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THE ANALYST

Micro-analysis of Silicate Rocks

Part V. Spectrophotometric Determination of Alumina*

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Alumina is determined spectrophotometrically as the 8-hydroxyquinolinate after ligand exchange in the organic phase with aluminium acetylacetonate extracted into benzene from aqueous medium at pH 6 to 7. Interfering elements such as iron, titanium, vanadium and zirconium are removed by extraction into *o*-dichlorobenzene from M hydrochloric acid, but beryllium is not removed by this procedure.

THE direct determination of alumina in silicate rocks has long been a problem in rock analysis, and the several methods proposed tend to be rather lengthy. The British Ceramic Research Association method[1] separates aluminium as the hydroxo complex, and deals with any residual iron by reducing it and forming its bipyridyl or phenanthroline complex before precipitating aluminium 8-hydroxyquinolinate. Milner and Woodhead[2] make a chloroform extraction of the cupferron complexes of iron, titanium, etc., and determine aluminium by addition of excess of EDTA, boiling to complete complex formation, and back-titration of the excess with iron(III); salicylic acid is used as indicator. Miller and Chalmers,[3] working on the micro scale, preferred *o*-dichlorobenzene as solvent for the cupferron extraction, and separated aluminium (and beryllium) by extraction of the acetylacetonate into ether, following this by back-extraction into 6 M hydrochloric acid and precipitation as aluminium 8-hydroxyquinolinate at pH 5 to separate it from any beryllium. Sajó,[4] who has proposed an EDTA titration of iron and aluminium in the same solution, says nothing about the behaviour of titanium. Přibil and Veselý[5] have proposed methods for the determination of iron, aluminium and titanium together, but do not deal with the problem of zirconium. Kiss[6] proposed a DCTA titration of aluminium after extraction of iron and titanium, but a correction was required for co-titrated elements such as manganese. Voinovitch,[7] after making a critical examination of six methods, concluded that the most accurate results were obtained by removal of silica, reduction of iron(III), precipitation of aluminium and titanium as the basic benzoate, and correction for the titanium present. He considered that the colorimetric method with 8-hydroxy-7-iodoquinoline-5-sulphonic acid needed more study of interferences, and gave a rather larger error. None of the colorimetric methods so far proposed is particularly reliable, except for the 8-hydroxyquinoline method based on the work of Gentry and Sherrington,[8] and that developed by Riley and Williams,[9] but a preliminary separation of iron would still be required.

For routine use in the "rapid" methods of rock analysis, a colorimetric method would be desirable. When Růžička and Starý[10] published their paper on ligand exchange in the organic phase, it occurred to us that the acetylacetonate extraction method[3] could be converted into a colorimetric one if the aluminium acetylacetonate could be transformed into the 8-hydroxyquinolinate in the organic phase. The acetylacetonate itself is not suitable for colorimetric work because the absorption peaks of the reagent and complex are close together and occur in the ultraviolet region; we therefore examined this ligand-exchange system.

* Paper presented at the Second SAC Conference 1968, Nottingham.

For details of Part IV of this series, see reference list p. 632.

Experimental

Choice of reaction conditions—

In the original gravimetric method[3] diethyl ether had been used as solvent for the acetylacetonate extraction, but as this solvent is rather too volatile for use in colorimetric methods, we therefore sought other suitable extracting solvents. A standard 0·2 M aluminium solution was prepared from high-purity aluminium metal and standardised gravimetrically with 8-hydroxyquinoline.[11] Aliquots were adjusted to about pH 7 after addition of acetylacetone, and extracted[3] with various solvents. The extracts were stripped with 6 M hydrochloric acid and the aluminium determined.[3] Carbon tetrachloride, chloroform, benzene, toluene and pentyl acetate all gave 99·9 to 100·1 per cent. recovery of 2·68 mg of aluminium in replicate experiments. Of these, carbon tetrachloride and chloroform are inconvenient if the extractions are made in stoppered tubes and the upper layer is withdrawn with the aid of Witt's apparatus, and were not considered further. The number of extractions and the duration of shaking needed were found by determining the amount of aluminium extracted in a single extraction (to ensure complete removal of the organic phase, the walls of the tube were rinsed with small amounts of solvent, and the washings were added to the extract, but there was no intimate mixing of the wash liquid with the aqueous phase). It was found that 3 ml of benzene extracted 95·3 to 95·6 per cent. of the aluminium present in 2 ml of aqueous phase, whether the shaking time was 1 or 5 minutes. The amounts of aluminium taken ranged from 2·68 to 13·40 mg. When three extractions were made (3 ml, 3 ml, 1 ml) with 2 minutes of shaking, recovery of 2·68 to 13·40 mg of aluminium was 99·9 to 100·1 per cent.

The compatibility of the acetylacetonate extract and the 8-hydroxyquinoline exchange solution was next examined. Toluene seemed a more desirable solvent to use than benzene because of its lower volatility but, when a toluene solution of 8-hydroxyquinoline was mixed with a toluene extract, a turbidity often appeared before the exchange reaction was complete; it was later found that the turbidity depended on the grade of toluene used, and that toluene could be satisfactorily used as solvent. No turbidity appeared when benzene was used as solvent, but the exchange reaction was slow, taking 48 hours to reach completion. The slow rate of exchange could be accounted for if the exchange reaction proceeds via an SN_1 mechanism, as steric hindrance would prevent access of 8-hydroxyquinoline to the aluminium ion and use of the empty $3d$ orbitals of the latter for further co-ordination, and a further retarding effect might occur if the aluminium acetylacetone were polynuclear. The reaction will be slowed by the presence of the excess of acetylacetone used in the extraction step. The stability constants of the two complexes are sufficiently different ($\log \beta_3 = 22{\cdot}3$ for the acetylacetonate,[12] $\log 1/K_{sp} = 36$ for the 8-hydroxyquinolinate[13]) for exchange to be complete. The rate of reaction was accelerated by the classic expedient of heating the solution, and equilibrium was reached (constant absorbance) after heating for 15 minutes on a water-bath. The absorbance remained unchanged even after 2 hours' heating, and on cooling the solution to room temperature remained stable for several days.

The necessary volume of 8-hydroxyquinoline solution for complete exchange was found by applying the procedure to a fixed amount of aluminium (160 μg) and acetylacetone and by using various amounts of exchange solution. A plot of absorbance against volume of exchange solution added showed that the absorbance became constant when a 4-fold molar excess of 8-hydroxyquinoline had been added. The excess of reagent did not interfere with the spectrophotometric measurement of the aluminium complex at the absorption maximum 395 nm if an accurately measured amount was added. A Beer's law plot was linear over the range 0 to 200 μg of aluminium in 50 ml of solution, and was highly reproducible. The slope (measured under the conditions described in Procedure) was $0{\cdot}208_8$ ml μg^{-1}.

Interferences—

It was known from previous work[3] that of the elements commonly found in rocks, only beryllium was co-extracted with aluminium as the acetylacetonate, and beryllium occurs usually only in traces. As might have been expected, beryllium was also found to give an exchange reaction with 8-hydroxyquinoline. Attempts to mask either aluminium or beryllium in the aqueous phase, or to strip one of them preferentially from the organic phase, by use of fluoride, hydroxide or EDTA, all failed, and if beryllium is present in significant amounts a correction must be applied for it.

Freedom from other interferences was established by preparing synthetic silica-free "rock-sample" solutions (solution B in the Shapiro and Brannock scheme[14]) containing the equivalent of 12 per cent. of MgO, 16 per cent. of CaO, 2·8 per cent. each of TiO_2, P_2O_5, CoO, MnO, NiO and Cr_2O_3, and amounts of aluminium ranging from 10 to 23 per cent. The recovery of aluminium was 99·8 to 100·0 per cent.

Method

Reagents and apparatus—

As described in Part IV,[3] except that the 8-hydroxyquinoline solution is 2 per cent. w/v n benzene, and benzene is used instead of ether for the extraction.

Procedure—

Prepare a Shapiro and Brannock[14] "Solution B" and take an aliquot corresponding to not more than 200 μg of aluminium (400 μg of alumina). This will usually be equivalent to a 1-mg rock sample. If a sufficiently precise microbalance is available (and a sample ground finely enough) a sample can be weighed out and decomposed as described earlier,[3] and the solution used direct. Proceed with the cupferron and acetylacetone extractions as described in Part IV, but use benzene instead of diethyl ether. Transfer the benzene extract of the aluminium acetylacetonate into a 50-ml standard flask and add by safety-pipette 10 ml of 8-hydroxyquinoline solution in benzene. Mix, then heat the flasks on a steam-bath for 30 minutes, cool them to room temperature, dilute to volume with benzene, mix, and measure the absorbance at 395 nm in 10-mm cells against a reagent blank.

Results

The method was applied first to synthetic samples as already described, and then to various rock samples and standards. Because of the notorious uncertainty of the results of alumina determinations in silicate rocks,[15,16,17] recovery was checked by adding known amounts of standard aluminium solution to the aliquots taken for analysis. Results for the samples, spiked and unspiked, are shown in Table I, together with such comparison results

Table I

Alumina in standard rock samples

Sample	Weight used, mg	Al_2O_3 Found, per cent.	Al_2O_3 Certificate or other value, per cent.	Al^{3+} Added, μg	Al^{3+} Found, μg
Olivine basalt	1·045	12·4, 12·6	12·6[3,18]	30·9	30·9
Felspar 70*	1·038	18·1	17·8[3] 18·0*	46·4	46·3
S1 Syenite†	1·205	9·4, 9·4	9·0[19] 9·6†	30·9	31·0
BCR1 Basalt‡	1·459	13·1, 13·2	13·8‡§	30·9	30·9
T1 Tonalite§	1·038	16·3, 16·3	16·3[19] 16·5‖	61·7	61·8
G2 Granite‡	1·044	14·9, 14·9	15·5‡§	46·2	46·3
GSP1 Granodiorite‡	1·002	15·5, 15·5	15·4‡ 14·5§	15·4	15·4
GR Granite‖	1·006	14·2, 14·2	14·75¶ 14·5[19]	15·4	15·4
AGV1 Andesite‡	1·169	16·0, 16·1	17·2‡§	15·5	15·4
		16·0, 16·0		53·6	53·6, 53·6
PCC1 Peridotite‡	11·22	0·73	0·69‡ 0·91§	—	—
DTS1 Dunite‡	14·26	0·21	0·28‡ 0·39§	—	—

* U.S. Bureau of Standards.
† Canadian Association of Applied Spectroscopy.
‡ U.S. Geological Survey.
§ Geology Department, University of Aberdeen.
‖ Tanganyika Survey.
¶ Centre de Recherches Petrographiques et Geochimiques, Nancy.

as were available. The recovery in spiked samples (amounts of alumina added ranged from 2·5 to 11·0 per cent.) is good, and the results agree reasonably well with those obtained by other methods, except for the Andesite AGV1. Analysis of AGV1 by the Ceramic Research Association method[1] gave a still lower result than that obtained by the method described

here. We offer no explanation of this, although, as we had only about 1 g of sample to work with, the possibility of segregation or sampling error cannot be ruled out.

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Note—Reference 3 is to Part IV of this series.

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An Automatic Analytical Procedure for the Colorimetric Determination of Molybdenum in Steel

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A spectrophotometric method for the determination of molybdenum in steel, based on the colour reaction with thiocyanate in the presence of tin(II) chloride and a titanium catalyst, has been adapted for the Technicon AutoAnalyzer. The method is suitable for most steels, but small corrections may be needed in the presence of high concentrations of chromium, cobalt and vanadium. Nickel, manganese and silicon at the usual levels do not interfere. For low-alloy steels the initial solution is identical with that required for an existing automatic method for manganese and phosphorus, but is modified when required to deal with high-speed steels containing alloying additions of tungsten. Results on standard steels are presented, and a statistical survey of long-term reproducibility is given.

AN important factor in the development of rapid steelmaking during recent years has been the application of analytical techniques based on physical methods, for example, automatic ultraviolet and X-ray fluorescence spectrometry. Such physical methods depend on the availability of standard specimens accurately analysed by chemical procedures, which are also needed for the analysis of specimens of non-standard size or metallurgical condition. It is also prudent and necessary to check a proportion of the physical analyses by chemical means in order to detect errors and drifts in both systems. The automation of chemical analysis offers advantages in the use of skilled manpower, economy in time or reagents and improved reproducibility. For these reasons apparatus for automatic chemical analysis was installed in our laboratories nearly 2 years ago as an auxiliary to conventional automatic emission-spectrographic analysis.

Publications by Scholes and Thulbourne have described spectrophotometric analysis of steelmaking slags[1] for total iron and the oxides of aluminium, manganese and phosphorus, and steels[2] for phosphorus, manganese and silicon, with the Technicon AutoAnalyzer. More recently Gale, George and Williams[3] have described a method of using the instrument for the rapid determination of soluble aluminium in steel.

We have successfully used Scholes' method for manganese and phosphorus in steel in our laboratories and have extended the procedure to include the determination of molybdenum. The method is based on the satisfactory, but now superseded, British Standard Method B.S. 1121 : Part 34 : 1955, in which a sulphuric acid solution of the steel is prepared, molybdenum and iron(III) thiocyanates are formed, and the former is measured photometrically, after the latter has been selectively reduced by tin(II) chloride, in aqueous solution.[4]

EXPERIMENTAL

For economy in time and reagents it is desirable to use aliquots from the same solution of steel as prepared for the determination of phosphorus and manganese. The determination of phosphorus requires "reflux fuming" of the solution to convert phosphorus into orthophosphoric acid, and chromium is at the same time oxidised to chromate. It is necessary to reduce

this ion by a suitable reductant, and because iron is necessary for the formation of the molybdenum thiocyanate complex, the natural choice is an addition of iron(II) sulphate during the mechanised manipulation.

Preliminary "static" experiments with a Spekker absorptiometer showed that determinations made by the British standard procedure gave a stable colour after about 1 minute, although the procedure specifies a standing time of 15 minutes. This has an important practical bearing on the amount of delay required in the manifold designed for automatic analysis.

The first manifold was designed to pump reagents at rates proportional to the additions used in the B.S. method and with a sample input of 0·8 ml per minute. The recording colorimeter gave straight plateaux and base-line under these conditions, but was oversensitive, 0·5 per cent. of molybdenum giving an optical density of 1·3.

Use of a smaller sampling tube to reduce sensitivity produced problems in the segmentation of the reagents by air bubbles. Initially it was not possible to add enough air to maintain a satisfactory bubble pattern throughout the train but, when a second addition of air was made, interference of the two trains of bubbles produced unsatisfactory recorder traces. The problem was solved by adding a diluent acid at the point where the sample solution and air were mixed; the number of reagent additions was minimised by including the titanium catalyst in the diluent acid. However, an attempt to include the ammonium thiocyanate in the same reagent was unsuccessful because of the slow liberation of hydrogen sulphide during storage, followed by interaction with tin(II) chloride and precipitation of tin(II) sulphide in the AutoAnalyzer manifold. A further attempt to eliminate one mixing coil by combining ammonium thiocyanate and titanium sulphate solutions immediately prior to injection into the manifold was unsuccessful because base-lines and plateaux were not stable.

Eventually considerable modifications had to be made to all the reagent concentrations to obtain a sample solution procedure compatible with the published methods for manganese and phosphorus in steel,[2] covering the desired range of molybdenum content, and giving stable recorder traces. Much interaction was found between the reagent concentration variables, and the method that follows was reached after considerable experimentation.

Method

The manifold found to be satisfactory is illustrated in Fig. 1. It requires the following reagents.

Reagents—

Titanium sulphate - acid mixture—Dissolve 1·5 g of titanium metal in 200 ml of sulphuric acid (1 + 7) and cool. To 200 ml of water add cautiously and with stirring 160 ml of perchloric acid (sp.gr. 1·54) and 100 ml of sulphuric acid (sp.gr. 1·84), and cool. Add the titanium sulphate solution and dilute to 1 litre.

Iron solution—Dissolve 10 g of pure iron in hydrochloric acid (sp.gr. 1·16), oxidise by dropwise addition of nitric acid (sp.gr. 1·42), add 130 ml of perchloric acid (sp.gr. 1·54) and evaporate to fumes of perchloric acid. Cover, and allow to reflux for 5 minutes. Cool, redissolve in water, filter if necessary, and dilute to 1 litre.

Titanium - iron dilution solution—Mix 150 ml of the titanium sulphate - acid mixture, 100 ml of iron solution and 100 ml of water, then add 2·3 g of ammonium iron(II) sulphate, shake to dissolve and dilute to 500 ml.

Ammonium thiocyanate solution—Dissolve 200 g of ammonium thiocyanate in water and dilute to 1 litre.

Tin(II) chloride solution—Transfer 40 g of tin(II) chloride ($SnCl_2.2H_2O$) to a 400-ml beaker, add 10 ml of hydrochloric acid (sp.gr. 1·16) and digest until a clear solution is obtained. Add 120 ml of water, cool, filter and dilute to 200 ml.

Solvent acid—Add 100 ml of nitric acid (sp.gr. 1·42) to 80 ml of water, cool, add 800 ml of perchloric acid (sp.gr. 1·54) and dilute to 1 litre.

Standard molybdenum solution—Dissolve 0·5 g of pure molybdenum in 15 ml of nitric acid (sp.gr. 1·20) and 10 ml of hydrochloric acid (sp.gr. 1·16). Cool and dilute to exactly 500 ml.

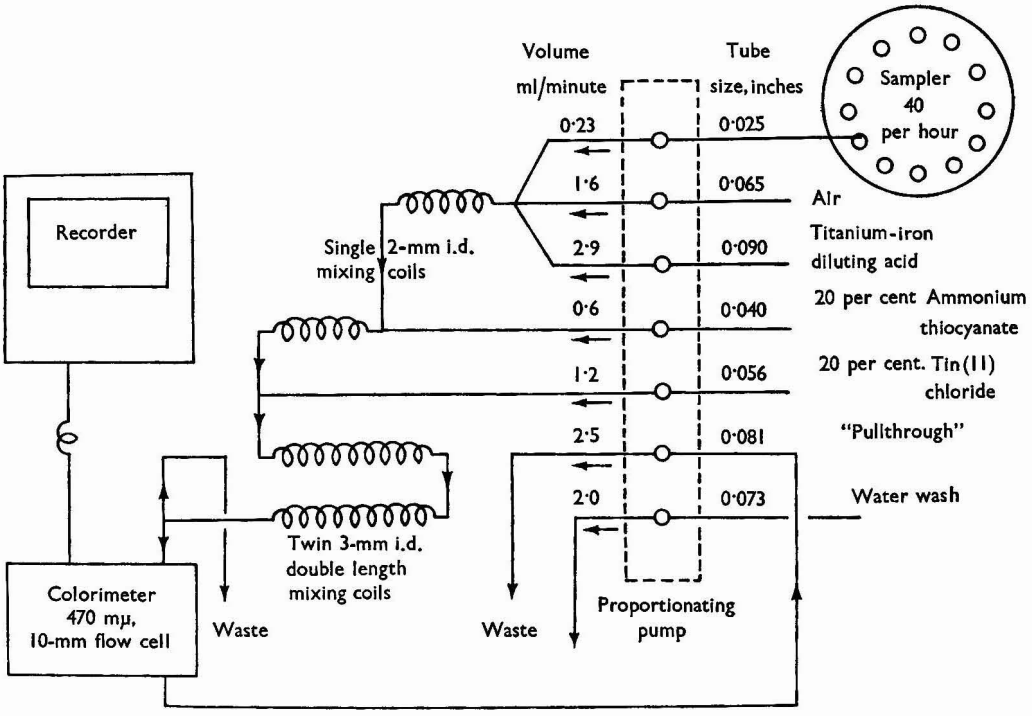

Fig. 1. AutoAnalyzer flow system for the determination of molybdenum in steel

CALIBRATION—

Weigh 1-g portions of pure iron into eight 350-ml conical flasks. Reserve one specimen as a blank, and to the remaining flasks add 1·0, 2·5, 3·5, 5·0, 6·5, 8·0 and 10·0 ml of standard molybdenum solution, equivalent to 0·10, 0·25, 0·35, 0·50, 0·65, 0·80 and 1·00 per cent. molybdenum, respectively. Follow the procedure described and use the peaks on the recorder trace to construct a calibration graph.

PROCEDURE FOR DISSOLVING THE SAMPLE—

(*a*) *Low alloy steels*—Weigh 1 g of steel sample into a 350-ml conical flask and dissolve it in 15 ml of solvent acid. Evaporate to fumes of perchloric acid and allow to reflux for 4 to 5 minutes. Cool, re-dissolve in 50 ml of water, transfer to a calibrated flask and dilute to 100 ml.

(*b*) *Highly alloyed steels not containing tungsten*—These can be dissolved in aqua regia and treated with 13 ml of perchloric acid (sp.gr. 1·54), then fumed as before. The automated additions contain sufficient iron(II) sulphate to deal with steels containing up to 25 per cent. of chromium after perchlorate oxidation.

Aliquots from the solution of steel prepared as described in (*a*) and (*b*) are suitable for AutoAnalyzer determination of phosphorus and manganese by published methods,[2] provided that the original step for arsenic separation, depending on volatilisation with hydrobromic acid, is re-inserted if phosphorus is to be determined.

(*c*) *Steels containing alloying amounts of tungsten*—Dissolve 0·5 g of sample in hydrochloric acid and digest until a clear solution is obtained, then oxidise by dropwise addition of nitric acid (sp.gr. 1·42). Add 10 ml of sulphuric acid (1 + 1) and evaporate to fumes, fume gently for 2 minutes, cool, add 30 ml of water and boil until the salts have re-dissolved. Cool, add 10 ml of ammonium citrate solution (500 g per litre), 1 ml of saturated aqueous sulphur dioxide solution, and neutralise with ammonia solution (sp.gr. 0·91), adding 3 to 4 ml

excess to dissolve the tungstic acid, if necessary warming the solution. Neutralise the solution with sulphuric acid (1 + 1) with litmus paper as indicator, and add 25 ml in excess. Cool, transfer to a 100-ml calibrated flask, dilute to the mark and mix. (This solution is unsuitable for phosphorus and manganese determinations.)

PROCEDURE FOR DETERMINING MOLYBDENUM—

Assemble the manifold shown in Fig. 1 and set the repetition rate at 40 samples per hour. If the steel solution is not clear, because of suspended particles of silica, filter a small portion through a dry filter-paper when filling the sample cups for the automatic sampler. Read the absorption of each sample from the recorder chart and convert molybdenum content into percentage by reference to a calibration graph prepared from calibration solutions analysed in a similar manner.

INTERFERING ELEMENTS

Elements occurring in steel that may cause interference include chromium, nickel, vanadium and cobalt. The effect of these and other elements has been investigated. Correct results for molybdenum at zero or 0·50 per cent. level were obtained from synthetic solutions containing additional reagents to simulate the presence of up to 30 per cent. of nickel, 30 per cent. of manganese or 3 per cent. of silicon. The interference from the background colour caused by tervalent chromium was small and can usually be neglected for low-alloy steels, 1 per cent. of chromium being equivalent to 0·001 per cent. of molybdenum. If a background compensation is needed, however, blank determinations can be obtained by substituting water for 20 per cent. ammonium thiocyanate solution at the manifold, and repeating the passage of the solutions to be analysed.

The text of B.S. 1121: Part 34: 1955, as amended in 1961, states that a small correction is needed if cobalt is present (0·005 per cent. of molybdenum per 1 per cent. of cobalt). The following results in Table I show the effect of cobalt additions to a 0·48 per cent. standard molybdenum steel. The error corresponds to an apparent rise in molybdenum content of 0·002 per cent. per 1 per cent. of cobalt present.

TABLE I

EFFECT OF COBALT ADDITIONS ON APPARENT ANALYSIS OF 0·48 PER CENT. MOLYBDENUM STEEL

Cobalt addition, per cent.	0	10	20	30
Molybdenum per cent.	$0{\cdot}47_5$	0·49	$0{\cdot}51_5$	0·54
Found, per cent.	0·48	0·51	0·52	0·54

The British Standard method also states that vanadium causes interference in a rather complex manner depending on both the molybdenum and the vanadium contents of the steel. Our experiments gave the results shown in Table II when vanadium was added to two standard steels and to pure iron.

TABLE II

EFFECT OF VANADIUM ADDITIONS ON APPARENT ANALYSES OF MOLYBDENUM STEELS

	Vanadium added, per cent.				
	0	0·20	0·40	0·80	1·00
Pure iron	0	0·005	0·008	0·010	0·013
B.C.S. 320 (0·22 per cent. of molybdenum)	0·23	0·23	0·235	0·23	0·23
Dunford Hadfields Ltd. steel ($0{\cdot}48_5$ per cent. of molybdenum)	0·49	0·50	0·50	0·50	0·52

The interference seems to be rather unpredictable, but barely significant when vanadium is less than 0·20 per cent., which it will be for most low-alloy steels. At higher levels the calibration solutions should be matched to the type of alloy to be analysed.

RESULTS ON STANDARD STEELS—

Table III shows the results of analyses for molybdenum with the AutoAnalyzer compared with the certificate value of several steels supplied by the Bureau of Analysed Standards Ltd.

TABLE III

RESULTS OBTAINED FOR MOLYBDENUM IN MILD AND LOW-ALLOY STEELS WITH THE AUTOMATED PROCEDURE

Steel No.	Steel type	Molybdenum, per cent. Auto-Analyzer	Molybdenum, per cent. Certificate value
B.C.S. 320	Mild steel	0·22	0·22
B.C.S. 321	with	0·07	0·068
B.C.S. 322	residuals	0·045	0·045
B.C.S. 324	series	0·10	0·10
B.C.S. 324		0·17	0·17
B.C.S. 325		0·16	0·16
B.C.S. 342	Ferritic stainless	0·695	0·69
B.C.S. 254	Low alloy*	1·30	1·29
B.C.S. 241/1	Tungsten	0·52	0·52
B.C.S. 241/2	high-speed	0·53	0·53
B.C.S. 481	steels with	$0·22_5$	0·22
B.C.S. 482	chromium,	0·27	0·27
B.C.S. 483	cobalt and	0·18	0·17
B.C.S. 484	vanadium †	1·09	1·07
B.C.S. 485		0·68	0·67

* 0·5 g of steel *plus* 0·5 g of pure iron.
† 0·5 g of steel dissolved by Procedure (*c*).

REPRODUCIBILITY—

Results that have been obtained over a period of 7 months on four standard steels, which have accompanied batches of routine determinations of molybdenum by the Auto-Analyzer procedure, have been submitted to statistical examination and are summarised in Table IV.

TABLE IV

LONG TERM REPRODUCIBILITY OF THE AUTOMATED PROCEDURE

Steel No.	Steel type	Number of results	Molybdenum, per cent. Range of results, minimum to maximum	Molybdenum, per cent. Standard deviation	Molybdenum, per cent. Mean results	Molybdenum, per cent. Accepted value
B.C.S. 320	Mild steels with	48	0·19 to 0·23	0·006	$0·21_9$	0·22
B.C.S. 324	residuals	23	0·16 to 0·19	0·006	$0·17_3$	0·17
B.C.S. 342	Ferritic stainless	20	0·67 to 0·70	0·014	$0·68_8$	0·69
Dunford Hadfields standard	1·5% Cr, 0·7% Ni	21	0·48 to 0·51	0·011	$0·49_2$	$0·48_5$

Typical coefficients of variation are found to be 2 to 3 per cent. of molybdenum content; these are similar to the variation from conventional "static" colorimetric methods. With the present method, 1·0 per cent. molybdenum gives an optical density of about 0·85, and the exponential response enables 0·01 per cent. molybdenum to be readily observed. However, by appropriate changes in pumping rates or in sample weight or dilution, the manifold could be adapted to measure molybdenum content in steel over smaller or greater ranges.

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The Fluorescence Quantum Efficiencies of Some Analytically Useful Chelate Complexes

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A computer method is described for calculating the quantum efficiencies of fluorescent compounds and chelate complexes in both aqueous and organic solvents. The quantum efficiency values can be incorporated into a sensitivity factor, *S*, which may be used to evaluate and assess analytically useful fluorescent complexes.

Limits of detection in spectrofluorimetric analysis depend to a large extent on the techniques and apparatus used and, therefore, a true measure of the sensitivity of a fluorimetric reagent cannot be obtained merely by comparison of limits of detection derived from diverse sources. An instance of this is the determination of the alkaline earth metals, calcium and magnesium. Here, the common occurrence of these elements frequently affects the magnitude and the reproducibility of the blank and, consequently, the limits of detection quoted by various authors ar reflections of the extent to which the solvents have been purified. Thus, a reliable g de to the sensitivity of an organic, fluorimetric reagent can only be obtained from physical measurements of fluorescence quantum efficiency, extinction coefficient and half-band width of the fluorescence-emission spectrum.

The total fluorescence-emission intensity of a species present in a dilute solution is given by—

$$F = 2{\cdot}303\ \mathrm{K}\phi I_0 \epsilon . c . d \qquad (1)$$

where K is a constant depending on instrumental factors, ϕ is the quantum efficiency of fluorescence, ϵ is the molar extinction coefficient of the fluorescent species at the wavelength of excitation (which need not correspond to a maximum value in the excitation spectrum), c is the molar concentration of the species, d is the excitation path length and I_0 is the incident radiation intensity in units of quanta.

Only the values of ϕ and ϵ are affected by the choice of spectrofluorimetric reagent. However, it is normal analytical practice to observe only a narrow band width of the total fluorescence-emission spectrum, hence the shape of the spectrum may be allowed for by introducing H, the half-band width of the emission spectrum. The sensitivity factor, S, for the metallofluorescent reagent is then given by—

$$S = \frac{\epsilon \phi}{H} \qquad (2).$$

Parker and Rees[1] have suggested the use of such a sensitivity index, for comparison of fluorescence, but have used values of H in wavenumber units of cm^{-1} and ϵ in units based on mass (μg per ml). As most commercial spectrofluorimeters are now calibrated in wavelength units of nm, the authors recommend the use of H in units of nm. Also, the use of the molar extinction coefficient ϵ would appear to have more significance than a mass-based ϵ. If sufficient instrumental parameters are available, the absolute fluorescence signal in microamps can be calculated.[2] Further, if a noise factor can be calculated or assumed, the limit of detection of the compound can be obtained.[2] For both of these calculations a knowledge of the S factor is necessary.

