THE ANALYST

EDITORIAL

Notice to Authors

A REVISED version of our standing instructions to those offering Papers or Notes for inclusion in *The Analyst* appears under this heading on the last two text pages of this issue.

The continually increasing cost of production is making it more imperative than ever that the length of papers be kept to a minimum. Certain revisions in the Notice to Authors are intended to help this. For example, detailed descriptions of preliminary work are no longer held to be justified, and the recommended scheme of presentation now omits a specific experimental section. Any mention of preliminary experiments is to be included in the introduction, together with the bare minimum of historical background necessary to introduce the new work. Often a simple statement will be sufficient to indicate the object of the investigation in which the method was developed. Though credit must always be given to the originators of ideas, references to other workers who may have developed some particular method should be restricted to those whose work directly inspired the author's proposed method.

PROCEEDINGS OF THE SOCIETY FOR ANALYTICAL CHEMISTRY

ORDINARY MEETING

AN Ordinary Meeting of the Society was held at 6.30 p.m. on Wednesday, October 2nd, 1957, in the meeting room of the Royal Society, Burlington House, London, W.1. The Chair was taken by the President, Dr. J. H. Hamence, M.Sc., F.R.I.C.

The following papers were presented and discussed: "The Analysis of 'Ferrites' by Means of EDTA," by D. G. Timms, B.Sc., A.R.I.C.; "The Determination of Mercury by Direct Distillation in its Compounds and Preparations," by H. E. Brookes, B.Sc., F.R.I.C., and L. E. Solomon, B.Sc.; "A System for the Determination of Certain Trace Metals in Crops," by W. D. Duffield; "Some Applications of X-ray Spectrography," by H. I. Shalgosky, B.Sc., A.R.I.C.

DEATHS

WE record with regret the deaths of

Archibald Prideaux Davson
James Gray
Adrian Joseph Clifford Lickorish.

MIDLANDS SECTION

AN Ordinary Meeting of the Section was held at 6.30 p.m. on Thursday, September 12th, 1957, in the Mason Theatre, The University, Edmund Street, Birmingham, 3. The Chair was taken by the Vice-Chairman of the Section, Dr. S. H. Jenkins, F.R.I.C., F.Inst.S.P.

A discussion on "The Determination of Some Inorganic Substances in Trade Effluents," was opened by N. T. Wilkinson, F.R.I.C.

The Volumetric Determination of Tin in Titanium and its Alloys

By H. J. G. CHALLIS and J. T. JONES

Procedures have been established for the determination of tin in titanium and its alloys. For alloying amounts, the method involves the direct reduction of tin with hypophosphite and volumetric determination with potassium iodate. Tests with solutions containing known amounts of titanium, tin and other metals indicate that recovery is satisfactory for amounts of tin above 0·2 per cent. and that no interference is caused by likely alloying metals. The recommended procedure is simple, rapid and particularly suitable for control analysis. No difficulty has been experienced in applying the method to a wide range of titanium - tin alloys.

For impurity amounts of tin below 0·2 per cent., a modification is necessary and it involves the preliminary separation of tin as sulphide in the presence of a carrier and then by reduction with hypophosphite and volumetric determination of the separated tin. Experiments have proved that recovery of tin is satisfactory for amounts as small as 0·002 per cent.

Titanium alloys containing tin and aluminium are of increasing importance when higher creep resistance is required, and consideration has therefore been given to the determination of tin in the presence of large amounts of titanium.

When this investigation was commenced, no published methods were available and a review of possible methods indicated that the hypophosphite reduction procedure[1,2] offered the most promising approach. Subsequently, however, Norwitz and Codell[3] recommended a method for alloying amounts of tin in titanium alloys based on the preliminary separation of tin as sulphide, but they found that copper and molybdenum (above 0·5 per cent.) interfered with the determination. A later method,[4] in which tin is reduced directly by iron powder, is stated to be ineffective in the presence of copper, and chromium and vanadium at concentrations above 5 per cent. definitely interfere.[5]

The experimental work described in this paper has led to the development of a rapid and direct method for tin in titanium - tin alloys, and reference is made to a modification suitable for the determination of tin present as an impurity in titanium metal.

Experimental

Effect of titanium—

During preliminary tests on the reduction of tin in the presence of titanium and diluted hydrochloric acid (1 + 1), the addition of sodium hypophosphite resulted in the formation of a dense flocculent precipitate. However, it was found that, when the solution was boiled, the precipitate dissolved to leave a clear pale-blue solution in which, after cooling, the reduced tin could be titrated with potassium iodate and the starch - iodine end-point easily observed.

Further tests on solutions containing the equivalent of 0·2 or 0·5 g of titanium with various amounts of tin added, indicated that the recovery of tin by the proposed method was satisfactory over the range of 0·1 to 25 per cent., as shown by the results in Table I.

Effect of other metals—

From previous work on copper-base alloys,[2] it was known that aluminium, iron, lead, manganese and nickel do not interfere with the volumetric determination of tin, and interference by precipitated arsenic, selenium and tellurium can be avoided by filtration. As titanium - tin alloys usually contain aluminium, confirmation was obtained of the non-interference of up to 10 per cent. of aluminium. In addition, possible interference by other metals likely to be present in titanium alloys, namely chromium, molybdenum or vanadium, was determined for a series of solutions containing the metal under investigation with 1 to 10 per cent. of tin and the appropriate amount of titanium to bring the total sample weight to 0·2 g. In addition, the effect of copper and tungsten, which may be present as impurities, was studied and the results of these tests are given in Table II.

TABLE I

RECOVERY OF TIN ADDED TO SOLUTIONS CONTAINING TITANIUM

Tin added		Tin recovered,
g	%	%
In presence of 0·2 g of titanium—		
Nil	Nil	Nil
0·0010	0·50	0·49
0·0020	1·00	1·00
0·0030	1·50	1·42
0·0050	2·50	2·45
0·0100	5·00	4·90
0·0200	10·00	9·98
0·0500	25·00	25·00
In presence of 0·5 g of titanium—		
Nil	Nil	Nil
0·0005	0·10	0·12
0·0010	0·20	0·20
0·0015	0·30	0·26
0·0025	0·50	0·49
0·0050	1·00	1·03
0·0100	2·00	2·00
0·0250	5·00	4·97

TABLE II

EFFECT OF METALS ON THE RECOVERY OF TIN

Metal	Amount of metal present		Titanium present		Tin present		Tin recovered,
	g	%	g	%	g	%	%
—	Nil	Nil	0·180	90·0	0·0200	10·00	9·98
Aluminium	0·02	10·0	0·178	89·0	0·0020	1·00	1·01
Chromium	0·02	10·0	0·178	89·0	0·0020	1·00	1·01
	0·02	10·0	0·160	80·0	0·0200	10·00	9·96
	0·06	30·0	0·120	60·0	0·0200	10·00	9·94
Vanadium	0·02	10·0	0·178	89·0	0·0020	1·00	1·01
	0·02	10·0	0·170	85·0	0·0100	5·00	5·04
	0·02	10·0	0·160	80·0	0·0200	10·00	9·98
	0·06	30·0	0·120	60·0	0·0200	10·00	9·97
Molybdenum	0·02	10·0	0·178	89·0	0·0020	1·00	1·01
	0·02	10·0	0·170	85·0	0·0100	5·00	4·99
	0·02	10·0	0·160	80·0	0·0200	10·00	9·99
	0·06	30·0	0·120	60·0	0·0200	10·00	9·97
Copper	0·001	0·5	0·197	98·5	0·0020	1·00	1·02
	0·001	0·5	0·179	89·5	0·0200	10·00	10·05
	0·002	1·0	0·178	89·0	0·0200	10·00	10·27
	0·010	5·0	0·170	85·0	0·0200	10·00	10·33
Tungsten	0·004	2·0	0·196	98·0	Nil	Nil	Nil
	0·004	2·0	0·194	97·0	0·0020	1·00	1·00
	0·004	2·0	0·186	93·0	0·0100	5·00	5·01
	0·008	4·0	0·192	96·0	Nil	Nil	Nil
	0·008	4·0	0·190	95·0	0·0020	1·00	1·01
	0·008	4·0	0·182	91·0	0·0100	5·00	4·99
	0·008	4·0	0·172	86·0	0·0200	10·00	9·97

The results indicated that up to 30 per cent. of chromium, molybdenum and vanadium had no effect on the determination of tin in the range of 1 to 10 per cent. A slight yellowish pink colour was noted in the presence of molybdenum, but the end-point could be easily detected. Addition of copper resulted in some fading of the end-point with consequent over-titration to the extent that 5 per cent. of copper introduced a positive error of 0·3 per cent. on 10 per cent. of tin. However, in commercial practice, only impurity amounts of copper are encountered and, as indicated in Table II, amounts as large as 0·5 per cent. had no appreciable effect.

With tungsten, a deep-blue solution was produced on reduction with sodium hypophosphite, but the end-point could still be discerned and the recovery of tin was satisfactory.

Method

Reagents—

Potassium iodate solution, 0·02 N—Dissolve 0·7134 g of potassium iodate and 10 g of potassium iodide in water. Add 25 ml of sodium hydroxide solution (about 0·05 *N*) and dilute to 1 litre.

1 ml ≡ 1·187 mg of tin (theoretical value).

Standardise the solution against a known amount (about 0·04 g) of high-purity tin in the presence of 0·160 g of pure tin-free titanium by the recommended procedure.

Mercuric chloride solution, saturated—Dissolve 6 g of mercuric chloride in 100 ml of boiling water, allow to cool and decant from the crystals formed.

Starch solution, 1 per cent.—Make a paste of 1 g of soluble starch with cold water, pour it into 80 ml of boiling water, boil the solution for a few minutes, cool and dilute to 100 ml. This solution should be freshly prepared.

Procedure—

For tin contents exceeding 0·5 per cent., weigh 0·2 g of sample and, for 0·2 to 0·5 per cent., weigh 0·5 g of sample; transfer it to a 100-ml beaker. Add 30 ml of dilute sulphuric acid (1 + 4) and warm gently to assist solution (see Note). Maintain the level of solution by the addition of water. When the reaction ceases, cool and oxidise with a slight excess of nitric acid, sp.gr. 1·42, added dropwise, and heat again until tin is dissolved. Evaporate to fumes of sulphur trioxide, cool, and transfer to a 500-ml conical flask, using about 50 ml of water.

Add 50 ml of hydrochloric acid, sp. gr. 1·18, 1 ml of saturated mercuric chloride solution and 5 g of sodium hypophosphite. Insert a rubber bung provided with a delivery tube leading into 40 per cent. w/v sodium bicarbonate solution and boil the sample solution gently for 15 minutes. Cool to room temperature with the outlet of the delivery tube maintained below the surface of the bicarbonate solution. Add 2 g of potassium iodide, replace the rubber bung and delivery tube with an ordinary rubber bung or glass stopper, shake until the iodide has dissolved, add about 5 ml of starch solution and titrate with standard 0·02 *N* potassium iodate solution to the characteristic starch - iodine blue end-point.

Calculate the tin content of the sample.

Note—

Alternatively, the sample may be dissolved in 50 ml of hydrochloric acid, sp.gr. 1·18, in a 500-ml conical flask. This method of solution has the advantage that evaporation to fumes of sulphur trioxide is avoided, but care is necessary to avoid loss of hydrochloric acid, and the drillings must be finely divided.

Application of proposed volumetric method

The proposed procedure was applied to the determination of tin in a number of experimental titanium - tin alloys nominally containing from 5 to 27·5 per cent. of tin. The results, shown in Table III, indicate that the method provides reasonably reproducible figures.

Table III

Application of the method to titanium - tin alloys

Alloy mark	Nominal tin content, %	Tin content by volumetric method, %
A	5·00	4·56, 4·55
B	7·50	7·52, 7·49
C	10·00	9·54, 9·34
D	12·50	12·60, 12·30
E	17·50	17·12, 17·38
F	21·80	21·75, 21·81, 21·67
G	22·50	22·48, 22·75
H	25·00	24·83, 24·79
I	27·00	26·77, 26·94
J	27·50	27·45, 27·45, 27·45

DETERMINATION OF SMALL AMOUNTS OF TIN IN TITANIUM—

Experience indicated that, with a sample weight of 0·2 g, the proposed direct method was only suitable for amounts of tin in excess of about 0·5 per cent. By increasing the sample weight to 0·5 g, amounts as small as 0·1 per cent. could be determined, but the end-point became increasingly difficult to detect.

For amounts of tin smaller than about 0·2 per cent., a modification involving the separation, or concentration, of tin was considered advisable. A tentative method was drawn up in which a larger weight of sample (up to 10 g) was used, with a single precipitation of tin as sulphide in the presence of added cadmium to act as a carrier. Preliminary tests were made to ascertain if the separated tin could be titrated with 0·005 *N* iodate, particularly in the presence of any small amounts of titanium that might remain owing to incomplete separation.

Various amounts of tin in increments from 0·1 to 10 mg were titrated with 0·005 *N* iodate both in the absence of titanium and in the presence of 10 to 50 mg of titanium. The end-points were satisfactory and the results, which are shown in Table IV, indicate that recovery of tin was sufficiently accurate, provided at least 10 mg of titanium were also present. Accordingly, in the proposed method; the addition of 10 mg of titanium is recommended just before the reduction with hypophosphite.

TABLE IV

EFFECT OF TITANIUM ON DETERMINATION OF SMALL AMOUNTS OF TIN, 0·005 *N* IODATE BEING USED

Titanium present in solution, mg	Tin taken, mg	Tin determined, mg	Difference, mg
Nil	0·10	0·12	+ 0·02
	0·20	0·11	− 0·09
	0·50	0·47	− 0·03
	1·00	0·34	− 0·66
	10·00	7·95	− 2·05
	10·00	7·49	− 2·51
10	0·10	0·12	+ 0·02
	0·20	0·22	+ 0·02
	0·50	0·50	Nil
	1·00	1·02	+ 0·02
	10·00	9·98	− 0·02
20	10·00	9·94	− 0·06
50	10·00	10·04	+ 0·04

As it had been proved that amounts of tin as low as 0·1 mg could be reduced by hypophosphite and then determined satisfactorily by titration with 0·005 *N* iodate, the full procedure, including the sulphide separation, was applied to a series of solutions containing titanium and the equivalent of 0·002 to 0·05 per cent. of tin. Ammonium citrate was added

TABLE V

RECOVERY OF SMALL AMOUNTS OF TIN FROM SOLUTIONS CONTAINING TITANIUM AND COPPER

Weight of titanium, g	Tin added, %	Tin recovered, %
(*a*) *In absence of copper*—		
1·0	0·05	0·050
2·0	0·05	0·051
2·0	0·02	0·020
5·0	0·0040	0·0039
10·0	0·0040	0·0039
10·0	0·0020	0·0018
10·0	0·0020	0·0017
(*b*) *In presence of copper equivalent to* 0·1 *per cent.*—		
1·0	0·10	0·098
2·0	0·050	0·049
5·0	0·020	0·020
5·0	0·010	0·010
10·0	0·0020	0·0020

to prevent precipitation of titanium hydroxide during neutralisation of the excess of acid with ammonia solution and 10 ml of diluted sulphuric acid (1 + 1) per 400 ml were added before the precipitation of tin by hydrogen sulphide. The modification for small amounts of tin under 0·1 per cent., as detailed later, was found to be reasonably convenient and the results (see Table V (*a*)) were sufficiently accurate to a lower limit of 0·002 per cent.

Any copper present would be co-precipitated as the sulphide and, if in sufficient concentration, would interfere subsequently with the end-point. Some tests were carried out in which copper, equivalent to 0·1 per cent., was added to a further series of solutions containing titanium together with the equivalent of 0·01 to 0·10 per cent. of tin. In these tests, tin was separated from the combined sulphide precipitate with ammonium sulphide and subsequently determined in the copper-free solutions. The results, shown in Table V (*b*), indicate that this additional step effectively eliminated the interference by copper and its use is recommended when the presence of copper is known or suspected from the colour of the sulphide precipitate.

Modified procedure for tin contents below 0·2 per cent.

Reagents—

Titanium sulphate solution—Dissolve 0·5 g of pure tin-free titanium in 30 ml of dilute sulphuric acid (1 + 4), cool and dilute with water to 50 ml.

Potassium iodate solution, 0·005 N—Transfer 25 ml of 0·02 *N* potassium iodate (see p. 660) to a 100-ml calibrated flask and dilute to the mark with water,

1 ml ≡ 0·297 mg of tin (theoretical value).

Cadmium sulphate solution—A 10 per cent. solution of cadmium sulphate, $3CdSO_4.8H_2O$, in water.

Procedure—

Dissolve the recommended weight of sample in the appropriate amount of dilute sulphuric acid (1 + 4), as follows—

Tin present, %	0·05 to 0·2	0·02 to 0·05	0·005 to 0·02	< 0·005
Weight of sample, g	1	2	5	10
Dilute sulphuric acid (1 + 4) required, ml	50	100	200	300

Warm gently to assist solution and maintain the level of the solution by the addition of water. Oxidise with a slight excess of nitric acid, sp.gr. 1·42, added dropwise, and then boil for 2 to 3 minutes to remove nitrous fumes and ensure dissolution of tin. Dilute to about 300 ml, add 2 ml of cadmium sulphate solution and 5 g of ammonium citrate (10 g for the 10-g sample). Neutralise with diluted ammonium hydroxide (1 + 1), using methyl red as indicator, acidify with 10 ml of diluted sulphuric acid (1 + 1) and dilute to 400 ml. Warm to 80° C and pass hydrogen sulphide through the solution for 30 minutes.

Set the solution aside at room temperature for at least 4 hours, then collect the precipitate on a Whatman No. 42 filter-paper and wash it once with cold water containing a small amount of hydrogen sulphide (see Note). Dissolve the precipitate from the filter-paper with 50 ml of warm hydrochloric acid, sp.gr. 1·18, into a 500-ml conical flask, wash the filter-paper with 50 ml of water, add 1 ml of titanium sulphate solution, 1 ml of saturated mercuric chloride solution and 5 g of sodium hypophosphite. Reduce the tin as described in the method for tin in titanium alloys and finally titrate with 0·005 *N* potassium iodate.

Calculate the tin content of the sample.

Note—

At this stage it will be apparent if a significant amount of copper is present. If it is known or suspected that the amount of copper is greater than about half the tin content, dissolve the tin sulphide through the paper with 10 ml of warm diluted ammonium sulphide (1 + 1) into a 500-ml conical flask. Wash the copper sulphide residue with 50 ml of water containing hydrogen sulphide. Add 50 ml of hydrochloric acid, sp.gr. 1·18, to the filtrate and then 1 ml of titanium sulphate solution, 1 ml of saturated mercuric chloride solution and 5 g of sodium hypophosphite, and continue as described above.

Conclusions

The direct volumetric method described on p. 660 is satisfactory for the determination of more than 0·2 per cent. of tin in titanium metal and alloys. Other alloying elements likely to be present, such as aluminium, chromium, iron, manganese, molybdenum and vanadium, and also impurity amounts of copper and tungsten, cause no interference; consequently the

method has definite advantages over other published methods[4,5] in which copper, molybdenum and vanadium interfere.

After solution of the sample, the proposed procedure for alloying amounts of tin takes only about 30 minutes and, being simple and direct, it can be recommended particularly for routine control analysis. The method has now been in use in a routine laboratory for over 2 years and no difficulties have been encountered, even when used by inexperienced personnel.

For amounts of tin less than 0·2 per cent., preliminary sulphide separations are recommended, followed by reduction and volumetric determination of tin. This modified procedure obviously increases the operational time, but experiments have proved that it is satisfactory for tin contents as low as 0·002 per cent.

References

1. Evans, B. S., *Analyst*, 1931, **56**, 17.
2. Bayley, W. J., *Chem. & Ind.*, 1950, 34.
3. Norwitz, G., and Codell, M., *Anal. Chim. Acta*, 1954, **11**, 33.
4. Dupraw, W. A., *Anal. Chem.*, 1954, **26**, 1642.
5. S. Tour & Co., "Final Technical Report on Research and Development of Methods of Chemical Analysis for Titanium Metal and Alloys," R10780, May, 1954.

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April 16th, 1957

The Use of Paper Chromatography for the Detection and Determination of Microgram Amounts of Inorganic Fluoride

BY R. J. HALL

A chromatographic method is described for the isolation, detection and determination of microgram amounts of inorganic fluoride. Recoveries of 89 per cent. were obtained for amounts of less than 20 μg. A staining method for as little as 1 μg of isolated fluoride (as calcium fluoride) is described.

THE determination of fluorine as inorganic fluoride presents one of the most interesting problems to the analyst. Of the methods in current use, by far the commonest is that of distillation as fluorosilicic acid, introduced by Willard and Winter,[1] and subsequent titration of the fluoride with thorium nitrate in the presence of an indicator such as alizarin S (sodium alizarinsulphonate) to form a thorium lake. The procedure for organically bound fluorine is to ignite the substance to be analysed with metallic sodium or potassium in a Parr-type bomb at 500° to 600° C and then to continue with the distillation. This method is tedious and difficult and not easily applicable to small amounts. During some recent work in this laboratory, it was necessary to show the presence of microgram amounts of fluoride in a mixture of other inorganic and organic substances and to be able to determine these small amounts with a reasonable degree of accuracy. Hence the technique described, which makes use of paper chromatography and the extreme insolubility of calcium fluoride, has been developed.

Method

REAGENTS—

Calcium chloride solution, 3 per cent. w/v aqueous.

Calcium chloride - thorium nitrate solution—A 3 per cent. w/v solution of dried analytical-reagent grade calcium chloride containing 0·12 per cent. w/v of thorium nitrate, $Th(NO_3)_4.4H_2O$.

Developing solution—A mixture of 85 parts of analytical-reagent grade acetone, 3 parts of glacial acetic acid and 12 parts of distilled water by volume.

Ammonium hydroxide, 0·1 M.

Boric acid solution, 0·1 N.

Monochloroacetic acid buffer solution—Prepared by dissolving 22·7 g of monochloroacetic acid in 800 ml of distilled water, then slowly adding 120 ml of *N* sodium hydroxide and adjusting the volume to 1 litre with distilled water.

Solochrome brilliant blue BS staining solution—Prepared by dissolving 0·1 g of Solochrome brilliant blue BS in 85 ml of distilled water and adding 15 ml of monochloroacetic acid buffer solution.

Silver perchlorate solution, 10 *per cent. w/v.*

Sodium hydroxide, 0·04 N.

Perchloric acid, 0·04 N.

Alizarin S solution, 0·01 *per cent. aqueous.*

Buffered alizarin S solution—Prepared by adding 10 ml of monochloroacetic acid buffer solution to 20 ml of 0·01 per cent. alizarin S solution and adjusting the volume to 100 ml with distilled water.

Thorium nitrate solution, 0·002 N.

Hydrochloric acid, 0·1 N.

Bromophenol blue indicator solution, 0·04 *per cent. aqueous.*

Standard lithium fluoride solution—A solution containing 10 μg of fluorine per ml, prepared by dissolving 2·7305 mg of spectrographically pure lithium fluoride (obtained from Johnson, Matthey & Co. Ltd.) in 200 ml of distilled water.

Standard fluoride solution for application to chromatogram—A solution of 44·21 mg of sodium fluoride or 27·305 mg of lithium fluoride in 20 ml of water.

0·01 ml of either solution $\equiv$ 10 μg of fluoride.

The monochloroacetic acid buffer solution, standard lithium fluoride solution and the buffered alizarin S solution are best stored in polythene bottles.

PROCEDURE FOR DETECTING FLUORIDE—

Sheets of Whatman No. 531 filter-paper, 23 cm $\times$ 15 cm, are cut and marked for descending-solvent chromatography. It is convenient to have the starting line some 9 to 10 cm from the end. With a Pasteur pipette, calcium chloride - thorium nitrate solution is applied to the starting line so as to form a band some 1½ to 2 inches wide. This is allowed to dry in the air. The filter-papers should not be heat dried, as this produces uneven impregnation of the paper with subsequent distortion of the chromatogram. Spots of 0·005 to 0·02 ml of a solution containing 1 to 20 μg of fluorine as inorganic fluoride are applied to the paper, which is then allowed to dry.

The chromatogram is developed in the descending-solvent manner for 1 to 2 hours at room temperature, after which it can be dried in the air and examined in ultra-violet light; this is useful for detecting certain components. Fluoride, calcium chloride and thorium nitrate are not themselves seen in ultra-violet light. The filter-paper is quickly rinsed in tap water and then immersed in 0·1 *M* ammonium hydroxide for 1 minute. It is then removed, again quickly rinsed in tap water, blotted dry and stained with Solochrome brilliant blue BS staining solution for 2 to 5 minutes. Fluoride is seen as bright violet-blue spots at the point of application. The excess of Solochrome brilliant blue BS staining solution is washed out with 0·1 *N* boric acid to leave a white background. Spots stained and washed in this way remain stable for a long while and as little as 1 μg of fluoride can easily be detected.

PROCEDURE FOR REMOVING PHOSPHATE AND OXALATE—

Several interfering radicals, including phosphates and oxalates, can be removed by precipitation with silver perchlorate, a reagent employed by Armstrong[2] to immobilise chloride during the distillation of fluorosilicic acid. The technique adopted for up to 3 mg of fluoride in the presence of about 30 mg of interfering substances is to add 0·5 ml of 10 per cent. w/v silver perchlorate solution to 2 ml of the sample in a 5-ml calibrated flask. The mixture is made to the mark with acetone, shaken well and then centrifuged. To 4 ml of the clear supernatant solution, transferred to another 5-ml calibrated flask, is added 0·1 ml of saturated potassium chloride solution; the mixture is diluted to the mark with water, vigorously shaken and again centrifuged. Suitable volumes of the clear supernatant solution can now be used for chromatographic separation.

