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Report No. 12. Determination of Ascaridole.
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The Determination of Small Amounts of Arsenic in Organic Matter.

Sub-Committee on the Determination of Unsaponifiable Matter in Oils and Fats and of Unsaponified Fat in Soaps:

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Report No. 1. Assay of Lobelia (*Lobelia Inflata*)
Report No. 2. Assay of Gelsemium.
Report No. 3. Assay of Aconite.
Report No. 4. Assay of Yohimba.
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Report No. 6. Assay of Ephedra and of Ephedrine in Nasal Sprays.

Sub-Committee on Vitamin Estimations:

Report on the Microbiological Assay of Riboflavine and Nicotinic Acid.
The Determination of Carotene in Green-Leaf Material. Part 1. Fresh Grass.
The Determination of Carotene in Green-Leaf Material. Part 2. Green-Leaf Materials other than Grass. (Gratis.)
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The Microbiological Determination of Thiamine.
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Examination of Detergent Preparations.

Meat Products Sub-Committee (formerly Meat Extract Sub-Committee:

Analysis of Meat Extract.
Determination of Gelatin in Meat Extract and Meat Stocks: Interim Report.
Nitrogen Factors for Pork *and* Nitrogen Content of Rusk Filler (as one reprint).

Pesticides Residues in Foodstuffs Sub-Committee:

Determination of Small Amounts of Total Organic Chlorine in Solvent Extracts of Vegetable Material.

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FOUNDED 1874. INCORPORATED 1907.

THE objects of the Society are to encourage, assist and extend the knowledge and study of analytical chemistry and of all questions relating to the analysis, nature and composition of natural and manufactured materials by promoting lectures, demonstrations, discussions and conferences and by publishing journals, reports and books.

The Society includes members of the following classes:—(*a*) Ordinary Members who are persons of not less than 21 years of age and who are or have been engaged in analytical, consulting or professional chemistry; (*b*) Junior Members who are persons between the ages of 18 and 27 years and who are or have been engaged in analytical, consulting or professional chemistry or *bona fide* full-time or part-time students of chemistry. Each candidate for election must be proposed by three Ordinary Members of the Society. If the Council in their discretion think fit, such sponsorship may be dispensed with in the case of a candidate not residing in the United Kingdom. Every application is placed before the Council and the Council have the power in their absolute discretion to elect candidates or to suspend or reject any application. Subject to the approval of Council, any Junior Member above the age of 21 may become an Ordinary Member if he so wishes. A member ceases to be a Junior Member on the 31st day of December in the year in which he attains the age of 27 years. Junior Members may attend all meetings, but are not entitled to vote.

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Scientific Meetings of the Society are usually held in October, November, December, February, April and May, in London, but from time to time meetings are arranged in other parts of the country. Notices of all meetings are sent to members by post.

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THE North of England, Scottish, Western and Midlands Sections were formed to promote the aims and interests of the Society among the members in those areas. The Microchemistry, Physical Methods and Biological Methods Groups have been formed within the Society to further the study of the application of microchemical, physical and biological methods of analysis. All members of the Society are eligible for membership of the Groups.

The Sections and Groups hold their own meetings from time to time in different places. There is no extra subscription for membership of a Section or Group. Application for registration as a member should be made to the Secretary.

THE ANALYST

PROCEEDINGS OF THE SOCIETY FOR ANALYTICAL CHEMISTRY

ORDINARY MEETING

AN Ordinary Meeting of the Society was held at 7 p.m. on Wednesday, October 10th, 1962, in the meeting room of the Chemical Society, Burlington House, London, W.1. The Chair was taken by the President, Dr. A. J. Amos, O.B.E., B.Sc., F.R.I.C.

The subject of the meeting was "Recent Developments in Polarography" and the following papers were presented and discussed: "Pulse Polarography," by H. M. Davis, B.Sc., A.Inst.P.; "Differential Cathode Ray Polarography," by H. I. Shalgosky, B.Sc., A.R.I.C.; "Analytical Aspects of Radio Frequency Polarography," by Dr. H. W. Nürnberg.

DEATH

WE record with regret the death of

Frederick Cecil Barron Marshall.

Applications of Reversed-phase Partition Chromatography of Uranium*

By T. J. HAYES

(*United Kingdom Atomic Energy Authority, Springfields Works, Salwick, Preston, Lancs.*)

and A. G. HAMLIN

(*United Kingdom Atomic Energy Authority, Capenhurst Works, nr. Chester, Cheshire*)

Reversed-phase partition chromatography is shown to be a convenient and simple method for the quantitative separation and recovery of uranium either from impure solutions of unknown origin or from solutions of uranium alloys.

The separation process has been studied and shown to obey a Freundlich-type isotherm from both nitric acid and hydrochloric acid media.

Applications of the technique to specific problems in the analysis of uranium are briefly outlined.

The analysis of alloys of uranium with zirconium, titanium, molybdenum, vanadium, etc., can often be simplified by an initial separation of the uranium, and the determination of trace impurities in such alloys can also be improved by a similar separation.

Hamlin *et al.*[1] have applied the technique of reversed-phase partition chromatography, with the selective reagent tri-n-butyl phosphate, to the extraction of uranium from wastes and residues of unknown origin. This technique has the advantage of simplicity compared with continuous or counter-current extraction with the same reagent. After the sample solution has been adjusted to optimum acidity with nitric acid, it is passed through a column of inert lyophobic microporous particles impregnated with tri-n-butyl phosphate and previously conditioned with nitric acid solution. The uranium is retained as a band at the top of the column and the impurities are removed by washing with nitric acid solution. The uranium itself is then eluted with water. The separation is affected only by the few elements that partition strongly into the selective reagent tri-n-butyl phosphate.

This chromatographic technique has now been applied to the precise determination of uranium in complex alloys. By providing a simple means of removing uranium with the minimum number of transfers and analytical operations, high accuracy is easily achieved. Alloying constituents and impurities can also be determined in the column effluent if so required.

Experimental

Preparation of tri-n-butyl phosphate—

One volume of commercial-grade tri-n-butyl phosphate was steam-distilled with 1·5 volumes of 0·5 M sodium hydroxide until the distillate was free from the odour of butanol. The tri-n-butyl phosphate was then separated from the aqueous alkaline phase, and washed with water and nitric acid.

Preparation of uranium solutions—

Uranyl nitrate solutions for column experiments were prepared from the analytical-reagent grade salt, and adjusted to the required molarity with nitric acid.

For precise work, spectrographically pure U_3O_8 was ignited at 920° C for 1½ hours; when cool, the required amount was weighed and then dissolved in nitric acid.

Uranyl chloride solutions were prepared by dissolving pure UO_3 in hydrochloric acid followed by repeated evaporations with distilled water to remove the excess of acid.

Apparatus—

Preliminary experiments established the approximate size of the column required. Experiments with solutions containing 10 to 50 mg of uranium were carried out with a chromatographic tube of the same dimensions as that used by Hamlin *et al.*[1]

* Presented at the meeting of the Society on Thursday and Friday, October 5th and 6th, 1961.

This size of tube will hold 1·5 g of support. For solutions containing up to 1 g of uranium, a tube containing 12·5 g of support, with an effective column length of approximately 20 cm, was used. The choice of column dimensions was governed by the initial and displaced uranium band lengths required for each particular separation.

CHOICE OF COLUMN MATERIAL—

The application of the reversed-phase partition technique depends on the availability of a suitable support material. In this work it was expected that the solutions would be moderately concentrated in nitric acid and that they would frequently contain fluoride; for these reasons a chemically inert support material was sought. The first choice was "Kel-F" moulding powder, previous experience having shown that the particular grade used ("Kel-F" 300 low density)* had a large capacity for stationary phase. Kel-F behaved satisfactorily and examination of several batches of the particular grade used indicated that its properties were reproducible. Other support materials, both organic and inorganic, have been examined,[1] but none showed as satisfactory retention of tri-n-butyl phosphate as Kel-F. Before use, the Kel-F was reduced to the required size either in a micro-hammer mill or, after freezing with solid carbon dioxide, in a coffee-mill. The required range of particle size was obtained by sifting.

The reason for the outstanding behaviour of Kel-F moulding powder has not been explained. The surface area of the 100- to 200-mesh material, measured by nitrogen and krypton gas adsorption, is of the order of 1·5 sq. metres per g, which is much less than that of chromatographic grade alumina and charcoal supports. It has been observed however that powder heated accidentally to near sintering point during grinding loses its absorptive capacity and produces an inefficient column.

COMPOSITION OF THE COLUMN—

The pure solvent was used as stationary phase in all experiments in order to obtain the most favourable partition coefficient for uranium. Equal weights of tri-n-butyl phosphate and Kel-F were used, as solvent was lost at an excessive rate on increasing the solvent loading much beyond this ratio. The apparently crude method of coating the support by slurrying with tri-n-butyl phosphate appeared to be as efficient as any other.

As tri-n-butyl phosphate has an appreciable solubility in both 5 M nitric acid and water, the life of the 1·5-g column is limited to about 30 separations. There is a visible check on the efficiency of a column in that excessive spreading of the uranium band indicates approaching exhaustion.

The Kel-F powder can be recovered for further use by solvent extraction with acetone and carbon tetrachloride. The Kel-F is slurried with acetone and then sucked dry on a Buchner filter; the last traces of tri-n-butyl phosphate are removed by extraction with carbon tetrachloride in a Soxhlet apparatus. Incomplete removal of tri-n-butyl phosphate caused trouble on one occasion owing to degradation on drying the Kel-F. This was noticed as an odour of butyric acid, and columns prepared from this batch gave poor separations from iron and zirconium.

COMPOSITION OF THE AQUEOUS PHASE—

It is known that the optimum partition of uranium into 20 per cent. tri-n-butyl phosphate in kerosene occurs when the aqueous phase is approximately 5·5 M in nitric acid. The extraction behaviour of both nitric acid and uranyl nitrate from the column was found to be similar to the conventional liquid - liquid system. When the column was conditioned with excess of 5·5 M nitric acid, a (1 + 1) nitric acid - tri-n-butyl phosphate complex was formed; when the column was saturated with uranyl nitrate the limiting state was found to be a (1 + 2) uranyl nitrate - tri-n-butyl phosphate complex. The use of pure tri-n-butyl phosphate dispersed on an inert support of Kel-F does not, therefore, involve any fundamental changes in the system, such as Pierce[2] found for the dithizone - copper reversed-phase system.

Experimentally, by determining the retention volumes for uranium solutions at different molarities of nitric acid, the optimum acidity for the extraction of uranium was found to be 5·5 M. The relationship between uranium partition and nitric acid molarity in the aqueous phase is shown in Fig. 1, curve A.

* Obtained from the Minnesota Mining and Manufacturing Co. Ltd.

For hydrochloric acid solutions the maximum extraction of uranium occurs between 3·3 and 6·0 M; 5·0 M hydrochloric acid was used as mobile phase for all further extraction experiments. At this acidity the equilibrium mole ratio of hydrochloric acid to tri-n-butyl phosphate was found to be 0·33, in agreement with the work of Kertes[3]; for 6·0 M hydrochloric acid the equilibrium mole ratio becomes 0·5.

The relationship between uranium partition and the molarity of hydrochloric acid is shown in Fig. 1, curve B.

EXTRACTION ISOTHERMS FOR URANIUM—

In order that suitable column characteristics could be derived theoretically rather than empirically, the reversed-phase extraction of uranium from both nitric and hydrochloric acid solutions was studied in some detail.

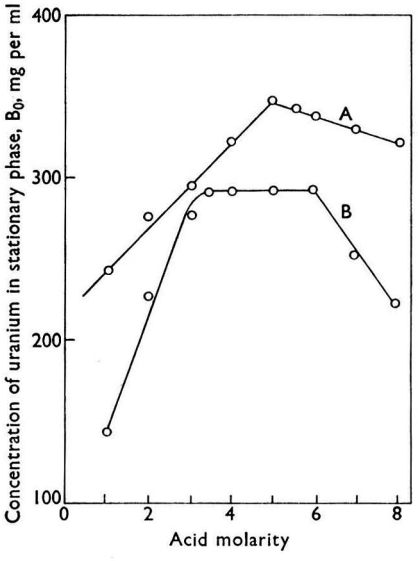

Fig. 1. Effect of acidity on the concentration of uranium in stationary phase, B_0, at a constant concentration of uranium in the mobile phase, C_0, of 28·3 mg per ml: curve A, nitric acid; curve B, hydrochloric acid

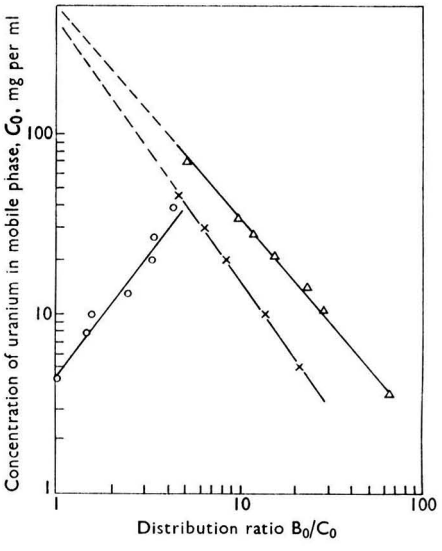

Fig. 2. Distribution of uranium between stationary and mobile phases for different values of C_0: △, 5·5 M nitric acid; ×, 5·0 M hydrochloric acid; O, water

Solutions of uranyl nitrate were adjusted to 5·5 M in nitric acid and passed through 12·5-g columns of Kel-F slurried with an equal weight of tri-n-butyl phosphate. The total volume of uranyl nitrate required to make the uranium front reach the bottom of the column was measured. This has been referred to by Tiselius as the "retention volume." The uranium distribution was then calculated from the equation—

$$B_0/C_0 = (V - S)/P$$

where B_0 = concentration of uranium (g per ml) in the tri-n-butyl phosphate phase,
C_0 = concentration of uranium (g per ml) in the mobile phase,
V = retention volume,
S = interstitial volume and
P = total weight of solvent in the static phase.

$(V - S)$ is the "corrected retention volume" and $(V - S)/P$ is the specific retention volume, numerically equal to the distribution ratio.

To determine which of the accepted isotherms was applicable, retention volumes at different concentrations of uranium were measured. It was found that a plot of $\log B_0$

against $\log C_0$ was linear, which indicates that the extraction of uranium follows a Freundlich isotherm—

$$B_0 = aC_0^n$$

The value of n was measured from the slope of the log - log plot and log "a" was obtained from the intercept on the $\log B$ axis at $C_0 = 1$.

For the extraction of uranium from 5·5 M nitric acid, the corresponding constants were found to be—

$$B_0 = 245\ C_0^{+0.15}$$

Retention volumes for uranyl nitrate in water were also measured, these being equivalent to the elution of uranium from the column. It was found that once again a Freundlich isotherm was followed, the corresponding constants being—

$$B_0 = 0{\cdot}4\ C_0^{-0.35}$$

Similar treatment of the data for the extraction of uranium from 5 M hydrochloric acid showed that the extraction could be expressed by—

$$B_0 = 66\ C_0^{+0.32}$$

The plotted isotherms for 5·5 M nitric acid, 5·0 M hydrochloric acid and water are shown in Fig. 2. The limiting figure of 440 mg per ml for B_0 represents saturation of the tri-n-butyl phosphate with respect to uranium.

Having identified the mechanism of partition it is possible to calculate such details as initial band lengths and the positions of the leading and trailing edges of the developed bands for different concentrations of uranium. Since both adsorption and elution follow a Freundlich isotherm, complete recovery of uranium is theoretically impossible. However, over the range 5 μg to 5 g this effect has not been in evidence.

Effect of acid concentration on extraction—

The influence of acid concentrations has been mentioned previously in connection with the choice of optimum acidity for the extraction of uranium. Retention volumes were measured for a fixed concentration of uranium at various concentrations of acid.

For nitric acid solutions up to 5·5 M, increasing acidity enhances the extraction of uranium according to the equation—

$$[U_{org.}] = 175\ ([U_{aq.}]\ [HNO_3]^2)^{0.15}$$

where [U] = concentration of uranium in mg per ml and
$[HNO_3]$ = molarity of the nitric acid mobile phase.

Above 5·5 M the extraction of uranium decreases and can be expressed as—

$$[U_{org.}] = 287\ ([U_{aq.}]\ [HNO_3]^{-1})^{0.15}$$

For hydrochloric acid solutions up to 3·2 M, extraction of uranium increases with increasing acidity according to the equation—

$$[U_{org.}] = 51\ ([U_{aq.}]\ [HCl]^2)^{0.3}$$

Between 3·2 and 6·0 M, extraction of uranium remains constant, but above 6·0 M extraction falls according to the equation—

$$[U_{org.}] = 573\ ([U_{aq.}]\ [HCl]^{-3})^{0.3}$$

Forward extraction from nitric acid can thus be expressed in terms of uranyl nitrate. The extraction mechanism above 5·5 M acidity, however, requires one mole of uranium to react with one mole of nitric acid in the aqueous phase. This ratio would require displacement of $UO_2(NO_3)^+$ by H^+. A kinetic mechanism for extraction via the $UO_2(NO_3)^+$ species has been proposed by Keisch.[4]

In hydrochloric acid solutions, extraction proceeds in the ratio 1 mole of uranium to 2 moles of chloride ion, *i.e.*, extraction of uranyl chloride. The reverse extraction, which occurs above 6 M acidity, occurs in the ratio 1 mole of uranium to 3 moles of hydrochloric acid, indicating formation of $H(UO_2Cl_3)$ in the aqueous phase.

The activity coefficients of hydrogen ion in the organic phase can also be related to the integers occurring in the distribution data.

DETERMINATION OF THEORETICAL COLUMN EFFICIENCY—

The number of theoretical partition stages obtained with a 12·5-g column was measured by two techniques; first, by following the break-through of a uranium solution in 5·5 M nitric acid and secondly by plotting the water elution curve of a narrow band of uranium. Both techniques gave a value of approximately N = 55 for a column length of 18·5 cm at a flow rate of 5 ml per minute.

APPLICATION TO ANALYTICAL PROBLEMS—

It was found that, as a general rule in nitric acid systems, two interstitial volumes of water were required to remove the excess of acid, a further two interstitial volumes of water being required to remove the uranium.

When adsorbed uranium is washed free from impurities, most of the common elements appear in the sample volume; two further interstitial volumes remove impurities completely from the column. Elements such as zirconium^{IV}, thorium^{IV}, plutonium^{IV} and cerium^{IV}, which have a measurable partition into tri-n-butyl phosphate from nitric acid, are not removed efficiently. The elution curves for several typical impurities have already been described.[1]

Hydrochloric acid is not as selective an aqueous medium as nitric acid for extracting uranium, but has the advantage of giving a better separation of uranium from thorium and also under suitable conditions from plutonium. Of the elements so far examined, Fe^{III}, Co^{II}, V^{V}, Mo^{VI}, Cr^{VI} and Cu^{II} are extracted wholly or partly from 2 to 6 M acid.

USE OF THE 1·5-g KEL-F COLUMN—

Use of the 1·5-g column was limited to those separations involving less than 50 mg of uranium. Wastes, liquors and effluents were conveniently handled by this technique and the separations were made from 5·5 M nitric acid to give maximum specificity. Recovery of uranium was investigated over a wide range of concentrations and by a variety of analytical procedures. The results obtained are summarised in Table I.

TABLE I

RECOVERY OF URANIUM WITH 1·5-g KEL-F COLUMNS

Uranium level	Analytical technique	Recovery, %
5 μg	α-counting	90 to 100
	Absorptiometric—	
5 μg	Dibenzoylmethane	90 to 100
5 μg	PAN	90 to 100
5 to 50 mg	Thiocyanate	100
5 to 50 mg	Sodium carbonate - hydrogen peroxide	99
5 to 50 mg	Thioglycollic acid - EDTA	99
	Volumetric—	
50 mg	Ceric titration of U^{IV}	99·9

USE OF 12·5-g KEL-F COLUMNS—

For application to weights of uranium in the range 100 mg to 1 g, columns of different dimensions containing between 10 and 25 g of Kel-F were examined. For general purpose work, a column containing 12·5-g of Kel-F packed to a length of 20 cm was a suitable compromise between maximum uranium loading and efficiency of washing. Kel-F (44 to 100 mesh) could also be substituted for the 100- to 200-mesh material used on the smaller columns.

Recovery of uranium over the range 0·25 to 1 g was first demonstrated. The eluted uranium was determined either gravimetrically as ammonium uranyl phosphate or volumetrically by dichromate or ceric titrimetry after reduction to U^{IV}. Mean recovery for the gravimetric finish was 100·04 per cent. and for the volumetric finish 100·07 per cent. The results were sufficiently encouraging to consider application of the technique to the determination of uranium in alloy systems.

ANALYSIS OF URANIUM - ZIRCALOY II ALLOYS—

Alloys of uranium with zirconium are of interest in fuel-element production. One such alloy is made from uranium and an alloy of zirconium, known as Zircaloy II. Zircaloy II contains 1·0 to 1·5 per cent. of tin, and iron, nickel and chromium to the sum of another 0·4 per cent.; traces of copper, tungsten, etc., are also present. Although reversed-phase separation of uranium from zirconium is not good from pure nitric acid, a satisfactory separation was achieved from a nitric - hydrofluoric acid mixture. The nitric acid molarity was adjusted to 5·5 M and the hydrofluoric acid molarity was between 1·3 and 1·5 M. The excess of hydrofluoric acid was complexed by addition of boric acid or aluminium nitrate. Boric acid was preferred as it was found to give shorter sharper uranium bands. The presence of zirconium and boric acid did not change the uranium distribution coefficient from that found in pure aqueous nitric acid.

Application of the technique to samples varying from pure uranium to 5 per cent. w/w uranium - Zircaloy II mixtures gave a mean recovery of 100·1 per cent., with a coefficient of variation of 0·14 per cent. Uranium was determined in the eluate by precipitation as ammonium uranyl phosphate in the presence of EDTA (ethylenediaminetetra-acetic acid). A similar method of analysis based on a continuous solvent-extraction technique required a much higher standard of analytical practice to reach a similar precision, and was biased low by 1 to 2 parts per thousand.

By using 350 ml of nitric acid to wash the column, quantitative recovery of all the alloying elements in 5 g of uranium - Zircaloy II is obtained, without loss of uranium. It was found convenient to determine other elements present, such as iron and nickel in column effluents, thus saving time and reducing the amount of sample required.

Operational details of the 12·5-g column used are shown in Table II.

TABLE II

DETAILS OF 12·5-g KEL-F COLUMN

Internal diameter of tube, mm	12·5
Effective Kel-F length, cm	20
Initial band length (275 mg of U), cm	3·0
Volume of nitric acid wash, ml	350
Final band length, cm	6·5
Volume of water just to elute the uranium, ml	80
Total volume of water used, ml	200
Flow rate, ml per minute	5

ANALYSIS OF URANIUM - TITANIUM ALLOYS—

After the successful application of reversed-phase separation to the uranium - Zircaloy system, the technique was applied to other alloys. One such alloy contained titanium in the range 1 to 10 per cent. by weight.

With the 12·5-g column it was possible to remove 0·5 g of uranium from solutions of uranium - titanium alloy in hydrochloric - nitric acid mixture at a total acidity of 5 M. For quantitative elution of the titanium it was necessary to wash with an additional 100 ml of 5·5 M nitric acid. At the higher level, titanium was determined volumetrically by addition of an excess of EDTA and titration of the excess with standard copper solution at pH 4·5, with PAN (1-(2-pyridylazo)-2-naphthol) as indicator. Smaller amounts of titanium were determined absorptiometrically with hydrogen peroxide in 10 per cent. sulphuric acid.

Recovery of 25 mg of titanium in the presence of 0·5 g of uranium gave a mean value of 100·0 per cent. with a standard deviation of 0·4 per cent.

Comparison of results obtained by this procedure with gravimetric results obtained after separation of titanium with cupferron also showed close agreement over the range 1 to 10 per cent. of titanium in uranium - titanium alloys.

APPLICATION TO GENERAL URANIUM ALLOYS—

Over the past year, the reversed-phase partition separation has been applied to alloys of uranium containing molybdenum, vanadium, chromium, niobium and aluminium, in the determination of either uranium or the alloying constituents, or both.

It has also proved useful as a means of preparing solutions free from uranium for spectrographic examination. Elimination of uranium eases considerably the task of reading

spectrographic plates by removing the complex background due to uranium. Trace elements in the column effluent are converted to phosphates and examined spectrographically by the rotating-disc technique. Quantitative recoveries at the 100 p.p.m. level, with a coefficient of variation of 5 to 10 per cent., have been obtained from aluminium, cobalt, copper, chromium, iron, magnesium, manganese, molybdenum, nickel, lead, bismuth, cadmium, scandium, titanium and vanadium[V]. Zirconium and niobium are also eluted quantitatively from a mixed nitric acid - hydrofluoric acid medium.

ALTERNATIVE REVERSED-PHASE SYSTEMS—

The solvent extraction of uranium from sulphate solutions with long-chain amines has now become well established. The corresponding reversed-phase separation with tri-n-octylamine supported on Kel-F has already been reported,[1] but, in this instance, recovery of uranium proved to be difficult. It has subsequently been found that Amberlite LA-1, a secondary amine described by the manufacturers as a liquid ion-exchange resin, can also be used as static phase supported on Kel-F. Extraction from either 0·5 M sulphuric acid or 9 M hydrochloric acid solutions with back-extraction into M nitric acid gives 98 to 100 per cent. recovery of uranium. Among other systems briefly examined, triphenylphosphine oxide also extracts uranium from nitric acid solution.

CONCLUSIONS

The purification of uranium before its determination can conveniently be carried out by solvent extraction from either nitric or hydrochloric acid by reversed-phase partition chromatography with tri-n-butyl phosphate as static phase. The method is simple and highly selective. No prior knowledge of the composition of the sample is required and the disadvantage of the slowness of the chromatographic step is offset both by this fact and by the many separations that can be handled concurrently by one operator.

The principle appears to be of general analytical application provided that suitable solvent and aqueous phases are available. Quantitative studies of solvent - solute relationships are also facilitated, as demonstrated by the separations described in this paper.

We thank Mr. J. C. C. Stewart, Managing Director, Production Group, U.K.A.E.A., for permission to publish this paper.

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DISCUSSION

MR. R. FRANKLIN said that the technique described had been extended by them to the determination of trace impurities in uranium compounds. The impurities were separated from uranium on the tributyl phosphate - Kel-F column and then subjected to spectrographic examination by the rotating-disc technique. Quantitative recoveries for 26 common elements had been obtained with a coefficient of variation of 5 to 6 per cent. at the 25 p.p.m. level.

MR. E. J. MILLETT asked if Kel-F had shown the same superiority as a support over the other materials tested for all the solvents mentioned.

MR. HAYES replied that other support materials, such as polythene, poly(vinyl chloride) and polytrifluoroethylene, had been tested, but primarily for use with tri-n-butyl phosphate. It was convenient to use Kel-F as a general support for other systems, and it had proved satisfactory for tri-n-octylamine, Amberlite LA-1 and triphenylphosphine oxide.

MR. D. F. WOOD asked if Mr. Hayes would give the advantages of this method applied to the determination of uranium over the method in which uranium was determined with dibenzoylmethane after solvent extraction with tributyl phosphate and fural, since the latter gave good results over a wide range of uranium concentrations, was almost specific and appeared to be quicker than the chromatographic procedure.

MR. HAYES said that the major advantages of the reversed-phase technique were that (*a*) it was applicable over a wide range of uranium concentrations (5 μg to 1 g), (*b*) large numbers of determinations could be carried out concurrently by relatively unskilled operators, (*c*) other elements were recovered quantitatively in the effluent and (*d*) there was a greater efficiency of separation from elements such as zirconium by reason of the greater number of theoretical stages.

If the specific determination of small amounts of uranium was required in material of known composition, a direct absorptiometric procedure, such as that suggested by Mr. Wood, would have been preferable.

MR. D. F. WOOD agreed that zirconium was also extracted, but that the amount was insufficient to cause interference in the subsequent colorimetric determination of, say, 20 to 100 p.p.m. of uranium based on a 0·5 g sample.

MR. R. FRANKLIN said that if uranium was left on the tributyl phosphate - Kel-F column for several hours it was extremely difficult to elute it with water. He asked if Mr. Hayes could offer any explanation of why this was so.

MR. HAYES said that, although they had not encountered this particular difficulty, they had noted appreciable degradation of tri-n-butyl phosphate on a column left in contact with 5 M nitric acid. The degradation products that caused trouble had not been identified, but they appeared to be eluted if the column was washed with sodium carbonate or ethylenediamine solution.

