THE ANALYST

Recommended Methods of Assay of Crude Drugs

PREPARED BY THE JOINT COMMITTEE OF THE PHARMACEUTICAL SOCIETY AND THE SOCIETY FOR ANALYTICAL CHEMISTRY ON METHODS OF ASSAY OF CRUDE DRUGS

The Determination of the Capsaicin Content of Capsicum and its Preparations

Second Report of the Panel

Introduction

Since the publication of the Panel's first Report[1] recommending methods for determining the capsaicin content of capsicum and its preparations, considerable additional experience has been gained in their routine use. In the light of this further collaborative work by the Panel,* certain modifications to the original published methods have been found desirable. Each of the suggested amendments is discussed below, and, for ease of reference, the methods incorporating them are published as Appendix I (see p. 382). The preparation of pure capsaicin is not necessarily required, but the method of preparing it can be referred to in Appendix II of the first Report.[1] With the development of a colorimetric method the need for alternative standards no longer arises and has not been further pursued. A report on the detection and determination of synthetic capsaicin analogues, work carried out by two members of the Panel (A.J.M. and G.I.S.), is published as Appendix II (see p. 387).

Report

Spectrophotometric characteristics of capsaicin—

When the first Report was prepared only two samples of pure capsaicin had been available to the Panel; since then further batches have been prepared and the spectrophotometric characteristics of each have been determined. Results indicate that these later samples were somewhat purer than the earlier ones, and, in the spectrophotometric difference method, the differences between the extinction values of the acid and alkaline solutions of capaicin, at both maxima, are amended as shown below—

At 248 mμ, the $E^{1\%}_{1\,cm}$ difference is **313** (not 308 as formerly).
At 296 mμ, the $E^{1\%}_{1\,cm}$ difference is **127** (not 116 at 294 mμ as formerly).

Assay of capsicum B.P.C., oleoresin of capsicum B.P.C. and tincture of capsicum B.P.C.—

Preparation of sample—The extraction of the ground sample of Capsicum B.P.C. by shaking it with cold 96 per cent. ethanol, as previously recommended, is open to criticism on the grounds that the filtered residue still retains traces of capsaicin that give a residual pungency after the residue has been washed with ethanol. In view of this it was decided to recommend the exhaustive extraction of the sample by absolute methanol in a Soxhlet apparatus, and to use methanol throughout the ensuing assay.

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Amount to be taken for assay—In all instances the amounts to be taken for assay have been more closely defined so that the concentration of capsaicin in the final methanolic solution is always approximately the same. Within the limits shown in Table VII (see p. 382), the readings obtained by the spectrophotometric difference method would be expected to lie between 0·3 and 0·9 at 248 mμ, and between 0·125 and 0·375 at 296 mμ. For Gibbs' colorimetric method, the net readings at 595 mμ would be expected to lie between 0·2 and 0·7.

Separation of capsaicin: chromatographic method—Difficulty in meeting the specification for absolute methanol by purification through an alumina column led to one member (G.M.W.) comparing methods for its purification (*a*) by passing it through an alumina column and (*b*) by boiling it under reflux over silver oxide for 3 hours with subsequent distillation. Two samples, from different sources, were investigated, and the absorption of each at 280 mμ was reduced to an acceptable figure only when procedure (*b*) was used. In view of this, the specification for the reagent was amended (see p. 384).

With regard to the aluminium oxide used for the column, it was decided to specify the generally available Merck acidic alumina, or the equivalent grade I on the Brockmann scale, and the specification for this reagent was amended (see p. 384).

In the "Procedure," to reduce the risk of impurities or haze, it was decided to reject the first 20 ml of filtrate before determining the capsaicin content (not 10 ml as previously recommended).

Ether - alkali extraction method—In view of the decision to use absolute methanol throughout the recommended methods, all references to ethanol have been deleted. With regard to the diethyl ether used, it was decided not to specify "anaesthetic" ether, since this contains a stabiliser that might affect the results: further, since the presence of peroxides might yield significantly high results, it was decided to specify that the reagent should be "analytical-reagent grade: peroxide-free, as tested by the B.P. method for 'Anaesthetic Ether'."

With regard to the carbon used, it was decided that for this method it was not necessary to specify a proprietary material of the purity required for the chromatographic method, activated carbon that had been washed with methanol being sufficient. In a series of determinations, one member (G.I.S.) found that the addition of 0·1 g of purified carbon (as previously recommended) to the methanolic solution before spectrophotometric evaluation, although improving the "disparity" figures between readings at 248 and 296 mμ, resulted in a significant loss of capsaicin. It was decided that, as it is necessary to add some carbon finally to remove traces of colour and other interfering substances, the amount of purified carbon to be added should be reduced to 0·05 g. Further, the volume of filtrate to be rejected after treatment with carbon was increased from 5 to 20 ml.

Determination of capsaicin: direct spectrophotometric method—This procedure is so rarely applicable that the information has been transferred to Note 4 at the end of Appendix I.

Spectrophotometric difference method—The procedure has been defined more closely, and because there is now little difference between the reliability of the results obtained at both wavelengths, it was decided that, when these are within 5 per cent. of each other, then the results of the assay shall be quoted as the mean of the figures obtained.

OINTMENT OF ~~CAPSAICIN~~ CAPSICUM B.P.C.—

A speedier and more reliable method has been devised, and the details given in Appendix I replace those previously published.

CAPSICUM WOOL B.P.C.—

The previously published Report[1] did not include methods for the assay of capsicum wool. These have now been devised and the details are given in Appendix I. At the time of the collaborative tests, capsicum wool complying with the B.P.C. 1959 specification was not commercially available, and a proprietary brand was used. The results are significantly higher than would be obtained with B.P.C. material.

ALTERNATIVE COLORIMETRIC METHOD FOR DETERMINING CAPSAICIN

The dangers of preparing dry diazonium salts for use in the diazo colorimetric method[2] were emphasised in Note 5 of the Panel's first Report. Because of this and in view of the poor reproducibility of the colour given by the diazotised sulphanilic acid reagent with capsaicin, it was decided to investigate whether any other methods would give acceptable extinction values, thereby obviating the necessity for preparing a standard calibration curve

for each determination, and one of the Panel members (G.I.S.) undertook the preliminary investigation.

Since the papers by Schulte and Krüger[3] and Spanyár, Kevei and Kiszel[4] appeared, Holló, Gal and Sütö[5] have published work on the determination of capsaicin with Gibbs' phenol reagent.[6] Two variations on the 4-aminophenazone method for phenols have been published by Ochynski[7] and Ashton,[8] although capsaicin was not one of the phenols tested.

In the preliminary investigation the two 4-aminophenazone procedures were tested with a standard solution of capsaicin in water. Pink colours were obtained in both, but the coloured compound was not sufficiently soluble in water. An opalescence was first produced and, when the solution was set aside or was filtered, solid pink particles separated. These dissolved when an organic solvent such as methanol, acetone (one phase) or butanol (two phases) was added. However, these methods were not investigated further because the departure from the published procedure would have had to be investigated and, also, the $E^{1\%}_{1\,cm}$ values obtained were only about one-fifth of those obtained by the diazo method and about one-seventh of those obtained with Gibbs' reagent.

TABLE I

OPTICAL DENSITIES OF CAPSAICIN SOLUTIONS OBTAINED WITH GIBBS' REAGENT

For Determination I a standard capsaicin solution containing 0·0213 g per 50 ml was used and for Determination II, which was carried out 3 months after Determination I, a standard capsaicin solution containing 0·0199 g per 50 ml was used

Determination I—					
Capsaicin present, μg	0	106·5	213	319·5	426
Optical density (E) in 1-cm cell at 595 mμ ..	0·036	0·134	0·232	0·332	0·429
$E - E_{blank}$	—	0·098	0·196	0·296	0·393
Determination II—					
Capsaicin present, μg	0	99·5	199	298·5	398
Optical density (E) in 1-cm cell at 595 mμ ..	0·046	0·137	0·235	0·335	0·423
$E - E_{blank}$	—	0·091	0·189	0·289	0·377

The results obtained with the last-named reagent (2,6-dichloro-*p*-benzoquinone-4-chloroimine) were the best of any colorimetric method used so far by the Panel for the determination of capsaicin. Table I shows results obtained on two occasions at an interval of three months.

The two curves obtained by plotting the net optical densities ($E - E_{blank}$) against concentration obey the Beer - Lambert law, and are separated by 0·010 at $E = 0{\cdot}400$; that is, they differ by 2·5 per cent.

The procedure described by Holló, Gal and Sütö,[5] in which the corresponding dibromoquinone-chloroimide reagent is used, is lacking in some details. Thus, it is simply stated that 1·0 mg of dibromoquinone-chloroimide in an alcoholic solution is added; neither the kind of alcohol nor its volume being stated. It is also stated that the contents of the 50-ml flask are diluted to the mark, and set aside for 30 minutes; thus it could be inferred that the diluting liquid could be water, buffer solution or alcohol. Preliminary trials with each alternative showed that the presence of alcohol greatly reduced the sensitivity of the reaction as well as affecting the pH. One millilitre of alcohol was therefore chosen as the smallest convenient volume, and the reagent used had a concentration of 0·1 per cent. w/v. The effect of the alcohol was minimised by deferring the addition of the reagent until the aqueous capsaicin solution had been diluted to 48 to 49 ml with aqueous buffer solution. The use of a larger volume of buffer solution not only gives a more rapid and convenient procedure, but it also follows more closely the original method recommended by Gibbs.[6]

Another criticism of Holló's method, as published, was to be found in the instability of the dibromoquinone-chloroimide reagent. Even after a few hours in the dark, the alcoholic solution had changed from yellow to red, and reproducibility of results was poor. This was contrary to experience with Gibbs' dichloro reagent used in the assay of pyridoxin hydrochloride tablets described in the U.S. Pharmacopoeia XVI. The stabilities of 0·1 per cent. solutions of the two reagents were therefore examined in methanol, 96 per cent. ethanol and isopropanol. The most stable was the dichloroquinone-chloroimide (2,6-dichloro-*p*-benzoquinone-4-chloroimine) in methanol. The two sets of results in Table I were obtained with

the same reagent solution originally prepared from dichloro reagent that had been recrystallised, dried and dissolved in analytical-reagent grade methanol.

TABLE II

RESULTS OF COLLABORATIVE ASSAYS OF CAPSICUM B.P.C.

Capsaicin was separated by the ether - alkali extraction procedure

Laboratory	Test No.	Capsaicin found by spectrophotometric difference method— at 248 mμ, %	at 296 mμ, %	mean, %	Capsaicin found by Gibbs' method, %
A	1	0·95	1·00	0·98	1·00
	2	0·97	1·00	0·99	1·03
B	1	0·99	1·05	1·02	1·02
	2	1·04	1·03	1·04	1·06
C	1	0·98	1·02	1·00	1·00
	2	1·06	1·02	1·04	0·96
D	1	0·97	1·01	0·99	0·91
	2	0·97	1·00	0·99	0·91
E	1	1·03	1·05	1·04	1·01
	2	0·99	1·01	1·00	1·01
			Mean ..	1·01	0·99

TABLE III

RESULTS OF COLLABORATIVE ASSAYS OF OLEORESIN OF CAPSICUM B.P.C.

Capsaicin was separated by the ether - alkali extraction procedure

Laboratory	Test No.	Capsaicin found by spectrophotometric difference method— at 248 mμ, %	at 296 mμ, %	mean, %	Capsaicin found by Gibbs' method, %
A	1	10·27	10·09	10·18	10·7
	2	10·35	10·17	10·26	10·8
B	1	10·25	10·15	10·20	10·4
	2	10·40	10·35	10·38	10·85
C	1	10·73	10·65	10·69	10·75
	2	10·44	10·29	10·36	10·50
D	1	10·73	10·68	10·70	10·12
E	1	10·08	9·94	10·01	10·0
	2	10·53	10·44	10·49	10·8
			Mean ..	10·35	10·54

TABLE IV

RESULTS OF COLLABORATIVE ASSAYS OF TINCTURE OF CAPSICUM B.P.C.

Capsaicin was separated by the ether - alkali extraction procedure

Laboratory	Test No.	Capsaicin found by spectrophotometric difference method— at 248 mμ, %	at 296 mμ, %	mean, %	Capsaicin Gibbs' method, %
A	1	0·047	0·047	0·047	0·049
	2	0·046	0·046	0·046	0·048
B	1	0·049	0·048	0·049	0·047
	2	0·046	0·044	0·045	0·044
C	1	0·041	0·040	0·041	0·045
	2	0·043	0·043	0·043	0·045
D	1	0·047	0·046	0·047	0·044
	2	0·048	0·046	0·047	0·044
E	1	0·046	0·046	0·046	0·043
	2	0·043	0·043	0·043	0·043
			Mean ..	0·045	0·045

As a result of the preliminary experimental work, a method was examined and subjected to considerable collaborative trial. The method finally adopted is that recommended in Appendix I.

In addition to the better reproducibility compared with the assay in which diazotised sulphanilic acid is used, the use of Gibbs' reagent offers slightly increased sensitivity, the possibility of less interference when applied to the assay of coloured solutions (since measurement is at 595mμ instead of at 495 mμ) and a simpler and quicker procedure, with fewer reagents required.

As a result of many determinations on standard solutions of capsaicin, the Panel recommends that the $E^{1\%}_{1\,cm}$ value by this method should be 470.

Collaborative assays

The results of a series of assays by the 5 collaborating laboratories are shown in Tables II to VI.

Table V

Results of collaborative assays of ointment of capsicum B.P.C.

Capsaicin was separated by the ether - alkali extraction procedure

Laboratory	Test No.	Capsaicin found by spectrophotometric difference method— at 248 mμ, %	at 296 mμ, %	mean, %	Capsaicin found by Gibbs' method, %
A	1	0·136	0·136	0·136	0·143
	2	0·154	0·147	0·150	0·157
B	1	0·147	0·146	0·147	0·162
	2	0·144	0·140	0·142	0·160
C	1	0·151	0·154	0·153	0·161
	2	0·151	0·157	0·154	0·164
D	1	0·150	0·148	0·149	0·142
	2	0·143	0·142	0·143	0·142
E	1	0·148	0·148	0·148	0·148
	2	0·154	0·150	0·152	0·150
			Mean ..	0·147	0·153

Table VI

Results of collaborative assays of capsicum WQOL*

Capsaicin was separated by the ether - alkali extraction procedure

Laboratory	Test No.	Capsaicin found by spectrophotometric difference method— at 248 mμ, %	at 296 mμ, %	mean, %
A	1	0·133	0·127	0·130
	2	0·128	0·120	0·124
B	1	0·130	0·120	0·125
C	1	0·120	0·110	0·115
	2	0·120	0·110	0·115
D	1	0·130	0·130	0·130
	2	0·130	0·120	0·125
E	1	0·128	0·134	0·131
	2	0·129	0·135	0·132
			Mean ..	0·125

* A proprietary brand, not B.P.C., was used.

Recommendations

The methods, as now amended, have been found to give consistently good results in the laboratories of the Panel members. It is recommended that, for all routine purposes, the sample of capsaicin oleoresin, tincture, ointment or wool, should be prepared and extracted as described and that the capsaicin content of the resulting test solution should preferably be determined by the spectrophotometric difference method or, if a suitable ultraviolet spectrophotometer is not available, by Gibbs' colorimetric method.

Appendix I

RECOMMENDED METHODS FOR THE DETERMINATION OF CAPSAICIN

PRINCIPLES OF METHODS—

After preliminary preparation of the sample, the capsaicin is extracted either chromatographically or by an ether - alkali extraction method. The capsaicin is then determined by a spectrophotometric difference method or by one of two colorimetric methods: (*a*) based on the coupling reaction with diazobenzenesulphonic acid or (*b*) the reaction with Gibbs' phenol reagent, 2,6-dichloro-*p*-benzoquinone-4-chloroimine. Of these, the spectrophotometric difference method gives the most reproducible results. If a colorimetric end-determination is required, Gibbs' method is preferable.

APPLICABILITY—

The methods may be used for the evaluation of ~~Capsaicin~~ Capsicum B.P.C., Oleoresin of Capsicum B.P.C., Tincture of Capsicum B.P.C., Ointment of Capsicum B.P.C. and Capsicum Wool B.P.C., or similar preparations.

Interfering substances—Capsaicin is accompanied by sterols, fatty acids and colouring matters, which are mostly removed on the chromatographic column, but a small proportion frequently passes through the column and renders the results invalid (see Note 4). In the event of a coloured eluate being obtained, this must not be passed through a second column, owing to preferential adsorption of capsaicin from the purer solution.

If the results obtained from this eluate by the spectrophotometric difference method agree within 5 per cent. of each other, they may be considered satisfactory; if they do not, the original sample should be assayed by the ether - alkali extraction method.

TABLE VII

AMOUNTS TO BE TAKEN FOR ASSAY

Sample	Expected range of capsaicin contents, %	Amount to be taken for assay	Equivalent capsaicin in final methanolic solution, μg per ml
~~Capsaicin~~ Capsicum B.P.C.	0·5 to 1	5 g	25 to 50
Oleoresin of Capsicum B.P.C. ..	8 to 12	3 g	48 to 72
Tincture of Capsicum B.P.C. ..	0·025 to 0·05	10 ml	25 to 50
Ointment of Capsicum B.P.C. ..	0·14 to 0·16	3 g	42 to 48
Capsicum Wool B.P.C.	0·03 to 0·07	10 g	30 to 70

A. PREPARATION OF SAMPLE

The amounts of sample to be taken for assay are shown in Table VII.

REAGENTS—

Methanol, absolute—Analytical-reagent grade. If subsequent separation of capsaicin by the chromatographic method is employed, the methanol must be purified as described in Section B under "Chromatographic Method."

Diethyl ether—Analytical-reagent grade: peroxide-free as tested by the B.P. method for "Anaesthetic Ether."

CAPSICUM B.P.C.—

Accurately weigh approximately 5 g of the sample in moderately fine powder (all to pass a 40-mesh sieve), and extract it in a Soxhlet apparatus for not less than 6 hours (or until exhausted) with absolute methanol. Transfer the extract to a 100-ml calibrated flask, and dilute it to the mark at 20° C with absolute methanol.

Proceed to the separation of capsaicin (Section B) as described below.

(*i*) Transfer a ~~100-ml~~ 10·0-ml portion of the solution to the 5-cm layer of methanol above the alumina column—*for the chromatographic method:*

or

(*ii*) Transfer a ~~100-ml~~ 10·0-ml portion of the solution to a 150-ml separating funnel, add 15 ml of absolute methanol and 15 ml of distilled water—*for the ether - alkali extraction method.*

OLEORESIN OF CAPSICUM B.P.C.—

Dissolve an accurately weighed sample (approximately 3 g) in absolute methanol, add, if necessary, 1 ml of diethyl ether to assist dissolution (see Note 1), and adjust the volume to 250 ml with absolute methanol.

Proceed to the separation of capsaicin (Section B) as described below.

(*i*) Transfer a 5·0-ml portion of the solution to the 5-cm layer of methanol above the alumina column—*for the chromatographic method:*

or

(*ii*) Transfer a 5·0-ml portion of the solution to a 150-ml separating funnel, and add 20 ml of absolute methanol and 15 ml of distilled water—*for the ether - alkali extraction method.*

TINCTURE OF CAPSICUM B.P.C.—

(*i*) Evaporate to dryness on a water-bath about 10 ml, accurately measured, of the tincture. Dissolve the residue in about 10 ml of absolute methanol, and add, if necessary, 1 ml of diethyl ether to assist dissolution. Transfer the whole of the solution to the 5-cm layer of methanol above the alumina column, and proceed to the chromatographic method of separating capsaicin (Section B):

or

(*ii*) Measure 10·0 ml of the tincture into a 150-ml separating funnel, add 15 ml of absolute methanol and 15 ml of distilled water, and proceed to the ether - alkali method of separating capsaicin (Section B).

OINTMENT OF ~~CAPSAICIN~~ CAPSICUM B.P.C.—

To 3 g of sample, accurately weighed, add 30 ml of distilled water, and heat on a steam-bath to melt waxes. Add 10 ml of freshly prepared 3 per cent. w/v barium hydroxide solution, and bring the mixture to the boil, with constant stirring. Immediately cool the mixture, and filter it through a moistened 9-cm Whatman No. 1 filter-paper. Return the residue and filter-paper to the flask, and repeat the above process twice more. Wash the filter-paper finally with a further 50 ml of distilled water. Combine the filtrates, adjust the pH to 7·0 to 7·5 with hydrochloric acid (use a pH meter or phenol red as internal indicator), and proceed to the ether - alkali extraction method of separating capsaicin (Section B) from "Extract the adjusted aqueous solution . . .," at the beginning of the second paragraph.

CAPSICUM WOOL B.P.C.—

Accurately weigh approximately 10 g of the sample, and extract it in a Soxhlet apparatus for not less than 6 hours (or until exhausted) with absolute methanol. Test organoleptically for complete extraction (the wool may still be slightly coloured owing to the presence of dye). Reduce the volume of methanolic solution to approximately 10 ml by heating it on a steam-bath. Proceed to the separation of capsaicin (Section B) as described below.

(*i*) Transfer the whole of the solution to the 5-cm layer of methanol above the alumina column—*for the chromatographic method:*

or

(*ii*) Transfer the whole of the solution to a 150-ml separating funnel, add 15 ml of absolute methanol, which is used to effect the transfer, and add 15 ml of distilled water—*for the ether - alkali extraction method.*

B. SEPARATION OF CAPSAICIN

Chromatographic Method

APPARATUS—

*A "Quickfit" No. C.R.*32/10 *chromatographic tube,* 20 cm long, internal diameter 18 mm, and fitted with a sintered-glass plate.

*A "Quickfit" No. D*2/42 *funnel,* 500-ml capacity, fitted with a DA23 adapter, as a solvent reservoir.

REAGENTS—

Methanol, absolute—Purify by distillation over silver oxide as described below. Dissolve 5 g of analytical-reagent grade sodium hydroxide in 3 litres of absolute methanol, and add 5 g of analytical-reagent grade silver nitrate. Boil the mixture under reflux for 3 hours. Cool the mixture slightly, and distil it, discarding the first 200 ml of distillate. Check the optical density of the distillate at 280 mμ in a 4-cm cell against distilled water; the optical density must not exceed 0·010. Store the purified methanol in amber-glass bottles.

Aluminium oxide—Acidic alumina (obtainable from Merck Sharp & Dohme Ltd., Hoddesdon, Herts.) or the equivalent grade I of the Brockmann scale.

Activated carbon—F.W. grade activated carbon (obtainable from Thomas Hill-Jones Ltd., Junction Works, Bow Common Lane, London, E.3).

Kieselguhr—Super-Cel grade kieselguhr (obtainable from Johns-Manville Co. Ltd., 20 Albert Embankment, London, S.E.11).

PROCEDURE—

Preparation of the column—On the glass sinter prepare first a column of 12 g of aluminium oxide made into a slurry with absolute methanol (see Note 2). On top of this add a mixture of 0·9 g of activated carbon and 0·5 g of kieselguhr also made into a slurry with methanol. Drain the column until a 1-cm layer of methanol remains on top of the column, then place a disc of filter-paper and finally a small plug of cotton-wool on top of the column. Pass absolute methanol from the reservoir through the column, under gravity, until the methanol eluate exhibits no interference at 280 mμ (approximately 100 ml is required). Leave a 5-cm layer of methanol above the cotton-wool plug.

Elution of capsaicin—Transfer the test solution, prepared as described in Section A, to the column, and elute it with methanol, under gravity, always keeping a layer of methanol above the cotton-wool plug. Collect 450 ml of the eluate (in about 4 to 5 hours; see Note 3), evaporate it on a steam-bath to approximately 50 ml, transfer to a 100-ml calibrated flask, and dilute to the mark with methanol at 20° C; mix the solution thoroughly. If necessary, filter it through a Whatman No. 542 filter-paper, rejecting the first 20 ml of filtrate.

Determine the capsaicin content of this solution by any of the methods given in Section C.

Ether - Alkali Extraction Method

REAGENTS—

Light petroleum, boiling-range 80° to 100° C—Analytical-reagent grade.

Diethyl ether—Analytical-reagent grade: peroxide-free, as tested by the B.P. method for "Anaesthetic Ether."

Methanol, absolute—Analytical-reagent grade.

Methanol, 60 per cent. v/v, aqueous—Diluted analytical-reagent grade.

Hydrochloric acid, approximately 0·1 N.

Sodium hydroxide, approximately 0·1 N.

Sodium chloride.

Purified carbon—Shake 10 g of activated carbon with 100 ml of absolute methanol, collect the solid by filtration in a sintered-glass funnel, and dry it at 105° C.

PROCEDURE—

To the methanolic solution prepared as described in Section A add 2 g of sodium chloride and 5 ml of sodium hydroxide solution, and shake the solution well. Extract it with three 10-ml portions of light petroleum. Wash the combined light-petroleum extract with two 5-ml portions of 60 per cent. methanol, and add the washings to the aqueous fraction. Filter the combined aqueous fractions through a small plug of cotton-wool into a beaker-flask, washing the filter with 10 ml of 60 per cent. methanol. Remove the methanol by evaporation to approximately 5 ml on a water-bath, dilute the solution to 50 ml with distilled water, and adjust the pH to 7·0 to 7·5 with hydrochloric acid (use a pH meter or phenol red as internal indicator).

Extract the adjusted aqueous solution with six 20-ml portions of diethyl ether. Wash the combined ethereal extracts with 10 ml of distilled water, and discard the washings. To the combined ethereal extracts in a beaker-flask add 20 ml of absolute methanol, and

evaporate the solution to approximately 1 ml on a water-bath in a fume cupboard. Add absolute methanol to the residue, transfer the solution to a 100-ml calibrated flask, and dilute to the mark with absolute methanol at 20° C. Add 0·05 g of purified carbon, shake the mixture well, and filter the solution through a Whatman No. 542 filter-paper, rejecting the first 20 ml of filtrate.

