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Volume 90, No. 1076 November, 1965

Summaries of Papers in This Issue

Quantitative Gas - Liquid Chromatographic Analysis with Detectors having a Non-linear Response

It is shown that if, in the gas - liquid chromatographic analysis of a mixture of two compounds, the ratio of the heights or areas of the two peaks is found to be constant regardless of the size of the sample injected, this cannot be taken as evidence that the response of the detector is linear with respect to vapour concentration, as is sometimes assumed. Such a result will be obtained, in general, if the detector response is proportional to a power of the vapour concentration, the value of the exponent being the same for both compounds; this situation can arise with the argon ionisation detector over appreciable concentration ranges at suitable voltages. A method is described for carrying out quantitative analyses in these circumstances, and is illustrated by results obtained by using mixtures of geraniol and nerol.

B. DUDLEY SULLY and P. L. WILLIAMS

A. Boake, Roberts & Co. Ltd., Carpenters Road, Stratford, London, E.15.

Analyst, 1965, **90**, 643–648.

The Identification and Determination of Chlorinated Pesticides Residues

Gas - liquid chromatography provides a method of extreme sensitivity for the detection and determination of organochlorine pesticides residues. A single retention-time cannot, however, be regarded as a reliable identification of an unknown compound. A "clean-up" procedure is described by which interference is virtually eliminated, and a technique is proposed in which the change in retention-time after simple chemical reactions provides confirmation of the identity of a pesticide.

J. H. HAMENCE, P. S. HALL and D. J. CAVERLY

Dr. Bernard Dyer & Partners, Peek House, 20 Eastcheap, London, E.C.3.

Analyst, 1965, **90**, 649–656.

The Control of Particle Characteristics in Precipitation and the Inter-relationship of Size-analysis Techniques

Varying size ranges of calcium carbonate are produced by controlled precipitation in a stirred batch reactor.

The application to calcium carbonate of various methods of determining particle-size distributions and surface areas is considered, and inter-relationships between these methods are established.

A relationship is established between final particle-surface area and power input to the slurry and an explanation is put forward in terms of the effect of turbulence on nucleation and crystallisation in the precipitation.

R. M. MORRIS

Department of Chemical Engineering, University of Natal, King George V Avenue, Durban, Natal.

Analyst, 1965, **90**, 657–663.

The Determination of Methanol by Oxidation to Formaldehyde and Polarographic Reduction

Small amounts of methanol can be determined by oxidation to formaldehyde with potassium permanganate in orthophosphoric acid, with a subsequent polarographic reduction of the formaldehyde at 50° C. Optimum conditions have been found for each stage of the determination. Oxidation is best effected with a solution containing 35 g of potassium permanganate and 300 ml of orthophosphoric acid per litre and allowing the reaction to proceed for 10 minutes at 0° C.

MOJMIR RANNY
Research Institute of Fat Industry, Rakovnik, Czechoslovakia.

Analyst, 1965, **90**, 664–673.

The Polarographic Determination of Methyl Esters

Methyl esters of fatty acids, in the presence of sugar esters and of solvents such as dimethylsulphoxide and dimethylformamide, can be determined by saponification, distillation of the liberated methanol, and then oxidation and polarography as described in a previous paper. Optimum conditions have been established, and ways have been found for avoiding interference due to dimethylformamide.

MOJMIR RANNY
Research Institute of Fat Industry, Rakovnik, Czechoslovakia.

Analyst, 1965, **90**, 674–680.

An Absorptiometer for the Sugar Industry

Measurement of optical density is a commonly used test of purity in the sugar industry. Difficulty arises when the test is made on solutions of refined sugar because the optical density due to dissolved impurity is very small and because it is liable to be masked by traces of suspended turbid matter.

Among recommendations designed to overcome the difficulty the best is considered to be that of Gillett, Meads and Holven, who suggested the definition: colour index = 1000 $(a^{\bullet}_{420} - 2a^{\bullet}_{720})$. For reasons that are stated a modified definition, colour index = 1000 $(a^{\bullet}_{420} - a^{\bullet}_{690})$, is proposed here. The modified index should be well correlated with the concentration of residual dissolved impurity and with the departure from whiteness of the solution.

An automatic absorptiometer has been developed to measure the colour index directly. Its readings have been compared with estimates made by a panel of visual observers who classified samples of "fine liquor" and solutions of refined sugar according to order of whiteness. Satisfactory correlations were obtained.

S. HILL and J. T. RUNDELL
Tate & Lyle Refineries Ltd., Keston, Kent.

Analyst, 1965, **90**, 681–691.

The Determination of Moisture in Plain Cakes by a Microwave Attenuation Technique

A procedure based on microwave attenuation measurement with an A.E.I. Ltd. moisture meter has been developed for the rapid determination of moisture in plain cakes. The main factors influencing attentuation, apart from moisture, are temperature, sample preparation and cake texture. Under the conditions described, linear relationships were obtained between moisture content, attenuation and temperature for a variety of cakes. At moisture levels ranging from 18 to 25 per cent., 95 per cent. of the results obtained by the microwave method were within $\pm 0{\cdot}6$ per cent. of results obtained by a Brabender oven method.

A. D. INCE and A. TURNER
Chemists' Department, Cadbury Brothers Limited, Bournville, Birmingham.

Analyst, 1965, **90**, 692–696.

The Micro-determination of Glutathione and Cysteine in the Presence of Each Other

Short Paper

M. WROŃSKI
Department of Chemical Technology, University of Łódź, Nowotki 18, Poland.

Analyst, 1965, **90**, 697–698.

A Comparison of Fibrous-residue Determinations by the Official (A.O.A.C.) Method and the Dimethylsulphoxide Method

Short Paper

H. ZENTNER
Bread Research Institute of Australia, Epping Road, North Ryde, N.S.W., Australia.

Analyst, 1965, **90**, 698–699.

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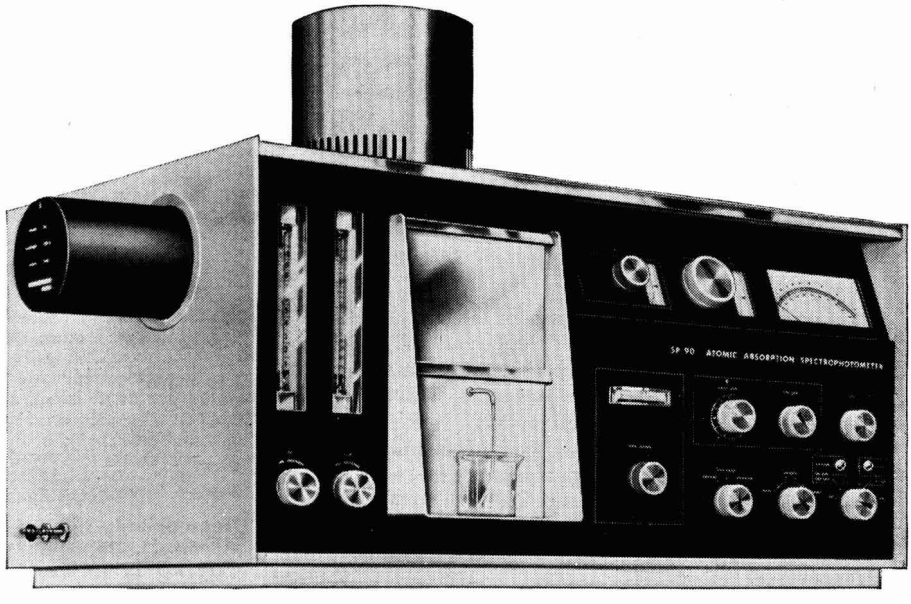

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AEI SCIENTIFIC APPARATUS
BULLETIN NO. 4

IMPROVED ACCURACY IN THE ANALYSIS OF SOLIDS BY SPARK SOURCE MASS SPECTROMETRY

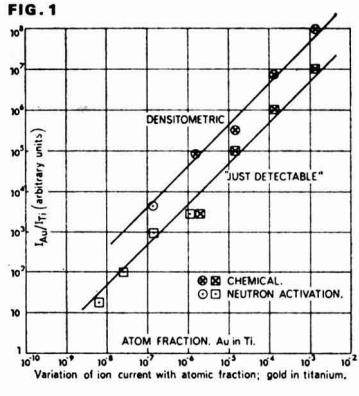
Since the possibility of using spark source ionization for the analysis of solids was first recognised ten years ago, the design of the double focusing mass spectrometer using Mattauch geometry has been considerably improved. And today one instrument—namely the AEI MS7—is capable of detecting impurities at levels as low as 1 part in 10^9. As a result the MS7—which, incidentally, was the first commercially available double focusing instrument to be built expressly for the analysis of solids—has found wide applications particularly where overall coverage of all elements and comparison analysis without standards are valuable.

Now the MS7 technique has been further improved by the careful control of certain parameters, and very good reproducability and accuracy can be guaranteed. This bulletin reviews the parameters affecting analytical accuracy and outlines the methods of control developed by AEI engineers.

It has been shown that the sensitivity of the majority of elements differs from some standard such as iron by no more than a factor of 3. In other words, most relative sensitivity factors lie between 0.3 and 3. In the case of the MS7, the determination of relative sensitivity factors is considerably simplified by the fact that the response of the instrument is linear over a very large range of concentration. Indeed Hannay & Ahearn[1] established linearity over the range 10^4 to 1 using doped silicon samples. More recently, W. A. Wolstenholme (AEI Consultant Lab.) has reported on the investigation of gold doped titanium samples covering a concentration range from 0.5% to .02 ppm by weight; a range of more than 10^5 to 1.

These samples were chosen because of their suitability for neutron activation and wet chemical analysis. Figure 1 shows the relative ion intensity of gold plotted against the concentration determined by chemical or neutron activation techniques. The "just detectable" line is an individual assessment for ion intensity and the "densitometric" line indicates that a microdensitometer was used to scan the spectral lines; the two graphs have been displaced for the sake of clarity. Matrix effects are generally very small, as is illustrated by the relative sensitivities for copper and steel standards reported in tables 1 and 2.

FIG. 1



Variation of ion current with atomic fraction; gold in titanium.

In copper and steel relative sensitivities are very similar for chromium (1·8 and 1·4) and for tin (1·3 and 1·1). Only for some low BP elements is there a marked dependence on the matrix, e.g. (1·4 and 2·6) for lead.

NOTE:

$$\text{Relative Sensitivity} = \frac{\text{uncorrected MS7 value}}{\text{known value}}$$

Homogeneity of standards and samples

Care has to be taken to use homogeneous standards or alternatively to increase the rate of consumption of sample above the usual 5 to 10 milligrams.

As it happens, however, the possibility of inadvertently using an inhomogeneous sample has been materially reduced by the introduction of more reliable methods of sample preparation.

TABLE 1
RELATIVE SENSITIVITIES AND REPRODUCIBILITY OF R.F. SPARK SOURCE ANALYSIS OF COPPER: JOHNSON MATTHEY CA2

30kV r.f.; 19.5kV accel. volts; pulse length and repetition rate varied in the analytical plates.

Impurity	Concentration given	p.p.m wt MS7	Relative sensitivity	Standard deviation % 8 separate analyses	Standard deviation % 15 repeat exposures
Bi	130	110	0.85	31	13
Pb	100	140	1.4	30	11
Sb	120	130	1.1	32	18
Sn	120	160	1.3	25	9.2
Ag	150	210	1.4	23	21
Ga	80	250	3.1	27	13
Cr	60	110	1.8	20	21

FOOTNOTE:

1 Hannay and Ahearn (1954) Anal. Chem 26 1956.

Reproducibility

The most important improvements in reproducibility have been achieved by careful control of certain instrumental parameters. As a result of recent investigations[2] it is now clear that the most important factor is one that is comparatively simple to control; namely, the ion accelerating voltage. When this is always set to the same value, and other source conditions are kept as constant as possible, reproducible analytical results are obtained. The principal reason is the improved constancy of the relative sensitivity factors for different elements. Table 2 shows how close agreement with known values is obtained when such relative sensitivity factors are measured and used to correct observed concentrations.

Variations in Photoplate

The standard deviations on identical exposures on a photoplate using a homogeneous aluminium standard indicate that the best standard deviation obtained for the different elements is about 10%. The standard deviation on isotope ratios, i.e. where the relative sensitivity factor is not involved and where a limited area of plate is used, show better figures of 3 to 4%. This indicates the likely variation due to the plate itself, and represents a limit that would remain even if a calibration spectrum of a standard was placed on the same photoplate as the sample to be analysed.

Reproducibility of Analyses

When different photoplates are used in separate analyses the standard deviation increases.

In repeat analyses different photoplates will be used and also source conditions may vary slightly. To cover the full range of exposures (10^7 to 1) it is sometimes necessary to vary the pulse length and repetition rate of the spark which might also contribute to the variations. An internal standard, although not essential, eliminates the need for such a wide range of exposures.

When the spark pulse-length and repetition rates for the analysis of a copper standard shown in Table 1 were held constant throughout the series of successive analyses of one of the standards, the minimum standard deviation did in fact decrease towards the minimum value previously obtained for repeat exposures (about 10%).

Accuracy of Analysis

The accuracy which can be attained with the MS7 when all parameters are properly controlled is perfectly exemplified by the data set forth in Table 2. In this case, of course, a standard of reasonable homogeneity has been used to establish relative sensitivity factors for the impurities in the given matrix.

It will be noted that only one standard is used, and that the levels of concentration in the standard do not correspond too closely to those in the unknown. A steel matrix has been taken as an example because studies of source conditions indicated that it seemed the most likely (compared with the aluminium and copper standards) to give poor results should the source conditions change. It is clear, however, that the results once corrected for relative sensitivity factors are in excellent agreement with those given by chemical analysis.

FOOTNOTE:

Halliday, Swift and Wolstenholme; Quantitative Analysis by Spark Source Mass Spectrometry, International Mass Spectrometry Conference, Paris 1964.

TABLE 2

ACCURACY OF ANALYSIS AFTER CALIBRATION: BUREAU OF ANALYSED STANDARDS LTD. MILD STEEL RESIDUAL SERIES SPECTROGRAPHIC STANDARD SS14

Pulse length 100 microsecs; 300 pulses/sec.: 30kV r.f.; 19.5kV accel. volts.
Concentration % wt

Impurity	MS7, average of eight analyses	MS7, corrected using SS12	Given Spectrographic value
Cr	0.26	0.18	0.185
Co	0.19	0.18	0.19
Ni	0.10	0.13	0.13
Cu	0.047	0.029	0.04
Zr	0.017	0.007	(0.005)
Nb	0.072	0.025	(0.05)
Mo	0.053	0.060	0.07
Sn	0.019	0.017	0.02
Pb	0.018	0.007	(0.0075)

( ) not certified—approximate

THE ANALYST

Quantitative Gas - Liquid Chromatographic Analysis with Detectors having a Non-linear Response

BY B. DUDLEY SULLY AND P. L. WILLIAMS

(*A. Boake, Roberts & Co. Ltd., Carpenters Road, Stratford, London, E.*15)

It is shown that if, in the gas - liquid chromatographic analysis of a mixture of two compounds, the ratio of the heights or areas of the two peaks is found to be constant regardless of the size of the sample injected, this cannot be taken as evidence that the response of the detector is linear with respect to vapour concentration, as is sometimes assumed. Such a result will be obtained, in general, if the detector response is proportional to a power of the vapour concentration, the value of the exponent being the same for both compounds; this situation can arise with the argon ionisation detector over appreciable concentration ranges at suitable voltages. A method is described for carrying out quantitative analyses in these circumstances, and is illustrated by results obtained by using mixtures of geraniol and nerol.

THE argon ionisation detector, introduced in 1958 by Lovelock,[1] has found wide acceptance owing largely to its excellent stability and insensitivity to external influences; in these respects it compares favourably with most other gas - liquid chromatographic detectors at present in use. Unfortunately, it has proved to be of only limited value in quantitative work because its response to a varying vapour concentration is markedly non-linear, except over a very limited range of operating conditions. The theoretical reasons for this were discussed in Lovelock's original paper cited above; the practical effects are well demonstrated in a recent paper by Fowlis, Maggs and Scott,[2] who studied the response characteristics of the detector over a wide range of conditions and found that the response was linear only over a small concentration range at one particular voltage, the value of which varied from one substance to another.

In general, therefore, the argon ionisation detector can be used for accurate quantitative work only if the instrument is first calibrated over the working range by using known vapour concentrations or mixtures of known composition. In this paper we describe a method of calibration that has been found conveniently applicable to the analysis of mixtures of components that give similar, though non-linear, response characteristics in the argon ionisation detector.

THEORY

This method depends on the assumption that the response of the detector is proportional to a power of the concentration of vapour in it, thus—

$$D = \sigma X^{\phi} \qquad (1)$$

where D is the detector response (that is, the signal in millivolts indicated by the recorder), X is the vapour concentration, and σ and ϕ are constants. The constant, ϕ, was termed the "response index" by Fowlis and Scott,[3] who described a method for determining its value, together with other detector characteristics, by using a vapour dilution apparatus. If the detector response is linear, ϕ will in general be sensibly constant only over limited ranges of vapour concentration.

The way in which ϕ varies with vapour concentration and with the applied voltage may be seen from the results of Fowlis, Maggs and Scott.[2] In their paper, examples are shown of the graphs obtained by plotting the logarithm of the detector response against the logarithm of the vapour concentration for a number of compounds at several voltages, when

flame ionisation and argon ionisation detectors are used. The slope of such a graph is equal to ϕ. With the argon ionisation detector, the graphs were curves whose slope varied appreciably over the range of concentrations studied; but this range was a very wide one (up to four powers of ten). Over a more restricted range of, say, one power of ten, which is of more interest for practical purposes, it would usually be possible to select a voltage such that the slope of the curve could be regarded as constant.

The method of calibration also requires that ϕ should be the same for all those components of the mixture being analysed whose relative concentrations it is desired to determine. This means that there may be difficulty in finding suitable operating conditions for the analysis of mixtures of compounds having very different ionisation characteristics, as, for example, mixtures of hydrocarbons with halogenated hydrocarbons.

When these two requirements (ϕ constant and the same for all components) are fulfilled, the system has special properties that tend to conceal any non-linearity of the detector response. In discussing this it will be assumed that the form of the elution curve is Gaussian; that is, the concentration of vapour in the detector, X, varies with time according to an equation of the form—

$$X = \frac{a}{\sqrt{2\pi}} \text{ exp. } \left[- \frac{(t - t_0)^2}{2b^2} \right] \quad .. \quad .. \quad .. \quad .. \quad (2)$$

where t = time, t_0 = the time corresponding to the maximum vapour concentration, and a and b are constants. In practice, the elution curve never has precisely this ideal form, because, apart from any lack of symmetry due to imperfect equilibrium conditions in the column, there is also asymmetry that arises from the fact that the width of the elution peak increases continuously, even while the peak is emerging from the column. However, under suitable conditions the curve is a reasonably close approximation to the Gaussian.

From equation (2) it follows that the maximum vapour concentration, when $t = t_0$, is given by—

$$X_{\text{max.}} = \frac{a}{\sqrt{2\pi}} \quad .. \quad .. \quad .. \quad .. \quad .. \quad (3)$$

and by integration the total amount of vapour, c, is equal to—

$$c = \int_{-\infty}^{+\infty} X dt = ab \quad .. \quad .. \quad .. \quad .. \quad .. \quad (4)$$

The constants a and b are related, respectively, to the height and breadth of the peak. The value of b depends on the column efficiency and on the retention-volume of the compound, but is independent of the size of the sample injected; the value of a is proportional to the latter amount.

If the detector response conforms to an equation of the form of equation (1), the variation in response, D, with time will be given by—

$$D = \sigma \left(\frac{a}{\sqrt{2\pi}} \right) \text{ exp. } \left[- \phi \frac{(t - t_0)^2}{2b^2} \right] \quad .. \quad .. \quad .. \quad (5)$$

This is still the equation of a Gaussian curve, no matter what the value of ϕ; thus there is no possibility that non-linearity of this form could be detected by an inspection of the the shape of the peak.

From equation (5), the peak height, H, is given by—

$$H = D_{\text{max.}} = \sigma \left(\frac{a}{\sqrt{2\pi}} \right)^{\phi} \quad .. \quad .. \quad .. \quad .. \quad (6)$$

and the peak area, A, by—

$$A = \int_{-\infty}^{+\infty} D dt = \frac{\sigma a^{\phi} b}{(2\pi)^{\frac{\phi - 1}{2}} \phi^{\frac{1}{2}}} \quad .. \quad .. \quad .. \quad .. \quad (7)$$

Suppose, now, that a sample of a mixture of two compounds is placed on the column, and that the respective amounts of the two compounds in the sample are c' and c''. Let the corresponding values of the parameters in equations (6) and (7) be, respectively, H', A', σ', a', b' and H'', A'', σ'', a'', b''. It is postulated that ϕ is the same for both components. Then—

$$\frac{H'}{H''} = \frac{\sigma'}{\sigma''}\left(\frac{a'}{a''}\right)^{\phi} \quad .. \quad .. \quad .. \quad .. \quad .. \quad (8)$$

and

$$\frac{A'}{A''} = \frac{\sigma' b'}{\sigma'' b''}\left(\frac{a'}{a''}\right)^{\phi} \quad .. \quad .. \quad .. \quad .. \quad (9)$$

From equation (4), $c' = a'b'$ and $c'' = a''b''$. Therefore $a'/a'' = c'b''/c''b'$, and by substituting in equations (8) and (9),

$$\frac{H'}{H''} = \frac{\sigma'}{\sigma''}\left(\frac{b''}{b'}\right)^{\phi}\left(\frac{c'}{c''}\right)^{\phi} \quad .. \quad .. \quad .. \quad (10)$$

and—

$$\frac{A'}{A''} = \frac{\sigma'}{\sigma''}\left(\frac{b''}{b'}\right)^{\phi-1}\left(\frac{c'}{c''}\right)^{\phi} \quad .. \quad .. \quad .. \quad (11)$$

Therefore, if it is assumed that b', b'' are independent of the sample size, it follows that the ratios H'/H'' and A'/A'' are functions only of the ratio c'/c'', and do not vary with the absolute values of c' and c'' provided that c'/c'' remains constant. In other words, if a series of samples of different sizes was placed on the column, the ratios of the heights or areas of the two peaks would be constant. Thus, invariability of such ratios with varying sample size cannot, as is sometimes assumed, be taken as a criterion of linearity of detector response. It can, however, be taken as evidence that the non-linearity is of the form assumed here, and therefore that the method of calibration described below is valid.

Calibration procedure

Equations (10) and (11) may be put into the form—

$$\log\left(\frac{H'}{H''}\right) = \log\left(\frac{\sigma'}{\sigma''}\right) + \phi\log\left(\frac{b''}{b'}\right) + \phi\log\left(\frac{c'}{c''}\right) \quad .. \quad .. \quad .. \quad (12)$$

and—

$$\log\left(\frac{A'}{A''}\right) = \log\left(\frac{\sigma'}{\sigma''}\right) + (\phi - 1)\log\left(\frac{b''}{b'}\right) + \phi\log\left(\frac{c'}{c''}\right) \quad .. \quad .. \quad .. \quad (13)$$

and the graph of either log (H'/H'') or log (A'/A'') against log (c'/c'') will be a straight line of slope ϕ. This is the basis of the calibration method.

It is first necessary to select the operating conditions so that when a suitable mixture, containing the two components in such proportions that their peaks differ considerably in height, is examined chromatographically, the ratio of the heights or areas of the peaks is for practical purposes independent of the sample size. The primary requirement is then fulfilled and ϕ will be effectively constant and the same for both components. Normally the procedure will be to vary the detector voltage until a suitable value is found. If the required conditions cannot be fulfilled at any detector voltage, the present method of calibration is not applicable, and indeed it is unlikely that any convenient, accurate method of calibration could be derived in these circumstances.

A calibration equation of a form analogous to equation (12) or (13) is then obtained from measurements on a series of chromatograms of standard mixtures having accurately known values of c'/c''. The equation may be derived graphically or calculated by a least-squares method; it is advisable in any event to plot the graph in order to verify that the linear relationship holds over the entire range of composition that is of interest. The equation or the graph may then be used to determine the composition of unknown samples.

For mixtures containing more than two components, it is necessary to obtain a calibration equation for each independent pair of components; that is, the number of equations required is one less than the total number of components to be determined. Alternatively, if it is

required to determine the content of only one component in a multi-component mixture, it may be more convenient to add a known proportion of another compound as a marker and to obtain the calibration equation of that component against the marker. Other variations of well known techniques may be used.

The theory of this method is based on assumptions concerning the peak shape and the constancy of the response index, which are in practice likely to be only approximately justified. However, the method has been found to work well in several instances; the example below gives the results obtained with mixtures of geraniol and nerol, which are, respectively, the *trans-* and *cis-* isomers of 3,7-dimethylocta-2,6-dien-1-ol.

Experimental conditions—

The instrument used was a Pye Argon chromatograph with a strontium-90 detector, operated under the following conditions—

Column dimensions—Internal diameter, 4 mm; packed length, 4 feet.

Column packing—Ten per cent. polyoxyethylene glycol phthalate on 85- to 100-mesh Embacel. The stationary phase was made by an alcoholysis reaction between Carbowax 1000* and dibutyl phthalate, catalysed by triethanolamine, with subsequent removal of low molecular weight material in a molecular still.

Column temperature—120° C.

Argon flow-rate—60 ml per minute. The argon was dried by molecular sieve.

Sensitivity setting—×10 (minimum).

Sample injection—By Hamilton microlitre syringe. The sample sizes required ranged down to 0·01 μl, which was too small for reliable injection by the syringe. The procedure adopted was therefore to dilute the sample, usually in the ratio 1 to 5, with a non-volatile silicone oil (Midland Silicones Fluid 702). The silicone fluid remained permanently on the column, but the performance of the latter was not significantly affected by it during the period covered by the experiments.

Chart speed—15 inches per hour.

Under these conditions, nerol and geraniol were well resolved on the chromatogram, the respective emergence times being approximately 32 and 40 minutes (measured from the point of injection to the apex of the peak). Although the absolute emergence times varied appreciably from day to day owing to small changes in gas flow-rate, the ratio of the emergence times for the two compounds remained constant; the average value for a random sample of 20 chromatograms was 1·257 with a standard deviation of 0·003. This variation was largely attributable to the error in measuring the charts. The constancy in the ratio of the emergence times was evidence that there were no significant short-term variations in operating conditions that could have influenced the relative peak heights. The relative widths of the peaks, measured at half the height, also showed no significant variation.

Peak heights were measured with a steel scale graduated in hundredths of an inch.

Results

Preliminary experiments at detector potentials of 1000 and 1250 volts showed that, at the lower of these two potentials the ratio of the peak heights for nerol and geraniol depended markedly on the sample size for a given mixture, indicating that the detector response was decidedly non-linear. At 1250 volts the ratio was almost independent of sample size, and this voltage was adopted for subsequent work. Five mixtures of accurately known composition were examined chromatographically several times each, the sample size being varied. The results are shown in Fig. 1, in which nerol peak height is plotted against geraniol peak height. The graphs approximate closely to straight lines passing through the origin, although the deviations from these lines are not entirely random, especially at the two extreme compositions.