MR. R. SCHOLEY asked (*a*) if the Kel-F was saturated in pure tributyl phosphate and (*b*) why, since some zirconium accompanied the uranium from 5·5 M nitric acid solution, did it not cause interference in the phosphate finish.

MR. HAYES replied (*a*) the Kel-F was 100 per cent. loaded, *i.e.*, equal weights of tri-n-butyl phosphate and Kel-F were slurried together and (*b*) the uranium was precipitated in the presence of EDTA, which effectively complexed the traces of zirconium present.

DR. J. HASLAM said it had been stated that, when small amounts of uranium were involved, recoveries were of the order of 97 per cent., whereas with larger amounts recoveries of 100 per cent. were claimed. He asked whether the figures were comparable. It would be expected that the gravimetric method used for the larger amounts would be much more accurate than the colorimetric test used for the smaller amounts.

MR. HAYES replied that all evidence so far obtained, particularly that by radiochemical techniques, indicated that uranium was quantitatively recovered. The figures quoted for absorptiometric methods were probably a function of the reagent used.

The Spectrographic Determination of Silicon and Other Impurities in Gallium Arsenide*

BY J. H. OLDFIELD AND D. L. MACK

(*Admiralty Materials Laboratory, Holton Heath, Poole, Dorset*)

A method has been developed for determining traces of silicon and other impurities in gallium arsenide. The sample is dissolved by treatment with hydrochloric acid and bromine in the presence of carbon tetrachloride, and the reaction mixture is evaporated to small bulk. The arsenic is thus completely removed. The gallium chloride is extracted with di-isopropyl ether. The impurities are then concentrated in the presence of sulphuric acid, dried on a carbon electrode and excited in a d.c. arc, copper being used as the internal standard.

THE investigation described here deals primarily with the determination of silicon in gallium arsenide. At low concentrations, silicon is electrically significant as a donor element, and, when silica vessels are used in the preparation of gallium arsenide, it is a likely contaminant that is not easily removed by the usual methods of purification.

The simultaneous determination of other elements was also investigated when it was found that silicon could be determined after chemical concentration by a spectrographic procedure basically similar to that used for the determination of many elements in pure acids.[1]

During the investigation, considerable difficulty was experienced in reducing the silicon blank value. This blank value originally came both from the reagents and from airborne contamination. The reagent blank value was reproducible and could be estimated, but the adventitious contamination was variable and had to be eliminated. This was done by carrying out the chemical concentration procedure in a closed chamber into which only filtered air was allowed to enter.

EXPERIMENTAL

PURIFICATION OF REAGENTS—

Pure water—To determine the amount of silicon in the de-ionised water a preliminary working curve was obtained from silicon standards prepared in a manner similar to that described later under "Preparation of Standard Solutions," p. 779. Copper was used as the internal standard, and the standards were examined by the spectrographic procedure developed for the analysis of pure acids.[1] The range plotted was from 0·02 to 2·0 μg of silicon. (It had been previously ascertained that, at this level, the silicon blank on 0·1 ml of standard solution would not be significant.) Portions (100 ml) of de-ionised water were examined by the spectrographic procedure,[1] and the water was found to contain 0·02 μg of silicon per ml. At this level of concentration its use in the preparation of reagents, etc., would have resulted in an unacceptably high blank value. Other methods of purification were therefore investigated. A summary of these methods and the results obtained are shown in Table I.

TABLE I

REDUCTION OF SILICON BLANK VALUE IN WATER

	Procedure	Concentration of silicon, μg per ml
1.	Double-distilled water passed through Elgastat de-ioniser	0·02
2.	Double-distilled water re-distilled in platinum	0·007
3.	Double-distilled water re-distilled in tin-lined still	0·003

Attempts were made to purify the water by passing it through Amberlite IRA-400, as recommended by Sonnenschein,[2] but the results were not as satisfactory as those obtained

* Presented at the joint meeting of the Midlands Section and the Physical Methods Group with the Birmingham and Midlands Section of the Royal Institute of Chemistry on Tuesday, May 1st, 1962.

by procedure 3 in Table I. Any further reference in this report to pure water will therefore imply that it had been prepared in this way.

Hydrochloric acid—By using silicon standards prepared from pure water, commercially available specially prepared 11 N hydrochloric acid was analysed and found to contain 0·2 μg of silicon per ml. Acid (8 N) prepared by isothermal diffusion[3] was purer, containing 0·1 μg of silicon per ml, but, as this procedure was slow and inconvenient for the preparation of more than small amounts, the more rapid procedure described below was investigated. Hydrogen chloride gas from a cylinder was passed through pure water contained in a 5-litre polythene bottle surrounded by ice, the gas being first passed through a trap of hydrochloric acid contained in a polythene bottle also surrounded by ice. By this procedure 3 litres of 11 N acid could be obtained in about 3 hours. The resulting acid was analysed; results were: <0·001 p.p.m. of beryllium, <0·002 p.p.m. of manganese, chromium, bismuth, nickel, molybdenum, indium, titanium, cobalt, gallium and vanadium, 0·01 p.p.m. of zirconium, <0·02 p.p.m. of iron and lead, 0·005 p.p.m. of magnesium, 0·02 p.p.m. of calcium and 0·1 p.p.m. of silicon. It can be seen that the silicon content is similar to that of acid prepared by isothermal diffusion. Attempts to improve on this value failed, and the limitations imposed by the magnitude of this blank value were therefore at this stage accepted. (A considerable amount of work was done with this acid, but subsequently it was found that 8 N acid containing only 0·02 μg of silicon per ml could be prepared by passing hydrogen chloride gas obtained by warming AnalaR hydrochloric acid first through a cold trap at −30° C and then through pure water. This acid has been used in some later tests.)

Sulphuric acid—The analysis of specially prepared commercial acid diluted with pure water to 0·1 N was satisfactory except for silicon (0·4 μg per ml). Acid of greater purity was prepared by drawing the fumes (under suction) from oleum—via a polythene hood and tubing—into pure water in a polythene bottle. The evolution of the fumes was maintained by gentle warming. Three-hundred millilitres of 1·5 N acid were prepared in about 3 hours, and the silicon content of 0·1 N acid was found to be 0·02 μg per ml.

Isopropyl ether—The peroxides were removed as described below. One litre of ether was shaken with and separated from—

(*a*) three successive 40-ml portions of a ferrous sulphate solution (60 g of ferrous sulphate and 6 ml of concentrated sulphuric acid made up to 120 ml with water);

(*b*) 40-ml portions of 5 per cent. aqueous potassium permanganate until there was no further reduction of the permanganate, as indicated by the absence of a colour change;

(*c*) three 30-ml portions of 5 per cent. aqueous sodium hydroxide;

(*d*) three 50-ml portions of pure water.

The ether was then dried over calcium chloride. In practice it was allowed to stand overnight and then distilled over sodium hydroxide immediately before use.

The ether was tested by analysing 8 N hydrochloric acid that had been saturated with it. There was no increase in the silicon content above that already known to be in the acid.

Carbon tetrachloride and bromine—Bromine (AnalaR) and carbon tetrachloride ("special for spectroscopy") were tested together by shaking them with pure hydrochloric acid. The acid was then analysed spectrographically and it was found that there was no increase in impurities due to the bromine and carbon tetrachloride.

PREPARATION OF STANDARD SOLUTIONS—

Silicon—A standard solution containing 1 mg of silicon per ml was prepared by fusing 0·535 g of silica with 5 g of sodium carbonate. The melt was extracted with 20-ml portions of pure water, and the extracts were made up to 250 ml. This solution, which contained 1000 μg of silicon per ml, was used as a master standard from which a series of standards ranging from 0·02 to 10 μg of silicon per ml was prepared by dilution with pure water.

To ensure that similar conditions existed between standards and samples, the effect of sodium on the emission of silicon was investigated. This was carried out by comparing the excitation of a standard solution containing sodium with that of a standard solution from which the sodium had been removed on a cation-exchange column. There was no difference between the spectrograms in so far as the relative intensities and the relative-intensity ratios of the silicon and copper lines were concerned.

Other elements—The standards used to determine the other elements were not specially prepared for this investigation. They had been prepared for general requirements in the spectrographic analysis of semi-conductor materials and contained zinc, aluminium, lead, cadmium, bismuth, beryllium, indium, vanadium, titanium, nickel, tellurium, zirconium, iron, manganese, magnesium, mercury, chromium, cobalt, tin and molybdenum. Separate solutions of these elements each containing 0·1 g of the metal in 100 ml of 7 N hydrochloric acid (the concentration of acid recommended for the extraction of gallium chloride with di-isopropyl ether[4]) were prepared, the first twelve listed above by solution of the Specpure metal in hydrochloric acid (solution of tellurium and zirconium being facilitated by the addition of a few drops of nitric acid), and the next seven from Specpure chloride solutions. The molybdenum standard was prepared from ammonium molybdate. A composite solution containing 10 ml of each solution was then diluted to 250 ml with 7 N hydrochloric acid. Dilution of this solution, which contained 40 μg of each element per ml, with the appropriate amount of 7 N hydrochloric acid provided a series of standards covering the concentration range required.

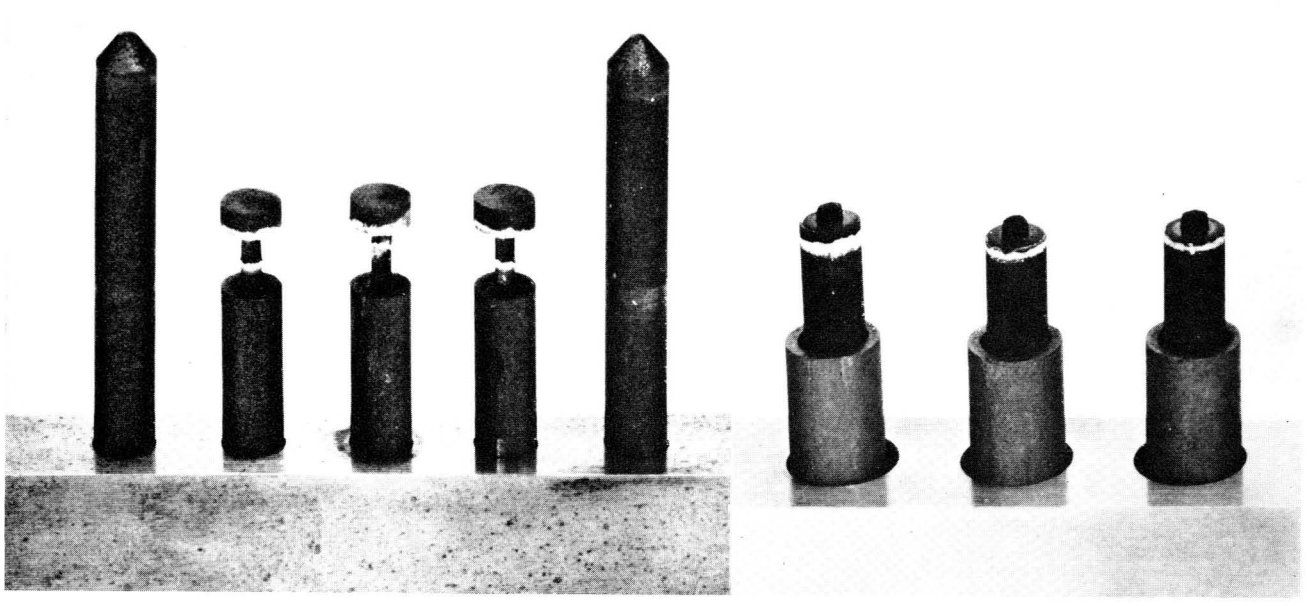
Copper sulphate—A standard solution of copper sulphate was used to provide the internal standard (copper). It was prepared by dissolving Specpure copper sulphate in pure water to give a solution containing 1 mg of copper per ml. One millilitre of this solution was added to the samples immediately before evaporation to small bulk after the arsenic and gallium had been removed.

PREPARATION OF ELECTRODES—

A solution technique has been described in which the solution was dried on an undercut graphite electrode.[1] It has been found, however, that the degree of porosity of the graphite varies, and that the solution seeps through into the neck of the electrode. This is shown in Fig. 1 (*a*). The variation in porosity results ultimately in a variation in sensitivity, since the amount of residue excited will vary. The use of carbon electrodes was therefore investigated. To test their porosity, standard solutions were dried on carbon electrodes that had been previously pre-arced at 4 amps for 2 seconds to remove surface contamination. The degree of seepage is shown in Fig. 1 (*b*). There is some slight difference in the appearance of the residue on the electrodes, but tests made on sliced sections of the electrodes showed that the solution did not seep further into the electrode than is indicated by the ring of residue seen about 1 to 2 mm below the surface. During arcing this 2 mm was burned completely away. These observations were confirmed by falling-plate tests. The tests were made on both carbon and graphite electrodes and the results are shown in Fig. 2. There are marked fluctuations in the density of the spectrograms from the graphite electrodes, even after 75 seconds' exposure. It was noted that these fluctuations corresponded to the excitation of the residue in the neck of the electrode and that these conditions were not reproducible. Further tests were made by subjecting two sets of electrodes, each consisting of one carbon and one graphite electrode, to the treatment described below. A 0·2-ml portion of copper sulphate (5 mg of copper per ml) in 0·1 N sulphuric acid was dried on each electrode, but for one set of electrodes this was followed by the addition of 0·1 ml of water. All the electrodes were then excited under the previous conditions, and the density of Cu 2882 line was measured in each spectrogram. The results were—

Graphite and copper sulphate	density 1·1
Graphite and copper sulphate + water	density 0·45
Carbon and copper sulphate	density 1·1
Carbon and copper sulphate + water	density 1·0

As a result of these tests it was decided to use carbon electrodes. It is generally considered that a disadvantage of carbon is that it is not as easy to machine as graphite. However, it was found that, owing to the low thermal conductivity of carbon, the temperature at the head of the electrode could be maintained without having recourse to undercutting, as had been necessary with graphite. The form of the electrode is shown in Fig. 1 (*b*), in which electrodes are seen supported in the graphite cups used to hold them (*i*) while the solution is dried on them and (*ii*) in the arc stand while the residue is being excited. The carbon electrodes are prepared by cutting suitable lengths from a 6·5-mm rod. The projecting tip is 2 mm in diameter and 2 mm long. Tests for silicon were made on several carbon electrodes



(*a*) (*b*)

Fig. 1. Electrodes : (*a*) graphite; (*b*) carbon

[To face page 780

by drying 0·1 ml of N sulphuric acid on them, and then arcing them under the conditions described under "Method." There was no increase in the silicon above that known to be in the acid. The incandescent carbon electrodes produced a heavy background above 3300 A. This was reduced by interposing a screen having an aperture permitting only radiations from the analysis gap to reach the spectrograph between the source and the slit of the spectrograph.

METHOD

CHEMICAL CONCENTRATION—

To prepare pieces of suitable size for analysis, the gallium arsenide crystal was attached to a ceramic base with Walkden wax, and cut into 1-mm thick slices with a diamond-impregnated copper wheel. The wheel operated under a copious flow of Honilo (Wakefield - Dick) lubricant. The wax was then softened by warming, and the slices were removed, any residual

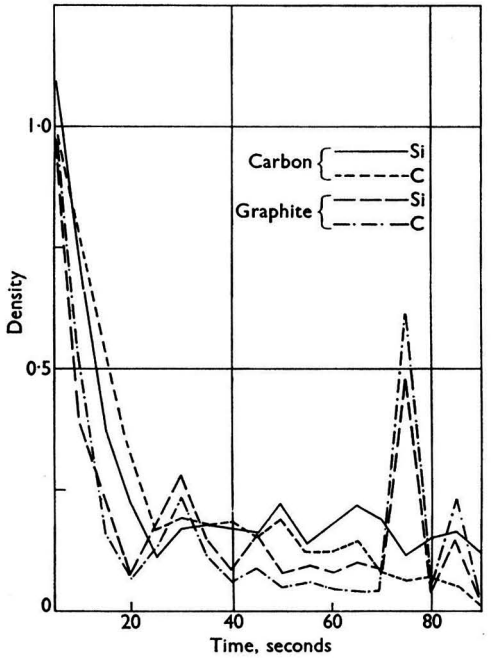


Fig. 2. Emission curves

wax being dissolved in boiling ethanol. The gallium arsenide was thoroughly cleaned with 11 N hydrochloric acid, washed with pure water, and dried. Approximately 1 g of sample was weighed into a polytetrafluoroethylene beaker that had been cleaned with aqua regia and boiling hydrochloric acid. A second beaker was similarly cleaned for the reagent blank solution, which was taken through the procedure with the sample. To the sample was added, successively, 5 ml of 8 N hydrochloric acid, 5 ml of carbon tetrachloride and 2 ml of bromine. The reaction between the bromine and the gallium arsenide was moderated by the carbon tetrachloride, in which the bromine dissolves. After the sample had dissolved, the solution was evaporated to small bulk, until it had a syrupy appearance (it was shown spectrographically that all the arsenic had been removed at this stage). The gallium chloride was then extracted with di-isopropyl ether as described below. The solution was transferred to a stoppered 50-ml polythene bottle with 6 ml of 8 N hydrochloric acid, 6 ml of ether were added, and the whole was vigorously shaken for 5 minutes. The separation of the two layers was facilitated by spinning in a centrifuge and drawing off as much as possible of the ether layer with a polythene pipette. The aqueous phase was then transferred to an 8-mm

diameter polythene tube, which facilitated the removal of the remaining ether, since, by reducing the intersurface area, it increased its depth. Three such extractions reduced the amount of gallium to less than 200 μg, an amount that could be tolerated in the spectrographic procedure.[5] Finally, the reduction of the acidity by the addition of 2 ml of water caused more ether to separate. This was removed as before. The acid layer was returned to the polytetrafluoroethylene beaker (cleaned as before), and 1 ml of 0·1 N sulphuric acid and 1 ml of copper sulphate solution were added. The solution was evaporated to approximately 0·1 ml in a dust-free chamber. It was then transferred dropwise to the carbon electrode supported in a graphite cup placed in a stainless-steel block (see Fig. 1 (*b*)). The block was on a hot-plate beneath a 250-watt infrared reflector lamp. The lamp was placed 20 cm above the electrode head, which was maintained at a temperature of about 90° C.

SPECTROGRAPHIC PROCEDURE—

The sample was excited in a controlled atmosphere in the previously described[1] excitation chamber, which is shown diagrammatically in Fig. 3. It consists of a brass cylindrical chamber that can be screwed down into an O-ring seal in a Tufnol base. The cylinder is provided with a quartz window to transmit the light to the spectrograph and a ruby-glass

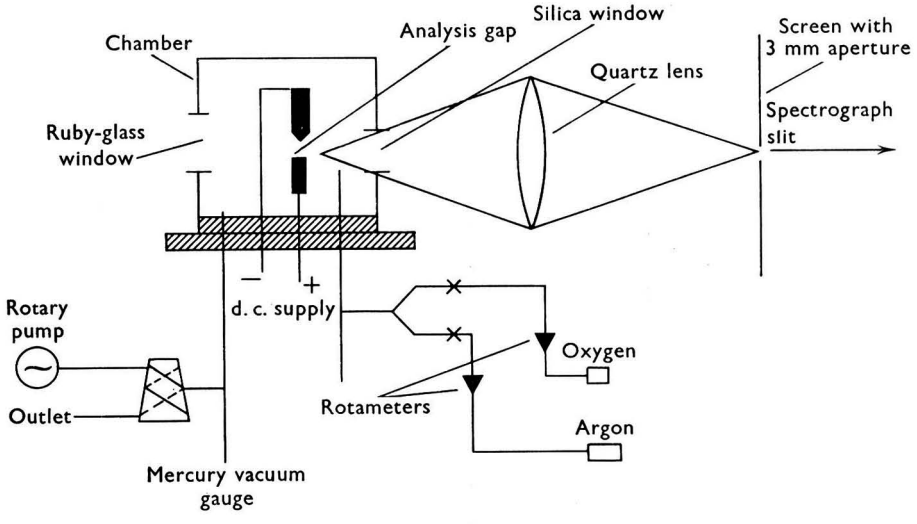


Fig. 3. Controlled atmosphere and optical system

window for inspection purposes. The base carries the electrode holders and the ports for evacuating the chamber and admitting the mixed gases. The graphite cup supporting the impregnated carbon electrode was placed in the lower positive electrode holder, a 6-mm diameter graphite rod ending in a blunted 80° cone (see Fig. 1 (*a*)) being used as a counter electrode. The analysis gap width was adjusted to 3 mm by focussing an image of the gap at unit magnification on to a tile with a horizontal aperture 3 mm wide located on the optical axis of the spectrograph and 30 cm from the slit. The aperture acted as a gauge for adjusting and aligning the gap. It permitted light from the 3 mm wide analysis gap to reach the spectrograph, but masked the light from the incandescent electrodes. The arrangement is shown in Fig. 3. The excitation chamber was evacuated to a pressure of 0·5 mm of mercury and then filled by a steady flow (2 litres per minute) of a (1 + 2) mixture of argon and oxygen. The sample was arced for 90 seconds at 12 amps, the spectrum being recorded on a Kodak B10 plate. The spectrographic conditions are shown in Table II.

The spectrogram was measured on a non-recording densitometer, galvanometer deflections being obtained for the element and internal standard lines and their adjacent backgrounds. Characteristic curves of the emulsion were obtained at the appropriate wavelengths from

TABLE II

SPECTROGRAPHIC CONDITIONS

Spectrograph	Hilger E478
Waveband	2700 to 4300 A
Slit width	10 μ
Slit length	3 mm
Source to slit distance	1 : 1 image focussed on tile 30 cm from slit
Upper electrode	Graphite 80° truncated cone (see Fig. 1(*a*))
Lower electrode	Carbon (as shown in Fig. 1(*b*))—positive
Analysis gap width	3 mm
Source	12-amp d.c. arc (spark initiated) in controlled atmosphere—(1 + 2) argon - oxygen mixture
Exposure time	90 seconds
Plate	Kodak B10
Development	Kodak D19b at 20° C for 3 minutes
Step-sector spectrum	Fe - Fe arc, 4 amps, 10·15 seconds
Line pairs	Si 2881·6 A - Cu 2882·9 A. For others see reference 1.

an iron arc step-sector spectrogram exposed on the same plate as the sample. The deflection values were converted to Seidel densities, background corrections were made, and the values of log(RI imp./RI Cu) were converted to micrograms from previously prepared working curves. The silicon working curve is shown in Fig. 4.

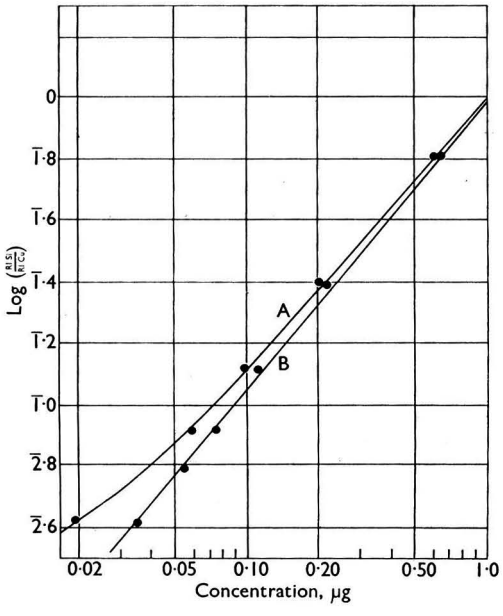


Fig. 4. Silicon working curve (line pair: Si 2881·58 - Cu 2882·93): curve A, uncorrected; curve B, corrected

TEST OF THE METHOD

RECOVERY FROM CHEMICAL PROCEDURE—

The 20 element standards as well as the silicon standards were used to test the recovery of the impurities from the chemical procedure. This was determined by making standard additions of impurities at concentrations of 0·5, 1·0 and 2 μg to samples of gallium arsenide. These samples, together with untreated samples, were analysed by the proposed method and the amounts of elements recovered were determined by deducting the results on the

untreated samples from those on the treated samples. The results for the elements successfully recovered were—

Element	Si, Mg, Mn, Cr, Ni, Bi, Al, Be	V, Zr, Co
Recovery, %	80 to 100	60 to 80

(Under the spectrographic conditions shown in Table II, mercury and tellurium could not be determined.) There was no significant difference between the recoveries at the various concentrations; the recovery values have therefore been expressed as percentages.

The recovery of cadmium (50 per cent.), although low, was reproducible, but the recoveries of zinc, molybdenum and indium varied and were usually low. Lead and titanium appear to be recovered, but their determinations were vitiated by high blank values. Iron and tin were not recovered.

Reproducibility—

Owing to the shortage of material available and the nature of the method, it was not feasible to test the reproducibility by a number of repeat exposures. However, the reproducibility of the spectrographic procedure was already known for all the elements except silicon.[1] The reproducibility for silicon was determined by making repeat exposures of the standards at 1- and 0·04-μg levels on different plates. The coefficient of variation was found to be 5 per cent. at the 1-μg level and 10 per cent. at the 0·04-μg level. The reproducibility of the recovery values determined on several samples indicated that the chemical procedure was reproducible within the limits set by the spectrographic procedure.

Application of the method—

Several samples have been analysed; the results are shown in Table III. Each result is the average of two or more determinations. The samples were from various sources of

Table III

Analysis of gallium arsenide

The concentrations of all elements are given in p.p.m. by weight

Sample No.	Si	Mg	Mn	Cr	Ni	Bi	Al	V	Zr	Co	Cd	Be
SC42	1	0·2	0·04	—	0·05	<0·05	0·3	<0·1	<0·1	<0·02	<0·40	<0·02
SC43	1	1	0·1	1·0	0·3	<0·05	0·4	<0·1	<0·1	<0·02	<0·40	<0·02
SC46A	2·5	0·2	0·02	<0·02	0·3	<0·05	0·5	<0·1	<0·1	<0·02	<0·40	<0·02
SC46B	2	0·2	0·02	0·05	0·5	<0·05	0·3	<0·1	<0·1	<0·02	<0·40	<0·02
SC46C	1	0·2	0·03	0·05	1·2	<0·05	0·3	<0·1	<0·1	<0·02	<0·40	<0·02
SC47	3	0·25	0·03	<0·02	0·5	<0·05	0·4	<0·1	<0·1	<0·02	<0·40	<0·02
SC50	2	0·7	0·1	0·1	2	<0·05	>50	<0·1	<0·1	<0·02	<0·40	<0·02
SC51	0·3	0·2	0·04	0·04	0·07	<0·05	0·6	<0·1	<0·1	<0·02	<0·40	<0·02
Typical blank ..	0·3	0·2	0·02	<0·02	<0·01	<0·05	0·2	<0·1	<0·1	<0·02	<0·40	<0·015

supply. Those marked SC46A, B and C, were from a single crystal, A and B representing the purified zones and C a contaminated zone. Sample SC50 was forwarded primarily for the determination of aluminium, since it had been made in an aluminium nitride container. Sample SC51 was from a pure single crystal of gallium arsenide that had been supplied to laboratories participating in the joint investigation into the analysis of gallium arsenide.

Conclusions

The spectrographic sensitivity, in terms of the minimum amount in micrograms on the electrode that can be detected, is—

Element	Be	Mg, Co	Cr, Ni, Mn, Si	Bi	Zr, Al, V	Cd
Sensitivity, μg ..	0·005	0·01	0·02	0·05	0·10	0·20

The amount that can be determined will depend on the recovery of the element and its presence in the reagents. The sensitivity, in p.p.m., can to some extent be increased by taking

a larger weight of sample, but, as this would involve the use of larger amounts of reagents, the potential increase in sensitivity for a particular element will therefore have to be considered with reference to the reagent blank value for that element.

Throughout the investigation the blank value, particularly for silicon and aluminium, has proved a most vexatious factor limiting the sensitivity of the method, and the fear of misleading results being obtained owing to adventitious contamination can only be eradicated by working in an efficiently air-controlled laboratory.

We acknowledge much helpful discussion with Mr. W. T. Rees and Mr. W. Barnes, and we thank the Superintendent of the Admiralty Materials Laboratory for permission to publish this paper.

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Polarographic Determination of Azinphos-methyl Residues in Certain Crops

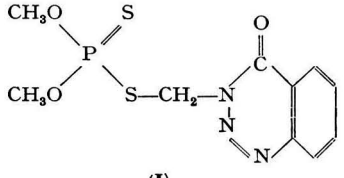
By J. A. R. BATES

(*Plant Pathology Laboratory, Ministry of Agriculture, Fisheries and Food, Hatching Green, Harpenden, Herts.*)

A method is described for determining azinphos-methyl residues in apples, pears, cucumbers and tomatoes by cathode-ray polarography. Interfering substances are removed on a column of magnesium oxide, and the polarographic procedure is carried out in a potassium chloride - acetic acid base electrolyte. Residue values are given for tomatoes and cucumbers treated experimentally with azinphos-methyl.