Determine the capsaicin content of this solution by any of the methods given in Section C.

C. Determination of capsaicin

Spectrophotometric Difference Method

Reagents—

Sodium hydroxide, approximately 0·1 N—Prepare this solution freshly as required.
Hydrochloric acid, approximately 0·05 N.
Methanol, absolute—Analytical-reagent grade.

Procedure—

Transfer by pipette 10·0 ml of the methanolic solution of capsaicin, prepared as described in Section B, to a 25-ml calibrated flask, add 5·0 ml of sodium hydroxide solution, mix well, and dilute the solution to the mark with absolute methanol; mix thoroughly (*Solution A*).

Similarly, treat a further 10·0 ml of the solution with 5·0 ml of the hydrochloric acid (*Solution B*).

Similarly, prepare blank solutions of alkali (*Solution C*) and acid (*Solution D*), omitting the methanolic solution of capsaicin. Measure the optical density of *Solution C* against *Solution D* at 248 mμ and at 296 mμ.

Measure the optical density of *Solution A* against *Solution B* at 248 mμ and at 296 mμ, and deduct the blank readings before calculation.

At 248 mμ, the $E^{1\%}_{1\,cm}$ difference is 313.

At 296 mμ, the $E^{1\%}_{1\,cm}$ difference is 127.

Calculate the capsaicin content from the difference obtained at both wavelengths and, if the disparity is less than 5 per cent., the result shall be the mean of the two findings. When, however, the disparity is greater than 5 per cent., the wavelengths of maximum absorption should be re-determined for the instrument; if the disparity is confirmed, the results are not valid.

Diazo Colorimetric Method

Reagents—

Methanol, absolute—Analytical-reagent grade.
Hydrochloric acid, dilute, approximately 0·25 N.
Sodium hydroxide, approximately 0·4 N—Keep this solution ice-cold.
Diazobenzenesulphonic acid—Dissolve 2 g of sulphanilic acid in 15 ml of 8 per cent. w/v sodium hydroxide solution and 4 ml of 20 per cent. w/v sodium nitrite solution; add this solution slowly, with rotation, to 4 ml of ice-cooled hydrochloric acid, sp.gr. 1·18, in a flask immersed in an ice-bath. Wash the precipitated diazobenzenesulphonic acid, on a previously cooled grade 3 sintered-glass filter, successively with 100 ml of ice-cold distilled water, 25 ml of methanol and 25 ml of diethyl ether (see Note 5).

> The diazonium salt when dry is liable to explode, and special care must be exercised when transferring the dry salt from the filter and during weighing. When the salt is in solution it is harmless. Any dry salt remaining after the required weight has been taken should immediately be washed down the sink. Alternatively, the filter can be weighed before and after filtration; the known weight of salt can then be dissolved off the filter with 0·25 N hydrochloric acid and the solution diluted to the correct volume.

Diazobenzenesulphonic acid solution—A 0·4 per cent. w/v solution of the diazonium salt, prepared as above, in approximately 0·25 N hydrochloric acid. Prepare this solution freshly as required and keep it ice-cold (see Note 6).

Sodium iodide solution, 0·33 per cent. w/v, aqueous.

Standard solution of capsaicin—Prepare a solution containing 130 μg of pure capsaicin per ml in absolute methanol. Details of the procedure for preparing pure capsaicin were given in Appendix II of the Panel's first Report.[1]

PROCEDURE—

Transfer by pipette a portion of the methanolic capsaicin solution (containing between 350 and 700 μg of capsaicin; it may be necessary to concentrate the solution), prepared as described in Section B, to a 20-ml calibrated flask immersed in an ice-bath, and adjust the volume to 10·0 ml with methanol if necessary; add 2·0 ml of sodium hydroxide solution and, after an interval of 10 minutes, 2·0 ml of diazobenzenesulphonic acid solution. Shake the mixture well, and set it aside at room temperature for 15 minutes. Add 2·0 ml of sodium iodide solution and then 2·0 ml of dilute hydrochloric acid, shake the flask well, and heat the contents by immersing the flask in a water-bath at 60° to 70° C for 15 minutes. Cool the solution, and dilute to the mark with sodium hydroxide solution. (If, when cooled, the solution is turbid, add not more than two drops of diethyl ether before diluting to the mark.) If necessary, filter the solution through a Whatman No. 542 filter-paper, rejecting the first 5 ml of filtrate.

Measure the optical density of the solution in a 1-cm cell at 480 mμ with distilled water in the comparison cell.

At the same time, dilute 3·0-, 4·0- and 5·0-ml portions of the standard capsaicin solution each to 10 ml with absolute methanol, and carry out duplicate determinations on each solution by the procedure described above for the test solution. Construct a graph relating the optical densities to the number of micrograms of capsaicin, and from this read the capsaicin content of the test solution.

Gibbs' Colorimetric Method

REAGENTS—

Methanol, absolute—Analytical-reagent grade.

Buffer solution, pH 9·4—Dissolve 3·1 g of analytical-reagent grade boric acid and 3·7 g of analytical-reagent grade potassium chloride in 750 ml of distilled water, and adjust the pH to 9·4 with sodium hydroxide (about 32 ml of N sodium hydroxide are required), and adjust the volume to 1 litre with distilled water.

Gibbs' reagent—Dissolve about 1 g of 2,6-dichloro-*p*-benzoquinone-4-chloroimine in 50 ml of acetone. Filter the solution, and precipitate the reagent by adding small amounts of distilled water, with constant stirring; use a total of 200 ml of water. Collect the crystals on a Buchner funnel, rapidly air-dry them by suction, and finally dry them in a desiccator. Store the reagent in a sealed bottle in a refrigerator.

Solution of Gibbs' reagent—A 0·1 per cent. w/v solution of the recrystallised reagent in absolute methanol. This solution must be prepared immediately before use.

PROCEDURE—

Transfer by pipette 5·0 ml of the methanolic solution of capsaicin, prepared as described in Section B, to a 50-ml calibrated flask. Add 40 ml of buffer solution, and shake the flask well; adjust the temperature of the contents of the flask to 20° C by immersion in a water-bath for 15 minutes, and then add 1·0 ml of the solution of Gibbs' reagent. Shake the flask to mix the contents, and dilute to the mark with buffer solution. Set the flask and contents aside in the dark for *exactly* 25 *minutes after the addition of the reagent*, and immediately read the optical density at 595 mμ in a 2-cm cell with distilled water in the comparison cell.

Prepare a blank solution, with 5·0 ml of methanol in place of the capsaicin solution, and proceed in an exactly similar manner as for the test solution, reading the optical density against distilled water.

At 595 mμ, the $E^{1\%}_{1\,cm}$ value is 470.

NOTES—

1. *Solubility of Oleoresin of Capsicum B.P.C.*—A freshly prepared oleoresin of capsicum is normally readily soluble in methanol. This solubility decreases with age, and it may be necessary to use up to 1 per cent. of diethyl ether to assist dissolution. When difficulty is still experienced in obtaining a clear solution, the insoluble matter should be exhaustively extracted with small portions of methanol. If this procedure has been found necessary, a portion of this solution should be evaporated to dryness and the residue transferred to the chromatographic column with absolute methanol.

2. *Packing of the column*—Since the most convenient method of mixing the activated carbon with kieselguhr is by making a slurry with methanol, the whole column is prepared by this method.

3. *Speed of percolation*—The conditions for percolation as defined should be adhered to closely, as it is found that hastening the time of percolation by applying positive or negative pressure increases the amount of interfering substances in the eluate.

4. *Spectrophotometric characteristics of capsaicin*—The optical density of a methanolic solution of capsaicin, determined in a 1-cm cell at 270, 280 and 290 mμ with methanol in the comparison cell, is a maximum at 280 mμ, the $E^{1\%}_{1cm}$ value at this wavelength being 102.

The ratios of the optical-density readings at 270 mμ/280 mμ and 290 mμ/280 mμ for capsaicin are 0·60 and 0·53, respectively. If solutions give figures differing widely from these values, the presence of interfering substances is indicated.

5. *Laboratory risks*—The diazonium salt is liable to explode when dry, but members of the Panel have never found this to occur unless undue friction was used in handling it.

To avoid any possibility of explosion, after the precipitated diazobenzenesulphonic acid has been washed with 100 ml of ice-cold water and allowed to drain, dissolve 0·8 g (approximately) of the moist salt in 100 ml of 0·25 N hydrochloric acid. This solution should be freshly prepared and kept ice-cold and may be used in place of the 0·4 per cent. solution described in the method.

6. *Colour intensity*—It is important to note that the intensity of the colour obtained by the diazo method increases with increasing concentration of the diazobenzenesulphonic acid solution. The Panel found that a concentration of 0·40 per cent., although not producing maximum colour, gave reproducible results, which were not obtained if larger excesses of reagent were used. It is essential to prepare the standard curve at the same time as the test and to use identical reagents.

Appendix II

REPORT ON THE DETECTION AND DETERMINATION OF SYNTHETIC CAPSAICIN ANALOGUES

Capsaicin exists essentially as the vanillylamide of isodecylenic acid, although there is some evidence that other amides of vanillylamine do occur in smaller amounts in natural sources of capsaicin.[9] Amides of vanillylamine and acids, such as nonanoic, are easily and cheaply synthesised and could be used as adulterants or as a means of fortifying less pungent or exhausted natural sources of capsaicin or its preparations. This reason, and the possibility that these synthetic materials may be undesirable in food flavours, makes their detection desirable. The Panel has considered some methods for the detection and determination of the possible synthetic analogues, since they are not distinguished by the general method proposed for the assay of capsaicin.

The methods considered were—

(*a*) Organoleptic;
(*b*) Paper chromatographic;
(*c*) Gas chromatographic;
(*d*) Infrared;

and short summaries of the work done on each technique are given below.

ORGANOLEPTIC—

Early individual work done by the Panel showed that both the synthetic nonanoic acid amide of vanillylamine and capsaicin itself were oxidised by potassium dichromate, peracetic acid, potassium permanganate and bromine water to non-pungent compounds.

The basis used for most of the collaborative work was the test proposed by Todd.[10] Correspondence with Todd took place, but in spite of additional suggestions by him, and a collaborative test of his method, the Panel was satisfied that oxidation with potassium chromate in acetic acid solution was useless and could give completely misleading results.

PAPER CHROMATOGRAPHY—

One worker reported on experiments made by Kowalewski's paper-chromatographic method.[11] He concluded that neither capsaicin, nor its nonanoic analogue, nor their sulphanilic diazo dyes could be separated by one-dimensional paper chromatography.

GAS CHROMATOGRAPHY—

The paper by Todd and Perun[9] was considered, but no work has been reported on this method.

INFRARED METHODS—

Early work with Nujol mulls showed capsaicin to be free of adsorption at 849 cm^{-1}, whereas the synthetic nonylic amide showed a peak of absorption at this frequency. This was later shown to be due to the crystalline structure alone, and disappeared in solution. It was also shown that capsaicin alone exhibited peak absorption at 970 cm^{-1} owing to the unconjugated *trans* double bond in the fatty-acid portion of the natural capsaicin molecule.

Later, Datta and Susi[12] published similar findings applied to pure compounds, and such work as was done was concentrated on whether this method could be applied to the cruder extracts obtained from galenicals. One method proposed was to extract chillies with 70 per cent. methanol, evaporating to dryness, with subsequent treatment of the extract with absolute ethanol to remove water and extraction of the residue with chloroform. The chloroform solution so obtained was used for measurement of the absorption at 970 cm^{-1}. Another member of the Panel expressed some doubt as to whether this purification would be satisfactory in all instances.

The Panel found that neither of its methods of purification for ultraviolet work yielded sufficiently pure capsaicin for qualitative work by infrared methods, but if a sufficiently large sample were available and the ether - alkali method described on p. 384 was applied to this larger sample, with slight modification, the final extract would clearly show qualitative differences between the native and synthetic substances. One member obtained quantitative results of reasonable accuracy working with two genuine oleoresins, one of which had been adulterated with a synthetic compound.

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The Absorptiometric Determination of Iron in Boiler Feed-water

Part I.* Method for Determining "Reactive"† Iron

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A method has been developed for determining "reactive" forms of iron in boiler feed-water and similar high-purity waters. The iron is extracted into isopentanol as the red iron^{II} - bathophenanthroline complex, and its concentration is determined absorptiometrically. When 200-ml samples were used, the criterion of detection, as defined by Roos, was about 0·4 μg of iron per litre, and the standard deviation of results varied from about $\pm$0·2 to $\pm$0·5 μg of iron per litre in the range 0 to 50 μg of iron per litre; the calibration curve was linear in this range. No appreciable interference was caused by other impurities likely to be present in feed-water, and a batch of 10 samples may be analysed in about 100 minutes. This method did not give the total iron content of several different feed-waters because of the presence of relatively insoluble iron compounds. The efficiency of different techniques for dissolving such "non-reactive" forms of iron has been investigated, and the results are given in Part II. The development of a method for determining the total iron content of feed-water is described in Part III.

THE determination of iron in the feed-water of high-pressure boilers is important because it indicates the amount of corrosion occurring in the condenser and feed-system. The concentration of iron is therefore one of the factors determining which method of feed-water treatment to use. The concentrations of interest are extremely small. For example, it has been recommended[1] that, for drum-boilers operating at or above 1500 p.s.i., the concentrations of iron and copper in the feed-water should together not exceed 10 μg per litre.

The Central Electricity Research Laboratories were asked to provide a method that could be used at power stations for determining iron in feed-water. The method had to be capable of giving results with a standard deviation of 0·5 to 1·0 μg of iron per litre in the range 0 to 10 μg per litre, and a limit of detection of about 1 μg of iron per litre was also desirable.

Absorptiometric techniques were considered most suitable for use in power-station laboratories. Calculations indicated that the formation and measurement of a coloured species in the aqueous sample would be inadequately sensitive when reasonably specific reagents were used. Accordingly, it was decided to use a solvent-extraction absorptiometric technique to concentrate the iron in an organic solvent. Two reagents appeared to be particularly suitable on the basis of sensitivity and specificity, namely 4,7-diphenyl-1,10-phenanthroline (bathophenanthroline) and 2,4,6-tri-pyridyl-1,3,5-triazine. The molar extinction coefficients of the ferrous complexes with these reagents in nitrobenzene are reported[2] as 23,300 and 24,100, respectively. From the literature, there appeared to be little to choose between these two reagents. Bathophenanthroline was chosen because it appeared possible that its ferrous complex was more readily extracted by organic solvents.

The use of bathophenanthroline for determining iron was first reported by Smith, McCurdy and Diehl,[3] and the reagent has subsequently been recommended many times[4 to 8] for analysing feed-waters. However, no detailed investigation of the optimum experimental conditions for good precision and robustness of the technique could be found. The results of such an investigation are presented in Part I of this Paper.

* For details of Parts II and III of this series, see reference list, p. 401.

† In this series of Papers, the term "reactive" iron is used to denote those forms of iron that are determined by the method described in Part I; this point is dealt with under "Discussion of the Method" in Part I; (see p. 400).

A complication that arises in determining iron in feed-water is that large proportions of the iron may exist in particulate and colloidal form.[9,10,11] Many workers[5, 7 to 10] have reported that quite vigorous treatment with mineral acids is necessary to convert all the iron to forms that react with bathophenanthroline. This point is considered in detail in Part II of the Paper,[12] but it seemed probable that the sample would require heating with acid before the total iron content could be determined.

Experimental

Materials—

All chemicals were of analytical-reagent grade whenever possible, and all reagents were prepared as described under "Method" except where stated otherwise. Re-distilled hydrochloric acid, acetic acid and isopentanol were used throughout. De-ionised water was obtained by passing distilled water through a small mixed-bed de-ionisation column. Sufficient de-ionised water for each batch of experiments was collected in one polythene bottle to ensure homogeneity.

The solvent extractions were carried out in 250-ml or 500-ml Pyrex-glass separating funnels, and all optical-density measurements were made with a Unicam SP600 spectrophotometer. During the work described in Part I, the laboratory temperature varied between 18° and 25° C. Whenever appropriate, the analysis of a batch of samples was carried out in random order. All other experimental conditions and techniques were as described under "Method," p. 395.

Preliminary investigations—

The method described by the American Society for Testing Materials[8] was used as a basis for preliminary investigations. From these investigations, a technique was established for detailed study. The details of this preliminary work are not reported, but some of the conclusions are summarised below.

Firstly, the absorption peak of the iron^{II} - bathophenanthroline complex was at 534 mμ, and this wavelength was used throughout. Repeated extraction of solutions containing known amounts of iron and combination of the extracts from each solution showed that the molar extinction coefficient of the iron^{II} - bathophenanthroline complex was 22,200 $\pm$ 300 (95 per cent. confidence limits). This result is in good agreement with that reported by Diehl and Smith.[2]

Secondly, attempts to simplify the technique by not diluting the final isopentanol extract to the mark were unsuccessful because the residual volume of isopentanol showed appreciable variations. Further, removal of water from the isopentanol extract by passage through filter-paper also gave rather variable results owing to both contamination from, and partial removal of the iron^{II} - bathophenanthroline complex by, the filter-paper.

Thirdly, the bathophenanthroline should be added to the sample in such a way that all, or nearly all, the solvent used for the bathophenanthroline dissolves in the aqueous phase. If this was not done, the time required for complete formation of the iron^{II} - bathophenanthroline complex was much greater, presumably because most of the bathophenanthroline remained in the organic phase, and was not readily available for reaction with ferrous ions in the aqueous phase.

Fourthly, a pH indicator was used so that the final desired pH, *i.e.*, about 3·8, could be obtained reproducibly and conveniently. It was envisaged that the method finally developed would be used for determining the iron content of samples after some treatment to dissolve the "non-reactive" iron. As this treatment might have been, for example, evaporating an acidified portion of the sample, the final acidity could have been quite variable, and hence some guide to the amount of ammonia solution to add in subsequent neutralisation was required. For determining "reactive" iron, the indicator may be omitted as conditions may then be simply standardised to give a satisfactory final pH.

Finally, the sensitivity obtained when 50-ml samples were analysed was inadequate, and the volume of sample used for analysis was increased to 200 ml.

The technique chosen for further detailed study is described below. To a 200-ml portion of the sample in a 250-ml separating funnel were added 2 ml of a 10 per cent. solution of hydroxylammonium chloride, 10 drops of a 0·02 per cent. solution of bromophenol blue,

and 10 ml of an acetate buffer (prepared as described under "Method," p. 395). Ammonia solution, sp.gr. 0·88, was then added until the indicator just changed colour. Thereafter, the technique was as described under "Method."'

EFFECT OF pH—

Solutions containing different concentrations of ferrous and cupric ions were analysed as above, except that no indicator was added and three different pH values of the aqueous phase were used. One determination was made for each condition tested, and the results are given in Table I.

TABLE I

EFFECT OF pH ON THE EXTRACTION OF THE IRON^{II} - BATHOPHENANTHROLINE COMPLEX

Concentration of metal, μg per litre—		Optical density, measured in 4-cm cuvettes pH of solutions		
iron	copper	3·5 to 3·6	4·1 to 4·2	4·5 to 4·7
0·0	0·0	0·033	0·036	0·029
5·0	0·0	0·094	0·098	0·090
50·0	0·0	0·657	0·658	0·648
0·0	500·0	0·040	0·047	0·036
5·0	500·0	0·107	0·110	0·104
50·0	500·0	0·664	0·663	0·578

The results show that pH was not critical in the range 3·5 to 4·7 when copper was absent. However, the relatively large concentration of copper caused markedly low results for 50 μg of iron per litre at the highest pH value. This effect is probably caused by the formation of the colourless copper^{I} - bathophenanthroline complex,[2] with consequent depletion of the amount of the reagent available for reaction with iron. At the two lower pH values, copper caused a small positive bias, the average effect being equivalent to about 0·7 μg of iron per litre. This effect was considered adequately small, and no attempt was made to reduce its size. A pH value of 3·6 to 4·0 was used in all further work except where stated; this pH was obtained by adding ammonium hydroxide to the solution until the indicator just changed colour.

EFFECT OF AMOUNT OF BATHOPHENANTHROLINE—

Solutions containing different concentrations of ferrous and cupric ions were analysed as above, except that different volumes of the bathophenanthroline reagent were used. The total volume of isopentanol added to the sample was kept constant by adjusting the volume added for the final extraction. Duplicate determinations were made under each condition, and the mean results are given in Table II.

TABLE II

EFFECT OF VOLUME OF BATHOPHENANTHROLINE REAGENT ON THE EXTRACTION OF THE IRON^{II} - BATHOPHENANTHROLINE COMPLEX

Concentration of metal, μg per litre—		Optical density, measured in 2-cm cuvettes Volume of reagent added, ml			
iron	copper	1·0	2·0	4·0	6·0
0·0	0·0	0·015	0·017	0·018	0·021
0·0	500·0	0·020	0·024	0·027	0·027
50·0	0·0	0·329	0·323	0·330	0·330
50·0	500·0	0·072	0·324	0·339	0·343

The results show that 1·0 ml of the bathophenanthroline reagent was sufficient when copper was absent. However, when copper was present the results were extremely low with 1 ml of the reagent and slightly low with 2 ml. On this basis, 4 ml of the reagent and 21 ml of isopentanol appeared to be suitable amounts.

EFFECT OF TIME OF REACTION—

The rate of formation of the iron^{II} - bathophenanthroline complex was checked by analysing solutions containing different concentrations of ferrous and cupric ions. The method used was as above except that different times were used for (*a*) the preliminary shaking of the sample after adding the bathophenanthroline reagent, and (*b*) the time elapsed between the end of the preliminary shaking and the addition of isopentanol. One determination was made under each condition tested, and the results in Table III show that the preliminary equilibration greatly increased the amount of the iron^{II} - bathophenanthroline complex formed. However, the durations of the shaking and standing periods were not critical, and a shaking time of 1 minute and a subsequent reaction time of not less than 2 minutes were suitable.

TABLE III

EFFECT OF TIME OF REACTION ON THE FORMATION OF THE IRON^{II} - BATHOPHENANTHROLINE COMPLEX

Shaking time, minutes	Standing time, minutes	Optical density, measured in 2-cm cuvettes. Concentrations of metals, μg per litre— iron 0 copper 0	iron 0 copper 500	iron 50 copper 0	iron 50 copper 500
0	0	0·018	0·024	0·079	0·059
0·5	5	0·020	—*	0·339	0·350
1·0	2	0·022	0·036	0·342	0·352
1·0	5	0·019	0·032	0·338	0·355
1·0	10	0·020	0·034	0·339	0·349
1·0	20	0·019	—*	0·336	—*
1·0	40	0·019	—*	0·337	—*
2·0	2	0·019	0·033	0·337	0·349
2·0	5	0·020	0·032	0·338	0·348

* Not determined.

EFFECT OF DURATION OF SHAKING TIME ON THE EXTRACTION OF THE IRON^{II} - BATHOPHENANTHROLINE COMPLEX—

Solutions containing 0 and 50 μg of iron per litre (as ferrous ions) were analysed as above, except that the shaking time for the final solvent extraction was varied; the rate of shaking in these and all subsequent tests was about 200 shakes per minute. Duplicate determinations were made under each condition, and the results in Table IV show that no more iron was extracted after shaking for 1 minute; a shaking time of 2 minutes should therefore be suitable.

TABLE IV

EFFECT OF SHAKING TIME ON THE EXTRACTION OF THE IRON^{II} - BATHOPHENANTHROLINE COMPLEX

Length of shaking time, seconds	Mean optical density, measured in 2-cm cuvettes. Concentration of iron, μg per litre: 0	50
30	0·021	0·302
40	0·021	0·322
50	0·022	0·328
60	0·020	0·337
120	0·021	0·338

Other tests showed that the volume of isopentanol used for the solvent extraction was not critical, because no significant difference was found when 16 ml were used instead of 21 ml. It was also shown that variations between 2 and 5·5 ml in the volume of industrial methylated spirit used for washing the separating funnels caused no significant effects.

The results in Tables III and IV indicate that the amount of iron extracted tended to a value equivalent to an optical density of 0·319 ± 0·002. From the molar extinction coeffient of the iron^{II} - bathophenanthroline complex, the optical density corresponding to complete extraction of the iron is 0·318 ± 0·004 (95 per cent. confidence limits). Thus, the single extraction was sufficient to extract essentially all of the iron from the aqueous phase.

EFFECT OF TEMPERATURE—

Effect of temperature on the optical density of the iron^{II} - bathophenanthroline complex—Solutions containing 50 μg of iron per litre (as ferrous ions) were analysed as above except that the final extracts were either cooled or warmed before their optical densities were measured. The measurements were repeated as the solution in the cuvette either warmed up or cooled down to room temperature, and the temperature of the solution was also measured at intervals. The results showed that the optical density decreased by about 0·3 per cent. per 1° C rise in temperature in the range 15° to 32° C.

Effect of temperature on the extraction of the iron^{II} - bathophenanthroline complex—Solutions containing 0 or 50 μg of iron per litre (as ferrous ions) were analysed as above except that they were either cooled or warmed before they were placed in the separating funnels. The temperatures of the aqueous phases were measured immediately before and after both the initial and final shaking periods; the mean temperatures are given in Table V. Duplicate determinations were made under each condition; the temperatures of all the extracts during measurement of their optical density were within 1° C of room remperature. The mean results in Table V show that temperature was not critical in the range tested, although results were about 3 per cent. low at the lowest temperature.