These almost linear plots do not, however, imply that the detector response also was almost linear; this is shown by the fact that the ratio of peak heights was not proportional to the ratio of the weights of the two components in the mixtures. The actual values are given in Table I.

* Polyoxyethylene glycol of mean molecular weight 1000, manufactured by the Union Carbide Corporation.

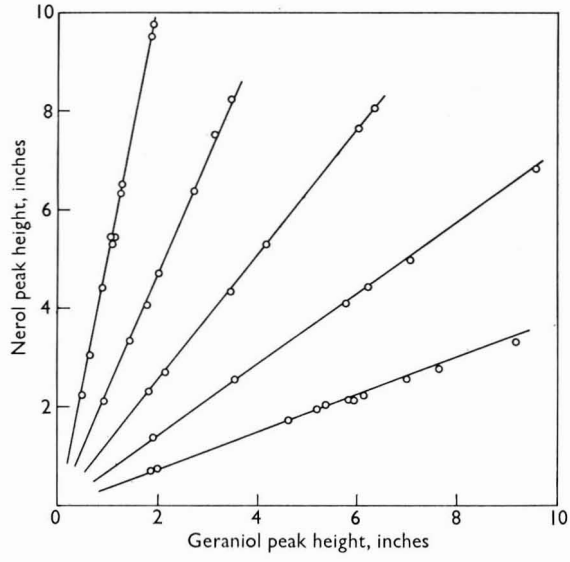


Fig. 1. Graphs of nerol *versus* geraniol peak height for five mixtures.

TABLE I

RATIO VALUES

Nerol - geraniol peak-height ratio (mean value)	Nerol - geraniol weight ratio	Weight ratio / Height ratio
0·373	0·336	0·901
0·713	0·607	0·851
1·260	0·996	0·791
2·323	1·694	0·729
4·884	3·317	0·697

These results, according to the preceding theoretical reasoning, imply that the detector response was approximately of the form described by equation (**1**), in which ϕ was not equal to 1. This was confirmed by plotting the logarithm of the peak-height ratio against

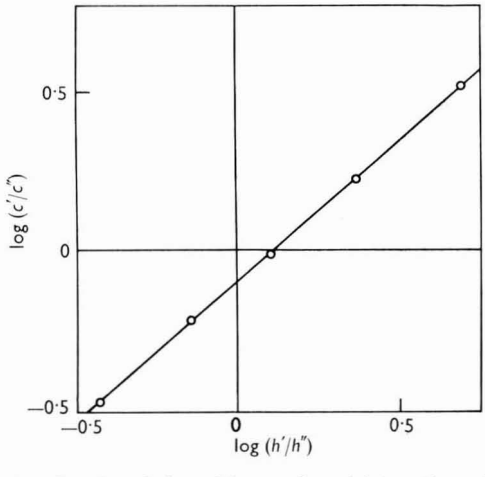


Fig. 2. Graph of logarithms of weight ratios plotted against logarithms of peak-height ratios for nerol and geraniol.

the logarithm of the weight ratio, when a closely linear plot was obtained (see Fig. 2). The results were fitted by a regression line having the equation—

$$\log\left(\frac{H'}{H''}\right) = 0{\cdot}1033 + 1{\cdot}124 \log\left(\frac{c'}{c''}\right) \qquad .. \quad .. \quad .. \quad (14)$$

in which H' and c' refer to nerol and H'' and c'' to geraniol. This equation is analogous to equation (12), and the value of ϕ is thus 1·124.

To provide an illustration of the use of this calibration method in quantitative analysis, two further nerol - geraniol mixtures of accurately known composition (not coinciding with any of the original five) were made up. Peak heights were measured on several chromatograms of each, and the compositions were calculated from equation (14). The results are given in Table II.

TABLE II

RESULTS FOR MIXTURES OF ACCURATELY KNOWN COMPOSITION

Mixture	Run number	$\frac{H'}{H''}$	$\frac{c'}{c''}$ from equation (14)	True $\frac{c'}{c''}$	Nerol, per cent. by gas - liquid chromatography	Nerol, per cent. (true)
1	1	3·116	2·224	2·204	68·98	68·79
	2	3·119	2·226		69·00	
	3	3·109	2·220		68·94	
2	1	1·027	0·829	0·834	45·32	45·46
	2	1·037	0·836		45·53	
	3	1·024	0·827		45·25	
	4	1·024	0·827		45·25	

For each mixture, the greatest deviation of the calculated value of c'/c'' from the true value was about 1 per cent. of the latter. In the last two columns of Table II the results are expressed in the more usual manner, as percentages of nerol; in this form, the discrepancy between the calculated and true values did not exceed 0·21 per cent. The agreement was within the limits of error associated with the instrumental and measuring techniques used.

APPLICATION OF THE METHOD TO DETECTORS OTHER THAN THE ARGON IONISATION TYPE

The method is obviously applicable to any detector system of which the response approximates sufficiently closely to the required form. This may happen, for example, with the flame ionisation detector, for which Fowlis, Maggs and Scott[2] found that the response index was constant over wide concentration ranges and that its value varied from 0·96 to 1·13, depending on the substance chosen and on the gas flow-rates used. The method has in fact been applied successfully to the analysis of mixture of isomeric ionones with a flame ionisation detector which showed marked non-linearity (A. J. Montgomery, private communication).

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The Identification and Determination of Chlorinated Pesticides Residues

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Gas - liquid chromatography provides a method of extreme sensitivity for the detection and determination of organochlorine pesticides residues. A single retention-time cannot, however, be regarded as a reliable identification of an unknown compound. A "clean-up" procedure is described by which interference is virtually eliminated, and a technique is proposed in which the change in retention-time after simple chemical reactions provides confirmation of the identity of a pesticide.

Gas - liquid chromatography is now well established for the determination of residues of organochlorine pesticides. The usual procedure involves solvent extraction of the pesticides, followed by a choice of "clean-up" procedures and, finally, detection and determination of the pesticides by gas - liquid chromatography with an electron-capture detector.

In the course of the examination of large numbers of wild-life specimens, a "clean-up" procedure has been developed that virtually removes all interfering matter, and a new technique has been devised to confirm the identity of the compounds giving a response in the electron-capture detector.

In the work described by Goodwin, Goulden and Reynolds[1] a simple extraction and "clean-up" procedure was used, involving a preliminary acetone extraction followed by partition into hexane. This procedure has been successfully applied to animal tissue by Taylor.[2]

Subsequently it has been found that, although results can rapidly be obtained with a minimum of initial clean-up, too much extraneous matter can cause interference and curtail column-life, as much as possible should therefore be removed.

Recently de Faubert Maunder *et al.*[3] have proposed a more elaborate procedure involving a hexane - dimethylformamide partition. It has been found in this laboratory, however, that a procedure based on the hexane - acetonitrile partition of Jones and Riddick[4] yields a cleaner extract substantially free from interfering matter, and resulting, incidentally, in a much prolonged column life.

It has also been recognised that, in the interpretation of the chromatogram, a single retention-time by itself is frequently not an adequate identification of a pesticide residue and it is common practice to use paper and thin-layer chromatography to confirm the identity of pesticides detected by gas chromatography. These techniques are less sensitive than gas chromatography.

Goulden, Goodwin and Davies[5] have described a multi-column gas chromatograph providing a "spectrochromatogram" characteristic of each substance. This, however, requires a major modification to the gas chromatograph.

A simpler and more rapid procedure is now proposed in which the unknown substance is converted by a simple chemical reaction into a derivative. The change in retention-time after conversion, together with the original retention-time, provides evidence as to its identity. By this means, substances that are normally unresolved by ordinary gas chromatography can be separated and identified. Only one gas chromatograph is required since the whole procedure is carried out on the same column.

Experimental

Gas chromatography—

The procedure and conditions described by Goodwin *et al.*[1] were satisfactory and were used throughout.

Clean-up—

The clean-up procedure proposed by Goodwin *et al.*[1] and subsequently Taylor[2] leaves a substantial part of the lipid fraction in the final extract. In order to prolong column life it is

desirable that as much extraneous matter as possible should be removed. Also, a high lipid concentration may exert a masking effect on the response of the electron-capture detector.

De Faubert Maunder's procedure[3] aims at providing a cleaner extract and introduces a hexane - dimethylformamide partition, designed to remove most of the lipids, followed by chromatography.

We have found acetone efficient in the initial extraction of animal viscera and convenient in subsequent operations. The hexane - acetonitrile partition described by Jones and Riddick[4] is also a more efficient means of removing unwanted lipids. The cost of the acetonitrile used is small in relation to the cost of the whole analysis.

Two further stages have been introduced—

A wash with weak aqueous alkali—This removes residual fatty acids and other acidic constituents. It was thought that this procedure might be criticised as being liable to lead to loss of DDT, but experiment shows that, if a light petroleum solution of DDT is shaken vigorously for 5 minutes with aqueous alkali under the conditions described, no detectable amount of DDE is formed and no loss of DDT is observed.

A final chromatographic clean-up using a very short weak alumina column—The efficiency of this clean-up procedure is sufficient to provide a base-line response on the gas chromatograph from 10 g of animal viscera, containing no pesticides, concentrated to a final volume of only 1 ml.

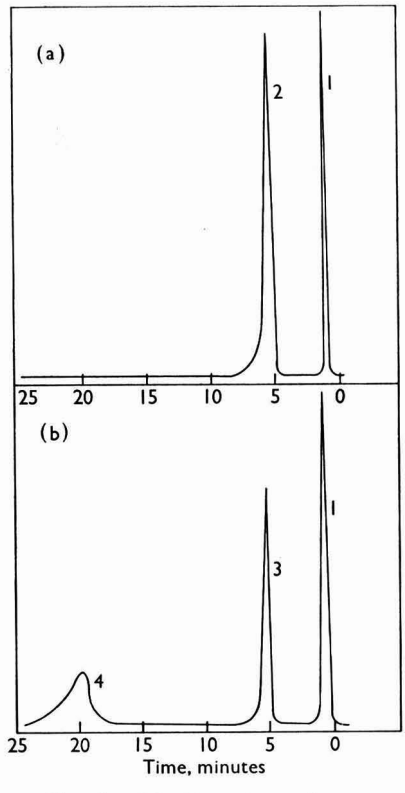

Fig. 1. (*a*) Gas chromatogram of a mixture of dieldrin and DDE. (*b*) The same mixture after reaction with hydrobromic acid reagent at 20° C.

Peak 1, solvent; peak 2, dieldrin and DDE mixture; peak 3, unchanged DDE; peak 4, dieldrin derivative

CONFIRMATORY REACTIONS—

Several reactions have been investigated and three have been selected as being adequate for most purposes.

Reactions with hydrobromic acid—O'Donnell, Johnson and Weiss[6] have shown that, on treatment with a hydrobromic acid - acetic anhydride mixture, the epoxide ring of dieldrin is opened. Our investigations showed that the products of the reaction gave a double peak on the gas chromatogram with a longer retention-time than the parent substance. Since mixtures of dieldrin and DDE are not resolved by the procedure for gas chromatography described by Goodwin *et al.*,[1] this reaction provides a solution to this problem. Unfortunately, under the conditions described by O'Donnell *et al.*,[6] some DDE appears to be lost, although no derivative is detected.

However, if the reaction is carried out at room temperature instead of at 120° C, as described by O'Donnell *et. al.*, dieldrin forms a single derivative, still clearly separated from DDE, and the latter is recovered unchanged without significant loss.

Chromatograms of a dieldrin and DDE mixture before and after treatment with hydrobromic acid reagent are shown in Fig. 1.

Of the common chlorinated pesticides, only dieldrin and the isomeric endrin react under these conditions. If the reaction is carried out at 120° C, however, all the cyclodiene pesticides react.

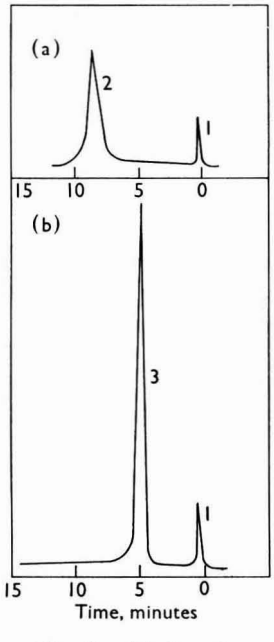

Fig. 2. (*a*) Gas chromatogram of *pp'*-DDT. (*b*) Gas chromatogram of *pp'*-DDT after reaction with alcoholic potassium hydroxide.

Peak 1, solvent; peak 2, *pp'*-DDT; peak 3, DDE

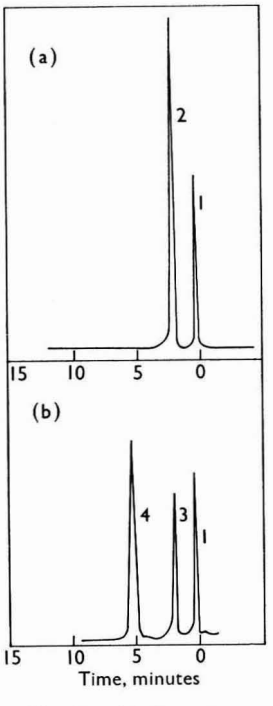

Fig. 3. (*a*) Gas chromatogram of a mixture of aldrin and a natural impurity having the same retention time. (*b*) The same mixture after reaction with chlorine.

Peak 1, solvent; peak 2, mixture of aldrin and natural impurity; peak 3,natural impurity unchanged; peak 4, aldrin derivative

Reaction with alcoholic potassium hydroxide—The reactions of DDT and BHC with cold alcoholic potassium hydroxide are standard methods of assay. DDT yields DDE which has a shorter retention time on the gas chromatogram. Chromatograms of DDT before and after treatment with alcoholic potassium hydroxide are shown in Fig. 2. TDE (Rothane) undergoes an analogous reaction. BHC yields products indistinguishable from the solvent peak on the gas chromatogram.

Reaction with chlorine—Of the commoner chlorinated insecticides, only aldrin yields a derivative with chlorine. The chlorine adds to the $C_{(6)}$ double bond in aldrin, yielding a product with a much longer retention-time.

The natural impurity observed by Goodwin *et al.*[1] to have a similar retention-time to aldrin does not react, and aldrin may be separated from it by this means. This is illustrated in Fig. 3.

Method

Apparatus—

Separating funnels, 1-litre, 250-ml and 100-ml capacity.
Hartley funnel, 7 cm in diameter.
Chromatographic column, 6 mm in diameter × 20 cm.
Test-tubes, 6-inch × $\frac{5}{8}$-inch, glass-stoppered.
Oil-bath, controlled at 120° C.
Gas chromatograph with electron-capture detector.

Reagents—

Acetone.
Light petroleum (boiling range 40 to 60° C).
Acetonitrile saturated with light petroleum (boiling range 40 to 60° C).
Sodium sulphate, anhydrous.
Sodium sulphate—Saturated solution.
Sodium hydroxide, 0·5 N.
Alkaline sodium sulphate solution—Mix equal volumes of saturated sodium sulphate solution and 0·5 N sodium hydroxide solution.
Activated alumina—B.D.H. alumina for chromatography, de-activated by addition of water to give an activity equivalent to Brockmann grade V.[7]
Hydrobromic acid reagent—To 10 ml of 48 per cent. hydrobromic acid in a glass-stoppered flask cooled in ice, carefully add 20 ml of acetic anhydride. Stopper the flask, mix and allow to stand for 30 minutes.
Alcoholic potassium hydroxide, 0·5 N.
Chlorine reagent—Suspend 0·25 g of bleaching powder in 10 ml of water in a 50-ml separating funnel. Add 10 ml of chloroform and 0·5 ml of concentrated hydrochloric acid. Shake thoroughly and allow the layers to separate. Run the chloroform layer through 0·5 g of anhydrous sodium sulphate into a small stoppered flask. This reagent should be freshly prepared before use.
Hexane—Low in aromatics.

Procedure—

Transfer about 5 to 10 g of flesh or viscera to a homogeniser. Add 100 ml of acetone and macerate for 5 minutes. Filter through the Hartley funnel, return the residue to the homogeniser and macerate again with 50 ml of acetone. Filter through the same filter paper and transfer the combined filtrates to a 1-litre separating funnel.

Dilute the contents of the separating funnel to 600 ml with water and add 40 ml of saturated sodium sulphate solution.

Extract the aqueous acetone solution successively with 100-ml and 50-ml portions of light petroleum. Pass the light petroleum extract through a small filter containing 15 g of anhydrous sodium sulphate. Wash the filter and sodium sulphate with a few millilitres of light petroleum, collecting the light petroleum in a 250-ml flask. Discard the aqueous acetone.

Transfer the flask containing light petroleum to a water bath and gently evaporate to a volume of about 20 ml. Remove the flask from the water bath and continue the evaporation with the aid of a stream of dry air until the volume is reduced to about 5 ml.

Transfer to a dry 100-ml separating funnel with the aid of sufficient light petroleum to give a total volume of 20 ml. Extract the light petroleum solution with 10, 10 and 5-ml portions of acetonitrile. Separate each portion in turn and wash it with the same 15 ml of light petroleum in a second separating funnel.

Combine the acetonitrile fractions and transfer them to a 250-ml separating funnel containing 90 ml of water, 10 ml of saturated sodium sulphate solution and 20 ml of light petroleum. Shake vigorously and allow the layers to separate completely. Separate the light petroleum phase and repeat the extraction of the aqueous phase with a further 15 ml of light petroleum. Reject the aqueous phase. Wash the light petroleum fractions by gently agitating each in turn with the same 5 ml of alkaline sodium sulphate solution and then with 5 ml of water.

Filter the light petroleum fractions in turn through 3 g of anhydrous sodium sulphate and evaporate the combined fractions to a volume of about 1 ml by the procedure previously described.

Prepare a slurry of 2·0 g of Brockmann grade V activated alumina in light petroleum and transfer it to the chromatographic tube. Allow the light petroleum level to sink just to the surface of the alumina and transfer the light petroleum extract to the column, rinsing the flask with 1 to 2 ml of light petroleum. Wash the column with light petroleum, collecting the first 15 ml of eluate.

Evaporate the eluate just to dryness with the aid of a stream of dry air, dissolve the residue in exactly 1 ml of hexane and use this solution for gas chromatography.

Gas chromatography—

Inject a 5-μl aliquot of the hexane solution of the extract, diluted if necessary, through the inlet port of the gas chromatograph.

Make a preliminary identification of the pesticides present by comparing the retention-times with those of standard solutions of pesticides.

By reference to Table I select one or more of the following reactions to provide confirmatory evidence of identity. Compare retention times before and after reaction with those of standard solutions similarly treated. Assess the proportion of pesticide present by comparing peak areas with standards at similar concentrations.

Reaction 1—Transfer a suitable aliquot of the hexane extract to a 6-inch × $\frac{5}{8}$-inch glass-stoppered test-tube. Gently evaporate just to dryness with the aid of a stream of dry air. Add 0·5 ml of hydrobromic acid reagent and set the mixture aside at room temperature for 30 minutes. Add 5 ml of water and 1 ml of saturated sodium sulphate solution and shake cautiously. Add 1 ml of hexane and shake vigorously. Remove the aqueous layer by syphoning. The organic layer may be used without further treatment for gas chromatography.

Reaction 2—Treat an aliquot in the same manner as in Reaction 1, but, after adding the hydrobromic acid reagent heat the test-tube in an oil-bath at 120° C for 30 minutes.

Reaction 3—Transfer an aliquot of the hexane extract to a stoppered test-tube and evaporate just to dryness as before. Add 0·5 ml of 0·5 N alcoholic potassium hydroxide solution to the residue, and set aside at room temperature for 5 minutes. Add 5 ml of water, 1 ml of saturated sodium sulphate solution and 1 ml of hexane, and shake the test-tube vigorously. Remove the aqueous layer by syphoning. Examine the organic layer by gas chromatography.

Reaction 4—Evaporate an aliquot of the hexane extract to dryness as before. Dissolve the residue in 0·5 ml of chloroform and add 0·1 ml of chlorine reagent. Set aside for 5 minutes at room temperature. Gently evaporate the chloroform, dissolve the residue in 1 ml of hexane and examine this solution by gas chromatography.

Discussion of the method

Retention times, relative to aldrin, of some of the more common pesticides before and after treatment by reactions described above are shown in Table I. It will be seen that hot hydrobromic acid - acetic anhydride reacts with all cyclodiene compounds, but not with BHC or with DDT and its allied compounds. Aldrin, dieldrin and endrin appear to form two derivatives, each showing a double peak on the chromatogram. Reaction at room temperature provides only one product with dieldrin and endrin, having a retention time

corresponding to the second of the two peaks provided by the hot reaction. Alcoholic potassium hydroxide, on the other hand, reacts with BHC, DDT and TDE, but not with DDE or the cyclodienes. Chlorine gives a derivative with aldrin only.

The proposed clean-up procedure yields an extract giving a base-line response on the gas chromatograph and this extract does not contain any artifacts that give detectable derivatives after treatment by the reactions described. The gas chromatogram of an extract, produced according to the method described from the viscera of an oystercatcher, is shown in Fig. 4(*a*). The specimen contained **16·7** p.p.m. of TDE, **10·2** p.p.m. of DDE, **9·7** p.p.m. of dieldin and a trace of BHC. The separation of the DDE and dieldrin in this extract by reaction with hydrobromic acid reagent is illustrated in Fig. 4(*b*).

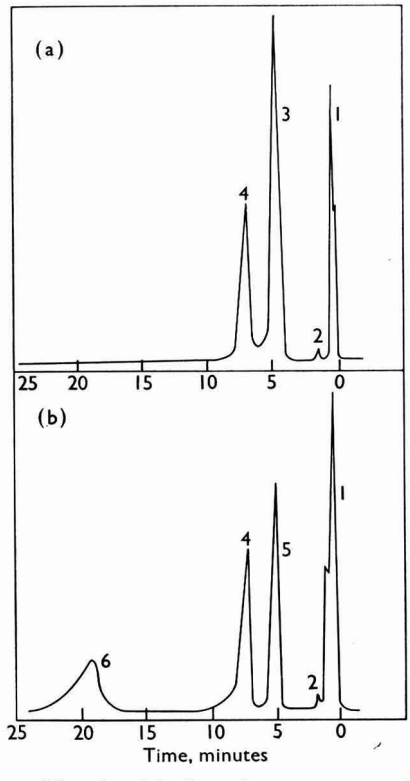

Fig. 4. (*a*) Gas chromatogram of an extract from the viscera of an oystercatcher. (*b*) The same extract after treatment with hydrobromic acid reagent at 20°C.

Peak 1, solvent; peak 2, BHC; peak 3, dieldrin and DDE mixture; peak 4, TDE; peak 5, unchanged DDE; peak 6, dieldrin derivative

It will be seen that the proposed procedure not only confirms the identification of a pesticide by gas chromatography, but also provides a simple method for the separation of compounds having similar retention times.

This technique clearly has wider application. Other simple reactions can be devised to deal with specific problems; for example, nitration has been used in the determination of residues of trichlorobenzoic acid, and reduction for the determination of pentachloronitrobenzene.

Further work, which we hope to report later, indicates that the clean-up procedure described may form the basis of a satisfactory general procedure for the determination of organochlorine and organophosphorus pesticide residues. It is equally satisfactory for the treatment of vegetable and foodstuff samples.

The retention times of parathion and malathion before and after reaction are shown in Table I. Malathion has a retention time similar to that of aldrin, but is easily distinguished from the latter by its reactions with alcoholic potassium hydroxide and with chlorine.

TABLE I

RETENTION TIMES OF COMMON PESTICIDES BEFORE AND AFTER REACTION REFERRED TO ALDRIN AS 1·0

Pesticide	Untreated	Treated with			
		Hydrobromic acid - acetic anhydride		Alcoholic potassium hydroxide	Chlorine
		Hot	Cold		
Aldrin	1·0	3·2 4·9	1·0	1·0	2·5
Dieldrin	1·9	7·6 6·1	7·6	1·9	1·9
Endrin	2·4	5·9 3·9	5·9	2·4	2·4
Heptachlor	0·8	1·1	0·8	0·8	0·8
Heptachlor epoxide	1·3	3·9	1·3	1·3	1·3
BHC	0·5	0·5	0·5	0*	0·5
pp'-DDT	3·3	3·3	3·3	1·9	3·3
op'-DDT	2·6	2·6	2·6	1·5†	2·6
DDE	1·9	1·9	1·9	1·9	1·9
TDE	3·0	3·0	3·0	1·6	3·0
Parathion	1·25	0*	1·25	1·25	1·50
Malathion	1·15	0*	1·15	0*	1·15

* 0 indicates that the original compound is destroyed and the products are either not detected or are indistinguishable from the solvent peak.

† The reaction is incomplete, a substantial proportion of *op'*-DDT remaining unchanged under the conditions described.

RECOVERY EXPERIMENTS—

Recovery experiments have been carried out in which the various pesticides, in the form of standard solutions in hexane, were added to animal viscera. The viscera were then subjected to the whole of the extraction and clean-up procedure. The results are shown in Table II. It will be noted that the recovery of aldrin is not entirely satisfactory. Aldrin is, however, found only infrequently in animal remains, since it is converted fairly rapidly into dieldrin.

The result of a recovery experiment with parathion is included in Table II. A gas chromatograph was used in this experiment, but experiments show that the extract is highly suitable for the chemical determination of phosphorus, giving control blanks comparable with those of existing procedures.

TABLE II

RECOVERIES OF PESTICIDES ADDED TO ANIMAL VISCERA

Pesticide	Level of addition, p.p.m.	Recovery, %
BHC	0·1	90
BHC	0·5	90
pp'-DDT	5·0	75
pp'-DDE	5·0	75
pp'-TDE	1·0	82
Dieldrin	0·1	83
Dieldrin	0·5	85
Endrin	0·5	83
Aldrin	0·5	40
Heptachlor epoxide	0·1	75
Heptachlor epoxide	0·5	90
Parathion	10	85

NOTE—

Our attention has been drawn to a paper by Beckman and Berkenkotter[8] in which a chemical reaction technique is used for the characterisation of organochlorine pesticides. The reaction involves use of sodium in liquid ammonia as a reagent and results in complete de-chlorination, so that the sensitivity of the electron-capture detector is not available. These authors indicate that aliquots containing at least 10 μg of pesticides are required for gas chromatography. This would not, therefore, provide a satisfactory procedure for the levels of pesticide in which we are currently interested.

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The Control of Particle Characteristics in Precipitation and the Inter-relationship of Size-analysis Techniques

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Varying size ranges of calcium carbonate are produced by controlled precipitation in a stirred batch reactor.

The application to calcium carbonate of various methods of determining particle-size distributions and surface areas is considered, and inter-relationships between these methods are established.

A relationship is established between final particle-surface area and power input to the slurry and an explanation is put forward in terms of the effect of turbulence on nucleation and crystallisation in the precipitation.