ALTHOUGH most methods used for determining pesticide residues are colorimetric, there has recently been a marked increase in the use of physical measurements for this purpose. The work reported here is part of a programme designed to utilise in the determination of pesticide residues the excellent reproducibility and versatility of the cathode-ray polarograph. Polarographic methods are usually rapid; the time needed to prepare the solutions can also be reduced considerably, as the inherent high sensitivity and resolving power of the cathode-ray polarograph often reduces the amount of purification necessary for the application of other techniques.

Although ketonic groups generally undergo reduction at a dropping-mercury electrode only at a very negative potential, the presence of certain groups or structures in the molecule can alter the electron deficiency with a subsequently easier acceptance of electrons from the electrode. The structure of azinphos-methyl [*S*-(3,4-dihydro-4-oxobenzo[*d*]-[1,2,3]-triazin-3-ylmethyl) dimethyl phosphorothiolothionate], **I**, suggests that the carbonyl group might be easily reduced polarographically, so forming the basis for an analytical method.



(**I**)

Azinphos-methyl is an insecticide and acaricide officially approved in the United Kingdom for the control of *Cydia pomonella* (codling moth), *Tortricidae* (tortrix moth caterpillars), *Metatetranychus ulmi* (fruit tree red spider mite) and *Bryobia spp.* (bryobia mites) on apples and pears. It is also used to control *Tetranychus telarius* (red spider mite) on cucumbers and tomatoes grown under glass.

The methods so far reported for determining azinphos-methyl residues in crops are colorimetric. Residues in cottonseed were determined by Giang and Schechter[1] by hydrolysing with hydrochloric acid, the formaldehyde liberated from the methylene group being distilled and determined colorimetrically with chromotropic acid. Recoveries of 94 to 99 per cent. of azinphos-methyl added to crushed cottonseeds were claimed. Alkaline hydrolysis of azinphos-methyl gives anthranilic acid, which Meagher, Adams, Anderson and MacDougall[2] diazotised and coupled with 1-naphthylethylenediamine dihydrochloride to produce a coloured complex. Suitable clean-up procedures have been described for cottonseed, fruits, chlorophyll-containing crops and milk, the final colour development being similar to Averell and Norris's method[3] for determining parathion. Azinphos-methyl can also be hydrolysed to a diazonium compound, which, when coupled with *N*-phenyl-1-naphthylamine gives a blue-violet colour.[4] This test is claimed to be specific to azinphos-methyl, but has not yet been applied to residues. Laws and Webley's general method,[5] which depends on the determination of phosphorus by the molybdenum-blue method after separation of the organo-phosphorus insecticide from interfering substances on a column of alumina, is applicable to azinphos-methyl in vegetables.

Experimental

Polarography of azinphos-methyl—

Preliminary investigations showed that azinphos-methyl gave an analytically useful peak in a base electrolyte of 0·05 M potassium chloride and 0·1 N acetic acid in 60 per cent. v/v acetone - water mixture. This electrolyte has a pH of 3·8, which is suitable for the reduction and avoids hydrolysis of the ester during the electrolysis. Slight variations in the base electrolyte were tried without any improvement in the form of the peak. With the start potential at —0·6 volt for cathodic reduction and a scale factor of 0·4, the peak potential was —0·83 volt against a saturated-calomel electrode. The relationship between peak height and concentration of azinphos-methyl was linear over the range 1 to 20 μg of azinphos-methyl per ml on the direct circuit. Typical cathodic reduction peaks for azinphos-methyl are shown in Fig. 1 (*a*).

Interfering substances—

In the development of a satisfactory method for determining residues, consideration must be given to the possible presence of metabolites and other decomposition products. The most probable principal metabolite of azinphos-methyl is its oxygen analogue. Metabolic studies[6] have shown that this compound is not formed in cotton, and throughout this investigation no secondary peak was detected that could be attributed to it or any other break-down product of azinphos-methyl.

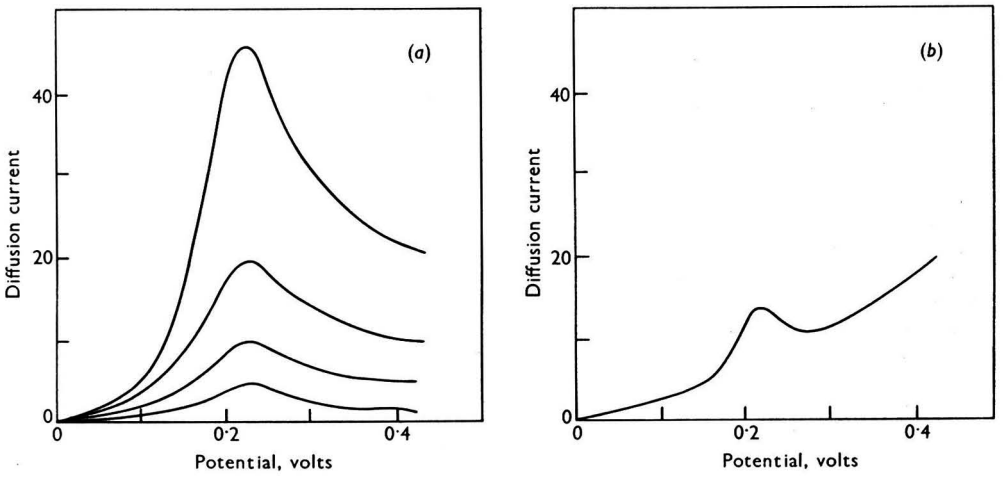


Fig. 1. Cathode-ray polarograms recorded at 25° C in 0·05 M potassium chloride – 0·1 N acetic acid base electrolyte in 60 per cent. v/v acetone – water at a start potential of — 0·6 volt against a saturated-calomel electrode: *(a)* solutions containing 2,4,8 and 20 μg of pure azinphos-methyl per ml (direct circuit; scale factor 0·4); *(b)* solution prepared from a tomato extract containing 4·3 μg (equivalent to 1·1 p.p.m. in tomatoes) of azinphos-methyl per ml (direct circuit; scale factor 0·4)

Azinphos-methyl was satisfactorily extracted from apples, pears, tomatoes and cucumbers as described below. The tissue was macerated in an excess of acetone, and the insoluble material was removed by filtration. The acetone solution was diluted with water, and the azinphos-methyl residues were extracted from the acetone - water phase with successive portions of chloroform; one 100-ml and four 50-ml portions were sufficient to extract 96 per cent. of the azinphos-methyl present. Two further extractions had little effect on the over-all recovery. The combined extract was unsuitable for direct polarographic examination owing to interference from the co-extracted plant chemicals, some of which were reduced at about the same voltage as azinphos-methyl. After examination of several adsorbent - solvent systems, azinphos-methyl was finally separated from interfering materials on a column of magnesium oxide, with benzene as initial solvent and eluting agent. After evaporation of the benzene the residue was dissolved in acetone and the supporting electrolyte was added for polarography.

APPLICATION TO CROP RESIDUES—

The method has so far been applied only to the four crops on which azinphos-methyl is currently used in this country, namely, apples, pears, tomatoes and cucumbers. To determine the over-all recoveries of the chemical from crops, aliquots of a standard solution of azinphos-methyl in acetone were thoroughly mixed with the macerate before extraction. In each test a 100-g sample of the crop was taken and the azinphos-methyl added was equivalent to 0·5 or 1·0 p.p.m.

TABLE I

RECOVERY OF AZINPHOS-METHYL ADDED TO APPLES, PEARS, TOMATOES AND CUCUMBERS

	Recovery of 50 μg of added azinphos-methyl, %	Recovery of 100 μg of added azinphos-methyl, %
Insecticide placed on column	100	87
Apple (Italian; variety unknown)	105	80
Apple purée (canned; Bramley)	100	96
Pear (South African; variety unknown) ..	120	80
Tomato	100	82
Tomato purée (canned)	100	99
Cucumber	100	82

METHOD

APPARATUS—

K1000 cathode-ray polarograph (Southern Analytical Ltd.), average drop mass = 6·98 mg of mercury.

The temperature of the water bath was controlled at 25° ± 0·25° C.

REAGENTS—

All reagents must be of recognised analytical grade.

Acetone.

Chloroform.

Benzene.

Sodium sulphate, anhydrous.

Magnesium oxide—The grade supplied for chromatographic analysis.

Potassium chloride, 0·25 M.

Acetic acid, 0·5 N.

Mercury, triple distilled.

Nitrogen, oxygen-free.

Standard azinphos-methyl solution—Recrystallise fairly pure technical azinphos-methyl several times from light petroleum, boiling range 60° to 80° C, until colourless leaflets (uncorrected m.p. 74·3° C) are obtained. Prepare a solution containing 100 μg of the product per ml, and take aliquots or dilute as required.

PREPARATION OF CALIBRATION CURVE—

By pipette, place different aliquots of the standard solution of azinphos-methyl in acetone in separate 25-ml calibrated flasks so that the final concentrations are in the range 1 to 20 μg of azinphos-methyl per ml. Add to each 5 ml of 0·5 N acetic acid and 5 ml of 0·25 M potassium chloride, and make up to the mark with acetone. Record polarograms on 5 ml of each solution with the starting potential at −0·6 volt and scale factors of 0·4 and 0·25. Plot the peak heights against concentration of azinphos-methyl.

EXTRACTION PROCEDURE—

Macerate a 100-g sub-sample of the crop with 100 ml of acetone for 5 minutes at high speed. (For recovery experiments add, at this stage, an aliquot of standard azinphos-methyl solution, and set aside for 15 minutes.) Add 100 ml of acetone, and mix for 5 minutes at low speed. Filter the mixture by suction through nylon cloth on a Buchner funnel. If the first portion is turbid, filter again. The resultant filtrate should be crystal clear.

Rinse the macerator jar with two 50-ml portions of acetone, and use the rinsings to wash the solid on the filter-pad. Transfer the filtrate to a 2-litre separating funnel, and add 300 ml of distilled water. Extract with five successive portions of chloroform (100 ml

for the first and 50 ml for each subsequent extraction). Shake gently during the first extraction to avoid the formation of stable emulsions. Wash the combined chloroform extracts with 200 ml of water. Separate the water, wash it with two 25-ml portions of fresh chloroform, and add the washings to the main chloroform extract. Dry the extract over anhydrous sodium sulphate, filter, and distil to small volume in a rotary film or Kuderna - Danish evaporator. Transfer to a beaker and remove the last traces of chloroform on a steam-bath in a current of air.

CHROMATOGRAPHIC PROCEDURE—

Dissolve the residue from the chloroform extract in 10 ml of benzene. Prepare a column, 1·5 cm in diameter, from a slurry of 5 g of magnesium oxide in benzene. Transfer the benzene solution to the column, and elute with 100 ml of benzene at about 4 ml per minute. Collect the first 30 ml in a graduated cylinder. Transfer the eluate to a beaker, and evaporate to dryness on a water bath; use a stream of air to assist evaporation. Dissolve the residue in 5 ml of acetone.

POLAROGRAPHIC PROCEDURE—

Transfer the acetone solution to a 25-ml calibrated flask. Wash the beaker with a further 5 ml of acetone, and transfer the washings to the calibrated flask. Add 5 ml of 0·5 N acetic acid and 5 ml of 0·25 M potassium chloride, and make up to the mark with acetone. De-gas 5 ml of this solution with oxygen-free nitrogen for 10 minutes, and then record a polarogram with a starting potential of −0·60 volt and scale factors of 0·4 and 0·25, or less if necessary to obtain a measurable wave height (see Fig. 1 (*b*)). Measure the concentration of azinphos-methyl in the sample by comparison of the peak height with the calibration curve.

RESULTS

APPLICATION TO CROP RESIDUES—TOMATOES AND CUCUMBERS—

During 1961, analyses were carried out on samples of glasshouse tomatoes and cucumbers treated with azinphos-methyl aerosols according to commercial practice. The rate of application was 1·1 g of azinphos-methyl per 1000 cu. feet of glasshouse space. The carrier solvent was acetone.

TABLE II

AZINPHOS-METHYL RESIDUES IN TOMATOES AND CUCUMBERS

	Date of application	Days after application: Picking	Days after application: Analysis	Azinphos-methyl residue found, p.p.m.
Tomatoes	12.8.61	2	3	0·6
	18.8.61	3	17	1·1
	4.9.61	2	10	0·6
Cucumbers	4.9.61	2	4	0·8
	15.9.61	4	19	not detected
	26.9.61	2	14	0·1

Approximately four pounds of tomatoes or six cucumbers were picked for each sample, and were held in deep-freeze storage before analysis. These were sub-sampled in the laboratory. Analyses were performed in duplicate and blank determinations on untreated crops and over-all recoveries of azinphos-methyl added to untreated crops were carried out at the same time. Average recoveries are shown in Table I. In none of the blank tests was there a detectable peak in the region of −0·83 volt. The peak potential varied a little when crop samples were analysed, but this did not interfere with the interpretation of results.

DISCUSSION OF RESULTS—

Although the proposed method could be described as reasonably specific in that it depends on a characteristic property of the >C=O group in the whole azinphos-methyl molecule, it will not distinguish between azinphos-methyl and its ethyl analogue, azinphos-ethyl. Specificity may not always be desirable if the compound can be oxidised or metabolised

in the plant, but for azinphos-methyl all the evidence so far indicates that it is not systemic and the oxygen analogue is not formed during weathering of the initial deposit. A sub-sample of the treated cucumbers containing 0·8 p.p.m. of azinphos-methyl was peeled, and the rind and pulp were analysed separately. No azinphos-methyl could be detected in the pulp and the total residue was found to be concentrated on or in the rind.

The azinphos-methyl peak is measured against the background or blank curve produced by the electrolyte and the tolerated interfering substances. The latter, in general, were not present in sufficient amount to give a harmful background, but no explanation has been found for the high recovery of 0·5 p.p.m. of azinphos-methyl from pears (see Table I). The average recovery of azinphos-methyl from crops for all results was 95 per cent. with a standard deviation of $\pm$12·2 per cent. When the 120 per cent. recovery was excluded from the calculations, the average became 93 per cent. with a standard deviation of $\pm$10·0 per cent.

Vegetables and fruits containing high concentrations of plant pigments and waxes may present additional clean-up problems, and further work may be necessary before the proposed polarographic method can be applied to crops other than apples, pears, cucumbers and tomatoes.

The limit of detection of the method was 0·1 μg of azinphos-methyl per ml, which corresponds to 0·1 p.p.m. on a 100-g crop sample. However, since the measurements were carried out at sensitivities of 0·4 and 0·25 and the highest sensitivity of the instrument was 0·004, it would appear that much smaller amounts of azinphos-methyl could be detected if the clean-up were more thorough. A further advantage in using the cathode-ray polarograph is that the method of standard addition is easily applied. The increase in peak height can be quickly measured and the concentration of azinphos-methyl in the test solution calculated. This is of value when the concentration is near the limit of detection.

I thank Miss P. A. Sanders for technical assistance and Mr. W. H. Read of the Glasshouse Crops Research Institute, Rustington, for providing the treated samples of cucumbers and tomatoes.

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A Paper-chromatographic Method for the Identification of Food Dyes

By Y. YANUKA, Y. SHALON, E. WEISSENBERG and I. NIR-GROSFELD

(Institute for Standardization & Control of Pharmaceuticals, Ministry of Health, Jerusalem, Israel)

A new approach is described to separating and identifying twelve food dyes in use in Israel. A single solvent system of butanol, ethanol and water in equal proportions has been successfully used for separating and identifying these dyes in all possible combinations. The dyes are identified from specific graphs obtained by running chromatographs of eight spots of each dye simultaneously at graded concentrations of two acids, one base and a buffer solution at pH 7.

Separating and identifying synthetic organic colouring matter added to foods constitutes a difficult problem for the analyst. Most of the dyes in use to-day are sulphonated azo-derivatives or other complex compounds, of which practically none is available in a chemically pure form. These dyes are added to food in minute amounts, and their determination therefore requires selective micro methods of great sensitivity.

The methods generally used for determining the dyes are paper or column chromatography, spectrophotometry and electrophoresis. Paper chromatography is regarded as the most useful technique available, and Thaler and Sommer[1] used it for identifying colouring matter added to food. Many papers have been published[2,3,4,5] describing the application of paper chromatography to food dyes, the main variation in the techniques reported being the use of different kinds and numbers of solvents. Verma and Dass[6] used thirteen solvents, six alkalis, three acids and three neutral compounds for determining forty-four dyes in foods; Sadini[7] used about one hundred and fifty solvents for determining the thirteen food colours listed in Italy. Tilden[8] also applied paper chromatography to determining F.D. & C. colours, employing over twenty different solvent systems.

In all except one[4] of the paper-chromatographic procedures mentioned, the identification of the dye was based on the determination of a specific R_F value. It is, however, extremely difficult to obtain reproducible results of absolute R_F values, as these depend on several factors, *e.g.*, type of paper and solvent systems, age of solvents, pH of the starting material, temperature and other variables not always strictly controllable. Further, as more than one colour is usually present in any one food, many solvents have to be used.

The use of several solvents makes the identification technique tedious and complicated, especially for unknown dyes.

In this paper a simple and reliable method is described that, by employing the ascending-solvent paper-chromatographic technique with only one solvent system, permits the identification and determination of twelve food colours in any possible mixture. The twelve dyes chosen are the ones listed for use in Israel.*

Method

The identification of the dyes is based on a characteristic curve composed of eight spots, instead of on a single R_F value. This curve is obtained by simultaneously running eight chromatograms of the dye under investigation at eight different pH values in one single solvent system. Two acids, one base and one buffer solution are used at different concentrations to produce the various chromatograms.

Reagents and apparatus—

Perchloric acid, N, 0·1 N *and* 0·01 N.
Sulphuric acid, N.
Potassium hydrogen carbonate, N, 0·1 N *and* 0·01 N.
Buffer solution, pH 7 (*Beckman* 3581), *diluted* (1 + 49).
Solvent system—n-Butanol, ethanol and water in equal parts by volume.

* In the meantime a new list of dyes permitted for use in food has been published by the health authorities. From this list Red 6B, Red FB, Naphthol yellow S and Green S were deleted and to it Erythrosine added.

Filter-paper—Whatman No. 1 sheets, 47 cm high × 35 cm wide.

Glass tanks—All-glass air-tight Shandon tanks, 50 cm × 50 cm × 22 cm, were used.

Fluorescent lamp—Black Ray Long Wave Ultra-violet (obtained from Ultra-violet Products Inc., U.S.A.) were used.

Micropipette—A 0·1-ml pipette graduated in 0·001-ml intervals.

FOOD DYES TESTED—

The twelve food dyes tested were—

Ponceau 4R	Tartrazine
Carmoisine	Naphthol yellow S
Amaranth	Sunset yellow FCF
Red 6B	Green S
Red FB	Blue VRS
Ponceau SX	Indigotine

The dye solution was aqueous, containing about 1 mg of dye per ml.

PROCEDURE—

Prepare sheets of filter-paper as described below. Cut slots 3 mm wide and 30 cm long, beginning 4 cm from the bottom of the sheet and finishing 10 cm from the top, leaving $3\frac{1}{2}$ cm of paper between slots. One sheet of eight strips of paper each 30 cm high and $3\frac{1}{2}$ cm wide is needed for identifying one dye sample. On each strip mark with a pencil two parallel lines, one $4\frac{1}{2}$ cm and the other $6\frac{1}{2}$ cm from the bottom of the sheet. In the centre of each line on each of the eight strips mark a point. Apply from a micropipette the aqueous dye solution to be tested to the eight points on the upper ($6\frac{1}{2}$ cm) lines. Repeat the application until a distinctly coloured area is obtained. The spot should contain approximately 10 μg of dye (limits 5 to 20 μg) and be not more than 1 cm in diameter. If more than one dye is present in a sample, the spot should contain 10 μg of each dye. Allow the spots to dry between the applications—a jet of hot air can be used for this. To each of the eight dye spots add, in order, working from left to right, 10 μl of one of the solutions listed below—

(1) N potassium hydrogen carbonate
(2) 0·1 N potassium hydrogen carbonate
(3) 0·01 N potassium hydrogen carbonate
(4) buffer solution, pH 7
(5) 0·01 N perchloric acid
(6) 0·1 N perchloric acid
(7) N perchloric acid
(8) N sulphuric acid.

On the lower ($4\frac{1}{2}$ cm) lines, place, in the same order, the same amounts of solutions 1 to 8 as on the upper ($6\frac{1}{2}$ cm) lines; do not place any dye solution on this line. Allow all the spots to dry.

Run the chromatograms for about 20 hours. Remove the sheets from the tank, and dry in a hood at room temperature. Inspect the sheets in ultraviolet light to see whether or not there are any fluorescent dyes present.

This procedure can be applied directly to commercial dyes. For determining dyes in food, the dyes must first be isolated and purified.

RESULTS

Chromatograms were prepared by the proposed method for the twelve dyes listed above; these chromatograms are shown in Fig. 1. The chromatograms present specific graphs demonstrating the rate of migration of each dye with solutions 1 to 8. The acids and base at the concentrations mentioned were chosen because they produced pronounced differences in the rates of migration of the dyes tested and thus provided characteristic curves for each dye. No such differences resulted when phosphoric, citric, tartaric, trichloroacetic or *p*-toluene sulphonic acids was tried, and no reproducible results could be obtained with hydrochloric

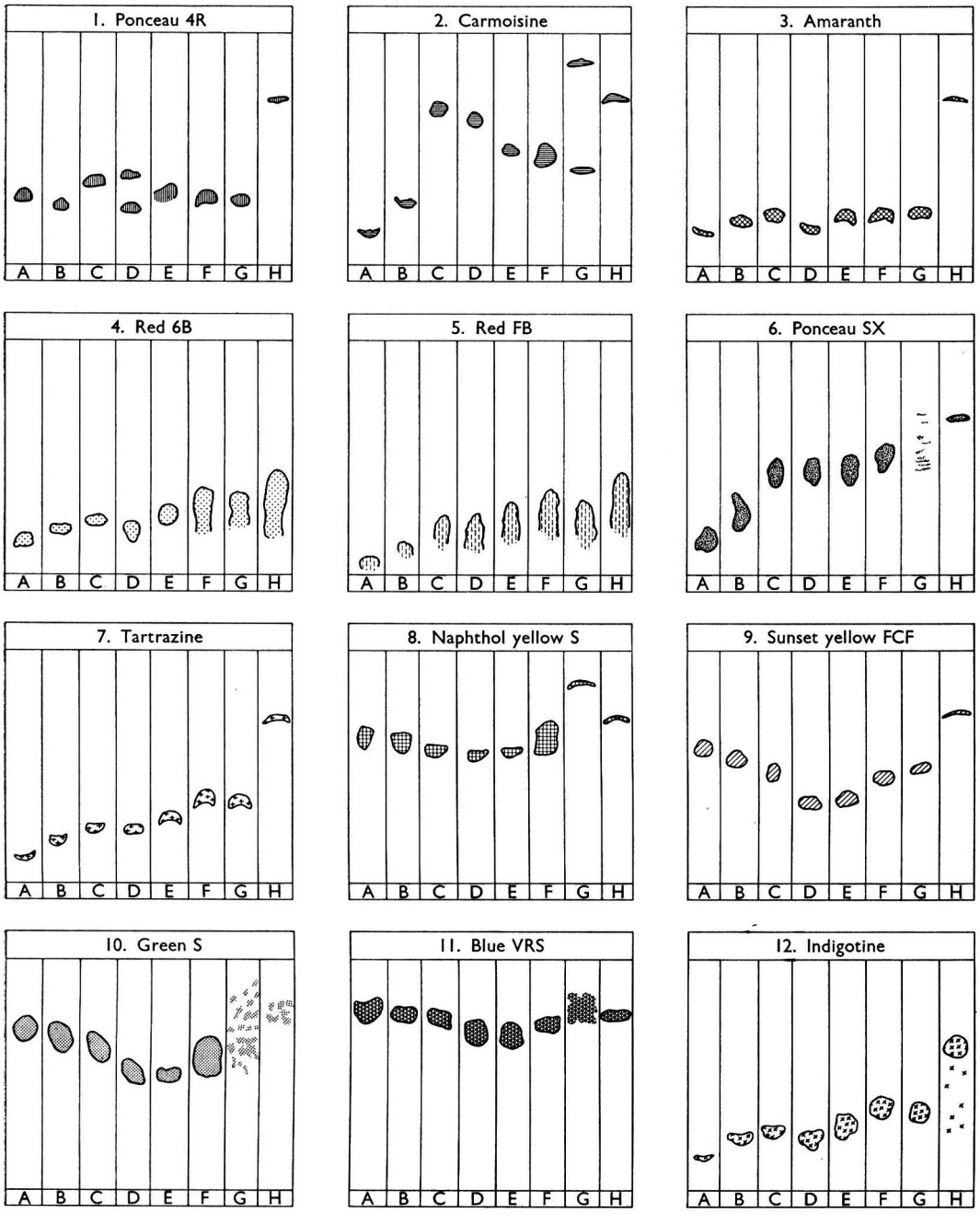

Fig. 1. Chromatograms of twelve dyes showing migration of dyes with: A, N potassium hydrogen carbonate; B, 0·1 N potassium hydrogen carbonate; C, 0·01 N potassium hydrogen carbonate; D, buffer solution pH 7; E, 0·01 N perchloric acid; F, 0·1 N perchloric acid; G, N perchloric acid; H, N sulphuric acid

or acetic acid, apparently on account of their volatility. The three concentrations of perchloric acid used appeared adequate to cover all significant differences in the rates of migration of the dyes, and no further advantage was gained by applying higher or other concentrations of the acid.

The 0·1 and 0·01 N concentrations of perchloric acid produced almost identical migration rates of the dyes as did corresponding concentrations of sulphuric acid, but the N concentrations of these acids yielded two distinctly different migration rates for the same dye spot.

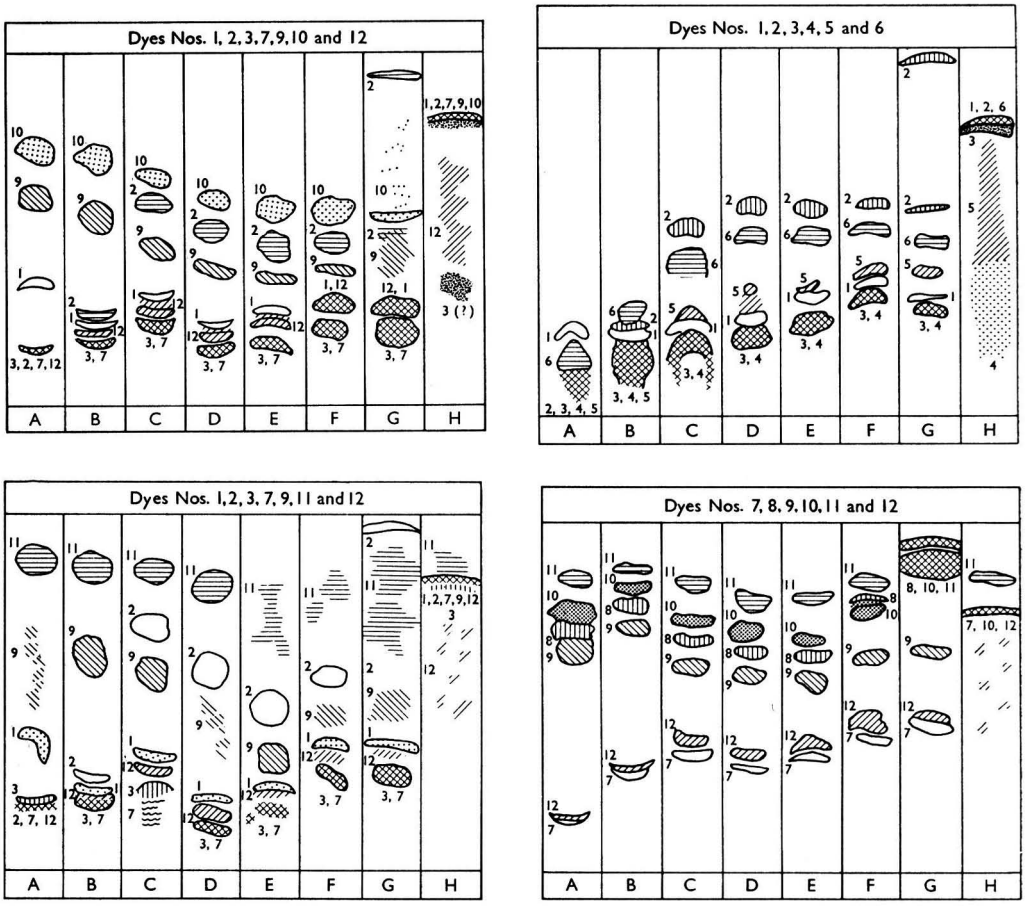

Fig. 2. Chromatograms of dyes in various mixtures (for explanation of numerals and letters, see Fig. 1)

Among the bases, potassium hydrogen carbonate was found to be the most suitable. Other bases tried were sodium hydrogen carbonate, sodium hydroxide, potassium carbonate ammonium hydroxide, pyridine and triethanolamine. However, the kind of base used does not seem to be critical in determining the migration rate of the dye.

