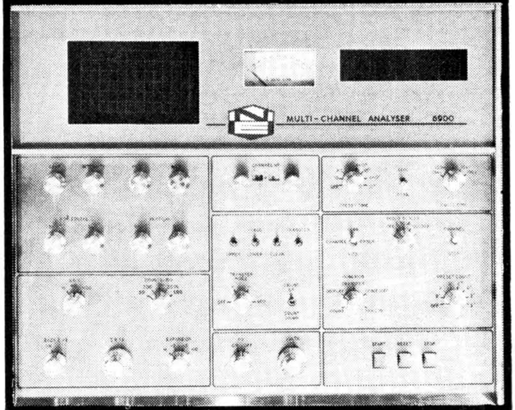
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A Simple Diffusion Method for the Determination of Fluoride

A method is described for the determination of sub-microgram amounts of inorganic fluoride. Diffusion on to a thin layer of solid alkali is used to separate fluoride from any contaminants. The diffused fluoride may be determined with an ion-selective fluoride electrode or by spectrophotometric methods.

Phosphate and sulphate do not interfere. The interference of halides, carbonate, nitrate and acetate is discussed. Organic material was found not to interfere with the recovery of fluoride except in determinations involving human serum. High-temperature ashing of organic samples was found to lead to loss of fluoride.

When used with the ion-selective electrode the method is applicable over the range 0·05 to 200 μg of fluoride.

J. L. STUART

Department of Preventive Dentistry, Turner Dental School, The University of Manchester, Manchester, M15 6FH.

Analyst, 1970, **95**, 1032–1038.

An Improved Synthesis of Alizarin Fluorine Blue

The ideas of Hellmann and Opitz concerning optimum condition for aminomethylation reactions are applied to the synthesis of alizarin fluorine blue. The yield is increased from 13 to 61 per cent. by using an aqueous ethanolic solvent of minimum alkalinity.

K. AL-ANI and M. A. LEONARD

Department of Analytical Chemistry, Queen's University, Belfast, BT9 5AG.

Analyst, 1970, **95**, 1039–1040.

Mixed-solvent Systems for the Flame Analysis of Petroleum Materials

Flame methods (emission, fluorescence and atomic-absorption spectroscopy) are particularly valuable in the petroleum industry because they permit petroleum products in organic solvent systems to be analysed directly although the benefits of these methods for oil analysis have been partly offset by various difficulties. In this paper we indicate how three of these difficulties can be avoided by selecting appropriate solvent mixtures.

S. T. HOLDING and J. W. NOAR

Shell Research Ltd., Thornton Research Centre, P.O. Box 1, Chester, CH1 3SH.

Analyst, 1970, **95**, 1041–1042.

A Method for Decreasing the Atomic-absorption Sensitivity of Calcium by Using the Germanium 422·657 nm Line

A method for decreasing the atomic-absorption sensitivity of calcium by using the overlap of the germanium 422·657 nm non-resonance line profile with the calcium 422·673 nm resonance line profile is described.

Almost linear calibration graphs were obtained with concentrations of up to 1500 $\mu g\ ml^{-1}$ in the air - acetylene flame (sensitivity 8·3 $\mu g\ ml^{-1}$), and up to 1000 $\mu g\ ml^{-1}$ in the nitrous oxide - acetylene flame (sensitivity 6·0 $\mu g\ ml^{-1}$).

K. C. THOMPSON

Southern Analytical Ltd., Frimley Road, Camberley, Surrey.

Analyst, 1970, **95**, 1043–1044.


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The Determination of Dimetridazole in Animal Feeds.
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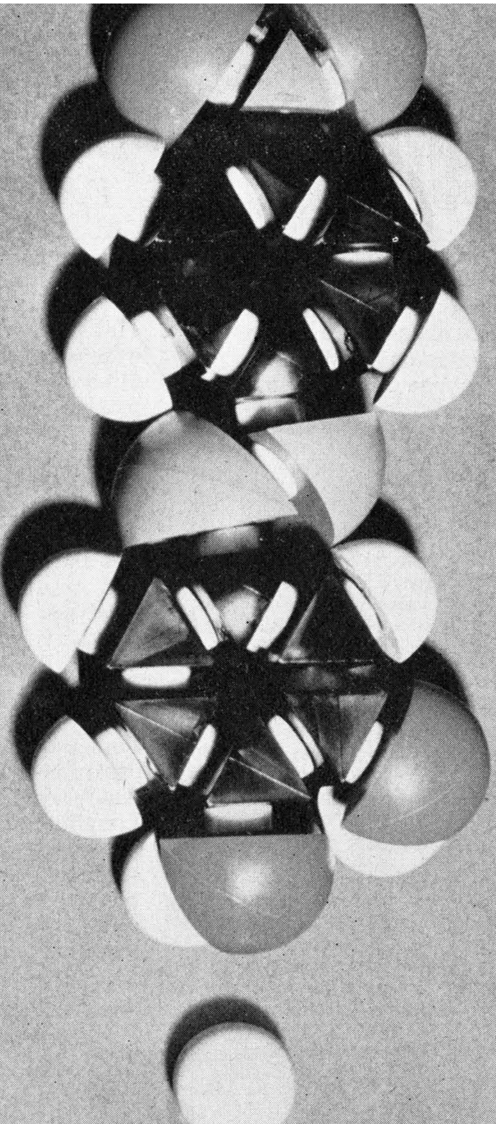
The Determination of Amprolium, Sulphaquinoxaline and Ethopabate when Present together in Animal Feeds (*Gratis*).



