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ERRATA:

VOL. **89**, 1964:

p. 378, line 42. *For* "OINTMENT OF CAPSAICIN B.P.C." *read* "OINTMENT OF CAPSICUM B.P.C."

p. 382, line 12. *For* "Capsaicin B.P.C." *read* "Capsicum B.P.C."

p. 382, 1st sample in Table VII. *For* "Capsaicin B.P.C." *read* "Capsicum B.P.C."

p. 382, 6th line under CAPSICUM B.P.C. *For* "100-ml" *read* "10·0-ml."

p. 382, 9th line under CAPSICUM B.P.C. *For* "100-ml" *read* "10·0-ml."

p. 383, line 22. *For* "OINTMENT OF CAPSAICIN B.P.C." *read* "OINTMENT OF CAPSICUM B.P.C."

VOL. **91**, 1966:

p. 42. *For second author* "G. Catanzaro" *read* "E. W. Catanzaro."

p. 202, 4th and 5th line under *Determination of the half-life of the isolated radio-elements. For* "36·88 hours" *read* "35·88 hours," *and for* "36·87 hours" *read* "35·87 hours."

p. 349, 1st reference. *For* "Analyst, 1965, **88**, 280" *read* "Analyst, 1965, **90**, 199."

p. 790, caption to figure. *For* "Fig. 1. Chromatogram of a Scotch all-malt whisky on polyethylene glycol 200" *read* "Fig. 1. Chromatogram of a cognac brandy on diethyl tartrate."

p. 792, caption to figure. *For* "Fig. 2. Chromatogram of a cognac brandy on diethyl tartrate" *read* "Fig. 2. Chromatogram of a Scotch all-malt whisky on polyethylene glycol 200."

p. 794, reference 2. *For* "1965, **26**" *read* "1964, **27**."

VOL. **92**, 1967:

p. 65, correction to p. 30 of "Official, Standardised and Recommended Methods of Analysis." *For* "*Dilute standard copper solution*," *read* "*Strong standard copper solution*."

p. 108, 7th line. *For* "*Potassium permanganate*, N" *read* "*Potassium permanganate*, 0·1 N."

p. 141, 7th line from the bottom. *For* "hexane ozonide" *read* "hexene ozonide."

p. 187, 12th line. *For* "solution,[3]" *read* "solution,[4]".

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

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

p. 194, 3rd line. *For* "*Kieselguhr GF*254" *read* "*Kieselgel GF*254."

p. 194, 22nd line. *For* "Kieselguhr GF254" *read* "Kieselgel GF254."

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

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

p. 688, 3rd line above Table I. *For* "1·003 for iron(III)" *read* "0·997 for iron(III)".

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THE ANALYST

Activation Analysis

A Review*

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and T. B. PIERCE
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Summary of contents

Activation analysis is one of the most rapidly developing analytical techniques at the present time. It is difficult to obtain a comprehensive and accurate picture of the state of the art because the papers are widely scattered throughout the literature of many different scientific disciplines. In this review an attempt has been made to indicate the diverse fields from which developments in technique have come; to evaluate their significance for the analyst and show how they have been used in practice and, for some, their potential in the near future.

In order that this review will be of use to the newcomer to this subject, as well as the specialist, a section briefly outlining the theoretical basis for the subject is included.

The paper has concentrated on work published in the last 3 years, and earlier material is included only if especially significant. Rather than merely list all the papers on a topic, a few key references have been selected, and, wherever possible, they are those that have improved the technique or evaluated carefully the effect of interferences, precision and accuracy, or compared activation analysis with other methods.

It is felt that the reviewers have a duty to comment on the potential of new developments and, further, if first-hand knowledge is available to recommend a particular approach to a problem. This is necessarily subjective, but it is hoped it will guide the less experienced workers in this field.

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

NUCLEAR INTERACTIONS

Simple nuclear interactions used as a basis for activation analysis may be represented by the following equations—

$$\mathrm{A} + \mathrm{a} \longrightarrow \mathrm{A} + \mathrm{a} \qquad \ldots \quad \ldots \quad \ldots \quad \ldots \tag{1}$$

$$\longrightarrow \mathrm{A}^* + \mathrm{a} \qquad \ldots \quad \ldots \quad \ldots \quad \ldots \tag{2}$$

$$\longrightarrow \mathrm{B} + \mathrm{b} \qquad \ldots \quad \ldots \quad \ldots \quad \ldots \tag{3}$$

where A and B are the target or residual nuclei, and a and b are the bombarding or emitted particles or photons. Processes (1) and (2) are both known as scattering. In reaction (1) the kinetic energy of reactants and products is the same, whereas in reaction (2) the total kinetic energy of the products is less than that of the reactants, the difference being used to raise the nucleus A to an excited state. Process (1) is known as elastic scattering, process (2) as inelastic scattering. Reaction (3) represents the transmutation of one element to another and one or more particles or γ-photons being emitted. The residual nucleus B may be formed in ground or excited states, the latter usually decaying very rapidly to the ground state by the emission of γ-radiation, and the ground state of nucleus B itself may be unstable and may decay to the ground or excited states of yet another nucleus, for example, by emission of α or β-radiation.

A further form of interaction that may occur between a charged particle and a target nucleus, coulomb excitation, has not hitherto been applied to activation analysis to any significant extent and will not be considered further here.

An arbitrary distinction is sometimes drawn between prompt and delayed radiation. Prompt decay refers to processes that occur rapidly after nuclear interaction, while delayed radiation is measurable over a much longer period of time so that the sample can usually be removed from the place of irradiation before counting.

The Q-value or energy balance of a reaction as calculated from the masses of reactants and products shows whether the process is endo-ergic or exo-ergic. Theoretically, an exo-ergic reaction can occur with a zero energy particle assuming no coulomb barrier restriction, while for endo-ergic reactions with an energy balance of —Q, an amount of energy equal to Q must be supplied by the kinetic energy of the incoming particle for the reaction to occur. As reaction between a stationary target nucleus and a bombarding particle will impart momentum to the compound nucleus, the reaction threshold will be greater than the Q value.

The rate of a nuclear reaction is given by

$$R_{\mathrm{R}} = f\sigma n$$

where f is the particle flux;
σ is the reaction cross section; and
n is the number of target atoms.

A counter observing prompt radiation emitted from a target during irradiation will yield a count-rate, C_{P}, which is proportional to the rate of reaction, *i.e.*,

$$C_{\mathrm{P}} = K_1 R_{\mathrm{R}} = K_1 f\sigma n \qquad \ldots \quad \ldots \quad \ldots \quad \ldots \tag{4}$$

where K_1 is a constant that depends upon the efficiency and geometry of the detector and the proportion of the decay emitting the radiation to be determined.

When the product is a radioactive nuclide, this will have a rate of decay $R_{\mathrm{D}} = \lambda N$, where N and λ are the number of atoms and the decay constant of the nuclide, respectively. The net rate of growth of a radioactive nuclide will be—

$$\frac{dN}{dt} = R_{\mathrm{R}} - R_{\mathrm{D}} = f\sigma n - \lambda N,$$

and thus the disintegration rate $D(t)$ after irradiation for time t will be given by—

$$D(t) = f\sigma n\,(1 - e^{-\lambda t}) \qquad \ldots \quad \ldots \quad \ldots \quad \ldots \tag{5}$$

As the half-life, $T_{\frac{1}{2}}$, of a radionuclide and the decay constant are related by the expression $\lambda = 0{\cdot}693/T_{\frac{1}{2}}$, equation (5) becomes—

$$D(t) = f\sigma n\,(1 - \mathrm{e}^{-0{\cdot}693t/T_{\frac{1}{2}}}) \qquad \ldots \quad \ldots \quad \ldots \quad \ldots \tag{6}$$

Thus for a sequence of nuclear reactions in which a compound nucleus is de-excited by prompt decay to form a radionuclide, the disintegration rates yielding prompt and delayed radiation will only be equal at saturation. After the completion of the irradiation, the decay rate of the radionuclide will then decay exponentially with time.

Prompt counts can be accumulated indefinitely by extending the duration of the irradiation, (*i.e.*, from equation (4) the total counts registered in time t, C_t, will be $C_t = K_1 f\sigma n t$) but in practice a useful lower limit will be imposed by counts from other sources that interfere with the determination. Radionuclides of a sufficiently long half-life can be removed from the place of irradiation and chemically separated from other active elements before counting.

IRRADIATION FACILITIES

NUCLEAR REACTORS—

The nuclear reactor is the most prolific source of neutrons for activation analysis, although only a limited number of reactors have been designed specifically for this purpose. The neutron flux available for the irradiation of samples ranges from 10^{11} to 10^{14} neutrons per second per cm^2, with most research reactors having a maximum flux of about 10^{12} to 10^{13}. The method of loading samples varies according to the installation, but generally two methods are required: (*a*), a pneumatic tube for rapidly transferring samples from the laboratory to the reactor core for periods of seconds to about an hour; and (*b*), a facility for long irradiations of up to about a week, which can operate more slowly and must be made of radiation-resistant materials.

The reactor is a source of neutrons of widely varying energy from thermal neutrons of about 0·02 eV up to fast neutrons of greater than 10 MeV. Usually, no special effort is made to select the energy of neutrons for irradiation of samples; it is, however, essential to have a knowledge of the energy spectrum because of possible interferences discussed below. Furthermore, there is often a rapid change in flux over short distances in the irradiation position, and significant errors may result if this is ignored. It is essential that the methods of transporting samples are reproducible, and often there is a variation in flux throughout the sample carrier requiring carefully packed samples.

Activation analysis is not usually an absolute method, although Girardi, Guzzi and Pauly[1] have shown that the nuclear constants involved are usually sufficiently well known to allow many elements to be determined absolutely with an accuracy of better than 10 per cent. The more usual method is to irradiate the sample simultaneously with known amounts of a pure material. If the sample and standard are then counted under similar conditions the unknown quantity of the element in the sample is readily calculated. Care must be taken in the selection of materials for standards; it is not unusual to find pure chemicals containing traces of elements of high neutron cross section that can seriously affect the validity of the standard.

If a great many samples are to be analysed for many elements then the simultaneous irradiation of standards each time would be very time consuming. The comparator method requires that only one standard is irradiated each time to monitor the neutron flux, and the activity of the samples is compared with standards separately irradiated at a known neutron flux. A single flux monitor cannot, of course, detect changes in neutron spectrum, and it is essential that this remains reasonably constant over long periods of time. A study of the errors that can occur with this method, particularly in reactors with a high fast-neutron flux, has been made by Girardi, Guzzi and Pauly.[2]

There are many compilations of sensitivity for thermal neutrons; possibly the most comprehensive and also indicating all of the interfering reactions is the handbook by Koch.[3]

The presence of fast neutrons in a reactor neutron spectrum demands that careful consideration be given to interfering reactions; it is possible to produce nuclides with atomic number 1 or 2 less than the matrix elements by (n,p) and (n,α) reactions. Durham, Navalkar and Ricci[4] discuss these interferences and also indicate certain examples in which fast neutrons can be utilised to advantage. Borg, Segal, Kienle and Campbell[5] showed that by surrounding samples with boron and cadmium it was possible to improve the detection limit of manganese in tissue by a factor of 7 because of the greater suppression of sodium-24 activity compared with manganese-56. Yule[6] experimentally determined the sensitivity of 28 elements for reactor fast neutrons. He showed that for 5 elements the sensitivity is greater than for thermal neutrons and is only slightly less for 15 others, and hence may be superior in some matrices.

In the TRIGA reactor it is possible to obtain, safely and reproducibly, neutron pulses of about 30-millisecond duration with peak fluxes of 5×10^{16} neutrons per second per cm^2, as well as a continuous flux of 10^{13} neutrons per second per cm^2. Lukens[7] showed that there is an increase in sensitivity for elements with half-lives of less than 50 seconds by pulsed operation of the reactor.

The interaction of the nucleus with a bombarding particle is independent of the matrix, but in practice the matrix can exert an influence by perturbing the neutron flux seen by different parts of the sample. This self-shielding effect has been studied by many workers; most of this work has been summarised by Hogdahl,[8] who has tested the accuracy of the calculations by experiment and has also suggested methods by which the effect can be calculated.

If the cadmium ratio for any nuclide is greater than 50, the effect of absorption of epithermal and fast neutrons can be ignored. Kamenoto's[9] simple equation, $\sigma W/A \simeq 0{\cdot}03$, for a 10 per cent. correction owing to absorption, where σ is the cross section in barns, W the weight of sample in grams and A the atomic weight of the nuclide, can be used to test quickly the need to consider self-shielding effects in more detail.

With the availability of very high flux reactors for activation, other second-order reactions can cause significant interferences. Ricci and Dyer[10] have found 42 instances in which interferences can occur, and in 23 of these they were able to compute the interference from currently available nuclear data.

So many elements in a wide variety of materials have been determined by thermal neutron activation it would be foolish to quote just a few examples. The bibliographies of Bock-Werthman,[11] published at regular intervals by the A.E.D. Information Service, have element and matrix indexes and provide an excellent reference source for published papers of this type.

ACCELERATOR NEUTRON SOURCES—

So that the advantages of activation analysis can be applied to laboratories without access to a reactor and to extend further the range of the technique, alternative sources of neutrons have been studied. In many nuclear reactions a neutron is the product of bombardment, but for practical purposes only, three reactions provide a useful neutron flux with reasonably priced accelerators.

(*i*) $^{3}H + {}^{2}H \rightarrow {}^{4}He + n + 17{\cdot}6$ MeV.

(*ii*) $^{2}H + {}^{2}H \rightarrow {}^{3}He + n + 3{\cdot}3$ MeV.

(*iii*) $^{9}Be + {}^{2}H \rightarrow {}^{10}B + n + 4{\cdot}4$ MeV.

Reaction (*i*), usually referred to as the DT reaction, is the most prolific source of neutrons for accelerators capable of generating voltages of less than 500 kV. The neutron energy is approximately 14 MeV, and is sufficient to produce (n, 2n), (n,p) and (n,α) reaction products with almost all elements. Reaction (*ii*), the DD reaction, has only found limited application in activation analysis. The neutron yield is much lower than for the DT reaction and the neutron energy of 2·5 MeV is generally insufficient to produce (n, 2n) reactions and only a limited number of elements can react to produce (n,p) and (n,α) products. The reaction has been used in preference to the DT reaction if the latter formed the same product from two elements and only one reaction was energetically possible with DD neutrons. Reaction (*iii*) produces a broad energy spectrum of neutrons up to about 6 MeV, and is the most prolific neutron source for accelerators rated at 1 MeV or above.

All types of accelerators can be used as sources of thermal neutrons by surrounding the target area with a water - paraffin moderator. It is not possible, however, to operate in a region of high thermal flux that is free from fast neutrons, and interference from fast neutron reactions must be carefully considered.

A recent survey[12] conveniently tabulates most of the data concerning accelerators commercially available for neutron production. Most laboratories have found that 150 to 200-keV accelerators that use the DT reaction for neutron production are the most useful at reasonable cost (£6000 to £8000). The small sealed-tube accelerators are particularly well suited to routine use in a factory for monitoring a specific product.

The shielding of a neutron generator is often an expensive item, particularly if a reasonably large space is enclosed; several installations are briefly described by Strain.[13] Neutron tubes have been installed in a small hole in the ground and then covered with polythene chips. This provides a shield that only costs about £500 and includes a fluidiser to allow the tube to be easily inserted and withdrawn. However, there must be a higher thermal neutron flux around the sample which may cause interference in certain determinations.

The inability to manufacture tritium targets which will maintain a high yield of neutrons over a long period has slightly limited the use of the neutron generator. In some earlier accelerators the target life was controlled by the loss of tritium by over-heating and the deposition of films of carbon on the target surface. By suitable design these problems can be overcome, but the loss of tritium by exchange with deuterium from the beam and subsequent diffusion of tritium out of the target is unavoidable. In most accelerators the neutron output from a titanium tritide target decreases by a factor of 2 after 60 to 100 minutes at about 800-μA beam current.[13] Erbium tritide targets, although possessing greater thermal stability, have a shorter life.

To avoid the necessity of replacing used targets, regeneration is sometimes used.[14] The deuteron beam is temporarily switched to a tritium beam that replaces the lost tritium from the target. Some sealed tubes[15] accelerate a mixture of deuterium and tritium, and a constant neutron output of 10^{10} neutron per second can be maintained for about 100 hours.

Most manufacturers of accelerators supply a transfer system to allow the sample to be moved rapidly from the neutron target to the counter. For reliable analysis it is essential that the system is capable of accurately locating the sample in a position of high flux each time. Because of the limited region of high neutron flux many workers do not simultaneously irradiate sample and standard, which is the common practice with reactor irradiations. Each is irradiated separately, and the change in neutron flux is monitored and normalised by measuring the flux with boron trifluoride counters, fission chambers, cooling water activity or a plastic scintillator. Iddings[16] has compared most of these methods and shown that the comparator method, involving the simultaneous irradiation of sample and standard, is the most accurate, but that it must inevitably lead to a decrease in sensitivity of the method.

The difficulty of determining oxygen by other methods and the high specificity of fast-neutron activation for oxygen under certain conditions has prompted many groups to study this determination extensively. Coleman[17] has shown that, by correcting for γ-absorption by the sample, it is possible to use one primary standard for oxygen determination in a variety of materials, which does not rely on samples analysed by other techniques. Satisfactory agreement with the vacuum fusion method for a variety of metals was achieved. Anders and Briden[18] improved the accuracy by rotating samples while irradiating and counting, and showed the need for neutron-absorption corrections if a large sample is irradiated. Mott[19] further reduced the experimental error (1 standard deviation) to about 0·4 per cent. The apparent increase in oxygen content arising from nitrogen-16 recoils from the air can be avoided by using nitrogen in the pneumatic transfer system,[20] or by rapid etching of the sample after irradiation and before counting.

Fast-neutron activation is finding application in other analytical problems, particularly for light elements. Nitrogen has been measured in rubber by Walker and Eggebraaten,[21] but in many materials copper-62 produced by the reaction, ^{63}Cu(n,2n)^{62}Cu, is a serious interference having the same half-life and decay mode as nitrogen-13. Blackburn[22] has determined fluorine in pure organo fluorine compounds with errors of about 1 per cent. by using the ^{19}F(n,2n)^{18}F reaction. The rapid and sensitive reaction ^{19}F(n,α)^{16}N is seriously interfered with by the formation of nitrogen-16 from oxygen if the sample is irradiated with 14-MeV neutrons. Anders[23] used neutrons from the deuteron bombardment of beryllium, and Steele[24] used neutrons from the DD reaction; in both instances neutrons are sufficiently energetic for the fluorine reaction but are below the threshold level for oxygen. The ^{28}Si(n,p)^{28}Al reaction permits the trace determination of silicon in many matrices, the only likely interference being phosphorus. Vogt[25] has used this reaction for the precise determination of silicon in small meteorites. Gorski[26] has determined the copper content of ores at the rate of several hundred per day.

Tables of calculated values for the sensitivity of fast-neutron activation have been published by Gillespie and Hill[27] and also by Coleman.[28] In practice it is found that the limits of detection are usually about 100 times larger than calculated, particularly if the active species is measured by γ-spectroscopy.

ISOTOPIC NEUTRON SOURCES—

Isotopic neutron sources have not been used extensively in activation analysis because of their low neutron flux, but nevertheless there are applications in which this is not a serious disadvantage. Many combinations of α-emitter and γ-emitter with light elements produce satisfactory neutron sources. In Table I, some typical sources supplied by the Radiochemical Centre, Amersham, are listed.

TABLE I

ISOTOPIC NEUTRON SOURCES

Source	Output, neutron per second per C	Maximum activity available, C	Cost for maximum activity
^{124}Sb - Be	$5{\cdot}2 \times 10^6$	10	£110
^{241}Am - Be	$2{\cdot}5 \times 10^6$	5	£400
^{210}Po - Be	$2{\cdot}5 \times 10^6$	50	£80
^{228}Th - Be	2×10^7	5	~£800

A new type of neutron source has been developed by Amiel[29] in which an α-emitter, usually americium-241 or thorium-228, is surrounded by oxygen-18 in a gaseous form such as carbon dioxide. As the gas can be readily separated from the α-source, the neutrons can be turned on and off, greatly simplifying shielding problems during transportation and storage of the source. Californium-252, which emits 10^9 neutrons per second per mg, will shortly be produced at the rate of 1 g per year in the high flux reactor at Oak Ridge. It should then be possible to obtain very small sources with a high neutron output.

De and Meinke[30] have made a general evaluation of 1 to 5-C antimony - beryllium sources for activation analysis. Isotopic sources can be useful in the rapid assay of samples in which conventional methods are rather tedious; for example, Grunewald[31] has determined iodine in organic compounds, and Bakes and Jeffery[32] have determined fluorine in fluorite ores; for both, the accuracy of the measurement was within 1 per cent.

The absorption of neutrons from isotopic sources by elements of high neutron cross section has been utilised by Strain and Lyon[33] for analysing flowing streams and batch samples containing cadmium and boron.

CHARGED PARTICLES—

The activation applications of charged-particle techniques have been limited by the relatively short distances that charged particles can penetrate into matter and the high heat dissipation that can occur if large beam currents are used. Nevertheless, a variety of charged particle methods have been developed for light elements that cannot be conveniently determined after irradiation by reactor neutrons; as few radioisotopes suitable for activation analysis can be produced from these elements, reaction products are limited to a few radionuclides, for example, carbon-11, nitrogen-13 and fluorine-18.

The coulomb barrier will restrict the reaction of low energy charged particles to elements of low atomic number, thus simplifying methods for the determination of light elements, but as particle energies are increased and the thresholds of additional reactions are crossed, the problem of nuclear interferences becomes more acute. Also, the matrix effect caused by variations in stopping power of different targets complicates standardisation. The preparation of synthetic standards for geochemical use has been found to be possible by Sippel and Glover,[34] and Ricci and Hahn[35] have advocated the use of an average cross section when calculating charged particle results.

Particle energies of 1 MeV or less can be conveniently obtained with a Cockcroft - Walton voltage multiplier or by other types of high voltage rectifiers. These machines are relatively cheap and can yield high ion currents.

Van de Graaff generators provide higher accelerating voltages (approximately 6 MeV for a single-stage machine) and for both Van de Graaff and Cockcroft - Walton accelerators, targets can be conveniently placed at the end of the flight-tube, with or without a thin window to isolate the irradiation chamber from the rest of the evacuated volume of the machine.

Higher particle energies can be attained with multiple-stage Van de Graaff generators, cyclotrons or linear accelerators.

Targets irradiated inside a cyclotron usually intercept the beam only at an edge, and therefore the use of an external beam is to be preferred when lower beam currents are acceptable.

Relatively few activation procedures have been published which are based on charged particle irradiation, and there has therefore been little incentive to develop an accelerator for activation analysis other than for the production of neutrons. However, recent interest in helium-3 has prompted Markowitz and Mahony[36] to propose a 9·2-inch cyclotron specifically for helium-3 irradiation at 8 MeV. A 30-inch general-purpose machine has been designed which is suitable for activation analysis; the cost[37] is likely to be less than $200,000.

Isotopic particle sources have found little application to activation analysis because of their low intensity and limited flexibility, but their small size is an advantage when space and weight is restricted, and curium-242 has been evaluated as an α-source for analysing lunar surfaces.[38]

The low depth of penetration of charged particles into matter, which may be accompanied by high heat dissipation, limits not only the type and form of sample that can be irradiated, but also the method of containment. Silicon samples, 3 × 3 × 40 mm, have been encapsulated in tantalum foil[39] for cyclotron irradiation, platinum[40] has been used to contain targets to be irradiated with helium-3 ions, and hydraulically pressed discs[41] without any container have been held in the accelerator vacuum for the measurement of prompt radiation.

More recent activation work with charged particles has been concerned with the use of helium-3 as an irradiating particle, as the helium-3 nucleus, which has a low binding energy, reacts exo-ergically with many elements; in particular, sensitive methods of analysis are available for a number of light elements.[36]

Oxygen has been determined in a number of matrices by the reaction, $^{16}O(^{3}He,p)^{18}F$ and $^{16}O(^{3}He,n)^{18}Ne \xrightarrow{\beta^-} {}^{18}F$, and the sensitivity has been estimated at a few parts per 10^9 by Mahoney.[42] However, the low depth of penetration of helium-3 ions necessitates the very careful removal of surface contamination. The reaction $^{12}C(^{3}He,\alpha)^{11}C$ can be used as a basis for the determination of carbon, and the sensitivity of the method has been reported[43] as being better than 1 part per 10^9. Analysis of the decay curves permits carbon and oxygen to be determined simultaneously. By using auto-radiography after irradiation of samples with helium-3 ions, Holm[44] studied the location of concentrations of oxygen and carbon. Beam inhomogeneities and surface contamination complicated the technique, but concentrations at grain boundaries were visible.

Carbon and oxygen have also been determined after irradiation with α-particles by Engelmann.[45] The product, fluorine-18, of the reaction $^{16}O + \alpha$ has a sufficiently long half-life to permit easy chemical separation but interference is experienced from the reaction $^{19}F(\alpha,\alpha n)^{18}F$. Carbon determinations have been based on the reaction $^{12}C(\alpha,\alpha n)^{11}C$, and by using a 10 μA per cm² beam of 44-MeV α-particles, the estimated sensitivities for carbon and oxygen are 0·01 and 0·001 p.p.m., respectively. Oxygen has been determined in high purity silicon by α-particle activation.[46]

The reaction $^{11}B(p,n)^{11}C$, induced by 20-MeV protons has been used by Gill[39] to detect as little as 0·003 p.p.m. of boron in silicon. Measurement of phosphorus-30 produced by the reaction $^{30}Si(p,n)^{30}P$ was used to standardise the reaction. There are several reactions that interfere with this determination[47]; even at low proton energies the reaction $^{14}N(p,\alpha)^{11}C$ occurs. The reaction between nitrogen and protons, *i.e.*, $^{14}N(p,\alpha)^{11}C$, has been used for the determination of nitrogen[48] in graphite at the 0·01 p.p.m. level.

Boron can also be determined by deuteron irradiation with the aid of the reactions $^{10}B(d,n)^{11}C$ and $^{11}B(d,2n)^{11}C$, but once again nitrogen interferes, this time by the reaction $^{14}N(d,\alpha n)^{11}C$.[49]

Charged particles emitted as a result of reactor neutron irradiation can sometimes be used to induce secondary nuclear reactions of analytical interest and permit relatively cheap reactor irradiations to be used. In particular, the sequence $^{6}Li(n,\alpha)T$, $^{16}O(t,n)^{18}F$ has been applied to the determination of oxygen in beryllium,[50] gallium arsenide,[51] on surfaces[52] and to the determination of lithium[53] in the presence of alkali metals. Aumann and Born[54] assessed the oxygen-18 content of samples by the $^{18}O(p,n)^{18}F$ reaction induced by recoil protons, and deuterium[55] in deuterated organic compounds has been measured by the reaction $^{12}C(d,n)^{13}N$.

γ-PHOTONS—

γ-Photons provide an alternative penetrating radiation to neutrons for inducing nuclear reactions, and they have been used mainly for the determination of those elements that cannot be measured satisfactorily after irradiation with reactor neutrons.

A variety of particles may be emitted as a result of photo-disintegration of a nucleus but most γ-ray activation techniques are based on the (γ,n) reaction. Photoneutron thresholds usually exceed 5 MeV, but photons of much higher energy are normally used to obtain adequate sensitivity and therefore samples are usually irradiated with bremsstrahlung from a betatron or linear accelerator. The penetration of γ-photons permits easy encapsulation of targets, and pneumatic transfer systems[56] can be used to move samples in and out of the irradiation position. Deuterium and beryllium exhibit low neutron binding energies of 2·2 and 1·7 MeV, respectively, and γ-rays from isotopes such as antimony-124 have been used by Goldstein[57] and many earlier workers to induce the (γ,n) reaction in beryllium.

Engelmann[58] has described the use of γ-ray activation for several important light elements such as carbon, nitrogen and oxygen. The sensitivity of the method was better than 1 μg for the three elements. Schweikert and Albert[59] have considered the potential use of γ-photon activation for heavier elements. Twenty-four elements were irradiated with bremsstrahlung from a linear accelerator and the induced activity counted; sensitivities were found to be better than 1 μg for many of the elements.

γ-Activation techniques have been used to analyse ores[60] and concentrates for light elements, and for copper, zinc and zirconium. Samples of 5 to 500 g were irradiated and the induced activity detected by γ-ray scintillation spectrometry; interferences were reduced by careful choice of photon energy. Mulvey, Cardarelli, Meyer, Cooper and Burrows[61] determined iodine by γ-activation by using a 22-MeV linear accelerator, and detecting the 0·386 and 0·650-MeV γ-rays of iodine-126; 1 μg was measured with a coefficient of variation of 0·56 per cent.

Large accelerators are required for most γ-photon activations, and it may not be either desirable or feasible to allocate most of the machine time to analytical work. Under these circumstances, γ-photon activation can be used as a referee method[62] to supplement more conventional methods of analysis.

MEASUREMENT OF INDUCED ACTIVITY

The method chosen for the measurement of the activity of an irradiated sample is governed by the decay characteristics of the active species and the relative activity of the element to be determined to the total activity of the sample. There is an increasing tendency towards non-destructive methods, which require specific counting methods such as γ-spectrometry.

RADIOCHEMICAL SEPARATIONS—

Although instrumental methods of activation analysis have received considerable attention over recent years, the high separation factors that can be achieved by chemical techniques still result in chemical methods being used in many activation procedures, particularly when a small yield of activity of one element is sought in the presence of a highly active matrix or the maximum sensitivity is required. Chemical separations are usually devised to yield the elements to be determined, either in a radiochemically pure form that can be counted by simple counting equipment, or into several groups which can then be further examined by γ-ray spectrometry. Inactive carriers are usually present during solution of the sample to enable a correction to be made for losses occurring during chemical processing, but treatment of the sample before irradiation has been carried out in a limited number of activation schemes when losses are expected to be small.[63] Surface etching after irradiation can remove impurities from suitable samples.

The radiochemistry of a great many elements is summarised in a series of monographs issued by the U.S. National Academy of Sciences, Nuclear Science Series NAS–NS, and many references are quoted. In addition, several laboratories publish their own separation procedures.