The best conditions for separating the dye were obtained when the acid was applied both directly on to the dye spot and also 2 cm beneath it. The base, for the sake of uniformity of the procedure, was placed in the same positions as the acid.

The optimal amount of dye for running a chromatogram was found to be 10 μg. Less than 5 μg did not produce clear spots, and more than 20 μg caused local spreading of the dye.

In the search for a neutral solvent system, water was first tested; it produced little separation of the dyes. On reducing the rate of migration by adding butanol to the water, a solvent system was obtained in which the dyes dissolved easily and gave a fairly good separation

of the spots. To ensure a high concentration of water in the butanol, ethanol was added. The final solvent solution used consisted of butanol, water and ethanol, in equal parts, and was quite stable. No significant change in the ratio of composition occurred, even after constant use for long periods of time.

The chromatograms observed in ultraviolet light showed that carmoisine, Red 6B, Red FB, Ponceau SX and tartrazine were fluorescent and Ponceau 4R, amaranth, Green S, Blue VRS and indigotine were non-fluorescent. Naphthol yellow S and Sunset yellow FCF, although non-fluorescent, changed their appearances in ultraviolet light, the former appearing as a grey-red and the latter as a dark red spot.

Chromatograms of some dyes in various mixtures are shown in Fig. 2.

Discussion of the method

In previous work on the paper chromatography of food dyes, the identification of the dyes was based on determining their absolute R_F values. As already pointed out, the variability of the many factors influencing the R_F values renders their determination extremely difficult. The R_F values become even less reliable when determined on dyes extracted from food, since traces of extraneous matter that influence their migrations are usually co-extracted. The many results given in the literature for the various R_F values have thus been of little value to other workers in this field. In the proposed method, the identification of the dye is not based on a single R_F value, but on a characteristic curve composed of eight heights of dye spots obtained by running chromatograms of the dye with two acids, a base and a buffer solution at different concentrations.

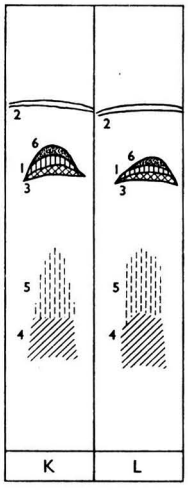

Fig. 3. Chromatograms of a mixture of dyes 1 to 6 showing migration with: K, N perchloric acid on the upper line and N sulphuric acid on the lower line; L, N sulphuric acid on the upper line and N perchloric acid on the lower line

The acid used should have a rate of migration similar to that of the dyes to be developed, since the migration rate of the acid directly influences that of the dye. Perchloric and sulphuric acids were found to be the most suitable, since dyes with a high migration rate move with the former, whereas dyes with a lower migration rate move with the latter. On the other hand, dyes with a high affinity to the filter-paper stay behind. Other acids tested were found to have migration rates too fast to be suitable, since they separated from the dyes at the beginning of the chromatographic run. This kind of separation also occurred with most of the dyes tested at a concentration of perchloric acid higher than 1 N. Concentrations of perchloric acid as high as 5 N caused a depression of the migration rate of the dye and separated from the dye spot at the beginning of the run. This lowering effect could

be explained by the diffusion pressure exerted at the high concentration of perchloric acid. Such an effect was not observed with similar or even higher concentrations of sulphuric acid.

A mixture of perchloric and sulphuric acids can also be applied to good advantage. To explore this possibility the six red dyes from our list were spotted, as described above, on the 6½-cm lines with 1 N sulphuric acid; on the lower 4½-cm line 1 N perchloric acid was applied. A clear separation, according to their migration rates, was obtained with two groups of dyes, as shown in Fig. 3. Appropriate combinations of various acids may provide an answer to the problem of separating mixtures of several food dyes into distinct groups, and further studies on this problem would seem worthwhile.

The acids used have to be strong so as to convert the dyes, which are salts of weak acids, to the undissociated form; otherwise, acid salts of the dyes would migrate in two forms, the undissociated and ionic form—thereby occasionally causing the appearance of double spots.

On the other hand, the addition of alkalis converts the acids to their salts, which have a great affinity for the paper, and consequently low rates of migration are obtained. As different concentrations of bases affect the migration rate differently, it would appear that the over-all migration rate is dependent on both the concentration of the base and its "ionic strength."

Certain correlations could be established between the chemical structure of the dye and the kind of graph obtained. Dyes such as Ponceau 4R and amaranth, each possessing three sulphonic groups, produced the same forms of graphs.

Tartrazine, containing the same number of polar groups as Ponceau 4R (two sulphonic and one carboxylic acid groups), also produced a similar graph. In contrast, carmoisine, which contains only two sulphonic groups and has therefore a pronouncedly less hydrophylic character than Ponceau 4R, produced a graph consisting of higher spots.

On the other hand, Red 6B and Red FB gave low and flat curves on account of their high hydrophylic character due to the polar groups in their molecules.

Green S and Blue VRS produced flat curves of high migratory rates with both acids and bases, since these dyes are present in the ammonium form in both acid and alkaline ranges.

A study of the graphs obtained for the twelve dyes tested showed that they can be divided into four distinct groups. They are distinguishable as indicated below.

1. Dyes with a high affinity for the paper produce low flat graphs (Red 6B and Red FB).

2. Dyes with three or more polar groups (sulphonic or carboxylic) produce low rates of migration in the presence of an alkali and high rates of migration in the presence of sulphuric acid (Ponceau 4R, tartrazine and amaranth).

3. Dyes with two polar groups (sulphonic) produce high rates of migration in the presence of an alkali and perchloric acid (Sunset yellow and Naphthol yellow).

4. Dyes with both basic and acidic groups give rise to high rates of migration in the presence of acids and bases, resulting in flat graphs (Blue VRS and Green S).

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Vermiculite as an Analytical Aid with Special Reference to the Extraction of Lycopene in the Determination of Tomato Solids in Tomato Products

By G. J. HOLLAND*

(*City Laboratories Service, Shortly Road, Coventry*)

Vermiculite has been found to be of considerable value in a general analytical laboratory; it has applications in the separation of oil and fat from compounded food products and in the isolation of caffeine from liquid coffee extracts. The general utility of this material in the examination of certain pharmaceutical products is discussed, and special reference is made to its use in extracting lycopene in the determination of tomato solids in tomato products.

Various chemical formulae have been proposed for vermiculite, and they differ appreciably.[1] One put forward as the average for 7 true vermiculites from different parts of the United States is—

$$22MgO.5Al_2O_3.Fe_2O_3.22SiO_2.40H_2O.$$

In the industrial uses of vermiculite, which include the manufacture of lightweight concrete, plasters, thermal and acoustic insulators, and in horticulture, it is the special physical properties of the material that are of consequence and similarly in the proposed use of this material as an analytical aid.

Many types of vermiculite are known; all are monoclinic hydrated ferro-magnesium alumino-silicates. The characteristic lattice structure of vermiculite has been investigated by X-ray analysis, by differential thermal analysis and by reference to its property of cation exchange. Gruner[2] has described vermiculite as being made up of biotite or talc-like silicate layers interspersed with layers of water molecules.

The name vermiculite is derived from the Latin *vermiculari* (to breed worms). This name was first given to a mineral occurring at Milbury, near Worcester, Massachusetts, by T. H. Webb in 1824; it arises from the curious property of the material in developing into worm-like threads when heated. The vermiculite minerals all have a laminated structure, in which respect they somewhat resemble micas, but behave differently from mica on heating. When heat is applies to vermiculite the material expands considerably. This swelling out effect is called exfoliation and is due to the pressures developed when water is driven out by heating. The thickness of the native material can be increased as much as twenty-fold by heating.

The facility with which exfoliated vermiculite absorbs water and aqueous solutions has been made use of in the selective extraction of water-insoluble materials from natural or compounded mixtures. In many instances the direct extraction of fat or oil from solid materials can readily be accomplished by extraction with a suitable solvent. With some materials, for example, meat and meat products, it is essential to carry out a preliminary treatment with strong acid (Werner - Schmidt) to ensure the release of intramuscular fat before its separation with an organic solvent.

Between these two extremes there are many products of a liquid, semi-liquid or pasty consistency for which the direct separation of oil or fat presents certain difficulties, typical examples being ice-cream, salad cream and lemon curd. It is practicable to adopt special procedures to minimise emulsification difficulties, which are sometimes accentuated by the presence of emulsifying agents incorporated in the product, when the direct method of separation of fat or oil from such mixtures with organic solvent is used. It is also possible to convert these liquids into a mass suitable for Soxhlet extraction by the use of anhydrous sodium sulphate. The resulting mass compacts together in such a manner that long periods of boiling under reflux in the Soxhlet apparatus are necessary, and even so extraction may be incomplete.[3]

* Present address: Chemical and Bacteriological Laboratories, Health Department, Civic Centre, Bolton.

The special properties of vermiculite appeared to present a possible alternative desiccant.

A suitable mixture of vermiculite and moist sample is friable and does not compact together. Its physical structure ensures ready penetration by organic solvents, so that with it extraction is both quicker and more complete than when anhydrous sodium sulphate is used as desiccant. For a given weight of moist sample a lesser weight of vermiculite is required than of anhydrous sodium sulphate. Unless an extremely large amount of sodium sulphate is used, it is not practicable to dilute the emulsion with water before desiccation. In many instances such dilution of the sample with water is desirable before adsorption on to vermiculite. Such dilution is practicable and has the advantage that a more even distribution of oil throughout the mass is obtained and viscous materials are much more easily absorbed after dilution. However, if the emulsion is diluted to such a degree that "splitting" occurs, then the liberated oil may so coat the vermiculite surface that absorption of water is hindered. As extensive dilution with water before desiccation is rarely required, this difficulty would seldom vitiate the procedure.

The method was found to be extremely useful for the extraction of fat and oil with organic solvents, and it was shown that, with slight modifications, it had even more general applications.

In general, any procedure involving the isolation of one or more of the constituents from an aqueous mixture by a non-miscible solvent would appear to be capable of modification for solvent extraction on vermiculite. For example, in the extraction of caffeine from samples of soluble coffee extracts the vermiculite procedure presents distinct advantages. The modification in this instance simply involves making alkaline a portion of the sample, adsorbing it directly on to a suitable mass of vermiculite and extracting the caffeine with chloroform in a Soxhlet apparatus. A Soxhlet thimble containing vermiculite will float in chloroform in the extractor; weighting of the thimble with large glass balls (marbles) placed on the surface of the vermiculite over a layer of cotton-wool is therefore recommended when chloroform or similarly dense solvents are used. The chloroform extract of relatively crude caffeine so obtained may then be washed with aqueous alkali and purified in the usual way. The advantage of this method for coffee extracts is that it is a simple matter to extract a series of samples simultaneously in ordinary Soxhlet extractors.

The utility of this procedure suggests its extension into many different applications in examining food or drug samples. For example, the isolation of alkaloids, etc., from compounded medicines could proceed along similar lines to the caffeine extraction. Isolation of organic bases from cough mixtures can with advantage be attempted in this manner, particularly when they contain emulsifying ingredients, for which simple extraction in a separator frequently leads to emulsions difficult to break. Similarly, acidic substances may be extracted by prior acidification of the sample before incorporation on to vermiculite.

Vermiculite used—

A grade of horticultural exfoliated vermiculite was used.* This was screened to give a fairly uniform grade of material. The portion retained on a 16-mesh sieve was rejected, as also were the fines passing a 25-mesh sieve. The selected material was then heated at 250° C for 2 hours to remove any free or semi-combined moisture, cooled in a desiccator and stored in air-tight bottles for use.

General method for extracting oils and fats

Procedure—

For such materials as ice-cream and salad cream, weigh accurately about 2 g of the sample into a 100-ml beaker containing a small stainless-steel spatula. Add, successively, three 1-ml portions of distilled water, stirring thoroughly after each addition. Incorporate in the liquid 5 g of the prepared vermiculite by successive small additions until the whole has been added and a friable non-adherent mass obtained. Transfer to a Soxhlet thimble, removing the last traces of vermiculite from the beaker with a plug of cotton-wool. Place the cotton-wool on top of the vermiculite, and place the thimble in the Soxhlet extractor.

* Collite Brand Vermiculite (fine seed sowing grade). Pan Britannica Industries Limited, Waltham Abbey, Essex, and Geo. A. Palmer Limited, Wykin, Hinckley, Leicester.

Rinse the beaker several times with the solvent (usually light petroleum, boiling range 40° to 60° C, for fat extractions). Carry out the extraction at a fairly rapid rate of reflux. The period required for extraction will vary to some extent with the type of sample being examined, but 1 hour should be sufficient; 3 hours will probably be adequate for any sample. After extraction, remove the solvent, and weigh the oil or fat in a previously tared flask. If a mixture of diethyl ether and light petroleum was used for the extraction, remove the solvents and weigh the flask. To obtain the weight of true fat, remove the fat from the contents of the flask with light petroleum alone, and reweigh. It has been found that the fat or oil recovered from samples by this process is extremely clean in appearance and free from extraneous matter.

RESULTS—

A comparison of some results by the proposed and an earlier method are shown in Table I.

TABLE I

COMPARISON OF RESULTS

Sample	Total fat content found by— Rose - Gottlieb method, % w/w	proposed vermiculite method, % w/w
Ice-cream	10·47	10·60
Ice-cream	10·42	11·00
Ice-cream	11·50	11·20
Ice-cream	13·01	12·95
Ice-cream	8·68	8·61
Ice-cream	8·16	8·62
Tomato soup	3·24	3·48
Tomato soup	4·60	4·94
Tomato soup	3·47	3·57
Salad cream	34·4	34·4
Lemon curd	4·6	4·3
Marzipan	13·8*	13·4

* Determined by the Werner - Schmidt method.

VERMICULITE AS AN AID IN THE EXTRACTION OF LYCOPENE IN THE DETERMINATION OF TOMATO SOLIDS IN TOMATO PRODUCTS

The determination of tomato solids in tomato products by a method involving the separation of lycopene and its determination by absorption spectroscopy has been described by Stock.[4]

After the sample has been mixed with sand, this method involves preliminary drying of the material in a vacuum desiccator at room temperature, the drying period recommended being at least 16 hours. The material is then powdered and transferred to a Bolton extractor. Acetone is used as solvent, and, after removal of acetone, the lycopene is dissolved in light petroleum before the absorption measurements are carried out.

The use of vermiculite in place of sand obviates entirely the necessity for a lengthy preliminary drying period. After incorporating the diluted tomato product on a suitable amount of vermiculite, the mass can be extracted directly with acetone in a Soxhlet apparatus.

It was recorded by Stock that direct extraction of the sand support with light petroleum was not successful, allegedly owing to a form of chromatographic separation taking place on the sand.

Similarly, direct extraction of the vermiculite column with light petroleum also gave incomplete extraction of lycopene (approximately 68 per cent.). After an initial direct extraction with light petroleum, it was found that further lycopene could be extracted with acetone. The total lycopene recovered in these two fractions was 97·2 per cent. of the amount obtained by direct extraction with acetone.

PROPOSED PROCEDURE—

In the examination of a tomato ketchup, sauce or purée, proceed as described under the general method for the separation of oils and fats. The beaker should be rinsed with acetone, which is the most suitable solvent for extracting lycopene. A rapid rate of reflux should

be maintained for a period of 1½ hours. During the extraction and subsequent distillation, the liquids should be protected from bright sunlight. Remove the bulk of the acetone by distillation and the remaining acetone by blowing off in a current of air or, preferably, nitrogen at room temperature. Add about 20 ml of light petroleum, boiling range 80° to 100° C, heat to boiling on a water bath, cool, and make up to 100 ml with light petroleum.

To determine the lycopene content of the solution prepared as described above, or a standard dilution of it, it is only necessary to measure the absorption at 505 mμ. If it is required to characterise the lycopene, the full absorption curve can be plotted. It is also useful to measure E_{max} 475 mμ and to calculate the ratio of $E_{max.}$ 505 mμ to $E_{max.}$ 475 mμ. Absorption readings should be made in duplicate.

It was found that most of the lycopene was extracted immediately when the cold acetone was poured on to the tomato - vermiculite mass, but to ensure reproducible results the solvent was boiled under reflux for 1½ hours. Extended periods of extraction will result in some loss of lycopene, and results are not reproducible after short extraction periods. The method gives good agreement between results on the same sample and by different workers.

$E^{1\%}_{1\,cm}$ at 505 mμ for lycopene in light petroleum has been taken as 2000 and the $E^{100\%}_{1\,cm}$ value is calculated for the material being examined.

(In relating the tomato solids content of sauces to the tomato purées used by the manufacturers, the salt content of the purées was not separately measured, the total solids content being taken as the tomato solids content.)

RESULTS—

To test the value of the method, a series of commercial tomato concentrates made available by a tomato-sauce manufacturer were examined for lycopene content (see Table II). Two types of tomato sauce produced by this manufacturer were then examined for tomato solids content; the results are shown in Table III.

From the results in Table II it would be reasonable to assume that, if the purées used were typical of the products currently available, 1 g of tomato solids contains 2000 μg of lycopene. This value was used in determining the tomato solids content of sauces made by the manufacturer who supplied the concentrates.

TABLE II

LYCOPENE CONTENT OF TOMATO PURÉES

Sample	Total solids, %	Lycopene found per g of sample, μg	Lycopene per g of total solids, μg
Italian triple concentrate	36·0	680	1889
Italian double concentrate	29·8	733	2460
Italian double concentrate	30·0	629	2097
Portuguese double concentrate ..	29·0	453	1562
Italian double concentrate	27·7	669	2415
Italian triple concentrate	37·3	783	2100
		Average ..	2087

TABLE III

TOMATO SOLIDS CONTENT OF TOMATO SAUCE

Sample	Tomato solids content— according to manufacturer's formulation, %	Tomato solids content— found by proposed method, %
Tomato sauce type I	18	17·7
Tomato sauce type II	9	9·1, 9·6

The effect of the action of light on the lycopene content of the two types of tomato sauce was investigated by means of lycopene determinations; the results are shown in Table IV. These results indicate that there is no substantial deterioration of lycopene in these tomato sauces as a consequence of lengthy normal illumination or intense short-term ultraviolet irradiation.

It may be noted that the water-soluble dye in tomato sauce type II appeared to have been retained in the Soxhlet thimble. No artificial dye was present in the light petroleum solution of the extracted lycopene. Tomato sauce type I was entirely free from added artificial colour. From both sauces the lycopene isolated exhibited a typical lycopene absorption curve.

TABLE IV

EFFECT OF LIGHT ON THE LYCOPENE CONTENT OF TOMATO SAUCE

	Tomato solids content—			
Sample	claimed by manufacturer, %	found after 3 days' exposure to ultraviolet light in a closed cabinet, %	found after 6 months' exposure to normal laboratory illumination, %	found after storage in the dark for 6 months and then 3 days' exposure to ultraviolet light, %
Tomato sauce type I ..	18	17·1	—	—
Tomato sauce type II ..	9	—	9·9	8·6

The method used has also been applied to a variety of different types of tomato products, including tomato juice, soup, ketchup and chutney. The determination of the tomato solids content by reference to the potassium content is unrealistic in that many tomato products contain potassium other than that originating from the tomato content. In such products the lycopene content appears to be the most satisfactory indication of the tomato solids content.

SUMMARY AND CONCLUSIONS

Vermiculite has been shown to be of value in facilitating the solvent extraction of oil and fat from certain food and drug samples. It is expected that the procedure can be adapted to cover a wide range of such samples. It has been further shown that the water retention properties of vermiculite are such that the procedure can be extended beyond the use of water-immiscible solvents, such as light petroleum and chloroform, and that a water-miscible solvent, such as acetone, can be used to extract an aqueous mixture adsorbed on to a vermiculite support. This feature has been made use of in the determination of lycopene for evaluating the tomato solids content of tomato products. The determination of lycopene in such materials has been greatly facilitated by a technique involving the use of vermiculite, which completely obviates the necessity of drying the product before extraction. This modification reduces possible transfer losses associated with powdering of dried material, and minimises the possibility of oxidation changes that might be engendered during drying.

The dilution of the sample with water before adsorption on vermiculite ensures that the lycopene can be readily extracted from the mass. The lycopene content of the commercial tomato purées examined has been shown by this technique to be appreciably greater than that indicated for similar purées by earlier extraction methods. The factor hitherto advocated for the conversion of micrograms of lycopene to grams of tomato solids differs appreciably from the factor applicable here. This suggests that the extraction is more thorough than that given by earlier methods. Special types of extractors are unnecessary; a normal Soxhlet apparatus is satisfactory.

The analytical procedure is simpler than existing ones; consistent results can be obtained, and the time taken to carry out an analysis is substantially reduced.

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Quantitative Analysis by Thin-film Chromatography

By S. JEAN PURDY and E. V. TRUTER
(*Textile Chemistry Laboratory, The University, Leeds, 2*)

It is shown that, in thin-film chromatography, the square root of the area of a spot is a linear function of the logarithm of the weight of the material present. Two experimental methods for quantitative determination are described; both require a sample of the pure compound as a standard. The accuracy attainable under the best conditions is 100 ± 2 per cent. in both adsorption chromatography and in partition chromatography. The analytical procedure has been tested with mixtures of organic compounds and a mixture of inorganic cations.

Methods of quantitative analysis by thin-film chromatography can be divided into two categories. In one, the compound is removed from the adsorbent before it is determined and in the other, the recovery of the compound is unnecessary. In the methods in which the compound is recovered, its subsequent determination is usually carried out by spectroscopic methods[1,2]; gravimetric methods have not been examined, mainly because the weights of material (~50 μg) require the use of a special balance. There are two inherent disadvantages in the methods requiring recovery of the compound. First, even if the relevant precautions are taken, impurities may still be extracted from the adsorbent. Secondly, the recovery may be incomplete, especially if the compound is strongly adsorbed. To overcome the first difficulty, the compound can be submitted to a specific colour reaction. The second difficulty can be avoided if the compound is not removed from the adsorbent until the chromogenic reaction has been carried out. For example, Gänshirt, Koss and Morianz[3] recommend that the chromogenic reaction be carried out while the compound is still adsorbed on the silica gel; subsequently, the silica gel is spun in a centrifuge, and the optical density of the supernatant liquor is measured.

If the determination were to be based on a function of the area of the spot formed by the compound on the chromatogram, substantial advantages would accrue, namely, the simplification of the technique and possibly also an improvement in the accuracy of the determination. Privett and co-workers,[4,5] in developing the method first devised by Hefendehl,[6] found that, if a thin-film chromatogram bearing an organic compound is sprayed with concentrated sulphuric acid and heated, an integrated function of the size and optical density of the spot is proportional to the weight of the compound. The main disadvantage of this technique is that, in addition to the variables in the thin-film chromatographic process, a new set of variables is introduced, namely, the factors affecting the degree of carbonisation. This depends mainly on the nature of the compound itself, and to a lesser extent on the amount of sulphuric acid used and the temperature and duration of the subsequent heat treatment.

The simplest method is based on the relation between the weight of material and the size of the spot it forms, irrespective of the intensity of the colour. Seher[7,8] suggested that samples of equal volumes of the unknown and of a series of standards of different concentrations should be chromatographed simultaneously. After the chromogenic reaction, the areas of the spots are measured and the areas of the standard samples are plotted as a function of their weights. By reference to the weight - area curve, the weight of an unknown sample is determined from its known area. Later, Purdy and Truter[9] reported that the square root of the area of a spot is a linear function of the logarithm of the weight of material it contains, and they outlined a simple analytical procedure that does not require simultaneous calibration as does Seher's method. These last two methods have the advantage that they do not require special apparatus, such as a spectrophotometer or an optical densitometer, and they are much more rapidly carried out.

In paper chromatography, Fisher and co-workers[10,11,12] found that the logarithm of the weight of material is proportional to the area of the spot it forms. Although some workers[13,14] have used this relationship, Stahl[15] and Shimi, Nour el Dein and Imam[16] doubt its general validity. Fowler[17] suggests that it is only an approximation, at low loads, for the more general linear relationship between the logarithm of the weight and the logarithm of the area.

For thin-film chromatography it has been reported that the logarithm of the weight is a linear function of the square root of the area; the evidence for the validity of this relationship is presented in this paper. Because the literature for paper chromatography suggests that several empirical relationships may exist, we have examined three possibilities, namely, the variation of the logarithm of the weight with (*a*) the area, (*b*) the logarithm of the area and (*c*) the square root of the area.

To determine which relationship would be the most useful for the subsequent analytical work, a series of observations was made with seven test compounds. For each compound, equal volumes of each of six solutions of different concentrations were chromatographed together, and the areas of the resulting spots were measured. All observations were made in replicate (usually duplicate); the areas of replicate spots never differed by more than $\pm 0{\cdot}5$ sq. mm from the mean. Graphical presentation of the observations for each compound in each of the three forms showed that the two relations $\log W/\sqrt{A}$ and $\log W/\log A$ were straight lines, but $\log W/A$ was curved. The best straight lines for each relationship were calculated by the method of least squares, and from the appropriate equations the values of—

$$\sqrt{\frac{1}{n}\sum\left\{\frac{A_{obs.}-A_{calc.}}{A_{obs.}}\right\}^2} \quad \text{and} \quad \sqrt{\frac{1}{n}\sum\left\{\frac{W_{obs.}-W_{calc.}}{W_{obs.}}\right\}^2}$$

were determined. The results are shown in Table I, together with the results of calculations based on observations taken from the literature. It is apparent that the relationship affording the most accurate determination of the weight of a sample from its area is $\log W \propto \sqrt{A}$.

TABLE I

ROOT MEAN SQUARE DEVIATIONS

Calculated, as percentages, from weights and areas obtained from different relationships

Compound	No. of determinations	$\sqrt{\frac{1}{n}\sum\left\{\frac{A_{obs.}-A_{calc.}}{A_{obs.}}\right\}^2}$ calculated via—			$\sqrt{\frac{1}{n}\sum\left\{\frac{W_{obs.}-W_{calc.}}{W_{obs.}}\right\}^2}$ calculated via—		
		A	$\log A$	$\sqrt{A}$	A	$\log A$	$\sqrt{A}$
K^+	6	4·65	1·86	2·83	6·06	2·71	3·81
Mg^{2+}	6	5·14	3·15	1·34	5·14	3·91	1·64
Hexadecanol	6	2·42	1·60	0·76	5·38	4·43	2·17
Cholesterol	6	5·44	3·03	0·77	7·27	6·24	1·64
Palmitic acid	6	1·90	1·37	1·03	10·75	3·27	2·96
Cholesteryl laurate	6	1·52	1·83	0·99	3·29	3·92	1·88
Phenylazo-2-naphthol	6	7·90	4·52	1·37	8·83	6·95	2·65
R.m.s. for above 7 compounds	42	4·73	2·69	1·46	7·06	4·71	2·49
Indole alkaloid[15]	6	8·28	2·84	4·69	11·63	4·14	5·62
Butylhydroxyanisole[7]	6	6·54	2·33	2·15	3·97	5·07	4·27
Glycine[18]	6	6·94	8·98	4·57	15·48	19·50	9·00
R.m.s. for above 3 compounds	18	7·29	5·30	3·98	11·41	11·87	6·60

For the determination of a particular compound in a mixture, the empirical relationship can be used in one of two ways, but both methods require that all chromatograms be prepared under identical conditions, and that a sample of the chromatographically pure compound be available for comparison.

GRAPHICAL METHOD—

In the first method, the pure compound is used for the preparation of a calibration graph of $\log W$ against $\sqrt{A}$ (or, as in Seher's method, weight may be plotted against area) from which the results for the unknown can be read. The main disadvantage of the method is that it requires a separate calibration for each set of chromatographic conditions, which include the variables listed below:

(1) The characteristics of individual adsorbent films.
(2) The volume of solution spotted on to the film.
(3) The nature of the solvents used in developing the chromatogram.
(4) The distance the compound has travelled along the adsorbent.

By careful attention to the details of the experimental technique, it is possible to standardise the chromatographic process to such an extent that the observations from several chromatograms can be combined, but the results are not very reliable. Normally, the calibration and test samples will be processed simultaneously on the same chromatoplate. It follows, therefore, that each determination requires its own calibration and this, in turn, means that relatively large amounts of chromatographically pure reference compounds are required.