TABLE V

EFFECT OF TEMPERATURE ON THE EXTRACTION OF THE IRON^{II} - BATHOPHENANTHROLINE COMPLEX

Temperature, °C	Mean optical density, measured in 2-cm cuvettes: Concentration of iron, μg per litre	
	0	50
30	0·027	0·347
24·5	0·027	0·348
12 to 15	0·023	0·333

STABILITY OF THE IRON^{II} - BATHOPHENANTHROLINE COMPLEX—

Solutions containing 50 μg of iron per litre (as ferrous ions) were analysed as above except that the optical densities of the final extracts were measured at different times after the final extraction. Duplicate determinations were made for each time, and the mean results, given below, show no appreciable effect for times between 5 and 60 minutes.

Time after extraction, minutes	2	5	10	20	30	60
Mean optical density, measured in 4-cm cuvettes	0·682	0·687	0·689	0·693	0·687	0·687

Further tests were made in which the entire analysis (apart from the final measurements) was carried out in extremely bright August sunlight. The solutions used contained 0, 5 and 50μg of iron per litre, and the optical densities of the final extracts were measured at different times during the period 1 to 60 minutes after extraction. Room temperature during these analyses was 30° C, and all extracts were stable, within $\pm$0·001 optical-density units, over a period of 1 hour. Thus, satisfactory results should be obtained if measurements are made within 1 hour of the solvent extraction.

TABLE VI

EFFECT OF THE AMOUNT OF ACETATE BUFFER

Concentration of metal, μg per litre—		Optical density, measured in 2-cm cuvettes: Volume of buffer, ml		
iron	copper	5	10	20
0·0	0·0	0·018	0·020	0·021
50·0	0·0	0·334	0·335	0·339
0·0	500·0	0·029	0·027	0·029
50·0	500·0	0·348	0·350	0·342

EFFECT OF THE AMOUNT OF ACETATE BUFFER—

Solutions containing different concentrations of ferrous and cupric ions were analysed as above except that the amount of the acetate buffer was varied. One determination was made under each condition tested, and the results in Table VI show that the amount of buffer

was not critical. There is an indication that the interference of copper was slightly smaller when the largest amount of buffer was used, but the decrease was so small that it was not thought worthwhile to use greater amounts of the buffer.

REDUCTION OF FERRIC IONS BY HYDROXYLAMMONIUM CHLORIDE—

Several workers[5,13] have reported that some of the iron in feed-water samples may deposit on the walls of sample containers unless the samples are acidified. It was decided therefore that samples should be collected into sufficient hydrochloric acid to make the final acidity 0·1 N. Under these conditions it was thought possible that a large proportion of the iron in samples would be in the ferric form. It was necessary therefore to check that ferric ions were quantitatively reduced by the hydroxylammonium chloride.

Solutions containing different concentrations of ferric and ferrous iron were analysed as above except that the amount of hydroxylammonium chloride was varied; all solutions were 0·1 N with respect to hydrochloric acid. Duplicate determinations were made for each condition, and the mean results in Table VII show that the amount of hydroxylammonium chloride was not critical, and was sufficient to reduce ferric ions quantitatively.

TABLE VII

EFFECT OF THE AMOUNT OF HYDROXYLAMMONIUM CHLORIDE ON THE REDUCTION OF FERRIC IONS

Volume of hydroxylammonium chloride reagent, ml	Optical density, measured in 2-cm cuvettes, with—		
	Reagent blank value	50 μg of iron^{II} per litre	50 μg of iron^{III} per litre
1·0	0·020	—	0·326
2·0	0·020	0·326	0·328

In all further work, sufficient re-distilled hydrochloric acid was added to 200-ml portions of solutions for analysis to adjust their final acidity to 0·1 N.

EFFECT OF INTERNAL pH INDICATOR—

Although the colour change of the bromophenol blue indicator was convenient for adjusting the pH, it was found that the pH of the aqueous phase had a marked effect on the contribution of the indicator to the optical density of extracts. For example, these contributions were 0·021, 0·039 and 0·051 optical-density units at pH values of 3·4, 3·8 and 4·2, respectively. It was thought preferable to eliminate this effect by using another indicator. However, it seemed likely that any indicator with a colour change at about pH 3·8 would show a similar effect. Investigation of several indicators indicated that *m*-cresol purple would be suitable, since its colour change from red to yellow occurred at about pH 2·1, and the subsequent addition of 10 ml of the acetate buffer, adjusted to pH 4·0, gave a final pH of 3·8 to 3·9. Further, the optical density due to the indicator in the final extracts (about 0·03 units) was independent of pH in the range 3·7 to 4·4, and increased by only 0·006 optical-density units (in 4-cm cuvettes) at pH 3·5. The procedure adopted on this basis was, therefore, as described under "Method," p. 395, and this was used for all further work reported here.

DETERMINATION OF IRON IN THE WATER USED FOR REAGENT BLANK SOLUTIONS—

If the water used for reagent blank solutions contains iron, the blank value will be falsely large; the iron content of this water must therefore be determined. A convenient method of doing this appeared to be to extract 200- and 400-ml portions of the water identically, and to take the difference in optical density between the two extracts as equivalent to the iron content of 200 ml of water. However, preliminary tests showed that this simple approach was invalid because (*a*) the residual volume of isopentanol after extraction was much smaller for 400- than 200-ml samples and (*b*) the distribution of bathophenanthroline and *m*-cresol purple between the aqueous and organic phases was affected by the volume of the aqueous phase. Therefore, the technique was modified to that described under "Method," p. 395. The efficiency of this modification for determining iron in the water used as the reagent blank solution was tested with the results given in Table VIII; each result is the mean of duplicate determinations.

The results show that the technique was adequate for recovering small concentrations of iron added to the water, and the results for the iron content of the water were also consistent.

TABLE VIII

DETERMINATION OF IRON IN THE WATER USED FOR REAGENT BLANK SOLUTIONS

Volume of sample, ml	Iron added, μg per litre	Optical density, measured in 4-cm cuvettes— measured	calculated for 25-ml final volume	Iron recovered, μg per litre
200	0·0	$0{\cdot}021_5$	0·021	—
400	0·0	0·031	$0{\cdot}020_5$	—
200	2·0	$0{\cdot}045_5$	$0{\cdot}043_5$	1·9
400	2·0	$0{\cdot}101_5$	0·066	1·9

During the work described here, the iron content of the water used for reagent blank solutions varied between <0·1 and 0·3 μg per litre.

METHOD

APPARATUS—

Detergents should not be used for cleaning apparatus.

Separating funnels, 250- and 500-ml—Pyrex-glass funnels should be used. To clean these adequately, soak them in a cleaning solution of chromic acid overnight, and then wash them with tap water and de-ionised water. Extract 200-ml portions of de-ionised water in each funnel as described under "Procedure: reagent blank determinations," p. 397, and then discard these solutions. Rinse the funnels with de-ionised water; they should now be ready for use. If funnels with taps are used, the taps should not be greased.

Results have indicated that different amounts of iron may be leached from different types of separating funnel during a determination. Therefore, it is recommended that the results from all funnels should be examined to see if any funnels give systematically different results from others.

Calibrated flasks, 25-ml—Clean the flasks in the same way as the separating funnels up to the first wash with de-ionised water. Then rinse each flask with a little analytical-reagent grade acetone, and dry them in an oven.

Polythene sampling bottles—Polythene bottles are suitable for collecting and storing samples, but they must first be cleaned by soaking them in diluted hydrochloric acid (1 + 1) until they are adequately clean.

Pyrex-glass reagent bottles—Clean these bottles and their stoppers in the same way as the 25-ml calibrated flasks, but omit the treatment with acetone.

REAGENTS—

Hydrochloric acid, approximately 6 N—To decrease the reagent blank values, it is recommended that re-distilled hydrochloric acid be used. To prepare this, distil 800 ml of diluted analytical-reagent grade hydrochloric acid (1 + 1) in an all-glass apparatus, and reject the first 50 and last 100 ml of distillate. Determine the normality of the distillate by titration against sodium hydroxide.

Ammonia solution, sp.gr. 0·88—Analytical-reagent grade was suitable; it should be stored in a Pyrex-glass bottle.

m-*Cresol purple solution*, 0·02 *per cent. w/v*—Dissolve 0·020 g of *m*-cresol purple in about 80 ml of de-ionised water and 2 drops of ammonia solution, sp.gr. 0·88, with heating. When all the indicator has dissolved, cool the solution and dilute it with water to 100 ml. This solution was adequately stable for at least 5 weeks.

Isopentanol—Use re-distilled analytical-reagent grade material.

4,7-*Diphenyl*-1,10-*phenanthroline* (*bathophenanthroline*) *solution*, 0·1 *per cent. w/v*—Dissolve 0·50 g of bathophenanthroline in 500 ml of isopentanol, and store the solution in a Pyrex-glass bottle. It is convenient to prepare this solution the day before it is required, but dissolution may be speeded, if necessary, by standing the stoppered bottle in warm water. This solution was adequately stable for at least 5 weeks. Many different batches of

the reagent from different suppliers have been used, but none significantly affected the sensitivity and precision of results.

Hydroxylammonium chloride solution, 10 per cent. w/v—Dissolve 50 g of analytical-reagent grade hydroxylammonium chloride in 500 ml of de-ionised water. This solution normally contains appreciable amounts of iron, and these should be removed by using the procedure described below. Add ammonia solution, sp.gr. 0·88, dropwise with stirring until the pH is about 4 (narrow-range indicator paper is suitable for assessing this pH value). Transfer the solution to a 1-litre separating funnel, add 4 ml of the bathophenanthroline solution, and shake the mixture for 1 minute. Set the funnel aside for at least 2 minutes, and then add 25 ml of isopentanol, and shake the funnel for 2 minutes. Allow the phases to separate, and run off the lower, aqueous layer into another separating funnel, and discard the alcoholic phase. Repeat this extraction procedure until the alcohol phase is colourless or only slightly pink. After the final extraction, set the funnel aside for at least 8 hours, and then run the aqueous phase into a Pyrex-glass bottle. This reagent solution was adequately stable for at least 5 weeks.

Acetic acid, glacial—To decrease the reagent blank values, it is recommended that re-distilled analytical-reagent grade glacial acetic acid be used.

Acetate buffer solution, pH 4·0—Dissolve 60 g of analytical-reagent grade ammonium acetate in 200 ml of de-ionised water and transfer the solution to a separating funnel. Add 2 ml of the hydroxylammonium chloride solution, 4 ml of the bathophenanthroline solution, and repeat the extraction as described for purifying the hydroxylammonium chloride solution. Set the funnel aside for at least 8 hours after the final extraction. Run the aqueous phase into a 1-litre Pyrex-glass beaker, add 550 ml of de-ionised water, 200 ml of acetic acid, and adjust the pH of the solution to $4 \pm 0{\cdot}05$ by adding ammonia solution or acetic acid. Store the solution in a Pyrex-glass bottle. This reagent solution was adequately stable for at least 5 weeks.

Ethanol—Industrial methylated spirit, 74° O.P.

Water—It is desirable that water with an extremely low "reactive" iron content (less than 0·5 μg per litre) should be available. Water containing less than 0·2 μg of "reactive" iron per litre has been consistently obtained by passing distilled water through a small mixed-bed de-ionisation column. The "reactive" iron content of the water used for reagent blank solutions must be determined as described under "Procedure." The container of this water should be shaken vigorously before any water is withdrawn from it.

Standard iron solution A—Dissolve 1·000 g of pure iron by warming it gently in a covered 250-ml glass beaker with 40 ml of diluted hydrochloric acid (1 + 1). When all the iron has dissolved, add 1·0 ml of analytical-reagent grade concentrated nitric acid cautiously in small portions down the side of the beaker, cover the beaker, and warm it until the dark brown colour in the solution has been dispelled. Cool the solution, add 150 ml of re-distilled hydrochloric acid, and dilute the solution with water to 1 litre in a calibrated flask.

1 ml of solution ≡ 1·0 mg of iron.

Standard iron solution B—Place 100·00 ml of standard iron solution A by pipette in a 1-litre calibrated flask, and dilute the solution to the mark with de-ionised water. Store this solution in a polythene container; the solution is stable for at least 6 months.

1 ml of solution ≡ 100 μg of iron.

Standard iron solution C—Place 4·00 ml of standard iron solution B by pipette in a 1-litre calibrated flask, and dilute it to the mark with de-ionised water. Prepare this solution freshly before use.

1 ml of solution ≡ 0·4 μg of iron.

PROCEDURE—

Sample collection—Put sufficient of the approximately 6 N hydrochloric acid into the container in which the sample is to be collected to ensure that the final acid concentration will be 0·1 N (±0·005 N); polythene containers are suitable. Make allowance for the acid contained in the sample when measuring the volume required for analysis.

Analysis of samples—Shake the sample vigorously, and then transfer a volume, equivalent to 200 ml of feed-water, to a separating funnel. Care should be taken that small glass chippings from pipettes, stoppers, etc., do not enter the funnel or falsely high results may

be obtained. Add 2·0 ml of the hydroxylammonium chloride solution, precisely 1·0 ml of the *m*-cresol purple solution, and swirl the funnel to mix its contents. Add ammonia solution, sp.gr. 0·88, dropwise with swirling until the indicator just changes to a yellow colour free of any tinge of pink; care should be taken not to add more than 1 or 2 drops of ammonia solution in excess. Add 10 ml of the acetate buffer solution, and mix the solutions by swirling the funnel. Add 4·0 ml of the bathophenanthroline solution, shake the funnel vigorously (about 200 shakes per minute) for 1 minute, set the funnel aside for between 2 and 40 minutes, and then add 21 ml of isopentanol and shake the funnel vigorously for 2 minutes. Set the funnel aside for between 10 and 60 minutes, and then discard the lower, aqueous phase, leaving as little water as possible in the separating funnel. Run the isopentanol through a small, glass filter-funnel into a dry 25-ml calibrated flask, leave the funnel and flask in place, and wash the walls of the separating funnel with 5 ml of industrial methylated spirit, allowing this to drain into the flask. Dilute the solution in the flask to the mark with industrial methylated spirit, and shake the flask until any water droplets have been dispersed. Wash a 4-cm cuvette with a little of the extract, and measure the optical density of the extract at 534 mμ against a reference cuvette filled with isopentanol. Let the measured optical density be A_S.

Reagent blank determinations—A reagent blank solution should be tested with each batch of sample determinations. For this, place 200 ml of water of known iron content (see below) in a separating funnel, and then add sufficient of the approximately 6 N hydrochloric acid to make the final acid concentration 0·1 N ($\pm$0·005 N); the acid should be from the same batch as that used for collecting samples. Analyse this solution exactly as described above. Let the measured optical density be A_B.

Determination of iron in the water used for reagent blank solutions—Add 200 ml of the water to be used for reagent blank solutions to one separating funnel, and add that amount of approximately 6 N hydrochloric acid used for the reagent blank determination. Add 2·0 ml of the hydroxylammonium chloride solution, and then the same amount of ammonia solution used for the reagent blank solution. Add 10 ml of the acetate buffer, 1·0 ml of the bathophenanthroline solution, shake the funnel vigorously for 1 minute, and set the funnel aside for at least 2 minutes. Add 24·0 ml of isopentanol, shake the funnel vigorously for 2 minutes and set the funnel aside for at least 10 minutes before discarding the aqueous phase. Run the upper, isopentanol layer into a dry 25-ml stoppered measuring cylinder, and wash the walls of the funnel with 5 ml of industrial methylated spirit, which is also collected in the cylinder. Insert the stopper in the cylinder, disperse all water droplets by vigorous shaking, read the volume of the solution (to the nearest 0·1 ml), and then measure the optical density as described above. Let the optical density be A_T, and the volume of the extract be V_T.

Repeat this test with 400 ml of the same water, but with exactly the same amounts of all reagents as specified for the 200-ml portion of water. Let the optical density be A_F, and the volume of the extract be V_F. Then the optical density, A_C, due to iron in the 200 ml of water used for the reagent blank solution is given by the expression—

$$A_C = \left(\frac{V_F}{25} \times A_F\right) - \left(\frac{V_T}{25} \times A_T\right)$$

Calculation of results—The optical density, A_A, due to iron in the sample is given by the expression—

$$A_A = A_S - A_B + A_C$$

and the concentration of iron in the sample can then be determined from the calibration curve.

PREPARATION OF CALIBRATION CURVE—

To a series of separating funnels, transfer 200, 198, 195, 190, 185, 180, and 175 ml of water whose iron content is low, and then add 0·00, 2·00, 5·00, 10·00, 15·00, 20·00 and 25·00 ml, respectively, of standard iron solution C. Treat these solutions as described under "Reagent blank determinations," and repeat the determinations until the calibration curve is defined with adequate precision.

Subtract the mean optical density of the reagent blank solution from the mean optical density of each of the other solutions, and plot the corrected optical densities against the concentration of iron added to the solutions. The calibration curve was linear (within 2 per cent.) up to at least 50 μg of iron per litre in samples. When measurements were made in

4-cm cuvettes with the Spekker absorptiometer and Ilford No. 604 filters, the calibration curve was linear to about 40 μg of iron per litre; for concentrations less than this, the optical densities were about 90 per cent. of those obtained by measuring at 534 mμ.

Results

Sensitivity and precision—

On each of 10 days, duplicate analyses were made in random order by the proposed method at concentrations of about 0, 2, 10, 25 and 50 μg of iron per litre. These samples were freshly prepared each day as described under "Preparation of Calibration Curve." The same reagent solutions were used throughout, and the analyses were made over a period of 2 weeks.

Analytical results are obtained by subtracting the optical density of the reagent blank solution from that of the sample; the precision of these corrected results was therefore calculated by allowing for the variability of both the blank solutions and the samples. The results were analysed by calculating the standard deviations corresponding to variations occurring within and between batches of analyses. The "within-batch" standard deviation for reagent blank solutions was also calculated, and a summary of all the results is given in Table IX. The total standard deviation was calculated as the square root of the sum of the variances of the "within-" and "between-batch" variations.

Table IX

Sensitivity and precision of iron determinations

Concentration of iron added, μg per litre	Mean optical density— corrected for blank value	Mean optical density— per 10 μg of iron per litre	Standard deviation, μg of iron per litre— within batches	between batches	total
0·00	—	—	$\pm 0{\cdot}14$	—	—
2·10	$0{\cdot}029_1$	$0{\cdot}138_5$	$\pm 0{\cdot}19$	0·00*	$\pm 0{\cdot}19$
10·36	$0{\cdot}133_8$	$0{\cdot}129_8$	$\pm 0{\cdot}30$	0·04*	$\pm 0{\cdot}30$
25·90	$0{\cdot}331_5$	$0{\cdot}127_8$	$\pm 0{\cdot}30$	0·12*	$\pm 0{\cdot}32$
51·87	$0{\cdot}649_6$	$0{\cdot}125_2$	$\pm 0{\cdot}31$	0·31	$\pm 0{\cdot}44$

Each "within-batch" and "between-batch" standard deviation has 10 and 9 degrees of freedom, respectively.

* "Between-batch" variations not significantly (95 per cent. confidence limits) greater than "within-batch" variations.

As a test of the stability of the reagents used for these checks of precision, a further batch of analyses was made 5 weeks after the reagents were first prepared. The mean optical densities (after subtracting the reagent blank value) for the solutions containing 2, 10, 25 and 50 μg of iron per litre were 0·031, 0·132, 0·331 and 0·647, respectively. These results agree well with those obtained (see Table IX) during the first 2 weeks after the reagents had been prepared.

Accuracy—

Analysis of feed-water—A sample of feed-water was collected at a power station and portions (to some of which were added known amounts of iron) were analysed as under "Method," p. 395. As a further test for interfering materials in the sample, other portions of the sample were diluted (1 + 1) with de-ionised water (containing less than 0·1 μg of iron per litre), and 200-ml portions of these diluted solutions were analysed as under "Method." The results are given in Table X.

Interferences—The effects of several other impurities were tested at each of two concentrations of iron, *viz.*, 0 and 20 μg per litre. The analyses were made singly by the proposed method, and solutions containing iron only were analysed with each batch of analyses. The results are shown in Table XI.

A few other tests showed that low results were obtained when 2 drops of ammonia solution, sp.gr. 0·88, in excess were added to samples containing 50 μg of iron per litre and 100 μg of chromium^{III} per litre. Results varied between 85 and 95 per cent. of the true value, but quantitative results were obtained when no excess of ammonia solution was added. This effect was caused by chromium, but the mechanism was not investigated. No such

TABLE X

ACCURACY AND PRECISION OF THE DETERMINATION OF IRON IN FEED-WATER

Sample	Iron added, μg per litre	Optical density	Iron found, μg per litre	Recovery* of added iron, per cent.
Reagent blank solution	—	0·066, 0·063	—	—
Feed-water	—	0·165, 0·162 0·162, 0·165	8·0	—
Feed-water	51·87	0·793, 0·805	59·1	98·7 (±1·2)
Diluted feed-water†	—	0·116, 0·112	4·0	—
Diluted feed-water†	51·87	0·757, 0·771	56·4	101·0 (±1·2)

* The limits given for the recoveries are for 95 per cent. confidence limits, and were calculated from the standard deviations shown in Table IX.

† Feed-water diluted (1 + 1) with de-ionised water containing less than 0·1 μg of iron per litre.

TABLE XI

EFFECT OF OTHER IMPURITIES

Impurity	Concentration of impurity, μg per litre	Iron recovered,* μg per litre— 0·0 μg per litre added	Iron recovered,* μg per litre— 20·7 μg per litre added
Tin^{II}	100	0·2	18·3
$Nickel^{II}$	1000	1·0	21·1
$Copper^{II}$	1000	0·8	20·9
$Manganese^{II}$	1000	0·3	20·8
$Zinc^{II}$	1000	0·0	21·0
$Aluminium^{III}$	1000	1·4	21·6
$Chromium^{III}$	1000	0·1	20·4
$Vanadium^{IV}$	100	0·0	21·1
$Molybdenum^{VI}$	1000	−0·1	20·7
$Vanadium^{V}$	100	0·5	21·6
$Tungsten^{VI}$	100		
$Titanium^{IV}$	100		
Tin^{IV}	100		
$Cobalt^{II}$	100		
$Calcium^{II}$	10,000	0·5	20·9
$Magnesium^{II}$	10,000		
Silicate	10,000		
Orthophosphate	10,000		
Fluoride	1000	−0·2	20·7
Sodium	10,000	0·1	20·3
Potassium	10,000		
Sulphate	20,000		
Nitrate	15,000		
Hydrazine	1000	0·2	21·4
Morpholine	10,000	0·1	20·8
Cyclohexylamine	10,000	0·2	21·0
Octadecylamine	1000	−0·3	21·1
Fulvic acids†	5000	2·0	22·1
Detergents‡	6000	0·5	12·3
Detergents‡	1200	0·2	18·8
Detergents‡	300	0·0	19·9

* The ranges of recoveries expected assuming no interference from impurities were calculated from the results given in Table IX; these ranges were (95 per cent. confidence limits)—

$0·0 \pm 0·4$ when 0·0 μg of iron per litre was added;
$20·7 \pm 0·8_5$ when 20·7 μg of iron per litre was added.

† Prepared as described previously.

‡ Omo, Daz, Surf, Dreft, Blue Tide and Quix (1000 μg of each per litre) were used.

effect was found when aluminium, nickel or copper were used in place of chromium. If large concentrations of chromic ions are likely to be present in samples, care should be taken not to add an excess of ammonia solution.

Discussion of the method

Calibration curve—

The results given in Table IX show that the calibration curve was linear (within ±2 per cent.) for concentrations between 10 to 15 μg of iron per litre. The average optical density obtained for solutions containing 2 μg of iron per litre was significantly greater than expected from the other solutions. However, this deviation was equivalent to only 0·2 μg of iron per litre, and was not further investigated.

A few experiments indicated that at least 98 per cent. of the iron in solutions containing 100 μg per litre was extracted. Thus, the range of the method may probably be extended if necessary.

Precision and sensitivity—

Separate determinations were made of the standard deviations of optical-density measurements and volumetric measurements. From these estimates, the "within-batch" standard deviation expected for analytical results from measurement errors alone was calculated to be 0·12 μg per litre, and was independent of the concentration of iron in the sample. This calculated value is less than all the values found experimentally (see Table IX). It appears, therefore, that the analytical technique introduces more variations. No attempt was made to define the source of these variations more closely, since the precision was adequate. However, contamination from apparatus and the general surroundings is considered the most likely source of "within-batch" variations.

The significant "between-batch" variations found at 50 μg of iron per litre were probably caused by a tendency for the extraction of iron to be slightly incomplete. The size of these variations was, however, small; the standard deviation was equivalent to only 0·6 per cent. of the iron concentration.

Although no specific investigation was made of means of increasing the sensitivity of this technique, the results indicate that it should be easily possible to extract iron from much larger volumes of solution. The results in Table VIII indicate that 400-ml portions of sample could be used instead of 200-ml portions. However, although the sensitivity could probably be improved, it does not necessarily follow that the precision of results would also be improved.

Criterion of detection—

Table IX shows that the "within-batch" standard deviation of the reagent blank value was 0·14 μg per litre. The criterion of detection (for 95 per cent. confidence limits), defined by Roos[14] as 2·326 times the standard deviation, is thus approximately 0·35 μg per litre.

Accuracy—

Table XI shows that few of the other impurities tested significantly affected results. The concentrations of those impurities that did cause significant effects were much greater than those expected to occur in feed-water, and it was concluded that the method was sufficiently specific for analysing feed-water. Markedly low results were caused by some detergents, and this effect could be extremely serious in waters containing sufficiently large concentrations of these detergents; for such water further investigation of the effect is desirable. The interference of fulvic acids probably arises from their solubility and colour in isopentanol; the effect could probably be eliminated by extracting the acidified sample with isopentanol before any other reagents are added. Table X also indicates satisfactory accuracy and precision when a sample of feed-water was analysed.

Speed of analysis—

The method has been found to be convenient to use, and 10 samples may be analysed in about 100 minutes. One sample could be analysed in about 10 to 15 minutes at the expense of a small loss of precision.

