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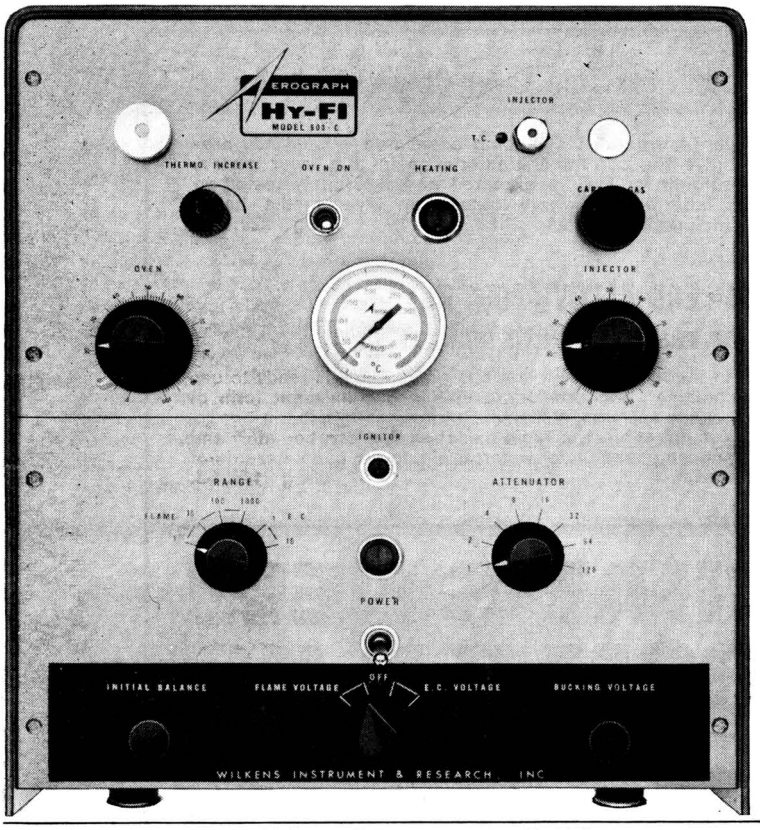

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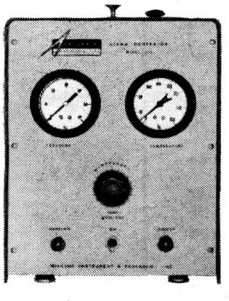

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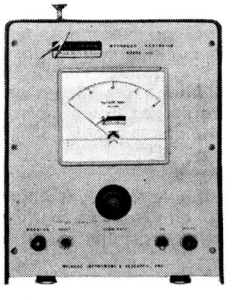

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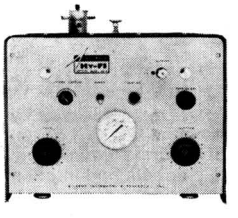

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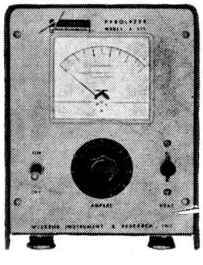

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THE ANALYST

PROCEEDINGS OF THE SOCIETY FOR ANALYTICAL CHEMISTRY

ORDINARY MEETINGS

AN Ordinary Meeting of the Society was held at 3 p.m. on Thursday, November 7th, 1963, in the Wellcome Building, Euston Road, London, N.W.1.

The subject of the meeting was "Gas Analysis." At the afternoon session the Chair was taken by the President, Dr. D. C. Garratt, Hon. M.P.S., F.R.I.C., and the following papers were presented and discussed: "Some Recent Developments in Gas Chromatography and Infrared Gas Analysis," by D. W. Hill, M.Sc., Ph.D., F.Inst.P., A.M.I.E.E., A.R.I.C.; "Some Problems Associated with the Analysis of Town Gas by Means of Gas Chromatography," by G. R. Boreham, B.Sc., A.R.I.C.; "Some Methods for Moisture Determination in Gases," by J. H. Scawin; "The Use of the Electrolytic Hygrometer for the Determination of Moisture in Gases," by J. E. Still, B.Sc., F.R.I.C. The Chair at the evening session was taken by Dr. S. G. Burgess, B.Sc., F.R.I.C., F.Inst.Pet., and the following papers were presented and discussed: "Sonic Gas Analysers—Uses and Limitations," by A. E. Martin, Ph.D., D.Sc.; "Some Electrochemical Methods of Gas Analysis," by J. H. Glover, B.Sc., F.R.I.C.; "The Use of a Slow Injection Syringe for Calibration in the Volumes per Million Range," by H. F. Downing, B.Sc.; "Apparatus for Handling Gas Samples and for the Preparation of Gas Mixtures of Known Composition," by J. E. Still, B.Sc., F.R.I.C.; "Dispersive and Non-dispersive Infrared Analysers for In-line Measurements," by A. W. Hough-Grassby, B.Sc., Ph.D.

AN Ordinary Meeting of the Society was held at 7 p.m. on Wednesday, December 4th, 1963, in the Meeting Room of the Chemical Society, Burlington House, London, W.1. The Chair was taken by the President, Dr. D. C. Garratt, Hon.M.P.S., F.R.I.C.

The subject of the meeting was "Differential Thermal Analysis" and the following papers were presented and discussed: "Differential Thermal Analysis and its Application to Soil and Plant Materials," by R. C. Mackenzie, D.Sc., Ph.D., F.R.I.C., F.G.S., F.R.S.E. (presented by B. D. Mitchell, B.Sc., A.R.I.C., and followed by a short film dealing with the method of differential thermal analysis as it is performed at the Macaulay Institute); "The Use of Differential Thermal Analysis in the Mineralogical Investigations of Building Materials," by H. G. Midgley, M.Sc., Ph.D., F.G.S.; "The Application of Differential Thermal Analysis to Polymeric Materials," by D. E. Eaves, B.Sc., Ph.D.

NEW MEMBERS

ORDINARY MEMBERS

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NORTH OF ENGLAND SECTION and PHYSICAL METHODS GROUP

A Joint Meeting of the North of England Section and the Physical Methods Group with the Modern Methods of Analysis Group of the Sheffield Metallurgical Association was held at 7 p.m. on Tuesday, October 22nd, 1963, in the Conference Room of the British Iron and Steel Research Association, Hoyle Street, Sheffield, 3. The Chair was taken by the Chairman of the Physical Methods Group, Dr. W. Cule Davies, F.R.I.C.

The following papers were presented and discussed: "The Determination of Oxygen in Metals by Fast-neutron Activation Analysis," by A. L. Gray, B.Sc.; "High-pressure Plasmas as Emission Sources," by S. Greenfield, L.I.M., I. L. Jones, D.L.C., A.R.I.C., and C. T. Berry, A.R.T.C.S., A.R.I.C. (see summaries below).

The Determination of Oxygen in Metals by Fast-neutron Activation Analysis

Mr. A. L. Gray described the technique of activation analysis and gave a short account of recent developments that made the technique practicable for routine plant analytical work. He discussed the convenience of the method for the determination of oxygen in metals by use of fast neutrons and described the development of a prototype automatic equipment, the "Analox."

The speaker described the assessment of the prototype instrument and discussed the correlation of the results obtained on a wide variety of samples with those by other methods, and outlined potentialities of the instrument. The paper concluded with a brief description of the fully developed version of the instrument and the considerations involved in its use in a steel works.

High-pressure Plasmas as Emission Sources

Mr. I. L. Jones described high-pressure thermal plasmas and compared them with low-pressure plasmas. He discussed the production of both direct-current arc and radio-frequency induced plasmas. He then described the application of plasmas as emission sources for spectrographs and flame spectrophotometers.

The speaker discussed the sensitivity and variance and the absence of interference from non-volatile compounds (as compared with flame sources) of plasma sources on flame spectrophotometers. He mentioned future applications of these sources, including the possibility of the use of direct calibration methods (obviating the need for internal standards) in emission analysis and the introduction of powdered samples into the plasma.

NORTH OF ENGLAND SECTION

An Ordinary Meeting of the Section was held at 2.15 p.m. on Saturday, November 23rd, 1963, at the Old Nag's Head Hotel, Lloyd Street, Manchester. The Chair was taken by the Chairman of the Section, Mr. C. J. House, B.Sc., A.R.C.S., F.R.I.C.

The following paper was presented and discussed: "The Chemistry of Wines and Spirits," by E. C. Barton-Wright, D.Sc., F.R.I.C., M.I.Biol.

SCOTTISH SECTION

An Ordinary Meeting of the Section was held at 7.15 p.m. on Friday, November 8th, 1963, at the Royal Society of Edinburgh, George Street, Edinburgh. The Chair was taken by the Chairman of the Section, Dr. R. A. Chalmers.

The following paper was presented and discussed: "Atoms and the Analyst—Some Confessions of an Unqualified Chemist," by J. M. A. Lenihan, M.Sc., Ph.D., A.M.I.E.E., F.Inst.P.

A JOINT Meeting of the Scottish Section with the Glasgow Section of the Society of Chemical Industry was held at 6 p.m. on Friday, November 22nd, 1963, at the Royal College of Science and Technology, Glasgow. The Chair was taken by the Chairman of the Scottish Section, Dr. R. A. Chalmers.

The following papers were presented and discussed: "The Presentation of Scientific Papers," by J. D. Nisbet, M.A., B.Ed., Ph.D., F.B.P.S.; "Editing a Scientific Journal," by J. B. Attrill, M.A., F.R.I.C.

MIDLANDS SECTION

AN Ordinary Meeting of the Section was held at 7 p.m. on Thursday, November 14th, 1963, at the Lanchester College of Technology, Cox Street, Coventry. The Chair was taken by the Chairman of the Section, Mr. W. H. Stephenson, F.P.S., D.B.A., F.R.I.C.

The following paper was presented and discussed: "Some Observations on the Use of Ion Exchange in Analytical Chemistry," by J. E. Salmon, B.Sc., Ph.D., F.R.I.C.

PHYSICAL METHODS GROUP

A SPECIAL General Meeting of the Group was held at 6.30 p.m. on Tuesday, September 10th, 1963, in the Meeting Room of the Chemical Society, Burlington House, London, W.1. The Chair was taken by the Chairman of the Group, Dr. W. Cule Davies, F.R.I.C.

Mr. S. G. E. Stevens was elected Honorary Auditor in place of Dr. D. C. Garratt, who had ceased to be eligible on being elected to the Presidency of the Society.

BIOLOGICAL METHODS GROUP

AN Ordinary Meeting of the Group was held at 7 p.m. on Wednesday, October 23rd, 1963, in the Meeting Room of the Chemical Society, Burlington House, London, W.1. The Chair was taken by the Chairman of the Group, Mr. W. A. Broom, B.Sc., F.R.I.C.

The subject of the meeting was "Assay of Virus Vaccines" and the following papers were presented and discussed: "The Control Testing of Vaccines," by D. I. Magrath, B.A., Ph.D.; "Manufacture and Control of Poliomyelitis Vaccine," by A. J. Beale, M.D., Dip.Bact.

Thermogravimetric Analysis

A Review*

BY A. W. COATS AND J. P. REDFERN

(*Chemistry Department, Battersea College of Technology, London, S.W.*11)

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Introduction
Techniques
Kinetic studies
Apparatus
Applications—
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THERMAL methods of investigation, generally referred to as thermo- or thermal analysis or thermo-analytical techniques, have found wide application in recent years.[1] These may be defined as experimental methods for characterising a system (element, compound or mixture) by measuring changes in physico-chemical properties at elevated temperatures as a function of increasing temperature.[2] The two chief methods are (*a*) differential thermal analysis,[3] in which changes in "heat content" are measured as a function of increasing temperature and (*b*) thermogravimetric analysis, in which changes in weight are measured as a function of increasing temperature. Other methods that come within this definition involve the use of changes in gas volume or pressure; changes in solid volume; changes in electrical resistance; changes in ultraviolet, visible or infrared transmission or reflectance.[4]

The two techniques, (*a*) and (*b*), provide information relating to certain physical and chemical phenomena, which are listed below—

Physical Phenomena	Chemical Phenomena
Crystalline transition	Chemisorption
Second-order transition	Desolvation (especially dehydration)
Fusion	Decomposition
Vaporisation	Oxidative degradation
Sublimation	
Absorption	Solid-state reactions
Adsorption	Solid - gas reactions (*e.g.*, oxidation or reduction)
Desorption	

The basic instrumental requirements for thermogravimetric analysis are a precision balance and a furnace that is programmed for a linear rise of temperature with time. Thermogravimetry can provide information on all the phenomena listed above, except crystalline transitions, fusions and those solid-state reactions that occur without change in weight.

Although information can be obtained by carrying out the weighing operations manually, nowadays automatic continuous recording of the weight and temperature are usual; the continuous record of weight and temperature ensures that no features of the weight loss - temperature curve are overlooked. The results from a thermogravimetric run may be presented by—

(*i*) weight (corrected weight—see below on "Corrections", p. 908), *versus* temperature (or time) curve, referred to as the thermogravimetric curve, see Fig. 1 (*a*), or

(*ii*) Rate of loss of weight *versus* temperature curve, referred to as the differential thermogravimetric curve, see Fig. 1 (*b*).

In (*i*), the weight axis may be scaled in one of several ways, *e.g.*, (*a*) as a true weight scale, (*b*) as a percentage of the total weight, (*c*) as a percentage of the total weight loss or as a fraction

* Reprints of this paper will be available shortly. For details, please see p. 984.

of the total weight lost, (*d*) in terms of molecular-weight units, or (*e*) expressed in terms of α (where α = fraction decomposed). (When the molecular weight of the compound is known, method (*d*) affords a convenient method of plotting the results, since it is easy to extract data relating to lost fragments, for example, in the study of an inorganic complex—it also forms an easy method of comparison of a family of such compounds.) The use of method (*e*) should be limited to a single-stage process, *i.e.*, a special instance of (*c*).

The following features of the thermogravimetric curve may be identified—

(i) A horizontal portion or plateau, which is indicative of constant weight.

(ii) A curved portion; the steepness of the curve is indicative of the rate of weight loss, and this will obviously pass through a maximum, giving an inflection with $\frac{dw}{dt}$ as a maximum. The shape of the curve is dependent on the variables discussed in the section on "Techniques" (*vide infra*).

(iii) An inflection (at which $\frac{dw}{dt}$ is a minimum, but not zero) may imply the formation of an intermediate compound. It may, however, be due to disturbances in the heating rate or in thermocouple response. It is necessary to ensure a regular rise of temperature,[5] and therefore it is desirable to have an independent temperature - time record to ensure the reliability of the results.[6]

The portion of the differential thermogravimetric curve lying on the line $\frac{dw}{dt} = 0$, see Fig. 1 (*b*), is equivalent to the horizontal portion of the thermogravimetric curve. The peak of the differential thermogravimetric curve corresponds to the curved portion of the thermo-

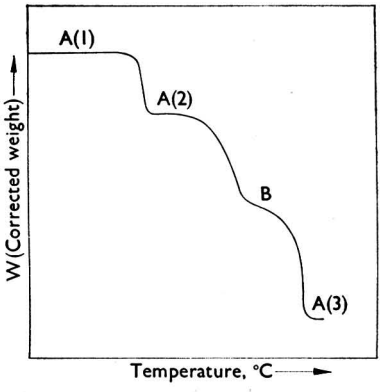

Fig. 1(*a*). Thermogravimetric curve: A (1), (2) and (3) are plateaux in the decomposition curve of the material. B is a point of reflexion (at which $\frac{dw}{dt}$ is a minimum)

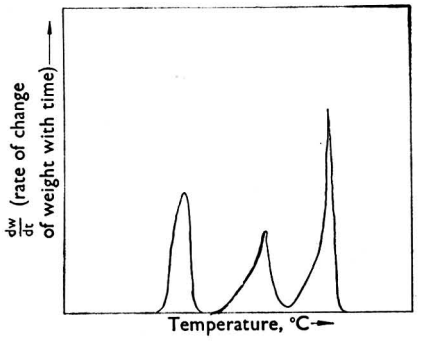

Fig. 1(*b*). Differential thermogravimetric curve: A (1), (2) and (3) correspond to the plateaux on the thermogravimetric curve (at which $\frac{dw}{dt}$ is zero). Trough B corresponds to the point of inflexion on the thermogravimetric curve (at which $\frac{dw}{dt}$ is a minimum)

gravimetric curve, whereas the peak maximum of the former is identical with the point of maximum slope of the latter. A trough on the differential thermogravimetric curve corresponds to an inflection at which $\frac{dw}{dt}$ is a minimum on the thermogravimetric curve. The height of the trough above the line $\frac{dw}{dt} = 0$ affords some measure of the stability of the intermediate and the extent to which the two consecutive reactions (corresponding to the peaks on either side of the trough) overlap. The differential thermogravimetric curve offers certain advantages over the thermogravimetric curve in the matter of presentation. Features in the thermogravimetric curve that are not readily discerned are more clearly seen in the differential thermogravimetric curve, *e.g.*, any change in the rate of weight loss may be seen immediately

as a trough, indicating two consecutive reactions, or as a shoulder to the peak, indicating two almost overlapping reactions or as a tail to a peak, which is probably an indication of strong adsorption of the volatile product on the new solid phase.[7] Differential thermogravimetric curves often show considerable similarity to differential thermal analysis curves or permit a ready comparison to be made.[8,9,10]

The use of thermogravimetric results for evaluating thermal stability has focused attention on finding a workable definition for a suitable standard for describing decomposition. The temperature at which a reaction begins in any particular thermobalance run is dependent on many variables, of which the rate of heating is perhaps the most important. This temperature is neither a true decomposition temperature, below which the reaction rate is zero, nor is it a transition temperature.[11] In fact, there is often little correlation between results from isothermal runs and non-isothermal runs.[5] Newkirk[12] argues that knowledge of this temperature is useful and the term "procedural decomposition temperature" used by Doyle,[13] in his polymer studies, stresses the dependance of this temperature on the powerfully influential procedural details. Pellon[14] in his work on the stability of phosphorus-containing polymers uses a temperature T_{10} (a temperature at which the cumulative weight change reaches 10 per cent.) as a means of defining thermal stability. A comparison of decomposition temperatures has often led to controversy owing to a lack of appreciation that the value is a function of method, apparatus and procedure. A clear statement of conditions used should be stated when quoting decomposition temperatures. Guiochon[11] comments: "Thermogravimetric measurements are easy to carry out, but somewhat difficult to account for."

A survey of the history of thermogravimetric analysis up to 1940 has recently been published by Duval,[15] in which he traces the design and construction of the first thermobalance back to Nernst and Riesenfeld[16]; Wache[17] has written an article on Chevenard. The second edition of Duval's book, which is now available,[18] is divided into a discussion of (*a*) "The Thermobalance" and (*b*) "The Thermolysed Substances." Review articles have been published on recording balances and instrumentation[19,20] and on thermogravimetry.[21 to 27] Several symposia have been held, notably in the United States.[28,29] The inception of the first Thermoanalysis Institute held at the Fairleigh-Dickinson University, New Jersey,[30] occurred in 1962. A survey of literature, published about four times a year, commenced in May, 1962.[1]

Techniques

This section is concerned with two aspects of thermogravimetry. The first arises from the dynamic nature of the method, and the second is concerned with the factors influencing the shape of the thermogravimetric curve, and these must be taken into account to obtain meaningful and reproducible results, so that thermogravimetric data on different compounds can confidently be compared.

Corrections—

If a crucible, that is known not to change weight, is heated when empty, there is, generally, an apparent change in weight with increasing temperature (see Fig. 2), *e.g.*, a

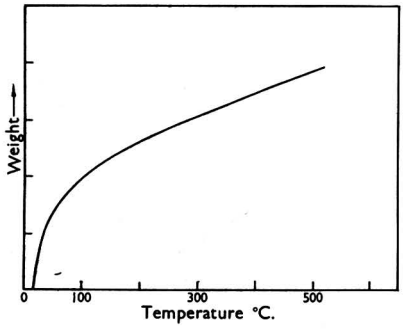

Fig. 2. Typical correction curve

crucible weighing 2 g would be expected to have an apparent change in weight at 1000° C of, say, 0·1 per cent. of the crucible weight; this would amount to an apparent change in weight of 2 per cent. if the sample weighed 0·1 g. This apparent weight change is caused by the interplay of a complex combination of several factors, such as air buoyancy, convection effects within the furnace, crucible geometry, radiation effects, the atmosphere in the furnace and the fact that the crucible support is subject to a temperature gradient within the furnace. It is necessary to use the apparent weight change of the empty crucible as a correction curve to arrive at the actual weight change occurring in a sample. This is considered to be more satisfactory than the use of a tare crucible, as has been proposed,[31] in a separate furnace, in view of the possible non-uniformity of the hot zones in the two furnaces.

Since several factors contribute to this correction curve it is found that the corrections to be applied differ with different rates of heating. Various workers[12,32,33] have discussed the factors affecting the correction curves, and Duval[34] and Newkirk[12] have suggested the use of vents at the top of the furnace to reduce the size of the correction. This has been criticised[32] as amounting to critical damping of the hot-zone volume. Empirical equations have been derived[35,36,37] for the correction curves, but because of the complexity of factors involved it seems simpler to use a correction curve determined practically under conditions identical to those of the actual experiment.

FACTORS AFFECTING THE RESULTS OBTAINED—

There are several factors that may, to a greater or less extent, influence the shape of a corrected thermogravimetric curve for a particular compound.

Heating rate—For a single-stage endothermic reaction, where T_i is the procedural decomposition temperature and T_f is the temperature at which the reaction is completed, it has been shown[5,38,39,40] that—

(*i*) $(T_f)_f > (T_f)_s$,

where the subscripts f and s refer to a fast and slow rate of heating, respectively,

(*ii*) $(T_i)_f > (T_i)_s$ and

(*iii*) $(T_f - T_i)_f > (T_f - T_i)_s$.

Newkirk[12] has shown that, at any given temperature, the extent of decomposition is greater at a slow rate of heating than for a similar sample heated at a faster rate. If the reaction involved is exothermic, the sample temperature will rise above the furnace temperature; it has been shown (in unpublished work by us) that the difference between the furnace temperature and the sample temperature is greatest for the faster rate of heating when a reaction is occurring. When successive reactions are involved, the rate of heating may well determine whether or not these reactions will be separated. The appearance of a point of inflection in the thermogravimetric curve at a fast heating rate may resolve into a plateau at a slower heating rate.[23] The importance of heating rates was stressed by early workers[41,42] and has been much studied.[43 to 47]

Sample—The sample weight can affect the thermogravimetric curve in three ways—

(*i*) The extent to which endo- or exothermic reactions of the sample will cause the sample temperature to deviate from a linear rise; in general, all other factors being equal, we have found that the greater the weight of sample, the greater will be the deviation.

(*ii*) The degree of diffusion of the product gas through the void space around the solid particles; under static conditions, the environmental atmosphere (*i.e.*, the atmosphere immediately surrounding the reacting particles) will be somewhat governed by the bulk of the material in the crucible.

(*iii*) The possible unevenness of the temperature throughout the sample, particularly if it has a low thermal conductivity.

Thus the use of as small a weight of sample as possible, within the limits of the sensitivity of the balance, is to be preferred.

The state of sub-division of the sample is important. The use of large crystals may result in spitting, as described by C. J. Keattch in a private communication. The sample may foam and bubble.[48] Most thermogravimetric studies have been carried out on powders, and the effect of particle size or surface area has been widely studied.[41 to 52] The smaller the

particle size the greater the extent to which equilibrium is reached, and at any given temperature the extent of decomposition is greater.

The pre-history of the sample is also important. Wendlandt[53] has discussed the effect of precipitation technique on the thermogravimetric curve of ammonium molybdophosphate. Recent work on magnesium hydroxide from different sources provides a good illustration of this factor.[54]

Crucible—The geometry of the crucible used will profoundly affect the shape of the thermogravimetric curve. Crucible design has been studied,[55,56] and the use of a crucible fitted with a piston (where reactions take place under a pressure of 1 atmosphere of the liberated gas) or an on open tray (at the partial pressure of the gas in the atmosphere) have been compared. This latter arrangement has been used by other workers.[57,58] The design of a crucible to overcome possible condensation on the support rod or decrepitation before decomposition has also received attention.[59,60] In certain instances the material of which the crucible is constructed affects the decomposition pattern.[61]

Atmosphere—

(*i*) *When the atmosphere does not take part in the reaction*—The use of an inert atmosphere in thermogravimetric analysis has been discussed.[59] The rôle of the gas is to remove gaseous products from the vicinity of the sample, to ensure that the environmental atmosphere is kept as constant as possible throughout the experiment and to prevent reaction occurring between the sample and air as normally employed,[62,63] or between the volatile components and the atmosphere.[5,59] Other workers[64 to 68] have used vacuum conditions to achieve the same results. The dependence of the shape of thermogravimetric curves on the pressure of the gas in the reaction chamber has been studied.[69]

(*ii*) *When the atmosphere is involved in the reaction*—Some work has been carried out with atmospheres either of humidified air or high pressure steam[12,70,71]; a dynamic air atmosphere has been used to study the roasting of copper sulphide[43] and the oxidation of reduced iron catalyst.[72] A comparison of some six atmospheres in metal - gas reactions has been made by Markowitz and Boryta.[73,74] Reducing atmospheres, *e.g.*, hydrogen, have been used.[75,76,77] Other atmospheres, in which reactions occur between the gas and the sample, that have been studied include hydrogen sulphide[78] and carbon dioxide.[79]

Kinetic studies

Thermogravimetric data can be used to evaluate kinetic parameters of reactions involving weight loss (or gain) of the following four types—

$$A_s \longrightarrow B_s + C_g \text{ [80,81]}$$
$$A_s + B_s \longrightarrow C_s + D_g$$
$$A_s + B_g \longrightarrow C_s \text{ [73]}$$
$$A_s \text{ or } A_l \longrightarrow A_g \text{ [82]}$$

The advantages of determining kinetic parameters by thermogravimetric methods rather than by conventional isothermal studies are (*i*) considerably less data are required than in the isothermal method, (*ii*) the kinetics can be probed over an entire temperature range in a continuous manner and (*iii*) when a sample undergoes considerable reaction in being raised to the required temperature, the results obtained by an isothermal method are often questionable. To these reasons may be added the advantage of using one single sample in the study.

It is important to know the temperature accurately and to ensure that endo- or exothermic effects do not cause the rate of heating to depart from its constant value.[73] The use of small samples, within the limits of sensitivity of the balance, is therefore necessary. It should be remembered that kinetic parameters derived from thermogravimetric experiments are dependent on the procedural details, *e.g.*, crucible shape and material, particle size, prehistory of sample and heating rate.[47,54]

The earliest attempt to use thermogravimetric curves for kinetic data appears to have been made by Van Krevelen, Van Heerden and Huntjens.[83] They derived an approximate equation, from which it was possible by graphical methods to determine the order of reaction (they differentiated between orders of 0, 1 and 2). They studied the pyrolysis of coal and showed that the primary decomposition was first order. On this assumption they developed graphical methods for determining the activation energy and frequency factor, knowing the rate of heating, temperature of maximum rate of decomposition and the half-value width of the differential thermogravimetric curve.

Kaesche-Krischer and Heinrich[47] used a similar approach in their study of the pyrolysis of poly(vinyl alcohol) in a vacuum, and Schnitzer, Turner and co-workers[84,85] studied the organic matter of Podzol soils. Turner, Hoffman and Chen[54] modified Van Krevelen's approach to accommodate a two-thirds order in their work on the thermal decomposition of magnesium hydroxide.

Freeman and Carroll[80] derived the equation—

$$-\frac{E/2{\cdot}3\mathrm{R}\Delta T^{-1}}{\Delta \log W_r} = -x + \frac{\Delta \log(dw/dt)}{\Delta \log W_r} \quad .. \quad .. \quad .. \quad (1)$$

where E = activation energy, R = general gas constant, T = absolute temperature, x = order of reaction and $W_r = (W_c - W)$, where W = weight loss at time t, and W_c = weight loss at completion of the reaction. This equation was derived by assuming a rate expression—

$$-\frac{dX}{dt} = kX^x \quad .. \quad .. \quad .. \quad .. \quad .. \quad (2)$$

where X = amount of A at time t and the rate constant, k, is given by the simple Arrhenius expression—

$$k = Ze^{-E/RT} \quad .. \quad .. \quad .. \quad .. \quad .. \quad (3)$$

where Z = frequency factor. Either by plotting a graph of $\frac{\Delta \log(dw/dt)}{\Delta \log W_r}$ *versus* $\frac{\Delta T^{-1}}{\Delta \log W_r}$ or by other suitable rearrangement of equation (1)[64,81,86] it is possible to derive values for both E and x.

This derivation has been used to study several dehydration reactions.[7,58,80,81,87 to 91] Barrer and Bratt[92] have used an adaptation of equation (1) in their study of non-stoicheiometric hydrates. The decomposition reactions of calcium oxalate,[80,81] thorium, lanthanum and uranium oxalates,[93] calcium carbonate,[80,81] chromium arsenate[58] and 12-tungstomanganic acid[94] have all been studied. Bear and Wendlandt[95] have studied the effect of added salts on the decomposition of trisethylenediamine and trispropylenediamine chromium^{III} chlorides and thiocyanates. Similarly, the pyrolysis of several polymers has been studied[64,86,96,97] However, several disadvantages appear to exist in the use of this derivation—

(*i*) in most practical instances the derivation seems to apply to an extremely limited portion of the decomposition curve;

(*ii*) there is considerable difficulty in obtaining a reliable value for the order of reaction;

(*iii*) none of the other methods of applying the derivation seems to overcome the difficulties; and

(*iv*) in certain reactions the method does not seem to yield an answer at all[87]—this may be due to the reaction itself and not inherent in the derivation.

In his method,[12] Newkirk assumes that all pyrolytic decompositions are first order, and his method involves calculating the first-order rate constants for a large number of temperatures, and then constructing the Arrhenius graph. In his study of Mylar (a plastic marketed by Du Pont Company Ltd.) he showed that the slope of the graph was independent of heating rate, but, as the rate of heating was increased, the graphs were displaced towards lower values of $1/T$, compared with the results obtained from isothermal studies. Smith[98] has used a similar approach to derive E_p (E_p denotes that E, the activation energy, may be somewhat dependent on the experimental procedure) for various polymers.

Other workers[44] have determined apparent activation energies by plotting the reciprocal of the final decomposition temperature against the logarithm of the heating rate.

Doyle[82] integrated a combination of expressions equivalent to equations (2) and (3), using an approximation. His technique is essentially one of curve fitting. He studied the zero-order volatilisation of liquid octamethylcyclotetrasiloxane and the first-order pyrolytic volatilisation of polytetrafluoroethylene. He confines himself to zero- or first-order reactions, but his approach cannot be used when the order is unknown.

Horowitz and Metzger[99] have recently developed another approach based on integration of a combination of expressions equivalent to equations (2) and (3) by an exact integral, using the substitution $(T - T_s) = \theta$ where T_s is the temperature at which $\frac{W}{W_o} = \frac{1}{e}$, ($W$ = weight of sample at time t, and W_o is the initial weight of the sample). The theory

shows that a plot of ln (ln $\frac{W_0}{W}$) *versus* θ should be a straight line of slope $\frac{E}{RT_s^2}$. These workers have studied four polymers and the dehydration of calcium oxalate monohydrate. In each reaction they assume a particular order of reaction. For an unknown order they suggest the use of the position of maximum rate since this is governed by the order. It is, however, not always easy to determine the position of maximum rate with accuracy.