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REAGENTS—

Quinine sulphate standards—These were prepared from quinine (British Drug Houses Ltd.), which was recrystallised from aqueous ethanol and dried to constant weight over phosphorus pentoxide before being dissolved in 0·1 M sulphuric acid to give a 1000 p.p.m. solution. More dilute solutions were prepared by dilution with 0·1 M sulphuric acid.

Aromatic hydrocarbons—These were obtained from a collection of purified specimens held by the Organic Chemistry Department, Imperial College.

Dyestuffs and indicators—B.D.H. Indicator grade were used without further purification.

Azomethine reagents—These reagents were prepared in this laboratory for a previous study.[3]

Calcein—As supplied by G. F. Smith Chemical Co., Columbus, Ohio.

All solvents used were of analytical-reagent grade purity; water was distilled from an all-glass apparatus.

APPARATUS—

Measurements were carried out with a Farrand spectrofluorimeter. The photomultiplier and exit monochromator were calibrated for spectral response by using a 1-kW tungsten lamp, run under specified conditions such that the filament operated at a colour temperature of 2854° K.

EXPERIMENTAL

Determinations of quantum efficiency were carried out by using the relative method described elsewhere in detail.[1,4] Quinine sulphate was used as the standard ($\phi = 0{\cdot}55$), and the correction procedure used was the same as outlined by the above authors.

The photomultiplier (RCA 1P 28) and exit monochromator were calibrated for their relative quantum spectral response by using a standard tungsten lamp (380 to 650 nm). Fluorescent screens of rhodamine B,[5] rhodamine B with acriflavine,[5] and fluorescein,[1] were used to calibrate the xenon arc lamp of the apparatus (250 to 400 nm); the xenon lamp was then used to calibrate the detector system over the lower wavelength region. A method similar to that previously described by Parker[6] was used. A calibrated xenon lamp is not necessary for the evaluation of the results; it is only used here as a secondary standard for calibration of the lower wavelength range of the photomultiplier and emission monochromator.

Fluorescence-emission spectra were obtained for quinine sulphate and unknown solutions by using the same wavelength of excitation. This eliminates the need for a calibrated excitation system and depends on the constancy of the quantum yield of the standard or unknown for excitation wavelengths lower than that of the long wavelength absorbance maximum. Absorbances were measured by placing the photomultiplier of the spectrofluorimeter so that the transmitted incident radiation was viewed directly. In this way, errors that might have arisen from differences in the spectral band widths of the spectrofluorimeter and absorption spectrophotometer were eliminated. Absorbances were measured on concentrated solutions (about 10^{-5} M), and the results used to calculate the absorbances of diluted solutions, assuming Beer's law was obeyed. Ten fluorescence spectra were obtained for each complex, covering a concentration range of two orders of magnitude. Where absorbances exceed 0·02, the filter effect on the excitation light was corrected for, by using a factor, f, which was empirically evaluated from standards. In instances when there is an overlap of excitation and emission spectra (*i.e.*, a filter effect on the emission, as well as excitation radiation), a plot of $\frac{1}{\phi}$ *versus* concentration would have given a shallow curve instead of a straight line parallel to the concentration axis. However, we did not observe any self-absorption of this nature with any of our dilute solutions. The values of $\log f$ were proportional to concentration, as would be expected from Beer's law. At the concentrations used, self-quenching by collisional de-activation can be assumed to be negligible[5] because of the large intermolecular distances involved. Oxygen quenching was considerable with some of the organic compounds listed in Table I, although none of the compounds in Table II, or quinine sulphate, exhibited this to any appreciable extent. Oxygen quenching was eliminated by bubbling nitrogen through the solutions for 10 to 15 minutes before measurement. The fluorescence of all of the solutions was measured at 20° C.

The complexes examined had metal-to-ligand ratios of 1 : 1 or 2 : 1. Because no complexes of the ratio 1 : 2 were formed, a large excess of metal ion could be used to ensure complete reaction of the chelating reagents (about 10^2 to 10^3-fold molar excesses of metal were used). The pH of aqueous alkaline solutions was adjusted to the recommended value with diethylamine. With non-aqueous solutions a few drops of diethylamine were added.

CALCULATION—

The equation used to derive the result from the experimental values is given below[1,4]—

$$\phi = \frac{F.(\epsilon.c.d)_q\phi_q}{F_q.(\epsilon.c.d)} \qquad (3)$$

where the subscripts, q, refer to the quinine standard, all other symbols having been defined earlier. F is the total fluorescence emission and is equal to the area under the corrected emission spectrum.

Fluorescence-emission spectra were digitised at equally spaced frequency intervals, and then corrected for spectral sensitivity variations in the detection system by using the correction factors described earlier. This is normally a tedious and time-consuming process, but was facilitated by the use of a computer. Input to the computer consisted of one card for each frequency. On the card were punched the frequency, the correction factor and up to ten intensity data points for that frequency, taken from different fluorescence spectra. Thirty-one cards constituted a set and the programme was designed to correct the data, integrate the corrected spectrum by using Simpson's rule, normalise the corrected and uncorrected data and finally print out the correct and uncorrected spectra, together with the result of the integration. Calculations were performed on the Imperial College I.B.M. 7090 computer, with a FORTRAN IV programme.[7] Drushel, Sommers and Cox[4] have described the use of a computer programme for the same purpose, although their programme differed considerably from ours. The results for F and F_q obtained from the computer were substituted into equation 3 to give ϕ.

Previous authors[8] have noted that comparisons of fluorescence between substances in different solvents must allow for effects arising from the different refractive indices of the media used. These corrections are only of second order importance and differ according to the optical system used. The main correction is to account for the difference in solid angle viewed by the exit monochromator; for our apparatus this was obtained by multiplying the quantum efficiency of fluorescence by the ratio $(n/n_q)^2$ where n is the refractive index of the solvent containing the unknown and n_q is the refractive index of 0·1 M sulphuric acid.

RESULTS AND DISCUSSION

To test the experimental technique and calculation, the results for the quantum efficiencies of some standard compounds are given in Table I. These results are for single determinations only. Several values obtained by both relative and absolute methods are quoted in the literature, and our results appear to be in good agreement. Values for rhodamine B given in the literature range from about 0·6 to nearly unity, and are probably a result of the different ionisation modes of this dyestuff. Reproducible values would only be expected when buffered, aqueous solvents are used. In addition, the dyestuffs rhodamine B and eosin are difficult to obtain in a pure form. Acriflavine, although a mixture of methylated aminoacridines, appears to give reproducible values of ϕ, whenever these have been quoted in the literature. In Tables I and II, $\phi_{corr.}$ values are those which have been corrected for refractive index of the solvent.

The results of quantum efficiencies of fluorescence for the magnesium - azomethine complexes are given in Table II. Standard deviations have been calculated after neglecting the highest and lowest values of ϕ within each set of ten. We have also corroborated earlier results[3] obtained in this laboratory by finding that the corresponding calcium complexes had ϕ values that were immeasurably small. The free reagents were also non-fluorescent, although they gave fluorescent hydrolysis products in aqueous solution. It is probable that the most important factor in determining the quantum efficiencies of these complexes is the energy of the n - π^* transition, relative to the π - π^* transition. If n - π^* transitions due to the azomethine nitrogen atoms occur at lower energies than the π - π^* transitions, a non-fluorescent or weakly fluorescent species will result as a consequence of increased inter-system crossing

to the triplet state.[9] On complexing the reagent with a non-transition metal, the π-π^* transition is generally unchanged relative to the π - π^* transition of the fully ionised reagent. However, the n - π^* transition is always shifted to higher energies and, if shifted sufficiently, a fluorescent species may result. This effect has even been observed on formation of hydrogen bonds and has been used to explain solvent-dependent fluorescence.[10] Because of the low extinction coefficients of n - π^* transitions (ϵ = 10 to 100), they can be experimentally observed only in certain instances. Other authors have used this mechanism to account for the fluorescence of the complexes of 8-hydroxyquinoline[11] and also for the fluorescence of this reagent in strongly acidic solutions.[12] In view of this, it is unlikely that the reagents are non-fluorescent at room temperature solely because of internal conversion de-activation processes caused by free rotation of the molecule; this would not account for the fluorescence of the chemically similar calcium complexes.

One more useful observation can be made from the results for the magnesium complexes listed in Table II. For several years the reagent bis-salicylidene ethylenediamine has been regarded as the most sensitive reagent for magnesium, no doubt because of the careful control of acidity and solvent purity.[13] Table II shows that other reagents may well approach, or even exceed, the limit of determination quoted by White and Cuttitta.[13] In addition, these reagents may have different properties with regard to selectivity, which may render them even more suitable for a particular application.

TABLE I

QUANTUM EFFICIENCIES OF SOME STANDARD ORGANIC COMPOUNDS

Compound	Solvent	Concentration, μg per ml	ϕ	$\phi_{corr.}$	Reference
Acriflavine	Water	4·0	0·57	0·57	0·56[14], 0·54[15]
Anthracene	Ethanol	0·5	0·28	0·30	0·27[5], 0·30[14]
			0·26	0·28	
Coronene	Chloroform	10·0	0·30	0·33	0·3[16]
Eosin	Sodium hydroxide, 0·1M	2·0	0·12	0·12	0·12[17], 0·15[18]
Fluorescein	Sodium carbonate, 0·1M	1·0	0·82	0·82	0·77[17], 0·79[17]
	Sodium hydrogen carbonate, 0·1M		0·81	0·81	0·85[1], 0·80[18]
Rhodamine B	Ethanol	1·0	0·66	0·70	0·69[1], 0·76[4]
Rubrene	Benzene	10·0	1·02	1·17	1·02[17]

TABLE II

QUANTUM EFFICIENCIES OF FLUORESCENCE OF SOME COMPLEXES COMMONLY USED IN THE DETERMINATION OF THE ALKALINE EARTH METALS

Complex	ϕ	$\phi_{corr.}$	δ $\times 10^{-3}$	δ per cent.	ϵ_λ $\times 10^3$	λ, nm	H, nm	S
Mg - SABF	0·21	0·23	8·7	4·1	23·0	492	47·2	112
Mg - SOPD	0·16	0·18	18	11	50·0	410	70·1	86
Mg - SED	0·39	0·45	23	5·9	13·5	360	85·6	114
Mg - Cal.	0·79	0·79	55	7·0	63·7	485	54·8	92
Ca - Cal.	1·01	1·01	80	8·0	52·2	485	43·5	130
Sr - Cal.	0·74	0·74	63	8·5	50·3	485	52·6	71
Ba - Cal.	0·81	0·81	77	9·0	52·2	485	54·4	78

SABF is *NN'*-bis-salicylidene-2,3-diaminobenzofuran in dimethylformamide.
SOPD is *NN'*-bis-salicylidene-*o*-phenylenediamine in dimethylformamide.
SED is *NN'*-bis-salicylidene ethylenediamine in dimethylformamide.
Cal. is calcein in water.
ϕ = quantum efficiency.
$\phi_{corr.}$ = quantum efficiency corrected for refractive index of solvent.
δ = standard deviation.
ϵ = molar extinction coefficient of fluorescent species at wavelength of excitation.
λ = absorption maximum of complex (not wavelength of excitation at which ϕ is measured).
H = half-band width of fluorescence-emission spectrum.
S = sensitivity factor $\left(\text{equal to } \frac{\epsilon\phi}{H}\right)$.

The generation of fluorescence in calcein on complexing with alkaline earth metals is more difficult to explain than with the azomethine reagents. This is mainly because of the

large number of ionisation modes of this reagent in the ground-state; each of these ground-state ionisation modes will have corresponding excited-state ionisation modes occurring at different pH values. Calcein is essentially a fluorescein molecule, substituted in the 2- and 4-positions with a carboxymethylaminomethyl group, and it is worth noting that the fluorescence efficiencies of the calcein complexes are of the same order as that of the fluorescein molecules. The sensitivity values quoted for the calcein complexes suggest that calcein would be the most sensitive fluorimetric reagent for determination of the alkaline earth metals. However, as we have already pointed out, the higher degrees of selectivity obtainable with other reagents may well prove to be the more important factors in analysis.

Since submitting this paper we have noted a similar comparison[19] of common reagents for aluminium, in which a sensitivity factor defined by $S = \epsilon\phi$ was used.

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An Evaluation of Some Methods for the Determination of Fluoride in Potable Waters and Other Aqueous Solutions

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Five spectrophotometric procedures for the determination of fluoride in water have been evaluated with respect to reproducibility, sensitivity, range, stability of coloured products and of reagents, specificity and effect of temperature. The thorium nitrate titration is briefly discussed, and the use of the Orion fluoride-ion electrode for pF measurement has also been investigated. Various samples of water containing natural or added fluoride have been analysed by four of the spectrophotometric methods, and the results compared with those obtained by titration and with the electrode. The electrode is shown to be less susceptible than the colorimetric methods to interference from other ions in solution, and it gives theoretical recoveries of fluoride added to several drinking water supplies.

FLUORINE is extensively distributed throughout nature, and has been detected in such diverse substances as water, rocks and minerals, fossils, teeth, foodstuffs and many biological specimens. Interest in the fluorine content of these materials arises chiefly from the toxic effects of prolonged ingestion of small amounts of fluorine (fluorosis), together with the detrimental results of sub-optimum levels in the diet. There is, therefore, a need for a method for the determination of trace amounts of fluorine, which is applicable to a wide variety of substances. Ideally, the method should also be simple and rapid, as many analyses may have to be carried out on a routine basis.

Methods of analysis vary with the nature of the starting material and the particular interfering elements to be removed. Thus urine,[1] the chief medium for the excretion of fluorine from the body, is first evaporated to dryness in the presence of alkali, the residue ashed to destroy organic matter, then the fluoride is distilled to separate it from interfering ions and finally determined in the distillate. On the other hand, samples of water[2] are frequently analysed directly for fluoride, although more accurate values can be obtained after prior distillation.

The distillation procedure is time consuming, potentially hazardous and can only be used by a skilled analyst, as it requires careful control to obtain reliable results. Recovery of fluoride depends on the design of the still, the concentration and nature of the acid used, temperature control and rate of distillation and the volume of distillate collected. Modern techniques are based on the studies and recommendations of Willard and Winter,[3] and are satisfactory as the recoveries of fluoride fall regularly in the range 95 to 100 per cent. An alternative apparatus, which incorporates a constant-temperature jacket of refluxing *sym*-tetrachloroethane, has been described by Samachson, Slovik and Sobel.[4] This method has also been used successfully by Glover and Phillips[5] in their studies on fossils. Some attention has been paid to diffusion,[6] as an alternative to steam-distillation, to effect the fluoride separation. This technique is hardly any less time consuming except, possibly, when many determinations have to be made concurrently.

Most colorimetric methods proposed for the final determination of fluoride depend on the bleaching action of fluoride ions on a particular organometallic dye complex.[7,8,9,10,11] The optimum reaction conditions have been investigated by many workers over the years, but the methods are subject to interference by other ions, which can also form stable complexes with either the metal or the fluoride ions present. A new principle was established in 1959 when Belcher, Leonard and West[12] reported the reaction between the red cerium(III) chelate of alizarin complexone and fluoride ions. The resulting complex is blue, and contains fluorine

and cerium - alizarin complexone in a 1:1 molar ratio.[13] This is the first positive colour-development reaction of the fluoride ion and, as a result of these investigations, a new colorimetric procedure was evolved.[14] This method, as modified by Greenhalgh and Riley,[15] has much greater sensitivity than conventional "bleaching" techniques but, unfortunately, is still subject to interference from several ions.

A further advance[16] is the recent development of an ion-specific electrode for fluoride which, it is claimed, is unaffected by a large excess of the common interfering ions and exhibits a Nernstian response over a wide concentration range. This electrode has been used by Raby and Sunderland[17] to determine fluoride in tungsten, following a simple fusion. Fluoride was then determined directly, without the need for any separation step. From this point of view, the electrode appears to be suitable for the determination of fluorides in water and other aqueous solutions.

In this work, the four most frequently used reagents for the determination of fluoride have been investigated. The spectrophotometric procedures are critically evaluated, with particular regard to linearity and range of calibration graph, reproducibility, sensitivity and limit of detection, stability of colour produced and of reagents, effect of temperature and interfering ions. The titration method is included as it is the official method of the Society for Analytical Chemistry. Finally, the use of the Orion ion-specific electrode with several aqueous solutions is reported, and the results compared with those obtained by using colorimetric procedures.

Methods examined are given below.

Method	Reagent	Procedure	Reference
1	Alizarin red S	Spectrophotometric	7
2	Alizarin red S	Titration	8
3	Eriochrome cyanine R (Solochrome)	Spectrophotometric (Cooke, Dixon and Sawyer's method)	9
4	Eriochrome cyanine R (Solochrome)	Spectrophotometric (Megregian's method)	10
5	SPADNS	Spectrophotometric	11
6	Alizarin complexone	Spectrophotometric	15
7		Ion-specific electrode	18

Experimental

Apparatus—

Optical density measurements were made in 10, 20 or 40-mm glass cells with a Unicam SP500 or SP600 spectrophotometer, de-mineralised water or the recommended reference solution being used in the compensating cell. An E.E.L. Spectra (Evans Electroselenium Ltd.) filter absorptiometer was also used; pF readings were obtained with the Orion, Model 94–09, electrode, with an E.I.L. (Electronic Instruments Ltd.) calomel reference electrode on a Pye "Dynacap" expanded-scale pH meter.

Reagents—

Analytical-reagent grade chemicals were used when possible.

The dyestuff reagents commercially available were of laboratory-reagent quality, and frequently contained considerable and varying amounts of inorganic and possibly other impurities. Samples were obtained from more than one manufacturer, but were purified only when specified in the method.

A fluoride stock solution was prepared by dissolving 0·221 g of dry sodium fluoride in de-mineralised water and making the solution up to 1 litre. This solution was stored in a polythene container.

A fluoride working solution was prepared by diluting 100 ml of the fluoride stock solution to 1 litre. This solution was also stored in a polythene container and found to be stable for at least 3 months.

(1 ml of solution $\equiv$ 10 μg of fluoride.)

Technique—

Each method was assessed by at least two experienced analysts working independently. In the main, the work was carried out in two different rooms within the same laboratory, where the temperature varied over the range 15° to 25° C. The instructions laid down

originally for each method were closely followed, but the interpretation of these instructions varied between analysts in some instances. For example, one analyst added the reagents from burettes while another used pipettes. In one study the colour development was carried out in calibrated flasks, while in another graduated Nessler cylinders were used. When alternative procedures were possible in a method, both were examined. No attempt was made to modify the concentrations of the reagents to produce colour intensities nearer to the optimum sensitivity range of the spectrophotometer.

In a preliminary study of each method, a calibration graph was prepared and the wavelength of maximum absorption checked. Thus, familiarity with the manipulative techniques of the methods was achieved before attempting to assess the precision of each method.

Results with spectrophotometric methods

Linearity of standard graph—

A standard graph was prepared for each method after the reagents had aged for at least 4 hours. Additional checks were made after 1 day and again at longer intervals to determine the stability of the reagents. Methods 1, 5 and 6 gave a graph that was linear to within ±2 per cent. over the range stated in Table I, which also gives the path length

Table I

Linear range of fluoride standard graphs

Method	Linear range: Fluoride, μg	Linear range: Fluoride, p.p.m.	Path length of cell used, cm	$\lambda_{max.}$, nm
1. Alizarin (photometric) ..	0 to 250	0 to 2·5	1	535
	0 to 200	0 to 2·0	2	
3. Eriochrome cyanine R .. (Cooke, Dixon and Sawyer)	0 to 15	0 to 0·8	2	540
4. Eriochrome cyanine R .. (Megregian)	0 to 60	0 to 1·2	1	527·5
5. SPADNS	0 to 70	0 to 1·4	1	570
6. Alizarin complexone ..	0 to 25	0 to 1·25	1	620
	0 to 12	0 to 0·6	2	
	0 to 6	0 to 0·3	4	

of the cell used for the optical density measurements and the wavelength of maximum absorption found experimentally. Methods 3 and 4 showed a more marked deviation from linearity. Method 3 gave the least satisfactory standard graph, as all three analysts found that it was difficult to achieve reproducible results from day to day. Table II shows the range of optical densities obtained by one analyst, together with the mean value and the calculated standard deviation for each point on the graph. It can be seen that the variation was greatest for the 15-μg sample and least for the 25-μg sample. Application of the statistical F-test to the ratio of the variances determined for the 15 and 25-μg samples shows that the variation observed at the 15-μg level is not significantly different from that found at the other levels. However, the shape of the graph obtained from the mean values in Table II was a little closer to linearity than that published by the original authors.

Table II

Standard graph with Eriochrome cyanine R

	Method No. 3			
Fluoride, μg	Optical density range	Mean optical density	Standard deviation	Degrees of freedom
0	0·895 to 0·955	0·927	0·0180	11
5	0·720 to 0·801	0·759	0·0187	11
10	0·543 to 0·613	0·590	0·0174	11
15	0·377 to 0·440	0·419	0·0203	11
20	0·246 to 0·299	0·283	0·0171	11
25	0·153 to 0·203	0·179	0·0135	11
30	0·076 to 0·133	0·118	0·0191	11

In view of the observed variation with this reagent, it was decided to investigate the procedure described by Megregian,[10] by using the same reagents but at different concentrations. Further work in which Megregian's reagents were used was reported by Sarma.[19]

Megregian's results were confirmed by the present authors, but the reproducibility of methods 3 and 4 over the full range of the calibration graph appeared to be about the same.

With all the methods it was generally necessary to prepare a new standard graph for each batch of reagents prepared. However, the difference between successive standard graphs, even between those prepared by different analysts, in method 6 was very small. A check determination at a single concentration of fluoride is, therefore, probably sufficient when using this method for most applications, except when the highest degree of accuracy is required.

The standard graph obtained after 3 hours in method 1 differed significantly from those produced after the reagents had aged for 1 day, or more. It is considered, therefore, that the recommended ageing time of 1 hour for the reagents in method 1 is insufficient.

Stability of coloured products—

Table III illustrates the change in optical densities with increasing time of colour development for each method. Generally, the colour fades with time, but the rate of change is sufficiently slow to permit accurate determinations of fluoride content without too rigid a time limit. In method 1, however, the change is more marked, and it is recommended in

Table III

Changes in optical density with development time

	Method	Fluoride taken	Optical densities after development for: 0 min.	10 min.	15 min.	20 min.	30 min.	45 min.	60 min.	75 min.	90 min.	120 min.	240 min.
1.	Alizarin (photometric)	1·0 p.p.m.	—	—	—	—	—	0·288	0·296	0·304	0·313	0·324	0·336
		Nil*	—	—	—	—	—	0·417	0·426	0·433	0·438	0·446	0·451
3.	Eriochrome cyanine R (Cooke, Dixon and Sawyer)	10 μg	0·285	—	0·275	—	0·276	—	0·278	—	0·277	—	—
4.	Eriochrome cyanine R (Megregian)	1·0 p.p.m.	0·561	0·561	—	—	0·559	—	0·559	—	0·560	0·560	—
		Nil*	1·16	1·19	—	—	1·19	—	1·18	—	1·16	1·18	—
5.	SPADNS	0·8 p.p.m.	0·215	—	—	—	—	—	0·207	—	—	0·202	0·202
		Nil*	0·372	—	—	—	—	—	0·372	—	—	0·362	0·362
6.	Alizarin complexone	5 μg	—	0·231	—	0·230	0·230	—	0·226	—	0·222	—	—

* Blank determination.

the method that the optical densities be measured 60 ± 2 minutes after addition of the reagents to the sample. However, it can be seen in Table III that the *difference* in optical density between the sample and the blank changes only slowly with time.

Table IV

Stability of reagents on ageing

	Method	Fluoride taken	Optical densities after ageing for: 1 day	1 week	12 days	3 weeks	4 weeks	5 weeks
1.	Alizarin (photometric)	Nil*	0·42	0·41	—	0·40	—	0·40
		1·0 p.p.m.	0·28	0·27	—	0·27	—	0·27
		2·5 p.p.m.	0·10	0·09	—	0·08	—	0·09
3.	Eriochrome cyanine R (Cooke, Dixon and Sawyer)	10 μg	0·55	0·55	—	0·58	—	0·54
4.	Eriochrome cyanine R (Megregian)	Nil*	1·15	1·18	—	1·12	—	1·16
		1 p.p.m.	0·58	0·56	—	0·61	—	0·57
5.	SPADNS	1 p.p.m.	0·176	0·176	—	0·182	—	0·175
6.	Alizarin complexone	6 μg						
	(in daylight)		0·278	—	0·263	—	0·251	—
	(in the dark)		0·278	—	0·278	—	0·280	—

* Blank determination.

STABILITY OF REAGENTS—

It is convenient in routine analysis to be able to use a reagent that is stable for several weeks when prepared. As can be seen in Table IV, there is no evidence to suggest that instability of any of the reagents studied would give rise to serious errors over a period of a few weeks. The alizarin complexone combined reagent deteriorates slightly when kept on the open bench, but it is more stable when stored in the dark.

REPRODUCIBILITY AND SENSITIVITY—

Most methods for the determination of fluoride can only be used over a narrow concentration range (see Table I). In practice, measurements are made at or about the 1 p.p.m. level and, accordingly, the reproducibility of each method was assessed at this value alone. To enable the limit of detection to be determined, the reproducibility of the blank was also determined.

TABLE V

REPRODUCIBILITY AT A CONCENTRATION OF 1 P.P.M. OF FLUORIDE

	Method	Analyst	Mean optical density	Standard deviation	Degrees of freedom	Standard deviation/ sensitivity
1.	Alizarin (photometric)	A	0·158	0·0014	20	1·0
		B	0·280	0·0011	20	0·8
		C	0·217	0·0011	20	0·8
3.	Eriochrome cyanine R	A	0·273	0·013*	30	0·9
	(Cooke, Dixon and	B	0·61	0·043†	20	3·0
	Sawyer)	C	0·283	0·017*	20	1·1
4.	Eriochrome cyanine R (Megregian)	A	0·575	0·026	20	2·4
5.	SPADNS	A	0·195	0·007	30	1·8
		C	0·184	0·003	20	0·8
6.	Alizarin complexone	A	0·240	0·006	20	0·24
		B	0·220	0·007	20	—

* 20 μg of fluoride.
† 10 μg of fluoride, with an E.E.L. Spectra.

Optical densities of 20 to 30 replicate samples, each containing 1·0 p.p.m. of fluoride, were recorded over a period of 1 to 2 weeks. The standard deviations were then calculated in accordance with accepted practice and are shown in Table V. It can also be seen that methods 3 and 4 are considerably less reproducible than the remaining methods. However, as the sensitivities of the methods differ so widely (Table VI), it was felt that the magnitude of the standard deviation was not, on its own, a fair measure of the relative reproducibility of each method. Sensitivities were calculated from the slope of the standard graph, within

TABLE VI

SENSITIVITY AND LIMIT OF DETECTION

	Method	Sensitivity: From standard graph (optical density per cm per μg of fluoride)	Sensitivity: Change in optical density over the range 0·9 to 1·1 p.p.m. of fluoride	Limit of detection: Standard deviation of blank	Limit of detection: Degrees of freedom	Limit of detection: Fluoride, p.p.m.
1.	Alizarin (photometric)	0·001	0·015	0·0055	20	0·02
3.	Eriochrome cyanine R (Cooke, Dixon and Sawyer)	0·015	0·055* 0·120†	0·0188	11	0·08
4.	Eriochrome cyanine R (Megregian)	0·011	0·11	0·017	6	0·10
5.	SPADNS	0·004	0·040	0·0055	8	0·02
6.	Alizarin complexone	0·025	0·100	0·0021	10	0·02

* 10-ml sample.
† 20-ml sample.

the linear range, allowance being made for the path length of the cell used. It is considered that the value obtained by dividing the standard deviation by the sensitivity (Table V) represents a more accurate estimate of the true reproducibility of each method. The differences observed between methods 1, 3, 4 and 5 are probably not significant, but the results suggest that, on this basis, method 6 (alizarin complexone) is considerably more reproducible than the others at a level of 1 p.p.m. of fluoride.

In addition to the theoretical sensitivity calculated from the standard graph, Table VI gives the differences in optical densities between solutions containing 0·90 and 1·10 p.p.m. of fluoride that were actually observed under the conditions recommended for each method. These levels represent the permitted limits of dosing that should be maintained in the artificial fluoridation of potable waters. Methods 3, 4 and 6 are considerably more sensitive than methods 1 and 5 and would, therefore, more readily detect dosing fluctuations at this concentration.

The limit of detection is an important criterion when the fluoride content is very small or the amount of sample available for analysis is restricted. Following Wilson[20] and Roos,[21] it is usual to define the limit of detection in terms of the reproducibility of the sample and of the blank. The values obtained by using the expression derived by Roos[21] are shown in Table VI, but it should be remembered that other factors, such as the pre-treatment of the sample, distillation technique or interfering ions, may introduce more uncertainty in the detection of fluoride at very low levels.

Effect of interfering ions—

The effects of those ions most likely to be present in waters were studied individually, and the results are presented in Table VII. Aluminium and metaphosphate produce the most noticeable effects while, in most instances, sulphate, chloride and calcium hardness give rise to smaller inaccuracies. The effect of colour was not fully investigated, but it is unlikely to be a serious problem with most waters. Sample 2040, a river water (Table VIII), was deep yellow, with a Hazen figure of 125, and it contained only a trace of natural fluoride. Methods 1, 5 and 6 gave slightly high results with this sample, but the recovery of added fluoride was in all instances not less than 90 per cent.