PROCEDURE FOR DETERMINING FLUORIDE—

Whatman No. 531 filter-papers are impregnated with 3 per cent. w/v calcium chloride solution and 0·005 to 0·02 ml spots are put on the papers; the chromatograms are developed and dried as before. An area of the paper is now cut out at the point of application of the sample. It is convenient to use a stencil of Perspex having spaces of different areas. For 0·005 to 0·02-ml spots a 1-inch × 1-inch area is suitable. The paper is placed in a 6-inch × $\frac{7}{8}$-inch test-tube with 2 ml of 0·1 *N* hydrochloric acid and brought just to the boil. After cooling, the paper is removed and washed with about 5 ml of water. Sufficient 0·04 *N* sodium hydroxide is added to bring the pH to 2·9 to 3·0, after which the fluoride is titrated with thorium nitrate.

Preliminary titration to pH 2·9 to 3·0—A blank area of paper adjacent to one on which the fluoride is to be determined, but containing no fluoride, is cut out and extracted with 0·1 *N* hydrochloric acid as before. Two drops of bromophenol blue indicator solution are added and the contents are titrated with 0·04 *N* sodium hydroxide until the colour of the indicator, after the solution has been adjusted to 20 ml with water, matches a blank of 20 ml of water, 0·2 ml of 0·04 *N* perchloric acid and two drops of the bromophenol blue indicator solution. The tube containing a sample is treated in exactly the same way and should require the same volume of sodium hydroxide. This titration for the adjustment of the pH (a Doran pH meter was used to check the pH) is most important, since even small variations outside the narrow pH range affect the thorium titration. It is necessary to carry out this titration with care and to see that the colours of the blank and test solutions match exactly. A second blank area of paper, two or three test areas and two or three areas to which have been applied solutions of fluoride of known concentrations from 2 to 20 μg are now extracted and volumes of 0·04 *N* sodium hydroxide and perchloric acid equal to the preliminary titrations are added, but without bromophenol blue indicator solution.

Thorium titration—By means of a pipette, 5-ml portions of buffered alizarin S solution are placed in each tube. To the blank tube is added 0·15 to 0·20 ml of thorium nitrate solution from a 5-ml microburette graduated in 0·01 ml. The tube is stoppered and mixed by gentle inversion. The solution should now assume a pale-buff colour with a distinct pink hue. The unknown solutions are titrated slowly (not more than 0·05 ml of thorium nitrate being added at a time) until the colours match that of the blank; over-titration makes the solution pinker. The titration requires practice at first, but reproducible results should soon be achieved. The colours are best matched by holding the tubes at an angle of 60° and looking down the depth of the solution against the bright-white matt background of Whatman No. 1 filter-paper, preferably in bright daylight or fluorescent daylight lighting, but not in direct sunlight.

RESULTS

Typical recoveries of fluoride are shown in Table I. Since thorium titrations for fluoride are not strictly stoicheiometric, it is necessary to prepare a calibration curve by applying the method to spots of standard lithium or sodium fluoride solutions. A typical calibration graph is shown in Fig. 1.

TABLE I

RECOVERY OF FLUORIDE FROM WHATMAN NO. 531 FILTER-PAPER

Fluoride applied, μg	Fluoride recovered, μg	Recovery, %
2	1·56	78
5	4·16, 4·71 (4·43)	83·4, 94·0 (88·7)
10	8·8, 8·7, 8·8, 9·1 (8·85)	88·5
14	12·9, 12·9	92·2, 92·2
20	18·35, 18·35	91·7, 91·7

Development of the method

Although the detection and determination of small amounts of fluoride have been of important interest for many years, surprisingly little attention appears to have been paid to the use of paper chromatography. Lederer and Lederer[3] detected fluoride as a white spot on a red background when the paper was sprayed with ferric salts, Mitchell[4] reported fluoride to be a problem in the separation and identification of halides and Burstall, Davies, Linstead and Wells[5] described the separation on paper of fluoride, chloride, bromide and iodide with acetone containing 20 per cent. of water. This technique did not satisfactorily separate fluoride from our mixtures and a method was required that would isolate the fluoride for its subsequent determination. When fluoride is applied to paper impregnated with calcium chloride, calcium fluoride is formed, which is one of the most insoluble of the calcium compounds and quite insoluble in dilute acetic acid. Only the oxalate and (+)-tartrate appear to have comparable solubilities and are also insoluble in dilute acetic acid. Hence in the descending-solvent development of the chromatogram, the calcium fluoride remains at the place of application and other substances are eluted away from it by the acidified aqueous acetone.

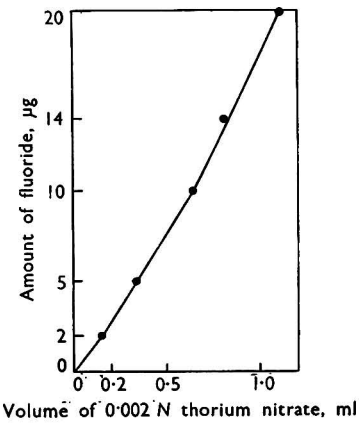

Fig. 1. Titration curve of fluoride spots from Whatman No. 531 filter-paper

The titration procedure was developed from the methods of Armstrong[2] and Hoskins and Ferris,[6] use being made of an improvement suggested by Buffa.[7] Solochrome brilliant blue BS, proposed by Milton, Liddell and Chivers[8] for the thorium titration, was found to be ideal for the qualitative application, but not preferable to alizarin S for the titration.

Of all the radicals that interfere with the thorium titration and that form slightly soluble calcium salts, only phosphate and oxalate were found to affect the chromatographic separation of fluoride as the calcium salt. This problem was overcome by using silver perchlorate in 50 per cent. acetone to remove phosphate in the presence of small amounts of fluoride. With as little as 60 μg of fluoride in a total of 6000 μg of phosphate, oxalate and citrate, 87 per cent. of the fluoride was recovered. When the fluoride content was 3 mg as sodium fluoride in a mixture of 30 mg of other interfering acid radicals, all the fluoride was recovered and no interfering substance could be detected in the final solution to be analysed.

The staining of the fluoride with Solochrome brilliant blue BS depends on the adsorption of thorium nitrate on the calcium fluoride. In a study of the staining of fluoride ions, this technique was found to be much more sensitive than any other method tried. Arsenate, tetraborate, citrate, silicate, tartrate and sulphate gave no reaction in amounts of 50 to 100 μg. In the absence of phosphate, even oxalate presented no difficulty, since it was found to be removed by treatment with 0·0005 N potassium permanganate in 2 per cent. acetic acid at 80° C for 2 to 5 minutes. This in no way affected the staining of the fluoride.

I acknowledge the constant interest that Sir Rudolph Peters, F.R.S., has shown in this work.

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AGRICULTURAL RESEARCH COUNCIL INSTITUTE OF ANIMAL PHYSIOLOGY
BIOCHEMISTRY DEPARTMENT
BABRAHAM, CAMBRIDGE

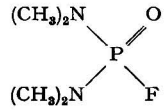
April 23rd, **1957**

The Determination of Dimefox Residues in Hops

BY K. FIELD AND E. Q. LAWS

A method is described for the extraction of dimefox from hops, its separation by chromatography from interfering substances and its subsequent determination as phosphorus.

THE compound tetramethylphosphorodiamidic fluoride (**I**) is known as dimefox. In commercial preparations it is also known as Hanane S·14, Terra-systam, BFPO, DIFO or Schrader's compound 13/28. It belongs to the class of compounds known as systemic insecticides, but differs from the majority of those at present in use by virtue of its comparatively simple molecular structure and the fact that the molecule contains a fluorine atom bonded to phosphorus.



($C_4H_{12}ON_2FP$, m.wt. 154)

(I)

The compound can be determined, after breakdown, as dimethylamine, inorganic fluoride or phosphoric acid. Although determination of dimethylamine would give the best quantitative results, it is not specific, as many plant substances decompose to give the amine. Determination of fluoride would be diagnostic of dimefox as contrasted with most other systemic insecticides, but it is not very sensitive; it has about one-tenth of the sensitivity of the phosphorus method. Determination of phosphorus is a standard procedure for this type of compound and is very sensitive but not diagnostic. The phosphorus method was selected as being the most convenient. A study of the chromatographic behaviour of dimefox shows that it is possible to separate the compound with relative ease from the other phosphorus compounds present. A simple determination of the phosphorus in the separated compound is then sufficient for its accurate quantitative evaluation.

EXPERIMENTAL

EXTRACTION OF DIMEFOX FROM HOPS—

Hops, on account of their resinous and oily nature, are difficult to prepare for the extraction of dimefox. A method described by Dupée, Heath and Otter[1] involves an extraction with oil, followed by vacuum-distillation of the oil containing the dimefox. In looking for a suitable chromatographic method, it was clearly desirable that a simpler approach should be used. The method of Walker,[2] originally designed for extraction of the hop resins, was found to be suitable. The method depends on the initial extraction of the dry hop material with methanol. One extraction is found to be sufficient, provided that on filtration of the vegetable residue the flask and residue are washed as indicated under "Method." After addition of light

petroleum to the extract, followed by ice-cold water, a partition is effected between methanol - water on the one hand and light petroleum on the other. The advantage of this system is that dimefox is retained in the aqueous part while the hop resins go into the light petroleum layer. The aqueous layer is extracted with chloroform to remove the dimefox, together with some of the plant substances. As the chromatography is best carried out in ether solution, the combined chloroform extracts are evaporated carefully to dryness and the solvent is replaced by ether. An extract thus obtained includes a high proportion of phosphorus-containing plant material, a relatively large amount of green colouring matter (chlorophylls), some yellow plant pigments and carotene, contained in ether solution free from other organic solvents. This is the solution for chromatographic separation.

THE CHROMATOGRAPHIC SEPARATION—

Schradan[3] has been separated on alumina columns from other phosphorus compounds by using chloroform and trichloroethylene as solvents. The same system may be used for dimefox,[4] but it is difficult to separate the compound from the vegetable extract. With a column of magnesia, the difficulties disappear and a satisfactory separation is rapidly obtained using ether as the eluent.

When the solution for chromatographic separation, referred to above, is placed on a column of 5 g of magnesia that has been prepared as described under "Method," chromatographic separation immediately takes place, the main portion of the colouring matter of the extract being retained at the top of the column. With hop extracts, the chromatogram obtained has numerous coloured zones. When 100 ml of ether have been run through the column, all the dimefox and a small amount of the yellow pigment is to be found in the eluate. Washing through with a further 100 ml of ether does not lead to any further recovery of dimefox, nor does the phosphorus content of the eluate increase. The yellow pigment (probably carotene) does not appear to have any phosphorus content, but in any event, it is removed in a subsequent procedure before the final determination of phosphorus. Although the method was designed for hops, it is also applicable to the chloroform extract obtained from the aqueous macerates of other vegetables, for example, brussels sprouts, cabbages and lettuces.

THE DETERMINATION OF DIMEFOX—

The ether solution from the chromatographic separation contains the dimefox that was originally present in the hops. After the removal of the solvent the dimefox is dissolved in water and is subsequently treated as in the method of Dupée, Heath and Otter.[1] The final determination of the phosphorus as molybdenum blue is based on the method of Berenblum and Chain,[5] with a modification suggested by Martin and Doty.[6] As the phosphorus concentration with which we are concerned is very low, the acidity and the salt concentration are factors of prime importance. The requirements are that the acidity of the final solution should lie between 0·5 and 1·5 *N*, and that neutral salts other than ammonium molybdate should not exceed 0·5 g in 10 ml of the test solution.

METHOD

The method consists in the extraction of dimefox from the plant material, its transfer to ether solution, chromatographic separation from interfering compounds and subsequent determination of the phosphorus by a spectrophotometric absorption measurement of the molybdenum-blue complex.

APPARATUS—

Unicam SP600 absorption spectrophotometer.
Household slicing and grating machine.
High-speed macerator.
Glass tubes for chromatography, 15 cm long and 1·5 cm diameter.

REAGENTS—

All the reagents used must be phosphate-free. Reagent blanks must be checked periodically.

Methanol, absolute.
Light petroleum, boiling range 40° to 60° C.

Sodium chloride—Analytical-reagent grade.
Ether—Use Anaesthetic Ether, B.P.
Magnesium oxide—The grade "for chromatographic analysis."
Perchloric acid, N.
Sulphuric acid, N.
Ammonium molybdate reagent—Dissolve 50 g of analytical-reagent grade ammonium molybdate in 400 ml of 10 *N* sulphuric acid and dilute to 1 litre with distilled water.
iso*Butanol - benzene mixture*—Mix equal volumes of *iso*butanol and benzene.
Stannous chloride solution, concentrated—Dissolve 10 g of analytical-reagent grade stannous chloride dihydrate in 25 ml of concentrated hydrochloric acid.
Stannous chloride solution, dilute—Dilute the concentrated solution 200-fold with *N* sulphuric acid. Prepare a fresh solution daily.
Ethanolic sulphuric acid—Mix 5 ml of concentrated sulphuric acid with 245 ml of absolute ethanol.
Standard dimefox solutions—We prepared these from dimefox of 90 per cent. purity (provided by Fison's Pest Control Ltd.). Prepare a standard solution by dissolving about 0·2 ml of the dimefox in approximately 1 litre of water and dilute the solution appropriately, so that it contains approximately 5 μg of dimefox per ml. Standardise the solution by comparison with standard phosphate solutions.

PROCEDURE—

Select a sample of dried hop material, and shred it into as fine a condition as possible. Macerate 10 g of the shredded material for 10 minutes with 100 ml of methanol, and set the solution aside overnight. Filter the solution through a Buchner funnel containing a Whatman No. 1 filter-paper, using slight suction. Wash the hops with a further 50 ml of methanol, also collecting the washings in the Buchner flask. It may be necessary to effect a second filtration. Transfer the solution into a 500-ml separating funnel, rinsing the Buchner flask with a further small volume of methanol and then with several small volumes of light petroleum, so that the total volume of light petroleum collected in the funnel is 100 ml. Shake the two layers together, add 200 ml of ice-cold water, and shake again. Add small amounts of sodium chloride to break up any emulsion. Transfer the lower aqueous methanol greenish yellow coloured layer into another 500-ml separating funnel and discard the upper dark green - brown coloured light petroleum layer. Extract the aqueous methanol layer five times with 50-ml portions of chloroform, collecting the lower chloroform layers after each separation. Collect the fractions in a conical flask. Combine the chloroform extracts and evaporate them to dryness; take care not to have the conical flask too hot. Immediately the chloroform has evaporated, run into the conical flask sufficient ether to form a solution. Evaporate the ether, taking similar precautions, and take up the residues in 50 ml of ether. The solution may appear cloudy.

THE MAGNESIA COLUMN—

Insert a cotton-wool plug into the bottom of a chromatographic tube and pour in 5 g of magnesia in a slurry with ether. Allow the magnesia to settle and then insert another cotton-wool plug on the surface of the column. Transfer the ether solution of the sample, with many rinses, on to the column. Collect the eluent in a 250-ml conical flask. Allow the green material to collect on the column before washing with ether. Wash the column with 100 ml of ether, first rinsing the original conical flask and then transferring the rinsings to the column. As the column is washed, a wide yellow band moves down and, as the washing proceeds, the front of this band splits into two smaller bands, followed by a wide band spreading from the top of the column. A greenish coloured band persists at the surface of the magnesia.

In some experiments the collected washings are colourless, in others quite yellow, depending on the extent to which the small yellow bands move downwards.

THE ELUATE—

Carefully distil off the ether, and immediately take up the residue in 10 ml of water. Stand the flask in a thermostatically controlled bath maintained at 25° $\pm$ 2° C, and add to the aqueous solution 4 ml of *N* perchloric acid. Set the solution aside for 20 minutes, with occasional shaking. Transfer the solution to a 100-ml separating funnel and extract it three times with equal volumes of chloroform. Discard the lower chloroform layers after

each extraction. Run the upper aqueous layer back into the conical flask, washing the separating funnel twice with small portions of water. To the total aqueous fraction add 0·5 ml of concentrated hydrochloric acid and 1 ml of concentrated nitric acid, and then evaporate the solution almost to dryness, until white fumes appear. Add a few millilitres of water and repeat the evaporation. Finally add a few millilitres of water and a few millilitres of ammonia solution, sp.gr. 0·880, and boil the solution for several minutes in order to remove the excess of ammonia. Allow the solution to cool and transfer it to a 100-ml separating funnel. Wash the conical flask with 3 ml of ammonium molybdate reagent and a little distilled water, transferring the washings to the separating funnel. Make the total volume of solution contained in the separating funnel 13 ml by adding more water if necessary. Shake the solution, and then add 10 ml of *iso*butanol - benzene mixture. Shake the mixture vigorously for 30 seconds, allow the layers to separate, and then discard the lower aqueous layer. Wash the remaining organic layer with 5 ml of *N* sulphuric acid, and again discard the lower aqueous layer. Finally wash the organic layer with 15 ml of dilute stannous chloride solution, discard the aqueous layer, and run the organic layer, which is blue if phosphorus is present, into a 10-ml measuring cylinder with a stopper, washing in with ethanolic sulphuric acid, and dilute to 10 ml with ethanolic sulphuric acid. Mix thoroughly and measure the optical density of the solution at 730 mμ, using 1-cm cells, against a blank solution consisting of *iso*butanol - benzene mixture and ethanolic sulphuric acid, in the same ratio as used in the experimental procedures.

Results

Recovery of dimefox from magnesia columns—

A recovery curve was prepared by taking a series of solutions with known concentrations of pure dimefox and treating them by a process similar to that described, namely, extraction of the aqueous dimefox solution with chloroform, transfer of the dimefox to ether solution and the chromatographic and analytical procedures. When the amounts of dimefox, in μg, were plotted against optical density, a straight line was obtained; it cut the optical density axis at a value equivalent to the reagent blank (see Fig. 1). The average recovery of dimefox over the range 0 to 5 μg was about 90 per cent.

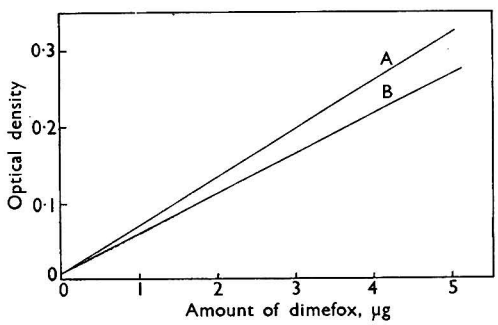


Fig. 1. Recovery of pure dimefox from aqueous solutions: curve A, theoretical recovery; curve B, recovery found

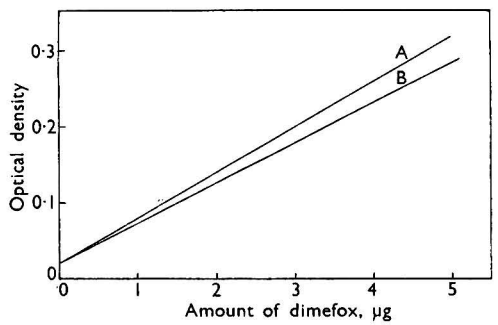


Fig. 2. Recovery of dimefox from dried hops: curve A, theoretical recovery; curve B, recovery found

Recovery of dimefox added to dried hops—

Aliquots of standard dimefox solution were run on to 10-g samples of dried shredded hops that were free from all insecticides. The samples were set aside for 10 minutes, then methanol was added and the mixture was macerated; after the mixture had been set aside overnight, it was treated by the proposed procedure. When the amounts of dimefox, in μg, were plotted against optical density, a straight line was obtained; it cut the optical-density axis at a value equivalent to the reagent blank (see Fig. 2). The average recovery of dimefox over the range 0 to 5 μg was about 90 per cent.

Samples of dimefox-treated hops examined for residues—

Eight samples of hops that had been the subject of soil treatment with dimefox were analysed by the chromatographic method and compared with an untreated sample. No

residue contained more than 0·13 p.p.m. and most contained much less. This is the type of result usually obtained for dimefox residues when the plants are taken for analysis some weeks after treatment. The available evidence suggests that break-down of this compound is rapid in the living plant.

Discussion of results

The recoveries quoted are derived from experiments in which known amounts of dimefox were added to dry hops that had received no phosphorus insecticide during growth. While this procedure simulates the conditions obtaining in the case of plants grown in dimefox-treated soil, it is not identical with field conditions. There may be unknown factors influencing the recovery from growing plants. Residue figures obtained by this method in treated crops are of the same order of magnitude as those obtained by the distillation method. The chromatographic separation has the advantage in simplicity and speed. In addition, the blanks obtained by the method are of the same order as the reagent blank. Recoveries are of the order of 85 to 95 per cent. of the amount added in the region 0·5 μg of dimefox on 10 g of dried hops.

We thank Dr. G. M. Bennett, C.B., F.R.S., the Government Chemist, for permission to publish this paper.

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Quantitative Determination of Traces of Carbon Dioxide in Water

By P. P. Jennings and E. M. Osborn

An improved method for the determination of traces of carbon dioxide dissolved in water is described. A sample of acidified water is aspirated with air free from carbon dioxide and the carbon dioxide evolved is absorbed in sodium hydroxide solution. The alkaline solution is titrated with 0·01 *N* acid, the titre between pH 8·3 and pH 5·0, measured with a glass electrode system, being a measure of the carbon dioxide present. The concentrations of carbon dioxide determined were in the range 0·01 to 0·10 mg per litre.

Investigations of the part played by carbon dioxide in the corrosion of boiler feed systems require the measurement of traces of carbon dioxide dissolved in the feed water. Previously published methods[1,2,3] were examined and found to be insufficiently sensitive, and a method has been developed whereby increased sensitivity has been achieved. Much attention has been given to the blank determinations in an endeavour to reduce the blank values to a minimum, and the size of the sample has been increased to increase the sensitivity.

The method involves the manipulation of the sample out of contact with atmospheric carbon dioxide, the carbon dioxide in the sample being aspirated with air free from carbon dioxide and absorbed in sodium hydroxide solution. The alkaline solution is then titrated in a closed circuit to a pH of 8·3 and then in a stream of air free from carbon dioxide to a pH of 5·0, a glass electrode system being used for the pH measurements.

Results with standard sodium carbonate solution over a range equivalent to 0·022 to 0·110 mg of carbon dioxide per litre gave results within $\pm$ 0·002 mg per litre of the concentration taken, with a standard deviation of 0·0016 mg per litre. The time taken for one determination is about 3 hours.

EXPERIMENTAL

In order to obtain maximum sensitivity the following sources of error have been considered and reduced to a minimum.

TRANSFER OF CARBON DIOXIDE FROM THE SAMPLE TO THE SODIUM HYDROXIDE SOLUTION—

Errors can be caused by carbon dioxide contained in the sodium hydroxide solution, carbon dioxide leaking in or diffusing through the polythene joints, and incomplete transfer of carbon dioxide from the sample to the sodium hydroxide in the titration cell.

The sodium hydroxide was originally prepared by electrolysing saturated brine with use of a mercury cathode and decomposing the amalgam with water free from carbon dioxide; this was later found to be unnecessary, sodium hydroxide "free from carbonate" being sufficiently pure for use. The purity of the sodium hydroxide solution can be easily tested in the apparatus to be described by the following method—

> A blank titration is carried out as described under "Procedure." Let the volume of acid used be B ml. The blank titration is then repeated with 2·0 ml of sodium hydroxide solution. Let the titre be C ml. Then $C-B$ is a measure of the carbon dioxide present in 1 ml of sodium hydroxide solution.

The second possible error is allowed for by the blank determination described in detail later.

The third possible error can be eliminated by continuing the aspiration in stages, and, as can be seen from Table I, p. 675, two aspirations were usually found to be sufficient.

TITRATION OF THE RESULTING SODIUM CARBONATE—

The carbon dioxide absorbed by the sodium hydroxide solution will be present mainly as carbonate at pH 11. Hence, 0·01 N sulphuric acid is added until the pH reaches 8·3, at which point the carbon dioxide will be present as bicarbonate; this part of the titration is carried out in a closed circuit to prevent loss of carbon dioxide from any local concentration of acid immediately on addition. The titration below pH 8·3 is carried out in a stream of air free from carbon dioxide, the sodium bicarbonate is decomposed, the carbon dioxide is removed to the atmosphere and the pH falls to 5·0, by which time the sodium bicarbonate is fully decomposed.

INTERFERING SUBSTANCES—

The preliminary concentration of the carbon dioxide by aspiration will eliminate interference by most substances found in boiler feed water. Non-volatile compounds, such as boiler salts, and all basic substances, *e.g.*, ammonia, hydrazine and amines, will be retained in the acidified sample. The only substance expected to interfere is sulphite by decomposition and aspiration of the sulphur dioxide. This effect might be reduced or eliminated by oxidation during aeration, but in view of the fact that no sulphite is present in the boiler feed water under examination this point has not, as yet, been investigated.

METHOD

It is essential that scrupulous attention should be given to all details of apparatus and procedure if reliable results are to be obtained.

APPARATUS—

The complete apparatus is shown in Figs. 1 and 2. The sample is collected and treated in a bottle, A, having a calibration mark at 10 litres. Through the rubber bung of the sample bottle are inserted (*a*) a glass tube, B, extending almost to the bottom of the sample bottle and having a polythene connecting tube at its upper end that can be closed by a screw clip, C; (*b*) a glass tube, D, having two No. 2 porosity sintered-glass discs and a three-way stopcock, S_1; (*c*) an outlet tube, E, having a three-way stopcock, S_2; and (*d*) a tap funnel of 100 ml capacity, F. It is important that all flexible connections should be as short as possible, that polythene - glass connections should be as tight as possible and, where manipulation allows, sealed with poly(vinyl chloride) cement, and that every effort should be made to eliminate leakage from the system.