Scope and application of the method—

Investigations by many workers and myself have shown that feed-waters may contain forms of iron that are not determined by the proposed method. It is probably impossible to define these forms unambiguously except by referring to the method of sampling and

analysis. Thus, the concentration found by the proposed method may be termed the "reactive" iron content of the sample. Clearly, this method should be used only when the "reactive" iron content is of interest. However, the method may also be used after the sample has been treated in some way in order to convert "non-reactive" forms of iron to "reactive" forms. An experimental investigation of methods of achieving this "activation" is described in Part II,[12] and the development of a method for determining the total iron content of feed-water is described in Part III.[15] When the "reactive" iron content of a sample is required, it should be easily possible to arrange conditions so that the final pH of the solution is obtained reproducibly without the need for an internal pH indicator. To do this, the method could be slightly simplified by omitting the *m*-cresol purple indicator.

This Paper is published by permission of the Central Electricity Generating Board. I thank Mrs. M. I. Ford who made most of the tests described in this Paper, and Mr. E. Bowler for providing the sample of feed-water.

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NOTE—References 12 and 15 are to Parts II and III of this series, respectively.

Received *December 23rd*, 1963

The Absorptiometric Determination of Iron in Boiler Feed-water

Part II.* Methods for Dissolving "Non-reactive"† Iron

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Boiler feed-water may contain "non-reactive" iron, and methods for converting this to a "reactive" form are reviewed. The efficiency of several methods has been investigated; of these, heating samples with thioglycollic acid is considered most suitable.

The method described in Part I of this Paper[1] is suitable for determining "reactive" iron in feed-water. However, many workers[2 to 7] have reported that feed-water may contain forms of iron that require some special treatment for dissolving them before they can be determined. Similar observations have been made in this laboratory and others of the Central Electricity Generating Board. The total iron content of feed-water is often of interest, and therefore a method was required for converting "non-reactive" to "reactive" forms of iron.

Little investigation of the nature of "non-reactive" iron appears to have been made. The only specific investigation of this point known to me is that of Bullen and Jeffery.[8] They examined material filtered from condensate and feed-water at one power station by optical and X-ray techniques. Quantitative analyses showed that this material contained approximately 20, 10 and 50 per cent. of Fe_3O_4, α-Fe_2O_3, and γ-FeO.OH, respectively; no other iron compounds were reported. Kirsch[9] has also discussed in detail the forms in which iron may exist in feed-water. It seems probable that "reactive" iron consists of ferrous ions and particulate and colloidal oxides and hydroxides that are converted to simple ionic species during sampling and analysis. "Non-reactive" iron in feed-water probably consists mainly of less readily soluble forms of oxides and hydroxides.

The dissolution of "non-reactive" iron is probably not in itself a difficult problem. For example, evaporation of the sample to dryness, and fusion of the residue with potassium hydrogen sulphate would almost certainly convert any type of "non-reactive" iron to a "reactive" form. However, practical difficulties arise with such a technique because of the small concentration of iron in feed-water. Thus, a large volume of sample may be required to achieve adequate precision; the time for evaporation is therefore long and this is inconvenient. Further, a lengthy evaporation increases the likelihood of variable contamination from the environment and apparatus. The environment of power-station laboratories is often such that contamination is likely to be a major source of error. Another difficulty is that increasing severity of the dissolution technique, *e.g.*, fusion or evaporation with acid, may lead to greater and more variable contamination of the sample by iron leached from the apparatus. Therefore, to minimise both random errors and the time required for analysis, it is desirable to use the most simple and rapid technique possible, consistent with obtaining efficient dissolution of "non-reactive" iron. For plant-control purposes, and if the concentration of iron is only a few micrograms per litre, it may even be preferable to use a method that dissolves only, say, 90 per cent. of the "non-reactive" iron, if small random errors can thereby be obtained. The relative efficiencies of different methods of dissolution are therefore of interest.

Many different methods for dissolving "non-reactive" iron have been reported. With one exception, all the methods rely on the action of mineral acids; they differ in details such as the nature and amount of the acid used, and the duration for, and temperature at, which the samples are heated. A summary of these methods of dissolution is given in Table I.

Table I shows that since the work of Ristroph and Yorkgitis,[2] there has been a tendency to use more vigorous acid-digestion techniques. However, I have been unable to find any published information on the relative efficiencies of these techniques. Only Klump and

* For details of Parts I and III of this series, see reference list, p. 410.

† The term "non-reactive" iron is used to denote those forms of iron in feed-water that are not determined by the method described in Part I.

Busch[14] have quoted results indicating the accuracy of a technique. They analysed a deposit (21 per cent. of iron) from a boiler-drum and an oxide layer (55 per cent. of iron) from a boiler-tube by two methods; these were (*a*) fusion and then a gravimetric determination, and (*b*) their proposed method. The latter method gave results 3 per cent. and 0·2 per cent. lower than those obtained gravimetrically.

TABLE I

METHODS FOR DISSOLVING "NON-REACTIVE" IRON IN FEED-WATER

Authors	Acidity to which sample is adjusted	Treatment of sample after addition of acid
Ristroph and Yorkgitis[2]	0·02 N, with hydrochloric acid	Heat the solution for 30 minutes at 60° C
Levendusky and Megahan[10] ..	0·15 N, with hydrochloric acid	Boil the solution gently for 15 minutes
A.S.T.M.,[11] British Standard[12] ..	N, with hydrochloric acid	Heat the solution for 1 hour at 60° C with hydroxylammonium chloride
Herre[6]	0·35 N, with sulphuric acid	Evaporate the solution nearly to dryness in presence of hydrogen peroxide
Kostrikin, Kalinina and Dzysyuk[7] ..	0·2 to 0·3 N, with hydrochloric acid	Evaporate the solution until only a few millilitres of sample remain
Vereinigung der Grosskesselbesitzer[13]	0·6 N, with hydrochloric acid	Distil the solution until a few millilitres of sample remain, add distillate to sample and repeat distillation; add distillate to sample
Klump and Busch[14]	2 per cent v/v with thioglycollic acid	Heat the solution for 30 minutes at approximately 90° C

Because of the lack of experimental results, the efficiencies of some of these methods of dissolution were investigated. The results obtained are described in Part II of this Paper.

EXPERIMENTAL

REAGENTS, APPARATUS AND TECHNIQUE—

The reagents used were as described in Part I of this Paper, except that the ammonia solution was purified by isopiestic distillation. For this, 400 ml of analytical-reagent grade ammonia solution, sp.gr. 0·88, were placed in the bottom of a desiccator, and 400 ml of water in a polythene dish were supported above the ammonia solution. The lid of the desiccator was replaced, and equilibrium was attained in 1 to 2 weeks, by which time the concentration of ammonia in the original water was approximately 9 N. The water used for reagent blank solutions was distilled water that had been re-distilled in an all-glass apparatus.

When different methods of dissolution were investigated, the dissolution was always done in Pyrex-glass apparatus except where stated. All other apparatus was as described in Part I. The Millipore filters were obtained from Thermal Control Ltd.

All samples of feed-water were collected in polythene bottles containing sufficient re-distilled hydrochloric acid to make the final acidity 0·1 N. Before any samples were withdrawn for analysis, the sample containers were shaken as vigorously as possible for approximately 1 minute initially, and for subsequent samples for approximately 10 seconds. The iron contents of solutions after different treatments were determined as described in Part I, except in some tests when the method of dissolution recommended by Klump and Busch[14] was used. All other techniques were as described in Part I except where stated. During the work, the temperature of the laboratory varied between 20° and 24° C.

Several different methods of dissolution were chosen for study, but before any samples of feed-water were analysed, the precision attainable with each technique was usually estimated by analysing portions of doubly distilled water. The precision attained in the first tests of each technique worsened as the acidity of the solution and the temperature and duration of the dissolution stage were increased. An important source of error was the leaching of variable amounts of iron from the glassware by the solution. Repeated analyses in the same glass flasks reduced the size of this contamination and of the standard deviation of results. Therefore batches of reagent blank solutions were usually analysed until the average optical density and standard deviation of results showed no systematic tendency to decrease. Precautions were also taken to reduce the size of air-borne contamination during the dissolution stage.

When results on reagent blank solutions were adequately precise, the recovery of known amounts of $iron^{III}$ added to reagent blank solutions was also checked for most methods of dissolution. The precisions and recoveries obtained are summarised under "Methods of Dissolution Investigated."

METHODS OF DISSOLUTION INVESTIGATED—

Method (*i*)—A 203-ml portion of the acidified sample of feed-water was placed in a 500-ml conical flask, and 2 ml of 10 per cent. hydroxylammonium chloride solution were added. The flask was warmed on a boiling-water bath for 30 minutes, and the contents were then cooled and analysed as in Part I, except that no further hydroxylammonium chloride was added.

For this and methods (*ii*), (*iii*) and (*iv*), the necks of the flasks were standard glass sockets. After the sample and reagents had been placed in the flask, a glass cone that carried an inverted U-tube was fitted in the neck of the flask. The aim of this device was to reduce air-borne contamination while steam and acid vapours were permitted to escape from the flask. The solutions in this method and methods (*ii*) and (*iii*) reached 60° C in about 10 minutes, and the temperatures after 30 and 60 minutes were about 85° C.

With this technique, the within-batch standard deviation for reagent blank solutions was approximately 0·3 μg of iron per litre of sample, and the recovery of 5 μg of iron was 100·0 $\pm$ 2 per cent. (95 per cent. confidence limits).

Method (*ii*)—This method was the same as method (*i*), except that before the solution was heated sufficient re-distilled hydrochloric acid was added to the sample in the flask to make the final acidity 0·5 N. The precision was the same as for method (*i*); the recovery of added iron was not checked.

Method (*iii*)—A 203-ml portion of the acidified sample was placed in a flask, sufficient re-distilled hydrochloric acid was added to make the final acidity N, and 8 ml of 10 per cent. hydroxylammonium chloride solution were added. The flask was then heated for 1 hour on a boiling-water bath, cooled and analysed as in Part I. The concentrations of hydrochloric acid and hydroxylammonium chloride are the same as those recommended by the American Society for Testing Materials[11] and the British Standards Institution.[12] The within-batch standard deviation for reagent blank solutions was approximately 1 μg of iron per litre of sample; the recovery of added iron was not checked.

Method (*iv*)—A 203-ml portion of the acidified sample was placed in a flask, and sufficient re-distilled hydrochloric acid was added to give the desired final acidity (0·1, 0·3, 0·5 or 1·0 N). The solution was then boiled on a hot-plate covered with asbestos paper until its volume was 5 to 10 ml. The flask was cooled, and its contents transferred quantitatively to a separating funnel, with sufficient water to make the final volume 200 $\pm$ 10 ml. The solution was then analysed as in Part I.

The within-batch standard deviations for reagent blank solutions were approximately 0·3, 0·4, 0·5 and 0·6 μg of iron per litre of sample for final acidities of 0·1, 0·3, 0·5 and 1·0 N, respectively. The recoveries of 5 μg of iron were 99·8 $\pm$ 2·4, 101·0 $\pm$ 3·6, and 101·8 $\pm$ 4·8 per cent. for final acidities of 0·3, 0·5 and 1·0 N, respectively.

For a few tests, the procedure was slightly modified by using 609-ml portions of samples, and 1-litre conical flasks were then used for the evaporation stage. The amount of acid was the same as for 203-ml samples, so that the initial acidity was reduced by a factor of 3. This modification did not significantly affect the precision of the results or the recovery of added iron.

Method (*v*)—One litre of the acidified sample was passed through a Millipore filter (pore size 0·45 μ), and the filtrate was collected and stored in a polythene bottle. The filter was held on a sintered-glass disc. The filter was then placed in a 100-ml beaker, and 0·5 ml of sulphuric acid were added dropwise on to the disc. Ten millilitres of re-distilled hydrochloric acid were then added, and the beaker was heated on a boiling-water bath for 30 minutes. The beaker was covered with a watch-glass whenever possible. After the heating period, the beaker was cooled, and its contents were added to the original filtrate from the sample; the beaker was washed twice with 5-ml portions of water, and the washings were also added to the filtrate. Finally, 207 ml of the combined filtrate were analysed as in Part I.

With this technique, the within-batch standard deviation for reagent blank solutions was approximately 0·4 μg of iron per litre of sample; the recovery of 5 μg of iron (added to the beaker before the filter) was 96·6 $\pm$ 3·2 per cent.

Method (vi)—This method was essentially that described by Klump and Busch.[14] However, slight modifications were necessary because the samples of feed-water contained hydrochloric acid (0·1 N). Thus, 91·5 ml of sample were placed in a calibrated flask, sufficient re-distilled ammonia solution was added to increase the pH to 9 ± 0·5, and 1·6 ml of thioglycollic acid were added. The amount of ammonia solution required was established by tests on a separate portion of sample. The flask was then placed in boiling water for 30 minutes, and allowed to cool in running tap water. Finally, 4 ml of analytical-reagent grade ammonia solution, sp.gr. 0·88, were added, the contents of the flask diluted to the mark with water, and the optical density of the solution was measured at 530 mμ in 10-cm cuvettes with a Uvispek spectrophotometer against a reference cuvette filled with water. Reagent blank solutions and standard iron solutions were analysed in the same way except that 90 ml of water or standard iron solution and 1·5 ml of re-distilled hydrochloric acid were used in place of the sample.

Ten micrograms of iron in the sample taken for analysis gave an optical density of 0·073. The within-batch standard deviation for reagent blank solutions was approximately 1·7 μg of iron per litre of sample; no specific recovery checks were made, since the standard solutions used for calibration purposes showed that the calibration curve was linear.

A few tests were also made in which the method of dissolution was carried out as above, except that 203-ml portions of sample were used; the amounts of all other reagents were increased proportionally. When the solutions had cooled, no additional ammonia solution was added, and the solutions were analysed by the method described in Part I. Reagent blank and standard iron solutions were analysed at the same time as the samples. No preliminary checks of precision were made, but the recovery of iron from the standard solution was 101·5 per cent. (probable 95 per cent. confidence limits estimated to be approximately ±3 per cent.).

Results

Comparison of methods (*i*) to (*v*)—

For these tests, portions of several samples were analysed after preliminary treatment by methods (*i*) to (*v*) as described above. In each batch of analyses, equal numbers of reagent blank solutions and samples were analysed. The same batch of re-distilled water was used for all reagent blank solutions, but no correction for the iron content of this water was made to results. This water contained 0·1 μg of "reactive" iron per litre. If only "reactive" iron were present, the results obtained with the different methods of dissolution would not be biassed with respect to each other. If "non-reactive" iron were present in the water, the most efficient methods of dissolution would tend to give falsely low results relative to the less efficient methods, but the size of this error cannot exceed the "non-reactive" iron content of the water. Subsequent work (see Part III[15]) indicated that the water contained a total of 0·3 to 0·5 μg of iron per litre. The mean results for each sample by each method are given in Table II together with the 95 per cent. confidence limits for the mean. These limits were calculated conventionally from the individual results for each sample by each method, and allowance was made for the variability of reagent blank determinations.

TABLE II

COMPARISON OF METHODS OF DISSOLUTION (*i*) TO (*v*)

Method used for dissolution	Average concentration of iron,* μg per litre—			
	Sample A1	Sample A2	Sample B1	Sample B2
None	2·3 ± 0·15 (8)	2·9 ± 0·17 (4)	4·8 ± 0·15 (8)	2·3 ± 0·19 (4)
Method (*i*)	2·4 ± 0·54 (8)	2·8 ± 0·44 (4)	5·4 ± 0·77 (4)	2·6 ± 0·60 (4)
Method (*ii*)	2·7 ± 0·39 (4)	—	6·8 ± 0·44 (4)	—
Method (*iii*)	2·7 ± 1·7 (4)	3·2 ± 1·4 (4)	4·9 ± 1·9 (4)	4·6 ± 0·92 (4)
Method (*iv*)†	3·9 ± 0·73 (8)	5·8 ± 0·57 (4)	9·5 ± 0·70 (8)	5·9 ± 0·76 (4)
Method (*v*)	—	2·4 ± 0·65 (6)	—	3·1 ± 0·77 (6)

* The limits quoted are for 95 per cent. confidence limits, and the numbers in brackets refer to the number of determinations (of samples and reagent blank solutions) upon which each mean is based.

† The acidity of samples was adjusted to 0·3 N before they were evaporated.

Table II shows that the apparent iron concentration of all samples was greatest when method (*iv*) was used. Therefore, methods (*i*), (*ii*), (*iii*) and (*v*) were not further considered, and the effect of experimental conditions in method (*iv*) was studied.

In all Tables, except Table I, samples are designated with a letter and a number; each letter is reserved for a particular power station, and each number refers to a different sample.

EFFECT OF ACIDITY AND EXTENT OF EVAPORATION IN METHOD (*iv*)—

For these tests, portions of samples and reagent blank solutions were analysed in equal numbers by using method (*iv*). However, the initial acidity of, and volume to which, solutions were evaporated were varied. One batch of water was used for all reagent blank solutions, but no correction for its iron content was made to results for samples. The mean results are given in Table III, and the 95 per cent. confidence limits were calculated as described in the previous section.

TABLE III

EFFECT OF ACIDITY AND EVAPORATION TIME IN METHOD (*iv*)

Initial concentration of hydrochloric acid, normality	Average concentration of iron,* μg per litre—			
	Sample A3	Sample B3	Sample C1	Sample D1
Solutions evaporated to 100 ml				
0·1	3·3 ± 0·58 (6)	—	—	—
0·5	3·6 ± 0·56 (6)	—	—	—
Solutions evaporated to 5 to 10 ml				
0·1	4·0 ± 0·58 (6)	4·1 ± 1·0 (4)	31·1 ± 1·6 (3)	—
0·5	4·0 ± 0·47 (6)	3·9 ± 0·46 (9)	34·0 ± 1·5 (3)	56·2 ± 1·5 (3)
1·0	3·9 ± 0·89 (6)	4·7 ± 0·67 (9)	34·1 ± 1·5 (3)	57·4 ± 1·5 (3)
Solutions not heated, but analysed as in Part I[1]	2·3 ± 0·65 (4)	1·9 ± 0·35 (4)	30·2 ± 0·38 (3)	—

* The limits quoted are for 95 per cent. confidence limits, and the numbers in brackets refer to the number of determinations (of samples and reagent blank solutions) upon which each mean is based.

The results given in Table III show that evaporation of samples to a volume of 100 ml gave lower results than when the evaporation was continued until only 5 to 10 ml remained. Accordingly, the less prolonged evaporation was not further considered. In general, when the evaporation was continued until the volume was 5 to 10 ml, the initial acidity appeared not to be critical. However, the results obtained by using an initial acidity of N were significantly greater than those obtained by using an initial acidity of 0·5 N for sample B3, and it was therefore decided that the best method of those studied for converting "non-reactive" to "reactive" forms of iron was method (*iv*), with an initial concentration of hydrochloric acid of N.

During these tests, the standard deviation of results on samples of feed-water was significantly greater than for standard solutions of iron (containing $iron^{III}$ in ionic form). It was thought that this effect was probably caused by particulate forms of iron in the samples of feed-water. Such forms would in general lead to sampling errors when portions of feed-water were removed from the sample container. To test this theory, two samples were filtered through a Millipore filter (pore size 0·45 μ), and portions of the filtered and unfiltered samples were then analysed by using method (*iv*), with an initial acidity of N. As a further check on the nature of the iron in the filtrates, the latter were analysed by the method given in Part I, and also by using method (*i*) described above. In each batch of analyses, equal numbers of feed-water and reagent blank solutions were analysed. During these tests, additional analyses of standard ("reactive") iron solutions were made as a check on precision. One batch of water was used for all tests, but no correction for its iron content was made to the results. The mean results are given in Table IV and the 95 per cent. confidence limits were calculated as before.

The results in Table IV illustrate several points. Firstly, the results for the standard iron solutions show how the precision is adversely affected when method (*iv*) is used as a preliminary treatment. Secondly, the precision of analysing the two unfiltered samples of feed-water by method (*iv*) was worse than for standard iron solutions. Thirdly, the precision was improved (by a factor of approximately 1·8) when the filtered samples were analysed, and

TABLE IV

EFFECT OF FILTERING SAMPLES ON PRECISION

Method of analysis	Average concentration of iron,* μg per litre— Feed-water: Sample A4	Feed-water: Sample A5	Concentration of standard solutions of iron, μg per litre—: 10	50
Unfiltered samples				
As in Part I	8·0 ± 0·38 (4)	12·1 ± 0·19 (4)	10·0 ± 0·10 (15)	50·1 ± 0·14 (15)
Method (*iv*)†	30·4 ± 0·78 (20) 11·8 ± 1·0 ‡(5)	27·1 ± 1·8 (5)	9·9 ± 0·34 (15)	50·1 ± 0·32 (15)
Filtered samples				
As in Part I	8·3 ± 0·21 (4)	12·0 ± 0·28 (4)	—	—
Method (*i*)	—	12·6 ± 0·35 (4)	—	—
Method (*iv*)†	11·8 ± 0·46 (20)	12·8 ± 1·0 (4)	—	—

* The limits quoted are for 95 per cent. confidence limits, and the numbers in brackets refer to the number of determinations (of samples and reagent blanks) upon which each mean is based.

† The acidity of sample was adjusted to N before evaporation.

‡ The sample-container was set aside overnight before withdrawal of the samples, which was achieved with the minimum disturbance to the contents.

TABLE V

COMPARISON OF METHODS OF DISSOLUTION (*iv*) AND (*vi*)

Method of analysis	Average concentration of iron,* μg per litre— Sample A6	Sample A7	Sample A8
Method (*iv*)†	19·6 ± 0·90‡ (8)	46·7 ± 0·50‡ (10)	27·1 ± 0·70 (12)
Method (*vi*)— Measuring iron^{II} - thioglycollate complex	22·8 ± 3·4 (4)	47·2 ± 2·1 (4)	—
Measuring iron^{II} - bathophenanthroline complex	20·3 ± 1·7 (3)	—	28·1 ± 3·2 (3)

* The limits quoted are for 95 per cent. confidence limits, and the numbers in brackets refer to the number of determinations (of samples and reagent blank solutions) upon which each mean is based.

† 40 ml of redistilled hydrochloric acid were added to all samples before evaporation, *i.e.*, sufficient to make the final acidity N when 203-ml samples were taken for analysis.

‡ 609-ml samples were taken for analysis.

a similar improvement was observed when portions of sample A4 that had been set aside overnight were analysed. For these last analyses, care was taken to disturb the contents of the sample container as little as possible during withdrawal of samples. Lastly, this method of taking portions of the sample gave the same results as the corresponding filtered sample. It seems clear, therefore, that (*a*) these two samples of feed-water contain particulate forms of iron of sufficient size that they cause sampling errors, and (*b*) these particles may settle out if the sample is set aside. It is of interest that "non-reactive" forms of iron passed through the Millipore filter with sample A4; this also occurred to a smaller extent with sample A5. However, the results for sample A5 indicate that these "non-reactive" forms are readily dissolved, since the relatively mild treatment of method (*i*) gave the same results, within experimental error, as method (*iv*). These results indicate that it would be practicable to devise a method in which a large volume of sample was passed through a Millipore filter, the filtrate analysed, and the residue on the filter attacked (*e.g.*, by a fusion technique) so that all iron was converted to a "reactive" form. Such a method would have the advantage that sampling errors due to non-homogeneous distribution of particles could be reduced.

COMPARISON OF METHODS (*iv*) AND (*vi*)—

The same general experimental design was used as in the other previous comparisons. Again, one batch of re-distilled water was used for all reagent blank solutions, but no correction for its iron content was made to results. The mean results are given in Table V, and the 95 per cent. confidence limits were calculated as before. The Table shows that the results obtained with methods (*iv*) and (*vi*) were not significantly different. The average ratio of results from method (*vi*) to those from method (*iv*) was 1·06 ± 0·19 (where the limits are for 95 per cent. confidence).

EFFICIENCY OF METHOD (*vi*) FOR DISSOLVING IRON OXIDES—

The results in the previous section indicated that either method (*iv*) or method (*vi*) was suitable for dissolving "non-reactive" iron in feed-water; of these two methods, method (*vi*) was preferred (see under "Discussion," p. 409). Therefore, further tests of the efficiency of this method were made by analysing 200-ml portions of water, to which were added known amounts of either ignited ferric oxide or precipitated ferroso-ferric oxide. The oxides were weighed into a small indentation on a thin, glass microscope slide, and the slide was then added to a flask containing 200 ml of water; experiments showed that negligible amounts of iron were leached from the slide during the subsequent treatment with thioglycollic acid. The weight of oxide taken for each determination was about 2·5 mg; tests showed that the standard deviation of the weighing operation was about 0·025 mg. The iron contents of the two oxides were determined volumetrically (by using the Zimmermann - Rheinhardt method) after both fusion with potassium pyrosulphate and dissolution in hydrochloric acid.

After the treatment with thioglycollic acid, the solutions were cooled, and diluted with water to the mark in 250-ml calibrated flasks. Portions (2 ml) of these solutions were added to separating funnels containing 198 ml of water, and the resulting solutions were analysed by the method given in Part I. In each batch of analyses, 200-ml portions of water were analysed as reagent blank determinations; a clean glass slide was added to each of these solutions. When portions of a standard iron solution containing 2·5 mg of iron were diluted to 200 ml, and analysed in this way, the recovery of iron was 100·7 ± 1·1 per cent. (95 per cent. confidence limits). By using this technique, the effect of (*a*) variations in the amount of thioglycollic acid added to samples and (*b*) the duration of the heating period were investigated. For these tests the temperature of the water in which the flasks were immersed was 95° C, and the results are given in Table VI.

