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The Quantitative Microanalysis of Carbonyl Compounds

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Several published methods for preparing 2,4-dinitrophenylhydrazones and determining them colorimetrically have been examined, and a practical system of analysis has been devised from them. The key step is the use of a 2,4-dinitrophenylhydrazine - 66 per cent. phosphoric acid column eluted with benzene, as well as light petroleum.

The results of an analysis in the difficult case of a butter fat are described.

In connection with flavour and other work a procedure was required for the quantitative microanalysis of complex mixtures of carbonyl compounds. Winter *et al.*[1] found that at least twelve carbonyl compounds were removed from butter by steam distillation. The amounts involved ranged from 18·8 p.p.m. for acetoin to less than 0·01 p.p.m. for hexanal, nonanal and nonan-2-one. Low concentrations[2,3] of monocarbonyl compounds have been implicated in the reversion flavours of edible oils[4,5] and in the fishy flavour of dairy products.[6,7,8]

Although other suggestions[9,10] have been made, the colorimetric determination of 2,4-dinitrophenylhydrazones was selected as being the most generally useful technique.

A great deal of work has been published on the chromatographic separation and spectrophotometric examination of 2,4-dinitrophenylhydrazones, but the strictly quantitative preparation of the derivatives with 2,4-dinitrophenylhydrazine has received relatively little attention. In a comparative study, Begemann and de Jong[11] showed that the reaction of a dilute ethereal solution of a carbonyl compound with 2,4-dinitrophenylhydrazine in mineral acid gave an incomplete conversion, the yield in homogeneous solution[12] being worse than that obtained when the solutions were partially[13] or almost completely immiscible.[14] Water was present in all three solutions, and unless sufficient carbonyl compound was present for the derivative to separate, the equilibrium mixture obtained presumably fell far short of complete conversion into the 2,4-dinitrophenylhydrazone.

Henick *et al.*[15,16] carried out the reaction under essentially anhydrous conditions in benzene with trichloroacetic acid as the catalyst. There seems to be no reason why this reaction should not go to completion; a Dean and Stark apparatus can be used to remove water if necessary.[17] However, the blank values were high (optical density in benzene - ethanolic potassium hydroxide, E = 0·35) and it was not clear whether this was due to excess reagent or to an artifact consequent upon the use of trichloroacetic acid. In addition, the presence of dicarbonyl compounds causes a large error in the calculation of carbonyl content, and therefore the procedure is largely useless for the empirical examination of oils for which it was originally proposed.[18]

Pool and Klose[19] claimed a quantitative reaction of aldehydes in benzene with 2,4-dinitrophenylhydrazine that had been adsorbed on to an alumina column, but their extinction coefficient (ϵ = 19,200 at 435 mμ in benzene - ethanolic potassium hydroxide at zero time) appears to include a correction factor; the now accepted value[18] (ϵ = 20,930 at 430 mμ after 10 minutes) is significantly higher. Begemann and de Jong[11] obtained a 90 per cent. conversion of heptanal into its 2,4-dinitrophenylhydrazone (assuming ϵ = 22,500 at 358 mμ in chloroform) but only 20 per cent. for nonan-2-one on this column. Keith and Day[18] obtained 75 per cent. yields with alkanals, 65 per cent. with alk-2-enals and 60 per cent. with alka-2,4-dienals.

The loading of this column is very low. Pool and Klose[19] recommended 0·05 to 0·50 μmole and Keith and Day[18] up to 1 μmole of carbonyl compounds for a 10-g column, although the amount of reagent present is about 25 μmoles. In addition, decomposition may occur; for example, Lea and Jackson[20] found that hydroperoxides give some carbonyl compounds under these conditions.

Forss *et al.*[21] appear to have been the first to prepare 2,4-dinitrophenylhydrazones by shaking 2,4-dinitrophenylhydrazine in 2 N hydrochloric acid with solutions of carbonyl compounds in light petroleum. Under these conditions the derivatives are obtained free from excess reagent in a surprisingly short time. Begemann and de Jong[11] found that the reaction with undecan-2-one was frequently complete in 4 hours. However, the results were more reproducible and the reaction still more rapid if the petroleum solution was percolated through a column of the aqueous phase supported on Celite. In this way, 0·3 to 5 μmoles of heptanal, heptan-2-one, non-2-enal, undecan-2-one, tridecanal and pentadecan-2-one gave almost complete conversion into their respective 2,4-dinitrophenylhydrazones in 1 hour with 2,4-dinitrophenylhydrazine (about 250 μmoles) in 7·5 ml of 2 N hydrochloric acid on 15 g of Celite.

Subsequently, Schwartz and Parks[22] suggested that the solution of 2,4-dinitrophenylhydrazine in 2 N hydrochloric acid could be replaced advantageously by 2,4-dinitrophenylhydrazine in 66 per cent. phosphoric acid. This column contained a relatively large amount of reagent (about 100 μmoles per g of stationary phase), from which impurities were removed by washing with benzene. A further advantage, particularly for the analysis of oils, is that the column does not decompose hydroperoxides to carbonyl compounds,[23] whereas the 2 N hydrochloric acid column[24] does (80 to 88 per cent.), either because hydrochloric acid is stronger than phosphoric acid, or because of the catalytic effect of the chloride ion.[25,26,27]

All workers in this field have stressed the importance of the ubiquitous nature of carbonyl compounds and the necessity for the careful purification of the solvents used.

Experimental

2,4-dinitrophenylhydrazine—

The commercially available material (*e.g.*, 2,4-dinitrophenylhydrazine AnalaR, British Drug Houses Ltd., m.p. 196° to 199° C) was suitable for most purposes, any impurities being removed from its solution in aqueous mineral acid by filtration and washing with carbon tetrachloride[28] or a carbonyl-free petroleum solvent. However, this method of purification was not possible for solutions in ethanol or benzene, and the reagent was purified as described under Method.

An alternative procedure, extraction with light petroleum in a Soxhlet apparatus,[11] gave material which still imparted a colour to the organic phase when distributed between 2 N sulphuric acid and purified light petroleum. Little purification could be effected by simple re-crystallisation from methanol.[17]

Cyclohexane and light petroleum—

Useful indications of the carbonyl contents of these solvents were obtained by shaking samples with a saturated solution of 2,4-dinitrophenylhydrazine in 2 N hydrochloric acid overnight, in a Griffin wrist-action flask shaker (Messrs. Griffin and George Ltd.). The resulting concentration of 2,4-dinitrophenylhydrazones in the organic phase was obtained by dividing the value of the optical density at its maximum (about 340 mμ) by the value of $\epsilon = 23{,}700$. It should be noted that not only is the adsorption maximum 20 mμ lower in hexane than it is in chloroform,[29] ethanol or benzene, but the extinction coefficient is 5 per cent. higher. We found that the lowest value for ϵ occurred in a 4 to 1 v/v mixture of benzene and ethanol. The heavily contaminated samples of cyclohexane and light petroleum gave precipitates which were taken up in benzene and determined after dilution with ethanolic potassium hydroxide.[17] The results obtained are shown in Table I.

Table I

Carbonyl compounds in some laboratory solvents

Solvent	Carbonyl concentration, μmolar
Hexane	3000
Hexane (spectroscopic grade)	14
Light petroleum (b.p. 60° to 80° C)	320
Light petroleum (b.p. 40° to 60° C)	330
Light petroleum (aromatic free)	17
Cyclohexane	37
Cyclohexane (spectroscopic grade)	10

Thus a good grade of cyclohexane or light petroleum was adequate for most purposes. By using the procedure described by Van der Ven and de Jonge[30] the carbonyl content of aromatic-free light petroleum was still further reduced to 2 μmolar.

BENZENE—

Carbonyl compounds in benzene were found to react incompletely when shaken overnight with a solution of 2,4-dinitrophenylhydrazine in mineral acid. They were therefore determined by adding 1 mg of purified 2,4-dinitrophenylhydrazine and 10 mg of trichloroacetic acid to a 10-ml sample, leaving to stand overnight and measuring the adsorption at 430 mμ after dilution with 1·8 per cent. ethanolic potassium hydroxide. The results of an investigation into the accuracy of this procedure are described in the next section.

It was found that consistent results could best be obtained by adding the benzene solution to an equal volume of freshly prepared 1·8 per cent. ethanolic potassium hydroxide (obtained by shaking 5 potassium hydroxide pellets with 25 ml of re-rectified ethanol at room temperature, and filtering) and reading the optical density with a self-recording spectrophotometer (Unicam SP800) after exactly 10 minutes. At low carbonyl concentrations, absorption due to unchanged reagent was too large to be ignored[31]; so measurements were also made at 360 mμ, and calculations performed after determining the appropriate extinction coefficients—

	$\epsilon_{360m\mu}$	$\epsilon_{430m\mu}$
2,4-Dinitrophenylhydrazine	1860	1180
2,4-Dinitrophenylhydrazones.. ..	3400	22,300

The last figure is significantly higher than that used by Keith and Day,[19] but is consistent with the values obtained by Jones *et al.*[32] if it is assumed that solvents, unlike oils, are liable to contain more ketones than aldehydes. The formulae derived from the above extinction coefficients were as follows—

$$\text{2,4-Dinitrophenylhydrazine} = (594\ E_{360} - 90\ E_{430})\ \mu\text{molar}$$
$$\text{2,4-Dinitrophenylhydrazones} = (49{\cdot}7\ E_{430} - 31{\cdot}4\ E_{360})\ \mu\text{molar}$$

The carbonyl content of sulphur-free benzene proved to be variable (10 to 500 μmolar), even with bottles labelled with the same batch number. One or two distillations from 2,4-dinitrophenylhydrazine - trichloroacetic acid[17] reduced this value to less than 4 μmolar. Details of this purification are given under Method.

REACTION OF CARBONYL COMPOUNDS WITH 2,4-DINITROPHENYLHYDRAZINE AND TRICHLOROACETIC ACID IN BENZENE—

An attempt was made to check the Henick procedure[15,16] by following the reaction with a model compound at room temperature. Purified 2,4-dinitrophenylhydrazine (2·6 mg, 12·9 μmoles), in 10 ml of carbonyl-free benzene, was added to nonan-2-one (101 μg, 0·72 μmole) and trichloroacetic acid (186 mg, 1·14 μmoles) in 15 ml of the same solvent. At intervals a sample was withdrawn, diluted with an equal volume of 1·8 per cent. ethanolic potassium hydroxide, and examined after 10 minutes in the self-recording spectrophotometer (time of scan was 2 minutes) against a blank of trichloroacetic acid in benzene - ethanolic potassium hydroxide. A control was run under the same conditions, the nonan-2-one being omitted. The net apparent quantities of saturated and unsaturated carbonyl 2,4-dinitrophenylhydrazones obtained are shown in Table II.

TABLE II

APPARENT EXTENT OF REACTION BETWEEN NONAN-2-ONE AND 2,4-DINITROPHENYLHYDRAZINE IN BENZENE IN THE PRESENCE OF TRICHLOROACETIC ACID

Time of reaction at 23° C	5 minutes	30 minutes	23 hours
Saturated carbonyls, μmoles	0·79	0·79	1·03
Saturated carbonyls, per cent.	110	110	143
Unsaturated carbonyls, μmoles.. ..	−0·05	−0·07	−0·24
Unsaturated carbonyls, per cent. ..	−7	−10	−33

The negative values are a consequence of the magnitude of the readings given by the control. In another experiment, purified 2,4-dinitrophenylhydrazine (2·5 mg, 12·5 μmoles) in 10 ml of carbonyl-free benzene was added to trichloroacetic acid (146 mg, 900 μmoles) in the same solvent. Samples were withdrawn and examined as before, and the optical densities obtained are shown in Table III.

TABLE III

REACTION BETWEEN 2,4-DINITROPHENYLHYDRAZINE AND TRICHLOROACETIC ACID IN BENZENE

Time of reaction at 23° C	E_{360}	E_{430}	E_{460}
5 minutes	0·63	0·30	0·18
30 minutes	0·61	0·30	0·18
6 hours	0·63	0·32	0·21
24 hours	0·68	0·39	0·26
47 hours	0·70	0·43	0·30
120 hours	0·75	0·51	0·37

The explanation of the high readings of the control appears to be that 2,4-dinitrophenylhydrazine reacts with trichloroacetic acid to give a pigment which is green in alkaline solution. It is possible to allow for this effect by assuming that the pigment has similar absorption (at 360 and 430 mμ) to 2,4-dinitrophenylhydrazine and by using the formulae given in the preceding section. This method for computing the results was applied to the original experiment and the results are set out below—

Time of reaction at 23° C		5 minutes	30 minutes	23 hours
Carbonyl 2,4-dinitrophenylhydrazones, μmoles	..	0·67	0·69	0·73
Carbonyl 2,4-dinitrophenylhydrazones, per cent.	..	93	96	101

Applied in this way the procedure gives reasonable results with much larger concentrations of carbonyl compounds. By using 2,4-dinitrophenylhydrazine (2·5 mg, 12·5 μmoles) and nonan-2-one (1·01 mg, 7·2 μmoles) under conditions similar to those described in the foregoing section, the results are shown below—

Time of reaction at 23° C		5 minutes	30 minutes	24 hours
Carbonyl 2,4-dinitrophenylhydrazones, μmoles	..	4·7	6·9	7·1
Carbonyl 2,4-dinitrophenylhydrazones, per cent.	..	65	96	99

For analyses involving subsequent fractionation of the derivatives by chromatography, however, the presence of artifacts from the reagents was clearly undesirable. For example, it was observed that a solution of 2,4-dinitrophenylhydrazine in 2 N aqueous trichloroacetic acid deposited a crystalline precipitate on standing at room temperature, and after 2 weeks the supernatant liquid was almost colourless.

REACTION BETWEEN 2,4-DINITROPHENYLHYDRAZINE IN AQUEOUS ACID AND NONAN-2-ONE IN VARIOUS SOLVENTS—

(*a*) *Light petroleum*—By using the wrist-action shaker under standard conditions it was possible to follow reactions in systems involving two immiscible liquids. The reaction between nonan-2-one (210 μg, 1·46 μmoles) in 5 ml of light petroleum and 2,4-dinitrophenylhydrazine (about 5 μmoles) in 5 ml of 2 N sulphuric acid proceeded as shown in Table IV.

TABLE IV

REACTION BETWEEN NONAN-2-ONE IN LIGHT PETROLEUM AND 2,4-DINITROPHENYLHYDRAZINE IN 2 N SULPHURIC ACID

Time, in minutes	Percentage conversion	Time, in hours	Percentage conversion
0	0	1	39
5	2·8	2	58
10	5·0	5	85
30	24·7	16	102

Thus the reaction was essentially (95 per cent.) complete in 6 hours, as was also the case with the carbonyl compounds studied by Forss *et al.*[21] and by Begemann and de Jonge[11] under similar conditions.

(*b*) *Hexane - benzene*—The reaction between nonan-2-one (470 μg, 3·3 μmoles) in 10 ml of hexane - benzene and 2,4-dinitrophenylhydrazine (about 4 μmoles) in 10 ml of 5 per cent. v/v sulphuric acid was studied in a similar manner with the ethanolic potassium hydroxide procedure to determine 2,4-dinitrophenylhydrazones in the presence of 2,4-dinitrophenylhydrazine. The results, set out below, clearly show the retarding effect of benzene on the reaction—

Benzene, per cent.	..	0	20	50	90
Percentage conversion in 16 hours	..	33	10·2	4·1	2·5
Percentage conversion in 65 hours	..	56	28	14·6	9·1

REACTION BETWEEN 2,4-DINITROPHENYLHYDRAZINE IN AQUEOUS ACID AND VARIOUS CARBONYLS IN LIGHT PETROLEUM—

Shaking experiments—A saturated solution of 2,4-dinitrophenylhydrazine (about 5 μmoles) in 5 ml of 2 N sulphuric acid was shaken with the carbonyl compound (1 to 3 μmoles) in 5 ml of light petroleum for a suitable time. A sample was withdrawn from the organic phase and examined in the spectrophotometer. The concentrations of the 2,4-dinitrophenylhydrazones of nonan-2-one, acetophenone and benzophenone were calculated, assuming $\epsilon = 22{,}600$, 24,100 and 28,300, respectively.[32]

With benzaldehyde, however, a precipitate separated at the interface; benzene was therefore added and the concentration of 2,4-dinitrophenylhydrazone was obtained by the ethanolic potassium hydroxide procedure, assuming $\epsilon = 33{,}300$.[32]

The results are set out in Table V. The relative rates of reaction were benzaldehyde > nonan-2-one > acetophenone > benzophenone. These results agree with those of Belcher and Fleet,[33] who found that benzophenone reacted very slowly with hydroxylamine in homogeneous solution.

TABLE V

REACTION BETWEEN 2,4-DINITROPHENYLHYDRAZINE IN 2 N SULPHURIC ACID AND VARIOUS CARBONYL COMPOUNDS IN LIGHT PETROLEUM

Carbonyl compound	Concentration (time = 0), μmolar	Time, in hours	Percentage conversion
Benzaldehyde	570	2	94
Nonan-2-one	197	2	61
Acetophenone	490	2	48
Acetophenone	490	16	63
Acetophenone	490	67	83
Benzophenone	268	16	9·9
Benzophenone	268	67	20

THE 2,4-DINITROPHENYLHYDRAZINE - 66 PER CENT. PHOSPHORIC ACID COLUMN—

This column was made up as described[22] and gave a quantitative conversion of benzophenone (3·9 μmoles and 0·39 μmoles) in 10 ml of cyclohexane into its 2,4-dinitrophenylhydrazone at a flow-rate of 22 ml per hour. Higher rates of flow gave lower yields, only 19 per cent. conversion being obtained at 97 ml per hour.

A second column, 1·0 × 18·5 cm, was made with 10·0 g of stationary phase, *i.e.*, at least 900 μmoles of 2,4-dinitrophenylhydrazine after washing. This column gave 73 per cent. conversion of 3·9 μmoles of benzophenone into its 2,4-dinitrophenylhydrazone at 8 ml per hour. Nonan-2-one (6·0 μmoles) was quantitatively converted at 20 ml per hour, which is the flow-rate laid down by Begemann and de Jonge[11] with a column of similar size containing 2,4-dinitrophenylhydrazine - 2N hydrochloric acid.

The reaction is reversible. A solution of butanone 2,4-dinitrophenylhydrazone (8·25 μmoles) in cyclohexane was decolourised completely by passage over a 1·0 × 9·5-cm column of Celite impregnated with 66 per cent. phosphoric acid. Octanol 2,4-dinitrophenylhydrazone (6·9 μmoles) was 33 per cent. hydrolysed at a flow-rate of 7 ml per hour, and decan-2-one 2,4-dinitrophenylhydrazone (6·0 μmoles) was 26 per cent. hydrolysed at 8 ml per hour.

The foregoing result suggested that, although a short chain carbonyl compound would react much more rapidly than benzophenone it might not react completely. Indeed, when a solution of 41 μmoles of acetone was percolated over the 2,4-dinitrophenylhydrazine column, the effluent had no more colour than a blank treated in the same way.

It seems probable, therefore, that the values given by Schwartz and Parkes[22] for the aliphatic monocarbonyl contents of various organic solvents do not include acetone, and it is not possible to say from the details given in their paper whether or not acetone is removed from solvents which have been percolated over a 2,4-dinitrophenylhydrazine - 66 per cent. phosphoric acid column.