Coats and Redfern[100] used a different approximation for integrating a combination of equations (2) and (3). The graph of

$$\log \frac{1 - (1 - \alpha)^{1-n}}{(1 - n)T^2} \textit{ versus } \frac{1}{T}$$

is a straight line of slope $- E/2{\cdot}3R$ for the correct value of n (α = fraction decomposed and n = order of reaction).

The activation energy can have a real meaning in solid-state kinetics, corresponding to the rate-determining step or steps, which might be the diffusion of the gaseous product out of the solid, or the transport of a particular ion, or the breakage of bonds. The order of reaction cannot presumably have the meaning attributed to it, as in a gas reaction, and may best be considered as a mathematical factor in the derived equations. However, geometric models of solid systems that lead to orders of reaction of $\frac{1}{2}$ and $\frac{2}{3}$ can be set up[101]; orders of 0 and 1 can also be justified.

Two points remain. The first is a plea for the use of standard symbols in the description of kinetic processes (see, for example, Garner[102]). The second is that it seems unlikely that the simple rate expression $\frac{d\alpha}{dt} = k(1 - \alpha)^n$, from which all the derivations quoted are ultimately derived, will be applicable to all solid-state decomposition reactions.

The use of computational methods for analysing thermogravimetric data is probably in wider use than the number of papers on this subject would suggest.[100,103] The advantages that these methods possess in assisting in the evaluation of kinetic parameters are obvious; their more widespread introduction will undoubtedly lead to a better coverage of some of the necessary studies on the effect of the variables, listed under "Techniques," on kinetic parameters.

Apparatus

The comprehensive review of Gordon and Campbell[19] covered the whole field of automatic and recording balances up to 1959. Many of these are directly applicable or readily adaptable for use in thermogravimetry. Lewin[20] discusses six makes of commercial thermobalances on the market in 1962 and lists another four firms manufacturing thermobalances. Duval[18] puts the number of thermobalance models on the market at fifty-two. Other firms[104,105,106] known to us are manufacturing thermobalances.

The essential components of a modern thermobalance are: (*i*) balance, (*ii*) furnace, and (*iii*) recorder. It is desirable to have a reaction chamber to permit work to be carried out under a wide variety of conditions, *e.g.*, inert, oxidising or reducing atmospheres or under vacuum, and to permit gas analysis to be carried out. The balances used can be grouped into two types. They are the null-point and the deflection types of instruments. The former incorporates a suitable sensing element that detects any deviation of the balance beam and the application of a restoring force, proportional to the change in weight, to return the beam to its original null-point. This restoring force is then recorded either directly or through a transducer. These null-point type instruments are often readily adaptable to working under vacuum conditions. Deflection instruments, *e.g.*, based on a conventional analytical balance, a helical spring, a cantilever beam, a strain gauge or a torsion balance, involve the conversion of deviations into a record of the weight change. The principles used in detecting and restoring deviations (in null-point balances), and in recording the changes in weight have been fully discussed.[19]

Furnace design and control is of great importance; it must be designed to provide a suitable smooth input so that it can maintain a linear heating programme or a fixed temperature, independent of any changes in external conditions. The comments on temperature control in differential thermal analysis[3] apply equally to thermogravimetric analysis. Control is generally achieved via a thermocouple situated as close to the furnace winding as possible.

The appearance of thermal pulsing effects owing to the periodic application of relatively large power increments leads to a ragged weight record,[32] and the use of some form of stepless control of power supply to the furnace through a saturable reactor is to be preferred.[107] Nichrome winding permits a maximum temperature of around 1100° C; platinum - rhodium winding permits a maximum of around 1450° C. Higher temperatures can be achieved by using a graphite tube furnace, but the associated control and measurement of temperatures raise considerable problems.

The recording system should be able to record both temperature and weight continuously and to make a periodic record of the time. The use of "$X_1 - X_2$"[105] or side-by-side[108] recording is to be preferred to the use of "X — Y" recording,[5] since these methods provide an independent record of both temperature and weight.

The prospective user has a variety of instruments available over a wide cost range. His choice should reflect the requirements of his particular field of study and the desiderata for good thermobalance design (see, for example, Lukaszewski and Redfern[23]), namely that—

(*i*) The thermobalance should be capable of continuously registering the weight change of the sample studied as a function of temperature (and time).

(*ii*) The furnace should be capable of reaching the maximum temperature desired. Most commercial instruments are marketed with a maximum of around either 1100° or 1500° C (some thermobalances are available and have been designed to work up to 2200°[105] or 2700° C[109]).

(*iii*) The rate of heating is truly linear and is reproducible.

(*iv*) The hot zone of the furnace is as near uniform as possible, and that the crucible is always located within this hot zone. This is particularly important in deflection instruments, since the crucible will move in relation to its initial position in the hot zone during the course of the run.

(*v*) The instrument is as versatile as possible, providing for—

- (*a*) variation in heating rate,
- (*b*) heating in a dynamic flow of a controlled atmosphere, inert, oxidising or reducing,
- (*c*) heating *in vacuo*,
- (*d*) variation in chart speed to aid interpretation and
- (*e*) the possibility of carrying out accurate isothermal studies.

(*vi*) Physical effects (*e.g.*, radiation and convection currents, and magnetic effects caused by the winding of the furnace) due to the functioning of the apparatus do not upset the balance mechanism. No interaction should occur between conducting or magnetic samples being studied and the furnace winding. Possible chemical attack, from the gases used or the gases evolved, can be eliminated either by the design of the reaction chamber or by the materials used. The balance mechanism is sufficiently protected from the furnace, so that its sensitivity remains constant throughout the whole of the experimental run.

(*vii*) The temperature of the sample is measured as accurately as possible.

(*viii*) The sensitivity of the balance mechanism allows the study of small samples.

Several workers have described the construction of relatively simple inexpensive instruments, *e.g.*, those suitable for teaching the principles of the subject or for preliminary studies.[22,110,111,112] Micro-recording,[113,114] vacuum or controlled-atmosphere[112,114,115,116] thermobalances have been described in detail. Recent papers, in which automatic thermobalances[117 to 121] are described, include details of models capable of operating up to 40[117] and 60[118] atmospheres. In view of the trends in current instrument design reviewed by Eastwood,[122] the development of an automatic digital-recording thermobalance is of great interest.[123] There is considerable activity in the development of instruments capable of performing both thermogravimetric and differential thermal analysis either on the same sample or under similar conditions.[46,107,124,125,126] However, it should be borne in mind that the conditions for producing meaningful results are not necessarily the same for both techniques, although comparison is often valuable and, in some instances, essential.

Several workers have described modifications to existing commercial recording balances or thermobalances. Thus the Ainsworth recording balance has been incorporated in a thermobalance design[31]; the Stanton thermobalance has been adapted for work in atmospheres of hydrogen[127] and for producing the differential thermogravimetric curve.[10]

It is possible to measure the sample temperature directly without affecting the performance of a conventional balance by means of extremely fine connecting wires (of about 0·001-inch diameter) between the balance arm and a suitably located terminal block. The thermocouple wires then run up the support rod the crucible, and the bead may be located in or close to the sample, depending on the design of the crucibles used.[46,108]

Applications

Analytical chemistry—

The widest application of thermogravimetric analysis to date has been in the investigation of analytical procedures: (*i*) in investigating suitable weighing forms for many elements; (*ii*) in testing materials that are actual or potential analytical standards; and (*iii*) in the direct application of the technique to analytical determinations.

Kobayashi[128] has reviewed the work of the Japanese School from 1925 to 1940 in the field of gravimetric analysis; from some twenty-seven references he listed about 300 precipitates and gave the recommended drying temperatures. In the first edition of his book,[129] Duval reported his studies on over 1000 gravimetric precipitates for nearly 70 elements. He concluded that only about 200 of these are suitable weighing forms for the elements. Erdey's book[130] on gravimetric analysis includes thermogravimetric, differential thermogravimetric and differential thermal analysis curves obtained on the Derivatograph[9] for each weighing form discussed. Two reviews have been published.[131,132]

There has been criticism of some of Duval's work on a number of grounds. Differences between other published work and that of Duval and his co-workers has been ascribed to different precipitating techniques,[53] to different washing techniques,[133] and to the use of high rates of heating.[134] Duval's criterion for rejecting a particular gravimetric precipitate as not being a suitable weighing form was that it did not give a plateau when heated at one particular rate of heating. Newkirk[12] refers to the investigation of zinc monosalicylaldoxime in which de Clercq and Duval,[135] using the method of Flagg and Furman[136] to prepare the precipitate, found no plateau on the thermogravimetric curve corresponding to the anhydrous compound. Detailed work by Rynasiewicz and Flagg[137] showed that the anhydrous form was stable from ambient temperature up to 285° C; further, that the plateau depended on the initial water content. Newkirk continues: "In as much as Duval has used a higher heating rate (ca. 380° C per hour), it is perhaps not surprising that he failed to find a plateau. The rejection of this precipitate as an analytical method for the determination of zinc on the grounds that it does not give a stable horizontal in the thermobalance at one particular rate of heating is clearly unwarranted. . . . The results suggest that when the thermobalance is used to study the drying of bulky precipitates that contain considerable water, it would be well to use very slow rates of heating."

The use of thermogravimetric data to interpret the best drying temperature (a constant temperature) must be made with a clear understanding of the dynamic nature of the technique. Regard must also be paid to all the factors mentioned in the section on "Techniques." It is clear that failure to do this may lead to considerable difficulty and controversy. To summarise—

(*i*) The lack of the appearance of a plateau, at one particular heating rate, is insufficient evidence on which to judge the suitability or otherwise of a particular weighing form.

(*ii*) The appearance of a plateau is not conclusive evidence that the weighing form is isothermally stable at all or any of the temperatures that lie on the plateau.[5]

(*iii*) It is evident that the most reliable information will be gained by using several different heating rates or, at least, a slow rate of heating, possibly with preliminary air drying.

However, there is much to be gained from a thermogravimetric investigation of a weighing form since the recommended temperatures for some procedures have been arrived at arbitrarily, and have frequently been quoted in the literature without any critical evaluation of the conditions required. Beamish and McBryde[138] have shown that certain instructions given

for the heating or ignition of materials in some gravimetric determinations were quite inadequate.

Recent investigations include the study of: ammonium molybdophosphate[53] and quinolinium molybdophosphate[139] as suitable weighing forms for phosphorus; several sulphides[140] and selenides[141] to confirm methods of drying and weighing; lanthanum carbonate[142] and lanthanum oxalate[143] to find suitable weighing forms for lanthanum; the use of *m*-nitrobenzoic acid as a precipitant for cerium^{IV} [144]; and various compounds of plutonium to find suitable weighing forms for it.[145,146] Picrates and styphnates of some organic bases, such as guanidine and related compounds, have been examined since these derivatives are constantly used in characterisation and analysis.[147]

Thermogravimetric studies have been made of many substances of use or of potential use as standards in analysis. Thus a series of papers by Duval has appeared on the use of this technique in conjunction with infrared spectra.[148,149] Investigations of potassium hydrogen phthalate,[150] ethylenediaminetetra-acetic acid (EDTA) and its derivatives,[151] some tetraphenylboron salts of oximes[152] and 5-substituted barbituric acids[153] have been carried out.

It has been shown that soils can be analysed thermogravimetrically for determining hygroscopic moisture, organic matter, and inorganic carbonates.[154,155,156] Dupuis and Dupuis[157] have used thermogravimetry for determining calcium and magnesium in dolomite rock. The use of thermogravimetry in the study of the composition of non-interacting binary mixtures has been outlined.[23] Erdey and co-workers,[158,159] using the Derivatograph, have determined calcium, strontium and barium in a single sample by their precipitation as the mixed hydrated oxalate, using the losses of weight of extraneous water and water of crystallisation, and loss of carbon monoxide and dioxide. Berlin and Robinson have used thermogravimetry in their determination of magnesium, potassium and lead with dilituric acid[160] and for determining ethylenediamine and quinine,[161] with the same precipitant. Fluorine has also been determined thermogravimetrically.[162]

INORGANIC CHEMISTRY—

In recent years the greatest number of papers on thermogravimetric analysis has appeared within the field of inorganic chemistry. In a review of this nature it is not possible to discuss the work in detail; however, an attempt has been made to collate recent work on the basis of the anion of the compound studied. Studies of inorganic compounds are concerned with their stability, decomposition and structure. The types of reaction that have been observed are—

(*i*) Loss of constituent water molecules; such dehydrations may be either single or multi-stage, and may involve the loss of the elements of water, *e.g.*, from hydroxyl groupings.

(*ii*) Decomposition reactions, which may be either of a disproportionation or of a degradation type. Brill,[163] in what was probably the first publication in the field of thermogravimetry, presented data of this degradation type. He studied the decomposition of magnesium, calcium and barium carbonates—

$$M^{II}CO_3 \longrightarrow M^{II}O + CO_2.$$

(*iii*) Degradation reactions specifically involving the atmosphere, for example, oxidative degradation. An example is provided by the oxidation in air of trispropylenediamine chromium^{III} chloride—

$$2[Cr(pn)_3]Cl_3 \xrightarrow{air} Cr_2O_3 + \text{volatiles.}^{164}$$

(*iv*) Loss of constituent volatile ligands from inorganic complexes. An example is provided by the loss of ethylenediamine from trisethylenediamine chromium^{III} chloride—

$$[Cr(en)_3]Cl_3 \longrightarrow [Cr(en)_2Cl_2]Cl + en.^{95}$$

Though it has been clearly demonstrated that the thermal decomposition of inorganic compounds is dependent on the nature of both anion and cation, there has been little attempt to relate thermal decomposition data to modern theories in inorganic chemistry. Wendlandt[165] found a linear relationship between the ionic radii of the alkali metal ions and the temperature at which their tetraphenylboron salts began to decompose. Others have proposed reaction schemes to account for the pattern of thermal decomposition.[58,95,166,167]

Anions derived from elements of Group III—Some borates of lithium and sodium have been studied.[168] The decomposition of some calcium aluminate hydrates[169] and calcium carbo-aluminate[170] has been investigated, that of the former under constant water vapour pressure.

Anions derived from elements of Group IV—

(*i*) *Carbonates*—Freeman[81] has studied the kinetics of the decomposition of calcium carbonate, comparing results obtained with results from isothermal studies. Lanthanum carbonate has been investigated as a suitable weighing form for lanthanum[142]; other lanthanon carbonates have been studied.[171,172] The effect of crucible design on the decomposition characteristics of lead carbonate has been studied.[173] Other recent studies on carbonates include ammonium scandium carbonate[174] and certain cobalt^{III} complex carbonates.[175] Thermogravimetric studies of solid-state reactions between cerium oxide, neodymium oxide or samarium oxide with either sodium carbonate or sodium sulphate,[176] and of the strontium carbonate - zirconium oxide and barium carbonate - zirconium oxide systems have been made.[177] Wilburn and co-workers[8,178] have studied the systems sodium carbonate - silica and calcium carbonate - silica, by both thermogravimetric and differential thermal analysis and discussed their behaviour in relation to glass manufacture.

(*ii*) *Formates, acetates, oxalates and other oxycarbon anions*—Copper^{II} formate[179] and aluminium acetate[180] have recently been investigated. However, oxalates have received a great deal of attention, *viz.*—those of copper,[67] beryllium,[181] magnesium,[182] calcium[5,80,81] zinc,[67] cadmium,[67] lead,[67] manganese^{II},[67] cobalt,[173] nickel,[67,182] scandium,[183] yttrium,[183] lanthanum,[68,93,143,183] other lanthanons,[68,172,184,185] thorium[93] and uranium,[93,186] complex cobalt oxalates,[175] oxalato-niobates[187] and other complex oxalates.[188] The additivity of the decomposition curves of a mixture of oxalates has been demonstrated,[182] and the thermal decomposition pattern of oxalates in different atmospheres has received attention.[5,189] Other compounds of oxycarbon anions that have been studied include potassium hydrogen phthalate,[190] caesium propionate, butyrate, and isovalerate[191] and lanthanum and cerium^{III} palmitate, laurate and stearate.[192]

(*iii*) *Silicates*—The dehydration behaviour of several silicates and silicate minerals has been investigated.[193 to 198] The use of thermogravimetry combined with infrared studies has been suggested to permit a distinction to be made between constitutional and adsorbed water.[199] The effect of alkaline-earth chlorides on the dehydration of silica Xerogel has been studied.[200]

(*iv*) *Others*—Thermogravimetry has been used to determine the conditions under which calcium orthoplumbate^{IV} is formed.[201]

Anions derived from elements of Group V—

(*i*) *Nitrogen*—The reaction between boron oxide and sodamide to yield boron nitride[202] and the oxidation of aluminium nitride[203] have been studied thermogravimetrically. The thermogravimetric behaviour of cerium^{III},[204] praseodymium, neodymium, samarium,[172] thorium,[205,206] plutonium[146] and nickel[207] nitrates has been investigated. Lead azide has been studied.[208]

(*ii*) *Phosphorus*—The resistance to oxidation of some transition metal phosphides[209] has been described. Phosphates of ammonium,[210] sodium,[211] beryllium,[212] calcium,[213,214] strontium,[214] cadmium,[214] aluminium,[215,216] antimony,[217] chromium[166] and iron,[215,216] and some halophosphates have been studied.[218,219,220] The decomposition of disodium dihydrogen pyrophosphate[221] and the reactions between magnesium pyrophosphate and strontium oxide or magnesium hydroxide[222] have been reported. Gerrard, Mooney and Rothenbury have used thermogravimetry in their investigation of polymers formed from chloroborazoles and phosphorus esters.[223]

(*iii*) *Other anions*—Arsenic acid[224] and arsenates of chromium,[58] cobalt[225] and nickel[225] have been investigated thermogravimetrically. Ammonium metavanadate,[226] some niobates and tantalates[227] and some oxalato-niobates[187] have also been studied.

Anions derived from elements of Group VI—

(*i*) *Oxides, peroxides and related compounds*—Thermogravimetric studies of oxides may be sub-divided into two main sections: (*a*) Dehydration reactions of hydrated oxides and subsequent disproportionation reactions—

$$M_xO_y.nH_2O_{(s)} \longrightarrow M_xO_{y(s)} + nH_2O_{(g)}$$ [7,228 to 235]

$$M_xO_{y(s)} \longrightarrow M_xO_{y-z(s)} + \frac{z}{2}O_{2(g)}$$ [229,230,232,236]

(*b*) Solid-state reactions, which may be either solid - solid reactions involving loss of volatiles,[8,176,177,178,202,222,237,238,239] or solid - gas reactions involving loss or gain in weight,[76,240,241] have also been reported.

The thermal dehydration of copper,[242] beryllium,[181] magnesium,[54] iron^{III} [243] and nickel[193] hydroxides has been investigated thermogravimetrically. On the basis of thermogravimetric and infrared methods several metal oxyhydrates have been classified into four groups.[244]

(*ii*) *Sulphides, sulphates and other anions of sulphur*—The oxidation and thermal decomposition of copper,[43,245] zinc,[245] cadmium,[245] mercury,[245] germanium,[246] tin,[245] lead,[245] and various nickel sulphides[247,248,249] has been described, and there have been two studies on pyrites.[50,250] Sulphates of the metals listed below have been studied: copper,[245,251] beryllium,[181] magnesium,[252] calcium,[253] zinc,[245] cadmium,[245,254] mercury,[245] indium,[255] tin,[245] lead,[245] nickel,[256] uranium,[257] lanthanum,[258,259] and other lanthanons,[172,259] as well as several double and complex sulphates.[252,260 to 263] Solid - solid[264] and solid - gas[265] reactions involving sulphates have also been reported. Rocchiccioli[266] has studied the decomposition of sulphamic acid and some metal sulphamates.

(*iii*) *Anions derived from the other elements of Group VI*—The selenides of arsenic, rhenium and mercury have been shown to sublime at definite temperatures.[141] The decomposition of magnesium selenates has been compared to the corresponding sulphates.[252] The thermal decomposition of neodymium and basic yttrium chromate has been studied,[267] as well as some isopolychromates of potassium.[268] Tungstic acid,[269] ammonium paratungstate,[269] several 12-heteropoly tungstates,[89,90,94] ammonium[53] and quinolinium molybdophosphate[139] have been investigated thermogravimetrically.

Anions derived from elements of Group VII—Thermogravimetric studies of mono-amine dichloro zinc[270] and basic aluminium chlorides[271] have been made. A study of the application of high temperature thermogravimetry of chlorides and sulphates to soil analysis has been reported.[272] $CdI_2.xNH_3$[273] and some tetra-iodomercurates[274] have been investigated. The thermal decomposition of the following compounds involving oxyanions has been reported: sodium chlorite,[275] barium bromate[276] and chlorate[276] and copper^{II} iodate,[276] together with the perchlorates of potassium,[48] magnesium,[277] calcium[277] and barium.[276,277] Binary systems of potassium perchlorate with either alkaline or alkaline-earth metal nitrates,[278] and the course of the reaction between potassium chlorate and manganese dioxide[279] have been followed by using thermogravimetric techniques.

Barium permanganate[280] and ammonium perrhenate[281] and the compounds $AgMn_2O_4$ and Ag_2MnO_2[282] have been studied by using a thermobalance.

Anions derived from elements of Group VIII—Alkali metal,[283] copper,[284] magnesium[285] and alkaline-earth[285] ferrocyanides have been examined by Seifer. Work on ammonium chloroplatinate[286] and bromoplatinic acid[287] has also been reported.

Complex inorganic compounds—Complex compounds of cobalt, chromium, nickel and platinum in which the ligand groups are wholly or partly either ammonia, ethylenediamine, propylenediamine or pyridine have been widely studied by Wendlandt and his school[95,175,288 to 292] and also by others.[167,293,294] The 8-hydroxyquinolines of many bivalent metals,[295,296] uranium[297] and plutonium[145] have been studied. Bivalent metal anthranilates,[298] substituted anthranilates of lanthanum and lead,[299] and other related chelate complexes[88,300,301] have all been studied thermogravimetrically. Other work on complexes has been carried out by Wendlandt and co-workers,[302,303,304] Charles[305] and Dhar and Basolo.[306] Clathrates of the type $Ni(CN)_2.NH_3.X$, where X = benzene, thiophen, pyrrole, furan or phenol,[307] have been examined by using a quartz-spring thermobalance.

ORGANIC CHEMISTRY—

The application of thermogravimetric analysis to organic compounds is best discussed under two headings.

Synthetic organic polymers—These have been much studied in recent years, particularly with the advent of apparatus in which the sample can be heated in an inert atmosphere or under vacuum. Since the breakdown of polymers into the volatile monomer units is nearly always a simple single-stage process, these reactions have been used as standards in non-isothermal kinetics (see "Kinetic Studies").[64,82,96,97,99] H. C. Anderson has compared differential thermogravimetry with differential thermometry as a method for studying the pyrolysis of polymers.[308] Recently Doyle[309] has derived equations from which it is possible to determine the isothermal life of polymers from thermogravimetric data. Table I lists some polymers that have been studied by a thermogravimetric technique.

TABLE I

SYNTHETIC POLYMERS STUDIED BY THERMOGRAVIMETRY

Polymer	References— Kinetics studied	Kinetics not studied
Epoxide polymers	97	65, 310
Polyamides	311	—
Polyethylene	64, 99	312
Polymers based on methyl methacrylate	99	52
Polypyromellitimides	—	63
Polystyrene	64	—
Poly(t-butyl acrylate)	313	—
Polytetrafluoroethylene	82, 96	310, 314
Poly(vinyl alcohol), PVA	47, 315	—
Poly(vinyl chloride), PVC	316	62, 310
Other vinyl polymers	—	317
Phenolic polymers	—	318 to 320
Silicones	—	310
Urea - formaldehyde resins	—	321

Other organic compounds—Few simple organic compounds have been studied. Duval[322] has investigated the thermal stability of some organic compounds used as analytical standards. Other compounds studied include EDTA,[151] barbituric acid and related compounds.[153] Some organo-tin compounds have been investigated by thermogravimetry.[323] The pyrolysis behaviour of several coals, peats and bitumens has been studied[324 to 327]; their kinetics of decomposition has also received attention.[83,328] The mechanism of thermal decomposition of some organo-montmorillonites has been investigated.[329] Thermogravimetric and differential thermal analytical studies have been made of the pyrolysis of wood and of wood treated with inorganic salts. Some conclusions regarding the action of salts that are flame retardant are discussed.[330,331] There have been two studies on inclusion or clathrate compounds. McAdie[332] has studied the urea - n-paraffin inclusion compounds, and Gilford and Gordon[67] have examined the behaviour of some quinol clathrates under ambient and reduced pressures.

COMPLEMENTARY PROCEDURES

We have reviewed the various applications of thermogravimetric analysis, but there has been little stress on the fact that, in many of the papers referred to, other techniques have been used to complement the information gained from thermogravimetry. In certain investigations, it is essential to use a complementary technique if the interpretation of the course of a thermal decomposition is not to be pure guess-work; at least, chemical analysis of the solid removed from the thermobalance at an appropriate point on the thermogravimetric curve[58] or, alternatively of the evolved gas[316] should be carried out. Information gained from thermogravimetry becomes more meaningful or can readily be extended by the use of techniques that are applicable to solid-state chemistry.[25] It is not the object of this review, however, to discuss these procedures in detail but to offer a few pertinent comments.

DIFFERENTIAL THERMAL ANALYSIS—

This method is taken first, owing to its close affinity to thermogravimetry (see Introduction, p. 906). Both are dynamic methods, and both are very much dependent on procedural details.[3] It is not true that what is good technique for thermogravimetric analysis

is necessarily so for differential thermal analysis. There is no doubt that much useful and meaningful information can be obtained with equipment designed to carry out both thermogravimetry and differential thermal analysis at one and the same time on the same sample—the success of the Derivatograph[9,50,158,159,210,243] is proof of this. Nevertheless, there are limitations. For example, in differential thermal analysis the sample is generally packed down in the crucible[3] so that the energy changes occurring in the course of the reaction produce a "good" peak; hence, again, the use of higher rates of heating in differential thermal analysis. For quantitative thermogravimetry, however (particularly if evaluation of kinetic parameters of the reaction is to be attempted), both of these operations would not be considered good practice. Garn[173] has discussed various aspects of this problem. An apparatus, such as that described by Hodgson,[107] offers a compromise, in that both procedures are available to the operator but as completely independent operations. The use of the two procedures permits information to be obtained on all phenomena listed on p. 906. Examination of a material by both procedures permits crystalline transitions, second-order transitions and solid - solid reactions occurring without weight change to be distinguished from a typical decomposition reaction, or indeed any of the phenomena listed on p. 906 that give rise to a weight change.

OTHER PROCEDURES—

In many of the studies discussed, one of the main uses of thermogravimetry has been the investigation of the thermal stability of the material,[13,149,322] or to find a suitable drying temperature for the investigation of a weighing form of an element.[143 to 146] However by using thermogravimetry together with infrared studies, X-ray diffraction[21] or electron-diffraction studies, or magnetic measurements—say, by heating up to a suitable point on the thermogravimetric curve (up to a point where a reaction has occurred) and removing the sample for examination by one or more of these methods—information can be gained on what structural changes have occurred during the course of the reaction. Thus a combination of infrared studies and magnetic measurements with a detailed thermogravimetric examination of the dehydration of hexa-aquochromium^{III} phosphate and arsenate enabled Lukaszewski and Redfern[58,166] to propose a reaction scheme for the dehydration reactions. A thermogravimetric study, coupled with X-ray diffraction studies and a study of catalytic activity,[7] of a hydrated ruthenium dioxide provided preliminary information on some structural changes occurring on heating.

CONCLUSION

It is evident from the volume of literature now appearing[1] that both thermogravimetric and differential thermal analysis have found many varied applications both in analytical chemistry and in other fields. It is likely that the introduction of more sophisticated instrumentation (with facilities for operating in inert, oxidising or otherwise controlled atmospheres) will lead to further developments, particularly in the fields of organic and inorganic materials and to the use of thermogravimetry directly as an analytical method. It is to be hoped that prospective workers in the field will realise the dynamic nature of the method and of its dependence on the factors outlined in the section on "Techniques."

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Analytical Methods Committee

REPORT OF THE ADDITIVES IN ANIMAL FEEDING STUFFS SUB-COMMITTEE

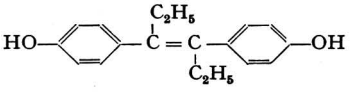
PART 2.* REPORT OF THE HORMONES PANEL

The Determination of Stilboestrol and Hexoestrol in Compound Feeding Stuffs

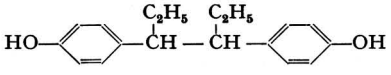
GENERAL INTRODUCTION

THE Hormones Panel was set up under the chairmanship of Dr. R. E. Stuckey, and its membership was: Mr. J. Allen, Mr. L. Brealey, Mr. J. A. Potter and Mr. W. L. Sheppard, with Miss A. M. Parry as Secretary. The Panel was appointed to consider methods for determining synthetic hormones included in animal rations as stimulants.

A number of synthetic hormones, both with an anabolic and an oestrogenic action, have been tried in practice as additives in animal feeding stuffs; they are stilboestrol, hexoestrol, dienoestrol, dianisyl hexane, dianisyl hexene, dianisyl hexadiene and dienoestrol acetate. However, only two—stilboestrol and hexoestrol—were considered by the Panel to be sufficiently important to warrant the detailed investigation necessary; their structures are shown below—



Stilboestrol



Hexoestrol

Although both these compounds possess phenolic groupings and can be assayed, when present in small amounts, by the use of a reagent that reacts with the phenol group, it was considered that some means of differentiating between the two compounds when present in feeding stuffs would be desirable.

However, early in the consideration of the assay methods available for these compounds, it was apparent that the methods of choice for stilboestrol and hexoestrol would be different. In fact, although the two compounds are chemically similar, the methods recommended are quite distinct and will be described separately.

STILBOESTROL

Stilboestrol has been known for many years, and methods for its determination, both in pharmaceuticals and in biological materials, have appeared in the literature; most of the chemical methods are based on reactions involving the phenolic group. Methods proposed include an ultraviolet-absorption method,[1] Folin's colorimetric methods,[2] a bromimetric method,[3] a nitroso method,[4] the irradiation method[5] and the antimony pentachloride method.[6]

The main problem in the chemical assay of stilboestrol in feeding stuffs lies in the extraction of the stilboestrol in a sufficiently pure form. Cheng and Burroughs,[7] working on a pre-mix containing 500 mg of stilboestrol per lb of soyabean meal, and a low-potency cattle diet supplement containing 5 mg of stilboestrol per lb in the same meal, described methods for determining stilboestrol in feeds. In these methods the stilboestrol was isolated by chromatography on a column of Celite, and the purified stilboestrol determined by measurement of the red colour produced by reaction with antimony pentachloride in ethylene chloride; alternatively, the extracted stilboestrol was determined by a method involving ultraviolet irradiation of a solution in 80 per cent. acetic acid and measurement of the resulting colour at 410 mμ. It was found that the colour in both methods obeyed Beer's law over the

* Part 1 (Report of the Antibiotics Panel) appeared in *The Analyst*, **1963**, **88**, 835.

range 5 to 30 μg of stilboestrol, although the authors reported that the irradiation method did not give consistent results on the diet supplements, and the results with the antimony pentachloride method were high, probably because interfering substances in the feeds were not completely removed by chromatography. For concentrations of stilboestrol less than 5 mg per lb, the authors preferred a biological assay—a mouse-uterine weight method.[8]

In 1956 Hedger and Manley,[9] after a study of several chemical assay methods, reported that they preferred the ultraviolet-irradiation method, and described a procedure with which they reported recoveries of 90 to 98 per cent. of known amounts of stilboestrol added to feeds. In 1958 Hanka and Lockhart[10] reported that stilboestrol exerted a marked and specific inhibitory effect on the growth of *Staphylococcus aureus* strain H, and that this effect could be used for determining stilboestrol microbiologically by broth or disc-plate techniques.