Table VII

Effect of interfering ions at a concentration of 1 p.p.m. of fluoride

Ion added	p.p.m.	Added as	Fluoride found by method 1 Alizarin (photometric)	3 Eriochrome cyanine R	5 SPADNS	6 Alizarin complexone	7 Ion-specific electrode
Aluminium	0·1	$Al(NO_3)_3.9H_2O$	0·94	0·93	0·96	0·90	0·98
	0·25		0·86	0·78	0·88	0·86	0·91
	0·50		0·73	0·72	0·79	0·75	0·87
Iron	0·1	$FeCl_3.6H_2O$	1·00	0·95	1·00	0·99	1·00
	1·0		1·00	1·00	0·99	1·03	1·00
	2·0		1·00	1·00	1·00	1·12	1·00
Calcium hardness	100	$CaCl_2$	1·00	0·86	1·00	0·93	1·00
	250		1·00	0·86	1·05	0·86	1·00
	500		1·00	0·90	1·06	0·79	1·00
Alkalinity	100	$NaHCO_3$	1·00	1·11	0·99	0·96	1·00
	250		0·98	1·16	1·05	0·96	1·00
	500		0·96	1·17	0·98	0·96	1·00
Sulphate	50	Na_2SO_4	1·00	1·08	1·02	1·00	1·00
	100		1·03	1·10	1·05	0·95	0·99
	200		1·11	1·25	1·07	0·95	0·98
Chloride	25	KCl	1·06	1·00	1·00	0·95	1·00
	100		1·00	1·03	0·96	0·93	1·00
	500		1·00	1·08	0·96	0·90	0·96
Phosphate	0·5	$(NaPO_3)_n.Na_2O$	1·11	1·07	1·08	0·94	1·00
	1·5		1·21	1·12	1·14	0·95	1·00
	2·5		1·42	1·14	1·20	0·94	1·00
Phosphate	0·5	KH_2PO_4	0·98	1·04	1·03	0·99	1·00
	1·5		0·98	1·04	1·02	1·03	1·00
	2·5		0·98	1·04	0·99	1·03	1·00

Public supplies of potable waters contain small residual amounts of free chlorine added for disinfection purposes, which are normally removed before analysis by reduction with sodium arsenite solution; a slight excess of the sodium arsenite solution does not disturb the reaction with fluoride.

TABLE VIII

DETERMINATION OF FLUORIDE IN VARIOUS WATERS

Sample		Fluoride, p.p.m., found by method					
		1	2	3	5	6	7
Laboratory number	Description	Alizarin (photometric)	Alizarin (titration)	Eriochrome (Cooke, Dixon and Sawyer)	SPADNS	Alizarin complexone	Ion-specific electrode
9	Ground waters, Derbyshire	1·39	1·4	1·40	1·48	1·45	1·50
12		0·59	0·5	0·60	0·61	0·63	0·62
27		0·40	0·7	0·50	0·50	0·44	0·45
51		0·10	0·1	0·10	0·15	0·14	0·14
2021	Borehole waters, London	1·89	2·5	2·40	2·12	2·00	2·00
2022		1·73	1·6	2·30	2·00	1·87	1·85
	River waters—						
17	R. Ouse	0·27	—	0·50	0·34	0·21	0·21
	(after fluoridation)	1·13	—	1·20	1·12	1·15	1·19
2040	R. Irthing	0·10	—	<0·1	0·14	0·12	<0·1
	(after fluoridation)	1·00	—	1·00	1·05	1·01	1·05
8	R. Thames at Windsor	Absent	—	0·29	0·23	0·28	0·17
	(after fluoridation)	1·02	—	1·21	1·07	1·09	1·15
	Fluoridated waters—						
656	Anglesey	0·92	0·9	1·02	0·93	1·07	0·96
657		0·20	0·3	0·36	0·14	0·21	0·20
658		0·96	1·1	1·08	0·98	1·06	0·93
661		0·80	0·7	0·96	0·85	0·83	0·79
663		0·82	0·7	0·94	0·88	0·98	0·81
705		0·94	0·8	0·99	1·01	0·93	0·91
636	Watford	0·90	1·0	1·06	1·10	1·06	1·06
637		1·09	0·9	1·14	1·18	1·11	1·06
638		0·97	0·9	1·09	1·18	1·11	1·08
639		1·05	1·0	1·20	1·19	1·12	1·08
	Eluates from dental cements—		Method 6 after distillation				
16		13·5	19·8	17·0	11·0	16·3	20·0
19		6·0	12·0	9·0	5·5	7·3	12·5
9		19·5	6·0	20·0	20·0	2·0	6·0
25		2·6	3·5	2·2	2·5	2·0	3·6

In methods 3 and 4, interferences by aluminium can be reduced by addition of excess of sodium hydroxide solution. Aluminium is then converted into the meta-aluminate state, when it is not able to complex fluoride ions in solution. On addition of reagent, reaction between fluoride and zirconium - dye occurs before hydrolysis of meta-aluminate to the ionic form, Al^{3+}.

Sulphate ions, when present in excessive amounts, can be precipitated as described in method 3, or the error can be corrected by using a nomograph, as in method 4. However, the only satisfactory methods for removing all of the interfering ions are distillation or diffusion. The present authors have no experience of diffusion, but distillation has proved satisfactory on a wide variety of samples, giving recoveries of fluorine of better than 95 per cent.

EFFECT OF TEMPERATURE—

Temperature has only a small effect on the colour developed in most methods, and the inaccuracies should be negligible under normal laboratory conditions. The Alizarin red S

and Eriochrome cyanine R reagents are the most sensitive to temperature changes and, for accurate work, the temperatures of samples and standards should be the same (±2° C). The change in optical density obtained within the range 15° to 25° C is shown in Table IX.

TABLE IX

EFFECT OF TEMPERATURE ON OPTICAL DENSITY

	Method	Solution	Optical density at 15° C	Optical density at 20° C	Optical density at 25° C
1.	Alizarin (photometric)	Blank	0·436	0·430	0·428
		1·0 p.p.m. of fluoride	0·300	0·299	0·299
		Difference	0·136	0·131	0·129
3.	Eriochrome cyanine R	10 μg of fluoride against reference	0·56	0·55	0·54
5.	SPADNS	1·0 p.p.m. of fluoride against reference	0·179	0·179	0·179
6.	Alizarin complexone	5 μg of fluoride against reference	0·218	0·215	0·216

TITRATION METHOD

In the titration procedure[8] (method 2), thorium nitrate titrant is added to the test solution until a faint permanent pink colour is observed in the presence of the indicator, Alizarin red S. The same volume of titrant is then added to a comparison solution, which becomes more pink than the test solution. The final titration is carried out with a standard fluoride solution that bleaches the comparison solution progressively to matching-point.

Previous experience with this method had shown that it was subject to considerable personal error because the end-point is rather indistinct, but consistent results can, however, be obtained with it after much practice in observing the end-point change. In the original report[22] of the Analytical Methods Sub-Committee, great stress was laid on the importance of experimental technique on the accuracy of the results obtained. A sample of evaporated milk was analysed by six members, all experienced in the method, and the results ranged from 5·8 to 6·8 p.p.m. of fluoride. What proportion of this error arose from the titration itself, as distinct from the preliminary treatment of the sample, is not stated, but clearly the percentage error in the titration will be greater at low levels of fluorine and will vary between analysts.

The titration is normally carried out in 100-ml Nessler cylinders. The optical density of the comparison solution, at a wavelength of 520 nm, decreases linearly with volume of fluoride titrant added. However, the change in optical density was found to be only 0·008 (4-cm cell) per ml of 10 p.p.m. fluoride titrant, and there is no change in λ_{max}. While a 100-ml Nessler cylinder has a colour depth of about 3½ times that of a 4-cm cell, the human eye is less sensitive than a spectrophotometer, and it is not surprising that many analysts find it difficult to obtain consistent results.

ORION ION-SPECIFIC ELECTRODE

Frant and Ross[16] have described a fluoride-sensitive electrode in which the potential developed across a lanthanum fluoride crystal is dependent on the ratio of the fluoride activities on either side of the crystal. As the internal fluoride activity is constant for all practical purposes, the potential developed depends only on the value of the fluoride activity in the external solution. In use, the electrode forms a cell with an external reference electrode, normally the calomel electrode.

The activity of fluoride ions in a test solution will depend on the total ionic strength of the solution. Thus, if the electrode is calibrated with standard fluoride solutions, errors in the concentration of fluoride will result when the unknown solutions contain appreciable amounts of ions other than fluoride. This effect is minimised by diluting the sample with an equal volume of a total ionic strength adjustment buffer, which can be purchased from the manufacturer of the electrode. This buffer also largely eliminates errors from pH changes and complexing agents such as aluminium. The buffer used in the present study was of an improved composition and contained phosphate, citrate and EDTA, and was recommended by workers at Electronic Instruments Ltd. It prevents serious interference from most ions present in water supplies, as can be seen from Table VII. Experiments in these laboratories have shown that an improved tolerance to aluminium, without loss of

sensitivity, can be obtained by increasing the strength of citrate in the buffer, although it it is not certain whether this would affect the life of the electrode.

The potential developed with the electrode is reproducible to ± 1 mV, and the response time was about that of a conventional pH electrode assembly. A waiting time of about 5 minutes is advisable when the concentration of fluoride in the sample is below 0·3 p.p.m. of fluoride, or when succeeding samples differ widely in fluoride content. Vigorous agitation of the sample solution reduces the time taken to attain equilibrium.

Changes in temperature affect the response of the electrode, as the values of both the slope and the intercept factors in the Nernst equation will change. Additionally, the solubility equilibrium of the saturated calomel electrode may be altered. Some correction of these effects can be built into the pH meter used with the electrode, but for precise work both standard and unknown solutions should be at the same temperature.

COMPARATIVE ANALYSIS OF WATERS—

Six of the methods were used to determine the fluoride content of a series of ground waters containing natural fluoride, together with several artificially fluoridated waters from the study areas at Anglesey and Watford. The results have been collected together in Table VIII. Three river waters were also examined to determine the natural fluoride content; 10 ml of a 10 p.p.m. standard fluoride solution were then added to 90 ml of each river water, and the determination was repeated to check the percentage recovery of fluoride. The results calculated from the figures given in Table VIII are shown below.

	Percentage recovery of fluoride by method				
	1	3	5	6	7
R. Ouse	91	83	86	97	100
R. Irthing	92	96	93	91	100
R. Thames	102	96	88	87	100

Method 7, the ion-specific electrode, is clearly the most satisfactory from this standpoint. This approach was extended to samples of water from several other public supplies, and in every instance the recovery of fluoride calculated from results obtained with the electrode agreed with theory. Further evidence of the reliability of the electrode can be seen in the results of the analysis of eluates of dental silicate cements that contain relatively large amounts of phosphate and aluminium, in addition to fluoride. The close agreement between method 6, after distillation to remove interferences, and method 7 (ion-specific electrode) can be seen from the results given in Table VIII.

DISCUSSION

The number of methods available to the analyst for the determination of fluorine is considerable and continually increasing. Simple comparator methods,[9,23,24] while of value for the routine testing of water supplies at the works, are far less sensitive than the photometric methods used in the laboratory. Further, these visual techniques are frequently associated with a large personal error and so were excluded from the present survey.* However, many of the conclusions listed under the appropriate reagent used with an instrumental procedure are likely to apply equally to the visual technique.

All of the instrumental methods have their own peculiar advantages and disadvantages, which have to be weighed one against the other in the light of the particular situation and requirements of the individual analysts. In general, little attention is paid to the purity of the dye reagents, and this may be partly responsible for some of the difficulties encountered in the use of these methods. The alizarin complexone procedure has many advantages over the bleaching methods, and is particularly suitable for samples containing only very small amounts of fluorine. However, the fluoride electrode surpasses all the colorimetric methods with regard to speed, accuracy and convenience, and is recommended for the routine monitoring of fluoridated water supplies. The indications are that it will also prove satisfactory for the determination of fluorine in a wide range of materials, following a simple pre-treatment to bring the fluorine into solution.

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* It is planned to examine these techniques at a later date.

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The Determination of the Di- and Mononitrates of Ethylene Glycol and 1,2-Propylene Glycol in Blood by Colorimetric and Gas-chromatographic Methods

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Methods are described for the determination of the nitrate esters, ethylene glycol dinitrate, propylene glycol 1,2-dinitrate, ethylene glycol mononitrate, propylene glycol 1-mononitrate and propylene glycol 2-mononitrate, in blood. These esters can be removed efficiently from blood by extraction with diethyl ether, and determination of the dinitrates can be carried out colorimetrically, after alkaline hydrolysis, by a diazotisation and coupling reaction. As the mononitrates are not determined by this procedure, they do not interfere with the determination of the corresponding dinitrate. Gas chromatography, with the electron-capture detector, provides a sensitive method for detecting and measuring the mononitrates, with adequate separation from the parent dinitrate, and the latter can also be determined by this method as an alternative to the colorimetric procedure. For both methods the effect of possible interferences is investigated, and the efficiency of recovery from blood over the range 0 to 25 μg of ester is reported.

The determination of nitrate esters of polyhydric alcohols in explosives, pharmaceuticals and air by colorimetric methods is well established.[1,2,3,4,5] These methods are relatively non-specific and do not always distinguish between individual nitrates in a mixture of esters. Recent gas-chromatographic procedures, especially those in which the electron-capture detector is used, have largely overcome the latter difficulty, and the identification of nitrate ester mixtures has been achieved.[6,7,8] Modifications of the above methods have been applied to the determination of nitrate esters of polyhydric alcohols in blood and other biological fluids.[9,10,11,12,13]

For the investigation of the toxicology and metabolism of organic nitrates in experimental animals, the non-specific colorimetric methods can be used for the determination of a free ester in blood when other esters are known to be absent. Thus, procedures can be used involving extraction of the blood with an organic solvent, followed by alkaline hydrolysis and colorimetric determination of the liberated nitrite. Although the individual steps of such an analysis are relatively simple, some of the published procedures involve time-consuming steps, such as prolonged solvent extraction or removal of solvent, thus requiring careful attention to prevent loss of ester. Of these procedures, that by Hasegawa, Sato, Yoshikawa, Sakabe, Yamaguchi and Hotta[13] for the analysis of ethylene glycol dinitrate is the simplest to apply, as it involves a simple extraction of the blood with diethyl ether and does not require any appreciable removal of solvent after the alkaline hydrolysis step and before the colorimetric determination. It is, therefore, suitable for the analysis of large numbers of samples encountered during metabolism studies. No assessment of this method was made regarding the recovery of ethylene glycol dinitrate from blood, the limits of detection, application to other nitrate esters, effects from metabolites in the blood produced during breakdown of ethylene glycol dinitrate or of the interference from normal blood constituents. Part of the present paper is therefore concerned with such an assessment of the method in a modified form, and of its application to the determination of propylene glycol 1,2-dinitrate.

It has been demonstrated during metabolism studies that ethylene glycol dinitrate breaks down in blood to give ethylene glycol mononitrate,[14] and propylene glycol 1,2-dinitrate to give propylene glycol 1-mononitrate and propylene glycol 2-mononitrate.[15] It is shown here

that these mononitrates are not detected by the colorimetric method described, and the remainder of this paper, therefore, describes a gas-chromatographic method for their determination. As adequate separation of the mononitrates from their parent dinitrates can be achieved on the gas-chromatographic column, this method also provides an alternative way of determining the latter.

Colorimetric method

Reagents—

All reagents should be of analytical-reagent grade unless otherwise stated.

Diethyl ether.

Ethanol, absolute.

Sodium hydroxide solution, N.

Hydrochloric acid solution, N.

Sulphanilic acid solution—Dissolve 10 g of sulphanilic acid in 2 N hydrochloric acid. Warm to dissolve, make up to 1 litre with 2 N hydrochloric acid and filter. Store in an amber-glass reagent bottle.

N-1-*Naphthylethylenediamine solution*—Dissolve 10 g of *N*-1-naphthylethylenediamine dihydrochloride in distilled water and make up to 1 litre. Filter and store in an amber-glass reagent bottle.

Ethylene glycol dinitrate, 1 per cent. w/v in ethanol.

Propylene glycol 1,2-dinitrate, 1 per cent. w/v in ethanol.

The last two solutions were supplied by I.C.I. Nobel Division. Working standard solutions when required are prepared by diluting 1 ml of the 1 per cent. solution to 100 ml with ethanol so that 1 ml $\equiv$ 100 μg of dinitrate; 1·0, 2·0, 3·0, 4·0, 5·0, 10·0, 15·0, 20·0 and 25·0 ml of this solution are each diluted to 100 ml with ethanol and are equivalent to 1·0, 2·0, 3·0, 4·0, 5·0, 10·0, 15·0, 20·0 and 25·0 μg per ml of dinitrate, respectively.

Procedure—

By pipette, introduce 1·0 ml of blood into a 50-ml separating funnel, followed by 1·0 ml of de-ionised water. Immediately add 10 ml of diethyl ether, gently shake the contents of the funnel for a few seconds and then release the pressure by easing the stopper cautiously. Shake the contents for 1 minute and allow the layers to separate for 4 to 5 minutes. Remove 5 ml of the upper ether layer with a pipette and transfer to a stoppered test-tube. Add 7 ml of ethanol, mix, and then add 1 ml of de-ionised water and 0·5 ml of N sodium hydroxide. Mix and heat the solution in a water-bath at a temperature of 40° C for 1 hour. Then pour the contents of the tube into a 25-ml beaker and gently evaporate off some of the ether on a hot water bath at about 50° to 60° C until the volume of liquid remaining is about 9 ml. Add 0·5 ml of N hydrochloric acid, allow the solution to cool, transfer it to a graduated tube and make up to 10 ml with ethanol. Add 1 ml of 1 per cent. sulphanilic acid, allow to stand for 15 minutes and add 1 ml of 1 per cent. *N*-1-naphthylethylenediamine. After 5 minutes measure the colour in a spectrophotometer at 540 nm in 1 or 4-cm cells against a reference solution prepared from 5 ml of ether and processed in the same way as the sample ether extract.

To calibrate the procedure, 1 ml of each of the working standard solutions is added to 5 ml of ether, 6 ml of ethanol are added and the standards then processed in the same way as for the samples, commencing from the addition of 1 ml of de-ionised water in the colorimetric procedure.

Calculation—

By using the above volumes of blood and ether the concentration of dinitrate in the blood, expressed in micrograms per millilitre, equals twice the number of micrograms of dinitrate read from the calibration graph.

Results and discussion

The colorimetric method gives a linear response up to 25 μg of either dinitrate. Propylene glycol 1,2-dinitrate gives a response equivalent to 0·45 of that of ethylene glycol dinitrate. Ethylene glycol mononitrate and the two propylene glycol mononitrates, in amounts up to 100 μg, give no colour when submitted to the procedure, even when the concentration of the sodium hydroxide used is 10 N.

RECOVERY FROM BLOOD—

To 1 ml of blood in a separating funnel, 1 ml of a standard solution of ethylene glycol dinitrate or propylene glycol 1,2-dinitrate in water was added and, immediately after mixing, analysis was carried out by the above procedure. In this way, twelve determinations were carried out at four levels of added dinitrate and the results tabulated (Table I).

TABLE I

RECOVERY OF ETHYLENE GLYCOL DINITRATE AND PROPYLENE GLYCOL 1,2-DINITRATE FROM BLOOD

Dinitrate added, μg	Ethylene glycol dinitrate			Propylene glycol 1,2-dinitrate		
	Recovered, μg		Recovery range, per cent.	Recovered, μg		Recovery range, per cent.
	Mean	Standard deviation		Mean	Standard deviation	
1	0·98	±0·04	95 to 107	0·97	±0·06	89 to 107
5	4·95	±0·18	94 to 106	4·90	±0·09	95 to 110
10	9·75	±0·12	95 to 99	9·70	±0·16	92 to 104
25	24·50	±0·18	97 to 100	24·20	±0·30	93 to 102

Each mean is the result of 12 determinations.

LIMIT OF DETECTION—

Blank determinations were carried out on twelve samples of rat blood. The mean value of the optical densities thus derived from the blank samples in the 4-cm cells was 0·002, with a standard deviation of 0·002. As the limit of detection has been calculated to be about three times the standard deviation of the blank,[16] then the corresponding values for ethylene glycol dinitrate and propylene glycol 1,2-dinitrate equivalent to an optical density of 0·006 are about 0·1 and 0·2 μg per ml, respectively.

INTERFERENCES—

Three sources of interference are possible: from natural constituents of blood; from mononitrates arising from the metabolism of the dinitrates in the blood[14,15]; and from inorganic nitrite also produced as a result of this metabolism.[14,15] It has been shown above that the blood blank is very small and is less than 0·1 μg of ethylene glycol dinitrate and 0·2 μg of propylene glycol 1,2-dinitrate per ml, and thus interference from blood constituents is minimal for concentrations of dinitrate above 1·0 μg per ml. It has also been shown above that the mononitrates do not hydrolyse to nitrite under the conditions of test, and even although they are extracted from blood with ether, as will be shown later, their presence will not influence the result of the colorimetric determination. Inorganic nitrite may be produced in relatively large concentrations in blood (up to 20 μg per ml) during *in vivo* studies of organic nitrates, and it is possible that some may be transferred into the ether layer at the extraction stage and so interfere with the colorimetric determination. To gauge the effect this might have, 1 ml of a standard solution of inorganic nitrite was added to 1 ml of blood and then immediately submitted to the analytical procedure. Six determinations with 25 μg of inorganic nitrite (as NO_2^-) gave results that did not differ from blood blank values, while six determinations with 50 μg of inorganic nitrite gave a mean result equivalent to 0·25 μg of ethylene glycol dinitrate per ml of blood. It is possible, therefore, that very high concentrations of inorganic nitrite (>25 μg per ml) may interfere with the results for low concentrations of organic dinitrate.

STABILITY—

Ethylene glycol dinitrate and propylene glycol 1,2-dinitrate are both rapidly broken down by blood to the corresponding mononitrates and inorganic nitrate *in vitro*.[14,15] Ether extraction of the blood should be carried out immediately the sample is withdrawn. Once either dinitrate has been extracted into the ether layer, the analysis may be delayed at this stage without fear of loss of dinitrate, provided the extract is stored at 4° to 5° C.

Conclusion

If present individually, the concentration of the nitrate esters, ethylene glycol dinitrate and propylene glycol 1,2-dinitrate, can be measured in blood samples by a simple procedure involving extraction with ether, alkaline hydrolysis and colorimetric determination by diazotisation and coupling. Inorganic nitrite and the mononitrates, ethylene glycol mononitrate, propylene glycol 1-mononitrate and propylene glycol 2-mononitrate, which may be present as a result of metabolism of the dinitrates in blood, do not interfere.

A method involving gas - liquid chromatography is now described, in which the mononitrates are determined and which, at the same time, provides an alternative means of determining the dinitrates, ethylene glycol dinitrate and propylene glycol 1,2-dinitrate.

Gas-chromatographic method

Reagents—

All reagents should be of analytical-reagent grade unless otherwise stated.

Diethyl ether—Analysis under the gas-chromatographic conditions described below should give no peaks corresponding to the nitrate esters tested.

Sodium sulphate, anhydrous.

Nitroglycerine solution, 1 μg per ml in ethanol.

Ethylene glycol mononitrate—This was prepared by the method of Ferris, McLean, Marks and Emmons[17]; a 0·1 per cent. w/v solution in ethanol was used.

Propylene glycol mononitrates—The two mononitrates, propylene glycol 1-nitrate and propylene glycol 2-nitrate, were prepared from the corresponding bromohydrins by using the method for ethylene glycol mononitrate[17]; 0·1 per cent. w/v solutions in ethanol were used.

Working standard solutions—One millilitre of a 0·1 per cent. mononitrate solution was diluted to 200 ml with ether; 1 ml of solution ≡ 5 μg of mononitrate. When required, 1·0, 2·0, 3·0, 4·0 and 5·0 ml of this solution are each diluted to 25 ml with ether and are equivalent to 0·2, 0·4, 0·6, 0·8 and 1·0 μg of mononitrate per ml, respectively.

Apparatus—

An Aerograph 1522 gas chromatograph, with a tritiated foil electron-capture detector and 5-feet × ⅛-inch o.d. stainless-steel column containing 30 per cent. E301 on 80 to 120-mesh acid-washed Celite, was used.

Operating conditions—

Column temperature, 100° C; injector, 140° C; detector, 135° C; nitrogen flow-rate, 60 ml per minute; and chart speed, 20 inches per hour (Honeywell recorder, 1 mV f.s.d.). Before commencing gas-chromatographic analysis, condition the column by injecting 5 μl of nitroglycerine solution 1 to 2 hours before the first sample injection (see Results and discussion).

Procedure—

By pipette, transfer 1·0 ml of blood into a 50-ml separating funnel, followed by 1·0 ml of de-ionised water. Immediately add 10 ml of diethyl ether, gently shake the contents of the funnel and release the pressure by easing the stopper. Shake the contents for 1 minute. Allow the layers to separate for 4 to 5 minutes. Remove as much of the ether layer as possible with a pipette and transfer it to a stoppered cylinder containing about 1 g of anhydrous sodium sulphate. Add 2 ml of ether to the remaining contents in the separating funnel, swirl and allow the layers to separate. Remove as much of the ether as possible and add it to that in the stoppered cylinder. Allow the ether extracts to remain in contact with the sodium sulphate for at least 2 to 3 hours and then transfer to a 15-ml calibrated flask. Wash the sodium sulphate with 2 ml of ether, add the washings to the flask and finally make up to volume with ether. Concentrate or dilute the extracts, if necessary, to a known volume of ether and inject 2 μl into the gas chromatograph.

Calibration graphs are prepared by injecting 2-μl portions of the standard solutions in ether and plotting the resultant peak areas against concentration over the range 0·2 to 1·0 μg per ml of nitrate ester.

CALCULATION—

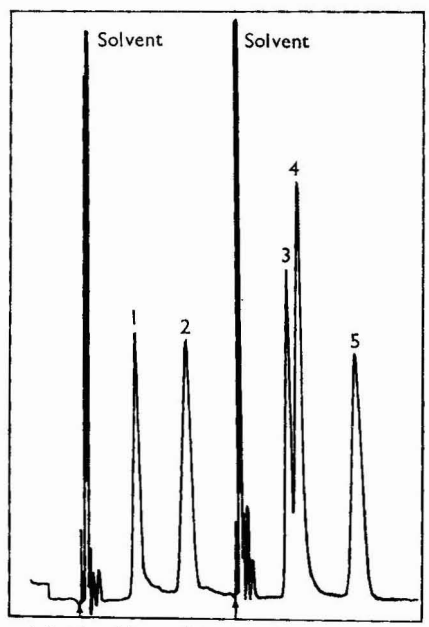
$$\text{Concentration in blood} = \text{concentration in ether extract} \times \frac{\text{ether volume}}{\text{blood volume}}.$$

RESULTS AND DISCUSSION

The sensitivity and reproducibility of the gas-chromatographic responses of the nitrate esters depend on the extent to which the column is conditioned to them. The injection of a nitroglycerine solution[11] helps to attain the maximum effect more quickly than by repeated injections of the nitrate ester under test. On a new column more than one injection of nitroglycerine is required before satisfactory sensitivity and reproducibility are attained. Once a column is being used regularly, one conditioning injection of nitroglycerine at the beginning of the day is sufficient. In my experience, the determination of ethylene glycol mononitrate depends on this conditioning effect more than the other esters.

The retentions of the five nitrate esters relative to one another on a 30 per cent. E301 column at 100° C under the conditions of test are given below.

Ester	Ethylene glycol mononitrate	Propylene glycol 1-mononitrate	Propylene glycol 2-mononitrate	Ethylene glycol dinitrate	Propylene glycol 1,2-dinitrate
Relative retention	1·00	0·96	1·17	1·83	2·17



1. Ethylene glycol mononitrate
2. Ethylene glycol dinitrate
3. Propylene glycol 1-mononitrate
4. Propylene glycol 2-mononitrate
5. Propylene glycol 1,2-dinitrate

Fig. 1. Chromatograms of 2-μl injections of mixtures in diethyl ether of: (*a*) ethylene glycol mononitrate and ethylene glycol dinitrate; and (*b*) propylene glycol 1-mononitrate, propylene glycol 2-mononitrate and propylene glycol 1,2-dinitrate

Fig. 1 shows the chromatograms obtained for the dinitrates compared with the corresponding mononitrates. The dinitrates gave an approximately equal response, and this

response is about double that obtained for the mononitrates. The operating conditions quoted in this paper are those required for adequate separation of the two propylene glycol mononitrates, but if this is not required then measurements at higher temperatures[14] will give sharper responses and more sensitivity.

Recovery from blood—

It has been shown in the section on colorimetric analysis that the extraction of ethylene glycol dinitrate and propylene glycol 1,2-dinitrate from blood with ether is virtually complete in the range 1·0 to 25·0 μg per ml. At the 0·2 μg per ml level, and with the gas-chromatographic finish, recoveries of over 90 per cent. are obtained. The recovery of ethylene glycol mononitrate and the two propylene glycol mononitrates from whole rat blood is shown in Table II. The recoveries are not as quantitative as for the dinitrates (Table I), presumably because of the relatively increased water solubility conferred by the hydroxyl group present in the mononitrates.

Table II

Recovery of ethylene glycol mononitrate, propylene glycol 1-mononitrate and propylene glycol 2-mononitrate from blood

Mononitrate added, μg	Ethylene glycol mononitrate: Recovered, μg Mean	Standard deviation	Recovery range, per cent.	Propylene glycol 1-mononitrate: Recovered, μg Mean	Standard deviation	Recovery range, per cent.	Propylene glycol 2-mononitrate: Recovered, μg Mean	Standard deviation	Recovery range, per cent.
0·5	0·41	±0·02	78 to 92	0·44	±0·02	84 to 94	0·45	±0·02	86 to 94
2·0	1·67	±0·08	78 to 93	1·80	±0·07	85 to 94	1·79	±0·06	85 to 93
5·0	4·15	±0·13	78 to 87	4·44	±0·20	83 to 94	4·51	±0·14	87 to 94
20·0	17·00	±0·65	80 to 90	18·80	±0·60	90 to 97	18·2	±0·40	88 to 94

Each mean is the result of 6 determinations.

Interference—

Blood from rats that had not received a dose of organic nitrate ester showed no gas-chromatographic peaks at the retention times of the five esters under review.