Precipitation is still widely used for scavenging, for separating one or more nuclides from several others, preparing sources suitable for counting and for chemical-yield determinations. Precipitation from homogeneous solution can improve the purity of the precipitate, and Salutsky[64] has reviewed this and other forms of precipitation.

The speed and versatility of solvent extraction makes the technique suitable for radiochemical separations; the book by Morrison and Freiser[65] is a valuable source of information, and Green[66] has reviewed the uses of liquid ion exchangers.

A monograph has been published on the use of high molecular weight amines in solvent extraction,[67] and a recent review by Morrison contains references to extraction procedures for many elements.[68]

Differential migration techniques have been extensively applied to radiochemical separations, and in particular to ion-exchange chromatography on columns of organic ion exchangers; the book by Samuelson is a useful guide for devising ion-exchange separations.[69] Inorganic ion exchangers have been applied to radiochemical separations less frequently, but Girardi, Merlini, Pauly and Pietra have reported their use in an extensive chromatographic separation scheme.[70] Reversed phase partition chromatography, in which the column material is an inert support retaining an organic extractant, has been developed more recently, but several workers have applied it to the radiochemical separation of a sample after irradiation. Towell, Volfousky and Winchester have determined rare-earth abundances in the standard granite G–1 and the standard diabase W–1 by neutron activation after separation of the elements on columns of di(2-ethylhexyl) hydrogen phosphate.[71] Paper chromatography has also been used in chemical separation schemes for neutron-activation analysis, and in certain cases the separation has been carried out before irradiation[72] although limitations are imposed by impurities in the paper and radiation damage of the chromatogram if this is irradiated.

Distillation can yield fast and clean separations of some elements and De Voe[73] has reviewed the technique in a short monograph. A simple apparatus has been described for vacuum distillation which permits the rapid distillation of small amounts of material.[74]

Other separation techniques applied to radiochemistry include electro-analytical methods, for example, controlled electro-potential deposition,[75] amalgam exchange[76] and the ring-oven technique.[77]

RAPID SEPARATIONS—

Separation of short-lived radionuclides must be effected rapidly after the completion of irradiation, and special techniques have been devised for this purpose. Kusaka and Meinke[78] have reviewed rapid radiochemical separation techniques, and Rengan and Meinke[79] have described a rapid separation of rare earths by elution from columns of cation exchanger with α-hydroxyisobutyric acid. Samsahl[80] has also carried out rapid separation with ion exchangers, but has used automatic equipment that permits many elements to be separated into several groups. Ruch, De Voe and Meinke[81] have demonstrated the speed of amalgam-exchange separations by separating indium from several elements in 11 minutes with a yield of higher than 95 per cent. The method was found to give more satisfactory results than the conventional bromide extraction.

Kemp and Smales[82] have used solvent extraction for the rapid separation of 3·76-minute vanadium from rocks and meteorites after neutron irradiation. The vanadium was extracted into chloroform as the cupferron complex in the presence of EDTA, and the activity was measured 13 to 14 minutes after the sample left the reactor.

Baker[83] has separated the 2·1-minute oxygen-15 isotope formed by the (γ,n) reaction on oxygen-16 by inert gas fusion in a graphite furnace at 2000° C, and by subsequent gas phase and solution chemistry. In this way oxygen has been determined in steels and other metals down to 1 p.p.m. after irradiation with 40-MeV bremsstrahlung.

Rapid radiochemical separations have recently been carried out on thin-layers of ion-exchange resin[84] by using centrifugal acceleration to reduce development time, and Wolf[85] has described apparatus for rapid radiochemical separation by co-precipitation with a carrier.

GROUP SEPARATIONS—

A chemical separation procedure may be devised to divide the constituents of a sample into several groups which are then examined by γ-ray spectrometry. This technique has been applied to a variety of samples. Samsahl, Brune and Wester[86] have described a scheme for the determination of 30 trace elements in cancerous and non-cancerous human tissue after chemical separation into 16 or 18 groups. The separation scheme was based on distillation and the use of organic and inorganic ion exchangers. For the determination of 62 elements in high purity beryllium, aluminium and iron, Ross[87] has combined non-destructive and chemical techniques. Forty-nine of the elements were determined after separation into 6 groups by distillation, precipitation and solvent extraction.

Coulomb and Schiltz[88] have used paper chromatography to provide a preliminary separation of elements derived from geochemical materials. After separation, the γ-spectra of different bands of the chromatogram were measured and analysed by computer techniques.

Aubouin and Laverlochere[89] have published a comprehensive ion-exchange separation for 30 elements and have determined impurities in several matrices. Many elements are separated individually, but the scheme provides a number of small groups which can be easily assayed by γ-ray spectrometry.

AUTOMATIC SEPARATION SYSTEMS—

Several workers have operated chemical separations automatically to reduce the effort required when many samples have to be processed. Girardi, Merlini, Pauly and Pietra[70] have devised an automatic system of chromatographic separation that is based on the use of two units: (*a*), a pump and programming unit; and (*b*), a fraction collector and motor-driven stopcock. By varying the number and arrangement of these units, constituents of a sample may be chromatographed through one or more columns and eluted with different solvents.

Group separations may be effected by sorption of the constituents of the sample on to several columns connected in series. Samsahl[80] has devised a pumping unit which automatically introduces solution to the solvent stream at points between columns to achieve the conditions necessary for selective sorption. The technique has been applied to the group separation of trace elements in biological material.[90] Comar and Le Poec[91] have reported an automatic method for the separation of iodine in biological fluids as part of a neutron-activation procedure.

SUBSTOICHEIOMETRY—

Activation procedures involving radiochemical separation usually require measurement of both the radioactivity and chemical yield of a radionuclide, but if a known or carefully controlled amount of the element can be sampled, only a measurement of activity is required. Sampling may be carried out by completely converting a known amount of reagent to a compound with the radionuclide and determining the activity of this compound; the amount of reagent added must be insufficient to combine with all of the radionuclide present in the system. This technique, known as substoicheiometry, has been described in detail by Růžička and Starý,[92] and procedures for many elements have been given.

If the reagent reacts with the radioactive element to be determined in preference to other elements in the system, a substoicheiometric step offers some selectivity which may be exploited to simplify and increase the speed of the separation procedure. Thus a substoicheiometric finish has been used for a determination of silver[93] based on measurement of the 2·3-minute silver-108 nuclide.

Růžička and Williams[94] have described a system for the continuous operation of substoicheiometric isotope dilution analysis, but the method can also be extended to activation analysis.

γ-SPECTROSCOPY—

The most widely used measuring technique in activation analysis is now γ-spectrometry; this generally involves absorption of the γ-rays in a sodium iodide crystal and then processing the pulses with a multi-channel analyser. Other crystal detectors, such as caesium iodide and calcium iodide, have been developed, but because of extra cost and, for the latter, technical difficulties in making large crystals, they have not found wide application.

For background information the book edited by Crouthamel[95] is useful; it also contains a compilation of γ-spectra that assists in identifying unknown species. More recently, Heath[96] has published an excellent two-volume report, which contains detailed information on his system and also over 200 spectra in graphical and digital form. These data can then be transferred to paper or magnetic tape and any selected spectrum read into the analyser, so allowing a direct visual comparison with the spectrum of interest. Other compilations have been produced specifically for activation analysis. Girardi, Guzzi and Pauly[97] have listed all the reactions normally used in thermal neutron activation which produce γ-emitting nuclides. The γ-spectrum is illustrated and additional information is provided to allow the activity to be rapidly determined for any weight of material from a combination of graphs of decay growth during irradiation and sensitivity. Aude and Laverlochere[98] have produced experimentally a similar compilation for fast-neutron products.

Before buying equipment for γ-spectrometry it is important to compare carefully the manufacturer's specifications with the experimental requirements. Crouch and Heath[99] have specified the standards required and the means of testing the equipment for accurate spectrometry by using computer methods of data analysis. The performance of analysers available early in 1965 is conveniently grouped together in a report,[100] but new and improved equipment is constantly coming on to the market. The quality-control methods of Covell[101] can be recommended for establishing and maintaining spectrometer stability.

If many samples are to be processed it is likely that an automatic sample changer will be required; most commercial equipment is unsuitable for γ-spectrometry inside a large shield, but changers that are suitable have been developed in several laboratories.[102,103]

With the advent of computer methods of analysis of γ-spectra, the method of transferring data from the analyser to the computer has been studied. Euler, Phelps and Covell[104] have described a fast punched-card read-out system, which collects data from a 256-channel analyser in 12 seconds and provides for the insertion of seven record and information words. The automated system of Wainerdi[105] transfers the data in less than 1 second. Although he has not applied it to γ-spectrometry, McNaught[106] has accumulated counting data on magnetic tape and then successfully transferred it into a computer via telephone wires.

As nuclear physics experimentation increases in complexity and vast amounts of data are produced there has been a tendency towards the use of small computers for data collection instead of fixed-wire analysers. Cohan[107] has used the N.B.S. system for activation analysis, and Spinrad[108] gives a general comparison of the merits of computers and analysers for multiparameter analysis. The use of small computers will increase in activation analysis, particularly when there is a need for on-line analysis, because the computer can be used for data processing as well as for a store. However, the conventional analyser is likely to provide the most economical answer for most routine operations for some time.

The control of drift in a spectrometer is of particular importance if accurate measurements are required from complex spectra by computer methods. Many systems have been described which monitor the position of peaks in the spectrum derived from a γ-emitter, α-emitter, a light source or a pulse generator. Comparatively few, however, control both the gain and threshold settings of the analyser, and in the authors' opinion both are liable to drift. Dudley and Scarpatelli[109] have a method that limits changes in gain and threshold to less than 0·1 per cent. and does not add unwanted peaks to the spectrum. The change in the system can also be corrected for by computer methods. Schonfeld's[110] programme allows for automatic data processing as well as for a store. However, the conventional analyser is likely to provide changes in gain and threshold and has been used most satisfactorily in the author's (R.F.C.) laboratory. Both of these last methods correct for all sources of drift, including the effect of high count-rates on the analyser and photomultiplier.

For quantitative measurement of nuclides it is usual to standarise the equipment by counting sources prepared from a known weight of irradiated material. Poor standards are a frequent source of error in activation analysis. The material used for standards should be free from impurities that could give rise to interfering activities, and the irradiation conditions, counting geometry and total amount of scattering material near the source should be as similar as possible for sample and standard. For the evaluation of simple spectra containing a few well resolved peaks the method of Covell[111] is widely used and is quite satisfactory. Yule[112] has applied convolution techniques to γ-spectra and then used a computer method to locate and determine the area of the photopeak for simple spectra. The direct determination of mixtures by complement subtraction, as proposed by Lee,[113] is possible with the accessories available with most analysers. This technique was further improved by Anders and Beamer[114] by using a small computer to normalise the spectra for decay, neutron flux, etc., before stripping.

To resolve and evaluate complex mixtures, a more sophisticated approach with larger computers is necessary. Zerby[115] attempted to calculate pulse-height distributions from a knowledge of basic interactions occurring in a detector by using Monte Carlo methods. Heath[116] was more successful, and empirically calculated the response function for any detector and experimental geometry. Many spectra of single γ-ray emitters are measured to characterise the system, and then, by suitable machine programming, any unknown spectrum can be generated from an empirical analytical function representing the pulse-height distribution and a knowledge of the decay scheme. The resolution of the complex spectrum is then achieved by least squares analysis. Salmon,[117] Trombka[118] and others have experimentally built up a library of standards and then used the least squares method. Perhaps it is surprising that although in activation analysis it is usual to measure several spectra over a period of time, few analysts have used this extra parameter, the decay constant, in the analysis of spectra. Nicholson, Schlosser and Brauer[119] have developed such a programme, and more recently Schonfeld[120] has applied this approach to activation analysis and demonstrated the improvement in precision.

Computer methods of analysis of γ-spectra have provided the key to rapid and economical routine analysis by activation methods. Automatic sample handling and irradiation facilities have been combined with computer methods of data analysis by Wainerdi, Menon and Fite[105] to provide a system capable of handling a great many of certain types of sample at low cost.

Other workers have attempted to increase the scope of instrumental methods of analysis through coincidence and anti-coincidence counting. Ljunggren[121] has used γ - γ coincidences for the measurement of several nuclides emitting γ-rays in cascade. Borg *et al.*[5] demonstrated the specificity of triple coincidence methods, two γ-rays and a high energy β-particle, by determining manganese in blood directly without first removing the sodium-24 activity.

The original method of Pierson,[122] which compensates for the Compton continuum by subtracting the spectrum produced by an anthracene crystal from the sodium iodide spectrum, has been greatly improved by De Soete and Hoste.[123] This reduction in the Compton contribution, by about 85 per cent. in the latter case, simplifies the task of interpreting and evaluating peak heights. Other methods of suppressing Compton pulses have been tried by surrounding the sodium iodide crystal with a large plastic scintillator,[124] a large sodium iodide crystal[125] or a caesium iodide crystal.[126] The most sophisticated and expensive device has been developed by Perkins,[127] and consists of two sodium iodide crystals surrounded by a 12-inch diameter sodium iodide detector. An extremely low background is achieved, and by measuring γ - γ coincidences with a multi-parameter analyser, very complex mixtures can be readily determined.

The use of sum coincidence γ-spectrometry has been demonstrated; Adams and Hoste[128] showed an improvement in sensitivity of a factor of 32 compared with the Compton compensated technique in the determination of antimony in lead. Wahlgren, Wing and Hines[129] have applied the method generally, and compare the sensitivity of a fast sum coincidence system with simple γ-spectrometry.

A relatively new and rapidly developing area of γ-spectrometry involves the use of lithium-drifted germanium. Currently, detectors are commercially available in sizes up to about 2·5 cm in diameter and 1 cm thick. Larger volumes have been made at Chalk River by Malm, Tavendale and Fowler[130] by using an axial drift technique; as these larger sizes become generally available they should find wide application in activation analysis. The resolution of such a detector is 4·8 KeV for 1·33-MeV cobalt-60 γ-ray, and gives complete separation of many photopeaks unresolved by sodium iodide. The necessity of operating germanium semiconductors in a vacuum and at 77° K is overcome by suitable design of equipment, and access to the detector is then quite easy. The application of these new detectors to activation-analysis problems has been demonstrated by Girardi, Guzzi and Pauly[131] in analysing hafnium in zirconium oxide and by Prussin, Harris and Hollander[132] in the non-destructive analysis of aluminium.

OTHER COUNTING METHODS—

β-Counting with Geiger or proportional counters is still frequently used for measuring the activity after a radiochemical separation that ensures purity of the source. Because of the high efficiency (up to 100 per cent.), such counters have advantages in achieving the maximum sensitivity possible by activation methods.

Cerenkov counters have found some application for the measurement of high energy β-particles. Lukens[133] counted β-particles from nitrogen-16 (maximum energy 10 MeV) and demonstrated the high efficiency of rejection of weaker β-particles, even from chlorine-38 (maximum energy 4·8 MeV). The Cerenkov counters were superior to sodium iodide crystals for the measurement of oxygen in some materials.

Fission counters have been used by Nilsson[134] for the accurate assay of small samples of uranium; 100 to 140-mg samples can be determined with a precision of 0·7 per cent. (two standard deviations).

Auto-radiography can be used for the quantitative measurement of boron[135] by counting recoil tracks after irradiating the sample and emulsion in a reactor. The method is useful in defining the position of impurities in a sample; for example, the study of segregation in metals by Makin.[136]

For a limited number of elements, neutron counting has provided a specific method of analysis. By using the delayed neutrons from fission, two groups of workers, Amiel[137] and Dyer, Emery and Leddicotte[138] have developed a rapid, routine method for uranium. In the author's (R.F.C.) laboratory, more than 100 urine and rock samples are analysed weekly, each measurement requiring less than 3 minutes. Thorium, oxygen and lithium can also be determined, but with lower sensitivity, by modifying the conditions of irradiation. Amiel[139] has further extended the value of neutron counting by using the photoneutrons emitted when high energy γ-rays bombard deuterium and beryllium for the analysis of sodium, manganese, sulphur and calcium.

PROMPT RADIATION TECHNIQUES—

Analytical methods based on the measurement of prompt radiation have received relatively little attention. Prompt radiation must be measured with the sample *in situ*, usually with the irradiation in progress, and chemical separation of the constituents of a sample between irradiation and counting cannot be used to isolate the elements to be determined from all others, or to etch and remove surface contaminants. Moreover, interference is likely from any contamination that might build up on the sample, for example, carbon during accelerator irradiation. Therefore, if pre-separation of the constituents of the sample is to be avoided, prompt radiation techniques must rely heavily on instrumentation to distinguish the radiation to be measured from others emitted by the samples and from machine background.

However, prompt techniques permit the radiation emitted during the decay of excited nuclear states to be counted, so avoiding the necessity of choosing a nuclear reaction producing a radioactive isotope as a basis for the determination. Thus, a 0·87-MeV γ-ray is emitted as a result of the irradiation of oxygen-16 with low energy deuterons, corresponding to the de-excitation of oxygen-17 produced by the (d,p) reaction, although both oxygen-16 and oxygen-17 are stable.

Analytical methods have been proposed that are based on the measurement of particles of γ-radiation. Energies of charged particles can be found with a magnetic spectrometer or with a semiconductor detector, but if thick targets are used, broadening of the particle lines caused by energy loss in the sample complicates spectral analysis. However, Patterson, Turkevich and Franzgrote[140] have examined the possibility of using Rutherford scattering and (α,p) reactions to analyse geochemical surfaces. Particle scattering can also be used to investigate thin films on surfaces,[141,142] particularly when a heavy element is present on a lighter matrix, and layers less than 1-μ thick can be measured.

Prompt neutrons have also been measured[143]; for example, deuterium in surface layers of Zircaloy-2 has been determined by measuring the neutrons emitted during the reaction $D(d,n)^3He$. Standardisation was effected by comparing the neutron yield from the target with that from a deuterium gas target cell.

Capture γ-rays emitted during neutron irradiation are often complex, but Greenwood and Reed[144] have discussed possible activation applications and compiled γ-spectra.[145] The high cross-section of the reaction $^{10}B(n,\alpha)^7Li$ for thermal neutrons feeding the 0·478-MeV level in ^7Li has been used by Isenhour and Morrison[146] as the basis of a method for determining boron.

Measurement of prompt γ-rays emitted during irradiation of a sample with neutrons from a neutron generator is complicated by the problem of shielding the γ-ray detector, and ring geometry, often used in nuclear physics experiments, requires a large sample which is not always convenient for analytical work. However, nitrogen has been determined in bulk material by the measurement of capture radiation with a polonium - beryllium source,[147] and carbon has been determined in coal by the inelastic scattering of 14·8-MeV neutrons.

Prompt γ-rays emitted during irradiation of a sample with charged particles provide analytical methods for the determination of many light elements,[148,149] but information is only obtained from a thin section of the sample near to the surface.

If a carefully collimated particle beam is used, localised areas of the surface of the sample can be examined.

APPLICATIONS

It is not possible to review critically all of the fields to which activation analysis has made a substantial contribution. However, it was considered desirable to attempt to select a few reviews and papers to illustrate the wide application of activation analysis, and to provide a starting point for a more extensive literature search.

HIGH PURITY MATERIALS—

The high sensitivity of activation analysis for many elements has resulted in the technique being applied to the determination of trace constituents of pure materials. Again, freedom from reagent blank during chemical processing enables the high sensitivity of the technique to be exploited, even when chemical processing of the sample is necessary between measurement and counting.

When the matrix is relatively insensitive to activation by the radiation with which the sample is being irradiated, trace elements may sometimes be determined non-destructively. Lightowlers[150] has determined small amounts of manganese, sodium and copper in natural diamonds by γ-ray spectroscopy after neutron activation, and Petit and Engelmann[151] have described non-destructive methods of beryllium analysis. However, it is more often necessary to take advantage of the high separation factors that can be achieved by chemically separating the activity to be measured from all others before measurement. Thus, Gebauhr and Martin[152] have described a scheme for the determination of thirty elements present at trace levels in high purity silicon. Auto-radiography was used to localise the impurities. A field of trace analysis to which activation analysis has been extensively applied has been the determination of trace-level impurities in semiconductor materials, and Cali[153] has described the methods used in detail.

Laboratories without direct access to suitable irradiation facilities may find value in using activation analysis as a referee method. Tellurium[154] has been determined in tellurium-doped gallium arsenide by neutron-activation analysis to check the validity of spectrophotometric measurements.

Extensive reference is made in bibliographies to the individual determination of many elements at trace levels in a variety of materials.

BIOLOGY—

The book by Bowen and Gibbons[155] provides an excellent starting point for obtaining details of biological applications of activation analysis. Most of the papers up to 1961 are reviewed, and also the reported values of elemental concentration in organisms and mammalian tissue are tabulated. Practical details of methods of analysis for twenty elements in biological tissue have been published by Bowen and Cawse.[156] Data on the elemental composition of blood as determined by activation analysis and many other methods have been collected by Bowen,[157] and indicate that large discrepancies occur for many elements. A biological reference material[158] has been prepared to permit intercomparison of analyses from different laboratories, and ultimately this should lead to an improvement in the reliability of analytical measurements.

The proceedings[159] of the biological symposium held at Saclay in 1964 demonstrate the wide variety of biological applications of activation analysis.

FORENSIC SCIENCE—

There has been considerable interest in the application of activation analysis to forensic science problems in the last few years, and useful reviews have been written by Guinn,[160] and by Smith and Lenihan.[161] Smith[162] has demonstrated the value of activation analysis in the investigation of arsenic poisoning. Ruch, Guinn and Pinker[163] have detected excess of antimony and barium on the hands of people who have recently fired a gun, and this method has already found some use in criminal investigations.

Much of the work in forensic science laboratories is aimed at establishing if two samples have a common origin. As has been indicated by several papers, *e.g.*, those by Ruch, Buchanan, Pinker, Guinn and Bellanca[164] and Bate, Emery, Leddicotte, Lyon and Pro,[165] activation analysis can be applied to this problem and so determine the trace-element content of many materials, such as hair, glass, rubber and plastics, that are of interest to forensic scientists. It is assumed that the variation in trace-element content of samples of different origin will appear significantly different, and only closely match in two samples of common origin. This is a dangerous assumption that is only justified if many samples have been analysed, indicating that a large variation in concentration does exist. Coleman[166] has investigated the trace-element content of a great many hair samples and has used a statistical method to assess quantitatively the value of this kind of evidence for identification purposes.

GEOCHEMISTRY—

Activation analysis has found application to the determination of major, minor and trace constituents in geochemical samples, but methods of trace-element analysis have found widest application, as the efficient separation of small amounts of activity from a complex and highly active matrix by chemical techniques can be effected without introducing a reagent blank, and correction can be made for losses that may occur during chemical processing. Mapper[167] has discussed in detail the application of radioactivation to geochemistry and has given many examples. The scope of activation analysis for geochemical determinations can be gauged from a summary of results recently obtained for the levels of different elements in the standard diabase W–1 and the standard granite G–1 in which reference is made to the determination of more than forty elements[168] by neutron-activation analysis.

Several authors have compared results found by activation analysis with those obtained by other analytical techniques. Thus Ball and Filby[169] obtained good agreement for the zinc content of some geochemical standards measured by neutron activation and X-ray fluorescence techniques, and Smales, Hughes, Mapper, McInnes and Webster[170] have compared results obtained for the rubidium and caesium content of stony meteorites by neutron-activation analysis and by an isotopic dilution method of mass spectrometry. For the latter, even at rubidium and caesium levels lower than 1 p.p.m., differences in results obtained by the two techniques exceeded 2σ only for one sample in every twelve.

Fast-neutron activation has been used to obtain figures for the silicon[171] and oxygen[172] contents of stony meteorites by using the reactions ^{28}Si(n,p)^{28}Al and ^{16}O(n,p)^{16}N, respectively. Measurements were non-destructive, and the precision for the silicon determinations was usually found to be better than ± 3 per cent.

The relatively small size of some neutron generators enables them to be used in portable instruments for geochemical analysis; Caldwell, Mills, Allen, Bell and Heath[173] have described the use of a pulsed fast-neutron source for the remote analysis of lunar and planetary surfaces. γ-Rays produced by three different processes are distinguished by their time of emission with respect to the neutron pulse; γ-rays from inelastic scattering are emitted during the pulse, capture radiation is emitted between pulses after the neutrons have been slowed down and γ-rays from isotopes, produced, for example, by (n,p), (n,2n) and (n,α) reactions, build up over a number of pulses. In addition, information about the composition of the target can be obtained from the rate at which the capture radiation, and hence the thermal neutrons, die away.

ON-LINE ANALYSIS—

Many analytical techniques are being adapted for process control, and activation methods can make a useful contribution in this field; Taylor's book[174] on activation analysis devotes a chapter to on-line analysis. A typical example has been the extensive study by Martin and co-workers[175] into coal analysis. Under laboratory conditions, carbon, oxygen, aluminium and silicon can be determined with an accuracy of 1 to 3 per cent. by using an accelerator neutron source and measuring γ-rays produced after inelastic scattering and from the decay of radionuclides. Further work is continuing in several laboratories to apply such techniques on a plant scale. The continuous measurement of perborate in detergents has been examined by Ljunggren and Christell.[176] Neutron absorption by boron-10 and also the measurement of γ-rays from the reaction ^{10}B(n,$\alpha\gamma$)^{7}Li can form the basis of the analytical method. A nuclear method of monitoring the addition of fluorine to drinking water by using a 14-MeV neutron source and counting the activation product, oxygen-19, in a special cell downstream from the neutron source has been demonstrated by Norgolwalla and Jervis.[177] On-line analysis would be of value in ore sorting plants; Ramdohr's work[178] on copper minerals is an example of the use of activation analysis.

SURFACE ANALYSIS—

Several nuclear methods are available for examining the surface of samples and these frequently involve the use of charged-particle irradiation. Charged-particle beams can be collimated relatively easily, and the depth of penetration of particles into the sample can be adjusted by careful control of particle energy; particle energy spectra can be obtained with a magnetic spectrometer or with a semiconductor detector assembly.

Elastic scattering techniques, which have been described by Rubin,[141] are capable of a high sensitivity, and can be used to examine layers of much less than 1 μ in thickness, although if thick targets are used the technique is most satisfactorily applied to the determination of a heavy element on the surface of a lighter matrix. Pliesach and Poole[142] have used a semiconductor detector to measure the scattered particles.

When there is a sharp resonance in the excitation function of a reaction of interest, the variation of yield with energy can provide useful information about the composition and thickness of surface films. Thus, Amsel and Samuel[179] have used sharp resonances in the reaction $^{18}O(p,\alpha)^{15}N$ to follow the growth of oxide films during anodic oxidation.

The use of auto-radiography after helium-3 irradiation to locate concentrations of light elements in surfaces has been mentioned. In addition to conventional radiographic techniques, charged-particle tracks in a variety of materials have been used to provide information about surfaces, in some cases after enlarging the damage trails by chemical attack. For example, α-particle tracks in plastic sheets have been used to study the inhomogeneous distribution of boron in the surfaces of metallurgical samples, and uranium has been determined in very small samples down to levels of 10^{-3} p.p.m. by fission track counting. Fleischer, Price and Walker[180] have discussed analytical applications of track counting in some detail. Leonhardt[52] has used tritons, emitted as a result of the reaction $^{6}Li(n,\alpha)T$, to produce fluorine-18 from surface oxygen by the reaction $^{16}O(t,n)^{18}F$, and was thus able to use a nuclear reactor as a radiation source. The amount of oxygen on the surface of foils was calculated from the annihilation radiation of the fluorine-18 after separation, and was found to vary from 1·4 μg of oxygen per cm^2 for platinum to 7·5 μg of oxygen per cm^2 for iron.

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The Spectrofluorimetric Determination of Magnesium with *NN'*-Bis-salicylidene-2,3-diaminobenzofuran*

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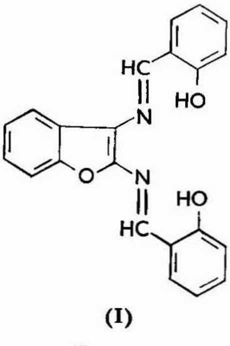
NN'-Bis-salicylidene-2,3-diaminobenzofuran, SABF, has been synthesised and is proposed as a stable and inexpensive spectrofluorimetric reagent for determining magnesium in the range 0·1 to 6 μg (2×10^{-3} to 1×10^{-1} p.p.m.). The method is both rapid and sensitive, and has a detection limit of about 10^{-3} p.p.m. in 50 per cent. aqueous methanol at an apparent pH of 10·5. The orange fluorescence (545 mμ) of the 1 : 1 complex is excited at 475 mμ and can be measured within 15 to 45 minutes of mixing the solutions. Few other ions yield a fluorescence with the reagent and those that do, or that interfere in other ways, may be tolerated in 100-fold molar proportions by simple addition of masking agents. The fluorescent magnesium - SABF complex can be extracted quantitatively into isobutyl methyl ketone without interference from 2000-fold amounts of calcium, thus providing an extremely useful method for determining magnesium in technical materials, etc. To demonstrate this, nine municipal water samples and fourteen blood plasma samples have been analysed successfully for magnesium by the proposed method in a 50 per cent. aqueous methanol medium.

THERE are few reagents currently available for the spectrofluorimetric determination of submicrogram amounts of magnesium in the presence of calcium. Lumomagneson IREA (2-hydroxy-3-sulpho-5-chloro-benzeneazo barbituric acid)[1] and 2,2'-dihydroxyazobenzene[2] give fluorescence reactions with magnesium, but both reagents form coloured calcium complexes which give rise to inner-filter quenching when calcium is present in greater than a 10-fold molar excess over magnesium. The magnesium complexes of these two reagents can only be extracted into organic solvents with difficulty. The most sensitive fluorimetric magnesium reagent proposed to date is *NN'*-bis-salicylidene ethylenediamine[3] which will detect about 10^{-5} p.p.m. of magnesium. However, the use of this reagent is restricted to anhydrous dimethylformamide solutions. This presents severe difficulties to its practical application and, in addition, calcium forms a coloured, non-fluorescent complex.

Other reagents that have been proposed for the spectrofluorimetric determination of magnesium include 8-hydroxyquinoline,[4] methyl salicylate[5] and calcein.[6] Although these reagents are sensitive, they also give a strong fluorescence reaction with calcium and the other alkaline earths.