The Panel finally decided that a chemical assay would be more generally useful than a microbiological or biological method. The irradiation method was in regular use by one of the members of the Panel, and it was decided that this method, modified from that of Hedger and Manley, should first be tried.

Experimental and results

The steps in the procedure used were—

(*i*) mixing of the feed, grinding if necessary, and sampling for analysis;
(*ii*) extraction of stilboestrol from the sample with chloroform;
(*iii*) washing of the chloroform extract with N sulphuric acid to remove impurities;
(*iv*) extraction of the purified chloroform extract with sodium hydroxide solution;
(*v*) adjustment of the sodium hydroxide solution to pH 9·0 to 9·5 with 2 N orthophosphoric acid, and re-extraction of the stilboestrol with chloroform;
(*vi*) evaporation of the purified chloroform extract to dryness, dissolution of the residue in glacial acetic acid, and irradiation of the solution with ultraviolet light;
(*vii*) measurement of the optical density at 420 mμ and calculation of the stilboestrol present in the original feed by reference to a calibration graph.

Collaborative tests by the method outlined above were first carried out on a sample of soyabean meal to which 11 p.p.m. (about 5 mg per lb) of stilboestrol had been added.

In the first instance the Panel members who had not previously used the method experienced trouble at the irradiation stage. It was found that the wavelength of the light used to irradiate the solutions was critical, the shorter wavelength of 254 mμ being satisfactory, whereas the colour of the solution was destroyed by radiation of wavelength 365 mμ. It was also found essential to adhere closely to the distances and times specified in the method. After this had been realised and the correct lamp used, no difficulty was experienced in developing the maximum colour. The first results obtained are shown in Table I.

Table I

Determination of stilboestrol in soyabean meal

Sample contained 11 p.p.m. of stilboestrol

Laboratory	Stilboestrol found, p.p.m.
A	8·8, 11·0
B	8·0
C	9·0
D	—

As an additional difficulty some samples of glacial acetic acid were found to be unsatisfactory, even when the procedure at the colour-development stage was carried out exactly as described in the method. Examination and fractionation of the acid suggested the presence, in some batches, of an inhibiting substance that was extremely difficult to remove. Careful fractionation sometimes produced a satisfactory product, but this did not always remain so on storage. Satisfactory samples of glacial acetic acid were circulated for use by the Panel members, and at this stage it was decided that a special test to assess the suitability of the acid used would have to be included in the reagent specification in the final method.

A further collaborative test was carried out on another sample of compound feeding stuff to which 10 p.p.m. of stilboestrol had been added. Considerable difficulty was again experienced in preparing glacial acetic acid satisfactory for the development of the colour by irradiation. At this stage, however, the United States Pharmacopoeia XVI was published, and it was noted that, in the official assay for stilboestrol,[11] phosphate buffer solution instead of glacial acetic acid was used as a solvent at the irradiation stage. A trial of this buffer solution showed that it was much preferable to glacial acetic acid and subject to none of its disadvantages. It was therefore agreed that this solvent should be substituted for glacial acetic acid at the colour-development stage. The results obtained in the collaborative test with the revised method are shown in Table II.

TABLE II

DETERMINATION OF STILBOESTROL IN A COMPOUND FEEDING STUFF
Sample contained 10 p.p.m. of stilboestrol

Laboratory	Stilboestrol found, p.p.m.	Remarks
A	(*a*) 8·1 8·9	Glacial acetic acid used
	(*b*) 8·8 7·3	Phosphate buffer solution used
	(*c*) 8·8	Phosphate buffer solution used on same extract as in (*a*)
B	9·4 9·7	Phosphate buffer solution used (colour developed more quickly)
C	(*a*) 8·4 9·1	Glacial acetic acid used (with a Hanovia lamp the colour was destroyed if the optimum time was exceeded)
	(*b*) 8·8	Phosphate buffer solution used
D	8·4	Phosphate buffer solution used

In view of the general agreement between the results and the possibility of incomplete incorporation of all the stilboestrol added during the preparation of the compound feeding stuff, it was considered by the Panel that this method was satisfactory and could be recommended; the method is described in detail in Appendix I.

HEXOESTROL

The determination of hexoestrol in pharmaceuticals and in biological materials has been described in the literature. The most common method and that described in the British Pharmaceutical Codex 1959 for determining hexoestrol in tablets depends on the measurement of the blue colour developed with sodium molybdophosphotungstate.

The use of hexoestrol in compound feeding stuffs is not as widespread as that of stilboestrol, and methods for determining hexoestrol specifically in feeding stuffs have not been described in the literature. Measurement of the colour developed with sodium molybdophosphotungstate is a sensitive method for determining hexoesterol, but the difficulty in applying it to feeding stuffs lies in isolating the hexoestrol in a sufficiently pure form.

After consideration of the methods available, the Panel decided to rely on the experience of one of its members, whose laboratory had devised a method for the chromatographic separation of hexoestrol and its subsequent purification.

EXPERIMENTAL AND RESULTS

The steps in the method initially tried by the Panel, but which was subsequently modified, were—

(*i*) mixing of the feed, grinding, sifting, and sampling for analysis;
(*ii*) extraction of hexoestrol from the sample with chloroform;
(*iii*) washing of the chloroform extract with N sulphuric acid to remove impurities;
(*iv*) extraction of the purified chloroform extract with sodium hydroxide solution;
(*v*) adjustment of the sodium hydroxide solution to pH 9·0 to 9·5 with 2 N orthophosphoric acid, and re-extraction of the hexoestrol with chloroform;
(*vi*) evaporation of a portion of the chloroform extract to dryness, and dissolution of the residue in a mixture of tetrahydrofuran, triethylamine and water;

(*vii*) removal of phenolic impurities by reversed-phase chromatography on a column of oleated cellulose powder;

(*viii*) elution of the hexoestrol from the cellulose with ether, and then extraction of the solution with sodium hydroxide solution;

(*ix*) acidification of the sodium hydroxide solution and re-extraction of the hexoestrol with ether;

(*x*) evaporation of the filtered ethereal extract, reaction with sodium molybdophosphotungstate reagent, measurement of the optical density of the coloured complex at 750 mμ and determination of hexoestrol by comparison with standards.

Collaborative tests were carried out by the method outlined above on a sample of soyabean meal to which 11 p.p.m. (about 5 mg per lb) of hexoestrol had been added.

In the first test, difficulties with the method were reported; the results obtained are shown in Table III.

TABLE III

DETERMINATION OF HEXOESTROL IN SOYABEAN MEAL

Sample contained 11 p.p.m. of hexoestrol

Laboratory	Hexoestrol found, p.p.m.
A	12·0, 10·4
B	7·2
C	8·8
D	—

Difficulties were experienced owing to the formation of emulsions during the sodium hydroxide - chloroform extraction stage and also to the fact that the final solutions for the colour measurement were often cloudy to different degrees. Incomplete purification, it was found, could also cause high results owing to the presence of extracted substances that reacted with the sodium molybdophosphotungstate.

It was decided that, at some stages, the assay procedure ought to be more precisely described, particularly at the stages relating to the extraction and the chromatography, and that the reagent grades ought to be specified.

A second collaborative test was carried out on a further sample of cattle feeding stuff to which 10 p.p.m. of hexoestrol had been added. During this second test one laborotory reported high results (about 15 p.p.m.), and it was suspected that fatty material from the chromatographic column might be responsible; this interference was subsequently minimised by describing in detail each step of the purification stage, by specifying a purified oil for the preparation of the oleated cellulose, and by removing any cloudiness in the final extract by filtration through sand. High results were also attributed to impurities in the solvent, but these could be avoided by distilling the ether before use. Other members reported occasional high results, which, it was considered, would be avoided if the ether were freshly distilled. Further, it was considered that any cloudiness occurring in the final extract should be removed by filtration through sand. The results obtained by the collaborating laboratories in a final collaborative test with the method described in Appendix II are shown in Table IV.

TABLE IV

DETERMINATION OF HEXOESTROL IN A COMPOUND FEEDING STUFF

Sample contained 10 p.p.m. of hexoestrol

Laboratory	Hexoestrol found, p.p.m.
A	8·3, 8·9, 8·6, 8·5
B	9·0
C	9·3, 9·2
D	10·9

The results were considered to indicate that the method was satisfactory for determining hexoestrol in compound feeding stuffs, and could be recommended (see Appendix II).

Appendix I

Recommended method for determining stilboestrol in compound feeding stuffs

Principle of method—

The stilboestrol is extracted from the feeding stuff with chloroform, then taken into sodium hydroxide solution, and, after the solution has been adjusted to pH 9·0 to 9·5 with orthophosphoric acid, is re-extracted with chloroform. The purified chloroform extract is evaporated, and the residue dissolved in phosphate buffer solution. The optical density is measured at 418 mμ before and after irradiation of the solution with ultraviolet light, and from the difference the amount of stilboestrol in the sample is calculated by reference to the optical-density difference when an extract to which a known amount of stilboestrol has been added is similarly treated.

Applicability—

The method is suitable for the analysis of feeding stuffs containing about 11 p.p.m. (about 5 mg per lb) stilboestrol.

Special apparatus—

Extraction apparatus—Any apparatus may be used provided it permits (*i*) the uniform percolation of the powdered feeding stuff with the extracting solvent and (*ii*) the regular flow of the solvent vapour around the percolator. A suitable form consists of a glass tube, about 4 cm in internal diameter and 15 cm in length, with a piece of coarse filter-paper covered with fine calico firmly tied over the lower flanged end. This percolator rests on a glass spiral inside an outer tube, about 6 cm in internal diameter and about 25 cm in length, the spiral being supported on the shoulder of a B24 standard joint sealed to the lower end of the outer tube. This standard joint fits into a flask of suitable size to contain the solvent, and a reflux condenser is attached to the top of the tube. An alundum crucible of medium porosity and suitable size also forms a satisfactory percolator.

Irradiation equipment—This consists of a mercury-discharge tube mounted horizontally in a suitable reflector, together with a holder capable of containing four silica cells, so arranged that the cells are perpendicular to the light source and fixed at 15 cm (6 inches) from it. The holder is also positioned so that a line through the liquid centres in the cells is level with the centre of the discharged tube, and the cells themselves, spaced about 1 cm apart, are centrally located relative to the ends of the discharge tube.

The Phillips Germicidal tube TUV (15 watt), together with a trough fitting, A7003, with the grill removed, is a suitable source of radiation.

Reagents—

Washed sand.

Chloroform—Analytical-reagent grade. The chloroform used must comply with the test described below.

Shake 35 ml of the chloroform with 70 ml of water, and then allow the layers to separate. To 10 ml of the aqueous extract add 40 ml of distilled water and 2 ml of Nessler's reagent, and set aside in the dark for 15 minutes. No colour or turbidity should be produced.

Sulphuric acid, N.

Sodium hydroxide, N.

Orthophosphoric acid, 2 N.

Sodium sulphate, anhydrous.

Ethanol, absolute.

Potassium phosphate solution—A 1·8 per cent. w/v solution of dipotassium hydrogen orthophosphate, K_2HPO_4, in distilled water.

Stock standard stilboestrol solution—Prepare a solution in chloroform to contain exactly 0·55 mg of stilboestrol B.P. per ml.

Working standard stilboestrol solution—Dilute 10 ml of stock standard stilboestrol solution to 100 ml at 20° C with chloroform;

1 ml $\equiv$ 55 μg of stilboestrol.

PROCEDURE

EXTRACTION OF SAMPLE—

Crush about 1 kg of the sample, mix and grind 100 g of the crushed material until not less than 95 per cent. passes a 30-mesh sieve. Weigh accurately about 40 g of the ground material, and mix with about 10 g of washed sand. Transfer the mixture to a stoppered flask, add about 100 ml of chloroform, shake vigorously, and set aside overnight. Transfer the contents of the flask to the percolator of the extraction apparatus, collecting the chloroform in the flask, and assemble the apparatus. Extract the solid material for 6 hours; use more chloroform if necessary. Filter the extract through a pad of cotton-wool into a 200-ml calibrated flask, washing the extraction flask and filter with chloroform, and finally dilute the combined extract and washings to 200 ml with chloroform.

PURIFICATION OF EXTRACT—

Transfer a 25-ml portion of the solution from the extraction of sample, together with 25 ml of chloroform, to each of two separators; add exactly 1 ml of working standard stilboestrol solution to the contents of one separator, and treat the contents of each separator as described below.

Add 25 ml of N sulphuric acid, swirl gently for 30 seconds, avoiding as far as possible the formation of emulsions, allow the layers to separate for 10 minutes, and transfer the lower chloroform layer to another separator. If emulsions form at this stage, it may be helpful to spin the mixture in a centrifuge. Add 10 ml of chloroform to the aqueous acid layer, shake gently for 10 seconds, still taking care to avoid emulsification, allow the layers to separate and add the lower chloroform layer to the initial chloroform solution. Repeat this last operation with two further successive 10-ml portions of chloroform, shaking vigorously, and then discard the aqueous acid liquid.

Shake the combined chloroform solutions carefully for 30 seconds with two successive 10-ml portions of N sodium hydroxide; set aside for 10 minutes each time before running off the lower chloroform layer. Discard the chloroform solution. Combine the sodium hydroxide extracts, add 5 ml of chloroform, shake for 5 seconds, allow the layers to separate, and transfer the lower chloroform layer to the empty separator that previously held a sodium hydroxide extract. Repeat the extraction of the combined sodium hydroxide extracts with two or three successive 5-ml portions of chloroform until a chloroform layer is obtained that, after having been shaken and allowed to separate, is colourless; add each chloroform layer to the first one. Add 5 ml of distilled water to the combined chloroform washings, shake, allow the layers to separate, and run off and discard the chloroform layer. Transfer the washed alkaline solution and the aqueous washings to a 50-ml beaker; rinse the empty separator with successive small portions of distilled water until the washings are free from all alkalinity, and add the rinsings to the contents of the beaker.

To the combined alkaline aqueous solution and washings, add 4 ml of 2 N phosphoric acid, and adjust the solution carefully to pH 9·0 to 9·5 with 2 N phosphoric acid; use a pH meter to make the measurements. Return the adjusted solution to the same separator, rinsing the beaker first with two successive 2-ml portions of distilled water and then with 15 ml of chloroform, and add the rinsings to the contents of the separator. Swirl the mixture carefully for 30 seconds taking care to avoid the formation of an emulsion, allow the layers to separate, and transfer the lower chloroform layer to another separator. Add to the chloroform extract 25 ml of distilled water, shake for 5 seconds, allow the layers to separate, and filter the lower chloroform layer through a 1-inch bed of anhydrous sodium sulphate in a sintered-glass funnel; collect the filtrate in a 50-ml calibrated flask. Repeat the extraction of the alkaline aqueous solution with two successive 15-ml portions of chloroform; wash both the chloroform extracts with the same 25 ml of water, and then filter the washed extracts through sodium sulphate as described above. Shake the aqueous washings with successive small portions of chloroform; use these chloroform extracts to wash the sodium sulphate and the funnel, and then add to the contents of the calibrated flask. Continue this process until the solution in the flask is adjusted to the mark, and mix.

DETERMINATION OF STILBOESTROL—

Transfer a 25-ml portion of the sample solution from the purification of extract to a 100-ml beaker previously rinsed with chloroform, and evaporate off the solvent with a gentle

current of air, warming the beaker on a water-bath until only the last traces of solvent remain. Dissolve the residue in 5 ml of absolute ethanol, warming gently, if necessary, to effect dissolution, and add 5 ml of potassium phosphate solution.

Measure the optical density at 418 mμ of the solution against water in a 1-cm cell with a spectrophotometer.

Transfer about 3 ml of the aqueous ethanolic solution to a silica cell, and irradiate with ultraviolet light for 10 minutes. Measure the optical density at 418 mμ of the irradiated solution against water in a 1-cm cell with a spectrophotometer. Re-irradiate the solution for successive 1-minute periods until the maximum optical-density reading is obtained. Repeat the irradiation on a further 3-ml portion of the aqueous ethanolic solution, irradiating for the period found previously to give the maximum optical density, and use this figure in the calculation.

Treat, in exactly the same manner, a 25-ml portion of the solution from the purification of extract obtained from the extract to which a known amount of stilboestrol has been added.

CALCULATION—

Calculate the amount of stilboestrol present in the sample from the expression—

$$\frac{A - a}{(B - b) - (A - a)} \times \frac{55}{2} \times \frac{8}{\text{Weight of sample}} \text{ p.p.m.,}$$

where A = optical density of sample solution after irradiation,
a = optical density of sample solution before irradiation,
B = optical density of sample solution with added stilboestrol after irradiation and
b = optical density of sample solution with added stilboestrol before irradiation.

Appendix II

RECOMMENDED METHOD FOR DETERMINING HEXOESTROL IN COMPOUND FEEDING STUFFS

PRINCIPLE OF METHOD—

The hexoestrol is extracted from the feeding stuff with chloroform, then taken into sodium hydroxide solution, and, after the solution has been adjusted to pH 9·0 to 9·5 with orthophosphoric acid, is re-extracted with chloroform. The purified chloroform extract is evaporated, the residue dissolved in an aqueous solution of tetrahydrofuran and triethylamine, and the hexoestrol separated by column chromatography. The hexoestrol is extracted with ether, the ethereal solution evaporated, the residue treated with a molybdophosphotungstic acid reagent, and the hexoestrol determined absorptiometrically.

APPLICABILITY—

The method is suitable for the analysis of feeding stuffs containing 10 p.p.m. (about 5 mg per lb) of hexoestrol.

SPECIAL APPARATUS—

Extraction apparatus—As described in the method for stilboestrol (see Appendix I, p. 929).

Chromatographic tube—A glass tube, about 1 cm in internal diameter and about 15 cm in length, drawn out at one end for a length of about 2 cm to terminate in a jet, about 3 mm in internal diameter, is suitable. In such a tube the adsorbent can be supported on a pledget of cotton-wool.

REAGENTS—

Washed sand.
Fine sand.
Cellulose powder.
Ether, redistilled—Anaesthetic ether B.P., freshly distilled.
Arachis oil solution—A 5 per cent. v/v solution of arachis oil B.P., in redistilled ether.
Chloroform—Analytical-reagent grade.
Sulphuric acid, N.
Sodium hydroxide solution, N.

Orthophosphoric acid, 2 N.

Sodium sulphate, anhydrous.

Tetrahydrofuran - triethylamine solution—A mixture of 5 volumes of triethylamine, 25 volumes of tetrahydrofuran and 70 volumes of distilled water.

Sulphuric acid, diluted—Mix carefully 1 volume of sulphuric acid, sp.gr. 1·83, with 9 volumes of distilled water.

Ethanol, 95 per cent. v/v.

Ethanol, diluted—Mix equal volumes of ethanol and distilled water.

Hydrochloric acid, diluted—Mix 1 volume of hydrochloric acid, sp.gr. 1·16 to 1·18, with 9 volumes of distilled water.

Molybdophosphotungstate reagent—Add 50 g of sodium tungstate, $NaWO_4.2H_2O$, 12 g of molybdophosphoric acid, $H_3PO_4.12MoO_3.24H_2O$, and 25 ml of orthophosphoric acid (88 per cent. w/w) to about 350 ml of distilled water in a round-bottomed flask. Boil the mixture under reflux for 2 hours, cool, and dilute to 500 ml with distilled water. Store in a well-stoppered bottle protected from light.

Sodium carbonate solution—A 10 per cent. w/v solution in distilled water.

Stock standard hexoestrol solution—Dissolve 50·0 mg of hexoestrol in diluted ethanol, and make up to 100 ml at 20° C with diluted ethanol.

Working standard hexoestrol solution—Dilute 5·0 ml of stock standard hexoestrol solution to 50 ml at 20° C with diluted ethanol;

1 ml ≡ 50 μg of hexoestrol.

Procedure

Extraction of sample—

Crush about 1 kg of the sample, mix, and grind about 100 g of the crushed material until not less than 95 per cent. passes a 30-mesh sieve. Weigh accurately about 40 g of the ground material, and mix with about 10 g of washed sand. Transfer the mixture to a stoppered flask, add about 100 ml of chloroform, shake vigorously, and set aside overnight. Transfer the contents of the flask to the percolator of the extraction apparatus, collecting the chloroform in the flask, and assemble the apparatus. Extract the solid material for 6 hours; use more chloroform if necessary. Filter the extract through a pad of cotton-wool into a 200-ml calibrated flask, washing the extraction flask and filter with chloroform, and finally dilute the combined extract and washings to 200 ml with chloroform.

Purification of extract—

Transfer a 50-ml portion of the solution from the extraction of sample to a separator, add 25 ml of N sulphuric acid, swirl gently for 30 seconds, avoiding as far as possible the formation of emulsions, allow the layers to separate for 10 minutes, and transfer the lower chloroform layer to another separator. If emulsions form at this stage, it may be helpful to spin the mixture in a centrifuge. Add 10 ml of chloroform to the aqueous acid layer, shake gently for 10 seconds, still taking care to avoid emulsification, allow the layers to separate, and add the lower chloroform layer to the initial chloroform solution. Repeat this last operation with two further successive 10-ml portions of the chloroform, shaking vigorously, and then discard the aqueous acid liquid.

Shake the combined chloroform solutions carefully for 30 seconds with two successive 10-ml portions of N sodium hydroxide; set aside for 10 minutes each time before running off the lower chloroform layer. Discard the chloroform solution. Combine the sodium hydroxide extracts, add 5 ml of chloroform, shake for 5 seconds, allow the layers to separate, and transfer the lower chloroform layer to the empty separator that previously held a sodium hydroxide extract. Repeat the extraction of the combined sodium hydroxide extracts with two or three successive 5-ml portion of chloroform until a chloroform layer is obtained that, after having been shaken and allowed to separate, is colourless; add each chloroform layer to the first one. Add 5 ml of distilled water to the combined chloroform washings, shake, allow the layers to separate, and run off and discard the chloroform layer. Transfer the washed alkaline solution and the aqueous washings to a 50-ml beaker; rinse the empty separator with successive small portions of distilled water until the washings are free from all alkalinity, and add the rinsings to the contents of the beaker.

To the combined alkaline aqueous solution and washings add 4 ml of 2 N phosphoric acid, and adjust the solution carefully to pH 9·0 to 9·5 with 2 N phosphoric acid; use a pH meter to make the measurements. Return the adjusted solution to the same separator, rinsing the beaker first with two successive 2-ml portions of distilled water and then with 15 ml of chloroform, and add the rinsings to the contents of the separator. Swirl the mixture carefully for 30 seconds, taking care to avoid the formation of an emulsion, allow the layers to separate, and transfer the lower chloroform layer to another separator. Add to the chloroform extract 25 ml of distilled water, shake for 5 seconds, allow the layers to separate and filter the lower chloroform layer through a 1-inch bed of anhydrous sodium sulphate in a sintered-glass funnel; collect the filtrate in a 50-ml calibrated flask. Repeat the extraction of the alkaline aqueous solution with two successive 15-ml portions of chloroform; wash both chloroform extracts with the same 25 ml of water, and then filter the washed extracts through sodium sulphate as described above. Shake the aqueous washings with successive small portions of chloroform; use these chloroform extracts to wash the sodium sulphate and the funnel, and then add them to the contents of the calibrated flask. Continue this process until the solution in the flask is adjusted to the mark, and mix.

ISOLATION AND DETERMINATION OF HEXOESTROL—

Preparation of adsorption column—Mix 2 g of cellulose powder with 6 ml of arachis oil solution, add more ether, and mix thoroughly, evaporating off the ether during the mixing. Make the residual oleated cellulose into a thin slurry with tetrahydrofuran - triethylamine solution, transfer to the chromatographic tube, and allow to drain.

Treatment of test solution—Transfer a 25-ml portion of the chloroform solution from the purification of the extract to a 100-ml beaker, and evaporate off the solvent with a gentle current of air, warming the beaker gently on a water-bath until only the last traces of solvent remain. Dissolve the residue in the minimum amount of tetrahydrofuran - triethylamine solution, and transfer the solution to the top of the prepared adsorption column. Allow the solution to percolate into the column until the surface of the liquid just disappears below the surface of the cellulose, stopper the upper end of the tube, and set aside for 1 hour. Transfer 10 ml of tetrahydrofuran - triethylamine solution to the top of the adsorption column, and allow the liquid to run through the column to remove impurities. When the flow has ceased, remove the residual liquid from the column by applying gentle suction at the bottom of the tube.

Transfer the cellulose column from the tube to a separator, wash out the tube with a little ether, and add the washings to the contents of the separator. Add 1 ml of N sulphuric acid and 20 ml of ether, shake, allow the layers to separate, and transfer the upper ethereal layer to another separator. Repeat the extraction of the acidified cellulose suspension with two successive 20-ml portions of ether, adding the ethereal layers to the ethereal extract in the second separator. To the combined ethereal extracts, add 10 ml of N sodium hydroxide, shake, and allow the layers to separate. Transfer the lower aqueous layer to another separator containing 5 ml of ether, shake, allow the layers to separate and transfer the lower aqueous layer to a third separator. Repeat the extraction of the combined ethereal extracts, first with a further 10-ml portion, and then with three successive 5-ml portions, of N sodium hydroxide; wash each aqueous alkaline extract with the same 5 ml of ether, and discard the ethereal solution and washings. Combine the washed aqueous alkaline extracts, acidify with diluted sulphuric acid, add 10 ml of ether, shake, and allow the layers to separate. Transfer the upper ethereal layer to another separator containing 5 ml of distilled water, shake, allow the layers to separate and transfer the upper ethereal layer to a 50-ml flask. Repeat the extraction of the acidified aqueous extracts, first with a further 10-ml portion, and then with three successive 5-ml portions, of ether; wash each ethereal extract with the same 5 ml of water before it is added to the first extract in the flask. Filter the combined ethereal extracts through a 1-cm layer of fine sand, wash the filter with several small portions of ether and evaporate the combined filtrate and washings to a volume of about 5 ml by gentle warming. Transfer the concentrated solution quantitatively to a 14-ml calibrated centrifuge tube with the aid of more ether, and carefully evaporate the ethereal solution to dryness.

Dissolve the residue in 0·5 ml of ethanol, add 0·5 ml of distilled water and then 0·4 ml of diluted hydrochloric acid, 0·8 ml of molybdophosphotungstate reagent and 5 ml of distilled water, mix well, and set aside for 10 minutes. Add 3 ml of sodium carbonate solution and

sufficient distilled water to produce 12 ml, mix, set aside for 1 hour, and spin in a centrifuge for 15 to 20 minutes. Measure the optical density at 750 mμ of the clear supernatant liquid with a spectrophotometer in a 1-cm cell against a blank solution prepared by adding 0·4 ml of dilute hydrochloric acid, 0·8 ml of molybdophosphotungstate reagent and 5 ml of water to 1 ml of diluted ethanol, and continue as described above, commencing at the words "mix well, and set aside for 10 minutes . . ."

Calculate the amount of hexoestrol in the solution by comparison of the optical density with that obtained by treating 0·8, 1·0 and 1·2 ml of working standard hexoestrol solution in the same manner, beginning with the addition of 0·4 ml of dilute hydrochloric acid in the previous paragraph; the optical densities of the test solution and of the chosen standard solution should not differ by more than 10 per cent. Hence calculate the amount of hexoestrol in the sample.

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PART 3. REPORT OF THE PROPHYLACTICS PANEL

The Determination of Nitrofurazone in Compound Feeding Stuffs

Introduction

The Prophylactics Panel was set up under the chairmanship of Dr. R. F. Phipers, and its membership was: Mr. C. W. Ballard, Mr. N. C. Brown, Dr. H. G. Dickenson, Mr. A. W. Hartley, Mr. A. Holbrook, and Mr. S. G. E. Stevens, with Miss A. M. Parry as Secretary; Mr. J. A. Stubbles and Mr. D. H. Mitchell were co-opted later. The Panel was appointed to consider methods of analysis for determining medicaments against coccidiosis and histomoniasis included in poultry rations.

The Panel turned its attention first to the determination of nitrofurazone, which is widely used as a poultry coccidiostat, and this report describes the results of collaborative tests that led to the recommended method being advanced.

There is a considerable volume of published information on the analysis of nitrofurazone in animal feeding stuffs, and it was not expected that there would be any difficulty in making a recommendation. However, the Panel's investigations showed that this was not so, and the method finally evolved contains various recommendations that, if ignored, will lead to inaccurate results being obtained.

Ells, McKay and Paul[1] described an extraction method for separating nitrofurazone from feeding stuffs, and they compared the optical densities of such extracts with the optical densities of similar solutions in which the nitrofurazone had been reduced by treatment with aqueous sodium dithionite solution. In recent years the complexity of the components used in the formulation of poultry feeding stuffs has increased; the introduction of grass meals and other vegetable materials yielding highly coloured extracts has rendered valueless simple methods of extraction and reduction. Buzard, Ells and Paul[2] attempted to overcome these difficulties by allowing the nitrofurazone extracted from medicated feeding stuffs to react with phenylhydrazine hydrochloride and measuring the red colour produced. Collaborative trials recorded by Puglisi[3] showed that this procedure was unreliable. van Zijl and Goosens[4] claimed that they could overcome interference from reducing and other materials, by oxidation of the sample solution with potassium permanganate. Tagaki and Uno[5] found that the addition of caustic alkali to nitrofurazone solutions led to an intensification of the colour, but the orange-red colour thus produced is unstable, and any determinations involving its use are difficult.

Cross, Hendey and Stevens[6] took advantage of some investigations by Porter,[7] who studied the colour reactions of certain nitro-compounds, and they adopted his use of dimethylformamide as a solvent. They found that the addition of alkali to solutions of nitrofurazone in dimethylformamide intensified the colour; this was of obvious value in spectrophotometric work, but they noted that the colour of the resulting solution required stabilisation before it could be used on a quantitative basis. Phenol was found to confer the desired degree of stability.

Cross, Hendey and Stevens[6] extracted a typical medicated poultry feeding stuff, *i.e.*, one containing about 0·01 per cent. of nitrofurazone, with light petroleum in a Soxhlet apparatus. Nitrofurazone is insoluble in light petroleum, and this first step permitted much interfering material to be removed. This extraction was followed by a similar treatment with carbon tetrachloride, in which solvent nitrofurazone also is essentially insoluble. The nitrofurazone was then extracted with acetone from the pre-extracted meal in a Soxhlet apparatus. After removal of acetone from the extract, the residue was dissolved in dimethylformamide. Suitable amounts of phenol, dissolved in dimethylformamide, and then aqueous alkali, were added. Two portions of the solution were taken, and one was treated with sodium dithionite. After centrifugation, the optical density of each solution was measured, and from the difference the amount of nitrofurazone present was obtained by reference to a calibration graph prepared by similarly treating known amounts of nitrofurazone.

Experimental and results

Information on the above method was given in a personal communication from Mr. S. G. E. Stevens early in the development of the method before the use of sodium dithionite had been introduced. A collaborative test with this early version of the method was carried out by members of the Panel on feeding stuffs of differing complexity. It will be seen from Table I, which gives the results obtained on meals of increasing complexity, that satisfactory results were obtained on the simple Meal A, but on the more complex meals, it is clear that the pre-extraction procedure failed to remove interfering materials.