TABLE VI

EFFICIENCY OF METHOD (*vi*) FOR DISSOLVING IRON OXIDES

Oxide	Volume of thioglycollic acid added, ml	Heating, time, minutes	Recovery of added iron, per cent.	Mean* recovery, per cent.
Fe_2O_3	1·0	30	91·0, 87·5	89·3
		60	95·0, 101·7	98·4
	2·0	30	92·6, 91·7	92·2
		60	98·2, 100·0	99·1
	3·5	30	95·7, 96·7, 95·5, 98·1	96·5
	5·0	30	91·9, 96·1	94·0
		60	99·8, 94·7	97·3
Fe_3O_4	1·0	30	94·8, 95·7	95·3
		60	105·3, 96·7	101·0
	2·0	30	100·8, 94·0	97·4
		60	100·8, 98·8	99·8
	3·5	30	102·5, 92·8, 98·9, 90·4	96·2
	5·0	30	106·8, 109·4	108·1
		60	95·5, 104·2	99·9
			Mean	97·5

* The 95 per cent. confidence limits (calculated from the results in the Table) are ±5·5 and 3·9 per cent. for the means of duplicate and quadruplicate analyses, respectively.

Other tests were made as above with 2 ml of thioglycollic acid and heating periods of 70 minutes, but the temperature of the water in which the flasks were immersed was decreased to 70° and 50° C. At the latter temperature, visible amounts of the oxides remained after

the heating period, and the determinations were not continued. At 70° C, the mean recoveries from quadruplicate determinations with both ferric and ferroso-ferric oxides were 95·3 and 103·2 per cent., respectively.

Discussion

Comparison of methods of dissolution (*iv*) and (*vi*)—

One limitation of the approach adopted in this investigation is that the nature of "non-reactive" iron may vary from one power station to another, and also from time to time within one station. Thus, a method of dissolution suitable for one sample may not be suitable for another. Further, the fact that two methods of dissolution give the same results, does not necessarily imply that both are dissolving all the "non-reactive" iron. However, any method that gives significantly lower results than another is clearly unsuitable for general use.

On this basis, the results in Table II show that of the methods in which samples are treated with hydrochloric acid, only those in which the acidified sample was evaporated to a small volume were worth further consideration. Table III indicates that the acidity of the sample should be adjusted to N with hydrochloric acid before the evaporation is commenced. The need for evaporation seems also to have been appreciated recently in Russia[7] and Germany[13] as two of the most recent methods of dissolution recommend evaporation of acidified samples to small volume. Kostrikin, Kalinina and Dzysyuk[7] use an initial acidity of 0·2 to 0·3 N, whereas Vereinigung der Grosskesselbesitzer[13] recommend a value of 0·6 N, both to be achieved with hydrochloric acid.

The results in Table V show that digestion of samples with thioglycollic acid as recommended by Klump and Busch[14] gave slightly, but not significantly, greater results than the acid-evaporation technique. Thus, on grounds of efficiency of dissolution of "non-reactive" iron, both methods appear to have equal merit. The technique of using thioglycollic acid was considered more suitable for general use. The reasons for this choice are discussed briefly in the paragraphs below, because the points are relevant to the determination of total iron no matter what method of dissolution is used.

Firstly, the effect of particulate "non-reactive" iron in samples must be considered; the results in Table IV show that a large proportion of the total iron may be particulate. This immediately raises the question of sampling accuracy. For example, sampling accuracy will be poor if analyses are made on 100-ml portions of a sample containing a few particles per litre. The magnitude of errors from this source depends on the size, number and iron content of individual particles in the sample, and also on the volume of sample taken for analysis. Estimates of the errors may be calculated on the basis of the binomial or Poisson distribution, and the coefficient of variation decreases as the volume of sample analysed increases. It is desirable, therefore, to be able to use large volumes of samples in the dissolution stage, if the precision of results indicates that this is necessary. The method of Klump and Busch[14] has the advantage over the evaporation technique that the heating period is essentially independent of the volume of sample; with the evaporation technique, the heating period is almost directly proportional to the volume of the sample.

Secondly, the vessel in which the evaporation is carried out must be open to the atmosphere to allow steam and acid vapours to escape; there is therefore the possibility of contamination from air-borne materials. Although the likelihood of this may be decreased, as shown by Vereinigung der Grosskesselbesitzer,[13] or by the results obtained with method (*i*) in this Part, it seems unlikely that it can be eliminated. However, during digestion with thioglycollic acid, the vessel can be effectively sealed against such contamination. This point would be of particular importance if large volumes of acidified samples had to be evaporated on hot-plates.

Thirdly, for sample volumes sufficiently large to give the required precision (several hundred millilitres), heating with thioglycollic acid is quicker than the evaporation technique. The greater simplicity and rapidity of the former technique also make it more amenable to automation than the evaporation technique.

Fourthly, considerable difficulty was experienced in adequately cleaning the Pyrex-glass flasks and other apparatus in which the evaporations were carried out. Prolonged boiling with hydrochloric acid, *i.e.*, for several days, was found to be necessary before the optical density and precision of reagent blank determinations decreased to stable values. Preliminary results, obtained when polythene bottles were used for the dissolution stage with thioglycollic acid, indicated that the bottles could be simply and quickly cleaned.

Lastly, with the evaporation technique there is the possibility that particles will deposit on the walls of the flask during evaporation. Thus, as evaporation proceeds such particles may remain in a "non-reactive" form, thus causing a low result for that sample, and possibly a high result for the next. This is not possible when thioglycollic acid is used as particles are immersed in the solution throughout the entire treatment.

The fact that the results obtained with methods of dissolution as dissimilar as methods (*iv*) and (*vi*) were not significantly different, indicates, but does not prove, that both are satisfactory for dissolving "non-reactive" iron. However, the tests made with known amounts of iron oxides were an attempt to establish the absolute accuracy of the method of digestion with thioglycollic acid. From the work of Bullen and Jeffery[8] and the discussion of Kirsch,[9] it seems likely that the least soluble forms of "non-reactive" iron are ferric and ferroso-ferric oxides. These oxides were, therefore, used in the experimental investigation. It is possible that other compounds of iron, that are less easily dissolved than these two oxides, may be present. For example, the solubilities of complex iron silicates and substituted spinels ($CuFe_2O_4$, $ZnFe_2O_4$), etc., have not yet been investigated. Nevertheless, it is thought that such materials are only likely to be present in minor amounts, if at all, in feed-waters of modern high-pressure boilers.

The tests made with iron oxides probably represent a rather extreme test of the efficiency of the method of dissolution. Thus, the amounts of oxides added corresponded to concentrations of about 9000 μg of iron per litre of sample, the particle sizes of the oxides were probably much larger than those present in feed-waters, and, for ferric oxide, the oxide had been ignited. Nevertheless, Table VI shows that when a heating period of 60 minutes was used, the average recoveries were 98·4 $\pm$ 3·0 and 100·2 $\pm$ 4·2 per cent. (95 per cent. confidence limits) for ferric and ferroso-ferric oxides, respectively. These results show that the technique is unlikely to have a negative bias of more than 5 per cent., and this was considered adequate. The results in Table VI and those reported in the text also indicate that small variations in the concentration of thioglycollic acid, the duration of the heating period and the temperature of the sample did not cause any appreciable effects.

It is concluded that a satisfactory method for dissolving "non-reactive" iron in feed-water is to add sufficient thioglycollic acid to the sample to give a final concentration of 1 per cent. v/v, and then to heat the sample for 1 hour at a temperature of at least 80° C, and preferably 90° to 95° C.

Klump and Busch[14] determined the concentration of iron after the treatment with thioglycollic acid by measuring the optical density of the iron^{II} - thioglycollate complex. This method of determination was insufficiently precise, since the within-batch standard deviation of reagent blank determinations alone was approximately 1·7 μg of iron per litre. However, it seemed likely that the precision could be improved by using the method of determination given in Part I after the treatment with thioglycollic acid. The use of this combination of methods has been studied; the results obtained and a method for determining the total iron content of feed-water are given in Part III.[15]

This Paper is published by permission of the Central Electricity Generating Board. I also thank Mrs. M. I. Ford who performed most of the analyses reported in this Part.

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NOTE—References 1 and 15 are to Parts I and III of this series, respectively.

Received *December 23rd*, 1963

8-Mercaptoquinoline as a Gravimetric Reagent for the Determination of Palladium and Nickel*

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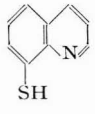
The investigation of 8-mercaptoquinoline as a gravimetric reagent for determining palladium and nickel is described. The sodium salt of 8-mercaptoquinoline is used because it is relatively stable towards aerial oxidation to the disulphide. The reagent is particularly suitable for determining palladium by precipitation from M hydrochloric acid as $Pd(C_9H_6NS)_2$. For the subsequent precipitation of nickel at pH 2 to 3 as $Ni(C_9H_6NS)_2$, the reagent is used in the presence of an excess of sulphur dioxide to prevent its aerial oxidation. After filtration the precipitates are washed with alcoholic wash-liquids to remove traces of absorbed reagent, and then they are dried at 120° C in air. Errors of less than ±0·2 per cent. are obtained in the analysis of standard solutions of palladium and nickel, separately and in mixtures of the two, up to ratios of about 1 to 10.

The selectivity of the reagent is described and some suggestions are made for masking interferences from other elements.

THE sulphur analogue of 8-hydroxyquinoline, 8-mercaptoquinoline—



has until recently received little attention as an analytical reagent. Its analytical applications, particularly as a colorimetric reagent, have been studied,[1 to 5] and the stability constants of some 1 + 1 bivalent metal complexes have been reported,[6] but no methods have been given for its use as a gravimetric reagent. This Paper describes an investigation of its use for the gravimetric determination of palladium and nickel. These metals were chosen to demonstrate the increased selectivity of 8-mercaptoquinoline, compared with 8-hydroxyquinoline, towards class (*b*) metals,[7] which tend to use the empty *d*-orbitals of the sulphur atoms to stabilise their complexes by back-donation of electrons.

METHOD

PREPARATION OF REAGENT—

Prepare 8-mercaptoquinoline from quinoline by the method described by Edinger.[8] The reagent is oxidised in air to the disulphide. For use as an analytical reagent, convert it to the sodium salt by mixing equimolar amounts of the red dihydrate and 50 per cent. aqueous sodium hydroxide solution. Recrystallise the crude product from ethanol - ether mixtures.

When stored in a well stoppered bottle, the sodium salt is stable towards air oxidation for at least several months. Prepare 1 per cent. solutions of the reagent in M hydrochloric acid as required, by filtering an aqueous solution of the sodium salt directly into hydrochloric acid. The reagent solution is stable for two or three days.

PROCEDURES—

Determination of palladium—Prepare a solution that contains between 2 and 100 mg of palladium^{II} per 100 ml and that is 1 to 1·5 M with respect to hydrochloric acid. Heat the solution to boiling, and add dropwise a 20 per cent. excess of the reagent solution. Set the solution aside to cool, with occasional stirring, to below 30° C, and then filter off the precipitate on a sintered-glass crucible of medium porosity. Wash the precipitate with about 100 ml of hot aqueous methanol (1 + 1) that has been acidified to about pH 1 with hydrochloric acid. Dry the precipitate at 120° C for 1 hour, and weigh it as bis-(8-mercaptoquinolinolato)-palladium^{II}, $Pd(C_9H_6NS)_2$, which contains 24·98 per cent. of palladium.

* Presented at a meeting of the Society, on Tuesday, April 9th, 1963.

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Determination of nickel—Prepare a solution that contains between 1 and 10 mg of nickel per 100 ml and that is slightly acid with respect to hydrochloric acid. Adjust the pH to between 2 and 3 with aqueous sodium acetate solution. Heat the solution to boiling, and add 10 ml of saturated sulphur dioxide solution. Add a 20 per cent. excess of the reagent dropwise to the hot solution, and cool rapidly to below 30° C with frequent stirring. Filter off the precipitate on a sintered-glass crucible of medium porosity. Wash the precipitate with about 50 ml of hot ethanol. Dry the precipitate at 120° C for 1 hour and weigh it as bis-(8-mercaptoquinolinolate)nickel^{II}, $Ni(C_9H_6NS)_2$, which contains 15·48 per cent. of nickel.

Palladium and nickel can be precipitated successively from the same solution. If this is done, palladium is precipitated, the excess of reagent is oxidised to the disulphide with hydrogen peroxide and the disulphide removed by filtration at pH 2 to 3 before nickel is determined.

Results and discussion

The oxidation of 8-mercaptoquinoline by air is the major difficulty in its use as a gravimetric reagent. Although the reaction—

$$2RSH \rightleftharpoons RSSR + 2H^+ + 2e$$

is dependent on the pH of the solution, the oxidation is appreciable even in M hydrochloric acid. Below pH 1 the disulphide is soluble, but at higher pH values it forms a fine, white precipitate. The reduction potential of the process was estimated to be about +0·3 volts, which is similar to that of the iodine - iodide system. For the determination of nickel at pH 2 to 3, the reagent was stabilised by adding a large excess of sulphur dioxide solution. These solutions are stable at the boiling-point and can be left overnight without formation of the disulphide.

It was observed that even in the presence of sulphur dioxide there was a gradual fading of the yellow colour of the excess of reagent in the supernatant solution after precipitation. The analysis of elements in the precipitates (see Table I), positive errors in the analysis of standard solutions (see Table II) and thermogravimetric evidence indicated that the excess of reagent gradually interacted with the precipitates. Various solvents were investigated to find liquids that could be used for washing the precipitates, and that would remove the adsorbed reagent preferentially from the precipitates after filtration. It was found that hot aqueous methanol (1 + 1), acidified to about pH 1 with hydrochloric acid, could be used for washing the precipitates of palladium, and hot ethanol for those of nickel. The volumes of the liquids used for washing should be between 50 and 100 ml.

Table I

Analysis of nickel and palladium complexes for elements

	Element found, per cent.				
	Nickel	Carbon	Hydrogen	Nitrogen	Sulphur
Required for Ni $(C_9H_6NS)_2$	15·5	57·04	3·2	7·4	16·9
Found in precipitates—					
washed with hot water	15·1	59·76	4·9	7·3	17·7
washed with hot ethanol	15·4	57·11	3·3	7·7	16·4
	Palladium	Carbon	Hydrogen	Nitrogen	Sulphur
Required for Pd $(C_9N_6NS)_2$	25·0	50·64	2·8	6·6	15·0
Found in precipitates—					
washed with hot water	—	50·15	2·8	6·1	15·9
washed with hot aqueous methanol (1 + 1), at pH 1	25·1	50·58	3·2	7·0	14·8

Analysis of standard solutions by these procedures gave errors of less than ±0·2 per cent. (see Table I). Similar errors were found for the determination of palladium and nickel by successive precipitation from the same solution, provided that the ratio of the metal concentrations did not exceed about 1 to 10. The improvement in the analysis of the elements in the precipitates (see Table I), reduction of errors in the analysis of standard solutions (see Table II) and thermogravimetric evidence confirmed that the excess of the absorbed material was removed preferentially by the liquids used for washing the precipitate.

Optimum conditions for the precipitation of the palladium and nickel complexes were investigated as a function of acidity (see Fig. 1), excess of reagent and metal-ion concentration. The last two variables are not critical. The wide difference in the acidity used for precipitating palladium and nickel, which is related to the difference in the stability of the complexes, demonstrated the marked selectivity of 8-mercaptoquinoline towards the class (*b*) metals. Attempts to determine the stability products of the 1,2-complexes by potentiometric titration in aqueous dioxan[9] were unsuccessful because of their extreme insolubility. However, comparison of the infrared spectra of cadmium, nickel and palladium complexes of 8-hydroxyquinoline[10] and 8-mercaptoquinoline showed peaks for the carbon - oxygen bond at 1105, 1115 and 1115 cm^{-1} and comparable peaks for the carbon - sulphur bond at 997, 1000 and 1015 cm^{-1}. The trends in these peaks indicate that the selectivity of 8-mercaptoquinoline is probably in the order palladium > nickel ~ cadmium, compared with the selectivity of 8-hydroxyquinoline, which is in the order palladium ~ nickel > cadmium.

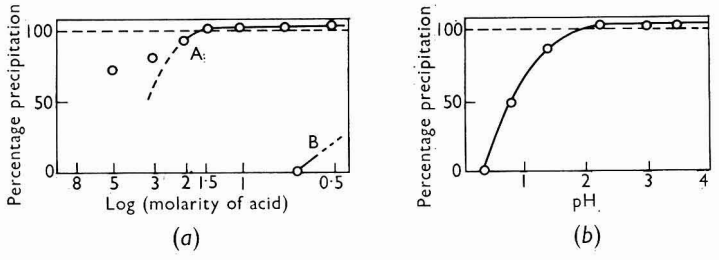

Fig. 1. Curves showing the effect of acidity on the precipitation of palladium and nickel complexes with a 10 per cent. excess of reagent. Fig. 1(*a*): curve A, palladium; curve B, nickel: Fig. 1(*b*), nickel

In strongly acidic solutions, above 2 M, palladium gives a pale-brown precipitate that is difficult to filter and decomposes on drying. This is probably a simple salt, which would account for the positive deviation from the dotted line shown in Fig. 1. In less acidic solutions, the pale-brown precipitate changes after digestion to the compact orange 1,2-complex that is easy to filter. The colour and ease of filtration of the nickel complex varies considerably. At low values of pH in the presence of a large excess of reagent, the material

TABLE II

RESULTS OF REPLICATE DETERMINATIONS OF NICKEL AND PALLADIUM

Metal	Liquid used for washing precipitate	Weight of metal found in precipitate, mg					True weight of metal, mg
Nickel	Hot water	27·54	27·47	27·44	27·55	27·54	27·20
Percentage error		*+1·2*	*+1·0*	*+0·9*	*+1·3*	*+1·2*	
Nickel	Hot ethanol	11·33	11·32	11·31	11·33	11·33	11·32
Percentage error		*+0·09*	*Nil*	*−0·09*	*+0·09*		
Palladium	Hot water	26·34	26·24	26·31	26·46	26·41	26·09
Percentage error		*+0·9*	*+0·6*	*+0·8*	*+1·4*	*+1·2*	
Palladium	Hot aqueous methanol at pH 1	26·13	26·10	26·11	26·12	—	26·09
Percentage error		*+0·15*	*+0·04*	*+0·08*	*+0·11*		

is compact and a bright crimson, but at higher values of pH with less reagent present, the precipitate is more voluminous and browner in colour. It was found that the nickel complex could be filtered more easily from solutions that had been cooled rapidly to below 30° C.

A qualitative study of the reactions of 8-mercaptoquinoline with several metal ions showed that many of the class (*b*) metals will interfere in the gravimetric determination of palladium and nickel, unless suitable masking agents are present.

The elements tested can be divided into three groups, as listed below—

(*i*) Those whose ions give precipitates with 8-mercaptoquinoline in M hydrochloric acid: copper^{I}, silver^{I}, gold^{I}, platinum^{IV}, rhenium^{III}, ruthenium^{III}, osmium^{III}, molybdate, mercury^{I}, mercury^{II}, tin^{II}, tin^{IV}, lead^{II}, lead^{IV}, antimony^{III}, bismuth^{III} and arsenite.

(*ii*) Those whose ions give precipitates with 8-mercaptoquinoline in the pH range 0·5 to 3: cobalt^{II}, iron^{II}, manganese^{II}, chromium^{III}, cadmium^{II}.

(*iii*) Those whose ions give no precipitate with 8-mercaptoquinoline below pH 3: vanadyl^{IV}, uranyl^{VI}, tungstate, titanium^{IV}, iron^{III}, aluminium^{III}, indium^{III}, magnesium^{II}, calcium^{II}, barium^{II}.

Bankovskiĭ *et al.*[1 to 5] have reported that thiourea forms stronger complexes than 8-mercaptoquinoline with the platinum metals, silver and gold, and has suggested its use as a masking agent. We have found that palladium precipitates as the 8-mercaptoquinoline complex in the presence of a hundred-fold excess of thiourea from M hydrochloric acid and that platinum does not precipitate under these conditions. It was also shown that interference from the other class (*b*) metals can be reduced with thiourea. Thus, in the presence of 8-mercaptoquinoline and thiourea no precipitates are obtained with gold^{I}, mercury^{I}, osmium^{III}, antimony^{III} and arsenite below pH 1 to 2, and none with silver^{I}, ruthenium^{III}, rhenium^{III}, bismuth^{III} and lead^{IV} below pH 2 to 4, and copper^{I} does not precpitate below pH 6 to 7.

It seems probable therefore that in the presence of thiourea as a masking agent, 8-mercaptoquinoline can be used as a selective reagent for determining palladium.

We are indebted to the Department of Scientific and Industrial Research for a Research Studentship (1959–61) and the Chelsea College of Science and Technology for a Research Assistantship (1961–62) for one of us (D.K.).

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Received *October 7th*, 1963

Differential Electrolytic Potentiometry

Part XIV.* An Examination of Electrode Parameters and Electrode Systems in Acid - Base Titrimetry

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The effects of variation of differentiating current density, ballast load, concentration of supporting electrolyte, atmosphere and the size and nature of the electrodes on the performance of differential electrolytic potentiometric titrations for different types of acid - base reactions are reported. The existence of the fine structure in the differential peaks for the antimony metal - metal oxide electrodes in oxygen-starved media is confirmed in titrations of highly diluted solutions at large current densities. Spontaneous differentiation is demonstrated for this class of reaction by using diverse electrode systems.

The performance of antimony electrodes in differential electrolytic potentiometric acid - base titrimetry in nitrogen atmospheres and their analytical accuracy and precision have been studied.[1] A theoretical interpretation of the current - temperature - potential response of twin polarised antimony electrodes in unpoised media of pH 7 under a low-oxygen atmosphere has been offered.[2] At other values of pH under the same conditions, the differential peak should split to give a trough with a minimum at the equivalence point.[3] A current-scan voltammetric technique was used for confirming this fine structure.[3] It appeared that the fine structure might be revealed by magnification of the abscissa by using high differentiating currents or low reagent concentrations, or both. Consequently, the survey of electrical variables was extended, and confirmation of the fine structure in actual titrations was achieved.

Experimental

Apparatus, reagents and procedures have been described.[1] Platinum-black electrodes were prepared as described below. A set of glass-sheathed bright-platinum electrodes having an exposed surface of 1 inch of 22 s.w.g. wire, or, for use in combination with antimony electrodes, of an exposed area equal to that of the antimony electrodes, 0·0315 sq. cm, were cleaned by immersing them in aqua regia and then thoroughly washing them. The leads were connected together, and the electrodes made cathodic to a separate platinum anode in an electrolyte of 0·05 M hexachloroplatinic acid containing a trace of lead acetate. A current density of 200 mA per sq. cm was applied for 60 seconds, and the electrodes were thoroughly washed and stored in water. *Water* as previously defined[1] was used throughout. The nitrogen used for preventing ingress of carbon dioxide to the titration vessel was of the ordinary grade. "White spot" nitrogen has too low an oxygen content for acceptable behaviour of antimony electrodes.

Results and discussion

Current density and reagent concentration—

The quality of titration and the peak height of the differential electrolytic potentiometric curve are functions of the chemical parameters[4] (c_t, the concentration of titrant; c_d, the initial concentration of titrand; v_t, the equivalence-point volume of titrant; Δv, the increment discrimination; and K, the reaction constant). When the parameters are such that the

* For details of earlier parts of this series, see reference list, p. 420.