A KNOWLEDGE of the particle-size distribution in a powder, slurry or aerosol is of vital interest to the chemical engineer. In recent years, technology has progressed to such a point that accurate quantitative information concerning the particle-size distribution is needed to control the product characteristics to a sufficient degree. This poses the problem of predicting the final particle characteristics from a knowledge of the system variables.

In addition to the complexity of the problem itself, examination of the particle-size distribution of a powder is further handicapped by the complicated techniques and expensive equipment involved. Several methods have widespread industrial use because of their simplicity and speed. They are reviewed and compared in several articles.[1 to 6] In this investigation, inter-relationships have been established between the more popular analytical techniques with a view to eliminating the need for conducting the more laborious ones.

The effect of power input to the impeller, temperature of the slurry and gassing rate on the final particle shape and size has been investigated and discussed.

Experimental

Calcium carbonate was precipitated from a slurry of calcium hydroxide by using a simulated flue gas (10 per cent. carbon dioxide). The reaction was carried out in a 10-litre, fully baffled tank, the slurry being aerated and agitated by means of a single-orifice gas sparger and a six-vaned, fully shrouded impeller. The reactor was similar to that described by Cooper, Fernstrom and Miller.[7]

Temperature was controlled by means of cooling coils and a 1-kW heating tape, and the gas flow was metered by means of calibrated rotameters.

The temperature of the slurry, the agitation rate, the concentration of calcium hydroxide and the gassing rate were chosen as potential variables of significance and a 2^4 factorial design was conducted initially, giving a range of particle sizes (see Table I). By Yates' method of analysis of variance,[8] the significant variables were obtained (see Table II).

Table I

Design of factorial experiment

Temperature of slurry, °C	Concentration of slurry, g of calcium hydroxide per litre	Speed of agitation, 1500 r.p.m. Period of gassing—		Speed of agitation, 2500 r.p.m. Period of gassing—	
		1 hour	3 hours	1 hour	3 hours
25	60	Run 1	Run 2	Run 3	Run 4
	100	Run 5	Run 6	Run 7	Run 8
35	60	Run 9	Run 10	Run 11	Run 12
	100	Run 13	Run 14	Run 15	Run 16

The specific surface area, determined by a nitrogen-adsorption technique,[9] was found to be dependent upon agitation speed at the 95 per cent. confidence level. It would therefore be expected that the effects of gassing rate and power input to the impeller would be confounded if the level of turbulence was a significant factor. From examination of the electron micrographs, one example of which is seen in Fig. 5, there appeared to be an ageing effect

TABLE II

VALUES FOR SIGNIFICANT VARIABLES

Run number	Specific surface, sq. metres per g, by— nitrogen adsorption	Specific surface, sq. metres per g, by— permeametry (mean value)	Mean diameter, d_m, in μ, determined by— nitrogen adsorption (*i*)	Mean diameter, d_m, in μ, determined by— permeametry (*ii*)	Mean volume surface - diameter, d_{vs}, in μ, determined by— sedimentation (*iii*)	Mean volume surface - diameter, d_{vs}, in μ, determined by— optical microscopy (*iv*)
1	24·23	5·23	0·09	0·42	1·40	11·04
2	11·65	2·36	0·19	0·96	1·41	7·25
3	18·53	3·57	0·12	0·62	1·86	10·40
4	13·00	2·70	0·17	0·82	1·42	7·20
5	18·01	3·25	0·12	0·68	1·74	9·51
6	15·34	2·89	0·14	0·77	1·03	13·04
7	14·09	3·09	0·16	0·72	1·42	6·21
8	14·91	3·17	0·15	0·70	1·30	10·68
9	18·27	4·20	0·12	0·53	1·61	7·66
10	12·02	2·54	0·18	0·87	1·75	6·91
11	15·18	3·14	0·15	0·71	1·34	5·32
12	13·38	3·11	0·17	0·71	1·06	9·31
13	19·65	3·55	0·11	0·62	1·67	12·47
14	10·05	2·34	0·22	0·95	0·80	9·30
15	20·02	4·30	0·11	0·51	1·57	6·81
16	12·36	2·88	0·18	0·77	1·13	9·61

Run number	Ratios (*ii*) to (*i*)	(*iii*) to (*iv*)	(*iii*) to (*ii*)	(*iii*) to (*i*)
1	4·65	0·13	3·30	15·33
2	5·05	0·19	1·47	7·41
3	5·20	0·18	3·01	15·65
4	4·83	0·20	1·73	8·35
5	5·54	0·18	2·55	14·11
6	5·31	0·08	1·34	7·11
7	4·57	0·23	1·97	9·01
8	4·72	0·12	1·86	8·79
9	4·36	0·21	3·06	13·31
10	4·73	0·25	2·01	9·51
11	4·83	0·25	1·90	9·18
12	4·31	0·11	1·50	6·44
13	5·32	0·13	2·67	14·73
14	4·30	0·09	0·85	3·64
15	4·63	0·23	3·06	14·17
16	4·30	0·12	1·47	6·32

TABLE III

DETERMINATION OF THE RELATIONSHIP BETWEEN PARTICLE SPECIFIC SURFACE AND SYSTEM VARIABLES

The temperature of each run was 25° C and the initial concentration of calcium hydroxide was 100 g per litre

Run number	Period of gassing, hours	Power input, *P*, to slurry, watts per litre of slurry	Specific surface area, *S*, of whiting, sq. metres per g determined by permeametry
17	3	80·92	3·07
18	2	81·30	3·31
19	1	83·18	3·55
20	3	50·06	2·95
21	2	56·36	2·96
22	1	58·04	3·26
23	3	31·96	2·67
24	2	32·58	2·60
25	1	30·24	2·73

of the crystal, and in the set of nine runs (see Table III) both gassing rate and speed of agitation were varied in order to determine the relationship between particle specific surface and system variables.

PARTICLE-SIZE ANALYSIS—

With minor exceptions, the methods of measuring the distributions of particle size may be classified into two main types: the direct linear measurement by microscopic means and the fluid dynamic measurements based on Stokes' law. A few other types, for example the X-ray method, have been developed but are not in wide use at present. In addition, there are several methods such as permeability and low-pressure gas adsorption that yield a mean particle size for the entire powder from a pseudo specific surface instead of a single distribution curve.

The methods used in this investigation include optical and electron-microscopic counting, sedimentation, permeability and low-pressure nitrogen adsorption.

Microscopy—The microscopic method consisted in dispersing the powder in de-agglomerated form in alcohol, upon a slide or membrane, depending on whether an optical microscope or electron microscope was used. Through magnification and microphotography, or comparison with a calibrated Porton-pattern eye-piece graticule, about 700 particles per slide were categorised.

In plotting the optical microscope counts on logarithmic-probability paper, curves rather than straight lines were obtained. These were straightened statistically by a "Limit Function" developed by Kottler.[10]

$$X = \frac{x - x_0}{x_\infty - x}(x_\infty - x_0) \qquad (1)$$

where X = "limit function" particle diameter,
x = particle diameter,
x_0 = smallest particle diameter,
x_∞ = largest particle diameter.

It appears, therefore, that these particles come from a universe with logarithmically distributed particle size. The small particles of calcium carbonate have a tendency to form agglomerates, whereas the larger particles may settle out rapidly during preparation of the suspension in making the slide. This, then, may account for the anomalous representation of the particle-size distribution.

The volume - surface mean diameter $\Sigma nd^3/\Sigma nd^2$ was evaluated from the Hatch and Choate equation[11]—

$$\log d_{vs} = \log d_g + 5{\cdot}7565 \log^2 \sigma_g \qquad (2)$$

where d_{vs} = mean volume - surface diameter,
d_g = geometric mean diameter,
σ_g = standard geometric deviation, *i.e.*, $d_{84{\cdot}13\%}d_{50\%}$.

This method is tedious and subject to serious sampling errors. In addition, the reproducibility that can be achieved in the de-agglomerated dispersion from slide to slide limits the reproducibility with which the count can be performed.

Sedimentation—The proportion of calcium carbonate particles above 25 μ is usually less than 5 per cent. when the particles are formed by precipitation from a liquid. Although exact knowledge of this proportion of coarse particles is important for some uses, sieving gives no useful information about the bulk of the powder; other methods for examining the particles are necessary. The Andreasen method[12] of test by sedimentation in water was successfully applied for the determination over the range 1 to 25 μ.

Since the agglomeration of particles is a handicap, several dispersing agents were tried. Good results were obtained with Dispersol T, a non-foaming water solution of the sodium salt of a formaldehyde - naphthalenesulphonic acid condensate (obtainable from Imperial Chemical Industries Ltd.). A millilitre of 50 per cent. Dispersol T solution was added to 600 ml of the suspending liquid.

The optimum concentration of calcium carbonate was found to be with a dilution of 0·2 per cent. by volume. This is in line with other investigations.[13]

The Hatch and Choate equation[11] was used to evaluate the volume - surface mean diameter according to the equation—

$$\log d_{vs} = \log d_g - 1{\cdot}1513 \log^2 \sigma_g \quad .. \quad .. \quad .. \quad (3)$$

Equations (2) and (3) differ in that the equivalent logarithmic values, in terms of statistical parameters of the distribution curves, are obtained by count and by weight, respectively.

Nitrogen adsorption—The method of determining the monolayer capacity of a powder for adsorbed gas molecules according to Brunauer, Emmett and Teller[9] was adopted. Nitrogen was absorbed on the surface of the carefully dried calcium carbonate sample at reduced pressure. The pressure in the system was of the order of 10^{-4} or 10^{-5} mm; a mercury diffusion pump was used to obtain this pressure. The experimental procedure was a volumetric one and was carried out in a system of burettes. The disadvantages of this technique are numerous, *viz.*, laborious preparation of the sample, trouble in elimination of leaks in the apparatus, involved analysis and evaluation of results.

The surface mean diameter, d_m, was evaluated from the equation by Carman[14]—

$$d_m = 6/S_0 \quad .. \quad .. \quad .. \quad .. \quad .. \quad (4)$$

where S_0 = specific surface, in sq. cm per cu. cm and
$d_m = \Sigma nd^3/\Sigma nd^2$

This is not to be confused[15] with the mean surface diameter, d_s, given by—

$$d_s = \sqrt{\frac{\Sigma nd^2}{\Sigma n}}$$

Air permeability—The specific surface area was determined by using the Carman apparatus.[14] It is generally accepted that the air-permeability method does not give reliable results with powders of specific surface beyond 5000 sq. cm per g and the error increases with the specific surface.[13] When air flows through a bed of fine particles, the dimensions of the capillaries approach in value the mean free path of the gas molecules, and the type of air flow through the bed changes from normal viscous flow to molecular streaming.

Carman and Malherbe[16] proposed an equation to allow for the slip correction due to the latter type of flow and the results obtained seemed to indicate that their method gave better results for specific surface. Calculations of the specific surface and surface mean diameter were based on the method by Carman.[14] This method is satisfactory in that the procedure is quick, the calculations are not involved and the error in not taking account of slip corrections is sufficiently small to be neglected.

Results

Microscopy—

Examination of the electron micrographs reveals that the shapes of most of the smaller whiting particles (up to 2 μ) approximate to cubes or needle-shaped crystals, but both the electron photomicrographs and the optical microscope analysis show the larger particles to be of irregular shapes, probably formed by clusters of smaller particles. These irregular shaped particles will clearly have a larger specific surface than would simple cubes of equal total volume.

Examination of the electron photomicrographs shows that in the final sample of each run no particles below the limit of the magnification used in the optical microscope counting ($\times 500$) were evident. It would appear that, beyond a certain stage in the run, further nucleation does not occur, but precipitation continues on the particles present. This is in accordance with the change that was noted in the size distribution of the calcium carbonate particles throughout the run. Small crystals may re-dissolve in a slightly supersaturated solution, and consequently large crystals, being more stable will tend to grow at the expense of the smaller ones. A nucleus under such conditions will only grow if it is sufficiently large for the solution to be supersaturated with respect to it. In counting, therefore, consideration was given only to the optical microscopy.

Optical analysis of the particles above 2 μ reveals that the ratio—

$$d_{min.}/d_{max}$$

where $d_{min.}$ is the minimum and $d_{max.}$ the maximum linear dimension of the particle, has

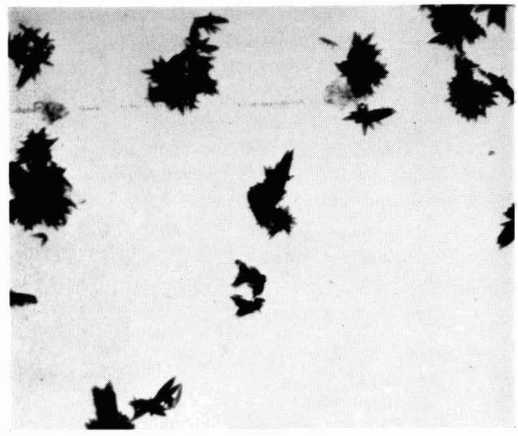

Fig. 1. Electron photomicrograph of aragonite crystals precipitated at 35° C in Run 15, indicating small unclustered particles. Magnification, × 5000

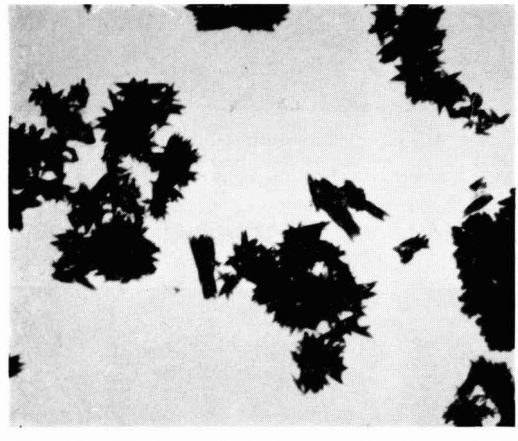

Fig. 2. Electron photomicrograph of sample from Run 13 showing residual calcium hydroxide particle and clustered aragonite particles. Magnification, × 5000

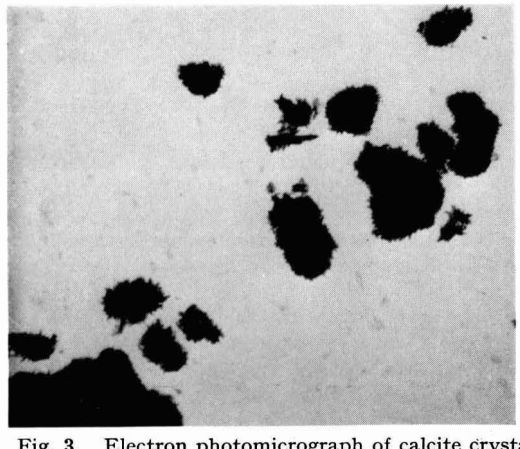

Fig. 3. Electron photomicrograph of calcite crystals from Run 7. Magnification, × 5000

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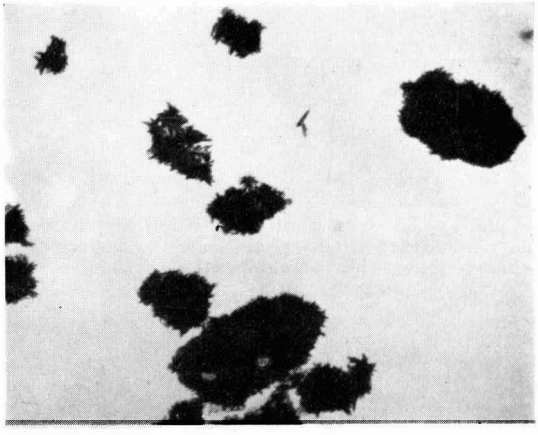

Fig. 4. Electron photomicrograph of calcite particles from Run 5 showing predominantly larger particles than in Fig. 3. Magnification, × 5000

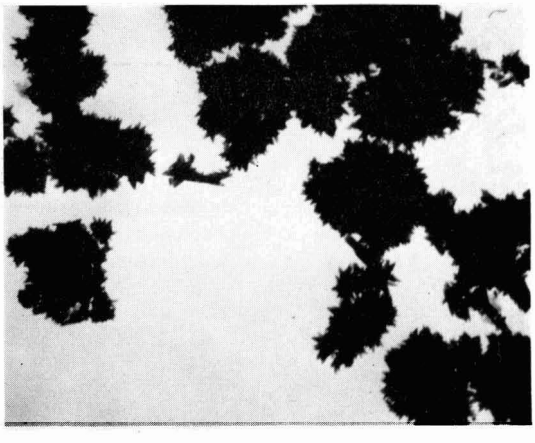

Fig. 5. Electron photomicrograph of calcite particles precipitated at 25° C at low gassing rates (Run 6) indicating thorn-like growths on the crystals presumably due to ageing. Magnification, × 5000

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a mean value of 0·54 with a standard deviation of 0·03 and a 95 per cent. confidence limit of 0·54 ± 10 per cent. for particles precipitated at 25° C, and a mean value for this ratio of 0·47 with a standard deviation of 0·04 and 95 per cent. confidence limits of 0·47 ± 10 per cent. for particles precipitated at 35° C. A hypothesis for taking a grand average for all values at 35° C and 25° C is invalid at the 0·05 significance level. It appears, therefore, that temperature of precipitation has a significant effect on the final particle shape, but no noticeable effect on the specific surface of the powder. Aragonite has been reported to precipitate as low as 35° C[17] and 30° C[18] (see Figs. 1 and 2), whereas calcite predominates at lower temperatures (see Figs. 3 and 4). This varying ratio, $d_{\text{min.}}$ to $d_{\text{max.}}$, at the two temperatures may be due to the transition from one crystal type to the other, and Meigen's reaction[19] showed a predominance of aragonite in the crystals precipitated at 35° C. The fact that there is no noticeable dependence of specific surface on temperature, and therefore crystal type, is readily explained by the high ratio of nitrogen adsorption specific surface to mean permeability specific surface in both crystal types, implying a high interstitial crystal area.

SURFACE-AREA MEASUREMENTS—

The results show that in every instance the area found by adsorption was larger than that found by air permeametry. An incorrect assumption in adsorption methods of the area occupied by a single nitrogen molecule may be made; in the present instance it has been assumed that the area is 17×10^{-16} sq. cm. However, Harkins and Jura[20] suggest $16{\cdot}2 \times 10^{-16}$ or $13{\cdot}8 \times 10^{-16}$ sq. cm. If the latter value had been used, the measured areas would have been 20 per cent. lower. A more feasible explanation for this difference is that in the air-permeability method the bed of powder behaves as a bundle of capillaries. In consequence, only the surface of the continuous paths through the bed will contribute to the measured specific surface area. The difference in areas therefore will be a measure of the area of the interstices. In the particles under study the "internal" surface area is of the order of four to five times the "external" surface area. Carman[21] emphasises the errors arising out of the lack of uniformity in compacting the bed to give a low void fraction especially with fine powders. The low mean deviation of ±3·4 per cent. in this investigation would exclude this as a possibility.

INTER-RELATIONSHIPS BETWEEN PARTICLE-SIZE ANALYSIS TECHNIQUES—

The particle-size analysis methods used were optical microscope counting, sedimentation, permeability and nitrogen adsorption. Variance analysis by Yates' method[8] of the ratios of the diameters obtained from these methods revealed that the first and the last two methods bore relationships to one another that were independent of precipitation variables.

A mean value of 4·77, with 95 per cent. confidence limits of ±5·45 per cent., was obtained for the ratio of the mean diameter, d_{m}, obtained by permeametry to the mean diameter obtained by the nitrogen-adsorption technique. For the ratio of the volume-surface mean diameters obtained by sedimentation and optical microscopy, the mean value of 0·17 was obtained with 95 per cent. confidence limits of ±17·6 per cent. The large degree of scatter in the latter instance would reflect the probable limitations of relating optical sizing to sizing by sedimentation due to the entirely random orientation of the particles on dispersing them upon the slide in contrast to the selected direction in which particles orientate themselves in sedimentation. A more realistic technique for preparing the slides would be to allow the particles to settle through a height of dispersing liquid on to the slide, thus enabling them to assume their orientation in free fall, which would eliminate the randomness in the technique of preparing the slide. Constant ratios with lower mean deviations may then result, which will suffice to eliminate the more tedious techniques and yet still furnish the required results in characterising the particle.

The ratios (*iii*) to (*ii*) and (*iii*) to (*i*), see Table II, were significantly dependent upon the gassing rate at the 95 per cent. confidence level, with mean values of 2·11 ± 19·2 per cent. and 10·19 ± 20·4 per cent., respectively. In the former instance, agglomeration of the crystals may be a cause, whereas the latter's dependence upon the gassing rate may be attributed to ageing of the crystals (see Fig. 5). This effect on surface area must, however, be small as gassing-rate did not appear as a significant effect in the Yates' analysis. Examination of the electron photomicrographs of calcite crystals precipitated at low gassing rates

(see Fig. 5) shows thorn-like growths on the crystals that may affect the sedimentation rates more than the specific surface. This effect would then be common to both ratios and would explain the apparent independence of specific surface on gassing rate.

NUCLEATION AND CRYSTALLISATION—

A log-log plot of the final particle surface area from the permeability technique, against power input to the slurry gave the following relationship based on a least-squares fit to the results of Table III at the 95 per cent. confidence level—

$$S = 2{\cdot}03\ P^{0.245}$$

where S = specific surface of powder, in sq. metres per g, determined by permeametry and
P = power input to the slurry, in watts per litre of slurry.

At a lower stirrer speed it is possible that a high degree of aggregation exists between the pre-nuclei favouring inter-growth. The low degree of turbulence and hence low degree of solute replenishment would imply that crystal growth occurs at that stage due to molecular diffusion.

On increasing the stirring speed, aggregation of the pre-nuclei would be hindered by the shorter periods of contact leading to a larger number of nuclei per mass of solute. Once the nucleus has reached a stable size where the solution is supersaturated with respect to it, crystal growth may occur due to surface renewal rather than molecular diffusion.

Garner[22] has enumerated the experimental evidence in favour of a laminar sublayer in contact with the particle in which turbulent eddies would be damped out. This has been supported by Frank-Kamenetskii[23] both theoretically and experimentally for mass transfer. If the existence of the sublayer is accepted then the only way in which molecular diffusion would become negligible in comparison with the bulk transfer is if there is an increase in the shear in the boundary layer.

From Frank-Kamenetskii's work—

$$v_x = V\left(\frac{f}{2} \cdot \frac{r}{r'} \cdot \frac{\rho}{\rho_L}\right)^{\frac{1}{3}} \quad .. \quad .. \quad .. \quad .. \quad (5)$$

$$r' = \frac{w}{\sigma} \quad .. \quad .. \quad .. \quad .. \quad .. \quad (6)$$

$$\epsilon = \frac{f}{2} \cdot \frac{\rho V^3 \sigma}{w} \quad .. \quad .. \quad .. \quad .. \quad .. \quad (7)$$

where v_x = relative velocity between particle and liquid,
V = velocity of the main flow,
f = resistance coefficient,
r = radius of the particle,
r' = scale of the main flow,
ρ = density of particle,
ρ_L = density of medium,
w = working volume of the liquid,
σ = friction surface,
ϵ = energy input per unit volume per unit time.

Equations (5), (6) and (7) can be solved simultaneously for the relative velocity, v_x—

$$v_x = \left(\frac{r\epsilon}{\rho_L}\right)^{\frac{1}{3}} \quad .. \quad .. \quad .. \quad .. \quad .. \quad (8)$$

This work was originally developed by Kolmgoroff[24] and subsequently expanded by Shinnar.[25]

From equation (8), therefore, the relative velocity between a particle of diameter 10 μ and a liquid power input of 83 watts per litre is 7·5 cm per second. This is infinitely larger than the terminal falling velocity of the particle in hindered settling and it appears therefore that above stirring speeds at which the particles are just in suspension, the crystal growth mechanism is controlled by shear in the boundary layer accompanied by an increase in the number of particles per unit mass of solute. In these tests there is, however, no indication of the upper limit of this effect.

Thus the dependence of particle surface area on power appears to be due to an increase in nucleation with increase in power with a subsequent shift in the size distribution towards the lower range. The absence of particles below 0·2 μ would preclude mechanical cleavage as a cause. Crystal growth above a certain minimum power input for complete suspension is probably due to solute replenishment in the laminar sublayer as a result of high relative velocities between solid and liquid.

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The Determination of Methanol by Oxidation to Formaldehyde and Polarographic Reduction

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Small amounts of methanol can be determined by oxidation to formaldehyde with potassium permanganate in orthophosphoric acid, with a subsequent polarographic reduction of the formaldehyde at 50° C. Optimum conditions have been found for each stage of the determination. Oxidation is best effected with a solution containing 35 g of potassium permanganate and 300 ml of orthophosphoric acid per litre and allowing the reaction to proceed for 10 minutes at 0° C.

THE determination of fatty methyl esters is important for the study of the synthesis of numerous surface active agents. For example, in the preparation of sugar esters by the reaction of fatty acid methyl esters with sucrose, the yield and the degree of substitution are indicated by the determination of residual methyl ester.

Most methods for determining methyl esters are based on a colorimetric determination of formaldehyde resulting from the oxidation of the methanol, liberated by hydrolysis of the esters.[1,2] The principal shortcoming of this procedure is insufficient standardisation of the conditions of oxidation of the methanol to formaldehyde, as well as poor reproducibility of the colorimetric formaldehyde determination.

In this study, I endeavoured to increase the sensitivity of the polarographic method for determining formaldehyde, and then to ascertain the optimum conditions for the oxidation of methanol with potassium permanganate.

THE POLAROGRAPHIC DETERMINATION OF FORMALDEHYDE

Most procedures for determining small amounts of formaldehyde are based on the colorimetric measurement of derivatives.[3,4,5,6,7] The spectrophotometric method of Eegriwe[8] is based on the reaction of formaldehyde with chromotropic acid, and has since been modified to include the reaction with J acid (7-amino-4-hydroxynaphthalene-2-sulphonic acid) or with the *N*-phenyl derivative of J acid[9]; the determination in which J acid is used is particularly sensitive. A relatively large error is associated with photocolorimetric analysis[11,12]; the solutions needed are stable only from a few hours to a few days at the utmost,[13,14] and it is necessary to calibrate daily. All these inconveniences can be avoided by using polarography. The formaldehyde is reduced, at a dropping-mercury electrode, to methanol giving, in an alkaline medium, a polarographic wave at −1·74 volts. The wave character is purely kinetic, that is to say, the wave height is directly proportional to the concentration. There is no dependence on the position of the mercury reservoir, but a pronounced one both on the temperature and the pH value of the solution. The polarographic determination of formaldehyde in disinfectants in concentrations of 0·2 mg in 10 ml of the supporting electrolyte has been described.[15] The sensitivity of this method is inadequate for determining methyl esters of fatty acids as the formaldehyde concentration is approximately one-tenth of that required by the method. However, the sensitivity can be increased considerably either by increasing the temperature, and thus accelerating the dehydration of the formaldehyde to polarographically active anhydrous formaldehyde, or by using the Smoler[16] capillary tube, which results in higher galvanometer sensitivity.