Table I

Effect of addition of grass meal on the recovery of nitrofurazone from a feeding stuff

Type of feed	Nitrofurazone present in sample, %	Recovery, %
Meal A	0·100	94
Meal A + 10 per cent. of grass meal	0·100	95
Meal B	0·010	110
Meal B + 10 per cent. of grass meal	0·010	135
Meal C	0·005	118
Meal C + 10 per cent. of grass meal	0·005	144

In subsequent assays the use of sodium dithionite to provide an empirical blank solution gave somewhat lower results more closely approximating to the theoretical values. It became apparent that, with some complex feeding stuffs, the extractives contained substances having reducing properties. This was demonstrated by measuring, at intervals of time, the optical density of the colour produced from an extract from a complex medicated meal containing nitrofurazone; the value decreased progressively.

The Panel then examined the use of potassium permanganate as described by van Zijl and Goosens,[4] and a modification of the method, in which the permanganate was added drop by drop, yielded the results shown in Table II.

Table II

Effect of treatment with potassium permanganate on the recovery of nitrofurazone from a complex feeding stuff

Type of feed	Laboratory	Nitrofurazone added, %	Recovery (mean of 3 results), %
Without grass meal	A	0·005	99
		0·010	98
	B	0·005	78
		0·010	83
	C	0·005	93
		0·010	94
	D	0·005	94
		0·010	93
With grass meal	A	0·005	99
		0·010	98
	B	0·005	100
		0·010	92
	D	0·005	83
		0·010	90

During attempts to reconcile the discrepancies that continued to appear in the collaborative tests, it became evident that the quality of the dimethylformamide was important; the purity of this reagent should be such that the colour developed in the phenol and sodium hydroxide solutions in the presence of nitrofurazone should be stable for at least 2 hours. It was also established that the sodium dithionite solution must be freshly prepared and that the solid sodium dithionite itself should not be more than 6 months old. Some members

also observed that if the meal were medicated by adding to it a portion of an acetone solution of the drug, low recoveries of the nitrofurazone were obtained. This point is illustrated in Table III.

TABLE III

EFFECT OF METHOD OF ADDING THE DRUG ON THE RECOVERY OF NITROFURAZONE

Laboratory	Nitrofurazone added, %	Recovery, %	Method of medication
A	0·005	97, 95	Solid drug added
	0·010	99, 102	
B	0·005	100, 97	Drug added in solution after extraction with carbon tetrachloride
	0·010	97, 100	
	0·010	52, 62	Drug added to feed as solution in acetone
		62	
C	0·005	96, 94	Solid drug added
	0·010	93, 97	
D	0·005	87, 86	Solid drug added
	0·010	87, 87	
	0·005	37, 33	Drug added as solution in acetone
	0·010	39, 39	
E	0·005	93	Solid drug added
	0·010	96, 95	
		99, 94	
		94	
	0·010	56, 57	Drug added as solution in acetone
		62	

Despite modifications to the experimental techniques, the Panel continued to obtain results lacking a reasonable degree of concordance. The factors detailed below were therefore examined.

(*i*) The suitability of dimethylformamide as a solvent in the development of colour.
(*ii*) The effect of time of centrifugation.
(*iii*) The effect of potassium permanganate on a solution of nitrofurazone in acetone.
(*iv*) The effect of potassium permanganate on extracts of feeding stuffs medicated with nitrofurazone.
(*v*) The extraction process.

Detailed investigation showed that the quality of the reagents and the extraction process were the only significant sources of variation.

A series of experiments demonstrated that the intensity of the colour produced was linearly proportional to the concentration of nitrofurazone in the range 0 to 0·0004 per cent. (Beer's law) and also that the calibration curves prepared by individual members of the Panel did not vary by more than ± 3 per cent.

By careful definition of the procedure, the Panel members were able to obtain better agreement, although the recoveries were in the range of only 80 to 90 per cent. For medicated feeds produced in bulk, two explanations were considered: (*a*) electrostatic deposition of the nitrofurazone in the mixing equipment and (*b*) the possibility of instability of the nitrofurazone in the feeding stuff. Experiments showed that the first of these possible explanations was not true when adequate earthing precautions had been taken. It was still not clear whether the observed losses were due to degradation of nitrofurazone in the feeding stuff itself or during the pre-treatment of the sample. It was shown that satisfactory results were obtained on fairly old samples examined by members of the Panel when only light petroleum was used for the preliminary extraction. However, if carbon tetrachloride was used as the only pre-extraction solvent, low recoveries were sometimes obtained. Further, it was shown that if such feeding stuffs were extracted with carbon tetrachloride before medication with nitrofurazone then the use of carbon tetrachloride as a pre-extraction solvent caused no losses. These findings appear to indicate that there is some interaction between the nitrofurazone and the materials either extracted from the feed or derived from carbon tetrachloride; possibly decomposition of nitrofurazone may also result from prior decomposition of carbon tetrachloride itself as a result of contact with other constituents in the feed. The Panel has not conducted an investigation into this degradation; they consider that it is sufficient to draw attention to it.

Therefore the original method, in which only light petroleum is used as the pre-extraction solvent, is recommended, and any subsequent use of carbon tetrachloride will be necessary only when results obtained by this method indicate a complexity in the feeding stuff such as to cause undue interference with the optical measurements.

The foregoing remarks are only applicable to the analysis of the compound feeding stuffs now commonly marketed and containing only nitrofurazone as the coccidiostat. The analyst should acquaint himself with changes in the composition of the feeding stuff, and he should consider the implications arising from the introduction of new components.

RESULTS OF COLLABORATIVE TESTS—

Members of the Panel carried out collaborative tests by the recommended method (see Appendix I). Two materials were examined, the first of these being a compound feeding stuff containing 0·0060 per cent. of nitrofurazone, prepared by a member of the Panel

TABLE IV

RESULTS OBTAINED BY THE RECOMMENDED METHOD ON A MEDICATED POULTRY FEED

Sample contained 0·0060 per cent. of nitrofurazone

Laboratory and Analyst	Nitrofurazone found, %	Recovery, %	Laboratory and Analyst	Nitrofurazone found, %	Recovery, %
B1	0·0060	100	E1	0·0059	98
	0·0060	100		0·0065	108
	0·0060	100		0·0060	100
C1	0·00600	100		0·0059	98
	0·00605	101	E2	0·0056	93
	0·00595	100		0·0056	93
C2	0·00565	94		0·0054	90
	0·00570	95		0·0057	95
	0·00565	94	E3	0·0058	97
D1	0·0063	105		0·0059	98
	0·0063	105		0·0063	105
	0·0061	102		0·0059	98
D2	0·0059	98	F1	0·00625	104
	0·0057	95		0·00645	108
	0·0057	95		0·00620	103
E1	0·0057	95		0·00625	104
	0·0057	95		0·00605	101
	0·0053	88		0·00625	104

TABLE V

RESULTS OBTAINED BY THE RECOMMENDED METHOD ON MEDICATED POULTRY FEEDS

Samples prepared by adding different known amounts of nitrofurazone to an unmedicated poultry feed

Laboratory and Analyst	Nitrofurazone added, %	Nitrofurazone found, %	Recovery, %
B1	0·0080	0·00808	101
	0·0098	0·00941	96
	0·0100	0·01080	108
C1	0·0052	0·00535	103
	0·0060	0·00595	99
	0·0062	0·00620	100
C2	0·0055	0·00565	103
	0·0079	0·00815	103
	0·0087	0·00895	103
D1	0·0060	0·0060	100
	0·0074	0·0072	97
	0·0076	0·0073	96
D2	0·0073	0·0070	96
	0·0080	0·0073	91
	0·0089	0·0083	93
E1	0·00658	0·00660	100
	0·00700	0·00680	97
	0·00709	0·00700	99
	0·00712	0·00695	98

experienced in feed compounding. The second sample was prepared by each worker from the same unmedicated feeding stuff to which he added a known amount of nitrofurazone within the range 0·005 to 0·01 per cent. The results on these two samples are shown in Tables IV and V, respectively.

Appendix I

Recommended method for determining nitrofurazone in compound feeding stuffs

Principle of method—

The nitrofurazone is extracted by a selective solvent-extraction procedure, and then treated with alkali in the presence of phenol. The stabilised colour developed is compared with that produced by a known amount of the drug, similarly treated.

Applicability—

The method is applicable to medicated pre-mixes and compound feeding stuffs, of the type marketed at the time this Report was prepared, containing nitrofurazone as the only coccidiostat.

Apparatus—

Soxhlet extractor—A 100-ml extractor fitted with a B34 socket and a B24 cone (B.S. 2071 : 1954).

Extraction thimble—Whatman single, 25 mm × 80 mm.

Reagents—

Light petroleum, boiling-range 40° to 60° C.

Acetone—Analytical-reagent grade.

Dimethylformamide—*N,N*-Dimethylformamide. Test the suitability of the reagent by developing the colour from nitrofurazone with solutions of phenol and sodium hydroxide (see "Procedure" below); the colour should remain stable for at least 2 hours.

Phenol solution—A 5 per cent. w/v solution in dimethylformamide.

Potassium permanganate solution, 0·1 N.

Sodium hydroxide solution, N.

Sodium dithionite solution—A 1 per cent. w/v solution of sodium dithionite, $Na_2S_2O_4$ (sodium hydrosulphite), (not more than 6 months old) in N sodium hydroxide. Prepare this solution immediately before use.

Nitrofurazone—Complying with the requirements of the British Veterinary Codex, 1953, p. 240.

Procedure

Preliminary extraction of sample—

Weigh accurately an amount of sample containing about 1 mg of nitrofurazone, and transfer it to the extraction thimble; cover the sample with a small pad of cotton-wool. Insert the packed thimble into the extractor, assemble the extraction apparatus, and extract the sample with light petroleum; use an electric pad as the source of heat, so adjusted that the solvent cycles twenty times in about 45 minutes, and sufficient solvent so that the volume in the flask throughout the operation is not less than 25 ml.

Remove the packed thimble, allow the solvent to drain, and carefully remove any residual solvent in a current of warm air at a temperature not exceeding 60° C.

Extraction of nitrofurazone—

Transfer the packed thimble to a clean extraction apparatus, and extract the sample with acetone; use a water-bath as the source of heat so that the solvent cycles twenty times in about 1 hour, and sufficient solvent so that the volume in the flask throughout the operation is not less than 25 ml. During this extraction, shield the apparatus from light with a cardboard cylinder containing a small inspection window, or by any other suitable means.

When the extraction is complete, rapidly cool the flask containing the extract to 20° C, and add 0·1 N potassium permanganate, drop by drop, until a faint pink colour is obtained

that is persistent for about 2 seconds (about 4 drops are required). Evaporate the extract on a water-bath to a volume of about 5 ml, shielding the extract from light. *It is important at this stage to avoid evaporating to dryness.*

Remove the flask from the water-bath, place an externally ribbed conical filter into the neck of the flask, and evaporate off the residual acetone by blowing a current of warm air (temperature not exceeding 60° C) across the top of the funnel in a way such that a slight turbulence is produced on the surface of the liquid in the flask.

DETERMINATION OF NITROFURAZONE—

Dissolve the residue in dimethylformamide, transfer the solution quantitatively to a 50-ml calibrated flask, suitably shielded from light, and dilute to the mark at 20° C with dimethylformamide. Transfer a suitable portion, containing about 0·3 mg of nitrofurazone, to each of two 50-ml calibrated flasks containing 5 ml of phenol solution.

To the contents of one flask add 2·5 ml of N sodium hydroxide, and dilute to the mark at 20° C with dimethylformamide; this is the *sample solution*. To the contents of the other flask add 2·5 ml of sodium dithionite solution, and dilute to the mark at 20° C with dimethylformamide; this is the *blank solution* and it should be a pale lemon-yellow colour free from any red or purplish tinge. Spin the solutions in a centrifuge, with a radius of 6 cm, at a speed of not less than 4000 r.p.m. for 2 minutes.

Measure the optical density at 530 mμ of the clear sample solution against the blank solution in 1-cm cells with a suitable spectrophotometer.

Obtain the amount of nitrofurazone present in the sample solution by reference to a calibration curve previously prepared by plotting the optical densities obtained when known amounts of nitrofurazone are treated as described above, beginning at the addition of a suitable portion to each of two 50-ml calibrated flasks containing 5 ml of phenol solution. Hence calculate the amount of nitrofurazone in the sample.

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Improvement of Identification in the Gas - Liquid Chromatographic Analysis of Agricultural Samples for Residues of some Chlorinated Pesticides*

Part I. Improvement of Resolution on Single Columns and Application of the Multi-column "Spectrochromatogram"

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Gas - liquid chromatography with detection by electron-capture ionisation has shown itself to be a technique of considerable value in the analysis of crops, soils and animal tissues for residues of the chlorinated pesticides. The chief limitation of the method, however, lies in incomplete certainty of identification of a pesticide based on retention time alone on a single column with the resolving power at present available.

Two methods of increasing the certainty of identification of a pesticide are described in Part I of this paper.

The first is by improvement of the resolution of a single packed column; the one described gives complete separation of at least 11 of the less volatile chlorinated pesticides in a run time of 30 minutes.

The second method involves similtaneous gas chromatography on five parallel columns holding stationary phases with differing gas - liquid chromatographic characteristics. In this system the eluate is fed to a single electron-capture ionisation detector. The multi-column "spectrochromatogram" produced has an appearance, and shows retention times, that are quite different and therefore highly characteristic for each individual pesticide. By this technique more certain identification is possible, *e.g.*, in such problem analyses as aldrin in interference-containing grain or soil, and dieldrin in admixture with *pp'*-DDE in animal tissues.

THREE years ago we indicated[1] that gas - liquid chromatography with detection by electron-capture ionisation was a technique with considerable potentialities in the field of pesticide-residue analysis, and followed this up by reporting procedures for the rapid identification and determination, on the nanogram scale, of the residues of several chlorinated pesticides in crops,[2] soils[3] and animal tissues[4] by this means. The exceptional sensitivity and selectivity of the electron-capture ionisation detector to such pesticides was also appreciated by Clark[5] and later by Watts and Klein,[6] by Moore[7] and by Taylor,[8] who successfully applied this technique to residue analyses on crops and animal products, foliage and soil, and animal and avian tissues, respectively.

Interest in residue analysis by electron-capture gas - liquid chromatography has since developed quickly in the laboratories of agricultural concerns, public health authorities and instrument manufacturers, particularly in the United States, and has resulted in a rapidly increasing volume of papers either just published,[9 to 15] or in the press.

There are several advantages of this technique over alternative methods of analysis of residues of chlorinated pesticides. Firstly, the gas - liquid chromatographic step makes possible simultaneous determination of two or more pesticides; secondly, the exceptional sensitivity of the detector allows analyses to be made, without cencentration of the extract, on samples much smaller than could be used previously; and lastly, the selectivity of the detector to compounds of high electron affinity permits residues of the chlorinated pesticides to be determined in extracts of samples of biological origin with the minimum of prior clean-up.

In common with all other analytical methods, however, the technique of electron-capture gas - liquid chromatograph has its own particular limitations. One of these relates to the

* A summary of this paper was presented at the Vth International Pesticides Congress, London, July 17th to 23rd, 1963.

characteristics of the electron-capture ionisation detector, whose originator has recently given a timely warning[16] of the care needed in its application and use, if the results obtained are to be fully valid.

A more general limitation concerns the gas - liquid chromatographic step in which identification of the pesticide being analysed is normally based on the retention time produced on a single gas-chromatographic column. The shortcomings encountered here are that two pesticides may have the same retention time on one particular column or that one pesticide may have the same retention time as a naturally-occurring co-extracted product either having high electron affinity, or being present in massive amount. These failings are of particular relevance in screening analyses on samples of unknown history, when valid control material is not usually available. During the last year our efforts have therefore been directed towards resolving or, at least, alleviating this problem.

TABLE I

RESOLVING POWER OF GAS - LIQUID CHROMATOGRAPHIC COLUMNS GIVING SATISFACTORY CHROMATOGRAPHY OF A WIDE RANGE OF CHLORINATED PESTICIDES ON THE NANOGRAM SCALE

Column length, feet	Column bore, inches	Celite mesh-size, B.S.S.	Stationary phase: Silicone oil, % w/w	Stationary phase: Epikote 1001, % w/w	Nitrogen flow-rate, ml per minute	Aldrin retention time, minutes	Aldrin - Telodrin resolution
2	0·065	100 to 120	2·5	0·25	35	3·25	None
2		120 to 150	2·5	0·25	80	4·25	None
4		60 to 72	10·0	1·00	100	7·30	0·75
6		100 to 120	2·5	0·25	40	6·90	1·00
10		60 to 72	2·5	0·25	45	7·50	1·15
4	0·095	60 to 72	5·0	0·50	120	4·40	0·95
4		60 to 72	2·5	0·25	30	6·50	0·95
6		60 to 72	2·5	0·25	115	4·45	1·10
2	0·125	100 to 120	2·5	0·25	60	4·00	0·55
2		100 to 120	5·0	0·50	115	4·70	1·10
4		60 to 72	2·5	0·25	100	4·55	1·10
4		100 to 120	2·5	0·25	100	6·00	1·30*
4		85 to 100	2·5	0·25	50	7·10	1·35
10		72 to 85	0·8	0·20	100	6·75	1·70
10		100 to 120	2·5	0·25	90	34·0	1·75
10		100 to 120	0·8	0·20	85	10·0	2·15
2	0·195	100 to 120	2·5	0·25	100	3·80	0·85†
2		100 to 120	2·0	0·50	100	3·40	0·90
4		100 to 120	0·8	0·20	100	4·50	0·90

* Illustrated in Fig. 1 B and Fig. 2.

† Illustrated in Fig. 1 A.

Resolution calculated from the expression

$$\text{Resolution} = 2 \times \frac{\text{difference between retention times}}{\text{sum of peak widths}}$$

There are three obvious ways by which certainty of identification of a pesticide can be increased in gas chromatography—

(*i*) improvement of column resolution,

(*ii*) application of stationary phases with differing characteristics or

(*iii*) complementary use of detectors having dissimilar responses.

Each of these approaches has been examined. The results of our work on the first two are reported in Part I of this paper, and on the last in Part II.

Experimental and results

Improvement of resolution on single columns—

Consideration of column design parameters—When considering ways in which the resolution of a packed gas-chromatographic column might be improved, attention was paid to the stationary phase, the supporting medium and also to the dimensions and material composition of the column itself.

For the stationary phase, no compound was found that would give a basic resolution superior to that of a non-polar silicone oil or elastomer.

As supporting medium, plain Celite, probably the most widely used material, was preferred. Although acid-washed Celite or other supports, *e.g.*, Diatoport, can be used with silicone oil alone, endrin may be at least partially decomposed at the nanogram level. With plain Celite, of course, the addition to the silicone of Epikote 1001, in 10 + 1 ratio, is necessary to avoid adsorption or decomposition effects. This small addition has no marked effect on the chromatographic characteristics of the silicone oil.

As a column material, copper has several advantages. It is cheap, robust and easily worked. It is certainly more reactive than borosilicate glass,[17] quartz[18] or polytetrafluoroethylene, but this disadvantage is obviated in the presence of the Epikote 1001 additive.

In developing conditions for improved resolution we imposed a limit of about thirty minutes for the gas - liquid chromatographic run-time, since protracted run-times reduce the attractiveness of the method and, moreover, increase the decomposition problem.

Run times can, of course, be reduced by decreasing the amount of stationary phase or increasing the column temperature or the gas flow-rate. The effect of marked reduction of the first would be to increase insecticide decomposition. This, coupled with increased vapour loss of the Epikote would also occur with the second. The gas flow-rate of 100 ml per minute at 163°C was already considered high by normal gas - liquid chromatograhic standards and was likely to be limited by increase in the drop of gas pressure when longer columns were used.

The two main variables remaining to be examined in the experiments on packed columns were, therefore, those of column length and bore.

Some consideration was also given to the application of capillary columns in this field of work. Such columns have found wide use in the petroleum and petrochemical industries because of their exceptional resolving power in the gas - liquid chromatography of relatively volatile materials, but little has been reported[19] on their value in pesticide residue analysis.

Evaluation of packed copper columns—Experiments were carried out to determine the resolution obtained, particularly for an aldrin - Telodrin* mixture, on packed copper columns varying in length from 2 to 10 feet and in bore from 0·065 to 0·195 inches. These columns were packed with various concentrations and ratios of silicone oil - Epikote 1001 mixtures as stationary phase, supported on plain Celite, ranging in mesh size between 60 to 72 and 120 to 150 (B.S.S.). The flow rate of nitrogen carrier gas was varied from 30 ml per minute to 120 ml per minute; the maximum inlet pressure used was 45 p.s.i.g. Column temperature was maintained at 163° C throughout.

The results obtained in these tests are summarised in Table I. Appraisal of these results and other relevant factors led to the conclusion that, within the run-time limit we imposed, the most suitable column for general use consisted of a 4-foot length of 0·125-inch bore copper tubing, packed with 2·5 per cent. w/w silicone oil plus 0·25 per cent. w/w Epikote 1001 supported on 100- to 120-mesh plain Celite maintained at 163° C and with a nitrogen flow-rate of 100 to 120 ml per minute.

A chromatogram of a mixture of aldrin, Telodrin, dieldrin and endrin run under the above conditions is shown in Fig. 1 B, and may be compared with a chromatogram of the same mixture, shown in Fig. 1 A, run on the standard 2 feet long × 0·195-inch bore type of column we were using[2] 2 years ago. It will be noted that with the new column, near-baseline resolution is achieved with both pairs of insecticides, though with an increase in run time of about 60 per cent.

The greater resolution of the new column permits satisfactory chromatography of a mixture of several more chlorinated pesticides (present only in nanogram amounts) than was

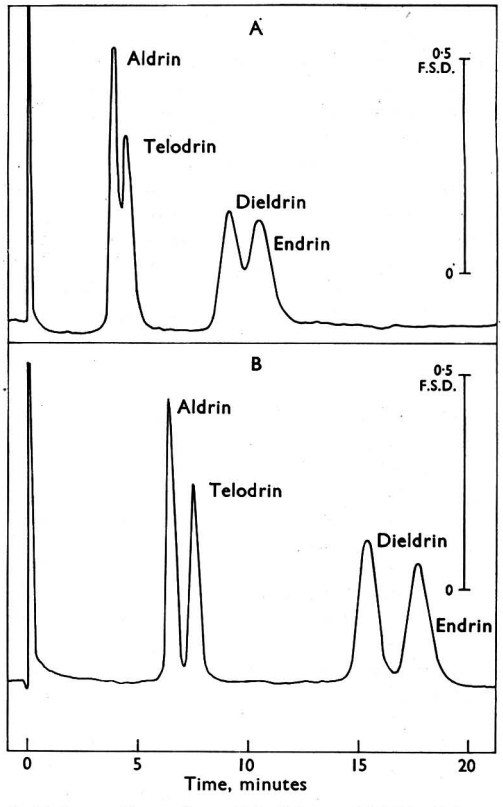
* Telodrin is the Shell Trade Mark name for 1,3,4,5,6,7,8,8-octachloro-1,3,3a,4,7,7a-hexahydro-4,7-methanoisobenzofuran.

previously possible. Fig. 2 shows a chromatogram in which thirteen of the less volatile pesticides are fully resolved in all but two instances.

Evaluation of copper capillary columns—Most of the work on capillary columns was with copper as column material. The attractions here were, again, cheapness and ease of working. For example, no difficulty was encountered in obtaining gas-tight joints: this was not so when polytetrafluoroethylene was used as capillary tubing.

The columns tested had a bore of 0·018 inches and varied in length from 25 to 125 feet. They were coated with silicone oil, with or without added Epikote 1001, by means of the dynamic method involving a plug of stationary-phase solution.

Because of the very low concentration of pesticides present in the extract used, splitting of the 1 to 8 μl sample on injection was not possible. A polytetrafluoroethylene insert was, therefore, located at the inlet of the injection port to reduce its dead volume and thereby minimise diffusion.



A: Column dimensions, 2 feet long, 0·195-inch bore
B: Column dimensions, 4 feet long, 0·125-inch bore

Fig. 1. Electron-capture gas chromatogram, showing resolution of chlorinated-pesticide pairs on packed columns

A temperature of 163° C was used throughout, with nitrogen carrier-gas flow-rate ranging from 2 to 20 ml per minute. For detection, a planar-source electron-capture ionisation cell of low (0·1 ml) internal volume was used, and was fed with nitrogen scavenger gas to maintain an adequate flow rate (50 ml per minute) through the detector.

When a mixture of chlorinated pesticides, present in nanogram amounts, was analysed on the gas - liquid chromatographic column, decomposition was found to be appreciable except when Epikote was present. Further, in most experiments, resolution was much inferior to that obtained on normal packed columns.

The best capillary column prepared was 125 feet long and was coated with a 2 per cent. w/v solution of silicone oil and Epikote 1001 in a 10 + 1 ratio in ethyl acetate. This column, however, had a resolution only slightly better than that of the standard packed column used to produce Fig. 1 A and, moreover, effected no decrease in run time.

APPLICATION OF THE MULTI-COLUMN "SPECTROCHROMATOGRAM"—

General considerations—In earlier papers[2,4] we drew attention to two ways in which uncertainty of identification of a pesticide, caused by the presence of interfering material of coincident retention time, might be overcome. These were to effect resolution of the two components by use of a second (polar) column, whose gas - liquid chromatographic characteristics differed from the standard (non-polar) column; or, alternatively, to remove the interfering component previously by liquid - liquid chromatography.

The latter technique is not always successful[14] and is of limited value in screening analyses on samples of unknown history, since loss of other sought-for pesticides or their metabolites may occur during the process.

The first-mentioned technique was pursued in the Tunstall Laboratories of "Shell" Research Ltd. by Robinson and Richardson,[20] who determined the relative retention of eight chlorinated pesticides on single columns of Apiezon L and Nonidet P40 as well as the silicone elastomer E301 and Epikote 1001 used by ourselves.

We therefore examined the usefulness of simultaneous gas - liquid chromatography on multiple parallel columns, each containing stationary phases of differing characteristics, as a method for increasing the certainty of identification of pesticides, particularly when these suffer interference from other pesticides or from naturally-occurring co-extracted material having coincident or near-coincident retention times on a standard (non-polar) column. The chromatograms so produced we have tentatively called "spectrochromatograms," because they are indicative of the spectrum of selectivity of the various stationary phases towards individual chlorinated pesticides.

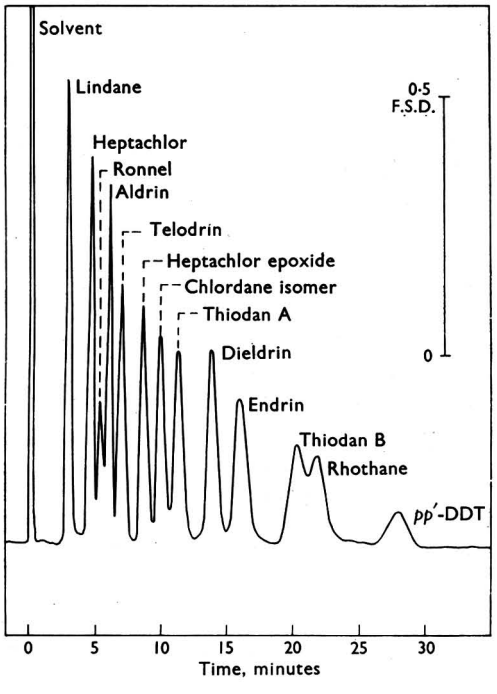


Fig. 2. Electron-capture gas chromatogram, showing resolution of thirteen chlorinated pesticides on a packed column: 0·4 nanograms of each pesticide

Requirements for, and evaluation of, stationary phases—It was decided to develop a multi-column system having five stationary phases; this was the maximum number that it was felt could reasonably be handled operationally. These five phases would ideally comprise two or three of proven general applicability in the chlorinated pesticide field, while the others would be present mainly because of their ability to resolve obvious problem pairs, *e.g.*, aldrin and grain interference or dieldrin and *pp'*-DDE mixtures.

The aim in searching for suitable stationary phases was that they should cover the widest possible range of polarity and structure, should be stable at the operating temperature of 163° C and, in particular, exhibit insufficient bleed to affect the highly sensitive electron-capture ionisation detector.

In addition to the silicone oil, Epikote 1001 and Apiezon L already referred to, twenty-one materials were evaluated as potential stationary phases on plain Celite for the gas - liquid chromatography of a test mixture of seven chlorinated pesticides. These pesticides were: indane, heptachlor, aldrin, Telodrin, dieldrin, endrin and *pp'*-DDT.

The stationary phases chosen are grouped below under three headings:

1	2	3
Behenic acid	Adipic acid	Diethylene glycol succinate
Calcium gluconate	Araldite	Dulcitol
D(+)Glucose	Mannitol	Nitrile silicone GE–XE–60
Mannitol hexa-acetate	Nitrile silicone XF–1150	Nitrile silicone XF–1112
Polyglycerol	Nonidet P40	Salicin
Sodium carboxymethylcellulose	P.E.G. 4000	D(−)Sorbitol
	P.E.G. 6000	
	P.E.G. 15–20,000	
	Zinc stearate	

The first group (1) was quite unsatisfactory (giving little or no chromatography); the second group (2) was partially successful (chromatography of 3 or 4 pesticides); while the last group (3) was the most successful (chromatography of 5, 6 or 7 pesticides).

It was clear from earlier work[2,20] that addition of Epikote 1001 to either the silicone or Apeizon stationary phases on plain Celite was essential if adsorption or decomposition of some of the chlorinated pesticides was to be avoided. The effect of this additive on salicin, nitrile silicone GE–XE–60 and diethylene glycol succinate was therefore examined. Only with the latter compound was there significant improvement in chromatography, although even here partial loss of heptachlor and total loss of *pp'*-DDT still occurred.

This work led to a consideration of other ways in which such adsorption - decomposition effects could be overcome. Pre-treatment of the stationary phase and supporting medium with tris-(2-biphenylyl) phosphate (Dow K–1110) was reported by Gunther, Blinn and Kohn,[21] but the success of this method was not confirmed by Beckman and Bevenue.[18] As an alternative to Epikote 1001, we found that Araldite, a proprietary epoxy resin adhesive, worked as well for the above purpose. The use of such epoxides resulted in experiments on the pre-treatment of plain Celite with epichlorhydrin under reflux. It was found that Celite so treated permitted the test mixture of the seven pesticides to be analysed by gas-liquid chromatography on the nanogram scale without decomposition, when silicone alone was used as stationary phase. This work has yet to be further investigated.

Design, composition and operation of the multi-column assembly—A multi-column assembly head was constructed in brass. The injection port led via a short capillary manifold to the inlets of five parallel U-shaped copper columns each 4 feet long with a 0·125-inch bore. The outlets of these five columns were then led via a second short capillary manifold, also located in the assembly head, to the inlet of a single planar-source electron-capture ionisation detector, whose internal volume was about 0·1 ml. All column connections were made with Simplifix fittings.

In preliminary tests severe decomposition of some pesticides was traced to contact with the metal of the assembly head. This decomposition was suppressed by washing the head out with a 2 per cent. w/v solution of silicone oil and Epikote 1001 mixture, in 4 + 1 ratio in ethyl acetate, followed by evaporation of the solvent before attaching the columns.

The final choice of five stationary phases, based on the requirements and evaluations given earlier, is given below, together with the concentrations used. These were carefully

chosen after appreciable experimentation to give peak resolution of as many chlorinated pesticides, metabolites and artifacts as possible.