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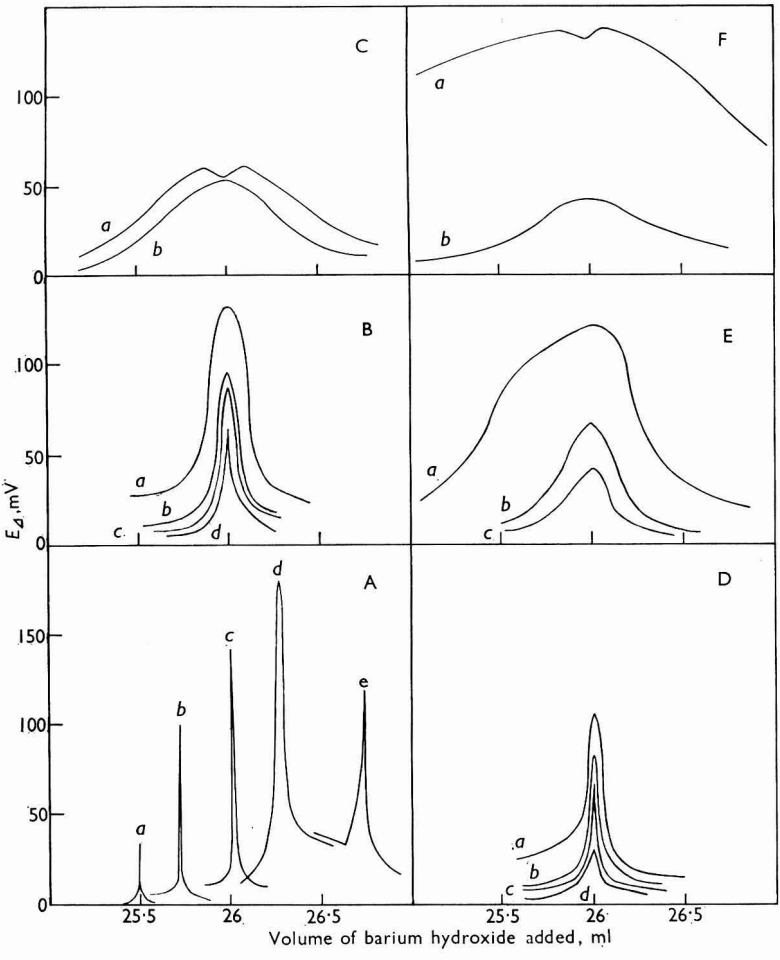

Fig. 1. The effect of varying the current density and the reagent concentration on the differential curves for strong base - strong acid and strong base - weak acid titrations in 0·03 M potassium chloride. Antimony electrodes; electrode area, 0·126 sq. cm

Family	Strength of acid	Strength of base	Current density, amps per sq. cm— (*a*)	(*b*)	(*c*)	(*d*)	(*e*)*
A	~0·0125 M $HClO_4$	~0·05 M $Ba(OH)_2$	0.953×10^{-6}	3.18×10^{-6}	4.76×10^{-6}	7.33×10^{-6}	0.878×10^{-6}
B	~0·00125 M $HClO_4$	~0·005 M $Ba(OH)_2$	3.97×10^{-6}	1.91×10^{-6}	0.953×10^{-6}	0.476×10^{-6}	
C	~0·000125 M $HClO_4$	~0·0005 M $Ba(OH)_2$	47.6×10^{-8}	9.53×10^{-8}			
D	~0·0125 M CH_3COOH	~0·05 M $Ba(OH)_2$	19.05×10^{-6}	11.9×10^{-6}	7.95×10^{-6}	3.97×10^{-6}	
E	~0·00125 M CH_3COOH	~0·005 M $Ba(OH)_2$	11.9×10^{-6}	3.97×10^{-6}	1.11×10^{-6}		
F	~0·000125 M CH_3COOH	~0·0005 M $Ba(OH)_2$	3.96×10^{-6}	0.953×10^{-6}			

* Platinum-black electrodes; electrode area, 0·571 sq. cm

›-current potentiometric step height exceeds 2 pB units for a 1-electron reaction, conditions said to be *favourable*.[4,5] Under *favourable* conditions, the pattern followed by the erential titration curves as the differentiating current, I_Δ, at fixed electrode area is ·eased, is as described below. For sufficiently low currents, no differentiation occurs, n a small blip appears at the equivalence-point and grows into a needle-sharp peak of siderable height, but of such small span that it is difficult to realise experimentally, ause an increment of titrant of 0·01 ml is sufficient for the curve to pass right through the k. The still-sharp peak continues to grow in height, and the span of the skirts increases :hat warning of the approach to the end-point improves until the two sides of the peak

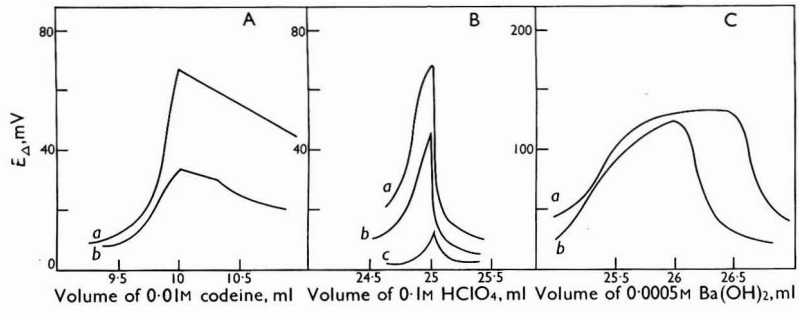

Fig. 2. The effect of current density, concentration, direction of titration and supporting electrolyte he differential curves for antimony electrodes of area 0·126 sq. cm

nily	Strength of acid	Strength of base	Current density, amps per sq. cm— (*a*)	(*b*)	(*c*)	
A*	~0·00125 M $HClO_4$	~0·01 M codeine	$11·9 \times 10^{-6}$	$3·97 \times 10^{-6}$		
B	~0·1 M $HClO_4$	~0·0125 M codeine	$7·95 \times 10^{-6}$	$3·97 \times 10^{-6}$	$0·953 \times 10^{-6}$	
C	~0·00125 M CH_3COOH	~0·005 M $Ba(OH)_2$	$11·9 \times 10^{-6}$			No supporting electrolyte
				$11·9 \times 10^{-6}$		Supporting electrolyte, 0·03 M KCl

* Note reversal of symmetry with change of direction of titration

:in to separate, and the breadth of the peak becomes detectable. Then comes the optimum dition, defined[6] as that providing the maximum peak-height potential without making the k broader than the volume discrimination required of the titration, *e.g.*, 0·01 ml for a ml titration. Thereafter the peak continues to grow in height and breadth, the top being irst sharp, then becoming rounded and finally flattened with steep S-shaped sides. Under *erse* conditions,[4,5] when the zero-current potentiometric step height lies between 2 and pB units, the sides of the peak retain their S-shape, but lose their steepness, and as ditions become more unfavourable the needle-sharp peak no longer appears. The aviour under *favourable* conditions is illustrated in Fig. 1 A and under increasingly *erse* conditions in Fig. 1 D, B, E, C, and F, and in Fig. 2. If the differentiating current ısity is maintained constant, and if (*i*) c_t is decreased, (*ii*) c_d is decreased, (*iii*) v_t is increased, Δv is decreased or (*v*) K is decreased, the conditions become increasingly *adverse*. Most :hese modes of variation are shown in Figs. 1 and 2. The empirical optimum conditions viously reported[1] were derived from such experiments.

MOSPHERE—

Carbon dioxide causes curve distortion, and even small amounts cause the top of the mal peak to be truncated.[1] It is also inimical to zero-current potentiometry. Extremely ıll traces are detectable (see Fig. 3 C), and it is possible to monitor carbon dioxide in gas process streams by observing the effect on the differential potential.[7] It is customary,

therefore, to use solutions that are free from carbon dioxide and are protected from the atmosphere by some other gas. Oxygen is normally used,[8] and ensures fast response and stable, reproducible potentials. Oxygen-free ("white spot") nitrogen leads to much larger differential potentials, but the electrodes become sluggish and the potentials erratic. Ordinary nitrogen[1] contains sufficient oxygen (about 1 per cent.) to give a satisfactory performance. However, at oxygen levels below about 10 per cent., the fine structure of the differential peaks[3] appears. It was deduced[3] that for extremely small (*adverse*) values of the discrimination Δv, the fine structure should occur in titrations. The volume scale could be so expanded by using extremely low concentrations of reagent or extremely high differentiating currents (see Fig. 1 C, curve *a* and Fig. 1 F, curve *c*). Substitution of oxygen for nitrogen destroys the fine structure and produces a smooth peak.

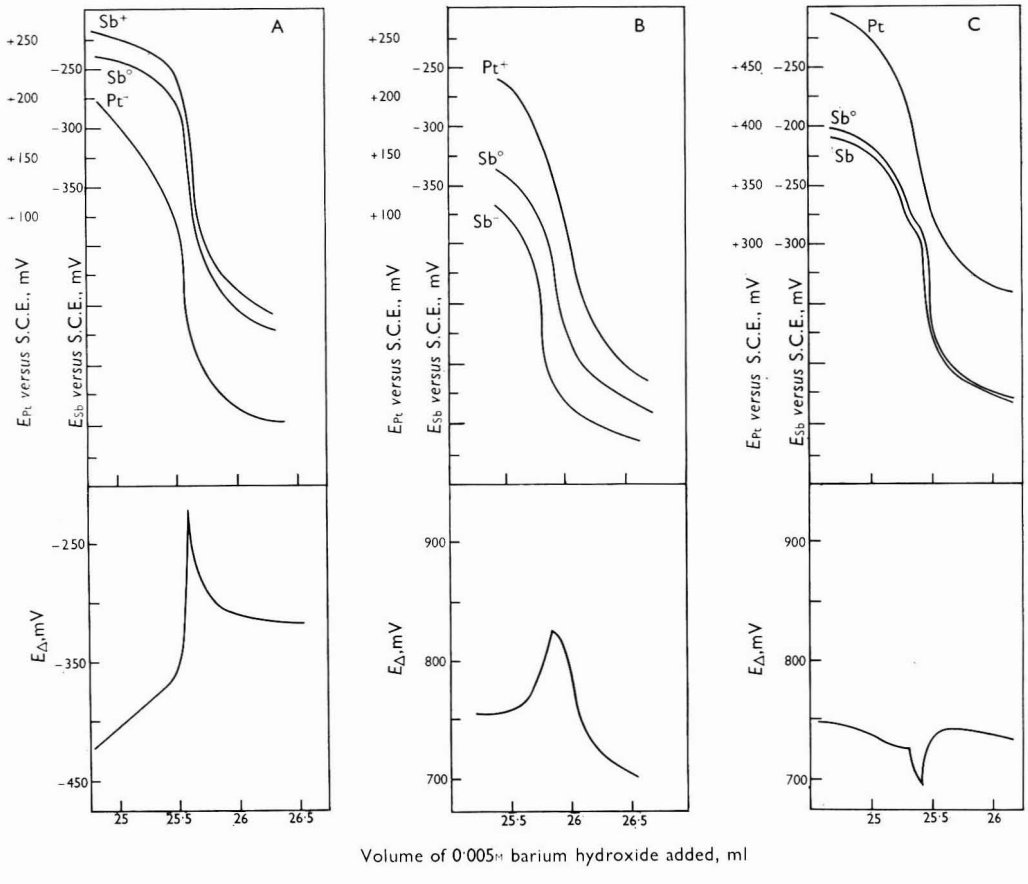

Fig. 3. Results obtained by using diverse electrode systems and spontaneous differentiation. Sb^+ = antimony anode; Sb° = antimony zero-current indicator electrode; Sb^- = antimony cathode; Pt^+ = platinum-black anode; Pt = platinum-black electrode; Pt^- = platinum-black cathode

Family	Current density, amps per sq. cm	Electrode area, sq. cm	Differential curve
A	$2{\cdot}54 \times 10^{-6}$	0·0315	Sb^+ vs. Pt^-
B	$2{\cdot}54 \times 10^{-6}$	0·0315	Pt^+ vs. Sb^-
C	Spontaneous differentiation, R_B = 100 megohms		Pt vs. Sb

SUPPORTING ELECTROLYTE—

A neutral supporting electrolyte is customarily provided,[1] typically 0·03 M potassium chloride previously tested for balance and neutrality. In the absence of this supporting electrolyte, the differential potential is increased, but becomes erratic, as shown in Fig. 2 C. An increase in the concentration of potassium chloride in *water* reduces the differential potential asymptotically to a minimum constant value at 0·04 M potassium chloride. At 0·03 M potassium chloride the potential is no more than a few millivolts above the minimum.

BALLAST LOAD—

Ballast load (source voltage, V_S, × ballast resistance, R_B) affects the differential potential,[9] and for antimony electrodes should be above 5×10^9 volt-ohms, otherwise current stabilisation is inadequate and the potentials are erratic, and below 10^{11} volt-ohms, under which conditions the electrode response begins to slow down and the potentials tend to drift.

ELECTRODE AREA—

If the other chemical parameters were fixed, variations of the area of the electrodes produced no change in the titration curves, provided that the ratio of the electrode areas, and the current density was kept constant. Antimony electrodes of equal area, ranging from 0·2 to 0·001 sq. cm, have been used without encountering any change below a critical area, such as was noted with silver electrodes.[10] Electrodes of different areas cause distortion of the curves, the electrode of smaller area giving a titration curve displaced farther along the volume axis,[1] resulting in an asymmetrical peak and displacement of the end-point from the equivalence-point. The error depends on the direction of titration, and with an alkaline titrant it would be negative if the cathode is smaller and positive if the anode is smaller. For currents near to optimum for the smaller electrode, this displacement is negligible if the electrode areas are within one order of each other, but at higher current densities the error increases with disparity in electrode area.

NATURE OF ELECTRODES—

Pairs of similar electrodes—Theoretically, any electrode system responsive to hydrogen, oxygen, hydrogen ion or hydroxyl ion could be used, but in practice the choice is restricted to metallic electrodes of reasonable response speed. (Glass electrodes, even if made of low-resistance uranium glass, do not offer an adequately conducting plane at which the necessary concentration polarisation can be produced by electrolysis.) Bright-platinum electrodes give a sluggish and erratic response.[8] Cathodic activation of bright platinum,[11] said to produce a surface with the activity of the platinised-platinum hydrogen electrode, is of no benefit.[8] Conventionally platinised platinum, prepared as described above, gave a response that had a slope considerably in excess of theory (see Fig. 3). Good differential peaks could be obtained (see Fig. 1 A, curve *e*), but the skirts showed unusual overpotential characteristics, and the response was too sluggish: 20 minutes were required for equilibrium to be reached. Tellurium electrodes, made like the antimony ones,[1] were even more sluggish than those made from platinised platinum.[12] Metal - metal oxide electrodes, especially antimony ones, have proved best.[8] Aberrations in absolute pH response[8,13] are without influence in differential electrolytic potentiometry, because the signal is the difference in potential between two similar electrodes.

Pairs of dissimilar electrodes—Electrodes, having differing normal potential, but similar response, should theoretically give the same type of curves as a pair of similar electrodes, but, instead of the potentials returning to zero on either side of the peak as in Fig. 1, the peak should be raised by a constant overpotential equal to the difference in normal potentials. Any difference in response slope should be revealed in asymmetry of the peak and of the over-potentials on either side of the peak, but should cause no displacement of the end-point. In practice, the electrodes will probably also differ in speed of response, which has been shown to sharpen differentiation,[14] and, in conjunction with electrolytic differentiation, to lead to second-differential curves.[6,14] Differential and individual electrode curves for titrations of dilute solutions by using antimony - platinum black and platinum black - antimony combinations are illustrated in Figs. 3 A and 3 B. In these experiments, sufficient time was allowed for a close approach to equilibrium potentials to be reached. The difference in slope for the two electrodes and its effect on the symmetry of the differential curves are apparent.

Spontaneous differentiation[5,6,14]—If two electrodes responding to the same ions have sufficiently different normal potentials or electrode slopes, they will constitute an electrochemical cell when they are combined. When this cell is short-circuited through a suitable resistor, the potentials of the two electrodes will tend towards equality, thus producing power that, if sufficient, and with an appropriate value of resistance, may cause electrolytic differentiation. This self-powered version of differential electrolytic potentiometry has been named spontaneous differentiation.[14] The antimony - platinum black combination constitutes such a system for acid - base reactions. If the short-circuiting resistance is too high, the current will be insufficient to cause differentiation; if it is too low, the potential difference will be too small to provide an adequate current. In a titration in dilute solution, with a short-circuiting resistance of 100 megohms and electrodes, 0·126 sq. cm in area, the platinum black (anodic) - antimony (cathodic) couple gave acceptable spontaneous differential curves, an example of which is shown in Fig. 3 C. Fig. 3 C also shows the effect of a trace of carbon dioxide. The sluggish response of the platinum-black electrode, however, diminishes its usefulness.

Conclusions

The behaviour of differential electrolytic potentiometry for acid - base reactions on variation of the electrical and chemical parameters extends and confirms the knowledge of that for reversible electrodes in ion-combination reactions, and for antimony electrodes closely approaches the ideal. Examination of the diverse pH-responsive electrode systems shows that the antimony - antimony oxide system is the most suitable for differential electrolytic potentiometry, and in favourable circumstances the speed of response is limited only by the time constants of the measuring instruments, so facilitating automation. The fine structure of the differential peak has been confirmed titrimetrically under conditions conducive to oxygen starvation of the electrodes. Combinations of different electrodes afford no advantage over paired antimony electrodes because of sluggishness of response, whether differentiation is spontaneous or applied.

We thank Electronic Instruments Ltd. for the loan of instruments and support of this work, and one of us (G.D.S.) is indebted to Electronic Instruments Ltd. and to the Company of Scientific Instrument Makers for maintenance grants during his tenure of the E.I.L. Fellowship.

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Note—References 1, 2, 3, 5, 6 and 9 are to Parts VI, VII, IX, XI, X and III of this series, respectively. Parts XII and XIII of this series appeared in *Anal. Chem.*, 1964, **36**, 726 and 730, respectively.

Received *July 10th*, 1963

Spectrophotometric Determination of Iron and Aluminium in Leaves of the Rubber Tree *(Hevea brasiliensis)*

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A rapid and accurate method is described for determining iron and aluminium by measuring the optical density of solutions of their hydroxyquinolinates in chloroform. The application of the method to the analysis of acid-digested leaves of the rubber tree is given; it is probably applicable also to the analysis of other plant materials. The method is sensitive to 3 p.p.m. of iron, and 1·5 p.p.m. of aluminium in solution, and its analytical precision is about 1 per cent. for iron, and 1·4 per cent. for aluminium. A considerably larger error, associated with the digestion process and subsampling was found in analyses of leaf samples, such that the coefficient of variation of a single determination on a given sample on any day, was 6·5 per cent. for iron and 3·1 per cent. for aluminium. The degree of heating during the digestion process appeared to influence the reliability of results, and special precautions were introduced to minimise this effect.

The rubber tree, *Hevea brasiliensis*, is grown mainly on leached, acidic tropical soils,[1] to which it seems well adapted by nature. However, it is suspected that in acidic soils the toxicity of iron and aluminium may directly or indirectly limit plant growth,[2,3] and it is possible that a study of the iron and aluminium contents of rubber leaves could illuminate this problem. Satisfactory methods for estimating iron in plants are already available,[4] but for aluminium sufficiently rapid and sensitive methods are unreliable. Before aluminium is determined, iron must usually be removed to prevent it interfering[4]; in the method described in this Paper, iron and aluminium are determined in the same solution, and since the preliminary determination removes iron, interference in the subsequent aluminium determination is avoided.

The proposed method is based on Gentry and Sherrington's studies of the properties of metallic hydroxyquinolinates[5,6]; but to attain satisfactory accuracy and precision, procedures for carefully controlling the pH during the extraction of iron and aluminium from an aqueous phase into chloroform, have had to be worked out.

Method

Before mineral elements are determined, the organic structure of plant samples must be destroyed, and for elements like iron and aluminium that are present in small amounts, this is better achieved by wet digestion than by dry ashing.[7]

Reagents—

The reagents listed should be prepared from pure chemicals and de-mineralised water.

Hydroxyquinoline - acetic acid solution—Prepare a 0·6 per cent. w/v solution of 8-hydroxyquinoline in 2 per cent. v/v aqueous acetic acid.

Ammonia solution, 5 N.

Ammonium acetate - acetic acid buffer solution—Prepare a 0·1 N solution of ammonium acetate in 5 N acetic acid, and adjust the pH to 2·85.

Hydroxyquinoline - chloroform solution—Prepare a 0·3 per cent. solution of 8-hydroxyquinoline in chloroform. This reagent should be protected from the light and used within a week of preparation.

Digestion—

Weigh a dry, finely ground, sub-sample of leaves, containing 0·02 to 0·08 mg of iron, and 0·008 to 0·032 mg of aluminium, into a 100-ml Kjeldahl flask. Add 5 ml of nitric acid, sp.gr. 1·42, for each 0·5 g of leaves and when the material has been thoroughly wetted by the acid, add 0·5 ml of perchloric acid, sp.gr. 1·70, and 0·5 ml of sulphuric acid, sp.gr. 1·84.

Heat the contents of the flask gently until the evolution of brown fumes slackens, and then more strongly until a vigorous reaction between the partly digested plant material and the perchloric acid takes place. When this reaction is complete, volatilise most of the excess of perchloric acid by heating the flask for a further 5 minutes; add 25 ml of water to the cooled digest and evaporate it cautiously until 5 to 6 ml of solution remain.

DETERMINATION OF IRON—

Transfer the cooled solution to a 50-ml, pear-shaped, all-glass separating funnel, using not more than 10 ml of water. Add 1 ml of the hydroxyquinoline - acetic acid reagent, and while continuously swirling the separating funnel, slowly neutralise the solution with 5 N ammonia solution, taking care to avoid local excess of the reagent; the hydroxyquinoline acts as an indicator, as will be explained later. Add 5 ml of ammonium acetate - acetic acid buffer to the funnel and mix the contents thoroughly. Add 10 ml of hydroxyquinoline - chloroform reagent, place the stopper in the funnel and shake it thoroughly. Allow the aqueous and chloroform phases to separate, and measure the optical density of the chloroform phase in a 1-cm cell by using a spectrophotometer set at a wavelength of 4700 Å against a reagent blank solution.[8] For purposes of neutralisation, the blank solution may be assumed to be of similar acidity to the sub-sample. If, after it has been removed from the separating funnel, the chloroform layer is opalescent, owing to the presence of a trace of moisture, immerse the optical cell in hot water for a few seconds, and then allow its contents to cool before measuring the optical density.

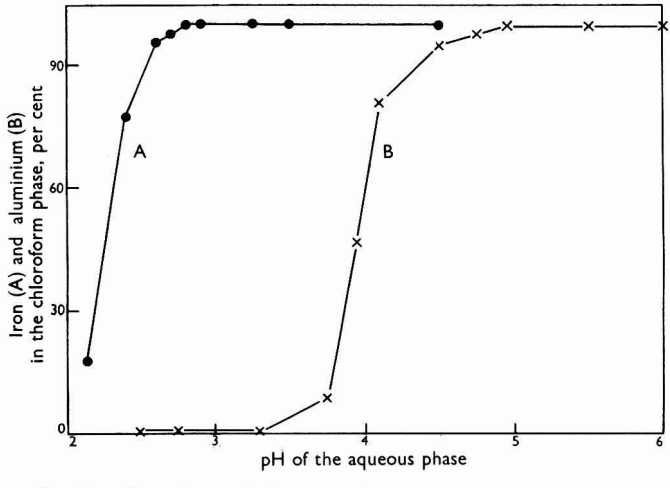

Fig. 1. The effect of the pH of the aqueous phase on the amount of iron (A) and aluminium (B) extracted into the chloroform

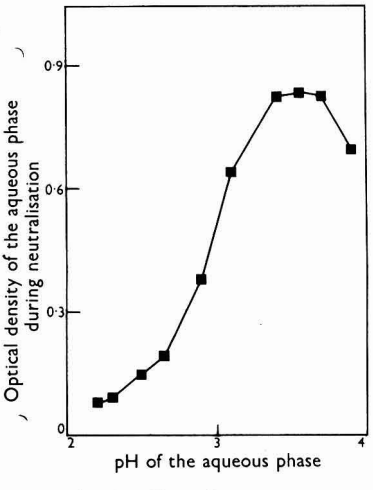

Fig. 2. The effect of pH on the development of colour as ferric hydroxyquinolinate in the aqueous phase

DETERMINATION OF ALUMINIUM—

Carefully run off any chloroform that remains in the funnel after the determination of iron and then slowly add 4·5 ml of 5 N ammonia solution while continuously swirling the solution. Add 10 ml of hydroxyquinoline - chloroform reagent, shake the stoppered funnel thoroughly, and after the chloroform phase has separated from the mixture, measure its optical density in a 1-cm cell at 3850 Å, as before, setting the instrument against a reagent blank solution.

CALIBRATION—

Tests with a standard solution, prepared from the ammonium alums of the two elements and used with the above procedures, showed concentrations of iron and aluminium (as mg of either metal in solution) and the optical densities of their hydroxyquinolines in chloroform E and E', respectively, are linearly related as given below—

$$\text{Iron content} = 0{\cdot}0936\ (\pm 0{\cdot}0009)\ E - 0{\cdot}0002\ (\pm 0{\cdot}0005)$$

and

$$\text{Aluminium content} = 0{\cdot}0363\ (\pm 0{\cdot}0003)\ E' + 0{\cdot}0001\ (\pm 0{\cdot}0002).$$

These least-square regressions show that the Beer - Lambert law is obeyed in both instances; moreover, an examination of residual error as described by Middleton and Westgarth,[9] indicated that the regressions were not significantly different from straight lines passing through the origin, and therefore that the simplified equations, *i.e.*, iron = 0·0933 E (at 4700 Å), and aluminium = 0·0365 E' (at 3850 Å), may be used for calculating the iron and aluminium contents for the concentration ranges specified.

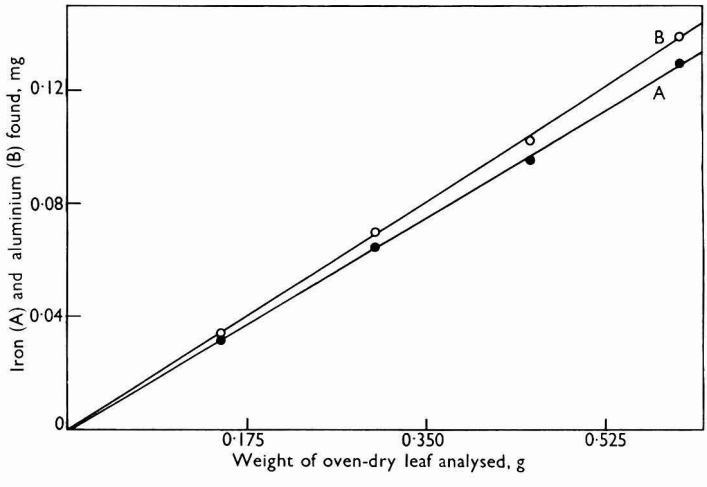

Fig. 3. The determination of iron (A) and aluminium (B) in different weights of rubber leaves.

Equation of (A): $y = 0{\cdot}2170\ (\pm 0{\cdot}0021)x - 0{\cdot}0012\ (\pm 0{\cdot}0009)$
Equation of (B): $y' = 0{\cdot}2323\ (\pm 0{\cdot}0037)x' - 0{\cdot}0007\ (\pm 0{\cdot}0015)$

The effect of pH in the aqueous phase upon the percentage iron and aluminium extracted as hydroxyquinolinates into the chloroform phase is shown in Fig. 1. Fig. 2 shows the development of colour in the aqueous phase as the dark green ferric hydroxyquinolate is formed with increasing pH values.

The effect of the mineral elements present in the leaf ash of *Hevea brasiliensis* on determinations by the proposed method was investigated in a factorial experiment, in which prepared solutions containing 4 levels each of iron and aluminium and 3 levels each of the elements considered most likely to interfere, namely phosphorus and manganese, were used. The other constituents were made to correspond to percentages commonly found in the leaves of the rubber tree, *i.e.*, potassium 1·20, magnesium 0·22, and calcium 0·65. A summary of the results of this investigation is given in Table I.

The accuracy of the method, when applied to the analysis of rubber leaves, has been assessed, as suggested by Youden,[10] by determining iron and aluminium in samples of differing weight taken from 3 different rubber trees; the assessment is given in Fig. 3 and in Table II.