THE 2,4-DINITROPHENYLHYDRAZINE - 2 N HYDROCHLORIC ACID COLUMN—

Gaddis *et al.*[34] prepared 2,4-dinitrophenylhydrazones in 2,4-dinitrophenylhydrazine - 2 N hydrochloric acid, followed by extraction with carbon tetrachloride and with benzene; both extracts were then washed with 2 N hydrochloric acid and with water. It appears from a

subsequent paper by Gaddis and Ellis[35] that the 2,4-dinitrophenylhydrazones of formaldehyde, acetaldehyde, acetone and butanone are extracted by the benzene but not by the carbon tetrachloride, and these authors commented that the quantitative aspects of the procedure required further study.

Begemann and de Jong[11] purified light petroleum, first by the method described by Van der Ven and de Jonge,[30] and then by passage over a 2,4-dinitrophenylhydrazine - 2 N hydrochloric acid column, followed by distillation. Begemann and de Jong stated that a very small amount of acetone was still present. We have found that this column gives a 95 per cent. yield of 2,4-dinitrophenylhydrazone with acetone, and an 84 per cent. yield with diacetone alcohol.

The column procedure is thus a considerable improvement over the simple extraction procedures, both for slow reactions and for reactions giving unfavourable equilibria. The use of benzene as the mobile phase would be unfavourable for slow reactions and would tend to strip the reagent from the column. However, its use by Gaddis and Ellis[34] suggested how the 2,4-dinitrophenylhydrazine - 66 per cent. phosphoric acid column, which has several advantages over the 2,4-dinitrophenylhydrazine - 2 N hydrochloric acid column, might be modified to give an improved yield with acetone.

METHOD

REAGENTS—

Cyclohexane and light petroleum—Cyclohexane for ultraviolet spectroscopy (as supplied by British Drug Houses Ltd. or Hopkin and Williams Ltd.) is used without further purification.

For more exact work, light petroleum (b.p. 40° to 60° C, aromatic free, Carless, Capel and Leonard Ltd.) is rendered carbonyl free in the following manner. 350 ml of fuming nitric acid, density 1·51, and 350 ml of sulphuric acid, density 1·84, are added, with stirring, to 3·5 litres of light petroleum, contained in a 5-litre flask fitted with a reflux condenser. The mixture becomes warm, and stirring is continued overnight. The mixed acid is removed by aspiration, and the organic phase is washed[22] twice with 700 ml of water, nine times with 700 ml of 20 per cent. potassium hydroxide and twice with 700 ml of water. The washed solvent is distilled over 350 ml of refined coconut oil (Van Den Berghs and Jurgens Ltd., Purfleet), and percolated firstly through a 3·5 × 22-cm column of 100 g of alumina (P. Spence, grade H) and then through a second column of alumina activated by heating at 800° C for 4 hours (a personal communication from H. J. Duin, H. W. A. E. Groeneweld and H. Van der Wel). In order to obtain a stable product it was found to be essential to remove both the carbonyl compounds and their precursors in the foregoing manner and to store in brown bottles in the dark.

Benzene—A mixture of 2·5 litres of thiophene-free benzene (Carless, Capel and Leonard Ltd.), 12·5 g of 2,4-dinitrophenylhydrazine and 2·5 g of trichloroacetic acid are refluxed for 4 hours under a Dean and Stark trap. The mixture is then distilled with constant stirring and use of a double splash head. If the mixture is not stirred, the reagent bakes on to the sides of the flask and is carried over into the receiver to give a coloured distillate. The distillation is repeated as necessary.

2,4-*Dinitrophenylhydrazine*—Commercial 2,4-dinitrophenylhydrazine and 100 ml per g of N hydrochloric acid are refluxed for 30 minutes, and the solution is then filtered. The filtrate is made alkaline with ammonia and cooled. The purified 2,4-dinitrophenylhydrazine is collected and re-crystallised from 200 ml per g of methanol to give leaflets of m.p. 195° C.

98 *per cent. neutral alumina*—A 2-ml portion of hydrochloric acid of density 1·16, and 98 g of alumina (P. Spence, grade H) are shaken together for 30 minutes and then allowed to stand overnight in a stoppered flask. The alumina should be used within 1 week.

1·8 *per cent. ethanolic potassium hydroxide*—Five potassium hydroxide pellets are dissolved in 25 ml of re-rectified ethanol by shaking mechanically in an Erlenmeyer flask. The solution is filtered through a Whatman No. 541 filter-paper and used within 2 hours.

PREPARATION OF 2,4-DINITROPHENYLHYDRAZONES—

Impregnate 10·0 g of analytical-grade Celite (Johns - Manville) with a solution of 2,4-dinitrophenylhydrazine (500 mg, 2·5 μmoles) in 6 ml of 88 to 93 per cent. orthophosphoric acid of density 1·75, diluted with 4 ml of water. Transfer this stationary phase to a column of 2·1-cm internal diameter, fitted with a B24 socket and cone and a sintered-glass plate,

containing cyclohexane and tamp it down to a height of 9·5 cm.[22] Wash with 50 ml of benzene to remove some of the reagent together with residual impurities. However, at least 2·2 μmoles of 2,4-dinitrophenylhydrazine will remain.

Fit this column to a swan-necked column[36] containing 35 g of 98 per cent. neutral alumina. Apply the sample (containing between 1 and 100 μmoles of carbonyl compounds) in 5 to 10 ml per g of cyclohexane to the celite column and elute with the same solvent at 20 ml per hour (total volume 175 ml), the 2,4-dinitrophenylhydrazones being retained on the columns. Up to 10 g of triglycerides and other non-polar materials may be present in the sample and are recovered in the eluate.

Elute both columns with 175 ml of benzene and examine an aliquot in the spectrophotometer, either directly or after adding to an equal volume of 1·8 per cent. ethanolic potassium hydroxide and leaving to stand for exactly 10 minutes. Approximate values for the wavelength maxima and extinction coefficients under neutral and basic conditions[19,35] (A. M. Parson, unpublished results) are given in Table VI.

TABLE VI

WAVELENGTH MAXIMA AND EXTINCTION COEFFICIENTS OF 2,4-DINITROPHENYLHYDRAZONES

2,4-Dinitrophenylhydrazone	$\lambda_{max.}^{Benzene}$, mμ	$\epsilon_{max.}$	$\lambda_{max.}^{KOH\text{-}C_2H_5OH}$, m$\mu$	$\epsilon_{max.}$
Alkan-2-ones	361	21,500	431	22,200
Alkanals	354	21,000	430	20,900
Alk-2-enals	372	27,900	460	30,000
Alka-2,4-dienals	388	36,000	480	41,000

When the various classes of carbonyl compounds are present together, their total concentrations may be obtained[29] with $\lambda_{430}^{KOH\text{-}C_2H_5OH} = 21{,}000$, or the separate concentrations of alkanals, alk-2-enals and alka-2,4-dienals may be obtained by measuring the absorptions at 430, 460 and 480 mμ and applying the equations derived by Keith and Day.[19] Unfortunately, the absorption maxima of alkan-2-one 2,4-dinitrophenylhydrazones are too close to those of alkanal 2,4-dinitrophenylhydrazones to permit separate determination by this means. However, by taking additional readings after 2 hours, further information may be obtained on this point, because only acetone and alkanal 2,4-dinitrophenylhydrazones fade appreciably under these conditions.[32,35]

For detailed analysis, the remainder of the benzene eluate may be fractionated into classes by adsorption chromatography on dry paper,[37] magnesium oxide,[38,39] or 92 per cent. alkaline alumina.[40] Separation according to chain length may then be achieved by partition chromatography on paper,[41,42] thin layers of Kieselguhr[43,44] or columns.[45,46,47] Numerous thin-layer adsorption systems have also been described.[43,44,48,49]

It is necessary to repeat the whole procedure, with omission of the sample, in order to allow for residual carbonyl compounds in the solvents and for artifacts produced on the columns. It is found, for example, that two pigments ($\lambda_{max.}^{benzene} < 320$ mμ) are obtained which are less polar than ketone 2,4-dinitrophenylhydrazones on adsorption chromatography. Winter *et al.* have noted several such artifacts[50] and have pointed out[51] that 2,4-dinitroaniline could arise from the reaction between acetoin and 2,4-dinitrophenylhydrazine.

For polar compounds when the 2,4-dinitrophenylhydrazine column is eluted with benzene, the 2,4-dinitrophenylhydrazones of polar carbonyl compounds are transferred to the 98 per cent. neutral alumina column, together with some unchanged reagent. The alumina column may subsequently be eluted with benzene containing between 1 and 10 per cent of ethanol and aliquots of the solutions examined in the spectrophotometer as before. The absorption maxima for acetoin[51] and for the two 2,4-dinitrophenylhydrazones of diacetyl[52] are shown in Table VII.

TABLE VII

WAVELENGTH MAXIMA AND EXTINCTION COEFFICIENTS OF DINITROPHENYLHYDRAZONES OF ACETOIN AND DIACETYL

2,4-Dinitrophenylhydrazone	$\lambda_{max.}^{Chloroform}$, m$\mu$	$\epsilon_{max.}$	$\lambda_{max.}^{KOH\text{-}C_2H_5OH}$, m$\mu$	$\epsilon_{max.}$
Acetoin	357	33,000	433	—
Diacetyl-mono	351	29,100	501	38,000
Diacetyl-bis	393	47,000	556	54,000
	435 (shoulder)	40,000	—	—

The reagent 2,4-dinitrophenylhydrazine has $\lambda_{max.}^{KOH - C_2H_5OH} = 360$ mμ, $\epsilon = 1{,}860$. There are, however, more satisfactory methods for determining acetoin and diacetyl which do not involve preparation of 2,4-dinitrophenylhydrazones.[53,54,55]

Results

Application of the method to model compounds gave good yields of products; even acetone gave 84 per cent.

Analysis of butter fat

The method also worked well with the molecular distillates of 200 g of butter fat obtained with a 2-inch wiped wall molecular still (Edwards High Vacuum Ltd.), 10–20 μ (b.p. 160° C), fitted with liquid nitrogen cooled traps. The values (Table VIII) agreed closely with those obtained after subsequent chromatography.

A 10-g sample of New Zealand butter fat was also examined directly, but in this case we were unable to separate the classes by chromatography. Schwartz *et al.* claim[56] that this separation can be performed after a preliminary fraction on a partition column but, as can be seen from the last column in Table VIII, the quantity of involatile carbonyl compounds in this fat is so large that it would place great demands on any technique to require it to carry out a separation at this stage.

Table VIII

Carbonyl content of butter fat and butter fat volatiles

	New Zealand volatiles, μmoles per kg	Danish volatiles, μmoles per kg	New Zealand butter fat, μmoles per kg
Alkan-2-ones and alkanals ..	2·2	8·1	730
Alk-2-enals	1·0	1·0	130
Alka-2,4-dienals	0·4	0·6	30

More recently, Schwartz and co-workers[57] have shown that keto-glycerides can be removed from butter fat by adsorption chromatography, and that these glycerides amount to no less than 0·045 per cent. by weight (550 μmoles per kg as keto-tripalmitin). We have removed polar material from 20 g of New Zealand butter fat in this way, and have converted the remaining carbonyl compounds to 2,4-dinitrophenylhydrazones by the method described above. The carbonyl compounds still amounted to over 120 μmoles per kg and, judging by their behaviour on subsequent chromatography, consisted largely of keto-glyceride 2,4-dinitrophenylhydrazones.

The polar fraction was also examined in the same way, except that a strongly acid (2 N perchloric acid) 2,4-dinitrophenylhydrazine column was used in order to decompose acid labile carbonyl precursors.[58] The 2,4-dinitrophenylhydrazones gave two bands on magnesium oxide,[38,39] in similar positions to those given by pentanal 2,4-dinitrophenylhydrazone and by dec-2-enal 2,4-dinitrophenylhydrazone. The spectral properties given in Table IX, however, indicate an alkanone and an alkanal fraction ($\lambda_{max.} = 430$ mμ in each instance, and the latter fading rapidly) amounting to 165 μmoles per kg and 36 μmoles per kg, respectively.

Table IX

Wavelength maxima and fading of dinitrophenylhydrazones of polar carbonyl compounds from butter fat

Fraction	$\lambda_{max.,\ m\mu}^{Benzene}$	$\lambda_{max.,\ m\mu}^{KOH - C_2H_5OH}$	Fading, per cent.
1	362	430	2·9
		540	3·4
2	360	430	15
		535	24

It therefore appears that, despite the use of a strongly acid reaction column, the carbonyl compounds still contained polar groups and were probably keto-glycerides and aldehydo-glycerides, respectively.

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Calibration of a Fisher Air-permeability Apparatus for Determining Specific Surface

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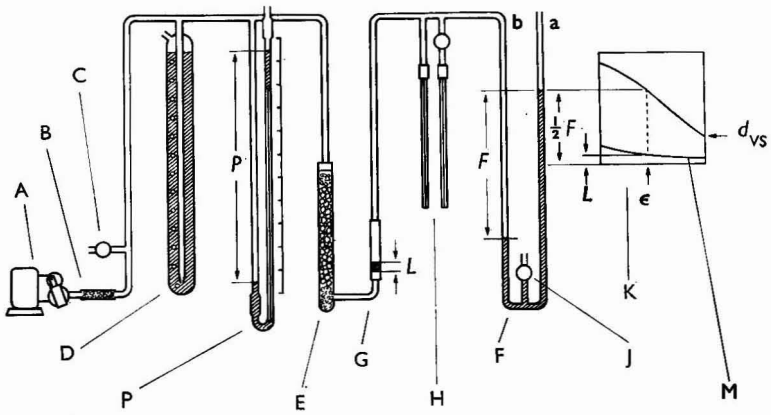
Calibrating the Fisher sub-sieve sizer at a single particle-size level does not ensure accuracy at other levels unless several variables are controlled. Instrument modifications that give better control of the several variables, and a calibration method that ensures accurate air-flow measurement without depending on a particle-size standard, are described. The modified instrument has good precision over the range 2 to 40 μ. A method for extending this range is given.

The air-permeability apparatus of Gooden and Smith,[1] available commercially as the Fisher sub-sieve sizer (Fisher Scientific Company, Pittsburg, Pa., U.S.A.; Kek Ltd., Ancoats, Manchester 12), is widely recognised as a convenient means of determining specific surface. Its advantages include an automatic calculator and the ability to give readings on a single sample compressed to successively lower porosities. We found considerable variation in the results for identical samples tested on different instruments. Some of the variation arises from fundamental errors in the usual method of calibrating and using the Fisher instrument. An alternative calibration method described by Dubrow[2] is indirect, and takes no account of some important factors.

The Fisher instrument, like other types of air-permeability apparatus[3,4,5,6] is a means of measuring flow-rate of air through a powder sample. In this paper it is shown how to modify and calibrate the instrument so as to ensure accurate flow-rate measurement.

The instrument (Fig. 1) consists of an air-supply section, a sample tube and a flow-meter. The water manometer, P, is not part of the standard instrument, but was added for the investigation and is now retained by us because it increases the instrument's accuracy and precision. The flow-meter consists of a capillary resistance, a water manometer, F, and a chart. A second flow-meter resistance can be opened to double the range.



A = Pump
B = Air filter
C = Pressure control
D = Standpipe
P = Water manometer
E = Drying tube
G = Sample tube
H = Flow-meter resistances
F = Flow-meter manometer
J = Zero control
K = Chart
M = Sample-height line

Fig. 1. Modified Fisher sub-sieve sizer

When the sample is compressed, a pointer indicates the sample height, L, and the corresponding bed porosity, ϵ, on the chart. After the sample tube is inserted in the air line, the water in one arm of the F manometer (a) rises to a level ($\frac{1}{2}F$) that depends on the flow-rate. This level is read directly as average particle diameter (d_{vs}) on the chart, which is mounted behind the manometer arm and on which there is a series of curves relating $\frac{1}{2}F$ and ϵ to d_{vs}. A rack-and-pinion device for compressing the sample and indicating L and $\frac{1}{2}F$ on the chart is not shown in Fig. 1.

The instrument equations

The Fisher instrument is based on the equation[1]—

$$d_{vs} = \frac{60{,}000}{14} \cdot \sqrt{\frac{\eta C \rho L^2 M^2 F}{(AL\rho - M)^3 (P - F)}} \quad .. \quad .. \quad .. \quad (1)$$

where d_{vs} is the volume-surface mean diameter in μ (specific surface in sq. cm per cc $= 60{,}000/d_{vs}$), η is the viscosity of air in poises, C is the conductance of the flow-meter resistance in cc per second unit pressure per drop, ρ is the sample density, L is the sample height in cm, M is the sample weight in g, A is the cross-sectional area of the sample tube in sq. cm, P is the air pressure entering the sample and F is the pressure drop across the flow-meter resistance, all pressures being expressed as centimetres of water.

The sample weight is standardised at a value numerically equal to ρ. Therefore, equation (1) can be simplified to the form—

$$d_{vs} = \frac{cL}{(AL - 1)^{3/2}} \cdot \sqrt{\frac{F}{P - F}} \quad .. \quad .. \quad .. \quad .. \quad (2)$$

where c is an instrument constant that combines factors from equation (1)—

$$c = \frac{60{,}000}{14} \cdot \sqrt{\eta C} \quad .. \quad .. \quad .. \quad .. \quad (3)$$

If the flow-meter resistance is a capillary tube of suitable dimensions, C is given by the simple form of Poiseuille's equation—

$$C = \frac{\pi r^4 g}{8 l \eta} \quad .. \quad .. \quad .. \quad .. \quad .. \quad (4)$$

where r is the radius and l the length of the capillary, g is the gravitational constant and η is the viscosity of air.

Construction of the chart

The base-line of the chart is graduated in values of ϵ from 0·80 to 0·40. The L ordinates (cm) of the sample-height line are given by—

$$L = \frac{1}{A(1 - \epsilon)} = \frac{0{\cdot}7893}{1 - \epsilon} \quad .. \quad .. \quad .. \quad .. \quad (5)$$

where A has the nominal value 1·267 sq. cm.

The equation for the d_{vs} ordinates, which are equal to $\frac{1}{2}F$ cm, is obtained by combining equations (2) and (5), and substituting the nominal values of $c = 3{\cdot}80$ and $P = 50{\cdot}0$ cm—

$$\tfrac{1}{2}F = 1 \Big/ \left(\frac{0{\cdot}3598\,(1 - \epsilon)}{d_{vs}^2 \epsilon^3} + 0{\cdot}04 \right) \quad .. \quad .. \quad .. \quad .. \quad (6)$$

Accuracy of the instrument

All the factors of equations (1) and (2) are possible sources of systematic error. It is apparent from the form of $\sqrt{F/(P - F)}$ in equation (2) that small errors in P or F can result in large errors in d_{vs}, especially when P and F are nearly equal. It will be shown also that c is not independent of P and F when the instrument is calibrated in the usual way. The remaining factors (A, L, M, η and ρ) are less important. The effect of error in measuring or standardising these factors on the apparent particle size indicated by the chart, d_{vs}', can be calculated after appropriate substitution in equations (1) or (2).

THE INSTRUMENT CONSTANT—

The flow-meter resistance in Fisher instruments is either a capillary tube whose resistance can be varied by a sliding wire in its lumen, or an adjustable needle-valve. Either type is usually calibrated against an artificial standard sample supplied with the instrument. This calibrator is a small ruby orifice-plate mounted in a sample tube. It is inserted in place of the normal sample, and the flow-meter resistance is then adjusted until the chart d_{vs} reading equals the value stamped on the calibrator; the instrument constant is then, nominally, equal to 3·80.