Experimental

Apparatus—

Polarograph—The Heyrovsky LP 55 polarograph was used with a galvanometer giving a deflection of 2×10^{-9} amp. per mm.

Capillary—A Smoler-type[16] was used, having an inside diameter of 0·06 mm, a length of 14 mm, and a capillary constant of $1{\cdot}43\ mg^{\frac{2}{3}}\ sec^{\frac{1}{4}}$.

Polarographic vessels—Novak type.

Thermostatically controlled bath.

Glass bath with a stand for Novak vessels.

REAGENTS—

All chemicals used were of analytical-reagent grade, and all solutions were prepared in distilled water.

Lithium hydroxide, N.

Distilled mercury.

Formaldehyde solutions—Prepared 3 hours before polarographic analysis; they contained 8·5, 17, 85 and 170 μg of formaldehyde in 5 ml of water.

PROCEDURE—

One millilitre of lithium hydroxide and 5 ml of the standardised formaldehyde solution were transferred by pipette into a Novak vessel with a mercury layer covering the bottom. After equilibration for 30 minutes at the chosen temperature, the solution was examined polarographically, beginning at −1·5 volts, without exclusion of air. The wave height was expressed for a 1-to-1 galvanometer sensitivity in microamps.

RESULTS—

The temperature region of 25° to 50° C was chosen for studying the influence of the temperature on the value of the limiting current. Higher temperatures cannot be used since the manipulation of hot vessels is difficult and, moreover, there could be formaldehyde losses due to evaporation.

The wave-height dependence on formaldehyde concentration is linear at all temperatures studied, and it can be used very well for quantitative formaldehyde determination (see Fig. 1). At 50° C, formaldehyde can be determined at a concentration of 7 μg in 5 ml (*i.e.*, about 1 mg per litre), because with the Smoler capillary, a galvanometer sensitivity as great as 1 to 2 can be used without excessive oscillations of greater amplitude. The wave height for this concentration and sensitivity is about 5 mm.

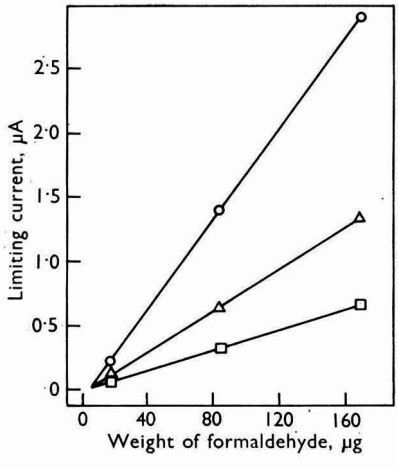

Fig. 1. Curves showing relationship between the limiting current and formaldehyde concentration in the polarographic determination: ○, at 50° C; △, at 35°C; □, at 25° C

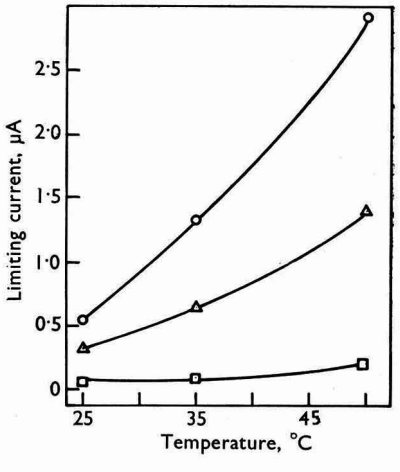

Fig. 2. Curves showing relationship between the limiting current and the temperature of the formaldehyde solution: ○, with 170 μg of formaldehyde; △, with 85 μg of formaldehyde; □, with 17 μg of formaldehyde

Fig. 2 shows the relation between the wave height and the temperature at constant concentrations. With a content of 170 μg in 5 ml, in the region of 50° C, the limiting current increases about 5 per cent. for 1° C. This means that a temperature deviation of $\pm$0·05° C causes an error of about 0·25 per cent. in the result. Since the accuracy of the polarographic method is about 1 per cent., the error is negligible.

It is known that aqueous formaldehyde solutions are not stable and deteriorate at room temperature. However, a solution containing 34 mg of formaldehyde in 1 litre, *i.e.*, 170 μg in 5 ml, was found to be sufficiently stable for about 2 days. It then started to deteriorate,

and after 12 days' storage at room temperature, formaldehyde was no longer detectable. It is therefore necessary, when preparing calibration curves, always to use fresh solutions of diluted formaldehyde. Formaldehyde is also unstable in alkaline solutions and at elevated temperatures; a series of experiments showed that the wave height was unchanged when the sample solutions were equilibrated for 25 to 60 minutes at 50° C.

The results mentioned above prove the polarographic procedure to be sufficiently accurate and sensitive for formaldehyde determination in such concentrations as occur in the determination of small amounts of methyl esters. The following conditions were found best for such determinations—

(*a*) The polarographic vessels should be equilibrated at 50·0° C for 30 minutes before the polarographic examination.

(*b*) Reservoir height, 85 cm; electromotive force applied to the potentiometer, −4 volts; speed, 400 mV per minute. The start of the curve is set at −1·5 volts.

(*c*) Galvanometer sensitivity, 1 : 2 to 1 : 30 according to the content of formaldehyde in the sample.

STUDY OF METHANOL AND FORMALDEHYDE DISTILLATION

The polarographic determination of methyl esters presupposes two distillations, namely one to separate methanol from the saponified sample, and the other to distil off the formaldehyde formed in the methanol oxidation. It is not possible to make a direct polarographic determination of formaldehyde in the oxidised sample, because the manganese wave obscures the formaldehyde wave. For the same reason it is not possible to work with an entire distillate from the oxidised mixture, since a portion of manganese salts is carried over into the distillate towards the end of the distillation. Preliminary experiments showed that it is necessary to leave about 5 ml in the distillation flask. These findings are based on experiments made in following the course of the distillation of methanol from a saponified sample of sucro-ester, and the formaldehyde losses in distilling solutions of different concentrations. The optimum temperatures for distilling both methanol and formaldehyde were found experimentally.

Experimental

Apparatus—

Semi-micro distillation apparatus—Fitted with ground-glass connections and a distillation flask of 250-ml capacity.

Constant-temperature bath—Having automatic control of temperature, sensitivity ±2° C, and filled with silicone oil.

Reagents—

All chemicals were of analytical-reagent grade, and solutions were prepared in distilled water.

Methanol solution, 0·2725 g per litre.

Formaldehyde solution, 0·0424 g per litre.

Oxidising solution—A mixture of 35 g of potassium permanganate and 300 ml of 84 per cent. orthophosphoric acid.

Sodium sulphite solution, saturated.

Procedure—

Construction of the calibration curve for methanol—Into 30-ml cylinders were transferred by pipette 1, 10 and 20 ml, respectively, of the standard methanol solution, and the volumes made up to 22·5 ml with water. Oxidation was carried out with 5 ml of oxidising solution under the conditions described at the end of the paper and quoted in the synopsis. After oxidation, and after reduction of the unused permanganate with sulphite solution, the volumes were made up to 30 ml. By using an oil-bath temperature of 160° C, distillation was carried out into a 25-ml measuring flask, leaving 5 ml of residue in the flask. The formaldehyde content was determined polarographically, and the results used to draw a calibration curve for methanol.

Study of methanol distillation—In the determination of the methyl esters in sucro-esters, the saponified sample from the reaction mixture is diluted with water to a 60-ml volume; therefore similar conditions were chosen for studying the methanol distillation. Forty

millilitres of the standard methanol solution and 20 ml of water were transferred by pipette into a distillation flask, porous pot was added and the liquid distilled off, the bath temperature being 150° C. The measuring cylinder, used as a receiver, was cooled in ice. The methanol content was determined polarographically in 5-ml fractions, after oxidation to formaldehyde.

Methanol losses during distillation—A blank determination was carried out with the standard methanol solution under identical conditions as for the above experiments. Portions (1, 10, 20 and 50 ml) of standard methanol solution were used. The methanol content was determined in 22·5 ml of distillate by the method indicated above.

Formaldehyde losses during distillation—Portions (1, 10 and 20 ml) of standard formaldehyde solution, respectively, were transferred by pipette into 25-ml measuring flasks, whose contents were then diluted with water. The formaldehyde content was then determined polarographically in each solution. Similar amounts of the standard formaldehyde solution were diluted to 30 ml, and distillation carried out as above, by using an oil-bath temperature of 160° C. In each instance 25 ml of distillate were collected. Polarographic determination was again carried out. The difference between the results obtained for the original solutions and for the solutions from distillation indicated the distillation losses, including the amount of formaldehyde remaining in the distillation residues.

Results

The results in the following diagrams are mean values from three separate determinations. When 60 ml of the methanol solution are distilled under the conditions given, nearly all the methanol distils with the first 25 ml of distillate (see Fig. 3). No methanol distils with the 25- to 30-ml fraction. In interpolating to 22·5 ml of distillate, the yield is found to be 99·7 per cent. of the theoretical.

The calibration curves (see Fig. 4) show that the losses of methanol during distillation are very small. The relationship between the amount of loss and the total methanol content is linear, an important consideration for the construction of calibration curves for the determination of methyl esters.

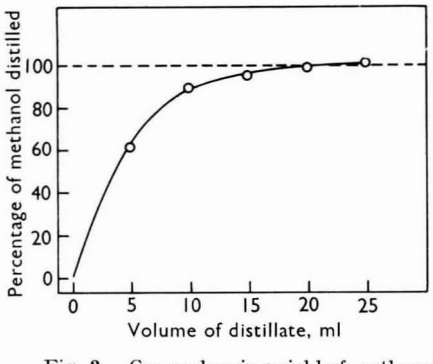

Fig. 3. Curve showing yield of methanol during distillation of 60 ml of solution and collection of 25 ml of distillate

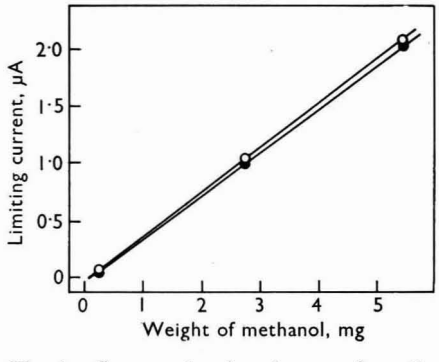

Fig. 4. Curves showing losses of methanol during distillation: ○, without distillation; ●, with distillation

Fig. 5 shows the formaldehyde losses in distilling 25 ml from 30 ml of formaldehyde solution. For all concentrations, about 85 ± 2 per cent. of the available formaldehyde distils off, which means that the distillation residue contains about 15 ± 2 per cent. of the formaldehyde.

Experiments showed that both methanol and formaldehyde can be distilled with sufficient accuracy for quantitative analysis, provided that constant conditions are observed, especially with regard to the distillation rate, the cooling of receivers and tightness of the distillation apparatus. The conditions for the distillation of methanol and formaldehyde can be summarised thus—

(*a*) Distillation of methanol from a sample of the saponified reaction mixture: 60 ml of the solution were distilled (the temperature of the oil-bath being 150 ± 2° C) and 22·5 ml of the distillate were taken for analysis. Distillation time was 15 ± 2 minutes.

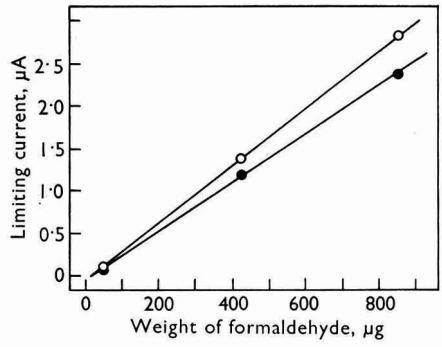

Fig. 5. Curves showing yield of formaldehyde during distillation of 30 ml of solution and collection of 25 ml of distillate: ○, without distillation, ●, with distillation

(*b*) Distillation of formaldehyde from the oxidised sample: 30 ml of the sample solution were distilled (the temperature of the oil-bath being 160 ± 2° C) and 25 ml of the distillate were collected. Distillation time was 16 ± 2 minutes.

THE OXIDATION OF METHANOL TO FORMALDEHYDE

Whereas the determination of formaldehyde is relatively simple and gives reasonably precise and accurate results, the determination of methanol is complicated, because oxidation of methanol to formaldehyde is influenced by many factors that required accurate standardisation.

Potassium permanganate in sulphuric or orthophosphoric acid medium is the oxidant most often used. Apart from this, hydrogen peroxide in alkaline solution, persulphate in sulphuric acid, chromic acid, lead chromate and other agents have been used.[17,18] Experiments other than with permanganate made in connection with this work were not successful. Most of these other oxidants either interfered with the polarographic determination of methanol, or gave formaldehyde yields that were too low. Attention was concentrated, therefore, on the study of potassium permanganate oxidation in phosphoric acid.

The published papers mostly deal with the oxidation of methanol to carbon dioxide[19,20] or the oxidation of methanol in admixture with ethanol.[4,13] Sharp[21] studied methanol oxidation with potassium permanganate in sulphuric acid at 45° to 60° C, and ascertained that the course of the oxidation depends strongly on the temperature, the amount of methanol and, to a lesser degree, on the proportion of methanol to oxidising agent. The whole course of the oxidation with permanganate can be illustrated by a series of reactions—

$$CH_3OH \longrightarrow HCHO \longrightarrow HCOOH \longrightarrow CO_2 + H_2O$$

The formaldehyde formed is converted relatively rapidly into formic acid, so that it is eliminated from the reaction mixture over a short period of time (normally 15 to 40 minutes). The speed of oxidation of formaldehyde depends on the excess of permanganate, on the temperature and other factors. Lapp and Reimers[4] reported that after 10 minutes' oxidation of mixtures of methanol and ethanol, 78 per cent. of the methanol is converted into formaldehyde, but at the same time 66 per cent. is oxidised further, so that only 16 per cent. of the formaldehyde that was formed remains in the oxidised sample. When they oxidised methanol alone, without ethanol, they found that under identical conditions only 3 per cent. of the formaldehyde remained.[4] In verifying Snell's procedure,[1] I ascertained that the conversion of methanol to formaldehyde depends not only on the temperature, but also on the initial amount of methanol present. After 4 minutes' oxidation, 3 to 18 per cent. of the formaldehyde was found, depending on the conditions of the oxidation. The oxidation results, according to my observations, are dependent, to a certain degree, also on the method used to stop the oxidation by neutralising the permanganate. Reduction with solid sodium sulphite does not give reproducible results.

In studying the oxidation of methanol, the effect of time and temperature, the amount of methanol present, and of the amounts of permanganate and orthophosphoric acid, were all investigated.

Experimental

Reagents—

All chemicals used were of analytical-reagent grade, and all solutions were prepared in distilled water.

Potassium permanganate.

Orthophosphoric acid, 85 per cent. w/v.

Sodium sulphite.

Methanol, anhydrous—Purify by distillation.

Procedure—

Statistical study to determine the influence of temperature, amount of methanol and composition of oxidation mixture—For the evaluation of the factors, a Graeco - Latin square[22] of 4 × 4 terms was used. The four variables studied and their levels and designations are set out in Table I. The combinations of variables actually used can be seen in Table II.

Table I

Variables studied in Graeco - Latin square experiment

Factor and units	Levels
Amount of methanol, mg*	I = 0·132, II = 2·64, III = 6·60, IV = 13·2
Oxidation temperature, °C	1 = 0, 2 = 20, 3 = 40, 4 = 60
Permanganate concentration, g per litre of oxidising solution	A = 9, B = 18, C = 27, D = 36
Orthophosphoric acid concentration, ml of 85% w/w H_3PO_4 per litre of oxidising solution	α = 100, β = 200, γ = 300, δ = 400

* 0·132 mg methanol is equivalent to 0·1 per cent. of methyl myristate in 1 g of sample.

Table II

Graeco - Latin square experimental design

Numbers in brackets indicate the random order in which the experiments were carried out; lower-case letters have been used for the evaluation of the experimental error

	I	II	III	IV
1	A α (11c)	D δ (13b)	B γ (6d)	C β (10a)
2	C γ (7b)	B β (16c)	D α (8a)	A δ (5d)
3	D β (15d)	A γ (9a)	C δ (14c)	B α (4b)
4	B δ (12a)	C α (2d)	A β (3b)	D γ (1c)

For example, the procedure used in experiment No. 1 was as follows—

A 22·5 ml portion of methanol in water containing 13·2 mg of methanol, was mixed with 5 ml of an oxidising agent containing 3·6 g of potassium permanganate with 30 ml of 85 per cent. orthophosphoric acid per 100 ml. Oxidation was carried out for 6 minutes at 60° C ± 0·1° C. Before being mixed, the solutions were brought to this temperature. In order to avoid a harmful temperature gradient inside the solution, caused by the exothermic reaction of permanganate with methanol,[4] the oxidised samples were thoroughly shaken once a minute. The oxidation was terminated by neutralisation of the excess of permanganate by titration with a standard solution of sodium sulphite. The neutralised solution was then diluted to 30 ml with water, 25 ml was distilled off and collected, and the formaldehyde determined polarographically. The method of statistical evaluation will not be described here: details can be found in the literature.[22]

Table III

Percentage of methanol oxidised to formaldehyde under different conditions

Temperature of oxidation	Methanol originally present, mg			
	0·132	2·6	6·60	13·2
0° C	24·2	15·3	14·2	10·2
20° C	21·0	8·8	8·1	8·3
40° C	16·1	7·3	7·4	5·3
60° C	9·1	4·5	3·3	3·3

Results

The amounts of formaldehyde found were calculated back to methanol and expressed as percentages of methanol oxidised. The results are set out in Table III.

It was found on examination of these results that only the influence of temperature and amount of methanol are significant (significance, 0·1),[22] whereas the influence of other factors can be attributed to experimental error; the average error on a single test amounts to ±1·8. The overall average value is 10·4 per cent.

The quantitative evaluation of the significant factors is expressed in Fig. 6. On the x-axis is shown the influence of the levels of methanol content, and of temperature, on the increase or decrease of the average amount of oxidation. Both from statistical evaluation and from the diagram it is evident that the influence of these factors is great. Although the effect of temperature on the oxidation is almost linear, the relationship between the amount of methanol and the conversion of the methanol to formaldehyde is much more complex. It should be realised that the correlations illustrated in the diagram are considerably influenced by the interaction of temperature and concentration of methanol and also by the oxidising agent.

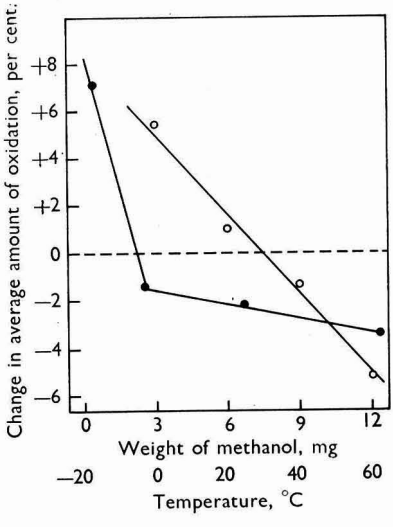

Fig. 6. Curves showing effect of temperature and concentration of methanol on the yield of oxidation: ○, effect of temperature; ●, effect of methanol

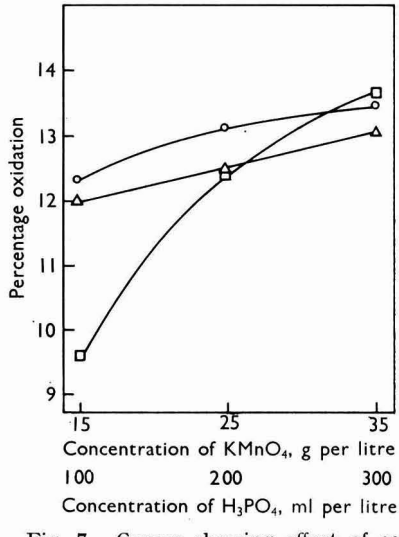

Fig. 7. Curves showing effect of composition of oxidizing solution on the yield of formaldehyde: ○, orthophosphoric acid in the presence of 35 g of potassium permanganate; △, potassium permanganate in the presence of 200 ml of orthophosphoric acid; □, orthophosphoric acid in the presence of 25 g of potassium permanganate

From the slope of the straight line that represents the influence of temperature, it can be calculated that a temperature of 1° C causes an increase in yield of formaldehyde of 0·16 per cent., *i.e.*, about 1·6 per cent. of the result. For this reason it is important to ensure accurate control of temperature. Maximum yields were obtained at 0° C, and therefore this temperature was used in all subsequent experiments.

The influence of wide variations in the concentration of the oxidising solutions is not large, and small fluctuations in the concentrations of both permanganate and acid may be ignored. These two factors, however, were further studied in a second experiment.

The influence of oxidising agent on the yield of formaldehyde—The influence of the composition of the oxidising solution was studied at a constant temperature of 0° C and with a standard amount of methanol of 1·8 mg in 22·5 ml of water. Combinations of three permanganate concentrations (15, 25 and 35 g per litre) and three orthophosphoric acid concentrations (100, 200 and 300 ml per litre) were used. The method of oxidation was as described in the preceding experiment, with an oxidation time of 10 minutes.

Results in Fig. 7 show that a decrease of 1 g per litre of permanganate in the oxidising agent leads to a decrease in formaldehyde yield of about 0·05 per cent. Decreasing the orthophosphoric acid level by 1 ml at a level of 25 g of permanganate per litre, results in a decrease in formaldehyde yield of 0·02 per cent., whereas with a concentration of permanganate of 35 g per litre, the same decrease in orthophosphoric acid content results in only 0·006 per cent. decrease in yield of formaldehyde. The stability of the oxidising solutions is also dependent on the concentration of permanganate. After 20 days, all samples lost their oxidising power with the exception of those that contained 300 ml of orthophosphoric acid per litre. The minimum fluctuation of results (±3·5 per cent.) was found after the oxidation of methanol with an oxidising solution comprising 35 g of permanganate and 300 ml of orthophosphoric acid per litre. This will be referred to as the recommended oxidising solution; the composition is quoted in the synopsis of the paper.

Influence of oxidation time—The relationship between oxidation time and methanol conversion was investigated. The procedure described for the preceding experiment was used with the change that the cylinders containing oxidation mixture were put into polyethylene bottles filled with ice and shaken continuously in laboratory shaking apparatus. The oxidation time was 1 to 15 minutes, and the oxidation temperature was 0° C.

TABLE IV

INFLUENCE OF OXIDATION TIME ON THE REPRODUCIBILITY OF THE METHANOL DETERMINATION

Methanol content, mg		Oxidation time, minutes				
		1	3	6	10	15
0·35	A	13·5	14·5	14·8	14·3	13·4
	B	5·8	5·1	5·6	2·9	3·3
	C	2·5	2·2	2·4	1·2	1·4
	D	18·5	15·2	16·2	8·4	10·4
1·75	A	11·8	13·5	13·8	13·7	12·1
	B	2·9	2·1	1·8	1·2	1·4
	C	1·2	0·9	0·8	0·5	0·6
	D	10·1	6·7	5·8	3·6	4·9
4·23	A	12·8	13·7	13·5	13·5	12·3
	B	2·3	3·3	1·5	0·8	0·7
	C	1·0	1·4	0·6	0·3	0·3
	D	7·8	10·2	4·4	2·2	2·4

A. Average oxidation of methanol to formaldehyde, per cent.
B. Range of values from five determinations (percentage of oxidation).
C. Standard deviation of percentage of oxidation.
D. Standard deviation as a percentage of A.

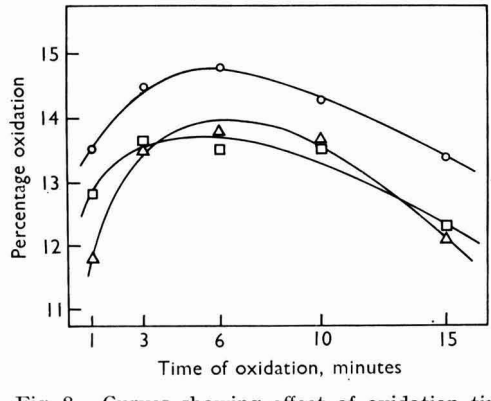

Fig. 8. Curves showing effect of oxidation time on the yield of formaldehyde: ○, 0·35 mg of formaldehyde; △, 1·75 mg of formaldehyde; □, 4·23 mg of formaldehyde

Results

From the nature of the curves in Fig. 8, it is obvious that the oxidation of methanol produces formaldehyde mostly in the initial period of the oxidation, the rate being dependent on the concentration of the oxidising solution. At a concentration of 25 g of permanganate per litre, maximum conversion is reached within 10 minutes, at higher permanganate concentrations (35 g per litre) within 3 to 6 minutes. Thereafter the content of formaldehyde in the oxidising solution decreases, owing to oxidation to formic acid or to carbon dioxide. The results in Fig. 8 are mean values of five determinations. The standard deviation is distinctly dependent on the oxidation time and on the methanol content (see Table IV).

From the results in Table IV, it can be seen that the best reproducibility was reached when the oxidation was carried out for 10 minutes.

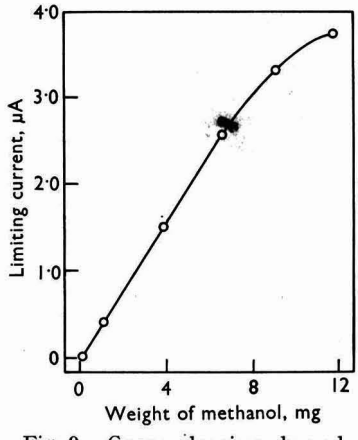

Fig. 9. Curve showing dependence of limiting current on the methanol concentration

Influence of methanol concentration—Samples were oxidised for 10 minutes with the recommended oxidising solution. The results (see Fig. 9) show that the limiting current is linearly related to the methanol concentration in the range 0·132 to 6·60 mg per 22·5 ml, which corresponds to 0·1 to 5 per cent. of methyl myristate in 1 g of sample. This means that when samples containing more than 5 per cent. of methyl esters are analysed, it is necessary to oxidise only an aliquot of the methanol solution.

SUMMARY OF THE STUDY OF THE OXIDATION OF METHANOL TO FORMALDEHYDE

The dehydrogenation of methanol to formaldehyde with permanganate is markedly dependent on temperature. Under the above mentioned conditions, there is a linear dependence of yields on temperature, indirectly proportional to temperature. The temperature of oxidation must be maintained accurately. Temperature changes of $\pm 1°$ C give rise to an error amounting to $\pm 1{\cdot}6$ per cent. of the resulting value. The optimum oxidation temperature in the quantitative determination of methanol is 0° C.

The influence of the composition of the oxidising solution, in a concentration range 15 to 35 g of permanganate and 100 to 300 ml of orthophosphoric acid per litre, on the formaldehyde yields is much greater than the influence of temperature. The greater the concentration of permanganate and of orthophosphoric acid, the greater the conversion of methanol to formaldehyde.