(*a*) 2·5 per cent. w/w silicone oil (May and Baker Ltd.) + 0·25 per cent. w/w Epikote 1001 (Shell Chemical Co. Ltd.).

(*b*) 1·0 per cent. w/w Apiezon L (A.E.I. Ltd.) + 0·2 per cent. w/w Epikote 1001.

(*c*) 1·0 per cent. w/w Epikote 1001.

(*d*) 3·3 per cent. w/w nitrile silicone fluid XF–1112 (General Electric Co., U.S.A.).

(*e*) 2·7 per cent. w/w nitrile silicone fluid GE–XE–60 (Applied Science Labs. Inc.; U.K. Agents, W. G. Pye & Co. Ltd., Cambridge).

The procedure for residue analysis with the multi-column assembly was essentially the same as that described previously for single column operation, except that injection volumes were increased fivefold to 25 μl to compensate for splitting of the charge into the parallel columns. The crop, soil or tissue sample was macerated with the minimum volume of

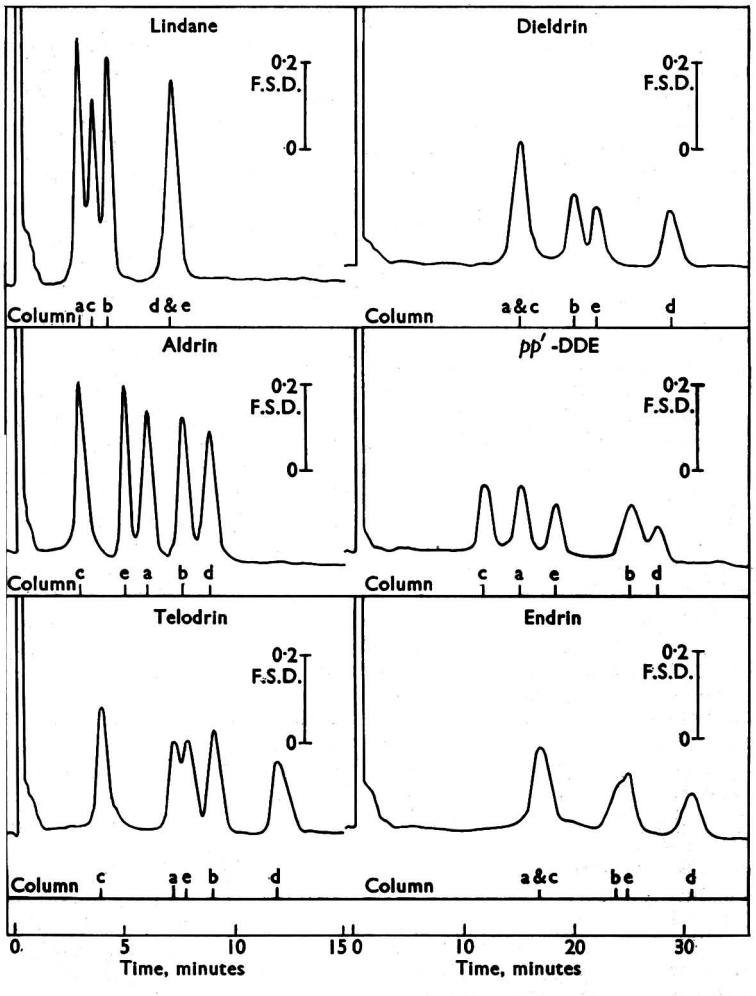


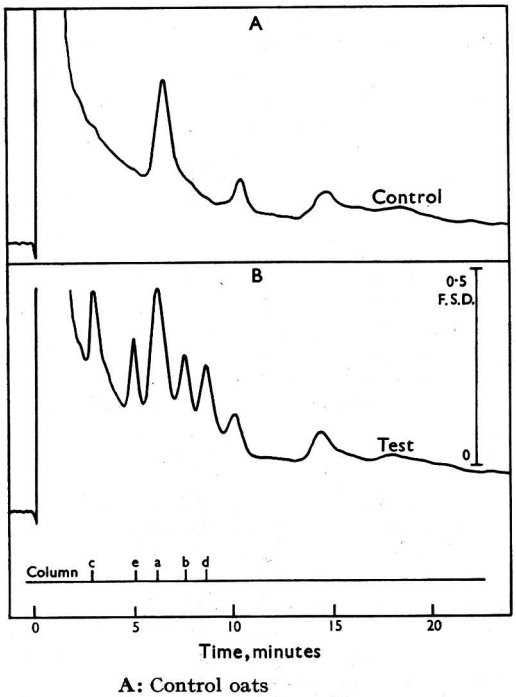
Fig. 3. Multi-column "spectrochromatograms" of some chlorinated pesticides: injection, 25 μl ≡ 2·5 nanograms of each pesticide

redistilled analytical-reagent grade acetone to give a fluid macerate that was filtered through sintered glass. The filtrate was made up to two volumes per unit weight of sample. A portion of this acetone extract was then shaken with twice its own volume of redistilled petroleum spirit (boiling-range 62° to 68° C), in the presence of excess aqueous sodium sulphate solution. This petroleum-spirit extract was used for the injection.

Column temperature was maintained at 163° C, with the nitrogen carrier-gas flow-rate through each column at the normal rate of 100 ml per minute. The flow-rate was set at this level to keep retention times as short as practicable, although this resulted in a very high combined flow-rate of 500 ml per minute to the detector, which the latter accepted without malfunction.

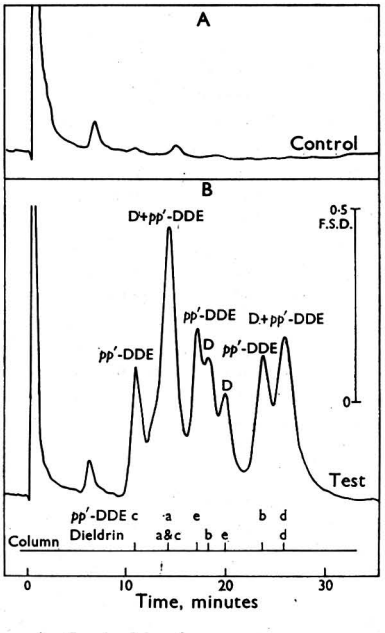
Evaluation of "spectrochromatograms" and multi-column performance—By using the above-mentioned system, multi-column "spectrochromatograms" were produced for a range of the less volatile chlorinated pesticides. Those for lindane, aldrin, Telodrin, dieldrin, *pp'*-DDE and endrin are shown together in Fig. 3. In general, 3 to 5 distinct peaks are obtained and show retention times which, considered together, provide good evidence for the identification of the compound present. For example, on our standard silicone and Epikote column of two years ago (see Fig. 1 A), aldrin was only partially resolved from Telodrin: in Fig. 3, however, it will be seen that the "spectrochromatogram" of aldrin is quite different both in appearance and indicated retention time from that of Telodrin. Similarly, on the standard column dieldrin was only partially resolved from endrin and not at all from *pp'*-DDE: Fig. 3 shows their "spectrochromatograms" to be appreciably different.

Most grain crops (wheat, oats, barley, maize and rice) have been found to contain materials that have the same retention time as aldrin on the standard column. The results of applying the multi-column system to this problem are shown in Fig. 4 in which, despite the presence



A: Control oats
B: Oats containing 0·25 p.p.m. of aldrin

Fig. 4. Multi-column "spectrochromatogram" of control and aldrin-treated oats: injection, 25 μl ≡ 6 mg of crop



A: Control beef
B: Beef containing 1·0 p.p.m. of *pp'*-DDE and 0·5 p.p.m. of dieldrin

Fig. 5. Multi-column "spectrochromatogram" of control beef and beef containing dieldrin and *pp'*-DDE: injection, 25 μl ≡ 6 mg of tissue

of the naturally-occurring interference, peaks whose appearance and retention times are characteristic of the aldrin "spectrochromatogram" are clearly evident in the aldrin-treated sample. Similar results were obtained on interference-containing soil samples.

Another problem is the one in which two insecticides that are unresolved on the standard column may well be present together in the test sample. Such an example is that of dieldrin and *pp'*-DDE in animal tissues. The "spectrochromatogram" produced for this mixture is shown in Fig. 5, in which it can be seen that there is sufficient characterisation to identify these two pesticides.

The reproducibility of "spectrochromatogram" retention times was found to be as good as on single columns. Reproducibility of splitting of the injection charge between the five parallel columns was, however, less satisfactory and was thought to be limited by the design of the particular assembly head used.

The life of the multi-column assembly was found to be restricted to about two weeks owing to ageing or vapour loss of some of the stationary phases, in particular the nitrile silicone XF–1112 and Epikote 1001. These effects resulted in an increase in pesticide decomposition with time coupled with changes in the retention times shown by the "spectrochromatogram" peaks. In consequence it was found advisable to determine the reference "spectrochromatograms" at the same period of time as those of the test samples were being run.

An indication of the value of the multi-column "spectrochromatogram" technique in screening work was obtained inadvertently when a wide variety of locally purchased "control" crops was being examined for use in work on the complementary detection system to be reported in Part II of this paper. Clearly-defined "spectrochromatograms" showed traces of identifiable chlorinated pesticide in one or two of these samples.

Discussion and conclusions

The development work on single packed columns described earlier in this paper produced a useful though not marked gain in resolution in a column having a general use for screening purposes. In consequence the 4 feet × 0·125-inch bore column is to be preferred to the 2 feet × 0·195-inch bore column used previously. It does not, however, greatly improve the certainty of identification of any particular pesticide. Against this may be set the fact that it had to be designed to give rapid chromatography of nanogram amounts of a very wide range of chlorinated pesticides (see Fig. 2). In more specialised circumstances, for example, when longer run times are no detriment, a valuable increase in resolution can be achieved by using 0·125-inch bore columns of much greater length.

The brief study of capillary columns proved disappointing, considering the known value of these columns in other fields of work. The main problem here lies in the extremely low dilution of the extract solution being analysed, rendering the stream splitting normally used in capillary work impracticable. There remains, nevertheless, much scope for further development in the application of capillary columns to pesticide-residue analysis, not only on solving the above-mentioned injection problem but in evaluating columns of other bores, lengths and materials and different stationary-phase systems.

As a means for improving the certainty of identification of residues of the chlorinated pesticides, the multi-column "spectrochromatogram" technique showed more promise than the others referred to, and the method could well be applied as a supplementary confirmatory technique for those samples that had given an indication on a short primary screening column of the possible presence of pesticide. The multi-column assembly is, of course, more troublesome to prepare, has a shorter working life than the single column and, at present, is only roughly quantitative. Against this, however, the method is capable of further improvement. Re-design of the assembly head, possibly in stainless steel, aluminium or glass may reduce the tendency for pesticide decomposition inside it, thus avoiding the need for the silicone and Epikote pre-treatment. At the same time, an improvement of the reproducibility of splitting of the charge into the parallel columns might be achieved. This would further increase certainty of pesticide identification by giving more reproducible "spectrochromatograms," and would also improve the extent to which the technique may be used quantitatively. A further contribution of value would be the introduction of alternative stationary phases; the two main requirements here are thermally stable and highly polar compounds.

We thank Mr. D. M. Barnett for much careful experimentation, Mr. G. F. Poulton for the design and construction of the multi-column assembly, and Dr. R. A. E. Galley, Director, and Mr. J. G. Reynolds, Associate Director of the Woodstock Agricultural Research Centre, for their interest and encouragement during the course of this work.

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Improvement of Identification in the Gas - Liquid Chromatographic Analysis of Agricultural Samples for Residues of some Chlorinated Pesticides*

Part II. A Halogen-sensitive Detector in Complementary or Alternative Use to an Electron-capture Ionisation Detector

By R. GOULDEN, E. S. GOODWIN and L. DAVIES

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Gas - liquid chromatography with detection by electron-capture ionisation has found considerable use in the analysis of crops, soils and animal tissues for residues of the chlorinated pesticides. The chief limitation of the method, however, lies in incomplete certainty of identification of a pesticide based on retention time alone on a single column of the resolving power at present available.

Two methods of increasing certainty of identification, the use of single columns of higher resolving power and the application of the multi-column "spectrochromatogram," were described in Part I of this paper.

In Part II, a third method based on simultaneous detection by two highly sensitive and selective detectors with dissimilar response characteristics to individual chlorinated pesticides is described. One of these sensors is the electron-capture ionisation detector, and the other is a halogen-sensitive cell of the type used in refrigeration leak detection. The last-mentioned detector has great potential value in residue analysis for the chlorinated pesticides. It is sensitive to the nanogram range, is more selective than the electron-capture ionisation type and has greater linearity of response. Moreover, it is relatively cheap and can be operated with simple circuitry.

Gas - liquid chromatography with detection by electron-capture ionisation has rapidly proved itself[1,2,3,4] to be a technique of particular value for detecting and determining residues of chlorinated pesticides in crops, soils and animal tissues. Its main advantages over alternative methods are greater sensitivity, greater selectivity and its ability to analyse for more than one component at the same time. These advantages result in a method in which extracts of small samples can be analysed rapidly for nanogram amounts of several pesticides without a concentration step, and with the minimum of prior clean-up.

Although care is necessary in the proper operation[5] of the detector itself, the main limitation of the method lies in the degree of certainty of identifying the individual pesticides, when this identification is based only on retention times obtained on a single column of the resolving power at present available. This factor is most relevant in screening analyses on samples of unknown history, when valid control material is not usually available.

In Part I of this paper,[6] two methods intended to overcome or reduce this problem were described: the use of single columns of higher resolving power than that of earlier versions, and the production of multi-column "spectrochromatograms" having an appearance and showing retention times highly characteristic of the individual pesticides being analysed.

A third approach, using highly sensitive detectors whose responses to the halogenated pesticides differ, is described in Part II. A detector is needed that has the unusual properties of exceptional sensitivity and high selectivity to the halogenated pesticide, but significantly different response characteristics from the electron-capture ionisation detector with which it could be used in tandem. Such a device is the halogen-sensitive element used in the Type HA leak-detector made by Associated Electrical Industries Ltd.

The idea of applying a halogen-sensitive device of this type to the analysis of pesticide residues is not new, for in 1952. C. A. Reilly, working in the Laboratories of the Shell Development Company at Emeryville, California, reported on a determined effort to use the General Electric Type H leak-detector of similar design to the Type HA for this purpose. This

* A summary of this paper was presented at the Vth International Pesticides Congress, London, July 17th to 23rd, 1963.

work, however, was not altogether successful, partly because the use of the detector was not preceded by a gas - liquid chromatographic step, and partly because of the electrical or electronic instability of the systems examined.

Much more recently the successful use of this type of halogen-sensitive element as a detector in the gas - liquid chromatography of volatile chlorinated hydrocarbons was briefly reported by Cremer, Kraus and Bechtold[7] in Germany. In this detailed study, high selectivity to chlorinated compounds was recorded, and such a sensitivity was developed that 0·3 nanograms of chlorine could be detected under favourable conditions.

This lead was followed in the Tunstall Laboratory of "Shell" Research Ltd. by A. Richardson, who showed that the A.E.I. halogen-sensitive element already referred to would function as a gas - liquid chromatographic detector, and could provide a relatively high sensitivity with the aid of only simple circuitry.

We therefore undertook a detailed evaluation of two versions of this halogen-sensitive device with the object of using it for determining residues of the chlorinated pesticides on the nanogram scale, and of operating it in tandem with an electron-capture ionisation detector as a method for increasing the certainty of identifying these compounds.

Experimental and Results

Description and operation of the halogen-sensitive element—

The A.E.I. Ozotron Type H halogen-sensitive element,[8] shown diagrammatically in Fig. 1, consists of a pair of concentric platinum cylinders mounted within a protective borosilicate glass envelope through which, in normal operation, the air to be tested for traces of halogenated compounds is drawn at a rate of about 150 ml per minute. The inner platinum

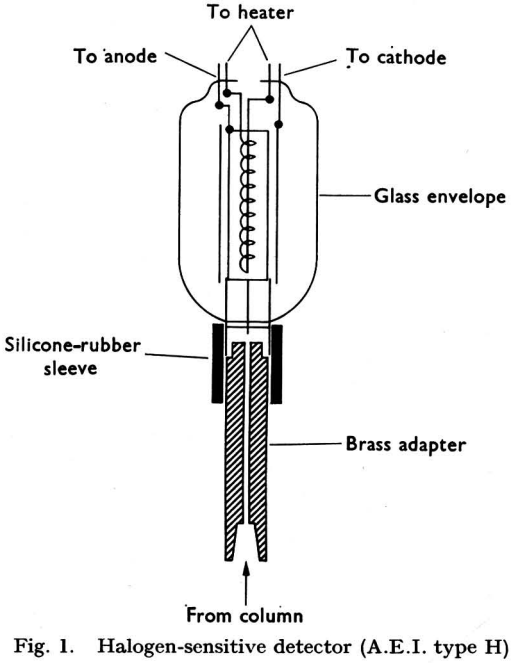


Fig. 1. Halogen-sensitive detector (A.E.I. type H)

cylinder, or anode, which has been sensitised by an alkali treatment, is indirectly heated by an internal platinum filament taking a current of 7·4 amps at 6 volts a.c., and operates at a temperature of about 800° C. An anode-to-cathode potential of 250 volts d.c. is normally used, resulting in the production of a positive-ion standing current that can be suitably amplified and presented. When air, containing halogen vapour, is drawn through the cell there is an apparent increase in the positive-ion current, whose magnitude is indicative of the concentration of halogen vapour present.

A newer version of the above detector has recently become available. This model, the Ozotron Type J, is basically the same as the Type H except that the element is mounted more rigidly in a two-piece ceramic envelope from which the platinum heater filament and electrodes are easily removed.

DESIGN OF CIRCUITS AND PERFORMANCE OF HALOGEN-SENSITIVE DETECTORS—

An Ozotron Type H halogen-sensitive element was connected up as detector to a 4 feet long × 0·125-inch bore silicone - Epikote column of the type described in Part I of this paper. The detector circuitry used is shown diagrammatically in Fig. 2(*a*). Power for the

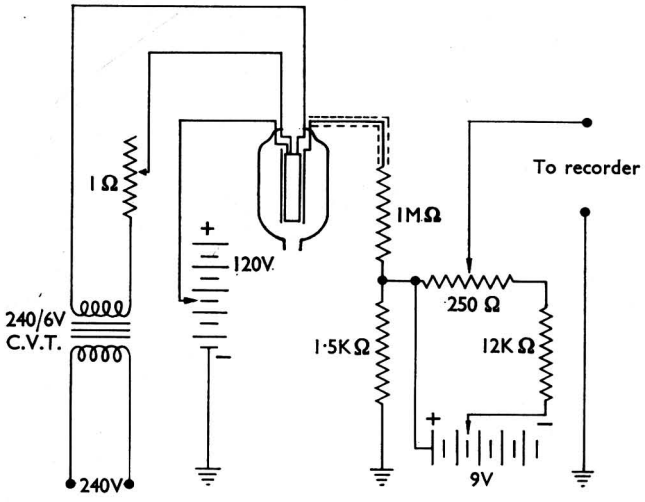


Fig. 2 (*a*). Simple circuit for halogen-sensitive detector

heater filament was supplied from a 240 to 6V step-down transformer connected in series with a 1-ohm sliding resistance, and the potential required for the anode was taken from 0 to 120 V high tension batteries. The signal from the cathode was led, via a 1-megohm load in series with a 1500-ohm input resistor (backed off by a 0 to 9 V grid-bias battery controlled by a wire-wound variable resistor), before being led directly into a 1-mV full-scale deflection chart recorder having a maximum input impedance of 1000 ohms and a pen-response time of 2 seconds.

An evaluation of detector performance was made under these conditions by injecting 1 to 10 μl volumes of dilute solutions of chlorinated pesticides in light petroleum (boiling-range 62° to 68° C) into the gas - liquid chromatographic apparatus at a column temperature of 163° C, while varying the operational parameters. Nitrogen was used as carrier gas at various flow-rates in the range 100 to 200 ml per minute, with and without the admission of air at the inlet of the detector.

It was found preferable to use separate leads to the anode and inner heater terminal instead of a common one as indicated by the manufacturer, as the latter procedure resulted in a higher electrical noise level.

Sensitivity, both to chlorinated pesticides and to light-petroleum solvent, increased with increase in heater voltage, but in favour of the solvent. In consequence it was impracticable to work at the highest heater voltage (6 volts), as the large solvent response obtained masked the early part of the chromatogram. Below about 4 volts, however, sensitivity rapidly decreased.

Similarly, increase in the potential applied to the anode of the cell resulted in an increase in both sensitivity and electrical noise level. Disproportionate increase of the latter made it desirable to operate at comparatively low potentials, although below about 36 volts d.c. a marked increase in peak tailing seriously affected the chromatograms obtained.

The time-constant of the cell was observed to be rather longer than that for the electron-capture ionisation type of detector, and in consequence, higher nitrogen flow-rates (greater

than 100 ml per minute) resulted in improved chromatogram peak shape, but at the expense of some sensitivity. Admission of air to the inlet of the detector to effect oxidative combustion of the pesticides rather than pyrolysis, produced a marked increase in the standing current of the cell and a reduction of sensitivity that became significant at air flow-rates in excess of about 5 ml per minute.

The level of sensitivity obtainable by using the above-mentioned simple circuitry and with a power input to the heater filament of 4·6 volts a.c. at 6·7 amps, an anode potential of +36 volts d.c. and a nitrogen carrier-gas flow-rate of 100 ml per minute at 163°C, corresponded to a limit of detection of about 2 nanograms for aldrin (approximately twice the noise level). Response of the detector *versus* pesticide load was observed to be linear up to recorder full-scale deflection, which for aldrin occurred with 100 nanograms.

This sensitivity, while encouraging, was not quite sufficient for our purpose, and a more sensitive version of the circuit, shown diagrammatically in Fig. 2(*b*), was therefore developed.

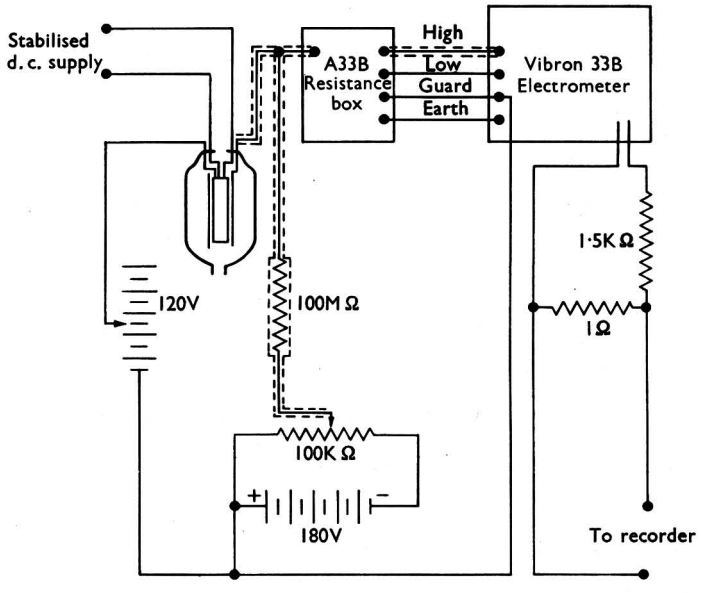


Fig. 2 (*b*). More sensitive circuit for halogen-sensitive detector

With this arrangement it was found essential to provide the heater filament with a more stable power supply than was given by the step-down transformer. Replacing the latter by a heavy duty 6 volt accumulator proved satisfactory, except that the high current consumption resulted in a gradual drift of the recorder baseline as the accumulator was discharged. The latter was therefore replaced by a comparatively inexpensive transistorised Zener-diode stabilised d.c. power supply, specially designed for the purpose.

In this more sensitive circuit the signal from the cathode was led through a 1-megohm input resistor in an Electronic Industries Ltd. model A33B current- and resistance-measuring bridge, backed off similarly to the one described above, and then passed into an E.I.L. Vibron model 33B vibrating-reed electrometer, before being fed via appropriate series - parallel matching resistors to the 1-mV recorder.

Under the same operating conditions as described above, the limit of detection for aldrin with this circuitry was about 0·1 nanograms at the 100-mV attenuation setting. Again, good linearity of response with increase in pesticide load was observed up to recorder full-scale deflection at the 1000-mV (maximum) attenuation setting, which for aldrin occurred with 30 nanograms.

A similar evaluation of the ceramic-protected Ozotron Type J halogen-sensitive element was also undertaken. The performance characteristics observed were similar in some respects to those outlined above.

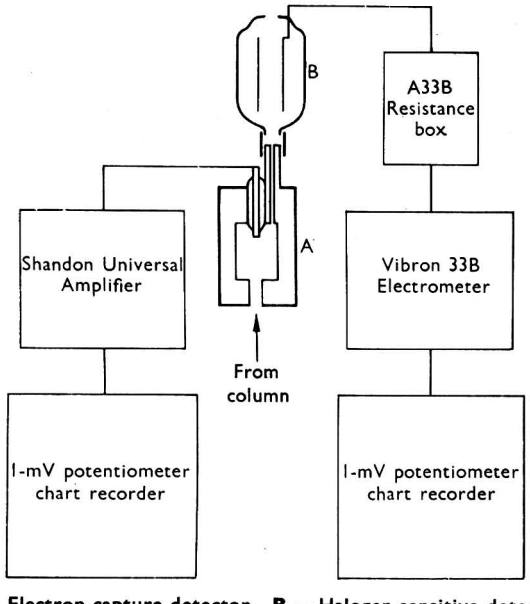
In this work it was found essential to ensure that all signal-carrying leads were fully screened by using co-axial cable, and that electrical connections, particularly the leads to the heater elements, were soundly made, *e.g.*, brazed or silver-soldered.

The two least satisfactory features of the Type H and J detectors when used in the manner described, were their general electrical instability and the need for conditioning them appreciably before high sensitivity could be achieved. The tendency towards electrical instability was considered to be caused partly by some lack of rigidity in the location of the concentric platinum electrodes (in the Type H) and partly by shorting of the electrodes, perhaps by carbonaceous deposits resulting from pyrolysis of the samples. Such deposits could easily be removed by cleaning the electrodes in acetone; this was facilitated for Type J by the ease with which the cell could be taken apart.

The conditioning of the cells and their maintenance in a state of high sensitivity was the biggest problem encountered, and has not yet been completely solved. A variety of conditioning processes were tried with five such cells, including pre-treatment with air, nitrogen or massive loads of chlorinated material, and washing the anode and gentle abrasive cleaning of the cathode in acetone. Of these, pretreatment with air shows promise of being the most effective. The results quoted above were obtained on the most sensitive of the detectors examined and this one (a Type H) was used to produce the halogen-detector chromatograms reproduced in this paper.

EVALUATION OF HALOGEN-SENSITIVE AND ELECTRON-CAPTURE IONISATION DETECTORS IN COMPLEMENTARY OPERATION—

An Ozotron Type H halogen-sensitive element was connected by means of a short brass capillary tube and silicone rubber sleeve on to the outlet port of an electron-capture ionisation detector. This, in turn, was connected to a 4 feet × 0·125-inch bore copper column that was packed with 2·5 per cent. w/w of silicone oil *plus* 0·25 per cent. w/w Epikote 1001, supported on 100- to 120-mesh plain Celite. The column was operated at a temperature of 163° C, and a nitrogen flow-rate of 100 ml per minute was maintained. For the Ozotron, the more



A = Electron-capture detector B = Halogen-sensitive detector

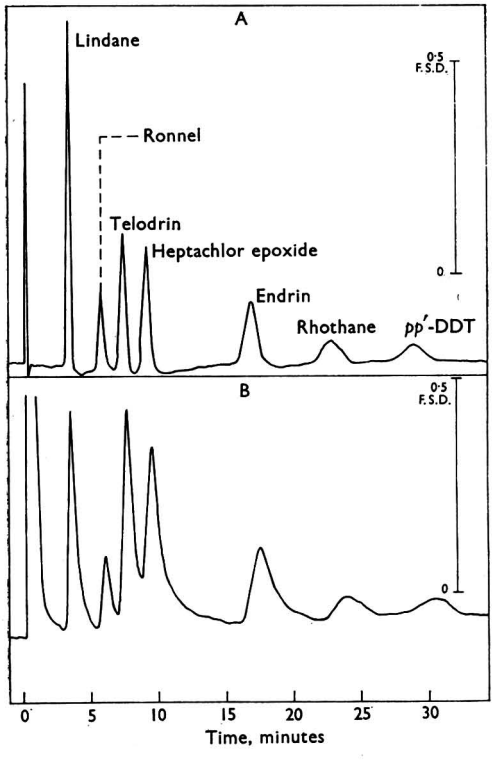
Fig. 3. Simultaneous complementary detection system

sensitive circuit was used, and the signals from both detectors after attenuation or amplification as appropriate were fed, as shown in Fig. 3, into separate 1-mV recorders running at the same chart speed. By this arrangement simultaneous chromatograms from these two different detection systems could be produced for solutions containing nanogram amounts of the chlorinated pesticides.

An example of this is illustrated in Fig. 4, in which the simultaneous halogen-sensitive and electron-capture chromatograms were produced on the injection of 2·3 μl of a petroleum spirit solution, containing 2·3 nanograms each of lindane, ronnel, Telodrin, heptachlor epoxide, endrin, Rhothane and *pp'*-DDT. It will be noted that the relative response of the two detectors is different, particularly for lindane, Telodrin and endrin.

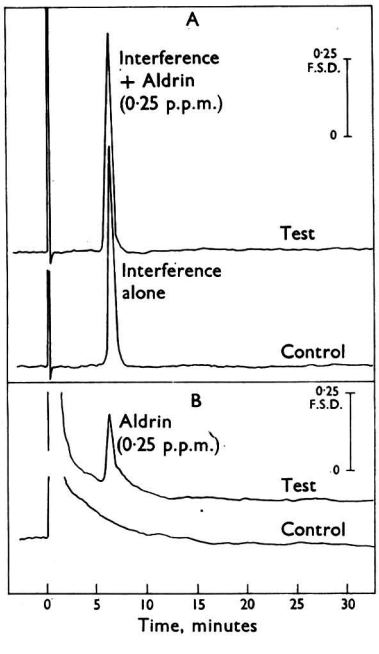
This difference in relative response can be more marked when sensitivity to chlorinated and non-chlorinated material is considered. In the top half of Fig. 5, the electron-capture chromatogram of "control" wheat before and after the addition of 0·25 p.p.m. of aldrin can be seen. As has been previously pointed out,[1] this control sample, typical of the grain crops, exhibits massive electron-capture interference from a naturally occurring co-extractive, whose retention time is the same as that of aldrin. In the simultaneous halogen-sensitive chromatograms, no such interference is evident. The complementary use of the two detectors could therefore be of much value in increasing the certainty of identification of any pesticide indicated as present in the sample.

The value of the difference in relative response of the two detectors to individual pesticides is shown in Fig. 6, in which, under standard operating conditions, reproducible relative responses (as measured by peak area ratios) provide good confirmatory evidence for the



A: Electron-capture detector
B: Halogen-sensitive detector

Fig. 4. Simultaneous complementary gas chromatograms of seven chlorinated pesticides: 2·3 nanograms of each pesticide.



A: Electron-capture detector
B: Halogen-sensitive detector

Fig. 5. Simultaneous complementary gas chromatograms of control and aldrin-treated wheat: injection, 5 μl ≡ 6 mg of crop.