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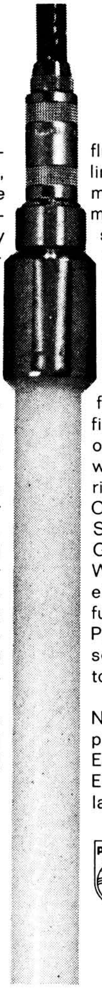

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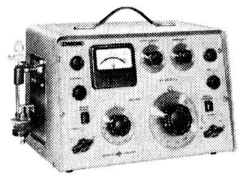

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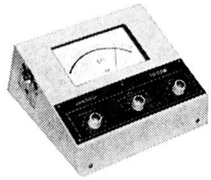

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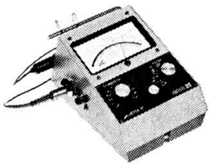

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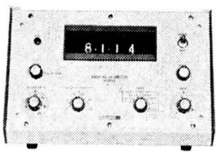

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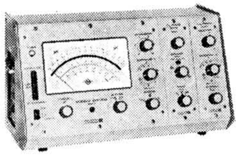

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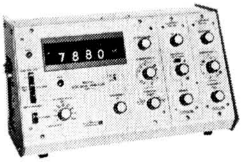

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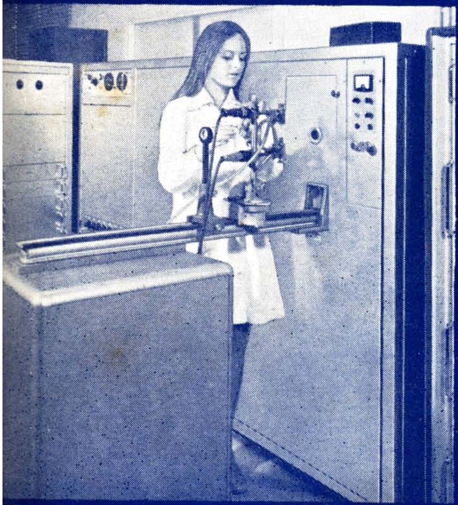

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Volume 95 No. 1137, Pages **977-1048** December, 1970

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ANALYTICAL CHEMISTRY

VOL. 95
1970

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ERRATA:

VOL. **92**, 1967:

p. 471, under ""CALCULATION". *For*

$$\frac{0{\cdot}008\,(A_x - A_b) \times W}{(A_s - A_b)} \quad \textit{read} \quad \frac{0{\cdot}008\,(A_x - A_b)}{(A_s - A_b) \times W}$$

VOL. **94**, 1969:

p. 968, main text, 13th line. *For* "is twice the standard deviation" *read* "is $\sqrt{2}$ times the standard deviation."
p. 971, 9th line from the bottom of the page. *For* "for amounts of calcium not in excess of 6 μg ml^{-1}" *read* "for amounts of calcium not in excess of 5 μg ml^{-1}."
p. 1136, Fig. 1, labelling at right of the flow diagram. *For* "0·6ml/min EDTA solution" *read* "0·6 ml/min sample"; *for* "1·2 ml/min sample" *read* "1·2 ml/min EDTA solution"; *for* "0·6 ml/min Water *read* "0·6 ml/min Air."

VOL. **95**, 1970:

p. 160, 2nd line. *For* "the range 0·5 to 100 per cent." *read* "the range 0·5 to 150 per cent."
p. 265, 3rd line and equation (4th line). These should read—
"Kimura and Miller were the first to use the well known reaction
$$Hg^{2+} + Sn^{2+} \rightarrow Hg^{0} + Sn^{4+}."$$
p. 266, 7th line. *For* "0·66 mm" *read* "0·06 mm."
p. 270, legend to Fig. 4, 4th line. *For* "$\phi\bar{x}=$" *read* "$\bar{x}=$."
p. 271, 19th line. *For* "F. Kartus" *read* "E. Kartus."
p. 315, 6th line of text. *For* "Dilute 30 and 200 ml" *read* "Dilute 30 ml to 200 ml."
p. 360, Table II. Under "*Seed dressing No.* 1," "*Seed Dressing No.* 3," "*Wheat bulb fly dressing No.* 1" and "*Wheat bulb fly dressing No.* 2," *for* "mineral filters" *read* "mineral fillers."