The ruby's labelled value is usually about 5·8 μ at porosity 0·75, corresponding to about 15 μ at porosity 0·5. Porosity has no real meaning in reference to an orifice; essentially, the ruby produces a constant F value on the flow-meter manometer when other factors are constant. This F value corresponds to different d_{vs} values, which depend merely on the porosity setting of the chart.

If the accuracy of this calibration is in doubt, a direct check of the ruby's nominal value is difficult. Since the orifice length-to-diameter ratio is small and the air flow turbulent, the flow-rate and its equivalent d_{vs} cannot be related to the orifice dimensions by any simple equation.

However, an accurate and constant value of c can be achieved by replacing the variable resistances with simple capillary tubes of dimensions calculated to give the required conductance. Substituting[6] $\eta = 0{\cdot}000183$ poise at 25° C and $c = 3{\cdot}80$ in equation (3) gives the normal-range conductance, $C = 0{\cdot}004296$ cc per second per cm pressure drop at 25° C. The conductance of the double-range capillary is three times that of the normal-range. Substituting in equation (4) gives the required length and radius.

Accurate resistances can be made from precision-bore tubing after selecting for uniformity of bore and determining the average radius by filling with mercury. Suitable nominal bore-diameters are 0·03 cm and 0·04 cm for the normal-range and double-range capillaries, respectively, with corresponding lengths of about 25 cm and 26 cm. Errors due to turbulence, molecular flow and end effects are negligible with these dimensions at the maximum pressure involved. Mounted vertically in the instrument, the capillaries are protected from chance contamination by dust and have maintained their constants during continual use for several years.

AIR PRESSURE—

The Fisher instrument's constant-pressure device consists of a control valve and a standpipe filled with water. In use, the valve is adjusted until a regular stream of bubbles rises through the water. Since the air pressure depends on the height of water (approximately 50 cm) and on the bubble rate, the standpipe is engraved with a water-level line by the manufacturer, and the bubble rate is directed to be adjusted to between 2 and 3 per second.

The reliability of this system was checked by means of the P manometer specially fitted for this purpose, as shown in Fig. 1. The P manometer is made from precision-bore tubing, with a narrow-bore section (1·5 mm), to damp oscillations caused by the pump, and two expansion bulbs to accommodate the initial surge before the standpipe begins to bubble. When the bubble rate was adjusted to 2·5 per second by careful timing with a stopwatch, the manometer gave a steady reading of 50·0 cm. But when different operators attempted to produce a steady bubble rate of 2 to 3 per second by unaided visual judgment, the reading varied between 49·5 and 51·0 cm. It is preferable, therefore, to standardise P by ignoring the bubble rate and adjusting the pressure control valve to give a manometer reading of 50·0 cm during each determination of particle size.

Gooden and Smith[1] adopted the standpipe from an earlier design by Traxler and Baum.[7] Although the latter claimed that such a pressure regulator is highly accurate, they included a manometer in the system and used its readings in their calculations. The real function of the standpipe is to reduce pulsation in the air flow by allowing a bubble to escape at each stroke of the pump. Since the stroke rate is usually about 4·5 per second, a steadier flow is obtained if the water level in the standpipe is lowered by about 0·5 cm, or until one bubble is given for each stroke while the manometer is at 50·0 cm.

The upper curve in Fig. 2 shows the error in indicated diameter at porosity 0·5 when $P' = 51$ cm instead of the standard 50·0 cm used to calculate the chart ordinates. The usual method of calibrating the variable flow-meter resistance against a standard sample

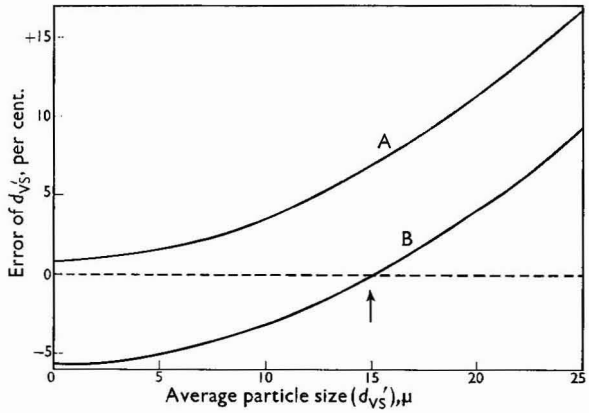


Fig. 2. Error of indicated particle size at porosity 0·5 due to error in air pressure (P′ = 51 cm) or to inequality of flow-meter manometer arm radii (r_a/r_b = 0·98): curve A, error uncompensated; curve B, error partially compensated by calibrating with a 15-μ standard

compensates the error in P' by introducing an equal and opposite error in the instrument constant, c. But the compensation is complete only at the chart level corresponding to the standard sample. The lower curve in Fig. 2 shows a residual error varying from −5·6 per cent. to +9·3 per cent. at other diameters when the original error is compensated with a standard equivalent to 15 μ at porosity 0·5. Thus a subtle disadvantage of the usual calibration method, when either the ruby standard or an actual fine-particle standard is used, is that it is a one-point calibration and gives the operator a false sense of accuracy.

ERROR IN MEASURING FLOW-METER PRESSURE DROP—

Manometer error—The Fisher method for determining $\frac{1}{2}F$ by observing the rise in one manometer arm, while ignoring the fall in the other arm, leads to error if the two arms are of unequal internal radius. Manometer-arm inequality has the same effect as error in standardising P'. Hence Fig. 2 also shows the effect of the manometer error when the arms have a radius ratio (r_a/r_b) of 0·98, corresponding to a maximum difference of 1 cm between the rise and fall in the two arms. Compensation occurs in the same way as for P'.

Inequality of r_a and r_b may be detected by fitting millimetre scales to both arms of the manometer. In this way, errors of more than 1 and 2 cm in F were found in two of the instruments mentioned in paragraph 1 above. It was not known whether the faulty tubes were original fittings, though the instruction manual refers to the need to use matched pairs.

Since the relationship of d_{vs} to F varies with ϵ according to equation (6), Fig. 2 is valid for any porosity if values of F corresponding to $\epsilon = 0{\cdot}5$ are substituted for d_{vs}' in the abscissa.

Chart error—The accuracy of the drawing and reproduction of the principal d_{vs} curves on the chart was checked by measuring the ordinates with a travelling microscope and comparing them with the values given by equation (6). The ordinates of the principal curves were found to have consistently 99·2 per cent. of the theoretical values at all porosities. The effect of this error on d_{vs}' at $\epsilon = 0{\cdot}5$ is shown in Fig. 3. As before, partial compensation occurs if the flow-meter resistance is calibrated against a standard sample. Values of F may be substituted for d_{vs}' in the abscissae as in Fig. 2. Some of the intermediate d_{vs} curves on the chart are less precisely drawn, in particular those from 3·1 to 3·3 μ at $\epsilon = 0{\cdot}47$.

Drainage error—Drainage error in setting the manometer zero and in taking the sample reading is difficult to differentiate from error due to manometer-tube radius. However, these errors are eliminated simultaneously if manometer tubing is selected so that a rise in one arm is equalled by a fall in the other under standard drainage conditions. In this way, and by applying corrections for measured chart errors, good accuracy is achieved for routine work.

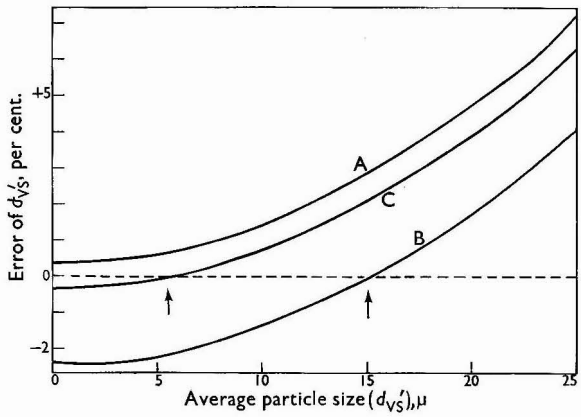


Fig. 3. Error of indicated particle size at porosity 0·5 due to — 0·8 per cent. error in the chart d_{VS} ordinates: curve A, error uncompensated; curves B and C, error partially compensated by calibrating with 15 and 5·5-μ standards, respectively

If greater accuracy and precision are required at the expense of convenience, the errors of the Fisher manometer - chart system can be eliminated by calculating d_{VS} via equation (2) from direct F readings on a separate manometer, so fitted that both arms can be read against a single millimetre scale.

VISCOSITY OF AIR—

If the viscosity of air is assumed to be constant within the range of pressures used, η cancels out when equation (4) is substituted in equation (1), and the instrument reading should be unaffected by temperature changes (*cf.* Lea and Nurse[3]). This was confirmed experimentally for constant ambient temperatures between 10° and 30° C and for flow-rates within the instrument range.

OTHER FACTORS—

The form of equation (1) is such that small percentage errors in A, L, M or ρ result in larger percentage errors in the apparent particle size, d_{VS}', indicated by the chart. The d_{VS}' errors are independent of particle size, but increase as porosity is reduced (see Table I).

Sample-tube diameter—The nominal cross-sectional area of the sample tube, $A = 1{\cdot}267$ sq. cm, corresponds to an internal diameter, $D = 1{\cdot}270$ cm. The internal diameters of the effective portions of three sample tubes measured with a travelling microscope were each found to be 0·31 per cent. high. Table I shows the factors by which this percentage error must be multiplied to give the corresponding percentage errors in d_{VS}'.

TABLE I

EFFECT OF ERROR IN SAMPLE TUBE DIAMETER, SAMPLE HEIGHT AND SAMPLE WEIGHT ON d_{VS}' AT DIFFERENT POROSITIES

Multiply the percentage error in D, L or M by the factors shown below to obtain the corresponding percentage error in d_{VS}'

Porosity	0·80	0·70	0·60	0·50	0·40
D	+3·8	+4·3	+5·1	+6·1	+7·6
L	+0·9	+1·3	+1·5	+2·0	+2·8
M	−1·4	−1·7	−2·0	−2·5	−3·3

Sample height—The sample height, L, is equal to the ordinate of the sample-height line on the chart, provided that the compression mechanism is properly adjusted in the usual way. The errors in the L-ordinates on a typical Fisher chart were found to vary from +0·2 to −0·7 per cent. over the porosity range 0·50 to 0·80, with a maximum error of −1·1 per cent. at porosity 0·40. Table I shows the factors by which these errors must be multiplied to give the corresponding errors in d_{VS}'.

Sample density and weight—The standard sample weight for the instrument is equal to the density of the sample material ($M = \rho$). Any systematic error in density, *e.g.*, by rounding-off, therefore results in a systematic error in sample weight, and the effect on d_{vs}' is increased by the factors shown in Table I. The factors are negative because an excess sample weight results in a low value of d_{vs}'.

Confirmation of accuracy of calibration

The flow-meter resistance—

A normal-range flow-meter resistance was made, as described previously, from selected precision-bore tubing. Substituting the measured radius and length in equation (4) gave the conductance as 0·004299 cc per second per cm pressure drop at 25° C, or 100·1 per cent. of the desired nominal value. The conductance at that temperature was measured by a bubble meter method[8] at constant pressures of about 50 cm as indicated on the P manometer. Four replicate determinations ranged from 0·004296 to 0·004320 cc per second per cm, the mean being 100·26 per cent. of the value by equation (4) or 100·33 per cent. of the desired nominal value of 0·004296 cc per second per cm. The same result was obtained for P values down to 5 cm.

From equation (1), d_{vs} is proportional to $\sqrt{C}$; it is therefore probable that the error of the calibration is not more than 0·16 per cent. in terms of d_{vs}.

Further confirmation was sought by using the capillary tube to determine the d_{vs}' of a standard Portland-cement sample No. 114j from the National Bureau of Standards, Washington, D.C., U.S.A. Chart errors were avoided by measuring F and L directly, as described above; the result was calculated from equation (2) after determining c from the measured dimensions of the capillary and giving A the value found by measuring the sample-tube diameter.

Duplicate results were 99·9 and 98·7 per cent. of the value, 5·755 μ at $\epsilon = 0{\cdot}500$, assigned to the sample by the Bureau, or 100·1 and 98·9 per cent. when calculated by the bubble meter calibration factor.

The bubble meter and cement results confirm the calibration accuracy within the limits of their respective experimental errors.

The ruby calibrator—

Several calibrators were checked with the calibrated capillary tube. Substituting measured values of F in equation (2) gave d_{vs} results about 2 per cent. lower than the rubies' labelled values. It is possible that this discrepancy is due to the makers' method of calibrating each production batch of rubies on a standard instrument that has itself been calibrated against a fresh N.B.S. Portland-cement sample. If the chart d_{vs} ordinates in the standard instrument have the −0·8 per cent. error reported above (Error in Measuring Flow-meter Pressure Drop), and that error is compensated at 5·5 μ by calibrating the instrument against cement of that value at porosity 0·5, curve C in Fig. 3 shows that the labelled value of a ruby, equivalent to 15 μ at porosity 0·5, will be 2·15 per cent. high.

Precision of the modified instrument

The over-all precision of d_{vs} determinations depends on the precision of measuring or standardising the factors previously mentioned. In the treatment below, it is assumed that when a quantity is measured to the nearest graduation on a linear scale, the measurement error is distributed rectangularly, and that the standard error is equal to the scale interval multiplied by $\sqrt{1/12}$ for a single reading, or by $\sqrt{1/6}$ for a measurement by difference.

Calibration by the mercury method—

The instrument constant, c, depends on the capillary radius and length. The precision of c therefore depends on weight and length measurements. The standard error of the calibration, in terms of d_{vs}', is $\pm 0{\cdot}17$ per cent. in the single-range, or $\pm 0{\cdot}08$ per cent. in the double-range, if weighings (of mercury) are made to the nearest 0·001 g, and length is measured to the nearest 0·002 cm (by travelling microscope).

Sample-tube diameter—

The error in d_{vs}', when D differs from its nominal value, can be corrected by applying factors calculated from the measured value of D. The standard error of the corrected d_{vs}', due to error in measuring D with a travelling microscope graduated to 0·002 cm, ranges from $\pm 0{\cdot}24$ per cent. at porosity 0·80 to $\pm 0{\cdot}47$ per cent. at porosity 0·40.

PRECISION OF d_{vs}' DETERMINATION—

The precision of d_{vs}', determined on a single modified instrument, with a single sample tube, depends on the precision of five successive operations: weighing the sample, standardising P at 50·0 cm, setting the pointer to the sample-height line, setting the reference bar of the pointer to the flow-meter meniscus, and reading the d_{vs}' indicated on the chart by the pointer.

Sample weight—Weighing to the nearest 0·001 g gives M a standard error of $\pm$0·03 per cent. when M = 1·5 g. This is smaller than the errors in other factors, but caution is necessary because this error must be multiplied by the factors given in Table I to obtain the standard error of d_{vs}'.

Air pressure—If the P-manometer levels are set to the nearest 0·1 cm, the standard error of P' will be $\pm$0·04 cm. This error has been used to calculate the d_{vs}' error shown in Fig. 4, but greater precision can be obtained in practice. Since the manometer has no adjustment for the effective volume of contained water, it is impossible to set the pressure so that both menisci will coincide with scale graduations; if one coincides exactly, the other will fall at random between two graduations. But the $\pm$0·04 cm standard error can be reduced to the extent that the operator can judge both menisci to be equally displaced from the appropriate graduations.

Sample height—The precision with which twenty operators could set the instrument pointer to the sample-height line was determined with a sensitive clock-gauge. The standard deviation from the mean setting was 0·003 cm, equal to one-fifth of the line thickness, or approximately equivalent to the limit of unaided visual resolution[9] at 15 cm. The same error occurs in setting the zero; combining both errors gives the standard error of L at porosity 0·5 as $\pm$0·27 per cent.

F-manometer setting—In a similar clock-gauge experiment the standard deviation of the meniscus setting was 0·009 cm. This is larger than that for the sample height, probably because the parallax error is greater. The equivalent standard error of F' is $\pm$0·026 cm.

Chart d_{vs}' reading—The spacing between d_{vs} curves on the chart is sufficient to permit visual estimation of the reading to the nearest one-fifth interval between successive curves. If we assume that such interpolation is accurate, the standard error of the reading will be $\pm 0{\cdot}2\sqrt{1/12}$ multiplied by the interval, in microns, between successive curves. This is the minimum theoretical error and has been used in the calculations for Fig. 4. After some training in recognising the appearance of the pointer in different positions between the curves, a careful operator can reduce the error to about 1·2 times the theoretical error.

The individual and combined effects of these errors on d_{vs}' at porosity 0·5 are shown in Fig. 4. The P, F and d_{vs} effects depend essentially on the F-manometer level. When the instrument is used in the double-range, a sample of given average particle size produces an F level equivalent to half the average size, and the chart reading must therefore be doubled. Hence the double-range error curve is obtained by doubling the abscissae of the single-range curve. The steps in the d_{vs} curve correspond to changes in the micron intervals between successive chart graduations.

The precision for samples over 40 μ can be increased, and the instrument range extended somewhat beyond 50 μ, by using an over-weight sample in order to obtain a lower F reading and so increase the value of $(P - F)$ in equation (2); the indicated ϵ and d_{vs}' can be corrected for the excess weight by means of the equations given in an earlier paper.[10] Since the curves intersect at 8 μ, it is advantageous to use the double-range for all samples over 8 μ at porosity 0·5.

The precision of the modified instrument was determined experimentally by testing four weighed samples from each of six procaine penicillin batches. The batches were prepared by grinding one lot of crystalline material at different pressures in a fluid-energy mill; they were therefore similar in general characteristics but differed in d_{vs}. Each batch was well blended to ensure uniformity between samples. Each weighed sample was tested at seven porosity levels obtained by compressing the same sample bed to successively lower porosities.

The detailed results for one batch are shown in Fig. 5 (*a*), and are typical of the results for each batch. They show two kinds of random error, an instrument error revealed by the small scatter of the points for a given sample about a smooth curve drawn through them, and a larger error between samples. The results for each batch, expressed as the mean of four samples, are shown in Fig. 5 (*b*), and show a trend towards a minimum d_{vs}' at porosities between 0·56 and 0·48.