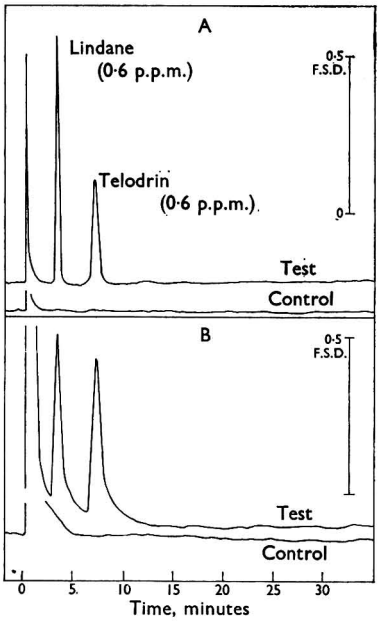
identification of the pesticides lindane and Telodrin, added to spring cabbage to the extent of 0·6 p.p.m.

In the last-mentioned chromatogram, the area of the halogen-sensitive detector peak for Telodrin (chlorine content, 68·8 per cent.) is greater than that for lindane (chlorine content, 73·1 per cent.), indicating that, for this detector, the response obtained is not simply a function of the chlorine content of the molecule.

Another example of simultaneous complementary halogen-sensitive - electron-capture gas - liquid chromatography is illustrated in Fig. 7, which shows the dual chromatograms produced by the addition of 1 p.p.m. of either dieldrin or *pp'*-DDE to "control" lamb. The retention times of these two pesticides on a single silicone - Epikote column are nearly coincident, with the consequent possibility of confusion between them. With the complementary detector system, however, the calculated peak area ratios, 1·3 and 1·1 for dieldrin and *pp'*-DDE respectively, could provide sufficient evidence to permit reasonable distinction between the two pesticides.

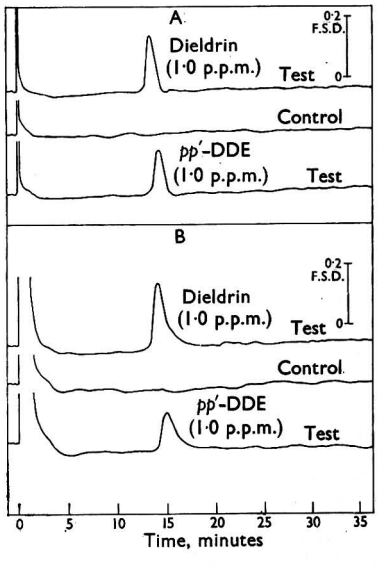
Discussion and conclusions

One of the chief merits of the halogen-sensitive detectors described lies in their exceptional selectivity towards the halogenated pesticides compared with natural materials co-extracted from crops, soils or tissues. There is evidence that this selectivity is significantly superior to that shown by the electron-capture ionisation type of detector. This is particularly marked in the example shown in Fig. 5. Certainly, in the screening of a wide range of crops with the halogen-sensitive detector, no sample containing non-pesticidal material gave a response greater than that for electron-capture ionisation detection, and there were indications that in general the superiority in selectivity was about tenfold.



A: Electron-capture detector (EC)
B: Halogen-sensitive detector (HS)

Fig. 6. Simultaneous complementary gas chromatograms of control and lindane - Telodrin treated spring cabbage: injection, 5 μl ≡ 6 mg of crop. Relative peak areas, HS/EC: lindane 1·1; Telodrin 2·5



A: Electron-capture detector (EC)
B: Halogen-sensitive detector (HS).

Fig· 7. Simultaneous complementary gas chromatograms of control lamb and lamb containing dieldrin or *pp'* - DDE: injection, 5 μl ≡ 2·5 mg of tissue. Relative peak areas, HS/EC: dieldrin 1·3; *pp'*-DDE 1·1

Another feature in favour of the halogen-sensitive element is that there is linearity of response with increase in load up to at least 100 nanograms. This could be of advantage in quantitative work, by reducing the need for repeated dilution and injection in the analysis of extracts of unknown pesticide concentration.

The cost of these detectors (£15 to £18) is not high, and in tests where the highest sensitivity (0·1 nanogram) is not required, simple and inexpensive circuitry that does not involve the use of either an electrometer or amplifier is adequate. This arrangement could be of immediate value in analyses in the near-residue range, *e.g.*, for chlorinated pesticides in field-strength dusts, fertilisers, wool, wood, plastics and hardboard, and after a tenfold concentration of the sample extract (by partition or evaporation, or both), could be applied to the low residue range. Indeed, it is probable that this concentration step can be eliminated simply by using a larger input resistor, and feeding the signal directly into a high-impedance 1-mV recorder.

The main disadvantages of these halogen-sensitive detectors, as has been already indicated, lie in their indifferent electrical stability under the conditions used, and the difficulty of conditioning the cells rapidly and maintaining them at high sensitivity. It must be remembered, however, that the detectors have been used in a manner quite unlike the one for which they were designed. It is possible, therefore, that design and development work on halogen-sensitive elements for use in gas - liquid chromatographic residue analysis could overcome both of these problems and, moreover, effect sufficient increase in sensitivity to make them of even greater value than the electron-capture type.

Most of the work reported here was performed with the Ozotron Type H detector, but the Type J shows considerable promise. The unglazed ceramic sheath of the latter, however, may not be as impervious as the glass of the former. Against this, the Type J is easier to clean and, perhaps, to condition since it can be taken apart. Further, it possesses a higher thermal capacity, which makes it somewhat less sensitive to slight fluctuations in the power supply to the heater filament.

In conclusion, it is considered that the halogen-sensitive detector when used in combination with the electron-capture ionisation detector in the manner described, provides a technique with a potential value at least comparable with that of the multi-column "spectro-chromatogram" (described in Part I of this paper), for improving the certainty of identifying chlorinated pesticides in analysis of residues. Apart from its complementary operation with the electron-capture ionisation type, it has great potential value as an alternative detector in gas - liquid chromatographic analysis of chlorinated pesticides, both on the residue and near-residue scales.

We thank Mr. A. Richardson of the Tunstall Laboratory of "Shell" Research Ltd. for focussing our attention on halogen-sensitive detectors, Mr. D. M. Barnett for much careful experimentation, Mr. G. Blunkell for the design and construction of the stabilised power pack, and Dr. R. A. E. Galley, Director, and Mr. J. G. Reynolds, Associate Director, of the Woodstock Agricultural Research Centre, for their interest and encouragement during the course of this work.

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The Determination of Tin in Beer

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A method is described for determining small amounts of tin in beer after wet-oxidation of the sample. The method has been made rapid and simple by making use of the advantages of subtractive cathode-ray polarography. A detection limit of 0·007 μg per ml of tin in beer has been obtained.

THE practice of supplying beer in tinned cans has increased greatly during the last year or two, and this has led to a demand for the determination of tin in beer samples. It has been reported that as little as 0·02 p.p.m. of tin can give rise to hazes in beer,[1,2,3] and with tin concentrations greater than 0·1 p.p.m. the degree of turbidity is aesthetically intolerable. It is necessary to use a method of high sensitivity for determining less than 0·1 p.p.m. of tin, and, because it is likely that several other metals will be present at similar concentration levels, the method should also be specific. Most of the colorimetric methods for determining tin lack the required sensitivity, and are prone to interference. For example, according to Sandell[4] no colour is produced with dithiol when less than 0·3 μg of tin per ml is present, and bismuth, copper, nickel, cobalt, mercury, silver, lead, cadmium and some other metals interfere. Polarography has been used for determining tin in foods; Godar and Alexander[5] determined tin after co-precipitation with aluminium hydroxide, and Markland and Shenton[6] introduced a simpler method involving no separation. Condliffe and Skrimshire[7] used a single cell cathode-ray polarograph for determining tin in various foodstuffs, but they did not report any results lower than 20 to 30 p.p.m. In general, the permissible tin content of foods is quite high, and it is only because of its deleterious effect on the appearance of the product that determinations of such high sensitivity are required for beer. Recent developments in polarography have led to the development of instruments with much higher sensitivity, and it was considered likely that the use of subtractive polarography[8] would give a lower detection limit than has previously been attainable.

Experimental

The detection limit for tin in the selected base electrolyte (5 N hydrochloric acid) was determined by measuring the peak obtained for tin on ten solutions containing 0·02 μg of tin per ml. The standard deviation, σ, obtained corresponded to 0·0028 μg per ml, which on a 2σ basis corresponds to a detection limit of 0·0056 μg per ml. These figures were obtained by

Table I

Recovery of added tin

Sample	Tin added, μg per ml	Tin found, μg per ml	Tin recovered, μg per ml
Light ale (bottled) ..	Nil	Nil	—
	0·020	0·019	0·019
	0·050	0·045	0·045
	0·10	0·10	0·10
	0·15	0·13	0·13
	0·20	0·19	0·19
Lager (canned)	Nil	0·062*	—
	0·050	0·11	0·048
	0·15	0·20	0·14
Guinness (bottled) ..	Nil	Nil	—
	0·020	0·020	0·020
	0·050	0·045	0·045
	0·10	0·098	0·098
	0·50	0·49	0·49

*Mean of 3 determinations

subtracting base-line effects, etc., 5 N hydrochloric acid being used in the reference cell of the polarograph. The improvement in sensitivity amply justifies the slightly increased complexity of the procedure. The improvement is illustrated in Figs. 1 to 4.

The wet-oxidation of beer samples was next investigated, and it was found that digestion with nitric, perchloric and sulphuric acids by the proposed procedure gave satisfactory destruction of the sample. Recoveries of added tin were investigated by making additions

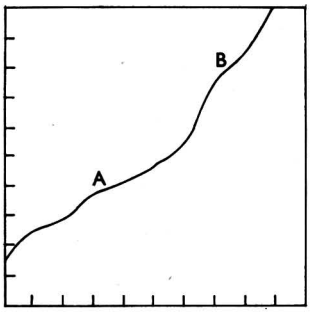

Fig. 1. Polarogram of beer containing: A, 0·031 μg of tin per ml; B, 0·18 μg of cadmium per ml. (Sensitivity $\frac{1}{25}$)

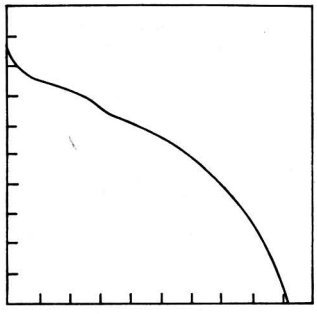

Fig. 2. Polarogram of 5 N hydrochloric acid base electrolyte, cell (II). (Sensitivity $\frac{1}{25}$)

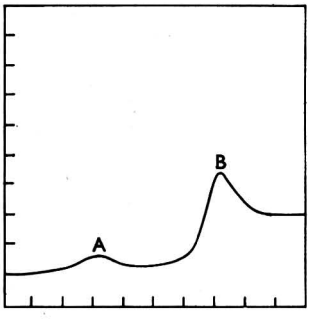

Fig. 3. Subtractive polarogram of beer containing: A, 0·031 μg of tin per ml; B, 0·18 μg of cadmium per ml. (Sensitivity $\frac{1}{10}$)

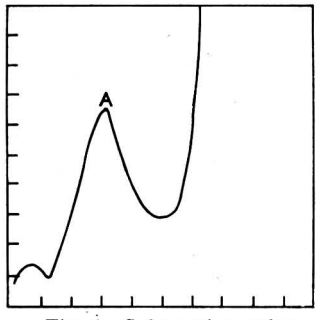

Fig. 4. Subtractive polarogram of beer containing: A, 0·031 μg of tin per ml. (Maximum sensitivity)

of stannic sulphate solutions to beer samples. The additions made, and the recoveries obtained, are shown in Table I. It was considered that these results indicated satisfactory recovery, and the method was applied to several beer samples. The results obtained on these samples are shown in Table II.

TABLE II

RESULTS OBTAINED ON VARIOUS BEER SAMPLES

Sample	Tin, μg per ml
Guinness (bottled)	<0·01, <0·01, <0·01
Light ale (bottled)	<0·01, <0·01, <0·01
Swiss lager (bottled)	<0·01, <0·01
Same Swiss lager (canned)	0·066, 0·058
Same Swiss lager (aluminium can) ..	0·044, 0·040
Swiss lager (canned)	0·10, 0·11
Swiss lager (bottled)	0·031, 0·026
Dutch lager (canned)	0·056, 0·061, 0·069

It is probable that the limit of detection of the whole analytical procedure will be at a higher concentration than that found for pure tin solutions, and this was found to be so in practice. It has been suggested[9] that the standard deviation of the blank value is likely to be the limiting factor in an analytical procedure, and the standard deviation of the blank value was therefore determined by analysing eight blank solutions over a period of 4 days; the

determinations were carried out by a single analyst. The standard deviation was found to be 0·009 μg of tin per ml in the cell, and by using the procedure described below this corresponds to 0·0036 μg per ml in the beer sample. The limit of detection of the method on a 2σ basis is considered to be about 0·007 μg per ml, and samples in our laboratory which give results indistinguishable from the blank value are reported as $<0{\cdot}01$ μg per ml.

Stannic chloride has a boiling-point of 114° C,[10] and use has been made of its volatility as a method of separation.[11] It is likely, therefore, that if samples rich in chloride are wet-ashed some tin may be lost by volatilisation; some of the samples investigated in the early part of this work gave low results when charred with nitric acid alone. It was found that when the sample was first charred with sulphuric acid, as in the procedure described below, satisfactory results were obtained.

In the 5 N hydrochloric acid base electrolyte used for determinating stannic tin, the only likely interfering substance will be lead. As the lead content of beer is usually low, the bulk of the lead present will probably come from the reagents, and will therefore be deducted with the blank value. Several samples had been investigated by making separate determinations of lead in a base electrolyte buffered to pH 4·6, at which tin is non-reducible, showed that the correction to be made for lead content was, in every experiment, negligible. No such correction is therefore incorporated in the proposed procedure, although if desired it can be made by using a separate determination of lead on a second portion of the solution. The statutory limit for the lead content of beer is 0·5 p.p.m., but this limit is seldom reached in practice. If substantial amounts of lead are present, then the shape of the reduction peak obtained will be altered; the tin^{IV} reduction peak is very much sharper than the $lead^{II}$ reduction peak, and little experience is necessary before the slightly more blunt combined tin - lead peak can be readily differentiated from a pure tin peak. Interference from arsenic is prevented, as it will have been oxidised to $arsenic^{V}$, and the presence of $germanium^{II}$, niobium, rhenium or thallium is unlikely. These are the only other elements that will give a reduction peak at $-0{\cdot}5$ volts.

Method

Reagents—

Perchloric acid, sp.gr. 1·54—Lead-free for foodstuffs analysis.
Nitric acid, sp.gr. 1·42—Lead-free for foodstuffs analysis.
Sulphuric acid, sp.gr. 1·84—Lead-free for foodstuffs analysis.
Hydrochloric acid, sp.gr. 1·18—Lead-free for foodstuffs analysis.

Apparatus—

All glassware including cover glasses should be of Pyrex or similar glass to minimise the lead blank values. The polarograph used for this work was the Southern Analytical A1660 Differential Cathode-Ray Polarograph.

Procedure—

Pour the well-shaken beer sample into an open beaker, and leave for about 20 minutes to liberate most of the dissolved gases. If this precaution is not taken, it is difficult to measure the sample by means of a pipette.

Transfer by means of a pipette 25 ml of the sample to a 100-ml beaker, cover the beaker, and evaporate almost to dryness; it will be necessary to heat gently until frothing ceases. Add 2 ml of sulphuric acid, and char the sample by gently heating. Allow to cool somewhat, and add 5 ml of nitric acid. Evaporate until fumes of sulphur trioxide appear; the sample should char again. Allow to cool, and add a further 5 ml of nitric acid and then 3 ml of perchloric acid. Evaporate gently until fumes of perchloric acid appear, and then until fumes of sulphur trioxide appear. Remove the cover from the beaker, and evaporate the solution to dryness. Allow the residue to cool, add 5 ml of hydrochloric acid, cover the beaker, and warm gently to dissolve the residue. Transfer the solution to a 10-ml calibrated flask and make up to the mark with distilled water (see Note 1). Transfer a portion of this solution to a polarograph cell, remove dissolved oxygen with a stream of nitrogen, and measure the tin peak at $-0{\cdot}5$ volts *versus* the mercury pool anode (see Note 2). It was found preferable to use 5 N hydrochloric acid in the reference cell of the instrument, for highest sensitivity,

and to measure the difference wave obtained. At least two reagent blank solutions should be processed, omitting the sample of beer, and these blank solutions should also be measured against the 5 N hydrochloric acid reference solution.

NOTES—

1. For high sensitivity polarography it has been reported[12,13] that de-ionised water can cause difficulties, and the use of distilled water for base electrolytes is recommended.

2. The polarograms obtained should contain two or perhaps three peaks, caused by copper at −0·25 volt, combined tin - lead at −0·5 volt and possibly cadmium at −0·7 volt. If this simple pattern is not obtained, or if large concentrations of copper or tin appear to be present, the wet-oxidation is probably incomplete and the determination should be repeated. It is necessary for the final evaporation to be carried out slowly, so that perchloric acid condenses on the walls of the beaker. The technique described has been successfully used on samples of lager, light ale and Guinness.

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The Polarographic Determination of Lead in Steels and Copper - Zinc Alloys

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Lead is separated from interfering elements by an ion-exchange medium. The difference in electrical conductivity between the lead-free and lead-containing eluates is used as a variable to effect the separation by automatic control. The separation technique and polarographic determination are described. Coefficients of variation for lead contents of 0·01 per cent. and 0·3 per cent. are ±4·25 per cent. and ±1·07 per cent., respectively.

THE object of this work was the development of a simple method for determining low concentrations of lead (about 0·01 per cent.) in steels and copper - zinc alloys. It was desirable that the method developed should use instrumentation already available and be sufficiently flexible to apply to both plain carbon and alloyed steels.

Published papers[1 to 7] did not appear to meet all these requirements, but Carson[8] has described an ion-exchange separation of lead and zinc from cobalt, in 2 M hydrochloric acid solution, and has suggested that this should be applicable to lead in steel. The work of Kraus and Nelson,[9] with anion-exchange resins, suggested that separation of lead from iron and most of the usual alloying elements in steel, and also copper, should be feasible at hydrochloric acid concentrations of either 9 M or 2 M.

Separation in hydrochloric acid solutions by anion exchange was attractive because this medium could act as solvent, eluent and polarographic base electrolyte. Further, if 2 M acid was chosen as the initial eluent, the change in electrical conductivity caused by the dilution of the eluting acid could be used to operate an automatic system for isolating the fraction containing the lead.

In view of these considerations, the method chosen involved an anion-exchange separation and determination of lead by linear-sweep cathode-ray polarography.

Experimental

Initial experiments using British Chemical Standard No. 212 (0·3 per cent. leaded steel) indicated that the ideal volumes and concentrations of eluting acid were 50 ml of 2 M, 100 ml of M and 300 ml of 0·005 M hydrochloric acid. The separation of iron and lead was further improved by allowing some degree of mixing between each concentration before entering the column.

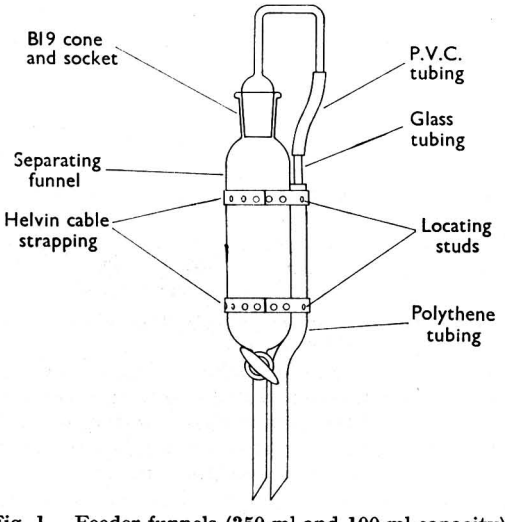

Fig. 1. Feeder funnels (350-ml and 100-ml capacity)

To introduce a degree of automation, a 100-ml burette, three-quarters filled with an analytical-reagent grade chloride-form strongly basic anion-exchange resin (De-Acidite FF), was fitted with a glass cup and a form of self regulating feed for the eluting acid (see Fig. 1). A conductivity cell was fitted at the base of the column so that the eluate passed through this cell and an anti-siphon tube to a two-way servo tap. This consisted of a spring-operated three-way tap with a relay (G.P.O. 2000 type) used as an electromagnetic catch. When the relay was energised, a spring-loaded arm was released and this rotated the tap-key,

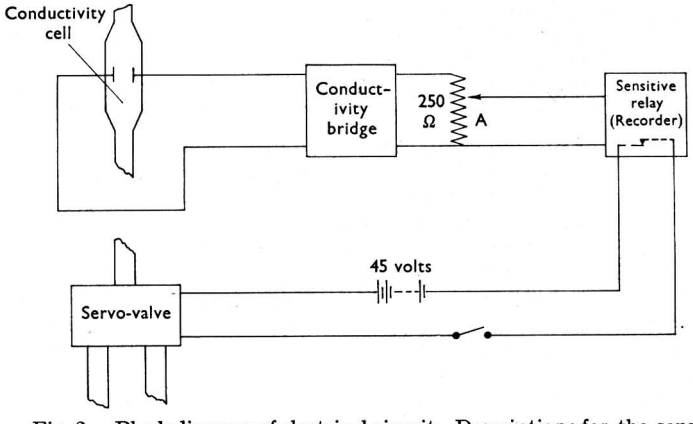

Fig. 2. Block diagram of electrical circuit Description: for the sensitive relay, a 50-mV electronic recorder with a microswitch on the pen-travel was used. By increasing the resistance of potentiometer **A** to 500 ohms, a sensitive electro-magnetic relay can be used in place of the recorder, which has a 50 μA operating current on a 2000 ohm coil

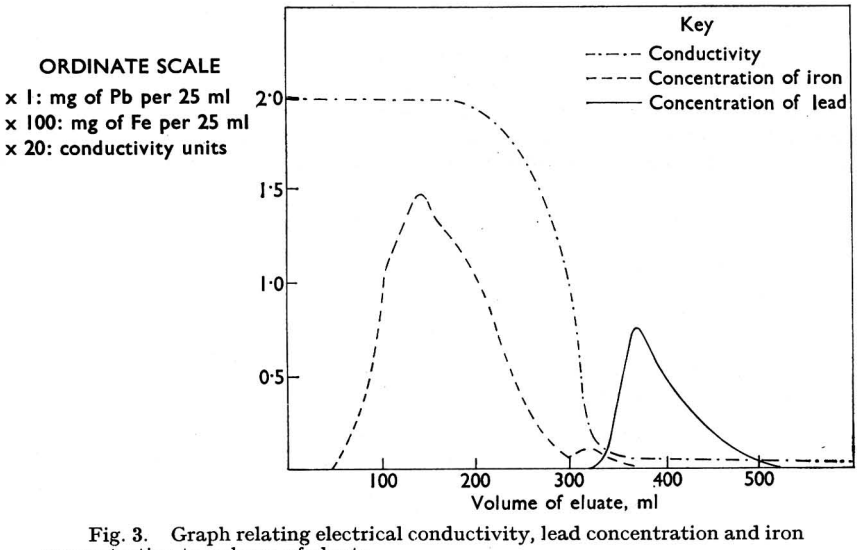

Fig. 3. Graph relating electrical conductivity, lead concentration and iron concentration to volume of eluate

diverting the eluate from waste to sample beaker. The tap was re-set manually between separations. The tap relay was controlled by the sensitive relay (in our case a recorder), which was coupled to the output of the conductivity bridge, so that when the conductivity of the eluate was the same as the bridge setting, the tap relay was energised (see Fig. 2).

The apparatus proved to be reliable and the efficiency of the separation is shown in Fig. 3. For this graph, iron was determined colorimetrically and the lead estimated by using a K 1000 polarograph.

Method

All reagents used should be of analytical-reagent grade.

Steels—

Dissolve 1 g of sample in 25 ml of diluted hydrochloric acid (1 + 1) with the minimum of heating, and add nitric acid, sp.gr. 1·42, dropwise until no further reaction occurs. Boil the solution free from nitrous fumes, cool, filter off any residue and dilute to 50 ml with water.

Regenerate the column with 350 ml of 2 M hydrochloric acid and introduce the sample solution into the cup at the top of the column. Set the servo tap to waste, and adjust the flow-rate through the column to 8 to 10 ml per minute. Add a further 50 ml of 2 M hydrochloric acid to the cup and fill the two eluant feeder funnels with 100 ml of M and 350 ml of 0·005 M hydrochloric acid. Open the taps on the feeder funnels, and switch on the conductivity bridge and associated circuits.

When the conductivity of the eluate falls to the bridge setting (see "Bridge Setting"), the servo tap diverts the eluate to a sample-collector and the lead-containing fraction of the eluate is collected. Evaporate this solution to approximately 30 ml, add 5 ml of 3 per cent. w/v hydroxylammonium chloride solution while still hot, and boil the solution to complete the reduction of iron. Cool, dilute with water to 50 ml, and record the peak-height on a K1000 polarograph at −0·45 volt, against a standard calomel electrode, using an initial potential of −0·35 volts. A blank experiment on all reagents should be performed, and the sample peak-height corrected for the blank value. Convert to percentage lead by reference to a calibration graph.

Copper - zinc alloys—

For lead contents between 0 and 1 per cent. dissolve 1 g of sample in 30 ml of diluted hydrochloric acid (1 + 1) and 1 ml of nitric acid, sp.gr. 1·42, with the minimum of heating, and proceed as for steels.

Calibration—

The calibration graph was prepared by analysing 1 g quantities of Specpure iron, to which were added 0, 1·0, 2·0, 3·0, 4·0 and 5·0 ml of standard lead solution (1·5980 g of Specpure lead nitrate, $Pb(NO_3)_2$, diluted to 1 litre). The quoted lead additions cover a range from 0 to 0·5 per cent. for a 1 g sample.

Bridge setting—

Obtain a graph relating conductivity, and iron and lead concentrations to volume of eluate (see Fig. 3) by performing an experiment on British Chemical Standard No. 212 (0·3 per cent. leaded steel) and analysing the eluate in 25-ml fractions. From this graph deduce the conductivity for the optimum separation of iron and lead. Set the conductivity bridge at this figure.

Application to carbon steel

Six 1-g samples of Specpure iron (Johnson Matthey & Co. Ltd.) containing 0·01 per cent. (added) lead impurity, together with a blank sample, were analysed as described in the method.

The results obtained after correction for the lead content ofthe blank sample showed that the mean value for the recovery of lead was 102 per cent., the standard deviation was $\pm 4{\cdot}34 \times 10^{-4}$ and the coefficient of variation was $\pm 4{\cdot}25$ per cent.

Suitable standard steel samples with a lead content between 0·01 per cent. and 0·05 per cent. were not available. Consequently, we tested the reliability of the method with an actual steel, British Chemical Standard No. 212 (leaded steel, certificate value 0·28 per cent. of lead). Six 1-g samples of this steel, together with a blank sample were analysed. Results showed that the mean value for the lead content was 0·282 per cent., the standard deviation was $\pm 3{\cdot}03 \times 10^{-3}$ and the coefficient of variation was $\pm 1{\cdot}07$ per cent. When British Chemical Standard No. 212/1 (leaded steel, certificate value 0·22 per cent. of lead) became available, it was also examined. Results showed that the mean value for the lead content was 0·215 per cent., the standard deviation was $\pm 3{\cdot}29 \times 10^{-3}$ and the coefficient of variation was $\pm 1{\cdot}53$ per cent.

The results show that in carbon steels, lead contents of 0·01 per cent. to 0·3 per cent. can be determined with reasonable accuracy.

APPLICATION TO ALLOY STEELS AND COPPER-ZINC ALLOYS—

Kraus and Nelson[9] showed that most interferences are eliminated by the column separation described, which should leave only zinc^{II}, cadmium^{II}, indium^{III}, antimony^{III}, and, possibly, molybdenum^{VI} and tin^{IV} ions in the lead fraction. In polarographic analysis with a dilute hydrochloric acid supporting electrolyte, zinc and cadmium have sufficiently negative half-wave potentials to prevent their interference with the determination of lead. In fact, zinc and cadmium in the lead-containing eluate could be quantitatively determined polarographically. It was most unlikely that indium would be present in steels or copper-zinc alloys, but if present probably would not interfere. Antimony would give a polarographic wave only in strongly acid conditions, but this wave is well removed from that of lead. The presence of molybdenum was shown to have no effect on the recovery of lead.

Any tin present in the lead-bearing eluate could seriously interfere with the polarographic determination as the waves of tin and lead in chloride media coalesce.

Small amounts of tin tended to remain on the column and were eluted with the lead in the next sample. This problem was solved for 1 g samples containing 0·2 per cent. of tin by using at least 350 ml of regenerant acid between separations (see Table I).

TABLE I

DETERMINATION OF LEAD IN BRITISH CHEMICAL STANDARD NO. 275 STEEL CONTAINING ADDED TIN

(Certificate value: 0·04 per cent. of tin, 0·005 per cent. of lead)

Tin added, per cent.	Total tin, per cent.	Lead found, per cent.	Recovery, per cent.
Nil	0·04	$0·004_9$	98
0·20	0·24	$0·005_2$	104
Nil	0·04	$0·004_9$	98
Nil	0·04	$0·005_1$	102
0·20	0·24	$0·005_4$	108
Nil	0·04	$0·005_0$	100

To determine the effect of tin in a copper - zinc alloy on the recovery of up to 0·2 per cent. of lead, we made a 60+40 copper - zinc alloy and added 0·2 per cent. of lead, and also we made a 60+40 copper - zinc alloy and added 0·2 per cent. of tin.

In one series of experiments (6 samples) we added 0·01 per cent. of lead, and found a recovery of between 94 per cent. and 105 per cent., with a mean value of 100 per cent. In the second series of experiments (4 samples), we added 0·2 per cent. of lead, and found a recovery of between 95 per cent. and 100 per cent., with a mean value of 97·5 per cent.

These experiments showed that lead, present to the extent of 0·01 per cent., could be determined accurately in carbon and alloy steels, and in copper - zinc alloys, provided that the tin content did not exceed 0·2 per cent.

CONCLUSIONS

The method has proved reliable for the routine determination of lead contents between 0·005 per cent. and 1 per cent. in a wide variety of steels and copper - zinc alloys, the only limitation being that the tin content for a 1-g sample should not exceed 0·2 per cent.

We thank the Director, A.W.R.E., Aldermaston, for permission to publish this report.

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SHORT PAPERS

The Determination of Glucose in the Presence of Maltose and Isomaltose by a Stable, Specific Enzymic Reagent

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During investigations on the degradation of starch by fungal enzymes, a simple and accurate micro method was required for determining glucose in the presence of other reaction products. The Barfoed reagent of Tauber and Kleiner[1] is specific for glucose in the presence of maltose, but reproducible results are obtained only if conditions are carefully standardised; even then, the reagent responds to isomaltose as well as to glucose.

Other methods were therefore investigated, and of these a modification of the glucose oxidase procedure was found most satisfactory. All the glucose oxidase enzyme preparations tested, unless prepared in tris buffer as described by Dahlqvist,[2] were found to react with maltose and isomaltose as well as with glucose. This enzyme reagent was further tested, and found to be highly specific for glucose, though it suffered from the disadvantage of relative instability. The

Table I

Estimation of glucose in the presence of maltose or isomaltose by glucose oxidase and barfoed reagent

Mixture contained— 50 μg glucose, *plus*	Glucose found (μg) by using— Glucose oxidase in phosphate	Glucose oxidase in tris buffer	Barfoed reagent
500 μg maltose	65	50	60
500 μg isomaltose	53·5	50	103

colour developed was linearly related to glucose concentration only up to 50 μg of glucose per ml of solution. The addition of up to 40 per cent. w/v glycerol, as recommended by Washko and Rice,[3] was found to increase the stability considerably, without altering the specificity of the Dahlqvist enzyme reagent. A number of colour-developing reagents were tested; of these *o*-dianisidine hydrochloride, at the specified concentration was found to be the most sensitive, particularly in presence of 5 N sulphuric acid.[3] These modifications have been found to increase the life of the enzyme reagent from a few days to over three months at 4° C, and to increase the linear response up to 100 μg of glucose per ml of test solution.