The lower the permanganate concentration in the oxidising solution, the greater the influence of orthophosphoric acid on the yield of formaldehyde. The most stable oxidising solution contains 35 g of permanganate and 300 ml of orthophosphoric acid per litre.

The rate of dehydrogenation of methanol is directly proportional to the concentration of the oxidising solution. The maximum yield of formaldehyde when oxidising methanol with the recommended oxidising solution is obtained after 4 to 6 minutes at 0° C. From the standpoint of reproducibility of results, the optimum oxidation time is 10 minutes.

The dependence of the limiting current on the concentration of methanol in the oxidation with the recommended oxidising solution is linear up to the concentration 6·6 mg of methanol in 22·5 ml, which corresponds to 5 per cent. of methyl myristate in 1 g of sample. When samples containing more than 5 per cent. of methyl ester are analysed, it is necessary to oxidise only an aliquot of the undistilled methanol solution.

The yield of formaldehyde under the recommended conditions is only about 14 per cent. All the variables must be controlled within narrow limits to maintain the yield at a fixed level.

SUMMARY—

Methyl esters of fatty acids may be analysed on the basis of liberating methanol by saponification of the sample, distillation of the methanol and oxidation of the methanol in the distillate to formaldehyde, which is then distilled off. A study of the oxidation shows the best conditions to be oxidation of methanol for 10 minutes at 0° C with an oxidising reagent containing 35 g of potassium permanganate and 300 ml of orthophosphoric acid per litre. Both methanol and formaldehyde may be distilled off with reproducible results. Small amounts of formaldehyde may preferably be determined polarographically at 50° C by means of a Smoler-type dropping electrode.

In the next communication[23] the application of this method will be described to the determination of methyl esters of fatty acids contained in sugar esters.

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The Polarographic Determination of Methyl Esters

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Methyl esters of fatty acids, in the presence of sugar esters and of solvents such as dimethylsulphoxide and dimethylformamide, can be determined by saponification, distillation of the liberated methanol, and then oxidation and polarography as described in a previous paper. Optimum conditions have been established, and ways have been found for avoiding interference due to dimethylformamide.

My earlier paper[1] described investigations carried out to find the optimum conditions for the oxidation of methanol to formaldehyde with potassium permanganate, and a procedure for determining small amounts of formaldehyde polarographically. The present report deals with the utilisation of these studies to the determination of methyl esters of fatty acids in reaction samples containing sugar esters, dimethylsulphoxide or dimethylformamide.

The analytical procedure in current use[2] requires solvent extraction of the sample before analysis. This procedure is tedious and, further, is not exact, since on evaporation of the solvent part of the methyl ester volatilises and is lost. Another disadvantage of this method is that incomplete saponification of the sample can occur in the distillation flask during the simultaneous saponification and distillation of methanol. The saponification, and the distillation of methanol, should be terminated in 30 to 45 and 90 to 120 minutes, respectively.[3] To decrease the methanol losses, saponification of the dried ester at 130° C in a sealed glass tube was proposed.[4] Disadvantages of this method are that heat-sealing of the tubes is tedious and they frequently crack while being oven dried.

These problems were eliminated by using a special stainless-steel pressure bomb.

EXPERIMENTAL

Determination of methyl ester in reaction solutions containing dimethylsulphoxide

Saponification of the methyl ester was carried out with sodium hydroxide, and the effect of saponification time and temperature, and of sodium hydroxide concentration was established.

Apparatus—

Bomb—A special bomb made of AKV Extra S stainless steel provided with a butyl rubber seal (see Fig. 1).

Reagents—

Reaction samples—Prepared by dissolving 1·02, 2·50 and 8·01 per cent. of methyl myristate, to which was added 3 per cent. of sucrose monomyristate (free from methyl ester), in 88 per cent. of dimethylsulphoxide, and making up to 100 per cent. with sucrose. The methyl myristate had a saponification value and an acid value of 232·9 and 0·5, respectively.

Sodium hydroxide, 3 N *and* 2 N.

Oxidising solution—A mixture of 35 g of potassium permanganate and 300 ml of concentrated orthophosphoric acid per litre.

Procedure—

A portion, 1 ± 0·05 g, of the reaction sample was weighed into the bomb and 5 ml of 2 N or 3 N sodium hydroxide were added. The saponification procedure was carried out for 1 or 2 hours at temperatures of 60°, 90° and 120° C. After the bomb had been cooled at −15° C for 30 minutes, its contents were quantitatively transferred into a distillation flask. Calcium chloride, 1 g, was added to prevent foaming during distillation. Methanol was determined according to the method described in the previous paper. All other details are summarised in Table I. Only one third of the distilled methanol originating from the sample containing 8·01 per cent. of methyl ester was oxidised, and the resulting limiting current value (wave height) was multiplied by 3.

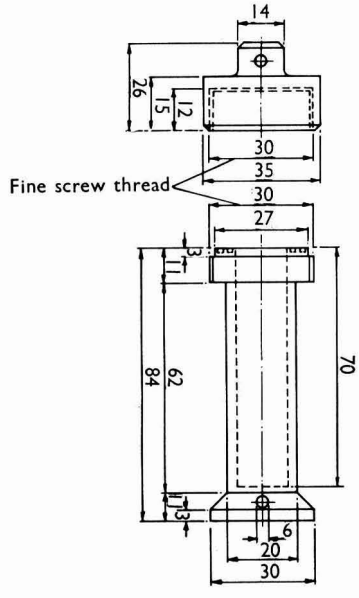

Fig. 1. Diagram of pressure bomb for the saponification of methyl esters. All dimensions are in millimetres

RESULTS—

From the results given in Table I, it can be seen that temperature is the most important factor, and saponification time and hydroxide concentration play a smaller rôle. For complete saponification it appears that the following conditions must be observed: saponification temperature should be 120° C; saponification time should be 2 hours (minimum); sodium hydroxide concentration should be 2 N.

TABLE I

EFFECTS OF VARYING SAPONIFICATION CONDITIONS UPON THE YIELD OF FORMALDEHYDE AS REPRESENTED BY THE LIMITING CURRENT

Methyl ester content of sample, per cent.	Saponification temperature, °C	Normality of sodium hydroxide solution	Limiting current (microamps) when saponification was for—	
			1 hour	2 hours
1·02	60	2	0·74	0·77
1·02	60	3	0·76	0·77
1·02	90	2	0·77	0·77
1·02	90	3	0·76	0·77
1·02	120	2	0·75	0·77
1·02	120	3	0·74	0·77
2·50	60	2	1·78	1·84
2·50	60	3	1·68	1·78
2·50	90	2	1·80	1·82
2·50	90	3	1·84	1·84
2·50	120	2	1·80	1·82
2·50	120	3	1·80	1·84
8·01	60	2	3·00	3·12
8·01	60	3	1·68	2·16
8·01	90	2	5·16	5·28
8·01	90	3	5·16	5·16
8·01	120	2	5·40	5·76
8·01	120	3	5·40	5·56

Analysis of samples of methyl esters of various fatty acids, and the reproducibility of results

Reagents used in the investigation—

Methyl ester of capric acid—Saponification value, 301·1; acid value, 0·5.
Methyl ester of lauric acid—Saponification value, 262·3; acid value, 0·1
Methyl ester of myristic acid—Saponification value, 232·9; acid value, 0·1.
Methyl ester of stearic acid—Saponification value, 189·6; acid value, 0·5.
Sucrose monocaprate—Free from methyl ester; saponification value, 114·3.
Sucrose monolaurate—Free from methyl ester; saponification value, 109·8.
Sucrose monomyristate—Free from methyl ester; saponification value, 103·2.
Sucrose monostearate—Free from methyl ester; saponification value, 94·2.
Sucrose.
Dimethylsulphoxide—Distilled.

Composition of samples analysed—

These samples were made up to contain 4 per cent. of sugar esters, 87 per cent. of dimethylsulphoxide and 9 per cent. of methyl ester *plus* sucrose. The methyl ester concentration was in the range of 0·08 to 8 per cent. The individual samples contained the methyl ester and sugar ester of the same fatty acid.

The analytical method is described fully on p. 678.

The relationship between the limiting current, the concentration of methyl ester, and the length of the hydrophobic methyl ester chain is shown in Fig. 2. Linear relationships were observed for all the methyl esters tested. By comparing the observed results to the known methanol contents, it was established that in all instances the limiting current is a function of the methanol content of the corresponding methyl ester.

To determine the reproducibility of results, samples containing different amounts of methyl myristate were analysed five times. The results obtained are shown in Table II.

Table II

Reproducibility of methyl ester determination in samples containing dimethylsulphoxide

Methyl ester content, per cent.	Analytical results, per cent.	Mean of five determinations, per cent.	Maximum error on individual test, percentage of methyl ester	Relative maximum error,* per cent.	Standard deviation	Relative standard deviation
0·08	0·10, 0·07 0·08, 0·11 0·07	0·084	+0·03	+37·5	±0·02	±20·0
1·14	1·10, 1·22 1·09, 1·13 1·13	1·13	+0·08	+7·0	±0·04	±3·5
3·68	3·50, 3·65 3·69, 3·56 3·73	3·63	−0·18	−4·9	±0·10	±2·7
8·01	8·13, 8·06 8·12, 7·94 7·99	8·05	+0·12	+1·5	±0·08	±1·0

* For individual tests, referred to the actual percentage methyl ester content of the samples.

Determination of methyl ester in reaction solutions containing dimethylformamide

Although the determination of methyl ester in samples containing dimethylsulphoxide and sucrose presents no special difficulties, the analysis of those containing dimethylformamide is much more complicated. During saponification, dimethylformamide is partially converted to dimethylamine, and this compound, which distils together with the methanol, subsequently

interferes with the polarographic formaldehyde determination. Further, formaldehyde is produced from this dimethylamine during the oxidation with potassium permanganate, according to the reactions—

$$CH_3 \diagdown \atop CH_3 \diagup NH \xrightarrow{[O]} CH_2 \diagdown \atop CH_3 \diagup N + H_2O \longrightarrow HCHO + CH_3NH_2$$

$$CH_3NH_2 \xrightarrow{[O]} CH_2{=}NH + H_2O \longrightarrow HCHO + NH_3$$

For this reason it is necessary to prevent volatilisation of dimethylamine during the methanol-distillation stage. This is possible by forming a non-volatile dimethylamine salt with a suitable acid. The presence of dimethylamine may have some influence on the course of saponification, since, in the formation of this compound, part of the sodium hydroxide is consumed. For this reason the amount of sodium hydroxide used for the saponification was doubled.

REMOVAL OF DIMETHYLAMINE—

Procedure—The reaction sample was prepared to contain 10 per cent. of sucrose monomyristate (free from methyl ester), 15 per cent. of sucrose and 75 per cent. of dimethyl formamide, and was saponified with 10 ml of 2 N sodium hydroxide and then transferred to a distillation flask. Before distillation, various amounts of orthophosphoric acid or tartaric acid *plus* 1 g of calcium chloride were added. Other details of procedure were identical to those used for the determination of methyl ester in dimethylsulphoxide.

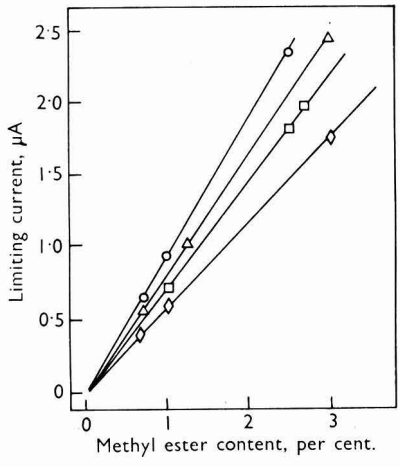

Fig. 2. Curves showing dependence of limiting current on the length of the hydrophobic chain: ○, C_{10}; △, C_{12}; □, C_{14}; ◇, C_{18}

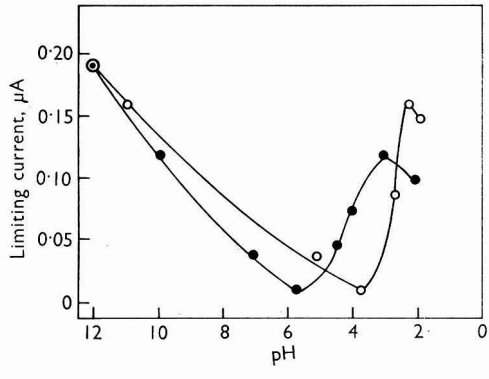

Fig. 3. Curves showing reduction of limiting current in blank solutions containing dimethylformamide by addition of ○, tartaric acid; ●, orthophosphoric acid

Results—Fig. 3 shows the relationship between the limiting current in the polarographic determination and the concentration of orthophosphoric or tartaric acid. It can be seen that both acids prevent the volatilisation of dimethylamine to an approximately equal degree. The minimum of the tartaric acid curve, however, lies at a lower pH (approximately 3·5) than that for orthophosphoric acid. The shapes of the curves are similar, and both branches of each of the curves are comparatively steep so that accurate measurement of the acid is necessary. It was impossible to totally prevent the volatilisation of dimethylamine by using these two acids, and the formaldehyde wave could be detected under all the conditions investigated.

In the tests without any addition of acid, the blank solution gave a formaldehyde wave corresponding to approximately 0·3 per cent. of methyl myristate (*i.e.*, 9 μg of formaldehyde). In tests in which the optimum addition of acid was used, the wave height corresponded to approximately 0·03 to 0·04 per cent. of methyl myristate.

It follows that the calibration curves for methyl esters will be shifted by approximately 0·015 μA in the direction of the ordinate.

On distilling samples neutralised to a pH higher than 5, with either orthophosphate or tartaric acid, turbid solutions are obtained. After oxidation with permanganate and reduction with sodium sulphite, a white, voluminous precipitate is formed.

Solutions neutralised to a pH lower than 5 are clear both after distillation and oxidation. Since the minimum of the orthophosphoric acid curve is situated at a pH exceeding 5, the use of tartaric acid is more satisfactory, the optimum amount being 2 g per g of sample (previously saponified with 10 ml of 2 N sodium hydroxide).

OXIDATION TO FORMALDEHYDE AND REPRODUCIBILITY OF THE RESULTS—

During distillation of the saponified samples, a proportion of the unsaponified dimethylformamide volatilises together with methanol and small amounts of dimethylamine; this decreases the oxidation yields. Preliminary tests indicated that under standard conditions, only 7 to 12 per cent. of methanol is oxidised to formaldehyde in comparison with 14 to 18 per cent. obtained with samples containing dimethylsulphoxide. For this reason, double the amount of oxidising solution was used.

Composition of the sample—A sample was prepared from 10 per cent. of sucrose ester, 75 per cent. of dimethylformamide, 0·2 to 3 per cent. of methyl myristate and made up to 100 per cent. with sucrose.

Procedure—The saponified sample was diluted with 45 ml of water, 2 g of tartaric acid were added and 22·5 ml distilled off. A 15-ml portion of the distillate was oxidised with 5 to 10 ml of oxidising solution. (The amount of distillate used is reduced when dimethylformamide is present).

Results—The calibration curves for the determination of methyl myristate reaction samples containing dimethylformamide and dimethylsulphoxide are shown in Fig. 4. For comparison, the calibration curve for methanol, calculated as percentage of methyl ester, is also plotted. It is apparent that the presence of dimethylsulphoxide increases and that of dimethylformamide decreases the degree of oxidation of methanol to formaldehyde. By increasing the amount of oxidising solution from 5 to 10 ml, the formaldehyde yields increase considerably and the calibration curve is linear in the whole range of the investigated concentrations. The results are given in Table III.

TABLE III

REPRODUCIBILITY OF THE DETERMINATION OF METHYL ESTERS IN SAMPLES

Methyl ester content, per cent.	Analytical results, per cent.	Average of five determinations, per cent.	Maximum error on individual test, percentage of methyl ester	Relative maximum error,* per cent.	Standard deviation	Relative standard deviation
0·20	0·20, 0·25 0·22, 0·19 0·24	0·22	+0·05	+23	±0·03	±14
1·02	1·00, 1·05 1·08, 1·06 1·00	1·04	+0·06	+6	±0·04	±4
3·03	2·97, 2·99 3·00, 3·02 3·04	3·00	−0·06	−2	±0·03	±1

* For individual results, referred to the actual percentage methyl ester content of the samples.

COMPLETE DESCRIPTION OF THE METHOD

APPARATUS—

Polarograph with a Smoler dropping-mercury electrode.

Novak polarographic cell.

Thermostatic glass bath with holder for cells.

Thermostat with pump.

Semi-micro distillation apparatus—Fitted with ground-glass joints.

Oil-bath—Thermostatically controlled for temperature range, 150° to 160° C, and having automatic control (accuracy ±2° C).

Laboratory shaking machine.

Stainless-steel bomb—See Fig. 1.

Distillation flask—Capacity, 250 ml.

Calibrated vessels—Capacity, 25 ml.
Cylinders—Glass stoppered, 30-ml capacity.
Polythene bottles—Wide necked, 1 litre capacity.

REAGENTS—

Mercury, distilled.
Lithium hydroxide, N.
Calcium chloride, anhydrous.
Oxidising solution—Prepare this by dissolving 35 g of potassium permanganate in 650 ml of water and 300 ml of 85 per cent. w/v orthophosphoric acid. After temperature adjustment, make the volume to 1000 ml with water. Set the solution aside for 24 hours, and filter it through a sintered-glass filter.
Sodium sulphite solution, saturated.
Sodium hydroxide, 2 N.
Dimethylsulphoxide.

PROCEDURE—

Weigh accurately 1 $\pm$ 0·05 g of the liquid sample to be analysed into the saponification bomb and add 5 ml of 2 N sodium hydroxide. (For samples containing dimethylformamide, the required amount is 10 ml. For powder samples, 0·2 $\pm$ 0·05 g of sample and 0·8 $\pm$ 0·05 g of a 10 per cent. sucrose solution in dimethylformamide are weighed into the bomb.) Carry out the saponification for 2 hours at 120° C.

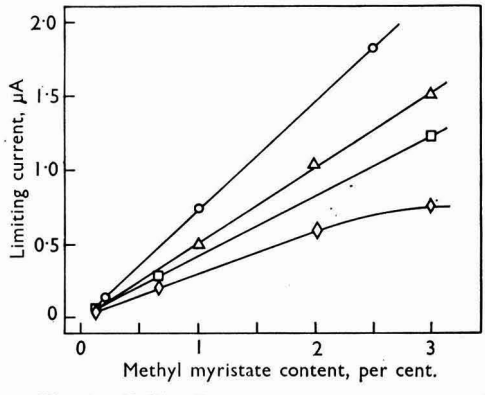

Fig. 4. Calibration curves for the determination of methyl myristate under various conditions: ○, dimethylsulphoxide; △, calibration curve for methanol, calculated as percentage methyl ester; □, dimethylformamide *plus* 10 ml of oxidising solution; ◇ dimethylformamide *plus* 5 ml of oxidising solution

Cool the bomb containing the saponified sample for 30 minutes at −15° C, and then wash the solidified contents quantitatively with 50 ml of water into a 250-ml distillation flask, to which have previously been added 1 g of calcium chloride and six glass beads. If the sample contains dimethylformamide, use only 45 ml of water for the washing and add 2 g of tartaric acid to the flask.

Distil the solution in a bath at 150 $\pm$ 2° C, and collect 22·5 ml of distillate in an ice-cooled graduated cylinder. Hold this cylinder containing distillate for 15 minutes in a polythene bottle filled with crushed ice. Then add 5 ml of oxidising solution, seal the bottle and fasten it in a horizontal position in a laboratory shaking machine.

For the oxidation of samples containing dimethylformamide use only 15 ml of distillate and add 10 ml of oxidising solution. For samples containing more than 4 per cent. of methyl ester, subject only part of the distillate to oxidation, the volume being made up to 22·5 ml with water, or 15 ml of water if dimethylformamide is present. Titrate the reaction mixture after 10 minutes $\pm$ 10 seconds with saturated sodium sulphite solution until the oxidised solution is colourless. Titration must not be continued beyond this end-point, since an excess of sodium sulphite would lower the results of the determination.

Dilute the decolorised solution with water to 30 ml, transfer the solution to a 250-ml distillation flask, into which glass beads are then added, and carry out distillation in a bath at 160° ± 2° C into a calibrated receiver immersed in an ice bath, and collect exactly 25 ml of distillate. Place, by pipette, 5 ml of N lithium hydroxide and 5 ml of distillate in a Novak cell having a layer of mercury in the bottom. Agitate the solution with a glass rod and place the cell in a holder of a thermostatically controlled bath. Mount the mercury electrode and after the temperature of the cell has been adjusted to 50° ± 0·05° C, carry out the polarographic determination without de-aeration, starting from −1·45 volts. The mercury reservoir height should be 85 cm, the input voltage to the potentiometric wire should be 0 to 4 volts, and the rate of scan of the polarographic drum should be 400 mV per minute. The sensitivity reduction depends on the methyl ester concentration of the sample. As a guide, the values quoted below can be used—

Methyl ester content of sample, per cent.	0 to 0·3	0·3 to 0·6	0·6 to 2	2 to 3	3 to 4
Sensitivity reduction of galvanometer	1-to-2	1-to-5	1-to-10	1-to-20	1-to-30

Reference to a calibration curve prepared by applying the same analytical procedure to known methyl ester samples, allows calculation of methyl ester contents from the wave-height readings. When a reaction sample contains methanol in addition to methyl ester, the free methanol content can be determined by distillation without saponification (1 g of sample is diluted with 50 ml of water for this). The result, calculated as a percentage of methyl ester, can be subtracted from the total result to give the actual methyl ester content.

Summary

A method has been developed for determining fatty acid methyl esters in liquid samples of sugar esters containing in addition dimethylsulphoxide or dimethylformamide.

Analysis for methyl esters in the presence of dimethylsulphoxide is comparatively simple, but the presence of dimethylformamide gives high results and adversely affects the final polarographic determination of formaldehyde. The effect of the presence of dimethylformamide, however, can be eliminated by adding tartaric acid before the stage of distilling off the methanol. The method itself has a good reproducibility, the accuracy of the results being within ±0·05 per cent. of methyl ester content.

References

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An Absorptiometer for the Sugar Industry

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Measurement of optical density is a commonly used test of purity in the sugar industry. Difficulty arises when the test is made on solutions of refined sugar because the optical density due to dissolved impurity is very small and because it is liable to be masked by traces of suspended turbid matter.

Among recommendations designed to overcome the difficulty the best is considered to be that of Gillett, Meads and Holven, who suggested the definition: colour index = 1000 $(a^{\bullet}_{420} - 2a^{\bullet}_{720})$. For reasons that are stated a modified definition, colour index = 1000 $(a^{\bullet}_{420} - a^{\bullet}_{690})$, is proposed here. The modified index should be well correlated with the concentration of residual dissolved impurity and with the departure from whiteness of the solution.

An automatic absorptiometer has been developed to measure the colour index directly. Its readings have been compared with estimates made by a panel of visual observers who classified samples of "fine liquor" and solutions of refined sugar according to order of whiteness. Satisfactory correlations were obtained.

IN the sugar refining industry measurement of the optical densities (absorbancies) of solutions of white sugar is extensively used for control purposes. Optical density can be measured comparatively rapidly and it is fairly closely correlated with the very small amount of impurity remaining in the sugar at the end of the refining process. In addition, because the visual appearance of refined sugar is important, determination of the departure from whiteness is a desirable test. "Fine liquor" (the purified sugar solution from which sugar is crystallised)† and solutions of refined sugar are difficult to measure because of their extremely small optical densities. If a measurement were made of the transmittancy of an unfiltered solution of refined sugar, sufficient turbid matter would be present to account for an appreciable proportion of the total attentuation of the light beam.

A procedure that eliminates the effect of turbidity is required. The basis of the procedure now in use at Tate & Lyle Refineries Ltd., and the design of an automatic absorptiometer[1] that has been developed for this purpose, will be described.

Previous work has been reported by Gillett, Meads and Holven,[2] Gillett and Heath[3] and Deitz.[4] Reference to the three papers by these authors will provide a fairly complete background to the subject.

REQUIREMENTS

The objects of "colour" measurement in the sugar refinery are—

(i) to obtain an index of the concentration of impurity,
(ii) to determine the departure from whiteness.

A system of optical-density specification and measurement for solutions of refined sugar should satisfy these requirements—

(*a*) The result of a measurement should be expressed as a single figure that is proportional to the concentration of colouring matter.

(*b*) Whiteness is a saleable attribute of refined sugar. When a measurement is made on a solution of refined sugar, the numerical value of the result should be closely correlated with the visual assessment of the departure from whiteness.

(*c*) The index of colour should have an absolute significance. It should be independent of any arbitrary series of colour standards and of the particular instrument used.

(*d*) The system of measurement must take into account the effects of turbidity.

(*e*) For routine measurements, a rapid and simple procedure is necessary. This rules out the removal of turbid matter by the slow process of filtration through membrane filters.

† It is not claimed that the method to be described is applicable to refinery intermediates in general but it can fairly be applied to "fine liquor" as well as to solutions of refined sugar.

In the method to be described, a rapid, vacuum-assisted filtration through paper is beneficial to the extent that it eliminates air bubbles, but it does not reduce turbidity appreciably.

(*f*) For reasons that will be stated in a later section the recommended procedure will require measurement of the optical density at each of two wavelengths. The instrument used for this purpose should perform automatically the necessary mechanical, electrical and arithmetical operations.

Requirement (*c*) indicates that the absorbancy index, *a*,† at a specified wavelength should be used as the index of colour. To satisfy this a photo-electric instrument, operating at a strictly defined wavelength (or wavelengths), will be needed.

CONSIDERATIONS CONCERNING THE SPECIFICATION OF COLOUR—

A typical absorption curve for a well filtered sugar solution is shown in Fig. 1, for which the absorbancy ($-\log_{10}T_S$) of a 4-cm depth of "fine liquor" is plotted logarithmically against wavelength. If the selected wavelength is to be in the visible part of the spectrum, errors of

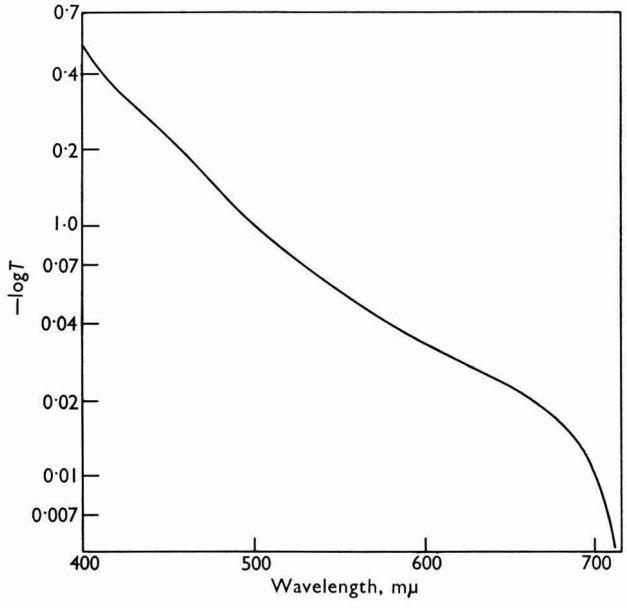

Fig. 1. Absorption curve for a typical "fine liquor," concentration 67 per cent.; 4-cm cells were used

measurement will be least at the violet end of the spectrum where the attenuation due to dissolved impurities is greatest relative to that due to turbidity.[2]

With these points in mind three methods of measurement will be considered.