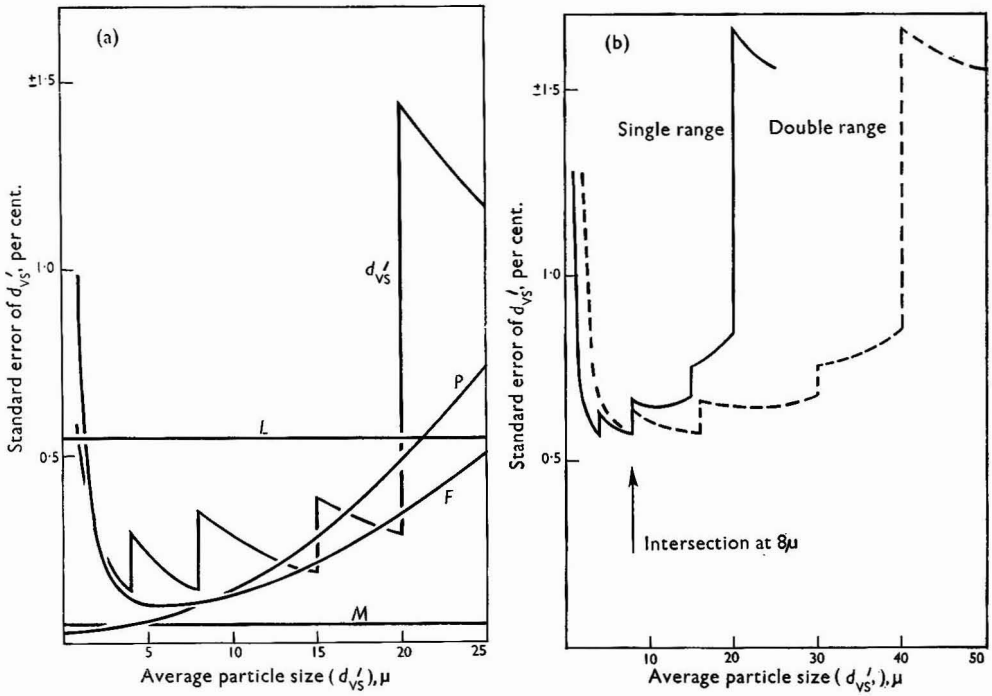


Fig. 4. Precision of a single determination at porosity 0·5 on a single modified instrument with a single sample tube as affected by the determined precision of: *M*, sample weight; *P*, air pressure; *L*, sample height; *F*, flow-meter manometer setting; d_{VS} chart reading. The effect of sample variability is not included.

(*a*) Individual effects of the five factors in the single range

(*b*) Combined effect (root sum of squares) of the five factors in the single and double range

The combined effect of the instrument error and the error between samples is shown by the standard deviations of the sample results from their corresponding batch means, calculated separately for each porosity and expressed as percentages of the over-all batch means—

Porosity	*0·60*	*0·58*	*0·56*	*0·54*	*0·52*	*0·50*	*0·48*
Over-all mean d_{VS}'	*7·96*	*7·80*	*7·70*	*7·70*	*7·71*	*7·72*	*7·72*
Standard deviation, per cent. ..	*4·73*	*3·13*	*2·99*	*2·74*	*3·17*	*2·98*	*3·49*

The several sources of variation are isolated by the analysis of variance summarised in Table II. The analysis is confined to the porosity range, 0·56 to 0·48, within which the porosity effect appears, from Fig. 5 (*b*), to be negligible.

TABLE II

ANALYSIS OF VARIANCE OF d_{VS}' RESULTS FOR 24 PROCAINE PENICILLIN SAMPLES AT FIVE POROSITY LEVELS ($\epsilon = 0{\cdot}56$ TO $0{\cdot}48$)

Source of variation	Degrees of freedom	Mean square	Significance level
Porosity	4	0·0023	not significant
Batches	5	25·4231	0·1 per cent.
Interaction	20	0·0021	not significant
Samples within batches	18	0·2696	0·1 per cent.
Error	72	0·0033	
Total	119		

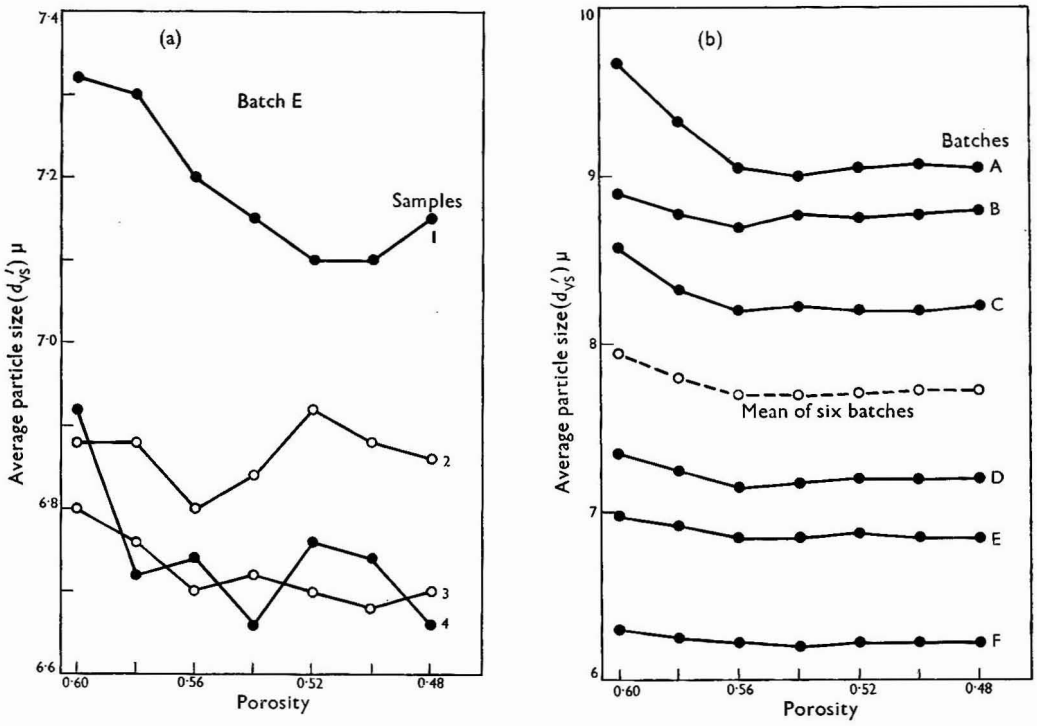


Fig. 5. Average particle size, d_{VS}', of six procaine penicillin batches as determined after compression of each weighed sample to successively lower porosities, (*a*) single determinations on four weighed samples of one batch, showing a small within-samples scatter and a larger between-samples scatter. Similar scatter occurred with the other batches: (*b*) means of four determinations on each of six batches

Instrument error—The square root of the error mean square (0·0033) is the standard error of the instrument (and the operator's ability to read it) after eliminating the sample error and the variation due to porosity, batches and their interaction. Expressed as a percentage of the over-all mean d_{VS}' for all batches in the porosity range, 0·56 to 0·48, it is ±0·74 per cent., which agrees well with the estimate in Fig. 4 (*b*) if the small effect of M is neglected.

Error between samples—The samples-within-batches mean square (0·2696) = 5 × samples variance + error mean square. Hence the samples standard error, excluding all other sources of variation and expressed as a percentage of the over-all mean d_{VS}' for all batches in the porosity range 0·56 to 0·48, is 3·0 per cent. This is too large to be explained as weighing error. It is probably due, not so much to inhomogeneity within batches of powder, as to variation in the uniformity of packing in successive sample beds as suggested by Rigden.[4]

The porosity effect—Fig. 5 (*b*) shows that d_{VS}' varies with porosity. The analysis of variance shows that the slight upward trend between porosity 0·56 and 0·48 is not significant. However, when the analysis was repeated over the range 0·60 to 0·48, the porosity effect was significant at the 0·1 per cent. level; the interaction between porosity and batches was also significant at the same level, indicating that the trend of d_{VS}' with porosity is not constant for all batches.

Discussion

The calibration method described above is absolute in the sense that it standardises the instrument variables in terms of c.g.s. units, without depending on an external particle-size standard. It therefore permits an accurate measurement of sample permeability. The

equation by which the Fisher instrument, like other apparatus such as those of Rigden or Lea and Nurse, relates permeability to mean particle size is adapted from the original Kozeny - Carman equation.[11] All such instruments, if properly calibrated, should therefore give the same result for mean particle size.

The question of the validity of the Kozeny - Carman relationship between permeability and particle size is not one of instrumental accuracy and is beyond the scope of this paper. In this respect, the N.B.S. Portland-cement sample is to be regarded as a permeability standard rather than a particle-size standard.

The modified instrument approaches the standard Lea and Nurse apparatus[3,6] in accuracy and precision, provided that the significant variables are standardised or calibrated as described. It has the additional convenience of permitting direct reading.

The ability to compress a single sample to successively lower porosities and obtain corresponding diameter readings is a further advantage of the Fisher instrument. Other workers[10,12] have observed a variation of apparent diameter with porosity, and it has been suggested that the minimum diameter may have special significance. The effect can be observed more readily with the Fisher apparatus than with apparatus that requires separate sample weighings for each porosity level; with the latter, the combined sampling and instrumental errors may obscure a trend in apparent diameter.

The porosity level, 0·56 to 0·54, at which the apparent particle size is a minimum and the wide range over which the size is almost constant in Fig. 5 (*b*), are characteristic of the particular batches used in this study; other batches and materials may show their minimum at a different level and over a narrower porosity range.[10]

I thank Mr. H. Gresley Grey for the analysis of variance, and Mr. J. W. Mitchell of the Fisher Scientific Company for information about their methods of calibrating standpipes and ruby orifices.

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A Study of the Macroscopic Distribution of Oxygen in a Steel Rod by Neutron-activation and Vacuum Fusion Techniques

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The distribution of oxygen was determined along the length of a steel rod. Neutron-activation and vacuum fusion techniques were used alternatively, and the relevant pieces of apparatus and methods are described. The over-all average oxygen content determined by neutron-activation analysis was 129 p.p.m., in excellent agreement with 128 p.p.m. found by vacuum fusion. The results further show the continuity between the two sets of results, and also a definite inhomogeneity in the macroscopic distribution of oxygen.

THE importance of oxygen determination in modern steelmaking has become increasingly apparent as the need for cleaner steels arose during the last few years.[1,2,3] It is the purpose of this study to show the variation of oxygen content along the length of a steel rod. Thereby, a picture is obtained of the degree of homogeneity that can be expected in a finished steel product. Two methods for oxygen determination have been applied, *viz.*, neutron-activation and vacuum fusion analysis. Concurrently with the oxygen distribution, an evaluation could therefore be made of the respective merits of the two methods.

Material and sampling

Selection of steel—

Because a high manganese content interferes with the vacuum fusion determination of oxygen in steel by gettering of carbon monoxide,[3,4,5,6,7] a steel with a very low manganese content was selected for this study. The composition of the steel was: 0·08 per cent. carbon, 0·16 per cent. manganese, 0·020 per cent. phosphorus, 0·021 per cent. sulphur and 0·01 per cent. silicon, and it was produced as a normal semi-killed grade in an 18-ton basic electric furnace from an all-scrap charge at the ISCOR steelworks in Pretoria. From the rolled stock of this heat a 50-mm square billet, corresponding to the central part of a 4-ton ingot, was selected, and one end was hot forged to a rod approximately 14 mm in diameter and 2·5 m in length.

Sampling—

The 14-mm rod was divided into 10 bars, numbered consecutively from 1 through to 10, and each bar was machined to 12-mm diameter. From each bar, 8 samples of approximately 18 g (19 mm) each, and then 10 samples of approximately 7 g (7 mm) each, were cut consecutively. The 18-g samples were analysed by neutron activation, and the 7-g samples by vacuum fusion techniques. Each sample was numbered according to its original position in the 2·5-m rod and the method of analysis utilised. Thus, the first sample cut from the rod would be 1N1, the second 1N2, and the last two samples 10V9 and 10V10, respectively. Just before analysis, each sample was etched for 1 minute in concentrated hydrochloric acid to remove surface oxide, rinsed consecutively in distilled water, ethanol and acetone, and finally dried in an air stream.

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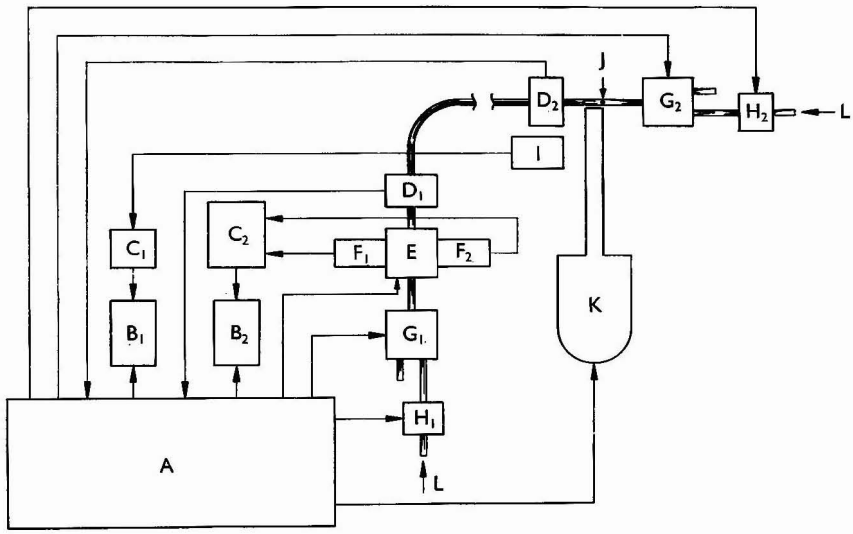
NEUTRON-ACTIVATION ANALYSIS

APPARATUS—

For the neutron-activation part of this research, an apparatus for general activation analysis at the Activation Analysis Research Laboratory (AARL), Texas A and M University, was adapted for possible routine use in a steel production plant for on-line analysis and in-process control. The analysis is based on the ^{16}O (n,p) ^{16}N reaction that has previously been proposed and used for oxygen determination.[8 to 17] The apparatus consists basically of a neutron source, a sample transfer sytem and detecting equipment (see Fig. 1).



A = Programmer
B_1, B_2 = Scalers
C_1 = Amplifier
C_2 = Amplifier discriminator
D_1, D_2 = Photocells
E = Detecting head
F_1, F_2 = Detectors with pre-amplifiers
G_1, G_2 = Transfer boxes
H_1, H_2 = Solenoid air valves
I = Flux monitor counter
J = Sample positioning pin
K = Neutron generator
L = Compressed air

Fig. 1. Block diagram of neutron-activation analysis system

Neutron source—A Texas Nuclear Corporation 150-kV, 1-mA maximum, Cockcroft-Walton deuteron accelerator with a tritiated titanium target is used as a neutron source. The ^3T (d,n) ^4He reaction yields an essentially isotropic and mono-energetic flux of 14·7-MeV neutrons. At full output, the generator, K, in the present case, is capable of producing a total flux of 10^{10} 14-MeV neutrons per second. To extend the target life the acclerator is, however, operated at a beam current of only 0·2 mA. A boron trifluoride counter, I, coupled through an amplifier, C_1, to a scaler, B_1, is used to monitor the neutron flux during irradiation.

Transfer system—The short half-life (7·35 seconds) of the product nuclide, nitrogen-16, makes rapid detection imperative. A pneumatic system was constructed for this purpose. It consists of a compressed air source which connects at L, a 16-mm i.d. plastic tube, solenoid air valves, H_1 and H_2, transfer boxes, G_1 and G_2, to direct the air flow,[18] a pin, J, and a detecting head, E, to position the sample at the irradiation and counting sides, respectively, and photocells, D_1 and D_2.

Detecting equipment—The 6·13 and 7·12-MeV γ-rays of nitrogen-16 are detected by two horizontally opposed and matched Harshaw Integral Line 3-inch × 3-inch thallium-activated sodium iodide crystal and photomultiplier assemblies with pre-amplifiers, F_1 and F_2. The remainder of the system consists of an amplifier discriminator, C_2, a scaler, B_2, and a programmer, A.

Shielding—The neutron-generator target is surrounded by 25 cm of paraffin wax. The generator is situated in a room with 60-cm concrete walls. The γ-ray detectors are enclosed

in a lead castle of wall thickness 7·5 cm. Between the detectors and the generator room, another 25 cm of paraffin wax and 60 cm of concrete are used to minimise activation of the crystals during irradiation.

PROCEDURE—

At the beginning of an analysis cycle, a sample is introduced into the pneumatic system at the transfer box, G_1, (Fig. 1). The rest of the cycle is controlled by the programming unit, A, in the following sequence. The sample positioning pin in the detector head, E, is raised for a short time and the sample blown through the pneumatic tube (air pressure 80 to 100 p.s.i.) against the sample stop pin, J, at the target. A signal from photocell D_2 turns on the neutron generator, K, and the flux monitor scaler, B_1, for an irradiation time of 20 seconds. At the end of irradiation the air flow is reversed by transfer boxes G_1 and G_2, and the activated sample is blown back against the positioning pin in the detector head, E. After a set delay time of 2 seconds, scaler B_2 is turned on for 20 seconds only if photocell D_1 has signalled the arrival of the sample at the detector. The amplifier discriminator, C_2, is set to pass only pulses corresponding to γ-ray energies above 4·6 MeV. A continuation of the air flow for a few seconds after arrival of the sample at both the target and the detector eliminates the possibility of the sample bouncing back from the respective positioning pins. The flow of air during the 2-second delay period purges the system of any activated air still present.

Standards—Weighed amounts (ranging from 3 to 1750 mg) of dry Specpure ferric oxide (Fe_2O_3), sealed in polythene vials, were used as standards. Sealed vials filled with air were run to determine the blank value. The possible increase in the blank value as a result of the absorption on the polythene of recoil nitrogen-16 nuclei, stemming from the activation of the air layer around the vial,[13] could be ignored at the level of activity in this experiment. The error introduced by assuming a constant volume of enclosed air in all the standards was insignificant.

General—The sharp edges of the steel samples were rounded with emery paper to facilitate their transport in the pneumatic system. Each sample and standard was irradiated and counted five times, and a background count, followed by a check determination on a standard, was made at hourly intervals. The linear distance between the detectors and the neutron generator is about 7 m with the shielding as described. There is still, however, a slight build-up of activity in the crystals during a day of continuous operation, which gradually increases the background. On a routine basis a single determination can be made in 1 minute and, allowing 1 to 2 minutes for the manual calculation, a result can be reported every 4 minutes for duplicate analyses.

VACUUM FUSION ANALYSIS

APPARATUS—

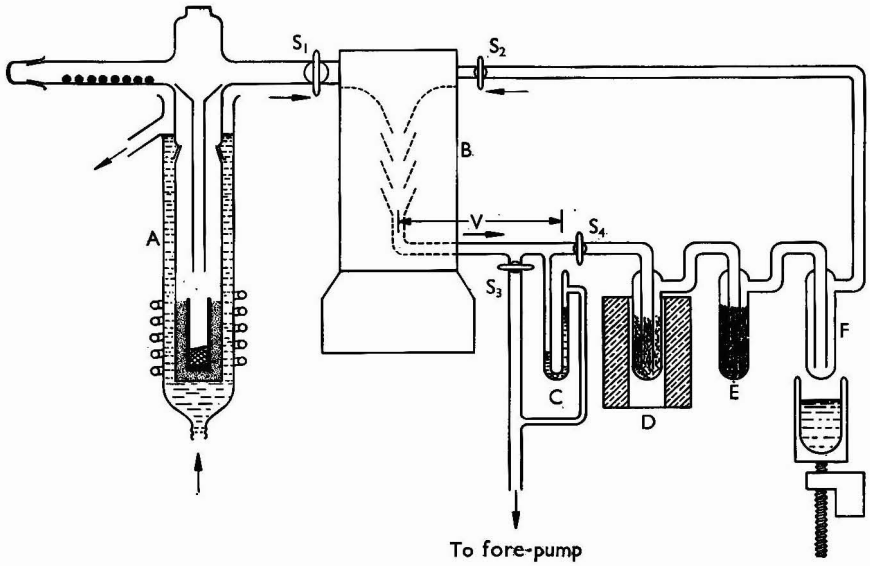
A slightly modified conventional vacuum fusion apparatus was constructed in the Research and Process Development Department of ISCOR, Pretoria. A schematic diagram is given in Fig. 2.