Method

Reagents—

Glucose oxidase, 10 *mg*—C. F. Boehringer und Soehne, Mannheim, Germany.

Horseradish peroxidase, 1 *mg*—Worthington Biochemical Corporation.

o-*Dianisidine hydrochloride*, 10 *mg*—Prepared from *o*-dianisidine.

Tris glycerol buffer, pH 7·0—Prepare by dissolving 61 g tris-(hydroxymethyl)aminomethane in 85 ml of 5 N hydrochloric acid, dilute the solution to 1 litre with water and add 660 ml of analytical-reagent grade glycerol. Adjust the pH to 7·0 if necessary.

"combined reagent – Dissolve 10 mg. of glucose oxidase, 1 mg of horseradish peroxidase and 10 mg o-dianisidine hydrochloride in 100 ml of tris glycerol buffer."

Procedure— Add 2 ml of the combined reagent to 1 ml of solution containing 0 to 100 μg of glucose. Mix the solutions and incubate them at 25° C for 1 hour. (Include standard glucose solutions.) After incubation, add 4 ml of 5 N sulphuric acid, mix, and measure the optical density at 525 mμ. (The colour is stable for at least 12 hours.) Prepare standards from a stock solution containing 2 mg per ml of analytical-reagent grade glucose in saturated aqueous sorbic acid. This solution keeps indefinitely. Dilute stock solution to 1 + 20 for a working standard, and use this standard for blank determinations when performing analyses.

Results

The effect of maltose and isomaltose on the determination of glucose by glucose oxidase (with phosphate or tris buffer) and Barfoed reagent is shown in Table I.

Fig. 1 shows the response curves for the various reagents.

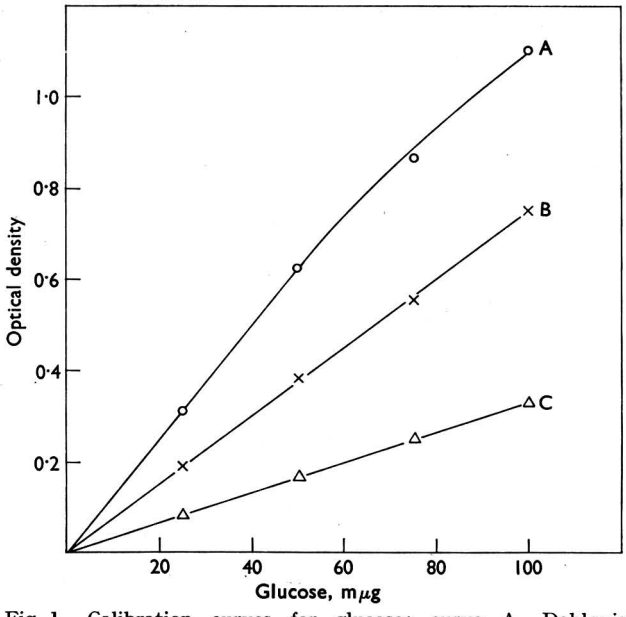

Fig. 1. Calibration curves for glucose: curve A, Dahlqvist reagent; curve B, modified reagent; curve C, Glucostat reagent

Discussion

As shown in Table I, the Barfoed copper reagent and the Glucostat glucose oxidase reagent, which contains phosphate buffer, are not specific for glucose in the presence of maltose and isomaltose. When the phosphate buffer is replaced by tris buffer at pH 7, the maltose and isomaltose activities are inhibited, and the reagent is specific for glucose. A further disadvantage of the Glucostat reagent is its low sensitivity compared with either Dahlqvist or the proposed reagent (see Fig. 1) both of which are specific for glucose. The proposed reagent although less sensitive than that of Dahlqvist, has a greater range and the additional advantage of being completely stable for at least six months.

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Improved Paper-chromatographic Separation of Sugar Phosphates by using Borate-impregnated Paper

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The borate-complexing technique for separating sugar phosphates has been successfully used in ion-exchange chromatography[1,2] and paper electrophoresis.[3,4] It has met with limited application in paper chromatography.[5,6,7] Cohen and Scott[5] used boric acid in neutral ethanol to achieve a sharp separation between aldopentose-5- and 3-phosphates, but the separation among the individual 5-phosphates or the 3-phosphates was not satisfactory. The solvent system failed to effect a satisfactory resolution among the hexose-monophosphates. According to Isherwood,[6] incorporation of boric acid in the alkaline n-propanol system readily effected a separation between glucose-1-phosphate and 6-phosphate. Baron and Brown[7] achieved success in separating ribose-2-, 3- and 5-phosphates by introducing boric acid in the propanol - ammonia solution system.

A scheme of paper-chromatographic separation of a mixture, containing essentially hexose-phosphates, by using borate-containing solvents has not been proposed. Nor has, to our knowledge, borate-impregnated paper been used in the separation. A simple and rapid method, in which borate-impregnated paper is used, has been developed by us, and this permits a fairly sharp separation of the components from a mixture of orthophosphate and five sugar phosphates.

Method

Procedure—

The samples of sugar phosphate in the form of sodium, potassium, or barium salts, obtained from recognised commercial sources, were de-cationised with a cation-exchange resin, in the hydrogen form, and the solutions neutralised to pH 7 with 0·1 M ammonia solution. The basic borate-free solvent system consisted of 60 volumes of t-butanol (this was superior to n-propanol[6]), 30 volumes of ammonia solution (sp.gr. 0·88) and 10 volumes of water. In the borate-containing solvent system, water was replaced by a solution of sodium tetraborate to give a final concentration of 0·01 M borate. Whatman No. 1 filter-paper strips (12 cm × 38 cm) were used, either without any treatment or after being dipped in borate solution (0·001 M to 0·01 M) and dried at room temperature. Where pre-complexing of esters with borate before application to paper was desired, stock solutions of the phosphate esters were made in 0·01 M borate solution. Portions of the solutions, containing 0·1 to 0·2 μmoles of sugar phosphates, were applied to the paper and dried. One-dimensional descending-solvent chromatograms were developed for about 24 hours at room temperature, the solvent being allowed to overflow and drip from the serrated edge of the paper. The air-dried chromatograms were treated with the spray reagent of Runeckles and Krotkov.[8]

Results and discussion

Spots were usually round or slightly pear-shaped. The migrations of various esters have been calculated as R_P values. This is the distance from the base line to the centre of each phosphate ester spot, as related to the corresponding distance of the orthophosphate spot, the latter being taken as 100 for convenience. Typical R_P values, obtained for various sugar phosphates under the influence of borate ions, are given in Table I.

There was little separation between the various sugar monophosphates in the non-borate system. The use of borate effected an alteration in the migratory behaviour, and the degree of alteration depended on several factors. The glucose-1-phosphate spot invariably moved faster than the other phosphates under all the conditions used, showing that this ester remained uncomplexed with borate.[1] The uncomplexed orthophosphate followed the glucose-1-phosphate under identical conditions. Glucose-1- and 6-phosphates separated from each other after merely introducing borate into the solvent system. This confirmed the observations of Isherwood,[6] but fructose-6-phosphate and ribose-5-phosphate were not separated from glucose-6-phosphate. The use of borate pre-complexed esters did not offer any significant advantage. Considerable improvement in resolution resulted when borate-impregnated paper was used instead of untreated paper. The concentration of borate critically affected the optimum separation. When 0·01 M borate was present, fructose-6-phosphate separated from the other two non-glucosidically phosphorylated monoesters, but ribose-5-phosphate and glucose-6-phosphate did not separate sharply. When

the concentration of borate was halved (0·005 M), a separation between glucose-6-phosphate and ribose-5-phosphate tended to occur. At 0·001 M borate-ion concentration, glucose-6-phosphate moved as fast as orthophosphate. Such behaviour is understandable if one assumes, as did Khym and Cohn,[1] that glucose-6-phosphate is capable of dual behaviour, changing from the highly complexed furanose form to the less complexed pyranose form at lower borate-ion concentration. Excellent separation of all the components was found to occur when 0·002 M borate was used for paper impregnation.

TABLE I

R_P VALUES OF SUGAR PHOSPHATES ON PAPER CHROMATOGRAMS UNDER THE INFLUENCE OF BORATE IONS

(R_P value for orthophosphate = 100)

System	R_P values for				
	Glucose-1-phosphate	Glucose-6-phosphate	Fructose-6-phosphate	Fructose-1,6-diphosphate	Ribose-5-phosphate
Non-borate system, control ..	120	116	114	59	114
Borate system, untreated paper:					
Irrigating solvent containing 0·01 M borate	121	106	111	65	107
Esters pre-complexed with 0·01 M borate	118	87	83	41	70
Borate-impregnated paper:					
0·01 M	117	58	83	40	63
0·005 M	125	61	79	31	55
0·004 M	127	65	63	27	50
0·003 M	121	66	73	32	47
0·002 M	125	86	76	31	41
0·001 M	140	97	86	40	62
Combination of techniques:					
Esters pre-complexed with 0·01 M borate, on 0·01 M borate-impregnated paper	114	61	79	40	61
Irrigating solvent containing 0·01 M borate, on 0·005 M borate-impregnated paper ..	108	64	89	58	69

Combinations of these techniques, for example, by using pre-complexed esters with impregnated paper, or borate-containing irrigating solvent with impregnated paper, did not satisfactorily separate all the components.

The results reported above for single components were also applicable when the components were present in a mixture. The present method represents a definite improvement on our earlier chromatographic method,[9] in which borate ions were not used.

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A Field Test for Aniline Vapour in Air

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The test recommended by H.M. Factory Inspectorate[1] for detecting and estimating aniline vapour in air involves the collection of the aniline in dilute hydrochloric acid, boiling this solution with bleaching powder and finally developing a permanent blue colour by reaction with ammoniacal phenol solution. The intensity of the colour is then estimated by comparison with liquid standards prepared from a blue dye or with Lovibond permanent glass standards.

In a recent investigation in this laboratory, it became necessary to determine low concentrations of aniline in air. The official method was used, but standards were prepared from carefully purified aniline hydrochloride rather than the blue dye. During the preparation of these standards, it was found that the intensity of the colour developed largely depended on the rate at which the solution was brought to the boil, and the length of time for which it was boiled. The variations in colour intensity were sufficient to vitiate the accuracy of the method. Even under controlled conditions, the resulting colours, when arranged in order of increasing intensity, frequently placed the standards in an incorrect order. We were unable to find any convenient means of obtaining reliably reproducible colours. Since the occurrence of such errors would not be apparent when the recommended types of colour standard were used, an alternative method, avoiding the necessity for boiling, appeared desirable for use in the field.

The method recently suggested by Hands[2] as a field test for nitrobenzene vapour in air was considered suitable for adaptation since it involved initial reduction of the nitrobenzene to aniline which was then estimated by a method very similar to the one described by Strafford, Strouts and Stubbings.[3] That part of the test involving the diazotisation and coupling of aniline with disodium 2-naphthol-3,6-disulphonate (R-salt) was shown by Hands to be superior to various alternatives under the conditions of the test, and is sufficiently sensitive for determining hazardous concentrations of aniline. Other reagents, described by Daniel,[4] give a greater sensitivity than is required here.

The adoption of Hands' method for estimating aniline has the great advantage that the same apparatus and reagents would be used in field tests for nitrobenzene, aniline and many other aromatic amines. Owing to the lower toxicity of aniline,[5] compared with nitrobenzene, a different range of standards must be prepared if the aniline test is to cover that concentration range that is of industrial interest. Alternatively, the original liquid or glass standards[2] could be used with a proportionately smaller air sample, and a correction made for the increased sampling efficiency. The bubbler collection efficiency in this instance proved to be 100 per cent., so that there was no need to apply a correction factor in the preparation of standards.

Method

Apparatus—

Bubbler—This should be an all-glass construction. The solvent container should be 9 cm deep and 1·3 cm in diameter, with a centrally blown bulb of diameter 2·5 cm. The internal diameter of the delivery tube should be 2 mm.

Pump—A metal hand-pump of 120 ml capacity or other form of suction device[6,7] capable of giving a rate of sampling of 1500 ml per minute should be used.

Trap—A suitable trap consists of a wide-necked bottle of about 100 ml capacity, fitted with a 2-hole bung and delivery tubes and connected between the bubbler and the pump.

Comparator tubes—13 mm × 8 cm standard tubes or rectangular cells.

Pipettes—2- and 5-ml dropping pipettes with rubber teats.

Reagents—

All chemicals used should be of analytical-reagent grade.

Hydrochloric acid—Dilute 5 ml of concentrated hydrochloric acid, sp.gr.1·18, to 100 ml with water.

Sodium nitrite solution—Dissolve 3·5 g of sodium nitrite in 100 ml of water. This solution should not be kept for more than one month.

Sodium carbonate solution—Dissolve 10 g of anhydrous sodium carbonate in 100 ml of water.

R-salt solution—Dissolve 0·8 g of the purified sodium salt of 2-naphthol-3,6-disulphonic acid in 100 ml of boiling water, adjust the pH to between 7·5 and 8·5 by adding M sodium carbonate, cool to room temperature and filter. This reagent should be stored in the dark, and should not be kept for more than one month.

Ammonia solution—Dilute 20 ml of ammonia solution, sp.gr. 0·88, to 100 ml with water.

COLOUR STANDARDS—

Dissolve 1·000 g of potassium dichromate in 1 litre of water; this is solution A.* Dissolve 70·26 g of cobalt sulphate septahydrate in 1 litre of water; this is solution B. Prepare the standards by placing the amounts of water and solutions A and B shown in Table I into 13-mm cells or comparator tubes.

TABLE I

COMPOSITION OF COLOUR STANDARDS

Aniline concentration, p.p.m. v/v	Solution A, ml	Solution B, ml	Water, ml
1	2·0	1·8	23
2·5	4·0	6·0	19
5	5·2	8	16
10	10	10	4

PROCEDURE—

Place 5 ml of the hydrochloric acid solution in a clean, dry bubbler and connect this to the pump via the trap. Take a 6-litre sample of the air under test at the rate of 1·5 litres per minute. (If the hand pump is used, 50 strokes at a rate of 5 seconds per exhaust stroke are required.) Detach the bubbler. Transfer its contents to a clean, dry 10-ml calibrated comparator tube. Wash out the bubbler with two successive 2-ml portions of the hydrochloric acid solution, transferring the washings to the calibrated tube. Make up the volume in the tube to 10 ml with the hydrochloric acid solution.

By means of the teat pipette, transfer 5 ml of this solution to another clean, dry 10-ml comparator tube, add 0·5 ml of the sodium nitrite solution, mix and allow to stand. After 2 minutes add 2 ml of the sodium carbonate solution, shake and then add 0·5 ml of the R-salt solution. Shake again to mix thoroughly and add 2 ml of the ammonia solution, shake and compare the colour of the solution with those of the colour standards.

NOTES—

1. Aniline concentrations outside the range 1 to 10 p.p.m. can be determined by varying the size of the air sample. The stated rate of sampling must not be exceeded if the collection efficiency of the bubblers is to be maintained.

2. The liquid colour-standards were prepared by matching the colour produced from standard concentrations of aniline hydrochloride in the hydrochloric acid solution against those of mixtures of the inorganic salt solutions.

3. The bubbler collection efficiency was checked by passing air containing aniline vapour through two bubblers in series. When the bubblers were operated under the stated conditions, no aniline hydrochloride could be detected in the second bubbler, either by the proposed colorimetric method or by ultraviolet absorption.

We thank Mr. R. A. Bragger for assistance with the experimental work.

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The Use of a Constricted Rubber Bulb for Obtaining an Average Sample of an Atmosphere During a Working Period

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The slow-sampling apparatus was primarily designed to draw 120 ml of air through a trichloroethylene indicator tube,[1] at a constant rate over a period of 8 hours, so that the indication obtained represents the average concentration of the contaminant during this period.

The method depends on the fact that the pressure within a deflated thick rubber bulb is reasonably constant, irrespective of the amount of deflation. The vacuum of the bulbs we use (Siebe Gorman sampling bulb, size 6) is equivalent to about 12 cm of mercury, and the capacity of the bulbs is about 120 ml. Against the suction head of about 12 cm of mercury, a sample flow of 15 ml per hour may be obtained by using a constriction made from 2 cm, 0·05-mm bore, tubing (polarograph capillary-tubing). We have used this type of constriction, protected from atmospheric dust by glass sinters fitted at either end. We have also found that a length of about 10 cm of electrode carbon-rod (Grade ENS, 13-mm diameter: Morgan Refractories Ltd.) is sufficiently porous to make a suitable constriction. Fig. 1 shows the slow-sampling apparatus in which the carbon-rod constriction is used. The carbon rod is fixed into the glass tube with Apiezon black wax, so that the peripheral space is completely filled with wax and the ends of the rods are clean.

The sleeve valve is constructed from a standard B24 cone and socket and this is lubricated with a heavy grease (Apiezon L is suitable). The actual sampling rate may be measured by deflating the bulb, closing the valve and then connecting the apparatus to a 1 ml graduated pipette by means of a short length of rubber tubing. The tip of the pipette is dipped in water, so that a bead of water is drawn in, and the time taken for the bead to rise through a volume in the pipette is measured.

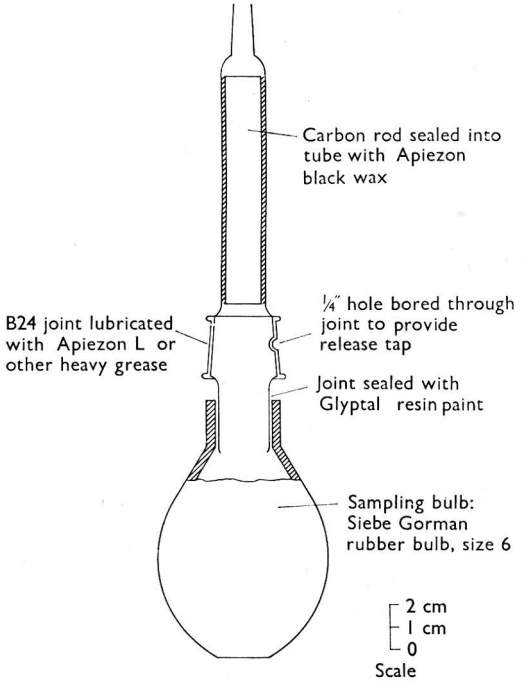

Fig 1. Constricted sampling bulb

If the sampling time is too long, it may be reduced by removing the rubber bulb and boring into the carbon by using a $\frac{5}{16}$-inch drill so that the effective length of the carbon rod is reduced. The rubber bulb is finally fixed permanently to the glass tube with Glyptal resin paint.

The calibration of the trichloroethylene indicator tube, for the normal sampling rate (4 minutes for 120 ml), remains valid at the slow sampling rate. With other indicator tubes it may be necessary to recalibrate when the sampling rate is changed.

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Titration of Weak Bases in Acetic Anhydride

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IN acetic anhydride the acidity of perchloric acid is markedly increased; the active "acidic" entity is probably the acetyl cation CH_3CO^+ (possibly solvated in the form $(CH_3CO)_3O^+$) rather than the solvated proton (which, in acetic acid, is in the form $CH_3COOH_2^+$). There is considerable evidence in support of this.[1 to 12]

Thus bases, such as amides and purines,[13,14,15] too weak to be titrated successfully in acetic acid, can be titrated potentiometically in acetic anhydride by using a modified glass - calomel electrode system. This led to the investigation of the basic properties of well-known derivatives of carbonyl compounds in this solvent. Since these are often prepared in the course of research in organic chemistry, it seemed desirable to have a method of direct titration. No satisfactory method has been reported concerning the direct titration of these derivatives as bases. The method described here is similar to that used by Wimer[16] for titrating amides, and is applied to semicarbazones, phenylhydrazones, 4-nitrophenylhydrazones and 2,4-dinitrophenylhydrazones.

METHOD

SAMPLES FOR ANALYSIS—

When commercial samples are available, the purest grades should be used without further purification, provided their melting-points agree with those quoted in reliable literature sources. Otherwise they should be purified by recrystallisation until the melting points are constant. Samples that are made should be purified likewise.

REAGENTS—

Acetic anhydride and glacial acetic acid—Good quality commercial grades.

Perchloric acid—72 per cent. aqueous solution, analytical-reagent grade.

The titrant—Prepare a 0·2 N perchloric acid in a mixture of equal parts of acetic anhydride and glacial acetic acid. Allow to stand for at least 12 hours before use. Although it may become a pale straw colour on storage it does not lose strength for at least two weeks (*cf.* Streuli[17]). Standardise it by potentiometric titration with analytical-reagent grade potassium hydrogen phthalate dissolved in glacial acetic acid, and check at frequent intervals.

APPARATUS—

Glass electrode—Cambridge wide-range type. Immersed in acetic anhydride for 12 hours before use.

Modified calomel electrode—Sleeve type; the aqueous solution being replaced by a 0·1 M solution of anhydrous lithium perchlorate in acetic anhydride as described by Wimer.[16]

Potentiometer—Cambridge pH meter, used as a potentiometer. Incorporate a Weston standard-cell into the circuit when necessary, to allow for reversed polarity of the electrodes in non-aqueous media.[18]

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PROCEDURE—

Dissolve 1 millimole of the sample (or a convenient quantity) in 40 ml of acetic anhydride using a glass-covered magnetic stirrer to assist dissolution. Cool the titration vessel to 0° C in a mixture of ice and salt (or, if rapidly soluble, dissolve at 0° C), and titrate with the 0·2 N perchloric acid. Take the usual precautions to exclude moisture. Plot a graph of e.m.f. in millivolts *versus* the volume of titrant added.

In this work it was desired to compare the basic strengths of the compounds under these conditions, and hence millimole quantities were weighed to within one milligram, and the temperature of the titration vessel was kept as constant as possible during the titrations. For substances sparingly soluble in acetic anhydride, the minimum amount of chloroform was sometimes used to dissolve them and the volume made up to 40 ml with acetic anhydride. It was found that amounts of chloroform up to 50 per cent. had little effect on the shape of the titration curve.

RESULTS

The percentage purity of the samples were calculated from the volume of titrant at the point of maximum slope of the curves.

SEMICARBAZONES—

Parent ketone or aldehyde	Percentage found from titration	Mean maximum slope, millivolts per ml
Ethyl methyl ketone	98·7, 98·0	500
Methyl n-propyl ketone	100·5, 99·5	800
Methyl isopropyl ketone	97·0, 98·5	800
t-Butyl methyl ketone	101·0, 101·0	400
Mesityl oxide	98·0, 98·0	1100
2-Methylcyclohexanone	98·0, 99·0, 97·8	520
Benzophenone	98·0, 98·3	135
Benzaldehyde	98·6, 98·0	Weakly basic*
Benzalacetone	98·0, 97·0	900
Acetophenone	96·0, 95·0†	—

* Solubility insufficient to allow of one millimole to be titrated.
† Required heating to dissolve; this may account for low result.

The semicarbazones of acetone and cyclohexanone gave low results, and those of aliphatic aldehydes showed weakly basic properties and gave very low results.

PHENYLHYDRAZONES—

Parent ketone or aldehyde	Percentage found from titration	Mean maximum slope, millivolts per ml
Acetophenone	99·0, 101·0	1000
Benzalacetone	98·0, 100·0, 100·2	1300
Dibenzalacetone	101·0, 101·0	200
Benzalacetophenone	99·0, 97·0	125
Benzophenone	100·0, 100·0	450
Cinnamaldehyde	100·3, 100·0	140

The aliphatic ketone and aldehyde phenylhydrazones were too unstable to be titrated successfully. The phenylhydrazones of benzaldehyde and cyclohexanone did not give stoicheiometric results.

4-NITROPHENYLHYDRAZONES—

Parent ketone or aldehyde	Percentage found from titration	Mean maximum slope, millivolts per ml
Acetone	100·0, 98·5, 98·3	1250
Cyclohexanone	98·0, 98·0	1250
Ethyl methyl ketone	98·8, 99·0	900
Methyl n-propyl ketone	98·0, 100·0, 98·5	900
Methyl isopropyl ketone	99·4, 100·8	750
t-Butyl methyl ketone	101·0, 101·8	560
Benzalacetone	99·8, 98·0	900
Acetophenone	98·0, 98·0	300
Benzophenone	98·0, 99·0	120
Cinnamaldehyde	99·4, 99·0	140
Dibenzalacetone	Non-basic	—

The 4-nitrophenylhydrazones of acetaldehyde, formaldehyde and butyraldehyde were non-basic, while that of benzaldehyde was so weakly basic that the end-point could not be determined with accuracy.

2,4-DINITROPHENYLHYDRAZONES—

Parent ketone or aldehyde	Percentage found from titration	Mean maximum slope, millivolts per ml
Acetone	100·0, 101·0	600
Cyclohexanone	98·0, 100·4	350
Ethyl methyl ketone	100·0, 98·5	550
Methyl n-propyl ketone	99·0, 98·0	500
Methyl isopropyl ketone ..	98·0, 97·0	200
2-Methylcyclohexanone	98·7, 98·0	300

The 2,4-dinitrophenylhydrazone of t-butyl methyl ketone was too weakly basic (slope 100) for the end-point to be determined accurately. The 2,4-dinitrophenylhydrazones of aromatic ketones were too sparingly soluble to be titrated and those of aliphatic aldehydes were non-basic.

OXIMES—

Oximes were non-basic in acetic anhydride with the exception of the weakly basic acetoxime but the curve was not stoicheiometric.

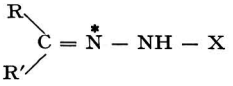
DISCUSSION

Although the method is rather limited in its application owing to the somewhat poor solvent power of acetic anhydride (with the addition, if necessary, of chloroform) the results obtained generally show a divergence of not more than 2 per cent. when millimolar amounts were used.

It was found that better and more consistent results were obtained when the titration vessel was maintained at 0° C, and the maximum slopes of the curves were usually greater than at room temperature. Further, the probability of acetylation and other reactions occurring with the solvent was reduced.

All the derivatives titrated acted as monoacidic bases in acetic anhydride and the mean maximum slopes of the curves show that the basic strength was profoundly affected by the groups attached to the original carbonyl group. Thus, in all experiments, the derivatives of aliphatic ketones were the most basic whereas, as might be expected, the presence of one or two aromatic rings markedly reduced their basic strength. The benzalacetone derivatives, however, showed remarkedly high basic strength. Aldehyde derivatives generally showed weak basic properties (slopes less than 200) or none at all. The reason for this is not understood. The method therefore cannot be recommended for the derivatives of aldehydes so far investigated.

In the general formula



where R and R′ are alkyl or aryl groups or H, and X is $CONH_2$, phenyl, 4-nitrophenyl or 2,4-dinitrophenyl, the substituent X had less influence on the basic strength than R and R′, even though the introduction of nitro groups into the phenylhydrazone derivatives did cause a progressive weakening of basic strength. It was therefore assumed that the nitrogen marked with an asterisk was the origin of the basic properties.

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Book Reviews

METHODS OF BIOCHEMICAL ANALYSIS. Volume XI. Edited by DAVID GLICK. Pp. x + 442. New York and London: Interscience Publishers, a division of John Wiley & Sons. 1963. Price 110s.

This volume opens with an article by J. B. Willis of Melbourne, Australia, on the analysis of biological materials by atomic absorption spectroscopy. It will be recalled that the Fraunhofer lines in the solar spectrum are caused by the absorption of radiation from the hot centre of the sun by sodium vapour in the outer parts of its atmosphere. Emission spectra have, for a long time, been much used in chemical analysis, but atomic absorption was neglected until A. Walsh in 1953 recognised the potentialities of the method and devised suitable techniques. At temperatures between, say, 2000° and 5000° K, only a small fraction of the (vaporised) atoms is raised above the ground state, *e.g.*, for the most easily excited metal (caesium) the fraction is about 3×10^{-2} at 4000° K, and for zinc it is only about $1{\cdot}5 \times 10^{-7}$. The difference explains why flame photometry works so much better for the alkali metals than for zinc or magnesium. The vast majority of the atoms in the vapour are in the ground state and can only absorb radiation corresponding to the wavelength of the resonance lines (*e.g.*, Na, 5890, 3303 and 2853 Å; Mg, 2852 and 2026 Å; Zn, 2138 and 1590 Å; P, 1775 Å.) In atomic absorption spectroscopy, the line corresponding to the transition from the ground state to the lowest excited state is generally used. It is necessary to have a source emitting a strong sharp line radiation that is passed through the atom-charged vapour and into a monochromator. Under optimum conditions the relation between intensity of absorption and concentration is linear. The chapter has a full account of technical procedures and an interesting comparison is made between flame photometry and atomic absorption spectrophotometry. The field is an interesting one in which important developments have occurred.

E. C. Horning, W. J. A. Van der Heuvel and B. G. Creech of Houston, Texas, write on the separation and determination of steroids by gas chromatography. In the last three years the preparation of thin-film columns has allowed temperature ranges around 200° to 220° C, and retention times of 10 to 20 minutes to be used with very small samples. The method permits gas chromatography of steroids and other substances of biochemical interest. Variations in steroid separations depend on thermostable liquid phases differing in structure, and displaying selective retention effects for different functional groups. Methyl-substituted siloxane polymers (methyl silicones) are non-selective, in that separation depends mainly on differences in molecular weight or shape. A methyl fluoralkyl silicone selectively separates alcohols, ketones and esters and some polyfunctional steroids, whereas methyl phenyl siloxane polymers show selective retention for carbon - carbon unsaturation. Polyesters such as neopentyl glycol succinate and adipate are also useful. In steroid work the thermal conductivity detection systems used with ordinary thick-film columns are replaced by ionisation detection systems, based on measuring changes in conductivity of the flowing gas stream as its composition changes. One such system, devised by Lovelock, depends on ionisation by argon. The cell contains a radioactive source (α- or β-radiation), and when a high-potential is applied in the presence of radiation, metastable argon atoms are formed, but the electrical conductivity remains low. The metastable argon leads to partial ionisation of organic substances entering the cell; current flow increases and is amplified and recorded. Other detector systems are described. This article reveals how rapidly advances are being made in the field. The experiments are technically exacting and require highly trained personnel, but the analytical possibilities are great.

I. E. Bush of Birmingham discusses advances in direct scanning of paper chromatograms for quantitative estimations. Effluent and eluate methods of chromatography are compared with scanning methods. Paper chromatograms obtained under the best conditions need not be inferior to column chromatography, and scanning leads conveniently to automation. The author stresses the importance of cleanliness and likens efficient paper chromatography to aseptic surgery. After many trials he thinks that as a general rule the 'cleaning' of filter-paper is now unnecessary and he does not advise washing before use, apart perhaps from cross-washing (*i.e.*, across the long paper strips). After chromatography, the substances being studied are treated to yield light-absorbing or fluorescent products. The application of reagents must be skilfully executed in quantitative work, and detailed instructions for preparing, drying and stabilising the paper strip must be followed.