ALGEBRAIC METHOD—

The second method is based on the linearity of the relation between log$W$ and $\sqrt{A}$. It has the advantage that it is self-calibrating, so that any set of conditions can be used.

From a solution of the mixture of known concentration, a more dilute solution is prepared. Replicate samples of equal volumes of these two solutions and a similar volume of a standard solution of the compound to be determined are spotted on to the same chromatoplate, and the chromatogram is developed in the usual way. (If time and materials permit, the best method is to chromatograph four samples; the unknown, a diluted sample of the unknown, the standard and a diluted sample of the standard.) The characteristics of the film, the solvent and the distance of development are no longer variables, because all samples undergo the same treatment. After the compounds have been located and the areas of the spots have been measured, the weight (and hence, the concentration and the percentage in the mixture) is determined.

As log$W$ varies linearly with $\sqrt{A}$, for the unknown solution—

$$\sqrt{A} = \mathrm{m}\log W + \mathrm{c} \quad .. \quad .. \quad .. \quad .. \quad (1)$$

For the dilute solution—

$$\sqrt{A_d} = \mathrm{m}\log(Wd) + \mathrm{c} \quad .. \quad .. \quad .. \quad .. \quad (2)$$

where the factor d accounts for the dilution. For the standard solution—

$$\sqrt{A_s} = \mathrm{m}\log W_s + \mathrm{c} \quad .. \quad .. \quad .. \quad .. \quad (3)$$

By eliminating m and c between equations (1), (2) and (3)—

$$\log W = \log W_s + \left\{\frac{\sqrt{A} - \sqrt{A_s}}{\sqrt{A_d} - \sqrt{A}}\right\} \log d \quad .. \quad .. \quad .. \quad .. \quad (4)$$

It is obvious that the reference compounds must be chromatographically pure, a standard that in thin-film chromatography is so exacting that many "pure" compounds can be shown to contain small amounts of impurities. The most satisfactory method for obtaining reference compounds of the required purity is to purify them by thin-film chromatography. Unfortunately, thin-film chromatography is a micro process; the amount of material that can be conveniently obtained is generally less than 2 mg. There are, therefore, circumstances that will require the analysis to be performed by comparison with extremely small amounts of standard compound. To conserve the supply of chromatographically pure reference materials, the analytical procedure described below has been suggested.[9] Its precision will be lower than that of the method outlined above, but it has the advantage that it can be used for quantitative analyses of compounds, perhaps still unidentified, that have been recovered from thin-film chromatograms.

From a solution of known concentration of the unknown mixture, two weaker solutions of the same concentration are prepared, to one of which is added a known weight of the pure compound. Replicate samples of equal volumes of each of the three solutions are spotted on to the same chromatoplate, and the chromatogram is processed in the usual way. For the first unknown solution and for the diluted unknown solution the relationships between $\sqrt{A}$ and log$W$ are expressed by equations (1) and (2). For the dilute solution containing a known added weight (a) of pure compound—

$$\sqrt{A_+} = \mathrm{m}\log(Wd + a) + \mathrm{c} \quad .. \quad .. \quad .. \quad .. \quad (5)$$

By eliminating m and c between equations (1), (2) and (5)—

$$\log\left\{\frac{Wd + a}{W}\right\} = \left\{\frac{\sqrt{A_+} - \sqrt{A}}{\sqrt{A_d} - \sqrt{A}}\right\} \log d \quad .. \quad .. \quad .. \quad .. \quad (6)$$

The only unknown factor in equation (6) is W, the weight of the compound in the spot.

For simplicity, the first method will be called the W_s method and the second the W_+ method.

Experimental

Preparation of films—

All films were prepared by mixing silica gel G with distilled water in the ratio 1 g of silica gel to 2 ml of water, and spreading the slurry over 20-cm × 15-cm glass plates (5 g of silica gel per plate; film thickness $\sim 250\ \mu$). The spreading device described by Stahl[19] was used. The chromatoplates were left to dry on the bench for 1 hour, baked at 105° C for 1 hour, and set aside to cool for 30 minutes at room temperature. They were then ready for use.

Spotting—

Accurately known volumes of solutions were spotted on to the films from an Agla micrometer syringe. To transfer the solutions from the micrometer syringe to the film in exactly the predetermined position, the syringe was clamped about 2 mm above the film, the solution was expressed from the syringe, and the drop was transferred to the film by gently tilting the plate until contact occurred. The solvent used in preparing the solutions affects the initial area of the spots and consequently the final area. Preliminary results with chloroform solutions showed a marked scatter that was attributable to the ease with which the chloroform evaporates during spotting; benzene and methanol were satisfactory solvents.

Development—

The solvents chosen for developing the chromatograms were able to move the material clear of the origin and not too near to the solvent front. Benzene containing various percentages of methanol was satisfactory for organic compounds, but for potassium and magnesium (as the acetates) a mixture of ethanol and methanol (1 + 1) containing 1 per cent. of acetic acid was used. Development was by the ascending-solvent technique, and the tank was lined with filter-paper saturated in the solvent to ensure that the atmosphere was saturated with solvent vapour.

Reversed-phase chromatography—

For chromatographing aliphatic alcohols and acids, the silica gel G films were prepared as described above, except that the chromatoplates were not baked. The films were impregnated with n-decane by allowing a 15 per cent. solution of decane in light petroleum (boiling range 40° to 60° C) to ascend to the top of the film in the way normally used for developing a chromatogram. Subsequently, the chromatoplates were exposed to the atmosphere for 15 minutes to allow the light petroleum to evaporate, leaving the silica gel impregnated with decane. After the samples had been spotted on to the film, the chromatogram was developed with a mixture of acetic acid and acetonitrile (1 + 1) for acids[20] or (1 + 3) for alcohols[20]; both solvents were 90 per cent. saturated with n-decane. For cholesteryl esters the impregnating solvent was 1 per cent. paraffin (boiling range 245° to 250° C) in ether, and the developing solvent was acetic acid.[21]

Chromogenic reagents—

Organic compounds were located by spraying the chromatograms with concentrated sulphuric acid and then heating the plate to 160° C for 10 minutes. Potassium and magnesium were located by spraying the film with 1·5 per cent. aqueous Acid violet 6BN.[22]

Area measurements—

After the compounds had been located, the areas of the spots were determined by laying a sheet of transparent paper on the film, tracing the outline of the spots, and measuring the areas of the figures by superposing the tracing on millimetre graph paper.

Observations

For quantitative determinations it is necessary to use the same volume for all spots, but first, the general validity of the relationship for spots of different volumes was examined. Different weights of sample were obtained by taking different volumes of the same solution. Two ways of spotting were tried; in one, the required volume was spotted on to the film by superposing several small drops, the solvent from one drop being allowed to evaporate before the next was added. Reproducibility was poor. In the other technique, the predetermined volume of the solution was expressed from the micrometer syringe in a single operation; larger areas were obtained and the reproducibility was much better. Both techniques gave straight lines when $\sqrt{A}$ was plotted against $\log W$.

As would be expected, when the compound was developed in the same solvent for different distances, the plots of $\sqrt{A}$ against $\log W$ gave a series of almost parallel straight lines. Fig. 1 shows the results obtained with a solution of phenylazo-2-naphthol in methanol spotted by the variable-volume single-drop technique, and also by the constant-volume technique.

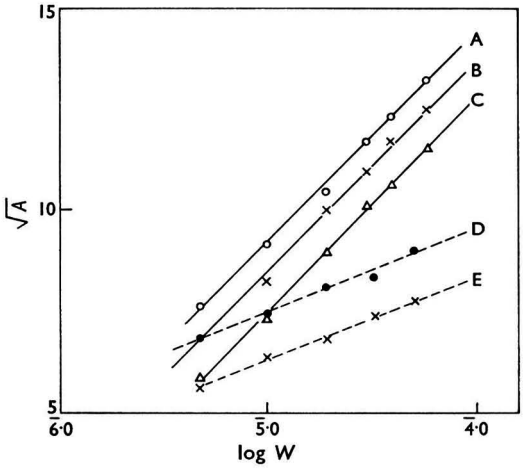

Fig. 1. Plots of $\sqrt{A}$ against $\log W$ for phenylazo-2-naphthol spotted on the film as different volumes (full lines) and as constant volumes (broken lines). Chromatograms developed for different distances : curve A, 10 cm; curve B, 7 cm; curve C, 5 cm; curve D, 10 cm; curve E, 5 cm

When samples of the same compound were developed to approximately the same distance, in different solvents, it was observed that the slope of the lines was not constant. Because the accuracy of the determination is to some extent dependent on the slope of the plot of $\log W/\sqrt{A}$, the developing solvent is a factor that can be used to improve the accuracy.

The accuracy of the graphical method was tested on colourless compounds that could not be located until the chromatograms had been treated with a chromogenic reagent. Constant volumes (4·0 μl) of solutions of different concentrations of cholesterol in benzene were spotted on to the film, and the chromatograms were developed in benzene containing 10 per cent. of methanol. To locate the cholesterol, the film was sprayed with a saturated solution of antimony trichloride in chloroform and heated to 105° C for 10 minutes. The areas of the violet spots of the cholesterol complex were determined, the calibration graph was spotted, and, from it, the cholesterol contents of three further solutions were determined. Similar experiments were conducted with n-hexadecanol and with a mixture of n-hexadecanoic acid and n-hexadecanol. After the components had been located, the weight in each spot was calculated by reference to the appropriate calibration graph.

The algebraic method was tested on samples (4·0 μl) of n-hexadecanoic acid, n-hexadecanol and phenylazo-2-naphthol made up as shown in Table III.

TABLE II

QUANTITATIVE RESULTS FOR BINARY TEST MIXTURES AND SINGLE COMPONENTS

Binary mixtures						Single compounds		
n-Hexadecanol			n-Hexadecanoic acid					
Area, sq. mm	Weight found, μg	Weight known, μg	Area, sq. mm	Weight found, μg	Weight known, μg	Area, sq. mm	Weight found, μg	Weight known, μg
29	4·6	4·6	29	4·5	4·2	*Cholesterol—*		
30	4·8		28	4·2		20	6·7	6·2
37	8·1	8·7	41	9·8	9·1	41	24·5	24·5
38	8·7		40	9·1				
46	13·7	13·8	51	16·2	16·3	56	51·3	51·3
45	13·2		51	16·2				
49	16·6	17·8	57	21·9	21·1	*n-Hexadecanol—*		
50	17·4		56	20·9		37	8·1	8·1
54	20·9	21·8	62	28·2	27·5	68	41·7	42·6
54	20·9		61	26·9				

Although the solutions were made up according to the W_+ method, the concentrations of the W_+ solutions are known, so that they can also be regarded as W_s solutions. Table IV shows the results calculated by both methods.

The results for the analyses of two binary mixtures by both the W_s and the W_+ methods (as separate determinations) are shown in Table V.

Members of an homologous series can be separated from one another by reversed-phase partition thin-film chromatography. First, the applicability of the W_+ method for determining

TABLE III

CONCENTRATIONS OF TEST SOLUTIONS

Solution	Unknown, g per litre	Diluted unknown, g per litre	Diluted and added, g per litre	Developing solvent
n-Hexadecanoic acid	23·37	4·674	20·104	Benzene + 20 per cent. methanol
n-Hexadecanol	10·06	2·012	7·972	Benzene + 10 per cent. methanol
Phenylazo-2-naphthol	13·15	2·630	11·020	Benzene + 5 per cent. methanol

a single compound was tested, and subsequently the method was extended to a binary and a quaternary mixture. The results, calculated by both the W_s and W_+ methods, are shown in Table VI.

Finally, a brief attempt was made to apply the W_+ method. It was used to determine the amount of cholesterol in two samples of wool-wax alcohols. Cholesterol in wool wax is most satisfactorily separated from its congeners by development of the chromatogram in a mixture of cyclohexane and acetone (9 + 1). Chromogenesis with concentrated sulphuric acid reveals at least 13 fractions, one of which, the aliphatic alcohols, tends to overlap the

TABLE IV

AREAS AND WEIGHTS FOUND BY ALGEBRAIC METHOD FOR SINGLE COMPOUNDS

	n-Hexadecanoic acid				n-Hexadecanol				Phenylazo-2-naphthol			
A, sq. mm	93	93	92	93	53	53	53	53	75	75	76	75
A_+, sq. mm	86	85	86	86	50	49	49	49	73	73	73	73
A_d, sq. mm	41	40	41	41	28	28	27	28	58	58	58	58
W_s, μg per 4·0 μl	96·8 (3·9)*				39·3 (3·2)*				53·8 (4·0)*			
W_+, μg per 4·0 μl	98·6 (5·8)*				39·1 (4·2)*				54·2 (4·2)*			
Weight known, μg per 4·0 μl ..	93·5				40·2				52·6			

* Root mean square deviations, in percentages, from the known values; 64 observations.

TABLE V

RESULTS OF ANALYSIS OF BINARY MIXTURES BY W_s AND W_+ METHODS

Mixture	Weight known, μg per μl	Weight found by W_s method, μg per μl	R.m.s., %	No. of observations	Weight found by W_+ method, μg per μl	R.m.s., %	No. of observations
n-Hexadecanol	18·61	18·38	2·6	128	17·82	4·6	64
Phenylazo-2-naphthol ..	17·50	17·47	2·0	112	17·56	0·3	64

Mixture	Weight known, μmole per 2 μl	Weight found by W_s method, μmole per 2 μl	R.m.s., %	No. of observations	Weight found by W_+ method, μmole per 2 μl	R.m.s., %	No. of observations
Potassium	0·6715	0·6661	0·8	54	0·6591	1·8	27
Magnesium	0·1224	0·1225	0·7	54	0·1209	1·2	27

cholesterol fraction. To minimise the ambiguity caused by the proximity of the aliphatic alcohol spot to the cholesterol spot, a differential chromogenic reagent was used, namely, the Liebermann - Burchardt reagent. By spraying the chromatogram with a solution prepared by cooling a mixture of chloroform (50 ml) and acetic anhydride (50 ml) in ice and slowly adding 10 ml of ice-cold concentrated sulphuric acid, only the steroidal components of the mixture were rendered visible.

TABLE VI

ANALYTICAL RESULTS FOR THIN-FILM PARTITION CHROMATOGRAPHY

	Weight known, μg	Weight found by— W_s, μg	Weight found by— W_+, μg	R.m.s. deviation for— W_s, %	R.m.s. deviation for— W_+, %	No. of observations
Single compound—						
n-Dodecanoic acid	10·04*	10·71*	10·14*	6·67	1·00	64
Binary mixture—						
n-Hexadecanol	4·76*	4·75*	4·70*	0·21	1·30	27
n-Octadecanol	4·64*	4·45*	4·41*	4·09	5·00	27
Quaternary mixture—						
Cholesteryl acetate	11·01†	11·04†	11·04†	1·91	4·04	216
Cholesteryl caprylate ..	5·44†	5·66†	5·71†	5·52	5·71	216
Cholesteryl laurate	10·00†	10·11†	9·97†	2·17	3·12	216
Cholesteryl stearate.. ..	5·33†	5·44†	5·18†	2·06	2·57	216

* In 4·0 μl. † In 1·0 μl.

For comparison, the cholesterol contents of the two samples were determined spectroscopically by the Liebermann - Burchardt technique and also by the digitonin method. The results are shown in Table VII.

DISCUSSION OF THE METHODS

Table I shows that, for the range of weights per spot encountered in thin-film chromatography (1 to 120 μg), the most suitable linear relationship between weight and area is

TABLE VII

CHOLESTEROL CONTENT OF WOOL-WAX ALCOHOLS

Sample No.	Cholesterol found by— W_+ method, %	digitonin, %	spectroscopy, %
1	30·2	30·4	27·8
2	29·3	31·1	27·4

$\log W \propto \sqrt{A}$. Rather surprisingly, however, $\log W$ is also a fairly good linear function of both A and $\log A$. The confusion in the literature for paper chromatography may arise from the fair degree of acceptability of several alternative relationships.

The root mean square deviations of all the determinations from the known values, classified according to the technique, are summarised below.

Graphical method (adsorption) ± 4·3 per cent. (25 observations)

Algebraic method (adsorption)

W_s ± 2·7 per cent. (540 observations)
W_+ ± 3·9 per cent. (374 observations)

Algebraic method (partition)

W_s ± 3·6 per cent. (982 observations)
W_+ ± 3·9 per cent. (982 observations)

As expected, the root mean square deviations are greatest for the graphical method and smallest for the W_s method. Comparison of the root mean square deviations for the W_s and W_+ methods shows that the W_+ method is less precise, but the difference is small enough to justify its use when only small amounts of chromatographically pure standards are available for comparison.

Although only a few compounds have been tested, their properties are distinctly different; they range from inorganic cations, to organic acids, alcohols and esters. It is possible, therefore, that the methods described here could be developed to embrace the analysis of every type of compound that can conveniently be subjected to thin-film chromatography. Volatile compounds will, however, present a special problem. Multi-component systems can be simultaneously analysed for each component provided (*a*) they can be adequately resolved by thin-film chromatography and (*b*) samples of chromatographically pure standards are available.

The accuracy of the simple algebraic method (either W_s or W_+) is similar to that of the best methods in which the compound is recovered from the adsorbent and determined spectroscopically.

We thank Professor J. B. Speakman for his interest and one of us (S.J.P.) thanks the Agricultural Research Council for financial assistance.

We thank Mr. B. Bush and Mr. C. O. M. Steward for the results shown in Table VII.

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The Gas-chromatographic Analysis of Mine Gases by a Zero Suppression Technique

By G. S. VIZARD and A. WYNNE

(*National Coal Board, North Eastern Division, Scientific Department, Golden Smithies Lane, Wath-upon-Dearne, Rotherham, Yorks.*)

A zero suppression technique has been employed in the recording of chromatograms of mixtures of permanent gases. This method has been used to study quantitatively the effects of small changes in (*i*) katharometer-bridge current, (*ii*) carrier-gas flow rate, (*iii*) the temperature of the chromatographic column and (*iv*) the temperature of the katharometer block.

The use of this technique, with the optimum carrier-gas flow rate and closely controlled bridge current and temperature, has resulted in considerable improvement in analytical accuracy. A comparison has been made of the accuracy of analyses by this method and that obtained by an experienced operator using the Haldane apparatus. The standard deviations for the chromatographic and Haldane methods are 0·02 and 0·04 per cent. v/v, respectively, for oxygen and 0·06 and 0·04 per cent. v/v for nitrogen, determined on synthetic gas mixtures.

The main advantage of gas chromatography is that it makes possible a more detailed analysis of complex gas mixtures than can be achieved by the chemical absorption and combustion methods. However, in some applications, for example, in the determination of oxygen and nitrogen, the accuracy given by the Haldane apparatus has so far been greater than could be obtained by gas chromatography.

The normal practice in gas chromatography is to produce, on a recording potentiometer, a chromatogram consisting of a trace of detector response against time.

When a differential type of detector is used, the complete peak corresponding to each component in the mixture is then recorded over a suitable millivolt range on the recorder. Commercially available potentiometers of this type commonly have chart widths between 6 and 11 inches, with a basic range of one millivolt. The accuracy with which the height of a peak on the recorder chart can be measured will set one of the practical limits to the ultimate accuracy attainable.

If it is assumed, provisionally, that this represents the only source of error in the chromatographic analysis of a sample of mine-air gases, then, for an oxygen peak 10 inches in height representing say 20 per cent. v/v of the mixture, the peak height would have to be measured to within $\pm 0{\cdot}01$ inch for the accuracy to be similar to that obtainable by an experienced operator using the Haldane apparatus. In the proposed method, all chromatograms are recorded on the one-millivolt range, and an accurately reproducible e.m.f. is applied to the recorder in opposition to the peak being recorded. For example, if the katharometer gives a response of 10·9 mV for the oxygen peak, a back e.m.f. of 10 mV is applied immediately the oxygen peak begins to emerge. Thus, only that portion of the peak in excess of 10 mV (in this instance 0·9 mV) is recorded, producing a peak approximately 10 inches in height on the 1-mV scale. This peak height will represent the difference between approximately 19 and 21 per cent. v/v of oxygen. For the accuracy in measurement of the peak to give a result comparable with that of a Haldane determination, it would now be sufficient to measure the peak to within $\pm 0{\cdot}1$ inch.

In practice, there are several other possible sources of error in a chromatographic analysis (*e.g.*, variations in katharometer-bridge current, in carrier-gas flow rate and in the temperatures of the column and katharometer). This method of recording chromatograms has been used, in the first place, to study quantitatively the effect of small changes in these variables and, secondly, to analyse synthetic gas samples and routine mine-air samples.

This technique was developed in the National Coal Board, North Eastern Division, Scientific Department, between August and November 1961. A subsequent more intensive search of the literature revealed prior publications[1,2,3,4] in which this method is referred to.

Apparatus

The apparatus used in this investigation incorporated a commercial katharometer detector of conventional design and a recording potentiometer having a pen-response time of $1\frac{1}{4}$ seconds. Details of the zero-suppression circuit are shown in Fig. 1. Argon was used as the carrier gas in conjunction with a chromatographic column packed with Linde molecular sieve.

To obtain a column that would give the maximum response for oxygen and nitrogen for a given size of sample and also complete resolution of these two components, a preliminary investigation into the behaviour of Linde molecular sieves types 5A and 13X was undertaken. The pellets of molecular sieve were ground in a pestle and mortar and dry sifted to between 30- and 60-mesh B.S. sieve. Portions of the sifted material were activated by heating in open silica dishes in a muffle furnace. The retention times, degree of separation and the peak heights for oxygen and nitrogen in samples of fresh air were noted, and a comparison was made, on this basis, between the relative merits of activating at different temperatures and for different periods of time. In all tests the column was packed immediately after removal of the hot molecular sieve from the furnace. The retention times were determined from the chromatograms by measuring from the point of injection to the peak maxima. The results are summarised in Table I.

Table I

Activation of Linde molecular sieves

Activation		Column length, feet	Retention time		Peak height	
Period, minutes	Temperature, °C		Oxygen, seconds	Nitrogen, seconds	Oxygen, chart divisions	Nitrogen, chart divisions
Molecular sieve type 13X—						
65	250	5	83	110	48	90
130	250	5	83	113	48	86
60	350	$4\frac{1}{4}$	90	140	46	68
110	350	$4\frac{2}{3}$	90	140	48	70
Molecular sieve type 5A—						
15	175	5	90*	110	37	79
15	250	5	113	193	45	58
75	250	5	120	223	43	50
65	350	$3\frac{3}{4}$	122	295	43	37

* Peaks not completely resolved.

It is clear that Linde molecular sieve type 5A requires less vigorous conditions of activation than does type 13X in order to obtain a similar degree of separation between oxygen and nitrogen. It is also clear that type 5A is affected more than type 13X by increasing either

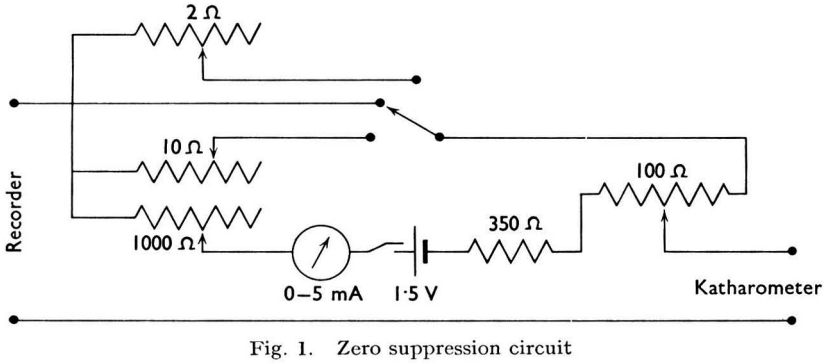

Fig. 1. Zero suppression circuit

the time or the temperature of activation. Finally, it can be seen that the retention time for nitrogen, and hence the sensitivity of the katharometer to this gas, is affected more by increasing the degree of activation of the molecular sieve than is the retention time for oxygen.

With a column, 5 feet in length, of internal diameter $\frac{3}{16}$ inch and packed with 30- to 60-mesh Linde molecular sieve, the optimum performance was obtained with type 13X molecular sieve activated at 250° C for one hour.

An alternative method of increasing the response of the katharometer, and hence the accuracy obtainable by this technique, was tried; hydrogen was used as the carrier gas and the bridge current was 350 mA. With the chromatographic column activated under the conditions already described and with a carrier-gas flow rate for hydrogen that had previously been found to give the best results, all peaks were eluted from the column much more rapidly than with argon as the carrier gas. By using the zero suppression technique under these conditions the response time of the recorder ($1\frac{1}{4}$ seconds) was found to be insufficiently rapid. It might have been possible to overcome this difficulty by using a considerably more active, or a longer, column, thus reducing the rate at which the peaks emerged, or by using a recorder having a faster pen-response time. With a column of molecular sieve 5 feet in length, oxygen and argon emerge together as a composite peak. When hydrogen is used as the carrier gas the katharometer will give a signal that is proportional to the percentage of oxygen and argon together. When argon is used as the carrier gas, however, the peak is proportional to the percentage of oxygen alone. This is an obvious advantage in the analysis of mine-air samples when the percentage of oxygen must be determined accurately. For this reason, the investigation with hydrogen as the carrier gas was not pursued any further.

Some results for the determination of oxygen in fresh air by the zero suppression technique were: 21·02, 20·83, 20·83, 21·23, 21·53, 21·22, 21·53, 20·43, 20·88, 20·42, 20·65, 20·90 and 20·64 per cent. v/v (mean, 20·93 per cent. v/v; standard deviation, $\pm$ 0·36 per cent. v/v). These results show the comparatively poor repeatability obtained at this stage.

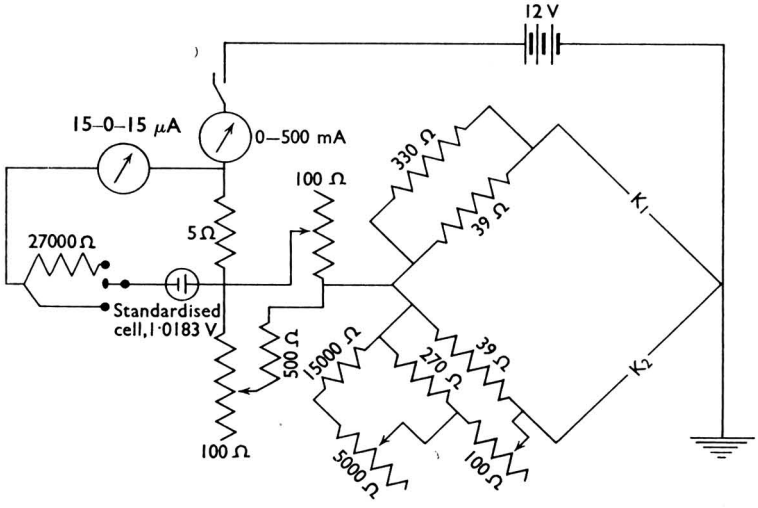

Fig. 2. Katharometer-bridge circuit and bridge-current standardisation

The sample pipette used up to this time consisted of a glass bulb with a two-way tap at each end. With this type of pipette the chromatograms were not accurately reproducible, owing to the fact that the sample was not swept on to the column in a compact undiluted "plug." These variations in response were more pronounced for the larger sample sizes. The substitution, for the pipette, of a sample loop of uniform narrow bore, fitted with a two-way tap at each end, reduced these variations.

Effect of changes in katharometer-bridge current

It was realised that slight variations in the katharometer-bridge current would produce comparatively large variations in the response of the detector. A means of standardising the bridge current against a Weston standard cell was therefore incorporated in the apparatus, as suggested by Keulemans.[5] Details of the circuit employed are shown in Fig. 2, together with the katharometer-bridge circuit.

Fig. 3 shows a plot of the change in response of the katharometer to oxygen, at the level present in fresh air, produced by small changes in bridge current; a change of 1 mA produced a change of almost 60 chart divisions (0·3 mV) in the oxygen response. With the

bridge-current standardisation circuit in use it has been found possible to standardise the bridge current to within $\pm 0{\cdot}01$ mA, the resulting variation in a peak representing 20·9 per cent. v/v of oxygen is one-sixth of a chart division (approximately $\pm 0{\cdot}001$ per cent. v/v on the oxygen peak).

To avoid the need for frequent standardisation of the bridge current, heavy duty (120 ampere/hour) car batteries were used. The voltage from these batteries is less steady when they are freshly charged and also when they are nearly discharged. A means of maintaining the batteries in a state of charge where the voltage remains most stable has been suggested by Dr. G. J. Pitt, National Coal Board, Central Research Establishment, Stoke Orchard, Cheltenham.

Details of the circuit, which was incorporated in our apparatus, are shown in Fig. 4.