Discussion

At equilibrium, the ratio of the solubility of aluminium hydroxyquinolate [tris-(8-quinolinolato)aluminium^{III}] in chloroform to its solubility product in water is large (about 9×10^{30}), and it may be predicted that when 10 equivalents of hydroxyquinoline are present for 1 equivalent of the metal, a single chloroform extraction will completely remove the hydroxyquinolinate from the aqueous phase.[11] In the proposed method over 50 equivalents of hydroxyquinoline are added for each equivalent of iron or aluminium. No published results are available to make a prediction for iron, but it may be easily shown experimentally that both hydroxyquinolinates are completely removed with one chloroform extraction.

As is well known, metallic hydroxyquinolinates may be selectively extracted from water into chloroform by regulating the hydrogen-ion concentration in the aqueous phase, and it may be inferred from Fig. 1 that there is an interval of more than half a pH unit between the complete extraction of ferric hydroxyquinolinate [tris-(8-quinolinolato)iron^{III}] and the start of the extraction of aluminium hydroxyquinolinate. Further investigation showed that this is so only when the extractions were made from solutions containing either iron or aluminium, and that when ferric hydroxyquinolinate is extracted from an aqueous solution containing both iron and aluminium at a pH value greater than 3, co-precipitation takes place, causing variable amounts of aluminium to accompany iron into the chloroform phase. Plant samples, digested in the manner described above, necessarily contain an excess of acid, and to ensure complete extraction of iron without appreciable loss of aluminium, their reaction must be adjusted between the narrow limits of pH 2·8 to 3·0.

Fig. 2 shows that when 5 N ammonia solution is added slowly to a diluted digest containing a small amount of hydroxyquinoline, an inflection in the curve takes place between pH 2·7 and 3·1, caused by the formation of ferric hydroxyquinolinate; this obviates the need for an acid - base indicator that could interfere in the subsequent colorimetric measurements. The hydroxyquinoline - acetic acid reagent therefore performs three functions—

(*i*) It acts as an indicator in the initial adjustment of the pH of an acidic digest.

(*ii*) It ensures that ferric hydroxyquinolinate is formed as the pH is increased and not a phosphate or an insoluble basic salt; this is a possible source of error.[11]

(*iii*) It facilitates the extraction into chloroform, which proceeds more rapidly if the metallic hydroxyquinolinate is formed before the organic solvent is added.[11]

It may be calculated that at pH 3 about one-fifth of the added hydroxyquinoline remains in the aqueous phase at equilibrium[12]; this amount is therefore available for the second and third functions described above, during the precipitation and extraction of aluminium.

It should be appreciated that the initial adjustment of the pH is vital for the accurate determination of both iron and aluminium, and to guard against accidental addition of excess of 5 N ammonia solution, 5 ml of the ammonium acetate - acetic acid reagent is added. This solution is strongly buffered at pH 2·85; subsequently, when 4·5 ml of 5 N ammonia solution is added, it forms a solution buffered at pH 5·5, which ensures complete extraction of aluminium hydroxyquinolinate without interference from manganese.[6] Objection has been made to the use of an acetate buffer for aluminium determinations because it is said to increase the optical density of hydroxyquinoline in chloroform.[8] In the proposed procedure no appreciable increase of this kind could be detected, and the objection is therefore not considered serious; an appreciable increase does, however, take place with ageing (and presumably decomposition) of the hydroxyquinoline - chloroform reagent.

Accuracy of the method—

As Youden has pointed out,[10] the relation between the weight of sample taken for analysis, x, and the weight of constituent found, y, can be used to detect bias in an analytical method. For the proposed method this relationship is summarised in Fig. 3; each point on the diagram is the mean of 12 measurements, *i.e.*, duplicate determinations for 2 separate days and for 3 different trees. An examination of residual error about the six individual regressions by using a procedure already described,[9] had demonstrated generally that least-square regressions did not differ significantly from lines passing through the origin. An examination of the standard errors of the parameters of the lines shown in Fig. 3 confirms that the overall result is similar, and clearly demonstrates that there can be no constant bias. If there were a

constant bias, the lines would make significant intercepts on the ordinate axis; it does not prove that there is no variable bias proportional to the amount of material taken for analysis.

Of the five major elements in the leaf ash of *Hevea brasiliensis*, phosphorus and manganese are most likely to bias determinations by the proposed method; this is because iron and aluminium can both form insoluble phosphates, and because bis-(8-quinolinolato)manganese[11], which is insoluble at pH 5·8, might co-precipitate with aluminium.[6] The results obtained with prepared solutions (see Table I) show in most instances no appreciable interference from phosphorus and manganese. Moreover, recoveries of both iron and aluminium rarely differ

TABLE I

PERCENTAGE RECOVERY OF IRON AND ALUMINIUM FROM PREPARED SOLUTIONS

In addition to iron and aluminium each solution contained potassium, magnesium and calcium

(Each figure is the mean of three determinations)

Metal in the prepared solutions, p.p.m.	Percentage recovery of metal: Manganese added, p.p.m. 0	5	10	Phosphorus added, p.p.m. 0	150	250	Minimum 5 per cent. significant difference between any mean and 100 per cent. recovery
Iron							
1·8	97	99	99	97	98	99	2·5
3·6	99	102	101	100	100	101	
5·4	99	103	102	101	101	102	
7·2	100	102	101	101	101	102	
Aluminium							
0·75	94	97	102	99	96	98	3·0
1·50	96	100	102	100	98	99	
2·25	98	101	102	99	101	101	
3·00	97	99	100	98	99	100	

TABLE II

IRON AND ALUMINIUM IN DIFFERENT WEIGHTS OF LEAF SAMPLES OF *Hevea brasiliensis*

Tree	Day	Sub-sample	Iron content, p.p.m. of oven-dry material: Weight taken for analysis, g 0·15	0·30	0·45	0·60	Day means	Aluminium content, p.p.m. of oven-dry material: Weight taken for analysis, g 0·15	0·30	0·45	0·60	Day means
A	1	1	299	312	297	296	296	195	195	191	190	192
		2	255	293	300	312		189	192	190	197	
	2	3	331	293	300	312	306	209	200	196	206	201
		4	293	303	300	314		199	200	196	198	
B	3	1	213	210	202	229	207	273	288	279	295	285
		2	200	197	204	200		283	290	276	298	
	4	3	200	200	198	173	192	273	275	276	273	276
		4	187	197	189	193		273	280	273	286	
C	5	1	151	142	138	144	139	217	227	215	214	215
		2	113	140	143	140		207	217	212	209	
	6	3	142	140	137	139	139	222	217	210	211	212
		4	139	137	142	138		202	204	217	213	
		Weight means	210	214	213	216		229	232	228	232	
Standard error of a mean ..				±3·6			±4·4		±1·6			±1·9
Minimum 5 per cent. significant difference between two means				10			13		5			6

significantly from 100 per cent. The generally lower recoveries obtained for the first level at which the two elements were added probably sets a limit to the sensitivity of the method; but where iron in solution exceeds 3 p.p.m. and aluminium 1·5 p.p.m., there is no significant interference by the 5 elements tested (except in one instance), indicating that analyses of

solutions similar in composition to digested rubber leaves are free from both constant and variable bias. This finding, combined with that summarised in Fig. 3, where results are within the sensitivity specified for for the method, demonstrates the absence of significant constant and variable bias in the proposed method as applied to the analysis of rubber leaves.

PRECISION OF THE METHOD—

The individual results summarised in Fig. 3 are given in Table II, and an analysis of variance of them (not given in this Paper) has permitted standard errors, and the minimum differences significant at the 5 per cent. level, to be computed for the mean values that are also shown in Table II. Highly significant differences between trees, and small differences between days are shown; the latter are significant in one instance for iron, and in two for aluminium. Large sub-sampling variations should be noted; sub-sampling variations are unavoidably confounded in these results with digestion errors, which are probably responsible for most of the variations. The statistical examination also indicates, as in the regression analysis (see Fig. 3), that results for different weights are generally similar.

The error associated with a single determination on a given sample on any day may be taken to define the precision of an analytical procedure for most practical purposes.[13] Components associated with sub-sampling, σ_s (which includes analytical error in this case), and with day-to-day variation, σ_d, have been obtained from the corresponding error mean squares in the analysis of variance referred to above. The standard error of a single determination, on a given sample, on any day was thus found to be—

(*a*) *For iron*—

$$\pm \sqrt{\sigma_s^2 + \sigma_d^2} = \pm \sqrt{156{\cdot}01 + 34{\cdot}29} = \pm 13{\cdot}79;$$

the coefficient of variation (expressed as a percentage of the mean iron content in p.p.m.) = 6·47 per cent.

(*b*) *For aluminium*—

$$\pm \sqrt{\sigma_s^2 + \sigma_d^2} = \pm \sqrt{29{\cdot}37 + 22{\cdot}47} = \pm 7{\cdot}20;$$

the coefficient of variation (expressed as a percentage of the mean aluminium content in p.p.m.) = 3·13 per cent.

The experimental error, computed from an analysis of variance of the results summarised in Table I, may be taken as an estimate of analytical error. The corresponding standard error for iron was ±1·15 (coefficient of variation = 1·15 per cent.), for aluminium it was ±1·72 (coefficient of variation = 1·73 per cent.). These estimates were checked by digesting 24 leaf samples of *Hevea brasiliensis*, taken from three different trees; after making the digests up to standard volumes, duplicate determinations were made on each solution by the proposed procedure. From the differences between these pairs, the analytical error for iron was found to equal ±1·73 (coefficient of variation = 0·97 per cent.), and for aluminium ±3·28 (coefficient of variation = 1·40 per cent.). These results are in good agreement with the previous estimates

THE DIGESTION PROCESS—

The method of digesting plant samples described in this Paper is based on a procedure given by Piper.[7] In order to facilitate neutralisation of digests, the amount of acid remaining at the end of the digestion should be made as small as possible; preliminary work had, however, shown that overheating digests during the final stages of evaporation, *i.e.*, after the vigorous reaction with perchloric acid had ceased, caused highly significant losses of iron and aluminium, possibly by conversion of the elements into partially ionised forms. Further work showed that such losses could be offset by adding 25 ml of water, and boiling the diluted digest until the volume was reduced to 5 or 6 ml. These effects are illustrated by the results listed below, which are means of two determinations expressed as p.p.m. of oven-dry material.

Iron—When the digested samples were not overheated, the value for unboiled digests was 181 and for boiled digests 185; when samples were overheated for 30 minutes, the corresponding figures were 86 and 189. The standard error of these means, computed from an analysis of variance of individual results, was ±6·5, and the minimum difference between means, significant at the 1 per cent. level, was 31.

Aluminium—Samples which were not overheated gave a mean value of **393** when not boiled, and **397** when boiled; for overheated samples the corresponding figures were **159** and **356**. The standard error of these means was $\pm 13{\cdot}5$ and the minimum 1 per cent. significant difference between the means was **64**.

Conclusions

The proposed method is economical in time and in the amount of material required, since iron and aluminium are both determined on the same digested sub-sample. It is accurate because statistical studies have shown such determinations to be essentially free from bias. The analytical precision is satisfactory, and is near to the expected value for a colorimetric procedure.[14] The overall precision, particularly for iron, is rather low, but the main source of error is associated with sub-sampling and digestion. These two components are inevitably confounded, and it is considered that the latter is the main cause of the variation.

I am grateful to the Director of the Rubber Research Institute of Malaya for permission to publish this Paper, to Miss Susie Oh and Mr. Chin Pong Tow for their careful work in compiling Tables I and II, and to Mr. R. Narayanan and Mr. D. R. Westgarth for statistical aid.

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Received *November 26th*, 1963

The Rapid Determination of Nitrogen in Apple Juice and Cider

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The Markham method for determining nitrogen was modified to allow titration of the distilled ammonia by using a pH meter. This modification permitted a reduction in the size of the sample and in the time required for a determination. Similar results were obtained by using the modified and original methods, and both methods gave satisfactory recovery of nitrogen added as glycine.

IN cider fermentations the determination of nitrogen is important because too high a content in the finished cider can contribute to biological instability, whereas too low a content in the apple juice can lead to arrested fermentation.[1,2,3]

Because cider-making is a seasonal activity, large numbers of samples need to be analysed at certain times of the year. Thus the rapidity of a method for determining nitrogen is an important factor in assessing its usefulness as a control measure.

Apple juices and ciders have a low nitrogen and a high sugar content compared with most biological materials. This tends to increase the possibility of losses of nitrogen during the digestion process. However, the absence of nitrogen compounds that resist digestion means that short digestions may be carried out in the presence of mercuric oxide, and thus these losses are minimised.

For some years members of the Cyder and Fruit Juices Section at the Long Ashton Research Station (and later the authors of this Paper) have used a method for apple juices and ciders that they based on Markham's distillation unit.[4,5,6] This method requires approximately 2 hours total time for batches of 6 determinations, and the cleared Kjeldahl digests are heated for 30 minutes only.

It appeared that a reduction in the size of the sample might reduce the digestion time. However, this involved a corresponding reduction in titration volume and a consequent increase in the relative titration error.

To overcome this difficulty, the titration was performed by using a pH meter (with a negligible drift) instead of the methyl red - bromocresol green indicator. Challinor and Burroughs found that by using this indicator the end-point could be determined to within 0·01 ml or less.[5] However, such accuracy calls for considerable skill, and we have not been able to work to such limits during the busy harvest period.

METHOD

APPARATUS—

Borosilicate glass test-tubes, 16 × 120 mm were used for the digestion.

Six holes, to take the 16-mm tubes, were drilled in the $1\frac{1}{4} \times 7\frac{1}{2}$-inch face of a $1\frac{1}{4} \times 2 \times 7\frac{1}{2}$-inch mild-steel block.

The arrangement of the apparatus is shown in Fig. 1. The collection of the Markham distillate in a stirred beaker containing the pH electrodes allowed the distillation and titration to be completed consecutively in the same apparatus. The meter was a Pye Dynacap pH meter, an instrument with good stability over long periods; it was used over the pH range 4 to 14 and had automatic temperature compensation.

The reservoir for the 2-ml burette was made by cutting a hole with a cork-borer in the bottom of a 250-ml polythene bottle to take a bung. This bung was removed when the apparatus was in use.

* Present address: Messrs. Munton & Fison Ltd., Stowmarket, Suffolk.

REAGENTS—

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

Sulphuric acid, concentrated—Low-in-nitrogen grade.

Sulphuric acid, N/140—Standardise the solution against sodium tetraborate by using Vogel's procedure.[7]

Ammonium sulphate, N/140—Prepare the solution from dried material.

Sodium hydroxide - sodium thiosulphate solution—Dissolve 400 g of sodium hydroxide and 50 g of sodium thiosulphate in 1 litre of water.

Boric acid solution—Dissolve 5 g of boric acid in 200 ml of industrial methylated spirit. Dilute to 1 litre with water and adjust the pH, if necessary, to 5·6 to 5·8 by using a minimum of 2 N sodium hydroxide.

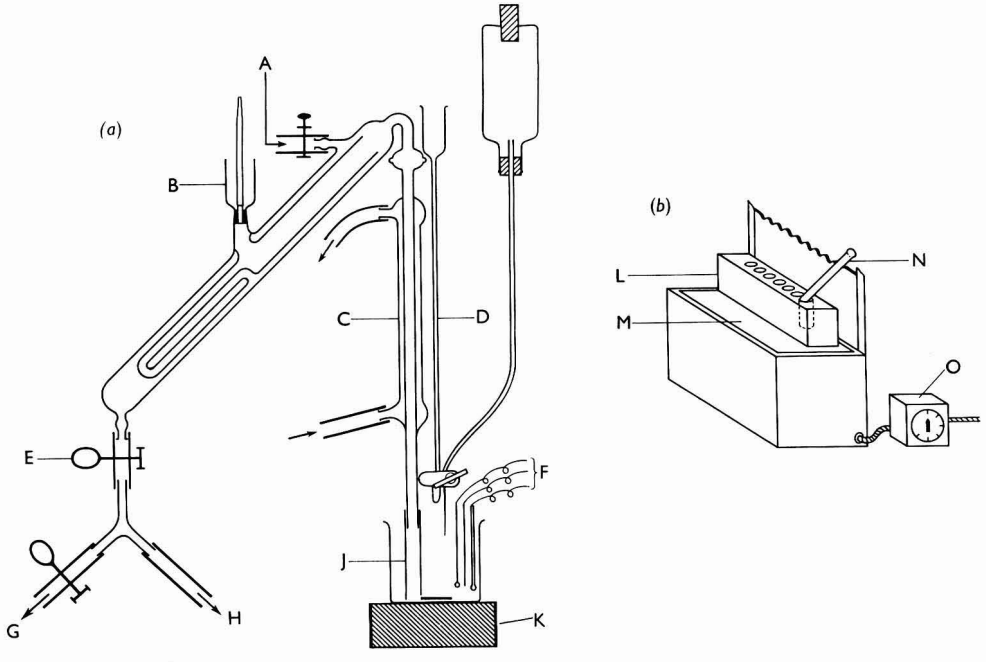

Fig. 1. (*a*) Distillation and titration apparatus; (*b*) digestion apparatus

Kjeldahl catalyst—Grind together 80 g of potassium sulphate, 20 g of copper sulphate, 0·34 g of sodium selenate and 10 g of mercuric oxide. Store the mixture in a glass-stoppered bottle containing a glass scoop of capacity 0·25 g.

Carborundum—20 to 40 mesh. (If the blank value is excessive this may be caused by the carborundum, which can then be digested with concentrated sulphuric acid before use.)

PROCEDURE—

Transfer with a pipette 2 ml of the cider sample, or 0·5 ml in the case of apple juice, into the digestion test-tube. Add one drop, 0·05 ml, of concentrated sulphuric acid, several grains of carborundum and 0·25 g of the catalyst.

Place the tube in an inclined position on top of the heated drilled block as in Fig. 1. Adjustment of the temperature of the heating-block with the Simmerstat ensures that the bubbles do not rise more than half-way up the tube. Evaporate the contents until the consistency of treacle is reached.

Remove the tube, add the appropriate amount of concentrated sulphuric acid and replace the tube vertically in the drilled block.

The total amount of sulphuric acid to be used, including the 0·05 ml added initially, is given by the formula—

Volume of sulphuric acid = 0·5 ml + 0·04 ml per 0·01 gm of soluble solids present,[8] and corrected to the nearest 0·1 ml.

Heat the tube for 15 minutes, adjusting the Simmerstat so that the contents become clear during the first 5 minutes.

After cooling the digests, transfer them to the Markham apparatus, add 5 ml of sodium hydroxide - sodium thiosulphate solution and distil the solution into 10 ml of boric acid solution. Note the pH before distilling the solution. After 3 minutes distillation, do not stop distilling, but titrate with N/140 sulphuric acid to restore the pH to its original value. Then—

1 ml of N/140 sulphuric acid ≡ 0·0001 g of nitrogen.

To clean the apparatus, close the steam clip, open the drain-clip and apply the vacuum to empty the beaker and the still. Refill the burette and wash both the beaker and the Markham cup twice with water. A reagent blank solution, comprising 0·5 ml of concentrated sulphuric acid, 0·25 g of Kjeldahl catalyst, carborundum and 5 ml of sodium hydroxide - sodium thiosulphate solution should be distilled before each set of determinations and should give a titration not greater than 0·05 ml.

For periodic checks on the distillation, or the combined digestion and distillation, 1 ml of N/140 ammonium sulphate is used together with the above reagents. Nitrogen recoveries should be within the range of 98 to 102 per cent.

TABLE I

COMPARISON OF RESULTS BY USING THE MARKHAM METHOD AND THE pH TITRATION METHOD

Sample	Titration, ml	Titration corrected for blank value, ml	Nitrogen recovery, per cent.
Established Markham method—			
Reagent blank solution ..	0·06	—	—
Glycine solution, 4·98 ml* ..	4·98, 5·20, 5·20	4·92, 5·14, 5·14	99, 103, 103
Glucose solution, 1 ml	0·09, 0·09, 0·10	—	—
Glycine solution, 4·98 ml* *plus* glucose solution, 1 ml	4·96, 4·98, 4·99	4·87, 4·89, 4·90	98, 98, 98
pH titration method—			
Reagent blank solution ..	0·03	—	—
Glycine solution, 1·02 ml*	1·03, 1·03, 1·05 1·07, 1·08, 1·07	1·00, 1·00, 1·02 1·04, 1·05, 1·04	98, 98, 100, 102, 103, 102
Glucose solution, 0·2 ml ..	0·06, 0·06, 0·06, 0·07	—	—
Glycine solution, 1·02 ml* *plus* glucose solution, 0·2 ml	1·08, 1·08, 1·05 1·04, 1·05, 1·06	1·02, 1·02, 0·99 0·98, 0·99, 1·00	100, 100, 97, 96, 97, 98

* Calibrated pipettes.

DISTILLATION TIME—

Complete recovery of ammonia (equivalent to 200 μg of nitrogen) from standard ammonium sulphate was obtained by distillation for 3 minutes. The recovery was not reduced by prolonging the distillation time to 8 minutes, though the pH reached 7·83; thus it appeared that this prolonged exposure to the atmosphere resulted in no loss of ammonia from the titration beaker by evaporation from the mildly alkaline solution.

RESULTS

COMPARISON WITH THE ORIGINAL METHOD—

A standard glycine solution, containing 0·000100 g of nitrogen per ml, was prepared from glycine obtained from The British Drug Houses Ltd. (assay ex nitrogen, not less than

99 per cent.). Nitrogen recovery was investigated by both the indicator and the pH titration methods.

The indicator titration method was that used by Challinor and Burroughs,[5] except that hydrogen peroxide was not added during the digestion and the whole of the digest was taken for the titration.

By using both methods the glycine solution was digested with and without the addition of analytical-reagent grade glucose added as a 10 per cent. solution in an amount that would represent the carbohydrate in a fairly dry cider. The results are shown in Table I.

For the digestion of glycine without carbohydrate, the average nitrogen recovery obtained by using either method was close to 100 per cent.

When glucose was added to the glycine a small loss of nitrogen (not more than 4 per cent.) apparently occurred.

Both methods were then used to analyse four cider samples and the results are shown in Table II.

TABLE II

NITROGEN CONTENT OF CIDERS BY USING THE MARKHAM AND THE pH TITRATION METHODS

Sample	Nitrogen found by using Markham method, per cent. w/v	Nitrogen found by using pH titration method, per cent. w/v
Cider A, without centrifugation ..	0·0034, 0·0035, 0·0035	0·0036, 0·0036, 0·0036
Cider B, without centrifugation ..	0·0039, 0·0039, 0·0039	0·0039, 0·0040, 0·0040
Cider A, with centrifugation	0·0013, 0·0014, 0·0014	0·0017, 0·0017
Cider B, with centrifugation	0·0015, 0·0014	0·0015, 0·0013

CONCLUSIONS

The reduction in the size of the sample and the use of pH titration give results in satisfactory agreement with those obtained by the established Markham semi-micro method for ciders and apple juices. The two methods gave results differing by as much as 0·0003 per cent. w/v of nitrogen for a sample of cider that had been spun in a centrifuge, see Table II. Because this error is relatively larger at low nitrogen levels of, say, 0·001 per cent., it is suggested that, for such low levels, the sample size would need to be increased, but this is not a serious limitation in cider analysis. The method has been in use for two years.

The pH titration method permits batches of six determinations to be carried out in less than 75 minutes.

In the possible application of this method to solid foods, difficulties in obtaining a representative small sample would presumably arise. Solid foods, for example, cereals, generally have much higher nitrogen contents than those for which pH titration is advantageous.

However, there would appear to be no obvious reason why the method should not be applicable to other beverages or biological samples where no heterocyclic nitrogen compounds that resist digestion are encountered.

We thank the Directors of Messrs. William Gaymer & Son Limited and Messrs. Showerings Limited for their encouragement and for permission to publish this Paper.

We thank particularly Dr. L. F. Burroughs of the University of Bristol Research Station, Long Ashton, for helpful criticism and advice, and Mrs. M. J. Macfarlane for assistance in preparing this Paper.

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Received *November 19th*, 1963

SHORT PAPERS

A Karl Fischer Titration Unit for Routine Use

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An electrometric procedure for direct titration of water with Karl Fischer reagent is the so-called "dead-stop end-point" method. This depends on the decrease in the apparent resistance between two platinum electrodes immersed in the solution when the end-point is reached. Carter and Williamson[1] devised a simple routine method involving the use of a circuit comprising a shunted galvanometer and a resistance in series with the electrodes. When a small e.m.f. is applied to the circuit, the current that flows on the addition of Karl Fischer reagent remains fairly constant during titration, but suddenly increases at the end-point.

The sensitivity of this method may be expressed in terms of scale usage, that is—

$$\frac{\text{(final reading)} - \text{(initial reading)}}{\text{(final reading)}}$$

If the apparent resistance between the electrodes falls from S to P ohms and the resistance in series with the electrodes has a value of R ohms, then the scale usage is given by—

$$\frac{S - P}{S + R}$$

Fig. 1 shows the variation of scale usage with the value of R, with the figures given by Carter and Williamson[1] for the fall from S to P ohms. With higher values of R, in which instances electrolysis may be neglected, scale usage becomes small, hence the method is insensitive at low currents.

If, however, variations in the voltage are used, rather than the variation in current, along the lines of the work by Brown and Volume,[2] a considerable improvement in scale usage can be obtained. The present application consists of removing the shunted galvanometer in the simple circuit described above and placing an extremely-high-resistance voltmeter in parallel with the cell to measure the potential difference across the cell instead of the current passing through it. The scale usage in this case is given by—

$$\frac{R\,(S - P)}{S\,(R + P)}$$

The variation of this expression with the value of R is also shown in Fig. 1, which shows that a value of 0·9 is reached when R is only 10 kilohms. Thus the method becomes more sensitive as the current is decreased and electrolysis avoided.