Stability of the mononitrates in blood—

Although the mononitrates break down relatively slowly in blood *in vitro*,[14,15] it must be remembered that during studies involving ethylene glycol dinitrate and propylene glycol 1,2-dinitrate the latter compounds may be present in the samples, and can rapidly break down to form the mononitrates. Thus, the remarks applied to the dinitrates concerning immediate extraction into ether after sampling apply equally well to the mononitrates.

Conclusions

Ethylene glycol dinitrate, propylene glycol 1,2-dinitrate, ethylene glycol mononitrate, propylene glycol 1-mononitrate and propylene glycol 2-mononitrate can be determined by gas - liquid chromatography in ether extracts from blood with adequate separation of the mononitrates from the dinitrates, and with no interference from blood constituents. Recovery of the mononitrates from blood is reproducible but slightly less quantitative than for the dinitrates.

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The Rapid Determination of Benzil, Benzoin and Hydrobenzoin in Mixtures by Quantitative Infrared Spectrophotometry

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From the infrared spectra of mixtures of benzil, benzoin and hydrobenzoin in solution in chloroform, benzoin and hydrobenzoin are determined by means of their absorption bands at 3460 and 3590 cm^{-1}, respectively. Benzil is determined from the carbonyl absorption at 1680 cm^{-1} after subtracting the contribution of benzoin to the absorbance at this frequency. The most suitable concentrations are 0·1 to 1·0 per cent. for benzil and benzoin and 0·1 to 2·0 per cent. for hydrobenzoin.

In a study of the reduction of aromatic diketones, a method was required for the rapid determination of benzil ($C_6H_5.CO.CO.C_6H_5$), benzoin ($C_6H_5.CHOH.CO.C_6H_5$) and hydrobenzoin ($C_6H_5.CHOH.CHOH.C_6H_5$). Several methods have been described in the literature for the determination of these substances singly, including titrimetric,[1,2] gravimetric,[2,3] polarographic[4] and fluorimetric[3] methods for benzil, and colorimetric methods[5,6] for benzil and benzoin, but none of these appeared to be readily adaptable to analysis of three component mixtures. Ultraviolet spectral results have been reported for benzil,[7] benzoin[8] and hydrobenzoin,[9] but because of overlapping of absorption peaks in a mixture, ultraviolet spectroscopy did not appear to be suitable for the present purpose.

Although infrared spectral results have previously been reported for benzil,[10] benzoin[11] and hydrobenzoin,[12] no quantitative study of mixtures of these substances appears to have been undertaken. It was therefore decided to investigate the possibility of quantitative analysis of benzil, benzoin and hydrobenzoin mixtures by infrared spectrophotometry.

Experimental

Infrared spectra were recorded by means of a Hilger & Watts Infrascan H900 double-beam spectrophotometer, with a grating and sodium chloride prism. A 1·00-mm path length, semi-permanent cell with sodium chloride windows was used throughout. To reduce, as far as possible, hydrogen-bonding effects for benzoin and hydrobenzoin, dilute solutions (about 0·1 to 2·0 per cent. w/v) of the materials in purified chloroform were used. The solvent used was analytical-reagent grade chloroform, which contains about 2 per cent. of ethanol as stabiliser. This was washed three times with an equal volume of water, dried over a mixture of anhydrous analytical-reagent grade sodium sulphate and analytical-reagent grade calcium chloride, distilled between 60·0° and 60·5° C and stored in amber bottles in the dark. Only 500-ml amounts were prepared at one time. Chloroform thus purified gave no detectable infrared absorption at 3460 cm^{-1} attributable to the hydroxyl group of ethanol. A small absorption at 3590 cm^{-1} remained, but the percentage transmission was shown by repeated checks to be constant, and therefore not liable to interfere in the procedure described below.

Absorbance measurements were made by using the "Baseline Technique," *viz.*, construction of a tangent from the troughs immediately on either side of the required absorption peak, followed by measurement of the vertical distance from the peak to the base-line or tangent.

CALIBRATION PROCEDURES AND BASIS OF QUANTITATIVE ANALYSIS—

Initially, separate calibration graphs were prepared for each component in the concentration range about 0·1 to 1·5 per cent. The peaks used were: for benzil, the carbonyl stretching absorption at 1680 cm^{-1}; for benzoin, both the carbonyl peak at 1680 cm^{-1} and the hydroxyl stretching absorption at 3460 cm^{-1}; and for hydrobenzoin, the hydroxyl stretching absorption at 3590 cm^{-1}. It will be noted that the hydroxyl peaks of benzoin and hydrobenzoin are separated by 130 cm^{-1} and are thus readily distinguishable. In all instances plots of absorbance against concentration were linear, and passed through or close to the origin. The reproducibility of the absorbance measurements was checked by running repeated spectra. Thus in a typical series of eight measurements of the absorbance at 1680 cm^{-1} given by 0·1 per cent. w/v of benzil, a mean value of 0·214 was obtained, with a standard deviation of 0·0015 and a coefficient of variation of 0·70 per cent.

Standard mixtures were then prepared containing benzil and benzoin in the concentration range 0·1 to 1·0 per cent. w/v and hydrobenzoin in the range 0·1 to 2·0 per cent. w/v. These concentration ranges were chosen primarily to give reasonable percentage transmissions on the recorded spectra. In particular, the intense carbonyl absorption at 1680 cm^{-1} renders the use of higher concentrations of benzil and benzoin unsatisfactory. With the standard mixtures, calibration graphs were plotted for benzoin and hydrobenzoin from the absorbances at 3460 and 3590 cm^{-1}, respectively; these graphs were almost identical with those for the single components. The 1680 cm^{-1} absorbance, however, now represents the combined contributions of benzil and benzoin. It is therefore necessary to use an indirect procedure for the benzil calibration. As in the spectrum of benzoin the absorbances at 1680 cm^{-1} and at 3460 cm^{-1} are linearly related to concentration, and hence to each other, it is a simple matter to calculate the contribution of benzoin to the 1680 cm^{-1} absorbance from the measured absorbance at 3460 cm^{-1}. Thus by subtraction of this contribution from the total absorbance at 1680 cm^{-1}, the absorbance caused by benzil at this frequency can be obtained. As a check on the validity of this procedure, the strict additivity of the contributions of benzil and benzoin to the total absorbance at 1680 cm^{-1} was confirmed by using a series of binary mixtures of these substances.

From a series of runs on the standard mixtures, linear calibration graphs passing through the origin were obtained in all instances. The actual absorbances corresponding to a concentration of 1·00 per cent. w/v of each component were as follows.

Hydrobenzoin at 3590 cm^{-1}	0·472
Benzoin at 3460 cm^{-1}	0·160
Benzoin at 1680 cm^{-1}, in absence of benzil	1·605
Benzil at 1680 cm^{-1}, after allowance for contribution of benzoin, when present	2·250

As a measure of the accuracy of the method, several synthetic mixtures were treated as unknown samples, and the results of these analyses are shown in Table I.

TABLE I

DETERMINATION OF BENZIL, BENZOIN AND HYDROBENZOIN IN SYNTHETIC MIXTURES

Benzil, per cent. w/v		Benzoin, per cent. w/v		Hydrobenzoin, per cent. w/v	
taken	found	taken	found	taken	found
0·10	0·10	0·10	0·09	0·25	0·27
0·10	0·11	0·20	0·19	0·80	0·82
0·20	0·21	0·40	0·39	0·40	0·40
0·25	0·26	0·10	0·09	0·25	0·26
0·30	0·29	0·30	0·30	0·40	0·40
0·30	0·28	0·40	0·37	0·80	0·84
0·40	0·39	0·10	0·09	0·60	0·63
0·40	0·40	0·20	0·18	0·60	0·62

DISCUSSION AND CONCLUSIONS

The method as described was found to be rapid and convenient once the initial calibrations had been carried out. The optimum concentrations were within the range 0·1 to 1·0 per cent. w/v for benzil and benzoin and 0·1 to 2·0 per cent. w/v for hydrobenzoin. More concentrated mixtures should, of course, be diluted to these concentration ranges.

The values for percentages found and taken agreed, in general, to within $\pm 0{\cdot}02$ per cent. at the lower concentrations and $\pm 0{\cdot}05$ per cent. at the higher concentrations, which was satisfactory for a rapid method for determining all three components. Greater deviations occurred occasionally, particularly when one component, not necessarily the one being determined, was present in excess of the recommended concentration range. The determination of benzil, by the indirect procedure involved, is particularly susceptible to error if the concentration of benzoin is substantially greater than that of benzil.

In spectroscopic methods of this type, errors can arise because of physical or chemical interactions in the solution, or because of uncertainties in the measurement of absorbances. In the present instance, although intermolecular hydrogen bonding is possible between –OH of benzoin and –OH of hydrobenzoin, or between these –OH groups and the >CO of either benzoin or benzil, it is unlikely to be pronounced at such low concentrations. Furthermore, such hydrogen bonding would be expected to cause an appreciable displacement of the absorption peaks, which were constant to within ± 2 cm^{-1} throughout.

Uncertainties in measurement of absorbances are a more likely source of error. Such errors are likely to be greatest with low percentage transmissions, which may be found particularly at the intense 1680 cm^{-1} peak, and when the spectral background causes uncertainties in the construction of the base-line for absorbance measurement. Therefore, calibration with standard mixtures of composition similar to those to be analysed is regarded as essential.

It is possible that greater accuracy could be achieved by means of a preliminary separation of all three components, *e.g.*, by thin-layer chromatography and subsequent elution. This aspect is currently being investigated, but any gain in accuracy will inevitably be accompanied by a substantial increase in the time required for the analysis.

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A Combustion Method with a Radiometric Finish for the Determination of Microgram Amounts of Sulphur in Light Petroleum

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A radiochemical technique has been combined with a modification of the surface combustion method of Schöberl to give a rapid method for the determination of sulphur in the range 1 to 10 p.p.m. in organic substances such as purified light petroleum. The sulphate formed is precipitated with barium chloride labelled with barium-133, and the amount of barium sulphate obtained determined by measuring its activity. A radiometric finish has the advantage of potential sensitivity, and barium-133 can be obtained with a specific activity of 1 Ci per g. Thus, a scintillation counter with an efficiency of 30 per cent. and a background of 20 counts per second (shielded) can detect $0{\cdot}4 \times 10^{-3}$ μg of sulphur, as barium sulphate, assuming no solubility losses.

The lowest detectable concentration of sulphur is dependent on the magnitude of the blank value, but because sulphate appears to be an almost universal contaminant it was found, under normal laboratory conditions, that it was impossible to reduce the blank value to much below the equivalent of 1 p.p.m. in the sample.

The various methods described in the literature for determining sulphur in organic substances involve either reduction of the sulphur compound to hydrogen sulphide or combustion to sulphur dioxide and absorption in hydrogen peroxide to give sulphuric acid. Both types of method must be combined with a suitable finish to determine the resulting compound.

The most popular reduction procedure appears to be Granatelli's[1] method with Raney nickel, which was modified by Reed.[2] The resulting hydrogen sulphide is converted into methylene blue by the method of Kriege and Wolfe[3] and measured spectrophotometrically. Detection down to 0·02 p.p.m. is claimed but the method is lengthy (2 to 3 hours), and there is evidence that some sulphur compounds are resistant to the Raney nickel reduction.

The combustion processes differ in the amount of sample that can be handled. In the Wickbold[4] method 50-g samples can be burnt in 15 minutes in an oxy-hydrogen flame produced with a special burner; with such high gas flow-rates (50 cu. ft. per hour) gas purification is difficult, and high blank values are obtained. The oxygen-flask method[5] appears ideal for handling small amounts when the sample has a high sulphur content but would not be suitable in the p.p.m. range. The Schöberl surface combustion method[6] falls between the two extremes. While primarily intended for milligram amounts, it can, as described later, be modified to enable 2-g samples to be burnt in about 15 minutes.

Methods for determining the resulting sulphuric acid include nephelometric titration with barium chloride (40·0 p.p.m.), a colorimetric method in which barium chloranilate[7] is used (0·06 p.p.m.) and flame molecular-emission spectroscopy[8] (6·4 p.p.m.). Because of the widespread occurrence of sulphate, high blank values are to be expected.

There do not appear to be reports of a radiochemical finish, yet such a method would be sensitive, providing that the radiochemical reagent could be obtained with a sufficiently high specific activity. Barium-133, in the form of barium chloride (Radiochemical Centre, Amersham), is an ideal tracer; it can be obtained with a specific activity of 1 Ci per g. It is a γ-emitter, giving a cascade of photons with an average emission of 1·36 per nuclear transformation, and this factor, together with an average energy of 0·3 MeV, makes detection with a scintillation counter easy and efficient; its half-life of 10 years results in small decay losses and a long shelf-life and, finally, it is comparatively cheap.

EXPERIMENTAL

In this work, a combustion process was selected because it is absolute. Providing the combustion is completely clean, as shown by absence of partly burnt products, it can be assumed that all of the sulphur compounds present have been converted into sulphur dioxide. Further, the hydrogen peroxide absorption appears to be quantitative down to very low concentrations and at high gas flow-rates. The sulphate in the hydrogen peroxide absorbent is then precipitated with the radioactive barium chloride reagent, and the amount thus obtained is determined by measuring the activity. Because of anticipated high blank values, the specific activity of the reagent, as purchased, was reduced by a factor of 350 by dilution with the inactive form to bring the sensitivity to the same order of magnitude as the anticipated blank values. Nevertheless, the method was still sensitive because it was found that each microgram of sulphur, in the form of sulphate, will precipitate sufficient barium chloride to give a count-rate of about 2250 counts per 10 seconds, with a counting efficiency of 30 per cent. The recovery of the barium sulphate must be quantitative and the precipitate completely free from excess of active barium chloride.

A limitation is imposed on this method by the solubility of barium sulphate in water. At room temperature, it varies from 3 mg per litre in neutral solution to 100 mg per litre in 2 N acid solution. Acid must be present to prevent the interference from carbonate, phosphate and silicate ions. Assuming a solubility of 10 mg per litre in weakly acidic solution, and a volume of 10 ml, the lowest limit for the detection of sulphur will be 13·0 μg, corresponding to 6·5 p.p.m. for a 2-ml sample.

In this work the solubility limitation was overcome by evaporating to dryness after decomposing the carbonate with acid, and the excess of barium chloride removed with anhydrous methanol; barium chloride is fairly soluble in this solvent, whereas barium sulphate is extremely insoluble, being about 0·3 mg per litre at room temperature. This would still permit interference by phosphate and silicate ions, but in some preliminary work it was found that both are easily removed with a solution of 1 per cent. hydrogen chloride gas in methanol, although this increases the solubility of barium sulphate to 0·5 mg per litre; these values were determined by a radiochemical method.[9]

Originally, it was intended to wash the barium sulphate with methanol in a centrifuge tube, spinning after each washing, and then measuring the activity by placing the tube on top of the sodium iodide crystal of a scintillation counter, but it was discovered that the barium sulphate precipitate adheres tenaciously to the bottom of the tube so that centrifuging was unnecessary between washings. Consequently, the thick-walled centrifuge tubes were replaced by thinner-walled Pyrex glass test-tubes, cut down to a convenient length.

The Schöberl surface combustion method was used for burning the sample. In this method, the sample is volatilised in a stream of air and the mixture passed through heated sintered-silica discs, where combustion occurs; the sulphur dioxide formed is absorbed in hydrogen peroxide solution. As this is a semi-micro method it required to be modified to enable 2-ml samples to be used.

DESCRIPTION OF THE APPARATUS, REAGENTS AND TECHNIQUE

COMBUSTION FURNACE—

Details of the apparatus are shown in Fig. 1. A tube furnace, fitted with a thermocouple pyrometer and thermostat, was used. The tube consisted of a piece of transparent quartz tubing 60 cm in length, external diameter 1·4 cm and wall thickness 0·1 cm. The section running through the furnace was loosely packed with fused quartz-wool, with the fibres, as far as possible, running longitudinally. This plug, 20 cm in length, provided a large surface area for the combustion, which was necessary for 2-ml samples, as opposed to the Schöberl method in which only 20 to 30-mg samples are used. The plug was held in position by means of a platinum wire, which had a loop at one end to retain the quartz plug, the other end being secured by bending it over the inlet end of the furnace tube.

Other furnace packings were tested, including narrow-bore thin-walled translucent silica tubing, but the combustion was not so efficient, and large variable blanks were obtained. The quartz-wool plug initially gave considerable blank values that were reduced by flushing out with air for 6 hours at 850° C. These furnace blanks were assumed to be caused by traces of sulphate volatilising from the silica. The possibility of silica volatilising from the

furnace was considered but barium silicate, unlike barium sulphate, does not adhere tenaciously to glass surfaces. A diffusion plug of quartz-wool was inserted into the inlet end of the furnace tube to homogenise the vapour - air mixture, and to contain any flash-back if the sample was introduced too rapidly. The furnace tube was connected to the other parts of the apparatus by using polythene bungs fitted around the tube at about 3 cm from the ends; B 19 ground silica cones fused to the ends of the furnace tube would have been preferred. The 25-cm length of tube emerging from the furnace provided some surface cooling of the effluent gases, but not sufficient for condensation of moisture to occur, which would carry sulphur dioxide down with it. The furnace temperature was controlled at between 800° and 850° C; at lower temperatures there was evidence of incomplete combustion.

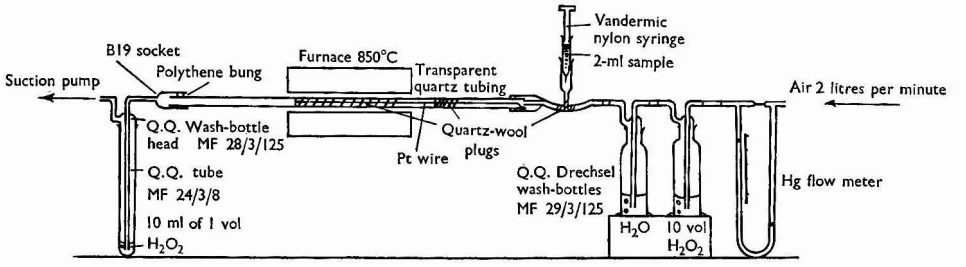

Fig. 1. Modified Schöberl surface combustion apparatus

INJECTION AND VOLATILISATION OF THE SAMPLE—

A 4-mm bore T-piece was used to inject the sample into the furnace. At about 3 cm from the end of one of the horizontal arms a B 19 socket was fused by using an internal seal; a 2-ml nylon syringe fitted with a hypodermic needle was connected to the vertical arm, and the sample was added, dropwise, from the syringe so that it fell on to a small quartz-wool plug placed at the junction of the T-piece. The sample was added at a rate such that the platinum wire adjacent to the mouth of the furnace glowed red hot, but not so fast that flash-back occurred. With an air flow-rate of 2 litres per minute the combustion time was 17 to 18 minutes. To ensure complete volatilisation of any traces of high-boiling fractions left on the plug, the T-piece was heated for a further 2 to 3 minutes to bring the total time to 20 minutes. To avoid this manual method of injection the T-piece could be replaced by a small Drechsel gas washing-bottle, the 2-ml sample being placed in the bottom and the stream of air bubbled through it, but with this method care must be taken because any flash-back passing beyond the diffusion plug would lead to an explosion in the bottle.

PURIFICATION OF AIR USED FOR THE COMBUSTION—

Air was drawn from outside the laboratory and purified by bubbling through 10 vol hydrogen peroxide contained in a 125-ml Drechsel gas washing-bottle; it was then passed through a similar bottle containing distilled water, PVC tubing being used to make the connections. Other methods of purification were tested. For example, the air was passed through sintered-glass discs supporting columns of reagents, such as alkaline permanganate as well as hydrogen peroxide, but no distinct improvements were obtained either in the magnitude or the constancy of the blanks. A possible improvement would have been to use cylinders of compressed air, which is of constant composition and would have placed the whole of the apparatus under a slight pressure instead of a slight vacuum. This would have prevented possible contamination from the laboratory atmosphere at any joints not completely air-tight.

ABSORPTION OF SULPHUR DIOXIDE—

To keep the blank from the absorbent as low as possible, only 10-ml portions of hydrogen peroxide were used at the lowest concentration possible. The vessel used for containing the absorbent consisted of the head from a Drechsel bottle fitted to a 2·5-cm diameter Pyrex test-tube that had a B 24 ground-glass socket; the bubbling tube of the Drechsel head was

extended to reach the bottom of the test-tube. A B 19 ground-glass socket was fused to the inlet tube of the head for making a connection to the polythene bung of the furnace tube. Considerable condensation occurred in the cylindrical part of this B 19 socket during the combustion, and it was always rinsed out with a portion of the hydrogen peroxide absorbent before being transferred for the radiochemical determination. It was found that 1 vol hydrogen peroxide was sufficient to absorb the sulphur dioxide completely, but there was about a 5 per cent. loss, which appeared to arise from carry-over by spray. Before use, the absorption vessel was well rinsed but not dried; after the combustion there was always a considerable increase in volume caused by condensation of water from the combustion. The final volume was found by weighing the absorption vessel, which avoided the rinsing out with distilled water that would have led to dilution.

The 1 vol hydrogen peroxide was prepared by diluting a specially purified non-stabilised grade of 100 vol hydrogen peroxide* with water obtained from alkaline permanganate solution by distillation with a fractionating column. All of the solutions were stored in plastic bottles. Blank determinations were repeatedly made on both the distilled water and the 1 vol hydrogen peroxide.

Radiochemical determination of the sulphate in the absorbent—

The volume of hydrogen peroxide absorbent used for the radiochemical determination will depend on the sulphur content of the sample. Ideally, the volume taken should be such that its sulphate content is equivalent to about 2 μg of sulphur, *e.g.*, if the petroleum sample contains about 10 p.p.m. of sulphur, then 1 ml of absorbent would be sufficient, but if the anticipated sulphur concentration is only 1 p.p.m., then at least 5 ml of the absorbent should be taken. The absorbent was introduced, by pipette, into a 3-cm diameter Pyrex glass test-tube, cut down to 6 cm in length and kept upright by standing in a small beaker. The appropriate amounts of radioactive barium chloride, to precipitate the sulphate, and hydrochloric acid, to decompose the carbonate, were added by using auto-zero pipettes. The solution was evaporated to dryness under a heat lamp, the time taken being about 15 minutes, but it could be reduced by using larger diameter tubes. The use of test-tubes has the advantage that the barium sulphate precipitate will always be located at the bottom of the radius of the tube, whereas with flat-bottomed containers the precipitate may be located anywhere on the base, and this would lead to inconsistent activity measurements. The active precipitate was washed free from excess of barium chloride reagent with small portions of anhydrous methanol until constant activity was obtained. The total blank activity was subtracted from this value and the resulting net activity expressed as micrograms of sulphur.

The scintillation counter used for the activity measurements was fitted with a 3-cm diameter sodium iodide crystal, and the test-tube containing the activity mounted directly above it. The γ-photons from the barium-133 are sufficiently energetic to penetrate both the wall of the test-tube and the aluminium can enclosing the crystal. In all of the activity measurements the time taken to record 10,000 counts was noted and results expressed as counts per 10 seconds. In this way the counting error caused by the random nature of decay was reduced to 1 per cent. The background of the counter was about 400 counts per 10 seconds and the crystal was not shielded.

Radioactive barium chloride reagent—

Barium-133 was selected for labelling because of both its long half-life and energetic γ-radiation; 100 μCi of barium-133 were purchased in the form of barium chloride, in a volume of 1 ml, the weight of barium being 70 μg. This solution was diluted to 250 ml with inactive 0·002 N barium chloride. For each determination, 0·1 ml of this solution was used, the amount of sulphate that it will precipitate being equivalent to 3·2 μg of sulphur.

The reagent was calibrated periodically against 0·1 ml of 0·001 N sulphuric acid; in this way a check was kept on the specific activity of the reagent. Dilution of the reagent does not affect its calibration, and for very low sulphur contents the reagent was diluted with twice its volume of water. With a half-life of 10 years, the decay correction is very small and, in any event, it is avoided by the calibration with sulphuric acid.

* Mallinckrodt Transistar Chemicals. Supplier Camlab (Glass) Ltd., Cambridge.

Health hazard and waste disposal are not serious problems as 0·1 ml of reagent contains only 0·04 μCi of activity; however, the usual precautions recommended when handling radioactive materials should be observed. The isotope is comparatively inexpensive and the cost of each determination is negligible.

Method

Flush the apparatus for 30 minutes with an air flow of 2 litres per minute, and a furnace temperature of 800° to 850° C. Rinse the absorption tube and head with distilled water and drain by shaking them. Add 10 ml of 1 vol hydrogen peroxide and connect the vessel to the furnace tube, moistening the polythene bung. Draw 2 ml of sample into the nylon syringe and fit it to the vertical arm of the T-piece injector. Initially, draw air through the furnace slowly and note that the bubbling rates in both the absorber and wash-bottles are the same, thus checking that the apparatus is air-tight. Set the air flow to 2 litres per minute and add the sample, dropwise, at a rate such that the platinum wire glows red hot but flash-back does not occur. When all of the sample has been added, heat the T-joint with a blast of hot air to volatilise any high-boiling fractions, the total time taken being 20 minutes. Weigh the absorption tube and head (previously weighed dry and empty) to the nearest decigram to give the total volume of absorbent, then rinse the inlet socket with some of the used absorbent and transfer the entire contents to a plastic sample bottle.

Transfer, by pipette, a volume of the absorbent, depending on the sulphur content of the sample, into the special test-tube, together with 0·1 ml of barium chloride reagent and 0·1 ml of 4 N hydrochloric acid, and evaporate to dryness under a heat lamp. Wash the residue with 1-ml portions of anhydrous methanol until constant activity is obtained.

Make a combined blank determination on the hydrogen peroxide absorbent, the air and the apparatus itself under exactly the same conditions. Determine the background activity of the counter.

Results

Heptane, to which sufficient *p*-toluene sulphonyl chloride was added to give 10 p.p.m. (w/v) of sulphur, was used. A 2-ml portion was burnt off, and a known volume of the hydrogen peroxide absorbent reacted with 0·1 ml of the radioactive barium chloride reagent, as previously described. All activities have been expressed as counts per 10 seconds.

Volume of absorbent from the combustion	13 ml
Net activity of barium sulphate from 1 ml of absorbent	4000 counts
Volume of absorbent from the blank	11 ml
Net activity of blank from 2 ml of absorbent	700 counts
From the calibration of the barium chloride reagent with 0·001 N sulphuric acid, 1 μg of sulphur	2250 counts

$$\text{Sulphur in 2 ml of heptane} = \frac{4000 \times \left(\frac{13}{1}\right) - 700 \times \left(\frac{11}{2}\right)}{2250}$$

$$= 21{\cdot}4\ \mu g$$

$$\equiv 10{\cdot}7 \text{ p.p.m. w/v.}$$

The discrepancy of 0·7 is caused by the heptane containing about 1 p.p.m. of sulphur.

Discussion

The lowest detectable amount of sulphur will be dependent on the magnitude of the blank, to which the quartz tube and packing make a considerable contribution. The grade of hydrogen peroxide used was the purest obtainable, but traces of sulphate and phosphate impurities were present. The distilled water also had a slight sulphate content, which possibly resulted from the evaporation in glass test-tubes. The purified air used for the combustion also had a blank value.

The average blank value corresponding to a determination on a 2-ml sample was equivalent to 1·8 to 2·0 μg of sulphur. The approximate contributions from each were: furnace tube and packing 2/5ths, the hydrogen peroxide, air and water 1/5th each of the total. This

value for the total blank gives the smallest amount of sulphur that can be determined with any degree of accuracy, which means that for a 2-ml sample the lowest detectable concentration is 1 p.p.m., but an approximate value could be obtained down to 0·25 p.p.m.

Analyses performed on a large number of samples of heptane, containing known amounts of sulphur in the form of either carbon disulphide or *p*-toluene sulphonyl chloride, disclosed that the accuracy of the method varied with the sulphur concentration. For those samples containing 10 p.p.m., the accuracy was better than ±5 per cent., but when the concentration was down to 1 p.p.m. the accuracy was no better than ±15 per cent. Similarly, the blank values had a spread of ±15 per cent. of the average value. This wide range for low-sulphur samples, and for the blanks, was attributed mainly to the random contamination from the laboratory atmosphere, and to a lesser extent to the furnace tube and packing releasing sulphur trioxide impurities in an irregular manner.

The author thanks the East Midlands Gas Board for assistance in the preliminary work and for providing the barium-133.

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The Automatic Determination of Original Gravity of Beer

Part I. Introduction and Determination of Reducing Sugar after Hydrolysis

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The determination of the "extract" gravity of sound beers by automatic means is presented and discussed. A method is proposed for correlating "extract" as determined by classical distillation methods with the total reducing sugar after hydrolysis as determined in an automatic analytical system operating at 30 samples per hour, and this is shown to have the form—

$E - 1000 = 4{\cdot}33\ S + 1{\cdot}2$, where E is the extract weight (expressed relative to water, sp. gr. 1000) and S is the percentage of sugar as anhydrous glucose. The use of the method as part of a screening technique for examination of samples against declaration is discussed.

Each year more than 20,000 samples of sound beer are examined in this laboratory for the purpose of controlling duty. This duty is based on the "original gravity" of the sugar solution, or wort, from which the beer was brewed. The method of determining this, as laid down by Statute,[1] requires that a measured volume of beer, taken at a fixed temperature, be distilled, and the distillate made up to the volume of the original sample at the same temperature and its specific gravity determined. A table in the Statute gives a value for the "gravity lost" which, when added to the gravity of the distillation residue, again at the original sample volume and temperature (residual sugar or "extract"), furnishes the "original gravity." As the determination of original gravity by the Statutory method is a relatively lengthy process, a rapid method of screening samples of beer against a declared value of original gravity is desirable. One such method, in which manual measurements of the physical constants, refractive index and present gravity of the beer are used,[2] is now in use. The potential operating speed and reduced operator attendance of automatic analysis systems offered an alternative to the rather tedious operation that gave laboratory juniors little satisfaction in their work.