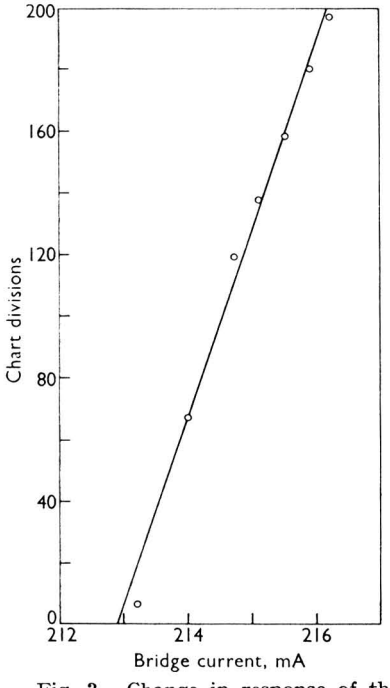

Fig. 3. Change in response of the katharometer to oxygen with small changes in bridge current

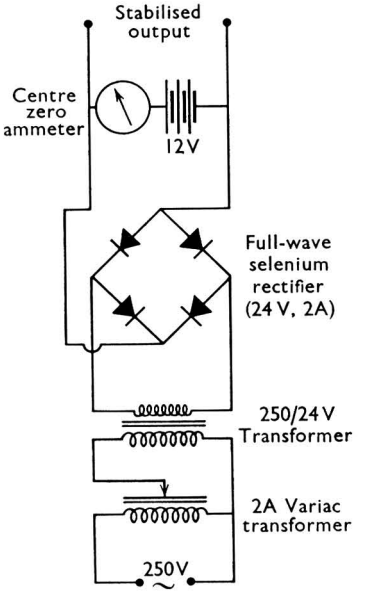

Fig. 4. Circuit for stabilising voltage output from 12-volt car batteries

Effect of changes in carrier-gas flow rate

Fig. 5 shows a plot of the change in response of the katharometer to oxygen, nitrogen and methane, due to changes in the carrier-gas flow rate. It will be seen that there is a different optimum flow rate for each component. It has also been found that the values of the optimum flow rates may be dependent, to a small extent, on the percentages of the component gases in the mixture.

In Fig. 5, the values for oxygen and nitrogen were obtained on samples of fresh air; those for methane from a sample containing 85 per cent. v/v of methane, 12 per cent. v/v of nitrogen, 2 per cent. v/v of oxygen and 1 per cent. v/v of carbon dioxide. With this sample it was noted that the optimum flow rate for oxygen was the same as determined with samples of fresh air, but the best flow rate for nitrogen had increased from 28 ml per minute (fresh-air samples) to 31 ml per minute.

For the analysis of samples of mine gases consisting mainly of oxygen and nitrogen in approximately fresh-air proportions, a flow rate of 31·5 ml per minute was selected. The values of these optimum flow rates will depend to a considerable extent on the dimensions of the column used and to some extent upon the geometry of the katharometer. It would, therefore, be a wise precaution to determine the best flow rates for each column and each apparatus used.

Effect of moisture in the sample

In the development of the technique the presence of small amounts of moisture was found to have an adverse effect on the repeatability. The samples were taken from outside the building and pumped into duralumin sample tubes fitted with Schrader valves,[6] which are widely used for the routine sampling of mine gases, and then transferred to the pipette system in the laboratory.

It was noticed that there was a significant difference in the peak heights, both for oxygen and nitrogen, between the earlier samples taken from a sample tube, at pressures between 200 and 300 lb per sq. inch, and those taken later from the same tube when the pressure had dropped below 50 lb per sq. inch. The difference in peak heights for oxygen between the first and last samples taken from a tube corresponded to a difference of approximately 0·2 per cent. v/v, *i.e.*, a drop from 20·93 to 20·73 per cent. v/v.

If an average value for the relative humidity outside the laboratory buildings is taken as 50 per cent., this would correspond to the presence of 1·08 per cent. v/v of water vapour in the air at 20° C. Assuming that there is a tendency for the water vapour to condense at the initial pressures (200 to 300 lb per sq. inch) within the sample tube, and that it is evolved in increasing amounts as the pressure within the tube is reduced, the differences found in the percentages of oxygen and nitrogen could be accounted for.

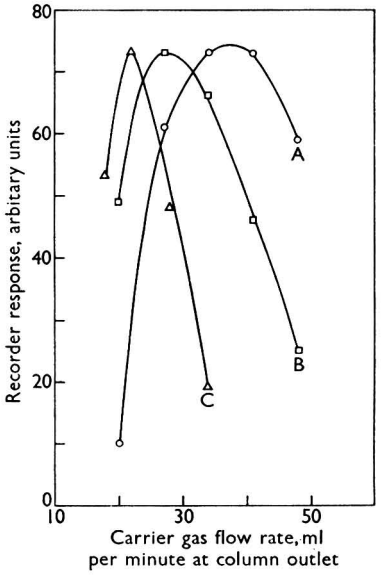

Fig. 5. Effect of changes in carrier-gas flow rate: curve A, oxygen; curve B, nitrogen; curve C, methane

In order to elucidate this point further, the amount of water evolved from a sample tube was determined by using a Flaschenträger micro absorption tube packed with anhydrous magnesium perchlorate between plugs of quartz wool. The results from a duplicate series of determinations are shown in Table II. The volume of air for each test, corresponding to a reduction in pressure of 25 lb per sq. inch, was 120 ml (at atmospheric pressure).

The results in Table II suggest that there is a direct quantitative relationship between the variation in the percentages of oxygen determined on a series of samples taken from a single sample tube and the dilution effect of water vapour, which is released in increasing amounts as the pressure within the tube is reduced.

This effect was overcome by introducing a small tube (1 inch $\times$ $\frac{3}{16}$ inch internal diameter) packed with anhydrous magnesium perchlorate before the sample loop. At this stage it was also decided to include a short packed length of soda lime in a similar guard tube, placed between the sample loop and the chromatographic column, to remove carbon dioxide, which would progressively de-activate the Linde molecular sieve.

TABLE II

RELEASE OF WATER VAPOUR FROM A SAMPLE TUBE AND THE CORRESPONDING DROP IN THE DETERMINED PERCENTAGE OF OXYGEN

Pressure in sample tube, lb per sq. inch	Weight of water evolved, mg	Oxygen found, % v/v
200	0·052	20·89
175	0·096	20·88
150	0·207	20·85
125	0·223	20·84
100	0·325	20·82
50	0·355	20·75
25	0·490	20·72

EFFECT OF CHANGES IN COLUMN TEMPERATURE

In the course of a day devoted to the analyses of fresh-air samples, the recorded response of the katharometer for nitrogen was found to drift. A typical set of results is shown in Table III.

It was thought that the drift in katharometer response could be due to variation in the temperature of the chromatographic column. Changes in the laboratory temperature would be expected to produce two opposing effects. Unless the temperature of the sample loop is thermostatically controlled, a rise in temperature will result in an effectively smaller sample being placed on the column (due to the normal expansion of the gases) and hence in a smaller recorded response to oxygen and nitrogen. A rise in temperature of the column will reduce the retention times for all components, thus tending to increase the response from the katharometer. From the figures quoted it can be seen that these two effects compensate each other for oxygen but not for nitrogen.

The effect of changes in column temperature was investigated by thermostatically controlling the temperature of the column (but not the sample loop) in a water bath to within $\pm 0{\cdot}05°$ C. A plot of the change in the recorded katharometer response to oxygen and nitrogen in samples of fresh air at different column temperatures is shown in Fig. 6. As already noted, the nitrogen response is affected more than is that of oxygen by changes in column temperature.

At this stage a sample volume of 3 ml was used and, for samples of fresh air, the recorded response for oxygen corresponded to 2·5 mm for 0·01 per cent. v/v, and for nitrogen 1 mm on the chart corresponded to 0·01 per cent. v/v. From Fig. 6 it can be seen that a change in column temperature of 0·1° C produced changes in response corresponding to approximately 0·004 per cent. v/v for oxygen and 0·5 per cent. v/v for nitrogen.

TABLE III

VARIATIONS IN RESULTS FOR OXYGEN AND NITROGEN OVER A PERIOD OF 7 HOURS

During the 7 hours the laboratory temperature rose 2° C

Oxygen found, % v/v	Nitrogen found (excluding other inert gases), % v/v	Oxygen found, % v/v	Nitrogen found (excluding other inert gases), % v/v
20·95	77·61	20·93	78·35
20·93	77·68	20·93	78·42
20·92	77·61	20·93	78·42
20·92	77·71	20·93	78·38
20·91	77·74	20·93	78·45
20·90	77·84	20·94	78·48
20·91	77·87	20·93	78·51
20·93	78·01	20·94	78·54
20·93	78·01	20·93	78·57
20·97	78·11	20·94	78·59
20·93	78·14	20·94	78·61
20·93	78·27	20·93	78·58

Effect of changes in temperature of the katharometer block

The effect of changes in the temperature of the katharometer block on its response to oxygen and nitrogen was found to be small. The katharometer temperature was raised, in stages, from 23° to 36° C, and then allowed to cool slowly to its original temperature. As the temperature of the katharometer rose, the resistance of the platinum filament increased slightly, resulting in a slight drop in bridge current. At each temperature the bridge current was therefore re-adjusted to its former level, all other conditions being maintained constant. With the samples of fresh air the net result on the katharometer response to oxygen, for a rise of 13° C, was an increase of 23 chart divisions, which would correspond to an increase of 0·115 per cent. v/v in the response to this component. The average increase in the response to oxygen over this temperature range would thus correspond to an increase of less than 0·01 per cent. v/v for each 1° C rise in temperature. The effect on nitrogen was considerably less than this.

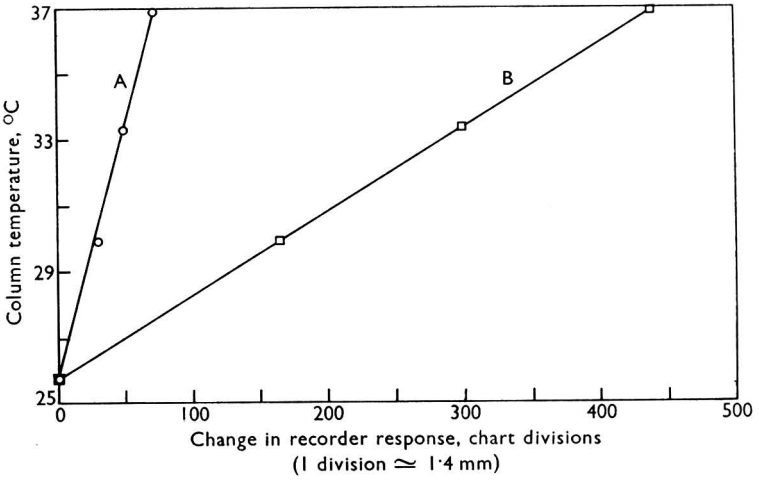

Fig. 6. Effect of column temperature on katharometer response: curve A, oxygen; curve B, nitrogen

It now appeared that the accuracy of the method had been sufficiently improved to justify joint tests, on synthetic gas mixtures, with the Haldane apparatus. The gas-chromatographic results obtained by the zero suppression technique and those obtained by an experienced operator using the Haldane apparatus are shown in Table IV, together with the calculated composition of the samples as made up.

The experimental conditions used in these and subsequent analyses were—

Column length—5 feet.
Column internal diameter—$\frac{3}{16}$ inch.
Column packing—Linde molecular sieve, type 13X, 30 to 60 mesh.
Column temperature—30° C.
Katharometer-bridge current—203 mA.
Carrier gas—Argon.
Carrier-gas flow rate (at column outlet)—31·5 ml per minute.
Pressure drop across the column—170 mm of mercury.
Sample volume—1·7 ml.

Joint tests on eleven routine samples of mine-air gases were also carried out, the results of which are shown in Table V.

A statistical analysis of the results quoted in Tables IV and V led to the conclusions listed below.

(1) SYNTHETIC MIXTURE (*Table IV*)—

From Table IV neither the gas-chromatographic nor the Haldane analyses show any bias, *i.e.*, there is no evidence of systematic errors.

The Variance Ratio Test shows that, on the oxygen determination, the gas chromatography variance is significantly less (at the 5 per cent. level) than that of the Haldane. The variance on the Haldane would, however, have been appreciably smaller but for the abnormal results on mixture No. 6.

(2) MINE-AIR GASES (*Table V*)—

There is no bias on any determination.

(3) CONCLUSIONS FROM STATISTICAL ANALYSIS—

Gas chromatography is a no less reliable method of analysis than is the Haldane apparatus.

TABLE IV

RESULTS OF JOINT TESTS ON SYNTHETIC GAS SAMPLES

	Oxygen			Nitrogen *plus* argon		
Mixture No.	As made up, % v/v	Found by gas chromatography, % v/v	Found with Haldane apparatus, % v/v	As made up, % v/v	Found by gas chromatography* (direct determination of nitrogen), % v/v	Found with Haldane apparatus† (by difference), % v/v
1	20·93	20·92 20·91	20·91 20·91	79·07	78·95 78·95	79·04 79·04
2	20·86	20·88 20·88	20·91 20·91 20·90	79·14	79·15 79·18	79·04 79·04 79·04
3	20·86	20·85 20·85	20·83 20·84 20·85	79·14	79·07 79·07	79·12 79·11 79·07
4	20·79	20·78 20·77	20·78 20·76	79·21	79·24 79·21	79·18 79·20
5	20·71	20·71 20·71	20·73 20·74	79·29	79·28 79·28	79·23 79·22
6	20·64	20·65 20·65	20·75 20·74 20·74	79·36	79·37 79·32	79·25 79·26 79·26
7	20·50	20·53 20·53	20·52 20·50 20·52	79·50	79·52 79·49	79·48 79·50 79·48
8	20·33	20·32 20·37	20·37 20·35 20·37	79·67	79·67 79·61	79·63 79·65 79·63
9	20·18	20·20 20·23	20·23 20·24 20·22	79·82	79·87 79·94	79·77 79·76 79·78
10	20·04	20·04 20·00	20·04 20·02 20·04	79·96	80·00 80·04	79·96 79·98 79·96
	Standard deviations.. ..	±0·02	±0·04		±0·06	±0·04

* The nitrogen figure in each test was 0·93 per cent. less than those quoted, this value being the argon content of air.

† In mixtures 1 to 5 inclusive, small percentages of carbon dioxide were found on the Haldane apparatus.

CONCLUSIONS

A technique has been developed whereby the accuracy of analyses of mixtures of permanent gases by gas chromatography has been greatly improved (particularly for the larger components in a mixture). It has been applied to the analysis of synthetic gas mixtures and

mine-air gases, and it has been shown that the accuracy of the method is equal to that obtainable by an experienced operator using the Haldane apparatus. The time taken for a single analysis of a sample is similar to that required on the Haldane apparatus. One of the advantages of the gas-chromatographic method lies in the fact that it is more specific; helium, argon and nitrogen can be determined separately, when required, whereas the Haldane apparatus can give only a single value (by difference) for the inert gases. Similarly, the chromatographic method permits the separate determination of hydrogen, methane, ethane

TABLE V

RESULTS OF JOINT ANALYSES ON MINE-AIR SAMPLES

Sample No.	Oxygen found— by gas chromatography, % v/v	Oxygen found— with Haldane apparatus, % v/v	Nitrogen *plus* argon found— by gas chromatography, % v/v	Nitrogen *plus* argon found— with Haldane apparatus,* % v/v	Methane found— by gas chromatography, % v/v	Methane found— with Haldane apparatus,† % v/v	Carbon dioxide found— by gas chromatography,* % v/v	Carbon dioxide found— with Haldane apparatus, % v/v
1	20·56	20·49	78·71	78·80	0·44	0·50	0·29	0·21
	20·58		78·71		0·44		0·27	
2	20·48	20·50	78·57	78·54	0·72	0·79	0·23	0·17
	20·49		78·61		0·72		0·18	
3	20·38	20·28	78·47	78·60	0·83	0·91	0·35	0·21
4	20·72	20·69	78·82	78·87	0·32	0·34	0·14	0·10
	20·71		78·79		0·32		0·20	
5	20·53	20·57	78·99	78·97	0·15	0·15	0·33	0·31
	20·53		78·97		0·15		0·35	
6	20·50	20·57	79·01	79·00	0·23	0·22	0·26	0·21
	20·50		79·01		0·23		0·26	
7	20·88	20·85	79·04	79·00	0·03	0·02	0·05	0·09
	20·88		79·04		0·04		0·04	
8	20·66	20·64	78·64	78·71	0·52	0·52	0·18	0·13
	20·66		78·71		0·52		0·11	
9	20·60	20·62	78·89	78·80	0·39	0·42	0·12	0·16
	20·60		78·89		0·39		0·12	
10	20·74	20·73	78·86	78·81	0·37	0·35	0·03	0·11
	20·74		78·84		0·37		0·05	
11	20·68	20·69	79·09	79·03	0·14	0·13	0·09	0·15
	20·68		79·11		0·14		0·07	

* Determined by difference.
† Combustible gas.

and higher homologues, whereas the Haldane apparatus is less reliable with this type of mixture. The other main advantage of the chromatographic technique lies in its versatility; the same apparatus can provide equally reliable analyses on samples differing widely in the number of components and in their percentage compositions.

It may be worthwhile to consider whether the zero suppression technique could also be applied to the gas-chromatographic analysis of organic liquids.

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The Determination of Thiocyanate in Coal-carbonising Plant Effluents, Sewage Works Influents and Effluents and Polluted Waters

By T. G. WHISTON and G. W. CHERRY
(*Central Laboratories, West Midlands Gas Board, Nechells, Birmingham, 7*)

It has been noted that some of the methods available for determining thiocyanate are subject to dilution errors and to interference from phenolic compounds, and that the various methods are each applicable over a limited range only.

With this in mind, these have been investigated and a method has been developed in which phenolic compounds are separated on a column of anion-exchange resin before the determination of thiocyanate.

The colorimetric determination of thiocyanate with ferric chloride reagent has been found to be both simple and applicable over a concentration range of 0 to 60 p.p.m., thereby considerably reducing dilution errors.

Methods suitable for the various ranges of thiocyanate concentrations are recommended.

In the carbonising industries the determination of thiocyanate is of great importance, since it is used as a basis for the assessment of pollution by carbonising plant effluents and for assessing the efficiency of biological plants treating these effluents before discharge to sewers or water courses. Samples on which the determination of thiocyanate is required in these laboratories fall into three groups—

(1) Gas-works effluents with thiocyanate concentrations of 400 p.p.m. and above.
(2) Partially treated gas-works effluents with thiocyanate concentrations of 50 to 400 p.p.m.
(3) Sewage works influents and effluents and polluted waters with thiocyanate concentrations of 0 to 20 p.p.m.

The methods at present available for determining thiocyanate in such samples are—

(1) The copper sulphate method, as described by Key.[1,2]
(2) The pyridine - pyrazolone method of Epstein.[3,4]
(3) Aldridge's method.[5]
(4) The mercuric nitrate method.[6]

As Aldridge's method makes use of benzidine, a carcinogenic substance, and has no advantages over the pyridine - pyrazolone method for determining low concentrations of thiocyanate, the latter is preferred.

The copper sulphate method is reliable only for concentrations of thiocyanate of 400 p.p.m. or more. The pyridine - pyrazolone method covers the concentration range of 0 to 2 p.p.m., so that for samples having thiocyanate concentrations within the range 2 to 400 p.p.m., dilution is necessary before the determination can be carried out by this method. It has been found, however, that phenolic compounds, which are normally present in relatively high concentrations in gas-works effluents, interfere in the pyridine - pyrazolone method; they give a slight colour with the reagents, and this interference can cause large errors in the results obtained, particularly when large dilution factors are involved.

The mercuric nitrate method is reliable for thiocyanate concentrations of 100 p.p.m. and above, but can only be used for samples whose chloride content is not more than 200 p.p.m. of Cl^-. This limits its use, since a normal gas-works effluent contains approximately 10,000 p.p.m. of Cl^-. There are, however, a few effluent liquors from certain parts of a gas-making plant that have low chloride contents; for these the mercuric nitrate method of determining thiocyanate is quicker than the copper sulphate method.

Because of the interference by phenolic compounds in the pyridine - pyrazolone method, a method was sought that would separate these compounds from the thiocyanate, and so permit its determination to be carried out without interference. A method in which De-Acidite E anion-exchange resin is used has been developed; it has been found that, by using this method, the final determination of thiocyanate can be carried out over the concentration range of 0 to 60 p.p.m. with ferric chloride reagent without any necessity for further dilution.

Experimental

The copper sulphate method—

The method depends on the precipitation of cuprous thiocyanate by the addition of copper sulphate solution, its decomposition by sodium hydroxide and the titration of the sodium thiocyanate formed with silver nitrate solution after acidification with nitric acid. It is rather time-consuming and gives results reliable to within ± 10 p.p.m. for thiocyanate concentrations of approximately 400 p.p.m. or above.

The mercuric nitrate method—

After the removal of chloride by the addition of bismuth nitrate and then filtration of the precipitated bismuth oxychloride, thiocyanate is titrated directly with standard mercuric nitrate solution. The method is relatively quick and simple, but its use is limited to samples with chloride contents of not more than 200 p.p.m. It gives results reliable to within ± 10 p.p.m. for thiocyanate concentrations of approximately 100 p.p.m. or above.

The pyridine - pyrazolone method—

The method depends on the production of cyanogen chloride, by the action of chloramine-T, and its reaction with pyridine and 1-phenyl-3-methyl-5-pyrazolone to give a blue colour. This blue colour gives a straight line calibration graph over the range 0 to 2 p.p.m. of SCN^- and results are reproducible to within $\pm 0{\cdot}1$ p.p.m. for thiocyanate concentrations in the range 0 to 20 p.p.m., *i.e.*, for dilution factors up to 10. Dilution errors, as well as interference from phenolic compounds, may occur at dilution factors greater than 10, as shown by the results listed below for thiocyanate in a standard solution containing 10 p.p.m. of SCN^- at different dilutions—

Dilution factor	5	10	20	100
Thiocyanate found, p.p.m.	10·3	10·4	12·2	25·0

From these results it should be noted that a dilution factor of 100 on a sample containing 10 p.p.m. of thiocyanate will result in a final solution containing only 0·1 p.p.m. of thiocyanate; such a low concentration can be difficult to determine accurately. In practice, it is preferable to choose a dilution factor that gives an instrument reading lying on the upper portion of the calibration graph. This may necessitate two determinations.

Aldridge's method—

The range of this method is similar to that of the pyridine - pyrazolone method. The cyanogen bromide produced by the action of bromine water reacts with benzidine in pyridine hydrochloride solution to give a pink colour.

Owing to the carcinogenic properties of benzidine, this method has been discontinued in these laboratories.

Separation of phenolic compounds from thiocyanate—

Attempts to extract the phenolic compounds with organic solvents, *e.g.*, ether, butyl acetate and isobutyl methyl ketone, were unsuccessful, since these solvents also extracted thiocyanate.

It was considered that thiocyanate might be determined by its absorption at 215 mμ, but it was found that phenols also absorbed in this region and that their prior removal was necessary.

The use of chromatographic columns was considered. A column of active charcoal proved to be useless, as thiocyanate was absorbed as well as phenols. Initial success was

obtained with a column of active alumina for the separation of various synthetic mixtures of phenols and ammonium thiocyanate. However, it was finally established that, whereas catechol and its homologues were retained on the column, resorcinol and its homologues were not retained and passed through with the thiocyanate. Thus, although a column of active alumina cannot be used for separating phenols from thiocyanate, it seems that it might find use in the separation of *o*-dihydroxyphenols from *m*-dihydroxyphenols. However, this point was not pursued.

It was finally found that the desired separation of phenolic compounds from thiocyanate could be achieved by the use of a column of De-Acidite E anion-exchange resin in the chloride form. The method depends on the absorption of the anions in the sample (chiefly thiocyanate and thiosulphate) on to a weakly basic anion-exchange resin, no absorption of phenols occurring. The anions are then eluted from the column by approximately 3 N ammonium hydroxide, and the thiocyanate is determined by any of the methods described.

A comparatively rapid method for determining thiocyanate after its elution from a column of De-Acidite E was found to be by the removal of ammonia by boiling and the addition of ferric chloride reagent, the red colour produced being measured with an absorptiometer. When a yellow filter (Ilford 606) was used, an almost straight line calibration graph was obtained over the range 0 to 200 p.p.m. of thiocyanate. When a green filter (Ilford 604) was used, the method was more sensitive and the calibration graph covered the range 0 to 60 p.p.m. Since the method involves a dilution factor of 2, this final determination can be carried out with use of a green filter on samples containing up to 120 p.p.m. of thiocyanate without further dilution.

Method

Reagents—

De-Acidite E anion-exchange resin (chloride form)—Wash well with water, and remove fines by decantation. Prepare a 1-inch × 4-inch column in the usual way, and wash with water until only a trace of chloride is present in the washings.

Ferric chloride reagent solution—Dissolve 100 g of hydrated analytical-reagent grade ferric chloride in water containing 50 ml of concentrated hydrochloric acid, and make up to 500 ml with water.

Ammonium hydroxide, approximately 3 N—Measure 170 ml of ammonium hydroxide, sp.gr. 0·880, and make up to 1 litre with water.

Procedure—

Measure 100 ml of the sample, and pass it through the column of anion-exchange resin at a rate of approximately 10 ml per minute. Wash through with 100 ml of water in a rapid stream. All phenolic compounds will pass through the column, but thiocyanate ions will be retained. Reject the solution that has passed through the column.

Elute the thiocyanate by passing 200 ml of approximately 3 N ammonium hydroxide through the column at a rate of approximately 10 ml per minute, and collect in a 600-ml beaker. (If the concentration of thiocyanate in the sample is 300 to 400 p.p.m., a further 100 ml of 3 N ammonium hydroxide must be passed through the column.) Wash through with 100 ml of water in a rapid stream. Boil the combined eluate and washings to approximately half their bulk or until all the ammonia has been boiled off. Cool, and make up to 200 ml in a calibrated flask.

If the thiocyanate concentration in the original sample is not more than 20 p.p.m., proceed with the pyridine - pyrazolone method. If the thiocyanate concentration in the original sample is in the range 20 to 400 p.p.m., proceed with the ferric chloride method, as described below—

> Measure a suitable portion from the 200-ml flask, and make up to 200 ml in another flask, so that the thiocyanate concentration of the diluted solution is in the range 10 to 60 p.p.m. Measure 100 ml of diluted solution into a 250-ml beaker, and add 10 ml of ferric chloride reagent solution. Prepare at the same time a blank solution of 100 ml of water and 10 ml of ferric chloride reagent solution, and compare in 1-cm cells the red colour produced; use an absorptiometer and Ilford 604 green filters. Apply the appropriate dilution factor in the calculation of the results.

After each determination, revivify the column. Pass 200 ml of diluted hydrochloric acid (1 + 9) through the column at a rate of approximately 10 ml per minute. Wash at a rapid rate with water until the washings contain only a trace of chloride.

Always keep the column of anion-exchange resin covered with water.

Results

The methods described have been applied to synthetic mixtures and routine samples, and typical results are shown in Tables I, II and III.

Table I

Determination of thiocyanate in synthetic solutions simulating partially treated gas-works effluents

Determinations were carried out by the proposed method in which De-Acidite E anion-exchange resin and ferric chloride reagent solution are used

Total monohydric and dihydric phenols present,* p.p.m.	40	100	160	190
Ammonium chloride present, p.p.m.	40	100	160	190
Thiosulphate present, p.p.m.	20	50	80	95
Thiocyanate added, p.p.m.	424	265	107	27
Thiocyanate found, p.p.m.	410	255	105	29

* The phenols present were phenol, *p*-cresol, 2,3-xylenol, 2,5-xylenol, catechol, resorcinol, 2-methylresorcinol, 5-methylresorcinol and quinol.

Table II

Comparison of results by the copper sulphate and mercuric nitrate methods

The samples used were of a gas-works effluent containing 100 to 150 p.p.m. of chloride

	Thiocyanate found in sample No.—				
	1, p.p.m.	2, p.p.m.	3, p.p.m.	4, p.p.m.	5, p.p.m.
Copper sulphate method	540	480	500	550	560
Mercuric nitrate method	530	490	520	560	550

Table I shows that the substances present in gas-works effluents do not interfere in the method with De-Acidite E and ferric chloride reagent, and Table II shows the degree of agreement between the copper sulphate and mercuric nitrate methods. The results in Table III show the errors in the determination of thiocyanate in biologically treated gas-works effluents, before separation of phenolic compounds, caused by increasing dilution when the

Table III

Comparison of results by the copper sulphate and pyridine - pyrazolone methods and the method in which De-Acidite E and ferric chloride reagent are used

Sample	Sample No.	Thiocyanate found by— copper sulphate method, p.p.m.	pyridine - pyrazolone method, p.p.m.	proposed method, p.p.m.
Gas-works effluent	6	520	—	500
Biologically treated gas-works effluent	7	—	107 † 114 ‡	95
	8	—	18·3 § 35·8 ‖ 60·0 ‡	18·0
	8*	—	124·3 ‡	105·0

* With 86·5 p.p.m. of thiocyanate added.
† Dilution factor 50.
‡ Dilution factor 100.
§ Dilution factor 10.
‖ Dilution factor 20.

pyridine - pyrazolone method is used. They also show that the proposed method returns results for added thiocyanate, and also that it gives results in agreement with the copper sulphate method.