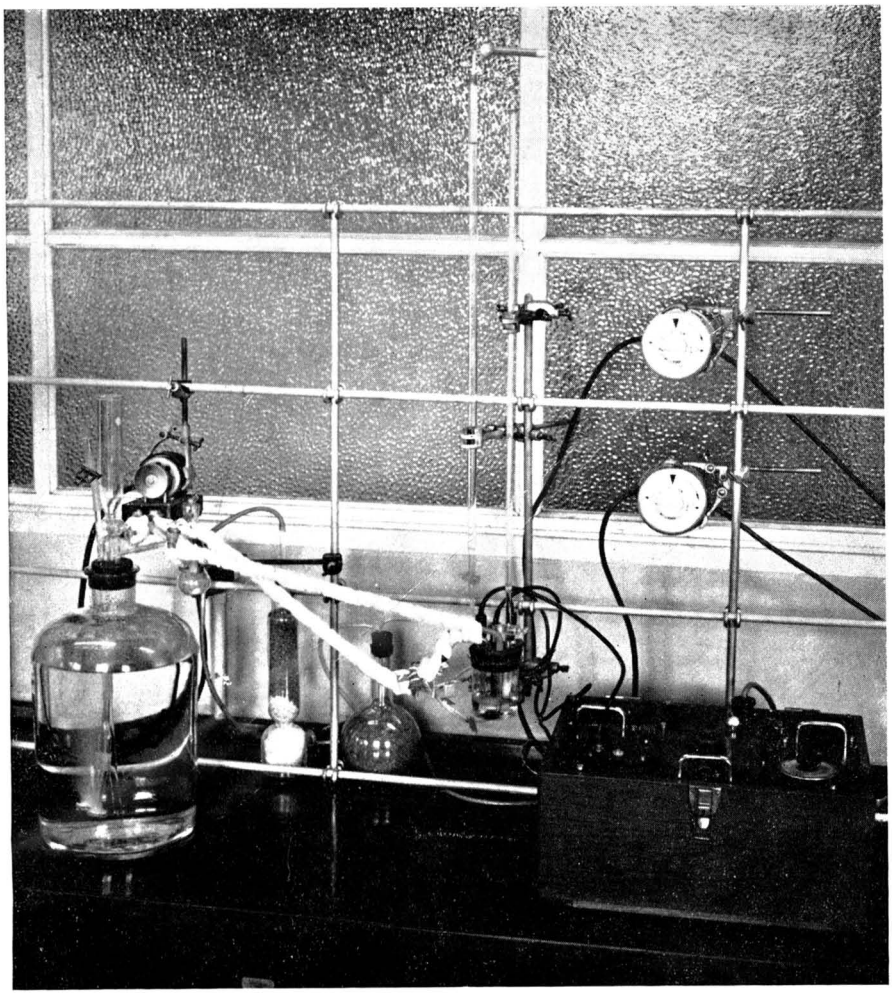
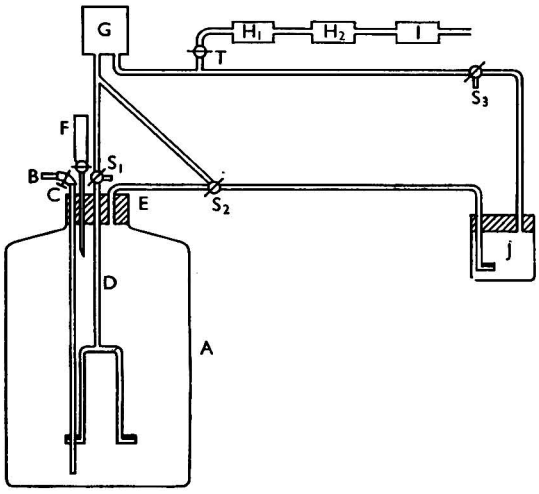


Fig. 1. Complete apparatus for determining carbon dioxide in water

[To face p. 672

Details of the titration cell are shown in Fig. 3; this consists of a 250-ml squat spoutless beaker having (*i*) a No. 2 porosity sintered-glass disc for the gas inlet; (*ii*) a glass electrode, a reference electrode and a temperature compensator; (*iii*) a gas outlet tube fitted with a three-way stopcock, S_3; and (*iv*) two burettes, one containing sulphuric acid and one containing sodium hydroxide, the latter being fitted with a capillary projecting beneath the surface of the liquid in the cell, as shown in Fig. 3 (*a*). It is important that the burette stopcocks should not be greased.



A = Sample bottle
B = Sample inlet
C = Screw clip
D = Air inlet
E = Air outlet
F = 100-ml funnel
G = Pump
H_1 and H_2 = Soda-lime containers
I = Silica-gel container
J = Titration cell
S_1, S_2 and S_3 = Three-way stopcocks
T = Two-way stopcock

Fig. 2. Sectional diagram of complete apparatus

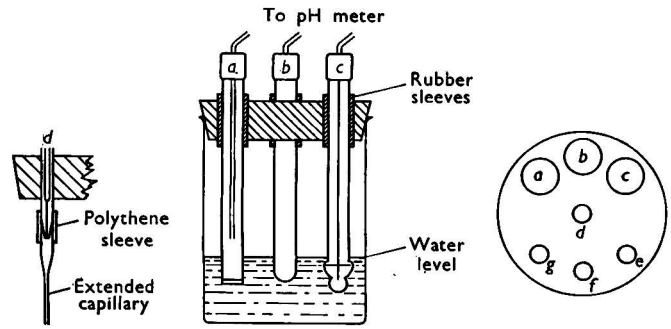


Fig. 3(*a*) Fig. 3(*b*) Fig. 3(*c*)

a = Calomel electrode
b = Temperature compensator
c = Glass electrode
d = Burette containing alkali
e = Burette containing acid
f = Gas inlet
g = Gas outlet

Fig. 3(*a*). Details of the burette containing the alkali
Fig. 3(*b*). Titration cell
Fig. 3(*c*). Plan of rubber bung

The pump used was a Dymax diaphragm-type compressor, manufactured by Charles Austin Ltd.

Two 90-cm heating tapes are wrapped round the lines from bottle to cell and from cell to pump. Control units are fitted to both tapes. The pH meter used was a Marconi mains-operated type TF717A.

REAGENTS—

Sodium hydroxide, 0·1 N—Free from carbonate.
Sulphuric acid, 0·01 N—Free o on dioxide.

Distilled water, free from carbon dioxide—Prepared by boiling laboratory distilled water for 30 minutes and cooling it under a guard tube.

Sulphuric acid, diluted (1 + 1), *free from carbon dioxide*—Prepared from the concentrated acid immediately before use.

Acetone—Analytical-reagent grade.

Soda lime—The self-indicating material.

Silica gel—The self-indicating material.

PROCEDURE FOR STANDARDISING THE pH METER—

As a standard buffer solution cannot be introduced into the titration cell while an analysis is being carried out, it is necessary to determine the residual e.m.f. of the particular glass electrode being used and to utilise this value in setting up the pH meter. This procedure is sufficiently accurate for this method of determination.

PROCEDURE FOR COLLECTING THE SAMPLE—

The sample bottle, A, is removed in such a way that stopcocks S_1 and S_2 are still connected to the bottle. The sample enters by way of tube B, the bottle is filled and water is allowed to overflow through funnel F and stopcocks S_1 and S_2. The contents of the bottle are displaced several times before the inlet and outlets are closed, and water is retained in the sampling tube.

PROCEDURE FOR DETERMINING CARBON DIOXIDE—

The sample bottle, A, is reconnected, stopcock T is opened to the atmosphere and stopcock S_2 is connected from the pump to the sample bottle; clip C is opened and water is pumped from the sample bottle by way of tube B and is replaced by air free from carbon dioxide. When the water level is almost down to the 10-litre mark, stopcock S_2 is turned to connect the sample bottle to the titration cell, stopcock S_3 being open to the atmosphere, and the water is allowed to syphon out of the sample bottle thereby reducing the pressure in the bottle. Clip C is closed when the correct level has been reached. A 50-ml portion of diluted sulphuric acid (1 + 1) is added through funnel F. Stopcock S_1 is opened to the atmosphere momentarily, to fill the line with air free from carbon dioxide, and is then closed.

The electrode system is thoroughly washed with acetone and then water (this was found to increase sensitivity by removing any grease and matter adhering to the surfaces). Approximately 100 ml of water free from carbon dioxide are placed in the titration cell and 2 to 3 ml of 0·01 *N* sulphuric acid are added; air free from carbon dioxide is then bubbled through and out by way of stopcock S_3 for 5 to 10 minutes to ensure freedom from traces of carbon dioxide. The liquid level in the titration cell is lowered to a position fixed by the electrode system by replacing the sulphuric acid burette by a syphon inserted to the required depth and momentarily closing stopcock S_3. It is important that the liquid should be adjusted to this level before each part of the determination. With air free from carbon dioxide flowing through the system, stopcock T, and then stopcock S_3, is closed, thus allowing air to circulate under slightly reduced pressure. Then 1 ml of 0·1 *N* sodium hydroxide is put into the titration cell and the sample bottle is brought into the circuit by adjusting stopcock S_1 to connect the pump to the sample bottle and also stopcock S_2 to connect the sample bottle to the titration cell. The heating tapes are switched on so as to maintain a temperature of approximately 40° C, which is sufficient to prevent any condensation from forming in the tubing. Aspiration is continued for 1 hour.

After aspiration of the sample, stopcock S_2 is adjusted so that the pump is connected directly to the titration cell and stopcock S_1 is closed, so that the sample bottle is cut out of the circuit. Then 0·01 *N* sulphuric acid is added to the titration cell until the pH is approximately 9. Increments of 0·1 ml of the same acid are added and the pH and burette reading are noted until the pH falls to just below 8·3. Stopcocks S_3 and T are then opened to allow air free from carbon dioxide to flush the system; further increments of acid are then added until the pH falls to below 5·0. The amount of acid required between pH 8·3 and 5·0 may be found by means of a graph—let this volume be *A* ml.

The sulphuric acid burette is removed and the liquid level is re-adjusted by means of the syphon.

A blank titration is now carried out. Stopcocks T and S_3 are closed, 1 ml of 0·1 *N* sodium hydroxide is run into the titration cell and the titration is again carried out. The

titre is the volume required under conditions in which carbon dioxide is absent and is usually about 0·4 to 0·5 ml of 0·01 *N* acid—let this volume be *B* ml.

The liquid is again syphoned to the required level, the sample bottle is brought back into the circuit and aspiration is continued for 30 or 60 minutes as required. Titrations are carried out as before to give titres of A_1 and B_1 ml. The blank titration should be repeated after each determination, owing to a very slight loss of sensitivity at the glass electrode; this should not be more than 0·05 ml of 0·01 *N* acid.

Aspiration 1 = A − (B + apparatus blank) = X ml.
Aspiration 2 = A_1 − (B_1 + apparatus blank) = Y ml.
Total 0·01 *N* sulphuric acid required = $X + Y$ ml.

The following decomposition takes place between pH 8·3 and pH 5·0—

$$NaHCO_3 + H_2SO_4 = NaHSO_4 + H_2O + CO_2.$$

Therefore 1 ml of 0·01 *N* $H_2SO_4 \equiv$ 0·44 mg of CO_2, *i.e.*, 0·044 mg of CO_2 per litre in a 10-litre sample.

PROCEDURE FOR DETERMINING THE APPARATUS BLANK—

The apparatus is allowed to run on a closed circuit with the sample bottle excluded for 1 hour and a titration is carried out, followed by a blank titration as described above and the difference, usually about 0·05 ml of 0·01 *N* acid per hour, is noted. This is believed to be due to diffusion or leakage of carbon dioxide through the flexible joints or pump diaphragm and the result, although very small, must be included in the above calculation.

TABLE I

DETERMINATION OF CARBON DIOXIDE IN BOILER FEED WATER

	Aspiration No.	Acid used, ml	Total acid, ml	Carbon dioxide found, mg per litre
Station 1—				
A	1	0·25	0·29	0·013
	2	0·04		
B	1	0·39	0·42	0·018
	2	0·03		
C	1	0·78	1·06	0·047
	2	0·26		
	3	0·02		
Station 2—				
A	1	0·77	0·87	0·038
	2	0·10		
B	1	0·65	0·65	0·029
	2	0·00		

RESULTS

The method has been applied to the determination of carbon dioxide in boiler feed water, and some typical results obtained at power stations having boilers operating at 900 lb per sq. inch are given in Table I. Results of determinations of the apparatus blank were as follows—

Volume of 0·01 *N* acid required per hour, ml ..	0·03	0·05	0·02	0·06	0·08*	0·03
Equivalent of carbon dioxide in sample, mg per litre	0·0013	0·0022	0·0009	0·0026	0·0035	0·0013

The method has also been checked with standard sodium carbonate solutions. The apparatus was assembled with the sample bottle filled to the 10-litre mark with acidified water aspirated free from carbon dioxide. A measured volume of 0·01 *N* sodium carbonate was added by means of a pipette and the carbon dioxide was determined by the proposed method. The results are given in Table II.

* This determination was made after the pump had run continuously for over 48 hours.

TABLE II

TESTS WITH CONTROL SOLUTIONS

Volume of 0·01 *N* sodium carbonate taken, ml	Aspiration No.	Acid used, ml	Total acid, ml	Carbon dioxide taken, mg per litre	Carbon dioxide found, mg per litre	Difference, mg per litre
1·0	1	0·42	0·47	0·022	0·021	−0·001
	2	0·05				
1·0	1	0·51	0·53	0·022	0·023	+0·001
	2	0·02				
1·0	1	0·53	0·53	0·022	0·023	+0·001
	2	0·00				
2·0	1	0·87	0·96	0·044	0·042	−0·002
	2	0·09				
2·0	1	0·96	1·04	0·044	0·046	+0·002
	2	0·08				
2·0	1	0·96	1·04	0·044	0·046	+0·002
	2	0·08				
5·0	1	2·02	2·53	0·110	0·111	+0·001
	2	0·46				
	3	0·05				

DISCUSSION OF RESULTS

It will be seen from the results in Table II that there is close agreement between the calculated and determined concentrations of carbon dioxide.

The standard deviation of the carbon dioxide found from the carbon dioxide taken was 0·0016 mg per litre and the standard deviation of the apparatus blank was 0·0009 mg per litre, which indicates that the apparatus blank is the largest factor in the residual error. No determination below 0·110 mg per litre showed an error greater than 0·002 mg per litre.

Work was also carried out with 2·2 and 4·4 mg of carbon dioxide, but it was found that the titration - pH curve in the region of pH 8·3 was very flat and led to errors in the volume of acid, *A*.

The results indicate that the method is capable of determining carbon dioxide in boiler feed waters with an accuracy of ± 0·003 mg per litre on a 10-litre sample provided that the sample does not contain more than 1 mg of carbon dioxide.

We thank Mr. R. Wolforth, Divisional Chief Chemist, for his encouragement and advice and the Controller, Central Electricity Authority, Yorkshire Division, for permission to publish this paper.

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CENTRAL ELECTRICITY AUTHORITY
YORKSHIRE DIVISION
DIVISIONAL CHEMICAL LABORATORY
SKELTON GRANGE POWER STATION
LEEDS

April 12th, 1957

The Influence of Chloride on the Dichromate-value Test

By W. M. CAMERON and T. B. MOORE

The suitability of dichromate for the determination of oxygen absorbed, by polluting matters, is confirmed. However, it is shown that errors develop in the presence of chloride and of chloride and nitrogen occurring together. These errors have a multiple effect. Correction is possible, but not precise.

THIS paper is a record of tests carried out for the purposes of an investigation on the dichromate-value test by Panel I of a Joint Committee of the Association of British Chemical Manufacturers and the Society for Analytical Chemistry, which was set up to enquire into analytical methods applicable to trade effluents.

For the determination of oxygen absorbed (O.A.) W. A. Moore *et al.* employed dichromate in a method involving heating under reflux[1] and later incorporated the use of silver as a catalyst,[2] as recommended by Muer.[3] They obtained results near to the calculated value for the oxygen required to oxidise a large number of selected organic compounds in pure solution.

According to Moore *et al.*, chloride, when present, is 100 per cent. oxidised, so that its oxygen requirements can be calculated to yield a true correction.

In the present work, the method of Moore *et al.* was examined, and four main points emerged from the investigations, as follows—

(*i*) The method gives results much nearer to theoretical values than the conventional British method based on permanganate, when pure solutions are being dealt with.

(*ii*) When the silver salt is added before the chloride is oxidised, silver chloride may be precipitated in a coagulated form difficult to oxidise.

(*iii*) The chloride present is not completely oxidised and, if this is ignored, errors may develop for which a precise correction cannot be made.

(*iv*) Correction is more difficult when chloride and nitrogen occur together, because the nitrogenous portion may be oxidised by the free chlorine developed during the oxidation of the chloride.

We recommend that sufficient dichromate should be added to oxidise the chloride, in addition to the normal 25 ml required to deal with the sample, and a period of heating under reflux should be carried out before the addition of the silver. We also suggest approximate corrections for the presence of chloride, and of chloride and nitrogen occurring together.

These modifications have been incorporated into the method put forward by Panel I of the Joint Committee.

Experimental

Oxidation in pure solution—

Some comparative dichromate and permanganate values were first found for solutions, in distilled water, of pure organic compounds. Results are given in Table I, from which it will be seen that they conform with the general thesis.

Table I

Comparison of dichromate and permanganate values

Compound	Calculated O.A., p.p.m.	Determined O.A. with— $N/8$ $KMnO_4$, p.p.m.	Determined O.A. with— $N/8$ $K_2Cr_2O_7$, p.p.m.	Ratio determined O.A. / calculated O.A. for— $N/8$ $KMnO_4$	Ratio determined O.A. / calculated O.A. for— $N/8$ $K_2Cr_2O_7$
Acetic acid	512	Nil	485	Nil	0·947
Phenol	499·6	396	512	0·793	1·024
Glycerophosphoric acid	504	220	526	0·437	1·044
Ethanol	498	129·6	496	0·260	0·995
Glycerol	497·8	268·8	498	0·540	1·00
Ammonium thiocyanate	498·5	392	480	0·786	0·963
Cresol	478·1	267·2	508	0·559	1·062

OXIDATION OF CHLORIDE IN PRESENCE OF SILVER—

Chloride is commonly a constituent of natural waters and is oxidised by dichromate. Moore *et al.* recommended that a correction should be applied for the presence of chloride, based on 100 per cent. oxidation of the chloride.

In the course of the present work, it became apparent that the degree of oxidation of chloride was generally not so complete.

It was found that chloride in high concentration was not readily oxidised, since silver chloride was precipitated in a coagulated form. It was necessary under those conditions to continue the period of heating under reflux until the precipitate was wholly dissolved, in order to complete the oxidation. In some tests 2 hours was found to be the minimum period.

For example, a solution of monohydric phenols having a theoretical oxygen absorbed of 259 p.p.m. and a sodium chloride content equivalent to 1750 p.p.m. of chlorine gave the following results, the oxygen absorbed being based on the assumption of 100 per cent. oxidation of chloride—

Time of heating under reflux, hours ..	½	1	2
Oxygen absorbed, p.p.m.	205	215	250

More satisfactory results were obtained with gas liquor, as shown by the following results—

Sample A, 2 per cent. of 10-oz gas liquor in potable water. After 1 hour's boiling, values for the oxygen absorbed were 316 and 310 p.p.m., giving a mean of 313 p.p.m.

Sample B, 2 per cent. of 10-oz gas liquor in potable water. After 1 hour's boiling, values for the oxygen absorbed were 380 and 387 p.p.m., giving a mean of 383 p.p.m.

Sample B, with added sodium chloride (equivalent to 120 p.p.m. of chloride). After 1 hour's boiling, the oxidation of chloride was incomplete, as judged by persistent turbidity. After 2 hours' boiling, the value for the oxygen absorbed was 381 p.p.m.

REMOVAL OF CHLORIDE—

Consideration was given to the removal of chloride by precipitation and filtration. However, experiments demonstrated, as was expected, that co-precipitation occurred in the presence of colloidal matter, *e.g.*, fatty acids and proteins. The oxygen-absorbed value was thereby reduced. Some results are shown in Table II. The results "with correction for chloride" were obtained by following the recommended method of the Joint Committee. The results "with precipitation of chloride" were obtained after the chloride had been precipitated and removed by filtration.

TABLE II

RESULTS FOR OXYGEN ABSORBED WITH ADDED CHLORIDE

Compound	O.A. value, p.p.m.	Chloride added, p.p.m. of oxygen absorbed	Oxygen absorbed with correction for chloride, p.p.m.	Oxygen absorbed with precipitation of chloride, p.p.m.
Cresylic acid	400	400	400	405
Soap	360	800	356	140
Soap	740	800	732	350
Soap	300	400	296	160
Glue	190	500	182	137

OXIDATION OF CHLORIDE IN ABSENCE OF SILVER—

Another probability was that oxidation of the chloride would be readily achieved if the addition of the silver salt was delayed.

Experiments with a solution of sodium chloride, silver being absent, demonstrated that maximum, but not complete, oxidation was achieved in ½ hour. The incompleteness of oxidation became a major point in later work, but for the sake of clarity is discussed at this stage.

In order to demonstrate the point more fully a series of tests was performed in which the back-titrations were carried out with $N/80$ ferrous salt. Solutions having various concentrations of sodium chloride were heated under reflux, 25-ml portions being taken, for 2 hours with excess of dichromate, but without the addition of silver sulphate. The results are given in Table III.

The incomplete oxidation of chloride, if ignored, leads to low oxygen-absorbed values, especially in the presence of high concentration of chloride. Hence if C is the true oxygen

equivalent of the chloride present, S the oxygen-absorbed value of the organic pollutant, both in the same terms, and P the percentage error due to the assumption of 100 per cent. oxidation of chloride, then the observed oxygen-absorbed value of the sample contains an error of $C/S \times P$ per cent. It follows that, unless P can be accurately determined, S cannot be exactly calculated for an unknown degree of organic pollution.

TABLE III

OXIDATION OF CHLORIDE IN ABSENCE OF SILVER

Chloride taken, p.p.m.	Equivalent volume of $N/8$ $AgNO_3$, ml	Volume of $N/8$ $K_2Cr_2O_7$ added, ml	Volume of $N/80$ $Fe(NH_4)_2(SO_4)_2$ required, ml	Oxidation, %
225	1·25	2·0	6·3	109·7
558	3·15	5·0	18·75	99·2
1058	6·10	10·0	40·10	98·1
2112	11·90	15·0	34·40	97·4
4224	23·80	30·0	67·90	97·4
5310	29·90	40·0	118·75	94·0

Hence the applicability of the dichromate-value test is limited in the presence of high chloride concentrations. However, if the test is confined to the use of $N/8$ dichromate, as recommended by the Panel, then it can be shown, by application of the above formula, that the concentration of chloride for organic oxygen-absorbed values of 250 to 500 p.p.m. should not exceed 1000 to 2000 p.p.m., if the resulting oxygen-absorbed value of the pollutant is to be within 5 per cent. of the true value.

It further follows that, if the concentration of chloride reaches 10,000 to 20,000 p.p.m., the errors developed might be equivalent to oxygen-absorbed values of 112 p.p.m. and 224 p.p.m. Hence the test would be valid when such values are appreciable fractions of the accompanying oxygen-absorbed value of the pollutant.

OXIDATION OF LACTIC ACID AND ALANINE—

The dichromate value of pure lactic acid in pure solution and in the presence of chloride was determined by using the method recommended by the Joint Committee. Typical results are given in Tables IVA and IVB, from which is seen that a serious depression in the amount of oxidation occurs at high concentrations of chloride.

TABLE IVA

DETERMINATION OF THE DICHROMATE VALUE OF LACTIC ACID

	Oxygen absorbed, ml of $N/8$ $K_2Cr_2O_7$	Theoretical oxygen absorbed, ml of $N/8$ $K_2Cr_2O_7$	Amount of oxidation, %
Series I—			
(*a*) Lactic acid	7·6	12·0	63·0
(*b*) Lactic acid + silver sulphate	9·6	12·0	80·0
(*c*) Lactic acid + silver sulphate + 12,600 p.p.m. of chloride	4·4	12·5	35·4
(*d*) Lactic acid + silver sulphate + 200 p.p.m. of chloride	9·2	12·5	73·5
Series II—			
(*a*) Lactic acid + silver sulphate	10·2	12·0	85·0
(*b*) Lactic acid + silver sulphate + 12,425 p.p.m. of chloride	3·65	12·0	30·4
(*c*) Lactic acid + silver sulphate + 12,425 p.p.m. of chloride	5·26*	12·0	43·8

* Mean of two tests, after aeration at the end of each test to remove any volatile oxidising agents.

If the differences in absorption, in ml of $N/8$ potassium dichromate, between that of the pure lactic acid and those obtained when chloride was present are ascribed to an error in the oxidation of chloride, additional oxidation of lactic acid is obtained as follows—

Series I—

(*c*) $\frac{5·2}{12·5} = 41·8$ per cent., to give a total of 76·8 per cent.

(*d*) $\frac{0{\cdot}4}{12{\cdot}5}$ = 3·2 per cent., to give a total of 76·7 per cent.

The value for pure lactic acid is 80 per cent.

Series II—

(*b*) $\frac{6{\cdot}56}{12{\cdot}0}$ = 54·7 per cent., to give a total of 85·1 per cent.

(*c*) $\frac{4{\cdot}94}{12{\cdot}0}$ = 41·2 per cent., to give a total of 85·0 per cent.

The value for pure lactic acid is 85 per cent.

The calculation of the error and the extent of oxidation of the chloride are seen from Table IVB.

