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Applicants should possess a science degree and/or the Associateship of the Institute of Chemistry.

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THE ANALYST

PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

AN Ordinary Meeting of the Society was held at 6 p.m. on Wednesday, February 6th, at the Chemical Society's Rooms, Burlington House, London, W.1, with the President, Dr. G. W. Monier-Williams, in the chair. The following papers were presented and discussed: "Notes on the Selective Oxidation of Vinegar," by F. A. Lyne, B.Sc., F.R.I.C., and T. McLachlan, D.C.M., A.C.G.F.C., F.R.I.C.; "The Determination of Auxins in Soils, including a Note on Synthetic Growth Substances," by J. Hubert Hamence, M.Sc., Ph.D., F.R.I.C.; "Application of the Intermittent Arc Technique of Spectrographic Analysis," by J. A. C. McClelland, B.Sc., Ph.D., A.R.I.C.

NEW MEMBERS

Arthur Alcock, A.R.I.C.; Charles Henry Allen; Russell James Laurence Allen, M.Sc, (Melbourne), Ph.D. (Cantab.), A.R.I.C.; Jack Basil Brewster Burch, M.A. (Oxon.); Eric H. England; Edwin Ernest Fitzgerald, B.Sc., A.R.I.C.; Jack Harry Glover, B.Sc. (Lond.). A.R.I.C.; William Peatman Hayes, B.Sc. (Lond.), A.R.I.C.; Norman Ronald Lock, B.Sc. (Bristol); Roy William Pearman, B.Sc. (Lond.), A.R.C.S., A.R.I.C.; James Waywell, A.M.C.T., A.R.I.C.

DEATHS

WE record with regret the deaths of the following members:

Gordon Westland Edwards — Maximilian Nierenstein
Alfred Douglas Heywood — Kapilram Hardevram Vakil

The Determination of Chloral in Technical Chloral

BY T. HARRINGTON, T. H. BOYD AND G. W. CHERRY

CHLORAL has recently come into prominence owing to its use as a starting material in the technical production of the insecticide D.D.T. It is produced by chlorinating alcohol to the chloral alcoholate stage and then distilling the product from concentrated sulphuric acid and is purified by redistillation, either alone or from calcium carbonate.

All known methods of determining chloral are based on alkaline hydrolysis, represented by reaction (1).

$$CCl_3.CHO + OH' \longrightarrow CHCl_3 + H.COO' \quad .. \quad .. \quad (1)$$

The hydrolysis may be quantitatively measured in three ways[1]: (*a*) determination of the amount of alkali consumed; (*b*) determination of the amount of formate produced; (*c*) measurement of the chloroform produced.

Although a method of type (*c*) does exist, we have not studied this type of method owing to the possibility of water-insoluble impurities being present in technical chloral; but we have developed three methods based on types (*a*) and (*b*). The results returned by all three methods are in good agreement and therefore probably very near the truth. Each method, with its development, is discussed below.

METHODS OF ANALYSIS

(i) THE ALKALIMETRIC HYDROLYSIS METHOD—In this method a neutralised aqueous solution containing 5 g. of sample is hydrolysed by swirling with *N* NaOH solution for exactly 2 minutes, and is then immediately titrated back to phenolphthalein, with *N* acid solution.[1]

The method was found to return 100% of chloral with pure chloral and recrystallised chloral hydrate, but when applied to technical material it gave results which varied with the time and temperature of hydrolysis (Tables IX and X). The possibility of chloroform being hydrolysed by the excess of alkali according to the equation

$$CHCl_3 + 4OH' \longrightarrow H.COO' + 3Cl' + H_2O \quad .. \quad .. \quad (2)$$

was considered, and it was shown that while some such hydrolysis did in fact occur, it was not sufficient to account for the variations observed (Table XI).

The effect of hydrolysing at 0° C. was tried in order to see if chloral could be completely hydrolysed at that temperature without hydrolysis of the impurities and chloroform. It was established that at 0° C. purity results are obtained which are independent of the time of hydrolysis, provided that sufficient time (about 5 minutes) is allowed for complete reaction (Table XII).

There still remained the possibility that the purity figure thus obtained included readily hydrolysable impurities. Chlorinated compounds and esters are the most likely compounds to interfere in this way, and it was considered that the application of a correction for increase of chloride during hydrolysis, based on reaction (3),

$$R.Cl + OH' \longrightarrow R.OH + Cl \quad .. \quad .. \quad .. \quad (3)$$

would very largely eliminate the effect of the former. No simple method of correcting for the possible hydrolysis of esters suggested itself.

It was found (Table XIII) that the application of such a chloride correction renders the method independent of reaction temperature over the range 0° to 20° C., but that above 20° C. the chloride correction does not take into account all the errors introduced.

Another effect noticed was that if a sample taken from the middle of an industrial chloral distillation is examined immediately, it usually gives no turbidity when dissolved in water, but that after the sample has stood for upwards of 24 hours a turbidity is nearly always produced. Samples taken from the first and last runnings of a distillation nearly always give a turbidity in water even if examined immediately. This turbidity is probably largely due to chloral polymer together with small amounts of water-insoluble liquid impurities, and since chloral polymer returns 70% of chloral by hydrolysis (Table V), it was decided to modify the method further by dissolving the chloral sample in water and filtering before carrying out the analysis.

Results then obtained (Tables I, II and XIV) were most satisfactory and no further modifications were found necessary. The final method is as follows.

Procedure—Weigh accurately, by means of a Lunge-Rey pipette or other suitable means, about 10 g. (w g.) of the sample into 50 ml. of distilled water contained in a 100 ml. stoppered standard flask. Shake to dissolve, dilute to 100 ml. with distilled water and filter.

(a) Pipette 25 ml. of the clear filtrate into a titration flask and titrate with $N/10$ sodium hydroxide solution, using methyl red as indicator (a ml.). Retain the solution.

(b) To the solution from (a) above add 20 ml. of 2 N nitric acid, excess of $N/10$ silver nitrate solution from a pipette or burette (x ml.), 5 ml. of nitrobenzene and 5 ml. of half-saturated ferric alum solution. Shake vigorously and titrate back with $N/10$ potassium thiocyanate solution to the appearance of the red ferric thiocyanate colour (b ml.).

(c) Pipette 50 ml. of the original filtered solution into a stoppered conical flask and cool to below 5° C. Pipette 50 ml. of N sodium hydroxide into a flask, cool to below 5° C. and add the cold alkali solution to the chloral solution, rinsing out the flask with 25 ml. of water of temperature below 5° C., and shake the mixture vigorously and continuously for not less than 5 minutes. Then immediately titrate back the residual alkali with N sulphuric acid, using phenolphthalein as indicator (c ml.). Retain the solution.

(d) Examine the solution from (c) in the same way as in (b) above (titre = d ml. of $N/10$ potassium thiocyanate for y ml.* of $N/10$ silver nitrate added).

Calculations:

$$\text{Apparent \% of chloral from the hydrolysis} = \frac{0{\cdot}1475\,(50-c)\times 100}{0{\cdot}5\,w}$$

$$\text{Correction due to acidity (as \% of chloral)} = \frac{0{\cdot}1475\,a\times 100}{2{\cdot}5\,w}$$

$$\text{Correction due to increase in chloride content (as \% of chloral)} = \frac{0{\cdot}1475\,(y-d)\times 100}{5\,w} - \frac{0{\cdot}1475\,(x-b)\times 100}{2{\cdot}5\,w}$$

$$\text{Thus corrected \% of chloral} = 2{\cdot}95\,[10(50-c) - 2(a+b-x)+d-y]/w.$$

* Since the acidity of the sample is largely due to the presence of HCl, a very acid sample requires much more $AgNO_3$ than a normal sample. Volumes of the order of (a + 10) ml. and (2a + 10) ml. will usually be found convenient for x and y respectively.

(ii) THE MERCURIC CHLORIDE METHOD—In this method, as at first proposed, the formate produced by the hydrolysis of chloral (reaction [1]) was estimated by the method of Auerbach and Plüdemann.[2] A portion of sample (0·2–0·3 g.) was weighed, by difference from a Lunge-Rey or similar pipette, into saturated lime-water and the liquid stirred and boiled to remove chloroform. The solution was acidified to dissolve any precipitated calcium salts and then neutralised. A buffered mercuric chloride solution was then added and the solution allowed to simmer for two hours, after which the mercurous chloride produced according to reaction (4) was filtered, washed and weighed.

$$H.COOH + 2HgCl_2 \longrightarrow Hg_2Cl_2 + 2HCl + CO_2 \quad .. \quad .. \quad (4)$$

The method was found to give fairly satisfactory results when applied to technical chloral (Table XV), though the repeatability was not good and disappointing results were sometimes obtained under routine conditions. Several modifications were found to be necessary.

(*a*) It was found that even under the most rigorously controlled conditions losses by evaporation during the weighing out of 0·2–0·3 g. amounted to as much as 2·5%. This error can be most conveniently eliminated by quickly weighing 10 g. of the sample into 1 litre of water and taking an aliquot part for analysis.

(*b*) When applied to chloral polymer, the method returned 71% of chloral (Table V) and so filtration of the aqueous solution is necessary before analysis (Table XVI).

(*c*) In the development of the alkalimetric hydrolysis method, the extent to which chloroform was hydrolysed by *N* sodium hydroxide was determined (see p. 104). A similar hydrolysis occurs with saturated lime-water, the extent depending upon the time taken to bring the solution to the boil, and with a normal bunsen burner, taking about 3 minutes to do this, the chloroform is hydrolysed sufficiently to return 0·5–1·0% of chloral.

(*d*) It has been established that the mercurous chloride precipitate is soluble, either by decomposition or actual solubility, in the final solution obtained in an estimation to an extent equivalent to a figure of 0·5% of chloral, and in the hot distilled water washings to the same extent, *i.e.*, results returned by the method are about 1% low owing to solubility effects. Thus, by a fortunate coincidence, the errors due to hydrolysis of the chloroform and to solubility of the precipitate tend to cancel each other.

(*e*) Reduction must be carried out by heating in a gently boiling water-bath, as it has been shown that simmering over a naked flame or on a hot-plate causes decomposition of the precipitate, presumably owing to local superheating. Under these conditions it has been confirmed that 2 hours are sufficient for the quantitative reduction of mercuric chloride by formic acid (Table XVII).

The final method, which minimises as far as possible the errors discussed above and gives very satisfactory results (Tables I and II), is as follows.

Procedure—Weigh accurately, by means of a Lunge-Rey pipette or other suitable means, about 10 g. (*w* g.) of sample into approx. 500 ml. of distilled water contained in a litre standard flask. Shake vigorously to effect solution and dilute to 1 litre. Filter and pipette 25 ml. of the clear filtrate into 100 ml. of saturated and filtered lime-water contained in a 500 ml. beaker. Swirl and bring to the boil as rapidly as possible over a full roaring bunsen flame and continue boiling for 10 minutes. Then cool somewhat, add a few drops of aqueous methyl red indicator and make acid with approx. *N* hydrochloric acid, adding sufficient acid to dissolve any precipitated calcium carbonate or hydroxide adhering to the sides of the beaker. When clear, make alkaline with approx. *N* sodium hydroxide and then just acid with one drop of approx. *N*/10 hydrochloric acid.

Dilute the solution to about 400 ml. with distilled water and add 25 ml. of buffered mercuric chloride solution.* Heat over a bunsen until the solution becomes cloudy and place in a gently boiling water-bath for 2 hours. Filter while hot into a tared G4 sintered glass crucible, wash three times with 30 ml. of hot distilled water (excessive washing should be avoided) and dry to constant weight at 110° C. (approx. 1–1½ hours). If the weight of mercurous chloride is P g.

$$\text{the \% of chloral in sample} = 1249\ P/w.$$

* The buffered mercuric chloride solution is made up by dissolving 200 g. of mercuric chloride, 300 g. of hydrated sodium acetate and 80 g. of sodium chloride, in distilled water, making up to 1 litre, leaving overnight and filtering.

(iii) THE PERMANGANATE METHOD—In this method, as at first proposed, 0·1–0·2 g. of sample was hydrolysed by $N/10$ sodium hydroxide at room temperature for 20 minutes, the solution was carefully neutralised and the chloroform produced was removed by boiling. The formate produced according to reaction (1) was then estimated with $N/10$ potassium permanganate according to the method of Jones.[3] As with the other two methods, several modifications had to be made before satisfactory results on technical chloral were obtained under routine conditions.

(*a*) Weighing errors occur similar to those which were found in connection with the mercuric chloride method and can be minimised in the same way.

(*b*) The method for formate specifies addition of excess permanganate solution at the boiling point. Results of experiments indicate that an excess of much more than 3 ml. of permanganate and prolonged boiling at this stage are to be avoided (Table XVIII). The oxidation is practically instantaneous and so back-titration of the excess permanganate may proceed immediately (Table XIX).

(*c*) Chloral polymer returned 75% of chloral by the permanganate method (Table V) and so filtration of the aqueous solution is again necessary before analysis (Table XX).

(*d*) The method of hydrolysis used in the mercuric chloride method, namely, hydrolysis with saturated lime-water followed by removal of chloroform by rapid boiling, proved to be much simpler and quicker than hydrolysis with $N/10$ sodium hydroxide. The hydrolysis of chloroform is reduced to a minimum by bringing to the boil rapidly over a powerful bunsen burner and results returned by the two methods of hydrolysis show good agreement (Table XXI).

The final method, which gives most satisfactory results (Tables I and II), is as follows:

Procedure—Weigh accurately, by means of a Lunge-Rey pipette or other suitable means, about 10 g. (*w* g.) of the sample into approx. 500 ml. of distilled water. Shake vigorously to effect solution and dilute to 1 litre. Filter and pipette 25 ml. of the clear filtrate into 100 ml. of saturated and filtered lime-water. Bring to the boil as rapidly as possible over a full roaring bunsen and boil for 10 minutes.

Add 50 ml. of 5% sodium carbonate solution, bring to the boil and during boiling add 50 ml. of $N/10$ potassium permanganate from a pipette. Allow to go off the boil and examine the meniscus of the liquid for a pink coloration due to excess permanganate. If no definite pink colour is observed add a further 2 ml. of $N/10$ permanganate solution from a burette, raise to the boil, allow to go off the boil and examine for excess permanganate as before. Repeat until the first definite pink colour is observed. Leave on the bench for ½–1 minute, add from a pipette 25 ml. of $N/10$ oxalic acid and then add 20 ml. of diluted sulphuric acid (1+4). If the solution does not become clear on standing for 1 minute add a further 5 ml. of the oxalic acid solution from a pipette. When the solution is clear, titrate at 60°–80° C. with $N/10$ permanganate from the burette used in making the 2 ml. additions. This minimises the number of burette readings.

Carry out a blank on all reagents, using 5 ml. of $N/10$ permanganate at the oxidation stage.

If: a = total volume of $N/10$ permanganate added;
a' = volume of $N/10$ permanganate added in the blank experiment;
b = total volume of $N/10$ oxalic acid added;
b' = volume of $N/10$ oxalic acid added in the blank experiment;

Then chloral % in sample $= 29{\cdot}52[(a-b) - (a'-b')]/w$.

COMPARISON OF THE THREE METHODS

Comparative results obtained in this laboratory by the three methods are given in Table I and results obtained on two samples of chloral by four independent laboratories are given in Table II.

TABLE I

Sample	Chloral (%) obtained by: Alkalimetric hydrolysis method	Mercuric chloride method	Permanganate method
1 A (first runnings) ..	82·5; 82·7	81·6; 81·1	81·8; 81·9; 82·0
1 B (middle fraction) ..	94·5	94·5; 94·8	94·5; 94·5; 94·7
1 C (last runnings) ..	96·0	94·3; 94·5	94·1; 94·1
Pure chloral	99·5	99·3	99·5
Recryst. chloral hydrate ..	100·0*	99·4*; 100·0*; 100·1*	99·6*

* Expressed as chloral hydrate.

TABLE II

(*a*) Crude Chloral before Final Distillation

Laboratory ..	A	B	C	D	Total range
Alkalimetric method ..	93·6; 93·6	93·5	93·2; 93·8	93·4	93·2–93·8
Mercuric chloride method	93·8; 94·1	95·8	93·3	93·1; 93·2	93·1–95·8
Permanganate method ..	93·5; 93·5	94·3; 94·2	94·7; 94·8	94·0; 94·0	93·5–94·8

(*b*) Technical Anhydrous Chloral

Laboratory ..	A	B	C	D	Total range
Alkalimetric method ..	96·7; 96·8	96·0; 96·2	97·9; 97·9	97·0; 97·0	96·0–97·9
Mercuric chloride method	97·3; 97·9	94·7; 95·1	96·1; 96·6	95·5; 95·5	94·7–97·9
Permanganate method ..	97·1, 97·2	97·2; 97·0	96·6; 96·6 96·9; 97·6	96·6; 96·6	96·6–97·6

The estimations in Table I were carried out by an operator with experience of the three methods and the agreement obtained is very satisfactory. The figure obtained by the hydrolysis method on the last runnings is somewhat high, owing no doubt to the presence of esters for which the method makes no allowance.

The results in Table II were obtained in different laboratories by operators to whom the methods were new. In view of this the results are considered to be satisfactory, and the improvement which has been effected in the methods is amply demonstrated.

APPLICATION OF THE THREE METHODS TO PLANT CHLORAL ALCOHOLATE

The methods described above were developed for the analysis of anhydrous chloral, but some work has been done to determine whether they are applicable to plant chloral alcoholate, which is also used as a starting material for the production of D.D.T.

The mercuric chloride method can be used without any further modifications and gives very satisfactory results (Table III).

When the permanganate method was applied to chloral alcoholate, trouble was at first experienced owing to oxidation by permanganate of impurities present, *e.g.*, alcohol and dichloroacetaldehyde. It was found, however, that if, at the chloroform removal stage, vigorous boiling was continued for 10 minutes, the effect of these impurities was much reduced, owing to their partial removal. Further, it was found that if a preliminary determination using not more than 3 ml. excess of permanganate is first carried out, followed by a controlled determination using a calculated 1 ml. excess of permanganate, satisfactory results, agreeing with those returned by the mercuric chloride method, are obtained (Table III). The modification of the permanganate method recommended for the analysis of plant chloral alcoholate is as follows.

Procedure—Weigh 10–12 g. (*w* g.) of the chloral alcoholate into 500 ml. of water in a 1000 ml. standard flask and shake vigorously for 5 minutes. Make up to 1000 ml. with distilled water and filter a portion through three No. 41 filter papers.

(*a*) *Preliminary permanganate oxidation*—Pipette 25 ml. of the filtered solution into 150 ml. of filtered saturated lime-water contained in a 500 ml. conical flask. Swirl to mix taining the solution at the boil* (see (*b*) below) add $N/10$ permanganate from a burette, at first rapidly and ultimately by 2 ml. increments, until the first definite pink colour is observed when the contents of the flask are allowed to settle. (Vol. of permanganate = X ml.) Allow to stand for 1 minute and add 25 ml. of $N/10$ oxalic acid followed by 20 ml. of diluted sulphuric acid (1+4). If the solution does not clear in 1 minute add 5 ml. portions of $N/10$ oxalic acid until it does. Titrate back the excess oxalic acid at 60°–80° C. with $N/10$ permanganate from the burette used in the first addition of permanganate.

If a = total volume of $N/10$ permanganate added;

b = volume of $N/10$ oxalic acid added.

Then the volume of $N/10$ permanganate consumed in the alkaline oxidation $= 5(a - b)/3 =$ A ml. (X − A) should not exceed 3 ml.

(*b*) *Controlled permanganate oxidation*—Treat a further 25 ml. of the filtered solution by the method just described as far as the point indicated by *. Then to the boiling alkaline solution add (A–1) ml. of $N/10$ permanganate and boil for a further 1 minute to remove carbon dioxide. Add a further 2 ml. of $N/10$ permanganate, bring just to the boil, leave

½ minute on the bench, add $N/10$ oxalic acid and sulphuric acid and titrate back as before. Carry out a blank determination on all reagents.

If a' = total volume of $N/10$ permanganate added;
a'' = volume of $N/10$ permanganate used in the blank experiment;
b' = volume of $N/10$ oxalic acid added;
b'' = volume of $N/10$ oxalic acid used in the blank experiment.

Then chloral % of sample = $29{\cdot}52\ [(a' - b') - (a'' - b'')]/w$.

Satisfactory results cannot be obtained by the alkalimetric hydrolysis method and figures are obtained which are from 2% to 5% higher than those returned by the other two methods (Table III). Possibly esters, which would be removed by the subsequent treatment for conversion of the chloral alcoholate to anhydrous chloral, are present in plant chloral alcoholate and the modifications introduced into the alkalimetric method do not eliminate all errors caused by these impurities.

TABLE III

COMPARATIVE ANALYSES OF PLANT CHLORAL ALCOHOLATE

Sample No.	Chloral % found by: Mercuric chloride method	Permanganate method	Alkalimetric method
CA/1	68·9	—	73·4
CA/2	69·7	—	71·7
CA/3	68·7	—	72·4
CA/4	68·7	68·8	73·0
CA/5	66·1	66·0; 66·1	71·8
CA/6	68·0	—	73·0
CA/7	68·6	69·0	74·2
CA/8	69·3	70·0	72·2
CA/9	68·8; 68·5	69·5	—
CA/10	—	67·0; 67·4	70·6
CA/11	69·3; 69·2	69·2	—

EFFECT OF THE IMPURITIES PRESENT IN TECHNICAL ANHYDROUS CHLORAL ON THE ANALYTICAL METHODS

An examination of the manufacturing process suggests that, in addition to chloral itself, the following impurities might be present in the final product: chlorinated paraffins; chloroethyl alcohols; dichloroacetaldehyde; chloroacetic acids and esters; chloroacetals, and chloral and dichloroacetaldehyde hydrates and polymers. Careful fractionation of 1 kg. of technical anhydrous chloral through a 5 ft. column packed with glass cuts yielded the fractions given in Table IV.

TABLE IV

Fraction	Weight (g.)	Distillation range, °C.	Identity
1	470	Up to 97	Chloral, chloral hydrate and dichloroacetaldehyde
2	500	97·0–97·5	Chloral
3	5	97·5–125	Chloral + fraction 4
4	12·5	125–180 (with slight break at 155)	Unknown, but containing 50% of alkali-hydrolysable material thought to be ethyl dichloroacetate
5	3·5	180	Dichloroacetal
6	10	Residue in flask	Unknown, but thought to contain trichloroacetal

TABLE V

Impurity	Apparent chloral % returned by the impurity when examined by: Mercuric chloride method	Modified alkalimetric method	Permanganate method
Dichloroacetaldehyde	1	8	45*
Fraction 4 of Table IV ..	5	55	5
Dichloroacetal	1	4	9
Trichloroacetal	0·5	4	3
Dichloroacetaldehyde polymer ..	5	10	35*
Chloral polymer	71	70	75

* Drifting end-point.

The effects of these impurities on the three methods of analysis are given in Table V. It would appear from Table V that the compound known as "chloral polymer" is not a true

polymer but is some form of condensation product. This observation is supported by total and alkali hydrolysable chlorine determinations, both of which give values of approximately 60% whereas the theoretical value for pure chloral polymer is 72·2%.

The effects of the high boiling impurities on the distillation range of technical chloral are given in Tables VI, VII and VIII. The distillations were carried out according to the method laid down in B.S. Specification 658/1936.

TABLE VI

EFFECT OF ETHYL DICHLOROACETATE ON THE DISTILLATION RANGE OF TECHNICAL CHLORAL

Added ethyl dichloroacetate, %	nil	0·36	0·83	1·6	4·7
Distillation temp. at first drop, °C.	94·3	94·2	94·3	94·3	94·3
Running temp., °C. (*a*) 5 ml. ..	95·7	95·8	95·6	95·6	95·6
(*b*) 95 ,, ..	98·1	99·1	100·6	104·3	116·0
Dry temp., °C.	99·6	108·8	116·3	125·3	157·0

TABLE VII

EFFECT OF DICHLOROACETAL ON THE DISTILLATION RANGE OF TECHNICAL CHLORAL

Added dichloroacetal, %	nil	0·54	1·07	1·46
Distillation temp. at first drop, °C.	95·5	95·5	95·5	95·0
Running temp., °C. (*a*) 5 ml. ..	96·5	96·25	96·0	95·5
(*b*) 95 ,, ..	98·0	100·5	104·0	111·5
Dry temp., °C.	100·0	124·0	124·0	130·0

TABLE VIII

EFFECT OF TRICHLOROACETAL ON THE DISTILLATION RANGE OF TECHNICAL CHLORAL

Added trichloroacetal, %	nil	0·55	0·93
Distillation temp. at first drop, °C. ..	95·5	95·25	95·25
Running temp., °C. (*a*) 5 ml. ..	96·5	96·25	96·0
(*b*) 95 ,, ..	98·0	100·25	103·0
Dry temp., °C.	100·0	125·0	150·0

PREPARATION OF PURE CHLORAL

The following method has been devised for the preparation of pure chloral from plant chloral alcoholate.

Procedure—Mix the chloral alcoholate with an approximately equal volume of conc. sulphuric acid and warm to not above 50° C. Swirl, allow to settle and run off the lower, sulphuric acid layer. Add excess of calcium carbonate and distil through a short fractionating column, collecting the distillate boiling at 96°–99° C. at 760 mm.

Form the hydrate by carefully mixing with a slight excess of water (18 g. of water per 100 g. of chloral will be found convenient). Allow to crystallise overnight, and filter at the pump and dry as thoroughly as possible by suction.

Roughly powder the hydrate and mix with an equal weight of conc. sulphuric acid and warm to a temperature not above 40° C. Run off the acid and stand the chloral over lead carbonate for about an hour. Transfer to another vessel by vacuum or pressure (to exclude contact with moist air) and add phosphorus pentoxide. Leave for about an hour and transfer in the same way to another vessel and fractionate through a very efficient column. Collect over a range of not more than 0·25° C. Moist air must be rigorously excluded from the apparatus.

The pure chloral used in this work was prepared by this method and had sp.gr. 1·5065 at 20°/4° C., and boiling range (760 mm.) 97·75° to 98·0° C.

EXPERIMENTAL

(i) THE ALKALIMETRIC HYDROLYSIS METHOD

(*a*) *Effect of time and temperature of hydrolysis*—5 g. of sample were weighed into 50 ml. of distilled water, the solution was neutralised to methyl red with *N*/10 NaOH solution, 50 ml. of *N* NaOH solution were added and the solution was swirled. The excess alkali was titrated back with *N* HCl solution. This procedure was carried out with the hydrolysing solution at different temperatures and with different times of swirling. Results are given in Tables IX and X. (See also p. 97.)

TABLE IX

CHLORAL % AFTER HYDROLYSIS FOR DIFFERENT TIMES AT ROOM TEMPERATURE

Time of hydrolysis, min.		1	2	7·5	15	60
Chloral %: Sample	2	92·9	93·3	—	96·0	99·0
,,	3	—	95·3	98·5	101·0	—

TABLE X

CHLORAL % AFTER HYDROLYSIS AT DIFFERENT TEMPERATURES

Temp. of hydrolysis, °C.		0	5	8	15	24	35	44
Chloral, %:								
Sample 4; hydrolysis time	2 min.	94·1	—	94·3	95·4	96·7	100·3	108·5
4; ,, ,,	5 ,,	—	95·1	—	—	—	—	112·1
2; ,, ,,	2 ,,	84·6	—	89·0	—	—	97·5	—

(*b*) *Hydrolysis of chloroform by normal alkali*—4 g. of chloroform (the approximate weight produced by the hydrolysis of 5 g. of chloral) in 50 ml. of distilled water were swirled for 2 minutes with 50 ml. of *N* NaOH solution at various temperatures followed by back titration of the excess alkali. The weight of chloroform hydrolysed was calculated in accordance with equation (2) (p. 97), 1 mol. of chloroform hydrolysed consuming 4 mols. of alkali. In the hydrolysis of chloral according to equation (1) (p. 97), 1 mol. of chloral consumes 1 mol. of alkali. Hence, calculated from alkali consumed, 1 mol. of chloroform corresponds to 4 mols. of chloral, or 1 mg. of chloroform corresponds to 4·94 mg. of chloral, which on a 5 g. sample is 0·1%. Hence every mg. of chloroform hydrolysed would increase the apparent chloral content of a 5 g. sample by 0·1%. The effect of this secondary hydrolysis at different temperatures is shown in Table XI.

TABLE XI

Temperature (°C.)	Gms. of $CHCl_3$ hydrolysed	% Chloral equivalent
0	nil	nil
19	0·003	0·3
40	0·02	2
60	0·15	15

(*c*) *Effect of time of hydrolysis at* 0° C.—Hydrolysis with *N* sodium hydroxide of the neutralised aqueous solution of the sample was carried out whilst cooling in ice. Results are given in Table XII.

TABLE XII

Sample No.		2	5
Chloral % after hydrolysis for	2 mins.	85·3; 87·9; 84·6; 86·3	80·4; 80·7
	3 ,,	—	93·6
	7 ,,	90·0	94·5
	10 ,,	90·9	—
	12½ ,,	—	94·5
	16 ,,	90·3	94·7
	20 ,,	91·1	—

(*d*) *Effect of the chloride correction*—5 g. of sample dissolved in 50 ml. of distilled water were neutralised with *N*/10 sodium hydroxide to methyl red indicator. The solution was acidified with 20 ml. of 2 *N* nitric acid and the chloride content determined by the Volhard method. A second 5 g. of sample in 50 ml. of distilled water was neutralised and hydrolysed by swirling for 5 minutes with 50 ml. of *N* sodium hydroxide, the excess alkali was titrated back with *N* sulphuric acid, and the chloride content was again determined as before. The hydrolysis was carried out at 0° C., 20° C. and 30° C., and Table XIII gives the % of chloral returned with and without the chloride correction.