Conclusions

From the results obtained, the recommendations given below can be made for the determination of thiocyanate in gas-works effluents, partially treated gas-works effluents, sewage works influents and effluents and polluted waters.

(1) For gas-works effluents, in which the concentration of thiocyanate is 400 p.p.m. or above, use the copper sulphate method.

(2) For gas-works effluents, in which the concentration of thiocyanate is 400 p.p.m. or above and the chloride concentration is not more than 200 p.p.m., use the mercuric nitrate method.

(3) For gas-works effluents, partially treated in biological plants, in which the concentration of thiocyanate is in the range 20 to 400 p.p.m., separate off the phenolic compounds by means of De-Acidite E anion-exchange resin (chloride form), dilute the eluted thiocyanate as necessary, and determine colorimetrically with ferric chloride reagent.

(4) For sewage works influents and effluents and for polluted waters, in which the concentration of thiocyanate is in the range 0 to 20 p.p.m., use the pyridine - pyrazolone method.

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Notes

THE RAPID DETERMINATION OF SEED-DRESSING INSECTICIDE RESIDUES IN ANIMAL RELICTA

THE rapid growth in the use of certain insecticides in agriculture has been accompanied by unforeseen side effects. The treatment of cereals with dressings containing aldrin, gamma-BHC, dieldrin or heptachlor against wheat bulb fly and/or wireworm has resulted in the death of seed-eating birds, principally pigeons, and occasionally of predators. It has not been easy to obtain positive evidence that the observed mortality of birds and animals was the direct or indirect result of exposure to these chemicals. This difficulty has been partly due to the lack of suitable analytical methods for dealing with biological material and partly to the large number of samples involved. Of the methods so far available, paper chromatography as developed by Mitchell,[1] McKinley and Mahon,[2] Mills[3] and Evans[4] has been the most favoured. Recently, the analysis of crop extracts for traces of chlorinated insecticides by gas - liquid chromatography and detection by electron capture has been reported by Goodwin, Goulden and Reynolds.[5]

The fate of these chemicals in animal bodies has been studied by several workers. Radomski and Davidow[6] have shown that, in the dog, heptachlor is converted to heptachlor epoxide, which is stored in the body fat. Bann, De Cino, Earle and Sun[7] have shown that aldrin is converted to dieldrin by several animal species. Dieldrin is apparently unchanged in the body and is stored as such. The fate of gamma-BHC in mammals is not certain. Fitzhugh, Nelson and Frawley[8] have shown that the various isomers of BHC when fed to rats and dogs are stored unchanged. When heptachlor or aldrin is fed to feral pigeons, only the epoxides are found. Analysis of seven pigeons fed aldrin resulted in dieldrin being found present in every case. In two of the birds aldrin was also present, but only in trace amount. When dieldrin or gamma-BHC is fed to animals, these compounds only are detected.

METHOD

APPARATUS—

M.S.E. homogeniser (Cat. No. 7700) fitted with 100-ml beaker.

Shandon Universal gas chromatograph fitted with 0 to 100 volt d.c. variable supply to the detector. A polytetrafluoroethylene jet is fitted at the column outlet for the detector to seat on.

A 1 mV recorder with 1 second response set to run at 6 inches per hour.

Hamilton micrometer syringes.

Separating funnels, 200 ml.

Calibrated flasks, 100 ml.

Column packing—Celite 100 to 120 mesh (J. Js. Ewell) impregnated with 2·5 per cent. of E 301 silicone elastomer (I.C.I. Ltd.) and 0·25 per cent. of Epikote resin 1001 (Shell Chemical Co. Ltd.) by weight. It was found that changes took place in freshly ground Epikote resin 1001 on storage, and it is advisable to pulverise just sufficient to prepare a batch of column packing. Any excess of column packing should be stored in an atmosphere of carbon dioxide or nitrogen until required. A 2-foot column kept at a temperature of 188° C was used for the work reported here. Larger columns may be used giving better separation but with less symmetrical peaks.

Other operating conditions were as described by Goodwin, Goulden and Reynolds.[5]

REAGENTS—

Acetone—Analytical-reagent grade acetone should be redistilled, and the first 5 per cent. rejected.

n-Hexane—Low in aromatics.

Sodium sulphate, anhydrous.

Standard solutions of aldrin, gamma-BHC, heptachlor, heptachlor epoxide and dieldrin—Containing 0·5 mg or less of the insecticide per 100 ml of n-hexane.

PROCEDURE—

Macerate 15 g of breast muscle or liver (with small animals use all the liver) with half this weight of anhydrous sodium sulphate and about 40 ml of redistilled acetone for 4 minutes in the homogeniser at high speed. Place the macerated tissue on a Whatman No. 1 filter-paper and

collect the filtrate in a 200-ml separating funnel; press the residue with a spatula to remove as much liquid as possible. Clean the homogeniser beaker, shaft and blades by washing with a further 40 to 50 ml of acetone, add the macerate from the filter funnel, and again mix the contents in the homogeniser. Again place the macerate on a Whatman No. 1 filter-paper and collect the filtrate in the separating funnel; use a further 20 ml of acetone to assist this transference, and press the residue as before. Shake the acetone extract vigorously in the separating funnel with 50 ml of n-hexane and 20 ml of water. Allow the n-hexane layer to separate, and run it off into a 100-ml calibrated flask. Extract the acetone - water solution with a further 40 ml of n-hexane, shake, allow the n-hexane layer to separate, and then run it off into the calibrated flask containing the previous hexane extract. Adjust the contents of the flask to the mark by adding n-hexane.

TABLE I

INSECTICIDES FOUND ON SPECIMENS FROM THE FIELD

Species	Dieldrin found—		Heptachlor epoxide found—	
	in flesh, p.p.m.	in liver, p.p.m.	in flesh, p.p.m.	in liver, p.p.m.
Woodpigeon (*Columba palumbus*)*	13·1	22·0	26·0	28·8
	26·0	28·8	9·5	9·2
	21·5	13·9	2·7	46·4
	14·4	n.e.		
			3·0	n.e.
			4·8	n.e.
	19·3	29·4		
			42·0	1·5
			16·7	19·8
			‡	10·0
	‡	‡		
	2·9	11·4		
	8·2			
			17·5	90·6
Pheasant (*Phasianus colchicus*)*	13·8	28·8	‡	25·4
			1·0	n.e.
			7·6	24·0
	6·1	n.e.		
	5·3	22·8		
	3·4	21·6	7·5	29·8
			‡	
			1·9	n.e.
Partridge (*Perdix perdix*)†	n.a.	46·3		
	6·9	n.e.		
			4·6	n.e.
Rat (*Rattus norvegicus*)			6·5	n.e.
			9·5	n.e.
			12·0	n.e.
Lapwing (*Vanellus vanellus*)	‡	n.e.		
Blackheaded gull (*Larus ridibundus*)	‡	n.e.		

n.e. = not examined.
n.a. = not available.
* Five samples gave negative results.
† Two samples gave negative results.
‡ Trace present (0·1 to 0·05 p.p.m.)

GAS - LIQUID CHROMATOGRAPHIC PROCEDURE—

Adjust the amplifier and recorder zero pre-set controls so that the base line is about 2 inches from the side of the recorder chart. Set the gain control on the amplifier unit at × 20, and switch to read. Adjust the backing-off voltage until the pen position coincides with that for amplifier and recorder zero. Start the chart drive. Inject a 5-μl n-hexane extract sample by means of a Hamilton micrometer syringe through the silicone-rubber disc at the inlet of the column. Identify any insecticide present by its retention time compared with samples of known insecticide solutions. By varying the volume of sample or the volume or strength of standard insecticide solution, obtain peaks of roughly the same magnitude, and, from measurement of peak heights, calculate the amount of insecticide in the sample.

Results

By the proposed procedure and with known amounts of insecticides added to pigeon flesh before maceration, recoveries of 78 per cent. of dieldrin, 69 per cent. of heptachlor epoxide and 63 per cent. of gamma-BHC were obtained at the 5 and 10 p.p.m. levels. Undoubtedly the recovery rate could be improved by more elaborate extraction, but only at the expense of speed. The procedure described was intended to cope with a large number of samples during the autumn sowing season of 1961. Fortunately, large numbers of casualties did not occur. The results are summarised in Table I. The significance of these results is discussed by Turtle, Taylor and Wright.[9] The absence of gamma-BHC in any sample is noteworthy; from experiments on laboratory birds it is known that, by using the method described, gamma-BHC if present is found.

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Infestation Control Laboratory
Ministry of Agriculture, Fisheries and Food
Tolworth, Surrey.

A. Taylor
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ACIDIMETRIC TITRATION OF METAL ACETATES IN NON-AQUEOUS SOLVENTS

A method was required for the rapid determination of the metal content of a number of hydrated acetates. Casey and Starke[1] have described the potentiometric titration in glacial acetic acid of the anhydrous acetates of eighteen metals, perchloric acid in acetic acid being used as the titrant. They claimed good reproducible end-points for all the metals examined except cobalt. In this investigation it was found that the acetates of sodium, calcium and strontium could be titrated satisfactorily in acetic acid, but those of zinc, cobalt and manganese gave poorly defined end-points. With methanol as the solvent, however, all these metals gave sharp reproducible end-points. The results obtained were in good agreement with those obtained by conventional methods and with the titration in acetic acid when direct comparison was possible. The hydrated salts were used, and no special precautions were taken to exclude moisture. The acetates of calcium and strontium are insoluble in methanol and were determined by adding excess of perchloric acid and titrating the excess with standard sodium acetate solution in methanol. The method could undoubtedly be extended to the other acetates examined by Casey and Starke and to salts of other organic acids.

Method

Reagents—

Perchloric acid solution in methanol, approximately 0·1 N—Slowly add the calculated amount of 60 per cent. aqueous analytical-reagent grade perchloric acid, with cooling, to 1 litre of analytical-reagent grade methanol, and dilute to 2 litres. This solution appeared to be stable and showed no change in strength on standing for a month at room temperature. The standardisation is most conveniently carried out by titration with standard aqueous 0·1 N sodium hydroxide, with methyl red as indicator.

Sodium acetate solution in methanol, approximately 0·1 N—Dissolve 13·6 g of analytical-reagent grade sodium acetate trihydrate in 1 litre of analytical-reagent grade methanol.

PROCEDURE—

Direct titration—Weigh accurately about 0·5 g of sample into a 250-ml beaker, and dissolve in 40 to 50 ml of analytical-reagent grade methanol. Insert the glass and calomel electrodes, stir magnetically, and titrate potentiometrically with the 0·1 N perchloric acid.

Indirect titration—Weigh accurately about 0·5 g of sample into a 250-ml beaker, and dissolve in 100 ml of the 0·1 N perchloric acid. Insert the glass and calomel electrodes, stir magnetically, and titrate potentiometrically with the 0·1 N sodium acetate in methanol.

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IMPERIAL CHEMICAL INDUSTRIES LTD.,
FIBRES DIVISION
HOOKSTONE ROAD
HARROGATE

R. B. RASHBROOK
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AN IMPROVED METHOD FOR PREPARING GLYOXAL BIS-(2-HYDROXYANIL)

KERR'S[1] spectrophotometric method for determining calcium, which is based on the work of Bayer[2] and of Goldstein and Stark-Mayer,[3] has been found satisfactory (see also Williams and Wilson[4,5]).

The method described below for preparing glyoxal bis-(2-hydroxyanil) avoids the tedious and unpleasant sublimation of *o*-aminophenol,[2,4] gives an improved yield and allows a larger amount of the reagent to be made conveniently at one time.

Put 22 g (0·1 mole) of *o*-aminophenol (Hopkin and Williams G.P.R. grade is suitable) into a 500-ml flask. Add 200 ml of methanol and 16 ml (19·3 g; 0·2 mole) of 30 per cent. w/w glyoxal. Heat the mixture under reflux, and continue heating for 90 seconds after the appearance of crystals. This takes only a few minutes; further heating does not increase the yield, but makes the product much darker.

Add 200 ml of distilled water at 60° C, mix, and cool. Filter off the crystals, and wash with successive small portions of methanol until the washings are almost colourless.

Dissolve the crystals in 100 ml of acetone by heating to boiling; filter if necessary. Add 50 ml of methanol, heat again to boiling, add 50 ml of water at 60° C, and set aside to cool. Filter off the crystals, wash with a little methanol, and dry at room temperature. The yield is 14 g (58 per cent.) of almost-white crystals, m.p. 203° C (Bayer[2] found 204° C).

The pure reagent is not particularly soluble in methanol. Crystals tend to separate from a 0·5 per cent. solution, so it is better to use a double volume of 0·25 per cent. solution in the analytical method. Acetone is a better solvent (7 ml will dissolve 1 g), but its use has not been investigated.

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MARCHON PRODUCTS LTD.
WHITEHAVEN, CUMBERLAND

J. DREWRY
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THE ABSORPTIOMETRIC DETERMINATION OF TIN BY MEANS OF DITHIOL

DURING the last year a number of analysts using the method for determining tin described by Dickinson and Holt[1] have reported turbidity in the final solution used for absorptiometric measurement of the colour. The method involves the use of Teepol X, which was originally suggested by Williams and Whitehead[2] as a suitable dispersant for preventing coagulation of the red tin - dithiol complex. As Teepol X is no longer manufactured, analysts have been using other dispersants that, owing to their unsatisfactory compositions, are the cause of the turbid solutions reported.

The use of a dispersant the composition of which might be altered as a result of a re-formulation or improved manufacturing technique presents a hazard, and therefore one conforming to a definite specification would be more reliable. Sodium lauryl sulphate has been stated to be a satisfactory dispersant for the tin - dithiol complex,[3] and it can be obtained in a fairly pure state as the B.P. material.

The solubility of sodium lauryl sulphate in water increases only slightly up to 21° C (krafft point), but above this temperature the solubility increases quickly. Sodium lauryl sulphate is precipitated if the temperature drops below the krafft point. When this happens it is only necessary to heat the solution to just above the krafft point to make it suitable for use.

Analysts should not experience trouble from turbidity when determining tin by the method described by Dickinson and Holt[1] provided that—

(*a*) Sodium lauryl sulphate, B.P., is used as the dispersant.

(*b*) The fusion mixture is weighed out to avoid excessive variation in the acidity of the final solution.

(*c*) The sentence, "To 5 ml of the solution . . . dithiol reagent," described in the method[1] is amended to read, "To 5 ml of the solution thus prepared add 1 ml of diluted hydrochloric acid (1 + 1), 4 drops of a 1 per cent. sodium lauryl sulphate solution and 0·3 ml of dithiol reagent."

(*d*) The 5 ml of solution is well shaken after the addition of each reagent.

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THE FRUIT AND VEGETABLE CANNING AND QUICK FREEZING RESEARCH ASSOCIATION
CHIPPING CAMPDEN, GLOS.

T. W. RAVEN
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AN IMPROVED EXTRACTION TECHNIQUE FOR SYNTHETIC ANIONIC DETERGENTS

IN the determination of anionic detergents by Slack's[1] modification of Longwell and Maniece's[2] method, difficulty was experienced in obtaining consistently reproducible results. The method has been slightly simplified and automatic shaking introduced. The gentle shaking of the separating funnel twice a second for 1 minute, as recommended, was found to be tedious and time-consuming, and gave poorer reproducibility as a result of incomplete extraction. The proposed method has been found most satisfactory for routine determinations.

PROCEDURE—

Prepare the samples and separating funnels in accordance with Longwell and Maniece's procedure.[2]

To the first series of separating funnels add 50-ml portions of chloroform,[1] insert the stoppers firmly, and place in a mechanical shaker (120 oscillations per minute with an amplitude of 3 inches), and shake for 1 minute. Run off into the second series of funnels as much of the chloroform layers as possible, and repeat the shaking for 1 minute. Break the emulsions formed with a glass rod. If sufficient clear chloroform extract cannot be obtained for optical-density measurements by this method, break the emulsion by spinning the extract in a centrifuge.

Run approximately the first 5 ml of chloroform extract from the second series through a funnel plugged with a little non-absorbent cotton-wool, and discard the filtrate. Filter the remaining clear chloroform extract through the same funnel into a 50-ml calibrated flask. Measure the optical density of the extract against chloroform at 650 mμ with a spectrophotometer (or use a Spekker absorptiometer and No. 8 filter) without making the extract up to volume. Measure the optical densities against chloroform as soon as possible after extraction, as the colour changes on standing unless the solution is covered and stored in a cold dark place.

Results

For comparison of the two methods, nine 20-ml portions of a humus-tank effluent sample were analysed with use of both the manual and automatic shaking techniques. From the results shown in Table I it is evident that the reproducibility was improved by the automatic shaking procedure.

Table I

Comparison of results obtained from manual shaking and automatic shaking

Sample No.	Detergent found with manual shaking, p.p.m.	Detergent found with automatic shaking, p.p.m.
1	2·95	3·53
2	3·15	3·55
3	3·75	3·53
4	2·80	3·55
5	3·50	3·55
6	2·85	3·55
7	3·50	3·68
8	2·85	3·65
9	3·50	3·53
Co-efficient of variation	11·26%	1·56%

A test was carried out to determine the optimum period of vigorous shaking. Complete extraction was obtained after 1 minute under the conditions described.

The emulsion formed on shaking was not found to interfere with the results obtained.

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National Institute for Water Research
Council for Scientific and Industrial Research
P.O. Box 395, Pretoria, South Africa

D. W. Brink
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USE OF THE PINK FERROUS - NITROSONIUM COMPLEX AS A QUALITATIVE TEST FOR NITRATE

The pink colour obtained on adding a solution of ferrous sulphate in concentrated sulphuric acid to a solid salt or a solution of the salt in sulphuric acid has been used by English,[1] Swann and Adams[2] and Norwitz[3] for the quantitative determination of inorganic nitrate. In this Note the application of the method to qualitative analysis is described, and the chemistry of the colorimetric reaction is considered.

Detection of nitrate

In the absence of interferences, the test described below can be used. One millilitre of the solution containing the nitrate is placed in a test-tube, 3 ml of sulphuric acid are added, the solution is cooled under a tap, and 3 ml of ferrous sulphate solution are added. The ferrous sulphate solution is prepared by dissolving 2 g of ferrous sulphate ($FeSO_4.7H_2O$) in 30 ml of water containing a few drops of sulphuric acid, adding 100 ml of sulphuric acid, and cooling the solution to room temperature.

A study of interferences in this method was carried out with 0·1 mg of NO_3^- and 25 mg of the ion in question; it was shown that the anions acetate, arsenate, arsenite, borate, carbonate, citrate, formate, fluoride, hydroxide, hypophosphite, oxalate, perchlorate, phosphate, silicate, sulphate, sulphite and tartrate did not interfere. The amount of chloride specified interfered by catalysing the rapid oxidation of the ferrous ion to ferric ion, which produced a yellow colour with hydrochloric acid (3 mg of Cl^- did not interfere). Bromate, bromide, chlorate, hypochlorite, iodate and iodide interfered because of the yellow colour produced when sulphuric acid was added. Permanganate interfered because of the brown colour produced when sulphuric acid was added. Ferricyanide, ferrocyanide, sulphide, thiocyanate and thiosulphate gave precipitates when sulphuric

acid was added. The colours produced by chromate, dichromate, molybdate, tungstate and vanadate when the ferrous sulphate solution was added masked the pink colour. Persulphate and peroxide prevented the colour formation. Cyanide caused the production of hydrogen cyanide. Nitrite produced a pink colour identical to that obtained with nitrate.

No cations interfered except chromium^{III}, cobalt, copper and nickel, which masked the colour.

The interference from chloride, bromide, iodate, iodide, ferricyanide, ferrocyanide, sulphide, thiocyanate, thiosulphate, chromate, dichromate, molybdate, tungstate, vanadate, peroxide and cyanide was overcome by precipitating the silver salts. To obtain complete precipitation of the silver salts of iodate, thiosulphate, chromate, dichromate, molybdate, tungstate and vanadate, it was necessary to use a buffered acetate medium. Commercial silver salts contained significant amounts of nitrate; the silver perchlorate used in making the buffered reagent was therefore prepared by dissolving silver nitrate in water and heating to fumes with perchloric acid to drive off the nitrate.

Interference from chromium^{III}, cobalt, copper and nickel was overcome by adding sodium hydroxide to precipitate the hydrous oxides.

No way was found of overcoming the interference of nitrite, bromate, chlorate, hypochlorite and permanganate. Persulphate was destroyed in the evaporation after the addition of the sodium hydroxide.

The procedure when interfering ions are present is described below. Five millilitres of buffered silver perchlorate reagent are added, and the solution is filtered through a fine filter-paper. Five millilitres of 5 per cent. sodium hydroxide solution are added, the solution is filtered, and the filtrate is evaporated to 0·5 to 1 ml on a hot-plate. The solution is cooled in an ice-bath, and 5 ml of sulphuric acid and then 5 ml of ferrous sulphate solution are added. The buffered silver perchlorate reagent is prepared by dissolving 10 g of silver nitrate in water, adding 10 ml of perchloric acid, evaporating to fumes of perchloric acid without a cover lid, fuming for 5 minutes, washing down the sides, fuming again until the salts precipitate in a solid mass, dissolving the residue in 200 ml of water, adding 5 per cent. sodium hydroxide solution until a permanent precipitate appears, adding a 10 ml excess of sodium hydroxide solution and then acetic acid until the solution just clears, and diluting to 250 ml with water.

Chemistry of the colorimetric reaction

It has been stated[1,2,4] that the pink colour obtained in the recommended test and sometimes in the brown ring test and the brown colour usually obtained in the brown ring test are both due to ferrinitrosylsulphate, $FeSO_4.NO$ or $FeNOSO_4$. If this is so, it would seem possible to convert the pink colour to the brown colour and vice versa. This could not be done.

There are essentially three modifications of the brown ring test—

(*a*) The method as described by Scott,[5] whereby a solution of the sample in sulphuric acid ranging in concentration from (15 + 2) to (20 + 1) is treated with saturated ferrous sulphate solution.

(*b*) The method as described by Treadwell and Hall,[6] whereby a solution of the sample in diluted sulphuric acid (1 + 1) is treated with saturated ferrous sulphate solution.

(*c*) The method as described by Charlot,[7] whereby a crystal of ferrous sulphate is dissolved in sulphuric acid, and an aqueous solution of the sample is added.

Experiments showed that in Scott's method the ring was at first pink, but as more ferrous sulphate solution was added it turned brown. In Treadwell and Hall's method the colour was brown, never pink. In Charlot's method the colour was pink and did not change.

It seemed to me that the pink colour obtained in the proposed method and sometimes in the brown ring test must be entirely different from the brown colour usually obtained in the brown ring test, and that the valency of the nitrogen in the two species must be different. This seemed to be supported by a consideration of the titration methods used for determining nitrate. When the nitrate is titrated with ferrous sulphate in moderately concentrated sulphuric acid, the nitrogen is reduced to the bivalent state[8]—

$$6FeSO_4 + 2HNO_3 + 3H_2SO_4 \rightarrow 2NO + 3Fe_2(SO_4)_3 + 4H_2O.$$

However, when the titration is performed in concentrated sulphuric acid, the nitrate is reduced to the tervalent state[8]—

$$4FeSO_4 + 2HNO_3 + 2H_2SO_4 \rightarrow N_2O_3 + 2Fe_2(SO_4)_3 + 3H_2O.$$

Since N_2O_3 is the anhydride of nitrous acid, the last reaction has also been expressed as[9]—

$$2FeSO_4 + HNO_3 + H_2SO_4 \rightarrow HNO_2 + Fe_2(SO_4)_3 + H_2O.$$

From the above equations it seems logical that the brown colour formed in moderately concentrated sulphuric acid should contain Fe^{2+} and NO, and that, in the pink colour formed in rather concentrated sulphuric acid, the nitrogen should be in the tervalent state. The most plausible explanation of the pink colour is that nitrosonium ion, NO^+, is produced when N_2O_3 dissolves in sulphuric acid and that this ion then reacts with Fe^{2+} to form a co-ordination complex.

It is known that nitrous acid (N_2O_3) reacts with sulphuric acid, when the concentration of the sulphuric acid is greater than 65 per cent., to form nitrosylsulphuric acid[10,11]—

$$HNO_2 + H_2SO_4 \rightarrow HNOSO_4 + H_2O.$$

Also, it has been established that nitrosylsulphuric acid ionises in concentrated sulphuric acid to NO^+ and HSO_4^-.[12]

It can readily be seen why, in the brown ring test, the conditions at the interface in Charlot's method are satisfactory for the development of the pink colour. In Scott's method conditions are initially satisfactory for the development of the pink colour; however, as more ferrous sulphate solution is added the pink colour is destroyed by dilution with water and conditions are established for the development of the brown colour. In Treadwell and Hall's method conditions for development of the pink colour are not satisfied and only the brown colour appears.

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Pitman-Dunn Laboratories
Frankford Arsenal
Philadelphia 37, Pa., U.S.A.

George Norwitz

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DETECTION OF NITRATE AND NITRITE WITH TOFRANIL

The antidepressant drug *N*-(γ-dimethylaminopropyl)iminodibenzyl hydrochloride, Tofranil (imipramine), forms an intense blue colour with nitric and nitrous acids. Though the reaction is the same with both acids, it can be applied under suitable conditions to detect either in the presence of the other. The test is carried out by placing 1 drop of the test solution on a spotting tile and adding 1 drop of Tofranil reagent and then 2 or 3 drops of concentrated sulphuric acid. Nitrates and nitrites develop a blue colour. Alternatively, the test may be performed in the micro test-tube described in our earlier Note,[1] in which the use of *N*-1-naphthylethylenediamine reagent is discussed for the detection of the same radicals.

Test for nitrate in presence of nitrite

For the detection of nitrate in the presence of nitrite, the test is applied after completely destroying the latter by sulphamic acid. To 1 drop of the test solution are added 1 drop of a 5 per cent. solution of sulphamic acid, 1 drop of the reagent solution and then 3 or 4 drops of concentrated sulphuric acid.

When nitrate is tested in this way, nitrite in a concentration not exceeding 2 per cent. can be tolerated. The anions acetate, arsenate, bicarbonate, bismuthate, borate, carbonate, citrate, chloride, cyanide, fluoride, oxalate, phosphate, picrate, sulphate, sulphite, tungstate and tartrate do not interfere, even when present in 100-fold excess. Tartrate in excess becomes carbonised and the sensitivity of the test is reduced. The yellow colour of picrate affects the blue colour of the reaction, making it appear green. Bromide and molybdate in over 10-fold excess interfere.

Oxidising agents such as chlorate, bromate, iodate, chromate, vanadate, permanganate, persulphate or ferric salts form bluish colours and iodine is liberated from iodides. Hence the presence of any one of these ions masks the colour formed by nitrate. Thiocyanate and thiosulphate form no colour with the reagent, but prevent the formation of the blue colour.

Test for nitrite in presence of nitrate

If hydrochloric acid is used instead of sulphuric acid in the test, only nitrite will develop a blue colour. Nitrate does not interfere provided that the concentration does not exceed 1 per cent. The test is carried out by adding 1 drop of the reagent and then 4 or 5 drops of concentrated hydrochloric acid to 1 drop of the test solution. The anions acetate, arsenate, bicarbonate, bismuthate, borate, bromide, carbonate, citrate, cyanide, fluoride, molybdate, oxalate, persulphate, phosphate, picrate, sulphate, tartrate and tungstate form no colour and do not interfere, even when present in 100-fold excess. Thiocyanate prevents the formation of the blue colour. The oxidising agents, with the exception of persulphate, that interfere in the test for nitrate also interfere in the test for nitrite, as do compounds such as sulphite and thiosulphate, which destroy nitrite.

Discussion and applicability

The test for nitrite is more delicate than that for nitrate. For a positive result a 0·0005 M solution of nitrite or a 0·001 M solution of nitrate is required. The sensitivity of the test depends on the size of the drop used. If 0·05-ml drops are used the dilution limits are 1 in 50,000 for nitrate and 1 in 100,000 for nitrite, the corresponding limits of identification being 1 μg for nitrate and 0·5 μg for nitrite.