TABLE IVB

CALCULATION OF THE ERROR AND THE EXTENT OF OXIDATION

		Approximate chloride concentration, p.p.m.	Equivalent of $N/8$ $K_2Cr_2O_7$ found, ml	Volume of $N/8$ $K_2Cr_2O_7$ not absorbed, ml	Error, %	Oxidation, %
Series I	(*d*)	200	1·4	0·4	28·5	71·5
	(*c*)	12,600	71·0	5·2	7·3	92·7
Series II	(*b*)	12,425	70·0	6·56	9·4	90·6
	(*c*)	12,425	70·0	4·94	7·1	92·9

The dichromate value of pure alanine, taken as being indicative of nitrogenous compounds, was similarly determined. Results are given in Table V.

TABLE V

DETERMINATION OF THE DICHROMATE VALUE OF ALANINE

	Oxygen absorbed, ml of $N/8$ $K_2Cr_2O_7$	Oxidation, %
(*a*) Alanine + silver sulphate	12·0	94·8
(*b*) Alanine + silver sulphate..	12·55	99·2
(*c*) Alanine + 293 p.p.m. of chloride added as sodium chloride ..	12·85	101·5
(*d*) Alanine + 12,753 p.p.m. of chloride added as sodium chloride	3·5	27·5
(*e*) Alanine + 12,753 p.p.m. of chloride added as sodium chloride	4·9	38·7

The theoretical absorption for each test was equivalent to 12·65 ml of $N/8$ potassium dichromate.

The chloride content of (*c*) was equivalent to 1·65 ml of $N/8$ potassium dichromate and that of (*d*) and (*e*) to 71·85 ml of $N/8$ potassium dichromate.

If (*d*) and (*e*) are corrected in the same manner as was done for lactic acid, the following values for these oxidations are obtained, 12·3 being taken as the mean of (*a*) and (*b*)—

(*d*) $\frac{8{\cdot}8}{12{\cdot}65}$ = 69·6 per cent., to give a total of 27·5 + 69·6 = 97·1 per cent.

(*e*) $\frac{7{\cdot}4}{12{\cdot}65}$ = 58·5 per cent., to give a total of 38·7 + 58·5 = 97·2 per cent.

The oxidation of the chloride is then equivalent to—

(*d*) $\frac{71{\cdot}85 - 8{\cdot}8}{71{\cdot}85}$ = 87·8 per cent.

(*e*) $\frac{71{\cdot}85 - 7{\cdot}4}{71{\cdot}85}$ = 89·7 per cent.

The effect of high chloride content together with its variable oxidation is confirmed.

In this case, however, the opportunity was taken to examine the oxidation from the point of view of nitrogen. In order to do this, the titrations were performed with $N/8$ ferrous sulphate, which was stored in an atmosphere of hydrogen. The amount of nitrogen present was 1·89 mg in each test and the amounts recovered, in mg of nitrogen, were: (*a*) 1·14, (*b*) 1·14, (*c*) 1·31, (*d*) 0·42 and (*e*) a trace.

OXIDATION OF UREA: PRESENCE OF NITROGEN AND CHLORIDE TOGETHER—

The oxidation of the nitrogenous portion of the molecule indicated that the reaction was in the foregoing instance more complicated than had been supposed and further experiments were carried out to elucidate the problem.

Urea was chosen for this purpose, since it is of simple chemical constitution and is a natural constituent of domestic sewage.

A preliminary experiment demonstrated that some oxidation did occur directly with dichromate, but that it was enhanced in the presence of chloride. The direct oxidation was thought to follow a reaction of the type—

$$CO(NH_2)_2 + 3O \rightarrow CO_2 + 2H_2O + N_2,$$

from which it was calculated that 12·5 ml of $N/8$ potassium dichromate might directly oxidise 15·6 mg of urea. Accordingly, in a series of determinations a solution of urea containing 15·6 mg per 25 ml was used, together with various concentrations of chloride in the same volume. The results from these tests are given in Table VI.

TABLE VI

OXIDATION OF UREA IN PRESENCE OF CHLORIDE

25 ml of $N/8$ $K_2Cr_2O_7 \equiv 5{\cdot}2$ mg of urea

Chloride present, p.p.m.	Total volume of $N/8$ $K_2Cr_2O_7$, ml	Absorption as volume of $N/8$ $FeSO_4$, ml	Oxidation of urea, %
266	26·5	6·5	52
639	28·6	8·5	68
1278	32·2	9·7	77
6327	60·7	7·14	57
Nil	25·0	4·2	33·6

The amount of oxidation is very variable between the tests. It is in marked contrast to the experience with lactic acid and alanine, for with these chloride apparently causes decreased oxidation.

The nitrogen determinations gave the following results, the amount of nitrogen originally present being 7·28 mg per 25 ml—

Chloride present, p.p.m.	266	639	1278	6327	Nil
Nitrogen recovered, mg	1·944	2·184	2·160	2·040	4·57
Oxidation, %	73	70	70	72	37

Hence the higher degree of oxidation of urea appears to depend on the presence, but not the concentration, of chloride. It would seem that the chloride at the lower concentrations acts in a cyclic manner, forming free chlorine and chloride alternately, and so oxidising the urea and thereby reducing the dichromate.

There are several routes by which chlorine may oxidise organically combined nitrogen, but it is thought that each involves tervalent nitrogen, therefore that is the basis of the correction offered in the Joint Committee method (*i.e.*, 1 ml of $N/8$ $K_2Cr_2O_7 \equiv 0{\cdot}5833$ mg of nitrogen). However, it should be pointed out that the correction, although based on valency, is not precise, since the nitrogen may be simultaneously directly and indirectly oxidised. The correction is given, since oxidation tests of this nature are generally considered to be concerned with carbonaceous oxidation only.

TABLE VII

OXIDATION IN THE PRESENCE OF ORGANICALLY COMBINED CHLORINE

Compound	Oxidation with silver absent, %	Oxidation with silver present, %
Carbon tetrachloride	6·4	6·4
Trichloroethylene	20·0	20·0
Benzoyl chloride*	94·3	94·5
Benzyl chloride	65·2	62·4
Chlorobenzene	12·0	25·6

* Owing to hydrolysis this test becomes in effect a test of benzoic acid and hydrochloric acid.

OXIDATION IN PRESENCE OF ORGANICALLY COMBINED CHLORINE—

In an incidental manner the oxidation of chain and ring structures in which chlorine is organically combined was examined. Some results are given in Table VII.

CONCLUSIONS

It has been confirmed that the results obtained in the dichromate-value test are generally close to the theoretical value, which is in marked contrast to those obtained from the permanganate-value test.

It has also been shown that chloride, which is of common occurrence, may introduce errors that cannot be corrected in a simple manner. However, oxidation of the chloride by dichromate before the addition of silver sulphate usually reduces the error to negligible dimensions.

Correction of the error is more difficult when chloride and nitrogenous compounds occur together. Part of the unoxidised nitrogen may be directly oxidised by the dichromate and part simultaneously oxidised by the free chlorine developed from the chloride. An empirical correction is suggested for such conditions.

These corrections are the basis of the limitations placed upon the test by Panel I of the Joint Committee.

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MIDDLESEX COUNTY COUNCIL
MAIN DRAINAGE DEPARTMENT
MOGDEN WORKS
ISLEWORTH

NORTH THAMES GAS BOARD
TAR AND AMMONIA PRODUCTS WORKS
EAST HAM, E.6

April 30th, 1956

Recommended Methods for the Analysis of Trade Effluents

PREPARED BY THE JOINT A.B.C.M. - S.A.C. COMMITTEE ON METHODS FOR THE ANALYSIS OF TRADE EFFLUENTS

Determination of Oxygen Demand

Introduction

THE ultimate purification of an effluent from organic matter must be by a process of oxidation. The assessment of the amount of oxygen necessary for this purpose has been the subject of much research over many years, but it is still true to say that no one single procedure has been devised that will give a satisfactory and complete determination of the requirements for all effluents and in all circumstances. Many methods or variations of these have been suggested during the past hundred years, but only three selected methods are recommended and described; two are essentially chemical in character and one is biochemical. Mention should also be made of the theoretical calculation of the total oxygen requirement of an effluent, which can be derived from a knowledge of the contents of the elements in it existing in oxidisable forms. This quantity has been termed the "Ultimate Oxygen Demand" (U.O.D.). When the main elements involved are carbon and nitrogen, the expression U.O.D. $= 2{\cdot}67C + 4{\cdot}57N$ has been suggested, where C is the organic carbon content and N is the sum of the ammoniacal and organic nitrogen contents. It must be pointed out, however, that other elements may also exert an oxygen demand, *e.g.*, hydrogen, sulphur and phosphorus. For strict accuracy these should also be taken into account and allowance should be made for the oxygen already present in organic combination.

The methods of assessment of the oxygen demand described are the determination of—

(*a*) oxygen absorbed from boiling acid potassium dichromate (dichromate value),
(*b*) oxygen absorbed from acid potassium permanganate (permanganate value), and
(*c*) Biochemical Oxygen Demand (B.O.D.).

Although these are dealt with in detail in the following sections, it is convenient to summarise here the main considerations with regard to their applicability and limitations.

DICHROMATE VALUE—

The use of potassium dichromate in boiling acid solution for determining the chemical oxygen demand of effluents was introduced by Adeney and Dawson[1] in 1926 and much subsequent research has been devoted to it. It is convenient to adopt the term "Dichromate Value"* as an abbreviated title for this test. As in the permanganate procedure, it must be stressed that the chemical oxygen demand as determined by the test represents the requirement for only part of the organic matter; the carbonaceous matter may not be completely oxidised and the organic nitrogen is generally unoxidised, the proportion depending on the structure of the organic compounds present. Chemical oxidation of this nature does not differentiate between the biochemically unstable and stable organic matter; therefore, chemical oxygen demand values are not strictly correlated with biochemical oxygen demand.

Nevertheless, the dichromate-value test, which is a new feature in British practice, is recommended as a standard method, since, for trade effluents, it has considerable advantages over the permanganate-value test, particularly in regard to reproducibility and to its applicability to a wide variety of samples.

* In the tenth edition of "Standard Methods for the Examination of Water, Sewage and Industrial Wastes,"[2] the result of the dichromate-value test is expressed as the "Chemical Oxygen Demand" of the sample.

PERMANGANATE VALUE—

The oxygen absorbed from acid potassium permanganate is the oldest of the tests now in use. It is convenient to adopt the term "Permanganate Value" as an abbbreviated title for this test. Its continued use in the British Isles is due to its simplicity, which enables it to be carried out by operators without a high degree of skill, and it also gives a result that is available on the same day. It was one of the earliest methods used to assess pollution and therefore provides a continuing basis for comparison with the results obtained in previous years. In most cases it is suitable for routine control in any particular works, and is one of the methods that may be used when a B.O.D. determination is inadmissible, for example, by reason of toxicity to seeding organisms of the materials under examination.

The scope of the test is limited; it is an empirical measure of chemically oxidisable matter, although, as a general rule, nitrogenous substances remain unoxidised. The result is only very broadly related to either the ultimate biochemical or chemical oxygen demands, the relationship varying with the chemical substances present in the sample.

In order to ensure that all the results obtained are comparable, it is imperative that the conditions specified shall be rigorously observed.

BIOCHEMICAL OXYGEN DEMAND—

The B.O.D. test was devised as the result of the researches made in this country under the auspices of the Royal Commission on Sewage Disposal. It is, in fact, a biological procedure that attempts to simulate the natural process of purification of organic matter by oxidation as it occurs in a river or stream, where the dissolved oxygen in the water supplies the oxygen used by the organic matter and is itself replaced in the water by the absorption of oxygen from the air at the air - water interface or surface. The duration of the test is 5 days and it must be stressed that in that time only a part of the total oxygen required for the complete oxidation of the organic matter will be used up. Moreover, during the 5 days, the carbonaceous matter will be preferentially oxidised, since the oxidation of the nitrogenous portion to nitrite or nitrate generally occurs at a later stage. Particularly for river and stream surveys and also for sewage-effluent assessment this is a most useful test, but with industrial wastes great caution must be observed, since the up-take of oxygen in the B.O.D. test is due to biological oxidation of organic matter, and the presence of any substance that depresses or inhibits biological activity interferes. Such substances include chlorine, chloramines, certain metallic compounds and organic substances that may be bactericidal when present in concentrations above a particular level.

After careful consideration the Joint Committee concluded that, in the interests of uniformity and for convenience of operation in the many laboratories that require to assess biochemical oxygen demand on both trade and sewage effluents, it was desirable that the method recommended should, so far as is possible, be identical in detail with that recommended by the Ministry of Housing and Local Government Committee for the Analysis of Sewage and Sewage Effluents. Permission was therefore obtained from H.M. Stationery Office to reproduce this method, although it has, of course, been necessary to make certain deletions and additions to the text in order to cover the special problems associated with trade effluents. The Joint Committee, therefore, put forward the revised text that appears in a following section of their Recommended Methods—the deletions and additions to the Ministry method being duly indicated (see footnote, p. 691). These remarks also apply to the section on "Dissolved Oxygen," which is a necessary preliminary to the B.O.D. determination.

In addition, the following points merit special attention—

(*a*) The question of "seeding" is of great importance in a B.O.D. method for trade effluents, but it presents considerable difficulties; appropriate paragraphs on this subject are included in the text (see pp. 701 and 702).

(*b*) It has already been mentioned that substances that depress or inhibit biological action interfere with the B.O.D. test. A list of inorganic substances that have been reported to interfere in this way in the B.O.D. test for sewage

TABLE IA

EFFECT OF INHIBITORY SUBSTANCES ON THE DETERMINATION OF THE B.O.D. OF SEWAGE BY THE DILUTION METHOD

Interfering substance or ion	Concentration in B.O.D. bottle,* mg per litre	Effect on B.O.D. of sewage: Reduction, %	Effect on B.O.D. of sewage: Qualitative effect	Reference
Sodium arsenate (as Na_3AsO_4) ..	up to 100	—	Little effect	3
Chloramine	0·005	9†		4
	0·005	4‡		4
Tervalent chromium (as Cr)	1	10		5
	4	67		6
Sexavalent chromium (as Cr)	0·1	—	Inhibited nitrification	7
	0·3	—	Had little effect on carbonaceous oxidation	7
	0·45	8†		4
	0·45	10‡		4
	0·9	20		7
	1·0	10		5
	4·0	70		6
Cobalt chloride (as Co)	0·9	5		3
Copper (as Cu)	0·01	5		7
	0·05	20	Inhibited nitrification	7
	0·1	8†		4
	0·1	7‡		4
	0·4	42		3
Cyanide (as CN)	0·1	5		8
	0·3	5		9
	0·8	22		3
	1·0	40		8
	2·0	38		3
	25	100		9
Lead (as Pb)	0·2	6†		4
	0·2	5‡		4
Mercuric chloride (as $HgCl_2$)	0·025	20		5
	0·5	35†		4
	0·5	37‡		4
	1·0	90		3
	2·0	100		5
Sea water ..	—	—	Rate of biochemical oxidation of sewage appeared to be greatest at a salinity of 8·5 g per 1000 g (= about 25 per cent. of sea water), but in one experiment the rate was greatest at a salinity of 22·7 g per 1000 g	10
	—	—	5-day B.O.D. of polluted estuary water was usually greatest at a salinity of about 20 g per 1000 g, but with some samples was greatest at a salinity of below 5 g per 1000 g	11

* In the manometric method (see Table IB) the sample is usually undiluted, but in the dilution method the sample may be diluted 50 to 100 times with synthetic dilution water.

† Reduction in B.O.D. of a solution containing 300 mg of glucose per litre, synthetic dilution water being used.

‡ Reduction in B.O.D. of a solution containing 300 mg of glutamic acid per litre, synthetic dilution water being used.

is given in Tables IA and IB. Some organic compounds may be inhibitory and others may not respond to the test because their molecular structure renders them resistant to biological attack. Lists of organic compounds, including most of the active agents of synthetic detergents,[12,13,14,15] whose behaviour in the dilution and manometric B.O.D. tests has been examined, are to be found in the technical literature.[16,17,18,19,20,21]

TABLE IB

EFFECT OF INHIBITORY SUBSTANCES ON THE DETERMINATION OF THE B.O.D. OF SEWAGE BY THE MANOMETRIC METHOD

Interfering substance or ion	Concentration in undiluted sewage, mg per litre	Effect on amount of oxygen taken up by sewage in 5 days: Reduction, %	Effect on amount of oxygen taken up by sewage in 5 days: Qualitative effect	Reference
Cadmium (as Cd) ..	5	16		22
Cobalt (as Co) ..	5	24	Caused initial retardation	22
Copper (as Cu) ..	1	22		22
Tervalent chromium (as Cr)	up to 20	—	Little effect	6
	25	14		22
Sexavalent chromium (as Cr)	up to 20	—	Little effect	6
	10	3		22
	25	10		22
Ferric chloride (as $FeCl_3$)	5*	13†		23
Nickel (as Ni) ..	5	24	Caused an initial lag of 1 day	22
Zinc (as Zn) ..	5	12		22

* Plus sodium bicarbonate to adjust the pH to 7·8.
† In 6 days.

(*c*) In addition to the standard dilution method, manometric methods for determining B.O.D. are discussed in general terms (see p. 705). Such methods are especially useful for trade effluents.

(*d*) Consideration was given to oxygen absorption, which, although measured in the 5-day B.O.D. test, may not be biological in character but rather a direct chemical oxidation, *e.g.*, that of fine metallic particles or readily oxidisable inorganic compounds. If it is desired to differentiate between the true biological and chemical oxidation, this may be done by using chloroform as an inhibitor of biological action, and carrying out duplicate determinations with and without the addition of chloroform.

(*e*) Compounds that interfere with the determination of dissolved oxygen by the Winkler method also interfere with the B.O.D. test; these include oxidising and reducing compounds, which either liberate iodine from potassium iodide or reduce liberated iodine, and some other compounds that have been reported to cause interference, as follows—

Chromate—This liberates iodine from potassium iodide. Since the oxygen consumed in the B.O.D. test is a difference figure, Placak, Ruchhoft and Snapp[7] consider that this should not introduce an error, particularly when the concentration of chromate is low.

Gas liquor—Abbott and Fearn[24] state that the dissolved oxygen in dilutions of sewage plus gas liquor cannot be determined by the Rideal - Stewart modification (see p. 695); the azide method could be used instead.

Miscellaneous substances—Heukelekian[3] states that the following substances, at the concentrations stated, interfere seriously with the unmodified Winkler method (see p. 692)—

Substance	Concentration, mg per litre
Gasoline	..1000 (slightly at 100)
Phenol	.. 50 (slightly at 10)
Sodium arsenite	.. 8
Potassium dichromate ..	.. 100
Potassium chromate	.. 10
Strychnine	.. 100

Interference by sodium arsenite can be overcome by using the Rideal - Stewart modification.

Sulphur dioxide and sulphites—Tyler and Gunter[25] state that sulphur dioxide and sulphites interfere in the unmodified Winkler determination, and that the hypochlorite modification does not overcome the interference.

Dichromate Value

(*Oxygen absorbed from boiling acid potassium dichromate solution*)

Potassium dichromate in acid solution was first used by Adeney and Dawson[1] to determine the chemical oxygen demand of polluted waters. The method has since been studied and improved by Abbott,[26] Ingols and Murray,[27] Moore, Kroner and Ruchhoft[28] and by others[29,30,31,32,33] and, since the introduction of the use of silver sulphate,[32] the results obtained have been found to be very close to the theoretical value for a large number of organic compounds. This finding was in marked contrast to those obtained by the traditional method in which potassium permanganate is used.

The relevant Panel of the Joint Committee therefore considered the two methods in detail. Some comparative tests were carried out during this investigation and the details and results are reported in a paper by Cameron and Moore.[34]

The dichromate-value test has other advantages besides yielding results that are often close to the theoretical value. One of these advantages is that a considerably shorter time is required to carry out a test—2 hours at the most—than is required for determining the permanganate value. Again, because the test is carried out at the boiling-point, errors caused by temperature differences do not arise and there is no need for a thermostatically controlled bath. The apparatus used is slightly more complicated than that necessary for the permanganate-value test, but it is still of a type that is readily available in every laboratory.

Finally, it should be noted that the inclusion in a collection of recommended methods of a procedure for determining the dichromate value is an entirely new feature in British practice. This is done in the belief, supported by the experimental work carried out, that the test is worthy of greater notice than it has hitherto received.

DEFINITION—

The dichromate value is expressed as the number of milligrams of oxygen absorbed from standard dichromate per litre of sample.

RANGE—

(*a*) In the *absence* of chloride, for dichromate values down to 50 mg per litre.

(*b*) In the *presence* of chloride, the method is applicable when the number of milligrams of oxygen absorbed per litre (*i.e.*, dichromate value) is greater than one-quarter of the number of milligrams of chloride present per litre. (See Note 1.)

APPLICABILITY—

The method is generally applicable. However, chloride ion (if present in amounts greater than four times the dichromate value) and certain nitrogenous organic substances (*e.g.*, urea) interfere. Details are given at the end of the "Procedure" for determining the correction to be applied for the amount of nitrogen oxidised, and this should be determined each time when accurate results are required. However, for routine analyses of an effluent, a preliminary check need only be made to ascertain whether, and to what extent, the nitrogen has been oxidised: if the result is negative, then the method is applicable without modification.

REAGENTS—

Silver sulphate solution—A 1·25 per cent. solution in 50 per cent. v/v sulphuric acid.

Sulphuric acid, sp.gr. 1·84.

Potassium dichromate solution, 0·1 N.

Ferrous sulphate solution, 0·1 N—Dissolve 27·8 g of the pure salt, $FeSO_4.7H_2O$, in a mixture of 25 ml of sulphuric acid and 75 ml of distilled water, and dilute with distilled water to 1 litre. This solution must be standardised at frequent intervals against the potassium dichromate solution, and its factor determined.

Ferrous - 1 : 10-phenanthroline indicator solution—Dissolve 0·695 g of ferrous sulphate, $FeSO_4.7H_2O$, in 100 ml of distilled water, add 1·485 g of 1 : 10-phenanthroline monohydrate and shake until dissolved.

PROCEDURE—

First determine the chloride content of the effluent sample in terms of 0·1 *N* silver nitrate (see "Determination of Chloride (Chlorion)"*).

Transfer a suitable volume of the effluent sample (25 or 50 ml) to a 500-ml round-bottomed flask having a standard B24 ground neck, and add to it the volume of 0·1 *N* potassium dichromate solution equal to the volume of 0·1 *N* silver nitrate required to combine with the chloride in the same volume of sample; then add a further 25 ml of potassium dichromate solution. Add an amount of sulphuric acid equal to the total (aqueous) volume multiplied by 1·2. Fit a suitable reflux condenser to the flask. Mix the solution well and boil it under reflux for at least ½ hour. At the end of this period, add 10 ml of silver sulphate solution and allow the heating under reflux to continue for a further 1½ hours. (See Note 2.)

Dilute the solution with a volume of distilled water equal to the original (total) aqueous volume multiplied by 4·5. Titrate with the ferrous sulphate solution, using a ferrous - 1 : 10-phenanthroline indicator solution; let this titre be T_1.

Treat in the same manner a volume of distilled water equal to the volume of sample taken, but omitting that volume of potassium dichromate solution added to deal with chloride; let this titre be T_2.

From the titration difference $(T_1 - T_2)$, calculate the net volume of dichromate used by the oxidation and then calculate the dichromate value in terms of milligrams of oxygen absorbed per litre of sample.

1 ml of 0·1 *N* potassium dichromate solution ≡ 0·8 mg of oxygen.

MODIFICATION FOR INTERFERENCE BY NITROGENOUS ORGANIC SUBSTANCES—

A determination of the total unoxidised nitrogen is carried out on (*a*) the final solution obtained above after titration, (*b*) a fresh portion of the sample equal in volume to that originally taken and (*c*) the titrated blank. The difference between (*a*) and (*b*), after correction for the blank, gives the loss of nitrogen incurred during the dichromate-value test.

The loss of nitrogen may take place through more than one chemical route, but it appears that tervalent nitrogen is involved. Therefore—

1 ml of 0·1 *N* potassium dichromate solution ≡ 0·467 mg of nitrogen.

Procedure—Evaporate to white fumes of sulphuric acid the test and blank solutions obtained after titration, and continue heating until the solutions are clear green in colour.

Proceed to determine the total unoxidised nitrogen in both solutions, as described under "Determination of Total Unoxidised Nitrogen."†

In addition, determine directly the total unoxidised nitrogen in a fresh portion of the effluent sample equal in volume to that originally taken.

Correction to be applied—For each 0·467 mg of nitrogen lost, deduct 0·8 mg of oxygen from the total amount of absorbed oxygen determined as above.

NOTES—1. Chloride, which is present in most trade effluents, introduces two sources of error.

In the first place, dichromate oxidises chloride to free chlorine and it is shown[34] that this oxidation is neither complete nor consistent, although always of a high degree. A consistent correction cannot, therefore, be applied. It should be appreciated that an assumption of 100 per cent. oxidation of chloride transfers an error to the concurrent oxidations and the magnitude of this transferred error depends on the relationship of chloride content to that of other oxidisable matter.

In the second place, the chlorine developed in the foregoing oxidation oxidises nitrogenous matter to elementary nitrogen. Hence, nitrogenous matter may be simultaneously oxidised directly by dichromate and indirectly by the chlorine developed, the latter oxidation being the greater. There is some evidence that

* See *Analyst*, **1956, 81**, 721, or *Reprint No. 5*.
† See *Analyst*, **1957, 82**, 276, or *Reprint No. 8*.

tervalent nitrogen is involved and the nitrogen correction is based on this assumption: nevertheless, because of the simultaneous reactions taking place, the correction is not precise.