(*e*) *Effect of removing water-insoluble impurities*—Two solutions were prepared, each containing 10 g. of sample in 100 ml. of distilled water. Both were examined by the final hydrolysis method except that one solution was not filtered before analysis. Results with and without filtration are given in Table XIV.

(ii) THE MERCURIC CHLORIDE METHOD

Results obtained by the method as at first evolved, *i.e.*, without applying any modifications, are given in Table XV.

TABLE XIII

EFFECT OF CHLORIDE CORRECTION

Temperature of hydrolysis	Sample No.	Chloral, %	
		Without chloride correction	With chloride correction
0° C.	2	89·1	87·6; 87·7; 87·5; 87·4
,,	5	93·9	93·3
,,	6	88·7	87·5
,,	7	95·6	95·1
,,	8	96·5; 96·5	95·1; 95·0
20° C.	2	89·3; 90·1	86·6; 87·3
,,	5	95·6	93·7
,,	6	91·9; 93·8	88·5; 89·5
,,	7	97·8	95·6
,,	8	97·2; 98·4	95·2; 95·9
30° C.	2	97·0	89·8

TABLE XIV

Sample No.	% Chloral	
	With filtration	Without filtration
1 A (1st runnings)	82·5; 82·7	82·8
1 B (middle fraction)	94·5	94·7
1 C (last runnings)	96·0	97·1
9 A (1st runnings)	81·7; 82·2	86·2
9 B (middle fraction)	94·0; 94·7	94·5
9 C (last runnings)	96·4	97·1

TABLE XV

Sample No.	Chloral, %
5	91·4
6	83·6; 83·0; 84·5; 84·1
7	94·4
8	92·9
10 A (first runnings) ..	79·8; 79·9
10 B (middle fraction) ..	93·1; 92·3; 91·2
10 C (last runnings) ..	93·6; 93·4
11	84·6; 85·1; 86·0
12	88·0; 89·7
13	90·6; 93·7

(*a*) *Effect of removing water-insoluble impurities*—A solution containing 10 g. of sample in 1 litre of distilled water was prepared and analyses were carried out on 25 ml. portions, with and without filtration before analysis according to the method as at first evolved. Results are given in Table XVI.

TABLE XVI

Sample No.	Chloral, %	
	With filtration	Without filtration
1 A (1st runnings)	81·6; 81·1	83·0; 83·3; 83·3
1 B (middle fraction) ..	94·5; 94·8	94·9; 95·3
1 C (last runnings)	94·3; 94·5	95·3; 95·7
9 A (1st runnings)	81·3; 79·2	82·0; 80·3
9 B (middle fraction) ..	92·0; 90·9	92·8
9 C (last runnings)	92·0	92·9

(*b*) *Effect of time and temperature on the reduction of mercuric chloride by formic acid*—10 g. of Sample No. 1 A (1st runnings) were dissolved in 1 litre of distilled water, the solution was filtered, and 25 ml. were taken for each determination given in Table XVII.

TABLE XVII

Time of heating after addition of mercuric chloride solution, hours ..	2	3	5
Chloral by heating in a gently boiling water bath, %	81·1	81·0	81·9
Chloral by simmering on a hot plate, %	78·0	79·1	81·3

(iii) THE PERMANGANATE METHOD

(*a*) *Effect of boiling after adding excess of permanganate*—10 g. of sample were dissolved in water and made up to 1 litre and 25 ml. were taken and hydrolysed by standing for 20 minutes with 50 ml. of $N/10$ sodium hydroxide. The solution was carefully neutralised to methyl red and the chloroform was removed by boiling down to half bulk. Fifty ml. of 5% sodium carbonate solution were added, the solution was raised to the boil, and during boiling a slight excess of permanganate solution was added from a burette. The solution was allowed to stand for 20 minutes, a known excess of $N/10$ oxalic acid solution was added, the solution was acidified with 20 ml. of diluted sulphuric acid (1+4) and the excess oxalic acid was titrated with $N/10$ permanganate. Results obtained, using Sample No. 1 A (1st runnings) and continuing the boiling after different excesses of permanganate had been added, are given in Table XVIII.

TABLE XVIII

Time of boiling	Chloral, %	
	15 ml. excess $KMnO_4$	3 ml. excess $KMnO_4$
5–10 sec.	83·7	83·0
1 min.	84·5	—
5 „	86·9	84·5
10 „	88·0	—

(*b*) *Time required for complete oxidation*—Determinations on Sample No. 1 A (first runnings) and employing different times of standing after the addition of an excess of 3 ml. of permanganate, gave the results shown in Table XIX.

TABLE XIX

Standing time	Chloral, %
10 sec.	83·0
2½ min.	83·0
5 „	83·5

(*c*) *Effect of removing water-insoluble impurities*—Results obtained with and without filtration of the aqueous solution before analysis, and employing 3 ml. excess of permanganate for the oxidation, are given in Table XX.

TABLE XX

Sample No.	Chloral, %	
	With filtration	Without filtration
1 A (1st runnings)	81·8; 81·9; 82·0	82·6
1 B (middle fraction)	94·5; 94·5; 94·7	94·9; 95·1
1 C (last runnings)	94·1; 94·1	95·0; 95·3
9 A (1st runnings)	81·3; 81·5; 81·7	86·6
9 B (middle fraction)	92·8; 92·9	96·6
9 C (last runnings)	94·1; 94·3	97·5

(*d*) *Choice of hydrolysing agent*—Comparative determinations were carried out, using two methods of hydrolysis:

(1) standing 20 minutes with 50 ml. of $N/10$ sodium hydroxide;

(2) rapidly heating to the boil with 100 ml. of filtered saturated lime-water.

Results obtained are given in Table XXI.

TABLE XXI

Sample No.	Chloral % after hydrolysis with agent	
	50 ml. N/10 NaOH	100 ml. saturated lime-water
1 A (1st runnings)	82·3; 82·3; 82·4	82·2; 82·7; 82·7; 82·7
1 C (last runnings)	93·9; 94·2	93·9; 94·2
Chloral boiling range 0·25° C. ..	99·3; 99·4; 99·5	99·5
Chloral hydrate	99·6*	99·6*

* Expressed as chloral hydrate.

SUMMARY

1. Three methods have been developed for the estimation of chloral in technical chloral; the results obtained by all methods are in agreement. Of the three methods the Permanganate Method is the most rapid while the Mercuric Chloride Method, although occupying the most time, is probably the most specific. In technique the Alkalimetric Hydrolysis Method is the simplest.

2. The Mercuric Chloride and the Permanganate Methods can be applied to the analysis of plant chloral alcoholate.

3. A method is given for the preparation of pure chloral from plant chloral alcoholate.

The authors wish to express their thanks to the Chief Scientific Officer, Ministry of Supply, for permission to publish this work. Thanks are also due to the Chemists in Charge of the Ministry of Supply and I.C.I. laboratories who supplied useful information and co-operated by performing routine and comparative analyses.

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CHEMICAL INSPECTION DEPARTMENT
MINISTRY OF SUPPLY

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A Micro-Diffusion Method for the Estimation of Carbon Monoxide in Blood

BY C. H. GRAY AND MARJORIE SANDIFORD

THIS communication describes a convenient adaptation of two earlier methods for the detection and estimation of carbon monoxide in blood. Both methods depended upon reduction of palladium chloride by carbon monoxide to metallic palladium. Wennesland[1] employed dilute sulphuric acid to liberate carbon monoxide from blood, the gas then being absorbed in palladium chloride solution. This was effected in a simple diffusion apparatus consisting of two conical flasks, the mouths of which were joined by a short piece of rubber tubing. Excess palladium chloride was determined iodimetrically, and from the amount which had been reduced the carbon monoxide content of the blood could be calculated. It appeared to the present authors that the liberation of carbon monoxide and its absorption in palladium chloride solution could more conveniently and rapidly be performed in a Conway micro-diffusion unit.[2] The volumes of solution used, however, were then so small that, in our hands, the iodimetric determination of the excess palladium chloride was unsatisfactory and an alternative method for this determination had to be found. Christman and Randall[3] had previously described the determination of carbon monoxide in blood, using a simple but less convenient apparatus in which the carbon monoxide was liberated with ferricyanide and absorbed similarly in palladium chloride. The colloidal palladium was then flocculated with aluminium sulphate and filtered off, and the excess palladium chloride was determined colorimetrically by addition of gum ghatti and excess potassium iodide and comparison of the resulting red colour with a suitable standard. This method has been found suitable for determination of the excess palladium chloride if the liberation and absorption have been carried out in a Conway unit.

METHOD—*Reagents*—

(1) *N/50 palladium chloride solution:* 0·444 g. of palladium chloride is dissolved with heating in 25 ml. of 0·1 *N* hydrochloric acid. After cooling the solution is made up to 250 ml.
(2) 4 *N sulphuric acid.*
(3) 10% (*w/v*) *aluminium sulphate solution.*
(4) 15% (*w/v*) *potassium iodide solution.*
(5) 1% (*w/v*) *gum ghatti solution:* 1 g. of powdered gum ghatti is shaken occasionally during 48 hours with 100 ml. of water and the small amount of insoluble residue is then filtered off. On standing the solution becomes slightly turbid and is therefore always re-filtered just before use.

Procedure—0·25 ml. of 4 *N* sulphuric acid and 1·00 ml. (accurately measured) of palladium chloride solution are pipetted into the outer and inner chambers respectively of a Conway unit, the lid of which has been prepared with a ring of vaseline. Two ml. of a 1 in 4 dilution of blood in water are carefully measured into the outer chamber without mixing with the sulphuric acid. This is facilitated by tilting the unit slightly and by putting the acid at the higher and the blood at the lower part of the outer chamber. The lid is placed on the unit, the vaseline acting as a seal, and the contents of the outer chamber are mixed by gentle rotation.

After 1 hour the lid is removed and the surface of the palladium chloride solution examined in a good light against a white background. When the carbon monoxide of the undiluted blood exceeds approximately 0·5 vol. % a thin grey film of metallic palladium may be seen on the surface of the palladium chloride. With increasing quantities of carbon monoxide in the original blood the film of palladium becomes more and more pronounced until with about 3 to 5 vols. % the palladium forms a definite metallic mirror on the surface. With greater quantities of carbon monoxide the brightness of the metallic mirror increases, but it is then difficult to assess the amount of reduction by mere inspection.

Approximately 0·15 ml. (3 drops) of 10% aluminium sulphate solution is added to the inner chamber, the contents of which are stirred for 2 or 3 minutes with a capillary pipette which is then used to transfer and filter the palladium chloride quantitatively through a 7 cm. Whatman No. 40 filter paper into a 50 ml. measuring flask. The inner chamber, capillary pipette and filter paper are washed quantitatively with six separate, approximately 1 ml., quantities of distilled water. The filter paper is then washed with five 5 ml. quantities of water. One ml. of gum ghatti solution is added to the filtrate, with shaking, followed by 10 ml. of 15% (w/v) potassium iodide solution. The solution is then made up to 50 ml. with distilled water and excess palladium chloride determined either by comparing the red solution with a suitable standard in a colorimeter or by measuring its light absorption in a photoelectric apparatus.

Absorptiometry—Most of the work described in this paper has been carried out with a Hilger Spekker photoelectric absorptiometer with an Ilford Spectral Yellow Green No. 605 filter, although Wratten 61 or Chance No. 6 filters have been found equally suitable. The absorption spectra of the red solutions show a marked increase of optical density with decreasing wavelength. There is a maximum at approx. 490 mμ and a minimum at approx. 470 mμ, but this "kink" in the curve is small. Almost any filter transmitting light of wavelength less than about 550 mμ may therefore be used. A calibration curve may be constructed by measuring 1, 2, 3, 4, 5 and 10 ml. of *N*/500 palladium chloride into 50 ml. measuring flasks, to each of which are then added 25 ml. of water, 1 ml. of gum ghatti solution, 10 ml. of 15% potassium iodide solution and water to 50 ml. The optical density of each solution is then measured and a calibration curve constructed. Since Beer's Law is closely followed it is adequate for most purposes to measure the optical density of a solution containing 1 ml. of *N*/50 palladium chloride suitably made up with gum ghatti, potassium iodide and water to 50 ml. In plotting ml. of *N*/50 palladium chloride against optical density a straight line may then be drawn from the origin to the point representing the absorption density of this single solution. Thus a solution consisting of 1 ml. of *N*/50 palladium chloride prepared in this way gives an optical density of 0·540 for a 1 cm. thickness and using an Ilford No. 605 filter combined with Chance's Calorex.

Colorimetry—The red colour of the final solution may be compared in a visual colorimeter with the most suitable of the standards prepared for the construction of the calibration curve. Since Beer's Law is so closely followed, for many purposes it may be adequate to compare with only the strongest of these standards, which are stable for many hours.

Calculations—Under the conditions of the experiment about 2% of the palladium chloride is adsorbed on the filter paper. Christman and Randall attempted to reduce the magnitude of this adsorption by washing the filter paper with the potassium iodide used to form the red colour, but if this is done we find the increase in optical density of the filtrate is greater than can be accounted for by elution of adsorbed palladium chloride from the filter, and above that obtained when the solutions are not filtered. This cannot be countered by filtering the standards as well since the increased light absorption produced by filtration of potassium iodide solution is variable in magnitude.

Thus the blank gives an optical density corresponding to that given by 0·98 ml. of *N*/50 palladium chloride diluted with the reagents to 50 ml.

If, in an estimation, the final absorption corresponds to A ml. of $N/50$ $PdCl_2$, (0·98 − A) ml. of $N/50$ $PdCl_2$ will have been reduced. From the equation

$$PdCl_2 + CO + H_2O = CO_2 + 2HCl + Pd$$

1 ml. of $N/50$ $PdCl_2$ solution is equivalent to 22·4/100 = 0·224 ml. of CO at N.T.P. Since 0·5 ml. of blood (2 ml. of 1 in 4 dilution) is used, the CO content of the original blood

$$= (0{\cdot}98 - A) \times 44{\cdot}8 \text{ ml. at N.T.P. per 100 ml.}$$

Similarly with a colorimeter, the CO content of the original blood is given by the expression:

$$(0{\cdot}98 - B \times S/U)\ 44{\cdot}8,$$

where S = colorimeter reading of standard,
U = ,, ,, ,, unknown,
B = ml. of $N/500$ palladium chloride from which standard was prepared, divided by 10.

RESULTS—A series of bloods of various degrees of saturation with carbon monoxide have been analysed both by this method and by the manometric method of Horvath and Roughton[4] with the results shown in Table I. It is clear that the accuracy of the method, although

TABLE I

COMPARISON OF RESULTS OF CARBON MONOXIDE DETERMINATIONS BY MANOMETRIC, PALLADIUM-COLORIMETRIC AND PALLADIUM-ABSORPTIOMETRIC METHODS

Ml. of Carbon Monoxide at N.T.P. per 100 ml. of Blood

	Micro-diffusion method	
Manometric method	Absorptiometric measurement	Colorimetric measurement
18·1	17·9	18·8
17·6	17·3	17·5
15·7	15·5	15·7
12·8	12·2	13·5
11·6	11·3	10·8
10·2	9·8	9·0
5·4	6·2	5·4
5·5	4·9	5·4
4·7	4·6	4·8
2·5	1·8	4·0

adequate for many purposes for which carbon monoxide measurement in blood may be required, is not so good as was suggested by the precision with which palladium chloride may be determined. Even with photoelectric measurement of absorption of the final solution, measurements are only accurate to within about 1 volume per cent. With high levels of saturation of blood with carbon monoxide ordinary colorimetry gives results very little less accurate, but with low levels, ordinary colorimetry is unsatisfactory since the result will depend upon two colorimeter readings differing only slightly from each other.

DISCUSSION—Most of the results obtained with the method here described are low compared with those given by the Horvath and Roughton method, but the magnitude of the discrepancy is not constant and, in fact, occasionally the reverse relationship may hold. Carbon monoxide in blood is held partly in physical solution but mainly as carboxyhaemoglobin, and it is possible that the discrepancy may be due to sulphuric acid liberating carbon monoxide only from the carboxyhaemoglobin, a significant quantity of carbon monoxide probably being left in physical solution in the 2·25 ml. of liquid in the outer compartment of the micro-diffusion unit. This physically dissolved carbon monoxide would take a longer time to diffuse into the liquid of the outer chamber than the bulk of the carbon monoxide liberated by the sulphuric acid into the air space of the unit. The effect of this factor will clearly depend on the prevailing temperature, but we have no opportunity of continuing our investigations on this point. In view of the magnitude of the discrepancies, and the fact that discrepancies in the reverse direction are occasionally encountered, this cannot be the sole factor.

Figure 1, which demonstrates the effect of varying the strength of the sulphuric acid, shows that, of those investigated, 4 N sulphuric acid is optimal, maximal reduction of the palladium chloride then occurring within 40 minutes. Sulphuric acid stronger than 6 N

forms with the diluted blood a thick coagulum which appears to occlude carbon monoxide. No experiments have been performed using acid ferricyanide to liberate carbon monoxide, since Wennesland has shown that the reagents alone cause slight reduction of palladium chloride under the conditions of the estimation.

The intensity of absorption of palladium chloride - potassium iodide solution is not significantly altered by minor variations in the concentration of potassium iodide nor by the presence of small amounts of aluminium sulphate or gum ghatti (or gum acacia). These reagents therefore do not require precise measurement. Potassium oxalate as an anti-coagulant in the quantities usually employed has been found to yield no measurable carbon monoxide under the conditions of the experiment. Hydrogen sulphide will interfere, but the authors have had no opportunity of investigating ways in which this source of error could be eliminated.

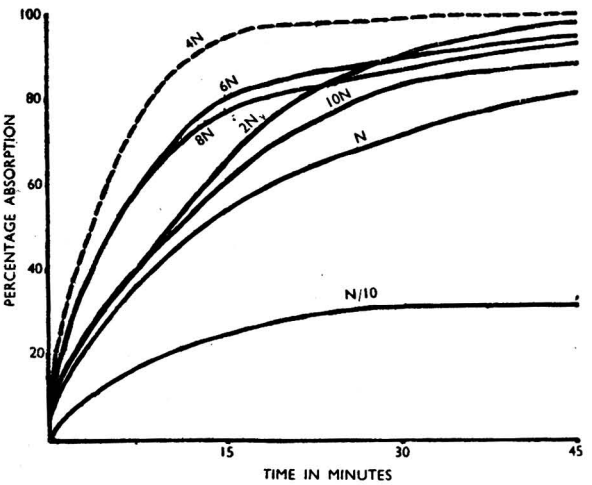

Fig. 1. The effect of varying concentrations of acid on diffusion and absorption of carbon monoxide from blood in Conway units.

APPLICATIONS OF THE METHOD—In spite of the limited accuracy of the method, it may prove of value in forensic as well as industrial medicine, the advantage of requiring only 0·25 or 0·5 ml. of capillary blood being of importance in dealing with workers who may resent venepuncture. Thus in a factory where water gas was produced on a semi-large scale, none of twenty office workers (smokers and non-smokers) showed a blood carbon monoxide content greater than 1 vol. per cent., while, of 12 workers likely to be exposed to carbon monoxide, 5 showed concentrations below 1 vol. per cent. and the remaining 7 showed concentrations between 1 and 2 vols. per cent.

The method is obviously useless for the determination of haemoglobin by the carbon monoxide capacity unless the sources of inaccuracy can be eliminated or compensated, but we have not been successful in doing this. Rough measurements of methaemoglobin might be possible by measuring the carbon monoxide content of blood saturated with carbon monoxide before and after reduction, the amount of methaemoglobin then being calculated from the difference between the two carbon monoxide combining capacities. Stoke's reagent (freshly prepared with a minimum of ammonia) or, possibly better still, a freshly prepared solution of ascorbic acid in methylene blue phosphate buffer solution (Vestling[5]) would be suitable reducing agents.

SUMMARY—1. A simple and convenient method, using a Conway micro-diffusion unit, for the detection and estimation of carbon monoxide in blood is described.

2. Its limitations and possible applications are discussed.

One of us (C. H. G.) is indebted to the University of London for the purchase of a Hilger Spekker absorptiometer with grants from the Central Research Fund and the Thomas Smythe Hughes Medical Research Fund.

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The Determination of Auxins in Soils: Including a Note on Synthetic Growth Substances

By J. HUBERT HAMENCE

(*Read at the Meeting of the Society on February 6th*, 1946)

A REVIEW of the literature indicates that three methods have been described for the detection and the determination of auxins or plant growth substances in soils.

The first was described in 1940 by Parker-Rhodes[1] and is essentially as follows. The soil is dried overnight in an oven at 120° C. and then ground to a fine powder. It is then extracted with an equal weight of cold water and the auxins in the aqueous extract are determined by observing the effect on the osmotic pressure of the root hair cells of wheat seedlings. The results for total auxins, expressed as β-indolyl acetic acid, obtained by Parker-Rhodes for soils on different plots at Long Ashton Experimental Station, ranged from 0·200 to 0·062 μg. per kg,* according to the manurial treatment of the plots. The method was employed also to study the effect of sterilisation upon the auxin content of greenhouse soils.

The second method was described in 1942 by Stewart and Anderson.[2] The technique adopted here is to extract the air-dried soil with water to which has been added sufficient lime water to give the final solution a *p*H of about 7·5. After filtration the extract is evaporated on a water-bath, the residue is treated with water and filtered and the filtrate is extracted with ether. The ether extract is evaporated to a low bulk and the residue incorporated into agar for the Avena test. Samples of different types of American soils, which had been stored in the air-dried condition for periods varying from 3 months to 4 years, were examined by these workers, and gave results for total auxins expressed in terms of β-indolyl acetic acid ranging from 0·072 μg. per kg. to nil.

In 1944 the present author[3] described methods for the detection and determination of auxins in organic manures and stated that one of the methods described,—the cold lime water process,—could be employed for the determination of auxins in soils.

Since then the author has made a large number of determinations of auxins in soils, not only for the purpose of ascertaining the total auxin content of different soils but also in the course of a study of the rate of production of auxins from organic manures added to soils.

Procedure for cold lime water process—For nearly all these determinations the cold lime water process was employed and was adapted to soils in the following manner. The soil was prepared by passing it through a sieve having circular apertures of one-sixteenth of an inch diameter to remove stones; in some cases it was found necessary to air-dry the soil partly before it reached a condition in which it could be screened satisfactorily.

Six hundred to 1000 g. of prepared soil were then weighed out into a dry flask, and 1 g. of powdered calcium oxide and 100 ml. of water were added for every 100 g. of soil taken. The flask was stoppered and the contents were well shaken at intervals for 1 hour and then allowed to stand overnight. Next morning the flask was shaken several times, the solution was filtered and the auxins in the filtrate were extracted as previously described.[3] The auxins in the final extract were determined by the Went Pea Test.

Part of the prepared soil was reserved for a moisture determination (by drying at 100° C.), and allowance was made for the moisture present in the soil when the total auxin content was calculated.

Table I shows typical results which have been obtained, representing total auxins expressed as β-indolyl acetic acid and calculated on the stone-free soil dried at 100° C.

TABLE I

Soil	Total auxins as β-indolyl acetic acid, mg./100 g.
Light loam	0·0025
Clay	0·0020
Glasshouse soil	0·0045
Heavy loam (meadow)	
top, 9 ins.	0·0016
second, 9 ins.	0·0008
third, 9 ins.	less than 0·0008

* 1·0 μg per kg. = 0·0001 mg. per 100 g.

If the sub-soils are disregarded it will be seen that the results obtained above range from 45 to 16 μg. per kg. of dried stone-free soil. Most of the soils examined have fallen within this range, and it will be noted that these figures are approximately 100 times greater than the results obtained by the previous workers mentioned.

In view of these results it is pertinent to compare the different methods of analysis in order to find out any significant differences which might account for the wide variation of the results obtained.

Stewart and Anderson Process—Consideration of the method of Stewart and Anderson (*loc. cit.*) immediately reveals that the true auxins are not determined by this process. In this method the auxins are extracted by treatment with water containing sufficient calcium hydroxide to give a final *p*H of about 7·5, and are therefore extracted as the calcium salts. The solution of the calcium salts is then evaporated to dryness; the residue is treated with a small quantity of water, the solution obtained is extracted with ether and the auxins in the ethereal extract are determined. The weakness of this method lies in the fact that the solution is not acidified before extraction with ether and, therefore, as auxins are nearly all acidic bodies, they would remain behind in aqueous solution as calcium salts and would not be extracted by the ether.

This point was proved by determining the auxins in a light loam soil by the cold lime water process, in which the aqueous solution is acidified before ether extraction, and also by the method of Stewart and Anderson. The results obtained are shown in Table II.

TABLE II

Method	Total auxins as β-indolyl acetic acid, mg./100 g.
Hamence cold lime water	0·0020
Stewart and Anderson	.. No detectable amount

As a confirmation of the cause of the breakdown of this latter process the lime water solution after ether extraction was acidified with hydrochloric acid and then re-extracted with ether, following the technique employed in the lime water process. The whole of the auxin was recovered in this extract.

It is clear from these experiments that only growth substances or auxins of a neutral or a basic nature could be determined by the Stewart and Anderson process, but it has yet to be proved that such bodies exist and that they would be extracted by the weak lime water that these workers employ.

In this process a second point which calls for criticism is the evaporation of the lime water solution. When this operation is conducted over a water-bath as directed by the authors there is always the possibility of part of the auxins,—those sensitive to hot alkalis,—being destroyed. The comparative tests described above indicate that no appreciable auxin was lost during this evaporation, as might have been expected, so that possibly with soils this may not be such a serious matter as it is with other materials.

These tests explain the very low auxin activity found in soils by these workers, and the number of negative results they obtained.

Parker-Rhodes Process—I am unfortunately not familiar with the osmotic pressure technique of Parker-Rhodes for the final assessment of the amount of auxin present in soil extracts, and have therefore been unable to check the whole of this process. But a number of comparative experiments have been made to compare the Parker-Rhodes extraction process with the cold lime water extraction process. In each the soil extract was acidified and the auxins were extracted with ether and finally assessed by the Went Pea Test. The results obtained are shown in Table III.

TABLE III

	Process	Total auxins found, as β-indolyl acetic acid, mg./100 g.
1.	Cold lime water process	0·0033
2.	Soil oven-dried 18 hours at 120° C. and extracted with water for 1 hour (*p*H of extract 7·5)	0·0014
3.	Soil oven-dried 18 hours at 120° C. and extracted with lime-water for 1 hour	0·0016
4.	" " " " " " " " 18 hours	0·0041

These results indicate that 1 hour is not a sufficiently long time of extraction to remove all the auxins from the soil, but they do not explain the wide divergence between the results obtained by Parker-Rhodes and the author. It is interesting to note that in process 4, which is in reality the cold lime water process carried out on the soil dried at 120° C., more auxin was found than was obtained from the undried soil. Recent work appears to indicate that this is due to the heating process causing a small increase in the auxin content.

The results shown above indicate that with the type of soil under examination approximately 40% of the auxin may be extracted from the oven-dried soil by cold water extraction for 1 hour. It should be mentioned that the *p*H value of the water extract from this soil was 7·5, and that less auxin may be extracted from an acid soil.

As a result of these experiments it is obvious that the wide discrepancy between the results obtained by Parker-Rhodes and those of the author is due only to a small extent to the method of extracting the soil. The method of assessment of the active substances in the final extracts may be the principal cause of the discrepancy. Growth substances do not all react similarly towards the Avena and the Pea Tests; for instance, some substances highly reactive in the Pea Test are almost inactive towards the Avena Test, and it is, therefore, possible that such a difference may exist between the Went Pea Test and the osmotic pressure test of Parker-Rhodes.

There is also one other aspect to be considered. In the Went Pea Test interfering substances are removed before the test is applied, whereas the osmotic test is applied directly to the soil extract, and it does not appear to have been demonstrated that substances which would interfere with the osmotic pressure test are absent in the water extract of a soil.

Efficacy of the Cold Lime Water Process—As with organic manures, the efficacy of the process for soils was checked by adding known amounts of β-indoyl acetic acid to samples of soils and determining the auxin added.

The following (Table IV) are typical of the results obtained; in each instance the total auxins were determined in the final extract by the Went Pea Test.

TABLE IV

Expt.	β-Indolyl acetic acid added mg./100 g.	Total auxins found, as β-indolyl acetic acid, mg./100 g.	β-Indolyl acetic acid recovered, mg./100 g.
1	nil	0·003	—
2	0·017	0·021	0·018
3	0·083	0·110	0·107

Hamence Perchloric Test for β-indolyl Acetic Acid—In view of the very small proportion of growth substances normally present in soils it is necessary to obtain the extract from at least a kilo of soil before applying this test for β-indolyl acetic acid. Experience has shown, however, that from such a large quantity of soil extract it is impossible to remove completely substances which interfere with the test and with some soils the results may be difficult to interpret at this level of activity. The principal interfering substance, which has already been referred to in a previous paper, gives a yellowish brown coloration in the perchloric acid test and traces of the yellow colouring matter are subsequently extracted when the solution is shaken with chloroform.

This interfering substance appears to be a pyrrole derivative, which has proved to be exceedingly difficult to separate from β-indolyl acetic acid and its homologues. It yields a red coloration when a solution of it in water is treated with Erhlich's aldehyde reagent and the mixture is boiled for 10 seconds.

In fractionation experiments, made with different solvents and precipitants with a view to purifying and identifying this interfering substance, it has been found difficult to separate it from substances which react in the Went Pea Test, and this raises the question whether or not it is itself one of the natural growth substances of the soil. Moreover, it is not readily extracted by cold water extraction of the soil and is only extractable by the cold lime water process; the greater activity found by the cold lime water process compared with the cold water process, shown in Table III, may therefore be due to this body.