Both nitrate and nitrite can be detected in the presence of large amounts of organic matter, such as protein and carbohydrate, provided that they are not intensely coloured. In many tests of this type in which other reagents are used, nitrate and nitrite are identified by the formation of a yellow or red colour; without sacrificing sensitivity, this cannot be detected in presence of the original yellow or brown colour of the organic matter. The blue colour formed with Tofranil reagent can be detected with comparative ease in presence of such interference without sacrificing much sensitivity. The interfering radicals could be selectively eliminated before applying the test, but that would render the test complex and less sensitive.

The test is applicable for the detection of Tofranil, which, when allowed to react with concentrated nitric acid or a mixture of concentrated sulphuric and nitric acids, forms a blue colour. The intensity of the blue colour formed is a measure of the amount of Tofranil present.

Tofranil is cheap and readily available. The reagent, which is a 2 per cent. w/v aqueous solution of Tofranil, is colourless, and when protected from light is stable almost indefinitely. The sulphuric, hydrochloric and sulphamic acids used must be of good quality and free from nitrate.

We thank Suhrid Geigy Trading Limited for the generous gift of Tofranil.

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State Forensic Science Laboratory
Madras-3, India

A. Irudayasamy
A. R. Natarajan
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THE LIMIT OF DETECTION OF ANALYTICAL METHODS

This Note is a comment on A. L. Wilson's interesting and valuable Note[1] on "The Precision and Limit of Detection of Analytical Methods."

The apparent amount of impurity in the sample, C, is given by A — B, where A and B are the amounts corresponding to the analytical responses obtained from the sample and the "blank," respectively. As C increases in magnitude, so does the probability that the impurity is present.

The analyst may decide that for all values of C above a certain critical value, the "criterion of detection," he will conclude that the impurity is present; in doing so he will be taking a certain risk, α, of falling into an "error of the first kind," *i.e.*, of concluding that the impurity is present when it is not, and another risk, β, of falling into an "error of the second kind," *i.e.*, concluding that the impurity is absent when it is not. Wilson, having assumed that the standard deviations of A and B are equal, has given in his Table I values of the "limit of detection" in terms of various probabilities of an error of the first kind; he does not, however, appear to have realised that this will not have the same value as a "limit of detection" in terms of the risk of making an error of the second kind.

If it is assumed, with Wilson, that C has a normal distribution with standard deviation S_C, and if the risk, α, of making an error of the first kind is to be 5 per cent. (the conventional limit of significance), then the "criterion of detection," k (0·05), is 1·645 S_C. If the risk, β, of making an error of the second kind is to be 5 per cent., the "limit of detection," p (0·05; 0·05), is $2 \times 1{\cdot}645\ S_C$. The analyst must decide for himself in each particular instance how he will define his "limit of detection," but it is important that he should realise that it may be defined in terms of either type of risk and that for a given probability of risk one "limit of detection" is twice the other if $\alpha = \beta$.

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RIJKSZUIVELSTATION
VREEWIJKSTRAAT 12b
LEIDEN
THE NETHERLANDS

J. B. Roos
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Apparatus

A SIMPLE OXYGEN GENERATOR FOR USE IN THE DUMAS - UNTERZAUCHER METHOD

IT is well known[1] that the classical micro-Dumas method for determining nitrogen in organic compounds gives unsatisfactory results with a wide variety of nitrogen-containing compounds that are difficult to burn. Unterzaucher[2] showed that, if oxygen was injected during the combustion, satisfactory results could be obtained with some of these compounds. This observation has been confirmed and extended to a wider range of such compounds by other workers.[3,4,5,6] In Unterzaucher's modification, oxygen of the required purity was prepared by passing carbon dioxide through a solution of hydrogen peroxide in which was suspended finely divided platinum. This method was shown to be unsatisfactory for general use, the main difficulty being that of controlling the amount of oxygen entering the combustion train.[3] As a result, alternative methods for the preparation and injection of oxygen have been sought. Medicinal oxygen,[5] oxygen prepared from potassium permanganate[4] and oxygen evolved by electrolysis[3,7,8] have all been used, but the systems devised were rather complicated.

A simple oxygen generator has been designed; it is based, as was Unterzaucher's, on the catalytic decomposition of hydrogen peroxide. The apparatus, shown in Fig. 1, consists of a tube, A, approximately 70 mm long by 25 mm wide, with an outlet at the bottom connected by poly(vinyl chloride) tubing to a 50-ml graduated separating funnel, C, which acts as a reservoir. The funnel is fitted with a spring-loaded tap, B. Through a rubber or glass stopper in the mouth of tube A pass (1) an inlet tube from the carbon dioxide supply, (2) an outlet tube connected to the combustion train and (3) a glass rod to which is attached a square piece of silver gauze, the bottom of the gauze being about 50 mm from the mouth of tube A. A piece of gauze 20 mm × 40 mm folded to form a 20-mm square is used, the fold forming the bottom edge of the square. The two sides are welded together to prevent any loose metal threads from detaching themselves during use. If any such pieces were to fall into the hydrogen peroxide they would generate oxygen at times when it is not required.

The apparatus is set up as shown in Fig. 1, funnel C being about 30 cm above the top of tube A. With tap B closed, funnel C is filled with 50-volume hydrogen peroxide. Tap B is then opened, and hydrogen peroxide is allowed to run into tube A until the level is just below that of

the silver gauze; tap B is then closed. In this position no oxygen is generated. To generate oxygen, tap B is opened, and the level of hydrogen peroxide is allowed to rise in tube A until the gauze is partially immersed; tap B is then closed. As soon as the hydrogen peroxide comes into contact with the gauze, oxygen is liberated in an amount proportional to the area of gauze immersed. With the gauze totally immersed, about 20 ml of oxygen per minute are produced. It is an easy matter, by using the graduated markings on funnel C, to calibrate the apparatus for obtaining flow rates of from 1 to 20 ml of oxygen per minute. To stop the production of oxygen, the reservoir is lowered below the level of tube A, tap B is opened, and the level of hydrogen peroxide in tube A is allowed to fall until it is just below the gauze; tap B is then closed.

Once the oxygen generator has been connected to the nitrogen apparatus, it will function for a long time without needing to be dismantled. All that is necessary is that, every 2 or 3 days, the hydrogen peroxide must be replaced with a fresh solution.

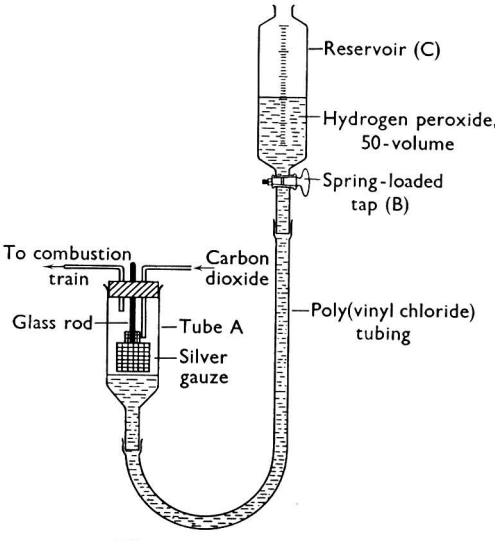

Fig. 1. Oxygen generator

Results

The nitrogen contents of various compounds have been determined by using oxygen from this generator at a rate of 5 ml per minute during the combustion, which took 10 minutes. The sections of the combustion apparatus containing copper oxide, copper and hopcalite were maintained at 900° ± 10° C, 550° ± 25° C and 120° ± 5° C, respectively.[9] The combustion products were swept through the apparatus into the azotometer over a period of 15 minutes.

Table I

Nitrogen content of various compounds

Compound	Nitrogen content— found, %	Nitrogen content— theoretical, %
S-Benzylthiouronium chloride (M.A.S.)	13·6, 13·9	13·8
p-Nitroaniline (M.A.S.)	20·2, 20·1	20·3
Trifluoroacetanilide (M.A.S.)	7·3, 7·3	7·4
Phenylthiourea (M.A.S.)	18·5, 18·4, 18·5, 18·4	18·4
Acetanilide (M.A.S.)	10·5, 10·4	10·4
Phenyl-s-benzothiazole	6·5, 6·5	6·7
2-(2-Hydroxy-5-methylphenyl) benzotriazole	18·7, 18·6	18·6

The nitrogen contents shown in Table I for the various compounds are satisfactory, the values found being within ±0·2 per cent. of theory and the standard error of a single determination being ±0·09 per cent. These results show that the oxygen generator is providing oxygen of the required purity.

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IMPERIAL CHEMICAL INDUSTRIES LIMITED
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HARROGATE

R. MCGILLIVRAY

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AUTOMATIC GAS-CHROMATOGRAPHIC SAMPLING FROM STATIC SYSTEMS OF SUB-ATMOSPHERIC PRESSURE

IN order to follow a chemical reaction occurring between two gaseous components at sub-atmospheric pressure in a static system of constant volume, an automatically operating gas chromatograph was devised.

The apparatus described here in detail performed the operations listed below consecutively every 30 minutes—

(1) Opened the sample loop of the sampling cell to a vacuum pump so that the loop was evacuated, and then closed the loop.

(2) Turned on a continuously recording strip chart recorder.

(3) Opened the evacuated sample loop to the reaction vessel for a pre-designated period, and then closed it.

(4) Opened the sample loop to the carrier-gas stream.

(5) Altered the degree of attenuation as various chromatographic peaks were recorded.

(6) Closed the sample loop to the carrier gas, and shut off the recorder chart drive.

A schematic representation of the flow system is shown in Fig. 1. D is a Gow-Mac stainless-steel thermal-conductivity cell with Pretzel geometry and tungsten filaments operated at ambient temperature, the filaments being heated by a current of 250 mA.

V_1 and V_2 are Hoke A431 bellows valves mounted in pneumatically actuated Research Control valves type 75, so that they are either on or off, depending on whether or not the Hoke B95A344 solenoid valves, SV_1 and SV_2, are actuated. All of the pneumatic valves were operated from a 15 lb per sq. inch compressed air supply.

V_3 is a Greenbrier Instrument Co. linear sampling valve, type C-2, pneumatically actuated. In the unactuated position the sample loop is isolated from the carrier-gas stream by a series of O-rings, which have been found to be pressure and vacuum tight. Solenoid valve SV_3 (identical to SV_1 and SV_2) controls the air supply to V_3 in an on - off manner.

V_4, V_5 and V_6 are Hoke 1252 open - closed toggle valves, and are used to facilitate a change in the type of chromatographic column, C_1 or C_2, to be used in the system.

A Welch Duo-Seal single-stage rotary vacuum pump mounted in the cabinet with the remainder of the instrument is used to evacuate the sample loop.

Fig. 2 is a schematic representation of the electrical circuit used.

T_1 is a six-cam Series MC electrical timer obtained from the Industrial Timer Corporation, driven by a 4 revolution per hour synchronous motor coupled to an A_6 gear chain, which effects one complete timer cycle every 30 minutes. Each cam is set to close its microswitch at an appropriate point in the cycle and to keep it closed for a pre-designated interval.

A_1 and A_2 are Daven 5000-ohm 12-step attenuators obtained from the F and M Corporation. These are pre-set before the beginning of the experiment, but which one is in the circuit during any part of the cycle is controlled by the timer.

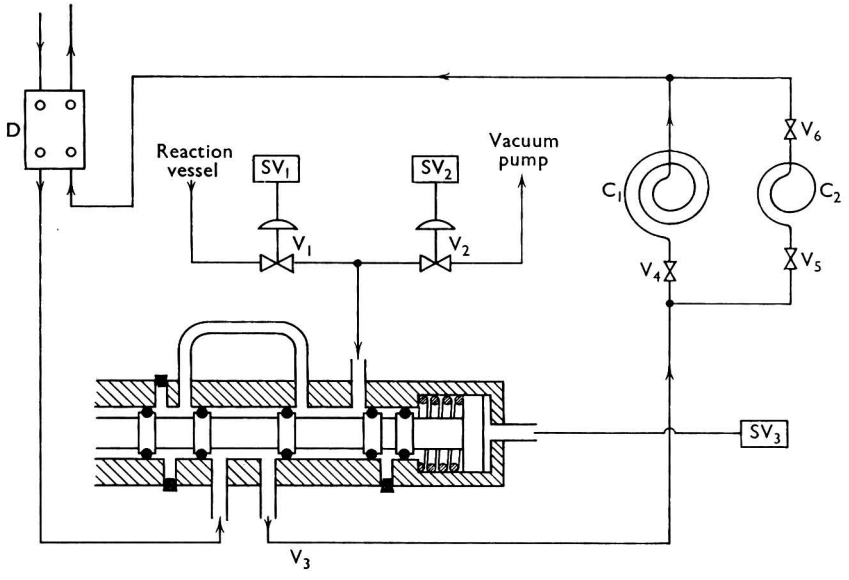

Fig. 1. Pneumatic circuit

Switches S_1, S_2 and S_3 control the solenoid valves (SV_1, SV_2 and SV_3 of Fig. 1) so that the various valves of the system can, should the need arise, be operated independently of the timer. This manual control is useful when calibrating the gas chromatograph for the gases present during the reaction being studied.

A Sorensen 12-volt d.c. power supply, model QM12–0·32, provides the 250-mA current required to heat the filaments of the Gow-Mac conductivity cell.

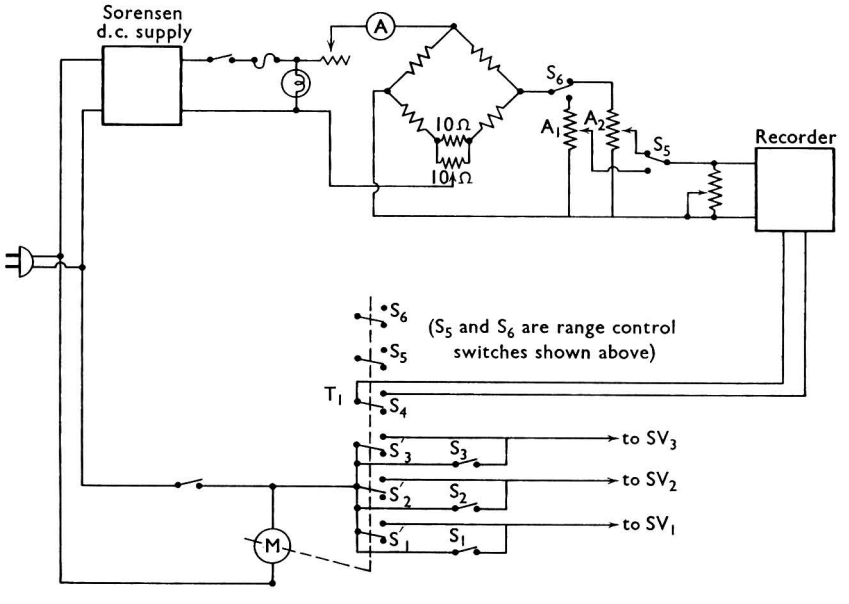

Fig. 2. Electrical circuit

OPERATION—

The unit performs in a most satisfactory manner all the operations listed above. Reproducible samples have been taken from reaction mixtures at many pressures of reactants. In normal operation small samples are taken from a non-flow system, the pressure of which varies from 10 to 1 cm of mercury. The reliable performance of the unit is further illustrated by the fact that it has now operated for over 7000 hours without any maintenance apart from a replacement of V_4.

DU PONT OF CANADA LIMITED
RESEARCH CENTRE
KINGSTON, ONTARIO, CANADA

K. CASEY
F. H. C. EDGECOMBE
D. A. JARDINE
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Book Reviews

CHEMICAL ANALYSIS: AN ADVANCED TEXT AND REFERENCE. By HERBERT A. LAITINEN. Pp. xiv + 611. New York, Toronto and London: McGraw-Hill Book Company Inc. 1960. Price $12.50; 97s.

This is a really important book that stresses the fundamental principles of analytical chemistry and is written to a standard aimed at advanced undergraduate or postgraduate level. It begins with a discussion of ionic equilibria and deals concisely with problems of activity coefficients before turning to a thorough account of acid - base equilibria in aqueous solutions. This leads naturally to a welcome chapter on the corresponding equilibria in non-aqueous solutions, which concludes with an account of the Hammett acidity function, H_0. The preparation of standard acids and bases and typical applications occupy some 20 pages.

The next main section deals with the solubility of precipitates. Here one encounters a really up-to-date account of the processes leading to the formation of precipitates, the various theories of nucleation and growth, a study of colloidal properties and aging of precipitates, which leads naturally to considerations of contamination through "occlusion" in its widest sense, co-precipitation, post-precipitation, and to homogeneous precipitation. The chapter on precipitation titrations (17 pp.) follows after an unexpected interpolation on thermal decomposition and volatilisation, which, excellent in itself, rather breaks the continuity.

An important section (25 pp.) discusses the theory of complexiметric titrations in considerable detail and leads to Chapter 14 (28 pp.) covering the use of organic reagents for the precipitation and solvent extraction of metals. Here again the theory is fully discussed with many relevant examples. There is a useful introduction to the use of phosphine oxides and long-chain amines and amine salts as extractants.

Electro-analysis is introduced by a concise account of the vexed question of the "convention of signs," and the author gives good reasons for adopting the recommendations of the IUPAC Commission of 1953. Discussions of reversible and irreversible electrode reactions lead on to practical aspects of electrolytic separation and electro-analysis at controlled potential or current. Titrations with coulometrically generated titrants are mentioned briefly. The theory of redox titrations is next discussed and the important Chapter 18, entitled "Prior Oxidation and Reduction," goes into the question of what is involved in bringing a sample into some specified oxidation state before analysis. Subsequent chapters deal with the uses of permanganate (19 pp.), ceric (15 pp.), iodine (32 pp.), various oxyhalogen compounds (14 pp.), dichromate etc. as oxidants, and Ti^{III}, Cr^{II}, V^{II}, Hg^{I}, ascorbic acid and hydroquinone as reductants.

The topic of reaction rates in chemical analysis is one commonly ignored in text-books of theory. One might suppose that reactions are either "fast enough to be practicable" or "too slow to be of use." Chapter 24 (20 pp.) of this volume indicates how measurements of rates can sometimes give information about concentrations even though the rate law is unknown. The uses of reaction rates in studying mixtures is examined, and a thorough treatment is given of catalysed, induced, and "coupled" reactions. There is a widespread tendency among many analysts to avoid reactions known to be subject to the influence of added materials; a perusal of this chapter would indicate some of the advantages to be gained by exploiting this very phenomenon.

Under the general title "Multistage Separation Processes" the author deals with fractional distillation, liquid - liquid extraction, gas - liquid chromatography, adsorption chromatography, paper chromatography, electrophoresis and the use of ion-exchange resins. The last two chapters deal with sampling (15 pp.) and statistics in quantitative analysis, the analysis of variance and the design of experiments (41 pp.).

Throughout the text there are worked examples, and each chapter concludes with a set of numerical problems for which answers are provided. The copious references are well chosen and right up-to-date. To my knowledge no other book covers so wide a range of topics with such authority. It is surely an essential purchase for every analyst concerned with the basic theory of his subject and a "must" for every student. H. IRVING

RAPID RADIOCHEMICAL SEPARATIONS. By YUSURU KUSAKA and W. WAYNE MEINKE. Pp. vi + 125. Nuclear Science Series NAS–NS–3104. Washington, D.C.: U.S. Department of Commerce, Office of Technical Services. 1961. Price $1.25.

This is one of a series of monographs, written by specialists, devoted to radiochemical techniques. It reviews in 39 pages methods that have been used for separating short-lived radionuclides, defined as nuclides having a half-life of less than 20 minutes; this criterion has not been rigidly applied, since polonium-210 and caesium-137 having half-lives of 138 days and 28 years, respectively, have been included.

The greater part, 67 pages, is occupied by a tabular presentation of separation methods for 242 nuclides ranging from helium-6 to mendelevium-255 documented by 273 references covering the period 1934 to 1961.

Although the techniques employed—distillation, precipitation, solvent extraction, etc.—are those with which every chemist will be familiar, their success depends in a large measure on the careful choice of chemical and physical form of the "target" material and on the mechanisation of operations, for example, the use of pneumatic-tube systems for speedy transport of samples from place to place. Only minute amounts of material have to be handled, yet engineering often plays as large a part in the work as chemistry or physics. D. A. LAMBIE

BIOCHEMICAL APPLICATIONS OF GAS CHROMATOGRAPHY. By H. P. BURCHFIELD and ELEANOR E. STORRS. Pp. xviii + 680. New York and London: Academic Press Inc. 1962. Price 157s.

While 20 years have elapsed since the possibilities of gas chromatography were first suggested, only 10 have passed since it began to be practised, and, to use a modern idiom, what a breakthrough it has made, particularly in analytical chemistry. There is no corner of the analytical field that it has not pervaded. In so many directions has this pervasion occurred, and with so many applications in each direction, that the time has become ripe for sub-divisional review and collation. In the present volume this has been ably done for the field of biochemistry. The book is so comprehensive in this field that an alternative title might be "What the intelligent (and imaginative) biochemist needs to know about gas chromatography." It is stimulating reading. The opening chapter, comprising about one-fifth of the book, is devoted to general principles, techniques and instrumentation. Rapidly advancing new techniques are apt to accumulate a babel of terms for the same concepts; the analytical section of IUPAC has proposed to bring some order into gas chromatography by recommending standard terms and units. These are usefully set forth and used throughout the volume. Operating parameters for the different determinations described are set forth in standard form, which simplifies search on the part of the analyst. Although the theory or the process is not dealt with in detail, the practical mathematical consequences are lucidly explained, *e.g.*, the calculation of retention values. The general design and variations thereof in the basic chromatograph, types of detectors, preparation of the columns, introduction of the sample, choice of carrier gas and general operation for both qualitative and quantitative analysis receive detailed treatment. This consideration of basic principles and techniques is followed by eight chapters dealing with the special problems of the biochemist, which largely resolve themselves into problems in inorganic and organic chemistry. A fundamental property of the material to be chromatographed is volatility, so that non-volatile substances need to be converted to volatile derivatives. Thus the preparation of the sample, its isolation from

biological tissues and fluids and possibly its preliminary fractionation are concomitant problems, particularly for the biochemist, to be solved before gas chromatography starts. These are thoroughly covered in the various chapters in a stimulating way. These chapters deal successively with permanent gases and organic vapours, volatile constituents of tissues and biological fluids, cyclic compounds, essential oils, resin acids, lipids, non-volatile components of tissues and miscellaneous applications, *e.g.*, organic analysis (elements and structure), pesticide and pharmaceutical analysis. These chapter headings give little idea of the wealth of information contained in the chapter. Thus the lipid chapter deals with aliphatic hydrocarbons, fatty acids, alcohols and aldehydes, alkyl glycerols, steroids and synthetic derivatives of lipids. Each chapter contains tables of retention data.

The book is well produced; the only typographical errors observed were "methene" and "methadiene" on p. 386 for "menthene" and "menthadiene." This is a book that should be in the hands of every analytical and research biochemist, the gas-chromatographic techniques being so eminently suited to the minute amounts of material usually available. J. I. M. JONES

INTRODUCTION TO CHEMICAL INSTRUMENTATION, ELECTRONIC SIGNALS AND OPERATIONS. By EDWARD J. BAIR. Pp. viii + 349. New York, Toronto and London: McGraw-Hill Book Company Inc. 1962. Price 83s. 6d.

The analytical chemist of to-day who is completely ignorant of electrical, electronic and optical principles is at a severe disadvantage. It is sad that he must seek his own salvation in this because there is little or no time or place for electronics in degree courses at universities, and it is also sad that few universities include courses on the principles of instrumentation. Instrumentation is now so much a part of chemistry, and particularly analytical chemistry, that black boxes are inevitable. Simply to twiddle the knobs and read a magic dial is perhaps a fit function for the unqualified assistant, but the chemist must know more. Much chemical and analytical information is in the form of electrical signals, and very little is not readily convertible into such. It is necessary to know what happens to chemical information on transduction and electrical and electronic processing to apply instruments intelligently, to make full use of the investment they represent and to design equipment for particular purposes. Modern books on electronics, information theory, servo systems, etc., are fearsome. Classical radio texts, such as Scroggie's "Foundations," on which many of us were brought up, require a wide vision to encompass and have no bearing on chemistry. We cannot look for a Faraday to straddle the gamut of both fields, so we must wait for a chemist who has painfully explored the field of electronics to the point of expertise to take us by the hand and lead us through this territory, explaining in terms understandable to us the delights and possibilities of this most useful and necessary adjunct to our own science.

Professor Bair of Indiana University has made the essay in this book, and has been, on the whole, very successful. In parts, the writing is quite brilliant; in others it is a little difficult to decide whether any one with no previous acquaintance with the subject would follow and benefit. The author's disclaimer that "The book is not comprehensive, or even extensive . . ." disarms criticism, and his continuation ". . . but is felt to be a representative collection of topics . . ." may be judged from the contents. Introduction—Instrumentation Signals, effectively done: Chemical Signal Sources, an excellent interpretation, though the glass electrode on p. 100 is rather queer and the abjuration to observe the voltage at constant potential on p. 116 is startling: Operation of Electronic Instrument Components, a clear and simple treatment, capacitor symbols should have included electrolytics, the second paragraph on p. 120 requires study before it reads properly, and tube circuits give no indication of how the output is taken: The Oscilloscope, though much praised, is dismissed in seven pages: Non-linear Operations and Signals, a valuable discussion of trigger and gating circuits that omits the popular Müller - Lingane trigger: Analysis of Small-signal Electronic Circuits, deals with linear valve and transistor circuits, feedback, stability and frequency response: Noise, Bandpass and Information is a clear and useful treatment of sensitivity and error: Instrumentation Laboratory Practice treats in a down-to-earth and simple fashion the use of soldering iron and pliers, the design and layout of circuits, strays, shielding, earthing, trouble shooting and the general know-how of building and repairing equipment: Examples of Chemical Instrumentation illustrates the text by discussing the principles of certain instruments, such as an automatic-recording potentiometer, a potentiometric null spectrophotometer, an optical null spectrophotometer, a time-of-flight mass spectrometer, a controlled potential and derivative polarograph and chemical-modulation spectroscopy.

Some of the phraseology is not too happy, for example, a four terminal network with three terminals (p. 213) is not immediately comprehensible, there are a number of errors, *e.g.*, the slope of 0·05195 for the glass electrode (p. 100), and the proof reading has missed several mistakes such as Bed for Bedi on p. 209. Nevertheless, the good features of this book heavily outweigh the bad. It is virtually the first in a wide open field and should be eagerly welcomed by those who seek to understand the instruments they use as well as by those who seek intelligently to design equipment to fit their own purposes. The unfortunate but inevitable price is offset by the excellent production, and this is a worthy addition to the publishers' series in advanced chemistry.

E. BISHOP

Publications Received

NAMING ORGANIC COMPOUNDS: A GUIDE TO THE NOMENCLATURE USED IN ORGANIC CHEMISTRY. By E. H. TINLEY, B.Sc., F.P.S. Pp. vi + 48. London: Alchemist Publications. 1962. Price 12s. 6d.

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PRACTICAL CLINICAL BIOCHEMISTRY. By HAROLD VARLEY, M.Sc., F.R.I.C. Third Edition. Pp. viii + 689. London: William Heinemann Medical Books Ltd.; New York: Interscience Books Inc. 1962. 50s.

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ENCYCLOPAEDIC DICTIONARY OF PHYSICS. Volume 4. Editor-in-chief: J. THEWLIS. Pp. x + 836. Oxford, London, New York and Paris: Pergamon Press. 1961. Price per set of 9 volumes £106; $298.

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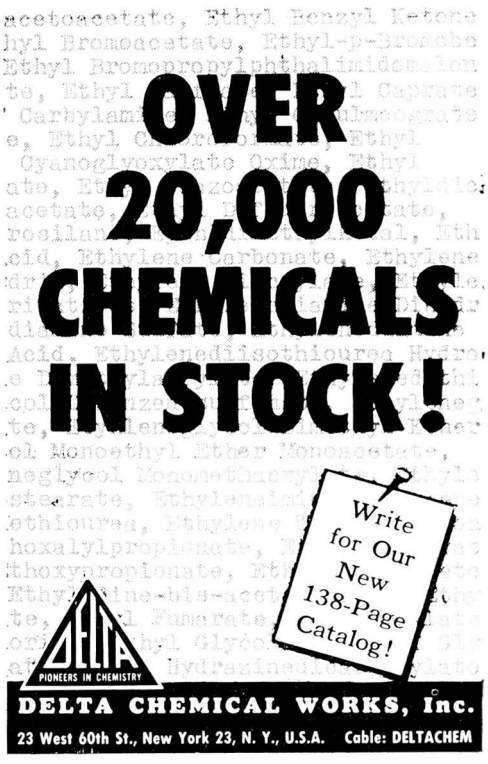

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