This indirect oxidation, although depending on the presence of chloride, is independent of the chloride concentration; the chlorine is reduced to chloride in performing the oxidation, thereby generating a cyclic reaction so long as nitrogenous matter responsive to this oxidation remains.

2. Experience may show that for some effluents the second reflux time may be reduced to 30 minutes.

Permanganate Value

(Oxygen absorbed from acid potassium permanganate solution)

The concentration of the permanganate solution used is usually N/80, but for trade wastes a concentration of *N*/8 is sometimes used (see Note, p. 691). The standard time for the test is 4 hours, but for some purposes a supplementary 3-minute test is made, and the relation between the 4-hour and the 3-minute tests may be a useful guide to the type of waste being examined.

It is desirable to use the same amounts of permanganate and of acid in all tests, the volume of sample being adjusted according to its estimated strength; also to make up the total volume used in any test to a fixed volume with water, and to adjust the volume of the sample so as to leave about one-half the permanganate unused. In practice, reductions between 30 and 70 per cent. are admissible.[35] The volume recommended is 50 ml of *N*/80 potassium permanganate, and the following figures show the volumes of sample usually necessary for a range of estimated permanganate values—

Permanganate value, mg per litre	25	50	100	125	250	500
Volume of sample, ml	100	50	25	20	10	5

When a small volume of sample is indicated, it is advisable to add it as a larger volume of a suitable dilution in order to ensure a representative distribution of suspended solids.

REAGENTS—

Potassium permanganate solution, approximately N/80—Dissolve 4·0 g of potassium permanganate in 1 litre of distilled water in a covered beaker and heat the solution to 90° to 95° C for 2 to 3 hours. Dilute to 10 litres with distilled water and store in the dark for some days to oxidise organic matter and to allow precipitated manganese dioxide to settle. Siphon or decant the supernatant liquid without disturbing the sediment, or filter through glass-wool or acid-digested and washed asbestos, or through a sintered-glass filter; the use of filter-paper is inadmissible. This solution must be stored in the dark, or in dark-glass bottles, and away from the risk of airborne dust or organic contamination.

Potassium permanganate solution, approximately N/8—Dissolve 4·0 g of potassium permanganate in 750 ml of hot water and heat to 90° to 95° C for 2 to 3 hours. Cool and dilute to 1 litre, and store in the dark for some days. Decant the supernatant liquid without disturbing the sediment or filter through glass-wool, acid-digested and washed asbestos, or through a sintered-glass filter. Store in the dark or in dark-glass bottles.

Potassium iodide solution, 10 per cent. w/v—Dissolve 10 g of potassium iodide in 100 ml of distilled water and store in a dark bottle. It is important that this solution shall remain colourless during the whole period that it is in use.

Potassium iodate solution, N/40—Dry analytical-reagent grade potassium iodate at 120° C, dissolve 0·892 g in distilled water and dilute to exactly 1 litre. This solution is stable for long periods if stored in a glass-stoppered bottle.

Sodium thiosulphate solution, approximately N/4—Dissolve 63 g of sodium thiosulphate, $Na_2S_2O_3.5H_2O$, in 1 litre of copper-free freshly boiled and cooled distilled water. Stabilise the solution by the addition of 1 ml of chloroform or 10 mg of mercuric iodide and allow to stand for several days before use.

Sodium thiosulphate solution, approximately N/80—Dilute 50 ml of *N*/4 sodium thiosulphate solution to 1 litre with copper-free freshly boiled and cooled distilled water, adding 1 ml of chloroform or 10 mg of mercuric iodide. Although reasonably

stable if kept in a dark-glass bottle, it requires frequent standardisation against potassium iodate as follows—

Mix 5 ml of potassium iodide solution and 10 ml of dilute sulphuric acid and add 20 ml of $N/40$ potassium iodate solution, in that order, in a glass-stoppered flask. Add about 100 ml of distilled water. Titrate immediately with approximately $N/80$ sodium thiosulphate solution until the colour is pale yellow; add 2 or 3 drops of starch indicator solution and continue the titration until the blue colour just disappears.

1 ml of $N/80$ sodium thiosulphate solution $\equiv$ 0·1 mg of oxygen.

Sulphuric acid, diluted (1 + 3)—Add gradually and cautiously 1 volume of sulphuric acid, sp.gr. 1·84, to three volumes of distilled water, mix and cool. Add $N/80$ potassium permanganate solution until a permanent faint pink colour is observed.

Starch indicator solution—Grind 1 g of soluble starch into a smooth paste with a little cold distilled water, and pour it into 1 litre of boiling distilled water with constant stirring. Boil for 1 minute, and allow to cool before use. This solution should be freshly prepared.

Stabilised starch indicator solution can be made by grinding 10 mg of mercuric iodide with the starch before adding the cold water, and then proceeding as described above: this solution can be used for some time after it is prepared. Alternatively, 0·1 g of thymol may be added to the boiling water used for making the solution.

NOTE—As an alternative to starch solution, sodium starch glycollate solution, which is stable for many months, may be used. Between 1 and 2 ml of a 0·5 per cent. solution in cold distilled water may be added at the start of the titration, the approach to the end-point being shown by change from green to intense blue. At the end-point, which is sharp, the solution becomes colourless.

PROCEDURE FOR PERMANGANATE VALUE, BY 4-HOUR TEST—

Measure into a 12-oz glass-stoppered bottle 10 ml of diluted sulphuric acid and 50 ml of $N/80$ potassium permanganate solution. Select the volume of effluent sample to be used, and subtract it from 100 ml; add this final volume of distilled water to the acidified permanganate, raise the temperature of the solution to 27° C and add the volume of sample chosen, also at 27° C, and mix by gentle rotation of the bottle. Maintain the mixture at 27° C in a water bath or, after first raising the temperature to 27° C, in an incubator for 4 hours, re-mixing after 1 hour if much suspended matter is present.

After exactly 4 hours, add 5 ml of potassium iodide solution or approximately 0·5 g of potassium iodide, mix, and titrate with N/80 sodium thiosulphate, adding a few drops of starch indicator solution near the end-point. Continue the titration until the blue colour disappears, and ignore any blue colour that may return.

Carry out a blank determination, using distilled water instead of the sample.

Calculation—

$$\text{Permanganate value (4-hour), mg per litre} = \frac{(\text{Vol. of } N/80\ Na_2S_2O_3 \text{ for blank, ml} - \text{vol. of } N/80\ Na_2S_2O_3 \text{ for test, ml}) \times 100}{\text{Vol. of sample taken, ml}}.$$

PROCEDURE FOR PERMANGANATE VALUE BY 3-MINUTE TEST—

Follow the same procedure as for the 4-hour test; it is essential to warm the effluent sample, the reagents and the distilled water to 27° C before mixing. Measure the permanganate and the acid into the bottle to be used for the test, and measure the requisite volumes of sample and distilled water into a separate container. Add the sample and the distilled water to the reagents, and mix by rotation. Maintain at 27° C for exactly 3 minutes, add the potassium iodide as in the 4-hour test, titrate, and calculate the permanganate value (3-minute) in the same manner.

INTERFERENCES—

Presence of nitrite—The test is affected by nitrite. A correction may be made by estimating the nitrite present and subtracting from the permanganate value a

figure equivalent to the nitrite nitrogen × 1·14. If the amount of nitrite present is significant, the result should be returned as "permanganate value corrected for nitrite." It is, however, preferable to destroy the nitrite by the following procedure—

Acidify the sample and the blank; add 1 g of urea to each and allow the solutions to stand for 5 minutes. Then add the appropriate volume of potassium permanganate solution and continue as in the "Procedure."

Presence of chromate—In the presence of acid and potassium iodide, chromates are reduced and decrease the permanganate value. A preliminary titration may be made with $N/80$ sodium thiosulphate after acid and potassium iodide solution have been added to a known volume of the sample, and the equivalent oxygen-absorption value added to the ascertained permanganate value.[36]

Presence of chloride—The use of phosphoric acid instead of sulphuric acid has been recommended in the presence of high concentrations of chloride.[37]

NOTE: DETERMINATION OF THE PERMANGANATE VALUE BY USING $N/8$ POTASSIUM PERMANGANATE SOLUTION AT 27° C—

When determining the permanganate value of trade wastes for calculation of the McGowan strength,[38] it is necessary to use $N/8$ potassium permanganate solution. There has, in the past, been some latitude in procedure, but the method in general has been in line with the recommendations of the Royal Commission on Sewage Disposal,[38] *i.e.*, 10 ml of $N/8$ potassium permanganate, 10 ml of 10 per cent. sulphuric acid and a volume of the sample that leaves about one-half of the permanganate unabsorbed. The volume of sample generally used is 25 ml, or a smaller volume of sample made up to 25 ml; it is not customary to dilute to 100 ml, as in the 4-hour test. The volumes used in this test should be precisely stated.

After tests for permanganate value have been carried out, all bottles should immediately afterwards be washed with chromic acid, then with tap water and finally with distilled water.

Dissolved Oxygen*

PRINCIPLE OF METHOD—

The methods to be described for the determination of dissolved oxygen in water **or trade effluents** are based on the procedure originally devised by Winkler.[39] In this method the precipitation of manganous hydroxide is brought about in a glass-stoppered bottle completely filled with the sample under test. Any oxygen present in solution then quickly combines with the manganous hydroxide to form higher hydroxides, which, on subsequent acidification in the presence of iodide, liberate iodine in an amount chemically equivalent to the original dissolved-oxygen content of the sample. The iodine is then determined by titration with a standard solution of sodium thiosulphate.

The observance of adequate precautions when taking samples . . . . . is particularly important when they are required for dissolved-oxygen determinations.

SAMPLING AND APPARATUS—

Bottles—The bottle used should be of good quality, with a narrow neck and a well fitting ground-glass stopper, and should be of about 250 ml capacity. It is a great convenience if each bottle and its stopper is etched with a distinguishing number. The base of the stopper should be of such a shape that when the bottle is completely filled with water the stopper can be firmly inserted without trapping air bubbles. The bottles should be cleansed with chromic acid mixture (not soap or synthetic-detergent solutions) and then washed out several times with clean water.

* The methods described here for the Determination of Dissolved Oxygen and of Biochemical Oxygen Demand up to (but excluding) "Manometric Methods" (p. 705), but including "General Observations" (on p. 706), have been taken by permission of the Controller, H.M. Stationery Office, from "Chemical Analysis as Applied to Sewage and Sewage Effluents," Second Edition (1956), published by H.M.S.O. for the Ministry of Housing and Local Government. Departures from the text of the Ministry's methods (other than changes of a purely editorial nature made to ensure consistency with other methods in this series) are indicated by the use of **sans-serif type for reworded and new material,** and by groups of five dots (. . . . .) for omission.

Sampling—The method of collecting the sample should involve the least possible disturbance of the liquid. This is best accomplished with the aid of a special sampling arrangement (see "Sampling"*), which ensures a several-fold displacement of the liquid in the sampling bottles without agitation with air bubbles.

If the percentage saturation of the sample with oxygen is to be determined, the temperature of the liquid at the moment of sampling and, if necessary, the barometric pressure should be noted.

Sampling apparatus—The sample must be collected without causing any change in the concentration of dissolved oxygen. Sometimes the liquid can be siphoned through a glass or rubber tube into the sample bottle; the tube should deliver to the bottom of the bottle and the liquid should be allowed to flow until the contents of the bottle have been changed several times.

The type of sampler . . . . . in common use . . . . . is the displacement sampler, consisting of a cylindrical container with a lead ring at the bottom, inside which the sample bottle is placed. The lid carries two tubes, one at the centre through which the water enters the bottle and another through which the displaced air leaves the container. These tubes are closed by small bungs attached to cords made fast to a line. While the sampler is being lowered to the desired depth, its weight is taken by a spring, also attached to the line. When the desired depth is reached, the line is jerked, thereby removing the bungs. Water then flows through the bottle until the container is full and the bottle is therefore washed out several times before the sample is taken. . . . .

When this displacement sampler is to be used at only a shallow depth, it is unnecessary to close the inlet and outlet tubes with bungs.

. . . . .

The temperature of the water should always be recorded immediately the sample is brought to the surface.

It is important to fix the dissolved oxygen in a sample immediately it is taken, as changes in the concentration of dissolved oxygen may occur rapidly both when the sample is sub-saturated and when it is super-saturated.

In the manipulations to be described in the ensuing sections, it is *extremely unwise* to suck the required amounts of reagents into pipettes, especially the alkaline iodide and sulphuric acid reagents. Dipping pipettes or pipettes fitted with rubber balls or teats should be used, and these will be found of very great convenience for field work. . . . .

DIRECT DETERMINATION AND MODIFICATIONS—

The Winkler method of determining dissolved oxygen is applicable only to relatively pure water. Many substances can interfere with the determination, and suitable modifications must be adopted to attain a reasonably accurate result. The commonest interfering substances are nitrites, ferrous salts, organic matter, sulphites, residual chlorine and suspended solids, and very considerable errors can be introduced in the determination if special precautions are not taken.

The two principal modifications of the original Winkler method are—

(*a*) The Alsterberg[40] or sodium azide procedure; this prevents interference by nitrite, but not by easily oxidised organic substances or inorganic substances (*e.g.*, ferrous iron, if more than 1 mg per litre is present).

(*b*) The Rideal - Stewart[41] or permanganate modification; this prevents interference by nitrite, by ferrous iron and by easily oxidised organic matter.

THE WINKLER METHOD

REAGENTS—

Manganous sulphate solution—Dissolve 500 g of manganous sulphate, $MnSO_4.4H_2O$, in distilled water, filter if necessary and dilute to 1 litre. . . . . The salt used should be free from ferric and manganic salts, *i.e.*, no iodine should be liberated when a portion of the salt is added to acidified potassium iodide solution.

* See *Analyst*, 1956, **81**, 492, or *Reprint No.* 3.

Alkaline iodide solution—Prepare a solution containing 500 g of sodium hydroxide (or 700 g of potassium hydroxide) and 150 g of potassium iodide (or 140 g of sodium iodide) per litre. The alkali solution should be virtually free from carbonate, and the following method of preparation may be used. Dissolve the required weight of pure sodium hydroxide in its own weight of distilled water. When cool, transfer the solution to a bottle preferably coated internally with paraffin wax, close with a rubber stopper, and allow to stand quiescent for some days, during which any carbonate present sinks to the bottom. Decant the clear supernatant liquid. Now add the required quantity of potassium iodide dissolved in a small quantity of cold freshly boiled distilled water; dilute the mixture to the desired concentration with cold freshly boiled distilled water and mix.

Sulphuric acid, sp.gr. 1·84.

Potassium iodate solution, N/40—See p. 689.

Sodium thiosulphate solution, approximately N/4—Stock solution, see p. 689.

Sodium thiosulphate solution, approximately N/80—Working solution, see p. 689.

Starch indicator solution—See p. 690.

PROCEDURE—

The following procedures apply to sample bottles of nominal capacity 250 ml. If a different size is used, the amounts of reagents must be adjusted accordingly.

To the sample of liquid taken as previously described, add 1 ml of manganous sulphate . . . . . solution, followed by 1 ml of alkaline iodide solution, the tips of the pipettes in each case being inserted well below the surface of the liquid. (The fine points of pipettes may be cut off so that they empty reasonably quickly.) Replace the stopper carefully so as to avoid inclusion of air bubbles, and thoroughly mix the contents by inverting and rotating the bottle several times.

The precipitate flocculates and settles fairly rapidly, but after the first mixing it will rarely be found that the top liquid is clear. A second period of inverting and rotating is necessary, both to clarify the liquid and to ensure that all the dissolved oxygen in the upper part of the bottle is absorbed. With saline liquids, a prolonged period of mixing may be necessary.

The precipitate is white if the sample was devoid of oxygen at the time of sampling, but becomes increasingly brown with rising oxygen content of the sample.

In the absence of organic matter the determination of the dissolved oxygen by acidification and titration may be postponed at this stage, provided that the bottles are kept in the dark. This is particularly convenient when the sampling for dissolved oxygen forms part of a survey in the field: the labelled bottles can be stored until the return to the laboratory. It is, of course, vital that air be excluded from the bottle during the period between precipitation and acidification; this should be assured if bottles with well fitting stoppers are used, but even then the period should not exceed a few hours.

Now add by pipette 1·5 ml of sulphuric acid, sp.gr. 1·84, re-stopper and well mix the contents by rotation. Some analysts prefer to acidify with 3 ml of 50 per cent. sulphuric acid, in order to avoid manipulations with the concentrated acid. Since solution of the precipitate is not rapid, allow sufficient time for this to take place. If necessary, continue to agitate by rotation until the precipitate dissolves before opening the bottle.

Transfer by pipette into a conical flask a suitable volume (say 100 ml from a 250-ml bottle) of the solution and immediately titrate the iodine with *N*/80 sodium thiosulphate solution, using as indicator 2 ml of starch indicator solution, which should be added towards the end of the titration. For very precise work an amperometric method may be used to detect the end-point in the titration of iodine with thiosulphate. Whichever method is used, however, it must be remembered that iodine is volatile and therefore the titration of its solution must be carried out as expeditiously as possible and with the minimum of exposure to the air.

. . . . .

CALCULATION—

$$\text{Dissolved oxygen, mg per litre} = \frac{\text{Volume of } N/80 \text{ sodium thiosulphate, ml} \times 100}{\text{Volume of sample titrated, ml}}.$$

There are two slight sources of error in this simplified calculation. One is due to the volume of sample displaced from the bottle by the reagents added, usually 3·5 ml for the 250-ml bottle. If great accuracy is required, the volume of the added reagents can be deducted from the volume of the bottle in making the calculation. The other source of error is that due to the presence of dissolved oxygen in the added reagents. This, however, has been determined[42,43] and is so small that it is negligible in the examination of trade effluents, although it may have to be taken into account in, for example, the examination of boiler-feed water.[44]

PRESENCE OF CERTAIN TYPES OF ORGANIC MATTER—

If the sample contains organic matter that is capable of direct oxidation by dissolved oxygen at pH values of about 12, corresponding to the degree of alkalinity produced when the alkaline iodide solution is added to a sample, then the sample should be acidified immediately the manganese hydroxide precipitate has settled sufficiently to give a layer of clear liquid near the top of the bottle. This procedure will give reliable results in the presence of organic matter corresponding to 1000 mg of dextrose or peptone per litre. If, say, about 5000 mg of such organic materials are present per litre, the samples should be acidified immediately after agitation, without waiting for the precipitate to settle.[45] As a guide to the time required for agitation, it may be mentioned that with 1 ml of manganous sulphate solution the time required for complete fixation of oxygen is 40 to 50 seconds; with double the quantity the time is halved.

THE ALSTERBERG OR SODIUM AZIDE MODIFICATION

When more than a trace of nitrite is present in a sample of liquid, it is not possible to obtain a satisfactory end-point to the iodine - thiosulphate titration, since nitrite in acid solution catalyses the liberation of iodine from iodide by dissolved oxygen. Hence the Winkler method is not directly applicable to waters containing nitrite. When nitrite, or ferrous iron at a concentration of less than 1 mg per litre, are the only interfering substances present, the Alsterberg modification is recommended for the determination of dissolved oxygen. In biochemical oxygen demand work, nitrites, even if absent at the start, may be formed during the incubation period. They may generally be expected in the effluents from biological oxidation processes.

Since nitrite is probably the most frequently encountered interfering substance, it is of great convenience for routine work if the reagent to eliminate it—sodium azide—is added in combination with the alkaline iodide solution. If nitrite is absent, the addition of azide is no disadvantage.

If the sample contains ferric salts, these may cause an error by interaction with the iodide. This can be avoided by carrying out the final acidification with 4 ml of 85 per cent. phosphoric acid.

The actual removal of nitrite takes place only after the acidification stage, *i.e.*, the reaction is between nitrous acid and hydrazoic acid—

$$HNO_2 + HN_3 = N_2 + N_2O + H_2O$$

The azide and its decomposition products are indifferent to mild oxidising agents. It is essential that mineral acid be present, for in neutral or acetic acid solution the following reaction occurs—

$$2NaN_3 + I_2 = 2NaI + 3N_2$$

PROCEDURE—

Carry out the determination in exactly the same way as described for the Winkler method, but replace the alkaline iodide reagent by a solution containing 500 g of sodium hydroxide, 150 g of potassium iodide and 10 g of sodium azide per litre. Prepare the sodium hydroxide as previously described. Dissolve the potassium

iodide and sodium azide separately in small amounts of distilled water. Add the iodide solution to the sodium hydroxide solution and dilute to about 950 ml. When cool, add the azide solution with stirring and adjust the total volume to 1 litre.

THE RIDEAL - STEWART OR PERMANGANATE MODIFICATION

When ferrous iron in excess of 1 mg per litre, or easily oxidisable organic matter, is present in a sample, the preliminary treatment known as the Rideal - Stewart or permanganate modification should be followed. This treatment also removes nitrites. It should be noted that 1 mg of ferrous iron per litre causes the dissolved oxygen value to be 0·14 mg per litre too low if the unmodified Winkler method is used.

Whereas ferrous salts, should their removal be neglected, will cause an apparent loss of dissolved oxygen, ferric salts may cause an unduly high result to be obtained, owing to release of iodine from iodides in the final acidification stage, especially on long standing. . . . . .

When samples contain drainage from mines, acid stream water or other waters that might contain considerable amounts of ferrous and ferric salts, the permanganate modification together with the potassium fluoride treatment should be followed.

The Rideal - Stewart modification is inappropriate for samples of sulphite wastes . . . . . , or for samples containing heavy suspensions of mud or activated sludge, and should therefore not be used in these cases.

REAGENTS (for the Rideal - Stewart preliminary treatment)—

Potassium permanganate solution, approximately N/8—Dissolve 3·95 g of potassium permanganate in 1 litre of distilled water.

Potassium oxalate solution—Dissolve 2 g of potassium oxalate, $(COOK)_2.H_2O$, in 100 ml of distilled water.

PROCEDURE—

Add exactly 0·7 ml of sulphuric acid, sp.gr. 1·84, to the sample, and follow with sufficient potassium permanganate solution so that, after being well mixed, the liquid retains a definite red-violet tinge for 20 minutes. With unknown samples, the correct amount may have to be ascertained by trial beforehand with another portion of sample, or by adding 1 ml of potassium permanganate solution to the bottle and observing its reaction. If noticeable fading takes place, another 1 ml must be added. If more than 2 ml are required, then a stronger solution should be used. With normal samples, 1 ml is usually sufficient.

After the 20-minute oxidation period has elapsed, remove the excess of permanganate by reaction with oxalate, using not more than the minimum amount of oxalate, since excess leads to results that are lower than the true value. Add 0·5 ml of potassium oxalate solution first, and if the permanganate colour persists after 5 to 10 minutes, add another 0·5 ml, leaving the bottles until complete decolorisation has taken place, otherwise the traces of manganic compounds remaining will react later with the iodides, to give falsely high results. The length of time for the decolorisation to be completed is noticeably dependent on the temperature; . . . . .

It has been pointed out[46] that if iron salts greater in amount than 1 mg per litre are present, then the decolorisation should be allowed to proceed in the dark, because, in the presence of oxalates, light easily reduces ferric salts to the ferrous condition.

When the contents of the bottle are completely decolorised, add 1 ml of manganous sulphate solution, followed by 3 ml of alkaline iodide solution (see pp. 692 and 693). Well mix the contents of the bottle by inversion and rotation, and proceed as described for the Winkler method. **The small error in the calculation given for the Winkler test (due to the displacement of sample by reagents) will be greater in the present case, but can, if desired, be allowed for by the correction given at the top of p. 694. The error due to dissolved oxygen present in the added reagents will again be negligibly small.**

When the iron content of the sample is greater than about 10 mg per litre, interaction between ferric salts and iodide may introduce an error. This may be avoided by carrying out the final acidification with 4 ml of 85 per cent. phosphoric

acid; alternatively, 2 ml of a 40 per cent. solution of potassium fluoride can be added to the sample at any stage before final acidification.

Special procedures

Polythionate, thiosulphate and sulphite wastes—

It has been stated that, when the interfering substances present include polythionates, thiosulphates or sulphites, permanganate treatment is inadvisable, as it cannot be relied upon to transform these compounds completely into sulphates, and unless this is done, substances will still be present that will interfere with the Winkler procedure. A method of dealing with such samples by preliminary oxidation with alkaline hypochlorite, and subsequent removal of excess of chlorine by means of sulphurous acid, has been suggested.[47] The latter part of this treatment is specified for samples containing residual chlorine, which of course must be removed before the Winkler procedure is undertaken.

Probably in any case of this sort, however, research would be necessary to establish the validity of the method, with use of, for example, the gasometric method of determination as a reference.

Sludges and muds—

The suspended matter of trade effluents, sludge or river mud, when present in amounts sufficient to give an apparent immediate oxygen demand, prevents the Winkler procedure from giving a true result. In such cases the following preliminary treatment is necessary. Collect a sample of about 1 litre in a glass-stoppered bottle, with the usual precautions against aeration. Add 10 ml of a 10 per cent. solution of aluminium potassium sulphate, and follow with 1 to 2 ml of ammonium hydroxide, sp.gr. 0·880. Re-stopper the bottle and rotate it for about 1 minute. After the floc has settled for about 10 minutes, siphon the clear supernatant liquid into the reaction bottle, taking the usual precautions to ensure at least two displacements of liquid, and follow the appropriate modification of the Winkler procedure.[48]

Activated-sludge mixed liquors—

If it is desired to determine the dissolved oxygen present in a liquid containing large amounts of easily oxidisable organic matter, as, for example, that flowing from the aeration tanks and channels of an activated-sludge plant or a bio-aeration plant, the direct procedure cannot be employed, because the suspended matter rapidly removes oxygen from solution as it settles. The bottles in which the sample is taken must therefore contain some inhibiting reagent that stops de-oxygenation at the moment of sampling. Ruchhoft and Placak[49] propose an inhibiting reagent of sulphamic acid, copper sulphate and acetic acid, prepared as follows. Dissolve 32 g of sulphamic acid in 475 ml of distilled water and add a solution of 50 g of copper sulphate, $CuSO_4.5H_2O$, in 500 ml of distilled water and 25 ml of glacial acetic acid. Heat should not be used to dissolve the sulphamic acid, nor should the mixed reagent be exposed to heat at any time.