Erhlich's Aldehyde Test—Although the pyrrole derivative which produces the red coloration in this test has not been identified, nevertheless, for the purpose of records, its response to the test has been quantitatively assessed in the following manner.

An aliquot portion of the final extract from the cold lime process is placed in a test tube and sufficient water added to make the bulk up to 5 ml. One ml. of a 2% solution of *p*-dimethylaminobenzaldehyde in diluted hydrochloric acid (1+1) is then added and the mixture is boiled for ten seconds. It is then rapidly cooled and the red coloration produced is matched immediately in a 1 cm. cell in a Lovibond tintometer. The colour of the solution rapidly fades on standing.

The results are expressed as the number of red units given by 100 g. of air-dried stone-free soil. Norman soils usually give a value between 15 and 50 red units for 100 g. of soil.

When more than the usual proportions of growth substances are present in a soil,—as during the decomposition of an organic manure,—and it is possible to apply the test to a smaller quantity of soil, then the influence of the interfering factor largely disappears and the perchloric test becomes quantitative. Definite evidence of the formation of β-indolyl acetic acid in soil treated with heavy dressings of herring meal has been obtained by this test.

SYNTHETIC GROWTH SUBSTANCES—Of recent years a number of synthetic growth substances have been prepared and are in use for different agricultural or horticultural purposes.

Two of these compounds typical of the different groups of substances in use were selected and tested in order to find out if they could be extracted from soil by the cold lime water process. α-Naphthalene acetic acid and 2 : 4-dichlorophenoxyacetic acid were the two compounds selected.

Known quantities of each were added to portions of soil and the total auxins determined by the Went Pea Test after the cold lime water extraction process. The limiting values in the Went Pea Test for 2 : 4-dichlorophenoxyacetic acid and α-naphthalene acetic acid were found by experiment to be 0·0005 mg. in 10 ml. and 0·001 mg. in 10 ml. respectively, and these figures were used in calculating the results shown below.

TABLE V

	Expt.	Growth substance recovered, mg./100 g.
1.	Soil with 0·167 mg./100 g. 2:4-dichlorophenoxyacetic acid added ..	0·104
2.	„ „ „ α-naphthalene-acetic acid added	0·125

The recoveries of growth substances shown in the above Table are short of the theoretical. But having regard to the fact that in practice it would be impossible to calculate the true proportion of growth substance present unless the identity of the growth substance was known, the cold lime water process followed by the Went Pea Test will serve to detect the presence of new growth substances in soils.

Synthetic growth substances containing chlorine may be detected by testing the final extract obtained by the cold lime water process for the presence of organically combined chlorine.

SUMMARY

Three processes for the determination of auxins in soils have been considered. The cold lime water process followed by the Went Pea Test has been shown to give higher results for the auxin content of soils than either of the other two methods.

Investigation of the process described by Stewart and Anderson shows that the method in its present form is incapable of determining the total auxins present in soil.

Experiments show that differences between the results obtained by the author and by Parker-Rhodes are only partly due to the method of extraction, and therefore it is possible that the different methods employed to determine the auxins in the final extracts are the principal cause.

The cold lime water process has been shown to be a satisfactory method for the extraction of auxins from soils prior to their assessment by the Went Pea Test. Moreover, satisfactory recoveries of added auxins (added as hetero-auxin) have been obtained by the process.

The auxin content of different soils as determined by the cold lime water process, and subsequently assessed by the Went Pea Test, have been found to vary between 45 and 16 μg. per kg. of stone-free air-dried soil.

In view of the possible alteration of auxin content of soil samples on storage the author recommends that the examination of soils for auxins should be carried out as soon as possible after sampling.

A compound found present in most soils may in sufficient quantity interfere with the

Hamence perchloric acid test for β-indolyl acetic acid. It gives a red coloration on heating with Erhlich's aldehyde reagent, and there is indication that it may be a natural soil growth substance responsible for part of the auxin activity as measured by the Went Pea Test. A procedure is also described for giving this test a quantitative significance.

The cold lime water process followed by the Went Pea Test is also capable of detecting traces of synthetic growth substances such as α-naphthalene acetic acid and 2 : 4-dichlorophenoxyacetic acid in soils.

I wish to thank Mr. George Taylor, F.R.I.C., for his interest and helpful criticism throughout this investigation and also Dr. A. H. Lewis for supplying the specimen of dichlorophenoxyacetic acid used.

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Notes on the "Auxin Balance" in Soils

The cold lime water method for the determination of auxins in soils has been employed in our laboratory for studying the effect of the application of organic manures containing auxins and also of pure hetero-auxin on the auxin content of soils.

β-Indolyl Acetic Acid—Pot experiments have shown that when fairly heavy doses of β-indolyl acetic acid, of the order of 1 mg. per 100 g., in the form of a solution of the sodium salt, are applied to soils, as much as 95 per cent. of the added auxin is destroyed in the first 24 hours, and after several days the auxin content of the soil has returned to its original value. The rate of destruction of added pure β-indolyl acetic acid is not affected by sterilisation of the soil but may be retarded by heavy applications of lime.

This rapid destruction of β-indolyl acetic acid may explain the widely varying results of its effect on plant growth reported by different workers.

Organic Manures—In general the effect of application of a normal dressing of an organic manure containing auxins to a soil is to increase its auxin content, and experiments in glass houses and in the open ground have shown that this increase may be maintained over a period of months.

Heavy Dressings of Organic Manures—The effect of heavy dressings of organic manures, such as dried blood and fish meal, have been studied for the purpose of finding out whether or not β-indolyl acetic acid is produced during their decomposition in the soil. The dressings employed were heavier than those normally employed in practice, but local heavy concentrations of fertiliser, similar to those used in these experiments, inevitably occur when dried blood is used as a top dressing in glasshouse work. These experiments have shown that the amount of auxin produced is partly governed by the water content of the soil and that, under suitable conditions, part of these auxins are present in the form of β-indolyl acetic acid.

These experiments have also brought to light a new phenomenon. Under suitable conditions of moisture content the auxin content of a soil which has been heavily dressed with dried blood or fish meal rapidly rises well above the normal value for soils and then begins to fall. The diminution continues and, in some instances, the auxin content may fall below the original auxin content of the soil. Moreover, it has been found that the red coloration given in the Ehrlich test also increases and diminishes with the total auxins, and when the auxin content falls below the original value for the soil no appreciable colour is given in this test. In due course, after standing for some time, the soil regains its original auxin content and at the same time positive reactions are given in the Ehrlich test. At the stage when the Ehrlich test becomes negative nitrites are found in the soil, and these subsequently disappear when the soil returns to its normal auxin content. These results on the effect of heavy dressings of organic manures have been obtained in experiments in pots and also in the open ground.

Lime-washed Soil—The auxin content of soil may be lowered by applying a heavy dressing of lime and washing copiously with water. A soil so treated slowly returns to the normal.

Auxin Balance—As stated in the main body of this paper on the determination of auxins in soils, the value for the auxin contents of the soils which have so far been examined have been found to fall within certain fairly narrow limits (from 45 to 16 μg. per kilogram of air-dried stone-free soil). The usual effect of a normal dressing of an organic manure containing auxins is to increase the auxin content of the soil towards the upper limit shown above.

Although by the addition of pure hetero-auxin or heavy dressings of organic manures such as dried blood, it may be possible to produce in the soil a temporary considerably enhanced auxin content, nevertheless this exists only for a short time, and sooner or later the auxin content falls to a value within what we have described as the normal range of auxin contents.

It appears that there are therefore two processes taking place continuously in soils—one producing auxins and the other eliminating or absorbing them. The net results of these two processes is to keep the auxin content within certain fairly narrow limits. We suggest that this indicates the existence of an "auxin balance" in the soil. Whether the difference in auxin content as represented by the upper and lower limits of the auxin range have any significant difference on plant growth still remains to be studied.

The full experimental details and results of this work will be published in due course.

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DISCUSSION

The PRESIDENT asked whether the soil auxins had their origin primarily in animal matter or whether, as products of bacterial metabolism, they were also present in purely vegetable material such as leaf mould.

Mr. L. EYNON asked whether the equilibrium auxin content of soils was the same for all soils or had characteristic values for different types of soils.

Mr. A. E. PARKES asked if the author had examined compost made on the "Indore" principle for auxins and, if so, how it compared with farmyard manure and peat. He also asked if anything was known about the origin of auxins and if the amounts present in manures and soils varied with the bacterial activities.

Mr. C. G. DAUBNEY asked if the author could say why the surface soil had a higher auxin content than the soil one or two "spits" below; would not the auxins, being soluble, tend to be washed downwards by rain water? Possibly the observed distribution is associated with bacterial activity, which is mainly confined to the top soil levels.

Mr. E. C. WOOD asked whether, in the Went pea test, it was customary to determine the limiting dose of β-indolyl acetic acid each time an assay was performed, or whether this was assumed to be constant from assay to assay. By analogy with other biological methods it would appear desirable to put up a series of standard dilutions simultaneously with each series of test dilutions.

Mr. D. M. FREELAND observed that, in view of the author's statement that the auxins were generally acidic, it appeared that the pH of the soil must influence their action, and presumably they would afford less stimulation of plant growth in heavily limed soils. In the author's estimations was allowance made for variation in the moisture content of soil samples? As auxins like β-indolyl acetic acid, or those contained in manures added to soils were almost entirely destroyed before a natural auxin balance was attained in a soil, was it not likely that the residual auxins were different constitutionally from those deliberately added?

Mr. A. L. BACHARACH asked whether the author could give some information about what was meant by a "sterile" soil. It was difficult to understand the fluctuations in auxin content reported by the author, were all biological processes in the soil absent, as one would expect if it were sterile.

Mr. F. W. J. GARTON said that the author had described a control experiment in which β-indolyl acetic acid was added to a soil and subsequently determined by his own method. Had he carried out a similar experiment using the American method in order to confirm the failure of that method when known amounts of this acid were present?

Dr. HAMENCE, in reply, said that the origin of soil auxins was not clearly established. While it was possible that manures containing tryptophan may produce some β-indolyl acetic acid, recent work had shown that by far the greater part of the soil auxins are metabolic products of soil bacteria. At the present time there was insufficient experimental data available to say whether the auxin range was the same for all soils or varied from one soil to another. As yet no composts prepared on the "Indore" principle had been examined, but usually in a well-rotted compost the auxin content was similar to that of well-rotted farmyard manure. The nature of the manure largely governed the effect of bacterial activity on its auxin content. For instance, when dried blood and fish meal decomposed, the auxin content increased, whilst urine and sewage sludge usually showed a reverse effect. The auxins were not all water-soluble, and therefore were only partly washed down by rain, but presumably the soil bacteria made good such losses as did occur in this manner quite quickly. In using the Went Pea Test it was not the speaker's custom to check the limiting value with pure β-indolyl acetic acid more often than once a fortnight unless a new batch of peas was used. Undoubtedly more frequent checks would be preferable, but so far no serious variations in the limiting value had been obtained. Very little work had so far been done on the effect of pH on the auxin content of soils, and this was a point which might be profitably studied in future. The growth of fungi would be greatly restricted in a heavily lime soil, and this would conceivably affect the auxin content. It was possible that the true soil auxins were different constitutionally from β-indolyl acetic acid; but at present little was known of their constitution. It was the speaker's practice to calculate the auxin content on the basis of the air-dried stone-free soil.

A sterilised soil was not sterile in the sense that the term was used by the bacteriologist. The process of soil sterilisation was not properly understood, and its ultimate effect in most cases was to cause a more prolific growth of certain soil bacteria. A check determination on the Stewart and Anderson method had been made, in which a known amount of pure β-indolyl acetic acid had been added.

Some Physicochemical Methods in Microchemistry: Viscosity, Surface Tension and Refractive Index

By CECIL L. WILSON

(*Read at a Joint Meeting of the Microchemistry Group of the Society and the Manchester Branch of the Royal Institute of Chemistry, on Friday, 25th May,* 1945)

THE existence of microchemical modifications of ordinary methods of chemical analysis is by now familiar to most chemists. It is not, perhaps, so generally realised that methods for the determination of many physical properties of use to the analyst have also been devised for very small scale work. In this paper representative methods for determining three of the more important physical properties on very small samples are reviewed briefly; they are illustrative of the ingenuity which has been applied to this end.

No attempt is made to distinguish between the micro and semi-micro methods. Many of the processes, by alteration in quantities and dimensions, can be included in either group. Other methods fall in the ill-defined border-zone, so that it is difficult to make a hard and fast classification.

VISCOSITY

Merz[1] gives a review of viscosity methods which will handle volumes of liquid ranging from 5 ml. to 15 ml. However, in my view, microchemistry is concerned with amounts considerably smaller than this.

The use of a rolling ball to determine viscosity, adapted from the well-known macro procedure, has been applied by Schneider and McConnell[2] (see Fig. 1). The jacketed tube

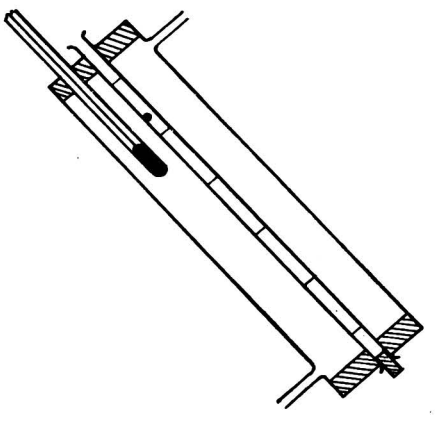


Fig. 1

Fig. 2

Fig. 3

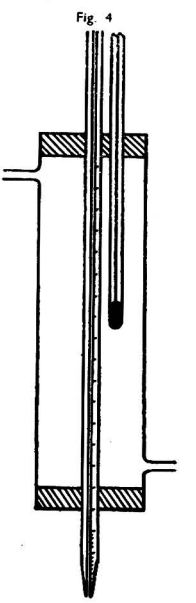


Fig. 4

of the viscometer is inclined at an angle of 60° to the horizontal, and the viscometer itself is made from a capillary tube, of total capacity about 2 ml. and internal diameter about 5 mm., etched at intervals of 4 cm. A steel ball, with a diameter approximately one-half that of the tube, is allowed to roll down, and is timed between two of the marks. By appropriate selection of the marks it is possible to take times of fall from 8 to 300 seconds for values of viscosity ranging from 0·7 to 35 poises. If only the lower marks are used, as little as 0·5 ml. of liquid may suffice. For any two marks, viscosity is proportional to the time of fall.

An adaptation of the ordinary U-tube viscometer of Ostwald is described by Cannon and Fenske[3] (Fig. 2). The wider tubing has a diameter of 2·5 mm. in both legs of the instrument, thus avoiding surface tension effects. The bore of the narrow capillary may be anything

between 0·5 and 2·0 mm., depending on the liquid being dealt with. A small bridge of solid glass lends the apparatus rigidity. The marks between which the flow is timed are 4 cms. apart, and the viscometer will handle 0·25 ml. of liquid. A reproducibility within ±0·2 per cent. is claimed.

Other methods for the determination of viscosity based on the rate of flow of liquid through a capillary are numerous. One of the simplest, that of Levin,[4] is readily applicable to liquids whose surface tensions do not differ markedly. As a consequence, it has been used for lubricating oils. The capillary viscometer (Fig. 3) is drawn from a 30-cm. length of tubing having a 1-mm. bore and a 5-mm. outer diameter, to give an 8-cm.-long tip with a uniform bore of about 0·2 mm. Three marks are made, one at 25 mm. from the tip and the others at 13-mm. intervals. A fixed amount of liquid (usually 15 mg., but as little as 5 mg. may be used) is measured into the cup by means of a capillary pipette, and the fine tip of the capillary viscometer is lowered into this. The times for the capillary rise of the liquid from the tip to the three marks are noted. The time to the middle mark is used for the actual viscosity determination, the others serving simply as a check on the conditions. Given calibration with a liquid having a similar surface tension, the viscosities are proportional to the times. The method is rapid, but, as one would imagine, not capable of high accuracy.

The apparatus of Lidstone[5] is much to be preferred for accurate work, since it corrects for surface tension variations. A uniform capillary tube (Fig. 4) is ground to a pointed tip at one end, and graduated in cms., the three lowest of which are graduated in mms. By just dipping the tip into the liquid, it is possible to measure the capillary rise in the tube. The liquid is then sucked up in the tube, and allowed to fall back, the time being taken for the liquid to pass two of the calibrations. When the time has been noted, the top of the tube is plugged, and the liquid is allowed to drain, so that the meniscus will now rise. When this rise has ceased, a reading is made which permits the calculation of a drainage error.

The drainage correction $\beta = (l-d)/l$ (= 0·9 approx.) where l = length of travel of meniscus, and d = drainage rise.

Using this, together with the time of flow, the length of path, and the capillary rise, it is possible to calculate the viscosity of the liquid from the relation

$$\text{Viscosity, } \eta = \frac{r^2 g \rho t}{8\beta(h_1 - h_2 + c \log h_1/h_2)}$$

where c = capillary rise; h_1 = original height—capillary rise; h_2 = final height—capillary rise.

Lidstone's apparatus deals with quantities of the order of 0·05 ml.

Another apparatus which handles amounts of the same or slightly smaller order has been devised by Bowman.[6] The apparatus may be used for absolute determinations of viscosity, as may Lidstone's apparatus, but it is more satisfactory for comparative determinations relative to a standard liquid. A small drop of liquid is drawn up to the lowest mark of the uniform (25- to 500-μ bore) jacketed capillary (Fig. 5), and the end of the capillary is then wiped clean. This gives a constant volume of liquid. The liquid in the capillary is now drawn up above the top mark, the upper end is opened to the air, and the time of fall of the upper meniscus from the top to the second mark is noted. Surface tension effects are avoided by drawing the length of liquid up the tube at approximately the same rate at which it will subsequently fall, so that similar films of liquid coat the walls both before and behind the falling drop. The tube is too fine for gravity to produce any observable alteration in the shapes of the two menisci. Errors due to the viscosity of the air will arise either if a liquid of low viscosity is being handled, or if the capillary is very fine. But either of these may be corrected for. Over the range 50 to 1000 secs. flow it is possible to obtain a straight-line curve by plotting time of fall against viscosity/time. For absolute determinations of viscosity, the formula given is

$$\text{Kinematic viscosity, } k = \frac{g r^2}{8L} t,$$

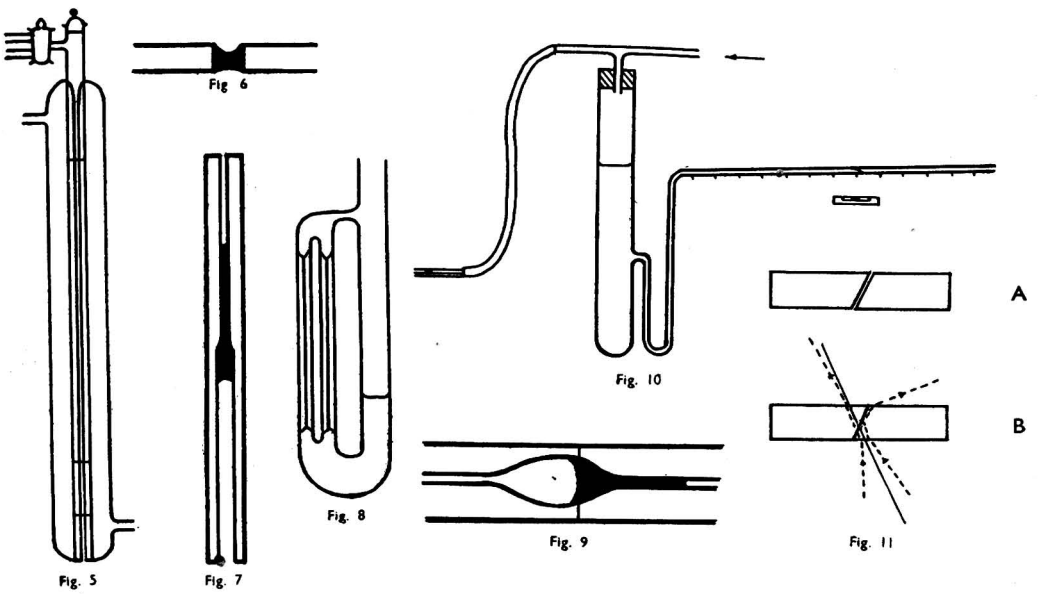
where g = gravity acceleration; r = radius of tube; L = distance between first and second marks; t = time taken for meniscus to fall between these two marks.

Surface Tension

Methods of measuring the surface tension of liquids, on the micro as on the macro scale, may give either an absolute or a relative figure. To obtain an absolute figure, on a small

scale, it is possible, as has been shown by, *e.g.*, Porter,[7] to calculate surface tension directly from the dimensions—the height and radius—of a drop of the liquid lying on a plane surface. But this method is very limited in its practical applications, and probably its greatest interest is from the theoretical point of view. The drop-weight method of determining surface tension has been applied to the measurement of the property in, *e.g.*, colloidal solutions.[8] A drop is held at full extension over a period of time by means of a plunger on a microscope extension. This permits accurate measurement of the drop by a cathetometer.

An absolute value may be obtained by the method of Naggiar.[9] For this, a disc with plane parallel sides, with a hole bored in it (shown in section in Fig. 6) is used. A drop of liquid is placed in the hole, and the radii of curvature of the top and bottom menisci are measured by microscopes placed on either side of the disc. The thickness of the drop along



its axis is also measured by the microscopes, and from these values it is possible to calculate the surface tension, since

$$2n\left(\frac{1}{R_1}-\frac{1}{R_2}\right)=\rho g x$$

where R_1 and R_2 are the radii of curvature, and x is the thickness of the drop. An accuracy within 1 per cent. is obtained, and the apparatus may be enclosed to enable such factors as the change of surface tension with temperature to be studied.

The measurement of a capillary rise, using a vertical tube built up from two capillaries of different bores joined end to end (Fig. 7), is capable of a precision of $\pm 0{\cdot}5$ per cent., using 0·1 ml. of liquid, but the results are found to be lower in general than those determined on the macro scale.[10,11]

Capillary rise is also used in Sugden's method,[12] as modified by Bowden.[13] In this case, the difference in rise between two capillaries of different bores, placed side by side (Fig. 8), gives a value from which surface tension can be derived.

For results which are essentially comparative, though they may be read directly in absolute units, the apparatus of Mouquin and Natelson[14] is probably the most ingenious and satisfactory. This apparatus is dependent on the fact that the pressure required to drive a liquid along a horizontal tube of conical bore is completely dependent on the radii of curvature of the two ends and the surface tension of the liquid. In effect, this is the same principle which is applied in Naggiar's method, replacing gravity by an applied and variable pressure. If the radius of curvature factor is kept constant—this is done by maintaining a constant ratio between the radii of the two ends—then the pressure and the surface tension are directly related.

A suitable conical tube is provided by the small bulb at the top of a broken thermometer, as indicated in Fig. 9. The capillary has a bore of about 0·23 mm. A mark is etched across the bulb to fix the wider radius, the other radius being that of the capillary, so that the relation between the two is constant if the wider meniscus is maintained at the mark, regardless of any evaporation from, and consequent movement of, the narrower end. The tube is kept horizontal, and the meniscus observed under a low-power microscope. The pressure is measured accurately by a horizontal manometer (Fig. 10), of which the arm is 60 cm. long and 0·35 cm. in bore, and is inclined at about 3° to the horizontal. Pressure is applied by a rubber bulb compressed by a screw clip.

The apparatus is calibrated with known liquids, which are measured simultaneously by the du Nouy method. A straight-line curve relates the applied pressure to the surface tension. Consequently it is feasible to alter the slope of the manometer slightly, so that each scale division corresponds to 1 dyne/cm. It may be maintained at this slope by means of the spirit level mounted on the same board.

A method which has some similarity to that just described has been proposed by Ferguson and Kennedy.[15] In this, the applied pressure is used to bring the liquid to the end of a *uniform* capillary tube. The pressure which just eliminates the curvature of the meniscus, and renders the surface of the liquid plane with the end of the tube, is related directly to the surface tension of the liquid. The point at which this occurs is observed by using the meniscus as a mirror. Light from a source is reflected into a low-power microscope, and the change from the normal concave to planar is noted.

Refractive Index

Wright[16] reviews methods for determining the refractive index of a drop of liquid, and recommends for very accurate measurements the formation of a 30° liquid prism between two optically plane glass plates held at the correct angle in a suitable clamp. For microscopic measurement, two plates of highly refracting glass (refractive index, 1·92) are prepared as shown in section in Fig. 11A, the bevelled edges being inclined at 60°, and the upper one polished, while the lower one has a matt finish. Liquid is placed between the bevelled edges, which are pressed together. Light on one side of the full line (Fig. 11B) will be reflected, while that on the other side will be transmitted, giving a field of view divided into dark and light portions by a well-defined edge. Under the microscope the position of this sharp demarcation is measured, using a micrometer eyepiece which has been calibrated empirically with substances of known refractive index.

The application of the well-known striation test for refractive index has been described in an extensive series of papers,[17] and also in Emich's standard work.[18] However, this method suffers from the drawback, not in general found in other refractive index methods, that the liquid being measured must be mixed with standard liquids, thus presenting a recovery problem which may be serious in cases where only small amounts of valuable samples are available.

The measurement of the refractive index of both liquids and solids by the immersion method, using the microscope, is of long standing, and too well known to require detailing here. For solids, many ranges of standard liquids have been proposed. Various authors have also suggested mixtures of liquids, suitable for special purposes. Mixtures, for example, of liquids like α-bromonaphthalene and *n*-butyl phthalate, which have approximately the same vapour pressures, are recommended,[19] as maintaining the same composition over long periods of time. For higher refractive indices, solutions of iodoform, antimony iodide, sulphur and arsenious sulphide in methylene iodide have been proposed, and for some purposes eutectics of such compounds as camphor, salol, thymol and α-naphthylamine have their uses.[20]

More recently, the simultaneous determination of refractive index and melting point of organic solids by the immersion method has been recommended as a useful aid to characterisation. For this purpose, a series of twenty-four glass powders, with refractive indices differing by steps of about 0·01 unit, and covering the range 1·43 to 1·67, are immersed in the molten solid.[21,22]

The microscope may be applied to the measurement of the refractive indices of liquids by the method of real and apparent depths. A description of one cell used for this purpose, that of Kirk and Gibson,[23] will suffice to illustrate the method. A hole, 1 mm. in diameter, is drilled to within 1 mm. of the bottom of a sheet of plate glass (Fig. 12), and the bottom of the cell so formed is polished, and then has a small scratch made on it. The microscope is

focussed on the scratch with a cover-slip in position over the cell, both without and with a liquid in the cell. The difference in reading is noted on the fine adjustment of the microscope. The cell is calibrated against a series of standard liquids, plotting fine adjustment against refractive index, and the value for an unknown liquid is read from this graph.

Another microscopic method employing a different principle is that devised by Nichols,[24] and also described by Alber and Bryant.[25] Two glass prisms of the same refractive index are fixed side by side to a microscope slide, in the centre of a cell which holds 5 to 7 cu. mm. of liquid. Side and top views of the cell are shown in Fig. 13. The cell is filled with liquid, and the double deviation of the light beam is measured by a microscope, actually by measuring the apparent distance between the two halves of a line running under the prisms. The deviation is read on a calibration curve, obtained from standard liquids, relating its value to refractive index.

Two such cells, one with glass prisms of refractive index 1·52, and the other with prisms of refractive index 1·75, will cover the refractive index range 1·33 to 2·0, with an accuracy within ±0·001 for white light, and ±0·0005 for sodium light.

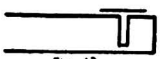
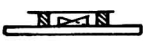


Fig. 12



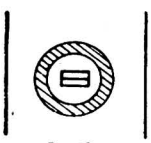


Fig. 13

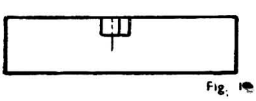


Fig. 14

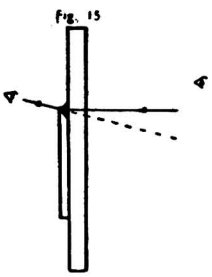


Fig. 15

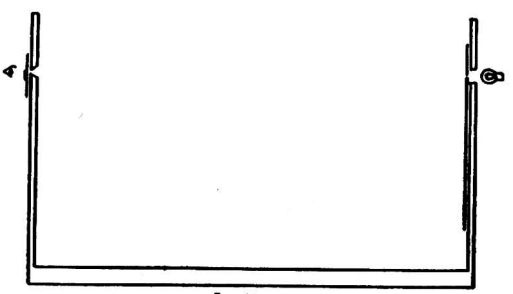


Fig. 16

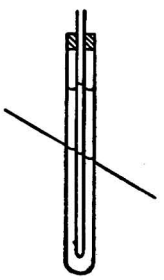


Fig. 17

A variation of this method, which makes use of more easily obtainable materials, although it is not quite so accurate, allows the liquid itself to form the prism. A small cell of triangular cross-section is constructed from a microscope slide and two pieces of a cover-slip, as shown in section in Fig. 14. The deviation of the light beam, by a prism formed by the liquid itself, is then measured by noting the apparent distance between the actual and observed positions of a line drawn under the liquid cell, and extending beyond one side of it.

Apart from the microscopic methods for refractive index, some of the recognised methods for determining refractive index instrumentally, such as, for instance, the use of the Abbe refractometer, may legitimately be included as micro methods, since this latter will determine the value on 50 to 100 cu.mm. of liquid with an accuracy within ±0·0001. However, refractometers have been devised to use even smaller amounts, down to 0·1 to 5 cu.mm. The basis of a number of these is the Jelley refractometer,[26] of which several commercial models were available before the war.