Preliminary indications of the possible use of automatic analytical techniques in this field appeared in published methods by the Brewing Industry Research Foundation.[3,4,5] These papers, in which methods for the automatic determination of reducing sugars and alcoholic content of beers were described, appeared to form the basis of the measurement of the two parameters necessary to determine original gravity.

The major portion of the distillation residue in beer samples is a mixture of polysaccharides and the fragment sugars from hydrolysed starches together with any residue from added cane and other priming sugars. A survey of the relationship between "extract weight" and reducing value after hydrolysis was carried out in this laboratory by using manual techniques; the correlation between these two factors was sufficiently encouraging to allow the development of automatic techniques to be pursued. Preliminary reports of the results of this work have been described elsewhere.[6]

This paper is concerned with the development of the technique of determining the reducing value of the residual sugars in beer in such a way that the results could be used to calculate the extract weight of the beer.

DETERMINATION OF REDUCING VALUE—

The "extract" specific gravity for the majority of beers of all types lies between the limiting values 1·004 to 1·048, which are about equivalent to a range of reducing values after acid hydrolysis of 1 to 10 per cent., expressed in terms of glucose. As the colour of beer varies from a pale straw colour for lagers to dark red - black for stouts, any colorimetric method applied to the whole range of samples must allow for these extremes of colour. Automatic methods with dialysis techniques were found to be unsatisfactory because of the varying amounts of coloured materials that dialysed from widely different samples.

Methods of determining reducing sugars in solution by manual techniques are based on the use of alkaline oxidants such as complexes of copper salts, *e.g.*, Fehling's solution and the many variants of this formulation; those most frequently encountered involve the use of gravimetric or titrimetric finishes.[7,8] Several variants in colorimetric finish of these exist; the Somogyi[9] modification of Nelson's[10] reagent with molybdoarsenate reagent to measure reduced copper in suspension has been used in both manual and automated methods.[11,12] These modifications are used with sugar solutions at a final concentration of 0·001 to 0·008 per cent. of dextrose, which is attained with a dilution ratio of 1 : 1250 for all beers. At this dilution the optical density of the dark stouts is in the region of 0·005 per cm at 500 to 550 mμ and may be regarded as analytically significant if an optimum optical density range of 0 to 0·3 per cm is used in the colorimetric determination. Furthermore, it has been stated[11] that at the upper levels of sugar concentration the reagent may be prone to deposit copper oxide. Intermittent precipitation of this nature cannot be tolerated in the automatic analytical system, as particulate matter in suspension produces extremely noisy traces and promotes base-line drift because of deposition of solid matter on flow-cell windows. For these reasons the copper reduction methods were not considered further.

Alkaline ferricyanide was first proposed as an oxidising agent for sugars by Gentele[13] and, subsequently, interest was revived by the introduction of the reagent in the analysis of blood sugars by Hagedorn and Jensen.[14] Colorimetric versions of the technique were proposed by Hoffman,[15] who determined loss of ferricyanide colour, and by Folin,[16] who determined the Prussian blue formed on addition of an acid iron(III) salt to the reduced ferricyanide. Automatic analytical systems with the Hoffman mode of analysis were proposed by Hill and Kessler,[17] and reports of various modifications of this method have appeared from time to time.[18,19] The "colour loss" technique is limited, however, as measurement of optical density is made at 420 mμ with solutions at concentrations in the range of 0·0006 to 0·007 per cent.; again the colour variation of the diluted samples has analytical significance with these methods, as a 1000-fold dilution of a dark beer yields a solution with an optical density of 0·02 per cm at 420 mμ. Automatic methods based on the formation of coloured species from the ferricyanide produced in solution have been described by Fuller,[20] who used Prussian blue formation and by Fingerhut, Ferzola and Marsh,[21] who used a molybdophosphate complex of ferrocyanide in acidic solution. These two approaches were considered to be worthy of further investigation as finishes in the sugar determination. It was necessary to develop a satisfactory finish technique before a study on the conditions of hydrolysis could be carried out and initially the Prussian blue technique, which we found satisfactory, was used, but for reasons given below this was later abandoned in favour of the molybdophosphate finish.[21] Methods given that combined acid hydrolysis with a colorimetric determination, such as those based on sulphuric acid anthrone,[22,23] were rejected because of the sample colour variation and the undesirability of 27 N sulphuric acid for routine use.

EXPERIMENTAL

In all our operations on the AutoAnalyzer with colorimetric finishes our aim has been to optimise the chemistry of the finish to produce a solution with an optical density within the range 0 to 0·3 referred to blank reagents at the working wavelength and with one of the standard flow cells in the system. This mode of operation has been used so that the 50 to 100 per cent. transmission portion of the recorder chart could be expanded to full scale by the use of a $\times 2$ range expansion. The resulting calibration graph is then spread over the maximum chart width and produces a calibration approaching a straight line with most methods.

APPARATUS—

The components of the AutoAnalyzer assembly used were as follows.

Voltage stabiliser; Sampler II operating at 30 samples per hour with de-ionised water wash between samples, the ratio of sample time to wash time being 2:1; two proportionating pumps (two speed) with accommodation for 15 tubes on each pump; one heating bath, set to operate at 95° C, with two delay coils and a colorimeter with 8-mm flow cell equipped with 600 mμ or 480 mμ filters; and a two-pen recorder with range expander on each amplifier.

PRINCIPLES OF MANIFOLD DESIGN—

A manifold has been designed to combine dilution, acid hydrolysis, reaction with alkaline ferricyanide, and subsequent reaction with a colour-forming species, to measure the hydrolysed sugars in the full range of beer samples. In order to obtain a suitable optical-density scale with the Prussian blue finish for the beer samples a dilution of 25,000 is necessary through the four stages of the method, which is more than required for removal of beer colour variation. The manifold design has been successfully developed by particular attention to detail in assembly of components to give the minimal flow path lengths for unsegmented liquid streams. Re-cycling and dilution stages are carried out by introduction of polythene tube (0·03 × 0·48 inch) into a close-fitting PVC insert in the lower limb of all C5 de-bubblers. The tube is inserted directly into a pump tube cut off immediately in front of the end block stop. A further length of polythene tubing is used to carry the unsegmented stream after pumping from a shortened end of the pump tube to the metal insert H3 type of connecting piece (Fig. 1). The full manifold layout is indicated in Fig. 2, together with details of the

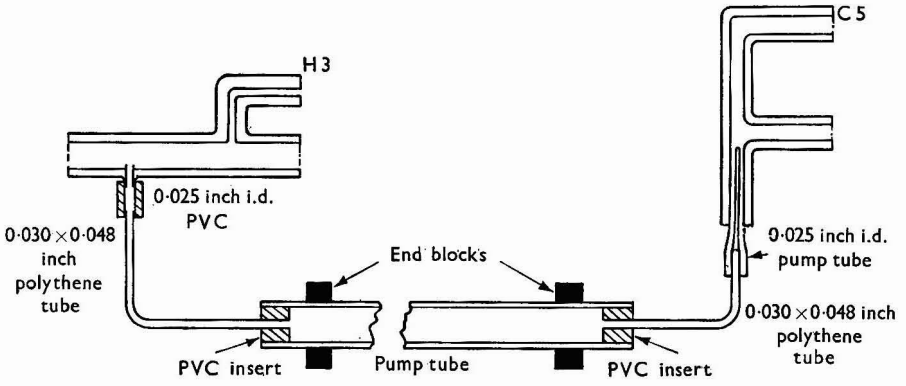


Fig. 1. Connection of debubbler to pump tube and return to manifold for dilution

reagents used. Observation of these basic principles of operation is essential for successful application of a multiple dilution method at sampling rates of 30 per hour with maintenance of adequate sample discrimination as shown in Fig. 3.

PRUSSIAN BLUE FINISH—

A typical calibration obtained over the range 1 to 11 per cent. of dextrose with the Prussian blue finish is indicated in Fig. 4; the method shows a pronounced curvature of response with cut-off at sugar concentrations lower than 1 per cent. Experimentation with the reagent system shows that the cut-off level can be adjusted by altering the sugar dilution or by adjusting the iron(III) ion concentration of the colour-forming reagent. An increase in iron(III) ion produces a colour within the "toe" of the curve but the same reagent produces a precipitate at higher sugar levels (*i.e.*, higher resultant ferrocyanide); similar effects are obtained by increasing the sugar concentration in the oxidising solution.

Comparison of this standard curve with that obtained with the molybdophosphate procedure detailed below, indicates that the threshold limit on the product $[Fe^{3+}]_4[Fe(CN)_6^{4-}]_3$ is influenced by the complex formed with free $[Fe^{3+}]$ by the phosphoric acid used as the

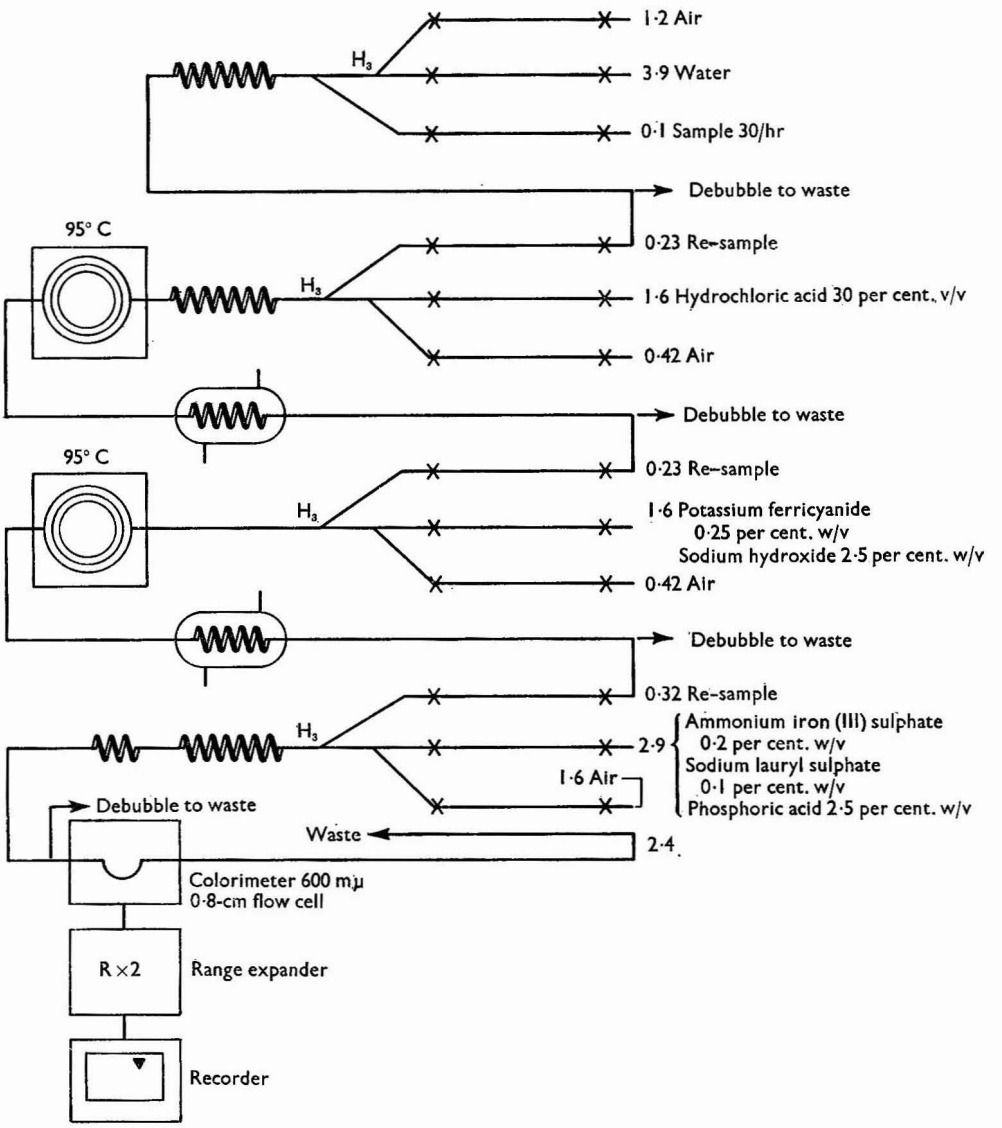


Fig. 2. Manifold for the total hydrolysable sugars in beer by the Prussian blue method

acidifying medium, and the solubility product is exceeded only when an amount of ferrocyanide is produced equivalent to the oxidation of sugar at the 1 per cent. level in the original sample. The reagent system shown is the best compromise solution operating over the range 1 to 11 per cent. of sugar in the original samples taken. The early report of this work[6] describes the use of the Prussian blue technique for the sugar determination; subsequently, this finish was abandoned in favour of the molybdophosphate determination. The principal reason for this change is that the threshold limit, and hence the calibration curve shape has been found to be susceptible to the quality of the phosphoric acid used in preparing the ammonium iron(III) sulphate reagent. The use of different batches of analytical-reagent grade phosphoric acid (especially from one manufacturer) produced precipitation of Prussian blue to varying degrees in the upper part of the calibration scale. Furthermore, in studying the variation of acid concentration necessary to give optimum hydrolysis conditions, it has

been found that precipitation also occurs at the colour finish stage when the hydrolysis is carried out with hydrochloric acid at concentrations over 25 per cent. v/v.

Although the curved response line could be overcome by addition of an appropriate amount of ferrocyanide to the ferricyanide reagent, the susceptibility to what appears to be extraneous iron contamination could not be readily eliminated. The molybdophosphate reagent system appears to tolerate those variations in reagent quality that were troublesome in the Prussian blue method.

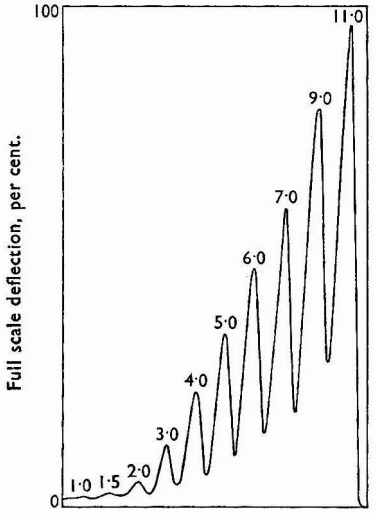


Fig. 3. Dextrose standards, Prussian blue method

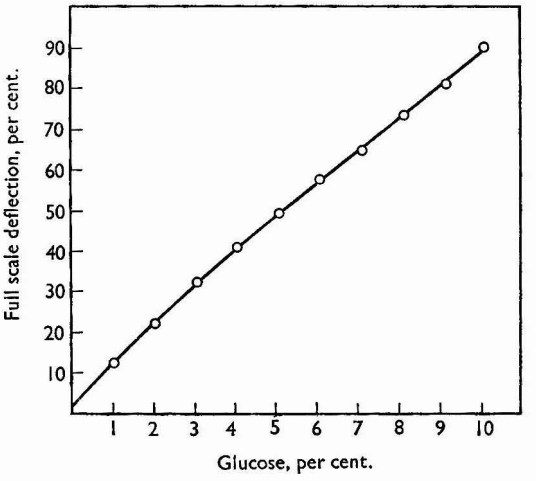


Fig. 4. Calibration curve, Prussian blue finish, of sugar as glucose, per cent. w/v, against full scale deflection, per cent.

MOLYBDOPHOSPHATE FINISH—

The method of Fingerhut, Ferzola and Marsh,[21] which is based on the reaction between molybdate ion and ferrocyanide in acidic solution,[24,25,26] has been adapted to satisfy the conditions required for analysis of beers containing 1 to 10 per cent. of reducing sugar after hydrolysis. A manifold is described by Fingerhut in which molybdophosphoric acid is added directly to the alkaline ferricyanide oxidation stream directly on emergence from the 95° C heating bath and the resulting colour measured at 450 mμ. We have found that if this course of action is followed the traces on the recorder chart are not suitable for application to our method of analysis. A typical set of traces is illustrated in Fig. 5. The curves owe their irregularity to three major faults. Firstly, 4 ml per minute of reagent are added to an existing reaction mixture that is pumped at 1·2 ml per minute, causing momentary stoppages

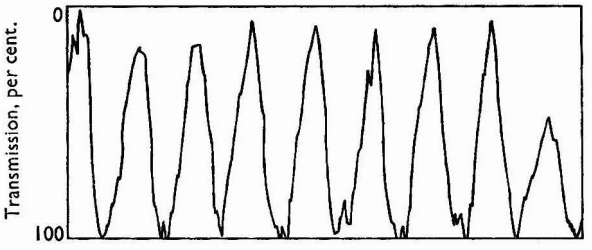


Fig. 5. Response, per cent. of dextrose by the original method

and surges in the main reaction stream. Secondly, direct addition of the acidic reagent to the alkaline reaction stream causes initial local concentration variations in the solution before mixing takes place, and at acid pH values and high ferrocyanide concentrations precipitation from solution of the reaction product occurs; this precipitate forms around the liquid junction and is detached in fragments by the flowing stream. Thirdly, at the wavelength chosen for measurement (450 mμ) the reagent system at zero sugar concentration has an appreciable optical density, as is shown in Fig. 6. Irregularities in the addition of reagent are amplified by measurement at such a point on the spectrum. A modified system designed to avoid these features has been assembled and the layout is indicated in Fig. 7; this manifold is the one finally used for the sugar determination in the beer samples.

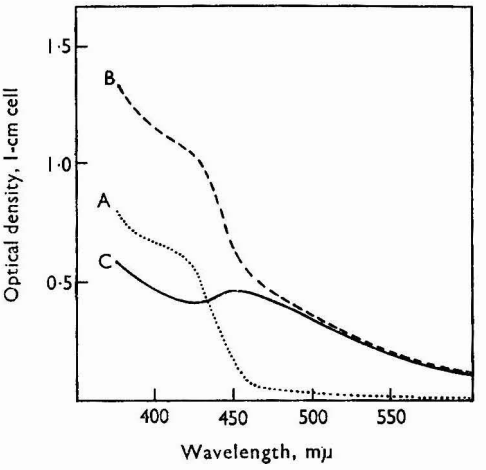


Fig. 6. Spectrum plots of molybdophosphoric acid - ferrocyanide system for: A, reagents against water; B, reaction mixture for 10 per cent. sugar against water; C, reaction mixture against reagents

DETERMINATION OF OPTIMUM ACIDITY FOR HYDROLYSIS—

The choice of acid concentration and time of hydrolysis of samples to obtain maximum reducing sugar yield for a given set of samples are dictated by the geometry of the apparatus and by the selection of suitable solution pumping rates. Choice of pumping rate is pre-determined by that ratio of liquid to segmenting air which will provide a satisfactory bubble pattern with good sample discrimination, after allowing for expansion and contraction of the air bubble on entering and leaving the heating bath. The slowest rate of pumping to satisfy these conditions is about 2 ml per minute total volume, with a liquid-to-air ratio of 4:1. In these circumstances the time in a standard coil in the heating bath is about 6¾ minutes per sample, which can be modified by varying the air segmentation rates from about 3 to over 8 minutes, but at both extremes the liquid segmentation is erratic.

Initial chromatographic examination of samples after acid hydrolysis with varying concentrations of hydrochloric acid indicated that an almost complete hydrolysis of higher polysaccharides is obtained with a working acid concentration of 2·3 N. The results of a further examination of various representative samples of beer with 15 to 40 per cent. v/v hydrochloric acid and an acid-to-sample ratio of 16:3, and with the full colorimetric finish, are indicated in Fig. 8. The graphs presented show that an optimum yield is obtained with most beers when the hydrolysis acid has an initial concentration in the region of 30 to 40 per cent., the highest reducing value for primed beers is obtained with 30 per cent. v/v acid while the lagers require an acid concentration approaching 40 per cent. v/v. The concentration chosen is a compromise value to include the whole range of beer types within

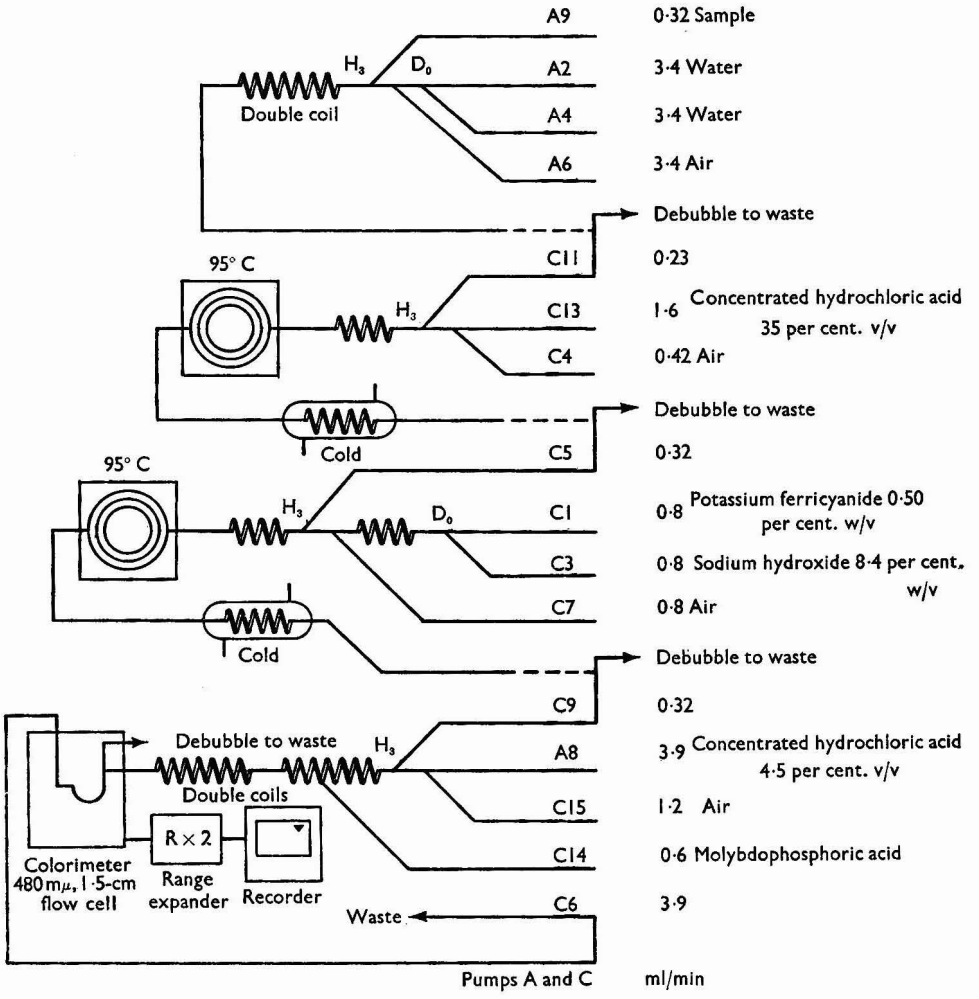


Fig. 7. Manifold for total hydrolysable sugars in beer

one screening method. If, however, the method is to be used with a single type of beer, then a hydrochloric acid concentration suitable for that beer type can be chosen.

Method

Reagents—

Hydrochloric acid for hydrolysis—Dilute 3·5 litres of analytical-reagent grade concentrated hydrochloric acid to 10 litres with distilled water.

Hydrochloric acid, dilute—Dilute 450 ml of analytical-reagent grade concentrated hydrochloric acid to 10 litres with distilled water.

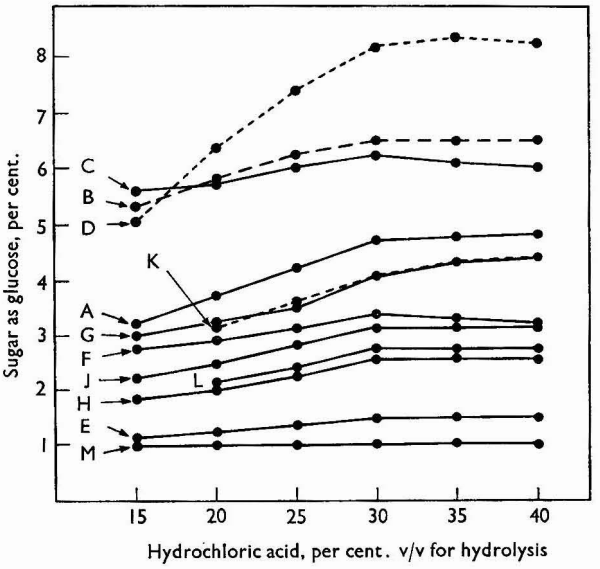
Potassium ferricyanide—Dissolve 25 g of analytical-reagent grade potassium ferricyanide in 5 litres of distilled water.

Sodium hydroxide—Dissolve 420 g of analytical-reagent grade sodium hydroxide in 5 litres of distilled water.

Molybdophosphoric acid reagent—Add 350 g of analytical-reagent grade molybdenum trioxide and 50 g of analytical-reagent grade sodium tungstate (hydrated) to 1 litre of distilled water in a 5-litre beaker, stir, add 200 g of sodium hydroxide, and boil the mixture for 20 to 30 minutes. Cool the solution, add 1 litre of distilled water and cautiously add

1·25 litres of 88 per cent. analytical-reagent grade orthophosphoric acid, cool and dilute to 5 litres.

Standard solutions—Prepare standard solutions, in the range of 1 to 10 per cent. (w/v) in unit steps, of analytical-reagent grade anhydrous glucose. In practice these standards contain similar unit volumes of alcohol for the alcohol method reported in Part II of this series (p. 680).



A = Continental beer
B = Bitter ale
C = Danish Pilsner
D = Pale ale
E = Export ale
F = Polish lager
G = Brown ale
H = Stout
J = Milk stout
K = Milk stout
L = Milk stout
M = Strong ale

Fig. 8. Variation of sugar determined after hydrolysis with different acid concentrations

PROCEDURE—

The samples for analysis are de-gassed by rousing and filtration through a Postlip filter-paper (18·5 cm, Evans Edlard and Co.) contained in a polythene funnel. The first runnings are rejected and the bulk sample is collected in a squat 250-ml beaker. A series of standard solutions is placed in 10-ml sample cups and placed in position in the Sampler II, and a series of samples is then placed in rotation following the standards. Individual check standard solutions are then placed at the rate of one in every twenty samples.

The sampler is operated at the rate of 30 samples per hour; in normal operation this feeds a double manifold for sugar and alcohol but consideration of the alcohol method has been omitted at this stage. An aliquot for sugar determination at 0·32 ml per minute is fed into air-segmented water at 6·8 ml per minute; the stream is mixed and re-cycled to the pump. This procedure conveniently dilutes out the residual dissolved gases and reduces the alcohol content of the samples to less than 0·5 per cent. v/v. An aliquot of this dilution is then pumped at 0·23 ml per minute into an air-segmented stream of hydrochloric acid (35 per cent. v/v). The streams are mixed and passed to a standard length delay coil in the heating bath at 95° C, the emergent stream of hydrolysed sugars is cooled, de-bubbled and an aliquot is pumped at 0·32 ml per minute into an air-segmented mixture formed by 0·8 ml per minute

sodium hydroxide (2·1 N), 0·8 ml per minute potassium ferricyanide (0·5 per cent.) and air at 0·8 ml per minute. The resultant alkaline mixture is then passed to the second standard delay coil in the heating bath where the reducing sugars are oxidised; the stream is then cooled and re-cycled for the final colorimetric stage. A 0·23 ml per minute aliquot of the

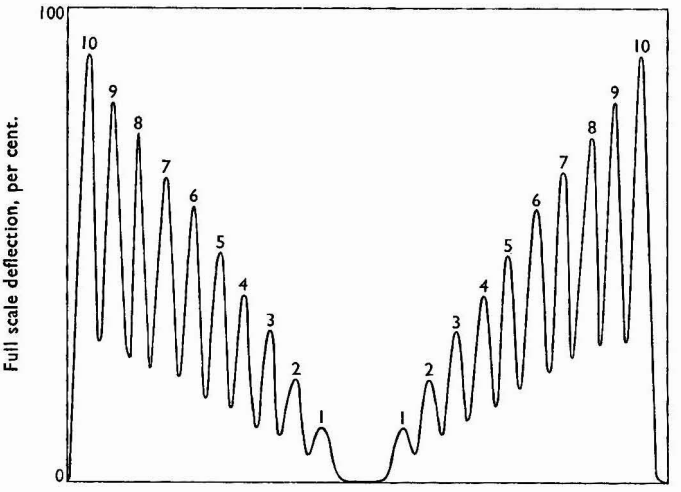


Fig. 9. Response peaks of dextrose, molybdophosphate method

ferricyanide - ferrocyanide couple is first diluted and acidified by passing it into an air-segmented stream of hydrochloric acid (4·5 per cent.) at 3·9 ml per minute. This step is essential to avoid the localised precipitation of the reaction product with molybdophosphate referred to under Molybdophosphate finish. The mixed and diluted ferricyanide - ferrocyanide then receives an addition of molybdophosphate reagent that is pumped at 0·6 ml per minute. After mixing, the resulting stream is de-bubbled, and a fraction of the liquid stream is pumped through an 8-mm flow cell in the colorimeter, which is equipped with

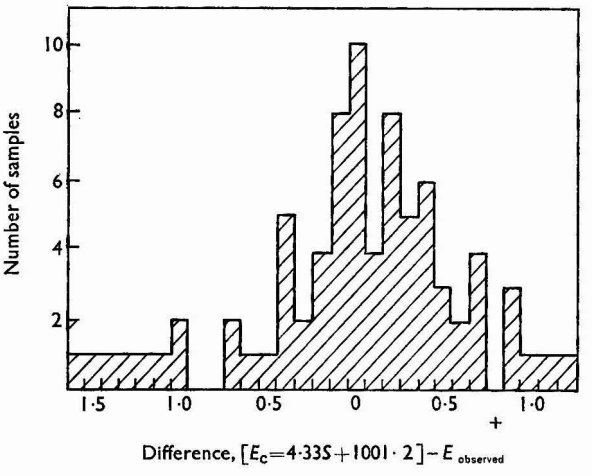


Fig. 10. Difference between calculated extract and observed extract samples used in preparing regression equations

480-mμ narrow band filters. Fig. 9 indicates the normal response peaks obtained with this method, and Fig. 10 indicates the plot of sugar concentration against peak height readings with a $\times 2$ range expansion on the recorder.