NN'-Bis-salicylidene-2,3-diaminobenzofuran, SABF, (I) was first prepared in this laboratory[7] and contains the same reactive groupings as *NN'*-bis-salicylidene ethylenediamine.[8]



(I)

Investigations showed that SABF gave a fluorescence reaction with magnesium in aqueous methanol solutions, and that the fluorescence intensity was only slightly influenced by the degree of reagent hydrolysis. This latter factor is of prime importance with this type of

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* Paper first presented by R. Smith at the meeting of the Society at Bristol on May 4th, 1966.

Schiff-base reagent, because hydrolysis occurs easily and the reaction products are fluorescent, thus giving high blanks. The detection limit for magnesium with this reagent (10^{-3} p.p.m.) is at least as good as that obtained with lumomagneson,[1] and probably better than that obtained with 2,2′-dihydroxyazobenzene, although Diehl, Olsen, Spielholtz and Jensen[2] have not quoted sensitivity figures for the latter. Similar excesses of calcium can be tolerated with both of these reagents and SABF. In contrast, however, SABF is easily prepared and purified. Further, traces of magnesium can be extracted quantitatively from aqueous solution into isobutyl methyl ketone. This extraction procedure is unique in that only a single extraction is necessary, *cf.* dihydroxyazobenzene, and that a non-fluorescent calcium complex is extracted, *cf.* 8-hydroxyquinoline. The extraction of the coloured calcium - SABF complex can be suppressed by the addition of strontium - EDTA.

METHOD

APPARATUS—

A Farrand Optical Co. spectrofluorimeter (Catalogue No. 104244)—This was fitted with a 150-watt d.c. xenon arc lamp and R.C.A. IP28 photomultiplier. The excitation monochromator was used with 20-mμ or 10-mμ half band-width slits and the emission monochromator was used with 10-mμ half band-width slits. Fused quartz cells (10 × 20 × 30 mm) were used throughout. The spectral response curves for the lamp and photomultiplier have been given elsewhere.[9]

A Unicam SP900A atomic-absorption / flame-emission spectrophotometer—This was used with an air - acetylene flame at 2852 Å.

Calibrated flasks—These were coated periodically with silicone "Repelcote," a 2 per cent. solution of dimethyldichlorosilane in carbon tetrachloride (Hopkin and Williams Ltd.), to minimise adsorption of ions on the glass.

REAGENTS—

NN′-*Bis-salicylidene*-2,3-*diaminobenzofuran, SABF*—De-aerate a mixture of 50 ml of salicylaldehyde and 80 ml of ethanol by passing nitrogen through it for 20 minutes, and then add 15 ml of concentrated ammonia solution. A white or pale lemon precipitate is formed immediately. Place the mixture in an ice-bath and, while stirring, add to it 6 g of potassium cyanide dissolved in 9 ml of water. During the course of the reaction the precipitate changes from lemon to orange - yellow, and after about two hours sets to a solid mass. Break up the mass and add 50 ml of ethanol. After a further 90 minutes add 15 ml of concentrated ammonia solution and continue stirring for a further 30 minutes. Filter the yellow or orange product and wash it well with 50 per cent. v/v water - methanol solution to remove the cyanide, followed by methanol, until the filtrate is clear yellow. Remove the residue from the filter, triturate with about 300 ml of methanol and filter. Dry in a vacuum over calcium chloride. [Yield 2·95 g (90 per cent. based on potassium cyanide). M.p. 181° to 183° C.]

Although the product obtained above is satisfactory for analytical purposes, the compound can be recrystallised from benzene - light petroleum or dioxan - water. [M.p. 182° to 183° C.]

Microanalysis results obtained for $C_{22}H_{16}N_2O_3$ (molecular weight = 356)

Found, per cent.	C 74·0	H 4·4	N 7·8
Calculated, per cent.	C 74·2	H 4·5	N 7·8

Molecular weight 356 (mass spectrum).

Dissolve 0·2 g of the reagent in 1 litre of dioxan (general-purpose reagent). This solution is stable for several months.

pH 10·5 *buffer*—Add 60 ml of diethylamine (general-purpose reagent) to 500 ml of 50 per cent. v/v methanol - water solution and adjust the apparent pH to 10·5 ± 0·1 with about 25 ml of concentrated hydrochloric acid.

Standard magnesium solution—Dry analytical-reagent grade magnesium oxide in an oven at 120° C for 3 hours. Dissolve 1·663 g in the minimum amount of 2 N hydrochloric acid and dilute the solution to 1 litre with de-ionised water. This gives a solution containing 10^{-3} p.p.m. of magnesium. Prepare by dilution a stock solution containing 100 p.p.m. of magnesium from which working solutions of 1 p.p.m. or 0·1 p.p.m. can be obtained. Solutions containing 1 p.p.m. or less of magnesium should not be stored for longer than two weeks because of losses caused by adsorption on the walls of the vessel.

Methanol, analytical-reagent grade.
Isobutyl methyl ketone, general-purpose reagent grade.
Pyridine, general-purpose reagent grade.
Diethylamine, general-purpose reagent grade.

Distilled water from an all-glass distillation apparatus was de-ionised by passing it through a 15 × 5-cm column of Zeo-Karb 225 (Permutit Co. Ltd., London) cation-exchange resin in the hydrogen ion form.

PROCEDURE—

Development of fluorescence in 50 per cent. methanol—To each 50-ml calibrated flask add 25 ml of methanol, 1 ml of pH 10·5 buffer and a solution containing between 0·1 and 6 μg of magnesium in about 15 ml. Add masking agents where necessary (see Interferences) at this point and allow the solutions to stand for 5 minutes. Add 5 ml of 0·02 per cent. SABF reagent in dioxan and dilute to the mark with de-ionised water. Measure the fluorescence intensity at 545 mμ, with an excitation wavelength of 475 mμ, between 15 and 45 minutes after the addition of reagent.

Analysis of blood plasma samples—The procedure above was followed by using 50 μl of untreated blood plasma and standard calibration solutions containing 0, 1 and 2 μg of magnesium. No masking agent was used, and it was not found necessary to de-proteinate the samples.

Analysis of water samples—The above procedure was followed by using 0·5, 0·25 or 0·05 ml of water and calibration solutions containing 0, 2, 4 and 6 μg of magnesium. Use 2 ml of strontium - EDTA masking agent, made by adding 50 ml of 10^{-1} M EDTA solution to 100 ml of 10^{-1} M strontium bromide, to overcome interference arising from the calcium.

Extraction procedure—To about 25 ml of aqueous solution containing 0·1 μg to 6 μg of magnesium in a 250-ml separating funnel, add masking agent as required (see Interferences), followed by 2 ml of pyridine and 1 ml of diethylamine. Add 25 ml of isobutyl methyl ketone and 2 ml of 0·1 per cent. SABF reagent in dioxan. Shake the mixture thoroughly for 1 minute, separate and discard the aqueous layer. Dilute the organic layer to 50 ml in a graduated flask and measure the fluorescence intensity at 555 mμ, with an excitation wavelength of 525 mμ.

RESULTS AND DISCUSSION

SPECTRAL CHARACTERISTICS—

All Schiff bases hydrolyse to some extent in aqueous solution and the effects of hydrolysis have been carefully evaluated for SABF. The uncorrected fluorescence excitation and emission spectra for the complex and blank are shown in Fig. 1. Correction curves for spectral variations

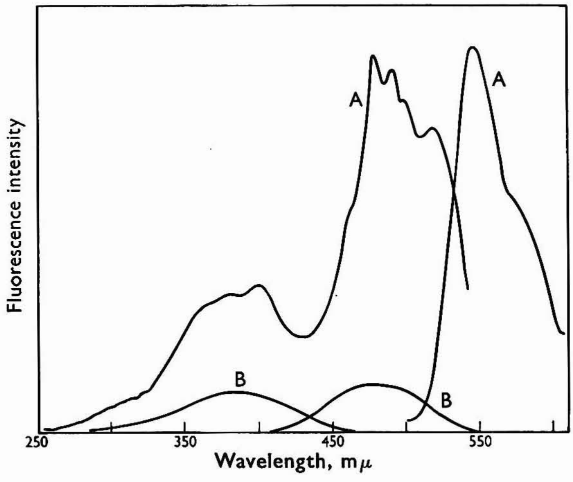

Fig. 1. Fluorescence emission and excitation spectra for: A, 1 ml of 10^{-4} M magnesium solution in the presence of 5 ml of 0·02 per cent. reagent; B, reagent blank measured in 50 per cent. aqueous methanol at an apparent pH of 10·5

in lamp emission and photomultiplier response have been given elsewhere.[9] The fluorescence of this blank was measured in the presence of EDTA and is, therefore, caused entirely by the hydrolysis products rather than by the presence of foreign cations. The emission maximum of the blank fortuitously coincides with the excitation maximum of the magnesium complex and, as a result, the blue fluorescence of the hydrolysis products does not interfere with the measurement of the orange fluorescence of the magnesium complex. The corrected fluorescence excitation spectrum for the magnesium - SABF complex is compared with the absorption spectrum for the complex in Fig. 2.

The fluorescence excitation maximum for the isobutyl methyl ketone extract is at 525 mμ. This peak is not the same as that used for excitation in 50 per cent. aqueous methanol where it occurs at 485 mμ (Fig. 1). The fluorescence emission maximum is shifted from 545 mμ in 50 per cent. aqueous methanol to 555 mμ in the ketone extract. These may be genuine solvent effects or may be the result of inner-filter quenching caused by the excess of reagent.

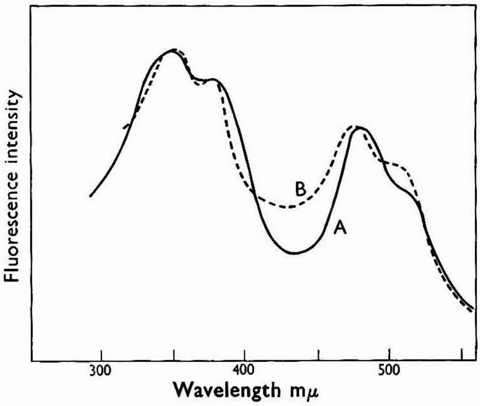

Fig. 2. Comparison of A, the fluorescence excitation spectrum (after correction for spectral variations in xenon arc lamp output) with B, the absorption spectrum of the complex by using 1 ml of a 10^{-2} M magnesium solution and 5 ml of a 0·02 per cent. reagent solution

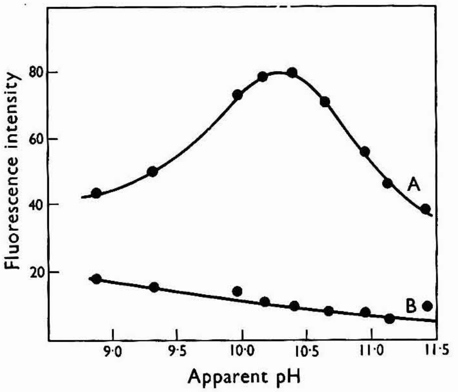

Fig. 3. Variation of fluorescence intensity with apparent pH in a 50 per cent. aqueous methanol medium for: A, magnesium complex; B, reagent blank

INFLUENCE OF pH—

The fluorescence intensity was measured over a range of apparent pH values for 50 per cent. aqueous methanol. The pH was adjusted by the addition of diethylamine and hydrochloric acid (Fig. 3). The corresponding blanks were obtained by adding one drop of 10^{-1} M EDTA solution to each of the solutions, after the fluorescence intensity of the magnesium complex had been measured. Maximum fluorescence intensity was obtained at pH 10·5 ± 0·2. This pH value can be adequately maintained with the buffer described and the fluorescence intensity is independent of the volume of buffer used.

REAGENT CONCENTRATION—

Maximum fluorescence intensity was obtained by using 4 to 10 ml of a 0·02 per cent. solution of the reagent in dioxan. The recommended volume of reagent, 5 ml, corresponds to a concentration of about 5·6 × 10^{-5} M in the final solution. This represents a 10-fold molar excess over the highest point on the calibration curve, *i.e.*, 6 μg of magnesium in 50 ml of solution. There is a tendency for precipitation to occur when larger excesses of reagent are used or if the pH falls below about pH 10.

EFFECT OF METHANOL—

Methanol has been used with other reagents[1,2] to improve their sensitivity towards magnesium. These reagents were found to give results that were dependent on the methanol concentration. With SABF, however, there is no difference in fluorescence intensity for methanol concentrations between 80 and 40 per cent. Below 40 per cent. of methanol, precipitation of the reagent occurs. The use of methanol concentrations higher than 50 per cent. reduces hydrolysis of the excess of reagent.

DEVELOPMENT TIME—

The fluorescence intensity increases during the first 10 minutes after addition of the reagent. The heat generated on mixing methanol and water is sufficient to raise the temperature of the solution to above 30° C. As the solution cools, the fluorescence intensity increases as a result of the negative temperature coefficient which is common to most fluorescence systems. A temperature effect also applies to the hydrolysis of the excess of reagent. In this instance the speed of hydrolysis increases with increase of temperature. As the reagent becomes hydrolysed, its inner-filter effect is reduced and the fluorescence intensity rises. If the solutions are allowed to reach room temperature before the addition of reagent, the rate of hydrolysis of excess of reagent is slow. As a result of these two opposing temperature effects, no meaningful temperature coefficient could be evaluated for the system. If no cooling time is allowed, the fluorescence intensity, relative to a quinine sulphate standard, is constant for between 15 and 45 minutes after addition of the reagent.

With the extraction procedure, on the other hand, the fluorescence intensity may be measured immediately after dilution and the fluorescence is stable for at least 2 hours.

INTERFERENCES—

The ions that react with SABF to form complexes have been summarised in a previous communication from this laboratory.[7] At pH 10·5 in 50 per cent. aqueous methanol, only magnesium, zinc and aluminium form fluorescent complexes with SABF. Many metals of ionic radius of less than 1 Å react to give coloured complexes which give rise to inner-filter action. The theoretical background to this has been discussed already[7] and, therefore, is not repeated here.

The effects of 100-fold molar excesses of foreign ions on the fluorescence intensity produced by 5 ml of 10^{-5} M magnesium solution (about 1·2 μg) were examined. The limiting error was taken empirically as ±5 per cent. of a magnesium standard containing no foreign ion. Marginal interferences were examined in more detail.

The following ions caused no interference in the 50 per cent. aqueous methanol procedure: As(III), As(V), Ba, Be, BO_3^{3-}, Cr(VI), Ga, Ge(IV), Hg(II), K, Mo(VI), Na, Sr, Ti(IV), Tl(I) and V(V).

In the absence of masking agents, 100-fold amounts of Ca, Cd, Co(II), Cr(III), Cu(II), Fe(II), Fe(III), Mn(II), Ni, Pd(II) and Zn interfered by formation of coloured, non-fluorescent complexes, which gave rise to low results.

Aluminium formed a fluorescent complex at this pH; the complex was only weakly fluorescent, but gave rise to high results. The even more weakly fluorescent zinc complex gave rise to low results because the large excess of zinc over reagent prevents the formation of the magnesium complex.

The following ions interfered by precipitation as chlorides or hydroxides: Ag, Hg(I), Pb, Sn(II), Sn(IV), Th and Zr.

In addition, Bi, In, La, Sb(III), Se(IV), Te(IV) and Y gave results that were low by about 10 per cent., although they did not show any visible signs of precipitation or colour formation.

The effects of various masking agents on selected interferences were, therefore, examined. Calcium could be tolerated in a 10-fold molar excess without the use of masking agents, and the interference arising from larger amounts of calcium was completely suppressed by a mixture of 10^{-1} M strontium and EDTA solutions in the ratio 2 to 1. Two hundred-fold molar excesses of calcium could be tolerated when 2 ml of the above mixture was used as a masking agent. The interference of 100-fold excesses of lanthanum and yttrium was also prevented in this way. Some metals that form very stable EDTA complexes in slightly acid solution, *e.g.*, aluminium, transition metals, etc., were not masked with strontium - EDTA because of the high pH of the determination.

The interferences arising from Ag, Cd, Co(II), Cu(II), Ni and Pd (II) were completely suppressed by the addition of 1 ml of 3 per cent. potassium cyanide, but Fe(II), Fe(III) and Zn were not masked in this way.

The addition of 5 ml of a 10^{-1} M sodium fluoride solution completely suppressed the interference of 100-fold excess of selenium(IV) or tellurium(IV). However, if this masking agent is used in the presence of large amounts of calcium (about 100 μg) co-precipitation of calcium fluoride and magnesium fluoride occurs and low readings are obtained.

The interference arising from 100-fold excesses of iron(III), aluminium or indium could be reduced by about 50 per cent. by using 0·5 ml of triethanolamine as a masking agent. This reagent may, however, be useful for complete suppression of the effects of lesser amounts of these metal ions. Manganese could not be prevented from interfering.

The effects of other masking agents were considered. One hundred-fold molar excesses of oxalate, citrate, tartrate, nitrilotriacetic acid, *trans*-1,2-diaminocyclohexane-*NNN'N'*-tetra-acetic acid and EDTA reduced the fluorescence intensity of the magnesium complex to below the blank value. A 100-fold molar excess of orthophosphate gave a reduction in signal of about 40 per cent. No interference, however, was noted from acetate, bromide, chloride, cyanide, fluoride, iodide, nitrate, perchlorate, sulphate or sulphide when present in 100-fold molar excess over magnesium. Similar concentrations of oxidising agents such as chromate or vanadate caused no interference.

Of these materials interfering in the 50 per cent. aqueous methanol method, no interference was noted in the extraction procedure from aluminium, mercury(I), manganese(II), lead and antimony(III) in 100-fold molar excess over magnesium. All the other interfering metal ions resulted in low recoveries. Various masking agents, *e.g.*, thiourea, tiron, sodium sulphide, *o*-phenanthroline, sodium fluoride and potassium cyanide, were used in an attempt to improve the selectivity of the extraction. Cyanide prevented the interference of copper only, and the other masking agents proved ineffective. However, with strontium - EDTA solution, calcium could be tolerated in 2000-fold molar excess over magnesium. This is a considerable improvement on the 50 per cent. aqueous methanol procedure.

Large amounts of the above interfering ions could probably be quantitatively removed by one or two pre-extractions from aqueous solution with either cupferron or sodium diethyldithiocarbamate.[10]

STRUCTURE OF THE COMPLEX—

A spectrofluorimetric study of complex formation with solution techniques[7] indicated the formation of a 1:1 magnesium - SABF complex. Further examination of the solid complex by infrared spectroscopy and thermogravimetry[7] indicated the structure as $[Mg(H_2O)_2(SABF)]$. This accounts for the slight solubility of the complex in pyridine, diethylamine, dimethylsulphoxide and dioxan, and its insolubility in chloroform, carbon tetrachloride and aromatic or aliphatic hydrocarbon solvents. Presumably the process of dissolution in any of these solvents corresponds to replacement of the water molecules by polar solvent molecules. No precipitation occurs when saturated solutions of the complex in polar solvents $[Mg(solvent)_2(SABF)]$ are added to light petroleum, despite the fact that the solid complex $[Mg(H_2O)_2(SABF)]$ is completely insoluble in this solvent. The structure proposed also accounts for the necessity of the presence of pyridine in the extraction procedure.

APPLICATIONS TO PRACTICAL SAMPLES—

Water samples—Nine water samples have been analysed for magnesium by the procedure described. The results (Table I) have been compared with those obtained by flame photometry on undiluted samples. The approximate calcium concentration of the samples was determined by EDTA titration with Acid Alizarin black SN as indicator.

Plasma samples—Fourteen plasma samples were analysed for magnesium by using 50 μl of sample and without subjecting this to any pre-treatment. The results are summarised in Table II and some flame-photometric results are also included. The flame-photometric method, however, requires 1 ml of sample and because of this, analysis of all the samples by this method was not possible. It is probable that the fluorimetric procedure could be adapted to the direct analysis of other biological materials.

The extraction procedure cannot be used for this determination because of protein precipitation during extraction.

Table I

Determination of magnesium in blood plasma

Sample	Found, µg per ml			Flame photometry, µg per ml
1	28	28	26	28
2	30	28	23	30
3	23	21	22	22
4	27	26	27	—
5	26	26	27	—
6	25	23	25	—
7	21	26	24	—
8	29	25	27	—
9	29	29	—	—
10	24	26	—	—
11	14	15	—	—
12	19	20	—	—
13	25	26	—	—
14	27	27	—	—

Table II

Analysis of water samples

Origin	Magnesium, µg per ml Flame photometry	Magnesium, µg per ml Fluorimetry	Calcium, µg per ml EDTA titration
Witney	5·8	5·8	170
Gloucester	14·2	14·6	115
Pontypool	6·3	5·8	36
Marlbrook (treated well-water) ..	1·8	1·5	32
Clacton	46	46	210
London	6·8	6·9	260
Bagshot	7·5	7·7	135
Marlow	2·0	2·3	110
Exeter	6·0	6·0	64

We thank Dr. I. Macintyre of the Postgraduate Medical School, Hammersmith, for supplying blood plasma samples, and to the Science Research Council for the award of a Research Studentship to one of us (R.S.). Thanks are also given to I.C.I. (Mond Division) for the loan of the Farrand spectrofluorimeter used in this work.

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The Catechol Violet Colour Reaction for Tin(IV) Sensitised by Cetyltrimethylammonium Bromide

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The sensitivity of the colour reaction between tin(IV) and catechol violet has been greatly increased by the sensitising action of cetyltrimethylammonium bromide ($\epsilon_{662\text{m}\mu} = 95{,}600$). The sensitisation is also accompanied by a considerable bathochromic shift (107 mμ) of the wavelength of maximum absorption. Full colour development occurs within 5 minutes at pH 2·2, and Beer's law is obeyed down to 0·2 p.p.m. of tin(IV).

THE most widely used methods for the spectrophotometric determination of tin are based on reactions with dithiol (4-methyl-1,2-dimercaptobenzene)[1,2,3,4,5] and phenylfluorone (2,3,7-trihydroxy-9-phenyl-6-fluorone)[1,6,7] in the presence of dispersing agents such as gelatin. Neither of these reagents has proved entirely satisfactory with respect to specificity and sensitivity, and the non-reproducibility of the dispersing agents adds further difficulties. Other spectrophotometric methods, based upon the reducing action of tin(II), include those with cacotheline,[8,9] and heteropoly molybdic acids.[1]

Recently, Ross and White[10] have described the use of catechol violet as a reagent for tin. These authors report the formation, at pH 2·5, of a red 2 : 1 complex of the reagent and tin(IV) with a high molecular extinction coefficient ($\epsilon_{555\ \text{m}\mu} = 65{,}000$). They also produced some evidence for the formation of a 1:2 complex ($\lambda_{\text{max.}} = 620$ mμ). Recently, Malát[11] has also investigated the use of catechol violet as a reagent for tin and observed that in the presence of gelatin there was a considerable bathochromic shift in the absorption maximum of the complex from 555 to 640 mμ. He was apparently unable to obtain the sensitivity reported by Ross and White, even in the presence of gelatin, which has a sensitising effect on the reaction, and the results he reports correspond to a $\epsilon_{640\text{m}\mu}$ value of 38,000 for the blue complex.

These observations prompted us to investigate this phenomenon and to study the effects of other dispersing agents on the catechol violet - tin complex, as the use of gelatin leaves much to be desired. From these investigations, cetyltrimethylammonium bromide was found to produce a more pronounced bathochromic shift of the absorption maximum (555 mμ to 662 mμ), and to increase greatly the sensitivity of the system ($\epsilon_{662\text{m}\mu} = 95{,}600$).

EXPERIMENTAL

REAGENTS—

Tin(IV) solution, 10^{-3} M—Dissolve 0·1187 g of tin metal (analytical-reagent grade) in 20 ml of concentrated sulphuric acid (analytical-reagent grade). After cooling, add the solution carefully to about 250 ml of ice-cold water, and add a further 60 ml of the sulphuric acid. Dilute to 1 litre.

Tin(IV) solution, 10^{-4} M—Prepare by appropriate dilution of 10^{-3} M tin(IV) solution when required.

Catechol violet, 10^{-3} M—Dissolve 0·286 g of catechol violet (Hopkin and Williams Ltd.) in distilled water and dilute to 1 litre.

Cetyltrimethylammonium bromide solution—Dissolve, with warming, 0·1 g of cetyltrimethylammonium bromide (obtainable from British Drug Houses Ltd.) in 100 ml of distilled water.

APPARATUS—

Spectrophotometer—Beckman DB with 1-cm glass cuvettes.

pH Meter—E.I.L. Vibron, model 39A.

PREPARATION OF CALIBRATION GRAPH—

Transfer by pipette aliquots increasing by 1 ml each time, from 1 to 10 ml, of 10^{-4} M tin(IV) solution and 5-ml aliquots of 10^{-3} M catechol violet solution into a series of 100-ml beakers. Add 2 ml of 0·1 per cent. cetyltrimethylammonium bromide solution and dilute each to about 50 ml. Adjust the pH of each solution to 2·2 with dilute ammonia solution by using a pH meter. Transfer the solutions to 100-ml calibrated flasks and dilute to the mark. After 10 minutes, measure the absorbances in 1-cm cuvettes against a reagent blank at 662 mμ. A plot of absorbance against tin concentration is a straight line that passes through the origin.

RESULTS AND DISCUSSION

SPECTRAL CHARACTERISTICS—

Fig. 1 curves A and B show the absorption spectra of catechol violet and its tin complex at pH 2·2, respectively, while curve C shows the effect of the addition of 0·002 per cent. of cetyltrimethylammonium bromide to the complex, as represented in curve B. It will be noted that the effect is a pronounced one, and that the absorbance of the reagent is small at the wavelength of maximum absorption of the sensitised tin complex (662 mμ), thus promoting conditions that are nearly ideal for analytical measurement.

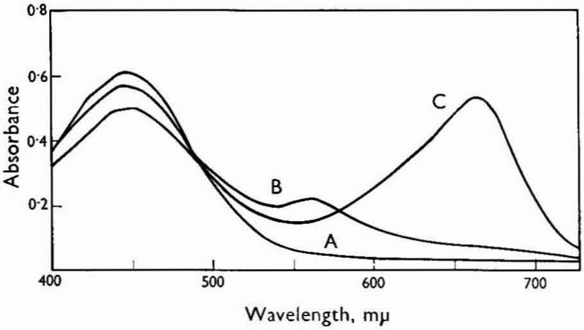

Fig. 1. Absorption spectra of tin(IV) - catechol violet system; curve A, 5 ml of 10^{-3} M catechol violet solution diluted to 100 ml at pH 2·2, measured against distilled water: 1-cm cuvette; curve B, as A but with the addition of 5 ml of 10^{-4} M tin(IV) solution before dilution to 100 ml; and curve C, as B but with the further addition of 2 ml of 0·1 per cent. cetyltrimethylammonium bromide solution

OPTIMUM CONDITIONS FOR COLOUR DEVELOPMENT—

Buffering experiments over the pH range of 0·5 to 4·0, *cf.* Fig. 2 curve A, revealed that maximum sensitivity was obtained at pH 2·2. This differs only slightly from the values quoted by the previous authors, *viz.*, pH 2·3[11] and 2·5.[10] The curve reveals that the reaction is dependent on the pH, but not critically so.

The maximum response was obtained with a 3-fold (molar) excess of catechol violet reagent relative to tin, *cf.* Fig. 2 curve B. The effect of using a greater concentration of reagent was to reduce the sensitivity of the reaction slightly, but this effect was insufficiently marked to place any practical restrictions on the proposed procedure.

Variation of the cetyltrimethylammonium bromide concentration in the range of 0·001 to 0·006 per cent. revealed, Fig. 2 curve C, that the maximum absorbance was obtained at about 0·002 per cent., and that a further increase in concentration produced an effect similar to that produced by excess of reagent.

The maximum absorbance of the complex was found to develop within 5 minutes and to remain constant for about 2 hours. After 2 hours a gradual decrease in absorbance was observed.

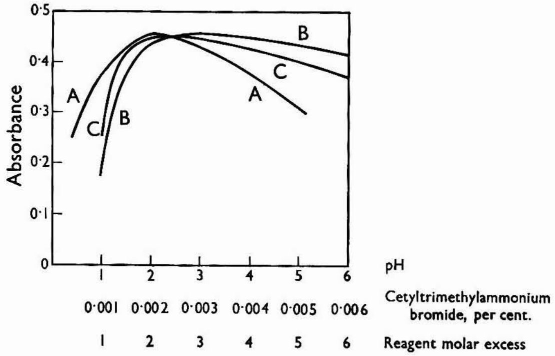

Fig. 2. Effects produced by variation of pH or of concentrations of catechol violet or cetyltrimethylammonium bromide: curve A, 5 ml of 10^{-4} M tin(IV), 5 ml of 10^{-3} M catechol violet and 2 ml of 0·1 per cent. cetyltrimethylammonium bromide solutions buffered at varying pH values, diluted to 100 ml; curve B, 5 ml of 10^{-4} M tin(IV), 2 ml of 0·1 per cent. cetyltrimethylammonium bromide buffered at pH 2·2 and varying excesses of catechol violet solution, diluted to 100 ml; and curve C, 5 ml of 10^{-4} M tin(IV), 5 ml of 10^{-3} M catechol violet solution buffered at pH 2·2 and 1·0 to 6·0 ml of 0·1 per cent cetyltrimethylammonium bromide solution, diluted to 100 ml

With the established optimum conditions, a calibration graph over the range 10^{-6} to 10^{-5} M tin concentration was prepared. Beer's law was obeyed and a molecular extinction coefficient of 95,600 was obtained at 662 mμ.

NATURE OF THE COMPLEX—

A continuous variation plot measured at 662 mμ exhibited a clear indication that a 2:1 catechol violet - tin complex was formed. Measurements at higher and lower wavelengths indicated that no other species were formed. A mole-ratio plot for the same system also indicated a 2:1 complex.