An instrument with similar principles, which can be made fairly easily in the laboratory, has been described by Edwards and Otto.[27] A cell to contain the liquid is formed by bevelling the edge of a cover-slip—actually a bundle of cover-slips is bevelled, and a perfect one selected and cut into three—and cementing the slip to a slide (Fig. 15). An illuminated slit, observed through the prism formed by the liquid (Fig. 16) is apparently deviated along a scale which has been constructed with reference to several standard liquids over a range of 1·3 to 1·85. The accuracy of the instrument is within ±0·3 per cent., and as little as 0·001 ml. of liquid is required for a determination. Although the accuracy is not so great as by the method of real and apparent depths, the apparatus has the advantage in the amount of liquid used.

A similar cell is incorporated in an eyepiece described by Frediani.[28] It is claimed to give an accuracy within 0·002 unit over the range 1·3 to 1·9. By incorporating a heating unit in the eyepiece, and placing a few crystals of solid in the cell instead of a drop of liquid,

the refractive index may be measured at the melting point. If the material is not very volatile, 2 mg. suffice for the double determination.

Finally, yet another principle will be found in the method of Pfund,[29] first applied to the determination of the constant for solid rods of glass, but capable of a simple extension to liquids. The liquid is placed in a glass capillary, which is immersed coaxially in a second tube containing a standard liquid, as is indicated diagrammatically in Fig. 17. The two filled tubes thus form a sort of lens system. Behind them is an inclined wire, and the slope of the centre part of the wire, as viewed through the system, is measured. The accuracy is within about 0·1 to 0·2 per cent., and the outstanding advantage of the method is that there is no upper limit to the refractive index which can be measured.

This survey of the field with respect to three properties will show that the measurement of physical constants on the micro scale is not an inordinately difficult process. I would stress, above all, that the survey is by no means exhaustive, and that it has rather endeavoured to be representative of the different ways of attacking one problem which have been devised by different minds.

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Micro-Filtration—A Review

BY G. H. WYATT

(*Read at a Meeting of the Microchemistry Group at Newcastle-on-Tyne, September 14th*, 1945)

THE operation of filtering a solution is so common in the chemical laboratory that one readily passes it by without consideration, except in those instances where a result is required urgently and its achievement seems to be postponed indefinitely by a choked filter. Yet it is in filtration that microchemical methods gain part of their increased speed compared with the classical ones; there is a comparatively small bulk to filter and the process is more rapid, even though the area of the filter itself is also reduced. Moreover, suitable changes in the technique of

filtration are generally made, which accentuate this difference. In qualitative microanalysis and in preparative microchemistry filtration may be avoided more frequently than in macrochemistry; for example, the separation of solution from a precipitate on the microscope slide, the withdrawal of mother liquor from crystals by means of a capillary, and the similar removal of solution from a precipitate after centrifuging. These procedures really correspond with large-scale decantation, and attention is drawn to their usefulness, although they cannot properly be regarded as filtrations.

Gas Filtration

It is frequently necessary to provide a current of dust-free air for accelerated evaporation or for stirring a liquid by a stream of bubbles. The air is most conveniently filtered by passing it through a plug of cotton-wool (Fig. 1A), as recommended by Pregl[1]; the cotton-wool may be supported on a sintered glass disc[2] if so desired. Hecht and Donau[2] also suggest using a sintered glass filter of porosity grade 3 (Fig. 1B), through which the air stream passes from

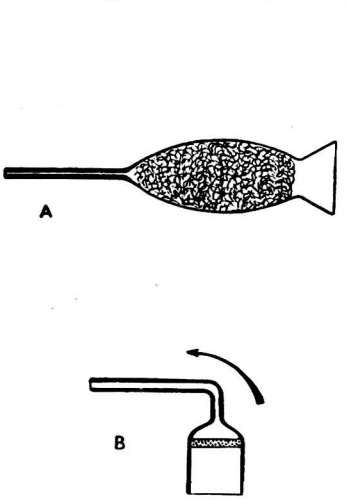

Fig. 1

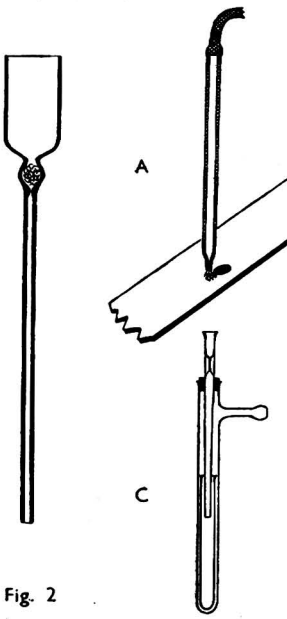

Fig. 2

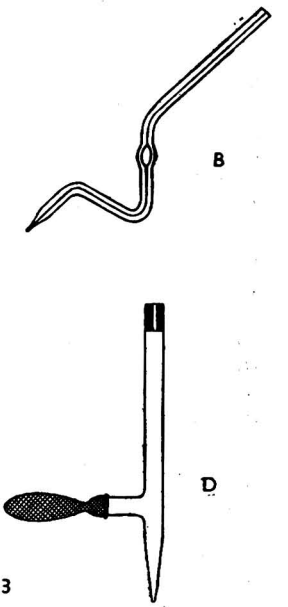

Fig. 3

below. When cotton-wool is used as the filtering medium, care should be taken that channelling does not occur; the writer has known calcium chloride dust to be carried through a 1-inch layer. It should also be remembered that the pad may yield a dust of very short fibres. Glass wool is readily disintegrated and is more difficult to pack into a tube without formation of channels, but once a satisfactory filter has been made it remains comparatively unchanged under moderate conditions.

Before leaving this brief account of gas filtration attention is drawn to the special purpose filters devised by Briscoe and Matthews.[3] These are pads of volatile solids, *e.g.*, benzoic acid, naphthalene or anthracene, or soluble solids such as salicylic acid, contained in stainless steel wire gauze within ebonite holders. They were used for collecting airborne dust, which was isolated for microanalysis by volatilisation of the filter medium or by its solution in absolute alcohol. Avy and Raillères[4] prefer to use tetrachloronaphthalene, dissolving it in benzene; if it is necessary to avoid organic solvents the filter may be made of sucrose and dissolved in water.[5] A summary of this work has been given by Matthews.[6]

Filtration of Liquids

The materials available for liquid filtration include those mentioned above but, owing to changed conditions, other criteria of their usefulness must also be considered.

Glass wool has been used for macro-filtration since 1878,[7] but it finds little application in microanalysis. Clemence[8] used cotton-wool in a macro-filter in 1887; in microchemistry it is very convenient, for example, in Pregl's[1] "micro-funnel" (Fig. 2), which may be made by

drawing down a test tube. This filter provides rapid clarification of a solution when the suspended matter is not required for further examination or weighing. It may be used with gentle suction. A cotton-wool pledget is easily pushed into the bulb and is removed by twisting on to a roughened wire.

Without doubt, paper is the most widely used filtering material; it is universally available and so cheap that it may be used once and discarded, in contrast with sintered glass, porcelain and platinum sponge. However, it suffers from important limitations where quantitative work is concerned. Fibres are liable to become detached, from both pulp and sheet, and then pass into the filtrate; they may be included in the weight of a precipitate subsequently formed. Moreover, apart from loss of fibres, it is very difficult to obtain reproducible weights of pieces of filter paper owing to marked dependence upon humidity and drying methods. This trouble also occurs on the macro scale and standardised treatment of the filter and a "blank" was suggested by Rüdorff[9] as early as 1890 (see also Smith[10]); similar procedure has been described[11] for microanalysis, but, in the writer's opinion, it is better to avoid the weighing of filter paper. An interesting feature of paper filtration is the capillary separation which occurs and which finds important application in spot test technique. This, however, is a subject on which many papers have been published and it cannot be discussed here; a good account, with literature references, is given by Feigl.[12]

Another fibrous material finding wide application for filtration is asbestos—first described in macro-analysis by Gooch.[13] Tendency to loss of fibre from a well prepared mat is negligible and there is no difficulty in drying the material to constant weight. It has the additional advantage that it may be ignited and that the weight then remains constant (volatile matter is, of course, lost during the first ignition). However, ignited asbestos is brittle and cannot be handled satisfactorily, so that it is usual to make the filter pad before ignition. The outstanding difficulty with asbestos is to free it from soluble material which would otherwise be leached out during the filtration and subsequent washing of the precipitate, with consequent change in weight of the filter. It is customary to use for microanalysis the so-called "Gooch asbestos," the coarser pieces being rubbed down. It is usually sufficient to digest this material with hydrochloric acid and then to wash free from chloride by water; occasionally an alkali digestion is also necessary. After the filter has been prepared, however, it should invariably be treated with the reagents and solvents to be used in the analysis; alternate treatment and drying should be continued until the filter has attained a constant weight. In some analytical procedures prolonged pre-treatment of the filter is required before this condition is acquired, *e.g.*, see Drew, Tress and Wyatt.[14] Some samples of asbestos are unsuitable for use in filters because of decomposition by hydrochloric and sulphuric acids.

Filters employing the above materials are of temporary character, but there are also permanent forms constructed from sintered glass[15,16] and quartz, unglazed porcelain[17] and alundum (fused alumina), and from platinum sponge. Briscoe and Lowe[18] have described the laboratory construction of sintered glassware but standard items may be purchased from laboratory furnishers and special apparatus may be built up from sintered glass discs. Accounts have also been published describing how to seal unglazed porcelain[19] or alundum[20-22] into glass to form filters; for the laboratory production of porcelain filters see Sweeney and Quam.[23] Glass and porcelain suffer from the disadvantage that they are attacked by alkaline solutions; after being weighed they should not come into contact with even dilute caustic alkali or ammonia, and more concentrated solutions of these reagents will change the filtering characteristics by increasing the pore size. Quartz filters are said[24] to be attacked inappreciably by alkaline solutions. It is better to confine a given filter to one kind of precipitate if possible; the bulk of the latter may then be dissolved in an appropriate solvent. A slow acting glass filter may be improved by the careful use of diluted hydrofluoric acid,[25,26] and choked porcelain often improves after ignition, but a time comes when both glass and porcelain filters become too slow for further use. Platinum sponge has the advantage that when the pad is spoilt in this manner it may be replaced without undue cost, since the precious metal retains its value as scrap and the main body of the filter is retained. The sponge may be prepared[27] as required by ignition of ammonium chloroplatinate crystals or platinum-iridium sponge may be purchased; in either case the filter is acid- and alkali-resistant and may be ignited gently without loss in weight.

The remainder of this paper describes the various forms of apparatus proposed to incorporate the above filtering media. For convenience it is divided into two sections: filters for qualitative and quantitative procedures respectively.

Qualitative filtration—Filtration on the microscope slide[28,29] may be effected by placing at the edge of the drop a small pledget of paper pulp on which rests the tip of a simple pipette (Fig. 3A); gentle suction results in the collection of the filtrate in the pipette. This method is attributed (without a reference) by Emich[30] to Hemmes.[31] A small piece of filter paper is used in a similar manner by Johnstone,[32] who designed a capillary pipette (Fig. 3B) to fill automatically by the action of surface tension. Other workers prefer to transfer the solution to a vertical filter in the conventional manner, for example, Feigl[28] recommends an Emich filter-stick (see p. 127) inverted and used in conjunction with a suction tube (Fig. 3C). A special filter was designed by Caley[33] in which the solution and precipitate are placed on a disc of filter paper placed over a short capillary tube at the top (Fig. 3D); the jet is closed and the solution sucked through the paper by means of a rubber teat, it may then be transferred to another vessel *via* the jet.

When larger volumes (up to about 1 ml.) are to be filtered, a wide range of filters is available. Possibly the simplest is Pregl's cotton-wool filter tube mentioned above (Fig. 2). This resembles the macro-filter suggested by Gibbs and Taylor[34] in 1867, but on the classical scale of working the filter tube has not become so popular. A similar micro form is Strzyzowski's funnel[35] (Fig. 4A), in which a small pad of asbestos fibre is supported upon a

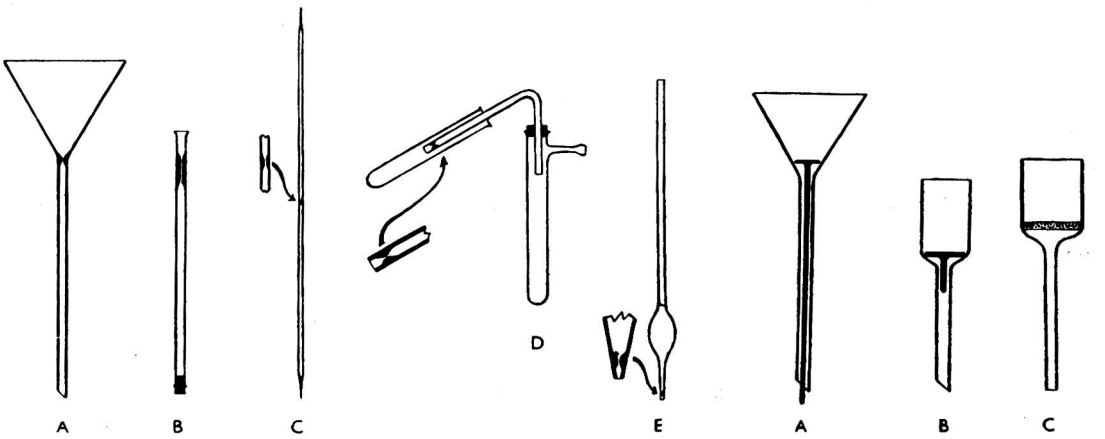

Fig. 4

Fig. 5

constriction in the stem. This method of supporting an asbestos filter has been widely applied, notably in Pregl's centrifuge filter[1,36] (Fig. 4B) and in Emich's sealed capillaries[30] (Fig. 4C), which are also used in conjunction with a centrifuge. The constriction has also been employed by Emich (*loc. cit.*) at the end of a tube connected with a suction vessel (Fig. 4D), and he recommends that the asbestos plug should be partly fused into the glass to secure it in position. A somewhat similar device is the filter pipette described by Wilson[37] (Fig. 4E), in which suction is applied by means of a rubber teat. Wilson also suggested that a number of filtering tips may be ground to fit a single pipette and that the asbestos may be replaced by a sintered glass mat.

A simple form of gravity filter is the Willstätter "nail" type (Fig. 5A), described by Palfray and Chanoine[38] and by Erdös and László[39] and attributed by the latter (without a reference) to Diepholder. The "nail" is made from glass rod and supports a pad of filter paper pulp or asbestos, or a disc of filter paper having a diameter somewhat greater than that of the head of the nail, so that its upturned edge fits against the side of the funnel. A short-stem glass "nail" was also used in the filter of Craig and Post[40,41] (Fig. 5B), but in this case the edge of the nail is ground to fit the glass funnel and the ground joint serves as the filtering medium. Sintered glass has also been applied to micro-filtration (see further below), for example, the small funnel (Fig. 5C) used by Erdös and László.[39] Sintered glass and unglazed porcelain have also been incorporated in apparatus designed for centrifuging,[42-45] and other rather elaborate devices have also been proposed for the centrifuge technique.[46-48] Attention may be drawn at this point to the rapid method of quantitative analysis by centrifuging a precipitate in a narrow tube and measuring its depth.

The filter shown in Fig. 6A is particularly useful in micro- and semi-micro-preparations. It has been recommended by Pregl[1] and Emich,[30] who both attribute it to Schwinger. It is somewhat similar to a macro-filter due to Mason[49] and consists of a small glass funnel and a glass capillary tube, the ends of both being ground flat and polished. The tubes are held in contact by a sliding rubber sleeve lubricated with glycerol; sandwiched between the polished faces is a piece of filter paper. An early form of this filter was described by Haushofer,[50] who held the glass pieces together with a clamp and applied suction through a side tube fused into the lower portion, the bottom of which was closed by a glass stopper or a rubber bung. According to Stähler,[51] a similar device was designed by Hirschwald (original literature by Schwinger and Hirschwald has not been traced). It should be noted that attempts to remove the filter paper (by tweezers) while suction is still being applied may result in vibration of the paper and scattering of the crystals or precipitate.

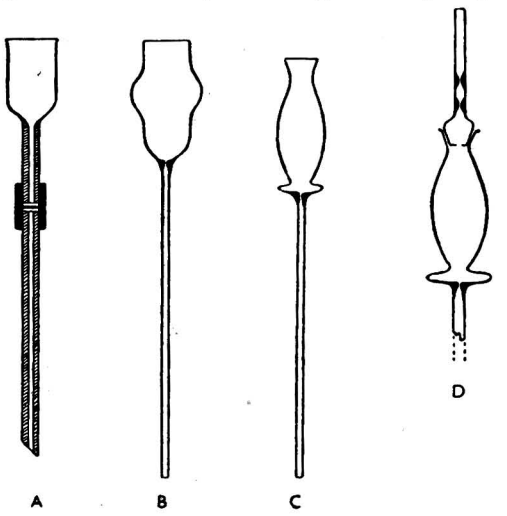

Fig. 6

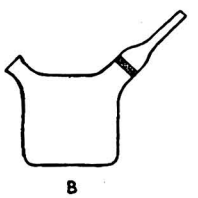

Fig. 7

Quantitative filtration—Of the filters designed for gravimetric analysis, the one most closely resembling those already described is Pregl's filter tube.[1] The early form (Fig. 6B) had a pad of asbestos supported by a small platinum spiral, a device used in a macro-filter by Drown[52] in 1891. In the later, more widely known form (Fig. 6C) Pregl (*loc. cit.*) dispensed with the spiral and formed the filter mat in a flattened bulb over a constriction in the stem. In this way swelling of the mat and its separation from the glass wall were eliminated. Drew and Porter[33] suggested the addition of a ground-in stopper with a capillary vent (Fig. 6D) to reduce access of the atmosphere to sensitive precipitates. Pregl[1] also described a filter tube in which the asbestos mat is supported upon a coarse-grained sintered glass disc fused into the tube (Fig. 6E)—*cf.* Lieb and Soltys.[54] For sintered glass filters without asbestos, see Erdös and László[39] (Fig. 5C). It may be noted that the so-called "glass Gooch" of macro-analysis was devised[15,16,55,56] in 1924 and 1925.

In the filters described above it is necessary to transfer the precipitate from another vessel on to the filter, and some loss of material may occur in the process. This is usually obviated in part by use of Pregl's feather,[1] which corresponds with the "policeman" of macro-analysis, but the transference itself is avoided in the composite vessel (Fig. 7A) designed by Kirk and Craig.[57] The precipitation is carried out in the upper vessel, after which the ground-in plug is withdrawn and the filtration performed by suction through asbestos supported on a perforated platinum disc; only the tube carrying the filter is weighed. Obviously it is essential that all of the precipitate shall be rinsed from the reaction chamber into the filter tube. Even this transference is avoided in the filter-beaker of Schwarz von Bergkampf[58]; here the precipitation occurs in the vessel itself (Fig. 7B), which is tilted to permit filtration through the sintered glass disc by suction. After washing and drying in the usual manner the whole vessel is weighed. Not only is it unnecessary to remove particles adhering to the walls of the filter-beaker, but it is a definite advantage not to have to transfer the precipitate

to the filter disc in that risk of choking of the latter is reduced. The outlet tube may be connected by a rubber sleeve to the inlet of a second filter-beaker so that a further determination may be made upon the filtrate. The filter-beaker resembles an earlier and more clumsy vessel described by Gartner[59] and has itself been modified; *e.g.*, Dworzak and Reich-Rohrwig,[60] and Dworzak and Ballczo[61] used glass stoppers when weighing volatile liquids or hygroscopic precipitates, while Hecht[62] recommends interchangeable ground glass joints. For a summary of the applications of filter-beakers see Hecht and Donau.[2]

The Gooch crucible,[13] so well known in macro-analysis, also has its micro counterpart. Probably the most commonly used micro form is the porcelain crucible with a porous, unglazed porcelain base (Fig. 8A), generally known as the "porcelain micro-Neubauer" crucible; it is provided with a small porcelain dish to protect the base from contamination. The micro-Neubauer crucible itself is made of platinum and, like the macro form,[63,64] has a layer of platinum sponge on the perforated bottom (Fig. 8B); a close-fitting platinum cap protects the latter. (For the preparation of the filter mat see Snelling.[27]) A special, smaller variation of the platinum filter crucible is the Donau dish, which is formed from thin platinum foil, using a glass rod and half-bored rubber bung as a mould (Fig. 8C). A number of

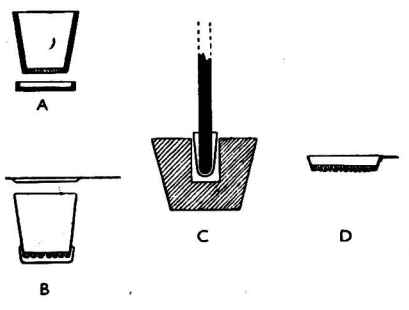

Fig. 8

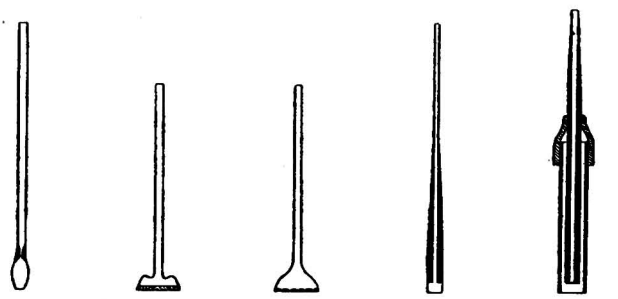

Fig. 9

fine holes is then pricked in the bottom of the dish, which is finally covered with a layer of asbestos or platinum sponge.[65-67] The vessel commonly known as the Donau dish is a later form having a wire gauze base to support the platinum sponge (Fig. 8D). Summaries of the application of this filter are given by Donau,[68] by Emich[69] and by Hecht and Donau.[2] This set of crucibles, unlike the filters in the preceding paragraphs, may be ignited. The platinum crucibles have a small heat capacity and may be weighed comparatively soon after ignition. The usual rubber gasket used during suction may be avoided by grinding the outside of a glass crucible to fit the top of the filter flask or other receiver.[70-72]

In micro-filtration the immersion filter, or "filter-stick" has assumed a leading position owing to its convenience and to the fact that there is no transference of the precipitate. It was popularised by Emich,[30] the earliest form (Fig. 9A) consisting of a glass tube with an expanded lower end, in which is placed a pad of asbestos supported upon a small platinum spiral. The preparation is described in the standard text-books[2,30,73] as well as in the original literature.[74,75] The filter was attributed by Emich (*loc. cit.*) to Stähler,[51] but a similar arrangement, for use in a filter tube, was described in 1891 by Drown.[52] Precipitation is carried out in a micro-beaker or crucible which has been tared together with the prepared filter-stick. After sucking off the solution through the filter-stick and washing, beaker (or crucible) and filter-stick are dried and weighed together. The platinum spiral may be replaced by any suitable rigid material, *e.g.*, a piece of glass or porcelain,[73,74] and Benedetti-Pichler[76] has described a special model carrying a double asbestos layer. To permit ignition of the filter and precipitate, the main body may be made from fused quartz,[74,75] and Schwarz von Bergkampf[58] suggested a sintered quartz disc fused into the base of the filter-stick. Similarly, sintered glass, unglazed porcelain (Fig. 9B) and platinum sponge (Fig. 9C) filters have been adapted to this technique. Even cotton-wool has been proposed,[77] but it seems doubtful if this would be satisfactory for quantitative work. The stream-line filter also appears as a micro filter-stick; Schwarz von Bergkampf[78] made a glass model having a cylindrical base, in which was placed a roll of filter paper and Miller[79] has described how to make an improved

form (Fig. 9D). A less satisfactory proposal consists of a straight glass tube, on the lower end of which is placed a paper cylinder resembling a small Soxhlet thimble; the original paper[80] shows how the latter may be folded from a circular paper. The most convenient method of using filter paper is in the form of a small disc cut by means of a sharpened cork-borer; these are used in the King[81] filter-stick (Fig. 9E). This consists of a glass capillary tube having a polished lower end and sliding within a glass tube to which it is attached by a sleeve of rubber tubing. The paper is placed in the small recess formed by the two tubes and it may be pushed out by lowering the capillary. In an "improved" form the inner capillary is replaced by a tube having a sintered glass disc at the lower end; it is said that a filter paper or asbestos pad is still necessary.

The technique of micro-filtration is adequately described in the text books quoted in the references, where original literature is also noted concerning transference of solution and precipitate, and suction apparatus for use with filter tubes, filter-beakers and filter-sticks,[39,58,62,65,82-89] as well as supporting stands,[61,90] forceps[90] and driers.[58,82,91-94]

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Applications of the Intermittent A.C. Arc Technique of Spectrographic Analysis

BY J. A. C. MCCLELLAND

(*Read at the meeting of the Society on February 6th*, 1946)

1. INTRODUCTION

THE intermittent A.C. arc technique is described in detail in the paper dealing with its construction and use in the analysis of zinc and zinc alloys for die-casting[1]; therefore no further description of the circuit (see Fig. 1) will be included here, beyond the statement that an Admiralty pattern quenched spark gap, as described in the Admiralty Handbook of Wireless Telegraphy, mentioned as a modification of the original circuit,[1] has been used in this work.

The ease and accuracy with which this new technique solved the problem of the determination of trace impurities in high purity zinc, and the enthusiasm for this type of circuit shown by all laboratories co-operating in that work, suggested that it was capable of a wider application. In fact in some laboratories it has already taken its place as a permanent piece of spectrographic equipment. The authors in the first account of work carried out by means of this mode of excitation suggested that it might be applicable to the analysis of materials not obtainable in metallic form, and following on this suggestion, the results now described have been obtained.

The high sensitivity of the intermittent A.C. arc raised the possibility that certain specific determinations common in some laboratories could be solved by this means; for example, the determination of lead present in tinned food to the extent of 1 part per million. Further, this technique, in view of its precision, was considered likely to replace with advantage the more common D.C. arc form of excitation which has been found in this laboratory to give only poor reproducibility.

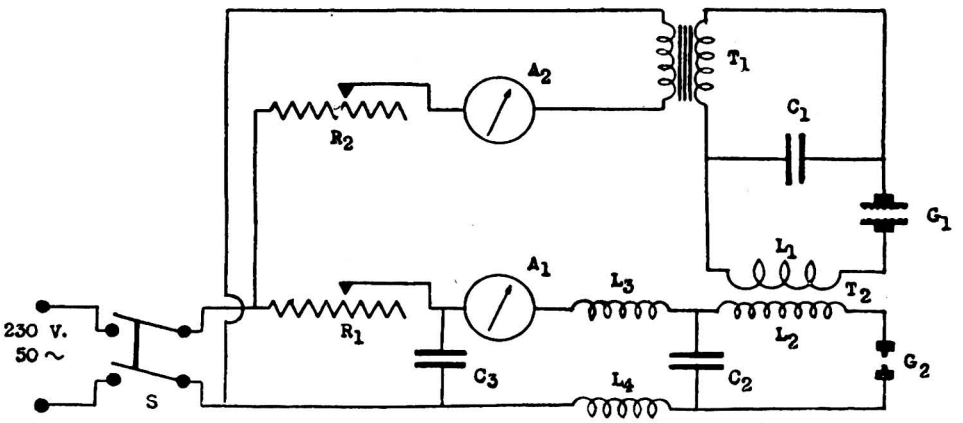

Fig. 1. Circuit diagram.

T_1 = High voltage transformer, 200-5000 V.
T_2 = Tesla coil.
C_1 = 0·005μ F.
C_2 = 0·01μ F.
C_3 = 48μ F.
G_1 = Auxiliary spark gap. Admiralty pattern 8310.
G_2 = Analysis spark gap.
L_3, L_4 = Inductances.

The accuracy claimed for types of A.C. arc which are widely used in the United States gave an indication that the intermittent arc would give better results. In particular, Hess, Owens and Rheinhardt,[2] working with a high voltage A.C. arc, obtained a high degree of sensitivity and reproducibility in the determination of several elements in the ashed residues of organic and biological materials.

The broad principles of the technique, which these workers have adapted from that of Forster, Langstroth and McRae,[3, 4] in the preparation of the electrodes and the use of a spectrographic buffer (see below), have been followed in the present work. Their method has now been modified for use with the different type of excitation circuit that has been developed. Hess, Owens and Rheinhardt give details for the determination of several elements in concentrations from 0·0001% to 0·02% and they mention the possibility of their method being applicable to larger concentrations. The scope of the method has been considerably broadened in the work described here in that, not only are analyses carried out in this range of concentration, but details are also given for the determination in one operation of higher percentages of about a dozen elements, using one internal control element, in such samples as inorganic residues of organic materials, inorganic powders, precipitates, and aqueous extracts.

2. Apparatus and Details of Technique

To make use of the intermittent A.C. arc in replacing the more common D.C. arc the major problem involved was the production of electrodes which would, as far as possible, simulate those of a metal or alloy.

The first method described in this account, Method A, is chiefly useful in determining small quantities of one or two elements in a residue or ash of a material, the matrix of which

has been largely removed. The second, Method B, is designed for the determination of a large number of minor constituents (from about 0·02 to 2%) in a variety of inorganic salts and precipitates; in fact it is intended largely to replace the D.C. arc mode of excitation when precision is required.

2A. METHOD A—This method has been particularly applied to the determination of lead and tin in various samples and of chromium in filter pads used for the sampling of air in the neighbourhood of chromium plating baths, but it is not suggested that its applicability is limited to these three elements. Provided that the material, or the relevant elements in the material, may be brought into solution with nitric acid in such a manner that large quantities of other elements are absent, and that a sufficiently sensitive line of the element is present in the region of the spectrum free from excessive background, the method should be applicable to a wide variety of elements.