The furnace assembly, A, consists of a water-cooled quartz tube joined, via a ground-glass joint, to a Pyrex-glass cross. A graphite crucible, thermally insulated from the quartz with coarse graphite powder (about 0·5-mm granular size), is heated inductively by a 15-kW radio-frequency unit. A four-stage, high speed, high backing pressure, mercury diffusion pump, B, is used for gas extraction, circulation and collection. The all-glass gas analysis equipment consists of a manometer, C, with dibutylphthalate as manometric fluid, a furnace-heated (400° C) vessel, D, containing cupric oxide (CuO) in wire form, a vapour trap, E, filled with granular magnesium perchlorate ($Mg(ClO_4)_2$), and a cold trap, F. A Dewar flask with liquid nitrogen for cooling F is raised and lowered by means of a motor-driven hoist. Stopcocks S_1 to S_4 and the mechanical fore-pump, which connects as indicated, complete the apparatus.

METHOD—

Principle of analysis—The oxygen in the sample is reduced by carbon at 1600° C and evolved as carbon monoxide together with nitrogen and hydrogen. The carbon monoxide and hydrogen are oxidised to carbon dioxide and water, respectively, by cupric oxide at 400° C. The water vapour is trapped in magnesium perchlorate and the pressure of the

carbon dioxide and nitrogen mixture measured. The carbon dioxide is then frozen out at −190° C by liquid nitrogen, and the pressure of the nitrogen alone is measured. The pressure of the carbon dioxide is determined by a difference calculation.



A = Furnace assembly
B = Diffusion pump
C = Manometer
D = Furnace heated copper oxide container
E = Vapour trap (magnesium perchlorate)
F = Cold trap (liquid nitrogen)
S_1, S_2, S_3, S_4 = Stopcocks

Fig. 2. Schematic diagram of vacuum fusion analysis equipment

Calibration—The collection volume (V) was calibrated by introducing a known volume of pure nitrogen at room temperature and pressure at the cross arm of the furnace assembly, and expanding it through the diffusion pump into the volume V. The pressure in V was measured on the manometer, which has a scale calibrated in millimetres of mercury. By arbitrarily assuming normal room temperature (25° C) in volume V, most of which is incorporated in the lower part of the diffusion pump at an unknown temperature, V was calculated from the ideal gas equations.

PROCEDURE—

A maximum of fifteen 5 to 10-g samples at a time are etched, weighed and introduced into the sample loading arm of the furnace assembly, together with about 30 g of nickel. The whole system is then evacuated with the crucible at 1800° C until the blank value is less than 1 per cent. of the expected gas content of a steel sample; this takes about 2 hours.

The crucible temperature is then lowered to 1600° C, and the nickel (in part or in total) introduced into the crucible through the quartz funnel (Fig. 2) with a magnet. This nickel is necessary for quick de-gassing and quantitative reduction of aluminium oxide.[5] After blank measurement the samples are dropped into the crucible and analysed. The evolved gas is extracted for 2 minutes and then circulated through the hot cupric oxide and the magnesium perchlorate for 1 minute to remove hydrogen. A pressure reading (nitrogen and carbon dioxide) is taken to the nearest 0·02 mm of mercury. The cold trap is subsequently immersed in liquid nitrogen and the final pressure (nitrogen alone) read after 3 minutes. To conclude the analysis, the cold trap is heated to room temperature in an air stream and the whole system evacuated by the diffusion pump (backed by the fore-pump) for 2 minutes, during which time the oxygen content of the sample is calculated. The determination, complete with calculation, is thus completed in 8 minutes.

Results and discussion

Activation analysis—

After correcting for background, the observed counts for the five activation analyses of each sample were normalised with respect to the neutron flux and the average and standard deviation calculated.

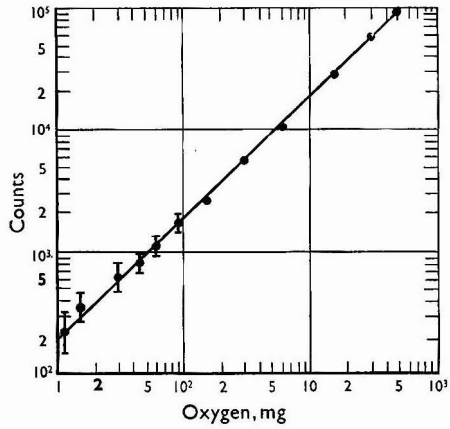


Fig. 3. Neutron-activation analysis calibration curve

The calibration curve obtained with the ferric oxide standards is shown in Fig. 3. The straight line, calculated by the least squares method, gives a calibration constant of 189 counts per mg of oxygen, and fits the results points very well throughout the entire range, *i.e.*, from 1 to 525 mg of oxygen.

Table I

Oxygen found by neutron-activation analysis

Bar:	1N		2N		3N		4N		5N	
Sample	Oxygen, p.p.m.	σ, per cent.	Oxygen, p.p.m.	σ, per cent.	Oxygen, p.p.m.	σ, per cent.	Oxygen, p.p.m.	σ, per cent.	Oxygen, p.p.m.	σ, per cent.
1	137	10	135	6	115	14	114	4	125	7
2	137	5	125	5	119	8	123	6	138	6
3	136	4	103	6	113	2	122	6	139	5
4	145	2	106	4	118	4	116	3	143	4
5	145	9	116	6	107	3	111	4	132	2
6	151	12	120	4	129	5	122	7	145	5
7	172	13	122	4	113	4	114	7	151	7
8	136	5	131	7	102	10	117	6	147	5
Average of bar (σ in p.p.m.)	145 ± 12		120 ± 11		115 ± 8		117 ± 4		140 ± 8	

Table I—*continued*

Bar:	6N		7N		8N		9N		10N	
Sample	Oxygen, p.p.m.	σ, per cent.	Oxygen, p.p.m.	σ, per cent.	Oxygen, p.p.m.	σ, per cent.	Oxygen, p.p.m.	σ, per cent.	Oxygen, p.p.m.	σ, per cent.
1	152	7	—	–	170	9	145	7	166	20
2	—	–	—	–	143	4	112	6	129	6
3	—	–	—	–	140	6	107	5	120	6
4	139	2	—	–	125	8	121	7	123	9
5	163	12	—	–	123	4	111	5	122	7
6	157	12	136	3	145	11	111	7	131	6
7	133	5	136	3	111	10	102	6	143	12
8	147	4	135	4	112	8	114	9	130	3
Average of bar (σ in p.p.m.)	149 ± 11		136 ± 1		134 ± 20		115 ± 13		133 ± 15	

Over-all average: 129 p.p.m. ± 17 p.p.m.

The oxygen content and percentage standard deviation for each sample are listed in Table I, and are grouped according to the sampling sequence described in the paragraph on sampling. The oxygen content varies from 102 to 172 p.p.m. Samples with visible cracks due to the forging of the original steel billet were not analysed (cf. bars 6N and 7N).

Typically, 625 counts and a background of 64 counts were observed per sample. The standard deviations quoted for the samples in Table I compare favourably with an expected value of 6 per cent., and the reproducibility is sufficient for routine analysis. In a few instances, somewhat higher standard deviations were observed. This fact is attributed to local inhomogeneity in the samples concerned.

In Table I a value is also given for the average oxygen content of each bar. The standard deviation in this case was calculated from the oxygen contents of the samples constituting the particular bar, and it is given directly in p.p.m. of oxygen.

To test the validity of the calibration with ferric oxide, 26 apparently homogeneous steel samples were analysed independently by two other laboratories, namely at Texas Nuclear Corporation and at Kaman Nuclear. In the first laboratory, titanium with a known oxygen content (certified by the N.B.S.) was used for calibration, whereas a synthetic sample consisting of a stack of alternating mylar and steel discs, was used as a standard at Kaman Nuclear.[14] The average oxygen content of the 26 samples (5 determinations on each) found by the Texas Nuclear Corporation and the Kaman Nuclear were 124 p.p.m. $\pm$ 11 p.p.m. and 123 p.p.m. $\pm$ 13 p.p.m., respectively, which are in excellent agreement with the value of 125 p.p.m. $\pm$ 14 p.p.m. found in our system (Table II), thus justifying the calibration with ferric oxide.

TABLE II

COMPARISON OF RESULTS OBTAINED IN THREE LABORATORIES FOR 26 HOMOGENEOUS SAMPLES

	Oxygen, p.p.m.		
Laboratory:	AARL	TNC	KN
Sample			
1N2	137	128	120
1N4	145	138	138
1N8	136	132	124
2N4	106	106	103
2N6	120	119	125
2N7	122	132	130
3N3	113	113	120
3N4	118	116	119
3N5	107	120	112
3N8	102	109	107
4N1	114	124	121
4N4	116	111	128
4N8	117	124	132
5N2	138	127	145
5N5	132	127	135
5N8	147	153	161
6N4	139	138	133
6N7	133	128	118
7N6	136	131	125
7N7	136	123	124
8N2	143	137	124
8N3	140	128	121
9N3	107	106	95
9N6	111	112	106
10N3	120	122	117
10N5	122	131	124
Mean	125 $\pm$ 14	124 $\pm$ 11	123 $\pm$ 13

In the three systems concerned there are significant differences in irradiation geometry. In the Kaman nuclear system a double-axis rotator is used to minimise the effect of inhomogeneity in the sample, whereas no sample rotation during irradiation occurred in the cases of the Activation Analysis Research Laboratory and the Texas Nuclear Corporation. Further, the minimum distance between sample and target is about 0·4 mm in the Texas Nuclear Corporation system (which was constructed for maximum sensitivity) as compared with about 6 mm for the other two. One would therefore expect sample inhomogeneity to

have a greater affect in the Texas Nuclear Corporation system and a smaller effect in the Kaman nuclear apparatus in comparison with the Activation Analysis Research Laboratory system. This is reflected clearly in the different standard deviations for 5 determinations on the same sample as found in the three laboratories, namely 13 per cent. (Activation Analysis Research Laboratory), 25 per cent. (Texas Nuclear Corporation) and 5 per cent. (Kaman nuclear) for an inhomogeneous sample, and 4, 8 and 2 per cent., respectively, for a homogeneous sample.

VACUUM FUSION—

As stated in the paragraph on the principle of analysis, one oxygen atom in each resultant carbon dioxide molecule originates from the sample being analysed. Thus, one gram mole of carbon dioxide (22,400 ml at S.T.P.) contains one gram atom (16 g) of sample oxygen. Assuming validity of the ideal gas equations, the oxygen content of a sample with mass M g is thus related to the carbon dioxide pressure reading by—

$$\text{p.p.m. of oxygen} = \frac{P}{M} \cdot V \cdot \frac{273}{298} \cdot \frac{10^6}{760} \cdot \frac{16}{22{,}400}$$

where V = calibrated collection volume in ml,

P = pressure of carbon dioxide in V (mm of mercury) (typically 4·2 mm of mercury).

The collection volume (V) was found to be 253 ml. Therefore, the oxygen content of the analysed sample is—

$$\text{p.p.m. of oxygen} = 218\frac{P}{M}.$$

The results of the vacuum fusion oxygen determinations are given in Table III. Again, the results are listed according to the sampling sequence, and a value for the average oxygen content of each bar is given, together with the standard deviation. There was only one sample containing a visible crack, and this was not analysed. The values range from 96 to 172 p.p.m.

TABLE III

OXYGEN FOUND BY VACUUM FUSION ANALYSIS

	Oxygen, p.p.m.				
Bar:	1V	2V	3V	4V	5V
Sample					
1	117	101	110	108	138
2	131	111	110	123	166
3	127	121	109	114	154
4	131	128	102	115	134
5	172	131	99	126	137
6	136	125	96	135	133
7	136	122	106	147	139
8	136	127	119	171	136
9	151	117	128	134	144
10	145	119	125	126	138
Average of bar	138 ± 15	120 ± 9	110 ± 11	130 ± 18	142 ± 10

TABLE III—*continued*

	Oxygen, p.p.m.				
Bar:	6V	7V	8V	9V	10V
Sample					
1	132	122	108	114	126
2	142	123	105	125	124
3	143	132	109	118	119
4	144	130	116	137	134
5	137	129	109	134	128
6	142	127	133	108	117
7	—	125	115	110	140
8	156	127	127	139	120
9	130	128	122	151	121
10	133	123	109	169	145
Average of bar	140 ± 8	127 ± 3	115 ± 9	131 ± 19	127 ± 9

Over-all average: 128 ± 15.

COMPARISON AND CONCLUSIONS—

The average of the 73 neutron-activation determinations is 129 p.p.m. ± 17 p.p.m., and is in striking agreement with 128 p.p.m. ± 15 p.p.m. found as the average for the 99 vacuum fusion analyses (Tables I and III). In Fig. 4, the two sets of results are combined and plotted as the oxygen content along the length of the original steel rod. The continuity between the two sets of results is evident. The continuous curve, drawn in Fig. 4 through the points representing the average values of the 20 sub-groups, illustrates the large-scale distribution of oxygen content around the over-all average for the complete rod. It clearly indicates an almost periodic fluctuation of the oxygen content around the over-all average for the complete rod, instead of the random scatter that would normally be expected in such a case.

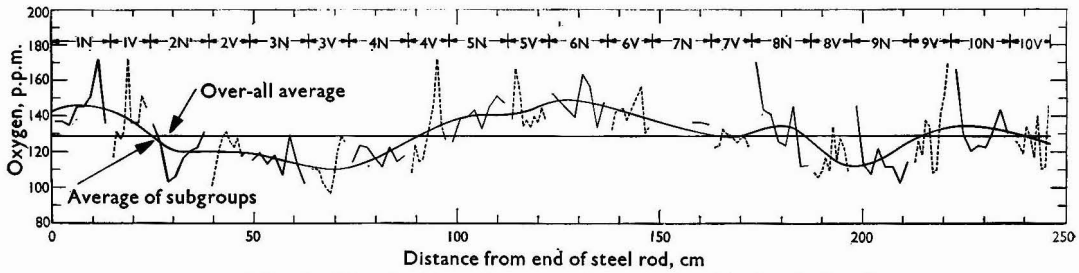


Fig. 4. Distribution of oxygen along the length of a steel rod

The figure shows that, in this rolled and forged steel rod which is a fair example of a finished steel product, there exists a definite inhomogeneity in the macroscopic oxygen distribution. This distribution can be determined with equal accuracy by both neutron-activation and vacuum fusion techniques, but faster, non-destructively and more conveniently by neutron activation.

The authors wish to express their thanks to the Activation Analysis Research Laboratory, Texas A and M University, and the Research and Process Development Department of the South African Iron and Steel Industrial Corporation, as well as their appreciation to Texas Nuclear Corporation and Kaman Nuclear for their kind co-operation described above.

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Quasi-quantitative Separation of Paraffins and Olefins

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By the addition of iodine monochloride to a mixture of paraffins and olefins, an easy chromatographic separation on silica gel of the two is made possible by virtue of the olefin adduct being much more strongly adsorbed. The olefins are regenerated by refluxing the halogenated derivative with ethanol and excess sodium iodide.

The efficacy of the method has been proved on the total aliphatics of low temperature tars and on pairs of pure n-paraffins and 1-olefins of the same carbon number. The small losses incurred are almost entirely in the olefins.

The problem of the quantitative separation of paraffins from olefins, and retention of the identity of the latter, arises in the examination of their mixtures in fractions from petroleum and coal tars. In the latter, saturated and unsaturated aliphatic hydrocarbons exist in quantity in low temperature tars and in those of a type intermediate between low and high temperature tars.[1]

Adsorption chromatography is well established as a method of determining hydrocarbon types, especially in lower boiling mixtures, *e.g.*, the fluorescent-indicator adsorption method.[2] Liquid chromatography has been used for the separation of paraffins and olefins, from C_9 or C_{10} up to C_{16}, from the lower boiling neutral portion of low temperature tars.[3,4] That the total neutral components in such tars could also be separated by this method was shown by Boyer *et al.*,[5] if three fractional distillation cuts were separately chromatographed. In the higher molecular weight hydrocarbons, where the lone double bond of the olefins was considerably "diluted" in the long chains, not more than 5 per cent. of paraffins contaminated the separated olefin fractions. Hydrocarbons up to about C_{33} were then determined by gas chromatography.

The original aim of the work reported here was the quantitative separation of paraffins and olefins from the total aliphatic hydrocarbons of low temperature tars, without prior fractionation of these hydrocarbons. The method devised, however, is applicable to such mixtures of hydrocarbons from a wide variety of sources. For example, we have applied it successfully to the analysis of hydrocarbons from coal extracts and to waxes from plants and soils.

Experimental

The total aliphatic hydrocarbons were first prepared by liquid chromatography on silica gel (column length, 20 cm; diameter, 2 cm; weight of charge, 2 g) of the neutral, light petroleum-soluble oil of a low temperature tar[1] from the carbonisation of Thoresby coal (National Coal Board classification No. 801) by internal heating of the charge with hot gas. The soft wax thus obtained contained n-, iso- and cyclo-paraffins and a corresponding series of olefins, the range being from C_{10} to C_{38}. In all subsequent chromatographic separations, the same ratios of column length to diameter were used, the actual dimensions being appropriate to the weight of charge. The bulk of the solvent was removed each time by atmospheric distillation on a steam-bath, the small amount remaining being eliminated by vacuum desiccation (5 mm of mercury) to constant weight of the cooled flask.

The basis of the method of separation of the paraffins from the olefins was the selective addition of iodine monochloride to the latter, the subsequent easy separation of the paraffins from these addition compounds by liquid chromatography, and the regeneration of the olefins. The scheme of analysis is shown in Fig. 1.

A 70 to 90 per cent. molar excess of Wijs' reagent (0·2 N iodine monochloride in glacial acetic acid) was added to a 2 per cent. solution in carbon tetrachloride of the whole aliphatic hydrocarbon fraction in a stoppered, brown glass bottle, and the whole allowed to stand for 30 minutes.

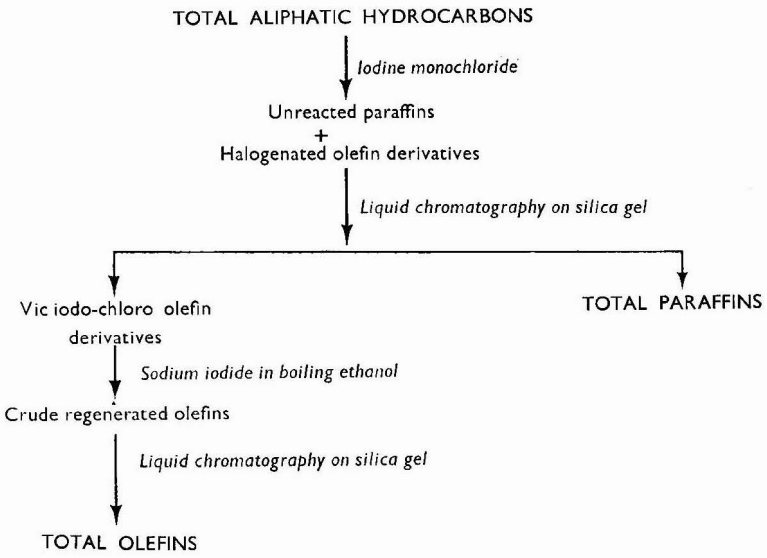

Fig. 1. Scheme for the separation of olefins and paraffins

At the end of the reaction period, excess of 15 per cent. potassium iodide solution was added to convert the unconsumed iodine monochloride reagent to iodine and potassium chloride; a large volume of distilled water was also added. The liberated iodine was finally titrated with sodium thiosulphate solution.