CONCLUSIONS

The bathochromic shift in the absorption spectrum of the catechol violet - tin(IV) complex, which may be caused by short range electrostatic forces operating on the surface of the micelle double layers of the cetyltrimethylammonium bromide,[12] provides the basis of a sensitive method of determining traces of tin(IV). The sensitivity of the method ($\epsilon_{662\ m\mu} = 95{,}000$) compares favourably with that reported by Ross and White[10] ($\epsilon_{555\ m\mu} = 65{,}000$), and, indeed, it will be seen from Fig. 2 curves A and B, that we have been unable to reproduce their results. The method also compares well with that reported by Malát[11] ($\epsilon_{640\ m\mu} \approx 38{,}000$) who used gelatin and is much more sensitive than standard methods based on toluene-3,4-dithiol ($\epsilon_{530\ m\mu} = 5800$)[13] and phenylfluorone ($\epsilon_{510\ m\mu} \approx 4000$), calculated from the data of Bennett and Smith.[14] The method is precise (with a percentage standard deviation of 0·6) and rapid.

The possibility of combining the selective solvent extraction of tin(IV) iodide with the cetyltrimethylammonium bromide sensitised reaction was investigated. The results obtained showed that the small amounts of iodide or iodine, or both, remaining in solution caused precipitation of the cetyltrimethylammonium bromide, thereby giving erroneous results.

Although we have not examined the method further, it would appear that the combination of this absorptiometric finish with a preliminary separation, such as distillation of the volatile bromide,[1] or precipitation of the tin as metastannic acid (with the aid of a carrier, if necessary, if the amount is small),[1] would provide a sensitive method for the determination of tin in commercial products.

We thank United Dairies Ltd., and the British Welding Research Association for financial aid in support of this work.

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Inorganic Thin-layer Chromatography

Part II.* Chromatography of Some First Row Transition Metals on Thin Layers of Substrates Impregnated with Tributyl Phosphate

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Several metal ions have been chromatographed on thin layers of cellulose and silica gel impregnated with tributyl phosphate at different concentrations of hydrochloric acid. The open nature of the cellulose results in the tributyl phosphate being held more firmly on this substrate than on the silica gel, and this in turn results in lower R_F values. The R_F values of the metal ions are also shown to vary with the concentration of the hydrochloric acid eluent.

LITTLE has been reported on the reversed-phase thin-layer chromatography of metal ions. Most authors have reported on the apparent lack of reproducibility of R_F values, which are seldom reported to a reproducibility greater than ±0·05 units. Markl and Hecht[1] attempted the reversed-phase thin-layer chromatography of some metal ions on thin layers of silica gel containing gypsum binder. Distances migrated by the solvent front and by the metal ions, rather than R_F values, were quoted for the systems tri-isooctylamine - mineral acid and tributyl phosphate (TBP) - mineral acid. Comparisons of the effectiveness of the several separations were not possible because these workers did not standardise the distance moved by the solvent front. The TBP-impregnated silica gel was heated for a total of 4 hours at 100° C before eluting with acid. It is, therefore, unlikely that sufficient TBP would be left in the silica gel to form a reversed-phase system. We have found that the vapour pressure of TBP at 100° C is sufficiently high to enable a glass plate "wetted" with TBP to become completely dry after heating at 100° C for 1½ hours in an air-oven, although the boiling-point of TBP is high. Hu[2] has reported the separation of titanium, thorium, scandium, niobium and uranium on thin layers of silica gel, previously eluted with TBP - benzene solution (30 + 70), with hydrochloric acid as eluent. This method of impregnation is known to give an uneven distribution of stationary phase over the layer,[3] and our experiments show that such a high impregnation coefficient does not give chromatograms suitable for comparative purposes. Hu and Liu used this system to separate selenium and tellurium in copper-anode slime,[4] and to separate niobium, tantalum and the noble metals.[5] Pierce and Flint[6] achieved the rapid separation of lanthanide mixtures on thin layers of corvic (a copolymer) impregnated with di-(2-ethylhexyl)orthophosphoric acid (HDEHP) and eluted with dilute hydrochloric acid.

Silica gel impregnated with HDEHP has also been used by Holzapfel and co-workers[7] to separate some rare earths. Brinkman and de Vries[8] separated metal ions on microscope slides dipped in silica gel slurried with a solution of a liquid ion exchanger, Amberlite LA-1.

Little systematic use has been made of the knowledge and technique of liquid - liquid extraction in the separation of trace amounts of metals by chromatographic processes. The reversed-phase chromatographic system may be regarded as a close approximation to a continual liquid - liquid extraction system,[9] and consequently there should exist a relationship between the stability constant of various metal complexes, their partition coefficients in a solvent system, and their R_F values in an analogous reversed-phase thin-layer chromatographic system.

The metals chosen for a study of this relationship were some of the transition metals. They were chromatographed by a reversed-phase thin-layer chromatographic technique with TBP and hydrochloric acid as the stationary and mobile phases, respectively.

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* For details of Part I of this series, see reference list, p. 35.

METHOD

REAGENTS—

Tributyl phosphate—General-purpose reagent, obtained from Hopkin and Williams, and purified.[10]

Hydrochloric acid solutions—Use analytical-grade reagent.

Carbon tetrachloride—Use "A" grade general-purpose reagent.

Solutions—Prepare aqueous solutions containing 5 mg per ml of analytical-reagent grade salts of Ti(IV), V(V), Cr(III), Mn(II), Co(II), Ni(II), Cu(II), Zn(II), Mo(IV), Ru(IV), Pd(II), Ag(I), Cd(II), In(III) and Sn(II).

Cellulose powder—Macherey-Nagel MN300HR, available from Camlab (Glass) Ltd., Cambridge.

Silica-gel powder—Macherey-Nagel MN Kieselgel N-HR, available from Camlab (Glass) Ltd., Cambridge.

SPRAY REAGENTS—

2-(*Pyridylazo*)-2-*naphthol*, 0·1 *per cent. w/v solution in ethanol*—This solution was used to identify Fe(II), Fe(III), Ni(II), Cu(II), Zn(II), Mn(II), Co(II), Zr(IV), Mo(VI), Ru(IV), Rh(II), Pd(II), Cd(II) and Sn(II).

Molybdenum and rhodium were visible as dark spots under ultraviolet light.

p-*Diethylaminobenzylidene rhodanine*, 0·1 *per cent. w/v solution in ethanol*—This solution was used for the identification of silver. The complex was visible as a bright yellow fluorescent spot on a dark background when viewed under ultraviolet light.

8-*Hydroxyquinoline*, 2 *per cent. w/v solution in chloroform*—This solution was used to identify titanium and vanadium which appeared as black spots after the plate had been heated at 110° C for 20 minutes.

PROCEDURE—

A 15-g portion of cellulose powder was mixed with 70 ml of a 5 per cent. v/v solution of TBP in carbon tetrachloride to give a homogeneous slurry. This was spread as an even layer 0·3 mm thick on five 20 × 20-cm plates by using a thin-layer chromatographic spreading device available from the Shandon Scientific Co. The plates were air-dried for 1 hour and spotted with 1-μl quantities of the 5 mg per ml metal ion solutions. After air-drying the spots, the plates were eluted with hydrochloric acid at 25° C ± 0·5° C in a modified version of the developing chamber described in an earlier paper[11]; instead of the two coated plates described, one was replaced by an uncoated plate on which was placed a 20 × 20-cm sheet of chromatography paper wetted with eluent phase (hydrochloric acid). The cellulose wick was found to contain considerable iron impurity and was replaced by one of glass-wool, which could be used repeatedly if thoroughly washed with de-ionised water and dried.[12] The vertical ascending development technique used gave developing times ranging from 1 to 3 hours, depending on the acid concentration of the eluent phase.

When elution was completed, the plates were dried for 20 minutes at 110° C to remove any remaining acid and most of the TBP. The positions of the metal ions on the plates were determined by spraying with a suitable chromogenic reagent and exposing the layers to concentrated ammonia vapour. Silica-gel layers were prepared in a similar manner with 30 g of powder and 70 ml of a 5 per cent. v/v TBP - carbon tetrachloride solution. The slurry was spread as an even layer 0·25 mm thick, dried in air for 1 hour and spotted with the four metal ions, manganese, cobalt, copper and zinc, (1 μl of the appropriate solutions). The plates were eluted with hydrochloric acid in the manner already described for cellulose.

To achieve maximum reproducibility of R_F values, great care was taken to standardise the extramolecular factors that influence chromatographic behaviour.[13,14] Each chromatographic system was repeated several times, and the R_F values quoted for each metal species are the average of at least four values, each within the normally accepted tolerance ($\pm 0{\cdot}02$ R_F units) for work on thin-layer chromatography.

RESULTS

Tables I, II, and III show the results obtained. Table I gives the average R_F values of the metal ions chromatographed on cellulose and silica-gel layers impregnated with 0, 2·5 and 5·0 per cent. v/v TBP, by using 6 M hydrochloric acid as the eluent. Table II gives the average R_F values of manganese, cobalt, copper and zinc, chromatographed on silica gel impregnated with 5 per cent. TBP solution and eluted with various acid concentrations. The average R_F values for the same four metal ions on layers of cellulose impregnated with 5 per cent. TBP solution are shown in Table III: values are quoted for several acid concentrations.

TABLE I

R_F VALUES OF METAL IONS IN 6 M HYDROCHLORIC ACID ON TBP-IMPREGNATED SILICA GEL AND CELLULOSE, AND UNTREATED SILICA GEL AND CELLULOSE

Metal	Ti(IV)	V(V)	Cr(III)	Mn(II)	Co(II)	Ni(II)	Cu(II)	Zn(II)
Cellulose	0·92	0·96	0·99	0·98	0·98	0·98	0·95	T
Cellulose - 2·5 per cent. TBP	0·87	0·59 0·70 0·85	0·94	0·90	0·88	0·88	0·84	0·54
Cellulose - 5·0 per cent. TBP	0·89	T	0·91	0·89	0·85	0·88	0·82	0·47
Silica	0·84	1·00	1·00	1·00	1·00	1·00	1·00	T
Silica - 5·0 per cent. TBP ..	—	—	—	0·91	0·89	0·89	0·87	0·50

TABLE I—*continued*

Metal	Mo(VI)	Ru(IV)	Rh(II)	Pd(II)	Ag(I)	Cd(II)	In(III)	Sn(II)
Cellulose	T	0·89	0·90	0·76	T	0·90	1·00	T
Cellulose - 2·5 per cent. TBP	0·00	0·56 0·86	0·85	0·70	0·86	0·42	0·13	0·00
Cellulose - 5·0 per cent. TBP	0·00	0·45 0·86	0·84	0·67	0·80	0·32	0·06	0·00
Silica	T	1·00	1·00	1·00	T	1·00	1·00	T
Silica - 5·0 per cent. TBP ..	0·00	0·49 0·92	0·93	0·81	0·75	0·37	0·12	0·00

where T = tailed. Multiple values indicate two or more spots appear on spraying with indicator.

TABLE II

MEAN R_F VALUES FOR MANGANESE, COBALT, COPPER AND ZINC ON SILICA GEL - TBP (5 PER CENT.) LAYERS AT VARIOUS ACID CONCENTRATIONS

	Acid concentration, M										
Metal ions	1·0	2·0	3·0	4·0	4·5	5·0	6·0	7·0	7·5	8·0	9·0
Manganese(II) ..	1·00	0·98	0·97	0·97	0·97	0·97	0·91	0·86	0·86	0·84	0·82
Cobalt(II)	1·00	0·98	0·97	0·96	0·96	0·96	0·87	0·82	0·80	0·78	0·76
Copper(II)	1·00	1·00	0·98	0·96	0·96	0·96	0·87	0·85	0·83	0·83	0·82
Zinc(II)	0·71	0·34	0·30	0·34	0·38	0·45	0·50	0·55	0·61	0·66	0·71

TABLE III

R_F VALUES FOR MANGANESE, COBALT, COPPER AND ZINC ON CELLULOSE - TBP (5 PER CENT.) LAYERS AT VARIOUS ACID CONCENTRATIONS

	Acid concentration, M										
Metal ions	1·0	2·0	3·0	4·0	4·5	5·0	6·0	7·0	7·5	8·0	9·0
Manganese(II) ..	1·00	0·97	0·96	0·95	0·95	0·95	0·89	0·87	—	0·76	0·66
Cobalt(II)	1·00	0·97	0·96	0·95	0·95	0·95	0·85	0·83	—	0·72	0·65
Copper(II)	—	—	0·96	—	0·95	0·91	0·82	—	0·76	0·74	0·67
Zinc(II)	—	—	0·18	—	0·36	0·40	0·47	—	0·55	0·59	0·62

The R_F values have a reproducibility generally better than ± 0·02. The reproducibility was found to alter with acid concentration; with 9M hydrochloric acid it was slightly less than ± 0·02, at values between 7·5 and 3M it was ± 0·02, but between 3 and 0·1M the reproducibility of R_F values was about ± 0·01.

DISCUSSION OF RESULTS

ESTABLISHMENT OF EXPERIMENTAL CONDITIONS—

A series of runs with concentrations of TBP in carbon tetrachloride varying from 0 to 20 per cent. showed that a concentration of 5 per cent. was the highest that could be used to obtain chromatograms suitable for comparative purposes. Loading the plates with concentrations of TBP greater than this gave rise to the phenomenon of "double-fronting," *i.e.*, the appearance of two distinct eluent fronts rising up the plate. The lower of these two fronts was very uneven and generally caused considerable distortion of the spots. On drying the plates in an air-oven after development of the chromatogram, the area below the lower front was seen to dry much more quickly than the area between the two fronts, thus indicating the presence of a higher boiling substance in the latter area. The greater the concentration of the acid used, the greater was the distortion of the spots, and it is suggested that the hydrochloric acid mobile phase acts as a "molecular plough" sweeping the excess TBP off the cellulose or silica-gel layers, *i.e.*, the plates were overloaded. A similar effect with paper has been noted previously.[15]

Comparisons were made between plates impregnated with TBP pre-treated with eluent acid and those impregnated with untreated TBP. Differences in R_F values were within the experimental differences of $\pm 0{\cdot}02$, and so pre-treatment was not considered necessary.

The use of untreated TBP had the advantage that any impregnated plate could be used with any eluent acid. The fact that there was not a significant difference between R_F values obtained from plates impregnated with TBP which contained some eluent acid, and those obtained from plates impregnated with untreated TBP, can be taken as evidence that adsorption of the eluent by the TBP is a relatively fast process, and is complete by the time the eluent front has reached the spots at the point of application.

In general, R_F values are higher on silica-gel layers than on the corresponding cellulose layers (Tables I and III). Ideally, the substrate should act only as an inert support for the TBP stationary phase, but the results in Table I show that for cellulose this is probably not so. When the metal ions were chromatographed on untreated silica gel, they all travelled with the solvent front. On untreated cellulose, however, some of the metal ions were retained immediately behind the eluent front while others, namely zinc, ruthenium, palladium and cadmium, gave discrete spots separated from the solvent front.

During development of the chromatograms, the cellulose retained the TBP much more efficiently than did the silica gel. The latter was particularly susceptible to double-fronting, *viz.*, for acid concentrations above 6 M, slight double-fronting occurred on 5 per cent. TBP - silica gel layers, the degree increasing with increasing acid concentration. On 5 per cent. TBP - cellulose layers, however, no double-fronting occurred at any acid concentration and so the substrate structure is a significant factor in chromatographic behaviour. Honeyman[16] has shown from X-ray studies that cellulose fibres are a random mixture of highly organised regions, termed crystalline cellulose, and non-ordered regions, termed amorphous cellulose. In the former regions, strong inter-chain hydrogen bonding occurs, while in the latter regions the hydroxyl groups are available for hydrogen bonding with molecules other than cellulose. Thus the reactivity of the cellulose is associated with the amorphous structure, as are the properties of swelling and sorption of water and other oxonium systems, *e.g.*, TBP. As Consden[17] has pointed out, these properties of the amorphous regions are extremely important in chromatographic mechanisms.

It is suggested that the lower R_F values on cellulose - TBP layers compared with those obtained on silica gel - TBP layers may be explained on this basis, *i.e.*, there will be a greater tendency for the TBP to associate with the cellulose, possibly by hydrogen bonding in the amorphous regions, than with the silica gel. This suggestion can also account for the more ready formation of a double-front on silica gel - TBP layers than on cellulose - TBP layers. To check this view we carried out a number of runs on a crystalline grade of cellulose[18] (Thin-layer Chromedia), which has low absorption properties. The R_F values obtained by using this grade of cellulose were even greater than those obtained on silica gel, and the system was even more prone to double-fronting with 5 per cent. v/v TBP solution than silica gel. The greater tendency to double-fronting in all systems as the acid concentration increases is probably caused by the cleavage of the TBP - cellulose hydrogen bonds by the hydrogen ions in the mobile phase.

The variations in the R_F values of the four metal ions, manganese, cobalt, copper and zinc with increasing concentration of hydrochloric acid are shown for the system silica gel - TBP (5 per cent. v/v) in Table II, and for the system cellulose - TBP (5 per cent. v/v) in Table III. The effect of increasing acid concentration is similar for both, although the values in the former system are higher. For the metal ions manganese, cobalt and copper, the R_F values generally fall as the acid concentration increases from 1·0 to 9·0 M. For zinc, however, a minimum R_F value was observed with 3 M hydrochloric acid. It is of interest to note that the behaviour of those ions show a strong resemblance to their extraction behaviour in the hydrochloric acid - TBP system reported by Ishimori and co-workers.[19] Table I shows several good separations of the metal ions chromatographed. For example, copper, zinc, molybdenum, palladium, cadmium, indium and tin can all be separated from each other in the cellulose - TBP (5 per cent.) - 6·0 M hydrochloric acid system. The chromatographic behaviour of those metal ions in other acid concentrations is being studied.

The multiple spots shown for vanadium and ruthenium are probably caused by the presence of more than one extractable species.[20]

CONCLUSION

In the reversed-phase thin-layer chromatography of metal ions with tributyl phosphate as the stationary phase, the retention of the stationary phase by the substrate is related to the availability of active sites on the so-called inert support. The greater the availability of these active sites, the greater the retention of the stationary phase and the lower the R_F values.

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NOTE—Reference 12 is to Part I of this series.

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A Thin-layer Chromatographic Method for the Determination of "Quassin" in Cosmetic Preparations

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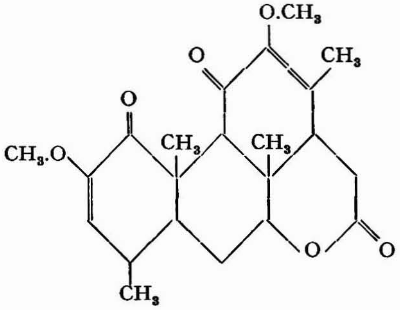
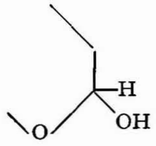
A method is described by which "quassin" may be determined when used as a denaturant for the alcohol contained in cosmetic preparations. The two bitter principles, neoquassin and quassin are determined together as quassin, after oxidation with a solution of sodium dichromate in glacial acetic acid. Perfume oils are first removed by solvent extraction and the aqueous phase is then evaporated to dryness and the residue oxidised. Further solvent-extraction steps yield a solution of quassin in chloroform which is applied to a thin-layer chromatographic plate. The spots are compared visually with standards prepared from an oxidised "quassin" solution to permit determinations within the range of 5 to 36 p.p.m. in samples.

"QUASSIN," extracted from the wood of the *Simarubaceae* family of trees, has been used for many years as a denaturant for the alcohol used in cosmetic preparations. It is odourless and intensely bitter. The concentration required in Great Britain for the partial denaturing of industrial methylated spirit preparations is about 20 to 30 p.p.m. The chemical structures of the two bitter principles of "quassin," *viz.*, quassin (**I**) and neoquassin (**II**), have recently been elucidated,[1,2,3] and are represented below.



(I)



(II)

Nothing appears to have been published on the analytical chemistry of "quassin." The term "quassin" used in this paper refers to the commercial material which generally contains quassin, neoquassin and other substances.

The efficacy of "quassin" when used as a denaturant is judged by the extent to which it produces an unpalatable bitterness, but it is desirable that this effect of bitterness should be related to a chemical assessment of the amount of "quassin" present. Two reactions appeared potentially useful. In the first, the lactone ring of quassin opens in alkaline solution[4,5] to give a compound no longer extractable by, for example, chloroform, whereas neoquassin is unaffected by alkalis; quassin can be recovered on acidifying the solution. Secondly, neoquassin is oxidised to quassin by a solution of sodium dichromate in glacial acetic acid.[4,6] Thin-layer chromatography was also considered as a possible means of isolating the bitter principles. This technique has been used by other workers[2,7] for the identification of quassin and related substances.

Pure specimens of quassin and neoquassin were prepared from commercial "quassin"[4,5] and were found to have about the same degree of bitterness. Four "quassin" samples were each shown by thin-layer chromatography to contain a total of about 90 per cent. of bitter material. Three of these samples, which were of British manufacture and presumably extracted from the wood of *Picrasma excelsa*, as specified in the British Pharmacopoeia (1953), contained a preponderance of neoquassin. The fourth sample was of French manufacture from the wood of *Quassia amara* and consisted predominantly of quassin.

The method studied for the examination of perfume samples involved extraction of the sample to remove essential oils, and oxidation of the residue obtained by evaporating the aqueous phase, followed by solvent extraction. The extracts were subjected to thin-layer chromatography and the quassin spots from the sample compared visually with those from standard solutions.

EXPERIMENTAL

Quassin is soluble in chloroform, ethyl acetate and benzene, and sparingly soluble in ether or light petroleum.[4] Aqueous solutions of quassin were extracted separately with carbon tetrachloride, ether and light petroleum (b.p. 40° to 60° C); only light petroleum failed to extract a significant amount of quassin and this solvent was therefore chosen for the extraction of the essential oils. Normally, a 5-ml volume of perfume would be diluted with water before extraction and the aqueous phase afterwards evaporated to dryness.

OXIDATION—

The oxidant used was a 10 per cent. w/v solution of sodium dichromate in glacial acetic acid,[6] with a reaction time of 45 minutes at 25° C, or 1 hour at 20° C. Pure quassin was unaffected and could be recovered completely, but neoquassin invariably suffered a loss of about 15 per cent. This loss was not diminished by reducing the sodium dichromate concentration to 2 per cent. w/v, nor by changing the time or temperature of reaction. Oxidation was incomplete when the oxidant was diluted with an equal volume of N sulphuric acid, and when the glacial acetic acid was replaced by 80 per cent. v/v acetic acid. A solution of alkaline hydrogen peroxide in acetone had no effect on neoquassin. Potassium permanganate, whether in acetone solution or in alkaline aqueous solution, left no detectable amounts of either neoquassin or quassin.

Solutions containing about 1 per cent. w/v of "quassin" in industrial methylated spirit are available commercially and are similar to those used for denaturing purposes. Five such solutions were each diluted with ethanol to give a concentration of 1 mg of "quassin" per ml (nominal) and an aliquot (0·1 ml) of each solution was evaporated to dryness; each residue was dissolved in 0·25 ml of chloroform and aliquots of the chloroform solutions were chromatographed as described in the Method. The spots that developed after spraying with potassium permanganate solution were assessed visually by comparison with spots of quassin and neoquassin standards on the same plate. Further 0·1-ml aliquots of the solutions containing 1 mg per ml of "quassin" were evaporated to dryness and oxidised with sodium dichromate solution as in the Method. Each sample was then diluted with 50 ml of water and extracted with chloroform as described, but omitting the pH adjustments and the preliminary extraction with carbon tetrachloride. Aliquots of the final chloroform solutions were chromatographed as before, and the developed spots compared visually with spots of quassin standards on the same plate.

The recoveries were reasonably consistent at 70 per cent. and warranted the use of oxidised "quassin" solution for chromatographic standards to compensate for oxidation losses when the method was applied to cosmetic samples.

The oxidation step was valuable in that it decomposed perfume residues remaining after the initial extraction and thus reduced possible interference effects on the chromatogram. Neither the extraction nor the oxidation alone gave an adequate degree of purification for chromatography.

EXTRACTION OF QUASSIN—

If the oxidised sample is made alkaline with sodium hydroxide solution, quassin is converted to sodium quassinate[4] and more impurities may be removed by extraction with carbon tetrachloride. Chloroform was found to extract a portion of the quassin despite the presence of an excess of sodium hydroxide. The addition of dilute acid decomposed the sodium quassinate with the regeneration of quassin, which could then be extracted by chloroform. In some experiments chromatographic spots were found which corresponded with neither neoquassin nor quassin. They were probably caused by hydrolysis products that resulted from temperature rises during pH adjustments; the action of heat on alkaline solutions of quassin is to cause hydrolysis with the formation of a stable acid.[2,4,5] Subsequently, to minimise temperature rises, acid and alkali solutions were cooled in an ice-bath before use, which eliminated the appearance of spurious spots.

CHROMATOGRAPHY—

Silica gel used as adsorbent on thin-layer plates was found to give better separations of neoquassin and quassin than alumina or cellulose because of its stronger adsorptive properties. Many solvent combinations were examined for developing the chromatograms on silica gel; the best of these were found to be—

A. Ether - glacial acetic acid - water (65 + 25 + 10). Approximate R_F values: neoquassin 0·75; quassin 0·65.

B. Chloroform - methanol (90 + 10). Approximate R_F values: neoquassin 0·65; quassin 0·75.

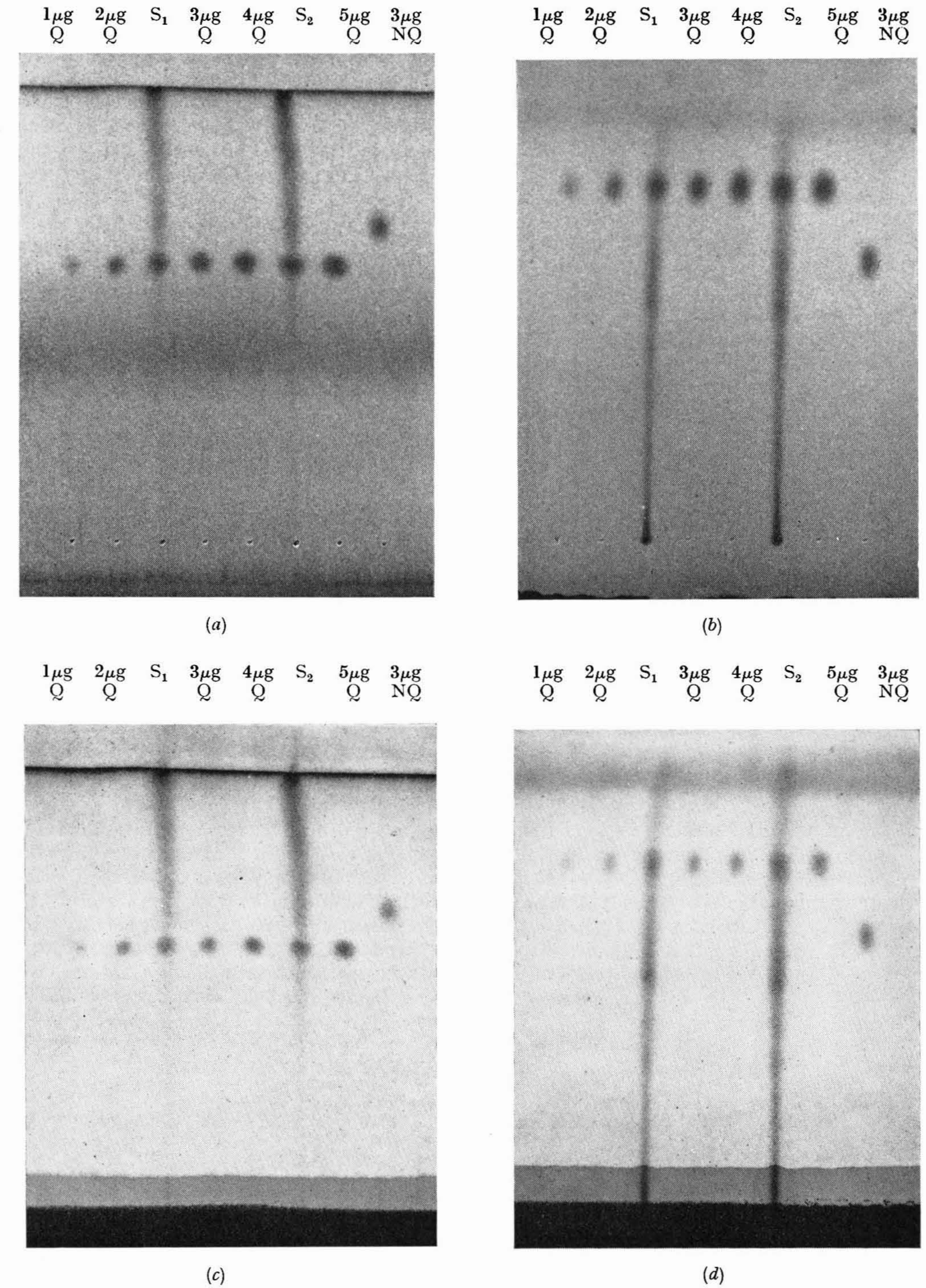
This solvent has been used in a study of extracts of the wood of *Picrasma excelsa*.[8]

The chromatograms are illustrated in Fig. 1. The order of elution was different in these two systems, a fact that was useful in confirming the identity of doubtful spots from cosmetic samples. Both neoquassin and quassin were strongly adsorbed from chloroform solution at the point of application on the plate so that the volume of the solution applied was not important unless other strongly adsorbed materials were also present. Solvent mixture A was generally preferred because, with cosmetic samples, most of the foreign matter was carried beyond the quassin spot. With solvent mixture B, streaks were frequently found over most of the chromatogram although the quassin spots were usually clear and capable of assessment.

Several spray reagents were examined, but none was found to give a selective reaction with quassin and neoquassin. The simplest method of spot location was to incorporate an inorganic additive in the silica gel, which caused it to fluoresce when irradiated with ultraviolet light (wavelength 254 mμ). Neoquassin and quassin each absorb light strongly at this wavelength and show as dark spots. A similar effect may be obtained by spraying an ordinary silica gel plate with rhodamine B solution in ethanol and examining the absorption of fluorescence at 254 mμ. Alternatively, the plate can be sprayed with 0·5 per cent. potassium permanganate solution, dried at 60° C for 10 minutes, and then the excess permanganate eluted with water[9]; neoquassin and quassin leave brown spots on a white to pale buff background. Other spray reagents that reacted with the two bitter materials were concentrated sulphuric acid and a solution of molybdophosphoric acid in ethanol, after heating at 110° C in both instances. Ammoniacal silver nitrate reacted with neoquassin, but not quassin, after heating at 110° C. The fluorescence - absorption method and the potassium permanganate method were considered to be best, each giving a well graded series of standard spots from 1 to 5 μg with both neoquassin and quassin; the gradation from 5 to 8 μg was slightly poorer. The fluorescence - absorption method showed less foreign matter from cosmetic samples than the potassium permanganate method, but the latter was considered better for spot matching.