The normal treatment of samples examined usually results in a concentration of the elements to be determined spectrographically; thus, foodstuffs for lead and tin are wet-ashed, waters are evaporated, and cotton wool filter pads are ashed.

When organic matter has to be destroyed, a dry ashing may suffice, but a slight loss of lead occurs in the dry ashing of cotton wool filter plugs unless the cotton wool is first moistened thoroughly with dilute sulphuric acid. Therefore, wet combustion is preferable, provided perchloric acid is used instead of sulphuric acid; the latter is not easily removed, and moreover, is liable to give trouble owing to the insolubility of the resulting sulphates. After evaporation to dryness with perchloric acid, the residue is dried again with nitric acid containing a standard quantity of a spectrographic buffer. The spectrographic buffer is added for the following reasons: (*a*) to facilitate the collection of the small quantity of ash, (*b*) to ensure that an even coating of solid is applied to the electrode, (*c*) to eliminate the effect of minor variations in the composition of the material under investigation, and (*d*) to stabilise and assist as far as possible the excitation. For this purpose a 10% solution of sodium nitrate is used. The dry residue is then dissolved in a solution containing the internal control element.

When the greatest sensitivity is required the final volume should of course be as small as possible. It has been found inconvenient to work with smaller volumes than 1 ml., and in this volume 1 μg. of lead may be determined. The sensitivity ultimately obtained in percentage of the element present in the original sample will depend generally on the quantity of sample taken for analysis; for instance, if the analysis of a sample of water is required, it is quite simple to evaporate a litre or more to dryness. If this residue is finally made up to 1 ml., 1 μg. of lead may be determined in the original litre of water, which is equivalent to one part in 10^9. Similarly in the examination of the atmosphere for contamination by lead, 1 μg. of lead may be determined in a cotton wool filter, the lead having been collected from the volume of air that has passed through the filter; if therefore the passage of 1 cu.ft. of air is insufficient to record the presence of lead, it is possible, though perhaps inconvenient, to pass a larger volume, *e.g.*, 10 cu.ft., through the filter. On the other hand, it is not practicable in the laboratory to wet-ash large quantities of tinned foodstuffs, the usual quantity taken being 1–10 g. The time occupied in wet ashing 10 g. is considerable, and 1 g. is adequate for testing to a lower limit of 1 p.p.m. of lead.

From the foregoing it will be obvious that when larger amounts of the element under investigation are present an aliquot proportion of the material should be used.

Having thus obtained a solution containing the ashed material, the spectrographic buffer, and the internal control element, electrodes are prepared for spectrographic analysis as follows. Supporting electrodes of graphite about 1·5 cm. long are turned on a lathe to give a depression of approximately hemispherical shape and 1·5 mm. depth. The surface of the crater is polished with a piece of stiff paper while the electrode is still in the lathe. This prevents the solution penetrating the electrode. A drop of solution is placed in the crater by means of a suitable dropping pipette. (With practice the drops can be controlled to be of approximately constant volume.) The drop of solution on the electrode is evaporated to dryness as rapidly as possible by indirect heat, the electrode being held in a pair of tongs and the latter heated in a flame; care is necessary to prevent the liquid running down the sides of the electrode. This procedure gives a smooth, firmly adhering, crust of the salt. Both electrodes are thus treated. Fresh electrodes are used for each exposure, but the unused end of a once used electrode may be employed provided that care has been taken to avoid contamination.

Experimental Details—Standard conditions for analysis to a lower limit of 1 μg. of lead per ml.

Analysis solution: The dry residue, after wet combustion or ashing, is dissolved in 1 ml. of a 10% solution of sodium nitrate in 2 *N* nitric acid and evaporated to dryness. The residue is dissolved in 1 ml. of a solution containing 0·05 g. of H.S. bismuth per litre of 2 *N* nitric acid.

Electrodes: H.S. graphite rods 6·5 mm. diameter, 1·5 cm. long, are turned and polished on a lathe to give a crater 1·5 mm. deep. These are treated with 1 drop of test solution which is then evaporated to dryness.

Standard Solutions: The standard solutions should be made up to contain approximately the equivalent quantity of a pure salt or mixture of salts which constitute the major constituent of the ash, together with the same quantity of the buffer solution and varying amounts of lead (or other elements that are being determined) to give an appropriate range of standards. The standard solutions are evaporated to dryness and dissolved in 1 ml. of standard bismuth solution. These solutions should cover the range of sensitivity of the elements under examination; in the case of lead, tin and chromium these are 1 to 25 μg./ml. for lead, 5 to 50 μg./ml. for tin and 1 to 25 μg./ml. for chromium.

Spectrograph: The medium quartz instrument with the appropriate condensing lens is used. (The large quartz instrument may be used with a slight variation in the details of setting, etc.)

Distance of source from slit: 38 cm.

Slit width: 0·0175 mm. (7 divisions).

Electrode separation: 4 mm. preferably set by optical means.

Plate: Ilford Special Rapid. Developed 4 min. in I.D.2 at 65° F.

Exposure: 1 min. (no preliminary).

Microphotometer slit width: 2 mm.

Recommended line pairs: Pb 2833·1A - Bi 2898·0A
Sn 2863·3A - Bi 2898·0A
Cr 2835·6A - Bi 2898·0A.

The line Fe 2832·4A does not interfere with Pb 2833·1A unless gross amounts of iron are present. When this is the case it will be necessary either to remove the iron or to use the large spectrograph.

2B. METHOD B—Further experiments, designed to apply the previous technique to a greater variety of materials, have been carried out, and this second method is applicable to most elements that can be brought into solution by treatment with nitric acid. It has been developed to include as large a number of elements as possible in ranges of concentration from 0·01–0·05% to about 2%. The procedure is similar to that of the previous method in that a solution of sodium nitrate in nitric acid is used as the spectrographic buffer solution, and, after evaporation to dryness, the residue is dissolved in a solution of the internal control element, which in this case is iron.

The choice of iron for the internal control element was made owing to the necessity of having a large number of lines, well distributed in the spectrum and of suitable density, for comparison by the internal standard method. Furthermore, small quantities of iron are common in most materials likely to be examined, and it is an element which may usually be determined rapidly and accurately by chemical means. By the use of the iron lines a method of plate calibration could be devised which would be useful when large numbers of similar materials are examined.

It is recommended that standard solutions used for comparison should be of approximately the same composition as the samples under investigation with respect to major constituents.

The preparation of the sample for spectrographic analysis may necessitate some chemical treatment in order that it may be brought into solution in nitric acid; *e.g.*, large quantities of silica may be removed by hydrofluoric acid. This requirement does to some extent limit the applicability of the method to materials which can be brought into solution, or homogeneous suspension, in dilute acid.

The concentration ranges of the elements determined (see below) are governed by the sensitivity of the available lines of the element in question in the intermittent A.C. arc discharge. A standardised technique is outlined for concentrations of elements most likely to be of use in analytical chemistry, particularly in laboratories dealing with a wide range of different materials.

The details for preparing the electrodes and carrying out the spectrographic process are similar to those outlined for method A, but it is much more important in this method to endeavour as far as possible to obtain exposures of constant density throughout the plate. The most significant adjustment, controlling variations in exposure between samples, is the electrode separation, and the care with which this setting should be carried out cannot be over-emphasised if good reproducibility is required. The necessity of maintaining the exposure as constant as possible is due to the fact that, with some elements, exposure drift is very marked; that is, if large variations in exposure have occurred, the ratio D_{line}/D_{Fe} will vary according to the density of the iron line. This causes serious errors with a few elements only.

By this method the following elements have been determined in synthetic mixtures with the limits of accuracy referred to in a later section: Mg, Mn, Pb, Sn, Cr, Al, V, Mo, Cd, Cu, Zn, Co, Ni, Sr.

The method is also further limited to elements whose spectral lines are of adequate sensitivity in the region of the spectrum employed, this region being that which is free from the spectrum background caused by the graphite arc, *i.e.*, that of wave length less than 3500A.

The following common elements have not been successfully determined by this technique for the reasons given.

Ba No sufficiently sensitive lines are present in the spectrum below 3500A unless the Ba content is greater than 2%.

Ca No sufficiently sensitive lines are present in the spectrum below 3500A. (The Lundegårdh technique is eminently satisfactory.)

W, Ag } These elements are not suitable for the solution technique owing to precipitation of their salts.

Ti, Si } These elements are not suitable for the solution technique and also are often present in small amounts in the graphite electrodes.

Fe This element is used as the internal control element.

Experimental Details

Analysis solution: 0·02 g. of substance is evaporated to dryness with nitric acid and dissolved in 0·5 ml. of a 10% solution of sodium nitrate in 2 *N* nitric acid; this solution is re-evaporated and the residue redissolved in 1 ml. of a solution containing 1 g. H.S. Fe per 100 ml. of 2 *N* nitric acid.

Electrodes: As for Method A.

Standard solutions: Three series of standard solutions are prepared from mixtures of salts containing varying proportions of the elements under investigation, each being of such strength that 1 ml. of the final solution containing the internal control element, after addition of the spectrographic buffer, contains 0·02 g. of the solid mixture. Each of the first two series comprises elements which do not mutually interfere; the third series, which is prepared by mixing equal quantities of the first and second series, may be used for samples containing elements of both series except where overlapping of spectral lines occurs. The first series is composed of the elements Mg, Mn, Pb, Sn, Cr, Al, Zn, Ni, and Sr, and the second of Mg, V, Mo, Cd, Co, and Cu. Five standards are prepared of each series, of such strengths as to cover the ranges of sensitivity governed by the quantity of an element that will produce a line of sufficient density on the one hand, and not too intense on the other, for microphotometric measurement. Four standards are prepared of the third series. The standard solutions are evaporated to dryness after addition of an equivalent quantity of the spectrographic buffer and dissolved in 1 ml. of the standard iron solution.

Spectrograph: Large quartz. Setting 2550–4000A. No lens used.

Distance of source from slit: 35 cm.

Slit width: 0·01 mm. 1 division.

Electrode separation: 4 mm. Preferably set by optical means.

Plate: Ilford Isozenith. Developed 5½ mins. in I.D.2 at 65° F.

Exposure: 90 sec. (no preliminary). (Note: correct exposure should give a density for the iron line 3091A of 0·5–0·7.)

Microphotometer slit width: 0·2 mm.

Recommended line pairs:

Mg 2802·7A - Fe 2804·5A	For Mg less than 0·2%.
Mg 2779·9A - Fe 2804·5A	For Mg greater than 0·1%. Masked by Sn.
Mn 2798·3A - Fe 2804·5A	
Pb 2833·1A - Fe 2845·6A	
Sn 2840·0A - Fe 2845·6A	
Cr 2849·8A - Fe 2845·6A	
Al 3082·2A - Fe 3091·6A	Masked by V.
V 3110·7A - Fe 3091·6A	Recommended for V greater than 0·25%.
V 3185·4A - Fe 3091·6A	Recommended for V less than 0·5%.
Mo 3158·2A - Fe 3091·6A	
Cd 3261·1A - Fe 3254·4A	
Zn 3345·0A - Fe 3450·3A	Mo 3344·75A is only just separated from Zn 3345·0A.
Co 3405·1A - Fe 3450·3A	
Ni 3461·7A - Fe 3450·3A	
Sr 3464·5A - Fe 3450·3A	
Cu 3274·0A - Fe 3286·8A	This is only satisfactory for very low percentages of copper. A better procedure for copper concentrations between 0·03% and 0·5% is to use a shorter exposure time or alternatively to use a special rapid plate instead of the isozenith and to expose separately for copper.

3. Results

3A. Method A—In the determination of lead, tin and chromium the lowest limits of detectability are 1 μg., 5 μg. and 1 μg. per ml. respectively, using the lines listed above. The tin line 3175A is slightly more sensitive but is not so conveniently situated in the spectrum. With concentrations greater than 25 μg. per ml. of Pb, 50 μg. per ml. of Sn and 25 μg. per ml. of Cr the densities of the lines of these elements become too intense for convenient measurement on the microphotometer and, if determination of higher percentages is required, dilution will be necessary.

By means of this technique, chromium has been determined in cases of suspected atmospheric pollution by compounds of that element; the contaminated air is drawn through a cotton wool filter and the chromium determined in the residue of the filter pad after ashing. As an example of the effect of other constituents in the determination of chromium the following example is cited. Two results are given, one obtained from standards made up without the residue from an equivalent amount of chromium-free cotton wool and the other in which the equivalent amount of cotton wool was used in the preparation of the standard solutions.

	Using standards without cotton wool	Using standards with cotton wool
Sample 1	2 μg. Cr/ml.	2·3 μg. Cr/ml.
„ 2	1·8 „	2·3 „

This difference is due to a variation of the major constituent and, though present, is shown to be quite small.

This method has also been used for the determination of 1 to 20 parts of lead per million in food products such as tinned meat, tinned soup and processed cheese; of 10 μg. of lead per litre and 20 μg. of tin per litre in distilled water; of 0·005–0·03% of lead in roofing felt; and of 1 μg. to 25 μg. of lead per cubic foot of air in cotton wool filters from atmospheric tests.

The following table gives an example of the determination of lead and tin.

Determination of Lead			Determination of Tin		
Pb added*	Ratio D_{Pb}/D_{Bi}	Pb found from curve R/Concn.	Sn added*	Ratio D_{Sn}/D_{Bi}	Sn found from curve R/Concn.
2·0	1·55	2·2	5·0	1·84	5·5
	1·475	1·9		1·89	5·0
5·0	0·91	5·0	10·0	1·37	9·5
	0·91	5·0		1·48	9·0
10·0	0·53	9·8	20·0	0·73	20·0
	0·47	11·1		0·715	20·5
16·0	0·345	16·2	36·0	0·41	36·0
	0·35	15·9		0·41	36·0
25·0	0·24	23·5	50·0	0·32	50·2
	0·23	24·3		0·325	50·0

* Concentrations expressed in μg./ml.

3B. METHOD B—The second method has been studied with the object of obtaining the widest possible applicability, and provided that interference of the lines of one element by the close proximity of those of another, as indicated in the previous section, does not take place, or that steps can be taken to overcome it, some fourteen elements can be determined in one operation.

Of the elements tested, the best results have been obtained for manganese, lead, tin, aluminium, cobalt, nickel and strontium and satisfactory results for magnesium, chromium, vanadium, molybdenum and cadmium; for zinc and copper the problem has not been quite so successfully solved owing to the low sensitivity of zinc and the exceptionally high sensitivity of the two persistent lines of copper. An example of the use of this technique, when confirmatory chemical analysis has been carried out, is given below. The analysis was made on an acid extract of a soil suspected of causing the death of birds. The chemical analysis was performed by routine methods and no attempt was made to obtain a greater accuracy chemically than the problem warranted.

	Spectrographic %	Chemical %
Lead	0·07	0·06
Copper	0·02(5)	0·03
Zinc ..	less than 0·1	0·07

Examples are given below of determinations carried out with the first two series of mixtures referred to in the preceding section, the first example is given for the determination of Mg, Mn, Pb, Sn, Cr, Al, Zn, Ni and Sr; the second for Mg, V, Mo, Cd and Co.

In order to obtain the greatest percentage range possible, it was found that, at the lower percentages of some of the elements, the line of the element was only just visible on the plate. The precision of the results, which can be roughly assessed from the figures given below, will be somewhat inferior at the lower percentages than at intermediate values. Curves of Ratio against % are, however, as a rule, steep at low concentrations and fairly wide variations of ratio do not grossly affect the percentage of elements as determined from the graph.

A detailed study of reproducibility is dealt with in the next section.

1ST SERIES

% Mg taken	Ratio D_{Mg}/D_{Fe}	% Mg from curve	% Mn taken	Ratio D_{Mn}/D_{Fe}	% Mn from curve	% Pb taken	Ratio D_{Pb}/D_{Fe}	% Pb from curve
0·012	1·77	0·009	0·03	1·75	0·03	0·03	1·53	0·03
	1·67	0·013		1·72	0·03		1·50	0·03
0·025	1·45	0·021	0·062	1·26	0·065	0·062	1·30	0·060
	1·25	0·029		1·275	0·062		1·28	0·064
0·05	0·92	0·049	0·125	0·815	0·127	0·125	1·01	0·128
	0·885	0·051		0·84	0·122		1·03	0·120
0·1	0·55	0·094	0·25	0·56	0·257	0·25	0·765	0·255
	0·485	0·112		0·575	0·242		0·77	0·250
0·2	0·345	0·196	0·5	0·455	0·45	0·5	0·48	0·50
	0·335	0·202		0·43	0·56		0·485	0·505

% Sn taken	Ratio D_{Sn}/D_{Fe}	% Sn from curve	% Cr taken	Ratio D_{Cr}/D_{Fe}	% Cr from curve	% Al taken	Ratio D_{Al}/D_{Fe}	% Al from curve
0·062	1·45	0·06	0·075	1·48	0·075	0·062	2·88	0·06
	1·435	0·07		1·48	0·075		3·02	0·05
0·125	1·17	0·125	0·15	1·25	0·15	0·125	2·51	0·125
	1·17	0·125		1·25	0·15		2·48	0·130
0·25	0·89	0·25	0·3	0·99	0·30	0·25	1·76	0·26
	0·895	0·25		0·99	0·30		1·84	0·24
0·5	0·56	0·51	0·6	0·68	0·56	0·5	1·11	0·50
	0·57	0·50		0·66	0·62		1·11	0·50
1·0	0·365	1·00	1·2	0·475	1·05	1·0	0·705	1·00
	0·36	1·02		0·425	1·34		0·70	1·05

% Zn taken	Ratio D_{Zn}/D_{Fe}	% Zn from curve	% Ni taken	Ratio D_{Ni}/D_{Fe}	% Ni from curve	% Sr taken	Ratio D_{Sr}/D_{Fe}	% Sr from curve
0·125	—	—	0·062	1·44	0·07	0·125	1·405	0·125
				1·50	0·05		1·44	0·120
0·25	1·51	0·195	0·125	1·19	0·125	0·25	1·225	0·21
	*1·42	0·26		1·19	0·125		1·13	0·27
0·5	1·17	0·53	0·25	0·865	0·25	0·5	0·85	0·50
	1·195	0·50		0·865	0·25		0·85	0·50
1·0	0·875	1·03	0·5	0·50	0·51	1·0	0·56	1·01
	0·91	0·97		0·51	0·50		0·565	1·00
2·0	0·53	2·10	1·0	0·305	0·97	2·0	0·305	2·07
	0·57	1·92		0·295	1·03		0·32	1·97

2ND SERIES

% Mg taken	Ratio D^{Mg}/D^{Fe}	% Mg from curve	% V taken	Ratio D_V/D_{Fe}	% V from curve	% Mo taken	Ratio D_{Mo}/D_{Fe}	% Mo from curve
0·125	2·72	0·125	0·062	3·18	0·06	0·062	2·69	0·06
	2·75	0·11		3·16	0·06		2·66	0·06
0·25	2·32	0·31	0·125	2·53	0·125	0·125	1·90	0·13
	2·37	0·28		2·67	0·11		2·03	0·115
0·5	1·87	0·45	0·25	1·65	0·24	0·25	1·49	0·23
	1·83	0·47		1·585	0·255		1·40	0·255
1·0	1·24	1·06	0·5	0·99	0·50	0·5	0·86	0·54
	1·265	1·03		1·00	0·495		0·99	0·45
2·0	0·78	2·09	1·0	0·63	0·93	1·0	0·63	0·93
	0·85	1·88		0·55	1·04		0·59	1·04

% Cd taken	Ratio D_{Cd}/D_{Fe}	% Cd from curve	% Co taken	Ratio D_{Co}/D_{Fe}	% Co from curve
0·125	1·58	0·125	0·062	1·21	0·06
	1·59	0·125		1·21	0·06
0·25	1·39	0·25	0·125	0·855	0·12
	1·39	0·25		0·815	0·13
0·5	0·985	0·49	0·25	0·525	0·25
	0·975	0·50		0·525	0·25
1·0	0·665	1·00	0·5	0·255	0·51
	0·65	1·02		0·27	0·485
2·0	0·39	1·95	1·0	0·12	1·02
	0·37	2·06		0·135	0·96

An investigation of the combination of the first and second series was carried out in order to ascertain the effect, if any, of the elements composing the one series upon those of the other. The effects of the elements of the first series upon the determination of the elements of the second series, and of the latter on the determination of the former, were not found to be such as to produce serious errors and no systematic effects were detected. Minor variations in ratio of a somewhat greater magnitude than that found in the investigation of the reproducibility of the method did sometimes occur but, as comparison was being made between two different solutions, the additional errors in the preparation of the solutions would be present which are absent in the determination of the standard deviation on a single solution.

Where, however, a line of one element is superimposed upon one of another element, results will not be reliable. Such occurrences have been recorded.

4. ACCURACY

Statistical tests have been carried out, using both method A and method B, in order to ascertain the degree of precision likely to be obtained in determinations by this process.

A rough estimate of the repeatability of the method can be made by an inspection of the

variation of duplicate exposures such as those given in the previous section, but in order to obtain more definite evidence of the reproducibility a series of repeat exposures of one standard mixture have been made on one plate, thereby obtaining a value for the maximum and standard deviation from the mean of the ratio of analysis line to internal standard line. From this the maximum and standard deviation of percentage can be obtained by reference to a typical curve.

4A. METHOD A—In order to determine the reproducibility of this method, a solution containing 5 μg. of lead per ml. and 20 μg. of tin per ml. was used. The solution did not contain any added material apart from the spectrographic buffer and the internal control element. When carrying out other determinations, on the ash of cotton wool filters for instance, small quantities of such elements as calcium, magnesium, and iron are present. Minor variations in the amounts of these elements, if they are present to approximately the same extent in standards and samples alike, would not adversely affect the reproducibility.

The reproducibility test on method A was carried out by making twenty repeat exposures of the solution of strength given above on the same plate. The following results were obtained. The standard deviation of ratio, σ_R, is expressed as $\sqrt{\frac{\Sigma d^2}{n-1}}$

Element	No. of exposures	Mean ratio	σ_R	Standard deviation, %	Maximum deviation, %	At concentration of element
Pb	20*	0·855	0·03	±6	±10	5 μg.
Sn	20	0·71	0·028	±4	± 8	20 ,,

* One exposure was obviously contaminated by lead and therefore omitted.

4B. METHOD B—The reproducibility tests on this method were carried out in a similar manner to that for Method A, namely by exposing the same standard mixture a large number of times on the same plate.

Three such tests have been performed, the first two for one sample of each of the two series of standards (series I and series II) and the third for one sample of the combined series; the last test gives values for the standard deviation which are in good agreement with those obtained on series I and series II separately.

The percentages of each element in the solution used vary according to the sensitivity of the element. Thus for the first series 22 exposures were made on a solution containing the following percentages of the elements: Mn 0·125%, Pb 0·125%, Sn 0·25%, Cr 0·3%, Al 0·25%, Zn 0·5%, Ni 0·25%, Sr 0·5%, and for the second series 22 exposures on a solution containing the following percentages of the elements: Mg 0·5%, V 0·25%, Mo 0·25%, Cd 0·5%, Co 0·25%. For the combined series fourteen exposures were made, which was considered sufficient to confirm the findings of the tests made on the two series separately.

To summarise, the detailed results of these statistical tests show that a precision of approximately ±5% can be obtained for the elements Mn, Pb, Sn, Al, Co, Ni and Sr; of approximately ±10% for Mg, Cr, V, Mo and Cd; and of ±10–20% for Zn.

The following table gives the results that have been obtained on the two series and the combined series of mixtures.

1ST SERIES

Element	No. of exposures	Mean ratio	σ_R	Standard deviation, %	Maximum deviation, %	At % of element
Mn ..	22	0·855	0·042	± 6	±12	0·125
Pb ..	22	1·025	0·037	± 7	±15	0·125
Sn ..	22	0·89	0·028	± 6	±12	0·25
Cr ..	21	0·94	0·046	±10	±15	0·30
Al ..	22	1·79	0·072	± 7	±15	0·25
Zn ..	20	1·22	0·049	±12	±20	0·50
Ni ..	22	0·84	0·026	± 5	±10	0·25
Sr ..	22	0·785	0·026	± 6	±12	0·50

2ND SERIES

Element	No. of exposures	Mean ratio	σ_R	Standard deviation, %	Maximum deviation, %	At % of element
Mg ..	22	2·28	0·140	±10	±20	0·50
V 3110.7A ..	21	1·535	0·10	±10	±20	0·25
Mo ..	22	1·385	0·085	± 8	±15	0·25
Cd ..	22	1·01	0·055	± 5	±12·5	0·50
Co ..	22	0·53	0·022	± 4	± 8	0·25

COMBINED SERIES: 1ST SERIES AND 2ND SERIES

Element	No. of exposures	Mean ratio	σ_R	Standard deviation, %	Maximum deviation, %	At % of element
Mn ..	14	0·80	0·028	± 6	±15	0·125
Pb ..	14	0·975	0·026	± 5	± 8	0·125
Sn ..	14	0·78	0·032	± 6	±10	0·25
Cr ..	14	0·99	0·045	± 7	±16	0·30
V 3185.4A ..	14	1·335	0·065	± 6	±10	0·125
Mo ..	14	1·97	0·12	±10	±16	0·125
Cd ..	14	1·305	0·055	±10	±16	0·25
Co ..	14	0·835	0·022	± 6	± 8	0·125
Ni ..	14	0·845	0·015	± 4	± 6	0·25
Sr ..	14	0·875	0·045	± 5	±12	0·50

5. CONCLUSION

The foregoing description outlines two techniques that may be used for the determination of either very small quantities of certain elements by method A or most minor impurities likely to be present in miscellaneous inorganic materials by method B. Further, it gives results within specified limits for these determinations. It is not suggested that precisely the same technique will necessarily be the best for the solution of all problems of this nature; modification of the general details outlined will suggest themselves in providing a solution to any specific problem. A slight alteration of the details described such as the choice of a different internal control element, for instance, might for a different set of circumstances produce more satisfactory results. A solution technique using the intermittent A.C. arc could be applied for the determination of minor impurities in metals and alloys in which the effects of segregation are such as to raise doubts about the efficacy of using solid electrodes. It should be noted, however, that, unless segregation or errors in sampling are likely to cause serious discrepancies in the results obtained using solid electrodes, the use of solutions involving chemical preparation, with the possibility of incidental contamination, cannot be recommended.

The suggestion originally made that the intermittent A.C. arc mode of excitation could, and indeed would, find a much wider application in spectrographic analysis than the mere determination of traces of impurities in metals is borne out in this paper. It is the experience of this laboratory that to the proved superiority of the Intermittent A.C. arc over the D.C. arc in reproducibility is added a wide applicability which should go far to make this circuit a useful addition to spectrographic equipment.

SUMMARY—The intermittent A.C. arc technique has been applied to materials in which the elements are not present in the metallic state; it was considered likely to give satisfactory results in the spectrographic analysis of samples of this nature. Two variants are described, one for very small quantities of one or two elements in simple conditions, the other for larger amounts of a number of common elements. In general, an accuracy of about ±5–10% of the proportion of the element present is attainable by either method.

I wish to thank the Government Chemist for permission to publish this work.

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DEPARTMENT OF THE GOVERNMENT CHEMIST
CLEMENT'S INN PASSAGE, STRAND, LONDON, W.C.2

November, 1945

DISCUSSION

Mr. D. M. SMITH said he was particularly interested in Dr. McClelland's paper, partly as a fellow-member of the B.S.I. panel, but more so because for the past year he also had been extending the application of the intermittent A.C. arc method. In view of the success of the method in analysing high purity zinc it had been tried and adopted for the analysis of the well-known "H.S." and "Specpure" substances for traces of impurities. Encouraging results had been obtained with metal rods, the globule (graphite arc) technique and the direct arcing of powders on graphite electrodes. The reproducibility of the results, judging by duplicate spectra, appeared to be very satisfactory in many instances, but he and his colleagues had not yet found time or opportunity for statistical tests such as those quoted by Dr. McClelland. Such tests were, of course, very desirable. The sensitivity of the method in the detection of traces had been

found to be high. He agreed with the view that modifications of the technique are necessary for the analysis of different types of material; one type of excitation source would not suffice for the many and varied types of material which they were called upon to analyse. Further modifications would no doubt be developed, but the method of analysis remained basically sound.

Mr. F. W. J. GARTON said that Dr. McClelland's use of iron as a control element was of considerable interest. In 1938 the speaker's Department published an account, in "*Spectrographic Analysis in Great Britain*" by A. C. Candler, of a D.C. arc method for the analysis of paint ashes and similar materials in the form of dry powders, using a flux consisting of equal parts of ferric sulphate and ammonium sulphate. The function of the ferric sulphate was two-fold, namely, to act as a spectrographic "buffer" and to act as a control element for line comparisons. As originally designed, the method was intended for the determination of Pb, Mn, Ni, Co and Ca only, but since then it had been extended to include some twenty or so more elements, and the accuracy had been improved by the use of a microphotometer.