Bush goes on to discuss optical and instrumental requirements, methods of recording and the automation of scanning procedures. His existing machine CASSANDRA (Chromatogram

Automatic Scanning and Digital Recording Apparatus) is a highly complicated and successful arrangement. [Incidentally, Cassandra, daughter of Priam and Hecuba, was endowed with the gift of prophecy, but it was ordained that none should believe her predictions!]

Some specific procedures for reducing steroids, 17-ketosteroids and amino acids by the ninhydrin method, etc., illustrate how productive the automatic apparatus can be made. It can serve the needs of several busy research groups without being overworked.

Morton K. Schwartz and O. Bodansky of the Sloan-Kettering Research Institute discuss automated methods for determining enzyme activity. In their introduction they mention that "certain safeguards must be taken lest automatism on the part of the research worker replace automation by the machine," and they regard "over-automation" as quite a danger.

These authors consider three stages of automation: (1) procedures in which reaction mixtures are prepared manually but enzyme activity is recorded on an instrument; (2) automated preparation of reaction mixture as well as recording of activity; (3) procedures (not yet fully developed) incorporating feedback devices and built-in computers. "The economics of handling the increased demands by the clinician for enzyme determinations and . . . for all diagnostic biochemical determinations will be burdensome unless automation in its final or third stage is realised." As examples, many assays culminate in measurement at 340 mμ of oxidation of NAD or NADP, and when recorded on a spectrophotometer this represents a stage 1 operation. The Robot Chemist made in California is a stage 2 effort, as also is the Astra enzyme assayer and the AutoAnalyser. The latter lends itself to rapid colorimetric determinations, and the authors proceed to describe a number of standard problems successfully tackled in this way.

I. J. Kopin of Bethesda, Maryland, deals with the estimation of magnitudes of alternative metabolic pathways. This is a discussion of principles, illustrated in terms of the metabolism of adrenaline and noradrenaline. The methods involve examination of urinary metabolites of endogenous and administered labelled precursors. This is a neat and convincing treatment of an important problem.

F. L. Crane and R. A. Dilley of Lafayette, Indiana, review the determination of co-enzyme Q (ubiquinone). This is an excellent article giving the essential background of the ubiquinone story with details of extraction and spectrophotometric assay procedures. Methods based on paper chromatography are also described.

R. P. Davis of New York contributes an article on the measurement of carbonic anhydrase activity. There is no standard method, but the author describes the preparation of the enzyme and discusses manometric methods and indicator methods, as well as electrometric and histochemical problems. The information given should allow investigators to choose the best method for their particular needs.

O. H. Müller of Syracuse, N.Y., contributes a substantial chapter on polarographic analysis of proteins, amino acids and other compounds by means of the Brdička reaction. It was found in 1933, by Brdička, that certain amino acids, polypeptides and proteins, dissolved in a cobalt-containing buffer of suitable pH, produce a reaction at the dropping-mercury electrode. The currents involved in the Brdička reaction are catalytic and distinct from the non-catalytic diffusion currents that form a basis for quantitative analysis by ordinary polarography. The Brdička reaction was applied earlier to the diagnosis of malignancy, but more recent work indicates that, although the test is not specific for cancer, it is nevertheless useful in biochemical analysis. (The results should be reported in terms of current density μA per sq. mm of electrode surface.) Characteristic protein double-waves are not obtained in a cobalt-free buffered solution of serum. The Brdička effect is due to a catalytic reduction of hydrogen ions from the buffer and has been traced to cystine and cysteine in protein hydrolysates or —SH— and —S—S— groups in protein. (One molecule of cystine is reduced at the dropping-mercury electrode to two molecules of cysteine.)

The author describes how to obtain a master curve for catalytic waves of cysteine in a given buffer. This serves to calibrate determinations of cysteine in protein hydrolysates and cystine in urine. Cysteine forms a stable complex with iodoacetate ($RS.CH_2COO^-$) and in mixtures this eliminates the contribution of cysteine leaving that of cystine unchanged. The Brdička reaction has also been studied for glutathione and acids containing a sulphydryl group. Disulphides like thioctic acid are reduced first at the dropping-mercury electrode.

The work on the reaction applied to native and denatured proteins has been reviewed, and a number of practical (clinical) applications have been noted. An unusual effect has been developed into a versatile analytical procedure.

Professor Glick has over the years shown excellent judgement in selecting topics for review and in finding the right contributors. Biochemical analysis seems, from the portents of this volume, to be needed on a vast scale which requires more and more instrumentation, and indeed automation. The effort in terms of money, time and ingenuity which is now being put into highly sophisticated analytical work leaves one a little breathless. No doubt the information gained will be digested and properly interpreted. In the past a great many advances in Science have come from somewhat amateurish insights. We are now giving a fair test to the notion that aspects of the process of discovery can be mechanised, and it may be that by processing overwhelming masses of factual information great new ideas will emerge. Who can say? R. A. MORTON

ORGANIC CHEMISTRY. Volume I. THE FUNDAMENTAL PRINCIPLES. By I. L. Finar, B.Sc., Ph.D., A.R.I.C. Fourth Edition. Pp. xvi + 853. London: Longmans, Green & Co. Ltd. 1963. Price 50s.

Learning organic chemistry becomes, with the advance of the science, less and less the memorising of types of compounds and methods of preparation and more and more one of acquiring knowledge of the underlying principles of molecular structure and reaction. With such knowledge, the modern organic chemist engaged, say, in drug research, finds it almost easier to make his complicated compounds than to name them.

Finar's "Organic Chemistry" is, and has been for some time, a standard text-book for degree and honours students; four editions and ten impressions in 12 years preclude any doubt on this point. A review might therefore seem supererogatory, the merits of the work being so well recognised. However, a comparison of the various editions shows how the need for new editions arises from the intrusion of new ideas into the science. These intrusions become, in time, some of the fundamental principles, the description and application of which is the aim of the work. Such comparison also high-lights the amount of labour involved in keeping a text-book apace with new developments; it is far from being a matter of corrections.

The arrangement of the subject matter is conventional. After an introduction and a chapter on the structure of the atom (which deals to an equal extent with the molecule and its orbitals), aliphatic compounds are dealt with in fifteen chapters, alicyclic in one, aromatic compounds in ten and heterocyclic in two. One of the aliphatic chapters is devoted to compounds of sulphur, phosphorus, arsenic and silicon and one to organo-metallic compounds.

The distinctive features of the work are the dissertations, interspersed at appropriate places in the chapters, on the fundamental principles involved. Particular stress is laid on molecular structure, and reaction mechanisms are considered from the point of view of electronic theory as, for example, in the electrophilic, nucleophilic and free-radical mechanisms of aromatic substitution. New matter includes, among other subjects, molecular shape and reactivity, transition state theory of reactions, neighbouring group participation and molecular over-crowding. With the ever expanding range of new compounds being made, some organised system of nomenclature becomes essential and this is dealt with appropriately in each chapter and an appendix.

Although the book is primarily intended as a students text-book, up-to-dateness in fundamental theory makes it equally useful for the trained organic chemist, though it naturally has its limitations with respect to the types of compound described, *e.g.*, heterocyclic compounds are restricted to six-membered rings and condensed aromatic rings to three-ring systems. These, however, are matters not introducing new principles and more appropriate to text-books on advanced organic chemistry.

One of the best tributes to the work was its adoption for his students by the late Professor T. S. Wheeler, himself part author of a one-time best seller among text-books on the same subject.
J. I. M. JONES

GAS CHROMATOGRAPHY. Edited by NATHANIEL BRENNER, JOSEPH E. CALLEN and MARVIN D. WEISS. Pp. xxiv + 719. New York and London: Academic Press Inc. 1962. Price 157s.

This book comprises the proceedings of the 3rd International Symposium on Gas Chromatography held at Michigan State University under the auspices of the Instrument Society of America, from June 13th to 16th, 1961.

The first and main part of the book contains the 37 papers that were presented. These are followed by an Appendix recording the Discussion and finally a Bibliography on Gas Chromatography is given. This is a continuation of two previous bibliographies and covers the period June, 1960, to October, 1961.

A short introduction is written by Golay, in which he emphasises the important developments that have taken place in column and detector design, and he stresses the need for still more sensitive detectors and suitable column improvements that will enable us to detect large organic substances.

The papers appear in a sequence such that those dealing with topics having something in common are grouped together. The Discussion follows a similar pattern and is arranged to deal with groups of papers at the same time. The first 4 papers deal mainly with a study of the effect of the nature of the liquid phase and the amount used on the solid support on separations. The nature of the solid support has been studied and the elimination of tailing effects by treatment of the support to render its surface inactive is clearly demonstrated. The advantages gained by using a lower column temperature, with liquid loading on the inert support drastically reduced, are demonstrated in a paper dealing with efficiency of columns. Programmed-temperature gas chromatography has not been neglected, and in one paper a theoretical study of the method has been made. There is also an interesting paper on a study of the effect of various factors on column permeability.

Halasz, who is well known for his work on the use of capillary columns for the quantitative analysis of hydrocarbon mixtures, presents a further paper on this subject. In an interesting paper on the performance of coated capillary columns, Desty, Goldup and Swanton express surprise that the application of these columns has not been more widely appreciated. In the opinion of the reviewer this lack of more general use in quantitative work may be due to the high cost or unavailability of the special integrating and recording apparatus. Two further papers deal with the application of gas chromatography to relatively high-boiling petroleum and tar oils and polyphenyl ethers.

After a specialised paper on the measurement of absorption isotherms by high temperature gas chromatography, there follows a series of 9 papers on the role of detectors in gas chromatography. Most attention seems to have been given to the now well known flame-ionisation detector, with particular emphasis to its response to functional groups. The plot of carbon number against response (relative area per molecule) is shown to be linear for a wide range of classes of compounds. Similar investigations for the argon detector are reported.

It is evident that the search for new types of detector still continues. In one paper a novel method based on the use of ultrasonics is put forward, and it shows that an "ultrasonic whistle" holds promise. The use of a low-current electric-discharge detector has been explored, and a detector based on a thermionic diode operating in an inert gas at atmospheric pressure has been examined.

The originator of the argon detector, Lovelock and co-author Gregory, have contributed a paper on their most recent work with the electron-capture ionisation detector.

Probably the most novel paper is one that deals with the development of a Lunar Gas Chromatograph designed to telemeter information on the composition of possible organics and volatiles present on the moon's surface. By means of a probe, a suitable sample would be removed from the moon, hydrolysed and the products submitted to gas chromatography.

Sample-introduction and fraction-collection systems are considered in other papers, and finally there are 10 papers dealing with miscellaneous applications. These include the analysis of hydrocarbon mixtures, fluoralcohols, fluoracrylates and flavours.

It is doubtful whether the Bibliography at the end of the book serves a useful purpose for any worker who possesses the Gas Chromatography Abstracts issued by the Gas Chromatography Discussion Group (Butterworth & Co., London).

The book is well produced and should certainly be included in the library of all those interested in gas chromatography.

A. F. WILLIAMS

HANDBOOK OF ANALYTICAL CHEMISTRY. Edited by LOUIS MEITES. Pp. xvii + 1806. New York, Toronto and London: McGraw-Hill Book Co. Inc. 1963. Price £18 8s.

Someone has said that science is measurement, which is, perhaps, a particularisation of Horace's "Est modus in rebus"—there is measure in everything. This needs no elaboration for the analytical chemist who is faced not only with the daily task of measurement, but with the equally frequent need, if he is to complete his task daily, to refer to measurements made in the past by others of his profession. It is easy to understand this need in these days of electrometric, nuclear-magnetic and thermo-analytical techniques, but not so readily appreciated that it was already felt more than 80 years ago.

It was met some time before 1880 by the publication of the "Chemiker Kalendar" which by 1930 contained some 1500 pages of the kind of quantitative information required by the practising chemist. Then, about the time of the First World War, we had, in English, Atack's Chemists Handbook and the American Handbook of Chemistry and Physics with its current 4000 pages covering a wider and more general field. In more sectional fields were the Landolt - Bernstein physico-chemical tables and Seidell's solubility tables. Now, in the present volume the analytical chemist comes into his own in a compendium extending to over 1800 pages of the fundamental data and techniques useful in his work. The compilation is the work of some 140 expert chemists and is published by a firm who ought to know what they are about, having published seventy-five such handbooks from plumbing, piping and petroleum to physics and chemistry.

To review such a varied collection of data as is presented in this volume is impracticable; one can but mention some of the topics to illustrate the scope of the work. Thus, the section on qualitative analysis contains, besides the classical H_2S scheme of cation identification, eight alternative schemes, with another nine for anion identification and sixteen covering organic qualitative analysis. The fifteen sections into which the book is divided include sections on electrometric, optical, nuclear and magnetic, thermo-analytical and biological (mainly microbiological) techniques. Section 3, on inorganic gravimetric and volumetric analysis, includes acid - base titrations in non-aqueous media, precipitation, redox and complexometric titrations with exhaustive tables, for example, of 116 redox titration methods, fifty-four pages describing indicators used in complexometric titrations and a 26-page table of methods for determining inorganic cations by visual EDTA titrations.

One of the most interesting sections (179 pages) is that on techniques of separation, covering solvent extraction, distillation, the various forms of chromatography, electrophoresis and ion exchange. Another is that (294 pages) on methods for the analysis of technical materials. Working details are, of course, not given, but the principles, reagents and references are given for metals and alloys, cement, fuels, fertilisers, oils and fats, paints, pesticides, petroleum and pharmaceutical products, soap and detergents, plastics, water and sewage.

Such a compendious volume needs a good index and this need is adequately supplied. Of the task of compilation, our French colleagues would say "C'est formidable," and the editor and his contributors are to be congratulated. The book is a valuable reference work for the analytical laboratory, particularly for the occasional special sample which is outside the field of its normal work. The amount of information contained is no doubt worth the price charged, but this price, at the same time, is likely to limit its sale to the industrial analytical laboratory with a wide range of practice.

J. I. M. JONES

STRUCTURAL CARBOHYDRATE CHEMISTRY. By E. G. V. PERCIVAL, D.Sc., Ph.D., F.R.I.C. Revised by ELIZABETH PERCIVAL, D.Sc., Ph.D., F.R.S.E., with a Foreword by E. L. HIRST, C.B.E., F.R.S. Second Edition. Pp. xvi + 360. London: J. Garnet Miller Ltd. 1962. Price 40s.

This important text-book of carbohydrate chemistry was first published by the late Dr. E. G. V. Percival some twelve years ago. Since that time there has been an enormous growth in the volume of knowledge in the carbohydrate field, and Dr. Percival's wife has now extensively revised this book.

In the first four chapters some minor but important changes have been made. For example, Hudson's isorotation rules and a new section on sugar conformation are included, together with some comments on the structure of phenylozazones, and a worthwhile and timely section on nitrogen-containing or amino deoxysugars. In other chapters, particularly on new disaccharides, a good deal of more recent information has been provided, and with this is included, for the first time in a text-book I believe, the recent syntheses of disaccharides.

A completely new chapter on oligosaccharides has been provided and an enormous amount of knowledge in this ever-expanding subject has been put into a concise form. Included in the oligosaccharide chapter is new knowledge of oligosaccharides that are derived from sucrose, the important human-milk oligosaccharides and the enzyme-produced Schardinger dextrins. Considerable new information is provided on the very important nitrogen glycosides and on the polyhydroxy compounds, the inositols, which are related to carbohydrates.

The whole of the section on the polysaccharides has been rewritten, and those of great biological importance have been included. It is difficult to get enough information in a short space on the complex polysaccharides to give to students the right kind of picture of these important compounds,

but by concise writing and by picking out the most significant information which only the specialist can do, it has been possible to make the whole subject quite exciting. Much knowledge is provided on the enzymic synthesis and breakdown of polysaccharides and important sugars of animal origin, especially those containing nitrogen and sulphur, and known as the "mucopolysaccharides," have been dealt with in a concise and orderly fashion.

This is a text-book written for advanced students in chemistry and it does achieve its purpose in a remarkable way. It gives, in a relatively small space, a well presented and orderly fashioned account of all important groups of the saccharides. It is beautifully printed and well supplied with subject index, and the formulae are clear and well drawn. Altogether this book can be recommended for all libraries and for all advanced and research students in chemistry. The book continues the high tradition set by the late Professor Sir Norman Haworth and his School of Chemistry at Birmingham, of which the Percivals were prominent members. M. STACEY

INORGANIC ISOTOPIC SYNTHESES. Edited by ROLFE H. HERBER. Pp. x + 249. New York: W. A. Benjamin Inc. 1962. Price $7.50.

The availability, both of ample supplies of radioactive isotopes of most of the elements and of an increasing number of mass spectrometers, has led to a revival of interest in isotopic-dilution analysis and other tracer techniques. For these, a prerequisite is a supply of the compounds of interest, isotopically "labelled."

Although in many instances nowadays suitable labelled compounds are commercially available, some workers still prefer to prepare their own: when the compounds are not otherwise available they have, of course, no alternative. Some of the information required by the chemist who undertakes isotopic syntheses, is gathered in book form; for example, "Isotopic Carbon" by Calvin *et al.*, but the bulk of it is scattered in journals.

The book under review is intended as a guide to part of the field, that of the preparation of inorganic compounds labelled with deuterium, tritium, nitrogen-15, oxygen-18, phosphorus-32, sulphur-35, chlorine-36 and iodine-131. It is claimed that in many instances enough detail is provided to make consultation of the literature unnecessary, and in other instances, especially when "unstable or esoteric substances" are involved, reference is given to the appropriate original publications.

The first chapter, on General Principles, deals with such subjects as Radiochemical Manipulations, Radioassay and Mass Spectrometry. Statements here cast doubt on the Editor's claim to have inveigled the best qualified person in each field to contribute. For example, in discussing the radiations likely to be encountered by a user of radioisotopes, the implication (p. 3) that gamma radiation is less likely from fission products than from nuclear-reactor produced nuclides is, at the very least, misleading and the statement (p. 6) that in a Geiger - Müller counter complete breakdown of the gas occurs with each ionising event, is untrue.

Succeeding chapters, which are in effect separate monographs, deal in turn with synthesis of compounds of each of the isotopes listed above.

The book has been produced from typescript by photolithography, possibly to save time, as haste is evident in the many irritating errors and omissions. Although some of these, such as the writing of NaO_2 for sodium peroxide (p. 25) and AgF_2 for silver fluoride (p. 48) are admittedly trivial, the omission of dimensions from Fig. 1 (p. 123), when the text specifies operating conditions for a cell of "the dimensions given in Fig. 1," is more tiresome. Even more tiresome andun helpful, and certainly not in line with the declared aim of the Editor, is the provision of a reference to a "private communication" as the only information on the preparation of one compound (potassium carbonate-^{18}O by exchange, on p. 132).

No reference is made to the need for compounds of high, or at least known, purity for accurate results in, for example, isotope-dilution analysis, although other sources of error, such as the isotope effect, are mentioned. As it cannot be assumed that the compounds produced will always be of high purity, some information on purity checks might be expected; it is all but absent.

However, in spite of its faults, many of which could have been eliminated by a more careful revision, the book would be a valuable addition to the library of anyone engaged in isotopic syntheses. D. A. LAMBIE

ENCYCLOPAEDIC DICTIONARY OF PHYSICS. Editor-in-Chief, J. THEWLIS. Volumes I to VI: Vol. I, pp. xvi + 800; Vol. II, pp. x + 880; Vol. III, pp. x + 894; Vol. IV, pp. x + 836; Vol. V, pp. x + 782; Vol. VI, pp. x + 883. Oxford, London, New York and Paris: Pergamon Press. 1962. Price per set of 9 volumes, £106; $298.

In 1922 the Dictionary of Applied Physics, edited by Sir Richard Glazebrook, was published in five volumes, each of which was devoted to a defined branch of the subject. Forty years have now passed, during which time many fundamental advances in physics, some having the most far-reaching practical consequences, have been made.

The publication of a new Encyclopaedic Dictionary of Physics, of which the first six volumes are available for review, is therefore opportune. Its appearance will be welcomed by all who have occasion to use a technical library. The tendency towards unification of different branches of science, in spite of their increasing complexity, has led to the inclusion of much subject matter on the borders of, and outside, physics. Of particular interest to readers of *The Analyst*, apart from articles on the well-known physical tools of their calling, will be the contributions devoted to matters in the fields of physical and structural chemistry, crystallography, physical metallurgy and radiation chemistry.

The order of entries throughout the sequence of volumes is strictly alphabetical, although the order of words in the title of each entry is sometimes chosen so as to place related topics in contiguity. Thus consecutive entires in the fifth volume deal with pump, axial; pump, centrifugal; pump, circulating; pump, diffusion; pump, getter-ion and so on. This simple and useful device of arrangement is to be commended. The actual entries range from three or four lines of definition to half-a-dozen well illustrated pages for topics of major significance. The long monographs characteristic of Glazebrook have inevitably been greatly reduced in length. Friction, for example, is adequately treated in four pages compared with the sixty-three pages under this heading in the earlier dictionary. The value of the articles in the present work is, however, enhanced by suitable cross-reference to other entries and by useful bibliographies.

Essential features of a good encyclopaedic dictionary are authority, readability and "findability." A sampling technique, ranging between the limits at present provided—Abbé refractometer to Stellar luminosity—indicates that the first two requirements are amply fulfilled. Judgment about the third criterion must, for the present, be held in suspense. The publishers' foreword and the editor's preface both stress the importance of consulting the Index Volume before searching in the dictionary, because so many items "are not given separate entries, but are covered in other relevant articles." This advice is repeated in a footnote on every alternate page of each volume. In the absence of the index volume one cannot therefore report on "findability." Again using a sampling technique, only some 50 per cent. of a group of arbitrarily chosen topics were found by alphabetical search. The promised index is thus essential, and will form an integral part of the whole.

The publishers, Dr. Thewlis (editor-in-chief), his associate editors, the consulting editors and the many contributors are to be congratulated on the production of this outstanding work. The courage and vision displayed in undertaking this onerous task have been fully justified by its realisation.

B. S. COOPER

Publications Received

GAS PHASE CHROMATOGRAPHY. VOLUME 1. GAS CHROMATOGRAPHY. By RUDOLF KAISER. Translated by P. H. SCOTT. Pp. viii + 199. London: Butterworth & Co. (Publishers) Ltd. 1963. Price 42s.

GAS PHASE CHROMATOGRAPHY. VOLUME 2. CAPILLARY CHROMATOGRAPHY. By RUDOLF KAISER. Translated by P. H. Scott. Pp. x + 120. London: Butterworth & Co. (Publishers) Ltd. 1963. Price 35s.

GAS PHASE CHROMATOGRAPHY. VOLUME 3. TABLES FOR GAS CHROMATOGRAPHY. By RUDOLF KAISER. Translated by P. H. SCOTT. Pp. x + 162. London: Butterworth & Co. (Publishers) Ltd. 1963. Price 40s.

INFRA-RED SPECTROSCOPY AND MOLECULAR STRUCTURE: AN OUTLINE OF THE PRINCIPLES. Edited by MANSEL DAVIES. Pp. xiv + 468. Amsterdam, London & New York: Elsevier Publishing Company. 1963. Price 75s.

MISES AU POINT DE CHIMIE ANALYTIQUE ORGANIQUE, PHARMACEUTIQUE ET BROMATOLOGIQUE. Edited by J.-A. GAUTIER and P. MALANGEAU. Onzième Série. Pp. 252. Paris: Masson et Cie. 1963. Price 55 NF.

L'ANALYSE QUALITATIVE ET LES RÉACTIONS EN SOLUTION. By G. CHARLOT. Fifth Edition. Pp. xvi + 442. Paris: Masson et Cie. 1963. Price (paper) 65 NF; (cloth) 75 NF.

THE MODERN STRUCTURAL THEORY OF ORGANIC CHEMISTRY. By LLOYD N. FERGUSON. Pp. viii + 600. Englewoods Cliffs, N. J.: Prentice-Hall, Inc.; London: Prentice-Hall International, Inc. 1963. Price 104s.

INFRARED BAND HANDBOOK. Edited by HERMAN A. SZYMANSKI. Pp. xii + 484. New York: Plenum Press. 1963. Price $35.

RECENT PROGRESS IN MICROCALORIMETRY. By E. CALVET and H. PRAT. Edited and translated from the French by H. A. SKINNER. Pp. xii + 177. Oxford, London, New York and Paris: Pergamon Press. 1963. Price 60s.

SPEKTRALE ZUORDNUNGSTAFEL DER INFRAROT-ABSORPTIONSBANDEN. By Dr. WALTER OTTING. Pp. 18. Berlin, Göttingen and Heidelberg: Springer-Verlag. 1963. Price. DM 7·50.

BORON HYDRIDES. By WILLIAM N. LIPSCOMB. Pp. x + 275. New York and Amsterdam: W. A. Benjamin, Inc. 1963. Price $15·40.

METAL IONS IN AQUEOUS SOLUTION. By JOHN P. HUNT. Pp. xii + 124. New York and Amsterdam: W. A. Benjamin, Inc. 1963. Price $6·05.

Errata

MARCH (1963) ISSUE, pp. 162, 163 (twice), 164 and 165, references. *For* "British Standard 3406 : Part 2 : 1962" *read* "British Standard 3406 : Part 2 : in the press."

IBID., p. 174, last line, and p. 175, 13th line. *For* "British Standard 3406 : Part 3 : 1962" *read* "British Standard 3406 : Part 3 : 1963."

IBID., p. 179, 7th line. *For* "British Standard 3406 : Part 4 : 1962, p. 196" *read* "British Standard 3406 : Part 4 : in the press."

OCTOBER (1963) ISSUE, p. 768, penultimate line. *For* "specific gravity" *read* "specific activity."

NOVEMBER (1963) ISSUE, p. 829, author's address. *For* "64–68" *read* "64–78."

IBID., p. 833, reference 10. *For* "26" (the page number of the reference) *read* "36."

IBID., p. 834, reference 23. *Delete* "Berry, M. M., and Kent, A."

IBID., p. 899, reference 2. *For* "71" (the page number of the reference) *read* "76."

The Editor regrets the errors in D. R. Curry's Review Paper on Information Retrieval, which resulted from a misinterpretation of some corrected proofs.

Reprints from *The Analyst*

Analytical Methods Committee Report: "Determination of Penicillin, Chlortetracycline and Oxytetracycline"

THE Report prepared by the Antibiotics Panel of the Additives in Animal Feeding Stuffs Sub-Committee, "The Determination of Penicillin, Chlortetracycline and Oxytetracycline in Diet Supplements and Compound Feeding Stuffs," reprinted from *The Analyst*, **1963**, **88**, 835–850, is now available from the Secretary, The Society for Analytical Chemistry, 14 Belgrave Square, London, S.W.1, price to members 1s. 6d. each; to non-members, 2s. 6d. each.

Review Paper "Thermogravimetric Analysis"

REPRINTS of the Review Paper, "Thermogravimetric Analysis," by A. W. Coats and J. P. Redfern, published in this issue of *The Analyst*, will soon be available from the Secretary, The Society for Analytical Chemistry, 14 Belgrave Square, London, S.W.1, at 5s. per copy, post free.

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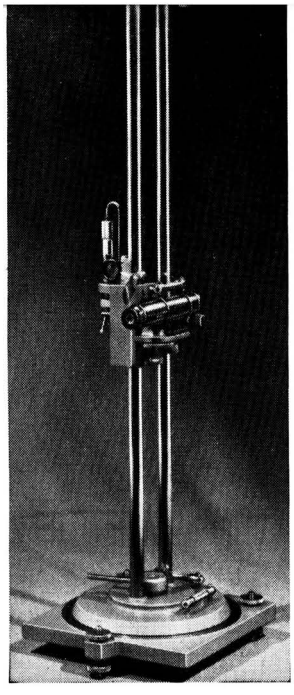

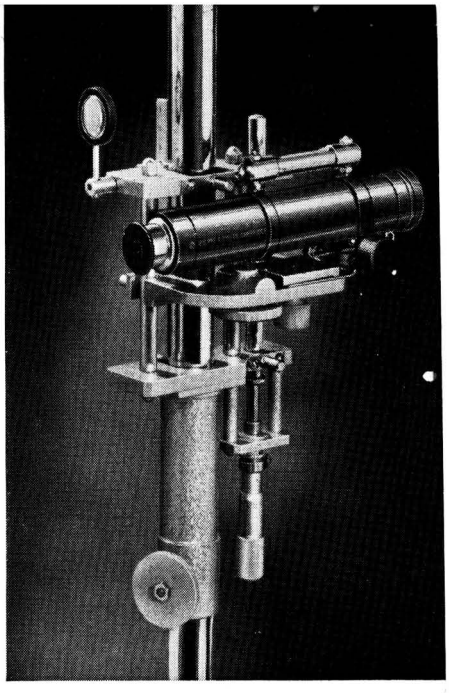

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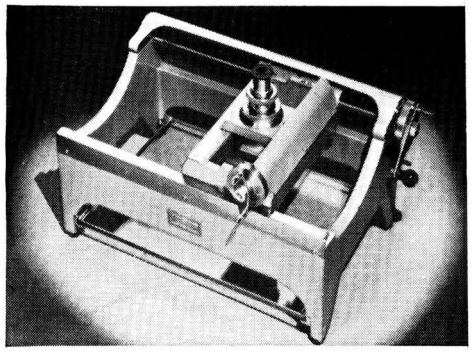

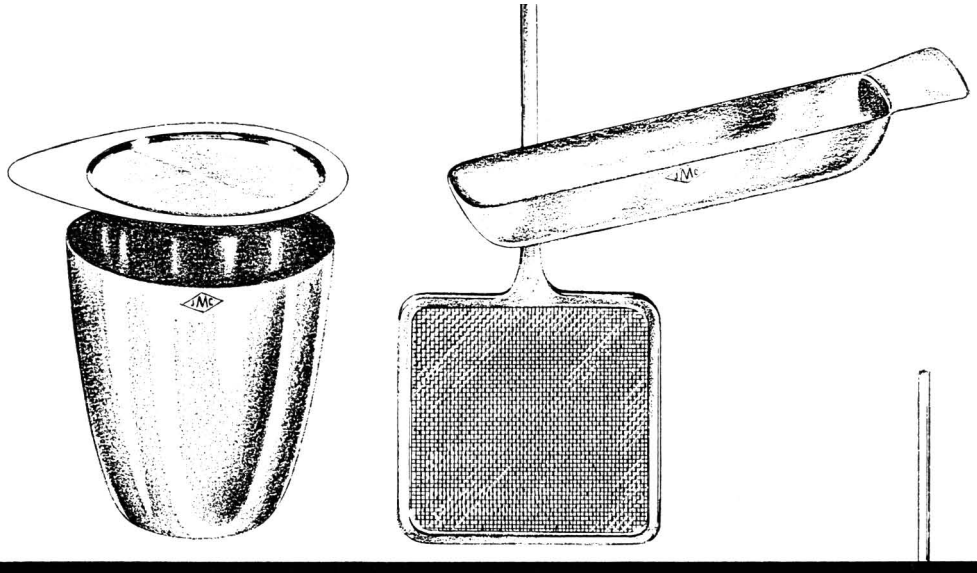

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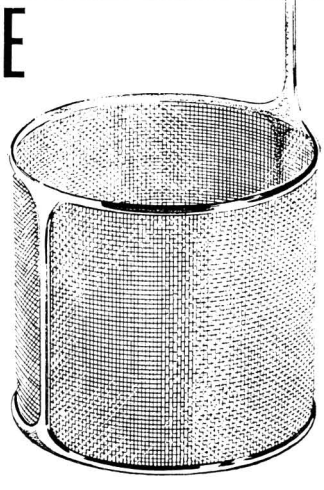

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