PREPARATION OF STANDARDS—

Pure neoquassin and quassin were separated from commercial "quassin" and purified by published methods.[1,4,5] Neoquassin was separated by crystallisation from methanolic potassium hydroxide solution diluted with 3 volumes of water. It was purified by treatment with charcoal, followed by a second crystallisation, and finally, by crystallisation from dilute methanol (1 + 3). The product melted at 228° C and showed no impurities by thin-layer chromatography. Quassin was recovered from the mother liquor by saturating it with carbon dioxide, and purified by chromatography on an alumina column with chloroform as eluent. The alumina was mixed with an inorganic fluorescent agent (Woelm, 2 per cent. w/w) and packed in a fused silica tube. The quassin could be seen moving as a dark band in ultraviolet light (254 mμ). Subsequent treatment with charcoal and crystallisation from dilute methanol (1 + 3) yielded crystals that showed no impurity by thin-layer chromatography, and that melted at 222° C. Because of the low content of quassin in most commercial "quassins," a second sample of quassin was prepared, after oxidation of the "quassin" with sodium dichromate in acetic acid solution[4] to convert neoquassin to quassin. Preparative-layer chromatography was also found to be feasible on plates coated with a 2-mm layer of fluorescent silica gel (Merck kieselgel HF_{254}, without binder). Five successive developments were necessary in a solvent mixture of chloroform and methanol (95 + 5), with a loading of about 5 mg of "quassin" per cm width of plate. Standard solutions for chromatography were prepared by



(*a*) (*b*)

(*c*) (*d*)

Fig. 1. Thin-layer chromatograms run in: (*a*) and (*c*), ether - glacial acetic acid - water (65 + 25 + 10 v/v); (*b*) and (*d*), chloroform - methanol (90 + 10 v/v). Q = quassin, NQ = neoquassin, S_1 and S_2 are 10 and 15 μl, respectively, of a perfume extract. Plates (*a*) and (*b*) are photographed in ultraviolet light (254 mμ), and plates (*c*) and (*d*) after spraying with potassium permanganate solution and eluting the excess of reagent

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dissolving 10 mg of pure quassin or neoquassin in 10 ml of chloroform. Ethanol was used as solvent in standard solutions to be added to cosmetic samples for recovery tests.

Standard quassin solutions for routine analysis are prepared from oxidised "quassin." The "quassin" should be the commercially pure material which normally contains about 90 per cent. of total quassin and neoquassin, and is white to pale yellow in colour; solutions should be colourless to straw coloured. Some foreign "quassin" powders have been found to be diluted and to contain 10 per cent., or less, of total quassin and neoquassin. A 1 per cent. w/v ethanolic solution of "quassin" is standardised by diluting with water to 10 p.p.m. of "quassin" and measuring the absorption on a spectrophotometer against a 1 per cent. v/v ethanol blank.

Correction for absorption by impurities may be made by use of a method similar to that used by Morton and Stubbs[10] in the determination of vitamin A. At the peak wavelength of 257·5 mμ which is used in this correction, the optical density of neoquassin is about 4 per cent. higher than that of quassin. This type of correction has been criticised by Wilkie[11] who proposed measurements based upon the difference in absorption at two wavelengths on the slope of the spectral curve. Neither type of correction shows any marked advantage over the other when applied to "quassin" solutions, so the Morton and Stubbs' type was adopted as it has been used for several years in conjunction with potability tests.

METHOD

PREPARATION OF STANDARD QUASSIN SOLUTION

Evaporate a 5-ml portion of the "quassin" solution (1 per cent. w/v) just to dryness on a water-bath. Add 5 ml of ethanol and again evaporate to dryness. Dissolve the residue in ethanol to make 50 ml of solution. Dilute 10 ml of this solution to 1 litre with water and measure the absorption at 245, 257·5 and 270 mμ in a 1-cm cell against a 1 per cent. v/v ethanol solution. Calculate the corrected value for $E^{1\%}_{1\,\text{cm}}$ as follows—

$E^{1\%}_{1\,\text{cm}}$ at 257·5 mμ $= a$; at 245 mμ $= b$; and at 270 mμ $= c$.

The correct $E^{1\%}_{1\,\text{cm}} = 2{\cdot}148\ [2a - (b + c)]$.

$E^{1\%}_{1\,\text{cm}}$ for crystalline "quassin" at 257·5 mμ is 302.

$$\text{"Quassin" content of sample} = \frac{\text{corrected } E^{1\%}_{1\,\text{cm}} \times 100}{302}, \text{ per cent.}$$

Calculate the volume of solution required to yield 1 mg of quassin from the known concentration of "quassin"; make a correction for the loss of 30 per cent. found after oxidation, as shown in Table I. Evaporate this volume to dryness and oxidise the residue with 2 ml of the 10 per cent. w/v sodium dichromate solution as described for the extraction of quassin. Dilute the solution with 50 ml of water and, omitting the pH adjustments and carbon tetrachloride extraction, extract the quassin with chloroform as described below. Evaporate the chloroform to dryness and dissolve the residue in exactly 1 ml of chloroform to give a quassin concentration of 1 μg per μl.

TABLE I

RECOVERY OF QUASSIN FROM "QUASSIN" AFTER OXIDATION

Sample	Quassin found, μg	Neoquassin found, μg	Total, μg	Quassin found after oxidation, μg	Recovery, per cent.
1	25	62	87	60	69
2	75	41	116	83	71
3	33	50	83	58	70
4	33	50	83	58	70
5	25	58	83	58	70

EXTRACTION OF QUASSIN

REAGENTS—

Light petroleum (b.p. 40° to 60° C).

Carbon tetrachloride—Use analytical-reagent grade.

Chloroform—Use analytical-reagent grade.

Sodium hydroxide, N.

Sulphuric acid, N.

Sodium dichromate solution—Dissolve 1 g of sodium dichromate dihydrate in 10 ml of glacial acetic acid.

PROCEDURE—

Place 50 ml of water in a 100-ml separating funnel, add 5 ml of sample, and mix. Add 20 ml of light petroleum (b.p. 40° to 60° C) and shake the funnel for 1 minute. Transfer the lower aqueous phase to a second 100-ml separating funnel. Wash the walls of the first separating funnel with a few millilitres of water and transfer the washings to the second separating funnel. Shake the aqueous phase again with 20 ml of light petroleum and run the lower layer into a 250-ml beaker. Wash the walls of the separating funnel with a few millilitres of water and run this into the beaker. Evaporate the aqueous solution to dryness on a steam-bath and cool. Add 2 ml of sodium dichromate solution, cover the beaker and allow it to stand for 45 minutes at 25° C, or 1 hour at 20° C. Add 40 ml of N sodium hydroxide solution that has been cooled in an ice-bath, to give a yellow solution showing a strongly alkaline reaction to pH test-paper, and transfer the solution to a 100-ml separating funnel. Shake it twice with 20-ml portions of carbon tetrachloride for 1 minute each and discard the lower layers. Add 10 ml of N sulphuric acid that has been cooled in an ice-bath, to produce a colour change to the orange dichromate; the solution is buffered at about pH 7 by the sodium acetate present. Shake the solution with three 20-ml portions of chloroform for 1 minute each, and run each lower layer in turn through a dry filter-paper into a 250-ml beaker. Evaporate the combined extracts to dryness, dissolve the residue in 1 ml of chloroform, and transfer to a glass-stoppered test-tube, about 100 mm long and 15 mm in diameter. Wash the beaker successively with two 1-ml portions of chloroform and add them to the test-tube. Evaporate the contents of the tube carefully to dryness on a steam-bath, cool, and dissolve the residue in exactly 0·25 ml of chloroform. This is the sample extract ready for thin-layer chromatography.

THIN-LAYER CHROMATOGRAPHY

REAGENTS—

Silica gel—Kieselgel GF_{254} (E. Merck and Co.).

Standard solution of quassin, 1 µg per µl in chloroform—(see "Preparation of standard quassin solution").

Spray reagent—Dissolve 0·5 g of potassium permanganate in 100 ml of water.

Developing solvent—Mix 65 ml of diethyl ether, 25 ml of glacial acetic acid and 10 ml of water.

PROCEDURE—

Shake 30 g of silica gel with 60 ml of water for 2 minutes. Spread the mixture immediately over a row of 5 plates, 20 × 20 cm, to a thickness of 0·25 mm. Allow the plates to dry in air, and activate them by heating in an oven at 120° C for 1 hour. Store them until required over silica gel in a desiccator; plates should be re-activated daily before use. Line the inside walls of a chromatographic tank, about 22 cm long by 9 cm wide and 20 cm high, with a sheet of filter-paper and add 100 ml of developing solvent 30 minutes before use.

Apply with a capillary micro-pipette, spots of 10 and 15 µl of the sample extract, in 5-µl aliquots, about 1·5 cm above the bottom of the plate. Allow each aliquot to dry before applying the next. Also apply spots of standard quassin solution to the plate to contain 1, 2, 3, 4 and 5 µg of quassin. Place the plate in the tank and allow the solvent to rise about 10 cm; this requires about 45 minutes. Remove the plate, allow it to dry in air and then in an oven at 120° C for 10 minutes. Allow the plate to cool and compare the sample spots visually with the standards under ultraviolet irradiation at 254 mµ. The spots are dark against a green fluorescent background. Glass or plastic goggles, or ordinary spectacles should be worn to protect the eyes from the harmful effects of the ultraviolet light. The spots may be assessed to the nearest 0·5 µg of quassin. Alternatively, spray the dried plate evenly with 0·5 per cent. w/v potassium permanganate solution until visibly damp. Warm the plate in an oven at 60° C until just dry (about 10 minutes), and then place it in a tank containing a 1-cm layer of water. Allow the water to rise until the front has carried the excess of potassium permanganate past the developed spots, and dry the plate in an oven at 120° C. The silica-gel layer can be protected by covering with a clean glass plate. Multiply the assessed quassin value by 1·4 to obtain the equivalent amount of "quassin."

RESULTS AND DISCUSSION

"Quassin" is generally added to cosmetic preparations of British manufacture as one of the denaturants when alcoholic formulations are used; the concentration of "quassin" required for denaturing may vary according to the different regulations governing different types of products, and may also vary with the alcohol content. The samples investigated during the development of this method were expected to contain 15 to 20 p.p.m. of bitter material, expressed as quassin after allowance had been made for alcohol content and for the purity of the "quassin." Commercial "quassins" have usually been found to contain not more than 90 per cent. w/v of total quassin and neoquassin. The method described was applied to some typical cosmetic samples and to perfumes denatured in the laboratory by known additions of "quassin." The results are shown in Table II.

TABLE II

DETERMINATION OF "QUASSIN" IN COSMETIC PREPARATIONS

Sample	"Quassin" present, p.p.m.	"Quassin" found, p.p.m.
Hair lacquer	20	22
Eau de Cologne I	—	16
Eau de Cologne II	—	16
Toilet water I	—	18
Toilet water II	17	18
Perfume I	—	15
Perfume II	—	18
Synthetic perfume I	15	16
Synthetic perfume II	15	16
Synthetic perfume III	12	11

The synthetic perfumes were compounded in this laboratory from a sample of mixed perfume oils. The values shown in column 3 are theoretical "quassin" contents based on known alcohol contents in these samples. The other samples would be expected to contain from 15 to 20 p.p.m. of "quassin," depending upon alcohol content. Some further samples gave results lower than the accepted minimum. These were analysed again after the addition of standard "quassin" solution to test for completeness of recovery.

TABLE III

RECOVERY OF "QUASSIN" FROM COSMETIC SAMPLES

Sample	"Quassin" found in sample, p.p.m.	"Quassin" added, p.p.m.	Total "quassin" found, p.p.m.	Recovery of added "quassin," per cent.
Toilet water ..	12	17·5	29	98
After-shave lotion I	11, 11	17·5	25	88
After-shave lotion II	12, 12, 11	17·5	29	98
Hair tonic	11, 11	17·5	29	102
Perfume	11, 11	17·5	27	95

The recoveries of added "quassin," shown in Table III, confirmed that the original samples were low in "quassin" and that no appreciable losses had occurred that could be attributed to failure of the method. However, two cosmetic preparations failed to yield results by the method described. One of these was a beer hair-setting lotion, which left a gummy residue on evaporation of the aqueous solution after the initial extraction with light petroleum. This residue did not dissolve in the oxidising solution and little oxidation occurred. Heavy interference was found on chromatograms in each of the two developing solvents. The other sample was a bath essence that contained 55 per cent. of detergent that was probably anionic. Extractions with both light petroleum and carbon tetrachloride led to emulsions which had to be spun in a centrifuge to recover the aqueous phase. The chloroform extraction also emulsified, the chloroform phase forming a gel.

The lower limit of determination by the method described is 1 μg of quassin; this is equivalent to 1·4 μg of the standardised "quassin," or 5 p.p.m., on a 5-ml sample. Lower concentrations of "quassin" may be determined for most samples by applying larger aliquots to the chromatographic plate; by using less chloroform to dissolve the final extract residue; or by taking a larger initial sample. The accuracy of the method is governed by the accuracy of the spot matching. Spots are usually assessed to the nearest 0·5 μg, which limits the maximum error to 10 per cent. on the 5-μg standard and to 50 per cent. on the 1-μg standard. Sample aliquots are generally chosen so that matching with the upper end of the standard range is possible. The accuracy of the method was found to be adequate for the purpose of this investigation.

"Quassin" solutions may be examined directly by chromatography after evaporation and dissolution of the residue in chloroform; the sample spots are compared with standard ranges of both quassin and neoquassin.

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The Determination of Niobium in Metals and Alloys

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A method is described for the determination of niobium in metals and alloys. Niobium is separated from the matrix elements by adsorption on to Dowex 1 anion-exchange resin from a solution of hydrofluoric acid and nitric acid. The niobium is eluted in a mixture of hydrofluoric and nitric acids and determined by the spectrophotometric measurement of the blue complex formed between niobium(V) and bromopyrogallol red.

The method has been applied to the determination of niobium in iron, steel, tantalum and zirconium.

CURRENT interest in the mechanical properties of low carbon steel containing small amounts of niobium has resulted in the need for a simple method of analysis for this element. The problem posed requires for its solution, first, a simple quantitative method for separating the niobium from the matrix, and secondly, an accurate and precise method for determining the niobium.

The separation of niobium from steel has been a difficult problem, especially when tantalum is present. Methods in which tannin is used for the fractional precipitation of niobium and tantalum in complexes with oxalic acid and tartaric acid,[1,2] and the behaviour of the ammonium fluoro complexes of niobium and tantalum in a cellulose column with ketone solutions[3] are complicated by the presence of large amounts of other ions.

For some time past in this laboratory, work has been carried out with anion-exchange resin and eluents of mixtures of hydrofluoric and nitric acids at various strengths. This has led to the development of simple techniques for the separation of elements such as niobium and tantalum from each other, and from iron, iron alloys, cobalt, chromium, nickel and zirconium. The advantage of the procedure evolved is that the niobium is separated "cleanly" in a solution which can be evaporated to leave a negligible residue, apart from the niobium. Also, no errors caused by hydrolytic precipitation occur in the hydrofluoric acid - nitric acid mixtures used.

The only alloying elements found in steel that are retained on the anion-exchange resin, together with niobium, are tantalum and boron. Tantalum remains adsorbed when the niobium and boron are eluted. Boron is volatilised and completely removed during subsequent evaporation of the hydrofluoric acid - nitric acid eluate. The only other elements known to be adsorbed under the conditions described, are rhenium, iridium and tellurium.[4] The separation procedure can be used to concentrate small amounts of niobium from large amounts of sample, thus enabling amounts of niobium as small as 1 p.p.m. to be determined.

The technique in which reactions that form colours are used, followed by spectrophotometric measurement, is accepted as the most suitable for determining small amounts of niobium.[5] Until recently only one colorimetric method was known that gave accurate results, namely that with hydrogen peroxide, followed by measurement of the absorption in the ultraviolet range. This method is not very sensitive, and the presence of titanium causes interference. A method in which PAR is used has also been published.[6,7]

Determination by measurement of the optical density of the yellow thiocyano complexes formed between niobium(V) and thiocyanate is inaccurate because the decomposition products of thiocyanic acid absorb under the same conditions, and vanadium, tungsten, molybdenum and titanium cause interference.[8] The spectrophotometric determination of niobium(V) with bromopyrogallol red has recently been described.[9] This colour system is stable and reproducible, and the reaction seems to be the most sensitive spectrophotometric method in the visible range so far proposed for niobium.

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COLOUR FORMATION—

Belcher, Ramakrishna and West[9] gave the following experimental conditions for the formation of the blue niobium(V) - bromopyrogallol red complex. The tartaric acid solution containing niobium(V) ions was nearly neutralised with sodium hydroxide solution until it was within the pH range of 5·7 to 6·6. It is within this pH range that the reaction between niobium(V) and bromopyrogallol red takes place. The colour-forming reaction was specific for niobium in a solution containing an acetate buffer that was made 0·3 per cent. w/v with respect to EDTA. It was found that colour development was complete after 90 minutes in the presence of EDTA. On measuring the optical density 3½ hours later, no change was observed. Gelatin was added to act as a protective colloid to prevent the colour complex settling out of solution as a blue precipitate.

Belcher *et al.*[9] showed that appreciable amounts of many elements could be tolerated by the reagent in the presence of EDTA. They also state that for good reproducibility, the reagent, which is subject to oxidation, should be prepared afresh every 7 days.

EXPERIMENTAL

A suitable amount of sample was dissolved in a solution that was 7 M with respect to nitric acid, and 5 M with respect to hydrofluoric acid. The residue, if any, was filtered off on a Whatman No. 542 filter-paper and discarded.

The niobium was separated from the matrix by adsorption on to Dowex 1 × 8 anion-exchange resin, 200 to 400 mesh, from a solution that was about 7 M with respect to nitric acid, and 5 M with respect to hydrofluoric acid. After eluting the matrix with nitric acid (7 M) - hydrofluoric acid (5 M) solution, the final runnings were checked for iron content with thioglycollic acid, when iron or steel was the matrix, and the niobium was eluted with a solution that was 5 M with respect to nitric acid, and 0·2 M with respect to hydrofluoric acid.

Tantalum, when present, was adsorbed with the niobium, but it had formed a strong complex and did not elute with the niobium. Tantalum was removed from the anion-exchange resin by eluting with a solution that was 12 M with respect to nitric acid and 5 M with respect to hydrofluoric acid. Slight degradation of the resin occurs in the acid mixture at this strength. This procedure gave a simple separation of niobium from tantalum, which was useful when analysing steels containing both niobium and tantalum.

The eluate containing the niobium was evaporated to remove nitric and hydrofluoric acids. The residue contained a small amount of anion-exchange resin that was removed by ignition which left niobium pentoxide. Fusion with potassium hydrogen sulphate enabled the niobium to be dissolved in a tartaric acid solution.

COLOUR MEASUREMENTS—

Measurements of optical densities were made at wavelength 610 mμ with a Unicam SP500 spectrophotometer with 4-cm cells, or by using a "Spekker" absorptiometer fitted with a tungsten lamp, Ilford 607 filters and 4-cm cells. The spectrophotometer calibration graph was constructed in the range 0 to 40 μg of niobium, and a straight-line relationship was obtained. The absorptiometer calibration graph was a straight line in the range 0 to 40 μg of niobium, but became curved from 40 to 60 μg of niobium.

NIOBIUM IN TANTALUM—

The determination of niobium in tantalum required a different procedure at the initial separation stage. The sample was dissolved in a nitric acid and hydrofluoric acid mixture. The strength of the acid was adjusted so that it was 5 M with respect to nitric acid and 0·2 M with respect to hydrofluoric acid. The tantalum was adsorbed on to Dowex 1 anion-exchange resin. Finally, the niobium was eluted with an eluent that was 5 M with respect to nitric acid and 0·2 M with respect to hydrofluoric acid, and the tantalum then eluted with an eluant that was 12 M with respect to nitric acid and 5 M with respect to hydrofluoric acid.

RESULTS

The procedure described was applied to steels of the British Chemical Standards series that contained niobium. It was also applied to a sample of tantalum, and to two zirconium - niobium binary alloys. The results are given in Table I.

These results show that the procedure developed for determining niobium after anion-exchange separation is quantitative, and possesses the required precision.

TABLE I

RESULTS OBTAINED FOR NIOBIUM BY THE PROPOSED METHOD

Sample	Niobium content, per cent.	Sample weight, g	Volume of test solution, ml	Aliquot taken, ml	Niobium in aliquot, µg	Niobium found, per cent.
B.C.S.	0·0003	2·0000	50	50	9·0	0·0004$_5$
No. 273		0·5000	50	50	2·5	0·0005
Mild steel		0·5000	50	50	2·5	0·0005
B.C.S.	0·035	0·5000	100	10	18·0	0·036
No. 275		0·5000	100	15	27·0	0·036
Mild steel		0·5000	100	20	35·5	0·035$_5$
		0·5000	100	10	17·5	0·035
		0·5000	100	15	27·5	0·035$_7$
		0·5000	100	20	35·5	0·035$_5$
		0·5000	100	10	17·3	0·034$_6$
		0·5000	100	15	26·8	0·035$_7$
		0·5000	100	20	34·5	0·034$_5$
B.C.S.	0·02	0·5000	100	20	21·5	0·021$_5$
No. 277		0·5000	100	20	21·6	0·021$_6$
Mild steel		0·5000	100	20	21·5	0·021$_5$
		0·5000	100	20	21·6	0·021$_6$
B.C.S.	Niobium	0·2500	100	0·5	14·5	1·16
No. 312	+ Tantalum	0·2500	100	1·0	30·5	1·22
P.M.A.	= 1·33	0·2500	100	1·5	45·2	1·21
	Tantalum	0·2500	100	1·0	29·2	1·17
	= 0·13	0·2500	100	1·5	44·7	1·19
B.C.S.	Niobium	0·2500	250	2·5	16·7	0·67
No. 261	+ Tantalum	0·2500	250	2·5	15·9	0·64
18/12	= 0·71	0·2500	250	5·0	33·0	0·66
Stainless	Tantalum	0·2500	250	5·0	33·0	0·66
steel	= 0·04	0·2500	250	7·5	50·8	0·65
		0·2500	250	7·5	50·8	0·65
ALA 13	0·10	0·5000	100	5	26·3	0·105
(2 per cent.		0·5000	100	5	26·0	0·104
manganese		0·5000	100	5	26·3	0·105
steel)		0·5000	100	5	26·3	0·105
		0·5000	100	5	26·0	0·104
		0·5000	100	10	51·0	0·102
		0·5000	100	10	51·7	0·103
ALA 15	0·10	0·5000	100	5	26·5	0·106
(2 per cent.		0·5000	100	5	26·0	0·104
manganese		0·2500	100	10	26·5	0·106
steel)		0·2500	100	10	26·3	0·105
Tantalum 1	0·0012	1·0000	50	10	2·5	0·0012$_5$
		1·0000	50	10	2·5	0·0012$_5$
		1·0000	50	25	5·5	0·0011
Zirconium 1	0·5	0·1140	200	10	31·8	0·56
Zirconium 2	1·0	0·0885	250	7·5	27·0	1·02

METHOD

APPARATUS AND MATERIALS—

A Unicam SP500 spectrophotometer or Spekker absorptiometer with tungsten lamp is used.

Ion-exchange column—This consisted of a polythene tube, 30 cm long by 1 cm internal diameter, narrowed at the outlet end and fitted with a polythene funnel at the inlet. The column was charged with 10 g of resin.

Resin—Dowex 1 × 8, 200 to 400 mesh, chloride form. Before use, wash with nitric acid (12 M) - hydrofluoric acid (5 M) solution.

Polythene, PTFE and platinum apparatus are required for handling hydrofluoric acid solutions.

REAGENTS—

Bromopyrogallol red solution—Dissolve 0·14 g of bromopyrogallol red in 500 ml of high purity ethanol and dilute to 1 litre with water. Use fresh solution every 7 days.

Buffer solution, pH 6·0—Dissolve 80 g of high purity ammonium acetate in water, add 6 ml of glacial acetic acid and dilute to 1 litre.

EDTA solution—Dissolve 50 g of EDTA (disodium salt dihydrate) in water and dilute to 1 litre.

Gelatin solution—Dissolve 1 g of gelatin in warm water, cool and dilute to 100 ml.

Use high purity grade for all other reagents.

PROCEDURE—

Dissolve the sample in 20 ml of a solution that is 7 M with respect to nitric acid and 5 M with respect to hydrofluoric acid. Warm the solution to expel oxides of nitrogen, cool, filter if necessary through a Whatman No. 540 filter-paper, and add it to the anion-exchange column. Elute the matrix by adding in small increments 90 ml of a solution that is 7 M with respect to nitric acid and 5 M with respect to hydrofluoric acid. Discard the eluate containing the matrix. Elute the adsorbed niobium with 200 ml of a solution that is 5 M with respect to nitric acid and 0·2 M with respect to hydrofluoric acid.

Evaporate the eluate containing the niobium to dryness and ignite the residue. Fuse the residue with 0·3 g of potassium hydrogen sulphate, cool and extract the melt with 10 ml of 20 per cent. w/v tartaric acid solution. Dilute the solution to 80 ml with water and adjust the pH to 6·0 with 20 per cent. w/v sodium hydroxide solution. Transfer the solution to a 100-ml calibrated flask, dilute to the mark and mix.

Place a suitable aliquot of the test solution in a 100-ml calibrated flask. Add 6 ml of EDTA solution, 10 ml of bromopyrogallol red solution, 10 ml of buffer solution and 2 ml of gelatin solution. Mix, allow the solution to stand for 90 minutes, dilute it to 100 ml, and mix. Measure the optical density of the blue complex against a reagent blank prepared in a similar way, in 4-cm cells at 610 mμ on a Unicam SP500 spectrophotometer or on a Spekker absorptiometer with Ilford 607 filters.

CALIBRATION—

Preparation of standard solution A—Fuse 0·1431 g of niobium pentoxide with 3·5 g of potassium hydrogen sulphate. Extract the melt with 20 ml of 20 per cent. w/v tartaric acid solution and add another 80 ml of tartaric acid solution. Dilute to 1 litre with water.

1·0 ml of standard solution A ≡ 100 μg of niobium.

Preparation of standard solution B—Take 20 ml of standard solution A, add 38 ml of 20 per cent. w/v tartaric acid solution and dilute to 180 ml with water. Adjust the pH of the solution to 6·0 and dilute to 200 ml.

1·0 ml of standard solution B ≡ 10 μg of niobium.

Transfer aliquots of 1, 2, 3, 4 and 5 ml to 100-ml calibrated flasks and continue as described in the last paragraph of the procedure. Plot optical density against micrograms of niobium.

NIOBIUM IN TANTALUM—

Dissolve the sample in a mixture of 5 ml of nitric acid and 5 ml of hydrofluoric acid, and evaporate the solution until recrystallisation just starts. Dissolve the residue in 25 ml of a solution that is 5 M with respect to nitric acid and 0·2 M with respect to hydrofluoric acid. Add it to the anion-exchange column and elute the niobium with 200 ml of 5 M nitric acid - 0·2 M hydrofluoric acid solution. Continue as described in the second paragraph of the procedure.

REGENERATION OF THE ANION-EXCHANGE RESIN—

Tantalum absent—Pass 25 ml of a solution that is 7 M with respect to nitric acid and 5 M with respect to hydrofluoric acid through the resin bed.

Tantalum present—Pass 200 ml of a solution that is 12 M with respect to nitric acid and 5 M with respect to hydrofluoric acid through the resin bed. Then pass 50 ml of a solution that is 5 M with respect to nitric acid and 0·2 M with respect to hydrofluoric acid.

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The Determination of Boron in Fertilisers

An Account of Investigations by the Analytical Committee of The Fertiliser Manufacturers' Association*

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Some of the work by the Analytical Committee of the Fertiliser Manufacturers' Association on the determination of boron is presented. One method is recommended for the determination of boron at levels usually found in fertilisers, and another is accepted as suitable for the determination of boron at lower levels.

AS part of its programme of investigating methods for the determination of additives in fertilisers, the Analytical Committee of the Fertiliser Manufacturers' Association considered methods for determining boron.

The determination of boron by titrating orthoboric acid solution with standard alkali in the presence of a polyhydroxy compound is a standard method. Many of the substances commonly found in fertilisers interfere with this determination and preliminary separations are usually necessary, although Lang[1] reported that the removal of phosphate, ammonia and carbon dioxide was not necessary. Taylor[2] used lead nitrate to remove phosphate and, after bringing the solution to a pH of 6·3, titrated it with standard alkali to the same pH in the presence of mannitol. Wilson and Pellegrini[3] used bismuth nitrate for removing phosphate. A titrimetric method and two spectrophotometric methods for determining boron were considered by Boden[4] in a paper on the determination of trace elements in feeding stuffs and in fertilisers. In the titrimetric method, the boron was separated from interfering substances by distillation from methanol for a period in excess of 1 hour. One of the spectrophotometric methods involved the use of carmine and was based on Hatcher and Wilcox's procedure.[5] Boden[4] found that a standing period of 7 hours was necessary for complete colour development. The other spectrophotometric method involved the use of 1:1-dianthrimide and followed the recommendations of Ellis *et al.*[6] Hayes and Metcalfe[7] studied the reaction between orthoboric acid and curcumin, and Gattorta and Servello[8] applied this reaction to the determination of boron in fertilisers after the boron had been separated by ion exchange. Reynolds[9] described the use of Victoria violet for the determination of boron.

Other instrumental techniques have been used to determine boron. Parker and Barnes[10] used benzoin as a reagent to determine boron fluorimetrically; Fornwalt[11] determined boron in plating-bath liquors by using a flame photometer; Newton *et al.*[12] used an isotope dilution method for determining boron in silicon; and Coleman[13] showed that boron could be determined by fast-neutron activation analysis.