In their laboratory the standard deviations obtained were of a similar order to those claimed by Dr. McClelland for his A.C. arc solution technique, the main differences lying in the fact that certain elements, *e.g.*, Pb, Sn and Al, gave better results by Dr. McClelland's method than by theirs, and other elements were better by their method. Attempts to modify their method by using A.C. arc excitation had hitherto been unsuccessful, owing to the rather disruptive nature of this type of discharge and to its considerably lower sensitivity; he would be interested to learn the results of Dr. McClelland's experiments on dry material. Comparison of the two methods of excitation for powder analysis was not at present possible, but it should be realised that in the production of a spectrum of this type of material such factors as chemical composition, and physical properties such as state of aggregation, melting point and relative volativities of the constituents (even in presence of a "buffer") may have a much larger effect on reproducibility and accuracy than the means of excitation used. Another point to which he would like to draw Dr. McClelland's attention was his use of graphite electrode. Hitherto only two types of graphite electrodes had been obtainable in this country—the extremely pure type (Hilger's Catalogue Number F.1100) costing over £1 per rod, which show no spectral impurities whatever after a short pre-arc, and the ordinary "H.S." brand graphite rods. The latter showed traces of various impurities, apparently unevenly distributed. Could Dr. McClelland say which type of electrode he used, and if he used the less pure type, had he met any interference due to this cause, bearing in mind the common experience of various workers that certain substances (more particularly the alkali metals) had the property of causing enhancement of the strengths of lines owing to impurities present in the electrodes? Lastly, could Dr. McClelland give further details of the amounts of iron, etc., added to the solution?

Dr. MCCLELLAND, in reply, said that he frequently used the D.C. arc method referred to by Mr. Garton but, though the sensitivity was as good, they at the Government Laboratory had not been able to obtain a reproducibility as satisfactory as that obtainable by the intermittent A.C. arc technique. While admitting that errors were likely to arise as a result of difference in physical properties of the sample, he was of the opinion that with samples of a similar nature the intermittent A.C. arc would give an appreciably higher reproducibility than the D.C. arc, using copper electrodes, and a substantially better reproducibility if graphite electrodes were used in the D.C. arc method. At present the technique outlined in the paper had only been fully developed for samples which could be treated in such a manner that they could be applied to the electrode in solution form. Tests had been made, using dry powders; at present the work was still in the experimental stage. It could be stated, however, that comparative tests in the analysis of TiO_2 for impurities, with both D.C. arc on copper and intermittent A.C. arc on graphite, showed the reproducibility of the latter to be definitely superior. The graphite electrodes employed were the ordinary H.S. brand. Blank tests, using buffer and internal control element only, were always performed; usually, however, the concentrations of the elements under investigation were such that the presence of traces of these elements in the electrode would not cause significant errors in the results.

Notes

NOTE ON COMMERCIAL PAPAVERINE

MANY of the colour reactions attributed to papaverine are due to impurities with which the alkaloid is often contaminated. Of particular interest is the observation of Pictet and Kramers,[1] who found that pure papaverine gave no colour with cold concentrated sulphuric acid and that the brilliant purple reaction frequently observed was due to cryptopine. The work of these authors has no doubt resulted in the appropriate monographs of the British Pharmaceutical Codex, the French Codex and the German Pharmacopoeia stating that the alkaloid and its hydrochloride give no colour with cold sulphuric acid.

During the war commercial papaverine yielding no colour with cold sulphuric acid has been almost unobtainable, and this led to an investigation in our laboratories of the cryptopine colour reaction. For this purpose some commercial papaverine was first purified by fractional crystallisation of the acid oxalate.[1] The alkaloid recovered from the salt still contained a trace of cryptopine, which was finally removed by passing a chloroform solution of the alkaloid through a column of aluminium oxide. In this way papaverine giving no colour with cold sulphuric acid was obtained.

Preliminary work with cryptopine showed that 0·01 mg. or less could be detected when treated with 1 ml. of sulphuric acid. By mixing chloroform solutions of papaverine and cryptopine and evaporating to dryness, a series of samples of known composition was prepared and 10 mg. of each was treated with 1 ml. of sulphuric acid. It was found that 0·2 per cent. of cryptopine was easily detectable or, in other words, papaverine which was 99·8% pure gave a colour with cold sulphuric acid.

It would seem that a standard requiring no colour with sulphuric acid, although satisfactory for synthetic papaverine, is too stringent for the natural product. The National Formulary VII has compromised by requiring the colour obtained, when 50 mg. of papaverine hydrochloride are dissolved in 2 ml.

of sulphuric acid, shall be no more than pale pink. A more satisfactory standard would result, however, if the colour produced were required to be less than that of a standard prepared from a stated amount of cryptopine.

Thebaine gives an orange red colour under the conditions of the test but, in our experience, the major impurity in natural papaverine is cryptopine.

I am indebted to the Directors of The Wellcome Foundation for permission to publish this note.

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THE WELLCOME CHEMICAL WORKS
DARTFORD

G. E. FOSTER
December, 1945

THE ESTIMATION OF CODEINE IN PRESENCE OF CREOSOTE

CODEINE is a common ingredient of mixtures used to alleviate coughing, and is invariably incorporated as a salt with substances of a syrupy nature. Creosote is often included on account of its expectorant properties, and this makes the estimation of the alkaloid somewhat tedious. It is thought that a method which has been applied successfully might be of general interest.

Codeine is not a phenol and is not soluble in alkalies. It is soluble in 120 parts of water, in 20 parts of ether, and readily in chloroform, and the last solvent is employed in its extraction, although sometimes a mixture of chloroform and ether is used.

The following method has been employed, chiefly with viscous liquids.

Transfer a known weight of the substance under test to a separator, using as little water as possible, and shake with an appropriate volume of 2% sodium hydroxide solution. The main bulk of the creosote, which consists largely of phenolic substances, is dissolved by combination with the alkali. Extract the alkaloid with chloroform in successive quantities of 25 ml., 15 ml. 10 ml. and 5 ml., and wash each of the extracts with one and the same quantity of 5 ml. of water. In both these stages vigorous shaking is not only unnecessary but is to be avoided, as an emulsion is very liable to be produced thereby.

Distil off the chloroform from the combined extracts, and treat the residue, consisting of codeine and some creosote, with $N/1$ sulphuric acid, which forms a soluble salt with the alkaloid and leaves the creosote unchanged; filter. Treat the filtered solution with an excess of $N/1$ sodium hydroxide and carry out a similar process of extraction; repeat these operations twice, when the codeine should be obtained entirely free from creosote. Dry the final chloroform extract with anhydrous sodium sulphate and filter, taking care to wash well with further quantities of chloroform to recover the last traces of alkaloid. Finally dry the residue at 100° C. to constant weight. Good results have been obtained using a vacuum desiccator.

Calculate results as anhydrous codeine, and check volumetrically by dissolving the weighed residue in a known volume of $N/10$ sulphuric acid and titrating the excess of acid with $N/100$ sodium hydroxide, using methyl red as indicator.

1 ml. of $N/100$ sulphuric acid consumed ≡ 0·0029917 g. of anhydrous codeine.

In an actual experiment, a solution in Simple Syrup was prepared, containing 0·023% of codeine and 0·130% of creosote. 51·37 g. of the sample were treated with 50 ml. of 2% sodium hydroxide solution, and the alkaloid was extracted with chloroform in the manner described above. The alkaloidal residue was treated with $N/10$ sulphuric acid and filtered, and after addition of excess of $N/10$ sodium hydroxide was re-extracted with chloroform, and this process was repeated twice more, when the residue obtained on removal of the solvent was apparently free from creosote. The weight of residue obtained was 0·01156 g., equivalent to 0·0225% of anhydrous codeine in the original product. The result by titration of the weighed residue was 0·0224%.

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November, 1945

THE FLECK AND WARD METHOD OF DETERMINING ELEMENTAL SULPHUR

WHILST studying an application of the Fleck and Ward method of determining sulphur,[1] my attention was drawn to a statement by Donnelly,[2] unsupported by experimental evidence, that above 15° C. this method gives high results owing to decomposition of the formaldehyde bisulphite compound. Although this is contrary to experience in these laboratories it was considered to warrant investigation. A series of tests were carried out, for simplicity, on synthetic assay solutions prepared by mixing $N/10$ sodium thiosulphate solution (25 ml.) and 8% w/v sodium sulphite solution (20 ml.). The solutions were titrated at the temperatures given in Table I, from which it is evident that Donnelly's statement is not confirmed.

TABLE I.

25 ml. $N/10$ thiosulphate ≡ $\begin{cases} 24{\cdot}90 \text{ ml. } N/10 \text{ Iodine} \\ 24{\cdot}95 \end{cases}$

Temperature of solution, °C.	$N/10$ iodine used, ml.
17	24·90
18	24·90
23	24·80
25	24·85
26	24·80
27	24·80
32	24·70

One possible error more liable to occur in serial analyses should be mentioned. The method directs that after addition of formaldehyde and acetic acid the titration should be carried out "forthwith." If an assay is allowed to stand before titration, particularly if dilution is deferred, a negative error will be obtained, as may be seen from Table II, which gives the results of titrations carried out on aliquot portions from a sulphur determination.

TABLE II

Time of standing, min.	$N/10$ Iodine used, ml.
0	36·35
2	35·85
5	34·60 end-point poor
36	30·0 ,, ,,

I wish to thank the directors of Messrs. Cooper, McDougall & Robertson for permission to publish this note.

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THE COOPER TECHNICAL BUREAU
BERKHAMSTED, BERKS.

D. A. LAMBIE
November, 1945

THE DETERMINATION OF CITRATE AND TARTRATE

STREBINGER and Wolfram[1] described a method depending on iodate oxidation for the determination of tartrate and Pirrone[2] gave a modified procedure for the determination of relatively small quantities of citrate and for tartrate. Pirrone's method was found by Lampitt and Rooke[3] to give high results for citrate. We have found that this happens with all three methods; our results, obtained with the procedures given in the literature were:

Material	Method	Quantity taken, g.	Quantity found, g.	Recovery, %
A.R. Citric acid monohydrate*	Pirrone	0·0343	0·0361	105·2
	Strebinger and Wolfram	0·2437	0·2594	106·4
A.R. Tartaric acid	Pirrone	0·0505	0·0584	115·3
	Strebinger and Wolfram	0·2988	0·3290	110·1

* The following reaction was assumed:

$$5C_6H_8O_7 + 18KIO_3 + 9H_2SO_4 = 9K_2SO_4 + 30CO_2 + 9I_2 + 29H_2O.$$

These high results are due to a reaction between the sulphuric acid and the potassium iodate. This was established as follows: 0·1 g. of potassium iodate was heated with known quantities of water and 30 ml. of sulphuric acid in a beaker over a moderate Bunsen flame for a known length of time and then treated as in Strebinger and Wolfram's method. The following results were obtained.

Water present, ml.	Time of heating mins.	KIO_3 recovery %
0·5	30	44·6
0·5	10	86·0
15	15*	94·6
15	15†	95·6

* Dense white fumes just produced. † on 0·05 g. KIO_3.

Hence it would appear that in a determination of either citrate or tartrate, an error not greater than 0·5% is obtained if (1) an excess of about 10% of potassium iodate and not less than 15 ml. of water are used and (2) the time of heating is not greater than 15 mins.

The following modification of Strebinger and Wolfram's method was therefore adopted—To 0·3 g. of tartaric acid or 0·24 g. of citric acid (or the equivalent as tartrate or citrate) add 1 g. of potassium iodate, 15 ml. of water and 30 ml. of concentrated sulphuric acid. Heat over a moderate flame until dense white fumes begin to be evolved (10–15 mins.) and allow to cool. Transfer to a 750 ml. flask containing 200 ml. of water, boil until free from iodine, cool, add 1–2 g. of potassium iodide and titrate with $N/10$ sodium thiosulphate, using chloroform as indicator.

1 ml. of $N/10$ $Na_2S_2O_3 \equiv 0{\cdot}003567$ g. of KIO_3
1 g. of $KIO_3 \equiv 0{\cdot}3507$ g. of $C_4H_6O_6$
$\equiv 0{\cdot}2727$ g. of $C_6H_8O_7.H_2O$
$\equiv 0{\cdot}2493$ g. of $C_6H_8O_7$.

The following results are typical of those obtained by this method.

Material	Quantity taken, g.	Error, mg.	Recovery, %
A.R. Citric acid	0·2366	+1·4	100·6
	0·1002	+0·6	100·6
	0·0493	+1·1	102·3
A.R. Tartaric acid	0·2980	+2·1	100·7
	0·1008	+1·0	101·0
	0·0504	+2·5	105·3
A.R. Sodium citrate	0·3520	−1·1	99·7
	0·3505	0·0	100·0

For the smaller quantities of citrate or tartrate, quantities of potassium iodate and sulphuric acid in proportion are used.

Interfering substances include reducing agents and acetates. Nitric acid or nitrates interfere, but the effect is negligible up to 15 ml. of $N/10$ solution. Halides interfere but can be removed by precipitation with silver in sulphuric acid solution; however, somewhat low recoveries, varying from 97–99% are then obtained. This may be due to co-precipitation of silver citrate or tartrate with the halide, silver alone having no effect on the estimation. For amounts of halide requiring not more than 15 ml. of $N/10$ silver solution, silver citrate may be used as the precipitant, but for larger amounts of halide, ammoniacal silver sulphate is recommended, the halide solution being kept at least 2 N with respect to sulphuric acid.

Substances not interfering include ammonium salts, phosphates and most metals.

Citrates and tartrates may sometimes be separated from interfering substances by a preliminary precipitation as follows—To 15 ml. of solution neutralised to phenolphthalein a small excess of lead nitrate in 5 ml. of water is added, the precipitate is filtered off on a No. 41 Whatman paper, washed with a small quantity of cold water, and transferred back to the original vessel with 15 ml. of water. The estimation is then completed normally except that the precipitated lead sulphate is filtered off on a paper pulp pad before the addition of potassium iodide. Determinations on sodium citrate (0·35 g.) and on sodium tartrate (0·50 g.) by this method gave recoveries of 99·2 and 99·4% respectively.

We wish to thank the Managing Director of the International Chemical Co., Ltd., for permission to publish this work.

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October, 1945

THE CONTROL OF DELIVERY FROM BURETTES

JUDGING from trade literature, it is apparent that, during the last decade, considerable improvements have been made in the methods of manufacture and accuracy in calibration of volumetric apparatus in general and of burettes in particular. Accordingly, it is surprising that possible improvements to the burette stopcock have not attracted more attention. As is only too well known, such control as is permitted by an ordinary stopcock functions within a small fraction of a complete rotation, so that even the most expert operator occasionally overshoots an end-point. In our attempts to spread the control of flow over a greater angular rotation, several possible methods, of which two are described below, were investigated.

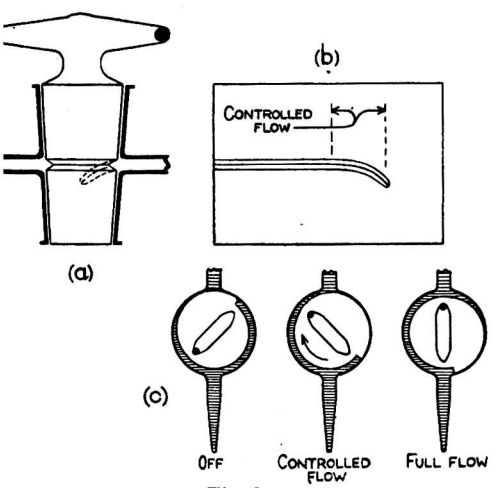

Fig. 1.

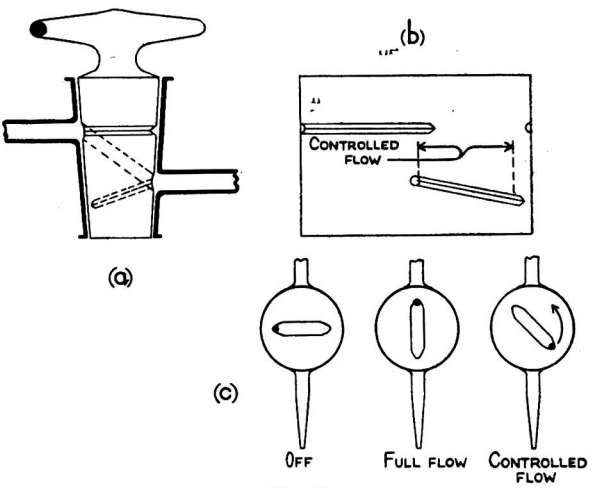

Fig. 2.

One method of obtaining an enhanced degree of control is shown in Fig. 1. The plug of the stopcock is replaced by another without a way (*i.e.*, undrilled), but having one end of the key marked by a bead of blue glass. The blind plug is carefully ground into the barrel so that a trace of lubricant permits free rotation. By means of a sharp triangular file, a V-shaped groove approximately 1·5 mm. wide is cut to extend halfway round the plug, as shown by full lines in Fig. 1*a*. The position of the groove with respect to the key of the plug should be noted, and the ends of the groove should register with the ways in the barrel. On inserting the plug into the barrel with the key vertical, flow of liquid controlled only by the bore of the jet should occur. Starting at the end beneath the blue bead on the key, the groove is extended a further 90° round the plug, initially making an angle of a few degrees with the original direction and then curving further away, as shown by broken lines in Fig. 1*a*. The shape should be such that when the plug is inserted with the key vertical and blue bead uppermost and then rotated anti-clockwise through 90° (all directions are taken facing the key of the plug), the rate of flow should steadily decrease and finally cease. A projection of the conical surface of the plug is shown in Fig. 1*b*.

The mode of action is depicted in Fig. 1*c*. As the plug is rotated clockwise from the "off" position, the effective cross section of the way, and hence the flow of liquid, increases steadily until the "full flow" position is reached. The control of the flow is thus spread over more than 80°, instead of the usual 10° or so with a stopcock of normal design (*vide infra*).

The second method makes use of a stopcock with an oblique bore. In this design, "full flow" takes place through the drilled way in the plug, the grooves cut on the conical surface being used in conjunction with the way for fine control only. As before, the plug is carefully ground in and one end of the key is marked. Two grooves 1 mm. wide are cut on the surface of the plug as shown in Fig. 2*a*. One commences at the upper end of the way and extends a little more than half way round; it is cut squarely, *i.e.*, in a plane at right angles to the axis of the plug. The second groove, which is cut on the opposite side of the plug to the first, starts at the lower end of the way and extends a little less than half way round. It is cut obliquely as shown by the treble broken lines in Fig. 2*a*. A projection of the conical surface of the plug is shown in Fig. 2*b*.

The plug is inserted in the "off" position as shown in Fig. 2*c*. On turning it clockwise through 90°, normal stopcock action giving "full flow" occurs. If the plug is rotated anti-clockwise from the "off" position, very slow flow commences when the marked end of the key is downwards and increases to "full flow" as the rotation is continued through a further half turn. Alternatively, increasing flow with clock-wise rotation may be obtained by altering the positions of the grooves.

Of the two methods, the second is preferable, since a standard stopcock is used, the control is spread over about 150°, and, despite the two grooves, cutting is simpler. Curve A, Fig. 3, shows the degree of control obtainable, and should be compared with Curve B, obtained with a stopcock of normal type. The stopcocks were each provided with a jet and sealed on to a 10-ml. burette, to the top of which a funnel device with water feed and overflow was fitted to give a constant head of 32 cm. above the stopcock. The rotations of the plug were measured by a circular protractor affixed to the small end of the plug. The water issuing from the jet was collected over timed intervals and weighed. The scale of Fig. 3 is too small to illustrate the degree of control obtainable in the range 0 to 30°. This is shown by the results in Table I.

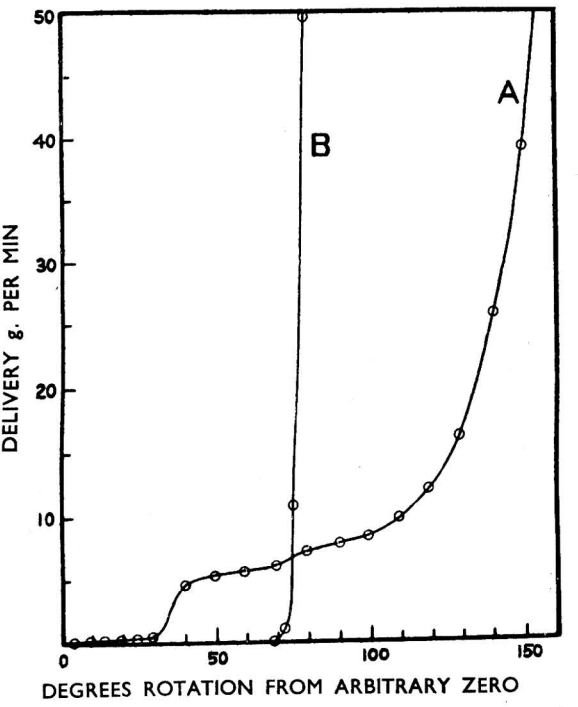

Fig. 3.

TABLE I

Rotation, degrees	Delivery, g. per min.	Time per drop, min.
1	0·0014	24·0
2·5	0·0019	19·0
5	0·0024	14·2
10	0·0038	11·5
15	0·0079	5·0
20	0·0150	3·0
25	0·0430	1·1
30	0·2600	0·18

In practice, the change in shape of curve A in the 30–40° region, due to a slight irregularity in the hand-cut oblique groove in the stopcock plug, is of little disadvantage. Machine cutting would largely eliminate such irregularities, and exponential or similar rotation-delivery responses could be obtained if desired.

Owing to the ease with which the drop speed may be varied, it is expected that burettes fitted with a fine-control stopcock will be useful in potentiometric titration.[1]

The stopcock should be generally useful in the control of flow of liquids. It should also have applications in connection with gases, and indeed a simpler form of the "nicked" plug is used in Pregl's micro Dumas apparatus for nitrogen determinations.[2]

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J. T. STOCK
M. A. FILL

November 28th, 1945

A VACUUM-OPERATED CIRCULATING PUMP

The pump (Fig. 1), which is of the pulsating type,[1,2,3] was developed for circulating water from a constant-temperature bath through a refractometer or similar jacketted instrument. Since the "piston" consists of a column of mercury, construction is comparatively simple, no close limits being involved. The pump is designed to be mounted in the bath itself. It is self-starting and will deliver water at any temperature up to about 97° C., even against considerable back-pressure.

The pumping action depends upon the oscillations of the mercury in the U-tube. As the column of mercury in limb A descends, water is drawn in through foot valve C. When the direction of movement of the column is reversed, valve C closes and water is delivered by way of head valve D. Oscillation of the mercury column is maintained by the automatic build-up and break of vacuum in limb B. Side tube E is connected to a water pump by way of a safety bottle.

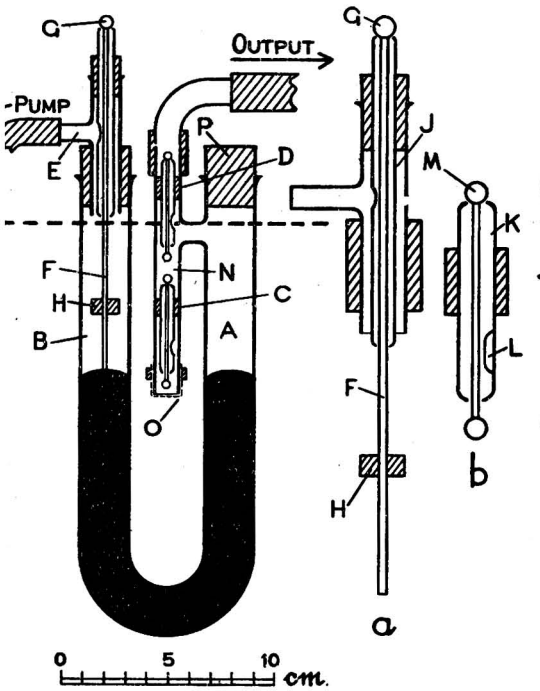

Fig. 1.

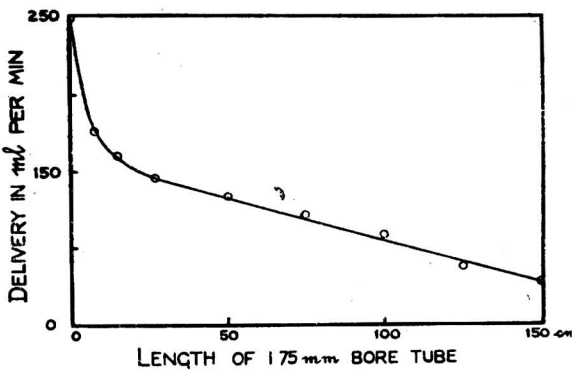

Fig. 2.

The valve motion resembles that used in the micro-stirrer previously described.[4] Owing, however, to the much slower strokes due to the inertia of the moving masses of liquid, a "hold-off" motion has been added to the "impact" principle already developed. Valve rod F is light and tends to float when the level of mercury in B has risen about 5 mm. above its rest position. Floating is prevented by the atmospheric pressure upon valve G, keeping the latter upon its seat. As the mercury continues to rise in B, it reaches rubber bumper H, suddenly increasing the upward thrust and breaking the vacuum by opening valve G. The buoyancy of the valve rod now exerts itself, and it continues to rise although movement of the mercury column may have ceased. The valve rod now floats in the mercury and follows the latter downwards until valve G closes, when the cycle of operations is repeated. By this means, long, steady strokes are obtained.

Details of the vacuum valve assembly are shown enlarged at (*a*) in Fig. 1. Valve rod F is of 3-mm. glass rod, which is fused into a 5-mm. diameter ball at the upper end to form valve G. The latter is lightly ground into the upper end of guide J; both ends of the latter are constricted to a bore of 4 mm. Bumper H is a 10-mm. length of 13-mm. diameter rubber pressure tubing which slides stiffly upon F.

Valve units C and D are identical, and their construction is shown at (*b*) in Fig. 1. The design allows ready replacement and gives an accurately controlled lift (5 mm.). Water enters guide K through the large oval hole L. The upper ball M is *lightly* ground into its seating, and the ends of K are constricted symmetrically so that the valve stem has a 2-mm. clearance.

To introduce the valve units into the chamber N, the bore of the latter is lightly smeared with glycerin. The units are then introduced with a screw-like motion, and pushed into position as shown in Fig. 1. A piece of tubing such as a blunt corkborer is useful for this purpose. If the rubber sleeves do not fit tightly, a drop of petrol may be applied. Thimble O, of copper gauze, is useful to prevent ingress of foreign particles.

The vacuum valve assembly is fitted and 70 ml. of mercury are placed in the U tube. Valve rod F should dip about 20 mm. into the mercury when the latter is at rest, valve G being closed. The distance x from the underside of H to the mercury surface should be about 35 mm. For a given suction, the amplitude of the oscillations, and hence the delivery of water, is roughly proportional to this dimension. Limb A is

completely filled with water, when stopper P is carefully inserted so that air is not entrapped. On applying suction at E, pumping commences.

With the greatest suction obtainable from an average water-jet pump fed from a fluctuating main, the output ranges from 200 to 300 ml. per min. as distance x is increased from 5 to 35 mm. The normal rate of action is 80 to 100 strokes per min. With the smallest suction consistent with regular operation, the delivery is from 40 to 200 ml. between the same limits of x.

As is shown by the results in Fig. 2 obtained by inserting various lengths of 1·75-mm. bore tubing horizontally in the delivery line, the device will deliver against a considerable resistance. (Dimension x was 25 mm. in this case.) It will also raise water at least 50 cm. above the level of the bath.

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M. A. FILL

November 26th, 1945.

Ministry of Food

STATUTORY RULES AND ORDERS*

1946—No. 157. The Food Standards (Self-raising Flour) Order, 1946. Dated Jan. 31, 1946. Price 1d.

This Order, which is to be read with the Food Standards (General Provisions) Order, 1944, replaces the Food Standards (Self-raising Flour) Order, 1944, with the following modifications:

(1) The minimum amount of available carbon dioxide required to be present in self-raising flour is reduced from 0·45% to 0·40%;
(2) The maximum figure for the total carbon dioxide is deleted;
(3) A more detailed method for determining the amount of available carbon dioxide is prescribed.

The Order came into force on February 11th, 1946.

The prescribed method of determining the available carbon dioxide is from the difference between the total and residual carbon dioxide determined respectively as follows.

(*a*) *Total Carbon Dioxide*: Shall be determined by ascertaining the weight thereof evolved when the self-raising flour is treated with excess of dilute sulphuric acid, the evolution being completed either by boiling for five minutes or by means of reduced pressure.

(*b*) *Residual Carbon Dioxide*: Shall be determined by taking not less than 5 g. of the self-raising flour, which shall be mixed to a smooth paste with distilled water, and a further quantity of distilled water amounting in all to not less than 20 times the weight of the flour shall then be incorporated. The liquid shall be heated in a boiling water bath for 30 minutes, being vigorously stirred for the first 5 minutes and thereafter for approximately half a minute at intervals of approximately 5 minutes. The liquid shall forthwith be boiled for 3 minutes, being vigorously stirred during the whole of such period, and then transferred to an apparatus for determining carbon dioxide, through which carbon dioxide-free air shall be passed for not less than 10 minutes. The residual carbon dioxide is the weight thereof evolved when the self-raising flour so treated is further treated with excess of dilute sulphuric acid, the evolution being completed either by boiling for 5 minutes or by means of reduced pressure.

— **No. 265. Order, dated February 20, 1946, amending the Flour Order, 1945.** Price 1d.

This amending Order provides for the rate of extraction of national flour to be increased from 80% to 82½% as from Sunday, 24th February, 1946.

— **No. 278. The Canned Fruit and Vegetables Order, 1946. Dated February 28, 1946.** Price 4d.

This Order revokes and replaces the Canned Fruit and Vegetables Order, 1945, as amended. It also revokes the Canned Fruit (Prohibition of Retail Sales) Order, 1945, with effect on March 3, 1946. Maximum prices are now provided for imported canned fruit and tomatoes and canned home-produced plums.

In this Order "Degrees Brix" in relation to any syrup means the percentage by weight of cane sugar or beet sugar contained in such syrup.

Spaghetti (cooked, in tomato sauce) is now included amongst the vegetables for which min. quantities per can are specified in Schedule I.