For each 100 ml of sample to be taken, measure 1 ml of this reagent into the sampling bottle, then fill the bottle to overflowing and insert the stopper. Thoroughly mix the contents, allow settlement of sludge to take place, and then siphon the top liquid into a dissolved-oxygen bottle. Then use the sodium azide modification of the Winkler method, as previously described.

Expression of results—

Dissolved oxygen is reported as milligrams per litre. If the result is required as ml of oxygen gas (at 0° C and 760 mm pressure) per litre of sample, the figure representing milligrams per litre of dissolved oxygen must be multiplied by 0·70.

In addition, it is customary and helpful to give the percentage saturation of the sample. For this, reference must be made to the Table of solubility data (Table II), which sets out the solubility of oxygen in fresh water, in mixtures of fresh water and sea water, and in sea water of stated degrees of salinity at various temperatures when in equilibrium with air containing 20·9 per cent. of oxygen under a pressure of 760 mm.

It should be noted that values appreciably in excess of 100 per cent. saturation are sometimes obtained, particularly with samples drawn from localities where oxygen-forming plants (*e.g.*, algae) are flourishing.

Oxygen is less soluble in salt water than in fresh water, and when it is desired to calculate the percentage saturation of oxygen in a sample of saline liquid, for example, the salinity of the sample must be known.

The solubility of oxygen in water

The solubility of oxygen in water has been investigated by the Water Pollution Research Laboratory,[50] who have obtained figures up to 4 per cent. lower than previously accepted values. Their results are related by the empirical equation—

$$C_o = 14{\cdot}161 - 0{\cdot}3943T + 0{\cdot}007714T^2 - 0{\cdot}00006467T^3,$$

where C_o is the saturation concentration of oxygen in mg per litre at temperature T° C. The formula applies to water in equilibrium with air at a pressure of 760 mm and containing 20·9 per cent. of oxygen.

Table II

Oxygen solubility

Temperature, ° C	Solubility of oxygen in water in equilibrium with air at 760 mm, mg per litre	Correction to be subtracted for each degree of salinity, g of total salts per 1000 g of saline water
0	14·16	0·08405
1	13·77	0·08153
2	13·40	0·07908
3	13·05	0·07671
4	12·70	0·07440
5	12·37	0·07218
6	12·06	0·07002
7	11·76	0·06795
8	11·47	0·06595
9	11·19	0·06402
10	10·92	0·06217
11	10·67	0·06039
12	10·43	0·05869
13	10·20	0·05706
14	9·98	0·05551
15	9·76	0·05404
16	9·56	0·05263
17	9·37	0·05130
18	9·18	0·05005
19	9·01	0·04887
20	8·84	0·04777
21	8·68	0·04674
22	8·53	0·04579
23	8·38	0·04491
24	8·25	0·04410
25	8·11	0·04338
26	7·99	0·04272
27	7·86	0·04214
28	7·75	0·04164
29	7·64	0·04121
30	7·53	0·04085

The effect of salinity on the solubility of oxygen was also investigated and the following formula accounted for the results—

$$C_s = C_o - S(0{\cdot}0841 - 0{\cdot}00256T + 0{\cdot}0000374T^2),$$

where C_s is the solubility at a salinity S (g of total salts per 1000 g of saline water) at temperature T° C.

The results were consistent with the commonly accepted assumption that the solubility at a given temperature varies lineally with the salinity.

The Table of solubility data (Table II), has been calculated from the above equations and is recommended for general use. The first column gives the temperature, the second the solubility in water containing no salts and the third a correction factor, to be subtracted from the solubility, to obtain the figure appropriate to water of salinity 1 g per 1000 g. For other salinities a proportionate correction factor should be used.

An exact correction for salinity can only be made for solutions containing inorganic salts of the same composition and in the same relative concentrations as are found in sea water, since the solubility of oxygen in solutions containing other salts, or the same salts in different proportional concentrations, has not been determined. In cases when the correction does apply, for example to estuary waters, the salinity is determined by the standard international method.[51]

EFFECT OF ORDINARY VARIATIONS IN BAROMETRIC PRESSURE ON CONCENTRATION OF DISSOLVED OXYGEN—

If the barometric pressure at the time of sampling is not 760 mm, then the saturation values at the actual pressure will vary slightly from those given in Table II, according to the formula—

$$S_x = \frac{SP_x}{760},$$

where S_x is the solubility at pressure P_x, S is the solubility at 760 mm, and P_x is the observed pressure in mm.

Most ordinary observations of barometric pressure at sea level in the British Isles fall within the range of 760 ± 20 mm. The following Table shows the variation in solubility over this range—

Temperature, °C	Concentration of dissolved oxygen in equilibrium at 760 mm, mg per litre	Assumed variation in barometric pressure		Variation in concentration in dissolved oxygen, mg per litre
		mm	%	
10	10·92			± 0·29
20	8·84	± 20	± 2·63	± 0·23
30	7·53			± 0·20

The total variation (*i.e.*, twice the variation from the mean) would be easily detectable in accurate measurements and might be large enough to have an influence on observations in the field.

EFFECT OF HEIGHT ABOVE SEA LEVEL ON CONCENTRATION OF DISSOLVED OXYGEN—

If the temperature of the atmosphere at different heights above sea level be taken as constant, it can be shown that for a gas that obeys Boyle's law the relation between the altitude, A, and the barometric height, H, in relation to the barometric height, H_0, at sea level is given by—

$$A = K \log \frac{H_0}{H}.$$

For measurements of A in feet, logarithms to the base 10 and a temperature of 0° C, $K = 60{,}370$. The following table gives some values calculated from this formula—

EFFECT OF ALTITUDE ON BAROMETRIC PRESSURE

Altitude, feet	1000	5000	10,000	16,404 (= 5000 metres)
Calculated barometric pressure,	731	628	519	408

Since the temperature of the atmosphere is not in fact constant at all altitudes, the above relationship is not exactly obeyed. In one particular year, however, the observed mean barometric pressure at an altitude over the **British Isles** of 5000 metres was reported as 403 mm, compared with the calculated 408 mm, indicating that deviations up to that height were not large.

In the British Isles it may be assumed that few . . . . . works or polluted rivers are at a height above sea level of more than 1000 feet, and the taking of this and the normal pressure variations into account might diminish the figures (Table II)

by up to 6 per cent. or increase them by up to 3 per cent. Only rarely would this make an important difference to the value of percentage saturation. It might, however, be worth while introducing a permanent correction for height for regular sampling points more than 500 feet above sea level.

At heights greater than 1000 feet such a correction becomes essential.

Biochemical Oxygen Demand (B.O.D.)*

When polluting organic matter is discharged into a watercourse or lake, a natural purifying action tends to set in, owing to the action of certain micro-organisms, which utilise the . . . . . oxygen dissolved in the water to oxidise the polluting substances. The length of time required for complete purification depends upon many conditions, including temperature and the nature of the organic matter.

The Royal Commission on Sewage Disposal, with which the names of Adeney, Letts, McGowan and Frye are widely associated, proposed that the weight of dissolved oxygen required by a definite volume of liquid for the process of biochemical oxidation during 5 days at 65° F be taken as a measure of the quality of the liquid. This test was first known as the "Dissolved Oxygen Absorption Test" or the "Royal Commission Test"; of late years "Biochemical Oxygen Demand" has superseded the early names, and now the abbreviation "B.O.D." has become almost universal.

The "Dilution Method" of determining B.O.D. is the one most generally used and the details of this, as specified in the Ministry of Housing and Local Government's booklet on Methods of Chemical Analysis as Applied to Sewage and Sewage Effluents, are quoted in the following paragraphs. Another procedure is the "Manometric Method," which has, up to the present, been used mainly for research, but which has many advantages and may well prove to be useful for works control. A following section deals with the general principles of this test, but for working details of the procedure to be adopted readers are referred to the original literature on the subject, for which references are given.

THE DILUTION METHOD

The principle of the test is simple. The dissolved-oxygen content of the liquid, with or without dilution, is determined before and after incubation for 5 days at the standard temperature, the difference giving the oxygen demand of the sample, allowance being made for the dilution, if any, that the sample received.

In practice, several points must be watched carefully in order to obtain concordant results.

Preservatives must not be added to samples intended for the determination of biochemical oxygen demand. So far as is possible the B.O.D. test should be proceeded with directly the sample is available; if kept at ordinary room temperature for several hours, a very appreciable change may occur in the B.O.D., depending on the character of the sample. In some instances it may decrease and in others it may increase. The decrease at room temperature has in a few cases been found to be as much as 40 per cent. during the first 8 hours of storage.

If samples cannot be dealt with at once for the determination of B.O.D., they should wherever practicable be stored at about 5° C. In the case of a composite sample representing a 24-hour (or other long-period) flow it is desirable to keep all the individual hourly samples at about 5° C until the composite sample can be made up for the B.O.D. determination.[52]

It is necessary that excess of dissolved oxygen be present during the whole period of incubation and desirable that at least **30 per cent.** of the saturation value remains after 5 days. Since only about 9 mg of dissolved oxygen per litre can be present in a saturated water at the temperature of incubation, it follows that samples that absorb more than about 5 mg per litre during incubation for 5 days require more oxygen than they can themselves dissolve. This is the case with . . . . . many waste liquids and . . . . . polluted river waters. The additional oxygen is supplied by diluting the sample with clean well aerated water, the amount of dilution depending upon the nature of the sample.

* See footnote, p. 691.

DILUTION WATER—

There has been much controversy about the nature of the water to be used for dilution, and the subject has been investigated at length. The logical diluent for an ..... effluent would appear to be the river water into which it is discharged, but this method could be adopted only in special cases, and obviously breaks down when effluents from widely differing localities are dealt with in one laboratory. Moreover, the river water may itself have a considerable oxygen demand.

Distilled water alone is unsatisfactory. In this country many laboratories have used tap water that has been well aerated and stored for some time at the incubator temperature. As tap waters differ very much in their content of inorganic salts, and as most of them are now chlorinated, it is recommended that a synthetic dilution water be employed, particularly for determinations the results of which might be required as evidence in a court of law. For less vital determinations a well aerated and stored tap water could continue to be employed, preferably after it has been established that the results were generally comparable with those given with the synthetic dilution water now to be described.

The synthetic dilution water recommended[53] is prepared by adding small quantities of four solutions to good quality distilled water. The latter should be prepared from stills of block tin, hard-glass or cast iron with a condenser of hard-glass, stainless steel, block tin or other suitable material. Copper stills or condensers should not be used, for the water must contain less than 0·01 mg of copper per litre, which otherwise would exert an inhibitory action on the biochemical processes. The distilled water is aerated at the temperature of incubation to remove excess of carbon dioxide and to saturate the water with air. Water approximating to distilled water in composition may now be cheaply prepared by ion-exchange methods, but little experience is yet available in this country on the suitability of such water as a diluent in the B.O.D. test, and more work on the subject is clearly desirable. In its absence the general use of such water cannot be recommended: it has been stated that it can prove unsuitable.

The following stock solutions are required—

Ferric chloride solution—Dissolve 0·25 g of ferric chloride, $FeCl_3.6H_2O$, in 1 litre of distilled water.

Calcium chloride solution—Dissolve 11·0 g of calcium chloride (or the equivalent if the hydrate is used) in 1 litre of distilled water.

Magnesium sulphate solution—Dissolve 10·0 g of magnesium sulphate, $MgSO_4.7H_2O$, in 1 litre of distilled water.

Phosphate buffer stock solution—Dissolve 34 g of potassium dihydrogen phosphate in 500 ml of distilled water. Add 175 ml of *N* sodium hydroxide. This should give a solution of pH 7·2. Add 1·5 g of ammonium sulphate and dilute to 1 litre.

The dilution water is prepared with freshly distilled water, which should be collected in a vessel previously cleaned with chromic acid mixture and well washed. To each litre of distilled water are added 0·5 ml of ferric chloride solution, 2·5 ml of calcium chloride solution, 2·5 ml of magnesium sulphate solution and 1·25 ml of phosphate buffer stock solution. The water should then be well aerated, stored at the incubation temperature and used as soon as possible. Any remaining unused after 1 week should be discarded and the bottle cleaned with chromic acid mixture and well washed. Stocks of dilution water should never be "topped up" with fresh solution.

These precautions in the use of standard dilution water are necessary, because it has been observed that, if kept over a long period, nitrification takes place and, if the water is then used for the B.O.D. test, an inaccurate value is obtained.

It has also been stated that, when dilution water is incubated alone under standard conditions, it should not absorb more than 0·2 mg of oxygen per litre. Cases have, however, occurred when substantially higher figures have been obtained for no apparent reason. It is desirable, therefore, to use as little dilution water as possible consistent with (*a*) at least 30 per cent. of the dissolved oxygen being present

at the end of the test and (*b*) the actual dilution being below the bacteriostatic level. With river waters, it may not always be necessary to use dilution water.

BOTTLES—

It is recommended that narrow-mouthed glass-stoppered bottles of a nominal capacity of 250 ml be used for the determination of B.O.D., as for the determination of dissolved oxygen, and that they be cleaned in the same way with chromic acid mixture.

Some analysts, especially those who have to deal regularly with a very large number of samples, prefer to use bottles of about 125 ml capacity, thus reducing the incubator space required. There is reason to believe, however, that with some types of samples the size of bottles (*i.e.*, the ratio of the glass surface to the volume of liquid) may have some influence on the result obtained. The analyst wishing to use small bottles must therefore satisfy himself that such procedure gives the same results as with the use of standard-size bottles. In all determinations in connection with disputes or disagreements standard-size bottles should be used.

TEMPERATURE—

To conform with international practice, it is recommended that the temperature of incubation for the B.O.D. test be 20° C. A water bath, or water-cooled incubator, or constant-temperature room, thermostatically controlled, is usually employed for carrying out the test. The limit of error of the temperature should be $\pm$ 0·5° C.

INCUBATION—

The period of incubation in the standard test is 5 days, which means 120 $\pm$ 1 hours.

In special cases, or for research work, other periods of incubation are sometimes selected, usually 1, 2, 10 and 20 days, but 5 days' incubation is always taken for the standard test.

Incubation should be carried out in the dark. Some waters may contain green plants, which, if incubated in the light, would give off oxygen by photosynthesis and thus interfere with the B.O.D. determination. The polluting effect of an effluent on a stream may, of course, be considerably altered by the photosynthesis of green plants present, but it is quite impossible to determine this effect quantitatively in incubation experiments.

PROCEDURE—

Unless the pH value of the sample is within the range 6·5 to 8·2, add sufficient alkali or acid to bring it within that range. The amount of acid or alkali to be added should be determined first on a separate sample.

Some samples of . . . . . trade wastes may be sterile and will need inoculation with fresh micro-organisms before incubation. It is usually recommended, and it is often convenient and satisfactory, to use as an inoculum a fresh sewage effluent of good quality obtained from a settling tank, after an aerobic biological process of purification. If this is done, to each litre of dilution water add 5 ml of the sewage effluent. If necessary, the effluent should be settled in a cylinder (not filtered) until the supernatant liquid contains less than 30 mg of suspended solids per litre and has a B.O.D. not exceeding 20 mg per litre.

The question of the uniform seeding of sterile discharges before incubation is not such a simple matter as might appear, for different sewage effluents may contain different bacterial flora, and even the same effluent may vary from hour to hour and from day to day, and, for some trade wastes, sewage effluent might not be suitable as an inoculum until it has been suitably conditioned.

When the B.O.D. value found by the standard test, with sewage effluent as an inoculum, is substantially less than about two-thirds of the chemical oxygen demand from dichromate, an anomalous behaviour must be suspected. This may arise from one of three causes (i) the sample may contain compounds having molecular structures resistant to biological breakdown, (ii) the constituents of the waste may be amenable to oxidation, but the organisms present in the inoculum

are of an unsuitable type or require acclimatisation, and (iii) toxic or bacteriostatic compounds may be present, exerting an inhibiting effect at the concentration employed for the test.

Compounds constitutionally resistant to breakdown are not potentially polluting when the oxygen depletion of the receiving waters is the sole criterion. Substances amenable to breakdown given suitable conditions will, however, generally contribute to the pollution load, for organisms will probably be present in the receiving stream capable of effecting their destruction.

There is no completely satisfactory method of preparing an active inoculum for test purposes. Sewage organisms can, however, often be suitably conditioned by aerating mixtures of settled sewage and waste liquor for periods of 1 to 7 days. Air should be supplied through a sintered-glass diffuser. It may be necessary to use initially a diluted sample of liquor, the concentration being increased stepwise until the test concentration is reached.

The need for a conditioned seed may be found by determining B.O.D. values for periods longer than 5 days. Frequently a conditioned seed develops during the first few days of incubation with a consequent rise in the rate of oxygen depletion towards the end of the 5-day period. Care should, however, be exercised in differentiating between this increase in rate and that normally resulting from the development of nitrifying activity.

Many trade effluents contain constituents that repress biological oxidation in concentrations higher than well defined threshold values. When this limiting concentration is lower than that prevailing in the B.O.D. test bottle, the sample must be diluted until a maximum value for the B.O.D. on an undiluted basis is obtained.

The advantageous use of the manometric technique for the study of inoculum activity and inhibiting action is described later under "Manometric Methods."

It is possible that some samples may be supersaturated with dissolved oxygen, especially waters in which algal growths are flourishing. Such samples should be well shaken in a partly filled bottle, or suction may be applied to remove excess of gas, if the liquid is to be incubated without dilution. If the sample is to be diluted, then this may be done with partly de-aerated water.

Unless the B.O.D. of the sample is already known approximately, the required degree of dilution will not be known and more than one dilution will have to be set up. A useful guide to the strength of the sample may be obtained by first determining the chemical oxygen demand, either by the 4-hour permanganate test or, better, by the dichromate method. If the general nature of the trade waste is known, an approximate estimate of the order of magnitude of the ratio of biochemical-oxygen demand to chemical oxygen demand may be made, and hence a very rough estimate of the expected B.O.D. If the expected B.O.D. is D mg per litre, then 1 volume of sample would require about $D/5$ volumes of dilution water and, when an unknown sample is being examined, this dilution would be set up, with at least one higher and one lower dilution.

Mix the sample and dilution water (containing when necessary the inoculum of sewage effluent) in a graduated cylinder or other suitable vessel. Thorough mixing may be accomplished with the aid of a plunge type of rod without entraining air in the mixture. Violent shaking should be avoided.

For each dilution of the sample, carefully pour the mixture into bottles, one for the initial determination of dissolved oxygen, and one (or two if a duplicate is desired) for incubation, avoiding the entrainment of air. At the same time fill two bottles completely with dilution water, containing, if necessary, an inoculum of sewage effluent. Leave the bottles for 2 to 3 minutes, when, by tapping the neck with a stopper, the few minute bubbles which sometimes form during filling can be displaced. Then firmly insert the stoppers.

Determine immediately the initial concentration of dissolved oxygen in one bottle of the mixture of sample and dilution water, and in one of the bottles containing only dilution water, using, in both cases, the Alsterberg method (see p. 694) or, if necessary, the Rideal - Stewart modification (see p. 695). Place the other bottles (those containing the mixture of sample and dilution water, and that containing

the plain dilution water to act as a blank) in an incubator. After incubation at 20° C for 5 days, determine the dissolved oxygen in the diluted samples and the blank.

. . . . .

CALCULATION OF RESULT—

If 100 ml of mixture require x and y ml of $N/80$ sodium thiosulphate before and after incubation, respectively, if the dilution water itself shows no consumption of dissolved oxygen and if a volumes of dilution water were used to one volume of sample, then—

$$\text{B.O.D.} = (x - y)(a + 1) \text{ mg per litre.}$$

If the dilution water consumes oxygen in the 5 days equivalent to z ml of $N/80$ sodium thiosulphate per 100 ml of the water, then the formula becomes—

$$\text{B.O.D.} = \left(x - y - \frac{az}{a + 1} \right) (a + 1) \text{ mg per litre.}$$

The initial dissolved-oxygen content of the mixture to be incubated may also be calculated from the separate dissolved-oxygen contents of the sample and of the dilution water. This method is appropriate for samples having an immediate dissolved-oxygen demand.

It is sometimes convenient to carry out the dilution of samples for B.O.D. determination by adding appropriate volumes of sample directly to bottles of known capacity that already contain sufficient dilution water so that together with the sample the liquid reaches the top of the neck of the bottle. The sample is added carefully by means of a pipette well below the surface of the water, the bottle is stoppered and the contents thoroughly mixed. Another bottle is completely filled with the dilution water at the same time, so that the dissolved oxygen at the start of the test may be calculated. The bottle containing the diluted sample is incubated as before, and the dissolved oxygen is determined after the 5-day period. This method is particularly useful for determining the B.O.D. of samples that have an *immediate* demand for dissolved oxygen. Such a demand is not included in the ordinary method, in which the initial dissolved oxygen content is found after the sample has been diluted in a cylinder and then poured into bottles.

In this case calculation is a little more difficult and an example is given.

A 2·5-ml portion of **sample** was added at the bottom of a 260-ml bottle filled with dilution water. After 5 days at 20° C, 100 ml required 4·1 ml of $N/80$ sodium thiosulphate; 100 ml of the dilution water used required 8·9 ml of $N/80$ sodium thiosulphate before incubation and 8·8 ml after incubation.

Since 260 ml of mixture contained 2·5 ml of **sample**, 100 ml of mixture contained 99·0 ml of dilution water, which had an initial dissolved-oxygen equivalent to $8{\cdot}9 \times 0{\cdot}99 = 8{\cdot}81$ ml of $N/80$ sodium thiosulphate. The oxygen demand of this volume of dilution water was equivalent to 0·1 ml of $N/80$ sodium thiosulphate and hence the initial dissolved-oxygen content of 100 ml of mixture may be regarded as equivalent to 8·7 ml of $N/80$ sodium thiosulphate.

The oxygen consumed by the **sample** in 100 ml was equivalent to $8{\cdot}7 - 4{\cdot}1 = 4{\cdot}6$ ml of $N/80$ sodium thiosulphate.

Therefore, B.O.D. of **sample**, mg per litre $= 4{\cdot}6 \times$ dilution

$$= \frac{4{\cdot}6 \times 260}{2{\cdot}5} = 480 \text{ mg per litre.}$$

When the dilution is small, an appreciable error may be introduced if it is assumed, as in the above example, that the sample contains no dissolved oxygen. The method has its chief value, however, when large dilutions are required, in which cases the sample itself is unlikely to contain much oxygen, and when there is an immediate oxygen demand, in which case the sample is unlikely to contain any oxygen at all.

Dilutions sensibly greater than 1 in 100, such as may be needed for . . . . . **some** trade wastes, should be made by diluting the sample first in a calibrated

flask or measure, and then adding the appropriate amount of this dilution to the incubation bottles containing the final dilution water. . . . . .

Coarse suspended matter in some **trade effluents** may cause difficulty, since the distribution of the solids may be uneven when the sample is made up into dilutions. Apart from the appearance of the sample, this unevenness may be shown by discrepancies in the results from different dilutions or duplicate dilutions. In these cases some means of disintegration and dispersion of suspended matter is needed in order to give a certain minimum degree of homogeneity to the sample. Since there is little doubt that artificial disintegration of solid matter leads to a higher oxygen demand than would otherwise be measured, and since the problem described does not normally arise, such processing of samples cannot be advocated in general. Moreover, there is no simple procedure capable of easy standardisation. In those cases in which dispersion of solids is necessary, however, an attempt should be made to use a method that gives a reproducible degree of disintegration.

CHLORINATED EFFLUENTS—

Some . . . . . trade wastes contain either residual chlorine or the products resulting from the action of chlorine on certain constituents. Such liquids cannot be used as received for the determination of B.O.D. because of the bactericidal effect of the chlorine or of its products, and also because the chlorine would introduce an error into the determination of dissolved oxygen.

If the sample gives a positive reaction with neutral starch - iodide, this normally indicates the presence of chlorine or chlorinated compounds. These substances may be removed by treating a portion of the sample with the calculated amount of sodium bisulphite, so that there remains in the liquid neither chlorine nor sulphite; this treated portion may then be used for the B.O.D. test after inoculation with suitable micro-organisms, as described in a previous section.

If a positive result is obtained, it is probable that this procedure will give reasonably good results . . . . . , but in the case of other effluents . . . . . it is possible that the chlorine will have combined with organic compounds present, producing substances that, although giving no test for chlorine with starch - iodide, are inhibitory to biochemical oxidation processes or are even bactericidal. It must be admitted that the B.O.D. as determined by this technique is generally lower than would be expected, having regard to the organic content as measured by other tests.

There is a lack of knowledge on the effect of chlorination on many compounds, which also adds to the difficulty of determining the actual oxygen-absorbing capacity of samples containing such substances.

Should it be desirable to obtain a figure for B.O.D. of a chlorinated effluent, notwithstanding the uncertainty of the interpretation of the test in these circumstances, the following procedure should be used. Add a crystal or two of potassium iodide to a convenient volume of sample, depending on the concentration of residual chlorine, and titrate the liquid with a solution of sodium bisulphite, using a few drops of fresh starch indicator solution. If the sample is alkaline to phenolphthalein, bring it to the acid side of this indicator (*i.e.*, to a pH value of about 7·0) by the addition of dilute sulphuric acid, before titration of the chlorine. Approximately $N/80$ is a convenient concentration of bisulphite solution to use, or $N/40$ for samples containing heavy doses of chlorine. To discharge the residual chlorine in 1 litre of sample containing for example 2 mg per litre the addition of 4·5 ml of $N/80$ sodium bisulphite is required.