In the methods depending on the titration of orthoboric acid, several techniques have been used to remove carbon dioxide.[2,3,14,15] The most usual method involves boiling the solution under slightly acidic conditions.[2,3,14] In particular, Taylor[2] boiled the solution containing the orthoboric acid for a few minutes with an excess (0·3 to 0·5 ml) of 0·5 N hydrochloric acid, and Wilson and Pellegrini[3] heated the solution containing the orthoboric acid to 90° C and stirred vigorously at a pH of about 5.

Many of these methods for determining boron are either lengthy, require expert manipulation or are performed on expensive instruments. The Analytical Committee of the Fertiliser Manufacturers' Association examined three titrimetric methods based on the titration of orthoboric acid, and a spectrophotometric method in which carmine was used.

One Committee member had previously modified Taylor's method[2] in that ammonia was completely removed, washing of the lead phosphate precipitate was avoided, and it was shown that the reaction between orthoboric acid and the titrant was stoicheiometric, thus obviating the need for special standardisation of the titrant. This method is hereafter referred to as the "lead nitrate method."

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In a preliminary collaborative experiment, it was shown that the statutory method of preparing a water-soluble extract for the determination of water-soluble phosphate[16] gave a solution that did not contain all of the boron.

All of the collaborative determinations of boron were made on solutions prepared by some form of acid treatment of the sample. The procedures for removing carbon dioxide given by the authors were followed when using Taylor's method,[2] and that of Wilson and Pellegrini.[3] In the "lead nitrate method," Taylor's procedure[2] for removing carbon dioxide was retained.

EXPERIMENTAL

The collaborative experimental work was conducted in a series of schemes culminating in a test embracing twenty laboratories in the fertiliser industry.

A sample of solid boronated fertiliser was analysed in three of the laboratories represented on the Committee by three methods. The sample was of a commercial preparation which had been ground to a suitable fineness and well mixed, and was ready for analysis as received by the laboratories. The laboratories were asked to analyse the sample for boron in true duplicate by three specified methods, those by Taylor,[2] Wilson and Pellegrini,[3] and a spectrophotometric method with carmine (SP1). In method SP1 the sample was moistened with water, calcium oxide was added and the mixture was dried and ignited at 500° C. The salts were dissolved in dilute hydrochloric acid, heated gently and filtered. A suitable aliquot was treated with concentrated sulphuric acid and the carmine reagent (0·1 per cent. w/v in concentrated sulphuric acid) was added. After 45 minutes' standing, the percentage transmission was read at 580 mμ against a reagent blank. The results are given in Table I.

TABLE I

COMPARISON OF THREE METHODS FOR BORON DETERMINATION

	Solid samples Boron, per cent.		
Method	Taylor	SP1	WP
Laboratory 1	0·17 0·18	0·18 0·18	0·19 0·18
Laboratory 2	0·19 0·19	0·19 0·19	0·19 0·19
Laboratory 3	0·15 0·15	0·14 0·14	0·17 0·17
Mean	0·172	0·170	0·182

SP1 = First spectrophotometric method; WP = Wilson and Pellegrini method.

Further samples were analysed for boron in five laboratories by the Wilson and Pellegrini[3] method and a spectrophotometric method, SP2. In method SP2 the reagent was a 0·025 per cent. w/v solution of carminic acid in concentrated sulphuric acid, and the coloured complex

TABLE II

COMPARISON OF WILSON AND PELLEGRINI METHOD AND SPECTROPHOTOMETRIC METHOD (SP2)

	Percentage of boron in				Boron, p.p.m. in			
	Solid sample A		Solid sample B		Liquid sample C		Liquid sample D	Liquid sample E
Method	WP	SP2	WP	SP2	WP	SP2	SP2	SP2
Laboratory 1 ..	0·17	0·17	0·29	0·27	66	105	23	11·1
	0·18	—	0·28	—	125	105	24	10·7
Laboratory 2 ..	0·18	0·17	0·27	—	94	96	—	—
	0·18	—	0·27	—	92	82	—	—
Laboratory 3 ..	0·18	0·19	0·28	0·29	82	75	23	9·6
	0·19	0·18	0·27	0·28	79	82	25	9·5
Laboratory 4 ..	0·18	0·15	0·28	0·23	82	93	19	6·1
	0·18	0·16	0·27	0·23	77	88	20	6·2
Laboratory 5 ..	0·19	0·17	0·27	0·22	—	88	23	8·9
	0·19	0·15	0·28	0·18	—	85	24	9·6
Mean	0·182	0·168	0·276	0·243	87	90	23	9·0
Nominal value	0·2		0·25		100		25	10

SP2 = Second spectrophotometric method; WP = Wilson and Pellegrini method.

was allowed to stand for 2 hours for colour development. The optical density was read at 625 mμ. The method was the same as SP1 in all other respects. Of the five samples, two were solids and three were liquids. The solid samples and the liquid sample with the highest boron content were analysed by both methods. The two liquid samples with the lower boron contents were analysed only by the spectrophotometric method (SP2).

The solid samples had been ground and mixed and were ready for analysis as received by the participating laboratories. The laboratories were asked to determine the boron content of each of the samples in true duplicate by the specified methods. The results are given in Table II.

At the level of boron present in the solid samples, the spectrophotometric method gives lower results than does the Wilson and Pellegrini[3] method, and the variation between laboratories is slightly greater.

The use of bismuth nitrate to remove phosphate requires the subsequent removal of the excess of bismuth. This extra filtration is avoided by the use of lead nitrate as in Taylor's method, and in the "lead nitrate method." Four samples were analysed for boron content in six laboratories by three methods. Two of the samples were solids with nominal boron contents of 1·0 per cent. and 0·4 per cent. They were ground and ready for analysis as supplied to the participating laboratories. The other two samples were liquids with calculated boron contents of 416 and 167 p.p.m. w/w. The two solid samples were analysed by the Wilson and Pellegrini[3] method and by the "lead nitrate method." The liquid sample with the higher boron content was analysed by the Wilson and Pellegrini[3] method and by a spectrophotometric method (FSP). The liquid sample with the lower boron content was analysed only by method FSP. Each laboratory was asked to make duplicate determinations on each sample by the required methods. Method FSP, which was similar to SP2 with some increase in the detail of the instructions, was finally recommended for the determination of low levels of boron in fertilisers, and is described later. The results are given in Table III.

TABLE III

COMPARISON OF THE "LEAD NITRATE," WILSON AND PELLEGRINI AND SPECTROPHOTOMETRIC METHODS

	Boron, per cent. in				Boron, p.p.m. in		
	Solid sample A		Solid sample B		Liquid sample C		Liquid sample D
Method	LN	WP	LN	WP	WP	FSP	FSP
Laboratory 1	0·93	0·88	0·35	0·24	376	382	141
	0·94	0·90	0·35	0·24	387	391	141
Laboratory 2	0·93	0·85	0·33	0·22	370	440	180
	0·93	0·82	0·33	0·24	370	450	180
Laboratory 3	0·91	0·87	0·31	0·29	352	393	144
	0·91	0·87	0·31	0·28	382	392	141
Laboratory 4	0·96	0·82	0·31	0·25	345	366	108
	0·95	0·82	0·32	0·26	347	368	111
Laboratory 5	0·93	0·85	0·29	0·24	394	427	174
	0·91	0·86	0·30	0·25	371	447	164
Laboratory 6	0·91	0·89	0·34	0·26	—	—	—
	0·91	0·87	0·34	0·27	—	—	—
Mean	0·927	0·858	0·323	0·253	369	406	148
Nominal value ..	1·0		0·4		—		—
Calculated value	—		—		416		167

LN = "Lead nitrate method"; WP = Wilson and Pellegrini method; FSP = Final spectrophotometric method.

The agreement between laboratories for both the "lead nitrate method" and the Wilson and Pellegrini[3] method is good but, for each sample and for each laboratory, the results obtained by the "lead nitrate method" are closer to the nominal values. The results on liquid sample C by the spectrophotometric method are closer to the known value than the results obtained by the Wilson and Pellegrini[3] method.

Two liquid samples, one containing 100 p.p.m. of boron and the other containing 5 p.p.m. of boron were analysed in the laboratories of six of the Committee members by the spectrophotometric method (FSP). The results are given in Table IV.

TABLE IV

COLLABORATIVE ANALYSIS OF BORON BY THE SPECTROPHOTOMETRIC METHOD (FSP)

	Boron, p.p.m.			
Laboratory	Liquid sample A		Liquid sample B	
1	95	100	5·7	5·6
2	115	99	7·5	6·2
3	103	—	6·8	7·1
4	115	109	7·4	7·1
5	100	101	4·8	4·8
6	94	97	3·7	3·8

The method has been accepted by the Committee for the determination of low levels of boron in fertilisers although the precision is relatively poor.

A further comparison between the "lead nitrate method" and the Wilson and Pellegrini[3] method was made. In this experiment three samples were analysed in six laboratories by both methods. One of the samples (C) was a commercial boronated fertiliser. This sample was ground and suitable for analysis as supplied to the participating laboratories. The other two samples were artificially prepared to minimise any between-sample variation. Each laboratory was supplied with a portion of a sample of boron-free fertiliser and a solution of known strength of orthoboric acid. They were asked to prepare solutions of the fertiliser containing amounts of orthoboric acid equivalent to 0·4 and 1·0 per cent. of boron in the solid fertiliser. Aliquots of these solutions were then to be used for boron determinations in duplicate by each method. The results are given in Table V.

TABLE V

COMPARISON OF "LEAD NITRATE" AND WILSON AND PELLEGRINI METHODS

Samples A and B are solid boron-free fertiliser with liquid orthoboric acid; Sample C is a commercial solid boronated fertiliser

	Boron, per cent.					
	Sample A		Sample B		Sample C	
Method	WP	LN	WP	LN	WP	LN
Laboratory 1	0·37	0·40	0·95	0·98	0·24	0·25
	0·37	0·38	0·95	1·00	0·23	0·25
Laboratory 2	0·36	0·42	0·99	1·01	0·25	0·26
	0·36	0·41	0·99	0·98	0·23	0·26
Laboratory 3	0·36	0·39	0·91	0·99	0·23	0·24
	0·38	0·41	0·91	0·99	0·23	0·24
Laboratory 4	0·36	0·40	0·96	1·01	0·26	0·26
	0·37	0·40	0·95	0·99	0·25	0·26
Laboratory 5	0·36	0·43	0·92	1·04	0·24	0·26
	0·36	0·45	0·91	1·04	0·23	0·26
Laboratory 6	0·35	0·40	0·96	1·01	0·23	0·25
	0·36	0·39	0·95	1·00	0·23	0·25
Mean	0·363	0·407	0·946	1·003	0·238	0·253
Calculated boron content	0·40		1·00		—	

WP = Wilson and Pellegrini method; LN = "Lead nitrate method."

Both methods show good precision within and between laboratories. The "lead nitrate method" gives results which are closer to the calculated values for the two samples for which these values are known, and for each sample gives a higher result than does the Wilson and Pellegrini[3] method which is biased on the low side. It was decided to adopt the "lead nitrate method" as the preferred method for fertilisers of boron content greater than 0·1 per cent.

A ground sample of boronated fertiliser was then circulated more widely by the Committee to twenty laboratories in the fertiliser industry. Each laboratory was asked to analyse the sample in true duplicate by the "lead nitrate method." In some of the participating laboratories more than one analyst carried out the determinations. The results are given in Table VI.

TABLE VI

COLLABORATIVE ANALYSIS OF BORON BY THE "LEAD NITRATE METHOD"

	Boron, per cent.					
Laboratory	Analyst 1		Analyst 2		Analyst 3	
1	0·18	0·18	0·18	0·18	—	
2	0·18	0·18	—		—	
3	0·17	0·18	0·18	0·18	—	
4	0·20	0·19	—		—	
5	0·18	0·18	0·19	0·19	—	
6	0·19	0·19	0·19	0·19	—	
7	0·19	0·19	0·18	0·18	—	
8	0·19	0·19	—		—	
9	0·18	0·18	0·19	0·18	0·18	0·17
10	0·18	0·18	0·18	0·17	—	
11	0·18	0·18	0·18	0·18	—	
12	0·18	0·18	0·18	0·19	0·19	0·19
13	0·20	0·21	0·19	0·19	—	
14	0·19	0·18	0·19	0·18	—	
15	0·19	0·19	0·19	0·19	—	
16	0·19	0·19	0·17	0·16	0·15	0·16
17	0·24	0·25	—		—	
18	0·17	0·17	0·17	—	0·17	—
19	0·19	0·18	—		—	
20	0·18	0·17	—		—	

In the collaborative work reported in Table VI, two of the laboratories returned results that did not agree with the general pattern. The range of results from laboratory 16 is excessive, and the mean result from laboratory 17 is high. When these results are excluded, the precision of the method measured by the duplicate results of each analyst is equivalent to a standard deviation of 0·004, and the standard deviation of a single result by one analyst from one laboratory is 0·008, giving a coefficient of variation of 4·4 per cent.

"LEAD NITRATE METHOD"

REAGENTS—

All reagents are of AnalaR quality when this grade is available.

Phenolphthalein solution, 0·1 *per cent. in* 50 *per cent. ethanol.*

Methyl red solution, 0·1 *per cent. in water.*

Hydrochloric acid, concentrated, 0·5 N *and* 20 *per cent. v/v.*

Sodium hydroxide solution, 0·5 N *and* 0·05 N. Prepare the 0·05 N sodium hydroxide solution from a 50 per cent. w/w solution which was stored out of contact with air until the insoluble carbonates had settled. Use distilled water that has been boiled and cooled for dilution, and store the 0·05 N solution in a polythene bottle that is protected from the atmosphere by a guard tube containing an absorbent for carbon dioxide.[17] Fit the bottle with a syphon for withdrawing the solution.

Sodium carbonate, anhydrous.

Mannitol, neutral.

Lead nitrate solution, 10 *per cent. w/v.*

APPARATUS—

The glassware should preferably be boron-free.

Potentiometric apparatus suitable for titration, with a glass electrode, and fitted with means of stirring the solution mechanically during the titration is used.

PROCEDURE—

Weigh 2 g of the sample if it contains 0·5 per cent. or less of boron, or 1 g of the sample if it contains from 0·5 to 1 per cent. of boron, on a balance capable of weighing to 1 mg. Transfer the weighed sample to a 400-ml tall-form beaker. Add 150 ml of water and a few drops of phenolphthalein solution. Add sodium carbonate to make the solution slightly alkaline and boil gently. Keep the solution just alkaline by further additions of sodium carbonate until all of the ammonia has been evolved.

Cool the solution and cautiously add 12 ml of a 50 per cent. v/v solution of hydrochloric acid. Add 20 ml of lead nitrate solution for each 12 per cent. of phosphate present as P_2O_5 in the sample if an aliquot of 2 g has been used, and 10 ml of lead nitrate solution for each 12 per cent. of phosphate present as P_2O_5 in the sample if an aliquot of 1 g has been used. Heat just to boiling, remove from the source of heat and carefully make just alkaline by adding solid sodium carbonate, avoiding excessive frothing. Stand the mixture on a boiling water bath for 5 minutes. Cool, transfer to a calibrated flask and dilute to 200 ml with water. Mix well and filter through a 24-cm Whatman No. 42 filter-paper, rejecting the first 10 to 20 ml of the filtrate.

Transfer 100 ml of the filtrate to a 250-ml beaker. Discharge the pink colour of the phenolphthalein indicator by the addition of the dilute hydrochloric acid solution. Add a few drops of methyl red solution followed, if necessary, by more of the dilute hydrochloric acid solution until the test solution is just acid to methyl red. Heat almost to boiling and stir vigorously to remove carbon dioxide. Add a little more of the dilute hydrochloric acid solution if the colour changes to orange or to yellow. Neutralise the test solution to methyl red with 0·5 N sodium hydroxide solution and then make just acid with 0·5 N hydrochloric acid solution. Cover with a watch-glass and boil gently for 5 minutes to expel remaining carbon dioxide. Cool rapidly and add rinsings from the underside of the glass to the beaker.

Place the electrodes of the potentiometric titrating apparatus in the beaker and adjust the pH to 6·3 by adding 0·05 N sodium hydroxide solution. Add 10 g of mannitol and titrate with 0·05 N sodium hydroxide solution until the pH is again 6·3. Add a further amount of mannitol and, if necessary, continue the titration with 0·05 N sodium hydroxide solution to a pH of 6·3. Further additions of mannitol should not alter the pH. Let x ml of 0·05 N sodium hydroxide solution be used for the titration owing to the addition of mannitol.

If a similar fertiliser free from boron is available, submit the appropriate amount of this fertiliser to the same procedure to determine the value of the blank. Let y ml of 0·05 N sodium hydroxide solution be used for this titration. If a similar fertiliser to that under test is not available for the determination of the blank let the value of y be taken as 0·1 ml of 0·05 N sodium hydroxide solution.

$$\text{Boron in sample} = \frac{0{\cdot}1082\,(x - y)}{\text{Weight of sample taken}}\ \text{per cent.}$$

SPECTROPHOTOMETRIC METHOD

REAGENTS—

All reagents are of AnalaR quality when this grade is available.

Carminic acid solution—0·025 per cent. w/v in concentrated sulphuric acid.

Calcium hydroxide—Free from boron.

Hydrochloric acid solution, 6 N.

Sulphuric acid, sp.gr. 1·84.

Standard boron solution—Dissolve 1·9047 g of orthoboric acid in water and dilute to 1 litre. 3 ml of solution ≡ 1·0 mg of boron.

APPARATUS—

Glassware must be free from boron.

Unicam SP600 spectrophotometer or its equivalent.

PROCEDURE—

Transfer a weight of sample containing between 16 and 160 μg of boron to a platinum or silica dish. Add 1 g of calcium hydroxide and 20 ml of water. Mix well and evaporate the mixture to dryness. Make sure that the mixture is alkaline to litmus throughout the evaporation and add a further amount of calcium hydroxide if necessary. Ash the residue in a muffle furnace at 450° to 500° C for 3 hours. Cool and carefully add the hydrochloric acid solution until the reaction is just acidic. Add 5 ml of the hydrochloric acid solution in excess and digest the mixture at 70° C for 15 minutes. Cool and transfer the contents of the dish to a 25-ml graduated flask, rinsing the dish with water. Make up to the mark with water. Mix the contents of the flask well and filter the liquid through a dry filter-paper. Discard the first 5 ml of the filtrate and transfer 3 ml to a 100-ml flask. Cautiously add, with cooling, 15 ml of concentrated sulphuric acid. Swirl the flask to mix and add 10 ml of carminic acid solution. Cool the flask rapidly to room temperature, mix well and allow to stand for exactly 2 hours. Measure the optical density of the coloured complex at 625 mμ with a 1-cm cell and against a blank which has been taken through all the stages of the determination.

Calculate the boron content of the sample from the weight of the sample and by reference to a calibration graph relating micrograms of boron to optical density. Obtain the calibration graph by treating 5, 10, 15, 20 and 25 μg of boron contained in 3 ml of standard solution as described above, starting at ". . . transfer 3 ml to a 100-ml flask." Prepare standard boron solutions such that 3 ml contain *x* μg of boron by diluting *x* ml of the standard boron solution to 1 litre.

CONCLUSION

In the collaborative work organised by the Analytical Committee of the Fertiliser Manufacturers' Association, the "lead nitrate method" for the determination of boron in fertilisers has been shown to be precise and accurate at levels of boron between 0·1 and 1 per cent. This range covers the amounts of boron normally added to fertilisers.

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Determination of Sulphur in Carbons and Cokes by Gas Chromatography

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A combined combustion - chromatographic method for determining sulphur in amorphous carbons and cokes is described. The method consists essentially in burning the sample in a stream of oxygen, separating the sulphur dioxide produced by using silica gel as adsorbent, and determining the concentration of sulphur dioxide with a thermal conductivity detector.

The method is rapid, is eminently suitable for small samples and covers a wide range of sulphur contents (0·2 to over 20 per cent.). The reproducibility of the method is comparable with the accepted tolerances of the British Standard methods for determining sulphur in coke. The difficulties experienced with cokes of high ash content are discussed, and suggestions made for eliminating these difficulties.

IN the course of studies of the formation and properties of carbon - sulphur complexes,[1] the need arose for a satisfactory method for determining 0·2 per cent. to some 20 per cent. of sulphur in small samples of sulphurised carbons. The present paper describes the experimental basis of the method, which may be of general interest, because of the ease and rapidity with which sulphur contents of small samples can be determined.

The method depends on the chromatographic measurement of the sulphur dioxide produced on combustion of the sample. This was a novel application when the work was commenced, but a gas - liquid chromatographic method has since been described.[2]

Preliminary experiments in which various adsorbents were used, such as silica gel, alumina, active charcoal and molecular sieves, showed that the most satisfactory results were obtained with a silica-gel column at about 100° C. In the early work, in which nitrogen was used as a carrier gas, the sample was burnt in a separate combustion tube and admitted to the column either as a whole or as samples extracted by a hypodermic syringe and injected into the carrier-gas stream through a serum cap.

For ease in routine analysis this method was replaced by a flow system in which oxygen is used as carrier gas and the sample is burnt in a combustion tube placed in the gas line before the column.

METHOD

APPARATUS—

The chromatographic column is made of $\frac{1}{4}$-inch PTFE tubing wound into a coil, of 2-inches diameter, around an electrically heated aluminium block, and mounted inside a container filled with vermiculite and lagged with asbestos string. In the present work a column length of 6 inches gives adequate separation and the column temperature is controlled, by the use of a Variac transformer, at 100° C.

The silica-gel adsorbent (chromatographic-analysis grade 25 to 52 B.S. mesh size) is pre-dried at 120° C in an oven for 2 hours. The column is packed by vibrating it until there is no further diminution in the volume occupied by the adsorbent, which is finally dried at 120° C *in situ*, by using a 50 ml per minute stream of oxygen, dried by passage through magnesium perchlorate.

A differential thermal conductivity detector comprising two tungsten filaments (Gow-mac W2) in a stainless-steel cell is used. It is enclosed in a thermostatically controlled box (30° ± 0·1° C), together with the components of the associated Wheatstone bridge. The current (140 mA) for the Wheatstone bridge circuit is supplied by a constant-voltage supply unit, and a pen recorder (1-mV full-scale deflection) measures the out-of-balance e.m.f. of the bridge. The flow-rate of the oxygen carrier gas is 50 ml per minute.

The sampling system consists of a transparent silica combustion tube (15 cm long × 1 cm diameter) with a B7 socket at one end. This tube is placed in the gas line immediately before a phosphorus pentoxide moisture trap and the silica-gel column.

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PROCEDURE—

Weigh (to an accuracy of ±0·005 mg) between 1 to 10 mg of the sample, depending upon the sulphur content, into a small silica boat, and place in the centre of the combustion tube. Steady conditions, as represented by a good recorder base-line, are attained after about 3 minutes, when the sample is rapidly burnt with a Teclu burner. The sulphur dioxide is eluted from the column, after the carbon dioxide, in about 5 minutes, and the time taken for a complete determination is about 10 to 12 minutes.

The system is calibrated by using pure carbon - sulphur mixtures together with sulphurous carbons and cokes (of low ash content) in which the sulphur has been determined previously by British Standard methods. Well defined linear relationships between the sulphur content and either the area under the chromatogram or the peak height are obtained. For ease of measurement in routine procedure the peak height is used and the sulphur content in the sample being analysed is computed from an appropriate calibration graph. No evidence was found of any sulphur trioxide formation during combustion.

RESULTS AND DISCUSSION

The results in Table I for a variety of sulphur - carbons, *e.g.*, sulphurised cellulose or poly(vinylidene chloride) carbons, show that the repeatability of the method is satisfactorily comparable with the accepted tolerances of the British Standard methods for determining sulphur in coke.

TABLE I

REPEATABILITY TESTS WITH SULPHUROUS CARBONS

Sample No.	Percentage of sulphur Determination (1)	Determination (2)	Difference (1–2)
1	0·55	0·53	0·02
2	0·73	0·76	−0·03
3	0·98	0·92	0·06
4	0·54	0·56	−0·02
5	1·17	1·19	−0·02
6	0·83	0·83	0·00
7	0·40	0·40	0·00
8	0·69	0·74	−0·05
9	1·15	1·17	−0·02
10	0·93	0·98	−0·06
11	1·58	1·65	−0·07
12	16·04	16·02	0·02
13	5·19	5·13	0·06
14	8·83	8·89	−0·06

Good agreement is obtained between the chromatographic and standard methods with sulphurous amorphous carbons, for which this method was devised, but with coke samples the present method tends to give low results compared with the standard high temperature method (Table II), apparently because of the influence of mineral constituents and fixation of sulphur in the residue as indicated by the results in Table III.

Cokes A, B, C, D and E were prepared from one parent coal, washed and cleaned to different ash contents. With cokes of low ash content the agreement between the present method and a standard method (high temperature method, British Standard 1016 : Part 7 : 1959) is good, but with cokes of high ash content the chromatographic method gives low results, the discrepancy increasing with increasing ash content. It should be emphasised that the samples must be finely ground (−240 B.S. mesh), both from the point of view of reducing sampling errors in weighing small amounts, and of ease of obtaining complete combustion.

It is apparent that for cokes a combustion temperature, higher than that attainable with the Teclu burner used in the present work, is necessary to obtain complete conversion of the total sulphur to sulphur dioxide and, where desirable, a small electric furnace may be suitable for this purpose. In this context a layer of finely divided alumina covering the sample should also prove to be beneficial.

TABLE II

COMPARISON WITH STANDARD METHODS

Sample	Percentage of sulphur found Chromatographic method (*A*)	 Standard method* (*B*)	Difference (*A–B*)
Sulphur carbon No. 1	3·35	3·47	−0·12
No. 3	3·72	3·86	−0·14
No. 4	3·72	3·49	0·23
No. 7	3·88	3·57	0·31
Coke No. 1	0·53	0·65	−0·12
No. 2	0·73	0·86	−0·13
No. 3	0·95	1·04	−0·09
No. 4	0·57	0·54	0·03
No. 5	1·18	1·31	−0·13
No. 6	0·83	1·02	−0·19
No. 7	0·40	0·45	−0·05
No. 8	0·72	1·00	−0·28
No. 9	1·16	1·34	−0·18
No. 10	0·96	1·07	−0·11
No. 11	1·62	1·58	0·04
2-Mercaptobenzothiazole	37·8	38·3‡	−0·5
S-Trithiane	65·6	69·6‡	−4·0
1,2-Benzodiphenylene	14·6	13·9‡	0·5
Sulphanilic acid†	18·1	18·5‡	−0·4

* High temperature method, British Standard 1016 : Part 7 : 1959.

† AnalaR quality. Other organic compounds were not of AnalaR quality.

‡ Theoretical value.

TABLE III

EFFECT OF ASH CONTENT OF COKE

Coke sample	Ash, per cent.	Percentage of sulphur found Chromatographic method (1)	 Standard method (2)	Difference (1–2)
A	0·60	0·71	0·65	+0·06
B	0·96	0·64	0·65	−0·01
C	2·54	0·80	0·86	−0·06
D	4·52	1·03	1·16	−0·13
E	8·88	1·04	1·24	−0·20
F	6·87	0·84	1·14	−0·30
G	7·58	0·83	1·12	−0·29

Organic compounds give reasonable agreement with the theoretical sulphur content, but care is needed in the heating procedure, in particular in the rate of heating, in order to avoid premature volatilisation and ensure complete combustion; some compounds being more difficult than others in this respect. Controlled electric heating and possibly a lower temperature of combustion may overcome this difficulty.

The work described formed part of the research programme of the British Coke Research Association, and the authors are indebted to Dr. H. E. Blayden for his helpful interest, and to the Director of the Association for permission to publish this paper.

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A Simple Low Cost Gas Chromatograph for the Determination of Fatty Acids

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The construction of a chromatograph, conductivity cell and recording conductivity meter, and their application to the determination of the lower fatty acids, formic to caproic acids, is described. The effect of operating variables on the detector and the relationship between acid concentration and detector response are discussed. Limits of detection of 0·001 µg of formic acid and 0·03 µg of caproic acid were found.

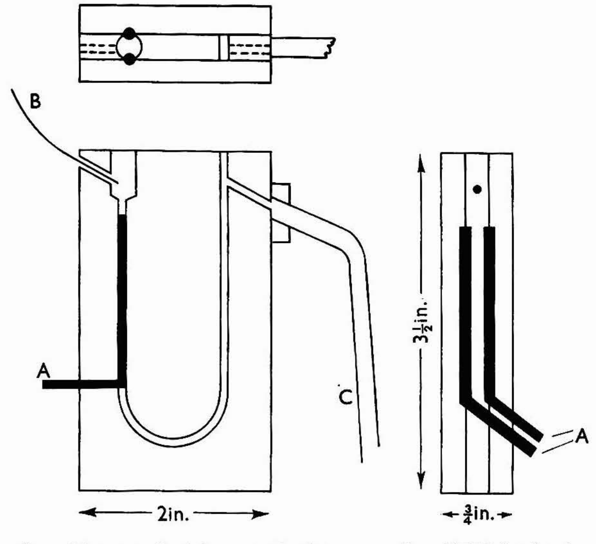
THE simple gas chromatograph described by James and Martin[1] has been used in this laboratory for several years for the analysis of fatty acids within the range formic acid to caproic acid, which occur as corrosion products and in decayed textile wrappings. Manual titration of the eluted acids with dilute sodium hydroxide solution was used in place of the original automatic titrator of James and Martin.

Recently, a simple, compact, low cost apparatus has been constructed in this laboratory with electrical conductivity cells based on Nash impingers[2,3] as detectors with a recording milliammeter, which it is thought will be of interest to other workers.

EXPERIMENTAL

APPARATUS—

The column is in the form of a glass helix, wound from 6-mm o.d. Pyrex tubing, that has a total length of 4 feet and consists of five turns of 8 cm diameter with a depth of 7 cm, the ends of which carry B7 ground-glass sockets. The column was packed with the stationary phase by placing a plug of quartz-wool into one end, applying a water-pump to this end to cause suction, and inserting the packing through a small funnel into the other end of the tube; by tapping the helix, a good dense packing could be achieved in a few minutes. The effluent gas passes into the conductivity cell through a short tube electrically heated ($4\frac{1}{2}$ watts)



A = 16 s.w.g. Stainless-steel wire B = PTFE feed tube
C = Drain tube

Fig. 1. Flow through conductivity cell

to prevent condensation of the higher acids, the gas impinging on the surface of the flowing water absorbent.