(1) Peters and Phelps[5] suggested a single measurement in the yellow-green (approximately 560 mμ) with filtration to minimise turbidity. This method, although difficult, can give satisfactory results, but it is not acceptable for routine use on account of the length of time required for the filtration. Moreover, at 560 mμ the optical density of a solution of refined sugar is too small for easy measurement.

(2) The need for filtration is eliminated by the two-wavelength method suggested by Gillett, Meads and Holven.[2] A main measurement is made in the violet (at 420 mμ), where

† The symbols used in this paper are as under—

T_S = transmittancy of a solution.
A = $-\log T_S$ in the absence of turbid matter.
A^* = $-\log T_S$ in the presence of turbid matter.
t = transmittancy of unit depth of a solution at unit concentration.
a = absorbancy index = $-\log t$ in the absence of turbid matter = A/bc.
a^* = $-\log T_S$ in the presence of turbid matter.
b = cell length, in cm.
c = concentration, in g per ml, of sucrose in solution.

the optical density is from 6 to 12 times that in the yellow-green (at 560 mμ). At 420 mμ the effect of turbidity is relatively small, but to take account of turbidity, a subsidiary measurement is made in the red (at 720 mμ) where the optical density due to dissolved impurity is substantially zero. Fig. 1 shows that fine liquor may have a ratio of A_{420} to A_{720} that is of the order of 100 to 1.

Gillett, Meads and Holven state that the component of optical density due to turbidity is twice as great at 420 as at 720 mμ and this is substantially supported by the results of Hibbert and Dickman.[6] On this basis the colour index may be defined as—

$$1000\ (A^{*}_{420} - kA^{*}_{720})$$

in which the factor 1000 is included to make the final figure more convenient, and the factor $k = 2$. According to this definition a solution of refined sugar would have an absorbancy ndex of the order of 12.

(3) Deitz[4] has used a modification of the Adams formula to determine the extent of the visible departure of sugar solutions from the condition of zero optical density. Departure of a colour from whiteness normally requires a three-figure specification—the trichromatic coefficients. However, spectrum absorption curves of sugar solutions show a considerable degree of uniformity, and Deitz has shown that, for practical purposes, the optical densities at 420 and 560 mμ sufficiently define the departure from whiteness.

Deitz has prepared charts (see Fig. 2) that indicate the departure from whiteness, in National Bureau of Standards units (ΔE_{NBS}), when A^{*}_{420} and A^{*}_{560} are known.

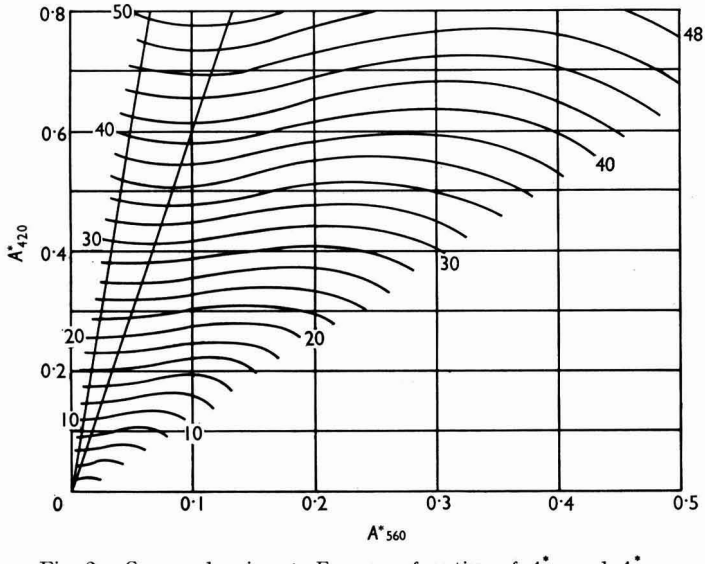

Fig. 2. Curves showing $\triangle$ E_{NBS} as a function of A^{*}_{420} and A^{*}_{560}

In Deitz's chart provision is made for values of A^{*}_{420}/A^{*}_{560} in the range 1·25 to 13. Measurements made in this laboratory have shown that, for filtered solutions of refined sugar, A_{420}/A_{560} lies between 6 and 12. These limits are indicated by the two radial lines in Fig. 2. White sugar (at 50 per cent. concentration in a 16·3-cm cell)† has a value of A_{420} that does not exceed 0·2. For these values of A_{420} and A_{560}, ΔE_{NBS} depends mainly on A_{420}. The curves A and B in Fig. 3 show the relationship between ΔE_{NBS} and A_{420} for values of A_{420} between zero and 0·8. Curves A and B represent the two instances in which $A_{560} = \frac{1}{6}A_{420}$ and $\frac{1}{12}A_{420}$, respectively. The effect of A_{560} is negligible and ΔE_{NBS} is a nearly linear function of A_{420}. It follows that for a light-coloured solution, the method of Gillett, Meads and Holven, which determines A_{420} by measuring A^{*}_{420} and accounting for tubidity by the measurement in the red, is a satisfactory means of determining departure from whiteness.

† This cell length has been chosen because 16·3 ml of a 50 per cent. solution contains 10·0 g of sucrose. The factor, *bc*, for a 50 per cent. solution in a 16·3 cm cell is 10.

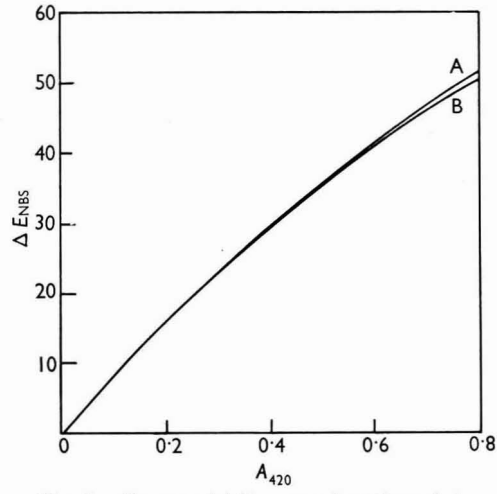

Fig. 3. Curves of ΔE_{NBS} as a function of A_{420}

Departure from whiteness is approximately proportional to concentration of colouring matter when the concentration is small.

RECOMMENDATION—

The two-wavelength method can be recommended, but it is necessary to make an independent determination of the value of k, the "red factor," in the equation—

$$\text{Colour index} = 1000\ (A^{*}_{420} - kA^{*}_{720})/bc.$$

DETERMINATION OF THE "RED FACTOR"—

Hibbert and Dickman's method[6] was used to evaluate the factor k. Fifty per cent. solutions were filtered through five filtering media of different pore diameter.

Readings of A^{*}_{420} and A^{*}_{720} were made before and after filtration through double Whatman No. 42 filter-papers and through sintered-glass discs of porosities 2, 3, 4 and 5.

Progressive extraction of turbid matter by filtration reduces A^{*}_{420} and A^{*}_{720} in the ratio k to 1. When A^{*}_{420} is plotted against A^{*}_{720} the slope of the line is the factor k.

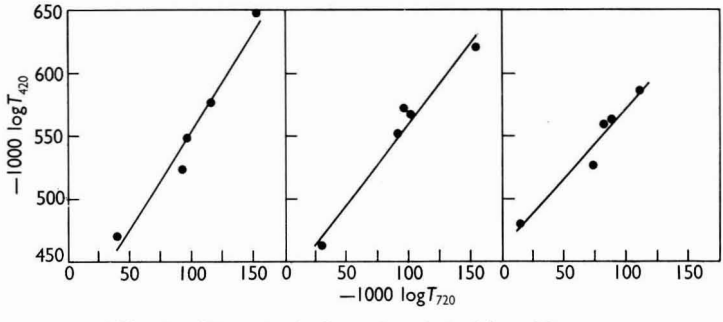

Fig. 4. Three typical graphs, plotted in arbitrary units, for determining the "Red factor," k

Fig. 4 shows three typical graphs (from a total of 22) of A^{*}_{420} against A^{*}_{720}, plotted in arbitrary units. The mean value of the slopes is 1·33 with a standard error of 0·10. This result disagrees with the values of Gillett, Meads and Holven and of Hibbert and Dickman, but these authors do not claim that their value of approximately 2 for the red factor is necessarily universal.

The instrument designed to perform the measurements at the two wavelengths should contain only one photo-electric cell, but no photocell having adequate sensitivity at both 420 and 720 mμ is commercially available. The best photocathode for this purpose is probably

the bismuth - silver oxide - caesium cathode, but even this has an undesirably low response at 720 mμ. However, it has adequate sensitivity at 690 mμ. At 690 mμ the turbid matter absorbs slightly more strongly than at 720 mμ and, in addition, there is at 690 mμ a very weak absorption by the colouring matter itself. Absorption by dissolved colouring matter at 690 mμ is very small, about 4 per cent. of the absorption at 420 mμ (see Fig. 1). Consequently we propose, for our own use, that the turbidity should be accounted for by a measurement at 690 mμ and that a "red factor" of unity be adopted.

This arrangement has the advantage that unity is the factor that is most easily introduced into an automatic instrument. However, in the instrument to be described, provision can be made for any reasonable value of the "red factor" that other users of the instrument may require.

An estimate of the error introduced by the compromise procedure can be obtained by the following considerations. A_{720} is negligible (see Fig. 1) and A^*_{720} (due to turbidity) is usually in the range 5 to 20 per cent. of A^*_{420}. If $A^*_{720} = 0{\cdot}1\ A^*_{420}$, the correct amount to be subtracted from A^*_{420} is $0{\cdot}133\ A^*_{720}$, so the correct result is $0{\cdot}867\ A^*_{420}$. Now it is reasonable to suppose that the change of absorption due to turbidity is approximately linear between 720 and 420 mμ.[2] The absorption at 690 mμ will then be $0{\cdot}1\left(1 + \frac{3}{30} \times 0{\cdot}33\right) A^*_{420}$, due to turbidity, *plus* $0{\cdot}04\ A_{420}$ (approximately), due to dissolved impurity (see Fig. 1). According to the compromise procedure this is subtracted from A^*_{420}, so we have—

$$A_{420} = A^*_{420}\ (1 - 0{\cdot}1033) - 0{\cdot}04\ A_{420}.$$

Hence—

$$A_{420} = 0{\cdot}862\ A^*_{420}.$$

Applying a similar argument to the instances in which A^*_{720}/A^*_{420} is equal to 0·05, 0·15 and 0·20 we have—

A^*_{720}/A^*_{420}	0·05	0·10	0·15	0·20
A_{420}/A^*_{420} (correct)	0·934	0·867	0·800	0·734
A_{420}/A^*_{420} (compromise) ..	0·912	0·862	0·812	0·763

To summarise, the colour index can be defined as

$$1000\ (a^*_{420} - a^*_{690}) = 1000\ (\log t_{690} - \log t_{420}) = 1000 \log\ (t_{690}/t_{420}).$$

Description of the Absorptiometer

In Fig. 5, S is a 4-watt, concentrated filament lamp supplied by a stabilized current source. Lens L_1 projects an image of the filament on to a 0·6-mm aperture in diaphragm A_1

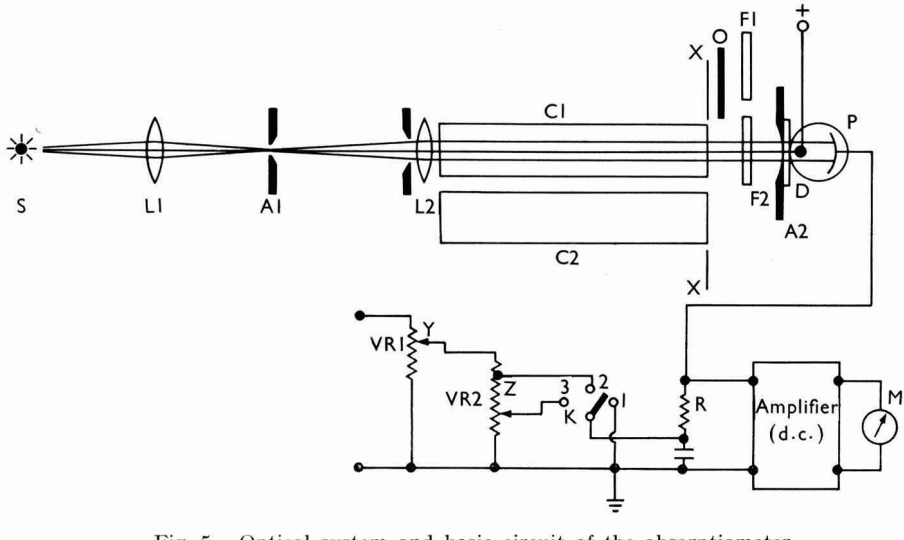

Fig. 5. Optical system and basic circuit of the absorptiometer.

and an image of the aperture is formed in the plane XX by lens L_2. C_1 and C_2 are interchangeable U-section sample cells, which contain water and the test sample, respectively. The outer faces of the exit plates are in the plane XX, and, by making the diameter of the image in this plane equal to 4 mm and stopping the aperture of L_2 to the same diameter, the beam is confined within a 4-mm cylinder. An internal cell width of 1·3 cm then gives ample clearance for the beam. The remaining components are an opaque stop O, which can be placed in the beam as required, interchangeable colour filters, F_1 and F_2,† an emission-type photocell, P, with a 7-mm aperture, A_2, designed to cut off forward-scattered light, and a diffusing screen, D, which reduces the effect of shift of the beam due to imperfections in the sample cells. P has a bismuth - silver oxide - caesium cathode, which is reasonably sensitive at 420 mμ and 690 mμ. F_1 is a narrow-band interference filter centred at 420 mμ and F_2 is a relatively cheap high pass filter with a pass band above 670 mμ. The transmission curve of F_2 combines with the sensitivity curve of the photocell to give an effective peak transmission at 690 mμ.

The transmission of cell C_2 is compared with that of the reference cell, C_1. At the low level of illumination used the voltage developed across resistance R is accurately proportional to the flux of light on the photocell. It is measured by introducing into the direct current amplifier a neutralising voltage from a calibrated potentiometer (VR_2 in Fig. 5). The ratio of the neutralising voltages corresponding to C_2 and C_1 is the transmission, T, of the solution in C_2.

The basic circuit for the measurement of transmission is also shown in Fig. 5. First consider measurement at a single wavelength. Meter M has a centre zero and is mechanically biassed so that the meter indicates zero when the input to the d.c. amplifier is also zero. With the optical stop, O, in the beam and switch K in position 1, the reading of the meter is reduced to zero by adjusting the amplifier bias. Next, with the stop withdrawn, tube C_1 and an appropriate colour filter in the beam, and the switch in position 2, the voltage developed across R by the photocell current is applied to the amplifier. The meter reading is again restored to zero by a stabilized but variable neutralising voltage from the potentiometer VR_1, applied to the amplifier via the fixed point Z. Finally, with tube C_2 and the colour filter in the beam and the switch in position 3 the tapping from the linear potentiometer VR_2 is adjusted to restore the meter reading again to zero. The transmission, T, is the ratio of this tapped resistance of VR_2 to the resistance of that portion of VR_2 that lies between switch terminal 2 (the point Z) and earth. VR_2 is provided with a logarithmic scale that indicates $-1000 \log T$ directly. The zero of this scale corresponds to the fixed tapping point Z.

However, we have defined the optical density of a sugar solution as—

$$1000\ (A^{\bullet}_{420} - A^{\bullet}_{690}) = 1000 \log \left(\frac{T_{690}}{T_{420}} \right)$$

The sequence of operations required for the measurement of this quantity is given below in tabular form. Each of the five operations is terminated by adjusting a potentiometer so that the reading of meter M is restored to zero.

Operation	Position of switch K	Components in optical beam	Adjustment to be made
1	1	Stop	Pre-set potentiometer (d.c. amplifier bias)
2	2	Tube C_2, 690-mμ filter	VR_1 (sensitivity potentiometer)
3	3	Tube C_1, 690-mμ filter	VR_2 (measuring potentiometer)
4	3	Tube C_1, 420-mμ filter	VR_1
5	3	Tube C_2, 420-mμ filter	VR_2

After operation 3 the position of the variable tapping point of VR_2, which is then between Y and Z, corresponds to the quantity $1/T_{690}$. This point is the zero point for operations 4 and 5, so that the final position of the variable point of VR_2 (between Y and earth) corresponds to (T_{420}/T_{690}).

In the final circuit the fixed tapping on VR_2 is replaced by a fixed connection to a voltage divider that is connected in parallel with VR_2.

If a "red factor" not equal to unity is to be used, a second variable potentiometer with a resistance characteristic appropriate to the value of k can be connected in parallel with

† The colour filters used were a red Ilford 609 and a violet Barr & Stroud metal-dielectric interference filter with 25 per cent. peak transmission at 4200 Å.

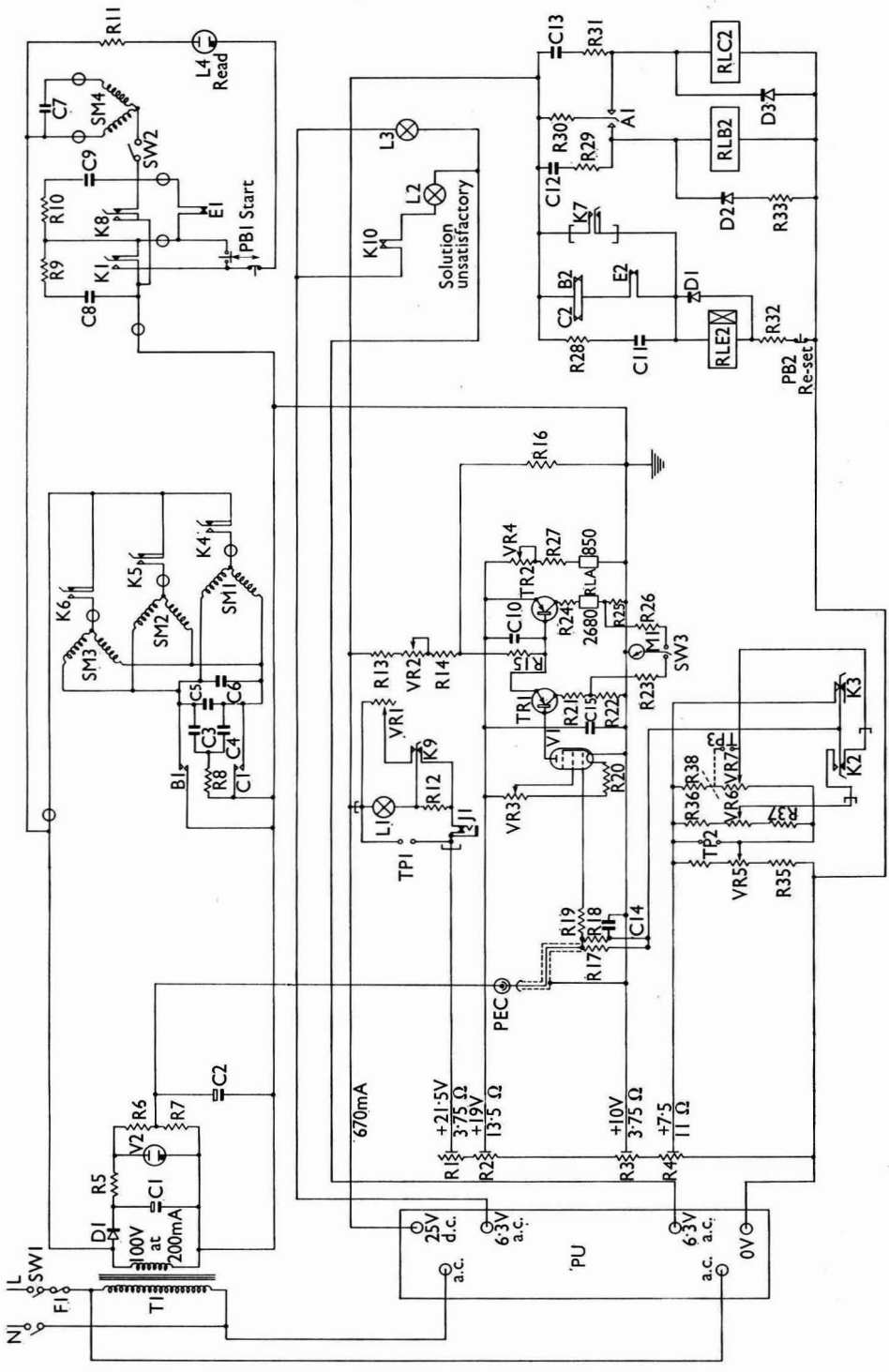

Fig. 6. Circuit diagram. For details of components, see Appendix, p. 690

VR_2 and ganged to it. By means of a suitable switching sequence this potentiometer is active for operation 3. It is switched out in favour of VR_2 for operations 4 and 5.

Automatic operation can be arranged by including in the circuit a servo system that restores the input to the d.c. amplifier, and hence the reading of the output meter, to zero after each operation. Ten cams, mounted on a motor-driven camshaft, control the sample cell carrier, the optical stop, the colour filters, and a set of micro-switches. Three of the micro-switches constitute the switch K of Fig. 5. The remainder control the pre-set, sensitivity and measuring servo-motors (see Fig. 6). With the camshaft in its initial position a zero-setting servo-motor is driven to the balance point. After each movement of the camshaft the photocell current energises the amplifier and the output meter is displaced from zero, the appropriate motor is switched in and the corresponding potentiometer is driven in the correct direction to the balance point. The camshaft servo-motor is then energised, and the camshaft revolves for one sixth of a revolution, setting up the conditions for the next stage in the measurement cycle. The sensitivity and measuring potentiometers approach the balance point rapidly and usually overshoot it, but two-speed reduction gears between the potentiometers and the motors ensure that balance is re-approached at reduced speed, and the motor stops without overshoot. Interlocking switches prevent the initiation of one operation before the previous one is complete. If the test solution has too dense a colour the measuring potentiometer will revolve continuously, and a red light will draw the attention of the operator who can restore the camshaft to its starting position by means of a "Reset" switch.

An illuminated transparent graticule is mounted on the shaft of VR_2; its graduations are projected on to a ground-glass screen on the front panel of the instrument. The indicated figure is the value of 1000 log (T_{690}/T_{420}). The time required for the instrument to perform its measurement cycle is about $1\frac{1}{2}$ minutes.

Performance tests

Reproducibility—

This was tested by taking ten readings on each of five samples. The results are given in Table I.

Linearity—

This was estimated by means of sets of ten readings on water and each of four solutions of a sample of soft sugar. The solutions were prepared by making a stock solution and diluting to $\frac{3}{4}$, $\frac{1}{2}$ and $\frac{1}{4}$ of its original concentration. A graph of the results is shown in Fig. 7.

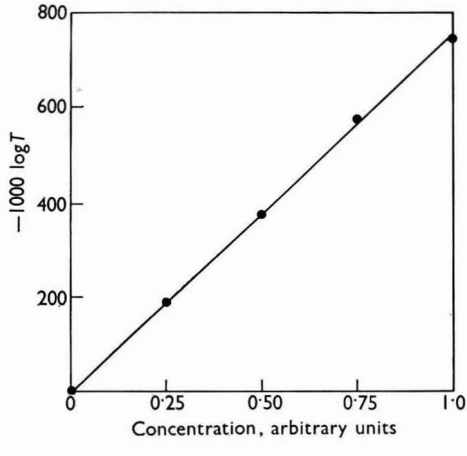

Fig. 7. Results of linearity test

Ability of the absorptiometer to estimate whiteness—

This was tested by measuring the colour indices of twelve 50 per cent. solutions of refined sugar in 16·3-cm cells. The samples were chosen to cover the whole colour range of granulated

sugar from Tate & Lyle and they were arranged in order of whiteness by ten independent observers who viewed a white background through a 60-cm depth of each solution. Observers' rankings are shown in the centre panel of Table II and in the last two columns are the instrumental and average visual rankings.

TABLE I

REPRODUCIBILITY RESULTS

	Value of 1000 log (T_{690}/T_{420}) Sample				
	1	2	3	4	5
	−1	218	490	598	700
	−2	218	492	596	700
	−1	218	492	602	705
	−1	218	492	602	695
	−2	217	490	602	705
	−2	218	492	602	700
	−3	218	490	596	710
	−1	218	494	602	705
	−3	218	492	598	705
	−3	220	492	602	695
Mean	*−1·9*	*218·1*	*491·6*	*600·0*	*702·0*
Standard deviation ..	*0·87*	*0·74*	*1·3*	*2·7*	*4·8*

TABLE II

GRANULATED SUGAR

Sample	Absorptiometer reading	Observers A	B	C	D	E	F	G	H	I	J	Average	Ranks: Absorptiometer	Ranks: Visual
b	52	3	3	3	4	3	3	1	1	1	3	2·5	1	3
c	54	2	1	2	2	1	2	4	3	2	2	2·1	2	2
l	58	1	2	1	1	2	1	2	2	3	1	1·6	3	1
d	62	4	4	4	3	4	4	3	4	5	6	4·1	4·5	4
j	62	5	7	5	6	5	5	5	5	6	7	5·6	4·5	5
f	70	6	6	7	5	6	6	6	7	8	5	6·2	6	7
i	72	8	8	8	8	8	8	8	8	7	8	7·9	7	8
k	78	7	5	6	7	7	7	7	6	4	4	6·0	8	6
a	101	9	9	9	9	9	9	10	9	9	10	9·2	9	9
e	103	10	10	10	10	10	10	9	10	10	9	9·8	10	10
g	119	12	12	12	11	12	12	12	12	12	12	11·9	11	12
h	128	11	11	11	12	11	11	11	11	11	11	11·1	12	11

Coefficient of concordance of assessors = 0·956.
Rank correlation coefficient (absorptiometer/visual) = 0·809.

TABLE III

"FINE LIQUOR" SAMPLES

6 from Pittsburgh carbon, 5 from bone charcoal

Sample	Absorptiometer reading	Observers A	B	C	D	E	F	G	H	I	J	Average	Ranks: Absortiometer	Ranks: Visual
b†	74	2	2	2	2	2	2	1	2	2	1	1·8	1	2
h	77	1	1	1	1	1	1	2	1	1	2	1·2	2	1
c	88	3	3	3	3	3	3	4	3	3	3	3·1	3	3
a†	97	5	4	4	4	4	4	3	4	5	4	4·1	4	4
i	116	4	5	5	5	5	5	5	5	4	5	4·8	5	5
d†	121	7	7	6	7	7	7	7	7	7	7	6·9	6	7
j	127	6	6	7	6	6	6	6	6	6	6	6·1	7	6
f†	147	9	8	8	8	8	8	9	8	8	8	8·2	8·5	8
e	147	8	9	9	10	10	9	8	9	10	9	9·1	8·5	9
g†	156	11	10	10	9	9	10	10	11	9	11	10·0	10	10
k	170	10	11	11	11	11	11	11	10	11	10	10·7	11	11

Coefficient of concordance of assessors = 0·977.
Rank correlation coefficient (absortiometer/visual) = 0·917.
† Liquors from bone charcoal.