The carbon tetrachloride layer was separated from the aqueous acid, which was then further extracted with carbon tetrachloride to ensure complete removal of the sample. The combined carbon tetrachloride solutions were washed with distilled water until free of acid, dried over anhydrous sodium sulphate, and the solvent distilled off.

The residual brown wax, a mixture of paraffins and halogenated olefins, was dissolved in sufficient light petroleum (40° to 60° C) to make a 10 per cent. solution; this was then poured on to an activated silica gel (100 to 200 mesh) chromatographic column. Elution was continued with light petroleum until the faint purple-brown colour, the front of the halogenated olefin derivatives, approached the bottom of the column. The colourless first eluate contained the total paraffins, which were recovered by evaporation of the solvent.

The coloured, halogenated olefins were completely eluted with ethanol and the original olefins regenerated by boiling the solution gently under reflux with excess sodium iodide. Most of the ethanol was removed by evaporation under reduced pressure, and the regeneration mixture partitioned between light petroleum (40° to 60° C) and dilute aqueous sodium thiosulphate solution: the latter removed the iodine which had been liberated, while the regenerated olefins passed into the light petroleum phase. After washing the light petroleum solution with distilled water and drying over anhydrous sodium sulphate, the solvent was evaporated off if a determination of the crude olefins was required.

To obtain the pure product, a 10 per cent. solution of the crude olefins in light petroleum (40° to 60° C) was chromatographed on silica gel with the same solvent as eluant. The olefins were recovered by distilling off the light petroleum from the total eluate.

For comparison, paraffin contents were also determined by sulphuric acid extraction. A 20 per cent. w/v solution of the sample in cyclohexane was extracted repeatedly at room temperature with equal volumes of 98 per cent. sulphuric acid until no further colouration was imparted to the acid layer (about 10 extractions). The acid extracts were combined, washed twice with cyclohexane, and then discarded. The combined cyclohexane solution and washings were evaporated to give an approximately 10 per cent. solution of paraffins and then chromatographed on silica gel with cyclohexane as eluant. The pure paraffins were then recovered from the total eluate by evaporation of the solvent.

Infrared absorption spectroscopy was used throughout as a guide to the completeness of separation of the olefins from the paraffins.

The results of a separation by the iodine monochloride method of 10 g of the aliphatic hydrocarbon fraction from the tar are given in Table I.

TABLE I

RESULTS FOR THE SEPARATION INTO ITS PARAFFINIC AND OLEFINIC COMPONENTS OF THE ALIPHATIC HYDROCARBON FRACTION FROM A LOW TEMPERATURE TAR

	Percentage by weight
Paraffins	49·1
Purified olefins	43·9
Losses	7·0
Paraffins (by removal of olefins with sulphuric acid)	51·1

G. Clubb and M. Vahrman, in this laboratory, working on a different aliphatic fraction from a low temperature tar, investigated the effect on the separation by this method of using different amounts of iodine monochloride, and ascertained the reproducibility of the results with the optimum excess of reagent (Table II).

TABLE II

RESULTS OF SEPARATIONS OF ALIPHATIC HYDROCARBONS FROM TAR BY THE IODINE MONOCHLORIDE METHOD

Molar excess of iodine monochloride, per cent.	Weight of starting material, g	Weight of paraffin recovered, g	Weight of olefin recovered, g	Paraffin, per cent.	Olefin, per cent.	Recovery, per cent.
10*	2·4916	1·8070	0·4760	72·4	19·1	91·5
30*	2·5085	1·6872	0·5637	67·2	22·4	89·6
50	2·5110	1·5138	0·6088	60·4	24·2	84·6
67	2·4751	1·5732	0·6366	63·6	25·8	89·4
80	2·4936	1·5757	0·7069	63·2	28·4	91·6
80	2·4694	1·5536	0·6232	63·2	25·2	88·4
80	2·4957	1·5632	0·6845	62·8	27·4	90·2
80	2·5299	1·5982	0·6942	63·1	27·4	90·5
80	2·4863	1·5572	0·6489	62·5	26·0	88·5
80	2·5138	1·5802	0·6611	62·9	26·3	89·2
80	2·4588	1·5480	0·6552	63·0	26·7	89·7
80	2·4688	1·5602	0·6509	63·2	26·4	89·6

* The infrared spectra of the recovered unreacted materials showed the presence of olefinic unsaturation. Paraffin content (by extraction with 98 per cent. sulphuric acid): 63·0 per cent. w/w.

The iodine monochloride addition method, with 80 per cent. excess of reagent, was tested on binary mixtures of n-paraffins and 1-olefins of the same carbon number (Table III).

TABLE III

RESULTS OF SEPARATIONS BY THE IODINE MONOCHLORIDE METHOD OF BINARY MIXTURES OF PURE PARAFFINS AND OLEFINS

Hydrocarbons	Percentage by weight in synthetic mixture	n_D^{20}	Percentage by weight of paraffin or olefin recovered	n_D^{20}
n-Tetradecane	52·3	1·4272	51·9	1·4269
Tetradec-1-ene	47·7	1·4342	45·3	1·4341
n-Eicosane	72·1	1·4306	72·0	1·4308
Eicos-1-ene	27·9	1·4363	25·8	1·4362

DISCUSSION

A minimum of 50 per cent. excess of iodine monochloride was necessary to effect complete separations of olefins from paraffins: 80 per cent. excess is considered to give a safe margin. The recovery of paraffins is virtually complete; the losses, mainly in the olefinic fraction, are probably due to irreversible substitution reactions with the halogen reagent, together with the retention of olefins on the column during purification. Losses diminish with increase in weight of original sample. No further yields of olefins could be obtained with ethanol and sodium iodide from the material left on the chromatographic column after removal of the pure olefins.

As both the addition and elimination of halogens in ethylenic compounds are specifically trans, and result in the regeneration of the original isomers, the method can be used if the structures of the olefins are to be investigated. That no isomerisation of the olefins occurred by the method described was confirmed from the infrared absorption spectra of the original aliphatic material and of the separated, regenerated olefins.

The authors are grateful to Rexco Research and Development Company Ltd., for a grant to one of them (J.A.S.), and for samples of their tar.

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A Study of the Determination of Thiamine in Breakfast Cereals

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The method of determining thiamine that involves the purification by base-exchange on sand, is satisfactory for materials of the "breakfast cereal" type. Traces of materials responsible for errors in the direct determination remain after treatment, but the errors are greatly reduced and oppose one another, so that they may reasonably be neglected.

Processes such as pressure cooking and subsequent baking, when applied to many cereal foods, result in the destruction of thiamine. At the same time, materials are produced that give a high fluorescence on treatment with sodium hydroxide and subsequent extraction with isobutanol ("blank fluorescence"[1]), and also substances that interfere with the fluorescence of thiochrome.[2] These effects rendered the "direct"[1] determination of thiochrome in unpurified extracts of such foods of little value until a more detailed study was made. The "blank" used was usually slightly greater than the total fluorescence that was developed after oxidation of the thiamine to the thiochrome, and although the blank and interferences opposed one another the relative magnitudes of the effects were unknown.

Recently, the addition of synthetic thiamine to such products during manufacture has become common practice, and a more detailed and prolonged study of one such product has been made which has given interesting results. Confirmatory results of a much less detailed nature have been obtained with five other products.

Method

Apparatus—

The apparatus used has been previously described.[3,4] In addition, 100-ml conical flasks were used.

Reagents—

Extract—This is prepared as described for flour,[1] but with 30 g of the product in a 1000-ml conical flask and 750 ml of 0·2 N acid. Large amounts are used to compensate for the probable uneven distribution of thiamine; 30 g is a convenient approximation to 1 oz, and the rate of fortification of flour is commonly expressed in terms of mg per oz. The extract is filtered next day through a large fluted filter-paper.

Thiamine additions for recovery experiments—Place 5 ml of a 200 μg per ml solution of thiamine (stage 1 in preparation of standards[1]) in a 100-ml calibrated flask and fill to the mark with the extract. Mix the solutions by emptying the flask turbulently, inverting it in a dry 200 or 250-ml conical flask equipped with a glass stopper. Shake the flask, as vigorously as is possible without the formation of a lasting foam, for about 3 minutes. Return the liquid to the measuring flask, and repeat this process at least twice more. Dilute 10 ml of this solution to 100 ml with the extract. Mix the solutions as before, then dilute 10 and 20 ml of this solution to 100 ml with the same precautions, to give extracts with +0·1 and +0·2 μg per ml of added thiamine.

Reagents, standards and procedure—

The same as those used for the "direct" method as described.[1]

The same as those used for the "sand" method, as previously described,[3] omitting those that were only used for digestion (unless this is to be undertaken).

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Results

At first, recoveries with the sand technique seemed disappointingly erratic but, after four sets had been examined (eight additions), it was found that the mean recovery in the eight results was 98·8 per cent., although extreme results were 108 per cent. and 90 per cent. One difficulty in recovery experiments is that each result expresses the sum of two errors of determination, and it was later decided that the mixing of solutions was still a source of error, in spite of the stringent precautions laid down previously, and hence the shaking in a conical flask, as described above, was prescribed as an additional precaution.

Duplicate eluates, washed with 3 portions of isobutanol, gave a higher mean recovery, but the results were not less erratic.

Examination of these earlier results suggested that both fluorescent and interfering substances were held on the column, probably with varying tenacities. Some were possibly held by base exchange, others only by loose adsorption. To test this hypothesis a large uniform bulk of extract was prepared and, to portions of this, additions of thiamine were made in the manner described above. A set of 8 sand columns was prepared for each solution (+0·0, +0·1, +0·2 μg per ml of added thiamine) and 10 ml of solution were applied to each column (a total of 24 columns).

Four columns of each set were then washed with 200 ml of 0·2 N hydrochloric acid, and the remainder with 500 ml of this acid. All the columns were then eluted, giving six sets of eluates. Two eluates from each four were then washed with 4 portions of 25 ml of isobutanol. The eluates were then arranged in the order 1,2,3,4, corresponding to Table I, oxidised and extracted with four portions of isobutanol and the fluorescence measured on 3 successive days as already described.[3] With the isobutanol-washed eluates, the fluorescence found was multiplied by a factor of 1·09 to allow for the increase in volume of the isobutanol owing to the smaller loss of isobutanol into the isobutanol-saturated aqueous phase. The fluorescence of the washings was measured separately. The results are given in Table I.

Table I

Effect of washing the column with acid and the eluate with isobutanol

I. Eluates not washed with isobutanol

Order of measuring fluorescence	200 ml of acid-wash of column.						500 ml of acid-wash of column					
	1			3			2			4		
Added B_1	+0·0	+0·1	+0·2	+0·0	+0·1	+0·2	+0·0	+0·1	+0·2	+0·0	+0·1	+0·2
B_1, μg per ml	0·362	0·456	0·540	0·365	0·458	0·554	0·362	0·457	0·558	0·359	0·462	0·555
Percentage recovery	—	94	89	—	93	95	—	95	98	—	103	98
	Mean 92·75						Mean 96·0					

II. Eluates washed with isobutanol

B_1, μg per ml	0·340	0·453	0·547	0·355	0·454	0·548	0·355	0·454	0·554	0·360	0·453	0·565
Percentage recovery	—	113	104	—	99	97	—	99	100	—	93	103
	Mean 103·25						Mean 98·75					

It will be seen that the washing of the columns with 500 ml of acid results in a slight improvement in the recovery. This indicates the slow removal of adsorbed interfering substances.

The washings from the eluates, when examined in the fluorimeter, showed a remarkably constant level of 0·026 μg per ml with a maximum deviation of $0{\cdot}001_3$, mean $0{\cdot}000_5$. The blank was reduced to 0·017 on eluates from both the 200-ml and 500-ml acid-washed columns. The blank on the isobutanol-washed eluates was reduced to 0·007 μg per ml.

These figures support the view that the column holds traces of both fluorescent and interfering bodies with varying degrees of tenacity, and that these small variations may contribute to the erratic recovery. Washing the columns with 500 ml of acid will improve the recovery but it will give much the same basic level, because of the balancing opposition of blank and interference.

In order to demonstrate still further the validity of these concepts, the isobutanol extracts were concentrated by distillation *in vacuo* by using an oil pump. Solid carbon dioxide and alcohol were used as a condensing refrigerant, and a trickle of carbon dioxide was passed from a Kipp's apparatus into the distilling liquid to prevent bumping and also to render

the alkali less soluble in the isobutanol. When the contents of the distillation flask had been reduced to a paste, it was stirred with dry isobutanol and spun in a centrifuge. The supernatant liquid was poured into a small flask. The solid was extracted three times in all in this way, and the combined extracts were then distilled again amost to dryness. The distillation residue was extracted with 4 portions of 0·5 ml of dry isobutanol, which were then transferred to an ignition tube and stored in a desiccator containing a small beaker of dry isobutanol to maintain a saturated atmosphere. The liquid was then chromatographed on paper previously extracted with flowing wet butanol for 48 hours and dried. The chromatogram was developed with water-saturated butanol in descending flow. The isobutanol extract of a 200 μg per ml thiamine solution was used as a marker without concentration. The chromatograms when developed and dried were exposed to ultraviolet light and the fluorescence photographed. Fig. 1 is composed from two such chromatograms, and shows the results of chromatographing extracts from: (I), autoclaved wheat; (II), autoclaved wheat after the addition of thiamine; (III), autoclaved wheat after final toasting; (IV), pure thiamine.

In order to show more clearly the nature of the interferences remaining after sand purification, 500 ml of the isobutanol washings of the eluates before oxidation were concentrated to 2 ml without being spun in a centrifuge as described above. The chromatogram of the liquid is shown in Fig. 2, A1; that of the liquid after stirring in some solid is shown in Fig. 2, A2. Fig. 2, A3 is the chromatogram of a pure thiochrome marker, and A4 is the washing of an eluate from sand alone. The chromatogram shows the presence of fluorescent material (blank) in the washings of the cereal eluates. The subsidiary spots on the pure thiochrome line are attributed to a repeating pair of faint spots that were noticed in such runs from time to time, and have not yet been explained.

The dark spots near the origin are due to the quenching of the fluorescence. The chromatogram was then sprayed with the highly fluorescent isobutanol extract of an oxidised thiamine standard containing 200 μg per ml, and then photographed with a shorter exposure. Thus the quenching effects are developed more clearly, as in Fig. 2, B. The same chromatogram was then sprayed with dipicrylamine to show the location of potassium on the paper (Fig. 2, C) and finally with potassium ferrocyanide to show the location of ferrous iron (Fig. 2, D). These ions are important, not for themselves but as carriers of the chloride ion which is a notorious quenching agent. The four photographs together show the quenching on the paper due to each ion, and also the presence of discrete spots which are presumably due to the presence of organic components from the washings. These are the quenching components which persist throughout the determination. The presence of both blank fluorescence and quenching materials are thus shown clearly. It can be seen that traces of iron in the washing from the eluates of the breakfast cereals run further than in the sand blank, where they remain at the origin. This appears to be due to the presence of organically combined iron.

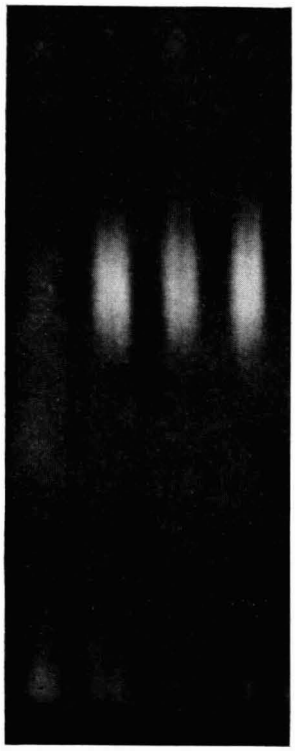
In the isobutanol extracts from oxidised eluates, iron is completely, or almost completely, removed by precipitation with sodium hydroxide, and the potassium (or sodium) chloride concentration is reduced by greater aqueous dilution. However, traces of potassium or sodium chloride will remain, but the quenching that arises from this is extremely small, and acts as compensation to the blank.

It has been found with this particular product, after frequent examination for about two years by both the direct and sand methods, that the direct method gives much the same result as the sand method if *the value for the blank is not deducted.* This was observed previously with uncooked wheat products,[5,3] and it is interesting that it should be true with the much higher blank obtained with this cooked material. In the examination of 63 extracts of 52 samples of one wheat product it was found that the blank had a mean value of 0·083 μg per

Fig. 1. Chromatograms of extracts from: (I), autoclaved wheat; (II), autoclaved wheat after the addition of thiamine; (III), autoclaved wheat after final toasting; (IV), pure thiamine

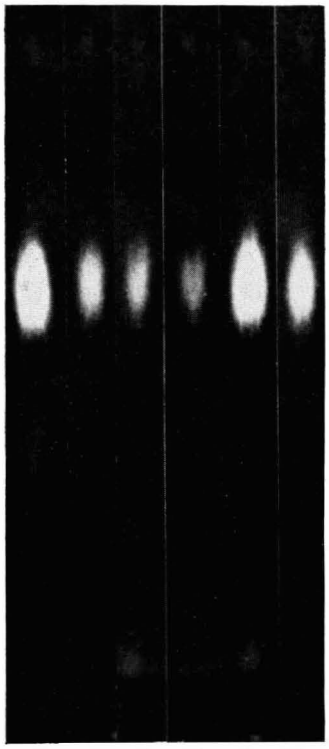
Fig. 3. Chromatograms of isobutanol extracts: E, pure thiamine; F, G, H, foods prepared from wheat; J, food prepared from rice; K, food prepared from maize

Fig. 2. Chromatograms of isobutanol washings of eluates before oxidation: A, run in water-saturated isobutanol; B, sprayed with thiochrome; C, sprayed with hexanitro-diphenylamine (dipicrylamine); D, sprayed with potassium ferrocyanide after spraying with dipicrylamine. I. Eluate washings, solution alone; II. solution and solids; III. 200 μg per ml of B; IV. washing of eluate from sand alone



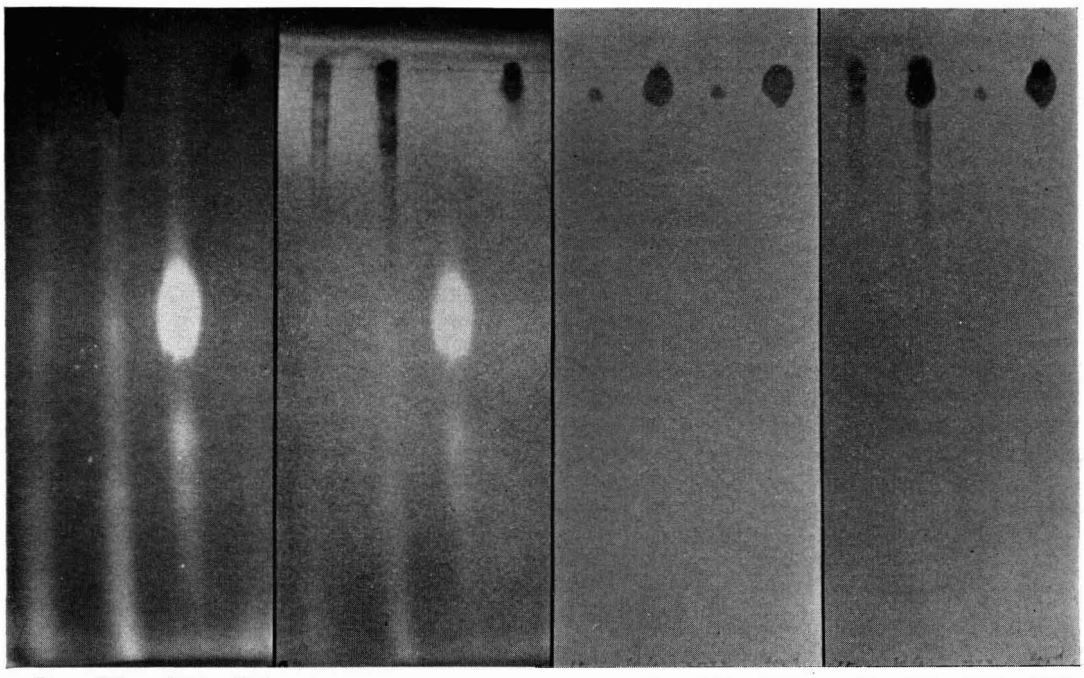
(I) (II) (III) (IV)

Fig. 1



E F G H J K

Fig. 3



(I) (II) (III) (IV) (I) (II) (III) (IV) (I) (II) (III) (IV) (I) (II) (III) (IV)

A B C D

Fig. 2

ml (the maximum value was 0·120 and the minimum value 0·060). Where the thiamine content was in the region of 0·4 μg per ml, the value, as determined by the sand method, was on the average 0·015 μg per ml higher than that determined by the direct method (mean of 2) *without deduction of the blank value*. The maximum differences were +0·046 and −0·022 μg per ml. In three tests, however, when the thiamine content rose to 0·5, 0·6 and 0·7 approximately, the corresponding excesses of the sand values were 0·030, 0·087 and 0·133. It appears likely that with high interferences the quenching is proportional to the square of the thiamine concentration, as with bran.[2] Therefore, at certain concentrations of this material, a measure of the direct value without deducting the blank value could be a valuable rapid routine check on the thiamine content. However, the balance of errors is not the same with other foods, even if they are prepared from wheat (see Table II). The matter should be carefully checked by the sand method with many samples.