To another portion of sample, sufficient to carry out the B.O.D. test, add the requisite amount of dilute sulphuric acid (when necessary), followed by the calculated volume of sodium bisulphite solution as determined by the previous titration. After thoroughly mixing, set aside for several minutes, then check the absence of chlorine by testing a small portion of the treated sample with neutral starch - iodide; likewise check the absence of excess of bisulphite in another portion by means of starch indicator solution and a drop of $N/80$ iodine solution, which should develop a blue colour.

Make up the dilutions with inoculated water and proceed as for unchlorinated samples.

SLUDGES AND MUDS—

The assessment of the B.O.D. of samples of sludges and muds requires a special procedure. The degree of dilution will naturally depend on the nature of the sludge or mud and, unless there is definite guidance from previous experience, several dilutions will have to be set up.

The determination of the initial dissolved oxygen of the dilution, and of the final dissolved oxygen after incubation, may be carried out after the liquid has been clarified, if necessary, by the charcoal and aluminium hydroxide procedure described under "Nitrogen Present as Nitrite,"* ("alum flocculation").

It should be borne in mind that mud may have an immediate oxygen demand.[54]

During the incubation period, the solid matter must be kept in suspension and not allowed to remain at the bottom of the bottle all the time. This requires the use of some device to ensure that the bottles are kept in slow rotation and inversion.

MANOMETRIC METHODS†

One of the disadvantages of using the standard method for determining the B.O.D. of industrial wastes, particularly those containing a substantial concentration of organic matter, is that, because of the low solubility of oxygen in water, the waste may have to be diluted with a very high proportion of water to provide sufficient oxygen to give a result in the test. Also the single value for oxygen absorbed in 5 days gives no indication of changes in rate of up-take of oxygen during the period of incubation, or of the possible effects of inhibiting substances, lack of adequate nutrients or lack of acclimatised seeding organisms. These factors can be studied to a limited extent by setting up large numbers of dilutions under different conditions, but, when they are likely to be of importance, a more satisfactory method is to observe directly in a respirometer the up-take of oxygen by the sample from an atmosphere of air or oxygen. In such an apparatus, either the undiluted trade waste, or any desired dilution, can be studied and the progress of absorption of oxygen by a single sample can be continuously followed.

Respirometers were originally devised for studying the respiration of biological tissues, but are suitable for the examination of any system in which a gaseous reactant is evolved or absorbed over a period of hours or days with a consequent change in pressure or volume of the gaseous phase. For the purpose of studying biochemical reactions in which oxygen is absorbed and carbon dioxide produced, the respirometer consists essentially of an absorption flask in which the sample to be studied is placed and in which there is a separate section for holding potassium hydroxide solution for absorption of carbon dioxide. The flask is connected to a manometer and the free space above the sample is filled with a known volume of air or oxygen. Multiple units are normally used and are mounted on a frame so that the whole can be mechanically shaken with the flasks immersed in a constant-temperature water bath. Several different systems are used for measuring the change in volume or pressure of the gaseous phase. The pressure of the gas can be brought to a constant value at each reading and the change in volume observed, or the change in pressure in relation to a compensating flask may be used to calculate the change in volume, as in the Barcroft differential respirometer; or the gas may be brought to constant volume and the change in pressure observed, allowance being made for changes in barometric pressure, as in the Warburg respirometer. For details of design, calibration and use of respirometers, the original literature should be consulted.[55,56,57] The Warburg type is the simplest from the point of view of calculation of the results and is probably the most widely used in the British Isles. In the respirometers mentioned the volume of sample is usually about 3 ml, but with suitable flasks, volumes up to 50 ml may be used.[58] For much larger samples (up to 750 ml) a differential apparatus with internal stirring instead of mechanical shaking may be used.[59] In using a respirometer, it is essential that the size and nature of sample taken, and the degree of shaking or stirring, should be such that the rate of transfer of oxygen from the

* See *Analyst*, 1957, **82**, 276, or *Reprint No.* 8, at p. 283.

† This section has been prepared by the Joint A.B.C.M. - S.A.C. Committee, and is *not* taken from the Ministry of Housing and Local Government's handbook (see footnote, p. 691).

gaseous to the liquid phase should be considerably greater than the rate of utilisation of oxygen, as otherwise false results will be obtained.

When substances inhibitory to biological action are present in an industrial waste, it is generally found, when the waste is diluted, that there is a critical concentration below which the inhibitory action becomes negligible. By setting up a number of different dilutions in a series of respirometers and plotting the rate of up-take of oxygen in each case, the minimum dilution required to achieve the maximum B.O.D. can be found and can be used as a guide to the minimum dilution that could be used in the standard B.O.D. test to obtain reliable results.

In a similar manner the effect of addition of compounds of nutrient elements, for example, nitrogen, phosphorus and potassium, on the rate and degree of up-take of oxygen in biological oxidation of a trade waste can be studied.

In the standard dilution test for B.O.D. it is specified that samples of trade waste likely to be sterile should be seeded by adding a small proportion of sewage effluent containing a mixed flora of micro-organisms. In respirometer experiments seeding of sterile samples is also necessary. The inoculum may not be suitable for biological oxidation of the trade waste, but may be amenable to adaptation or acclimatisation to produce a flora that will bring about oxidation. In this case determination of the B.O.D. by the standard dilution technique would give false and probably non-reproducible results, but a curve of oxygen absorption against time, plotted from the results of a respirometer experiment, would show a lag period during which little or no oxygen was absorbed, followed by a rising curve of oxygen absorption when the flora had become adapted to the new conditions. If liquid from the respirometer reaction flask were then used to inoculate a further sample of trade waste in a second experiment, the lag period might be shortened or might disappear.[60] When, by successive inoculations, the lag period had been eliminated, a normal oxygen up-take curve would be obtained, giving a result for the B.O.D. more in accord with conditions of oxidation that would obtain in a biological treatment plant, or in a river into which a trade waste was being continuously discharged.

When applied to domestic sewage, the biochemical oxygen demand measured by the manometric method has been stated to be higher by 15 per cent.[56,61] or more[62] than the value obtained by the standard dilution technique. In recent experiments at the Water Pollution Research Laboratory, however, results obtained by the two methods have been found to be in good agreement. When applied to an industrial waste, the result may be higher or lower than that obtained by the dilution method.[56] It is not certain, therefore, whether the manometric method could be put forward as an alternative to the standard dilution method, but it is recommended for studying and rectifying conditions that may invalidate the dilution test if applied indiscriminately to trade wastes. Although the manometric technique is no simpler than the dilution technique, under the right conditions it might possibly be used to obtain an indication of the biological oxidisability of a trade waste in a shorter period of test than 5 days.

General observations*

In assessing the B.O.D. of various types of waste waters, it is often desirable to draw the oxidation curve of the sample, *i.e.*, to plot a graph of the absorption of oxygen against time during the incubation period, as described under "Manometric Methods." Such a graph will reveal whether the liquid contained immediately oxidisable substances, and also whether there was any departure from the logarithmic curve given by a pseudo-unimolecular reaction. For example, nitrification during incubation would cause such a departure.

The subject of nitrification of samples during incubation in the test for B.O.D. in 5 days has caused some controversy in recent years. The oxidation of carbonaceous matter to carbon dioxide, and of nitrogenous matter and ammonia to nitrates, are separate processes, and it is thought by some that the oxygen requirements of the former only should be included in the oxygen demand, and that the inclusion of

* From the Ministry of Housing and Local Government's handbook, with changes (see footnote, p. 691).

the latter leads to unfairly high results when the effect of the liquid on a stream is considered.

It is probable that in the determination of the B.O.D. in 5 days of crude and settled sewages, and of the majority of industrial wastes, nitrification is not important, but it may easily be a serious factor in the oxygen demands of purified . . . . . effluents, especially those that have received a high degree of treatment. In such cases it is very likely that nitrification sets in shortly after incubation has begun and proceeds simultaneously with carbonaceous oxidation. . . . . .

Attempts have been made to suppress nitrification during incubation, but so far a completely successful method has not been developed, since carbonaceous oxidation tends to be suppressed by substances that suppress nitrification.

There has been much discussion as to whether the oxygen absorbed by nitrogenous compounds in an effluent should properly be included in the value for B.O.D. in 5 days as reported. It is recommended that in the present state of knowledge the oxygen demand due to nitrification processes should be included in the value given and, if, in special investigations, nitrification is suppressed during incubation, this should be clearly stated when reporting the results. If proof exists that nitrification is responsible for a substantial up-take of oxygen during incubation, this also should be stated: it must then be shown that the content of nitrogen in the form of nitrite plus nitrate in the liquid after incubation was greater than in the corresponding liquid before incubation.

During the last 25 or 30 years, the test for B.O.D. in 5 days has been universally adopted as one important measure of the quality of a liquid containing biologically oxidisable organic matter. **The warning should be repeated, however, that particularly in regard to its application to trade effluents the test is by no means infallible; the results should always be considered in conjunction with the results of other determinations. Mention should also be made of the fact that salinity may have an effect on the figure obtained for B.O.D.**[20]

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Notes

ANALYSIS OF CARBIDES EXTRACTED FROM STEEL

IN the development of alloy steels for improved creep resistance and other properties, a knowledge of the crystallographic form and chemical composition of the carbides present may be helpful in the interpretation of experimental results. Several workers have described methods for the extraction[1,2,3,4,5,6] and analysis[2] of carbides from steel.

During investigations that have extended over several years, we frequently noticed that the totals of the constituents of the analyses of carbide residues extracted from creep-resistant and precipitation-hardening steels failed to reach 100 per cent. by an amount that greatly exceeded the experimental error likely to arise in the semi-micro methods used.

Part of the discrepancy was traced to the difficulty of removing absorbed water, since the residues have a very small particle size and are readily decomposed by heating in air at relatively low temperatures insufficient to remove moisture. It is now our practice to dry carbide extracts before analysis by heating to 350° C in an evacuated Pyrex-glass chamber, the vacuum being produced by a two-stage mercury-vapour diffusion pump protected by cold traps.

Three possible sources of error have been considered, namely—

(*a*) incomplete determination of the steelmaking elements present,
(*b*) oxidation of the residue after preparation and
(*c*) oxidation of the carbide during electrolytic separation.

The first possible source of error was ruled out by calculations based on the yield of residue and the analysis of the original steels, which showed that unsuspected elements could not be present in sufficient amounts to account for the observed discrepancies.

The second possible source of error was investigated by comparing the analyses of carbides prepared in the normal way, and weighed in air after drying in a vacuum, with results on specimens that had been dried in a vacuum and sealed in ampoules before being weighed. A typical comparison is shown by the results in Table I.

TABLE I

COMPARISON OF THE ANALYSES OF CARBIDE RESIDUES

Analysis	Carbide residue No. R100/1A weighed in air	Carbide residue No. R100/1B weighed in vacuum
Carbon, %	14·2	n.d.
Silicon, %	trace	trace
Manganese, %	0·9	1·0
Chromium, %	5·2	5·3
Nickel, %	0·1	0·1
Vanadium, %	62·2	62·1
Iron, %	1·4	1·3
Total	84·0	

n.d. = not determined.

If the residues were diluted by atmospheric oxidation, the analyses should differ appreciably, but no such effect was observed.

If the deficiencies of 5 to 30 per cent. were due to oxidation, examination of the carbides by X-ray diffraction would be expected to show the patterns of oxides; these, however, were never observed, but, when a prepared mixture of carbide and its oxidation product was examined, the presence of oxide was at once recognised. It therefore seemed likely that the carbide particles were being given a very thin surface layer of oxide during the electrolytic extraction, and this was supported by the observation that the deficiency was always at a maximum in the members of series, given different heat-treatments, when the carbides would, from other evidence, be expected to have their smallest particle size. Electron micrographs of steels and of carbide particles prepared by Dr. J. Nutting (of Cambridge University) showed these carbides to have diameters as small as 160 A, *i.e.*, they would be about seventy atoms in diameter. An approximate calculation showed that a spherical particle of this size having a surface monolayer of oxide would contain 3 to 5 per cent. of oxygen, and for plate or dendritic particles the content could be considerably higher.

The substitution of 10 per cent. ethanolic hydrobromic acid for our customary electrolyte of 10 per cent. ethanolic hydrochloric acid often produced an improved yield and the total of the constituents by analysis was nearer the theoretical, presumably owing to a reduced chance of attack by nascent oxygen during electrolysis. An electrolyte containing 10 per cent. of hydriodic acid also gave improved results, but contamination of the carbides by iodine was difficult to remove.

Finally, it was decided to determine oxygen on a number of carbide extracts by a modification of the well-known vacuum fusion technique.[7] For this purpose, capsules having a diameter of $\frac{1}{2}$ inch and $\frac{1}{2}$ inch long were machined from a bar of steel of known low oxygen content. These capsules, together with close-fitting taper plugs of the same steel, were weighed on a microbalance before and after the introduction of 20 to 70 mg of carbide into the cavity, according to the amount of oxygen expected. The composite specimen was analysed by the normal vacuum fusion method, allowance being made for the oxygen in the capsule and a correction being applied for a blank determination on solid pieces of the same steel of approximately the same weight.

Table II shows the results recently obtained for the oxygen content of nine residues selected from materials examined over a period of 3 to 4 years.

The residues had oxygen contents ranging from 2·8 to 26·8 per cent. and, although the agreement between duplicates is not good, probably because of the many possible sources of error (*e.g.*, variability in steel capsules and carbide residues, small sample weights and long storage of residues), these determinations show that failure to reach 100 per cent. in the ordinary chemical analysis is mainly due to the presence of oxygen previously undetected by X-ray diffraction, but suspected from a consideration of the evidence of electron microscopy, analysis and influence of electrolyte. An interesting minor point is the reasonable agreement between the results of vacuum fusion and chemical methods of determining nitrogen in the residues.

TABLE II

RESULTS OF ANALYSES ON CARBIDE RESIDUES FROM STEELS

Residue No.	R7	R47	R102A	R133A	R147	R191	R192	R194	R206N
Extraction method	A	A	B	B	B	B	B	C	D
Phases present—									
major	$Cr_{23}C_6$	$Cr_{23}C_6$	VC	VC	VC	Fe_3C	$Cr_{23}C_6$	Fe_3C	Cr_2N - Mo_2C*
minor	Nb_4C_3	Nb_4C_3	—	—	$Cr_{23}C_6$	—	—	—	—
Analysis, %									
Carbon	5·3	5·4	5·0	9·5	11·8	10·6	4·5	7·5	7·8
Silicon	0·3	2·5	0·8	0·6	0·2	—	0·7	—	3·2
Manganese	1·7	1·0	0·5	0·6	2·1	—	—	—	—
Chromium	43·9	34·0	10·9	12·1	12·1	—	69·3	—	38·2
Nickel	0·8	—	—	—	0·2	—	—	—	nil
Vanadium	2·7	1·4	29·3	35·4	60·0	—	—	—	—
Molybdenum	5·4	4·2	8·7	8·3	—	—	—	—	39·4
Tungsten	—	—	10·0	12·1	—	—	—	—	—
Iron	16·0	12·2	3·2	5·6	4·2	83·4	17·4	88·2	—
Niobium	13·1	22·9	—	—	—	—	—	—	—
Titanium	0·1	0·2	—	—	—	—	—	—	—
Boron	—	3·1	—	—	—	—	—	—	—
Water	2·3†	—	—	—	—	—	—	—	—
Chlorine	1·6	—	—	—	—	—	—	—	—
Nitrogen	1·2	3·3	—	—	—	—	—	—	0·8
Total	94·4	90·2	68·4	84·2	90·6	94·0	91·9	95·7	89·4
Vacuum fusion analysis—									
Oxygen, %	6·9, 5·2	4·3	26·8	24·1	17·6, 13·3	2·9	3·4	2·8	6·8
Nitrogen, %	(1·1, 0·8)	(3·4)	0·2	0·2	0·8, 1·1	0·2	0·6	0·5	(0·9)
Total with oxygen and nitrogen,‡ %	100·5	94·5	95·4	108·5	107·0	97·1	95·9	99·0	96·2

NOTE ON TABLE—Extraction methods: A, electrolytic, with ethanolic hydrochloric acid; B, electrolytic, with ethanolic hydrobromic acid; C, electrolytic, with aqueous potassium iodide, citric acid and hydrochloric acid; D, extraction by Beeghly method[8] (methyl acetate and bromine).

* Cr_2N - Mo_2C is a new phase, reported elsewhere.[9]
† Air-dried only.
‡ Chemical result used when available.

I express my gratitude to Mr. E. W. Colbeck, Metallurgical Director of Hadfields Limited, for permission to publish this Note, and also to Dr. J. R. Rait for his interest in these experiments.

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HADFIELDS LIMITED
EAST HECLA WORKS
SHEFFIELD 9

J. D. HOBSON
May 7th, 1957

THE EFFECT OF COLLOIDAL ORGANIC MATTER ON THE PRECIPITATION OF BARIUM SULPHATE AND A MODIFIED METHOD FOR DETERMINING SOLUBLE SULPHATE IN SOILS

AMONG the published methods for the routine determination of soluble sulphate in soils, that described by Chesnin and Yien[1] is probably the most convenient and gives the most reproducible results. However, the extracting solution recommended, an acetic acid - sodium acetate buffer solution, also extracts considerable amounts of colloidal organic matter, which leads to erroneous

results of two different kinds. First, when low sulphate contents (0 to 10 p.p.m. of sulphur in solution) are involved, the organic matter acts as a protective colloid and causes low results and, secondly, at higher concentrations of sulphate the organic matter is co-precipitated with the barium sulphate, which makes it more bulky and causes the results to be too high. The latter error will be the greater the higher the sulphate concentration and will be particularly important when top-soils to which sulphate has been added are being analysed, for example, as part of a fertiliser experiment.

Experimental

The results for sulphate-sulphur found by the method of Chesnin and Yien after known amounts of calcium sulphate had been added to a forest top-soil previously shown to contain no sulphate were as follows—

SO_4-S added to soil, p.p.m.	0	5	10	30	50	100	200	300	400	500	1000	1500	2000	2500
SO_4-S found in soil, p.p.m.	0	0	2	71	104	183	400	633	1026	1258	1520	1872	2785	3810

Experiments have shown that this striking effect is due entirely to colloidal organic matter and is not due to the added calcium, the colour of the extract or to co-precipitation of nitrate, phosphate or bases.

A series of dilutions of the soil extract (total organic-nitrogen content 10 p.p.m. of nitrogen) was prepared with acetate buffer solution, and a standard amount of sulphate, containing 40 p.p.m. of sulphur, was added to each. The results for the determination of sulphate were as follows—

Nitrogen in solution, p.p.m. ..	0	0·001 to 0·009	0·01	0·05	0·10	0·20	0·30
SO_4-S found in solution, p.p.m. ..	40	40 to 45	46	46	47	51	55
Nitrogen in solution, p.p.m. ..	0·40	0·50	0·60	0·70	0·80	0·90	1·00 to 10·00
SO_4-S found in solution, p.p.m. ..	58	60	60	65	75	75	75 to 77

These results show that the co-precipitation effect is noticeable at such dilutions of organic matter as 0·1 p.p.m. of nitrogen. The results also show that for the effect to be progressive both nitrogen and sulphate must increase in concentration. Hence, in practice, either the organic matter or the sulphate will be a limiting factor.

Although the error in determining sulphate in the solution actually measured may seem small, it assumes a greater importance when multiplied up to the soil concentration. Hence, if the basic principles of the Chesnin and Yien method are to be retained, which in the interests of simplicity and ease of analysing large numbers of soils is desirable, then organic matter must be removed.

Shaking with activated carbon, although removing colour, did not remove all the colloidal matter and, as pointed out by Little,[2] the organic matter cannot be destroyed by oxidising agents, because some organic sulphur may also be oxidised. An investigation into the possible use of anion-exchange resins showed that such treatments, although effective, were far too tedious to be of use when many samples are to be analysed.

A method has therefore been developed whereby colloidal organic matter is co-precipitated with ferric hydroxide and subsequently removed by filtration before the precipitation of barium sulphate. The extra stage so introduced into the procedure takes very little time and not only does it result in the elimination of interfering colloids, but it removes colour and ferric ions at the same time. Both these factors interfere with the method and must in any event be removed, and so in practice the proposed new method is probably more convenient than that in current use.

In Table I are shown the results, from the two methods, of determining sulphate in solutions of calcium sulphate made up in acetate buffer solution and in buffer-solution extracts of a sulphate-free soil. These results show that the ferric hydroxide precipitate efficiently removes the interfering substances and eliminates the co-precipitation and protective-colloid effects.

Method

Reagents—

*Acetic acid - sodium acetate buffer solution.**

Gum acacia solution—A 0·25 per cent. aqueous solution.*

Barium chloride, crystalline—Ground to pass a 40-mesh sieve and be retained on 60 mesh.

Ferric chloride solution—A 5 per cent. aqueous solution of ferric chloride, $FeCl_3.6H_2O$.

Sodium hydroxide solution—A 40 per cent. aqueous solution.

Acetic acid, glacial.

* Prepared as described by Chesnin and Yien.[1]

PROCEDURE—

Prepare the soil extract as described by Chesnin and Yien, and put a 15-ml aliquot by pipette into a conical flask. Add 1 ml of ferric chloride solution and then 1 ml of sodium hydroxide solution dropwise swirling the flask during the addition. Remove the precipitated ferric hydroxide by filtration through a small Whatman No. 41 filter-paper and wash the flask and precipitate with exactly 7 ml of distilled water. Finally add 1 ml of glacial acetic acid to the filtrate.

TABLE I

SULPHATE MEASUREMENTS IN STANDARD SOLUTIONS MADE IN BUFFER SOLUTION AND SOIL EXTRACTS BY THE PROPOSED METHOD AND BY THAT OF CHESNIN AND YIEN

SO_4-S in buffer solution untreated, p.p.m.	SO_4-S in buffer solution treated with ferric hydroxide p.p.m.	SO_4-S in soil extract untreated p.p.m.	SO_4-S in soil extract treated with ferric hydroxide, p.p.m.
0	0	0	0
1·4	1·8	0	2·0
3·8	4·0	0	4·1
6·4	6·3	0	6·8
18·8	17·5	30·4	17·5
25·4	23·8	39·6	23·5
31·4	30·3	57·4	30·3
40·0	40·2	98·1	40·5

Place a suitable aliquot of the filtrate (now theoretically 25 ml), depending upon its sulphate content, by pipette in a 25-ml calibrated flask and complete the determination as in the Chesnin and Yien method.

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EAST AFRICAN AGRICULTURE AND FORESTRY RESEARCH ORGANISATION
KIKUYU, KENYA

P. R. HESSE
March 29th, 1957

(PRESENT ADDRESS:
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WELLING, KENT)

Apparatus

AN AUTOMATIC DIRECT-READING APPARATUS FOR DETERMINING THE SURFACE AREA OF POWDERS

MANY methods and apparatus assemblies are available for the determination of the particle-size distribution of powders. Those that evaluate the surface area in sq. cm per g have found valuable use in the control of finely divided materials in the ceramics and paint industries. In the cement industry the control of milling operations in respect of fineness of grinding of the finished cement takes account of the residue after sieving, usually on a B.S. 170-mesh sieve, the flour content in weight of particles usually below 30 μ, expressed as a percentage of the total, and finally the specific surface in sq. cm per g.

The British Standard specifications[1,2] have incorporated both the sieving test residue and the specific surface value in the specification tests for fineness, and the Lea and Nurse[3] apparatus has been standardised for the latter.

Surface-area methods in which the permeability to air is measured are quicker than those involving sedimentation or elutriation and have almost completely displaced these in cement testing.

The Lea and Nurse and the Rigden[4] instruments were developed from work carried out by Kozeny[5] and Carman[6] and may be used for powders, with the Rigden instrument, of between 500 and 50,000 sq. cm per g, and, with the Lea and Nurse instrument, of between approximately 1000 and 3500 sq. cm per g.

For routine testing, when numerous results are required, there are the disadvantages that measurements of time, pressure and air-flow have to be taken and that each result has to be separately calculated. In the cement industry, milling control is such that it is unlikely for any ground

cement sample to fall outside the range of 1500 to 4500 sq. cm per g, and the apparatus described has been designed to cover this range and at the same time to dispense with calculations and to some extent with operational errors.

Experimental

Description of the apparatus—

The apparatus measures, in effect, the permeability to air of a powder bed of fixed depth.

The smaller Rigden cell has been adapted for use by fitting a gauge to give a constant bed depth, but any suitable cell may be similarly adapted.

Fig. 1 shows the complete apparatus, and Fig. 2 shows the Rigden cell and the plunger. The completed unit is compact in size, being almost completely contained in a metal box measuring 12 inches × 12 inches × 4 inches. The U-tube containing mercury has two platinum contacts sealed into the glass at distances of 2 inches and 6 inches from the base, one in each arm. The lower contact completes the electrical circuit as the air pressure in the reservoir falls and the upper one breaks the circuit at the end of the operation.