Results and discussion

A study has been made of the correlation between the "extract weight" as determined by the distillation procedure with the 1000 notation for specific gravity, and the reducing sugar value after hydrolysis, expressed as anhydrous glucose. A total of 79 samples of different beers of all types with extract weights varying from 1008·0 to 1039·0 has been used to establish such a correlation having the form—

$$* E - 1000 = 4{\cdot}33\,S + 1{\cdot}2$$

where E = extract weight, expressed relative to water (sp.gr. 1000), and
S = percentage of sugar as anhydrous glucose.

The statistical constants obtained for this line are: regression coefficient = 0·996, standard error of the estimate of the extract weight = 0·62.

A histogram plot of calculated extract weight against observed extract weight for the samples used in deriving this equation is shown in Fig. 11.

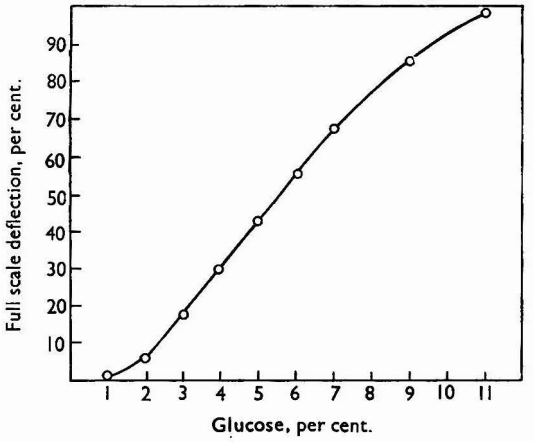


Fig. 11. Calibration curve, glucose, per cent., against full scale deflection, per cent.

A further series of samples was chosen on the basis of the difference between calculated and observed extract values to examine whether the deviations for a particular type of beer were constant and, if so, what significance could be placed on a "label correction." Values for these observations are indicated in Table I and probability levels of significance

Table I

Comparison of extract weight from sugar determinations with that obtained by distillation of various beers

Type of beer	Number of samples	Mean extract: Calculated from sugar	Mean extract: Observed on distillation	Mean difference between pairs calculated - observed	Standard deviation of differences
Milk stout	6	28·2	28·1	+0·1	0·24
Stout	5	26·3	24·8	+1·5†	0·22
Stout	5	16·7	16·4	+0·3‡	0·10
Continental lager ..	11	18·2	17·7	+0·5†	0·30
Continental lager ..	12	10·8	10·4	+0·3†	0·13
Continental lager ..	12	14·3	13·8	+0·5†	0·21
U.K. lager	12	15·4	14·6	+0·8†	0·25
Pale ale	12	16·6	16·3	+0·3‡	0·36
Strong ale	6	27·6	29·7	−2·1†	0·37
Brown ale	5	17·0	16·7	+0·3	0·45

† Significant at 0·1 per cent. probability level.
‡ Significant at 1 per cent. probability level.

* The equivalent correlation determined by manual hydrolysis and titration with Fehling's solution was $E - 1000 = 4{\cdot}32\,S + 1{\cdot}9$ for 39 samples.

obtained by Student's *t*-test are indicated. There is evidence of a deviation characteristic of a particular label within the proposed method. In the practical situation when the method is used as a screening test, this value would be weighed against any tolerance allowed within the laboratory for such a screening test.

A further examination of the calculated extract from duplicate determinations of sugar for various beer samples showed that for 131 pairs the mean extract difference is 0·27 and the standard deviation for the population is 0·29; this compares with a similar average figure for the standard deviation of differences between calculated extract and true extract. The larger value of standard error of the estimate of extract from sugar values obtained in the regression analysis confirms that the differences between observed and calculated values for specific samples shown in Table I are significant. The development of a method for the determination of "gravity lost" from the alcohol content of the various types of beer forms the basis of a further paper (Part II), which will also include a comparison of the determination of original gravity by distillation and by a combination of the two methods.

The authors wish to thank Miss L. M. Grisley for the manual determinations used in establishing the correlations shown, and Miss B. G. Cox for experimental work on the molybdophosphate method. The Government Chemist is also thanked for his permission to publish this paper.

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The Automatic Determination of Original Gravity of Beer

Part II.* The Determination of Alcohol and Gravity Lost

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The determination of gravity lost on samples of sound beers by an automatic distillation procedure, followed by determination of alcohol in the distillate, is presented and discussed. The correlation between alcohol content and gravity lost, determined by distillation, is assessed, and the resulting relationship is used to determine gravity lost in a series of samples. Summation of results with those obtained in Part I indicates the accuracy of the determination of total original gravity by the method proposed, and is shown to be in agreement within ± 2 units of original gravity with the distillation procedure; a mean standard deviation of 0·37 is obtained on replicate analysis.

The reasons for the development of an automatic method for the determination of original gravity of beers have been elaborated in a previous paper[1] concerned with the determination of the "extract gravity." This paper is concerned with the direct automatic determination of alcohol in the sample, the value for which is used to calculate the "gravity lost" during fermentation. The conversion of alcohol content via distillate gravity into "gravity lost," from the classical distillation method, is defined in the Mean Brewery Table, and is consolidated in the Customs and Excise Act.[2]

One method for the automatic determination of alcohol in samples of beer has been described by Ashurst.[3] A colorimetric method, based on the formation of red, solvent-soluble alcoholates of vanadium 8-hydroxyquinolinate, has been described by Tanaka,[4] and this method has been used for determinations of alcohol in blood.[5,6] Enzymatic methods based on the use of alcohol dehydrogenase for automatic determinations of blood alcohol have also been reviewed and reported.[7,8] The use of such enzymatic techniques was regarded as too costly for work on the scale proposed in our laboratory, and no further consideration was given to this method.

Preliminary experimental work—

The method of Ashurst, in which the red hexanitratocerate alcoholate colour was used, was considered initially, but the major disadvantage of this method was that a knowledge of the specific gravity of the beer sample was necessary so that the sample could be diluted manually to within a fixed gravity range. This procedure was necessary because carbohydrates interfered with the hexanitratocerate reaction. Dilution to a fixed specific gravity range reduced the residual sugar concentration of the sample to a level at which almost complete oxidation of the reducing sugars with alkaline ferricyanide occurred before the reaction with the alcohol reagent removed the interference.

The procedure depended on the dialysis of alcohol away from the ferricyanide reaction mixture, used for oxidation of sugars, before the reaction with hexanitratocerate. It was considered that the manual step required in this procedure negated some, if not most, of the advantages of an automatic technique. Attempts were made to modify this procedure to allow samples to be treated wholly by the automatic technique. It was found, however, that, at the increased concentration of alkaline ferricyanide necessary to oxidise the reducing sugars in the high gravity beer samples, a fraction of the ferricyanide reagent dialysed across the dialyser membrane together with the alcohol. This ferricyanide concentration was sufficient to cause variable reaction with the hexanitratocerate; the extent of reaction was inversely proportional to the amount of ferricyanide used in the sugar oxidation. Transfer of colour across the dialyser membrane was also evident with dark samples. For these reasons further work on this method was discontinued.

* For details of Part I of this series, see reference list, p. 687.

Further attention was then paid to the Tanaka method, which depends on the transfer of alcohol from the aqueous phase into a solvent containing vanadium 8-hydroxyquinolinate where the red alcoholate is formed from the blue - black reagent. Under the conditions necessary to measure alcohol derived from beer in any of the solvents (nitrobenzene, dichlorobenzene, chloroform) compatible with the system, it was necessary to heat the two-phase reaction mixture to 50° C to produce a measurable colour in the organic phase in a practicable time. On separation of the phases following the heating cycle, removal of dissolved water from the organic phase was incomplete and this caused noisy traces. Addition of acetone or dioxan to the organic layer after separation rendered the water droplets miscible with the solvent but also introduced residual sample colour.

The use of automatic vapour sampling techniques has been described in the determination of hydrogen fluoride after distillation,[9] metabolic carbon dioxide[10] and carbon dioxide derived from antibiotic assays with micro organisms.[11] The method of continuous distillation of volatile aldehydes and ketones described by Duncombe and Shaw[12] has been adapted to suit our needs.

Experimental

Apparatus—

In addition to the units elaborated in Part I,[1] the apparatus required for the operation of the combined manifold comprises a distillation unit mounted in a 95° C heating bath, a proportioning pump, a colorimeter with an 8-mm flow cell and 610-mμ filters, a range expander to second pen on a two-pen recorder and two standard delay coils.

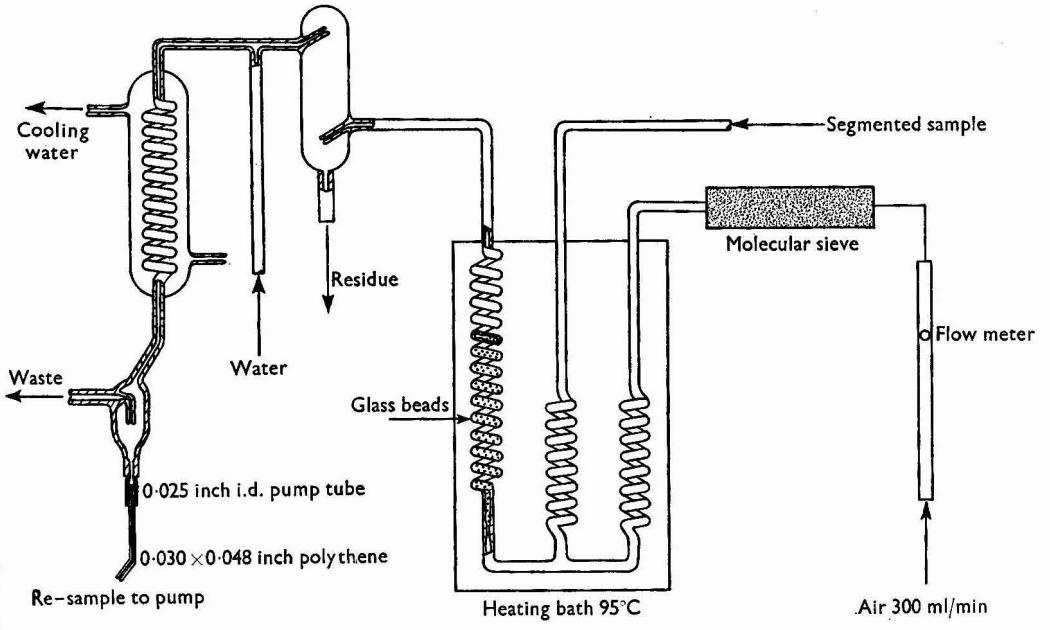

Fig. 1. Distillation flow path

The distillation unit developed is illustrated in Fig. 1. The assembly consists of three vertically mounted glass coils, the lower ends of which meet at a T-junction in a 95° C oil-bath. A stream of nitrogen is introduced via one coil and meets a hot segmented stream of liquid set up from a series of samples in the second coil. A flash distillation of alcohol is achieved, and the vapour is carried through the third beaded coil to the outside of the heating bath. The vapour and residue are then separated in the glass separator illustrated, and a sample of vapour is carried by the gas stream to a condensing train fed at a T-junction with distilled water. The undistilled residue and some gas are rejected to waste. The sample vapour is condensed in a standard coiled condenser and the emergent gas and liquid are separated

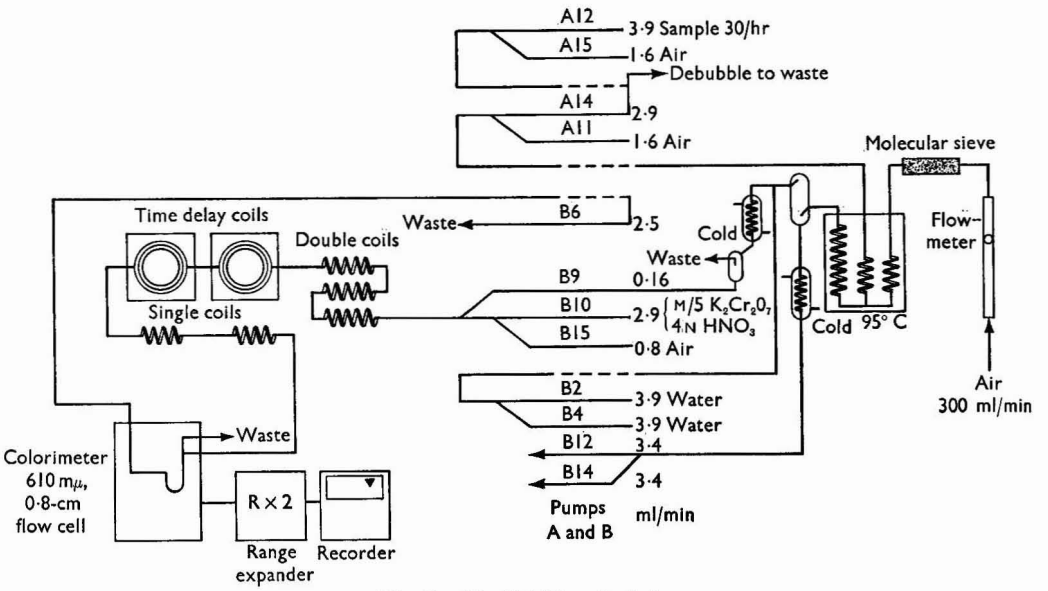

Fig. 2. Manifold for alcohol

at a modified Technicon B2 micro trap; all the gas and some condensate are rejected to waste and a sample aliquot is pumped back into the manifold for colorimetric analysis with an acid dichromate finish. In normal operation the wash chamber of the sampler is supplied with distilled water. This ensures that the distillation unit is cleaned between samples and that no insoluble residue is deposited in the glass coils. A diagram of the manifold as a separate assembly is shown in Fig. 2. In the full method a combined manifold incorporating the

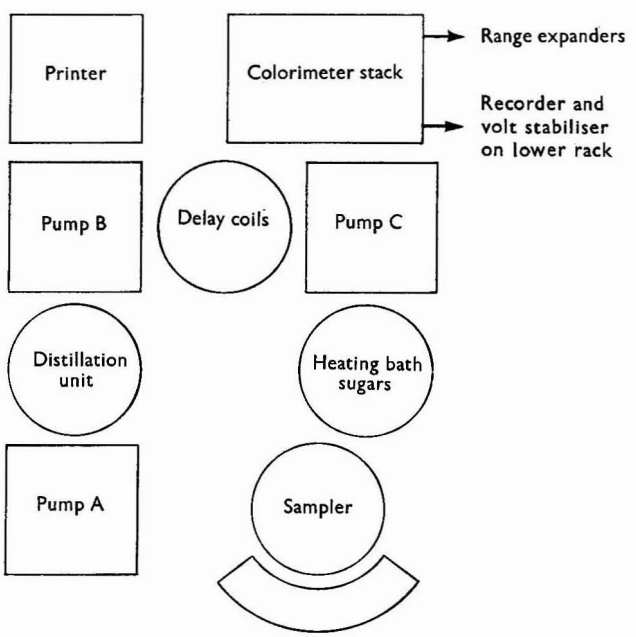

Fig. 3. Block schematic layout of operating units of AutoAnalyzer assembly for combined manifold

sugar determination is used and operated from a common sample line to a stream splitter; the distribution of pumping tubes between three pumps, A, B and C, is indicated. A block schematic layout of the various units is shown in Fig 3; pump A controls the sampling and primary diluting stages, pump B controls the alcohol end method, pump C controls the sugar end method. The principles of manifold design elaborated in Part I are also applied in this method to obtain good discrimination at 30 samples per hour.

Method

Reagents—

Acid dichromate—Suspend 600 g of analytical-reagent grade potassium dichromate in 6 litres of distilled water; add to this 2·5 litres of concentrated analytical-reagent grade nitric acid. Shake to dissolve and dilute to 10 litres with distilled water.

Standard solutions—Prepare standard solutions from analytical-reagent grade absolute ethanol in the range 2 to 10 per cent. v/v ethanol. The specific gravity of a 10 per cent. v/v solution, $0{\cdot}98659 \left(\frac{60° \text{ F}}{60° \text{ F}}\right)$, is used as a check on quality. In practice these standards also contain sugar for the sugar method.

Procedure—

The samples are prepared as indicated in Part I[1]; an aliquot of the sample is taken from a stream splitter, which is connected to the sample probe of the sampler and to the sugar manifold. The aliquot for alcohol analysis is pumped at the rate of 3·9 ml per minute; this is immediately re-cycled via a de-bubbler to remove the air pulse obtained when the probe is out of solution. A 2·9 ml per minute aliquot is taken, segmented and passed to the 95° C distillation unit (Fig. 1). The sample stream is met by an air stream at 300 ml per minute, which is obtained from a cylinder supply through a reducing valve at 40 p.s.i. and stabilised by passing through a molecular-sieve filter. The vapour generated in this system is passed through an ascending coil filled with glass beads and emerges at the lower jet of the separator as a spray that is directed to the lower part of the bulb. The undistilled residue is withdrawn with some gas from the bottom end of the separator via a cooling coil and pumped to waste at 6·8 ml per minute. The separated gas and vapour stream is collected at the jet, directed towards the dome of the separator, and is passed to a T-piece where it meets a stream of de-ionised water pumped at 7·8 ml per minute. This mixed stream is condensed and passed to a second gas - liquid separator, which is a modified Technicon B2 micro separator unit. The excess of gas is vented to waste via the internally sealed jet together with most of the condensed liquid. A sample for analysis is withdrawn from the

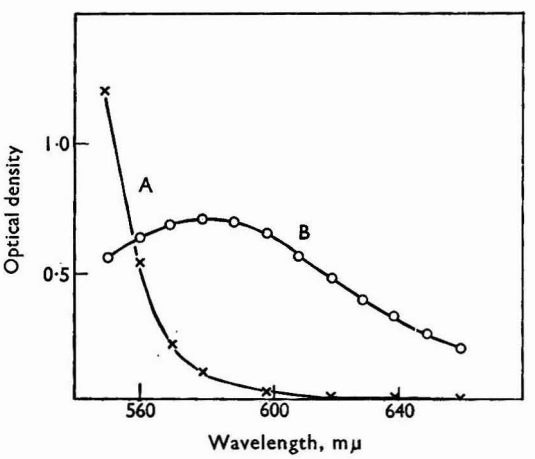

Fig. 4. Spectrum of alcohol reagent system for A, reagents against water; B, reagents *plus* 11 per cent. of ethanol against water in a 1-cm cell

pool of liquid at the lower end of the separator. This pool is of small volume (less than 0·2 ml) and is continuously flushed by the condensate and gas to the waste stream; the concentration of alcohol remains representative of the original samples in sequence. A sample aliquot is withdrawn at 0·16 ml per minute and this is passed via an H_3 connector to an air-segmented stream of acid dichromate pumped at the rate 2·9 ml per minute, and the colour is developed and read at 610 mμ in an 8-mm flow cell. Fig. 4 illustrates the spectra of the reagent-sample mixtures and indicates that at the wavelength chosen the reagent system alone has minimal absorbance. The reaction time path is adjusted to give near coincident emergence

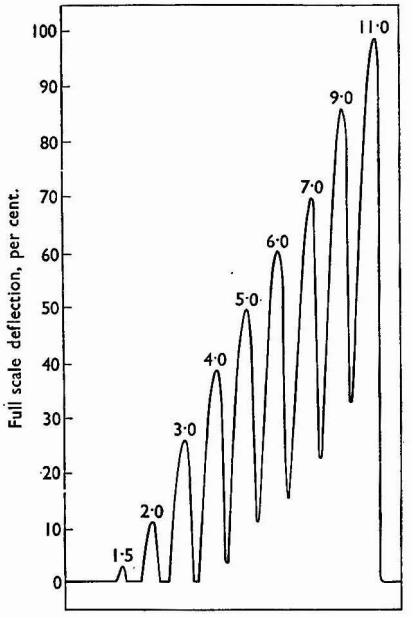

Fig. 5. Ethanol standards

of the alcohol and sugar peaks for each sample, the overriding factor being the time taken for the sugar method. A series of standard ethanol peaks is illustrated in Fig. 5, and a typical calibration on $\times 2$ range expansion is shown in Fig. 6. A total analysis time of 25 minutes from sampler to recorder is required for both channels.

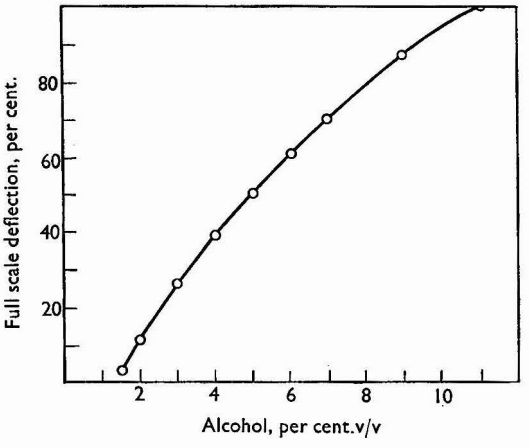

Fig. 6. Calibration curve of ethanol, per cent., against full scale deflection

Results

With the manifold assembly detailed operating at optimum sampling speed of 30 samples per hour, a study has been made of the correlation between the "gravity lost," as determined by the distillation procedure, and the alcohol, as determined by our method. A total of 80 samples of different beers was used with a range of "gravity lost" from 13·8 (2·2 per cent. v/v of alcohol) to 54·9 (8·6 per cent. v/v of alcohol) and the relationship between "gravity lost" and percentage of alcohol found to be—

$$G = 6{\cdot}56\,A - 0{\cdot}3$$

where G = gravity lost expressed in the 1000 notation and
A = percentage of alcohol.

The statistical constants found for this line are regression coefficient $r = 0{\cdot}996$ and standard error of the estimate of gravity lost from alcohol = 0·79. The range of differences between calculated gravity lost and the observed figure by the distillation procedure for the set of samples was −1·7 to +1·5.

Table I

Comparison of results of the determination of gravity lost (G) by the proposed and the official methods

Type	Number of samples	Mean gravity lost: Calculated from alcohol	Mean gravity lost: Observed by distillation	Mean difference between pairs calculated - observed	Standard deviation of differences
Milk stout	6	28·1	28·9	−0·8*	0·37
Stout	5	20·0	19·6	+0·4	0·20
Stout	5	17·3	16·8	+0·5†	0·13
Brown ale	5	16·6	16·4	+0·2	0·19
Continental lager	11	34·2	33·8	+0·4	0·48
Continental lager	12	18·4	18·8	−0·4†	0·13
Continental lager	12	28·1	27·9	+0·2	0·32
U.K. lager	12	29·3	29·5	−0·2	0·29
Pale ale	12	29·4	28·6	+0·8†	0·37
Strong ale	6	58·9	57·3	+1·6*	0·46

* Significant at 1 per cent. probability level.
† Significant at 0·1 per cent. probability level.

Following the scheme outlined in Part I, a series of samples was chosen to test the difference between calculated and observed values for particular varieties, and the results of this examination are shown in Table I, together with the standard deviation for the differences in each series. Probability levels of significance of differences obtained by use of Student's t-test are also indicated. Again there is evidence of a difference characteristic

Table II

Comparison of results of the determination of original gravity by the proposed and the official methods

Type	Number of samples	Mean original gravity: *Calculated from sugar and alcohol	Mean original gravity: Observed by distillation	Mean difference between pairs calculated - observed	Standard deviation of differences
Milk stout	6	56·3	57·0	−0·7†	0·34
Stout	5	46·3	44·4	+1·9‡	0·33
Stout	5	34·0	33·2	+0·8‡	0·19
Brown ale	5	33·6	33·0	+0·6	0·46
Continental lager	11	52·4	51·5	+0·9†	0·50
Continental lager	12	29·2	29·3	−0·1	0·23
Continental lager	12	42·4	41·7	+0·7‡	0·34
U.K. lager	12	44·7	44·1	+0·6‡	0·40
Pale ale	12	46·0	44·9	+1·1‡	0·58
Strong ale	6	86·5	87·0	−0·5‡	0·17

* Significant figures on the 1000 notation scale are quoted (*e.g.*, 56·3 ≡ 1056·3).
† Significant at 1 per cent. probability level.
‡ Significant at 0·1 per cent. probability level.

of a particular label. The sums of the two component parameters of the original gravity determination derived from those values shown in Part I for "extract weight" and for "gravity lost" are shown in Table II, together with the standard deviation of the differences between the original gravity by direct distillation and original gravity calculated from sugar and alcohol contents.

The sum of the two parameters lies between $\pm 2{\cdot}0$ units of original gravity in all these cases, and the weighted mean standard deviation of the differences is 0·37.

As may be expected, a few types of beer fall outside the limits $\pm 2{\cdot}0$ units of original gravity indicated above; these are types that are infrequently met in practice and include such extreme varieties as the dry diabetic lagers and some high gravity porters and stouts. These samples are again consistent in their behaviour and due allowance can be made in any screening technique applied.

TABLE III

RESULTS OF ANALYSIS OF ROUTINE SAMPLES BY THE TWO METHODS

Type	Number of samples with same label	*Nominal gravity	*Gravity found by traditional method		*Gravity found by proposed method	
			Range	Average	Range	Average
Continental lagers	10	30	29·2 to 30·0	29·7†	28·5 to 29·5	29·3
	1	30	—	28·6†	—	27·9
	1	42	—	42·4	—	41·8
	3	30	30·3 to 30·8	30·5	29·5 to 30·3	30·0
	1	48	—	42·8	—	42·1
	4	81	77·9 to 79·9	78·6†	78·6 to 80·4	79·5
	4	44	42·5 to 43·5	43·0	43·6 to 44·8	44·4
U.K. lager	11	44	43·5 to 45·1	44·5	43·5 to 44·6	44·0
Pils	3	45	43·8 to 44·4	44·1†	43·1 to 44·1	43·6
Brown ale	10	33	31·8 to 33·8	32·8	31·9 to 33·5	32·8
	3	34	33·0 to 33·2	33·1	34·7 to 35·1	34·9
Stout	1	39	—	40·7	—	39·1
	1	37	—	36·6	—	35·1
	2	57	55·6 to 57·6	56·6	54·5 to 56·2	55·4
Ales	6	44	43·2 to 44·1	43·4	42·9 to 44·3	43·5
	4	38	36·1 to 37·4	36·5	36·2 to 37·2	36·8
	1	48·7	—	46·8	—	46·6
	20	55	52·7 to 55·4	54·3	53·2 to 55·0	54·0
	1	45	—	45·6	—	44·5
	3	42	41·3 to 42·0	41·8	41·9 to 42·9	42·4
	1	41	—	40·6	—	41·3
	1	56	—	55·9	—	57·1
	1	68	—	66·4	—	67·5
	2	48	46·5 to 47·6	47·0	47·9 to 48·5	48·2
	1	47	—	47·8	—	46·9
	3	45·5	44·2 to 45·0	44·5	44·4 to 45·4	44·9
	2	35	34·7	34·7	34·4 to 34·7	34·6
	1	37	36·6	—	—	37·1
	1	45·5	43·1	—	—	42·9
	2	40	39·8 to 40·0	39·9	40·0 to 40·4	40·2
	2	44	43·7 to 43·9	43·8	44·0 to 44·2	44·1
	1	56	—	55·9	—	57·1
	1	63	—	62·4	—	61·6
	1	76	—	75·8	—	74·4
	1	87	—	85·8	—	86·6
	1	100	—	99·9	—	96·4

* Significant figures on the 1000 notation scale are quoted (*e.g.*, 30 ≡ 1030).
† Determined by distillation.

Table III shows a summary of a typical set of analyses on 112 routine samples examined by the proposed method and by the normal distillation or refractometric[13,14] methods of analysis; declared values of original gravity are also shown for comparison. With one exception, a heavy gravity ale, the agreement between the two methods is within the limits expected; it is also noteworthy that the two methods of analysis indicate the one sample in the series for which declaration is grossly in error.

Further work on the automatic reading of peak heights and automatic calculation of the results of analysis is in hand and will be reported in a further paper.

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NOTE—Reference 1 is to Part I of this series.

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Consistency of R_{st} Values of Six Organophosphorus Pesticides Resolved by Thin-layer Chromatography in the Presence of Plant Extracts without Elaborate Clean-up

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This paper reports statistical analyses of the ratio of the distance travelled by each of five organophosphorus pesticides to that travelled by parathion in the presence of plant extracts, without elaborate clean-up. Mixtures of azinphos-methyl, carbophenothion, diazinon, ethion, malathion and mevinphos in the presence of apple, beet, carrot, lettuce or pea extracts were evaluated with reference to the parathion internal standard.

GALANOS and Kapoulas[1] enumerated the sources of variation in the determination of R_F (the distance travelled by a compound divided by the distance travelled by the solvent front) values of chemical compounds. Dhont and de Rooy,[2] and Severin,[3] found that the R_{st} values of some 3,5-dinitrobenzoates on plates coated with silica gel were consistently and conveniently obtained—

$$R_{st} = \frac{\text{Distance travelled by the studied substance } X}{\text{Distance travelled by the reference standard } st}.$$

Our observation that R_F values were greatly affected by the changes in room humidity concurred with that of Reichel[4]; the R_F values obtained were low in winter and high in summer even at constant temperature of the chromatographic tanks. In addition, the same compound spotted on a thin-layer chromatographic plate at different positions along the origin line gave slightly different spot locations, although the solvent front moved evenly.[5] Because of such limitations, R_{st} values were calculated to substantiate further the identity of the pesticides in plant extracts without comparing each spot with the pesticide standards. As the compounds used belonged to different organic classes, R_{st} was used instead of R_F°.[1] Parathion was chosen as the standard reference because its migration rate was intermediate between the slow and fast-moving compounds; its R_F value is 0·41. In addition, it is stable and sensitive to the enzyme-detection technique.[5] R_{st} values of azinphos-methyl, carbophenothion, diazinon, ethion, malathion and mevinphos were calculated in the absence or presence of extractives from beets, apples, carrots, lettuce and peas. The R_{st} values were evaluated to determine the degree of variation in the migration pattern of the six pesticides on plates with a 450-μ thick silica gel layer.