TABLE II

THIAMINE DETERMINATION ON BREAKFAST CEREALS

30 g per 750 ml acid extracts

Cereal:	B8619 Maize		B8620 Wheat		B8623 Wheat	B8624 Wheat	B8625 Rice	
Direct determination—								
μg per ml	0·289	*0·316**	0·11	*0·104**	0·093	0·075	0·311	*0·290**
Blank	0·105	*0·092*	0·070	*0·055*	0·070	0·070	0·070	*0·082*
Addition + 0·1 μg per ml ..		*0·408*		*0·201*	—	—		*0·386*
Addition + 0·2 μg per ml ..		*0·492*		*0·277*	—	—		*0·460*
mg per 100 g blank deducted	0·46	*0·56*	0·11	*0·12*	0·06	0·01	0·60	*0·52*
mg per 100 g blank not deducted	0·72	*0·79*	0·28	*0·26*	0·22	0·19	0·78	*0·72*
Sand determination—								
μg per ml	0·230	*0·249*	0·087	*0·073*	0·072	0·030	0·284	*0·209*
Addition + 0·1 μg per ml ..		*0·345*		*0·165*	—	—		*0·305*
Addition + 0·2 μg per ml ..		*0·425*		*0·263*	—	—		*0·410*
Washed eluates, μg per ml ..	0·242		0·081		—	—	—	
Washings, μg per ml	0·041		0·035		—	—	—	
mg per 100 g	0·57	*0·62*	0·22	*0·18*	0·18	0·08	0·71	*0·51*
Manufacturers claim, mg per 100 g	0·60						0·60	

* Figures in italics were obtained from different extracts of the sample, and lack of correspondence of these values with the first determinations are indicative of an erratic distribution of vitamin in the original sample.

The value of taking a large amount (30 g) for the extract was shown by examining duplicate extracts of 26 samples of one brand of cereals for 15 months. Mean deviations of the results from means of their respective pairs was 0·02 on a general level of 0·9 mg per 100 g, with a maximum deviation of 0·07. Six extracts of each of 5 samples were prepared with only 2 g. Mean deviation from the means was 0·07 mg per 100 g with a maximum of 0·17.

Two 5-g portions of a single sample of one fortified breakfast cereal were digested, purified by base exchange on Decalso, and the thiamine determined by using the method described by the Aneurine Panel of the Analytical Methods Committee.[6] The mean of the two results obtained was 0·86 mg per 100 g. The same fluorimeter readings interpreted from a curve prepared from five standards, instead of by calculation from a single 0·2 μg per ml standard as prescribed by the Analytical Methods Committee method, gave a mean result of 0·93. The mean value obtained from two 30-g portions examined by the sand technique was 0·95 mg per 100 g. The low result by the above calculation is due to variation with concentration of the response of the fluorimeter used, as is shown by the sigmoid form of the calibration curve.[1] Thus, if the calculation is performed with the reading from the 0·4 μg per ml standard in the five mentioned above, a mean value of 0·90 is obtained.

Single samples of five other brands of "breakfast cereal" have been examined by both the direct and sand methods. The results are given in Table II. As one of these brands was a wheat product that was believed to have had only part of the cooking and treatment given to the brand most studied, and two of the other brands were maize and rice products, respectively, these three were examined a second time with additions, and the results from these are included in Table II (the columns in italics). As the distribution of thiamine when added to these products tends to be erratic, repeat figures on different extracts are valueless as

a guide to reproducibility of the method. The recovery results are the best guide to this, as these are based on one extract with and without additions.

The isobutanol extracts from the original determinations of these five products were concentrated and separated by chromatography. Fig. 3 has been composed from the two chromatograms so prepared, and shows: E, pure thiamine; F, G, H, foods prepared from wheat; J, food prepared from rice; K, food prepared from maize; the last two being fortified in manufacture.

It is concluded that the method involving the purification by base exchange on sand is satisfactory for cereal foods that have been manufactured by pressure cooking and subsequent baking or toasting. Traces of fluorescent material are held on the column, apparently by base exchange. Traces of fluorescence-quenching substances and possibly also traces of light-absorbing materials, which may be the same as the quenching substances,[2] are held on the column in a less firm and reproducible manner. These last effects oppose the blank however, and both being small are advisedly neglected, as attempts to remove them by washing the eluates with isobutanol are somewhat tedious and liable to give rise to small errors owing to partition and volume effects. It was found that the procedure previously laid down for mixing solutions prepared in measuring flasks,[1] stringent though it was, was quite inadequate for the somewhat viscous solutions, and this appears to have been the main cause of erratic recoveries. Hence the more extreme methods laid down in this paper were found to be essential.

The direct method may have value as a rough routine check, but the balance of blank and interference varies according to the treatment and nature of raw material.

I thank Mr. K. H. Willis for carrying out the analyses and the photography.

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SHORT PAPERS

The Assay of Neomycin

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Commercial neomycin consists of true neomycins, which are glycosides yielding ribose on hydrolysis, with some admixture of the relatively inactive neamine, which yields no ribose.

Neomycin is normally assayed microbiologically. However, various methods have been described for the chemical determination.[1,2,3] Most of these are based on the fact that when neomycins are heated with a strong mineral acid, furfural is formed from the ribose as one of the decomposition products.

A method has been developed which is less time consuming than the other published methods and yet is of the same order of reproducibility. The determination is based on the reaction of ribose with phloroglucinol in a mixture of concentrated hydrochloric acid and glacial acetic acid.[4]

Optimum conditions for the determination of neomycin are found in heating a solution of neomycin with a mixture of 110 ml of glacial acetic acid, 4 ml of concentrated hydrochloric acid, 1 ml of a 0·8 per cent. aqueous solution of glucose and 5 ml of a 5 per cent. alcoholic solution of phloroglucinol in a boiling water-bath for 15 minutes, and measuring the extinction at 552 mμ. If a higher concentration of hydrochloric acid is used the blank becomes much darker.

When neomycin is heated with glacial acetic acid and concentrated hydrochloric acid in the ratio used in the reagent, the maximum amount of furfural is formed after heating for 45 minutes at 100° C. Therefore it seemed unlikely that the determination depended on the formation of furfural. This is confirmed by the fact that furfural does not react with the reagent.

Hydrolysis of neomycin with 1·5 N hydrochloric acid and determination of the released ribose with the phloroglucinol reagent gave very erratic results for the ribose content. Hence it is more satisfactory to carry out the hydrolysis and development of the colour with phloroglucinol simultaneously.

5-Hydroxymethylfurfural has been used as a qualitative test for sugars, and it was thought that this might be used for determining neomycin. However, although ribose reacted with 5-hydroxymethylfurfural under the conditions outlined by Love[5] to give a straight line relationship between extinction and concentration of ribose, neomycin did not react with 5-hydroxymethylfurfural.

Method

Reagents—

Glacial acetic acid, analytical-reagent grade, fractionally distilled.

Concentrated hydrochloric acid, analytical-reagent grade, sp.gr. 1·18.

Glucose solution—Prepare a 0·8 per cent. w/v solution in distilled water.

Phloroglucinol solution—Prepare a 5 per cent. w/v solution in 95 per cent. alcohol just before use.

Phloroglucinol reagent—Mix together in a stoppered flask 110 ml of glacial acetic acid, 4 ml of concentrated hydrochloric acid, 1 ml of glucose solution and 5 ml of phloroglucinol solution. This solution must be freshly prepared.

Neomycin standard—The International Reference Preparation for Neomycin obtained from the National Institute for Medical Research was used. 1 mg of this preparation is equivalent to 680 international units.

Neomycin samples—These were dried over phosphorus pentoxide in a vacuum desiccator for 24 hours.

Procedure—

Transfer by pipette 1 ml of test solution containing about 200 μg of neomycin sulphate per ml into a glass-stoppered test-tube. Add 10 ml of the phloroglucinol reagent, mix the solutions, and place the tube in a boiling water-bath with the water level above the liquid level in the tube,

for 15 minutes. After this time cool the tube and immediately measure the extinction at 552 mμ against a blank prepared with water instead of test solution.

Prepare a series of standard solutions containing between 80 and 400 μg per ml of neomycin sulphate, and carry them through the procedure at the same time as the sample solutions.

Determine the amount of neomycin sulphate in the samples by referring to the standard curve.

Results

The results obtained from separate assays on samples of neomycin sulphate powder are shown in Table I. These are compared with the results obtained by using the methods of Korchagin

Table I

International units of neomycin per mg of sample

Sample	Proposed method	Method of Korchagin *et al.*	Method of Brooks, Forist and Loehr
1	651	641	652
	655	622	734
	665	683	662
	687	652	642
	662	645	—
	648	—	—
2	694	694	655
	687	700	829
	683	710	717
	662	703	637
	656	689	—
	637	—	—
3	699	711	856
	708	682	704
	694	707	683
	695	700	668
	714	699	—
	707	—	—
4	655	650	802
	668	648	661
	658	659	805
	638	628	728
	674	641	—
	637	—	—

et al., and Brooks, Forist and Loehr on the same solutions. Both the latter methods take longer to carry out owing to the greater hydrolysis time required and the need to make up to a known volume after hydrolysis. The over-all time required to carry out an assay by the proposed method is about 1½ hours, as compared with about 2 hours by the method of Korchagin *et al.*, and about 3 hours by the method of Brooks, Forist and Loehr.

The method as proposed is not specific for neomycin because other amino sugar antibiotics yielding ribose on hydrolysis, *e.g.*, paramomycin, will also react with the phloroglucinol reagent.

The author thanks the Government Chemist for permission to publish this paper.

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Removal of Polyphenolic Compounds Interfering with Carbohydrate Determinations in Plant Extracts with an Insoluble Polyphenol Adsorbent

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It has long been recognised that catechins give most of the reactions characteristic of carbohydrates.[1] Further, it has been reported that clarification of plant extracts with lead acetate[2] is not sufficient to remove all the catechins and related polyphenolic compounds contained in extracts of plant tissues which contain high concentrations of these compounds.[3,4] During the course of our recent investigations of carbohydrates in tea plants[5] we have confirmed the fact that catechins and related compounds interfere in the quantitative determination of carbohydrates, and that clarification of extracts of tea-plant tissues with neutral lead acetate[2] is not effective in reducing this interference to negligible amounts.

We have devised a rapid and effective method for removing interfering catechins and related polyphenolic compounds from plant-tissue extracts which is based on the polyphenol absorbing properties of Polyclar AT,[6] an insoluble cross-linked poly(vinyl pyrrolidone) manufactured by Antara Chemicals Division of General Aniline & Film Corp., New York, N.Y., U.S.A. The method as applicable to tea shoot tips is described as follows. Extract the plant material with 80 per cent. (v/v) ethanol and clarify with neutral lead acetate in the usual way.[2] The clarified extract is made up to volume and 1 g of Polyclar AT per g of dry weight tissue is added. The mixture is stirred for 30 minutes, after which the Polyclar AT and adsorbed polyphenolic compounds are removed by centrifugation. Re-treatment of the extracts is made with 0·2 g of Polyclar AT per g of dry weight tissue in the same manner as previously indicated. Carbohydrates are determined in the Polyclar AT treated extracts by any convenient method (we use the method of Somogyi[7]).

The quantitative nature of the interference caused by catechins in the determination of carbohydrates and the effectiveness of Polyclar AT treatment in removing this interference is shown in Table I. Further, these results show that the Polyclar AT treatment does not interfere with the determination of the carbohydrates themselves.

TABLE I

THE QUANTITATIVE NATURE OF CATECHIN INTERFERENCE IN THE DETERMINATION OF CARBOHYDRATES, AND THE REMOVAL OF THIS INTERFERENCE WITH POLYCLAR AT TREATMENT

Carbohydrates were determined by the method of Somogyi.[7] Polyclar treatment was made by stirring 5·0 g of Polyclar AT per 100 mg of sugar (or catechin) in the solution being tested for 30 minutes

Weight of chemical compound	Titre before Polyclar treatment (ml of 0·05 N thiosulphate)	Titre after Polyclar treatment (ml of 0·05 N thiosulphate)	Significance of change in titre due to Polyclar treatment
Glucose, 125 μg	0·94	0·95	none
Glucose, 250 μg	1·93	1·92	none
D-Catechin, 100 μg	0·36	0·01*	significant change ($p < 0·01$)
D-Catechin, 200 μg	0·73	0·02*	significant change ($p < 0·01$)
Glucose, 125 μg + D-Catechin, 100 μg ..	1·28	0·98†	significant change ($p < 0·01$)
Ribose, 200 μg	0·98	0·98	none
Fructose, 250 μg	1·90	1·85	none
Sucrose, hydrolysed, 500 μg ..	3·81	3·74	none

* Not significantly different from zero.
† Not significantly different from titre for glucose only.

The inadequacy of the usual lead acetate clarification procedure[2] for removing the catechins, and related polyphenolic compounds present in three different plant tissues is shown in Table II.

The results also show that Polyclar AT treatment was effective in each case in removing these interfering compounds, and this was further verified by examination of the various fractions by using paper chromatography.[8]

TABLE II

EFFECT OF POLYCLAR TREATMENT ON THE CATECHIN AND THE "APPARENT" CARBOHYDRATE CONTENTS OF LEAD ACETATE CLARIFIED BY EXTRACTS OF THREE PLANT TISSUES

Carbohydrates were determined by the method of Somogyi.[7] Catechins were determined by the concentrated sulphuric acid - vanillin method of Swain and Hillis.[10]

Treatment	Tea (*Camellia sinensis*, L.) Shoot tips Catechins	Tea (*Camellia sinensis*, L.) Shoot tips Carbohydrates	Tea (*Camellia sinensis*, L.) Roots Catechins	Tea (*Camellia sinensis*, L.) Roots Carbohydrates	Ash plantain (*Musa paradisiaca*, L.) fruit Catechins	Ash plantain (*Musa paradisiaca*, L.) fruit Carbohydrates
	(mg per g of dry weight tissue)					
Extract, 80 per cent., v/v	240	—	34	—	10·6	—
After clarification with neutral lead acetate[2]	15·7	17·1	2·86	2·35	0·50	8·33
After first Polyclar treatment (1·0 g per g of dry weight tissue) ..	0·23	5·66	0·00	1·49	0·00	6·44
After second Polyclar treatment (0·2 g per g of dry weight tissue) ..	0·00	5·09	0·00	1·54	0·00	6·33
After third Polyclar treatment (0·2 g per g of dry weight tissue) ..	0·00	5·00	—	—	—	—

The method described in this paper is especially important in tea biochemistry because of the high concentration of catechins and related polyphenolic compounds present in these tissues,[9] but, as shown by our work on ash plantain fruit, the method may also be applied with advantage in carbohydrate studies on other plant tissues.

We thank Mr. P. Kanapathipillai for assistance with the statistical analysis of our results.

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The Detection of Dinitro and Trinitro Aromatic Bodies in Industrial Blasting Explosives

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THE detection and identification of nitro-bodies is a common requirement in the explosives laboratory. Janowski's reaction[1] provides an adequate test for dinitro and trinitro aromatic compounds by means of the characteristic colours developed in alkaline acetone. In the forensic laboratory, however, these compounds normally have to be detected as minor constituents in small samples

of industrial blasting explosives. For this purpose Janowski's reaction has been found unsatisfactory, particularly when applied as a spot test. The reason for this is that the test is insufficiently sensitive, and so an attempt has been made to effect an improvement.

English[2] has described the use of 50/50 acetone - alcohol as a solvent. It has been found that this gives better results with forensic samples than any other of a range of organic solvents tested. The alkali normally used for this reaction is potassium hydroxide, 5 per cent. being the commonly recommended strength. It was found, however, that the sensitivity of the test was increased if stronger alkali was used. This agrees with the observations made by English on alcoholic sodium hydroxide. The limit of detection for 2,4-dinitrotoluene was found to be 20 μg with 10 per cent. potassium hydroxide, improving to 1 μg with 50 per cent. potassium hydroxide.

Because very strong alkali is not a convenient reagent for bench use, alternatives were considered. It was known that coloured complexes are formed between some nitrobodies and organic bases,[3] and this reaction forms a convenient technique for colouring trinitrotoluene in crystal structure investigations.[4] A range of organic bases was therefore studied, and three emerged as giving good colour reactions with di- and trinitrotoluene at low concentrations. These were tetramethylammonium hydroxide, diethylene triamine and tetraethylene pentamine. For use as a bench reagent, 25 per cent. aqueous tetramethylammonium hydroxide was found to be the most convenient since it is a clear mobile liquid that can be used satisfactorily with a dropping bottle.

The reaction can be readily carried out on a spot plate. The addition of a 5 to 10-mg specimen of one drop of acetone - alcohol, and one drop of tetramethylammonium hydroxide produces a blue colour with dinitrotoluene and a dark red with trinitrotoluene. The initial colour should be observed since changes occur with time. A slight yellow is produced with nitroglycerine so that if this is present the colour produced with dinitrotoluene is green. The test has been applied to a wide range of obsolete and current industrial blasting explosives, and it has been found that none of the other components present interfere with the reaction. The limits of identification are 4 μg for *m*-dinitrobenzene, 2 μg for 2,4-dinitrotoluene and 1 μg for 2,4,6-trinitrotoluene.