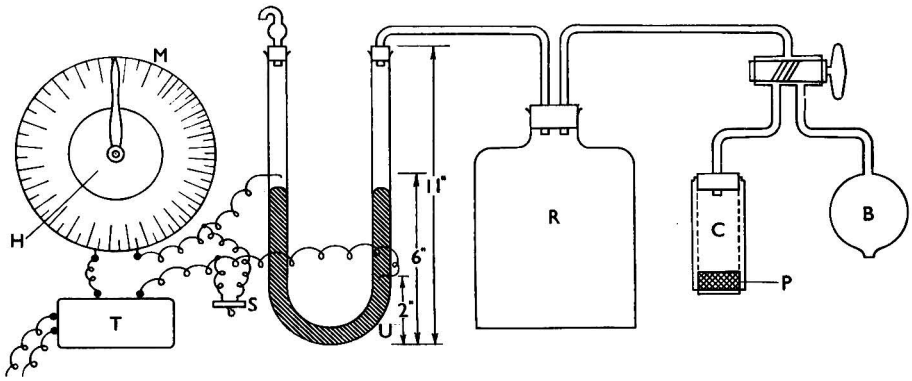

Fig. 1. Diagram of complete apparatus

B = Rubber bulb
C = Permeability cell
H = Spring-loaded pointer for resetting by hand
M = Clock, with 50-V mechanism (1 revolution ≡ 5 minutes)
P = Powder bed
R = Air reservoir of capacity 1 litre
S = Reset switch
T = Transformer, with a 230-V input and a 50-V output
U = U-tube containing mercury, with platinum contacts

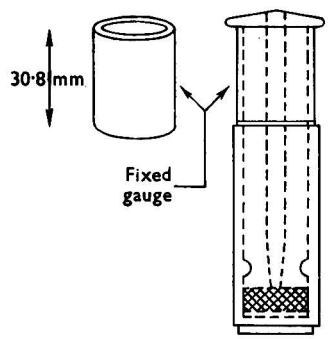

Fig. 2. Permeability cell and plunger

A reset switch has been incorporated as well as the spring-loaded hand-resetting pointer to permit the final adjustment to zero to be made by the motor under normal working load. The motor is a single-phase constant-speed synchronous clock motor, which gives one revolution in 5 minutes.

A slot is cut in the front of the metal box opposite the U-tube so that the mercury level may be observed as the air pressure in the reservoir is raised by means of the blow bulb to the working pressure.

PROCEDURE—

Prepare the permeability cell by fitting a Whatman No. 40 filter-paper over the perforated disc. Weigh 10 g of powder and transfer to the cell; place a cork in the top, and consolidate the powder by allowing the cell to fall a distance of roughly 2 inches on to a hard surface four times in all.

Insert the plunger fitted with the cylindrical gauge and prepare the bed as for the Rigden or Lea and Nurse cells. Connect the cell to the apparatus. Turn the stopcock to allow a free flow from the blow bulb to the air reservoir and pump until the mercury in the U-tube has reached a point approximately $\frac{1}{2}$ inch below the lower platinum contact.

With the pointer set to the start mark on the dial and the instrument connected to the mains and switched on, turn the stopcock to allow air to pass through the permeability cell.

Read the value for the specific surface on the scale when the pointer has come to rest.

CALIBRATION—

The instrument may be calibrated directly against either the Rigden or the Lea and Nurse assemblies when the samples for test are of uniform specific gravity.

The apparatus described has been calibrated for a cement of sp.gr. 3·26, which gives a bed of porosity 46·3 per cent.

For powders of a different uniform specific gravity an initial weight to give the same porosity may be taken and the recorded values corrected for the difference in specific gravity or the apparatus may be calibrated to suit.

The specific surface, as with the Rigden apparatus, is a function of $T^{\frac{1}{2}}$, where T is the time in seconds. For the particular apparatus described, the specific surface $= T^{\frac{1}{2}} \times 246{\cdot}0$.

This simple equation applies, since the weight of powder used has been fixed at 10 g, the solid density is constant at sp.gr. 3·26 and the powder-bed depth is fixed at 1·13 cm. Hence the fractional voids are always the same and the only variable is T.

The constant may be determined by calculation from the mean of not less than twelve points around the scale, and from it the direct-reading scale may be prepared. The simplest way of carrying this out follows.

PROCEDURE FOR PREPARING THE DIRECT-READING SCALE—

Attach a blank paper circle to the clock dial. Mark a "start" point at approximately "12 o'clock." Treat powders of known surface area as described under "Procedure" and plot their values on the scale. When a sufficient number have been plotted (twelve to twenty spaced fairly evenly around the scale), set the pointer at approximately 1 inch before the start mark and, by means of the reset switch and a stop-watch, time the pointer movements between start and the plotted values.

Calculate K values from the following expression—

$$K = \frac{S}{T^{\frac{1}{2}}},$$

where K is a constant, S is the surface area in sq. cm per g and T is the time in seconds. Eliminate any erroneous results, which may be easily seen as fluctuations from the general average of value K, and take the mean of not less than 12 results for the ultimate value of K.

Calculate values of T between 1500 and 4500 sq. cm per g in steps of 100. Multiply values of T by $1{\cdot}2 \left(\frac{360°}{300''}\right)$ to convert to degrees of a circle.

Prepare the scale from these major values. Mark off intermediate values linearly. (This will give only insignificant errors.)

NOTE—

Values of surface area between 4260 and 4500 sq. cm per g will take more than one complete revolution of the dial and will occupy the space that would have been taken by values between zero and 1500.

Conclusions

For daily or hourly checking of milling efficiency this apparatus has been found to save time and eliminate some operational errors, and its chief appeal is in the simplicity of operation and the direct reading.

I thank the Directors of the Lafarge Aluminous Cement Co. Ltd. for permission to publish this work.

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Lafarge Aluminous Cement Co. Ltd.
Fondu Works,
West Thurrock, Grays, Essex

F. J. Spillane
April 10th, 1957

British Standards Institution

NEW SPECIFICATION*

B.S. 976: 1957. Density—Composition Tables for Aqueous Solutions of Hydrochloric Acid. Price 7s. 6d.

AMENDMENT SLIPS*

Printed slips bearing amendments to British Standards have been issued by the Institution, as follows—

PD 2870—Amendment No. 2 (August, 1957) to B.S. 572: 1950. Interchangeable Conical Ground Glass Joints.
PD 1871—Amendment No. 2 (August, 1957) to B.S. 1132: 1952. Automatic Pipettes.
PD 2873—Amendment No. 3 (August, 1957) to B.S. 1583: 1950. One-mark Bulb Pipettes.
PD 2881—Amendment No. 1 (August, 1957) to B.S. 593: 1954. Laboratory Thermometers.
PD 2882 Amendment No. 2 (August, 1957) to B.S. 612: 1952. Nessler Cylinders.

Book Reviews

Aspects of River Pollution. By Louis Klein, M.Sc., Ph.D., F.R.I.C., M.Inst.S.P., with J. R. Erichsen Jones, Ph.D., D.Sc., and H. A. Hawkes, M.Sc., A.M.Inst.S.P. Pp. xii + 621. London: Butterworths Scientific Publications; New York: Academic Press Inc. 1957. Price 84s.; $14.50.

During this century the control of river pollution has become a problem of ever increasing importance and difficulty, a fact that has found Parliamentary recognition by the passing of the Public Health (Drainage of Trade Premises) Act, 1937, the River Boards Act, 1948, and the Rivers (Prevention of Pollution) Act, 1951. As a consequence, this specialised subject is one of considerable significance to analysts and all concerned will agree with the reviewer in giving a special welcome to this valuable book. In a Foreword Dr. B. A. Southgate (Director of the Water Pollution Research Laboratory) observes that Dr. Klein has not only called upon his long experience in this field, but "has included in his book references to more than 1300 original papers which means that he must have considered and rejected many times this number."

After an interesting historical introduction the first two chapters give an excellent summary of legal aspects from the appointment of the Royal Commission on Rivers Pollution of 1865 up to the Act of 1951 already mentioned. There follow chapters entitled: Nature and Effect of Pollution; Causes of River Pollution; Uses of River Water; Biochemical and Physical Aspects of Pollution.

The next chapter of 32 pages on Fish and River Pollution has been written by a biologist, Dr. J. R. E. Jones, and among much useful material contains a table giving the lethal concentrations of about 70 polluting chemical substances towards certain typical fish. There follow 61 pages on the Biological Aspects of River Pollution by Mr. H. A. Hawkes, in which the influence of contamination on the general fauna and flora of rivers is authoritatively discussed. These two chapters are of great value to the chemist who may have to advise on the toxicity of trade effluents,

* Obtainable from the British Standards Institution, Sales Department, 2 Park Street, London, W.1.

because, in making assessments, the effect on fish food as well as on the fish themselves must be considered and the general character of the stream needs also to be taken into account.

The next 166 pages are concerned with the analysis of river water and effluents and consist of a survey of methods with references, the chapter headings being: Detection and Measurement of River Pollution; Significance and Interpretation of Chemical Tests; Physical Characteristics of Rivers. This portion of the book alone embodies 473 references.

The Abatement of Pollution is discussed in two chapters occupying 121 pages, the one dealing with Sewage Disposal and the other with Disposal and Treatment of Trade Wastes. To many consultants the second of these may prove to be the most important part of this treatise. By the nature of the problem each individual case has to be solved in its own particular way, but a preliminary study of this part of Dr. Klein's book and a follow-up of the appropriate references out of a total of 166 given for this section will surely direct the harassed consultant along the right path.

After discussing Standards for Rivers, Sewage Effluents and Trade Effluents, the book concludes with a short chapter on the Present and Future Status of River Pollution.

In writing this most distinguished treatise, Dr. Klein has successfully combined a valuable presentation of specialised scientific knowledge with a masterly appreciation of social requirements in an expanding industrial community. NOEL L. ALLPORT

STATISTICAL METHODS IN RESEARCH AND PRODUCTION WITH SPECIAL REFERENCE TO THE CHEMICAL INDUSTRY. Edited by OWEN L. DAVIES, M.Sc., Ph.D. Third Edition. Pp. x + 396. Edinburgh and London: Oliver and Boyd Ltd., *for* Imperial Chemical Industries Ltd. 1957. Price 45s.

My review of the first edition of this product of a team of I.C.I. scientists began (*Analyst*, 1948, **73**, 53) with the words "This excellent book . . . ." The adjective was deliberately chosen. Nevertheless, the third edition leaves me vainly seeking another that will fittingly connote the improvement achieved. This is no mere touching-up; four chapters only remain substantially unchanged, the remainder being either re-written or altogether new. Emphasis is laid throughout on the economic aspects of sampling and testing procedures, so that the chemist who absorbs the teachings of this book may demonstrate to his employers the value of his work in the way that appeals to them most—in terms of money saved. The older techniques of making decisions after tests of significance, though of course fully explained, are somewhat overshadowed by the emphasis laid on the use of confidence limits, which the authors consider are of more practical value; they may well be right. The chapter on sampling, which I previously mildly criticised, has been completely re-cast and modernised, with much consequent improvement. A particularly praiseworthy section is that dealing with relationships between two variables—though it might perhaps be pointed out, in the interests of accuracy, that the statement on p. 191 that, when the correlation coefficient is zero, "the variables are independent" is not strictly true; it can only be said that they are not *linearly* related.

The style and clarity of the exposition is throughout admirable; a wealth of fully worked out numerical examples, all based on chemical and physical testing procedures, will appeal to those many readers who prefer the concrete illustration to the abstract generality. Any chemist who has already grasped the simple fundamental concepts of statistics and wants to learn how to apply statistics in practice cannot do better than acquire and study "this excellent book." After all, there is no more suitable adjective. E. C. WOOD

PHYSICAL METHODS IN CHEMICAL ANALYSIS. Volume III. Edited by WALTER G. BERL. Pp. xii + 652. New York and London: Academic Press Inc. 1956. Price $15.00; 107s.

The first and second volumes of this work were published in 1950 and 1951, respectively. That it has been found necessary to plan a further and entirely new volume only 6 years later is in itself an indication of the analytical revolution through which we are passing.

The chapter headings comprise: Gas Chromatography, Electrochromatography (Zone Electrophoresis), Electroanalytical Methods in Trace Analysis, High-frequency Method of Chemical Analysis, Field Emission Microscopy, Theory and Principles of Sampling, Flame Photometry, Microwave Spectroscopy, Analytical Applications of Nuclear Magnetic Resonance, Fluorescent X-Ray Spectrometric Analysis, Analytical Distillation, Neutron Spectroscopy and Neutron Interactions in Chemical Analysis. The only British author is C. S. G. Phillips, who reminds us that it was as recently as 1952 that the classic paper of James and Martin on gas chromatography appeared. It was indeed at the Oxford Congress that year that Dr. Martin demonstrated the

technique. This and some of the other techniques hardly heard of in 1952, *e.g.*, nuclear magnetic resonance, are now in use and suitable apparatus is available commercially.

The sections on gas chromatography and on flame photometry will perhaps have the widest and the most immediate appeal; both make their contribution to the accepted tasks of the analyst of separation, identification and measurement. The scope of each goes far beyond mere stimulation of interest, which is often enough all that books of this type can hope to achieve. They are authoritative and critical, and their value is enhanced by a generous bibliography and by the inclusion of examples of practical applications.

The treatment of the other subjects is equally good; there is an excellent review of the technique and applications of X-ray fluorescence analysis, and it should be noted that the last section on neutron spectroscopy and neutron interaction in chemical analysis runs to 170 pages, the longest section in the book.

The message is clear and is summed up in the Editor's preface, "The scope of the analyst's function is changing rapidly and fundamentally. Apart from identifications and quantitative estimations the problems of shape and structure on the molecular and on grosser levels have been added to his province." In this volume the high standard of the series is more than maintained. It can be highly recommended to all who wish to keep abreast of modern analytical developments.

R. C. CHIRNSIDE

METHODS OF BIOCHEMICAL ANALYSIS. Volume IV. Edited by DAVID GLICK. Pp. x + 362. New York and London: Interscience Publishers Inc. 1957. Price $8.50; 68s.

This volume opens with an article by E. M. Bickoff on the determination of carotene. The general account of technique covers extraction and separation of pigments, measurement of purified carotene fractions and estimation of stereoisomerides. More detailed procedures are given for determining carotene in fresh or dried plant materials, in vegetable oils, milk and in whole dried eggs. The determination of β-carotene stereoisomerides in alfalfa by a combination of chromatographic and spectrophotometric methods is described fully.

The determination of vitamin A is discussed by N. D. Embree, S. R. Ames, R. W. Lehman and P. L. Harris of Distillation Products Industries. This article reviews the chemistry of vitamin A and related substances and goes on to consider bio-assays under the headings: growth, liver storage, vaginal smear and other biological responses. Physicochemical procedures are then described and detailed procedures for various materials are given.

The measurement of poly-unsaturated fatty acids is dealt with by R. T. Holman of Minnesota. This article is mainly about spectrophotometric methods involving alkaline isomerisation. Most of the naturally occurring polyethenoid acids are unconjugated and do not exhibit selective absorption of ultra-violet light. Heating with potash in glycol effects partial isomerisation to conjugated diene, triene and polyene acids; the process can be reproducible under controlled experimental conditions. As Holman says "alkaline isomerisation is the best method we now have for this kind of analysis and it can be used to gain very useful information."

Just before the book went to Press it was reported that 5 per cent. potassium *tert.*-butoxide causes isomerisation to occur in a few hours at the boiling-point of *n*-butanol (90° C) and that there are fewer side-reactions than with potash in glycol at 170° to 180° C. The empirical approach has however been taken so far that only a truly stoicheiometric reaction is likely to replace the recommended methods.

Holman also describes briefly the application of paper chromatography to the separation of poly-unsaturated acids.

R. H. Silber and C. C. Porter of the Merck Therapeutic Institute at Rahway discuss the determination of 17:21-dihydroxy-20-oxo steroids in urine and plasma. The phenylhydrazine - sulphuric acid reaction on which the authors reported in 1954 is effective for various corticosteroids. Details are given for analytical procedures applicable to plasma, sweat and urine.

Under the title "The pH-STAT and its Use in Biochemistry," C. F. Jacobsen, J. Leonis, K. Linderstrøm-Lang and M. Otteson, of Copenhagen, review recent developments in the automatisation of the control and measurement of biochemical reactions at constant pH. A suitable instrument is called a pH-STAT and is described fully. The article also expounds the theoretical background for the study of such rate processes in biochemistry: examples are given that are both effective and interesting in themselves.

The assay of sulphatases is reviewed by K. S. Dodgson and B. Spencer of the University of Wales. The authors were trained at Liverpool and gained their research experience under R. T.

Williams; it therefore gives special pleasure to the reviewer to read a highly competent article by them on a subject they have made their own.

W. H. Fishman and H. M. Davidson of Tufts University deal with the determination of acid phosphatases in serum. The procedure is based on the hydrolysis of phenyl phosphate measured in the presence and in the absence of L-tartrate. The difference found in the amount of phenol liberated is believed to represent the inhibition of acid phosphatase of prostatic origin and so to reflect the concentration of the latter enzyme in serum. The phenol is measured by the Folin - Ciocalteu reagent. The problem is important in the diagnosis of prostatic disease.

E. F. Gale of Cambridge contributes an article on the determination of amino acids by the use of bacterial amino-acid decarboxylases. Specific enzymes act on an amino-acid substrate to yield an amine and to liberate carbon dioxide; under suitable conditions the procedure can be quantitative and Warburg manometers or Conway units can be used. The enzymes, which attack only the L-isomers, are prepared from suitable micro-organisms (coliforms, clostridia and faecal streptococci) and are reasonably stable as acetone powders or in some cases as cell suspensions. The estimation of histidine is described in detail as an example of the method and notes are given about special points in the estimation of other amino acids by decarboxylase preparations.

T. P. Singer and E. B. Kearney discuss the determination of succinic dehydrogenase activity. The primary dehydrogenase may be determined by using phenazine methosulphate as acceptor. Use of the Warburg apparatus leads to excellent results and use can also be made of the great difference in ultra-violet absorption at 387 mμ (pH 7·6) between the oxidised and reduced forms of the dye. The ferricyanide method of providing an acceptor is also described fully.

The assay of the succinic dehydrogenase complex is best made by a variant of the methylene blue method.

This volume, like its predecessors in the series, serves a dual purpose; the different articles help the non-specialist to see each problem in perspective and they also provide the biochemist with clearly described instructions based on actual experience at the bench.

The Editor allows the authors freedom to write in their own way (subject to uniformity in the technical aspects of presentation), and there is consequently rather more variety of approach than might have been expected.

R. A. MORTON

Publications Received

THROUGH ALCHEMY TO CHEMISTRY. A PROCESSION OF IDEAS AND PERSONALITIES. By JOHN READ, F.R.S. Pp. xviii + 206. London: G. Bell & Sons Ltd. 1957. Price 18s. 6d.

A LABORATORY MANUAL OF SEMI-MICRO INORGANIC QUALITATIVE ANALYSIS. By E. T. THOMPSON, B.Sc., A.K.C. Pp. 48. London: Edward Arnold (Publishers) Ltd. 1957. Price 4s.

METHODEN ZUR BESTIMMUNG PFLANZLICHER WUCHSSTOFFE. By HANS LINSER and OSWALD KIERMAYER. Pp. viii + 181. Vienna: Springer-Verlag. 1957. Price 56s.

CALENDAR OF THE PHARMACEUTICAL SOCIETY OF GREAT BRITAIN, 1957–1958. Pp. vi + 308. London: The Pharmaceutical Press. 1957. Price 20s.

BRITISH NATIONAL FORMULARY 1957: ALTERNATIVE EDITION. Pp. 245. London: The British Medical Association *and* The Pharmaceutical Press. 1957. Price 7s. 6d. (interleaved copies, 10s. 6d.).

This Edition is based on a pharmacological classification.

COMPLEXOMETRIC TITRATIONS. By GEROLD SCHWARZENBACH. Translated and revised in collaboration with the author by HARRY IRVING, M.A., D.Phil., D.Sc., F.R.I.C. Pp. xviii + 132. London: Methuen & Co. Ltd. 1957. Price 21s.

ANNUAL REPORT, 1956–7. Pp. 272. London: British Standards Institution. 1957. Price 7s. 6d.

COLLECTIVE INDEX OF THE JOURNAL OF THE INSTITUTE OF BREWING, 1946 TO 1955. Compiled by W. H. BIRD. Pp. iv + 218. London: The Institute of Brewing. 1957. Price 105s.

ANALAR' STANDARDS FOR LABORATORY CHEMICALS. By THE BRITISH DRUG HOUSES LTD. *and* HOPKINS & WILLIAMS LTD. Fifth Edition. Pp. xvi + 397. Poole, Dorset: British Drug Houses Ltd.; Chadwell Heath, Essex: Hopkins & Williams Ltd. 1957. Price 21s.

Notice to Authors

THE Society publishes papers on all aspects of analytical chemistry, inorganic and organic, including chemical, physical, biological, bacteriological and micro methods. Papers on these and allied subjects, by members of the Society or by non-members, may be submitted for publication; they may record—

(1) proposals for new methods and the investigations on which the proposals are based;
(2) the results of original investigations into known methods or improvements therein;
(3) analytical results obtained by known methods where these results, by virtue of special circumstances, such as their range or the novelty of the materials examined, constitute useful analytical information;
(4) the application of new apparatus and new devices in analytical technique and the interpretation of results;
(5) minor investigations or kindred matter and descriptions of new apparatus and its applications for publication under their respective section headings.

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Reprints—Twenty-five reprints, or a maximum of fifty if there is more than one author, are supplied gratis. Additional reprints may be obtained at cost if ordered directly from the printers, W. Heffer & Sons Ltd., Hills Road, Cambridge, at the time of publication. Details are sent to authors with the proofs.

NOTES ON THE WRITING OF PAPERS FOR *The Analyst*

Be brief: a page contains, on average, 800 words and costs £15 to publish.

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The title should be brief but descriptive.

Conciseness of expression should be aimed at; clearness is increased by adopting a logical order of presentation, with suitable paragraph or section headings.

Descriptions of new methods should be supported by experimental results showing their precision and selectivity.

Generally, the best order of presentation is as indicated below—

(*a*) Synopsis.
(*b*) Statement of object of investigation and, if necessary, historical introduction and account of preliminary experimental work; these must be no longer than is strictly necessary for the understanding of the new material.
(*c*) Description of method. Working details of proposed methods are most concise and clear when given in the imperative mood, and should normally be given in this form, *e.g.*, "Dissolve 1 g of sample in 10 ml of water and add . . ." Well known procedures must not be described in detail.

(*d*) Presentation of results.
(*e*) Scope and validity of results.
(*f*) Conclusions and, if required, a short summary of the principal results.

Tables, diagrams, etc.—The number of tables should be kept to a minimum. Column headings should be brief. Tables consisting of only two columns may often be arranged horizontally. No lines should be ruled in tables in the manuscript. Tables must be supplied with titles and be so set out as to be understandable without reference to the text.

Tables or graphs may be used, but not both for the same set of results, unless important additional information is given by so doing.

In general, graphs should have a reasonable number of co-ordinate lines, and not only the two main axes. Graphs consisting of straight lines passing through the origin, such as calibration curves, should not be submitted: instead, an equation should be given in the text.

Diagrams and graphs should be drawn in Indian ink on Bristol board, stout paper or tracing cloth, not larger than foolscap size and with at least 1-inch margins all round. The use of squared paper should be avoided if possible; red, orange or brown ruled paper must not be used, as these colours reproduce in block-making. If it is necessary to use ruled paper, blue or green rulings may be used. All lettering should be inserted lightly in black lead pencil at the appropriate place on the diagram, and will be replaced by type in block-making. All lines in Indian ink should be firmly drawn and sufficiently thick to stand reduction.

Drawings should be specially prepared for submission to *The Analyst*, as they cannot normally be returned, and may be modified or cut in the course of block-making. The duplicate manuscript may be accompanied by photographic or dyeline copies of the figures, or by pencil sketches, for transmission to the referee; there is no need to prepare Indian-ink duplicates.

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Abbreviations—Normality and molarity are generally expressed as decimal fractions (*e.g.*, 0·02 *N*, 0·375 *M*). Abbreviational full stops are omitted after the common contractions of metric units (*e.g.*, ml, g, μg, mm) and after ° C, ° F, μ, A and other units represented by symbols; litre and metre, when without prefixes, are printed in full.

Abbreviations other than those of recognised units should be avoided in the text; symbols and formulae are not used instead of the names of elements and compounds in the text, but may be used in addition to names when they are necessary to avoid ambiguity, *e.g.*, to specify crystalline composition, as in $CuSO_4.5H_2O$, to show structure, or in equations.

Percentage concentrations of solutions should be stated as "per cent. w/w" (alternatively "g per 100 g"), as "per cent. w/v" (alternatively "g per 100 ml") or as "per cent. v/v." Concentrations of solutions of the common acids, however, are often conveniently given as dilutions of the concentrated acids; they should then be given in the form "diluted hydrochloric acid (1 + 4)," which signifies 1 volume of the concentrated acid mixed with 4 volumes of water. This avoids the ambiguity of 1 : 4, which might be equivalent to *either* 1 + 4 *or* 1 + 3.

References—References should be numbered serially in the text by means of superscript figures, *e.g.*, Dunn and Bloxam[1] or Mitchell,[2] and collected in numerical order under "REFERENCES" at the end of the paper. They should be listed, with the authors' initials, in the following form—

1. Dunn, J. T., and Bloxam, H. C. L., *J. Soc. Chem. Ind.*, **1933, 52,** 189T.
2. Mitchell, C. A., *Editor*, "Allen's Commercial Organic Analysis," Fifth Edition, J. & A. Churchill Ltd., London, 1932, Volume 9, p. 149.

For books, the edition (if not the first), the publisher and the place and date of publication should be given, followed by the volume or page number, or both if required.

The entry of "personal communications" in the reference list is not justified; full acknowledgment to such unpublished sources should be made in the text or in the acknowledgments at the end of the paper.

Authors must, in their own interests, check their lists of references against the original papers; second-hand references are a frequent source of error. The number of references must be kept to a minimum.

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Further useful advice to authors is contained in the Royal Society's publication entitled "General Notes on the Preparation of Scientific Papers," published for the Royal Society by the Cambridge University Press, price 2s. 6d.