The chromatograph helix and a mercury thermometer are supported by rubber bungs on an aluminium disc clamped to the flange of a wide-mouthed flask of 700-ml capacity (Quickfit and Quartz Part No. FK700F) which is filled with water. The flask is heated by two 450-watt smoothing iron elements that are connected in series and clamped around the outside, the temperature being controlled by a "Simmerstat."

The conductivity cell, which was constructed from three pieces of Perspex sheet of $\frac{1}{4}$-inch thickness, is shown in Fig. 1; the electrodes were of stainless-steel wire (16 s.w.g.), 5 cm long, and were held in the bevelled edges of the U-shaped liquid channel in the central piece. The absorbent, water, flows at the rate of 0·2 ml per minute from a small constant head device through a length of PTFE tubing of 0·02 inch diameter into the cup at the top of the cell block; the column effluent impinges on the water surface, the jet being a few millimetres above this surface. The absorbent flows down the channel past the measuring electrodes and runs to waste over a dam, maintaining a constant level in the impinger cup.

All of these components can be mounted on a wooden base (9 × 9 inches), as shown in Fig. 2.

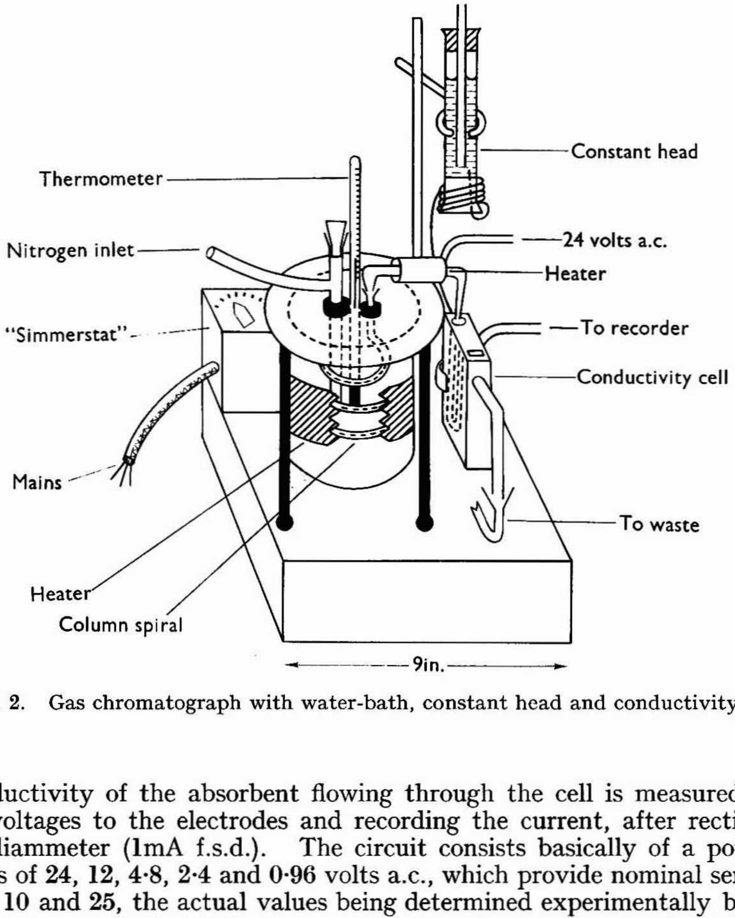


Fig. 2. Gas chromatograph with water-bath, constant head and conductivity cell

The conductivity of the absorbent flowing through the cell is measured by applying various a.c. voltages to the electrodes and recording the current, after rectifying it, on a recording milliammeter (1mA f.s.d.). The circuit consists basically of a potential divider giving outputs of 24, 12, 4·8, 2·4 and 0·96 volts a.c., which provide nominal sensitivity ratios times 1, 2, 5, 10 and 25, the actual values being determined experimentally by using dilute acetic acid solutions. To facilitate the examination of unknown samples, two broad-band ranges were incorporated having non-linear responses based on the commercial ohm-meter principle, in which a fixed resistor is placed in series with the supply so that full-scale deflection is given when the cell is short-circuited. One range ("BL," 24 volt, 24 kΩ) gives better sensitivity to small signals, and the other ("BH," 12 volt, 12 kΩ) gives better height resolution for large signals. The relationship between the current recorded on a broad-band range and that given at the maximum sensitivity setting is expressed by the equation—

$$\frac{1}{i} = \left(\frac{1}{i_s} - 1\right) V$$

where i is the current obtained in the absence of a fixed resistor,
i_s is the current obtained in the presence of a fixed resistor
and V is the applied potential.

This equation has been solved for the two ranges used, and the results necessary to construct conversion curves (preferably on log/log scales) are given in Table I.

TABLE I

RESULTS FOR CONSTRUCTING CURVES FOR "BH" AND "BL" RANGES FOR RECORDING CHROMATOGRAPH

Indicated current, mA	Current for times 1 sensitivity, mA	
	Range "BH"	Range "BL"
0·01	0·020	0·010
0·02	0·041	0·020
0·05	0·105	0·052
0·10	0·220	0·111
0·20	0·500	0·250
0·30	0·86	0·43
0·40	1·33	0·67
0·50	2·00	1·00
0·60	3·00	1·50
0·70	4·70	2·36
0·80	8·00	4·00
0·90	18·5	9·20
0·95	40	18·5
1·00	∞	∞

APPLICATION TO DETERMINATION OF FATTY ACIDS—

The column is filled with "Celite" (B.D.H. Ltd.) containing half its own weight of a mixture of MS500 silicone oil (80 per cent.), stearic acid (10 per cent.) and glacial phosphoric acid (10 per cent.); a temperature of 85° C has been found satisfactory, but slightly different temperatures may be required for different batches of Celite to obtain resolution of formic and acetic acids. The carrier gas used was white-spot nitrogen at a flow-rate of 25 ml per minute for the first 30 minutes, when formic and acetic acids are eluted, and subsequently at a flow-rate of 50 ml per minute; the relative retention volumes were found to be in good agreement with the values obtained by James and Martin[1] for a temperature of 100° C.

For textiles and similar samples, the fatty acids are separated by steam-distilling the sample, extracting into ether and, after making just alkaline with dilute sodium hydroxide solution, distilling off the bulk of the ether until about 1 ml remains. The residue of ether and alkaline aqueous phases is transferred to a 5-ml stoppered test-tube and made just acid with dilute sulphuric acid, and the liberated fatty acids are extracted into the ether phase. This ethereal solution is drawn into a hypodermic syringe and introduced into the column through a rubber septum. The ether has no effect on the behaviour of the column and has a virtually zero retention time.

For solid corrosion products the sample is moistened with a few drops of dilute sulphuric acid, and the fatty acids are extracted into 1 ml of ether, which is introduced into the column as described above.

DISCUSSION

The apparatus described has given satisfactory chromatograms, similar to those obtained on conventional equipment, just detecting 0·001 μg of formic acid and 0·03 μg of caproic acid.

The sensitivity of the detector is dependent on the flow-rate of the absorbent and a flow-rate of 0·2 ml per minute, controlled by a constant head, was adopted. As the fatty acids are weak electrolytes, the conductivity of their solutions should be proportional to the square root of the concentration; this was confirmed over the range acetic to nonoic acids;

that of formic acid, being a stronger electrolyte, was proportional to the 0·6th power of the concentration. The relative weight sensitivities to the acids was found to be proportional to the reciprocal of the number of carbon atoms in the molecule after passing through the column. A complete calibration could be obtained from a run on a single acid injection, if required.

The performance of the apparatus was found to be satisfactory without resorting to thermostatic control of the conductivity cell.

The relative retention volumes were found to be in good agreement with the results obtained by James and Martin,[1] the retention time for butyric acid being 73 minutes under the conditions given above.

Acknowledgment is made to the Engineer-in-Chief of the General Post Office and to the Controller of Her Majesty's Stationery Office for permission to publish this paper.

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The Determination of Trace Amounts of Chloride by a Rapid Evolution - Conductimetric Procedure

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A procedure for determining microgram and submicrogram amounts of chloride is described, in which a conductimetric detector is used after the chlorine is evolved as hydrogen chloride. The relationship between conductivity and chloride present and the effect of possible interferences is discussed. Results obtained for organochloro compounds after oxygen-flask combustion and for an analytical-reagent grade sodium carbonate are presented.

In an investigation requiring the determination of microgram and submicrogram amounts of chloride in 1-ml volumes of 10 per cent. sodium carbonate solutions, serious poisoning of the silver electrodes was experienced in the differential electrolytic potentiometric procedure[1] used in this laboratory for the determination of chloride in electrolytic capacitor electrolyte. This was overcome by the use of a cation-exchange resin, but this, and the subsequent evaporation of the solution to low bulk that was necessary, introduced large erratic blanks and an alternative procedure was sought.

The concentration cell procedures of Roburn[2] and Emmott[3] were studied, but these gave inadequate resolution for total amounts of less than 1 μg. The elegant procedure by Emmott, whereby hydrogen chloride is evolved from 80 per cent. sulphuric acid solution, was studied by using a Nash-type impinger conductivity cell[4,5] in conjunction with a simple recording conductivity meter.[6] A comparable procedure for the determination of phosphine in air has been described by Greenfield, Moule and Perry.[7]

EXPERIMENTAL

APPARATUS—

The evolution vessel, or generator, used was similar to that demonstrated by Emmott,[3] which has a 25-ml three-necked pear-shaped semi-micro flask with a plug of fine quartz-wool in the delivery tube to hold back any acid spray. This flask is immersed in a beaker of water at 80° C. The impinger cell was constructed from a 5-cm length of $\frac{1}{2}$-inch i.d. Perspex tube, the ring of four electrodes consisting of 20 s.w.g. stainless-steel wires held in a rubber bung or Perspex plug; opposite pairs were connected together, their lengths being such that their upper ends were just below the meniscus when 1·0 ml of water was placed in the cell. The effluent gas from the generator passed through a jet 0·5 mm in diameter, 1 to 4 mm above the water surface.

The recording conductivity meter[6] is used at a chart speed of 6 inches per hour.

PROCEDURE—

Ten ml of 80 per cent. sulphuric acid are placed in the generator and 1·0 ml of distilled water in the conductivity cell; white-spot nitrogen is passed through at a rate of 100 ml per minute until no further increase in conductivity occurs. The water in the cell is replaced, the sample introduced into the flask and nitrogen is passed through until there is no further increase in conductivity.

The volume of hydrogen chloride evolved is then read off from a calibration graph prepared by adding known amounts of chloride (as a solution of sodium chloride) to the generator.

If the sample volume is greater than 1 ml, 90 per cent. sulphuric acid can be used to ensure that the final concentration of the sulphuric acid is not less than 60 per cent., although 80 per cent. is preferable.

RESULTS AND DISCUSSION

It was found that an impinger cell had twice the recovery efficiency of a simple bubbler, and that whereas the bubbler showed a continuous loss of material from water containing a few p.p.m. of hydrogen chloride, the impinger cells showed no loss, constant conductivity being obtained for considerable periods of time.

The evolution of hydrogen chloride is rapid from 60 to 90 per cent. sulphuric acid, being complete in less than 10 minutes with nitrogen flow-rates of 75 to 150 ml per minute. The end-point is better defined if the generator is heated to 80° C, and this temperature and a nitrogen flow-rate of 100 ml per minute were adopted for most of this work. Lower gas flow-rates gave incomplete scrubbing of the acid and higher flow-rates gave carry-over of acid spray.

During a run the conductivity is constant until the hydrogen chloride is evolved, when a rapid increase occurs. The value should level off when all the chloride has been evolved, but, in practice, a slow increase is obtained and the end-point is taken as that point where the gradient becomes constant; this can be easily seen after a little experience, as shown in Fig. 1.

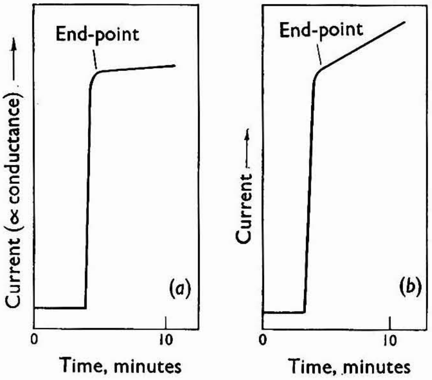

Fig. 1. Typical current against time recordings
(*a*) chloride alone, (*b*) in presence of nitrate

The calibration graphs, in which the change in conductivity is plotted against the chloride added to the generator, were straight lines over the range 0·5 to 20 µg, but were curved over longer ranges (0 to 100 µg). In this laboratory, where the temperature is reasonably stable, adequate results were obtained without the complication of thermostatic control of the measuring cell.

No interference is to be expected from phosphoric or sulphuric acids, and none has been found from them, nor from boric acid nor carbon dioxide. Tartaric and citric acids were also found to cause no interference when 60 per cent. sulphuric acid is used in the generator; at higher concentrations some sulphur dioxide is evolved. Nitric acid is evolved from nitrates under the conditions described; the evolution rate is much lower than that of hydrogen chloride from chlorides, and good results are obtained when the nitrate content of the sample is less than twenty times the chloride content. If the ratio is greater, the end-point on the conductivity curve is difficult to locate. Acetic acid is also evolved, but the interference is only slight; satisfactory determination of 1 µg of chloride in the presence of 10,000 times as much (10 mg) acetic acid has been achieved and its determination in the presence of larger proportions of acetic acid should be possible.

As a check on the procedure, the chloride content of a batch of analytical-reagent grade sodium carbonate was determined by using the evolution method and by neutron-activation analysis. For the evolution procedure, two calibration graphs were prepared to cover the range 0 to 1 µg of chloride, one by adding solutions of sodium chloride, and the other, by adding solutions of chloride containing 10·0 mg of sodium carbonate. Two straight parallel lines were obtained, their displacement giving a mean result of 0·0065 per cent. chloride. The neutron-activation figures were 0·0062, 0·0065 and 0·0064 per cent.

When the procedure was used after combusting various organochloro compounds by the oxygen-flask method, the results shown in Table I were obtained.

TABLE I

CHLORINE CONTENTS OF ORGANIC COMPOUNDS

Compound	Nominal chlorine, per cent.	Amount combusted	Fraction for determination	Chlorine found, per cent.
p-Chlorobenzoic acid ..	22·6	49·8 mg	10 µl from 20 ml	22·1, 22·8
Dichloroacetic acid ..	54·3	10 µl	10 µl from 20 ml	52·5, 53·8
Barium chloroanilate ..	20·35	47·8 mg	10 µl from 20 ml	19·25

These results show that the evolution - conductimetric procedure gives results in good agreement with those obtained by other methods and has the advantages of rapidity and simplicity, thereby reducing the possibility of contamination. It could be advantageously used in many applications where the determination of traces of chloride is required.

While a full statistical study of the procedure has not been made, the results obtained with duplicate and triplicate analyses, and in the preparation of calibration graphs, suggest that the coefficient of variation will be about 2 to 5 per cent.

Acknowledgment is made to the Engineer-in-Chief of the General Post Office and to the Controller of Her Majesty's Stationery Office for permission to publish this paper, to Dr. D. Gibbons of the Wantage Research Laboratory (A.E.R.E.) for the neutron-activation analysis and to Mr. W. E. Stace of this laboratory who carried out much of the experimental work.

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A Rapid Method for Determining Nitrogen in Uranium Nitrides

By B. L. TAYLOR and B. S. PERRETT

(*Analytical Sciences Division, Atomic Energy Research Establishment, Harwell, Berks.*)

An inert-gas fusion method for the determination of nitrogen in uranium nitrides has been developed. It is more rapid than the existing Dumas[1] and Kjeldahl[2] methods, and is comparable with these with regard to accuracy and precision. The sample, encapsulated in platinum tubing, is heated to about 1900° C in a graphite resistance furnace, and helium is used to sweep out the gaseous reaction products. The hydrogen and carbon monoxide fractions of this gas are oxidised to water and carbon dioxide by passage through copper oxide at 450° C. After drying, the gas is passed through a multi-loop trap cooled with liquid nitrogen to remove the carbon dioxide. If required, this can be measured later to obtain the oxygen content of the sample.[3] The helium, now containing only nitrogen, is passed through a U-tube containing molecular sieve 5A, 40 to 60 mesh, cooled to −196° C. The nitrogen is absorbed on to the molecular sieve. At the end of the reaction the U-tube is connected to a vacuum system and the helium is pumped away. The molecular sieve is then heated with a hot air blower to release the nitrogen which is transferred to a gas burette by a mercury diffusion pump. This pump, an Edwards 2M4A, has a high backing pressure (30 to 35 mm), and the volume of the burette is large enough for this not to be exceeded with the sample weights used (10 to 50 mg). Each determination by this method takes about 20 minutes, and the oxygen content of the sample can be obtained simultaneously. For uranium nitrides ($UN_{1\cdot0}$ to $UN_{1\cdot7}$) the results generally agree to within 1 per cent. of those obtained by the Dumas method. The precision is in the range 0·5 to 1 per cent. coefficient of variation.

The method has also been applied satisfactorily to aluminium nitride.

Further details are to be published in AERE–R 5247, H.M.S.O.

REFERENCES

1. Milner, G. W. C., Row, D. H., Foster, E., and Phillips, G., *U.K. Atomic Energy Research Establishment Report* AERE–R 4644, H.M. Stationery Office, London, 1964.
2. Milner, G. W. C., Jones, G., Crossley, D. R., and Phillips, G., *U.K. Atomic Energy Research Establishment Report* AERE–R 4713, H.M. Stationery Office, London, 1964.
3. Taylor, B. L., Prior, H. A., Perrett, B. S., and Bird, P., *U.K. Atomic Energy Research Establishment Report* AERE–R 5190, H.M. Stationery Office, London, 1966.

Received *July 22nd*, 1966

Separation of Mustard Gas and Hydroxy Analogues by Thin-layer Chromatography

By F. G. STANFORD

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A method was required for determining the radiochemical purity of sulphur-35-labelled mustard gas ($\beta\beta'$-dichloroethyl sulphide), which would give the percentage of "half-mustard" (β-chloro-β'-hydroxyethyl sulphide) and thiodiglycol ($\beta\beta'$-dihydroxyethyl sulphide). Thin-layer chromatography has proved satisfactory as there is no significant loss of sample from the plate during spotting, elution, air drying and scanning of the radioactivity distribution after elution at a laboratory temperature of 20° C.

Samples of 20 μg in 0·005 ml of ethanol are applied to 0·25-mm of Merck silica gel G layers on 20 × 20-cm plates, previously activated at 110° C for 1 hour. The eluent is chloroform - acetone, 50 + 40 v/v, with 15 cm travel of eluent front. R_F values are mustard gas, 0·80; half-mustard, 0·60; and thiodiglycol, 0·33. In addition, the method will separate oxidation products of stored samples.

The radioactivity distribution is scanned by an argon - methane windowless proportional counter connected to an amplifier and potentiometric pen recorder, and down to 0·5 per cent. of each compound can be detected in a mixture. The plates are scanned directly the eluent has evaporated; storage of the developed plates involves a 1 to 2 per cent. loss of mustard gas after 12 hours, and 5 per cent. after 24 hours.

Inactive reference spots are made visible by spraying with 1 per cent. of potassium permanganate in 6 per cent. sodium carbonate.

Received *June 24th*, 1966

Analytical Methods Committee

NOTIFICATION OF ERRATA

Official, Standardised and Recommended Methods of Analysis

THE Analytical Methods Committee's collection of its standardised methods of analysis was published in 1963 by W. Heffer & Sons Ltd., Cambridge, under the title "Official, Standardised and Recommended Methods of Analysis." To date, 5 errors, details of which are given below, have been discovered among the methods of analysis that comprise the first part of the book—

Page 30. *~~Dilute~~* Strong *standard copper solution*, line 1:
For "0·3926 g" *read* "0·393 g".

Page 114. REAGENTS. *Copper solution*, lines 2 and 3:
For "copper sulphate, $CuSO_4.5H_2O$" *read* "finely powdered crystalline copper acetate".

Page 132. DETERMINATION OF CREATININE. *Visual Colour-comparison Method*, line 2:
For "1-" *read* "7-".

Page 205. REAGENTS. *Mercuric chloride paper*, line 5:
After "in millimetres," *insert* "of 400 papers".

Page 214. REAGENTS. *Strong standard copper solution*, line 3:
For "100 ml" *read* "200 ml".

Determination of Trace Elements with Special Reference to Fertilisers and Feeding Stuffs

The Report of the Trace Elements in Fertilisers and Feeding Stuffs Sub-Committee to the Analytical Methods Committee was published in 1963 by W. Heffer & Sons Ltd., Cambridge, under the title "Determination of Trace Elements, with Special Reference to Fertilisers and Feeding Stuffs." To date, 2 errors have been discovered in this book, and details of them are given below—

Page 28. Line 6:
For "0·05 g" *read* "0·5 g".

Page 30. DETERMINATION OF NICKEL, line 7:
For "separate" *read* "stand".

Book Reviews

ATOMIC-ABSORPTION SPECTROPHOTOMETRY. By W. T. ELWELL and J. A. F. GIDLEY. Second (Revised) Edition. Pp. xii + 139. Oxford, London, Edinburgh, New York, Toronto, Sydney, Paris and Braunschweig: Pergamon Press. 1966. Price 42s.

When the first edition of this book was published in 1961, atomic-absorption spectrophotometry showed much promise as an analytical tool. Now this promise has been fulfilled, and the extensive developments that have taken place since that time are fully reflected in this completely revised second edition. Most of the text is new, and what has been retained from the previous edition has been extended in the light of recent advances. The size of the book, however, has only increased by 37 pages because a smaller type-size has been used.

The first four chapters deal with the theory, instrument components, interferences and comparisons with other instrumental techniques. The remaining half of the book deals with analytical applications. After a general consideration of the experimental variables and detailed descriptions of two typical procedures, a comprehensive account of the use of atomic-absorption spectrophotometry for the analysis of each of 36 elements is given, in alphabetical order. This compared with a discussion of the determination of only 10 elements in the first edition. The information given for each element includes details of flame compositions, wavelengths and sensitivities, followed by an account of the work that has been carried out for that element. There is also a set of references for each element, in addition to almost one hundred references keyed to the other parts of the text. Detailed procedures for each element are not given, because most atomic-absorption determinations follow one of the typical procedures previously outlined, and any additional details can be found in the section devoted to the particular element.

This is undoubtedly a book for the practical analyst. Material that does not have a direct bearing on the application of atomic absorption to analysis, such as flame chemistry and impractical light sources, are only briefly mentioned. The information supplied is accurate and up-to-date. Such innovations as long-path burners, high-intensity lamps and nitrous oxide - acetylene flames are included, and it is probably the first book to deal (albeit briefly) with the analytical applications of atomic fluorescence in flames. A valuable feature, also, is a large, pull-out sheet giving detailed descriptions of 17 commercially available atomic-absorption instruments, arranged so as to give a ready comparison of the instruments. Probably the only improvement that could be recommended is a somewhat more extended description of the principles of atomic-absorption spectrophotometry. At present, newcomers to the technique may find themselves embarking on the development and theory of the process before they have fully grasped its basic essentials.

For the last five years, Elwell and Gidley has been the only available monograph devoted to atomic-absorption spectrophotometry, and for the last three of those it has been out of print. This lack of comprehensive coverage has been aggravated by a reticence to include accounts of the technique in text-books and the longer review publications. Thus the appearance of the second edition of this popular book is very welcome. Its reasonable price should ensure that it will reach as wide an audience as its predecessor.

A. TOWNSHEND

ANALYSE DER METALLE. Volume I. SCHIEDSANALYSEN. Edited by CHEMIKERAUSSCHUSS DER GESELLSCHAFT DEUTSCHER METALLHÜTTEN UND BERGLEUTE. Third Edition. Pp. xii + 507. Berlin, Heidelberg and New York: Springer-Verlag. 1966. Price DM 57.

The prime purpose of this book is to make available a collection of authoritative German methods for the analysis of 35 commercially important metals, ranging alphabetically from aluminium to zirconium.

The recommended methods are, essentially, *classical*, and preference is given to referee methods. Procedures that are dependent on the provision of chemically analysed standards, as in spectrographic analysis, have not been included.

Special attention is given to the determination of major and some minor constituents present in the parent metals, and to qualitative and quantitative tests for the analysis of raw and intermediate materials, with due regard to their established usage and commercial significance.

The publication provides detailed information for the analysis of a very wide range of materials, hence some of the less common determinations have not been included, and these omissions, so far as the specialist is concerned, may restrict its appeal.

Frequent references are made to the *Deutsches Institut für Normen*, and full use is made of this important source of information, but only after a careful selection and assessment of DIN and other reliable methods had been made, was any method approved by the *Chemikerausschuss der Gesellschaft Deutscher Metallhütten und Bergleute*, for inclusion in this publication.

The expert may be disappointed at the lack of supporting literature references, and the choice of some of the methods given, but it must be appreciated that the recommendations do not represent isolated preferences, but a concensus of opinions drawn from a representative body of 50 experts with first-hand experience in their respective fields.

A publication such as this is likely to be most useful in providing agreed analytical methods for application on either a national or international basis. W. T. ELWELL

LABORATORY HANDBOOK OF TOXIC AGENTS. Second Edition. Edited by C. H. GRAY. Pp. x + 190. London: The Royal Institute of Chemistry. 1966. Price 24s.

"A serious toxic effect is not blunted because it was unexpected, nor is tragedy less painful because a danger was not anticipated."

This statement opens the introduction to the first chapter of the Royal Institute of Chemistry's Laboratory Handbook of Toxic Agents, edited by Dr. C. H. Gray, Professor of Chemical Pathology in the Kings College Hospital Medical School, and it is a statement with which few people associated with the chemical industry will disagree. The modern chemical laboratory is the home of a comprehensive selection of hazards ranging from the crude and corrosive mineral acids and caustic alkalis to the subtle and seductive dangers of organic ethers and chlorinated solvents; it is indeed surprising that the widespread practice of employing junior and unskilled personnel to perform the more menial tasks in our laboratories does not lead to more serious accidents than it does. The Laboratory Handbook of Toxic Agents, of which the first edition appeared in 1960, has been a valuable guide to laboratory managers in general, and especially so to those who do not have the benefit of resident medical services to call upon.

The second edition is substantially an expanded version of the first; forty new monographs have been added, and the general chapters have been revised. The first chapter drives home the lesson that all chemicals should be treated with caution, and that with some of them the effects of exposure may include damage to the tissues of the nerve system or of vital organs; damage which is not immediately apparent but which may be irreversible and may lead to such obscure effects as change of personality. The second chapter, which is headed "Precautions and Preventions," offers useful suggestions on laboratory planning and safety precautions; the dangers of consumption of food and drink in chemical laboratories are properly emphasised, as also are the possible effects of smoking in atmospheres containing chemicals which may produce toxic substances on pyrolysis. Chapter three concerns first aid in the laboratory, and has been re-written in the second edition to include Sylvestor's method of ventilating the lungs in cases where breathing has stopped. No doubt there will be circumstances in which the administration of first aid by other laboratory personnel is desirable, but one feels that the advice given on the first page of this chapter, to summon qualified medical attention at the earliest possible moment, is the soundest advice that can be given. Chapter four now contains monographs describing the toxic and harmful properties of about 250 reagents and the treatment that should be applied for immediate alleviation of the symptoms. Often the threshold limit value, as accepted by the American Conference of Governmental Hygienists, is quoted, but the importance that should be attached to the effects of protracted exposure to atmospheres containing contaminants at the low level of concentration represented by the threshold limit value has not been emphasised as strongly as it might have been, nor is there any mention of the methods by which laboratory atmospheres can be examined for very low concentrations of poisonous chemicals. Chapter five deals with precautions to be taken against radiations (*i.e.*, from X-ray and radioactive sources) and includes brief references to the effects of ultraviolet and infrared radiation.

The book is well bound and clearly printed, and the only typographical error noticed was on page vii where the acknowledgment is presumably to Dr. A. J. Amos, rather than to Dr. A. J. Amor.

Such minor criticisms apart, the handbook is an extremely valuable reference and should be made readily accessible to all laboratory chemists and managers. H. E. STAGG

THE ELECTRON MICROPROBE. Proceedings of the Symposium Sponsored by the Electrothermics and Metallurgy Division, the Electrochemical Society, Washington, D.C., October, 1964. Edited by T. D. MCKINLEY, K. F. J. HEINRICH and D. B. WITTRY. Pp. xvi + 1035. New York, London, Sydney: John Wiley and Sons. 1966. Price £10 10s.

The publication of symposium proceedings is always a controversial topic. When a symposium covers a wide range of subjects it is likely that only a small portion of the proceedings will be of interest to any one reader. On the other hand, when the subject of a symposium is restricted to a single process or technique then the proceedings should provide much useful information to the interested reader. The book under review comes in the second category.

Forty-three papers are published, together with an extensive bibliography covering all aspects of electron-probe microanalysis. The book is divided into four parts: Analysis of Light Elements (90 pages); Quantitative Analysis (320 pages); New Techniques and Instrumentation (83 pages); and Applications (336 pages); the bibliography takes up a further 190 pages.

The section dealing with "Analysis of Light Elements" contains six papers that clearly indicate the principles on which modern techniques are based, but because of the rapid strides that have recently been taken some of the detail is out of date. Anyone just entering this field of analysis, however, would find these papers a most useful introduction to the subject.

"Quantitative Analysis" is covered by twelve papers which include several correction procedures, both complex and simplified. Extensive tables (83 pages), based on computer calculations, are appended to one paper to help in the computation of correction factors. Probably one of the least satisfactory situations in correction procedures is the lack of reliable data on X-ray mass absorption coefficients. The paper by Heinrich reviews the situation and shows how internally consistent values can be obtained from published data. The most useful part of this work to the practising microprobe analyst is the provision of tables of mass absorption coefficients for the K_α and K_β lines (sodium - molybdenum), L_α and L_β lines (gallium - uranium) and M_α and M_β lines (ytterbium - uranium) in each of the elements of the periodic table from lithium to plutonium. These tables are included in the text (27 pages). As mass absorption coefficients are of importance in every correction procedure, tables of reliable coefficients are used frequently. It is unfortunate therefore that the tables have been reduced during printing so that it is not always easy to pick out the correct figure from the large mass of surrounding numbers. It is perhaps doubtful whether these tables should have been included in this book at all, rather than in a separate publication; the size of the book reduces the ease with which the tables can be used.