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The Use of Molecular Sieve 5A for Collecting Fractions from a Gas Chromatograph

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THE separation of the components of a mixture by gas chromatography, and examination of the appropriate fractions in a mass spectrometer is now recognised as one of the most useful ways of identifying the unknown compounds revealed on a gas-chromatographic record. In the most convenient procedure, the chromatograph column is attached directly to the inlet manifold of the mass spectrometer, into which the fractions are fed in turn, so that the mass spectrum is obtained without isolation of the unknown components; however, all the components emerging from the column are diluted with carrier gas, and when a compound is present in very small amounts, *i.e.*, as a trace impurity in the sample, the direct gas - liquid chromatography - mass spectrometer apparatus gives rather weak spectra, making identification more difficult. In order to obtain stronger spectra it is desirable to remove the excess of carrier gas, and although with hydrogen and helium this can be done by fractional diffusion through a fritted-glass tube,[1] or by the device described by Ryhage,[2] an alternative and simpler procedure is available whereby the component is trapped on an adsorbent and subsequently desorbed (by heating) into the mass spectrometer. Silica gel has been used for this purpose,[3,4] but we have found that this converts alcohols into olefins, and irreversibly adsorbs amines. We find that molecular sieve 5A is a very

useful adsorbent as it is free from these disadvantages. Under the conditions given below, the molecular sieve is not only a strong adsorbent for substances with short straight chain molecules, but is also a weak adsorbent for all types of substances with more complex molecules.

The molecular sieve is ground, and the 30 to 60-mesh fraction is heated *in vacuo* to 360° C immediately before use; 0·1 g of this material is loosely packed (and held in position by quartz-wool plugs) in glass tubes 8 cm long, a 3-mm bore drawn out at one end. A number of these adsorption tubes are prepared and held ready for use during the chromatographic run.

For the initial separation a packed analytical-scale gas - liquid chromatography column is used with a load of sample of about 1 mg, but the load can be increased when necessary, provided that the resolution of the required components is not too greatly impaired. A narrow bore T-piece is inserted between the chromatograph column and the flame ionisation detector so that about half the effluent passes to the detector and about half to the side pipe; when an unknown component of a sample begins to emerge from the column (as indicated by the beginning of a peak on the chromatograph chart), the narrow end of an adsorption tube is connected to this side pipe by silicone tubing, and this adsorption tube is removed when the component has been completely eluted. In this way a number of components can be collected from one chromatographic run; the adsorption tubes are then placed in sealed containers until they can be analysed on the mass spectrometer.

For examination in the mass spectrometer, the molecular sieve packing is transferred from the adsorption tube into a 2 × ¼-inch glass tube (closed at one end), which is then connected by means of a vacuum-tight joint to the inlet manifold of the mass spectrometer. The tube is evacuated (after cooling with crushed solid carbon dioxide if the compounds under investigation are very volatile), opened to the mass spectrometer, and heated with a small luminous bunsen flame until a satisfactory pressure or monitor reading (depending on the type of mass spectrometer used) is obtained; care is taken not to heat the tube too strongly, otherwise it may collapse. The tube is isolated from the mass spectrometer by closing the appropriate tap while the mass spectrum is being recorded, to prevent re-adsorption on the molecular sieve. This procedure has given spectra at least fifty times as strong as those obtained from the direct gas - liquid chromatography mass spectrometer apparatus.

Molecular sieve 5A has also been used successfully for adsorbing organic vapour from the atmosphere, for which purpose it has an advantage over the more usual gas - liquid chromatography[5] packings because it allows one to sample much larger volumes of air before "break-through" of the organic compound occurs.

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Book Reviews

NEWER REDOX TITRANTS. By A. BERKA, J. VULTERIN and J. ZÝKA. Translated by H. WEISZ. Pp. x + 245. Oxford, London, Edinburgh, New York, Paris and Frankfurt: Pergamon Press. 1965. Price 60s.

This is a translation, with a certain amount of revision, of the German edition of 1964 prepared from the original Czech edition of 1961. It deals succinctly but compendiously with the titrants alkaline permanganate (10 pp), manganese(III), copper(II), hexacyanoferrate(III), hypochlorite, hypobromite, chloramine-T (11 pp), bromine, *N*-bromosuccinimide, iodine monochloride (11 pp), periodate, lead(IV), vanadium(V) (11 pp), iron(III), cobalt(III), hydrogen peroxide, chromium(II) (16 pp), tin(II), arsenic(III), mercury(I), copper(I), molybdenum(V), molybdenum(III), tungsten(V), tungsten(III), uranium(IV), vanadium(II), vanadium(IV), ascorbic acid (11 pp), hydroquinone, hydrazine, nitrite and certain others. The longest chapters are indicated by the number of pages they occupy: the remaining chapters are quite short. Although in line of succession with older texts, this book has not the explanatory discussion of Böttger's "Neuer Methoden der Massanalyse," nor the critical appraisal and detail of Kolthoff and Belcher's "Volumetric Analysis," Volume III.

Such depth of treatment is not to be expected for reagents of recent vintage, and need not be repeated if there is nothing to add to the information in the older texts on longer established titrants.

Each chapter opens with a brief, indeed exiguous, introduction outlining the principles of the reactions of the reagent. This is followed by an account of the preparation and standardisation of solutions and some account of visual indicators that have been used with the reagent. The main part of each chapter is a review of the determinations that have been made with the reagent with a brief indication of working conditions. Finally, a bibliography is included covering the literature satisfactorily and fairly comprehensively up to 1962 or 1963. The introduction often amounts to no more than a simple statement, and is sometimes at fault. No account is taken of rates of reaction, nor of reaction mechanisms in the true sense. The survey of methods gives barely sufficient working detail and is uncritical. The most valuable feature of the book is the extensive bibliographies which the surveys of methods serve to annotate. The treatment will appeal to the analyst rather than to the analytical chemist, and will serve to bring to his attention many alternative or new methods of determination which may help to solve his particular problems. The real virtue of this book is that it offers a compact and compendious key to the literature of the less familiar electron-transfer titrimetric reagents. In this respect it will find a ready welcome from all engaged in this field. E. BISHOP

COMPUTER PROGRAMMING FOR CHEMISTS. KENNETH B. WIBERG. Pp. x + 269. New York and Amsterdam: W. A. Benjamin Inc., 1965. Price (cloth) in the U.S.A. $12.50; elsewhere $13.75.

The title of this book is misleading. It should read "FORTRAN programming for chemists." It deals only with FORTRAN II, with a note on the modifications incorporated in FORTRAN IV, which is now replacing the earlier version, a mnemonic language devised for use with IBM computers. This language, with certain modifications and restrictions, may also be used with a few other makes of computer provided with punched card input and output, if FORTRAN compilers have been written for them. For the most part the text is written around the large, 32,768 word, IBM-709, 7090, 7094 computers, but a few notes are added on the smaller IBM-1620 computer. In addition, the longest chapter deals with assembly programmes FAP (for 709 and 7090 FORTRAN II) and MAP (for 7090 and 7094 FORTRAN IV).

For users of IBM machines and for others prepared to work within the facilities and restrictions of FORTRAN II, here is a pretty comprehensive treatment of programming in this language. They will furthermore have the benefit of an account written by a chemist, so that the illustrations of programmes and programme segments have a chemical flavour and use mathematics of a type familiar to chemists. Most books on the subject either deal only with the specification of the language, or provide mathematical or engineering illustrations requiring some effort for assimilation before the programming aspects can be appreciated. The illustrations in this book are real working programmes of some length culled from the author's own interests in physical organic chemistry, and will be appreciatively received. The complete tyro can safely and confidently begin his study with this book, though perhaps more emphasis could have been placed on the general philosophy of computing and programming, and on the restrictions in the particular language under consideration. Programmers experienced in other languages will be able to assimilate the material quickly, but will find themselves asking questions which are not answered explicitly, and to them the advice would be: "if it is not there you cannot do it."

For users of machines with punched tape or film input and output provided with compilers for ALGOL or their own mnemonic code, the benefit of this book lies in the extensive illustrations with a chemical application. There is no doubt that a study of these programmes will be rewarding, but the treatment is exclusively FORTRAN, and direct translation will not produce the most economical programme in another language. For such programmers the very stiff price of the book will be an effective deterrant. E. BISHOP

OSCILLOMETRY AND CONDUCTOMETRY. By E. PUNGOR, D.Sc. Translated by T. DAMOKOS. Editor A. TOWNSHEND. Pp. xvi + 238. Oxford, London, Edinburgh, New York, Paris and Frankfurt: Pergamon Press. 1965. Price 70s.

The author observes in his introduction that the conductometric titration of solutions was one of the first instrumental methods of analysis to be developed, and that now the oscillometric technique makes possible the examination of liquids contained in a closed system. The book is divided into five parts. Part I gives an adequate description of the theory underlying the measurements of conductivity and dielectric constant of solution. In Part II are described instruments

and apparatus for these measurements, in a thorough description occupying nearly one-third of the total text. A consideration of a variety of applications in acid - base titrations in aqueous and non-aqueous media is given, and also titrations based on precipitation and complex formation. Part V contains a discussion of the other applications of conductometry and oscillometry to subjects such as kinetic studies on the hydrolysis of esters, chromatography and establishment of phase diagrams.

There is also a bibliography with 374 references and a separate author index. The latter is not completely error free, though in general mistakes appear to be nominal.

This book is well produced and is clearly readable. It will be of value to analytical chemists either specialising in this subject or to those who wish to know its scope. V. J. JENNINGS

STRUCTURE OF AQUEOUS ELECTROLYTE SOLUTIONS AND THE HYDRATION OF IONS. By O. YA. SAMOILOV. Pp. xiv + 185. New York: Consultants Bureau. 1965. Price $15.00.

In 1957, the Faraday Society held a Discussion on Interactions in Ionic Solutions, and one of the outstanding papers was that presented, *in absentia*, by Professor Samoilov of Moscow. To many of those present this was the first contact with the original and stimulating work of his Russian school. Since then a considerable effort has been made to bring the extensive results of their researches into the general orbit of English speaking peoples, and it has culminated in this excellent translation of Samoilov's book.

The book is concerned to give a clear and quantitative description of water and aqueous solutions, essentially in terms of the variable relaxation times of water molecules in the presence of ions and in the presence of other water molecules. The quasi-crystallinity of water resulting from the intense hydrogen bonding is shown to be disrupted by some solutes and enhanced by others (*cf.* the cluster models of Frank) and these features, referred to as negative and positive hydration, respectively, are related to observable properties such as fluidity and self-diffusion. This book is by far the best account of the properties of ions in aqueous solution, and it is devoid of much of the nonsense that is frequently presented under the heading of solvation. Even so, it perpetuates one or two attitudes with which this reviewer would quarrel. Once the word "structure" is involved, it inevitably leads to a model which is too rigid and which is likely to obscure the most prominent feature of all liquids, namely Brownian motion. Furthermore, the failure to realise that density is the most discriminating variable of a liquid (and not temperature) leads to a restricted view of the kinetic picture. Nevertheless, in spite of these shortcomings (and it has to be realised that the subject of aqueous solutions is very wide and complicated), the book has a vitality and freshness reminiscent of Gurney.

This English text has been prepared via the German text, and as such is third hand. However, the translator, D. J. G. Ives, is himself as authoritative as the original author, and a highly readable and accurate translation has resulted. There is no other comparable work and it is warmly recommended. G. J. HILLS

SPECTROCHEMICAL ANALYSIS: OPTICAL SPECTROMETRY, X-RAY FLUORESCENCE SPECTROMETRY, AND ELECTRON PROBE MICROANALYSIS TECHNIQUES, JUNE 1964 TO JUNE 1965. Edited by BOURDON F. SCRIBNER. Pp. x + 75. Washington, D.C.: U.S. Department of Commerce. 1965. Price 50 cents.

The Analytical Chemistry Division of the National Bureau of Standards intends to publish annual summaries of progress made by each of its sections during the preceding year. This first report of the Spectrochemical Analysis Section contains information in the fields of optical spectrometry, X-ray fluorescence spectrometry and electron-probe microscopy.

Examples of the subjects covered are excitation studies by arcs and sparks in controlled atmospheres and by the laser probe. The publication also includes sections on the measurement of arc-temperature, the use of continuum sources in atomic-absorption spectrometry and chemical and physical-enrichment methods for the analysis of high-purity platinum, tin and zinc. X-ray fluorescence spectrometric methods are dealt with for the analysis of silver and gold-base dental alloys and the determination of copper in brass by a solution procedure. An account of modifications made to a recently installed electron-probe microanalyser, and examples of some applications of the instrument are also given.

Workers in these fields who wish to keep up-to-date are always interested in developments in other laboratories, and these inexpensive publications should enable the progress of this well known spectroscopic group to be followed. D. M. PEAKE

QUANTITATIVE ELECTRON MICROPROBE ANALYSIS. By ROGER THIESON. Pp. iv + 170. Berlin, Heidelberg and New York: Springer-Verlag. 1965. Price DM 24.

In electron-probe microanalysis it is usual to make quantitative determinations by measuring characteristic X-ray intensities from the specimen and comparing these with similar measurements from high-purity elemental standards. A series of corrections must be applied to this crude ratio to give results of reasonable accuracy.

This book details a method for converting the X-ray intensity ratio into mass concentration, taking simultaneous account of electron penetration, X-ray emission efficiency, mass absorption and electron back-scatter. The first 25 pages give the background to, and development of the theory on which the corrections are based. Six tables, covering 144 pages, enable the corrections to be carried out readily.

The text is marred by a large number of typographical errors and incorrect equations that make development of the theory difficult to follow. This is the more unfortunate as the final equations differ significantly from those commonly used at present. Little evidence is given for the reliability of the proposed method, and this limits the usefulness of the book.

To operators of electron-probe microanalysis who wish to survey all the current correction theories, this book gives useful data for one theory, but until further evidence of reliability is forthcoming it cannot be recommended for general use. J. A. F. GIDLEY

MISES AU POINT DE CHIMIE ANALYTIQUE ORGANIQUE, PHARMACEUTIQUE ET BROMATOLOGIQUE. Douzième Série. Edited by J.-A. GAUTIER and P. MALANGEAU. Pp. ii + 239. Paris: Masson et Cie. 1964. Price Fr. 60.

MISES AU POINT DE CHIMIE ANALYTIQUE ORGANIQUE, PHARMACEUTIQUE ET BROMATOLOGIQUE. Treizième Série. Edited by J.-A. GAUTIER and P. MALANGEAU. Pp. 236. Paris: Masson et Cie. 1964. Price Fr. 66.

"*Mises au Point*" continues its annual publications of reviews on various aspects of analytical chemistry. The twelfth issue has six contributions dealing with analytical control in the cereal products industries, organic peroxides, methods for the determination of the double bond, new aspects of the chromatography of carbohydrates, analytical control in the chocolate and confectionery trades and, finally, the study of fatty acids by gas chromatography.

Moisture determination in cereal products is again given a detailed treatment with special emphasis on the Chopin multicellular drying-oven. Modern instrumental methods in cereal chemistry are by no means overlooked, as, for example, the electrophoresis of gluten proteins and even moisture determination by nuclear magnetic resonance spectroscopy. The Kjeldahl method for the determination of total nitrogen in cereals is briefly discussed, but there is little practical guidance on this subject.

Analytical control in the chocolate and confectionery trades is dealt with from the view-point of classical conventional methods, in spite of the title "Methodes analytiques recentes." Organic peroxides, the carbon - carbon double bond and their determination are treated quite fully.

Chromatography is represented by an article on the chromatography of sugars and carbohydrates, and by a study of gas chromatography of the fatty acids. The study of chromatography of sugars provides a useful starting point for the analyst unfamiliar with work in this field. Paper chromatography, thin-layer chromatography and gel-filtration separations of sugars are included, but not the gas chromatography of sugar derivatives such as the methylated sugars or trimethylsilyl ethers.

The article on the gas chromatography of fatty acids includes some useful results on the fatty acid composition of various oils and fats which are not, as yet, readily available in other reviews or reference books. The technique itself as applied to fatty acids is considered, particularly with reference to the problems likely to be encountered. The separation of linolenic and arachidic acids on the more polar polyester stationary phases is mentioned as a special difficulty, but is readily achieved by using the more sensitive ionisation detectors, more efficient columns and a less polar polyester stationary phase—a practice that is now a routine procedure in many laboratories specialising in this field.

The thirteenth issue of "*Mises au Point*" is entirely devoted to various aspects of food-colouring matters, both natural and artificial. The subject is treated from the view-point of French legislation, but food chemists in this country may well find it useful to have a fairly comprehensive source of information at hand on food colours. An unusual approach to the problem of the identification of food colours is the use of textile-dying techniques on various fibres, both natural and synthetic, in addition to the better known chromatographic methods. P. MORRIES

PAPER AND THIN LAYER CHROMATOGRAPHY AND ELECTROPHORESIS. A TEACHING LEVEL MANUAL. Second Edition. By IVOR SMITH, B.Sc., Ph.D., F.R.I.C., M.I.Biol., and J. G. FEINBERG, B.Sc., M.Sc., D.V.M., M.I.Biol. Pp. xvi + 241. London: Shandon Scientific Company Ltd., 1965. Price 36s.

The first edition of this book appeared in 1962 and was reviewed in *The Analyst*, 1963, **88**, 568. This second edition follows the same general pattern but is considerably enlarged. Two important new sections have been added: inorganic paper chromatography, written by the late Dr. F. H. Pollard and Dr. G. Nickless; and thin-layer chromatography written by Dr. I. Smith and Mrs. M. Smith. The original material has been improved, particularly by the inclusion of some additional experiments of biochemical interest.

Any student, or indeed any chemist new to these types of chromatography, who makes use of this book will acquire a good working knowledge of, and possibly even an enthusiasm for, the techniques described. For the teacher there are available silent loop films in colour for use in conjunction with each section of the book; suggested commentaries appear in an appendix.

Although the text is written around a proprietary set of apparatus, this in no way detracts from its value. Perhaps one of the avowed intentions of the book, *i.e.*, to be an "aid to the busy teacher" is somewhat overdone. For example, each experiment is described fully, resulting in considerable duplication; this in itself is not serious, but it should be noted that the price of the book is rather high for a paper-back production. This, however, is a minor criticism of a useful introduction to these important topics.

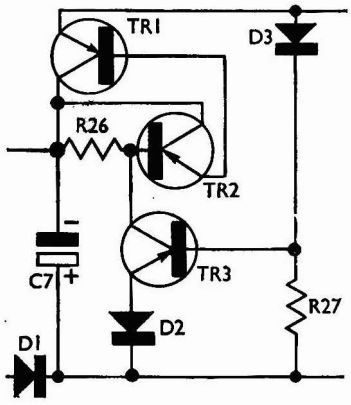
J. F. HERRINGSHAW

Errata

FEBRUARY (1965) ISSUE, p. 79, 1st line under REAGENTS. *For* "NN'-*Dimethylformamide*" *read* "N-*Dimethylformamide*."

AUGUST (1965) ISSUE, p. ~~497~~ 529, heading in middle of page. *For* "ESTABLISHED I.C.U.M.S.A. STANDARD METHOD I" *read* "TENTATIVE I.C.U.M.S.A. STANDARD METHOD I."

DECEMBER (1965) ISSUE, p. 707, Fig. 4. Transistors TR_1, TR_2 and TR_3 are shown wrongly connected. The correction published in the February (1966) issue was incorrect in that no junction was shown between R26, C7 and TR1. The circuit diagram between the secondary of transformer T_2 and capacitor C_8 should be replaced by—



FEBRUARY (1966) ISSUE, p. 124, 4th line. *For* "123 ml" *read* "123 g."