In a similar test with eleven samples of "fine liquor" the effect of different tints was investigated. The precise tint of fine liquor depends on the adsorbent used in the purification. Samples a, b, d, f and g were decolorised with bone charcoal; the remainder were decolorised with Pittsburgh granular carbon. Although the difference of tint was considerable the observers attained a high degree of concordance and their average ranking was fairly well approximated by the absorptiometer (see Table III). In the test 4·6-cm cells were used for the absorptiometer measurements; the observers used 60-cm cells.

Appendix

List of components

R_1, R_2, R_3, R_4	= 39-ohm, 100-watt, wire-wound, tapped resistors
R_5	= 6000-ohm, 2-watt, wire-wound resistor
R_6, R_7	= 150,000-ohm, ½-watt resistors
R_8, R_9, R_{10}, R_{22}, R_{24}, R_{25}, R_{29}, R_{31}	= 100-ohm, ½-watt resistors
R_{11}	= 33,000-ohm, ½-watt resistor
R_{12}	= 1·5-ohm, 3-watt, wire-wound resistor
R_{13}, R_{14}	= 220-ohm, ½-watt resistors
R_{15}	= 4700-ohm, ½-watt resistor
R_{16}	= 1-megohm, ½-watt resistor
R_{17}, R_{18}	= 1000-megohm, Welwyn, glass-sealed resistors
R_{19}	= 3·3-megohm, ½-watt resistor
R_{20}	= 680-ohm, high-stability resistor, 5 per cent. tolerance
R_{21}	= 3300-ohm, ½-watt resistor
R_{23}, R_{26}	= 68-ohm, ½-watt resistors
R_{27}	= 330-ohm, ½-watt resistor
R_{28}	= 47-ohm, ½-watt resistor
R_{30}	= 2000-ohm, 1-watt resistor
R_{32}, R_{33}	= 500-ohm, ½-watt resistors
R_{34}, R_{35}	= Resistors with a value according to sensitivity of the photocell (PEC)
R_{36}	= 47,000-ohm, ½-watt resistor
R_{37}	= 20,000-ohm, ½-watt resistor
R_{38}	= 1110-ohm resistor, adjusted in calibration
VR_1	= 50-ohm, 5-watt, wire-wound, variable resistor
VR_2	= 1000-ohm helical P.X. Fox PX4/H/10 variable resistor (set zero)
VR_3	= 200-ohm, 1-watt, wire-wound, variable resistor (coarse zero)
VR_4	= 1000-ohm, 5-watt, wire-wound, variable resistor (relay balance)
VR_5	= 1000-ohm P.X. Fox C 355 variable resistor (sensitivity setting)
VR_6	= 10,000-ohm, 5-watt, wire-wound, variable resistor (dummy zero, zero-point setting
VR_7	= 15,000-ohm precision P.X. Fox C 300 variable resistor (measuring potentiometer)
C_1	= 10-μF electrolytic capacitor, 250-volt working
C_2	= 100-μF electrolytic capacitor, 150-volt working
C_3, C_4, C_8, C_9, C_{10}, C_{11}, C_{12}, C_{13}	= 0·1-μF capacitors, 250-volt working (paper dielectric)
C_5, C_6, C_7	= 2-μF capacitor, 350-volt working (paper dielectric)
C_{14}	= 0·01-μF capacitor, 250-volt working (paper dielectric)
C_{15}	= 0·5-μF capacitor, 250-volt working (paper dielectric)
L_1	= Projector lamp, G27, 4·0-volt, 0·75-amp.
L_2	= Indicator lamp, 6·0-volt, 0·3-amp., M.E.S.
L_3	= Scale lamp, 6·0-volt, 0·3-amp., M.E.S.
L_4	= Mains indicator, neon, Arcolectric SL90N
V_1	= ME1403 valve
V_2	= 85A2 valve
TR_1	= OC 202 transistor
TR_2	= OC 43 transistor
RLA	= Carpenter type 51 B4/63 relay; coil resistances 2680 and 850 ohms
RLB, RLC	= 1 make, 1 heavy-duty make Post Office 3000-type relays with 1000-ohm coils
RLE	= 1 break, 1 break, 1½ inch toe slug Post Office 3000-type relay with 1000-ohm coil
M_1	= Milliameter, 0 to 1 mA
D_1	= Rectifier, RMO
D_2, D_3, D_4	= Suppressor diodes, OA 10
PB_1	= "Start" push-button, c/o contacts
PB_2	= "Re-set" push-button, break contacts
SM_1	= Servomotor, type T4FA1, 2 r.p.m. (set zero)
SM_2	= Servomotor, type T4FA1, 2 r.p.m. (set sensitivity)
SM_3	= Servomotor, type T4FA1, 2 r.p.m. (measuring)
SM_4	= Camshaft servomotor, type Q3FA1, 8 r.p.m.
K_1 to K_9	= Cam-operated microswitches, Burgess type V3 + VLR1

K_{10} = Cam-operated leaf switch
J_1 = Closed-circuit jack
PEC = Photocell, Cintel type VB39
SW_1 = Double-pole single-throw mains switch
SW_2 = Single-pole single-throw camshaft blocking switch
SW_3 = Single-pole change-over setting-up switch
PU = Advance DCT1 power unit, 30-volt, 1-amp.
T_1 = Transformer, Gardiners TS8000
F_1 = Mains fuse, 1-amp.
TP_1, TP_2, TP_3 = Test points

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The Determination of Moisture in Plain Cakes by a Microwave Attenuation Technique

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A procedure based on microwave attentuation measurement with an A.E.I. Ltd. moisture meter has been developed for the rapid determination of moisture in plain cakes. The main factors influencing attentuation, apart from moisture, are temperature, sample preparation and cake texture. Under the conditions described, linear relationships were obtained between moisture content, attenuation and temperature for a variety of cakes. At moisture levels ranging from 18 to 25 per cent., 95 per cent. of the results obtained by the microwave method were within $\pm 0{\cdot}6$ per cent. of results obtained by a Brabender oven method.

As a result of a need for a very rapid method for determining moisture in cakes, it was decided to investigate the possible application of a microwave moisture meter. At the time, although brief references had been made to the application of this technique to foodstuffs, the details available concerned building materials, for which the technique was originally developed, and also sand, tobacco and paper.[1,2,3] Even with these substances, however, information of practical interest to the analyst was scanty.

More recently, Taylor[4,5] has considered the technique and its limitations in greater detail, and has outlined some of these at a Society Meeting in May, 1964.[6] The results of our experiments, completed before the publication of this paper,[6] independently confirm his observations on some of the factors affecting microwave attenuation, *i.e.*, absorption, measurements. But, since our approach was that of the analyst rather than that of the electronics engineer, we believe that our more detailed results in applying this new approach to moisture measurements will help other analysts contemplating its use.

Microwave absorption and equipment

Molecular energy changes due to the absorption of energy in the microwave region of the electromagnetic spectrum, *i.e.*, wavelengths of 0·1 to 30 cm, can occur in a similar way to those resulting from the absorption of infrared radiation. Water is one of those substances whose molecular rotation spectrum includes natural frequencies in this region. At these frequencies, the attenuation by water is much greater than that of most dry materials, so that, once the relationship between moisture content and attenuation has been determined for a given material by calibration, the attenuation can be used as a direct measure of moisture content.

The instrument used in our experiments was an Associated Electrical Industries Ltd. X-band moisture meter operating at a frequency of 10,680 Mc/s. It is made up of two lightweight, portable units, the transmitter and the receiver. Basically, the transmitter consists of a klystron oscillator modulated by a 3·2 Kc/s square wave, terminating in a 3-inch square horn. A similar horn on the receiver is coupled to a precision attenuator and crystal detector. The signal from the latter is amplified and fed to a meter, or recorder if desired.

In use, the transmitter and receiver horns are adjusted at a set distance apart. This distance is determined by the size of the sample found to be necessary. The attenuator setting required to give a mid-scale deflection on the meter is determined with and without the sample inserted between the horns. The difference between the two settings is the attenuation of the sample, measured in decibels (dB).

When applied to powders or granular materials, wave-guides, tubes of small rectangular cross-section and variable length, may be used as containers. Preliminary experiments with cake and the use of such wave-guides were unsuccessful. The most satisfactory container was found to be a 4-inch section of 6-inch diameter Perspex tubing sealed on to a $6\frac{1}{2}$-inch square of $\frac{1}{8}$-inch Perspex sheet. Additional strips of Perspex were cemented to the underneath of the base so that the cell could easily be slotted on to the transmitter horn, which was at a constant distance of $4\frac{1}{4}$ inches from the receiver horn. The equipment and the cell are illustrated in Fig. 1.

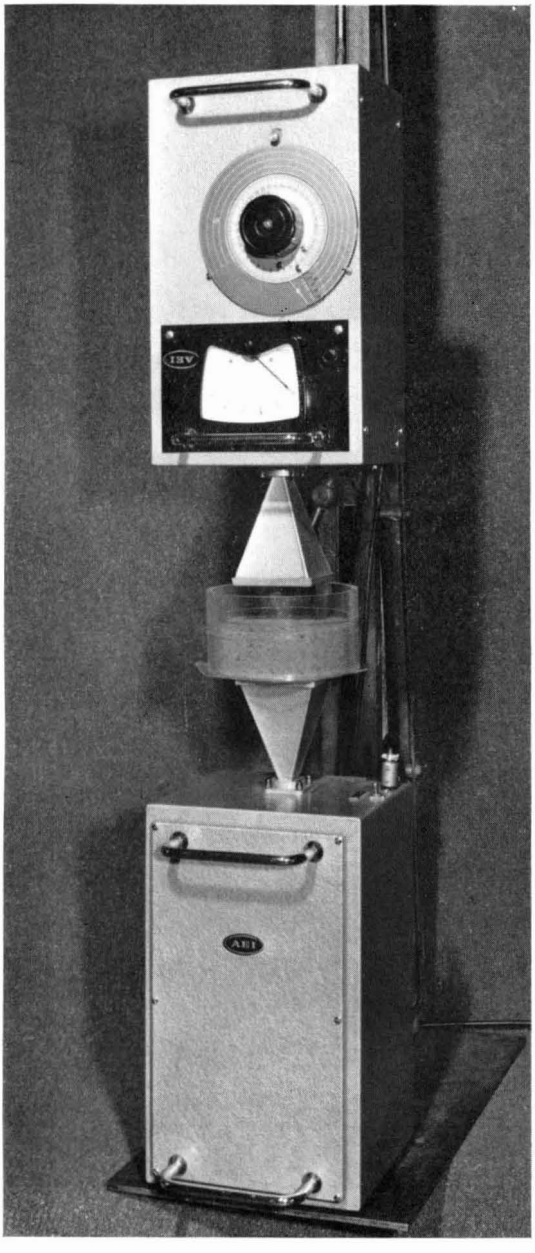

Fig. 1. General view of moisture meter

[To face p. 692

Experimental

Selection of a standard method for calibration—

In order to achieve reliable calibration of the instrument, it was essential to know more about the precision and accuracy of the conventional oven methods used in cake analysis, and a series of experiments was carried out in which the results obtained by drying in a Brabender oven at 105° C for 1 hour were examined statistically and compared with those obtained by drying for 4 hours in a steam-jacketed oven of the Imperial Tobacco Company's design. As might be expected, sampling affected the results greatly, and it was found that, for a plain cake, a difference of up to 1 per cent. of moisture (actual) could be expected from different parts of the same cake. However, by using well mixed samples that were allowed to reach moisture equilibrium in sealed tins, it was shown that the accuracy and precision of each method were practically identical, the latter being $\pm 0{\cdot}3$ per cent. of moisture. Thus it was decided to base the calibration on the Brabender oven method, which was the quicker of the two.

Effect of sample weight and compression—

Preliminary tests on one type of cake showed that attenuation varied linearly with sample weight and that in order to accommodate the expected range of moisture contents, a sample weight of 425 g was required. This corresponded to more than one cake, so that the technique initially adopted was to disintegrate three cakes in a Hobart mixer by using a paddle-type blade and to withdraw 425 g for the attenuation measurement.

In this manner, weighed samples of plain sponge-cakes were transferred to the Perspex cell and gently shaken to level the surface. It was found that the depth of cake in the cell varied from sample to sample, and so these results were compared with those obtained on the same samples, but after compression to a uniform depth of 2 inches. The standard deviation of a single reading for the loosely packed technique was 0·3 dB, and for the compressed samples 0·2 dB. Additionally, the attenuation of each sample increased when the sample was compressed, and was due to either an attenuation increase of the sample, or an increased loss of radiation by reflection at the air - cake interface. The slightly better precision obtained after compression was most likely attributable to the maintenance of a constant distance between this interface and the receiver horn so that reflection losses were constant.

Similar results were obtained with Madeira cake and gateau-type cakes, but the texture of ginger cake was such that 425 g could be contained in a 2-inch depth in the cell with little or no compression and so in this instance a compressed depth of $1\frac{1}{2}$ inches was used.

Effect of temperature—

Attenuation measurements were carried out on plain sponge cakes at temperatures varying from 15° to 25° C and the moisture contents determined by the Brabender oven method. By plotting graphs of attenuation against temperature for samples having the same moisture contents it was found that the attenuation increased by about 0·4 dB for each °C rise, thus confirming that the microwave method is temperature dependent.[4,5] This is illustrated in Figs. 2 (*a*) and (*b*) in which are plotted attenuations against moisture contents, uncompensated for temperature in Fig. 2 (*a*) and compensated to 20° C in Fig. 2 (*b*). The same conclusions held for other varieties of cake, such as chocolate sponge, Madeira and gateau-type.

Effect of crumb agglomeration—

Samples of plain sponge cake broken down in the Hobart mixer and which had been intentionally heated to above 30° C were found to have lower attenuations than expected after the temperature correction factor had been applied. Further, when a different sample disintegrator, which operated on a rotating-knife principle (a Turmix shredder) was used, higher than expected attenuations were obtained for the same variety of cake. The main difference between the prepared samples in these and previous experiments was in texture. The Hobart-mixed samples were coarse and larger in crum structure than the Turmix-prepared samples, which were small in crumb and light-textured. Further, the heated samples ($> 30°$ C) looked distinctly soggy.

The dependence of the attenuation - moisture relationship, temperature corrected, upon crumb size and agglomeration was confirmed by separate tests on Swiss roll samples. Here, uncreamed roll, freshly disintegrated in a Turmix, gave a different, but still linear, calibration from the same samples after they had been stored in sealed tins for a few hours. The stored samples were different in appearance to the fresh samples and had a more clinging crumb structure.

EFFECT OF SAMPLING ERRORS ON THE BRABENDER RESULTS—

After measurement of the attenuation, samples for the Brabender oven moisture determination were taken (*a*) directly from the cell and (*b*) from a polythene bag to which the contents of the cell had been transferred and thoroughly mixed by shaking for 1 minute. Statistical examination of the results revealed that the precision of the calibration was better with procedure (*b*) and this was ascribed to improved moisture distribution, so that the 10 g withdrawn for the Brabender test was more representative.

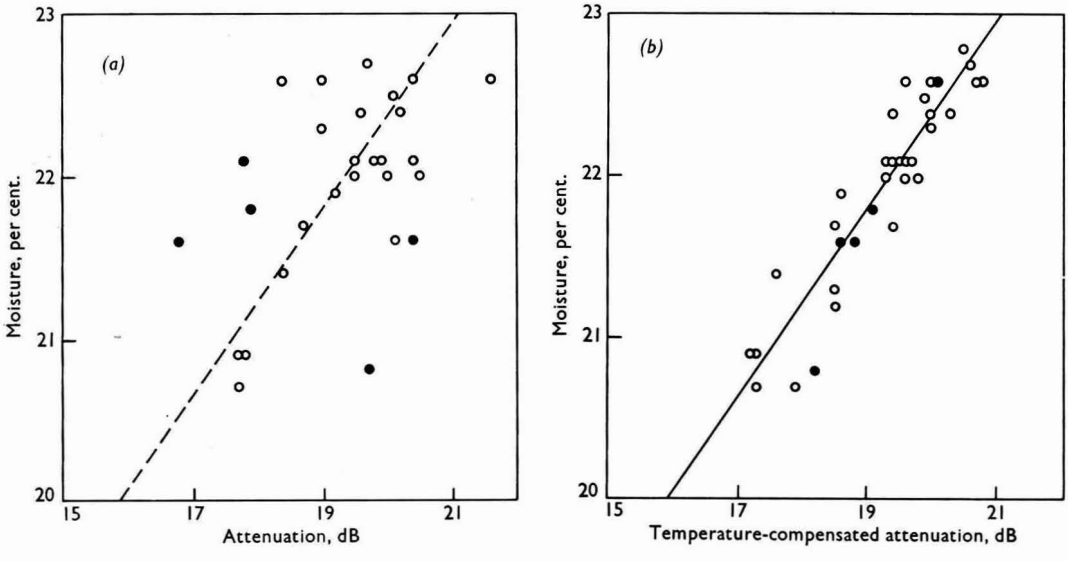

Fig. 2. The effect of moisture on the attenuation of cake samples (*a*) varying in temperature between 15° and 25° C and (*b*) after compensation for temperature to 20° C. Points ○, determined within the range 17° to 23° C; points ●, determined within the ranges 15° to 17° C and 23° to 25° C

METHOD

Two or three cakes, depending on the variety, are required to provide sufficient sample for the test, but their temperature must be less than 30° C before testing. Slice each cake into two, pass one portion from each cake successively through a Turmix shredder, and then shred the remaining halves in a similarly successive manner. In this way, a satisfactorily mixed bulk sample is obtained for the microwave measurement.

With the equipment set up as shown in Fig. 1, switch on at the mains and then the transmitter, and allow the latter to warm up for 5 minutes. Slot the appropriate Perspex cell fully home across the transmitter horn. For ginger cakes, use a cell of 1½-inch sample depth; for other cakes, *i.e.*, Swiss roll, Madeira, sponge and gateau, use a cell of 2-inch sample depth.

Adjust the attenuator control to give a mid-scale deflection on the meter. Align the zero line of the annular scale with the zero line on the decibel scale, and lock by means of the knurled screw. This setting should be checked every 45 minutes when the instrument

is in operation. Remove the cell and weigh into it 425 ± 1 g of prepared sample directly from the Turmix container. Gently shake the cell horizontally to obtain an even distribution of cake and compress the sample with a suitable plunger to the appropriate depth. Re-insert the cell between the horns, and regain mid-scale deflection of the meter by adjusting the attenuator control. Record the decibel reading coincident with the zero set line of the annular scale. This value is the attenuation of the sample. Take the temperature of the sample at three different positions, by using a calibrated thermistor-type thermometer and record the average temperature to the nearest 0·5° C.

CALIBRATION OF THE INSTRUMENT—

Carry out the procedure as described above, then transfer the sample from the cell into a dry polythene bag of suitable capacity, and shake the bag thoroughly for at least 1 minute to ensure a satisfactorily mixed sample for the conventional moisture determination. Weigh 10 g of sample, and determine the moisture content by using a Brabender oven and drying for 1 hour at 105° C. Select samples so that the entire range of results expected to be met in practice is covered by the calibration. At least 20 results, preferably more, are required to produce an accurate calibration.

DISCUSSION OF RESULTS

SWISS ROLL—

Twenty-one samples were tested by the microwave and Brabender procedures. Temperatures varied between 21° and 30° C, and the moisture contents ranged from 16 to 24 per cent. A statistical analysis of the results by the method of least squares produced the equation—

$$M = 0{\cdot}48A - 0{\cdot}18T + 14{\cdot}16$$

where M = moisture content of sample, per cent. by weight,
A = observed attenuation of 425 g of sample and
T = temperature of sample, in °C.

By using this relationship, the moisture content of the cakes relating to the determined attenuations were calculated, and the deviations from the actual Brabender results derived. All of the microwave results differed from the oven result by less than 0·6 per cent. of moisture, and 75 per cent. by less than 0·3 per cent. The mean deviation from the oven result was −0·15 per cent. Since the Brabender determination contributes to the errors, the microwave method could be more accurate than these results suggest, particularly when account is taken of the fact that the errors due to sampling will be less for this method because of the much larger sample weight.

PLAIN AND CHOCOLATE SPONGES, MADEIRA CAKES AND GATEAUX—

In this series of tests, 6 butter-sponge samples, 7 chocolate-sponge samples, 8 Madeira-cake samples and 16 gateau samples were examined, having moisture contents ranging from 21 to 25 per cent. It was statistically calculated that all four cakes were governed by the relationship—

$$M = 0{\cdot}59A - 0{\cdot}22T + 13{\cdot}54$$

Again, the deviations of the calculated result from the Brabender result were derived. Just under 95 per cent. of the microwave results differed from the Brabender result by less than 0·6 per cent. of moisture, and about 70 per cent. by less than 0·3 per cent. of moisture. The mean deviation between the two methods was zero.

GINGER CAKE—

The relationship for this cake was—

$$M = 0{\cdot}50A - 0{\cdot}27T + 16{\cdot}35$$

This regression equation was developed from only 14 results, which was insufficient to produce the most accurate equation. Nevertheless, the mean deviation from the Brabender result was only +0·15 per cent. and only one microwave result differed from the oven result by greater than 0·5 per cent. of moisture. The range of moisture content covered was again 21 to 25 per cent.

The three sets of results above for different varieties of cake are presented graphically in Fig. 3, which illustrates more clearly the different linear relationships.

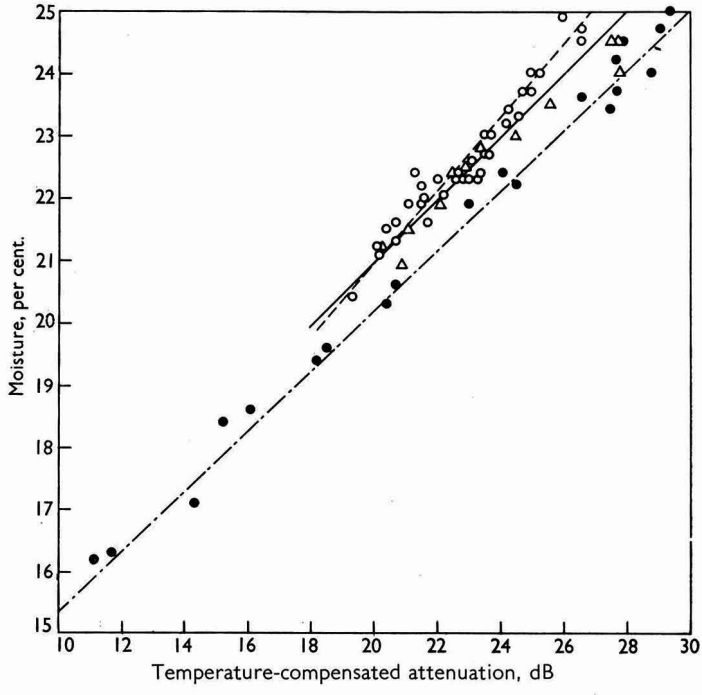

Fig. 3. The effect of cake texture on the linear relationship between attenuation and moisture content. Points ●, swiss roll; points △, ginger cake; points ○, sponges, gateaux and Madeira cake

Conclusions

We have found that, by following a standardised technique that takes into account factors affecting attenuation measurements, moisture can be determined within the accuracy required for control purposes in certain varieties of cake by using a microwave attenuation technique. The main factors affecting the method are temperature and texture of sample, the latter being influenced by sample preparation. Changes in the cake recipe, provided that the texture is not altered, appear to have little effect; in fact, in one instance, the same calibration held for four varieties of cake. Textural differences are more important and have a greater influence upon results.

The regression equations quoted apply specifically to the conditions and circumstances described, and it is recommended that other workers carry out individual calibrations along the lines indicated.

We thank Mr. J. G. Ross for his assistance in the statistical interpretation of the results and the Directors of Cadbury Brothers Ltd. for permission to publish this work.

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SHORT PAPERS

The Micro-determination of Glutathione and Cysteine in the Presence of Each Other

By M. WROŃSKI

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Knowledge of the concentration of reduced glutathione and cysteine in some products of living creatures is of prime importance for certain biochemical investigations.[1] This paper reports a method for determining both compounds in the presence of each other, in the absence of proteins, which can be removed by precipitation.

As has been already shown[2] cysteine reacts very rapidly with formaldehyde, forming probably a 5-membered ring, whereas the reaction with its *N*-acid derivatives is slow, if there are no amino groups in the molecule. Glutathione has an amino group, which can form only a 10-membered ring with formaldehyde. Consequently, the reaction between formaldehyde and glutathione is more rapid in comparison with the compounds that have no amino group at all. In acidic solutions containing 3 per cent. of formaldehyde, about 80 per cent. of the mercapto groups in glutathione are no longer detectable after 5 minutes at 20° C, probably because of the formation of 10-membered rings with $-NH.CH_2.S-$ bonds. In alkaline solutions the reaction is slow, whereas the reaction between cysteine and formaldehyde is very rapid in acidic as well as in alkaline solutions, and in this way both compounds can be distinguished.

The determination of cysteine and glutathione in trace amounts can be carried out by titration with 5×10^{-4} N *o*-hydroxymercuribenzoic acid in the presence of thiofluorescein in 0·05 N ammonia solution as indicator, with an accuracy of $\pm$0·05 ml of the reagent.

Some typical results are shown in Table I.

The same results were obtained by titrating cysteine in 0·05 N sodium hydroxide in the presence of dithizone, but under these conditions the titration of glutathione is less accurate and the results are about 3 per cent. too low. The use of thiofluorescein in 0·05 N sodium hydroxide is not convenient, because the end-point is not sharp, the change of colour lies in a range of 0·5 ml of 5×10^{-4} N *o*-hydroxymercuribenzoic acid, whereas in ammonia solutions the range is 0·2 ml of 5×10^{-4} N *o*-hydroxymercuribenzoic acid.

For the determination of cysteine and glutathione in the range 0·2 to 10 μmoles, the procedure given below is recommended.

Method

Reagent—

o-*Hydroxymercuribenzoic acid*—Dissolve *m* (about 0·16) g of *o*-hydroxymercuribenzoic acid anhydride in 10 ml of 0·1 N sodium hydroxide and dilute the solution to 1 litre. The normality, N_{HMB}, of the solution is given by—

$$N_{HMB} = \frac{m}{320 \cdot 71}$$

Procedure—

For cysteine plus *glutathione*—Add 1 ml of N ammonia solution to 20 ml of aqueous sample and titrate the mixture with the *o*-hydroxymercuribenzoic acid with thiofluorescein as indicator. Let this titre be V_1 ml. The titre of a blank solution containing indicator should be 0·2 ml.