EXPERIMENTAL

PREPARATION OF THE SAMPLE—

Each 50-g plant sample was extracted and partitioned according to McLeod, Mendoza, Wales and McKinley.[6] The combined partition fraction was fortified with azinphos-methyl at 1·00 p.p.m., carbophenothion at 0·50 p.p.m., diazinon at 0·10 p.p.m., ethion at 0·10 p.p.m., malathion at 8·00 p.p.m., mevinphos at 0·25 p.p.m. and parathion at 1·00 p.p.m. The fortified extract was made up to 200 ml with hexane; a portion was concentrated 20 times under a gentle flow of nitrogen.

THIN-LAYER CHROMATOGRAPHIC PROCEDURE—

Aliquots equivalent to 100, 150 and 200 mg of plant samples were spotted in duplicate on each thin-layer chromatographic plate, coated with a 450-μ thick layer of MN-Kieselgel G-HR (silica gel). The pesticides on the thin-layer chromatographic plates were resolved at 25° C in a glass tank with 15 ml of acetone diluted to 100 ml with hexane; the solvent front

was allowed to reach a distance of 15 cm. The thin-layer chromatographic - enzyme inhibition technique[5] was used to detect the pesticides on the plates.

STATISTICS—

The following model was used for the statistical analysis of the R_{st} values of the pesticide in the presence of plant co-extractives—

$$Y_{ijkl} = \mu + P_i + D_{j(i)} + L_{k(ij)} + \epsilon_{l(ijk)},$$

where Y_{ijkl} is the observed R_{st} value for each pesticide;

μ is the over-all mean;

P_i is the fixed effect of the i^{th} plant sample;

$D_{j(i)}$ is the random effect of the j^{th} day in the i^{th} plant sample;

$L_{k(ij)}$ is the random effect of the k^{th} plate within the j^{th} day in the i^{th} plant sample; and

$\epsilon_{l(ijk)}$ is the random variation between spots of the same pesticide on the same plate.

Because only one R_{st} value was observed on each plate for each standard pesticide (without plant extracts), a slightly different model was used for the analysis of these data—

$$Y_{ijk} = \mu + G_i + D_{j(i)} + R_{k(ij)},$$

where the effects μ and $D_{j(i)}$ are as defined before;

G_i is the effect of the i^{th} grouping (arbitrarily grouped according to plant sample); and

$R_{k(ij)}$ is the residual source of variation.

RESULTS AND DISCUSSION

The chromatograms showed that the distance travelled by the pesticides was not affected by plant extracts or by the size of the aliquots spotted.

Table I shows the mean R_{st} values of the standard pesticides and of the same pesticides in the presence of plant extracts. In all instances, the variance among the six values for each pesticide, within plates, is small.

TABLE I

MEAN $R_{parathion}$ VALUES OF SIX ORGANOPHOSPHORUS PESTICIDES*

	Pesticide						
	Mevinphos	Azinphos-methyl	Malathion	Parathion	Diazinon	Ethion	Carbophenothion
Standard†	0·32	0·52	0·76	1·00	1·11	1·22	1·47
With plant extractives‡	0·30	0·51	0·75	1·00	1·11	1·22	1·47

* $R_{parathion}$ = the distance travelled by a compound divided by the distance travelled by parathion; MN-Kieselgel G-HR, 450-μ thick layer, 15 ml of acetone diluted to 100 ml with hexane solvent system, 25° C room temperature.

† Each value is a mean of 20 determinations.

‡ Each value is a mean of 100 determinations, except that for diazinon, which is a mean of 99 values.

Table II shows that plant samples were not a significant source of variation but the days and the plates gave variation larger than that between individual pesticide spots on the same plate. The significant variation for days and plates may be attributed to changes in room humidity on different days, when the plates were made and used in chromatography.

Table III indicates that the groupings (arbitrarily grouped according to plant sample) did not give a significant source of variation for any pesticides, with the exception of malathion. The significant mean square for groupings for malathion was most likely caused by the low mean square for days within groupings. For ethion, the variation from day to day was only significant at the 5 per cent. level, and may be attributed to changes in room humidity on different days.

TABLE II

ANALYSES OF VARIANCE OF THE R_{st} VALUES OF THE PESTICIDE STANDARDS IN THE PRESENCE OF PLANT EXTRACTIVES

		Mean squares for each pesticide					
Source	d.f.	Mevinphos	Azinphos-methyl	Malathion	Diazinon	Ethion	Carbophenothion
Plant samples	4	0·012972	0·007275	0·001450	0·003225	0·001942	0·014200
Days/plant samples ..	5	0·010328*	0·016460†	0·004380*	0·004040†	0·003564*	0·023680*
Plates/days/plant samples	10	0·002237†	0·002270†	0·000920†	0·000370	0·000556	0·004570†
Between spots/days/plant samples ..	100‡	0·000367 (0·001352)	0·000663 (0·002112)	0·000190 (0·000599)	0·000227 (0·000557)	0·000380 (0·000660)	0·000675 (0·002915)

d.f. = Degrees of freedom.
* Significant at $P_{0·05}$.
† Significant at $P_{0·01}$.
‡ Degrees of freedom for diazinon = 99.
Figures in parentheses are sums of variance components.

TABLE III

ANALYSES OF VARIANCE OF THE R_{st} VALUES OF THE PESTICIDE STANDARDS

		Mean squares for each pesticide					
Source	d.f.	Mevinphos	Azinphos-methyl	Malathion	Diazinon	Ethion	Carbophenothion
Groupings	4	0·002712	0·001338	0·001220*	0·001580	0·000500	0·004150
Days/groupings ..	5	0·000895	0·001530	0·000180	0·000820	0·001060†	0·004545
Residual/days/groupings	10	0·000725 (0·000805)	0·000910 (0·001220)	0·000600 (0·000600)	0·000250 (0·000535)	0·000310 (0·000685)	0·001445 (0·002995)

d.f. = Degrees of freedom.
* Significant at $P_{0·05}$ when tested against the days, not significant when tested against the residual.
† Significant at $P_{0·05}$.
Figures in parentheses are sums of variance components.

The variance components, calculated from the R_{st} values of standards, shown in Tables II and III (figures in parentheses), were similar to those of the same pesticides in the presence of plant extractives. This similarity permitted the use of individual error terms from the first analysis to test the significance between individual standard values and means of the six values (per individual pesticide in the presence of plant extractives) per plate. Only five differences out of 120 observations were found greater than the 5 per cent. least significant difference values. This indicates that no significant difference exists between the R_{st} values of a pesticide standard and of the same pesticide in the presence of plant extractives, both determined on the same plate on the same day.

Analyses showed that R_{st} values for the six organophosphorus pesticides were consistent, regardless of the presence of plant extractives and of the location of the spot origin. The effect of the origin location was negligible when the test compounds and the parathion reference standard were contained in the same sample spot. When close control of humidity is a problem in the determination of R_F values, R_{st} values relative to parathion on the same path of migration can, therefore, be used to substantiate further the identity of the six pesticides studied.

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Procedure for Semi-quantitative Confirmation of Some Organophosphorus Pesticide Residues in Plant Extracts

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This paper describes the combination of carbon-column chromatography with thin-layer chromatographic - enzyme inhibition technique for semi-quantitative confirmation of organophosphorus pesticides. The combined procedure was evaluated for azinphos-methyl, carbophenothion, diazinon, disulfoton, malathion, mevinphos and parathion. Based on separation of compounds by elution from the column, two mixtures of standards were added to potato extracts and eluates and were analysed on thin-layer chromatograms.

McLeod, Mendoza, Wales and McKinley[1] reported a procedure for simultaneous clean-up and separation of pesticide residues in plant materials by using a carbon - cellulose column and three eluates. The organophosphorus pesticides were selectively eluted from this column by the first and second eluants. The eluates were analysed by gas - liquid chromatography with electron-capture detection.

Our previous study showed the application of a thin-layer chromatographic - enzyme inhibition procedure to screen for organophosphorus pesticides in extracts of plant samples.[2] The seven organophosphorus pesticides studied were distinctly and simultaneously detected when co-chromatographed with extractives from apple, beet, carrot, lettuce or pea (aliquots equivalent to 100 to 500 mg of sample).

However, the identity of the pesticides detected by both methods[1,2] must be confirmed. It was thought that the carbon-column procedure could be incorporated into the thin-layer chromatographic - enzyme inhibition technique for such confirmation. Elution from the carbon column would separate the pesticides into two known groups. Based on the sensitivity of the enzyme inhibition technique for each compound, appropriate aliquots of the eluates could then be chosen to detect only a certain compound or group of compounds.

Our recent experiment[2] also showed that, while apple, beet, carrot, lettuce and pea extracts had little or no interference, the interference from potato extracts prevented the thin-layer chromatographic resolution and identification of diazinon, parathion, malathion, azinphos-methyl and mevinphos. Thus, potato was chosen as the test sample to evaluate this combined procedure for semi-quantitative confirmation of pesticide residues and to show the applicability of this procedure to all six crops. As a continuation of the thin-layer chromatographic - enzyme inhibition technique,[2,3] this semi-quantitation aims to detect pesticide residues present in amounts near the tolerance levels.

Materials and methods

The procedure and equipment used for extraction, partition and elution of plant samples and for thin-layer chromatography, including the preparation of liver homogenate and chromogenic spray solutions, were outlined in recent papers.[2,3] The thin-layer chromatographic plates were developed in 20 ml of acetone diluted with hexane to 100 ml.

The potato samples were extracted with acetonitrile and partitioned with hexane.[1] Aliquots equivalent to 10 g of plant sample were eluted, at 4 to 6 ml per minute, from the carbon - cellulose column (0·7 g of Darco G–60 and 2·2 g of Solka Floc per column) by using two eluants successively; eluant A was 175 ml of 1·5 per cent. acetonitrile in hexane and eluant B was 200 ml of chloroform.

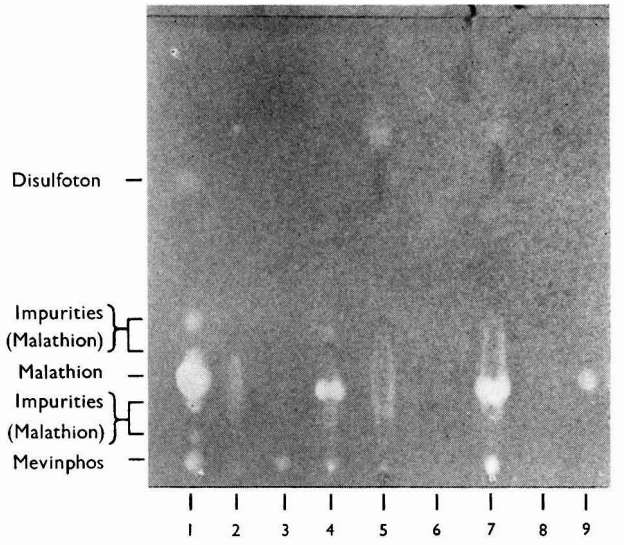

Fig. 1. Chromatogram of fortified and control extract A of potato and corresponding standards. (Concentrations are given in Table I.) 1, 40 μl of pesticide standard mixture at A_2 concentration; 2, control extract A equivalent to 320 mg of sample; 3, 80 ng of mevinphos; 4, 6·4 μl of extract A fortified with pesticide standard mixture at A_2 concentration (equivalent to 320 mg of sample); 5, control extract A equivalent to 2000 mg of sample; 6, 400 ng of disulfoton; 7, 40 μl of extract A fortified at A_2 concentration (equivalent to 2000 mg of sample); 8, control extract A equivalent to 2·7 mg of sample; 9, 54 μl of extract A fortified at A_1 concentration (equivalent to 2·7 mg of sample)

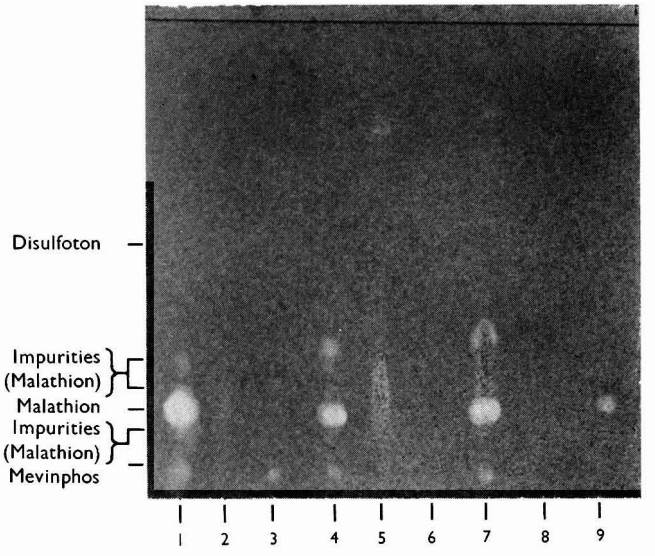

Fig. 2. Chromatogram of fortified and control eluate A of potato and corresponding standards. (Concentrations are given in Table I.) 1, 40 μl of pesticide standard mixture at A_2 concentration; 2, control eluate A equivalent to 320 mg of sample; 3, 80 ng of mevinphos; 4, 6·4 μl of eluate A fortified with pesticide standard mixture at A_2 concentration (equivalent to 320 mg of sample); 5, control eluate A equivalent to 2000 mg of sample; 6, 400 ng of disulfoton; 7, 40 μl of eluate A fortified at A_2 concentration (equivalent to 2000 mg of sample); 8, control eluate A equivalent to 2·7 mg of sample; 9, 54 μl of eluate A fortified at A_1 concentration (equivalent to 2·7 mg of sample)

[To face p. 692

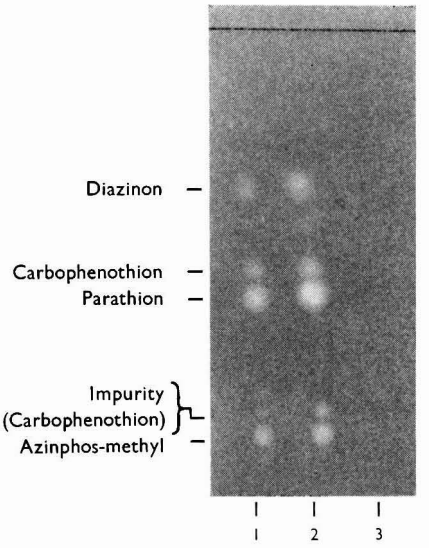

Fig. 3. Chromatogram of fortified and control extract B of potato and corresponding standard. (Concentrations are given in Table II.) 1, 10 μl of extract B fortified with pesticide standard mixture at B_2 concentration (equivalent to 10 mg of sample); 2, 10 μl of pesticide standard mixture at B_2 concentration; 3, 10 μl of control extract B (equivalent to 10 mg of sample)

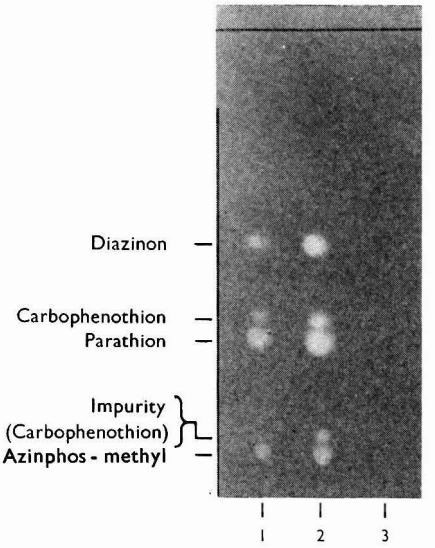

Fig. 4. Chromatogram of fortified and control eluate B of potato and corresponding standard. (Concentrations are given in Table II.) 1, 10 μl of eluate B fortified with pesticide standard mixture at B_2 concentration (equivalent to 10 mg of sample); 2, 10 μl of pesticide standard mixture at B_2 concentration; 3, 10 μl of control eluate B (equivalent to 10 mg of sample)

[To face p. 693

The extracts and the eluates were fortified with standard mixtures at the tolerance level (this connotes the amount tolerated in some crops, not necessarily in potato).[4] Standard mixture A (Table I) contained the compounds eluted by eluant A: disulfoton (Disyston),

TABLE I

CONCENTRATIONS OF PESTICIDES IN SOLUTIONS FORTIFIED WITH STANDARD MIXTURE A

	Disulfoton, μg per ml	Malathion, μg per ml	Mevinphos, μg per ml
Standard mixture A	2·0	80·0	2·5
Extract or eluate A (A_1*)	$1·00 \times 10^{-2}$	$40·00 \times 10^{-2}$	$1·25 \times 10^{-2}$
Extract or eluate A concentrated 1000 times (A_2)	10·0	400·0	12·5

* An equivalent of 10 g of sample was contained in 200 ml of A_1.

malathion and mevinphos (Phosdrin). Standard mixture B (Table II) contained the compounds eluted by eluant B: azinphos-methyl (Guthion), carbophenothion (Trithion), diazinon and parathion. One millilitre of standard mixture A or B was added to extracts or eluates (each equivalent to 10 g of sample). To simulate the presence of the seven pesticides in potato samples at levels indicated in Tables I and II, the extract and the first eluate were fortified with standard mixture A and were extract A and eluate A, respectively. The extract and the second eluate were fortified with standard mixture B and were extract B and eluate B, respectively. Eluates A and B were made up to final volumes of 200 ml with hexane and chloroform, respectively. Each 10-g aliquot of crude extract was diluted to 200 ml with hexane. Each extract or eluate had a corresponding unfortified control.

TABLE II

CONCENTRATIONS OF PESTICIDES IN SOLUTIONS FORTIFIED WITH STANDARD MIXTURE B

	Azinphos-methyl, μg per ml	Carbophenothion, μg per ml	Diazinon, μg per ml	Parathion, μg per ml
Standard mixture B	20·0	8·0	7·5	10·0
Extract or eluate B (B_1*) ..	$10·0 \times 10^{-2}$	$4·0 \times 10^{-2}$	$3·75 \times 10^{-2}$	$5·00 \times 10^{-2}$
Extract or eluate B concentrated 20 times (B_2)	2·0	0·8	0·75	1·0

* An equivalent of 10 g of sample was contained in 200 ml of B_1.

Both fortified and unfortified extracts and eluates were analysed by the thin-layer chromatographic - enzyme inhibition procedure, with 5-bromo-4-chloroindoxyl acetate as substrate. Based on the lowest limits of detection,[3] various volumes of extracts and eluates were spotted to detect selectively a single compound or group of compounds (Tables I and II).

RESULTS

Figs. 1 and 2 show chromatograms of extract A and eluate A, respectively, together with the corresponding controls and standards. White streaks were visible on the thin-layer chromatographic plates for the extract A and eluate A, and sometimes caused a distortion of the malathion spot (Fig. 1, Spots 4 and 7). However, the streaks did not interfere with the detection of malathion or mevinphos, even when the amounts of sample were increased to 320 (Figs. 1 and 2, Spot 4) and 2000 mg (Figs. 1 and 2, Spot 7). With this technique, it is possible to analyse an aliquot equivalent to only 2·7 mg of sample when it is fortified with malathion at 8 p.p.m.; malathion was the only compound detected with this aliquot size (Figs. 1 and 2, Spot 9). As expected, malathion was detected in a 320-mg sample (Figs. 1 and 2, Spot 4); both malathion and mevinphos were detected in a 2000-mg sample equivalent (Figs. 1 and 2, Spot 7). Mevinphos was detected in a 320-mg sample from either extract A or eluate A (Figs. 1 and 2, Spot 4). Disulfoton standard, 0·4 μg, was detected but the spot produced persisted for a few minutes only (Figs. 1 and 2, Spots 1 and 6). However, the same amount in a 2000-mg sample equivalent was not usually detected (Figs. 1 and 2, Spot 7).

Figs. 3 and 4 show chromatograms of extract B and eluate B, respectively, together with the corresponding controls and standards. Azinphos-methyl, carbophenothion, diazinon, and parathion in a sample equivalent to 10 mg gave distinct spots (Figs. 3 and 4, Spot 1). Spotting of samples equivalent to 54 and 62 mg gave no interference. An impurity in 0·05 μg of carbophenothion standard was detectable by thin-layer chromatography.

Discussion and conclusion

Thin-layer chromatograms of potato extracts were comparable with those of eluates, indicating that elution from the carbon - cellulose column gave no appreciable reduction of the inhibition caused by components extracted from potato. In marked contrast to the streaks previously obtained for pesticides in potato extract without elution from the carbon - cellulose column,[2] the spots produced by the pesticides when separated into two groups were distinct and definite (Figs. 1 and 2). Thus the spots were readily distinguished from the interference caused by the components extracted from potato (Fig. 1).

In combination with the carbon-column procedure, the thin-layer chromatographic - enzyme inhibition technique was shown to be applicable to the confirmation and semi-quantitation of some pesticides in extracts of potato. Separation into two groups both confirmed the identity of the pesticides detected and enabled the separation of smaller aliquots for semi-quantitation. The separation and smaller aliquots also favoured the elimination of interference.

Malathion, mevinphos, azinphos-methyl, carbophenothion, diazinon and parathion have been detected as distinct spots when present at tolerance levels in crude extracts of apples, beets, carrots, lettuce and peas (equivalent to 100 to 500 mg).[2] Thus, we can assume that the confirmatory and semi-quantitative procedure developed with potato extracts can be applied to these pesticides in extracts of apples, beets, carrots, lettuce and peas. The results show that disulfoton requires further study.

Acknowledgment is made to Dr. D. E. Coffin and Dr. N. P. Sen for review of the manuscript, and to Mr. H. Baird and Mr. B. Korda for the photography.

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Received *March 12th*, 1968

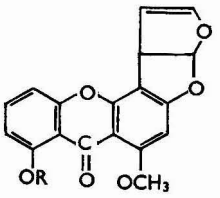
A Method for the Determination of Sterigmatocystin in Grain and Oilseeds

By L. J. VORSTER and I. F. H. PURCHASE

(*National Nutrition Research Institute, Council for Scientific and Industrial Research, Pretoria, South Africa*)

A sensitive method is described for the quantitative determination of the mycotoxin sterigmatocystin by thin-layer chromatography. A sensitivity of 0·0025 μg is achieved by conversion of sterigmatocystin into the mono-acetate, which fluoresces an intense light blue on thin-layer chromatoplates when viewed under ultraviolet light.

Sterigmatocystin is a mycotoxin that has gained increasing significance as a food or feed contaminant during the past 5 years. Mycotoxins are at present under suspicion as carcinogens, which may be responsible for the high incidence of liver cancer in Africa.[1] This possibility is linked with the poor storage facilities that exist in many of the affected areas and the ease with which stored grains can become contaminated by toxigenic fungi. Sterigmatocystin, in particular, has been proved carcinogenic by Dickens, Jones and Waynforth.[2] Holzapfel, Purchase, Steyn and Gouws[3] found that this toxin is produced in high yield by *Aspergillus nidulans* and by an undescribed strain of *Bipolaris*. Sterigmatocystin was first obtained by the extraction of cultures of *Aspergillus versicolor* by Davies, Kirkaldy and Roberts[4] and Bullock, Roberts and Underwood,[5] who proposed the structure, **I**, for the compound.



I R=H—

II R=CH_3CO—

Thin-layer chromatography of solutions containing sterigmatocystin reveals the presence of the toxin as a brick-red fluorescent spot when the plate is viewed under long wave ultraviolet light. The spot appears at R_F 0·8 when the plate is developed in chloroform containing 2 per cent. of methanol. The fluorescence is weak and difficult to detect in an impure extract of grain; this is especially so in extracts of sorghum, which contain several red fluorescent compounds. The lower limit of visual detectability has been established to be about 0·4 μg. This method is therefore one thousand times less sensitive than when applied to the aflatoxins.

The possibility of determining sterigmatocystin after converting it into a strongly fluorescent derivative was, therefore, investigated. It was found that benzoylation and acetylation gave products exhibiting the required intense fluorescence. Acetylation was preferred because this step could be readily adapted for an assay procedure. Sterigmatocystin can be converted efficiently into a diacetate by treatment with acetic anhydride and sodium acetate,[4] but the procedure is slow. Acetylation with acetic anhydride and pyridine, however, is rapid. Davies, Kirkaldy and Roberts[4] reported that the acetylation of sterigmatocystin with acetic anhydride and pyridine is anomalous in that a mixture of products is formed. Careful study of the reaction confirmed this, but it was found that a mono-acetate, **II**,* melting-point

* The mono-acetate (about 10 mg) is isolated from the reaction mixture by column chromatography on formamide-impregnated cellulose powder (sample weight 1 per cent. of weight of column material). The column is packed in hexane and eluted successively with hexane containing 15, 30 and 50 per cent. of benzene in portions of 100 ml each. The final elution is carried out with 100 ml of pure benzene. The eluted fraction containing the acetate is evaporated to dryness and the acetate recrystallised from anhydrous diethyl ether. The structure of the compound was deduced from mass and nuclear magnetic resonance spectroscopic results.

126° C, is constantly formed in yields of about 90 per cent. if close control is maintained over the conditions under which the reaction is allowed to proceed. The main reaction product has an intense light blue fluorescence on thin-layer plates.

Although the introduction of an acetylation reaction complicates the analytical procedure for mycotoxins, it forms the basis of the proposed method, as the lower limit of visual detectability for sterigmatocystin can be decreased to 0·0025 μg.

Analytical procedure

Weigh 20 g of the finely ground sample into an extraction thimble and extract for 5 hours in a Soxhlet apparatus with azeotropic chloroform - methanol (87 + 13). Alternatively, blend the sample with 100 ml of the solvent for 1 minute at high speed, decant the solvent and repeat the extraction with a fresh portion of 100 ml of solvent. Filter the combined extract.

Evaporate the extract obtained by either method to dryness and immediately dissolve the residue in 50 ml of 95 per cent. methanol. Transfer the solution to a separating funnel, rinse the flask with 50 ml of hexane and add it to the solution. Shake the mixture well and, after separation, run off the lower layer (methanol) into the flask.

Extract the hexane layer twice with 25-ml portions of 95 per cent. methanol and combine them with the methanol in the flask. Discard the hexane and pour the methanol solution into the separating funnel. Dilute the methanol to 50 per cent. with distilled water. Extract the methanol solution three times with 30-ml portions of chloroform and combine the extracts. Evaporate the solvent under reduced pressure to about 10 ml.

Transfer the concentrated chloroform extract quantitatively into a glass vial (2 × 1-inch diameter), and evaporate it to dryness on a water-bath.

As soon as the chloroform has evaporated dissolve the residue in 1 ml of pyridine and transfer the vial to a wax-bath or sand-bath maintained at 110° C in a fume cupboard. By pipette, introduce 1 ml of acetic anhydride into the vial and evaporate the liquid *slowly* (in about 20 minutes). Towards the end of the evaporation blow a stream of nitrogen gently into the vial to facilitate the evaporation of the last drops. After cooling the vial, dissolve the residue in 5 ml of chloroform and cap the vial. The solution of sterigmatocystin acetate is sensitive to daylight and should be stored in a dark cupboard. Proceed with the next step as soon as possible. The chromatography should be carried out in a darkened room.

Transfer, by pipette, 1 ml of a standard solution of sterigmatocystin (20 μg per 50 ml) into a vial, evaporate the solvent and proceed with the acetylation as described. Re-dissolve the reaction product in 1 ml of chloroform.

Make a trial determination of the concentration of sterigmatocystin acetate in the sample solution by spotting 1, 5 and 10 μl on an activated thin-layer plate. Develop the plate in chloroform containing 0·75 per cent. of ethanol, as stabiliser, and 2 per cent. of methanol.

When viewed under an ultraviolet source such as a HPW 125 W, Type 57202 E/70, Philip's lamp (peak emission about 360 nm), the presence of sterigmatocystin is revealed by a light blue fluorescent spot at R_F 0·6. From the intensity of the spots the analyst can decide on the necessary dilutions or concentrations of the solution required to reach the lower limit of visual detectability. We have determined that under the conditions in our laboratory it is 0·0025 μg.

Each plate evaluator should establish the lower limit of visual detectability of the acetylated standard for himself under the conditions prevailing in his laboratory.

Results and discussion

It was found that extraction by macerating the sample with the solvent in a Buhler blender was as effective as Soxhlet extraction. The filtration rate of the suspension from the blender may be rather slow, and this step can be substituted by centrifugation and by washing the precipitate with a little solvent. Efficiency of extraction by either method was determined by extracting the extracted meal for a further 6 hours in a Soxhlet. No sterigmatocystin could be detected in the extract. A mixture of equal volumes of chloroform and methanol was as effective as the azeotrope in extracting all of the sterigmatocystin. Extracted meals were combined and fed to ducklings as 30 per cent. of their ration until they were 14 days old. Their weight gain was equal to that of the controls, thus confirming that extraction was complete.

The aflatoxins are, in fact, extracted by this technique if they are present in the sample. As they are more polar than sterigmatocystin acetate they barely move on a thin-layer plate eluted with chloroform containing 2 per cent. of methanol, and thus do not cause interference in the detection of sterigmatocystin acetate.

Sterigmatocystin is partitioned between 95 per cent. methanol and hexane (de-fatting step) in the ratio of about 9:1. It is, therefore, necessary to re-extract the hexane twice with methanol.

Thin-layer plates prepared from Camag Kieselgel D–5, and activated for 2 hours at 105° C, were found to resolve the acetylated extract of maize and sorghum so clearly into its components that it should be possible to evaluate the chromatograms by the densitometric method described by Pons, Robertson and Goldblatt.[6]

We thank Dr. C. W. Holzapfel for determining the purity and confirming the structure of a sample of acetylated sterigmatocystin.

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Book Reviews

THE DETERMINATION OF PARTICLE SIZE. I. A CRITICAL REVIEW OF SEDIMENTATION METHODS. Prepared by the PARTICLE SIZE ANALYSIS SUB-COMMITTEE OF THE ANALYTICAL METHODS COMMITTEE. Pp. iv + 42. London: The Society for Analytical Chemistry. 1968. Price (Members) 25s.; (Non-members) 30s.