All fruit other than apples or other than fruit packed in an A10 can shall be packed in syrup of 29–32·5 Brix. Fruit (other than apples) packed in an A10 can shall be packed either (a) in syrup of 14–17·5° Brix. and marked "Packed in light syrup" or (b) in water and marked "Water pack." Apples (other than solid pack apples) shall be packed in syrup of 14–17·5° Brix. and marked "Packed in light syrup."

Particulars noted in ANALYST, 1945, **70**, 260, *for peas, spinach purée, macedoine and beans in tomato sauce have not been changed.*

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* Obtainable from H.M. Stationery Office. Italics signify changed wording.

Ministry of Health

STATUTORY RULES AND ORDERS*

1946—No. 10. The Milk (Special Designations) Regulations, 1946, dated January 2, 1946, made by the Minister of Health under the Food and Drugs Act, 1938. Price 1d.

These Regulations, which came into force on March 1, 1946, *make amendments in par.* 4 *of Part I* (C) *and par.* 7 *of Part III of the Third Schedule of the Milk* (*Special Designations*) *Order*, 1936, *and add two new Parts, IV and V, to the Third Schedule.*

The effect of these changes is to supersede the plate-count test, hitherto prescribed for "Pasteurised Milk" and "Tuberculin-Tested (*Pasteurised*) *Milk," by the phosphatase test and the methylene blue test in the form in which these tests have already been prescribed for Heat-Treated Milk in the Heat-Treated Milk* (*Prescribed Tests*) *Order*, 1944.

Any sample of "Pasteurised Milk" or "Tuberculin-Tested (*Pasteurised*) *Milk" taken after pasteurisation and before delivery to the consumer shall satisfy both tests.*

The phosphatase test shall be deemed to be satisfied by milk which gives a reading of 2·3 Lovibond blue units or less.

The methylene blue test shall be deemed to be satisfied by milk which fails to decolorise methylene blue in thirty minutes. The milk to be tested shall be kept at atmospheric shade temperature until it reaches the laboratory and shall there be kept at atmospheric shade temperature not exceeding 65° F. until the test is begun. The test shall be begun not earlier than nine and not later than ten in the forenoon on the day after the sample has been taken.

Details of the method of carrying out the tests are given in Parts IV and V of the Third Schedule and are the same as are given in the Schedule to the Heat-Treated Milk (*Prescribed Tests*) *Order*, 1944.

CIRCULAR 10/46

THIS explanatory Circular on the above Regulations was issued by the Ministry of Health on January 18, 1946. It gives as reasons for rescinding the plate-count test that a wide margin of error appears inevitable in arriving at the result of the count, and more particularly that the test takes account of heat-resistant organisms whose presence is of no material significance for the safety or the keeping quality of the milk. It draws attention to the importance of observing carefully the precautions recommended in the Addendum to Memorandum 139/Foods, respecting the phosphatase test issued by the Ministry of Health in March, 1943. It also points out that the details of the methylene blue test, as now prescribed for Pasteurised milk differ from those prescribed in the Milk (Special Designations) Regulations, 1936–1943, in relation to raw designated milks. Samples should not be packed in ice for transport to the laboratory, but should be kept at atmospheric shade temperature and protected from the direct rays of the sun. The methylene blue tablets referred to in Part V of the Third Schedule to the Regulations are the same as are used for the methylene blue test in relation to raw designated milks in accordance with paragraph 13 of Memorandum 139/Foods issued by the Ministry of Health in January, 1937.

ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

Food and Drugs

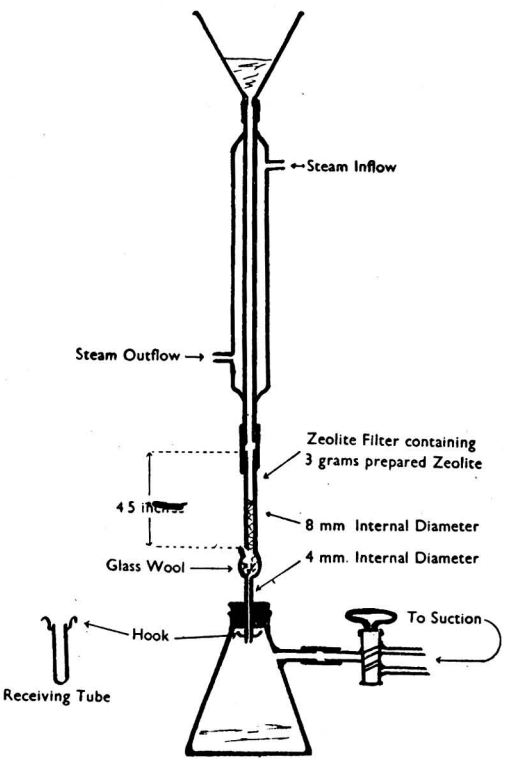
Simplified Colorimetric Determination of Aneurine in Cereal Products. M. Hochberg, D. Melnick and B. L. Oser (*Cereal Chem.*, 1945, **22**, 83)—This method relies on the formation of a red colour by the reaction of aneurine with diazotised *p*-aminoacetophenone. It is claimed to be applicable to materials of low aneurine content. No expensive apparatus is necessary and 12–15 tests can be made in 8 hours by one operator.

Reagents—Sulphuric Acid Solution: 0·05 *N*. *Sodium Acetate Solution:* 1·8 *M*. *Phosphatase Preparation:* takadiastase free from aneurine. *Zeolite:* approximately 50-mesh Decalso. The synthetic zeolite used for adsorption is prepared in bulk by stirring with four 10-volume portions of 3% acetic acid for 10 min. each; between the second and third acid washes a 15-min. treatment with 5-volumes of 25% potassium chloride soln. is introduced. The zeolite is finally washed thoroughly with water, alcohol, and ether, dried in air, and stored in a sealed bottle. *Salt Solution:* 25% potassium chloride solution in 0·1 *N* hydrochloric acid. *Standard Aneurine Solution:* 50 μg. of anhydrous aneurine hydrochloride per ml. in 25% alcohol at *p*H 1 to 2; stored in a refrigerator. *p-Aminoacetophenone Solution:* 6·35 g. of *p*-aminoacetophenone dissolved in 90 ml. of conc. hydrochloric acid diluted to 1000 ml. with distilled water; stored in an amber bottle and protected from direct sunlight. *Sodium Nitrite Solution:* 22·5 g. $NaNO_2$ dissolved in water, made up to 500 ml. and stored in a refrigerator; stable for 3 months. *Sodium Hydroxide - Sodium Bicarbonate Solution:* 40 g. of sodium hydroxide are dissolved in 1500 ml. of distilled water, 57·6 g. of sodium bicarbonate are added and sufficient distilled water to bring the volume to 2000 ml. *Diazotised p-Aminoacetophenone Solution:* 5 ml. of the *p*-aminoacetophenone solution (above) are pipetted into a 50 ml. graduated vessel surrounded with chopped ice and provided with a stirrer; 5 ml. of the sodium nitrite solution are added slowly and the resulting solution is stirred for 10 min. Then 20 ml. more of the nitrite solution are added slowly and the stirring is continued for a further 30 min. The temperature of diazotisation should not exceed 5° C. This solution should be used within 24 hr. after preparation; when not in use it should be kept below 5° C. *Aneurine Reagent:* 10 ml. of the above diazonium salt solution are added with stirring to 137 ml. of the sodium hydroxide - bicarbonate solution. The reagent is ready for use when the initial purple colour changes to a pale yellow, which usually takes 5 to 20 min. This reagent is prepared immediately before use. *Alcohol - Phenol*

* Obtainable from H.M. Stationery Office. Italics signify changed wording.

Solution: 15·6 g. of phenol are dissolved in sufficient 95% alcohol to make 2000 ml., and stored in an amber glass bottle. *Sodium Hydroxide Solution:* 1·0 *N*. *Thymol Blue Indicator:* 1% solution in alcohol. *Xylene.*

Extraction and De-phosphorylation—Weigh 10–20 g. of sample into a conical flask, add 150 ml. of 0·05 *N* sulphuric acid, and heat under reflux at 100° C. for 30 mins. Cool and add 10 ml. of sodium acetate solution to bring to *p*H 4·5. Add 1 g. of takadiastase and incubate overnight at 38° C. Cool to 20° C. and make up to 200 ml. Centrifuge and filter if necessary.



Adsorption and Elution of the Aneurine—The apparatus used for these operations is shown in the diagram. Pass 50 ml. of cold water through the column with suction. Pass a suitable quantity of the clear extract through the column at room temperature with mild suction at a rate of 3 to 4 drops per second. Release the suction. Pass steam through the jacket and pour 30 ml. of water on to the column. After half min. suck the hot water through the column. Release the suction. Elute the aneurine immediately by passing 10 ml. of the potassium chloride solution through the hot column, the eluate being collected in the receiving tube at a rate of 1 drop per 2 seconds. Apply suction for the last few drops. Remove the receiving tube and clean the column by passing through it 150 ml. of water with steam on and full suction. Follow this immediately with 50 ml. of water with the steam off, and full suction applied. The column is then ready for the next extract.

Colour Development and Readings—Transfer the eluate to a 100 ml. centrifuge tube and add 10 ml. of the alcohol - phenol reagent via the receiving tube to rinse out the latter. Add 2 drops of thymol blue indicator, followed by the sodium hydroxide solution until the first distinct blue colour is produced. Immediately add 25 ml. of the freshly prepared aneurine reagent, mix the solution and allow to stand in the dark for at least two hours. Then add 5–15 ml. of xylene and shake well for three minutes. Centrifuge. Remove the red xylene layer and match either visually or in an absorptiometer such as the Evelyn Photoelectric Colorimeter (520 μ filter) against a standard solution treated in the same way.

Typical Results—

Sample	Aneurine µg./g. Colorimetric method	Aneurine µg./g. Thiochrome method
White flour	0·99	1·00
Enriched flour	4·82	5·00
Whole wheat flour	4·88	4·50
Enriched bread (air-dried) ..	3·92	3·87
Barley	3·79	3·50
White rice	1·05	0·99
Dried yeast	711	714

W. M.

The Determination of Lipase in Pancreatin together with a Report on Commercial Pancreatins. K. Bullock (*Quart. J. Pharm.*, 1945, **18**, 234–244)—The British Pharmacopoeia method for the determination of the lipase activity of pancreatin is criticised on the grounds that it is too long, tedious to perform and variable in results. A method is described which is a modification of that due to Knaffl-Lenz (*Arch. exp. Path. Pharmak.*, 1923, **97**, 242) in which triacetin is used as substrate. *Method* —Add 6·5 ml. of triacetin and 0·2 ml. of bromocresol purple indicator solution to 95 ml. of water contained in a stoppered measuring cylinder, shake, neutralise with 0·05 *N* sodium hydroxide and dilute to 110 ml. Transfer 50 ml. of this solution to each of two large (3 × 20 cm.) test-tubes, *A* and *B*, each fitted with a two-holed rubber bung, one carrying a piece of glass tube through which passes a silk or cotton thread operating a glass stirring coil and the other the drawn-out tip of a burette. Place the tubes in a water-bath maintained at 30° C. and stir the contents until they attain the temperature of the bath. To tube *A* add 1 ml. of the enzyme solution (0·1 g. of pancreatin made up to 10 ml. with water) and to tube *B* add 1 ml. of the enzyme solution previously boiled. Adjust the reaction of the contents of both tubes to *p*H 6·2–6·4 by the dropwise addition of 0·05 *N* sodium hydroxide, comparing the colours with those of two standard tubes each containing 0·18 ml. of indicator solution, 15 ml. of buffer solution, in one case at *p*H 6·2 and in the other at *p*H 6·4, and water to make up 50 ml. Note the time and the burette readings of *A* and *B*. Continue to maintain the reaction of the digestion mixture between *p*H 6·2 and 6·4 by the dropwise addition of 0·05 *N* sodium hydroxide solution and note the burette readings after 10, 20 and 30 min., subtracting the readings for tube *B* from those for tube *A*. As a standard it is suggested that a 1% suspension of pancreatin should, under the conditions of the assay, liberate acid at a rate equivalent to 1 ml. of 0·05 *N* sodium hydroxide in 30 min. Since it is shown that the amount of acid liberated is approximately proportional to the time, it is not necessary to continue the test beyond 10 min. if at that time the amount of alkali then added is more than 0·33 ml. It is only essential to carry the test out for the full

time if an accurate assay is required or if the sample under examination is of border-line activity. It is estimated that a quantity of pancreatin just complying with the B.P. requirement for lipase would give a reading of approximately 0·2 ml. in the triacetin test, *i.e.*, the proposed standard is about five times the present standard. The indicator, shown by Bamann and Schmeller (*Z. physiol. Chem.*, 1931, **194**, 1) to be the most suitable, is prepared by dissolving 0·04 g. of solid bromocresol purple in 4 ml. of 0·05 *N* sodium hydroxide and diluting to 50 ml. with water. At *p*H 6·4 the reading of the blank tube *B* is less than 0·1 ml. for 30 min. digestion. A variation in the reaction of 0·1 *p*H unit has no appreciable effect on the result, and a temperature variation of 1° C. alters a titre of 1·0 ml. by less than 0·1 ml. The addition of activators to the substrate is considered unnecessary, and it is shown that the concentration of triacetin specified is the optimum for the test. A number of commercial samples of pancreatin have been examined for lipase activity and it is shown that the majority are of good but variable activity. The relationship between the lipolytic, amylolytic and tryptic activities of various samples has been investigated and the question of a standard of lipase activity is discussed.

J. A.

Notes on some Tests for Acetone and Ethyl Alcohol, with Special Reference to Methyl Alcohol. G. J. W. Ferrey (*Quart. J. Pharm.*, 1945, **18**, 193–200)—The tests for limits of ethyl alcohol and acetone in methyl alcohol in the British Pharmaceutical Codex are considered unsatisfactory, the first, the iodoform reaction, gives a turbidity with an amount of acetone insufficient to react with the second, Legal's nitroprusside test. The sensitivities of both have been examined and it is shown that Legal's test, when applied to methyl alcohol as described in the B.P.C., will detect 0·3% v/v of acetone, whereas the iodoform reaction as a test for ethyl alcohol in methyl alcohol is vitiated by 0·0025% v/v of acetone. It is suggested that a more satisfactory reagent for the detection of acetone in water-soluble alcohols would be Nessler's reagent and the following method is recommended:—Dilute 1 ml. of the alcohol to 50 ml. with water and add 5 ml. of alkaline solution of potassio-mercuric iodide; the turbidity at the end of 5 min. is not greater than that produced by treating similarly 1 ml. of a 0·05% v/v solution of acetone in water. The Nessler reaction could be used for the detection of acetone in acetone-free industrial methylated spirit in place of the indigotin reaction of Adams and Nicholls (ANALYST, 1929, **54**, 2) which, it is stated, gives ambiguous results with certain samples of methylated spirit, and also as a limit test for acetone in *iso*propyl alcohol, in which case the time between the addition of the Nessler's reagent and comparison of the turbidity should not exceed 5 min. For the detection of ethyl alcohol, the iodoform reaction has been modified to render it more sensitive. The following procedure, which produces a marked turbidity with 10 mg. of ethyl alcohol, is suggested:—To 5 ml. of methyl alcohol add 25 ml. of water, 25 ml. of 10% sodium hydroxide solution and 25 ml. of 0·1 *N* iodine. Heat in a water-bath at a temperature of 39° to 41° C. for not more than 10 min. with constant shaking. The presence of the methyl alcohol has no effect on the sensitivity, but the character of the precipitate is somewhat different and tends to disappear more quickly on standing. When testing for the presence of ethyl alcohol, any appreciable amount of acetone must be removed. Two methods are recommended—(1) To 5 ml. of the methyl alcohol add 10 ml. of saturated mercuric chloride solution and 10 ml. of 50% w/v sodium hydroxide solution and boil under a reflux condenser in an all-glass apparatus for 10 min. by immersion in a boiling water-bath. Wash down the condenser with 5 ml. of water and boil for a further 2 min. Repeat the washing and boil for 1 min. more. Allow to cool, add 20 ml. of water and leave for a few min. to allow the precipitate to settle. Decant as completely as possible through a No. 4 sintered-glass filter, add to the bright filtrate half its volume of 0·1 *N* iodine and heat at 40° C. as described above. Figures are quoted which indicate that acetone up to 20 mg. in 5 ml. of methyl alcohol is completely removed by this procedure. (2) To 5 ml. of the methyl alcohol add 25 ml. of water, 25 ml. of 10% sodium hydroxide solution and 25 ml. of 0·1 *N* iodine solution. If the acetone content is expected to be small, add 2 ml. of a 0·05% v/v aqueous solution of acetone before adding the iodine; this gives a coarsely crystalline precipitate of iodoform which is readily filtered. Allow to stand, with occasional vigorous shaking for 2 hr. Filter through a No. 4 sintered-glass filter under slight suction, add 15 ml. of 0·1 *N* iodine to the bright filtrate, heat to 40° C. and continue the test as above. By this procedure, 0·1% of ethyl alcohol in methyl alcohol can be detected. Using the quantities of reagents stated, the acetone content of the methyl alcohol should not exceed 0·05% v/v.

J. A.

Biochemical

Estimation of Small Amounts of Carbon Monoxide in Blood. F. J. W. Roughton and W. S. Root (*J. Biol. Chem.*, 1945, **160**, 123–133)—In this method the blood is laked and shaken in the Van Slyke - Neill apparatus with a sodium borate - hydrosulphite soln., and the extracted nitrogen quantitatively ejected. The carbon monoxide and carbon dioxide in soln. are then liberated by shaking with a de-aerated ferricyanide - acetate mixture, dissolved oxygen remaining bound by the hydrosulphite. After absorption of the carbon dioxide by de-aerated alkali the pressure of the residual carbon monoxide (together with a trace of nitrogen) is measured, and the percentage of carbon monoxide therein is measured by transferring to the Scholander - Roughton syringe capillary apparatus, where it is analysed by the usual procedure.

Evacuate 5 ml. of distilled water in the chamber of a Van Slyke apparatus with shaking, and lower the mercury reservoir so that the evacuated water is drawn down into the rubber connection between the chamber and the rest of the apparatus. Expel the water and liberated air, and draw 3 to 4 drops of caprylic alcohol into the chamber. Introduce 0·5 to 2·0 ml. of the blood into the Van Slyke cup and thence into the chamber, followed by 2 ml. of 1% saponin soln. in the same way. Leave for about a minute to ensure complete laking and then add 2 ml. of borate - hydrosulphite soln. (prepared each day by filling a 25-ml. test-tube almost to the top with 3% sodium borate soln. and then adding approx. 0·5 g. of sodium hydrosulphite; transfer the soln. to a 25-ml. burette with minimum contact with air). Close the tap and lower the mercury to the 50-ml. mark, covering the chamber with black paper. Evacuate the blood soln. by shaking for 1½ mins. and expel the extracted gases. Repeat the evacuation for 1½ mins. and again expel the gas.

Dry the cup with a roll of filter paper. Put 3 to 4 ml. of 32% potassium ferricyanide soln. into the cup and draw 1 ml. into the chamber. Close the tap, shake for 3 mins. at the 50-ml. mark, and absorb the carbon dioxide from the extracted gas by adding 1·5 ml. of de-aerated 4% sodium hydroxide soln. from the cup in the usual way, after drying the cup again before introducing the alkali. Record the pressure readings at the 2·0-ml. mark (p_{12}) and the 0·5-ml. mark ($p_{1·5}$). Fill the Van Slyke cup with 30% sodium chloride soln., and open the tap to allow a drop or two to enter and clear the mercury from the bore of the tap, then close the tap and press the cup of an inverted Scholander - Roughton syringe capillary apparatus, previously filled with 30% sodium chloride soln., against the bottom of the Van Slyke cup. Raise the mercury reservoir, close the lower tap, and then open the upper tap cautiously, so that the gas bubble passes through the bore and into the inverted cup of the syringe capillary, from which it is readily withdrawn into the capillary by means of the plunger. If the bubble is too big for the capillary, the excess can be ejected. Analyse the bubble for carbon monoxide according to the directions of Scholander and Roughton (*J. Biol. Chem.*, 1943, **148**, 551). Expel the rest of the gas from the Van Slyke chamber and record the manometer readings at the 2-ml. mark (p_{22}) and the 0·5-ml. mark ($p_{2·5}$) without any gas in the chamber. Clean the Van Slyke - Neill apparatus by filling the chamber with water, to which 2 or 3 ml. of 2% sodium hydrosulphite in 4% sodium hydroxide soln. have been added. The carbon monoxide content of the blood is calculated from the expression

$$(p_{12} - p_{22}) \times \frac{l_1 - l_2}{l_1} \times f_2$$

or

$$(p_{1·5} - p_{2·5}) \times \frac{l_1 - l_2}{l_1} \times f_{0·5}$$

where l_1 and l_2 are the lengths of the bubble in capillary divisions before and after absorption with Winkler's soln. in the Scholander - Roughton apparatus, f_2 and $f_{0·5}$ are the factors for the quantity of blood used at the 2-ml. and 0·5-ml. marks respectively. These are read off from the appropriate columns in table 30 of Peters and Van Slyke's "Quantitative Clinical Chemistry Methods," Baltimore, 1932. The error involved in the analysis of 0·05-ml. samples of blood containing 0 to 2 vols. per cent. of carbon monoxide ranged from −0·04 to +0·03 vol. per cent. With samples containing 2 to 5 vols. per cent., the error did not exceed 0·03 vol. per cent. F. A. R.

Estimation of Small Amounts of Carbon Monoxide in Air. F. J. W. Roughton and W. S. Root (*J. Biol. Chem.*, 1945, **160**, 135–148)— A new method for the estimation of concentrations of carbon monoxide in air below 0·05%, is described, and a modification of the method of Sendroy (*J. Biol. Chem.*, 1932, **95**, 599) for concentrations between 0·05 and 0·7%; this is more accurate than the original method and the blank correction has been eliminated.

Procedure with concentrations below 0·05%. Fill a tonometer with 30% sodium chloride soln. and displace the latter with the carbon monoxide mixture to be analysed, adjusting the final pressure to about 1½ atmospheres. Immerse in a water-bath at room temperature for a few mins., and measure the gas pressure by means of a mercury manometer. Introduce 50 ml. of Krogh's soln. (dissolve 16 g. of sodium hydrosulphite, 2 g. of sodium anthraquinone-β-sulphonate and 14 g. of potassium hydroxide in 100 ml. of water) into the tonometer and shake rapidly for 5 mins. to absorb the oxygen and carbon dioxide. After about 10 mins. run off the Krogh's soln. and wash with three 5-ml. portions of water. Next introduce 5 ml. of borated blood (mix together two drops of caprylic alcohol, 1 part of fresh human blood, 1 part of water, 1 part of 3% borax soln. and 2 parts of 1% saponin soln.) and rotate the apparatus in the dark for 1½ to 2 hrs. in a water-bath at room temperature. Allow the apparatus to stand vertically for 2 mins. and then run off the blood soln. into the cup of the Van Slyke - Neill apparatus and thence into the chamber. Rinse the tonometer into the cup with two or three 3-ml. portions of water and, after measuring the vol. of each washing in the cup, run it into the chamber. Add 2 drops of caprylic alcohol, and 1 ml. of borate-hydrosulphite soln., and proceed as described in the preceding abstract except that (*a*) the amount of ferricyanide soln. is increased from 1·0 to 1·5 ml., and of alkali soln. for absorption of carbon dioxide from 1·5 to 2·0 ml., and (*b*) the blood - ferricyanide soln. is shaken for 10, not 3, mins. Determine the carbon monoxide content of 5 ml. of the borated blood soln. beforehand. Calculate the carbon monoxide content of the blood soln. and washings at N.T.P. from the expression

$$(p_{12} - p_{22}) \times \frac{l_1 - l_2}{l_1} \times \frac{f_2}{100} \times \left(1 + \frac{S\alpha}{50 - S}\right)\left(1 + \frac{3·5\alpha}{50 - 3·5}\right)$$

or

$$(p_{1·5} - p_{2·5}) \times \frac{l_1 - l_2}{l_1} \times \frac{f_{0·5}}{100} \times \left(1 + \frac{S\alpha}{50 - S}\right)\left(1 + \frac{3·5\alpha}{50 - 3·5}\right)$$

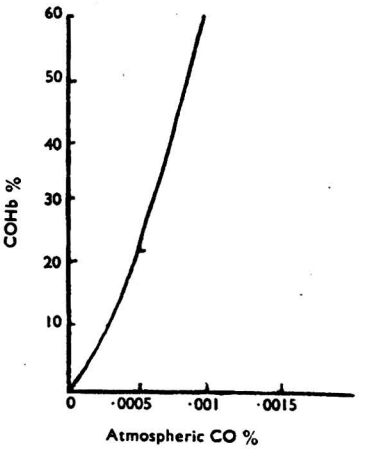
where p_{12}, p_{22}, $p_{1·5}$, $p_{2·5}$, l_1, l_2, f_2 and $f_{0·5}$ have the same significance as in the preceding abstract, S is the total vol.of soln. extracted in the Van Slyke chamber and α is the solubility coefficient of carbon monoxide in aqueous soln. at the temperature of the measurement. From the value so obtained, subtract the carbon monoxide content of the borated blood to obtain the vol. of carbon monoxide taken up by the blood soln. Then the % of carbon monoxide in the gas mixture less the carbon monoxide unabsorbed at equilibrium (A) equals

$$100 \times \frac{\text{vol. of CO taken up by blood soln.}}{\text{vol. of gas sample at N.T.P.}}$$

Calculate from the vol. of carbon monoxide taken up by the blood soln. its carboxy-haemoglobin content and read off from the Fig. (the carbon monoxide dissociation curve of 1 : 5 blood soln. at 25° C.), the % of atmospheric carbon monoxide corresponding to this value. Then the % of carbon monoxide in the original gas mixture is obtained by adding this value to A. The results ranged from 98·0 to 102·3% of the theoretical.

Procedure with concentrations of 0·04 *to* 0·7%. Fill a modified Hempel pipette with Krogh's hydrosulphite soln., the cup, the bore of the 3-way tap and the side-tube being filled with water. Connect the side-tube with the gas to be analysed and pass sufficient to displace the air in the apparatus. Turn the 3-way tap so as to connect the gas with Krogh's soln., and fill the Hempel bulb with the gas. Seal the tap with mercury and absorb the oxygen and carbon dioxide by shaking for 5 mins.

and then leaving for 5–10 mins. Evacuate 5 ml. of water in the Van Slyke - Neill chamber and shake for 1½ mins. Draw the evacuated water into the rubber connection between the chamber and the rest of the apparatus and expel the water and extracted air and introduce 4 drops of caprylic acid, 1–2 ml. of fresh human blood (preferably from a non-smoker), 2 ml. of 1% saponin soln. and enough



water to make 5 ml. Close the tap, lower the mercury to the 50-ml. mark, covering the chamber with black paper, shake for 4 mins., and expel the extracted gases. Close the tap and read the manometer with the mercury at the 50-ml. mark (p_1, temperature t). Adjust the pressure to slightly less than atmospheric and admit 10–40 ml. of gas from the Hempel pipette, according to the carbon monoxide content. Close the tap of the Van Slyke chamber, again set the mercury at the 50-ml. mark and read the manometer (p_2). Then the vol. of gas drawn into the chamber =

$$(\text{vol. of chamber} - \text{vol. of blood soln.}) \times \frac{273}{273+t} \times \frac{p_2 - p_1}{760}.$$

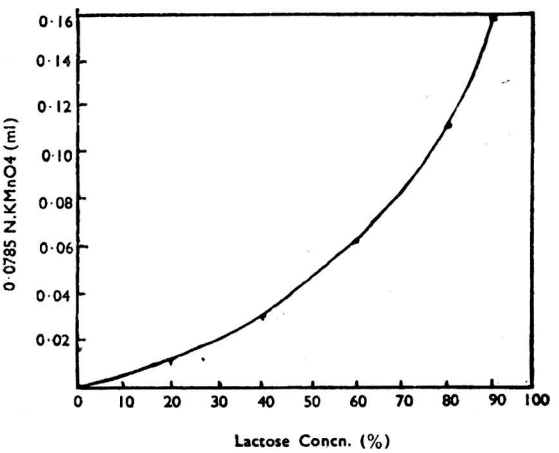
Shake for 30 mins., expel the gas quantitatively from the chamber, and introduce 2 ml. of the borate-hydrosulphite soln. Continue the procedure as described for the estimation of carbon monoxide in blood and calculate the % of carbon monoxide as described above, making allowance for the carbon monoxide remaining in the gas phase at equilibrium. This correction, however, is of less importance than with the lower range of concentrations. The results obtained by this method ranged from 97·5 to 101·5% of the theoretical.

F. A. R.

Organic

Determination of Glucose, Galactose and Lactose in their Mixtures. G. A. Ramsdell (*J. Dairy Sci.*, 1945, **28**, 671–676)—The method, designed to test lactose syrups, involves the determination of the sum of the two hexoses by means of Barfoed's modified reagent (Svanberg, *Z. physiol. Chem.*, 1930, **188**, 219) and the determination of the reducing power of the mixed sugars before and after destruction of the glucose with baker's yeast, according to the method of Shaffer and Somogyi, using reagent No. 50 with 5 g. of potassium iodide (*J. Biol. Chem.*, 1933, **100**, 695). The method used by Svanberg was modified in detail in order to make it applicable to the estimation of concentrated sugar solutions.