Method

Apparatus—

A feature of the Karl Fischer reaction is that there is a time lag of a few seconds between the addition of the reagent and the completion of the reaction. As the end-point is approached, premature end-points are indicated, excess of iodine being present for short periods. It follows that a high-resistance voltmeter constructed from a galvanometer with a series resistance would be extremely difficult and tedious to use because of its relatively long period. There remain two alternatives, a valve or a transistor voltmeter.

Commercial high-resistance voltmeters of this type are expensive and, for the purpose of this work, unnecessarily complicated. A simple, inexpensive unit has, therefore, been designed and constructed. It incorporates a 150 kΩ per V transistorised voltmeter of a standard bridge type.[3] The electrode system consists of two platinum wire loops as described by Carter and Williamson, but the leads from the titration unit are soldered directly to the platinum. Magnetic stirring is used.

PROCEDURE—

The procedure for setting up the indicating unit shown in Fig. 2 is given below. Put the sensitivity control, R_2, to its mid-position and turn the four-pole three-way switch to the second position, "set zero." The test cell is now short-circuited. Adjust the resistor, R_5, for zero meter

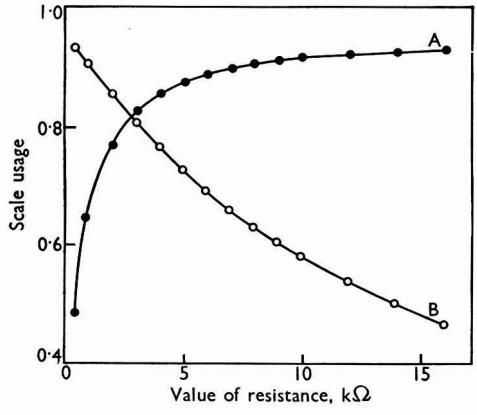
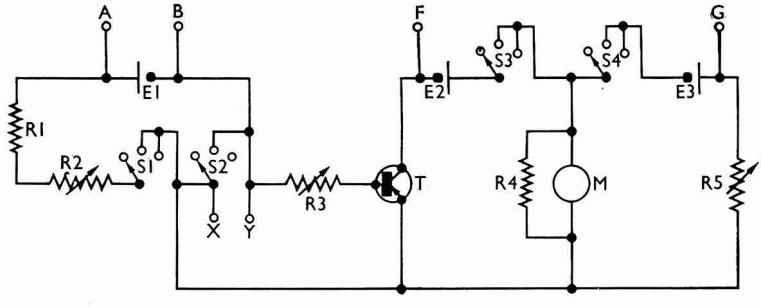


Fig. 1. The effect of the value of the resistance on the value of the scale usage when A, voltage change and B, current change is used for dead-stop end-point



A,B,F,G = Sockets for testing batteries on load
X,Y = Cell electrodes
E_1,E_2,E_3 = 1·5-V Ever Ready U2 batteries
S_1,S_2,S_3,S_4 = 4-pole 3-way wafer switches
M = 100-μA moving-coil meter
T = Mullard OC71 transistor
R_1 = 3300-ohm resistor
R_2,R_4 = 10,000-ohm resistors
R_3 = 0·5-megohm resistor
R_5 = 100,000-ohm resistor

Fig. 2. Circuit diagram of the Karl Fischer indicating unit

reading. Turn the four-pole, three-way switch to the final position, "run," and adjust the resistor, R_3, for full-scale deflection. Run Karl Fischer reagent into the cell, and adjust R_2 to give a suitable sensitivity. The value of R_2 need not be changed or tested again unless the type of sample or test cell is changed, but R_3 and R_5 may need slight adjustment before each titration.

The premature end-points appear as needle "kicks," the kicks becoming larger as the end-point is approached.

The unit shown in Fig. 2 has facilities for battery checks with an external voltmeter. In a later version, the indicating meter is used for this purpose and a "battery check" position provided on the multi-pole switch.

Results

The instruments are in continuous use and the readings that are given in Table I indicate the reproducibility possible with the method. Because the Karl Fischer reagent is unstable only the titrations within a day may be compared.

Table I

Titration of 5 ml of water with Karl Fischer reagent

Day	Volume of Karl Fischer reagent, ml	Mean, ml	Standard deviation[4]
1	2·55, 2·55, 2·65, 2·50, 2·55, 2·60, 2·50	2·56	0·06
2	5·35, 5·20, 5·20, 5·20, 5·25, 5·20, 5·15	5·22	0·07

The standard deviations of the titres are similar to those published by other workers[1,2] and the end-point is easily recognised by the operator.

Distilled water is used here as the primary standard to determine the water equivalent of the Karl Fischer reagent, and sodium acetate in dry methanol is used for check samples. The results given in Table II for the water content of sodium acetate indicate the accuracy of the method.

Table II

Water content of sodium acetate

Day	Water, per cent.	Mean	Standard deviation[4]
1	40·3, 40·0, 40·2	40·2	0·2
2	39·8, 40·3, 39·8	40·0	0·3
3	40·6, 40·3, 40·4	40·4	0·2
4	39·4, 40·0, 39·9	39·8	0·3
5	40·2, 40·7, 39·2	40·0	0·9

The theoretical value for the water content of sodium acetate trihydrate, $CH_3COONa.3H_2O$, is 39·75 per cent. These figures show that the unit gave results that were above the true value by, on average, less than 1 per cent. This is a satisfactory accuracy.

In the literature, reviewed by Mitchell and Smith,[5] the use of sodium acetate as a standard is sometimes criticised because of an alleged variability in moisture content, but no difference has been found here between bottles nor has sodium acetate been found to absorb moisture when kept in a bottle that is opened frequently. However, the alternative recommended standard material, sodium tartrate, has been tried for comparison purposes. It was found inconvenient to use because a quantitative transfer of the salt had to be made into the Karl Fischer reaction vessel. The results obtained are given in Table III.

Table III

Water content of sodium tartrate

Day	Water, per cent.	Mean	Standard deviation[4]
1	15·4, 15·5, 15·6	15·5	0·1
2	15·5, 15·5, 15·5	15·5	—

The theoretical value for the water content of sodium tartrate dihydrate, $[CH(OH)COONa]_2.2H_2O$,

is 15·65 per cent., so, again, the indicated accuracy is satisfactory.

I am indebted to Mr. D. H. Oliver for his help in testing the titration unit.

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Received *August 30th*, 1963.

Determination of Quaternary Ammonium Compounds as Phosphotungstates

By W. M. BANICK, jun., and J. R. VALENTINE

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Lincoln and Chinnick[1] have described a method for determining surface-active quaternary ammonium compounds that is based on precipitating the quaternary ammonium compound as a phosphotungstate.[2] After the precipitate had been dried at 105° C, it was shown to have the composition, $Q_3PO_4.12WO_3$, where Q is the quaternary ammonium cation.[1,2]

We have found a class of surface-active quaternary ammonium compounds that yield phosphotungstate precipitates whose composition appears to be $Q_2HPO_4.12WO_3$. This class of compounds may be represented by the general formula—

$$[CH_3(CH_2)_nCONH(CH_2)_3N(CH_3)_3]^+X^-$$

Experimental

Two members of the class of quaternary ammonium compounds were studied. They were—

(3-myristamidopropyl)trimethylammonium chloride—

$$[CH_3(CH_2)_{12}CONH(CH_2)_3N(CH_3)_3]^+Cl^- \quad .. \quad .. \quad .. \quad \textbf{(I)}$$

and

(3-lauramidopropyl)trimethylammonium methyl sulphate—

$$[CH_3CH_2)_{10}CONH(CH_2)_3N(CH_3)_3]^+CH_3SO_4^- \quad .. \quad .. \quad .. \quad \textbf{(II)}$$

The phosphotungstates of these compounds were precipitated, dried and ignited as described by Lincoln and Chinnick.[1] The dried precipitates were also analysed for carbon and hydrogen. The precipitations were found to be quantitative.

Results

Analytical results are summarised in Table I. The results indicate that the precipitate has the composition, $Q_2HPO_4.12WO_3$, and not the composition $Q_3PO_4.12WO_3$ found for the other quaternary ammonium compounds. The uniquely different composition is probably the result of the amido group in the quaternary cation.

Table I

Loss on ignition and carbon and hydrogen analyses of phosphotungstate precipitates

	Quaternary ammonium compound	Loss on ignition, per cent.*	Carbon, per cent.	Hydrogen, per cent.
(I)	$[CH_3(CH_2)_{12}CONH(CH_2)_3N(CH_3)_3]^+Cl^-$	19·83†	14·16†	2·69†
	Calculated for $Q_2HPO_4.12WO_3$	18·99	13·60	2·48
	Calculated for $Q_3PO_4.12WO_3$	25·84	18·67	3·37
(II)	$[CH_3(CH_2)_{10}CONH(CH_2)_3N(CH_3)_3]^+CH_3SO_4^-$	17·71†	12·67†	2·43†
	Calculated for $Q_2HPO_4.12WO_3$	17·68	12·44	2·29
	Calculated for $Q_3PO_4.12WO_3$	24·18	17·18	3·12

* The theoretical loss on ignition was calculated by using $HPO_4.12WO_3$ as the composition of the ignition residue.

† These results are the mean of duplicate determinations.

Purity of compounds—

The apparent molecular weight of compound (**I**), F.W. = 363·0, was found by macro chloride determinations to be 380·4, 381·1. The apparent molecular weight based on the loss on ignition of the phosphotungstate precipitate of composition $Q_2HPO_4.12WO_3$ was 380·2, 381·9.

The excellent agreement of the molecular-weight values, which in one instance was based on determination of the anion and in the other on the cation, convinced us that compound (**I**) was contaminated with an impurity similar in structure but with a higher molecular weight. We discovered that one of the reactants from which compound (**I**) was prepared was not pure. No attempt was made to purify compound (**I**).

An elemental analysis was carried out on compound (**II**) and the results listed below were obtained—

	Carbon	Hydrogen	Nitrogen	Sulphur
Calculated, per cent. ..	55·6	10·31	6·82	7·81
Found, per cent.	55·1	9·43	6·61	7·63

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Received *October 2nd*, 1963

The Detection and Estimation of Aflatoxin in Groundnuts and Groundnut Materials

Part III.* Classification of Aflatoxin B_1 Levels

BY TREVOR J. COOMES, P. C. CROWTHER, B. J. FRANCIS AND G. SHONE

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Two methods for assessing the aflatoxin levels in groundnuts and groundnut materials have been published.[1,2] Both the paper-chromatographic method of Coomes and Sanders[1] and the thin-layer chromatographic plate technique of Broadbent, Cornelius and Shone[2] dealt with the detection and subjective estimation of aflatoxin B, and both methods were based on the smallest weight of that compound giving an observable fluorescence in ultraviolet light ($\lambda = 365$ mμ).

Since more powerful chromatographic techniques have been used, it has now been shown[3] that the material originally called aflatoxin B actually consisted of two substances, aflatoxin B_1, the major component, whose structure is now known,[4] contaminated with aflatoxin B_2, the corresponding dihydro derivative,[5,6] and other impurities.

The two methods[1,2] have now been re-examined in the light of our present knowledge, particularly in view of the fact that it is not possible to effect the separation of aflatoxin B_1 and B_2 by either technique, although they may be separated from the related metabolites of *Aspergillus flavus* called aflatoxins G_1 and G_2.[7] From our own work and from that of Hartley, Nesbitt and O'Kelly,[5] we now know that the amounts of B_2 in both naturally and artificially produced aflatoxin are negligible. It follows that reliable estimates of the aflatoxin B_1 content are therefore possible with the procedures already described.[1,2] Further, since the toxicity of aflatoxin B_2 is much less than that of B_1 when day-old ducklings are used as test animals,[5] reliable estimates of potential toxicity may be based on a knowledge of the aflatoxin B_1 content of a given sample.

By using a Phillips type HPW, 125-watt fluorescent lamp ($\lambda = 365$ mμ) in a suitable starter unit, the smallest weight of pure aflatoxin B_1 that gives an observable fluorescence on Whatman No. 1 filter-paper after development in the solvent system described[1] and placed 30 cm from the excitation source, has been determined as 0·1 μg. Under the same excitation conditions, on alumina (M. Woelm; neutral) chromatographic plates, 740 $\pm$ 10 μ thick, the smallest weight of this compound that gives an observable fluorescence, after development under the prescribed conditions,[2] is 0·003 μg.

It is therefore recommended that the aflatoxin B levels set out in Table I (p. 211) of the paper-chromatographic procedure[1] and those contained in Table I (p. 216) of the thin-layer chromatographic procedure[2] be revised in terms of aflatoxin B_1 levels as shown in Table I of this Paper.

Both Coomes and Sanders[1] and Broadbent, Cornelius and Shone[2] have described a more definite determination of the aflatoxin content of a sample extract by noting the size of the smallest volume giving an observable fluorescence. This volume is now taken to contain 0·1 μg of aflatoxin B_1 if the former method is used, and 0·003 μg if the thin-layer technique is used.

It has been shown in this Institute that by using the technique whereby the extract is diluted until no fluorescence is observed on the alumina thin-layer plates an over-estimate of the aflatoxin

* For details of earlier parts of this series, see reference list, p. 437.

content may be made for certain samples. Those samples giving dark-coloured methanolic extracts, and in particular, meals that might have been subjected to high temperatures during the process of expelling the oil on the commercial scale have been found suspect in this connection.

TABLE I

CLASSIFICATION OF AFLATOXIN B_1 LEVELS

Size of portion used in—		Concentration of aflatoxin, p.p.m.		Aflatoxin B_1 level if fluorescence is observed
paper-chromatographic method	thin-layer chromatographic method	No fluorescence	Fluorescence	
6·25 μl from 5 ml	5 μl from 35 ml	1·0	1·0	Very high
25 μl from 5 ml	20 μl from 35 ml	0·25	0·25 to 1·0	High
125 μl from 5 ml	15 μl from 5 ml	0·05*	0·05 to 0·25	Medium

* Samples containing less than 0·05 p.p.m. of toxin are classified as of low or zero aflatoxin B_1 level.

A similar procedure to the published thin-layer procedure,[2] but with thin layers of kieselgel G, which have already been used[3] for exploratory purposes in connection with the toxic metabolites of *A. flavus*, has been found more sensitive. Current work on extracts of groundnuts with this adsorbent in thin layers has indicated that more satisfactory estimates of known aflatoxin B_1 content are feasible by using the technique of diluting the extract until no fluorescence is observed on the plates, although over-estimates are still possible with certain samples.

A fully quantitative assay procedure, based on non-subjective assessment of fluorescence, involving the use of thin layers of kieselgel G is now being studied in this Institute. In the meantime, the thin-layer procedure, in which aluminium oxide[2] is used, is recommended for category determination only, as indicated in Table I of this Paper.

We thank Dr. Brenda Nesbitt for the samples of pure aflatoxin B_1.

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NOTE—References 1 and 2 are to Parts I and II of this series, respectively.

Received *January 3rd*, 1964

Book Reviews

ZONE ELECTROPHORESIS IN BLOCKS AND COLUMNS. By H. BLOEMENDAL. Pp. viii + 219. Amsterdam, London and New York: Elsevier Publishing Company. 1963. Price 40s.

Since the introduction of starch-gel electrophoresis in the mid 'fifties by Smithies, many new media have been introduced to this technique, notably agar, silica, Sephadex and acrylamide. Almost every laboratory working with these techniques devised its own apparatus. Dr. Bloemendal's book therefore, is a welcome guide to a maze of miscellaneous information on what essentially remains an individualistic technique. This monograph presents the judgment of the author, who apparently has first-hand experience of these diverse techniques, and who is thus in a position to indicate their correct use in the fields for which they are most suited. Basic principles are given their mathematical treatment in a brief formulation that might be, perhaps, too brief for the uninitiated.

It is not a book for beginners, but a useful addition to the bookshelf of the analyst who uses these techniques or who is in search of new techniques. Not only is the book almost free of errors and misprints, but it seems entirely devoid of superfluous words, which in spite of its small format and its 200-odd pages, makes it good value for money. EINHART KAWERAU

CHROMATOGRAPHIC METHODS. By R. STOCK, B.Sc., Ph.D., F.R.I.C., and C. B. F. RICE, B.Sc., F.R.I.C. Pp. viii + 206. London: Chapman and Hall. 1963. Price 40s.

This book presents a treatment of the current techniques that are in use in the various methods of separating mixtures and that are included under the general title "Chromatography," and as such is an invaluable addition to the increasing amount of literature on these subjects. There are, in essence, 5 chapters in this book, each chapter attempting to provide a link with the other, to emphasise the complementary nature of chromatography, but each dealing with a specific aspect of the subject. Thus there are chapters on Classification—Chromatographic Methods; Liquid-phase Chromatography on Columns; Paper Chromatography; Gas Chromatography, and the final chapter describes model experiments in chromatographic techniques: there is a useful bibliography at the end of each chapter. In the first 4 chapters stress is laid on the general practical approach to the subject, with the obvious variations, and in the last chapter and the Appendix, recipe-type instructions are given.

Although practitioners of the art may disagree with the degree of stress that should be placed on a particular technique, each technique is adequately and concisely explained. Particularly noteworthy is a judicious blending of the use of experiments involving inorganic or organic solutes, which in turn reflects the interests of the authors. I feel, however, that criticism must be made of the experiments illustrating gas chromatography, since the gas-chromatographic chapter introduces the subject, and concerns itself with the practical side of the subject, detectors (including the latest ionisation types), the operation of capillary columns and the theory of gas chromatography (including the determination of partition coefficients). The experimental chapter seems to fail to illustrate these points in view of the importance and attention that is being paid to this technique, although the Appendix does include a brief account of the construction of a katharometer or flame ionisation detector.

In general, this is a book that should be read widely in teaching institutions, especially by students, since its cost should present little difficulty to their means. G. NICKLESS

MISES AU POINT DE CHIMIE ANALYTIQUE PURE ET APPLIQUÉE ET D'ANALYSE BROMATOLOGIQUE. Edited by J.-A. GAUTIER. Neuvième Série. Pp. 209. Paris: Masson et Cie. 1961. Price 40 NF.

MISES AU POINT DE CHIMIE ANALYTIQUE PURE ET APPLIQUÉE ET D'ANALYSE BROMATOLOGIQUE. Edited by J.-A. GAUTIER. Dixième Série. Pp. 257. Paris: Masson et Cie. 1962. Price 55 NF.

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

The 11th issue of these reviews marks not only a minor change in the title, but also, more significantly, a change in editorial policy. Future issues will devote more space to reaction mechanisms and fundamental physico-chemical aspects of chemistry, particularly organic chemistry, as well as contributions dealing with the applications of analytical chemistry in industry and technology. Professor Gautier in his foreword to the 11th issue sees this change as part of a general broadening of the definition and scope of analytical chemistry. He strongly deplores the present tendency to over-specialisation and the increasing sub-division of the sciences into separate compartments. To deal with analytical chemistry on this broad basis is to be welcomed, for no branch of human endeavour benefits by isolating itself.

Evidence of a change in policy can be seen in the previous issues (9th and 10th) and more so in the current (11th) issue, which includes reviews on electrochemical reactions in acetonitrile, coupling reactions of diazo compounds, analytical and biological aspects of food preservatives, a theoretical comparison of chromatography and fractional distillation, and the anthocyanines.

The determination of moisture and total solids in food and drug samples may still present the analyst with problems. These determinations were dealt with in previous issues, but are described at length in the 10th issue together with the Karl Fischer method and physical methods of moisture determination making use of dielectric properties.

Physical methods are well represented in the 9th and 10th issues by several contributions on infrared and Raman spectroscopy, and chromatography, including gas chromatography. The full potentialities of gas chromatography are not always fully developed, particularly with respect to the use of the more sensitive ionisation detectors.

An item of special interest in the 10th issue is one dealing with the "osmopile." Berton applied this name to a device by which small amounts of vapour are detected in a gas stream passing over an electrode in contact with an electrolyte that is reactive towards the vapour in the gas stream. The potential set up at the electrode serves to detect the presence of vapours in the gas stream. Typical electrolytes for organic vapours are strong oxidising mixtures, such as chromic acid - sulphuric acid mixture. The name "osmopile" appears to be derived from the Greek "osmein," meaning to smell. Examples are quoted of its use as a detector in gas chromatography, and for this purpose it may offer some advantages as a selective detector for various functional groups. Attention is directed to this contribution because accounts of this device do not appear to be readily accessible in English-language publications. P. MORRIES

TABLES OF SPECTROPHOTOMETRIC ABSORPTION DATA OF COMPOUNDS USED FOR THE COLORIMETRIC DETERMINATION OF ELEMENTS. Prepared by the Commission on Spectrochemical and Other Optical Procedures for Analysis of the International Union of Pure and Applied Chemistry. Pp. xvi + 626. London: Butterworth & Co. (Publishers) Ltd. 1963. Price 130s.

The text of this compilation reviews spectrophotometric data for 73 elements listed according to the alphabetical order of their chemical symbols. The coverage of the text is even wider than this suggests, because some of the headings incorporate sub-sections for different ionic species. Thus under "Nitrogen," classification exists for nitrite, nitrate and ammonia, whereas under "Chlorine" we find chlorine, chloride, hypochlorite, chlorine dioxide, chlorate and perchlorate. The report is the result of the work of analytical chemists in many countries, and is presented throughout in English, French and German. For unknown reasons, however, the introduction is presented bilingually in English and French only.

The information given in this text is usefully concise, and is of considerable value. The recorded data include, wherever possible, the structural formula of the reagent and its complex, the nature of the solvent, a graphical representation of the absorption spectra, the extinction coefficient, Beer's law range, minimum working instructions, interferences and key references.

Only the most well tried methods are considered, and these are arranged according to the oxidation state of the element and in alphabetical order of the trivial or systematic name of the reagent. The various oxidation states of each element are dealt with in order of increasing oxidation number. The coverage of the text extends up to work recorded in *Chemical Abstracts* for December, 1955, but in fact several of the authors of sections have included references up to 1959. It may give some idea of the scope if it is recorded here that for copper^{I} there are 41 entries (many of them 1,10-phenanthroline derivatives); for copper^{II}, 17; for palladium, 1; for nickel, 25; for titanium, 9; for lithium, 2; for iron^{II}, 17; for iron^{III}, 38; for aluminium, 5; for zinc, 10; for ytterbium, 1; and so on.

Altogether, this is a useful compilation to have in any laboratory concerned with trace-metal (and non-metal) analysis. Many of the interesting new reagents that have been developed recently as a result of work in the allied field of complexometric analysis are missing from these pages. This is probably inevitable in a co-operative book of this nature, and provided that this limitation is recognised by the user, it only detracts slightly from the value of the book. The volume itself is neatly bound and well set out, but the cost is somewhat high. T. S. WEST

FOOD COMPOSITION AND ANALYSIS. By HOWARD O. TRIEBOLD, Ph.D., and LEONARD W. AURAND, Ph.D. Pp. viii + 497. Princeton, N.J., New York, Toronto and London: D. Van Nostrand Company Inc. 1963. Price 95s.; $12.50.

This book has been written by two American Professors of Food Technology, and it seems to be designed mainly as a textbook for a course in their own and similar institutions. The preface claims that it is also of value to those doing research, but this is only true in the sense that it could provide useful information to a non-specialist enquirer; primarily it is a student's book.

The scope of the book is considerable, although it is not, and does not claim to be, comprehensive. The first chapter deals with food laws and standards, and gives a short, but useful, account of the working of the American system. The next two chapters deal with sampling, proximate analysis and physical methods. Procedures are given for density determination, refractometry, colorimetry and saccharimetry. Chromatography, ion exchange, polarography and other techniques of varying complexity up to nuclear magnetic resonance are discussed more briefly.

Chapter 4 consists of 95 pages on fat and fat products, and gives adequate and useful accounts of the analytical methods used and the interpretation of the results. The 75 pages of Chapter 5 on sugars comprise 17 pages of analytical conversion and correction tables, and one complete page is devoted to a single structural formula of raffinose. Lead in maple sugar is referred back to an electrolytic method for copper on page 241 (*sic*). The 48-page chapter on cereals is short in relation to the importance of the subject. The Pekar test is excellent as a student's exercise, but some mention at least should have been made of instrumental aids, such as the Kent-Jones and Martin grader or its American equivalent.

The chapter on milk contains a 5-page account of the use of the Hortvet cryoscope; the thermometer is standardised with 7 and 10 per cent. sucrose solutions. The chapter on meat and its products is extremely short.

Food additives are discussed in a balanced and reasonable way in Chapter 9, which deals with food contamination, deterioration, and preservation. Pesticides are mentioned only briefly. Food colours are dealt with separately in a short chapter, perhaps because any such account of food colours must be virtually out of date before it is published, owing to the rapid development of the subject. It should, however, have been possible to include the new Colour Index instead of the old edition in the list of references, and to have ensured that the table transcribed from the 7th edition of the Official Methods of the A.O.A.C. was correctly annotated in respect of oil-soluble colours.

The last chapter, on food flavours and flavouring agents, includes a section on flavour evaluation by taste panels, and sections on common spices, aromatic seeds and herbs. Photographs purporting to show the microscopical characteristics of starches are reproduced, but as so often happens, the results are not a complete success. The chapter also contains the usual methods for the chemical analysis of spices.

The authors, according to their preface, have set out to meet a need for a textbook for training food technologists, combining both lecture material and laboratory experiments. In this they have succeeded, and for students in the United States and in those countries that take their food laws and methods from the United States, they may have succeeded very well. The book, however, cannot be unreservedly recommended for the use of students in countries whose food laws, regulations and methods have developed differently.

E. I. JOHNSON

Erratum

MARCH (1960) ISSUE, p. 176, 1st line under "PROCEDURE FOR LARGE QUANTITIES OF SILVER."
For "stage 4" *read* "stage 9."