Particle size analysis is becoming increasingly important in countless applications. Methods and apparatus for size measurement are many and diverse, and the choice of equipment is bewildering. In the early 1960's the Society for Analytical Chemistry, with the help of its members, set itself the mammoth task of surveying and evaluating the methods available and, as a preliminary, listed 10 basic methods covering 74 types of apparatus. The present publication covers only one of these methods, namely sedimentation.

The book begins with a general description of the theory of sedimentation including the applicability of, and the departures from, Stokes' Law. The methods of analysis are classified under the headings of Gravitational and Centrifugal and are further divided into Two-layer and Homogenous methods. Hindered Settling is also covered. A brief description of the theory of each method is given, followed by details of apparatus and a critical discussion of its merits.

Not surprisingly, apart from the Micromerograph, in which air is used as the suspending fluid, all of the methods dealt with are concerned with liquid suspensions. Pipette, Hydrometer, Diver, Photosedimentation, Sedimentation Columns, and Balances, Manometric and Decantation methods are described under Gravitational Methods. Centrifugal Sedimentation methods include the Whitby Column, the I.C.I. - Joyce Disc Centrifuge, the Sharples Super Centrifuge, the Kaye Disc and the Simcar Analyser.

Although no diagrams of apparatus are given, the written descriptions are easily followed, and for those wishing to delve further a comprehensive bibliography is appended, with over 150 references. A particularly useful table listing the practical range of sizes covered by each apparatus is given.

The book is an extremely useful guide, not only to those who are already concerned with particle size analysis but also to those who are new to the subject, and succeeds admirably in condensing the knowledge and experience of many experts in the science of sizing particles.

J. DALMON

TITRATIONS IN NONAQUEOUS SOLVENTS. By WALTER HUBER. Pp. xiv + 252. New York and London: Academic Press. 1967. Price 100s.

This book discusses the theory and practice of the titration of organic compounds in solvents other than water. It is concerned with the application of the Brönsted - Lowry theory rather than the Lewis theory of acids and bases, as the former was worked out principally for the specific problem of acid - base reactions in solvent media not containing hydroxyl ions. This is certainly convenient as most organic "acids" and "bases" are proton donors or acceptors.

Not unnaturally there is a fairly heavy emphasis on methods for locating end-points, particularly in potentiometric, conductimetric, amperometric, spectrophotometric and thermometric procedures. The assessment of precision of titration is particularly useful in this area of the subject. Various commercial instruments are reviewed and also indicators and indicator methods. The small section dealing with the suitability of various solvents for certain types of analysis is a valuable feature of the text, and will be particularly useful to many users of the technique.

In the "Practical Section" finely detailed procedures are given for the determination of many acids and bases and for the resolution of mixtures, etc. There is an excellent selection of tabular data on pK values in many different media at the end of the book. The bibliography is extensive and there are detailed author and subject indices.

This book is a welcome addition to the rather sparse literature on non-aqueous titrimetry and should prove useful to most practitioners of the subject. It is well written and produced, although somewhat expensive.

T. S. WEST

THE CHEMISTRY OF THE RARER PLATINUM METALS (OS, RU, IR, AND RH). By W. P. GRIFFITH. Pp. x + 491. London, New York and Sydney: Interscience Publishers, a division of John Wiley & Sons Inc. 1967. Price 120s.

The chemical, physical and catalytic properties of the platinum-group metals and their compounds have never failed to attract attention, and the number of related published papers, especially over the past decade, shows a growing interest in the importance of the four members of the group covered by this book.

The book is primarily devoted to the co-ordination chemistry and molecular structure of osmium, ruthenium, iridium and rhodium and is, essentially, a reference book containing a comprehensive and critical assessment of published information, excluding patent literature, from the discovery of these metals in the early part of the nineteenth century up to the end of 1966.

The section on Analytical Chemistry takes up less than 2 pages, and this is unlikely to meet the needs of the analyst with an occasional interest in these metals, or the specialist, unless use is made of the further page of 26 well chosen references. However, the analyst in this highly specialised field, who may be involved in the multifarious problems that so often find their way into the analytical laboratory, could find useful information in the section Physical Measurements on Complexes of the Metals, contained in the chapter General Chemical Survey. This 5-page section deals with such subjects as Electron spin resonance spectra, Nuclear magnetic resonance spectra, the Mössbauer effect, Infrared and Raman spectra and Polarography.

For a book with this coverage to serve the best possible needs, adequate supporting references are essential throughout, and in this respect the book scores full marks. These references appear in their appropriate places in the text, and at the end of the book as a collated 30-page Author Index; the book also contains the usual Subject Index, and a Formula Index. W. T. ELWELL

ADVANCES IN PHARMACEUTICAL SCIENCES. Volume 2. Edited by H. S. BEAN, A. H. BECKETT and J. E. CARLESS. Pp. x + 329. London and New York: Academic Press. 1967. Price 80s.; $14.00.

This second volume, like its predecessor, contains four articles on four subjects. The method of treatment indicates that the book is intended to be of interest not only to advanced students but also to pharmacists and others in the pharmaceutical industry and in hospital practice. The articles are not solely reviews of recent advances, but contain, to a greater or lesser extent, summaries of the basic knowledge of each subject and are, therefore, of interest to the non-specialist as well as to those who wish to go more deeply. The first article in Volume 2 is on Kinetics and Mechanisms in Stability of Drugs by E. R. Garrett and gives an admirable summary of basic kinetics and relates the application of this to the deterioration of individual drugs. There are about 9 pages of references at the end of the article and such a long list of references is somewhat formidable, and some of them must be of doubtful value. This is not always indicated in the text.

The second article is on Particle-size Analysis by I. C. Edmundson. It can be easily read and understood by anybody not a specialist in this field, and although, as stated in the preface, it is essentially a general article, it is none the worse for this. It also has only 4 pages of references.

The third article on the Flow Properties of Powders is by Barbara S. Neumann. It is a comparatively brief article consisting of some 40 pages only, but provides an interesting introduction to the subject. The fourth article by C. A. Johnson deals with Water Determination and its Significance in Pharmaceutical Practice. Part of this article is closely related to that by E. R. Garrett, as it deals with the significance of water on the stability of pharmaceutical products. Mr. Johnson's wide experience in pharmaceutical analysis has ensured that the article contains an admirable critical survey of various methods of determining water.

The two volumes together provide a useful review of the application of science to pharmacy. It is obviously intended that there shall be further volumes. K. R. CAPPER

PHYSICAL CHEMISTRY. By FRANK T. GUCKER and RALPH L. SEIFERT. Pp. xvi + 827. London: The English Universities Press Ltd. 1967. Price 70s.

There are now several books, all American, covering what in Britain is an honours course in physical chemistry. The subject matter does not vary widely from book to book, so here emphasis will be given to what is different in the volume under review.

Quite extensive space is given to "fringe benefits" in the following form: mathematical introduction (30 pp.), problems (70 pp.), reading list and data sources (30 pp.) and, at length, index

(30 pp.). Such an allocation will suit only some readers. The same holds for the lead-in to the subject matter: chapter 2 *et seq.* comprise 75 pages on nuclei—including Mössbauer, but not (yet) n.m.r., spectroscopy—and radioactivity. One has, therefore, reached page 100 before the whole atom is broached.

To persist with this "quantitative analysis," atomic and molecular structure and spectroscopy occupy about 100 pages, gaseous behaviour 50, thermodynamics and chemical equilibria 90, solids 40, liquids 35, statistical mechanics 50, non-electrolyte solutions 40, electrolytes, electrochemistry and ionic equilibria 150, surfaces 35, kinetics 55 and heterogeneous equilibria 45 pages.

Wave mechanics is presented with a reasonable balance between description and mathematics. Although the bonding in diatomics up to O_2 is dealt with adequately, that in transition metal complexes is but briefly maltreated. Useful descriptions of n.m.r. and Mössbauer spectroscopy contrast with a rather brief outline of molecular spectroscopy.

The chapters on the gaseous state end with an unusual (*inter alia*) equation of state: it is useful to be reminded that these are still being generated at an appreciable rate. Thermodynamics includes the usual, necessarily specious, derivation of the second law from the first. Solid and liquid structures come out well, and the statistical mechanics is painless. The remaining sections seemed eminently readable when dipped into. The major criticism called for arises in connection with kinetics. It is a symptom of injudicious emphasis that the new, widely applicable, rapid-reaction techniques are not mentioned at all, in sharp contrast with the several pages given, for example, to Mössbauer spectroscopy.

The presentation generally manages to be both discursive and comprehensive. Over-simplification is no worse here than elsewhere. Sometimes, however, ten lines are taken to express two lines' worth of information. More space could have been given to simple derivations (instead of mere quotation) of expressions yielding, for example, dipole moments, magnetic or electric, from bulk measurements; here the mathematics can be both elegant and simple and worth many paragraphs of wordage. Extensive references to monographs and sometimes papers are not always helpful; that to Fuoss and Kraus (1957) on conductance theory scarcely leads directly on from the given text, nor does it give Fuoss's present views. Nearly all scientists quoted are referred to complete with Christian name, a very matey convention, but Debye suffers as Peter J. W. on pages 341 and 517, Peter B. J. on page 325 and Peter J. B. on page 359.

Units, dimensions and symbols are not happily treated, departing far from current recommendations (*Nature*, 1967, 1272, and 1968, 308). The statement "fugacity is usually expressed in atmospheres" (p. 395) is intended to mean that fugacity has the dimensions of pressure.

In conclusion, some readers may like the discursiveness and absence of heavy going often achieved by commendable verbal clarity; others, perhaps among them analysts using isotopes, may be attracted by the introductory emphasis on nuclear properties; but others will be put off by other aspects mentioned above. The page appearance is pleasing and no typographical errors were noted.

D. R. ROSSEINSKY

FOUNDATIONS OF COLLEGE CHEMISTRY. By MORRIS HEIN. Pp. xviii + 395. Belmont, California: Dickenson Publishing Company Inc. 1967. Price 74s. 6d.

Providing an introductory course for the student with no previous experience in chemistry, this book is a good example of an American elementary text, and well worth closer study. It is attractively produced, and the claims on the dust jacket seem substantially (though not perhaps entirely) correct. The development is concisely and coherently argued. There are numerous illustrations, problems and review questions organised systematically. The need to simplify has made some of the aspects seem more clear-cut than they really are, but the book moves from the elementary to the sophisticated rapidly, with a transition that is smooth and not abrupt. While few students here are in the same position as those in the U.S.A., for whom the book was written, the book will undoubtedly be a useful addition to the reference library in the sixth form, and to libraries in technical colleges and colleges of education, where what should be familiar can be approached in a fresh and intellectually satisfying way. Another possible outlet lies in the science content of liberal studies for non-specialists.

The chapters on Atomic Theory and Structure, the Formation of Compounds from Atoms, and the Periodic Arrangement of the Elements are commended. Electronic configurations are dealt with at the level of the average Advanced Level student: *s*, *p*, *d*, *f* orbitals are mentioned but *s* and *p* only are given pictorially. Elementary diagrams of atomic structures and the changes in electronic configuration on bond formation are particularly clear. Electronegativities and

unequal sharing of electrons are mentioned, but bonds are presented as distinct types without the merging of one into the other. Certain examples among those chosen to illustrate the ionic bond can be criticised for reasons that are self-apparent: NaCl, $MgCl_2$, NaF, LiCl, $AlCl_3$, MgO, Na_2S. Use of co-ordinate covalent bonding in sulphurous and sulphuric acids is neat and fits octet theory, but does not quite tally with bond lengths as measured. As^{5+} and Sb^{5+} and other highly polar and suspect ions are quoted. Oxidation number and nomenclature are clearly expounded, but $HgNO_3$, instead of $Hg_2(NO_3)_2$, is used for mercury(I) nitrate, and $Al(HCO_3)_3$, aluminium bicarbonate, which may possibly exist but the reviewer has not met it, is quoted as an example of a salt with more than one positive ion. NH_4HS, KHC_2O_4, etc., also appear in this table, which suggests that the caption needs modifying.

Hydrogen bonding is soon introduced into the study of water, which proceeds to consideration of the physical properties of liquids and solutions, molarity and normality. Brönsted - Lowry and Lewis theories are brought into the chapter on ionisation, with the elementary chemistry of acids, bases and salts leading to the study of electrolytes, pH and electrolysis. Arrhenius is twice misspelt in the index but is correct in the text. Ion - electron equations and oxidation numbers feature fully in Oxidation and Reduction. The essentials of radioactivity and atomic energy are explained lucidly at the introductory level but, in a book with few misprints (about 10), argon is given as A in a transmutation equation.

Examples of gases and solutions are used to introduce Chemical Equilibrium. No mention of the terms homogeneous and heterogeneous is made in this part of the book and it is simply not true to say

"In the reaction,

$$CaCO_3\,(g) \rightleftharpoons CaO\,(s) + CO_2\,(g)$$

an increase in pressure will slow down the forward reaction. . . ."

No units are quoted for equilibrium constants and solubility products.

In the survey of carbon and its compounds, including organic chemistry, I.U.P.A.C. nomenclature appears, and there are photographs of ball-and-stick and scale models of molecules. Aliphatic and aromatic hydrocarbons are described in modest terms and there is a glimpse of the range of other organic compounds. The other chapter at the end deals with the descriptive chemistry of selected elements, namely the alkali metals, halogens, the sulphur and nitrogen families. These two chapters comprise 72 pages but the treatment is about right in an introductory text and should generate interest in the subject. Topics (*e.g.*, Quantum Theory) and elements and/or their compounds (*e.g.*, aluminium) mentioned briefly in the text are excluded from the index, which otherwise seems perfectly adequate.

I welcome the addition of this type of book to reference libraries provided for students at advanced level. While, in my opinion, this book has the blemishes indicated above, the general impression is that it is lively, well put together and the work of an experienced teacher. Accordingly, it is recommended. B. J. Moody

Practical Chemistry. An Integrated Course. By J. W. Buttle, B.Sc., A.R.I.C., and D. J. Daniels, B.Sc., Ph.D., Dip.Ed., F.R.I.C. Second Edition. Pp. xvi + 315. London: Butterworths. 1967. Price 28s.

This attractively presented book, which aims at providing a continuous course of practical chemistry, suitable for Sixth Form and Ordinary National Certificate Students, first appeared in 1962 and is well worth close study.

There are three sections: inorganic and physical, organic and physical (probably to be studied in parallel) and qualitative analysis of simple inorganic and organic compounds. Nine appendices include examination questions, details of chemicals and apparatus required, first-aid notes and various tables. The new edition adds an introductory chapter on the experimental basis for the present view of the atom, some new examination questions, an extension of suggestions for further work and of modern nomenclature in the inorganic section.

Each experiment or preparation, for which instructions are clearly and concisely given, is followed by several short questions, some experimental, others theoretical in nature. Diagrams are clearly drawn and labelled. Those for preparations show proprietary glass-jointed apparatus on the small scale. Structural bond diagrams are shown for organic and inorganic compounds. Dative bonds are shown in oxo-salts, which is a matter of opinion. The treatment is comprehensive and reliable. The book is recommended for its purpose. B. J. Moody

Summaries of Papers in this Issue

Micro-analysis of Silicate Rocks
Part V. Spectrophotometric Determination of Alumina

Alumina is determined spectrophotometrically as the 8-hydroxyquinolinate after ligand exchange in the organic phase with aluminium acetylacetonate extracted into benzene from aqueous medium at pH 6 to 7. Interfering elements such as iron, titanium, vanadium and zirconium are removed by extraction into *o*-dichlorobenzene from M hydrochloric acid, but beryllium is not removed by this procedure.

ROBERT A. CHALMERS and MOHAMMED ABDUL BASIT
Chemistry Department, University of Aberdeen, Old Aberdeen, Scotland.

Analyst, 1968, **93**, 629–632.

An Automatic Analytical Procedure for the Colorimetric Determination of Molybdenum in Steel

A spectrophotometric method for the determination of molybdenum in steel, based on the colour reaction with thiocyanate in the presence of tin(II) chloride and a titanium catalyst, has been adapted for the Technicon AutoAnalyzer. The method is suitable for most steels, but small corrections may be needed in the presence of high concentrations of chromium, cobalt and vanadium. Nickel, manganese and silicon at the usual levels do not interfere. For low-alloy steels the initial solution is identical with that required for an existing automatic method for manganese and phosphorus, but is modified when required to deal with high-speed steels containing alloying additions of tungsten. Results on standard steels are presented, and a statistical survey of long-term reproducibility is given.

K. BRAITHWAITE and J. D. HOBSON
Dunford Hadfields Ltd., East Hecla Works, Sheffield S9 1TZ.

Analyst, 1968, **93**, 633–637.

The Fluorescence Quantum Efficiencies of Some Analytically Useful Chelate Complexes

A computer method is described for calculating the quantum efficiencies of fluorescent compounds and chelate complexes in both aqueous and organic solvents. The quantum efficiency values can be incorporated into a sensitivity factor, *S*, which may be used to evaluate and assess analytically useful fluorescent complexes.

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

Analyst, 1968, **93**, 638–642.

An Evaluation of Some Methods for the Determination of Fluoride in Potable Waters and Other Aqueous Solutions

Five spectrophotometric procedures for the determination of fluoride in water have been evaluated with respect to reproducibility, sensitivity, range, stability of coloured products and of reagents, specificity and effect of temperature. The thorium nitrate titration is briefly discussed, and the use of the Orion fluoride-ion electrode for pF measurement has also been investigated. Various samples of water containing natural or added fluoride have been analysed by four of the spectrophotometric methods, and the results compared with those obtained by titration and with the electrode. The electrode is shown to be less susceptible than the colorimetric methods to interference from other ions in solution, and it gives theoretical recoveries of fluoride added to several drinking water supplies.

N. T. CROSBY, A. L. DENNIS and J. G. STEVENS
Laboratory of the Government Chemist, Ministry of Technology, Cornwall House, Stamford Street, London, S.E.1.

Analyst, 1968, **93**, 643–652.

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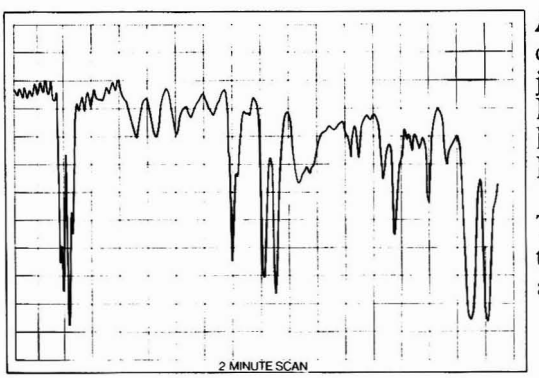

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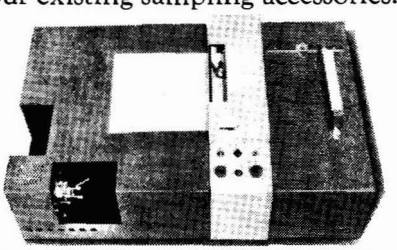

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THE QUEEN'S AWARD TO INDUSTRY

P. A. May 1874

The Determination of the Di- and Mononitrates of Ethylene Glycol and 1,2-Propylene Glycol in Blood by Colorimetric and Gas-chromatographic Methods

Methods are described for the determination of the nitrate esters, ethylene glycol dinitrate, propylene glycol 1,2-dinitrate, ethylene glycol mononitrate, propylene glycol 1-mononitrate and propylene glycol 2-mononitrate, in blood. These esters can be removed efficiently from blood by extraction with diethyl ether, and determination of the dinitrates can be carried out colorimetrically, after alkaline hydrolysis, by a diazotisation and coupling reaction. As the mononitrates are not determined by this procedure, they do not interfere with the determination of the corresponding dinitrate. Gas chromatography, with the electron-capture detector, provides a sensitive method for detecting and measuring the mononitrates, with adequate separation from the parent dinitrate, and the latter can also be determined by this method as an alternative to the colorimetric procedure. For both methods the effect of possible interferences is investigated, and the efficiency of recovery from blood over the range 0 to 25 μg of ester is reported.

M. H. LITCHFIELD

Imperial Chemical Industries Ltd., Industrial Hygiene Research Laboratories, Alderley Park, Cheshire.

Analyst, 1968, **93**, 653–659.

The Rapid Determination of Benzil, Benzoin and Hydrobenzoin in Mixtures by Quantitative Infrared Spectrophotometry

From the infrared spectra of mixtures of benzil, benzoin and hydrobenzoin in solution in chloroform, benzoin and hydrobenzoin are determined by means of their absorption bands at 3460 and 3590 cm^{-1}, respectively. Benzil is determined from the carbonyl absorption at 1680 cm^{-1} after subtracting the contribution of benzoin to the absorbance at this frequency. The most suitable concentrations are 0·1 to 1·0 per cent. for benzil and benzoin and 0·1 to 2·0 per cent. for hydrobenzoin.

P. R. FALKNER, G. DAVISON and G. B. STOKER

John Dalton College of Technology, Manchester.

Analyst, 1968, **93**, 660–662.

A Combustion Method with a Radiometric Finish for the Determination of Microgram Amounts of Sulphur in Light Petroleum

A radiochemical technique has been combined with a modification of the surface combustion method of Schöberl to give a rapid method for the determination of sulphur in the range 1 to 10 p.p.m. in organic substances such as purified light petroleum. The sulphate formed is precipitated with barium chloride labelled with barium-133, and the amount of barium sulphate obtained determined by measuring its activity. A radiometric finish has the advantage of potential sensitivity, and barium-133 can be obtained with a specific activity of 1 Ci per g. Thus, a scintillation counter with an efficiency of 30 per cent. and a background of 20 counts per second (shielded) can detect $0{\cdot}4 \times 10^{-3}$ μg of sulphur, as barium sulphate, assuming no solubility losses.

The lowest detectable concentration of sulphur is dependent on the magnitude of the blank value, but because sulphate appears to be an almost universal contaminant it was found, under normal laboratory conditions, that it was impossible to reduce the blank value to much below the equivalent of 1 p.p.m. in the sample.

E. V. GOODE

Chemistry Department, Derby and District College of Technology, Derby.

Analyst, 1968, **93**, 663–668.

The Automatic Determination of Original Gravity of Beer Part I. Introduction and Determination of Reducing Sugar after Hydrolysis

The determination of the "extract" gravity of sound beers by automatic means is presented and discussed. A method is proposed for correlating "extract" as determined by classical distillation methods with the total reducing sugar after hydrolysis as determined in an automatic analytical system operating at 30 samples per hour, and this is shown to have the form— $E - 1000 = 4{\cdot}33\ S + 1{\cdot}2$, where E is the extract weight (expressed relative to water, sp.gr. 1000) and S is the percentage of sugar as anhydrous glucose. The use of the method as part of a screening technique for examination of samples against declaration is discussed.

R. SAWYER and E. J. DIXON

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

Analyst, 1968, **93**, 669–679.

The Automatic Determination of Original Gravity of Beer Part II. The Determination of Alcohol and Gravity Lost

The determination of gravity lost on samples of sound beers by an automatic distillation procedure, followed by determination of alcohol in the distillate, is presented and discussed. The correlation between alcohol content and gravity lost, determined by distillation, is assessed, and the resulting relationship is used to determine gravity lost in a series of samples. Summation of results with those obtained in Part I indicates the accuracy of the determination of total original gravity by the method proposed, and is shown to be in agreement within ± 2 units of original gravity with the distillation procedure; a mean standard deviation of 0·37 is obtained on replicate analysis.

R. SAWYER and E. J. DIXON

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

Analyst, 1968, **93**, 680–687.

Consistency of R_{st} Values of Six Organophosphorus Pesticides Resolved by Thin-layer Chromatography in the Presence of Plant Extracts without Elaborate Clean-up

This paper reports statistical analyses of the ratio of the distance travelled by each of five organophosphorus pesticides to that travelled by parathion in the presence of plant extracts, without elaborate clean-up. Mixtures of azinphos-methyl, carbophenothion, diazinon, ethion, malathion and mevinphos in the presence of apple, beet, carrot, lettuce or pea extracts were evaluated with reference to the parathion internal standard.

C. E. MENDOZA, P. J. WALES and D. F. BRAY

Research Laboratories, Food and Drug Directorate, Ottawa 3, Ontario, Canada.

Analyst, 1968, **93**, 688–690.

Procedure for Semi-quantitative Confirmation of Some Organophosphorus Pesticide Residues in Plant Extracts

This paper describes the combination of carbon-column chromatography with thin-layer chromatographic - enzyme inhibition technique for semi-quantitative confirmation of organophosphorus pesticides. The combined procedure was evaluated for azinphos-methyl, carbophenothion, diazinon, disulfoton, malathion, mevinphos and parathion. Based on separation of compounds by elution from the column, two mixtures of standards were added to potato extracts and eluates and were analysed on thin-layer chromatograms.

P. J. WALES, C. E. MENDOZA, H. A. McLEOD and W. P. McKINLEY
Research Laboratories, Food and Drug Directorate, Ottawa 3, Ontario, Canada.

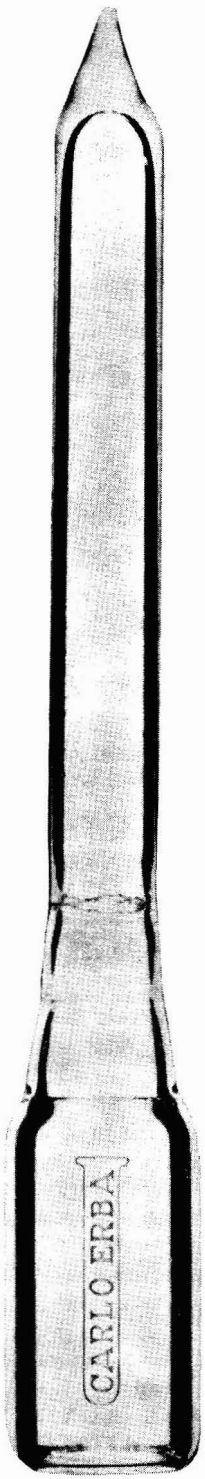
Analyst, 1968, **93**, 691–693.

A Method for the Determination of Sterigmatocystin in Grain and Oilseeds

A sensitive method is described for the quantitative determination of the mycotoxin sterigmatocystin by thin-layer chromatography. A sensitivity of 0·0025 μg is achieved by conversion of sterigmatocystin into the mono-acetate, which fluoresces an intense light blue on thin-layer chromatoplates when viewed under ultraviolet light.

L. J. VORSTER and I. F. H. PURCHASE
National Nutrition Research Institute, Council for Scientific and Industrial Research, Pretoria, South Africa.

Analyst, 1968, **93**, 694–696.



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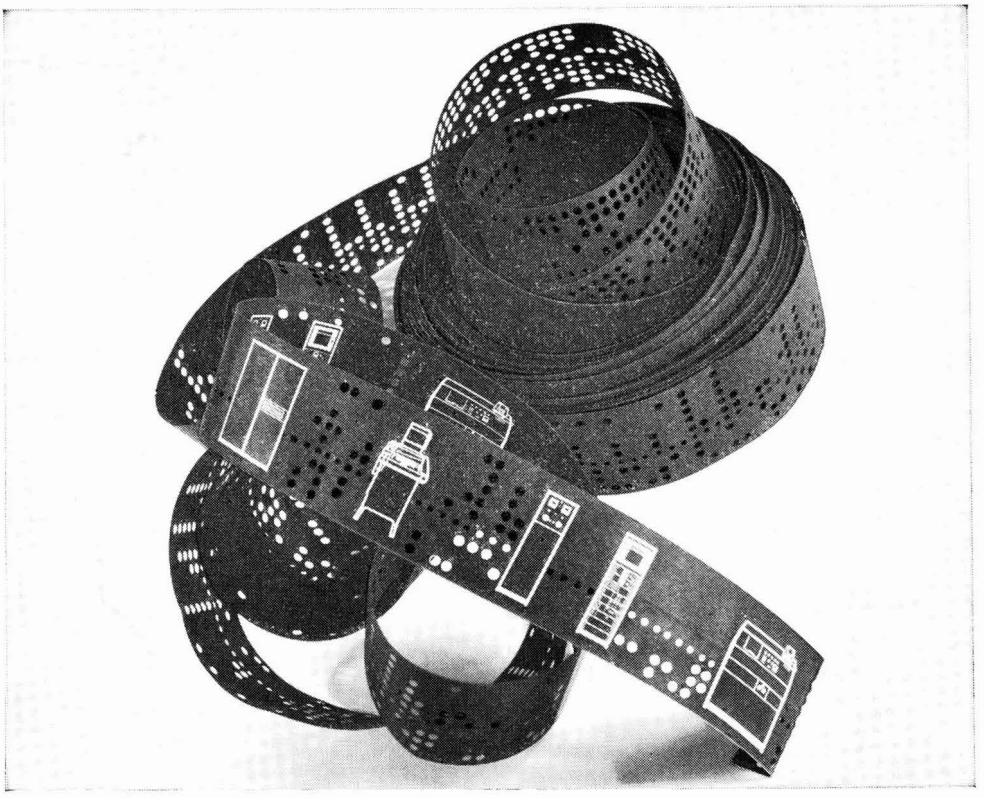

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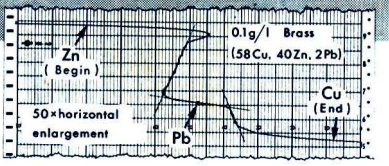

greatly increases the readability of a polarogram. **Below:** Normal DC-Polarogram of brass dissolved in KNO_3. The Pb-step is hardly discernible. **Above:** In tast polarography with controlled drop life, the step can be enlarged 50 times in a horizontal direction.

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3. Polarography with hanging mercury drop, with Kemula Electrode Assembly, type E69b. Metal ion concentrations down to 10^{-9} mol/l are measured.

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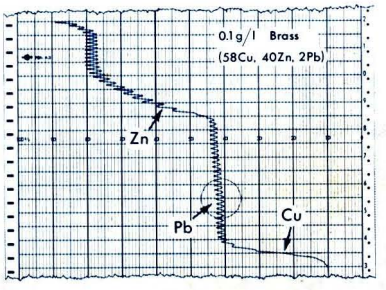

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