Pipette 5 ml. of the diluted syrup, containing approx. 10 mg. of sugar, and 15 ml. of buffered copper reagent (10·4 g. of glacial acetic acid, 66·5 g. of cupric acetate and 7·0 g. of sodium acetate made up to 1·5 litres with water) into 1 × 6 in. test-tubes, and saturate with washed hydrogen. Heat in a boiling water-bath for 10 mins. with the gas passing through the soln. at the rate of 30 bubbles per min. and then cool immediately to 50° C. Filter off the cuprous oxide with the aid of slight suction, wash with two 10-ml. portions of water at 60° C., dissolve the precipitate in a solution containing 200 g. of sulphuric acid and 50 g. of ferric sulphate per litre, and titrate the reduced iron with standard permanganate soln. The calculation is complicated. First estimate the (uncorrected) amount (A) of hexoses in the sample by reference to a standard curve obtained by treating pure solns. of a 1 : 1 glucose and galactose mixture in the same way. Then estimate the glucose on 4 mg. of syrup by means of Shaffer and Somogyi's method before and after fermentation, the amount (B) of glucose present being equivalent to the difference between the two results. The difference between A and B gives the (uncorrected) amount (C) of galactose. Next calculate from a standard curve the volume of thiosulphate equivalent to this amount of galactose and add the value so obtained to the volume of thiosulphate equivalent to B. This gives the volume equivalent to the glucose plus galactose. By subtracting this from the volume of thiosulphate required in Shaffer and Somogyi's method without fermentation, the volume of thiosulphate equivalent to the lactose is obtained, and from this



the amount (D) of lactose present is found by reference to a standard curve for lactose. Then the lactose content, per cent. of the total sugars

$$= \frac{D}{B + C + D} \times 100.$$

Since lactose is not entirely without effect on the modified Barfoed reagent, a correction must be applied to the result obtained on titration with permanganate. This is obtained from the Fig., the volume of potassium permanganate equivalent to the above lactose concentration being read off from the curve and subtracted from the original titre. Calculate the (corrected) amount (E) of hexoses from the result using the standard hexose curve

(see above) and then calculate the (corrected) amount (F) of galactose by subtracting B from E. The true values for the glucose, galactose and lactose contents are therefore represented by B, F and D respectively. F. A. R.

Inorganic

Mercurous Perchlorate as a Volumetric Reagent for Iron. W. Pugh (*J. Chem. Soc.*, 1945, 588–589)—Bradbury and Edwards (*J. Soc. Chem. Ind.*, 1940, **59**, 96T) have shown that mercurous nitrate reduces the ferric thiocyanate complex and have adapted the reaction to the direct titration of ferric solns. Mercurous perchlorate is now proposed as a suitable reagent as it yields a very stable soln. (An earlier report—*J. Chem. Soc.*, 1937, 1824—that the soln. loses strength is now corrected; solns. kept in the dark over mercury have remained almost unchanged for 7 years). The method is most successful when the 100–200 ml. of soln. titrated contain 3 to 5 g. of potassium or ammonium thiocyanate and not more than 5 ml. of conc. nitric or sulphuric acid or 2 to 3 ml. of conc. hydrochloric acid. Greater amounts of acid lead to high results, but their effect can be countered by using more thiocyanate or, better, by partial neutralisation. Under the conditions employed the reaction is not strictly stoicheiometric, and the reagent soln. should be standardised against a known ferric soln. The reaction is slow when near the end point if the soln. is cold, but erratic low results occur if the soln. is hot when titrated. Ferrous iron does not affect the titration. Phosphates and acetates cause high results and indistinct end-points.

Procedure—The mercurous perchlorate soln. is prepared as previously described (*loc. cit.*). Dissolve iron oxide ores in hydrochloric acid, adding stannous chloride soln. if necessary, and oxidise with bromine. Dissolve steels in hydrochloric acid, filter, and oxidise with bromine. Dissolve pyrites in conc. nitric acid and potassium chlorate and boil down three times with hydrochloric acid. Decompose bauxite with hot conc. sulphuric acid, dilute, ppt. the iron and aluminium with ammonia and dissolve the ppt. in hydrochloric acid. Adjust the solns. to the volume and acidity given above and add the thiocyanate. Titrate with approx. 0·1 *N* mercurous perchlorate soln., adding the titrant to the cold liquid until the colour fades from dark red to light orange (4 to 5 drops from the end-point), heat to 60–70° C., and finish the titration, shaking the flask well. L. A. D.

Applications of Vacuum Distillation to the Analysis of Alloys. J. W. Price (*J.S.C.I.*, 1945, **64**, 283–285)—The apparatus used is that of Hanson and Pell-Walpole (*Int. Tin Res. and Dev. Council Tech. Pub.*, A.96, 1940), in which the samples are placed in silica or alundum boats in a horizontal silica tube closed at one end. The other end is connected to a vacuum pump by means of a rubber bung sealed with a paste of alcohol and sealing wax. A trap placed between the tube and pump is found to be unnecessary. Pressures are measured with a simple gauge of the "Vacustat" type and are 0·05 mm. or less. The tube is heated to the desired temp. by means of an electric furnace. Weighed samples are inserted, heated as required, cooled to below 200° C., removed and re-weighed. After a successful treatment the samples form bright rounded buttons; thus if partitions are made in the boat with alundum cement several determinations may be made at one time.

Zinc in tin-zinc alloys—Most of the zinc distils at 750° C., but complete separation requires 850° C. (1) 92/8 tin-zinc—Determine the zinc by heating 3 g. for 30 min. at 850° C. (2) 69/29·5/1·5 tin-zinc-copper—Too rapid distillation of the zinc causes sputtering. Heat for 15 min. at 750° C. and then for 15 min. at 850° C. The copper does not interfere. *Lead in solders*—With pure lead-tin solders heat for 1 hour at 1000° C. to distil off the lead. Some slight loss of tin by penetration into the boat may occur at high temperature. If antimony is present it will distil slowly at 1000° C., and the alloy must be heated to constant weight (8 to 10 hours) at 850° C. Cadmium, if present, will distil completely, silver and bismuth partially. *Zinc in brass and gun-metal*—Copper loses weight if heated above 850° C. in vacuum and as most brasses and bronzes are solid at this temperature the rate of removal of lead or zinc depends on their rate of diffusion in the solid and the fineness of the sample. Fine division of the sample may introduce errors caused by oxidation. Enough tin (about equal in weight to the sample) is therefore added to form an alloy liquid at 850° C. Zinc may then be removed in 30 min. If phosphorus is present some of it distils in presence of tin (although pure phosphor copper containing 15% of phosphorus is unaffected). However, when working with metals containing about 0·05% of phosphorus, only about 0·02% is lost and the consequent error may often be neglected. When tin is added to a sample the latter should preferably be in one piece; if turnings are used they should be clean so that they alloy readily with the tin. Lead, if present, will also distil; arsenic and antimony will remain in the residue. *Phosphorus in phosphor-tin* (5%). Heat for 20 to 30 min. at 500° C. The phosphorus will distil readily at 250–300° C., but condenses in a finely divided yellow form and ignites on opening the tube. At 500° C. the deposit is black and does not ignite in air. *Lead in copper-lead and gun-metal*—Addition of tin does not have much effect on the rate of removal of lead. Heat the sample at 850° C. until it is constant in weight (1–2 hours). To determine lead and zinc together add tin as above. *Cadmium in copper-cadmium alloys*—Heat for 30 min. at 750–800° C., after adding tin. If tin is not added the distillation of cadmium is very slow, even at 850° C. L. A. D.

Physical Methods, Apparatus, etc.

Spectrophotometric Determination of Calcium. R. E. Scott and C. R. Johnson (*Ind. Eng. Chem., Anal. Ed.*, 1945, **17**, 504–506)—Several colorimetric methods of determining calcium are briefly reviewed with a view to adapting them to spectrophotometric or absorptiometric technique. The calcium oxalate-permanganate procedure is selected as being direct and convenient, and satisfactory conditions for making spectrophotometric determinations have been worked out. The preparation of the standard solns. and reagents used and other work done in investigating the method are described. It is found, using the Coleman 10–S–30 spectrophotometer, that potassium permanganate solns. obey Beer's law precisely at a wavelength of 529 mμ when the concentration (C) is between 0·2 and 2·0 mg. of manganese per 100 ml. When T is the percentage transmission and l cm. is the depth of the cell

$$C = (-2{\cdot}512 \log_{10} T + 5{\cdot}018)/l.$$

However, when a soln. of potassium permanganate

is partly reduced by known amounts of oxalate in presence of sulphuric acid the linear relationship does not apply; but with suitable buffers stable solns. of this type may be prepared conforming to highly reproducible calibration curves.

Reagents—(1) An oxalate soln. containing 5·738 g. of oxalic acid dihydrate or 6·099 g. of sodium oxalate per litre. (2) Potassium permanganate soln., 0·0910 *N*, 2·877 g. per litre, standardised against reagent 1 and adjusted if necessary so that 1 ml. contains 1·000 mg. of manganese. (3) Acetate buffer (*p*H 4·7): 68·1 g. of sodium acetate trihydrate and 31·3 ml. of 16 *N* acetic acid per litre. (4) Formate buffer (*p*H 3·7): 31·5 g. of ammonium formate and 20·8 ml. of 24 *N* formic acid per litre.

Preparation of sample solns.—Samples of solns. containing mainly calcium chloride or sulphate and most natural waters are suitable. If the chloride concn. is high methods 1 and 2 (below) cannot be used. In general the portions used for the analysis must be freed from high concns. of oxidising or reducing substances, anions forming insol. calcium salts and cations forming insol. oxalates. Under the best conditions the methods have an accuracy of about 0·3%. Two avoidable causes of error are the use of formates in the first two methods, and neglect of the layer of calcium oxalate formed on the electrodes during unnecessarily protracted *p*H measurements. After each pptn. of calcium oxalate keep the system at 90° C. for 10 min. and at room temp. for at least 30 min.

Method 1—Run a sample containing about 2 mg. of calcium into a 50-ml. beaker, add 1 ml. of acetate buffer and 2·00 ml. of reagent (1) and adjust the *p*H to 4·7 or lower if certain interfering substances are present. Digest, reducing the vol. to between 2 and 8 ml., and cool. Filter through a 15 ml. glass filter crucible of medium porosity and wash the ppt. and beaker with three 2-ml. portions of water. Collect the filtrate and washings in a 100 ml. flask under a bell jar with tubulures for the crucible and suction tube. Add 10 ml. of 18 *N* sulphuric acid (requiring not more than 2 mg. of manganese as permanganate per litre to give it a pink colour) and 2·00 ml. of reagent (2). When the colour change has occurred dilute to 100 ml. and measure the transmittance within 20 min. Read the result of the analysis from a calibration curve. The ppt. on the filter may be used to give a confirmatory result by method 3.

Method 2—Adjust the *p*H of the sample to 4·7 and run an aliquot portion containing 2 to 4 mg. of calcium into a 50-ml. volumetric flask. Add 1 ml. of acetate buffer and 4·00 ml. of reagent (1). Shake occasionally during the 10 min. at 90° C. and leave for 30 min. at room temp., the vol. at this stage being 8 to 20 ml. Dilute to 50 ml., mix quickly and centrifuge for 10 min. at about 3000 r.p.m. Pipette 25 ml. of the clear liquid into a 100 ml. flask, add 10 ml. of 18 *N* sulphuric acid and 2·00 ml. of reagent (2), dilute to 100 ml. and complete as in method 1.

Method 3—Pipette an aliquot portion containing about 1·7 mg. of calcium into a 50 ml. beaker and add 1 ml. each of formate buffer and saturated ammonium oxalate soln. Adjust the *p*H to 3·7, digest at about 95° C., reducing the vol. to about 20 ml., cool and leave for at least 30 min. Filter as in method 1, washing with four 1–2 ml. portions of water. Remove the crucible and wash the outside and bottom thoroughly. Dissolve the ppt. in the beaker and crucible in two 10 ml. portions of hot 9 *N* sulphuric acid. Collect the soln. and two 5-ml. water washings in a 100-ml. graduated flask, add 2·00 ml. of reagent (2) and complete as in method 1.

Method 3*a*—The range and precision may be increased, *e.g.*, by adapting the procedure of McComas and Rieman (*Ind. Eng. Chem., Anal. Ed.*, 1942, **14**, 929). Pipette an aliquot portion containing about 17 mg. of calcium into a 100-ml. beaker, add 5 ml. of 0·5 *M* formic acid and adjust to 30 ml. Heat to 95° C., and add 5 ml. of 0·5 *M* oxalic acid in 85 sec. Digest for 5 min. at 85 to 95° C. and cool to 25° C. in about 15 min. Add 15 ml. of 1·0 *M* ammonium formate, adjust the *p*H to 3·7 (if necessary) and digest for 30 min. at 25° C. Wash the ppt. as in method 3, dissolve in 4 to 8 10-ml. portions of hot 9 *N* sulphuric acid and dilute the soln. to 100 ml. Add 10 ml. of 18 *N* sulphuric acid and 2·00 ml. of reagent (2) to a 10 ml. aliquot portion, dilute to 100 ml. and complete as in method 1. Adjustments of *p*H are made with 0·1 and 1·0 *N* sulphuric acid and sodium hydroxide in all the methods. L. A. D.

Colorimetric Determination of Copper in Aluminium Alloys. R. F. Patridge (*Ind. Eng. Chem., Anal. Ed.*, 1945, **17**, 422–424)—Sodium diethyldithiocarbamate is used as the reagent for copper; the coloured compound formed is extracted with carbon tetrachloride, and the absorption of the greenish-yellow soln. is measured in a photoelectric absorptiometer. As solns. containing between 0·002 and 0·014 mg. of copper per ml. of carbon tetrachloride are convenient to measure, a value near the middle of this range (0·0064 mg. per ml.) is arbitrarily selected to represent 4% of copper in the sample. The method thus covers alloys containing between 0 and 8% of copper. The coloured soln. obeys the Beer - Lambert law up to 6% of copper, the remainder of the calibration curve up to 8% showing a deviation from the linear relationship. When the method is used as described accurate results are obtained on synthetic mixtures simulating solns. of aluminium alloys containing 1%, 4% or 8% of copper together with 11% of zinc, 10% of magnesium, 5% of tin, 4% of nickel and 2% each of iron, manganese, chromium, lead, titanium and bismuth, either separately or all together.

Reagents—(1) Acid mixture—475 ml. of water, 125 ml. of conc. sulphuric acid, 200 ml. of conc. nitric acid and 200 ml. of conc. hydrochloric acid. (2) Aluminium soln.—Dissolve 0·800 g. of pure aluminium in the acid mixture, evaporate to fuming and dilute to 1 litre. One ml. contains 0·80 mg. of aluminium. (3) Standard copper soln.—Dissolve 1·000 g. of pure copper in 10 ml. of nitric acid, add 5 ml. of conc. sulphuric acid and dilute to 1 litre. Dilute 10 ml. of this soln. to 1 litre. One ml. contains 0·01 mg. of copper. (4) Citric acid—100 g. in 1 litre of water. (5) Dimethylglyoxime soln.—1 g. in 100 ml. of conc. ammonia soln. (6) Sodium diethyldithiocarbamate soln.—1 g. per litre of water. Store in a brown bottle and do not keep for more than 1 week. *Method*—Weigh 0·2 g. of sample into a 250 ml. beaker and dissolve in 15 ml. of acid mixture. Evaporate to fuming, cool, add 60 ml. of hot water and boil until the residue dissolves. (A turbidity caused by suspended silica does not affect the method.) Cool and dilute to 250 ml. in a graduated flask. Pipette 10 ml. of the soln. into a 250 ml. beaker and add in order 10 ml. of citric acid, 5 drops of dimethylglyoxime soln. and 10 ml. of diluted ammonia (1+3). Allow to stand for 3 min. and if a ppt. forms filter, and wash with a small amount of

hot water. Transfer the soln. to a 500 ml. separating funnel and add 20 ml. of sodium diethyldithiocarbamate soln. Extract with two 25-ml. portions of carbon tetrachloride, draining into a dry 50 ml. graduated flask. Dilute to the mark with carbon tetrachloride and measure the optical absorption in a photoelectric instrument (Klett-Summerson 900–3), using a green filter with a maximum transmission at 540 mμ. Use a soln. prepared from pure aluminium soln. to set the zero of the instrument. Refer the measurement of the unknown to a calibration curve prepared from standards made from pure aluminium soln. and known amounts of standard copper soln. L. A. D.

Application of Multiplier Photo-Tubes to the Spectrochemical Analysis of Magnesium Alloys. G. A. Nahstoll and F. R. Bryan (*J. Opt. Soc. Amer.*, 1945, **35**, 646–650)—Replacement of the photographic plate in a spectrograph by a multiplier photocell sensitive to ultra-violet radiation (RCA type 1 P 28) enables photographic processing and microphotometry to be eliminated from the spectrographic procedure. A framework in the focal plane of the spectrograph carries exit slits in positions corresponding to selected analysis lines of the alloying constituents to be determined. The multiplier cell in its mount can be moved on a carriage to receive radiation, as required, from each of these slits. A second photocell in a fixed position receives radiation reflected from the focus of a magnesium line selected to serve as the internal standard. A ratio of the outputs of the two multiplier cells (receiving analysis line and internal standard line respectively) is obtained by using a valve voltmeter to indicate the difference of potential between the anodes of the two cells, when each is connected to a 20 megohm load. A 0·1 μF condenser across the voltmeter tends to average irregularities in the cell outputs caused by fluctuations in the spectrographic spark source. The voltmeter dial is calibrated from working curves to read directly the percentage composition of the sample under test. The constituents determined are aluminium, zinc, manganese, silicon, copper, nickel, iron and lead. These eight determinations can be made during a sparking time of about 4 min.; one operator can deal with 10 samples per hour as received from the foundry. The accuracy appears to be similar to that of conventional spectrographic methods. The principal disadvantage is that replacement of a photocell may require complete revision of the calibration owing to a change in spectral and total sensitivity. B. S. C.

Dilution Factor in Spectrochemical Analysis C. B. Post, D. G. Schoffstall and G. Hurley (*Ind. Eng. Chem., Anal. Ed.*, 1945, **17**, 412–416)—In the spectrographic analysis of ferrous alloys iron is often used as the internal standard. In certain steels with high nickel or chromium content for example, the decreased intensity of the iron spectrum necessitates the preparation of a fresh set of calibration curves for elements such as silicon, manganese, molybdenum, etc., based on standard samples of appropriate iron content. To avoid this elaborate procedure, the use of an "atomic dilution factor" allows spectrographic analysis of ferrous alloys covering a wide range of iron contents to be made by reference to only one set of calibration curves. *Procedure*—Determine the ratio of the unknown (X) and iron spectrum lines in the conventional manner. Multiply this ratio by the atomic dilution factor (100−Y)/100, where Y is the atomic percentage of alloying constituents other than X. This gives the corrected intensity ratio of X to iron. Use this ratio with a calibration curve prepared from iron alloys whose Y content can be ignored. This will then give the true percentage of X in the alloy. The applicability of the procedure has been studied and results have been found to agree with this theoretical treatment, using the special arc method of Hasler, Harvey and Dietert (Abst., ANALYST, 1943, **68**, 293). It is pointed out that the method is not applicable to all alloys. In the analysis of aluminium alloys, for example, an increase in magnesium or zinc content causes a depression of aluminium lines far in excess of that predicted by atomic dilution and not accompanied by a similar depression of some of the elements to be determined. B. S. C.

Reviews

REACTIFS POUR L'ANALYSE QUALITATIVE MINERALE. Deuxième Rapport de la Commission Internationale des Réactions et Réactifs analytiques nouveaux de l'Union Internationale de Chimie. Pp. xvi + 288. Basle: Wepf & Cie. 1945. Fr. 25 (paper), Fr. 28 (bound).

The First Report of the International Committee on New Analytical Reactions and Reagents of the Union Internationale de Chimie, published in 1938, provided a somewhat complicated reference book to the vast number of reagents for qualitative inorganic analysis discovered between the years 1910 and 1936 together with such older reagents as had been investigated during that period with regard to their application to problems of importance to the analyst.

The Second Report, now under review, presents the results of a critical study of the compounds mentioned in the First Report as well as those discovered between 1937 and 1943, although it is pointed out that the Committee were unable to consult many journals in the English language owing to the war. The Working Committee consisted of Prof. Dr. C. J. van Nieuwenburg of Delft, Prof. Dr. J. Gillis of Ghent and Prof. Dr. P. Wenger of Geneva, and the Report is edited by Prof. Dr. Wenger and Dr. R. Duckert of Geneva.

In order that the Report should not be unduly long, the number of reagents for each element has been limited to five, although in many cases fewer than this number are described. The criteria which have determined the choice are first, the sensitivity should be as great as possible, usually lying between the limits of dilution 1 in 10^4 to 1 in 10^6. However, a reagent of extreme sensitivity (*e.g.*, 1 in 10^8) is not generally recommended unless it has an unusually high degree of specificity. Secondly, the specificity should be as great as is attainable—wherever possible relative to all the other elements, but for the majority of reagents it is relative to all the ions in an analytical group. Preliminary separations enable some less specific reagents to be included, particularly for amphoteric elements. Thirdly, the reagents should be readily available commercially; methods of preparation of a few unusual reagents, that do not present any special difficulties, are described. Lastly, the chosen reagents should be stable: particulars of the stability of the few exceptions included are given.

After a Preface describing the constitution of the Committee and their terms of reference, and a Technical Introduction explaining the scope and purpose of the Report, the book is divided into two sections, devoted to Cations and Anions respectively. The metals are listed in four groups, first, those precipitated by hydrogen sulphide, then the elements of the ammonium sulphide group, followed by the alkaline earth metals and, finally, the alkali metals. A total of fifty-seven elements are discussed. To the rare earths are assigned three reagents, one for the group as a whole (oxalic acid) and one each for the cerium sub-group and the yttrium sub-group (ammonium succinate and lactic acid respectively). Following each of the main groups are very useful descriptions of recommended procedures for the qualitative separation and detection of the various elements in the group in presence of the others; these are undoubtedly the most valuable part of the Report, as they supply the analyst with a ready means of deciding which reagent to use in any particular set of circumstances.

The second section deals with thirty-one anions together with elementary chlorine, bromine and iodine, and is followed by a table giving a résumé of the reagents of choice for the qualitative separation of the anions previously discussed individually.

Each metal or ion is treated in a strictly uniform manner; its atomic number and atomic, or ionic, weight are given, together with the name of the Committee-member responsible for the examination of the proposed tests. The recommended reagents follow, and for each is indicated the valency state of the element to which it responds, its reference number in the First Report, its formula and its name, the latter, in the case of inorganic compounds, being given according to the nomenclature adopted by the "Commission de Réforme de la Nomenclature de Chimie inorganique, 1940" (*Helv. Chim.-Acta*, 1940, **23**, 1012, and *Bull. Soc. chim.*, 1941, (5), **8**, 814), while for organic compounds the modern French system is followed, the named used in "Beilstein" being quoted where it differs materially from this and, in order that reference to original literature may be facilitated, the trivial name employed by the authors is given even where, in the opinion of the editors, this may be erroneous. A very brief outline of the mechanism of the reaction follows adequate bibliographical references, and the technique of the test is described as simply as possible. The sensitivity of each reagent is expressed as a limit of dilution and its specificity is discussed with careful indications as to the effect of the presence of allied elements on the sensitivity.

The bibliography, collected at the end of the Report, refers the reader, where possible, to *Chemisches Zentralblatt* and *Chemical Abstracts* as well as to the original sources. A comprehensive index of reagents is provided.

One feels that to criticise a work such as this would be presumptuous. The care and skill that have contributed to its production are manifest even to the casual reader. There are few errors and the whiteness of the paper and clearness of the type make the book a pleasure to use. Although, comparing it with the trilingual First Report, the Editors regret that the circumstances of the last few years have made it necessary to restrict this Second Report to the French language, there can be few analysts in this country who will find this an inconvenience. All whose work may require the intelligent application of micro-methods to the detection of traces of inorganic compounds will find in this volume reliable guidance and wise counsel.

JOHN ALLEN

COLLOIDS, THEIR PROPERTIES AND APPLICATIONS. By A. G. WARD, M.A. Pp. 133. London: Blackie & Son, Ltd. 1945. Price 5s. net.

As Mr. Ward points out in his Preface, there is an adequate textbook and monograph literature dealing with colloid science, but no very recent more elementary introduction to

the subject for those meeting it for the first time. His little volume is an attempt to fill the gap.

In three parts, the text deals with The Nature of the Colloidal State, The Colloidal Systems, and Colloids in Industry and in Living Matter. Although the treatment is necessarily cursory, it is sound and full of interest. Important basic facts and principles are laid down and on them is raised the story of industrial colloidal materials.

The information is up-to-date and should encourage any young chemist to see in colloid science a scheme which links together numerous and diverse phenomena of fundamental importance and utility in industrial operations covering many fields.

WILLIAM CLAYTON

SCIENCE AND NUTRITION. By A. L. BACHARACH. Pp. xii + 142. 2nd Edition. London: Watts & Co. Price 5s.

The exigencies of the recent war period have awakened or developed in many of those who fall within the difficultly definable category of "the man in the street" an interest in various branches of science, and particularly in those which relate to comfort, protection and well-being. It is, therefore, a wise decision of the publishers to issue a second edition of "Science and Nutrition" which, as the author makes clear in his first chapter, is mainly intended for the layman.

The skeleton of the book, that is the titles and sequence of the chapters, has not been altered but the subject matter has been revised and brought up to date. The book is divided into five Sections which are entitled respectively: "The Experimental Basis; Classical Nutritional Science; Minerals; Vitamins; Diet and Human Health." Section I comprises an introduction and a chapter on the use of animal experiments. Section II consists of seven chapters devoted to the chemistry and metabolism of carbohydrates, fats and proteins. Section III reviews in three chapters the major mineral elements, the hormones and iodine, and the trace elements. Section IV, which contains five chapters, deals with the nature of vitamins, their detection, measurement and identification, and the diseases which can arise from an inadequate vitamin intake. Section V has two chapters dealing respectively with the necessity for, and the absence of, the optimal.

Those portions of the book which deal with the vitamins of the B group, vitamin P, vitamin K and iron have been considerably extended by the addition of information which has been brought to light by investigations conducted since the publication of the previous edition. Apart from the inclusion of new matter, however, very few alterations to the original text have been called for, a fact which speaks well for the soundness of an exposition written at a period when riboflavine was known as lactoflavine, when lists of the members of the vitamin B complex made no mention of pantothenic acid or pyridoxine, but included an eluate factor and a filtrate factor, when the composition of vitamin K was unknown and when even the identification of nicotinic acid as a vitamin was not fully established!

It is vastly more difficult successfully to present the facts of a scientific subject to the layman than it is to prepare for one's professional brethren an erudite treatise on the same subject. Obviously, in the book for the layman the author must take care to avoid statements or explanations which assume in the reader a knowledge that the average man in the street is not likely to possess, but there is more in it than this. A bald statement of facts cemented by cold reasoning will suffice for the learned text book—and indeed the interpolation of only a few touches of lightness will be frowned upon by some readers as unwarranted levity—but the layman requires that his book shall first and foremost be "readable." The reader for whom "Science and Nutrition" is intended cannot quarrel with the author on this score. Mr. Bacharach's style and punctuation make for easy reading, and here and there one meets a light touch of humour which adds to the appeal of the book.

Bearing in mind that the object of this book is to instruct the non-scientific reader, is the simile of gloves the best means of explaining the differing ways in which linkage between amino acids can occur? It is true that Mr. Bacharach has corrected the simile as it appeared in the first edition by cutting off and sewing up the surplus fingers, but the reviewer feels that the layman would probably acquire a clearer picture of the problem, and that more readily, from a simple diagrammatic illustration.

The contradictory views which have been expressed in the numerous articles on nutrition and dietetics that have appeared in the press and in popular journals during the past seven

years have, not unnaturally, confused many members of the public, and they are inclined to feel with Hilaire Belloc:

"And all the world is torn and rent
By varying views on nutriment."

Mr. Bacharach's book is admirably suited to dispel this erroneous impression.

A. J. AMOS

A SCIENTIST IN THE CRIMINAL COURTS. C. AINSWORTH MITCHELL, M.A., D.Sc. Pp. 144. London: Chapman & Hall, Ltd. 1945. Price 8s. 6d.

It would be difficult to name a book with so much to commend it and so little in need of commendation.

The simple facts are that it is an account of some of the most interesting criminal cases in which Dr. Mitchell has given evidence—cases covering a wide range of subjects, murder, forgery, divorce, poison-pens, spies, indeed specimens from the varied menu of the King's Bench, Chancery, Probate and Criminal Courts, and even the Judicial Committee of the Privy Council. Its scientific merit lies not merely in the recording of quite a lot of information acquired by the author in the course of long years of experience, on ink, paper, handwriting, special forms of photography, and the technique of methods of examination and detection. There is much more; it presents, in most readable form, guidance from an acknowledged authority on the spirit and manner in which scientific evidence should be given. Were all scientists to give their testimony with the moderation, fairness and lucidity of Dr. Mitchell, the old gibes about experts and expert witnesses would be completely forgotten, and they would bid fair to become the most sought after and trusted source of evidence.

The revival of the ancient Witchcraft Act as a means of stopping professional spiritism gives point to the inclusion of an account hitherto unpublished of experience in testing the so-called human aura and of spirit writings. Dr. Mitchell refers to the possibility of evidence on these matters being required in Court.

So it will be seen that there is much interest, food for thought, and instruction in the volume. Add to this the fact that the author is the much-beloved member of our Society who has edited our Journal for about thirty years, that it is presented in the charming way which he always has in writing, and that it is dedicated to our much missed M.B.E. The result is a book which we shall all feel the urge to possess, and, possessing it, will treasure.

H. E. COX

NOTICE

to intending Applicants for Public Analyst Appointments

BEFORE applying for Public Analyst appointments, candidates are urged to make themselves acquainted with the recommendations as to salaries and fees approved by the Society. Enquiries should be directed to the Honorary Secretary of the Society.

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