The short section on "New Techniques and Instrumentation" comprises six papers, two of which describe commercially available equipment. Inclusion of the "advertising" papers is of doubtful value as more up-to-date information concerning these instruments is available from the manufacturers' technical hand-outs.

The largest section (19 papers), covers the application of electron-probe microanalysis to problems in the metallurgical, electronic, semiconductor, non-conductor and medical fields. There is a wealth of information contained in these papers concerning detailed techniques. The final result may not be of direct interest, but the method of getting there may well be applicable to problems in a completely different field. The detail that may be of importance is unlikely to be indicated in the title of the paper, and use of the subject index is therefore necessary.

The bibliography, compiled by K. F. J. Heinrich, has already had a wide circulation in a loose-leaf form and it seems unnecessary to have included it in this volume. The size of the book, already large by virtue of the conference proceedings, has been increased by 20 per cent. by including the bibliography, and presumably the cost has been increased also.

This large volume contains much information that is of value to the practising electron-probe microanalyst and to such it is recommended. It is unfortunate, however, that the editors did not consider the separate publication of the tables of mass absorption coefficients and the bibliography, thus reducing the size, weight and, presumably, the price of the book. J. A. F. GIDLEY

THE MODERN ELECTROPLATING LABORATORY MANUAL. By REX CONDE ARMET. Pp. xviii + 382. Teddington: Robert Draper Ltd. 1965. Price 115s.

This book is just what it sets out to be, a laboratory manual for the use of those engaged in electroplating, describing concisely how to make the tests that are required in day-to-day working. The selection of methods is based on the author's experience in the field. As he remarks: "The whiff of brimstone which once hung thickly over the industry has generally speaking been blown away, and electroplating, far from being considered a black art, is now held to be a respectable business taking its natural place in the vast metal finishing industry." Bearing this in mind he has been at pains to put forward methods that are reliable and good, and, where possible, rapid as well; he has also included information on spot testing, the metallurgy of base metals, micro-examination and plating tests, and there are sections on the control of trade effluents and the identification of plastics. K. A. WILLIAMS

PHOTOELECTRIC COLORIMETRY IN CLINICAL BIOCHEMISTRY. By G. E. DELORY, M.Sc.,Ph.D. Pp. xii + 80. London: Hilger & Watts Ltd. Price 25s.

This simple book is based on an earlier work by the author, entitled "Photoelectric Methods in Clinical Biochemistry," published in 1949 and reviewed in *The Analyst*, 1949, **74**, 574. Indeed, it might be said to consist of the same text, re-written and slightly condensed. It includes fewer details of photoelectric instruments adapted for making biochemical determinations than did the former book, but the descriptions of the methods that the author has chosen to present are just as well done as before. A short chapter has been added on Sources of Information, and it is intended as an introduction to the use of the library; brief reference is made to the advent of automated methods of analysis. K. A. WILLIAMS

LES SÉPARATIONS PAR LES RÉSINES ÉCHANGEUSES D'IONS. By B. TRÉMILLON. Pp. viii + 400. Paris: Gauthier-Villars. 1965. Price 90 F.

The author's intention in writing this book has been to provide a guide to the way in which to carry out separations with ion-exchange resins. The book is divided into two parts. In the first part, the general properties of ion-exchange resins are discussed, a detailed account is then given of the effects of complex formation and the nature of the solvent on ion-exchange equilibria and, finally, the different ways in which ion-exchange resins can be used in the laboratory are classified and briefly described. In the second part of the book, the behaviour of ion-exchange columns and the principles underlying the different types of separation process are described in four chapters. A final chapter is devoted to electrochemical applications and ion-exchange membranes. A comparatively short account of experimental procedures is included as an appendix.

The book is one of the series published under the general title "Monographies de Chimie Minérale" and the emphasis is on the use of ion-exchange resins for the separation of inorganic ions; only a few examples are given of the applications of ion-exchange resins in organic chemistry and in biochemistry. A notable feature is the inclusion of experimental values for the limiting partition coefficients of various inorganic ions; these are presented in the form of diagrams at the end of the book. Unfortunately, it is not clear on what basis these coefficients have been calculated; reference to the original papers shows that some authors expressed concentrations in the resin phase in terms of the amount per gram of dry resin, others in terms of the amount per millilitre of swollen resin. From the original paper by Strelow it also appears that the results shown in the first diagram were obtained at comparatively high resin loadings.

Throughout the book emphasis has rightly been placed on the principles of ion-exchange behaviour and ion-exchange chromatography. It is true that many of the most striking ion-exchange separations were initially developed on a more or less empirical basis, and our knowledge of the behaviour of ions in solution and in the resin phase has not yet reached the stage (except in the simplest of cases) where their ion-exchange and chromatographic behaviour can be predicted. Even so, understanding of the basic principles is clearly essential for the proper application of ion-exchange techniques to separation problems.

This book, which fills a gap in the French literature on ion exchange, will be of interest to those who are engaged in research on the improvement or development of ion-exchange separation processes and who have had some experience with ion-exchange resins. Those who are unfamiliar with the use of ion-exchange resins may find that, although the detailed treatment of principles in this book is helpful, they would welcome more advice on experimental techniques. D. K. HALE

METHODS OF FORENSIC SCIENCE. Volumes I and II. Edited by FRANK LUNDQUIST. Volumes III and IV. Edited by A. S. CURRY. Pp. xii + 659 (volume I); xii + 302 (volume II); xiv + 342 (volume III); xii + 369 (volume IV). London and New York: Interscience Publishers, a division of John Wiley & Sons Inc. 1962; 1963; 1964; 1965. Prices 160s.; 89s.; 95s.; 105s.

The first volume of this work was published in 1962, since when three further volumes have followed, Volume IV completing the series.

Since the days of Conan Doyle's literary creations, when forensic science was considered to be within the orbit of the intelligent amateur, real-life forensic scientists have become increasingly specialised. This is reflected in the structure of this publication, which consists of thirty-four chapters with extensive references, each covering a different aspect of practical forensic science. They are each written by an internationally recognised expert with experience, not only of his subject matter, but also of its forensic applications (an important aspect of expertise sometimes overlooked by the expert witness).

Volume I deals with the identification of alkaloids and barbiturates, and with the estimation of carbon monoxide, with particular reference to analytical toxicology. There are also chapters on blood stains and serum haptoglobin in paternity cases, and one chapter dealing with fire-arms.

The second volume, published a year later, has three chapters on various aspects of documents (papers, inks, typewriters), uses of gas - liquid chromatography, toxicological determination of metals, grouping of dried and whole blood, determination of bone age and time of death.

Volume III has three chapters on various aspects of forgery, three on toxicology (lead, drugs in urine and cyanides). There are two chapters on activation analysis, and the use of the acid phosphatase test for semen.

Volume IV deals with recent advances in grouping of dried blood and secretion stains. Metabolites, infrared and alcohol determinations comprise the toxicology, together with a chapter on soap abortions. Thin-layer chromatography is dealt with (mainly its toxicological applications), and the forensic examination of glass and coloured fibres.

There has been little attempt to arrange related subjects according to volume, and consequently the later volumes sometimes augment earlier contributions. Thus Dr. Clarke in his chapter on alkaloid extraction and identification (Volume I) uses paper chromatography, and colour and microcrystalline tests in an admirably comprehensive coverage, but scarcely touches upon the several instrumental techniques now in general use. Neither Dr. Clarke nor Dr. Schmidt (in his work on barbiturates in Volume I) could have anticipated, for example, the rapid development of gas - liquid chromatography, dealt with by Dr. Cadman in Volume II.

There is some overlap of subject matter, as between Professor Schleger and Dr. Fiori, both of whom deal at length in different chapters with treatment of blood stains, and between Dr. Hensel (Urine Analysis for Drugs) and Dr. Turner (Searching for Drug Metabolites in Viscera). This is probably unavoidable in a work of this nature, as each chapter is a self-contained and usable account of a method, which that contributor has found to work. Excessive editing would have removed this most valuable feature.

Somewhat surprisingly there is no treatment of systematic identification of wood fragments, a technique that has yielded valuable evidence on several occasions. This is the only omission found in a work of very wide and comprehensive coverage.

This work is written primarily to assist the practising forensic scientist and those specialists, both scientific and legal, who are called upon from time to time to assess forensic scientific evidence. But although some of this work can have little value to those not engaged in these specialised fields, there is also an immense fund of information concerning analytical techniques that is not available elsewhere. To analytical chemists, therefore, irrespective of their special interests, no less than to forensic scientists, these volumes are warmly recommended. M. S. Moss

Errata

JUNE (1964) ISSUE, p. 378, line 42. *For* "OINTMENT OF CAPSAICIN B.P.C." *read* "OINTMENT OF CAPSICUM B.P.C.".

IBID., p. 382, line 12. *For* "Capsaicin B.P.C." *read* "Capsicum B.P.C.".

IBID., p. 382, 1st sample in Table VII. *For* "Capsaicin B.P.C." *read* "Capsicum B.P.C.".

IBID., p. 382, 6th line under CAPSICUM B.P.C. *For* "100-ml" *read* "10·0-ml".

IBID., p. 382, 9th line under CAPSICUM B.P.C. *For* "100-ml" *read* "10·0-ml".

IBID., p. 383, line 22. *For* "OINTMENT OF CAPSAICIN B.P.C." *read* "OINTMENT OF CAPSICUM B.P.C.".

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3 June 10.

Summaries of Papers in this Issue

Activation Analysis

A Review

SUMMARY OF CONTENTS

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  - Surface analysis

R. F. COLEMAN
Atomic Weapons Research Establishment, Aldermaston, Berks.

and T. B. PIERCE
Atomic Energy Research Establishment, Harwell, Berks.

Analyst, 1967, **92**, 1–19.

REPRINTS of this Review paper will soon be available from the Secretary, The Society for Analytical Chemistry, 14 Belgrave Square, London, S.W.1, at 5s. per copy, post free.

A remittance for the correct amount, made out to The Society for Analytical Chemistry, MUST accompany every order; these reprints are not available through Trade Agents.

The Spectrofluorimetric Determination of Magnesium with *NN'*-Bis-salicylidene-2,3-diaminobenzofuran

NN'-Bis-salicylidene-2,3-diaminobenzofuran, SABF, has been synthesised and is proposed as a stable and inexpensive spectrofluorimetric reagent for determining magnesium in the range 0·1 to 6 μg (2×10^{-3} to 1×10^{-1} p.p.m.). The method is both rapid and sensitive, and has a detection limit of about 10^{-3} p.p.m. in 50 per cent. aqueous methanol at an apparent pH of 10·5. The orange fluorescence (545 mμ) of the 1 : 1 complex is excited at 475 mμ and can be measured within 15 to 45 minutes of mixing the solutions. Few other ions yield a fluorescence with the reagent and those that do, or that interfere in other ways, may be tolerated in 100-fold molar proportions by simple addition of masking agents. The fluorescent magnesium - SABF complex can be extracted quantitatively into isobutyl methyl ketone without interference from 2000-fold amounts of calcium, thus providing an extremely useful method for determining magnesium in technical materials, etc. To demonstrate this, nine municipal water samples and fourteen blood plasma samples have been analysed successfully for magnesium by the proposed method in a 50 per cent. aqueous methanol medium.

R. M. DAGNALL, R. SMITH and T. S. WEST
Department of Chemistry, Imperial College, London, S.W.7.

Analyst, 1967, **92**, 20–26.

The Catechol Violet Colour Reaction for Tin(IV) by Cetyltrimethylammonium Bromide

The sensitivity of the colour reaction between tin(IV) and catechol violet has been greatly increased by the sensitising action of cetyltrimethylammonium bromide ($\epsilon_{662m\mu} = 95{,}600$). The sensitisation is also accompanied by a considerable bathochromic shift (107 mμ) of the wavelength of maximum absorption. Full colour development occurs within 5 minutes at pH 2·2, and Beer's law is obeyed down to 0·2 p.p.m. of tin(IV).

R. M. DAGNALL, T. S. WEST and P. YOUNG

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

Analyst, **1967, 92, 27–30.**

Inorganic Thin-layer Chromatography: Part II. Chromatography of Some First Row Transition Metals on Thin Layers of Substrates Impregnated with Tributyl Phosphate

Several metal ions have been chromatographed on thin layers of cellulose and silica gel impregnated with tributyl phosphate at different concentrations of hydrochloric acid. The open nature of the cellulose results in the tributyl phosphate being more firmly held on this substrate than on the silica gel, and this in turn results in lower R_F values. The R_F values of the metal ions are also shown to vary with the concentration of the hydrochloric acid eluent.

L. S. BARK, G. DUNCAN and R. J. T. GRAHAM

Department of Chemistry and Applied Chemistry, The Royal College of Advanced Technology, Salford 5, Lancs.

Analyst, **1967, 92, 31–35.**

A Tin-layer chromatographic Method for the Determination of "Quassin" in Cosmetic Preparations

A method is described by which "quassin" may be determined when used as a denaturant for the alcohol contained in cosmetic preparations. The two bitter principles, neoquassin and quassin are determined together as quassin, after oxidation with a solution of sodium dichromate in glacial acetic acid. Perfume oils are first removed by solvent extraction and the aqueous phase is then evaporated to dryness and the residue oxidised. Further solvent-extraction steps yield a solution of quassin in chloroform which is applied to a thin-layer chromatographic plate. The spots are compared visually with standards prepared from an oxidised "quassin" solution to permit determinations within the range of 5 to 36 p.p.m. in samples.

E. C. HUNT

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

Analyst, **1967, 92, 36–42.**

The Determination of Niobium in Metals and Alloys

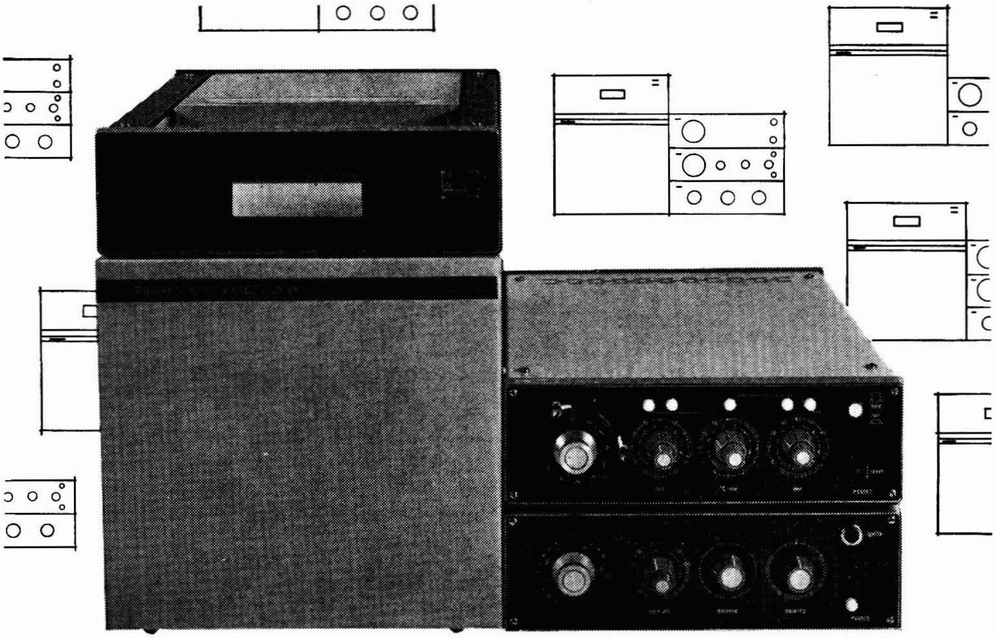
A method is described for the determination of niobium in metals and alloys. Niobium is separated from the matrix elements by adsorption on to Dowex 1 anion-exchange resin from a solution of hydrofluoric acid and nitric acid. The niobium is eluted in a mixture of hydrofluoric and nitric acids and determined by the spectrophotometric measurement of the blue complex formed between niobium(V) and bromopyrogallol red.

The method has been applied to the determination of niobium in iron, steel, tantalum and zirconium.

A. I. WILLIAMS

Metallurgy Division, National Physical Laboratory, Teddington, Middlesex.

Analyst, **1967, 92, 43–46.**



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The Determination of Boron in Fertilisers

An Account of Investigations by the Analytical Committee of The Fertiliser Manufacturers' Association

Some of the work by the Analytical Committee of The Fertiliser Manufacturers' Association on the determination of boron is presented. One method is recommended for the determination of boron at levels usually found in fertilisers, and another is accepted as suitable for the determination of boron at lower levels.

H. BORLAND, I. A. BROWNLIE and P. T. GODDEN

c/o The Fertiliser Manufacturers' Association Ltd., Alembic House, 93 Albert Embankment, London, S.E.1.

Analyst, 1967, **92**, 47–53.

Determination of Sulphur in Carbon and Cokes by Gas Chromatography

A combined combustion - chromatographic method for determining sulphur in amorphous carbons and cokes is described. The method consists essentially in burning the sample in a stream of oxygen, separating the sulphur dioxide produced by using silica gel as adsorbent, and determining the concentration of sulphur dioxide with a thermal conductivity detector.

The method is rapid, is eminently suitable for small samples and covers a wide range of sulphur contents (0·2 to over 20 per cent.). The reproducibility of the method is comparable with the accepted tolerances of the British Standard methods for determining sulphur in coke. The difficulties experienced with cokes of high ash content are discussed, and suggestions made for eliminating these difficulties.

F. M. W. OLDS, J. W. PATRICK and F. H. SHAW

The British Coke Research Assocation, Chesterfield, Derbyshire.

Analyst, 1967, **92**, 54–56.

A Simple Low Cost Gas Chromatograph for the Determination of Fatty Acids

The construction of a chromatograph, conductivity cell and recording conductivity meter, and their application to the determination of the lower fatty acids, formic to caproic acids, is described. The effect of operating variables on the detector and the relationship between acid concentration and detector response are discussed. Limits of detection of 0·001 μg of formic acid and 0·03 μg of caproic acid were found.

B. H. PRISCOTT

Birmingham Materials Section, Test and Inspection Branch, Post Office Engineering Department, Fordrough Lane, Birmingham 9.

Analyst, 1967, **92**, 57–60.

The Determination of Trace Amounts of Chloride by a Rapid Evolution - Conductimetric Procedure

A procedure for determining microgram and submicrogram amounts of chloride is described, in which a conductimetric detector is used after the chlorine is evolved as hydrogen chloride. The relationship between conductivity and chloride present and the effect of possible interferences is discussed. Results obtained for organochloro compounds after oxygen-flask combustion and for an analytical-reagent grade sodium carbonate are presented.

B. H. PRISCOTT

Birmingham Materials Section, Test and Inspection Branch, Post Office Engineering Department, Fordrough Lane, Birmingham 9.

Analyst, 1967, **92**, 61–63.

A Rapid Method for Determining Nitrogen in Uranium Nitrides

An inert-gas fusion method is described.

B. L. TAYLOR and B. S. PERRETT

Analytical Sciences Division, Atomic Energy Research Establishment, Harwell, Berks.

Analyst, 1967, **92**, 64.

Separation of Mustard Gas and Hydroxy Analogues by Thin-layer Chromatography

F. G. STANFORD

U.K.A.E.A., The Radiochemical Centre, Amersham, Buckinghamshire.

Analyst, 1967, **92**, 64.

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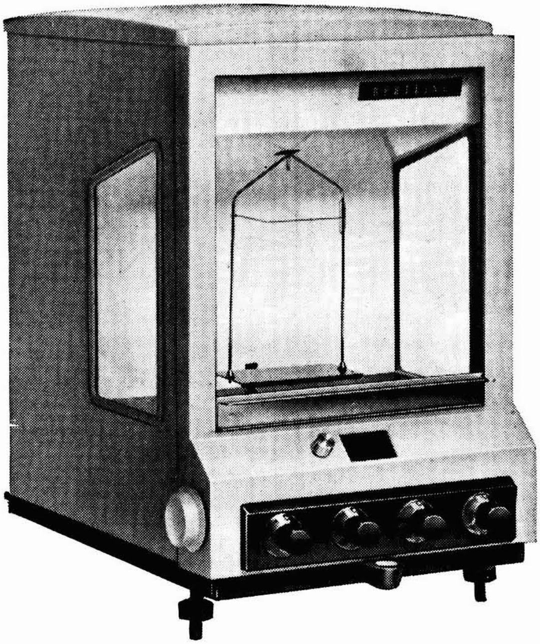

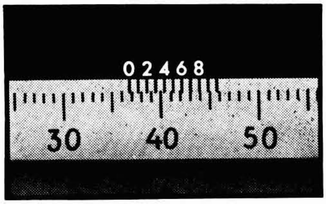
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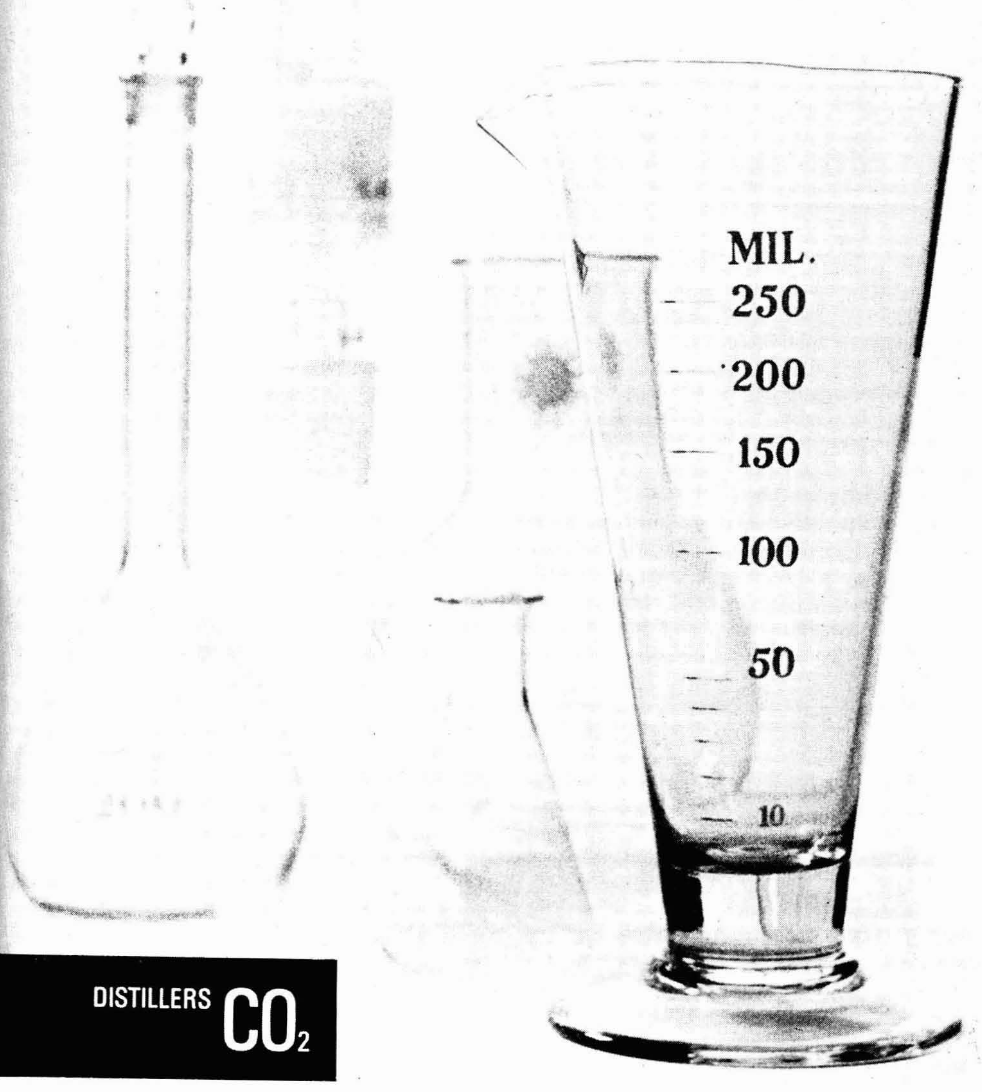
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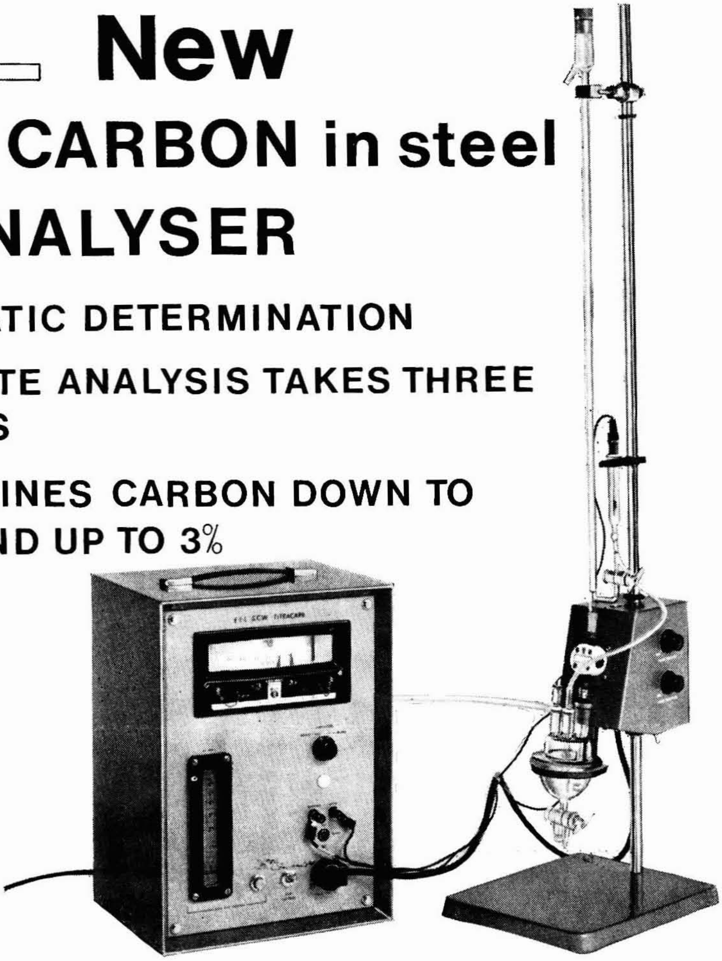

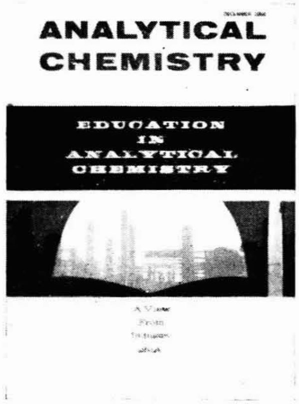
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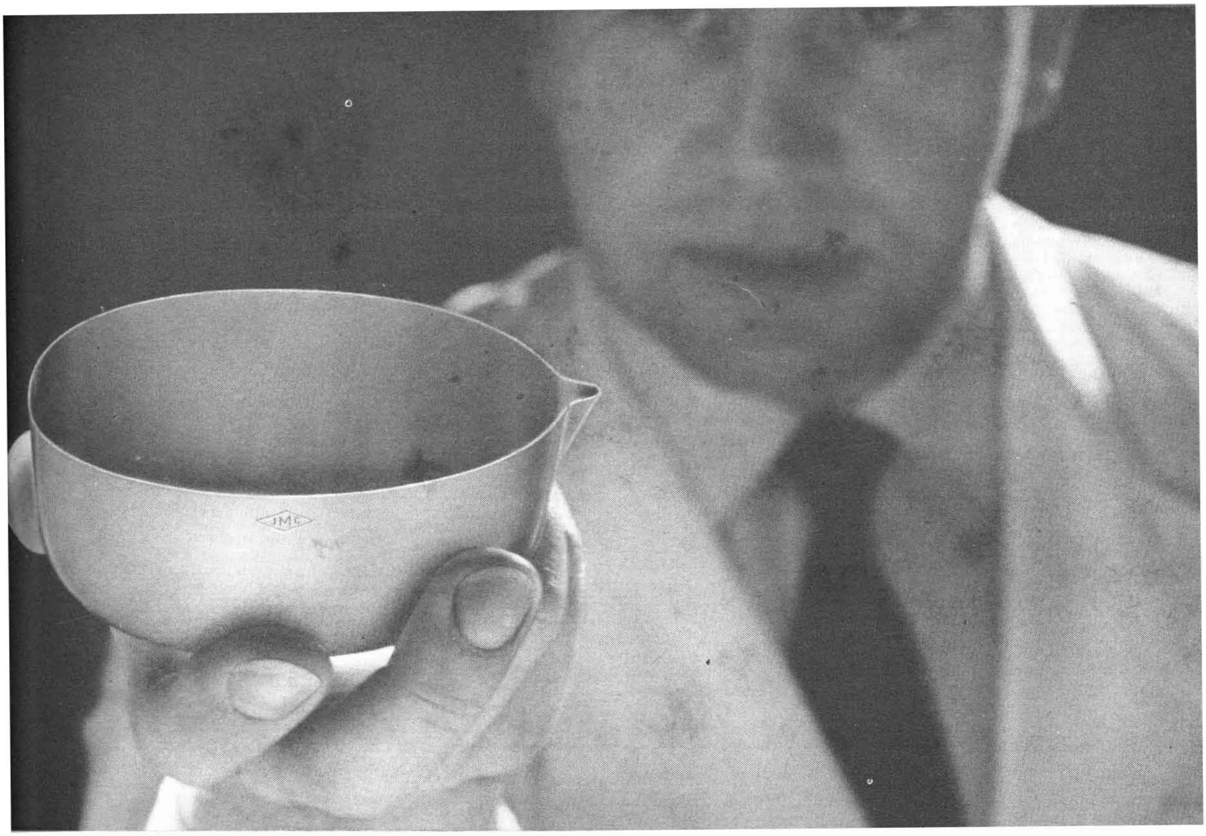

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