For glutathione—Add 1 ml of N sodium hydroxide and 5 ml of 3 per cent. w/v formaldehyde to 20 ml of aqueous sample. Set the solution aside for 1 minute and then titrate without delay with the *o*-hydroxymercuribenzoic acid with dithizone as indicator until the colour changes from yellow to purple. Let this titre be V_2 ml. The titre of a blank solution containing indicator should be 0·1 ml. Use the blank solution with the same amount of indicator and 0·1 ml of *o*-hydroxymercuribenzoic acid for comparison at the end-point. Only 6 per cent. of the mercapto groups in glutathione and more than 99 per cent. of the mercapto groups in cysteine, react under the

conditions used with formaldehyde. Consequently, the full correction for glutathione amounts to 9 per cent. as compared with titration in ammonia solution in which thiofluorescein is used as indicator. Calculate the results from the equations—

$$\text{Glutathione content, mmoles} = 1{\cdot}09\ (V_2 - 0{\cdot}1)\ N_{\text{HMB}}.$$
$$\text{Cysteine content, mmoles} = [V_1 - 0{\cdot}2 - 1{\cdot}09\ (V_2 - 0{\cdot}1)]\ N_{\text{HMB}}$$

TABLE I

DETERMINATION OF CYSTEINE AND GLUTATHIONE

Cysteine present, μmoles	Glutathione present, μmoles	Cysteine found, μmoles	Glutathione found, μmoles
4·30	0·000	4·27	0·025
0·000	1·08	0·012	1·07
0·000	2·16	—	2·19
0·000	4·32	0·035	4·28
2·66	2·25	2·60	2·27
1·28	3·38	1·33	3·26
5·15	1·13	5·10	1·15
2·57	1·13	2·47	1·13
4·30	0·86	4·26	0·87

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A Comparison of Fibrous-residue Determinations by the Official (A.O.A.C.) Method and the Dimethylsulphoxide Method

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A SIMPLIFIED method for the determination of fibrous residue in wheatmeals and in brown and wholemeal breads has been proposed.[1] This method, based on a single digestion of the sample with a mixture of dimethylsulphoxide and formic acid, gives identical results with the official method[2] up to a fibre content of about 4·5 per cent.

A series of determinations on a range of sample, containing from 0·45 to 13·6 per cent. of crude fibre was carried out by the official method and the dimethylsulphoxide method and the results compared (see Table I).

It was observed that the dimethylsulphoxide method gave consistently higher results than the official method on samples of a fibre content higher than 4·5 per cent.

The nitrogen determination on the fibre isolated by the dimethylsulphoxide - formic acid process showed that the fibre is almost nitrogen free. The higher results obtained could therefore not be attributed to the incomplete removal of proteinaceous matter by the new digestion procedure.

The fibre isolated from wheat products by the dimethylsulphoxide method is practically ash-free. (The fibre from a sample of grass contained about 6 per cent. of ash, half of which was silica, but the results in Table I have been reported on an ash-free basis.)

The fact that the results obtained by the dimethylsulphoxide method agree well with those obtained by the official method up to a certain content, but are higher for samples of higher fibre content (in the absence of nitrogen in the fibre) seems to indicate that partial hydrolysis of fibre material takes place in the official method. It is assumed that partial hydrolysis does not significantly influence the results in the lower fibre-range, but becomes significant in samples of high fibre content.

To test this assumption, 1-g lots of cotton-wool were digested by the official and the dimethylsulphoxide method. With the latter method a recovery of 97 per cent. of cotton-wool (on a dry basis) was obtained, whereas with the official method the recovery was only 86 per cent. The

filtrate of the acid digestion of cotton-wool by the official method gave an appreciable amount of precipitate of Cu_2O with Fehling solution indicating that some cellulose had been hydrolysed.

Similarly, the fibre isolated by the dimethylsulphoxide method from four determinations from the grass mixture was bulked and subjected to the procedure of the official method. A loss of 31 per cent. occurred and the filtrate of the acid-digestion step reduced Fehlings solution, indicating again that hydrolysis had taken place.

These findings agree with those reported previously by several authors. (For an excellent review see Weinstock and Benham.[3])

TABLE I

Sample	Crude fibre, percent., by— Official method	Crude fibre, percent., by— Dimethylsulphoxide method	Nitrogen, per cent., based on fibre weight	Recovery by official method as a percentage of that by dimethylsulphoxide method
Calf meal	13·64, 13·46	15·53, 15·83	0·0, 0·12	86
Cattle nuts ..	9·77, 9·59	13·23, 13·81	0·0, 0·12	72
Pollard	6·43, 6·48	7·53, 7·55	0·0, 0·0	86
Fine bran	9·58, 9·63	10·57, 10·55	0·0, 0·12	91
Coarse bran ..	11·30, 11·74	13·20, 13·51	0·12, 0·12	86
Grass mixture ..	23·22, 23·21	26·5, 26·9	0·0, 0·0	87
Animal feed ..	6·30, 6·48	7·31, 7·40	0·0, 0·0	87
Animal feed ..	4·74, 4·61	4·90, 4·63	0·0, 0·0	—
Sharps	2·01, 2·05	2·23, 2·09	—	—
Wholemeal bread..	1·59, 1·66	1·54, 1·58	—	—
Brown bread ..	0·45, 0·43	0·42, 0·49	—	—
Wheatmeal ..	2·17, 2·24	2·16, 2·25	—	—

Table I, column 4 lists the recovery of crude fibre by the official method, assuming the recovery of crude fibre by the dimethylsulphoxide method to be 100 per cent.

As the material isolated by the dimethylsulphoxide method has been washed with boiling 30 per cent. aqueous formic acid and with boiling water and furthermore as it contains practically no nitrogen, it may be assumed that it represents more accurately the fibrous material present in plants, than the material isolated by the official method, in which hydrolysis of cellulosic substance has been shown to occur.

The author gratefully acknowledges the technical assistance given by Miss H. J. Robinson.

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Received *April 13th*, 1965

Book Reviews

HANDBOOK OF X-RAY ANALYSIS OF POLYCRYSTALLINE MATERIALS. By LEV IOSIFOVICH MIRKIN. Pp. xx + 731. New York: Consultants Bureau. 1964. Price $35.00.

Dr. Mirkin's remarkable book was originally published in Russian in 1961, and the present volume is a translation by Dr. J. E. S. Bradley. It brings together an astonishingly comprehensive collection of those constants and data used in the production and interpretation of X-ray diffraction patterns from polycrystalline materials. Whilst the 464 references cover the world's literature, many are from relatively inaccessible Russian sources and this translation makes the material available to a wider public.

The data are presented for the main part in tabular, graphic and nomographic form. In a foreword the Editor, Professor Umanskii, stresses that the book is not intended to present the type of material to be found in textbooks, so that only brief statements are given as to the use of the tables and nomograms. Derivations of formulae and descriptions of methods are given concisely, if at all. The list of contents of the twelve chapters indicates how very fully the subject has been covered.

The book is divided into two parts. Part 1, comprising the first four chapters, is concerned with the initial stages of an X-ray analysis. In Chapter 1 X-ray spectra and the interaction of X-rays with matter are discussed, whilst chapter 2 deals with the production and measurement of X-ray patterns. Chapter 3 is about the indexing of X-ray patterns and the final chapter in this part deals with factors affecting line intensities.

In Part 2, chapter 5 is concerned with phase analysis and subsequent chapters include precision measurement of lattice constants, determination of macroscopic stresses, determination of crystallite sizes and microstresses, preferred-orientation textures, small-angle scattering, electron diffraction and neutron diffraction.

The first twenty pages of chapter 2 describe Russian X-ray equipment and the inclusion of this section in the present American edition is of doubtful value. In the same chapter the nomograms giving parameters for bent quartz crystal monochromators are an example of the detailed presentation accorded to much of the information in the book.

Chapter 3 is very long (220 pages) and covers the indexing of powder patterns for crystals in descending order of symmetry. The Hull–Davey charts for indexing patterns from hexagonal and tetragonal crystals included in this chapter have not been reproduced for almost forty years. The use of the theory of homology in indexing patterns for crystals of low symmetry is given an extensive treatment in this chapter.

Chapter 5 in the Russian edition contained 126 pages of data from the A.S.T.M. Powder Data File. These are omitted in the translation under review.

The last two chapters in the book are relatively short and are the ones concerned with electron and neutron diffraction. Their inclusion is hard to justify as these topics are not developed in the systematic way accorded to the X-ray method and the relatively small amount of material included is unlikely to interest those working in these areas.

In a book of this type it is important that the data should be readily available and in this espect the index, used in conjunction with the list of contents, appears to be adequate. Several minor inaccuracies are to be found in the references listed at the end of the volume.

The book is stoutly bound and clearly printed. It is highly recommended to those whose work involves some knowledge of powder diffraction methods. E. A. KELLETT

TABLES OF INTERATOMIC DISTANCES AND CONFIGURATION IN MOLECULES AND IONS: SUPPLEMENT 1956–1959. Edited by L. E. SUTTON, M.A., D.Phil., F.R.I.C., F.R.S. Pp. vi + 288. London: The Chemical Society. 1965. Price 84s.; $12.00.

This supplement of Special Publication No. 11 covers the period 1956 to 1959, inclusive, and gives details of structural parameters (bond lengths and angles) obtained by X-ray crystallography, electron-diffraction, or spectroscopic techniques. The compounds listed range from molecular hydrogen to vitamin B_{12} and include structural details of many of importance to the analyst, *e.g.*, acetylacetonates of beryllium, copper(II), nickel, vanadium(III), cobalt(III), iron(III) and thorium; bisdimethylglyoxime complexes of copper, nickel, palladium and platinum; bipyridyl; mercury dithizonate. It is noteworthy that in the EDTA complex, ammonium ethylenediaminetetra-acetato cobalt(III), the metal is 6-co-ordinated by the two nitrogen and four oxygen atoms of the ligand anion; but in dihydrogenethylenediaminetetra-acetato aquonickelate(II) one carboxyl group is free and octahedral co-ordination is completed by the water molecule. There are over 800 literature references and excellent indexes and tables of selected bond lengths in this invaluable and scholarly work. H. M. N. H. IRVING

THE EQUILIBRIUM THEORY OF CLASSICAL FLUIDS. By HARRY L. FRISCH and JOEL L. LEBOWITZ. Pp. xvi + 517. New York and Amsterdam: W. A. Benjamin Inc. 1964. Price (cloth) $11.00; (paper) $6.55.

This is a rather new kind of book and a very useful kind, too. It is one of a series called "Frontiers in Physics," is described as a "Lecture Note and Reprint Volume," and turns out to be a brilliantly compiled anthology of twenty-eight articles photographically reproduced either from published papers or from typescripts hot off the griddle, together with brief but pertinent comments and a useful bibliography. A few well chosen classics are reprinted. These are papers by Ornstein and Zernike (1914) and Zernike (1916); Kirkwood (1935); Guggenheim (1945); Mayer (1947); Van Hove (1950; here translated into English) and Nijboer and Van Hove (1952); and two papers by Yang and Lee (1952). Apart from these, all the papers were published between 1962 and 1964, or had not been previously published (indicated with asterisks). They include papers by Ruelle*; Groeneveld; Lebowitz and Penrose; Edwards and Lenard; Percus*; Stell*; Wertheim; Ree and Hoover; Kac, Uhlenbeck and Hemmer; Helfand*; and Fisher. To those who are already familiar with the field, these names will speak for themselves.

The anthologists are two of the best known members of the vigorous and gifted "New York School," which is leading a remarkable leap forward in statistical mechanics, particularly as applied to fluids. They say that "The main purpose of this book is twofold: (1) to serve as a

convenient reference book for scientists with an active interest in the field of classical fluids or in related fields (such as magnetism, the quantum mechanical many-body problem, and so on, and (2) to serve as a summary and guide to the present status of some active areas of research in this field." No matter that the reproduction is not always perfect, or that there are many signs of hasty preparation (such as the omission of a bracket in the sentence just quoted), or that the number of words on a page varies greatly from article to article. Every beginner in the field, as well as every expert, will wish to have this splendid collection on his desk or at his bed-side, even when, as is likely quickly to be the case, the anthologists themselves and their friends have made a good deal of it out of date.

M. L. McGLASHAN

RUBIDIUM AND CAESIUM. By F. M. PEREL'MAN. Translated by R. G. P. TOWNDROW, English Translation Edited by R. W. CLARKE. Pp. xvi + 144. Oxford, London, Edinburgh, New York, Paris and Frankfurt: Pergamon Press. 1965. Price 60s.

This book, the second edition of one first published in 1941, is one of the International Series of Monographs on Nuclear Energy and is Volume 2 of Division VIII, Materials.

Since the publication of the first edition, tremendous advances in the knowledge of these two rare elements of the alkali-metal group have been made and a book of this nature including the most up-to-date information is most welcome. The bibliography contains 346 references to papers published up to the end of 1960 and a supplementary list refers to papers published subsequently to the end of 1964.

The book is divided into six chapters dealing in turn with The Discovery and Occurrence of Rubidium and Caesium, Properties of Rubidium and Caesium and Their Compounds, Systems Formed by Rubidium and Caesium Salts, The Analytical Chemistry of Rubidium and Caesium, The Extraction of Rubidium and Caesium from Minerals and Ores, and The Preparation of The Metals and Various Compounds of Rubidium and Caesium. The chapter on analytical chemistry is divided into two sections covering qualitative and quantitative analysis, respectively, and is most comprehensive. The heavier atomic weight elements of the alkali-metal group have been notoriously difficult to separate and determine. The task has been simplified by the development of newer techniques such as flame photometry, use of radioactive tracers, colorimetry and paper chromatography, and these are considered in fair detail. With regard to the last of these methods, however, there seems to be some confusion of nomenclature. The methods described would seem to refer rather to electrophoresis since it is implied that a current is passed through the paper column in the presence of solvents. There is no reference to separation on a paper column by the use of solvents alone.

This book may be recommended to those interested in the alkali metals, particularly the rarer ones, which, as is implied in the preface, are becoming of increasing importance.

F. M. LEVER

PROCEEDINGS OF THE FIRST AUSTRALIAN CONFERENCE ON ELECTROCHEMISTRY. Edited by J. A. FRIEND and F. GUTMAN with the assistance of J. W. HAYES. Pp. xvi + 954. Oxford, London, Edinburgh, New York, Paris and Frankfurt: Pergamon Press. 1965. Price £10.

This weighty and well printed volume provides a record of about seventy papers given at the First Australian Conference on Electrochemistry held in 1963. They are divided into twelve sections—

1. Solid-State Chemistry.
2. Thermodynamics of Electrolytes.
3. Corrosion.
4. Theory of Double Layers.
5. Electroanalytical Methods.
6. Applications (Electroplating, Anodizing).
7. Non-aqueous Electrolytes.
8. Molten Salts.
9. Fuel Cells.
10. Electrode Processes.
11. Electrochemical Processes.
12. Electrowinning and Electrorefining.

This classification is somewhat arbitrary and seems to have been determined in part by the original planning of the conference. It is often difficult to make a rational arrangement of papers in advance, but there seems to be no good reason why this should be perpetuated in print.

For example, the paper by Bockris *et al.* is included in the curiously titled section 11 when it is an obvious choice for section 4. Similarly, the stimulating paper by Fleischmann *et al.* on the use of pulsed potentiostatic methods to control electrosynthesis is only incidentally concerned with non-aqueous solutions and should be in section 10 not 7.

Each section begins with a chairman's address (that of section 5 is unfortunately not printed) that varies from a general survey to something more like a contributed paper, according to personal views of the way in which the greatest contribution could be made. Although the relations between sections might have been clarified by a more rational grouping there is much of interest in each. The first section is somewhat anomalous; the connection between solid-state chemistry and electrochemistry is important and the last decade has seen great progress in building bridges between these subjects. It is particularly unfortunate that the relationship is hardly perceptible in this section and that there is no paper on the electrochemistry of semiconductors. The remaining sections are of a more conventional electrochemical character though it is notable that the full range from the most abstract theoretical type of paper to the practical recipe is covered in a single meeting. It is to be hoped that the resulting mutual stimulation is greater than the mutual frustration due to the difficulties of communication.

The production of handsome volumes at this sort of price always raises doubts in the mind of the reader who has to persuade his library to buy them. Is this the best way of publishing the proceedings of a conference? Many, though not all, of these papers could be published in the normal scientific journals (some of them have been, presumably because the authors were impatient of the two-year delay in publication) but, perhaps they gain from juxtaposition. However, all scientists would agree that one of the most important features of a conference is the discussion of the papers. While much of this is outside the formal sessions, that which is public is usually of great value and its inclusion in a published report provides the best justification for such a publication, in spite of the extra work it demands from the Editors. The complete absence of any report on the discussion is the biggest defect of the volume under review. ROGER PARSONS

DETERMINATION OF MOLECULAR WEIGHTS AND POLYDISPERSITY OF HIGH POLYMERS. By S. R. RAFIKOV, S. A. PAVLOVA and I. I. TVERDOKHLEBOVA. Pp. viii + 357. Jerusalem: Israel Program for Scientific Translations. Distributed in Great Britain and the Commonwealth, South Africa, Eire and Europe by Oldbourne Press, London. 1964. Price 99s.

This translation by Dr. J. Eliassaf and Dr. J. Schnorak is a readable review of the principal methods of molecular-weight determination, and shows relatively few signs of passage from Russian to English, apart from occasional awkward phraseology. On page 15, occurs "It is also necessary to take into account the possibility of the degradation of the chain of the dissolved polymer by the solvent or due to thermal effects, . . .", and on page 48, "Desreux recommended to deposit preliminarily the polyethylene on Celite or sand. . . ". A few other instances are to be found, but the meaning is always conveyed clearly enough.

Many of the methods described are claimed to have been used by the authors, but most appear to be quoted directly from the original sources, not always too critically. The statement in the introduction to the effect that Soviet sources have been more abundantly quoted, is slightly misleading, as less than a fifth of the many references are from Soviet sources—still a higher proportion than would appear in any Western treatise.

The most extensive treatment is given to fractionation, and the coverage of this field is very comprehensive in description of methods and apparatus, but theoretical considerations are dealt with somewhat summarily. Since the book is intended as a source of information on methods for workers in fields other than physical chemistry, use can easily be made of the very extensive references to locate more complete theoretical treatments.

Theoretical treatment is more comprehensive in the chapter dealing with light scattering, this chapter also giving much prominence to apparatus, and relatively little to actual measurements on polymer solutions.

Diffraction measurements and ultracentrifuge sedimentation are accorded short treatments, while osmometry and ebullioscopic, cryoscopic and isopiestic methods are described in very great detail, most of the many types of apparatus appearing in the literature before 1960 being described. Viscometry is given a similarly detailed treatment, and end-group assay and other methods an honourable mention.

The book is altogether a mine of information to any newcomer to the field of molecular-weight measurement, and contains much of interest to experienced workers in the field if only because it brings together in one volume most of the available methods of measurement. F. YEARSLEY

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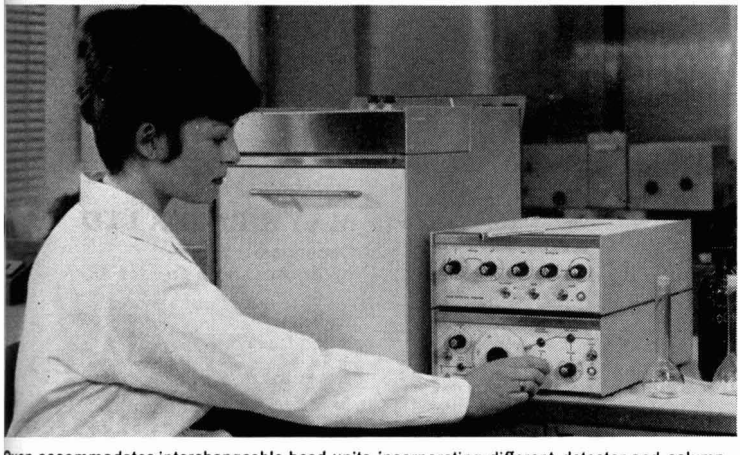

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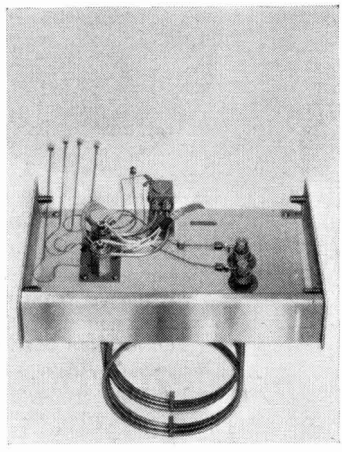

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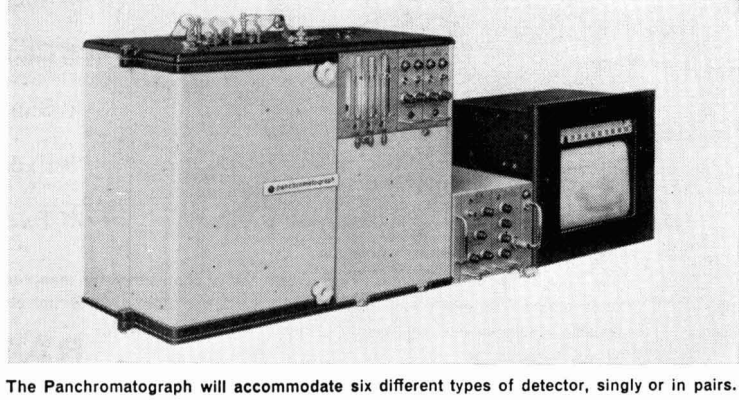

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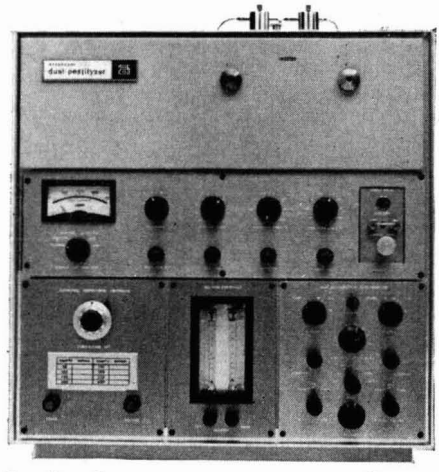
SCIENTIFIC PYE INSTRUMENTS

Pesticide Analysis



To do a good job you need good instrumentation. Wilkens has all you need and a little more: Experience and good Service

1. The Instruments

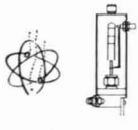
a) Model 205 – The Dual Channel Pestilyzer
This unit features the unique two-channel electrometer for simultaneous work on two identical or different detectors, i.e. EC/EC, Flame/EC, Flame/Thermionic etc. Two identical or different columns i.e. Dow 11/QF-1, glass or metal columns, linear temperature programmer (205-1) or isothermal oven controller (205-2). The most advanced instrument available for pesticide analysis.

b) Model 610 – A single column instrument with EC detector. The EC detector may be interchanged for a Flame or Thermionic detector. Ballistic or Isothermal temperature programs, addition of the model 328 – Isothermal Temperature Controller is recommended for analysis requiring a very accurate column oven temperature control.

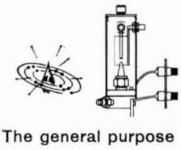
c) Model 682 – The Aerograph Pestilyzer for routine analysis. A low cost single column gas chromatograph with EC detector, built-in detector voltage controller and precision pyrometer.

2. The Detectors

The selective Pesticide detectors:



a) Electron Capture detector
A detector of cylindric design requiring no pulsed voltage.



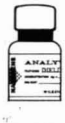
b) Thermionic Detector
A detector with specific sensitivity to phosphorous containing compounds.

The general purpose

c) Hydrogen Flame Ionization detector

Important: All detectors have the base in common and use the same electrometer and are therefore interchangeable without any modifications or extra accessories.

3. Pesticide Standards



Wilkens is the exclusive distributor of the Nanogens standards – pesticide standard solutions in nanogens per microliter. 249 different pesticides in alphabetical order from Alanap to Ziram can be delivered in concentrations from 0.1–1000 ppm in powers of ten.

4. The Pesticide Handbook

The Wilkens 125 page Pesticide Residue Analysis Handbook is the most complete and up-to-date handbook available on pesticide analysic by gas chromatography using the EC detector.
This book treats all phases of the analysis from Sample Preparation to Trouble-shooting.

Wilkens

Instrument + Research (U.K.) Ltd.

For further information contact:

Wythenshawe Civic Center
Manchester, England

ap

THE SOCIETY FOR ANALYTICAL CHEMISTRY

FOUNDED 1874. INCORPORATED 1907.

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

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

The Entrance Fee for Ordinary Members is £1 1s. and the Annual Subscription is £4. Junior Members are not required to pay an Entrance Fee and their Annual Subscription is £1 1s. No entrance Fee is payable by a Junior Member on transferring to Ordinary Membership. The entrance Fee (where applicable) and first year's Subscription must accompany the completed Form of Application for Membership. Subscriptions are due on January 1st of each year.

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

All members of the Society have the privilege of using the Library of The Chemical Society. Full details about this facility can be obtained from the Librarian, The Chemical Society, Burlington House, Piccadilly, London, W.1.

The Analyst, the Journal of the Society, which contains original and review papers, information about analytical methods and reviews of books, and has a world-wide distribution, and *Proceedings of the Society for Analytical Chemistry*, in which are reported the day-to-day activities of the Society, are issued monthly to all Ordinary and Junior Members. In addition, all Ordinary Members receive *Analytical Abstracts*, providing a reliable index to the analytical literature of the world.

Forms of application for membership of the Society may be obtained from the Secretary, The Society for Analytical Chemistry, 14 Belgrave Square, London, S.W.1.

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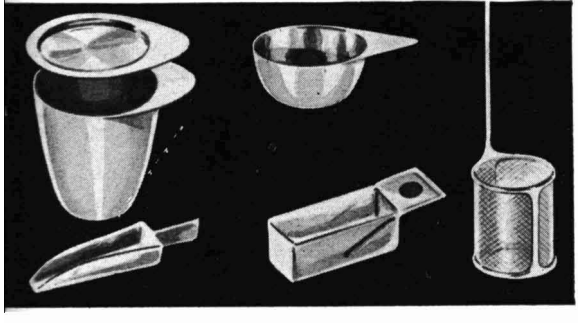
THE North of England, Scottish, Western and Midlands Sections were formed to promote the aims and interests of the Society among the members in those areas.

Specialised Groups within the Society are concerned with the study of various branches of analytical chemistry of specialised or topical interest. Groups dealing with such topics as Microchemical Methods, Biological Methods, Thin-Layer Chromatography, Atomic-Absorption Spectroscopy, Thermal Analysis, Automatic Methods and a Special Techniques Group, covering very new developments and specialised physical methods, are at present active—and further Groups are formed from time to time as the need arises.

Non-members of the Society may be non-voting members of a Group.

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

MACRO or micro



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